UNIVERSITY OF NAIROBI

COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF *ZANTHOXYLUM PARACANTHUM* AND *ZANTHOXYLUM CHALYBEUM* FOR CYTOTOXIC PRINCIPLES AGAINST DRUG SENSITIVE AND MULTIDRUG RESISTANT LEUKEMIA CANCER CELL LINES

BY

GACHERU MARTIN MBOGO

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OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE

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DECLARATION

This research thesis is my original work and has never been presented for a degree in any higher institution of learning or University.

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ABSTRACT

Cancer statistics show that 13% of all death globally results from cancer. In Kenya, about 80,000 new cases of cancer are reported each year. From these new cases, about 50 people die every day as a result of cancer.

To manage cancer, there are various treatment modes including; surgery, immunotherapy, chemotherapy, phototherapy among others. However, chemotherapy is the most common.

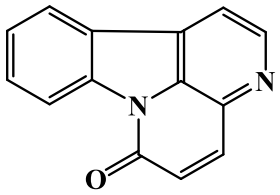
As much as chemotherapy is useful to the patient, it comes with adverse side effects like, nausea, vomiting, loss of appetite etc. To make it even worse there is emergence of multi drug resistance (MDR) cancer cell lines. This has made the treatment of cancer to be difficult due to treatment failures by contemporary drugs.

The extracts of *Zanthoxylum* species like *Z. gilletii* showed good activities against drug sensitive and drug resistant cancer cell lines and this motivated us to investigate other *Zanthoxylum* species. In this research work, the stem barks of *Zanthoxylum paracanthum* and *Zanthoxylum chalybeum* were examined for their chemical components and the compounds tested for their cytotoxicity against drug sensitive and MDR leukemia cell lines.

The stem bark material of *Z. paracanthum* was collected from Msambweni forest in Kwale County which is about 37 Km from Mombasa town, while that of *Z. chalybeum* was collected from Kakamega forest in Kakamega County which is about 10 Km from Kakamega town. The plant materials were identified by Mr. Patrick Mutiso a technologist with experience in plant identification techniques, from the School of Biological Sciences (SBS), University of Nairobi. The plant materials were then dried under shade for 1 week and then ground to fine particles. The ground materials of the two *Zanthoxylum* species were extracted exhaustively using 50% MeOH in CH_2Cl_2 to yield 97g (9.7%) crude extract of *Z. paracanthum* and 84g (9.3%) of *Z. chalybeum*.

The crude extracts were subjected to a combination of chromatographic techniques with varying solvent systems to yield four compounds from *Z. paracanthum* namely; an indole alkaloid, canthin-6-one (**1**), one benzophenanthridine alkaloid, dihydrochelerythrine (**2**), one terpenoid, lupeol (**3**) and one lignin, sesamin (**4**) and three compounds for *Z. chalybeum* namely; one new amide, 3-(1-isoprenoloxy)-4-methoxyfagaramide (**5**) and one known one, fagaramide (**6**) together with sesamin (**4**) that was also isolated from *Zanthoxylum chalybeum*.

The isolated compounds were tested for their cytotoxicity against drug sensitive and MDR leukemia cell lines using resazurin test (metabolic capacity of viable cells reduce resazurin dye to resorufin which is highly fluorescent). Canthin-6-one (**1**) exhibited the highest activities against both the drug sensitive and drug resistant leukemia cell lines, with only < 1% of the drug sensitive leukemia cells remaining viable (99% inhibition) and <3% resistant leukemia cells remaining viable (97% inhibition) at 10 µg/ml. The other compounds were considered inactive as they exhibited cell inhibition of less than 70% at 10 µg/ml in accordance to criteria evaluation of the cytotoxicity of pure compounds.



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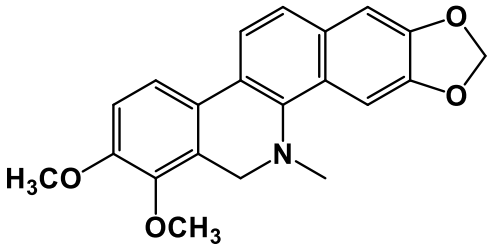

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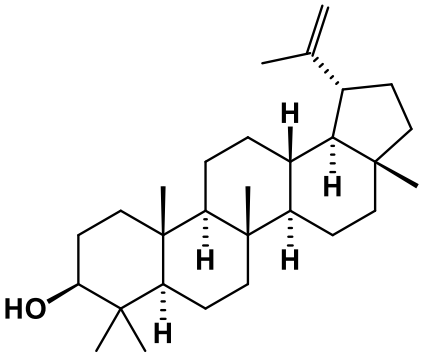

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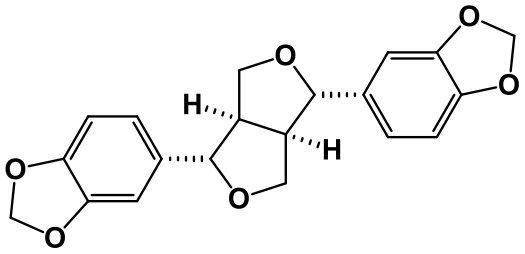

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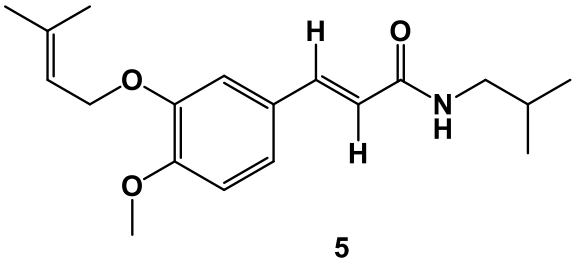

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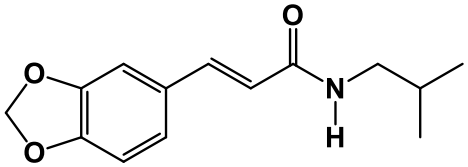

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DEDICATION

THIS THESIS IS DEDICATED TO MY LATE DAD AND MUM WHO DIED OF CANCER

ACKNOWLEDGMENT

I thank God Almighty for His grace throughout the entire project period. My gratitude goes to Dr. Leonidah K. Omosa, Professor Jacob O. Midiwo and Dr. Korir for their tireless intellectual guidance and support during the entire research period. My dear family; Ann, Brian and Christian, is highly appreciated for their moral support and encouragement in the writing of this project.

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In equal measure, Renee Munayi, Yosef Atilaw, Boniface Kamau, Ajuliu Kinyua, John Isemeki and Dickens Omondi are appreciated for their encouragement to carry out and complete the research work. Finally, but not least, special thanks to KEN02 for funding this project.

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LIST OF ABBREVIATIONS AND ACRONYMS

CC	Column Chromatography
$CDCl_3$	Deutrated trichloromethane
CH_2Cl_2	Dichloromethane
COSY	Correlation Spectroscopy
3D7	Artemether resistant strain
D6	Chloroquine resistant
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethylsulfoxide
DNP	Dictionary of Natural Product
EtOAc	Ethylacetate
GC/MS	Gas Chromatography/Mass Spectrometry
GDP	Gross Domestic Product
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Correlation
MHz	Mega hertz
IC_{50}	Half maximal inhibition Concentration
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
PTLC	Preparative Thin Layer Chromatography
SBS	School of biological sciences
TLC	Thin Layer Chromatography
TMS	Tetra methyl silane
UV	Ultra Violet
λmax	Maximum absorption wavelength
W2	Chloroquine sensitive
W.H.O	World Health Organization

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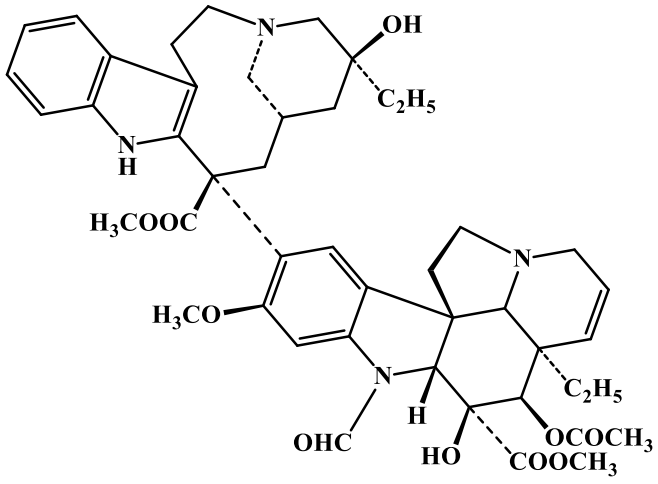
CHAPTER ONE

1.1 INTRODUCTION

Man have used natural products in dyes, flavors, and perfumes and also as the active components in traditional medicine mainly administered in form of tinctures, teas and herbal formulations (Balunas and Kinghorn, 2005). The most exploited practice of natural products remains in medicine [Osbourn and Lanzotti, 2009]. With advances in modern technology, it is conceivable to isolate and refine the active compounds from plants and use them as medicine either in their pure natural form or their derivatives (Ferguson *et al.*, 2004). As a result of their medicinal value, secondary metabolites mostly from plants which are traditionally used to manage tumors and related ailments are considered now as an essential source of anticancer drugs (Ferguson *et al.*, 2004). It is essential to note that the first anticancer drugs, the vinca alkaloids, vincristine (**7**) and vinblastine (**8**) were isolated from *Catharunun roseus* G. Don, a Mexican merigold plant leading to increased scientific interest in the quest of new anticancer medications templates from plants (Gueritte and Fahy, 2005).



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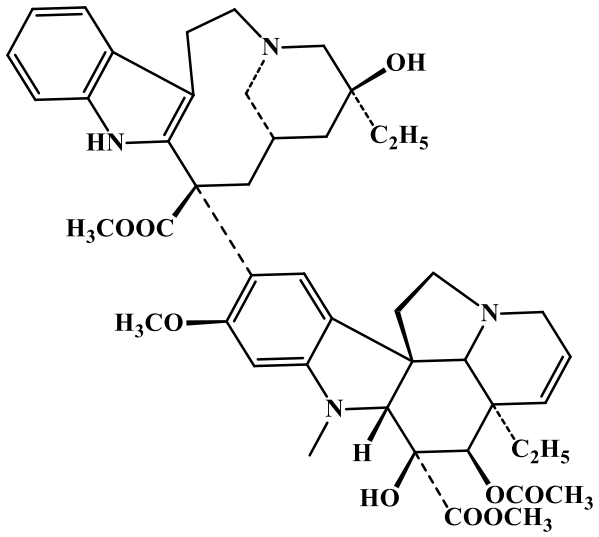

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In the early days, it was normal practice to refer to cancer as a hard swelling, calluses or warts (Cragg and Newman, 2005). Plants that have been used for management of certain tumors include *Bidens pilosa* (Kviecinski *et al.*, 2008) and *Cnidium monnierii* (Graham *et al.*, 2000). In Africa and most specifically Kenya, different communities use herbal medicine to manage numerous ailments including; tumors and related disorders presently referred to as cancer (Kareru *et al*, 2011). Incidentally, a very high percentage of these ethno medicinal trees and shrubs including; *Zanthoxylum parachanthum* and *Z. chalybeum* do not have scientific backup to support their traditional uses. Furthermore, their cytotoxicity and safety profiles are not known.

Traditional medicines were in the form of crude extracts contained in herbal formulations such as rations, tinctures and powders (Balunas and Kinghorn, 2005). Currently, man is inclining towards natural products since they are less toxic than synthetic forms.

There is a good distribution of the genus *Zanthoxylum* in humid and mild parts of the world accompanied by a good measure of their historical tradition medicinal use. There is proof of anti-malarial and anti-microbial activities in many *Zanthoxylum* species. *Z. acutifolium* (Mara *et al.*, 1992), *Z. chalybeum* (Gessler *et al.*, 1994), and *Z. rhoifolium* (Jullian *et al.*, 2006) have indeed shown credible anti-malarial activities. In Kenya alone, several *Zanthoxylum* species have been studied and remarkable results reported. From the previous studies, genus *Zanthoxylum* afforded benzophenanthridines, lignans, terpenoids and amides compounds among others. This current project investigated the anticancer profiles of the extracts and constituent compounds of *Z. paracanthum* and *Z. chalybeum* mostly administered traditionally by communities around the Coastal region of Kenya to cope with tumor and associated disorders. Bioassay was carried out against drug sensitive and multidrug resistant cancer cell lines.

1.2 STATEMENT OF THE PROBLEM

Recent researches have shown that cancer is among the most severe health complications in the world affecting both the developed and developing countries. The high costs in treatment and management of cancer as well as development of multidrug resistant (MDR) cancer that leads to recurrence of cancer, are challenges significantly felt by the developing countries. As a way of mitigating the menace, many patients have searched alternative cures from traditional herbal medicines. Though these herbal medicines are beneficial to cancer patients, it is important to investigate the cytotoxicity of the extracts against drug sensitive and MDR cancer cell lines. The study is thus a follow up on the ethno botanical survey of plants used by communities mostly along the coastal and western regions of Kenya to cure and or manage cancer and its related disorders.

1.3 JUSTIFICATION

Worldwide, due to effective prevention and early detection of cancer, there is notable achievement in the management of cancer in the last few years. However, available treatment options mainly chemotherapy have been rendered ineffective due to the unusual behavior of the cancerous cells to develop resistance to the treatment causing a major challenge (Prakash *et al.*, 2013). It has been recorded that most victims have recurrence of cancer even after a prior successful treatment using chemotherapy (Saraswathy and Gong, 2013; Prakash *et al.*, 2013). Natural products have thus been suggested as leads to overcome MDR in cancer cells (Kuete *et al.*, 2014). There is need to evaluate the potential of the stem barks of *Zanthoxylum parachanthum* and *Zanthoxylum chalybeum* used concurrently with other plant extracts by communities mostly in the coastal and western regions of Kenya to manage tumors and related ailments to treat MDR cancer.

1.4 OBJECTIVES

1.4.1 Main objective

To determine the cytotoxicity of constituent compounds of *Zanthoxylum parachanthum* and *Zanthoxylum chalybeum* towards drug sensitive and multidrug resistance cancer cell lines.

1.4.2 Specific objectives

1. To characterize the compounds from the stem bark of *Zanthoxylum parachanthum* and *Zanthoxylum chalybeum* using 1D and 2D NMR and Mass Spectroscopy.
2. To determine the cytotoxicity of the isolated compounds for anticancer potential against drug sensitive and multidrug resistant leukemia cell lines.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background Information on Cancer

Cancer can be broadly referred to as a group of diseases that are characterized by abnormal cell growth resulting into tumors. Tumors may be benign (do not affect the nearby tissue or spread to other tissues) or malignant. Where these tumors are malignant, they have the potential to metastasis and affect normal cells (Hunter, 2008).

The escalation in cancer cases has not been fully established but it has been attributed to diet, environment and genetic disposition where majority of cancer cases are linked to lifestyle changes including; cigarette smoking, dietary changes, excessive exposure to solar light, the existence of acrylamide, unnecessary use of synthetic aroma accompaniments, pigments and food seasoning in general have been advocated as potential contributors to growing cases of cancer and heart diseases (Pact Kenya Cancer Assessment in Africa and Asia, 2010). Since cancer is a group of diseases, there is no definitive list for cancer symptoms as they are varied and depend on the specific type of cancer that an individual has acquired (National Cancer Institute- US, 2015). Among the challenges developing nations face in combating cancer is inventing convincing strategies for building sustainable cancer control capacity. Cancer management goes outside the identification and treatment of cancer. It also embraces cancer prevention, regulation planning, surveillance and cancer records, early detection and reassuring care. Some of the cancer treatment methods that are commonly used include surgery, chemotherapy, radiotherapy and psychosocial support (Pact Kenya Cancer Assessment in Africa and Asia, 2010). Radiotherapy plays a key function in the management of cancer along with chemotherapy and surgery. Indeed, more than 50% of all cancer patients need radiotherapy at some point in the course of their illness, but regrettably, a probable unavailability of about 5,000 radiotherapy apparatus in developing nations, lead to 70% of cancer victims existing in these parts being unable to enjoy this critical therapeutic or pain relieving cure (AGaRT, 2009).

It is alarming to note that there are statistics estimate that cancer is an increasing health challenge and accounts for more deaths than HIV/AIDS, malaria and tuberculosis combined (Kerr and Midgley, 2010).

2.1.1 Estimated Incidence, Mortality and 5 Year Prevalence of Cancer

According to World Health Organization (2015) body (International Agency Research on Cancer) the estimated cancer incidences; mortality and 5 year prevalence in both sexes in the United States of America were as follows.

Incidence; lung-13%, breast-11.9%, colorectum-9.7%, prostate-7.8%, stomach-6.8%, liver-5.6%, cervix uteri-3.8%, oesophagus-3.2%, bladder-3.1%, others-35.3%, total: 14 067 894

Mortality; lung-19.4%, liver-9.1%, stomach-8.8%, colorectum-8.5%, breast-6.4%, oesophagus-4.9%, pancreas-4%, prostate-3.7%, cervix uteri-3.2%, others-32%, total: 8 201 575

5-year prevalence; breast-19.2%, prostate-11.9%, colorectum-10.9%, lung-5.8%, cervix uteri-4.8%, stomach-4.7%, bladder-4.1%, corpus uteri-3.7%, thyroid-3.7%, others-31.1%, total: 32 455 179.

Indeed, 60% of all deaths that occurred in 2005 were attributed to chronic ailments such as cancer, diabetes and heart sicknesses. About 80% of which occurred in low and middle income nations. An aggregate of 1,658,370 new cancer cases and 589,430 cancer demises were anticipated to happen in the United States in 2015 (American Cancer Society, 2015).

Africa, which is among the low and middle income countries, reported 715,000 new cases of cancer in the year 2008 (Ferley *et al.*, 2008). These numbers have been projected to double mainly due to the aging and growth of the population (Ferley *et al.*, 2008). In Kenya today, cancer deaths are at third place after communicable heart diseases causing 7% of the total national mortality yearly (National cancer control strategy, 2011-2016).

2.2 Carcinogenesis

Carcinogenesis simply is defined as a process through which a normal cell undergoes many changes and becomes a cancerous cell (Reddy, 2003). This involves permanent changes to DNA called mutations in a normal cell causing the cell to by-pass all natural defensive body mechanisms, multiply without control forming a mass of abnormal cells called a tumor (American Cancer Society, 2015). Benign tumors are non-cancerous growths unlike malignant tumors which damage nearby cells or organs thereafter establishing a secondary tumor through a process called metastasis

(Hunter, 2008). Carcinogenesis occurs in numerous stages including; tumor initiation, promotion and progression over a period of many years (Reddy, 2003).

2.3 Drug Sensitive and Multidrug Resistance in Cancer Cells

Normal cells ordinarily respond to cytotoxic drugs. However, cancer cells do not respond to cytotoxic but rather develop resistance. In most cases, combined treatment is prescribed to cancer patients, which unfortunately lead to occurrence of drug resistant cancer resulting to reduced ability of the present anticancer agents and hence therapeutic failure (Kuete *et al.*, 2013). Multidrug resistance (MDR) cancer can occur as a result of genetic disposition or through successive treatments leading to recurrence of cancer even after a previous successful treatment (Efferth, 2001). MDR through successive treatment is believed to largely occur because chemotherapy kills drug sensitive cancer cells reducing their number and in effect increasing the number of drug resistance cells (Kuete *et al.*, 2014).

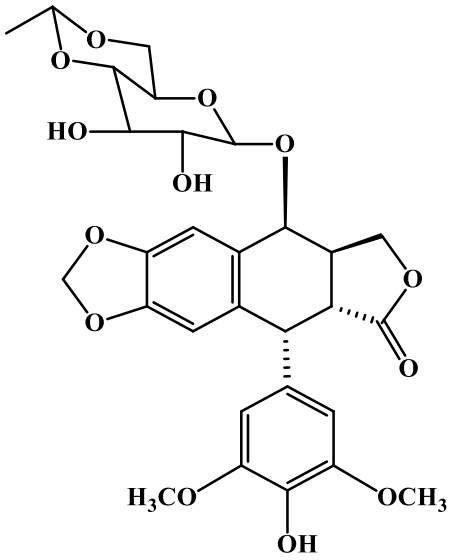
When the tumour starts to grow again, chemotherapy is no longer effective since the tumor cells are drug-resistant (Nature America Inc. 2000). Most chemotherapy use antibiotics, antitumor, antimetabolites, alkylating agents, natural anticancer agents and platinum analogues. There are several theories allied to development of MDR in cancer cells. However, the outstanding research associate protein transporters known as adenopsine triphosphate binding cassette (ABC) (Efferth, 2001). The main role of ABC in the body is detoxification (Szakacs *et al.*, 2006). It is suggested that MDR develops due to over-expression of an energy defendant efflux pump known as P-glycoprotein (P-gp) which is affiliated to the larger ABC transporters (Hyde *et al.*, 1990).

2.4 Natural Products Based Anticancer Agents

A substantial number of flora-derived anti-cancer agents are in clinical use today. A summary of some anticancer drugs from plant source in clinical use are shown in the Table 2.1 below.

Table 2.1: Plant based anticancer agents**.**

Anticancer agent	**Source**	**Mechanism of action**
Vincristine (**7**)	*Catharanthus rosea*	Interferes with microtubule assembly
Vinblastine (**8**)	*Catharanthus rosea*	Interferes with microtubule assembly
Etoposide (**9**)	*Podophyllum peltatum* semisynthetic	Topoisomerase II inhibitor
Teniposide (**10**)	*Podophyllum peltatum* semisynthetic	Topoisomerase II inhibitor
Flavopiridol (Alvocidib) (**11**)	Rohitukine semisynthetic, a flavonoid from *Dysoxylum binectariferum*	Inhibits cyclin-dependant kinases.
Rotenone (**12**)	Several *papilionoideae* plants	Used in combination with vinblastine
Docetaxel (**13**)	Paclitaxel derivative from *Yew weed*	Disrupts de-polymerization of microtubule.
Ixabepilone (**14**)	Epothilone B derivative from mycobacterium *Sporangium cellulosum*	Disrupts de-polymerization of microtubule.
Combretastatin A-4 (**15**)	*Cambretum caffrum*	Inhibits tubulin polymerization
Englerin A (**16**)	*Phyllanthus engleri*	Induction of necrotic cell death.
Doxorubicin (**17**)	*Streptomyces peucetius*	DNA intercalating agent



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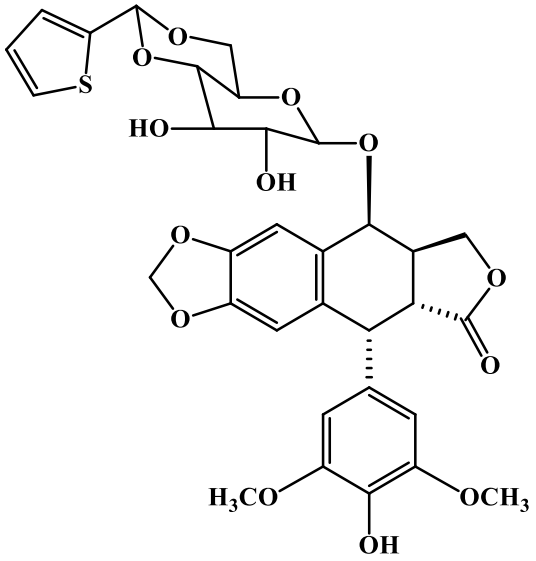

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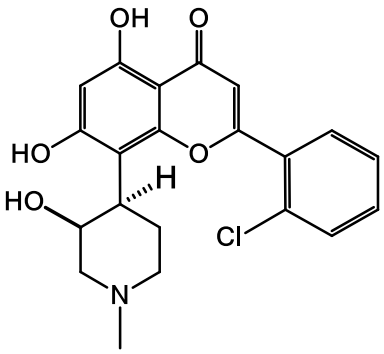

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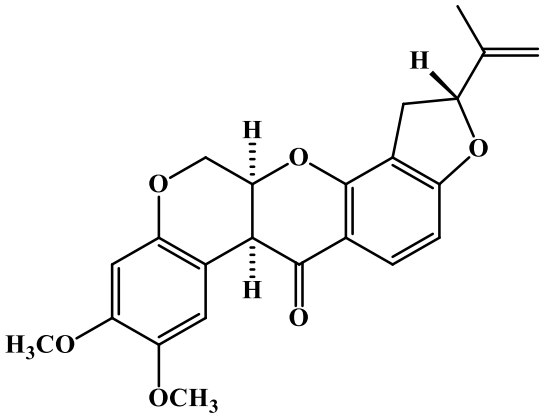

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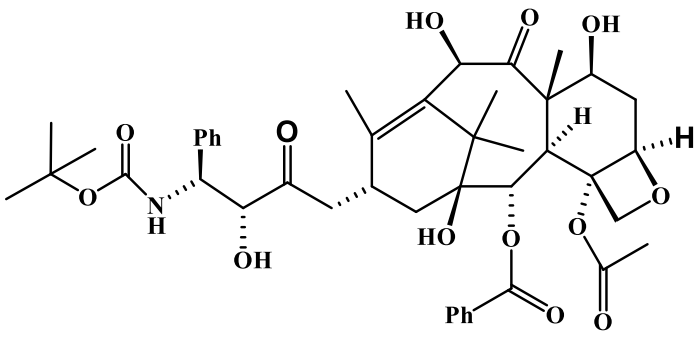

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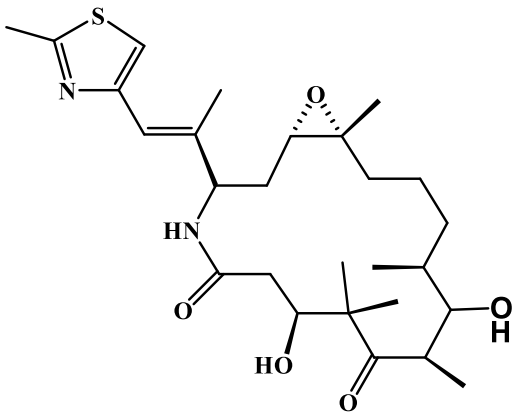

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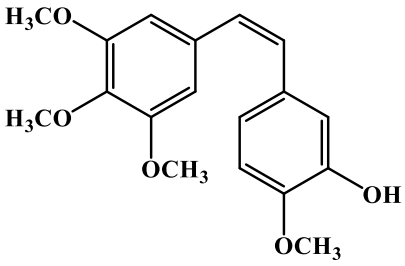

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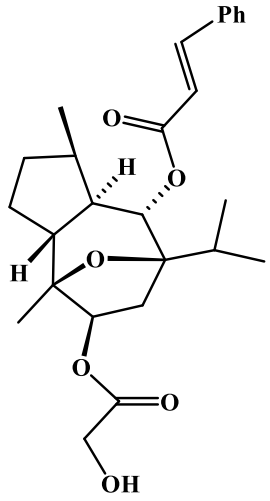

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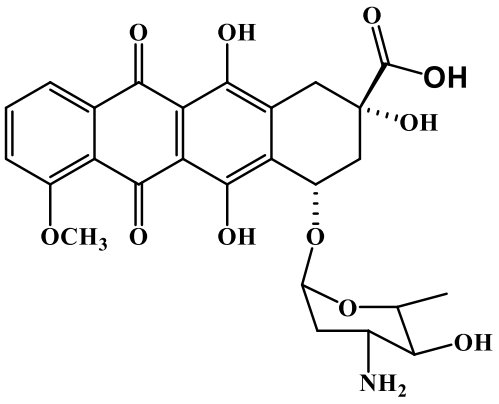

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2.5 The Genus *Zanthoxylum*

The generic name, *Zanthoxylum* came from the Ancient Greek word ξανθός (*xanthos*), meaning "yellow," and since most of these plants are trees and shrub the word ξύλον (*xylon*), meaning "wood" was added. *Z. paracanthum* and *Z. chalybeum* belongs to the genus *Zanthoxylum* belonging to the family Rutaceae, subfamily Rutoideae in the tribe Zanthoxyleae with two taxa- Fagara L and *Zanthoxylum*. The genus Fagara L contains over 240 species, while *Zanthoxylum* has only 15 species. In 1966, Hartley clustered *Zanthoxylum* and Fagara under the name of *Zanthoxylum*. Nevertheless, some writers still use the term Fagara (Chaaib, 2004).

2.5.1 Distribution of the genus *Zanthoxylum*

About 549 species of *Zanthoxylum* are spread globally mostly in humid and temperate regions (Global Biodiversity Information Facility, 2010). In Eastern Africa, seven species are reported which are mainly found in wet or dry woodlands and also in the vegetation near the sea. These species include; *Z. gilletii* (De wild), *Z. paracanthum* (mildbr) and *Z. rubescens* (Beentje, 1994), var *Z. chalybeum* (Engl.), *chalybeum*, *Z. holstzianum* (Engl.) Waterman, *Z. usamarense* (Engl.) Waterman, *Z. mildbraedii* (Engl.) Waterman. In Kenya, *Z. paracanthum* is largely found in Msambweni forest along the coastal region while *Z. chalybeum* is widely found in the western part of Kenya including Kakamega forest.

2.5.2 Ethno-botanical use of the genus *Zanthoxylum*

Traditionally, the *Zanthoxylum* genera has diverse ethnobotanical uses that have since been reported. In general, *Zanthoxylum* is used as sources of pharmaceutical and cosmetics raw material (Bafi-Yeboa *et al.*, 2003). In this genus, spices are prepared from *Z. simulans,* Z. *piperitum, Z. schinifolium,* Z. *bungeanum* among other species. Sichuan pepper is usually prepared by crushing the husks that surround *Z. piperitum* berries. *Zanthoxylum* fruits have several uses including; curing asthma and bronchitis, treating cardiovascular diseases, throat and teeth disorders, diarrhea and piles (Kritikar and Basu, 1983). The leaves and root bark of many species are used to prepare drugs to cure tooth-ache, stomachache, leprous ulcerations, coughs, rheumatism, urinary and veneral diseases (Oliver- Bever, 1982). The root bark and the leaves of *Zanthoxylum* species are exploited in the cure of different diseases which include; coughs, urinary infections, venereal diseases, upset stomach, toothaches, rheumatism and leprous ulcerations (Negi *et al.*, 2011). In brief, some of the traditional practices of the genus are summarized in Table 2.2 below.

Table 2.2: Main ethno botanical uses of some *Zanthoxylum* species

Species	Part used	Popular uses	Form of administration	References
Z. acanthopodium	Fruits	Used as spices for relieve of tooth ache and stomach ache	Oral route	Suryanto *et al.*, 2004
Z. ailantoides	Leaves	Used as relief of common cold, to treat rheumatism, arthralgia, stasis, snake bites and as blood circulation stimulant.	Local and Oral route	Cheng *et al.*, 2004
Z. armatum	Fruit and seed	Used as an aromatic tonic in the treatment of fever dyspepsia and cholera	Oral route	Sati *et al.*, 2011
Z. capense	Leaves	Used for the treatment of fever, tooth ache and epilepsy.	Oral route	Amabeoku & Kinyua, 2010
Z. chalybeum	Leaves	Used to relief snake bite, to treat oedema in kwashiorkor, severe colds and pneumonia.	Local and Oral routes	Dharani *et al.*, 2010; Kamikawa *et al.*, 1996
	Bark	Used for the treatment of malaria, colds, coughs and dizziness from decoctions. Chewed for the treatment of toothaches, asthma and tuberculosis.	Oral route	Kokwaro, 2009
	Roots	Used for the treatment of malarial, toothache, colds, headache and wounds.	Local and Oral routes	Nguta *et al.*, 2010
Z. chiloperone	Root bark	Used as anti-malaria and anti-rheumatism.	Oral route	Ferreira *et-al*, 2002
Z. elephantiasis		Used for the treatment of chest diseases, diarrhea, intermittent fever, teeth diseases and ear aches.	Oral route	Diequez *et al.*, 2003
Z. gilletii	Bark	Used for the treatment of stomachache, joint pain, toothache, fever, rheumatism, venereal	Oral route	Dharani *et al.*, 2010; Kokwaro, 2009

		infections and for washing wounds.		
Z. leprieurii		Used for treatment of gonorrhea, kidney pain and sterility	Oral route	Tatsadjieu *et al*., 2003
Z. limonela	Fruit oil	Used as anthelmintic and for gastro-intestinal stimulant effects, treatment of wounds and for digestion enhancement.	Oral route	Sati *et al*., 2011; Setzer *et al*, 2004
Z. pistaciiflorum	Leaves, Bark and fruit	Used to relieve headaches and for poisoning fish	Local Oral route	Chen *et al*., 2004
Z. rhoifolium	Bark	Used to treat earache, toothache, in the treatment of hemorrhoids, anti-tumor and as an anti-venom serum.	Oral route	da Silva *et al*., 2007a
Z. schinifolium	Leaves and ripe pericarp	Treat epigastric pain	Oral route or decoction powder	Cao *et al*., 2009 Cui *et al*., 2009 Chang *et al*., 1997

2.5.3 Biological activities of the genus *Zanthoxylum*

Several other studies have revealed that plants in the genus *Zanthoxylum* have useful bioactivities due to the presence of essential oils and alkaloids. Such bioactivities include: antiplasmodial, antiviral, larvicidal, antinociceptive, analgesics, anthelmintic, anti-viral, antioxidant, anti-fungal, antibiotic, and anti-inflammatory and cytotoxicity (Barnabas *et al.*, 2011; Chen *et al.*, 2007, 2008; Guo *et al.*, 2011; Lee and Lim, 2008; Amabeoku and Kinyua, 2010; Ross *et al.*, 2004; Chou *et al.*, 2011; Song *et al.*, 2010; Yang and Chen, 2008; Islam *et al.*, 2001a, b; Gansane *et al.*, 2010;). Some of the bioactivities are illustrated in Table 2.3 below:-

Table 2.3: Biological Activities of some *Zanthoxylum* species

Species	Plant part	Extract	Activity	Reference
Z. xanthoxyloides	Root bark	Purified alkaloids	Analgesic	Prempeh & Mensah-Attipoe, 2008
Z. capense	Leaves	Methanol and aqueous	Anticonvulsant	Amabeoku & Kinyua, 2010
Z. xanthoxyloides	Leaves	Acetone : water (70:30) and ethanol	Antihelmitic	Azando *et al.*, 2011; Barnabas *et al.*, 2011
Z. tetraspernum	Stem bark	Benzophenanthridine alkaloids	Anti-bacterial	Negi *et al.*, 2011; Tatsadjieu *et al.*, 2003
Z. rhoifolium	Leaves	Alkyl amides	Anti-tumor	Negi *et al.*, 2011; Tatsadjieu *et al.*, 2003
Z. integrifolium and *Z. avicennae*	Stem	Methanol	Anti-inflammatory	Chen *et al.*, 2008
Z. armatum	Fruit	Ethanol	Antibacterial	Panthi & Chaudhary, 2006
Z. chalybeum and *Z. usambarense*	Root and stem-bark	Aqueous, hexane and methanol extracts	Antibacterial	Matu & Staden, 2003
Z. americana	Whole plant	Extracts	Anti-fungal	Negi *et al.*, 2011; Tatsadjieu *et al.*, 2003
Z. syncarpum	Leaves	Alkamides	Anti-plasmodial	Ross *et al.*, 2004
Z. piperitum	Fruits	Methanol	Antioxidant	Yamazaki *et al.*, 2007

2.6 Phytochemistry of the genus *Zanthoxylum*

Scholarly research has confirmed the presence of minor metabolites which are alkaloids in nature from the genus *Zanthoxylum*. Other classes of compounds that are most common from this genus being; coumarins, amides, lignans, flavonoids, terpenes and sterols (Adesina, 2005; Patiño, 2004; Waterman and Grundon, 1983).

2.6.1 Alkaloids from the genus *Zanthoxylum*

Alkaloids in the genus *Zanthoxylum* manifest in all plant parts, but mostly in the root bark and stem (Dieguez *et al.*, 2003). These are secondary metabolites compounds which contain nitrogen and generally have low molecular weights. There are mainly two types of alkaloids isolated from the genus namely: quinolines and isoquinolines (Krane *et al.* 1984; Waterman & Grundon, 1983; Cordell, 1981). Below is a detailed discussion of the two types of alkaloids.

2.6.1.1 Quinoline

The presence of quinoline alkaloids in the genus *Zanthoxylum* are very common.

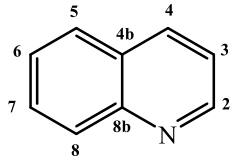
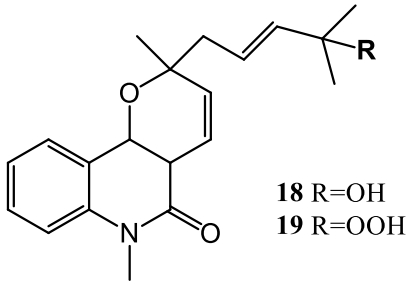


Figure 2.1: Basic structure of quinoline

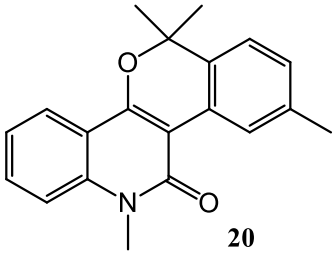
These alkaloids have been found to be of two types: pyranoquinolines and furoquinolines.

2.6.1.1.1 Pyranoquinolines

Pyranoquinolines alkaloids that have been isolated from *Zanthoxylum simulans* are simulenoline (**18**), peroxysimulenoline (**19**) and benzosimuline (**20**)



18 R=OH
19 R=OOH



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2.6.1.2 Isoquinolines

There are several types of isoquinolines isolated from the genus *Zanthoxylum*.

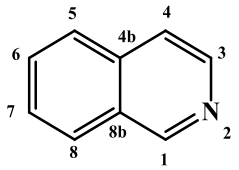


Figure 2.2: Basic structure of isoquinoline

The most common are discussed below.

2.6.1.2.1 Benzophenanthridine

The *Zanthoxylum* genera is very rich in benzophenanthridine which are useful alkaloids due to their varied biological activity including antitumor (Simeon *et al.*, 1989; Slaninová *et al.*, 2001; Tang *et al.* 2003; Eun & Koh, 2004; Nyangulu *et al.*, 2005; Dvorak *et al.*, 2006; Tillequin, 2007; Maiti & Kumar, 2007; Maiti & Kumar, 2009).

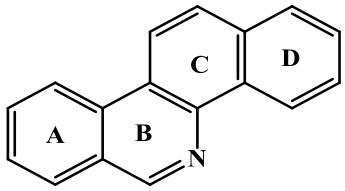
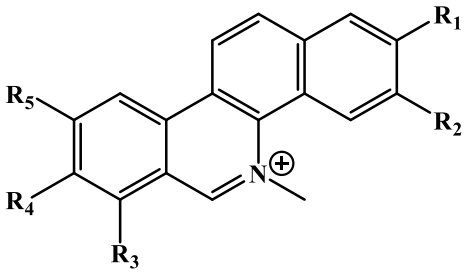
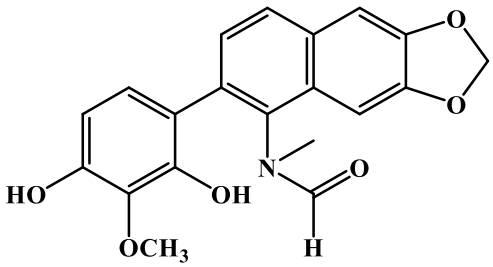


Figure 2.3: Basic structure of benzophenanthridine

Benzophenanthridine are represented by the following alkaloids; chelerythrine (**21**), fagaronine (**22**), sanguinarine (**23**), and nitidine (**24**). Interestingly, iwamide (**25**) and integriamide (**26**) are compounds with similar chemical structure and have found themselves categorized by diverse authors as benzophenanthridine alkaloids (Krane *et al.*, 1984).



21: R_1 =R_2 = OCH_2O; R_3 =R_4 = OCH_3; R_5 =H
22: R_1=OH; R_2=OCH_3; R_3=H; R_4=R_5=OCH_3
23: R_1 =R_2 =R_3 =R_4 = OCH_2O; R_5 =H
24: R_1 =R_2 = OCH_2O; R_3 = H; R_4 =R_5 = OCH_3



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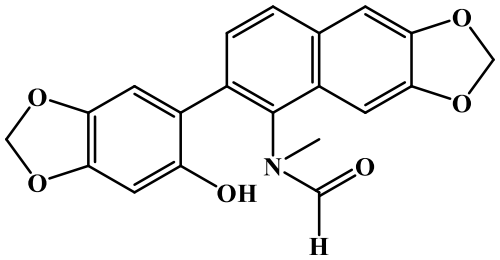

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2.6.1.2.2 Aporphine

Isoquinolines alkaloids comprises of aporphine skeleton which are derived from *L*-tyrosine (Kuo *et al.*, 2012). Approximately seven aporphine alkaloids have been isolated and characterized from the genus *Zanthoxylum* by year 2010 (DNP, 2011). Substitution on the basic structure takes place mainly at C-1 and C-2; positions 3, 8, 9, 10 and 11 mainly contain OCH_3 and OH (Kuo *et al.*, 2012). Some of the aporphine alkaloids of the genus *Zanthoxylum* are shown in table 2.4 below.

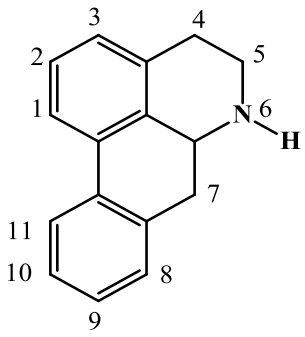
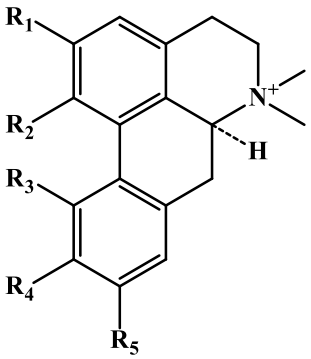


Figure 2.4: The core structure of aporphine

Table 2.4: Aporphine alkaloids of the genus *Zanthoxylum*

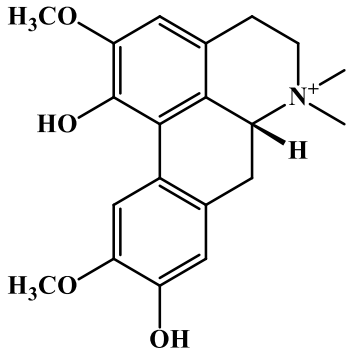
Compound	**Plant source**	**Reference**
Zanthoplanine (**27**)	*Z. planispium*	Ishii *et al.*, 1961
Laurifoline (**28**)	*Z. elephantiasis*	Hufford *et al.*, 1976
Methylcorydine (**29**)	*Z. nigrescens*	Hufford *et al.*, 1976
Liriodenine (**30**)	*Z. simulans*	Hufford *et al.*, 1976
N-acetyldehyderoanonaine (**31**)	*Z. simulans*	Chen *et al.*, 1996
N-Acetylanonaine (**32**)	*Z. bungeanum*	Chen *et al.*, 1996
Zanthoxoaporphine A (**33**)	*Z. parachanthum*	Fideli *et al.*, 2013

Zanthoxoaporphine B (**34**)	*Z. parachanthum*	Fideli *et al.*, 2013
Zanthoxoaporphine C (**35**)	*Z. paracanthum*	Fideli *et al.*, 2013



27: R_1, R_4, $R_5 = OCH_3$; $R_2 = OH$; $R_3 = H$

29: R_1, R_3, $R_4 = OCH_3$; $R_2 = OH$; $R_5 = H$



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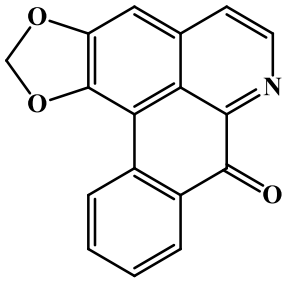

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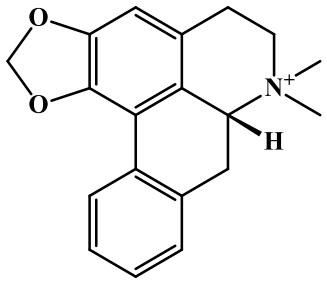

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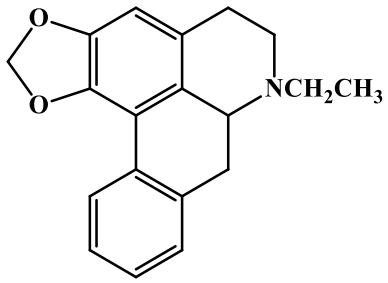

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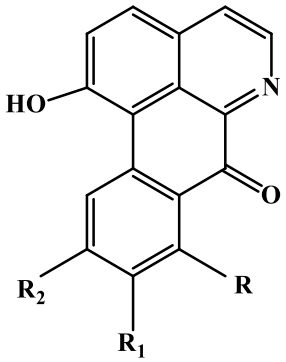


33: $R=R_1=H$, $R_2=OCH_3$

34: $R=R_1=R_2=H$

35: $R=OH$, $R_1=OCH_3$, $R_2=H$

2.6.1.2.3 Protoberberine

The protoberberine alkaloids are biogenetically derivative from tyrosine pathway with 5,6-dihydrodibenzo quinolizinuim ($C_{17}H_{14}N^+$) forming the skeleton of the quaternary protoberberine alkaloids (Lenka *et al.*, 2007; Liscombe *et al.*, 2005). Its basic structure is as shown in Figure 2.5 below.

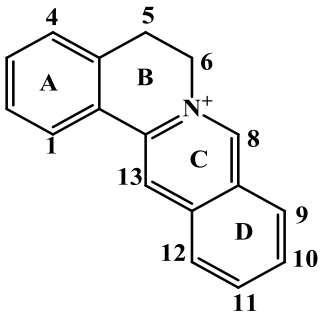
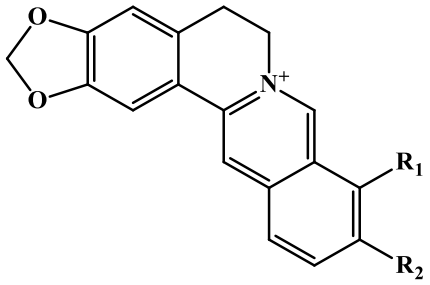


Figure 2.5: The basic structure of Protoberberines

Protoberberines have been isolated from many herbal families such as Fumariaceae Papaveraceae, Rutaceae, Menispermaceae, Berberidaceae, Annonaceae, Ranunculaceae, with other scarce instances in Magnoliaceae and Convolvulaceae (Bentley, 1999-2006) families. Berberine (**36**) is the main alkaloid that can be converted to many other protoberberine derivatives. Some of the notable examples of protoberberine alkaloids from the genus *Zanthoxylum* include; berberine (**36**) from *Z. chiloperone* (Ferreira *et-al*., 2002), berberrubine (**37**) and coptisine (**38**) from *Z. nitidine* (Jiang *et al.*, 2007). These alkaloids contain a methylenedioxy at C-2, C-3 on ring A and oxygenation takes place at C-9 on ring D.



36: R_1, R_2 = OCH_3

37: R_1, R_2 = OCH_2O

38: R_1 = OH; R_2 = OCH_3

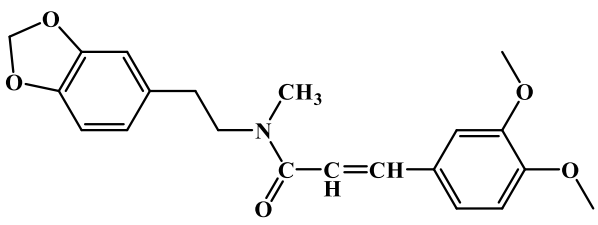
2.6.1.2.4 Amide

Amides in the genus *Zanthoxylum* have chemotaxonomic importance and are isolated from the pericarp of the fruit, roots and stems. Olefinic alkamides (unsaturated aliphatic acid amides) are common in the genus *Zanthoxylum* and are used as antitussive, sialagogues and analgesic (Adesina, 2005; Chaaib, 2004). Aromatic amides which are usually described as *trans*-cinnamoylamides have also been reported in this genus. These include *N*-methyl, *N*-(3, 4- methylenedioxyphenylethyl), 3',4-dimethoxycinnamoylamide (**39**), *N*-(3, 4-methylenedioxyphenylethyl)-3'-4'-dimethoxycinnamoylamide (**40**) and *N*-(3, 4-dimethoxyphenylethyl), 3', 4'-methylenedioxycinnamoylamide (**41**). From Z. *syncarpum*, an active compound against plasmodia

species named syncarpamide (**42**) was isolated. α-sanshool (**43**) is an example of an amide isolated from Z. *liebmannianum* species with anthelmintic properties (Navarrete & Hong, 1996). Other amide alkaloids isolated from the genus *Zanthoxylum* include; herclvine (**44**), lanyuamide (**45**), 3-methoxyaegeline (**46**), *O*-methyl tembamide (**47**) and (+)-tembamide (**48**).

Table 2.5: Amides of the genus *Zanthoxylum*

Compound	**Source plant**	**Plant part**	**Reference**
N-methyl, *N*-(3,4-methylenedioxyphenylethyl),3',4-dimethoxycinnamoylamide (**39**),	*Zanthoxylum rubescens*	Stem bark	Adesina, 1989
N-(3,4-methylenedioxyphenylethyl)-3'-4'-dimethoxycinnamoylamide (**40**)	*Zanthoxylum rubescens*	Stem bark	Adesina, 1989
N-(3,4-dimethoxyphenylethyl), 3',4'-methylenedioxycinnamoylamide (**41**)	*Zanthoxylum rubescens*	Stem bark	Adesina, 1989
Syncarpamide (**42**)	*Z. syncarpum*		Ross *et al.*, 2004
α-Sanshool **(43)**	*Z. liebmannianum*	Fruit	Navarrete & Hong, 1996
Herclvine **(44)**	*Zanthoxylum ssp*	Stem bark	Kashiwanda *et al.*, 1997
Lanyuamide (**45**)	*Z. integrifoliolum*	Stem bark	Sheng *et al.*, 1999
3-Methoxyaegeline **(46)**	*Z. syncarpum*	Leaves	Samir *et al.*, 2005
O-Methyl tembamide (**47**)	*Z. ailantoides*	Root bark	Cheng *et al.*, 2005
(+)-Tembamide **(48)**	*Z. ailantoides*	Root bark	Cheng *et al.*, 2005



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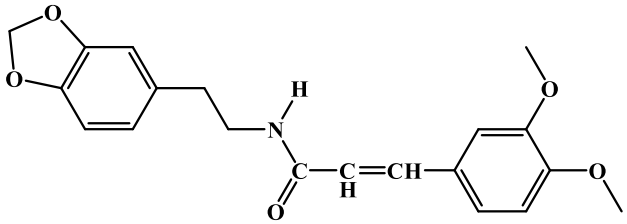

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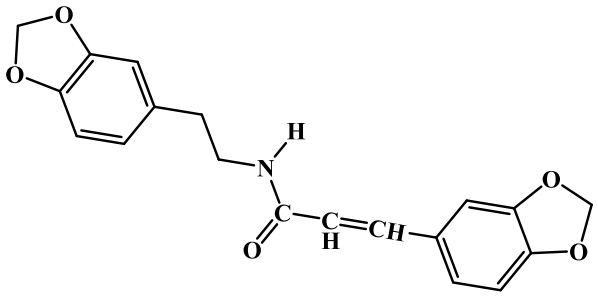

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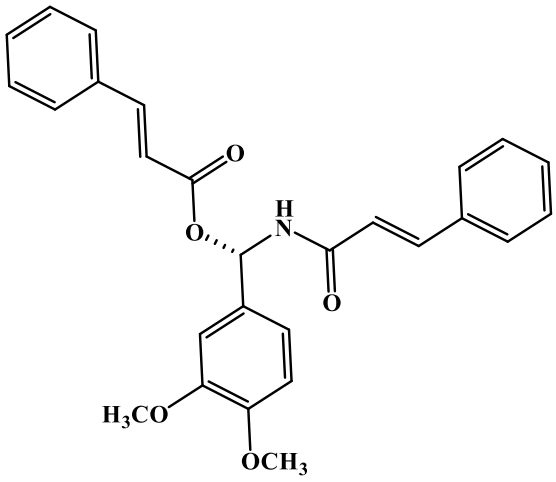

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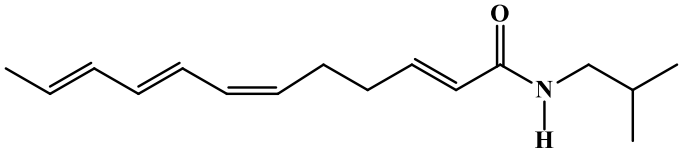

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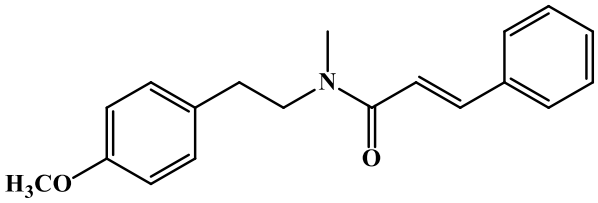

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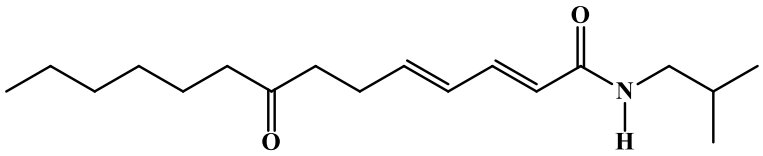

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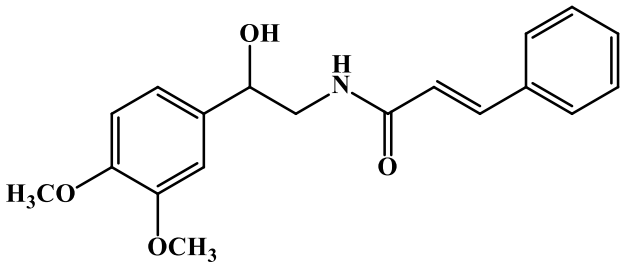

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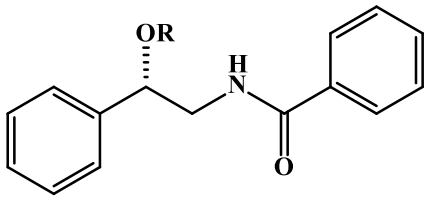
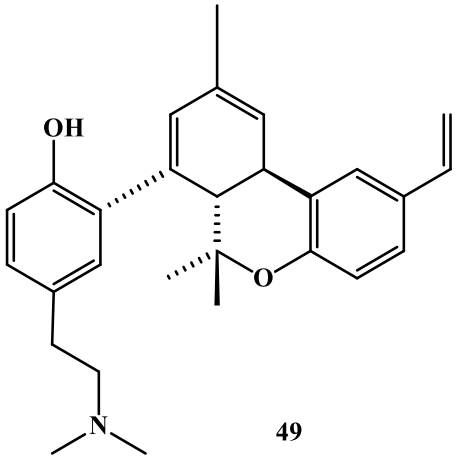


47: R = CH_3

48: R = OH

2.6.1.3 Other alkaloids from the genus *Zanthoxylum*

Bishoderninyl terpene (**49**), canthin-6-one (**1**), carbazole (**50)** and quinazoline (**51)** alkaloids are rare compounds in the genus *Zanthoxylum*. However, there is evidence of bishordeninyl terpene alkaloids isolated from the leaves of *Z. integrifoliolum* (Liu *et al*, 2000).



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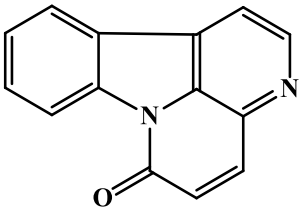

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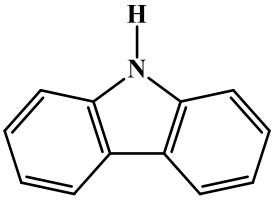

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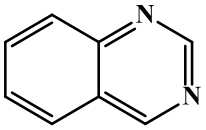

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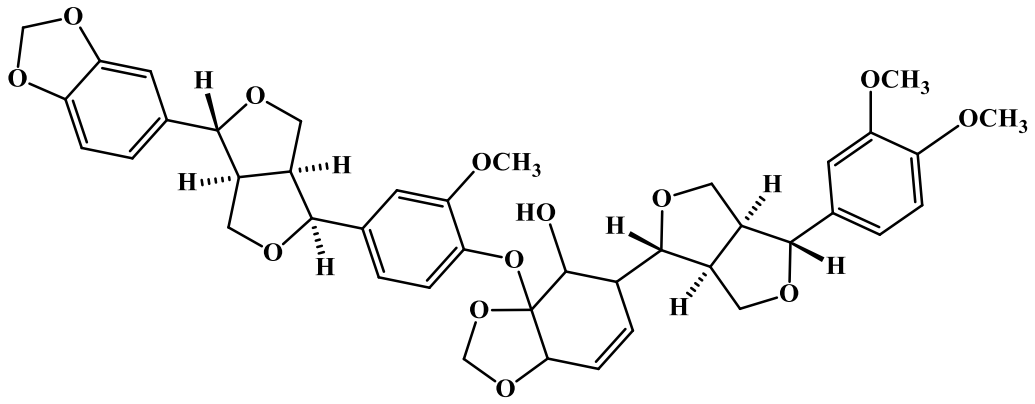
2.6.2 Lignans from the genus *Zanthoxylum*

Lignans are significant due to their variable biological activities such as; antioxidant, antiviral, microbial, antitumour, ant tuberculosis, insecticides and also their ability to inhibit specific enzymes (Chen *et al.*, 1996). The two types of lignans mainly reported from the genus *Zanthoxylum* are two, 6-diaryl-3, 7-dioxyabicyclo [3.3.0] octanes and diarylbutirolactones.

Table 2.6: Lignans from the genus *Zanthoxylum*

Compound	**Plant source**	**Reference**
Zanthpodocarpin **(52**)	*Z. podocarpum*	Zhou *et al.*, 2011

7,9′-Epoxylignan (**53**)	*Z. culantrillo*	Luis *et al.*, 1998
Eudesmin (**54)**	*Z. armatum*	Guo *et al.*, 2011
Magnone A (**55**)	*Z. podocarpum*	Xiao *et al.*, 2011
Syringaresinol (**56**)	*Z. quinduense* and *Z.monophyllum*	Patiño and Cuca, 2011
(+)-Sesamin (**4**)	*Z. budrunga (Rutaceae)*	Mukhlesur *et al* 2003
(-)-Simulanol (**57**)	*Z. simulans* (stem bark)	Yang *et al.*, 2002
(-)-Cubecin (**58**)	*Z.monophyllum*	Cuca *et al.*, 1998



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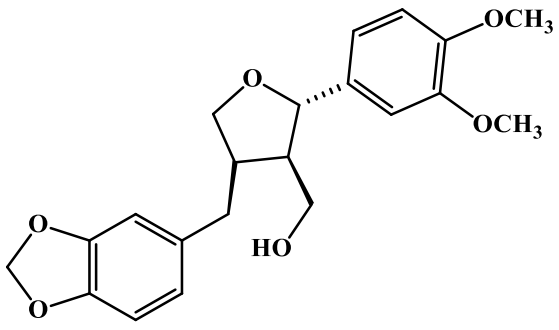

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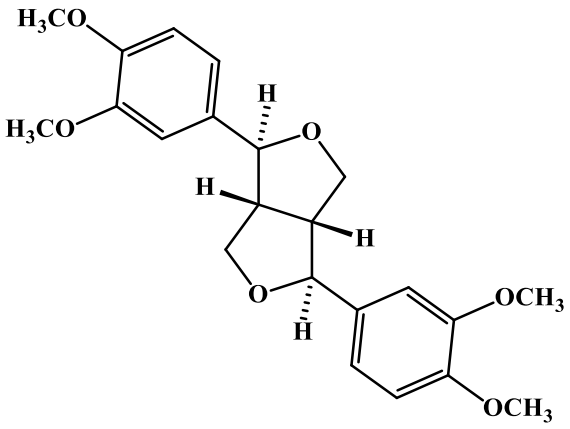

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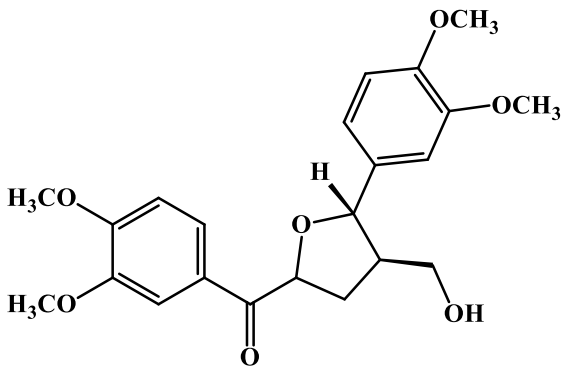

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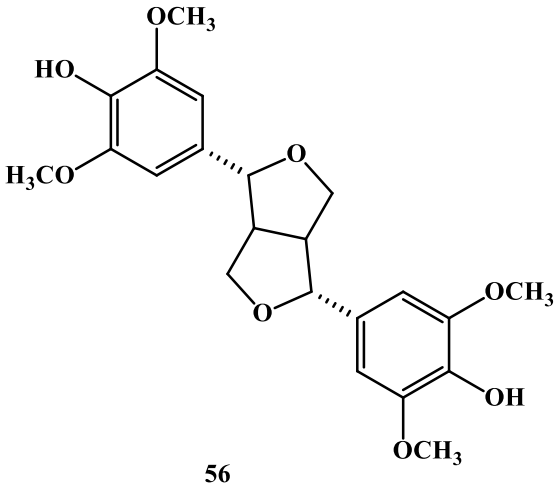

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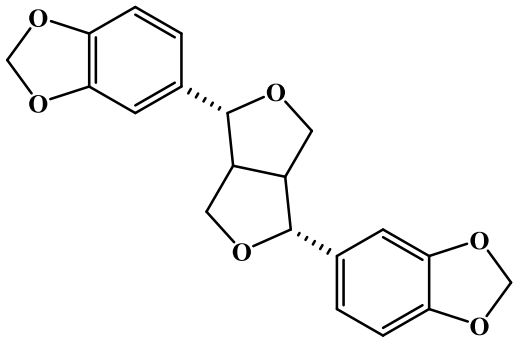

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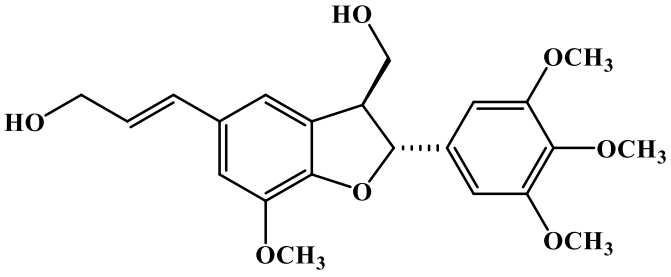

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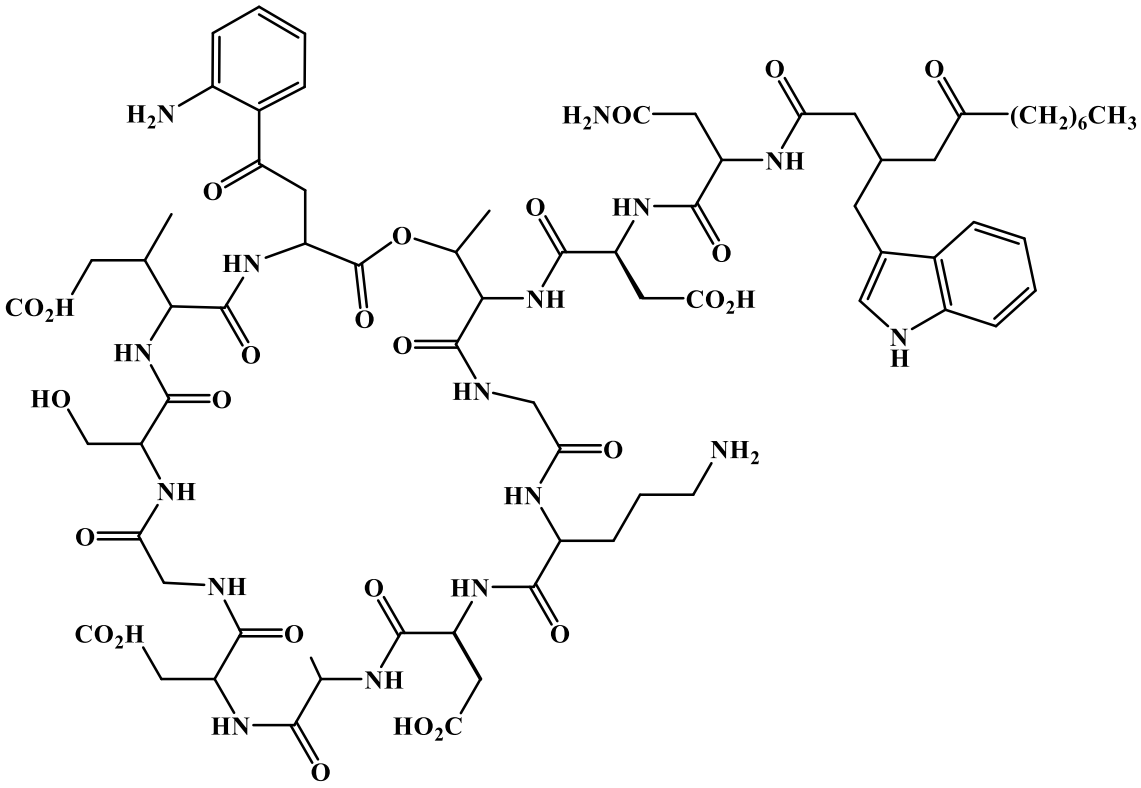
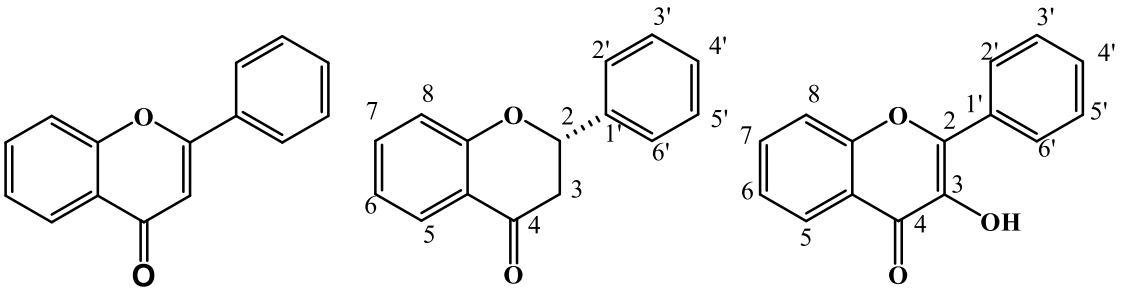


58

2.6.3 Flavonoids from the genus *Zanthoxylum*

Flavonoids are natural products which are phenolic in nature and are isolated from almost all *Zanthoxylum* plant parts. These class of compounds have been known to have antioxidants, antithrombotic, antitumour, antiviral, anti-inflammatory, antihypertensive, estrogenic and antiallergic activities ((Andersson *et al.* 1996; Harborne & Williams, 2000). Some of these flavonoids are shown in table 2.7 below.

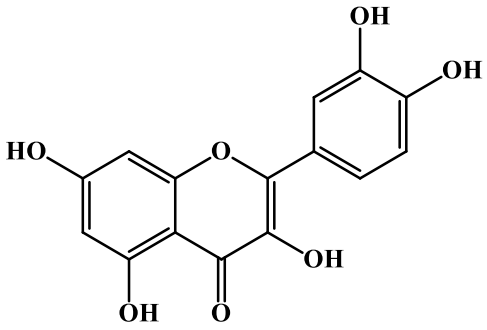
In this genus, there are mainly glycosides of flavones (**59**), flavanones (**60**) and flavonols (**61**). These isolated flavonoids are considered to be polymethoxylated (Waterman & Grundon, 1983). The shikimic acid pathway is essential in flavonoids biosynthetic pathway which utilizes cinnamoyl-CoA as a starter unit (Dewick, 2009).



59 **60** **61**

Table 2.7: Flavonoids of the genus *Zanthoxylum*

Compound	**Plant Part**	**Plant source**	**Reference**
Quercetin (**62**)	Stem bark	*Z. bungeanum*	Xiong *et al.*, 1994
5,2′-Dihydroxy-3-methoxy-7-*O*-β-D glycopyranoiside (**63)**	Roots	*Z. armatum*	Sati *et al.*, 2011
3,5-Diacetyl tambulin (**64**)	Stem bark	*Z. integrifoliolum*	Chen *et al.*, 1996
Diosmetin (**65**)	Leaves	*Z. avicennae*	Cho *et al.*, 2012
Apigenin **(66)**	Leaves	*Z. avicennae*	Cho *et al.*, 2012



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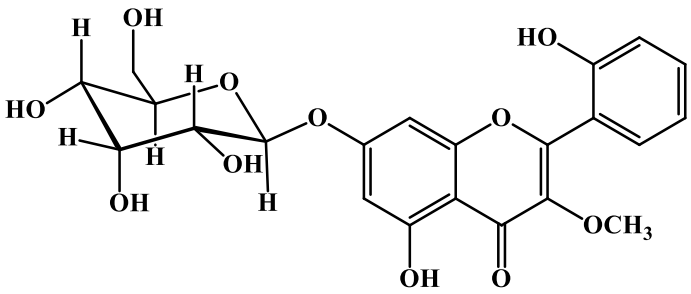

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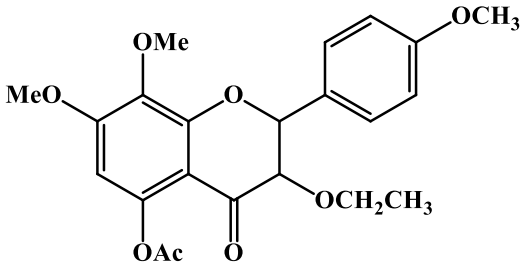

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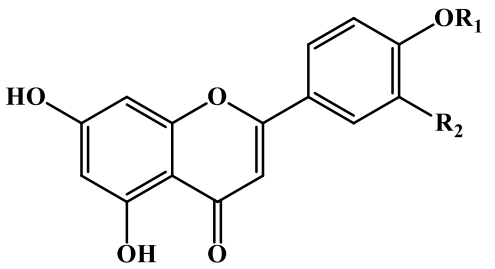


65: $R_1 = CH_3$; $R_2 = OH$

66: R_1, $R_2 = H$

2.6.4 Coumarins

Coumarins are biologically useful as many have shown anticoagulant activities, antitumour, vasodilatory (in coronary vessels) and antibacterial. Further observations have shown that coumarins have no lethal side effects but overdose leads to haemorrhages (Murray *et al.*, 1982). *Zanthozylum* genera have diverse kinds of coumarins including; simple, linear, furocoumarins, dihydrofurocumarins and pyranocoumarins. Psoralen (**67**) is a furanocoumarins isolated from berries of Z. *americanum* and was found to have cytotoxic activity against human tumour cells (Saquib *et al.*, 1990). The core structure of coumarins is shown in figure 2.6 below while table 2.8 shows some coumarins from the genus *Zanthoxylum.*

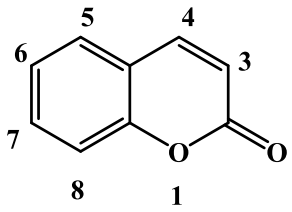
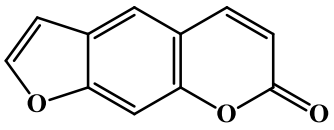


Figure 2.6: The basic structure of coumarins

Table 2.8: Coumarins from the genus *Zanthoxylum*

Compound	**Plant source**	**Plant part**	**Biological activity**	**Reference**
Psoralen (**67**)	Z. *americanum*	Berries	Antitumour	Saquib *et al.*, 1990
Larcinatin (**60**)	Z. *shinifolium*	Stem	Enzyme monoamine oxidase (MAO)	Tsai *et al.*, 2002
Auraptene (**61**)	Z. *shinifolium*	Bark	antiplatelet	Tsai *et al.*, 2000
Collinine (**62**)	Z. *shinifolium*	Bark	Inhibition of DNA replication in hepatitis B virus	Tsai *et al.*, 2000



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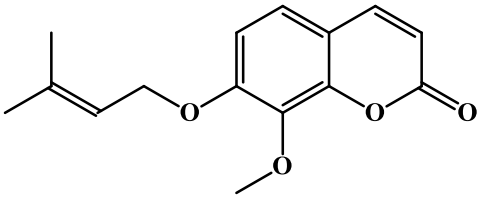

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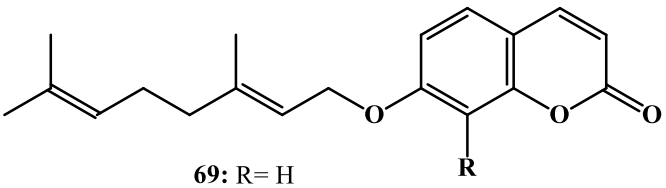


69: R= H
70: R= O$\mathbf{CH_3}$

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Material Collection

The stem barks of *Z. paracanthum* and *Z. chalybeum* were collected from Msambweni in Kwale County which is approximately 37 km from Mombasa city in the Coastal region of Kenya on 25th August, 2015. Both flora samples were positively identified by Mr. Patrick Mutiso who is a senior technologist with experience in plant identification, based in University of Nairobi herbarium, School of Biological Sciences (SBS) where voucher samples (labeled GMM/2015/09/ZC and GMM/2015/09/ZP) were deposited.

3.2 Apparatus and reagents used

In column chromatography (CC), Merck silica gel 60 (70-230 mesh) and Sephadex LH-20 were used as stationary phases. During purification of isolated compounds preparative thin layer chromatography (PTLC) (1.0 mm, 20 x 20 cm) were prepared using Merck silica gel 60 ($PF_{254-366}$) while to monitor the purity of the isolated compound, factory-made analytical aluminium TLC plates (silica gel 60 $F_{254,}$ Merck) were used. The resulting spots were visualized under UV light at 254 or 366 nm and where necessary the plates were sprayed with iodine reagent for the non UV active. To test for the presence of alkaloids, Dragendorff's reagent was used. The ^{1}H and ^{13}C NMR spectra were recorded using Varian-Mercury 200 MHz and Bruker- Avance 500 and 600 MHz spectrometers. Bruker software was used to get the NMR data. Chemical shifts of the compounds were measured in part per million (ppm) relative to the internal standard tetra methyl silane (TMS).

3.3 Extraction of *Z. paracanthum* and *Z. chalybeum*

The stem barks of *Z. paracanthum* and *Z. chalybeum* materials were separately dried openly under shade in the Department of Chemistry, School of Physical Sciences, College of biological and physical sciences, University of Nairobi, Kenya. This operation was done at room temperature for a period of seven days before being ground into fine particles using an electrically powered grinder. The dried and ground materials weighing 1000g of *Z. paracanthum* and 900g of *Z. chalybeum* were

separately exhaustively and sequentially extracted with sufficient amount of 50% methanol (MeOH) in dichloromethane (CH_2Cl_2) and subsequently with 5% water (H_2O) in MeOH. The resultant extracts were filtered to remove debris and the solvent was removed *in vacuo* using a rotary evaporator. This resulted into 97g of crude *Z. paracanthum* and 84g of crude *Z. chalybeum.* The two crude extracts were separately adsorbed onto equal amount of silica gel (97 g and 84g) and loaded onto 500 g of silica gel column packed under 100 % *n*-hexane a day earlier. The columns were run progressively with solvent systems of increasing polarity (*n*-hexane:EtOAc), starting with 1%, 3% and then increasing by 2% until 17% before adjusting the polarity increment again by 5% up to 100 % EtOAc. This yielded a total of 430 fractions of 150 ml each from *Z. paracanthum* and 220 fractions of 200 ml of *Z. chalybeum*. The fractions were concentrated *in vacuo* in a rotatory evaporator and spotted on analytical TLC plates. The fractions with similar TLC profiles were combined resulting into 25 fractions from *Z. paracanthum* and 12 fractions from *Z. chalybeum*.

3.4 Isolation of compounds from *Z. parachanthum*

The fractions of the major column of *Z. paracanthum* eluted between 1% and 5% did not yield much but gave oily substances. The fractions of the main column eluted with 7% and 9 % EtOAc in *n*-hexane yielded white amorphous solids of sesamin (**4**, 60mg). The subsequent fractions of the main column eluted with 11-15% EtOAc in *n*-hexane were crystallized in the conical flask and then filtered out *in vacuo* using a Buchner funnel before washing them thoroughly using 100 % CH_2Cl_2. Upon drying in open air it yielded crystals of colorless dihydrochelerythrine (**2,** 22 mg). The fractions eluted with 17% EtOAc in *n*-hexane when crystallized and washed yielded lupeol (**3,** 30mg). Subsequent fractions resulted to very small amounts that were difficult to analyse until the fraction of the main column eluted with 25-45% EtOAc in hexane were combined and solvent removed *in vacuo* with a rotatory evaporator and loaded on a Sephadex LH 20 column leading to isolation of white amorphous solids of Canthin-6-one (**1,** 80mg).

3.5 Isolation of compounds from *Z. chalybeum*

The fraction of the main column from *Z. chalybeum* eluted with 8% and 10 % EtOAc in *n*-hexane yielded white amorphous crystals of sesamin (**4,** 18mg). The fractions of the main column eluted with 14 -18% EtOAc in *n*-hexane were combined and solvent removed *in vacuo* with a rotatory evaporator and loaded on a Sephadex LH 20 column, affording white crystals of 3-(1-Isoprenoloxy)-4-methoxyfagaramide (**5,** 80mg) while the fractions of the major column eluted

between 20% and 30% were combined and solvent removed *in vacuo* with a rotatory evaporator and loaded on a Sephadex LH 20 column. When crystallized, it resulted to the isolation of creamy crystals of fagaramide (**6,** 28mg).

3.6 Structure Elucidation of Isolated Compounds

The profiles of the collected fractions were observed on thin layer chromatography using UV_{254} and iodine vapour. The structures of the isolated compounds were determined through ^{1}H-NMR, ^{13}C-NMR, HMBC, HSQC, COSY and NOESY spectroscopy and comparison with authentic samples. Mass spectroscopy was used to confirm the structure of the new compound.

3.7 Resazurin Assay

The isolated compounds were tested for their cytotoxicity against drug sensitive and MDR leukemia cell lines using resazurin test. In this test, resazurin dye was dissolved in biological buffers (resulting in a deep blue colored solution) and added directly to cells in culture in a consistent format (Duellman *et al.,* 2015). Viable cells with active metabolism reduce resazurin into the resorufin product which is pink and highly fluorescent. Incubation period needed to generate an acceptable fluorescent signal above background is usually between 1 and 4 hours. However, this period is dependent on the metabolic activity of the particular cells being tested, the cell density per well, and other assay environments including the type of culture medium used. The incubation period should be optimized and kept short enough to avoid reagent toxicity nevertheless, long enough to provide adequate sensitivity (Duellman *et al.,* 2015). Non-viable cells have no effect on resazurin dye. Under normal conditions, resazurin weakly fluorescents (blue).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Secondary Metabolites isolated from *Zanthoxylum paracanthum*

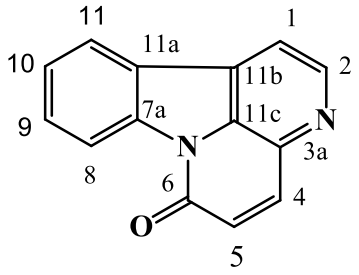
The dried and ground stem bark of *Z. paracanthum* extracted using methanol/dichloromethane (1:1) yielded three compounds which were characterized as: one indole alkaloid, canthin-6-one (**1**), one known benzophenanthridine alkaloid, dihydrochelerythrine (**2**), one steroids, lupeol (**3**) and one lignin, sesamin (**4**). The detailed spectroscopic characterization of these compounds are discussed below.

4.1.1 Canthin-6-one (1)

Compound **1** was isolated as yellowish crystals that were UV active with a melting point of 160-161.0 °C. The ^{1}H NMR (Appendix **1a**) exhibited eight well resolved peaks in the aromatic region which included; four sets of *ortho*-coupled doublets assigned as follows; δ_H 6.96 (*d, J*=9.76, 1H, H-5) and δ_H 8.03 (*d, J*= 9.76, 1H, H-4); δ_H 7.99 (*d, J*= 4.97, 1H, H-1) and δ_H 8.82 (*d, J*= 4.97, 1H, H-2); δ_H 8.64 (*d, J*= 8.16, 1H, H-8) and δ_H 7.74 (*td, J*= 8.31, 1H, H-9); δ_H 7.57 (*td, J*= 7.68, 1H, H-10) and δ_H 8.16 (*d, J*= 7.67, 1H, H-11). Furthermore, the ^{1}H NMR showed coupling between H-9 and H-10 resulting into a triplet of a doublet. From the ^{13}C NMR spectrum (Appendix **1b**), a total of fourteen (14) carbons were identified (Table 4.1). From these carbons, eight were methyne (-CH) and six quaternary, according to ^{13}C NMR spectrum. The eight methyne carbons appeared at the following chemical shift values δ_C (128.7, 139.7, 116.4, 145.8, 116.9, 130.7, 125.5, and 122.8) and were assigned to C-5, C-4, C-1, C-2, C-8, C-9, C-10 and C-11, respectively. Using Heteronuclear Single Quantum Coherence (Appendix **1f**) the following carbon atoms; C-1, C-2, C-4, C-5, C-8, C-9, C-10 and C-11 were found to be protonated. This was further confirmed by the correlations adduced from ^{1}H-H COSY (Appendix **1e**) which confirmed that H-1 and H-2 were coupling partners. Also found to be coupling partners were; H-4 and H-5, H-8 and H-9, H-9 and H-10 and finally H-10 and H-11. Using Heteronuclear Multiple Bond Connectivity (Appendix **1d**), H-1 correlated with C-11a and C-11c, H-2 correlated with C-11b, H-4 correlated with C-6 and C-11c while C-5 correlated with C-3. Other observed correlations were; H-8 with C-10 and C-11a, H-9 with C-7a and C-11, H-10 with C-8 and C-11a and finally H-11 with C-9, C-7a and C-11b.

From the ^{13}C NMR analysis, the six quaternary carbons appeared at δ_C (159.4, 124.5, 132.0, 130.0, 136.3, and 139.4) and were assigned to C-6, C-11a, C-11c, C-11b, C-3a and C-7a respectively. The

δ_C 159.4 was associated to a carbonyl group and was attributed to C-6 while the amine groups were assigned to position 3 of one of the aromatic ring and position 6a. The NMR assignments were equally reasoned from NOESY (Appendix **1g**) correlations which resembles COSY (Appendix **1e**). Cross peaks were observed on close protons.



Canthin-6-one (**1**)

Upon further check of the formula, $C_{14}H_8ON_2$, using index of hydrogen deficiency = $\frac{2n+2-m}{2}$

$$\frac{[(2x14)+2]-8+2}{2} = 2$$

This was satisfied by the two aromatic rings for 8 index of hydrogen deficiency and two non-aromatic rings for 4 hydrogen deficiency therefore the structure of the compound elucidated.

This compound was further subjected to mass spectroscopy to confirm the findings. From mass spectrum, the elemental composition was reported as *m*/z = 220.0637 (Appendix **1h**), while the theoretical mass was reported as 220.0684. The overall elemental composition was reported to have 14 carbons, 8 protons, 1 oxygen and 2 nitrogen ($C_{14}H_8N_2O$).

Mass spectroscopy was in agreement with the proposed structure and was therefore confirmed.

In view of the structural elucidation and comparison with literature it was logically concluded that the alkaloid compound whose NMR data is illustrated in table 4.1 below was canthin-6-one (**1**). A similar compound was earlier reported from *the* North American mushroom *Boletus curtisii* (Martin *et al*,. 2004*).*

Table 4.1: NMR data for canthin-6-one (**1**)

Position	δ_H (H, *m*, *J* in Hz)	δ_C, ppm	HMBC (^2J, ^3J)
1	7.99 (1H, *d*, *J*= 5.0)	116.4	C-11a, 11c
2	8.82 (1H, *d*, *J* = 5.0)	145.8	C-11b
3a		136.2 q	
4	8.03 (1H, *d*, *J*= 9.7)	139.7	C-11c, C-6
5	6.96 (1H, *d*, *J*= 9.8)	128.7	C-3a
6		159.4 q	
7a		139.4 q	
8	8.64 (1H, *d*, *J*= 8.16)	116.9	C-10, C-11a
9	7.74 (1H, *td*, *J*= 8.31)	130.7	C-7a, C-11
10	7.57 (1H, *td*, *J*= 7.68)	125.5	C-8, C11a
11	8.16 (1H, *d*, *J*= 7.67)	122.8	C-7a, C-9, C11b
11a	-	124.5 q	
11b	-	130.0 q	
11c	-	132.0 q	

1.2 Dihydrochelerythrine (2)

Compound **2** was isolated as colorless crystals which melted at 113-115 °C. Upon spraying with Dragendorff's reagent (test for alkaloid) it produced an orange coloration confirming that it was an alkaloid. Upon further analysis, a spot of this compound on Thin Layer Chromatography (TLC) plate fluoresced blue both under UV_{366} and UV_{254}. However, on prolonged exposure to air/light the spot turned yellow which is a characteristic of benzophenanthridine alkaloids (Kwok *et al.,* 1987). The ^{1}H NMR spectra data (Appendix **IIa** and Appendix **IIb**) showed two close peaks at δ_H 3.93 and 3.88 which were well pronounced, characteristics for methoxyl group. The δ_H 6.05 peak was associated with a methylenedioxy protons while the δ_H 2.58 singlet signal was attributed to *N*-methyl protons. Six peaks in the aromatic region were also evident in the ^{1}H NMR spectrum. One set of *ortho*-coupled doublets at δ_H 6.96 and δ_H 7.51 (J = 8.4 Hz) was assigned to H-3 and H-4, respectively. The other set of doublets at δ_H 7.73 and δ_H 7.48 (J = 9.0 Hz) was assigned to H-9 and H-10. The singlet at δ_H 7.12 was assigned to H-11 and that at δ_H 7.66 to H-14.

Furthermore, the ^{13}C NMR spectral data (Appendix **IIc**) showed 21 carbon peaks, sixteen of which were sp^2 carbons. This was attributed to a 4-ring system that is characteristic of a benzophenanthridine alkaloid skeletal structure (Nissanka *et al.,* 2001). The ^{13}C NMR spectrum, δ_C 61.0 and 55.9 were associated with two methoxyl groups which accounted for two of the four peaks for oxygenated carbon within the aromatic rings. Using the HMBC data (Appendix **IIe**) and HSQC data (Appendix **IIf**), it was evident that C-1 and C-2 were aromatic carbons attached to methoxyl

groups. On further analysis of ^{13}C NMR spectrum, C-12 and C-13 were found to be equally oxygenated by the methylenedioxy group hence accounting for the two oxygenated carbons peaks that were remaining. The methylenedioxy carbon was assigned the δ_C 101.7 while the *N*-methyl carbon was assigned to the δ_C 41.4. The ^3J correlations of both H-11 and OCH_2O protons with C-13 and between a δ_H 6.05 (OCH_2O) and the quaternary carbons C-12 and C-13 established the substitution pattern in this ring. Table 4.2 below has a summary of the NMR spectrum. In view of the structural elucidation and comparison with literature it was logically concluded that the alkaloid compound was dihydrochelerythrine (**2**). A similar compound was earlier reported from *Z. rubescens* species (Waterman *et al*., 1976)

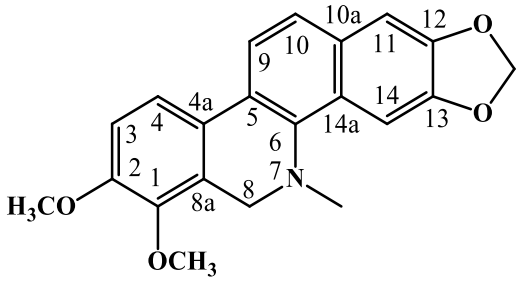


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Table 4.2: NMR data for dihydrochelerythrine (**2**)

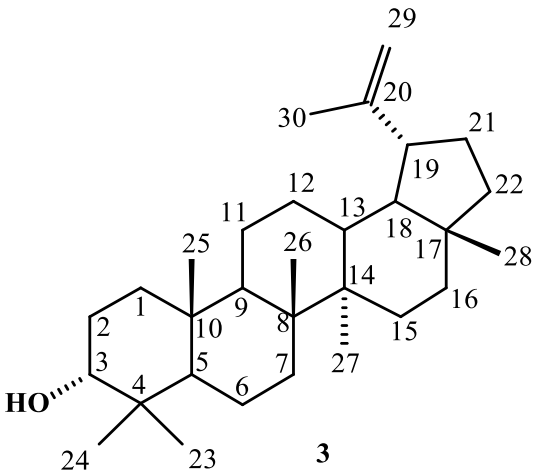
Position	δ_H (H, *m*, *J* in Hz)	δ_C, ppm	HMBC (^2J, ^3J)
1	-	146.0 q	
2	-	152.7 q	
3	6.96 (1H, *d*, *J* = 8.4)	111.4	C-4a, 1
4	7.51 (1H, *d*, *J* = 8.4)	118.8	C-8a, 5, 2
4a	-	126.4 q	
5	-	126.3 q	
6	-	145.0 q	
7	-	-	
8	4.30 (2H, *s*)	48.9	C-6, 4a, 1, N-CH_3
8a		124.6 q	
9	7.73 (1H, *d*, *J* = 9.0)	120.4	C-10a, 6, 4a
10	7.48 (1H, *d*, *J* = 9.0)	123.9	C-10a, 14a, 11, 5
10a	-	131.1 q	
11	7.12 (1H, *s*)	104.4	C-14a, 13, 10
12		148.4 q	
13		148.7 q	
14	7.66 (1H, *s*)	100.0	C-12, 10a, 6
14a		126.7q	
OCH_3	3.93 (3H, *s*)	56.8	C-2

OCH_3	3.88 (3H, *s*)	61.7	C-1
N-CH_3	2.58 (3H, *s*)	41.4	C-8, 6
OCH_2O	6.05 (2H, *s*)	101.7	C-13, 12

4.1.3 Lupeol (3)

Compound **3** was isolated as white shapeless solids which were UV_{254} inactive and had a melting point of 191-192 ˚C. However, upon exposing the spot on TLC to iodine vapor the spot was visible. The compound tested negative with Dragendorff's reagent hence confirmed it was not an alkaloid. The ^1H NMR spectrum (Appendix **IIIa**) clearly showed seven singlets characteristic of methyl peaks at δ_H 0.96, 0.88, 0.82, 1.03, 0.94, 0.79 attributed to methyl at C-23 to C-28 respectively while δ_H 0.68 was assigned to methyl at C-30. The two broad singlets were associated with olefinic protons for the methylene group which appeared at δ_H 4.68 and 4.56.

The ^{13}C NMR spectrum (Appendix **IIIb**) showed 30 carbons, characteristic of triterpenes. Furthermore, one of these carbons was oxygenated and therefore appeared downfield shifted at δ_C 79.2 and was assigned to C-3. The signal at δ_C 151.2 was assigned to a quaternary carbon for C-20 and that appearing at δ_C 109.6 was assigned to one of the olefinic carbon at C-29. The ^{13}C NMR spectrum also showed other quaternary carbons signals at δ_C 37.4, 39.1, 41.0, 43.0 and 43.2 were assigned to C-10, C-4, C-8, C14 and C-17 respectively. The peaks at δ_C 18.5, 21.2, 25.3, 27.6, 27.7, 30.1, 34.5, 35.8, 38.9 and 40.2 were assigned to methylene carbons at C-6, C-11, C-12, C-2, C-15, C-21, C-7, C-16, C-1 and C-22 respectively. Moreover, there were seven characteristic peaks for methyl carbons at δ_C 14.8 was assigned to C-27; δ_C 15.6, 16.4 and 16.2 assigned to C-24, C-25 and C-26 respectively while δ_C 18.2, δ_C 19.5 and δ_C 28.2 were associated with C-28, C-30 and C-23 respectively. Using the HMBC data (Appendix **IIIg, IIIh** and **IIIi**) and HSQC data (Appendix **IIIe** and **IIIf**), it was evident that six carbons were quaternary. The correlations observed vide HMBC, ^1H, H-COSY (Appendix **IIId**) and the above chemical shift values tabulated in table 4.3 below corroborated with those of lupeol that is characterized from a number of plants including *Z. rhoifolium* (Reynolds *et al.*, 1986).



3

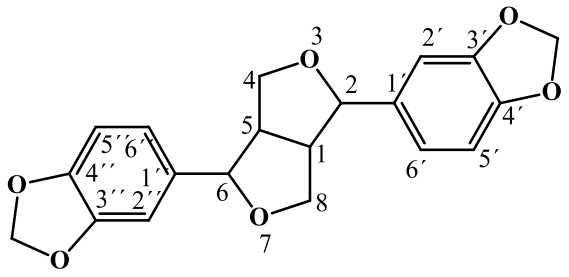
Table 4.3: NMR Data for lupeol (**3**)

Position	δ_H (H, *m*, *J* in Hz)	δ_H (H, *m*, *J* in Hz)	δ_C ppm	HMBC (^2J, ^3J)
1	0.9	1.67	38.9	
2	1.59	1.52	27.6	
3		3. 20 (1H, *m*)	79.2	C-1, 2, 24
4			39.1	
5		0.66 (1H, *s*)	55.5	
6	1.51	1.38	18.5	
7	1.38	1.38	34.5	
8			41.0	
9		1.28	50.6	
10			37.4	
11	1.41	1.25	21.2	
12	1.07	1.43	25.3	
13		1.62	38.3	
14			43.0	
15	1.03	1.68	27.7	
16	1.38	1.52	35.8	
17			43.2	
18		1.35	48.5	
19		2.45 (1H, *s*)	48.2	C-13, 17, 20, 29, 30
20			151.2	
21	1.32	1.97	30.1	C-18, 20, 22
22	1.03	1.41	40.2	C-16, 18,
23		0.96 (3H, *s*)	28.2	
24		0.88 (3H, *s*)	15.6	
25		0.82 (3H, *s*)	16.4	
26		1.03 (3H, *s*)	16.2	
27		0.94 (3H, *s*)	14.8	
28		0.79 (3H, *s*)	18.2	
29	4.60	4.73 (1H, *s*)	109.6	C-20, 19, 30
30		0.68 (3H, *s*)	19.5	

4.1.4 Sesamin (4)

Compound **4** was isolated as white unstructured solid which was UV active. The compound reportedly had a melting point of 121-122 °C. The ^{1}H NMR data (Appendix **IVa**) revealed a multiplet signal at δ_H 3.06 (*m*, 1H) attributed to the methyne protons appearing at C-1 and C-5. Furthermore, two sets of doublets were identified on the ^{1}H NMR at δ_H 3.88 (*dd, J* = 9.23 Hz, 2H) and δ_H 4.24 (*ddd, J* = 9.13 Hz, 2H) which were attributed to the methylene protons at C-4 (axial) and C-4 (equatorial). A doublet signal at δ_H 4.73 (*J* = 4.37 Hz) was also observed and assigned to H-2 and H-6. The downfield shifted signal appearing at δ_H 5.99 (*s*, 2H) was characteristic of methylenedioxy protons. The ^{1}H NMR spectrum also showed signals associated with four chemically and magnetically equivalent aromatic protons at δ_H 6.84 which was assigned to H-5′, H-6′ and H-5″, H-6″ while the signal at δ_H 6.89 was assigned to H- 2′ and H-2″.

The ^{13}C NMR spectrum (Appendix **IVb**) displayed peaks at δ_C 135.5 attributed to carbons at C-1′ and C-1″ and δ_C 86.0 was assigned to C-2 and C-6. The ^{13}C NMR signal at δ_C 101.2 was attributed to the methylenedioxy attached to the aromatic rings. The ^{13}C NMR spectrum further revealed two oxygenated carbons at δ_C 147.0 and δ_C 147.9 for C-3′, 3″ and C-4′, 4″ respectively. The other ^{13}C NMR peaks δ_C 106.4 was attributed to C-2′ and C-2″, δ_C 107.9 was assigned to C-5′ and C-5″ while δ_C 119.3 was associated with C-6′ and C-6. HMBC (Appendix **IVc**) and HSQC (Appendix **IVd**) spectra together with all NMR spectra values as recorded in table 4.4 below and were considered in the above structural elucidation. Evaluation with previously recorded literature values, compound **4** was identified as sesamin (Pelter, 1976) which was earlier isolated from the *Z. budrunga* species (Mukhlesur *et al* 2003).



4

Sesamin (**4**)

Table 4.4: NMR Data for sesamin (**4**)

Position	**δ_H (H, *m, J*, in Hz)**	**δ_C ppm**	**HMBC (^2J, ^3J)**
1	3.06 (1H, *m*)	54.4	C- 2, C-8, C-1′
2	4.74 (1H, *d*)	85.7	
4a	3.88 (1H, *dd, J* = 9.2)	71.7	C- 5, 6
4e	4.24 (1H, *dd, J* = 3.6)		C-5, 6
5	3.05 (1H, *m*)	54.4	C-6, 8a, 1″
6	4.74 (1H, *dd, J* = 3.7)	85.7	C-5, 4a, 2″, 6″
8a	3.88 (1H, *d, J* = 4.4)	71.7	C-1, 2
8e	4.24 (1H, *dd, J* = 3.6)		C-1, 2
1′	-	135.5	
2′	6.90 (1H, *d, J* = 1.4)	106.4	C-3′, 4′, 6′, 2
3′	-	147.0	
4′	-	147.9	
5′	6.82 (1H, *dd, J* = 8.0)	107.9	C-1′, 3′
6′	6.86 (1H, *d, J* = 1.4)	119.3	C- 4´, 2´, 2
1″		135.5	
2″	6.90 (1H, *d, J* = 1.4)	106.4	C-3″, 4″, 6″, 6
3″		147.0	
4″		147.9	
5″	6.82 (1H, *dd, J* = 8.0)	107.9	C-3″, 1″
6″	6.86 (1H, *d, J* = 1.4)	119.3	C-2″, 4″, 6
OCH_2O	5.99 (4H, *s*)	101.2	

Key:
a = axial
e = equatorial

4.2 Secondary metabolites isolated from *Zanthoxylum chalybeum*

One novel compound and two known ones were isolated from *Z. chalybeum*. However, one of the isolated compounds, sesamin (**4**) was also isolated from *Z. parachanthum*.

4.2.1 3-(1-Isoprenoloxy)-4-methoxyfagaramide (5)

Compound **5** was isolated as white crystals which were UV_{254} active. From the ^{1}H NMR spectrum (Appendix **Va** and **Vb**), there were three olefinic protons δ_H 7.5 (*d, J*= 15.52 Hz, 1H, H-7), δ_H 6.35(*d, J* =15.51 Hz, 1H, H-8) and δ_H 5.52 (*m*, 1H, H-2″). The coupling constant of *J* = 15.5 flanked by the two proton was evidence of a *trans*-configuration of the olefinic bond (Okorie, 1976; Kubo *et al.*, 1984). There was evidence of three aromatic protons at δ_H 7.09 (*d, J* = 8.2Hz, 1H, H-2), δ_H 7.11 (*dd*, 1H, H-6) and δ_H 6.9 (*d, J* = 8.23 Hz, 1H, H-5). The ^{1}H NMR displayed signals at δ_H 3.21 (2H, *m*), δ_H 1.3 (1H, *m*), δ_H 0.98 ($2CH_3$, *d, J* = 6.69 Hz) indicating the presence of an isobutyl amide functional group in the compound (Known and Horvath, 1981). There was evidence of one methoxy group δ_H 3.89 ($-OCH_3$, *s*) which were placed at C-4. Furthermore, the ^{1}H NMR spectrum showed two singlet signals which were shielded evidence of methyl groups δ_H 1.84 ($-CH_3$, *s*, C-2′). HMBC spectral data (Appendix **Vg** and **Vh**) showed two protons at δ_H 4.59 (*d, J*= 6.80 Hz, 2H, H-1″) were assigned to an oxygenated carbon by virtue of their strong correlation with the methylene protons a δ_H 5.39 (*t*, 1H, H-2″).

The ^{13}C NMR spectrum (Appendix **Vc**), showed 19 peaks assigned to 19 carbons. One of which was characteristic of the carbonyl group δ_C 165.7 assigned C-9. This carbonyl group was attached to amide functional group and an olefinic hydrocarbon C-8 and hence the observed chemical shift value. The olefinic hydrocarbon at C-7 is also attached to an aromatic ring. There was a signal at δ_C 46.9 assigned to $-CH_2$ that was bonded to a nitrogen atom while that at δ_C 65.6 was due to oxygenated methylene group. Four of the absorptions were attributed to the methyl groups δ_H 1.79 (3H, *s*, H4′, H5″).

The HMBC spectra of compound **5** (Appendix **Vg** and **Vh**) showed the following correlations; H-2 with C-2, C-6 and C-7; H- 5 with C-1, C-3 and C4. Other HMBC correlations included H-6 with C-2, C-4 and C-7; H-7 with C-1, C-2, C-6, C-8 and C-9. H-8 correlated with C-1, C-7 and C-9; H-1′ with C-2′, C-3′ and C-4′; H-1″ with C-2″ and C-3″. This is illustrated by figure 4.1 below.

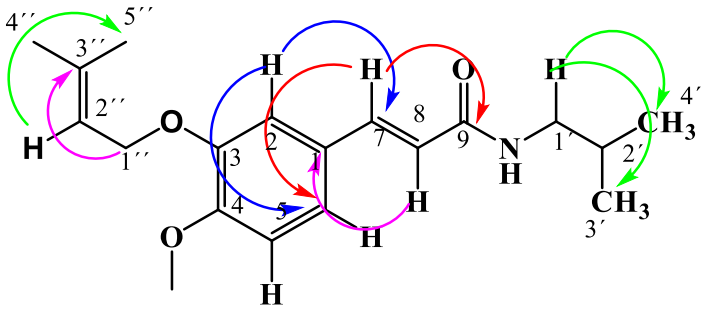


Figure 4.1: HMBC correlation of 3-(1-isoprenoloxy) 4-methoxyfagaramide (**5**)

The COSY (Appendix **Vd** and **Ve**) and NOESY (Appendix **Vi**) correlations showed that H-5 and H-6 were coupling partners; H-7 and H-8 were also coupling partners (figure 4.2).

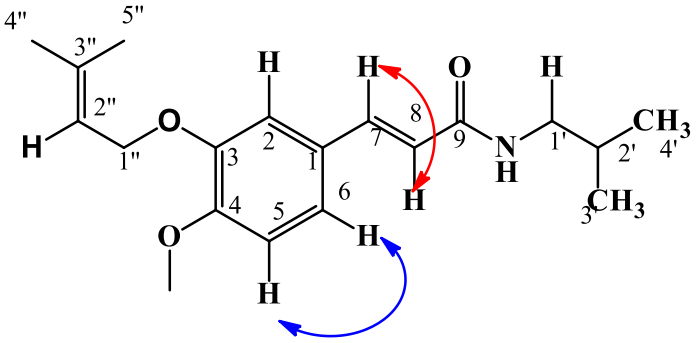


Figure 4.2: NOESY correlation of 3-(1-isoprenoloxy) 4-methoxyfagaramide (**5**)

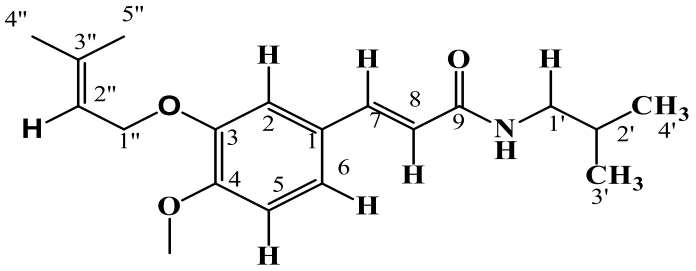
Using the formula, $C_{19}H_{27}O_3N$, index of hydrogen deficiency = $\frac{2n+2-m}{2}$

$$\frac{[(2x19)+2]-27+2}{2} = 7$$

This satisfied the one aromatic ring for 4 index of hydrogen deficiency and 3 double bond for 3 index of hydrogen deficiency. With the above NMR spectral data the structure of compound **5** was elucidated

This compound was further subjected to Mass Spectroscopy to confirm the findings. From Mass Spectroscopy, the elemental composition was reported as M/z = 317.1979, while the theoretical mass was reported as 317.1991. The delta (ppm) was reported as -3.9 while RDB equiv. was reported as 7.0. The overall elemental composition reported was 19 carbons, 27 protons, 3 oxygen and 1 nitrogen ($C_{19}H_{27}O_3N$)

On the basis of the above ^{1}H NMR, ^{13}C NMR and other spectral data summarized in table 4.5 below, the compound was therefore characterized as 4-(1-Isoprenoloxy)-3-methoxyfagaramide (**5**) as shown in figure 4.3 below.



5

Figure 4.3: Structure of 3-(1-isoprenoloxy) 4-methoxyfagaramide (**5**)

Table 4.5: NMR data for 3-(1-Isoprenoloxy)-4-methoxyfagaramide (**5**)

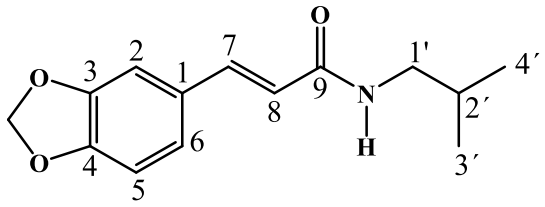
Position	**δ_H (H, *m*, *J* in Hz**	**δ_C ppm**	**HMBC**
1	-	127.7	-
2	7.09 (1H, *d*)	109.8	-
3	-	149.9	-
4	-	149.5	-
5	6.90 (1H, *d* , *J* = 8.23)	112.5	C-6
6	7.11 (1H, *dd* , *J* = 8.30)	121.6	
7	7.53 (1H, *d* , *J* = 15.52)	140.1	C-8
8	6.35 (1H, *d*, *J* = 15.51)	118.8	
9	-	165.7	
1′	3.21 (2H, *dd*)	46.9	C-2′
2′	1.84 (1H, *m*)	28.7	
3′	0.98 (3H, *d*)	17.9	-
4′	0.98 (3H, *d*)	17.9	-
OCH_3	3.89 (3H, *s*)	101.6	
N-H	5.80 (1H, *br s*)		
1″	4.59 (2H, *d*)	65.6	C-2″
2″	5.39 (1H, *dd*)	119.3	
3″	-	138.4	
4″	1.79 (3H, *s*)	19.9	
5″	1.79 (3H, s)	19.9	

4.2.2 Fagaramide (6)

The UV_{254} active compound **6** was isolated as creamy crystals and recorded a melting point of 113-115 °C. In the ^{1}H NMR spectrum (Appendix **VIa**), the large coupling constant (J = 15.4 Hz) signified the presence of olefinic protons at δ_H 6.32 (*d, J* = 15.0 Hz, 1H, H-8) and 7.51 (*d, J* = 15.0 Hz, 1H, H-7). This is consistent of *trans*-configuration of the olefinic bond (Okorie, 1976; Kubo *et al.*, 1984). A two proton singlet at δ_H 6.0 characteristic of the methylenedioxy group was assigned to C-3 and C-4. The ^{1}H NMR peaks at δ_H 3.2 ($-CH_2$, *m*), 1.87 (1H, *m*), 0.98 ($2CH_3$, *d, J* = 7.2 Hz) suggested the presence of an isobutyl amide functional group in the compound (Known and Horvath, 1981). Accurate analysis of this spectrum afforded presence of three mutually coupled aromatic proton at δ_H 6.97 (*d, J* = 2.0 Hz, H-2), δ_H 6.94 (*dd, J* = 8.0, 2.0 Hz, H-6), and δ_H 6.78 (*d, J* = 8.2 Hz, H-5) of a *tri*-substituted aromatic ring.

The ^{13}C NMR spectra data (Appendix **VIb**) showed signals for fourteen carbons. Among these peaks, six were identified as aromatic carbons which comprised of three sp_2 δ_C 119.3, 129.4 and 123.6 assigned to C-2, C-5 and C-6 respectively, and three quaternary carbons δ_C 139.9, 148.3 and 149.0 assigned to C-1, C-3 and C-4 respectively. The signal at δ_C 166.4, was attributed to the carbonyl group of the amide whereas the signal at δ_C 101.7 was attributed to methylenedioxy group. The olefinic hydrocarbons were recorded at δ_C 106.1 and 108.4 for C-7 and C-8. A signal for a CH_2 with δ_C 46.9 was attributed to be near a nitrogen atom. Two similar signals observed at δ_C 19.9 were ascribed to methyl groups and were assigned to C-3′ and C-4′, while a signal observed at δ_C 28.7 was attributed to C-2′

Using the ^{1}H NMR spectral data, ^{13}C NMR spectral data, analyzing the HMBC (Appendix **VIf** and **VIg**) data, COSY data (Appendix **VIc** and **VId**) and HSQC data (Appendix **VIe**) tabulated in table 4.6 below and upon comparing with literature values, compound **6** was characterized as fagaramide (Mbaze *et al.*, 2009). There is documented evidence of similar compound previously isolated from a number of plants including *Z. schinifolium.*



6

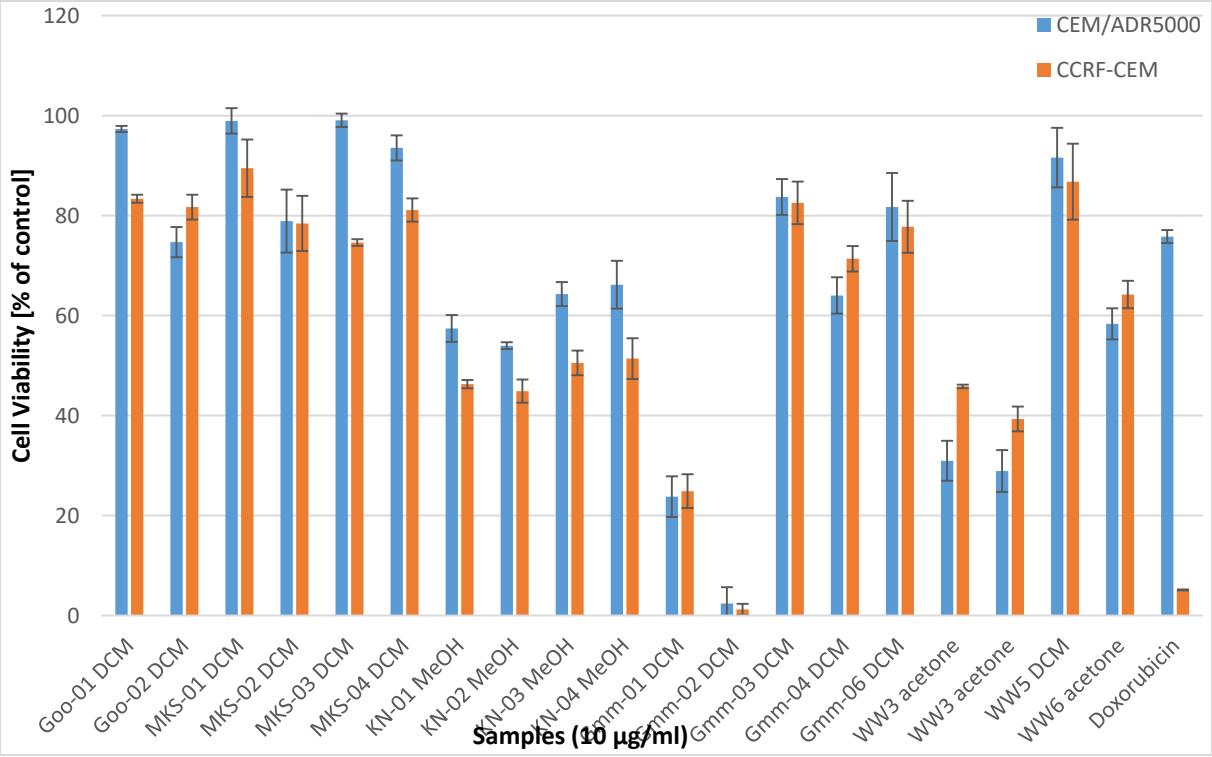
Table 4.6: NMR Data for fagaramide **(6)**

Position	**δ_H (H, *m*, *J* in Hz**	**δ_C, ppm**
1	-	139.9
2	6.85 (1H, *s*)	119.3
3	-	148.3
4	-	149.0
5	7.07 (1H, *d* , *J* = 8.0)	129.4
6	7.04 (1H, *d* , *J* = 8.0)	123.6
7	6.32 (1H, *d* , *J* = 15.0)	106.1
8	7.51 (1H, *d*, *J* = 15.0)	108.4
9	-	165.6
1′	3.22 (2H, *m*)	46.9
2′	1.87 (1H, *m*)	28.7
3′	0.99 (3H, *d*)	19.9
4′	0.97 (3H, *d*)	19.9
OCH_2O	6.03 (2H, *s*)	101.7
N-H	5.85 (1H, *br s*)	

4.3 Biological activity test results

The isolated compounds were tested for their cytotoxicity against drug sensitive and MDR leukemia cell lines using resazurin test. Resazurin dye is dissolved in physiological buffers (resulting in a deep blue colored solution) and added directly to cells in culture in a consistent format (Duellman, *et al.*, 2015). Viable cells with active metabolism reduce resazurin into the resorufin product which is pink and highly fluorescent. Incubation period needed to generate an acceptable fluorescent signal above background is usually between 1 and 4 hours. However, this period is dependent on the metabolic activity of the particular cells being tested, the cell density per well, and other assay conditions including the type of culture medium used. The incubation period should be optimized and kept short enough to avoid reagent toxicity nevertheless, long enough to provide adequate sensitivity (Duellman, *et al.*, 2015).

The results were as shown in figure 4.4 below.



Gmm 02: Canthin-6-one (**1**), Gmm 04: dihydrochelerythrine (**2**), Gmm 06: Sesamin (**4**), MKS 04: 3-(1-isoprenoloxy)-4-methoxyfagaramide (**5**), MKS 02: Fagaramide (**6**)

Figure 4.4: Bioassays results of different samples using doxorubicin as standard

The isolated compounds were tested for their potential to inhibit drug sensitive (CCRF-CEM) and drug resistance cell lines (CEM-ADR 5000) and doxorubicin was used as the reference drug (Figure 4.0). Compounds are considered active when they cause cell inhibition of more than 70% at 10 µg/ml leaving only 30% of the cells viable.

Based on the criteria for evaluation of active principles, canthin-6-one (Gmm **02**) exhibited the highest activities against both the drug sensitive and drug resistant leukemia cell lines, with only < 6% of the drug sensitive and resistant leukemia cells remaining viable (94% inhibition against CEM-ADR 5000 and 81% inhibition against CCRF-CEM) at 10 µg/ml. It is important to note that the activity of Canthin-6-one **(1)** was higher than that of the reference drug, doxorubicin at the tested concentration of 10 µg/ml. The rest of the compounds were inactive as they inhibited less than 70%. Fagaramide **4** (MKS **02**) showed cell viability of about 78.4% (21.6% inhibition) and 78.8% (inhibition 21.2%), against CEM-ADR 5000 and CCRF-CEM, respectively. Sesamin **4** (Gmm **06**) showed cell viability of about 77.8% (22.2% inhibition) and 81.7% (inhibition 18.3%), against CEM-ADR 5000 and CCRF-CEM, respectively. 3-(1-isoprenoloxy)-4-methoxyfagaramide (MKS **04**) on the other hand, showed cell viability of about 81.1% (18.9% cell inhibition) and 93.6% (6.4% cell inhibition) against drug sensitive-CCRF-CEM and resistance CEM-ADR 5000 cell lines. According to criteria used to evaluate the cytotoxicity of pure compounds they were then considered inactive.

CHAPTER FIVE

5.0 Conclusion and Recommendations

5.1 Conclusion

Seven compound were isolated. Four from *Z. paracanthum* namely; canthin-6-one (**1**), dihydrochelerythrine (**2**), lupeol (**3**), sesamin (**4**) and three from *Z. chalybeum* namely; 3-(1-Isoprenoloxy)-4-methoxyfagaramide (**5**), sesamin (**4**) and fagaramide (**6**). The compound sesamin (**4**) was common in both *Z. paracanthum* and *Z. chalybeum*.

One of the six compounds was novel (have never been isolated before from nature) namely; 3-(1-isoprenoloxy)-4-methoxyfagaramide (**5**).

Elucidation of compounds was successfully done using 1D, 2D NMR and Mass spectroscopy and comparison with known samples.

Canthin-6-one (**1**) isolated from *Z. paracanthum* showed good cytotoxicity and was even more active than the standard used, doxorubicin against the two leukemia cell lines at the concentration of 10 µg/ml. Rest of the compound were inactive.

5.2 Recommendations

Since the new compound Canthin-6-one which showed good activity was isolated in very low quantity it is recommended that the compound be re-isolated in order to determine the IC_{50} value and also test it against other cancer cell lines. In vivo studies against animal models for this active compound should be carried out to determine its bioactivity. Selective Index of this compound should be determined. The stem barks of *Z. paracanthum* and *Z. chalybeum* should be further investigated using modern separation techniques such as high performance liquid chromatography (HPLC) or prep HPLC to target minor compounds.

REFERENCES

Adesina, S. K. (2005). The Nigerian *Zanthoxylum*: Chemical and Biological Values. African Journal of Traditional, Complementary and Alternative medicines, 2, 282-301. ISSN: 0189-6016.

Advisory Group on increasing access to Radiotherapy Technology in low and middle income countries (AGaRT), (2009). PACT Programme Office, International Atomic Energy Agency- www.iaea.org

Amabeoku, G. J., Kinyua, C. G. (2010). Evaluation of the Anticonvulsant of Zanthoxylum capense (Thunb.) Harv. (Rutaceae) in Mice. International Journal of Pharmacology, 6 (6), 844-853. ISSN: 1811-7775.

American Cancer Society, (2015). CA Cancer J Clin, 65:5–29.

Andersson, C. M., Halberg, A., Hogberg, T. (1996). Advances in the Development of Pharmaceutical Antioxidants. Advances in Drug Research, 28, 65-180. ISBN: 0-12013326-1.

Azando, E. V., Hounzangbé-Adoté, M. S., Oloundadé, P. A., Brunet, S., Fabre, N., Valentin, A., Hoste, H. (2011). Involvement of tannins and flavonoids in the in vitro effects of Newbouldia laevis and Zanthoxylum zanthoxyloïdes extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. Veterinary Parasitology, 180, 292-297. ISSN: 0304-4017.

Bafi-Yeboa, N. F., Arnason J. T., Barker, J., Smith M. L., (2003). Anti-fungal constituents of Northern prickly ash, *Zanthoxylum americanum* Mill: Phyto medicine.12, 370-377.

Balunas, M. J., Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences* 78,431-44.

Barnabas, B. B., Man, A., Ogunrinola, T. S., Anyanwu, P. E. (2011). Screening for Anthelminthic Activities from Extracts of Zanthoxylum Zanthoxyloides, Neocarya Macrophylla and Celosia Laxa Against Ascaris Infection in Rabbits. International Journal of Applied Research in Natural Products, 3 (4), 1-4. ISSN: 19406223.

Beentje, H. J. (1994). Kenya trees shrubs and lianas. *Published by the national museums of Kenya, Nairobi.*

Bentley, K. W. (1999). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 16, 367–388.

Bentley, K. W. (2000). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 17, 247–268.

Bentley, K. W. (2001). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 18, 148–170.

Bentley, K. W. (2002). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 19, 332–356.

Bentley, K. W. (2003). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 20, 342–365.

Bentley, K. W. (2004). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 21, 395–424.

Bentley, K. W. (2005). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 22, 249–268.

Bentley, K. W. (2006). β-Phenylethylamines and the isoquinoline alkaloids. Nat. Prod. Rep. 23, 444–463.

Boulware, R. T. and Stermitz, F. R. (1981). Some alkaloids and other constituents of *Z. microcarpum* and *Z. procerum*: *Journal of Natural Product*, 44, (2), 200-205.

Cao, L. H., Lee, Y. J., Kang, D. G., Kim, J. S., Lee, H. S. (2009). Effect of Zanthoxylum schinifolium on TNF--induced vascular inflammation in human umbilical vein endothelial cells. Vascular Pharmacology, 50, 200-207. ISSN: 1537-1891.

Chaaib, K. F. (2004). Investigation Phytochimique d'une Brosse à Dents Africaine Zanthoxylum zanthoxyloides (Lam.) Zepernick et Timler (Syn. Fagara zanthoxyloides L.) (Rutaceae). Thèse de doctorat. Faculté des Sciences de l'Université de Lausanne. pp. 11-44

Chang, C. T., Doong, S. L., Tsai, I. L., Chen, I. S. (1997). Coumarins and Anti-HBV Constituents from Zanthoxylum schinifolium. Phytochemistry, 45 (7), 1419-1422. ISSN: 0031-9422.

Chen, I., Shwu, W., Yann, L., Inn, T. and Tian, W. (1996). Alkaloids from root bark of *Zanthoxylum simulans*: *Phytochemistry*, 42, (1), 2157-219.

Chen, J., Hung, H., Chang, D. and Ih, C. (2004). Cytotoxic Constituents from the Stem Bark of *Zanthoxylum pistaciiflorum*: *Journal of the Chinese Chemical Society*, 51,659-663.

Chen, J. J., Wang, T. Y., Hwang, T. L. (2008). Neolignans, a Coumarinolignan, Lignan Derivatives, and a Chromene: Anti-inflammatory Constituents from Zanthoxylum avicennae. Journal of Natural Products, 71, 212-217. ISSN: 0163-3864.

Cheng, M. J., Wu, C. C., Tsai, I. L., Chen I. S. (2004). Chemical and Antiplatelet Constituents from the Stem of Zanthoxylum beecheyanum Journal of the Chinese Chemical Society, 51, 1065-1072. ISSN: 0009-4536.

Cheng, M., Kuo, L., Ian, T. and Ih, C. (2005). Two new sesquiterpenoids and ant-HIV Principles from the root bark of *Zanthoxylum ailanthoides*: *Bioorganic and Medicinal Chemistry*, 13, 5915-5920.

Cho, J. Y., Hwang, T. L., Chang, T. H., Lim, Y. P., Sung, P. J., Lee, T. H., Chen, J. J. (2012). New coumarins and anti-inflammatory constituents from *Zanthoxylum* avicennae. *Food chemistry*, 135, 17-23.

Chou, Z. T., Chan, H. H., Peng, H. Y., Liou, M. J., Wu, T. S. (2011). Isolation of substances with antiproliferative and apoptosis-inducing activities against leukemia cells from the leaves of Zanthoxylum ailanthoides Sieb. & Zucc. Phytomedicine, 18, 344-348. ISSN: 0944-7113.

Cordell, A. G. (1981). Introduction to Alkaloids -A Biogenetic Approach. John Wiley & Sons. ISBN: 0471034789, New York. 509-517.

Cragg, Gordon, M., David J. N., (2005), Plants as a source of anti-cancer agents, Journal of Ethnopharmacology, 100, 72–79

Cuca, L. E., Martinez, J. C., Monache, F. D. (1998). Constituyentes Químicos de Zanthoxylum monophyllum. Revista Colombiana de Química, 27, 17-27. ISSN: 0120-2804.

Cui, H. Z., Choi, H. R., Choi, D. H., Cho, K. W., Kang, D. G., Lee, H. S. (2009). Aqueous extract of Zanthoxylum schinifolium elicts contractile and secretory responses via adrenoceptor activation in beating rabbit atria. Journal of Ethnopharmacology, 126, 300-307. ISSN: 0378-8741.

Da Silva, S. L., Figueredo, P. M. S., Yano, T. (2007a). Chemotherapeutic potential of the volatile oils from Zanthoxylum rhoifolium Lam leaves. European Journal of Pharmacology, 576, 180-188. ISSN: 0014- 2999

Dewick, P. M., (2009). Medicinal natural products. *Abiosynthetic approach*. ISBN: 978-0-470-74168-9.

Diéguez, R.., Garrido, G., Prieto, S., Iznaga, Y., González, L., Molina, J., Curini, M., Epifano, F., Marcotullio, M. C. (2003). Antifungal activity of some Cuban *Zanthoxylum* species. Fitoterapia, 74, 384-386. ISSN: 0367-326X.

D.N.P., (2011): Dictionary of Natural Product.

Dieguez, R., Garrido, G., Prieto, S., Iznaga, Y., Gonzalez, L., Molina, J., Curini, M., Epifano, F. and Marcotullio, M. C. (2003). Antifungal activity of some Cuban *Zanthoxylum* species: *Fitoterapia*, 74, 384-386.

Dharani, N., Rukunga, G., Yenesew, A., Mbora, A., Mwaura, L., Dawson, I., Jamnadass, R., (2010). Common anti-malarial trees and shrubs of east Africa: a Description of Species and Guide to cultivation and conservation through use. Dawson. World Agroforestry Centre (ICRAF), Nairobi Kenya. I ed, 73-78.

Duellman, S., Zhou, W., Meisenheimer, P., Vidugiris, G., Cali, J. J., Gautam, P., Wennerberg, K,. Vidugiriene, J., Bioluminescent, (2015). Nonlytic, Real-Time Cell Viability Assay and Use in Inhibitor Screening. Assay Drug Dev Tech.13 (8):456–65

Dvořák Z., Kubáň V., Klejdus B., Hlaváč J., Vičar J., Ulrichová J., Šimánek V. (2006). Quaternary Benzo[c]phenanthridines Sanguinarine and Chelerythrine: A Review of Investigations from Chemical and Biological Studies. Heterocycles, 68, 2403-2422. ISSN: 0385-5414.

Efferth, T. (2001). The human ATP-binding cassette transporter genes: from the bench to the bedside. Current Molecular Medicine, 1, 45–65.

Eun, J. P., Koh, G. Y. (2004). Suppression of Angiogenesis by the Plant Alkaloid, Sanguinarine. Biochemical and Biophysics Research Communications, 317, 618-624. ISSN: 0006-291X.

Ferguson, P., Kurowska E., Freeman D., Chambers A. and Koropatnick D. (2004). A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cells lines, 134, 1529-1535.

Ferlay, J., Shin H. R., Bray, F., Forman, D., Mathers, C., Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008, Int J Cancer. 127(12):2893-917. doi: 10.1002/ijc.25516

Ferreira, M. E., Rojas A. A., Torres, S. O., Inchausti, A., Nakayama, H., Thouvenel, C., Hocquemiller, R. and Fournet, A. (2002). Leishmanicidal activity of two canthin-6-one alkaloids, two major constituents of *Zanthoxylum chiloperone Var.angustifolium*: *Journal of Ethnopharmacology*, 80, 199-202.

Fidelis, N. S., Louis, P. S., Isaiah, O. N., Ahmed H., and Wilber L. (2013) Zanthoxoaporphines A–C: Three new larvicidal dibenzo[*de*,*g*]quinolin-7-one alkaloids from *Zanthoxylum paracanthum* (Rutaceae), Beilstein J Org Chem. 9: 447–452.

Gansane, A., Sanon, S., Ouattara, P. L., Hutter, S., Ollivier, E., Azas, N., Traore, A., Traore, A. S., Guissou, I. P., Nebie, I., Sirima, B. S. (2010). Antiplasmodial Activity and Cytotoxicity of Semi Purified Fractions from Zanthoxylum zanthoxyloides Lam. Bark of Trunk. International Journal of Pharmacology, 6 (6), 921-925. ISSN: 1811-7775.

Gessler, M. C., Nkunyah M. H. H., Mwasumbi L. B., Heinrich M., Tanner M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica* 56, 65-77

Graham, J. G., M. L. Quinn, D. S. Fabricant, N. R. Farnsworth (2000). Plants used against cancer – an extension of the work of Jonathan Hartwell, Journal of Ethnopharmacology, 73, 347 – 377.

Gueritte, F. and Fahy, J. (2005). The vinca alkaloids In Anticancer Agents from Natural Products, Psychology Press, chapter 7, 23.

Guo, T., Deng, Y. X., Hui X., Yao C. Y., Cai, C. C., Pan, S. l. and Yang-Lin, W. (2011). Antinociceptive and anti-inflammatory activities of ethylacetate fraction from *Zanthoxylum armatum* in mice. Fitoterapia, 82, 347-351.

Harborne, J. B., Williams, C. A. (2000). Advances in Flavonoid Research since 1992. Phytochemistry, 55, 481-504. ISSN: 0031-9422.

Hufford, C. D. (1976). Aporphine Alkaloids of Z. simulans and Z. nigrescens: Phytochemistry, 15, 1169.

Hunter, K. W., Nigel, P. S., Crawford and Jude A. (2008). Mechanisms of metastasis, Breast Cancer Research. 10 (Suppl 1): S2.

Hyde, S. C., Emsley P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher M. P., Gill, D. R., Hubbard, R. E., Higgins, C. F. (1990). Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. PubMed., 346(6282):362-5.

Ishii, H. (1961). Alkaloids of rutaceous plants XVI. Alkaloids of *Zanthoxylum* schinifolium. *Journal of Pharmacology. Soc. Jap*.81: 1633-1635.

Islam, A., Sayeed, A., Bhuiyan, M. S. A., Mosaddik, M. A., Islam, M. A. U., Astaq, M. K., (2001). Antimicrobial activity and citotoxicity of Zanthoxylum budrunga. Fitoterapia, 72, 428-430. ISSN: 0367-326X.

Jiang, H., Wei, Z., Yun, S., Chuan, Z., Lei, X., Run, L., Bin, W. and Xi, X. (2007). Alkaloids from *Zanthoxylum nitidum* (Roxb.) DC: *Biochemical Systematics and Ecology* 35, 114-117.

Jullian, V., Bourdy, G., Georges, S., Maurel, S. and Sauvain, M. (2006). Validation of use of a traditional antimalarial remedy from French Guiana, *Zanthoxylum rhoifolium* Lam: *Journal of Ethnopharmacology*, 106, 348–352.

Kashiwanda, Y., Chikashi, I., Hitoshi, K., Izumi, M., Katsuko, K., Suneo, N. and Yasumasa, I. (1997). Alkylamides of the Fruit of *Zanthoxylum S. S. P*: *Phytochemistry*, 44, (6), 1125-1127.

Kamikawa, T., Hanaoka, Y., Fujie, S., Saito, K.Yamagiwa, Y., Fukuhara, K., Kubo, I. (1996). SRS-A Antagonist Pyranoquinolone Alkaloids from East African Fagara Plants and their Synthesis.Bioorganic & Medicinal Chemistry, 4 (8), 1317-1320. ISSN: 09680896.

Kareru, P. G., Muthee, J. K., Gakuya, D. W., Mbaria, J. M., Mulei, C.M. Njonge, F. K. (2011), Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya Journal of Ethnopharmacology 135, 15-21.

Kerr, D. J. and Rachel Midgley, M.D. (2010). Can We Treat Cancer for a Dollar a Day? Guidelines for Low-Income Countries, The New England journal of medicine, 369, 801-803.

Kokwaro, J. O. (2009). Medicinal Plants of East Africa, Third Edition: *University of Nairobi Press*, 256-257.

Krane, B. D., Fagbule, M. O., Shamma, M. (1984). The Benzophenanthridine Alkaloids. Journal of Natural Products, 47, 1-43. ISSN: 0163-3864.

Kubo, I., Klocke, M. J. A., Kamikawa, T. (1984). Molluscicidal and insecticidal activities of isobutylamides isolated from Fagara macrophylla. Experentia 40, 340–341.

Kuete, V., Tankeo S.B., Saeed M.E.M., Wiench B., Tane P., Efferth T. (2014). Cytotoxicity and modes of action of five Cameroonian medicinal plants against multi-factorial drug resistance of tumor cells. Journal of Ethnopharmacology, 153(1), 207-219.

Kuete, V., Tchakam, P. D., Wiench, B., Ngameni, B., Wabo, H. K., Tala, M. F., Moungang, M. L., Ngadjui, B. T., Murayama, T., Efferth, T. (2013). Cytotoxicity and modes of action of four naturally occurring benzophenones: 2, 2′, 5, 6′-Tetrahydroxybenzophenone, guttiferone E, isogarcinol and isoxanthochymol. Phytomedicine, 20(6), 528-536.

Kuo, G., Jianxin, C., Huihui, Z., Hao, W., Bing, L., Wei, W. (2012). Distribution, Structures and Pharmacological Activities of Aporphine Alkaloids in various plant families. Topclass Journal of Herbal Medicine. 1(1), 001-028, 26.

Kviecinski, M. R., Felipe, K. B., Schoenfelder, T., Wiese, L., Rossi, M., Gonçalez, E., Felicio, J., Filho, D. and Pedrosa, R. (2008). Study of the antitumor potential of Bidens pilosa (Asteraceae) used in Brazilian folk medicine. J Ethnopharmacol 117, 69-75.

Kwok, M. N. G., Alexander, I. G. and Waterman, P. G. (1987). Benzophenanthridine Alkaloids from the Stem Bark of a *Zanthoxylum* species: *Phytochemistry*, 26, 12, 3251-3254.

Lenka, G., Jiri, D. and Radek, M. (2007). Quaternary Protoberberine Alkaloids: *Biochemical Systematics and Ecology*, 35, 114-117.

Liscombe, D. K., Benjamin, P. M., Natalia, L., Owi, I. N and Facchini, P. J. (2005). Evidence for the monophyletic evolution of benzylisoquinoline alkaloid biosynthesis in angiosperms: *Phytochemistry*, 66, 2500–2520.

Liu, S. L., Tsai, I. L., Ishikawa, T., Harayama, T, Chen, I. S. (2000). Bishordeninyl Terpene Alkaloids from Zanthoxylum integrifoliolum. Journal of Chinese Chemical Society, 47, 571-574. ISSN: 0009- 4536.

Luis, E., Cuca, S., Juan C., Martinez, V. and Franco D. M. (1998). 7, 9 Epoxylignan and other constituents of Zanthoxylum culantrillo. 47 (7), 1437-1439.

Maicon, R. K., Karina, B., Felipe, T. S., Luiz P. L., Wiese, M. H., Rossi, E. G., Joana D. F., Danilo, W. F., Rozangela, C. P. (2008). Study of the antitumor potential of Bidens pilosa (Asteraceae) used in Brazilian folk medicine, Journal of Ethnopharmacology. 117, 69–75.

Maiti, M., Kumar, G. S. (2007). Molecular Aspects on the Interaction of Protoberberine, Benzophenanthridine, and Aristolochia Group of Alkaloids with Nucleic Acid Structures and Biological Perspectives. Medical Care Research and Review, 27, 649695. ISSN: 1552-6801.

Mara, S. P., Fernandes, L. B., Vieira, P. C. M., Fatima, F. G. D. and Pirani, J. R. (1992). Chemistry of *Zanthoxylum rhoifolium*: A New Secofuroquinoline Alkaloid, *Biochemical Systematics and Ecology*, 20, (2), 173-178.

Matu, E. N., Staden, J. (2003). Antibacterial and anti-inflamatory activities of some plants used for medicinal purposes in Kenya. Journal of Ethnopharmacology, 87, 35-41. ISSN: 0378-8741.

Martin, G. B., Johannes D., Bert S., Wolfgang S., Yuekui W., Gerhard R. and Jörg, F. (2004). An Unusual Series of Thiomethylated Canthin-6-ones from the North American Mushroom Boletus curtisii, Eur. J. Org. Chem., 4856-4863.

Mukhlesur, M. R., Anwarul, I., Proma, K. and Alexander, I. G. (2003). Alkaloids and Lignans from *Zanthoxylum budrunga* (Rutaceae): *Biochemical Systematics and Ecology*, 33, 91–96.

Murray, R. D. H., Méndez, J., Brown, S. A. (1982). The Natural Coumarins. Occurrence, Chemistry and Biochemistry. John Wiley & Sons LTD. pp. 343-345. ISBN: 0471280577.

National Cancer Control Strategy 2011 – 2016 Ministry of Health, www.health.go.ke

National Cancer Institute at the National Institutes of Health, USA (2015)

Nature America Inc. (2000) Cancer multidrug resistance, http://biotech.nature.com

Navarrete, A., Hong, E. (1996). Anthelmintic Properties of ┘-Sanshool from Zanthoxylum liebmannianum. Planta Medica, 62, 250-251. ISSN: 0032-0943.

Negi, J. S; Bisht, V. K; Bhandari, A. K; Shingh, P and Sundriyal R. C. (2011). Chemical constituents and biological activity of the genus *Zanthoxylum*: A review: *African Journal of Pure and Applied Chemistry*, 5, (12), 412-416.

Nguta, J. M., Mbaira, J. M., Gakuya, D. W., Gathumbi, P. K., Kiama, S. G. (2010). Antimalarial herbal remedies of Msambweni, Kenya. Journal of Ethnopharmacology, 128, 424-432. ISSN: 0378-8741.

Nissanka, A. P. K., Veranja, K., Rutnayake, B. M. B., Vijaya, K., Tsutomu, N., Masatoshi, N., Akira, I., Tillekeratne, L. M. V., Wijesundara, D. S. A. and Leslie, G. A. A. (2001). Antimicrobial Alkaloids from *Zanthoxylum tetraspermum* and *caudatum*: *Phytochemistry*, 56, 857-861.

Nyangulu, J. M., Hargreaves, S. L., Sharples, S. L., Mackay, S. P., Waigh, R. D., Duval, O., Mberu, E. K., Watkins, W. M. (2005). Antimalarial Benzo[c]phenanthridines. Bioorganic & Medicinal Chemistry Letters, 15, 2007-2010. ISSN: 0960-894X.

Okorie, D. A. (1976). A new phthalide and xanthones from Anthocleista djalonensis and Anthocleista vogelli. Phytochemistry 15, 1799–1800.

Osbourn, A. E., Lanzotti V. (2009). "Plant derived natural products; synthesis, function and application." Springer Dordrecht Heidelbeg London New York, 3.

Pact Kenya Cancer Assessment in Africa and Asia report (2010) -www.cancer.iaea.org/newstory

Panthi, M. P., Chaudhary, R. P. (2006). Antibacterial Activity of Some Selected Folklore Medicinal Plants from West Nepal. Scientific World, 4 (4), 16-21. ISSN 1996-8949.

Patino, O. J. (2004). Estudio Fitoquímico Parcial de *Zanthoxylum* quinduensis (Rutaceae). Tesis de grado, Departamento de Química, Universidad Nacional de Colombia, 2-56 Bogota

Patiño, O. J., Cuca, L. E. (2010). Isoquinoline Alkaloids of Zanthoxylum quinduense (Rutaceae). Biochemical Systematic and Ecology, 38, 853-856. ISSN: 0305-1978.

Patiño, O. J., Cuca, L. E. (2011). Monophyllidin, a New Alkaloid L-Proline Derivative from Zanthoxylum monophyllum (Rutaceae). Phytochemistry Letters, 4, 22-25. ISSN: 18743900.

Pelter, A. and Ward, R. S. (1976). Revised structures of pluviatilol, methylpluviatilol and xanthoxlol. Tetrahedron, 32, 2784.

Prakash, A. G., Pankaj, P., Rajni, S., Manoj, K. and Suphla, G. (2013). A Promising Future for Anticancer Drug, Studies in Natural Products Chemistry, 40, 229-325.

Prempeh, A. B. A., Mensah-Attipoe, J. (2008). Analgesic Activity of Crude Aqueous Extract of the Root Bark of Zanthoxylum xanthoxyloides. General Medical Journal, 42 (2), 79-84.

Reddy, L, Odhav B, Bhoola KD. (2003), Natural products for cancer prevention: a global perspective, Pharmacol Ther. 99(1) 1-13.

Reynolds, W. F., Maclean, S., Poplawski, J., Enriquez, G., Escobar, I. and Leon, (1986). Total assignment of ^{13}C and ^{1}H spectra of three triterpenol derivatives by 2D NMR: An investigation of potential of ^{1}H chemical shift in structural investigation of complex natural products. *Tetrahedron*. 42, 3419.

Ross, S. A., Sultana, G. N. N., Burandt, C. L., ElSohly, M. A., Marais, J. P. J., Ferreira, D. (2004). Syncaparmide, a New Antiplasmodial (+)-Norepinephrine Derivative from *Zanthoxylum* syncarpum. Journal of Natural Products, 67, 88-90. ISSN: 0163-3864.

Samir, A. R., Mounirah, A. A., Kesanapalli, S. K., Fronczek, F. R. and. Burandt, C. L. (2005). Alkamides from the Leaves of *Zanthoxylum syncarpum*: *Journal of Natural Product*, 68, 1297-1299.

Sati, S. C., Manisha D. S., Rakesh, R. and Bandoni P. P. (2011). A new Flavanoidal Glucoside from stem bark of *Zanthozylum armatum:* IJPI'S journal of pharmacognosy and herbal Formulations Vol. 1:2(2011) 28-32.

Saraswathy, M., Shaoqin, G. (2013), Different strategies to overcome multidrug resistance in cancer, Research review paper, Biotechnology Advances, volume 31, page 1397–1407.

Saquib, Q. N., Hui, Y. H., Anderson, J. E., Mclaughlin, J. L. (1990). Bioactive Furanocoumarins from the Berries of Zanthoxylum americanum. Phytotherapy Research, 4, 216-219. ISSN: 1099-1573.

Setzer, W. N., Noletto, J. A., Lawton, R. O. and Haber, W. A. (2004). Leaf essential oil Composition of five *Zanthoxylum* species Monteverde, Costa Rica: *Molecular Diversity*, 9, 3-13.

Sheng, C; Tzu, C., Ya, C., Che, T. and Wei, L. (1999). Chemical Constituents and Biological Activity of the Fruit of Z. integrifoliolum: *Jaurnal of Natural Product*, 62, 833-837.

Simeón, S., Rios, J. L., Villar, A. (1989). Pharmacological Activities of Benzophenanthridine and Phenanthrene Alkaloids. Pharmazie, 44, 593-597. ISSN: 0031-7144.

Slaninová, I., Táborská, E., Bochořáková H., Slanina, J. (2001). Interaction of Benzo[c]phenanthridine and Protoberberine Alkaloids with Animal and Yeast Cells. Cell Biology and Toxicology, 17, 51-63. ISSN: 1573-6822.

Song, J. H., Chae, S. W., Yoon, K. A., Park, J. S., Choi, H. J. (2010). Antiviral Activity of Zanthoxylum Species against Porcine Epidemic Diarrhea Virus. Journal of Cosmetics and Public Health, 6 (2), 42-44.

Suryanto, E., Sastrohamidjojo, H., Raharjo, S., Trangongo. (2004). Antiradical Activity of Analiman (Zanthoxylum achanthopodium DC) Fruit Extract. Indonesian Food and Nutrition Progress, 11 (1), 15-19.

Szakács, G., Váradi, A., Ozvegy-Laczka, C., Sarkadi, B. (2008). The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Drug Discovery Today. 13(9-10):379-93.

Tang, W., Hemm, I., Bertram, B. (2003). Recent Development of Antitumor Agents from Chinese Herbal Medicines, Part I. Low Molecular Compounds. Planta Medica, 69, 97-108. ISSN: 0032-0943.

Tatsadjieu, L. N, Essia, N. J, Ngassoum, M. B., Etoa, F. X. (2003). Antibacterial and antifungal activity of *Xylopiaaethiopica*, *Monodora myristica*, *Zanthoxylum xanthoxylo¨ides* and *Zanthoxylum leprieurii* from Cameroon. Fitoterapia 74 (2003) 469–472.

Tillequin, F. (2007). Rutaceous Alkaloids as Models for the Design of Novel Antitumor Drugs. Phytochemical Reviews, 6, 65-70. ISSN: 1572-980X.

Tsai, I. L., Lin, W. Y., Teng, C. M., Ishikawa, T., Doong, S. L., Huang, M. W., Chen, Y. C., Chen, I. S. (2000). "Coumarins and Antiplatelet Constituents from the Root Bark of Zanthoxylum schinifolium". Planta Medica, 66, 618-623. ISSN: 0032-0943.

Waterman, P. G., Alexander, G. and Elizabeth, G. C. (1976). A comparative Study on the Alkaloids of *Zanthoxylum leprieurii, Z. lemailei* and *Z. rubescens* from Ghana: *Biochemical Systematics and Ecology*, 4, 259-262.

Waterman, P. G., Grundon M. F. (1983). Chemistry and Chemical Taxonomy of the Rutales Academic Press, ISBN: 0127376801.

World health organization, (2015). Summary of world cancer report 2012, ISBN 9759241564533.

Xiao, N., Ping, Z., Wei, L. and Hong, X. (2011). Effects of Chelerythrine, a Specific Inhibitor of Cyclooxygenase-2, on Acute Inflammation in Mice: *Fitoterapia*, 82, 620–625.

Xiong, Q., Dawen, S., & Mizuno. (1994). Flavanol glucosides in Pericarps of *Zanthoxylum bungeanum*: *Phytochemistry*, 39 (3), 723-729.

Yamazaki, E., Inagaki, M., Kurita, O., Inoue, T. (2007). Antioxidant activity of Japanese pepper (Zanthoxylum piperitum DC.) fruit. Food Chemistry, 100, 171-177. ISSN: 03088146.

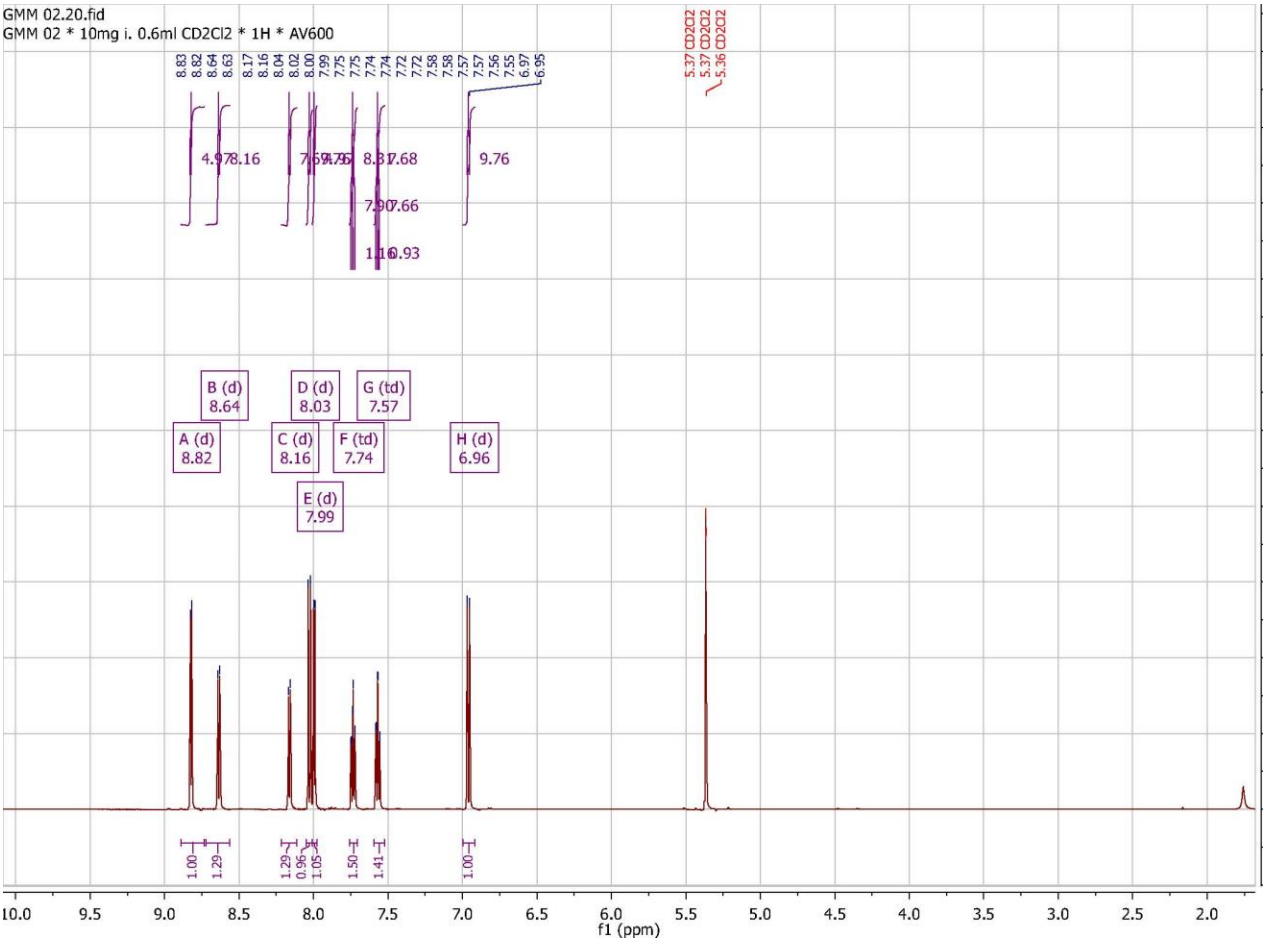
Yang, Y. P., Cheng, M. J., Teng, C. M., Chang, Y. L., Tsai, I. L., Chen, I. S. (2002). Chemical and anti-platelet constituents from Formosan *Zanthoxylum simulans. Phytochemistry* 61, 567–572.

Zhou, X. J., Chen, X. L., Li, X. S., Su, J., He, J. B., Wang, Y. H., Li, Y., Cheng, Y. X. (2011). Two dimeric lignans with an unusual a, b-unsaturated ketone motif from *Zanthoxylum* podocarpum and their inhibitory effects on nitric oxide production. *Bio-organic and medicinal chemistry letters*, 21, 373-376.

APPENDIXES

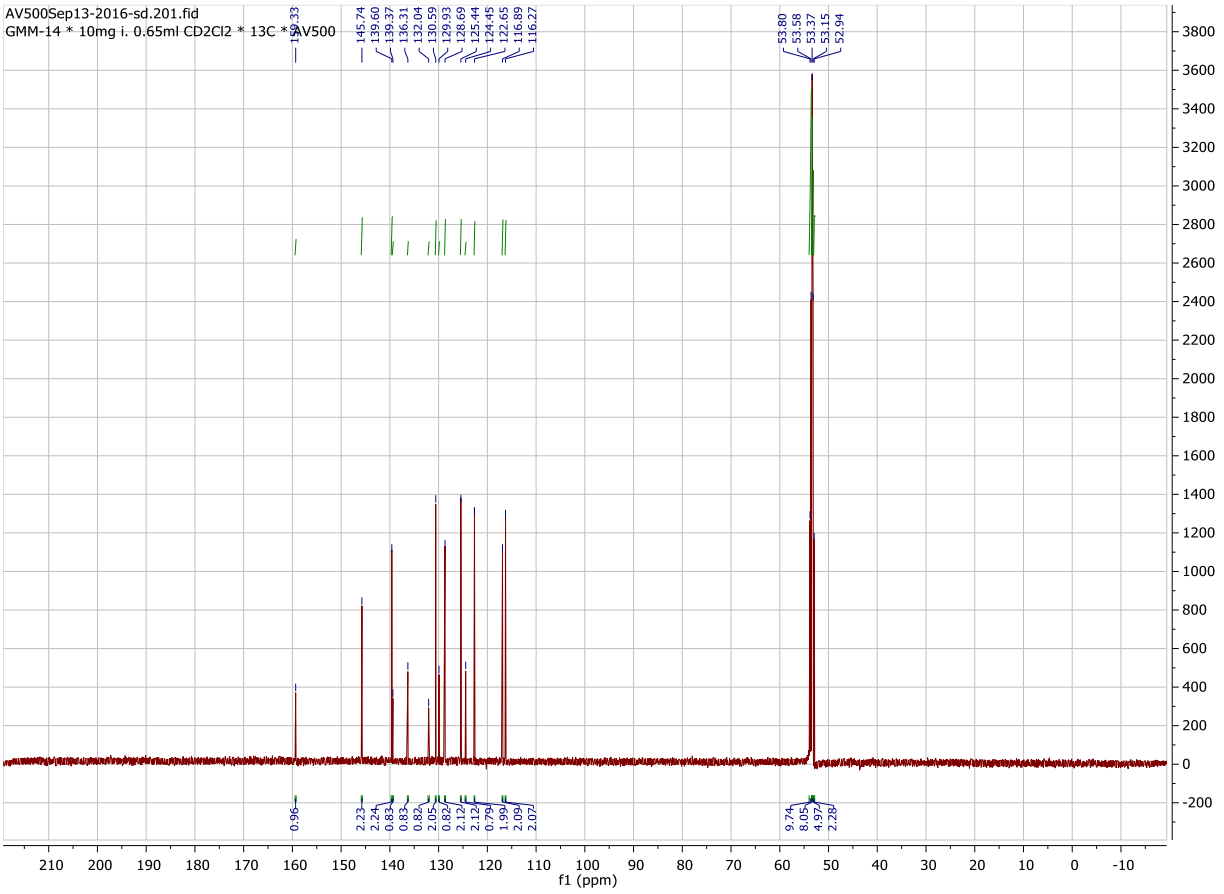
APPENDIX 1a

^{1}H NMR data of compound 1



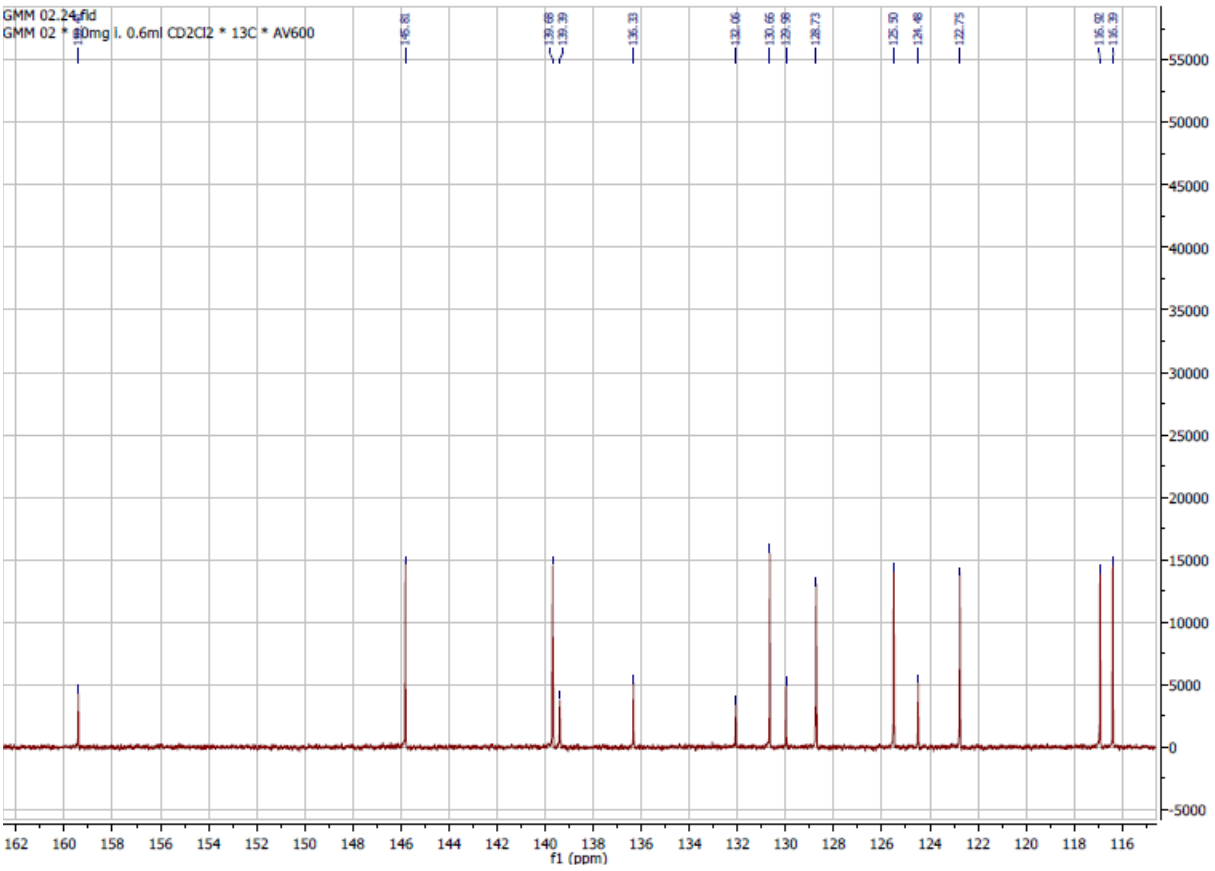
APPENDIX 2b

^{13} C NMR data of compound 1



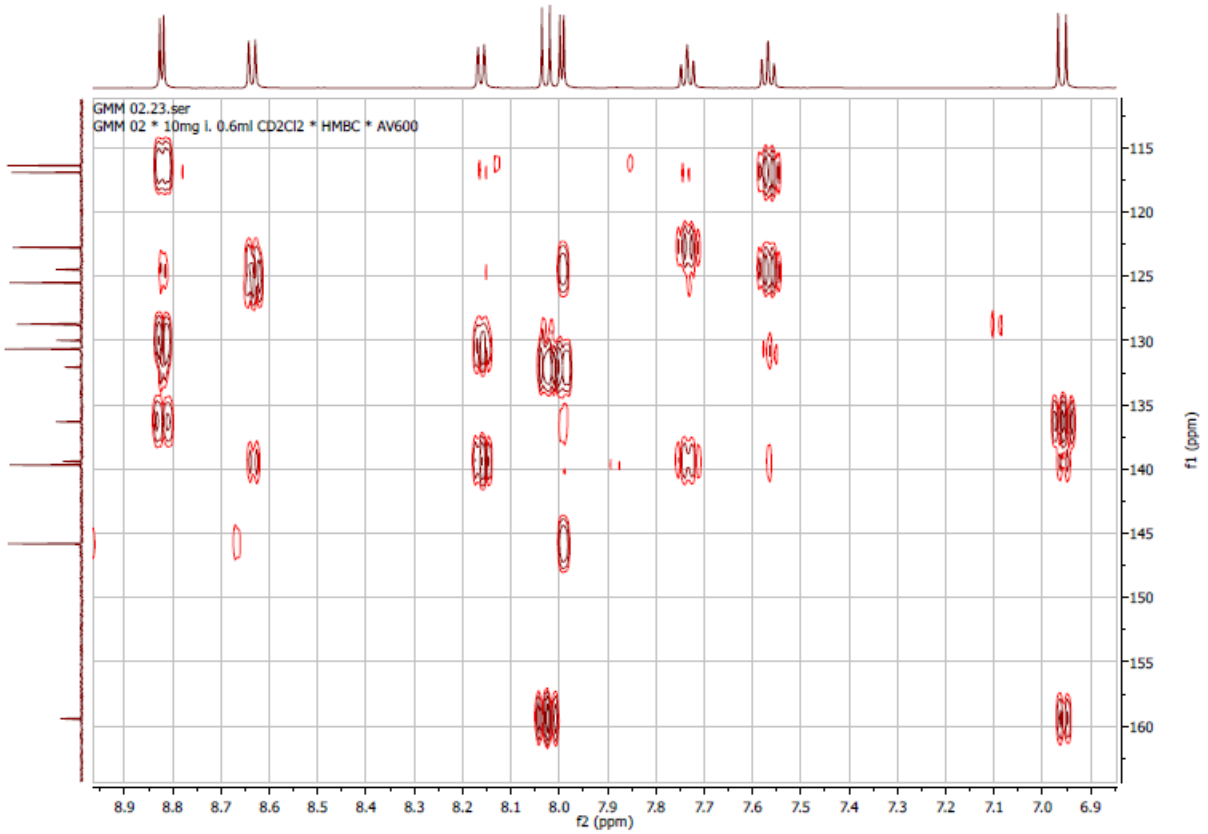
APPENDIX 3c

Expanded ^{13}C NMR data of compound 1



APPENDIX 4d

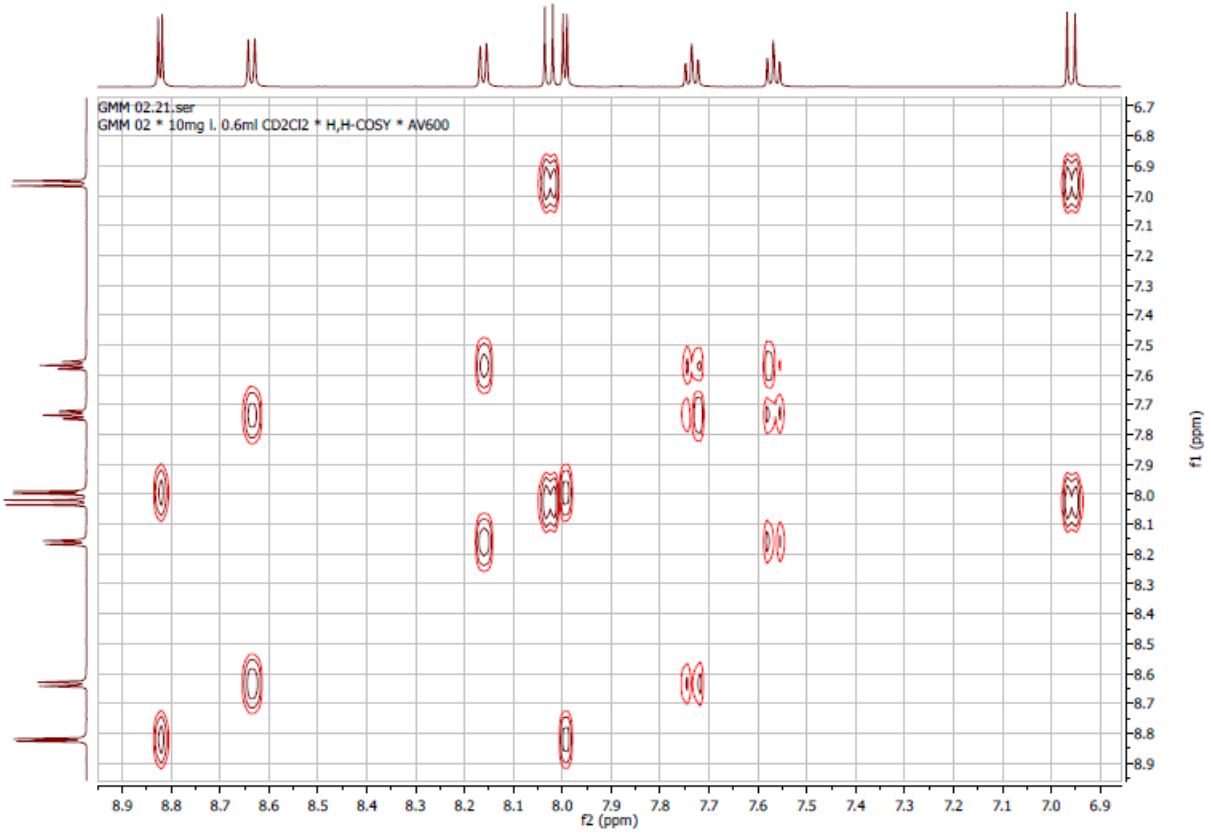
HMBC data of compound 1



1

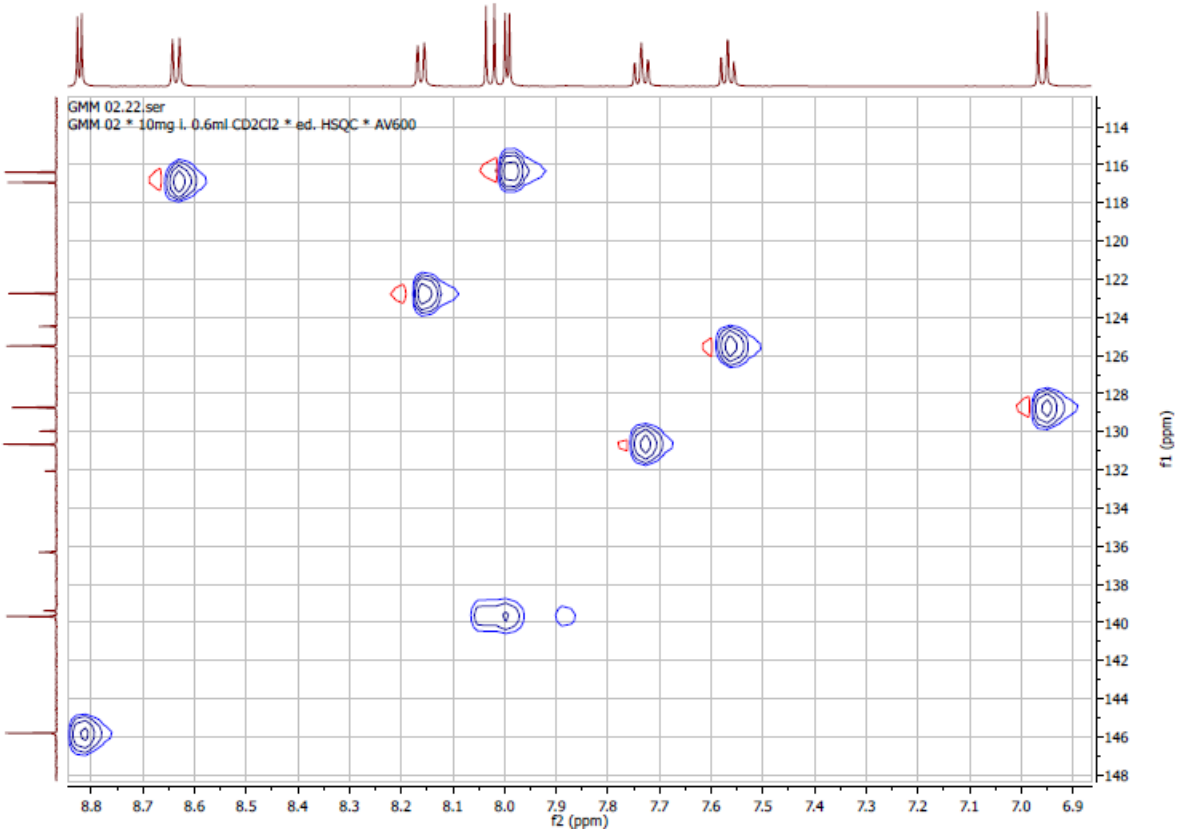
APPENDIX 5e

H,H-COSY data of compound 1



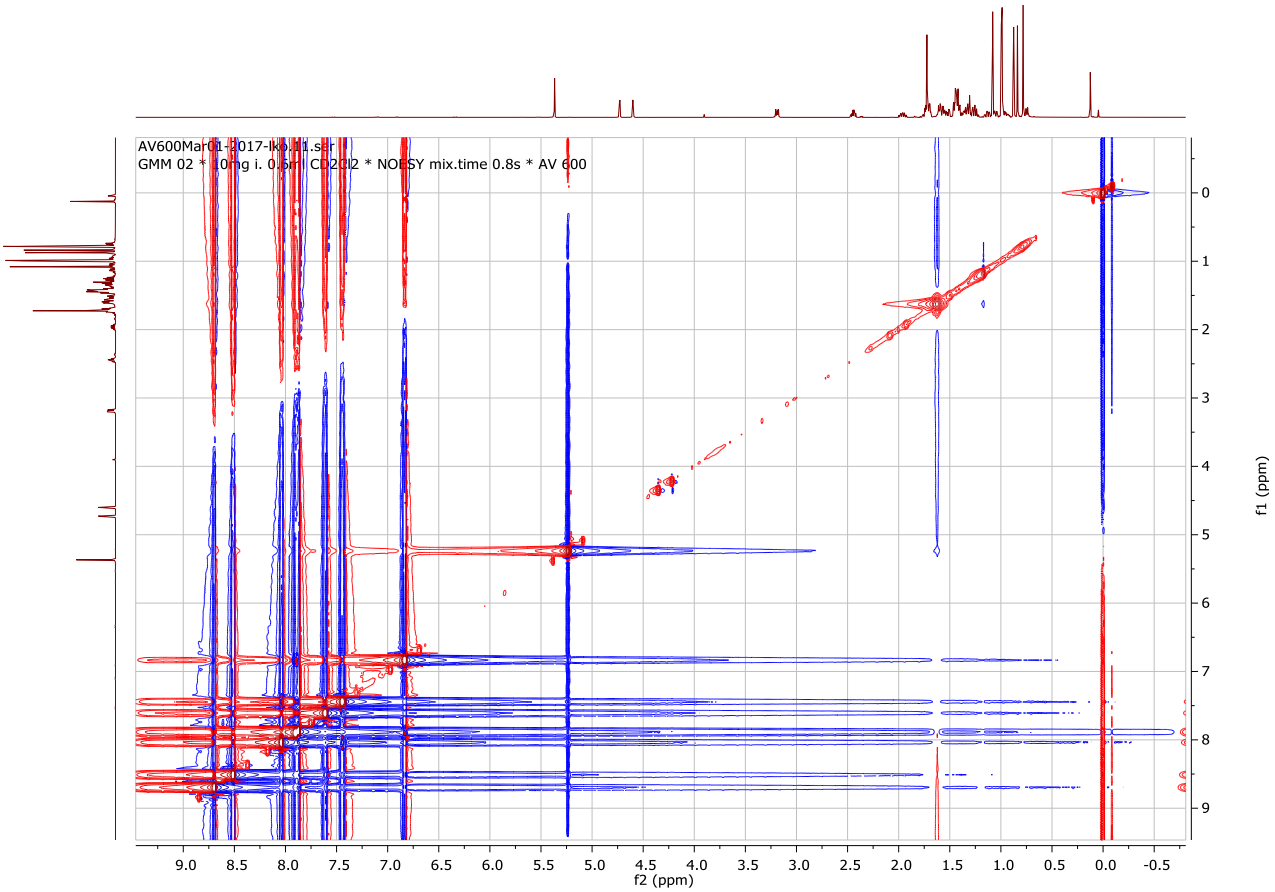
APPENDIX If

HSQC data of compound 1



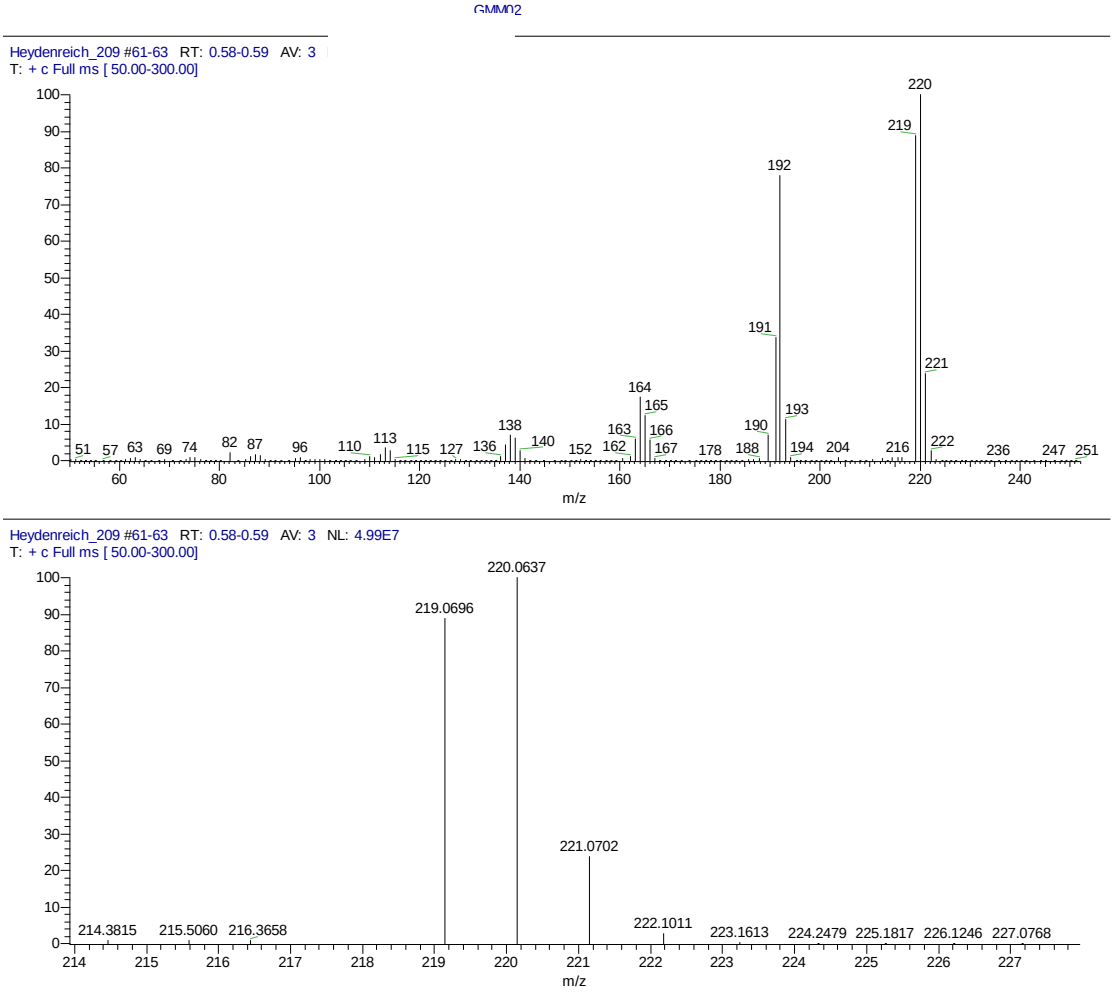
APPENDIX Ig

NOESY data of compound 1



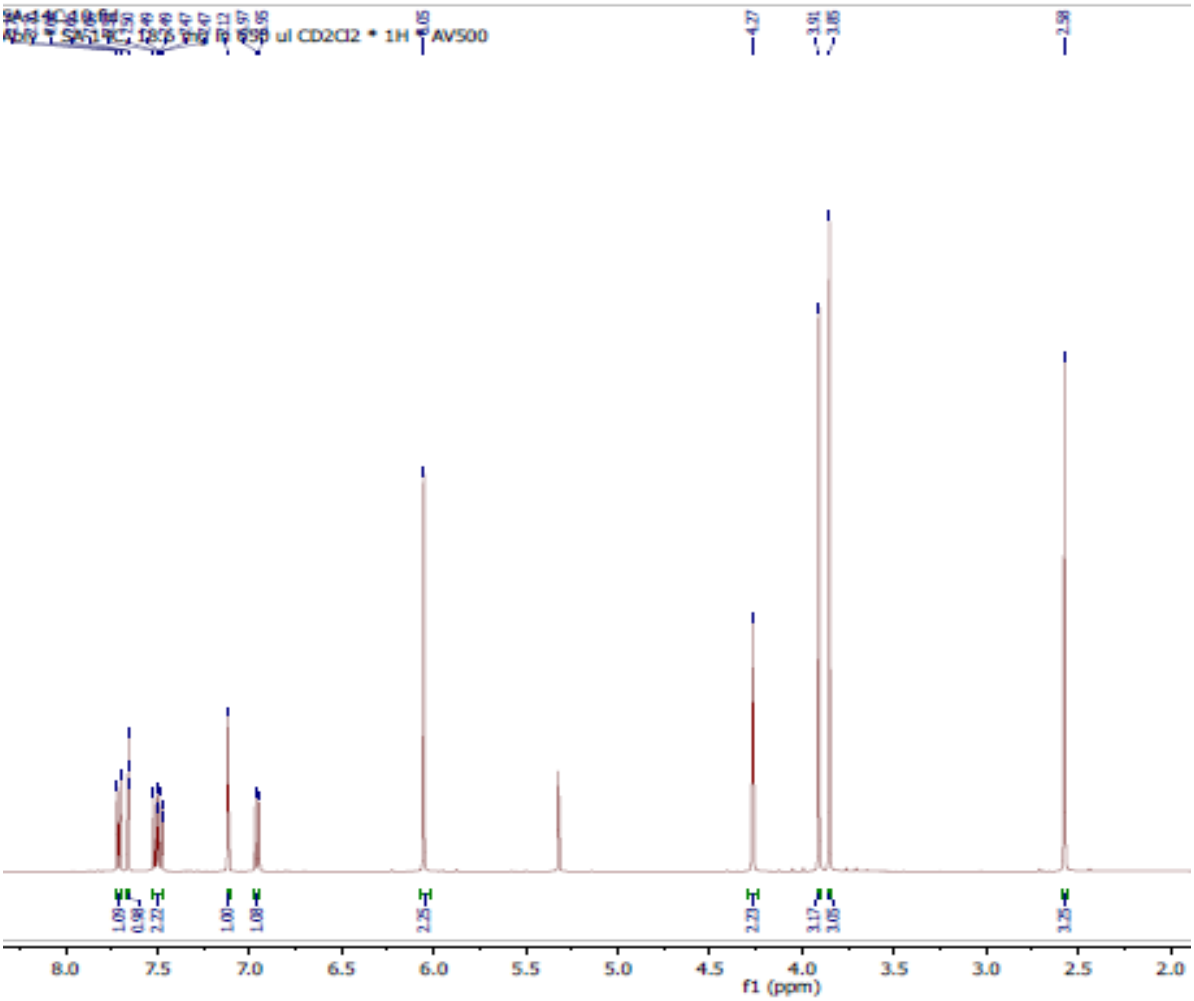
APPENDIX Ih

MS spectroscopy data for compound 1



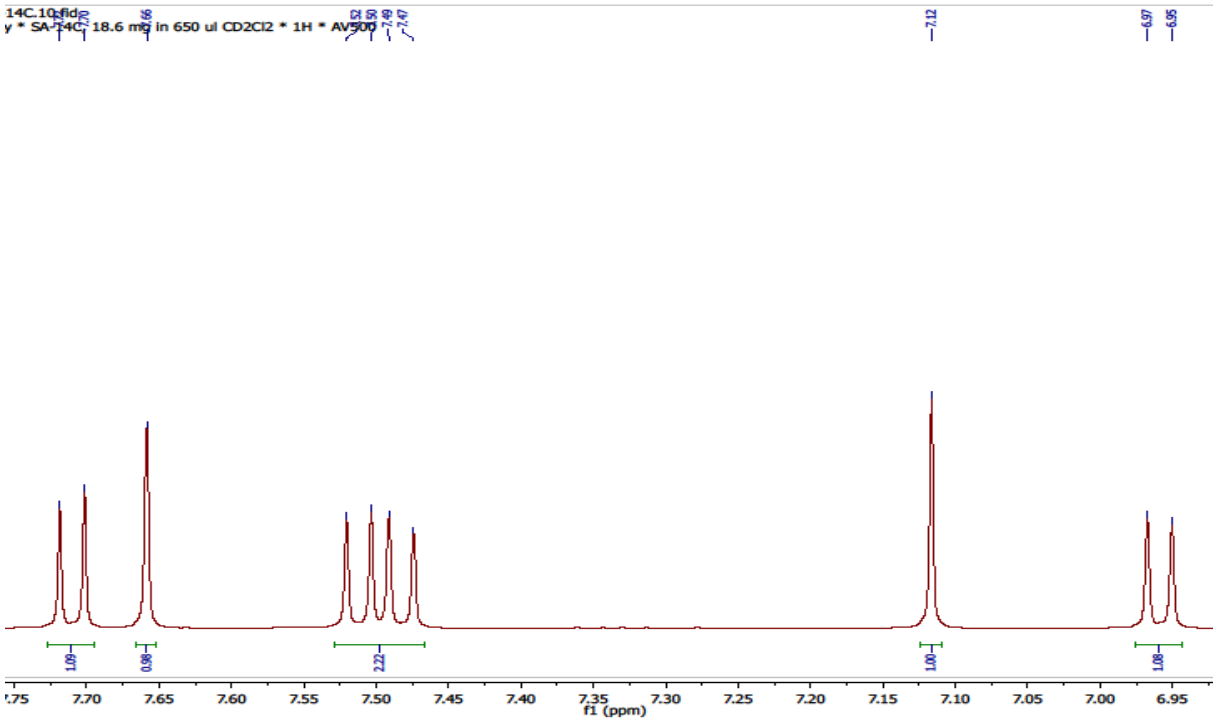
APPENDIX IIa

^{1}H NMR data of compound 2



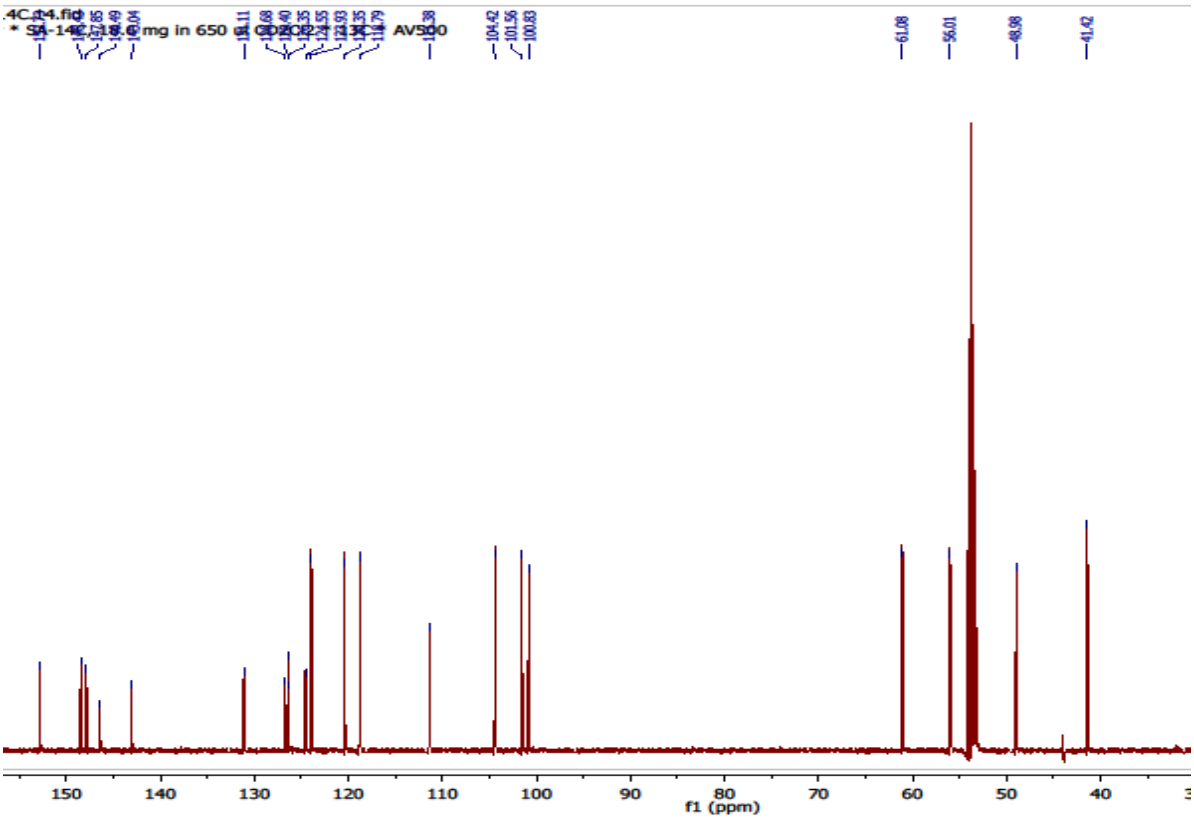
APPENDIX IIb

Expanded ^{1}H NMR data of compound 2



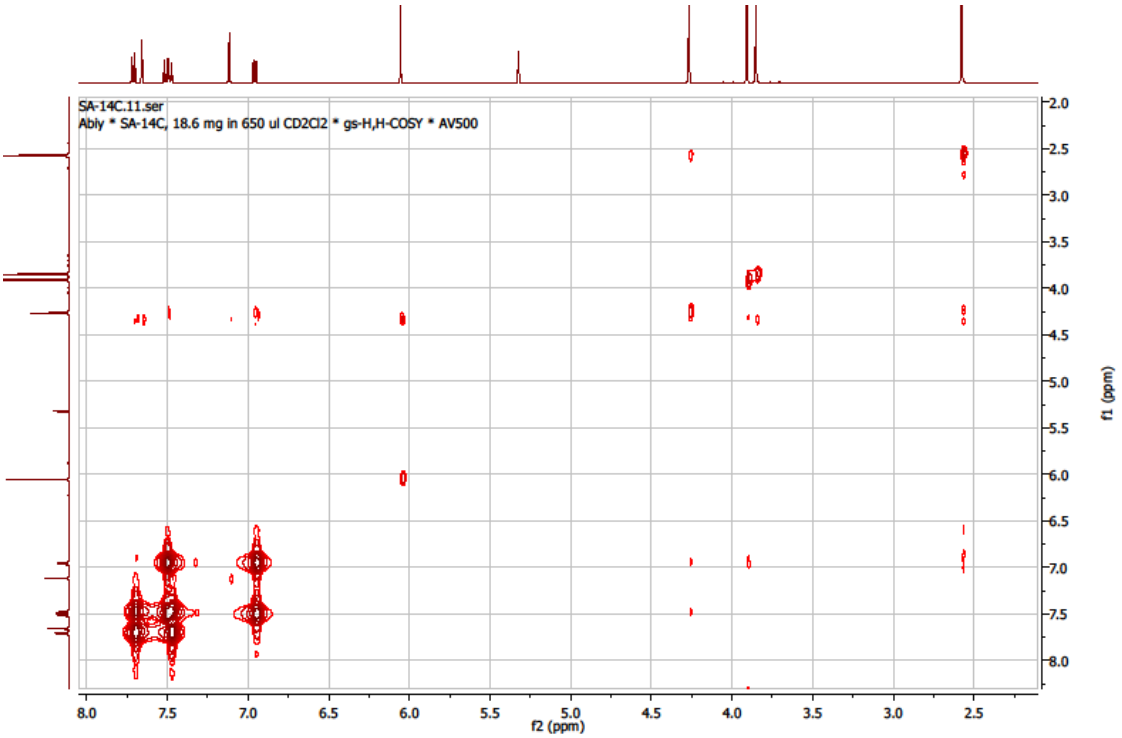
APPENDIX IIc

^{13}C NMR data of compound 2



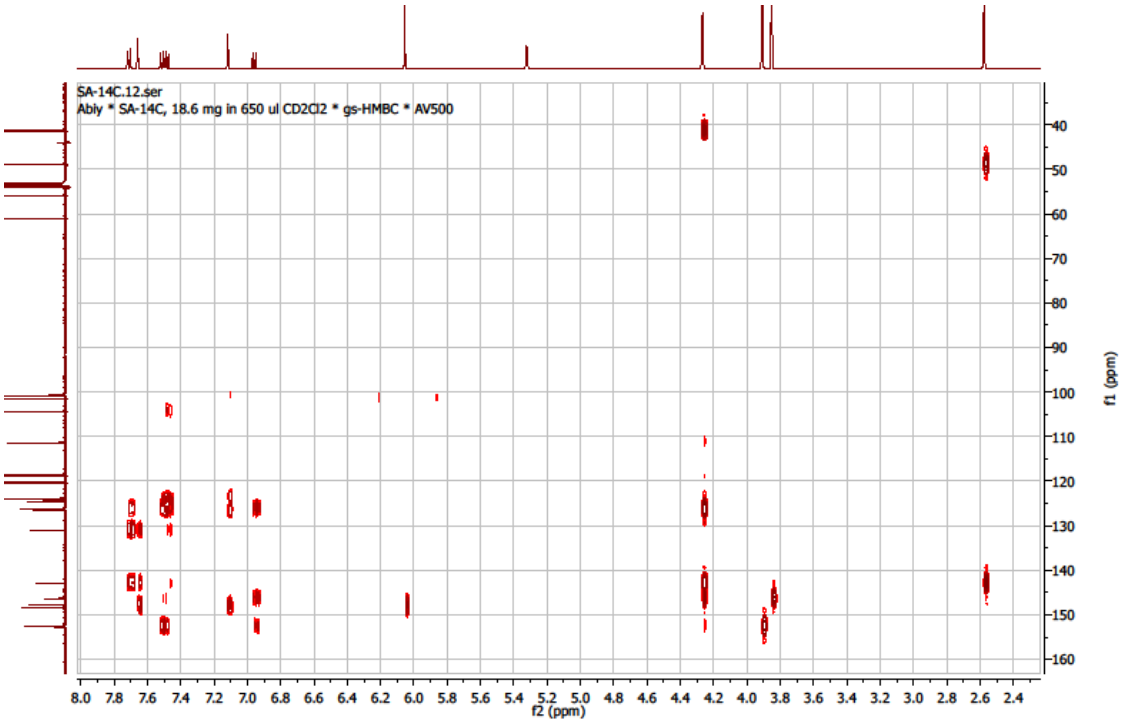
APPENDIX IId

H,H-COSY data of compound 2



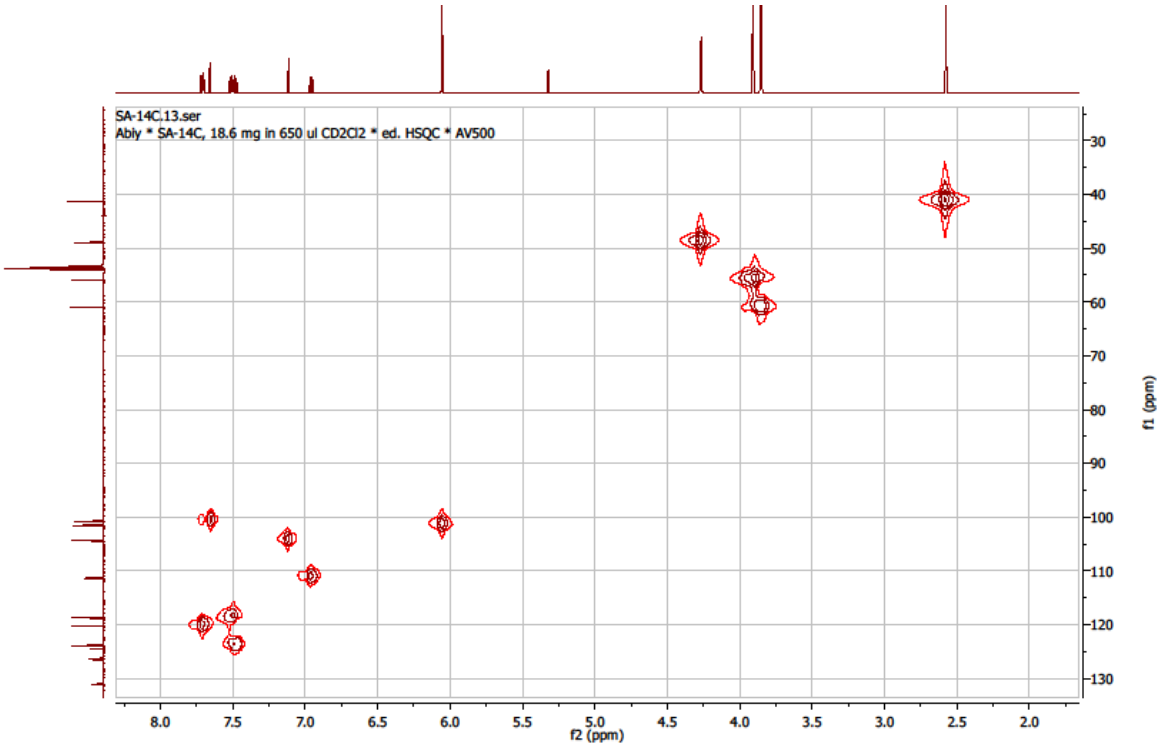
APPENDIX IIe

HMBC data of compound 2



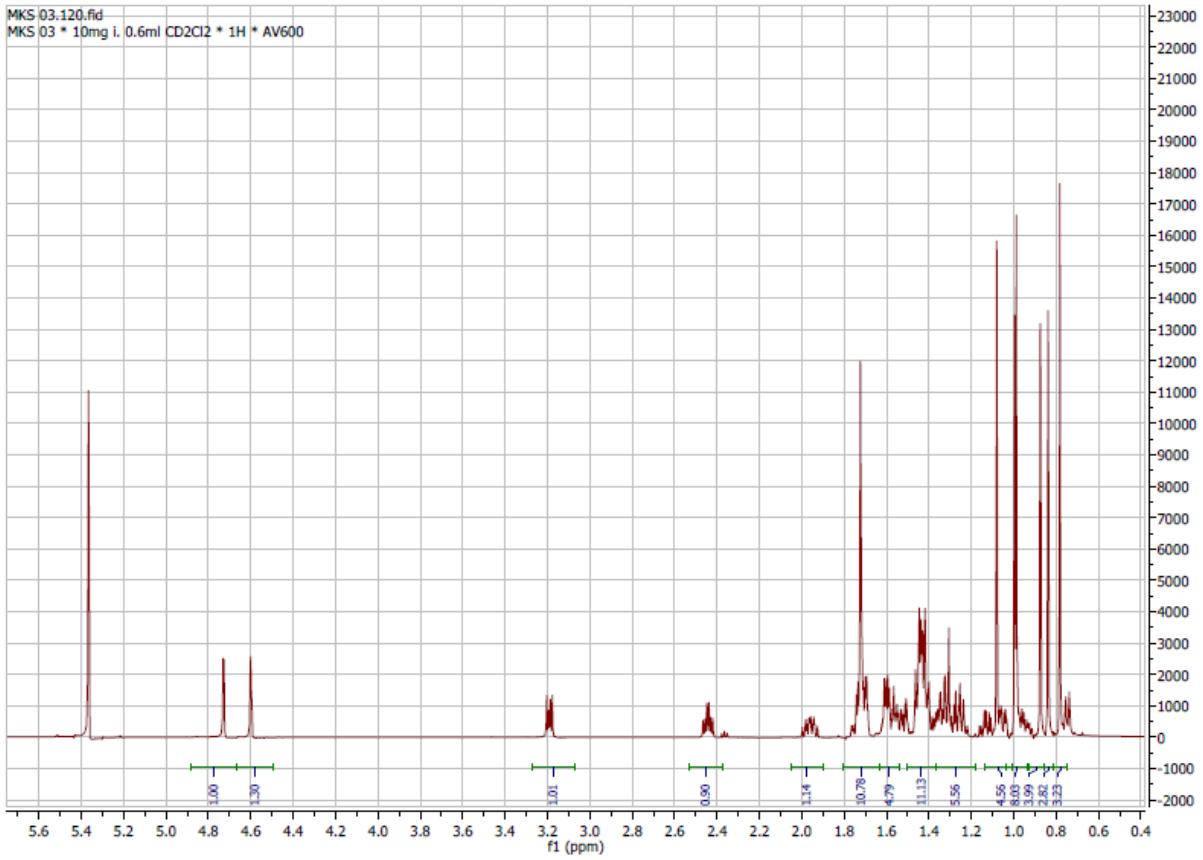
APPENDIX IIf

HSQC data of compound 2



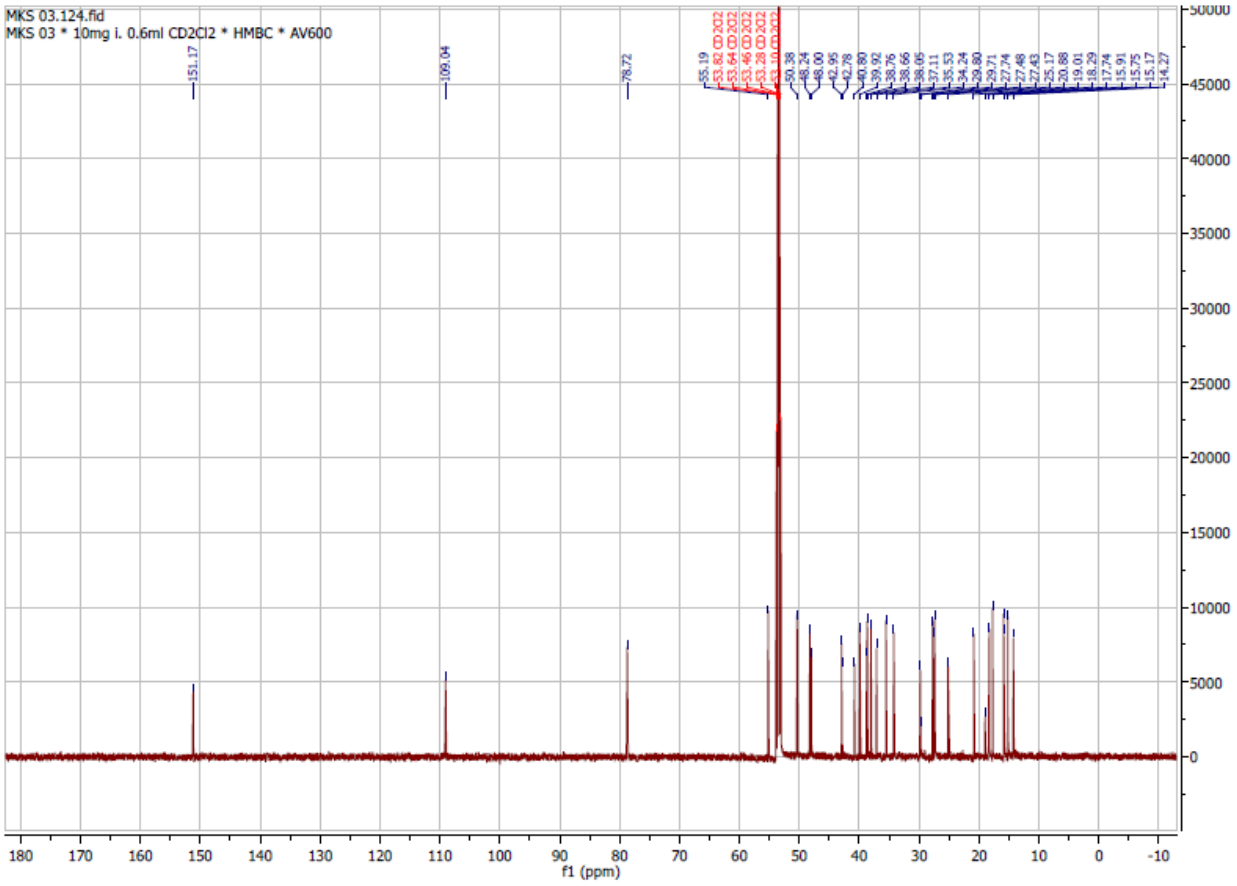
APPENDIX IIIa

^{1}H NMR data of compound 3



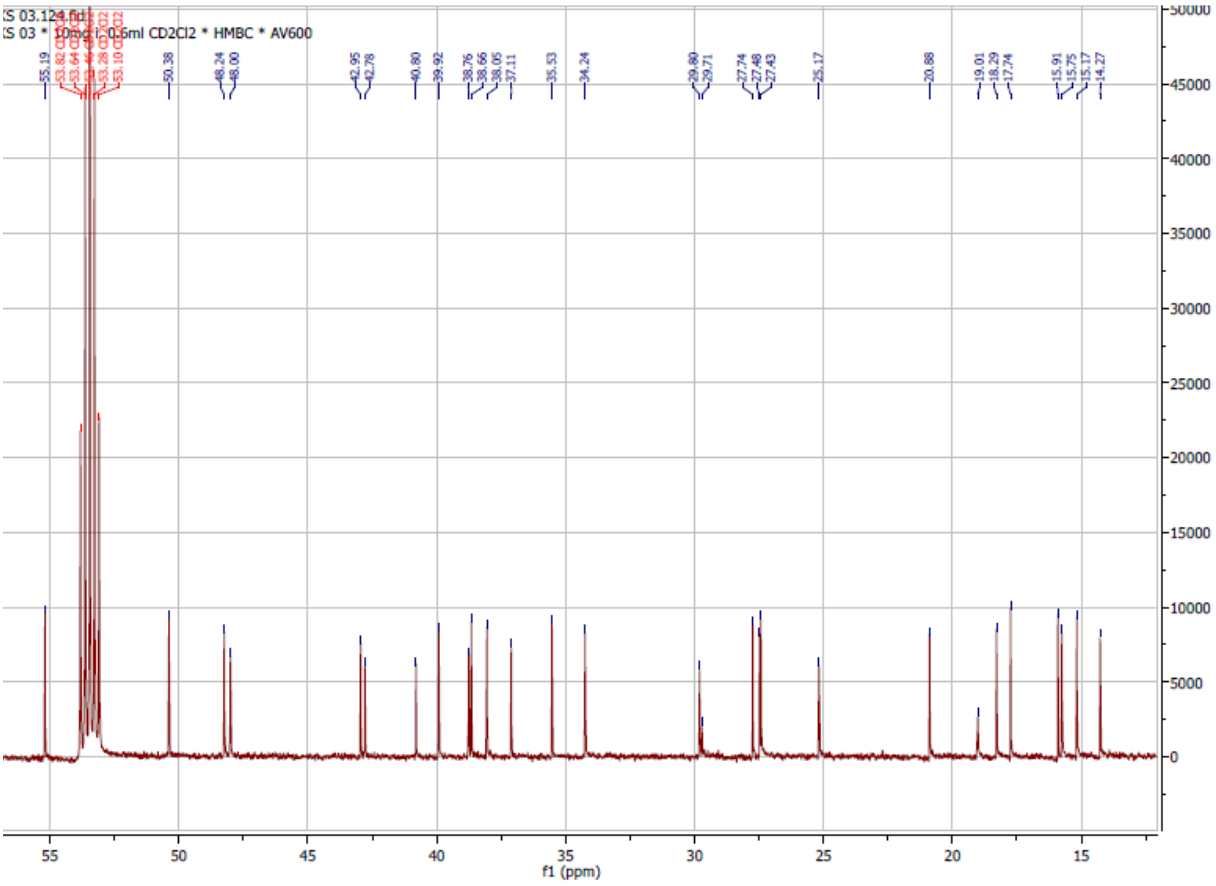
APPENDIX IIIb

^{13}C NMR data of compound 3



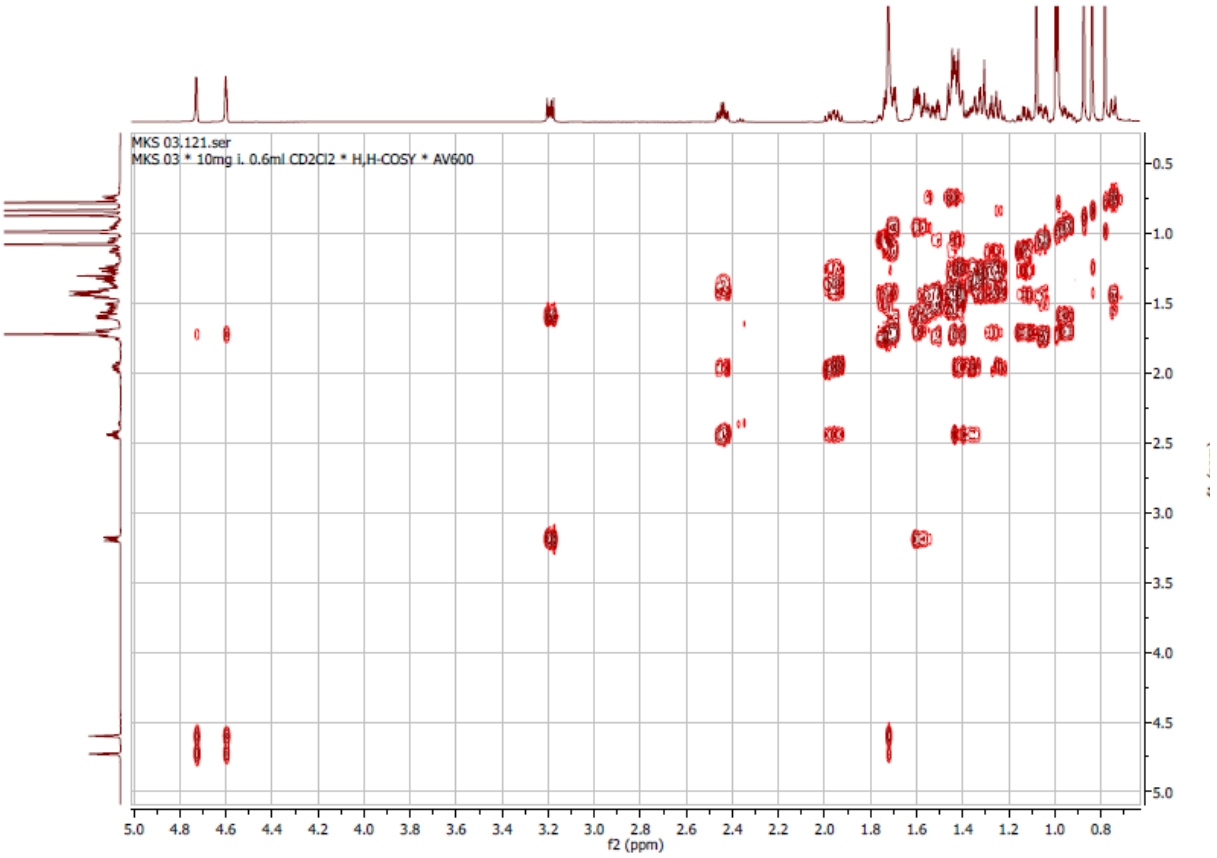
APPENDIX IIIc

Expanded ^{13}C NMR data of compound 3



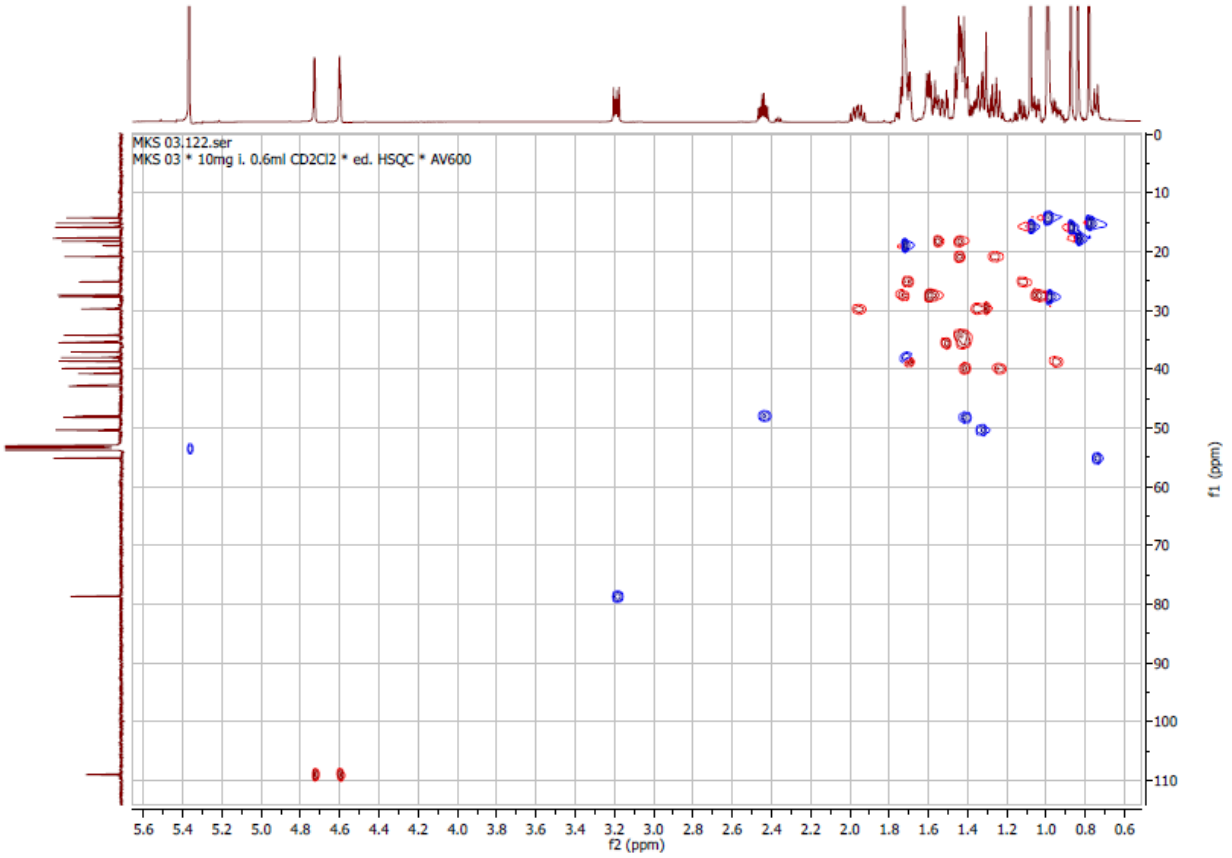
APPENDIX IIId

H,H-COSY data of compound 3



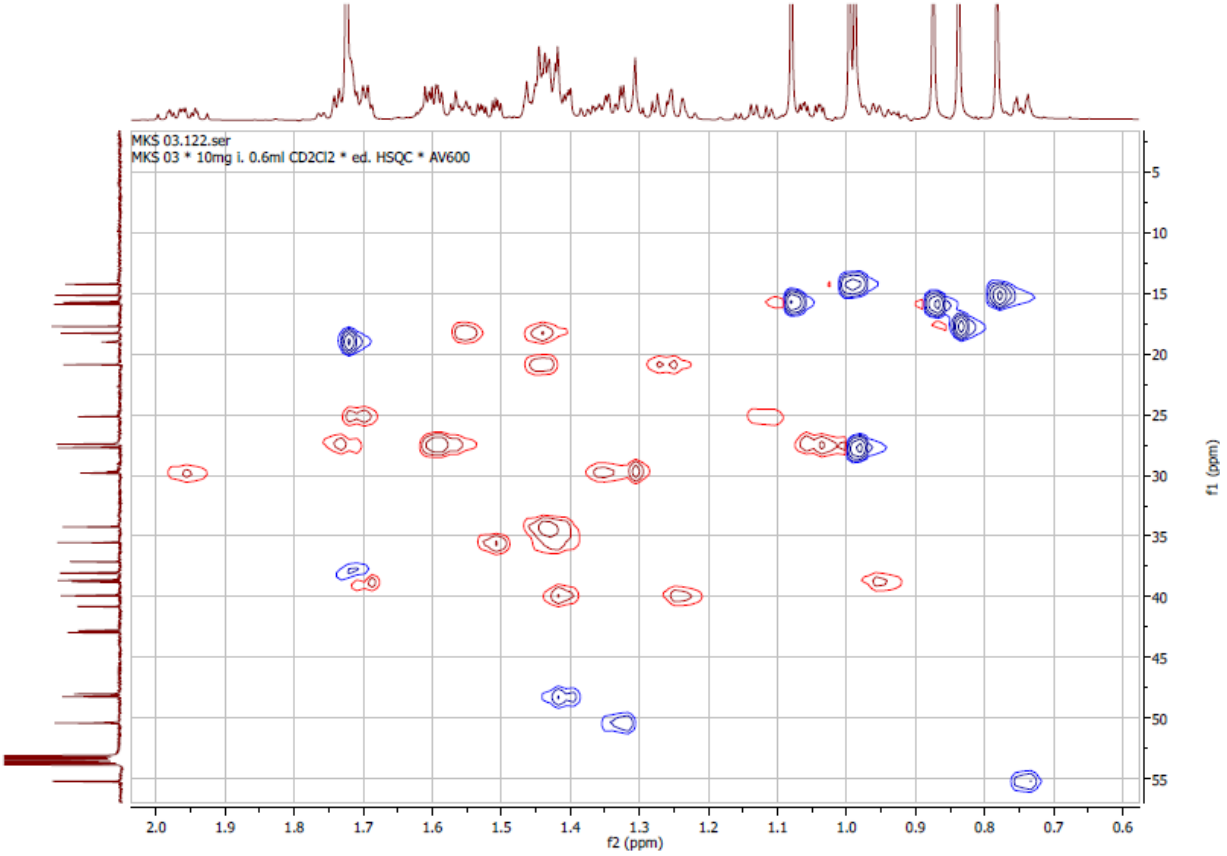
APPENDIX IIIe

HSQC data of compound 3



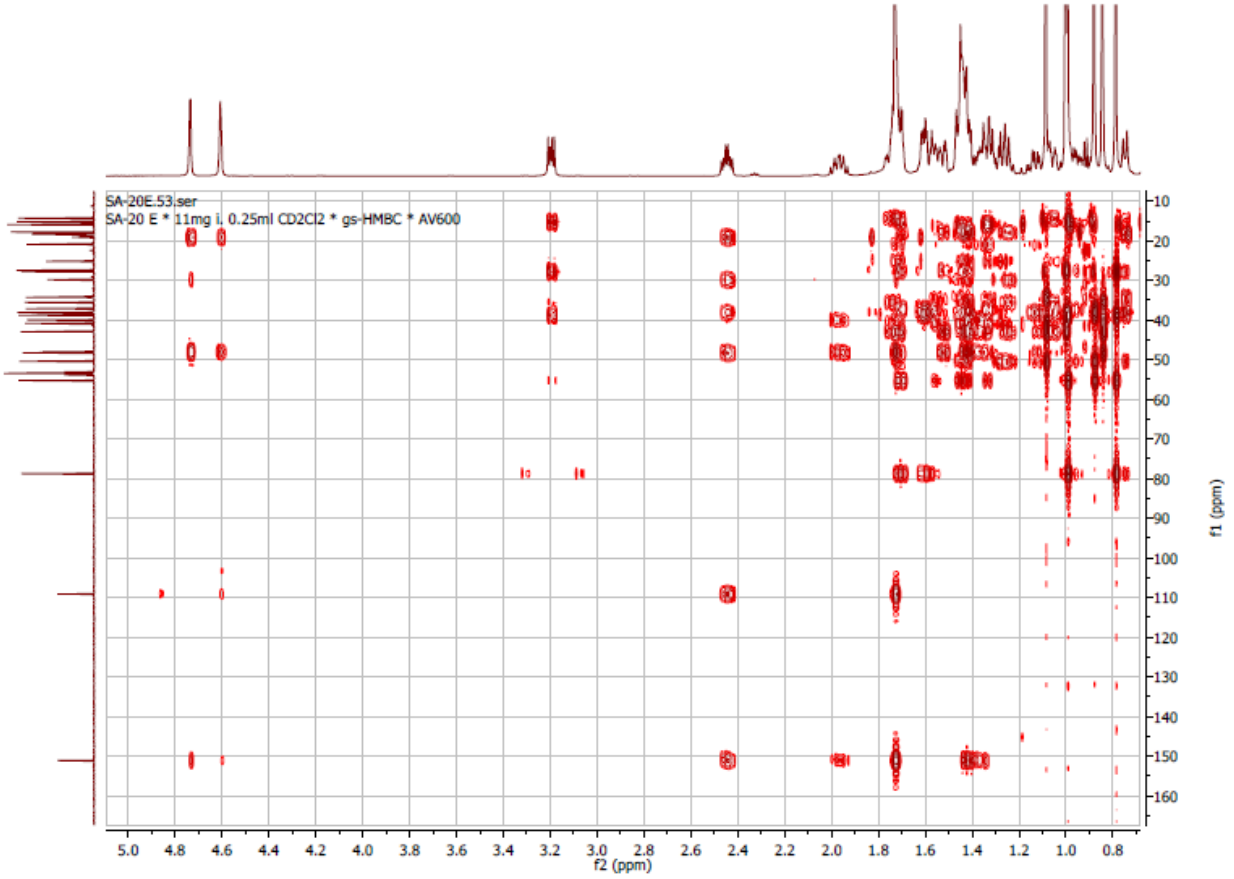
APPENDIX IIIf

Expanded HSQC data of compound 3



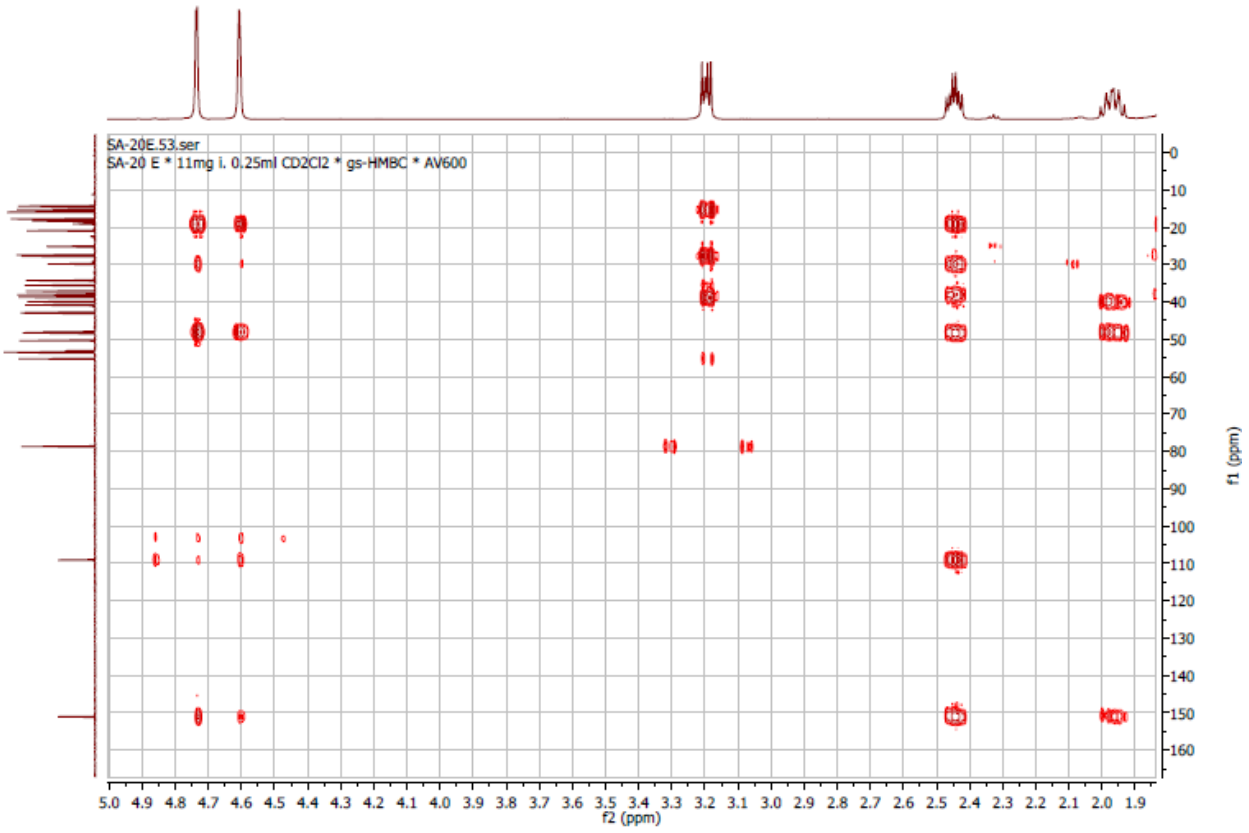
APPENDIX IIIg

HMBC data of compound 3



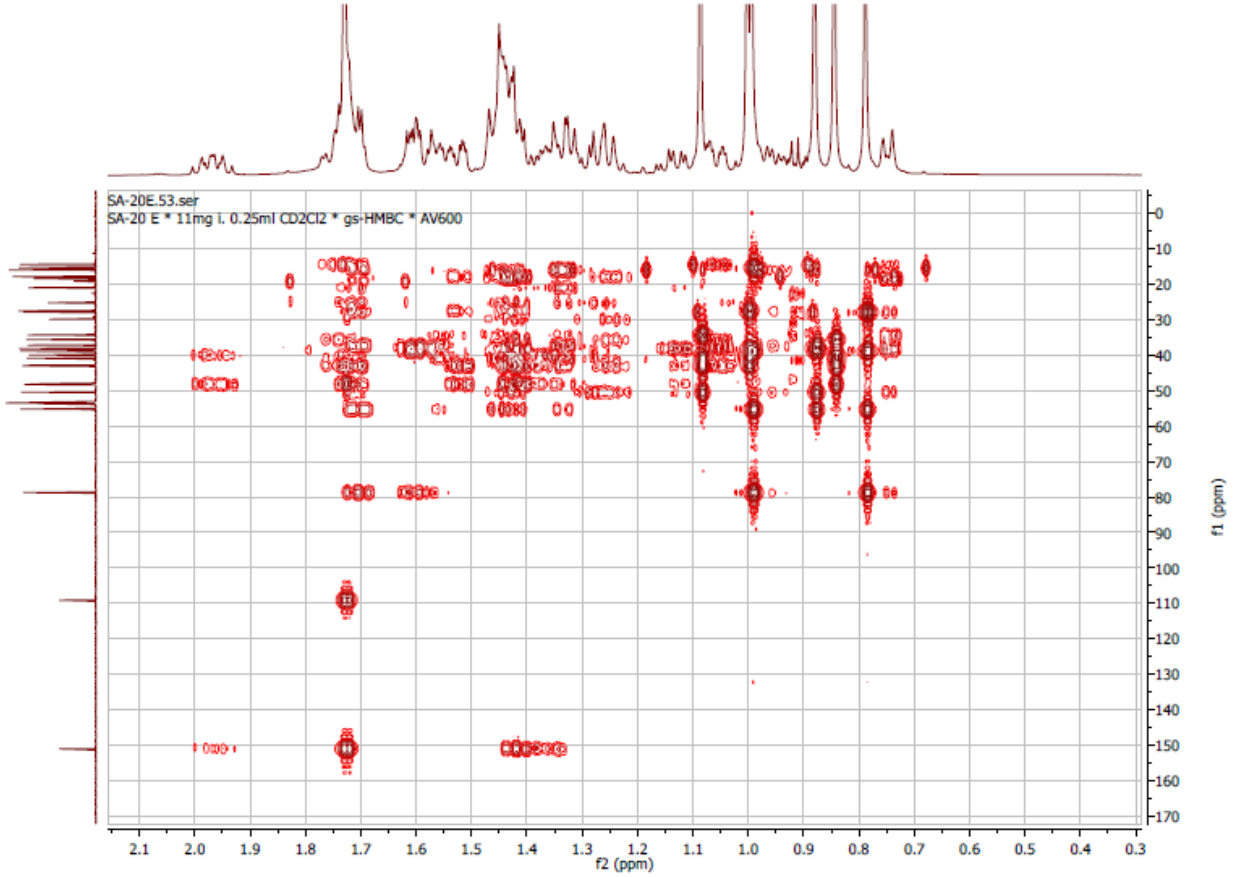
APPENDIX IIIh

Expanded HMBC data of compound 3



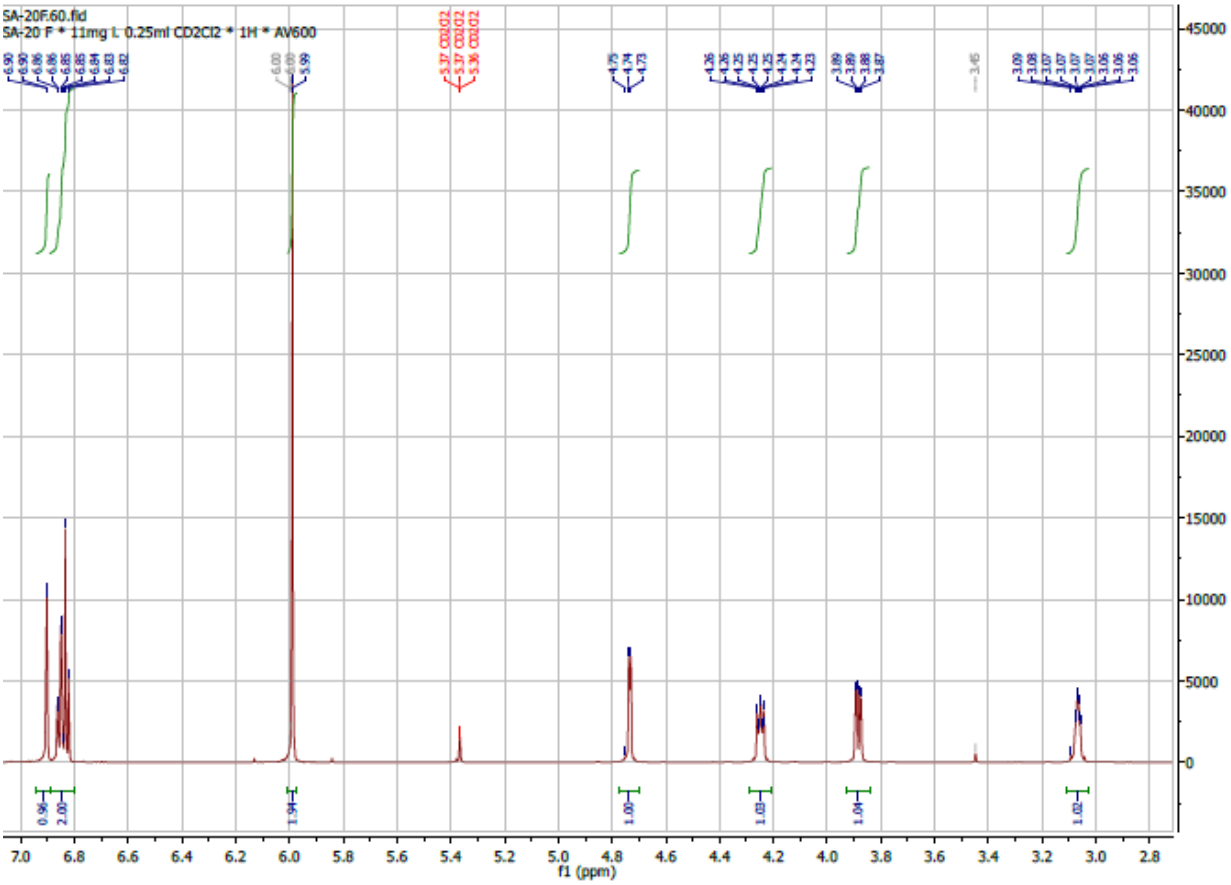
APPENDIX IIIi

Expanded HMBC data of compound 3



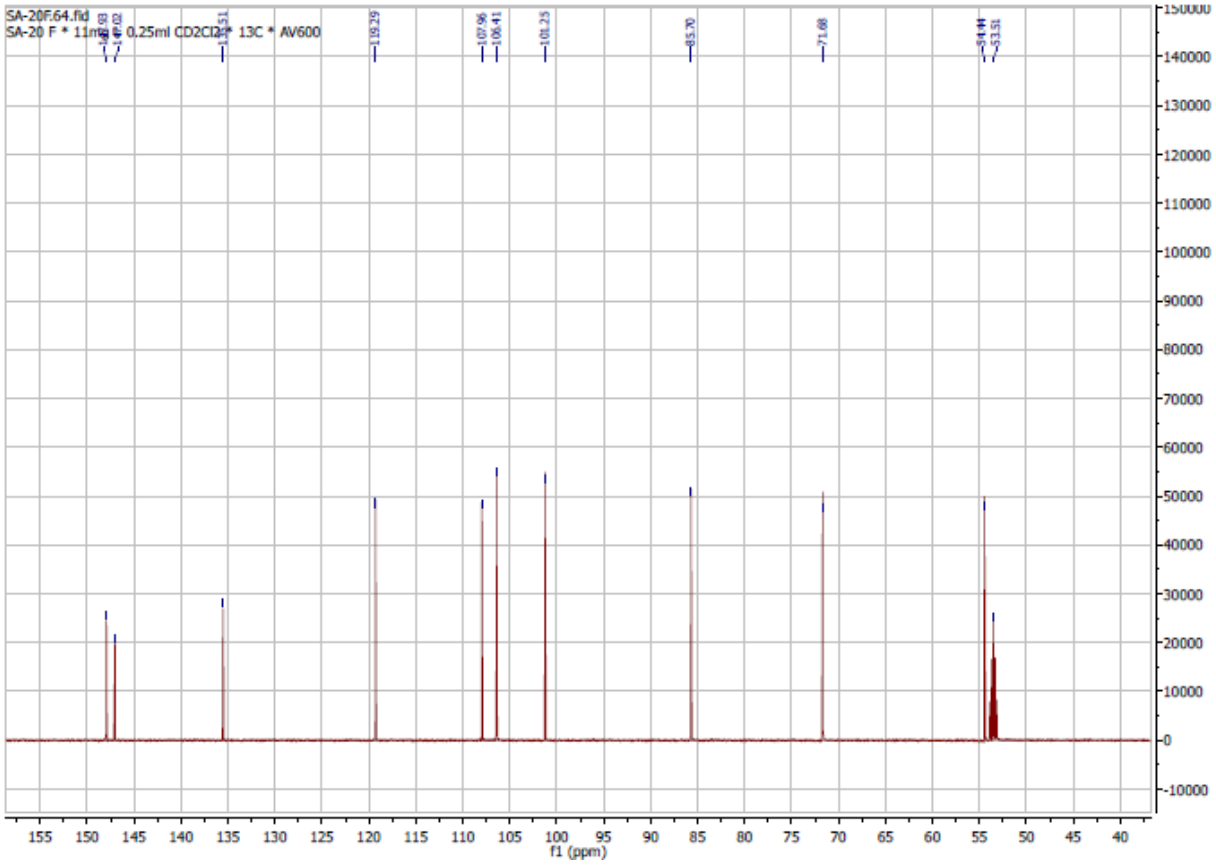
APPENDIX IVa

^{1}H NMR data of compound 4



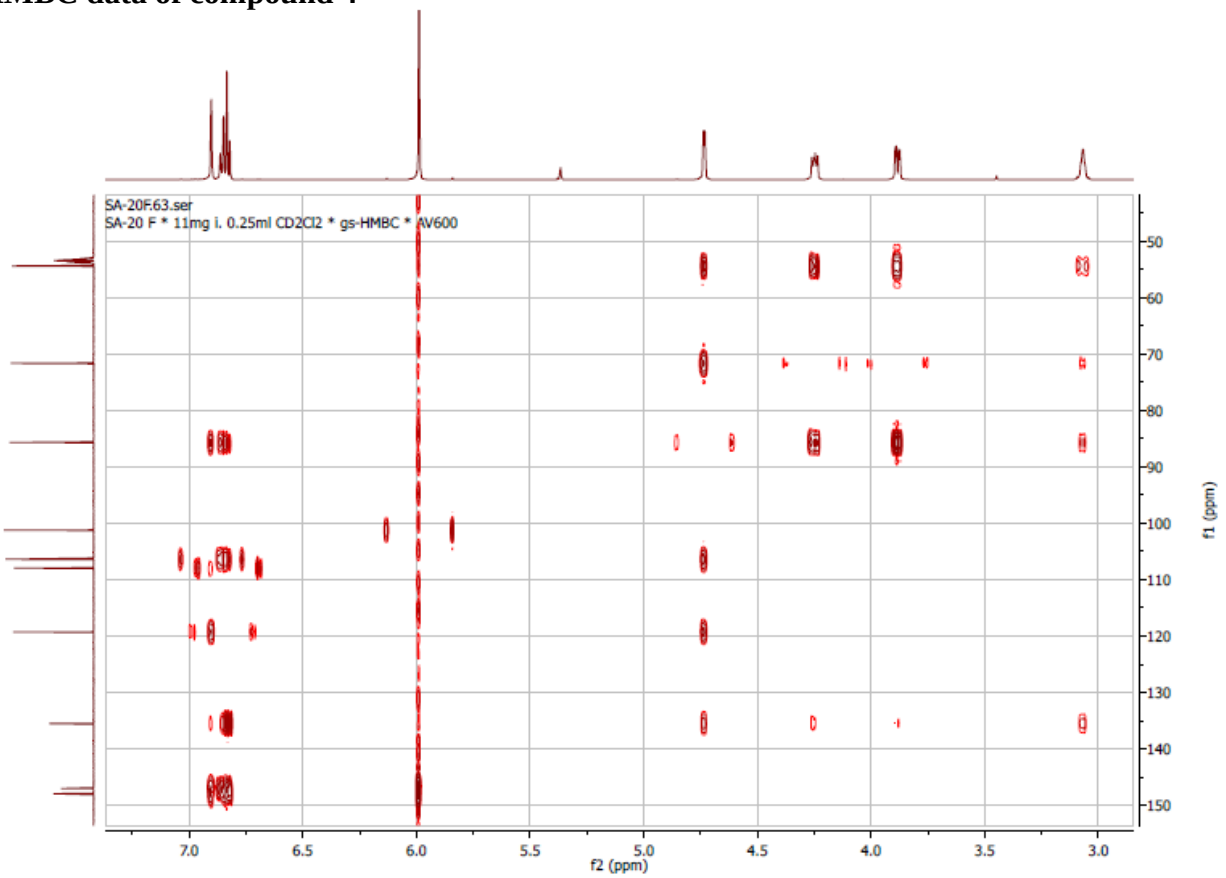
APPENDIX IVb

^{13}C NMR data of compound 4



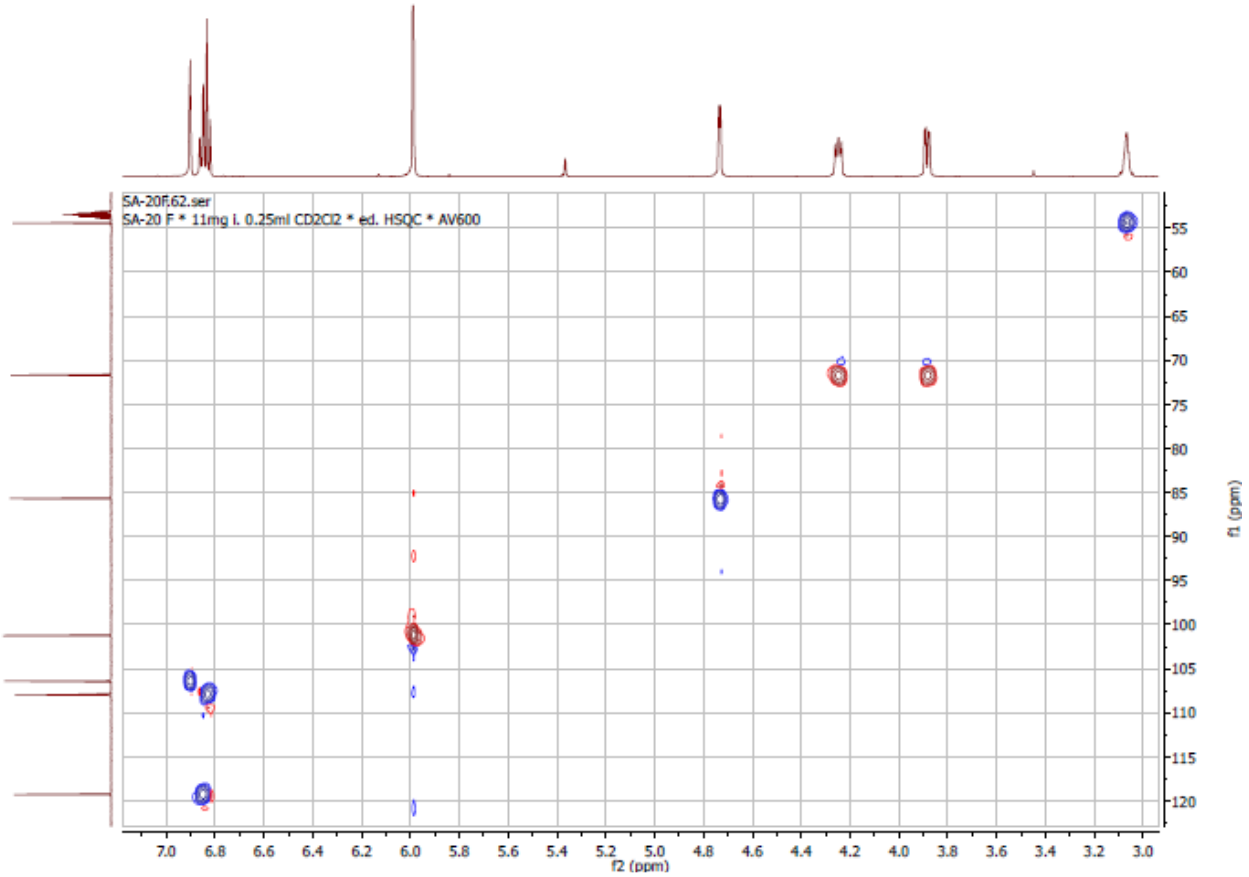
APPENDIX IVc

HMBC data of compound 4



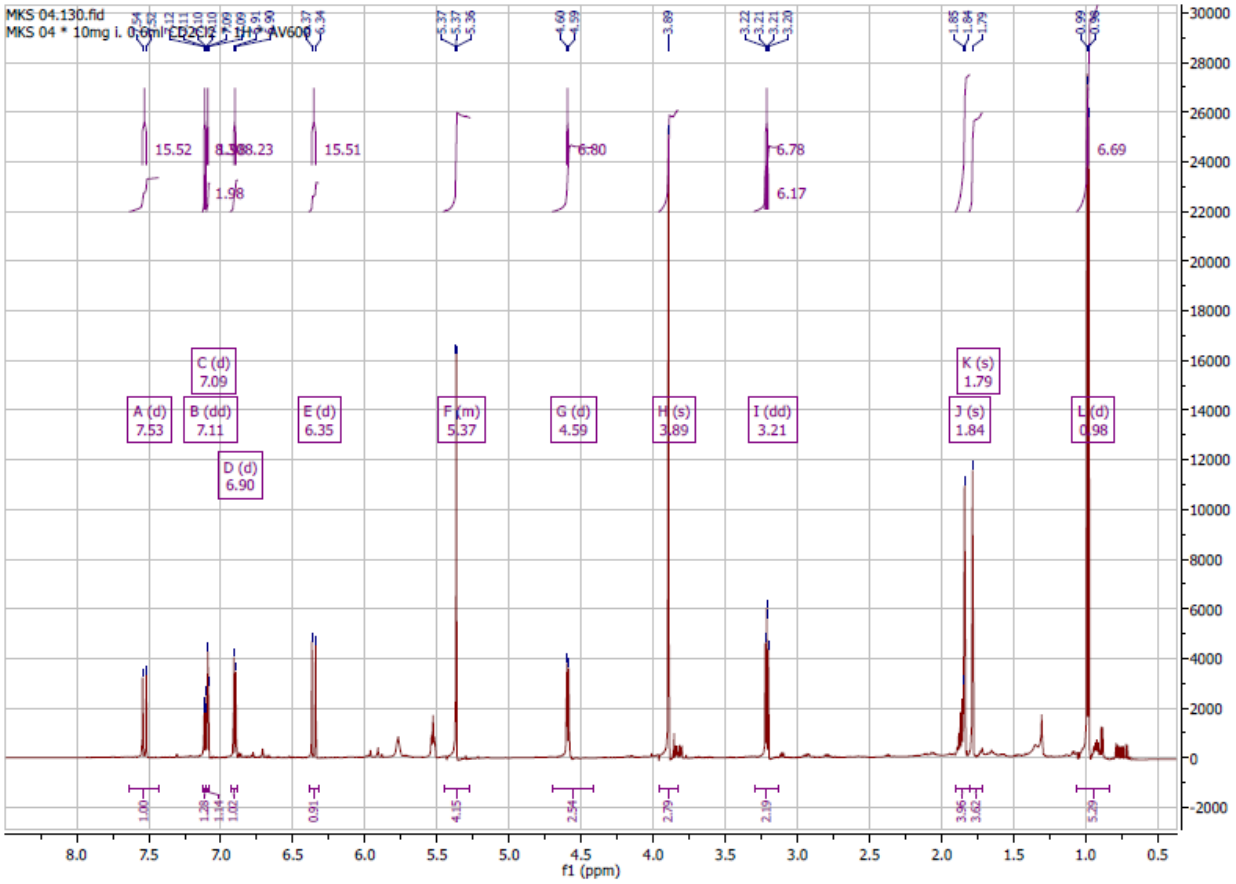
APPENDIX IVd

HSQC data of compound 4



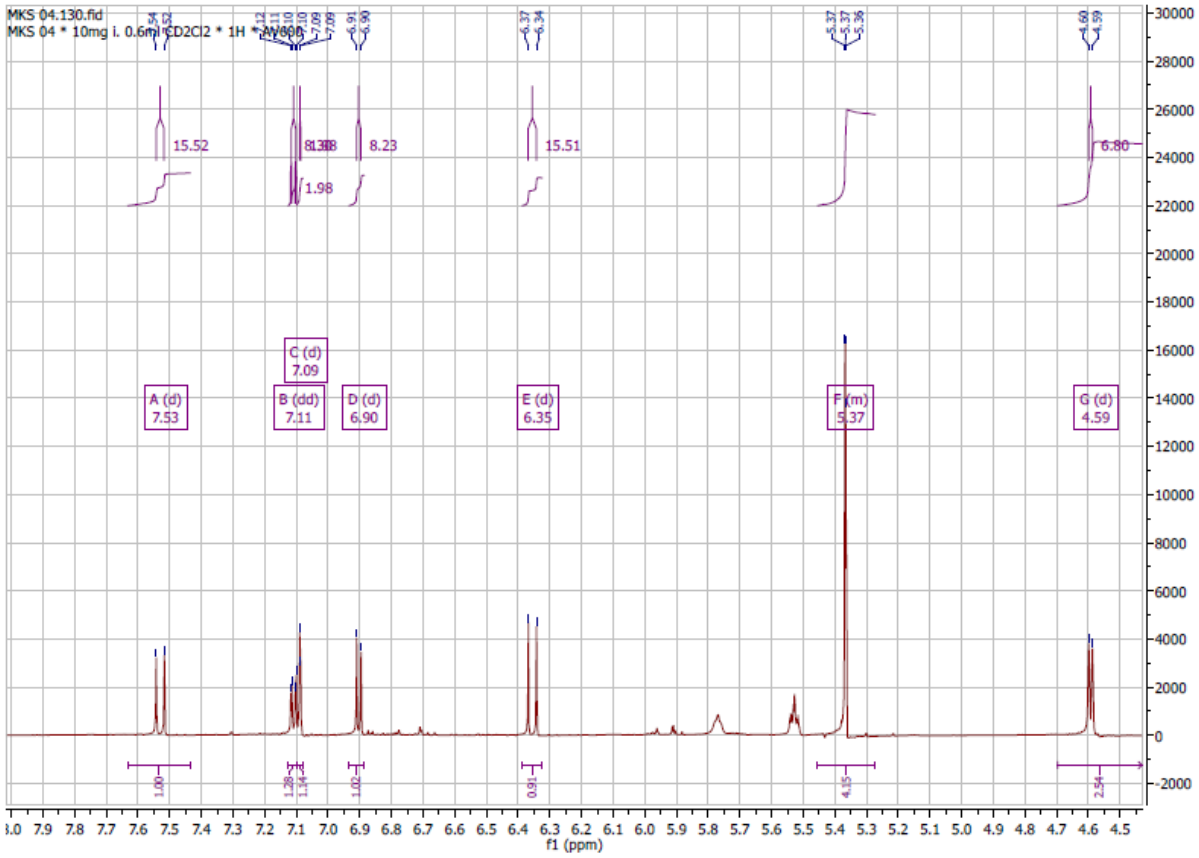
APPENDIX Va

^{1}H NMR data of compound 5



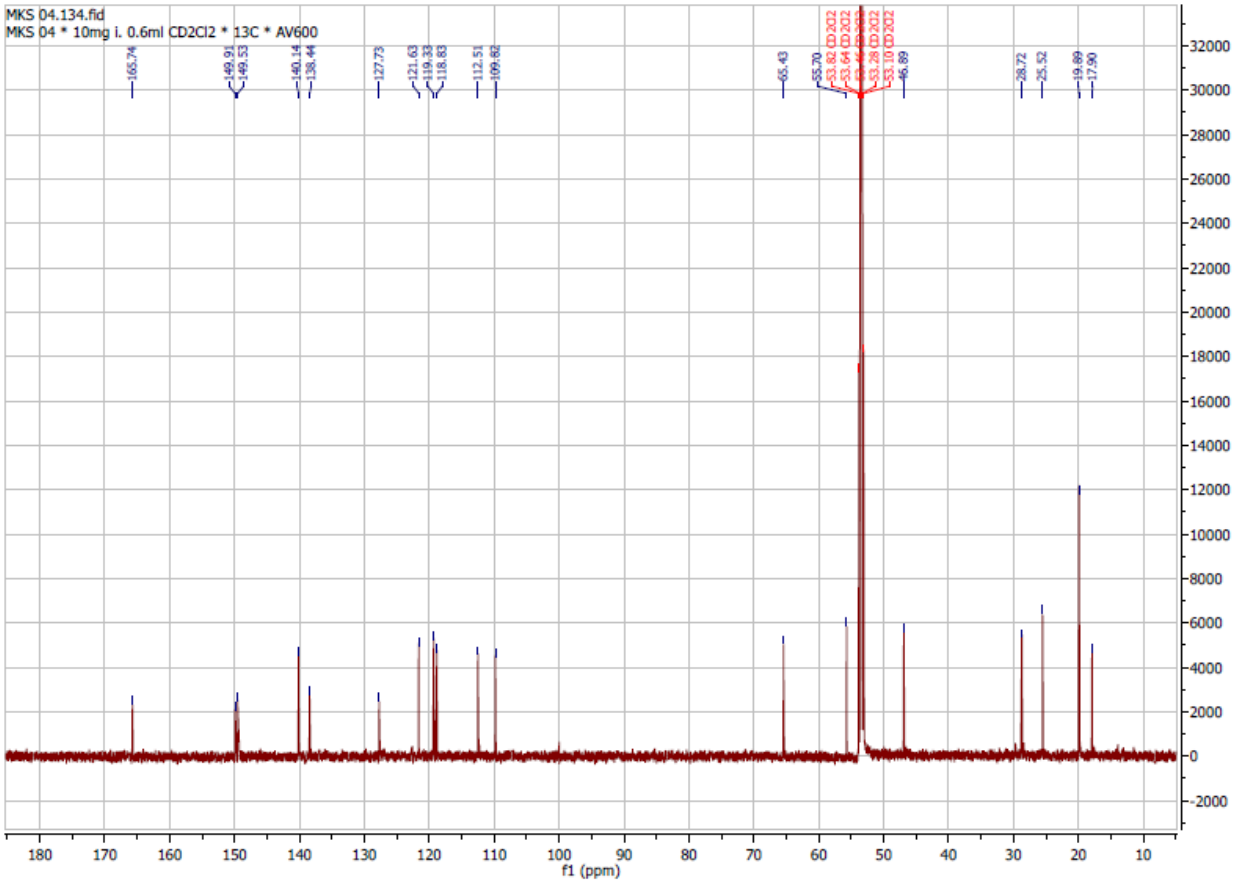
APPENDIX Vb

Expanded ^{1}H NMR data of compound 5



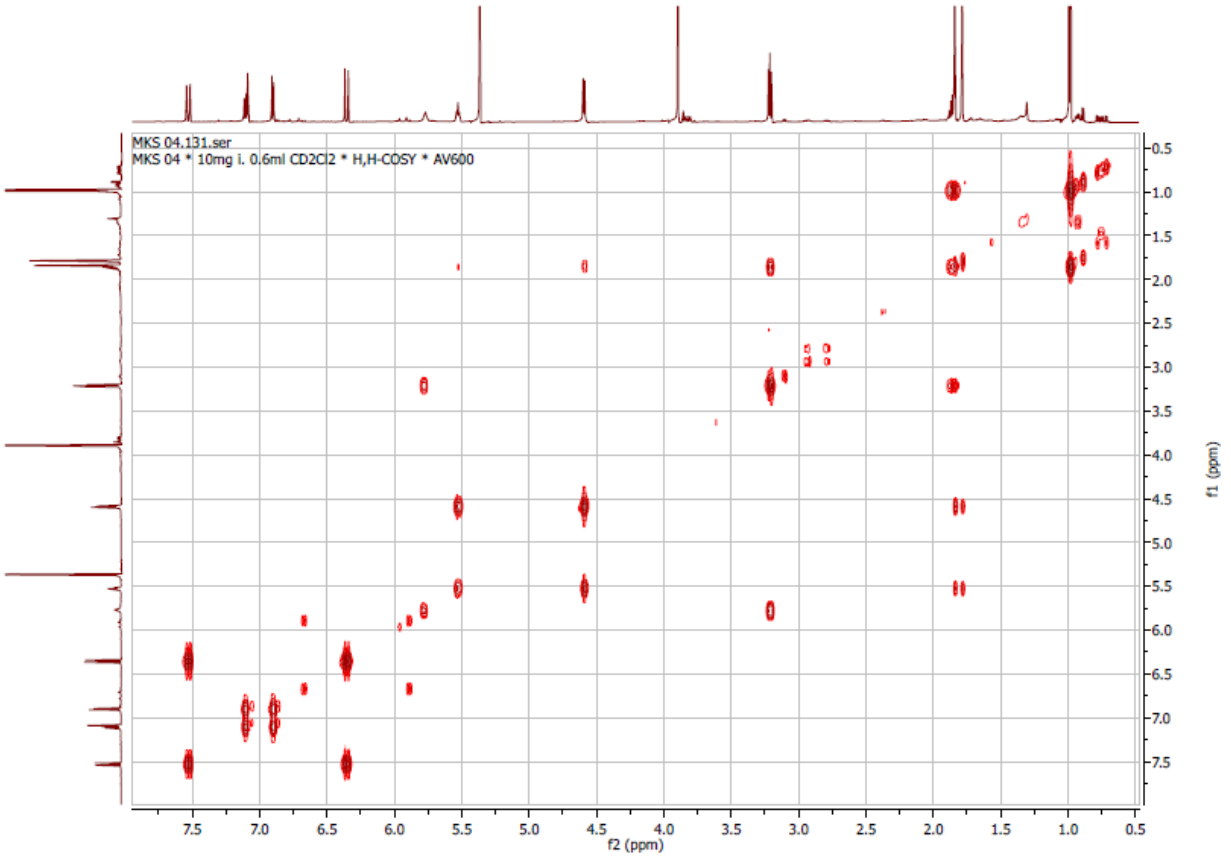
APPENDIX Vc

^{13}C NMR data of compound 5



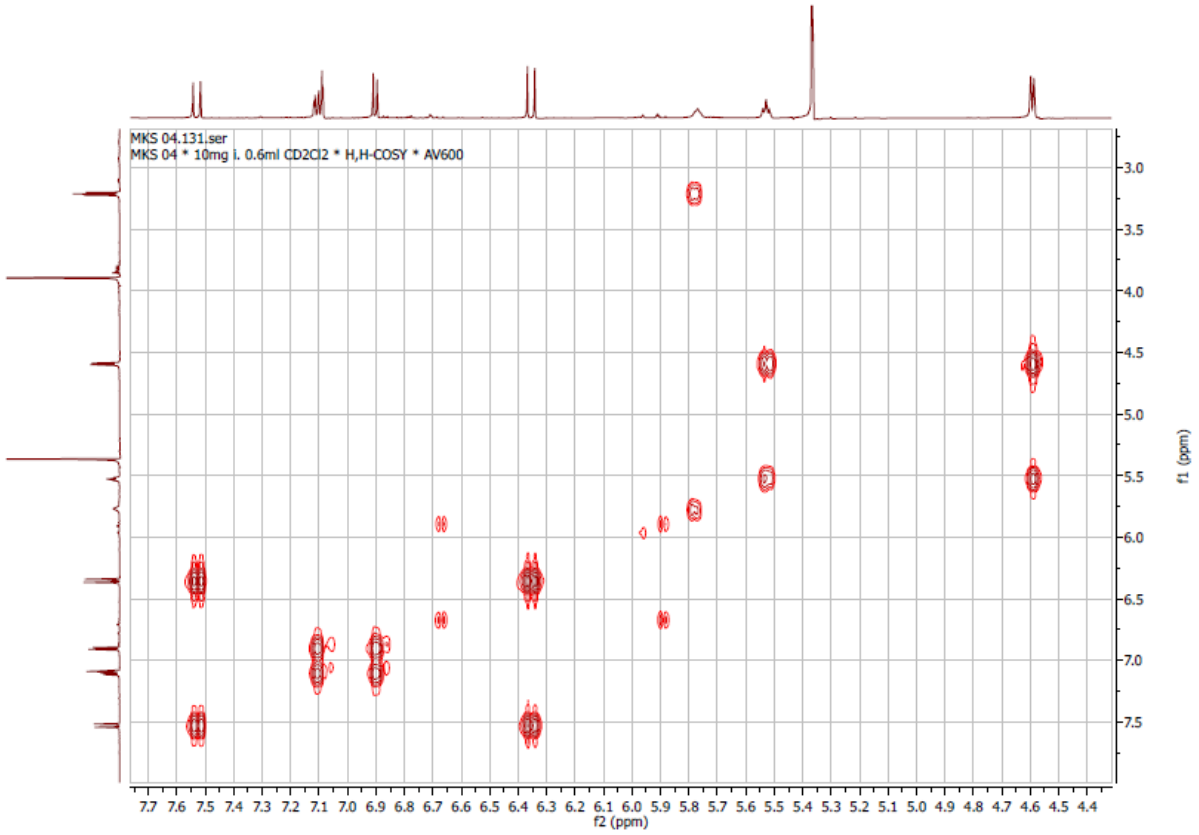
APPENDIX Vd

H, H-COSY data of compound 5



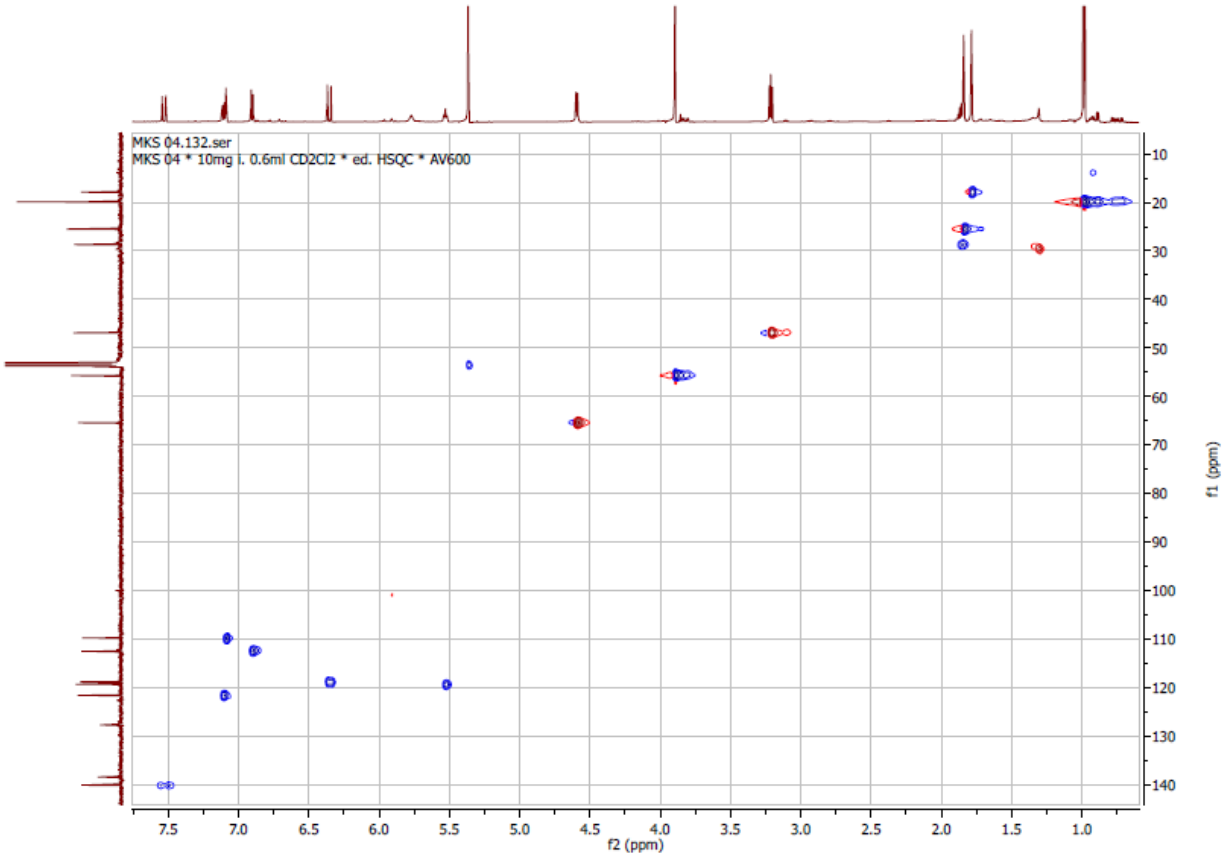
APPENDIX Ve

Expanded H, H-COSY data of compound 5



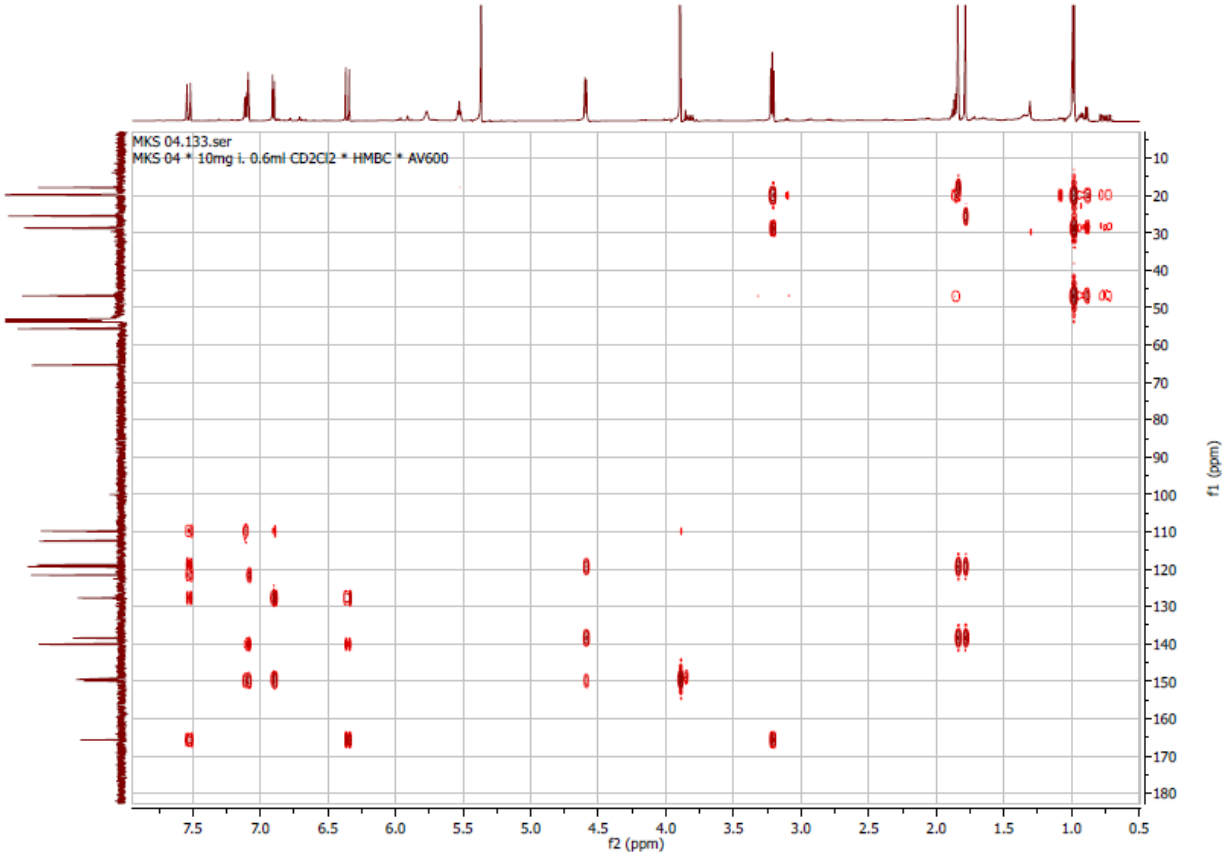
APPENDIX Vf

HSQC data of compound 5



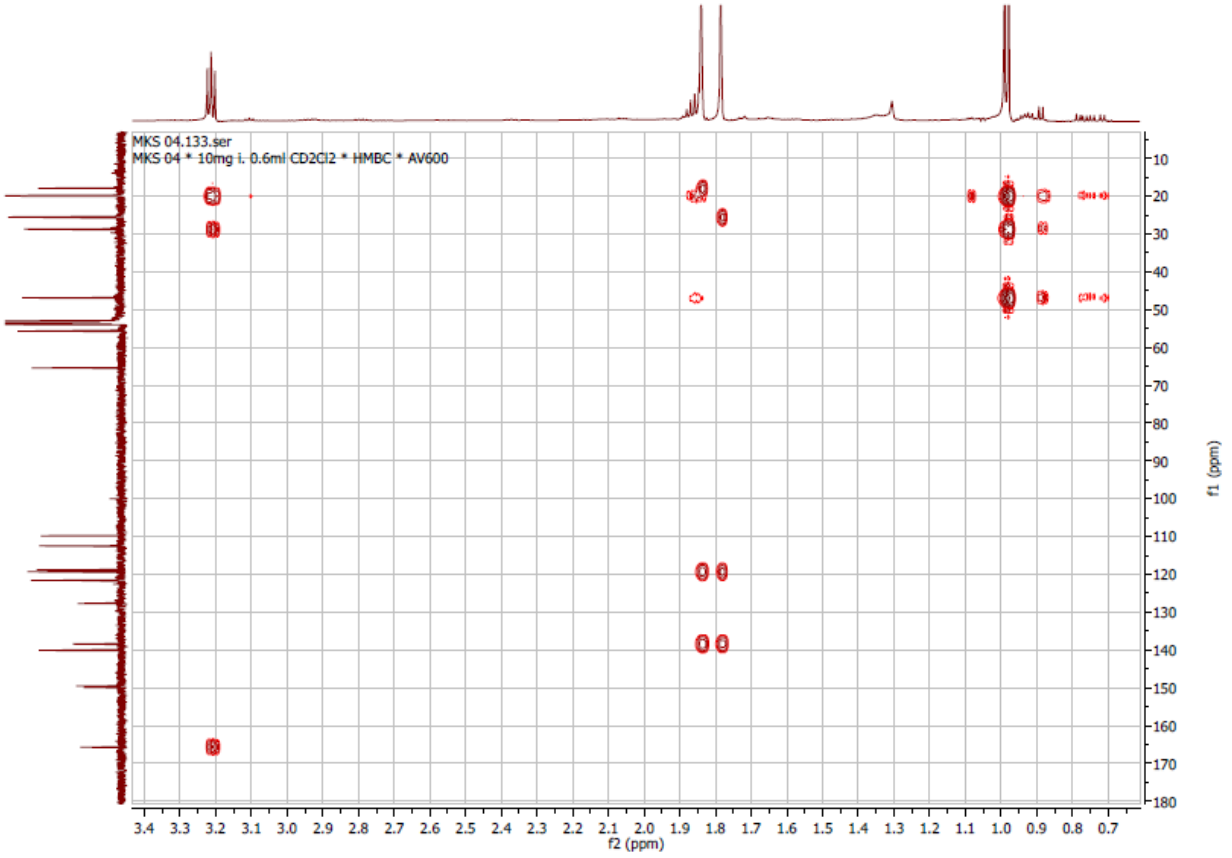
APPENDIX Vg

HMBC data of compound 5



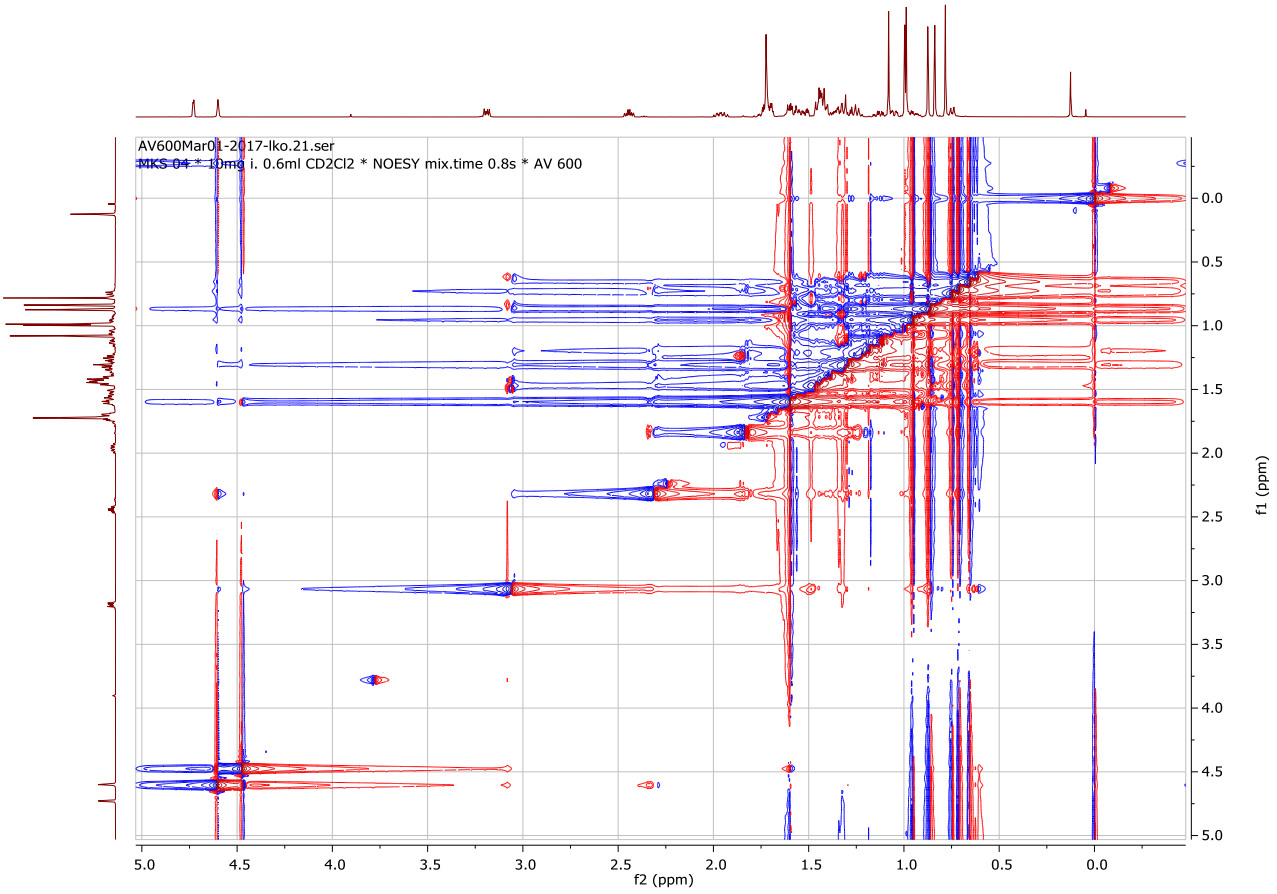
APPENDIX Vh

Expanded HMBC data of compound 5



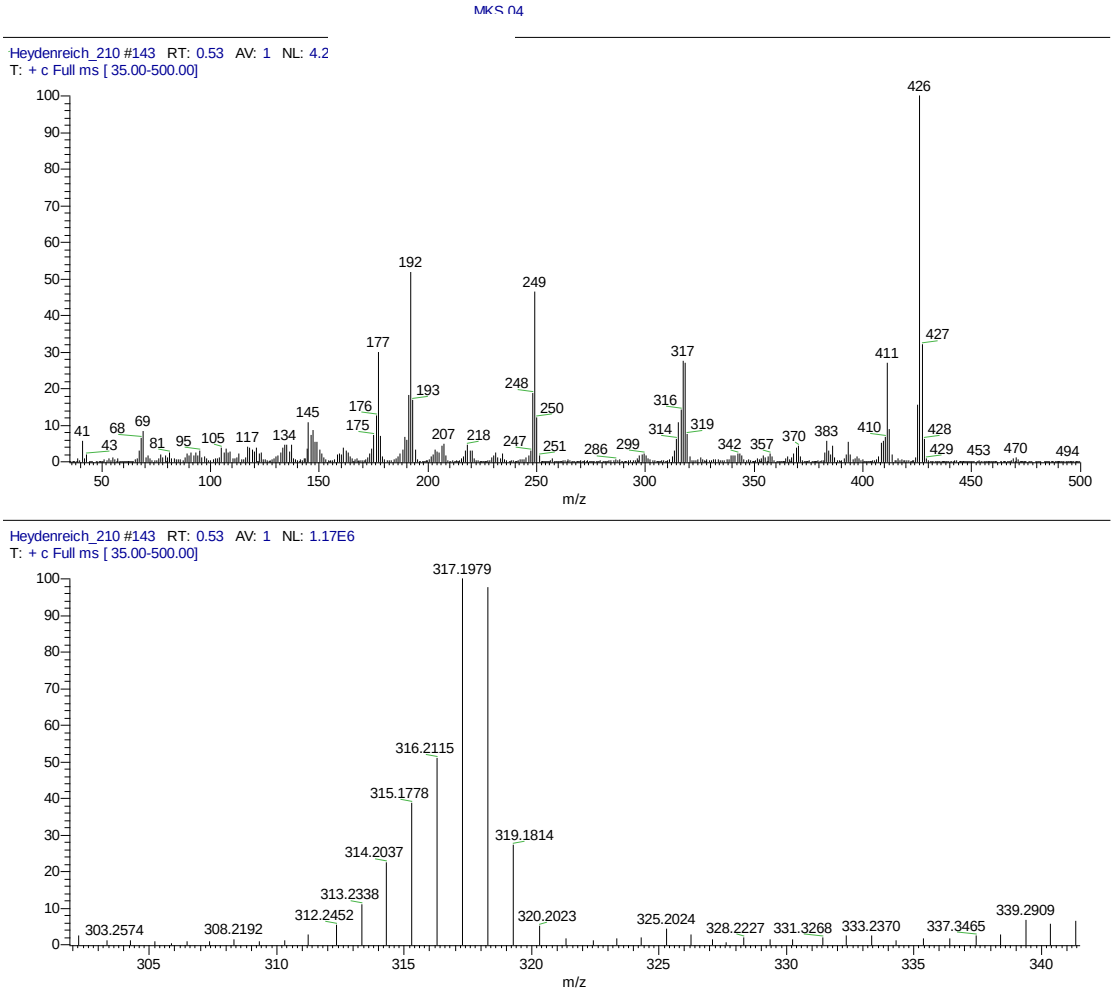
APPENDIX Vi

NOESY data of compound 5



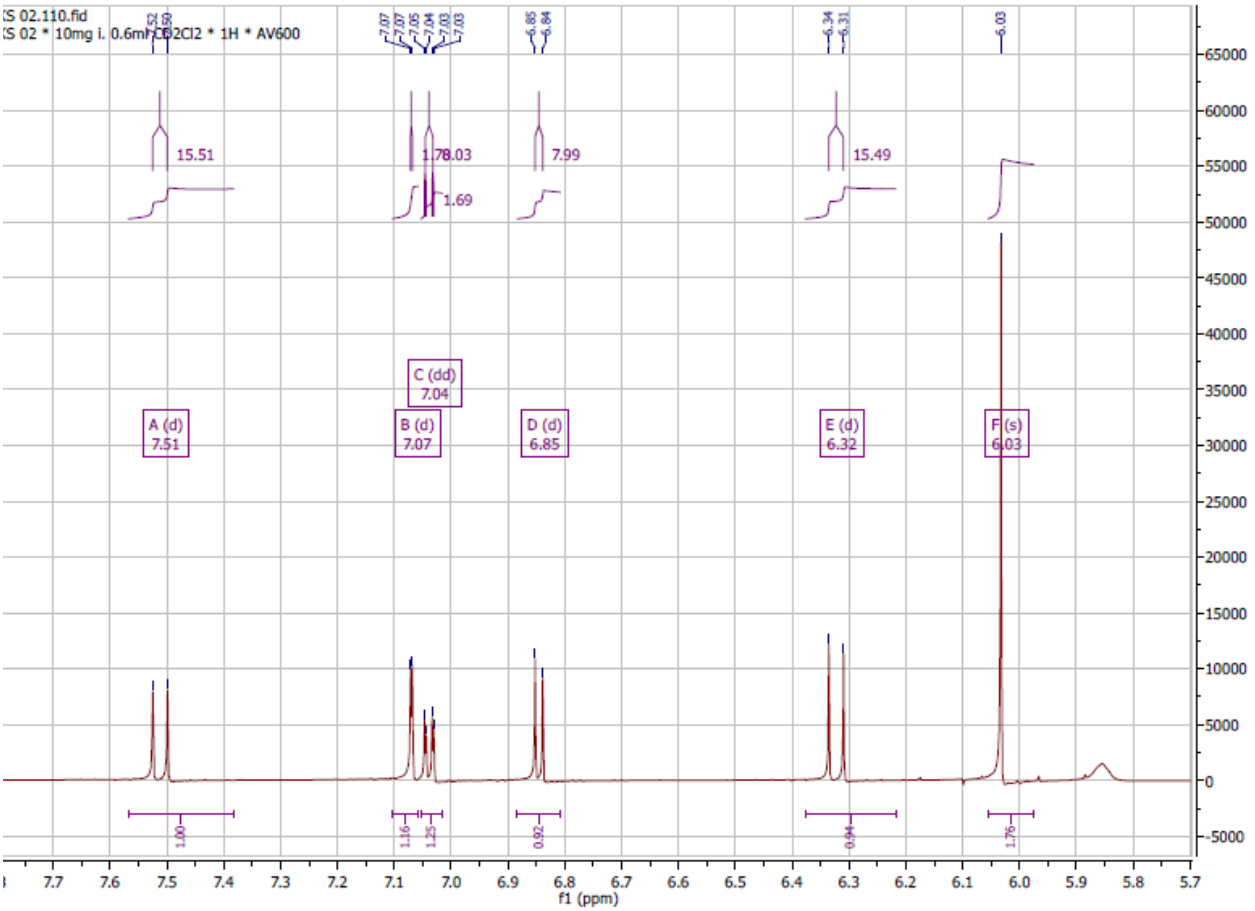
APPENDIX Vj

MS spectroscopy data for compound 5



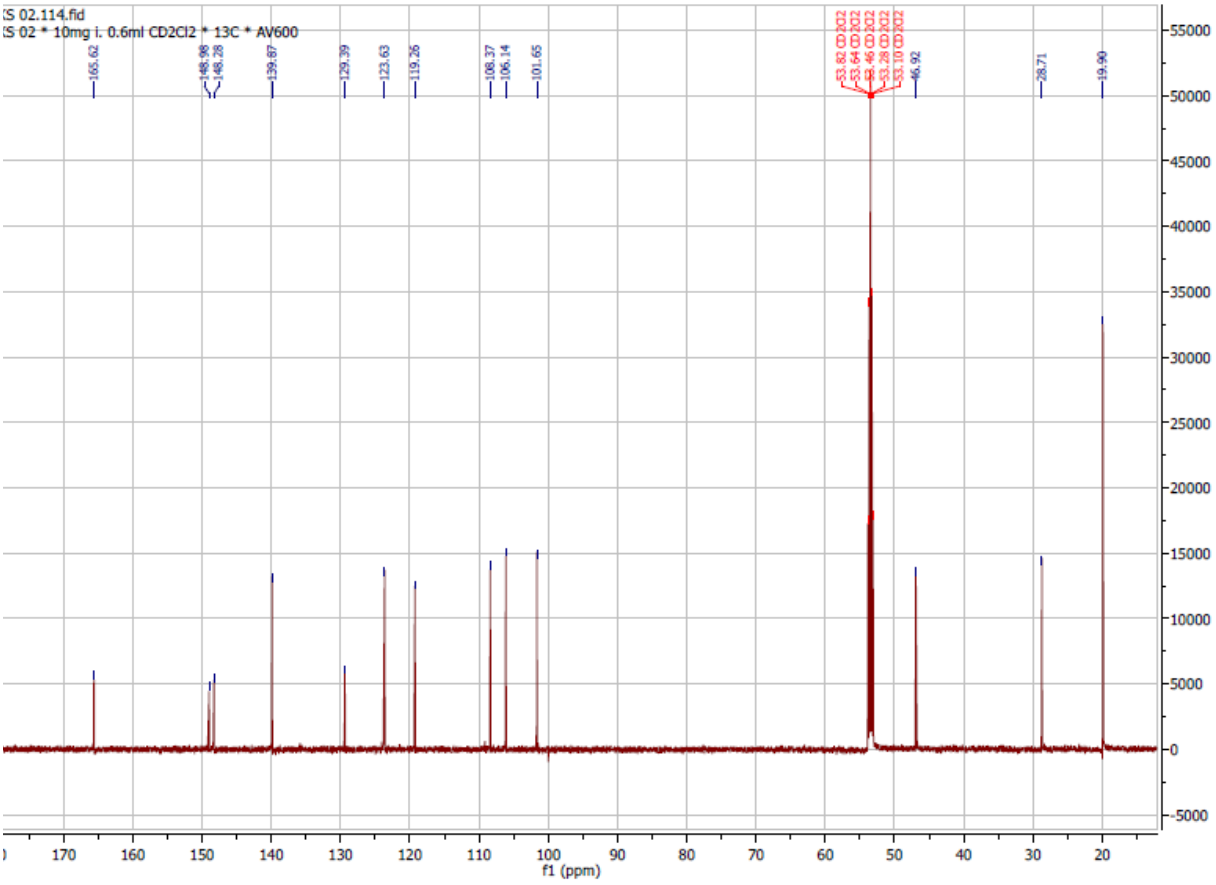
APPENDIX VIa

^{1}H NMR data of compound 6



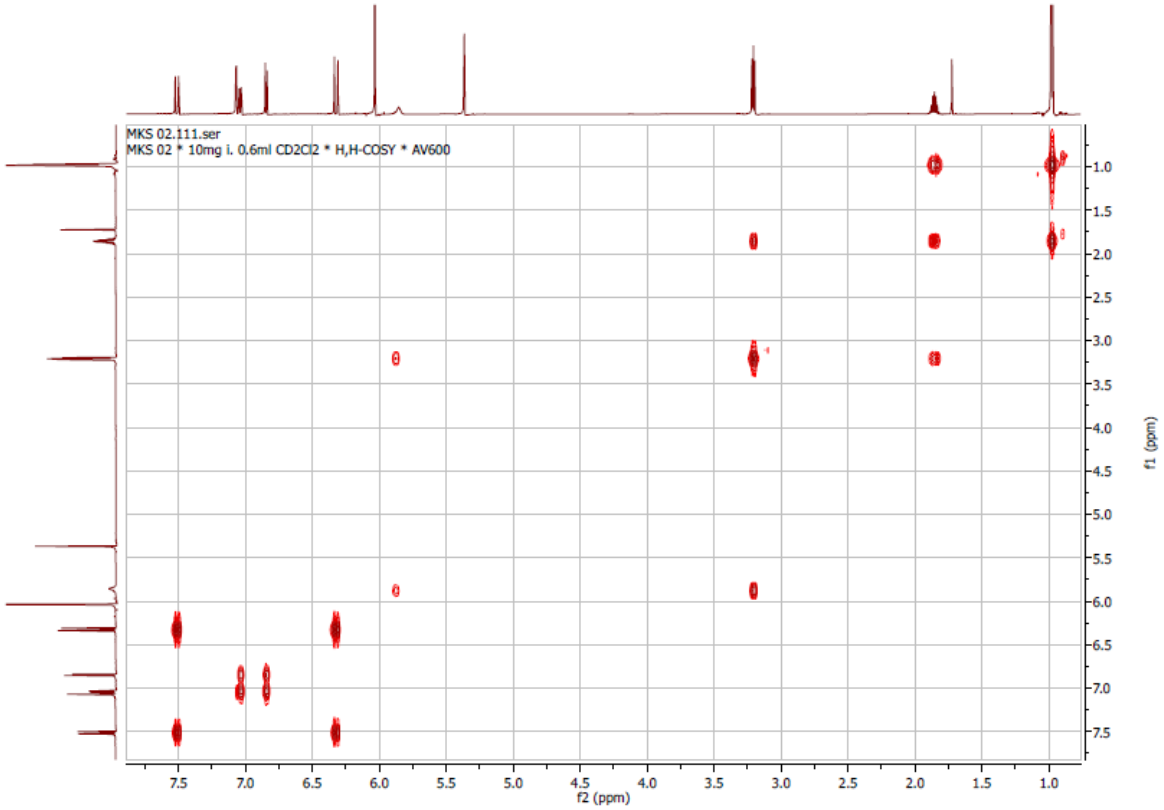
APPENDIX VIb

^{13}C NMR data of compound 6



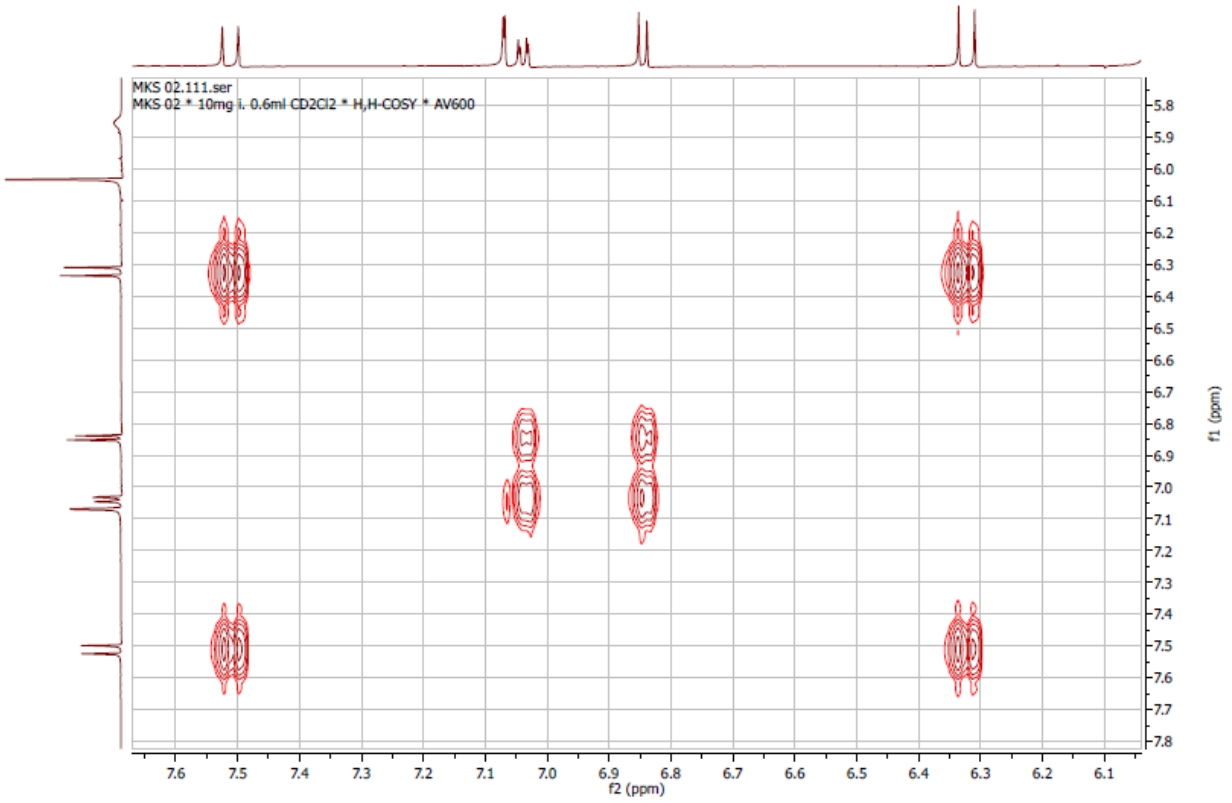
APPENDIX VIc

H, H-COSY data of compound 6



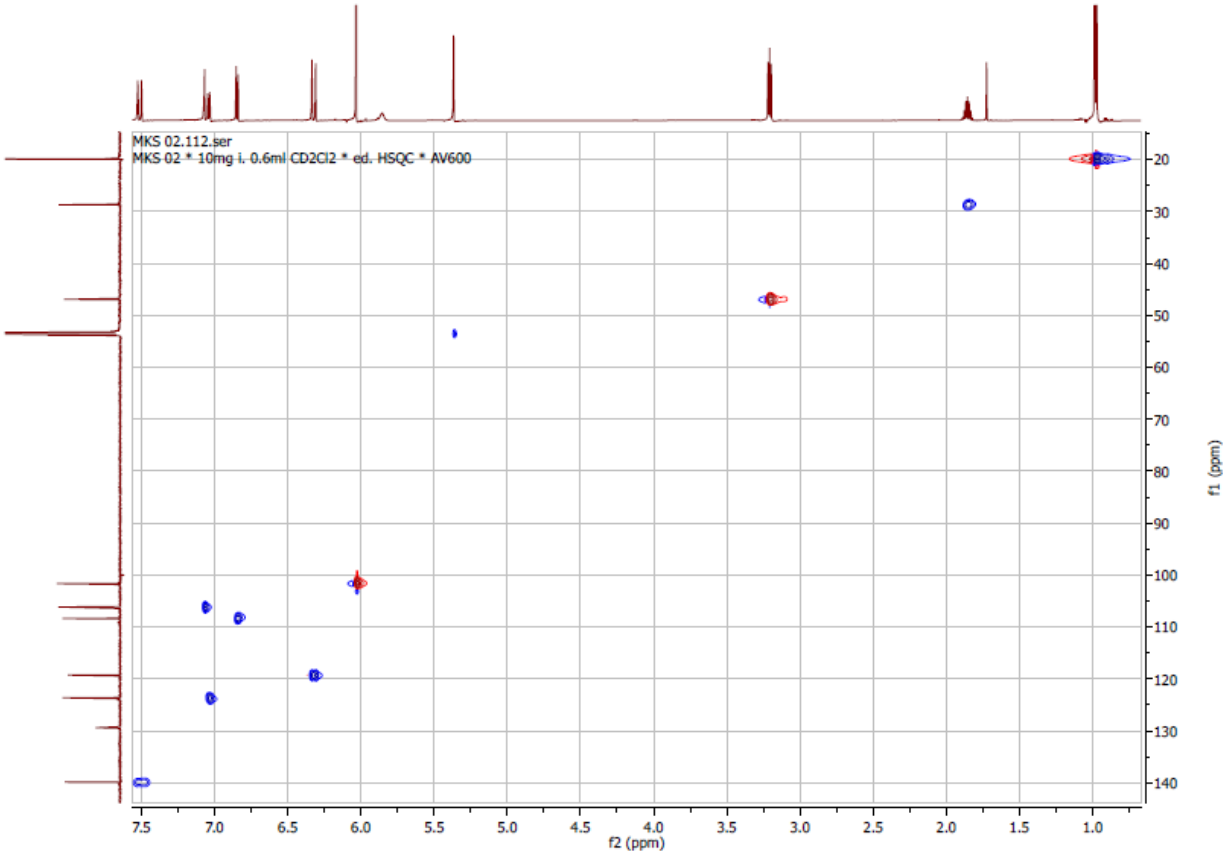
APPENDIX VId

Expanded H, H-COSY data of compound 6



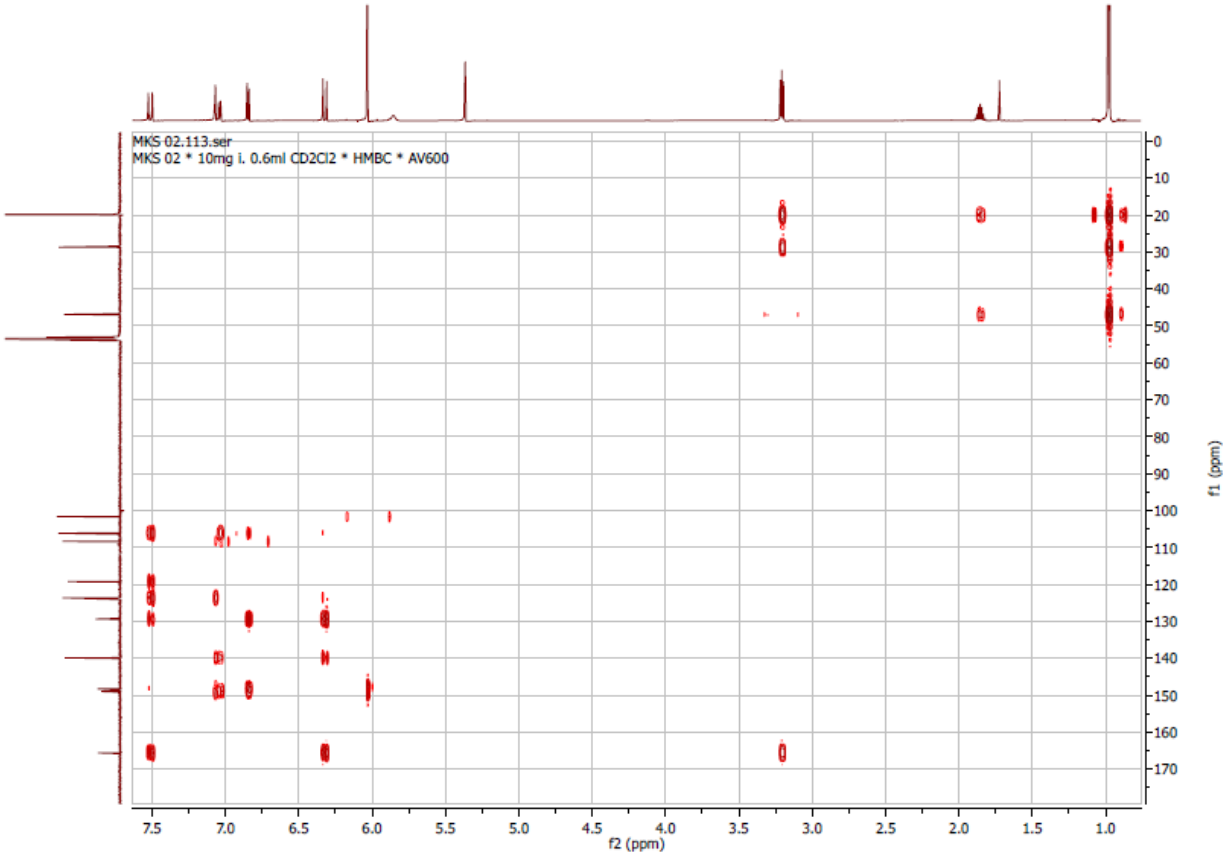
APPENDIX VIe

HSQC data of compound 6



APPENDIX VIf

HMBC data of compound 6



APPENDIX VIg

Expanded HMBC data of compound 6