UNIVERSITY OF NAIROBI
COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES
SCHOOL OF PHYSICAL SCIENCES
DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL ANALYSIS OF SELECTED PLANTS IN THE LEGUMINOSAE AND MORACEAE FAMILIES FOR ANTICANCER PRINCIPLES

BY

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A Thesis Submitted for Examination in Fulfillment of the Requirements for Award of the Degree of Doctor of Philosophy in Chemistry of the University of Nairobi

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements

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DEDICATION

This thesis is dedicated to my parents and my lovely son Minallah

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ABSTRACT

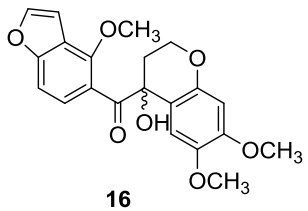
Cancer is an increasing public health problem in the world with about 17.5 million new cases and 8.7 million cancer deaths reported in 2015 alone. The development of drug resistant cancer cells represents one of the major challenges in cancer chemotherapy. The ever growing incidences of cancer, and the rapid development of drug resistance, has made it necessary to discover novel drugs to tackle this menace. A survey of current pharmaceutical drugs revealed that 60% of cancer therapeutics are derived from natural products. Due to drug resistance, the search for new anticancer agents has continued, especially among plant metabolites. Members of the family Leguminosae and Moraceae produce a broad variety of heterocycles compounds with a wide range of biological activities, including anticancer activities, indicating that these families could be sources of anticancer agents.

Chromatographic separation of the CH_2Cl_2/MeOH (1:1) extracts of six plants belonging to the Leguminosae (*Ormocarpum kirkii* S x Moore, *Derris trifoliata* Lour, *Lonchocarpus bussei* Harms, *Lonchocarpus eriocalyx* Harms) and Moraceae (*Dorstenia kameruniana* Engl and *Streblus usambarensis* (Engl) x CC x Berg) families resulted in the isolation of forty one compounds (**1-41**), of which nine are new. Thus a new rotenoid derivative, 7a-*O*-methyl-12a-hydroxyelliptonol (**16**), along with eight known compounds (a pterocarpan, five rotenoid derivatives and two isoflavones) were isolated from the roots of *Derris trifoliata*. Phytochemical investigation of the leaves of *Lonchocarpus bussei* resulted in the identification of a new isoflavone, 4'-prenyloxyvigvexin A (**24**) along with four known isoflavones. The stem bark of *Lonchocarpus eriocalyx* afforded a new pterocarpan, (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**33**) along with a known pterocarpan. The roots and twigs of *Dorstenia kameruniana* yielded three new benzylbenzofuran derivatives, 2-(*p*-hydroxybenzyl)-6-hydroxybenzofuran (**34**), 2-(*p*-hydroxy-benzyl)-6-hydroxy-7-methoxybenzofuran (**35**) and 2-(*p*-hydroxy-benzyl)-6-hydroxy-4ˈ-prenylbenzofuran (**36**) (named dorsmerunin A, B and C, respectively), along with a known coumarin known and a chalcone. Similar investigation of the roots and stems of *Streblus usambarensis* yielded three new unusual naphthobenzofuran derivatives, 2,3-(5'-hydroxy-naphtalene)-6,7-chromene-5-hydroxybenzofuran (**39**), 2,3-(5'-methoxy-naphthalene)-6,7-chromene-5-hydroxybenzofuran (**40**) and 2,3-(5'-methoxy-naphthalene)-6-methoxy-5-hydroxy-7-prenylbenzofuran (**41**) named usambarin A, B and C, respectively. The structures of the isolated compounds were elucidated by mass spectrometry and NMR (^{1}H NMR, ^{13}C NMR, COSY, NOESY, HSQC and HMBC) spectroscopy. The absolute configuration of some of the chiral compounds was determined by ECD spectroscopy.

The cytotoxicity of the isolated compounds was determined based on the resazurin assay using drug-sensitive and multidrug-resistant cancer cell lines. Among the tested compounds, 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**), osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) displayed IC_{50} values below 20 µM in both CCRF-CEM and CEM/ADR5000 cells, while 3',6,7-trimethoxyl-4',5'-methylenedioxyisoflavone (**27**) and durmillone (**28**) were active against leukemia CCRF-CEM cells; 4-hydroxylonchocarpin (**29**) and durmillone (**28**) against its resistant counterpart CEM/ADR5000 cells. Bergapten (**37**) and licoagrochalcone A (**38**) showed good activities (IC_{50} values of 7.17 µM and 5.16 µM, respectively) against CCRF-CEM leukemia cells. Usambarin B (**40)** had significant effects towards CEM/ADR5000 leukemia cells with IC_{50} value of 6.13 µM. Osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) had significant cytotoxic effects with IC_{50} values below or around 10 µM against 7 carcinoma cells and against the normal AML12 hepatocytes (4/7, 5/7 and 7/7). Durmillone (**28**) showed IC_{50} values below 10 µM against the resistant breast

adenocarcinoma MDA-MB231/*BCRP* cells and resistant gliobastoma U87MG.*ΔEGFR* cells. Licoagrochalcone A (**38**) also showed cytotoxicity against 7 sensitive or drug-resistant solid tumor cell lines (breast carcinoma, colon carcinoma, glioblastoma) with IC_{50} values below 50 µM, whilst bergapten (**37**) showed selective activity. Usambarin B (**40)** and usambarin C (**41**) had cytotoxic effects against the 7 tested carcinoma cell lines with IC_{50} values below 63 µM. Cytotoxicity of some isolated compounds was also assessed against human embryonic kidney cells (HEK293). The highest activity was observed for rotenone (**10**) an IC_{50} value of 0.82 ± 0.02 µM while the rotenoloids 7a-*O*-methyldeguelol (**12**) and 7-a-*O*-methylelliptonol (**18**) showed cytotoxicity with an IC_{50} values of 9.4 ± 0.25 µM and 7.1 ± 0.50 µM, respectively. The isoflavones, 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**) and osajin (**3**) showed comparable activity (IC_{50} 27.1 and 27.3 ± 2.0 µM, respectively) against this cell line.

Further studies were conducted to determine the modes of action of osajin (**3**) and 7,7"-di-*O*-methylchamaejasmin (**4**). The result showed osajin (**3**) and 7,7"-di-*O*-methylchamaejasmin (**4**) caused cell cycle arrest in G0/G1 phase as well as apoptosis with significant increase of cells in sub-G0/G1 phase. The activity of caspases in CCRF-CEM cells showed that the two compounds did not increase the activity of caspases 3/7, 8 and 9. These compounds (**3, 4)** induced apoptosis in CCRF-CEM cells mediated by MMP alteration and increased ROS production. Overall, the study has demonstrated that the plants investigated here elaborate diverse range of phenolics with some having unique structural features. These plants are also good sources of cytotoxic compounds with potential use in cancer therapy.



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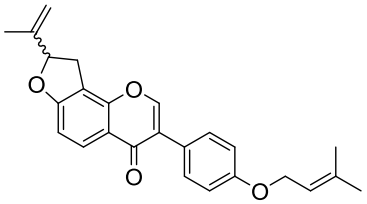

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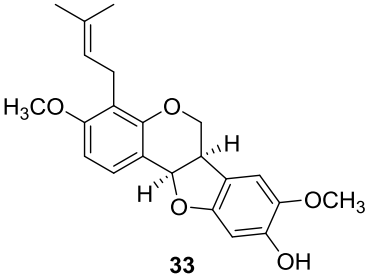

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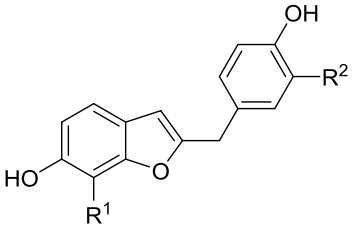
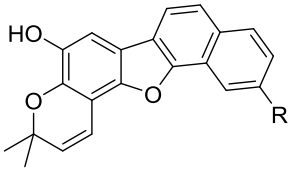


34 $R^1 = R^2 = H$
35 $R^1 = OCH_3$, $R^2 = H$
36 $R^1 = H$, R^2 = prenyl



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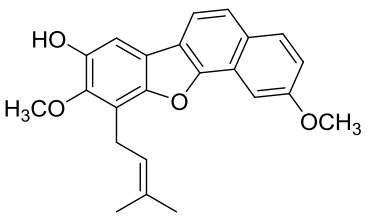

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LIST OF PUBLICATIONS

1. **Adem, F. A**., Kuete, V., Mbaveng, A. T., Heydenreich, M., Koch, A., Ndakala, A., *et al.* (2018). Cytotoxic flavonoids from two *Lonchocarpus* species. *Nat Prod Res*, 1-9.

2. **Adem, F. A**., Kuete, V., Mbaveng, A. T., Heydenreich, M., Ndakala, A., Irungu, B., *et al.* (2018). Cytotoxic benzylbenzofuran derivatives from *Dorstenia kameruniana*. *Fitoterapia, 128*, 26-30.

3. **Adem, F. A**., Mbaveng, A. T., Kuete, V., Heydenreich, M., Ndakala, A., Irungu, B., *et al.* (2019). Cytotoxicity of isoflavones and biflavonoids from *Ormocarpum kirkii* towards multi-factorial drug resistant cancer. *Phytomedicine, 58*, 152853.

4. **Adem**, F.A., Victor Kuete, Armelle T. Mbaveng, Matthias Heydenreich, Albert Ndakala, Beatrice Irungu, Thomas Efferth and Abiy Yenesew. Cytotoxic naphthobenzofuran derivatives from *Streblus usambarensis.* (manuscript in preparation).

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LIST OF ABBREVIATIONS AND SYMBOLS

δ	Chemical shift
CD	Circular Dichroism
COSY	Two-dimentional magnetic resonance
d	Doublet
dd	Doublet of doublet
CH_2Cl_2	Dichloromethane
DMSO	Dimethylsulfoxide
DHA	Dexamethasone
ECD	Electronic Circular Dichroism
EtOAc	Ethyl acetate
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
HPLC	High Performance Liquid Chromatography
HREIMS	High Resolution Electrospray Ionization Mass Spectrometry
H_2O_2	Hydrogen Peroxide
IR	Infrared
IC_{50}	50% inhibitory concentration
LCMS	Liquid Chromatography Mass Spectrometry
m/z	Mass to charge ratio
MeOH	Methanol
MMP	Mitochondrial Membrane Potential
MDR	Multidrug Resistance
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser and Exchange Spectroscopy
1D NMR	One Dimensional Nuclear Magnetic Resonance
PTLC	Preparative Thin Layer Chromatography
ROS	Reactive Oxygen Species
s	Singlet
t	Triplet
2D NMR	Two Dimensional Nuclear Magnetic Resonance
UV	Ultra Violet
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

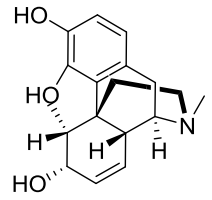
1.1 Background

Many plants have a long history of usage as therapeutic agents. In 1500 BC, the ancient Egyptian documented over 700 medicinal herbs in 'Ebers Papirus', including the use of Willow as tree an antipyretic agent (Viktorin, 1999). The antimalarial application of *Artemisia annua* in Chinese traditional medicine was documented in 'The handbook of prescription for emergencies' (Govindarajan *et al.*, 2005). The Indian Ayurvedic describes medicinal uses of approximately 1,000 herbs and shrubs (Aggarwal *et al.*, 2007). The Persian and Arab civilizations are credited for starting a healthcare system, rich in the use of traditional medicinal plants (Castleman, 2001). In many developing countries, herbal medicines remain important in the treatment of numerous ailments. According to World Health Organization, about 80% of the world population in developing countries still relis on traditional medicine for their primary health care (Ekor, 2013). The wide use of traditional medicine in primary healthcare and the rich biodiversity has made significant contribution to the development of modern medicine. Many of the current pharmaceutical drugs have their roots in herbal remedies. For example, morphine (**42**) which is used as a pain reliever was isolated from Opium. The common antipyretic and analgesic agent, aspirin (**43**), was derived from the natural compound, salicylic acid which was isolated from the bark of Willow tree *Salix alba* L (Viktorin, 1999); the antimalarial drug quinine (**44**) was obtained from the *Cinchona* species. Following the resistance of *Plasmodium falciparum* to the commonly used antimalarial drugs, the relatively new antimalarial drug artemisinin (**45**), was isolated from the Chinese traditional medicinal plant have, *Artemisia annua* (Lombardino and Lowe, 2004). Plants also played a significant role in the discovery of anticancer drugs.

Cancer is a group of associated diseases, which involves abnormal cell growth that are unable to perform normal cell function and can spread to the surrounding tissues and other body parts (Bunz, 2008). It is mainly caused by bacterial or viral infections, family history, and exposure to chemicals; environmental factors, and risky life-style also contribute as to increase of cancer incidebts in recent year (Anand *et al.*, 2008; Irigaray *et al.*, 2007; Perera, 1997). Cancer is the leading cause of deaths worldwide, after cardiovascular diseases, with about 17.5 million new cases and 8.7 million cancer deaths reported in 2015 alone (Fitzmaurice *et al.*, 2017).

Plant-derived cancer chemotherapeutic agents have remained an integral part of cancer therapy. The anticancer agent taxol (**46**) was derived from the bark of the pacific Yew tree, *Taxus brevifolia* (Wani *et al*., 1971); the vinca alkaloids, vinblastine (**47**) and vincristine (**48**), are naturally occurring antineoplastic agents, obtained from Madagascar periwinkle, *Catharantus rosesus*. The semi-synthetic derivatives vinorelbine (**49**) and vindezine (**50**) derived from these alkaloids are currently in use for the treatment of a variety of cancer types (Cragg and Newman, 2005; Sisodiya, 2013).

Despite the fact that there are advances in the treatment of cancer through the discovery of new cancer treatment agents, cancer still presents a major global burden mainly due to Multi–Drug-Resistance (MDR) incidences of cancer. MDR cells reveal cross- resistance to diverse drugs which are structurally and functionally unrelated, which leads to failure of cancer chemotherapy. Drug resistance is believed to cause about 90% MDR treatment failure in metastasis cancer patients (Longley and Johnston, 2005).



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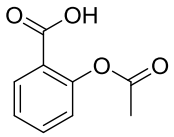

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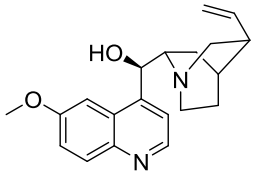

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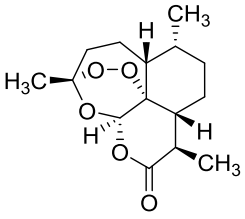

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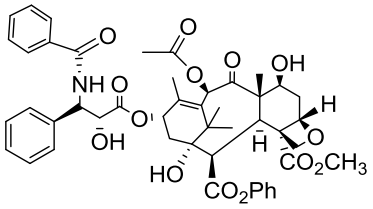

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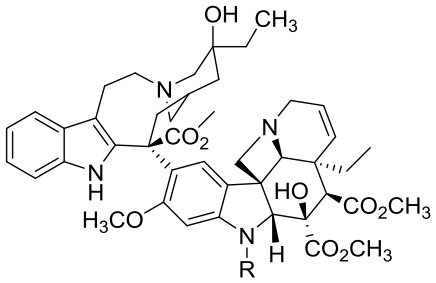
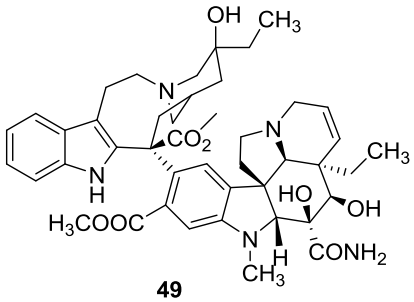


47 R = CH_3

48 R = CHO



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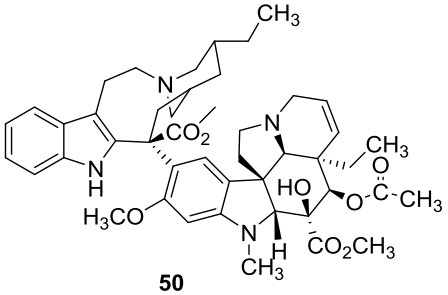

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1.2. Statement of the Problem

Cancer is an increasing public health problem in the world, with 17.5 million new cases and 8.7 million cancer deaths reported in 2015 worldwide (Fitzmaurice *et al.*, 2017).. To make the matter worse, it is expected that there will be 26 million new cancer cases and 17 million cancer deaths per year over the next two decades (Thun *et al.*, 2010). It has remained the main cause of death in economically developed countries and the second leading cause in developing countries (Jemal *et al.*, 2011). In Kenya, cancer is the third leading cause of mortality with 7% of total deaths each year attributed to cancer. It is estimated that 40,000 new cases and over 28,000 deaths occur annually in this country (Topazian *et al.*, 2016). Surgery, radiation and chemotherapy are the main choices of treatment of cancer. However,

in most cases such treatment options are effective only when the tumour is small in size and localized. In addition to this, most of the drugs currently available induce side effects by affecting the normal cells too. Resistance to chemotherapeutic drugs is the other main obstacle to effective cancer treatment with upto 80% of cancer patients developing resistance to drugs (Alfarouk *et al.*, 2015; Geretto *et al.*, 2017; Velingkar & Dandekar, 2010). Hence, the search for new anticancer drugs that successfully kill the cancer cells without significantly affecting normal cells has become of paramount importance in recent years.

1.3. Objectives

1.3.1. General Objective

The general objective of this study was to identify anticancer principles from selected plants of the Leguminosae and Moraceae families.

1.3.3. Specific Objectives

The specific objectives of the study were to:

i. Isolate and characterize compounds from *Ormocarpum kirkii*, *Derris trifoliata*, *Lonchocarpus eriocalyx*, *Lonchocarpus bussei*, *Dorstenia kameruniana* and *Streblus usambarensis;*
ii. Establish the anticancer activities of the isolated compounds against drug sensitive and multidrug resistant cancer cell lines;
iii. Determine the mode of action of some of the active compounds.

1.4. Justification

The use of medicinal plants is still widely practiced and has played an important role in the field of drug discovery. A survey of the currently used pharmaceutical drugs revealed that 60% of cancer chemotherapeutics are either natural products or their synthetic derivatives (Newman & Cragg, 2012). A similar study showed that, out of 175 natural anticancer agents

reported since 1940's to the end of 2014, 49% were approved as anticancer drugs (Newman and Cragg, 2016). Plants remain potential sources of first-line anticancer drugs, as exemplified by the development of vinblastine, vincristine, camptothecin, podophyllotoxin, paclitaxel and some of their derivatives that are obtained from medicinal plants (Cragg and Newman, 2005; Sisodiya, 2013). Thus, isolating and identifying the bioactive constituents from medicinal plants will have great contribution in addressing this global problem. Members of the families Leguminosae and Moraceae produce a broad variety of heterocyclic compounds with a wide range of biological activity including cytotoxic compounds. It is thus postulated that phytochemical studies on these families would lead to the discovery of anticancer lead compounds.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background Information on Cancer

Cancer is the uncontrolled growth of cells which disables the normal cell function. This rapid division leads to a solid mass of cells known as a tumour. The initial tumour, known as the primary tumour, is often not the cause of death, but secondary growth when cancerous cells break away from the primary tumour to the blood stream and lymphatic system and seeds new tumour elsewhere in the body, a phenomenon referred to as metastasis (Bunz, 2008). Most cancer cases result from exposure to an ever increasing number of chemicals in the environment, risky life-style, unhealthy diet, viral or bacterial infections and inheritance are also contributing factors to cancer (Anand, *et al.*, 2008; Irigaray, *et al.*, 2007; Perera, 1997).

There are several types of treatment modalities available for cancer. However, most cancer treatment practices combine two or more therapeutic methods. Surgery is the most common, where primary treatment of cancer is done by removing the tumour. It may be used alone or in combination with other treatment methods, such as radiation therapy which requires high energy irradiation to kill cancer cells with low level of harm to normal cells. This treatment is applied before, during or after treatment. Another method of cancer treatment is hormone therapy which involves the use of medicine to remove hormones, or block their actions, and as the result of the growth of cancer is arrested. Immunotherapy is a cancer treatment in which the body repairs the immune system to stop or slow the growth of cancer cells and to prevent the cancer cells from spreading. Chemotherapy is a drug based treatment and is usually recommended to all cancer patients, and chemotherapy can be administered intravenously or orally (Miller *et al.*, 1981; Palmer, 1982).

2.2 Cancer Chemotherapeutics Agents

Anticancer drugs are classified into several groups according to their mechanism of action. These include alkylating agents, antimetabolites, antimicrotubules, antibiotics and topoisomerase inhibitors (Sarah, 2008).

2.2.1 Alkylating Agents

Alkylating agents include classes of compounds comprising of nitrogen mustards, alkylsulfonates, piperazines, nitrosoureas, tetrazines and platinum compounds. They inhibit cancer cells growth by cross-linking DNA strands and are the most common antineoplastic chemotherapy drugs used for the treatment of Hodgkin's lymphoma and brain cancer (Celkan, 2013; McClean *et al.*, 1999).

2.2.2 Antimetabolites

Antimetabolites contain folic acid antagonistic, purine and pyrimidine analogues. They inhibit cell division by inhibiting nucleotide synthesis or interfering with DNA and preventing the synthesis of a new extension of the DNA strand. Methotrexate, fluorouracil and pentostatiol are some of the common antimetabolite drugs (Hillcoat *et al.*, 1978; Kaye, 1998).

2.2.3 Antimicrotubular Agents

Naturally occurring compounds such as vinca alkaloids and taxanes are the two main groups of anti-microtubule agents. The mechanism of action of the vinca alkaloids, vinblastine and vincristine, in the treatment of cancer is by binding to the protein tubulin and inhibiting polymerization and forming microtubules resulting in the blockage of cell proliferation whereas taxanes, paclitaxel and docetaxel act by inhibiting microtubule disassembly (Dumontet and Jordan, 2010). These classes of drugs are used mainly for the treatment of ovarian and breast cancer (Zeng *et al.*, 2000).

2.2.4 Antibiotics

Antineoplastic antibiotics are derived from streptomyces bacteria (Watve *et al.,* 2001), and act by binding to DNA and blocking the synthesis of RNA. These groups of agents include doxorubicin, plicamycin, bleomycin and mitomycin C (Davey & Tudhope, 1983).

2.2.5 Topoisomerase Inhibitors

Topoisomerases bind to DNA creating a DNA topoisomerase complex; they are involved in DNA-metabolism reaction, such as replication, transcription, recombination and chromosome segregation (Beretta *et al.,* 2013). Inhibition of topoisomerase function results in the cleavage of DNA topoisomerase complex, leading to cell death (Wozniak & Ross, 1983). Topoisomerase I inhibitors include camptothecin and its derivatives, topotecan and irinotecan which are used for the treatment of ovarian, lung and prostate cancer. Topoisomarase II inhibitors are used in the treatment of leukemia, lymphoma and breast cancer. They include several classes of compounds, such as anthracyclines, amsacrines and anthracenediones (Gordon *et al.,* 2001).

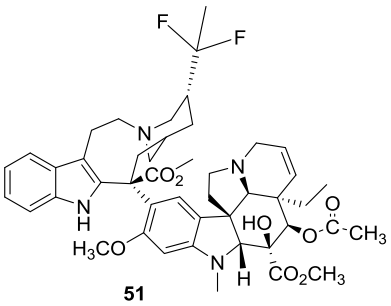
2.3 Plant based Anticancer Drugs

Several drugs of plant origin are used as anticancer agents. Some of these drugs and their use are described in Table 1.

Table 1: Anticancer drugs of plant origin used in modern medicine

Anticancer drugs	Source	Uses
Vinblastine (**47**)	*Cathrantahus roseus*	Breast cancer, lymphoma, germ cell tumour
Vincristine (**48**)	*Cathrantahus roseus*	Leukemia, Lymphoma, neuroblastoma, breast, lung cancer
Vinorelbine (**49**)	Derivative of vinblastine	Breast, osteosarcoma, lung cancer
Vinflunine (**51**)	Derivative of vincristine	Non-small cell lung cancer, breast carcinoma
Paclitaxel (**46**)	*Taxus brevifolia*	Ovary, breast, kaposi's sarcoma and non-small cell lung cancer
Docetaxel (**53**)	Derivative of paclitaxel	Breast, prostate and lung cancer
Cabazitaxel (**54**)	Semi-synthetic derivative of paclitaxel	Metastasis hormone-refractory prostate cancer
Topotecan (**52**)	Semi-synthetic derivative of campotothecin	Metastasis ovarian cancer, small cell lung cancer and pediatric cancer

(Haque *et al.*, 2016; Moudi *et al.*, 2013; Ojima *et al.*, 2016)



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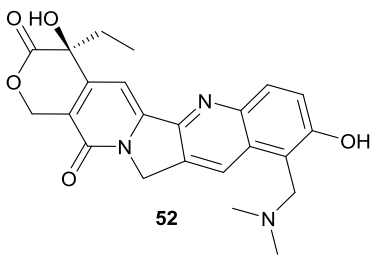

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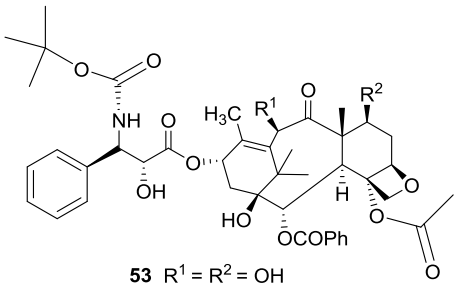


53 $R^1 = R^2 = OH$
54 $R^1 = R^2 = OCH_3$

2.4 Multi-Drug Resistance (MDR) in Cancer Chemotherapy

Resistance to chemotherapeutic drugs is one of the main challenges in effective cancer treatment. Multi-drug resistance occurs when cancer cells become resistant to different classes of drugs which are structurally and functionally unrelated and have different molecular targets (Gottesman, 1993). Cancer drug resistance may be intrinsic, where tumour cells initially susceptible to certain chemotherapies or acquired, resistance during the process of treatment through DNA mutation and metabolic change (Holohan *et al.*, 2013). Different genes and mechanisms contribute to multidrug resistance in cancer; these include change in the activity of specific enzyme systems involved in cell regulation such as glutathione-S-transferase and topoisomerase I/II (Hao *et al.*, 1994; Seitz *et al.*, 2010). Alteration of protein levels that control apoptosis can also reduce the effectiveness of anticancer drugs. The ATP-binding cassette (ABC) transporters family mediate multi-drug resistance through the hydrolysis of ATP-dependent drug efflux pumps (Holohan, *et al.*, 2013; Housman *et al.*, 2014). To address the problem of drug resistant, the development of new drugs that are cytotoxic towards multidrug resistance cancer cells is of high priority.

2.5 Botanical Information of the Leguminosae and Moraceae Families

2.5.1 The family Leguminosae

The family Leguminosae (commonly known as the legume, pea or bean family), with about 700 genera and over 18,000 species, is the third largest family of the flowering plants after the Asteraceae and Orchidaceae (Lewis, 2005). It belongs to the class of dicotyledons and is divided into three subfamilies known as Papilionoideae, Mimosoideae and Caesalpinioideae (Kaess & Wink, 1996). The subfamily Papilionoideae is the largest group in the legume and is classified into 33 tribes (Polhill, 1981). The family representatives range from giant plants

to ephemerals and distributed from the equator to the edge of the cold and hot deserts (Polhill, 1981).

2.5.1.1 The Genus Ormocarpum

The genus *Ormocarpum* belonging to the subfamily Papilionoideae tribe Dalbergieae comprises approximately 25 species, with 17 of which restricted to Africa (Lock, 1989). In the flora of East Africa, there are 8 species recorded, of which 6 species are found in Kenya (Gillett, 1971). *Ormocarpum kirkii* S x Moore is a shrub or a small tree (Figure 1) which is distributed in Somalia, Kenya, Tanzania, Malawi, Mozambique, Zaire, Zimbabwe and South Africa (Gillett, 1971). In Kenya, it is found in Machakos, Kajiado and Lamu counties (Gillett, 1971).

Figure 1: *Ormocarpum kirkii* S x Moore picture taken by Mr. Patrick Chalo Mutiso in Sep 2014

2.5.1.2 The Genus Derris

The genus *Derris* belongs to the tribe Millettieae, subfamily Papilionoideae, family Leguminosae. It consists of 50 species, with one costal species, *Derris trifoliata* Lour,

distributed in the tropical region of Asia and East Africa (Adema, 2003). In the flora of Kenya, the genus is represented by *Derris trifoliata* Lour, found in Kilifi county, coastal region of Kenya (Gillett, 1971).

2.5.1.3 The Genus Lonchocarpus

The genus *Lonchocarpus* belongs to the family Leguminosae, subfamily Papilionoideae and comprises of over 100 species found in tropical America, Africa and the Caribbean Islands (Magalhaes *et al.*, 1996). Some taxonomists consider the genus *Lonchocarpus* to be one of the complex genera within the tribe Millettieae, which also includes the genera *Derris* and *Millettia* (Lavin *et al.*, 1998; Polhill, 1981). Six *Lonchocarpus* species are recorded in the flora of tropical East Africa. In Kenya, the genus is represented by three species; *Lonchocarpus bussei* Harms, *L. eriocalyx* Harms and *L. kanurii* Brenanand Gillet (Beentje, 1994).

(I) (II)

Figure 2: Picture of *Lonchocarpus bussei* Harms (I) and *Lonchocarpus eriocalyx* Harms [taken by Mr. Patrick Chalo Mutiso in Kaya Muhaka forest, January 2018.]

2.5.2 The family Moraceae

The Moraceae is a family of flowering plants comprising 6 tribes with over 40 genera and about 1,000 species, is distributed in the tropics, subtropics and in temperate region (Corner, 1962). Most plants in this family are shrubs, trees, herbs, featuring woody stem, alternative or opposite leaves, unisexual flowers and fruits are multiple. Some of the species have a great agricultural and economic importance. The bark of *Broussonetia* (paper mulberry) is used for the manufacture of cloth and paper. Several species in the genera *Morus*, *Ficus*, and *Arthocarpus* (breadfruit and jackfruit) are cultivated for their edible fruits. The genus *Ficus* is also known for their latex and timber production (Mahbubur Rahman, 2013).

2.5.2.1 The Genus Dorstenia

The genus *Dorstenia*, consists of about 170 species, is the biggest genus of the family Moraceae (Mabberley, 1987), and is distributed in tropical Africa, the Middle-East, central and southern America (Abegaz & Ngadjui, 1999). There are 28 species recorded in the flora of East Africa, 13 of which are found in Kenya (Polhill, 1989). Among the Kenyan species, *Dorstenia Kameruniana* Engl grows in Gongoni, Mwele Mdogo and Cha Simba forest (Polhill, 1989).

2.5.2.2 The Genus Streblus

The genus *Streblus* comprises about 25 species and is mostly distributed in tropical and subtropical Asia (Roy, 2013). In Kenya, the genus is represented by *Streblus usambarensis* (Eng,) x CC x Berg (Beentje, 1994), which is a shrub and found in Mombasa and Kilifi counties (Beentje, 1994).

Figure 3: Picture of *Dorstenia kameruniana* Engl (I) and *Streblus usambarensis* (Eng,) x CC x Berg (II) [taken by Mr. Patrick Chalo Mutiso in Ukunda, Gongoni forest in January 2018.]

2.6 Ethnomedicinal Uses of Plants from the Leguminosae and Moraceae Families

Ethnomedicinal applications of some plants of the Leguminosae and Moraceae families are given in Table 2

Table 2: Ethnomedicinal uses of some plants of the Leguminosae and Moraceae families

Genus	Species	Ethinomedicinal uses	Reference
rmocarpum		The roots and leaves are used for treatment of abscess and cellulites.	
	O. kirkii	Boiled roots are drunk against fever, rheumatism, stomach troubles and infectious disease.	Maregesi *et al.*, 2007; Nyandat *et al.*, 1990
		The ash of the roots of this plant is used for allergic diseases and to decrease oedemas.	
	O. trichocarpum	The Leaves used for the treatment of stomach-related ailments.	
		The bark of this plant is used to induce vomiting for suspected poisoning.	Miller, 1997
erris			
	D. trifoliata	Different parts of this plant is used in the control of ticks and other ectoparasites in cattle and sheep.	Cheenpracha *et al.*, 2007
	D. indica	Different part of this plant is used in the treatment of bronchitis, cough, rheumatic joints and diabetes.	Koysomboon *et al.*, 2006
	D. scandnes	The dried stem is used for the management of cough, inflammation, urinary tract obstruction, muscle aches and pains.	Mahabusarakam *et al.*, 2004
ɔnchocarpus			
	L. eriocalyx	The bark is used in the treatment of blood pressure and to reduce sugar level.	Kareru *et al.*, 2006
	L. bussei	The roots and the stem bark is used in the management of fever and abdominal pain.	Chhabra *et al.*, 1990

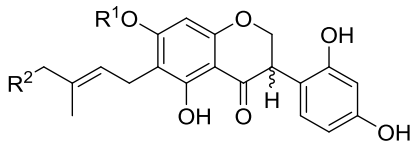
Dorstenia	*D. convexa*	The leaves-infusion is used as an enema for a children's disease called 'lunyama'.	Terashima, 1992
	D. kameruniana	The leaves is administrated for the treatment of cough, headache and stomach pain.	Abegaz *et al.*, 1998
	D. foetida	The juice of the aerial part used for treating skin diseases.	
		The roots are taken against leprosy, liver diseases and to remove intestinal worms.	Al-Fatimi *et al.*, 2007
	D. barteri	The leaves and twigs are used in the treatment of mumps, yaws and for infected wounds.	Tsopmo *et al.*, 1999
reblus	*S. asper*	Different parts of this plant are used for relief of fever, toothache, dysentery, gingivitis, wound decoction, epilepsy, cardiac disorder and oedema.	Rastogi *et al.*, 2006
	S. indicus	The bark is used for the treatment of inflammation and various rheumatoid diseases.	Zhao *et al.*, 1999

2.7 Phytochemistry and Biological Activities

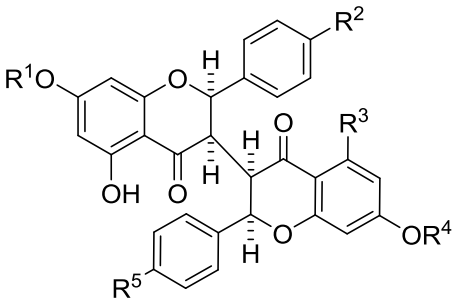
2.7.1 Phytochemistry and Bioactivity of Ormocarpum species

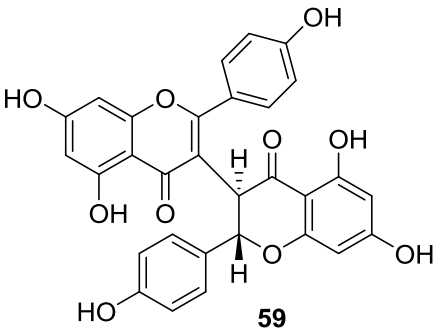
The presence of monomeric flavonoids have been reported from the genus *Ormocarpum*. For example, an isoflavone, 7-*O*-glucosyldiphysolone (**55**) was previously isolated from the roots of *Ormocarpum kirkii* (Xu *et al.*, 2012); an isoflavanone, 4"-hydroxydiphysolone (**56**) has also been isolated from the roots of this plant (Dhooghe *et al.*, 2010). Dimeric flavanones

having I-3,II-3 linkage (**57-58**), a biflavone with I-3,II-3 linkage (**59**) and a coumarin glycoside (**60-61**) have also been reported in *Ormocarpum kirkii* (Dhooghe *et al.*, 2010; Nyandat *et al.*, 1990; Xu *et al.*, 2012). The isolated compounds from *Ormocarpum kirkii* have been evaluated for antimicrobial, antileshmanial (against *Typanosoma cruzi* and *Typanosoma brucei)* and antiplasmodial (aganst the chloroquine-resistance K1 strain of *Plasmodium falciparum*) activities. The monomeric isoflavone 4"-Hydroxydiphysolone (**56**) showed no activity against all bacteria and parasites (Dhooghe, *et al.*, 2010). On the other hand the dimeric isoflavone Isochamaejsmin (**57**) revealed antiplasmodial activity with IC_{50} 7.3 ± 3.8 µM whereas apigeninyl-(I-3, II-3)-naringenin (**58**) showed activity against *T. rubrum* (IC_{50} 7.0 ± 6.4 µM) with cytotoxicity (CC_{50} 50.2 ± 16.3 µM) against MRC-5 cells. 5,5'-Di-*O*-methyldiphysin (**59**) showed moderate activity against all parasites and bacteria (Dhooghe *et al.*, 2010).



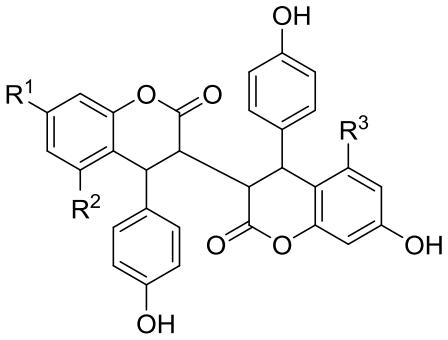
55 R^1= Glc R^2 = H
56 R^1= OH R^2 = H





59

57 R^1 = H R^2 = OH R^3 = OH R^4 = H R^5 = OH
58 R^1 = Glc R^2 = OH R^3 = OH R^4 = Glc R^5 = OH



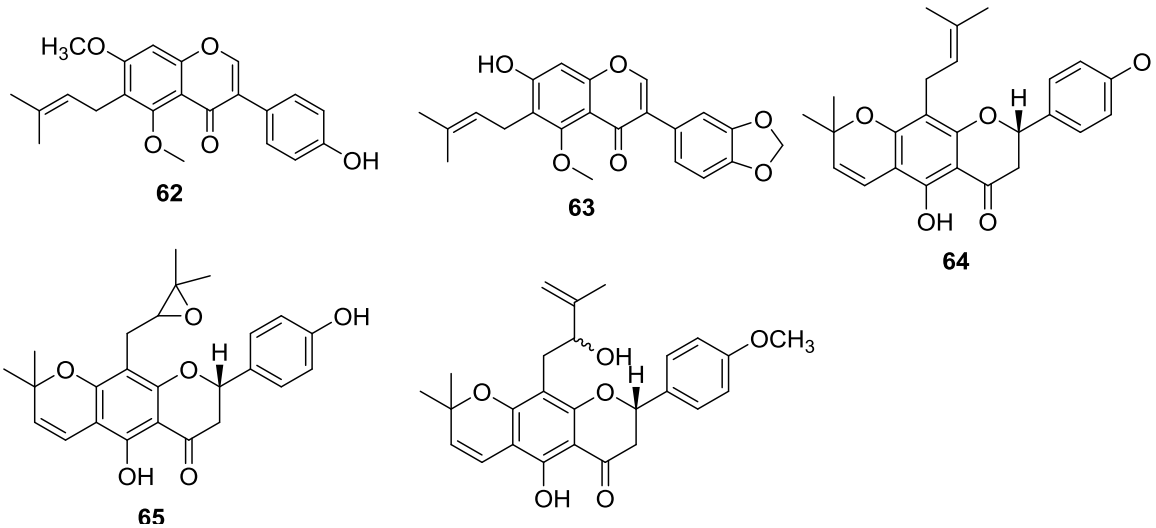
60 $R^1 = OCH_3$ $R^2 = OH$ $R^3 = OCH_3$
61 $R^1 = OH$ $R^2 = OCH_3$ $R^3 = OH$

2.7.2 Phytochemistry and bioactivity of Derris species

Many compounds including flavonoids, rotenoids, chalcones, pterocarpans and other constituents have been isolated from *Derris* species.

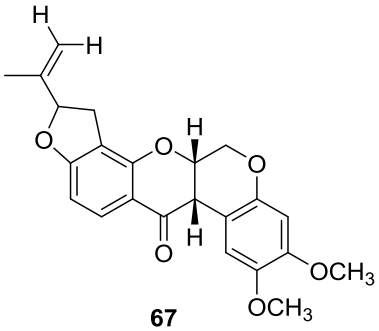
The major class of compounds of the genus *Derris* is the flavonoids including isoflavons; 4'-hydroxy-5,7-dimethoxy-6-(-3-methyl-2-butinyl)-isoflavone (**62**) and derrubon-5-methyl ether (**63**) reported from stems of *Derris eriocarpa* and possess antifungal activities against *Trichophyton mentagrophytes* with an MIC value of 25 µg/ml for both compounds and against *Microsporum gypsium* with MIC values 12.5 µg/ml and 25 µg/ml, respectively (Zhang *et al.*, 2014). A flavanone derivative, lupinifolin-4'-methyl ether (**64**) has been isolated from the seed pods of *Derris trifoliata* (Yenesew *et al.*, 2009). The seed-pods extract of *Derris trifoliata* was reported to be active against the D6 and W2 strains of *Plasmodium falciparum* with IC_{50} values of 12.2 ± 2.4, 13.4 ± 2.6 µg/ml, respectively, but was inactive (LC_{50} above 20 µg/ml) towards 2nd instar larvae of *Aedes aegyptii*. Lupinifolin-4'-methyl ether (**64**) isolated from this extract exhibited significant inhibitory effect against the D6 and W2 strains of *Plasmodium falciparum* with IC_{50} values of 12.9 ± 1.6 and 15.0 ± 2.5 µg/ml, respectively, but was inactive (LC_{50} above 20 µg/ml) towards 2nd instar larvae of *Aedes aegypti* (Yenesew, *et al.*, 2009). The flavanones, 2''', 3'''-epoxylupinifolin (**65**) and

dereticulatin (**66**) have been reported from the stem of *Derris reticulata* and inhibited P-338 cell line (0.4-0.5µg/ml) (Mahidol *et al.*, 1997).



Another type of constituents of the genus *Derris* are the rotenoids, represented by the common rotenoids, rotenone (**67**), deguelin (**68**) and toxicarol (**69**), which occur widely in the genus (Lu *et al.*, 2008; Yenesew *et al.*, 2006; Yenesew *et al.*, 2005). Rotenoids are well known for their anticancer activities; deguelin (**68**) and toxicarol (**69**) have been reported to show remarkable antitumor effects on mouse skin tumors in an *in vitro* two stage carcinogenesis test (Konoshima *et al.*, 1993; Udeani *et al.*, 1997). Rotenone, a compound isolated from the roots of *Derris trifoliata* has shown high larvicidal activity to the second instar larva of *Culex quinquefasciatus* (LC_{50} value 1.45 ± 0.1 µg/mL) (Yenesew, *et al.*, 2005). Apart from the usual rotenoids, rotenoid derivatives with unusual skeleton having an open ring C, 7a-*O*-methyl-12a-hydroxydeguelol (**70**), and the spiro rotenoid, spiro-13-homo-13-oxaelliptone (**71**), have been isolated from the seeds of *Derris trifoliata*. 7a-*O*-Methyldeguelol (**72**) and 7a-*O*-methylelliptonol (**73**) were also reported from the roots and stem of *Derris trifoliata* (Cheenpracha, *et al.*, 2007; Yenesew *et al.*, 2006; Yenesew *et al.*,

2005). 7a-*O*-Methyldeguelol (**72**) has been reported to exhibit cytotoxic effect against NCI-H187 cell lines (Cheenpracha, *et al.*, 2007).



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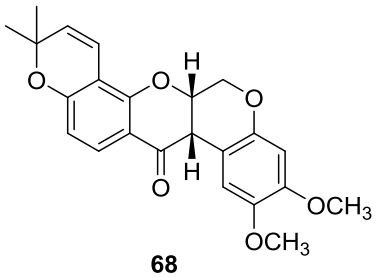

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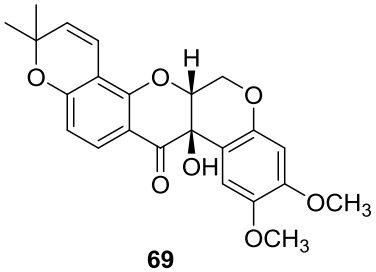

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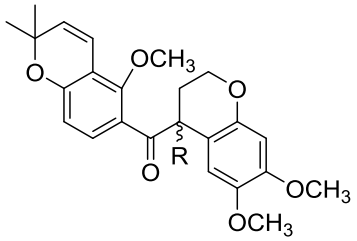
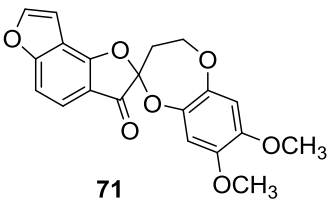


70 R = OH
72 R = H



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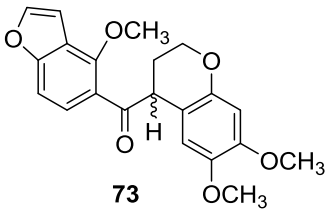

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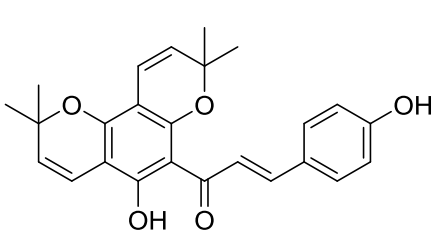
The chalcones laxichalcone (**74**) and derrichalcone (**75**) have been isolated from the roots of *Derris laxiflora* (Lin *et al.*, 1992). The prenylated chalcone, 2', 4'-dihydroxy-4-methoxy-3'-prenylchalcone (**76**) has been isolated from the leaves of *Derris malaccensis* (Siripaisarnpipat *et al.*, 2007). The chalcones, derrischalcone (**77**), tunicatachalcone (**78**), and obovatachalcone (**79**) isolated from the fruits of *Derris indica* showed cytotoxic activity with IC_{50} value in the range of 0.59 to 7.0 µg/ml and 2.6 to 5.3 µg/ml against cholangiocarcinoma cell line (M156) human hepatoma ($HEPG_2$) cells, respectively (Decharchoochart *et al.*, 2014).



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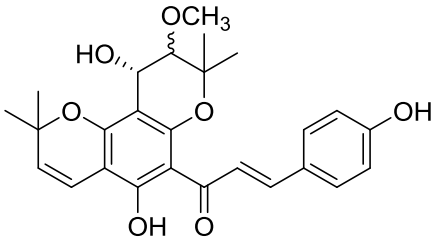

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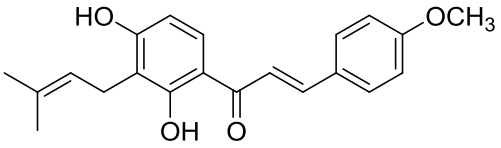

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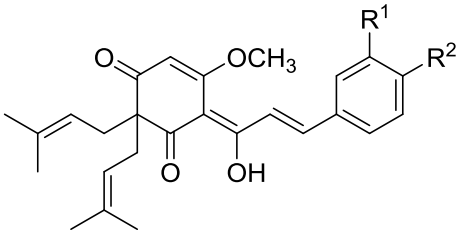
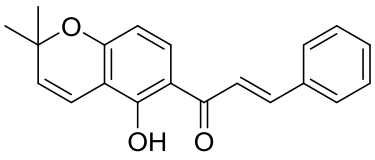


77 $R^1 = R^2 = -OCH_2O-$

78 $R^1 = R^2 = H$



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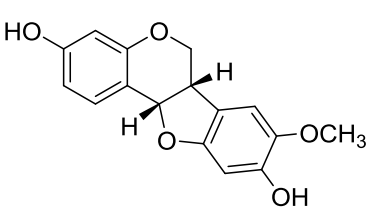
From the whole plant of *Derris laxiflora,* the pterocarpans, lespedezole (**80**) and derrispisatin (**81**) (Chien *et al.*, 2016) were reported. Addtional pterocarpans, pterocarpadiol A-D (**82-85**), were reported from twigs and leaves of *Derris robusta* (Li *et al.*, 2015). NOESY correlation was used to establish the relative configuration at C-6a and C-11a of compounds **80** and **81.** There are two possible isomers for these compounds: (6a*R*,11a*R*) or (6a*R*,11a*R*). To determine the correct absolute configuration for compound **80** and **81**, the CD spectra were measured and compared to the theoretical predicted CD spectra for the two isomers (Yenesew *et al*., 1998a).



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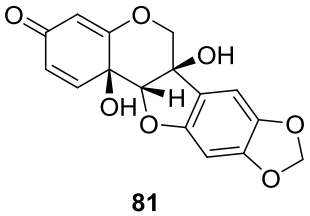

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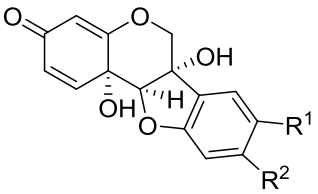
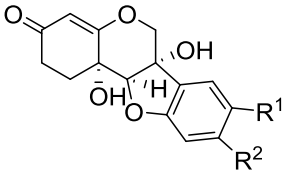


82 $R^1 = R^2 = OCH_2O$

83 $R^1 = H$ $R^2 = OCH_3$

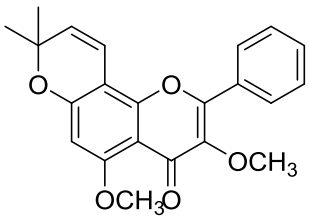


84 $R^1 = R^2 = OCH_2O$

85 $R^1 = H$ $R^2 = OCH3$

2.7.3 Phytochemistry and Bioactivity of Lonchocarpus species

Phytochemical studies of the genus *Lonchocarpus* revealed that the genus mainly elaborate flavonoids (including chalcones, pterocarpans and rotenoids), dibenzoylmethane and stilbenes. Chemical investigation of the roots of *Lonchocarpus latifolius* led to the isolation of a flavone, 3,5-dimethoxy-2",2"-dimethylpyrano-(5",6":8,7)flavone (**86**); flavanones, 3-methoxy-(2"-3":7,8)-furanoflavanone (**87**) and 3',4'-methylenedioxy-(2"-3":7,8)-furanoflavanone (**88**) and a flavan, (2,3-*trans*-3,4-*trans*)-3,4-dimethoxy-(2",3":7,8)-furanoflavan (**89**) (Magalhaes *et al.*, 2000). From the leaves of *Lonchocarpus xuul* and *Lonchocarpus yucatanensis* flavones including 5,4'-dihydroxy-3'-methoxy-(6:7)-2,2-dimethylpyranoflavone (**90**) and 5,4'-dimethoxy-(6:7)-2,2-dimethylpyranoflavonen (**91**) have been reported (Borges-Argaez *et al.*, 2002).



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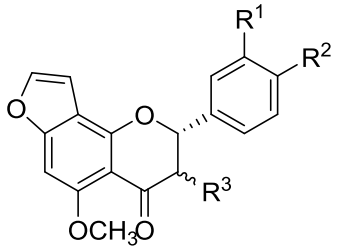
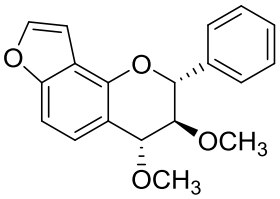


87 $R^1 = R^2 = H$ $R^3 = OCH_3$

88 $R^1 = R^2 = O\text{-}CH_2\text{-}O$ $R^3 = OCH_3$



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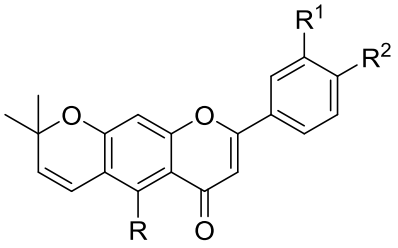
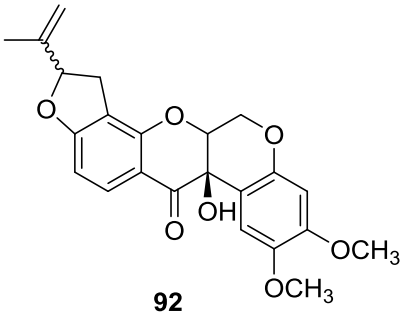


90 R = OH $R^1 = OCH_3$ $R^2 = OH$

91 R = OCH_3 R^1= H $R^2 = OCH_3$

The genus *Lonchocarpus* also contains rotenoid derivatives including 12a-hydroxyrotenone (**92**) and pterocarpans such as medicarpin (**93**) (Magalhaes, *et al.*, 2000; Magalhaes *et al.*, 1996).



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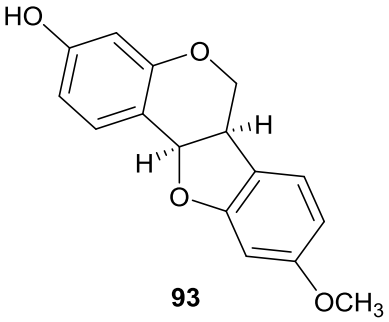
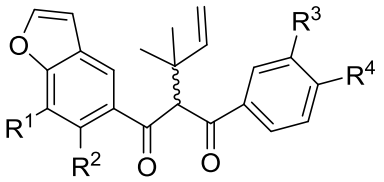


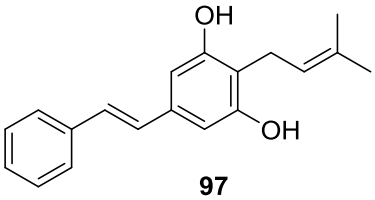
93

The other classes of compounds isolated from this genus are dibenzoylmethane and stilbenes. From the roots of *Lonchocarpus latifolius* and *L. muehlbergianus* 2'-methoxy-[2",3":4ˈ,3ˈ]-furanodibenzoylmethane (**94**), 3.4-methylenedioxy-2ˈ-methoxy-[2",3":4',4']-furanodibenzoylmethane (**95**) and 2',5',6'-trimethoxy-[2",3",4ˈ,3']-furanodibenzoylmethane (**96**) have been isolated (Magalhaes *et al.*, 1997). Prenylated stilbenes including chiricanines

A-C (**97-99**) have been reported from *Lonchocarpus chiricanus* (Ioset *et al.*, 2001). Chiricanines A (**97**) exhibited antifungal effects against *Cladosporium cucumerinus* with MIC value 5 µg/ml, but Chiricanines B-C (**98-99**) were not active against this fungus. Chiricanines A-C (**97-99**) have tested for larvicidal activity against *Aedes aegypti* of which **97** was active with IC_{50} values of 6 µg/ml, while **98** and **99** were inactive (IC_{50} >50 µg/ml) (Ioset *et al.*, 2001).



94 $R^1 = R^2 = R^3 = R^4 = H$
95 $R^1 = R^2 = H$ $R^3 = R^4 = OCH_2O$
96 $R^1 = R^2 = OCH_3$ $R^3 = R^4 = H$



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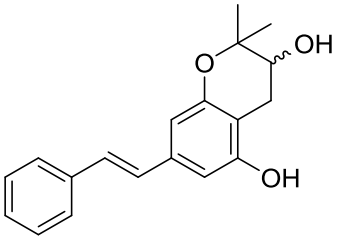

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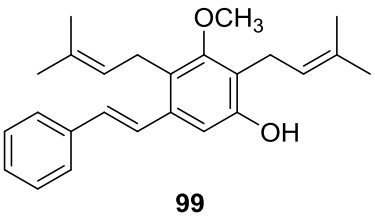
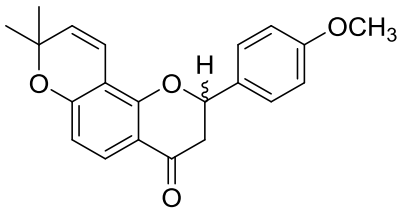


99

2.7.4 Phytochemistry and Bioactivity of Dorstenia Species

The genus *Dorstenia* is a rich source of prenylated and geranylated flavanones, flavones, flavonols, chalcones, coumarins, styrenes and benzofurans. Monoprenylated as well as diprenylated flavanones have been reported from *Dorstenia* species. Dorspoinsettifolin (**100**) was obtained from the twigs of *D. poinsettifolia* (Ngadjui *et al.*, 1999). The diprenylated flavanone, dinklagin A (**101**), has been reported from *Dorstenia dinklagei* (Ngadjui *et al.*, 2002). The authors did not establish the configuration of Dorspoinsettifolin (**100**) and dinklagin A (**101**) at C-2 of ring C.



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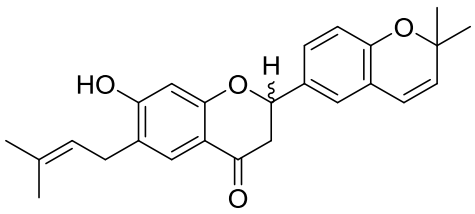
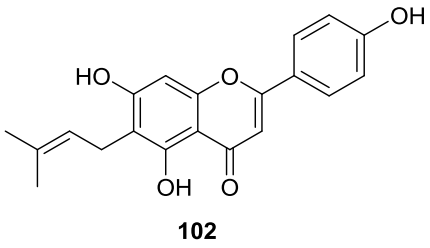


101

The flavones reported from *Dorstenia* species contain up to three prenyl groups. The monoprenylated flavones of *Dorstenia* include 6-prenylapigenin (**102**), isolated from *D. cilata*, *D. dinklagei* and *D. kameruniana* (Abegaz *et al.*, 1998; Ngadjui, *et al.*, 2002). 6-Prenylapigenin (**102**) has been tested against growth profiles and viability of HL-60 promyeloytic leukaemia cells and was not toxic at low concentration but showed high toxicity at 100 µM (Abegaz *et al.*, 1998). The 8-prenylated isomer, licoflavone C (**103**), has been reported from *Dorstenia poinsettifolia* (Tsopmo *et al.*, 1998). *Dorstenia psilurus* produced a number of triprenylated flavones such as dorsilurin F–H (**104-106**) (Tabopda *et al.*, 2008).



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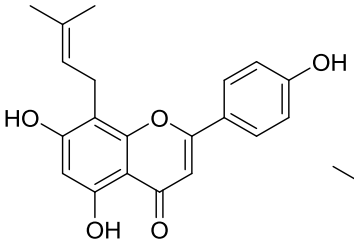

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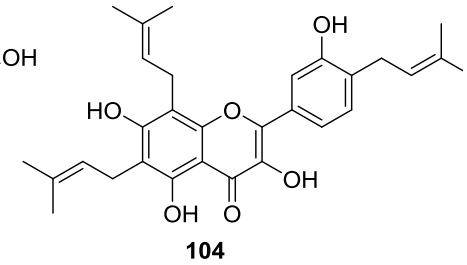

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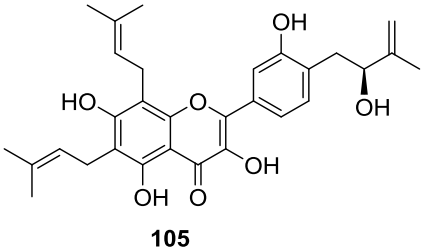

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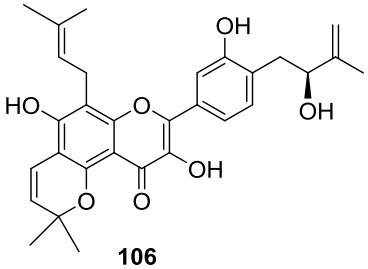
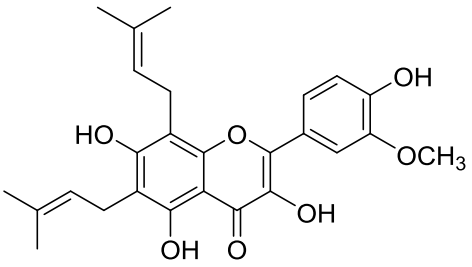


106

Flavonols with two prenyl groups at C-6 and C-8, dorsmanin D (**107**) and dorsilurin C (**108**) have been isolated from *Dorstenia mannii* and *D. psilurus,* respectively (Ngadjui *et al.,* 1998; Ngadjui, *et al.*, 1999). The geranyl substituted flavonol, dorsmannin C (**109**), has been isolated from *Dorstenia mannii* and *D. tayloriana* (Ngadjui, *et al.*, 1998). The anti-oxidant activity of prenylated flavonoids isolated from *Dorstenia mannii* have been reported and dorsmanin C (**109**) showed significant binding to copper ions (Dufall *et al.*, 2003).The geranylated flavonol derivative, poinsettifolin A (**110**), has been isolated from *Dorstenia poinsettifolia* (Tsopmo, *et al.*, 1998).



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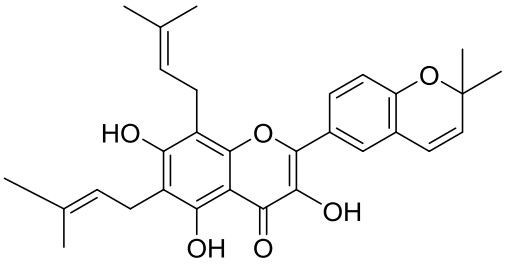

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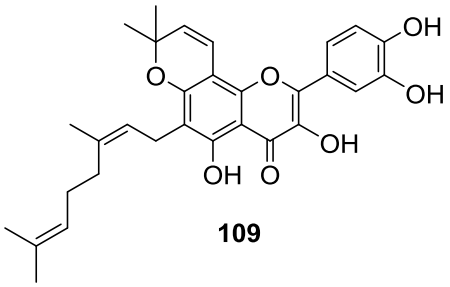

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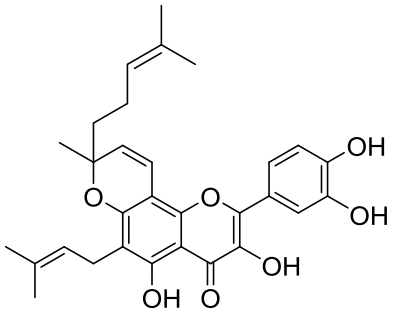
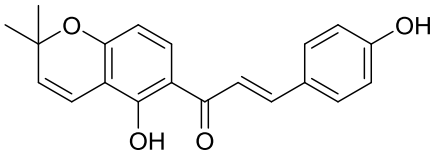


110

Chemical investigation of the twigs of *Dorstenia mannii* yielded the prenylated chalcone, 4-hydroxylonchocarpin (**111**) (Ngadjui *et al.*, 1998). Isobavachalcone (**112**) has been reported from *Dorstenia kameruniana* and *Dorstenia ciliata* (Abegaz *et al.*, 1998). A chalcone having two prenyl groups, stipulin (**113**), has been reported from *Dorstenia kameruniana*, *D. ciliate*, and *D. dinklagei* (Abegaz *et al.*, 1998; Ngadjui *et al.*, 1998). The cytotoxicity of stipulin (**113**) has been reported against promyeloytic leukaemia (HL-60) with ED_{50} of 50 µM

(Abegaz *et al.*, 1998). *Dorstenia kameruniana* and *Dorstenia mannii* have also yielded the bis-dihydropyran derivative (**114**) (Abegaz *et al.*, 1998; Ngadjui *et al.*, 1998). Investigation of the aerial part of *Dorstenia poinsettifolia* and *D. proropens*, resulted in the isolation of the geranylated chalcones, Poinsettifolin B (**115**) and proropensin (**116**) (Abegaz *et al.*, 2002; Tsopmo, *et al.*, 1998).



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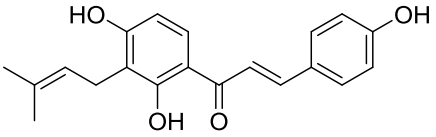

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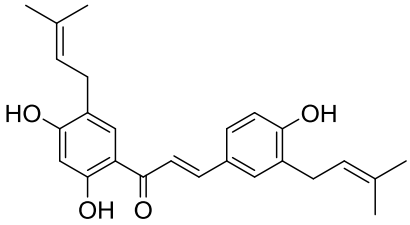

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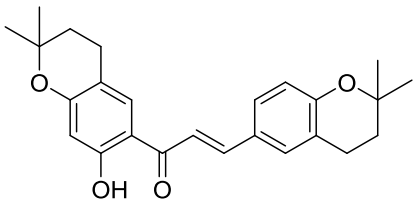

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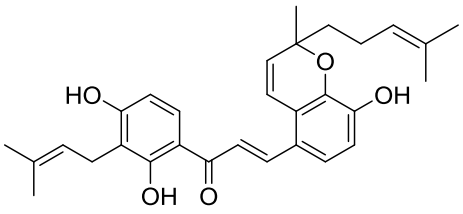

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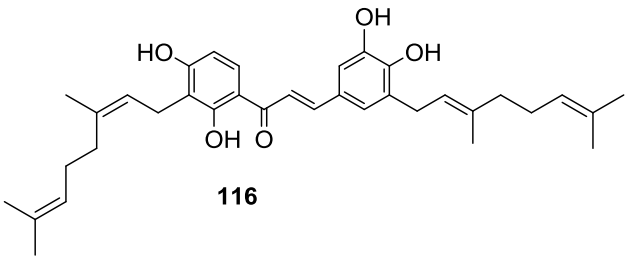
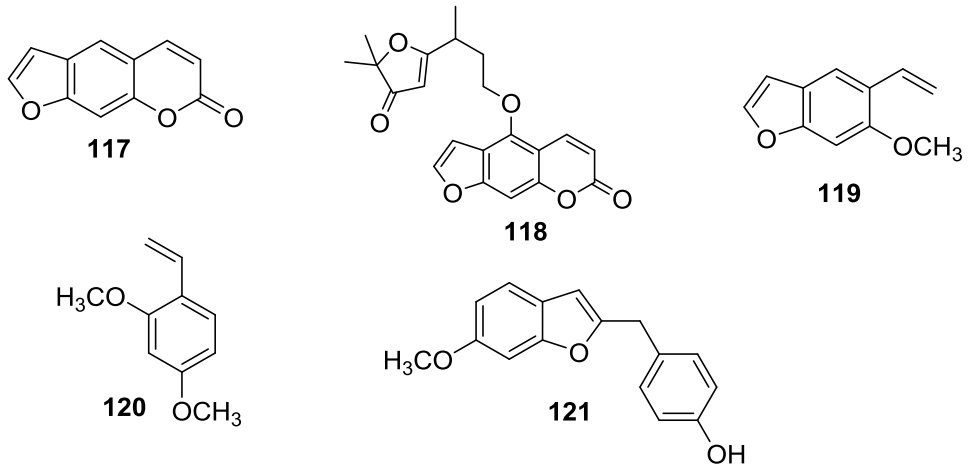


116

Phytochemical examination of the rhizomes of *Dorstenia psilurus* led to the isolation of the furanocoumarin derivative, psoralen (**117**) (Ngadjui, *et al.*, 1998). Psoralen (**117**) is recognized as cytotoxic, antitumour promoter, artemicide, antimutagenic, bacteriophagicide and viricide (Duke, 1992). Investigation of yet another *Dorstenia* species resulted in the isolation of an unusual furanocoumarin, *O*-[3-(2,2,-dimethyl-3-oxo-2H-furan-5-yl)butyl]bergaptol (**118**) from *Dorstenia caypiaa* which has also been isolated from *Dorstenia contrajerva* and *Dorstenia elliptica* (Terreaux *et al.*, 1995). Compound **118** was

tested for its antitumor properties, but did not show significant inhibitory activity (Terreaux, *et al.*, 1995).

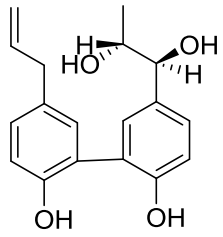
The first naturally occurring styrenes which contain a furan moiety, 6-methoxy-5-vinylbenzofuran (**119**) and 2,4-dimethoxystyrene (**120**) were reported from the roots of *Dorstenia barnimiana* (Woldu *et al.*, 1988). 6-Methoxy-5-vinylbenzofuran (**119**) and 2,4-dimethoxystyrene (**120**) have been tested against several microbes, but they did not show significant activity (Woldu, *et al.*, 1988). The benzofuran derivative, 2-(*p*-hydroxybenzyl)-6-methoxybenzofuran (**121**) reported from the leaves of *Dorstenia gigas*, exhibited antifungal activity in the *Cladosporium* bioassay (Franke *et al.*, 2001).



2.7.5 Phytochemistry and Bioactivity of Streblus Species

They are only few *Streblus* species which have been phytochemically investigated, most of the reports being on *Streblus asper*. From the heart wood of *Streblus asper*, lignans, erythro-strebluslignanol (**122**), threo-7ˈ-methoxylstrebluslignanol (**123**) and erythro-7'-methoxyl strebluslignanol (**124**) have been isolated and the anti-hepatitis B virus activity of these compounds have been reported with IC_{50} values between 131.23 µM and 156.75 µM (Li *et al.*, 2012). The other lignans reported from the roots of this plant include (7'*R*,8'*S*)-4,4'-dimethoxystrebuslignanol (**125**), 3'-hydroxy-isostrebluslignaldehyde (**126**), 3-3'-methylene

and bis-(4-hydroxybenzaldehyde) (**127**) (Nie *et al.*, 2016). It has been reported that compounds **125**-**127** showed good antimicrobial activity against *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Pseudomonal aeraginosa*, *Escherichia coli* and *Staphylococcus aureus* with MIC values ranging from 0.0183 to 0.0853 µM (Nie, *et al.*, 2016).



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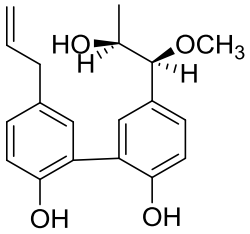

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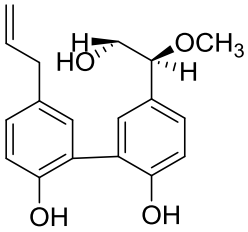

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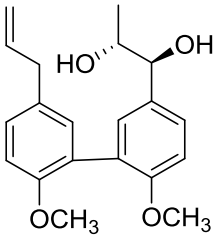

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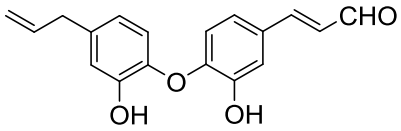

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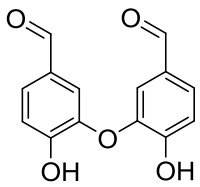
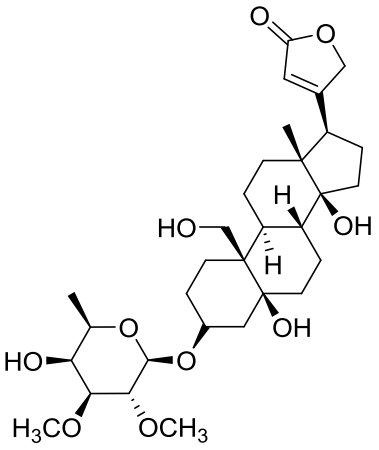


127

Cardiac glycosides, (+)-19-hydroxykamaloside (**128**), (+)-5-hydroxyasperoside (**129**) and (+)-3'-de-*O*-methylkamaloside (**130**) have been isolated from the stem bark of *Streblus asper* (Ren *et al.*, 2017). These compounds show potent cytotoxicity against MV4-11 and Kasumi-1 leukemia cell lines, and H1299 non small cell lung cancer cells with IC_{50} values ranging from 160 to 360 nM (Ren, *et al.*, 2017). Furthermore, the presence of cardiac glycosides from different parts of *Streblus asper* have been reported (Rastogi *et al.*, 2006a); (Vidhu *et al.*, 2012).



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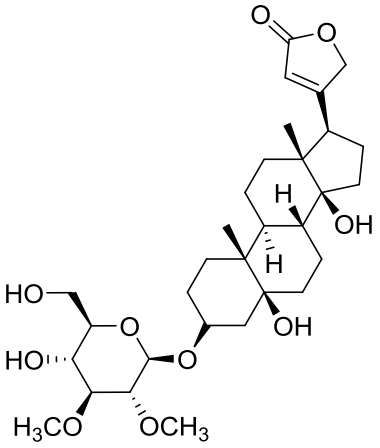

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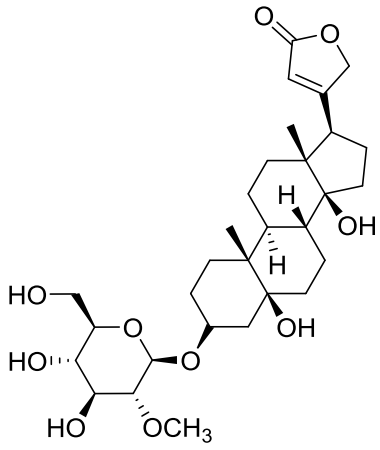
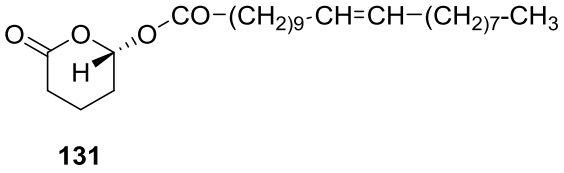


130

The root of *Streblus asper* also contains the aliphatic esters, hexacosenyl lactones (e.g. **131**), a fatty acid glycoside, cerotic acid glycoside (**132**) and salicylic glucoside ester, nonadecanyl salicylate glucoside (**133**) (Vidhu, *et al.*, 2012).



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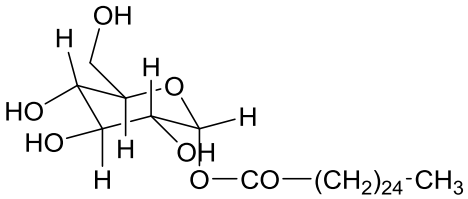
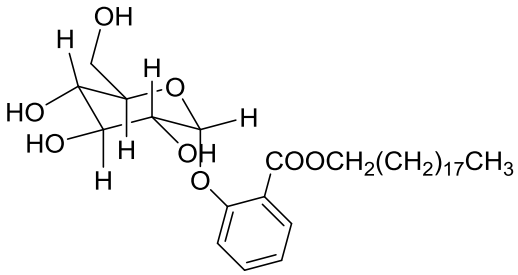
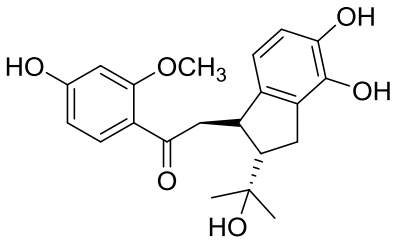


132



133

Phytochemical investigation of the bark of *Streblus indicus* yielded the cytotoxic 2,3-dihydro-1H-indene derivatives indidene A-C (**134-136**) with inhibitory activity towards human lung epithelial A549 and human breast carcinoma MCF-7 cells with IC_{50} values ranging from 2.2 ± 0.5 to 7.2 ± 0.9 µM (He *et al.*, 2016).



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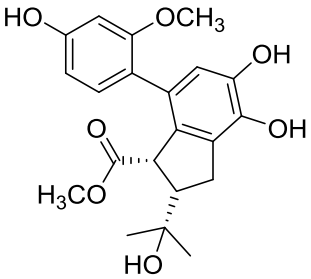

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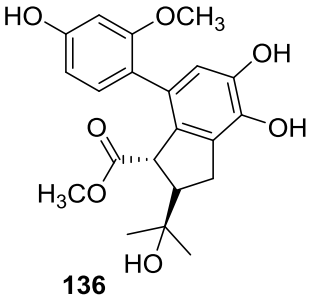

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Materials

3.1.1 Ormocarpum kirkii

The roots and stem bark of *Ormocarpum kirkii* were collected in September 2014 from Muthetheni location, Machakos County by Mr. Patrick C. Mutiso. The plant was identified and a voucher speciman (PBC 2016/004) deposited at the University Herbarium, School of Biological Sciences, University of Nairobi.

3.1.2 Derris trifoliata

The different plant parts of *Derris trifoliata* were collected at the mouth of river Sabaki in Malindi, Coast Province, Kenya, in August 2013. The plant was identified by Mr. Patrick C. Mutiso, of the Herbarium, School of Biological Sciences, University of Nairobi, where a voucher specimen was deposited.

3.1.3 Lonchocarpus bussei and Lonchocarpus eriocalyx

The plant materials of *Lonchocarpus bussei* were collected from Muhaka-Kaya forest, Kwale County, Kenya in January 2016. *Lonchocarpus eriocalyx* were collected from Muthetheni location, Machakos County, Kenya in January 2016. The plants were authenticated by Mr. Patrick C. Mutiso of the University Herbarium, School of Biological Sciences, University of Nairobi, where voucher specimens (PBC2016/005 for *Lonchocarpus bussei* and PBC2016/006 for *Lonchocarpus bussei*) were deposited.

3.1.4 Dorstenia kameruniana

The roots and twigs of *Dorstenia kameruniana* were collected in July 2016 from Gondoni forest, Kwale County, Kenya. The plant was identified and authenticated by Mr. Patrick C.

Mutiso from School of Biological Sciences, University of Nairobi where a voucher specimen (PBC 2016/007) was deposited.

3.1.5 Streblus usambarensis

The stem and roots of *Streblus usambarensis* were collected from Gondoni forest, Kwale County, in July 2016. The authenticity of the plant was confirmed by Mr. Patrick C. Mutiso from School of Biological Sciences, University of Nairobi, where voucher specimen (PBC 2016/008) was deposited.

3.2 Methods

3.2.1 General Methods

Column chromatographic separations were performed on Merck silica gel 60 (70-230 mesh). Further purifications were conducted on Preparative HPLC using Water 600 instruments loaded with an RP C8 Kromasil (250 mm x 55 mm) column, eluting with H_2O/MeOH solvent system. Gel filtration was done on Sephadex LH-20 eluting with CH_2Cl_2/MeOH (1:1). PTLC sparations were done with silica gel on 60 (20 cm x 20 cm) plates. In the cytotoxicity assay, fluorescence was measured on an Infinite 200 Pro TECAN plate reader. Cell cycle analysis, Annexin V/VI, JC-1 and H2DCFH-DA staining were measured on a flow cytometer LSR-Fortessa FACS analyser (Bacton-Dickinson, Heidelberg, Germany). NMR spectra were recorded on Varian VNMR-S 500, BRUKER AVANCE 600 and BRUKER AVANCE III HD 800 spectrometers. The NMR spectra were processed using MestReNova 10.0 software. The HRESIMS were acquired on GC-TOF micromass (Waters Inc.). LCESIMS was acquired on Perkin Elmer PESUEX API 150EX. UV spectra were acquired on specord S 600. Melting points were determined on SMP 10 apparatus. Electronic Circular Dichroism spectra were measured using JASCO J-815. Optical rotations were read on AUTOPOL IV. IR spectra were measured on Perkin Elmer UATR Two instrument.

3.3 Extraction and Isolation of Compounds

3.3.1 Extraction and isolation of compounds from the stem bark of Ormokarpum kirkii

The stem bark of *Ormokarpum kirkii* was air dried and crushed into powder. The powdered stem bark (1.5 kg) was extracted successively with CH_2Cl_2/MeOH (1:1) (4 x 2 L) and MeOH (4 x 2 L) by cold percolation. The combined extract (194 g) was partitioned between EtOAc and water. The EtOAc layer was collected and concentrated to yield 175 g of crude extract. A 100 g portion of the EtOAc extract was adsorbed on 100 g of silica gel and subjected to column chromatography over silica gel (800 g) and eluted with *n*-hexane containing increasing amounts of EtOAc. A total of 271 fractions (*ca*. 500 ml) were collected. Osajin-4-methylether (**1**) (10 mg) was obtained from the fractions eluted with 3% EtOAc in *n*-hexane after purification by column chromatography over Sephadex LH-20 (eluted with CH_2Cl_2/MeOH, 1:1) and crystallization. The precipitate obtained from 4% EtOAc in *n*-hexane eluent was filtered and washed with *n*-hexane to yield 5,7,-dihydroxy-4"-methoxy-6,8-diprenylisoflavone (**2**) (5 mg). The mother liquor was purified on Sephadex LH-20 eluting with CH_2Cl_2/MeOH (1:1) to yield yellow crystals of osajin (**3**) (20 mg). The fraction obtained from 6% EtOAc in *n*-hexane was subjected to column chromatography over silica gel (100 g) eluting with 5% EtOAc in *n*-hexane to yield more amounts of osajin (**3**) (9 mg). A white precipitate obtained from 10% EtOAc in *n*-hexane was filtered and washed with CH_2Cl_2 to afford 7,7"-di-*O*-methylchamaejasmin (**4**) (100 mg). A precipitate obtained from 15% EtOAc in *n*-hexane was filtered and washed with CH_2Cl_2 to afford chamaejasmin (**5**) (200 mg). In the same way purification of the fraction eluted with 50% EtOAc in *n*-hexane gave diphysin (**6**) (75 mg).

3.3.2 Extraction and isolation of compounds from the roots of Ormokarpum kirkii

The powdered roots of *Ormokarpum kirkii* was extracted as described above to give 205 g of a combined extract. The extract was partitioned between EtOAc and water. The EtOAc layer

was collected and concentrated on a rotary evaporator to provide 160 g of crude extract. Part of the extract (100 g) was subjected to column chromatography over silica gel (800 g). The column was eluted with *n*-hexane containing increasing amounts of EtOAc. A total of 130 fractions, 500 ml each, were collected. The fraction eluted with 10% EtOAc in *n*-hexane was purified over Sephadex LH-20 CH_2Cl_2/MeOH (1:1) and yielded osajin (**3**) (3 mg) and heptacosyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylate (**7**) (11 mg). A precipitate obtained from the fraction eluted with 15% EtOAc in *n*-hexane was filtered and rinsed with CH_2Cl_2 to afford 7,7"-di-*O*-methylchamaejasmin (**4**) (200 mg)**.** Fractional crystallization of the 30% EtOAc in *n*-hexane eluent gave chamaejasmin (**5**) (35 mg) and campylospermone (**8**) (50 mg). The 50% EtOAc in *n*-hexane fraction gave diphysin (**6**) (24 mg) as a precipitate.

3.3.3 Extraction and isolation of compounds from the roots of Derris trifoliata

The dried and ground roots of *Derris trifoliata* (1 kg) was extracted in CH_2Cl_2/MeOH (1:1) by cold percolation to yield 100 g of crude extract. The extract was partitioned between EtOAc and water. The organic layer was separated and concentrated on a rotary evaporator to give 25 g of extract. The extract was separated by column chromatography over silica gel (450 g) and eluted with *n*-hexane containing increasing amounts of EtOAc. A total of 55 fractions were collected and combined into 10 major fractions based on their TLC profiles. A brown oily fraction obtained from 8% EtOAc in *n*-hexane was purified using Sephadex LH-20 (eluted with CH_2Cl_2/MeOH, 1:1) to afford (-)-medicarpin (**9**) (3.3 mg) and a mixture of two compounds. This fraction was separated by PTLC (eluting with *n*-hexane/CH_2Cl_2/EtOAc, 4:2:1) followed by preparative HPLC (eluting with H_2O/CH_3OH, 15:85, 250 mm x 55 mm column for 54 minutes) and yielded rotenone (**10**) (2.7 mg). α-Toxicarol (**13**) (3.8 mg) was crystallized from the fraction eluted with 10% EtOAc in *n*-hexane. From the mother liquor of this fraction 7a-*O*-methyldeguelol (**12**) (3.2 mg) was isolated after purification by PTLC (eluted with *n*-hexane/CH_2Cl_2/EtOAc, 4:2:1). Purification

of the fraction obtained from 13% EtOAc in *n*-hexane by PTLC (eluted with *n*-hexane/CH_2Cl_2/EtOAc (4:2:1)) yielded a mixture of two compounds. These were separated by preparative HPLC (eluting with H_2O/CH_3OH, 90:10, 250 mm x 55 mm column, 42 and 46 minutes, respectively) to yield prunetin (**19**) (2.6 mg) and barbigerone (**20**) (2.5 mg). The fraction eluted with 15% EtOAc in *n*-hexane was separated over Sephadex LH-20 (eluted with CH_2Cl_2/MeOH; 1:1) to yield 7a-*O*-methylelliptonol (**18**) (3.2 mg) and a mixture of two compounds. This mixture was purified using preparative HPLC (eluted with H_2O/CH_3OH, 85:15, 250 mm x 55 mm, column at 35 and 37 minutes, respectively) and afforded 12a-hydroxyelliptonol (**15**) (3.7 mg) and 7a-*O*-Methyl-12a-hydroxyelliptonol (**16**) (1.1 mg).

3.3.4 Extraction and isolation of compounds from the stem of Derris trifoliata

The air dried and ground stem of *Derris trifoliata* (2 kg) was macerated overnight in CH_2Cl_2/MeOH (1:1) (5 x 3 L). The solvent was concentrated on a rotary evaporator to provide 147 g of extract. The extract was partitioned between EtOAc and water. The EtOAc layer was collected and the solvent was concentrated on a rotary evaporator under reduced pressure to give 35 g of an organic extract. The extract was subjected to column chromatography over silica gel (400 g) and eluted with *n*-hexane-containing increasing amounts of EtOAc to afford 78 fractions of 500 mL each. The fractions were analysed by TLC and pooled into 10 major fractions. The fraction eluted with 8% EtOAc in *n*-hexane was separated over Sephadex LH-20 column (eluted with CH_2Cl_2/MeOH, 1:1) to give rotenone (**10**) (2.7 mg)**.** The fraction eluted with 10% of EtOAc in *n*-hexane was further separated over Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to remove chlorophyll followed by purification using preparative HPLC eluting with CH_3OH /H_2O, 50:50, 250 mm x 55 mm column, at 52, 49, 38 minute respectively) to afford deguelin (**11**) (3.3 mg), tephrosin (**14**) (3.2 mg) and elliptone **(17**) (3.0 mg). The fraction eluted with 15% EtOAc in *n*-hexane was purified over Sephadex LH-20 using CH_2Cl_2/MeOH (1:1) as eluent and then on preparative

HPLC (eluted with H_2O/CH_3OH, 70:30, 250 mm x 55 mm column, at 42 and 40 minute) to afford dereticulatin (**22**) (2.5 mg) and 6,7-dimethoxy-4-chromanone (**23**) (3.9 mg). Lupinifolin (**21**) (3.1 mg) was isolated from the fraction eluted with 20% EtOAc in *n*-hexane by PTLC using *n*-hexane/CH_2Cl_2/EtOAc (4:2:1) followed by further purification using preparative HPLC (eluting with H_2O/CH_3OH gradient 65:35, 250 mm x 55 mm column, at 43 minutes).

3.3.5 Extraction and isolation of compounds from the leaves of Lonchocarpus bussei

The air dried and powdered leaves of *Lonchocarpus bussei* (2 kg) were extracted with CH_2Cl_2/MeOH (1:1) (3 x 2 L) at room temperature. A dark green crude extract (100 g) was obtained after removal of the solvent under reduced pressure. A portion of the extract (85 g) was subjected to column chromatography (CC) over silica gel (450 g, 80 × 4 cm) and eluted with *n*-hexane-EtOAc gradient (1-100%). A total of 75 fractions each *ca*. 500 mL were collected. The fraction eluted with 3% EtOAc in *n*-hexane was purified by column chromatography using Sephadex LH-20 as a stationary phase (eluent: CH_2Cl_2/MeOH, 1:1) to give 4'-prenyloxyvigvexin A (**24**) (15 mg) and maximaisoflavone (**25**) (17 mg). 7,2'-Dimethoxy-3',4'-methylenedioxyisoflavone (**26**) (25 mg) was crystallized from the fraction eluted with 5% EtOAc in *n*-hexane. The mother liquor of this fraction was purified by column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to give 6,7,3'-trimethoxylenedioxyisoflavone (**27**) (50 mg) and more amounts of 7, 2'-dimethoxy-3',4'-methylenedioxyisoflavone (**26**) (3 mg)**.** The fractions eluted with 10% EtOAc in *n*-hexane was passed through Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) and crystallised to give durmillone (**28**) (10 mg) and 4-hydroxylonchocarpin (**29**) (12 mg). Similar treatment of the fraction eluted with 20% EtOAc in *n*-hexane afforded colenemol (**30**) (22 mg).

3.3.6 Extraction and isolation of compounds from the roots of Lonchocarpus bussei

The air dried and crushed roots of *Lonchocarpus bussei* (2 kg) were extracted at room temperature with CH_2Cl_2/MeOH (1:1). The solvent was removed using a rotary evaporator to afford 84 g of crude extract. Part of this extract (75 g) was subjected to column chromatography on silica gel (400 g) and eluted with *n*-hexane containing increasing amounts of EtOAc to give a total of 50 fractions, of about 500 ml each. The fraction obtained with 15% EtOAc in *n*-hexane was separated by PTLC (eluent: *n*-hexane-CH_2Cl_2-EtOAc, 8:1:1) to afford (6a*R*,11a*R*)-maackiain (**31**) (5 mg) and (6a*R*,11a*R*)-edunol (**32**) (4 mg).

3.3.7 *Extraction and isolation of compounds from the stem bark of Lonchocarpus eriocalyx*

The dried stem bark of *Lonchocarpus eriocalyx* (2 kg) were crushed and extracted using CH_2Cl_2/MeOH (1:1) at room temperature. The extract was concentrated under reduced pressure to yield a brown crude extract (100 g). A portion of the extract (85 g) was subjected to column chromatography over silica gel (450 g) eluting with *n*-hexane containing increasing amounts of EtOAc to give a total of 100 fractions, about 500 ml each. The fraction eluted with 5% EtOAc in *n*-hexane was subjected to silica gel PTLC using *n*-hexane-CH_2Cl_2-EtOAc (8:1:1) to afford (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**33**) (20 mg). Purification of the fraction eluted with 10% EtOAc in *n*-hexane by column chromatography over Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) afforded (6a*R*,11a*R*)-edunol (**32**) (3 mg).

3.3.8 Extraction and isolation of compounds from the roots of Dorstenia kameruniana

The roots of *Dorstenia kameruniana* (1.5 kg) were air dried and crushed into powder. The powdered roots were extracted with CH_2Cl_2/MeOH (1:1) (4 x 2L) at room temperature. The extract was combined and the solvent removed using a rotary evaporator to give 90 g of crude extract. A portion of the extract (75 g) was subjected to column chromatography (CC) over silica gel (450 g) and eluted with *n*-hexane containing increasing amounts of EtOAc to give

71 fractions, *ca*. 500 ml each. Crystallization of the fraction eluted with 10% EtOAc in *n*-hexane gave bergapten (**37**) (100 mg). The mother liquor from this fraction was purified by column chromatography (silica gel, *n*-hexane//EtOAc, gradient) and by PTLC (eluent: *n*-hexane/CH_2Cl_2/EtOAc (8:1:1) to afford dorsmerunin A (**34**) (5 mg), dorsmerunin B (**35**) (20 mg) and dorsmerunin C (**36**) (4 mg).

3.3.9 Extraction and isolation of compounds from the twigs of Dorstenia kameruniana

The air dried and powdered twigs of *Dorstenia kameruniana* (2.5 kg) were extracted with CH_2Cl_2/MeOH (1:1) (4 x 2L) at room temperature. The extracts were combined and concentrated under reduced pressure to yield 64 g of crude extract. A portion of the extract (60 g) was subjected to column chromatography on silica gel (400 g) with increasing polarity of EtOAc in *n*-hexane (1-100%) to afford 52 fractions, *ca.* 500 mL each. The fraction obtained from 10% EtOAc eluent was crystallized to give bergapten (**37**) (55 mg). The mother liquor was concentrated and then purified on Sephadex LH-20 column using CH_2Cl_2/MeOH (1:1) as eluent to give two major sub-fractions (I and II). PTLC (silica gel, eluent: *n*-hexane/CH_2Cl_2/EtOAc, 8:1:1) separation of sub-fraction I yielded dorsmerunin A (**34**) (6 mg) and dorsmerunin B (**35**) (25 mg). Sub-fraction II was subjected to column chromatography over silica gel eluting with 5% EtOAc in *n*-hexane to provide dorsmerunin C (**36**) (3 mg). The 20% EtOAc in *n*-hexane eluent from the original column was further purified on Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to afford licoagrochalcone A (**38**) (18 mg).

3.3.10 Extraction and isolation of compounds from roots of Streblus usambarensis

The air dried roots of *Streblus usambarensis* (1.5 kg) was crushed into powder and extracted with CH_2Cl_2/MeOH (1:1) by cold percolation. The solvent was removed under reduced pressure to yield 56 g of crude extract. A portion of the extract (50 g) was chromatographed

on a silica gel (450 g) to yield a total of 58 fractions of 500 ml each. The fraction obtained from 1% of EtOAc in *n*-hexane elution was further separated over Sephadex LH-20 column (eluted with CH_2Cl_2/MeOH; 1:1) to yield usambarin A (**39**) (30 mg). The fraction eluted with 3% EtOAc in *n*-hexane was subjected to column chromatography over Sephadex LH-20 eluting with CH_2Cl_2/MeOH, followed by purification by preparative TLC using *n*-hexane/ CH_2Cl_2/EtOAc (2:1:0.5) to yield usambarin (**40**) (15 mg).

3.3.10 Extraction and isolation of compounds from stems of Streblus usambarensis

The dried and crushed stems (2 kg) of *Streblus usambarensis* were extracted with a mixture of CH_2Cl_2/MeOH (1:1) at room temperature. Concentration of the extract under reduced pressure yielded 100 g of extract. Part of this extract (90 g) was subjected to column chromatography over silica gel and the column was eluted with *n*-hexane containing increasing polarities of EtOAc to obtain 64 fractions of 500 ml each. The fraction obtained from 10% *n*-hexane-EtOAc elution was applied over silica gel column and eluted with 6% EtOAc in *n*-hexane followed by Sephadex LH-20 (eluted with CH_2Cl_2/MeOH; 1:1) to yield usambarin C (**41**) (25 mg).

3.4 Biological Assay

3.4.1 Cell cultures of drug sensitive and drug-resistant cancer cell lines

Various models of sensitive cell lines and their resistant counterparts were used. These were drug-sensitive CCRF-CEM leukemia and its multidrug-resistant P-glycoprotein-over-expressing subline CEM/ADR5000 (Efferth *et al.*, 2003; Gillet *et al.*, 2004; Kimmig *et al.*, 1990), MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-*BCRP* clone 23 (Doyle *et al.*, 1998), HCT116 ($p53^{+/+}$), colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$), U87MG glioblastoma cells and its resistant subline U87MG.Δ*EGFR* (Kuete *et al.*, 2013a; Kuete *et al.*, 2013b; Kuete *et al.*, 2013c). To compare tumor with

normal cells, HepG2 hepatocarcinoma cells and AML12 normal hepatocytes were used (Kuete *et al.*, 2013a; Kuete *et al.*, 2013b; Kuete *et al.*, 2013c).

3.4.2. Anticancer Assay against Drug sensitive and Drug-resistant Cancer Cell Lines

The resazurin reduction assay (O'Brien *et al.*, 2000) was performed to assess the cytotoxicity of isolated compounds and doxorubicin as control drug towards various sensitive and drug-resistant cancer cell lines, including the CCRF-CEM and CEM/ADR5000 leukemia, MDA-MB231 breast cancer cells and its resistant subline MDA-MB231/*BCRP*, HCT116$p53^{+/+}$ colon cancer cells and its resistant subline HCT116$p53^{-/-}$, U87MG glioblastoma cells and its resistant subline U87MG.*ΔEGFR* and HepG2 hepatocarcinoma cells and normal AML12 hepatocytes. The details have been described (Kuete *et al.*, 2017). The assay is based on the reduction of the indicator dye, resazurin, to the highly fluorescent resorufin by viable cells. Non-viable cells rapidly lose their metabolic capacity to reduce resazurin and, thus, do not produce fluorescent signals anymore. Briefly, adherent cells were detached by treatment with 0.25 % trypsin/EDTA (Invitrogen, Darmstadt Germany) and an aliquot of 1×10^4 cells was placed in each well of a 96-well cell culture plate (Thermo Scientific, Langenselbold, Germany) in a total volume of 200 µL. Cells were allowed to attach overnight and then were treated with different concentrations of compounds. For suspension cells, aliquots of 2×10^4 cells per well were seeded in 96-well-plates in a total volume of 100 µL. The studied compound was immediately added in varying concentrations in an additional 100 µL of culture medium to obtain a total volume of 200 µL/well. After 72 h, resazurin (Sigma-Aldrich, Schnelldorf, Germany) (20 µL, 0.01% w/v) in distilled H_2O was added to each well and the plates were incubated at 37 °C for 4 h. Fluorescence was measured on an Infinite M2000 ProTM plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was done at least twice with six replicates each. The viability was evaluated based on a comparison with untreated cells. IC_{50}

values represent the compound concentrations required to inhibit 50% of cell proliferation and were calculated from a calibration curve by linear regression using Microsoft Excel.

3.4.3 Anticancer Assay against HEK293 Cell Line

Human Embryonic Kidney cells (HEK293) were maintained in DMEM medium supplemented with 10% FBS. HEK293 cells were exposed to the compounds in TC-treated 384-wells plates (Falcon) as previously described (Fletcher and Avery, 2014). Plates were incubated for 72h at 37 °C, 5% CO_2, and then the media was removed from the wells and replaced with an equal volume of 44 µM resazurin. After an additional 5-6 hours incubation at standard conditions, the total fluorescence (excitation/emission: 530 nm / 595 nm) was measured using an Envision plate reader (PerkinElmer). Raw data was normalized using the in-plate positive and negative controls to obtain normalized % inhibition data, which was then used to calculate IC_{50} values, through a 4 parameter logistic curve fitting in GraphPad Prism v.6. The experiments were carried out in two independent biological replicates, each consisting of two technical replicates.

3.4.4. Mechanistic Studies

3.4.4. 1. Analysis of the cell cycle distribution and detection of apoptotic cells by flow cytometry

Isoflavonoid **3**, flavonoid **4**, and doxorubicin or DMSO (used as solvent control) at various concentrations were used to treat CCRF-CEM cells (1×10^6cells). The cell cycle was then analyzed after 24 h incubation in a humidified 5% CO_2 atmosphere at 37°C as previously described (Mbaveng *et al.*, 2018a; Mbaveng *et al.*, 2018b). The propidium iodide (PI) fluorescence of individual nuclei was measured using BD Accury C6 Flow Cytometer (BD Biosciences, Heidelberg, Germany). Assays were performed with three independent experiments each at least with triplicate parallel measurements.

3.4.4. 2. Assessment of apoptosis by annexin V/PI staining

Compounds **3, 4** and doxorubicin at various concentrations were used to treat CCRF-CEM cells (1×10^6; 1 ml) for 24 h (in humidified 5% CO_2 atmosphere at 37°C), and apoptosis was further assessed using fluorescein isothiocyanate (FITC)-conjugated annexin V/PI assay kit (eBioscience™Annexin V; Invitrogen, San Diego, USA) by flow cytometry under similar experimental conditions as reported earlier (Mbaveng *et al.*, 2018a; Mbaveng *et al.*, 2018b). Cells were analyzed using BD Accury C6 Flow Cytometer (BD Biosciences). Early and late apoptosis or necrosis was evaluated on fluorescence 2 (FL2 for PI) versus fluorescence 1 (FL1 for annexin) plots (Gerwirtz and Elmore, 2005; Samarghandian *et al.*, 2011).

3.4.4. 3. Effects of compounds on caspases activities

Compounds **3** and **4** at various concentrations were used to treat CCRF-CEM cells for 6 h. The activity of caspases was further determined using Caspase-Glo 3/7, Caspase-Glo 8 and Caspase-Glo 9 Assay kits (Promega, Mannheim, Germany) as previously reported (Kuete *et al.*, 2014a).

3.4.4. 4. Evaluation of the integrity of the mitochondrial membrane

Compounds **3** and **4** at various concentrations, as well as a reference mitochondrial gradient dissipation drug, valinomycin (positive control) were used to treat CCRF-CEM cells for 24 h. Their mitochondrial membrane potential (MMP) was then analyzed. The MMP was analyzed using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Biomol, Hamburg, Germany) staining as previously described (Kuete *et al.*, 2013d). Experimental conditions were similar to those reported earlier (Kuete *et al.*, 2013d; Mbaveng *et al.*, 2018a).

3.4.4. 5. Assessment of the production of reactive oxygen species (ROS)

Isoflavonoid **3** and flavonoid **4** at various concentrations, as well as DMSO (solvent control), or hydrogen peroxide (H_2O_2; positive control) were used to treat CCRF-CEM cells for 24 h. The production of ROS was further assessed as previously reported (Bass *et al.*, 1983; Cossarizza *et al.*, 2009; Kuete *et al.*, 2014b). 2´,7´-Dichlorodihydrofluorescein diacetate (H_2DCFH-DA) (Sigma-Aldrich) was used for detection of ROS under similar experimental conditions as reported earlier (Kuete *et al.*, 2016; Mbaveng *et al.*, 2018a).

3.5. Theoretical Calculations

Different conformations and configurations of compound **33** were optimized at the B3LYP/6-311G** (Becke, 1993; Lee *et al.*, 1988) level of theory without any restrictions. The ECD were computed using the Time Dependent DFT (TDDFT) (Autschbach *et al.*, 2002; Bauernschmitt & Ahlrichs, 1996) algorithm in the program package GAUSSIAN 09 (Frisch, 2009). The 6-31G* basis set was applied. 10 singlet and 10 triplet states were solved (keyword TD (NStates=10, 50-50). All GAUSSIAN results were analysed and the spectra display using the SpecDis 1.62 (Bruhn *et al.*, 2013). The molecules are displayed using SYBYL-X 2.1.1 ("SYBYL-X 2.1.1 Tripos a Certara Company, 1699 South Hanley Rd. , St. Louis, MO ", 2013).

CHAPTER FOUR

RESULTS AND DISCUSSION

Selected plants in the Leguminosae (*Ormocarpum kirkii*, *Derris trifoliata*, *Lonchocarpus bussei* and *Lonchocarpus eryocalix*) and Moraceae (*Dorstenia kameruniana* and *Streblus usambarensis*) families were investigated for secondary metabolites. The structure elucidation and cytotoxicity of the isolated compounds and the modes of action of two of these compounds (**3** and **4)** is discussed in this chapter. A total of forty one compounds including nine new compounds were isolated and characterized. These include, three isoflavones **(1**-**3**) and three biflavonoids **(4, 5** and **8**) and a dimeric coumarin **(6**) and an ester of ferullic acid with long alkyl chain **(7**) from *Ormocarpum kirkii*. A new rotenoid derivative, 7a-*O*-methyl-12a-hydroxyelliptonol (**16**) along with eight rotenoids (**10**, **11**, **12**, **13**, **14**, **15, 17** and **18**), a pterocarpan (**9**), isoflavones (**19**-**20**), two flavanones (**21**-**22**) and a chromanone derivative (**23**) from *Derris trifoliata*. A new isoflavone, 4′-prenyloxyvigvexin A (**24**) along with four isoflavones (**25**-**28**), a chalcone (**29**), a geranylated phenylpropanol (**30**) and two pterocarpans (**31**-**32**) were isolated from *Lonchocarpus bussei.* Also isolated was a new pterocarpan, (6a*R*,11a*R*)-4,9-dimethoxybitucarpin B (**33**) along with the known pterocarpan (**32**) from *Lonchocarpus eriocalyx*. Three new benzylbenzofuran derivatives (**34**-**36**) (named dorsmerunin A, B and C respectively), along with known coumarin (**37**) and chalcone (**38**) from *Dorstenia kameruniana* and new naturally unusual naphthobenzofuran derivatives, usambarin A, B and C (**39**-**41**) were isolated from *Streblus usambarensis*. The cytotoxicity of the isolated compounds was tested against various sensitive and their resistant cancer cell lines. In the cytotoxicity assay of compounds isolated from *Ormocarpum kirkii***, 5,7**-dihydroxy-4ˈ-methoxy-6,8-diprenylisoflavone (**2**), Osajin (**3**) and 7,7′′-di-*O*-methylchamaejasmin (**4**) displayed IC_{50} values below 20 µM against both CCRF-CEM and

CEM/ADR5000 cells while osajin (**3**) and 7,7′′-di-*O*-methylchamaejasmin (**4**) had significant cytotoxic effects with IC_{50} values below or around 10 µM against 7 carcinoma cells and a normal AML12 hepatocytes (4/7, 5/7 and 7/7). The compounds isolated from *Ormocarpum kirkii* were also tested against the HEK293 cell line. The isoflavones, 5,7-dihydroxy-4′-methoxy-6,8-diprenylisoflavone (**2**) and osajin (**3**) showed comparable activity against HEK293 with IC_{50} values of 27.1 and 27.3 ± 2.0 respectively. The biflavanones 7,7''-di-*O*-methylchamaejasmin (**4**) and chamaejasmin (**5**) displayed significant activity, with IC_{50} values of 20.8 ± 6.8 µM and 43.5 µM, respectively. The compounds (**9**-**23**) isolated from *Derris trifoliata* were evaluated for their cytotoxicity against HEK293 cells. Rotenone (**10**) exhibited the highest cytotoxic (IC_{50} value of 0.82 ± 0.02 µM) towards HEK293 cells. The rotenoloids 7a-*O*-methyldeguelol (**12**) and 7-a-*O*-methylelliptonol (**18**) showed cytotoxicity towards HEK293 cells (IC_{50} 9.4 ± 0.25 µM and IC_{50} 7.1 ± 0.50 µM, respectively). Isoflavones isolated from *Lonchocarpus* species showed significant cytotoxicity. 3′,6,7-Trimethoxyl-4′,5′-methylenedioxyisoflavone (**27**) and durmillone (**28**) were cytotoxic against leukemia CCRF-CEM cells; while 4-hydroxylonchocarpin (**29**) and durmillone (**28**) showed significant antiproliferative effects against its resistant counterpart CEM/ADR5000 cells with IC_{50} values below 20 µM. Durmillone (**28**) showed IC_{50} values below 10 µM against the resistant breast adenocarcinoma MDA-MB231/*BCRP* cells and resistant gliobastoma U87MG.*ΔEGFR* cells. The compounds isolated from *Dorstenia kameruniana* **34**-**38** displayed cytotoxicity against the sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cells, where bergapten (**37**) and licoagrochalcone A (**38**) had the highest activities (IC_{50} values of 7.17 µM and 5.16 µM, respectively) against CCRF-CEM leukemia cells. Licoagrochalcone A (**38**) also showed cytotoxicity against 7 sensitive or drug-resistant solid tumor cell lines (breast carcinoma, colon carcinoma, glioblastoma) with IC_{50} below 50 µM, whilst bergapten (**37**) showed selective activity. The new naphthobenzofuran

derivatives characterized from *Streblus usambarensis* **39-41** displayed cytotoxic effects in both sensitive CCRF-CEM and resistant CEM/ADR5000 leukemia cells with an IC_{50} values below 25 µM. Besides, Usambarin B (**40)** had significant effects towards CEM/ADR5000 leukemia cells with an IC_{50} value of 6.13 µM. Usambarin B (**40)** and Usambarin C (**41**) had cytotoxic effect in the 7 tested carcinoma cell lines with IC_{50} values below 63 µM. The study was extended to analyse the modes of action of osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**). The result showed that Osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) caused cell cycle arrest in G0/G1 phase as well as apoptosis with significant increase of cells in sub-G0/G1 phase. The activity of caspases in CCRF-CEM cells showed that the two compounds did not increase the activity of caspases 3/7, 8 and 9. These compounds (**3, 4)** induced apoptosis in CCRF-CEM cells mediated by MMP alteration and increased ROS production.

4.1. Compounds Isolated from *Ormocarpum kirkii*

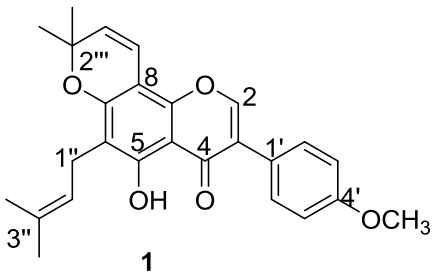
Chromatographic separation of the extract of the stem bark of *Ormocarpum kirkii* led to the isolation of four isoflavones [osajin-4′-methyl ether (**1**), 5,7-dihydroxy-4′-methoxy-6,8-diprenylisoflavone (**2**) and osajin (**3**)]; two biflavonoids [7,7′′-di-*O*-methylchamaejasmin (**4**) and chamaejasmin (**5**)]; a dimeric chromene [diphysin (**6**)**]** and an ester of ferullic acid with long alkyl chain [erythrinasinate (**7**)]. Compounds **3**, **4**, **5**, **6** and **7** along with campylospermone A (**8**) were also isolated from the roots of this plant**.**

4.1.1. Osajin-4'-methyl ether (1)

Compound **1** was isolated as yellow crystals. HRESIMS (*m/z* = 418.1764, $[M]^+$) and NMR data (Table 4) allowed the assignment of the molecular formula $C_{26}H_{26}O_5$. In the ^1H NMR spectrum, the up-field singlet at δ_H 8.28, corresponding to H-2 of ring C, is characteristic of isoflavones. The ^{13}C NMR spectrum (Table 4) showed 26 carbon signals, including a carbonyl carbon (δ_C 180.9), four oxygenated carbons and a de-shielded carbon signal (δ_C

153.5) corresponding to C-2. In the proton NMR spectrum, the presence of a pair of mutually coupled proton signals at δ_H 3.35 (2H, *d*, *J* = 7.4 Hz) and δ_H 5.25 (1H, *m*); and signals for two methyl groups at δ_H 1.83 (s) and δ_H 1.68 (s) showed the presence of a prenyl group. Additionally, the ^{1}H NMR spectrum showed two signals at δ_H 6.76 (*d*, *J* = 9.9 Hz, 1H, H-4''') and δ_H 5.78 (*d*, *J* = 10.0, 1H, H-3''') and two methyl signals at δ_H 1.52 clearly indicating the presence of 2''',2'''-dimethylchromene ring. The attachment of both groups in ring A was established from the HMBC spectrum (Table 4). Thus, the proton signal at δ_H 3.35 showed HMBC correlation with the oxygenated carbon δ_C 156.9 (C-7), showing the attachment of the prenyl group is at C-6 (δ_C 112.2). The two proton signals of the chromene ring showed HMBC correlation with C-8 (δ_C 100.7) and C-8a (δ_C 150.4), showing that this group is attached at C-7/C-8 of ring A.

In ring B, the ^{1}H NMR spectrum showed an AA'XX' spin system at δ_H 7.58 (for H-2'/6') and δ_H 7.03 (for H-3'/5') with methoxy group (δ_H 3.86 and δ_C 54.7) attached on C-4' (δ_H 159.8). Therefore, this compound was identified as osajin-4'-methyl ether, previously reported from *Derris scandens* and *Hedysarem scoparium* (Babu *et al.*, 2010; Chen *et al.*, 2007).



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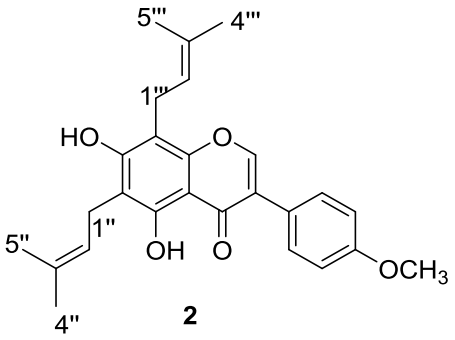
*4.1.2. 5,7-Dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**)*

Compound **2** (UV λ_{max} 270 nm) was isolated as yellow crystals with a melting point of 165─166 ^{0}C. The HREIMS showed a molecular ion peak *m/z* 420.1935 which suggested with the molecular formula $C_{26}H_{28}O_5$. In agreement with this, the ^{13}C NMR (Table 3) of compound **2** showed 26 carbon signals, including two prenyl and a methoxy group on an

isoflavone skeleton (Table 3). The NMR spectral data (Table 3) of **2** is similar to **1,** except that there are two prenyl groups (Table 3) in ring A at C-7 (δ_C 105.9) and C-8 (δ_C 111.3). The placement of the prenyl groups at C-6 and C-8 was confirmed from the HMBC spectrum which showed correlation of CH_2-1" and CH_2-1"' with C-6 (δ_C 105.9) and C-8 (δ_C 111.3), respectively. As in compound **1**, the ^{1}H NMR displayed an AA'XX' spin system with a methoxy group (δ_H 3.87, δ_C 54.7) placed at C-4'. Thus, compound **2** was identified as 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone, previously isolated from the roots of *Hedysarem scoparium* (Chen, *et al.*, 2007). This appears to be the first report of the isolation of this compound from the genus *Ormocarpum*.



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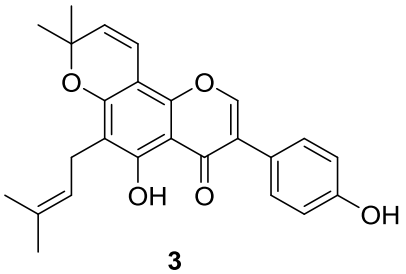

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Table 3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data along with HMBC correlations for compound **2** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC
2	8.28 (*s*)	153.3	C-3, C-4, C-8a
3		122.4	
4		181.1	
5		157.6	
5a		105.3	
6		105.9	
7		158.9	
8		111.3	
8a		153.5	
1'		123.5	
2'/6'	7.51 (*d*, *J* = 8.8 Hz)	130.2	C-3, C-3'/5', C-4'
3'/5'	7.01 (*d*, *J* = 8.8 Hz)	113.5	C-1', C-4'
4'		159.7	
1"	3.54 (*d*, *J* = 7.0 Hz)	21.4	C-6, C-7, C-2", C-3", C-

1'''	3.46 (*d, J* = 7.0 Hz)	21.4	4''/5''-CH_3, C-4'''/5'''CH_3, C-2''/2''', C-3''', C-7, C-8
2''/2'''	5.23 (*m*)	122.0	C-4''/5''CH_3, C-4'''/5'''CH_3, C-1''
3''		131.6	
3'''		131.8	
4''/4'''CH_3	1.85	17.1	C-2''/2''', C--4''/4''',
5''/5'''CH_3	1.68 (*s*)	25.0	C-2''/2''', C-5''/5''',
4'OCH_3	3.87 (*s*)	54.7	C-4'
5-OH	13.34 (*s*)		C-5, C-5a, C-6

*4.1.3. Osajin (**3**)*

Osajin (**3)** was isolated as yellow crystals, mpt. 170─171 ^{0}C. The molecular formula ($C_{25}H_{24}O_5$) was established based on NMR data (Table 4) and HR-EI-MS analysis (M^+ at *m/z* 404.1637). The UV (λ_{max} 285 nm) and NMR spectra (δ_H 8.30, s, for H-2; δ_C 153.5 for C-2) are consistent with this compound being an isoflavone derivative. Compound **3** is similar to **1**, with the only difference being that the methoxy group in compound **1** is replaced by hydroxy substituent at C-4' in ring B. Thus the ^{1}H NMR spectrum (Table 4) showed once again an AA'XX' spin system at δ_H 7.49 (*d, J* = 8.0 Hz, H-2'/6') and δ_H 6.93 (*d, J* = 8.7 Hz, H-3'/5'); these protons showed HMBC correlation with the oxygenated carbon peak at δ_C 157.5 (C-4'). In ring A, the placement of the prenyl and chromene groups was established based on HMBC correlations of δ_H 3.44 (*d, J* = 7.4 Hz, H-1'') with C-5 (δ_C 154.9) and C-6 (δ_C 107.2); Correlation of the olefinic proton in the chromene ring, δ_H 6.72 (*d, J* = 10.0 Hz, H-4''') with C-7 (δ_C 156.5) and C-8 (δ_C 105.6) and δ_H 5.80 (*d, J* = 10.0 Hz, H-3''') with C-8 established that the prenyl group is located at C-6 and the chromene group is fused to C-7 and C-8. Based on the above data compound **3** was characterized as osajin, previously reported from *Maclura pomofera* (Ribaudo *et al.*, 2017). This is the first report of the occurrence of osajin (**3**) in the genus *Ormocarpum*.

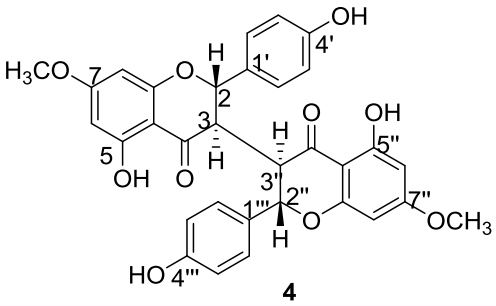
Table 4: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data along with HMBC correlations for compounds **1** and **3** (acetone-d_6)

Carbon No.	**1**			**3**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	8.28 (*s*)	153.5	C-2, C-4, C-8a	8.30 (*s*)	153..5	C-3, C-4, C-8
3		122.9			122.9	
4		180.9			181.2	
5		159.2			154.9	
5a		105.3			105.0	
6		112.2			107.2	
7		156.9			156.5	
8		100.7			105.6	
8a		150.4			154.6	
1'		123.2			121.9	
2'/6'	7.58 (*d, J* = 8.5 Hz)	130.2	C-3, C-3'/5', C-4'	7.49 (*d, J* = 8.0 Hz)	130.2	C-3, C-3'/5', C-4'
3'/5'	7.03 (*d, J* = 8.8 Hz)	113.6	C-1', C-4'	6.93 (*d, J* = 8.7 Hz)	115.1	C-1', C-4'
4'		159.8			157.5	
1"	3.35 (*d, J* = 7.4 Hz)	20.9	C-2", C-3", C-7	3.44 (*d, J* = 7.4 Hz)	20.9	C-6, C-3'', C-2'', C-5
2"	5.25 (*m*)	122.0	C-3"CH_3, C-1"	5.23 (*m*)	121.9	C-1'', C-4''/5''CH_3
3"		130.8			131.2	
2"'		77.9			77.8	
3"'	5.75 (*d, J* = 9.9 Hz)	128.3	C-8, C-8a, 2"' CH_3, C-2 "'	5.80 (*d, J* = 10.0 Hz)	128.3	C-2''' CH_3, C-2''', C-8
4"'	6.76 (*d, J* = 9.9 Hz)	115.3	C-8a, 2"' CH_3, C-2"'	6.72 (*d, J* = 10.0 Hz)	115.3	C-2''' CH_3, C-2''', C-7, C-8,
2"' $(CH_3)_2$	1.52 (*s*)	27.3	2"' CH_3, C-2"', C-3"'	1.5 (*s*)	27.4	C-2''' CH_3, C-2''', C-3'''
4" CH_3	1.83 (*s*)	17.1	C-2", C-3", C-4",	1.85 (*s*)	17.1	C-2'', C-3'', C-4'' CH_3,
5" CH_3	1.68 (*s*)	25.0	C-2", C-3", C-5",	1.69 (*s*)	25.0	C-2'', C-3'', C-5'' CH_3,
4'OCH_3	3.86 (*s*)	54.7	C-4'			
5-OH	13.46 (*s*)		C-5, C-5a	13.43 (*s*)		C-5, C-5a

*4.1.4. 7,7''-Di-O-methylchamaejasmin (**4**)*

Compound **4** was obtained as a colourless solid, mpt. 209─210 °C, $[\alpha]_D^{21}$ +198.7° (*c* 0.17, acetone). HREIMS showed molecular ion peak *m/z* 570.1518, which together with the NMR data (Table 5) is consistent with the molecular formula $C_{32}H_{26}O_{10}$. The ^{13}C NMR spectrum showed only 14 carbon signals indicating that the molecule is a symmetrical dimer. In agreement with this, the ^{1}H NMR spectrum showed signals at δ_H 5.90 (*d, J* = 11.9 Hz, H-2) and 3.00 (*d, J* = 11.8 Hz, H-3), typical of a 3,3''-biflavanone moiety (Dhooghe *et al.*, 2010; Nyandat *et al.*, 1990; Xu *et al.*, 2012). Furthermore the ^{1}H NMR spectrum showed two *meta*-oriented aromatic protons in ring A at δ_H 6.06 (*d, J* = 2.3 Hz) and 5.99 (*d, J* = 2.3 Hz) assigned to H-6 and H-8, respectively with the biogenetically expected oxygenation at C-5 (δ_C 164.1) and C-7 (δ_C 168.2). The substituent at C-7 was established to be a methoxy (δ_H 3.85) from the HMBC correlation of the methoxy protons and H-8 (δ_H 5.99) with C-7 (δ_C 168.2). In ring B, the ^{1}H NMR spectrum showed an AA'XX' spin system at δ_H 7.05 (*d, J* = 8.6 Hz) assigned to H-2'/6' whereas δ_H 6.88 (*d, J* = 8.6 Hz) to H-3'/5' is consistent with 4'-oxygenation (OH). In agreement with this, both sets of proton signals showed HMBC correlation with C-4' (δ_C 158.3).

The *trans* relationship of H-2/H-3 was suggested from large coupling constant of proton signals δ_H 5.90 (*d, J* = 11.9 Hz, H-2) and 3.00 (*d, J* = 11.8 Hz, H-3) suggestive of a 2*S**,3*R** relative configuration. The ECD spectrum (Fig 4) showed a positive and then negative Cotton effects at 310 nm and 285 nm, for $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions, respectively consistent with (2*S*,3*R*) absolute configuration (Dhooghe *et al.*, 2010; Xu *et al.*, 2012). Therefore the structure of compound **4** was characterized as (2*S*,3*R*)-7,7''-di-*O*-methylchamaejasmin, previously isolated from the aerial part of *Ormocarpum trichocarpum* (Chukwujekwu *et al.*, 2012), but has not been reported from any other source.



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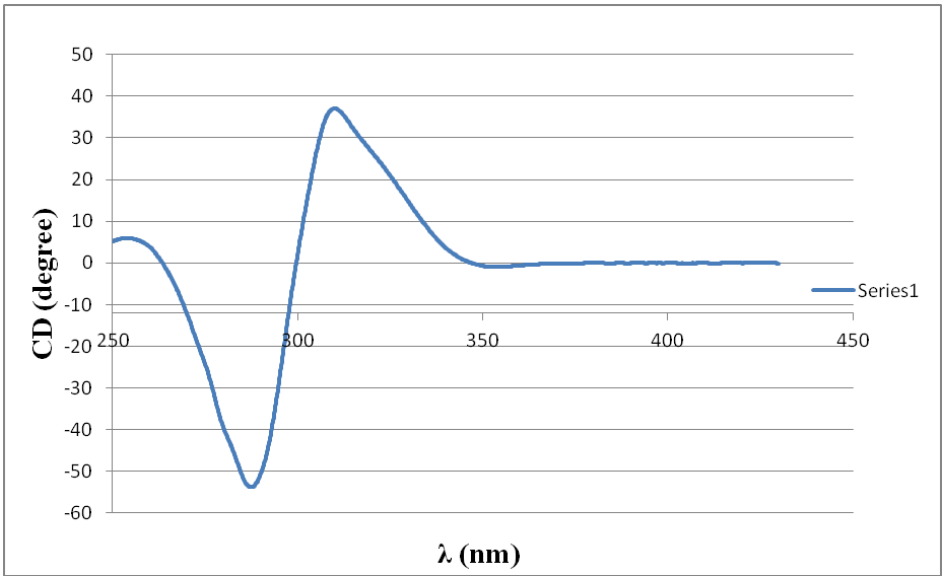
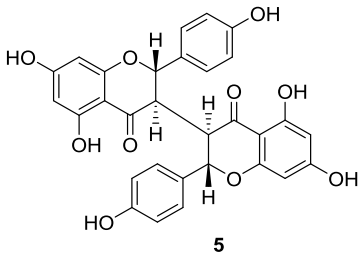


Figure 4: ECD spectrum of 7,7''-di-*O*-methylchamaejasmin (**4**)

*4.1.5. Chamaejasmin (**5**)*

Compound **5** was obtained as a colourless amorphous solid, mpt. 210-212 °C, $[\alpha]_D^{21}$ +138.9° (*c* 0.15, acetone). HR-EI-MS spectra showed the molecular ion peak at *m/z* 542.1215 $[M]^+$ corresponding to the molecular formula $C_{30}H_{22}O_{10}$. As in compound **5**, the ^{1}H NMR data displayed symmetrical flavanone resonance, the characteristic signals for 3,3'-biflavanone moiety was evident from the ^{1}H NMR signals at δ_H 5.87 (H-2) and 2.96 (H-3) with the corresponding (from HSQC spectrum) ^{13}C NMR peaks at δ_C 83.6 (C-2) and 49.6 (C-3). Two *meta* coupling aromatic protons δ_H 5.98 (*d*, *J* = 2.2 Hz) and 5.90 (*d*, *J* = 2.2 Hz) were assigned to H-6 and H-8, respectively of ring A with the biogenetically 5,7-dihydroxy substitution. This was confirmed by HMBC correlation of H-6 (δ_H 5.98) with C-5 (δ_C 164.4)

and C-7 (δ_C 166.6); H-8 correlated with C-7. In ring B, as in compound **5**, the ^{1}H NMR spectrum showed an AA'XX' spin system at δ_H 7.04 (*d*, *J* = 8.5 Hz) and at 6.88 (*d*, *J* = 8.6 Hz), assigned to H-2'/6' and H-3'/5' with 4'-hydroxy group. The large coupling constant of the proton signals at δ_H 5.87 (*d*, *J* = 11.9 Hz, H-2) and 2.96 (*d*, *J* = 11.8 Hz, H-3) and the ECD spectrum (Figure 5) which showed a negative Cotton effect at 285 nm and positive Cotton effect at 310 nm are consistent with (2*S*,3*R*) absolute configuration as in compound **4**. Therefore, compound **5** was identified as chamaejasmin (**5**), previously isolated from *Ormocarpum kirkii* and *Campylospermum mannii* (Dhooghe *et al.*, 2010; Elo Manga *et al.*, 2009; Nyandat, *et al.*, 1990).



5

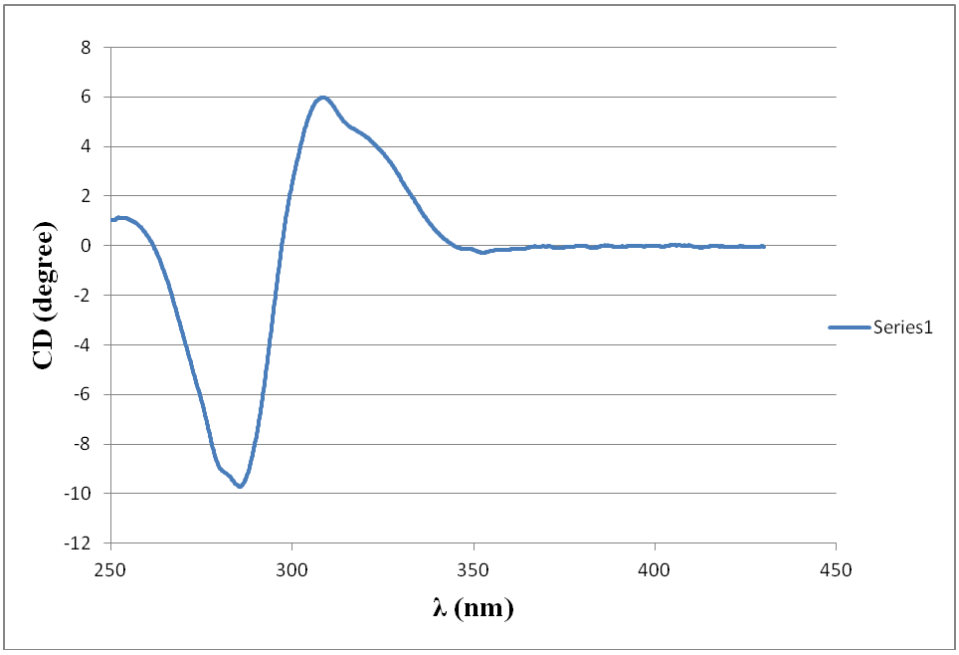


Figure 5: ECD spectrum of chamaejasmin (**5**)

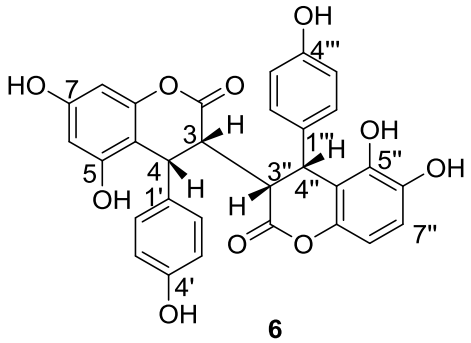
Table 5: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data together with HMBC correlations for **4** and **5** (acetone-d_6)

Carbon No.	**4**			**5**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	5.90 (*d*, *J* = 11.9 Hz)	83.7	C-3, C-2'/6', C-4	5.87	83.6	C-4, C-1'
3	3.00 (*d*, *J* = 11.8 Hz)	49.7	C-2, C-5a, C-1', C-4	2.96 (*m*)	49.6	C-2, C-4, C-1'
4		197.2			196.9	
5		164.1			164.4	
5a		102.8			102.3	
6	6.06 (*d*, *J* = 2.3 Hz)	94.8	C-4, C-5, C-5a, C-7, C-8	5.98 (*d*, *J* = 2.2 Hz)	96.1	C-5, C-5a, C-7, C-8
7		168.2			166.6	
8	5.99 (*d*, *J* = 2.3 Hz)	93.7	C-4, C-5a, C-6, C-7, C-8a	5.90 (*d*, *J* = 2.2 Hz)	94.9	C-4, C-5a, C-6, C-7, C-8a
8a		162.9			166.6	
1'		127.4			127.5	
2'/6'	7.05 (*d*, *J* = 8.6 Hz)	129.4	C-2, C-1', C-3'/5', C-4'	7.04 (*d*, *J* = 8.5 Hz)	129.4	C-2, C-3'/5', C-4'
3'/5'	6.88 (*d*, *J* = 8.6 Hz)	115.4	C-1', C-4'	6.88 (*d*, *J* = 8.6 Hz)	115.4	C-2'/6', C-4'
4'		158.3			158.3	
7-OCH_3	3.85 (*s*)	55.4	C-7			
5-OH	11.89 (*s*)		C-4, C-5, C-5a, C-6, C-7	11.94 (*s*)		C-5, C-5a, C-6

The NMR assignment is for one half of the symmetrical dimer

*4.1.6. Diphysin (**6**)*

Compound **6** was isolated as a white amorphous solid with melting point in the range of 325-326 °C; $[\alpha]_D^{21}$ −183.0° (*c* 0.125, acetone). HREIMS of **7** showed $[M]^+$ peak at *m/z* 542.1226 corresponding to the molecular formula $C_{30}H_{22}O_{10}$. The ESIMS fragment ion at *m/z* 271 as well as the NMR data (Table 6) account for one half of this dimer. An ester carbonyl δ_C 168.9 together with the ^{1}H NMR signals at δ_H 3.04 (*dd, J* = 3.49, 1.19 Hz) and δ_H 4.85 (*dd, J* = 3.2, 2.1Hz) assigned to H-3 and H-4 is typical of 4-aryldihydrocoumarin (Stermitz *et al.*, 1993). This was further confirmed by HMBC correlation of H-3 and H-4 with C-1' (δ_C 129.4). The AA'XX' spin system in the ^{1}H NMR spectrum at δ_H 7.21 (*d, J* = 8.7 Hz) and 6.78 (*d, J* = 8.7 Hz) were assigned to H-2'/6' and H-3'/5' of a 1,4-disubstituted benzene ring i.e. ring B. Two *meta* coupled aromatic protons at δ_H 6.15 (*d, J* = 2.3 Hz) and 6.18 (*d, J* = 2.3 Hz) of ring A correlating with the carbon signal at δ_C 95.1 (C-6) and δ_C 98.6 (C-8) in the HSQC spectrum, are characteristic of protons *ortho* to two oxygenated carbon atoms. Additionally, H-6 and H-8 showed HMBC correlation with C-5 (δ_C 152.4) and C-7 (δ_C 158.0) which confirmed oxygenation (hydroxy groups) at C-5 and C-7. The small coupling constants between H-3 (δ_H 3.04, *dd, J* = 3.49, 1.19 Hz) and H-4 (4.85, *dd, J* = 3.2, 2.1Hz) indicated the *cis* relative configuration at C-3/C-4 junction of the two units, similar to that previously reported for 5,5"-di-*O*-methyldiphysin and 3"-epydiphysin (Dhooghe *et al.*, 2010; Xu, *et al.*, 2012). The levorotatory nature of this compound ($[\alpha]_D^{21}$ −183.0°) is consistent with (3*S*,3"*S*,4*R*,4"*R*) absolute configuration (Chukwujekwu *et al.*, 2012; Xu *et al.*, 2012). Therefore, this compound was characterized as diphysin, first reported from *Diphysa robinoides* (Stermitz *et al.*, 1993) and later from *Ormocarpum trichocarpum* and *Ormocarpum kirkii* (Chukwujekwu *et al.*, 2012; Xu *et al.*, 2012).



6

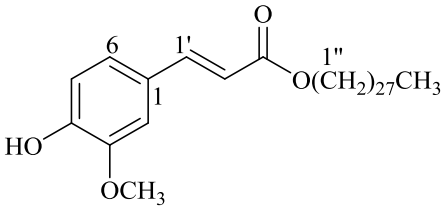
Table 6: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data together with HMBC correlations of **6** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC
2/2''		168.9	
3/3''	3.04 (*dd*, *J* = 3.5, 1.2 Hz)	35.9	C-2, C-3, C-1'
4/4''	4.85 (*dd*, *J* = 3.2, 2.1 Hz)	42.9	C-2, C-4, C-5, C-5a, C-8a, C-1'
5/5''		152.4	
5a/5a''		106.9	
6/6''	6.15 (*d*, *J* = 2.3 Hz)	95.1	C-5, C-5a, C-7, C-8
7/7''		158.0	
8/8''	6.18 (*d*, *J* = 2.3 Hz)	98.6	C-5a, C-6, C-7, C-8a
8a/8a''		154.6	
1'/1'''		129.4	
2'/6'/2'''/6'''	7.21 (*d*, *J* = 8.7 Hz)	129.2	C-1, C-3, C-3'/5', C-4'
3'/5'/3'''/5'''	6.78 (*d*, *J* = 8.7 Hz)	115.4	C-2'/6', C-4'

4.1.7. Erithrinasinate (7)

Compound **7** was isolated as a white solid, m.pt. 94-95 °C. The UV (λ_{max} 295 and 321 nm) and NMR data (Table 7) suggested the aromatic nature of **7**. The presence of three mutually coupled aromatic proton signals in the ^{1}H NMR spectrum at δ_H 7.12 (*m*, 2H, H-5/6) and 6.94 (*d*, *J* = 7.9 Hz, H-2) suggested compound **7** is a 1,3,4-trisubstituted benzene. A singlet integrating for three protons at δ_H 3.97 (*s*) and signals for quaternary carbons at δ_C 146.9 (C-3) and 147.9 (C-4) was indicative of methoxy and hydroxy substituents at C-3 and C-4. Additionally, two *trans*-oriented olefinic protons at δ_H 7.63 (*d*, *J* = 15.9 Hz, H-1') and 6.35 (*d*, *J* = 15.9 Hz, 1H, H-2') which showed HMBC correlation with the ester carbonyl (δ_C 167.0) together with multiplet (δ_H 1.20-1.50) and terminal methyl protons at δ_H 0.92 (*t*, *J* =

7.0 Hz) established the presence of a long alkenyl ester substituent at C-1 (δ_C 127.0). The length of the side chain was determined from the HREIMS which showed a molecular ion peak at *m/z* 586.4970. Hence, based on the above spectroscopic data, compound **7** was characterized as *erithrinasinate*. This is the first report of this compound from the genus *Ormocarpum*.



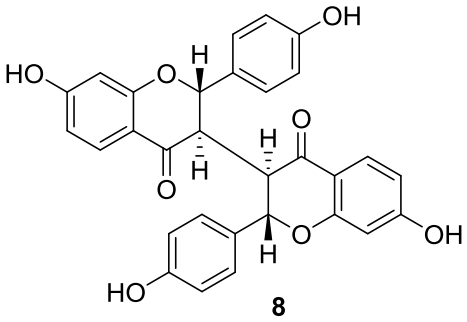
7

Table 7: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data together with HMBC correlations in compound **7** (CD_2Cl_2)

Carbon No.	δ_H	δ_C	HMBC
1		127.0	
2	6.94 (*d*, *J* = 7.9 Hz)	114.4	C-1, C-3, C-6
3		146.9	
4		147.9	
5	7.12 (*d*, *J* = 7.8 Hz)	122.9	C-4, C-6
6	7,12 (*d*, *J* = 7.8 Hz)	109.3	C-4, C-5, C-1'
1'	7.63 (*d*, *J* = 15.9 Hz)	144.2	C-1, C-5, C-6, C-2', C-3'
2'	6.35 (*d*, *J* = 15.9 Hz)	115.7	C-1, C-1', C-3'
3'		167.0	
1"	4.20 (app *t*, *J* = 6.7, 6.7 Hz)	64.4	C-3', C-2"
2"	1.73 (*m*)	26.01	C-3', C-3"
(3"-26")	1.20-1.50 (*m*)	22.7-35.9	
CH_3	0.92 (*t*, *J* = 7.0 Hz)	13.9	
3-OCH_3	3.97 (*s*)	55.9	C-3

*4.1.8. Campylospermone A (**8**)*

HR-EI-MS analysis of compound **8,** ($[\alpha]_D^{21}$ −182.3 °C (*c* 0.145, acetone), mpt. 300-302 °C), showed a molecular ion peak at *m/z* 510.1320 which is in agreement with the molecular formula $C_{30}H_{22}O_8$. As in compounds **4**, **5** and **6**, the ^{1}H and ^{13}C NMR spectra (Table 8) showed one half of a flavanone dimer. For each part of the molecule the ^{1}H NMR showed two proton signals at δ_H 6.00 (d, *J* = 12.0 Hz, 1H, H-2) and 2.75 (d, *J* = 11.8 Hz, 1H, H-3) with large coupling proton, characteristic of a 3-substituted 2,3-*trans*-flavanone (Dhooghe *et al.*, 2010; Xu *et al.*, 2012). The ring A aromatic proton signals with an AXY spin system, at δ_H 7.74 (d, *J* = 8.7 Hz), δ_H 6.60 (*dd*, *J* = 8.7, 2.3 Hz) and δ_H 6.34 (d, *J* = 2.3 Hz) was assigned to H-5, H-6 and H-8, respectively. The nature of ring A which is substituted with hydroxyl at C-7 was confirmed by HMBC correlation of H-6 (δ_H 6.60) with C-5a (δ_C 114.3) C-7 (δ_C 164.4) and C-8 (δ_C 102.5). In ring B, an AA'XX' spin system at δ_H 7.04 (d, *J* = 8.6 Hz, 2H, H-2′/6′) and δ_H 6.88 (d, *J* = 8.6 Hz, 2H, H-3′/5′) is consistent with oxygenation (hydroxyl group) at C-4' (δ_C 158.1). The large coupling constant (*J* = 12.0 Hz) between H-2 (δ_H 6.00) and H-3 (δ_H 2.75) indicated a *trans* relative configuration at the C-2/C-3 stereogenic centres. The ECD spectrum which showed a positive Cotton effect at 310 nm and a negative one at 290 nm (Fig. 6) was consistent with (2*S*,3*R*) absolute configuration. Thus, based on the above data and comparison with literature, this 3,3'-biflavanone was identified as campylospermone A (**8**), previously reported from *Campylospermum mannii* (Elo Manga *et al.*, 2009). However, campylospermone A (**8**) is being reported for the first time from the genus *Ormocarpum*.



8

Table 8: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data together with HMBC correlations in compound **8** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC
2	6.00 (*d*, *J* = 12.0 Hz)	84.1	C2'/6', C-8a, C-4
3	2.75 (*d*, *J* = 11.8 Hz)	50.9	C-2, C-1', C-4
4		190.7	
5	7.74 (*d*, *J* = 8.7 Hz)	129.2	C-8, C-6, C-8a, C-4
5a		114.3	
6	6.60 (*d*, *J* = 8.7, 2.3 Hz)	110.4	C-8, C-5a, C-7
7		164.4	
8	6.34 (*d*, *J* = 2.3 Hz)	102.5	C-5a, C-6, C-8a, C-4
8a		163.3	
1'		128.3	
2'/6'	7.04 (*d*, *J* = 8.6 Hz)	129.0	C-2, C-3'/5', C-4'
3'/5'	6.88 (*d*, *J* = 8.6 Hz)	115.3	C-2'/6', C-4'
4'		158.1	

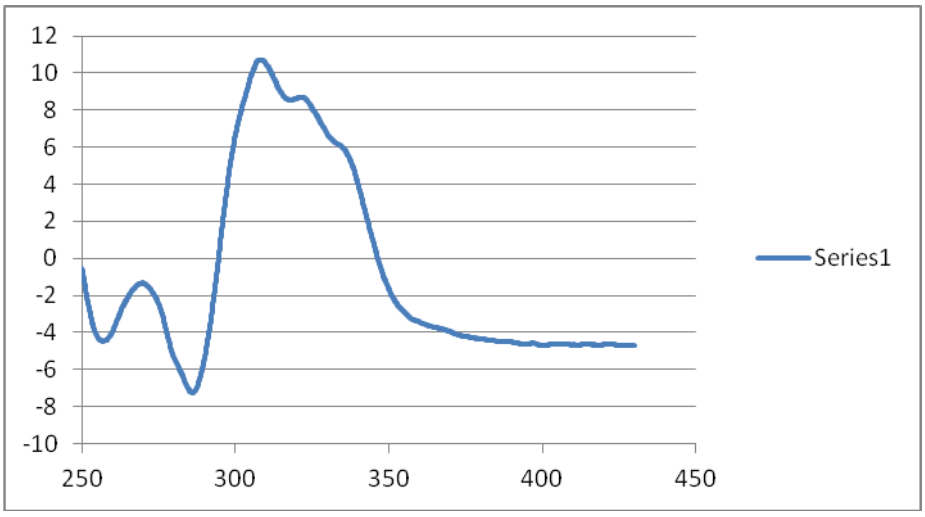


Figure 6: ECD spectrum of campylospermone A (**8**)

4.2. Compounds Isolated from *Derris trifoliata*

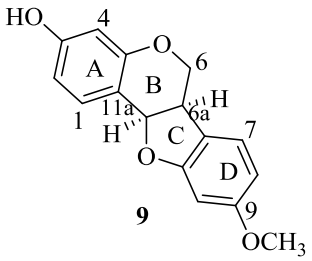
Chromatographic separation of the extract from the roots and stem of *Derris trifoliata* resulted in the isolation of 15 compounds. Isomedicarpin (**9**), rotenone (**10**), 7a-*O*-methyldeguelol (**12**), α-toxicarol (**13)**, 12a-hydroxyelliptonol (**15**), 7a-*O*-methyl-12a-hydroxyelliptonol (**16**), 7a-*O*-methylelliptonol (**18**), prunetin (**19**) and barbigerone (**20**) were isolated from the roots. Among these, 7a-*O*-methyl-12a-hydroxyelliptonol (**16**) is a new compound. A similar investigation of the stem afforded seven known compounds; namely rotenone (**10**), deguelin (**11**), tephrosin (**14**), elliptone (**17**), lupinifolin (**21**), dereticulatin (**22**) and 6,7-dimethoxy-4-chromanone (**23**). The characterization of these compounds is discussed below.

*4.2.1. Medicarpin (**9**)*

Compound **9** was obtained as a brown paste. Its molecular formula was determined as $C_{16}H_{14}O_4$ by LCMS analysis ($[M+H]^+$ *m/z* 271.7) and NMR spectra (Table 9). The ^{1}H NMR spectrum showed signals at δ_H 5.57 (*d*, 7.3 Hz, H-11a), 3.70 (*dd*, *J* = 11.1, 9.4 Hz, H-6α), 4.26 (*dd*, 10.9, 4.7 Hz, H-6β) and 3.64 (*ddd*, *J* = 9.4, 7.3, 4.7 Hz, H-6a) indicating a pterocarpan skeleton. An AXY spin system of proton signals at δ_H 7.34 (*d*, *J* = 7.8 Hz), 6.35 (*d*, 2.4 Hz) and 6.54 (*dd*, *J* = 8.4, 2.4 Hz) was assigned to the protons in ring A at C-1 (δ_C 132.8), C-2 (δ_C 103.5) and C-4 (δ_C 110.1), respectively. The nature of ring A was confirmed from HMBC correlation of H-1, H-2 and H-4 with C-3 (δ_C 158.9). Additional proton signals in the aromatic region with AXY spin system at δ_H 7.22 (*d*, *J* = 7.8 Hz), 6.42 (*d*, *J* = 2.3 Hz) and 6.49 (*dd*, *J* = 8.2, 2.3 Hz) were assigned for protons at C-7 (δ_C 125.5), C-8 (δ_C 96.8) and C-10 (δ_C 106.6) in ring D. In addition to these, a sharp singlet at δ_H 3.76 with corresponding carbon peak (from HSQC spectrum) at δ_C 55.6 showing an HMBC correlation with C-9 (δ_C 161.3) was assigned to the methoxy substituent at C-9 in ring B. The HMBC correlation of the methoxy protons, H-7, H-8 and H-10 with C-9 (δ_C 161.3) confirmed the placement of

methoxy group at C-9. The magnitude of the coupling constant between H-6a and H-11a (*J* = 7.3 Hz), is consistent with cis fusion at the B/C ring junction. The high negative specific rotation, $[\alpha]_D^{21}$ −196° (*c* 0.25, acetone), suggested (6a*R*,11a*R*) absolute configuration for compound **9** (Yenesew *et al.*, 1998c).

On the basis of the above spectroscopic data and comparison with literature, this compound was identified as (6a*R*,11a*R*)-3-hydroxy-9-methoxypterocarpan trival name medicarpin. This compound has been reported from *Millettia leptobotrya* (Zhi Na, 2013) and other plant in the family Leguminosae, however this is the first report from the genus *Derris*.



9

Table 9:^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compound **9** (CD_3CN)

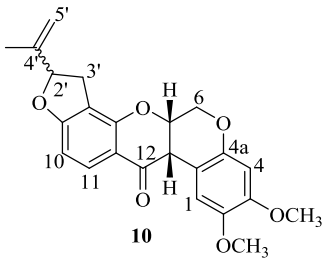
Carbon No.	δ_H	δ_C	HMBC
1	7.34 (*d*, *J* = 7.8 Hz)	132.8	C-1a, C-10, C-7a, C-9
1a		105.5	C-6a, C-6, C-5a, C-1, C-5, C-12
2	6.35 (*d*, *J* =2.4 Hz)	103.5	C-8, C-11a, C-9
3		158.9	
4	6.54 (*dd*, *J* = 8.4, 2.4 Hz)	110.1	C-10, C-11a, C-7a, C-9
4a		157.3	
6	3.70 (*dd*, *J* = 11.1, 9.4 Hz)	66.8	C-6a, C-1a, C-5a, C-7a
	4.26 (*dd*, 10.9, 4.7 Hz)		
6a	3.64 (*ddd*, *J* = 9.4, 7.3, 4.7 Hz)	39.9	C-6, C-5a, C-2a

7	7.22 (*d*, *J* =7.8 Hz)	125.5	C-6a, C-4, C-3
7a		120.1	
8	6.49 (*dd*, *J* = 7.8, 2.3 Hz)	96.8	C-2, C-5a, C-3
9	6.42 (*d*, *J* =2.3 Hz)	161.3	
10		106.6	C-4, C-5a, C-3
10a		161.6	
11a	5.57 (*d*, *J* = 7.3 Hz)	79.0	C-6a, C-6, C-11a, C-11, C-7a
9-OCH_3	3.76 (*s*)	55.6	C-3

*4.2.2. Rotenone (**10**)*

Compound **10** was isolated as a white amorphous solid. The molecular formula $C_{23}H_{22}O_6$ was established from LCMS ($[M+H]^+$ *m/z* 395.3) and NMR data (Table 10). The ^{1}H NMR [at δ_H 4.55 (*dd*, *J* = 12.3, 3.0 Hz, H-6), 4.22 (*dd*, *J* = 12.4, 1.0 Hz, H-6), 5.04 (*ddd*, *J* = 4.0, 3.0, 1.1 Hz, H-6a) and 3.97 (*m*, H-12a)] and the ^{13}C NMR [at δ_C 66.6 (C-6), 72.7 (C-6a) and 44.7 (12a)] are typical of a rotenoid skeleton. In the aromatic region of the ^{1}H NMR spectrum, a pair of *ortho*-coupled signals at δ_H 6.54 (*d*, *J* = 8.5 Hz) and 7.80 (*d*, *J* = 8.5 Hz) were assigned to H-10 and H-11 of ring D which is substituted at C-8 (δ_C 113.9) and C-9 (δ_C 167.7). This substituent was established to be 2'-isopropenyldihydrofuran group from the ^{1}H NMR signals at δ_H 2.96 (*dd*, *J* = 15.7, 7.8 Hz, H-3'α), 3.28 (*m*, H-3'β), 5.34 (*m*, H-2'), 4.95 (*d*, *J* = 1.6 Hz, H-5'α), 5.07 (*dt*, *J* = 1.9, 1.0 Hz, H-5'β), and methyl signal at 1.76 (*s*, 3H). The location of the isopropenyldihydrofuran group between C-8 (δ_C 113.9) and C-9 (δ_C 167.7) of ring D was confirmed by HMBC correlation of H-10, H-11 and H-3' with C-8 and C-9. In ring A, signals at δ_H 6.70 (s) and δ_H 6.50 (s) were assigned to H-1 and H-4, respectively, with C-2 (δ_C 150.4) and C-3 (δ_C 144.3) substituted by methoxy groups (δ_H 3.66 and 3.77). The placement of the methoxy groups was confirmed by HMBC correlation of methoxy signals at δ_H 3.66 to C-2

(δ_C 150.4) and δ_H 3.77 to C-3 (δ_C 144.3) and also correlation of H-1 (δ_H 6.70) and H-4 (δ_H 6.50) to C-2 and C-3. The absolute configurations at C-6a and C-12a were not established. However, the coupling constant between one of the protons at C-6 and H-6a are indicative of a cis–configuration for H-6aand C-12a. This was further supported by the chemical shift of H-1 δ_H 6.59 Hz (*s*) consistant with the B/C ring junction being *cis* configured (Ito *et al.*, 2004). Therefore, on the basis of the above evidence and comparison with literature data, compound **10** was identified as rotenone, previously reported from several plants belonging to the family Leguminosae including *Derris trifoliata* (Yenesew *et al.*, 2005).



10

Table 10: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compound **10** (CD_3CN)

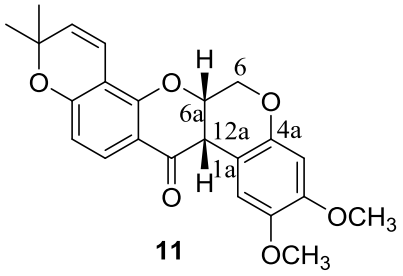
Carbon No.	δ_H	δ_C	HMBC
1	6.70 (s)	111.3	C-2, C-3, C-5a, C-12a
2		150.4	
3		144.3	
4	6.50 (s)	101.7	C-2, C-3, C-5a, C-12a
4a		148.3	
6α	4.22 (*dd*, *J* = 12.4, 1.0 Hz	66.6	C-5a, C-6a, C-12a
6β	4.55 (*dd*, *J* = 12.3, 3.0 Hz)		
6a	5.04 (*ddd*, *J* = 4.0, 3.0, 1.1 Hz)	72.7	-
7a		158.5	
8		113.9	

9		167.7	
10	6.54 (*d*, *J* = 8.5 Hz)	104.9	C-8
11	7.80 (*d*, *J* = 8.5 Hz)	130.0	C-7a, C-9, C-12
11a		113.5	
12		189.5	
12a	3.97 (*m*)	44.7	C-1a, , C-5a, C-11aC-12
2'	5.34 (*m*)	88.2	-
3'	2.96 (*dd*, *J* = 15.7.4, 7.8 Hz)	31.3	C-2', C-4', C-7a, C-8, C-9
	3.28 (*m*)		
4'		144.3	
5'	4.95 (*q*, *J* = 1.6 Hz)	112.3	C-2', C-4 '
	5.07 (*dt*, *J* = 1.9, 1.0 Hz)		
6'-CH_3	1.76 (*s*)	16.7	C-2', C-4', C-5'
2-OCH_3	3.66 (*s*)	56.3	C-2
3-OCH_3	3.77 (*s*)	55.9	C-3

*4.2.3. Deguelin (**11**)*

Compound **11** was obtained as a brown paste. The ^{1}H NMR spectrum displayed signals at δ_H 4.61 (*dd*, *J* = 12.3, 2.8 Hz), 4.22 (*d*, *J* = 12.3 Hz), 5.05 (*m*) and 3.88 (*d*, *J* = 4.0 Hz) with the corresponding ^{13}C signal at δ_C 66.0, 72.4 and 43.9 indicating that compound **11** is also a rotenoid derivative. As in compound **10**, the ^{1}H NMR spectrum showed two singlet aromatic protons at δ_H 6.70 (*s*) and 6.51 (*s*) allocated to H-1 and H-4, respectively, of ring A with two methoxy groups (δ_H 3.77 and 3.66) located at C-2 (δ_C 143.7) and C-3 (δ_H 149.8). The placement of the methoxy groups was confirmed from HMBC correlation of the methoxy proton signal at δ_H 3.77 with 143.7 (C-2), and the signal at δ_H 3.66 with 149.8 (C-3). A pair of *ortho*-coupled doublets at δ_H 6.46 (*d*, *J* = 8.7 Hz) and 7.71 (*d*, *J* = 8.7 Hz) were assigned to H-10 and H-11, respectively of ring D which is substituted at C-8 and C-9. A set of signals at

δ_H 6.64 (*d, J* = 10.1 Hz) and 5.73 (*d, J* = 10.1 Hz) together with two methyl signals at δ_H 1.37 (*s*) and 1.46 (*s*) showed that this substituent is a 2',2'-dimethylchromene or pyran group. The attachment of the pyran ring at C-8/C-9 of ring D was confirmed from HMBC correlation of H-10 and H-11 with C-8 (δ_C 109.0) and C-9 (δ_C 159.6), and by correlation of H-4' with C-8 and C-9. As in compound **10**, the configuration at C-6a and C-12a was assigned as cis configuration from the *J* value (Table 11) of CH_2-6 with H-6a and the chemical shift value of H-1 (δ_H 6.70). Thus compound **11** was identified as deguelin, a compound which also occurs widely in the family Leguminosae including the roots of *Derris trifoliata* (Yenesew, *et al.*, 2005).



11

*4.2.4. 7a-O-Methyldeguelol (**12**)*

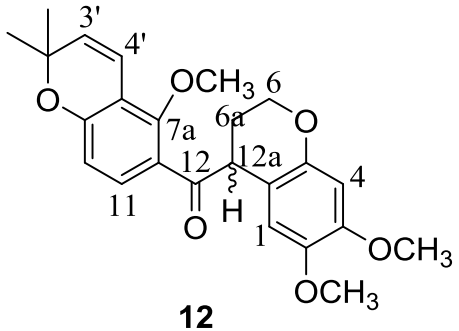
Compound **12** was isolated as a colourless paste and showed $[M+H]^+$ at *m/z* 411.3 in the LCMS analysis, corresponding to the molecular formula $C_{24}H_{26}O_6$. In the ^1H NMR spectrum, the presence of a methylene signal δ_H 2.16 (*ddd, J* = 7.9, 5.6, 4.0 Hz, 2H) along with oxymethylene at δ_H 4.08 (*ddd, J* = 11.0, 8.0, 5.0 Hz, 1H, H-6α) and 4.16 (*ddd, J* = 11.0, 4.1 Hz, 1H, H-6β), and methine at δ_H 4.66 (*m*, 1H, H-12a) was typical of a rotenoid derivative with an open ring C (a new subclass named as rotenoloid) (Yenesew *et al.*, 2005). As in compound **11,** two singlet aromatic proton signals at δ_H 6.47 (H-1) and δ_H 6.43 (H-4) were observed with the corresponding ^{13}C NMR peaks appearing at δ_C 114.2 and 101.5 of ring A. Furthermore, two sharp singlet proton signals at δ_H 3.59 (*s*), 3.77 (*s*) were assigned to methoxy substituents at C-2 (δ_C 143.5) and C-3 (δ_C 149.7) of ring A. The attachment of the

methoxy group in ring A was established by HMBC correlation of the methoxy signals δ_H 3.59 to C-2 and δ_H 3.77 with C-3. In support of this, the HMBC spectrum also showed correlation of the singal δ_H 6.47 (H-1) with C-3 (δ_C 149.7), and δ_H 6.43 (H-4) with C-2 (δ_C 143.5). In ring D, the presence of a methoxy δ_H 3.81(*s*) together with mutually coupled aromatic protons at δ_H 6.65 (*d*, *J* = 8.5 Hz, H-10) and 7.41 (*d*, *J* = 8.5 Hz, H-11) and a 2',2'-dimethylchromene substituent at δ_H 5.85 (*d*, *J* = 10.0 Hz), 6.67 (*d*, *J* = 10.0 Hz) and 1.46 (*s*) further confirmed that ring C in the rotenoloid moiety is open. The placement of the 2',2'-dimethylchromene ring at C-8 (δ_C 115.6) and C-9 (δ_C 157.8) in ring D was confirmed by HMBC correlation of H-10 (δ_H 6.65) with C-8 (δ_C 115.6), C-9 (δ_C 157.8), as well as H-11 (δ_H 7.41) with C-9 (δ_C 157.8). Therefore, based on the above spectroscopic evidence and comparison with literature data, compound **12** was identified as 7a-*O*-methyldeguelol; previously reported from *Derris trifoliata* (Cheenpracha, *et al.*, 2007; Yenesew *et al.*, 2005). The absolute configuration at C-12a was not determined.

Table 11: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compounds **11** and **12** (CD_3CN)

Carbon No.	**11**			**12**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	6.70 (*s*)	110.7	C-2, C-3, C-5	6.47 (*s*)	114.2	C-3, C-4a, C-12a
1a		104.9			111.2	
2		143.7			143.5	
3		149.8	C-2, C-3, C-5		149.7	
4	6.51 (*s*)	101.2		6.43 (s)	101.5	C-1a, C-2, C-4a, C-12a
4a		147.7			149.8	
6α	4.22 (*d*, *J* = 12.3 Hz)	66.0	C-6a, C-12a	4.08 (*ddd*, *J* = 11.0, 8.0, 5.0 Hz)	63.6	C-4a, C-6a, C-12a
6β	4.61 (*dd*, *J* = 12.3, 2.8 Hz)			4.16 (*ddd*, *J* = 11.0, 4.1 Hz)		C-4a, C-6a, C-12a
6a	5.05 (*m*)	72.4	-	2.16 (*ddd*, *J* = 7.9, 5.6, 4.0 Hz, 2H)	25.5	C-1a, C-6, C-12, C-12a,
7a		156.7			156.2	
8		109.0			115.6	
9		159.6			157.8	
10	6.46 (*d*, *J* = 8.7 Hz)	110.0	C-9, C-11, C-11a	6.65 (*d*, *J* = 8.5 Hz)	112.8	C-8, C-9, C-11a
11	7.71 (*d*, *J* = 8.7 Hz)	128.7		7.41 (*d*, *J* = 8.5 Hz)	131.8	C-7a, C-9, C-12
11a		112.8			126.0	

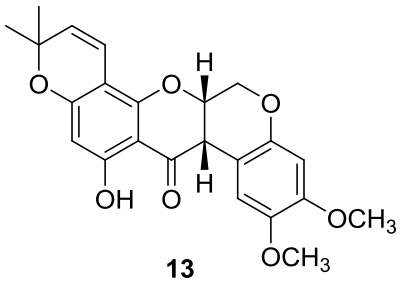
12		189.2			203.1	
12a	3.88 (*d*, *J* = 4.0 Hz)	43.9	C-4, C-1, C-12	4.66 (*t*, *J* = 5.1 Hz)	111.2	C-1, C-4a, C-5a, C-6, C-6a, C-12
2'		77.7			77.4	
3'	5.73 (*d*, *J* = 10.1 Hz)	129.6	C-2', C-4'	5.85 (*d*, *J* = 10.0 Hz)	131.0	C-CH_3 , C-2', C-8
4'	6.64 (*d*, *J* = 10.1 Hz)	114.8	C-2', C-8	6.67 (*d*, *J* = 10.0 Hz)	116.7	C-2', C-7a, C-9
2' $(CH_3)_2$	1.37 (*s*) 1.46 (*s*)	27.0 27.5	C- CH_3, C-2', C-3'	1.46 (*s*)	27.5	C- CH_3, C-2', C-3'
2-OCH_3	3.77 (*s*)	55.3	C- CH_3, C-2', C-3'	3.59 (*s*)	55.3	C-2
3-OCH_3	3.66 (*s*)	55.8	C-2	3.77 (*s*)	55.8	C-3
7a-OCH_3				3.81(*s*)	63.7	C-7a



12

*4.2.5. α-Toxicarol (**13**)*

Compound **13** was isolated as a brown paste, the molecular formula was determined as $C_{23}H_{22}O_7$ by LC-MS ($[M+H]^+$ peak at *m/z* 411.3) and NMR spectra (Table 12). The NMR spectra of **13** is similar to **11** except for the presence of a signal for intramolecularly bonded hydroxy group at δ_H 12.23 (C-11 δ_C 164.9) in ring D. That this compound is a rotenoid derivative was established from the ^{1}H NMR spectrum which showed characteristic peaks at δ_H 4.61 (*dd, J* = 12.4, 2.9 Hz, 1H), 4.22 (*m*, 1H), 5.01 (*m*, 1H) and 3.91 (*m*, 1H) corresponding to protons on C-6 (δ_C 66.3), C-6a (δ_C 72.5) and C-12a (δ_C 43.7). Ring A is identical to that of compound **11** which showed two singlet aromatic protons δ_H 6.81 (H-1) and 6.51 (H-4) with the corresponding ^{13}C NMR peaks (from HSQC spectrum) δ_C at 111.3 (C-1) and 101.7 (C-4), respectively. As in compound **11**, the ^{1}H NMR spectrum revealed the presence of 2',2'-dimethylpyran group [δ_H 5.62 (*d, J* = 10.1 Hz), 6.55 (*d, J* = 10.0 Hz), and two methyl signals δ_H 1.35 (s) and 1.44 (*s*)]. The attachment of the 2',2'-dimethylpyran moiety at C-8/C-9 in ring D was established from the HMBC correlation of H-10 (δ_H 5.93) with C-9 (δ_C163.0), and H-4' (δ_H 6.55) with C-8 (δ_C 106.2). The remaining singlet proton signals at 3.78 (s) and 3.69 (*s*) belong to methoxy groups at C-2 (δ_C 150.6) and C-3 (δ_C 144.4) of ring A. Thus, this compound was identified as α-toxicarol, previously isolated from the roots of *Derriis trifoliata* (Yenesew, *et al.*, 2005).



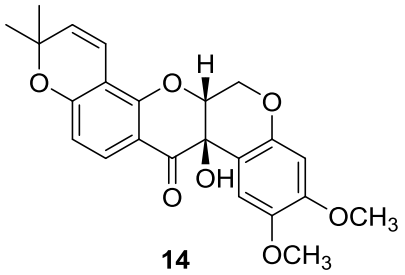
13

Table 12: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compound **13** (CD_3CN)

Carbon No.	δ_H	δ_C	HMBC
1	6.81 (s)	111.3	C-2, C-3, C-5
2		150.6	
3		144.4	
4	6.51 (s)	101.7	C-2, C-3, C-5
5		156.7	
5a		-	
6α	4.22 (m)	66.3	C-6a, C-12a
6β	4.61 (*dd*, *J* = 12.4, 2.9 Hz)		
6a	5.01 (m)	72.5	-
7a		156.7	
8		106.2	
9		163.0	
10	5.93 (s)	97.8	C-9, C-11, C-11a
11		164.9	
11a		101.7	
12		195.8	
12a	3.91 (m)	43.7	C-1, C-4, C-12
2'		78.9	
3'	5.62 (*d*, *J* = 10.1 Hz)	127.7	C-2', C-4'
4'	6.55 (*d*, *J* = 10.0 Hz)	115.2	C-2', C-8
2'-CH_3	1.35 (s)	27.8	CH_3, C-2', C-3'
2'-CH_3	1.44 (*s*)	28.2	CH_3, C-2', C-3'
2-OCH_3	3.78 (s)	55.9	C-2
3-OCH_3	3.69(*s*)	56.4	C-3
11-OH	12.23 (s)		C-7a, C-10, C-11a

*4.2.6. Tephrosin (**14**)*

Compound **14** was isolated as a light brown oil having the LC-MS fragment ion peak at *m/z* 393.3 $[M\text{-}H_2O+H]^+$ corresponding to $[C_{23}H_{21}O_6]^+$. The NMR spectra of **14** is similar to **11** except for the presence of a hydroxy substituent at C-12a in compound **14.** Thus the ^1H NMR spectrum showed signals at δ_H 4.65 (*m*, H-6α), 4.44 (*dd*, *J* = 12.3, 1.1 Hz, H-6β) and 4.68 (*m*, H-6a), with the corresponding ^{13}C NMR signals (from HSQC) appearing at δ_C 63.7 and 76.1, which is consistent with the presence of hydroxy group at C-12a. In the aromatic region of the spectrum, two singlet proton signals at δ_H 6.59 and 6.54 were assigned to H-1 and H-4 of ring A. The presence of two methoxy signals δ_H 3.78 (*s*) and 3.65 (*s*) were apparent and were placed at C-2 (δ_C 151.4) and C-3 (δ_C 143.7) in ring A. The position of the methoxy group was fixed from HMBC spectrum (Table 13). Additionally, *ortho* coupling signals at δ_H 6.48 (*d*, *J* = 8.7 Hz) and 7.71 (*d*, *J* = 8.7 Hz), and their HSQC cross peaks at δ_C 111.3 and δ_C 128.8 were assigned to H-10 and H-11 of ring D. The signals δ_H 5.72 (*d*, *J* = 10.2 Hz), 6.60 (*d*, *J* = 10.0 Hz) and methyl signals δ_H 1.36 (*s*) and 1.46 (*s*) were representative of the 2',2'-dimethylpyran group. The fusion of the 2',2'-dimethylpyran at C-8 and C-9 in ring D was established by HMBC correlation of H-10 and H-11 to C-9 (δ_C 160.0), as well as H-3' (δ_H 5.72) with C-8 and H-4' (δ_H 108.5) with C-9 (160.0). The relative configuration at the B/C ring junction was established from NMR as in the other rotenoids of this plant. Therefore, this compound was identified as tephrosin, previously isolated from seeds of *Mellettia dura* (Yenesew *et al.*, 2003) and several plants in the family Leguminosae.



14

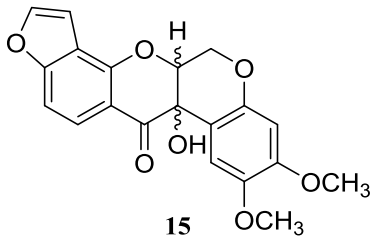
Table 13: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data and HMBC correlations for **14** (CD_3CN)

Carbon No.	δ_H	δ_C	HMBC
1	6.59 (*s*)	110.4	C-12a, C-1a, C-2, C-3, C-4a,
1a		108.9	
2		151.4	
3		143.7	
4	6.54 (*s*)	101.1	C-6a, C-12a, C-2, C-3, C-4a
4a		148.5	
6α	4.44 (*dd*, *J* = 12.3, 1.1 Hz)	63.7	C-6a, C-12a, C-4a, C-12, C-7a
6β	4.65 (*m*)		
6a	4.68 (*m*)	76.1	C-6, C-12a, C-12
7a		156.1	
8		108.5	
9		160.0	
10	6.48 (*d*, *J* = 8.7 Hz)	111.3	C-11a, C-9
11	7.71 (*d*, *J* = 8.7 Hz)	128.8	C-11, C-9, C-7a
11a		108.9	
12		190.9	
12a		67.6	
2'		77.9	
3'	5.72 (*d*, *J* = 10.2 Hz)	129.7	2'-CH_3, C-2', C-8, C-9
4'	6.60 (*d*, *J* = 10.0 Hz)	114.5	C-2', C-9
2-OCH_3	3.78 (*s*)	55.4	C-2
3-OCH_3	3.65 (*s*)	55.8	C-3
2'CH_3	1.36 (*s*)	21.7	C-2', C-3'
	1.46 (*s*)	27.6	

*4.2.7. 12a-Hydroxyelliptonol (**15**)*

Compound **15** was identified as a pale yellow oil. LCMS indicated the molecular ion peak at *m/z* 351.7 corresponding to the dehydration product with the molecular formula $C_{20}H_{15}O_6^+$. The ^{1}H and ^{13}C NMR signals at δ_H 4.67 (*dd*, *J* = 12.5, 2.5 Hz, H-6α), 4.51 (*dd*, *J* = 12.5, 1.1 Hz, H-6β) and 4.87 (*dd*, *J* = 2.5, 1.1 Hz, H-6a) with corresponding carbon peaks (from HSQC spectrum) appearing at δ_C 64.3 (C-6), 77.1 (C-6a) and 68.4 (C-12a), indicated that compound **15** is a rotenoid derivative having a hydroxyl substituent at C-12a (δ_C 68.4).

The ^{1}H NMR signals at δ_H 6.59 (s) and 6.55 (s) were assigned to H-1 and H-4, respectively of ring A, with methoxy groups (δ_H 3.77 (*s*) and 3.64) attached to C-2 (δ_C 152.0) and C-3 (δ_C 144.3) of ring A. The aromatic protons at δ_H 7.26 (*d*, *J* = 8.8, 0.9 Hz) and 7.87 (*d*, *J* = 8.8 Hz) together with ^{13}C NMR peak δ_C 107.2 (C-10) and 124.2 (C-11) were assigned to H-10 and H-11 in ring D, and further proton signals at δ_H 7.76 (*d*, *J* = 2.2 Hz) and 6.94 (*dd*, *J* = 2.2, 0.9 Hz) confirmed the presence of a 1,2,-disubstituted furan moiety in ring D. The placement of the furan ring between C-8 (δ_C 117.4) and C-9 (δ_C 160.7) in ring D was further confirmed by the HMBC correlation of protons of the furan ring (H-2' and H-3') and the aromatic ring proton (H-10 and H-11) with quaternary carbon peaks at C-8 (δ_C 117.4) and C-9 (δ_C 160.7). The 6aα,12aα (Ito, *et al.*, 2004; Yenesew, *et al.*, 2006) and the 6aβ,12aβ (Cheenpracha, *et al.*, 2007; Thasana *et al.*, 2001) configuration of 12a-hydroxylelliptone has been suggested. However the configuration at C-6a and C-12a in **15** has not been determined. Thus, this compound was identified as 12a-hydroxylelliptone, a compound previously reported from *Derris trifoliata* (Cheenpracha, *et al.*, 2007; Ito, *et al.*, 2004; Yenesew, *et al.*, 2006) and from *Derris malaccensis* (Thasana, *et al.*, 2001).

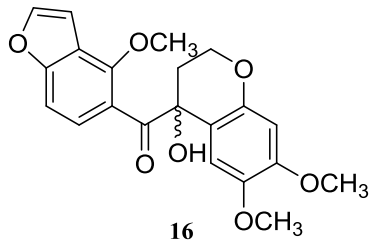


15

*4.2.8. 7a-O-Methyl-12a-hydroxyelliptonol (**16**)*

Compound **16** was isolated as a pale yellow oil. As in compound **15**, the ^{1}H and ^{13}C NMR spectra displayed signals corresponding to two aromatic proton singlets at δ_H 6.60 (H-1) and 6.48 (H-4) in ring A and two *ortho* coupled signals at δ_H 7.17 (*d*, *J* = 8.5, 1.0 Hz, H-10) and 6.93 (*d*, *J* = 8.4 Hz, H-11) in ring D. As in the other rotenoid derivatives, the methoxy groups which appeared at δ_H 3.64 (δ_C 56.3) and 3.80 (δ_C 55.9), were placed at C-2 and C-3,

respectively in ring A. The presence of mutually coupled proton signals at δ_H 7.77 (*d*, *J* = 2.3 Hz) and 7.12 (*dd*, *J* = 2.3, 0.9 Hz) was evidence for the presence of a furan moiety in ring D as in compound **15**. Furthermore, in the NMR spectrum two mutually coupled, methylene signals at δ_H 4.12 (*m*) and 4.24 (*m*) with the corresponding ^{13}C signal δ_C 63.3 (from HSQC) were observed and were assigned to CH_2 -6. The replacement of a methine [δ_H 4.87 (*dd*, *J* = 2.5, 1.1 Hz), δ_C 77.1] in compound **14** by methylene δ_H 2.04 (*m*) (δ_C 34.5) signals in **16** and the presence of an additional methoxyl (at C-7a) signal at δ_H 4.23 (*s*) (δ_C 60.6) clearly indicated that ring C of **15** is open at C-6a. Therefore, based on the above data this new compound was characterized as 7a-*O*-methyl-12a-hydroxyelliptonol (**16**). The absolute configuration at C-12a has not been determined.



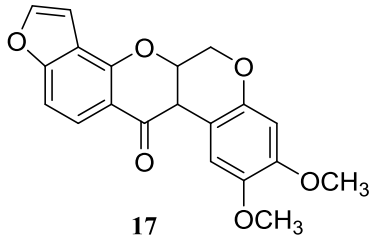
16

Table 14: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for **15** and **16** along with HMBC correlations in **15**

Carbon No.	**15**			**16**	
	δ_H	δ_C	HMBC	δ_H	δ_C
1	6.59 (s)	110.8	C-1a, C-2, C-3, C-4a, C-12a	6.60 (*s*)	111.5
1a		108.9			-
2		152.0			-
3		144.3			-
4	6.55 (s)	101.6	C-1a, C-2, C-3, C-4a, C-12a	6.48 (*s*)	101.5
4a		149.1			-
6α	4.51 (*dd*, *J* =12.5, 1.1 Hz)	64.3	C-4a, C-6a, C-12a	4.12 (*m*)	63.3
6β	4.67 (*dd*, *J* =12.5, 2.5 Hz)			4.24 (*m*)	
6a	4.87 (*dd*, *J* = 2.5, 1.1 Hz)	77.1	C-1a, C-12a	2.04 (*m*)	34.5
7a		155.6			-
8		117.4			-
9		160.7			-
10	7.26 (*dd*, *J* = 8.8, 0.9 Hz)	107.2	C-8, C-9, C-11a	7.17 (*d*,d *J* = 8.5, 1.0 Hz)	106.0
11	7.87 (*d*, *J* = 8.8 Hz)	124.2	C-7a, C-9, C-12	6.93 (*d*, *J* = 8.4 Hz)	125.0
11a		117.4			-
12		192.3			-
12a		68.4			-
2'	7.76 (*d*, *J* = 2.2 Hz)	146.8	C-3', C-8, C-9	7.77 (*d*, *J* = 2.3 Hz)	146.0
3'	6.94 (*dd*, *J* = 2.2, 0.9 Hz)	104.6	C-2', C-8, C-9	7.12 (*dd*, *J* = 2.3, 0.9 Hz)	105.9
2 -OCH_3	3.77 (*s*)	55.9	C-2	3.64 (*s*)	56.3
3-OCH_3	3.64 (*s*)	56.4	C-3	3.80 (*s*)	55.9
7a- OCH_3				4.23 (*s*)	60.6

*4.2.9. Elliptone (**17**)*

Compound **17** was isolated as a colourless paste. The ^{1}H and ^{13}C NMR of **17** is typical of a rotenoid and showed signals at δ_H 4.69 (*dd, J* = 12.5, 2.5 Hz, 1H, H-6β), 4.51 (*m*, 1H, H-6α), 4.87 (*dd, J* = 2.5, 1.1 Hz, H-6a) and 2.70 (*m*, H-12a), with the corresponding carbon peaks appearing at δ_C 64.3 (C-6), 77.1 (C-6a) and 49.4 (C-12a), respectively. In the ^{1}H NMR spectrum, two aromatic singlets at δ_H 6.59 (*s*) and 6.55 (*s*) were assigned to H-1 and H-4 of ring A with methoxy (δ_H 3.77 and 3.64) groups being at C-2 and C-3, respectively. HMBC correlation of the two methoxy protons with carbon peaks at C-2 (δ_C 152.0) and C-3 (δ_C 144.3) supported their placement at C-2 and C-3 as in the other rotenoids of this plant. Two mutually coupled protons at δ_H 7.26 (*dd, J* = 8.8, 1.0 Hz) and 7.87 (*d, J* = 8.8 Hz) were assigned to H-10 and H-11 of ring D which is substituted at C-8 and C-9. This substituent is a furan moiety as in compound **15** as shown by the ^{1}H NMR signals at δ_H 7.76 (*d, J* = 2.3 Hz) and 6.95 (*dd, J* = 2.3, 0.9 Hz). Therefore, this compound was identified as elliptone; previously reported from *Derris trifoliata* (Ito, *et al.*, 2004) and from *Millettia duchesnei* (Ngandeu *et al.*, 2008). The absolute configuration of **17** was not determined here, however in the previous report the relative configuration had been assigned as 6a*β*,12aβ (Ito, *et al.*, 2004).

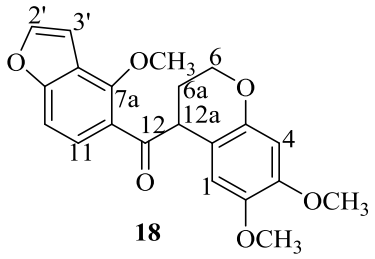


17

*4.2.10. 7-a-O-Methylelliptonol (**18**)*

Compound **18** was isolated as a yellowish paste with $[M+H]^+$ at *m/z* 369.2 from LCMS. This information along with NMR data (Table 15) allowed the assignment of molecular formula as $C_{21}H_{21}O_6$. The ^{1}H and ^{13}C data (Table 15) of **18** showed that it had identical A and D rings as

in elliptonol (**16**). The only difference is that the quaternary carbon signal for C-12a in compound **16** (δ_C 68.4) is replaced with signals for a methine in **18** δ_H 4.73 (*dd*, *J* = 6.2, 4.3 Hz), δ_C 46.1. Consequently, this compound was identified as 7-a-*O*-methylelliptonol, previously reported from *Derris trifoliata* (Cheenpracha, *et al.*, 2007). The absolute configuration at C-12a remains unresolved.



18

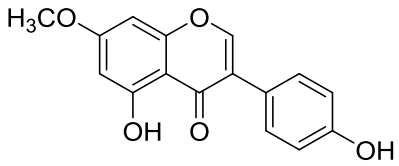
Table 15: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compounds **17** and **18** (CD_3CN)

Carbon No.	**17**			**18**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	6.59 (*s*)	110.8	C-1a, C-2, C-3, C-4a, C-12a,	6.49 (s)	114.2	C-12a, C-2, C-3, C-4a
1a		108.9			111.7	
2		152.0			143.5	
3		144.3			149.8	
4	6.55 (s)	101.6	C-1a, C-2, C-3, C-4a, C-12a,	6.44 (s)	101.4	C-1a, C-2, C-4a
4a		149.1			149.7	
6α	4.51 (*m*)		C-6a, C-12a, C-4a, C-12	4.11 (m)	63.6	C-6a, C-12a,
6β	4.69 (*dd*, *J* = 12.5, 2.5 Hz)	64.3		4.16 (m)		C-4a
6a	4.87 (*dd*, *J* = 2.5, 1.1 Hz)	77.1	C-12a, C-1a	2.18 (m)	25.5	C-12a, C-6a, C-1a, C-12
8		160.7			153.4	
9		-			118.4	
10		155.6			159.2	
11	7.26 (*dd*, *J* = 8.8, 1.0 Hz)	107.2	C-11a, C-9	7.30 (*dd*, *J* = 8.5, 1.0 Hz)	106.5	C-8, C-11a
11a	7.87 (*d*, *J* = 8.8 Hz)	124.2	C-7a, C-9, C-12	7.47 (*d*, *J* = 7.47 Hz)	126.6	C-7a, C-9, C-12
12		113.3			125.4	
12a	2.70 (*m*)	49.4		4.73 (*dd*, *J* = 6.2, 4.3 Hz)	46.1	C-6, C-1a, C-1
2'	7.76 (*d*, *J* = 2.3 Hz)	146.8	C-3', C-7a	7.82 (*d*, *J* = 2.3 Hz)	146.0	
3'	6.95 (*dd*, *J* = 2.3, 0.9 Hz)	104.6	C-2', C-7a	7.22 (*dd*, *J* = 2.3, 1.0 Hz)	106.2	C-2', C-8, C-9
2-OCH_3	3.77 (*s*)	55.9	C-2	3.65 (*s*)	56.3	C-2
3-OCH_3	3.64 (*s*)	56.4	C-3	3.77 (*s*)	55.8	C-3
7a-OCH_3				4.23 (*s*)	60.8	C-7a

*4.2.11. Prunetin (**19**)*

Compound **19** was isolated as a white amorphous solid; LC-MS analysis showed $[M+H]^+$ at *m/z* 285.0 which together with NMR data (Table 16) allowed the assignment of the molecular formula as $C_{16}H_{14}O_5$. In its ^1H NMR spectrum, a singlet at δ_H 8.08 (H-2), with the corresponding ^{13}C NMR peak (from HSQC spectrum) δ_C 154.3 (C-2) indicated compound **19** is an isoflavone derivative. The presence of downfield shifted proton (δ_H 12.94, for 5-OH), methoxy group (δ_H 3.91, δ_C 56.4), was evident from the NMR spectra. In the ^{13}C NMR spectrum (Table 16), the presence of five downfield shifted quaternary carbon signals are consistent with five oxygenated sp^2 hybridized carbon atoms. Two *meta* coupled aromatic proton signals at δ_H 6.40 (*d, J* = 2.3 Hz) and 6.56 (*d, J* = 2.3 Hz) with upfield carbon signals δ_C 98.6 (C-6) and 92.7 (C-8) assigned to H-6 and H-8, respectively in ring A, consistent with oxygenation at C-5 (OH) and C-7 as expected from biogenetic considerations. HMBC correlation of the methoxy protons with C-7 allowed its placement at this carbon. The substitution pattern in this ring was confirmed by HMBC correlation of the signal δ_H 6.40 (H-6) with C-5 (δ_C 163.0) and C-7 (δ_C 166.3), δ_H 6.56 (H-8) with C-7 (δ_C 166.3).

In ring B, an AA'XX' spin system at δ_H 7.42 and 6.91 was assigned to H-2'/6' and H-3'/5' respectively, with C-4' substituted with hydroxy group (δ_C 157.8). In agreement with this, the HMBC spectrum showed correlation of H-2'/6' and H-3'/5' with the carbon peak at δ_C 157.8 (C-4'). Therefore, compound **19** was identified as 4',5-dihydroxy-7-methoxyisoflavone, trivial name prunetin. This compound has been reported from *Dalbergia spinosa* and *Dalbergia sympathetica* (Nagarajan *et al.*, 2006; Narayanan & Nagarajan, 1988). This is however the first report of prunetin (**19**) from the genus *Derris*.



19

Table 16: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compound **19** (CD_3CN)

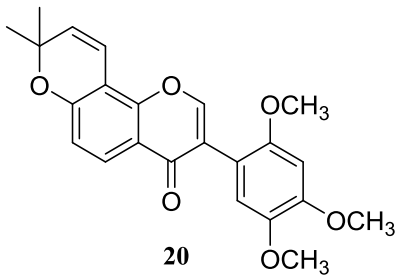
Carbon No.	δ_H	δ_C	HMBC
2	8.08 (*s*)	154.3	C-1', C-3, C-4, C-8a
3		123.8	
4		181.6	
5		163.0	
5a		106.5	
6	6.40 (*d*, *J* = 2.3 Hz)	98.6	C-5, C-5a, C-7, C-8
7		166.3	
8	6.56 (*d*, *J* = 2.3 Hz)	92.7	C-7, C-8a
8a		158.7	
1'		122.8	
2'/ 6'	7.42 (*d*, *J* = 8.5 Hz)	130.4	C-3, C-2'/6', C-4'
3'/ 5'	6.91 (*d*, *J* = 8.5 Hz)	115.7	C-3'/5', C-2', C-4'
4'		157.8	
7- OCH_3	3.91 (*s*)	56.4	C-6
5-OH	12.94 (s)		C-6, C-4a, C-5

*4.2.12. Barbigerone (**20**)*

Compound **20** was obtained as a white amorphous solid. LCMS analysis showed a $[M+H]^+$ peak at *m/z* 395.3, which together with NMR data (Table 17) is consistent with the molecular

formula $C_{23}H_{23}O_6$. That this compound is an isoflavone is shown from the ^{1}H NMR signal at δ_H 8.03 (*s*, H-2) and ^{13}C NMR signals at δ_C 154.6 (C-2), 122.5 (C-3) and 175.7 (C-4). The presence of a 2",2"-dimethylpyran [δ_H 5.89 (*d*, *J* = 9.9 Hz, H-4"), δ_H 6.87 (*d*, *J* = 10.7 Hz, H-3") and δ_H 1.51 (6H, *s*, $(Me)_2$-2"] and three methoxy groups (δ_H 3.76 (*s*), 3.78 (*s*) and 3.90 (*s*)) was evident from the ^{1}H NMR spectrum. In ring A, two *ortho* coupled aromatic protons at δ_H 7.93 (*d*, *J* = 8.6 Hz, H-5) and 6.87 (*d*, *J* = 8.5 Hz, H-6) with the corresponding carbon signals (from HSQC spectrum) appearing at δ_C 126.5 (C-5) and 115.0 (C-6) correspond to H-5 and H-6, respectively with C-7/C-8 substituted with a 2",2"-dimethylpyran moiety. The placement of the 2",2"-dimethylpyran ring at C-7/C-8 was confirmed from the HMBC correlation of H-5 (δ_H 7.93) with C-7 (δ_C 152.8), as well as the correlations of H-6 (δ_H 6.87) with C-7 (δ_C 152.8) and C-8 (δ_C 110.1).

In ring B, the presence of two singlet aromatic protons δ_H 6.75 (*s*) (H-3') and 6.92 (*s*) (H-6'), is consistent with the placement of the three methoxy groups at C-2' (δ_C 152.8), C-4' (δ_C 143.3) and C-5' (δ_C 150.7). The substitution pattern in ring B was confirmed by HMBC correlation of H-3' and H-5' with C-2' (δ_C 152.8), C-4' (δ_C 143.3) and C-5' (δ_C 150.7). Therefore, this compound was identified as barbigerone (**20**), previously reported from seeds of *Tephrosia barbigera* (Vilain, 1980) and *Millettia usaramensis* (Yenesew *et al*., 1998a). This is the first report from the genus *Derris*.



20

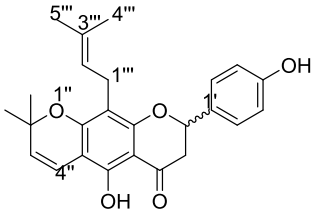
Table 17: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compound **20** (CD_3CN)

Carbon No.	δ_H	δ_C	HMBC
2	8.03 (*s*)	154.6	C-1', C-2', C-3, C-8a, C-4
3		122.5	
4		175.7	
4a		-	
5	7.93 (*d*, *J* = 8.6 Hz)	126.5	C-7, C-8a, C-4
6	6.87 (*d*, *J* = 8.5 Hz)	115.0	C-2'', C-7, C-8, C-8a
7		152.8	
8		110.1	
8a		157.6	
1'		112.9	
2'		152.8	
3'	6.75 (*s*)	99.0	C-1', C-2', C-3, C-4', C-5'
4'		143.3	
5'		150.7	
6'	6.92 (*s*)	116.7	C-1', C-2', C-3, C-4', C-5'
2''		78.3	
3''	5.89 (*d*, *J* = 9.9 Hz)	131.5	2'' CH_3, C-2'', C-7
4''	6.87 (*d*, *J* = 10.7 Hz)	115.4	C-2'', C-7, C-8, C-8a
2'-OCH_3	3.76 (*s*)	56.7	C-2'
4'-OCH_3	3.78 (s)	56.8	C-4'
5'-OCH_3	3.90 (*s*)	52.6	C-5'
2''-$(CH_3)_2$	1.51 (*s*)	27.7	C-2', C-3'

*4.2.13. Lupinifolin (**21**)*

Lupinifolin was isolated as a yellow oil with a molecular ion peak $[M+1]^+$ appearing at *m/z* 407.7 corresponding to $C_{22}H_{26}O_5$. The ^1H NMR spectrum revealed a flavanone skeleton with proton signals at δ_H 5.43 (*dd, J* = 12.8, 3.1 Hz, H-2), 3.15 (*dd, J* = 17.1, 12.8 Hz, H-3ax) and 2.81 (*dd, J* = 17.1, 3.1 Hz, H-3eq) with the corresponding carbon signals appearing at δ_C 79.3 (C-2) and δ_C 42.9 (C-3) of ring C. The proton signals δ_H 3.19 (*m*) and 5.15 (*m*) together with the methyl signals at δ_H 1.66 (6H, *s*) are indicative of the prenyl substituent in ring A. The presence of another set of proton signals at δ_H 5.63 (*d, J* = 10.0 Hz), 6.60 (*d, J* = 10.0 Hz) and methyl protons [δ_H 1.44 (*s*) and 1.45 (*s*)] are due to a 2",2"-dimethylpyrano ring. The placement of prenyl at C-8 and the 2", 2"-dimethylpyrano between C-6 and C-7 in ring A was established by the HMBC correlation of H-1"' (δ_H 3.19) to C-8 (δ_C 108.8); correlation of proton signals of the chromene ring H-3' (δ_H 5.63) to C-6 (δ_C 103.0) as well as H-4' to C-7 (δ_C 159.7). Furthermore, a strongly hydrogen bonded hydroxy signal at δ_H 12.42 is placed at C-5 (δ_C 156.8) of ring A which is fully substituted. The AA'XX' spin system at δ_H 7.37 (*d, J* = 8.5 Hz) and 6.89 (*d, J* = 8.5 Hz) was assigned to H-2'/6' and H-3'/5', respectively of ring B with hydroxy substituent at C-4'' (δ_C 157.8). These proton signals showed HMBC correlation with C-2 (δ_C 79.3) and C-4' (δ_C 157.8) confirming the nature of ring B.

Based on the above spectroscopic evidence and comparison with literature, compound **21** was identified as lupinifolin, a compound previously reported from *Derris reticulata* (Mahido, *et al.*, 1997) and *Derris trifoliata* (Yenesew *et al.*, 2009). The absolute configuration has not yet been established, but the large coupling constant between H-2 and one of the protons at C-3 (*J* = 12.8 Hz) indicates that H-2 is in an axial orientation and the B ring is in an equatorial position.

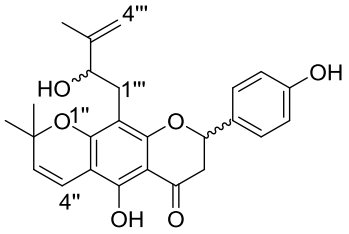


21

*4.2.14. Dereticulatin (**22**)*

Compound **22** was isolated as a brown paste. The LCMS spectrum displayed a molecular ion peak at *m/z* 423.1. Proton signals at δ_H 5.39 (*dd*, *J* = 13.3, 2.9 Hz), 2.78 (*m*, H-3α) and 3.14 (*m*, H-3β) and carbon peaks (δ_C 79.5 (C-2) and δ_C 42.6 (C-3) characteristic of a flavanone were displayed. The ^{1}H and ^{13}C NMR spectra of **22** were similar to compound **21**, the only difference was that the presence of a 2-hydroxy-3-methylbut-3-en-1-yl) group instead of a prenyl group at C-8. Thus the ^{1}H NMR signals at δ_H 2.78 (*m*, H-1'''), 4.21 (*m*, H-2'''), 4.69 (*m*, H-4'''), and 1.68 (*s*, 3'''-CH_3), together with the ^{13}C NMR peaks at δ_C 28.8 (C-1'''), 74.6 (C-2'''), 109.5 (C-4''') and 17.0 (C-3'''CH_3) represented the 2-hydroxy-3-methylbut-3-en-1-yl) group. Additional set of signals at δ_H 5.63 (*d*, *J* = 10.0 Hz), 6.61 (*d*, *J* = 10.0 Hz) and 1.47 (*s*, 2''CH_3) belong to the 2'',2''-dimethylpyrano ring. The placement of both substituents (2-hydroxy-3-methylbut-3-en-1-yl) and 2'',2''-dimethylpyrano) in ring A was confirmed by the HMBC correlation of H-1''' (δ_H 2.78) to C-7 (δ_C 159.6), C-8 (δ_C 105.6) and C-8a (δ_C 148.1), as well as H-3'' correlation to C-6 (δ_C 102.4), and H-4'' with C-5 (δ_C 156.6) and C-7 (δ_C 159.6). The AA'XX' spin system signals in ring B resonates at δ_H 7.38 (*d*, *J* = 8.6 Hz) and 6.89 (*d*, *J* = 8.5 Hz) is consistent with C-4' (δ_C 157.3) oxygenation. The nature of this ring was confirmed on the basis of HMBC correlation of the signals at δ_H 7.38 (H-2'/6') to C-2 (δ_C 42.6) and C-4' (δ_C 157.3), as well as correlation of H-3'/5' to C-1' (δ_C 130.2), and C-4' (δ_C 157.3). The absolute configuration at C-2 has not yet been established, but the coupling constant between H-2 and one of the protons (*J* = 13.2 Hz) suggested an axial and equatorial

position for H-2 and the B ring, respectively. Therefore, this compound was identified as dereticulatin; previously reported from the stem of *Derris reticulata* (Mahidol *et al.*, 1997).



22

Table 18: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations of compounds **21** and **22** (CD_3CN)

Carbon No.	**21**			**22**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	5.39 (*dd*, *J* = 13.2, 2.93 Hz)	79.5	C-1', C-2'/6'	5.43 (*dd*, *J* = 12.8, 3.1 Hz)	79.3	C-2'/6', C-1', C-4
3	2.78 (*m*) 3.14 (*m*)	42.6	C-2, C-1'	2.81 (*dd*, *J* = 17.1, 3.1 Hz) 3.15 (*dd*, *J* = 17.1, 12.8 Hz)	42.9	C-5a, C-4 C-2, C-1'
4		197.4			197.7	
5		156.6			156.8	
5a		102.2			103.0	
6		102.4			103.0	
7		159.6			159.7	
8		105.6			108.8	
8a		148.1			160.1	
2'/6'	7.38 (*d*, *J* = 8.6 Hz)	128.6	C-2, C-4'	7.37 (*d*, *J* = 8.5 Hz)	128.6	C-2, C-4'
3'/5'	6.89 (*d*, *J* = 8.5 Hz)	115.8	C-1', C-4'	6.89 (*d*, *J* = 8.5 Hz)	115.8	C-2, C-4'
1'		130.2			130.7	
4'		157.3			157.8	
2''		78.3			78.3	
3''	5.63 (*d*, *J* = 10.0 Hz)	127.0	2''CH_3, C-2'', C-6	5.63(*d*, *J* = 10.0)	127.1	2''CH_3, C-2'', C-6
4''	6.61 (*d*, *J* = 10.0 Hz)	115.4	C-2'', C-5a, C-5, C-7	6.60 (*d*, *J* = 10.0)	115.5	C-2'', C-5, C-7
1'''	2.78 (*m*)	28.8	C-2''', C-7, C-8, C-8a	3.19 (*m*)	21.6	C-8, C-2''', C-3''', C-7
2'''	4.21 (*m*)	74.6		5.15 (*m*)	122.8	4''' CH_3, 5''' CH_3
3'''					131.6	
4'''	4.69 (*m*)	109.5		1.66 (*s*)	17.5	5''' CH_3, C-3''', C-2'''
5'''				1.66 (*s*)	25.4	4''' CH_3, C-3''', C-2'''
2''CH_3	1.47 (*s*)	27.6	C-2''CH_3, C-2'', C-3''	1.44 (*s*) 1.45 (*s*)		2'' CH_3, C-2'', C-3''
3'''CH_3	1.68 (*s*)	17.0	C-3'''CH_3, C-4''', C-8a			
5-OH	12.47 (*s*)			12.42 (*s*)		C-5, C-5a

*4.2.15. 6,7-Dimethoxy-4-chromanone (**23**)*

Compound **23** was isolated as a colourless paste with $[M+1]^+$ peak in the LCMS analysis at *m/z* 209.2 corresponding to molecular formula $C_{11}H_{12}O_4$. That this compound is chroman-4-one derivative was evident from the NMR sectra (Table 19). Thus, the ^1H NMR spectrum showed two singlet aromatic signals at δ_H 7.24 (*s*) and 6.54 (*s*) assigned to H-5 and H-8, as well as two methoxy signals at δ_H 3.87 (*s*) and 3.81 (*s*) attached at C-6 (δ_C 156.8) and C-7 (δ_C 145.0), respectively. The oxymethylene protons and methylene proton signals at 4.15 (*m*) and 2.70 (*dd, J* = 6.9, 6.1 Hz) corresponding to protons at C-2 and C-3, respectively, which is consistent with a disubstituted chroman-4-one skeleton (Wangensteen *et al.*, 2005). The placement of methoxy group at C-6 was established from the HMBC correlation of the methoxy protons (δ_H 3.87) with a carbon peak at δ_C 156.8 (C-6), whereas the methoxy signal at δ_H 3.81 correlated to peak at δ_C 145.0 (C-7). HMBC correlation of H-5 (δ_H 7.24), oxymethylene (δ_H 4.15) and methylene (δ_H 2.70) protons with C-4 (δ_C 190.7) is consistent with the structre proposed. Hence, on the basis of this spectroscopic evidence and comparison with literature data, this compound was identified as 6,7-dimethoxy-4-chromanone, previously reported from *Sarcolobus globosus* (Aslclepiadaceae) (Wangensteen, *et al.*, 2005) and *Derris trifoliata* (Yenesew, *et al.*, 2006).

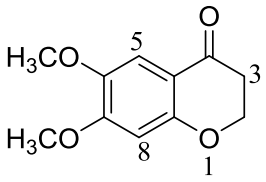


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Table 19: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data together with HMBC correlations in compound **23** (CD_3CN)

Carbon No.	δ_H	δ_C	HMBC
2	4.15 (*m*)	68.1	C-3, C-4, C-8a,
3	2.70 (*dd, J* = 6.9, 6.1 Hz)	37.4	C-2, C-4, C-5a
4		190.7	
5	7.24 (*s*)	107.1	C-4, C-5a, C-6, C-7, C-8a
5a		113.9	
6		156.8	
7		145.0	
8	6.54 (*s*)	100.8	C-5a, C-6, C-7, C-8a, C-4
8a		158.8	
6-OCH_3	3.87 (*s*)	56.4	C-6
7-OCH_3	3.81 (*s*)	56.1	C-7

4.3. Compounds Isolated from of *Lonchocarpus bussei*

A new isoflavone, 4'-prenyloxyvigvexin A (**24**) along with four known isoflavones: maximaisoflavone H (**25**), 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone (**26**), 6,7,3'-trimethoxy-4',5'-methylenedioxyisoflavone (**27**) and durmillone (**28**) were isolated from the CH_2Cl_2/MeOH (1:1) extract of the leaves of *Lonchocarpus bussei*. The roots extract of this plant gave a chalcone, 4-hydroxylonchocarpin (**29**); a geranylated phenylpropanol, colenemol (**30**) and two known pterocarpans, (6a*R*,11a*R*)-maackiain (**31**) and (6a*R*,11a*R*)-edunol (**32**). The characterization of these compounds is discussed below.

*4.3.1. 4'-Prenyloxyvigvexin A (**24**)*

Compound **24** was obtained as a colourless paste; HREIMS analysis showed a $[M+H]^+$ peak at *m/z* 389.1753, which together with NMR data (Table 20) allowed the assignment of the molecular formula $C_{25}H_{24}O_4$. The UV (λ_{max} 250 and 312 nm), ^{1}H NMR (δ_H 7.91 for H-2) and ^{13}C NMR (δ_C 152.0 for C-2, 124.9 for C-3, 175.9 for C-4) spectral data were characteristics

of an isoflavone moiety (Yenesew *et al.*, 1998b). Two *ortho*-coupled proton signals at δ_H 8.09 (*d*, *J* = 8.6 Hz) and 6.92 (*d*, *J* = 8.6 Hz) were assigned to H-5 and H-6, respectively, of ring-A which is substituted at C-7 and C-8. In the HMBC spectrum, H-5 (δ_H 8.09) correlated to C-4 (δ_C 175.9), C-7 (δ_C 165.2) and C-8a (δ_C 153.9); H-6 to C-7 (δ_C 165.2), C-8 (113.5) and C-4a (δ_C 119.1), consistent with a 7,8-disubstituted ring-A. The substituent was identified as 2-isopropenyl-2,3-dihydrofuran as shown from the ^{1}H NMR spectrum [δ_H 3.22 (1H, *dd*, *J* = 15.8, 9.9 Hz, H-1a"), 3.58 (1H, *dd*, *J* = 15.8, 7.9 Hz, H-1b"), 5.43 (1H, *brt*, *J* = 8.9 Hz, H-2"), 4.98 (1H, *m*, H-5a"), 5.13 (1H, *m*, H-5b") and 1.80 (3H, *s*, 4"-CH_3)]. The placement of the 2-isopropenyl-2,3-dihydrofuran ring at C-7/C-8 of ring-A was supported by HMBC correlation of CH_2-3" to C-7 (165.2), C-8 (113.5) and C-8a (153.9); and H-2" to C-7 (165.2) (Table 20).

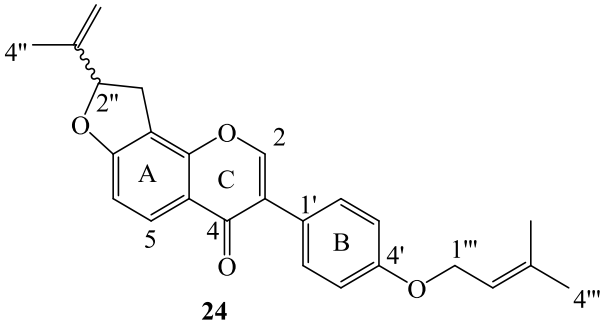
In the ^{1}H NMR spectrum, an AA'XX' spin system at δ_H 7.45 (*d*, *J* = 8.8 Hz) and 6.96 (*d*, *J* = 8.8 Hz) was assigned to H-2'/6' and H-3'/5', respectively, of 4'-oxygenated ring-B. In addition, the ^{1}H NMR spectrum revealed the presence of a prenyloxy group in ring B- based on the signals at δ_H 4.56 (2H, *d*, *J* = 6.7 Hz, CH_2-1'"), 5.49 (1H, *m*, H-2'"), 1.76 (3H, *s*, 4"'-CH_3) and 1.80 (3H, *s*, 5"'-CH_3) together with the corresponding ^{13}C signals at δ_C 65.2 (C-1'"), 120.0 (C-2'"), 138.2 (C-3'") and two methyl carbon signals at δ_C 18.2 (4"'-CH_3) and 25.8 (5"'-CH_3). The attachment of the prenyloxy group at C-4' (δc 159.2) was confirmed by the HMBC correlation (Table 20) of H-3'/5' (δ_H 6.96) to C-4' (δc 159.2). On the basis of the above data and comparison with related compounds (Derese *et al.*, 2014; Leu *et al.*, 2012), compound **24** characterised as 7,8-(2"-isopropenyl-2",3"-dihydrofuran)-4'-prenyloxyisoflavone, a new compound named 4'-prenyloxyvigvexin A. The absolute configuration at C-2" has not been determined.

Table 20: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data together with HMBC correlations in compounds **24** (CD_2Cl_2)

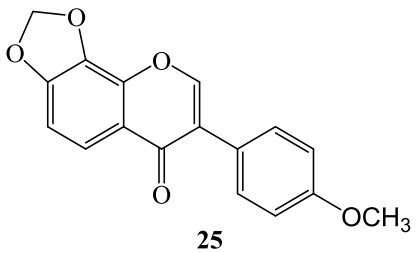
Carbon No.	δ_H	δ_C	HMBC (H→C)
2	7.91 (*s*)	152.0	C-3, C-4, C-8a, C-1'
3		124.9*	
4		175.9	
4a		119.1	
5	8.09 (*d*, *J* = 8.6 Hz)	128.0	C-4, C-7, C-8a
6	6.92 (*d*, *J* = 8.6 Hz)	108.6	C-4a, C-7 (w), C-8
7		165.2	
8		113.5	
8a		153.9	
1'		124.7*	
2'/6'	7.45 (*d*, *J* = 8.8 Hz)	130.5	C-3, C-1', C-2'/6', C-3'/5' (w), C-4'
3'/5'	6.96 (*d*, J= 8.8 Hz)	114.8	C-1', C-3'/5', C-4'
4'		159.2	
1"-α	3.22 (*dd*, *J* = 15.8, 9.9 Hz)	31.8	C-7, C-8, C-8a, C-2" (w), C-3"
1"-β	3.58 (*dd*, *J* = 15.8, 9.9 Hz)		C-7, C-8, C-8a, C-2" (w), C-3"
2"	5.43 (*br t*, *J* = 8.9 Hz)	88.2	C-7, C-3", C-5"', C-1", C-4"
3"		143.7	
5"-α	4.98 (*m*)	112.7	C-2", C-3" (w), C-4"
5"-β	5.13 (*m*)		C-2", C-3" (w), C-4"
1"'	4.56 (2H, *d*, *J* = 6.7 Hz)	65.2	C-4', C-2"', C-3"'
2"'	5.49 (*m*)	120.0	4"', 5"'
3"'		138.2	
4"	1.80 (*s*)	18.2	C-2", C-3", C-5"
4"'	1.80 (*s*)	25.8	C-2"', C-3"', C-5"'
5"'	1.76 (*s*)	18.2	C-2"', C-3"', C-4"'

*4.3.2. Maximaisoflavone H (**25**)*

Compound **25** was obtained as white crystals and had a melting point of 205-206 °C. LCMS analysis showed a molecular ion peak at *m/z* 296.0 corresponding to the molecular formula C_{17} H_{12} O_5. The UV (λ_{max} 261, 290 and 325 nm), ^{1}H NMR (δ_H 7.96 for H-2) and ^{13}C NMR (δ_C 151.7 for C-2, 124.1 for C-3 and 171.5 for C-4) spectra of compound **25** is characteristic of isoflavones. In ring A, *ortho* coupled protons at δ_H 7.87 (*d, J* = 8.5 Hz) and 7.02 (*d, J* = 8.6Hz) were assigned to H-5 and H-6, respectively of ring A which is substituted at C-7 and C-8. This substituent was identified as a methylenedioxy group as shown by the presence of a sharp singlet at δ_H 6.25 (2H, *s*) with the corresponding carbon appearing at δ_C 103.5. The placement of the methylenedioxy group was confirmed from the HMBC correlation of the signals at δ_H 7.87 (H-5) and 7.02 (H-6) with carbon signal at δ_C 141.2 (C-7) and 103.5 (C-8). In ring B, the aromatic proton signals at δ_H 7.01 (*d, J* = 8.6 Hz, for H-2'/6') and 7.51 (*d, J* = 8.8 Hz, H-3'/5') formed an AA'XX' spin system with C-4' (δ_C 159.7) substituted with a methoxy group (δ_H 3.88, δ_C 55.2). The nature of ring B was confirmed by HMBC correlation of H-2'/6' and H-3'/5' with C-4' (δ_C 159.7). Based on the above spectroscopic data and comparison with literature, compound **25** was identified as maximaisoflavone H; previously reported from *Millettia dura* (Yenesew *et al.*, 1996) and *Millettia ferruginea* (Dagne *et al.*, 1990).



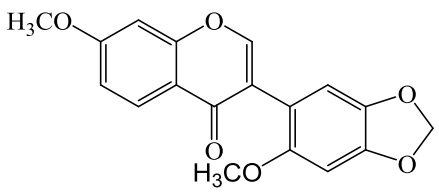
24



25

*4.3.3. 7,2'-Dimethoxy-3',4'-methylenedioxyisoflavone (**26**)*

Compound **26** was isolated as a white amorphous solid, mpt. 215-216 °C; it showed a molecular ion peak at *m/z* 326.0781 corresponding to the molecular formula $C_{18}H_{14}O_6$. The UV (λ_{max} 249, 273 and 306 nm), ^1H and ^{13}C NMR spectra (Table 21) displayed characteristics features of an isoflavone skeleton substituted with two methoxy groups (δ_H 3.96, δ_C 55.9 and δ_H 3.76, δ_C 56.6) and a methylenedioxy (δ_H 6.01, δ_C 101.5) groups. In ring A, the ^1H NMR spectrum showed an AXY spin system at δ_H 8.15 (*d*, *J* = 8.9 Hz, H-5), 7.03 (*dd*, *J* = 8.9, 2.4 Hz, H-6) and 6.93 (*d*, *J* = 2.39 Hz, H-8), with the corresponding carbon atoms (from HSQC spectrum) appearing at δ_C 127.3 (C-5), 114.3 (C-6) and 100.1 (C-8) respectively. With one of the methoxy groups fixed at C-7 (from HMBC correlation of methoxy protons with C-7), the second methoxy and the methylenedioxy group must be located in ring B. The ^1H NMR spectrum showed two singlet protons at δ_H 6.69 and 6.84 and assigned to H-3' and H-6', respectively for ring B. This suggested the placement of the methylenedioxy at C-4'/C-5' and the second methoxy at C-2'. In the HMBC spectrum, the two singlet protons at δ_H 6.69 and 6.84 showed correlation with C-2', C-4' and C-5' confirmed the substitution pattern in ring B. Therefore compound **26** was identified as 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone, a compound which has been isolated from the genus *Millettia* (Kapingu *et al.*, 2006; Marco *et al.*, 2017). This is the first report on the occurance of compound **26** in the genus *Lonchocarpus*.



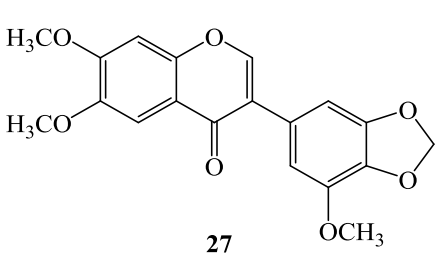
26

Table 21: ^{1}H (500 and 600 MHz) and ^{13}C (125 and 150 MHz) NMR data together with HMBC correlations in compound **25** and **26** (CD_2Cl_2)

Carbon No.	**25**			**26**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	7.96 (*s*)	151.7	C-3, C-4, C-8a	7.94 (*s*)	154.1	C-3, C-4, C-8a, C-1'
3		124.1			112.9	
4		171.5			175.3	
5	7.87 (*d*, *J* = 8.5 Hz)	120.5	C-4, C-6, C-7, C-8a	8.15 (*d*, *J* = 8.8 Hz)	127.3	C-4, C-7, C-8a
5a		-			118.3	
6	7.02 (*d*, *J* = 8.6 Hz)	107.1	C-6, C-7, C-8	7.03 (*dd*, *J* = 8.8, 2.4 Hz)	114.3	C-5a, C-7, C-8
7		141.2			163.9	
7/8	6.25 (2H, *s*)	103.5	C-8, C-8a		146.3	
8		134.6		6.93 (*d*, *J* = 2.3 Hz)	100.1	C-4, C-5, C-5a, C-8a, C-7
8a		152.2			157.9	
1'		124.3			121.9	
2'					152.9	
2'/6'	7.01 (*d*, *J* = 8.6 Hz)	113.7	C-1', C-4'			
3'				6.69 (*s*)	95.1	C-3, C-2', C-4', C-5'
3'/5'	7.51 (*d*, *J* = 8.8 Hz)	130.2	C-1', C-2'/6', C-4'		156.1	
4'		159.7			148.3	
4'/5'				6.01 (2H, *s*)	101.5	C-4', C-5'
5'					141.0	
6'				6.84 (*s*)	111.0	C-1', C-2', C-4' C-5'
4'OCH_3	3.88 (*s*)	55.2	C-4'			
2'OCH_3				3.76 (*s*)	56.6	C-6'
7 OCH_3				3.96 (*s*)	55.9	C-7

*4.3.4. 6,7,3'-Trimethoxy-4',5'-methylenedioxyisoflavone (**27**)*

Compound **27** was obtained as a white solid, mpt. 209-210 °C. The HRESIMS showed molecular ion peak at m/z 356.0914 which matches the molecular formula $C_{19}H_{16}O_7$. The UV spectrum of this compound exhibited the absorption maximum at 270 and 325 nm, which together with NMR data (Table 22) suggested an isoflavone skeleton. The presence of three methoxy (δ_H 3.99, 4.00 and 3.95) and a methylenedioxy (δ_H 6.03) groups were evident from the ^{1}H NMR spectra (Table 22). In ring A, the ^{1}H NMR spectrum further revealed the presence of two aromatic protons at δ_H 7.60 (*s*), 6.94 (*s*), assigned to H-5 and H-8, respectively, with C-6 and C-7 substituted. The two singlets in ring A correlated to C-6 (δ_C 147.9) and C-7 (δ_C 154.6) which in turn correlated with the signal for the methoxy protons. This therefore meant that one methoxy and the methylenedioxy groups are located in ring B. The ^{1}H NMR spectrum showed two *meta*-coupled protons at δ_H 6.81 (*d, J* = 1.5 Hz) and 6.77 (*d, J* = 1.4 Hz) assigned to H-2' and H-6' with the methylenedioxy group placed at C-4'/5' and the methoxy at C-3'. The chemical shift values of the oxygenated carbon atoms are consistent with oxygenation at C-3' (δ_C 143.5), C-4' (δ_C 135.2) and C-5' (δ_C 148.7). In agreement with this, in the HMBC spectrum, the methylenedioxy protons at δ_H 6.03 correlated with C-4' (δ_C 135.2) and C-5' (δ_C148.7). In the same manner, the two mutually coupled protons in ring B showed correlation with the sp^2 carbon signals for at C-3' (δ_C 143.5) C-4' (δ_C 135.2) and C-5' (δ_C 148.7). Therefore, this compound was identified as 6,7,3'-trimethoxyl-4',5'-methylenedioxyisoflavone. This compound was previously reported from the heartwood of *Cordyla africana* (Campbell *et al.*, 1969). However, this is the first report of its occurrence in the genus *Lonchocarpus*.



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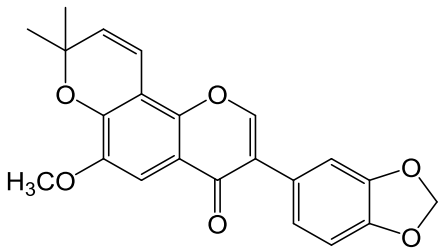

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*4.3.5. Durmillone (**28**)*

Compound **28** was isolated as a white amorphous solid, m pt. 195-196 °C. Its molecular formula was determined as $C_{22}H_{18}O_6$ (HREIMS ([M^+] at *m/z* 378. 1107) and NMR data (Table 22). The UV (λ_{max} 263, 230 and 348 nm) and NMR spectra of compound **28** is characteristic of an isoflavone. Thus ^{1}H and ^{13}C NMR spectra further showed singnals at δ_H 7.99 (for H-2) and δ_C 151.8 (for C-2, from HSQC spectrum). The AXY spin system [at δ_H 7.13 (*d, J* = 1.6 Hz, H-2ˈ), 6.92 (*d, J* = 7.9 Hz, H-5ˈ) and 7.02 (*dd, J* = 8.0, 1.7 Hz, H-6ˈ)], along with a methylenedioxy group (δ_H 6.04, δ_C 101.3) belong to ring B. In agreement with this, HMBC spectrum showed correlation of H-2' with C-3 (δ_C 124.0), C-1ˈ (δ_C 126.1), C-3ˈ (δ_C 147.5) and C-4ˈ (δ_C 147.5).

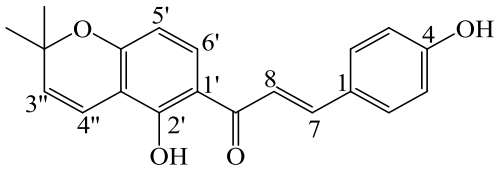
The presence of a singlet aromatic proton signal at δ_H 7.54 (*s*) indicates that ring A is tri-substituted with a methoxy (δ_H 3.98 and δ_C 56.1) at C-6 and a 2",2"-dimethylpyran [δ_H 5.81 (*d, J* = 10.0 Hz, H-3"), 6.85 (*d, J* = 10.0 Hz, H-4") and methyl protons δ_H 1.46 (*s*)] at C-7/C-8 groups. In agreement with this, the only proton in this ring H-5 (δ_H 7.54) showed HMBC correlation with the carbonyl carbon signal (δ_C 175.0), quaternary carbon at 117.4 (δ_C C-5a) and oxygenated carbon δ_C 147.2 (C-6). Similarly, the placement of the 2",2"-dimethylpyran group at C-7 (δ_C 147.2) and C-8 (δ_C 110.2) was confirmed by HMBC correlation of H-3" (δ_H 5.81) to C-8a (147.2), as well as H-4" to C-7 (147.2) and C-8 (110.2). This compound was therefore identified as durmillone, previously reported from *Millettia oblata* and *Millettia dura* (Dhooghe *et al.*, 2010; Yenesew, *et al.*, 1996).

Table 22: ^{1}H (600) and ^{13}C (150 MHz) NMR data along with HMBC correlations for compounds **27** and **28** (CD_2Cl_2)

Carbon No.	**27**			**28**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	8.00 (*s*)	152.1	C-3, C-4, C-8a	7.9 9 (*s*)	151.8	C-3, C-4. C-8a
3		124.2			124.0	
4		174.9			175.0	
5a		117.6			117.4	
5	7.60 (*s*)	104.5	C-5a, C-6	7.54 (*s*)	104.8	C-4. C-5a, C-6
6		147.9			147.2	C-6, C-7, C-8
7		154.6			147.2	
8	6.94 (*s*)	99.5	C-4, C-5a, C-6, C-7, C-8a,		110.2	
8a		152.2			147.2	
1'		125.5			126.1	
2'	6.81 (*d*, *J* = 1.5 Hz)	108.8	C-3, C-6', C-4', C-5'	7.13 (*d*, *J* = 1.6 Hz)	109.8	C-4', C-6'
3'		143.5			147.5	
3'/4'				6.04 (2H, *s*)	101.3	C-3'/4'
4'		135.2			147.5	
4'/5'	6.03 (2H, *s*)	101.6	C-4', C-5'			
5'		148.7		6.92 (*d*, *J* = 7.9 Hz)	108.1	C-1', C-3'
6'	6.77 (*d*, *J* = 1.4 Hz)	103.7	C-3, C-2', C-4', C-5'	7.02 (*dd*, *J* = 8.0, 1.7 Hz)	122'3	C-3, C-2', C-4'
2"					78.0	
3"				5.81 (*d*, *J* = 10.0 Hz)	130.4	2"-CH_3, C-4", C-8a
4"				6.85 (*d*, *J* = 10.0 Hz)	114.9	2"-CH_3, C-7, C-8
3'-OCH_3	3.95 (*s*)	56.6	C-3'			
6 -OCH_3	3.99 (*s*)	56.3	C-6	3.98 (*s*)	56.1	C-6
3"-CH_3				1.46 (2xCH_3, *s*)	27.7	2"-CH_3, C-2", C-3"

*4.3.6. 4-Hydroxylonchocarpin (**29**)*

Compound **29** was obtained as yellow crystals, m pt. 206-208 °C. The HREIMS showed $[M]^+$ peak at *m/z* 322.1215 suggesting the molecular formula $C_{20}H_{18}O_4$. The UV (λ_{max} 285, 311 and 363 nm), ^1H NMR (δ_H 7.88, *d*, *J* = 15.4 Hz, H-α; 7.78, *d*, *J* = 15.4 Hz for H-β) and ^{13}C NMR (δ_C 144.6 for C-α, 131.0 for C-β and 192.3 for C=O) spectra were consistent with an α,β-unsaturated moiety of a chalcone skeleton. The ^1H NMR spectrum further showed an AA'XX' spin system of aromatic proton signals at δ_H 7.77 (*d*, *J* = 8.0 Hz) and 6.96 (*d*, *J* = 8.7 Hz) assigned for H-2/6 and H-3/5 of ring A, respectively, which is substituted with a hydroxy group at C-4 (δ_C 159.5). Two mutually coupled aromatic proton signals at δ_H 6.40 (*d*, *J* = 8.9 Hz) and 8.08 (*d*, *J* = 8.9 Hz) represented for H-5' and H-6' of ring B, which is substituted with a hydroxy (δ_H 14.11) at C-2' (δ_C 160.8) and a 2'',2''-dimethylpyran group [δ_H 5.73 (*d*, *J* = 10.0 Hz), 6.73 (*d*, *J* = 10.0 Hz) and two methyl groups at δ_H 1.47 (*s*)] at C-3'/C-4'. The placement of this group was confirmed based on the HMBC correlation of H-3'' (δ_H 5.73) and H-4'' (δ_H 6.73) with C-3' (δ_C 109.0) and C-4' (δ_C 160.2). Hence compound **29** was characterized as 4-hydroxylonchocarpin, a chalcone previously isolated from *Millettia ferruginea* (Dagne *et al.*, 1990). This appears to be the first report from the genus *Lonchocarpus*.



29

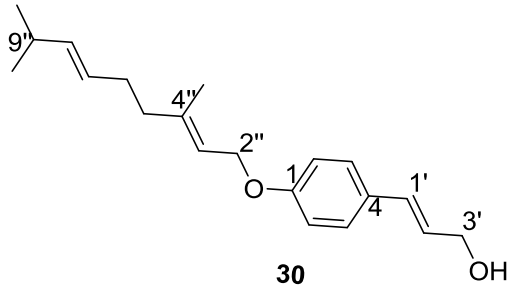
Table 23: ^{1}H (600) and ^{13}C (150 MHz) NMR data together with HMBC correlations in compound **29** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC
1		126.6	
2/6	7.77 (*d*, *J* = 8.0 Hz)	117.2	
3/5	6.96 (*d*, *J* = 8.7 Hz)	115.9	C-1, C-4
4		159.5	
7	7.88 (*d*, *J* = 15.4 Hz)	144.6	C-2/6, C-9
8	7.78 (*d*, *J* = 15.4 Hz)	131.0	C-1, C-9
9		192.3	
1'		114.0	
2'		160.8	
3'		109.0	
4'		160.2	
5'	6.40 (*d*, *J* = 8.9 Hz)	107.9	C-4', C-6', C-9 C-1', C-3
6'	8.08 (*d*, *J* = 8.9 Hz)	131.3	C-1', C-4', C-5', C-9
2"		77.5	
3"	5.73 (*d*, *J* = 10.0 Hz)	128.3	2-"CH_3, C-2", C-3', C-4'
4"	6.73 (*d*, *J* = 10.0 Hz)	115.4	2''-CH_3, C-2'' C-3', C-4'
2" $(CH_3)_2$	1.47 (*s*)	27.6	2"-CH_3, C-2", C-3"
2'-OH	14.11 (*s*)		C-2', C-3', C-4'

*4.3.7. Colenemol (**30**)*

Colenemol (**30)** was isolated as pale yellow paste, and showed UV absorption maximum at 273 nm. The HREIMS ($[M]^+$ at *m/z* 286.19939) together with NMR data suggested the molecular formula as $C_{19}H_{26}O_2$. The presence of a AA'XX' spin system at δ_H 7.43 (*d*, *J* = 8.6 Hz) and 6.90 (*d*, *J* = 8.8 Hz) assigned to H-2/6 and H-3/5, respectively, in the aromatic region of the ^{1}H NMR spectrum suggested that compound **30** is a 1,4-disubstituted benzene derivative. The ^{1}H NMR spectrum also displayed signals at δ_H 4.58 (*m*), 5.50 (*tdd*, *J* = 5.2, 2.5, 1.3 Hz), 1.78 (*s*), 2.12 (*m*), 2.18 (*m*), 5.15 (*tdd*, *J* = 5.6, 2.8, 1.4 Hz), 1.72 (*s*), 1.66 (*s*) indicating that one of the substituents is a geranyloxy group. Another set of proton signals at δ_H 6.58 (d, *J* = 15.9 Hz, H-1'), 6.28 (*dt*, *J* = 15.9, 5.9 Hz, H-2') and 4.30 (*dd*, *J* = 5.8, 1.5 Hz, H-3') were assigned to 3-hydroxy-1-propenyl group. HMBC correlation of H-1' and H-2' with

C-4 (δ_H 129.4) showed the position of the 3-hydroxy-1-propenyl moiety is at C-4; and the geranyloxy was then placed at C-1 (δ_C 156.8). This was supported by HMBC correlation of H-2/6 (δ_H 7.43) and H-3/5 (δ_H 6.90) to C-4 (δ_C129.1) and C-1 (δ_C 156.8). Hence, based on these spectroscopic data and comparison with literature, compound **30** was characterized as (E)-3-(4-(((E)-4,8-dimethylnona-3,7-dien-1-yl)oxy)phenyl)prop-2-en-1-ol, common name colenemol. This compound was first reported from *Coleonema pulchellum* (Brader *et al.*, 1997) and later from the genus *Millettia* (Deyou *et al.*, 2015).



30

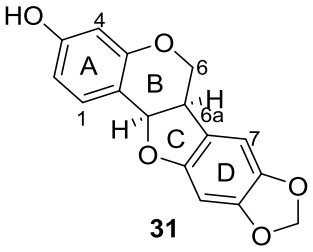
Table 24: ^{1}H (600) and ^{13}C (150 MHz) NMR data together with HMBC correlations for compound **30** (CD_2Cl_2)

Carbon No.	δ_H	δ_C	HMBC
1		156.8	
2/6	7.43 (*d*, *J* = 8.6 Hz)	127.4	C-1, C-3/C-5,C-4
3/5	6.90 (*d*, *J* = 8.7Hz)	114.3	C-2/C-6,C-4, C-1
4		129.4	
1'	6.58 (*d*, *J* = 15.8 Hz)	130.3	C-3', C-4
2'	6.28 (*dt*, *J* = 15.9, 5.86, 5.9 Hz)	126.5	C-3', C-4
3'	4.30 (*dd*, *J* = 5.8,1.4 Hz)	63.6	C-1', C-2'
2"α	4.58 (*m*)	64.9	5"-CH_3, C-4", C-6"
2"β			
3"	5.50 (*tdd*, *J* = 5.2, 2.5, 1.3 Hz)	119.5	5"-CH_3, C-7"
4"		25.3	
6"α	2.12 (*m*)	39.4	5"-CH_3, C-7"
6"β			
7"α	2.18 (*m*)	26.3	C-6", C-8", C-9"
7"β			
8"	5.15 (*tdd*, *J* = 5.6, 2.8, 1.4 Hz, 1H)	123.7	C-6", 10" -CH3, 11"-CH3
9"		131.8	
5"CH_3	1.78 (*s*)	16.3	C-6"
10"- CH_3	1.66 (*s*)	17.3	11"- CH_3
11"- CH_3	1.72 (*s*)	25.3	10"- CH_3

*4.3.8. (6aR,11aR)-Maackiain (**31**)*

Compound **31** was isolated as brown paste, $[\alpha]_D^{21}$ −180° (*c* 0.07, acetone). Its molecular formula was assigned as $C_{16}H_{12}O_5$ from HREIMS which showed a molecular ion peak at *m/z* 284.0694 and NMR spectra (Table 25). The UV spectrum showed absorption maximum at 280, 286 and 313 nm indicating a conjugated double bond or aromatic ring. The presence of signals for a pyranofuran moiety at δ_H 5.51 (*d, J* = 7.0 Hz), 4.29 (*m*), 3.64 (*m*) and 3.58 (*m*) in the ^{1}H NMR alongside sp^3 hybridized carbon peaks at δ_C 78.4 (C-11a), 66.0 (C-6) and 40.1 (C-6a) of a pterocarpan derivative. The NMR spectra further showed the presence of hydroxy (δ_C 158.7) and a methylenedioxy (δ_H 5.96, *d, J* = 1.1 Hz and δ_H 5.93, *d, J* = 1.1 Hz; δ_C 101.2) groups. The AXY spin system at δ_H 7.32 (*d, J* = 8.4 Hz, H-1), 6.58 (*dd, J* = 8.37, 2.4 Hz, H-2) and 6.38 (*d, J* = 2.4 Hz, H-4) with the corresponding (from HSQC spectrum) carbon signals appearing at δ_C 132.6 (C-1), 109.5 (C-2) and 103.0 (C-4) is consistent with a mono substituted (OH-3) ring A. HMBC correlation of H-1 (δ_H 7.32) and H-2 (δ_H 6.58) to an oxygenated carbon C-3 (δ_C 158.7) confirmed the identity of ring A. In addition, the ^{1}H NMR spectrum showed two singlet aromatic proton signals at δ_H 6.92 (H-7) and 6.42 (H-10) indicating that ring B is substituted with methylenedioxy group. This was confirmed by HMBC correlation of H-7 (δ_H 6.92) and H-10 (δ_H 6.42) with C-8 (δ_C141.5) and C-9 (δ_C 148.0).

The high negative specific rotation, $[\alpha]_D^{21}$ −180°, is consistent with (6a*R*,11a*R*) absolute configuration (Yenesew *et al.*, 1998c). This was further confirmed by the experimental ECD spectrum (Figure 7) which showed Cotton effects at 305 nm consistant with (6a*R*,11a*R*) absolute configuration (Adem *et al.*, 2018) (**33**). Therefore, compound **31** was characterized as (6a*R*,11a*R*)-9-hydroxyl-3,4-methylenedioxypterocarpan (trivial name (+) maackiain), which has been reported from the roots of *Onionis vaginalis* (Abdel-Kader, 2001). This is the first report of its occurrence in the genus *Lonchocarpus*.

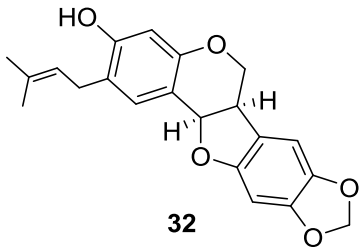


31

*4.3.9. (6aR,11aR)-Edunol (**32**)*

Compound **32** was isolated as a colourless paste, $[\alpha]_D^{21}$ −222° (*c* 0.125, acetone). The molecular formula $C_{21}H_{20}O_5$ was determined from HREIMS which showed $[M]^+$ *m/z* 352.1315 and NMR data (Table 25). As in compound **31**, the UV (λ_{max} 280, 286 and 311 nm) together with the ^{1}H NMR [δ_H 5.48 (*d, J* = 6.7 Hz, 1H, H-11a), 4.25 (*m*, 1H, H-6α), 3.57 (*m*, 1H, H-6β) and 3.53 (*ddd, J* = 10.3, 6.8, 4.3 Hz, 1H, H-6a)] and ^{13}C NMR [δ_C 78.6 (C-11a), 66.1 (C-6) and 40.2 (C-6a)] indicated that compound **32** has a pterocarpan skeleton. The ^{1}H NMR spectrum showed four singlet aromatic proton signals at δ_H 7.18, 6.90, 6.42 and 6.40 indicating a 2,3-disubstituted ring A, and 8,9-disubstituted ring D. The proton signals at δ_H 6.90 (δ_C 105.0) and δ_H 6.42 (δ_C 93.0) were assigned to H-7 and H-10 in ring B respectively, whereas δ_H 7.18 (δ_C 131.5) and δ_H 6.40 (δ_C 102.6) represented for H-1 and H-4 of ring A. Another set of proton signal at δ_H 3.31 (*d, J* = 7.4 Hz, δ_C 27.5), 5.37 (*m*, δ_C 123.0) and methyl at δ_H 1.77 (δ_C 16.9, 25.0) are due to the presence of prenyl group. The placement of the prenyl group at C-2 (δ_C 118.7) in ring A was determined by the HMBC correlation of the methylene protons (CH_2-1', δ_H 3.31) of the prenyl group to C-1 (δ_C 131.5), whereas H-2' (δ_H 5.37) correlated to quaternary carbon peak at δ_C 118.7 (C-2). The substituent in ring D was determined to be a methylenedioxy group from the proton signal at δ_H 5.94 (*s*) with the corresponding carbon appearing at δ_C 101.2. In addition to these, the HMBC spectrum showed the singlet aromatic proton correlating to C-8 (141.5) and C-9 (147.9). Additional proton signals δ_H 6.90 (H-7) and δ_H 6.42 (H-10) in ring B correlated to C-8 (141.5) and C-9 (147.9). The high negative specific rotation, $[\alpha]_D^{21}$ −222°, and the experimental ECD

spectrum (Figure 8) which showed a positive Cotton effect at 305 nm suggested a (6a*R*,11a*R*) absolute configuration (Adem *et al.*, 2018). Therefore, based on the above data and comparison with literature, this compound was identified as (6a*R*,11a*R*)-edunol, previously isolated from *Brongniartia podalyrioides* (Reyes-Chilpa *et al.*, 1994). This is the first report on the occurance of compound **32** from the genus *Lonchocarpus*.



32

Table 25: ^{1}H (600) and ^{13}C (150 MHz) NMR data together with HMBC correlations for compounds **31** and **32** (acetone-d_6)

Carbon No.	**31**			**32**		
	δ_H	δ_C	HMBC (H→C)	δ_H	δ_C	HMBC (H→C)
1	7.32 (*d*, *J* = 8.4 Hz)	132.6	C-4a, C-4, C-11a	7.18 (*s*)	131.5	C-4, C-4a, C-11a
1a		115.4			111.5	
2	6.58 (*dd*, *J* = 8.3, 2.4 Hz)	109.5	C-1a, C-3, C-4		118.7	
3		158.7			-	
4	6.38 (*d*, *J* = 2.4 Hz)	103.0	C-1a, C-2, C-4a	6.40 (*s*)	102.6	C-1a, C-4a
4a		156.8			154.4	
6	3.64 (*m*)	66.0	C-4a, C-6a, C-7a, C-11a	3.57 (*m*)	66.1	C-4a , C-6a, C-7a, C-10a,
	4.29 (*m*)			4.25 (*m*)		C-11a
6a	3.58 (*m*)	40.1	C-6, C-7a, C-10a	3.53 (*m*)	40.2	C-6, C-7a
6b		118.6				
7	6.92 (*s*)	105.0	C-6a, C-8, C-9, C-10	6.90 (*s*)	105.0	C-6a, C-8, C-9, C-10
8/9	5.96 (*d*, *J* = 1.0 Hz)	101.2	C-8, C-9	5.94 (2H, *s*)	101.2	C-8, C-9
	5.93 (*d*, *J* = 1.0 Hz)					
8		148.0			147.9	
9		141.5			141.5	
10	6.42 (*s*)	93.0	C-7a, C-8, C-9, C-10a	6.42 (*s*)	93.0	C-7a, C-8, C-9, C-10a
10a		154.4			154.6	
11a	5.51 (*d*, *J* = 7.0 Hz)	78.4	C-1a, C-1, C-4a, C-6, C-6a	5.48 (*d*, *J* = 6.7 Hz)	78.6	C-1, C-1a, C-4a, C-6, C-6a, C-7a
1'				3.31 (*d*, *J* = 7.4 Hz)	27.5	C-2', C-1
2'				5.37 (*m*)	123.0	CH_3, C-1', C-2
3'					131.4	
3' $(CH_3)_2$				1.77 (2 CH_3, *s*)	16.9	CH_3, C-2', C-3'
					25.0	

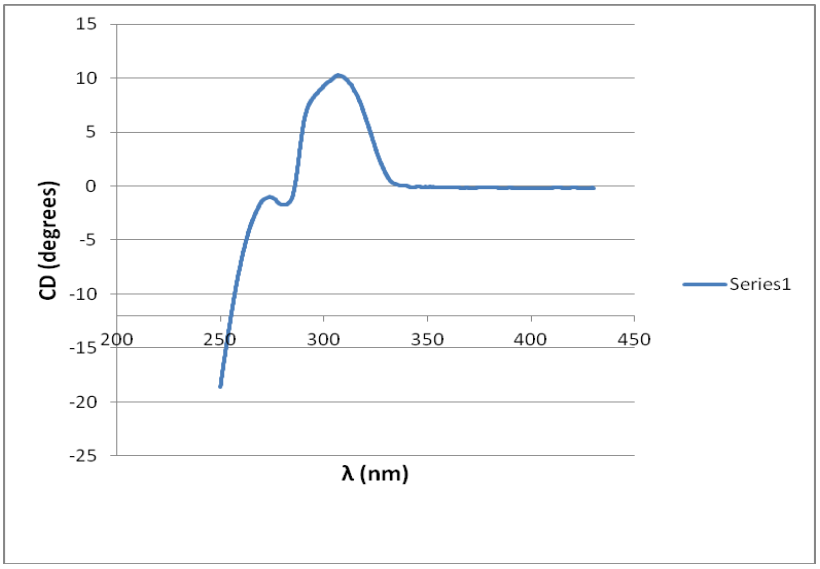


Figure 7: Experimental ECD spectra of (6a*R*,11a*R*)- Maackiain (**31**)

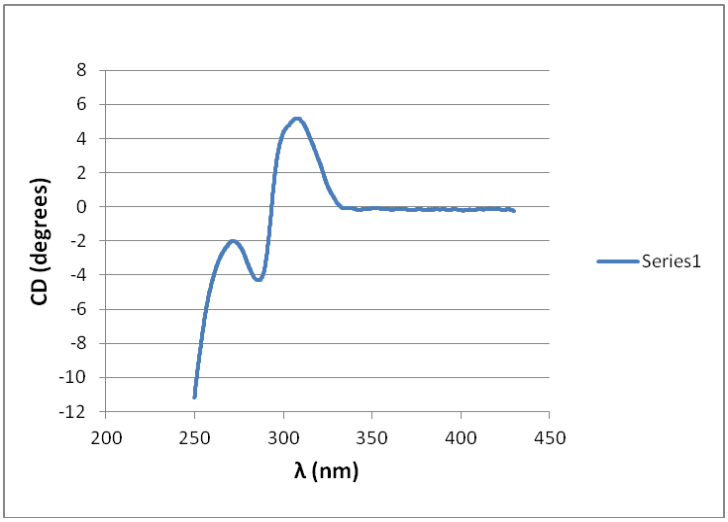


Figure 8. Experimental ECD spectrum of (6a*R*,11a*R*)-edunol (**32)**

4.4. Compounds Isolated from *Lonchocarpus eriocalyx*

Chromatographic separation of the stem bark extract of *Lonchocarpus eriocalyx* afforded a new pterocarpan, (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**33**) along with a known pterocarpan (6a*R*,11a*R*)-edunol (**32**).

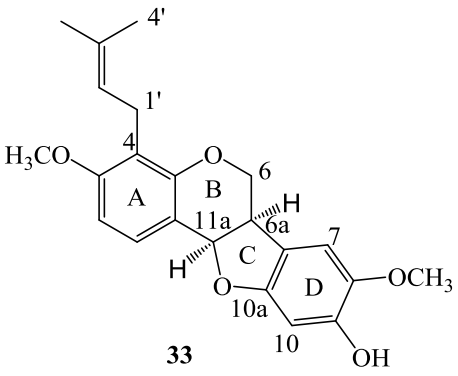
*4.4.1. (6aR,11aR)-3,8-Dimethoxybitucarpin B (**33**)*

Compound **33** was isolated as a pale yellow gum. The HREIMS of compound **33**, $[\alpha]_D^{22}$ −114°, showed a molecular ion peak at *m/z* 368.1626, which along with NMR data (Table 26) suggested the molecular formula $C_{22}H_{24}O_5$. The UV (λ_{max} 288 and 304 nm), ^{1}H NMR spectrum [δ_H 3.60 and 4.34 (CH_2-6), 3.58 (H-6a), and 5.48 (H-11a)], with the corresponding carbon signals (from HSQC spectrum) appearing at δ_C 68.3 (C-6), 41.9 (C-6a) and 80.0 (C-11a), suggested that compound **33** has a pterocarpan skeleton. The presence of a prenyl, two methoxy and a hydroxy substituent was also evident from the NMR spectra (Table 26). In ring-A, the ^{1}H NMR spectrum showed two *ortho*-coupled aromatic protons at δ_H 6.71 (*d*, *J* = 8.5 Hz) and 7.31 (*d*, *J* = 8.5 Hz), the latter of which showed HMBC correlation with C-3 (δ_C 159.7), C-4a (δ_C 155.6) and C-11a (δ_C 80.0), allowing the assignment of this signal (δ_H 7.31) to H-1 and its coupling partner (δ_H 6.71) to H-2. One of the methoxy protons (δ_H 3.84) showed NOE correlation with H-2 (δ_H 6.71), and HMBC correlation with C-3 (δ_C 159.7) and hence placed at C-3. The methylene protons of the prenyl group which showed HMBC correlation with C-3 (δ_C 159.7), C-4 (δ_C 118.9) and C-4a (δ_C 155.6) was consistent with its placement at C-4.

In ring-D, two singlet aromatic protons resonating at δ_H 7.02 and 6.33 were assigned to H-7 and H-10, respectively, indicating that ring D is 8,9-disubstituted with hydroxyl and methoxy groups. This was supported in the HMBC spectrum where both H-7 and H-8 ((δ_C 143.4) and C-9 (δ_C 149.2). The methoxy group (δ_H 3.80) in this ring showed NOE correlation with H-7

(δ_H 7.02) and HMBC correlation with C-8 (δ_C 143.4) and hence was placed on this carbon, leaving the hydroxy group to be located on C-9. Compound **33** was therefore characterised as 9-hydroxy-3,8-dimethoxy-4(3',3'-dimethylallyl)pterocarpan, named 4,9-dimethoxybitucarpin B.

The high negative specific rotation, $[\alpha]_D^{22}$ −114°, and the experimental ECD spectrum (Figure 8) which showed a positive Cotton effect at 300 nm suggested (6a*R*,11a*R*) absolute configuration (Yenesew *et al.*, 1998c). In order to ascertain this, the energies of different conformers of (6a*R**,11a*R**)-**33** were calculated and the one with minimum energy and two immediately above it with ΔE < 0.99 kcal/mol were identified (Figure 9). Then the ECD spectra for these conformations were calculated separately and compared with experimental ECD of **33**. The weighed Boltzmann sum of calculated for three energy minimum confirmations of (6a*R*,11a*R*)-**33** showed a positive cotton effect at *ca* 300 nm as in the experimental ECD spectrum (Figure 10), confirming this configuration. Therefore on the basis of the above evidence, this new compound was characterised as (6a*R*,11a*R*)-4,8-dimethoxybitucarpin B.



33

Table 26: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data and HMBC correlations in compound **33** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC (H→C)
1	7.31 (*d*, *J* = 8.5 Hz)	130.7	C-3, C-4a, C-11a
1a		115.4	
2	6.71 (*d*, *J* = 8.6 Hz)	106.1	C-1a, C-3, C-4, C-4a (w)
3		159.7	
4		118.9	
4a		155.6*	
6	3.60 (*m*)	68.3	C-4a, C-6a, C-11a
	4.34 (*m*)		C-4a, C-6a, C-6b, C-11a
6a	3.58 (*m*)	41.9	C-6, C-6b, C-10a
6b		118.6	
7	7.02 (*s*)	111.2	C-6b, C-8, C-9, C-10
8		143.4	
9		149.2	
10	6.33 (*s*)	99.3	C-6b, C-8, C-9, C-10a
10a		155.7*	
11a	5.48 (*d*, *J* = 6.6 Hz)	80.0	C-1, C-1a, C-4a, C-6, C-6a, C-6b
1'	3.30 (*m*)	26.3	C-3, C-4, C-4a, C-2', C-3'
2'	5.15 (*m*)	124.4	C-1a, C-4, C-1', C-4', C-5'
3'		131.8	
4'	1.73 (*s*)	18.5	C-2', C-3', C-5'
5'	1.60 (*s*)	26.5	C-2', C-3', C-4'
3-OCH_3	3.84 (*s*)	56.8	C-3
8-OCH_3	3.80 (*s*)	58.2	C-8

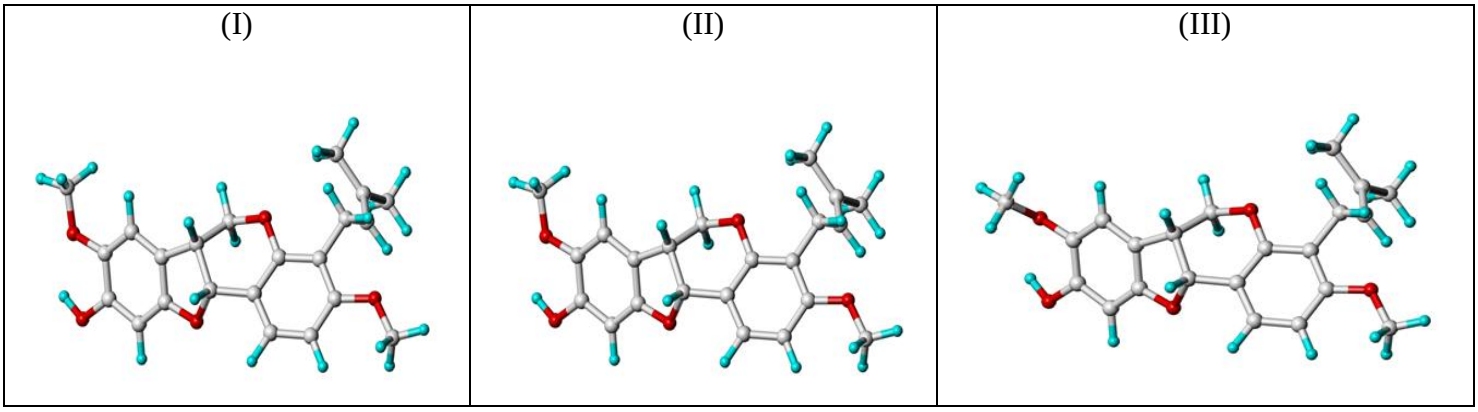


Figure 9: The calculated global energy minimum geometries of conformers of (6a*R*,11a*R*)-**33** (conformation I: global minimum, conformation II: + 0.10 kcal/mole, confirmation III: +0.99).

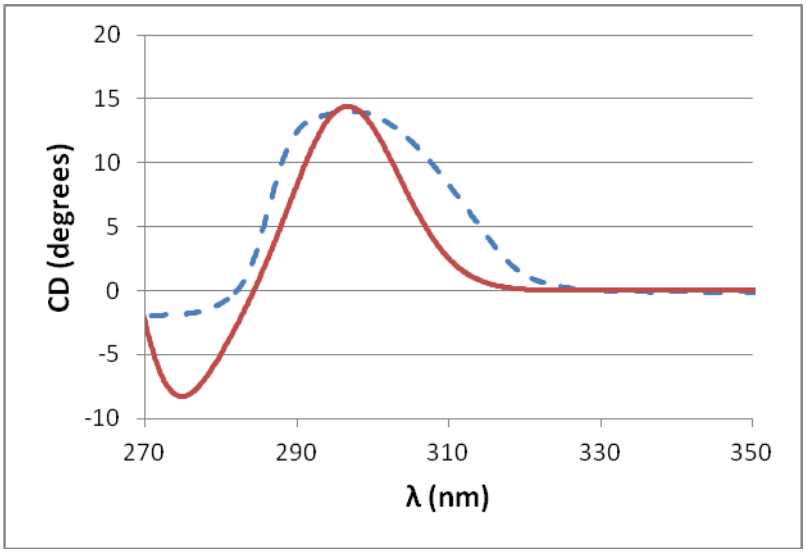


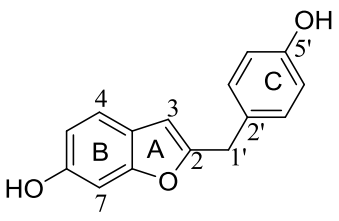
Figure 10: ECD spectra of 33: Experimental (blue broken line) and Boltzmann weighted calculation for the three energy minimum conformations of (6a*R*,11a*R*)-**33** (red solid line)

4.5. Compounds Isolated from *Dorstenia kameruniana*

Chromatographic separation of the CH_2Cl_2/MeOH extract of the roots of *Dorstenia kameruniana* yielded three new benzylbenzofuran derivatives, 2-(*p*-hydroxybenzyl)-6-hydroxybenzofuran (**34**), 2-(*p*-hydroxybenzyl)-6-hydroxy-7-methoxybenzofuran (**35**) and 2-(*p*-hydroxybenzyl)-6-hydroxy-4'-prenylbenzofuran (**36**) (named dorsmerunin A, B and C respectively), along with the known compound, bergapten (**37**). The twigs of this plant also produced compounds **34**-**37** as well as the known chalcone licoagrochalcone A (**38**).

*4.5.1. Dorsmerunin A (**34**)*

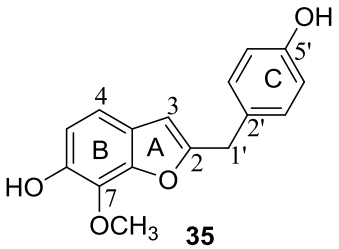
Compound **34** was obtained as a brown gum. HREIMS showed a molecular ion peak at *m/z* 240.0779, which together with NMR data (Table 27) allowed the assignment of the molecular formula $C_{15}H_{12}O_3$. The UV (λ_{max} 250, 258 and 287 nm), IR (Appendix 34D) and NMR data (Table 27) indicated that the compound is aromatic. That this is a benzyl benzofuran derivative (Franke *et al.*, 2001), was established from the NMR spectra: δ_H 6.33 for H-3; δ_C 158.7 for C-2, 104.1 for C-3 (for the furan ring) and δ_H 7.29 (*d*, *J* = 8.3 Hz), 6.74 (*dd*, *J* = 8.3, 2.1 Hz) and 6.87 (*d*, *J* = 1.8 Hz) assigned to H-4, H-5 and H-7, respectively, for ring B protons, which is substituted with a hydroxyl group at C-6 (ν_{max} at 3187 cm-1 for hydroxy band; δ_C 156.4 for C-6). The identity of ring B was confirmed from the HMBC spectrum (Figure 12) where the proton signals at δ_H 7.29, 6.74 and 6.87 showed HMBC correlation with the oxygenated carbon signal at δ_C 156.4 (C-6). The methylene protons signal at δ_H 3.97 (*s*) exhibited HMBC cross peak (Figure 13) with the signal at δ_C 158.7 (C-2), δ_C 104.1 (C-3) and δ_C 131.4 (C-3′) which was consistent with the attachment of benzyl group at C-2 (δ_C 158.7) of the furan ring. The AA'XX' spin system at δ_H 7.14 and δ_H 6.80 was assigned to H-3'/H-7' and H-4'/H-6', which indicates oxygenation (hydroxy group) at C-5' (δ_C 157.5) of ring C. Furthermore, the placement of the hydroxy substituent at C-5' was supported by the HMBC correlation of the proton signals at δ_H 7.14 (H-3'/H-7') and δ_H 6.80 (H-4'/H-6') with C-5′ (δ_C 157.5) (Figure 12). Thus, this new compound **34** was identified as 2-(*p*-hydroxy)-3-(3methylbut-2-en-1-yl)-benzofuran-6-ol, named dorsmerunin A.



34

*4.5.2. Dorsmerunin B (**35**)*

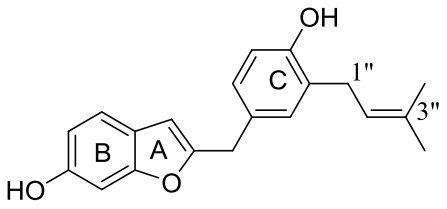
Compound **35** was isolated as a brown gum, and the molecular formula was established as $C_{16}H_{14}O_4$ from HREIMS analysis, which revealed a molecular ion peak at *m/z* 270.0891. The UV (λ_{max} 258 and 283 nm), and NMR spectra (Table 27) of **35** is similar to those of **34** indicating that compound **35** is also a benzylbenzofuran derivative. As in compound **34**, the presence of *p*-hydroxybenzyl group attached to C-2 of the benzofuran skeleton was evident from the NMR spectra (Table 27) and the MS fragment ion at *m/z* 107 ($[C_7H_7O]^+$). In ring B, the AMX spin system observed in compound **34** is now replaced by an AX spin system for H-4 (δ_H 7.00 (*d*, *J* = 8.3 Hz) and H-5 (δ_H 6.78 (*d*, *J* = 8.3 Hz), which indicated the presence of hydroxyl and methoxy (δ_H 4.01; δ_C 61.4) substituents at C-6 and C-7. The ^{13}C NMR chemical shift value of the methoxy group is down-field shifted (δ_C 61.4) which requires that it be *di-ortho*-substituted, placing this group at C-7 rather than C-6 or C-5' (typical value of such "free" methoxy group is at δ_C 54-56 ppm). In agreement with this, the HMBC spectrum (Figure 12) showed correlation of the methoxy protons (δ_H 4.01) with C-7 (δ_C 133.9). In addition, the HMBC correlation of the proton signal at δ_H 6.78 (H-5) to C-3a, C-6, C-7; and H-4 with C-3, C-7 and C-7a (Figure 12) confirmed the identity of ring B. Therefore, the second new compound from this plant (**35**) was characterized as 2-(*p*-hydroxybenzyl)-3-(3-methylbut-2-en-1-yl)benzyl)-6-ol, for which the trivial name dorsmerunin B was assigned.



35

*4.5.3. Dorsmerunin C (**36**)*

Compound **36** with molecular ion $[M]^+$ at *m/z* 308.1411 corresponds to the molecular formula $C_{20}H_{20}O_3$. This compound also showed UV (λ_{max} 251, 258 and 287 nm) and NMR (Table 27) spectra of benzylbenzofuran. Typical of such compounds, the ^{1}H NMR signal at δ_H 6.33 (*brs*) was assigned to H-3 with the corresponding carbon C-3 appearing at δ_C 104.0. The presence of two hydroxyl (at C-6 and C-5') and a prenyl group was evident from NMR spectra. As in compound **34**, the ^{1}H NMR spectrum of **36** exhibited an AMX spin system for ring B protons appearing at δ_H 7.29 (*d, J* = 8.3 Hz), 6.74 (*dd, J* = 8.3, 2.2 Hz) and 6.86 (*d, J* = 2.1 Hz) allocated to protons at C-4 (δ_C 121.9), C-5 (δ_C 113.0) and C-7 (δ_C 99.0) of ring B. The methylene protons (δ_H 3.94) of the benzyl moiety showed HMBC cross peak (Figure 12) with C-2 (δ_C 158.8) and C-3 (δ_C 104.0) confirming its connection to C-2 of the furan ring (ring A). In ring C, an AMX spin system appearing at δ_H 7.05 (*d, J* = 2.2 Hz), 6.78 (*d, J* = 8.1 Hz) and 6.95 (*dd, J* = 8.1, 2.3 Hz) was assigned to protons on C-3' (δ_C 131.6), C-6' (δ_C 116.4) and C-7' (δ_C 128.5), which requires substitution, (hydroxy and prenyl) at C-4' and C-5'. The attachment of the prenyl group at C-4' (δ_C 129.4) and hydroxy at C-5' (δ_C 155.1) was established from the HMBC correlation of H-3' (δ_H 7.05) to C-1" (δ_C 29.7); CH_2-1" (δ_H 5.32) with C-3' (δ_C 131.6), C-4' (δ_C 129.4) and C-5' (δ_C 155.1) (Figure 12). Thus, this new compound (**36**) was characterized as 2-(*p*-hydroxybenzyl)-6-hydroxy-4'-prenylbenzofuran, for which the trivial name dorsmerunin C was assigned.



36

Whereas several chalcones (Abegaz *et al.*, 1998) and furanocoumarins (Franke *et al.*, 2001; Heinke *et al.*, 2011) have been reported from the genus *Dorstenia*, only three

benzylbenzofurans (Franke *et al.*, 2001; Peniche-Pavia *et al.*, 2016) have been reported prior to the present report; 2-(p-hydroxybenzyl)-6-methoxybenzofuran from *D. gigas* (Franke, *et al.*, 2001) and dorsjervins A and B from *D. Contrajerva* (Franke *et al.*, 2001; Peniche-Pavia, *et al.*, 2016). It has been suggested (Franke *et al.*, 2001; Peniche-Pavia, *et al.*, 2016) that the biogenesis of benzylbenzofuran to be similar to the biosynthesis of aurones, presumably from chalcone precursor (Figure 11). The co-occurence of the chalcone licoagrochalcone A (**38**) with the benzylbenzofuran **34** may suggest that **34** is derived from **38**. Alternatively, compound **34** could have first been formed from the corresponding chalcone precursor (4,2',4'-trihydroxychalcone), then prenylation of **34** at C-4' may give compound **36**; while oxidation and methylation at C-7 of **34** produces compound **35** (Figure 11).

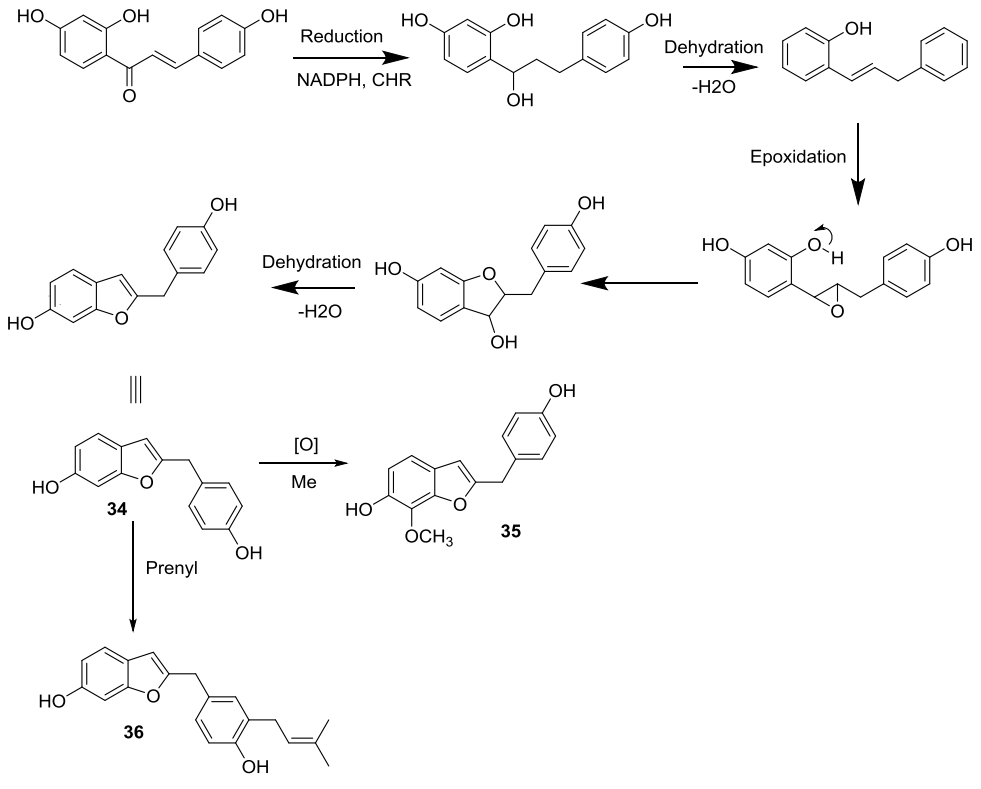


Figure 11: Proposed biogenesis of benzylbenzofurans **34─36**.

Table 27: ^{1}H (500 MHz) and ^{13}C NMR (125) spectral data of compounds **34-36** (acetone-d_6)

Carbon No.	**34**		**35**		**36**	
	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C
2		158.7		159.1		158.8
3	6.33 (*br s*)	104.1	6.33 (*t, J* = 0.9)	104.4	6.33 (*br s*)	104.0
3a		122.8		124.8		122.8
4	7.29 (*d, J* = 8.3)	121.9	7.00 (*d, J* = 8.3)	115.6	7.29 (*d, J* = 8.3)	121.9
5	6.74 (*dd, J* = 8.3, 2.1)	113.1	6.78 (*d, J* = 8.3)	113.8	6.74 (*dd, J* = 8.3, 2.2)	113.0
6		156.4		147.2		156.4
6-OH	8.33 (*br s*)*		7.76 (*s*)		8.39 (*s*)	
7	6.87 (*d, J* = 1.8)	99.0		133.9	6.86 (*d, J* = 2.1)	99.0
7a		157.6		148.3		157.5
1'	3.97 (*s*)	35.0	4.99 (*br s*)	35.0	3.94 (*s*)	35.2
2'		130.0		129.9		130.1
3'	7.14 (*d, J* = 8.6)	131.4	7.17 (*d, J* = 8.6)	131.3	7.05 (*d, J* = 2.2)	131.6
4'	6.80 (*d, J* = 8.6)	116.7	6.81 (*d, J* = 8.6)	116.8		129.4
5'		157.5		157.6		155.1
5'-OH	8.24 (*br s*)*		8.27 (*s*)		8.19 (*s*)	
6'					6.78 (*d, J* = 8.1)	116.4
7'					6.95 (*dd, J* = 8.1, 2.3)	128.5
7-OCH_3			4.01 (*s*)	61.4		
1''					3.30 (*d, J* = 7.4)	29.7
2''					5.32 (*m*)	124.4
3''						133.0
3''$(CH3)_2$					1.68 (6H, *m*)	26.5

* or reverse

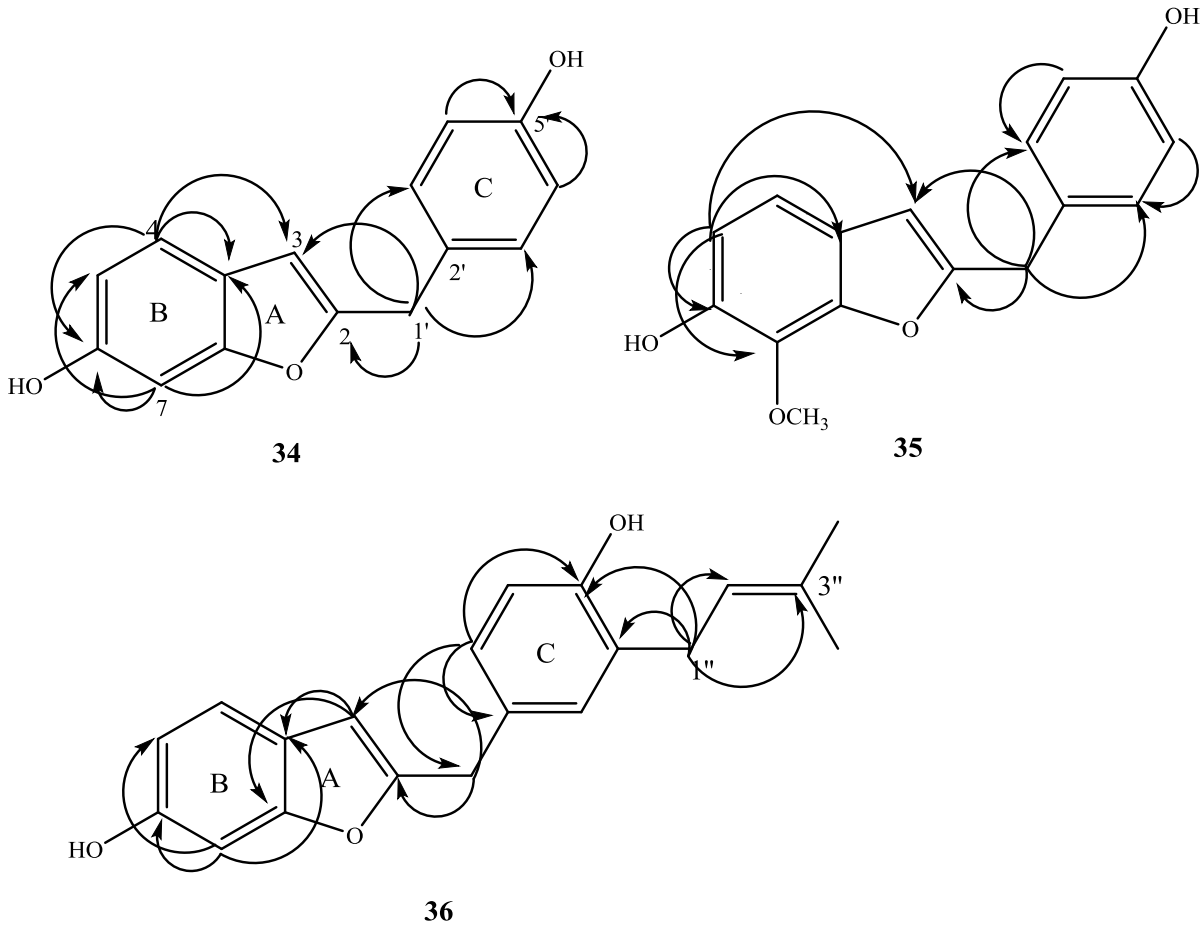
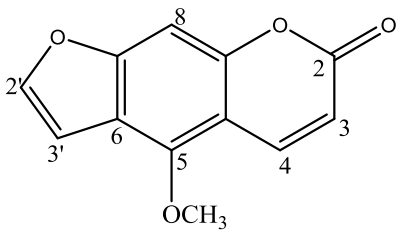


Figure 12:Important HMBC correlations in compounds **34- 36**.

*4.5.4. Bergapten (**37**)*

Bergapten (**37**) was crystallized from CH_2Cl_2/MeOH as white crystals (mpt. 197-198 °C). This compound exhibited UV absorption maxima at 242, 251, 261, 269 and 314 nm characteristic of coumarins (Choudhary *et al.*, 2002). The HREIMS showed a molecular ion peak at *m/z* 216.0415 suggesting a molecular formula $C_{12}H_8O_4$. The ^{1}H NMR spectrum showed singlet aromatic proton at δ_H 7.11 (H-8) and a pair of mutually coupled protons at δ_H 6.25 (*d*, *J* = 9.8 Hz, H-3) and 8.15 (*d*, *J* = 9.8 Hz, H-4) indicating a 6,7,5-trisubstituted coumarin skeleton. This was supported by HMBC correlation of the proton signal at δ_H 7.11 with a quaternary carbon peak δ_C 158.3 (C-7) and 112.5 (C-6). The signals of two other

protons at δ_H 7.03 (*d*, *J* = 2.4 Hz, H-2') and 7.60 (*d*, *J* = 2.4 Hz, H-3') together with the corresponding (from the HSQC spectrum) ^{13}C NMR peaks at δ_C 112.5 (C-6) and 158.3 (C-7) indicated the attachment of the furan ring at C-6 and C-7 of ring A. An additional sharp singlet proton signal at δ_H 4.28 indicated the presence of a methoxy group. The placement of methoxy at C-5 (δ_C 149.5) was established by HMBC correlation of H-4 (δ_H 8.15) with the ^{13}C NMR peak at δ_C 149.5 (C-5) (Table 28). In the ^{13}C NMR spectrum, five methine carbons, two quaternary carbons, three oxygenated carbons and one ester carbonyl carbon were observed. Therefore, the structure of this compound was established as bergapten (5-methoxypsoralen), previously isolated from *Dorstenia brasiliensis* and *Dorstenia contrajerva* (Kuster *et al.*, 1994; Terreaux *et al.*, 1995).



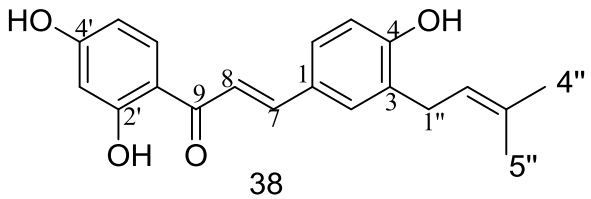
37

Table 28: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data and HMBC correlations for compound **37** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC
2		161.2	
3	6.25 (*d*, *J* = 9.8 Hz)	112.4	C-2, C-4a
4	8.15 (*d*, *J* = 9.8 Hz)	139.2	C-2, C-4a, C-5, C-8, C-8a
4a		106.3	
5		149.5	
6		112.5	
7		158.3	
5	7.11 (*s*)	93.7	C-4a, C-6, C-7, C-8a
8a		152.6	
2'	7.03 (*d*, *J* = 2.4 Hz)	105.0	C-3',C-6, C-7
3'	7.60 (*d*, *J* = 2.4 Hz)	144.7	C-2', C-6, C-7, C-8
4-OCH_3	4.28 (*s*)	60.0	C-5

*4.5.5. Licoagrochalcone A (**38**)*

Compound **38** was isolated as a yellow paste, and its HREIMS showed a molecular ion peak at *m/z* 324.1375 suggesting a molecular formula of $C_{20}H_{20}O_4$. The UV (λ_{max} 288 and 363 nm), ^{1}H NMR at δ_H 7.84 (*d*, *J* = 15.3 Hz) for H-α and 7.75 (*d*, *J* = 15.3 Hz) for H-β and ^{13}C NMR (δ_C 144.7 for C-α, δ_C 117.0 for C-β and δ_C 191.9 for C=O) suggested **38** to be a chalchone derivative (Markham, 1982). The presence of a prenyl (Table 29) and three hydroxy groups (one of which intramolecular hydrogen bonded) was evident from the NMR spectra (Table 29). In ring A, the ^{1}H NMR showed an AMX spin system at δ_H 7.64 (H-6), 7.59 (H-2) and 6.95 (H-5) suggested the placement of the prenyl group at C-3 with the biogenetically expected oxygenation (hydroxy group) at C-4. In agreement with this, in the HMBC spectrum H-2 showed corelation with C-4 (δ_C 157.8), and H-5 with C-1" (δ_C 28.2). In ring B, with the the hydrogen bonded hydroxy group being at C-2', the ^{1}H NMR showed another AMX spin system (Table 29) which requires the placement of the third hydroxy group at C-4' (which is biogenetically expected). Therefore this compound was identified as licoagrochalcone A, previously reported from the roots of *Ononis vaginalis* (Abdel-Kader, 2001). This is the first report on the occurance of compound **38** from the genus dorstenia.



38

Table 29: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data and HMBC correlations for compound **38** (acetone-d_6)

Carbon No.	δ_H	δ_C	HMBC
1		127.3	
2	7.59 (*dd*, *J* = 8.3, 2.3 Hz)	128.3	C-4, C-6, C-7
3		128.2	
4		157.8	
5	6.95 (*d*, *J* = 8.3 Hz)	115.4	C-1", C-1, C-4
6	7.64 (*d*, *J* = 2.0 Hz)	130.9	C-2, C-4, C-7
7	7.84 (*d*, *J* = 15.3 Hz)	144.7	C-1, C-6, C-8, C-9
8	7.75 (*d*, *J* = 15.3 Hz)	117.0	C-1, C-7, C-9
9		191.9	
1'		113.6	
2'		166.2	
3'	6.38 (*d*, *J* = 2.3 Hz)	102.8	C-1', C-4', C-5'
4'		164.6.	
5'	6.48 (*dd*, *J* = 8.9, 2.3 Hz)	107.7	C-1', C-3', C-4
6'	8.10 (*d*, *J* = 8.9 Hz)	132.3	C-1', C-2, C-3', C-9
1"	3.38 (*d*, *J* = 7.3 Hz)	28.2	C-2", C-2, C-4
2"	5.39 (*d*, *J* = 10.0 Hz)	122.4	CH_3
3"		132.3	
4"-CH_3	1.74 (*s*)	25.0	5"-CH_3, C-2"
5"-CH_3	1.76 (*s*)	17.0	4"-CH_3, C-2"
2'-OH	13.68 (*s*)		C-1', C-2', C-3'

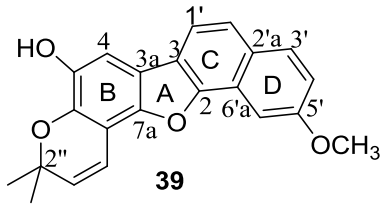
4.6. Compounds Isolated from the Roots and stem of *Streblus usambarensis*

Column chromatographic separation of the CH_2Cl_2/MeOH (1:1) extract of the roots of *Streblus usambarensis* yielded two new compounds, named usambarin A (**39**) and usambarin B (**40**). Similar investigation of the stem of this plant resulted in the isolation of a further new compound, usambarin C (**41**). The characterization of these compounds is discussed below.

*4.6.1. Usambarin A (**39**)*

Compound **39** was obtained as a brown gum. The UV (λ_{max} 270, 297 and 350 nm), IR (ν_{max} 3188, OH, 1468, 1219 and 860 cm^{-1}) and NMR spectral data (Table 30) suggested this compound to be aromatic. Its molecular formula was established as $C_{22}H_{18}O_4$ (*m/z* 346.1214 $[M]^+$) from HREIMS and NMR (Table 30) analyses. The ^{1}H and ^{13}C NMR spectra further suggested an unprecedented naphtho[1,2-b]benzofuran-2,8,9-triol skeleton. Thus, the singlet

at δ_H 7.39 (*s*) was assigned to H-4 in ring B of the benzofuran moiety which is substituted at C-5, C-6 and C-7. The proton signals at δ_H 5.83 (*d, J* = 9.9 Hz, H-3"), 7.08 (*d, J* = 9.86 Hz, H-4") and methyl signal δ_H 1.58 (6H, *s*) corresponds to a 2",2"-dimethylpyran substituent at C-6/C-7 with hydroxy group being at C-5 (δ_C 141.6). Consistent with this substitution pattern in ring B, the HMBC spectrum showed correlation of H-3" and H-4" with C-2", C-6, C-7 and C-7a; H-4 (δ_H 7.39) with C-3 (δ_C 120.3), C-6 (δ_C 145.9) and C-7a (δ_C 138.5) (Figure 13). The fusion of ring C of the naphthalene moiety at C-2 (δ_C 151.4) and C-3 (δ_C 120.3) of the furan ring was evident from the presence of two *ortho*-coupled signals at δ_H 7.68 (*d, J* = 8.32 Hz, H-1') and 7.75 (*d, J* = 8.30 Hz, H-2') in the ^{1}H NMR spectrum, which was supported by HMBC correlation H-1' with C-2 (δ_C 151.4), C-2'a (δ_C 127.6) and C-3 (δ_C 120.3) (Fig.14). An AMX spin system at δ_H 7.87 (*d, J* = 8.9 Hz), 7.66 (*d, J* = 2.6 Hz) and 7.19 (*dd, J* = 8.9, 2.6 Hz) was assigned to H-3', H-6' and H-4', respectively, of ring D which is substituted at C-5' (δ_C 158.1) with methoxy group (δ_H 4.05, δ_C 55.4). The placement of methoxy group was confirmed by HMBC correlation of H-3' (δ_H 7.87), H-4' (δ_H 7.19) and H-6' (δ_H 7.66) with C-5' (δ_C 158.1) (Figure13). Thus this new compound was characterized as 11-methoxy-3,3-dimethyl-3H-naphtho[2',1':4,5]furo[2,3-f]chromen-5-ol, and given the trivial name usambarin A.

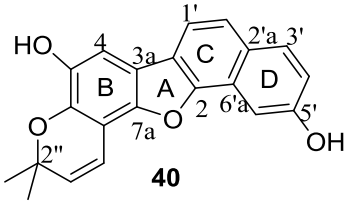


39

*4.6.2. Usambarin B (**40**)*

Compound **40** was isolated as a brown gum. Its molecular formula, $C_{21}H_{16}O_4$, was established from HREIMS (*m/z* 332.1053, [M^+]) and NMR analyses (Table 30). The UV (λ_{max} at 257 and 341 nm), IR (ν_{max} 3373, OH, 1444, 1165 and 840 cm^{-1}), NMR spectra (Table 30) indicated

that compound **40** is also has a naphtho[1,2-b]benzofuran-2,8,9-triol skeleton as compound **39.** In fact the only difference between the two compounds is that compound **40** has a hydroxy group instead of a methoxy at C-5' in ring D of the naphthalene moiety. Thus the ^{1}H NMR spectrum exhibited a singlet at δ_H 6.85 (*s*) for H-4, and signals for a 2",2"-dimethylpyran ring in ring B. The placement at C-6/C-7 of this ring was established by HMBC correlation of H-3' (δ_H 5.75) and H-4' with C-7 (δ_C119.3) as well as H-4 (δ_H 6.85) correlation with C-5 (δ_C140.2) and C-6 (δ_C137.8). Moreover, the proton signals at 7.60 (*d, J* = 8.30 Hz) and 7.25 (*d, J* = 8.32 Hz) corresponds to H-1' and H-2' respectively of ring C (Fig.14). The ABX spin system at 7.17 (*d, J* = 8.77 Hz, H-3'), 7.82 (*d, J* = 8.92, 2.56 Hz, H-4') and 7.25 (*d, J* = 2.5 Hz, H-6') corresponds to ring D protons with a hydroxy group at C-5' (δ_C 155.6). The substitution pattern in this ring was confirmed by HMBC correlation of H-3' and H-4' with C-5' (δ_C 155.6) (Fig.13). Thus the second new compound from this plant (**40**) was characterized as 3,3-dimethyl-3H-naphtho[2',1':4,5]furo[2,3-f]chromene-5,11-diol, and named usambarin B.

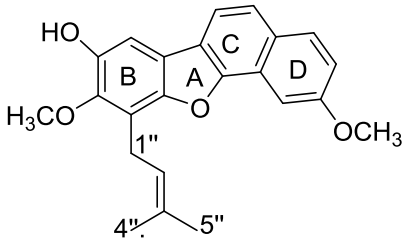


40

*4.6.3. Usambarin C (**41**)*

Compound **41** was isolated as white crystals, mpt.154-155 °C. HREIMS (*m/z* 362.1510, $[M]^+$) analysis together with the NMR data suggested the molecular formula $C_{23}H_{22}O_4$. The UV (λ_{max} 267, 273, 309, 321 nm), ^{1}H and ^{13}C NMR spectra (Table 30) displayed the presence of a prenyl and methoxy substituents on a naphtho[1,2-b]benzofuran-2,8,9-triol skeleton. Furthermore, the substitution pattern of this compound is also the same as in compounds **39** and **40**, with oxygenation (two methoxy and one hydroxy) at C-5, C-6 and C-5' and a C_5

(prenyl) substituent at C-7. In ring A, one of the methoxy groups (δ_H 3.95) showed HMBC correlation with C-6 (δ_C 144.3) indicating that it is attached to this carbon atom. Likewise, the second methoxy group was placed at C-5' (δ_C 158.1) from the HMBC spectrum which showed correlation of the methoxy group to C-5' (Figure 13). The substitution pattern in ring A was confirmed based on HMBC spectrum where the singlet at δ_H 7.41 (H-4), showed HMBC cross peak with C-3 (δ_C 120.9) and C-6 (δ_C 144.3); HMBC correlation of the proton signal at δ_H 3.83 (*d, J* = 7.21 Hz, H-1'') with C-7 (δ_C 119.0) (Figure 13). The NMR spectrum also showed identical rings C and D of the naphthalene group as in compounds **39** and **40** with two *ortho* coupled protons at δ_H 7.78 (*d, J* = 8.38 Hz) and 7.68 (*d, J* = 8.30 Hz) assigned to H-1' and H-2' in ring C. An ABX spin system were observed at δ_H 7.89 (*d, J* = 8.94 Hz, H-3'), 7.21 (*d, J* = 8.93, 2.65 Hz, H-4') and 7.69 (*d*, 2.40 Hz. H-5') for ring D protons with methoxy group placed at C-5'.The substitution pattern in ring D was confirmed by HMBC correlation of methoxy signal δ_H 4.06 (*s*) as well as AMX protons with C-6' (δ_C 158.1). Therefore, this new compound was characterized as 2,9-dimethoxy-10-(3-methylbut-2-en-1-yl)naphtho[1,2-b]benzofuran-8-ol and named usambarin C.



41

Table 30: ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) (acetone-d_6) spectral data of compounds **39**-**41**

Carbon No.	**39**		**40**		**41**	
	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C
2		151.4		135.0		151.9
3		120.3		126.4		120.9
3a		117.8		130.8		120.0
4	7.39 (*s*)	104.4	6.85 (*s*)	115.1	7.41 (*s*)	102.8
5		141.6		140.2		145.7
6		145.9		137.8		144.3
7		106.6		119.3		119.0
7a		138.5		145.1		149.1
1'	7.68 (*d*, *J* = 8.3 Hz)	122.8	7.60 (*d*, *J* = 8.3Hz)	109.0	7.78 (*d*, *J* = 8.3 Hz)	115.9
2'	7.75 (*d*, *J* = 8.3 Hz)	115.6	7.25 (*d*, *J* = 8.3 Hz)	126.4	7.68 (*d*, *J* = 8.3 Hz)	122.6
2'a		127.6		127.3		127.9
3'	7.87 (*d*, *J* = 8.9 Hz)	130.0	7.17 (*d*, *J* = 8.7 Hz)	118.2	7.89 (*d*, *J* = 8.9 Hz)	130.0
4'	7.19 (*dd*, *J* = 8.9, 2.56 Hz)	118.0	7.82 (*dd*, *J* = 8.9, 2.5 Hz)	129.1	7.21 (*dd*, *J* = 8.9, 2.6 Hz)	118.1
5'		158.1		155.6		158.1
6'	7.66 (*d*, *J* = 2.6 Hz)	98.9	7.25 (*d*, *J* = 2.5 Hz)	125.2	7.69 (*d*, 2.40 Hz)	99.3
6'a		122.2		130.8		122.3

1"					3.83 (*d*, *J* = 7.21 Hz)	24.0
2"		77.8		75.3	5.49 (*m*)	121.8
3"	5.83 (*d*, *J* = 9.8 Hz)	130.6	5.75 (*d*, *J* = 10.0 Hz)	130.6		132.5
4"	7.08 (*d*, *J* = 9.8 Hz)	116.5	6.41 (*d*, *J* = 9.9 Hz)	120.7		
2"-$(CH_3)_2$	1.58 (*s*)	27.9	1.48 (*s*)	26.6		
4"-CH_3					2.02 (*s*)	18.0
5"-CH_3					1.77 (*s*)	25.8
5'-OCH_3	4.05 (*s*)	55.4	4.05 (*s*)	55.4	3.95 (*s*)	62.2
6-OCH_3					4.06 (*s*)	55.2

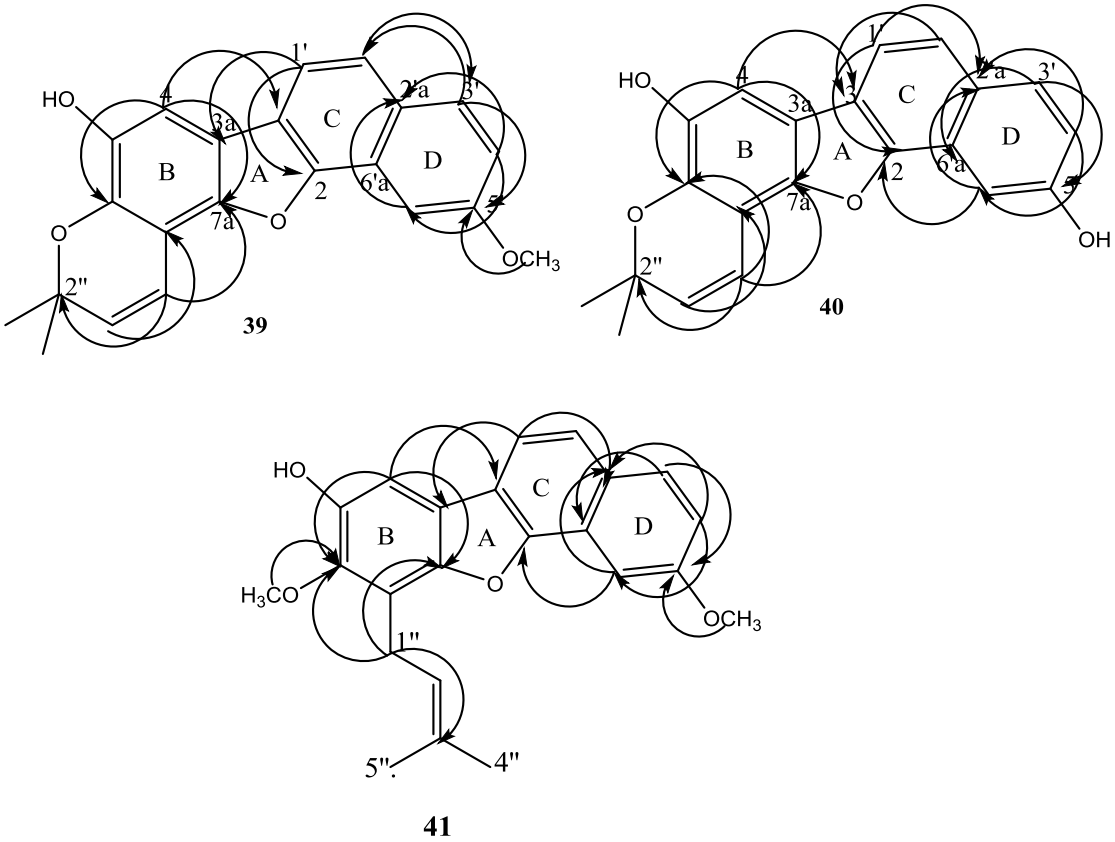


Figure 13: Important HMBC correlations in compounds **39-41**

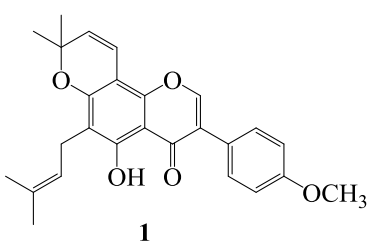
4.7. Bioactivity

The compounds isolated from the six plants in this study were tested for cytotoxicity against a panel of cell-lines. The results are presented and discussed in the following subsections.

4.7.1. Cytotoxicity of Compounds isolated from Ormocarpum kirkii

Compounds **2-8** were tasted for cytotoxicity on human embryonic kidney cells (HEK293) at 100 µM. Compounds **2-6** showed more than 75% inhibition at this concentration while compounds **7** and **8** were not cytotoxic (Table 31). The compounds were then tested at different concentrations and the IC_{50} values for the cytotoxicity determined (Table 31). Compounds **2-5** showed some degree of cytotoxicity with IC_{50} values less than 45 µM, while compounds **6-8** were inactive (IC_{50} > 100 µM), towards HEK 293 cells (Table 31). The isoflavones, 5,7-dihydroxy-4ˈ-methoxy-6,8-diprenylisoflavone (**2**) and osajin (**3**) showed comparable activity against HEK293 with IC_{50} values 27.1 and IC_{50} value 27.3 ± 2.0

respectively. Among the three biflavanones (**4**, **5** and **8**) assayed, 7,7''-di-*O*-methylchamaejasmin (**4**) and chamaejasmin (**5**) displayed significant activity, with IC_{50} values of 20.8 µM and 43.5 µM, respectively, while campylospermone A (**8**) was inactive (Table 31). This suggested that the presence of hydroxy group at C-5 in ring A may be important for the observed activities of the biflavanones **4** and **5**. Diphysin (**6**) and heptacosyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylate (**7**) did not inhibit HEK293 cells up to 100 µM. The activity of compounds **4**-**6** was previously reported against the D10 strain of *Plasmodium falciparum* without toxicity against Chinese hamster ovarian (CHO) cell line (Chukwujekwu *et al.*, 2012).



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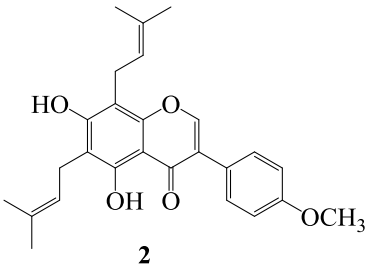

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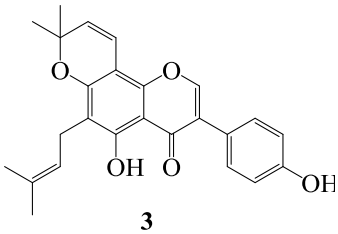

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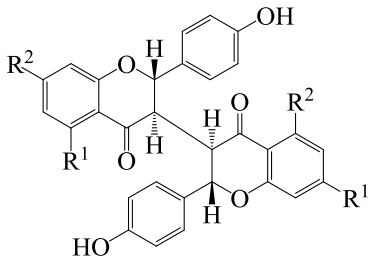
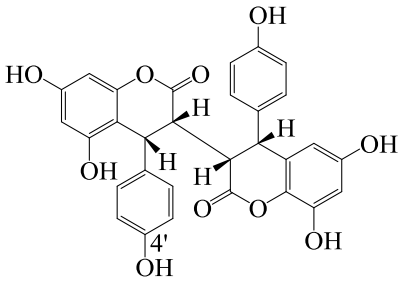


4 R^1 = OH R^2 = OCH_3
5 R^1 = OH R^2 = OH
8 R^1 = H R^2 = OH



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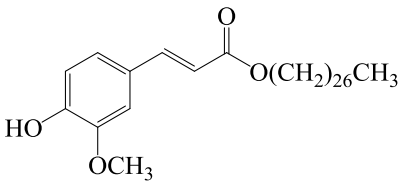

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Table 31: Cytotoxic activity of compounds **2-8** against HEK293 cell line

Compounds	HEK293 at top dose (100 µM)	HEK293 IC_{50} in µM
2	88.8 ± 1.58	27.1
3	101.9 ± 0.49	27.3 ± 2.0
4	101.8 ± 0.47	20.8 ± 6.8
5	78.6 ± 21.9	43.5
6	9.96 ± 14.6	> 100
7	-6.59 ± 14.47	> 100
8	-6.61 ± 17.75	> 100
DHA	92.3 ± 9.71	~10.6
Puromycin	98.3 ± 0.54	0.7 ± 0.13
Pyrimethamine	62.3 ± 3.6	~15.8
Pyronaridine	97.9 ± 0.21	4.9 ± 0.84

The cytotoxicity of compounds **2-8** isolated from *Ormocarpum kirkii* together with the standard doxorubicin was first determined in both sensitive CCRF-CEM leukemia cells and in drug resistant subline CEM/ADR5000 cells. 5,7-Dihydroxy-4ˈ-methoxy-6,8-diprenylisoflavone (**2**), Osajin (**3**) and 7,7"-di-*O*-methylchamaejasmin (**4**) and diphysin (**6**) were cytotoxic to both sensitive CCRF-CEM and resistant CEM/ADR5000 leukemia cells with IC_{50} values below 61 µM (Table 32). In both CCRF-CEM and CEM/ADR5000 cells, significant activity with IC_{50} values below 10 µM (Brahemi *et al.*, 2010; Kuete and Efferth, 2015) were obtained for osajin (**3**) and 7,7"-di-*O*-methylchamaejasmin (**4**) as well as doxorubicin. IC_{50} values below 10 µM were also obtained for 5,7-dihydroxy-4ˈ-methoxy-6,8-diprenylisoflavone (**2**) towards CEM/ADR5000 cells.

*4.7.2.Mode of action of osajin (**3**) and 7,7''-di-O-methylchamaejasmin (**4**)*

4.7.2.1.Cell cycle distribution and apoptosis

The distribution of cell cycle in CCRF-CEM cells treated with the isoflavonoids osajin (**3**) and the biflavonoid 7,7''-di-*O*-methylchamaejasmin (**4**) as well as the reference drug doxorubicin is depicted in Figure 14. Concentration-dependent modifications of cell cycle phases were observed with osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) as well as doxorubicin. Increase of cells in sub-G0/G1 phase was generally observed with all tested samples. Osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) induced cell cycle arrest in G0/G1 phase, meanwhile doxorubicin induced S and G2/M phase arrest. The amounts of cells in the sub-G0/G1 phase varied from 1.27% (1/4 × IC_{50}) to 35.61% (2 × IC_{50}) after treatment with osajin (**3**), from 3.27% (1/4 × IC_{50}) to 59.68% (2 × IC_{50}) after treatment with 7,7''-di-*O*-methylchamaejasmin (**4**), and from 4.81% (1/4 × IC_{50}) to 10.35% (2 × IC_{50}) upon treatment with doxorubicin. In non-treated cells, the percentage of cells in sub-G0/G1 phase remains at 1.78% (Figure 15). The increase of cells in sub-G0/G1 phase indicates that osajin (**3**)**,** 7,7''-di-*O*-methylchamaejasmin (**4**) and doxorubicin probably induced apoptosis in CCRF-CEM cells. A dose-dependent induction of apoptosis by the two compounds and the drug doxorubicin was further investigated by annexin V/PI staining and the results are shown in (Figure 16). At 2 × IC_{50} for example, osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) slightly induced late apoptosis with 4.4% and 4.4% annexin V (+)/PI (+) cells, respectively. They mostly induced necrosis with 51.8% and 66.5% annexin V (-)/PI (+) cells for osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**), respectively (Figure 15).

4.7.2.2.Activation of caspases

The activity of caspases in CCRF-CEM cells upon treatment with osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) is shown in Figure 16. It appeared that the two compounds did not increase the activity of caspases 3/7, 8 and 9.

4.7.2.3. Integrity of mitochondrial membrane

The effects of osajin (**3**), 7,7''-di-*O*-methylchamaejasmin (**4**) and standard drug valinomycin on the integrity of MMP in CCRF-CEM cells are depicted in (Figure 17). It can be seen that the two compounds considerably altered the MMP in CCRF-CEM cells from their IC_{50}. At 2 × IC_{50}, 79.4% and 94.9% alterations of MMP were obtained as the results of treatment with osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**), respectively, meanwhile valinomycin at 10 µM induced 45.9% alteration.

4.7.2.4. Production of reactive oxygen species (ROS)

The effects of osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) on ROS production in CCRF-CEM cells are given in Figure 18. The tested compounds caused dose-dependent increase of ROS in the range of 13.2% (1.30 µM) to 67.8% (10.34 µM) for osajin (**3**) and of 16% (0.90 µM) to 74.5% (7.16 µM) for 7,7''-di-*O*-methylchamaejasmin (**4**). The positive control, H_2O_2 increased the ROS levels to 92.8% at 50 µM, while ROS production in non-treated cells was 0.2%.

Hypersensitivity (degree of resistance or D.R. below 0.90) (Mbaveng *et al.*, 2017) of CEM/ADR5000 compared to its sensitive parental cell line CCRF-CEM was noted with 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**), osajin (**3**), chamaejasmin (**5**) and diphysin (**6**) (Table 32), suggesting that these compounds could be potential inhibitors of P-glycoprotein's expression (Mbaveng, *et al.*, 2017). With regards to their activities to the two leukemia cells, 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**), osajin (**3**), and 7,7''-di-

O-methylchamaejasmin (**4**) displaying IC_{50} values below 20 µM were selected and tested against 7 carcinoma cells and normal AML12 hepatocytes (Table 33). The four compounds showed cytotoxicity against the 7 carcinoma cell lines with IC_{50} values below 50 µM. Moreover, osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) had significant cytotoxic effects with IC_{50} values below 10 µM against 4/7, 5/7 and 7/7 tested carcinoma cells. Hence, the potential of these compounds in the fight against cancer should be further explored. Interestingly, hypersensitivity was observed with the four compounds towards resistant U87MG.Δ*EGRF* glioblastoma cells compared to its respective sensitive counterparts U87MG cells, and in some cases, with 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**)**,** in another resistant cell line (Table 33). It is also interesting to note that the selectivity indexes of 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**), osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) for the normal AML12 hepatocytes versus HepG2 hepatocarcinoma cells are around or below 1, suggesting their poor selectivity to liver cancer cells. Nonetheless, a keen look at IC_{50} values of compounds **2-4** in AML12 cells versus that of doxorubicin indicate that these compounds can be explored further towards cancer therapy.

Apoptosis is one of the likely modes of action of cytotoxic compounds in cancer cells. In this work, it was found that the isoflavone osajin (**3**) and the biflavonoid 7,7''-di-*O*-methylchamaejasmin (**4**) caused cell cycle arrest in G0/G1 phase as well as apoptosis with significant increase of cells in sub-G0/G1 phase (Figure 14). This is an indication of apoptosis, which was later confirmed by annexin V/PI staining, as osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) also caused increase in late apoptotic and necrotic cells (Figure 16). Activation of caspase-dependent apoptosis is involved in cell death in mammals (Fuchs and Steller, 2011) making them good therapeutic targets for modulators (Howley and Fearnhead, 2008; Mbaveng, *et al.*, 2017). Neither the isoflavone **3** nor the biflavonoid **4** induced the activation of caspases 3/7, 8 and 9 (Figure 16), suggesting that they are not caspases

modulators. The activity of caspases 3, 8, and 9 was previously measured in HeLa cells together with its effect on the phosphoinositide 3-kinase (PI3K)/Akt pathway (Qian & Li, 2017). These compounds induced apoptosis in HeLa cells mediated through the suppression of PI3K/Akt signaling cascades, but not by caspases activation (Qian and Li, 2017). Breakdown of the mitochondrial membrane potential causes cytochrome C release due to formation of channels in the outer mitochondrial membrane leading to apoptosis (Dejean et al., 2006). In this work, it was found that CCRF-CEM cells treated with osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) considerably deteriorated MMP with upto 79.4% and 94.9% alterations at 2 × IC_{50}, respectively (Figure 18). These compounds also induced high levels of ROS in CCRF-CEM cells with up to 67.80% for **3** and 74.50% for **4** at 2 × IC_{50} (Figure 19). These data suggest that MMP alterations as well as increased ROS production were involved in cell death induced by compounds **3** and **4**.

With regards to structure-activity relationship, it appears that both the isoflavones **2** and biflavonoid **4** were active. Within biflavonoids, it seems that the substitution of some hydroxy groups by methoxy positively influenced the cytotoxicity. In effect, compound **4** with 4 hydroxy- and 2 methoxy groups revealed an IC_{50} value below 10 µM in 9/9 cancer cell lines, whilst the biflavonoids **4** and **5** (with 4 and 6 hydroxy groups and no methoxy substituent, respectively) were less active (Tables 32). This is also the case with compounds **5** and **6** having 4 and 6 hydroxy groups and no methoxy substituent, respectively, that also showed poor cytotoxic effects (Table 32).

The overall data highlighted the possibility of using the tested natural compounds, especially, osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) to develop a novel drug to fight drug sensitive and resistant cancers.

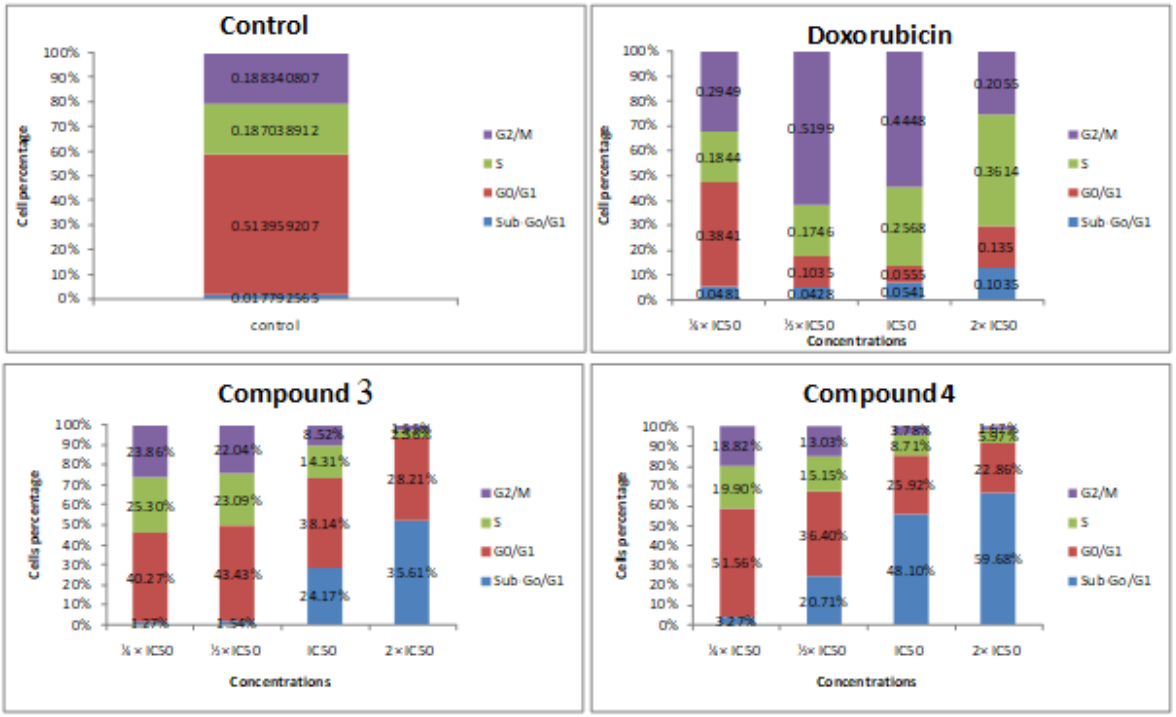


Figure 14: Effect of 24 h treatment of osajin (**3**), 7,7”-di-*O*-methylchamaejasmin (**4**) and doxorubicin on the cell cycle distribution of CCRF-CEM leukemia cells. The IC_{50} values were 5.17 µM for compound 3, 3.58 µM for compound 4 and 0.02 µM for doxorubicin.

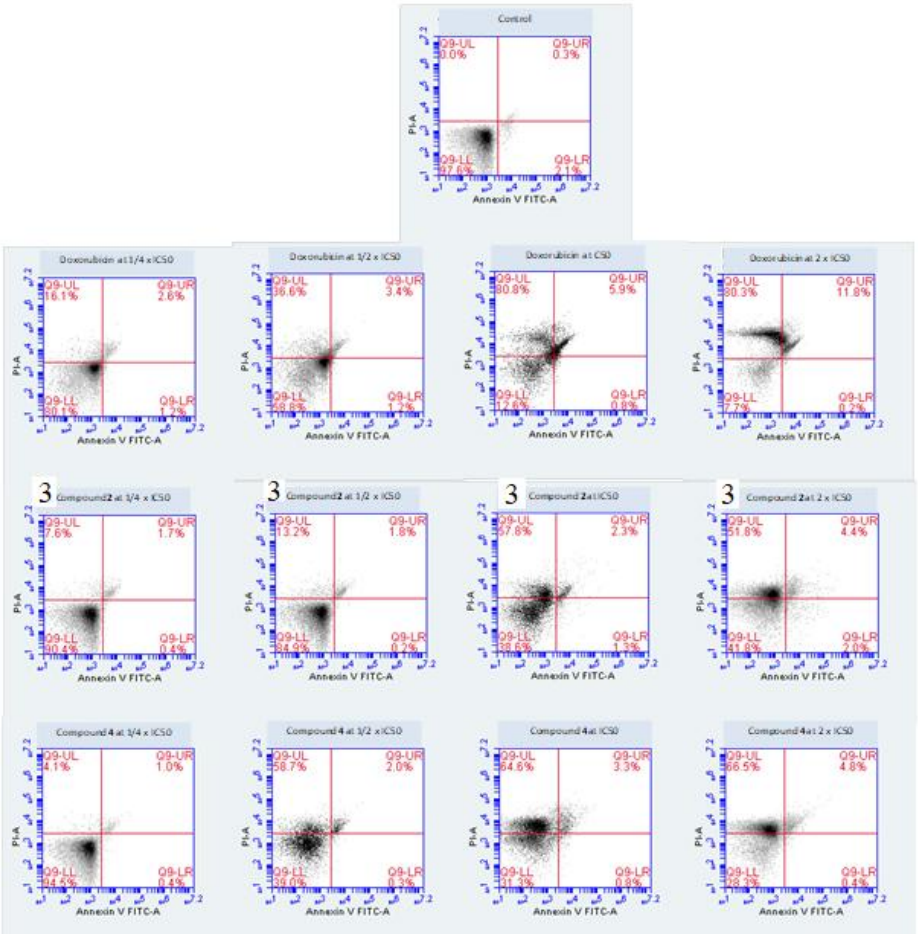


Figure 15: Apoptosis assessment of osajin (**3**), 7,7”-di-*O*-methylchamaejasmin (**4**) and doxorubicin on CCRF-CEM leukemia cells after 24 h as determined by annexin V/PI assay. Apoptosis was assessed by flow cytometry after annexin V-PI double staining. Necrotic cells lose membrane integrity, allowing PI entry. Q9-LL: viable cells exhibit annexin V (-)/PI (-); Q9-LR: early apoptotic cells exhibit annexin (+)/PI (-); Q9-UR and Q9-UL: late apoptotic cells or necrotic cells exhibit annexin V (+)/PI (+) or annexin V (-)/PI (+). IC_{50} values were 5.17 µM for compound 3, 3.58 µM for compound 4 and 0.02 µM for doxorubicin.

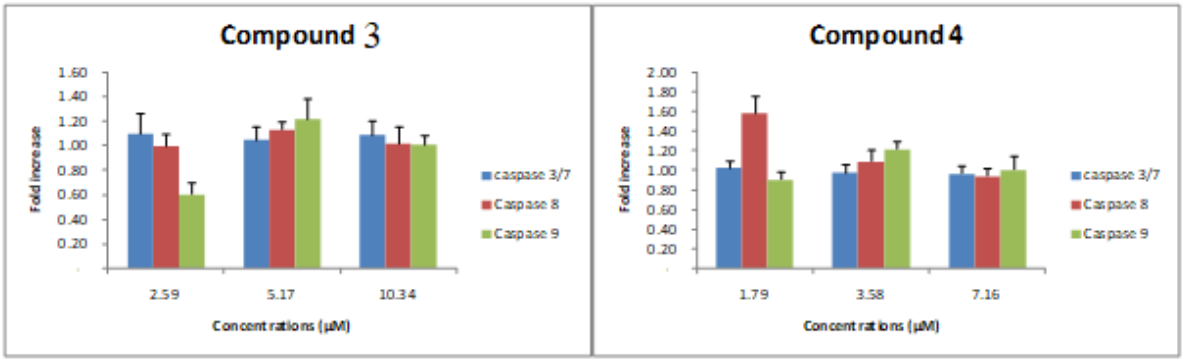


Figure 16: Activity of caspases in CCRF-CEM cells treated for 6 h with osajin (**3**) and 7,7”-di-*O*-methylchamaejasmin (**4**). The IC_{50} values were 5.17 µM for compound 2 and 3.58 µM for compound **4**. The concentrations tested corresponded to 1/2 × IC_{50}, IC_{50} and 2 × IC_{50}; Shown are mean ± SD of three independent experiments.

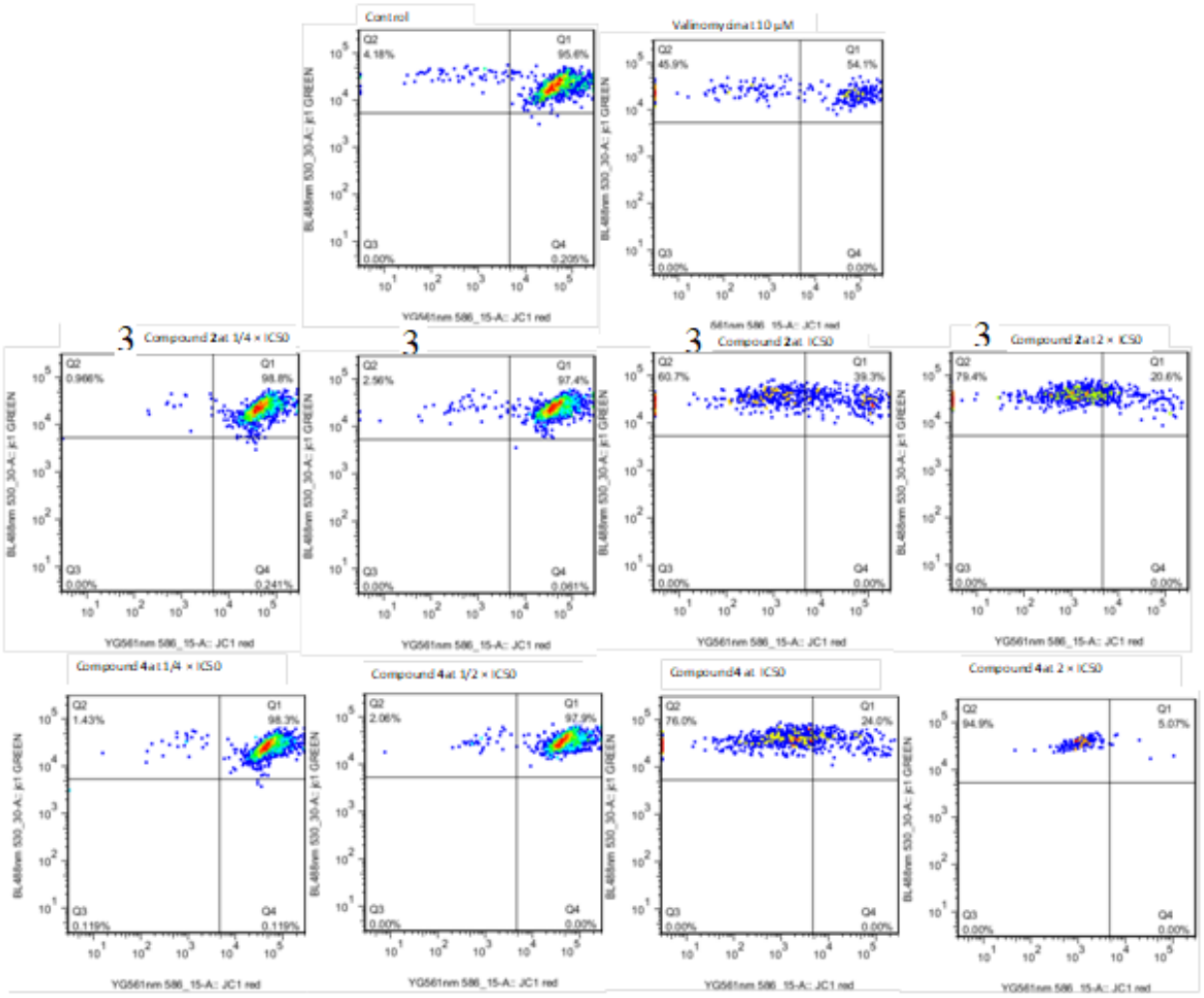


Figure 17: Effect of osajin (**3**), 7,7”-di-*O*-methylchamaejasmin (**4**) and valinomycin for 24 h on the mitochondrial membrane potential of CCRF-CEM cells. Intact cells (Q1), loss of MMP (Q2), ruptured cell membrane (Q3 and Q4). The IC_{50} values were 5.17 µM for compound 3 and 3.58 µM for compound **4**.

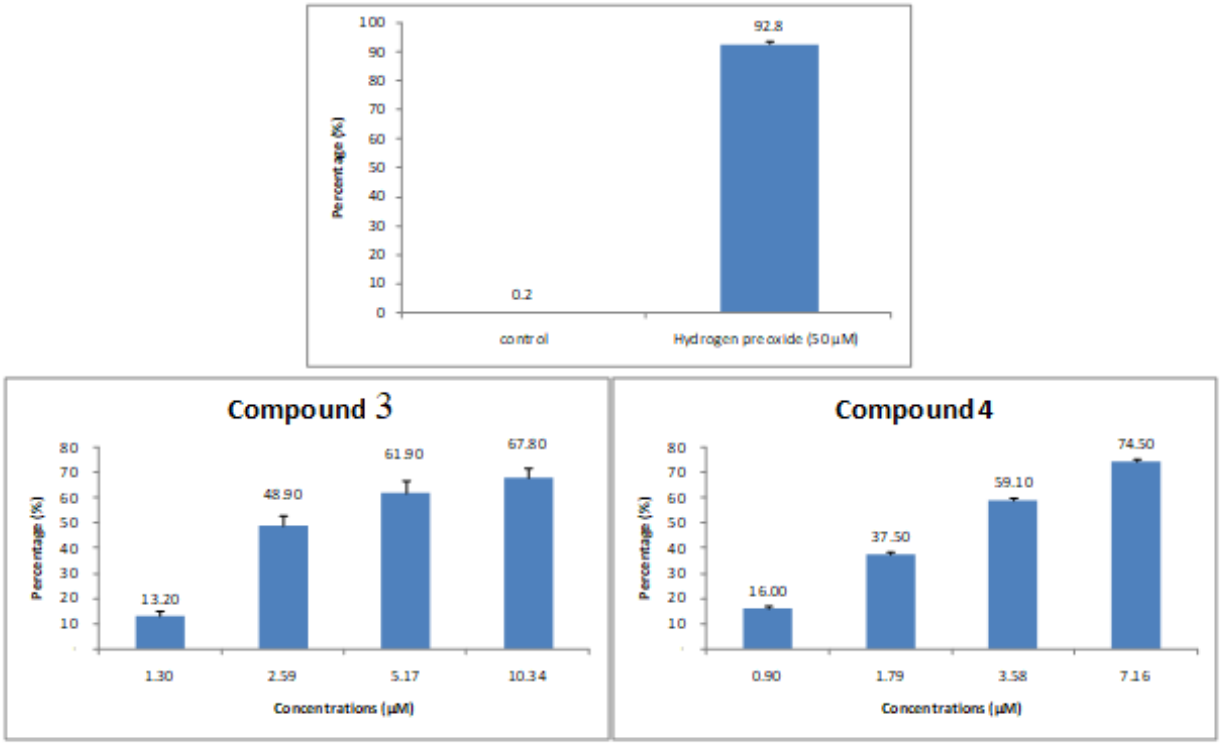


Figure 18: Reactive oxygen species (ROS) production in CCRF-CEM cells treated for 24 h with osajin (**3**), 7,7"-di-*O*-methylchamaejasmin (**4**), and hydrogen peroxide (H_2O_2). Shown are mean ± SD of three independent experiments. The IC_{50} values were 5.17 µM for compound 3 and 3.58 µM for compound 4.The concentrations tested corresponded to 1/4 × IC_{50}, 1/2 × IC_{50}, IC_{50} and 2 × IC_{50}.

Table 32: Cytotoxicity of compounds **2-8** and doxorubicin towards leukemia cells as determined by resazurin assay

Compounds	CCRF-CEM IC_{50} in µM	CEM/ADR5000 IC_{50} in µM	Degree of resistance*
2	15.05 ± 2.29	5.81 ± 0.02	0.38
3	5.17 ± 1.08	3.87 ± 0.44	0.74
4	3.58 ± 0.09	5.69 ± 0.51	1.58
5	> 73.73	65.28 ± 8.62	< 0.88
6	60.40 ± 9.63	47.74 ± 6.80	0.79
7	> 69.87	> 69.87	na
8	34.25 ± 0.84	> 78.85	> 2.30
Doxorubicin	0.02 ± 0.00	66.83 ± 2.20	3341

(*): The degree of resistance was determined as the ratio of IC_{50} value in multidrug- resistant CEM/ADR5000 cells divided by the IC_{50} in sensitive CCRF-CEM cells.

Table 33: Cytotoxicity of compounds **2**, **3** and **4** and doxorubicin towards drug sensitive and resistant solid tumor cell lines as determined by resazurin assay

Cell lines	IC_{50} values in µM and degree of resistance			Doxorubicin
	Compounds			
	2	**3**	**4**	
MDA-MB231	24.28 ± 5.38	9.95 ± 0.65	5.97 ± 0.46	0.07 ±0.00
MDA-MB231*/BCRP*	17.82 ± 2.61	14.44 ± 1.40	7.76± 0.30	0.43 ± 0.10
Degree of resistance*	0.73	1.45	1.29	6.14
HCT116*($p53^{+/+}$)*	19.67 ± 3.93	8.49 ± 2.47	6.02 ± 0.36	0.26 ± 0.01
HCT116*($p53^{-/-}$)*	23.15 ± 2.30	8.76 ± 0.30	5.48 ± 0.16	0.97 ± 0.02
Degree of resistance*	1.17	1.03	0.91	3.73
U87MG	42.23 ± 4.37	12.25 ± 5.61	6.16 ± 0.06	0.14 ± 0.01
U87MG.*ΔEGFR*	16.90 ± 0.92	7.85 ± 0.26	4.96 ± 0.22	0.53 ± 0.08
Degree of resistance*	0.4	0.64	0.80	3.79
HepG2	48.67 ± 7.15	12.86 ± 3.74	5.59 ± 0.61	2.15 ± 0.03
AML12	38.68 ± 6.64	7.96 ± 3.15	6.37 ± 0.63	0.48 ± 0.01
Sensitivity index**	0.79	0.61	1.13	0.22

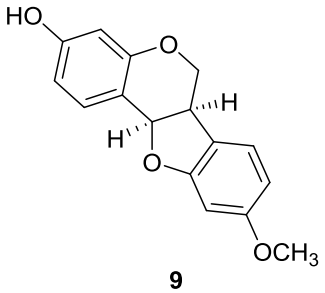
(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; MDA-MB-231-*BCRP*, HCT116 (*$p53^{-/-}$*) and U87MG.Δ*EGFR* were used as the corresponding resistant counterpart for MDA-MB-231-*pcDNA*, HCT116 (*$p53^{+/+}$*), U87MG respectively; (**): The selectivity index was determined as the ratio of IC_{50} value of normal AML12 hepatocytes divided by the IC_{50} of HepG2 hepatocarcinoma cells.

4.7.3. Cytotoxicity of compounds isolated from Derris trifoliata

The compounds (**9**-**23**) isolated from *Derris trifoliata*, except compound **16,** were evaluated for their cytotoxicity against HEK293 cells. Except for medicarpin (**9**) and 6,7-dimethoxy-4-chromanone (**23**) (IC_{50} > 100 µM against HEK cells) all the compounds tested showed activity with IC_{50} values less than 95 µM (Table 34). Rotenone (**10**) exhibited the highest cytotoxic (IC_{50} value of 0.82 ± 0.02 µM) towards HEK293 cells. Deguelin (**11**) and elliptone (**17**) showed weak activity with IC_{50} values of 38.9 and 84.0 µM against the HEK293 cells. The rotenoloids (rotenoid with an open ring C) 7a-*O*-methyldeguelol (**12**), showed better cytotoxicity towards HEK293 cells (IC_{50} 9.4 ± 0.25 µM). Similarly, the activity of 7-a-*O*-methylelliptonol (**18**) was higher than elliptone (**17**) (IC_{50} 7.1 ± 0.50 µM) (Table 34) suggesting that rotenoloids (rotenoids moiety with open ring C) have better activities compared to their rotenoid counter parts. The rotenoloids and rotenoids have earlier been reported for their cytotoxicity against KB, BC and NCI-H187 cell lines (Cheenpracha, *et al.*, 2007). The rotenoids (rotenone (**10**), deguelin (**11**) and α-toxicarol (**13)**) showed better cytotoxicity against KB, BC and NCI-H187 cell lines with IC_{50} values in the range of 0.05 to 1.8 µM, but rotenoloid 7a-*O*-methyldeguelol (**12**) showed selectivity (IC_{50} 4.1 µM) against NCI-H187 cell lines, while 7a-*O*-methylelliptonol (**18**) showed activity against KB, BC and NCI-H187 cells with IC_{50} values of 4.1, 3.8 and 3.0 µM, respectively (Cheenpracha, *et al.*, 2007). Among flavones (**19**-**22**) tested barbigerone (**20**) showed good cytotoxicity with an IC_{50} value of 2.1 ± 0.30 µM. Prunetin (19), lupinifolin (**21**) and dereticulatin (22) showed weak activity with IC_{50} values of 45.8, 39.7 and 93.8 µM, respectively against the HEK293 cells (Table 34).

Table 34: Cytotoxic activity of compounds **9**-**23** against HEK293 cell line

Compounds	HEK293 at top dose (100 µM)	HEK293 IC_{50} in µM
9	31.5 ± 4.68	> 100
10	88.5 ± 0.86	0.82 ± 0.02
11	65.3 ± 0.40	~38.9
12	91.6 ± 0.61	9.4 ± 0.25
13	77.4 ± 1.85	21.4 ± 3.79
14	83.3 ± 5.31	~11.5
15	75.4 ± 1.46	~8.4
17	61.3 ± 5.71	~84.0
18	83.6 ± 0.66	7.1 ± 0.50
19	61.1 ± 6.06	45.8 ± 7.41
20	89.0 ± 2.12	2.1 ± 0.30
21	100.9 ± 0.49	~39.7
22	50.7 ± 11.8	~93.8
23	17.7 ± 15.3	> 100
DHA	92.3 ± 9.71	~10.6
Puromycin	98.3 ± 0.54	0.7 ± 0.13
Pyrimethamine	62.3 ± 3.6	~15.8
Pyronaridine	97.9 ± 0.21	4.9 ± 0.84



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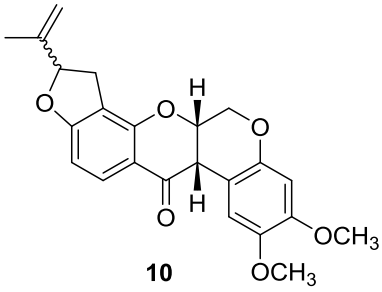

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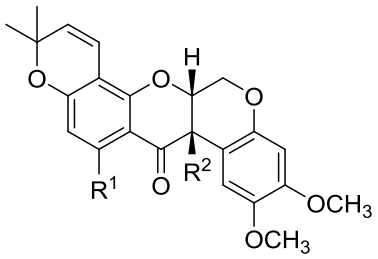

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13 R1 = OH R2 = H
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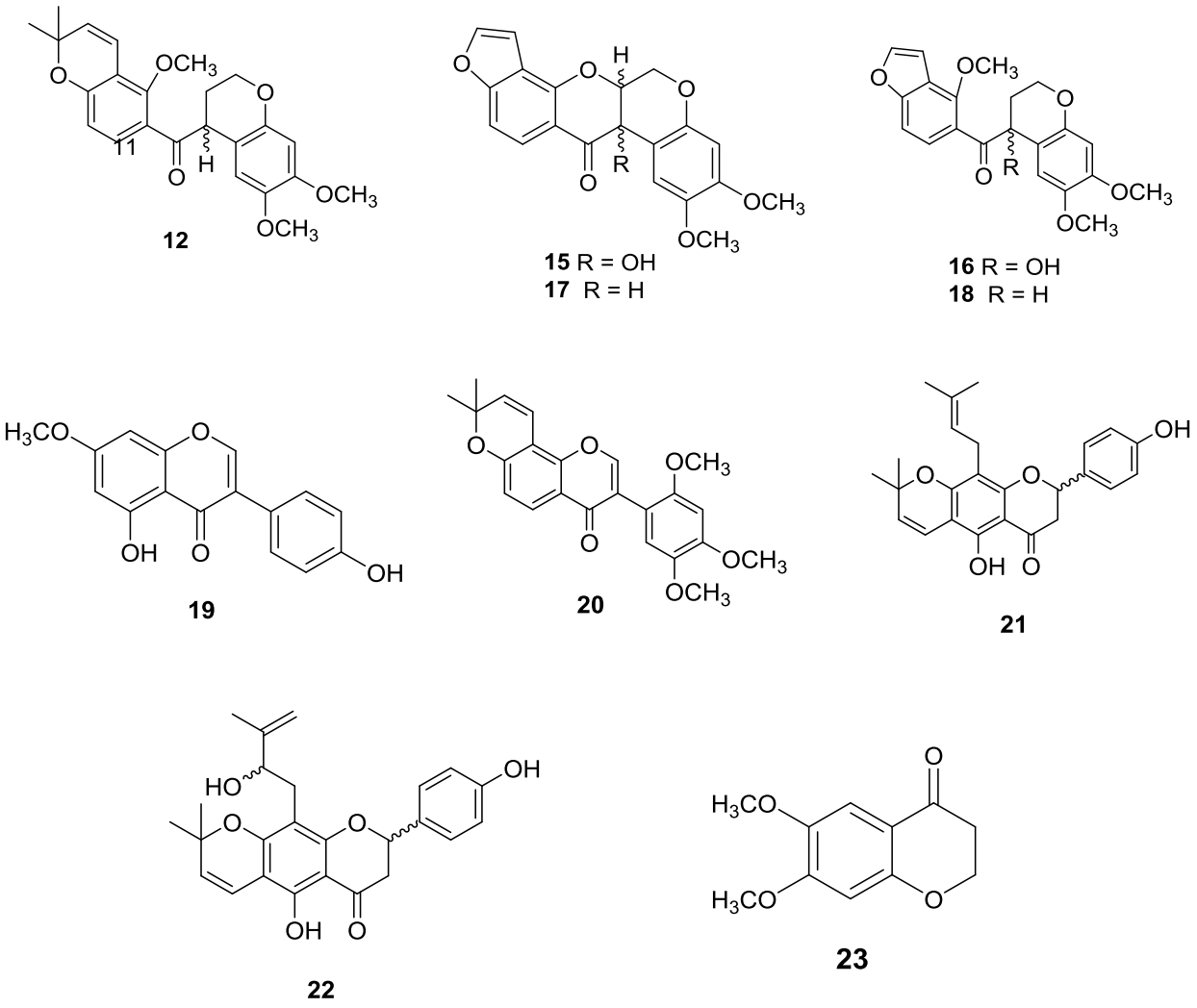


4.7.4. Cytotoxicity of compounds isolated from Lonchocarpus bussie and L. eryocalix

The cytotoxicity of compounds **24-33** as well as the standard doxorubicin was first determined against sensitive leukemia CCRF-CEM cells and the drug resistant subline CEM/ADR5000 cells. Apart from maximaisoflavone H (**26**) against CCRF-CEM and CEM/ADR5000 cells, and (6a*R*,11a*R*)-maackiain (**32**) against CCRF-CEM cells, all other compounds showed activities with IC_{50} values below 90 µM against the two cell lines (Table 35). Against the CCRF-CEM cells, significant activity with IC_{50} values less than 10 µM (Brahemi *et al.*, 2010; Kuete & Efferth, 2015), were observed for 6,7,3'-trimethoxy-4',5'-methylenedioxyisoflavone (**27**, IC_{50} 6.27 µM)

and durmillone (**28**, IC_{50} 0.54 µM). Similarly, 4'-hydroxylonchocarpin (**30**, IC_{50} 3.43 µM) and durmillone (**28**, IC_{50} 0.86 µM) showed moderate to good activities against the CEM/ADR5000 cells, respectively. It is worth noting that CEM/ADR5000 cells were highly resistant to the drug doxorubicin; on the other hand, all the tested compounds (except for compounds **25** and **31**) showed better activity than the standard against this cell-line. Hypersensitivity (degree of resistance or D.R. below 0.90) (Mbaveng *et al.,* 2017) of CEM/ADR5000 compared to it sensitive parental cell line CCRF-CEM was noted with (6a*R*,11a*R*)-4,9-dimethoxybitucarpin B (**33**), 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone (**25**), 4-hydroxylonchocarpin (**29**) and colenemol (**30**), suggesting that they might have inhibitory effect on P-glycoprotein's expression (Mbaveng *et al.*, 2017). With regards to their activity in leukemia cells, 4'-prenyloxyvigvexin A (**24**), 6,7,3'-trimethoxy-4',5'-methylenedioxyisoflavone (**27**) and durmillone (**28**) displaying IC_{50} values below 20 µM towards CCRF- CEM cells were selected and screened further against a panel of 7 carcinoma cells and normal AML12 hepatocytes (Table 36). Of the three compounds, the isoflavone durmillone (**28**) displayed IC_{50} values below 10 µM towards resistant MDA-MB231/*BCRP* cells (8.97 µM) and resistant glioblastoma U87MG.Δ*EGRF* cells (5.83 µM). Interestingly, hypersensitivity was obtained with the three selected compounds in U87MG.Δ*EGRF* cells as well as with 4'-prenyloxyvigvexin A (**24**) and durmillone (**28**) in MDA-MB231/*BCRP* cells compared to their respective sensitive counterparts U87MG cells and MDA-MB231 cells. It is also important to note that the selectivity indexes of 4'-prenyloxyvigvexin A (**24**), 6,7,3'-trimethoxyl-4',5'-methylenedioxyisoflavone (**27**) and durmillone (**28**) for the normal AML12 hepatocytes versus hepatocarcinoma HepG2 cells are below 1, suggesting their poor selectivity to liver cancer cells. However, a closer look at the IC_{50} values of the compounds against AML12 cells versus other cell lines indicated that higher selectivity can be achieved with

other cancer types. Overall, the study reveals the good activity of durmillone (**28**), with IC_{50} below 1 µM towards the two leukemia cells and against 2/7 tested carcinoma cells. This compound is a cytotoxic agent that can be explored further towards the development of an anticancer drug.

Table 35: Cytotoxicity of the studied compounds (**24**-**33**) and doxorubicin towards leukemia cells as determined by resazurin assay

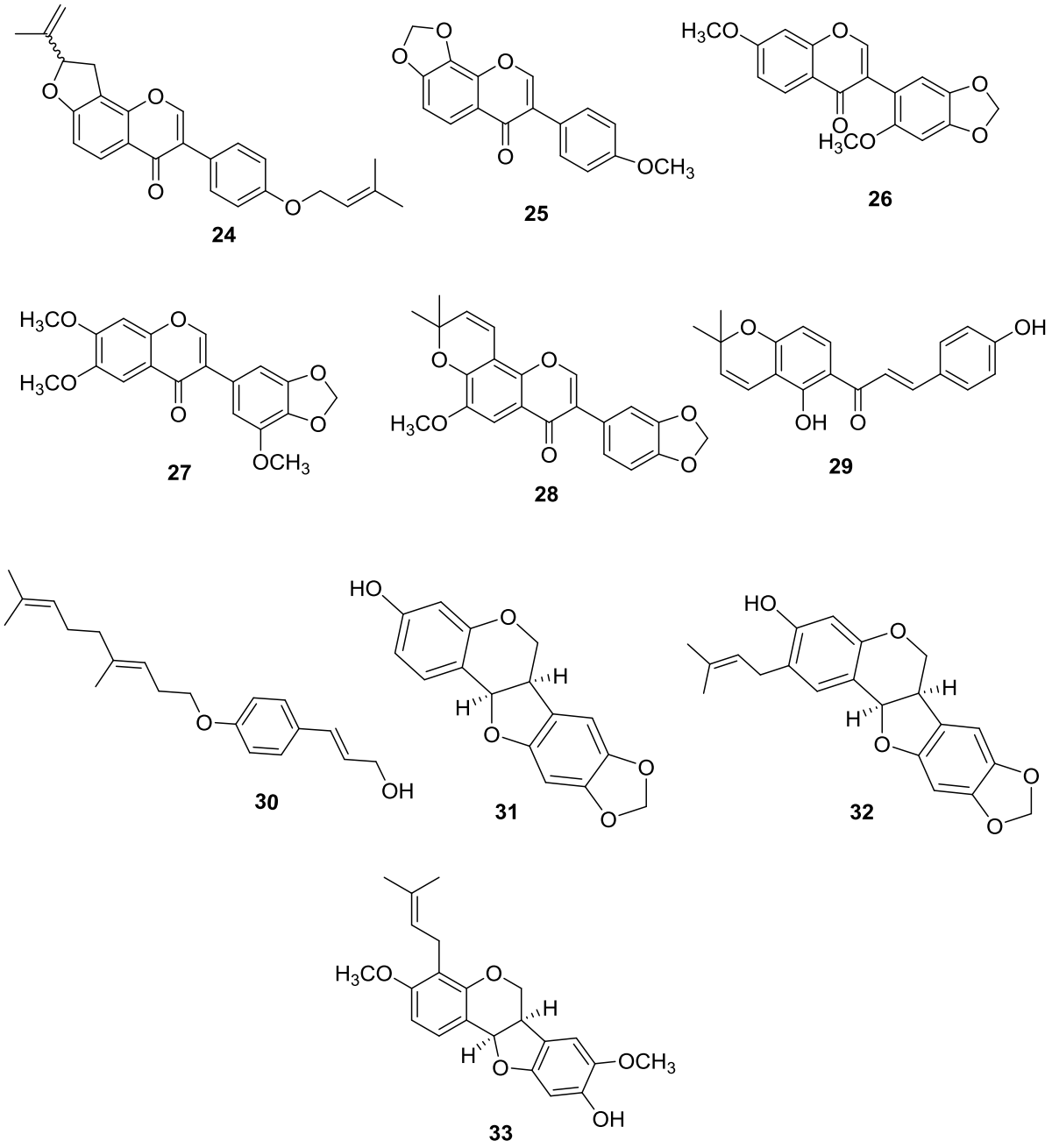
Compound	IC_{50} value in µM and degree of resistance		Degree of resistance*
	CCRF-CEM	CEM/ADR5000	
24	18.92±4.88	25.53±9.05	1.34
25	31.82 ±3.40	16.87±1.94	0.53
26	>135.10	>135.10	na
27	**6.27±1.41**[b]	29.51±3.75	6.70
28	**0.54±0.17**[b]	**0.86±0.02**[b]	1.59
29	49.49±11.27	32.47±5.53	0.65
30	88.91±1.18	**3.43±1.21**[b]	0.03
31	37.74±14.12	21.58±0.84	0.57
32	>140.70	81.34±4.10	>0.57
33	34.11±0.80	33.22±4.07	0.97
Doxorubicin	**0.02±0.00**[b]	66.83±2.20	3341

[b] Values in bold: Significant activity

Table 36: Cytotoxicity of compounds **24**, **27**, **28** and doxorubicin towards leukemia cells as determined by resazurin assay

	IC_{50} value in µM and degree of resistance			
	Compound			
Cell lines	**24**	**27**	**28**	Doxorubicin
MDA-MB231	>102.99	67.89 ± 23.34	27.14±4.73	**0.07±0.00**[b]
MDA-MB231/ BCRP	83.08 ± 0.29	>112.26	**8.97±3.83**[b]	**0.43±0.10**[b]
Degree of resistance	<0.80	>1.65	0.33	6.14
HCT116p53+/+	57.49±2.91	103.30±12.03	23.56±3.27	**0.26±0.01**[b]
HCT116p53-/-	67.42±7.01	>112.26	>105.71	**0.97±0.02**[b]
Degree of resistance	>1.17	>1.08	>4.48	3.73
U87MG	85.71±16.79	>112.26	14.92±1.85	**0.14±0.01**[b]
U87MG.ΔEGFR	56.31±5.90	80.19±16.53	**5.83±1.07**[b]	**0.53±0.08**[b]
Degree of resistance	0.65	0.71	0.39	3.79
HepG2	>102.99	>112.26	>105.71	**2.15±0.03**[b]
AML12	60.41±2.64	85.82±1.07	96.04±5.53	**0.48±0.01**
Sensitivity index	<0.58	<0.76	<0.90	0.22

[b] Values in bold: Significant activity



4.7.5. Cytotoxicity of compounds isolated from Dorstenia kameruniana

The cytotoxicity of compounds **34-38** as well as that of the standard doxorubicin was first determined against the drug-sensitive leukemia CCRF-CEM cells and its multidrug-resistant subline, CEM/ADR5000. Dorsmerunin A-C (**34-36**) and licoagrochalcone A (**38**) displayed

cytotoxic effects against both cell-lines with IC_{50} values below 80 µM (Table 37). Against CCRF-CEM cells, significant activity with IC_{50} values less than 10 µM (Brahemi, *et al.*, 2010; Kuete & Efferth, 2015) were obtained for bergapten (**37**) (IC_{50} = 7.17 µM) and licoagrochalcone A (**38**) (IC_{50} = 5.16 µM). Hypersensitivity (collateral sensitivity with a degree of resistance (D.R.) below 0.90) of CEM/ADR5000 cells compared to their sensitive parental CCRF-CEM cells was observed for dorsmerunin A (**34**) and dorsmerunun B (**35**) (Table 37). This indicates that the two compounds might be potential inhibitors of P-glycoprotein's expression (Mbaveng *et al.*, 2017). With regards to their activity in the sensitive CCRF-CEM leukemia cells, bergapten (**37**) and licoagrochalcone A (**38**) displaying IC_{50} values below 10 µM were selected and tested towards a panel of 7 solid tumor cell lines and normal AML12 hepatocytes (Table 38). licoagrochalcone A (**38**) showed cytotoxic effect against all 7 solid tumor cell lines tested with IC_{50} values below 50 µM, whilst bergapten (**37**) showed selective activity (Table 38). Interestingly, collateral sensitivity was observed for licoagrochalcone A (**38**) against drug-resistant U87MG.Δ*EGRF* glioblastoma cells (D.R. of 0.60) compared to the corresponding sensitive counterpart U87MG cells, Table 38). It is also important to note that the selectivity indexes of bergapten (**37**) and licoagrochalcone A (**38**) for normal AML12 hepatocytes *versus* HepG2 hepatocarcinoma cells were below 1, suggesting poor selectivity to liver cancer cells. Nonetheless, a closer look at the IC_{50} values of licoagrochalcone A (**38**) in AML12 cells compared to other cell lines allows to speculate that higher selectivity can be achieved with other cancer types such as HCT116 *($p53^{+/+}$)* colon carcinoma cells or U87MG.*ΔEGFR* glioblastoma cells. The overall data showed the potential of the tested compounds, especially bergapten (**37**) and licoagrochalcone A (**38**), towards the development of novel drugs to treat cancer.

Table 37: Cytotoxicity of compounds **34**-**38** and doxorubicin towards leukemia cells as determined by resazurin assay.

Samples	CCRF-CEM IC_{50} in µM	CEM/ADR5000 IC_{50} in µM	Degree of resistance*
34	77.15 ± 1.27	58.21 ± 0.38	0.75
35	35.13 ± 4.63	28.95 ± 2.64	0.82
36	29.07 ± 2.18	47.81 ± 4.56	1.64
37	7.17 ± 0.85	>185.02	>25.8
38	5.16 ± 1.40	17.38 ± 1.96	3.36
Doxorubicin	0.02 ± 0.00	66.83 ± 2.20	3341

(*): The degree of resistance was determined as the ratio of IC_{50} value in multidrug- resistant CEM/ADR5000 cells divided by the IC_{50} in sensitive CCRF-CEM cells.

Table 38: Cytotoxicity of compounds **37**, **38** and doxorubicin towards drug sensitive and resistant solid tumor cell lines as determined by resazurin assay.

Cell lines	IC_{50} value in µM and degree of resistance		Doxorubicin
	Compounds		
	37	**38**	
MDA-MB231	132.80 ± 3.40	34.84 ± 0.33	0.07 ±0.00
MDA-MB231/*BCRP*	158.55 ± 10.92	49.57 ± 0.44	0.43 ± 0.10
Degree of resistance*	1.19	1.42	6.14
HCT116($p53^{+/+}$)	111.61 ± 29.44	21.84 ± 4.68	0.26 ± 0.01
HCT116($p53^{-/-}$)	>185.02	31.25 ± 2.82	0.97 ± 0.02
Degree of resistance*	>1.65	1.43	3.73
U87MG	163.61 ± 12.17	37.63 ± 1.48	0.14 ± 0.01
U87MG.*ΔEGFR*	163.40 ± 15.67	22.75 ± 1.30	0.53 ± 0.08
Degree of resistance*	0.99	0.60	3.79
HepG2	>185.02	42.95 ± 2.82	2.15 ± 0.03
AML12	166.66 ± 7.06	37.16 ± 4.45	0.48 ± 0.01
Sensitivity index**	<0.90	0.86	0.22

(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; MDA-MB-231-*BCRP*, HCT116 ($p53^{-/-}$) and U87MG.Δ*EGFR* were used as the corresponding resistant counterpart for MDA-MB-231-*pcDNA*, HCT116 ($p53^{+/+}$), U87MG respectively; (**): The selectivity index was determined as the ratio of IC_{50} value of normal AML12 hepatocytes divided by the IC_{50} of HepG2 hepatocarcin

4.7.6. Cytotoxicity of compounds isolated from Streblus usambarensis

The cytotoxicity of compounds **39-41** as well as doxorubicin was first determined in both sensitive CCRF-CEM leukemia cells and in drug resistant subline CEM/ADR5000 cells. The three compounds displayed cytotoxic effects in both sensitive CCRF-CEM and resistant CEM/ADR5000 leukemia cells with IC_{50} values below 25 µM (Table 39). In CEM/ADR5000 cells, significant activity with IC_{50} values of 10 µM (Brahemi, *et al.*, 2010; Kuete & Efferth, 2015) was obtained for usambarin B (**40**), while doxorubicin had IC_{50} values of 0.02 µM in CCRF-CEM cells. Hypersensitivity (degree of resistance or D.R. below 0.90) (Mbaveng, *et al.*, 2017) of CEM/ADR5000 compared to its sensitive parental cell line CCRF-CEM was noted with usambarin B (**40**) (Table 39), suggesting that this compound could be potential inhibitors of P-glycoprotein's expression (Mbaveng *et al.*, 2017). In regards to their activity in the two leukemia cells, usambarin B (**40**) and usambarin C (**41**) displaying IC_{50} values below 20 µM were selected and tested towards a panel of 7 carcinoma cells and normal AML12 hepatocytes (Table 40). The two compounds had cytotoxic effect in the 7 tested carcinoma cell lines with below 63 µM. Usambarin B (**40**) and usambarin C (**41**) could be exploited in the fight against cancers. Interestingly, hypersensitivity was obtained with Usambarin B (**40**) towards resistant U87MG.Δ*EGRF* glioblastoma cells (D.R. of 0.42) compared to its respective sensitive counterparts U87MG cells, and with usambarin C (**41**) towards U87MG.Δ*EGRF* cells (D.R. of 0.73) and resistant MDA-MB231/*BCRP* breast adenocarcinoma cells compared to its sensitive counterparts MDA-MB231 cells (D.R. of 0.85). It is also important to note that the selectivity indexes of usambarin B (**40**) and usambarin C (**41**) for the normal AML12 hepatocytes versus HepG2 hepatocarcinoma cells are around or below 1, suggesting their poor selectivity to liver cancer cells; Nonetheless, a keen look of IC_{50} values of usambarin C (**41**) in AML12 cells versus

other cell lines indicate that higher selectivity can be achieved with other cancer types such as HCT116*(p53$^{+/+}$)* colon carcinoma cells or U87MG.*ΔEGFR* cells. The overall data highlight the possibility of using the tested phytochemicals and mostly usambarin B (**40**) and usambarin C (**41**) to develop a novel drug to fight drug sensitive and resistant cancers.

Table 39: Cytotoxicity of compounds **39-41** and doxorubicin towards leukemia cells as determined by resazurin assay.

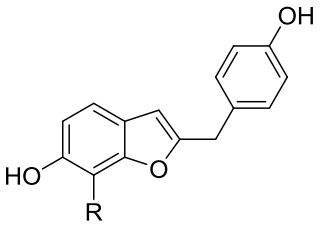
Samples	CCRF-CEM IC_{50} in µM	CEM/ADR5000 IC_{50} in µM	Degree of resistance*
39	22.84 ± 4.00	24.45 ± 2.91	1.07
40	16.02 ± 0.20	6.13 ± 0.22	0.38
41	18.93 ± 2.30	18.17 ± 1.00	0.95
Doxorubicin	0.02 ± 0.00	66.83 ± 2.20	3341

(*): The degree of resistance was determined as the ratio of IC_{50} value in multidrug- resistant CEM/ADR5000 cells divided by the IC_{50} in sensitive CCRF-CEM cells.

Table 40: Cytotoxicity of compounds **40**, **41** and doxorubicin towards drug sensitive and resistant solid tumor cell lines as determined by resazurin assay.

Cell lines	IC_{50} value in µM and degree of resistance		Doxorubicin
	Compounds		
	40	**41**	
MDA-MB231	23.31 ± 2.80	26.90 ± 10.65	0.07 ±0.00
MDA-MB231*/BCRP*	27.15± 9.25	23.12 ± 1.37	0.43 ± 0.10
Degree of resistance*	1.16	0.85	6.14
HCT116*(p53$^{+/+}$)*	19.60 ± 0.73	16.11 ± 0.31	0.26 ± 0.01
HCT116*(p53$^{-/-}$)*	29.59 ± 7.02	14.90 ± 2.97	0.97 ± 0.02
Degree of resistance*	1.50	0.92	3.73
U87MG	32.22 ± 0.08	22.28 ± 3.45	0.14 ± 0.01
U87MG.*ΔEGFR*	13.77 ± 0.20	16.46 ± 1.07	0.53 ± 0.08
Degree of resistance*	0.42	0.73	3.79
HepG2	62.76 ± 3.43	20.54 ± 2.17	2.15 ± 0.03
AML12	32.58 ± 4.06	21.54 ± 2.60	0.48 ± 0.01
Sensitivity index**	0.51	1.04	0.22

(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; MDA-MB-231-*BCRP*, HCT116 (*p53*$^{-/-}$) and U87MG.Δ*EGFR* were used as the corresponding resistant counterpart for MDA-MB-231-*pcDNA*, HCT116 (*p53*$^{+/+}$), U87MG respectively; (**): The selectivity index was determined as the ratio of IC_{50} value of normal AML12 hepatocytes divided by the IC_{50} of HepG2 hepatocarcinoma cells.



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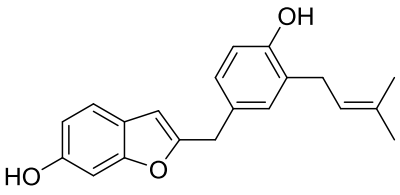

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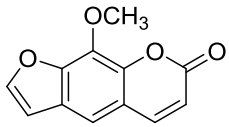

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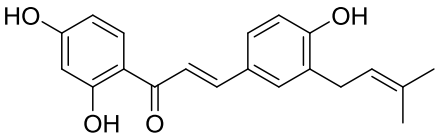

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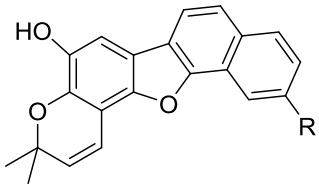

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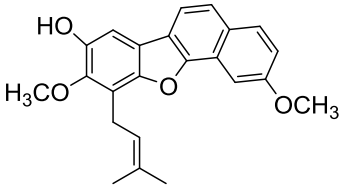

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Phytochemical study of plants from Leguminosae (*Ormocarpum kirkii*, *Derris trifoliata*, *Lonchocarpus bussei*, *Lonchocarpus eriocalyx*) and Moraceae families (*Dorstenia kameruniana* and *Streblus usambarensis*) led to the isolation of forty one compounds, of which 7a-*O*-methyl-12a-hydroxyelliptonol (**16**), 4'-prenyloxyvigvexin (**24**), (6a*R*,11a*R*)-4,9-dimethoxybitucarpinB (**33**) dorsmeruninA, B and C (**34**-**36**), usambarin A, B and C (**39**-**41**) are new.

Investigation for cytotoxicity of the isolated compounds, based on resazutine assay, on drug sensitive and multidrug resistant cancer cell lines showed that osajin (**3**), 7,7''-di-*O*-methylchamaejasmin (**4**) and durmillone (**28**) had significant cytotoxic effects with IC_{50} values below 10 µM against 7 carcinoma cells and a normal AML12 hepatocytes (4/7, 7/7 and 2/7 respectivelly); while rotenone (**10**), 7a-*O*-methyldeguelol (**12**), 12a-Hydroxyelliptonol (**15**), 7-a-*O*-methylelliptonol (**18**) and barbigerone (**20**) towards HEK293 cells.

The mechanistic studies of the most cytotoxic compounds were indicates that Osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) induced apoptosis in CCRF-CEM cells by MMP alteration and increased ROS production.

5.2. Recommendation

1. Synthetic derivatives of the active compounds, especially of osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) should be prepared and tested.
2. The X-ray data and biosynthetic pathway of the unique compounds usambarin A (**39**), usambarin B (**40**) and usambarin C (**41**) should be generated.

3. In vivo cytotoxicity or toxicology of the most active compounds; osajin (**3**) and 7,7''-di-*O*-methylchamaejasmin (**4**) should be established.
4. The mechanism of action of durmillone (**28**) shoud be explored.
5. Osajin (**3**), 7,7''-di-*O*-methylchamaejasmin (**4**) and durmillone (**28**) should be explored further towards the development of an anticancer drug.

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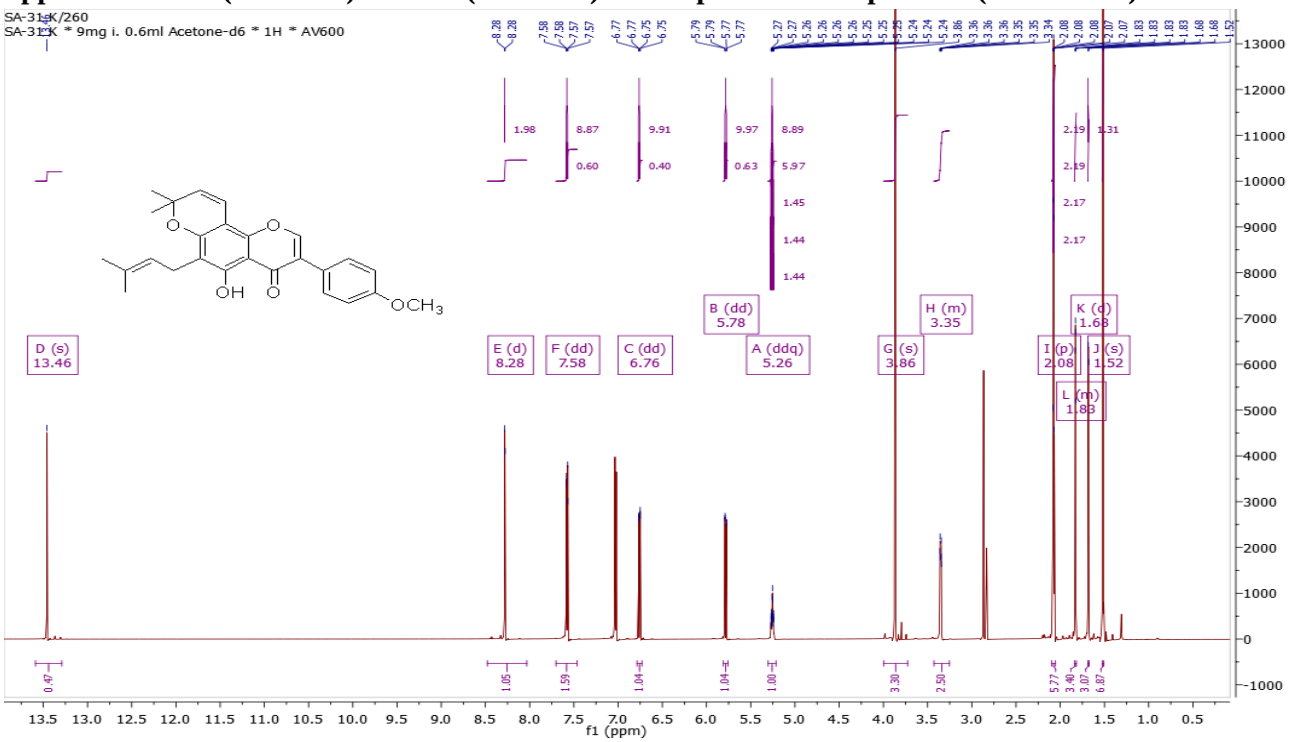
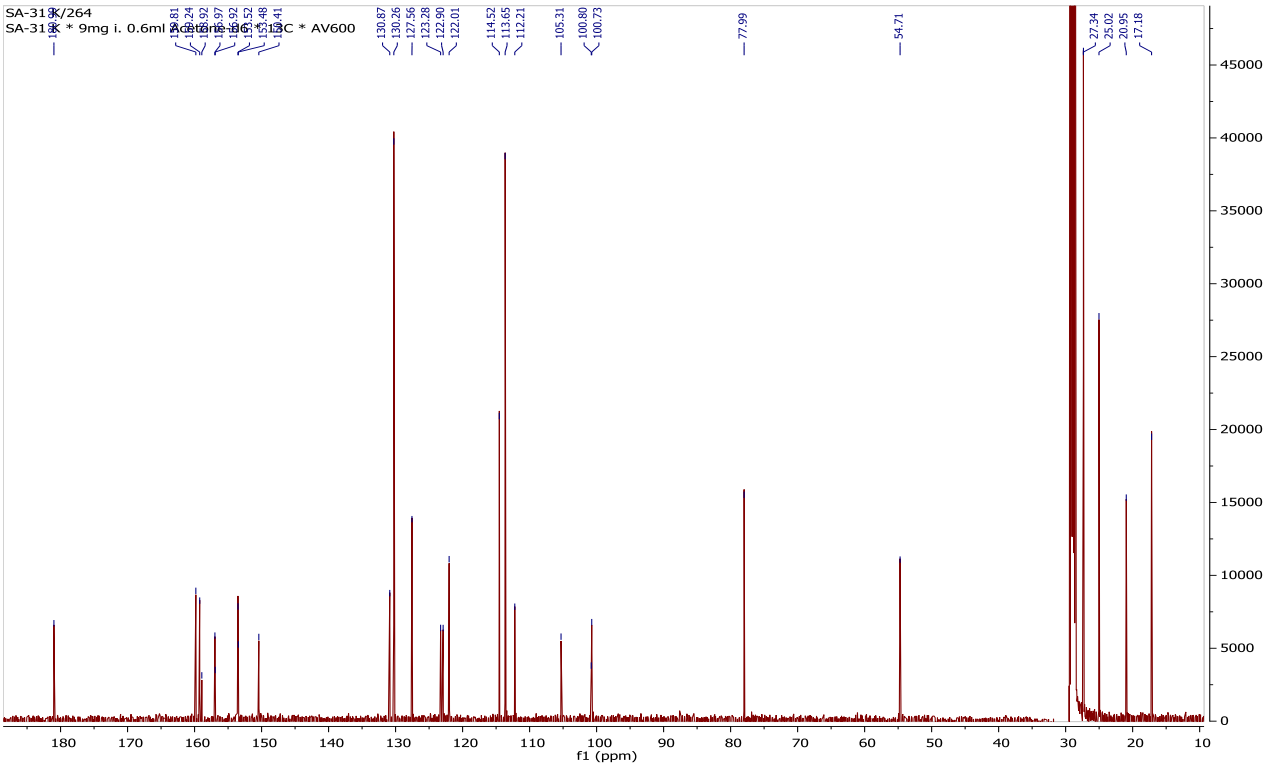
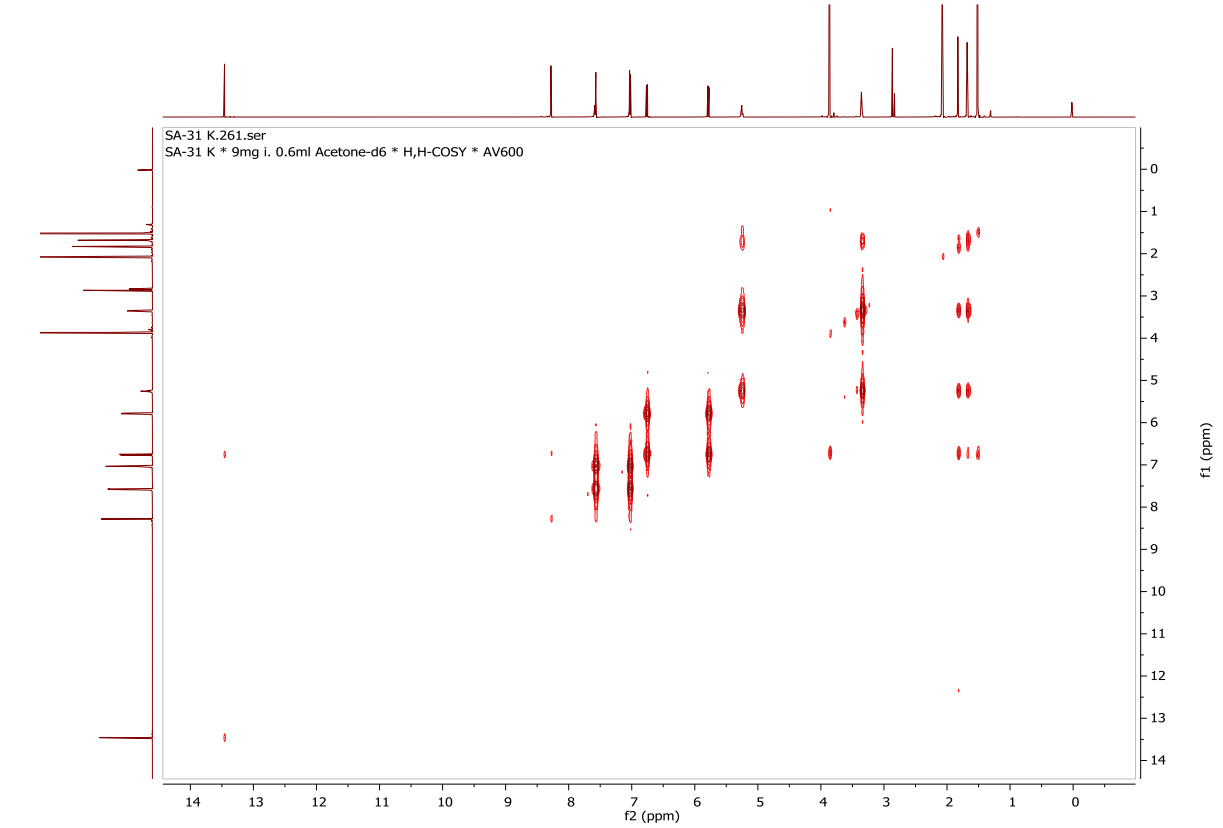
APPENDICES

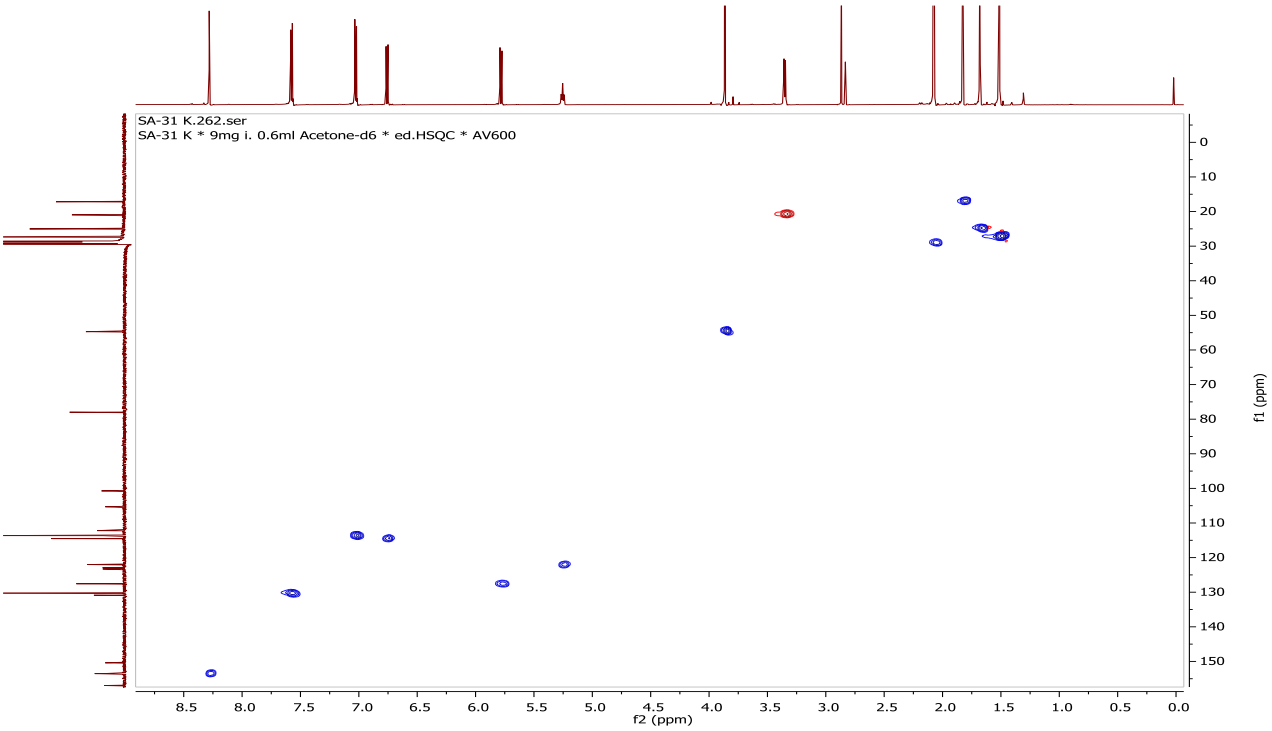
Appendix 1A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of compound 1 (Acetone-d6)



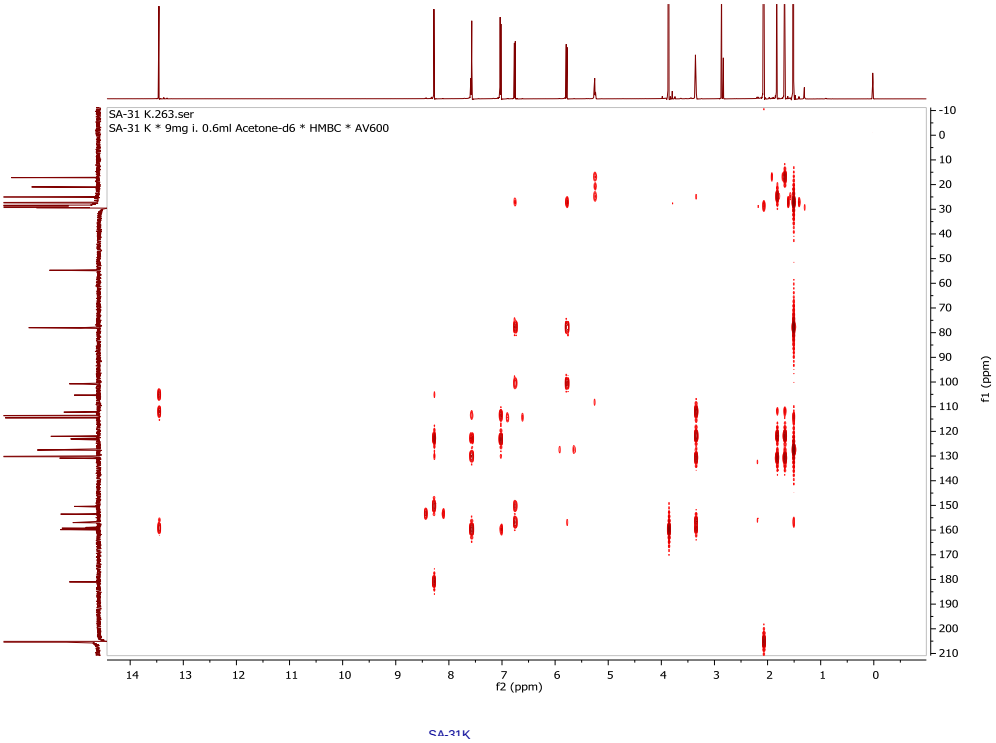


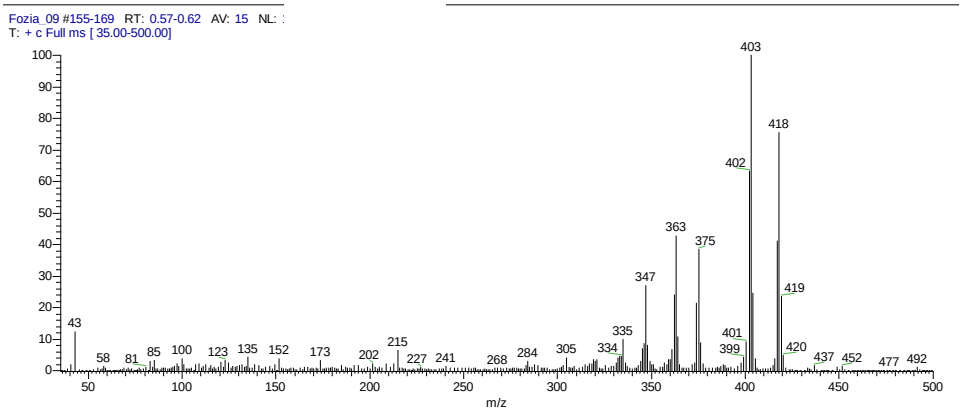
Appendix 1B: H,H-COSY and HSQC spectra of compound 1 (600 MHz, acetone-d6)

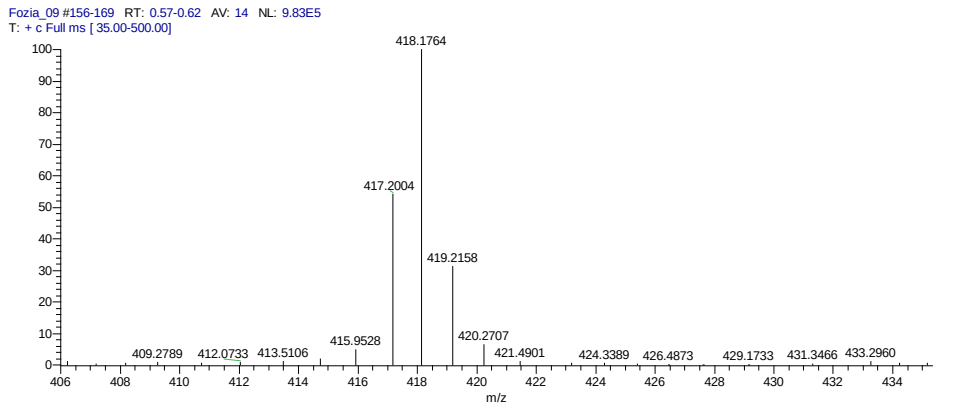




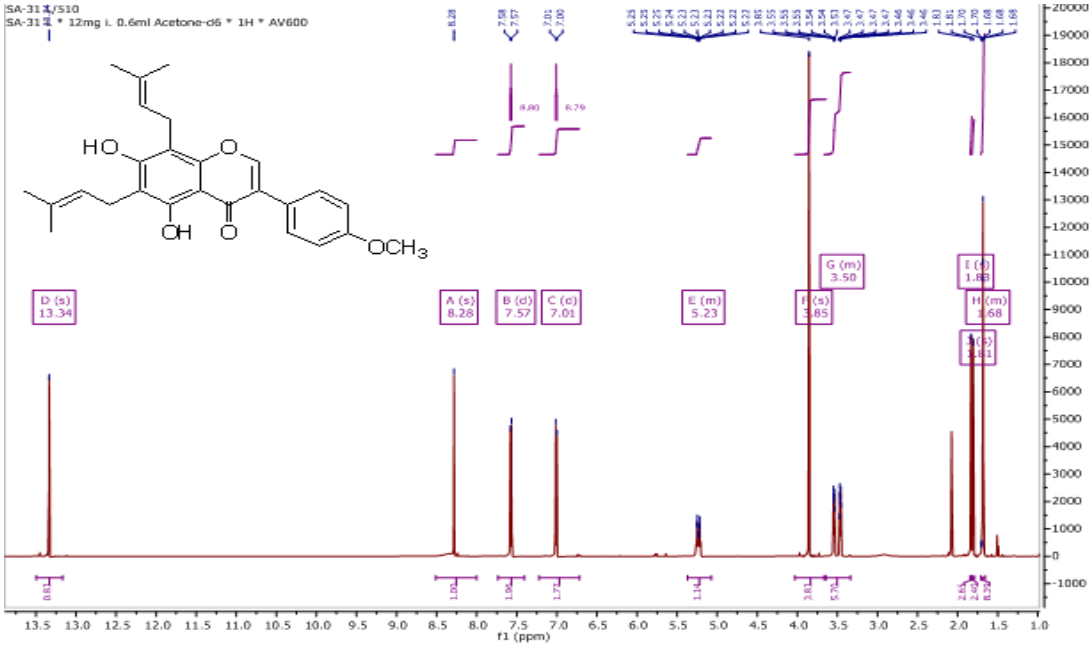
Appendix 1C: HMBC (600 MHz, acetone-d6) and HREIMS spectra of compound 1

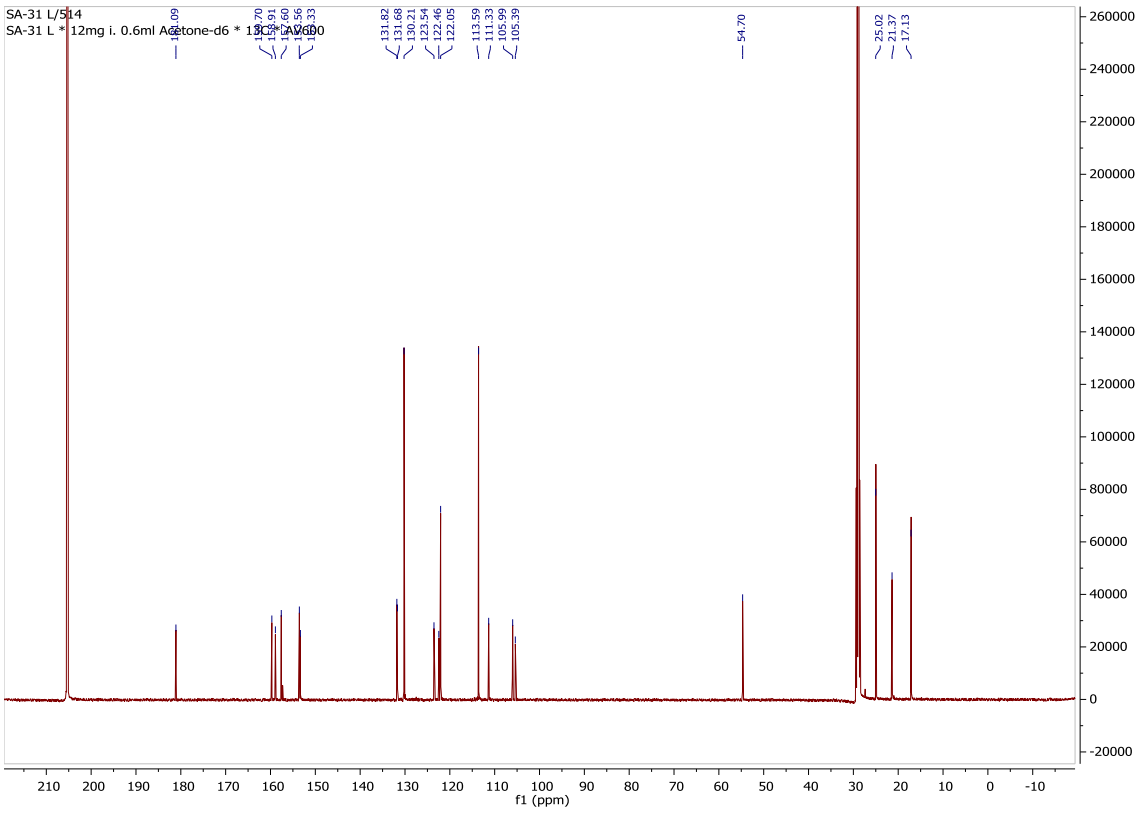




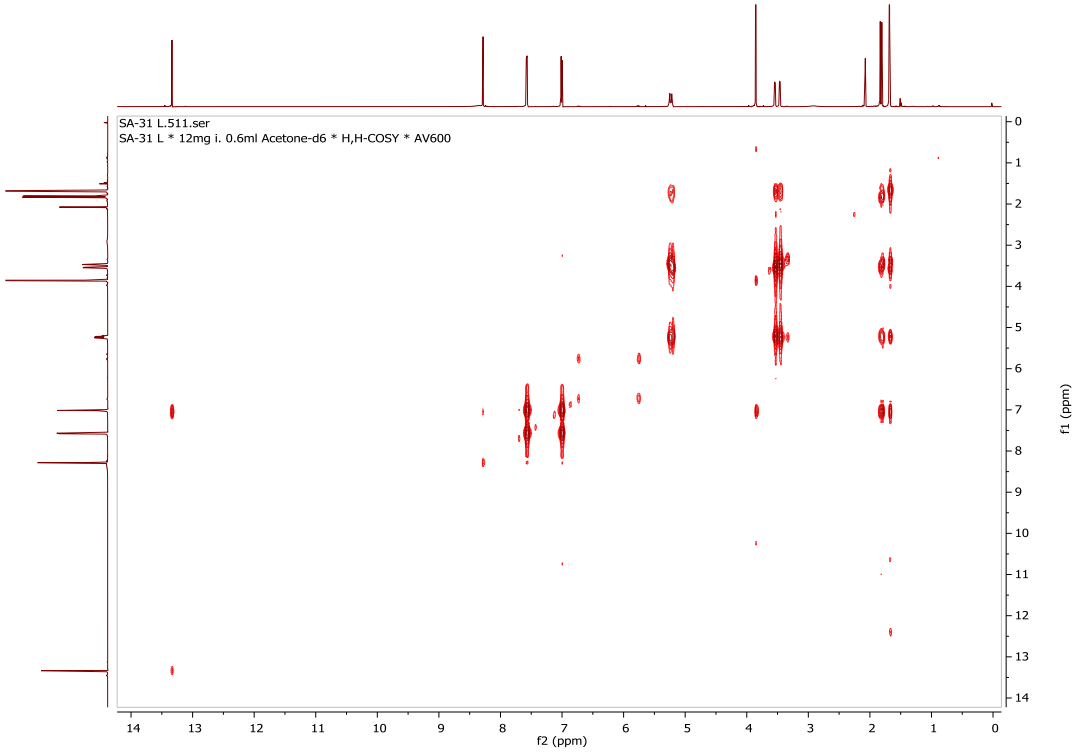


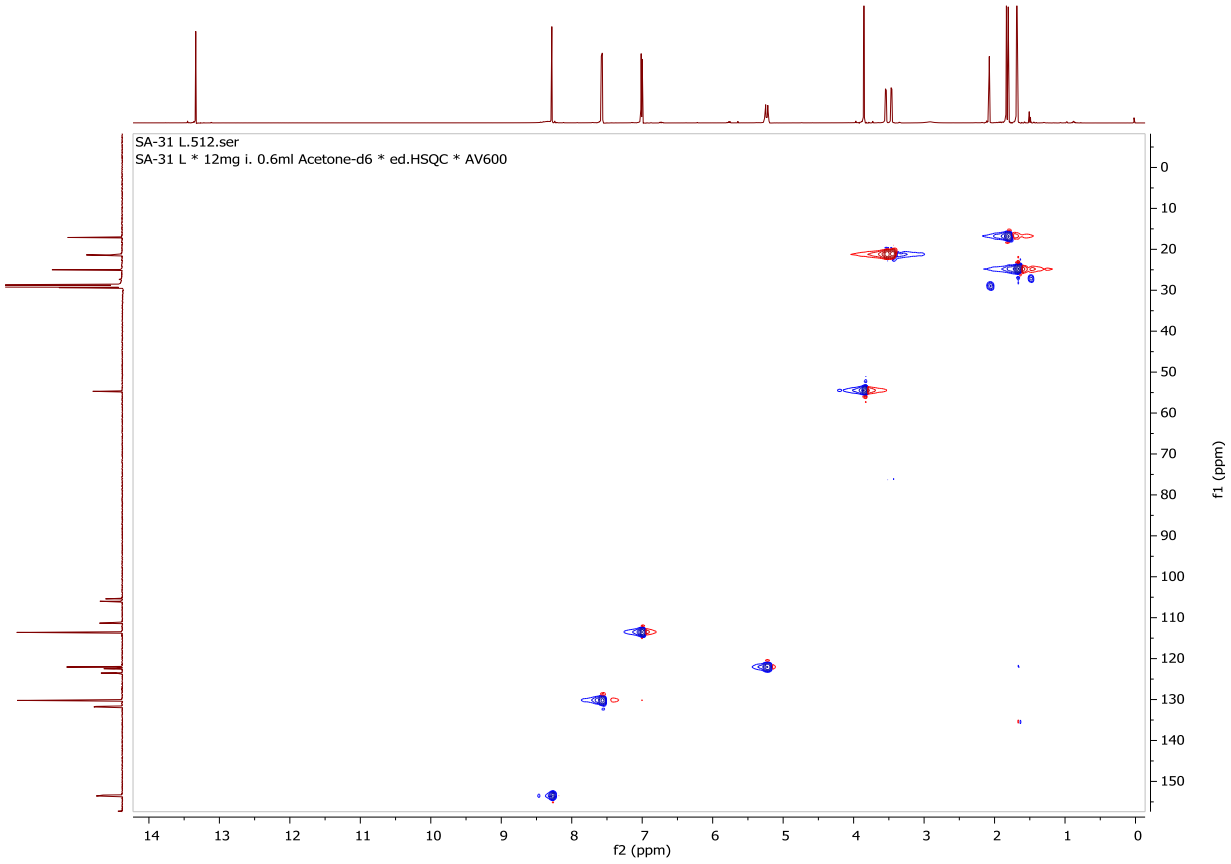
Appendix 2A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of compound 1 (Acetone-d6)



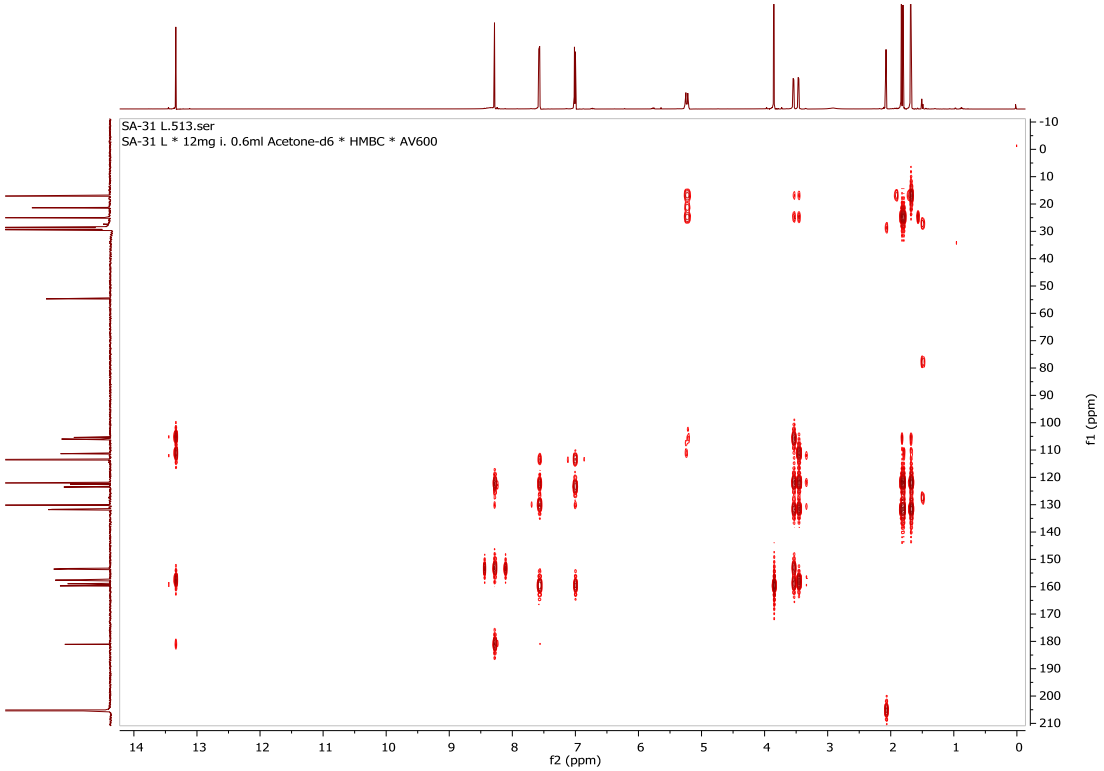


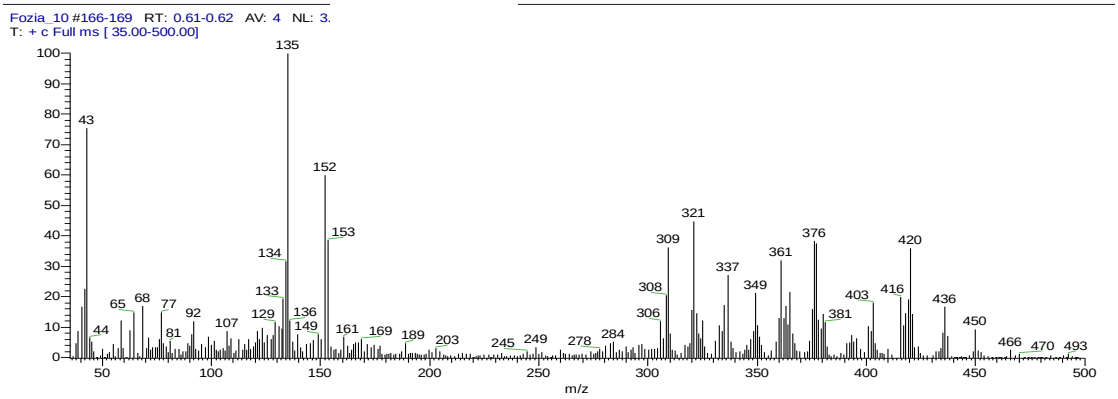
Appendix 2B: H,H-COSY and HSQC spectra of compound 2 (600 MHz, acetone-d_6)

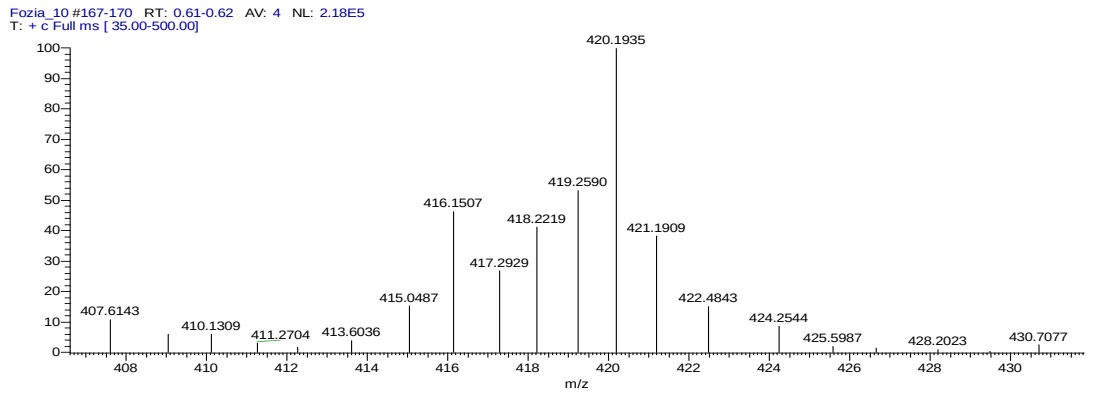




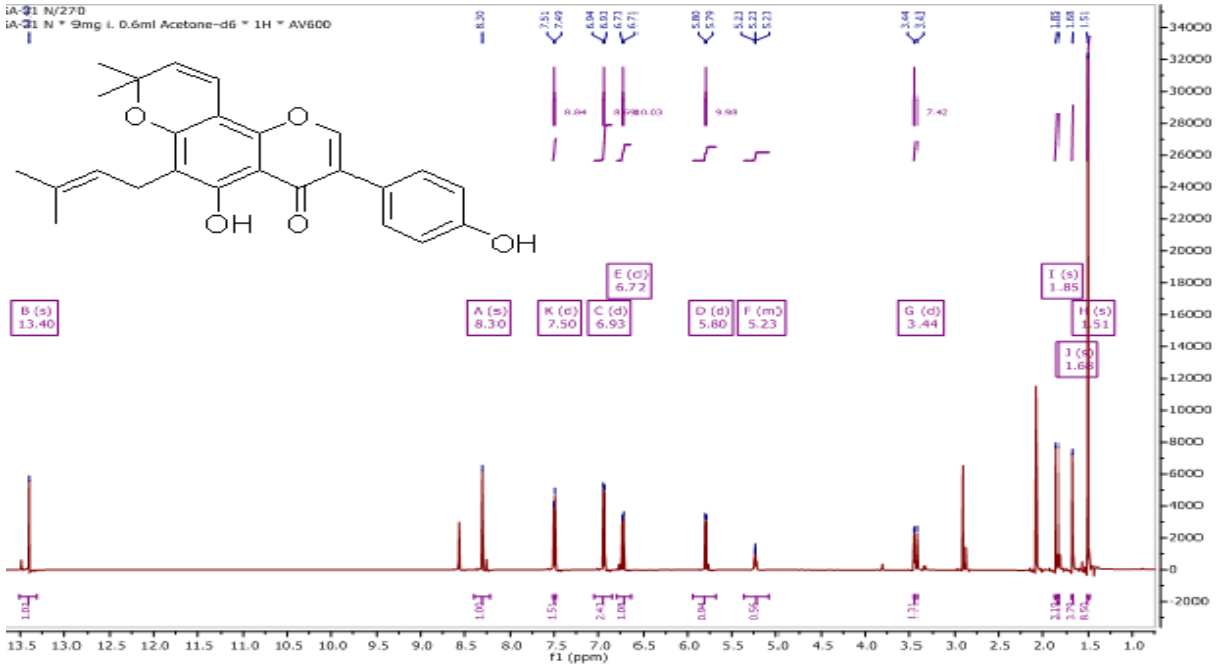
Appendix 2C: HMBC (600 MHz, acetone-d_6) and HREIMS spectra of compound 2

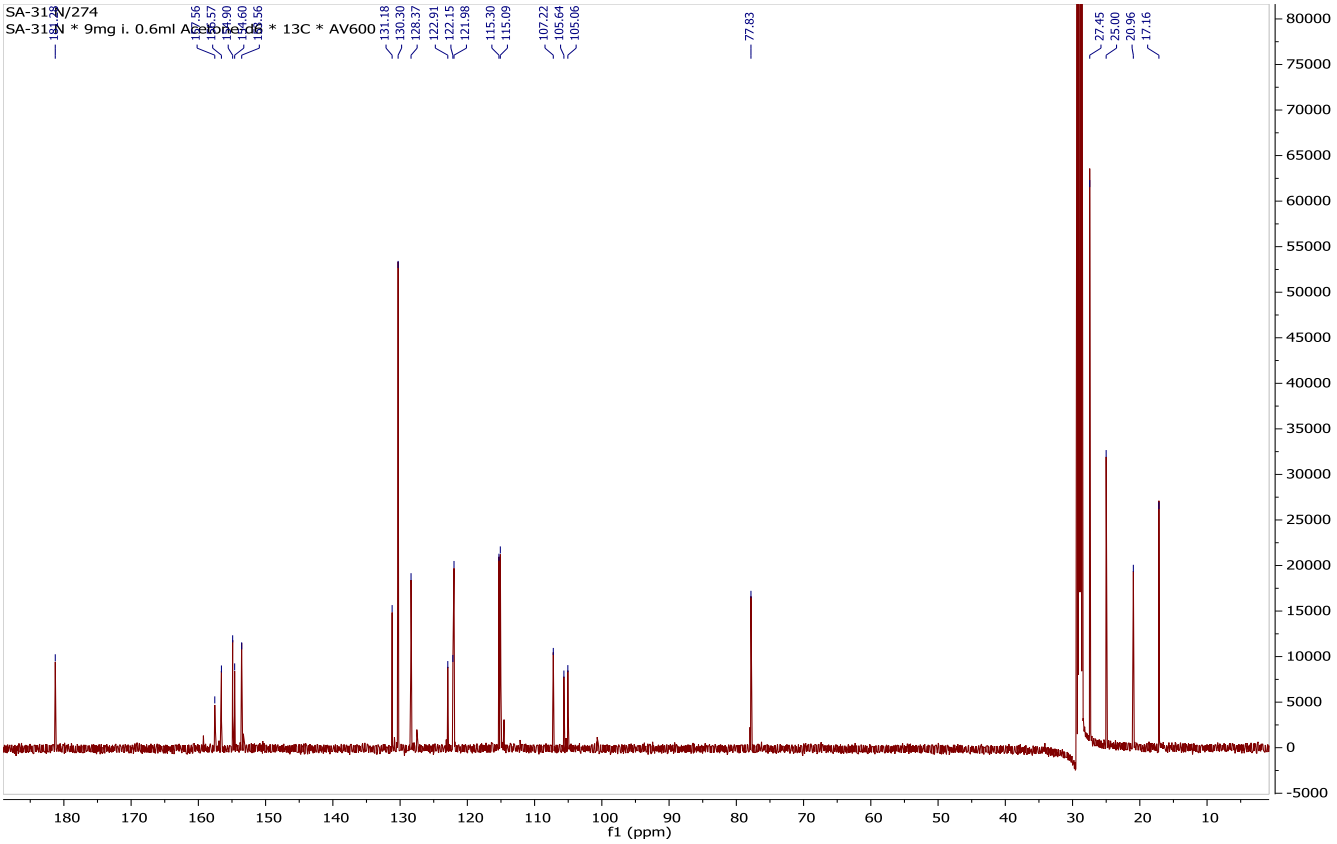




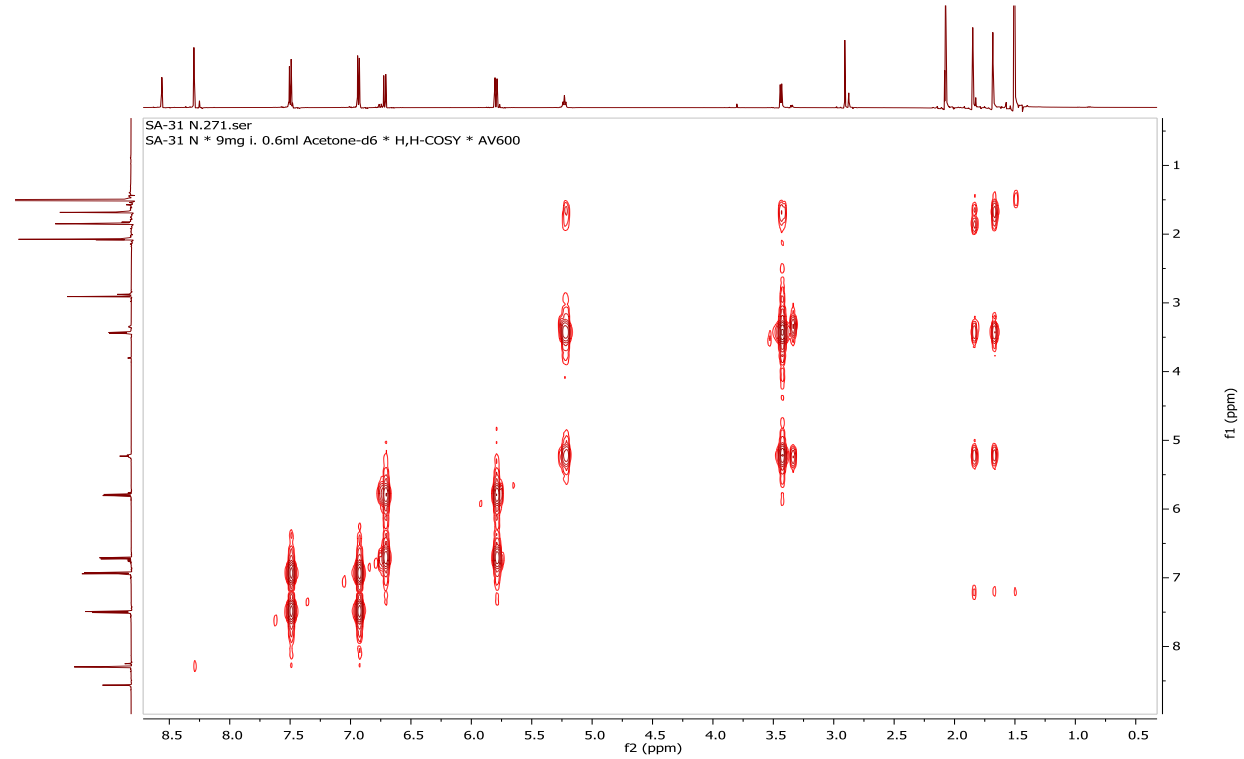


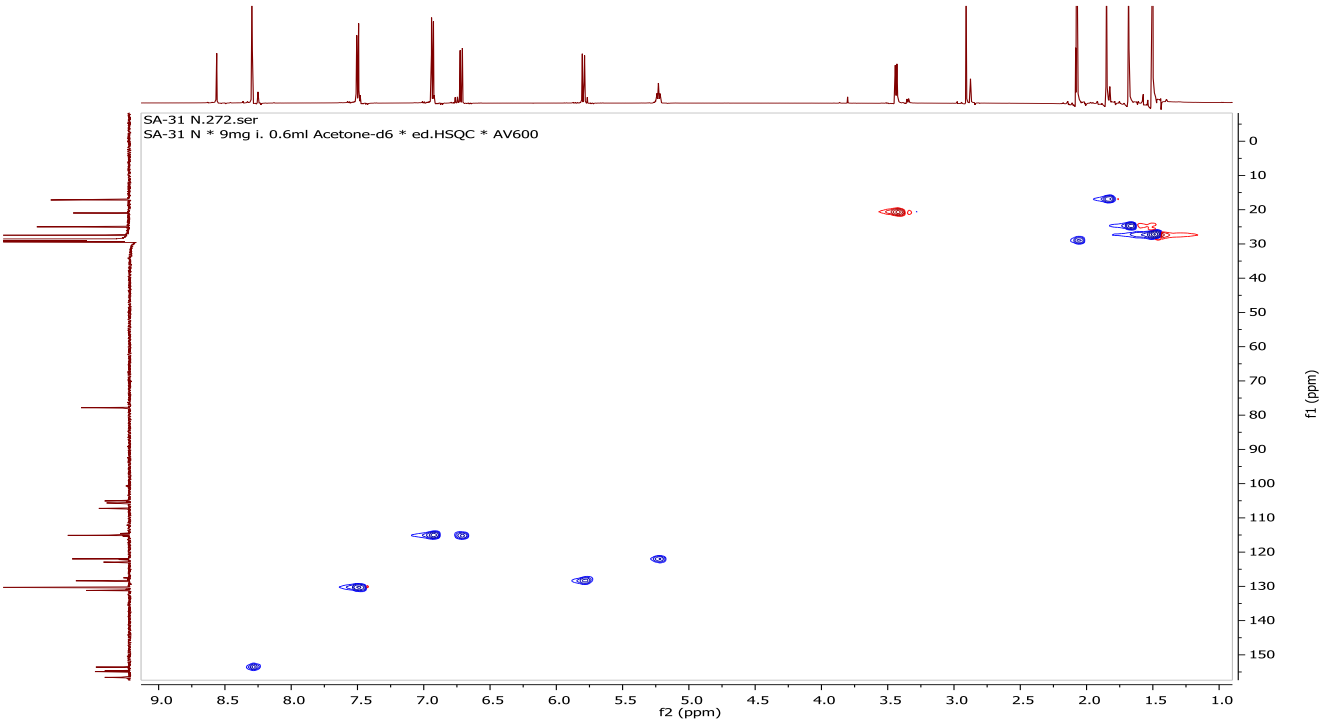
Appendix 3A: ^{1}H (600 MHz) and ^{13} C NMR (150 MHz) spectrum of compound 3 (Acetone-d_6)



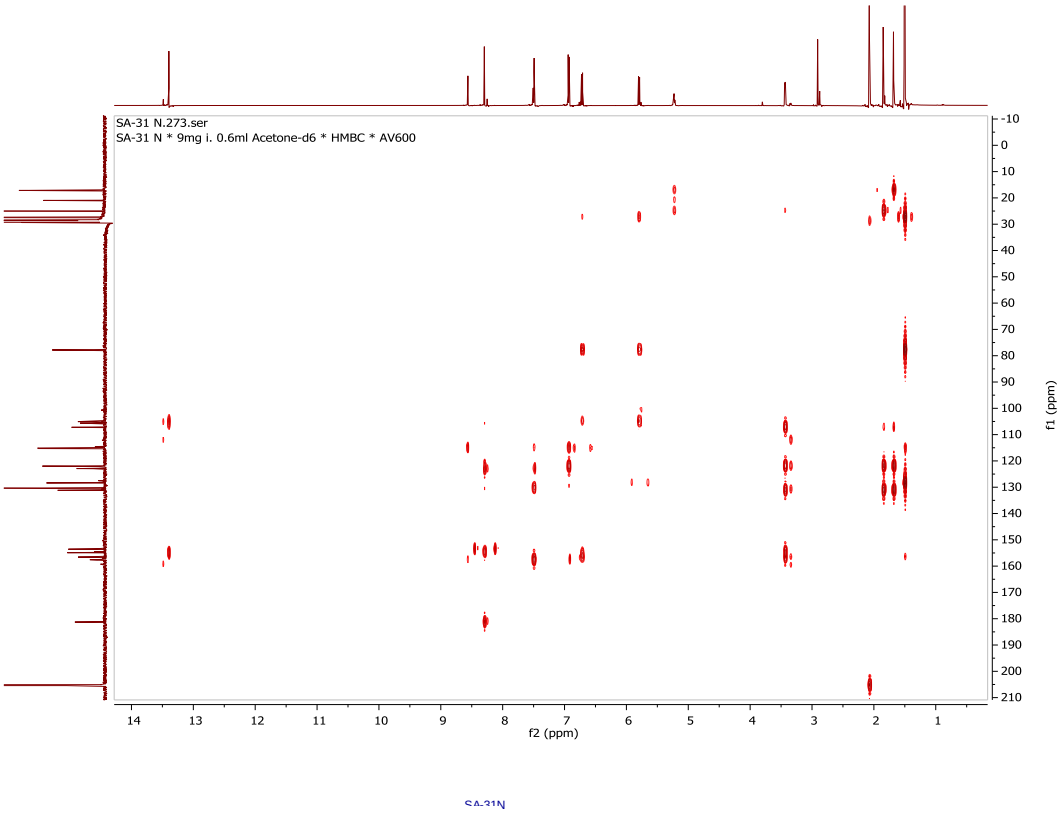


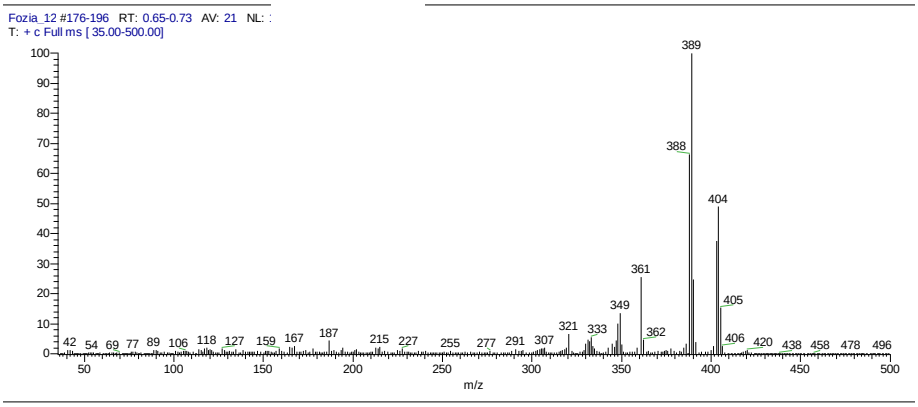
Appendix 3B: H,H-COSY and HSQC spectra of compound 3 (600 MHz, acetone-d_6)

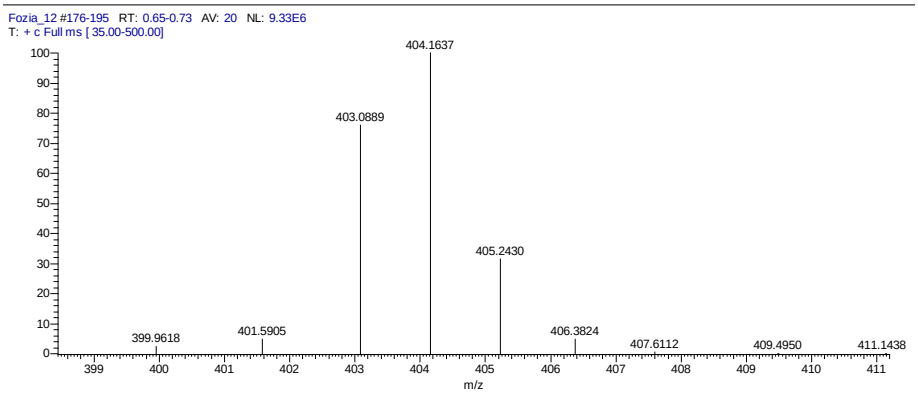




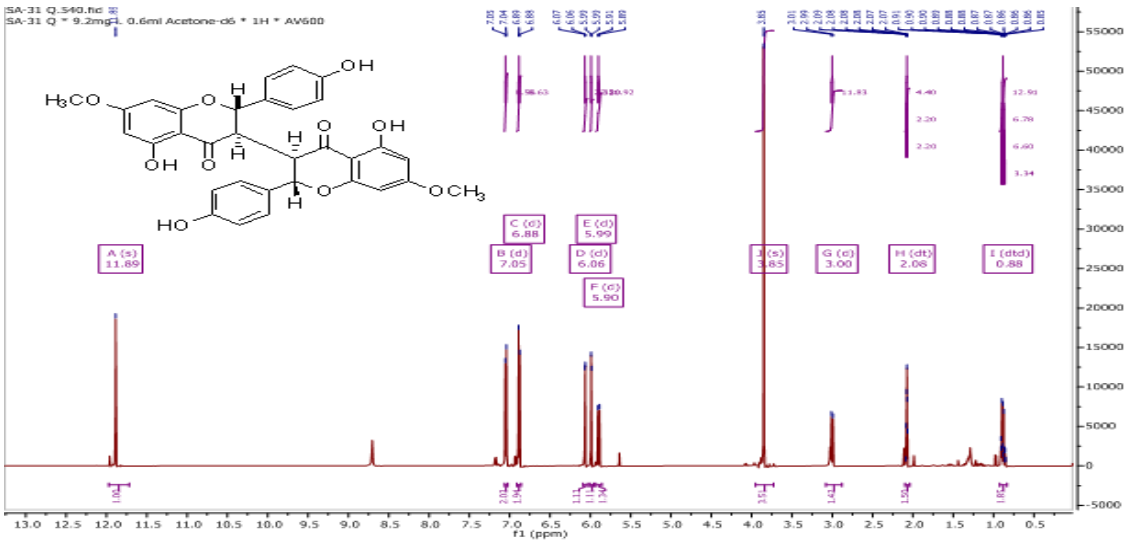
Appendix 3C: HMBC and HREIMS spectra of compound 3 (600 MHz, acetone-d_6)

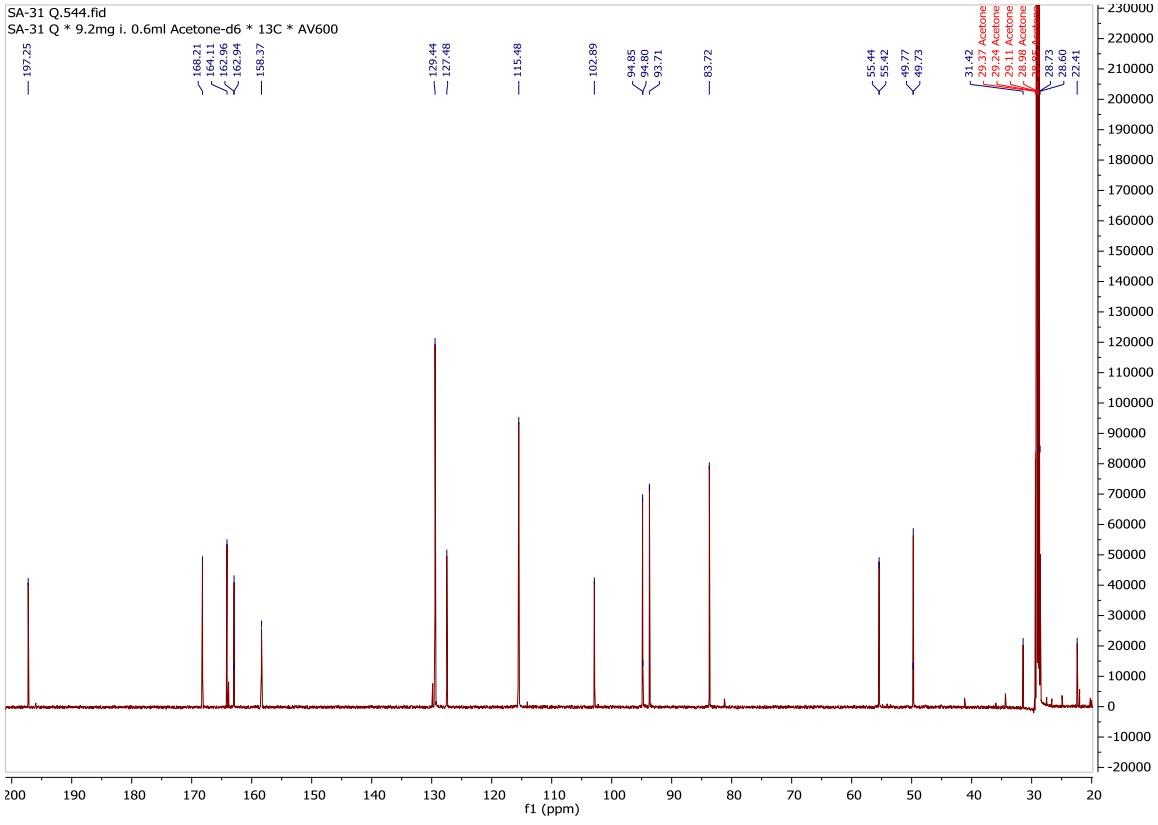




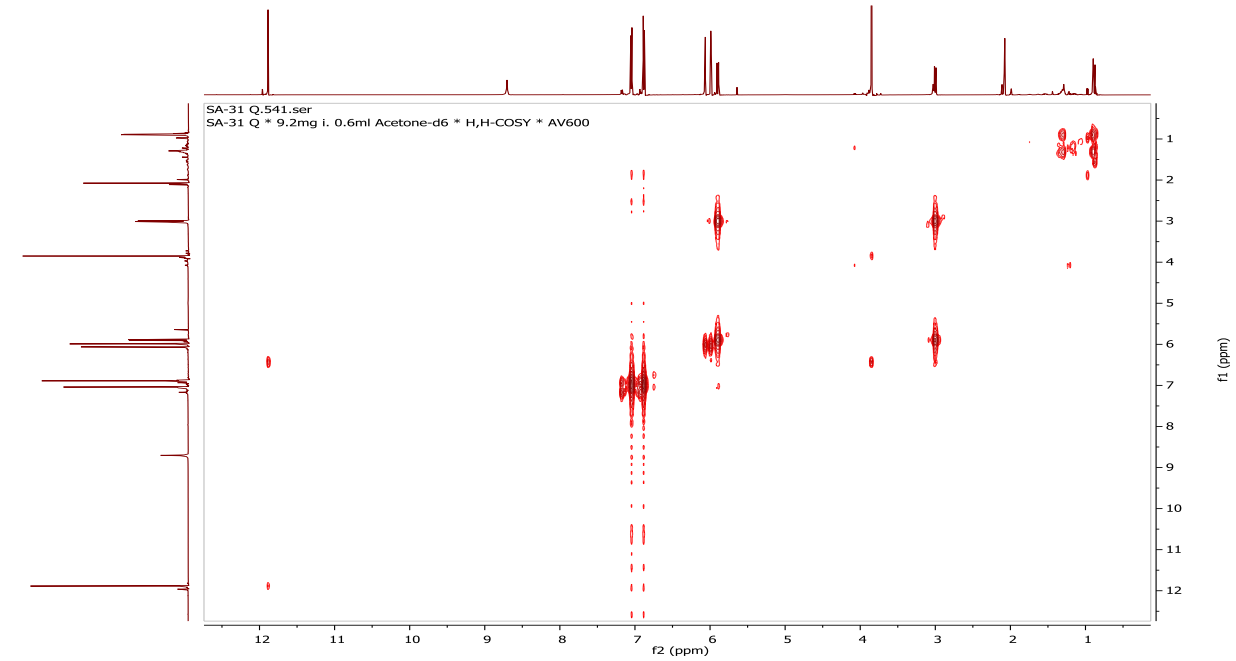


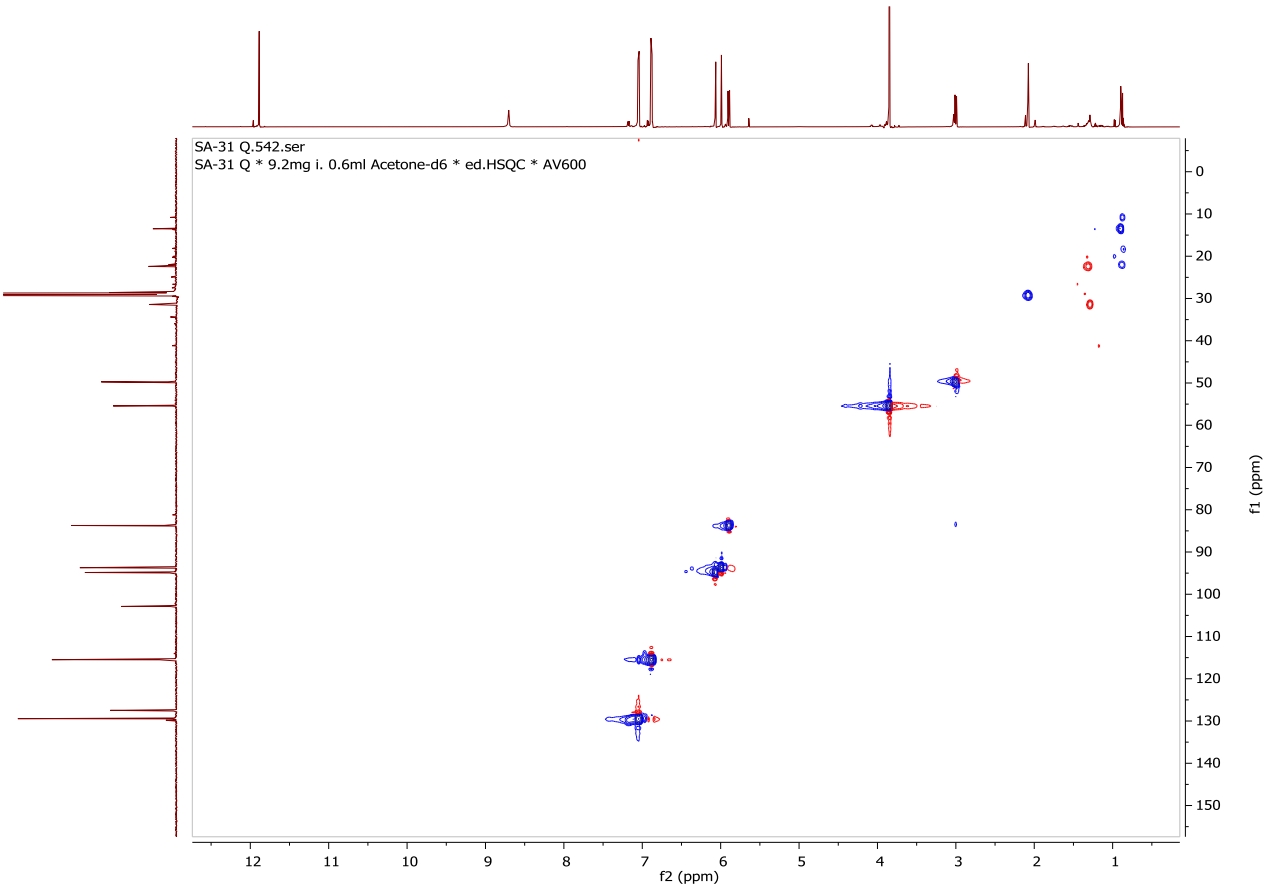
Appendix 4A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of compound 4 (Acetone-d_6)



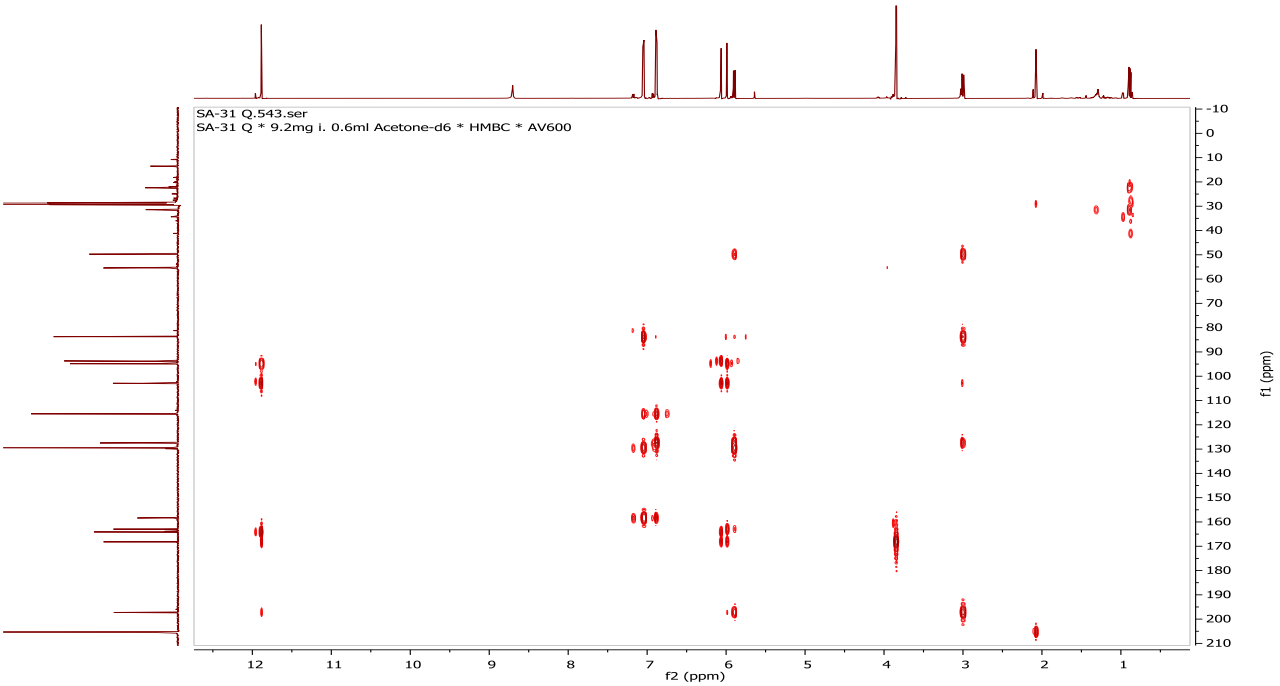


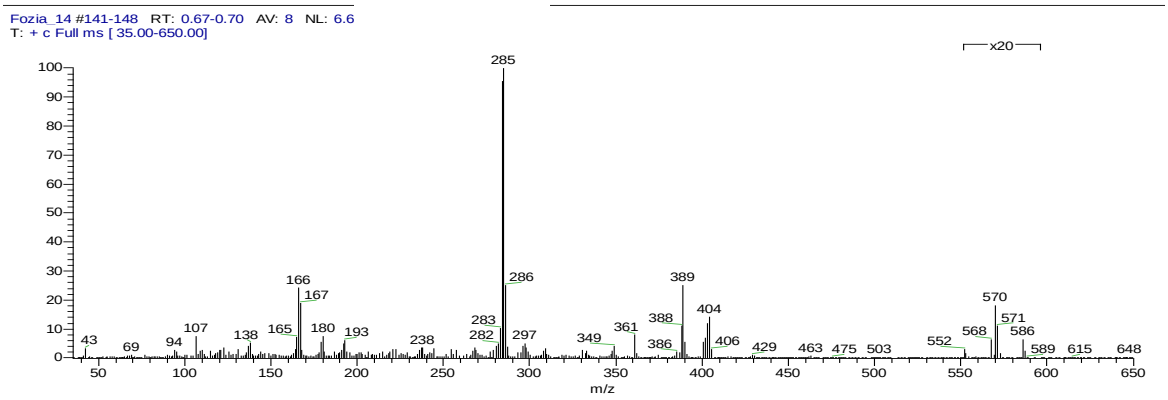
Appendix 4B: H,HCOSY and HSQC spectra of compound 4 (600 MHz, acetone-d_6)

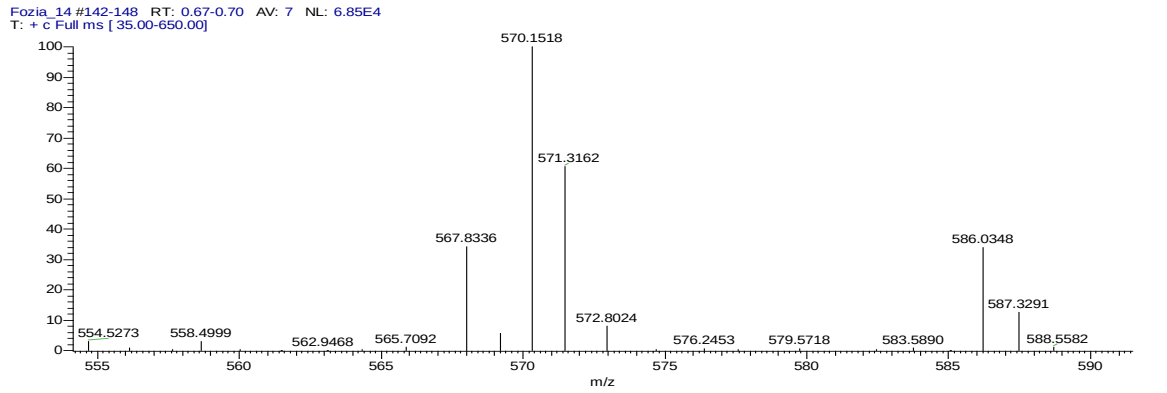




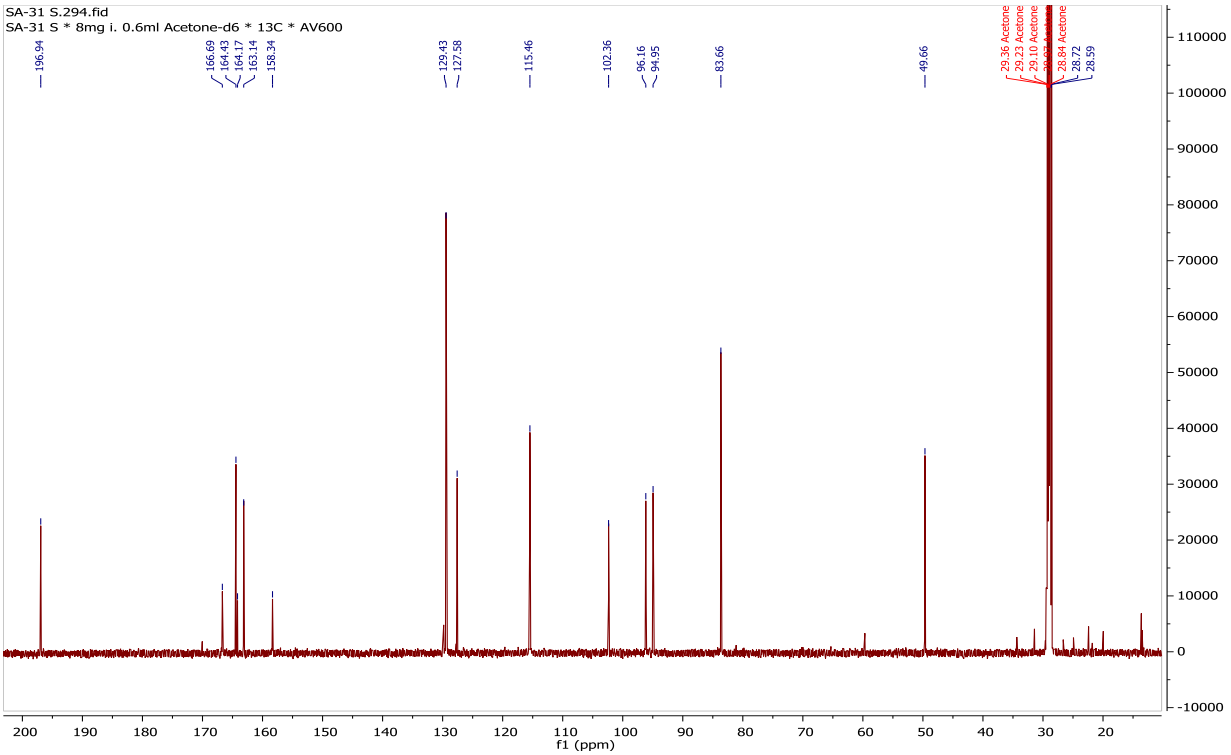
Appendix 4C: HMBC (600 MHz, acetone-d_6) and HREIMS spectra of compound 4



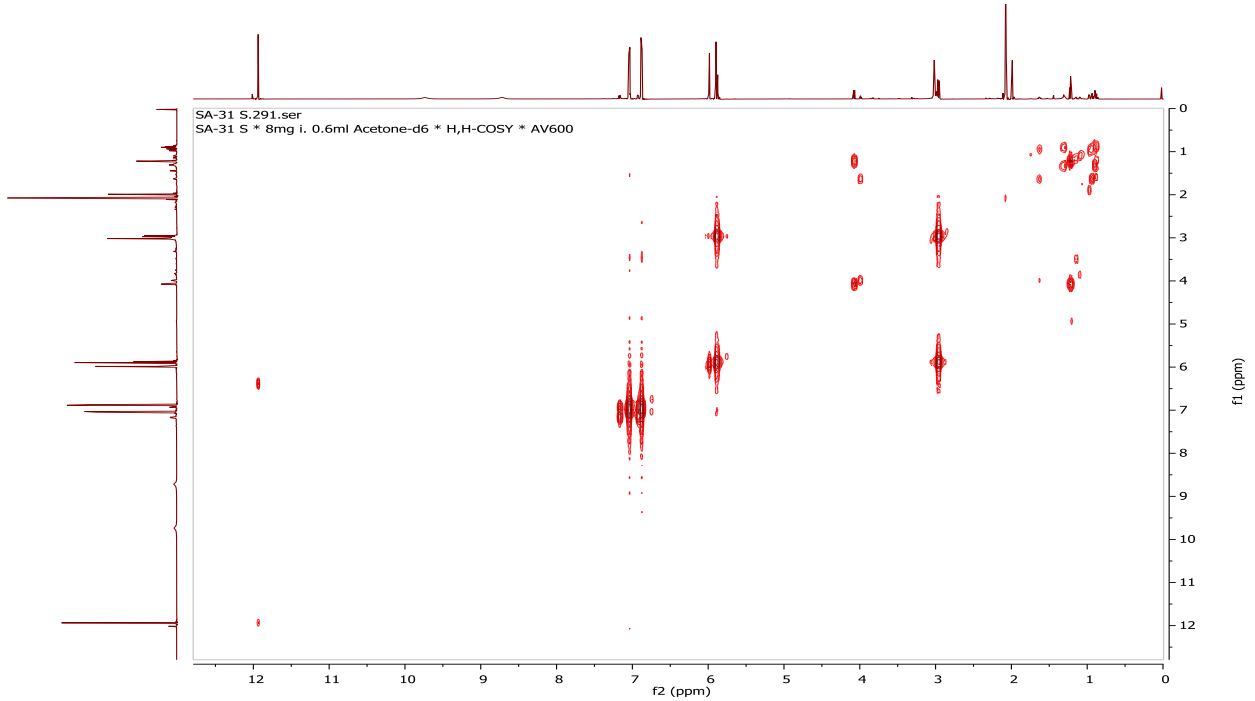


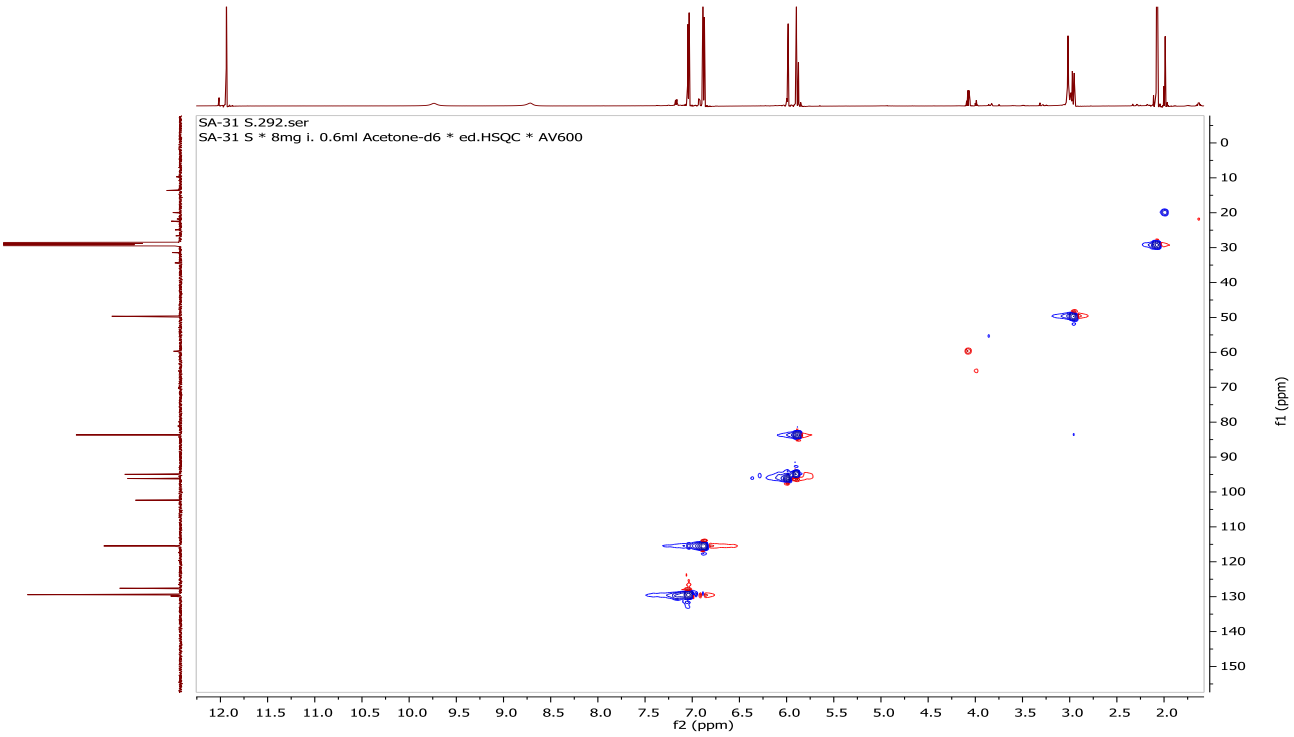


Appendix 5A: ^{1}H 600 MHz) and ^{13}C NMR (150 MHz) spectra of compound 5 (Acetone-d6)

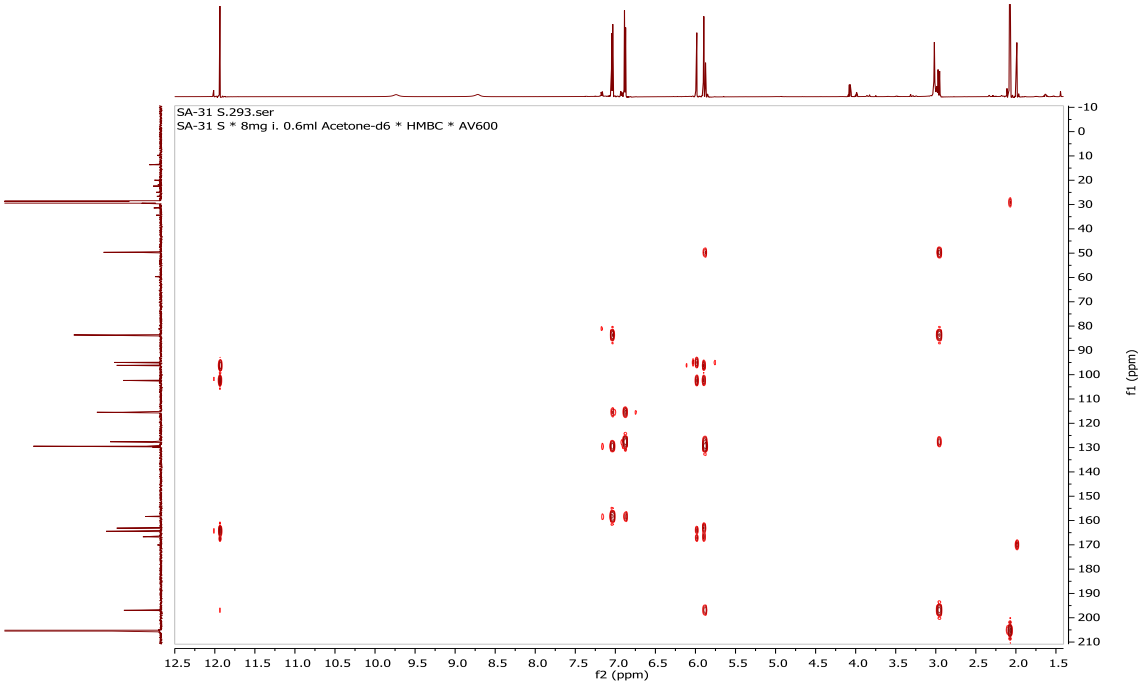


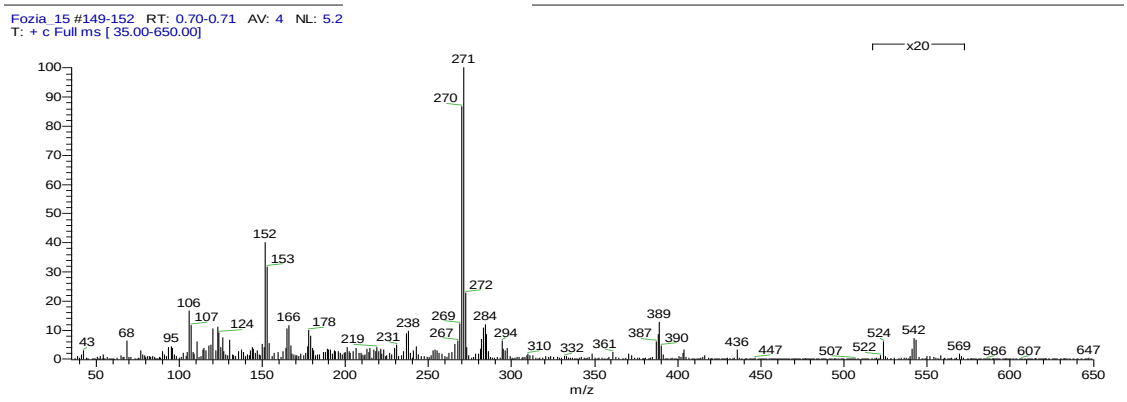
Appendix 5B: H,H-COSY and HSQC spectra of compound 5 (600 MHz, acetone-d_6)

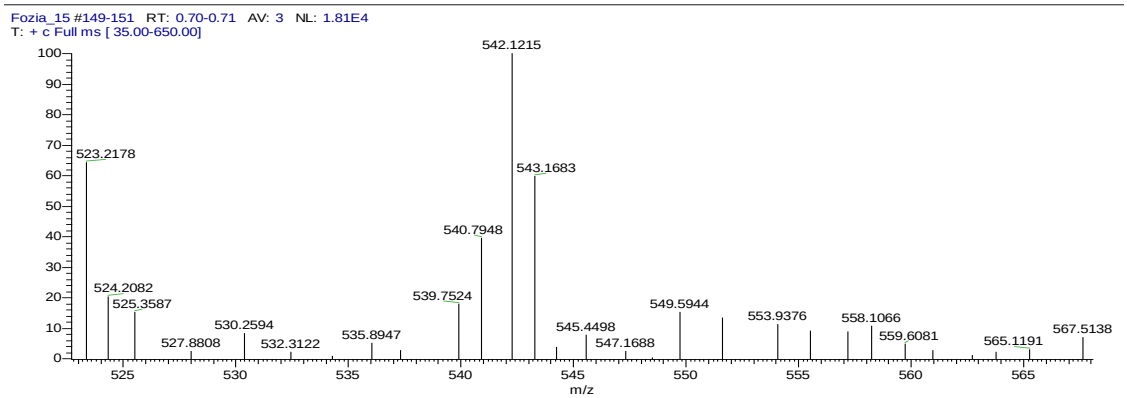




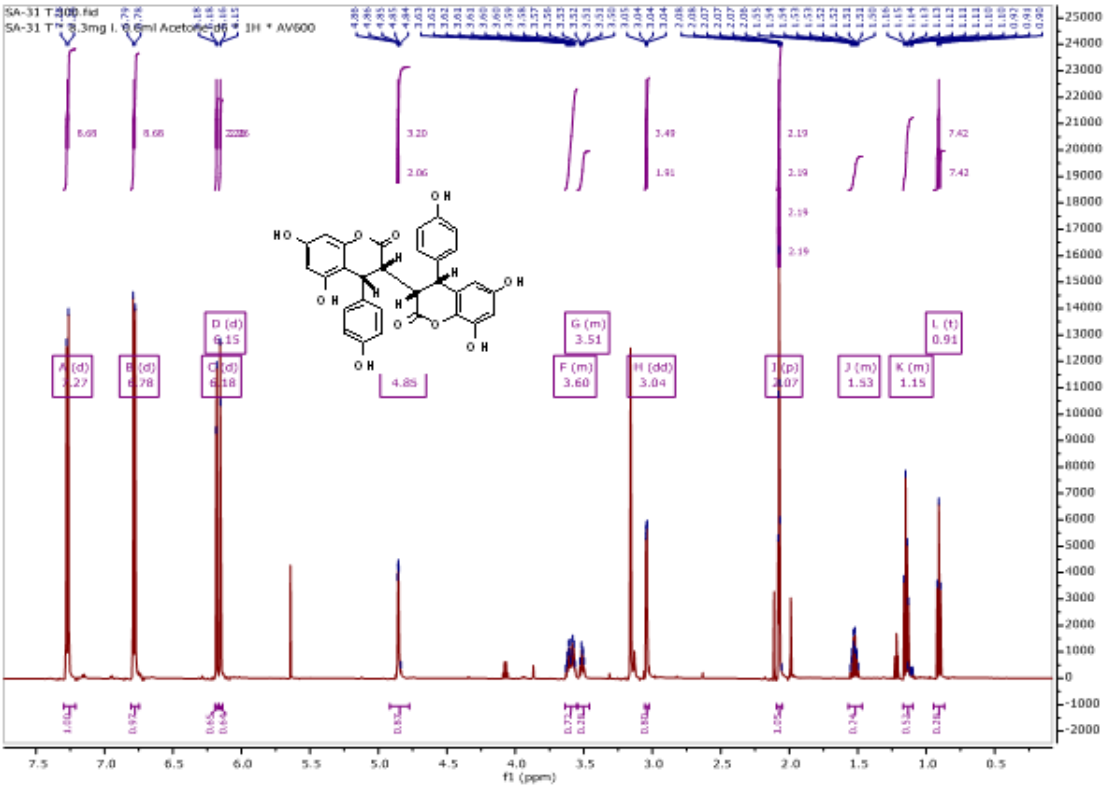
Appendix 5C: HMBC (600 MHz, acetone-d_6) and HREIMS spectra of compound 5

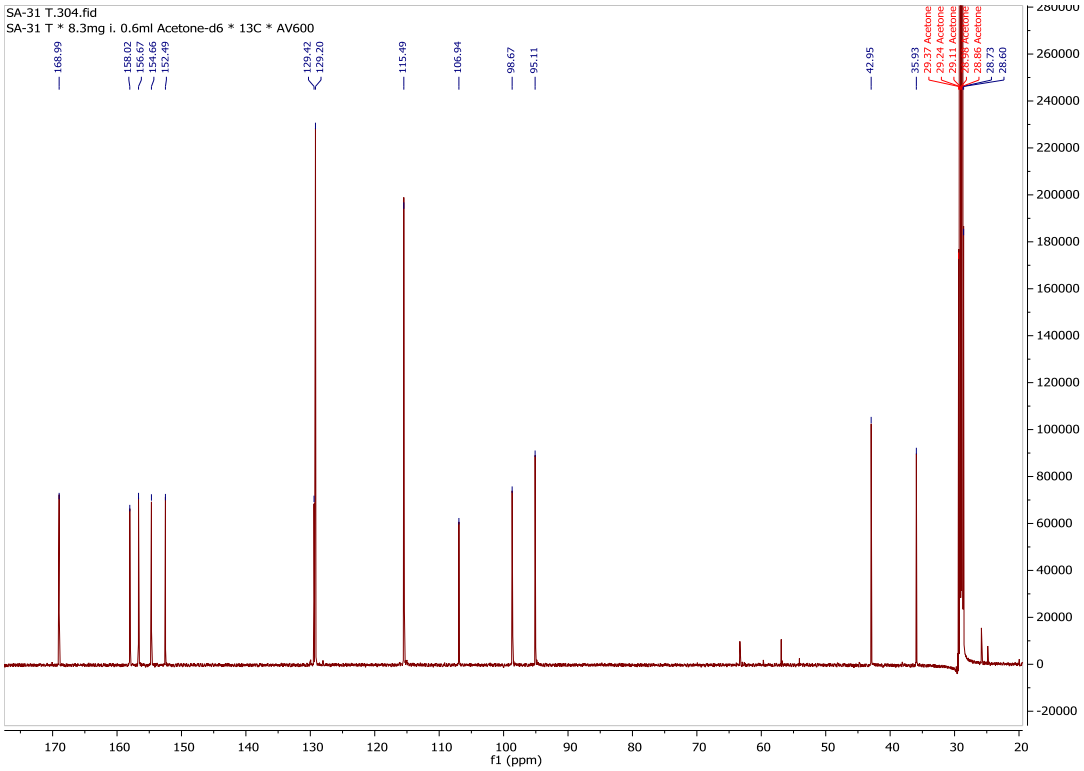




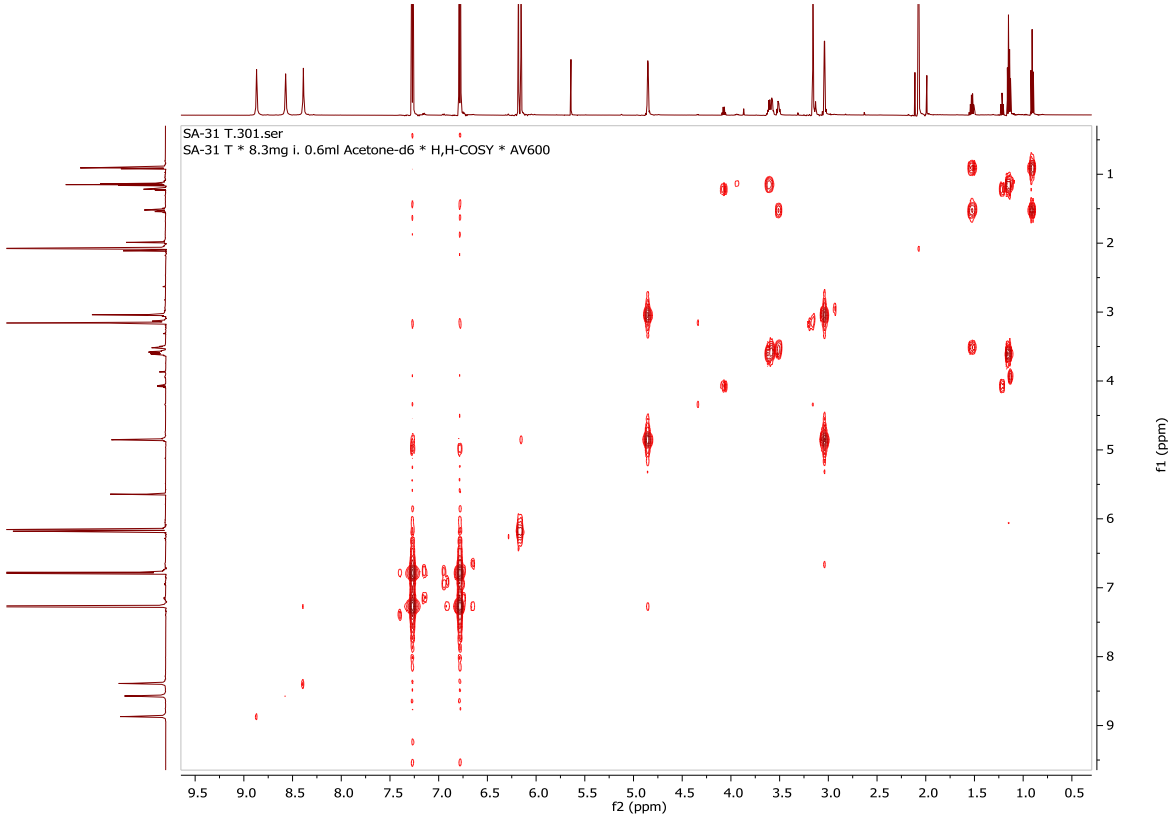


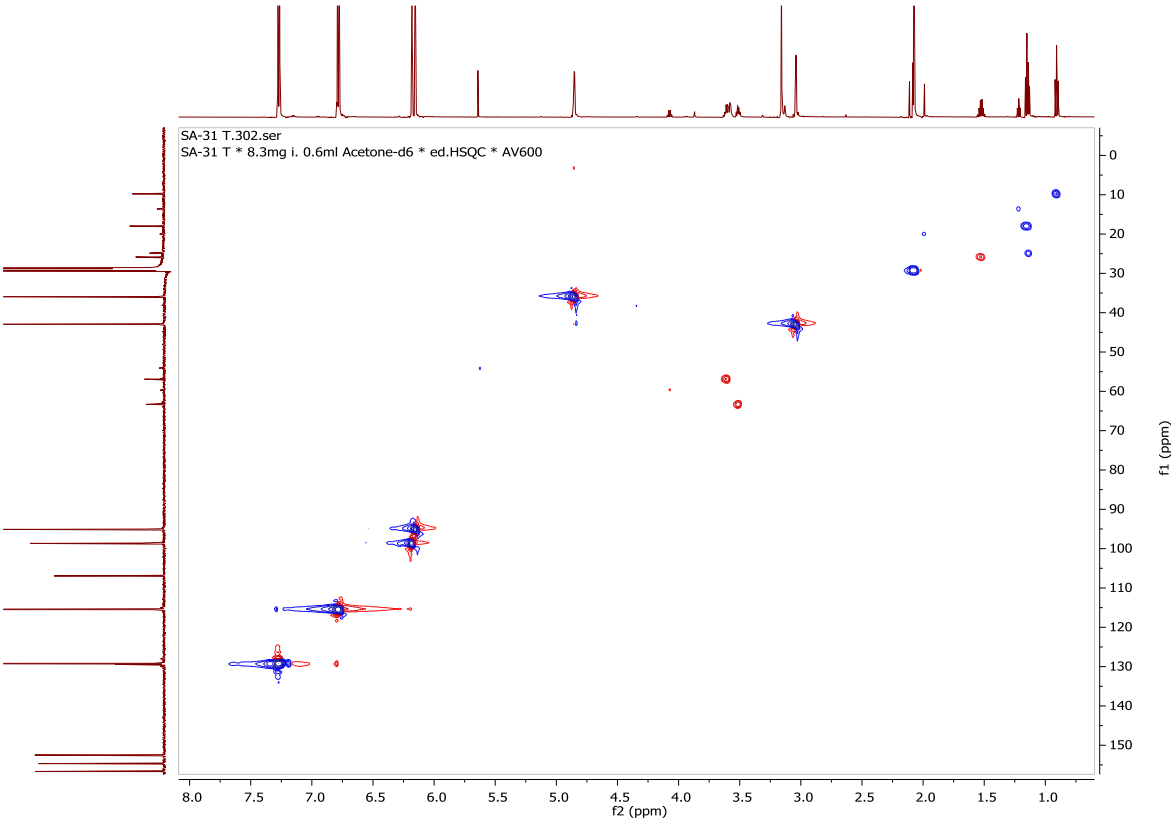
Appendix 6A: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz)spectra of compound 6 (Acetone-d_6)



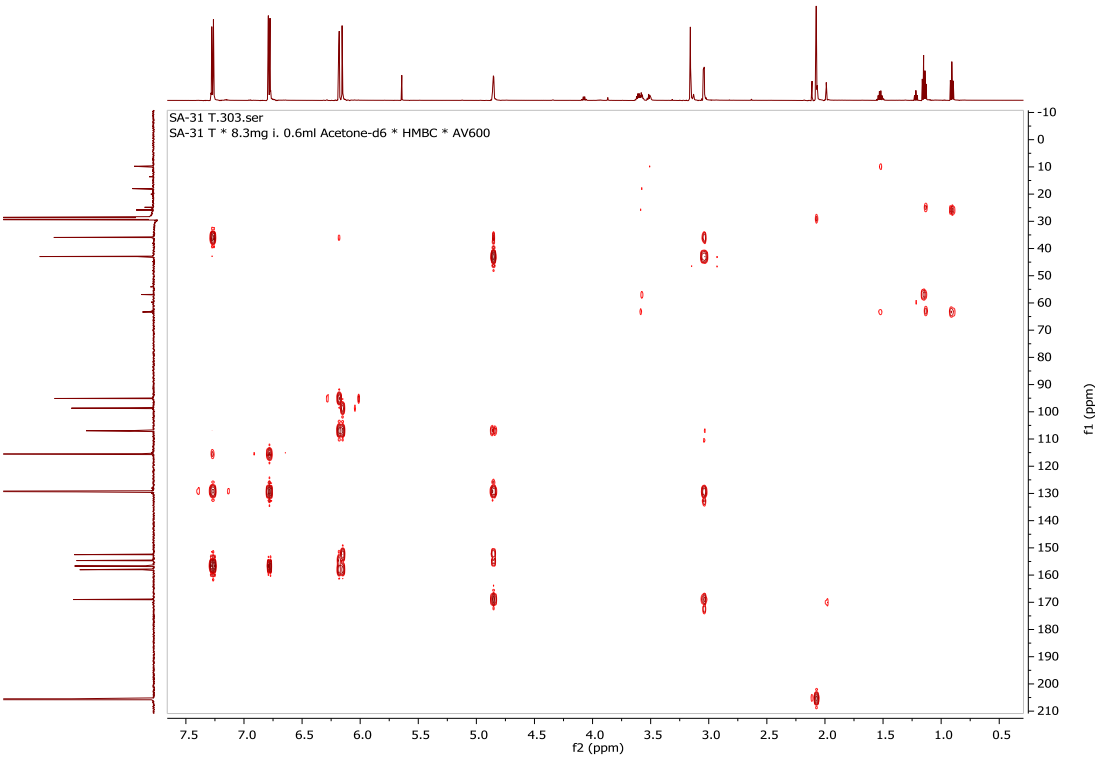


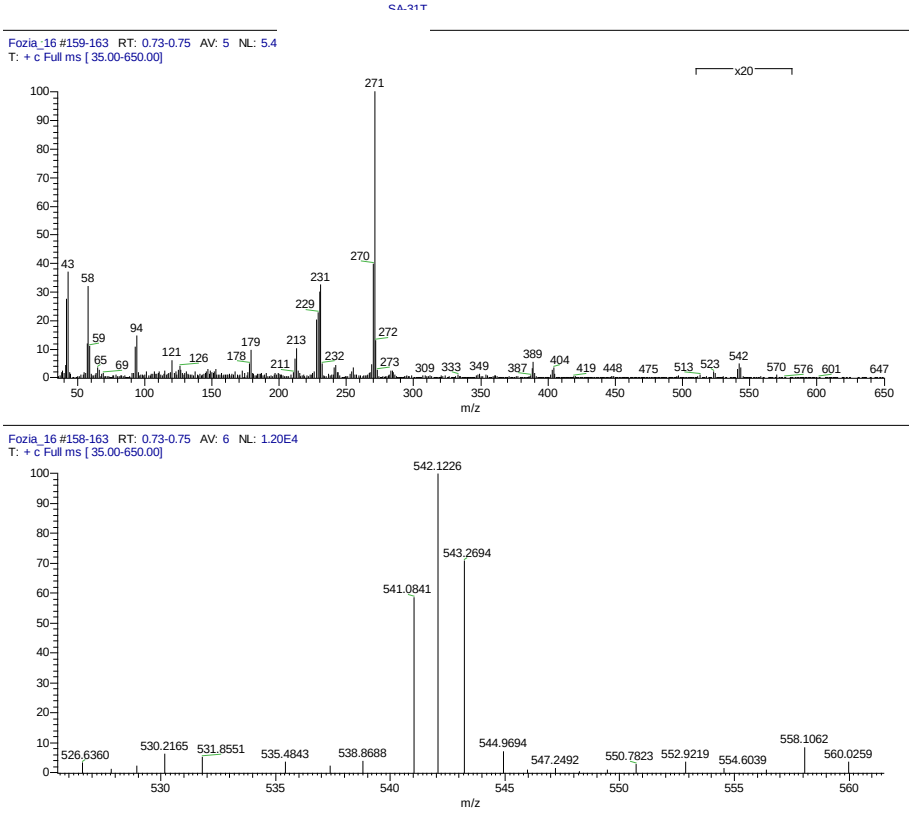
Appendix 6B: H,H-COSY and HSQC spectra of compound 6 (600 MHz, acetone-d_6)



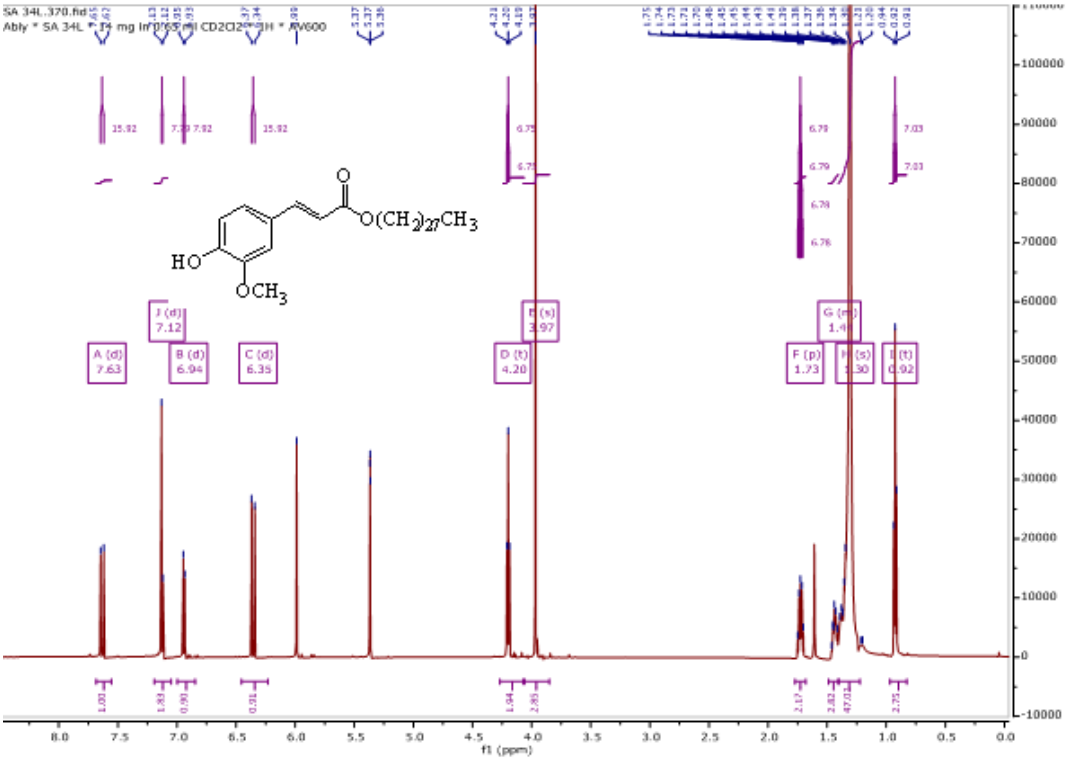


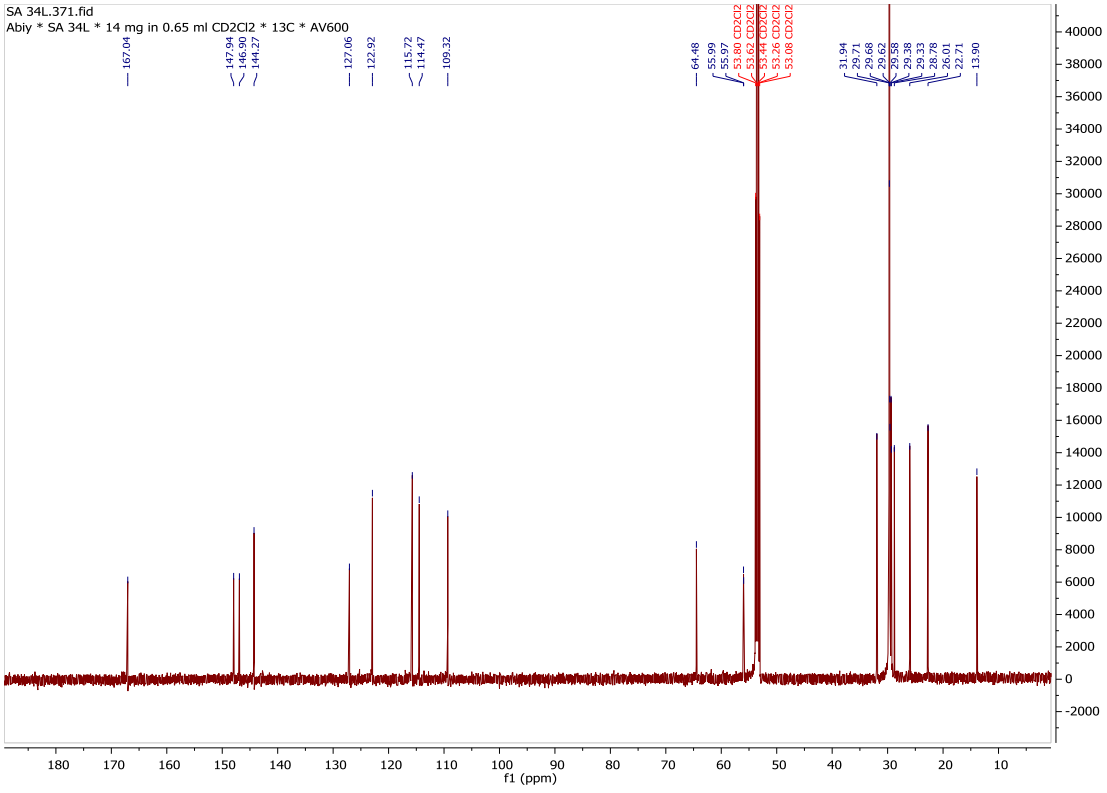
Appendix 6C: HMBC (600 MHz, acetone-d_6) and HREIMS spectra of compound 6



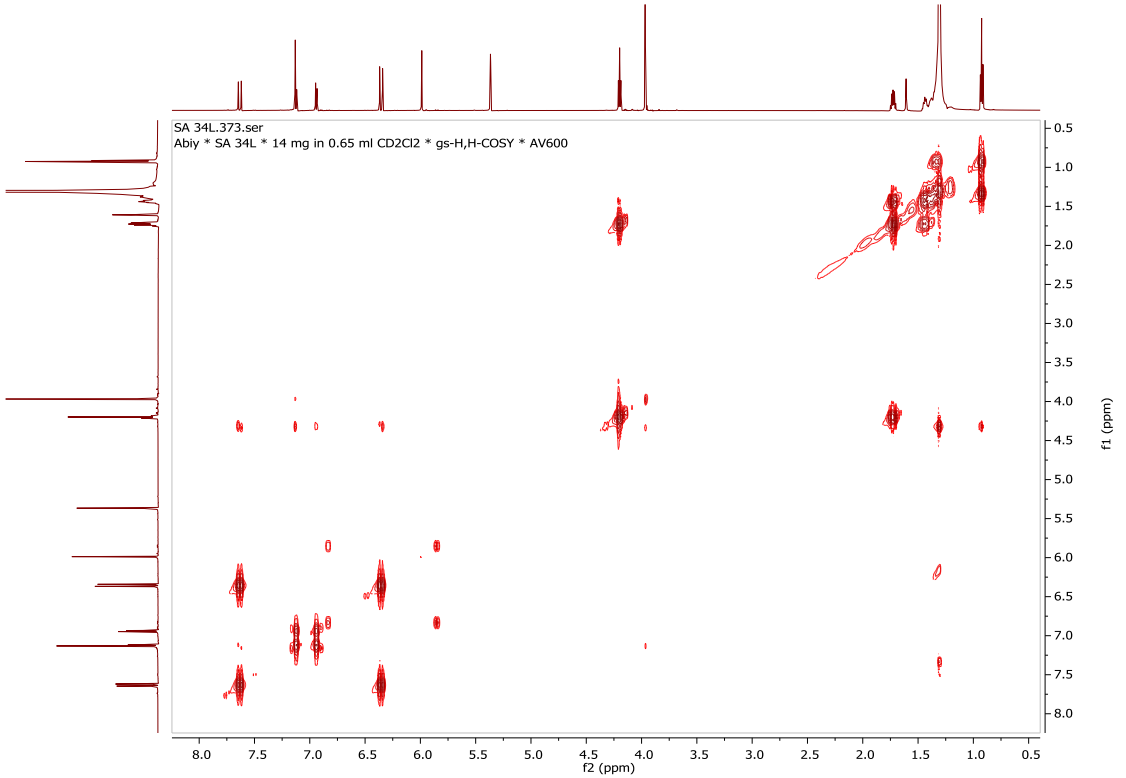


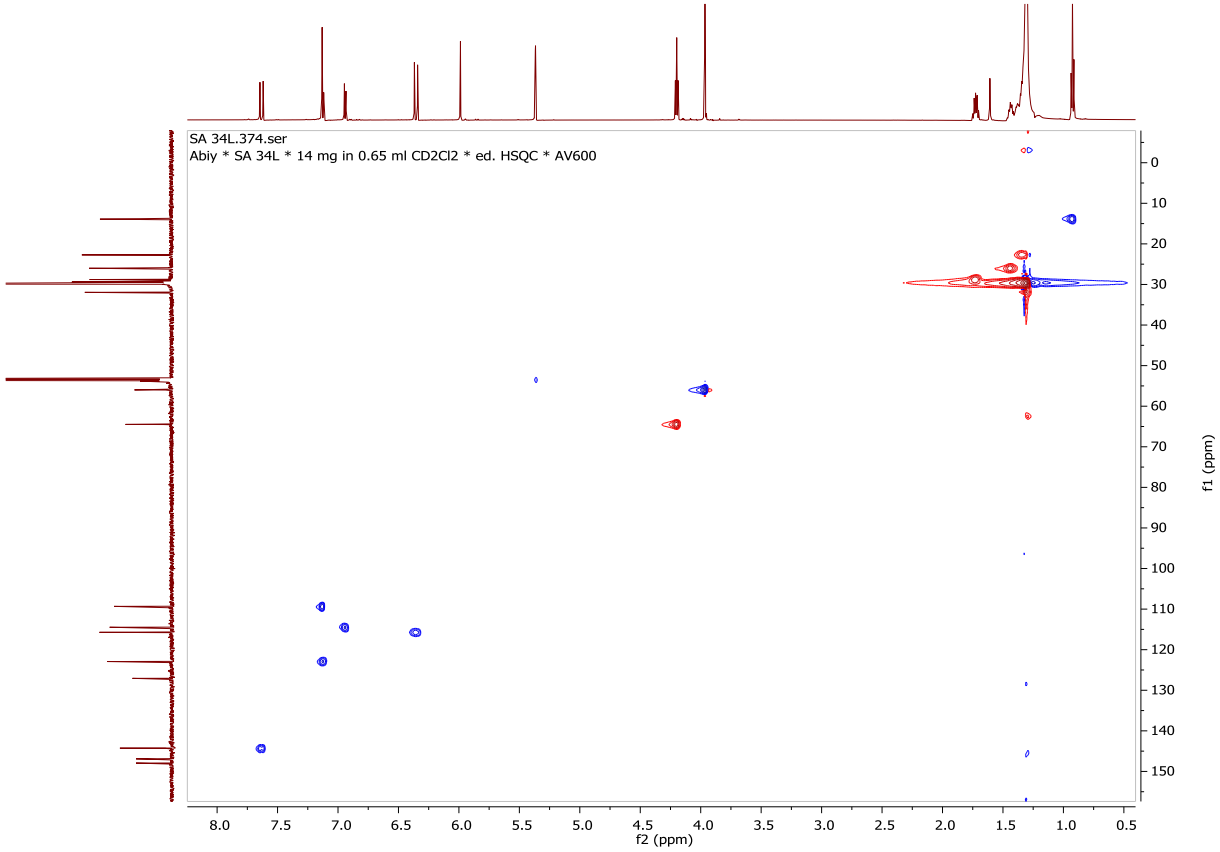
Appendix 7A: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra of compound 7 (Acetone-d_6)



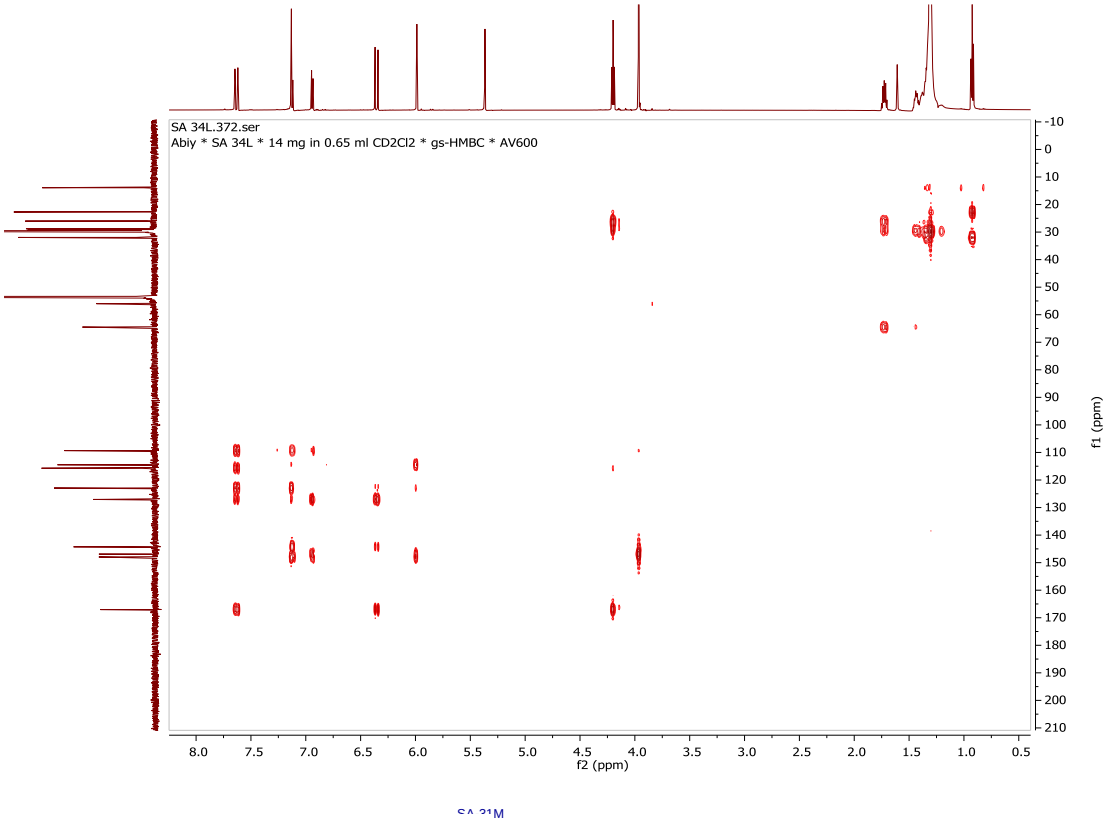


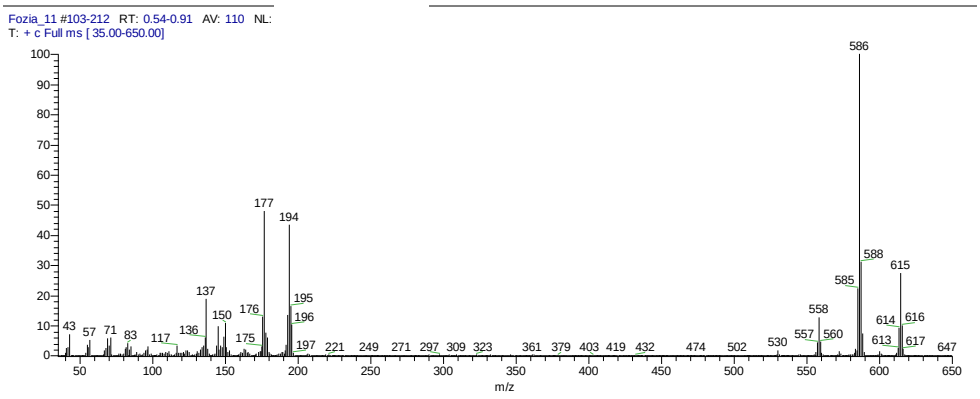
Appendix 7B: H,H-COSY and HSQC spectra of compound 7 (600 MHz, acetone-d_6)

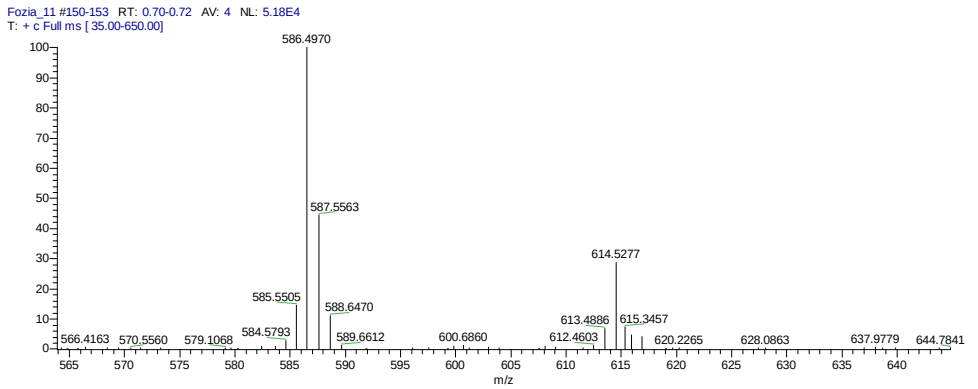




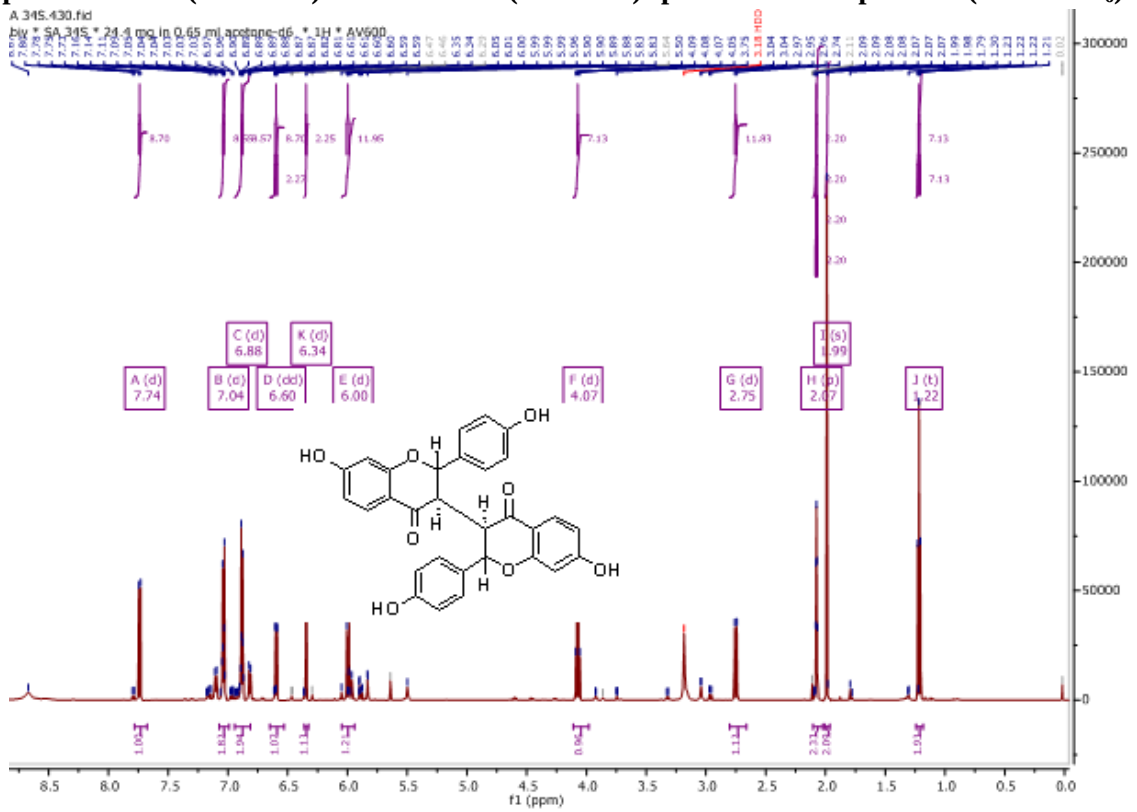
Appendix 7C: HMBC (600 MHz, acetone-d_6) and HREIMS spectrum of compound 7

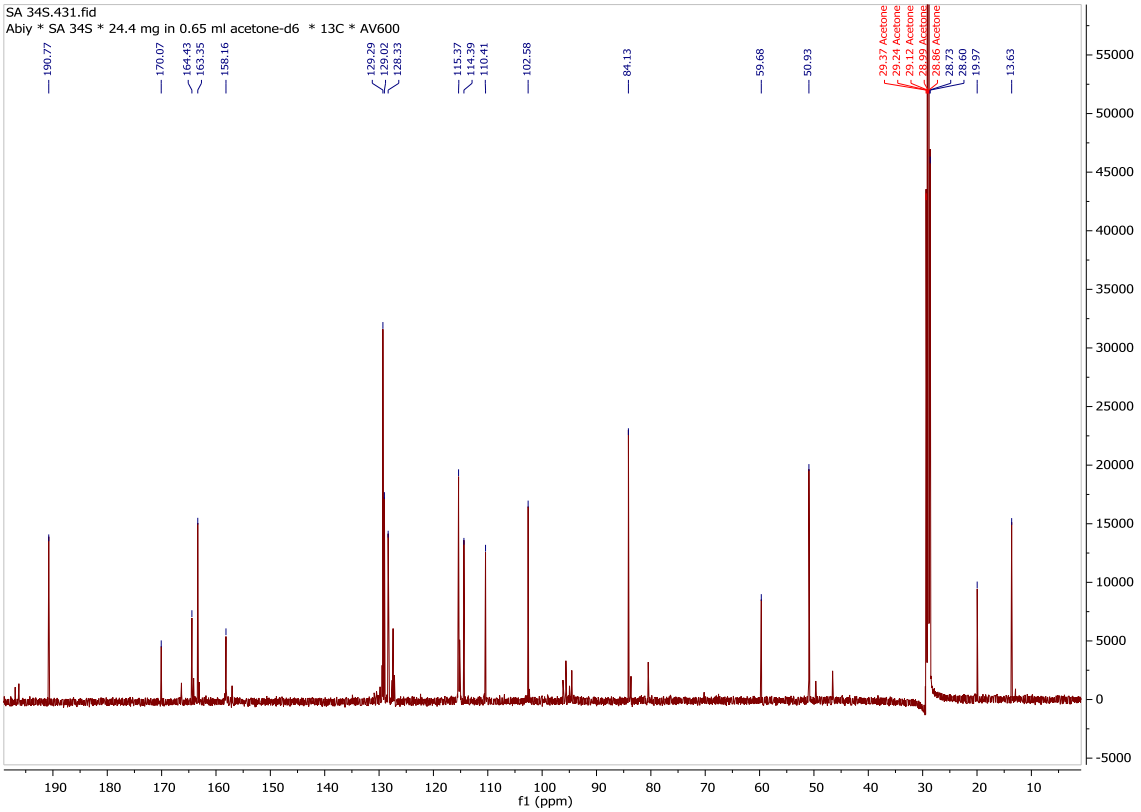




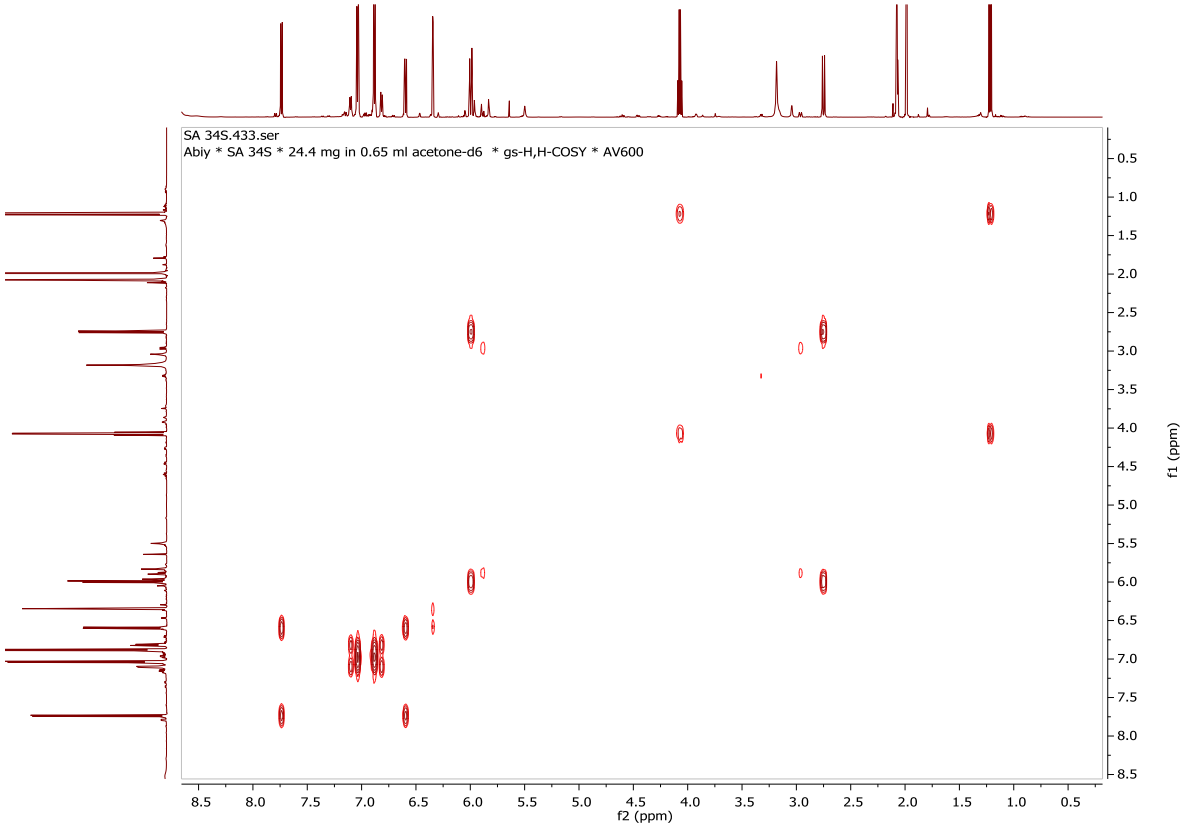


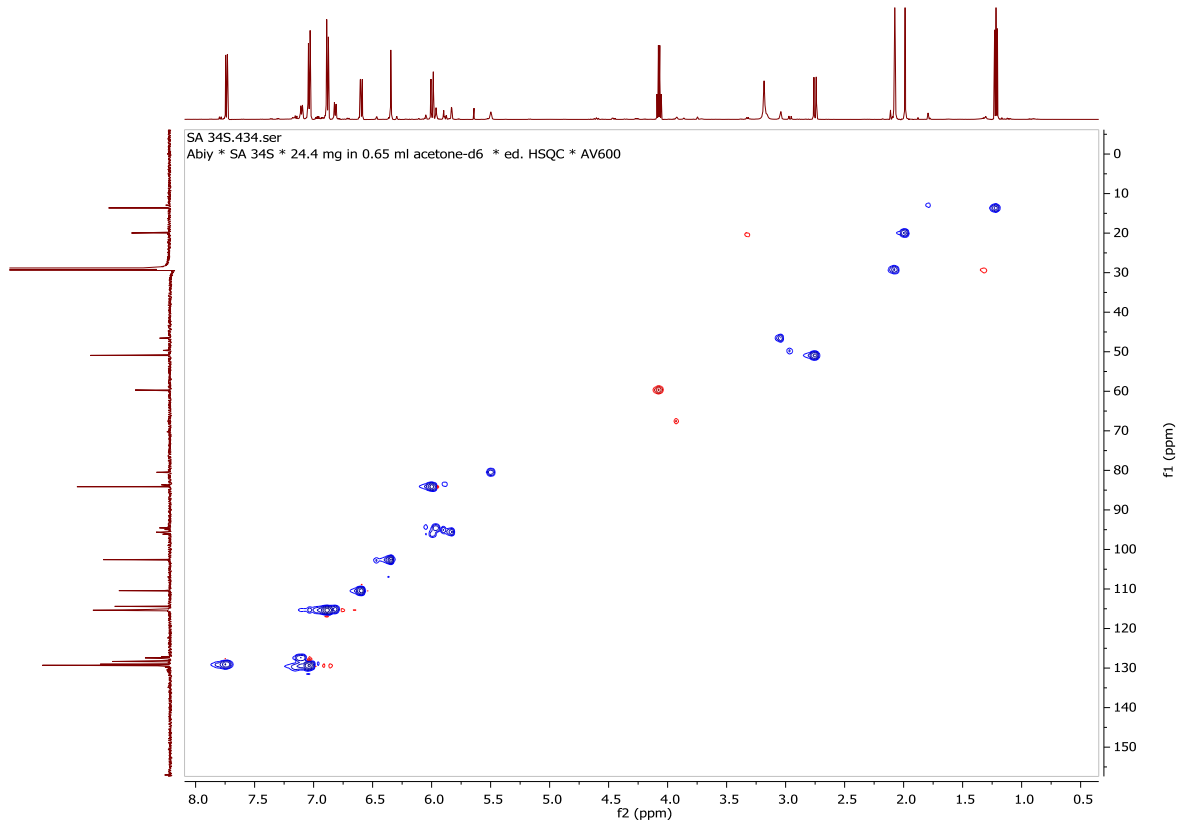
Appendix 8A: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectrum of compound 8 (Acetone-d_6)



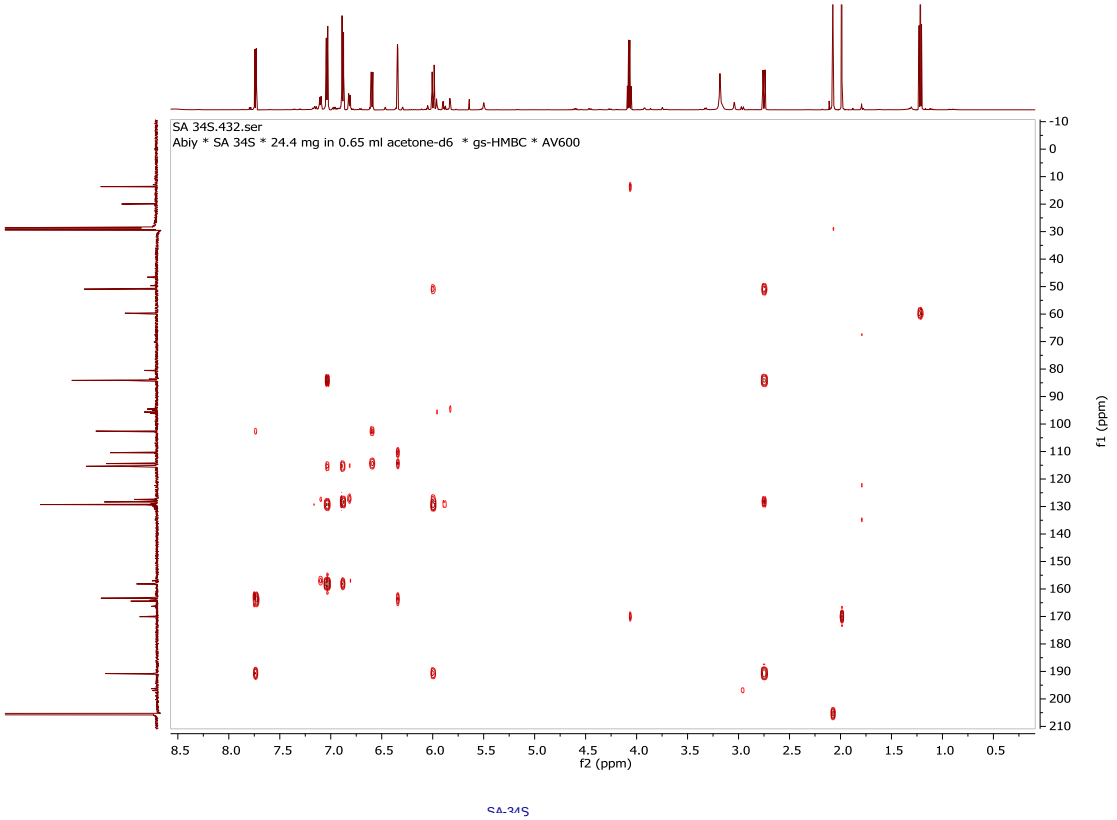


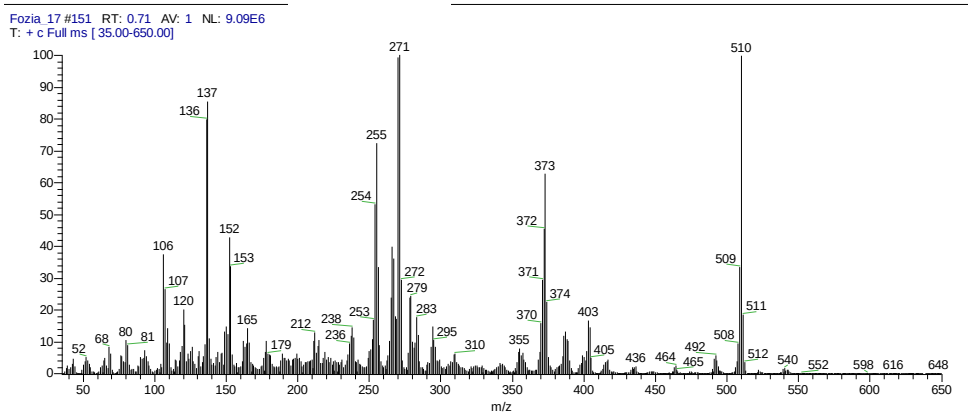
Appendix 8B: H,H-COSY and HSQC spectra of compound 8 (600 MHz, acetone-d6)

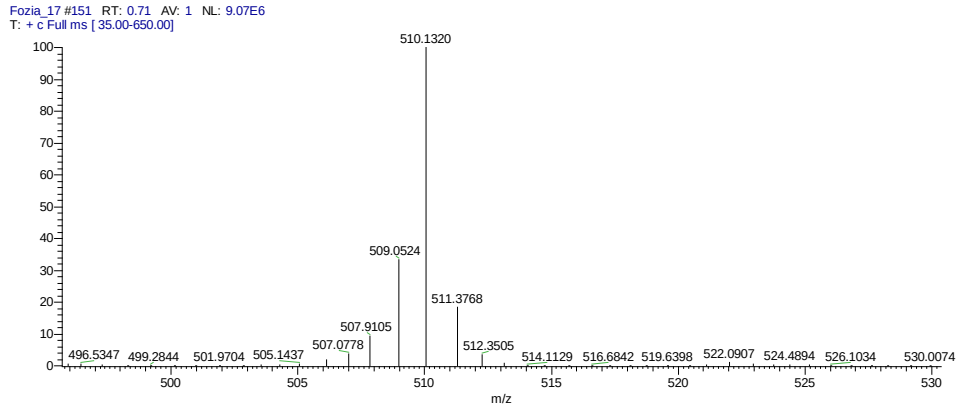




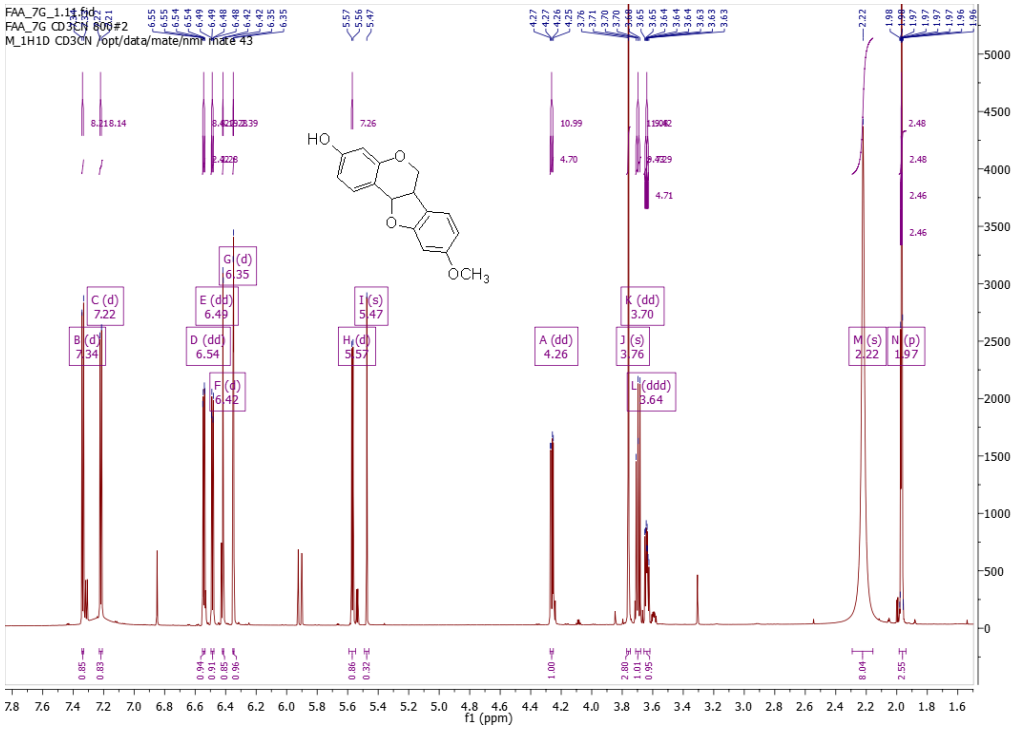
Appendix 8C: HMBC (600 MHz, acetone-d_6) and HREIMS spectra of compound 8

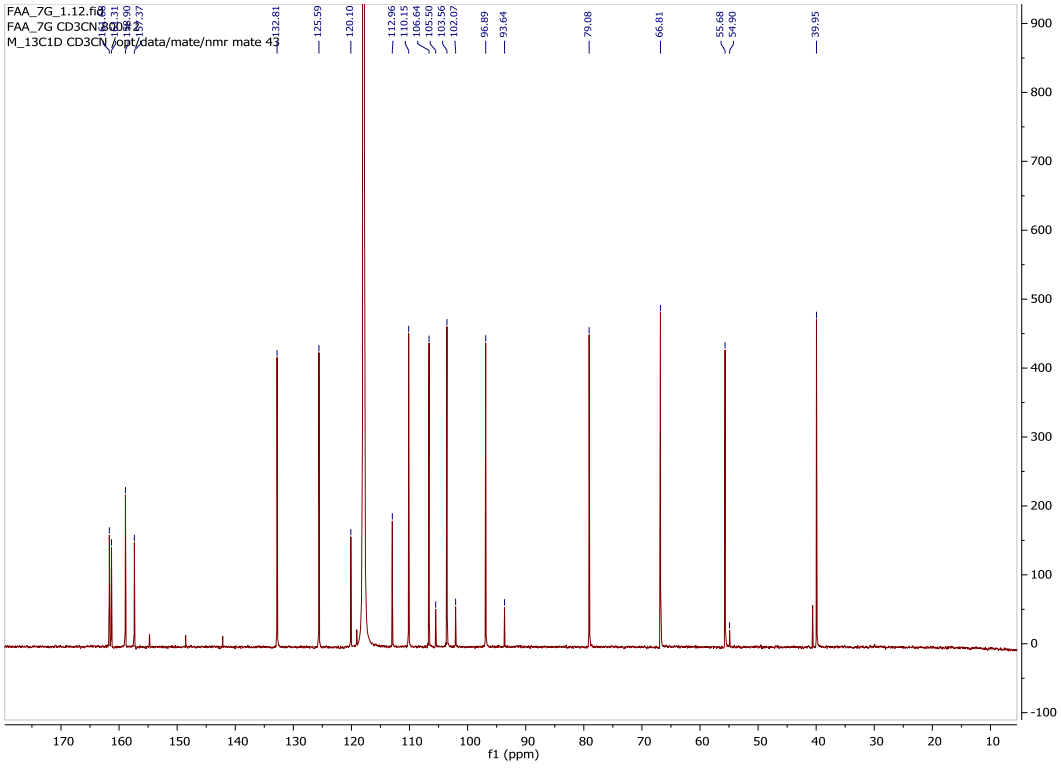




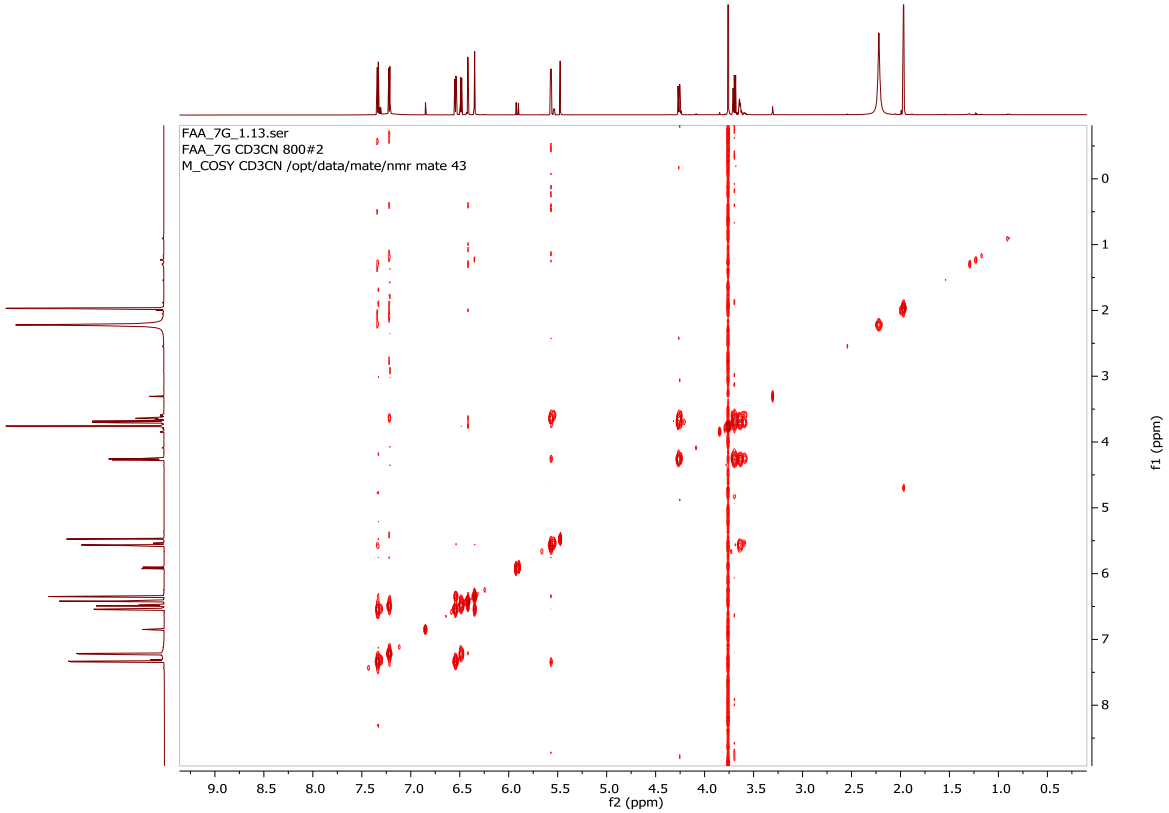


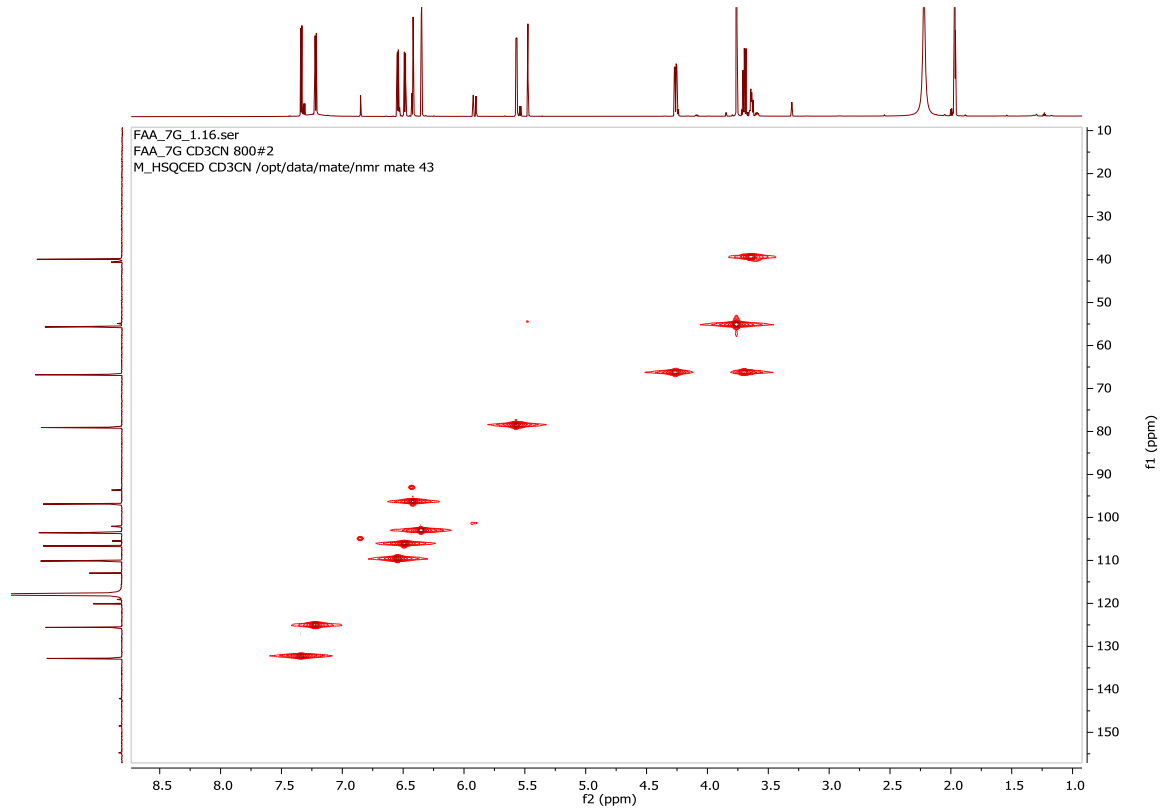
Appendix 9A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 9 (CD_3CN)



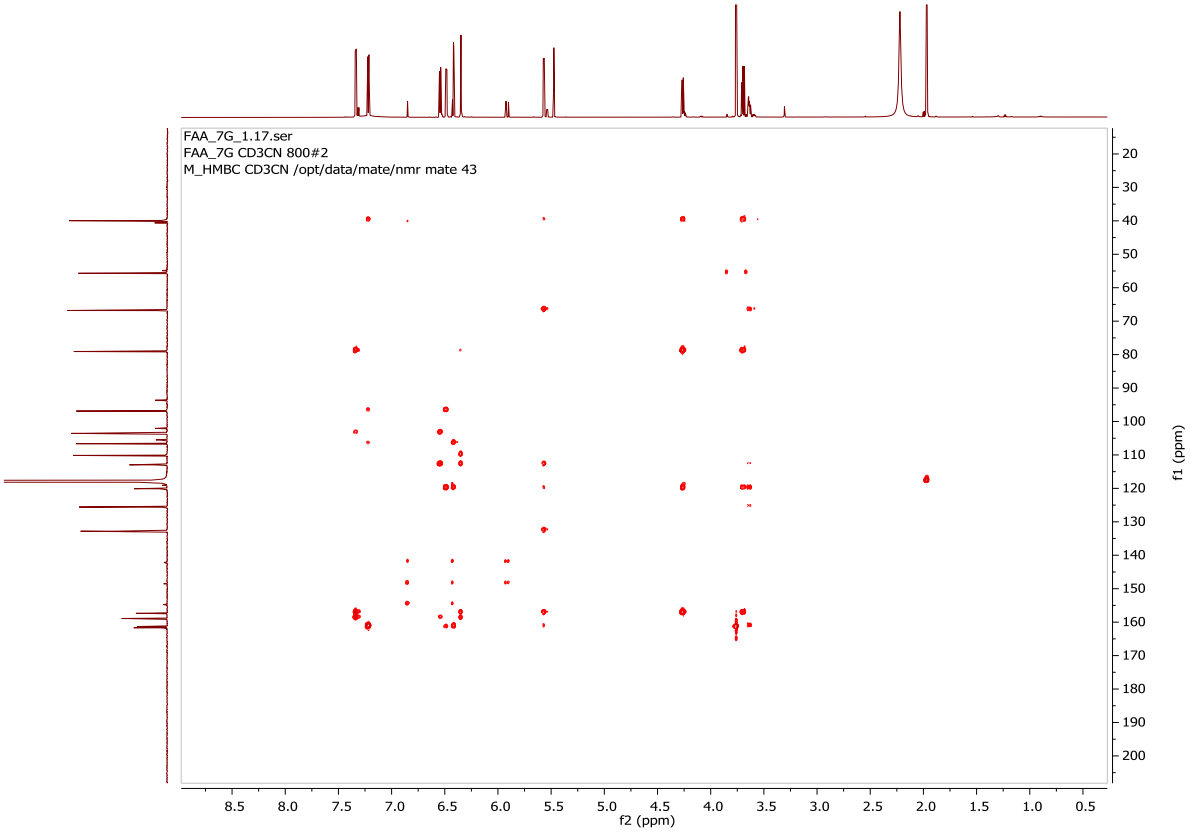


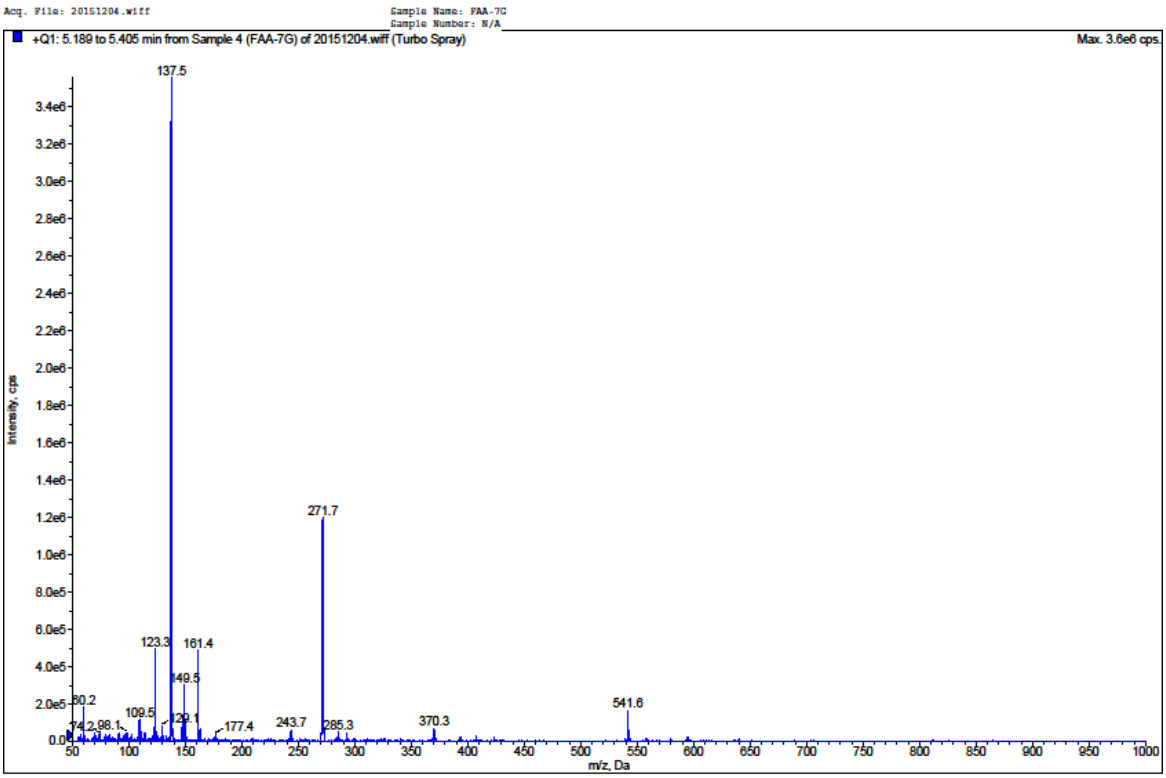
Appendix 9B: H,H-COSY and HSQC spectra of compound 9 (800 MHz; CD_3CN)



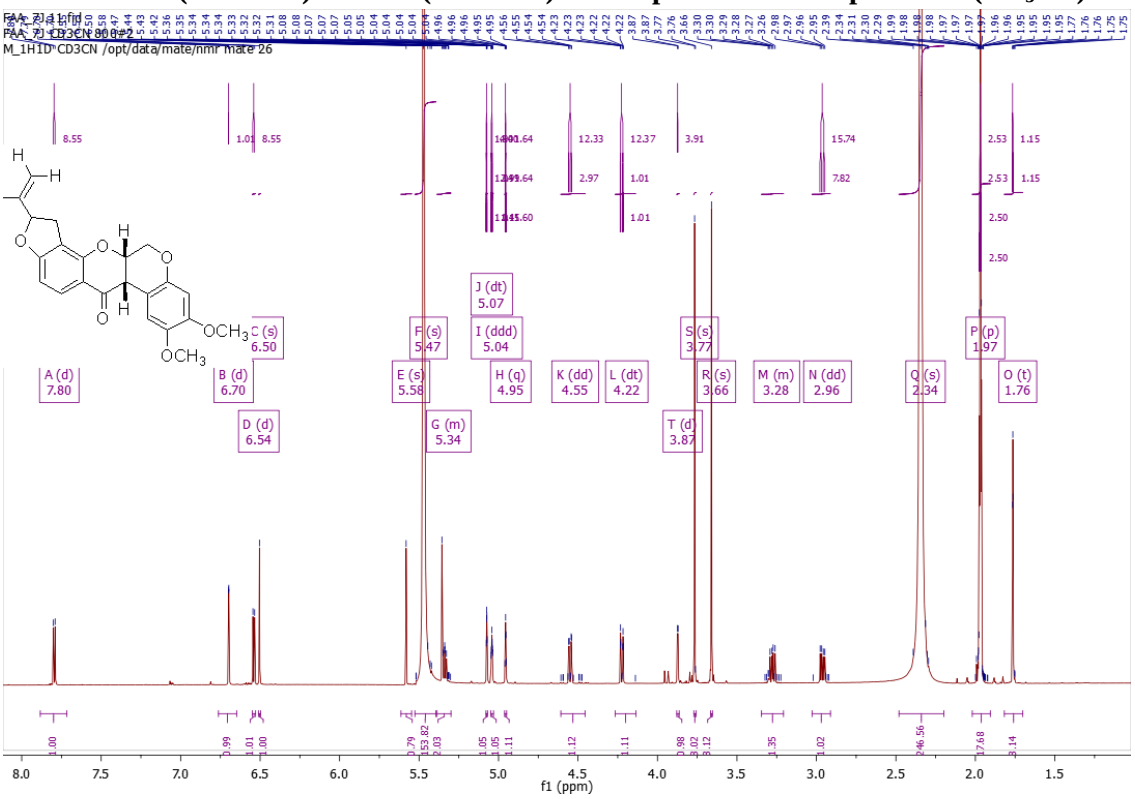


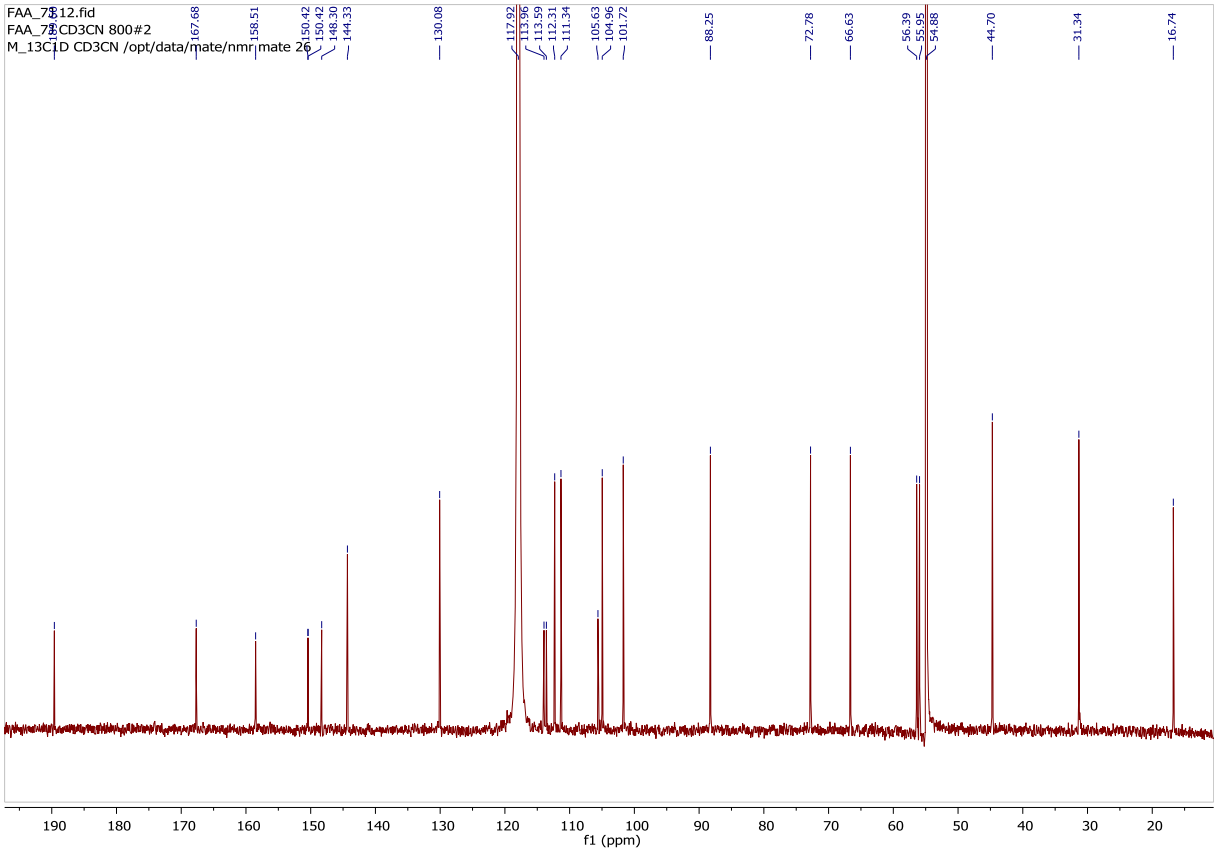
Appendix 9C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 9



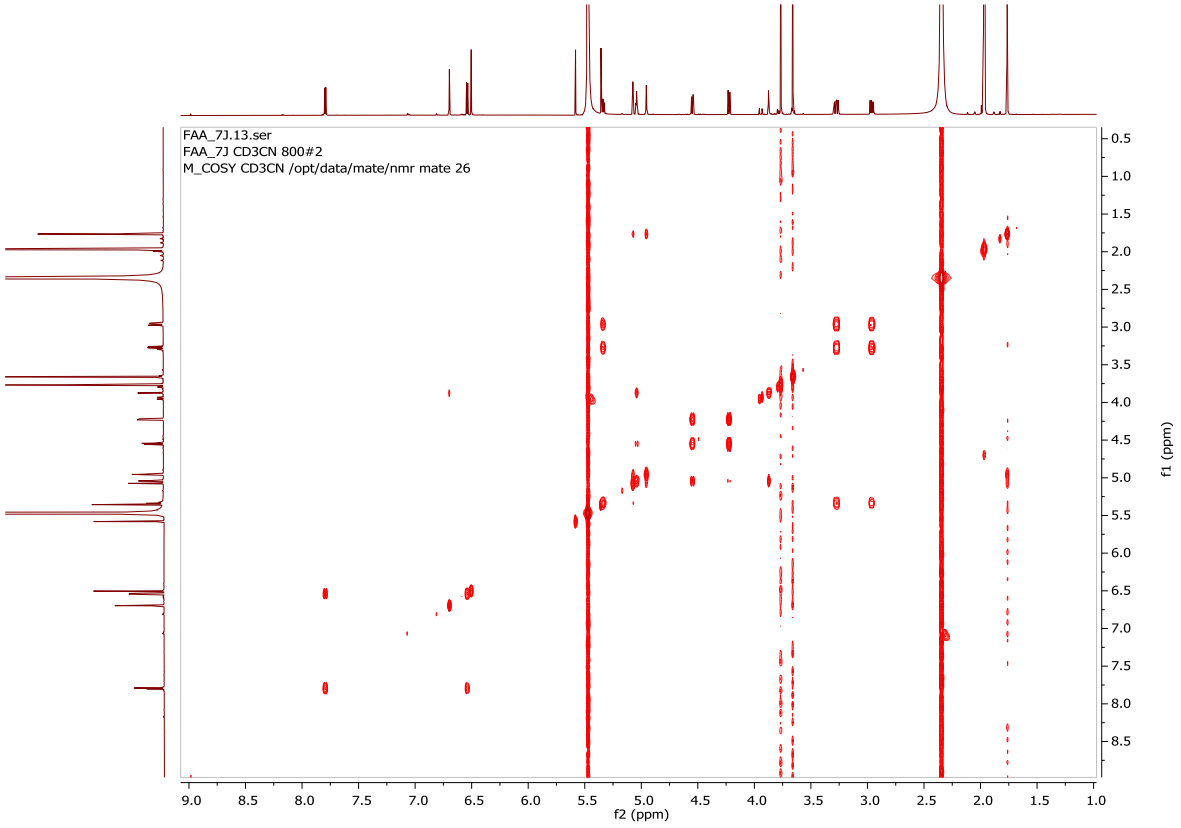


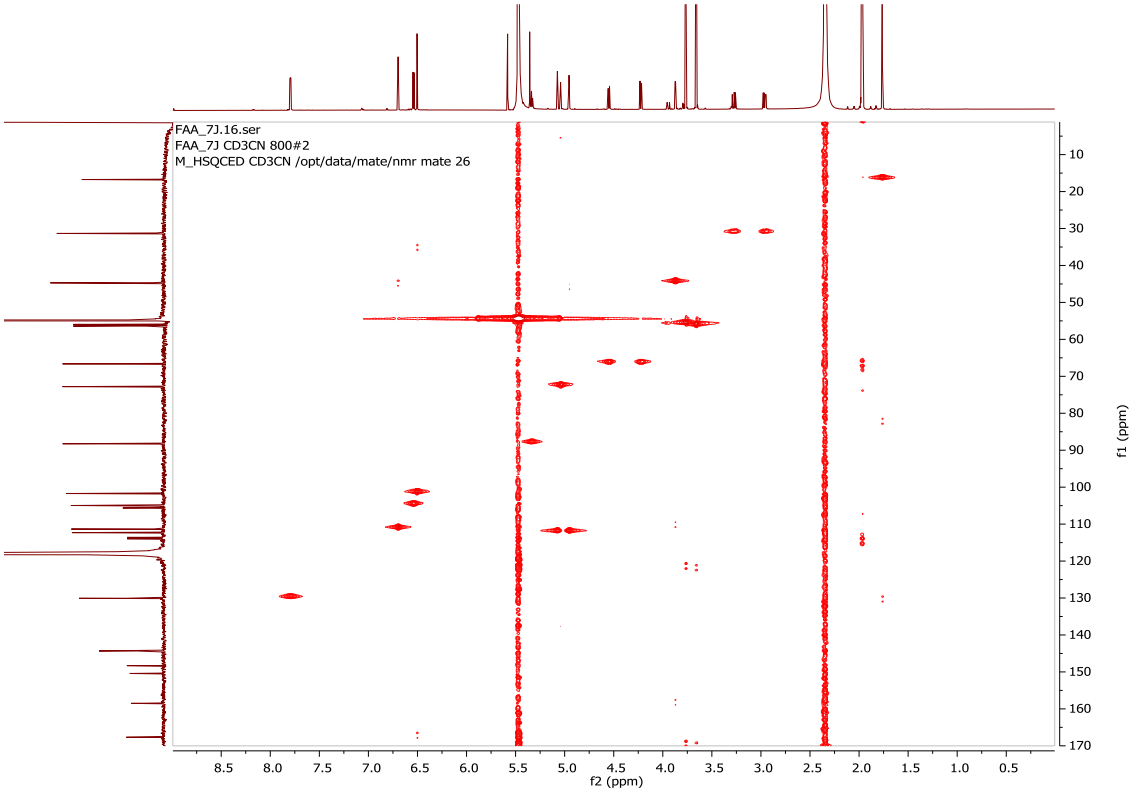
Appendix 10A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectrum of compound 10 (CD_3CN)



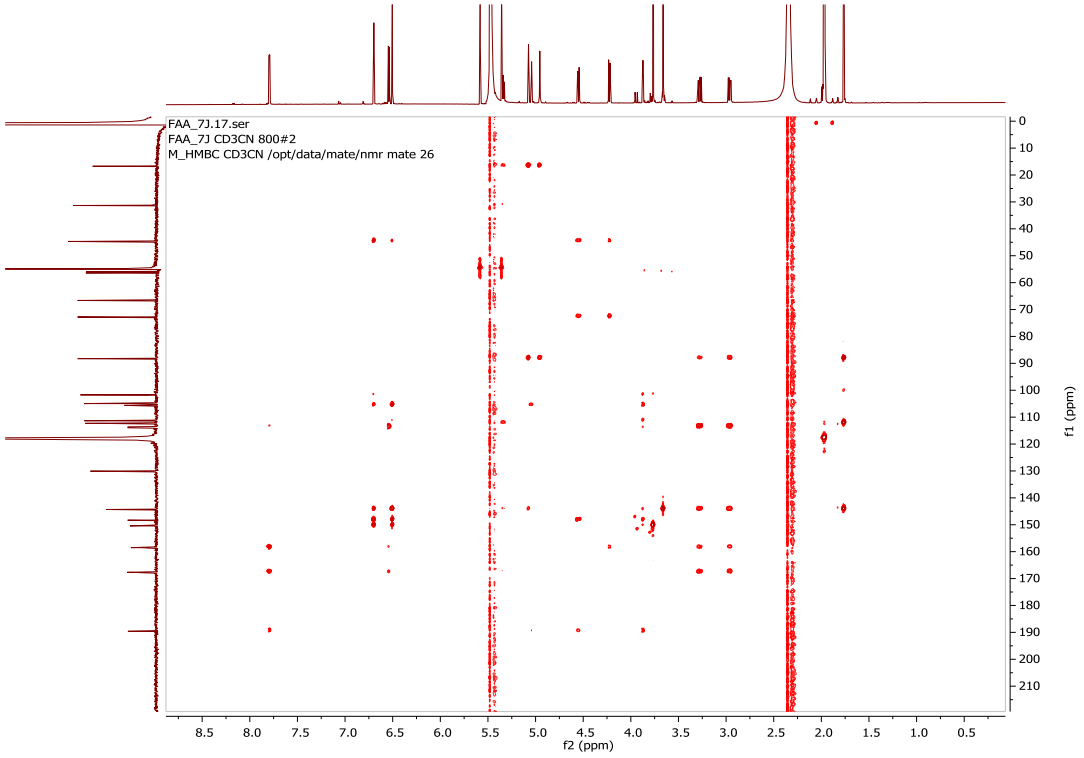


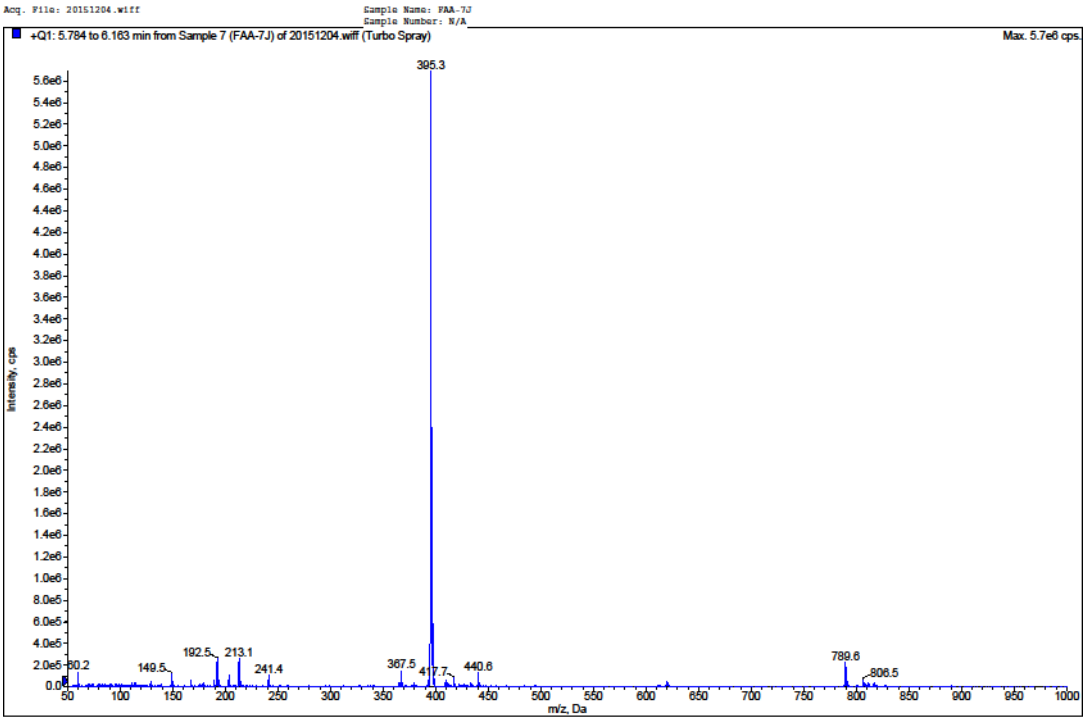
Appendix 10B: H,H-COSY and HSQC spectra of compound 10 (800 MHz; CD_3CN)



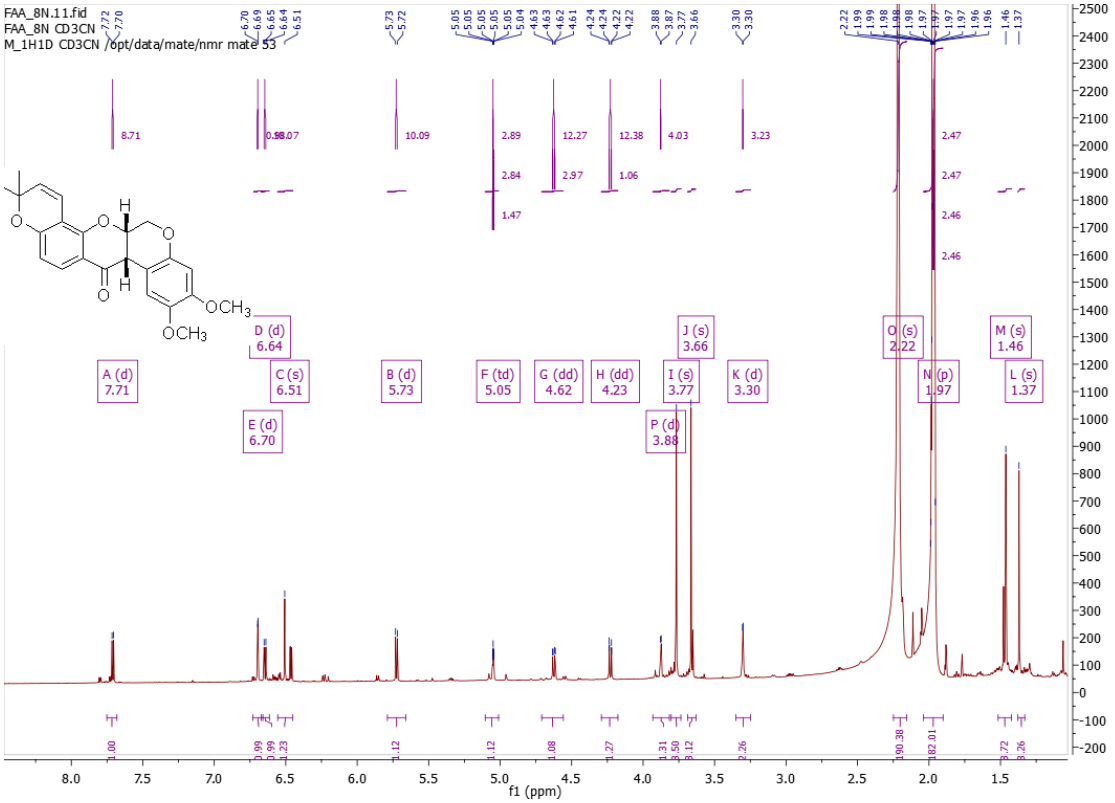


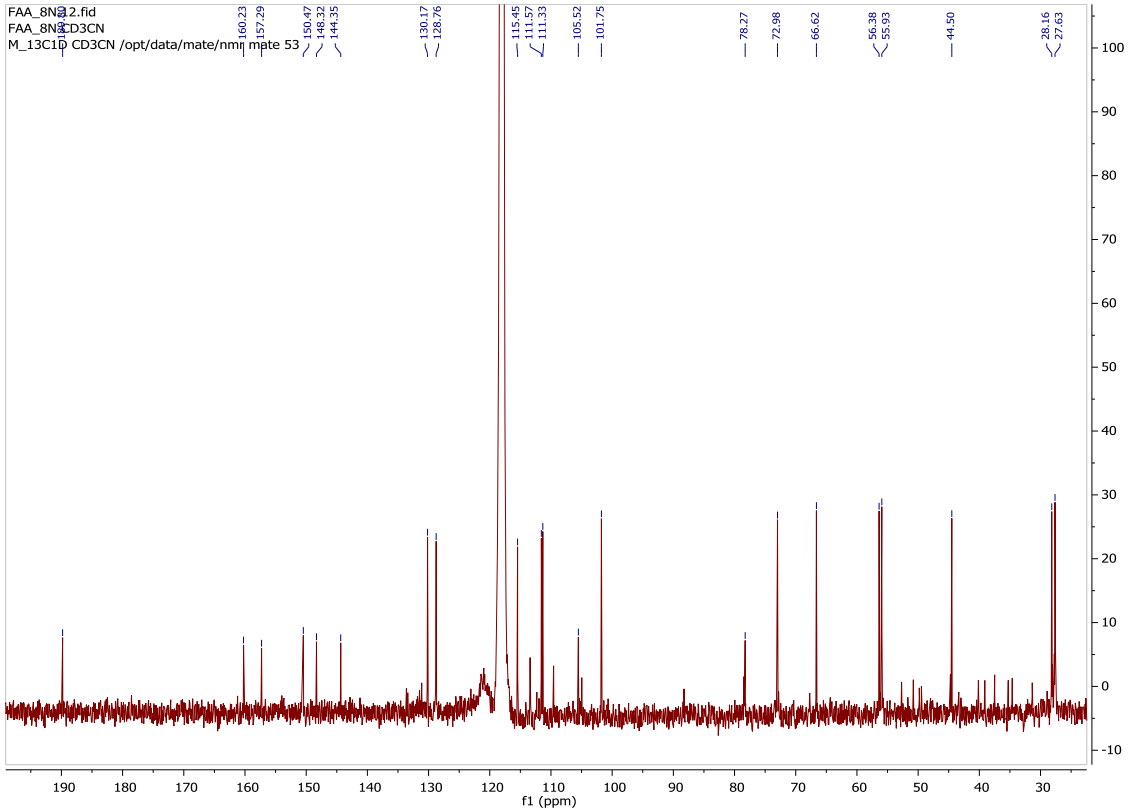
Appendix 10C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 10



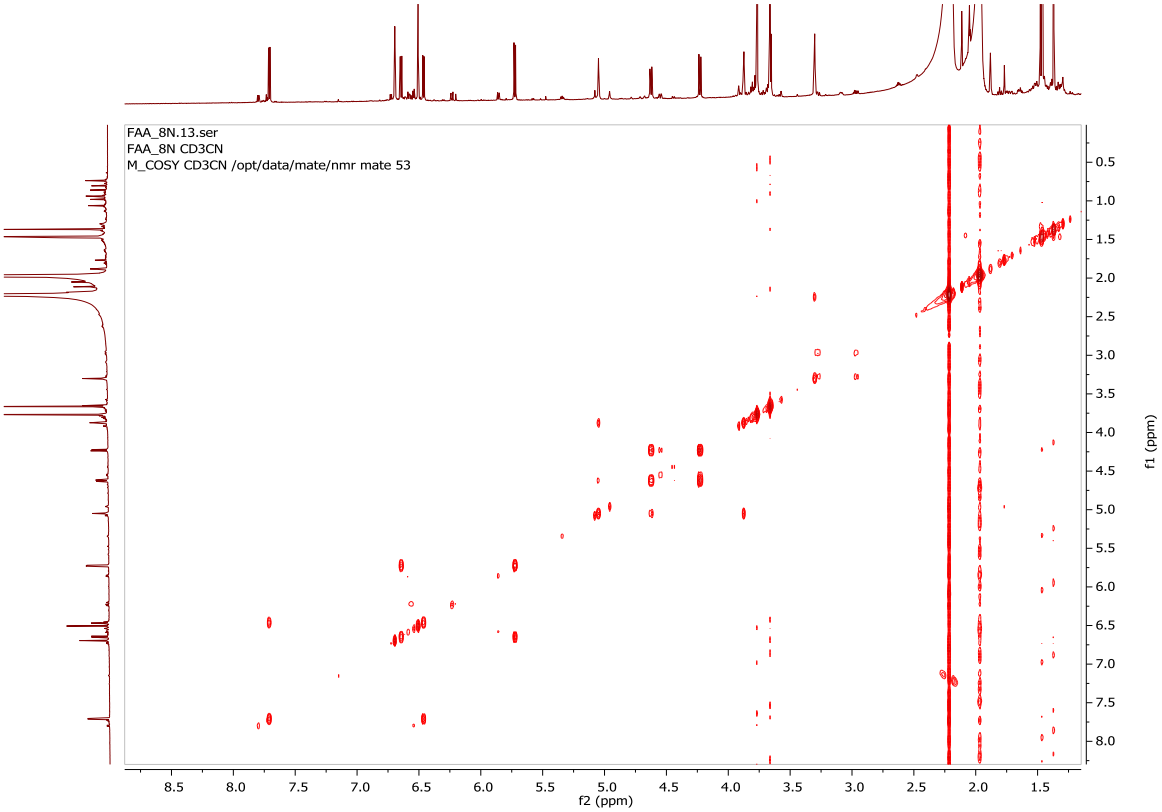


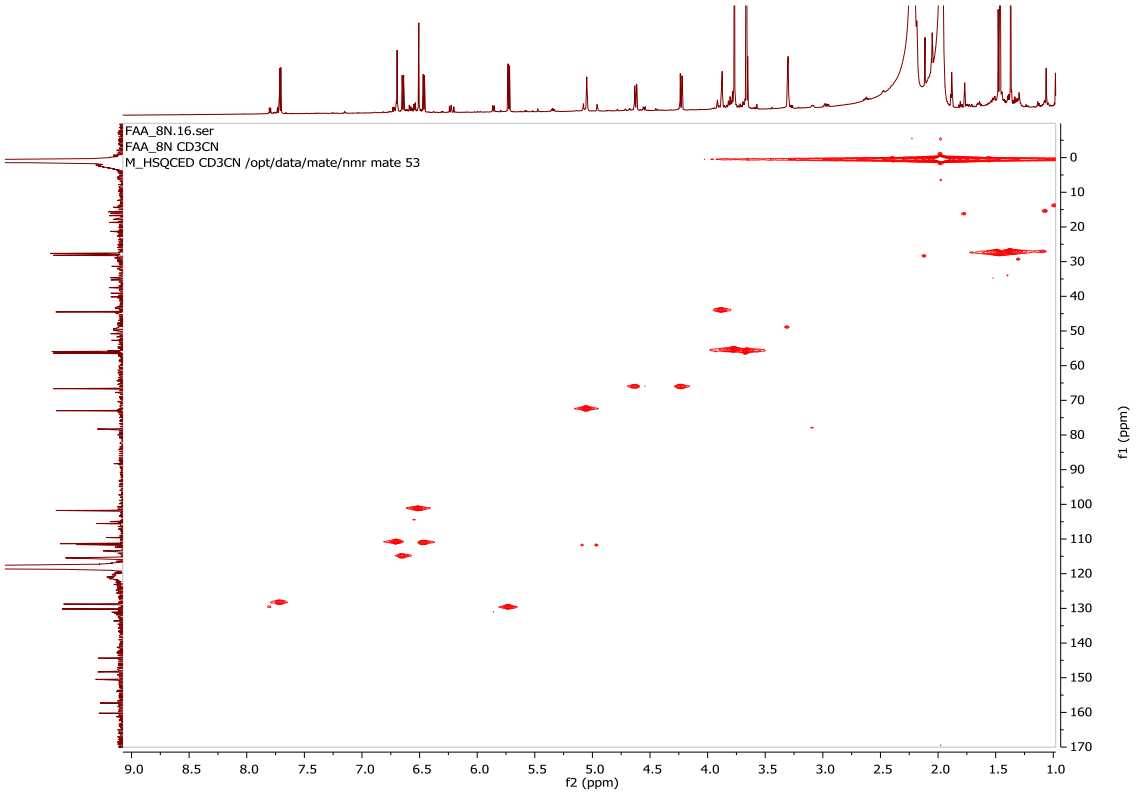
Appendix 11A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 11 (CD_3CN)



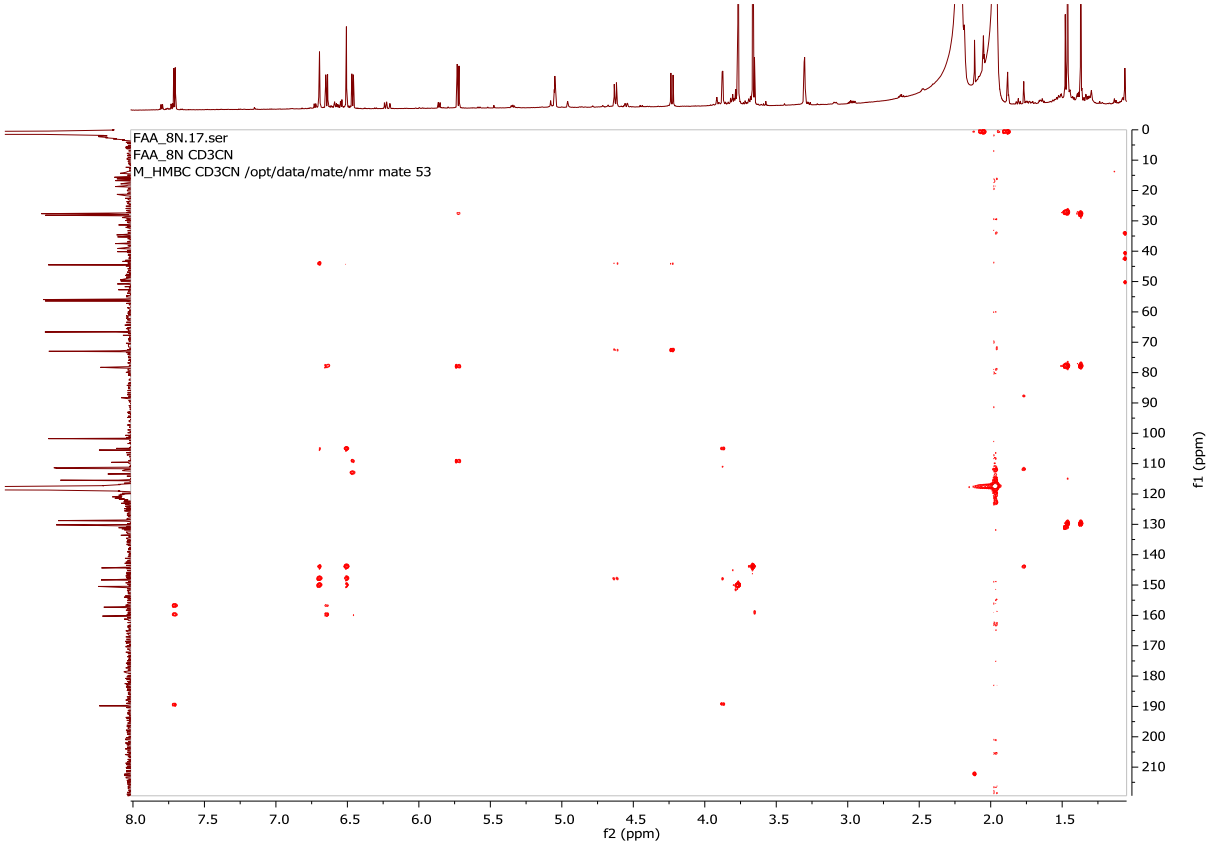


Appendix 11B: H,H-COSY and HSQC spectra of compound 11 (800 MHz; CD_3CN)

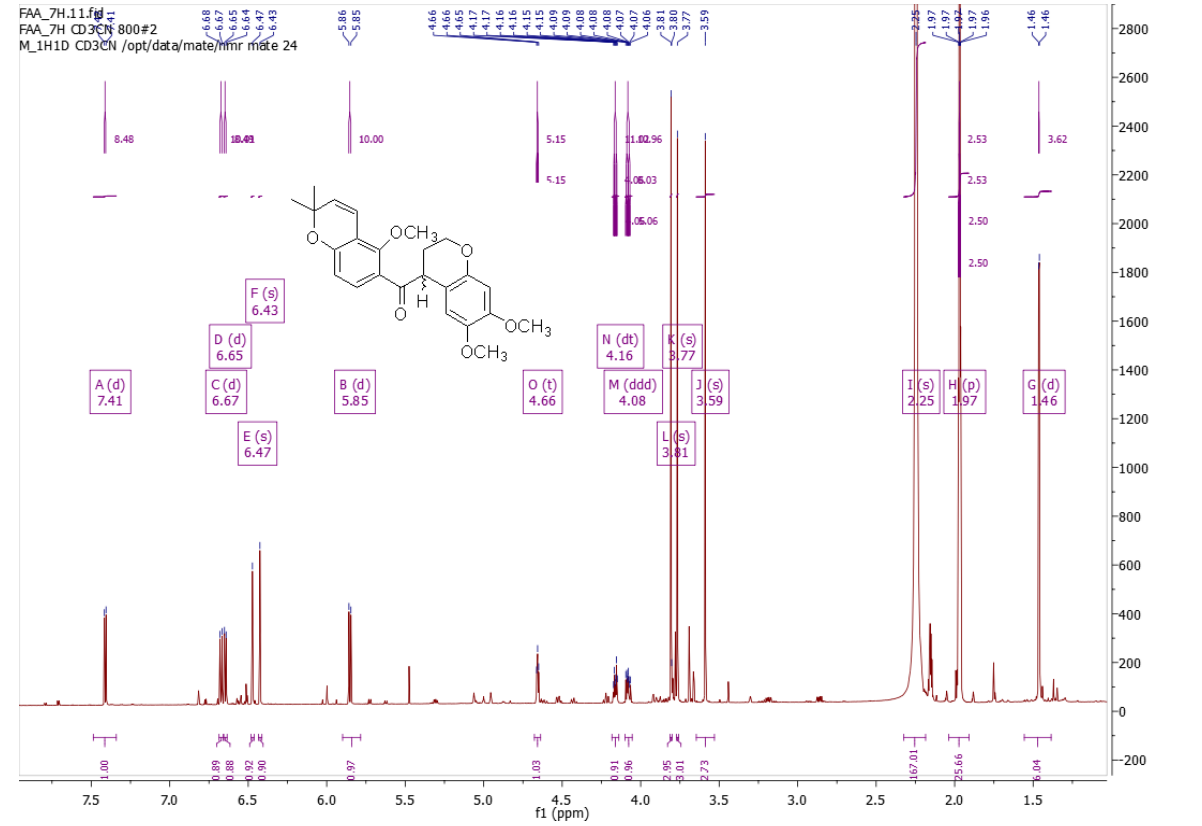


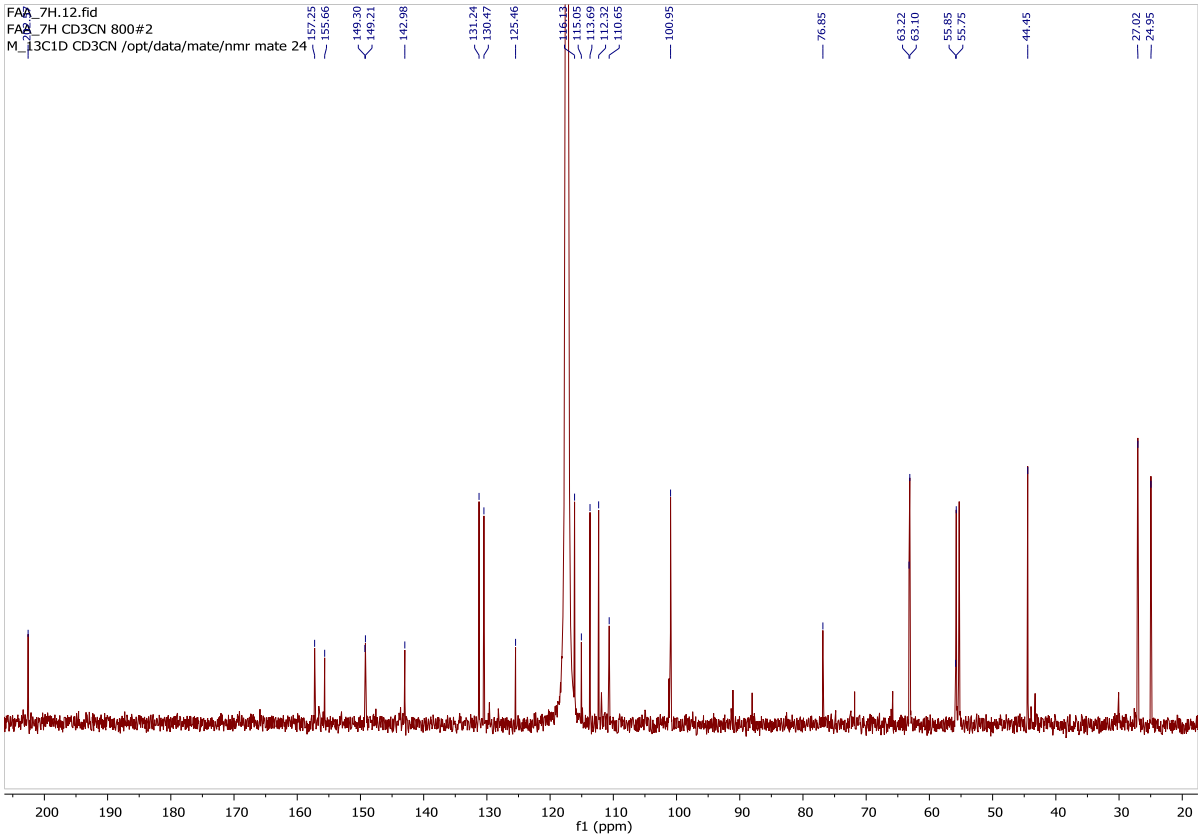


Appendix 11C: HMBC spectrum of compound 11 (800 MHz; CD_3CN)

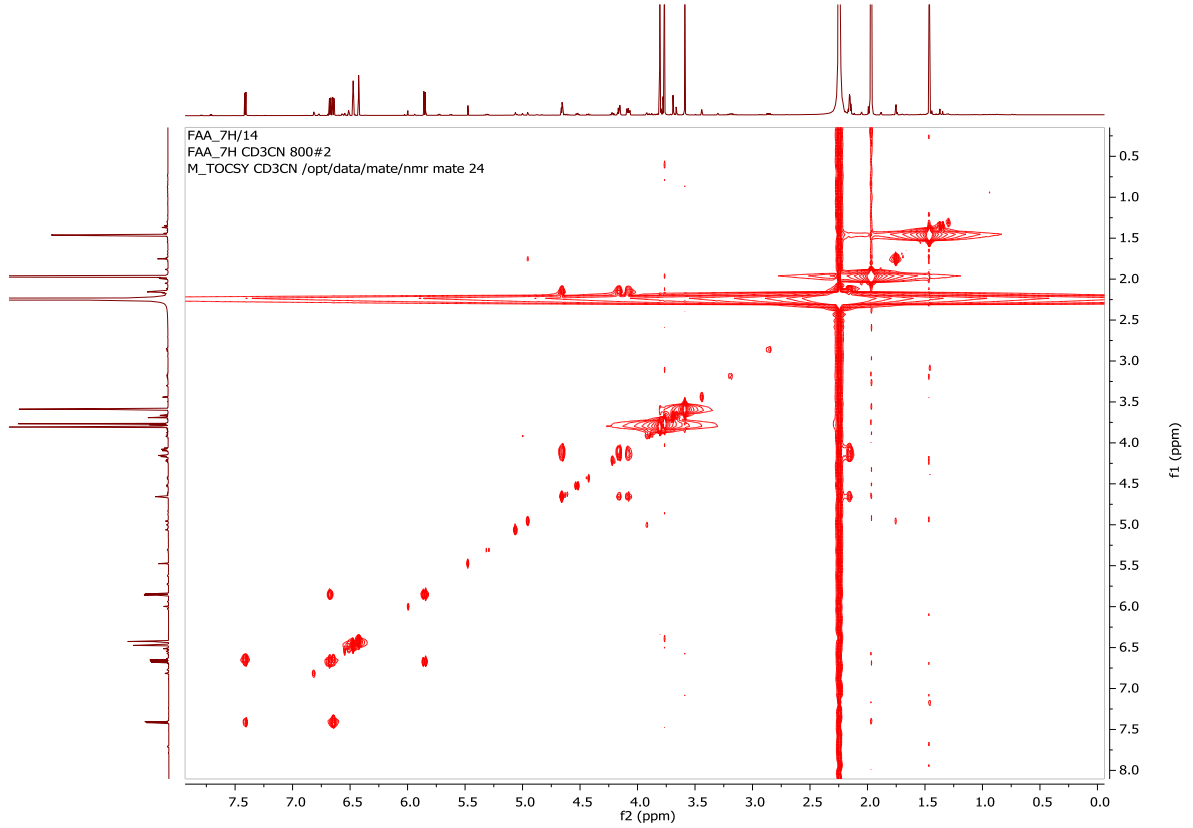


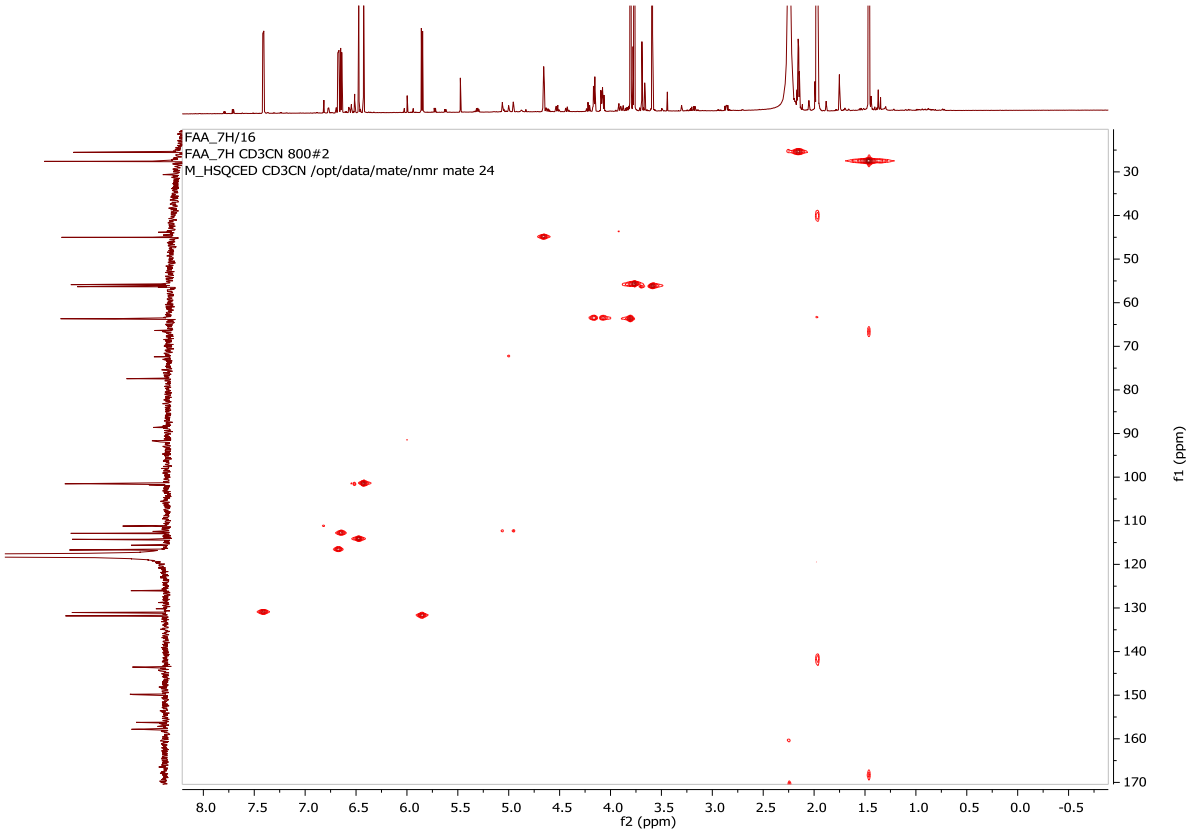
Appendix 12A: ^{1}H (800 MHz) and ^{13}C NMR (200 MHz) spectra of compound 12 (CD_3CN)



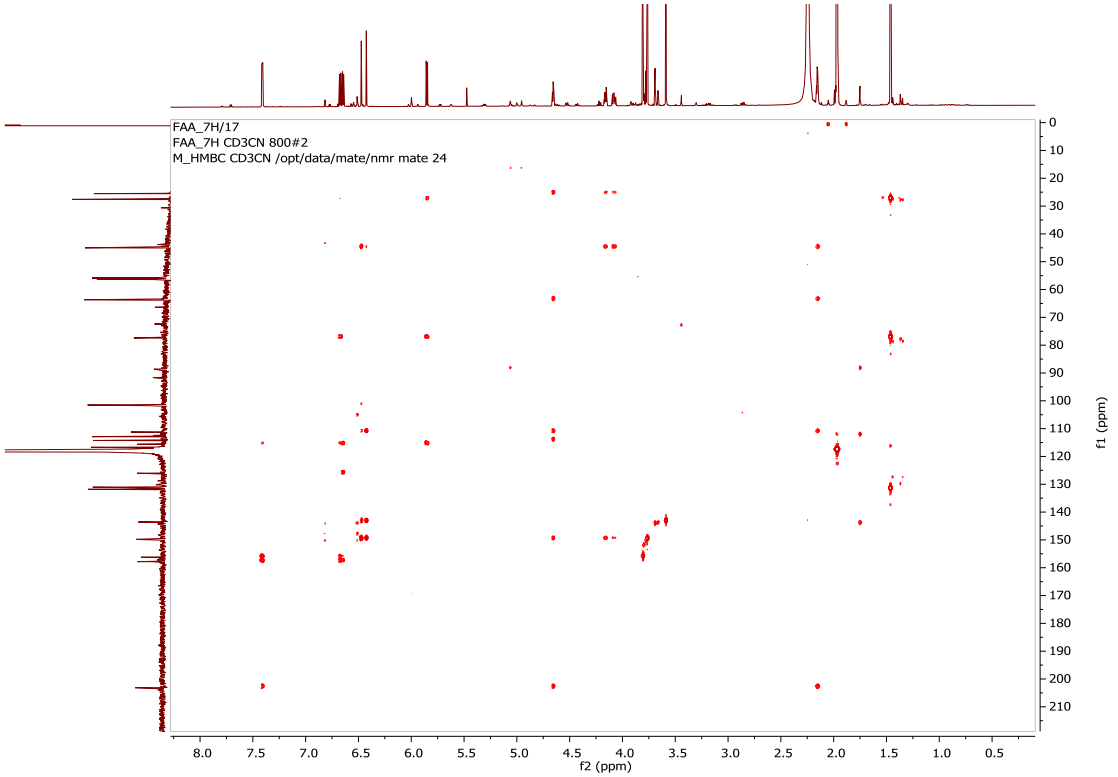


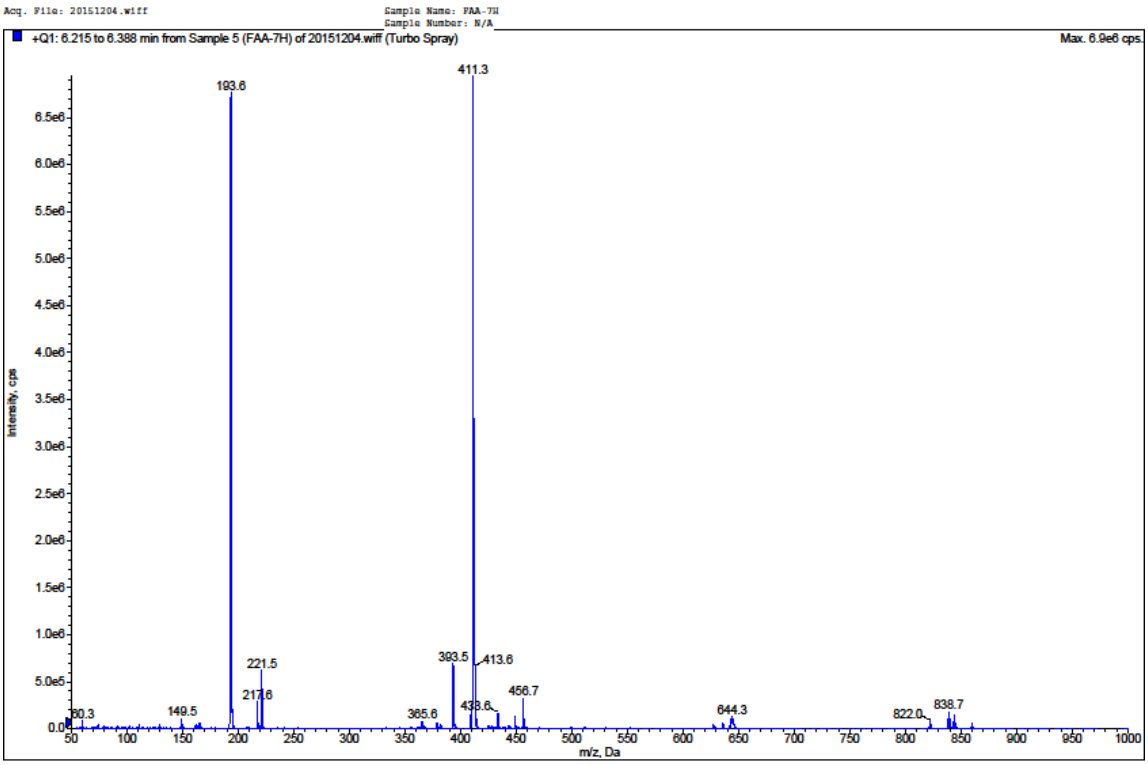
Appendix 12B: TOCSY and HSQC spectra of compound 12 (800 MHz; CD_3CN)



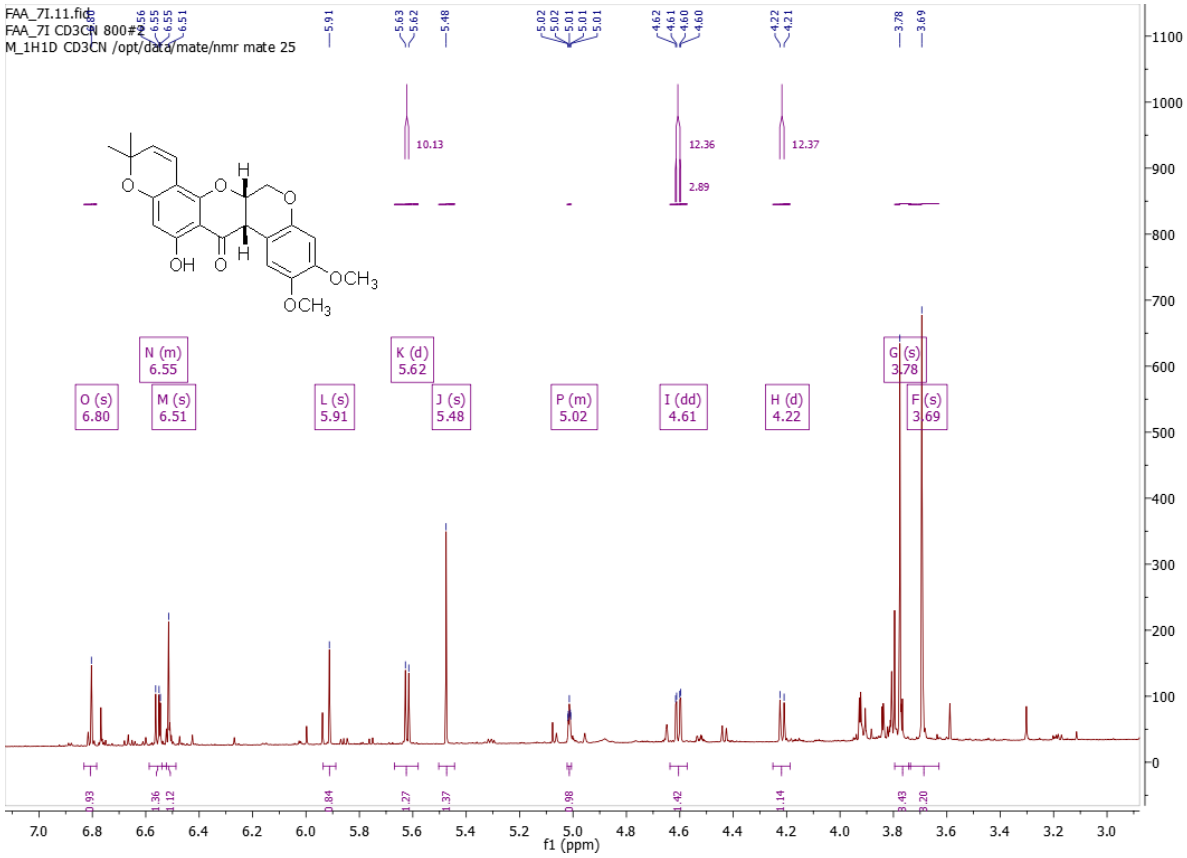


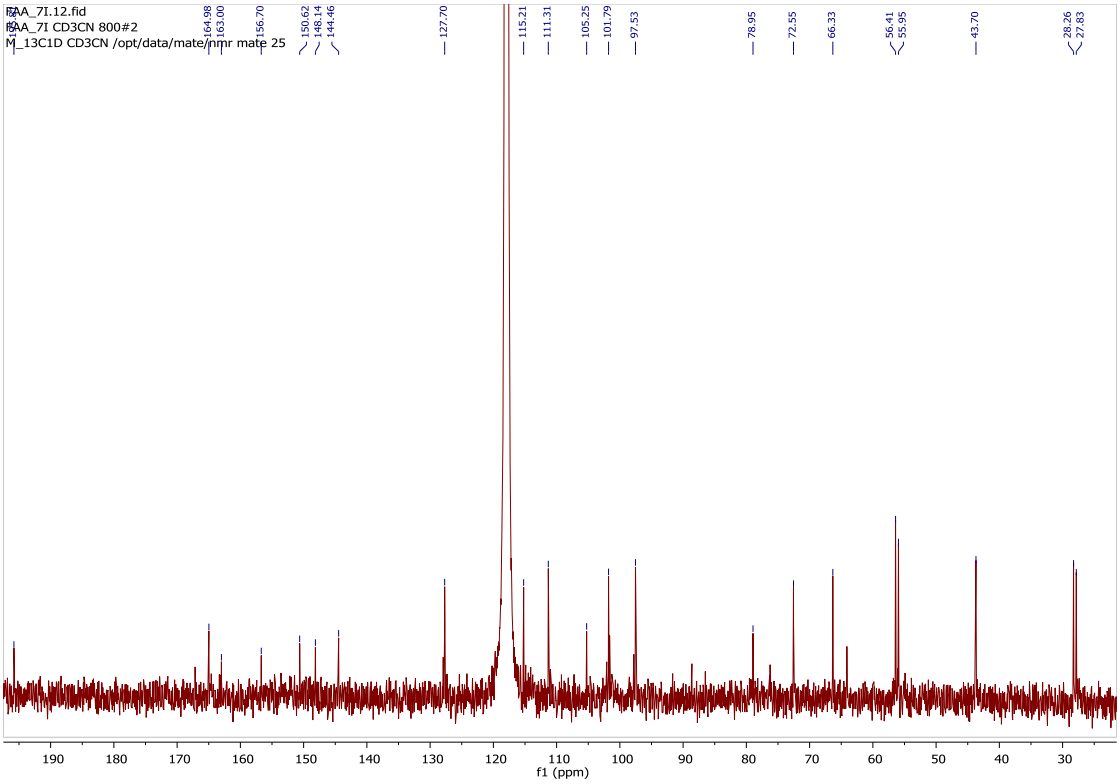
Appendix 12C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 12



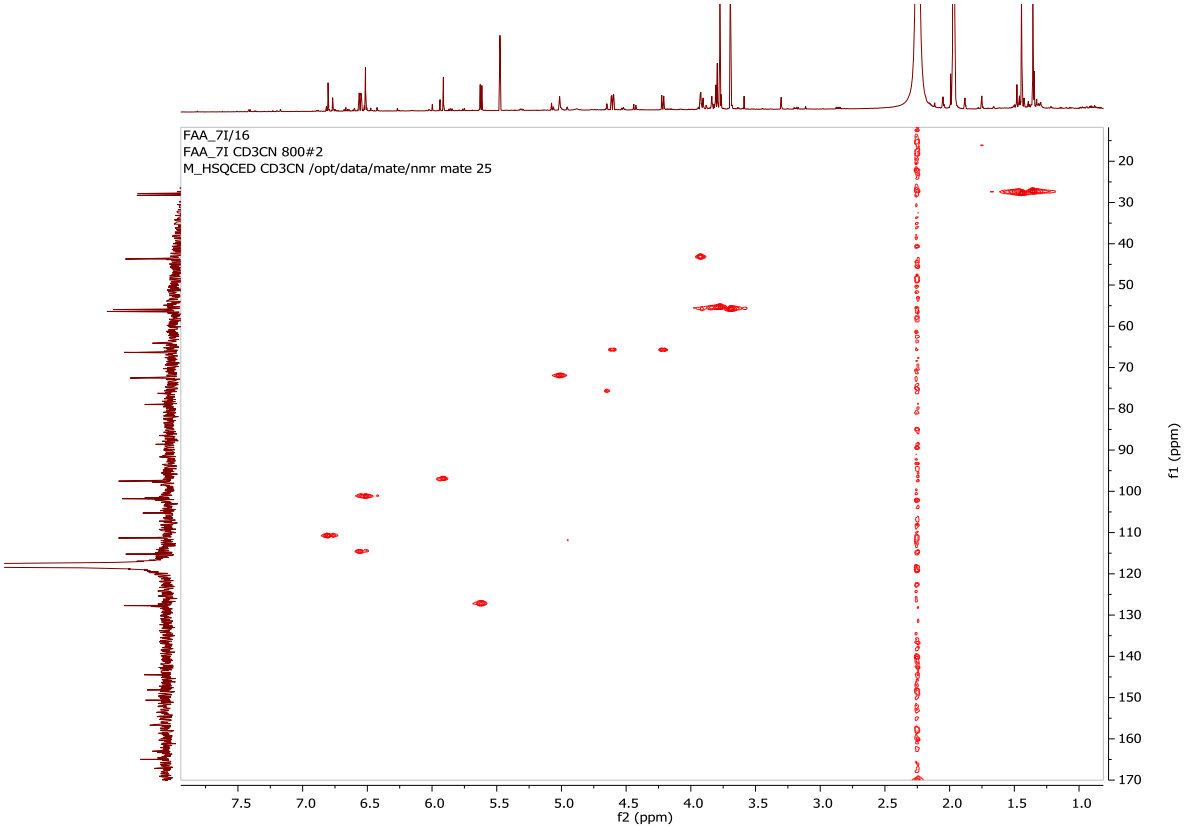


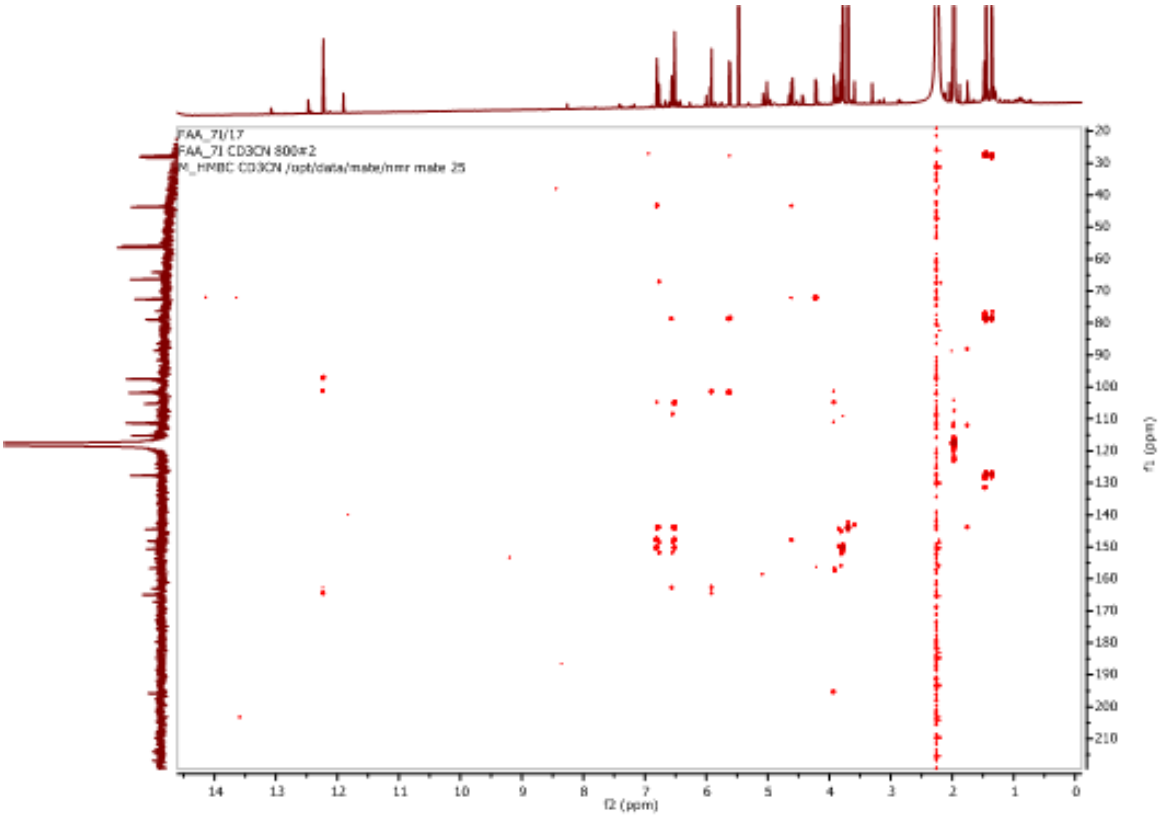
Appendix 13A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 13 (CD_3CN)



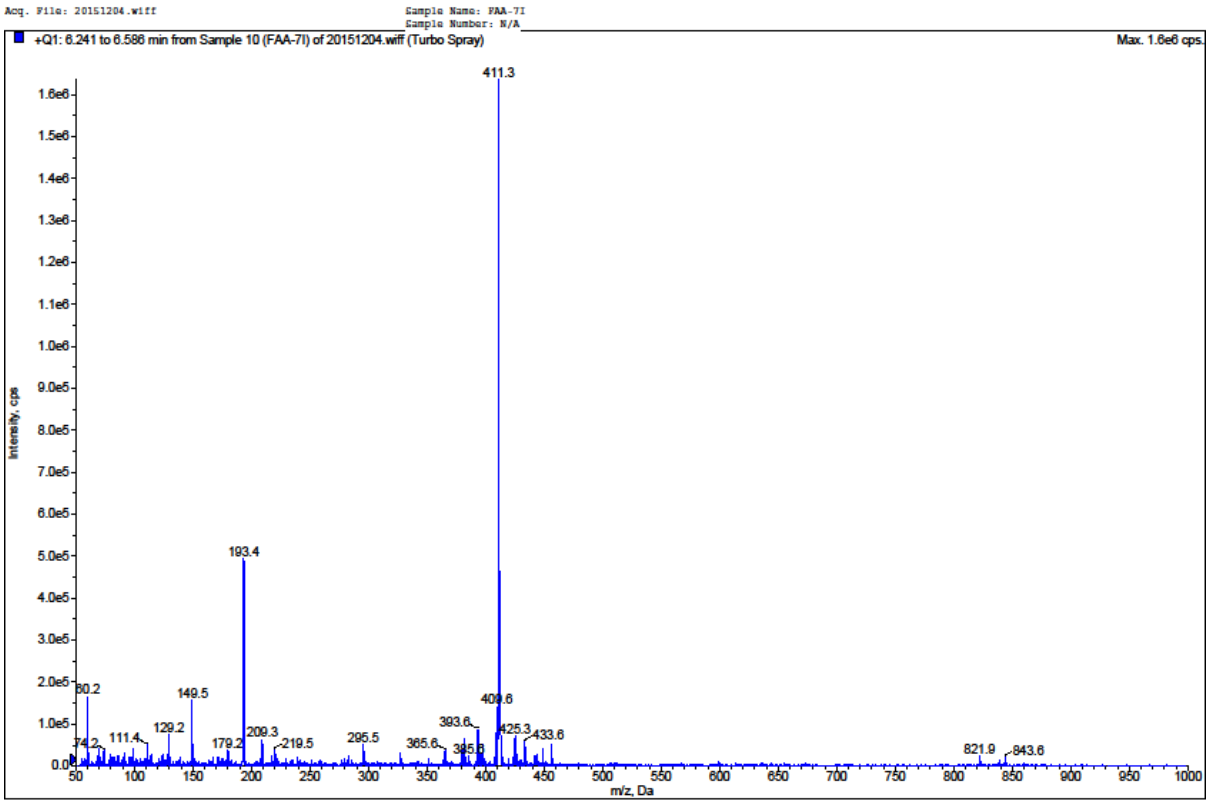


Appendix 13B: HSQC and HMBC spectra of compound 13 (800 MHz; CD_3CN)

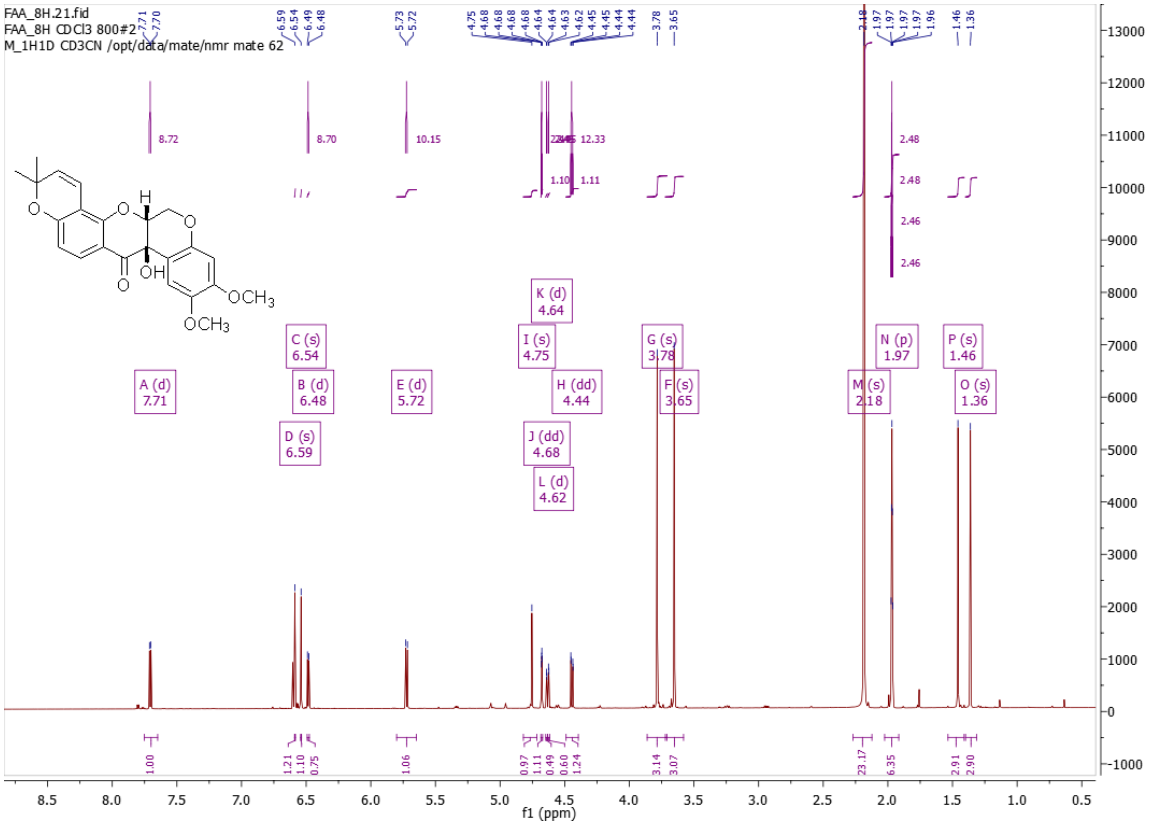


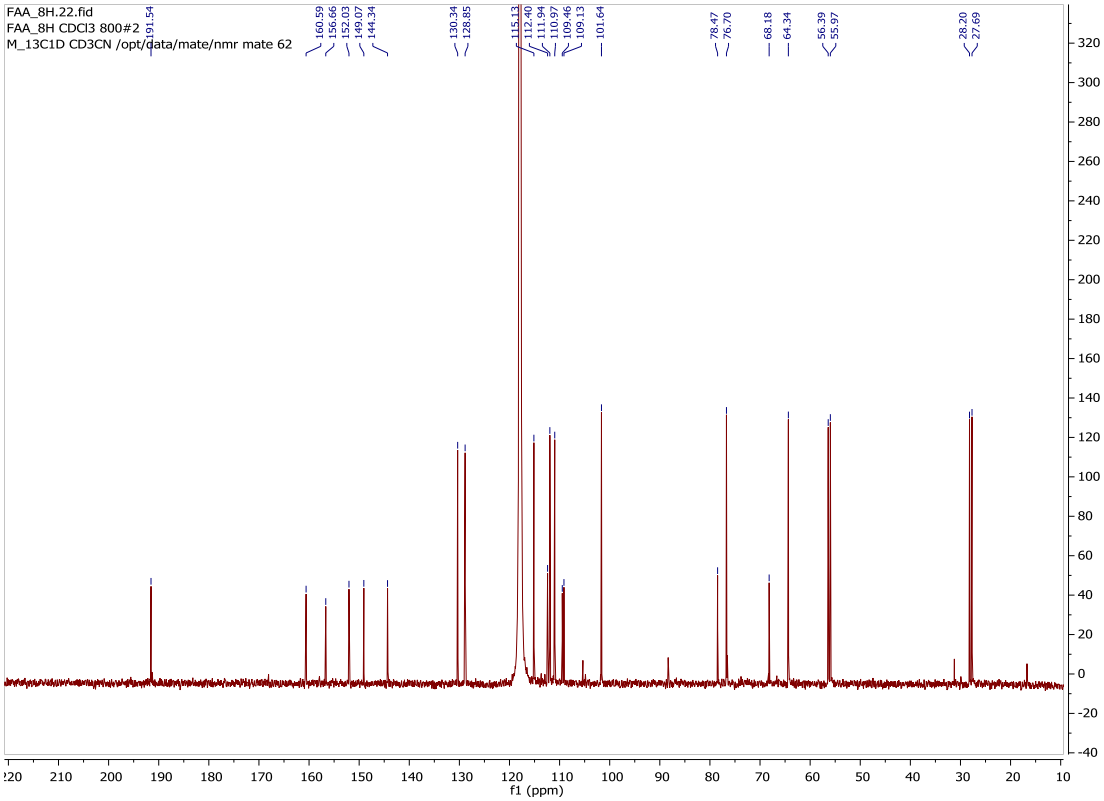


Appendix 13C: LCMS spectrum of compound 13

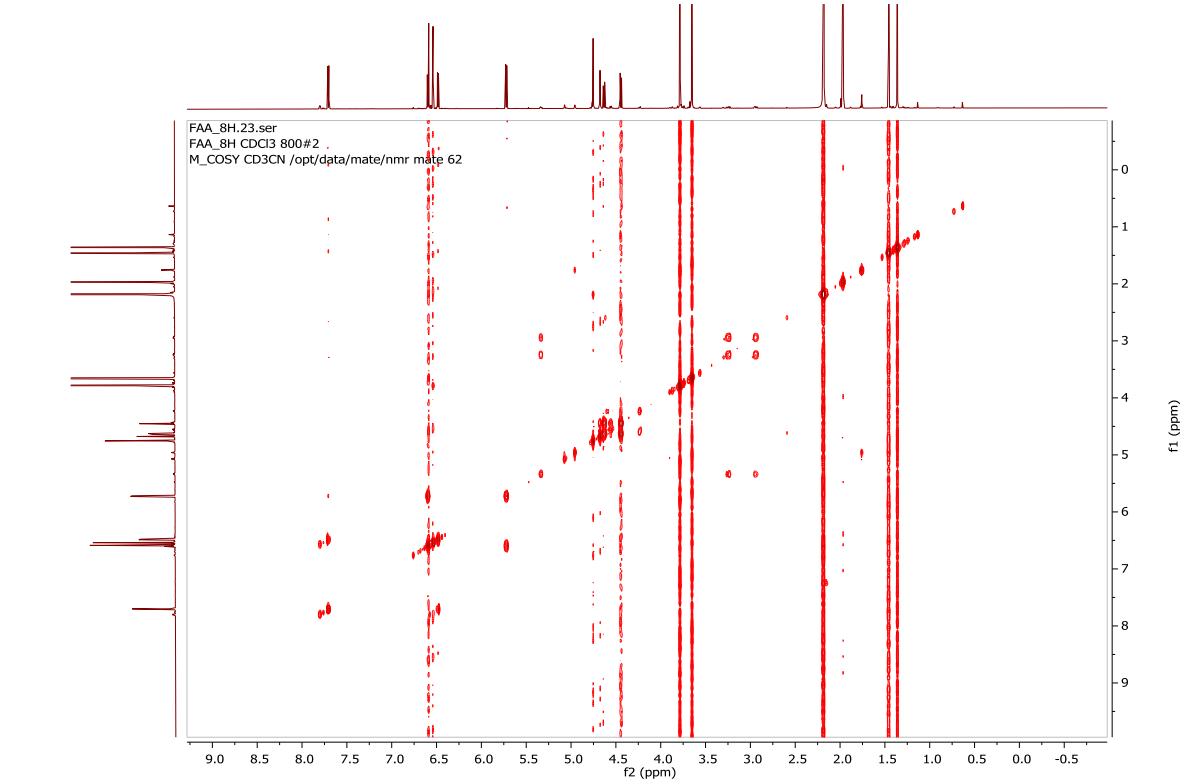


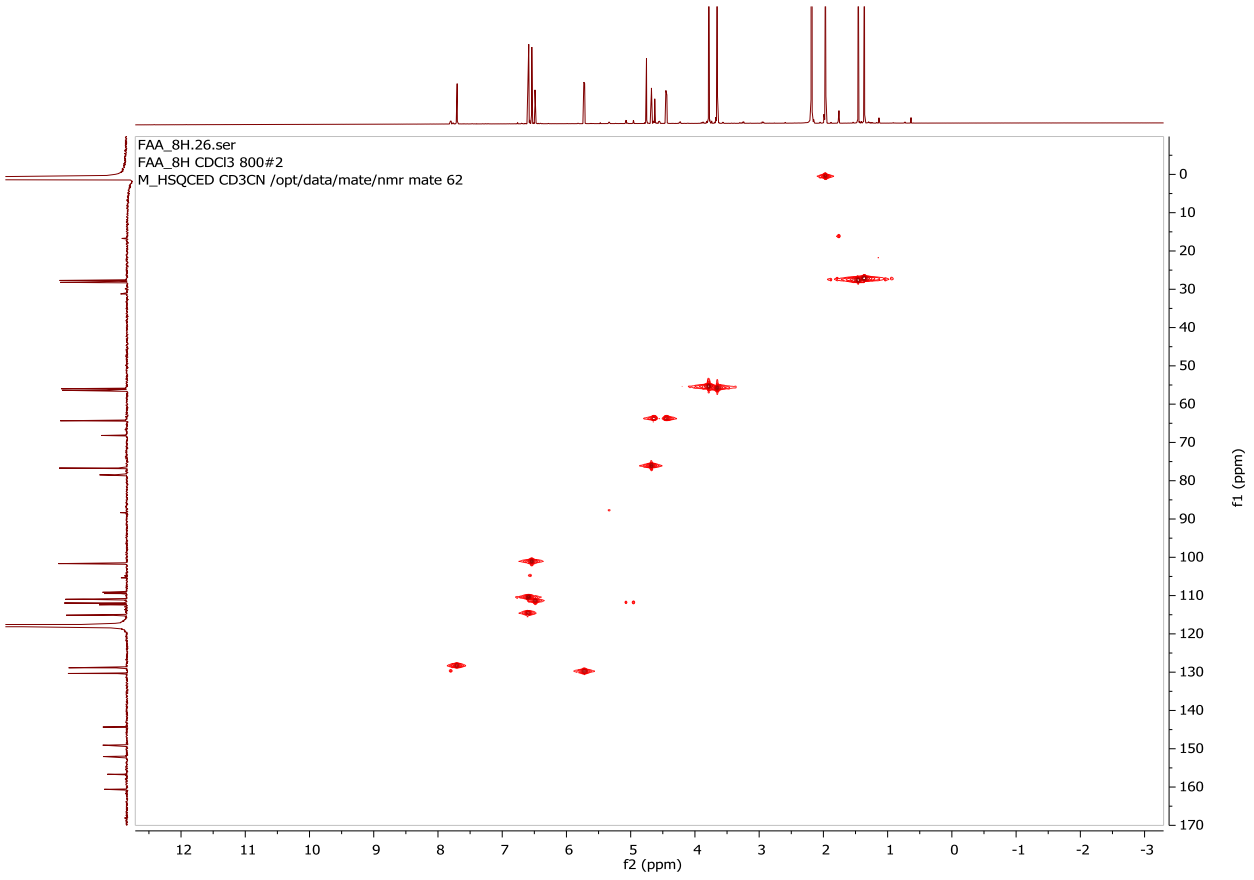
Appendix 14A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 14 (CD_3CN)



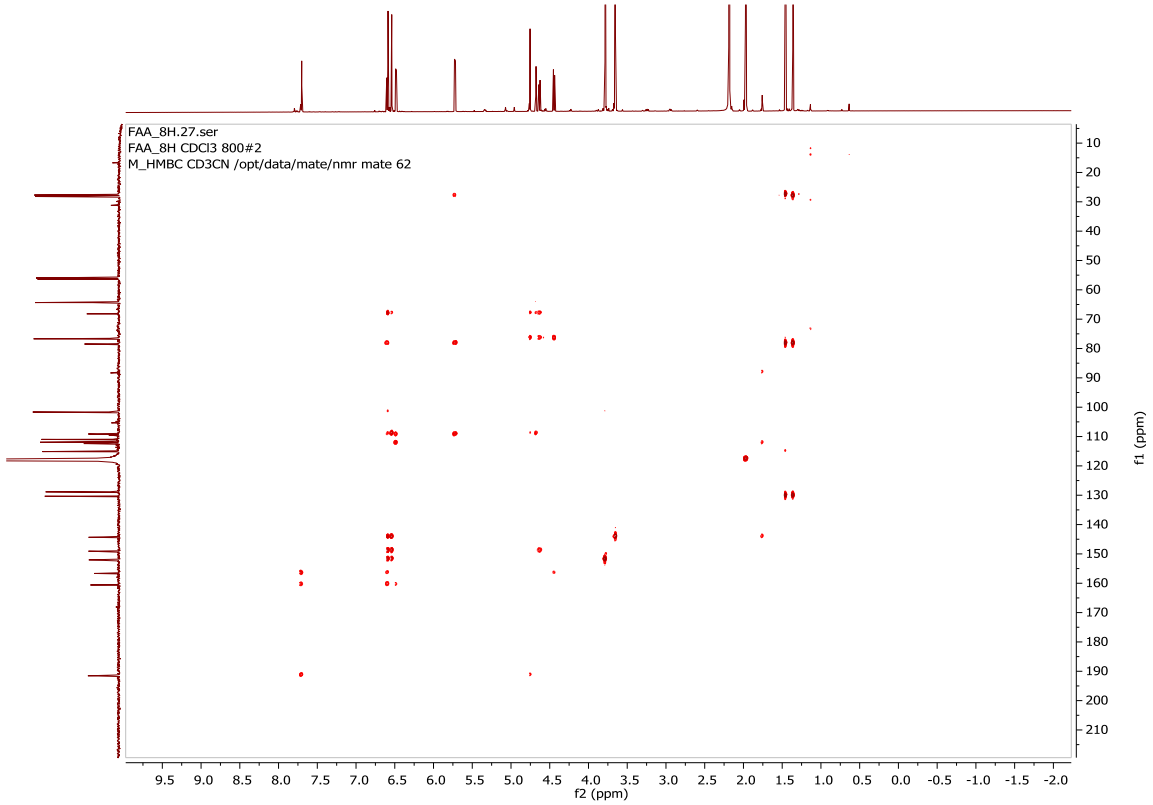


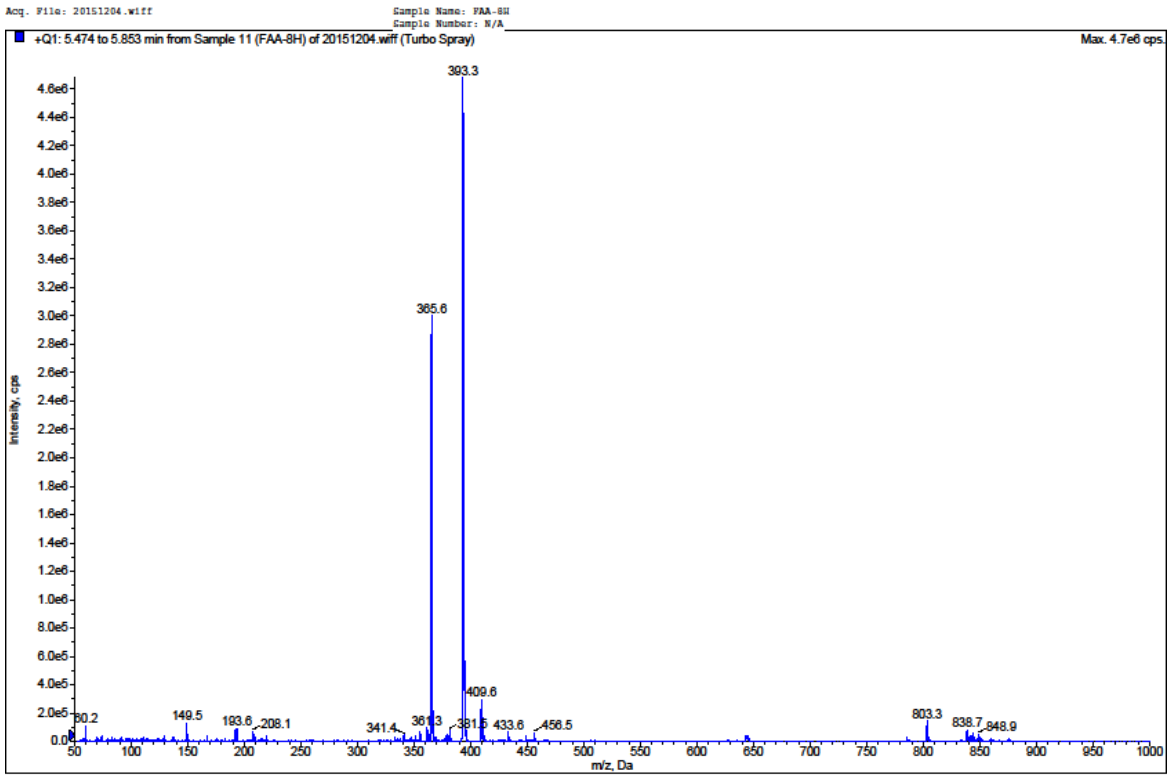
Appendix 14B: H,H COSY and HSQC spectra of compound 14 (800 MHz; CD3CN)



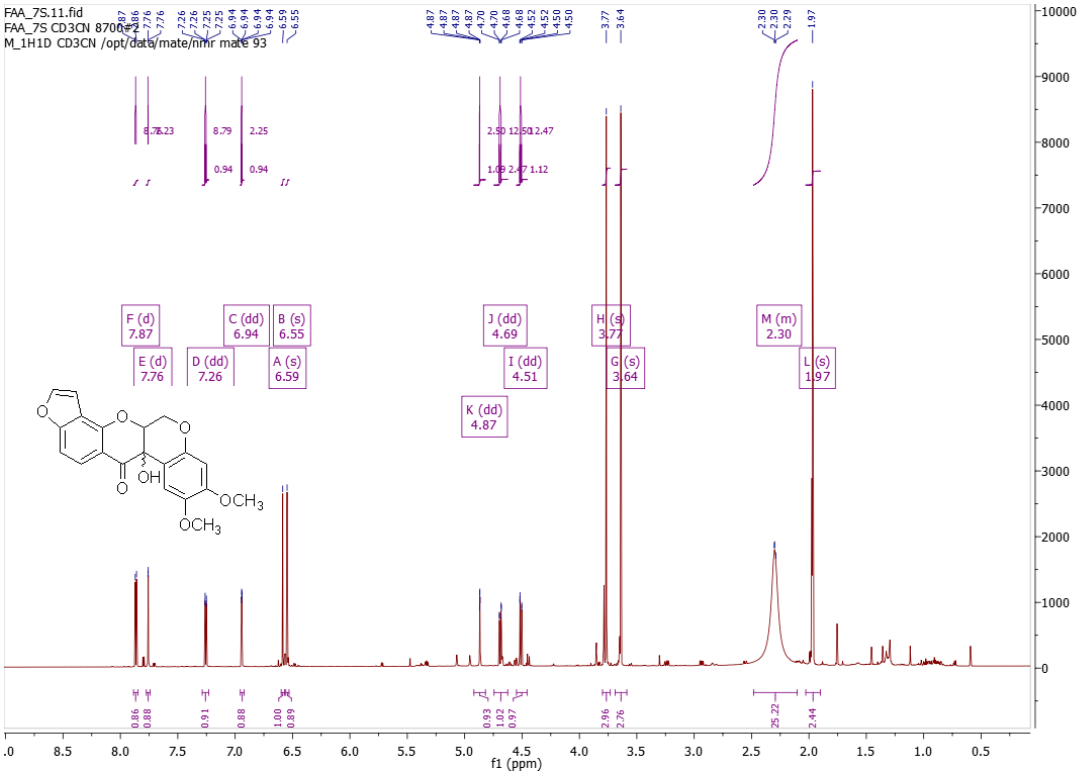


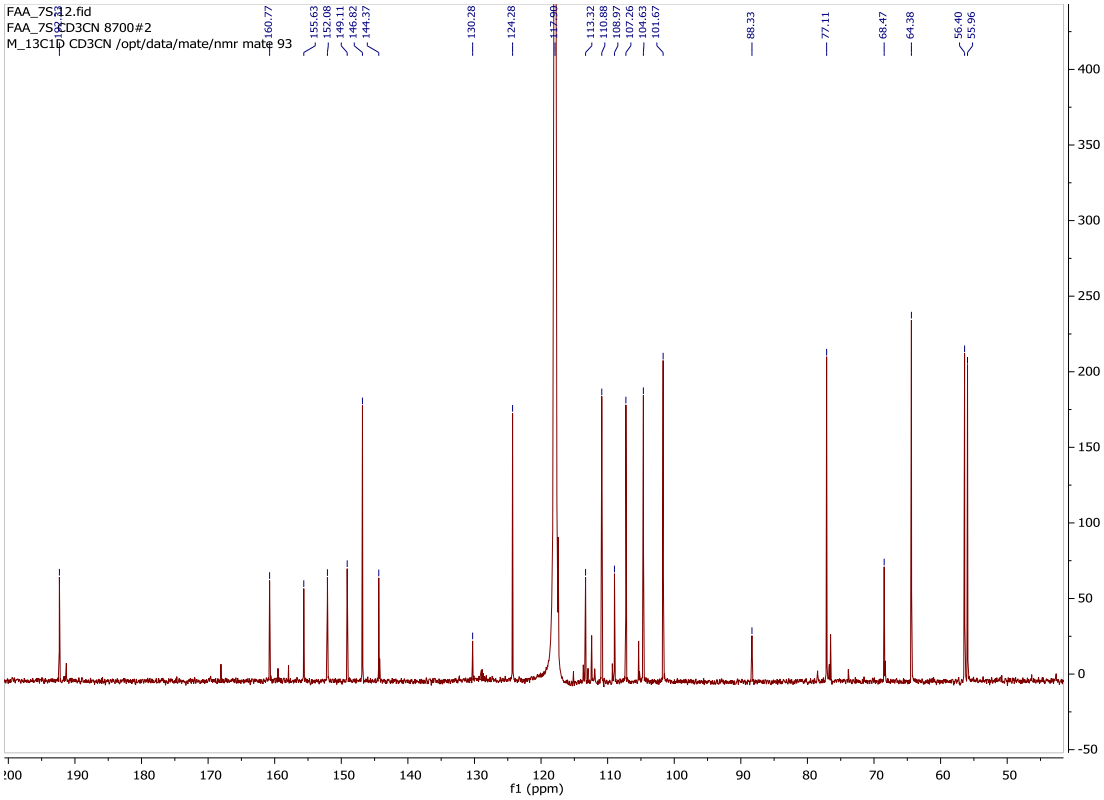
Appendix 14C: HMBC and LCMS spectra of compound 14 (800 MHz; CD_3CN)



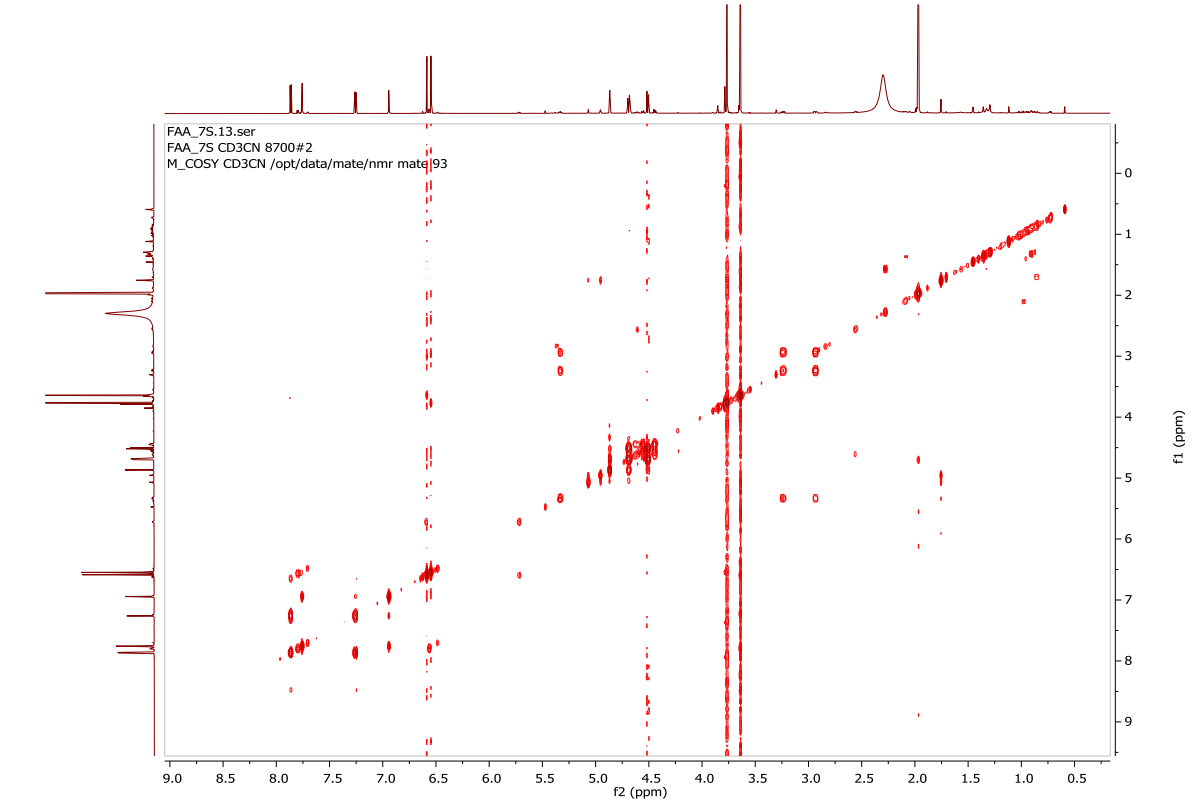


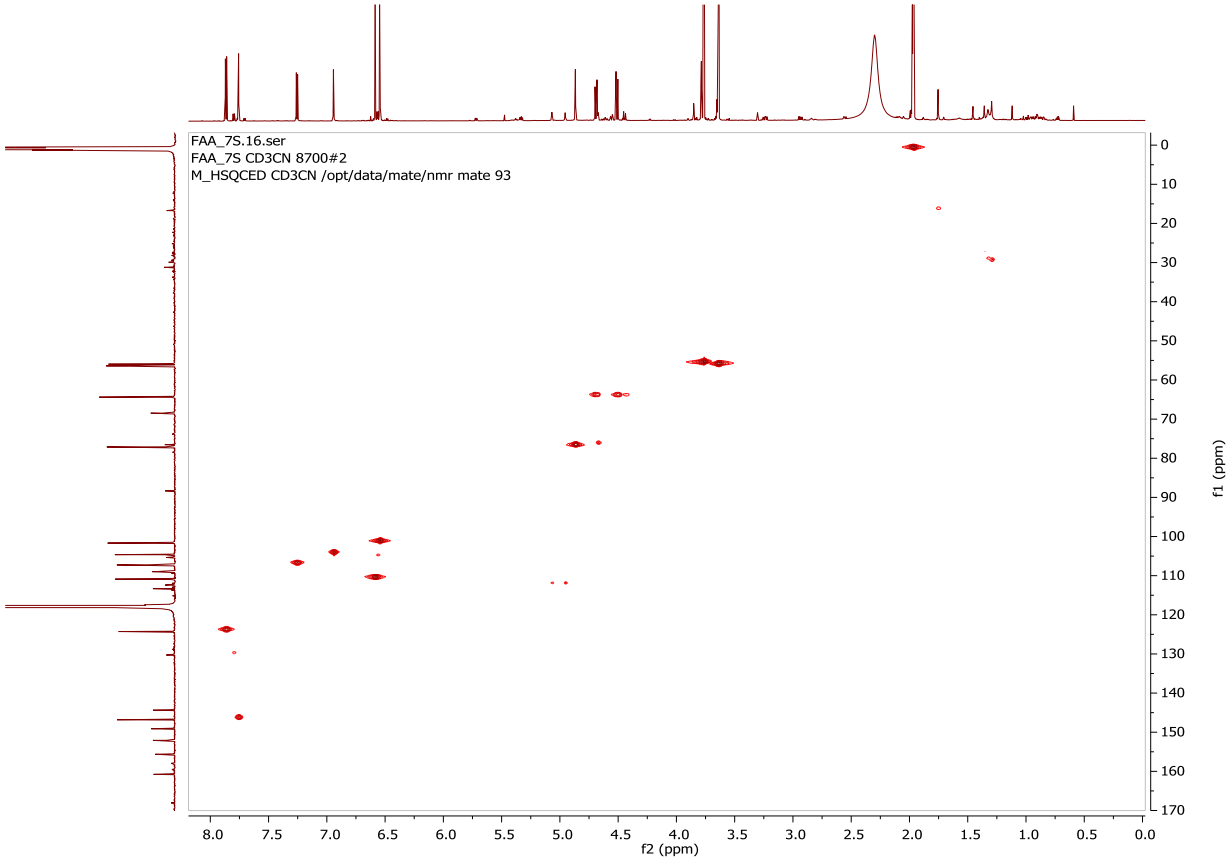
Appendix 15A: ^{1}H (800 MHz) and ^{13}C NMR (200 MHz) spectra of compound 15 (CD_3CN)



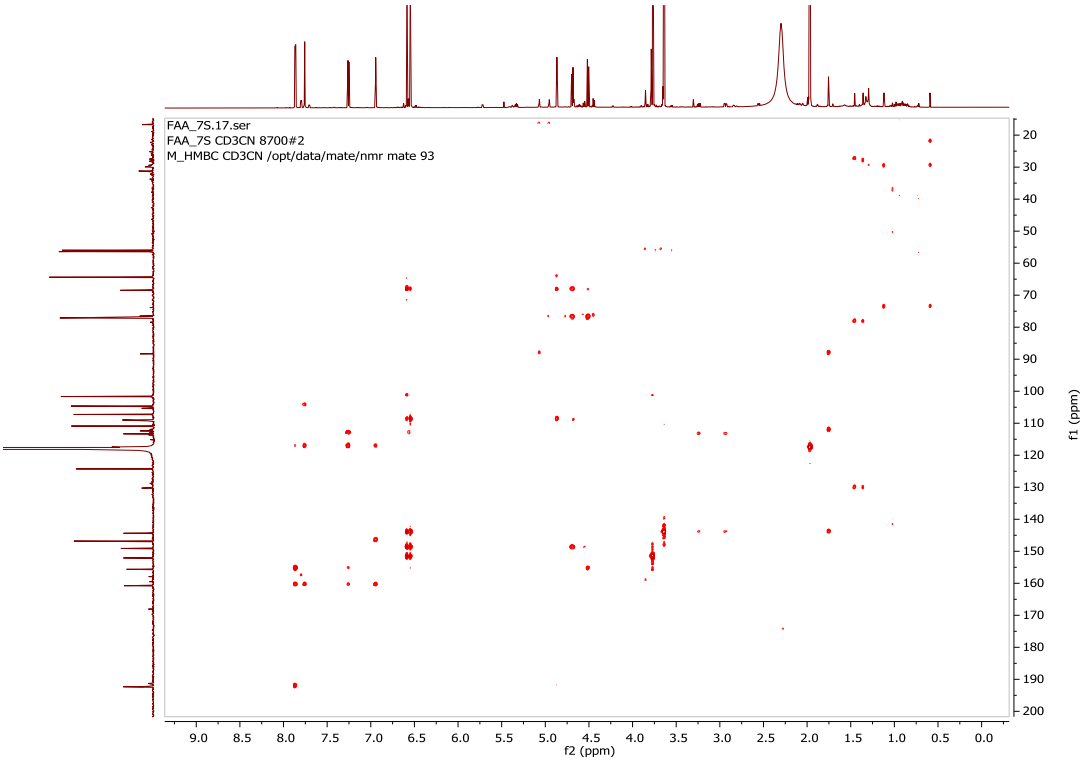


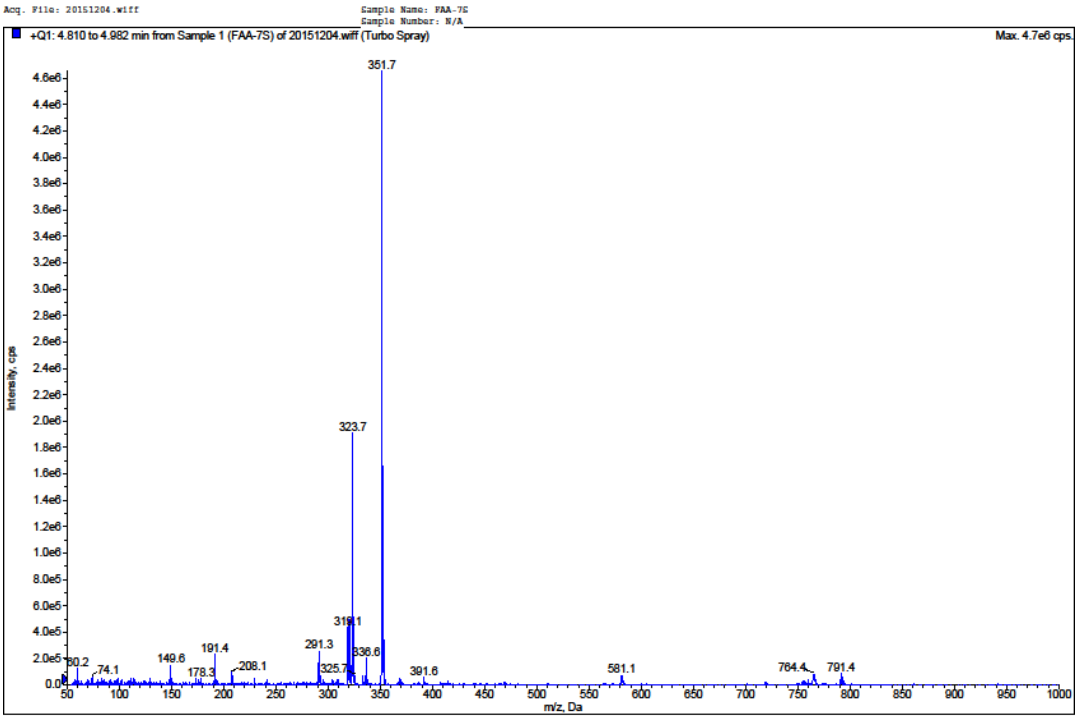
Appendix 15B: H,H COSY and HSQC spectra of compound 15 (800 MHz; CD_3CN)



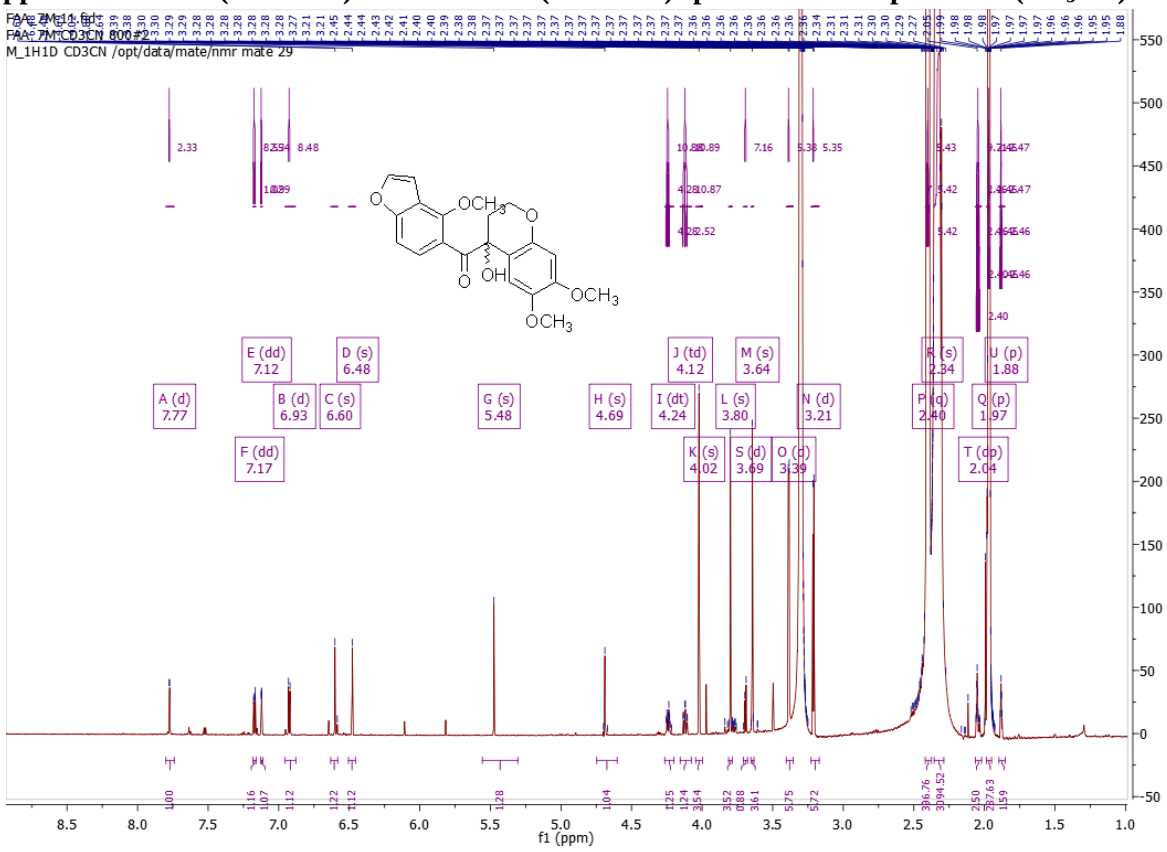


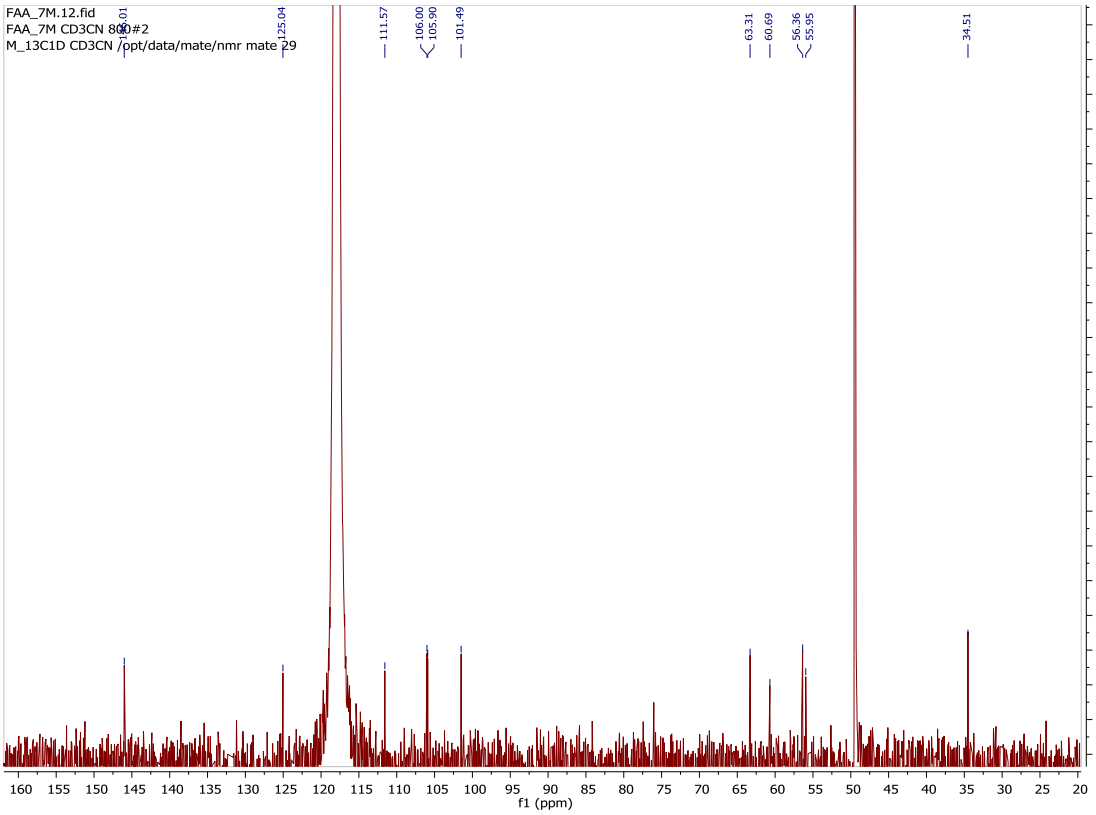
Appendix 15C: HMBC and LCMS spectra of compound 15 (800 MHz; CD_3CN)



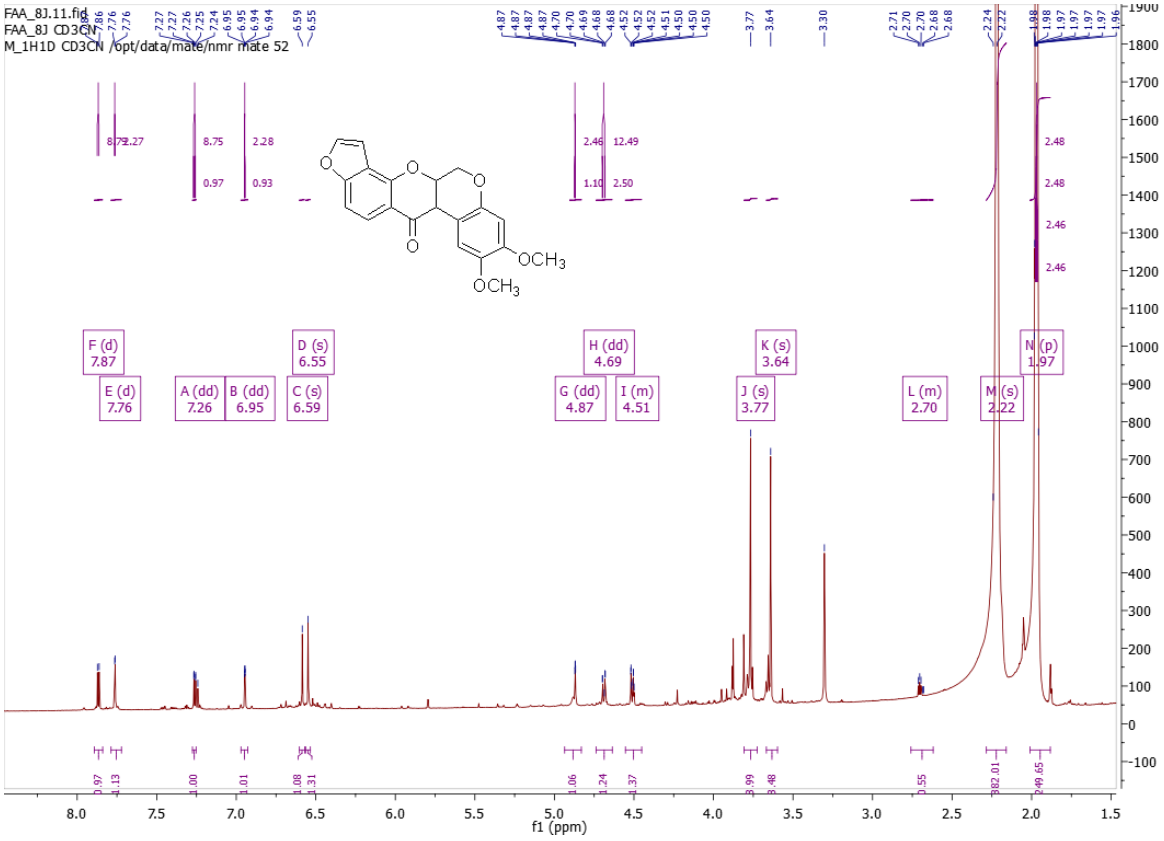


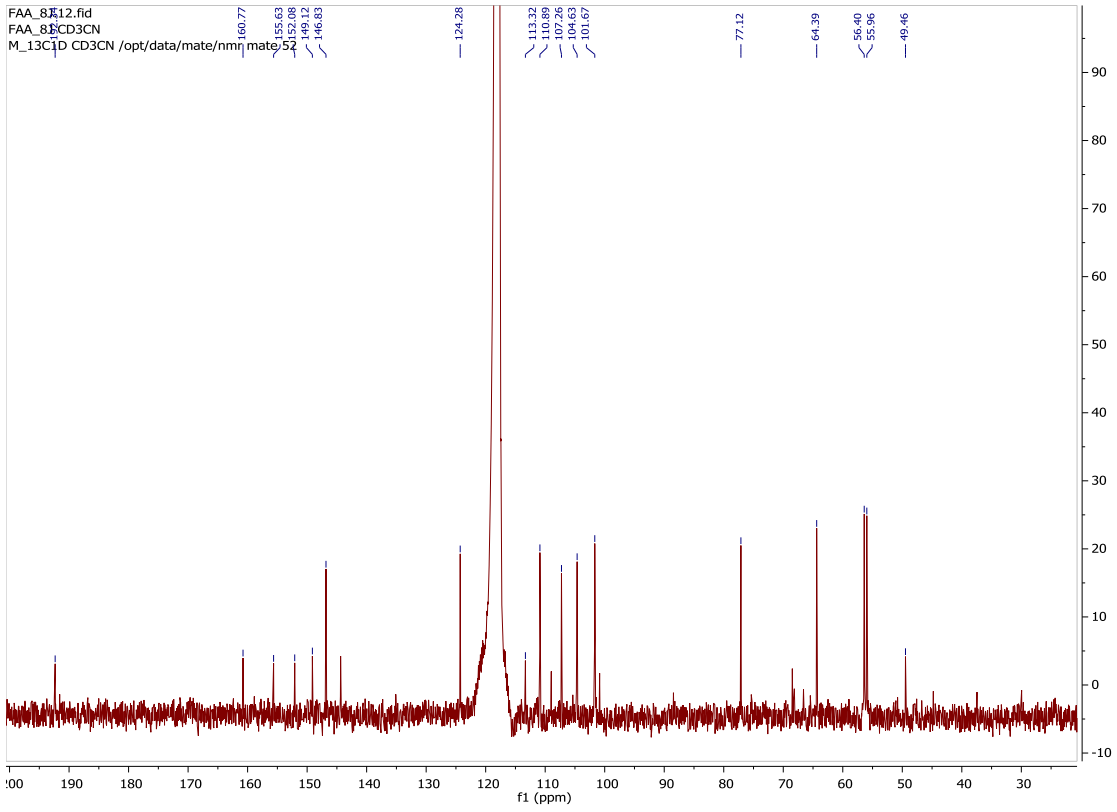
Appendix 16A: ^{1}H (800 MHz) and ^{13}C NMR (200 MHz) spectrum of compound 16 (CD_3CN)



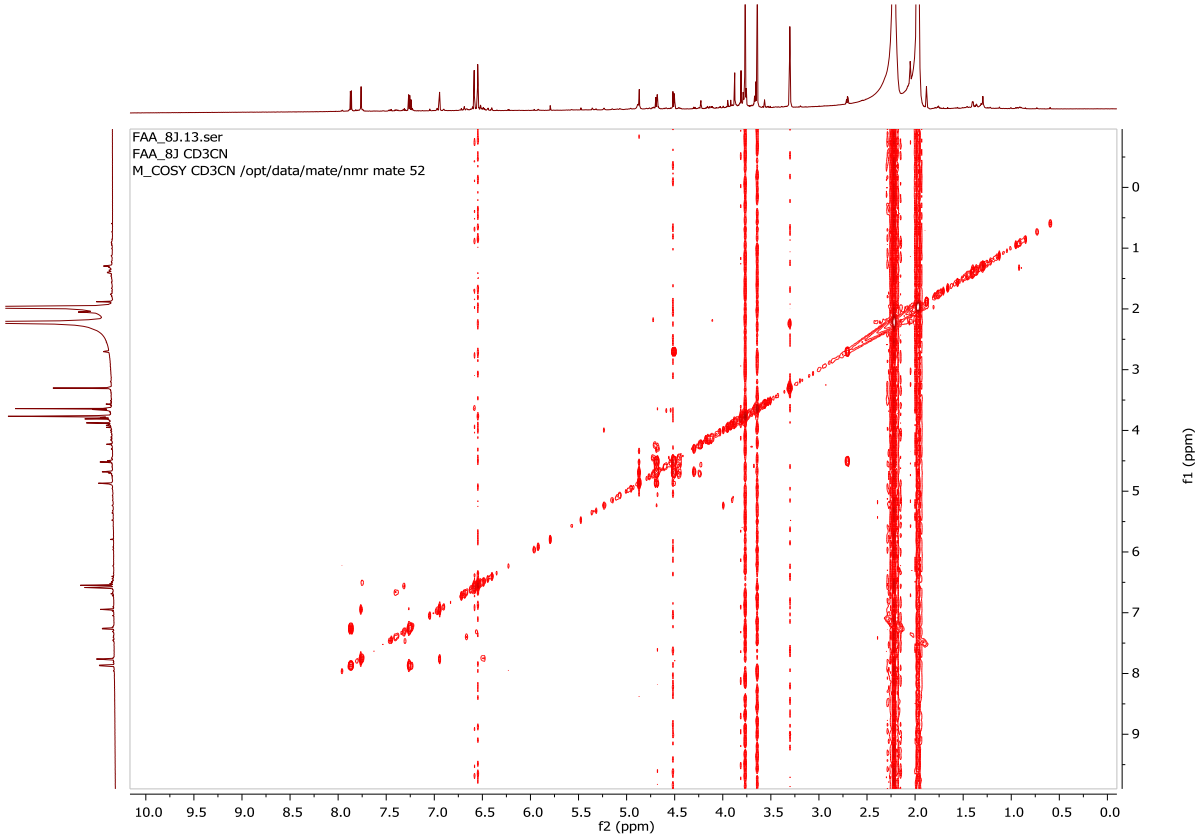


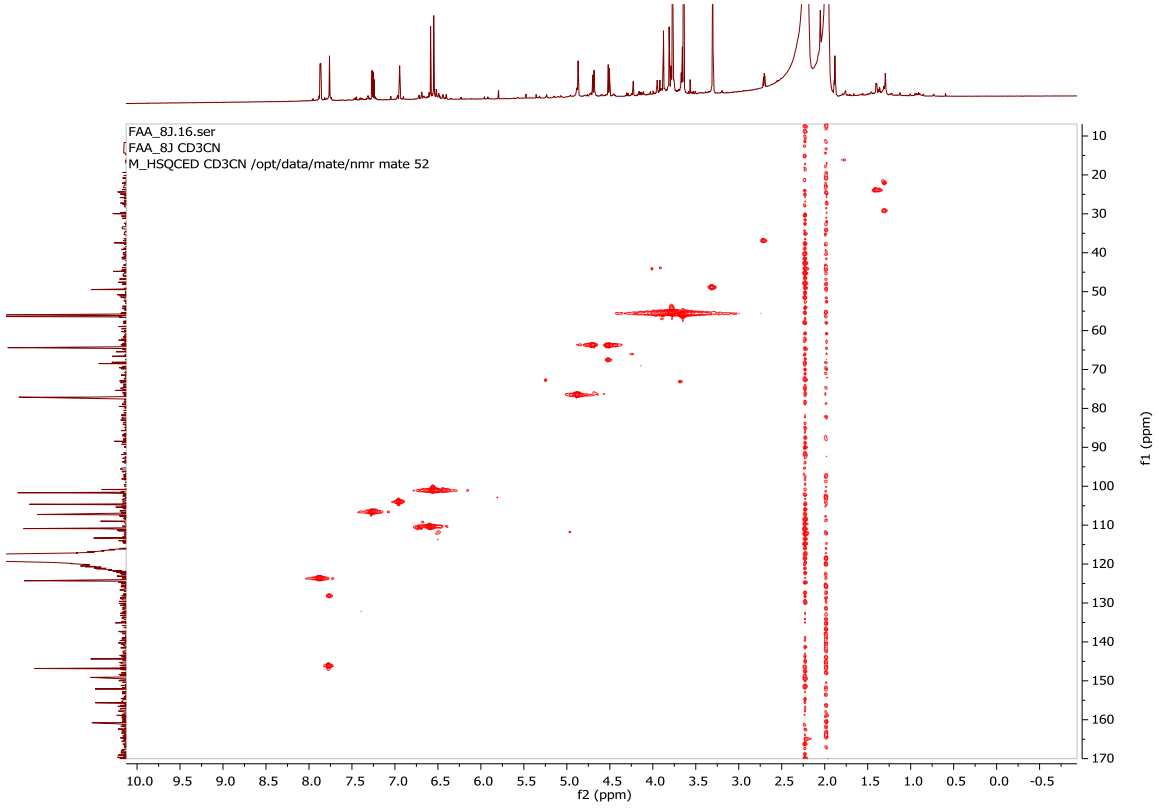
Appendix 17A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 17 (CD_3CN)



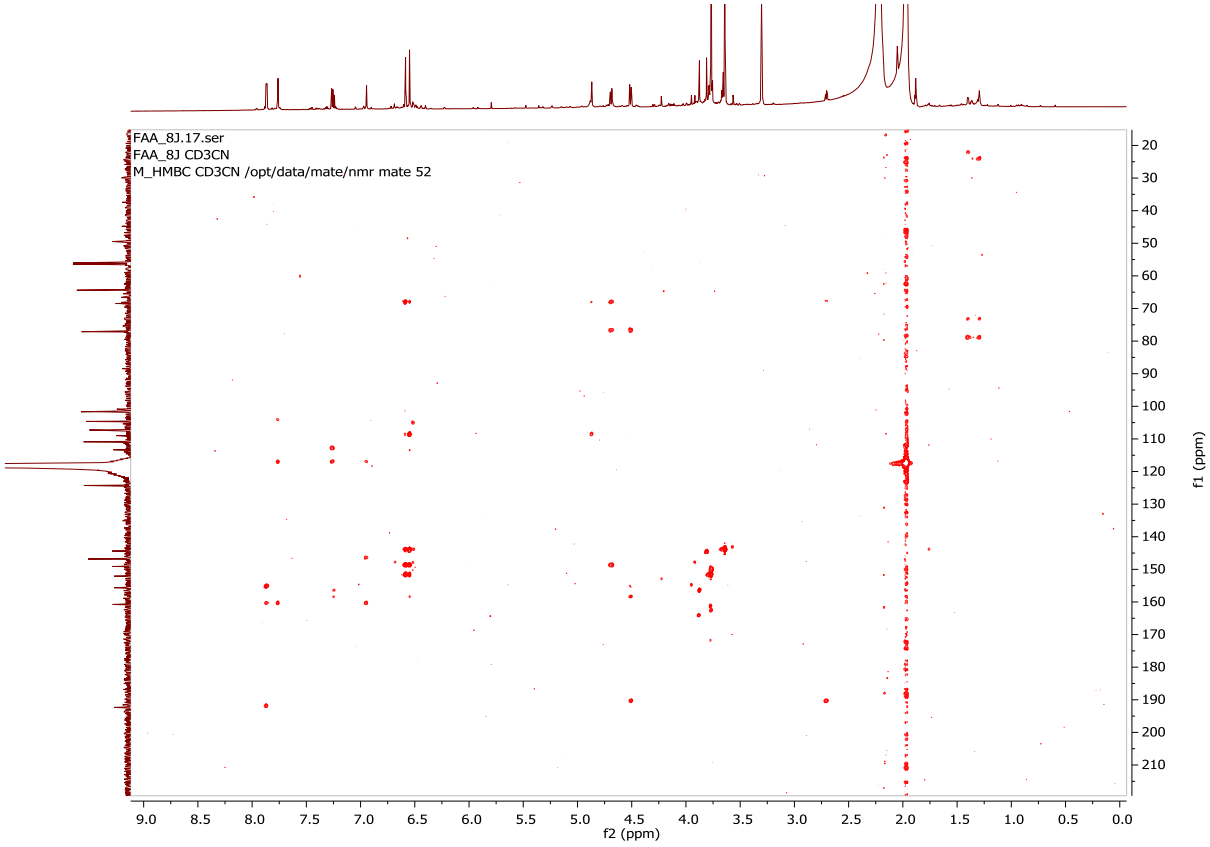


Appendix 17B: H,H COSY and HSQC spectra of compound 17 (800 MHz; CD_3CN)

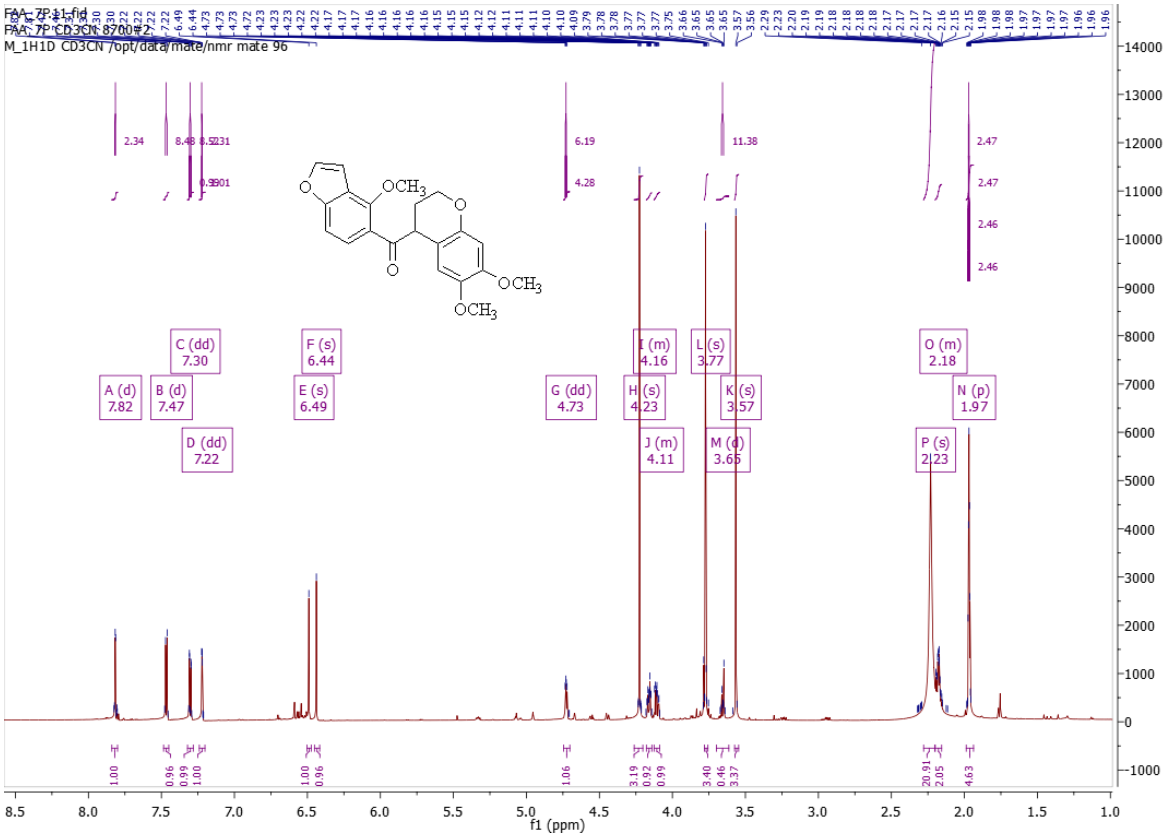


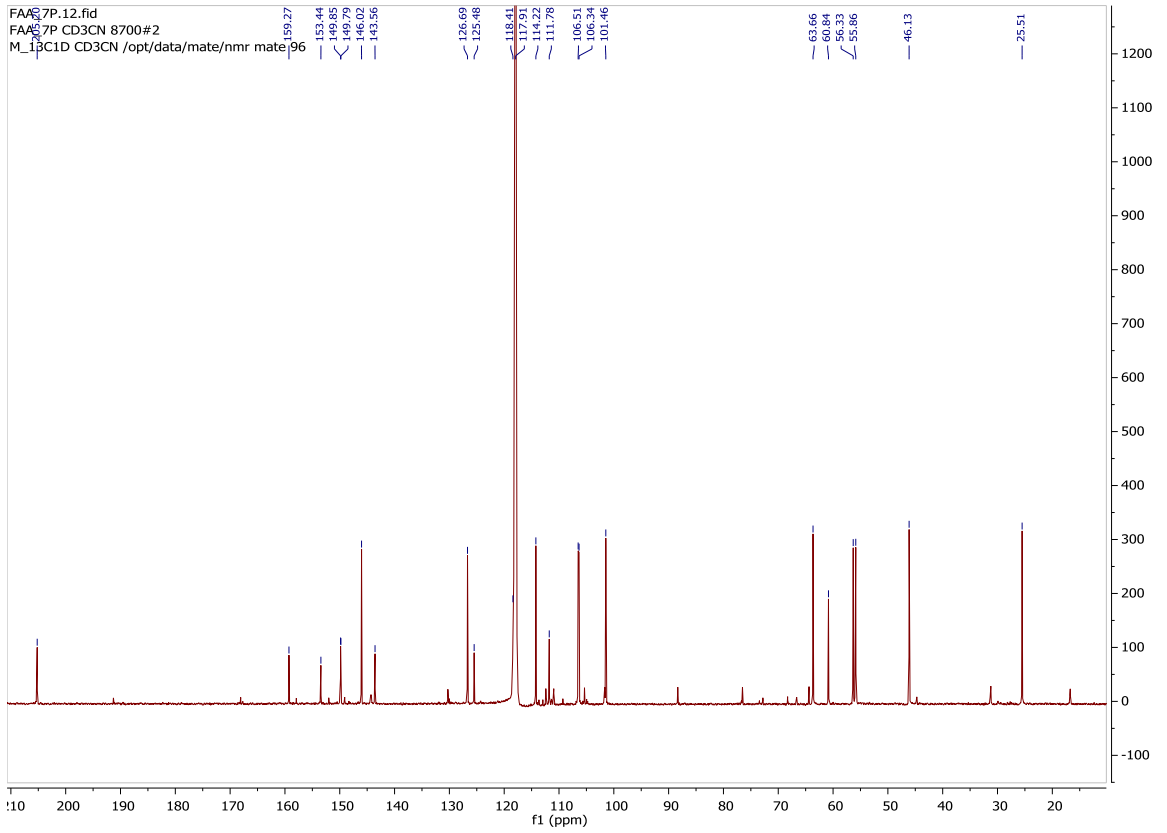


Appendix 17C: HMBC spectrum of compound 17 (800 MHz; CD_3CN)

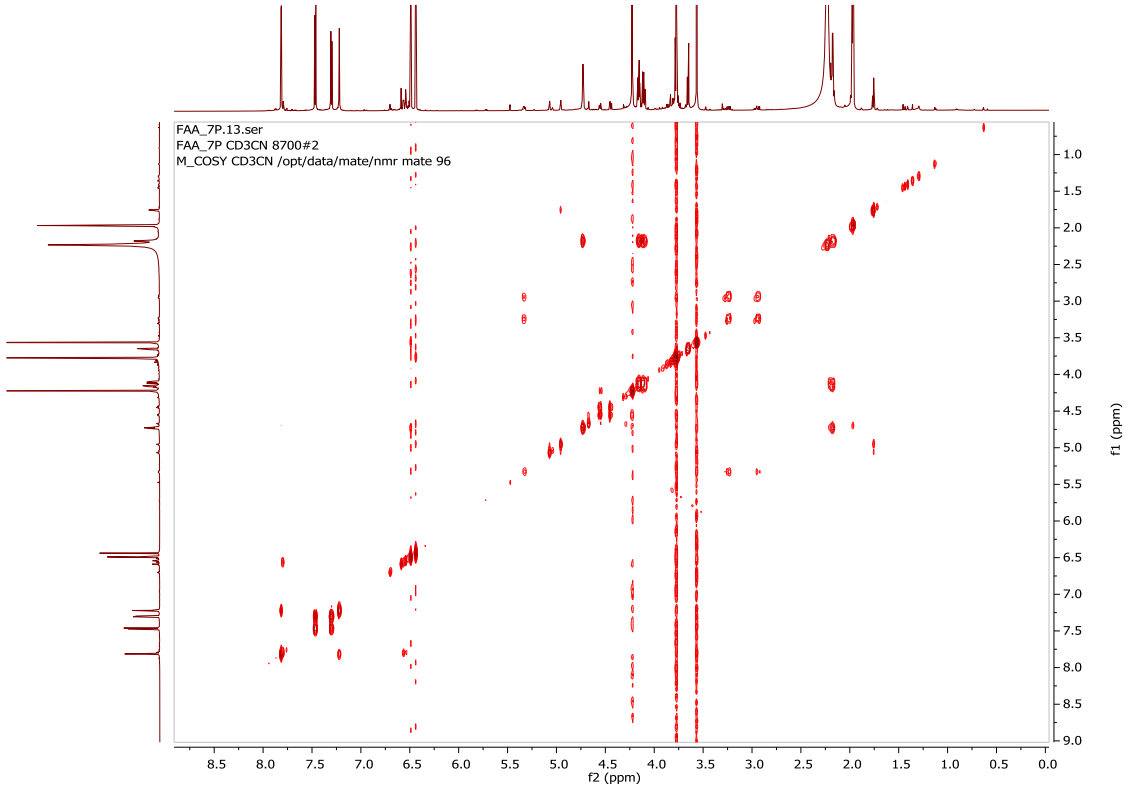


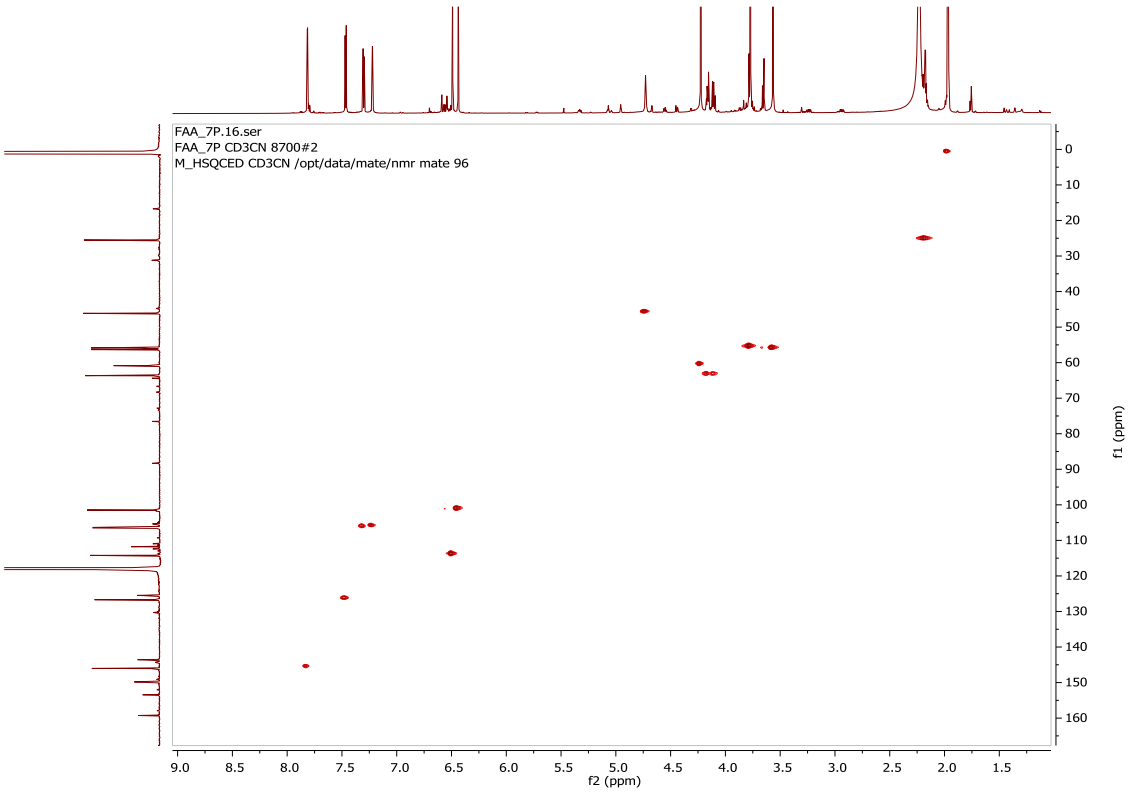
Appendix 18A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 18 (CD_3CN)



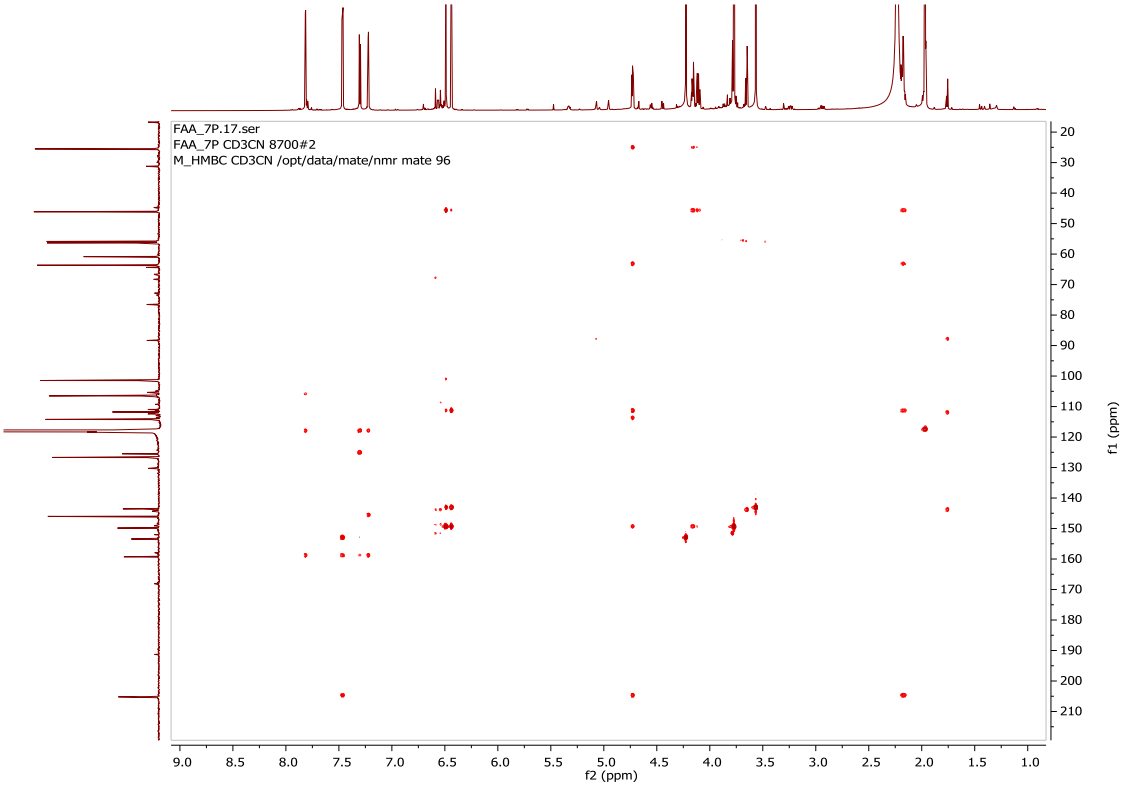


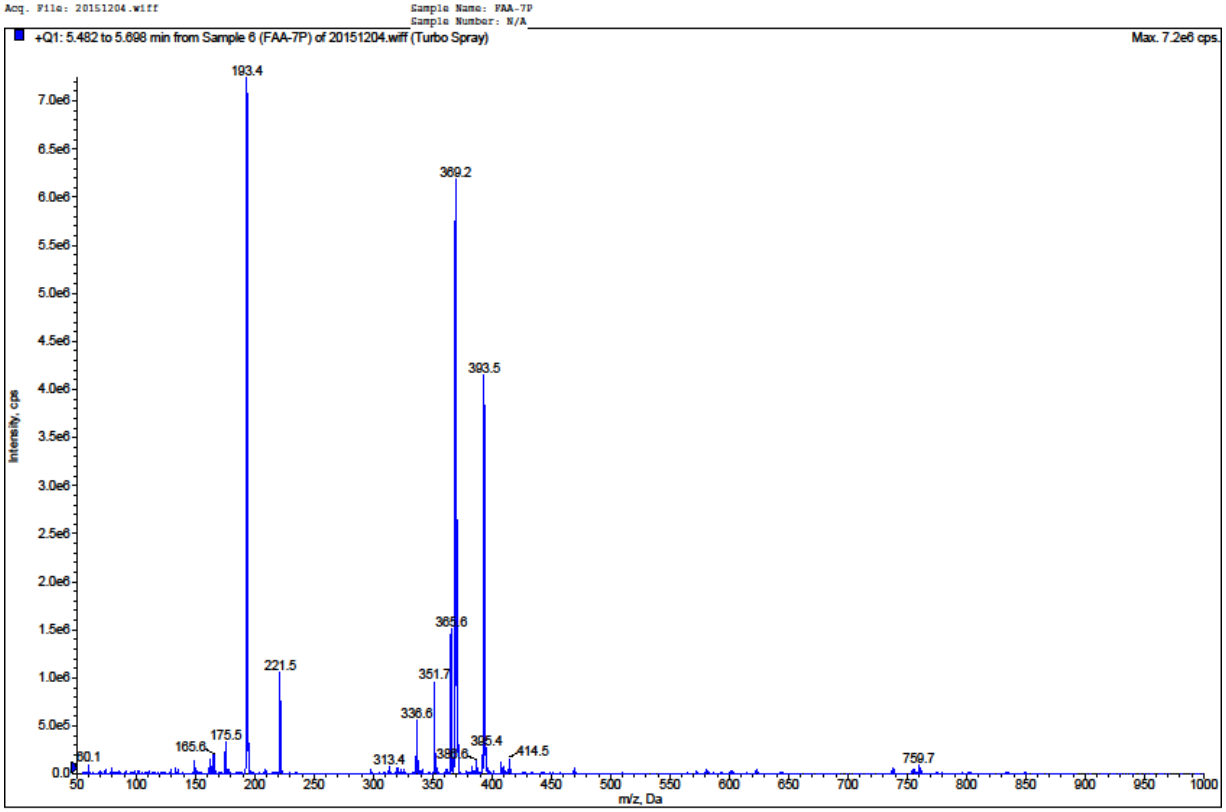
Appendix 18B: H,H COSY and HSQC spectra of compound 18 (800 MHz; CD_3CN)



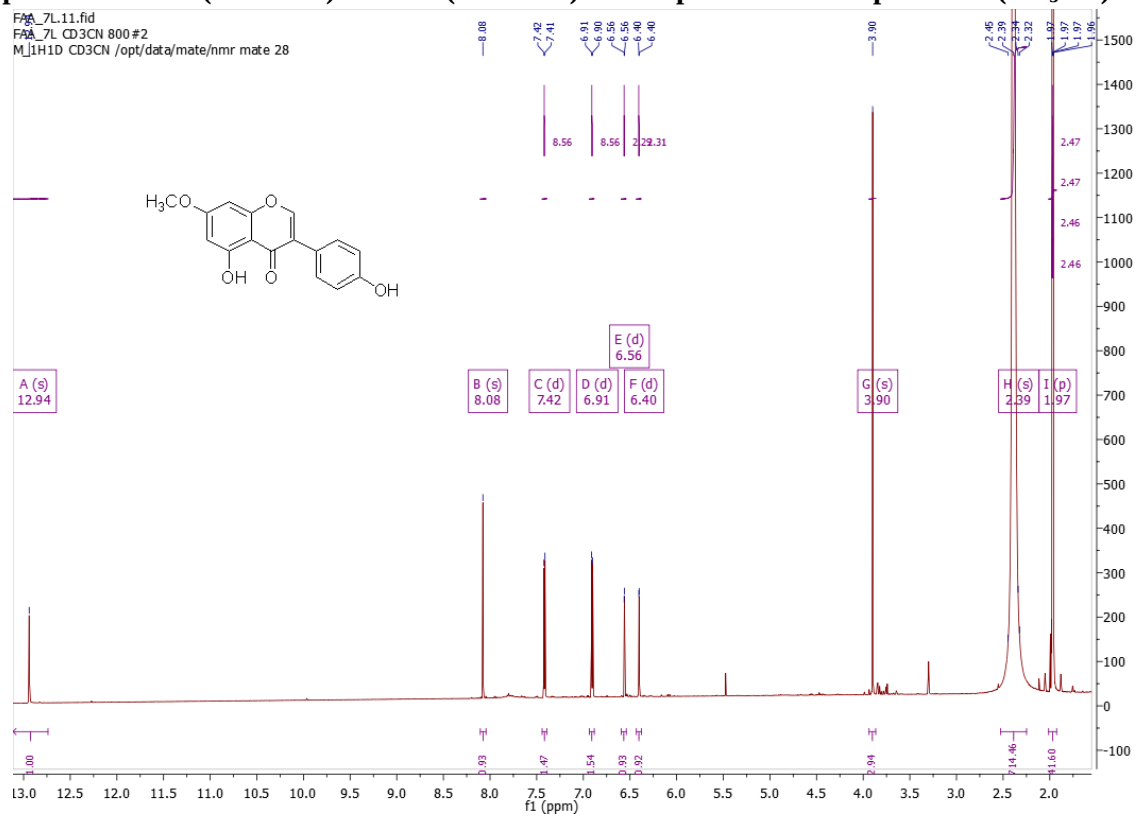


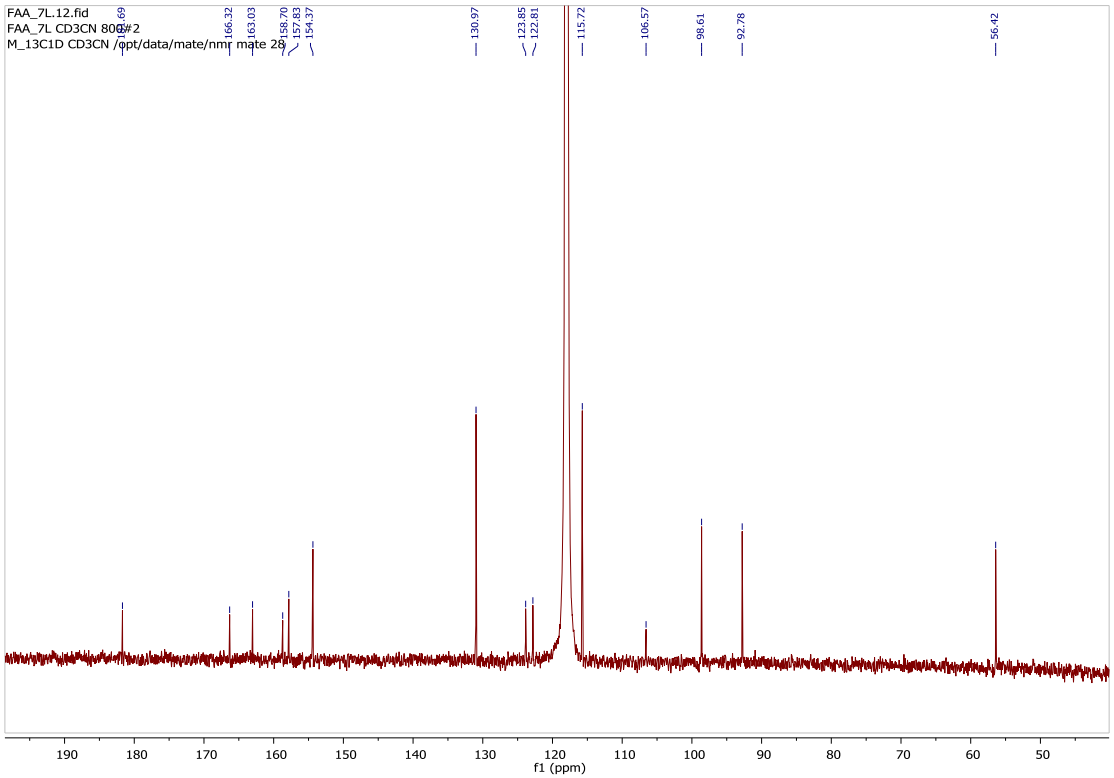
Appendix 18C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 18



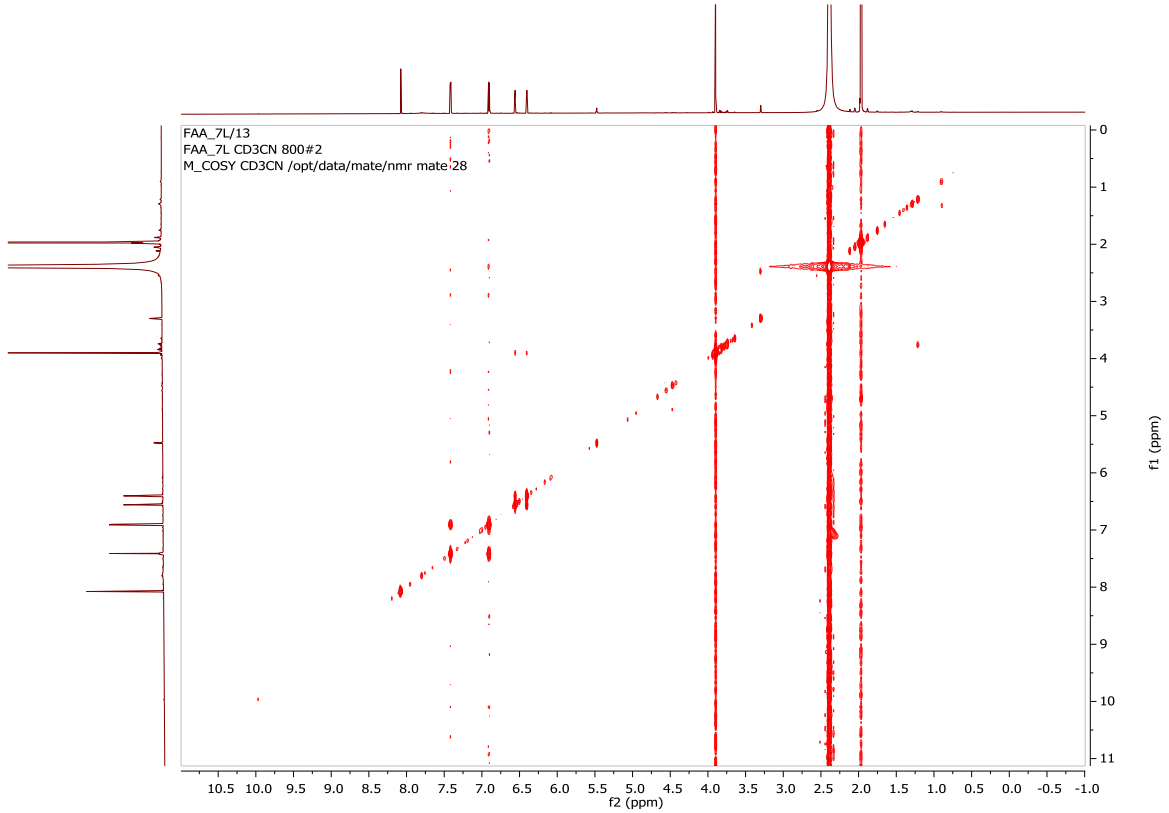


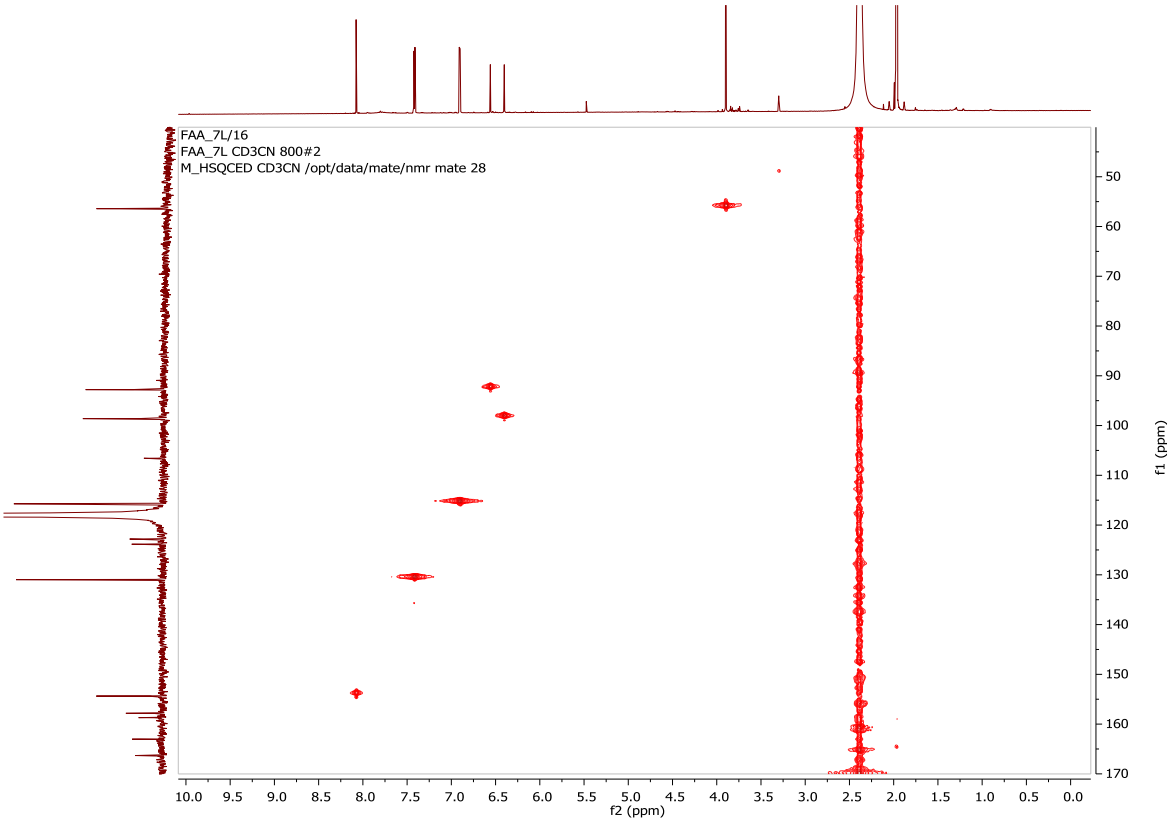
Appendix 19A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectrum of compound 19 (CD_3CN)



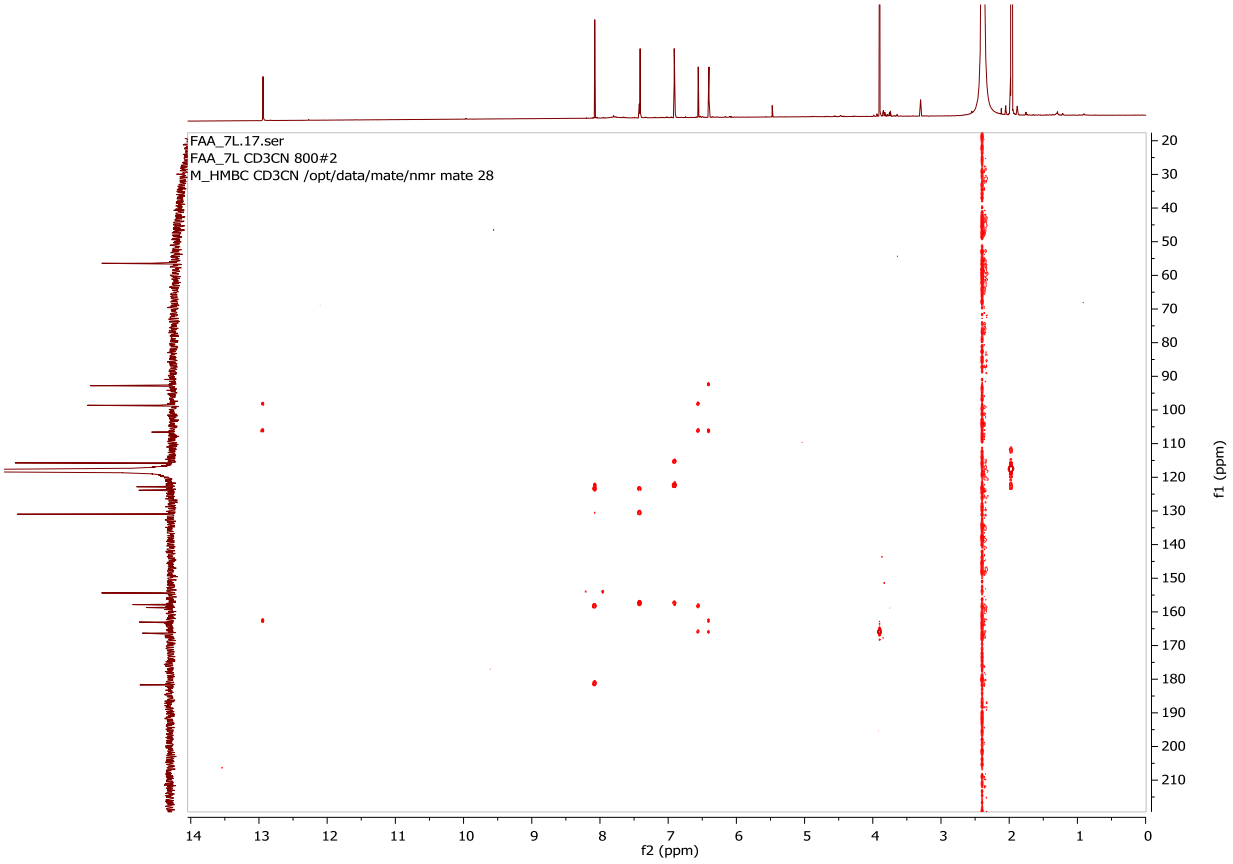


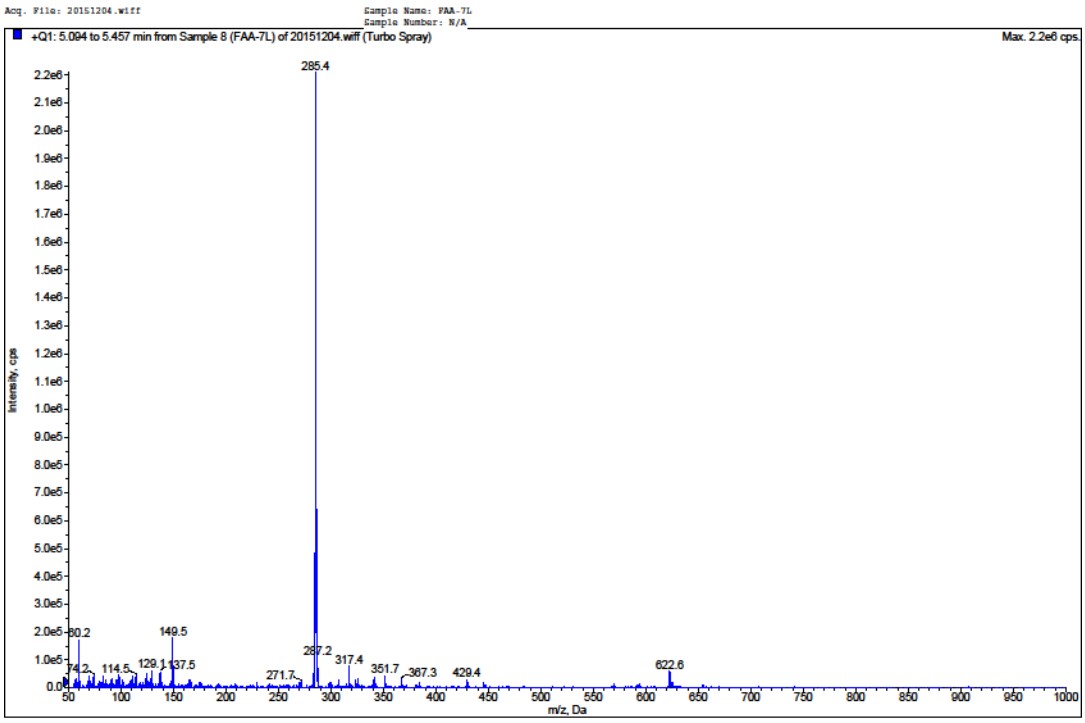
Appendix 19B: H,H COSY and HSQC spectra of compound 19 (800 MHz; CD_3CN)



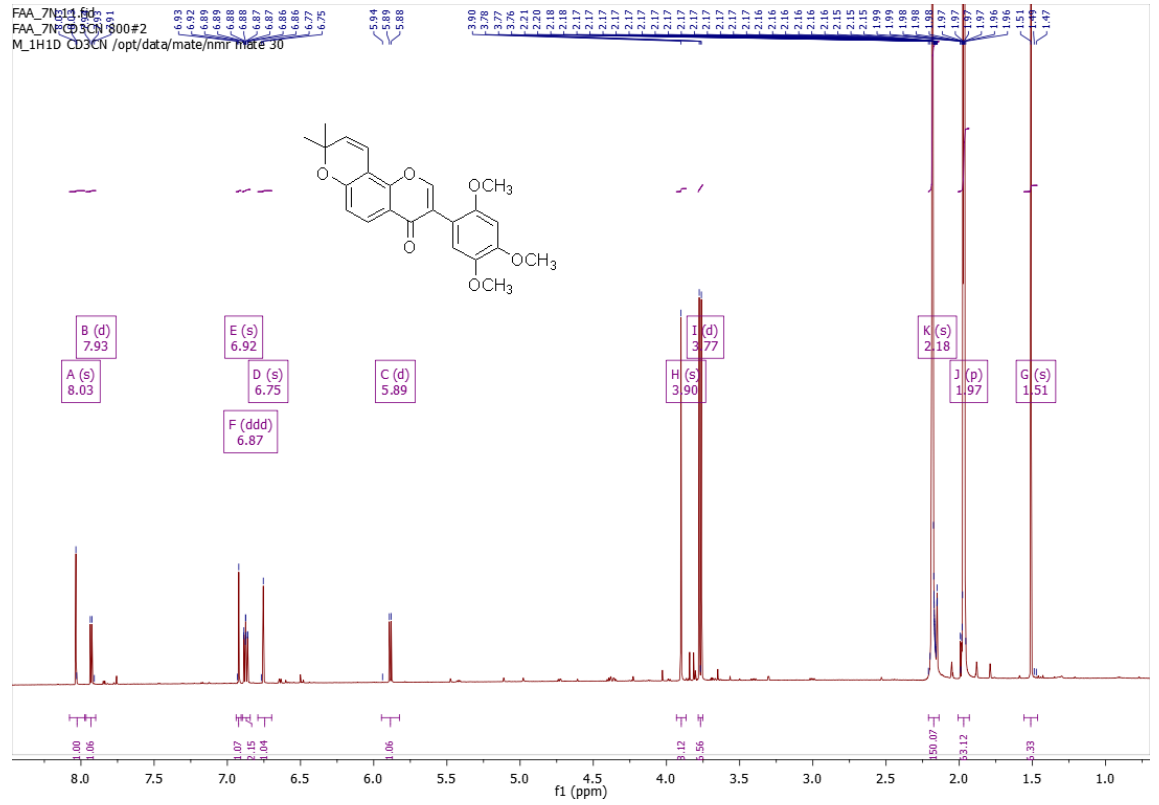


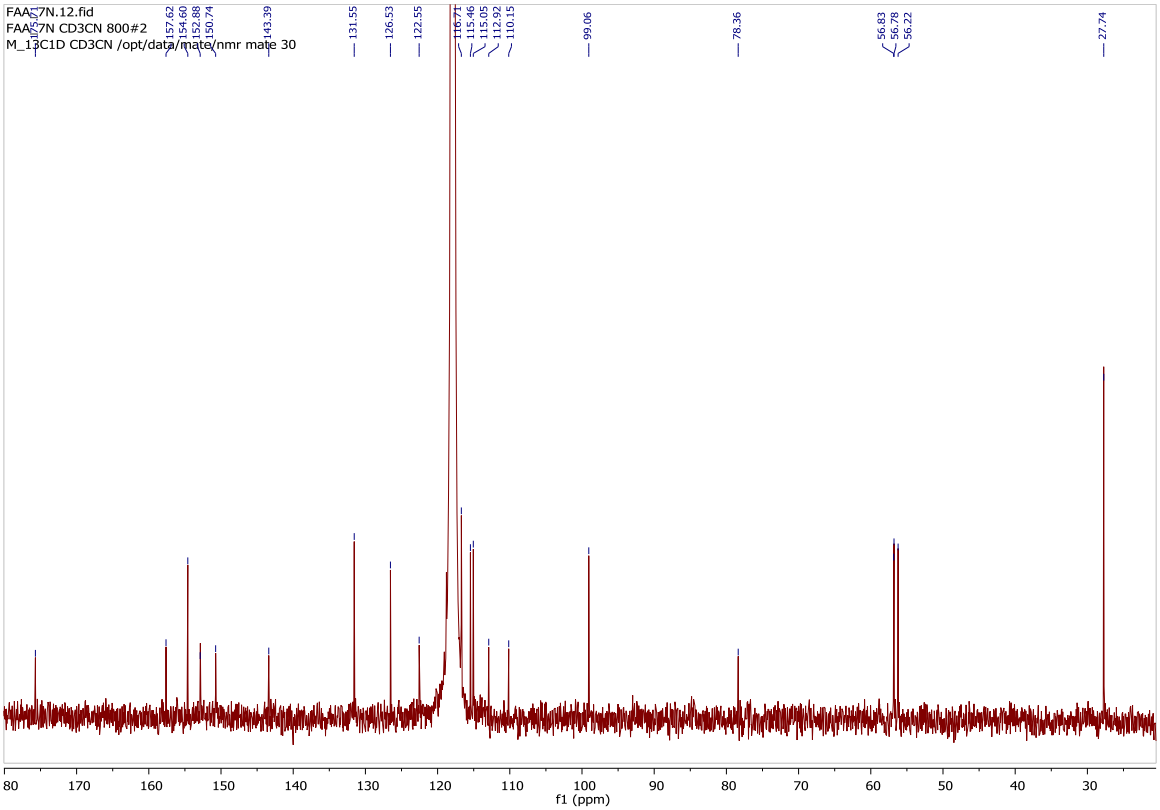
Appendix 19C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 19



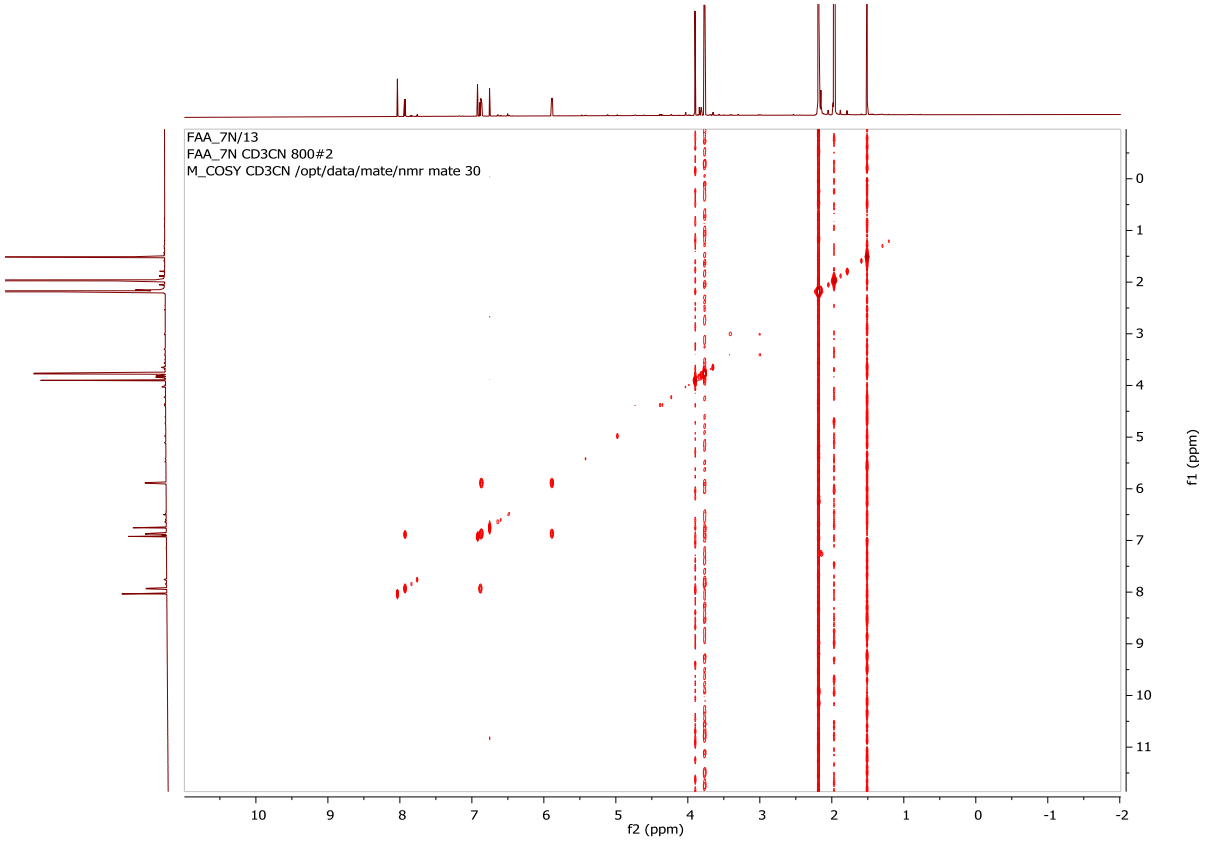


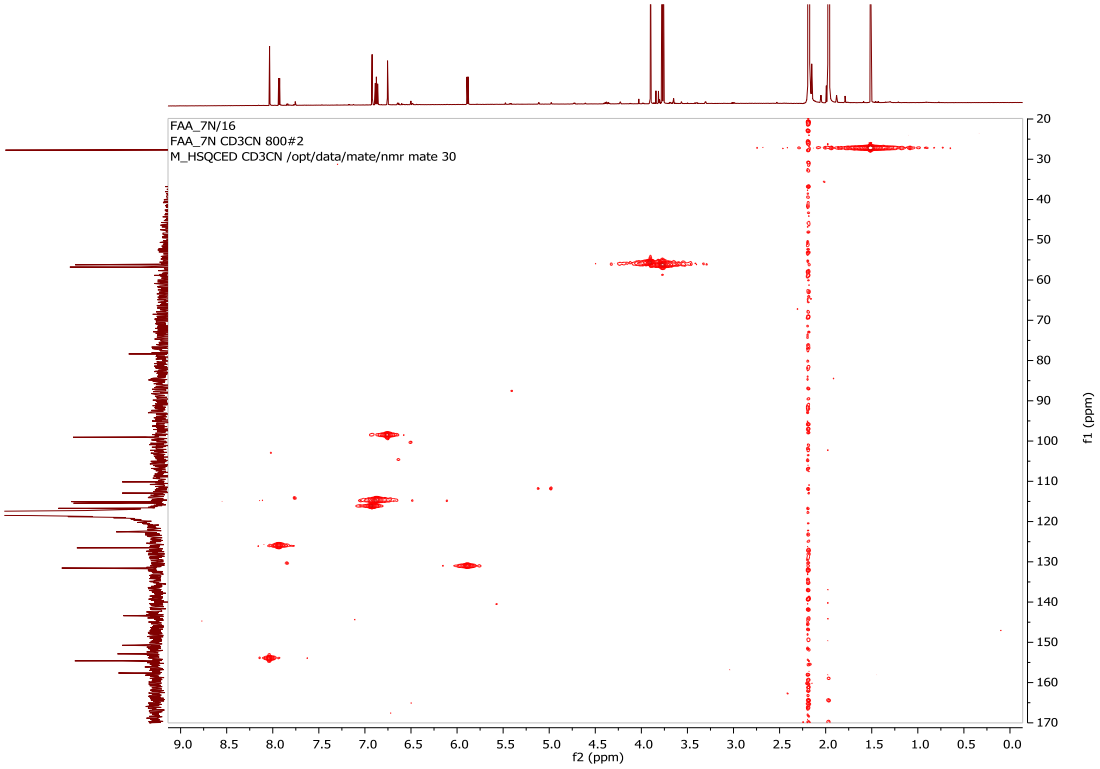
Appendix 20A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 20 (CD_3CN)



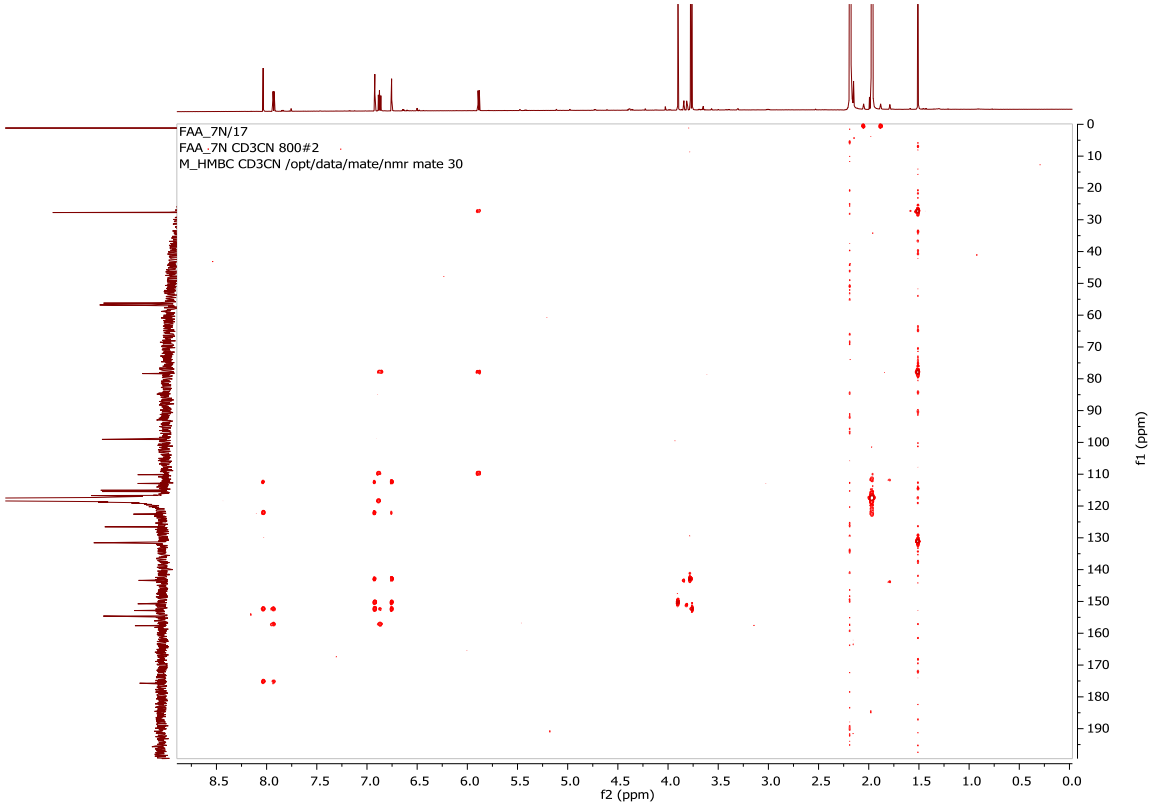


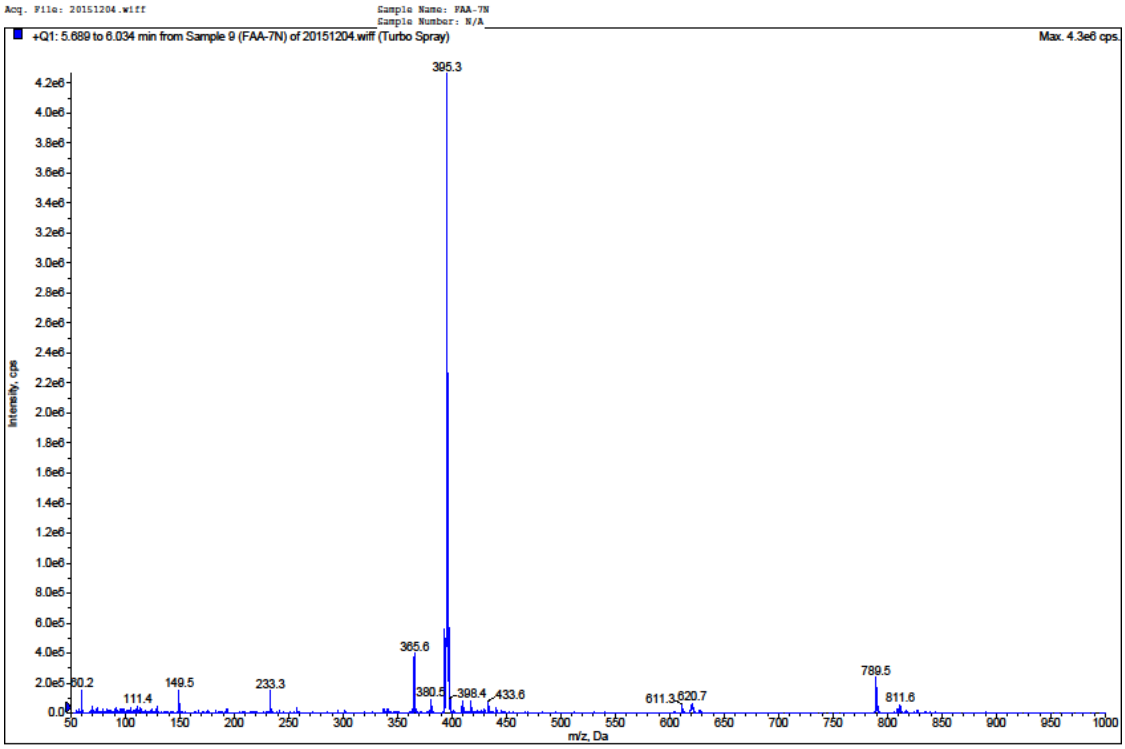
Appendix 20B: H,H COSY and HSQC spectra of compound 20 (800 MHz; CD_3CN)



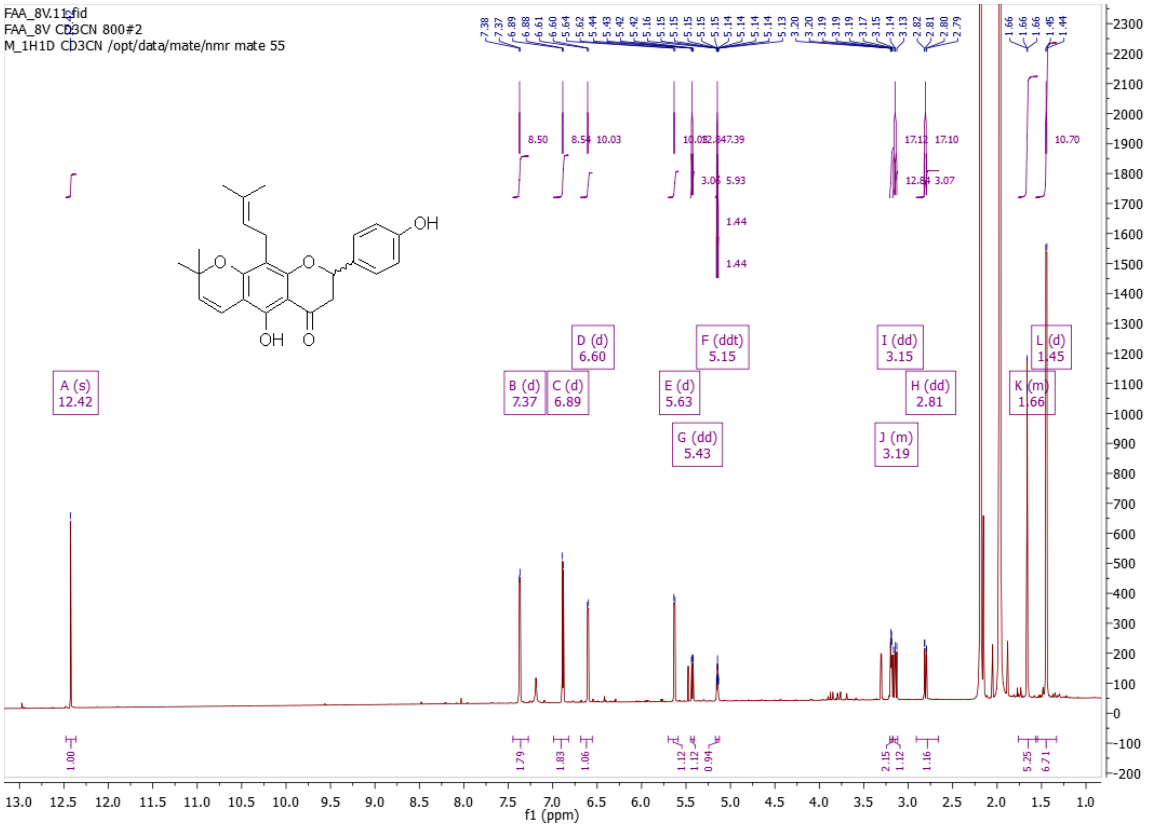


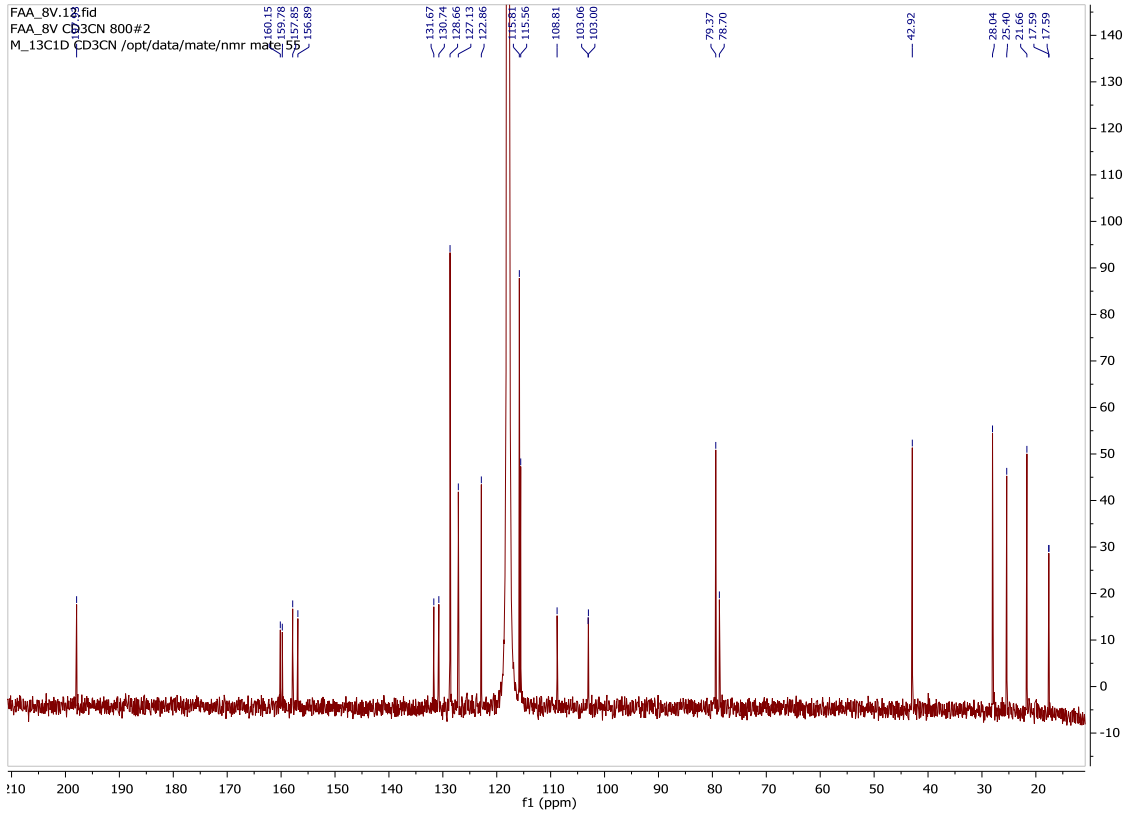
Appendix 20C: HMBC and LCMS spectra of compound 20 (800 MHz; CD_3CN)



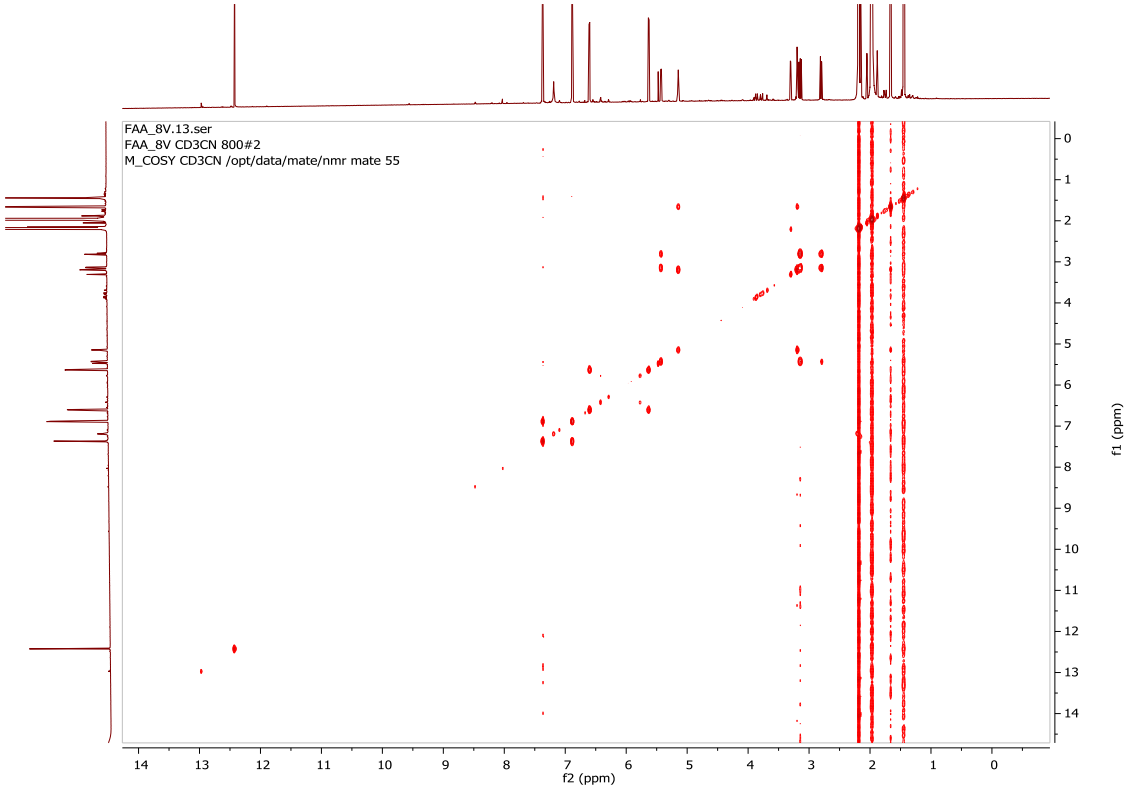


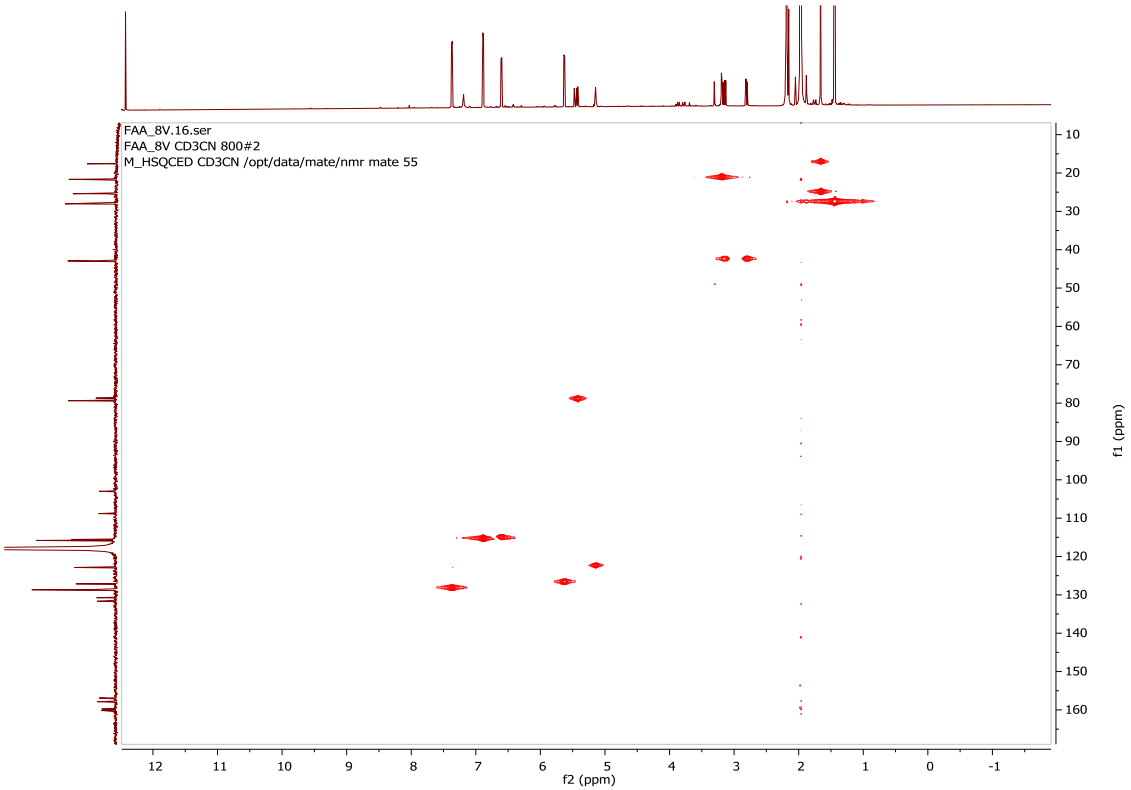
Appendix 21A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 21 (CD_3CN)



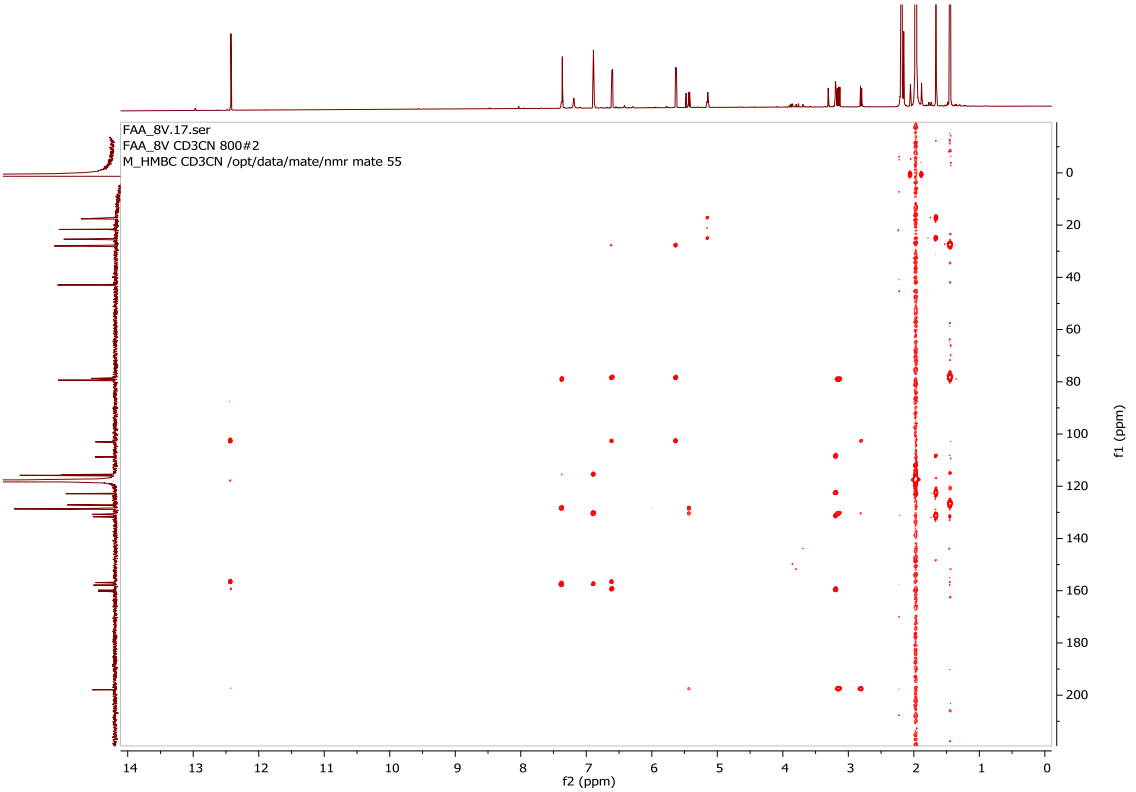


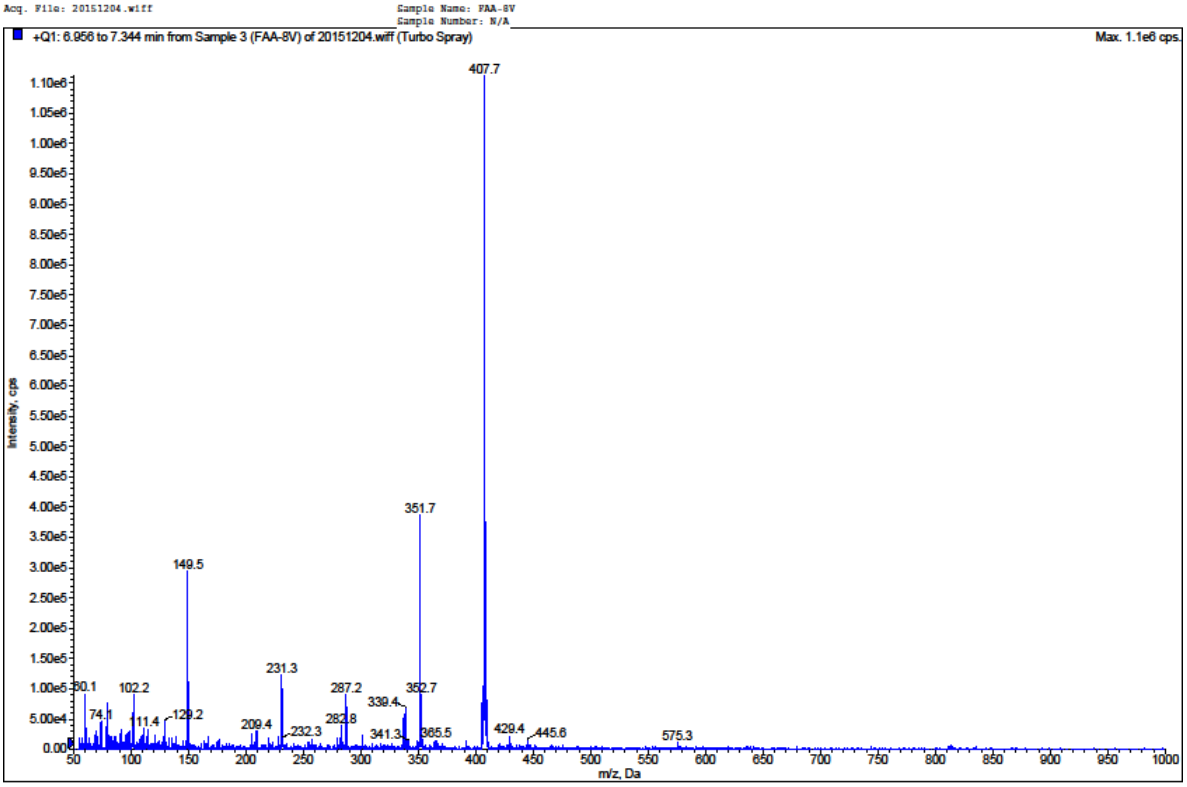
Appendix 21B: H,H COSY and HSQC spectra of compound 21 (800 MHz; CD_3CN)



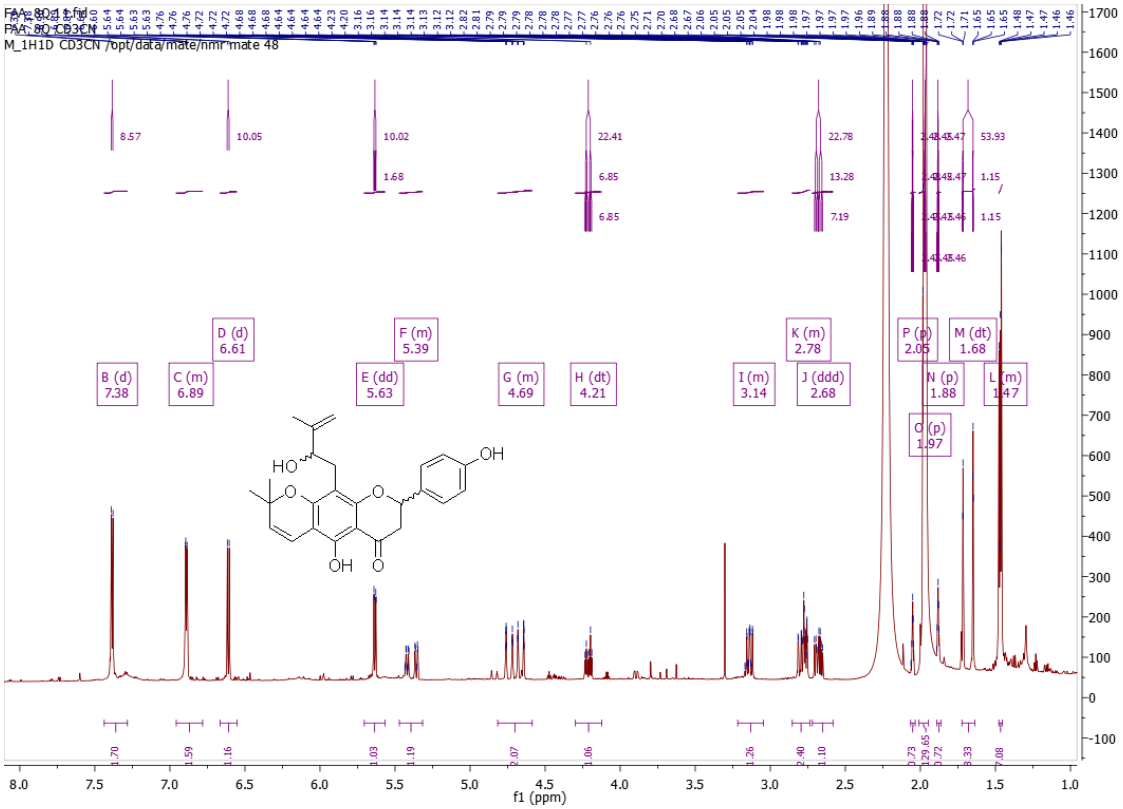


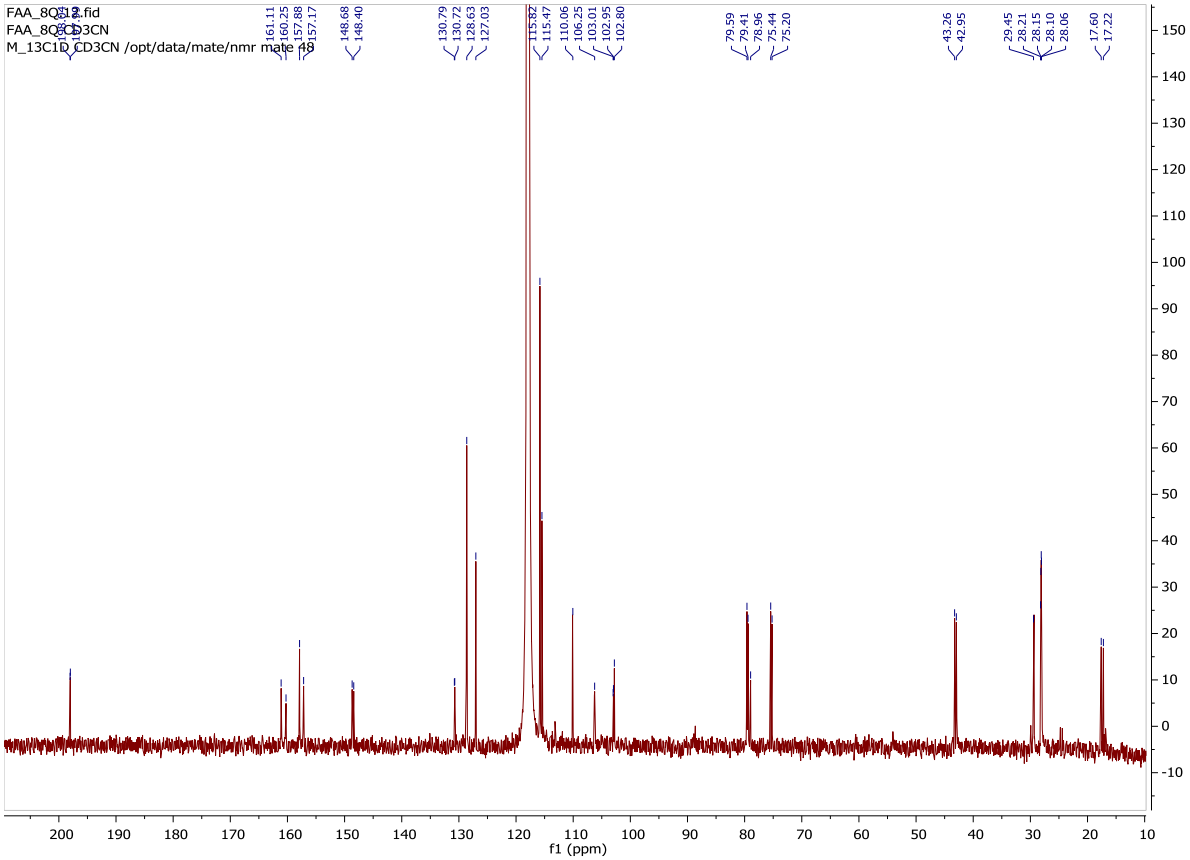
Appendix 21C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 21



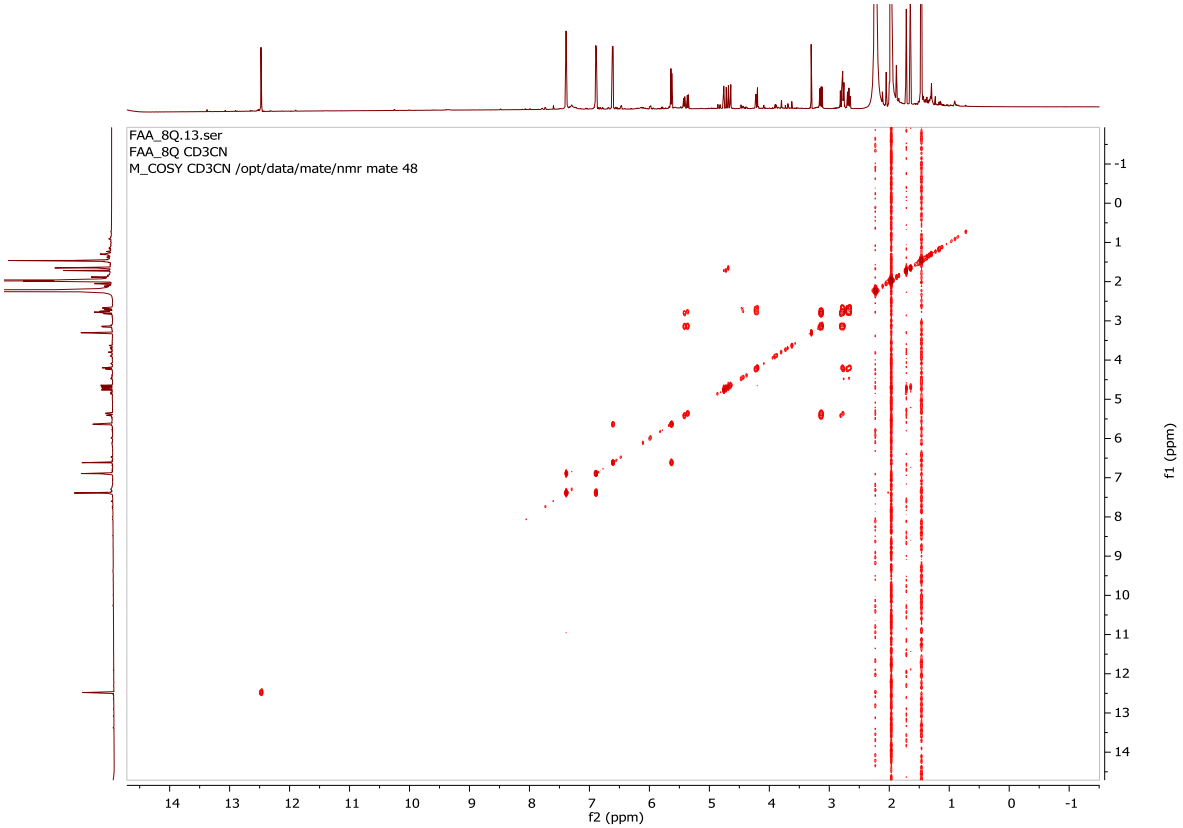


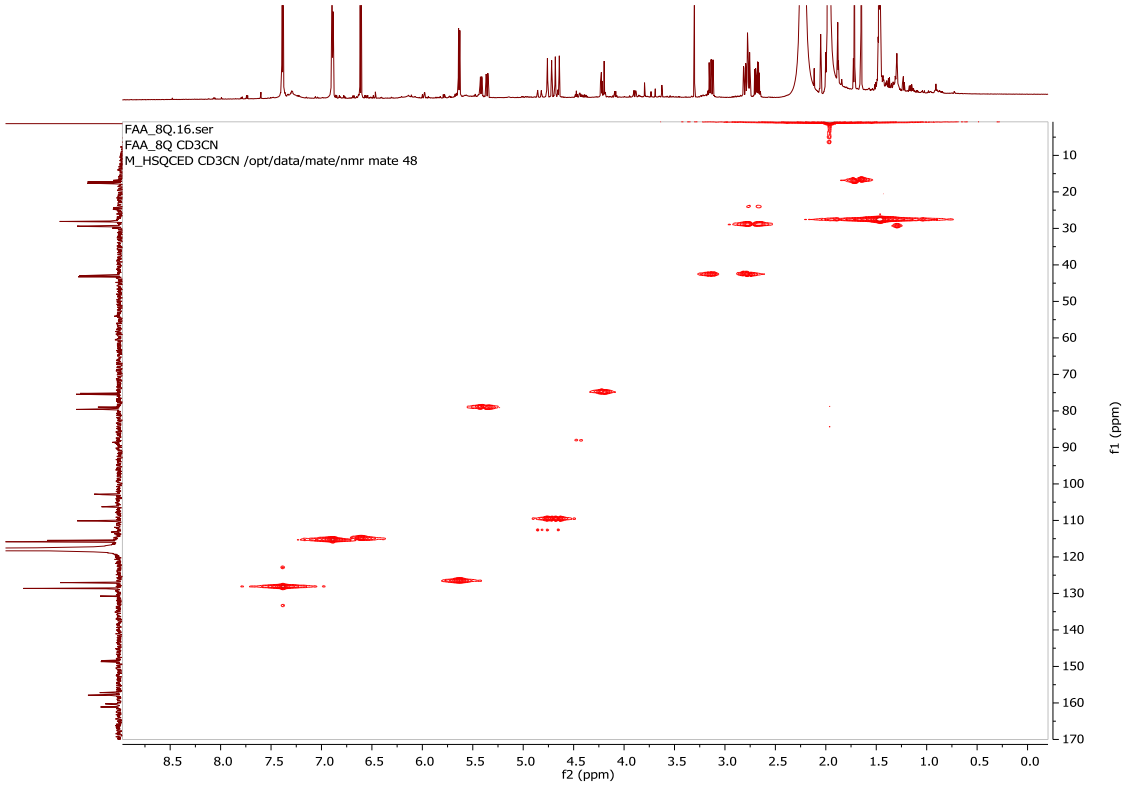
Appendix 22A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 22 (CD_3CN)



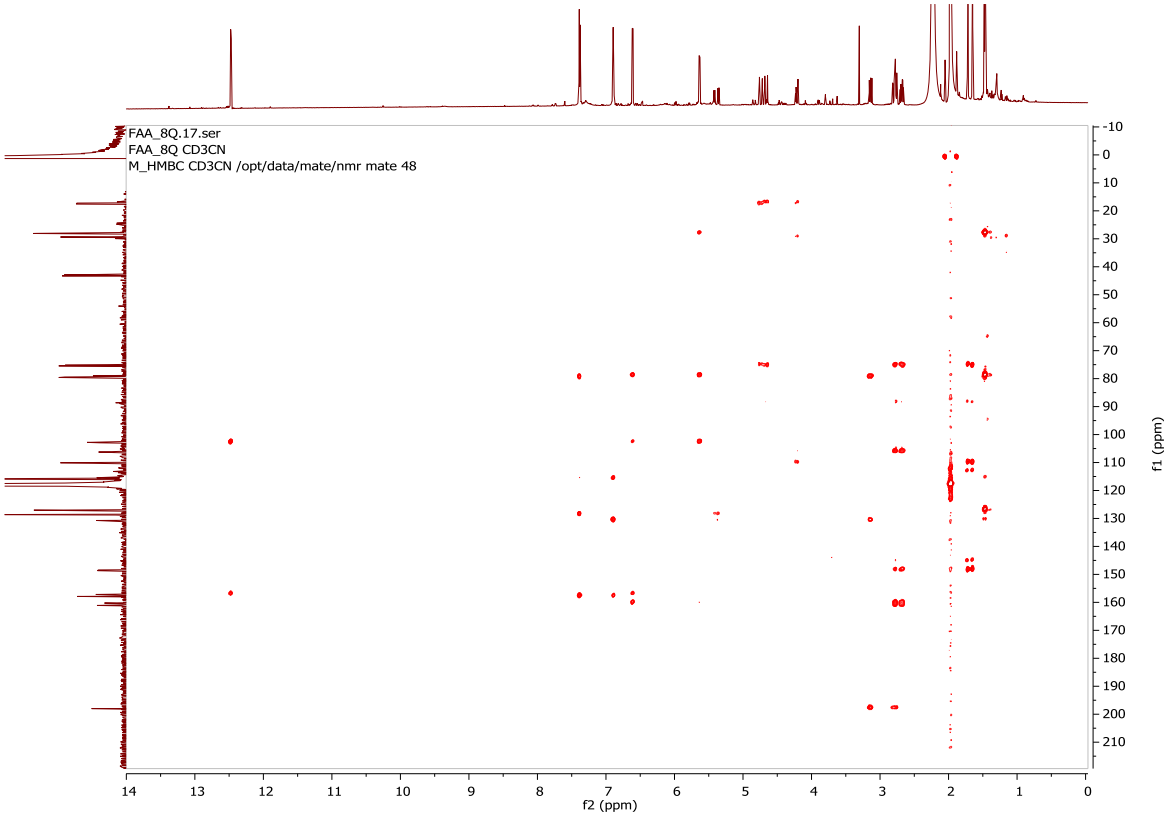


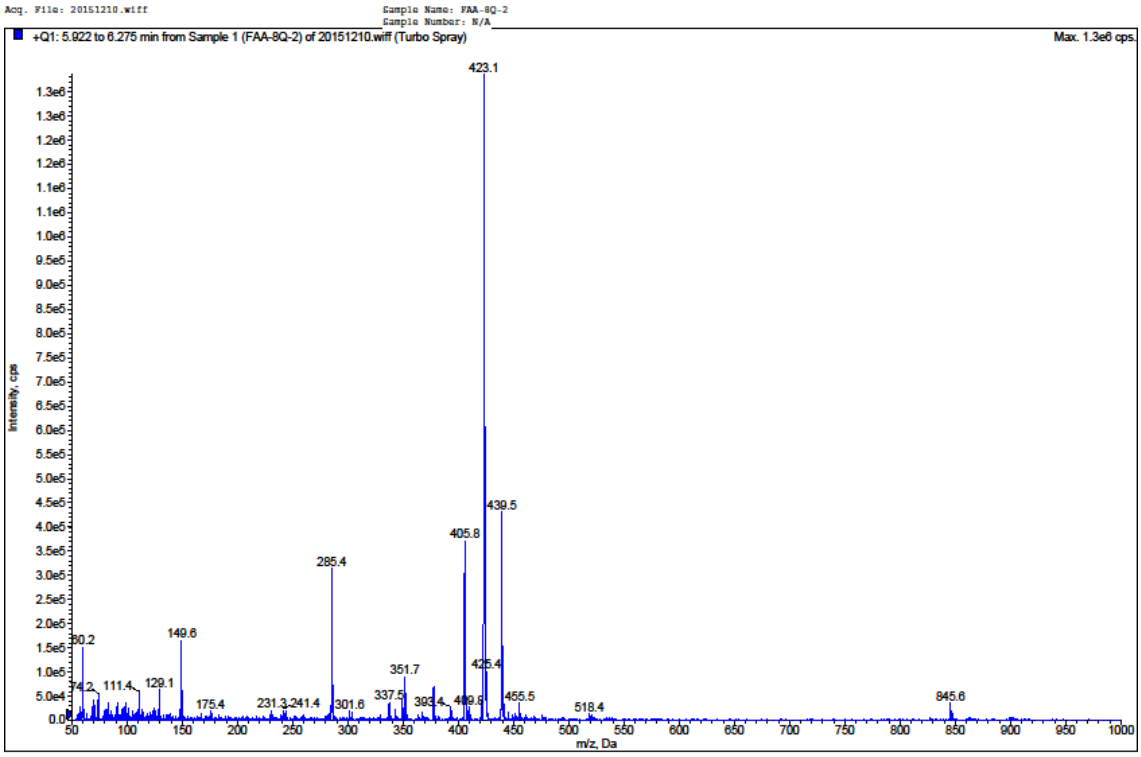
Appendix 22B: H,H COSY and HSQC spectra of compound 22 (800 MHz; CD_3CN)



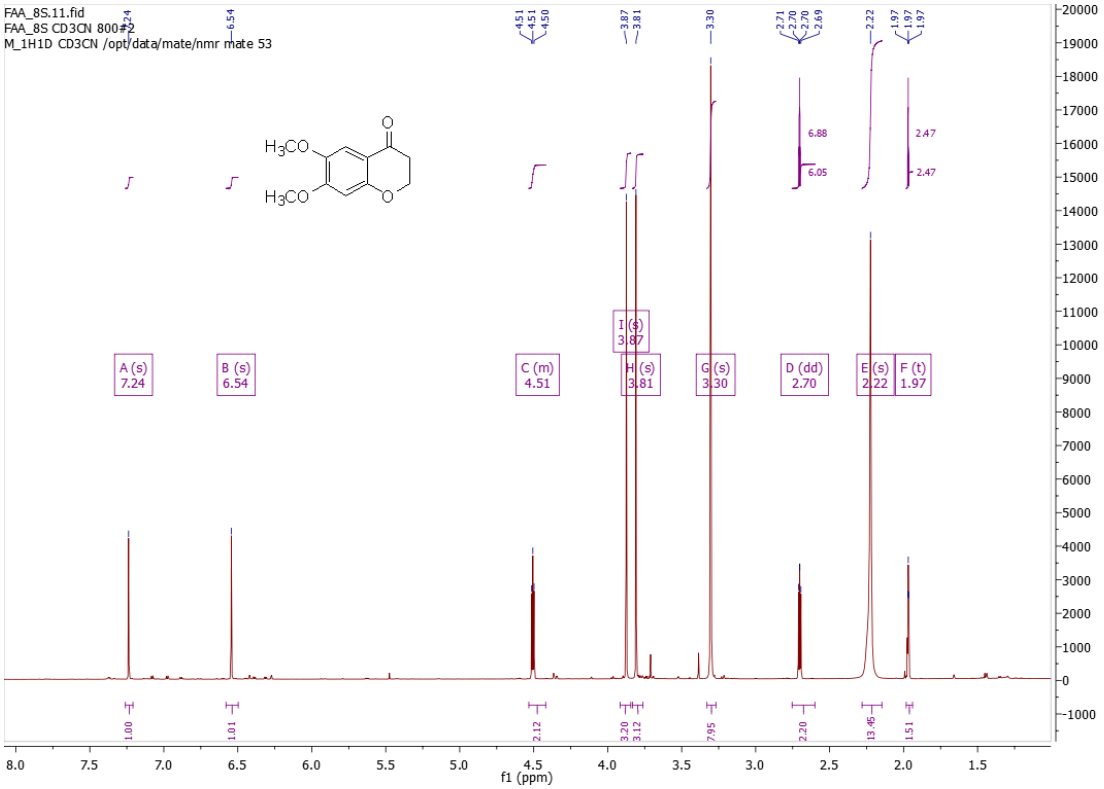


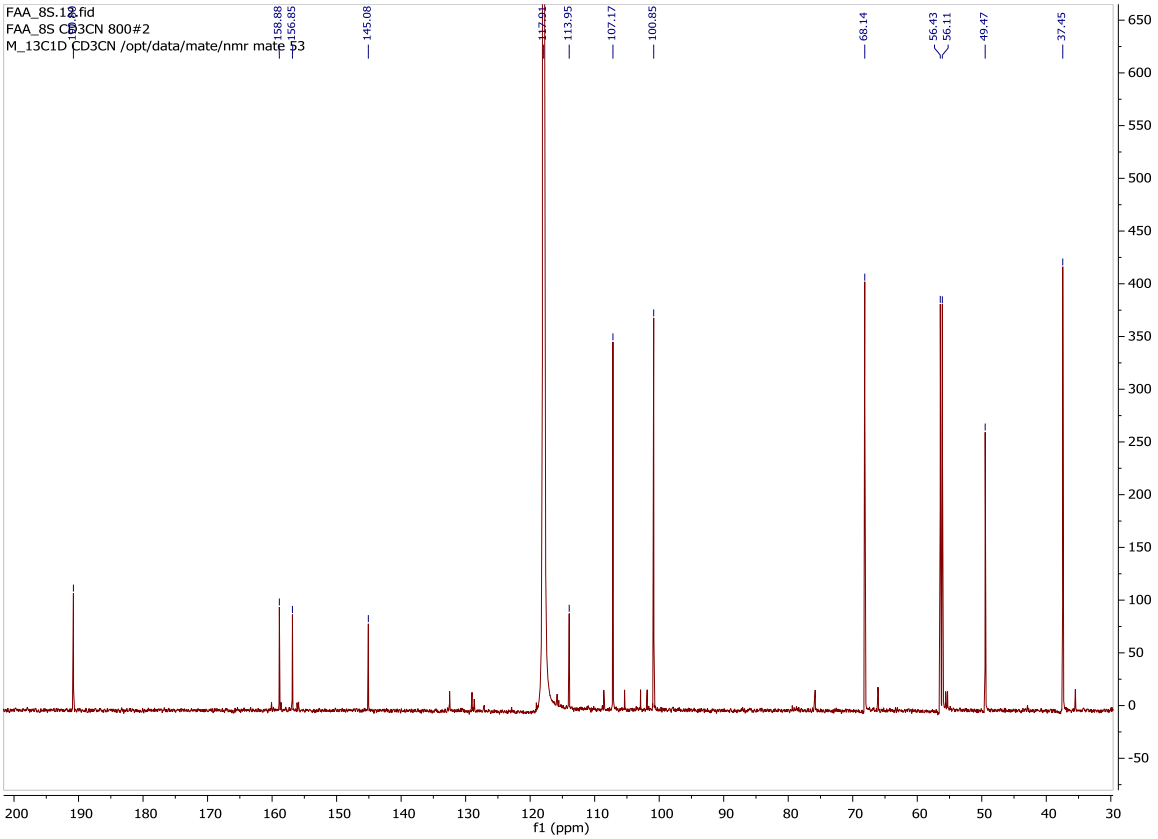
Appendix 22C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 22



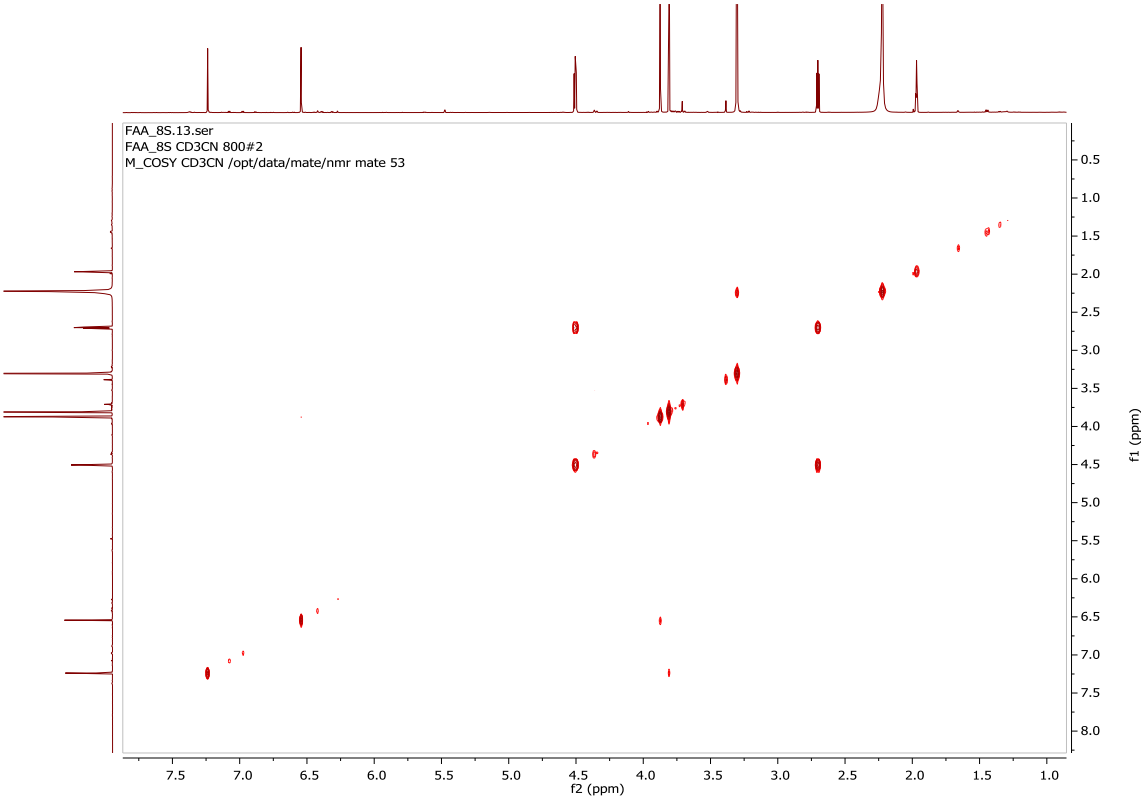


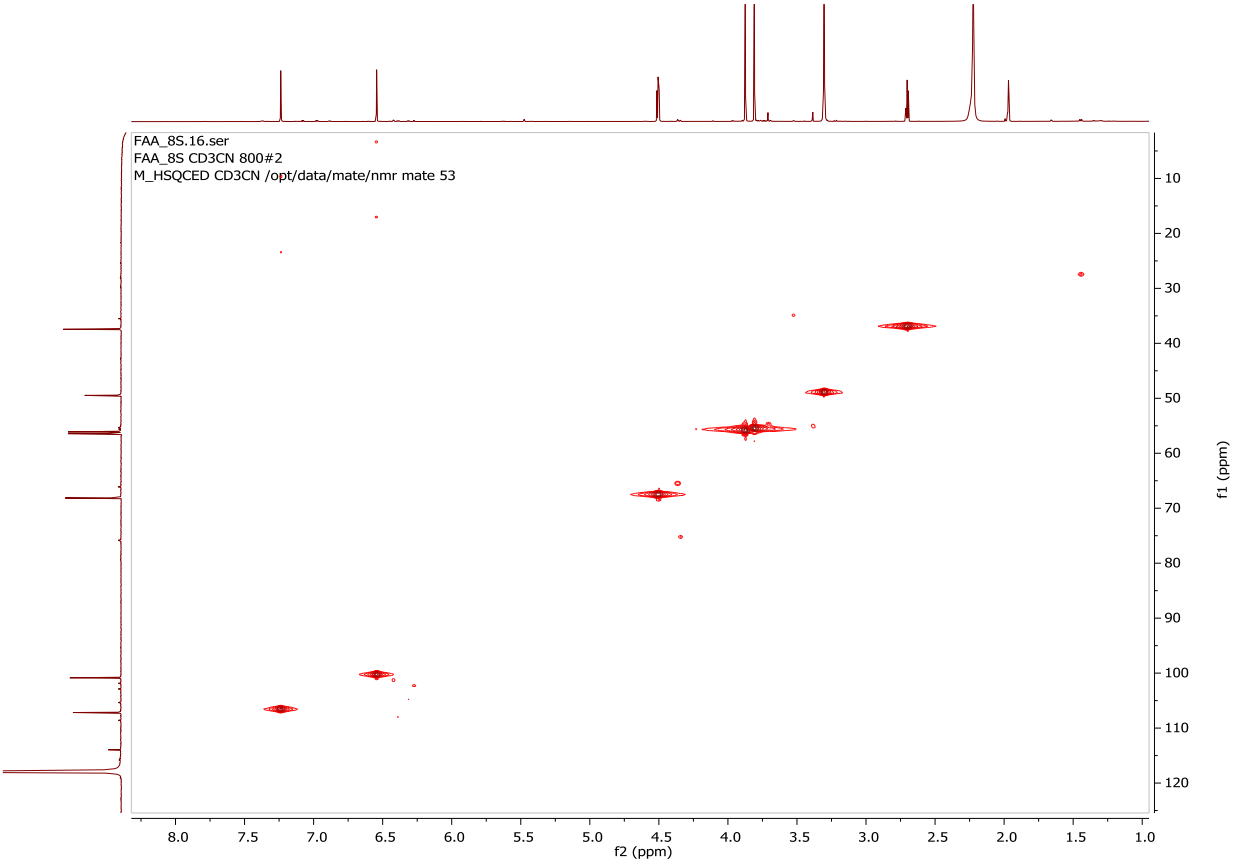
Appendix 23A: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of compound 23 (CD_3CN)



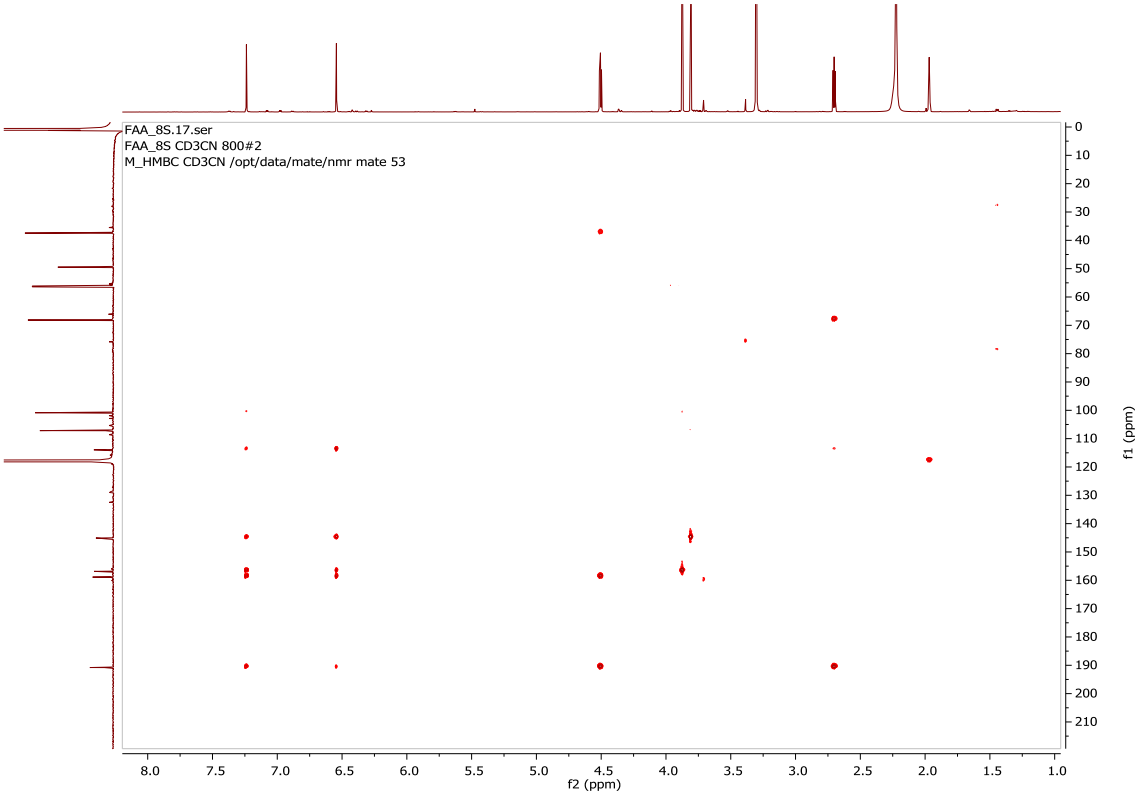


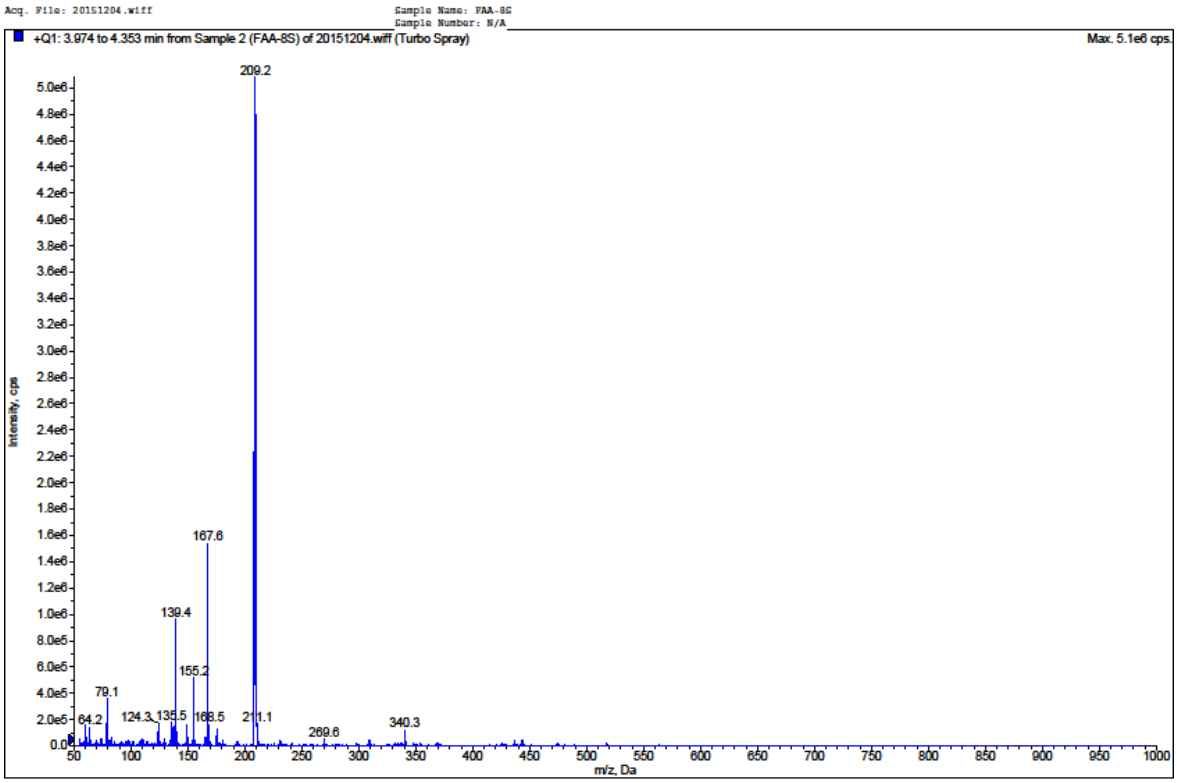
Appendix 23B: H,H COSY and HSQC spectra of compound 23 (800 MHz; CD_3CN)



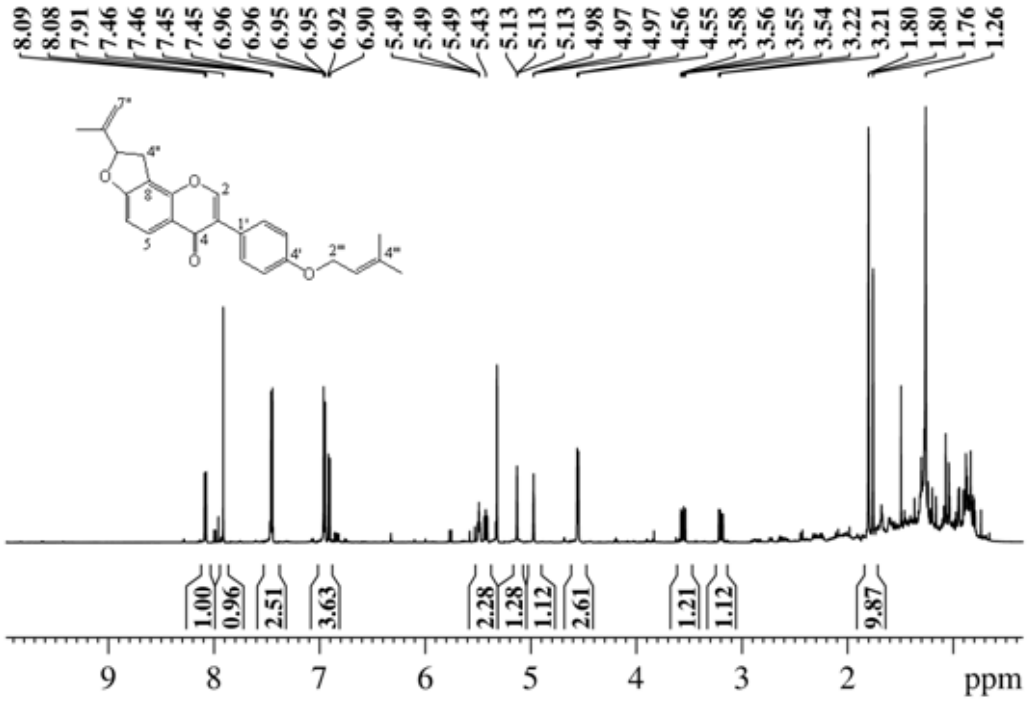


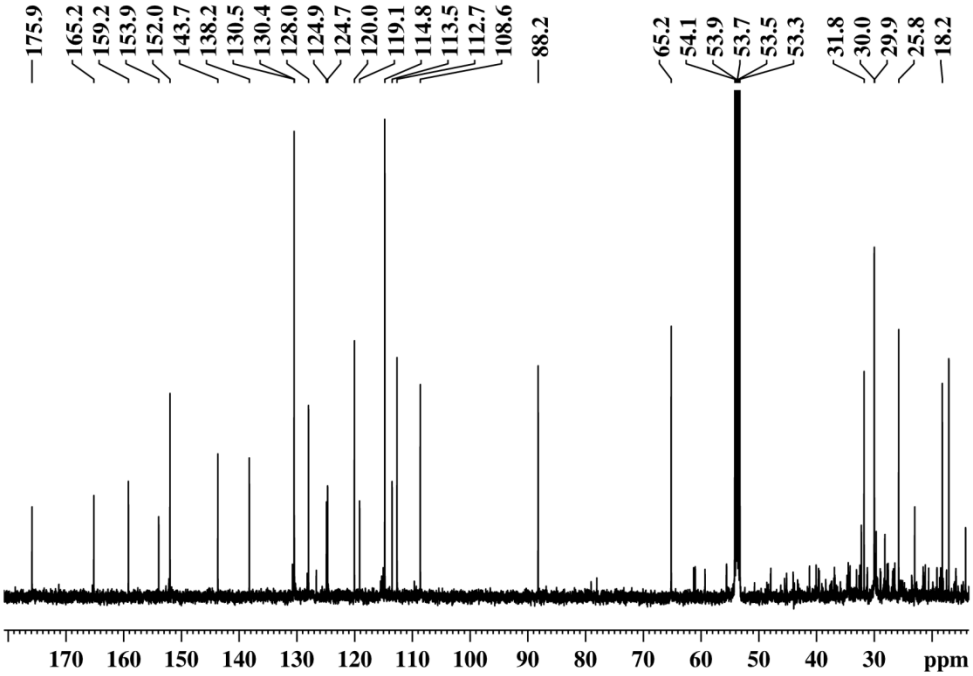
Appendix 23C: HMBC (800 MHz; CD_3CN) and LCMS spectra of compound 23



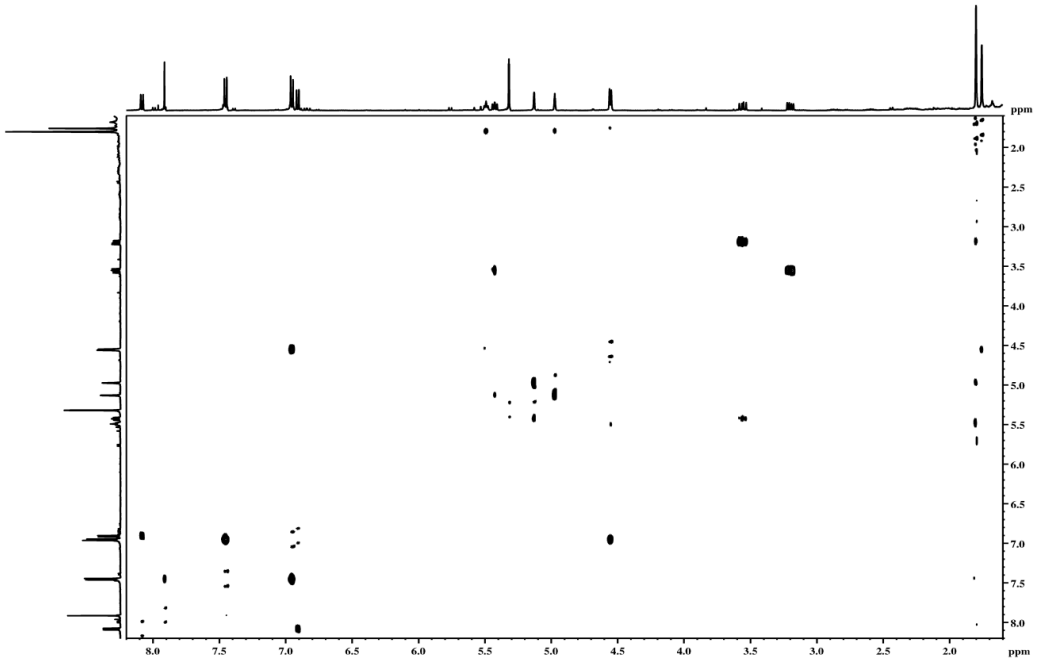


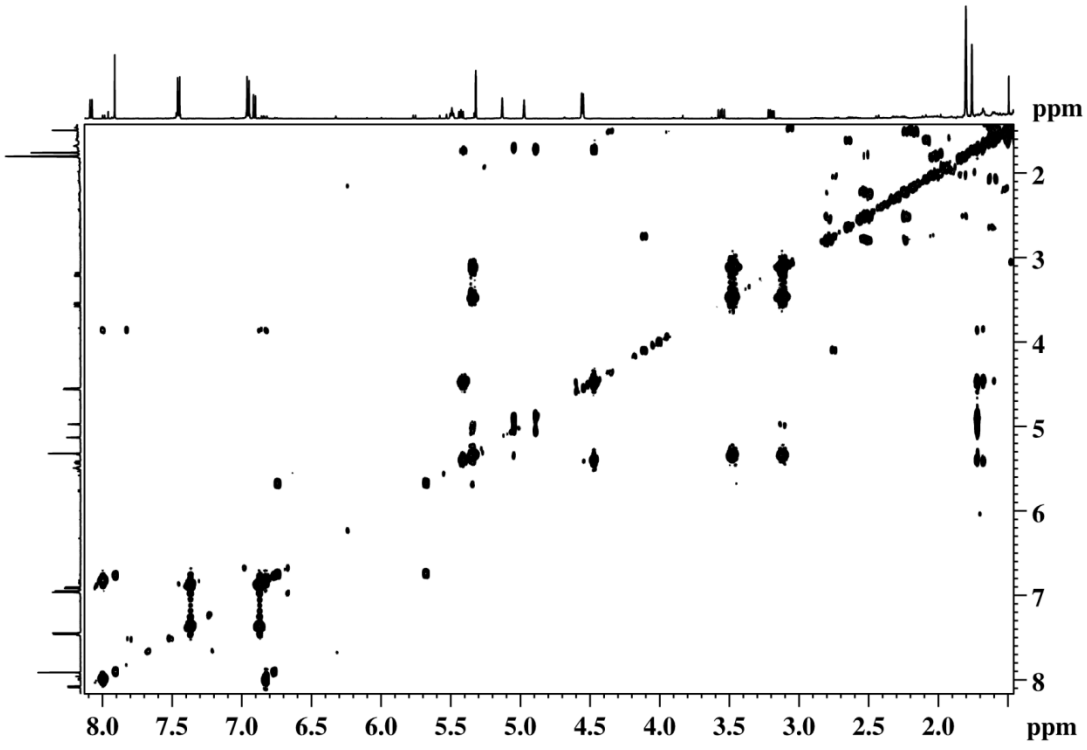
Appendix 24A: ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR spectrum of compound 24 (CD_2Cl_2)



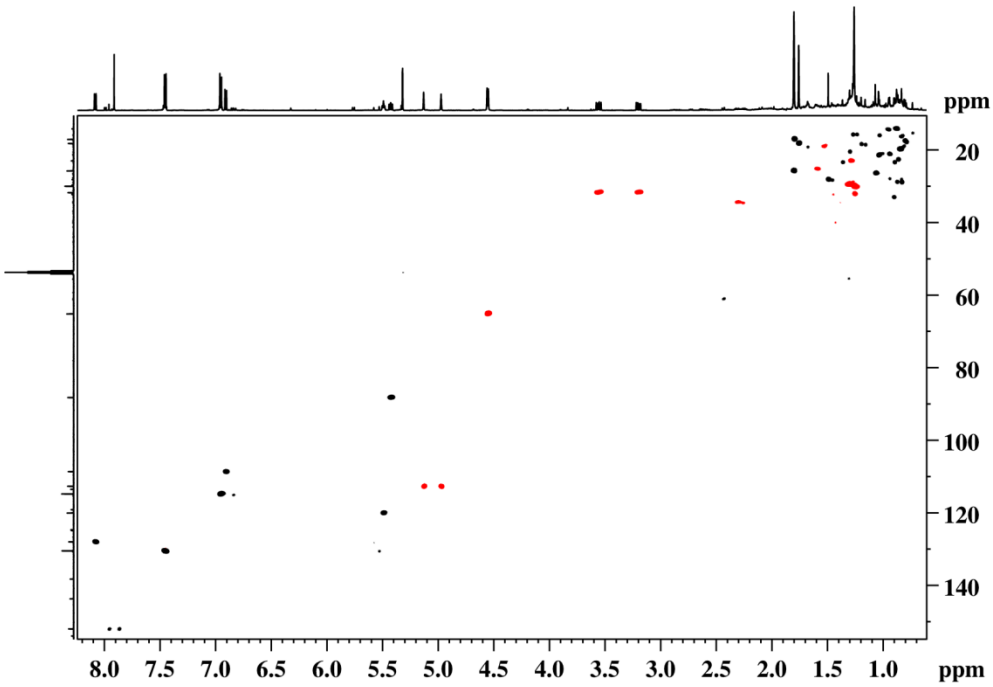


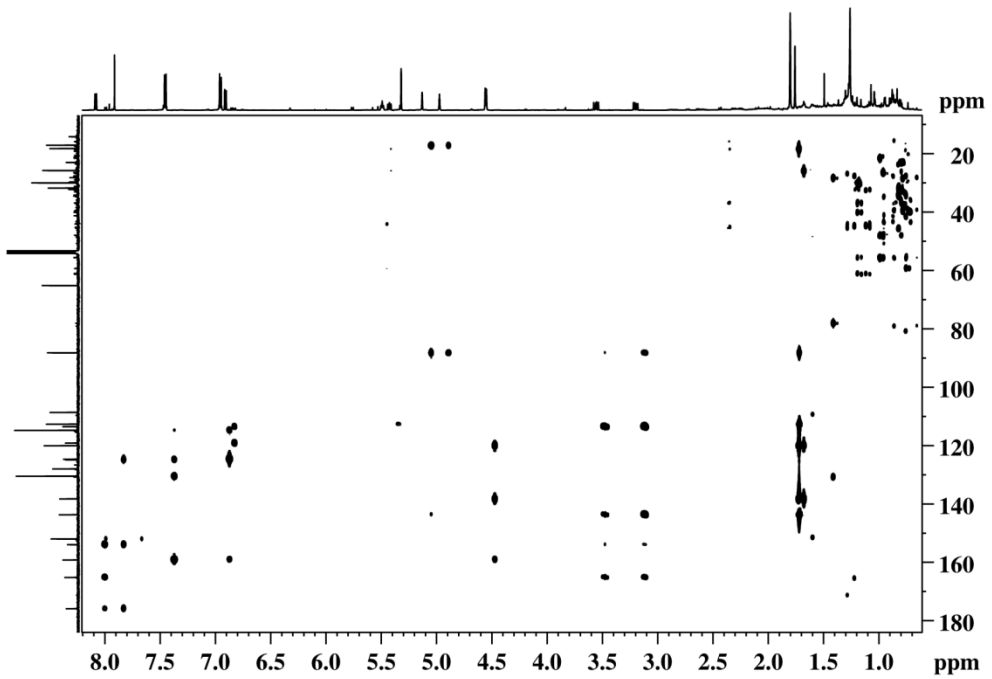
Appendix 24B: NOESY (500 MHz) and H,H-COSY (600 MHz) spectra of compound 24 (CD_2Cl_2)



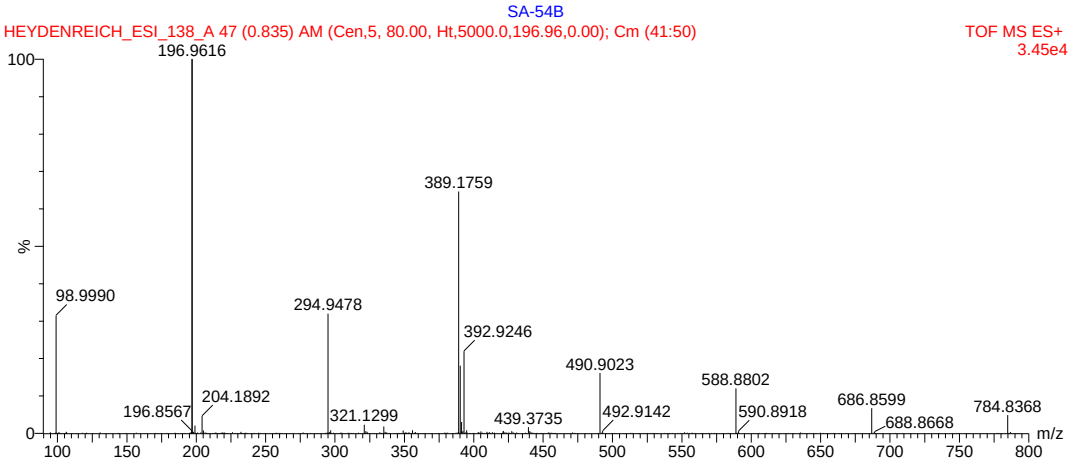


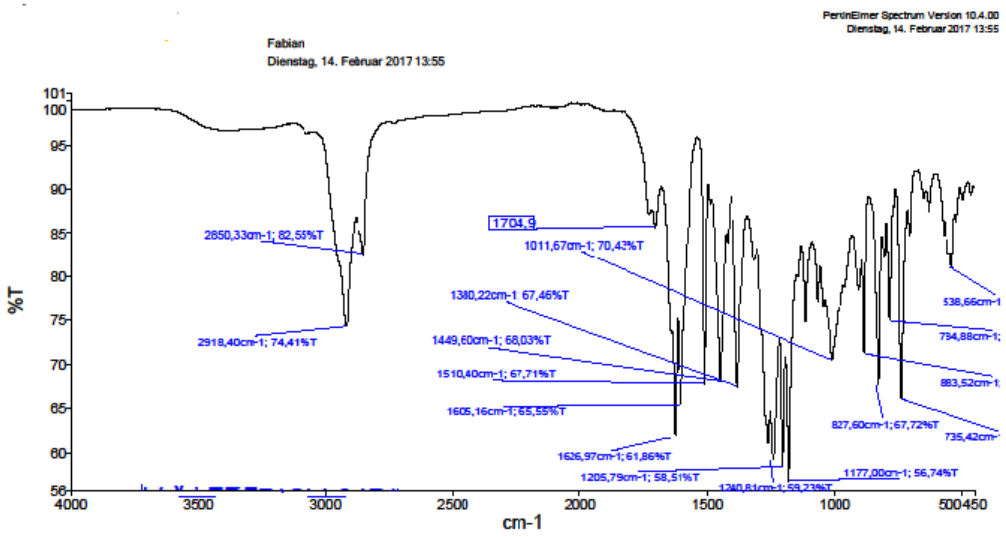
Appendix 24C: HSQC and HMBC spectra of compound 24 (600 MHz; CD_2Cl_2)

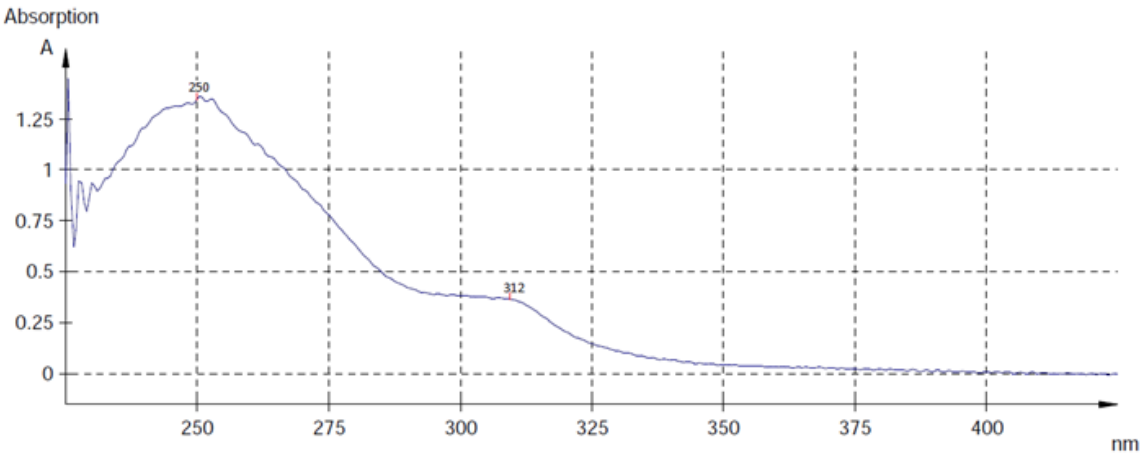




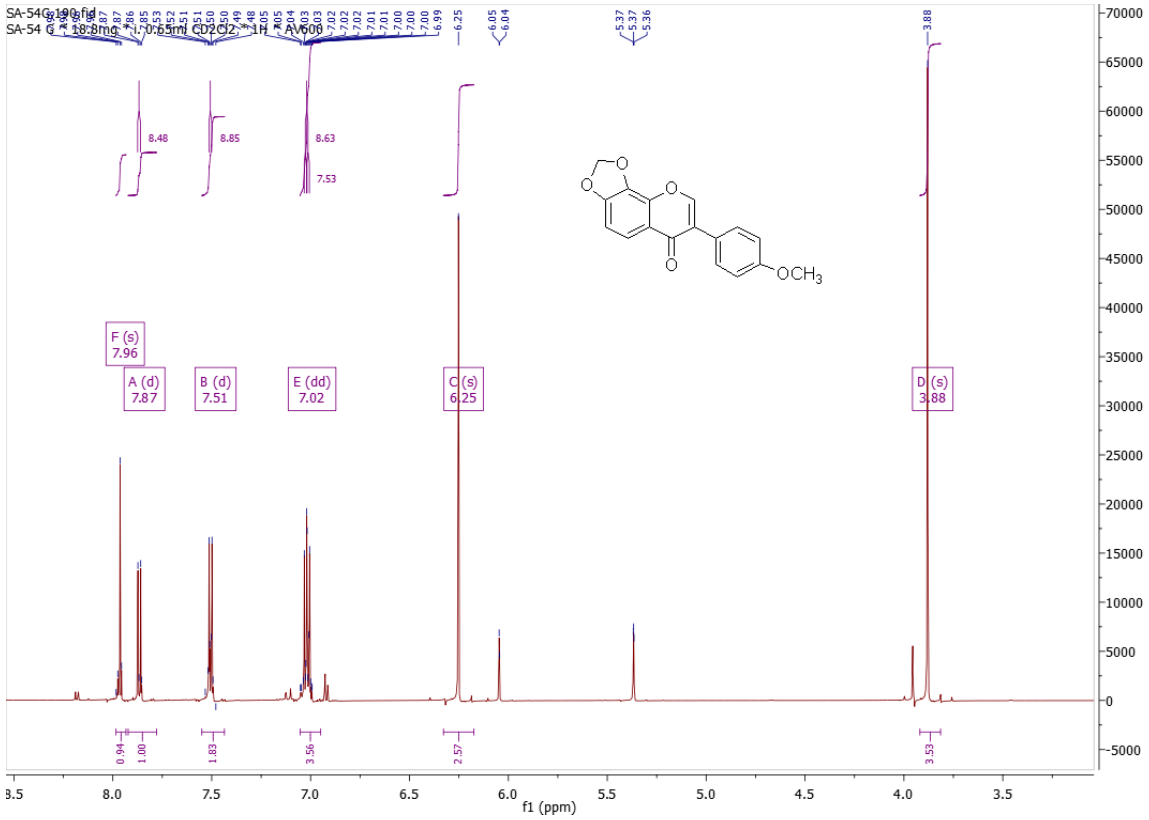
Appendix 24D: HREIMS, IR and UV spectra of compound 24

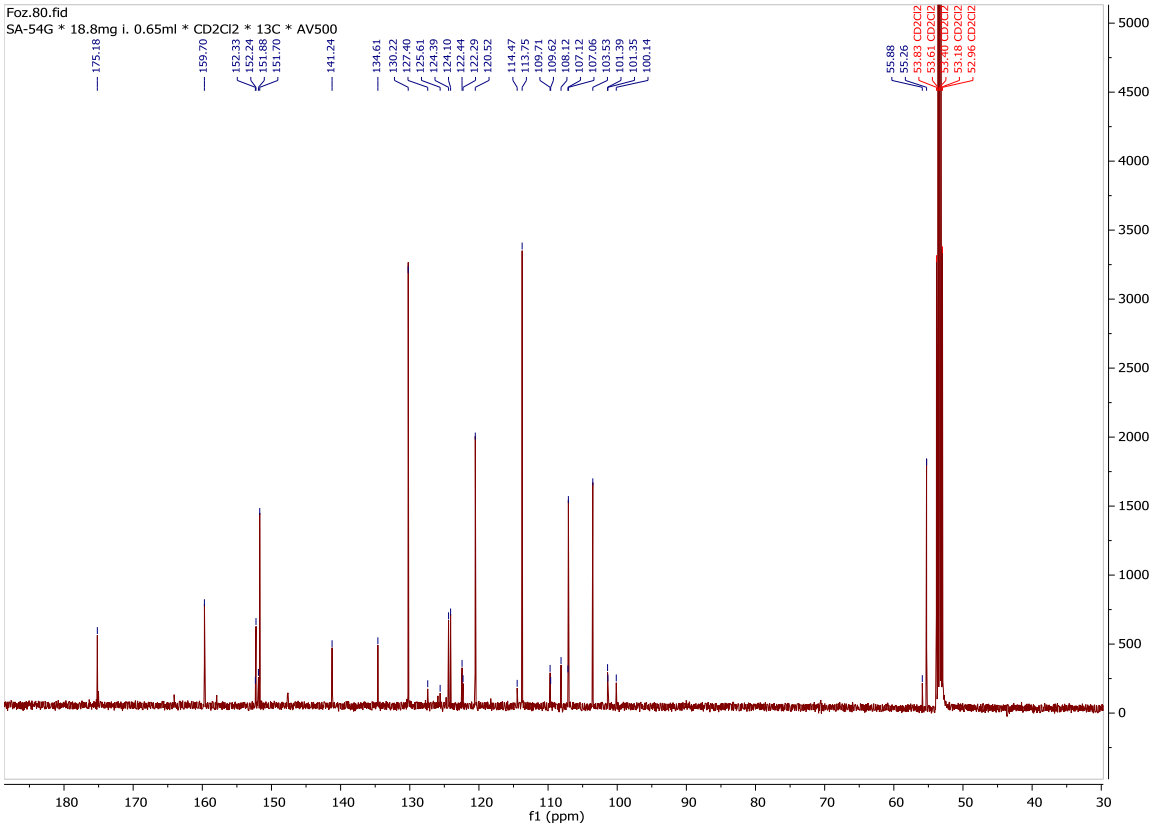




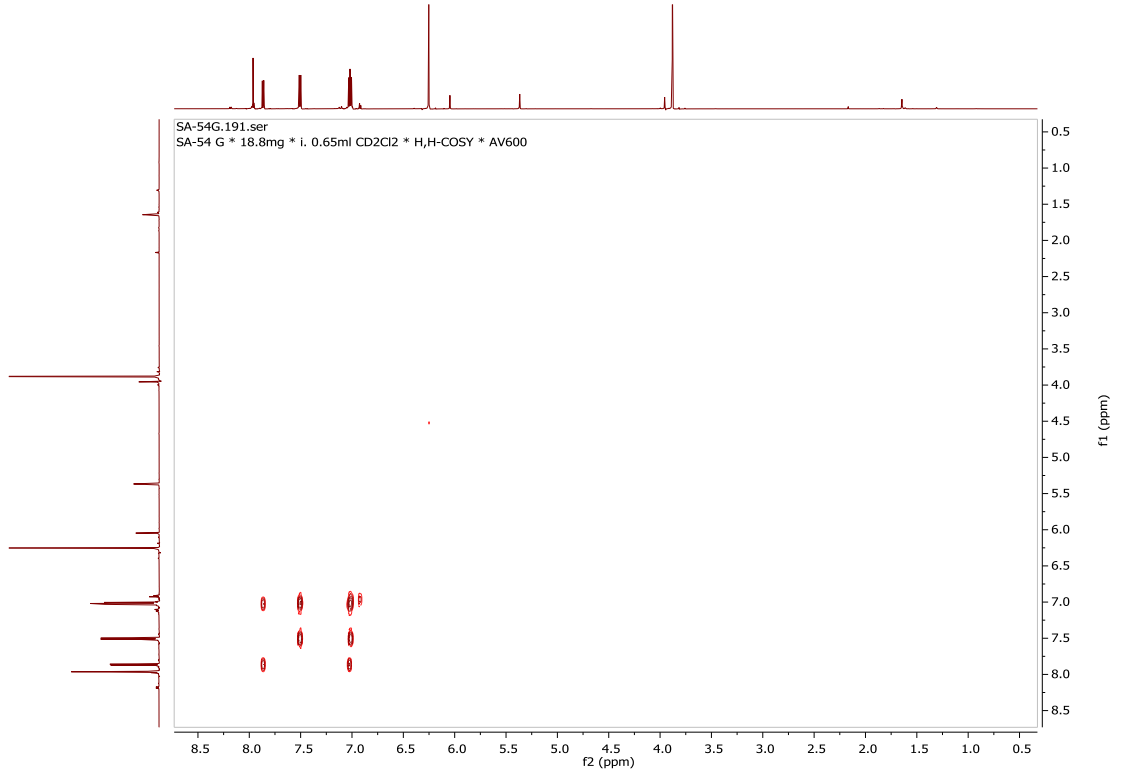


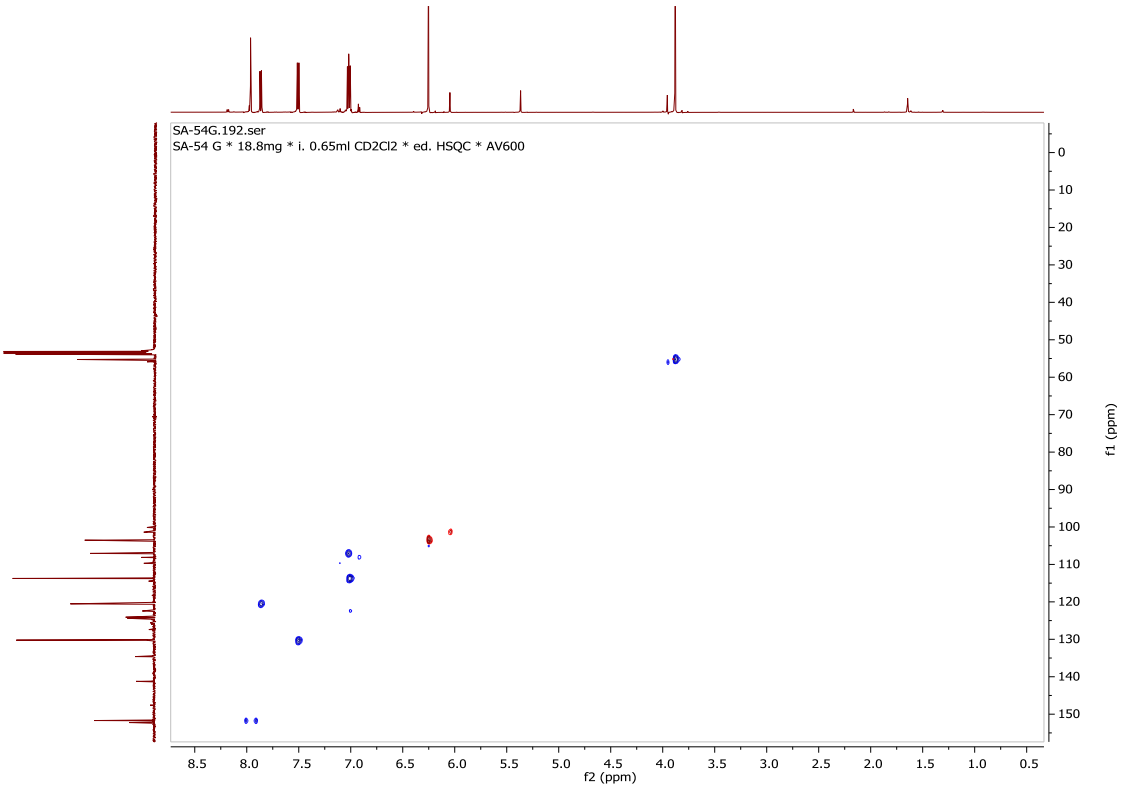
Appendix 25A: ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of compound 25 (CD_2Cl_2)



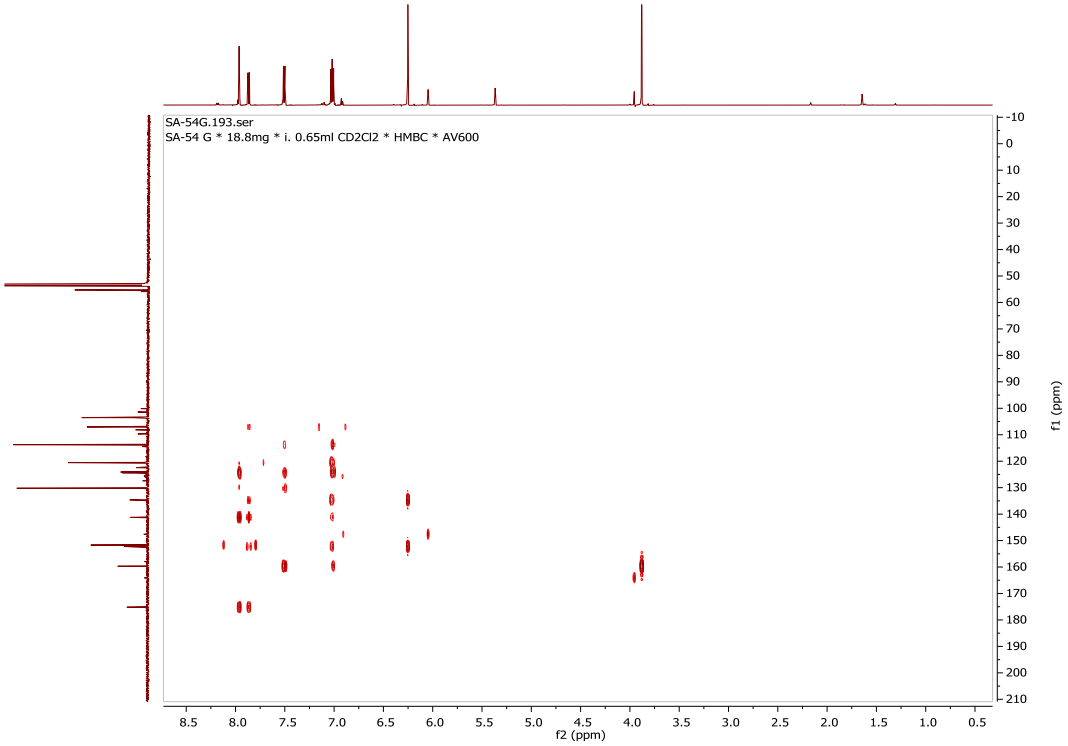


Appendix 25B: H,H-COSY and HSQC spectra of compound 25 (500 MHz; CD_2Cl_2)

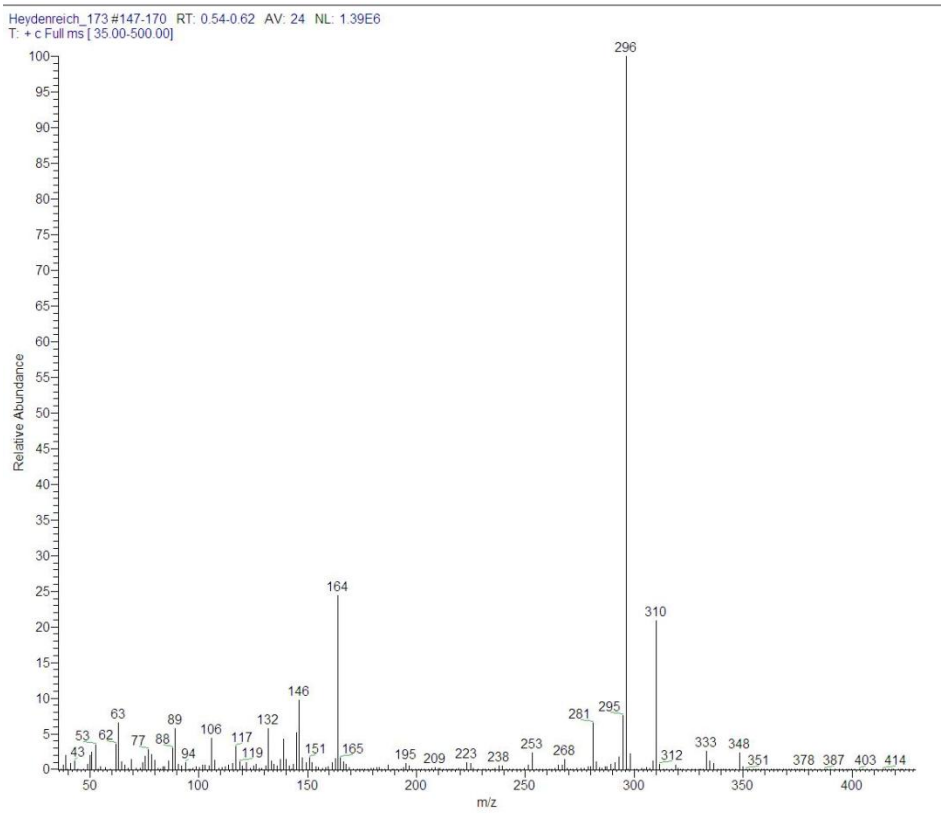




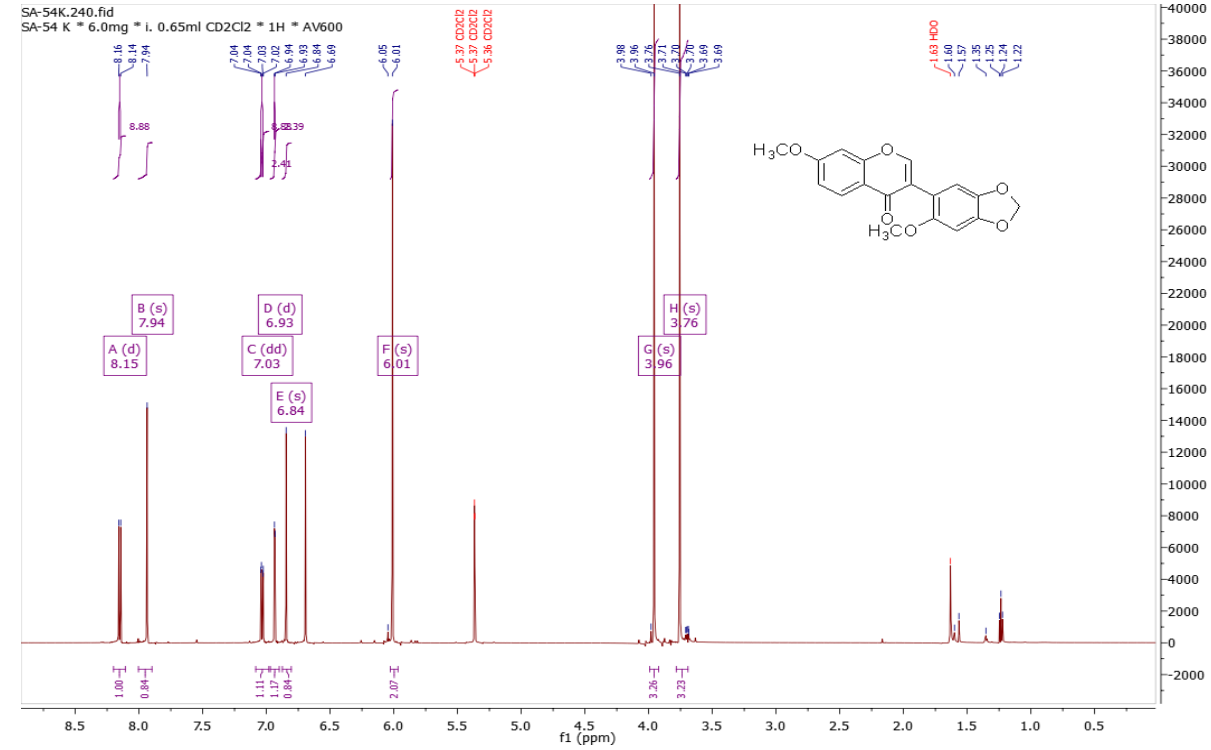
Appendix 25C: HMBC (500 MHz; CD_2Cl_2) and LCMS spectra of compound 25

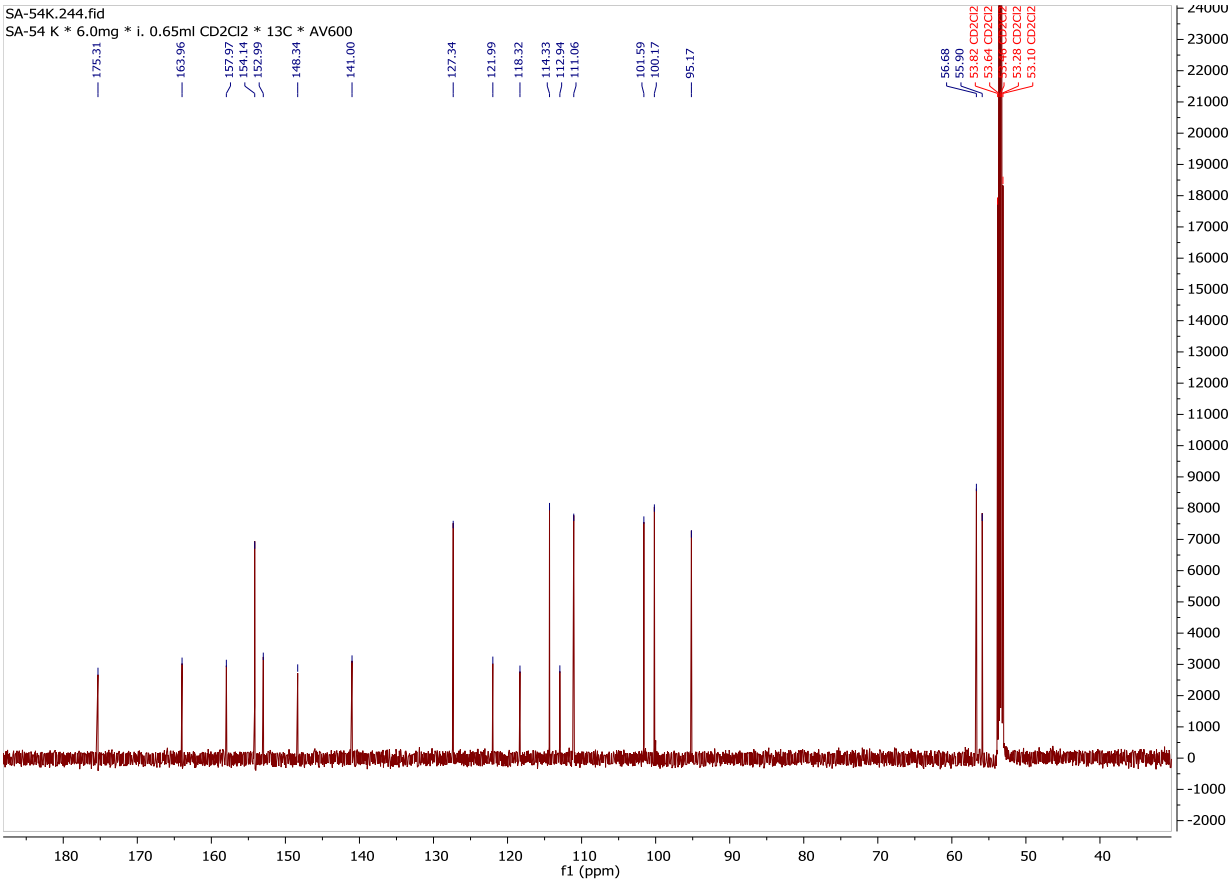


SA-54G

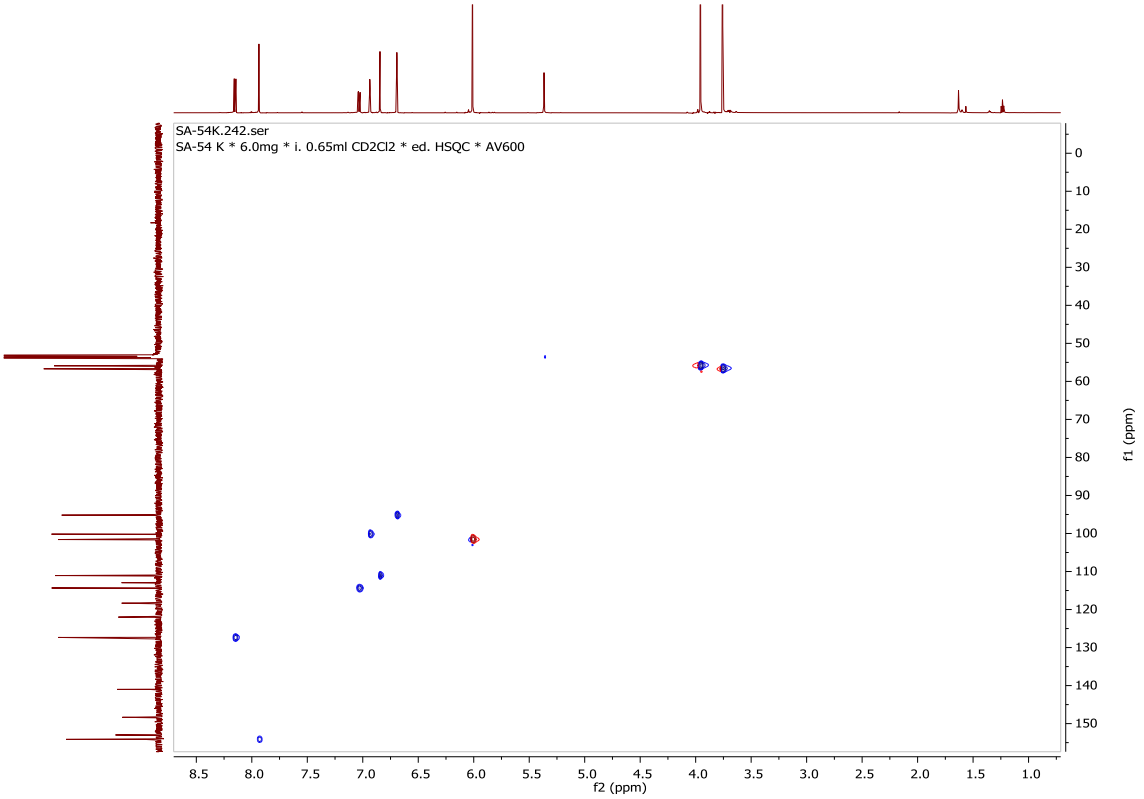


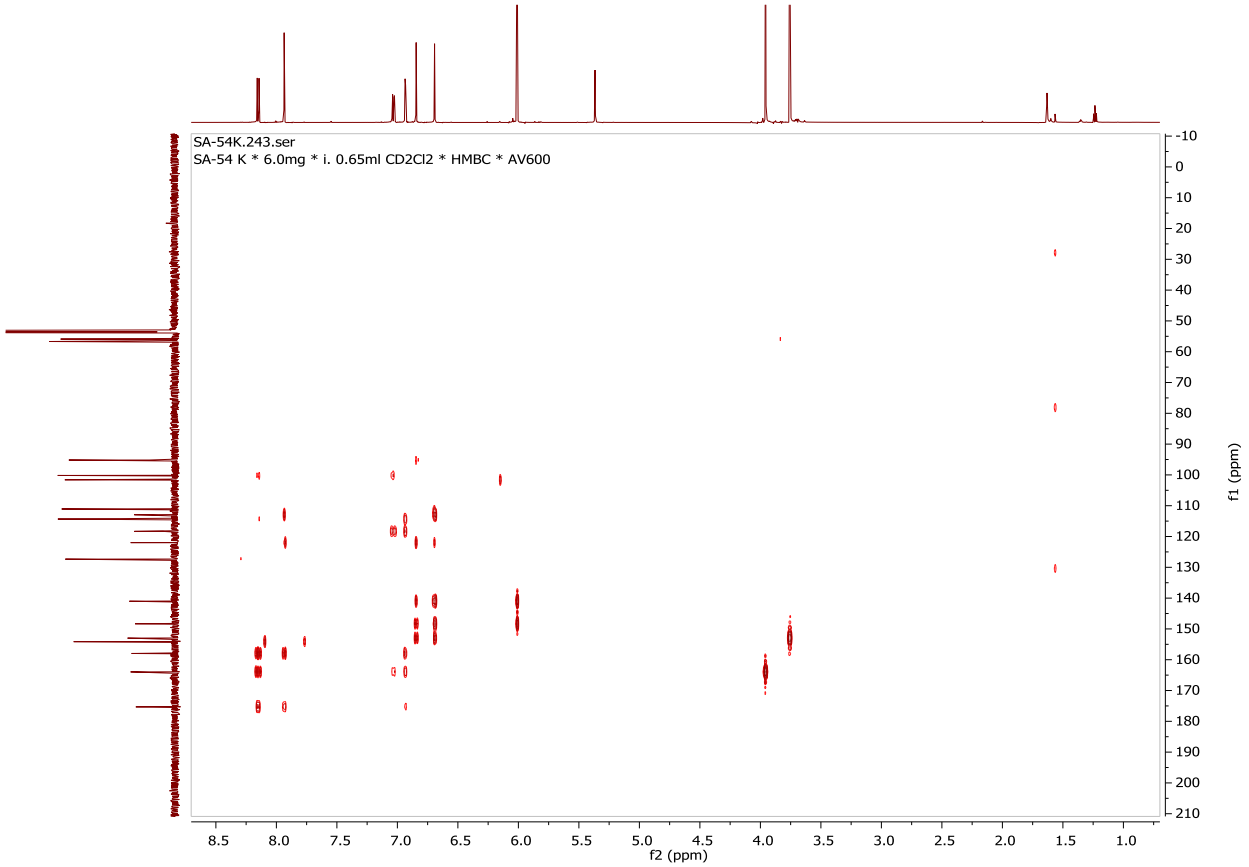
Appendix 26A: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra of compound 26 (CD_2Cl_2)



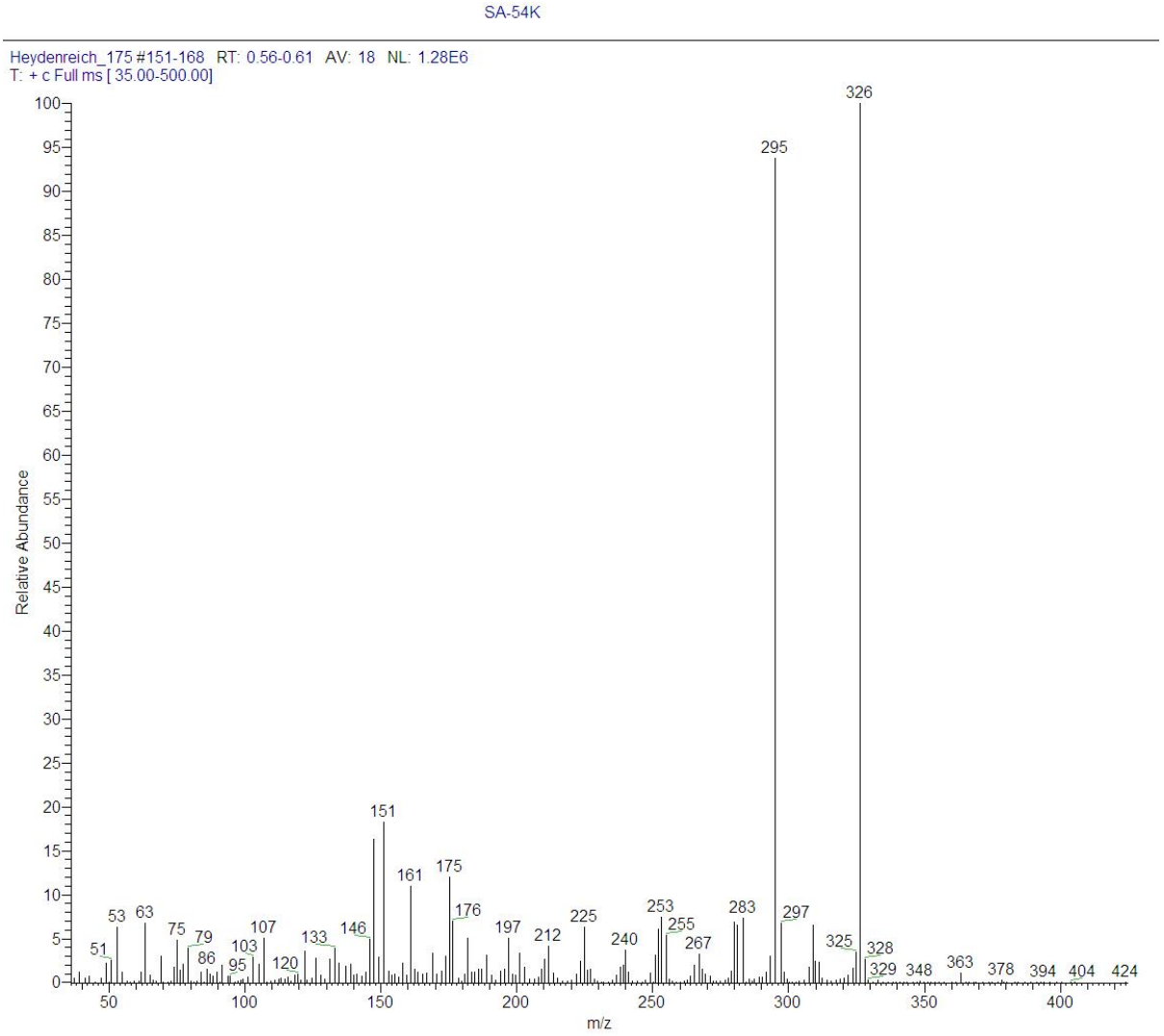


Appendix 26B: HSQC and HMBC spectra of compound 26 (500 MHz; CD_2Cl_2)

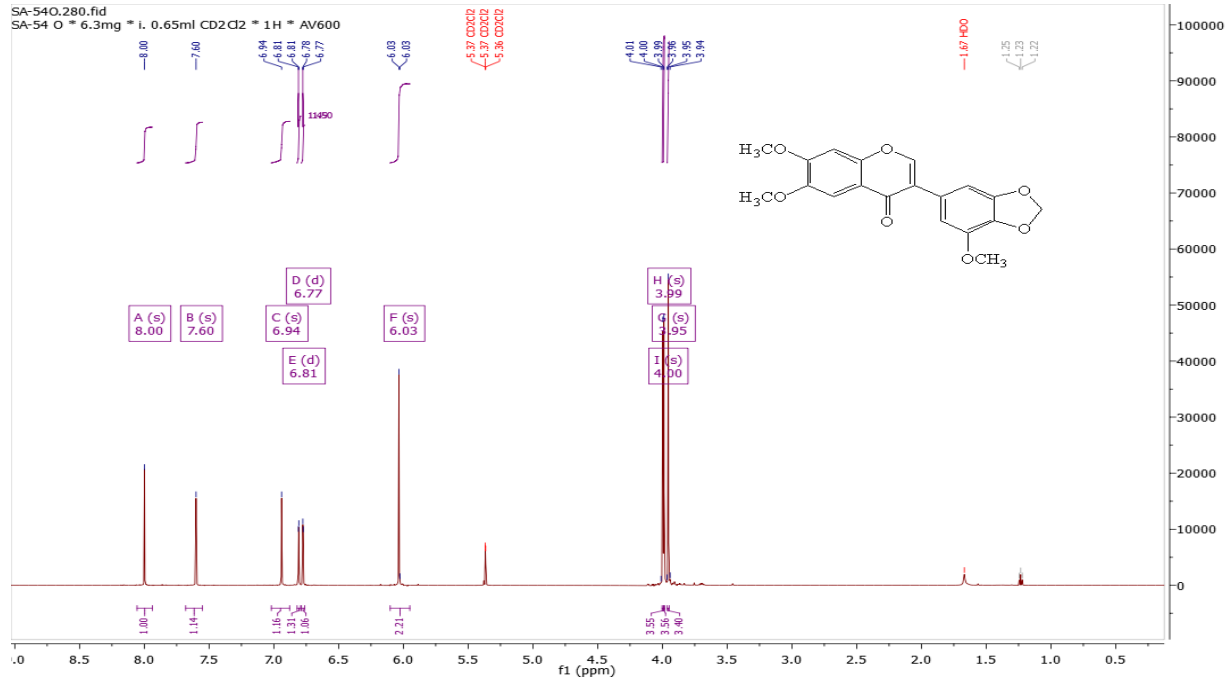


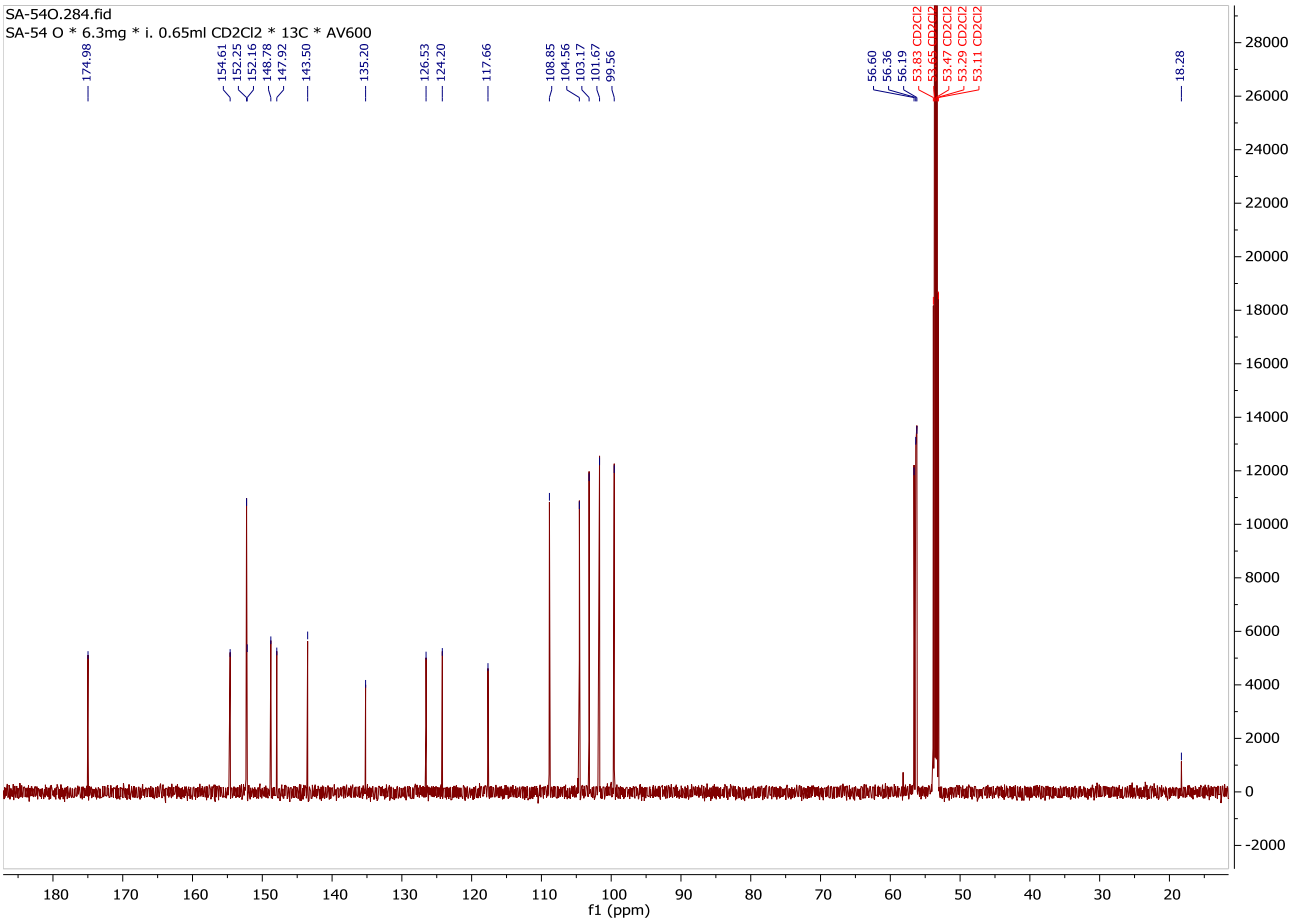


Appendix 26C: LCMS spectrum of compound 26E

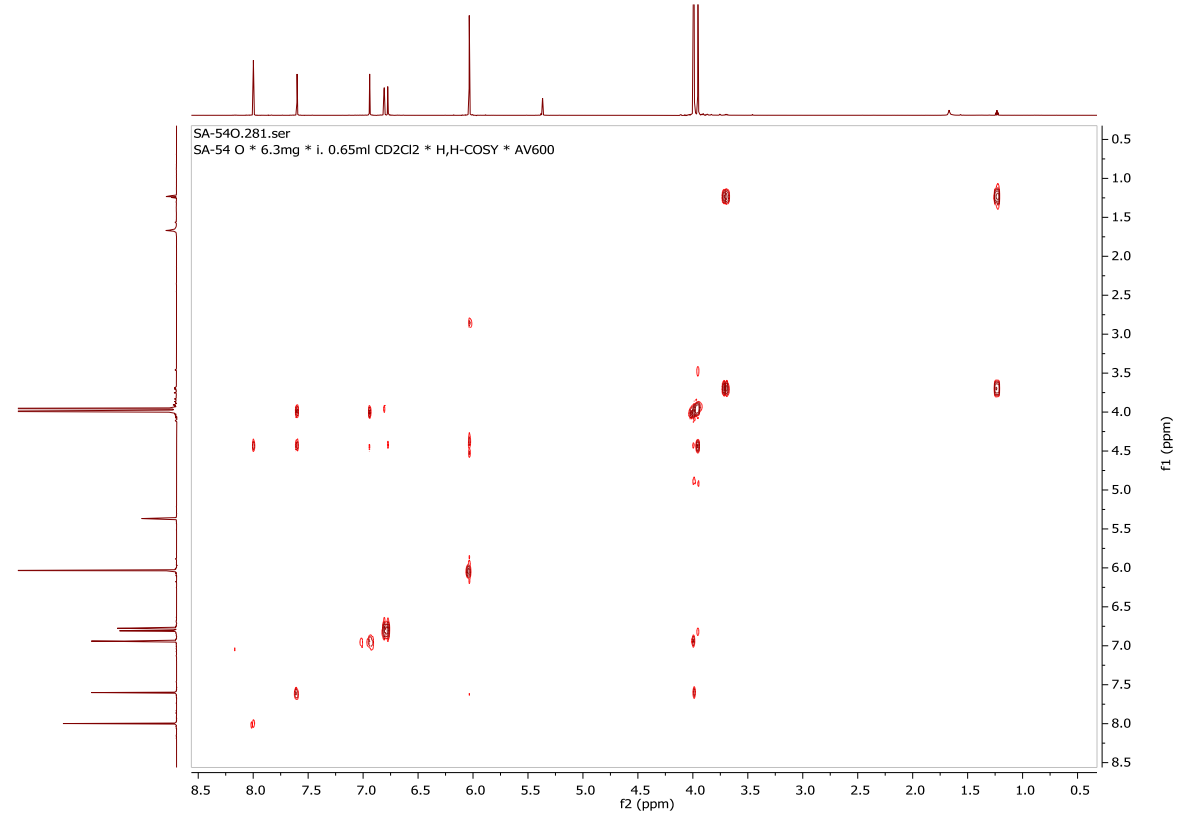


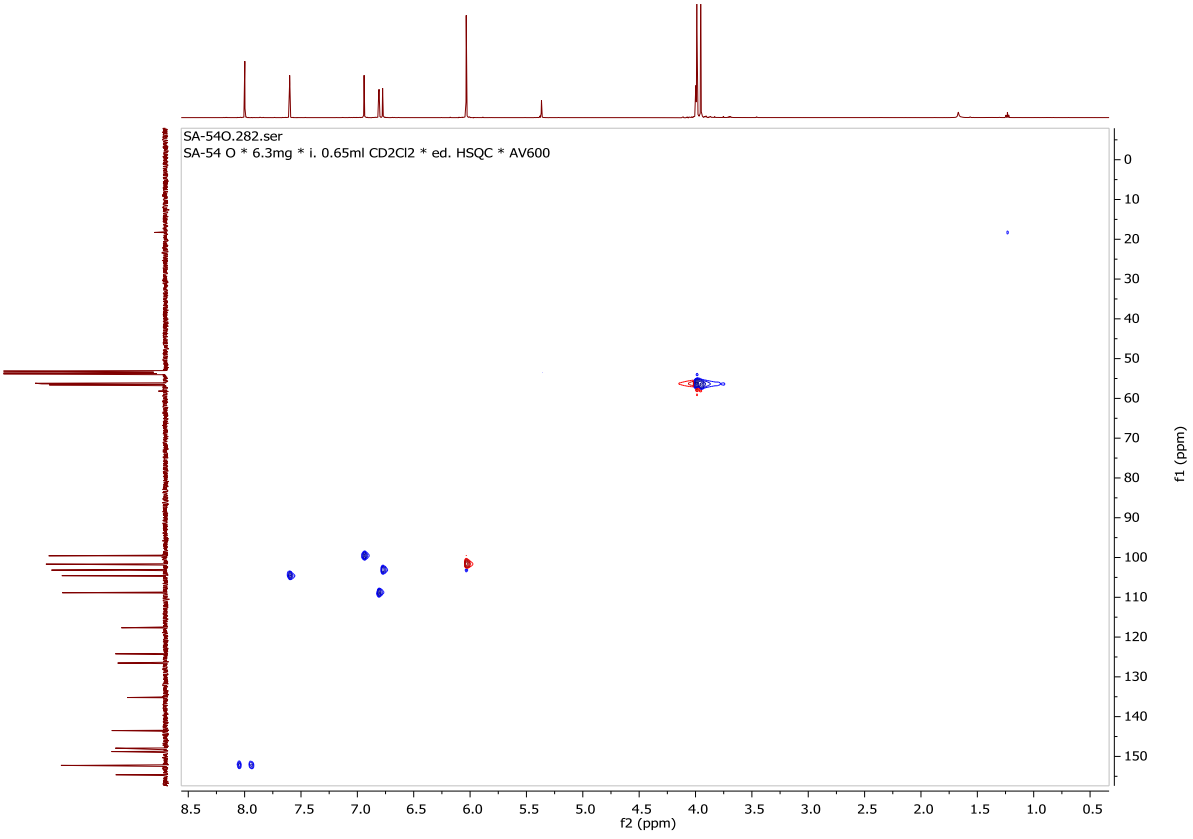
Appendix 27A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of compound 27 (CD_2Cl_2)



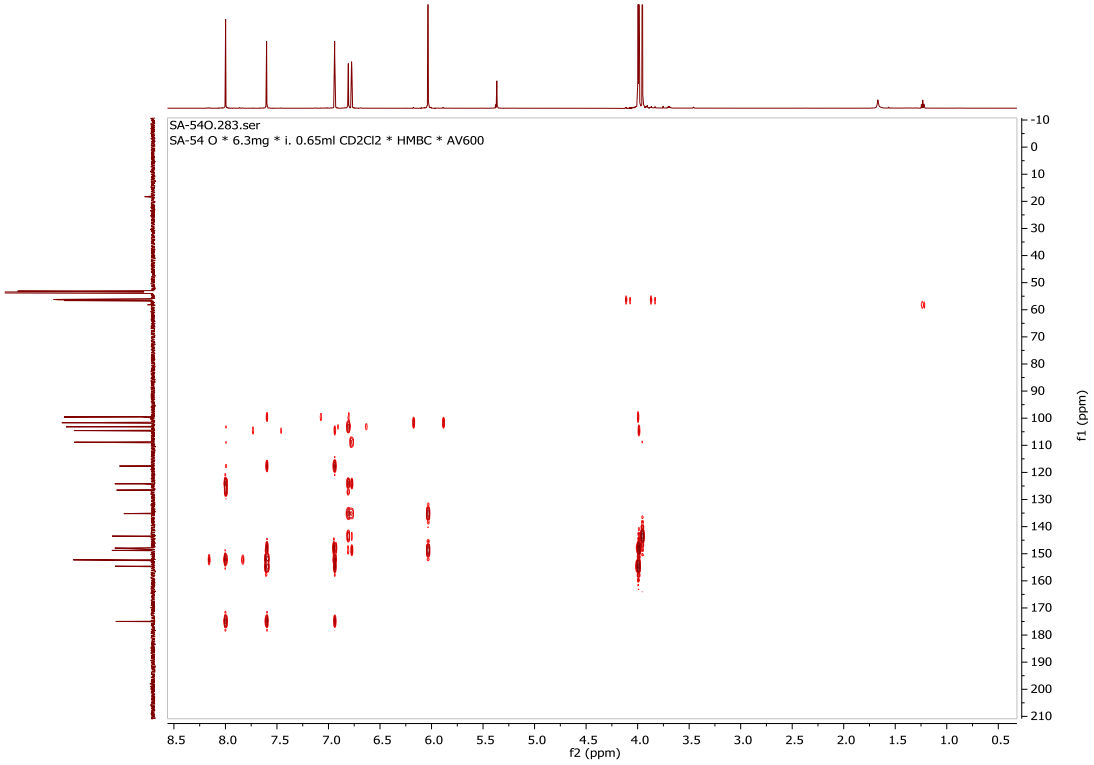


Appendix 27B: H,H-COSY and HSQC spectra of compound 27 (600 MHz; CD_2Cl_2)

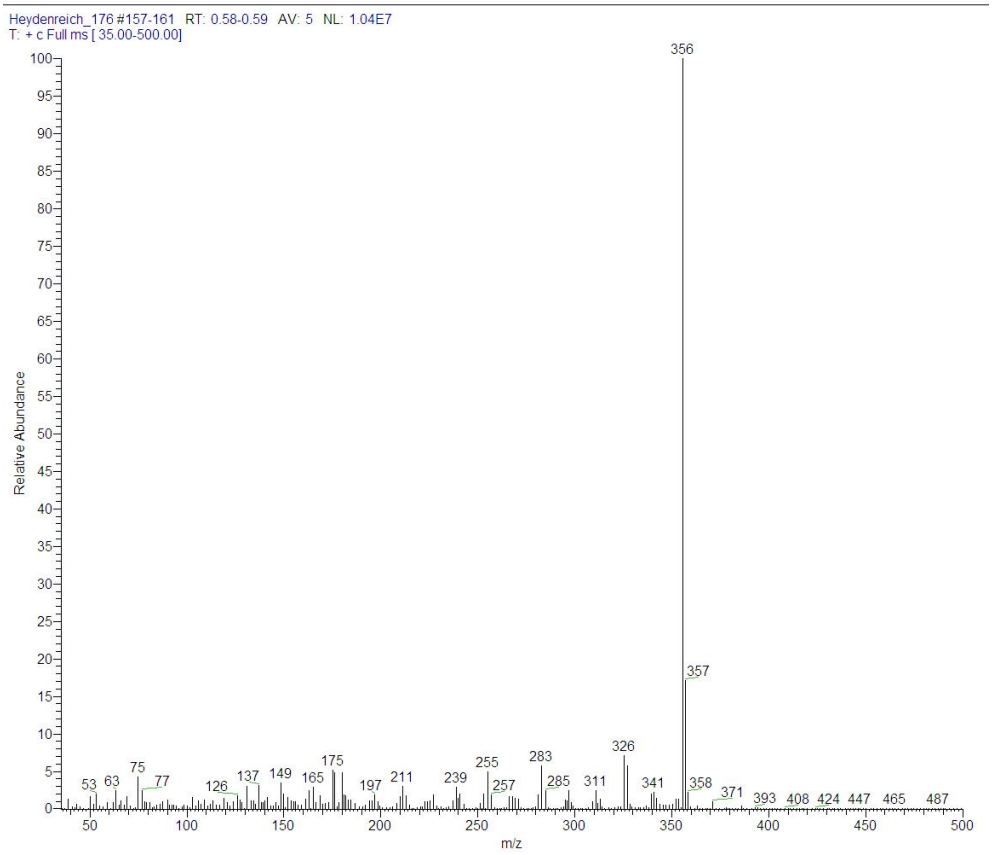




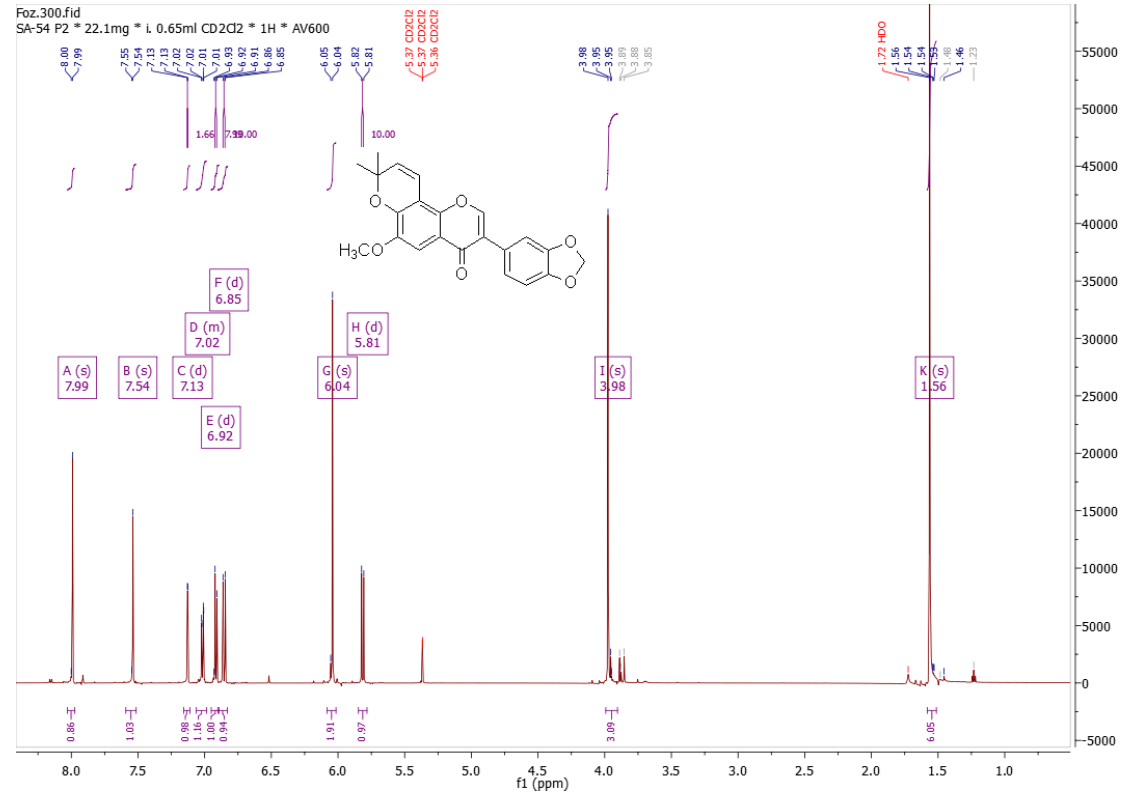
Appendix 27C: HMBC (600 MHz; CD_2Cl_2) and LCMS spectra of compound 27

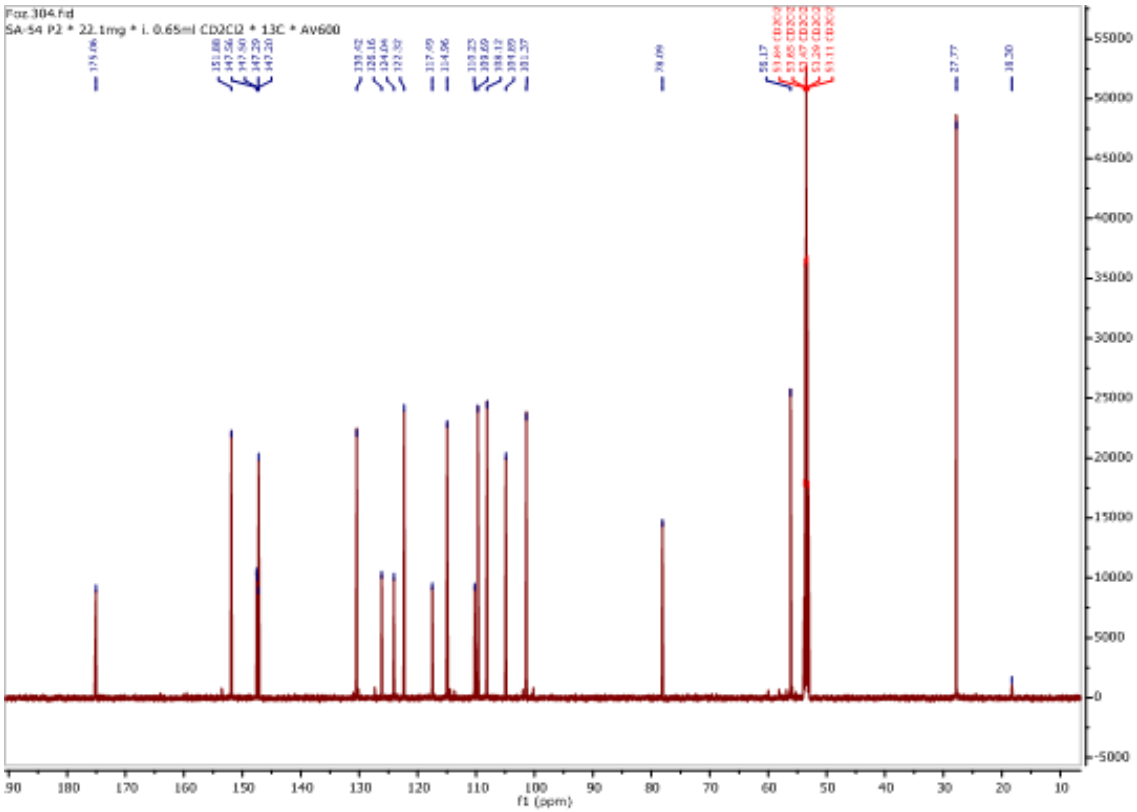


SA-54O

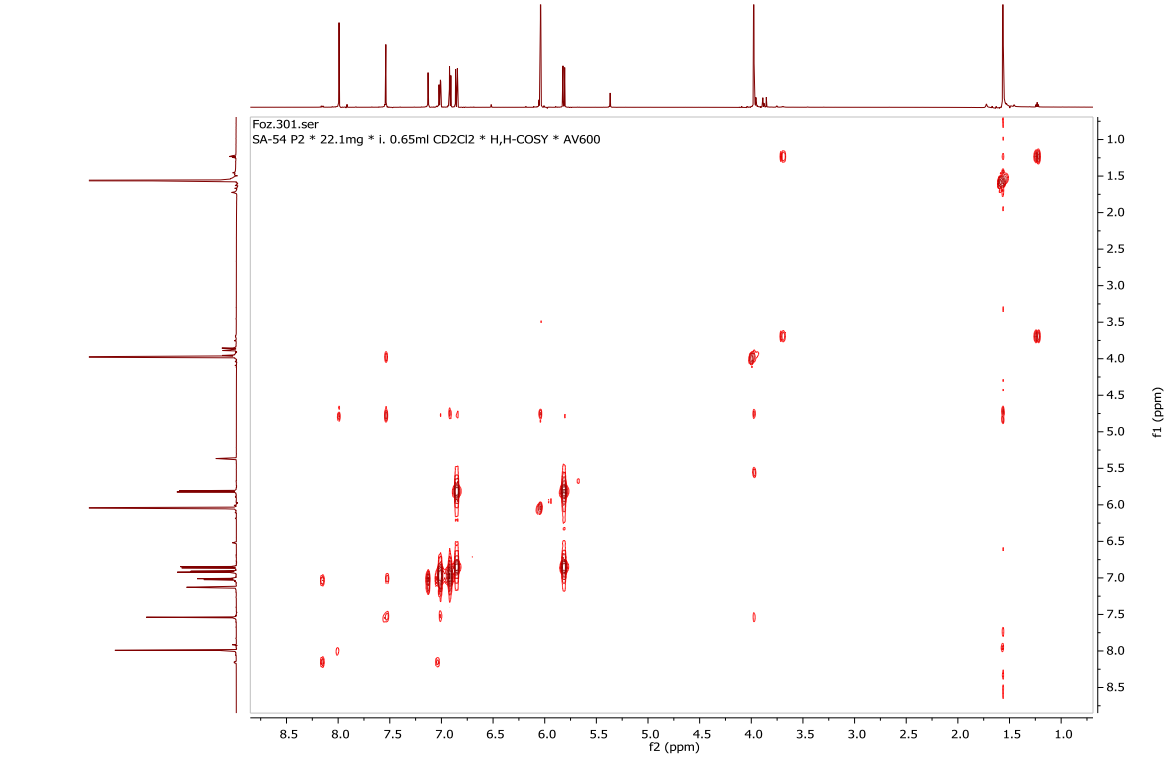


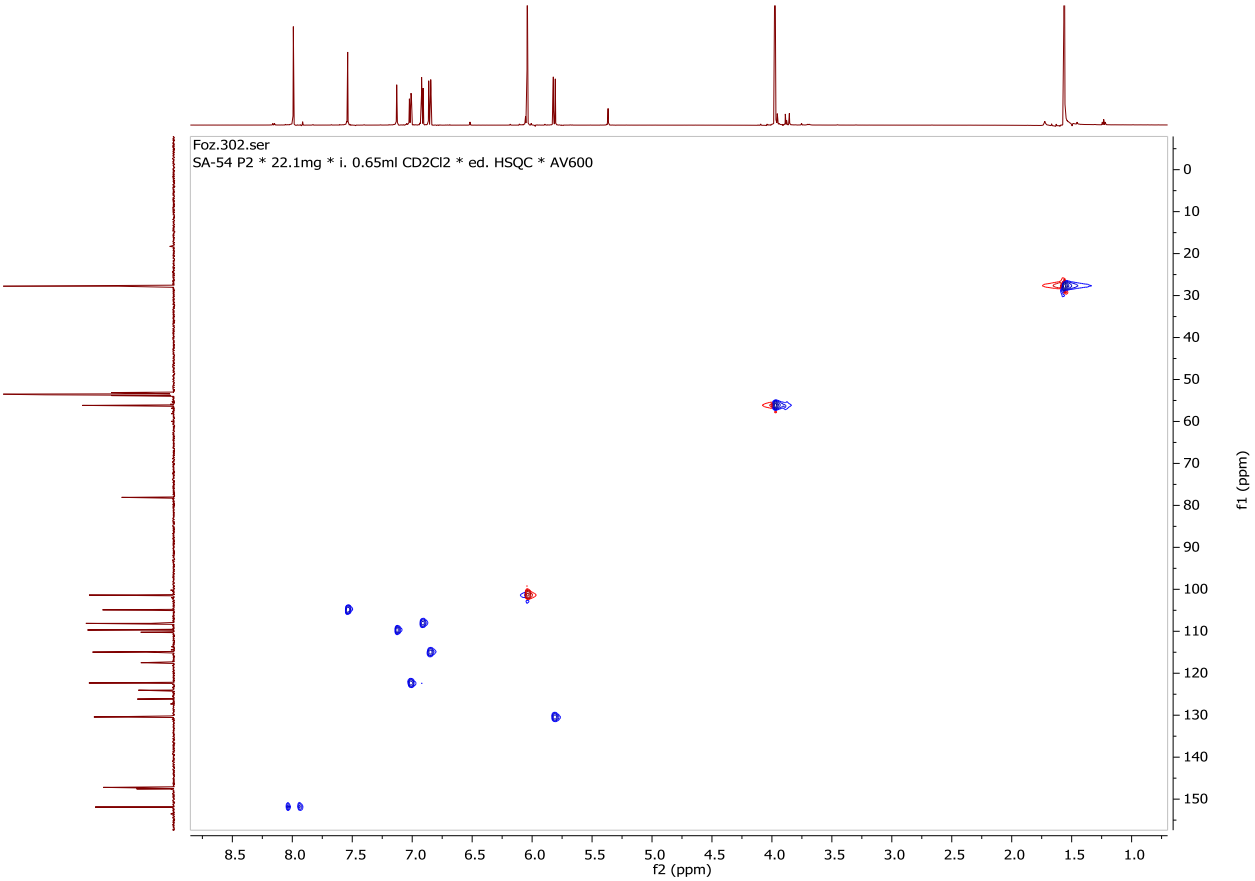
Appendix 28A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectrum of compound 28 (CD_2Cl_2)



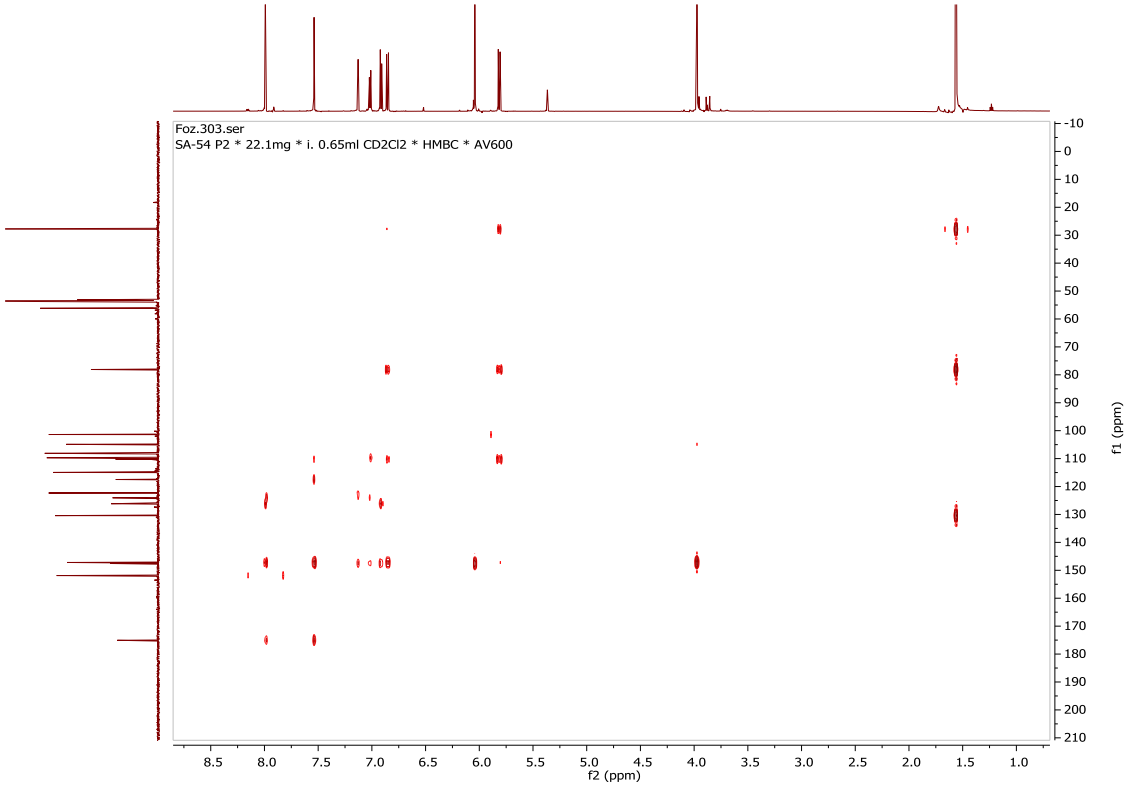


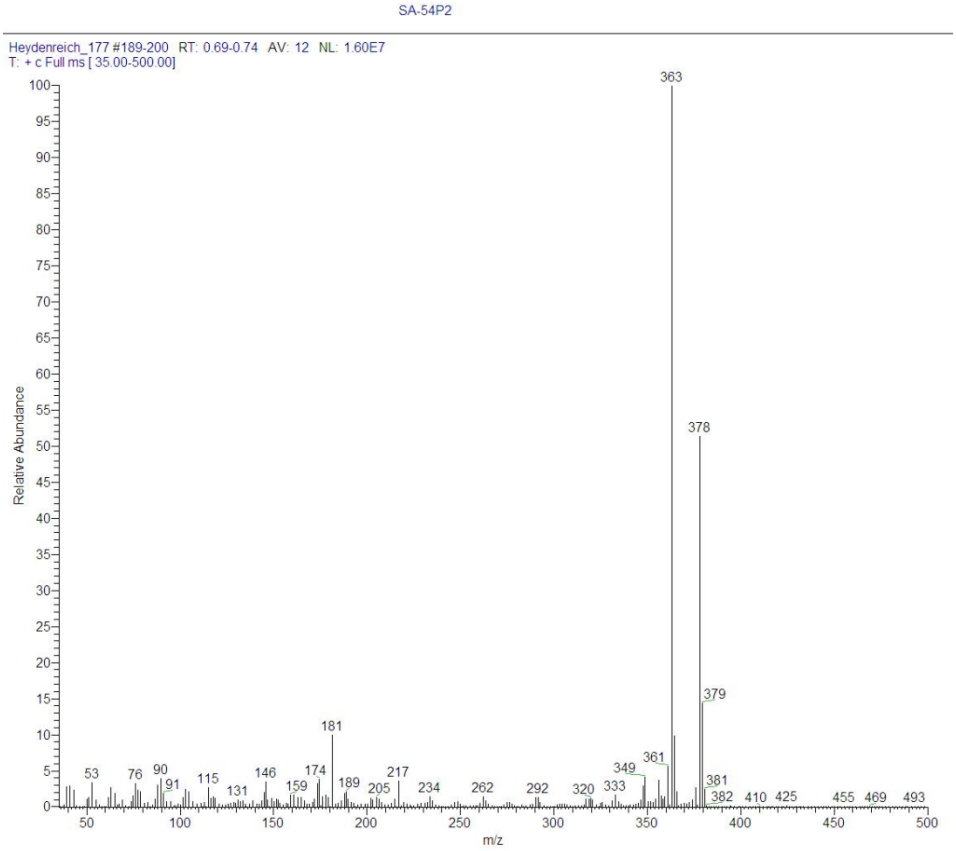
Appendix 28B: H,H-COSY and HSQC spectrum of compound 28 (600 MHz; CD_2Cl_2)



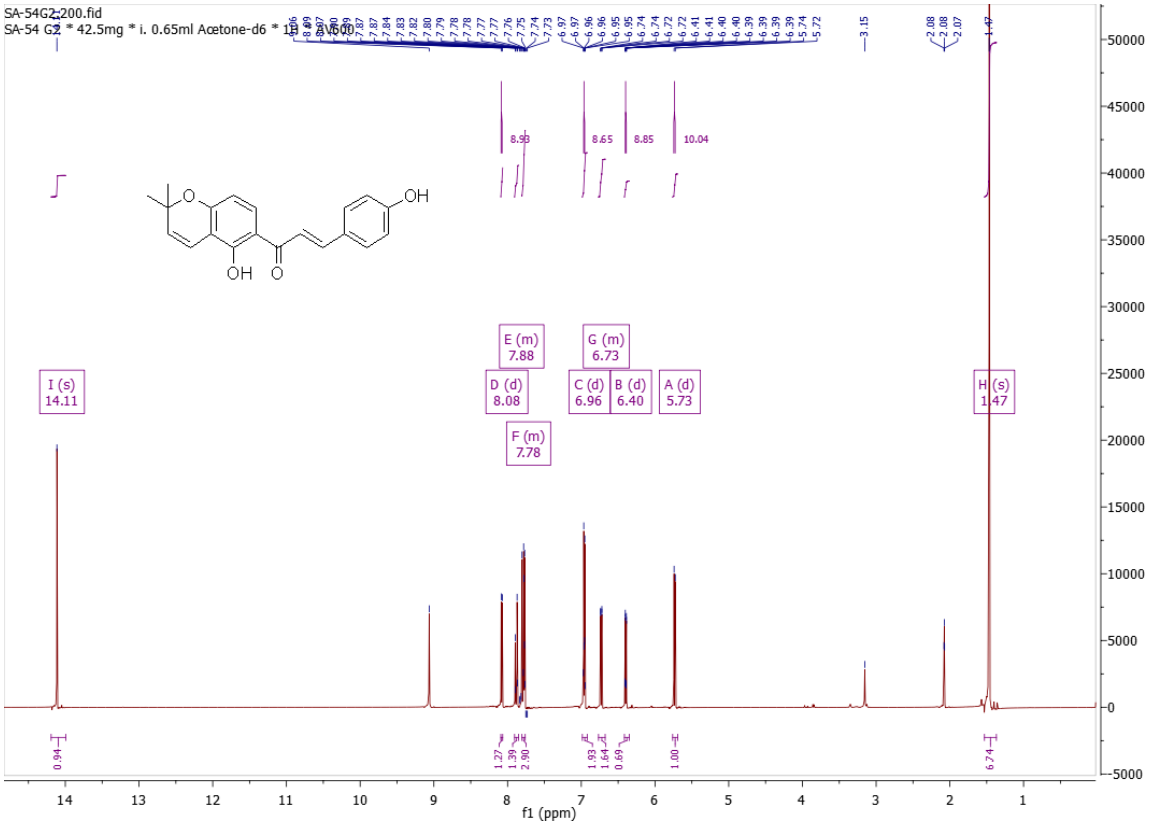


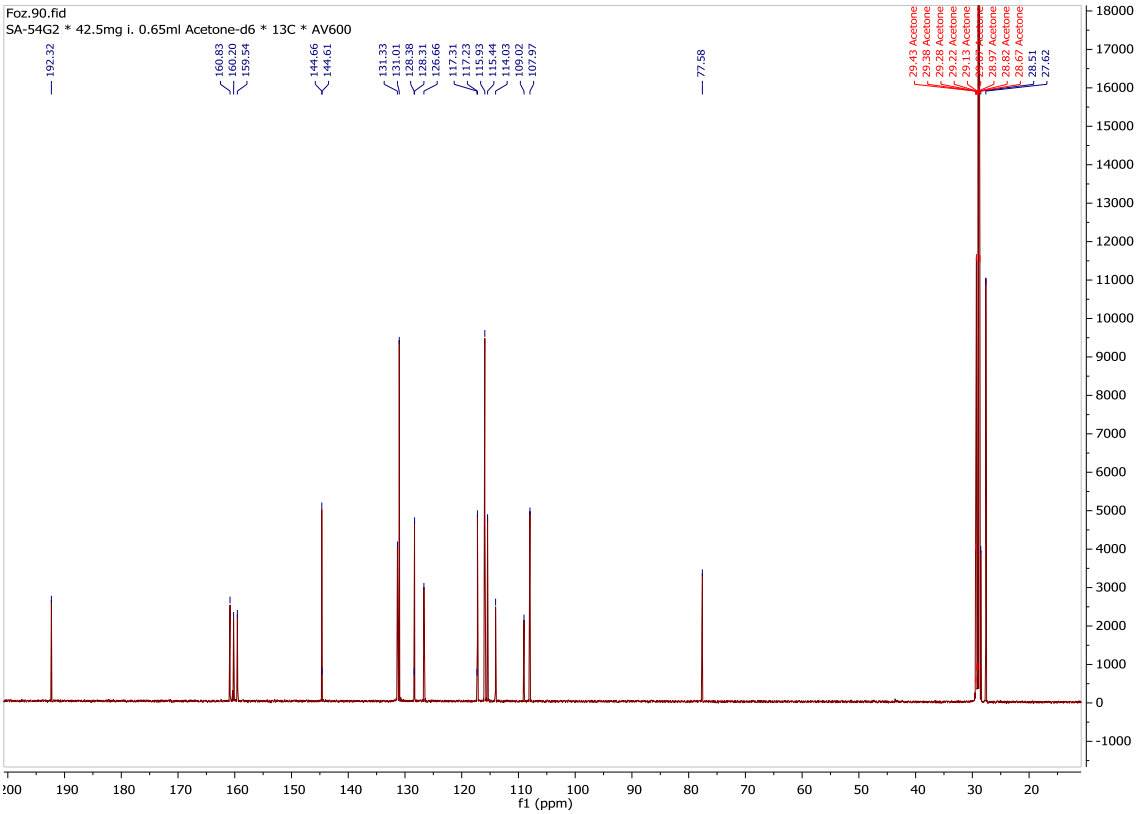
Appendix 28C: HMBC (600 MHz; CD_2Cl_2) and LCMS spectrum of compound 28



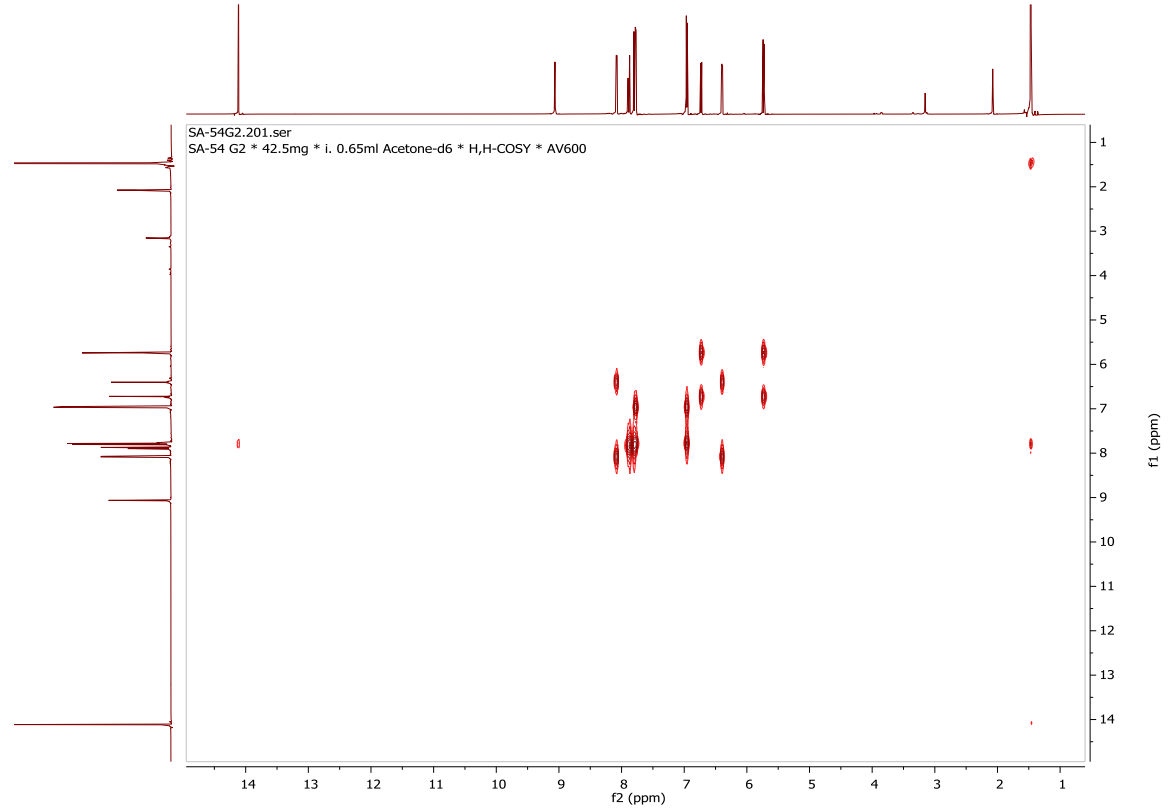


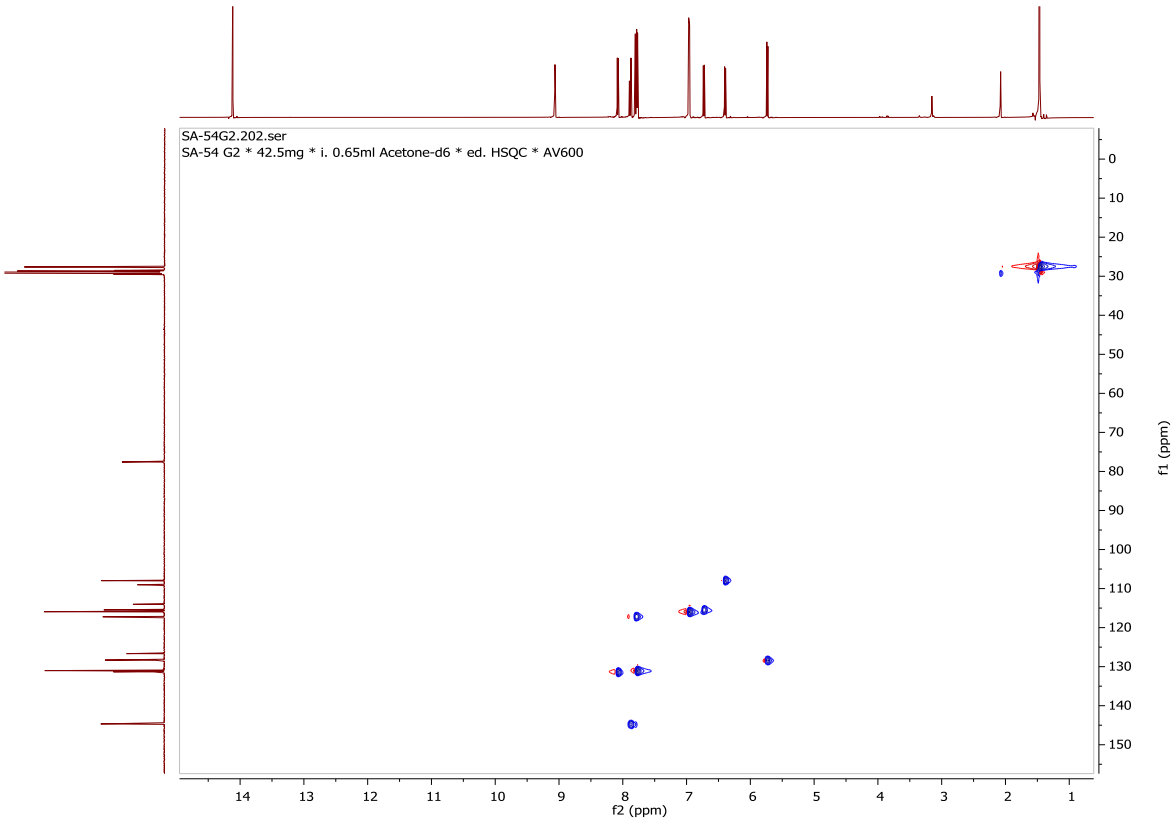
Appendix 29A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectrum of compound 29 (CD_2Cl_2)



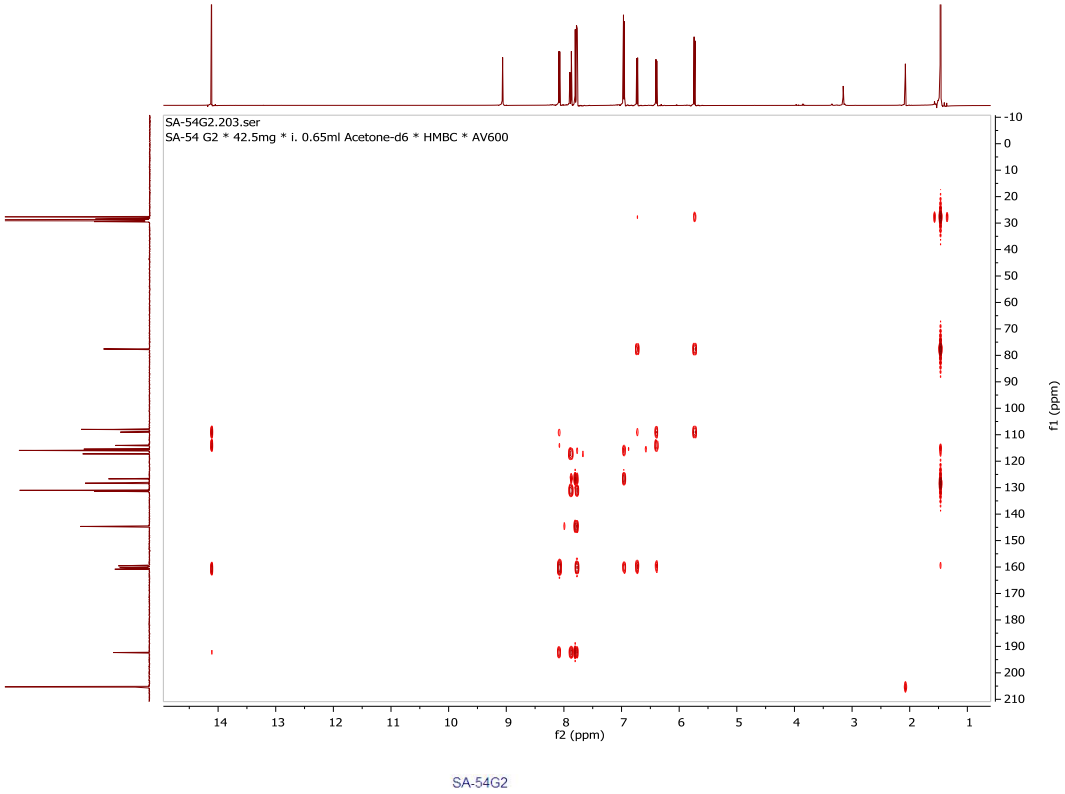


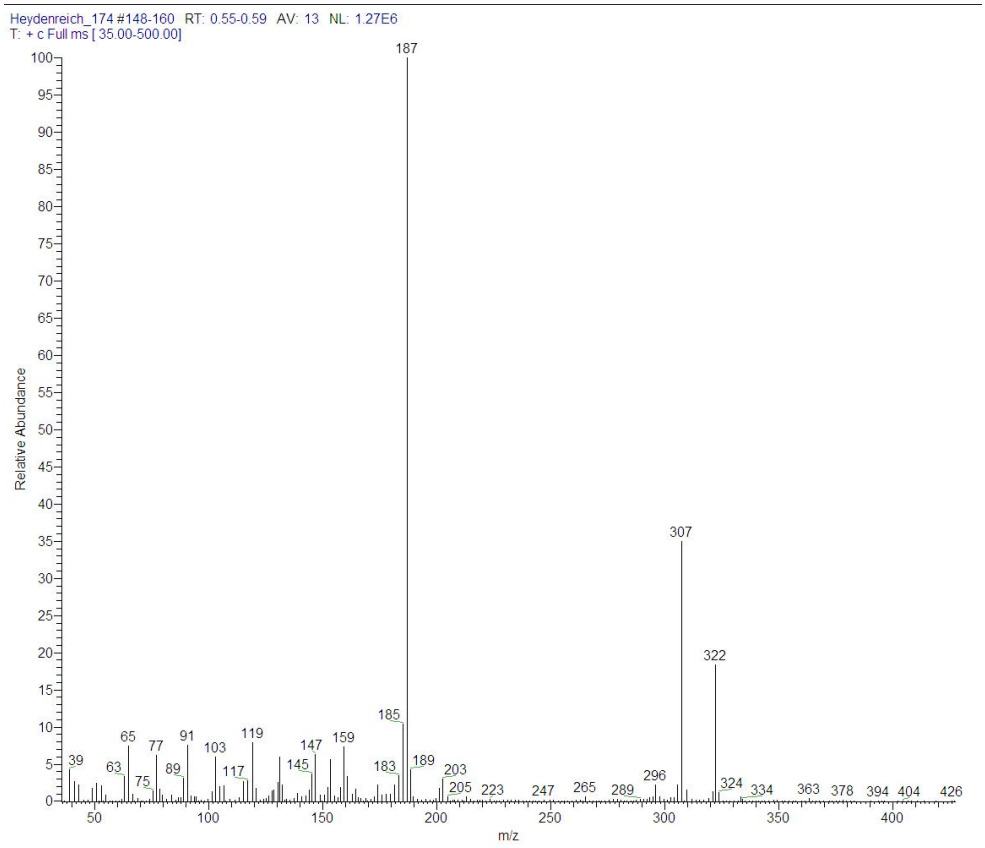
Appendix 29B: H,H-COSY and HSQC spectrum of compound 29 (600 MHz; CD_2Cl_2)



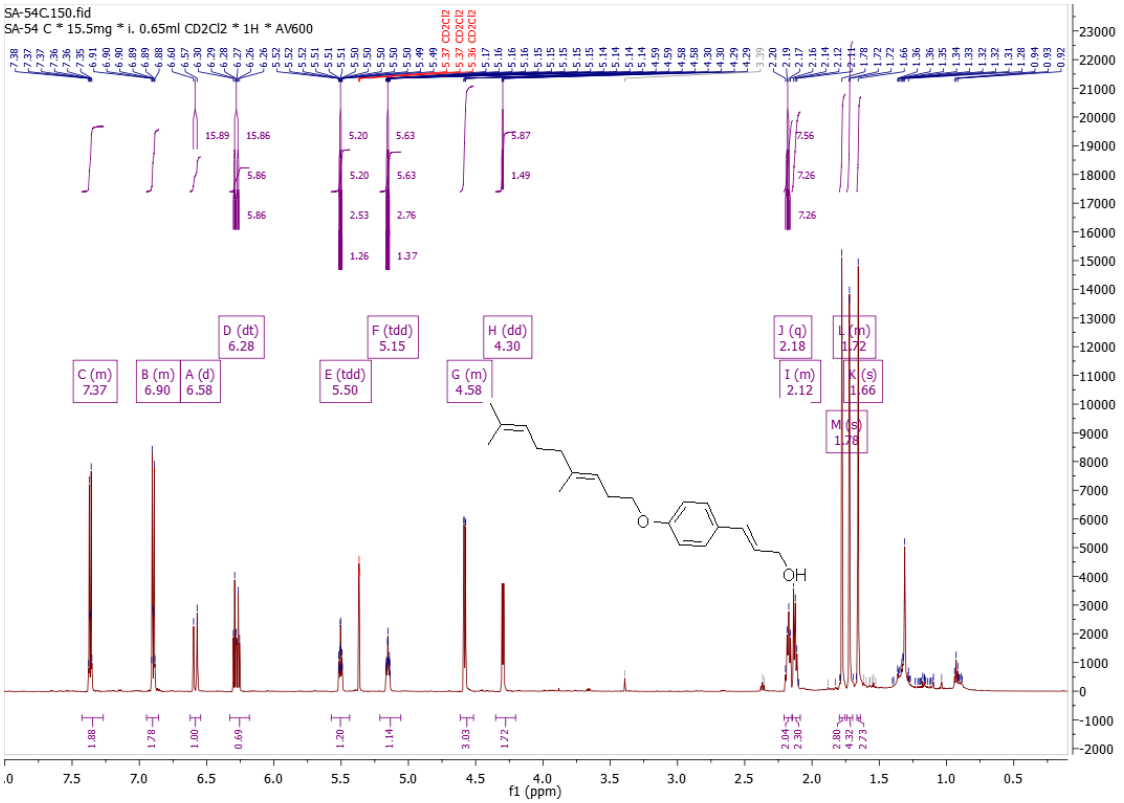


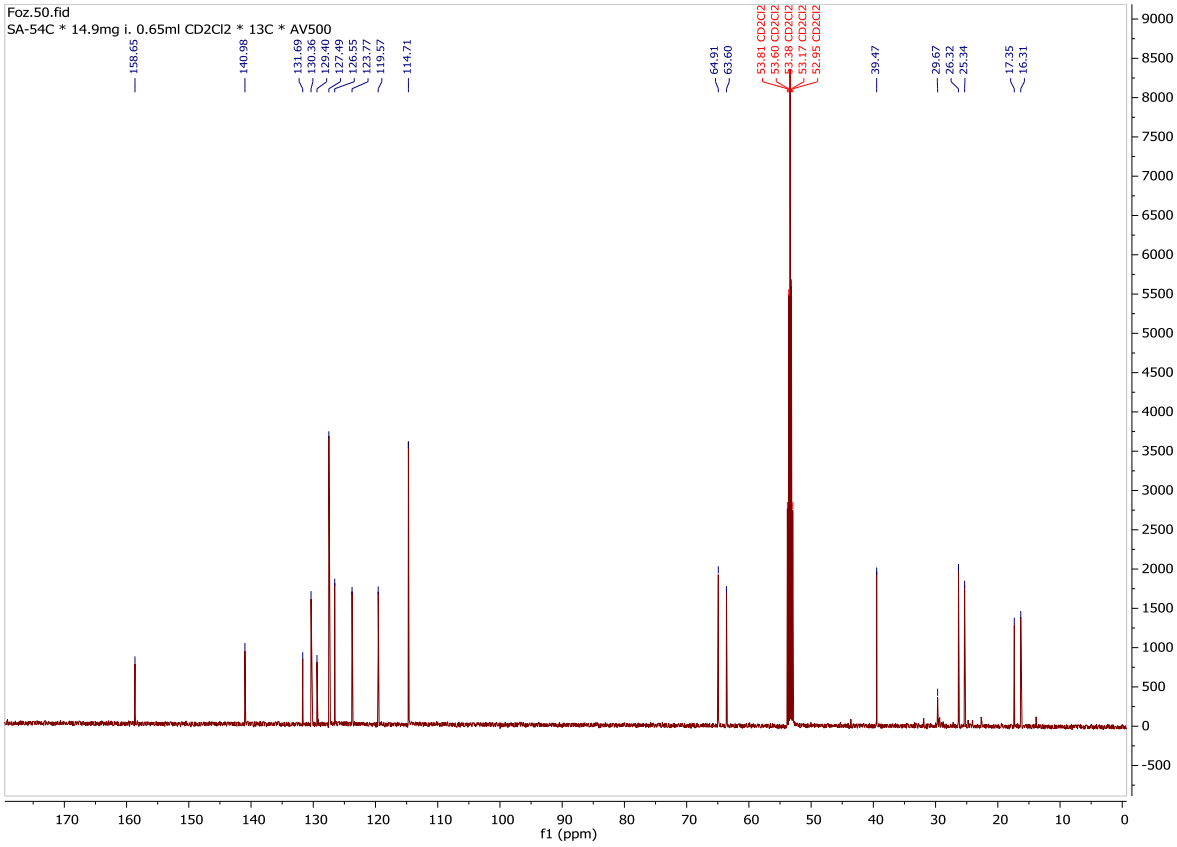
Appendix 29C: HMBC (600 MHz; CD_2Cl_2) and LCMS spectra of compound 29



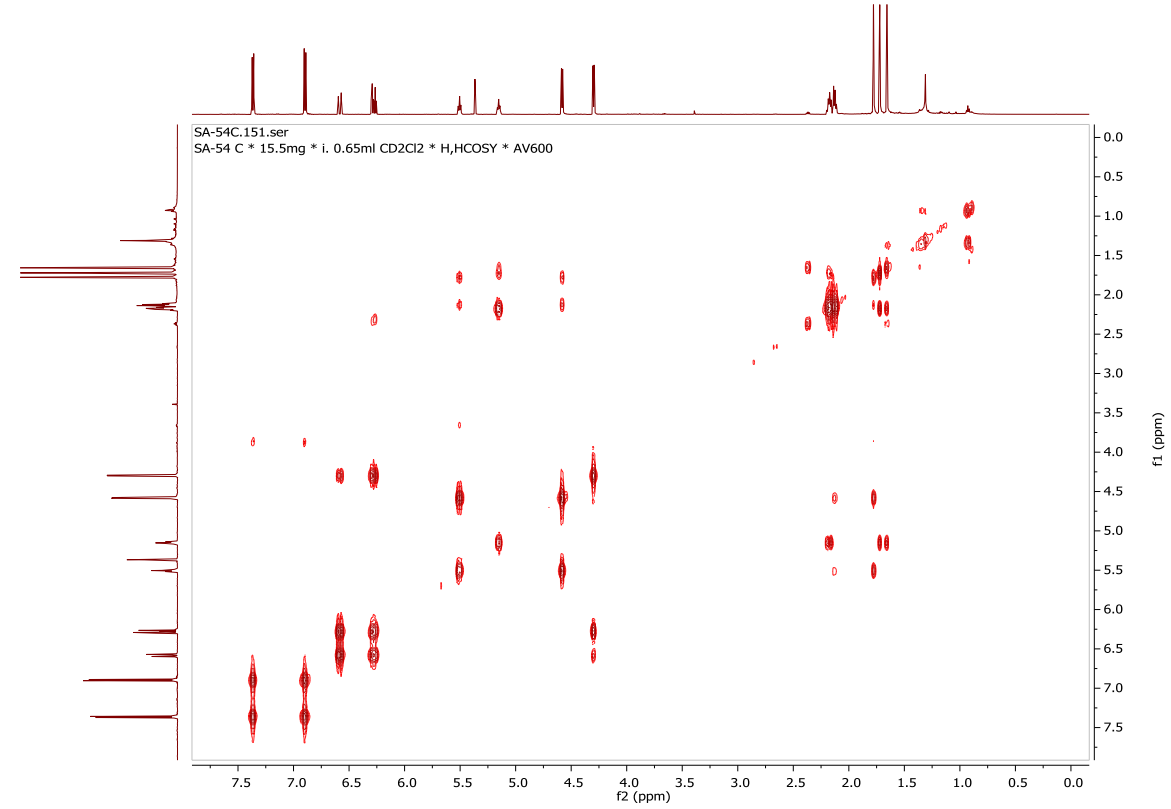


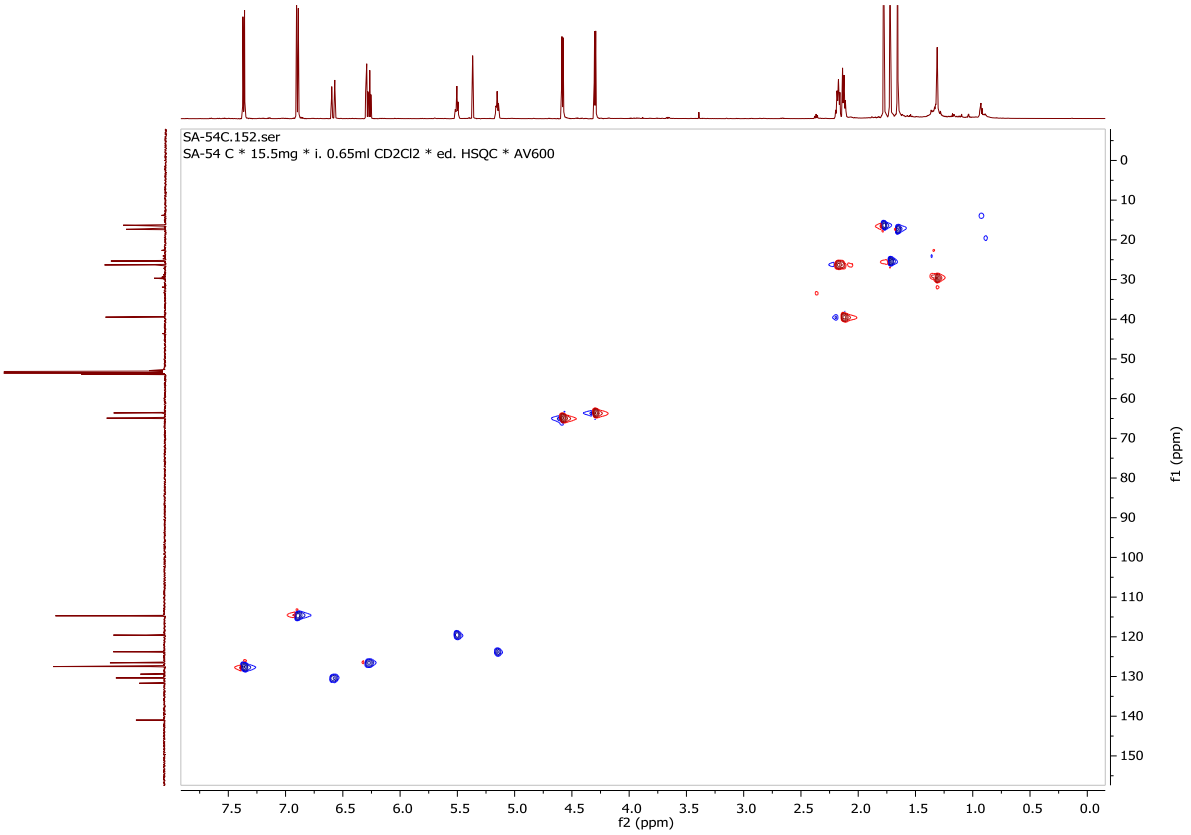
Appendix 30A: ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR spectra of compound 30 (CD_2Cl_2)



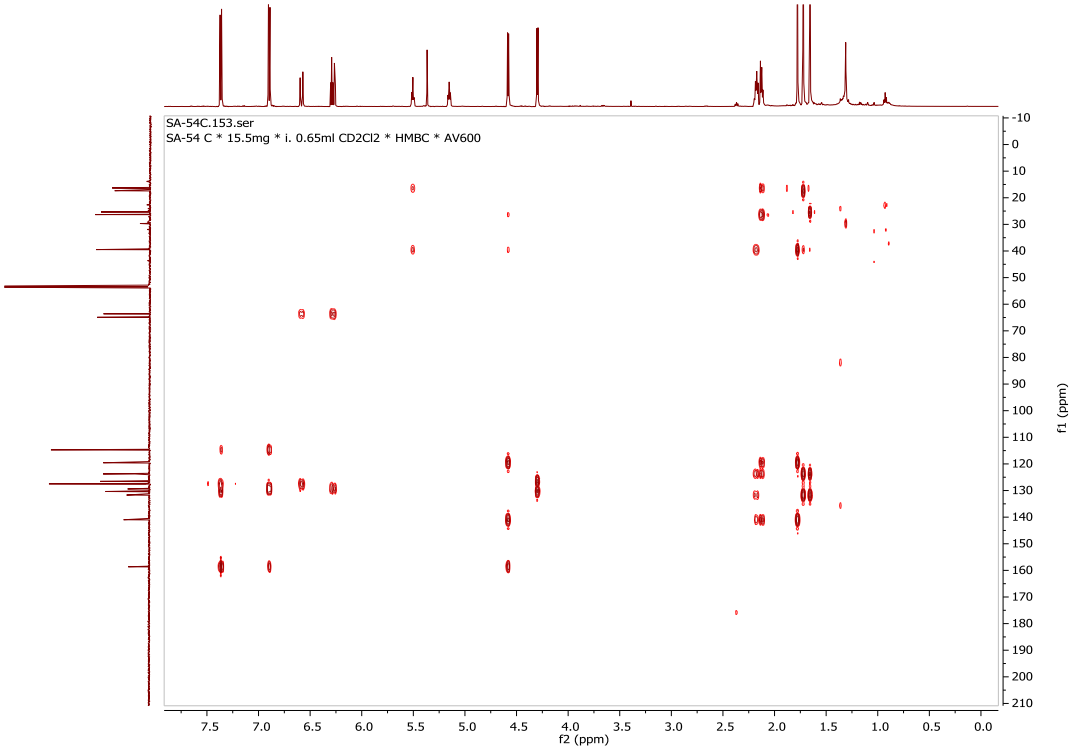


Appendix 30B: H,H-COSY and HSQC spectra of compound 30 (600 MHz; CD_2Cl_2)





Appendix 30C: HMBC (600 MHz; CD_2Cl_2) and LCMS spectra of compound 30



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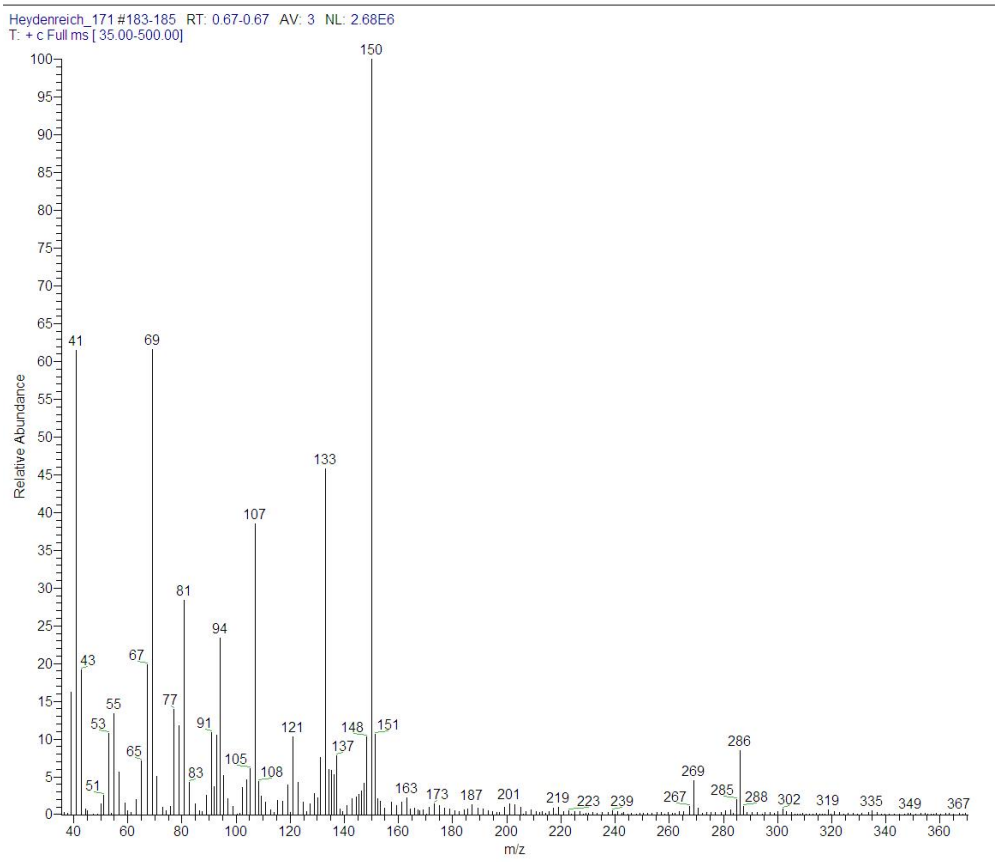
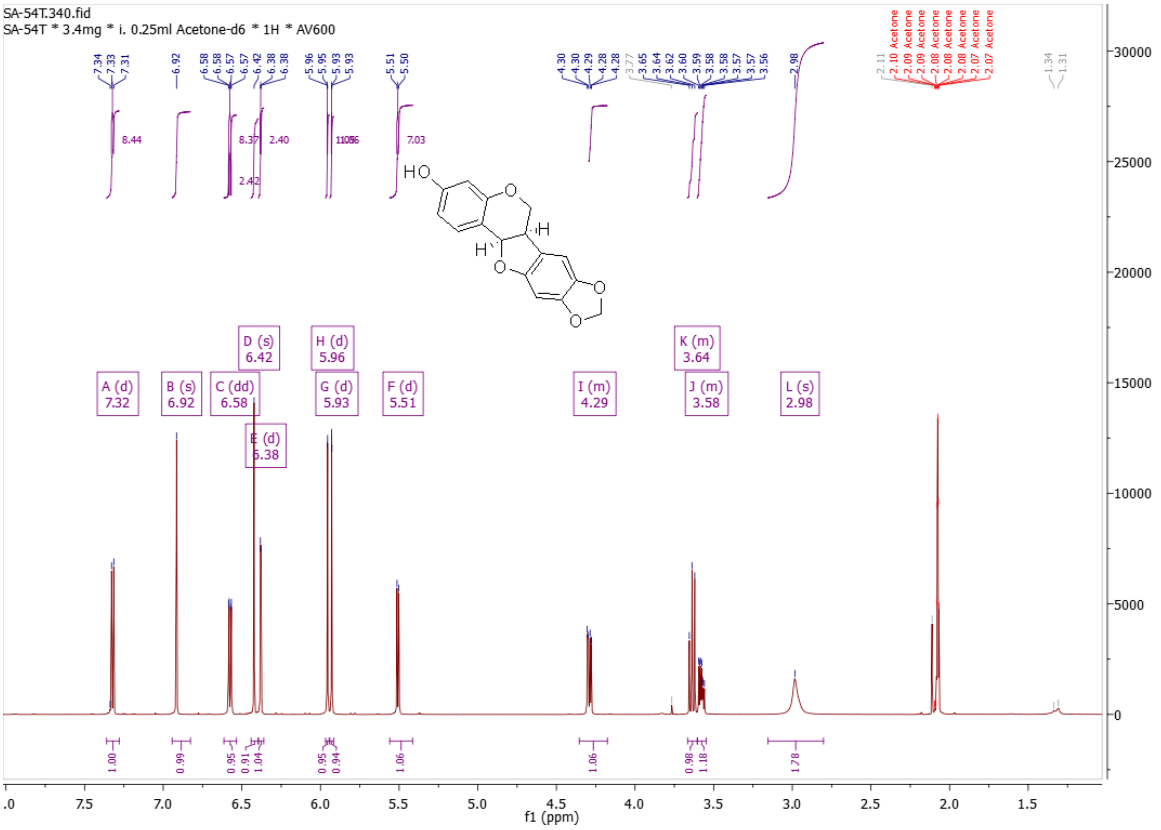
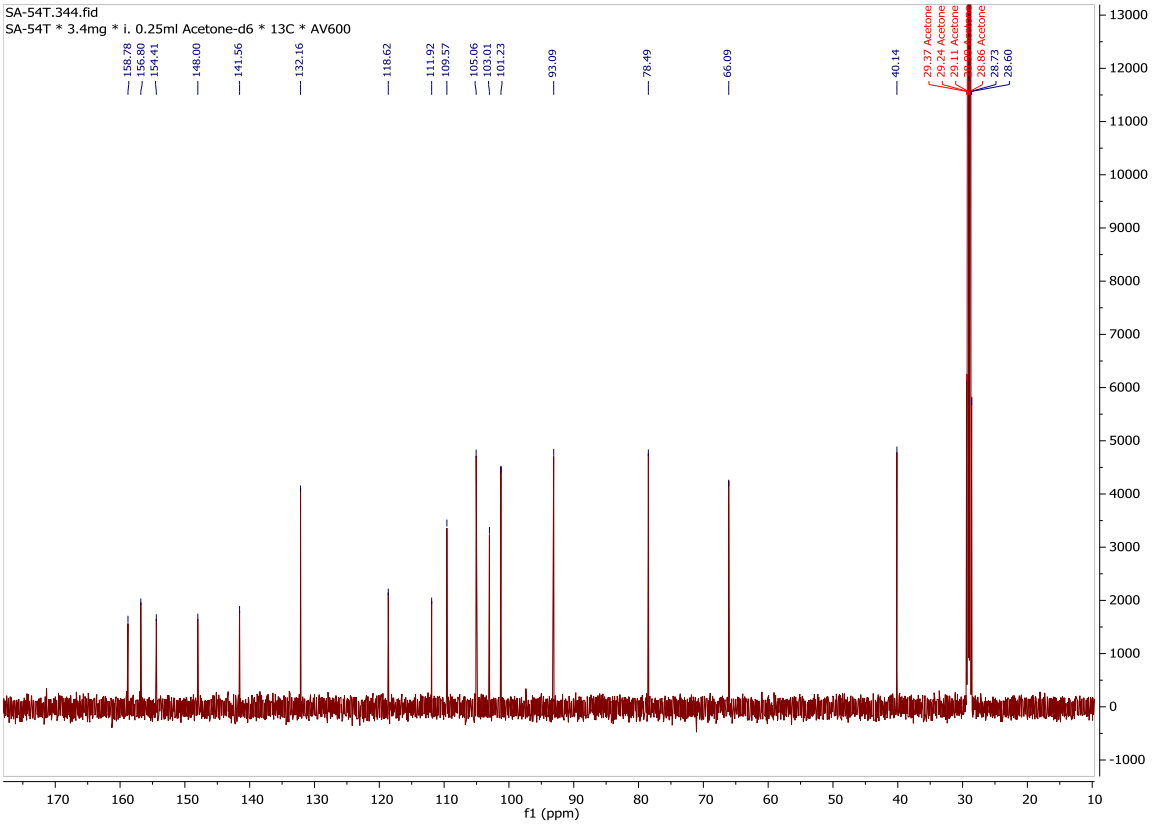
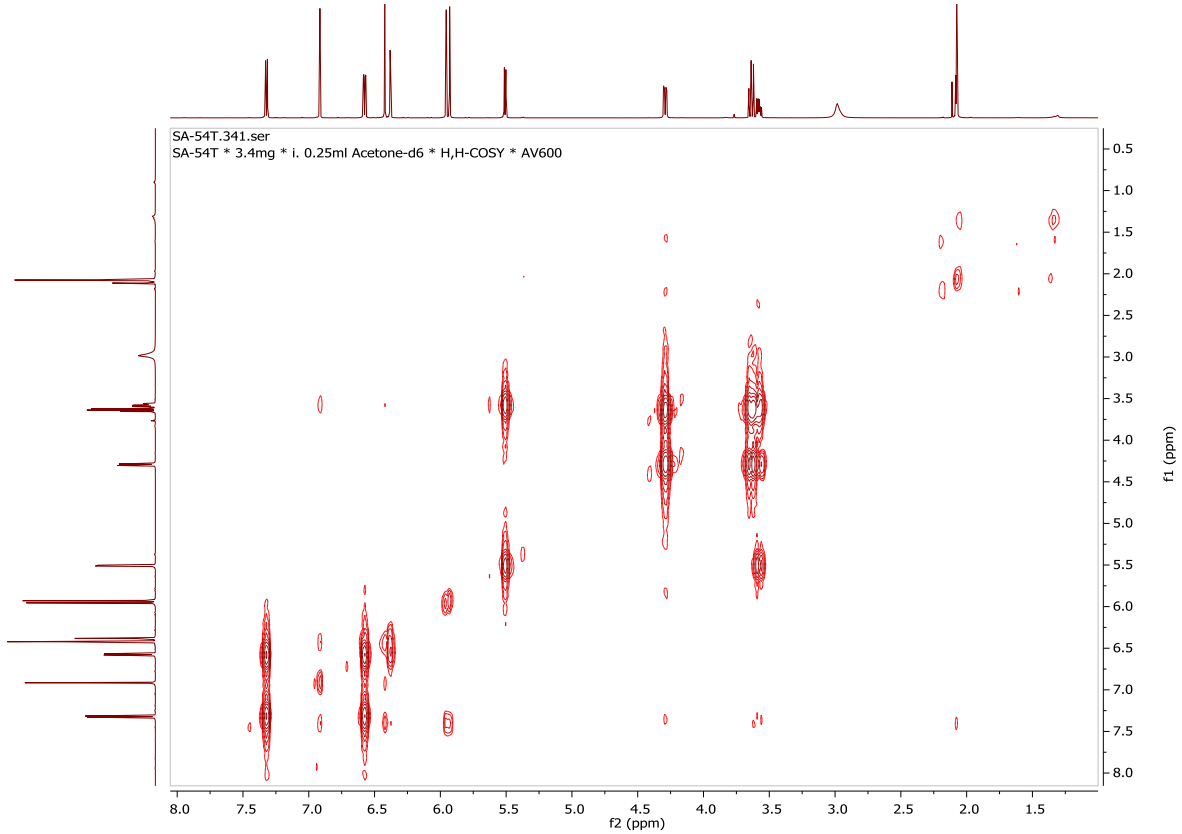


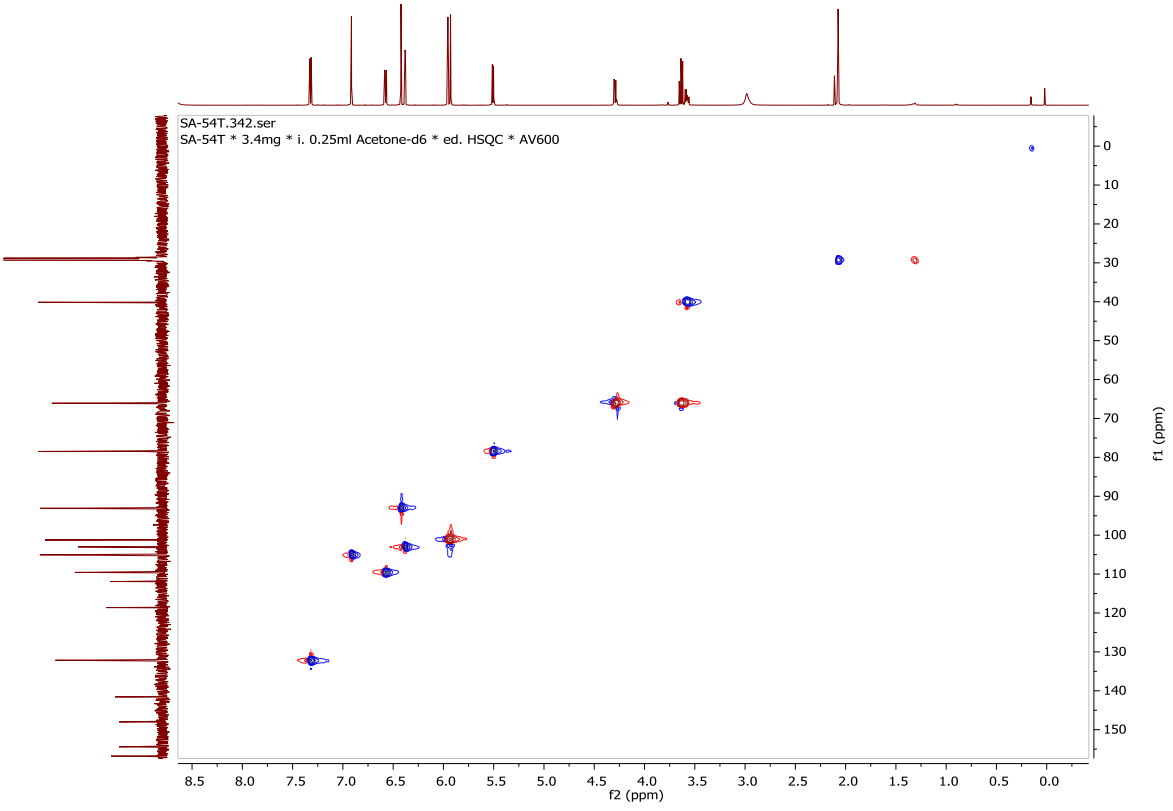
Appendix 31A: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra of compound 31 (CD_2Cl_2)



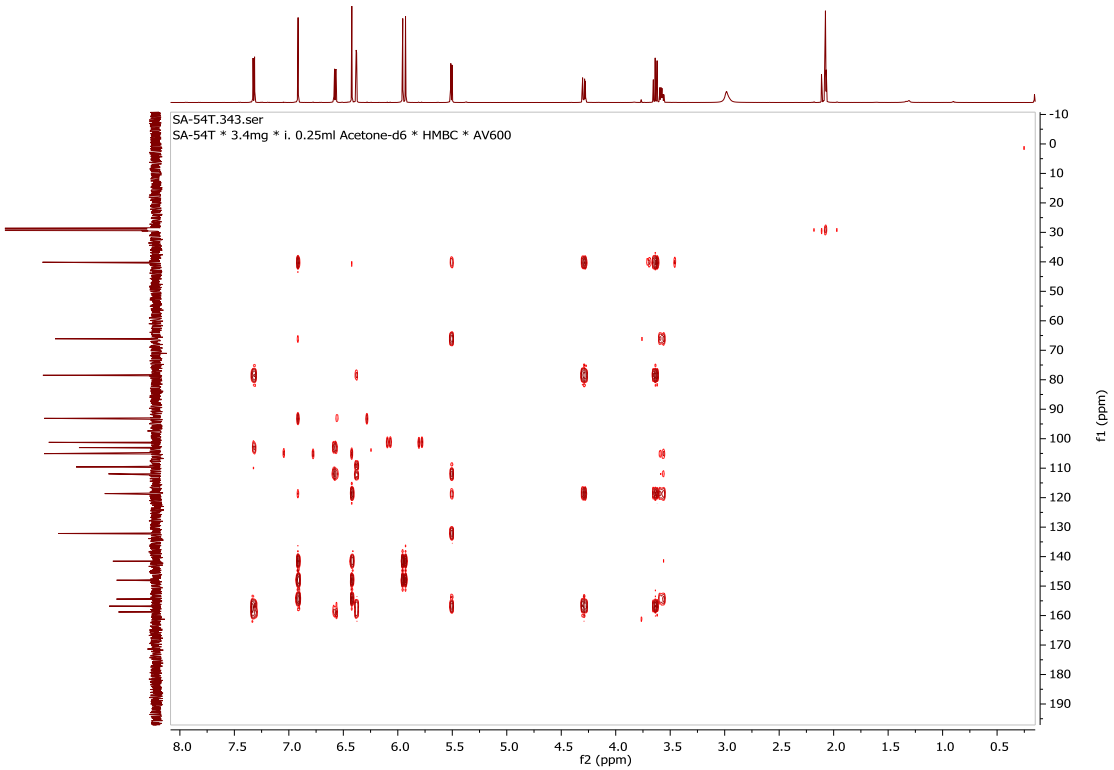


Appendix 31B: H,H-COSY and HSQC spectra of compound 31 (600 MHz; CD_2Cl_2)





Appendix 31C: HMBC (600 MHz; CD_2Cl_2) and LCMS spectra of compound 31



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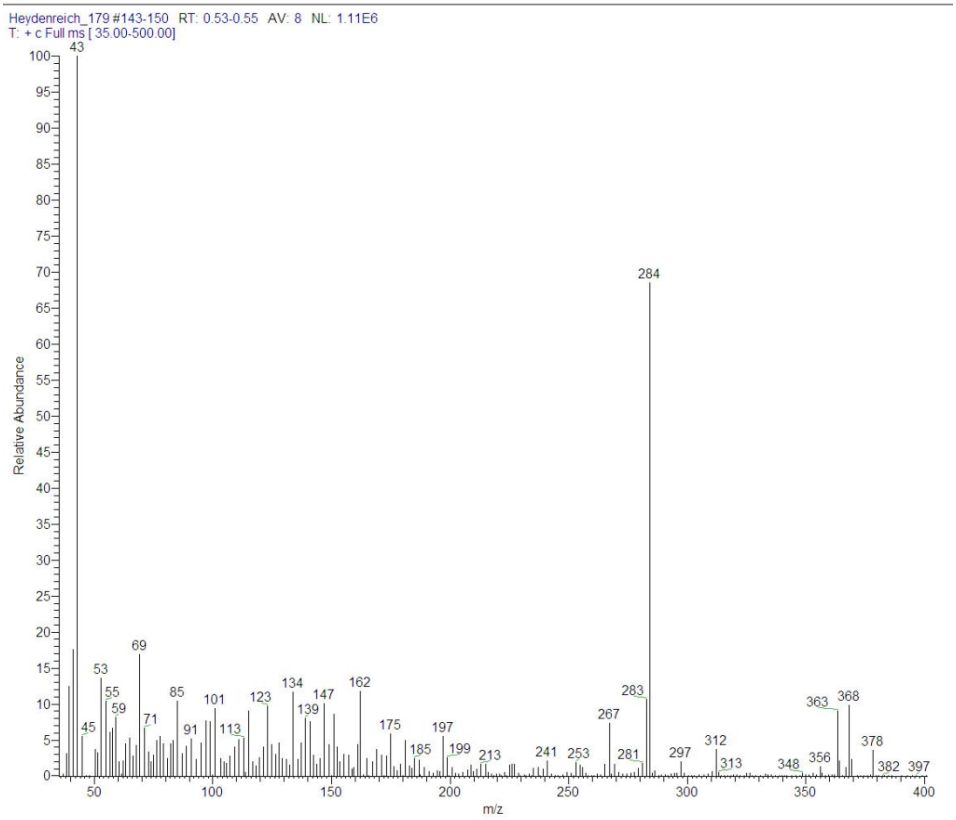
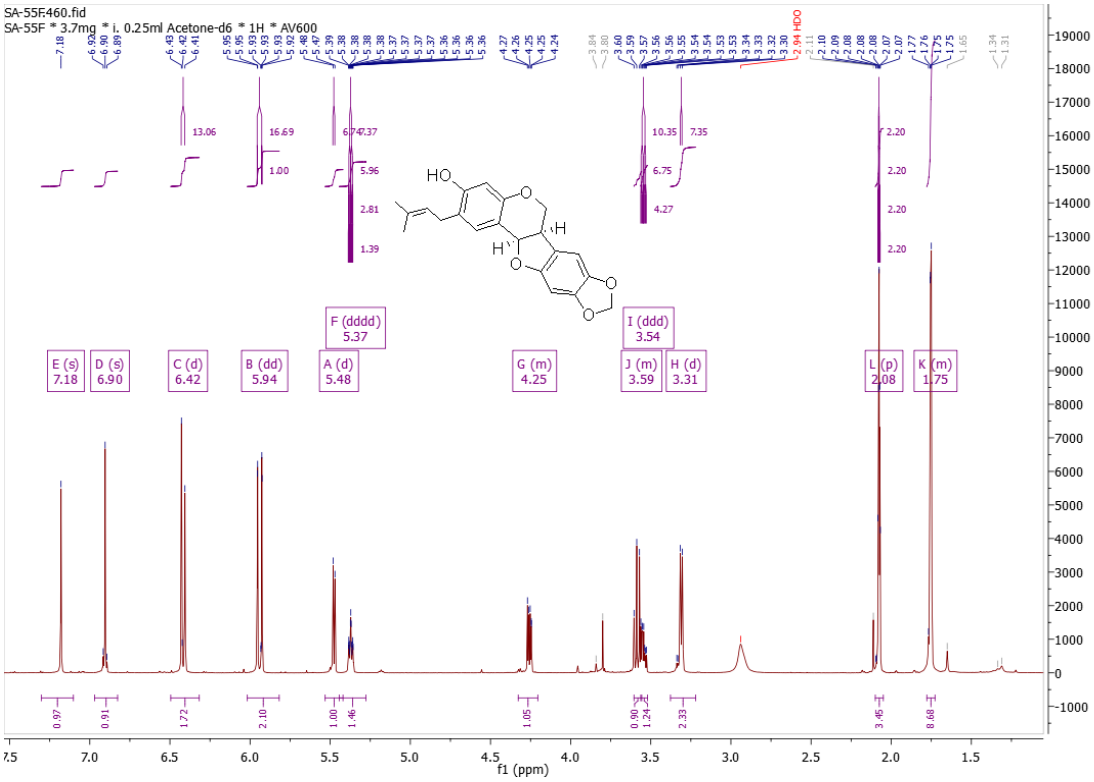
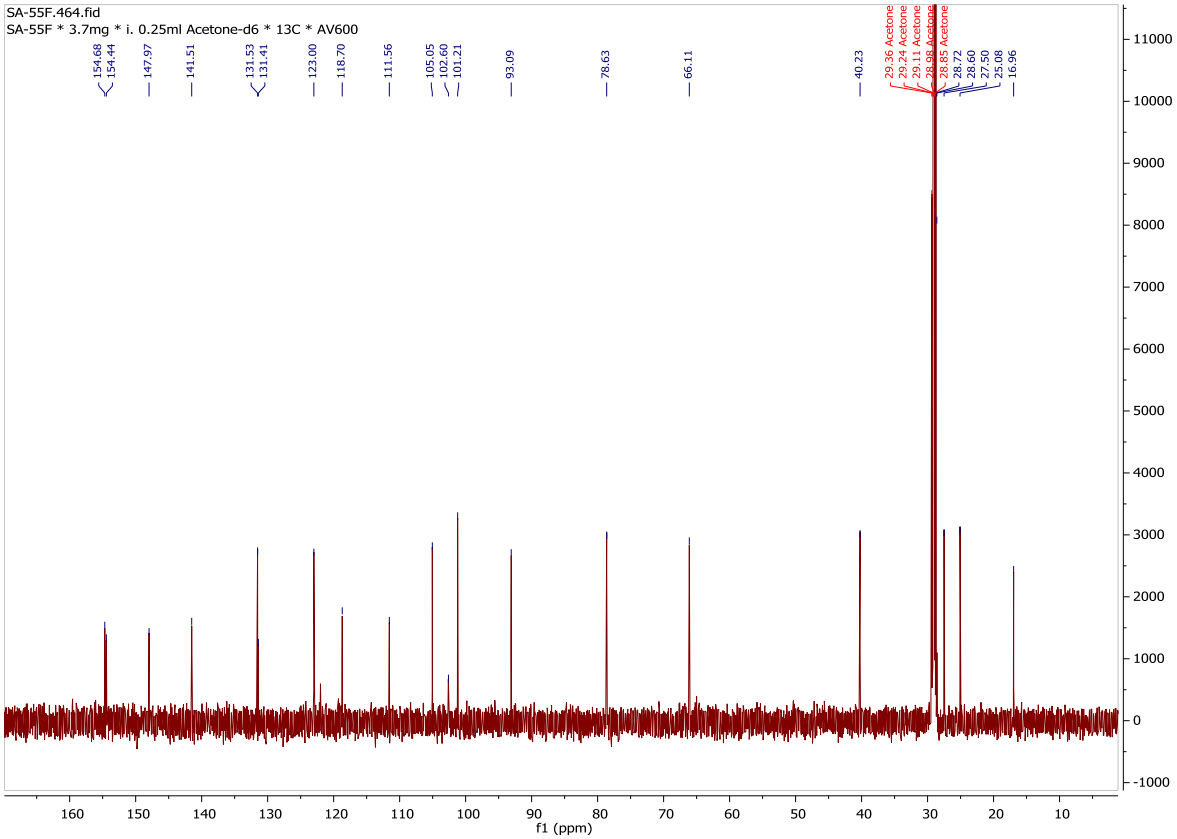
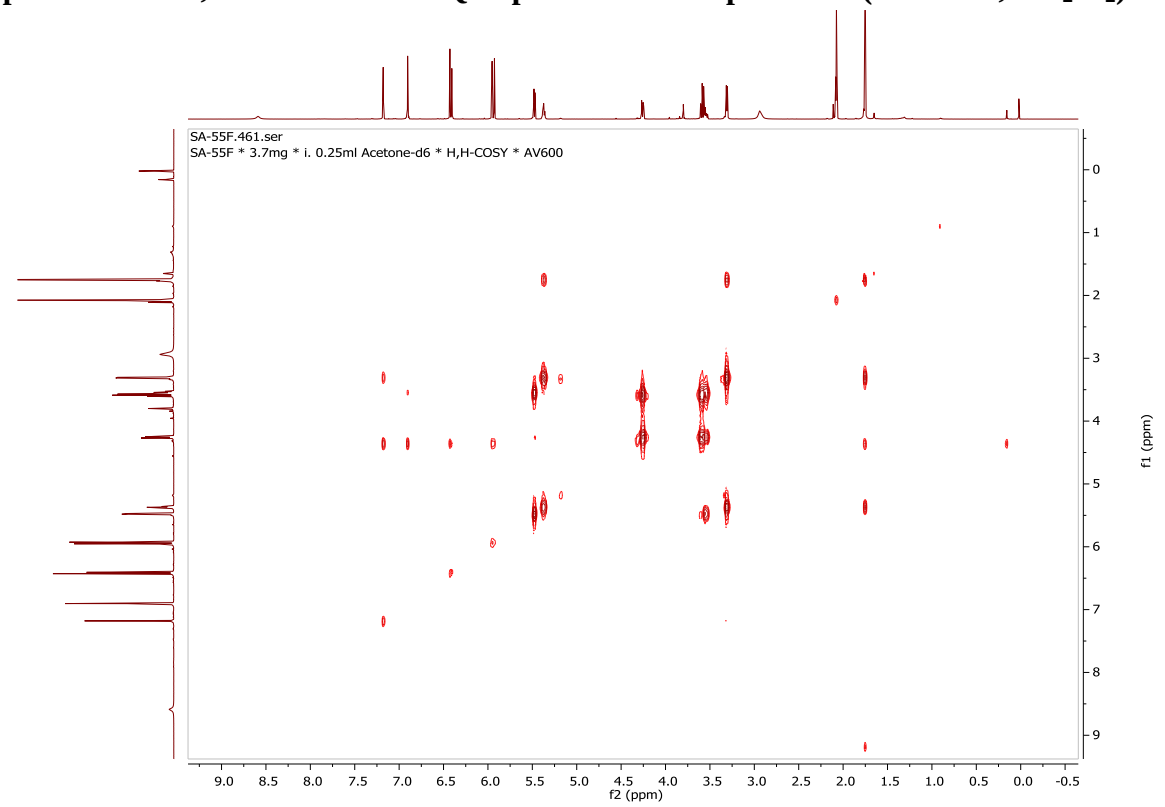


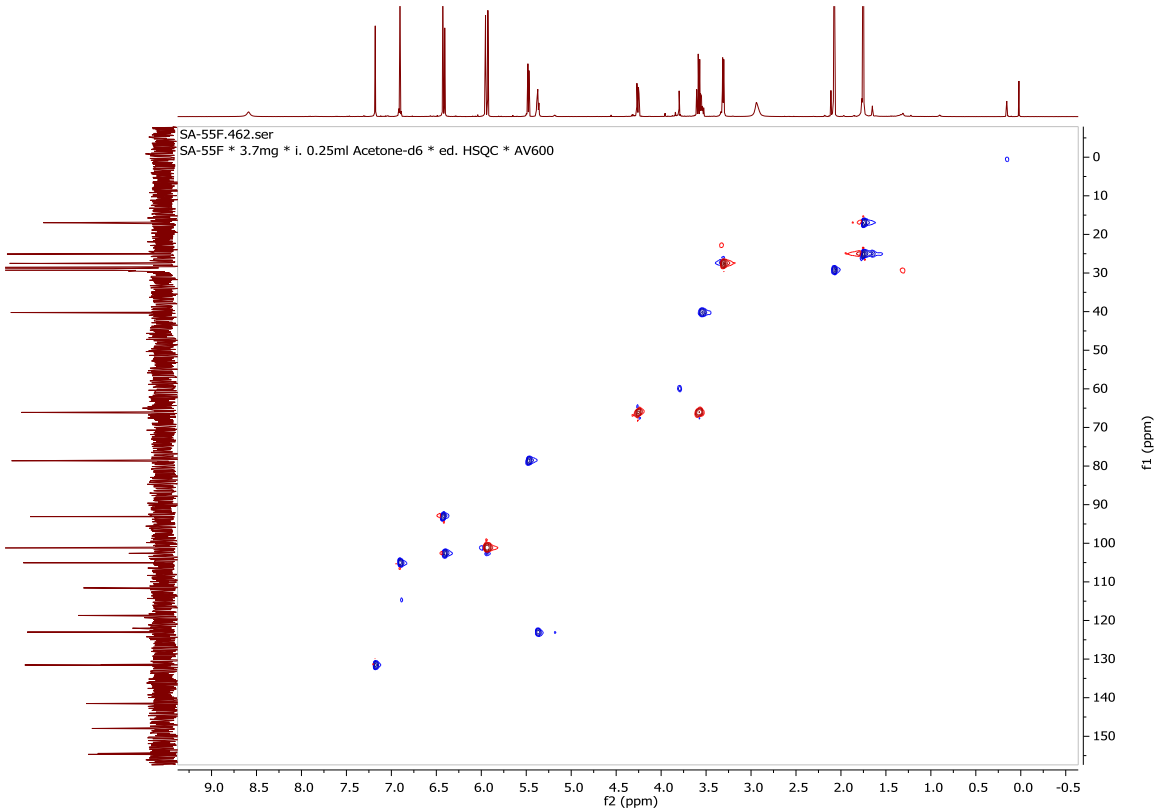
Appendix 32A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of compound 32 (CD_2Cl_2)



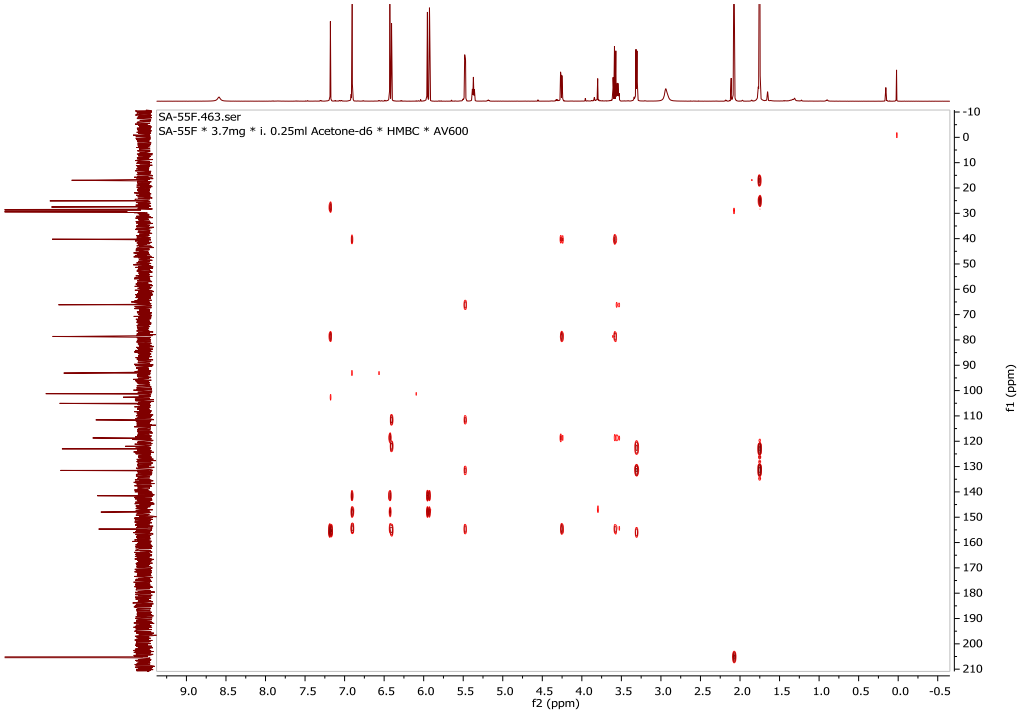


Appendix 32B: H,H-COSY and HSQC spectrum of compound 32 (600 MHz; CD_2Cl_2)





Appendix 32C: HMBC and LCMS spectra of compound 32 (600 MHz; CD_2Cl_2)



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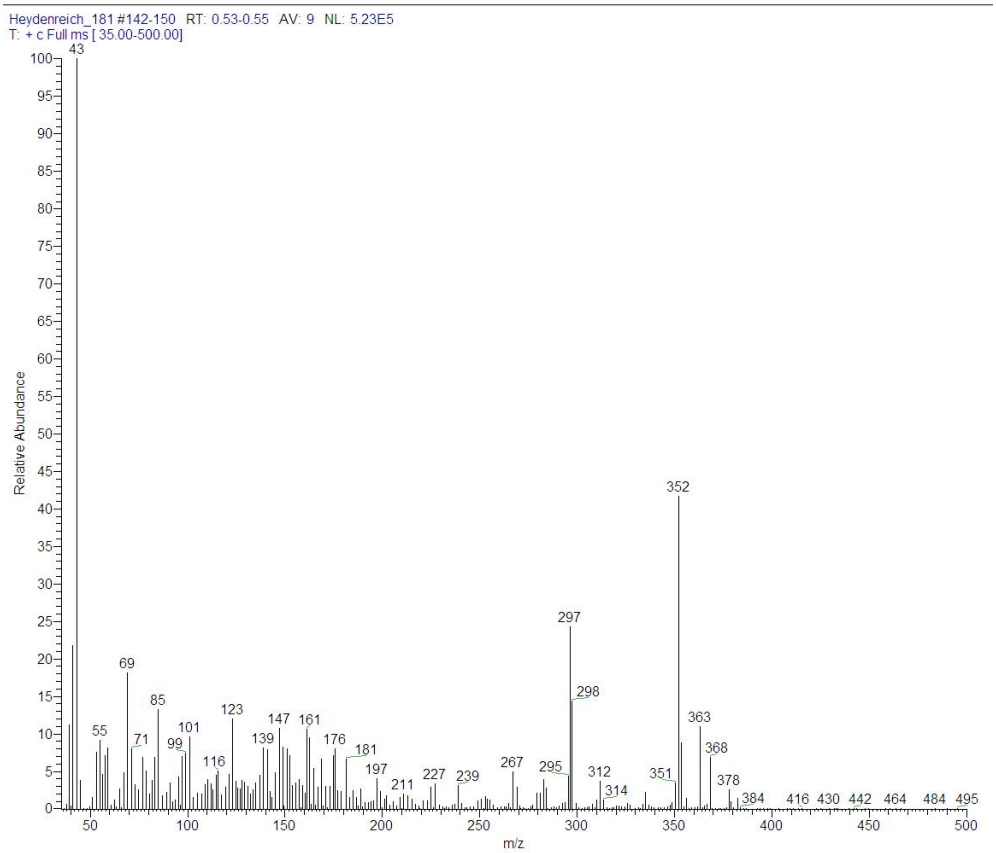
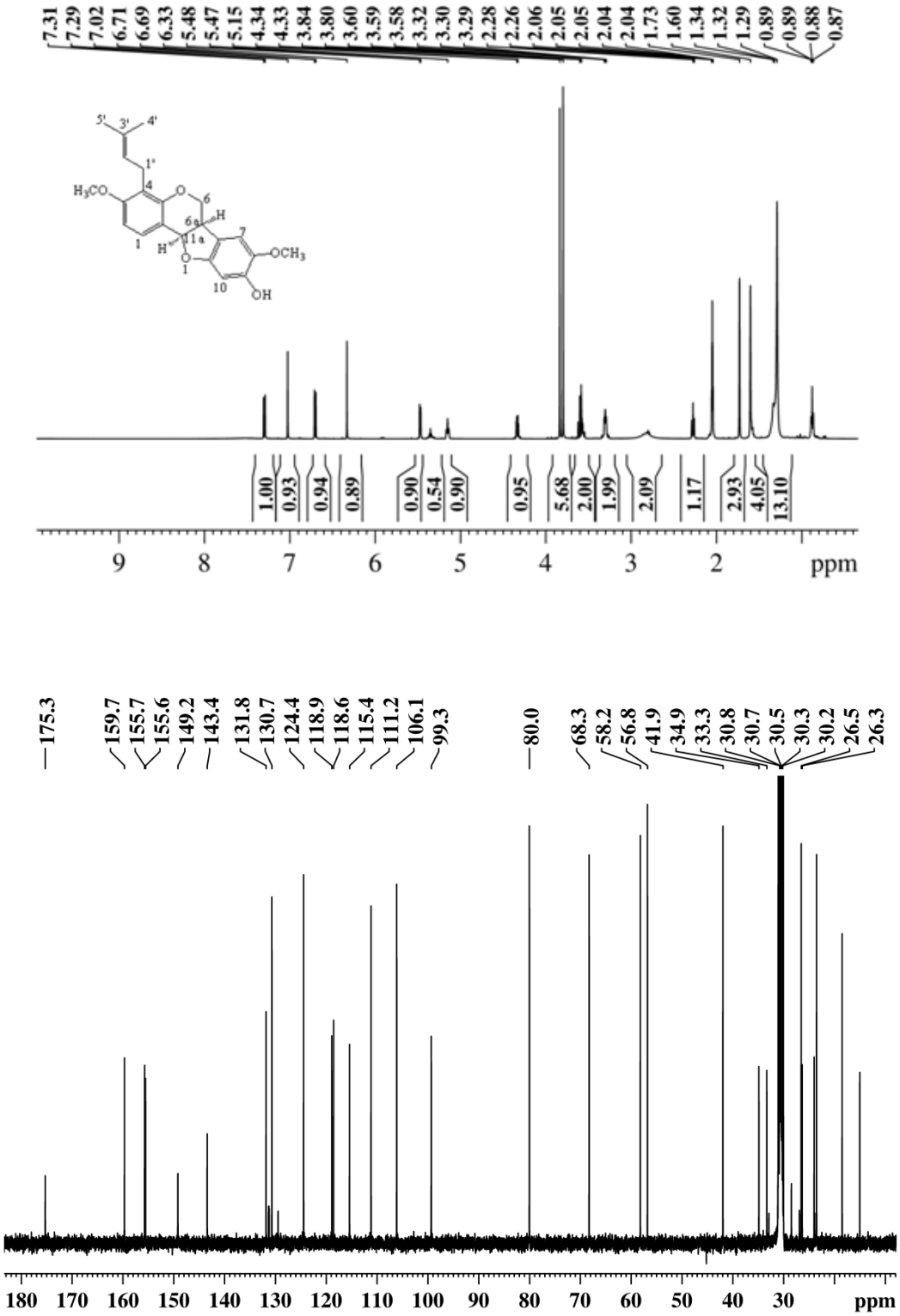
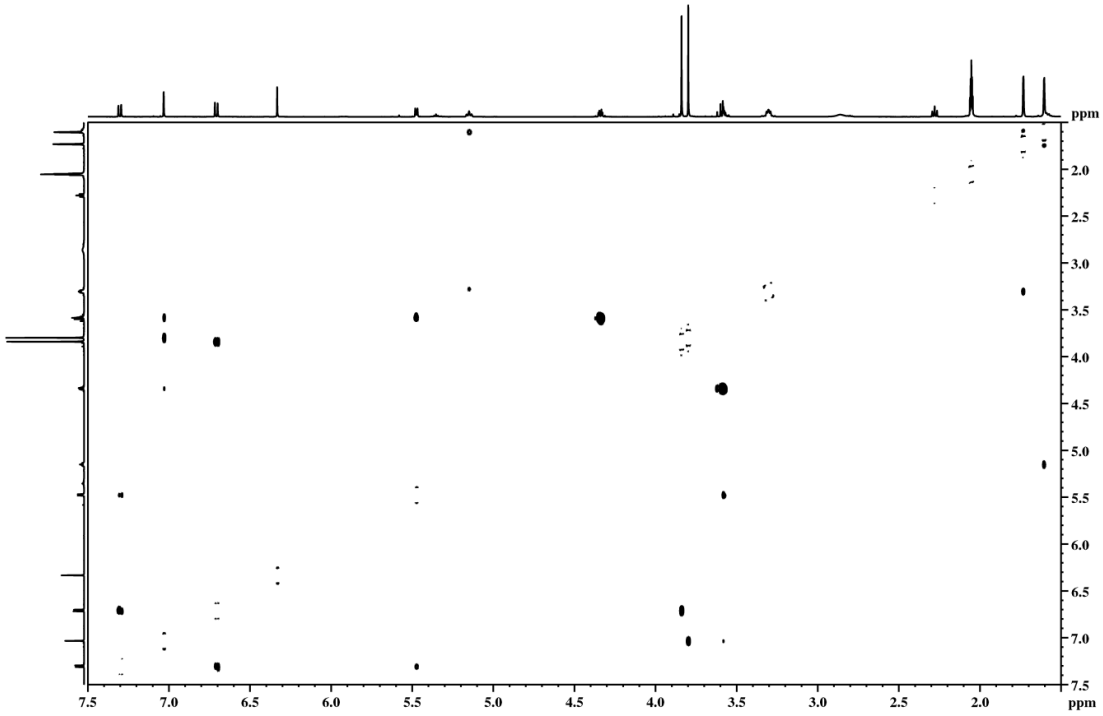
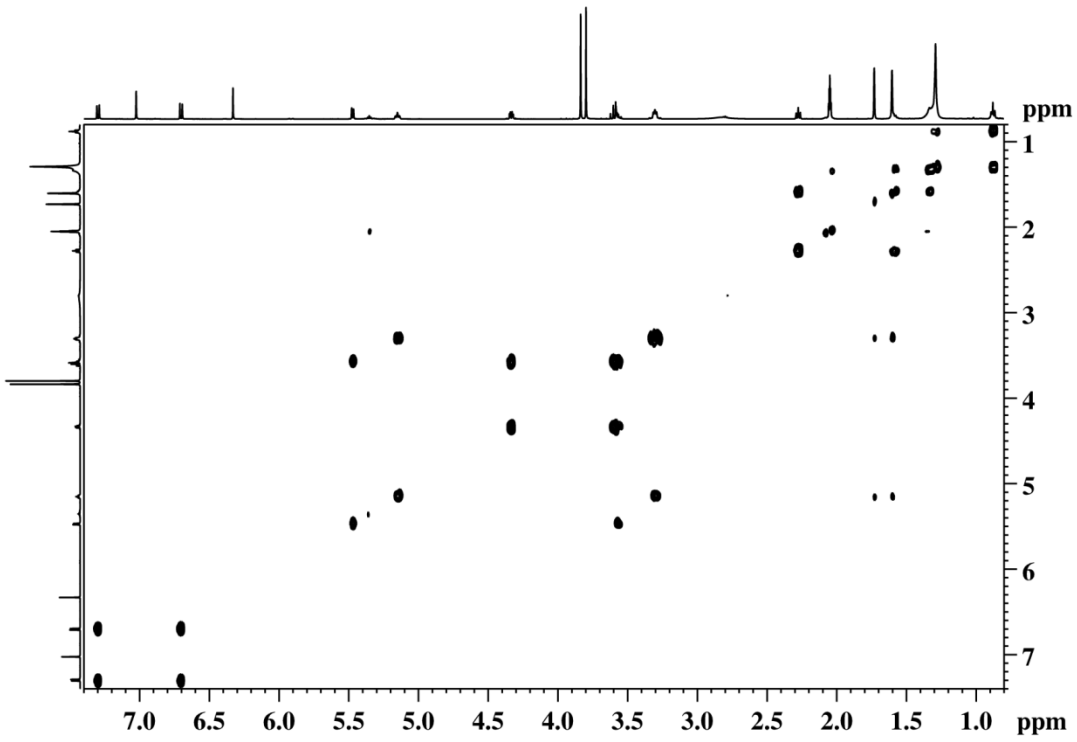


Appendix 33A: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra of compound 33 (CD_2Cl_2)

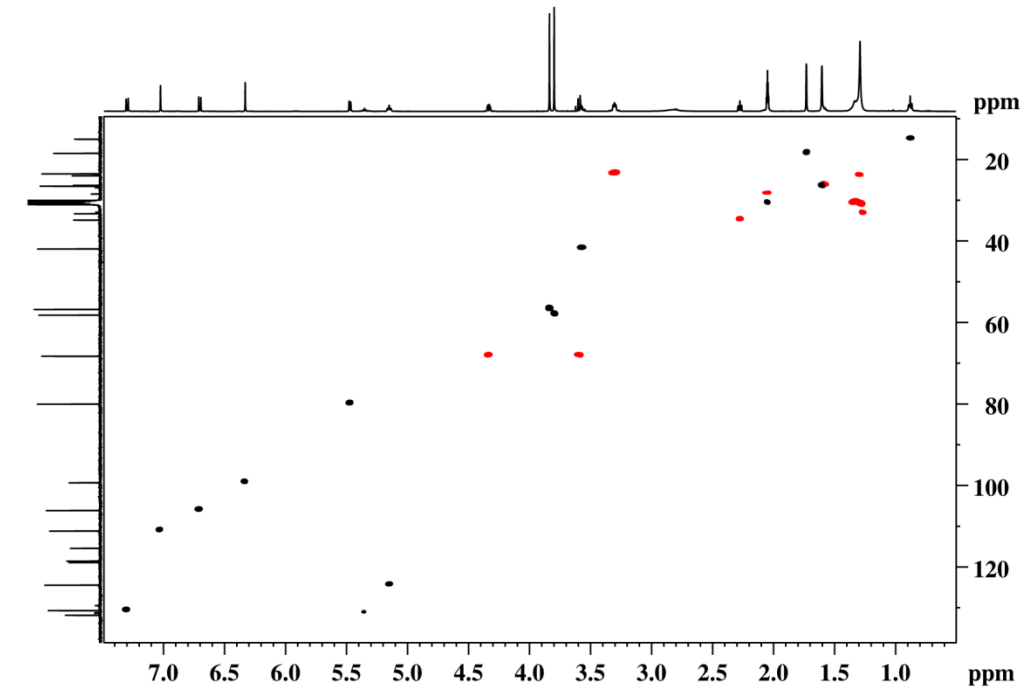


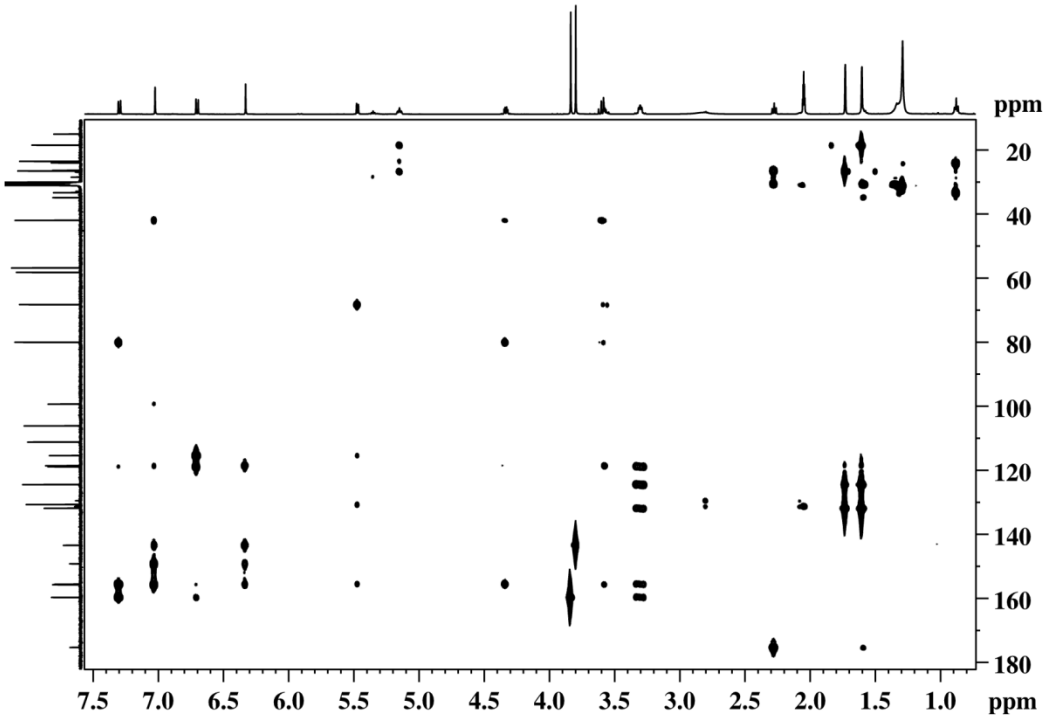
Appendix 33B: NOSEY (500 MHz) and H,H-COSY (600 MHz) spectra of compound 33 (CD_2Cl_2)



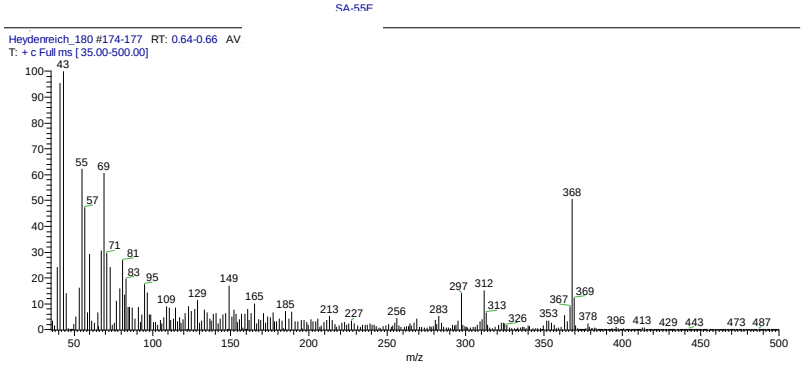


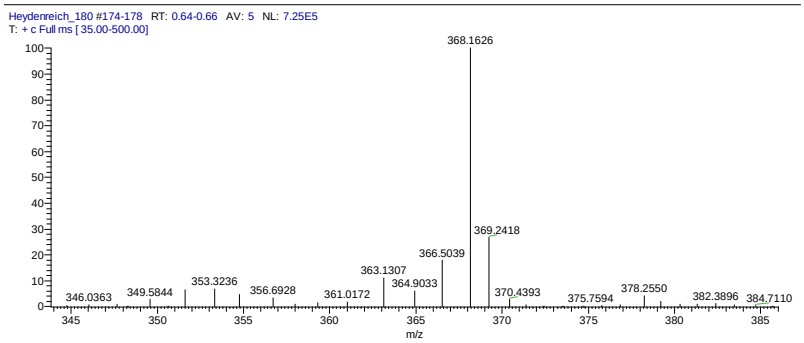
Appendix 33C: HSQC and HMBC spectra of compound 33 (600 MHz; CD_2Cl_2)

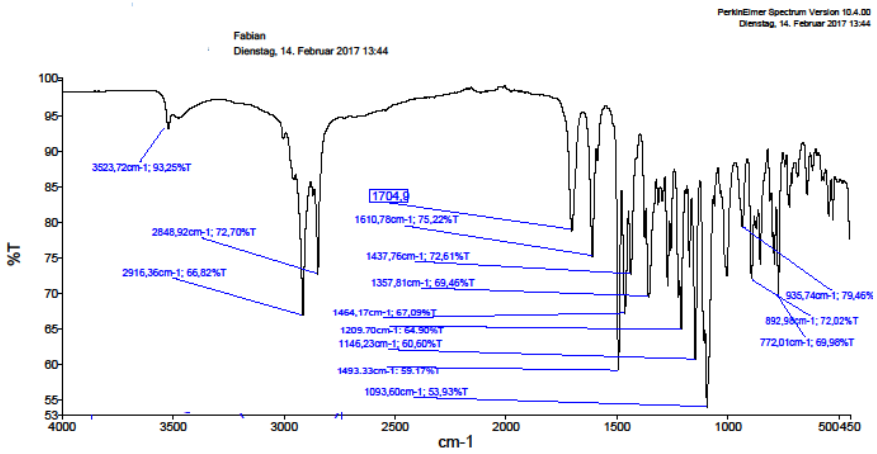


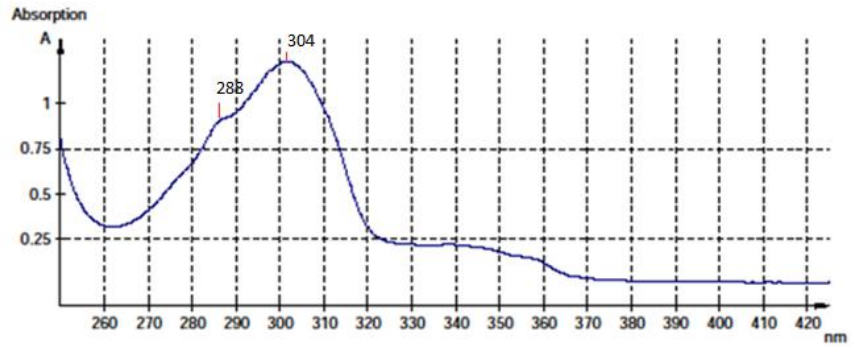


Appendix 33D: HREIMS, IR and UV spectra of compound 33

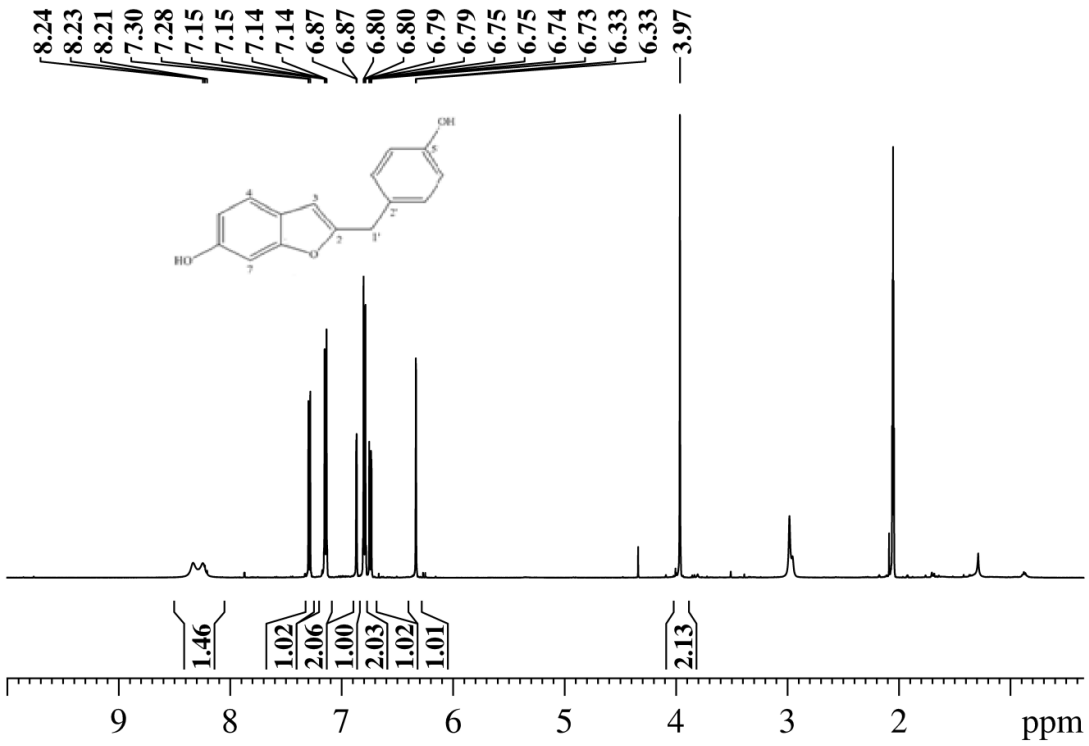


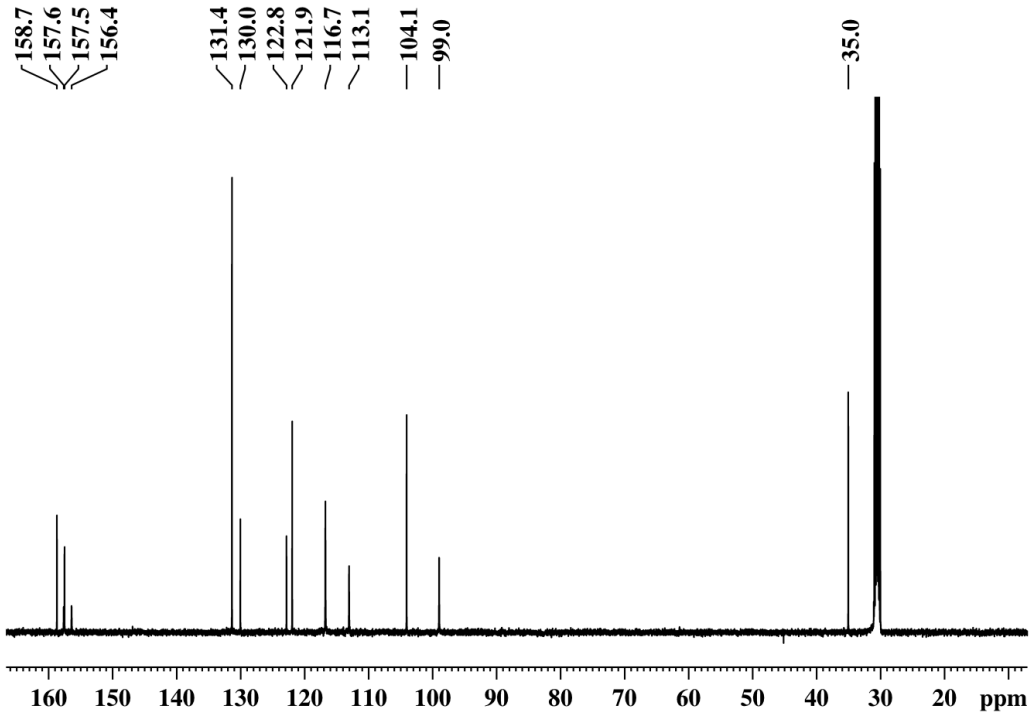




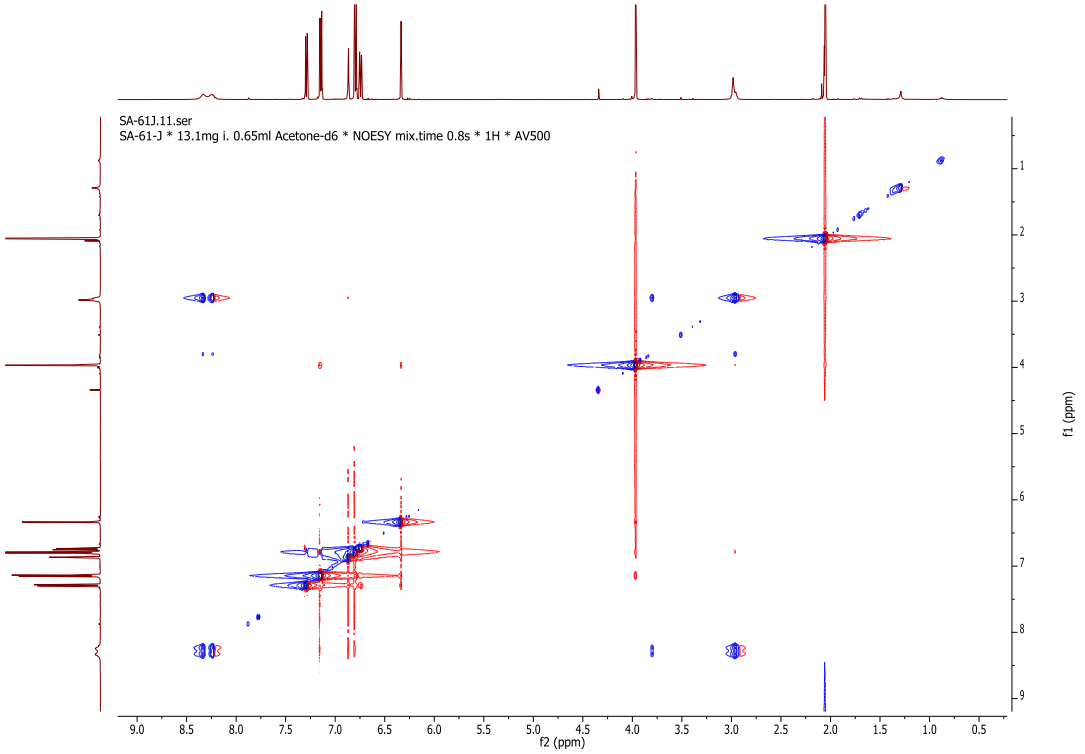


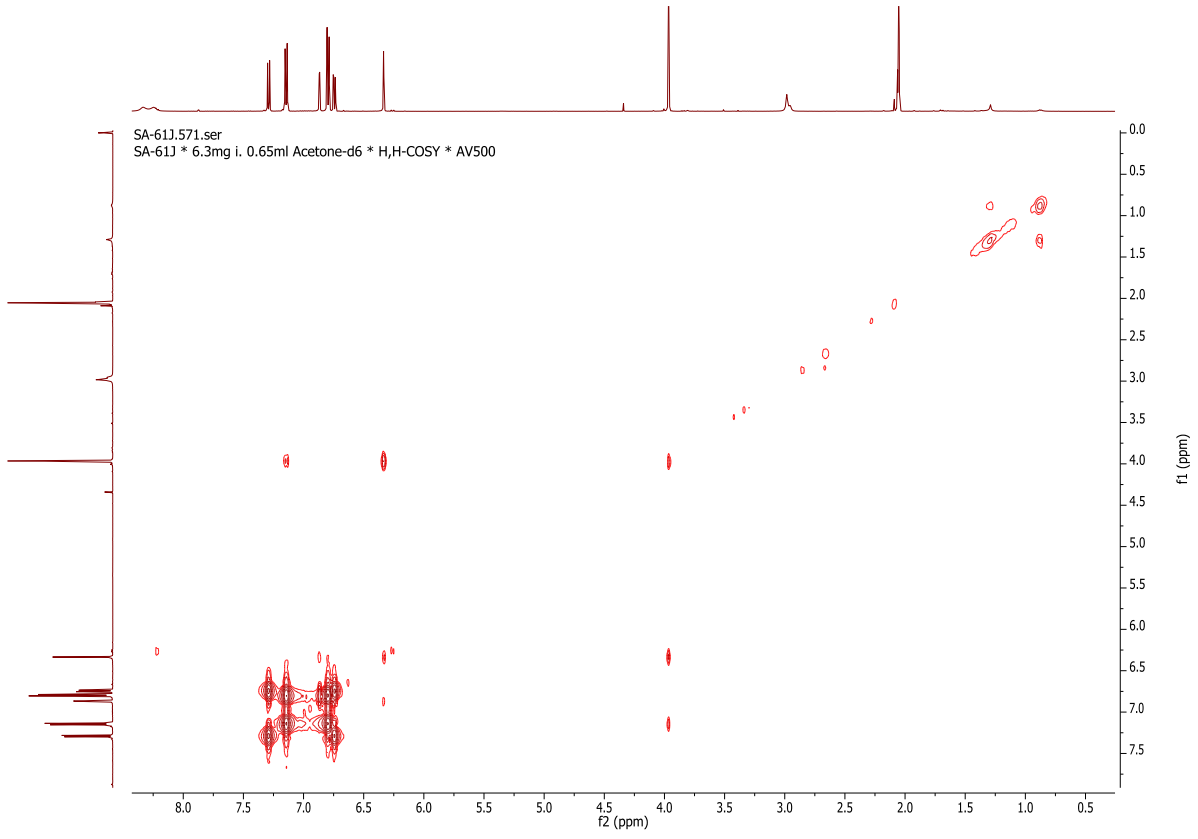
Appendix 34A: ^{1}H (500 MHz) and ^{13} C (125 MHz) NMR spectra of compound 34 (Acetone-d_6)



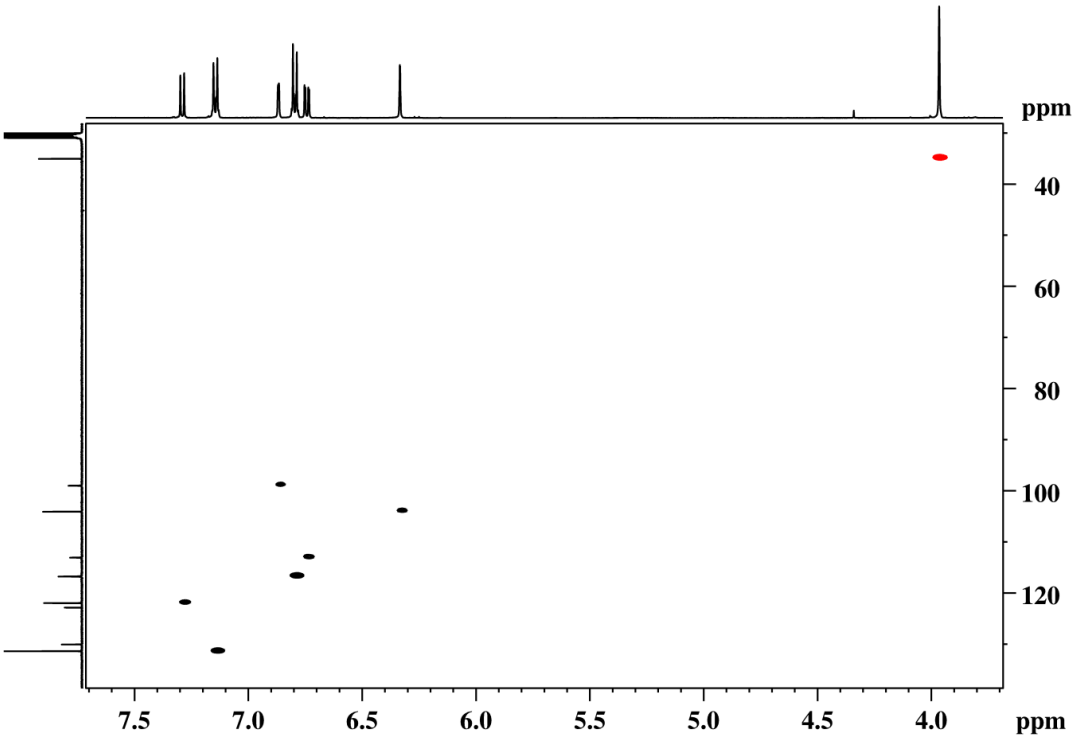


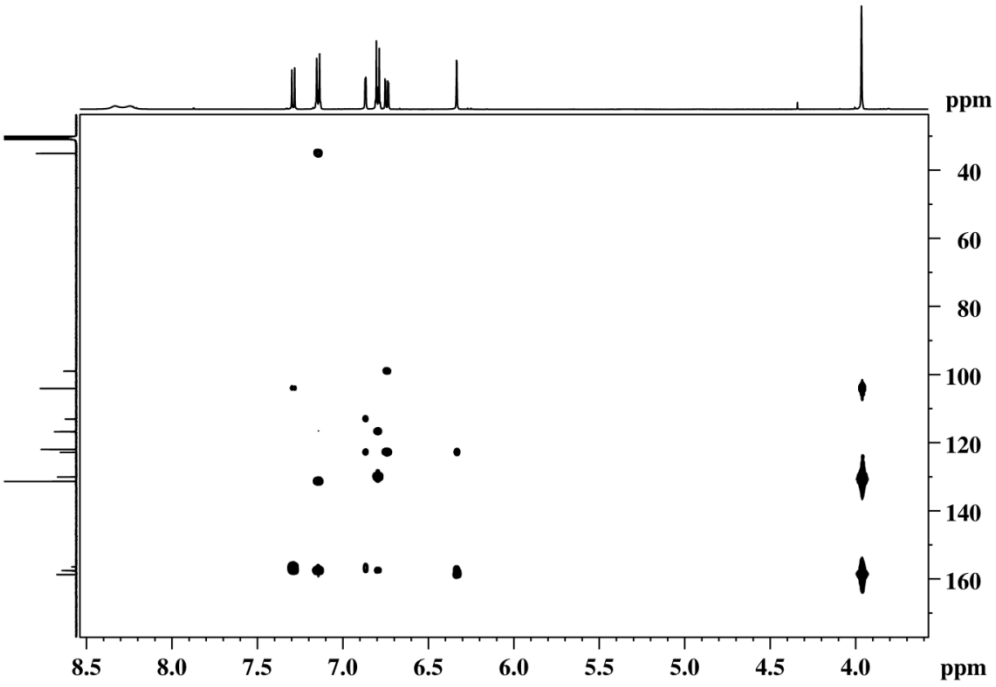
Appendix 34B: NOESY and H,H-COSY spectrum of compound 34 (500 MHz, acetone-d_6)



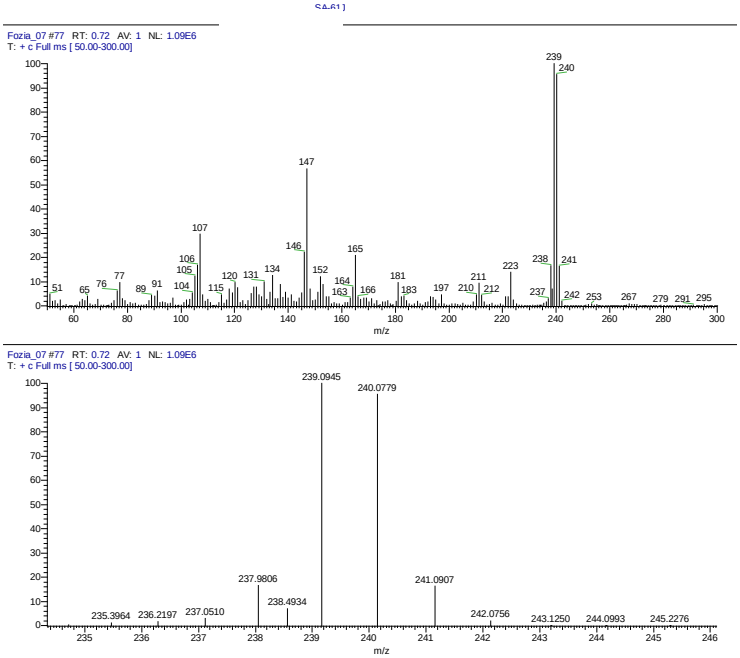


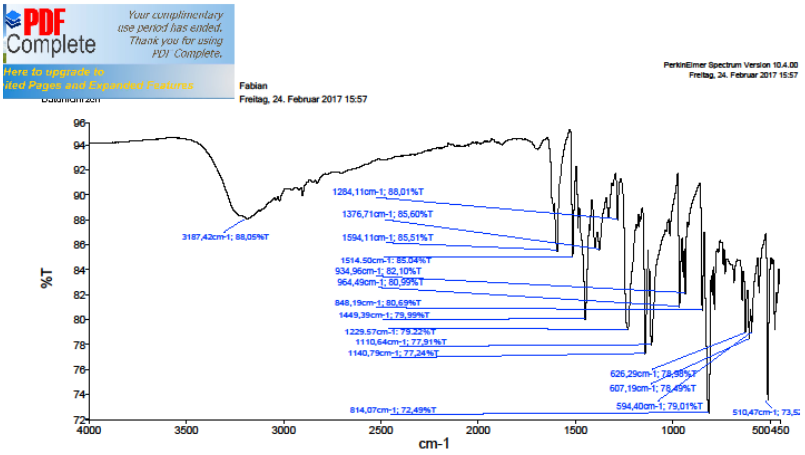
Appendix 34C: HSQC and HMBC spectrum of compound 34 (500 MHz, acetone-d_6)

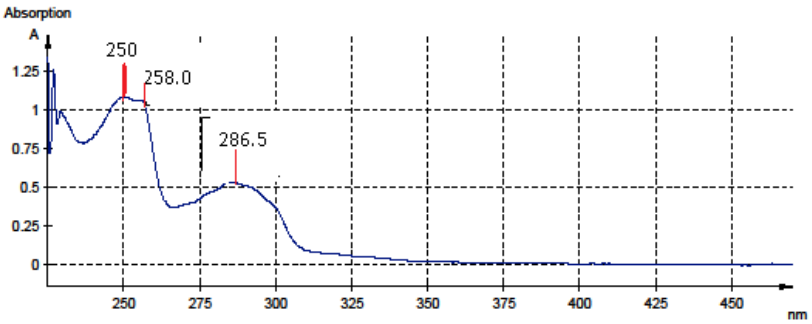




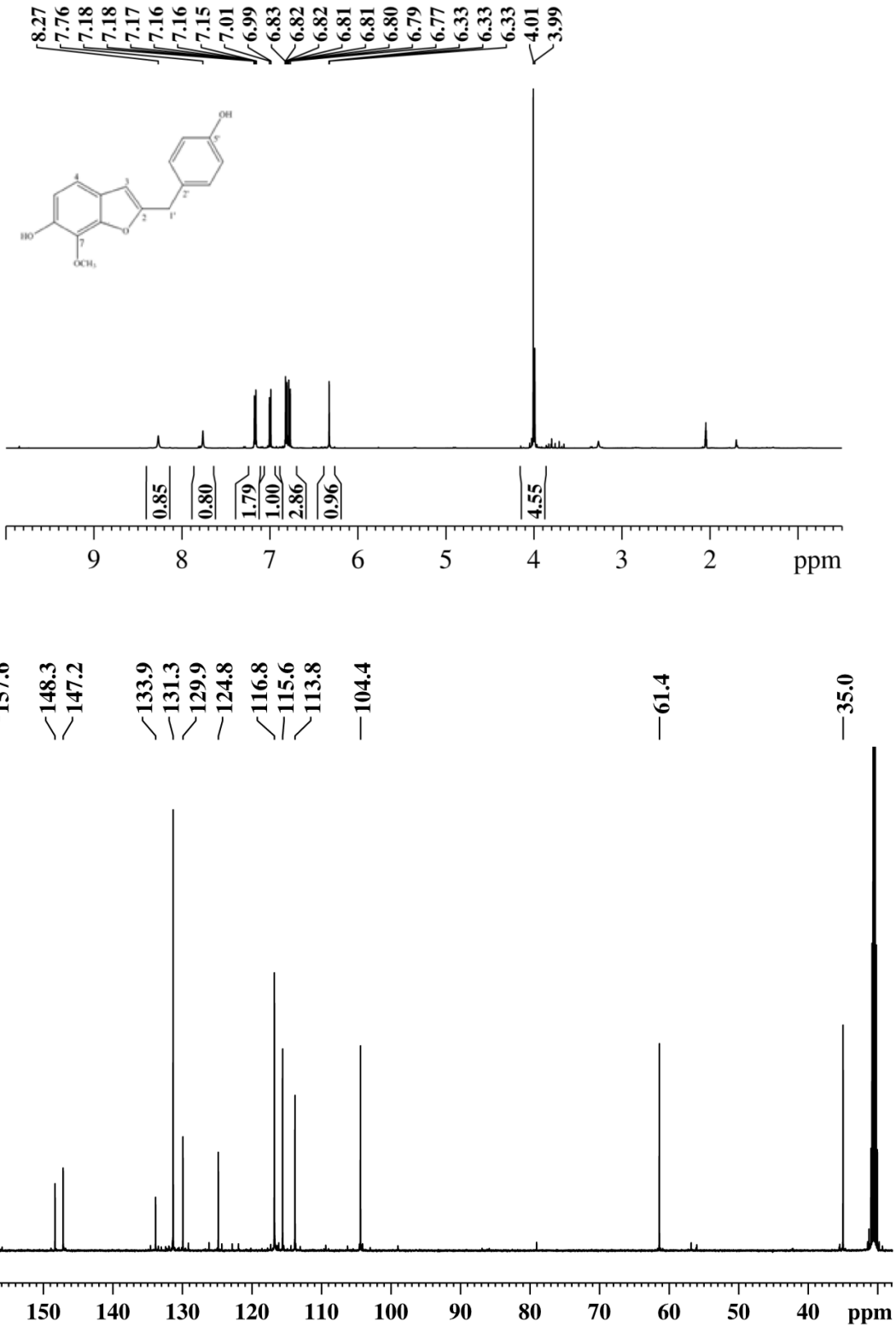
Appendix 34D: HREIMS, IR and UV spectra of compound 34



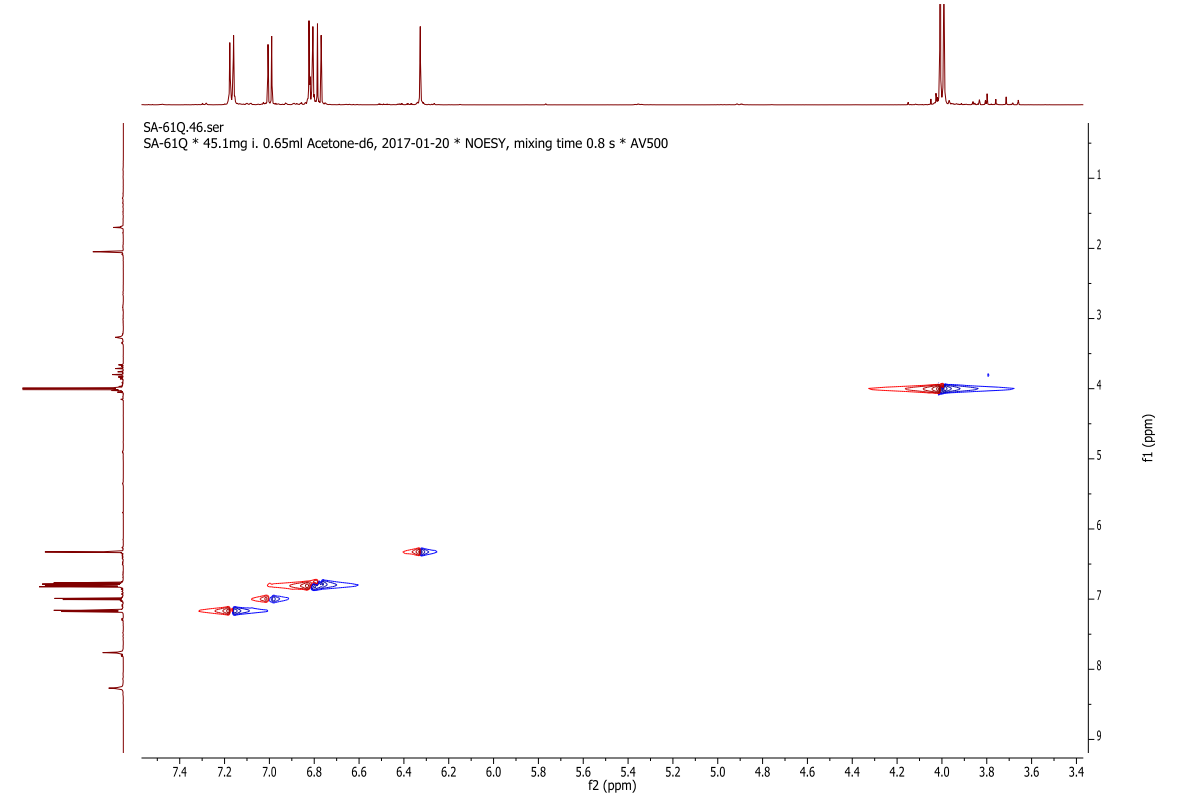


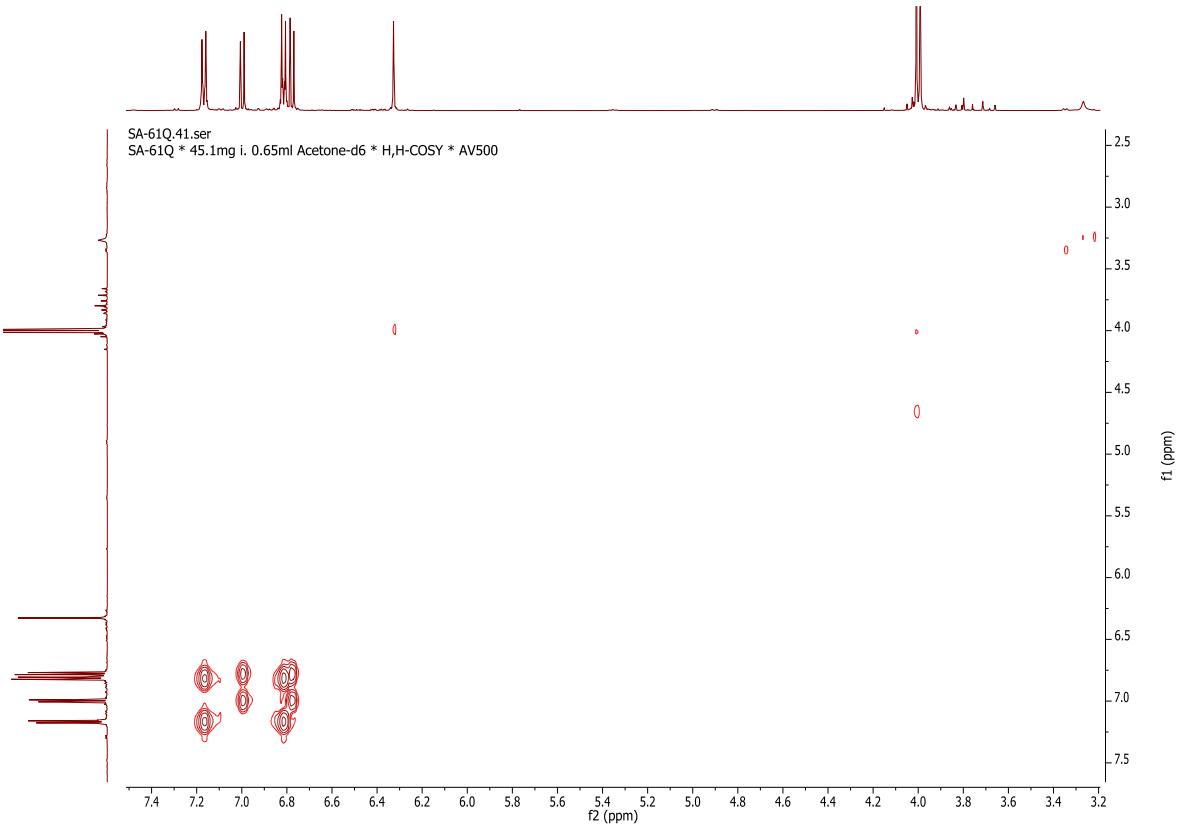


Appendix 35A: ^{1}H and ^{13} C NMR spectrum of compound 35 (500 MHz, acetone-d_6)

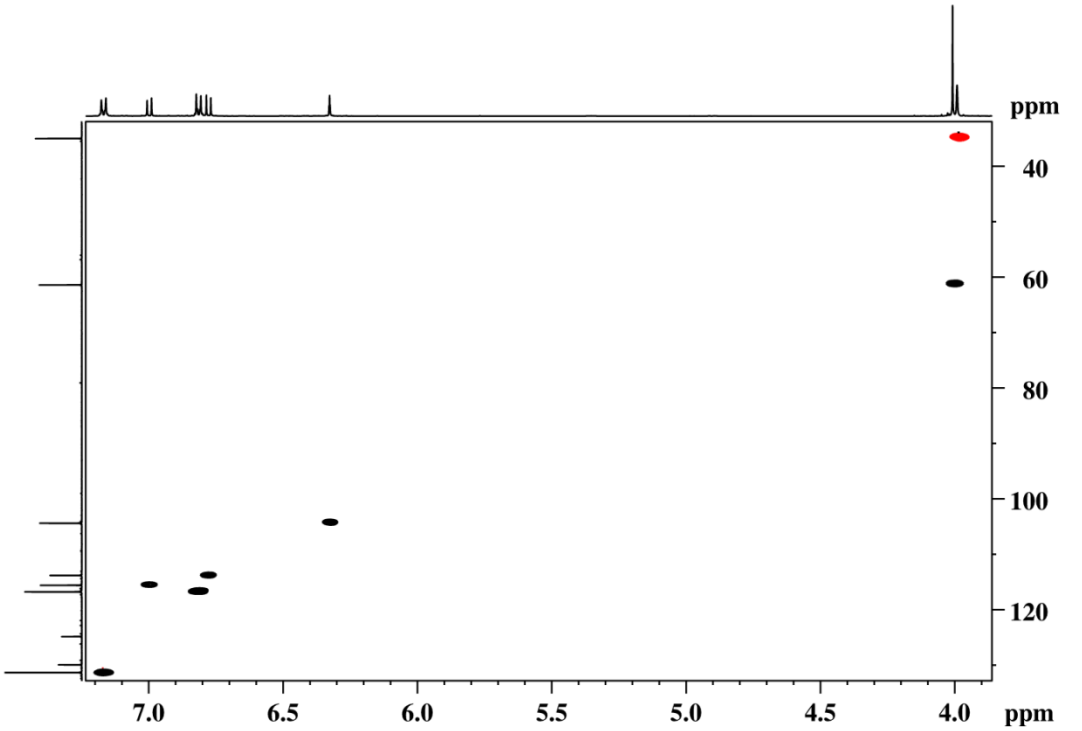


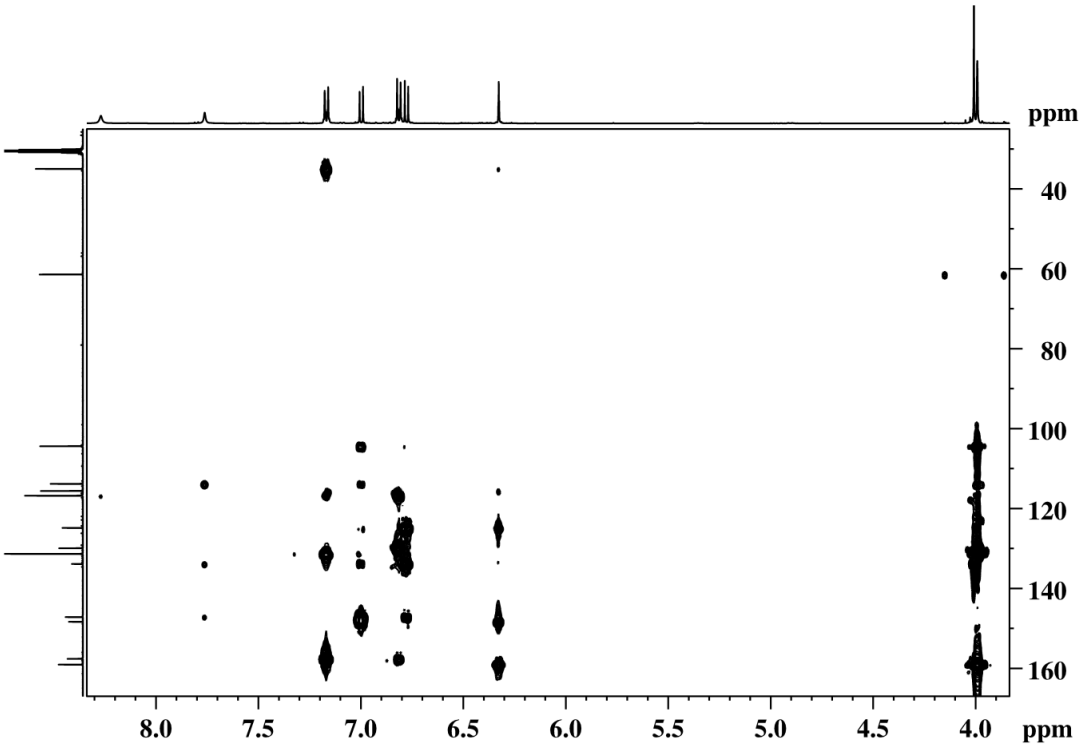
Appendix 35B: NOSEY and H,H-COSY spectra of compound 35 (500 MHz, acetone-d_6)



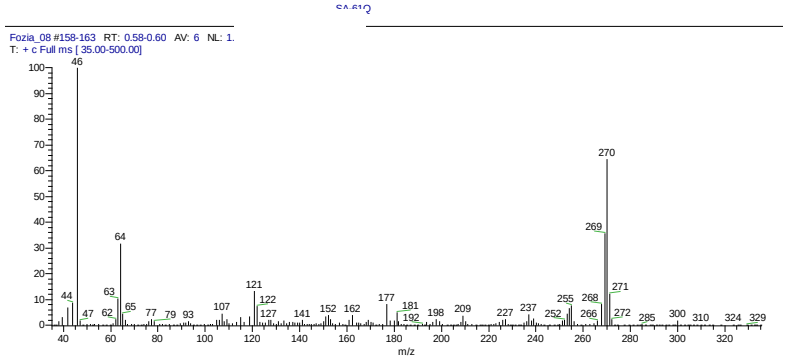


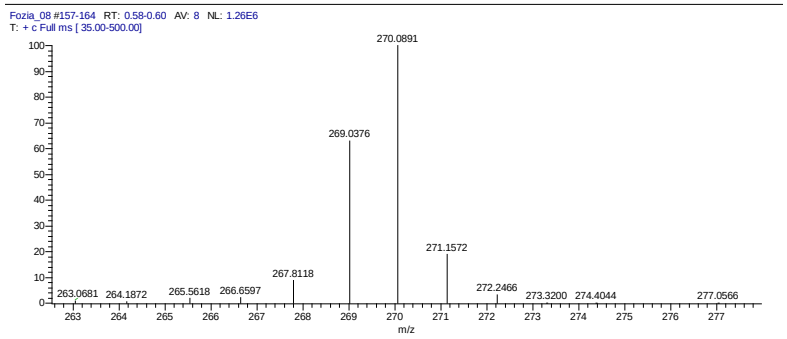
Appendix 35C: HSQC and HMBC spectra of compound 35 (500 MHz, acetone-d_6)

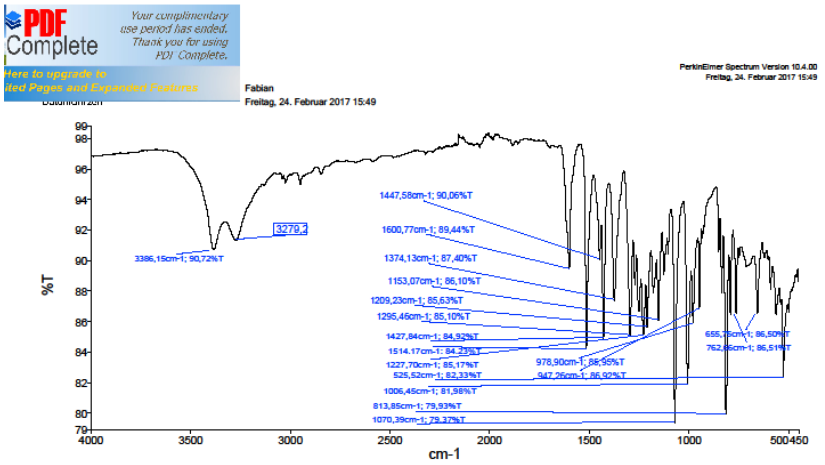


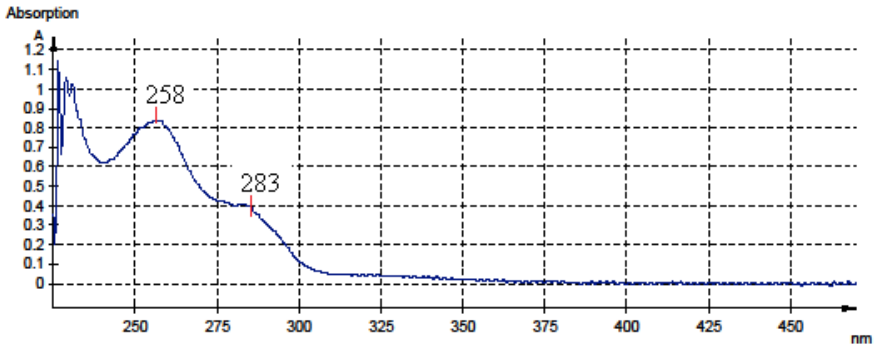


Appendix 35D: HREIMS, IR and UV spectram of compound 35

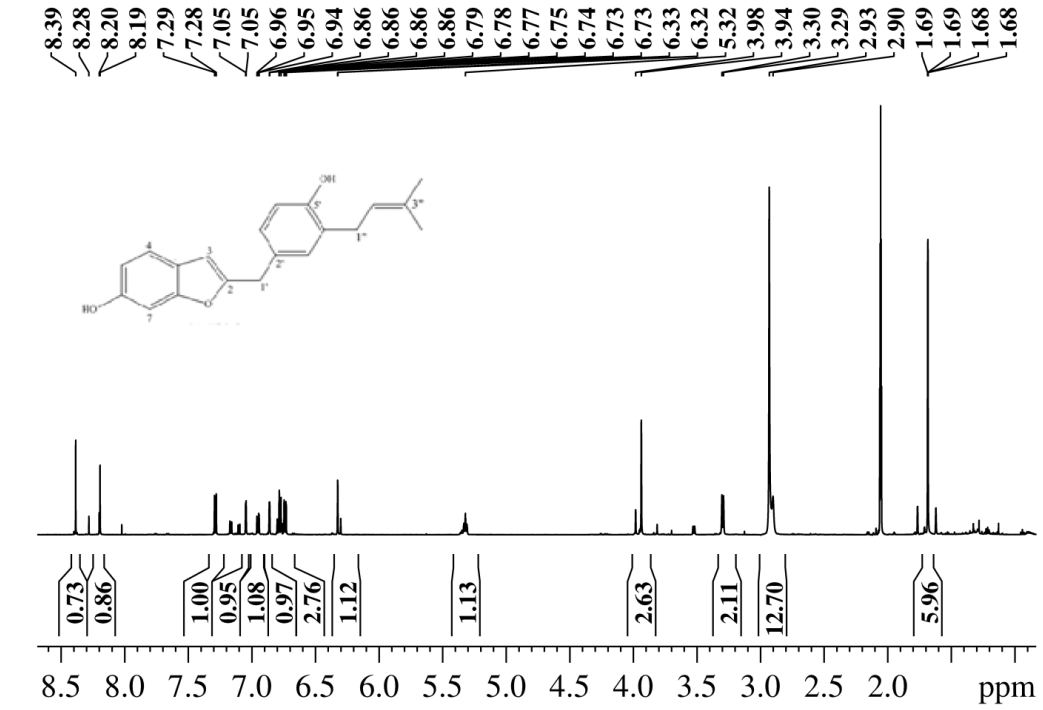


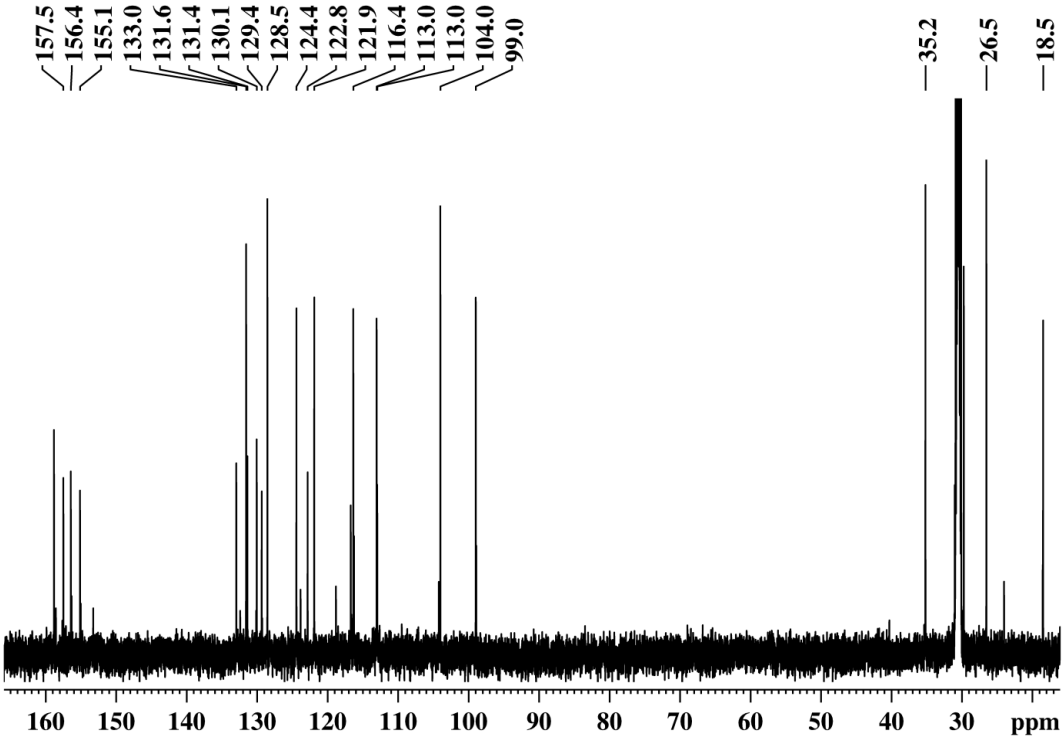




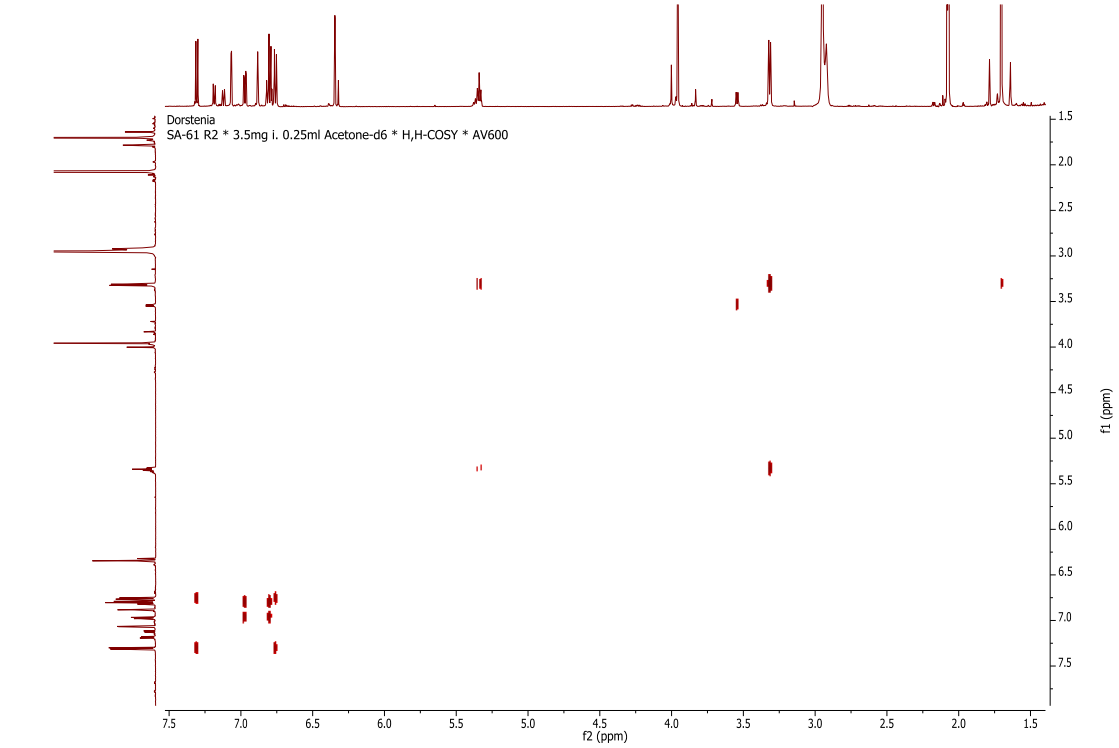


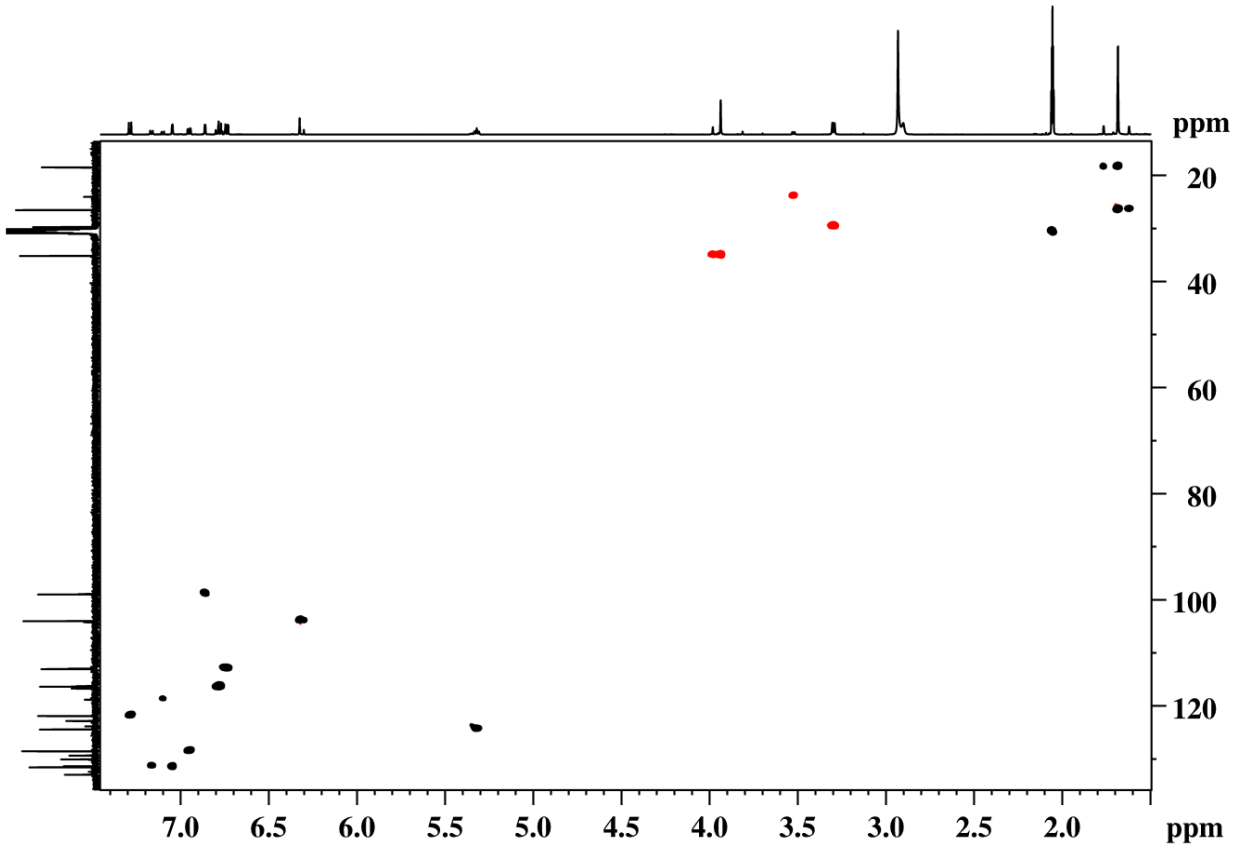
Appendix 36A: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of compound 36 (Acetone-d_6)



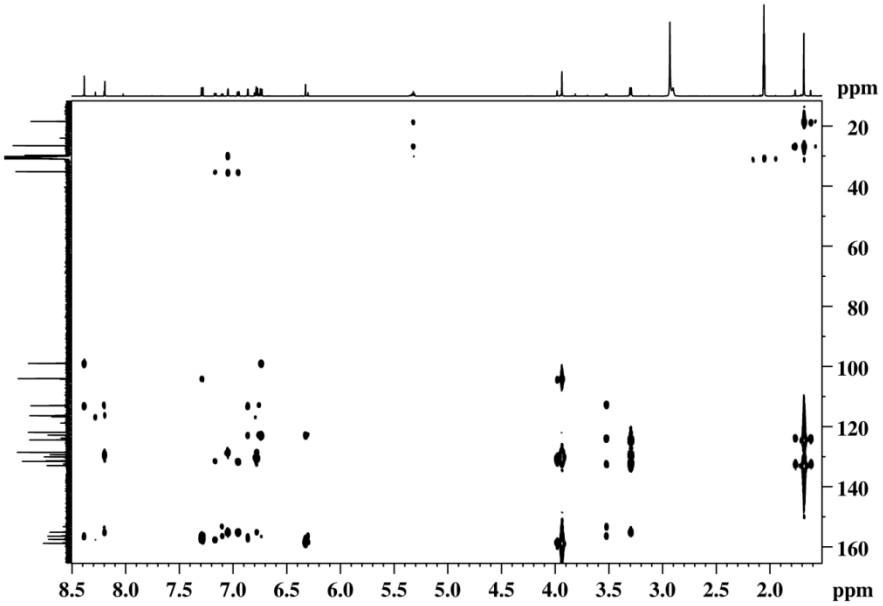


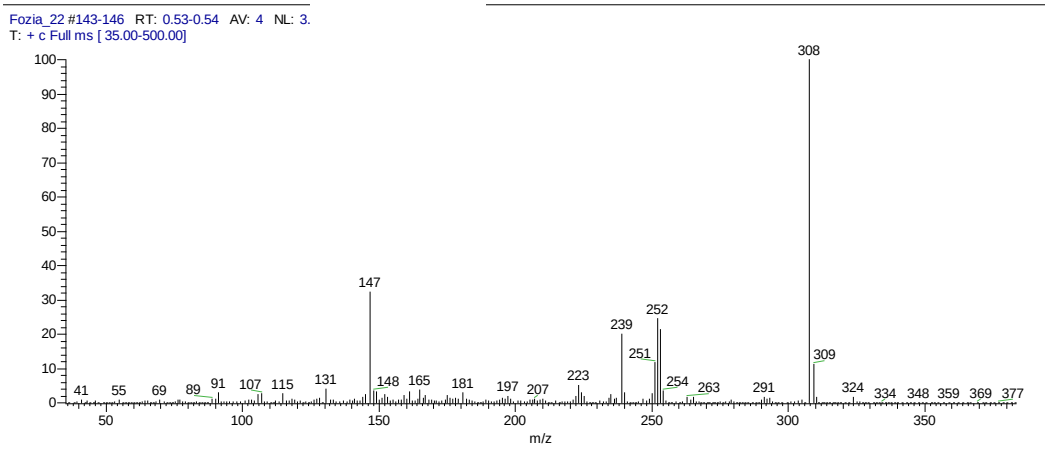
Appendix 36B: H,H-COSY and HSQC spectra of compound 36 (600 MHz, Acetone-d6)

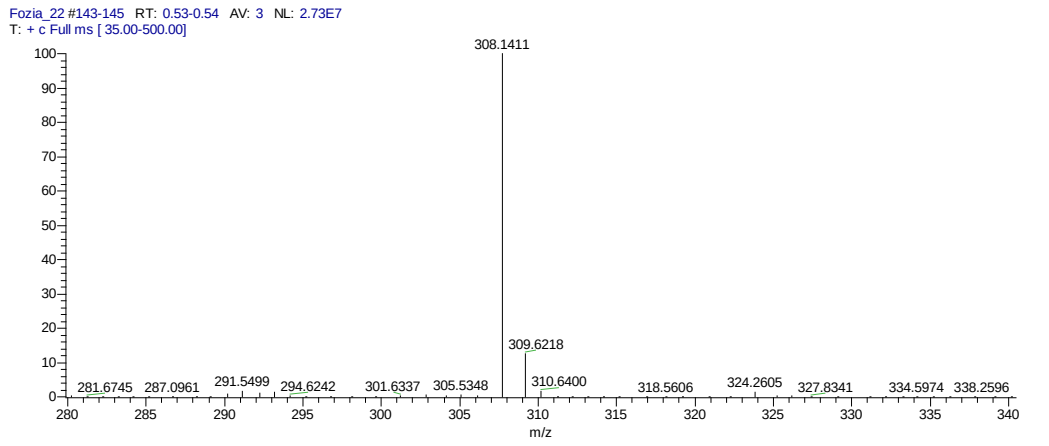




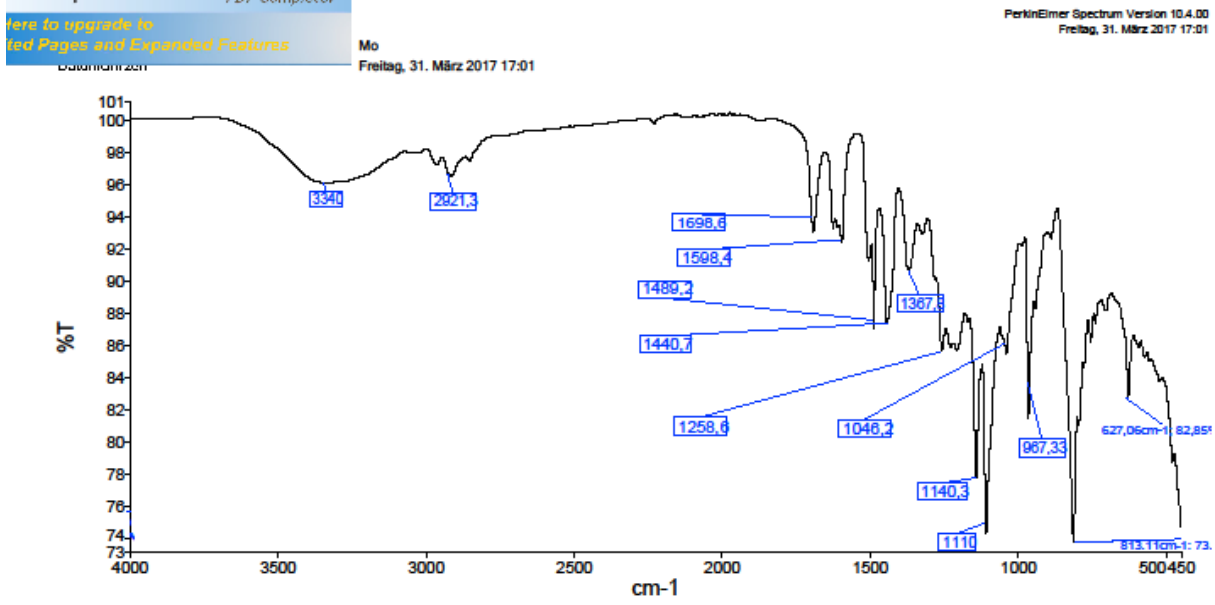
Appendix 36C: HMBC (600 MHz, acetone-d_6) and HREIMS spectra of compound 36

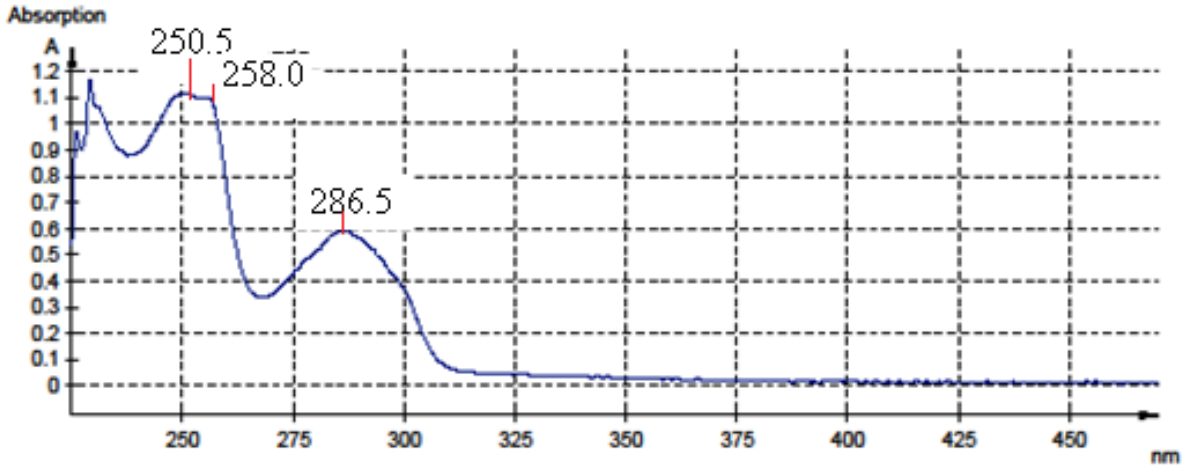




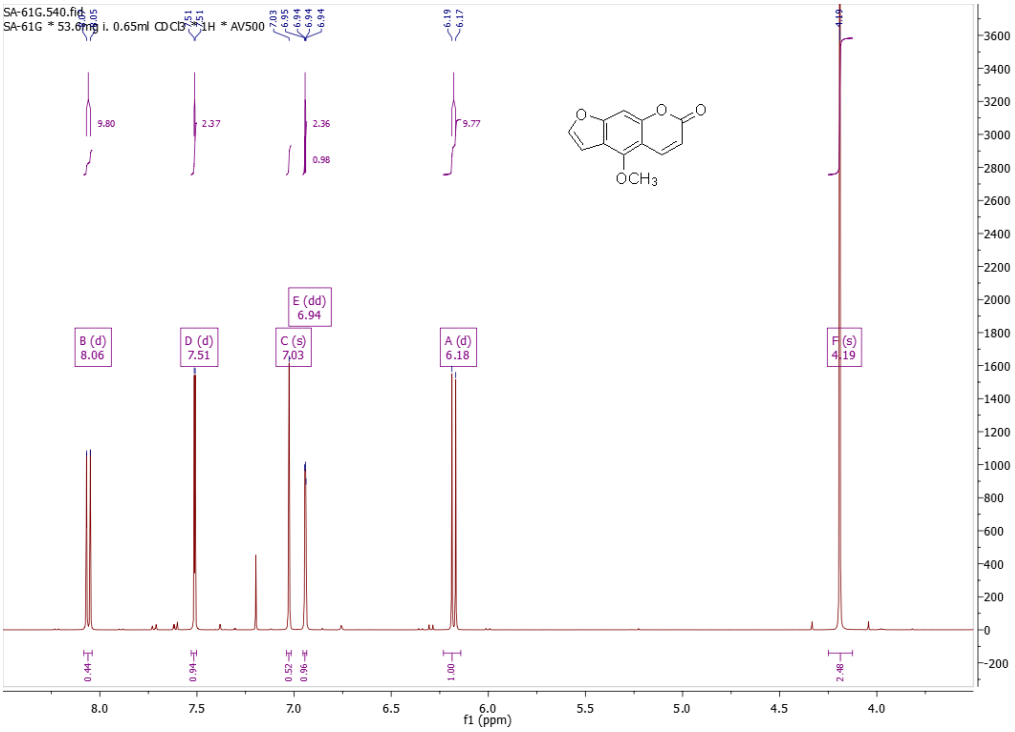


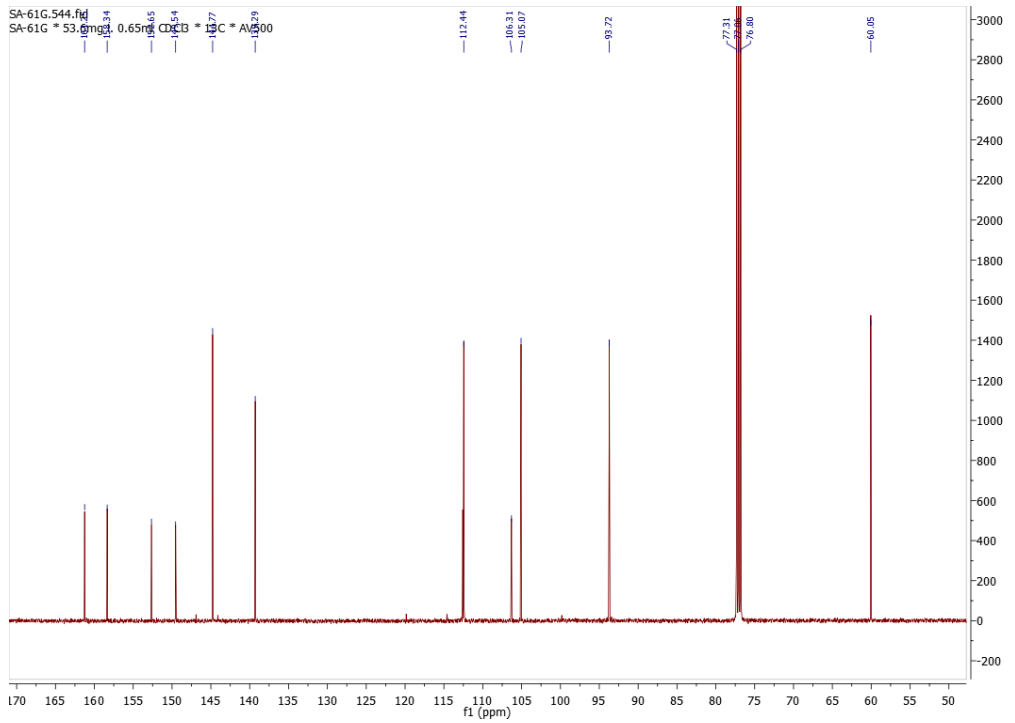
Appendix 36D: IR and UV spectra of compound 36



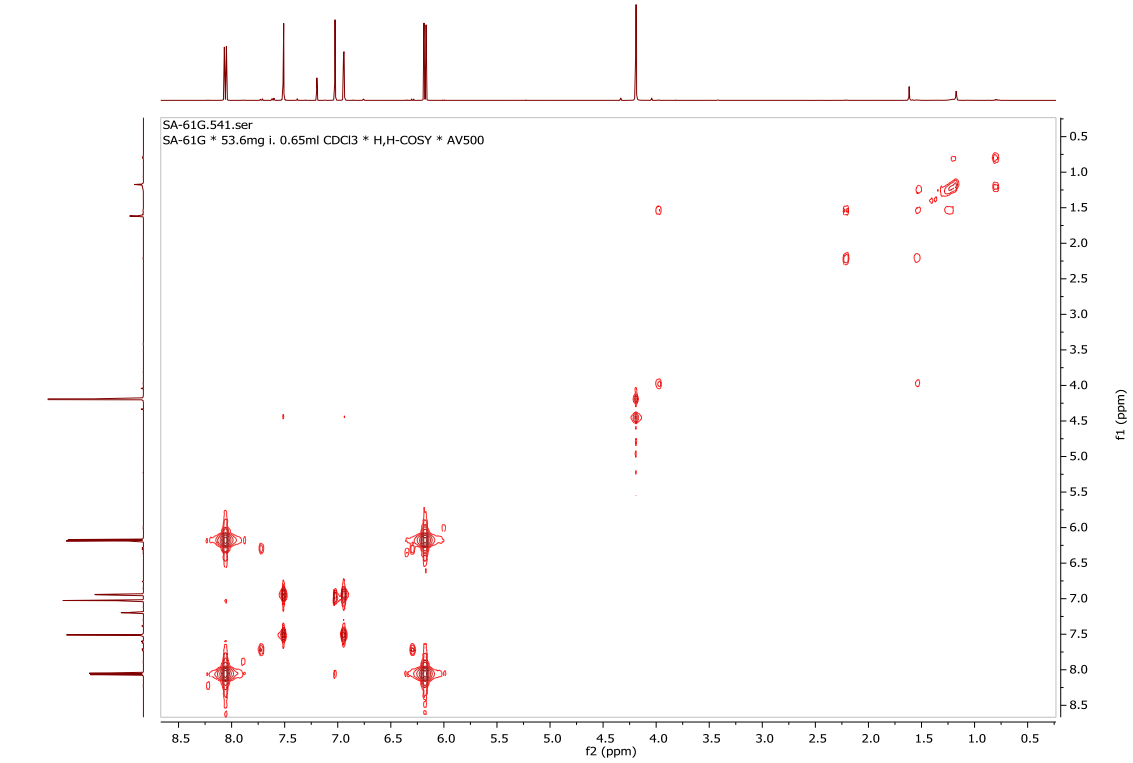


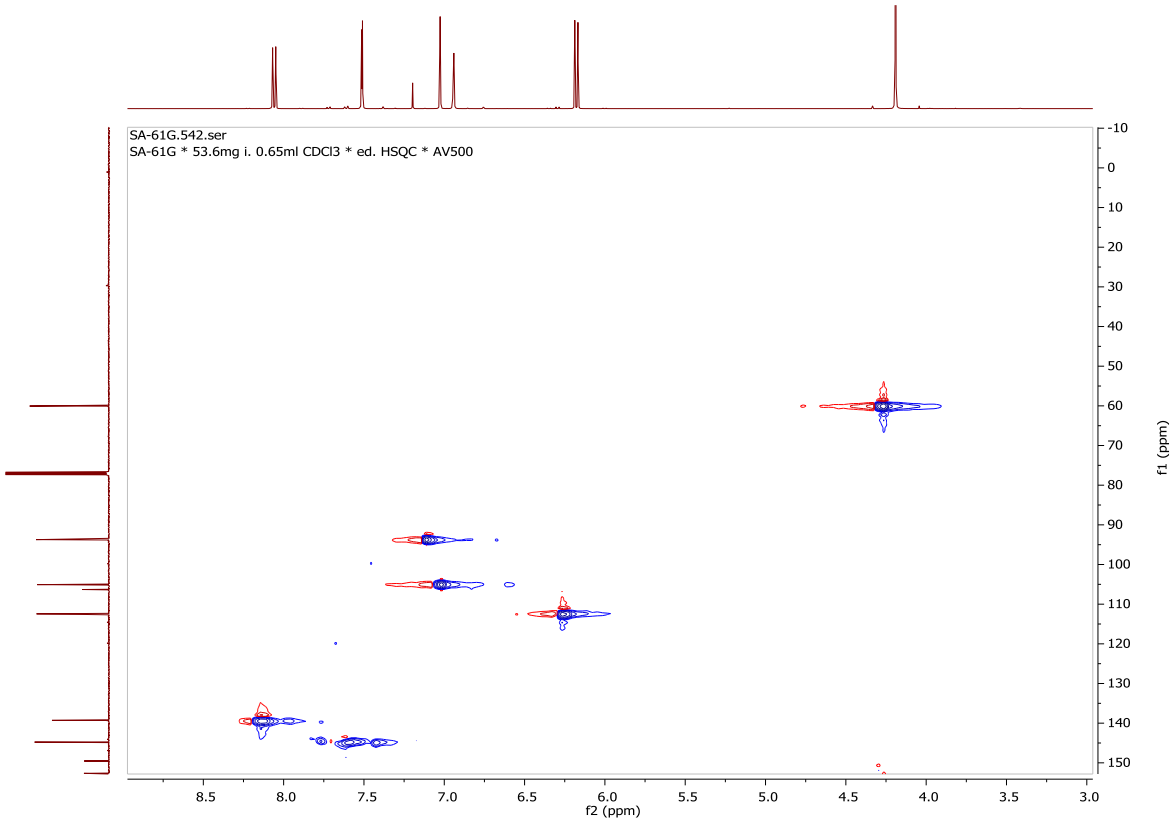
Appendix 37A: ^{1}H (500 MHz) and ^{13}C NMR (500 MHz) spectra of compound 37 (Acetone-d_6)



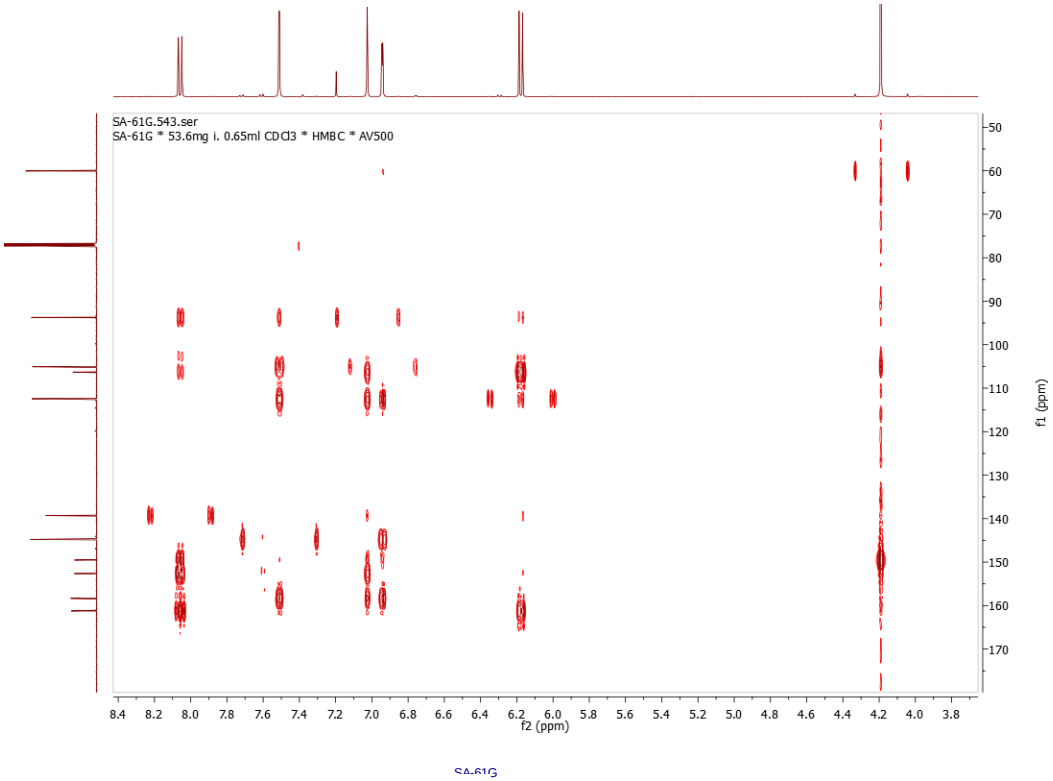


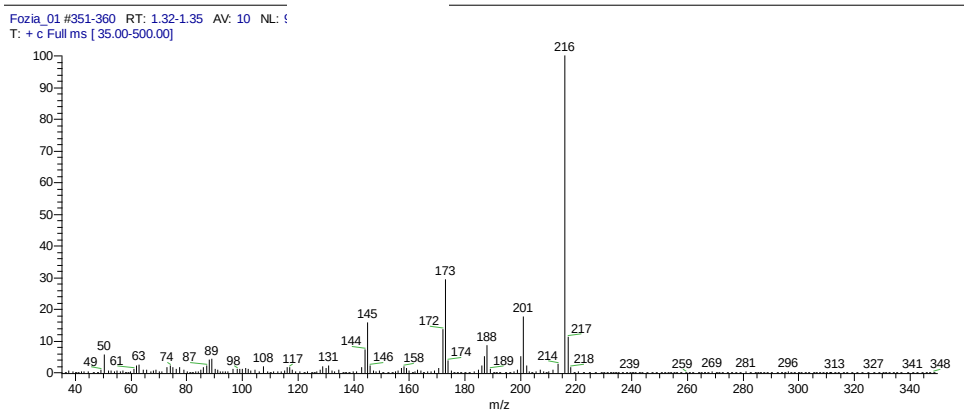
Appendix 37B: H,H-COSY and HSQC spectra of compound 37 (500 MHz, Acetone-d6)

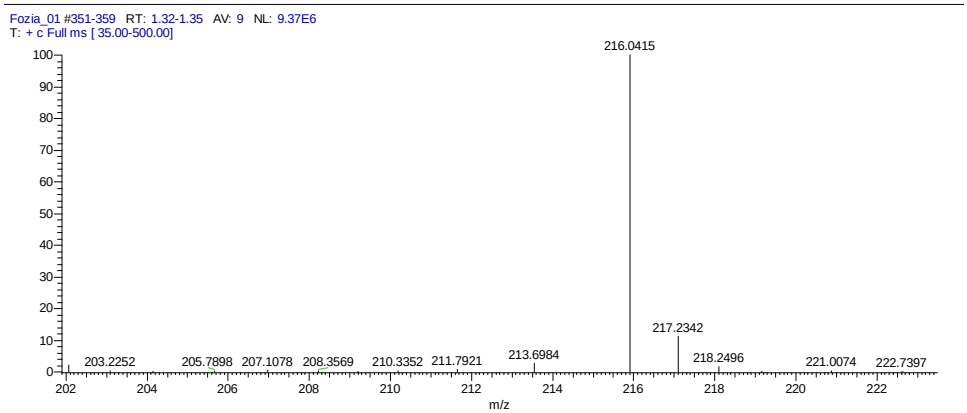




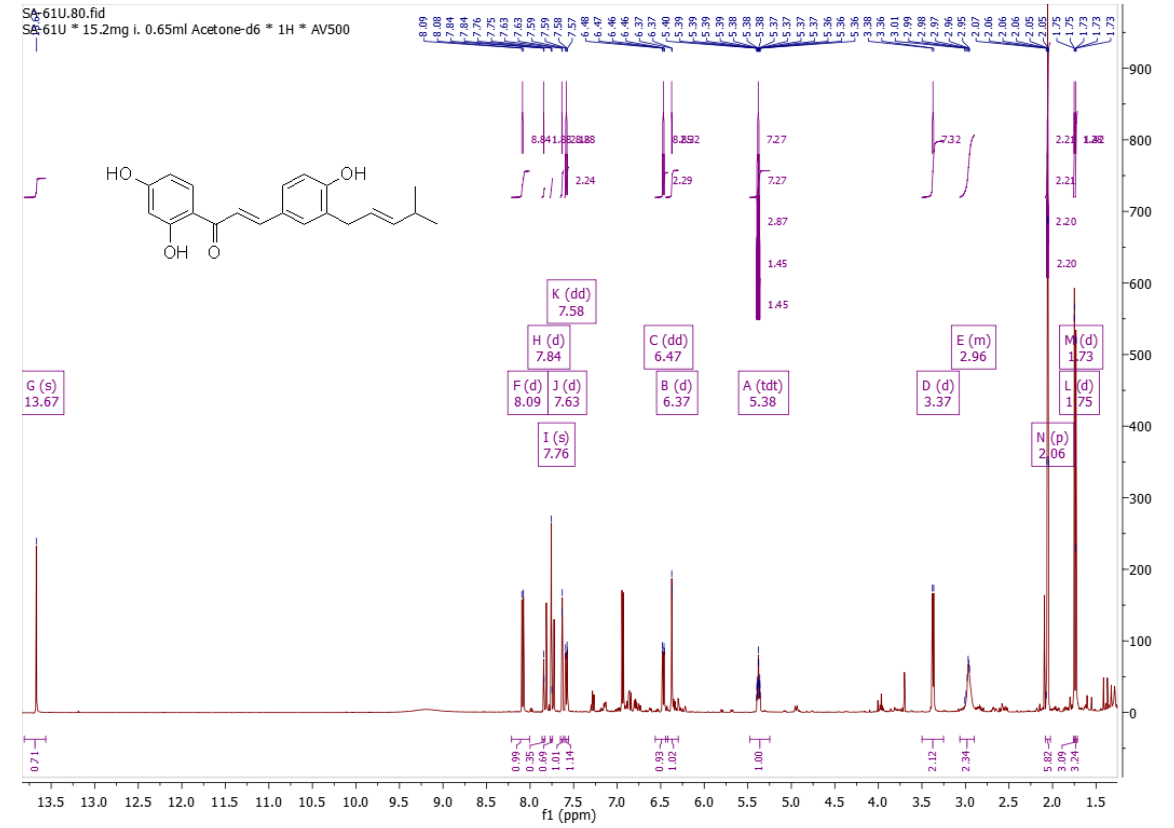
Appendix 37C: HMBC (500 MHz, Acetone-d6) and HREIMS spectra of compound 37

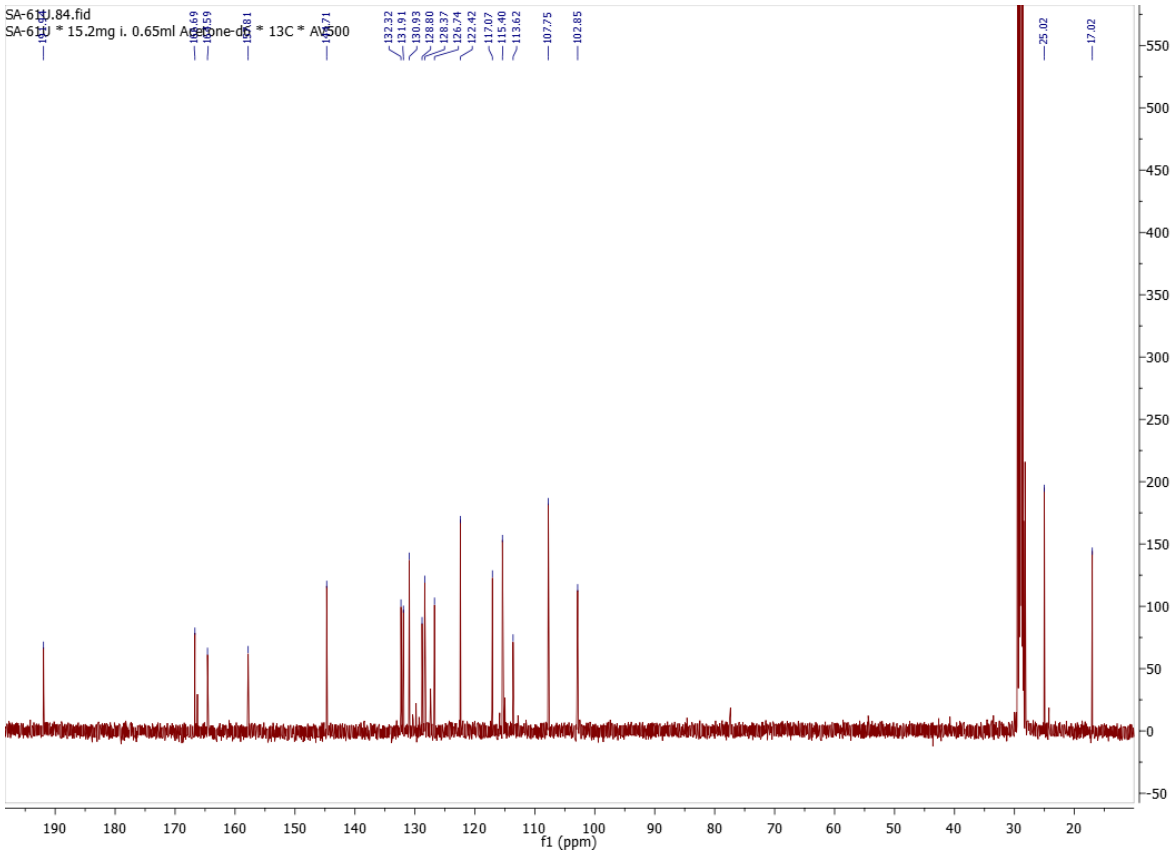




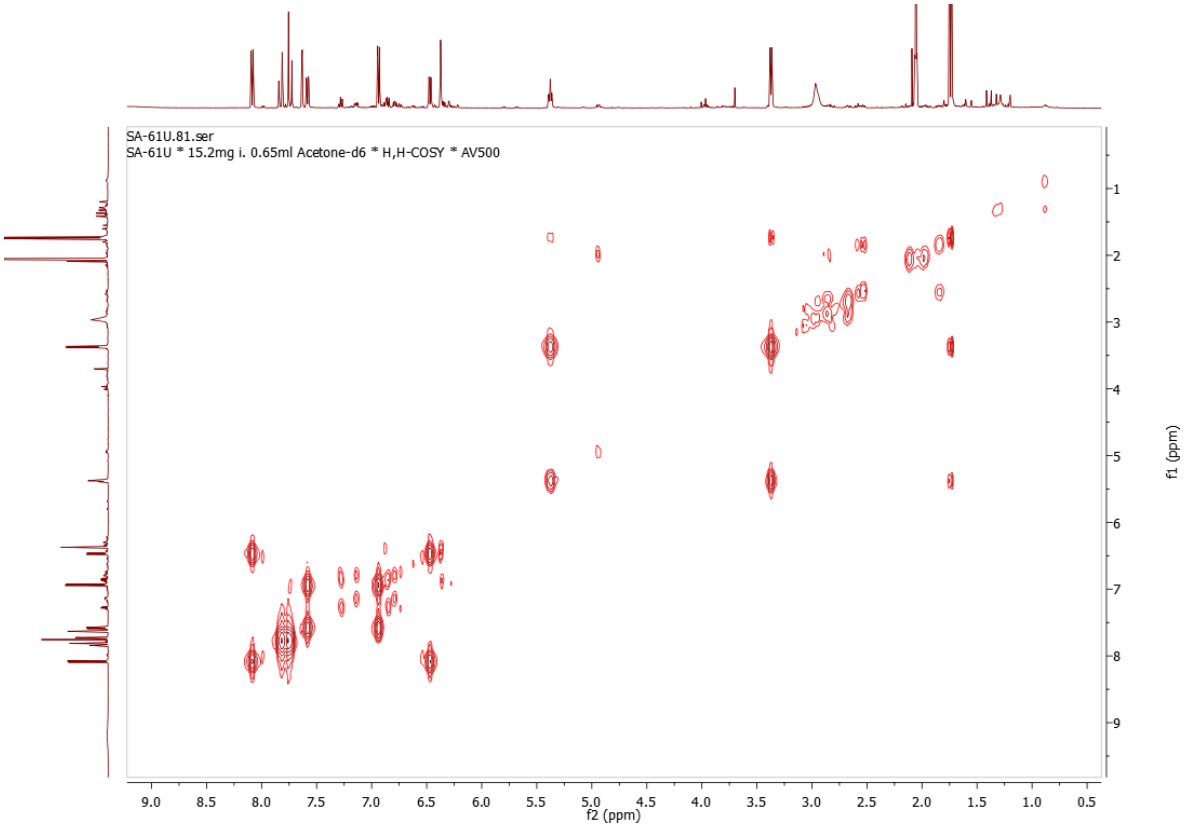


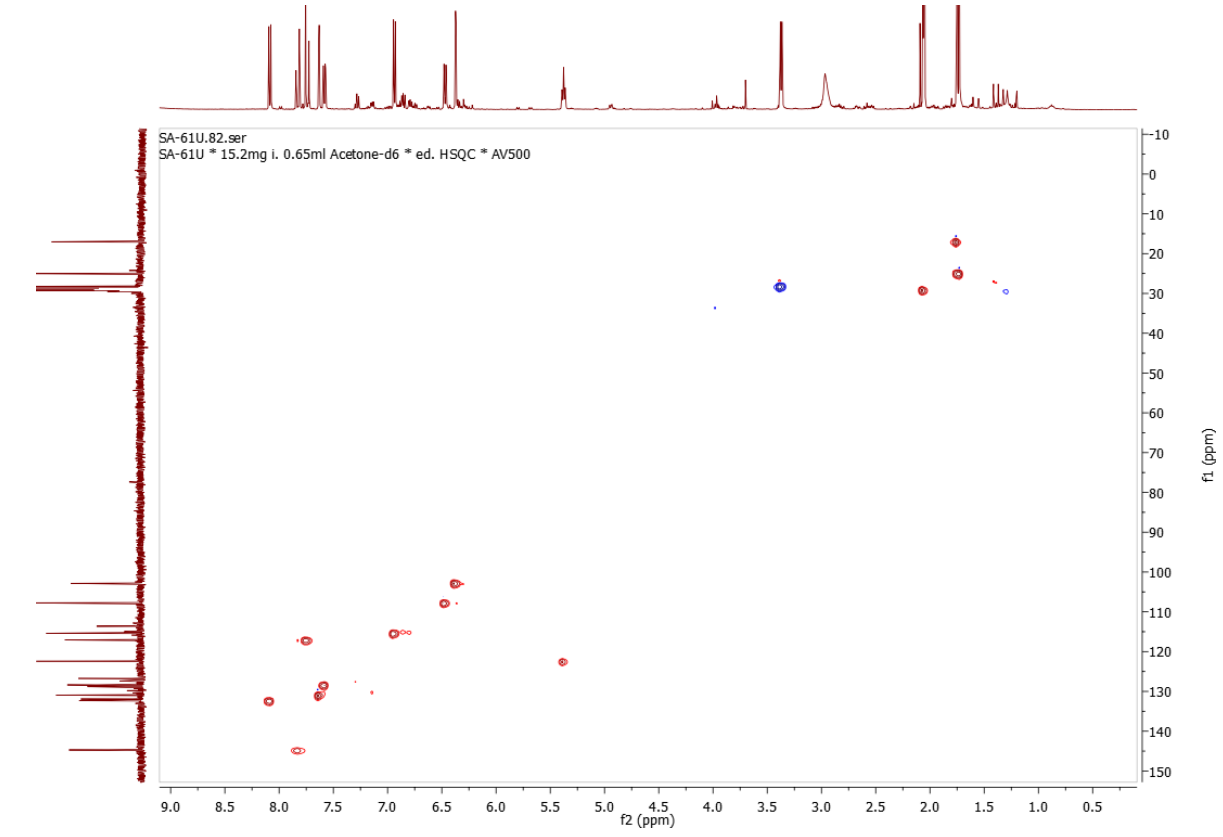
Appendix 38A: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra of compound 38 (Acetone-d_6)



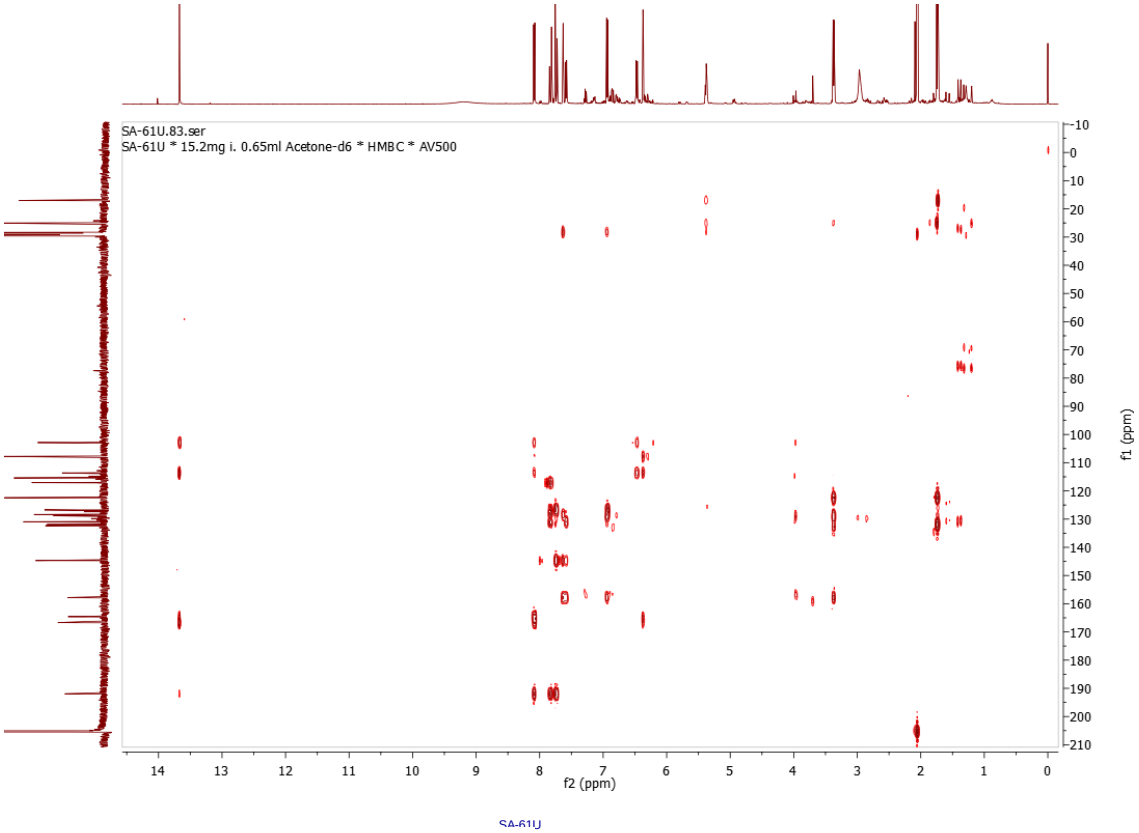


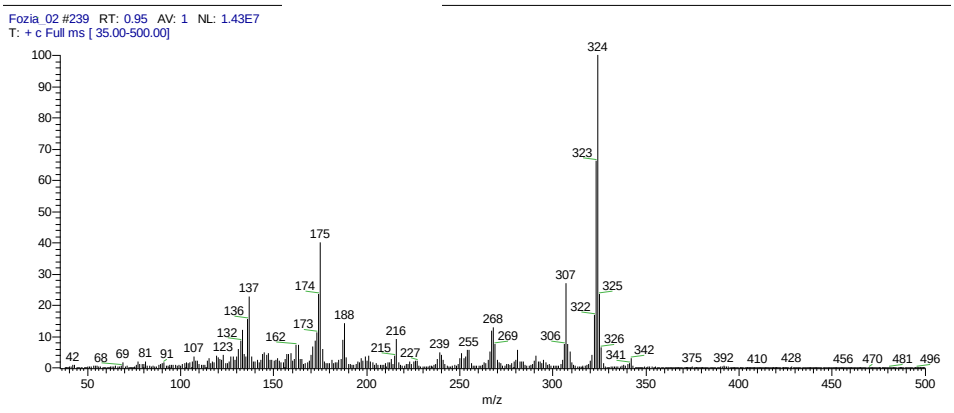
Appendix 38B: H,H-COSY and HSQC spectra of compound 38 (500 MHz, Acetone-d6)

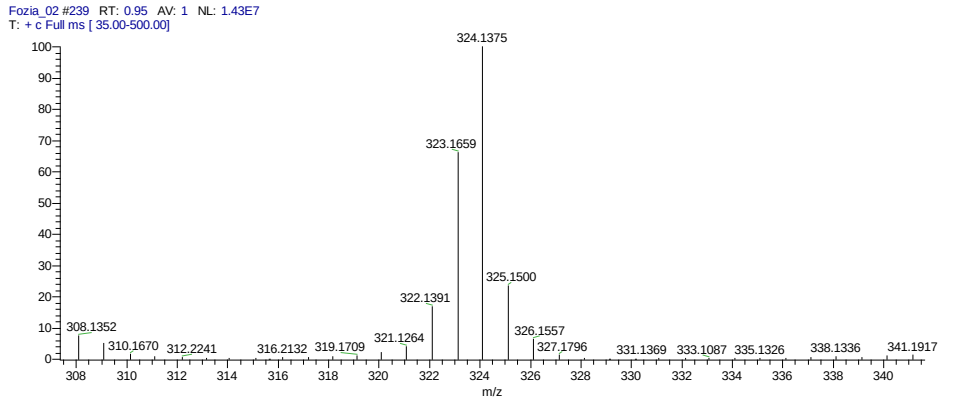




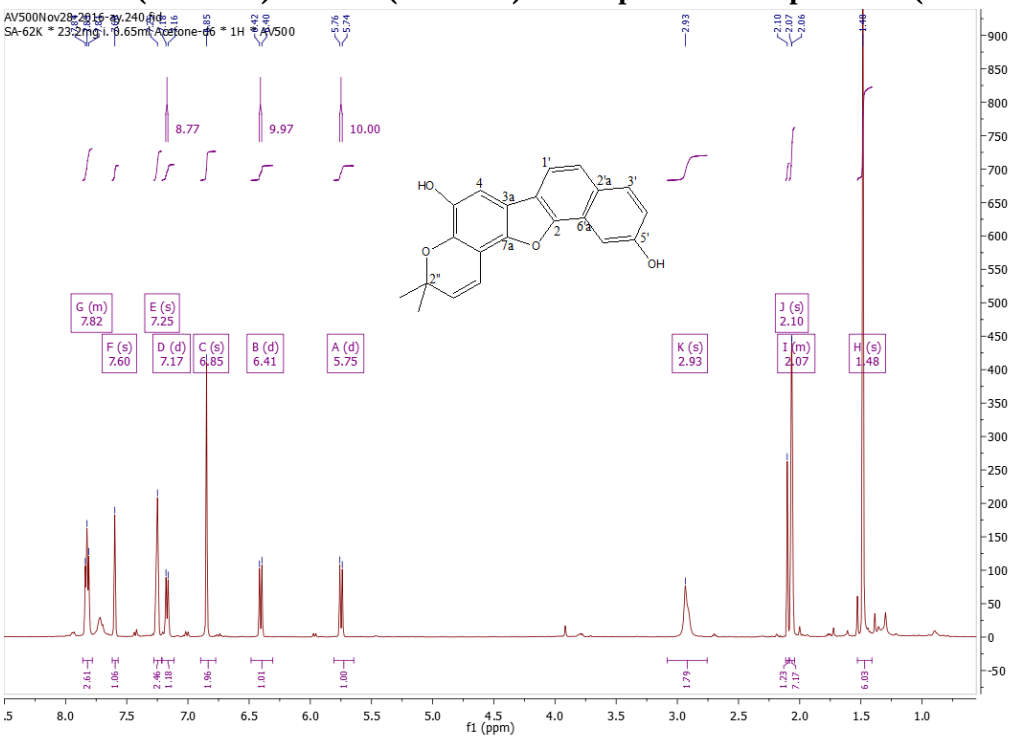
Appendix 38C: HMBC (500 MHz, Acetone-d6) and HREIMS spectra of compound 38

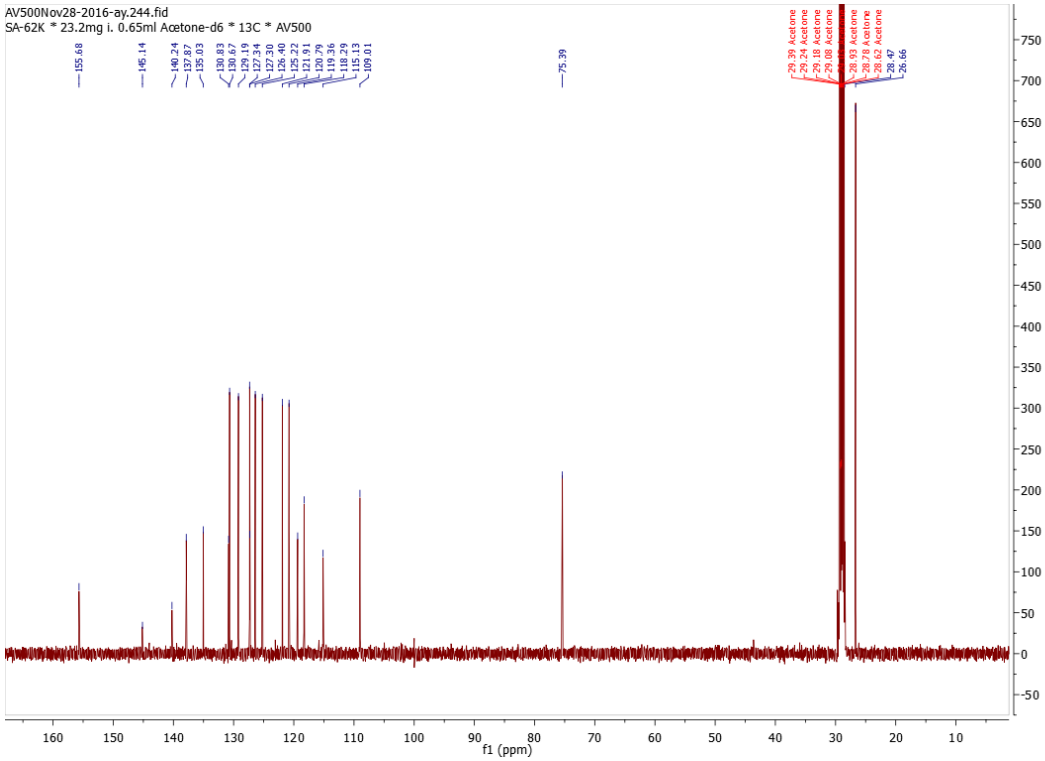




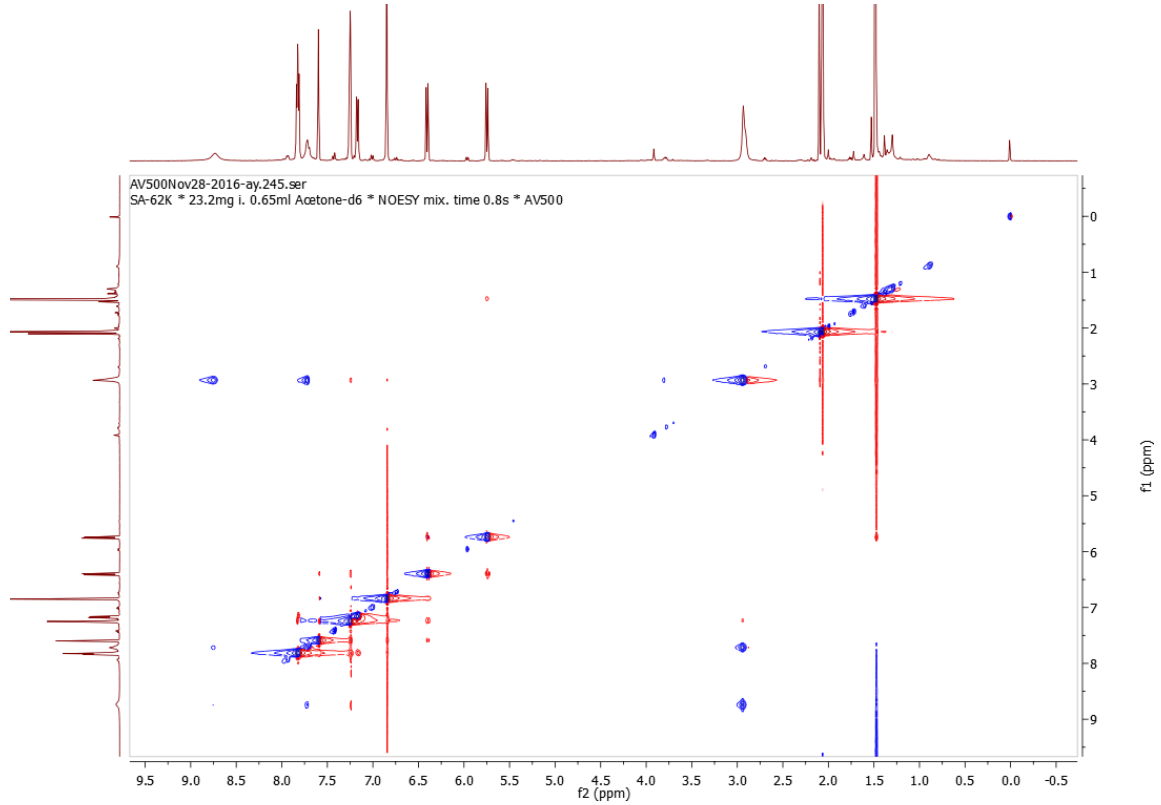


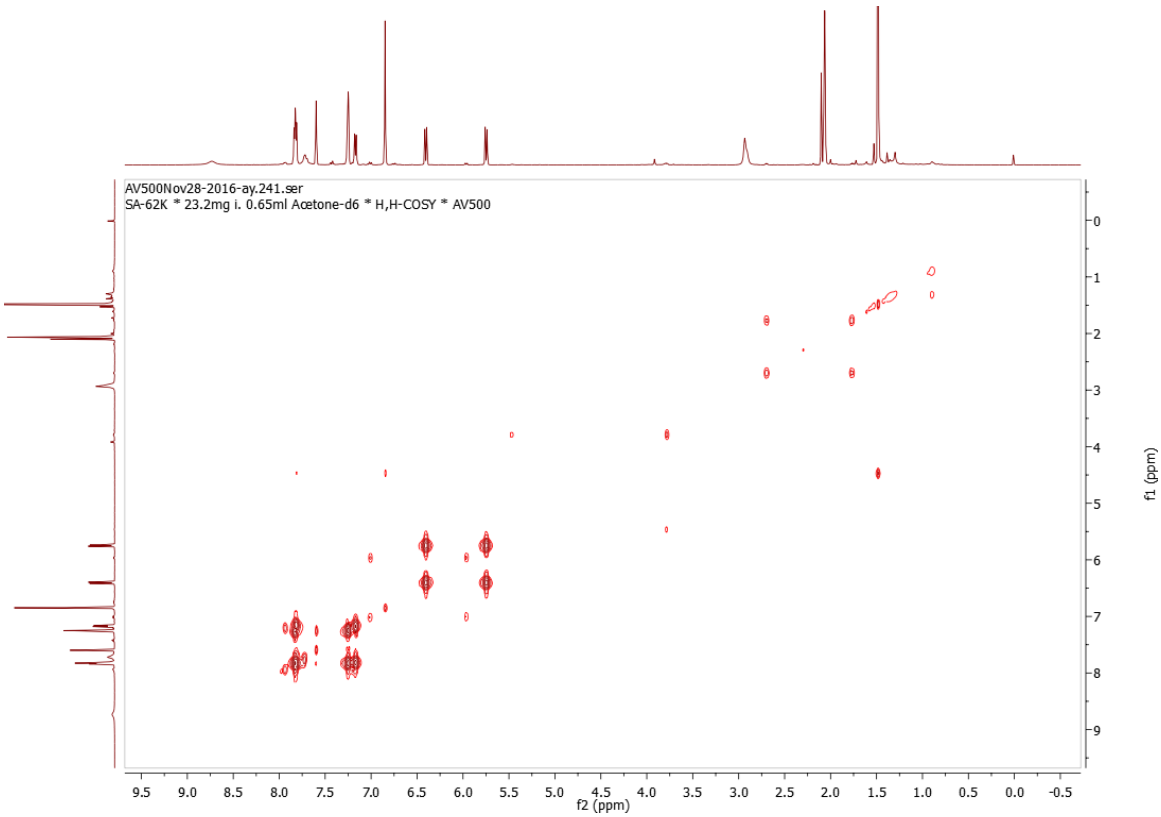
Appendix 39A: ^{1}H (500 MHz) and ^{13} C (125 MHz) NMR spectra of compound 39 (Acetone-d_6)



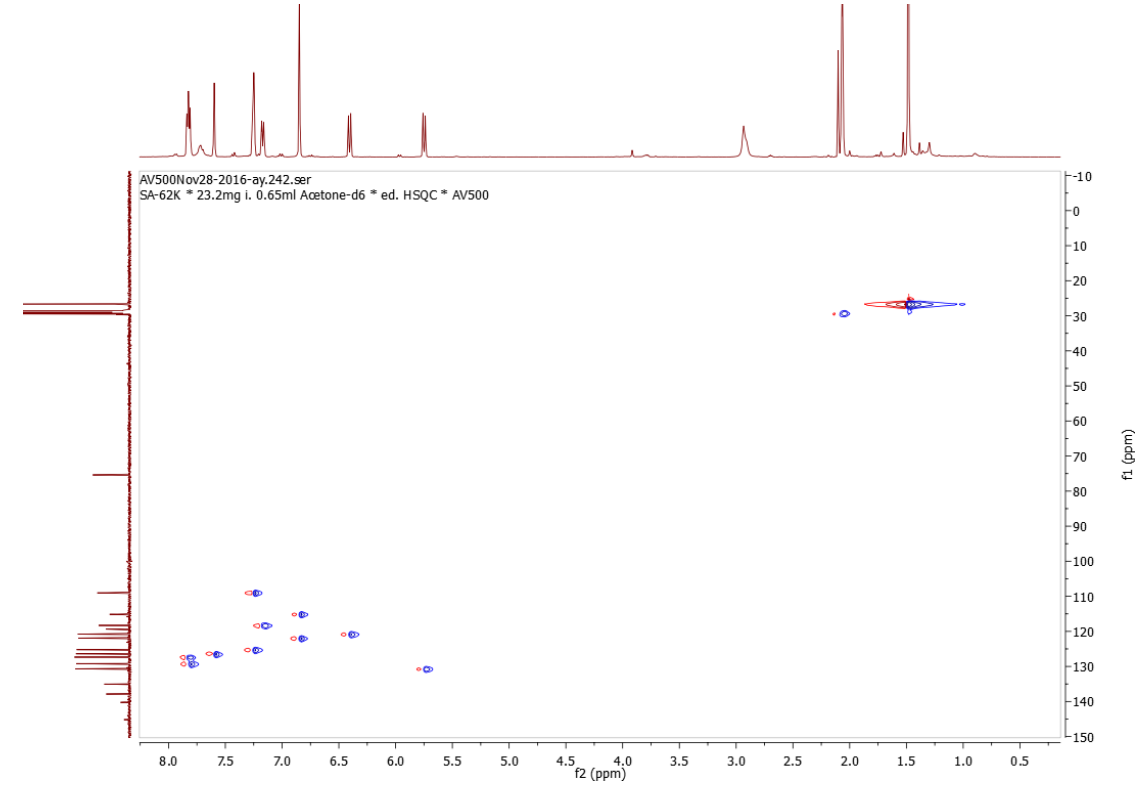


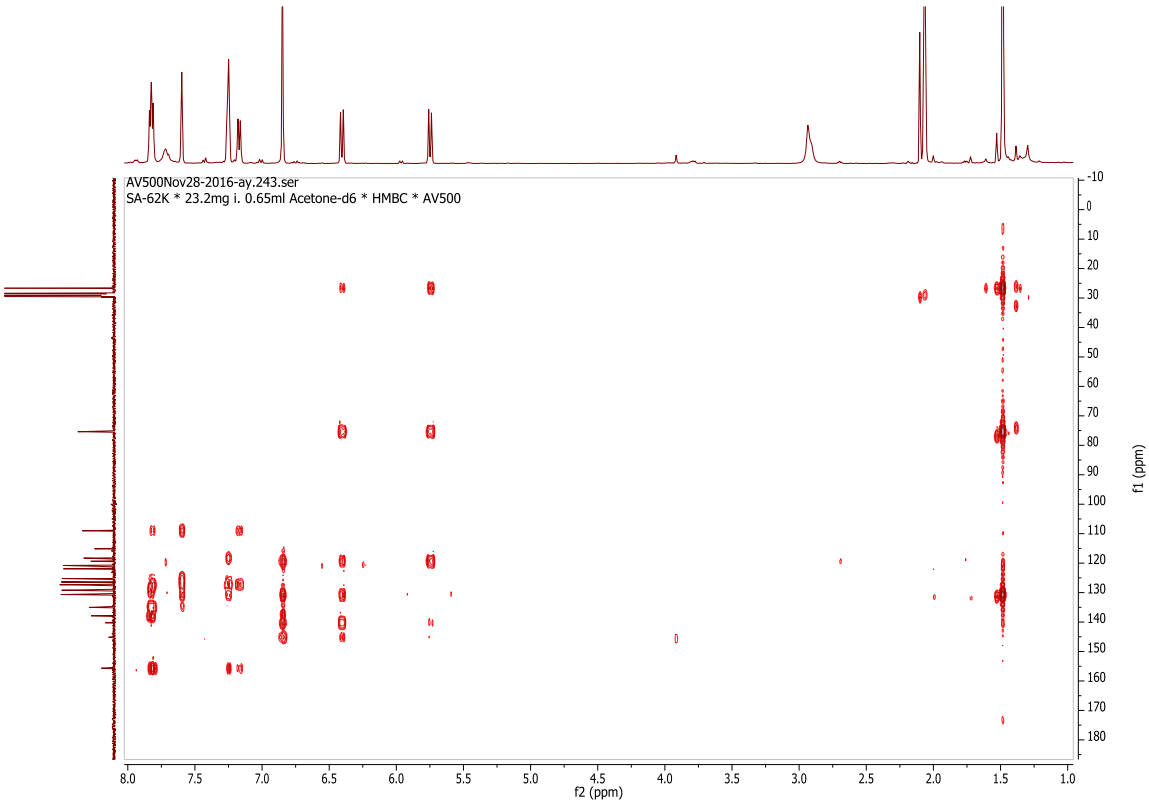
Appendix 39B: NOESY and H,H-COSY spectra of compound 39 (500 MHz, acetone-d_6)



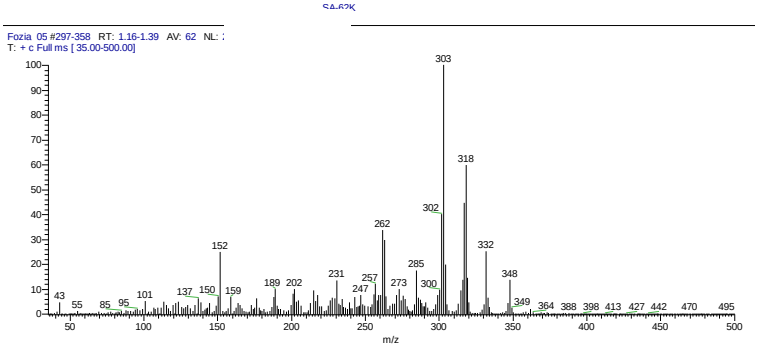


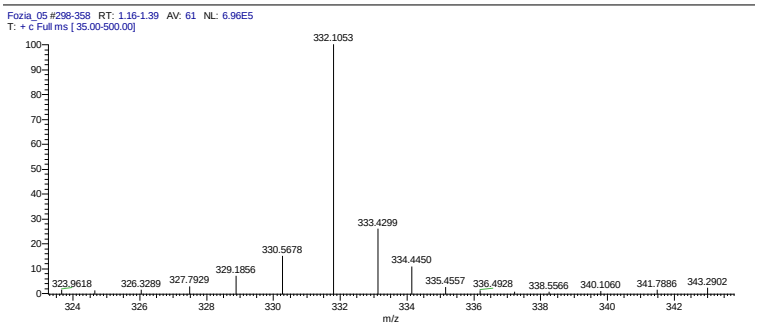
Appendix 39C: HSQC and HMBC spectra of compound 39 (500 MHz, acetone-d_6)

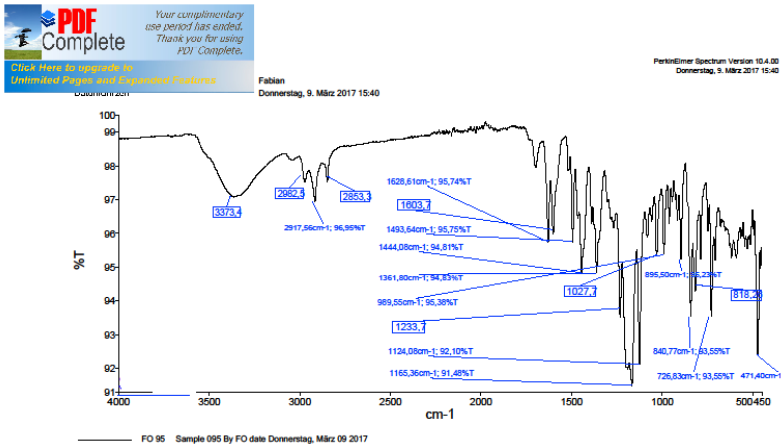


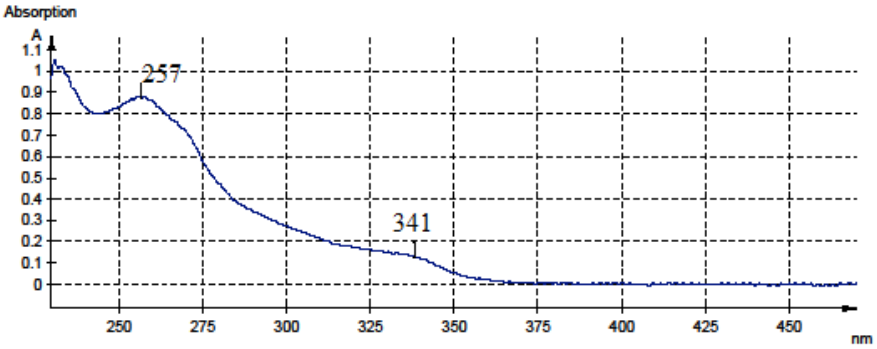


Appendix 39D: HREIMS, IR and UV spectra of compound 39

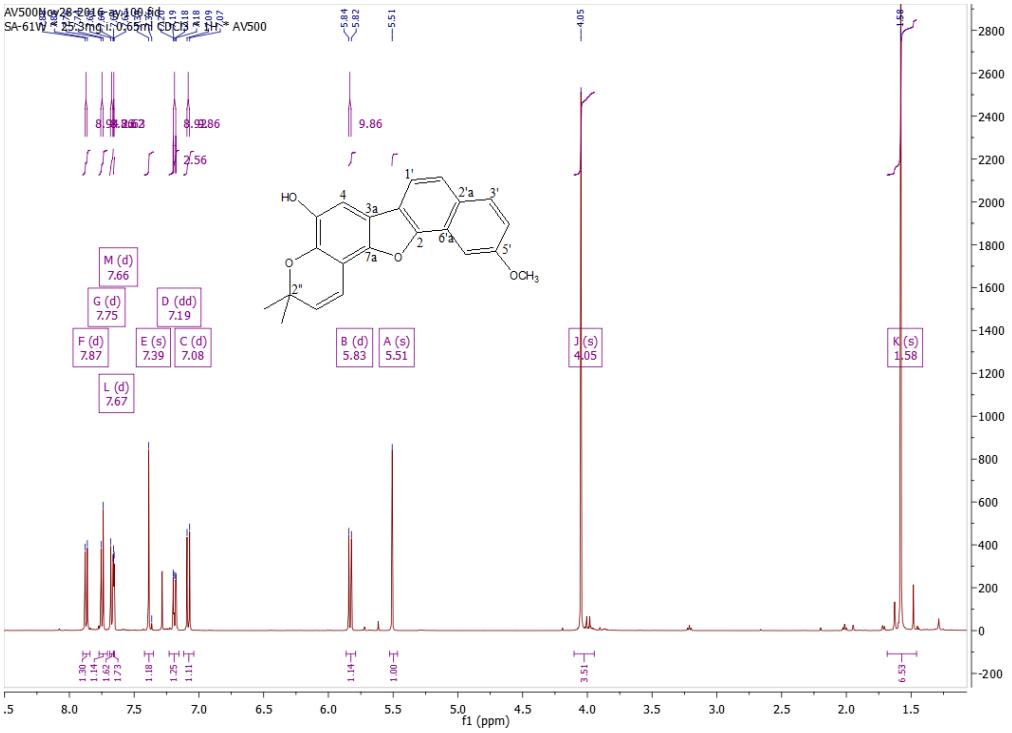


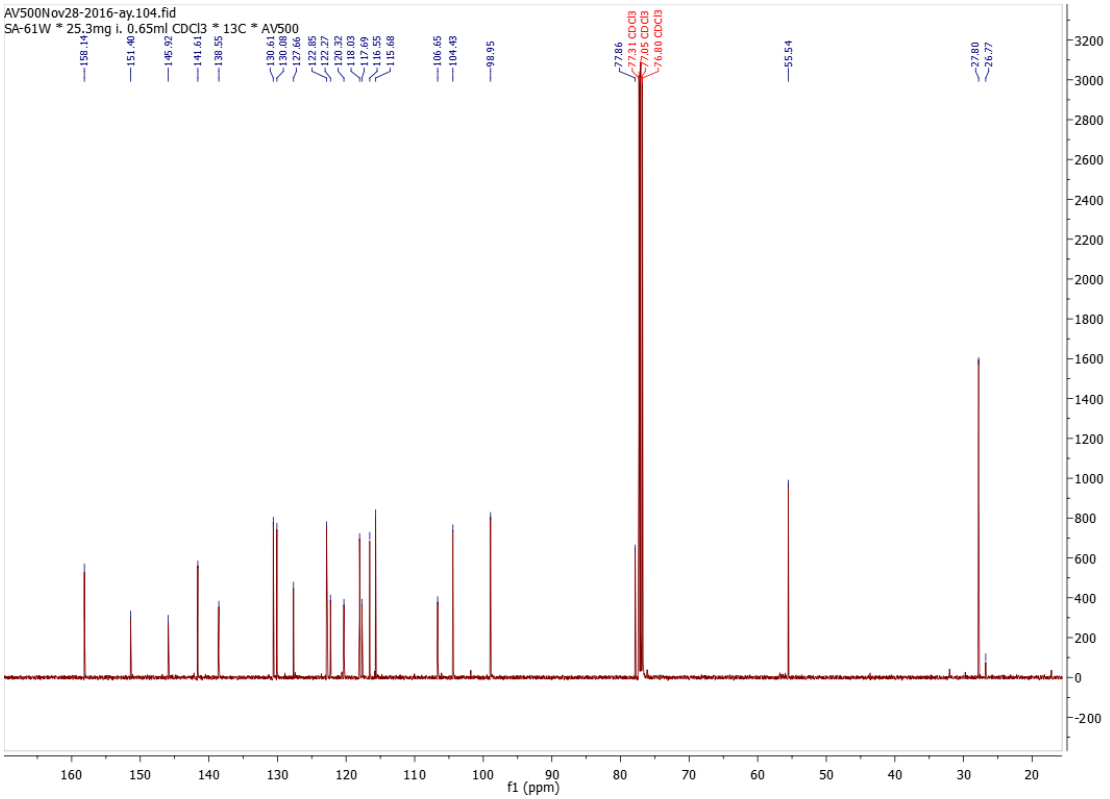




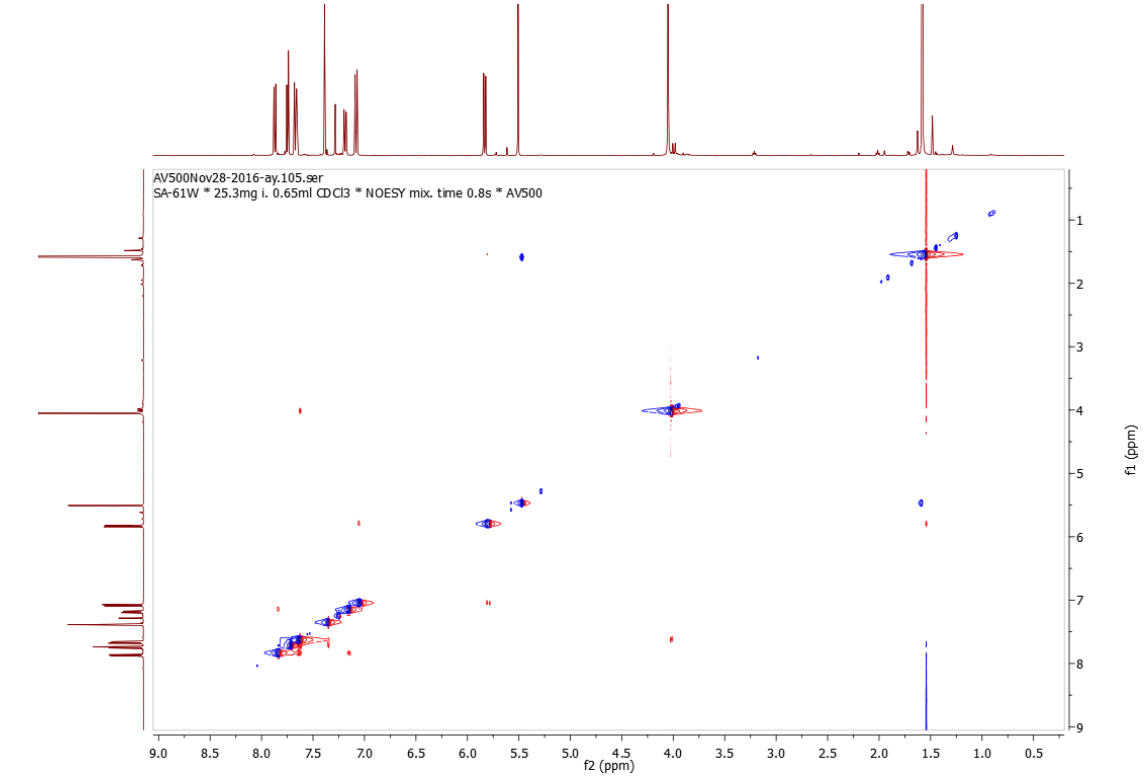


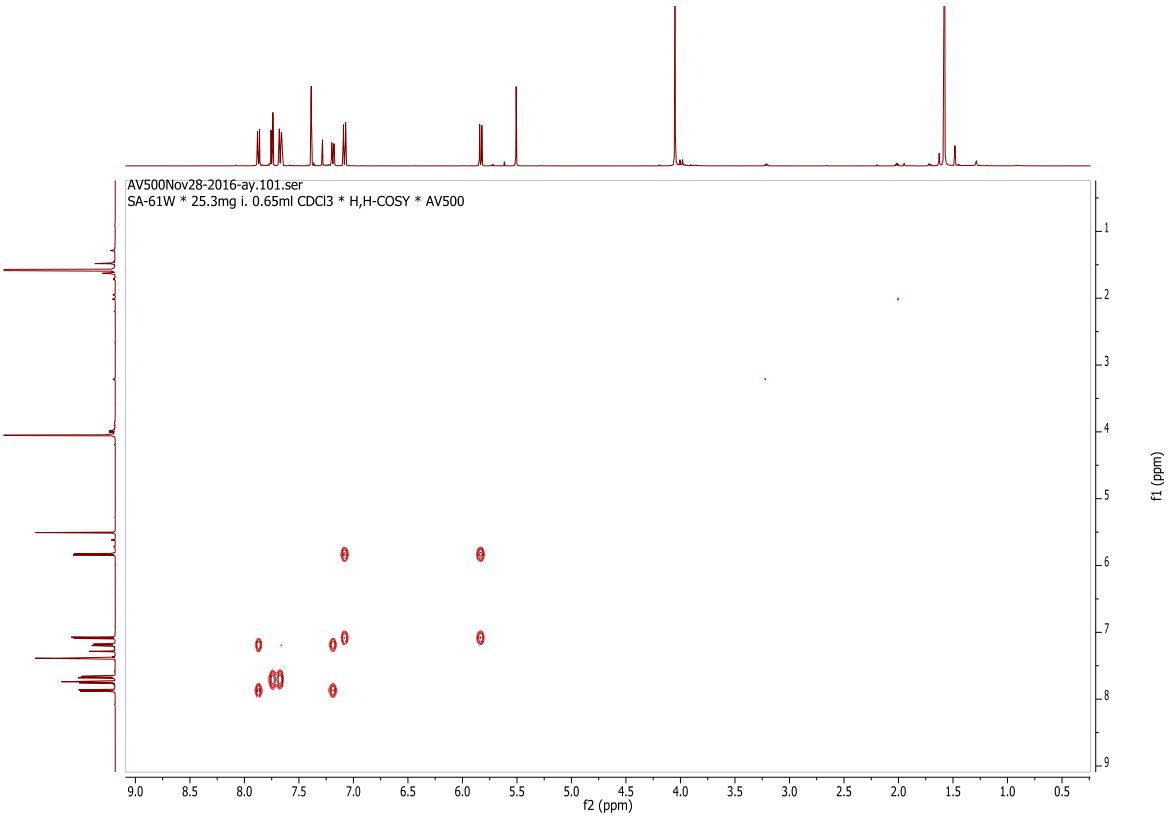
Appendix 40A: ^{1}H (500 MHz) and ^{13} C (125 MHz) NMR spectrum of compound 40 ($CDCl_3$)



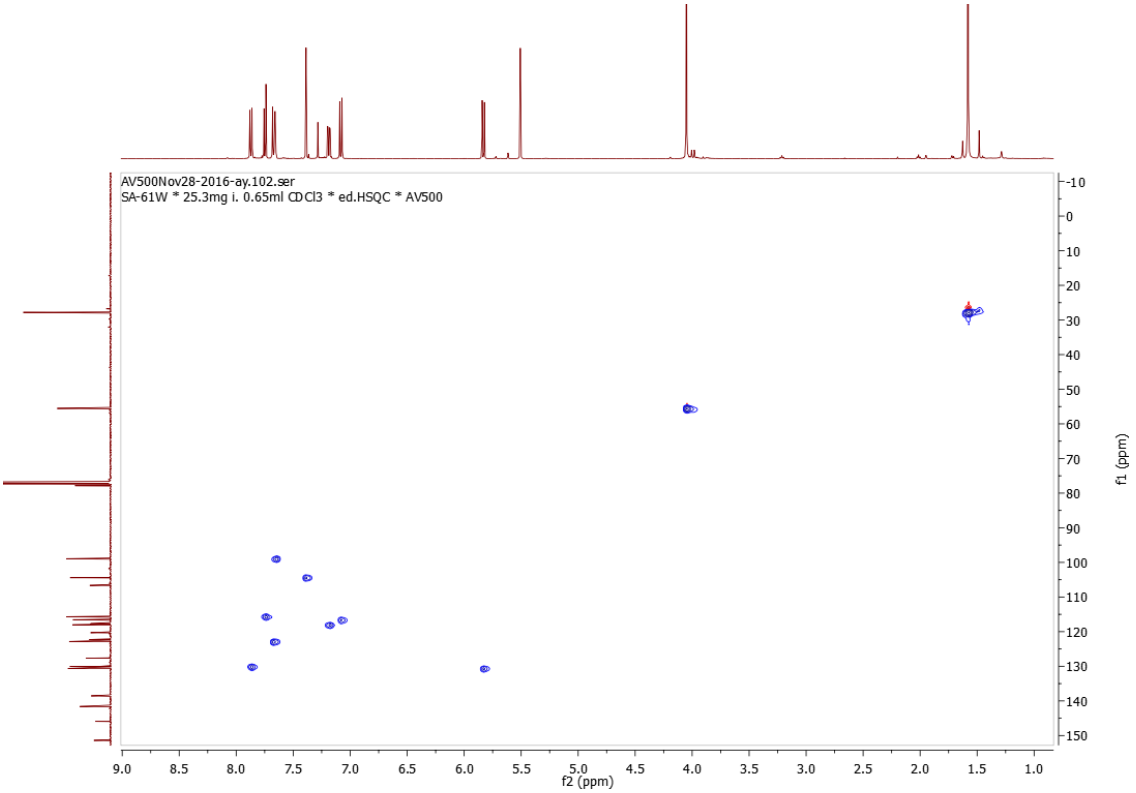


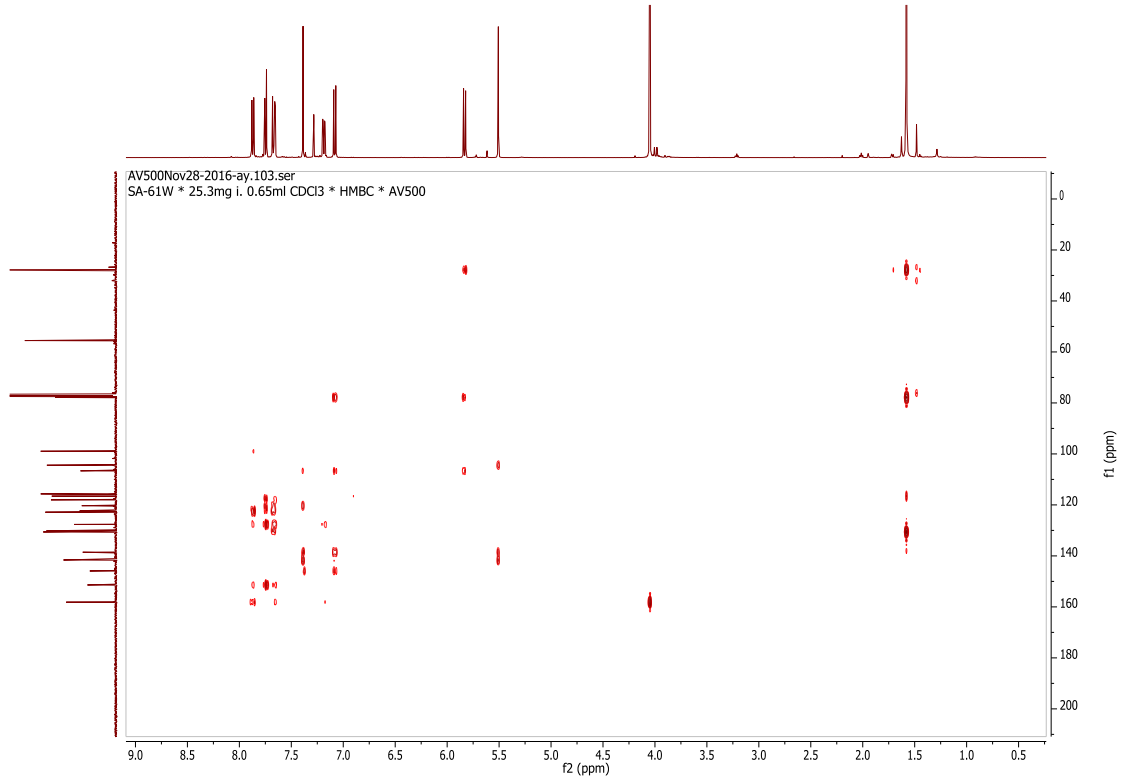
Appendix 40B: NOSEY and H,H-COSY spectra of compound 40 (500 MHz, $CDCl_3$)



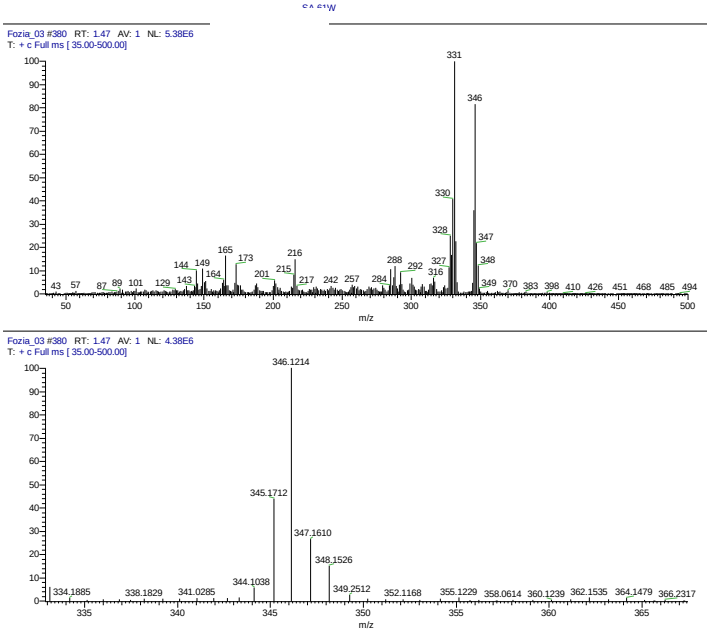


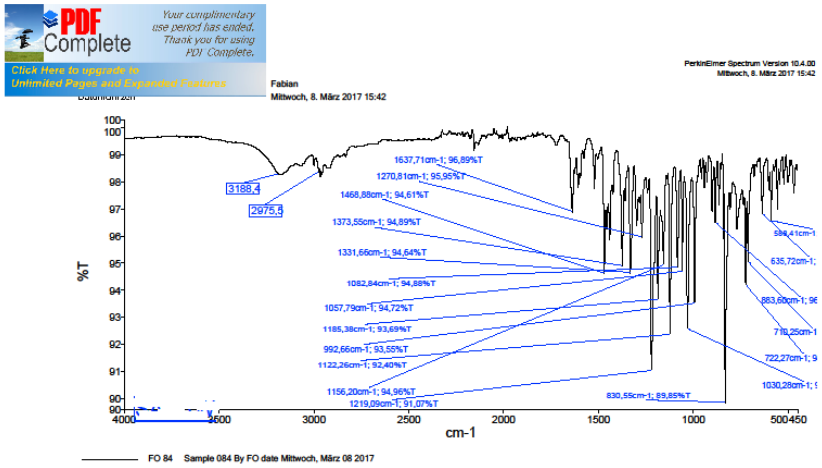
Appendix 40C: HSQC and HMBC spectra of compound 40 (500 MHz, $CDCl_3$)

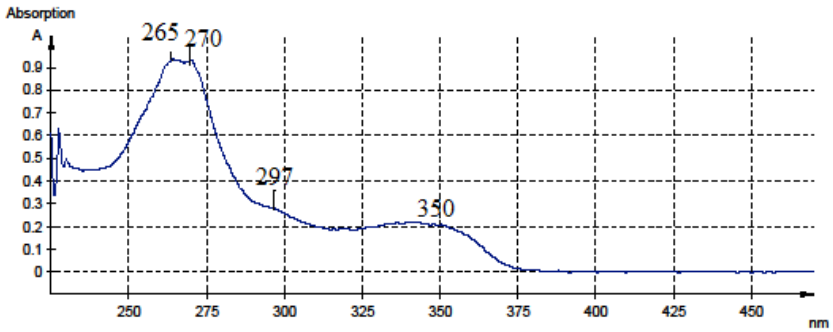




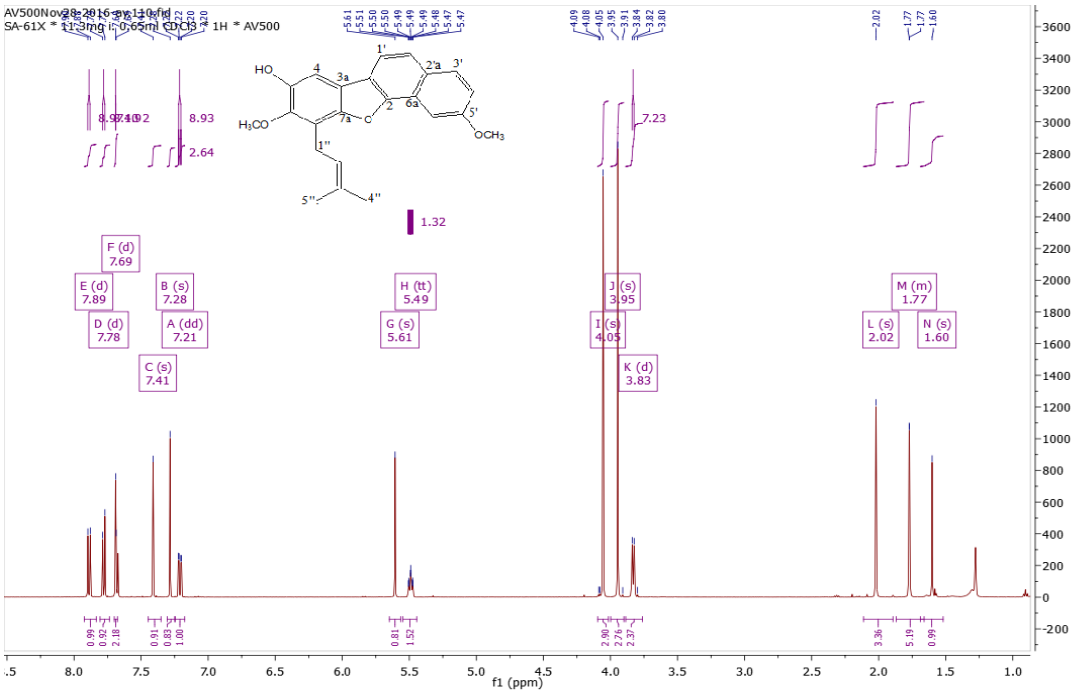
Appendix 40C: HREIMS, IR and UV spectra of compound 40

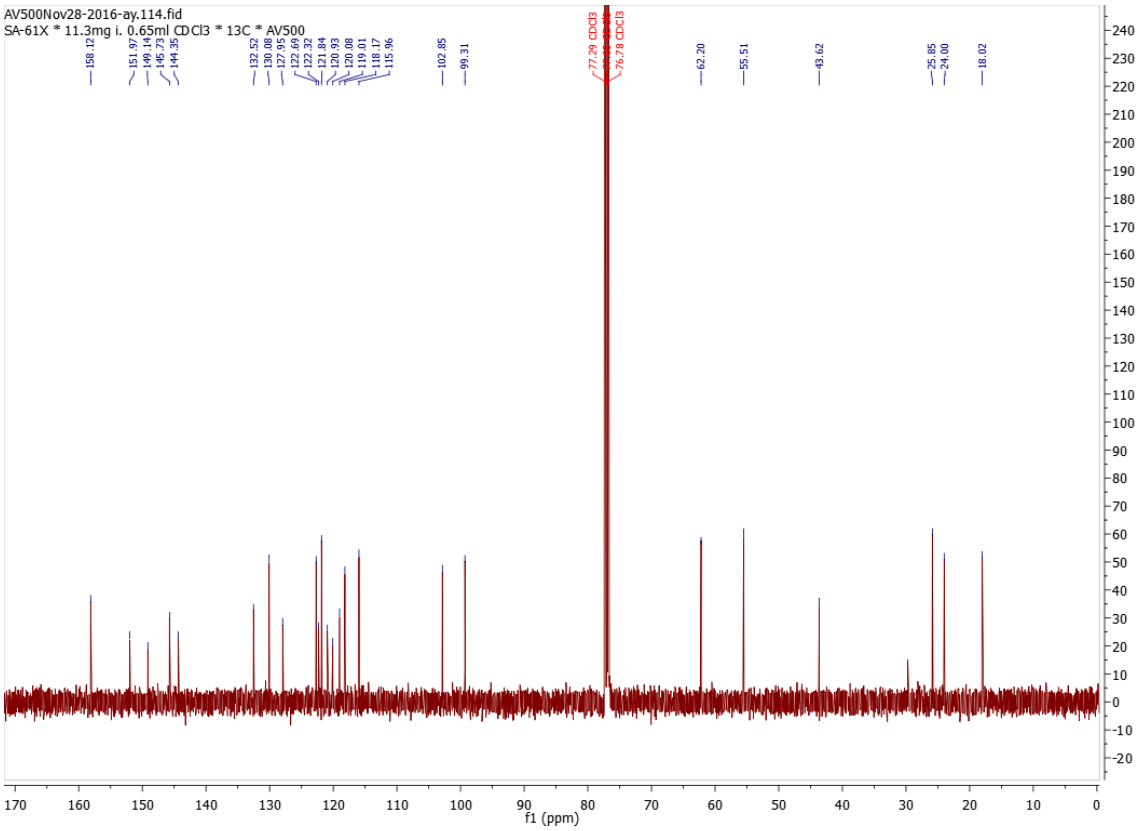




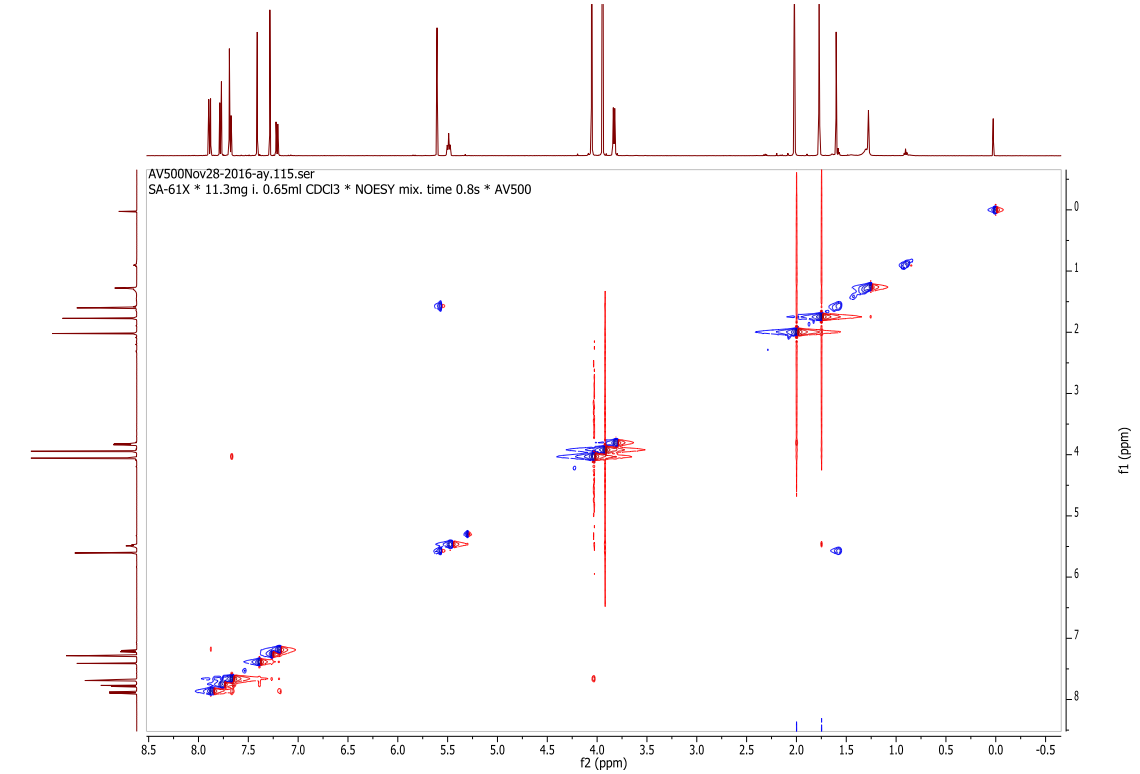


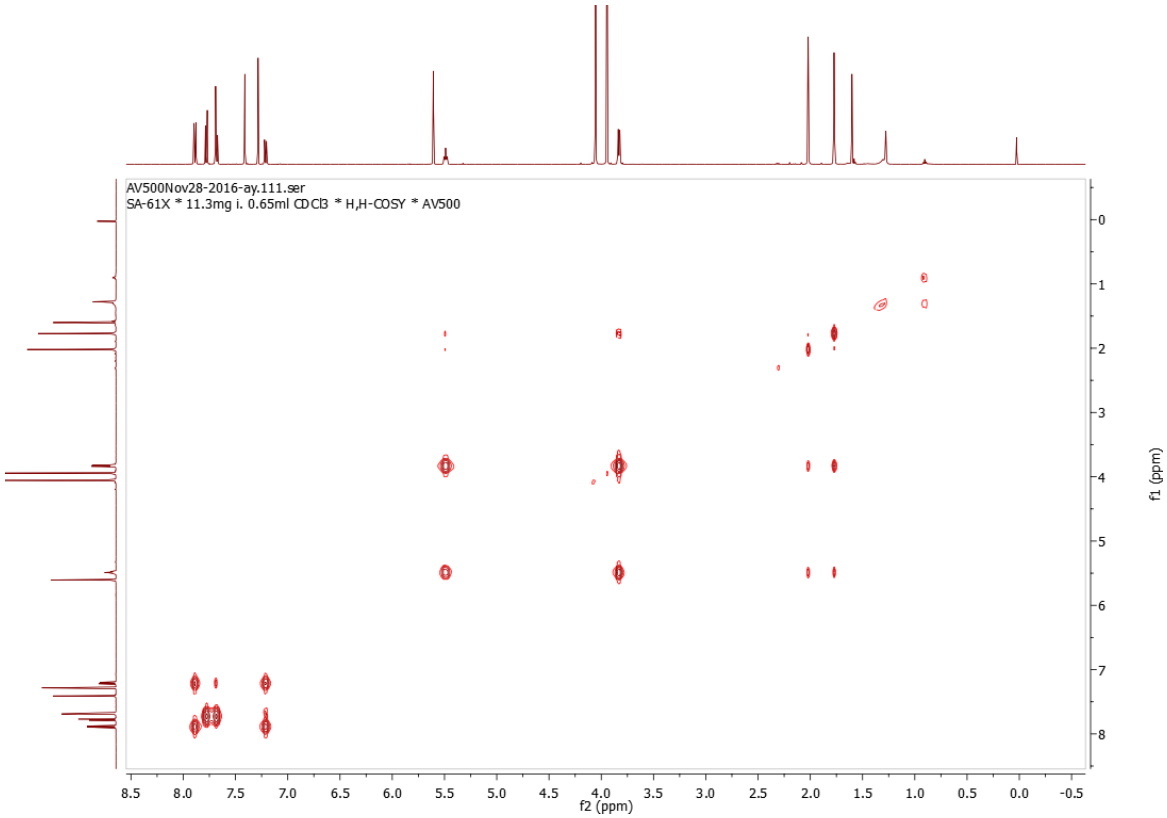
Appendix 41A: ^{1}H (500 MHz) and ^{13} C (125 MHz) NMR spectrum of compound 41 ($CDCl_3$)



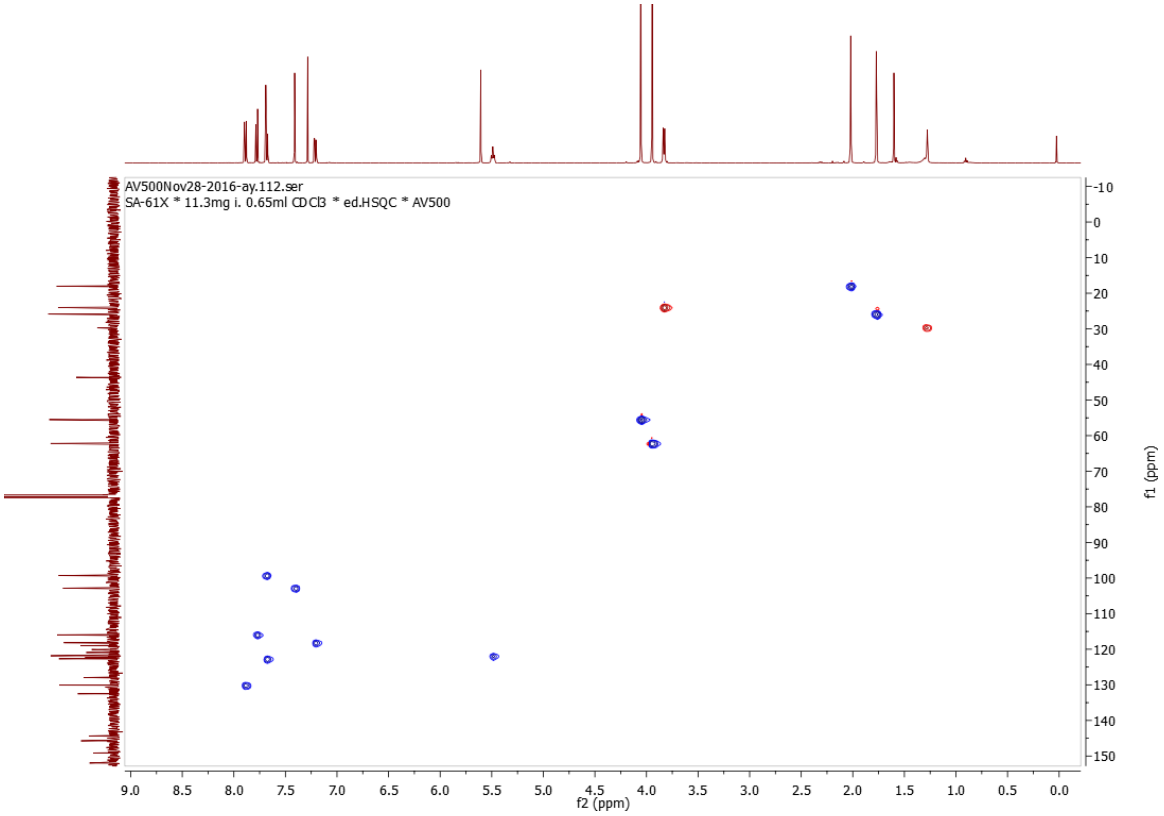


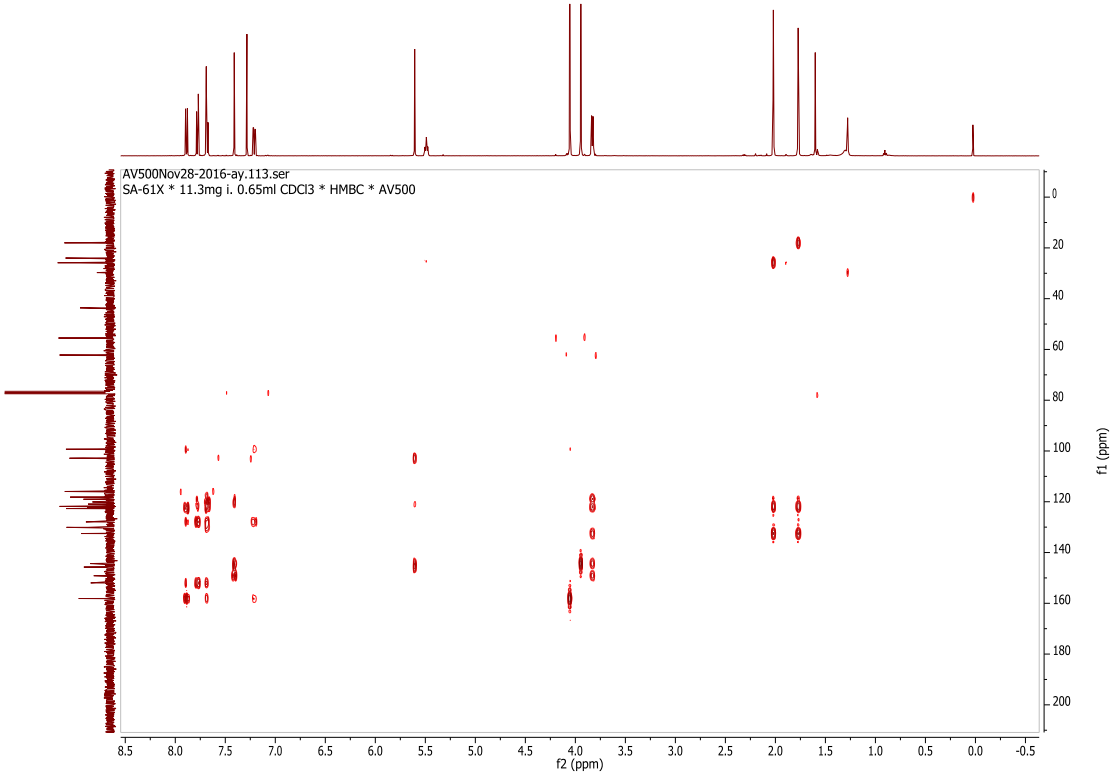
Appendix 41B: NOSEY and H,H-COSY spectra of compound 41 (500 MHz, $CDCl_3$)



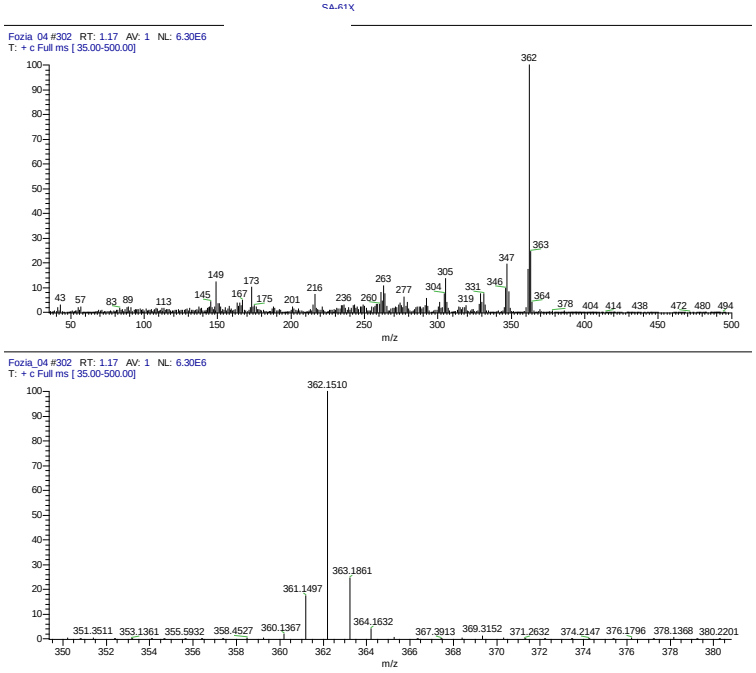


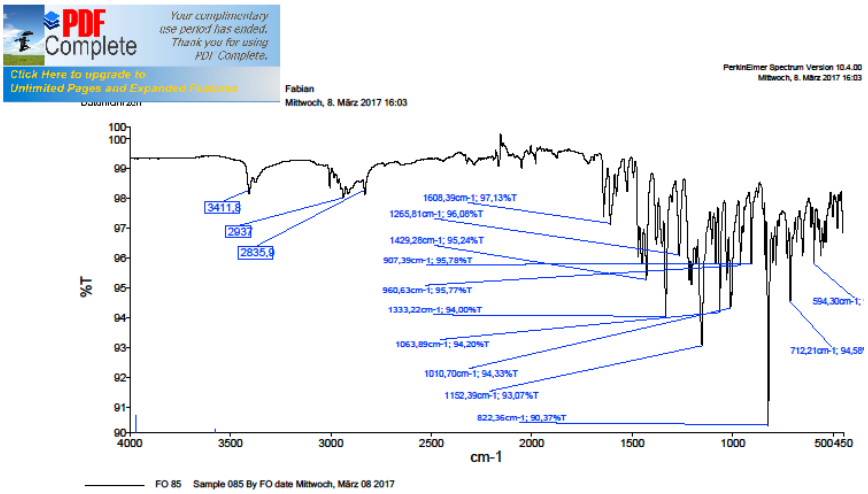
Appendix 41C: HSQC and HMBC spectra of compound 41 (500 MHz, $CDCl_3$)

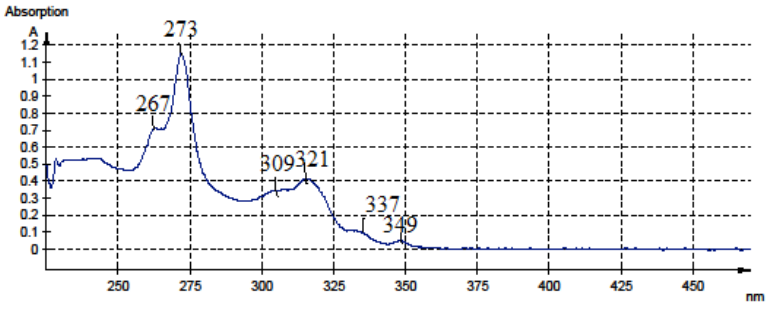




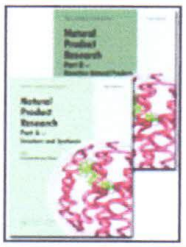
Appendix 41D: HREIMS, IR and UV spectra of compound 41







PUBLICATIONS



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Cytotoxic flavonoids from two Lonchocarpus species

Fozia A. Adem, Victor Kuete, Armelle T. Mbaveng, Matthias Heydenreich, Andreas Koch, Albert Ndakala, Beatrice Irungu, Abiy Yenesew & Thomas Efferth

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Cytotoxic flavonoids from two *Lonchocarpus* species

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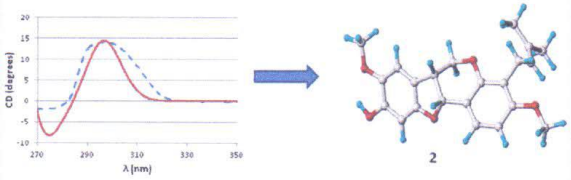
ABSTRACT

A new isoflavone, 4′-prenyloxyvigvexin A (**1**) and a new pterocarpan, (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**2**) were isolated from the leaves of *Lonchocarpus bussei* and the stem bark of *Lonchocarpus eriocalyx*, respectively. The extract of *L. bussei* also gave four known isoflavones, maximaisoflavone H, 7,2′-dimethoxy-3′,4′-methylenedioxyisoflavone, 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone, durmillone; a chalcone, 4-hydroxylonchocarpin; a geranylated phenylpropanol, colenemol; and two known pterocarpans, (6a*R*,11a*R*)-maackiain and (6a*R*,11a*R*)-edunol. (6a*R*,11a*R*)-Edunol was also isolated from the stem bark of *L. eriocalyx*. The structures of the isolated compounds were elucidated by spectroscopy. The cytotoxicity of the compounds was tested by resazurin assay using drug-sensitive and multidrug-resistant cancer cell lines. Significant antiproliferative effects with IC_{50} values below 10 μM were observed for the isoflavones 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone and durmillone against leukemia CCRF-CEM cells; for the chalcone, 4-hydroxylonchocarpin and durmillone against its resistant counterpart CEM/ADR5000 cells; as well as for durmillone against the resistant breast adenocarcinoma MDA-MB231/*BCRP* cells and resistant gliobastoma U87MG.Δ*EGFR* cells.



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Cytotoxic benzylbenzofuran derivatives from *Dorstenia kameruniana*

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Chalcone
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ABSTRACT

Chromatographic separation of the extract of the roots of *Dorstenia kameruniana* (family Moraceae) led to the isolation of three new benzylbenzofuran derivatives, 2-(*p*-hydroxybenzyl)benzofuran-6-ol (**1**), 2-(*p*-hydroxybenzyl)-7-methoxybenzofuran-6-ol (**2**) and 2-(*p*-hydroxy)-3-(3-methylbut-2-en-1-yl)benzyl)benzofuran-6-ol (**3**) (named dorsmerunin A, B and C, respectively), along with the known furanocoumarin, bergapten (**4**). The twigs of *Dorstenia kameruniana* also produced compounds **1–4** as well as the known chalcone licoagrochalcone A (**5**). The structures were elucidated by NMR spectroscopy and mass spectrometry. The isolated compounds displayed cytotoxicity against the sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cells, where compounds **4** and **5** had the highest activities (IC_{50} values of 7.17 μM and 5.16 μM, respectively) against CCRF-CEM leukemia cells. Compound **5** also showed cytotoxicity against 7 sensitive or drug-resistant solid tumor cell lines (breast carcinoma, colon carcinoma, glioblastoma), with IC_{50} below 50 μM, whilst **4** showed selective activity.

1. Introduction

The genus *Dorstenia* (family Moraceae) comprises about 170 species [1] and is distributed in tropical Africa, the Middle East, central and southern America [2,3]. In East Africa, 28 *Dorstenia* species have been recorded, of which 13 are found in Kenya [4]. The genus *Dorstenia* is a rich source of heterocyclic compounds and has long been of interest as a potential source of biologically active metabolites [5]. Some of the *Dorstenia* species are used in folk medicine for the treatment of different diseases. In Cameroon, the leaves of *Dorstenia psilurus* are used to treat cough, stomach pain and headache [6,7]. In the northern part of Ethiopia, the roots of *Dorstenia barnimiana* are used to treat leprosy, liver disease and to remove intestinal worms [8]. Previous phytochemical investigations on this genus led to the isolation of prenylated chalcones and flavonoids [7,9], furanocoumarins [10–12], styrenes [13], benzylbenzofurans [11,14] and benzofurans [6,11,13].

2. Results and discussion

From the CH_2Cl_2/MeOH (1:1) extract of the roots of *Dorstenia kameruniana*, three new benzylbenzofuran derivatives (**1–3**, Fig. 1) and the known furanocoumarin bergapten (**4**) [12] were isolated. Compounds **1–4** were also isolated from the twigs of *Dorstenia kameruniana* along with the known chalcone licoagrochalcone A (**5**) [15].

Compound **1** was obtained as a brown gum. HREIMS showed a molecular ion peak at *m/z* 240.0779, which together with NMR data (Table 1, Figs. S1-S7) allowed the assignment of the molecular formula $C_{15}H_{12}O_3$. The UV (λ_{max} 250, 258 and 287 nm), IR (Section 3.4) and NMR data (Table 1) indicated that the compound is aromatic. That this compound is a benzylbenzofuran derivative [11,14], was established from the NMR spectra (Table 1): δ_H 6.33 for H-3; δ_C 158.7 for C-2, 104.1 for C-3 (for the furan ring), and δ_H 7.29 (d, J = 8.3 Hz), 6.74 (*dd*, J = 8.3, 2.1 Hz) and 6.87 (d, J = 1.8 Hz) assigned to H-4, H-5 and H-7, respectively, for ring B protons, which is substituted with a hydroxy group at C-6 (ν_{max} at 3187 cm^{-1} for hydroxy band; δ_C 156.4 for C-6). The identity of ring B was confirmed from the HMBC spectrum (Fig. 2) where the proton signals at δ_H 7.29, 6.74 and 6.87 showed HMBC correlation with the oxygenated carbon signal at δ_C 156.4 (C-6). The methylene protons at δ_H 3.97 (s) exhibited HMBC cross peak with the signals at δ_C 158.7 (C-2), δ_C 104.1 (C-3) and δ_C 131.4 (C-3′) which was

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Cytotoxicity of isoflavones and biflavonoids from *Ormocarpum kirkii* towards multi-factorial drug resistant cancer

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Multi-drug resistance

ABSTRACT

Background: While incidences of cancer are continuously increasing, drug resistance of malignant cells is observed towards almost all pharmaceuticals. Several isoflavonoids and flavonoids are known for their cytotoxicity towards various cancer cells.

Purpose: The aim of this study was to determine the cytotoxicity of isoflavones: osajin (**1**), 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone (**2**) and biflavonoids: chamaejasmin (**3**), 7,7″-di-*O*-methylchamaejasmin (**4**) and campylospermone A (**5**), a dimeric chromene [diphysin(**6**)] and an ester of ferullic acid with long alkyl chain [erythrinasinate (**7**)] isolated from the stem bark and roots of the Kenyan medicinal plant, *Ormocarpum kirkii*. The mode of action of compounds **2** and **4** was further investigated.

Methods: The cytotoxicity of compounds was determined based on the resazurin reduction assay. Caspases activation was evaluated using the caspase-Glo assay. Flow cytometry was used to analyze the cell cycle (propodium iodide (PI) staining), apoptosis (annexin V/PI staining), mitochondrial membrane potential (MMP) (JC-1) and reactive oxygen species (ROS) (H_2DCFH-DA). CCRF-CEM leukemia cells were used as model cells for mechanistic studies.

Results: Compounds **1**, **2** and **4** displayed IC_{50} values below 20 μM towards CCRF-CEM and CEM/ADR5000 leukemia cells, and were further tested towards a panel of 7 carcinoma cells. The IC_{50} values of the compounds against carcinoma cells varied from 16.90 μM (in resistant U87MG.*ΔEGFR* glioblastoma cells) to 48.67 μM (against HepG2 hepatocarcinoma cells) for **1**, from 7.85 μM (in U87MG.*ΔEGFR* cells) to 14.44 μM (in resistant MDA-MB231/*BCRP* breast adenocarcinoma cells) for **2**, from 4.96 μM (towards U87MG.*ΔEGFR*cells) to 7.76 μM (against MDA-MB231/*BCRP* cells) for **4**, and from 0.07 μM (against MDA-MB231 cells) to 2.15 μM (against HepG2 cells) for doxorubicin. Compounds **2** and **4** induced apoptosis in CCRF-CEM cells mediated by MMP alteration and increased ROS production.

Conclusion: The present report indicates that isoflavones and biflavonoids from *Ormocarpum kirkii* are cytotoxic compounds with the potential of being exploited in cancer chemotherapy. Compounds **2** and **4** deserve further studies to develop new anticancer drugs to fight sensitive and resistant cancer cell lines.

Abbreviations: **1**, osajin; **2**, 5,7-dihydroxy-4'-methoxy-6,8-diprenylisoflavone; **3**, chamaejasmin; **4**, 7,7″-di-*O*-methylchamaejasmin; **5**, campylospermone A, **6**, diphysin; **7**, erythrinasinate; ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; BNIP-3, BCL2 Interacting Protein 3; DMSO, dimethylsulfoxide; D.R., resistance; EGFR, epidermal growth factor receptor; FITC, flourescein isothiocynate; H_2O_2, Hydrogen peroxide; H2DCFH-DA, 2′,7′-dichlorodihydrofluoresceine diacetate; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; IC_{50}, 50% inhibitory concentration; MDR, multidrug resistance; MMP, mitochondrial membrane potential; PARP-1, poly (ADP-ribose) polymerase 1; P-gp, P-glycoprotein; PI, propidium iodide; RIPK-1, receptor-interacting serine/threonine-protein kinase 1; ROS, reactive oxygen species

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