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PHYTOCHEMICAL INVESTIGATION OF *ALOE TURKANENSIS* FOR ANTICANCER ACTIVITY

BY

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for an award of a degree.

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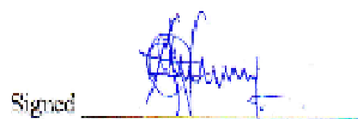
Signed  Date 4/6/2014.

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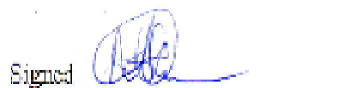
Signed  Date 04/6/2014

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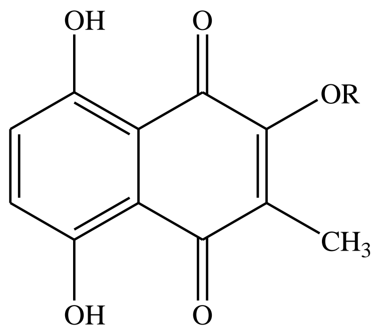
ABSTRACT

Cancer cases are on the increase all over the world including Sub-Saharan countries. In the search for new anticancer drugs, nature remains an excellent source of lead compounds. Among various classes of natural compounds quinones are well known for their anticancer activities some being drugs (e.g. daunomycin and doxorubicin). The genus *Aloe* including *Aloe turkanensis* is a rich source of quinones.

The dried and ground rhizomes and leaves of *Aloe turkanensis* were exhaustively extracted with dichloromethane/methanol (1:1) by cold percolation. The crude extracts exerted cytotoxic activity against human extra hepatic bile duct cancer cell line (TFK-1) by showing significant reduction in cell viability. The crude extracts were then subjected to chromatographic separations on silica gel, Sephadex LH-20 and preparative TLC, which resulted in the isolation of twelve compounds. The structures of the isolated compounds were determined using spectroscopic methods including UV, ^{1}H and ^{13}C NMR, COSY, NOESY, HMBC and HSQC. These compounds were two naphthoquinones [3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**)], seven anthraquinones [chrysophanol (**3**), aloesaponarin I (**4**), aloesaponarin II (**5**), laccaic acid D methyl ester (**6**) helminthosporin (**8**) aloe-emodin (**10**) and α-L-11-*O*-rhamnopyranosylaloe-emodin (**11**)], a preanthraquinone [aloesaponol I (**7**)] a pyrone derivative [feralolide (**9**)] and a benzoic acid derivative [3,4-dihydroxybenzoic acid (**12**)]. The naphthoquinones [3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) and 5,8-dihydroxy-2-methoxy-2-methylnaphthalene-1,4-dione (**2**)] is here reported for the second time from the genus *Aloe*. Furthermore, 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) is reported here for the first time from the family Asphodelaceae.

The *in-vitro* anticancer activities of the isolated compounds were conducted against the human extra hepatic bile duct carcinoma (TFK-1) and liver (HuH7) cancer cell lines. Based on the 3-[4,5-dimethylthiazol-2-yl]-2-,5-diphenyltetrazolium bromide (MTT) assay, the anthraquinone aloe-emodin (**10**) and the naphthoquinone 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**) exhibited high inhibition against TFK-1 cell lines with IC_{50} values of 6.0 and 15.0 µg/mL (in TFK-1 cells) and 31 and 20 µg/mL (in HuH7 cell line), respectively. The pre-anthraquinone aloesaponol I (**7**) inhibited the growth of TFK-1 cells with IC_{50} values of 10.0 µg/mL, and with 88.0 µg/mL against the HuH7 cells. The aloe-emodin glycoside, α-L-11-*O*-rhamnopyranosylaloe-emodin (**11**) and the anthraquinone aloesaponarin II (**4**) inhibited viability of TKF-1 cell line with IC_{50} values of 23.0 µg/mL and 34.0 µg/mL, respectively; and with IC_{50} values of 47.0 µg/mL and 55.0 µg/mL in HuH7 cell lines, respectively. Helminthosporin (**8**) also significantly inhibited the growth of TFK-1 cells with IC_{50} values of 46.0 µg/mL but did not inhibit the HuH7 cells at the tested concentrations. This is the first report on the anticancer activity of the isolated compounds against extra hepatic bile duct (TFK-1) and liver (HuH7) cancer cell lines. Further investigation on normal cell lines and their mechanism of action has to be estsblished before the potential of these compounds as anticancer drugs can be established.



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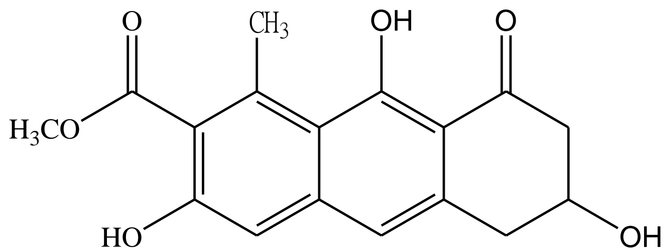

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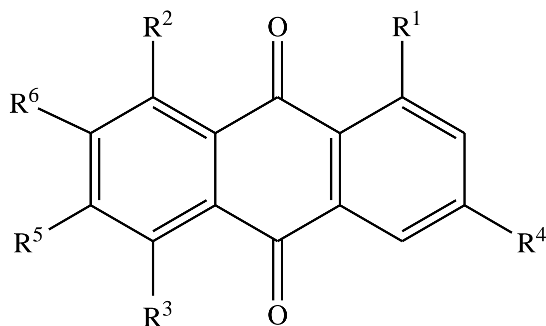


3 $R^1 = R^2 = OH$, $R^4 = CH_3$, $R^3 = R^5 = R^6 = H$

4 $R^1 = R^5 = OH$, $R^2 = CH_3$, $R^3 = R^4 = R^6 = H$

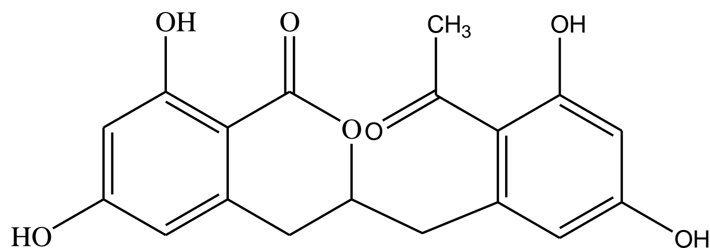
5 $R^1 = R^5 = OH$, $R^2 = CH_3$, $R^3 = R^4 = H$, $R^6 = COOCH_3$

6 $R^1 = R^4 = R^5 = OH$, $R^2 = CH_3$, $R^3 = H$, $R^6 = COOCH_3$

8 $R^1 = R^2 = R^3 = OH$, $R^4 = CH_3$, $R^5 = R^6 = H$

10 $R^1 = R^2 = OH$, $R^3 = R^5 = R^6 = H$, $R^4 = CH_2OH$

11 $R^1 = R^2 = OH$, $R^3 = R^5 = R^6 = H$, $R^4 = CH_2ORham$



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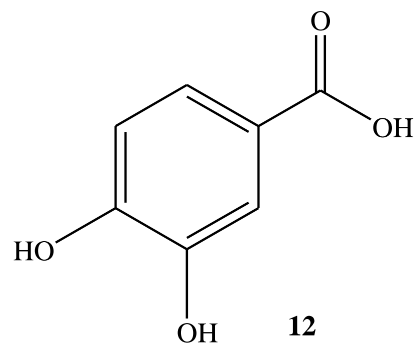

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DEDICATION

This thesis is dedicated to my brother, Kedir Ali Adem. I also dedicate this thesis to my memory at Saarland University, Germany.

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LIST OF ABBREVIATIONS

1D	One Dimensional
2D	Two Dimensional
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HRMS	High resolution Mass Spectroscopy
HCC	Hepatocellular Carcinoma
EHBDC	Extrahepatic Bile Duct Cancer
MS	Mass Spectrometry
MHz	Mega Hertz
m/z	Mass to Charge ratio
$[M]^+$	Molecular ion
NMR	Nuclear Magnetic Resonance
nm	Nanometer
UV	Ultra Violet
λ_{max}	Maximum wavelength of absorption
Hz	Hertz
J	Coupling constant
s	Singlet
d	Doublet
dd	Double doublet
t	Triplet
m	Multiplet
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
IC_{50}	Concentration of 50% inhibition
WHO	World Health Organization

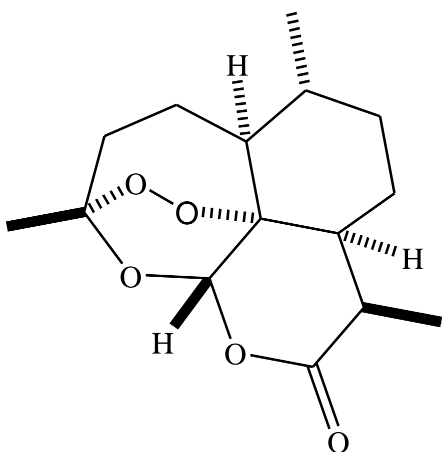
CHAPTER ONE

INTRODUCTION

1.1. Background of the study

Throughout history, human beings have used remedies from nature to improve their health or to cure illnesses. The first written proof of ethno-medical science is at least a thousand years old in countries such as China (Chang and But, 1986) and India (Kapoor, 1990). In modern times several communities, especially those in developing countries, still rely on traditional medicinal plant based treatment. This is mainly due to limited availability or affordability of conventional medicines in the developing countries. It is estimated that 80% of the population in developing countries depend on traditional medicine for their primary health care needs (WHO, 2002).

Based on the ancient traditional practices; scientists have been purifying the active constituents which are responsible for the treatment of different diseases. For example, the antimalarial agent artemisinin (**13**), was isolated from *Artemisia annua* - an old Chinese anti-malaria remedy called *quinhao* (Dewick, 2002). Furthermore, through the advances in the field of pharmacy and chemical sciences, the formulation of the first purely synthetic drugs, aspirin (**14**), was achieved through acetylation of salicylic acid (**15**); an active ingredient in *Salix alba* which is known for its pain-relieving property (Samuelson, 2004). The first agent to be advanced in cancer chemotherapy is taxol (**16**), isolated from the Indian traditional medicine *Taxus brevifolia*. This was shown to be effective in the treatment of cancer (Wani *et al*., 1971).



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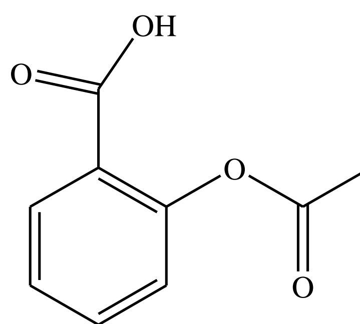

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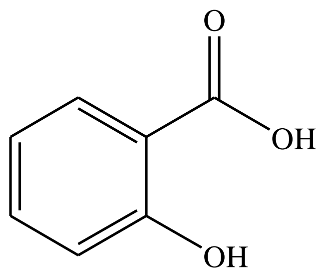

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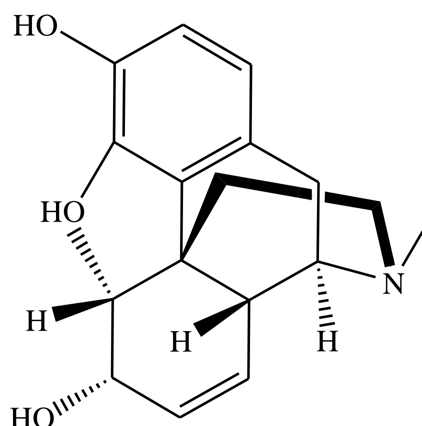

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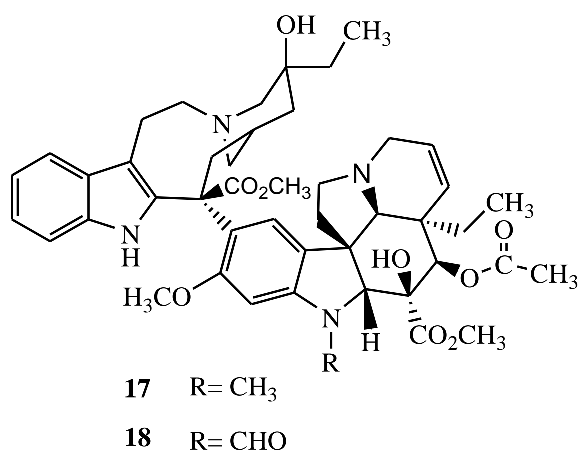
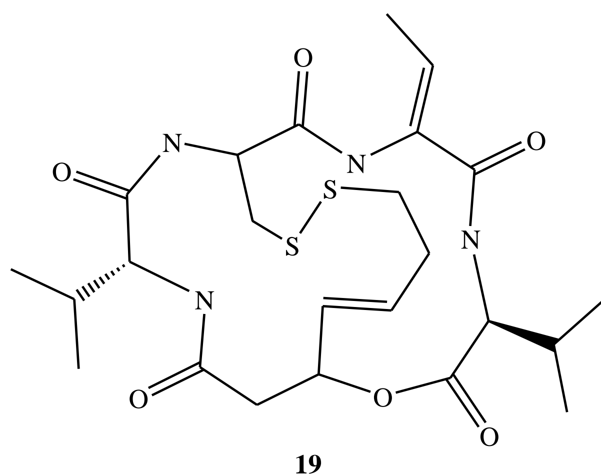
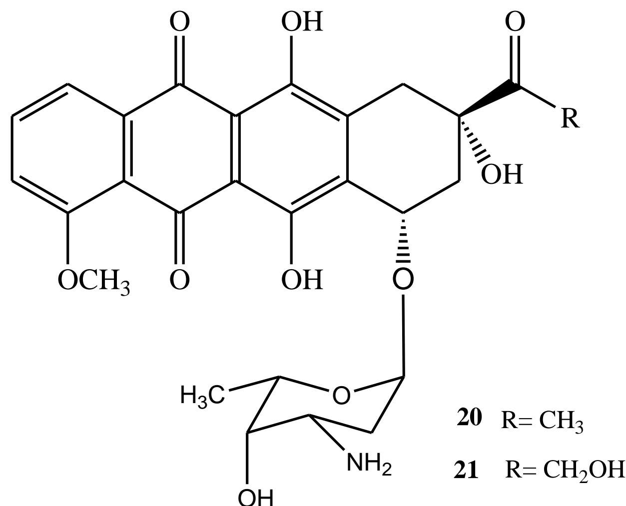
Cancer is a disturbed balance between the rate of cell division and cell death. Normal cells in the body follow a systematic path way of growth, division, and death. Programmed cell death is called apoptosis, and when this process fails, cancer begins to form. Unlike normal cells, cancer cells do not experience programmed death and instead continue to grow and divide. This leads to a mass of abnormal cells that grow out of control and can affect other organs of the body (Bright and Khar, 1994). The main risk factor of cancers are largely related to lifestyle choices; while certain infections, occupational exposures to harmful chemicals and rays and some environmental factors can also cause cancer (Buell and Bann, 1965; Kolonel and Wilkens, 2006).

Nature remains an ever evolving source of compounds of medicinal importance. In this regard the isolation and characterization of compounds from medicinal plants continue today. For example, the vinca alkaloids, vinblastin (**17**) and vincristine (**18**) from the Madagascar *Catharanthus roseus* (Apocynaceae), have been identified as a cancer chemotherapeutic agents (Stefania *et al*., 2009). More recently romidepsin (**19**), a bi-cyclic peptide, isolated from the bacteria *Chromo bacterium violaceum* strain 968, is cytotoxic against several human cancer cell lines; and currently it is undergoing clinical trials for the treatment of cancers (Haigentz *et al*., 2012).

Natural compounds containing quinone moiety are well-known as anticancer drugs. Among these daunomycin (**20**) was isolated from the bacteria *Streptomycin peucetius* var. *caesius* and its derivative doxorubicin (**21**) contain quinone moiety and is known for the treatment different solid tumor types as well as acute leukemia (Octavia *et al*., 2012).







It is then apparent that the genus *Aloe* including *Aloe turkanensis* may provide opportunities for seeking new anticancer therapeutic drugs because of the availability of compounds containing the quinone moiety.

1.2. Statement of the Problem

Cancer remains a major global public health problem that is responsible for almost 13% deaths. It is estimated that 12.7 million new cancer cases were diagnosed worldwide in 2008, with noted increase in developing countries (Ferlay *et al.*, 2008). It is suggested that cancer rates could be doubled by 2020, the increase will be higher in developing countries where 80% of the population depends on medicinal plants whose safety and efficacy is not well understood or documented (Murray and Lopez, 1996). Therefore, there is a need to develop safe, effective and affordable anticancer drugs and plant preparations.

1.3. General objective

This project is geared towards identification of cytotoxic constituents from *Aloe turkanensis*.

1.4. Specific objectives

i) To isolate the constituents of the leaves and rhizomes of *Aloe turkanensis*;

ii) To characterize the structure of the isolated compounds;

iii) To determine the anticancer activity of the isolated compounds and crude extracts against extrahepatic (TFK-1) and liver cancer (HuH7) cell lines.

1.5. Justification

The search for natural product derived anticancer drugs has been ongoing over the past decades that resulted in the isolation of vinka alkaloids from the plant *Vinca rosea*, and taxol from the bark of the Western yew *Taxus brevifolia*. These are some examples of cytotoxic compounds that are commonly used in cancer treatment. The role of quinones in the anticancer drug discovery either as natural products or their derivatives are known (Salustiano *et al*., 2009; Kovacic *et al*., 2011). The genus *Aloe* is good source of quinones. It was therefore reasonable to explore the cytotoxic constituents from *Aloe* species. *Aloe turkanensis* is widly used to treate different diseases. However, neither the phytochemistry nor the anticancer activity of this plant hase been investigated.

CHAPTER TWO

LITERATURE REVIEW

2.1. The Cancer Problem

The word cancer was coined by the father of medicine, Hippocrates; who used the Greek term *carcinos* to describe tumors. *Carcinos* is the Greek word meaning crab. Hippocrates thought the body of crab was to be similar to cancerous tumor. He also believed that an excess of the black bile in the body caused cancer (www.cancer.org).

Cancer cells multiply uncontrollably in a different way from normal cell growth and invade the normal cell function and in some cancers it results in the formation of an abnormal mass of tissue, called tumors (Khanna *et al.*, 2001; Castedo *et al.*, 2004).

Cancer has been labeled as western problem, but of late it is increasingly becoming a public health problem in developing countries. Its occurrence is mainly due to lifestyle - smoking, excess alcohol consumption and diet (Belpomme *et al.*, 2007); exposure to diverse physical, chemical and biological agents also play a major role in the occurrence of the disease (Epstein *et al.*, 1994, Sasco *et al.*, 2003 and Clapp *et al.*, 2006). According to Globocan, a WHO report; in 2008 alone there were 12.7 million new cancer cases worldwide; and it was suggested that it will increase to 17 million by 2020. This increase will be more in low and medium resource countries (Stewart and Kleihues *et al.*, 2003).

2.1.1. Hepatocellular cancer (HCC)

Hepatocellular cancer (HCC) is the most common primary malignant tumor of the liver. In the year 2007, there were about 711,000 new cause of HCC worldwide and women are more susceptible to HCC than men (American cancer society, 2007). HCC is the major case of all liver

cancer and is caused by hepatitis B or C viral infection or chronic alcohol consumption (Llovet *et al.*, 2003). Other possible risk factors associated with HCC are smoking, obesity, contamination of food with aflatoxins B_1 and type-2 diabetes. High incidence and mortality are reported in Eastern Asia and Sub-Saharan Africa (Parkin *et al.*, 1998) as the result of chronic hepatitis inflammation and dietary aflatoxin exposure (Wild *et al.*, 2002; Bosch *et al.*, 2004). In low HCC incidence areas such as Northern Europe and the US, chronic alcohol consumption plays an important role in the development HCC (Hassan *et al.*, 2002; Oshea *et al.*, 2010). Furthermore, obesity-related non-alcoholic fatty liver disease (NAFLD) is being considered as risk factors of HCC. NAFLD is the accumulation of fat in the liver cells and is called steatosis. The extreme form of NAFLD is known as non-alcoholic steatohepatitis (NASH) which causes cirrhosis and hepatocellular carcinoma (HCC) (Chalasani *et al.*, 2012).

With the aim of increasing survival rate in patients affected by HCC, several classification systems have been made to identify tumor stage of the liver to select the appropriate treatment strategy. These include the Okuda (Okuda *et al.*, 1985), Japan Integrated Staging (JIS) (Kudo *et al.*, 2003), Chinese University Prognostic Index (CUPI) (Leung *et al.*, 2002) and the Barcelona Clinic Liver Cancer (BCLC) staging systems.

The Barcelona Clinic Liver Cancer (BCLC) staging as approved by a European association for clinical management of HCC patients, recognizes four stages of tumor: early stage, intermediate stage, advanced stage and end-stage tumor (Bruix *et al.*, 2002).

Liver transplantation (LT) and hepatic resection are the best surgical modalities in the treatment of HCC with an early stage of HCC, resulting in improved survival rate (Bismuth *et al.*, 1999). Percutaneous ablation such as percutaneous ethanol injection (PEI) and radiofrequency ablation

(RFA) are the best treatment options for early stage tumors (Bruix *et al.*, 2011). Transarterial arterial chemoembolization (TACE) is widely used and is efficient in reducing tumor growth at the intermediate Barcelona Clinic Liver Cancer (BCLC) stage and also it increases in survival rate (Llovet *et al.*, 1999). Recently an emoblizing device, drug-eluting beads (DCB) has been developed for the treatment of HCC to decrease toxicity during TACE.

2.1.2. Extra hepatic bile duct cancer (EBDC)

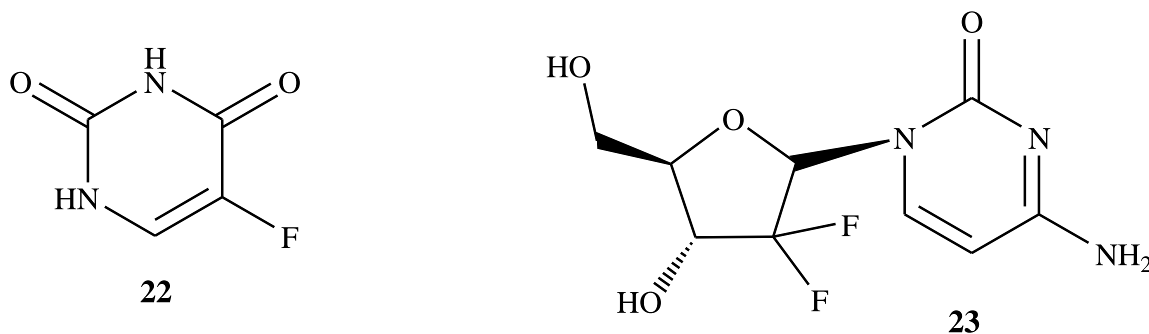
Extra hepatic bile duct carcinoma is an aggressive malignancy, accounting for 3% all of cases of gastrointestinal malignancies with poor chance (5-year) of survival rate as the result of advanced tumor stage detection (Khan *et al.*, 2005; Mizumoto *et al.*, 1993) and it is mainly due to primary sclerosing cholangitis, choledocholithiasis, and an abnormal pancreaticobiliary duct junction resulting chronic inflammatory state of the bile duct (Patel *et al.*, 2006). Other possible risk factors include inflammatory bowel disease, liver fluke, infection, hepatolithiasis, thorotrast, nitrosamines (Stewart and Kleihues, 2003).

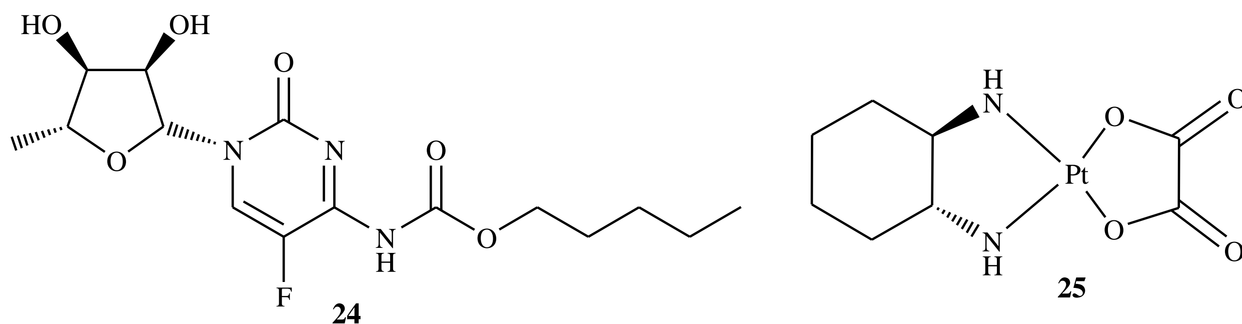
Tumor of the bile duct may happen intrahepatically or extrahepatically. Intrahepatic bile duct tumor grows in the bile duct branch within the liver. Previously the extrahepatic bile duct tumor was considered as a single tumor. However, in recent literature the extrahepatic bile duct tumor is classified into distal bile duct tumors and perihilar or hilar tumors, also called klatskin tumor (AJCC, 2011). Perihilar tumor comprises about two-third of all cases of extrahepatic tumor (De Groen *et al.*, 1999).

Predetermination of the size or spread of the tumor is important in order to identify the choice of treatment. The most commonly used staging system for EBDC are the Bismuth-Corlette staging system, which is designed for tumor spread along the biliary tree (Bismuth *et al.*, 1975); and the

American Joint Committee on Cancer (AJCC) staging system which is based on the size of the tumor as well as spread to different part of the body (Grenee *et al*., 2002). Surgical resection is the treatment of choice for patients with advanced EBDC with poor 5-year survival rate (Bismuth *et al*., 1992).

For the purpose of increasing the survival rate during surgical resection, gemcitabine chemotherapy (Murakami *et al*., 2009) and adjuvant radiotherapy (Stein *et al*., 2005) have been used after resection. Single chemotherapeutic agents such as 5-FU (Fluorouracil) (**22**) (Raderer *et al*., 1999; Hsu *et al*., 2004), gemcitabine (**23**) (Kubicka *et al*., 2001) and capsitabine (**24**) (Lozano *et al*., 2000) as well as the drug combinations, oxaliplatin/capecitabine (XELOX) (Nehls *et al*., 2003), gemcitabine/oxaliplatin (**25**) (GEMOX) (Andre *et al*., 2006) and gemcitabine/capecitabine (Iyer *et al*., 2005) are used to cure cholangiocarcinoma. Several studies have been conducted concerning the photodynamic therapy with the aim of increasing survival rate of patients with bile duct carcinoma (Berr *et al*., 2000; Zoepf *et al*., 2005; Ortner *et al*., 2003).





2.2. Cancer Treatment

The battle to control cancer has been fought on several fronts, including surgery, use of high energy rays, prophylactic chemotherapy as well as the use of antibody and vaccine related approaches; gene therapy to reverse, suppress, or prevent the development of cancer have also been used (www.cancer.org).

2.2.1. Cancer chemotherapy

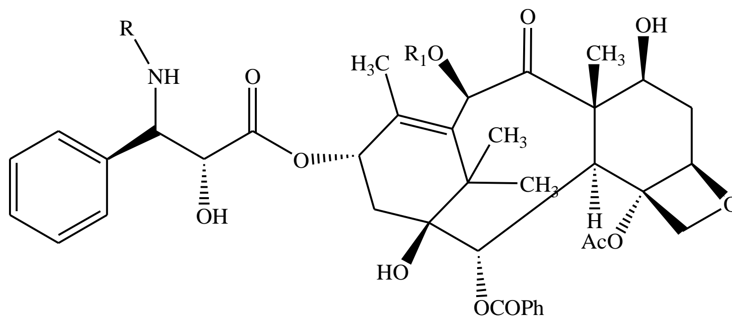
The principle governing the chemotherapy of cancer involves the use of drugs to selectively destroy a tumor or limit its growth. The concept of selective action of chemotherapeutic compounds must therefore be considered from both the qualitative and the quantitative aspect. The available anticancer drugs have a well defined range of action against different cancer cell lines. They also have a varying degree of activities against the different stages of cell cycle. Thus any assessment of the value of an anticancer compound must be based on an understanding the level of activities in relation to the various stages of cancer cells (Jaishree *et al*., 2009; Andrew *et al*., 2012).

2.2.1.1. Anticancer drugs from higher plants

Medicinal plants have played important roles in the discovery of anticancer drugs. Some of these are summerized below:

2.2.1.1.1. Paclitaxel and docetaxel

For many years, paclitaxel (**26**) and docetaxel (**27**) were considered to be the most important drugs for the treatment of cancer. Paclitaxel (**26**), isolated from the bark of Western *Taxus brevifolia,* is a diterpenoid having a taxane ring with a four-membered oxetane ring and an ester side chain at C-13. Docetaxel (**27**) is a semisynthetic derivative of a taxane and is highly effective in both mono-therapy and combination therapy for different tumor types (Ringel *et al.*, 1991; Hanauske *et al.*, 1992). Paclitaxel (**26**) and docetaxel (**27**) inhibit cell replication by forming rigid microtubular network blocking cells in the late G2-mitotic phase of the cell cycle. This inhibiting ability prevents the cells from duplicating by themselves (Kim *et al.*, 2001; Singla *et al.*, 2002). These are the most widely used drugs in treatment of solid tumors, including ovarian cancer, lung cancer and breast cancer (Nicolas *et al.*, 2006).

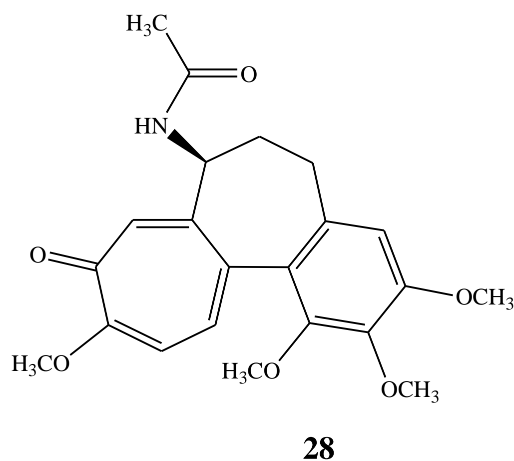


26 R = COPh $R_1 = CH_3$

27 R = $COOC(CH_3)_3$ R_1 = H

2.2.1.1.2. Colchicine

Colchicine (**28**), a tricyclic compound containing a tropolone ring was isolated from *Colchicum autumnale* plant (Liliaceae) (Nuki *et al.*, 2006). It is effective anticancer agent, however its medical use has declined due to side effects which include gastroenteritis, blood dyscrasias, dermatitis and neuromuscular toxicity (Kuncl *et al.*, 1987). To overcome the toxicity associated with colchicine, many derivatives have been chemically modified. For example *N*-methyl colchiceinamide, a semisynthetic derivative of colchicine, is slightly less toxic than colchicine (Lin *et al.*, 1988). The mechanism of action of colchicines (**28**) and its analogs is that it suppresses cell division by inhibiting mitosis by binding to tubulin and hence prevents its polymerization into microtubules.



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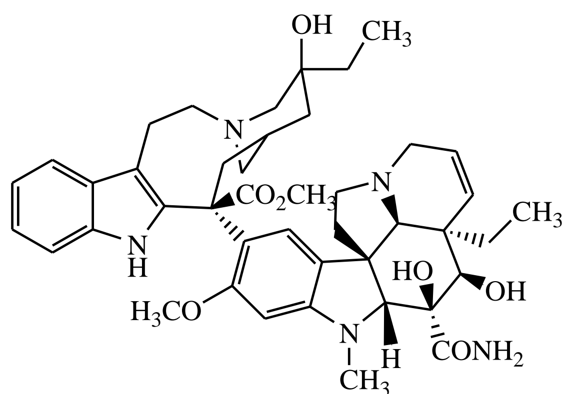
2.2.1.1.3. Vinca alkaloids and derivatives

The vinca alkaloids vinblastine (**17**) and vincristine (**18**) are dimeric molecules isolated from the Madagascar medicinal herbs *Catharanthus roseus* (Apocynaceae) and first reported as hypoglycemic agents (Johnson *et al.*, 1968). These vinca alkaloids and thier semi-synthetic derivatives have played an invaluable role in the treatment certain solid tumors by interacting

with tubulin, which leads to inhibition of mitosis at the meta phase (Jordan *et al*., 2004; Okouneva *et al*., 2003; Kavallaris *et al*., 2008).

Vinblastine (**17**) was recognized by FDA in 1961 as anti-cancer agent and is widely used for the treatment of lymphocytic and histiocytic lymphomas, Hodgkin’s disease, advanced breast carcinoma, Kaposi’s sarcoma and advanced testicular carcinoma (www.cdn.shopify.com). Vincristine (**18**) is cytotoxic against various malignancies such as Hodgkin’s disease, multiple myeloma, small cell lung cancer, breast cancer, brain tumors, cervical carcinoma, malignant melanoma, non-small cell lung cancer and Kaposi,s sarcoma (Gidding *et al*., 1999).

A semisynthetic derivative of vinblastine, vindesin (**29**), was synthesized by preferential hydrazinolysis of the C-3 ester of the vindoline moiety of vinblastine (**17**), followed by hydrogenolysis of the resulting deacetyl vinblastine hydrazide. Vindesin (**29**) is mainly used to treat melanoma and lung cancers and, in combination with other drugs, to treat uterine cancers. Vinorelbine (**30**), obtained through a modified Polonovski reaction, is used as a treatment for breast cancer and non-small cell lung cancer (Curran and Plosker, 2002; Gregory *et al*., 2000).



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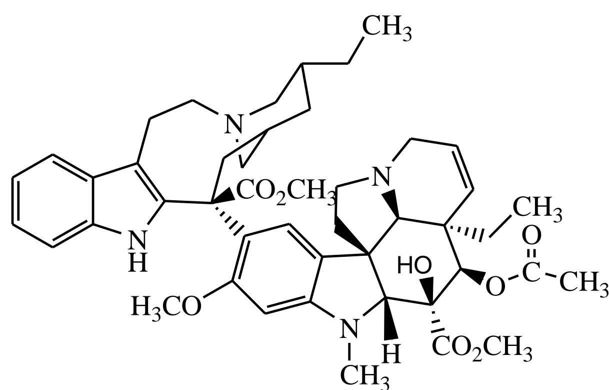

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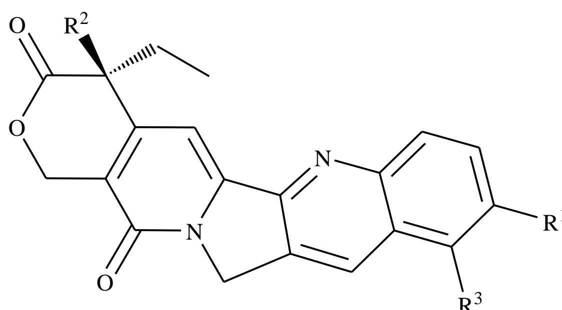
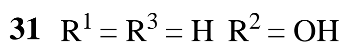
2.2.1.1.4. Camptothecin and topotecan

Camptothecin (**31**) is a pyranoindolizinoquinoline alkaloid isolated from the bark of *Camptotheca acuminate* (Nyssaceae) (Wall *et al*., 1966). The therapeutic value of camptothecin (**31**) is limited by its low solubility in water and serious side effect. Following its low solubility problems associated with camptothecin, a number of derivatives have been chemically prepared (Venditto *et al*., 2010). Topotecan (**32**), 10-hydroxy-9-dimethylaminomethyl-camptothecin is one of camptothecin analogues synthesized by the reaction of bis-(dimethylamino) methane with (*S*)-10-hydroxycamptothecin and it was approved by FDA as a treatment for advanced ovarian cancers and small-cell lung cancer (Armstrong *et al*., 2005).

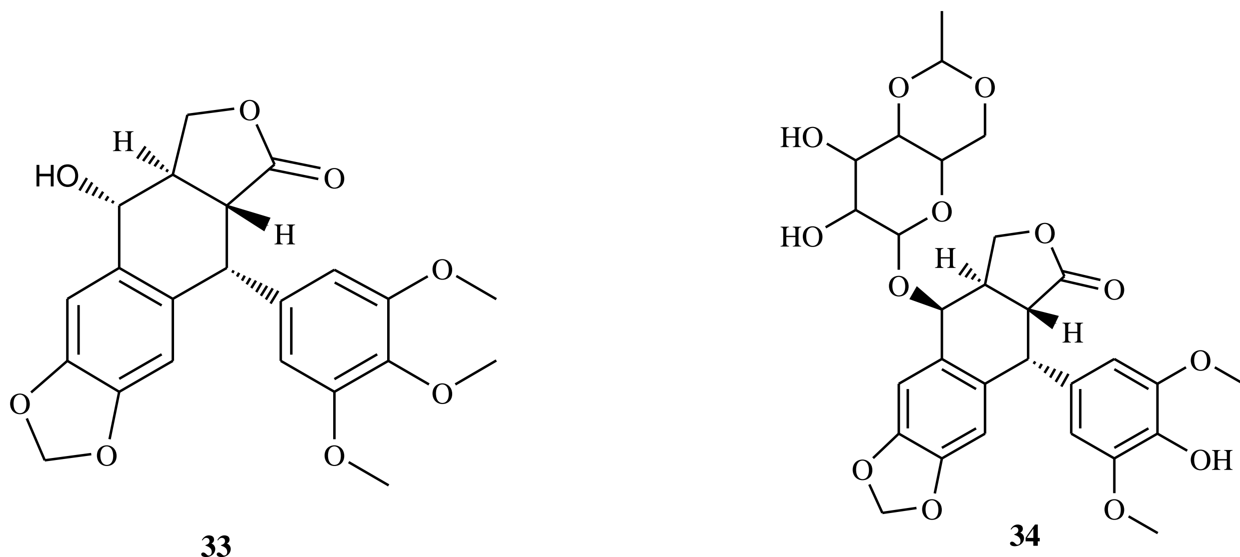




2.2.1.1.5. Podophyllotoxin and etoposide

Podophyllotoxin (**33**) is a dimeric phenylpropanoid lignan isolated from *Podophyllum peltatum* (Berberidaceae) (Gensler *et al*.,1966). Etoposide (**34**) is the synthetic analogue of podophyllotoxin (**33**), structurally modified at C-4 of podophyllotoxin (**34**), and is widely used for the treatment of small-cell lung cancer, testicular cancer, melanoma, acute leukemia, Hodgkin’s disease, non-Hodgkin’s disease, gastric cancer, breast cancer, and ovarian cancer (Fleming *et al*., 1989). Podophyllotoxin inhibits the assembly of microtubules and inhibits

mitosis by binding to the tubulin molecule but etoposide and related analogue prevent cells from entering mitosis and arrest cells in the late S-phase of the cell cycle by binding to topoisomarase II (Castro *et al.*, 2003).



2.3. Botanical Information on the Genus *Aloe*

The genus *Aloe* is taxonomically placed in the family Asphodelaceae and the sub-family Alooideae. The family Asphodelaceae is one of the families within the order Asparagales, and comprises about 17 genera and 800 species, and is subdivided into two sub-families (Asphodeloideae and Alooideae) based on vegetative and reproductive characteristics (Dahlgren *et al.*, 1985). Members of the family have mainly Old World distribution - from the Mediterranean area and Africa to central Asia and New Zealand, with the main center of distribution being southern Africa. The plants within the Asphodelaceae family are mostly herbs, shrubs, and sometimes arborescent, which grows into woody forms with trunks that can grow up to several meters high. The leaves are often thick and succulent with parallel venation (Adam *et al.*, 2000). The flowers are actinomorphic or zygomorphic. Fruits are always non-fleshy (except

in *Lomatophyllum*), in a dehiscent loculicidal capsule. The seeds can be winged or wingless; and contain a dry aril that arises as an annular invagination at the distal end of the funiculus (Judd *et al*., 1999; Watson and Dallwitz, 1992). Furthermore the family Asphodelaceae is chemically characterized by the presence of anthraquinones, lack of steroidal saponins, simultaneous microsporogenesis, atypical ovular morphology and presence of an aril (van Wyk *et al*., 1993; Smith and van Wyk, 1998).

2.3.2. The subfamily Alooideae

The subfamily Alooideae of the family Asphodelaceae is an Old World group currently comprising six genera: *Aloe*, *Astroloba*, *Chortolirion, Gasteria*, *Haworthia* and *Lomathophylum*. It contains 500 species and characterised by more or less distinctly succulent leaves, often with prickly or toothed margins, and a markedly bimodal karyotype n = 7 (Smith and van Wyk, 1998). Members of the subfamily also share some chemical characters, notably the presence of anthrone-*C*-glycosides in their leaves and 1-methyl-8-hydroxyanthraquinones in their roots (Smith and van Wyk, 1998).

2.3.3. The genus *Aloe*

The name "Aloe", derived from Arabic word 'Alloeh', means shining bitter substance (Dagne *et al*., 2002). The genus *Aloe* is the largest genus in Alooideae and it comprises approximately 400 species, ranging from grass to trees and is spread in several biodiversity hotspots, including Africa, Arabian Peninsula, Madagascar and islands in western Indian Ocean with highest distribution in Africa (Raynolds, 1950). There are about 83 *Aloe* species are found in East Africa, of which 60 species are grown in Kenya with 26 species being endemic (Carter, 1994; Wabuyele, 2006). The genus *Aloe* is distinguished from other Alooideae genera in that the leaves are tough, spiked or toothed margins with astringent/unpalatable juice, sunken stomata

and distichous leaf arrangement. The flowers are vivid yellow to red, sometimes bicoloured, size ranging from small to large, campanulate to tubular or gasteriform, radially symmetrical to bilabiate. The seeds are flattened, wind-dispersed and sometimes fleshy (Reynolds, 1966; Viljoen, 1999; Glen and Hardy, 2000; Klopper and Smith, 2007).

2.3.4. *Aloe turkanensis*

Aloe turkanensis is a shrub with stems up to 70 cm long (Figure 1) and is widespread in north-western Kenya and in the Karamoja district of Uganda. It grows in loose clumps up to 2 m diameter. Leaves are borne in a compact rosette, are erect to spreading with elongated whitish spots on both surfaces. The inflorescence is many-branched, up to 26 cm long and bright pink in colour (Bosch, 2006). The flowers are mostly second, laxly flowers, more widely spreading and directed back ward (Reynolds, 1966).

Figure 1: Picture of *Aloe turkanensis* (www.prota 4u.org)

2.4. Uses of *Aloe*

The genus *Aloe* is of commercial and medicinal importance which makes them is fascinating subject for research from chemical, pharmaceutical, economic and taxonomic points of view. The commercial *Aloe* is separated into two products; *Aloe* gel and *Aloe* latex. *Aloe* latex comes from under the skin of the leaves and is yellow in color which contains hydroxyanthracene derivatives such as the anthraquinone glycosides aloin A and B (Saccu *et al*., 2001). Aloe gel is the clear, jelly-like substance found in the inner part of the aloe leaf. Most of the health benefits of aloe are attributed to its bioactive constituents found in the gel of the leaves which is mainly composed of 99% water and the rest is polysaccharides, soluble sugars, proteins, enzymes, aminoacids, vitamins and anthraquinones (Liu *et al*., 2007).

Aloe polysaccharides play important role in functions as immunoregulation, anti-radiation, anti-ulcer, ant-ioxidation, hypolipidemic and hypoglycemic effects (Lee *et al*., 2001). The most common polysaccharide, acemannan, plays an important role in reducing opportunistic infections and stimulating the healing processes (Christiaki *et al*., 2010; Ni *et al*., 2004).

Aloe species contain phenolic compounds like aloin (an anthraquinone glycoside) which have laxative property to maintain digestive system functional (Grindlay *et al*., 1986), while the chromone derivative aloesin exerts anti-inflammatory effect (Park *et al*., 2009).

Besides being of pharmacological importance, *Aloe* is used as an active ingredient in cosmetic products such as cleaning and nursing, skin care, skin beautifying, and related products. For example, the juice from the leaves of *A. vera* is used as cosmetics additive in the production of creams, lotions, soaps, shampoos, facial cleansers and other products (Hamman *et al*., 2008);

Aloe vera is also used as an ingredient for health drinks and beverages, yogurt and some desserts (Boghani *et al.*, 2012).

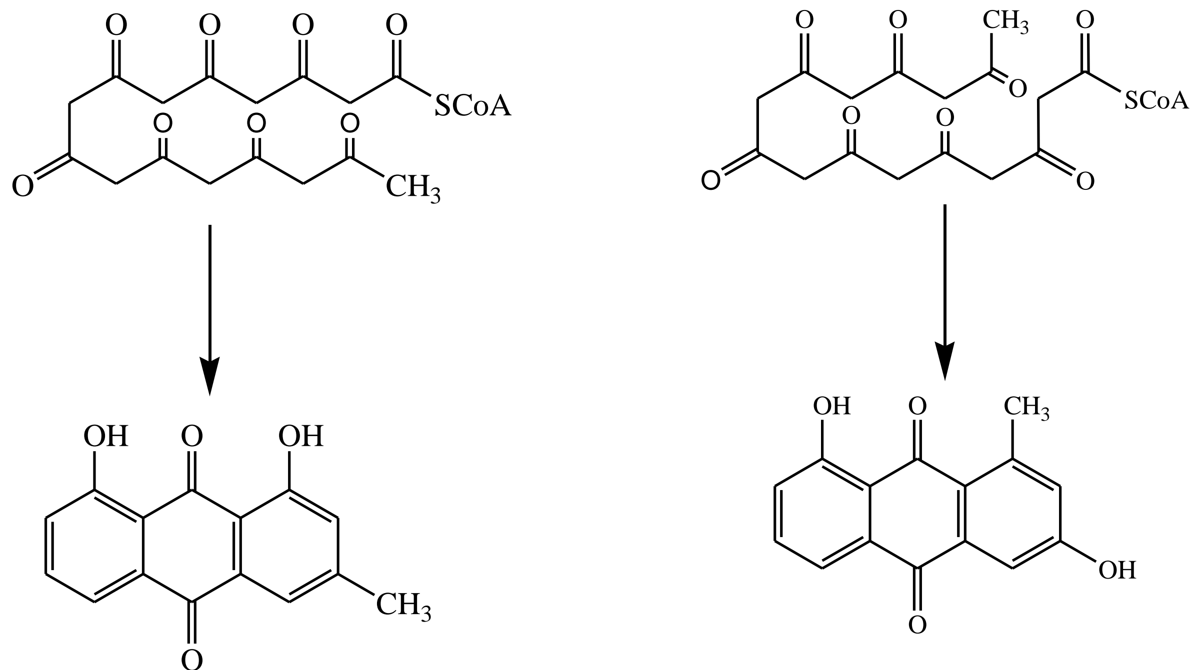
Aloes are also common in the general horticultural trade servicing gardeners and landscapers, particularly in Australia, the UK, the USA, and South Africa and Kenya to produce some spectacular garden plants which are commercialized (Capasso *et al.,* 1998).

2.5. Phytochemical Information on *Aloe* Species

Chemical investigations on *Aloe* species have revealed the presence of anthraquinones, pre-anthraquinones, naphthoquinones, anthrones, alkaloids, sterols, chromones, pyrones and flavonoides. Some of the compounds isolated from the genus *Aloe* are given in below.

2.5.1. Anthraquinones of *Aloe*

Anthraquinones consists of a tricyclic aromatic ring with 9,10-quinone skeleton and several substituent groups at different position. Biosynthetically anthraquinones of *Aloe* are derived from two parallale routes of the polyketide pathway leading to 1,8-dihydroxy-3-methylanthraquinones and 3,8-dihydroxy-1-methylanthraquinones depending on the folding of the octaketide chain (Fig. 2).

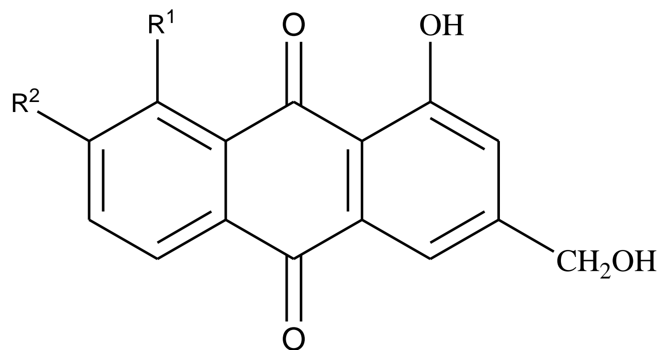


1, 8-Dihydroxy-3-methylanthraquinones (e.g. Chrysophanol (**3**))

3, 8-Dihydroxy-1-methylanthraquinones (e.g. Aloesaponarin II (**4**))

Figure 2: Different folding mechanism involved in the synthesis of anthraquinones.

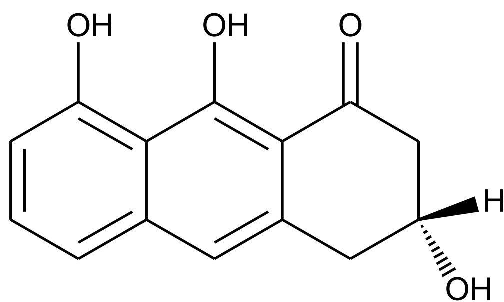
Anthraquinones are known by their wide range of biological activities. The anthraquinone: 2,8-dihydroxy-6-(hydroxymethyl)-1-methoxyanthracene-9,10-dione (**35**) isolated from *Aloe sincatana* has been reported to exhibit inhibitory effect on protein glycation (Elhassan *et al.*, 2012). Other anthraquinone; aloe-emodin (**36**) isolated from *Aloe vera* showed strong antibacterial activity (Ferro *et al.*, 2003). Aloe-emodin has also been shown to inhibit neuroctodermal tumor and lung squimous cell carcinoma cell lines (Pecere *et al.*, 2000; Lee *et al.*, 2001).



35 $R^1 = OCH_3$ $R^2 = OH$
36 $R^1 = OH$ $R^2 = H$

2.5.2. Pre-anthraquinones of *Aloe*

Biosynthetically pre-anthraquinones are considered to be precursors of anthraquinones. Pre-anthraquinones mainly occur in the roots and substerranean stem of the genus *Aloe*. Some of the isolated pre-anthraquinones are prechrysophanol (**37**) from roots of *Aloe graminacola* and aloesaponols I (**38**) and aloesaponols II (**39**) from the substerranean stem of *Aloe saponaria* (Yenesew *et al*., 1993; Yagi *et al*., 1974). Aloesaponols I and II occur widely in the roots of *Aloe* (van Wyk *et al*., 1995).



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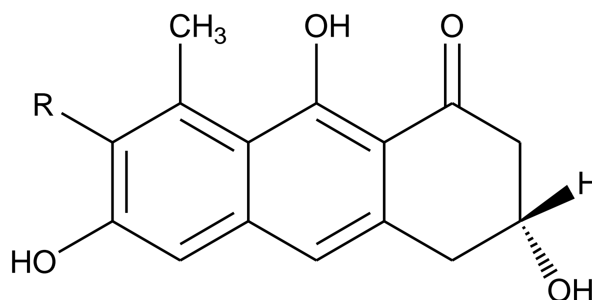
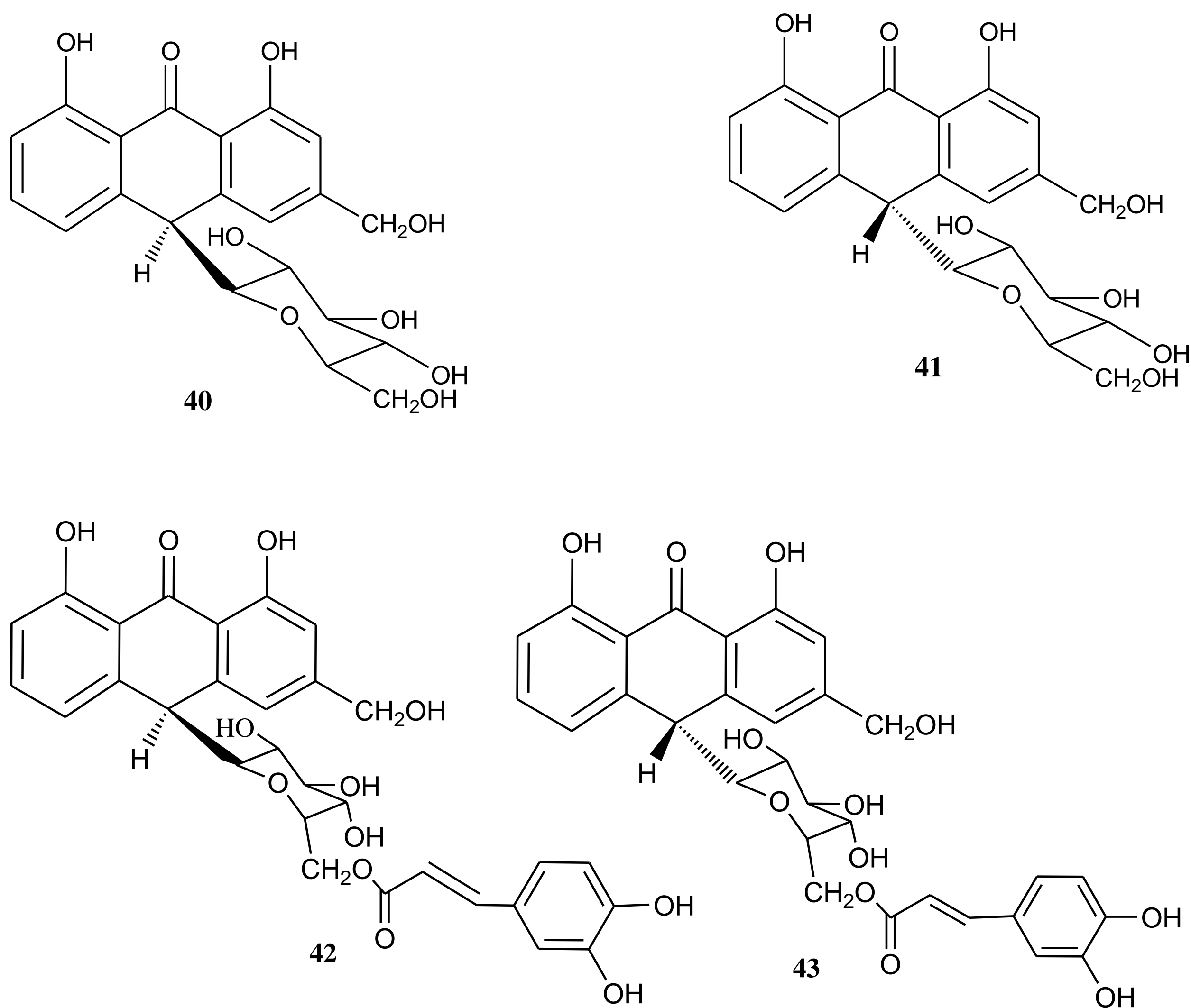
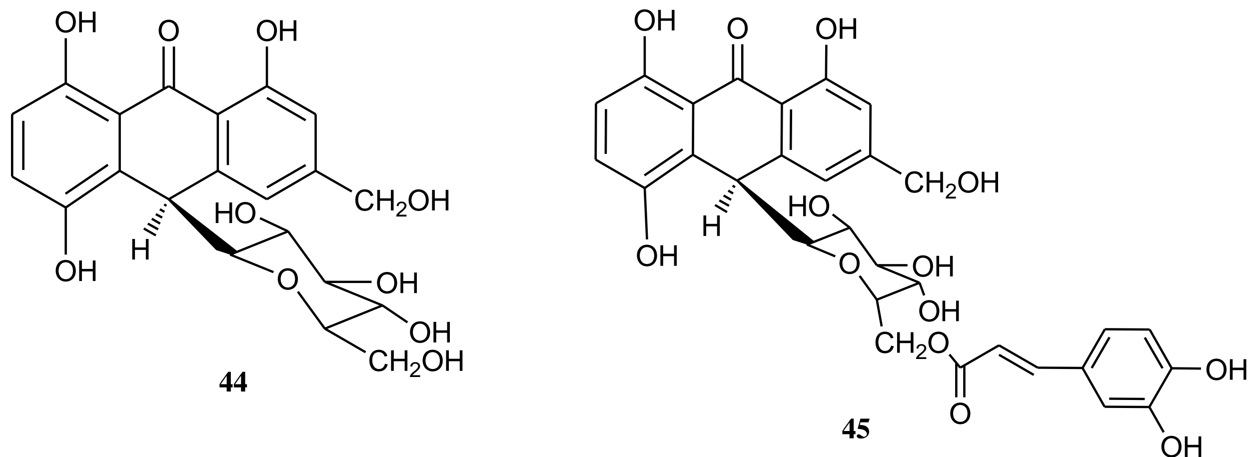


38 R = $COOCH_3$
39 R = H

2.5.3. Anthrones of *Aloe*

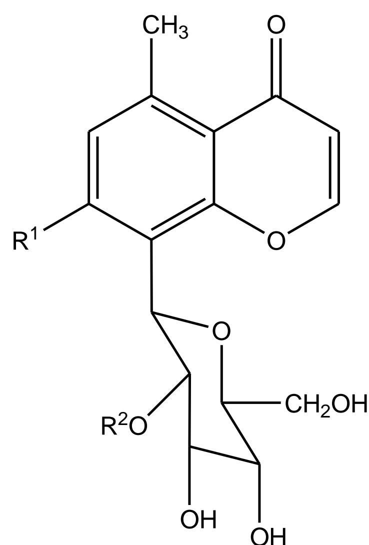
Anthrones are intermediate in the biosynthesis of anthraquinone from polyketide pathway (Fig. 2). They are known constituents of the leaves of *Aloe* and possess bitter and purgative properties (Dagne *et al*., 2000). The taxonomic distribution of aloin A (**40**) and aloin B (**41**) with microdontin A (**42**) and microdontin B (**43**) has been studied and showed anthrones to be the most widely found class of compound in *Aloe* species (Viljoen *et al*., 2001). In addition, 5-hydroxyaloin A (**44**) and microstigmin A (**45**) are some of the anthrones isolated from *Aloe marlothii* (Bisrat *et al*., 2000) and *Aloe microstigma* (Dagne *et al*., 1997), respectively.

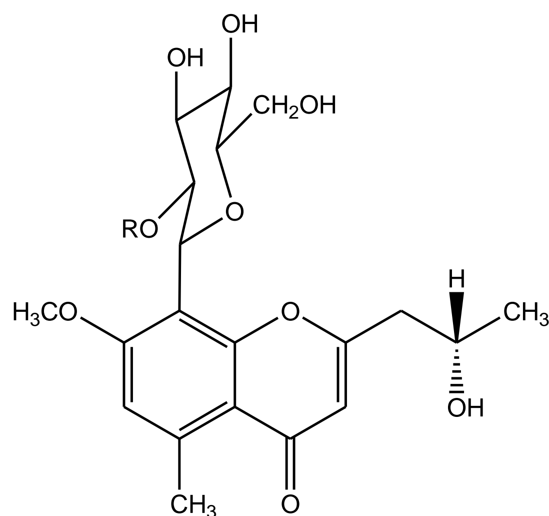




2.5.4. Chromones of *Aloe*

Chromones are derivatives of benzopyron with substitueted keto group on the pyran ring and they are found in *Aloe* leaves. Aloeresin A (**46**) and aloeresin B (**47**) were first identified by Hayne *et al*., (1970) as major constituents of the genus. Latter several chromone derivatives have been reported. Among these, 7-*O*-methylaloresin A (**48**) and 7-*O*-methylaloresin (**49**) were reported from leaf exudates of *Aloe marlothii* (Bisrat *et al*., 2000) and 8-*C*-glycosyl-7-*O*-methyl aloesol (**50**), isoaloeresin (**51**) and aloeresin E (**52**) from *Aloe vera* (Okamura *et al*., 1996) are some additional examples of chromones of *Aloe*.





46 R^1 = OH, R^2 = *p*-Coumaroyl
47 R^1 = OH, R^2 = H
48 R^1 = CH_3, R^2 = *p*-Coumaroyl
49 R^1 = CH_3, R^2 = H

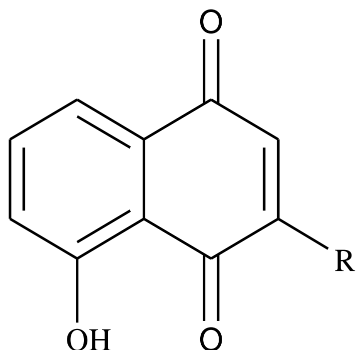
50 R = H
51 R = HO–C₆H₄–CH=CH–CO-
52 R = C₆H₅–CH=CH–CO-

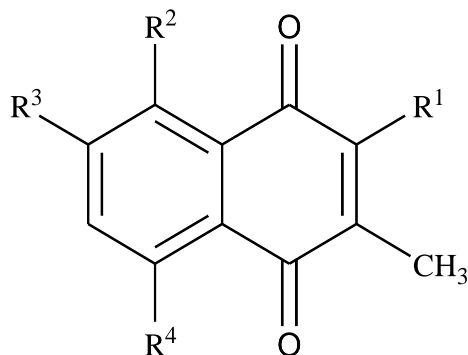
2.5.5. Naphthoquinones of *Aloe*

Naphthoquinones are widely found in the family Droceraceae, Ebenaceae, Ericaceae, Iridaceae, Juglandaceae, Plumbaginaceae and Proteaceae (Zenk and Leistner, 1968). They are known to have interesting biological effects. For example plumbagin (**53**) isolated from the family Plumbaginaceae has antiinflammatory, anticarcinogenic, immune-suppressive and antiatherosceloratic activities (Sugie *et al*., 1998; Itoigawa *et al*., 1991). In addition, the naphthoquinone; juglone (**54**) isolated from the Juglandaceae family has shown cytotoxic effects against various tumor cell lines. Juglone has also shown antifungal, antibacterial and antiviral activities (Kamei *et al*., 1998; Segura *et al*., 1992).

The occurence and cytotoxic effect of naphthoquinones; 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione (**55**) and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione

(**56**) have also been reported in the roots of *Aloe secondiflora* (Asphodelaceae) (Induli *et al*., 2012).



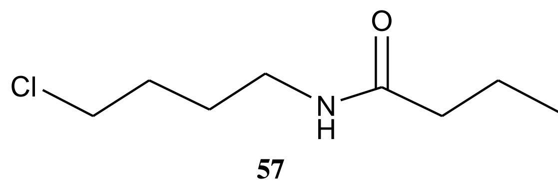


53 R = CH_3
54 R = H

55 $R^1 = R^3 = OCH_3$; $R^2 = OH$; $R^4 = H$
56 $R^1 = OCH_3$; $R^2 = R^4 = OH$; $R^3 = H$

2.5.6. Miscellaneous Compounds

Other constituents of *Aloe* present in minor quantities include alkaloids; *N*-4'-chlorobutylbutyramid (**57**) and, *N*,*N*-dimethylconiine (**58**) from *Aloe sabaea* (Blitzke *et al*., 2000). The Sterols; cholesterol (**59**) and β-sistosterol (**60**) from *A .barbadensis* and *A. arborescens* (Waller *et al*., 1978; Yamamato *et al*., 1991) and pyrones; alonin aglycone (**61**) and alonin acetal (**62**) from *Aloe nyeriensis* and *Aloe arborescens* (Conner *et al*., 1987; Woo *et al*., 1994) are among the minor metabolites of *Aloe*.



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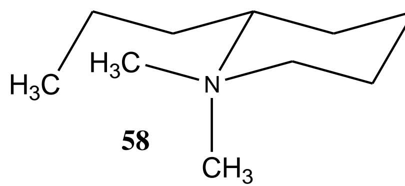

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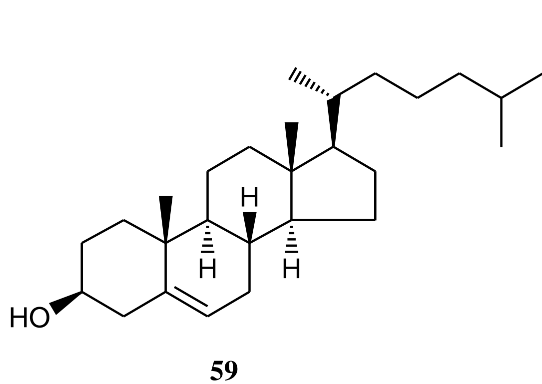

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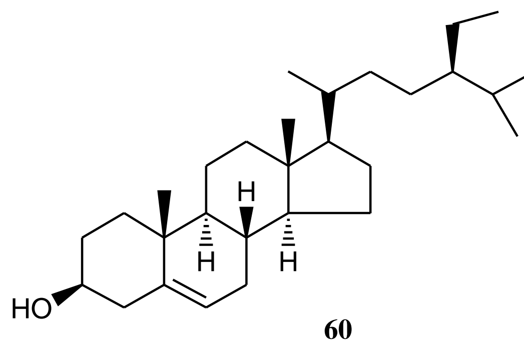

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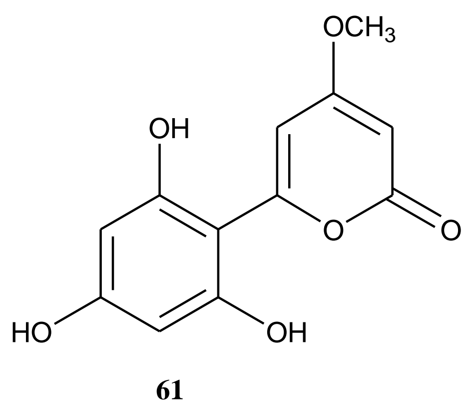

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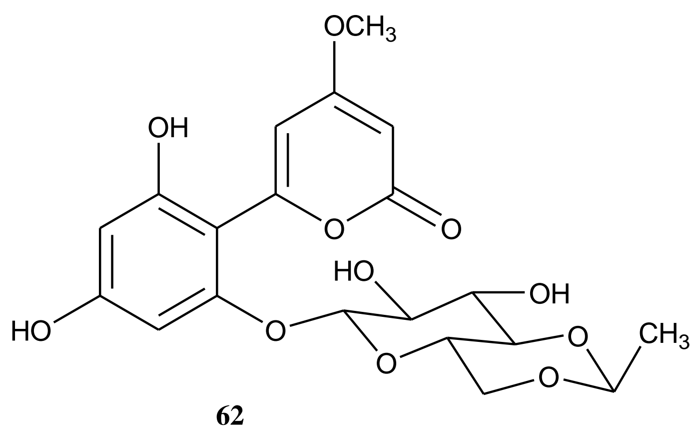

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CHAPTER THREE

MATERIALS AND METHODS

3.1. General

3.1.1. Instrumentation

NMR spectra were run at Department of Pharmaceutical Biology, Saarland University, Germany on Bruker Avance (500 MHz) spectrometer using residual solvent signals as a reference. Homonuclear Correlation Spectroscopy (COSY), Hetronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) were obtained using the standard Bruker software (Top spin 3.0 pl 3). The absorbance of purple formazan solution in living cells was recorded at Saarland University, Department of Pharmaceutical Biology, Germany using TECAN sunrise software XFluor4 at wavelength 550 nm and 690 nm. Column Chromatography (CC) were run on silica gel (70-23 mesh) and Sephadex LH-20 was used for purification of compounds. Analytical TLC using silica gel 60 F254 pre-coated plates (Merck).

3.2. Plant Material

The rhizome and leaves of *Aloe turkanensis* were collected from Marigat in Indao, Kenya in June 2012. The plant was identified by Mr. Simon Mathenge of the Herbarium, Botany Department School of Biological Science, University of Nairobi, where a voucher specimen has been deposited under deposite number FAA 2012/001.

3.3. Extraction and Isolation

3.3.1. Extraction and isolation from rhizomes of *Aloe turkanensis*

The rhizomes of *Aloe turkanensis* were dried under shade and crushed into powder. The powdered plant material (2 Kg) was extracted exaustively with CH_2Cl_2/MeOH (1:1) by cold percolation. The combined extract was then filtered and concentrated under reduced pressure to yield 30 g of crude extract. The extract was partitioned between ethyl acetate and water. The ethyl acetate layer was dried by rotary evaporator to give 20 g of crude extract. A 15 g portion of the ethyl acetate extract was subjected to column chromatography on oxalic acid deactivated silica gel (400 g) eluting with *n*-hexane containing increasing amounts of ethyl acetate to give 250 fractions each of *ca*. 250 mL. These were combined into 21 fractions based on their TLC profile.

Fraction 2 (1% of ethyl acetate in *n*-hexane) was further separated by CC on Sephadex LH-20 (eluting with CH_2Cl_2/MeOH, 1:1) to give 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**, 4.0 mg) and 3,5,8-trihydroxy-2-methyl naphthalene1,4-dione (**1**, 3.0 mg). Fraction 3 (3% ethyl acetate in *n*-hexane) was collected as a yellow solution, upon concentration of this fraction a yellow precipitate was formed, which was filtered and washed with *n*-hexane to yield chrysophanol (**3**, 6 mg). Fraction 6 (10% ethyl acetate in *n*-hexane) was obtained as orange solution which was filtered and washed with *n*-hexane to give aloesaponarin I (**4**, 25 mg). Purification of the filtrate of fraction 6 by Sephadex LH-20 (eluted with CH_2Cl_2/MeOH; 1:1) gave aloesaponarin II (**5**, 20 mg). After purification of a brown solution of fraction 12 (30% ethyl acetate in *n*-hexane) by CC on Sephadex LH 20 (eluting with CH_2Cl_2/MeOH; 1:1) laccaic acid D-methyl ester (**6**, 40 mg) was obtained. Fraction 16 (50% ethyl acetate in *n*-hexane) gave a

colorless precipitate which was filtered and washed with *n*-hexan/acetone mixture and yielded aloesaponol I (**7,** 20 mg).

3.3.2. Extraction and isolation from the leaves of *Aloe turkanensis*

The shade dried and powdered leaves of *Aloe turkanensis* (2 Kg) was extracted and concentrated as above to give 31 g of crude extract. The extract was then partitioned between ethyl acetate and water, removal of the organic solvent gave 25 g extract. A 20 g portion of the ethyl acetate extract was subjected to CC on oxalic acid impregnated silica gel (400 g) eluting with *n*-hexane containing increasing amounts of ethyl acetate. A total of 280 fractions each *ca*. 250 mL were collected and combined into 20 fractions. Fraction 2 (eluted with 1% ethyl acetate in *n*-hexane) was further purified by CC over Sephadex LH-20 (eluent: CH_2Cl_2/MeOH; 1:1) to give helminthosporin (**8**, 3 mg) and 3,5,8-trihydroxy-2-methylnaphthalene1,4-dione (**1**, 2.5 mg). Similar purification of fraction 3 (3% ethyl acetate in *n*-hexane) afforded chrysophanol (**3**, 5 mg). Fraction 6 (7% ethyl acetate in *n*-hexane) was eluted as dark green solution and purification of this fraction using Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) gave aloesaponarin I (**4**, 15 mg) and aloesaponarin II (**5**, 10 mg). Fraction 8 (15% ethyl acetate in *n*-hexane) showed two blue fluorescence spots under UV light and were separated by CC over Sephadex LH-20 (elution: CH_2Cl_2/MeOH; 1:1) yielding feralolide (**9**, 15 mg) and 3,4-dihydroxybenzoic acid (**12**, 12 mg). Aloe-emodin (**10**, 10 mg) and *α*-L-11-*O*-rhamnosyl aloe-emodin (**11**, 8.5 mg) crystallized from fractions 13 (40% ethyl acetate in *n*-hexane) and 16 (60% ethyl acetate in *n*-hexane), respectively.

3.4. Physical and Spectroscopic Properties of Isolated Compounds

3.4.1. 3,5,8-Trihydroxy-2-methyl naphthalene-1,4-dione (1)

Red amorphous solid. UV λ_{max} 300, 420, 480 nm. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 12.81 (1H, *s*, 5-OH), 11.48 (1H, *s*, 8-OH), 7.29 (1H, *d*, *J* = 9.5 Hz, H-6), 7.18 (1H, *d*, *J* = 9.5 Hz, H-7), 2.11(3H, *s*, 2-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 188.8 (C-1), 182.0 (C-4), 157.6 (C-5), 157.1 (C-8), 153.8 (C-3), 134.1 (C-6), 127.4 (C-7), 121.6 (C-2), 110.7 (C-5a), 110.1 (C-8a), 8.3 (2-CH_3).

3.4.2. 5,8-Dihydroxy-3-methoxy-2-methyl naphthalene-1,4-dione (2)

Red amorphous solid. UV λ_{max} at 300, 480 nm. EIMS *m/z* $[M]^+$ 234.9. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 12.72 (1H, *s*, 8-OH), 12.32 (1H, *s*, 5-OH), 7.24 (1H, *d*, *J* = 10.0 Hz, H-7), 7.22 (1H, *d*, *J* = 10.0 Hz, H-6), 4.14 (1H, *s*, 3-OCH_3), 2.11 (3H, *s*, 2-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 188.4 (C-1), 183.5 (C-4), 158.3 (C-3), 158.3 (C-5), 157.5 (C-8), 133.2 (C-2), 129.9 (C-7), 128.6 (C-6), 111.5 (C-8a), 111.1 (C-5a), 61.4 (OCH_3), 9.0 (CH_3).

3.4.3. Chrysophanol (3)

Orange needles. UV λ_{max} 300, 420 nm. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 12.11 (1H, *s*, H-8), 12.00 (1H, *s*, H-1), 7.82 (1H, *dd*, *J* = 1.0, 8.5 Hz, H-5), 7.68 (1H, *t*, *J* = 7.5 Hz, H-6), 7.64 (1H, *bs*, H-2), 7.29 (1H, *dd*, *J* = 1.0, 8.5 Hz, H-7), 7.09 (1H, *bs*, H-4), 2.46 (3H, *s*). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 192.5 (C-10), 181.9 (C-9), 162.7 (C-8), 162.4 (C-1), 149.3 (C-3), 136.9 (C-6), 133.6 (C-5a), 133.2 (C-4a), 124.5 (C-7), 124.3 (C-4), 121.3 (C-2), 119.9 (C-5), 115.8 (C-8a), 113.7 (C-1a), 22.2 (CH_3).

3.4.4. Aloesaponarin II (4)

Orange crystals. UV λ_{max} 260, 290, 400 nm. EIMS *m/z* $[M]^+$ 254.58. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 12.94 (1H, *s*, 1-OH), 7.68 (1H, *t*, *J* = 7.5 Hz, H-3), 7.58 (1H, *dd*, *J* = 7.5, 1.5 Hz, H-2), 7.41 (1H, *d*, *J* = 3.0 Hz, H-5), 7.29 (1H, *dd, J* = 8.5, 1.0 Hz, H-4), 6.99 (1H, *d*, *J* = 2.5 Hz, H-7), 2.66 (3-H, *s*, 8-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 189.2 (C-9), 182.1 (C-10), 162.2 (C-1), 161.4 (C-6), 145.2 (C-8), 136.7 (C-5a), 135.8 (C-3), 132.4 (C-4a), 116.3 (C-1a), 124.4 (C-7), 124.1 (C-2), 122.2 (C-8a), 118.1 (C-4), 111.9 (C-5), 23.5 (CH_3).

3.4.5. Aloesaponarin I (5)

Orange crystals. UV λ_{max} 260, 300, 410 nm.^{1}H NMR (acetone-d_6, 500 MHz): δ_H 12.87 (1H, *s*, 1-OH), 7.70 (1H, *dd*, *J* = 5.5, 2.5 Hz, H-4), 7.73 (1H, *s*, H-5), 7.68 (1H, *t*, *J* = 1.9, H-3), 7.29 (1H, *dd, J* = 7.5, 1.0 Hz, H-2), 3.95 (3H, *s*, OCH_3), 2.70 (3-H, *s,* 8-CH_3). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C 190.4 (C-9), 182.3 (C-10), 167.9 (C=O), 162.9 (C-1), 159.5 (C-6), 142.9 (C-7), 136.9 (C-5), 133.8 (C-5a), 133.6 (C-4a), 130.8 (C-8), 125.2 (C-8a), 124.4 (C-2), 119.2 (C-3), 117.9 (C-1a), 113.0 (C-4), 52.7 (OCH_3), 20.3 (8-CH_3).

3.4.6. Laccaic acid D methyl ester (6)

Orange crystals. UV λ_{max} 300, 410 nm. EIMS *m/z* $[M]^+$ 328.71. ^{1}H NMR (acetone-d_6, 500 MHz): δ_H 13.16 (1H, *s*, 1-OH), 7.72 (1H, *s*, H-5), 7.18 (1H, *d*, *J* = 2.5 Hz, H-4), 6.65 (1H, *d*, *J* = 2.5 Hz,H-2), 3.93 (3H, *s*, OCH_3), 2.70 (3-H, s). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C 189.3 (C-9), 182.7 (C-10), 168.1 (C=O), 166.2 (C-1), 165.1 (C-3), 159.3 (C-6), 142.3 (C-7), 138.0 (C-5a), 135.5 (C-4a), 130.9 (C-8), 109.2 (C-2), 108.1 (C-4), 113.1 (C-5), 111.7 (C-1a), 52.6 (OCH_3), 20.3 (CH_3).

3.4.7. Aloesaponol I (7)

Colorless solid. The TLC showed blue fluorescence under UV light (366 nm). UV λ_{max} 300, 380 nm. EIMS *m/z* $[M]^+$ 316.90. ^1H NMR (DMSO-d_6, 500 MHz): δ_H 15.27 (1H, *s*, 9-OH), 6.95 (1H, *s*, H-5), 6.92 (1H, *s*, H-10), 4.24 (1H, *m*, H-3), 3.83 (3H, *s*, OCH_3), 3.14 (1H, *dd*, *J* = 3.3,15.8 Hz, H-4), 2.96 (1H, *dd*, *J* = 3.3, 17.1 Hz, H-2), 2.90 (1H, *dd*, *J* = 6.8, 15.6 Hz, H-4), 2.70 (1H, *dd*, *J* = 1.8, 5.4 Hz, H-2), 2.70 (3H, *s*). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 203.7 (C-1), 168.2 (C=O), 165.9 (C-9), 140.8 (C-7), 137.2 (C-8), 136.6 (9a), 155.1(C-6), 125.4 (C-8a), 116.6 (C-5), 107.5 (C-10), 110.2 (C-1 0a), 64.4 (C-3), 52.1 (OCH_3), 46.4 (C-4), 37.5 (C-2), 20.8 (CH_3).

3.4.8. Helminthosporin (8)

Red needle. UV λ_{max} at 500, 580 nm. ^1H NMR (DMSO-d_6, 500 MHz): δ_H 12.83 (1H, *s*, 5-OH), 12.03 (2H, *s*, 1-OH and 8-OH), 7.65 (1H, *brs*, H-4), 7.44 (1H, *d*, H-7), 7.44 (1H, *d,* H-6), 7.26 (1H, *brs*, H-2), 2.50 (3H, *s,*3-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 189.9 (C-9), 186.3 (C-10), 161.7 (C-1), 157.1 (C-5), 156.4 (C-8), 149.1 (C-3), 132.8 (C-4a), 129.6 (C-7), 129.4 (C-6), 113.8 (C-1a), 124.3 (C-2), 120.2 (C-4), 112.6 (C-8a), 112.5 (C-5a), 22.1 (CH_3).

3.4.9. Feralolide (9)

Brown solid. UV λ_{max} 310 nm. EIMS *m/z* $[M]^+$ 344.75. ^1H NMR ($CDCl_3$, 500 MHz): δ_H 11.21 (1H, *s*, OH-8), 6.42 (1H, *d*, *J* = 1.9 Hz, H-7'), 6.37 (1H, *d*, *J* = 2.5 Hz, H-5'), 6.31 (1H, *d*, *J* = 1.5, H-5), 6.26 (1H, *d, J* = 1.9, H-7), 4.80 (1H, *m*, H-3), 3.08 (1H, *dd*, *J* = 5.5,14Hz, H-1'), 3.22 (1H, *dd*, *J* = 6.9,13.5 H-1‘), 2.94 (1H, *dd*, H-4), 2.92 (1H, *dd*, H-4), 2.57 (1H, *s*, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 203.9 ($COCH_3$), 170.3 (C-1), 165.2 (C-8), 165.0 (C-6), 160.3 (C-6'), 160.1 (C-4'), 142.9 (C-4a), 139.4 (C-2'),121.0 (C-3'), 111.7 (C-7'), 107.6 (C-5), 102.5 (C-5'), 101.9 (C-7), 101.8 (C-8a), 80.5 (C-3), 39.5 (C-1'), 33.1 (CH_3), 32.8 (C-4).

3.4.10. Aloe-emodin (10)

Orange crystals. UV λ_{max} at 260, 300, 420 nm. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 11.93 (2H, *s*, 1-OH, 8-OH), 7.81 (1H, *t*, *J* = 8.0 Hz, H-6), 7.71 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-5), 7.68 (1H, *d*, *J* = 1.6 Hz, H-4), 7.38 (1H, *dd*, *J* = 9.0, 1.1 Hz, H-7), 7.28 (1H, *d*, *J* = 1.6 Hz, H-2), 4.62 (2H, *s*,H-11). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 191.6 (C-9), 181.4 (C-10), 161.6 (C-1), 161.3 (C-8), 153.7 (C-3), 137.3 (C-6), 133.3 (C-4a), 133.1 (C-5a), 124.4 (C-7), 120.6 (C-2), 119.3 (C-5), 115.9 (C-8a), 114.4 (C-1a), 107.1 (C-4), 62.07 (C-11).

3.4.11. *α*-L-11-*O*-Rhamnopyranosyl aloe-emodin (11)

Orange crystals. UV λ_{max} 260, 300, 430 nm. EIMS *m/z* $[M]^+$ 416. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 7.82 (1H, *st*, *J* = 8.4 Hz, H-6), 7.73 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-7), 7.68 (1H, *d*, *J* = 1.6 Hz, H-4), 7.41 (1H, *dd*, *J* = 8.4, 1.1 Hz, H-5), 7.32 (1H, *d*, *J* = 1.6 Hz, H-2), 4.75 (1H, *d*, *J* = 14.0 Hz, H-11), 4.71 (1H, *d*, *J* = 1.6 Hz, H-1'), 4.61 (1H, *d*, *J* = 13.9 Hz, H-11), 3.73 (2H, *dd*, *J* = 3.4, 1.7 Hz, H-2'), 3.62-3.28 (33H, *m*, H-3'), 3.23 (2H, *s*, H-4'), 1.16 (3H, *d*, *J* = 6.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 191.6 (C-9), 181.4 (C-10), 161.4 (C-1), 161.3 (C-8), 148.8 (C-3), 137.4 (C-6), 133.4 (C-4a), 133.3 (C-5a), 124.4 (C-7), 121.8 (C-2), 119.3 (C-5), 117.7 (C-4), 115.9 (C-8a), 115.3 (C-1a), 99.9 (C-1'), 71.8 (C-4'), 70.4 (C-2'), 69.0 (C-3'), 66.9 (C-11), 70.7 (C-5'), 17.9 (CH_3).

3.4.12. 3,4-Dihydroxybenzoic acid (12)

Brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.53 (1H, *d*, *J* = 2.0 Hz, H-2), 7.47 (1H, *dd*, *J* = 6.5, 2.0 Hz, H-6), 6.89 (1H, *d*, *J* = 4.9 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 168.7 (C=O), 167.5 (C-3), 150.6 (C-4), 146.2 (C-1), 123.6 (C-6), 117.4 (C-2), 115.6 (C-5).

3.5. Biological Tests

3.5.1. *In vitro* anticancer activities

The cytotoxicity test was performed at Saarland University, Department of Pharmaceutical Biology, Germany by using colorimetric assay, also known as MTT assay as described by Heo *et al*., (1990).

3.5.1.1. Cell culture

Human extra hepatic bile duct carcinoma cell line TFK-1 was cultured in DMEM (#42460-025, Gibco, Germany) with 20% fetal calf serum (FCS) and 1% penicillin/streptomycin (10.000 units/ml/10mg/ml). Human hepatocelluar carcinoma cell line HuH7 were cultured in RPMI 1640 (#R8757, Sigma, Germany) with 20% fetal calf serum (FCS) and 1% penicillin/streptomycin (10.000 units/mL/10mg/mL). The cell line was incubated in a humidified incubator at 37°C (5% CO_2) (Hoe *et al*., 1990).

3.5.1.2. MTT assay

Cells were counted and $1x10^4$ cells per well were plated in 96-well plates. The two cancer cell lines were treated with different concentrations of the isolated compounds which were dissolved in DMSO and diluted with culture medium (1-100 µg/mL) and the treated cells were incubated for 48 hrs. After incubation, cell proliferation was determined by the 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) in PBS. A 150 µL portion of MTT solution was added to each well, followed by one hour incubation. After incubation, MTT-containing medium was aspirated and 80 µL of DMSO was added to each well to dissolve formazan crystals. The concentration of formazan was measured using TECAN sunrise software XFluor4 at 550 and 690 nm as control wave length (Fig. 3).

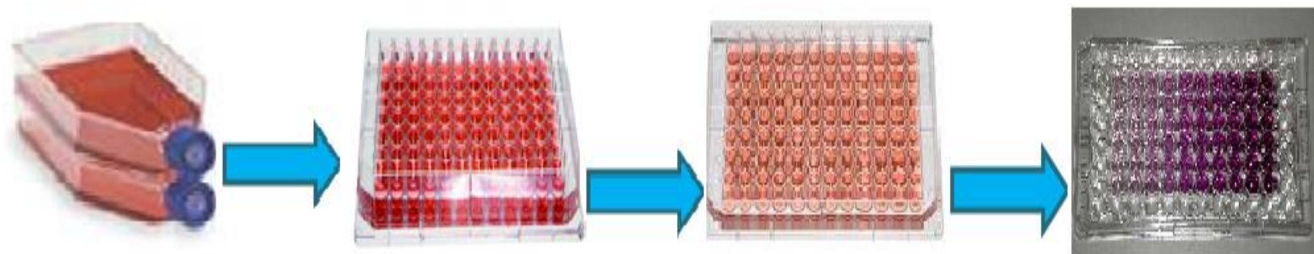


Figure 3: Picture of MTT assay

3.5.1.3. Statistical analysis

Data analysis were performed using Microsoft office excel software. All data were displayed as mean ± SEM. Statistical differences were estimated by independent two-sided two-sample t-test. Differences were considered statistically significant when *p* values were less than 0.05 (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Preliminary Test

Preliminary test showed that the crude extract of the rhizomes and the leaves of *Aloe turkanensis* exhibited significant reduction in cell viability against TFK-1 cell line. TLC analyses of the crude extracts showed the presence of colored spots which also absorb UV light (254 and 366 nm). The yellow spots changed red when exposed to ammonia vapor suggesting these are quinone derivatives. The compounds were isolated through chromatographic separation. Anticancer test on the isolated compounds were carried out on hepatoma carcinoma (HuH7) and extra hepatic bile duct carcinoma (TFK-1) cell lines. The characterization and anticancer activities of the isolated compounds from the rhizomes and leaves of *A. turkanensis* are discussed below.

4.2. Characterization of Compounds from the Rhizomes of *Aloe turkanensis*

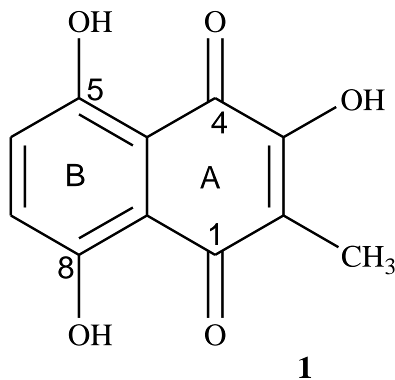
The rhizomes of *A. turkanensis* were extracted with dichloromethane/methanol (1:1) by cold percolation followed by partitioning between ethyl acetate and water. Chromatographic separation of the ethyl acetate extract resulted in the isolation of two naphthoquinones, four anthraquinones, a pre-anthraquinone, a chromone derivative and a benzoic acid derivative. The structures of the compounds were elucidated using spectroscopic techniques as discussed below.

4.2.1. 3,5,8-Trihydroxy-2-methylnaphthalene-1,4-dione (1)

Compound **1** was isolated as a red amorphous solid with the UV spectrum (λ_{max} 300, 420, 480 nm) being characteristics of a 3,5,8-trihydroxy substituted naphthoquinone (Bringmann *et al.*, 2011). In agreement with this, the ^{13}C NMR spectrum showed three oxygenated carbon atoms at δ_C 153.8, 157.1 and 157.6 for C-3, C-8 and C-5; respectively.

The ^{13}C NMR spectrum (Table 1) showed eleven carbon signals of which two were for carbonyl (at δ_C 182.0 and 188.8, for C-4 and C-1). Additionally, the NMR spectra showed a methyl carbon at δ_C 8.3 (δ_H 2.11) and two chelated hydroxyl (δ_H 11.48 and 12.81) substituents on the naphthoquinone skeleton. The ^{1}H NMR spectrum (Table 1) of **1** further revealed the presence of two *ortho*-coupled aromatic protons in ring B [at δ_H 7.18 (J = 9.5 Hz, δ_C 127.4) and 7. 29 (J = 9.5 Hz, δ_C 131.4)] which is substituted at C-5 and C-8 with hydroxyl groups.

In the HMBC spectrum, the aromatic proton at δ_H 7.18 showed correlation with C-8 and C-8a; the signal at δ_H 7.29 correlated with C-5 and C-5a; allowing the assignment of the signals at δ_H 7.18 and δ_H 7.29 to H-7 and H-6 respectively. The methyl group at δ_H 2.11 showed HMBC correlation with C-1, C-2 and C-3 being consistent with its placement at C-2. The structure of **1** was therefore characterized as 3,5,8-trihydroxy-2-methyl-1,4-naphthoquinone, trivial name 8-hydroxydroserone. This is the first report of compound **1** from the family Asphodelaceae, which has earlier been isolated from Droseraceae and Nepenthaceae families (Macbeth *et al.*, 1935); it was also reported from lyophilized cell culture of *Triphyophyllum peltatum* (Bringmann *et al.*, 2011).



4.2.2. 5, 8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2)

Compound **2** was isolated as a red amorphous solid, EIMS showed a molecular ion $[M]^+$ peak at *m/z* 234.91, corresponding to the molecular formula of $C_{12}H_{10}O_5$. The UV spectrum (λ_{max} at 300, 480 nm), is characteristic of a 1,4-naphthoquinone skeleton (Bringmann *et al.*, 2008). In agreement with this the ^{13}C NMR spectrum showed two carbonyl signals at δ_C 183.5 and 188.4 corresponding to C-4 and C-1, respectively of a1,4-naphthoquinone. The ^1H and ^{13}C NMR spectra of **2** (Tables 1) were found to be similar to those of **1**, except for the presence of a methoxy group at C-2 (δ_H 4.14, δ_C 61.4) instead of hydroxyl group, suggesting that **2** is a methyl ether derivative of **1**. Thus, the ^{13}C NMR spectrum (Table 1) showed twelve carbon signals including methyl carbon at δ_C 9.01 (δ_H 2.11). Additionally, the ^1H NMR spectrum showed the presence of two chelated hydroxyl proton at δ_H 12.32 and δ_H 12.72 corresponding to 5-OH and 8-OH. Two *ortho*-coupling aromatic protons in ring B at δ_H 7.24 (δc 129.9), δ_H 7.22 (δc 128.6) (J = 10.0 Hz) were assigned to H-6 and H-7. The substitution pattern in ring B was confirmed from HMBC spectrum (Table 1).

This compound was therefore identified as 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**), a compound which has been reported as a new compound recently from the roots of

Aloe secundiflora (Induli *et al*., 2012). However, this is the second report on the occurrence of compound **2** from the genus *Aloe*.

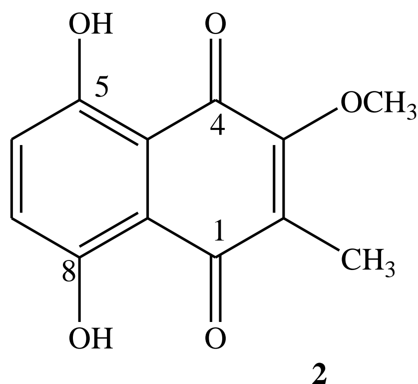


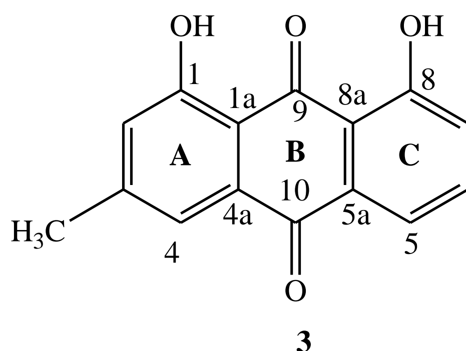
Table 1: ^{1}H (500 MHz), ^{13}C (125 MHz) and HMBC spectral data of compounds **1** and **2** ($CDCl_3$)

Carbon No.	Compound **1**			Compound **2**		
	^{1}H δ_H (*m*, *J* in Hz)	^{13}C	HMBC	^{1}H δ_H (*m*, *J* in Hz)	^{13}C	HMBC
1	-	188.8		-	188.4	
2	-	121.6		-	133.2	
3	-	153.8		-	158.3	
4	-	182.0		-	183.5	
5	-	157.6		-	158.3	
5a	-	110.7		-	111.5	
6	7.29 (*d,* 9.5)	127.4	C-5, C-5a	7.22 (*d*,10.0)	128.6	C-5, C-5a, C-8
7	7.18 (*d* , 9.5)	131.4	C-8, C-8a	7.24 (*d*,10.0)	129.9	C-8, C-8a, C-5
8	-	157.1		-	157.5	
8a	-	110.1		-	111.1	
2-CH_3	2.11 (*s*)	8.2	C-1, C-2, C-3	2.11 (*s*)	9.0	C-1, C-2, C-3
3-OCH_3				4.14 (*s*)	61.4	C-3
5-OH	12.81(*s*)	-	C-5, C-5a	12.32 (*s*)	-	C-5a, C-6, C-5
8-OH	11.48 (*s*)	-	C-8, C-8a	12.72 (*s*)	-	C-8a, C-7, C-8

4.2.3. Chrysophanol (3)

Compound **3** was isolated as an orange needles with UV absorption at λ_{max} 300, 420 nm, which is typical of 1,8-dihydroxyanthraquinones (Dagne *et al.*, 1994). This compound showed two chelated hydroxyl protons at δ_H 12.00 and 12.11 for 1-OH and 8-OH. The ^{13}C NMR spectrum (Table 2) showed fifteen signals including one methyl, five methine, nine quaternary carbons [of which two are oxygenated, at (δ_C 162.4 and 162.7) and two carbonyls (δ_C 181.9 and 192.5].

The ^{1}H NMR spectrum showed two broad singlet aromatic protons at δ_H 7.64 and δ 7.09 which were assigned to H-2 and H-4, respectively of ring A, with the biogenetically expected methyl group (δ_H 2.46, δ_C 22.6) being at C-3. The AMX pattern at 7.82 (1H, *dd*, J = 1.0, 8.5 Hz, H-5), 7.68 (1H, *t*, J = 8.5 Hz, H-6) and 7.29 (1H, *dd*, J = 1.0, 8.5, H-7) corresponds to ring C protons. Therefore this compound was identified as 1,8-hydroxy-3-methylanthraquinone, trivial name chrysophanol (**3**). Compound **3** is known from roots of *Aloe* and it has been reported from other genera of the family Asphodelaceae (Yenesew *et al.*, 1988).



4.2.4. Aloesaponarin II (4)

Compound **4** was isolated as an orange crystal exhibiting UV absorption at λ_{max} 260, 290, 400 nm, suggesting an anthraquinone skeleton (Yagi *et al.*, 1974). ESI analysis showed $[M]^+$ at 254.58 corresponding to the molecular formula $C_{15}H_{10}O_4$. The ^{13}C NMR spectrum (Table 2) of **4** showed fifteen carbon signals, two of which corresponding to carbonyl carbons (at δ_C 189.2 and 182.1, assigned for C-9 and C-10, respectively of an anthraquinone), and two oxygenated aromatic carbons (δ_C 162.2 and 161.4) and a methyl signal at δ_C 23.5. The ^1H NMR spectrum of this compound differs from compound **3** by showing only one chelated hydroxyl proton signal at δ_H 12.94 and a down-field shifted methyl proton at δ_H 2.66 which suggested that this compound is isomeric to compound **3.**

Two *meta*-coupled aromatic proton at δ_H 7.41 (1H, *d*, J = 3.0 Hz) and 6.99 (1H, *d*, J = 2.5 Hz) corresponding to H-5 and H-7, respectivelly of ring C. Furthermore the ^1H NMR showed the presence of an AMX spin system corresponding to three aromatic protons which resonated at δ_H 7. 58 (1H, *dd*, J = 7.5, 1.5 Hz), 7.68 (1H, *t*, J = 7.5 Hz) and 7.29 (1H, *dd,* J = 8.5, 1.0 Hz) corresponding to H-4, H-3 and H-2, respectivelly of ring A. This compound was therefore identified as aloesaponarin II (**4**), a compound first reported from *Aloe saponaria* (Yagi *et al.*, 1974) and latter known to occur in other *Aloe* species (Yenesew *et al.*, 1993; Dagne *et al.*, 1994). Compound **4** has been also reported as bacterial metabolite (Cui *et al.*, 2006; Bartel *et al.*, 1990; Fotso *et al.*, 2003).

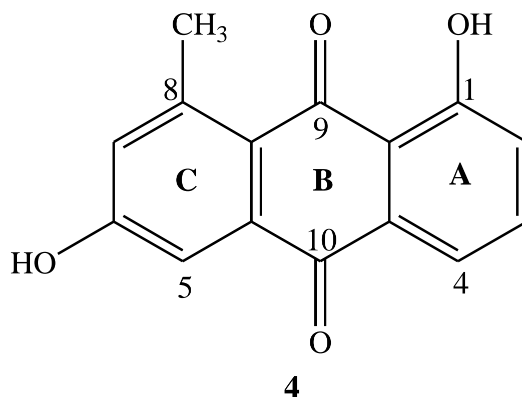


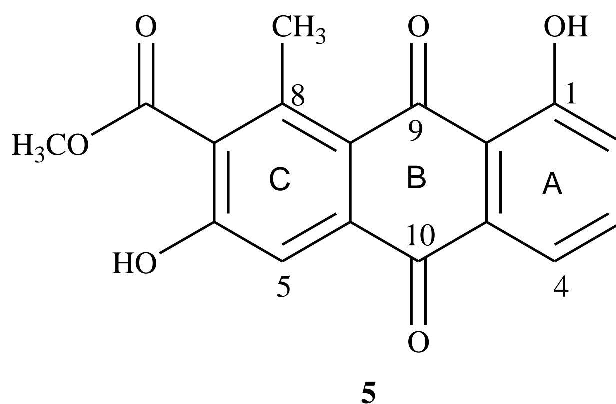
Table 2: ${}^{1}H$ (500 MHz) and ${}^{13}C$ (125 MHz) spectral data of compounds **3** and **4** (DMSO-d_6)

Carbon No.	Compound **3**		Compound **4**	
	${}^{1}H$ δ_H (*m*, *J* in Hz)	${}^{13}C$	${}^{1}H$ δ_H (*m*, *J* in Hz)	${}^{13}C$
1	-	162.4	-	162.2
1a	-	113.7	-	116.3
2	7.64 (*bs*)	121.3	7.29 (*dd, J* = 8.5, 1.0 Hz)	124.1
3	-	149.3	7.68 (*t*, *J* = 7.5 Hz)	136.7
4	7.09 (*bs*)	124.3	7.58 (*dd*, *J* =7.5, 1.5 Hz)	118.1
4a	-	133.2	-	132.4
5	7.82 (*dd, J* = 8.5, 1.0 Hz)	119.9	7.41 (*d*, *J* = 3.0 Hz)	111.9
5a	-	133.6	-	136.7
6	7.68 (*dd*, *J* = 7.5, 1.5 Hz)	136.9	-	161.4
7	7.29 (*dd*, *J* = 7.5, 1.5 Hz)	124.5	6.99 (*d*, *J* = 2.5 Hz	124.4
8	-	162.7	-	145.2
8a	-	115.8	-	122.6
9	-	181.9	-	189.2
10	-	192.5	-	182.1
1-OH	12.00 (*s*)	-	12.94 (*s*)	-
8-CH_3	2.46 (s)	22.6	2.66 (*s*)	23.5

4.2.5. Aloesaponarin I (5)

Compound **5** was isolated as an orange crystals; the UV spectrum showed absorption at λ_{max} 260, 300, 410 nm which is suggestive of an anthraquinone chromophore as in compound **4**. The ${}^{1}H$ and ${}^{13}C$ NMR spectra of **5** (Table 3) are similar to those of **4**, except for the presence of

additional signals corresponding to a methyl ester group (δ_H 3.95, δ_C 52.7 and δ_C 167.9) at C-7. Thus the ^{1}H NMR spectrum of **5** showed the presence of only one aromatic singlet at δ_H 7.73 which is assigned to H-5 of ring C. In ring A three mutually coupled aromatic protons at δ_H 7.70 (*dd*, *J* = 5.5, 2.5 Hz for H-4), 7.68 (*t*, *J* = 2.0 Hz, H-3), 7.29 (*dd, J* = 7.5, 1.0 Hz, H-2) are consistent with identical ring with that of compound **4**. The ^{13}C NMR spectrum showed 17 signals (Table 3). The data is in agreement that this compound is 1,6-dihydroxy-8-methylanthraquinone-7-carboxy methyl ester (trivial name aloesaponarin I). It has been reported from *Aloe graminicola* among other *Aloe* species (Yenesew *et al*., 1993; Dagne *et al*., 1994).

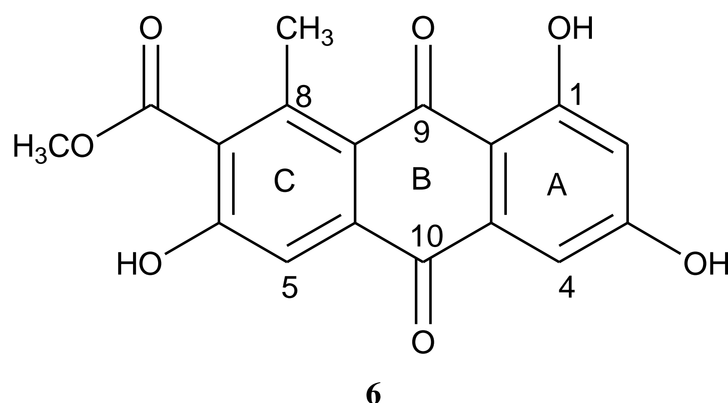


5

4.2.6. Laccaic acid D methyl ester (6)

Compound **6** was obtained as orange crystals; the molecular formula $C_{17}H_{12}O_7$ was deduced from the MS which showed a molecular ion peak at *m/z* 328.71. Compound **6** showed UV absorption at λ_{max} 300, 410 nm being consistent with a 9,10-anthraquinone chromophore. The ^{1}H and ^{13}C NMR (Tables 3 and 4) spectra of **6** were found to be similar to those of **5**, except for the presence of an additional hydroxyl group at C-3 (δ_C 165.1). Thus ^{1}H NMR spectrum of **6** showed the presence of two *meta*-coupled aromatic protons in ring A at δ_H 6.65 (*d*, *J* = 2.5 Hz, H-2), 7.18 (*d*,

J = 2.5 Hz, H-4) and one singlet aromatic proton in ring C at 7.72 (H-5). The presence of methyl ester (at C-6), methyl (at C-8), three hydroxyl substituents (at C-1, C-3 and C-6) substituents were established from NMR spectra (Tables 3 and 4). This compound was therefore identified as laccaic acid D methyl ester (**6**), a compound which has been reported from some *Aloe* species (Yagi *et al*., 1974; Dagne *et al*., 1992; van Wyk *et al*., 1995).



6

Table 3: ^{1}H (500 MHz, acetone-d_6) spectral data of compound **5** and **6**

Carbon No.	Compound	
	^{1}H δ_H (*m*, *J* in Hz)	
	5	**6**
2	7.29 (*dd*, 7.5, 1.0)	6.65 (*d*, 2.5)
3	7.68 (*t*, 1.9)	-
4	7.70 (*dd*, 5.5, 2.5)	7.18 (*d*, 2.5)
5	7.73 (*s*)	7.72 (*s*)
1-OH	12.8 (*s*)	13.16 (*s*)
8-CH_3	2.70 (*s*)	2.70 (*s*)
CO-OCH_3	3.95 (*s*)	3.93 (*s*)

Table 4: ^{13}C (125 MHz, acetone-d_6) spectral data of compound **5** and **6**

Carbon No.	δ_C	
	5	**6**
1	162.9	166.2
1a	125.3	111.7
2	124.4	109.2
3	119.2	165.1
4	113.0	108.1
4a	133.6	135.5
5	136.9	113.1
5a	133.8	138.0
6	159.5	159.3
7	142.9	142.3
8	130.8	130.9
8a	125.2	124.2
9	190.4	189.3
10	182.3	182.7
*C*O-OCH$_3$	167.9	168.1
CO-O*C*H$_3$	52.7	52.6
CH$_3$	20.3	20.3

4.2.7. Aloesaponol I (7)

Compound **7** was obtained as a colorless solid and showed blue fluorescence under UV light (366 nm). This compound exhibited UV absorption at λ_{max} 300, 380 nm which is typical of a pre-anthraquinone chromophore (Yagi *et al.*, 1974). ESIMS showed a molecular ion peak at *m/z* 316.90 corresponding to the molecular formula of $C_{17}H_{15}O_6$.

The ^1H and ^{13}C NMR spectra (Table 6) of **7** supported a pre-anthraquinone skeleton. Thus, the ^1H NMR spectrum showed two singlet aromatic protons at (δ_H 6.95 and 6.92 corresponding to H-5 and H-10) and a deshielded methyl group (at δ_H 2.70) placed at C-8. Furthermore, the ^1H NMR spectrum showed strongly chelated hydroxyl signal at δ_H 15.27 and methyl ester at δ_H 3.83 (δ_C =

52.1) suggesting that the compound is the precursor to aloesaponarin I. Moreover, compound **7** displayed aliphatic signals including a multiplet for oxymethine (δ_H 4.24) at C-3 and two methylene groups [δ_H 3.14 (*dd*, *J* = 3.3, 15.8 Hz); 2.96 (*dd*, *J* = 3.3, 17.1 Hz)]; and [2.90 (*dd*, *J* = 6.8, 15.6 Hz); 2.50 (*dd*, *J* = 1.8, 5.4 Hz)], corresponding to CH_2-2 and CH_2-4.

The ^{13}C NMR spectrum (Table 5) revealed the presence of an oxymethine carbon at δ_C 64.4 (C-3), two oxygenated sp^2 hybridized carbon atom, (δ_C 155.1 for C-9 and δ_C 165.9 for C-6) and carbonyl signal at δ_C 203.7 (C-1). Furthermore, the ^{13}C NMR spectrum showed the presence of an ester group [carbonyl at δ_C 168.2; methoxy at 52.1]. Therefore, compound **7** was identified as 3,6,9-trihydroxy-8-methyl-1-oxo-5,6,7,8-tetrahydroanthracene-2-carboxylic acid methyl ester, (trivial name aloesaponol I). The absolute configuration of this compound at C-3 is not determined here. However, (*R*)-configuration at C-3 has been reported (Dagne *et al*., 1992; Yenesew *et al*., 1993). The compound was first isolated from the subterranean stem of *Aloe saponaria* (Yagi *et al*., 1974) and latter from other *Aloe* species (van Wyk *et al*., 1995; Dagne *et al*., 1994).

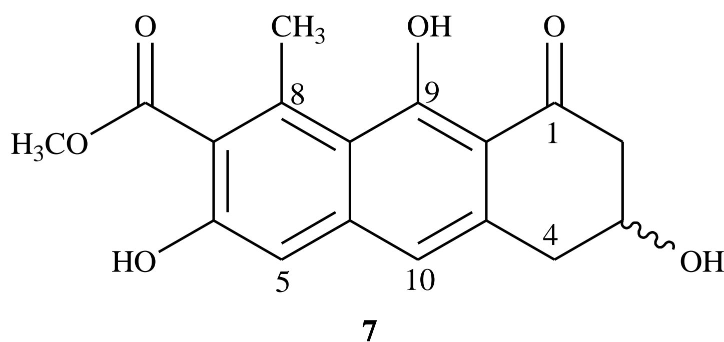


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Table 5: ^{1}H (500 MHz) ^{13}C (125 MHz) data of compound **7** (DMSO-d_6)

Carbon No.	δ_H	δ_C
1	-	203.7
CH_2-2	2.70 (*dd*, *J* = 1.8, 5.4 Hz) 2.96 (*dd*, *J* = 3.3, 17.1 Hz)	46.4
3	4.24 (*m*)	64.4
CH_2-4	2.90 (*dd*, *J* = 6.8, 15.6 Hz) 3.14(*dd*, *J* = 3.3,15.8 Hz)	37.5
5	6.95 (*s*)	116.6
5a	-	-
6	-	155.1
7	-	140.8
8	-	137.2
8a	-	125.4
9	-	165.9
9a	-	136.6
10	6.92 (*s*)	107.5
10a	-	110.2
OCH_3	3.83 (*s*)	52.1
$COOCH_3$	-	168.2
CH_3	2.70 (*s*)	20.8
9-OH	15.27 (*s*)	-

4.3. Characterization of Compounds from the Leaves of *Aloe turkanensis*

The air dried and powdered leaves of *A. turkanensis* were extracted with dichloromethane/methanol (1:1) by cold percolation. The crude extract was partitioned between ethyl acetate and water. The ethyl acetate layer was subjected to column chromatography on silica gel which resulted in the isolation of a naphthoquinone, five anthraquinones, a pyrone derivative and a benzoic acid derivative. Compounds **1**, **4** and **5** were isolated both from the roots and the leaves of *A. turkanensis*, and the structure elucidation has been discussed in section 4.2. The characterization of five additional compounds which were only isolated from the leaves is discussed below.

4.3.1. Helminthosporin (8)

Compound **8** was obtained as a red solid, showing UV absorbing at λ_{max} 230, 250, 500, 580 nm which is typical of 1,5,8-trihydroxyanthraquinone (Yagi *et al*., 1977). In agreement with this, the ^{1}H NMR spectrum (Table 6) showed three chelated hydroxyl protons at δ_H 12.03, 12.03 and 12.83 for 1-OH, 5-OH and 8-OH. The ^{13}C NMR specrum (Table 6) exhibited fifteen carbon signals of which two are for carbonyl (at δ_C 189.9 and 186.3) and one for methyl (δ_c 22.1; δ_H 2.50) groups. The ^{1}H NMR spectrum (Table 6) of compound **8** further exhibited two broad singlet aromatic protons at δ_H 7.26 (δ_C 124.3) and 7.65 (δ_C 120.2) and were assigned to H-2 and H-4 of ring A; and a singlet integrated for two protons at δ_H 7. 44 was assigned to H-6 and H-7 of ring C. Therefore, compound **8** was identified as 1,5,8-trihydroxy-3-methyl-9,10-anthraquinone (trivial name helminthosporin). This compound has been isolated from some *Aloe* species (Yagi *et al*., 1977; Yenesew *et al*., 1993; Dagne *et al*., 1994) and also from *Drechslera holmii* and *Drechslera ravenelii* (van Eijk and Roeymans, 1981).

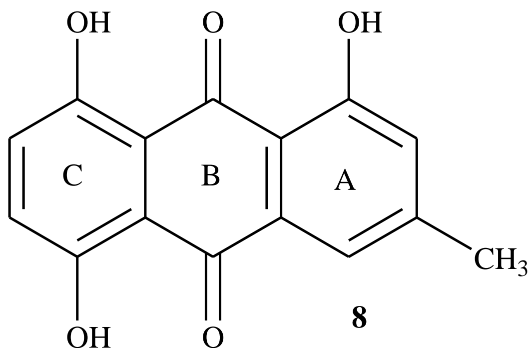

Table 6: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **8** (DMSO-d_6)

Carbon No.	**^{1}H δ_H (*m*)**	**^{13}C**
1	-	161.7
1a	-	113.8
2	7.26 (*brs*)	124.3
3	-	149.1
4	7.65 (*brs*)	120.2
4a	-	132.8
5	-	157.1
5a	-	112.5
6	7.44 (*brs*)	129.4
7	7.44 (*brs*)	129.4
8	-	156.4
8a	-	112.6
9	-	189.9
10	-	186.3
CH_3	2.50 (s)	22.1
OH	12.83	-
OH	12.83	-
OH	12.03	-

4.3.2. Feralolide (9)

Compound **9** was obtained as a brown solid showing blue fluorescence under UV light (366 nm). This compound exhibit UV absorbing at λ_{max} 310 nm. The ESIMS showed $[M]^+$ at m/z 344.75 corresponding to a molecular formula of $C_{18}H_{16}O_7$. The compound was identified as feralolide (**9**) based on comparison of spectroscopic data with literature (Speranza *et al*., 1993, Abd-Alla *et al*., 2009; Elhassan *et al*., 2012).

The ^{1}H NMR spectrum (Table 7) of **9** revealed the presence of two aromatic rings, each containing a pair of *meta*-coupled protons (δ_H 6.31, 6.26 for H-5, H-7) and (δ_H 6.37, 6.42 for H-5', H-7'). Furthermore the ^{1}H NMR showed a chelated hydroxyl proton at δ_H 11.21 (8-OH). The ^{13}C NMR spectrum revealed the presence of oxymethine carbon (δ_C 80.5, C-3) with

corresponding proton appearing as a multiplate at δ_H 4.80. Furthermore, the ^{13}C NMR spectrum showed two methylene carbon atoms (δ_C 32.8 and 39.5) with corresponding protons signals appearing at δ_H 2.94, 2.92 and 3.22, 3.08, each appearing as a doublet of a doublet, due to CH_2-4 and CH_2-1' respectively in the ^{1}H NMR spectrum. Additionally the ^{13}C NMR spectrum showed a lacton carbonyl (δ_C 170.3, C-1) and ketonic carbonyl signal at (δ_C 203.9). The compound was therefore identified as feralolide (**9**). The absolute configuration of at C-3 is not determined here. However (*R*)-configuration at C-3 has been previously reported for this compound (Speranza *et al.*, 1993).

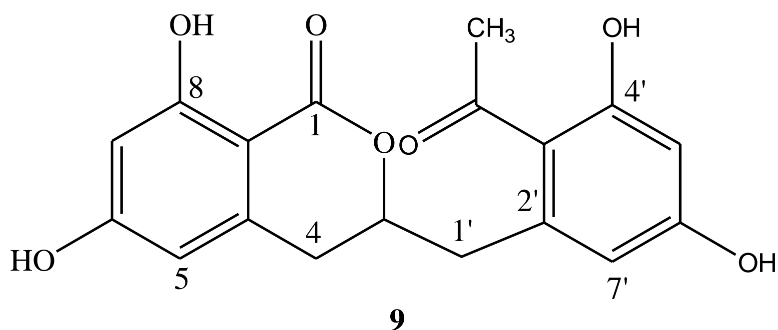

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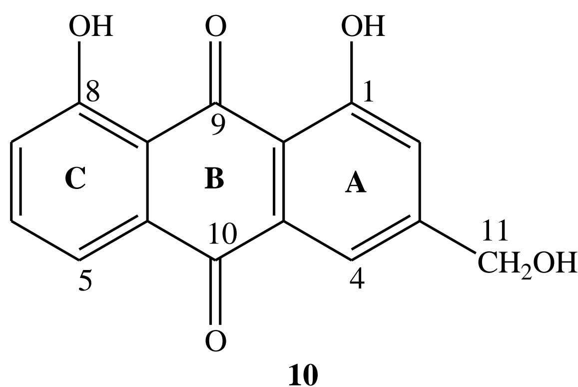
Table 7: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of compound **9** (acetone-d_6)

Carbon No.	^{1}H δ_H	^{13}C δ_C
1	-	170.3
3	4.80 (*m*)	80.5
4	-	32.8
CH_2-4	2.92 (*dd*, *J* = 6.9 Hz) 2.94 (*dd*, *J* = 6.9 Hz)	32.8
4a	-	142.9
5	6.31 (*d*, *J* = 1.5 Hz)	107.6
6	-	165.0
7	6.26 (*d*, *J* = 1.9 Hz)	101.9
8	-	165.2
8a	-	101.8
1'	-	39.5
CH_2-1'	3.08 (*dd*, *J* = 5.5, 14 Hz) 3.22 (dd, *J* = 6.9, 13.5 Hz)	39.5
2'	-	139.4
3'	-	121.0
4'	-	160.1
5'	6.37 (*d*, *J* = 2.5 Hz)	102.5
6'	-	160.3
7'	6.42(*d*, *J* = 1.9 Hz)	111.7
COCH_3	-	203.9
CH_3	2.57 (*s*)	33.1
8-OH	11.21 (*s*)	-

4.3.3. Aloe-emodin (10)

Compound **10** was isolated as an orange crystals, having UV absorbing band at λ_{max} at 260, 300, 420 nm which is typical of 9,10-anthraquinones. In agreement with this, the ^{13}C NMR spectrum (Table 9) showed two carbonyl signals at δ_C 191.6 and 181.4 corresponding to C-9 and C-10, respectively. The ^{1}H NMR spectrum (Table 8) of **10** revealed three mutually coupled aromatic protons at δ_H 7.38 (1H, *dd*, *J* = 9.0, 1.1 Hz, H-7), 7.81 (1H, *t*, *J* = 8.0 Hz, H-6) and 7.71 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-5) of ring C. In ring A, two broad singlet aromatic protons at δ_H 7.28 (1H, *d*, *J* = 1.6 Hz) and 7.68 (1H, *d*, *J* = 1.6 Hz) were assigned to H-2 and H-4, respectively with

oxymethylene (δ_H 4.61, δ_C 62.1) being at C-3 which must have been formed throuhgh oxidation of the methyl in compound **3**. Therefore, this compound was identified as aloe-emodin (**10**). The compound has been reported from some *Aloe* species (Reynols *et al.*, 1985; Conner *et al.*, 1990; ELhassan *et al.*, 2012).



4.3.4. α-L-11-*O*-Rhamnosyl aloe-emodin (11)

Compound **11** is a glycoside of aloe-emodin (**10**) with a molecular weight of 416, according to the ESI mass spectrum. This compound exhibited UV absorption at λ_{max} 260, 300, 430 nm. The ^{1}H NMR spectrum is similar to that of **10**, except that the presence of signals corresponding to a sugar unit (δ_H 3.23 - 4.71 ppm) attached at the oxymethylene position which appeared two mutually coupled protons at δ_H 4.75 (1H, *d*, J = 14.0 Hz) and 4.61 (1H, *d*, J = 13.9 Hz). The sugar unit was identified as L-rhamnose based on NMR spectra (Table 8) most noticeable of which methyl group on the sugar moiety at δ_H 1.16 (*d*, J= 6.2 Hz) (δ_c 17.9). Furthermore the ^{1}H NMR spectrum showed five oxymethine protons, of which the anomeric proton appeared at δ_H 4.71 (1H, *d*, J = 1.6 Hz); with the corresponding ^{13}C NMR signal being at δ_C 99.9 this indicating the sugar moiety was *α*-configuration (Elizabeth *et al.*, 1989). The $^1C_4$ conformation of the sugar unit was established from ^{1}H NMR spectrum the signal at 3.73 (2H, *dd*, J = 3.4, 1.7 Hz) for H-2'.

The ^{13}C NMR spectrum showed the presence of two carbonyls at δ_C 191.6 and 181.4 which corresponding to C-9 and C-10 respectivelly. The attachement of the sugar moiety was fixed at the oxymethylene position from the HMBC correlation (Table 8) of the anomeric protons with C-11, C-2‘ and C-3‘. Therefore this compound was identified as α-L-11-*O*-Rhamnosyl aloe-emodin, which has previously been reported from exudates of *Aloe rabaiensis*. (Conner *et al.*, 1989).

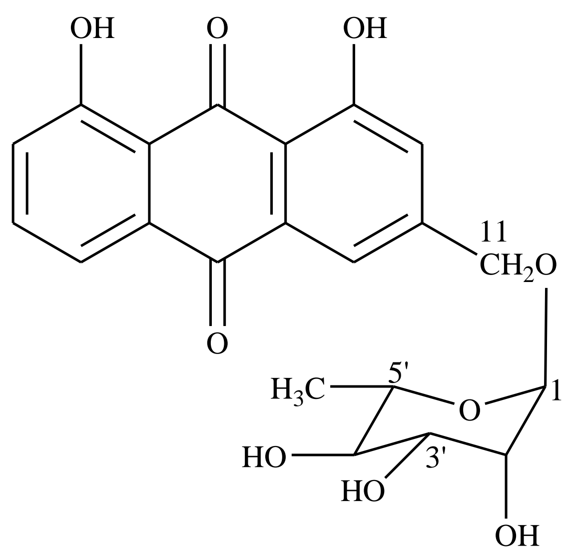


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Table 8: ^{1}H (500 MHz), ^{13}C (125 MHz) and HMBC (500MHz) spectra data of compound **10** and **11** (DMSO-d_6)

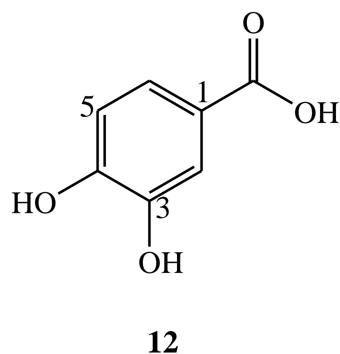
Carbon No.	Compound **10**			Compound **11**		
	^{1}Hδ_H (*m*, *J* in Hz)	^{13}C	HMBC	^{1}H δ_H (*m*, *J* in Hz)	^{13}C	HMBC
1	-	161.6		-	161.4	
1a	-	114.6		-	115.3	
2	7.28 (1H, *d*, *J* = 1.6 Hz)	120.6	C-1, C-1a	7.32 (1H, *d*, *J* = 1.6 Hz)	121.8	C-1a, C-4
3	-	157.3		-	148.8	
4	7.68 (1H, *d*, *J* = 1.6 Hz)	117.1	C-1a, C-1, C-2	7.68 (1H, *d*, *J* = 1.6 Hz)	117.7	C-1a, C-2, C-4a, C-10
4a	-	133.3		-	133.4	
5	7.71 (1H, *dd*, *J* = 7.5, 1.1 Hz)	119.3	C-7, C-8a, C-8	7.41 (1H, *dd*, *J* = 8.4, 1.1 Hz)	119.3	C-8a, C-5, C-8
5a	-	133.1		-	133.3	
6	7.81 (1H, *t*, *J* = 8.0 Hz)	137.3	C-5a, C-8, C-7, C-5	7.82 (1H, *st*, *J* = 8.4 Hz)	137.4	C-5a, C-5, C-8
7	7.38 (1H, *dd*, *J* = 9.0, 1.1 Hz	124.4	C-8a, C-8 , C-5	7.73 (*dd*, *J* = 5.0 Hz)	124.4	C-8a, C-7, C-8
8	-	161.3		-	161.3	
8a		115.9			115.9	
9	-	191.6		-	191.6	
10	-	181.4		-	181.4	
CH_2- 11	4.62 (*s*)	62.1		4.75 (1H, *d*, *J* = 14.0 Hz) 4.61 (1H, *d*, *J* = 13.9 Hz)	66.9	C-1', C-2, C-3, C-4
1’		-		4.71 (1H, *d*, *J* = 1.6 Hz)	99.9	C-11, C-2', C-3'
2’	-	-		3.73 (2H, *dd*, *J* = 3.4, 1.7 Hz)	70.4	C-3'
3’	-			3.62-3.28 (33H, *m*)	69.0	C-4'
4’	-	-		3.23 (2H, *s*)	71.8	CH_3, C-5'
5’	-	-		-	70.7	-
1-OH & 8-OH	11.93 (*s*)	-	-	-	-	-
CH_3	-	-	-	1.16 (*d*, *J* = 6.2 Hz)	17.9	C-5'

4.3.5. 3, 4-Dihydroxybenzoic acid (12)

Compound **12** was isolated as a brown solid. In the ^{1}H NMR spectrum three aromatic proton with an AXY spin system at δ_H 7.53 (*d*, *J* = 2.0 Hz) δ_H 6.89 (*d*, *J* = 4.9 Hz) and δ_H 7.48 (*dd*, *J* = 6.5 Hz, 2.0 Hz) corresponding to H-2 (δ_C 117.4), H-5 (δ_C 115.6) and H-6 (δ_C 123.6), respectively of a 1,3,4-trisubstituted benzene ring.

The ^{13}C NMR spectrum of **12** exhibited seven carbon signals including a carbonyl at δ_C 168.7 corresponding to carboxylic acid substituent at C-1 and two downfield shifted signals at δ_C 167.5 and δ_C 150.6 of the two hydroxyl substitutents at C-3 and C-4, respectively. Therefore, compound **12** was identified as 3,4-dihydroxy benzoic acid (trivial name protocatechuic acid). It has been previously isolated from the genus *Aloe* (Dagne *et al*., 1991) and from the family Ginkgoaceae, Hypericaceae and Rusaceae (Ellnain-Wojtaszek *et al*., 1997; Jurgenliemk *et al*., 2002 and Lee *et al*., 1994).

Table 9: ^{1}H (500 MHz) and ^{13}C (125 MHz) spectra data of compound **12** (acetone-d_6)



Carbon No	δ_H (*m*, *J* in Hz)	δ_C
1	-	146.2
2	7.53 (*d*. *J* = 2.0 Hz)	117.4
3	-	167.5
4	-	150.6
5	6.89 (*d*, *J* = 4.9)	115.6
6	7.47 (*dd*, *J* = 6.5, 2.0)	123.6
COOH	-	168.7

4.4. Chemotaxonomic Importance of the Isolated Naphthoquinones

Based on morphological characteristics of *Aloe*; the genus *Aloe* vary from grass to tree *Aloe* (Reynolds, 1966). *Aloe turkanensis* is placed in group 14 characterized by flowers secund. The morphological classification shows some variations between the species in the same groups.

Chemical constituents have made contribution to the classification system in *Aloe*. Previously, naphthoquinones have been reported from *Aloe secondiflora* which belongs to the same group (Induli *et al*., 2012). Reynolds (1996) reported the chromatographic comparison of the leaves exudates between *Aloe turkanensis* and *Aloe scabrifoli* showed that the two taxa are different. In this study, two naphthoquinones [3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**)] were isolated. This is the second report of the occurrence of naphthoquinones from the genus *Aloe*. Naphthoquinones may be a taxonomic marker for group 14 (*Aloe* with secund flowers). It is therefore will be interesting to explore the presence or absence of naphthoquinones on related species including *Aloe scabrifolia*.

4.5. *In vitro* Anticancer Activities

4.5.1. Effect of DMSO on cell viability

The cytotoxicity test on the extracts and pure compounds was carried out in the presence of DMSO. To determine the effect of DMSO on cell viability during the test, different concentrations of DMSO were used to decide the maximum amount of DMSO to be added without cytotoxic effect. The results suggest that DMSO did not show significant effect on cell viability at concentration less than 1 μg/mL (Fig. 4).

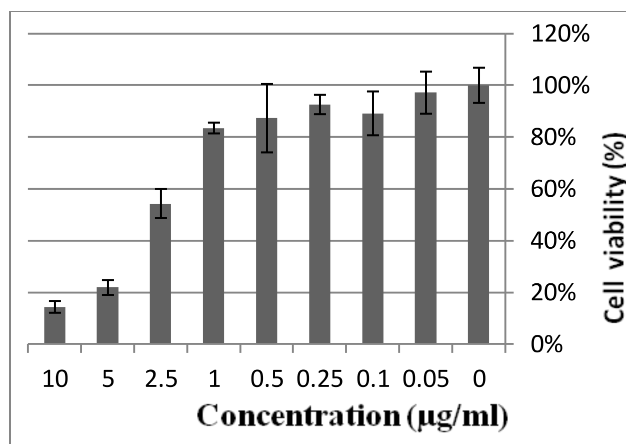


Figure 4- Effect of DMSO on TFK-1 cell viability

4.5.2. Anticancer test of crude extracts and compounds on TFK-1 cell line

Anticancer tests were carried out for the crude extracts and isolated compounds against human extra hepatic bile duct carcinoma cell line (TFK-1). The CH_2Cl_2/MeOH (1:1) extract of *Aloe turkanensis* rhizomes exhibited significant cytotoxicity within the concentration range of 50-100 µg/mL (Fig. 5) against human extra hepatic bile duct carcinoma cell line (TFK-1), while the leaves extract showed significant reduction in cell viability at 75 and 100 µg/mL against the same cell line. Interestingly, significant activities were also observed in the low concentrations of 2.5 and 10 µg/mL (Fig. 5), suggesting that the synergestic effect of compounds of the leaves extract increasing the activity at lower concentration.

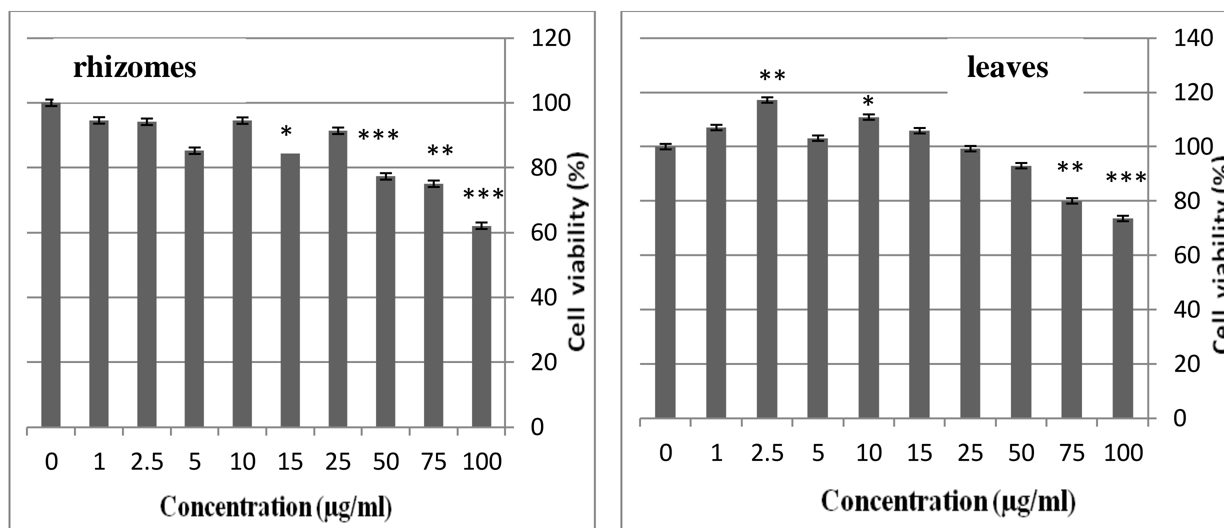


Figure 5: Effect of rhizomes and leaves extract on TFK-1 cell viability

Due to the observed cytotoxic effects of the crude extracts of the rhizomes and leaves (Fig. 5) of *A. turkanensis*, the twelve isolated compounds isolated from these extracts were tested against human TFK-1 cell line. Activities were observed for different classes of compounds *vis* anthraquinones, preanthraquinone and naphthoquinones.

Among the anthraquinones, aloe-emodin (**10**) and its glycoside, α-L-11-*O*-rhamnopyranosylaloe-emodin (**11**) had highest reduction of cell viability (Fig. 6) in the concentration range of 5-100 µg/mL ($p<0.001$). Aloesaponarin II (**4**) which has a methyl group *peri* to carbonyl (at C-8), strongly reduced cell viability (10-100 µg/mL) (Fig. 7). The isomeric structure chrysophanol (**3**) which has its methyl group at C-3 only showed significant cytotoxicity at concentration of 2.5 and 25 µg/mL ($P < 0.05$) (Fig. 8) indicating that the presence of methyl group at C-8, (*peri* to the carbonyl), in alosaponarin II (**4**) is important for the observed cytotoxicity. In agreement with this, two other anthraquinones, also having methyl group at C-8, *vis* aloesaponarin I (**5**) and laccaic acid D methyl ester (**6**), showed significant reduction of cell viability in 10-100 µg/mL and 50-100 µg/mL (Fig. 9) concentrations range. As shown in Fig. 8 helminthosporin (**8**)

significantly reduced cell viability at 5-100 μg/mL, better than chrysophanol (**3**), which only showed reduction in cell viability at 2.5 and 25 μg/mL (Fig. 8). The pre-anthraquinone aloesaponol I (**5**) reduce cell viability at a higher degree in concentration of 5-100 μg/mL (Fig. 7).

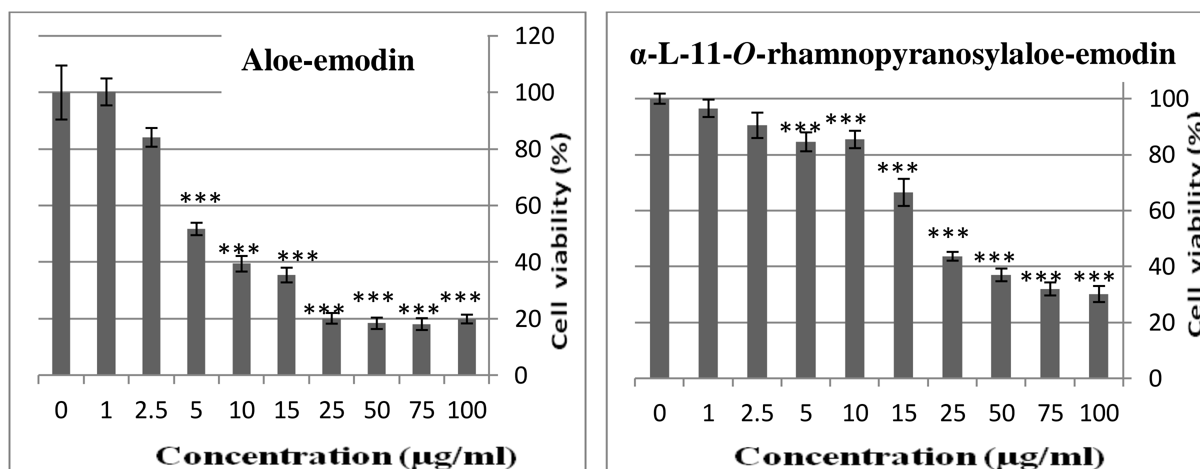


Figure 6: Effect of aloe-emodin and α-L-11-*O*-rhamnosyl aloe-emodin on TFK-1 cell viability

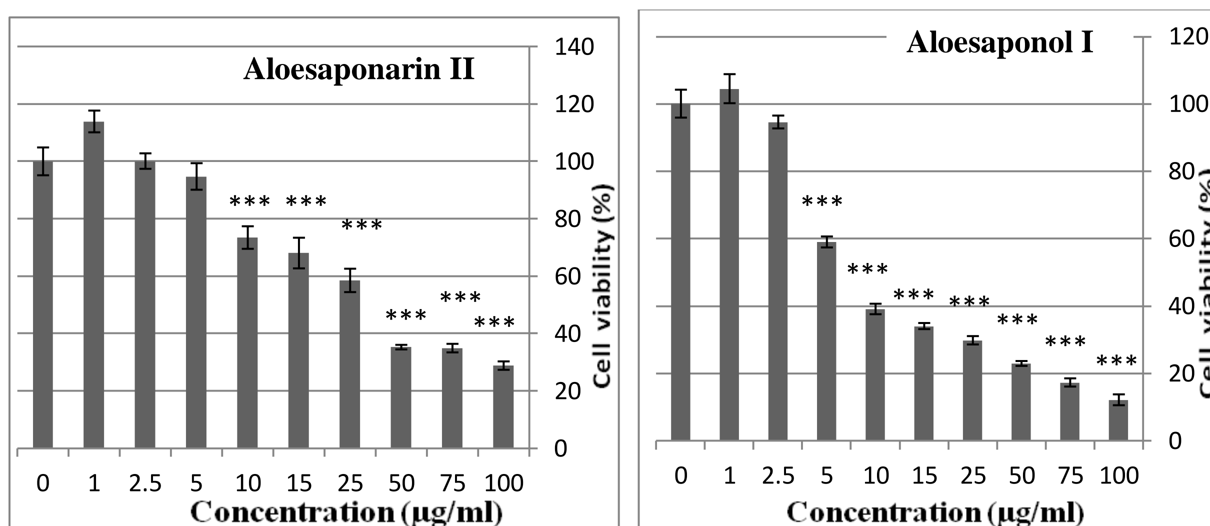


Figure 7: Effect of aloesaponarin II and aloesaponol I on TKF-1 cell viability

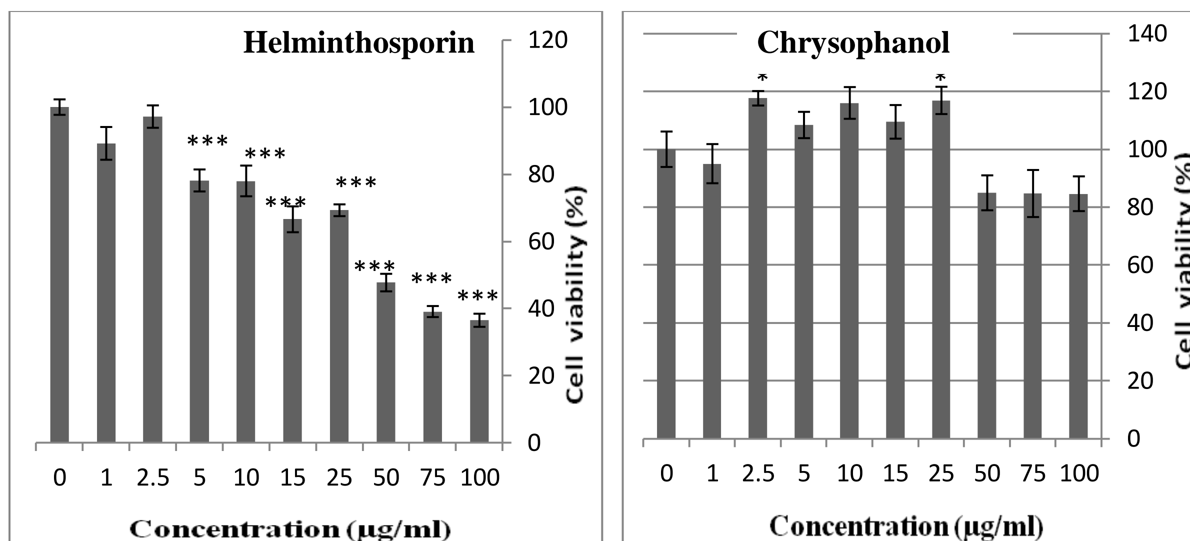


Figure 8: Effect of helminthosporin and chrysophanol on TFK-1 cell viability

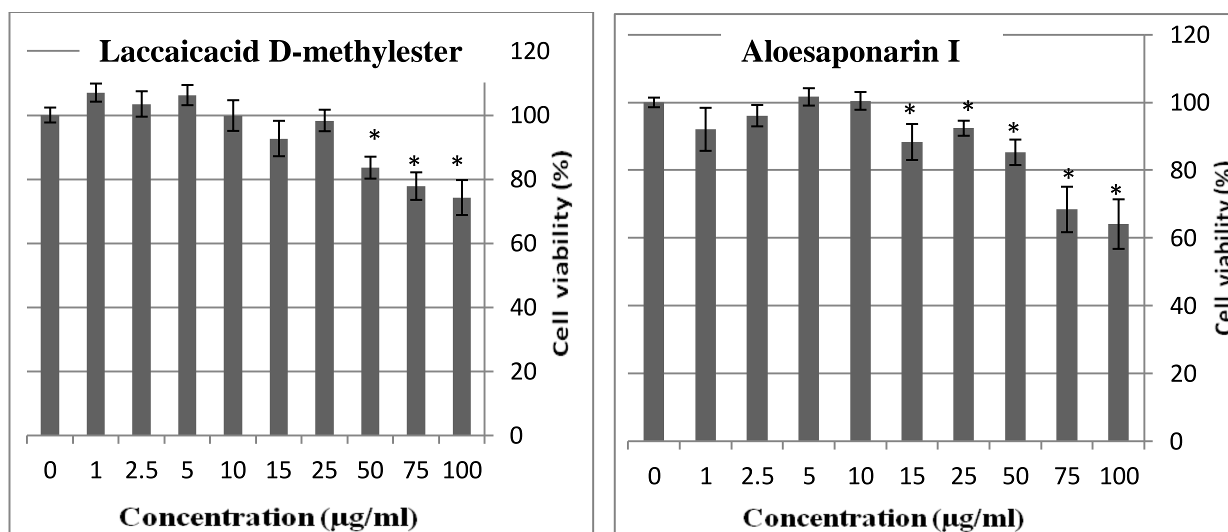


Figure 9: Effect of laccaic acid D- methy ester and aloesaponarin I on TFK-1 cell viability

The naphthoquinone 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**) revealed potent cytotoxicity at concentrations of 25-100 µg/mL (Fig. 10) with a reduction of cell viability over 90%. Furthermore, this compound even showed significant reduction of cell viability in the 5-15 µg/mL concentrations. The second naphthoquinone tested, 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**), also showed significant reduction in cell viability at 100, 75

and 5 µg/mL (Fig. 10). But still the methylated naphthoquinone (**2**) reduced cell viability to a greater degree than the non-methylated naphthoquinone (**1**).

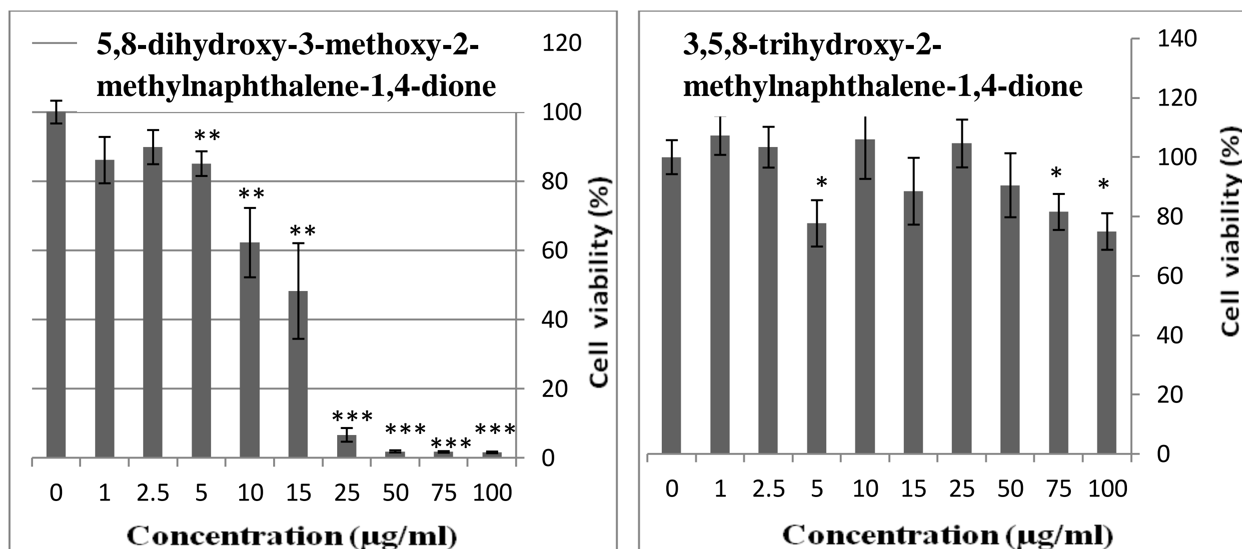


Figure 10: Effect of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1-4-dione and 3,5,8-Tri hydroxyl-2-methylnaphthalene-1,4-dione on TFK-1 cell viability

The benzoic acid derivative, 3,4-dihydroxybenzoic acid (**12**) had significant reduction of cell viability at 25 and 50 µg/mL ($p < 0.05$) (Fig. 11). The pyrone derivative, feralolide (**9**) also showed significant effect at 5 µg/mL ($p < 0.05$) against TFK-1 cell line in the tested concentrations (Fig. 11).

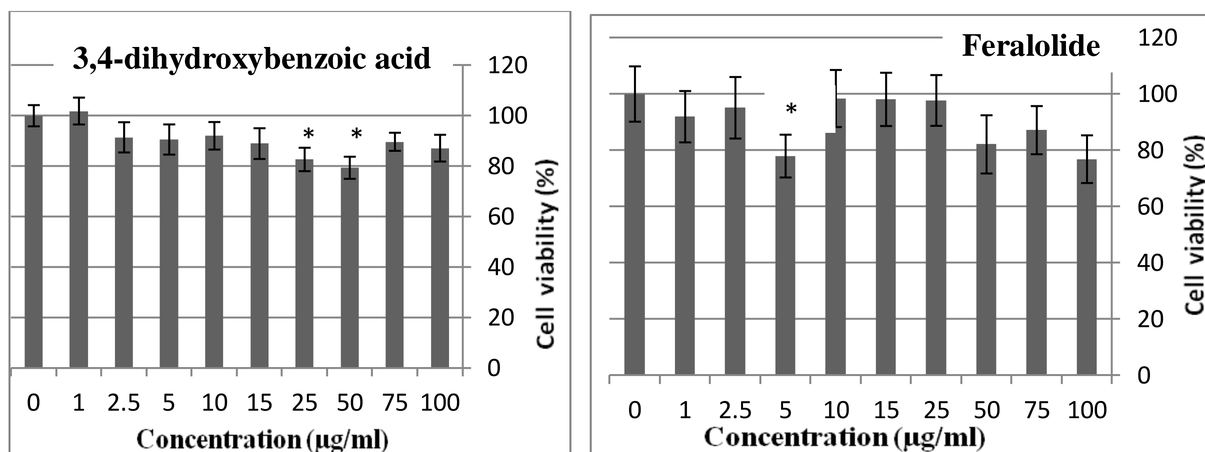


Figure 11: Effect off 3,4-dihydroxybenzoic acid and feralolide on TFK-1 cell viability

It appears these compounds are responsible for the significant reduction in cell viability of the crude extracts against TFK-1 cell line.

4.5.3. Anticancer test on selected compounds on HuH7 cell line

The most cytotoxic compounds against extra hepatic bile duct carcinoma cell line (TFK-1) were tested against human hepatoma carcinoma cell line (HuH7). Among these, the naphthoquinone derivative 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**) reduced cell viability to greater degree (between 80 to 97.5%) in the concentration of 25-100 µg/mL (Fig.12). This compound also significantly reduced cell viability (by 29.4%) at the concentration of 15 µg/mL(Fig. 12).

Aloe-emodin (**10**) and its glycoside α-L-11-*O*-rhamnopyranosyl aloe-emodin (**11**) showed higher decrease in cell viability in 25-100 µg/mL (Fig. 12 and Fig. 13). The pre-anthraquinone aloesaponol I (**7**) and the anthraquinone aloesaponarin II (**4**) highly reduced cell viability at higher concentrations (50-100 µg/mL) (Fig.14). Figure 11 showed that helminthosporin (**8**) did not significantly affect HuH7 cells in the tested concentrations.

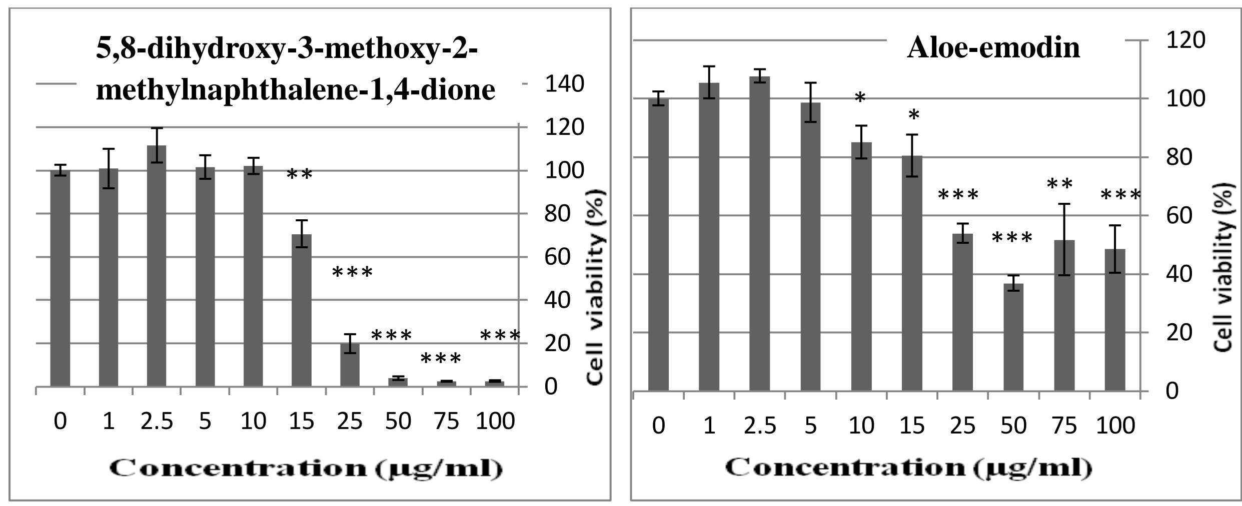


Figure 12: Effect of 5,8-dihydroxy-3-methoxy-2-methylnaphtalene-1,4-dione and aloe-emodine on HuH7 cell viability

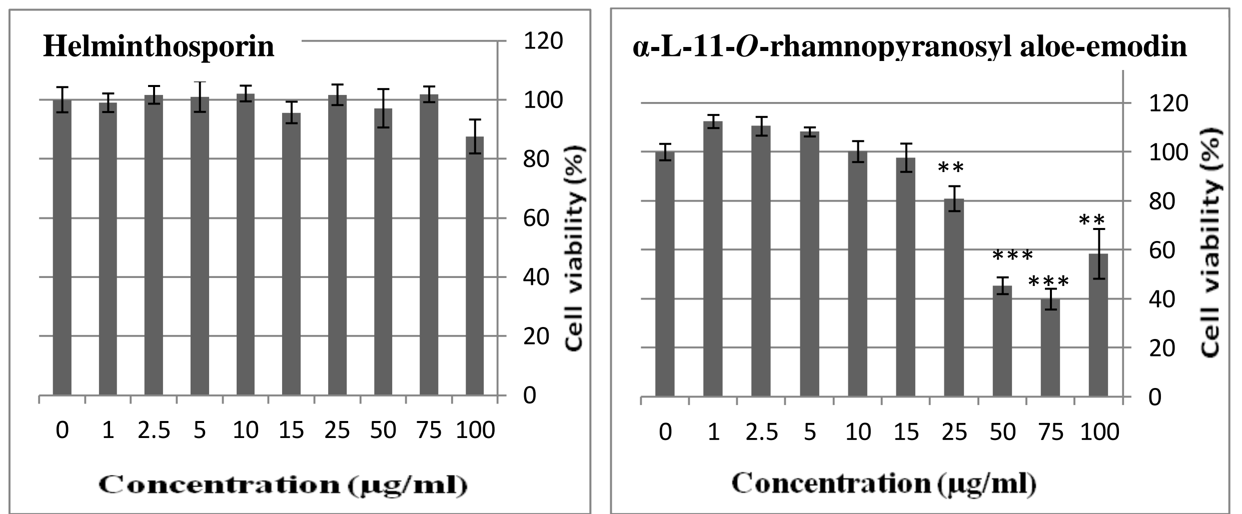


Figure 13: Effect of helminthosporin and α-L-11-*O*-rhampopyranosyl aloe-emodin on HuH7 cell viability

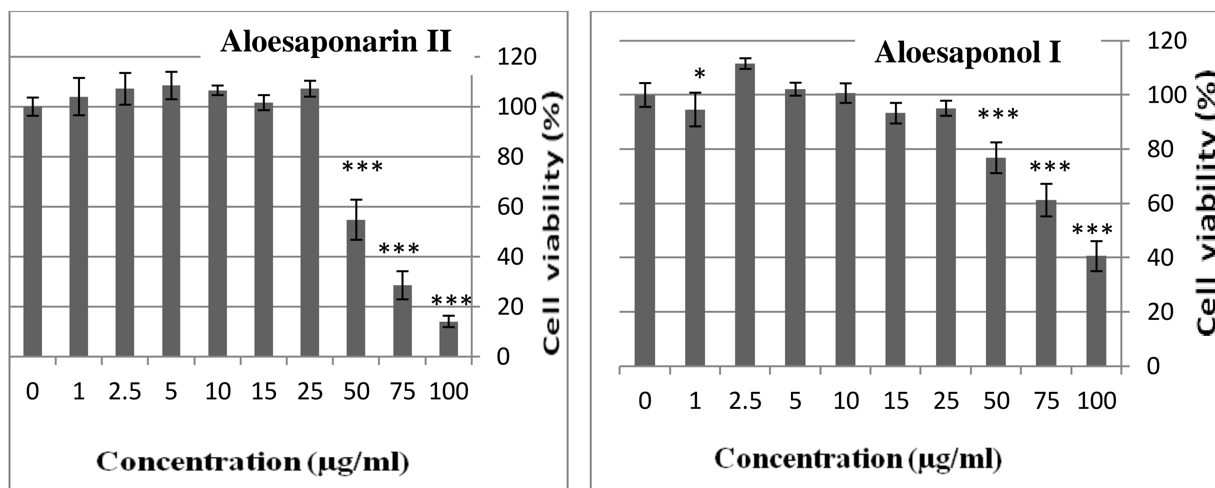


Figure 14: Effect of aloesaponarin II and aloesaponol I on HuH7 cell viability

The cytotoxicity results (Table 10) revealed that aloe-emodin (**10**) is the most potent inhibitor against TFK-1 (IC_{50} value of 6 µg/mL) and HuH7 (IC_{50} value 31 µg/mL) cell lines. The naphthoquinone derivative 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**) also exhibited high inhibition against TFK-1 (IC_{50} values of 15 µg/mL) and HuH7 (IC_{50} values of 20 µg/mL) cell lines. The pre-anthraquinone aloesaponol I (**7**) inhibited the growth of TFK-1 cells with IC_{50} value of 10 µg/mL. Aloe-emodin glycoside, α-L-11-*O*-rhamnopyranosyl aloe-emodin (**11**) and aloesaponarin II (**4**) inhibited viability of TKF-1 cell with IC_{50} values of 23 µg/mL and 34 µg/mL, respectively; while IC_{50} of 47 µg/mL and 55 µg/mL against HuH7 cell lines, respectively were observed. Helminthosporin (**8**) also significantly inhibited the growth of TFK-1 cells with IC_{50} values of 46 µg/mL but not on the HuH7 cells in the tested concentrations. All of the compounds that inhibited growth of cell line on TFK-1 had a lower cytotoxic effect on HuH7 cell line (Table. 10).

Table 10: Cytotoxcity (IC_{50} value) of pure compounds and crude extraces from *Aloe turkanensis* against human cancer cell lines (TFK-1 and HuH7)

compounds	IC_{50} (µg/mL)	
	TFK-1	HuH7
Crude extracts	>100	NT
3,5,8-Trihydroxy-2-methylnaphthalene-1,4-dione (**1**)	>100	NT
5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**)	15.0	20.0
Chrysophanol (**3**)	>100	NT
Aloesaponarin II (**4**)	34.0	55.0
Aloesaponarin I (**5**)	>100	NT
Laccaic acid D-methyl ester (**6**)	>100	NT
Aloesaponol I (**7**)	10.0	88.0
Helminthosporin (**8**)	46.0	NA
Feralolide (**9**)	>100	NT
Aloe-emodin (**10**)	6.0	31.0
α-L-11-*O*-Rhamnopyranosylaloe-emodin (**11**)	23.0	47.0
3,4-Dihydroxybenzoic acid (**12**)	>100	NT

NA = not active up to 100 µg/ml

NT = not tested

From the previous studies, the inhibitory growth effects of aloe-emodin have been reported on various cancer cell lines. For example, aloe-emodin showed inhibition of the growth of neuroectodermal tumors cells (Pecere *et al*., 2000), lung squamous cell carcinoma (Lee, 2001); aloe-emodin has also showed anticancer effects on the SCC-4 human tongue squamous carcinoma cells (Chiu *et al*., 2009). Recently, it has been reported that aloe-emodin suppresses the growth of prostate cancer by inhibiting mTORC2 activity (Liu *et al*., 2012).

Many structurally related analogues of naphthoquinones of synthetic and plant origin have been studied for anticancer potential against various human cancerous cell lines *in vitro,* and against animal tumor models. For example, plumbagin exhibited cell growth inhibition against human non small cell lung cancer cells, A549 (Hsu *et al*., 2006). Polyfluorinated derivatives of 1,4-naphthoquinone showed strong cytotoxicity against human myeloma RPMI 8226, human mammary adenocarcinoma MCF-7, mouse fibroblasts LMTK and primary mouse fibroblast cell line (PMF) (Zakharova *et al*., 2011). Recently Bringmann *et al*. (2011) reported the anti-tumor activity of synthetic and natural naphthoquinones in different cancer cell lines. However, this is the first report that describes the anticancer activities of the anthraquinones, preanthraquinone and naphthoquinone derivatives on human extra hepatic bile duct (TFK-1) and liver cancer (HuH7) cell lines.

CHAPTER FIVE

CONCLUSIONS AND RECCOMENDATIONS

5.1. Conclusion

Chromatographic separation of *Aloe turkanensis* lead the isolation of twelve compounds. These were identified as 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**), 5,8-dihydroxy-2-methoxy-2-methylnaphthalene-1,4-dione (**2**), chrysophanol (**3**), aloesaponarin I (**4**), aloesaponarin II (**5**). laccaic acid D methyl ester (**6**) and aloesaponol I (**7**). From the leaves of *Aloe turkanensis* eight compounds were isolated and characterised as helminthosporin (**8**), feralolide (**9**), aloe-emodin (**10**), α-L-11-*O*-rhamnopyranosyl aloe-emodin (**11**)] and 3,4-dihydroxybenzoic acid (**12**). Among these 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**), aloesaponarin I (**4**), aloesaponarin II (**5**) were isolated both from the rhizomes and the leaves. The naphthoquinone 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) is reported here for the first time from the family Asphodelaceae. The crude extracts showed significant reduction in cell viability against extra hepatic bile duct cancer cell line (TFK-1). Six compounds [5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**), aloesaponarin II (**5**), aloesaponol I (**7**), Helminthosporin (**8**), Aloe-emodin (**10**) and α-L-11-*O*-rhamnopyranosylaloe-emodin (**11**)] revealed very potent inhibition of TKF-1 cell line. Five compounds [5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**), aloesaponarin II (**5**). aloesaponol I (**7**), Aloe-emodine (**10**) and α-L-11-*O*-rhamnopyranosylaloe-emodin (**11**)] showed higher inhibition on HuH7 cell line. Helminthosporin (**8**) did not inhibit the HuH7 cells at the tested concentrations. This is the first report on the phytochemical and anticancer potential of the isolated compounds from against extra hepatic (TFK-1) and liver (HuH7) cancer cell lines.

5.2. Recommendation

- Further cytotoxic of the active compounds on normal cell lines should be conducted.
- The mechanism of action of the active compounds should be investigated.
- Chemotaxonomic survay of naphthoquinones should be investigated on related species.
- Further phytochemical investigation on *Aloe turkanensis* should be done using HPLC.

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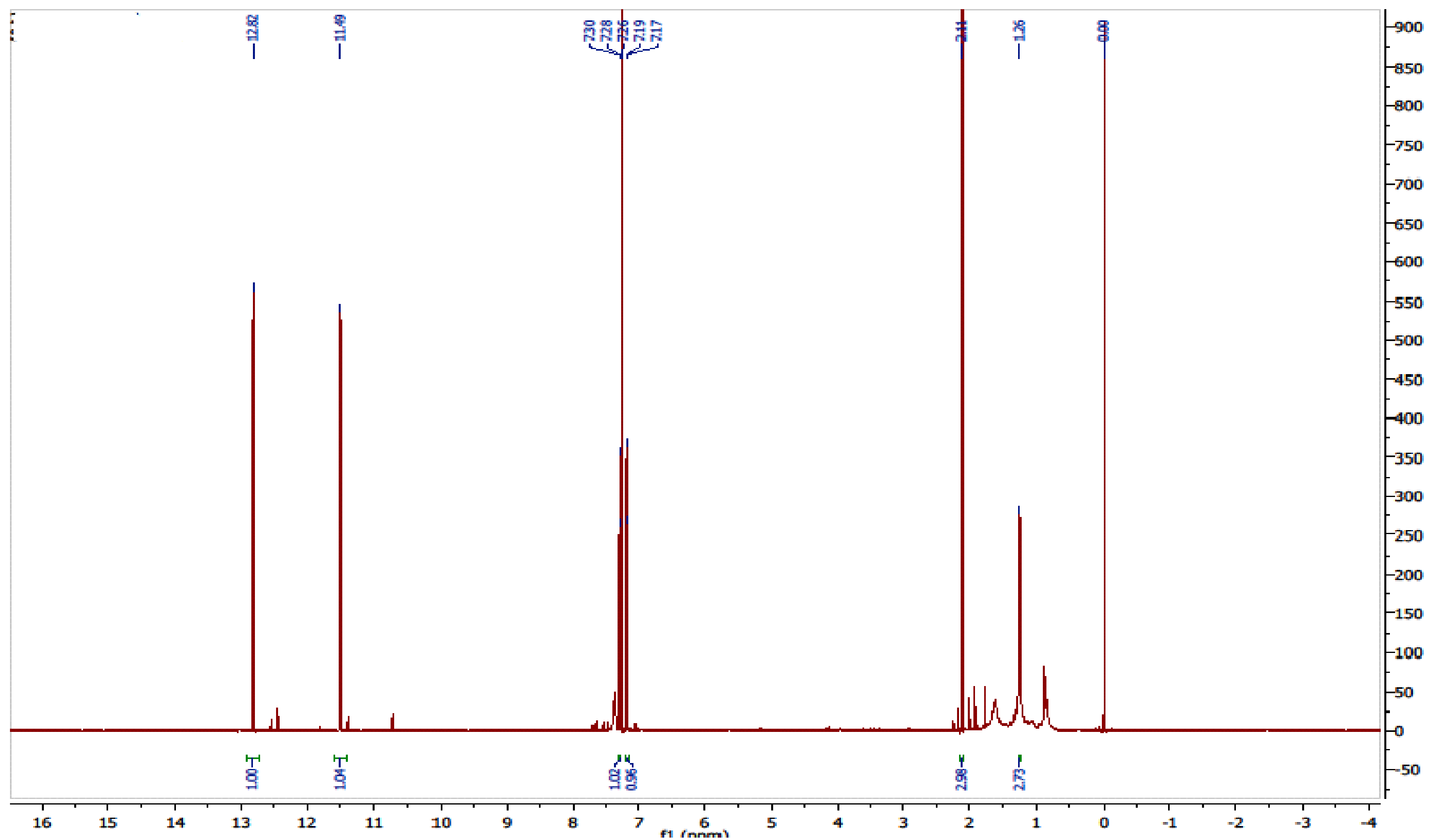
APPENDICES

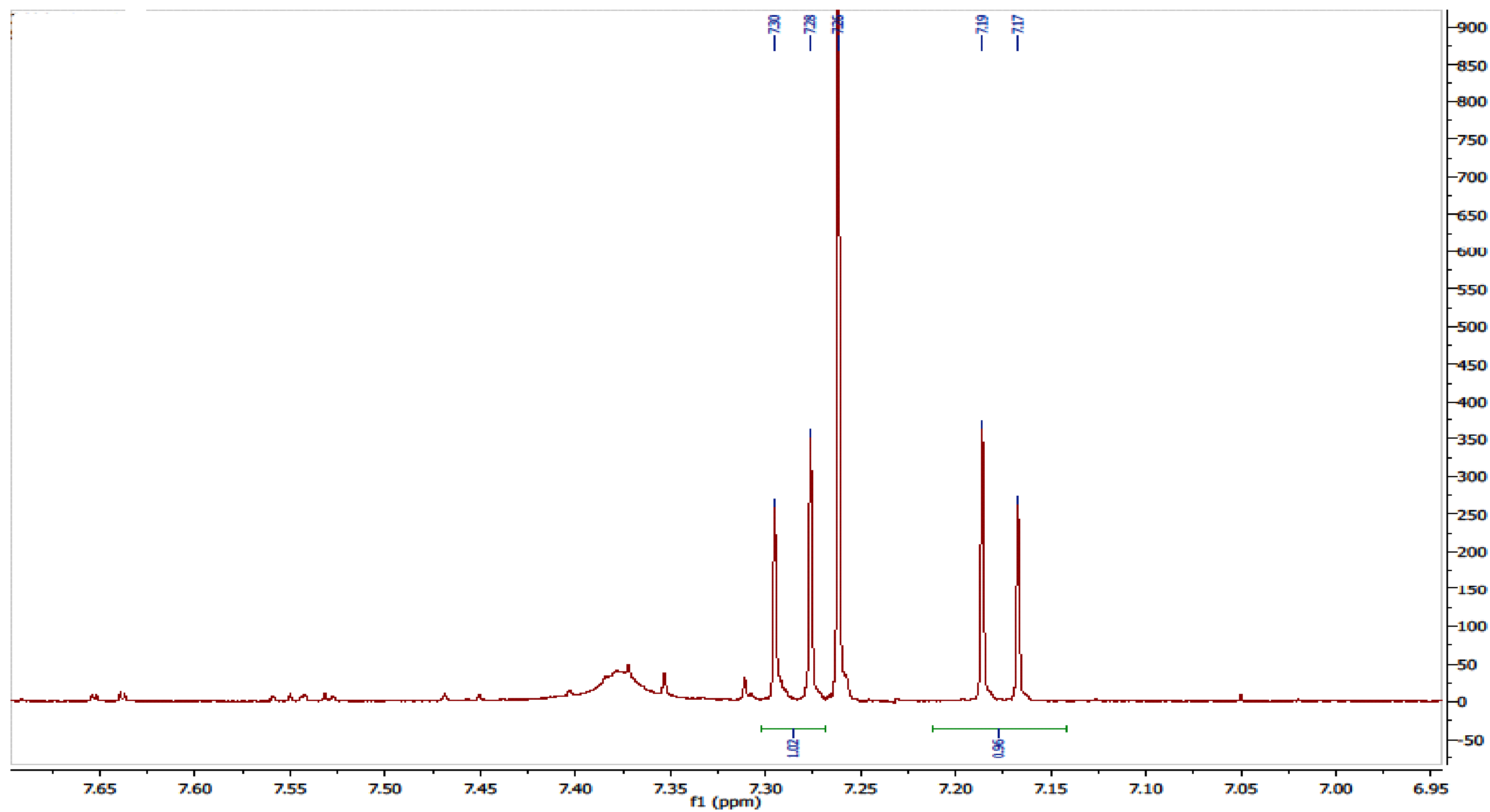
APPENDIX A

SPECTRA FOR COMPOUND 1

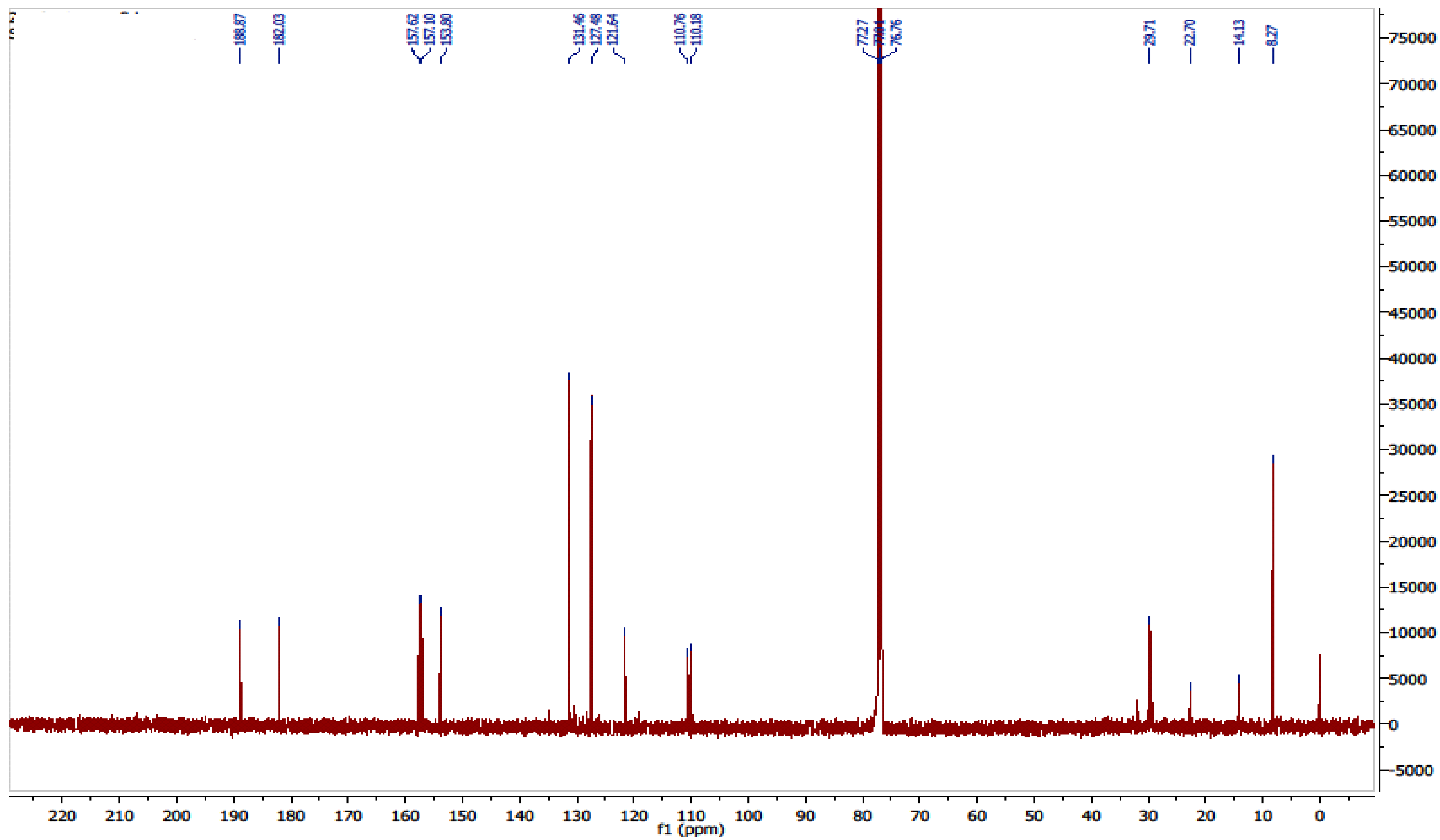
^{1}H NMR SPECTRUM FOR COMPOUND 1 ($CDCl_3$, 500 MHz)



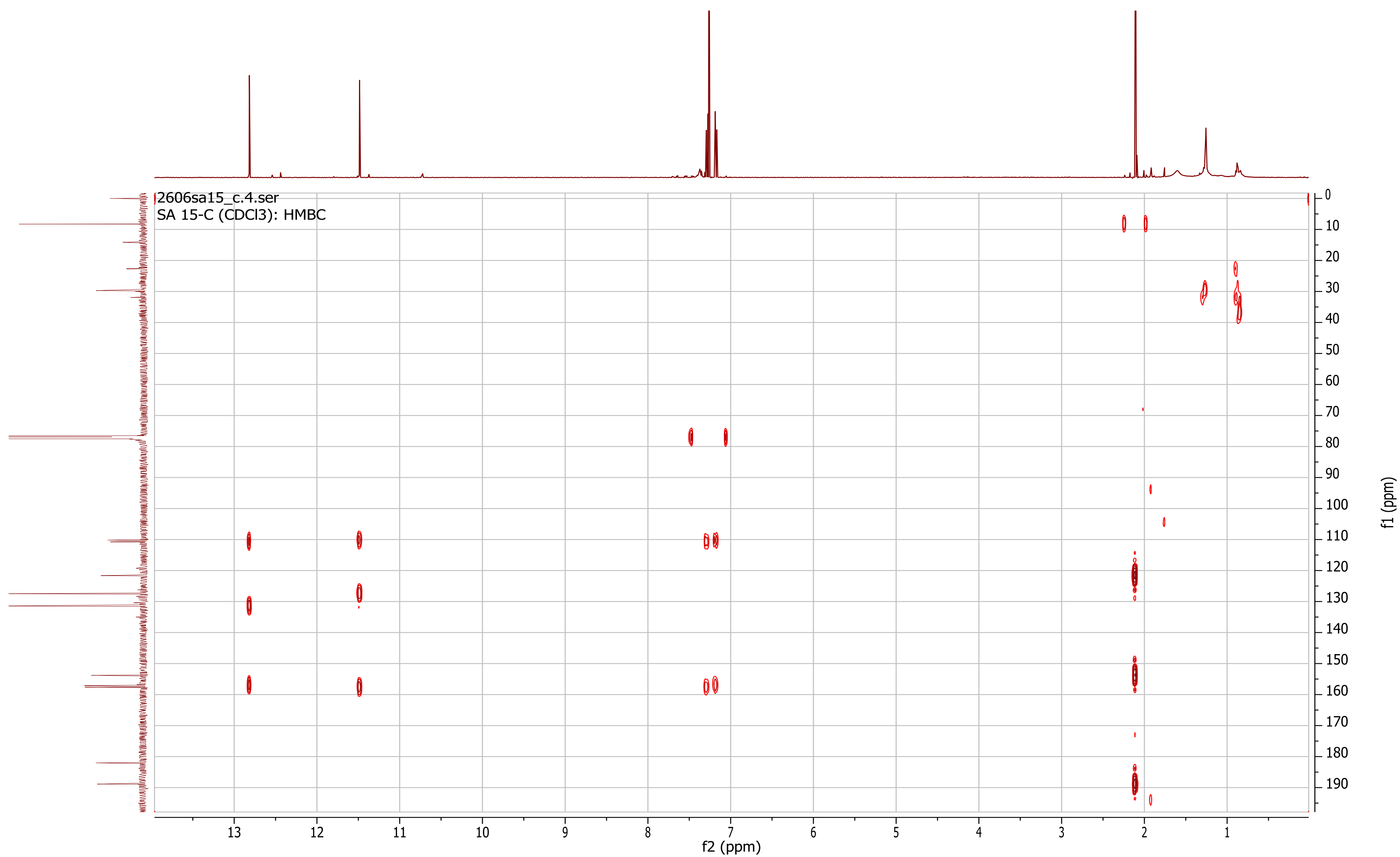
^{1}H NMR SPECTRUM FOR COMPOUND 1 ($CDCl_3$, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 1 ($CDCl_3$, 125 MHz)



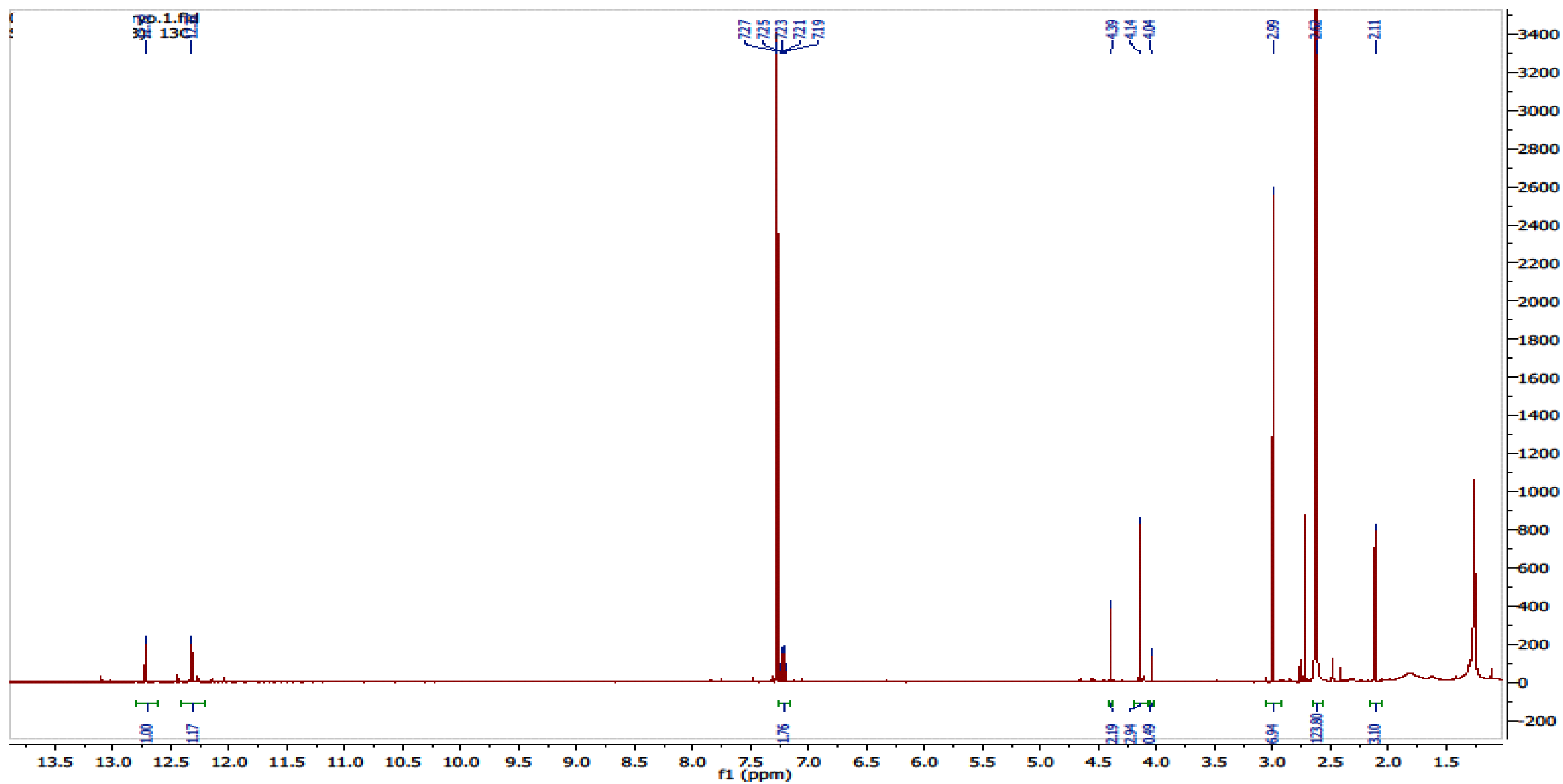
HMBC SPECTRUM FOR COMPOUND 1 ($CDCl_3$, 500 MHz)



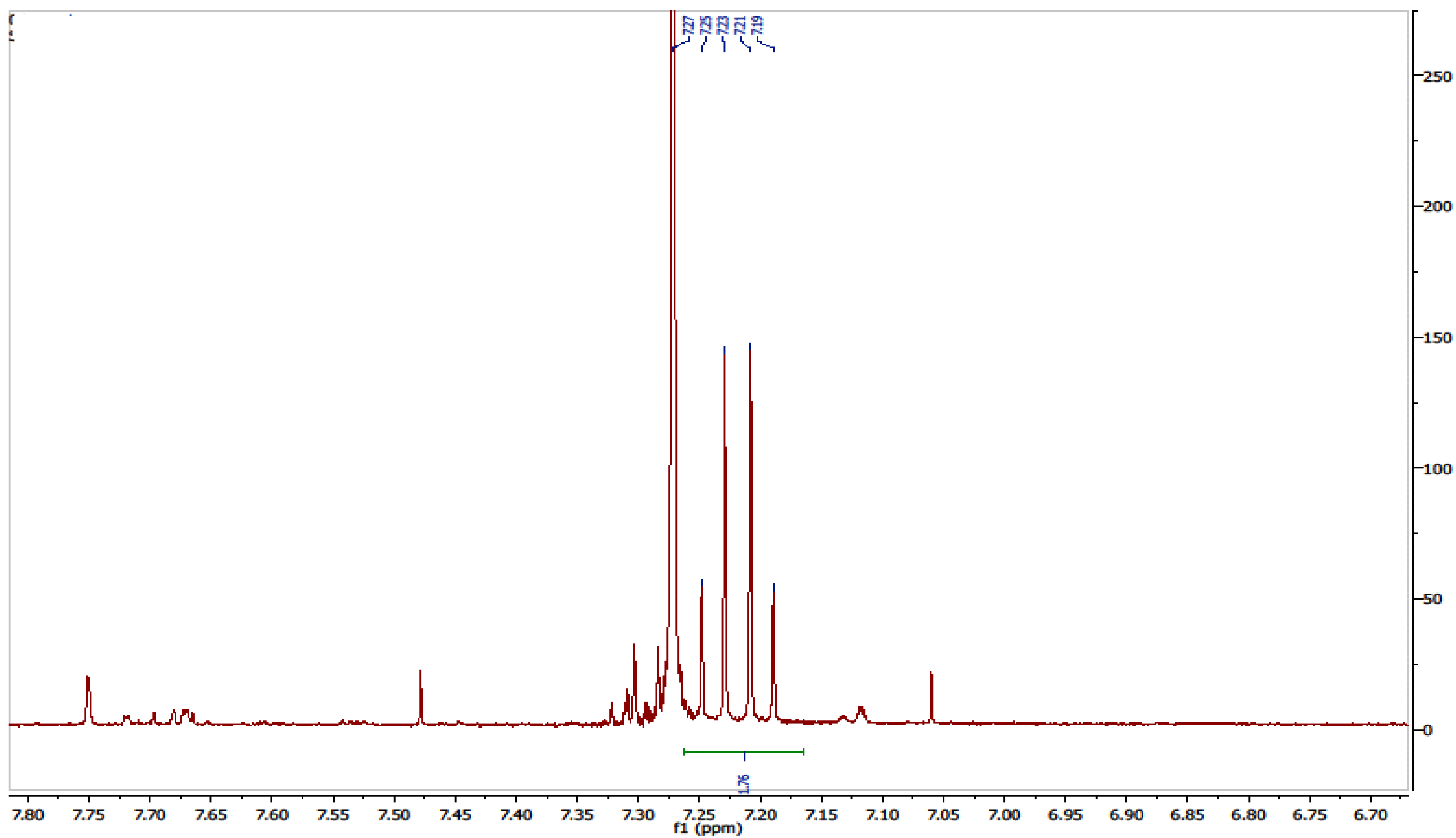
APPENDIX B

SPECTRA FOR COMPOUND 2

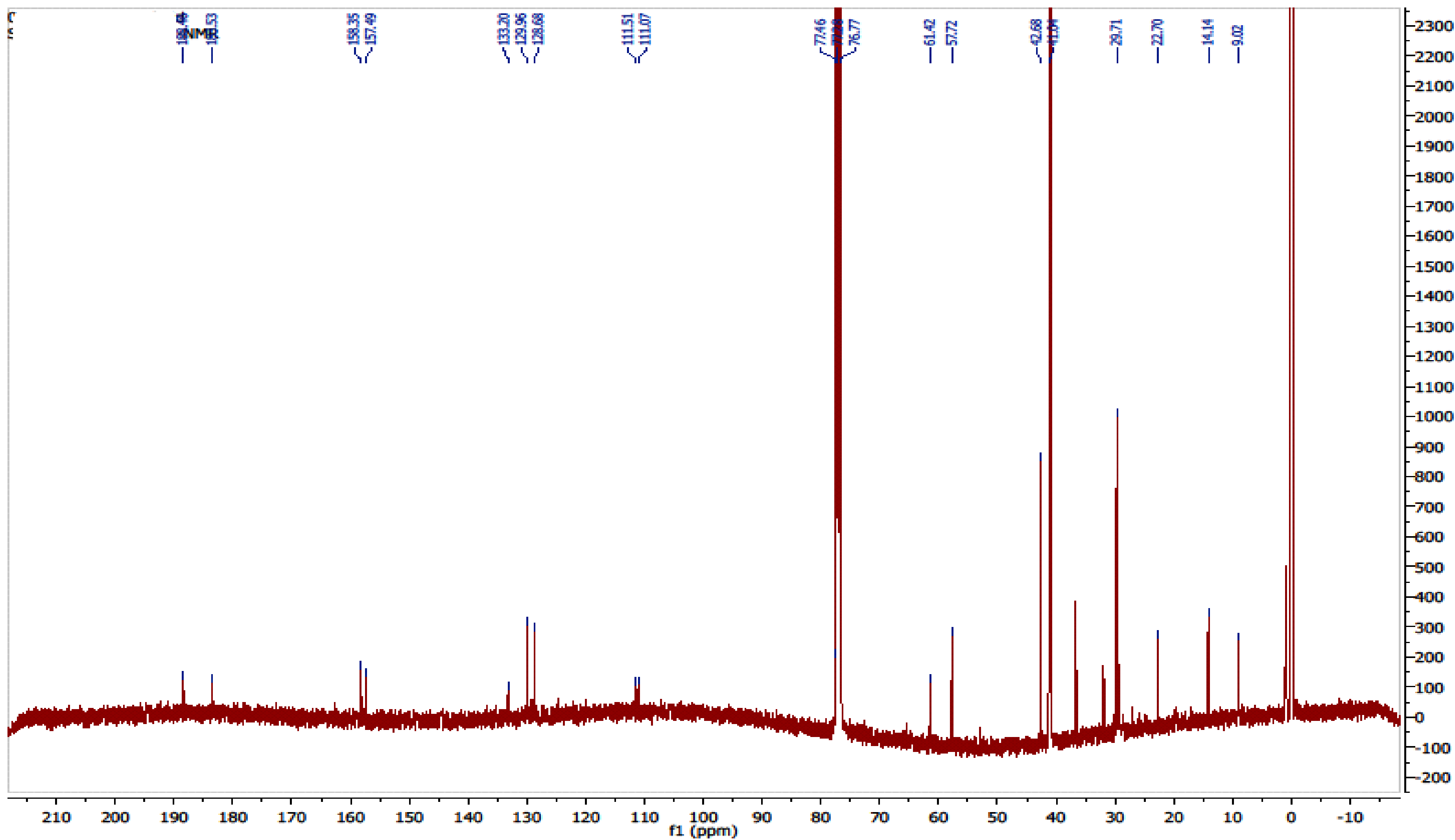
^{1}H NMR SPECTRUM FOR COMPOUND 2 ($CDCl_3$, 500 MHz)



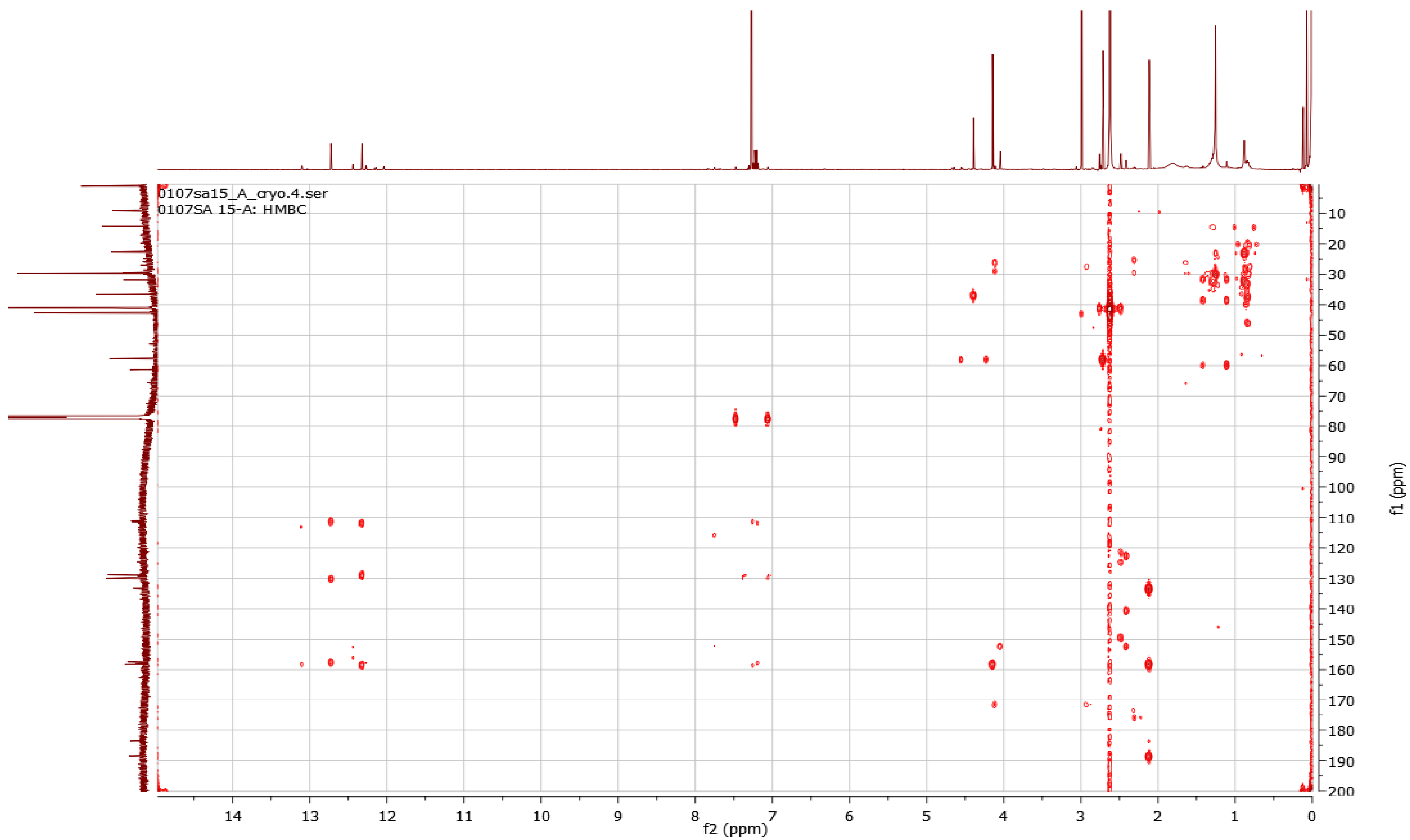
^{1}H NMR SPECTRUM FOR COMPOUND 2 ($CDCl_3$, 500 MHz)



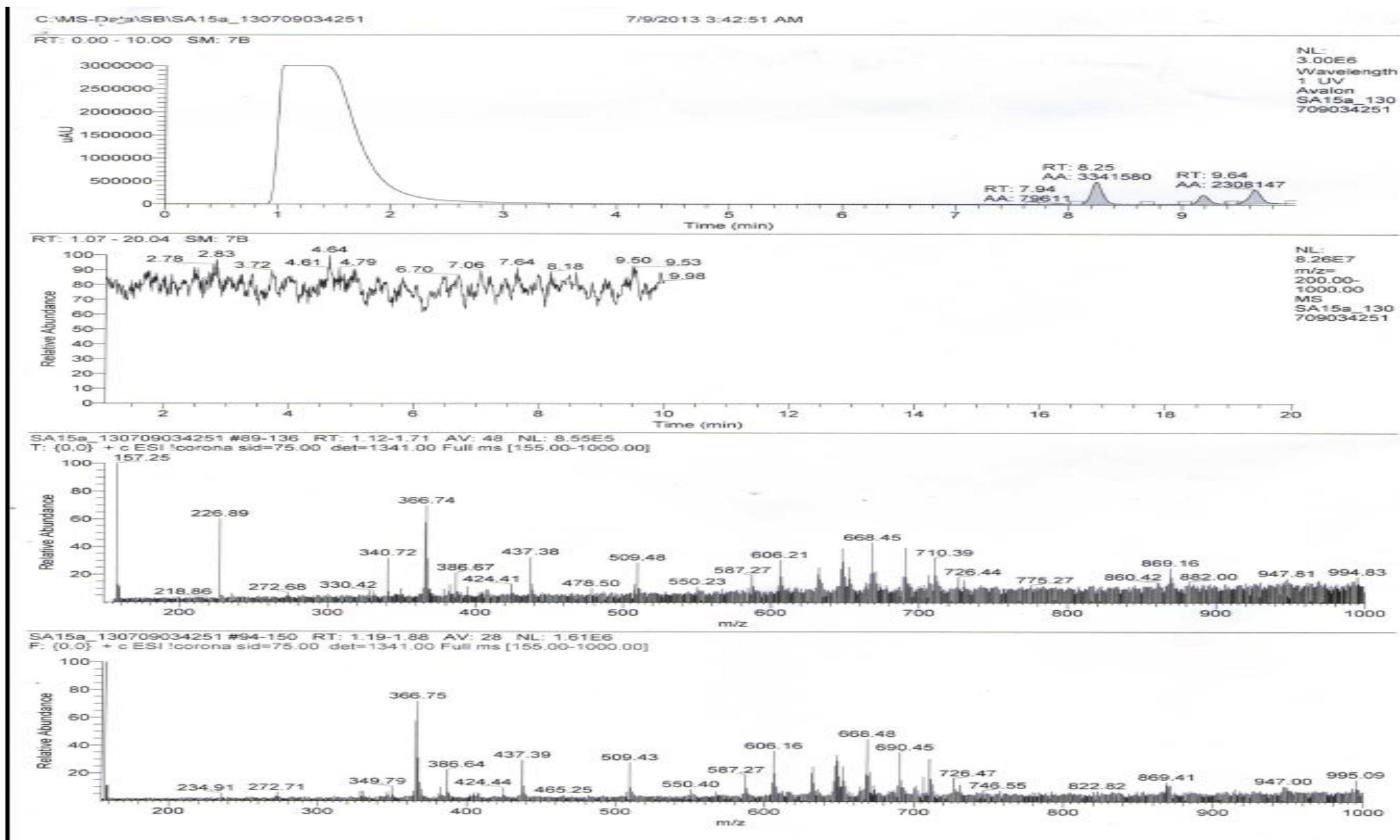
^{13}C NMR SPECTRUM FOR COMPOUND 2 ($CDCl_3$, 125 MHz)



HMBC SPECTRUM FOR COMPOUND 2 ($CDCl_3$, 500 MHz)



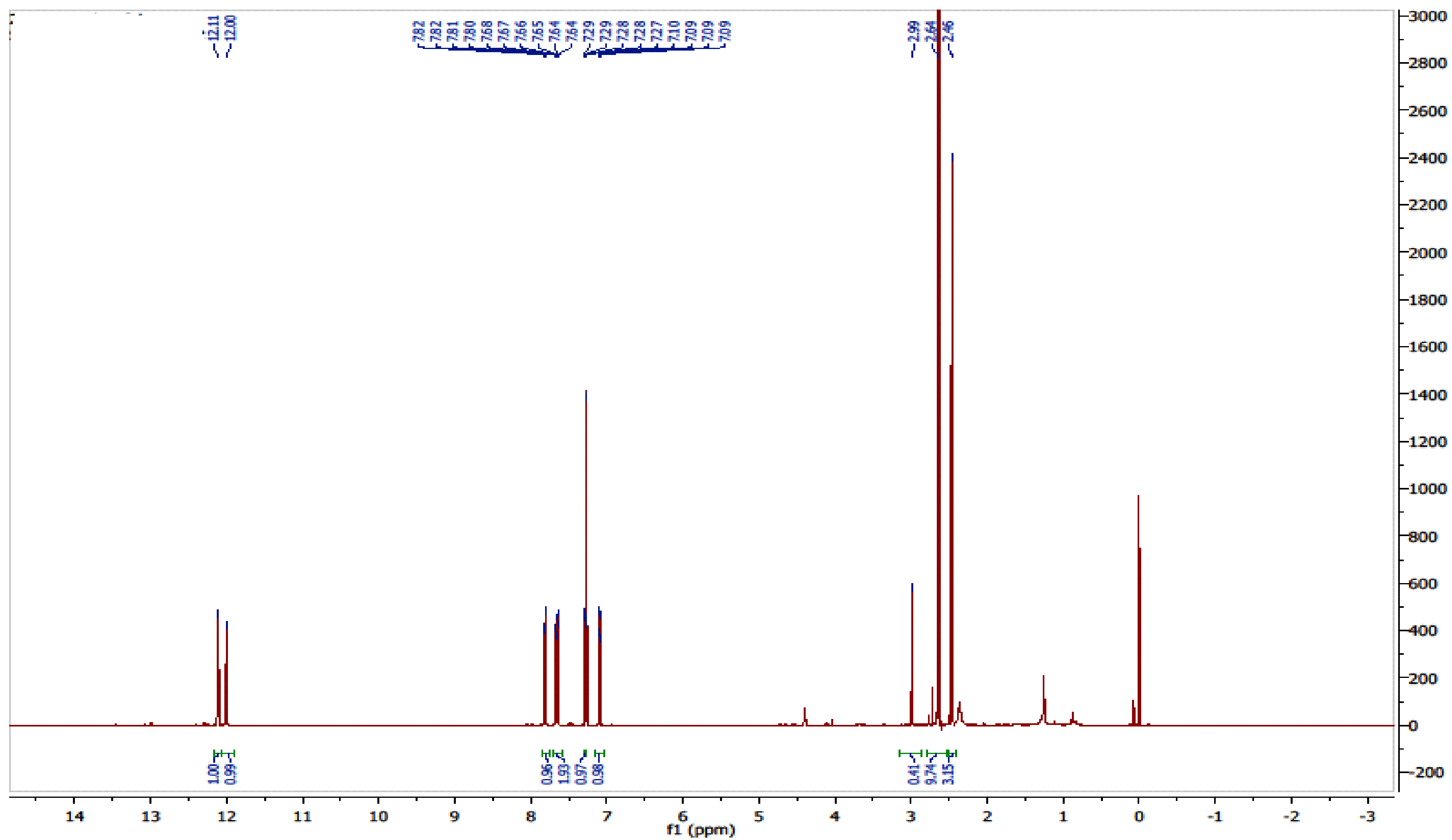
MASS FOR COMPOUND 2



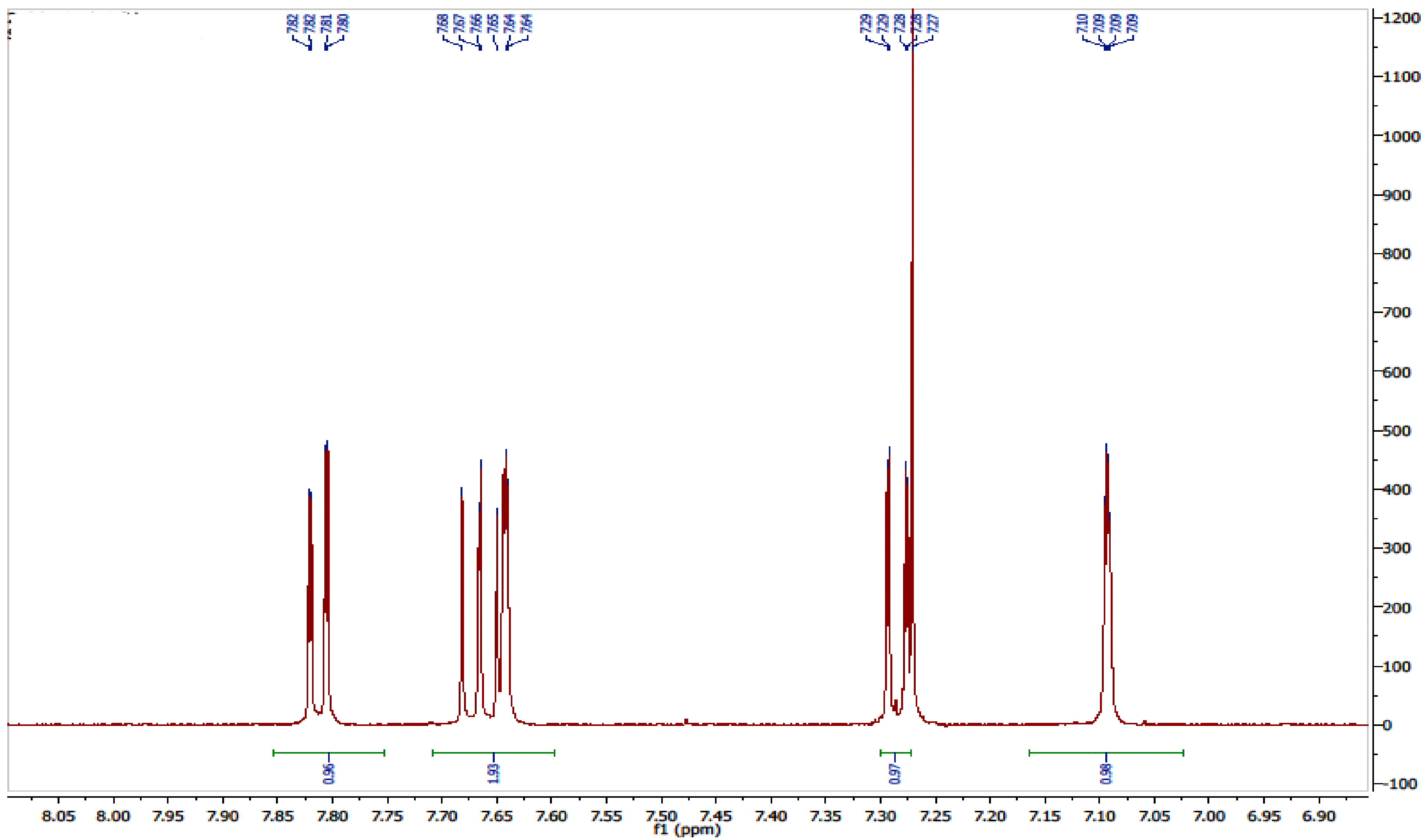
APPENDIX C

SPECTRA FOR COMPOUND 3

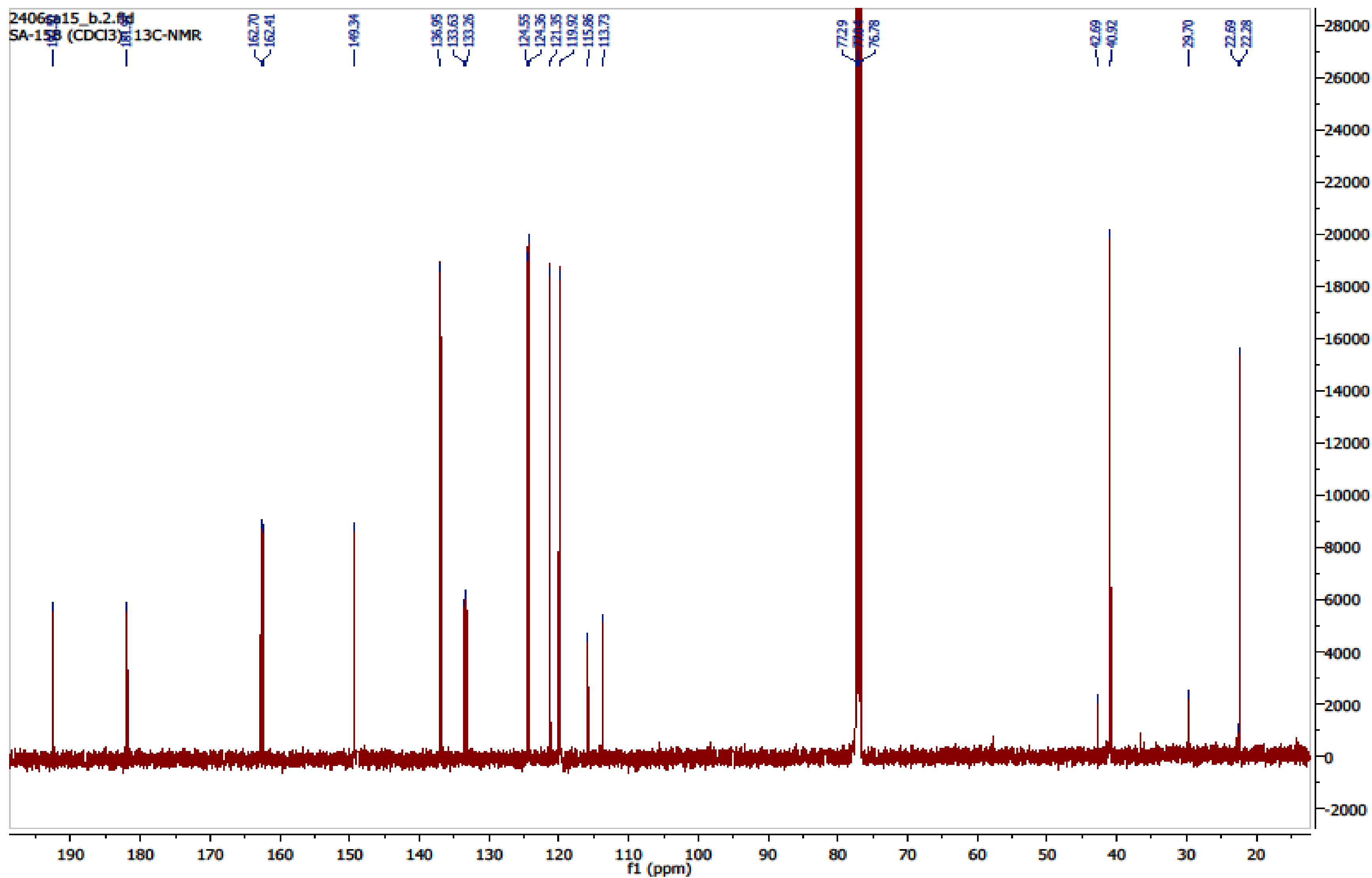
^{1}H NMR SPECTRUM FOR COMPOUND 3 ($CDCl_3$, 500 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 3 ($CDCl_3$, 500 MHz)



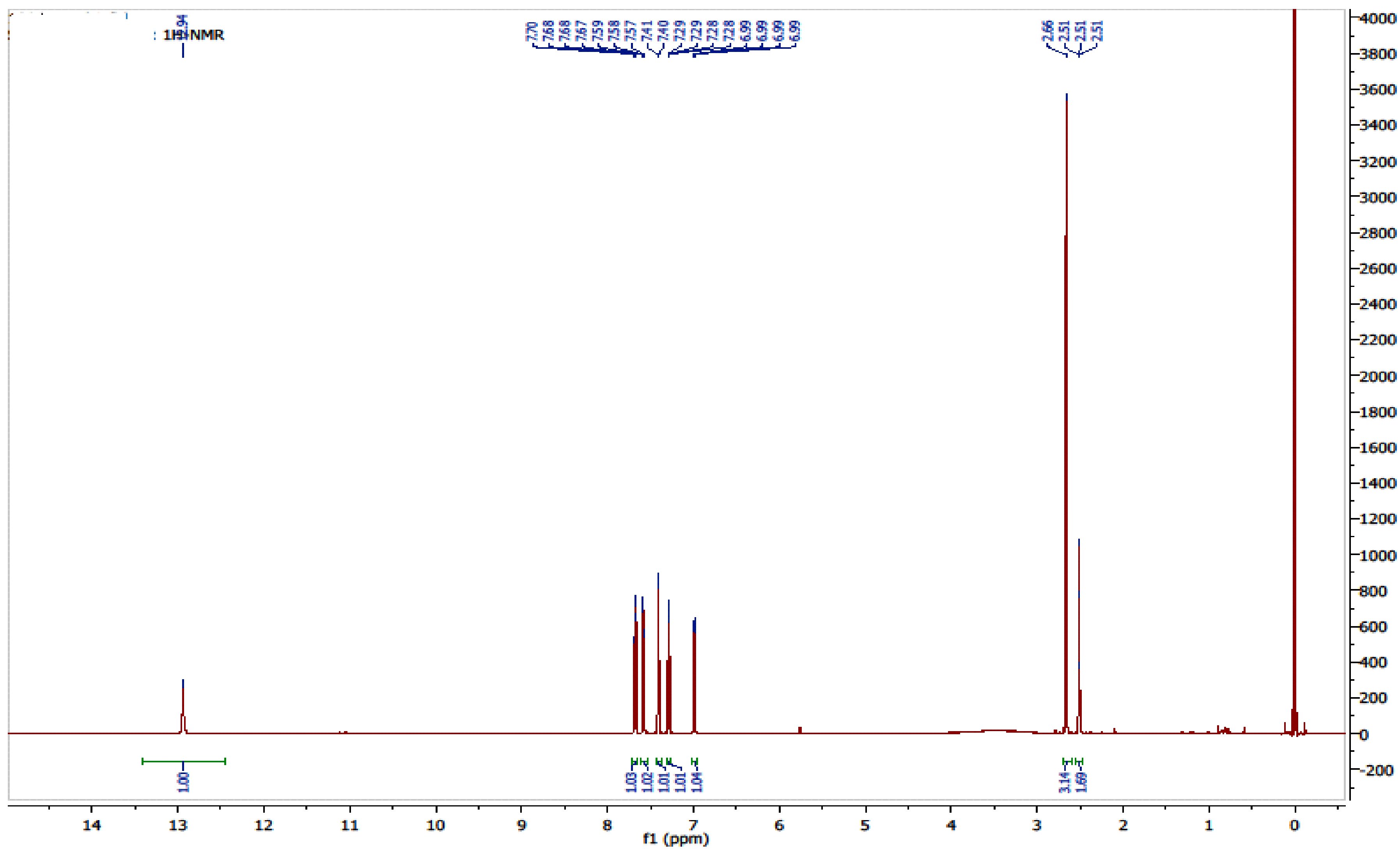
^{13}C NMR SPECTRUM FOR COMPOUND 3 ($CDCl_3$, 125 MHz)



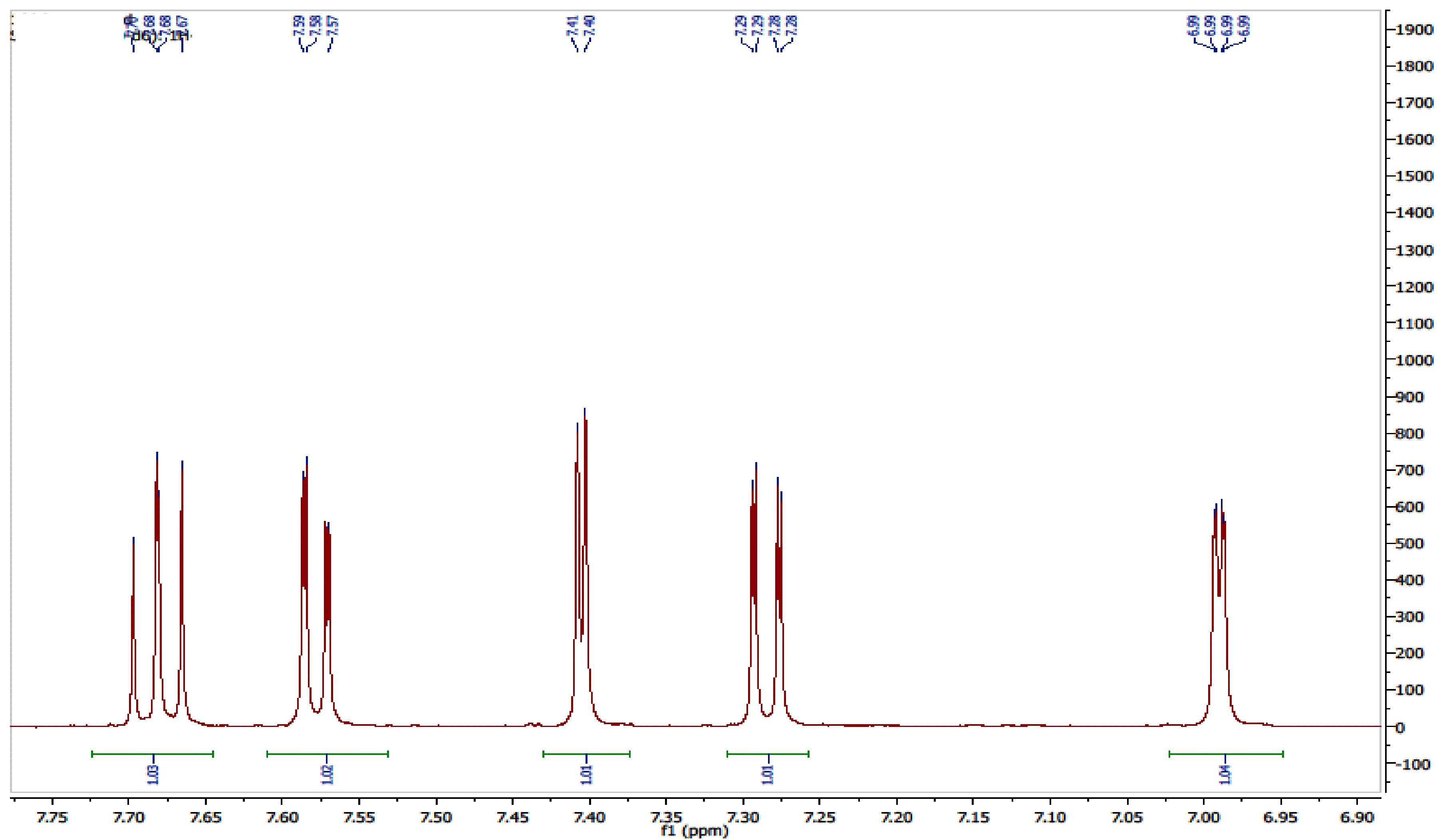
APPENDIX D

SPECTRA FOR COMPOUND 4

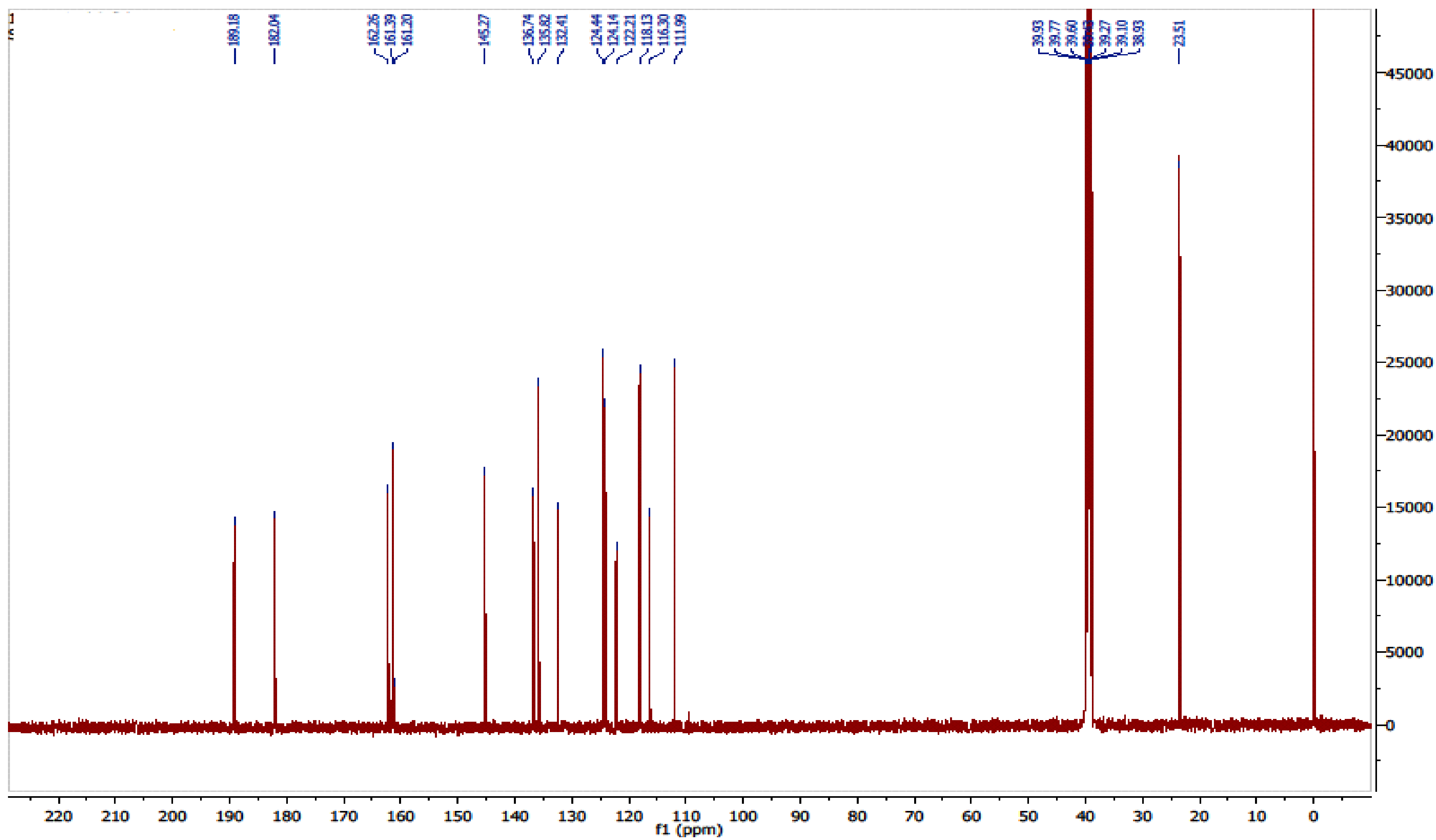
^{1}H NMR SPECTRUM FOR COMPOUND 4 (DMSO, 500 MHz)



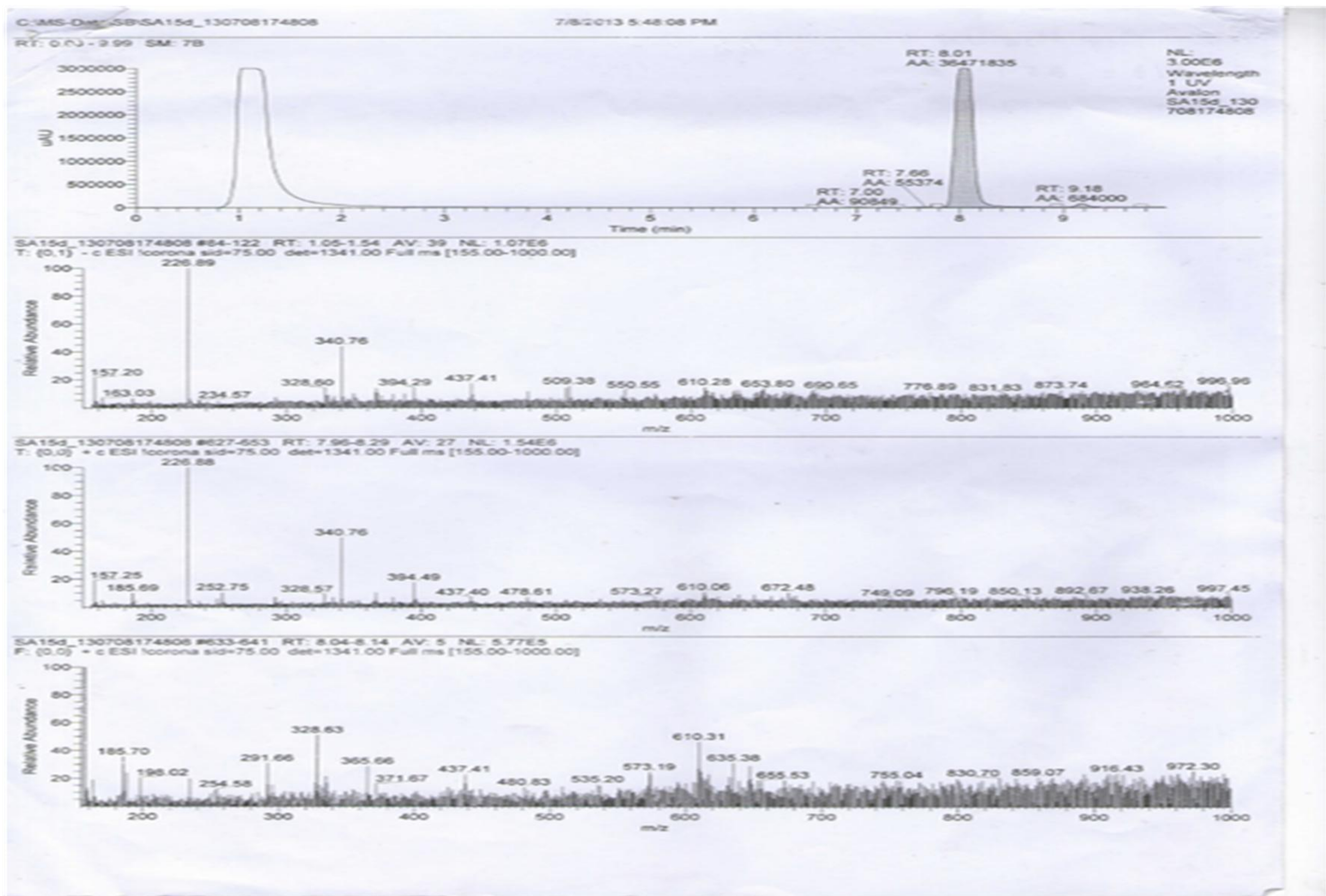
^{1}H NMR SPECTRUM FOR COMPOUND 4 (DMSO, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 4 (DMSO, 125 MHz)



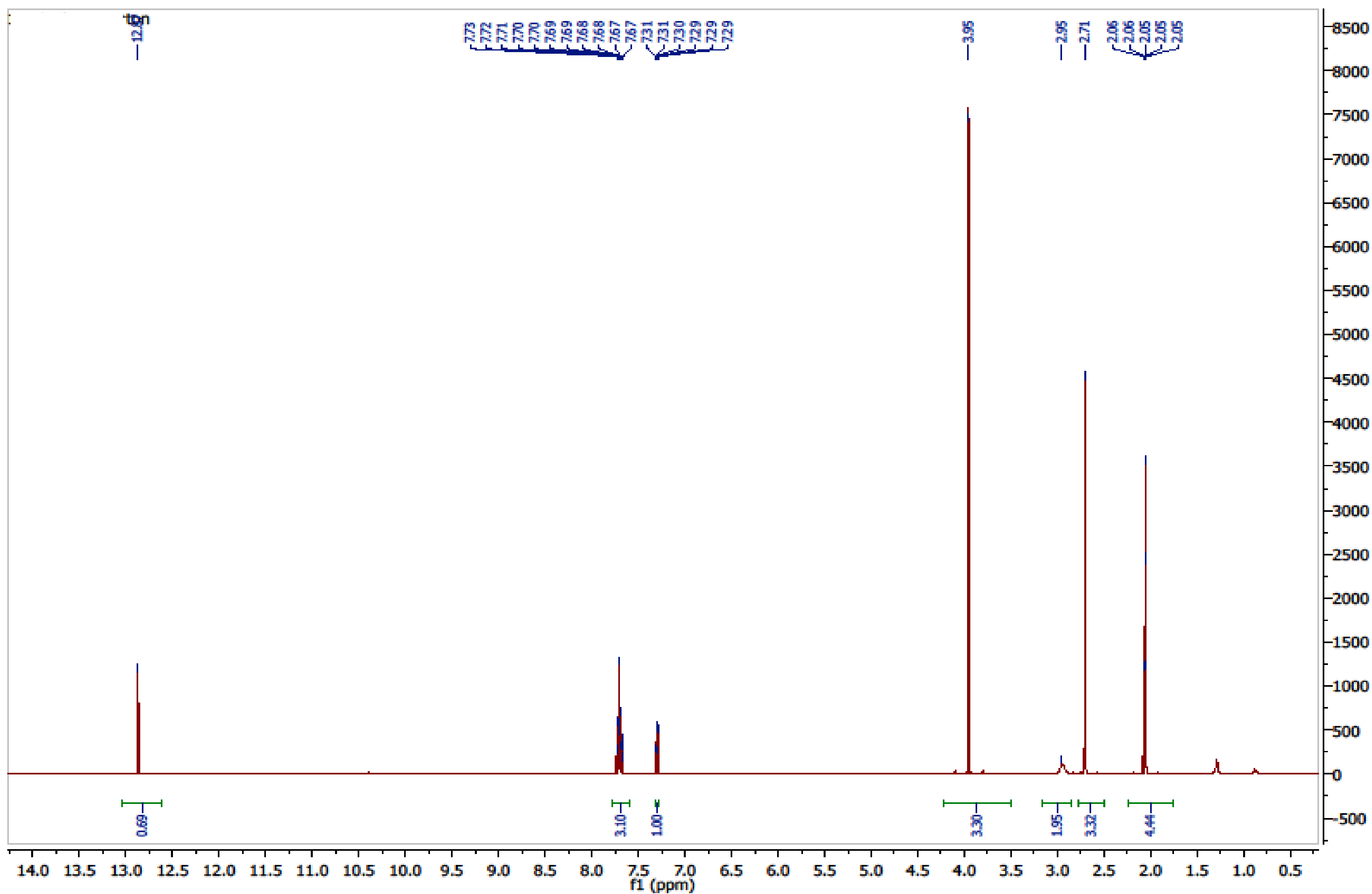
MS FOR COMPOUND 4



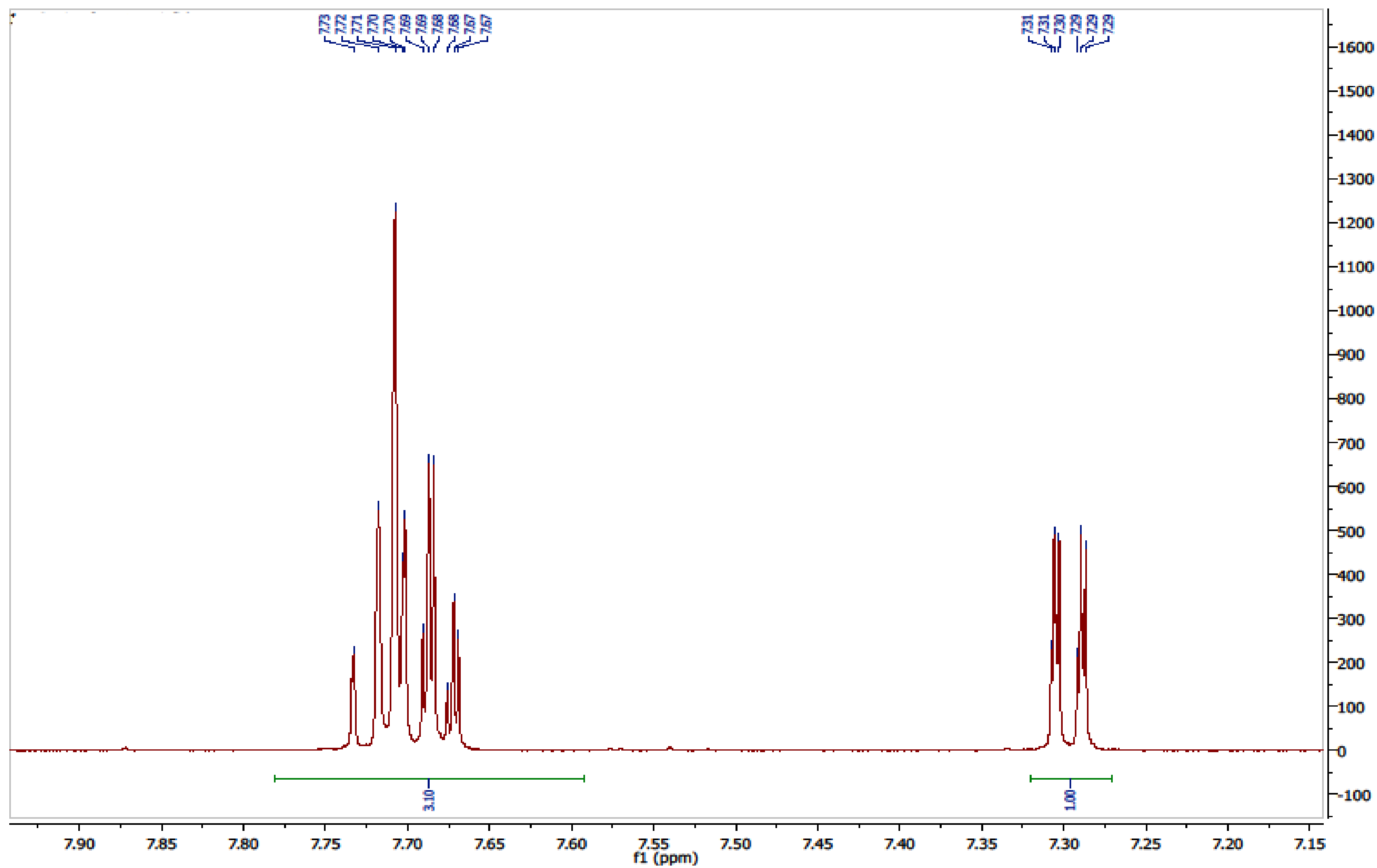
APPENDIX E

SPECTRA FOR COMPOUND 5

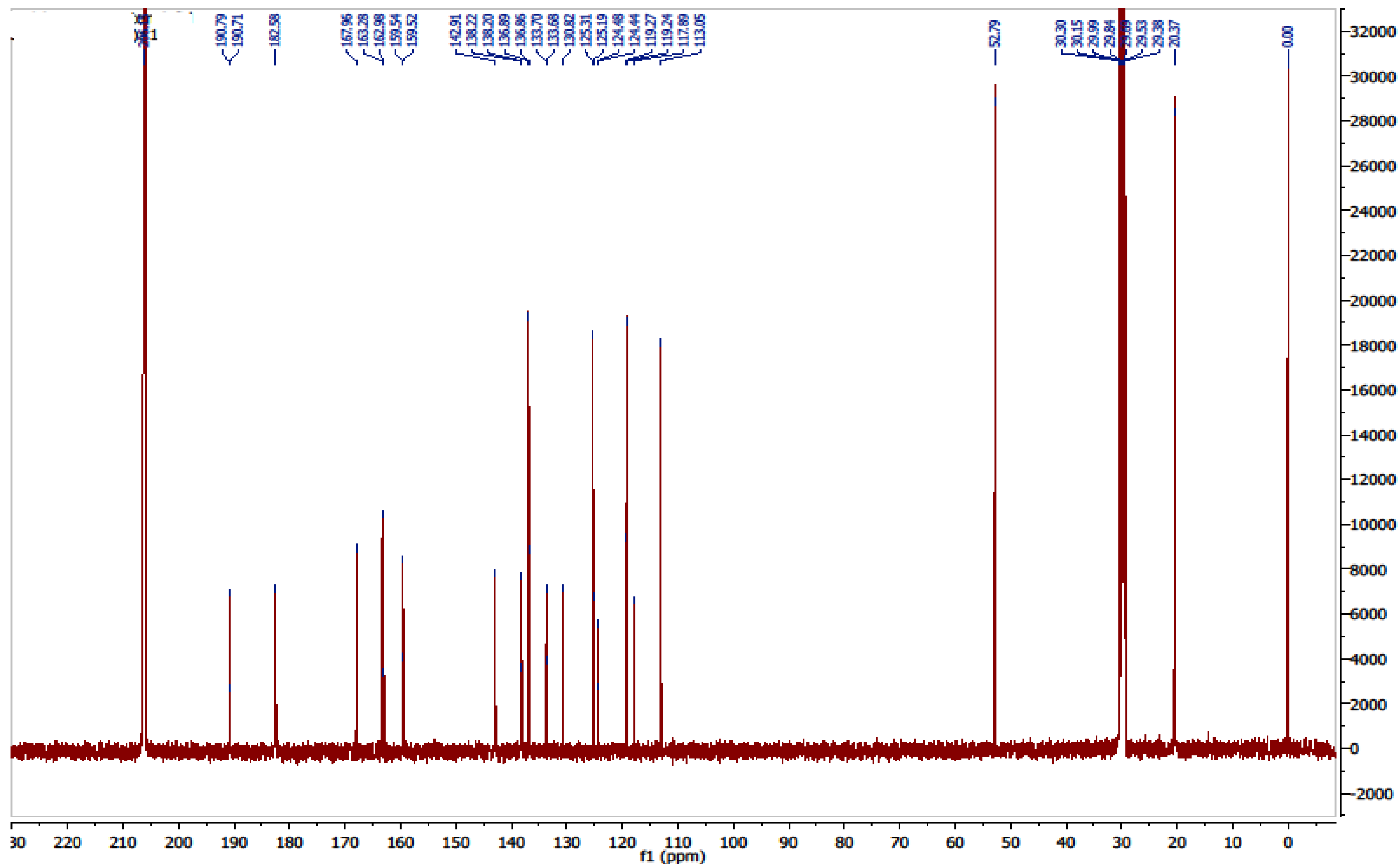
^{1}H NMR SPECTRUM FOR COMPOUND 5 (ACETONE, 500 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 5 (ACETONE, 500 MHz)



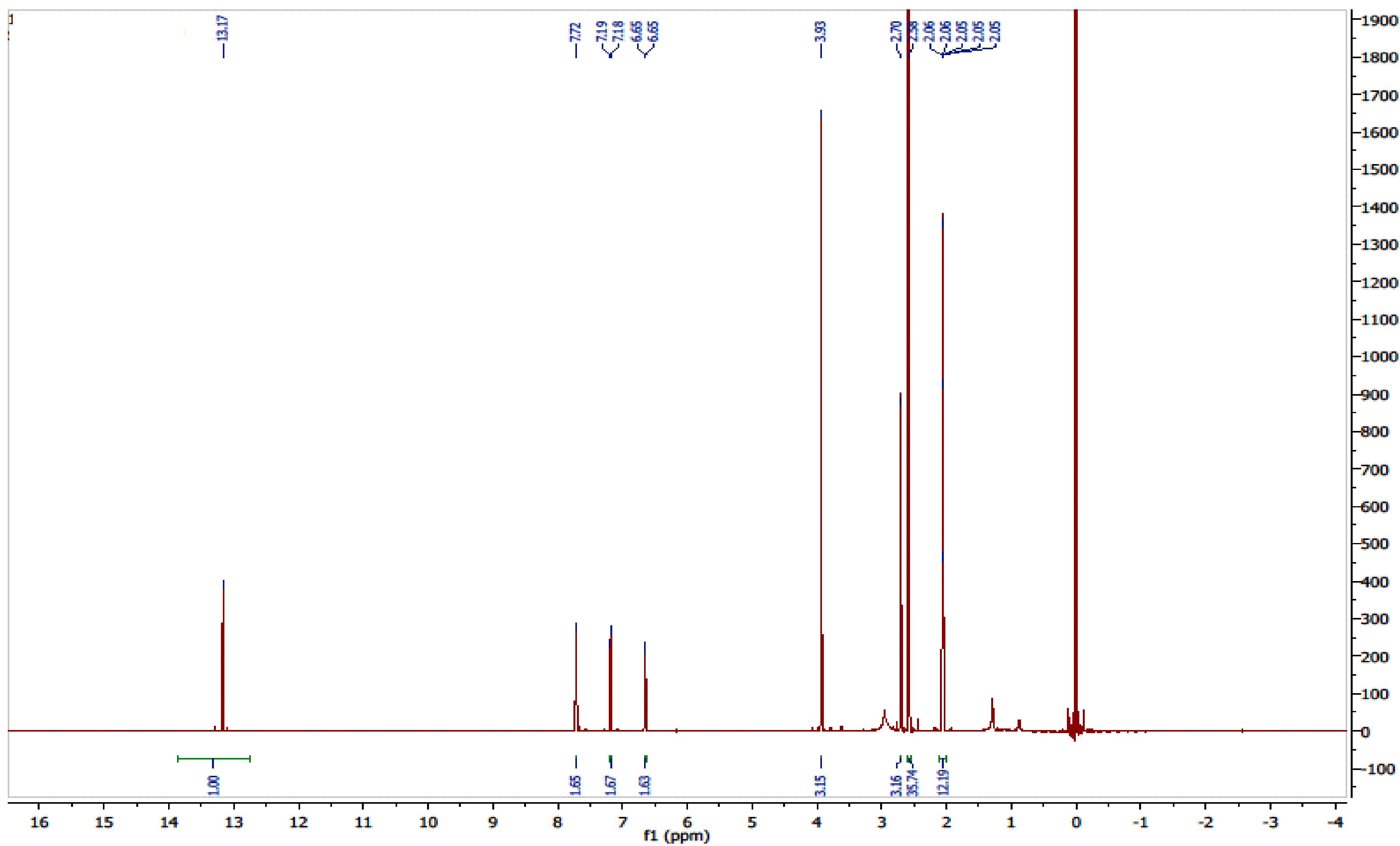
^{13}C NMR SPECTRUM FOR COMPOUND 5 (ACETONE, 125 MHz)



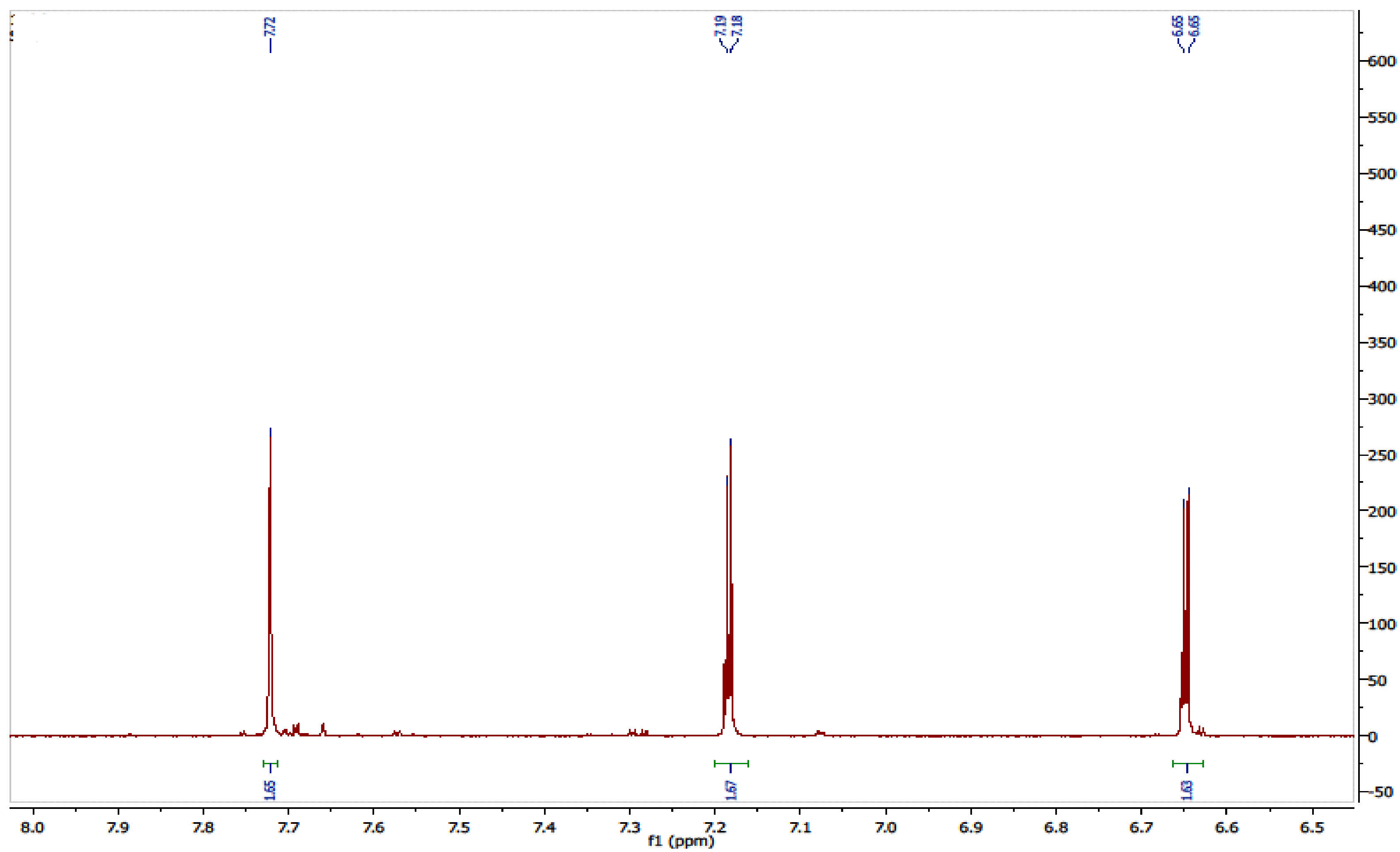
APPENDIX F

SPECTRA FOR COMPOUND 6

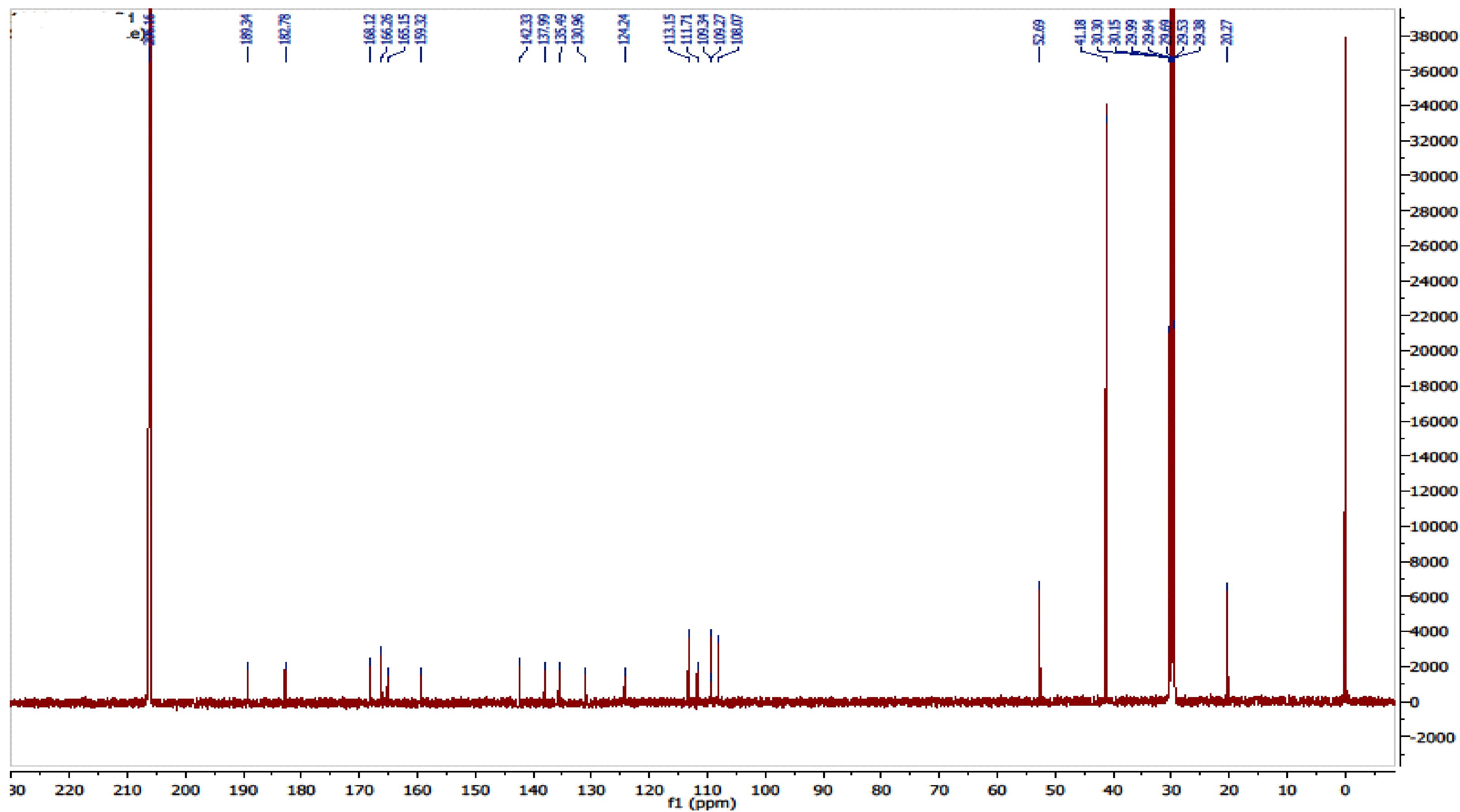
^{1}H NMR SPECTRUM FOR COMPOUND 6 (ACETONE, 500 MHz)



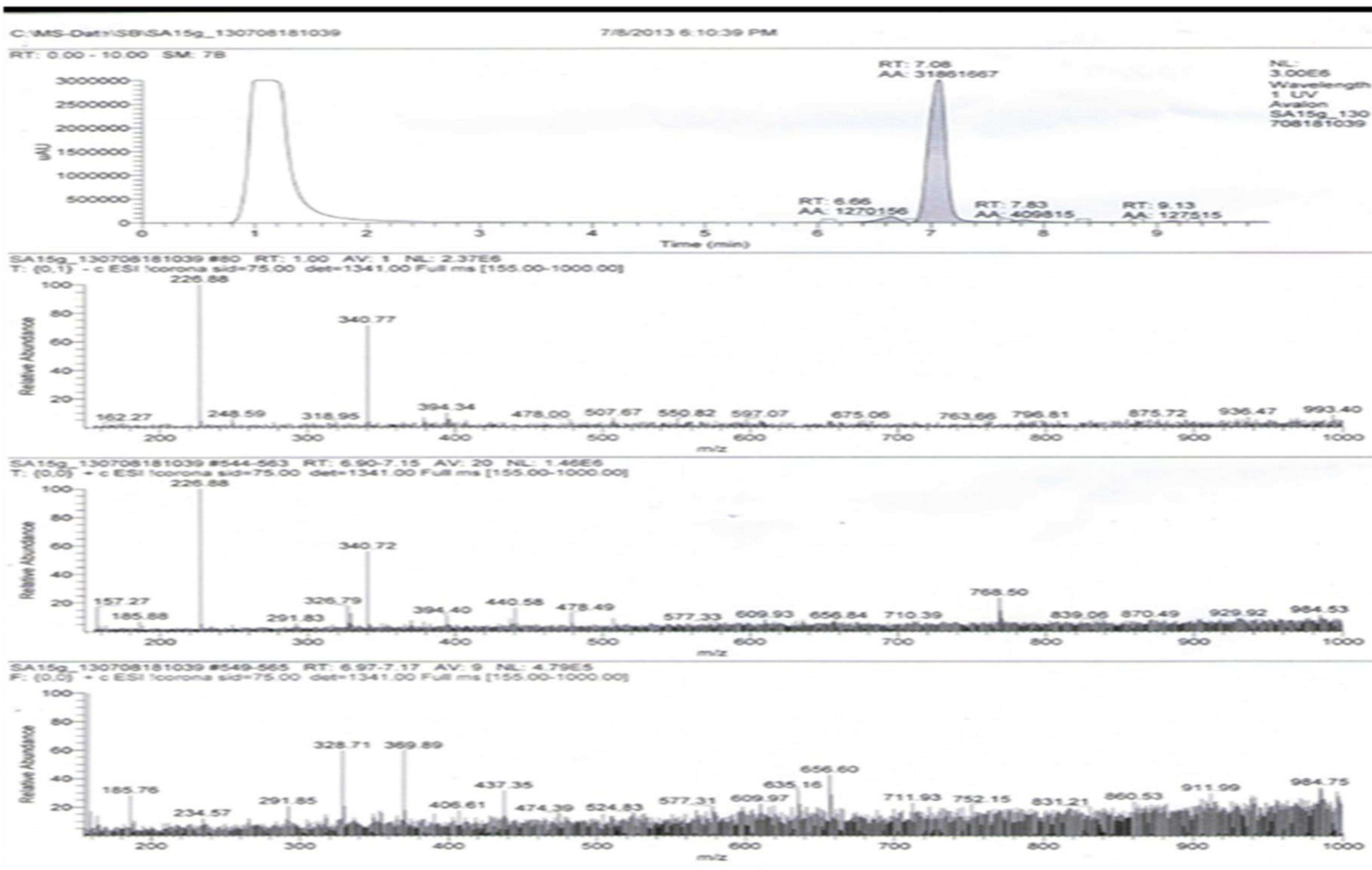
^{1}H NMR SPECTRUM FOR COMPOUND 6 (ACETONE, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 6 (ACETONE, 125 MHz)



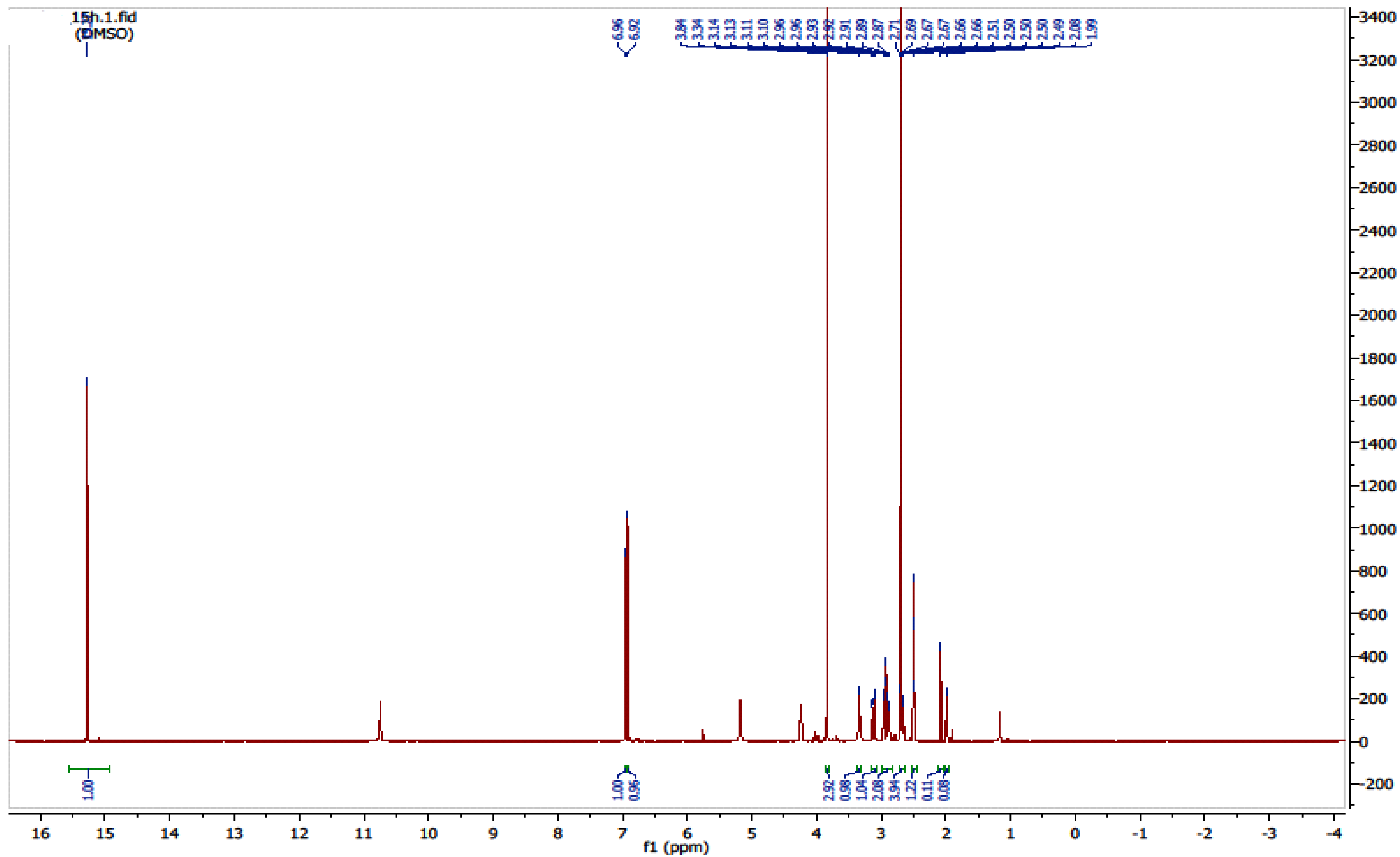
MASS FOR COMPOUND 6



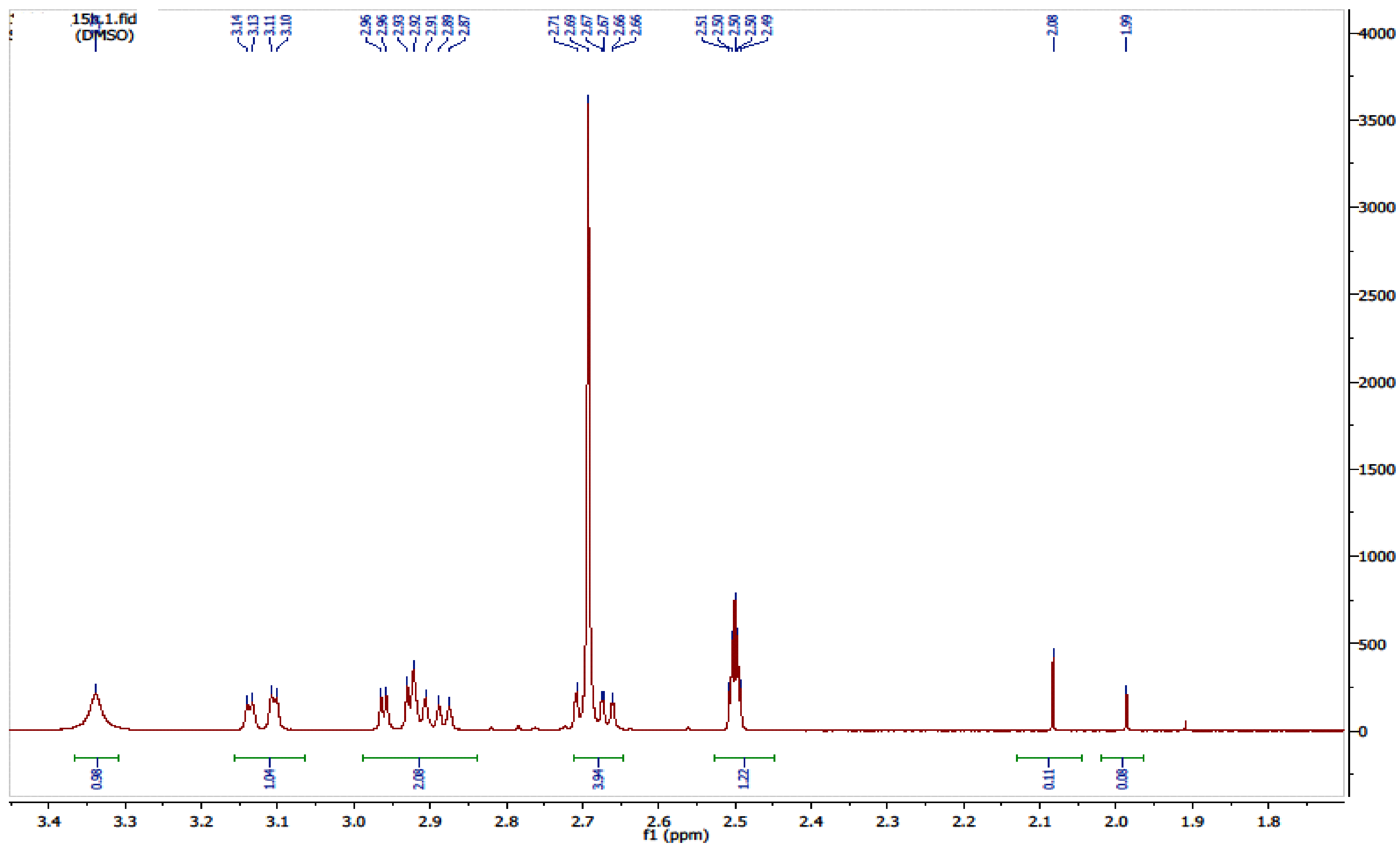
APPENDIX G

SPECTRA FOR COMPOUND 7

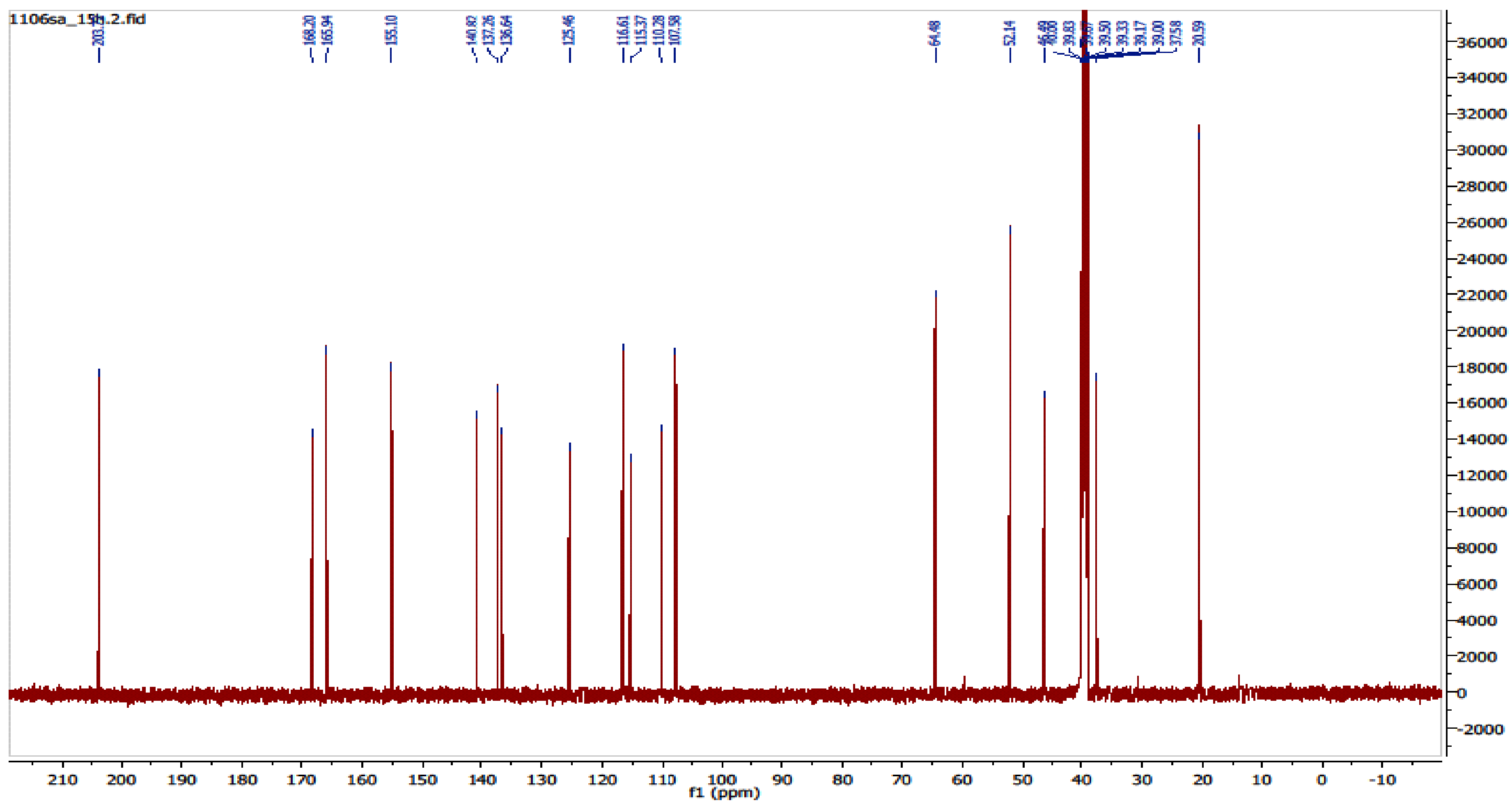
^{1}H NMR SPECTRUM FOR COMPOUND 7 (DMSO, 500 MHz)



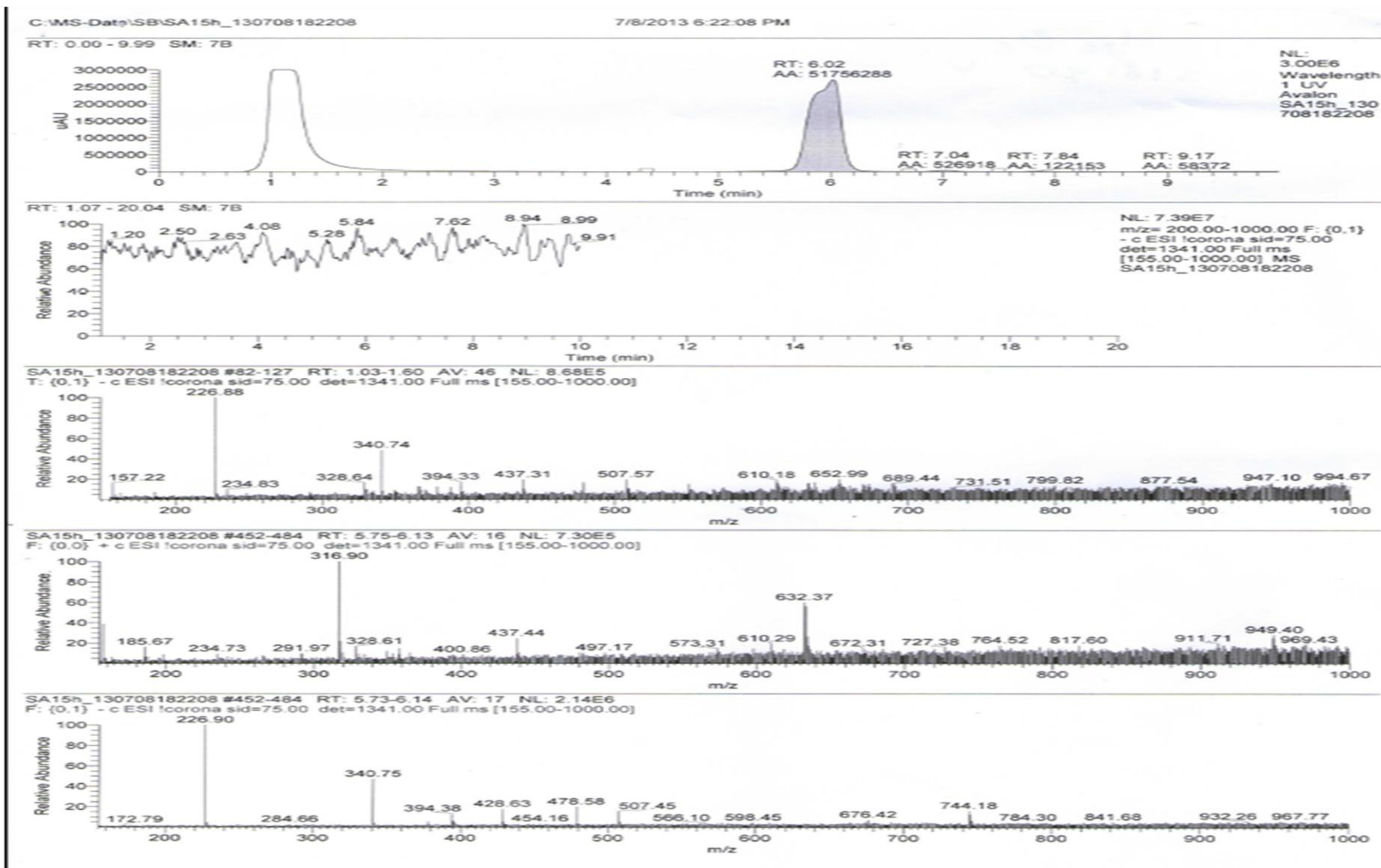
^{1}H NMR SPECTRUM FOR COMPOUND 7 (DMSO, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 7 (DMSO, 125 MHz)



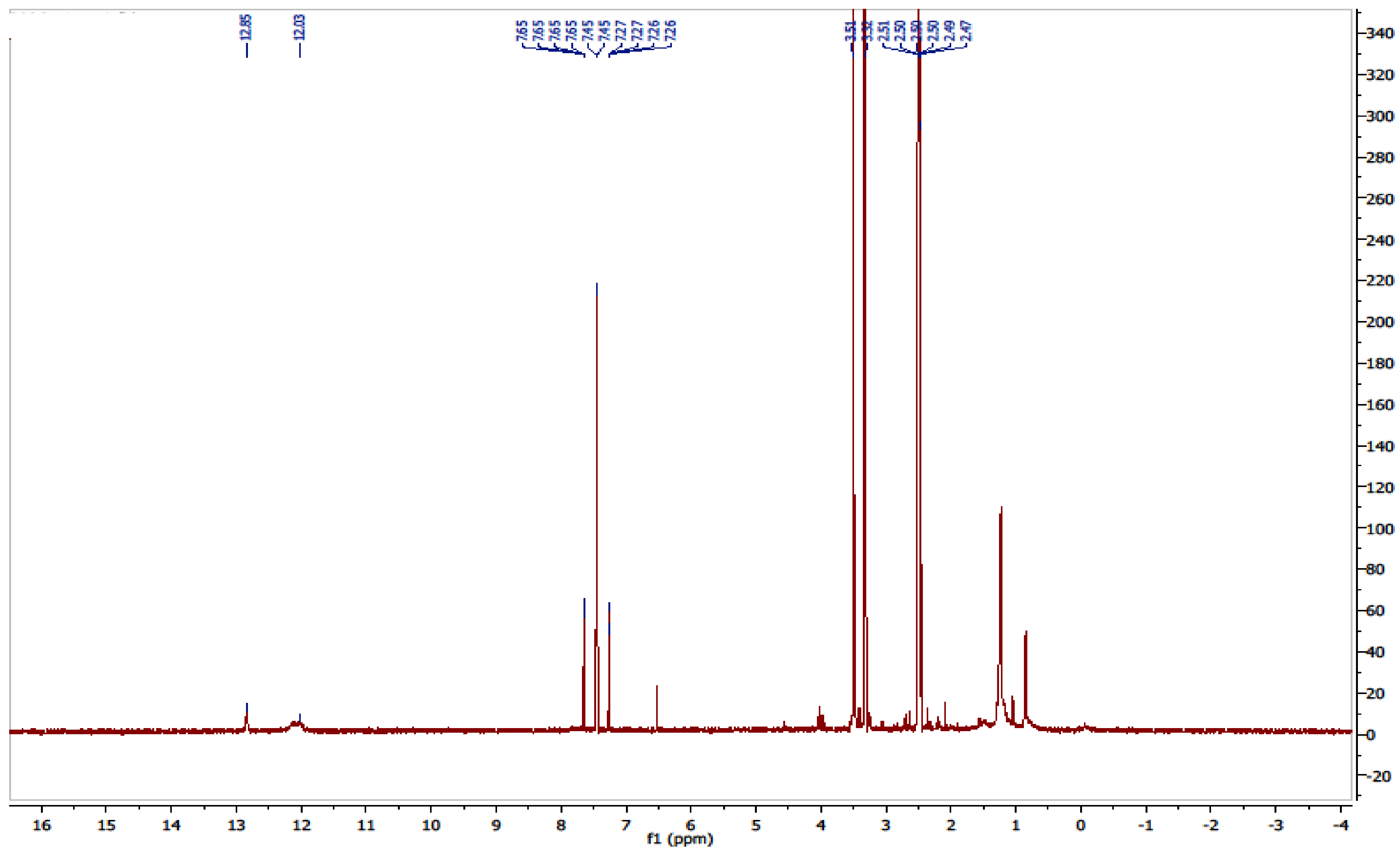
MASS FOR COMPOUND 7



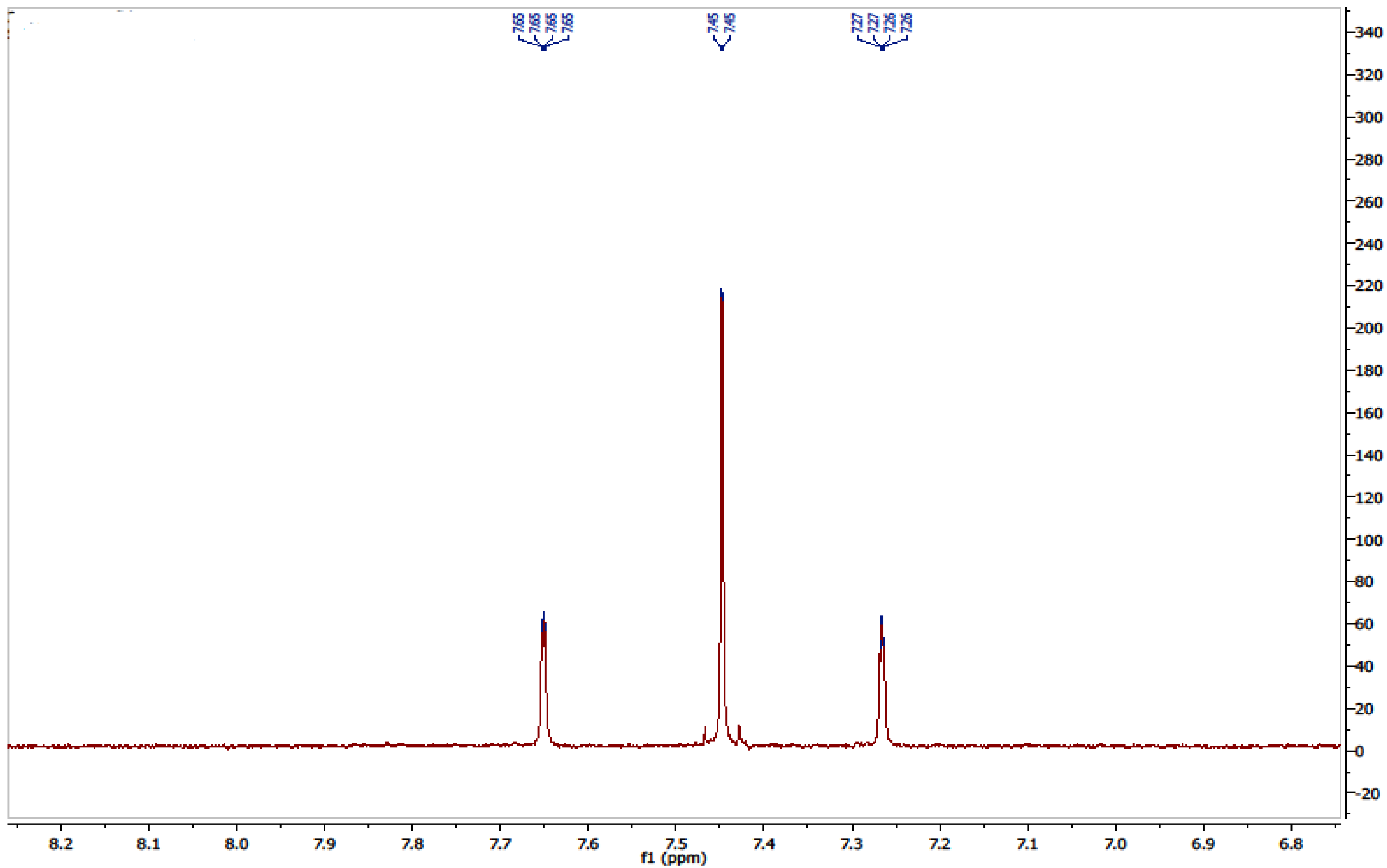
APPENDIX H

SPECTRA FOR COMPOUND 8

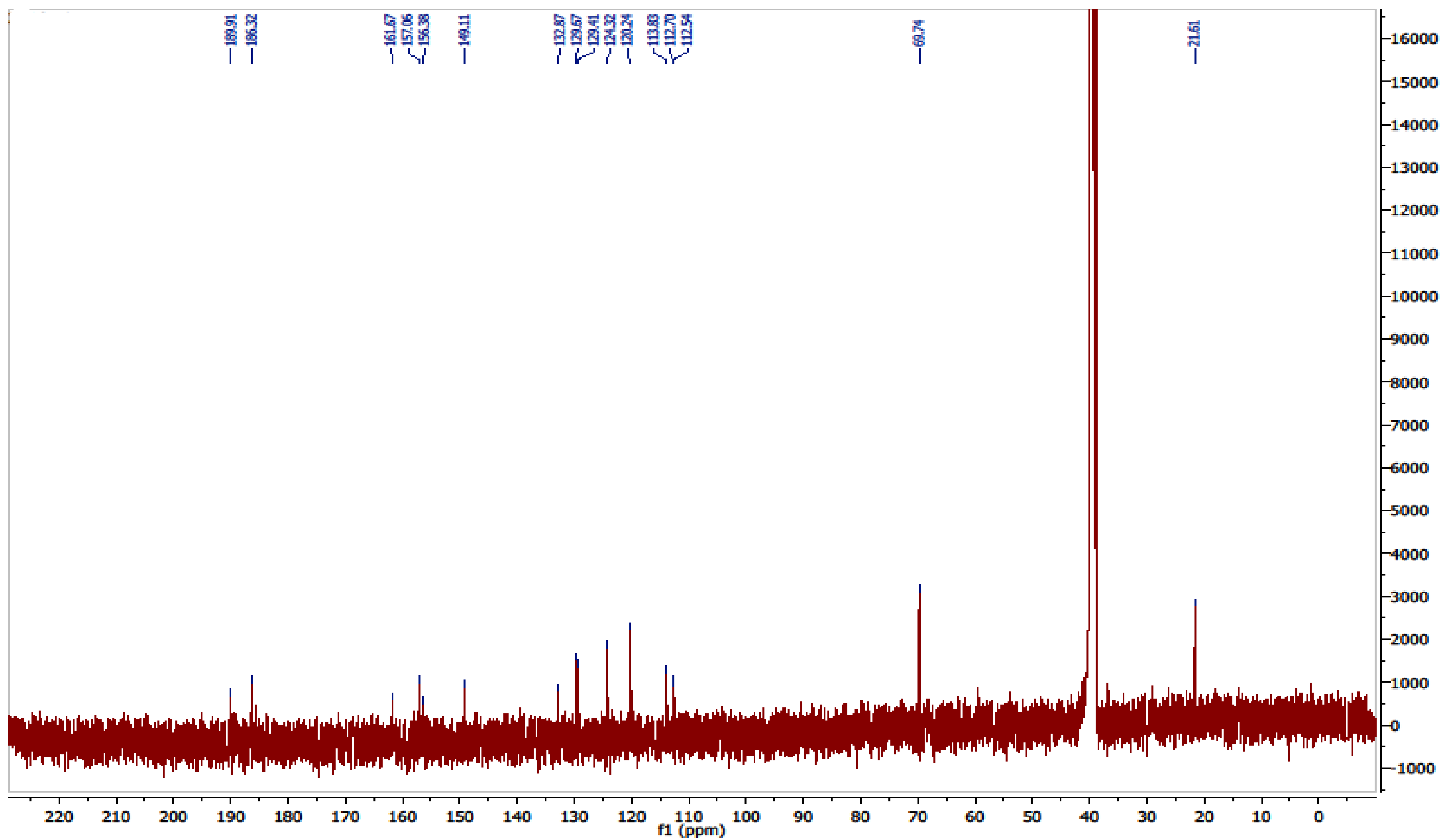
^{1}H NMR SPECTRUM FOR COMPOUND 8 (DMSO, 500 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 8 (DMSO, 500 MHz)



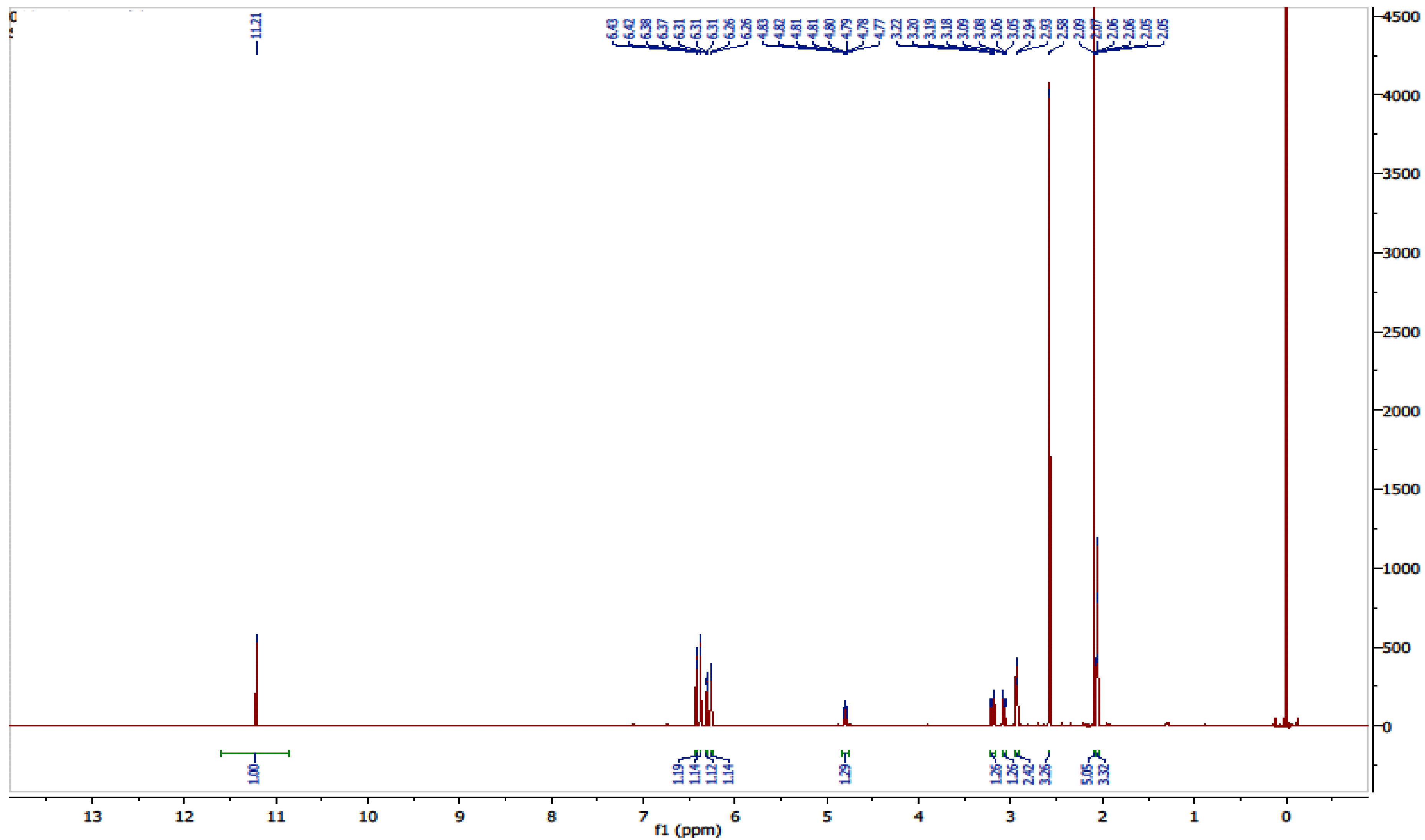
^{13}C NMR SPECTRUM FOR COMPOUND 8 (DMSO, 125 MHz)



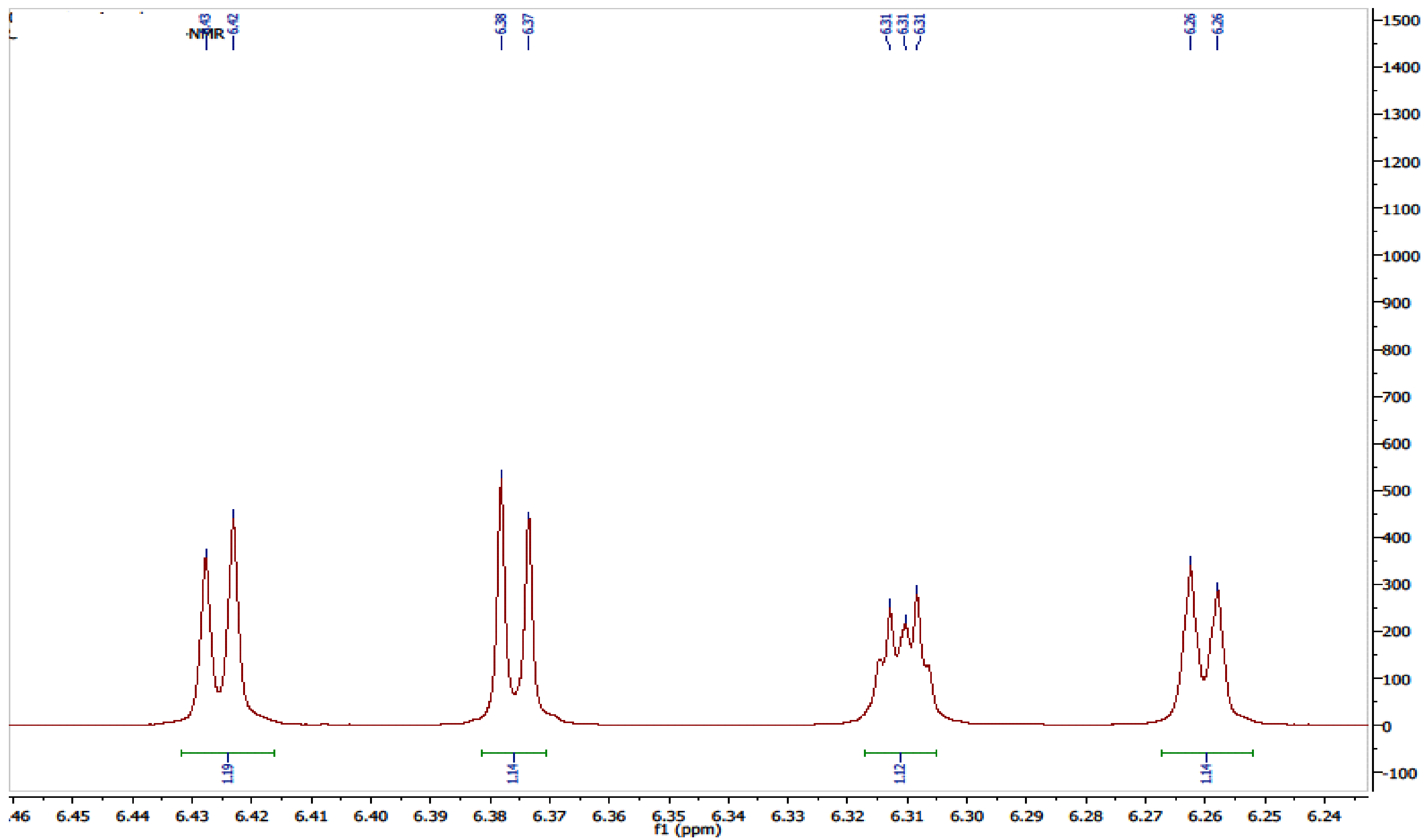
APPENDIX I

SPECTRA FOR COMPOUND 9

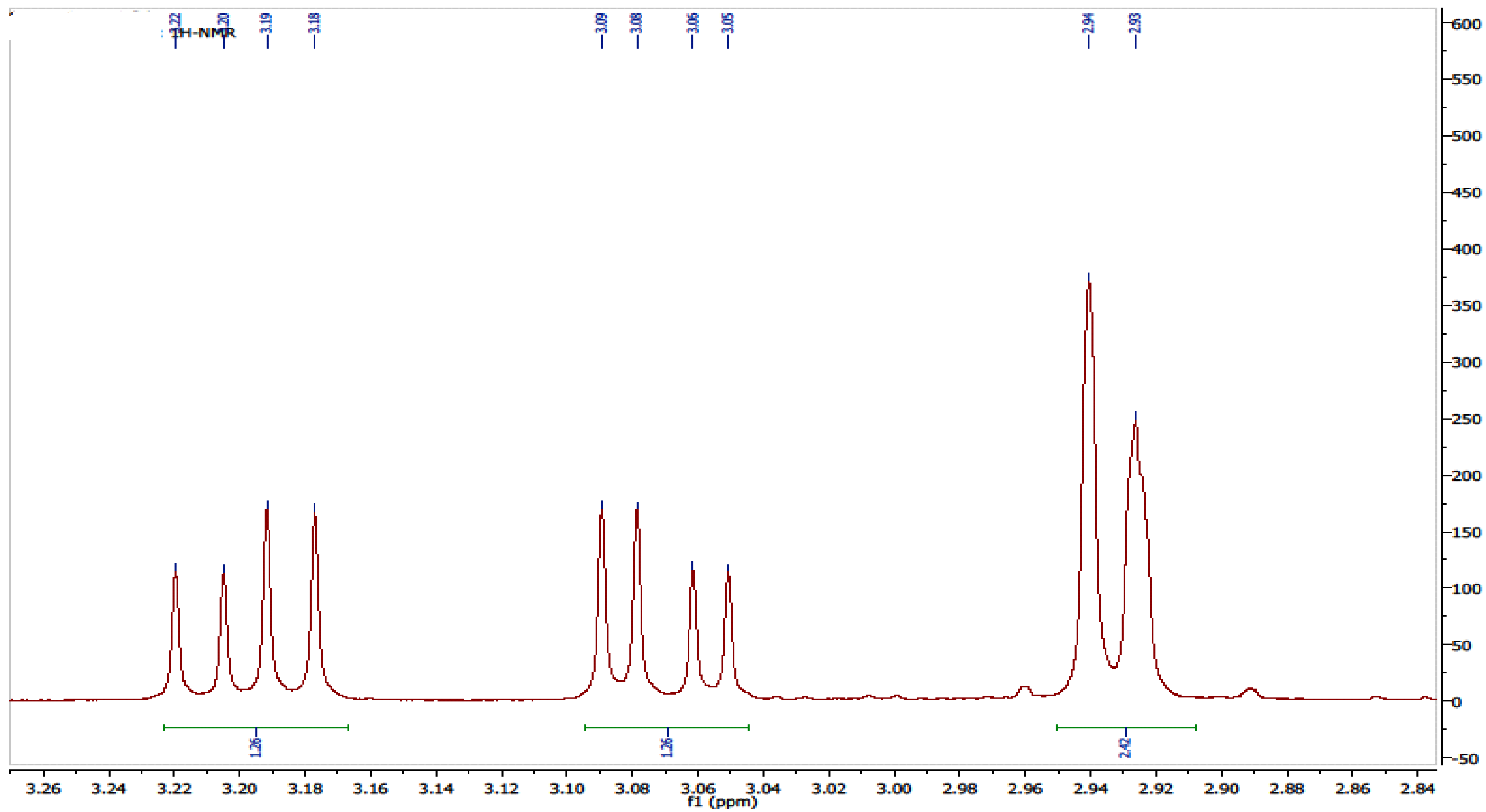
^1H NMR SPECTRUM FOR COMPOUND 9 (ACETONE, 500 MHz)



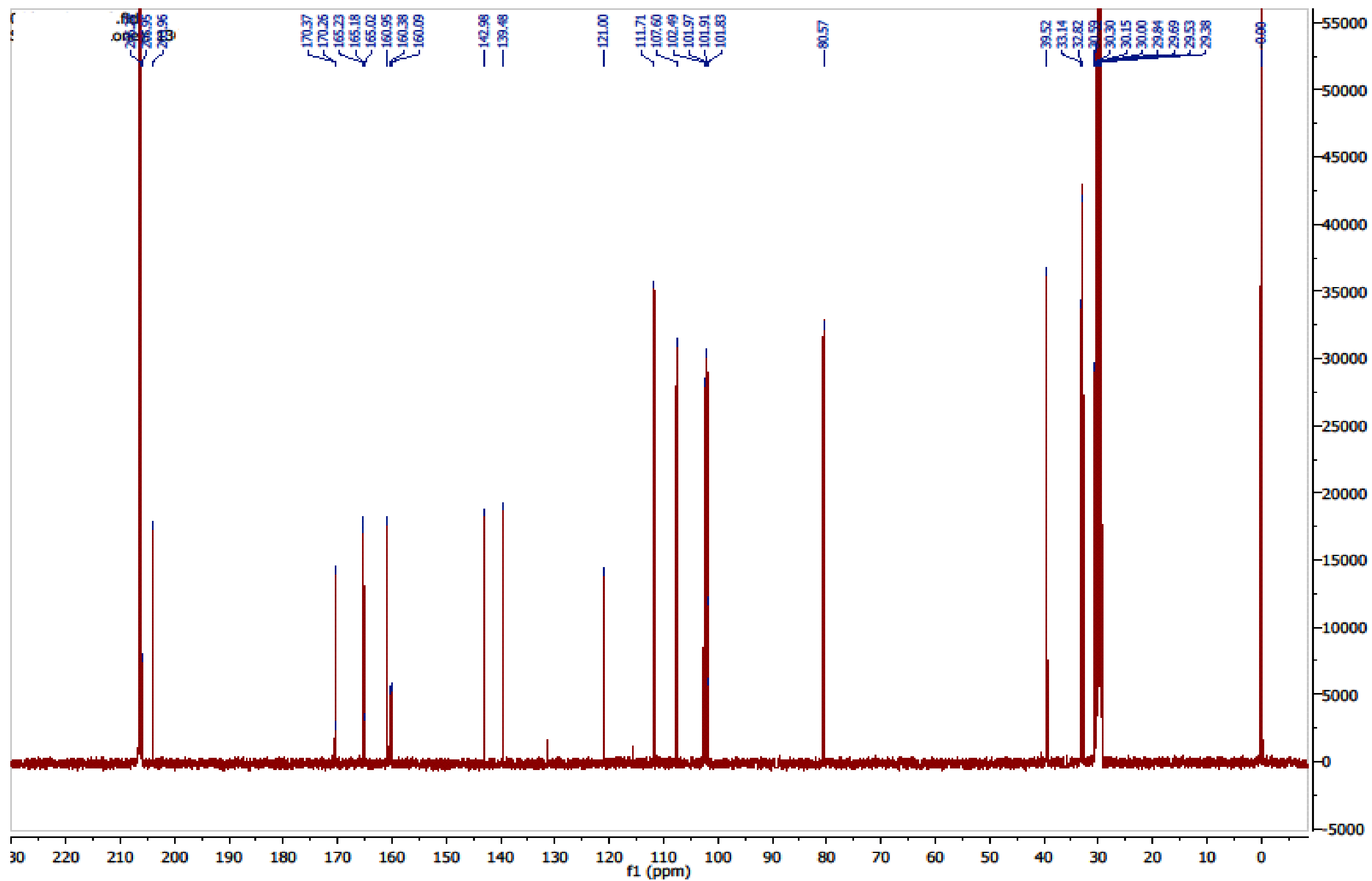
^{1}H NMR SPECTRUM FOR COMPOUND 9 (ACETONE, 500 MHz)



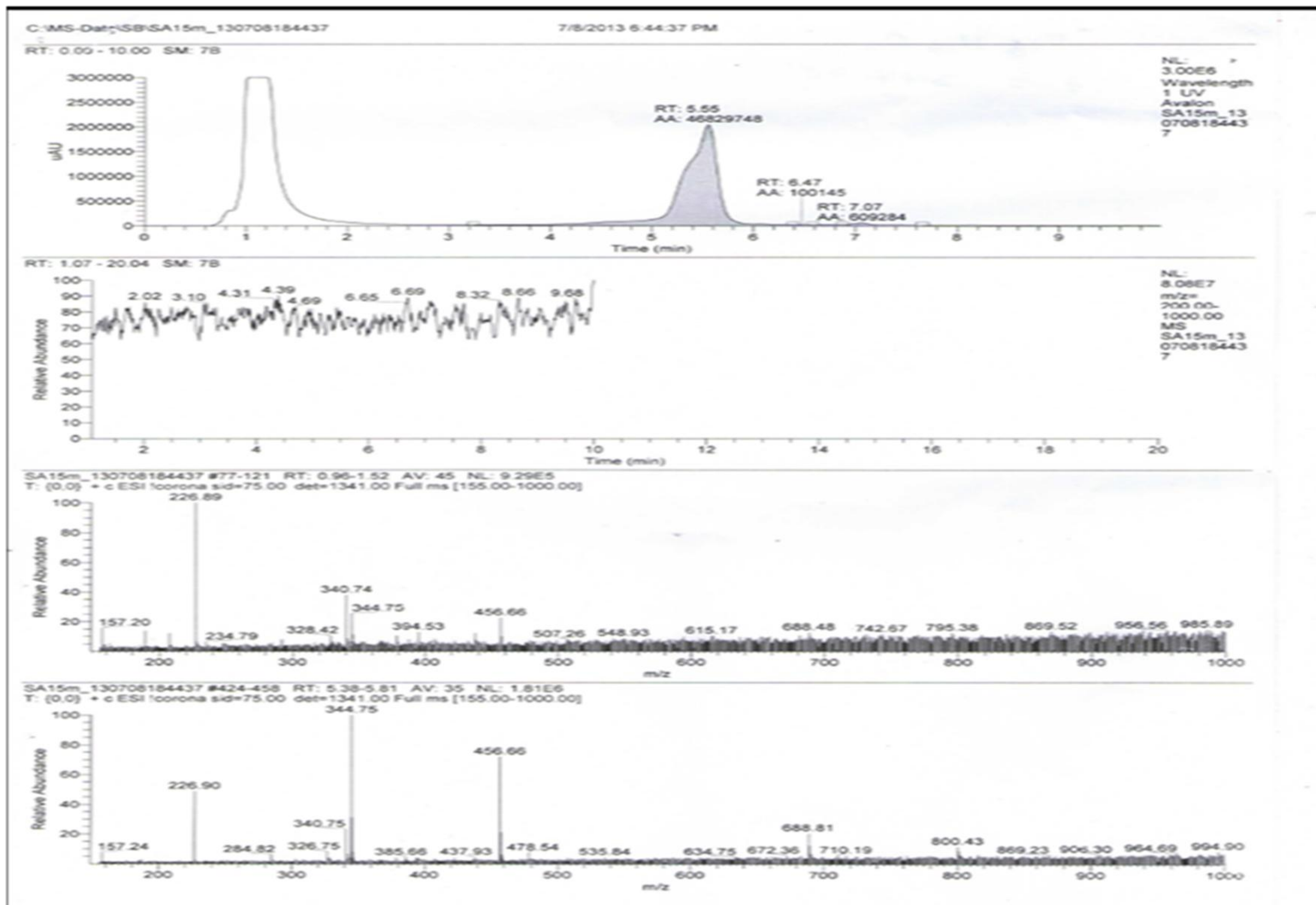
^{1}H NMR SPECTRUM FOR COMPOUND 9 (ACETONE, 500 MHz)



^{13}C SPECTRUM FOR COMPOUND 9 (ACETONE, 125 MHz)



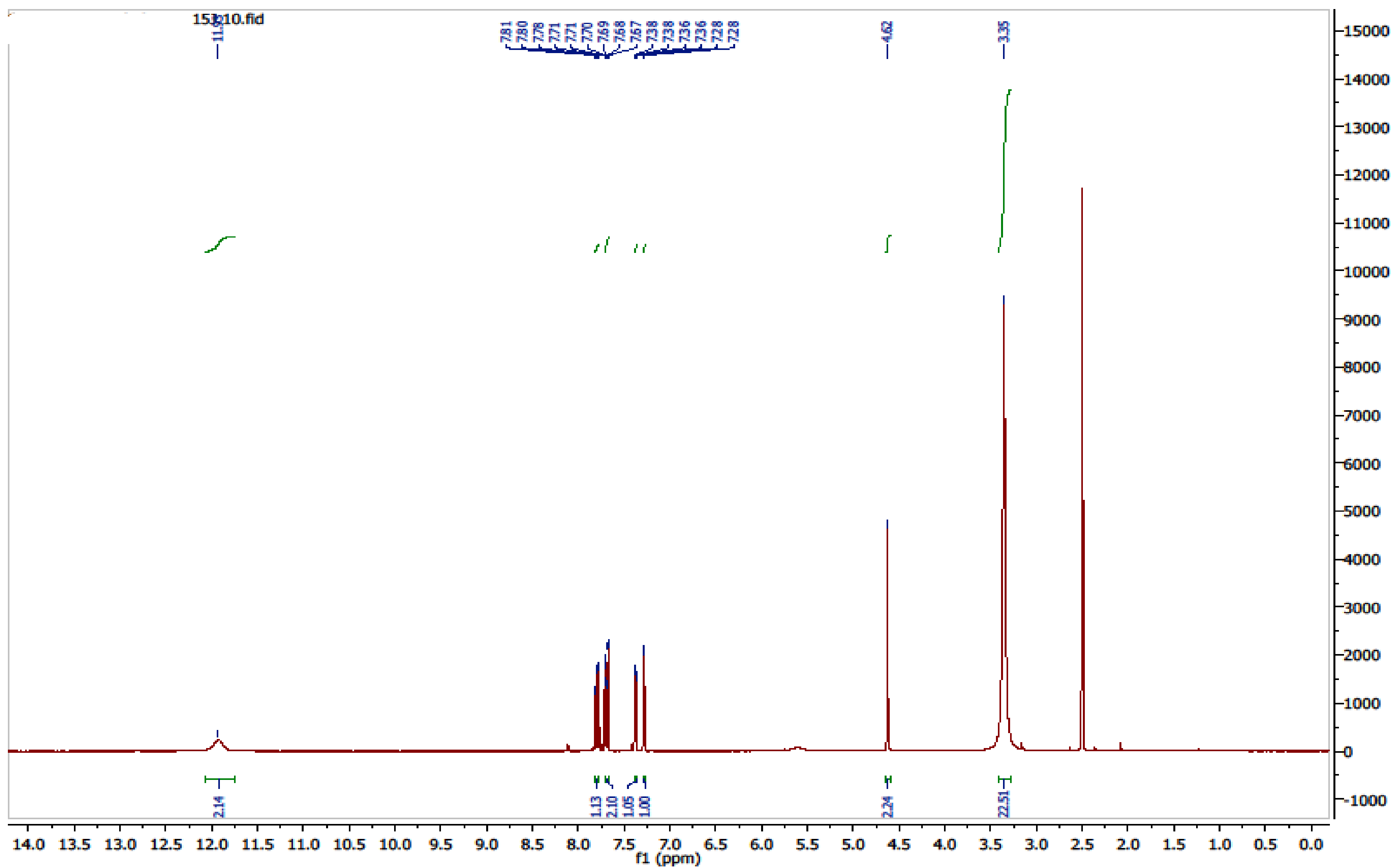
MASS FOR COMPOUND 9



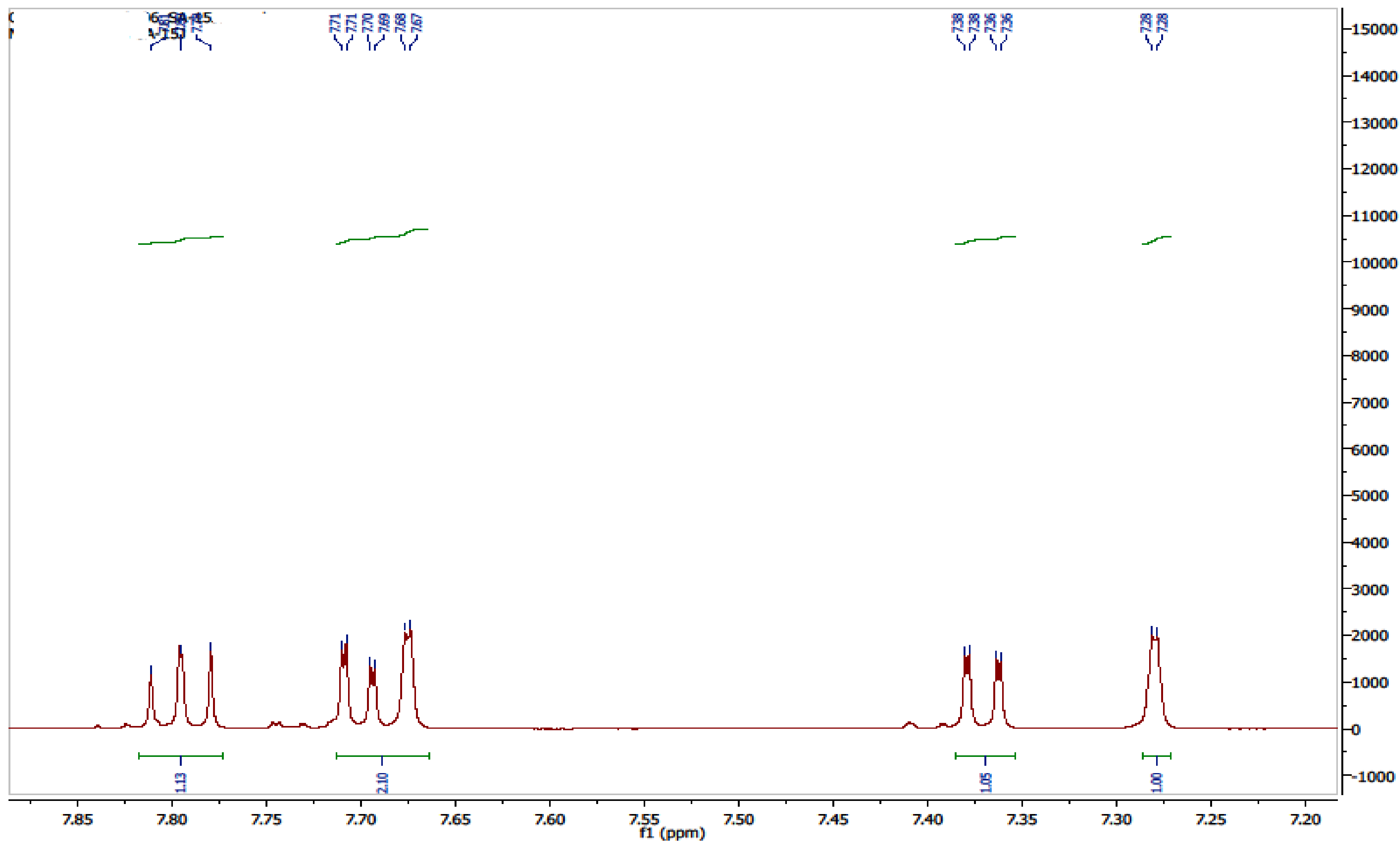
APPENDIX J

SPECTRA FOR COMPOUND 10

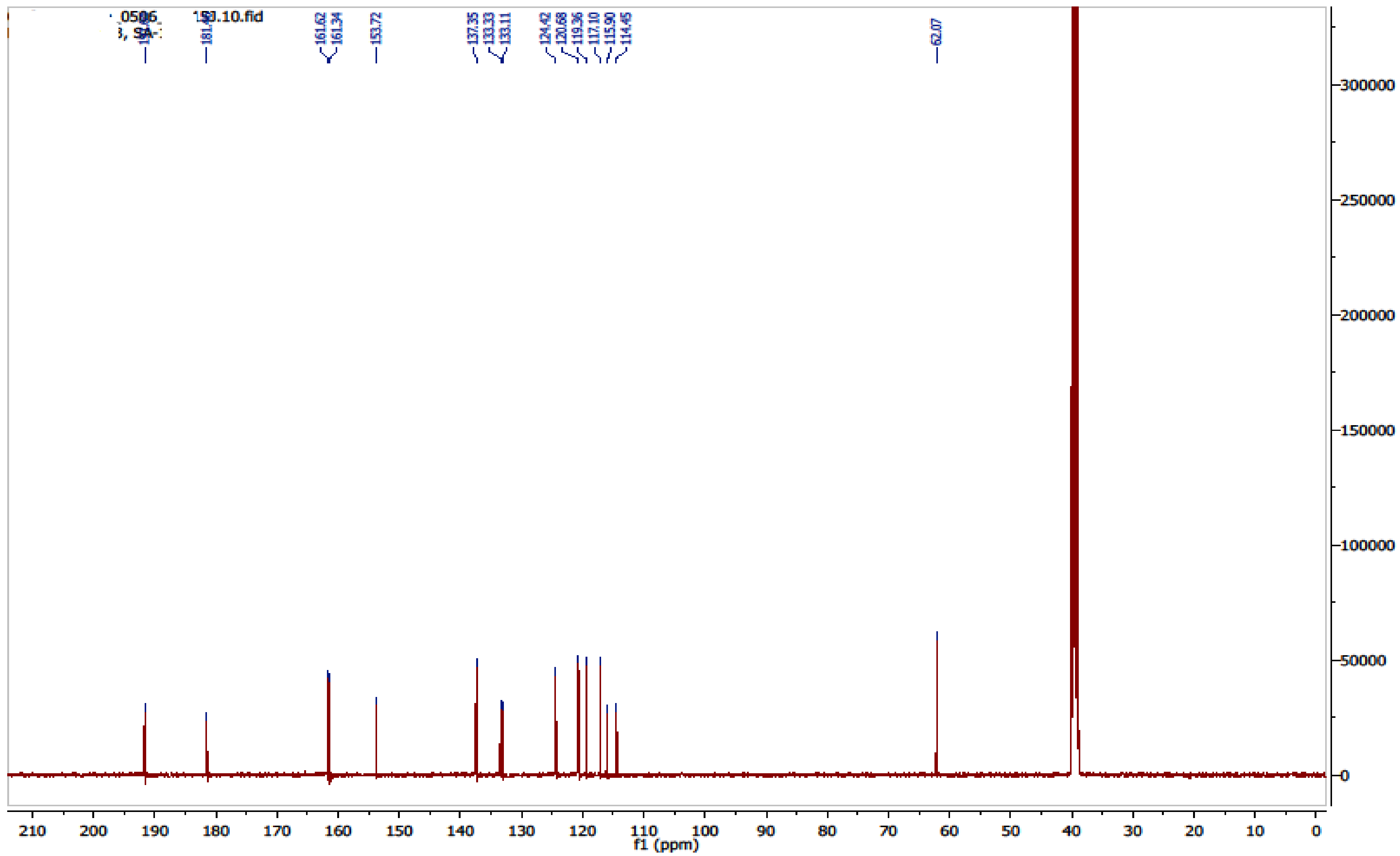
^{1}H NMR SPECTRUM FOR COMPOUND 10 (DMSO, 500 MHz)



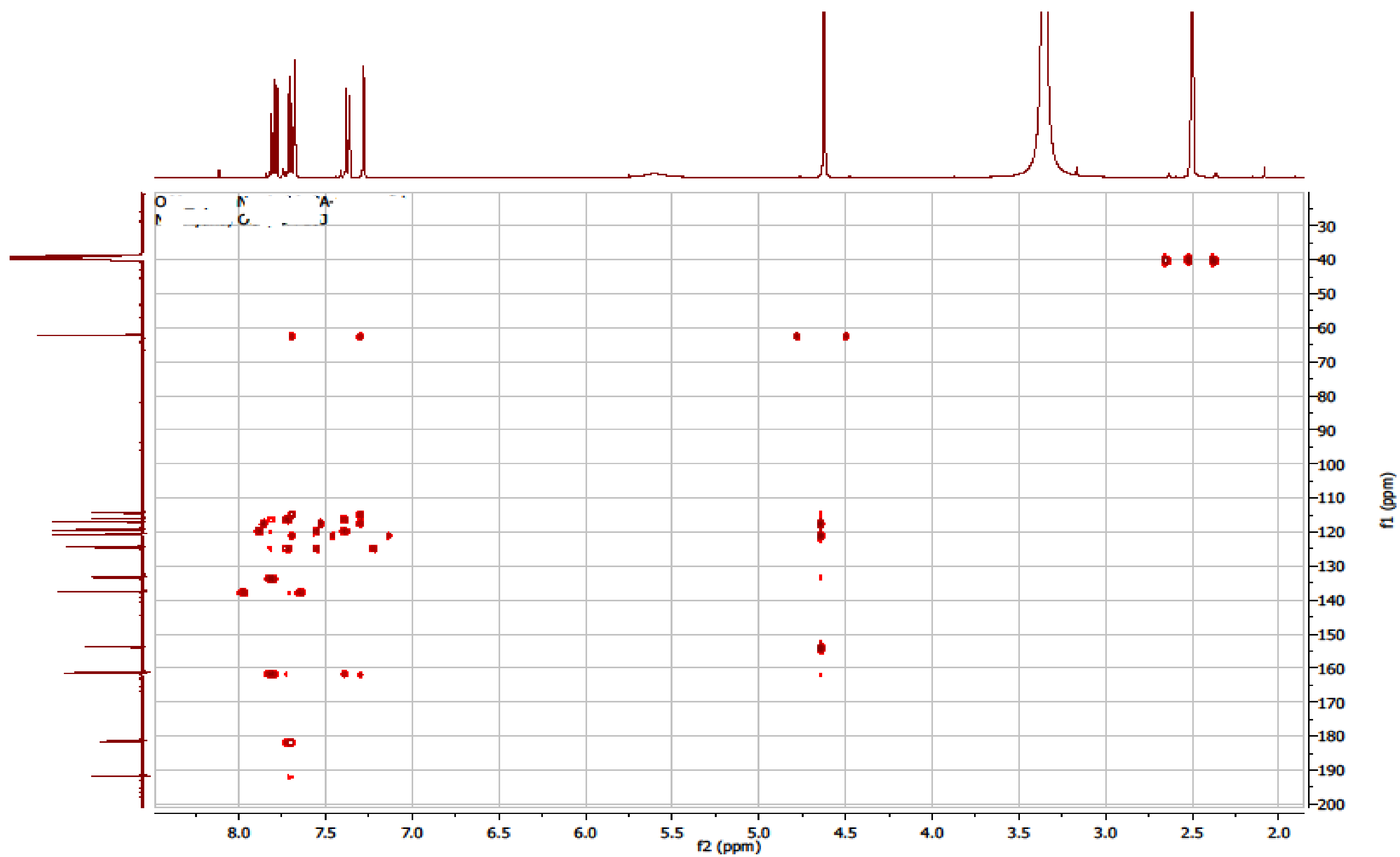
^{1}H NMR SPECTRUM FOR COMPOUND 10 (DMSO, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 10 (DMSO, 125 MHz)



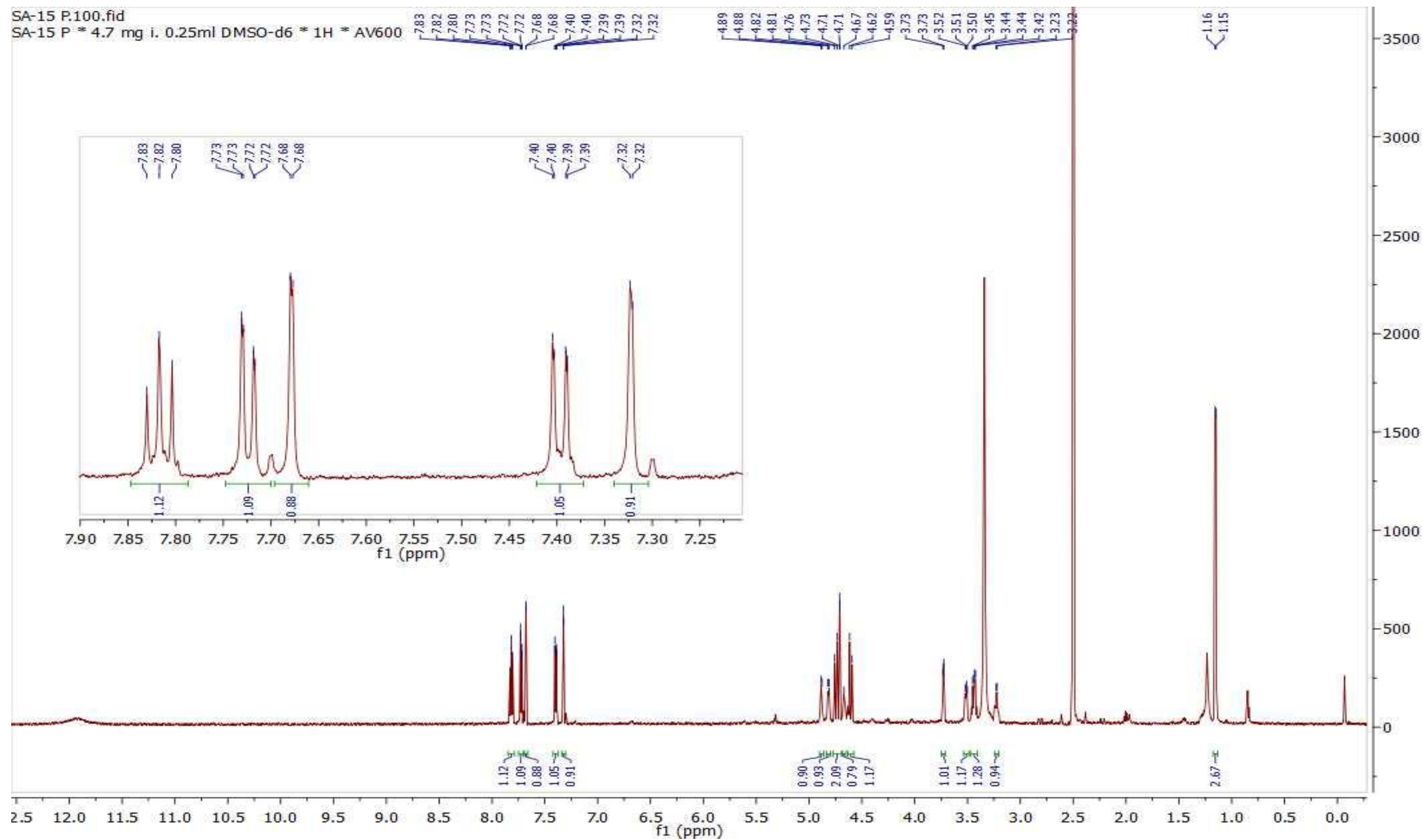
HMBC SPECTRUM FOR COMPOUND 10 (DMSO, 500 MHz)



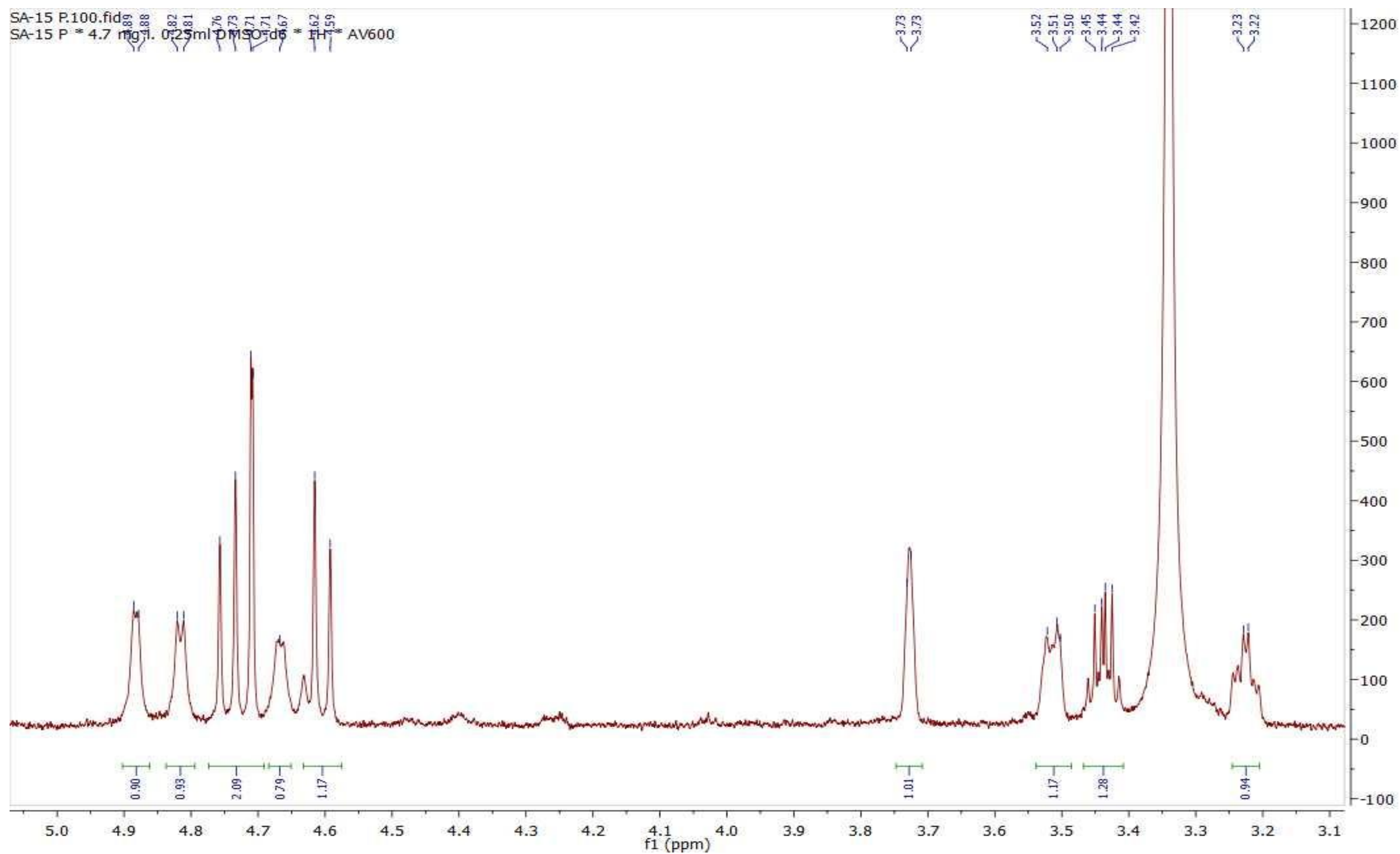
APPENDIX K

SPECTRA FOR COMPOUND 11

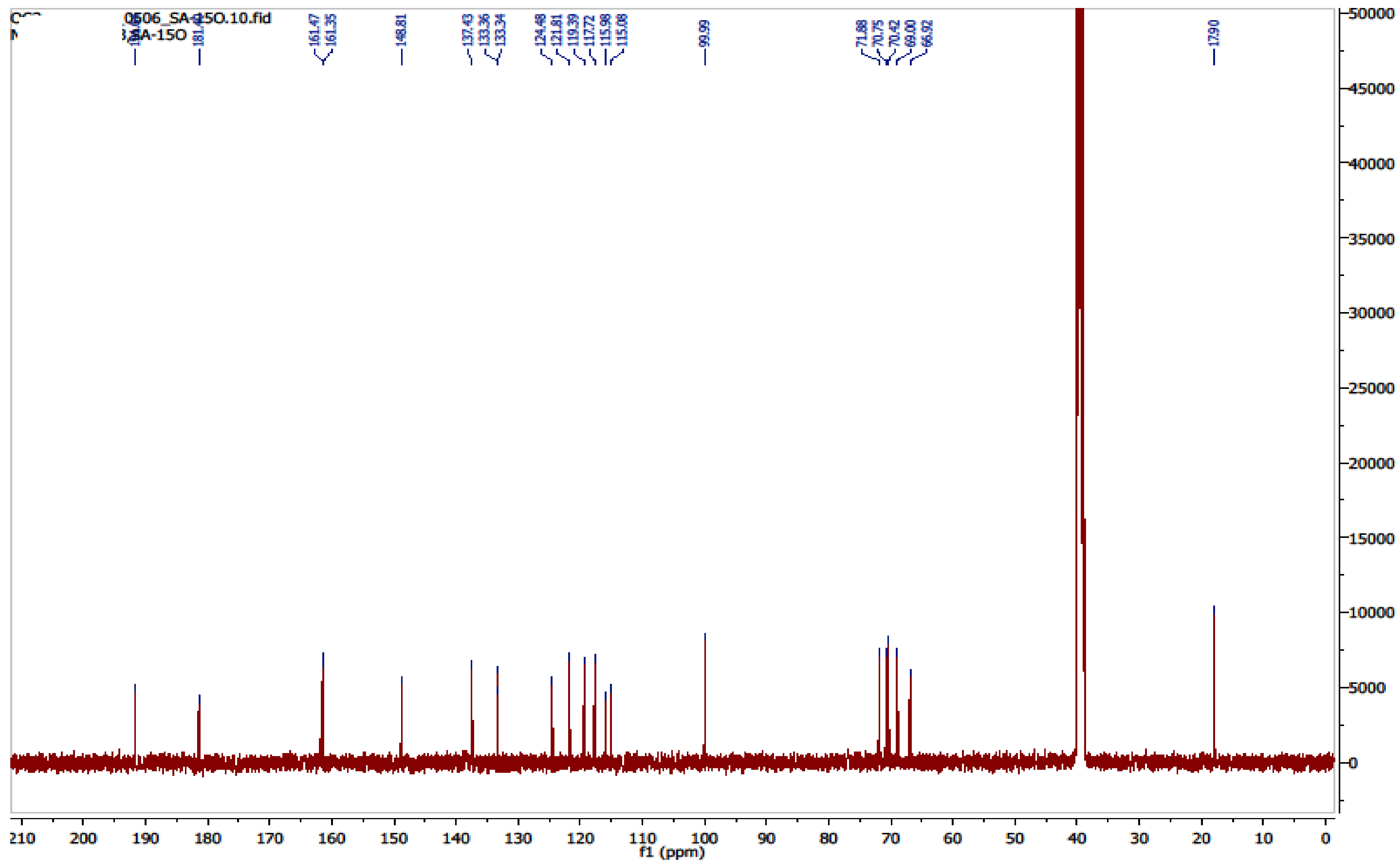
^{1}H NMR SPECTRUM FOR COMPOUND 11 (DMSO, 500 MHz)



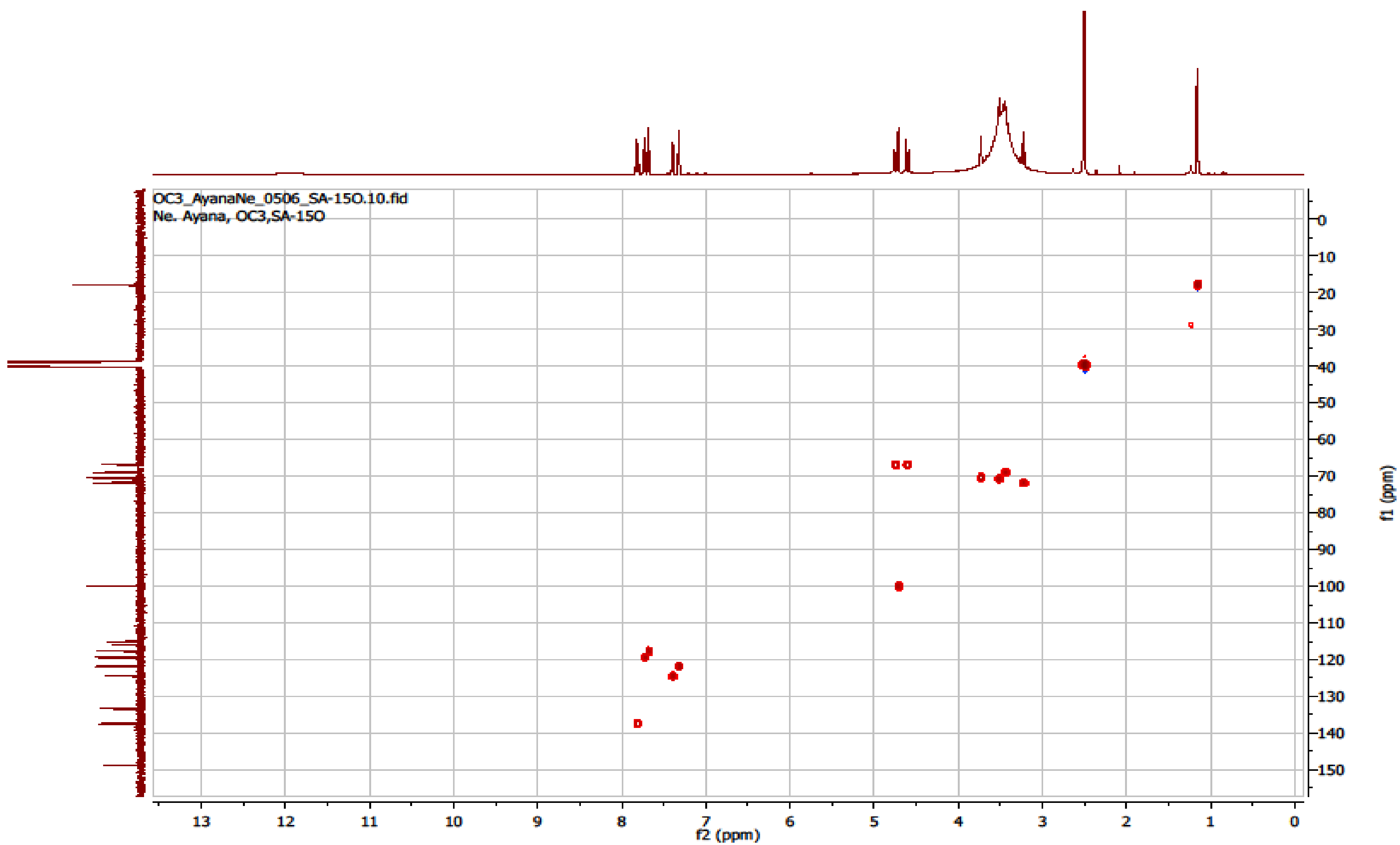
^{1}H NMR SPECTRUM FOR COMPOUND 11 (DMSO, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 11 (DMSO, 125 MHz)



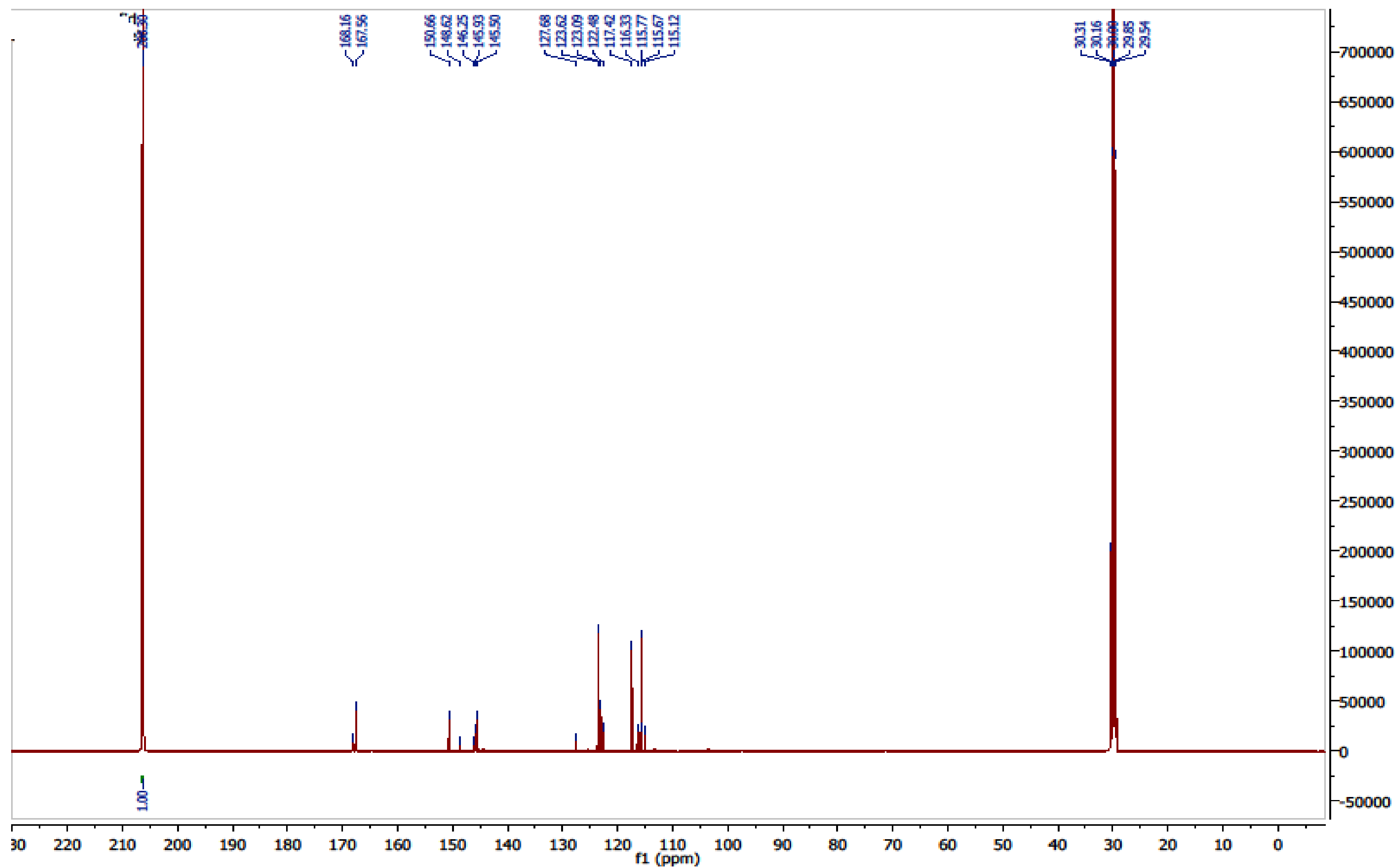
HMBC SPECTRUM FOR COMPOUND 11 (DMSO, 500 MHz)



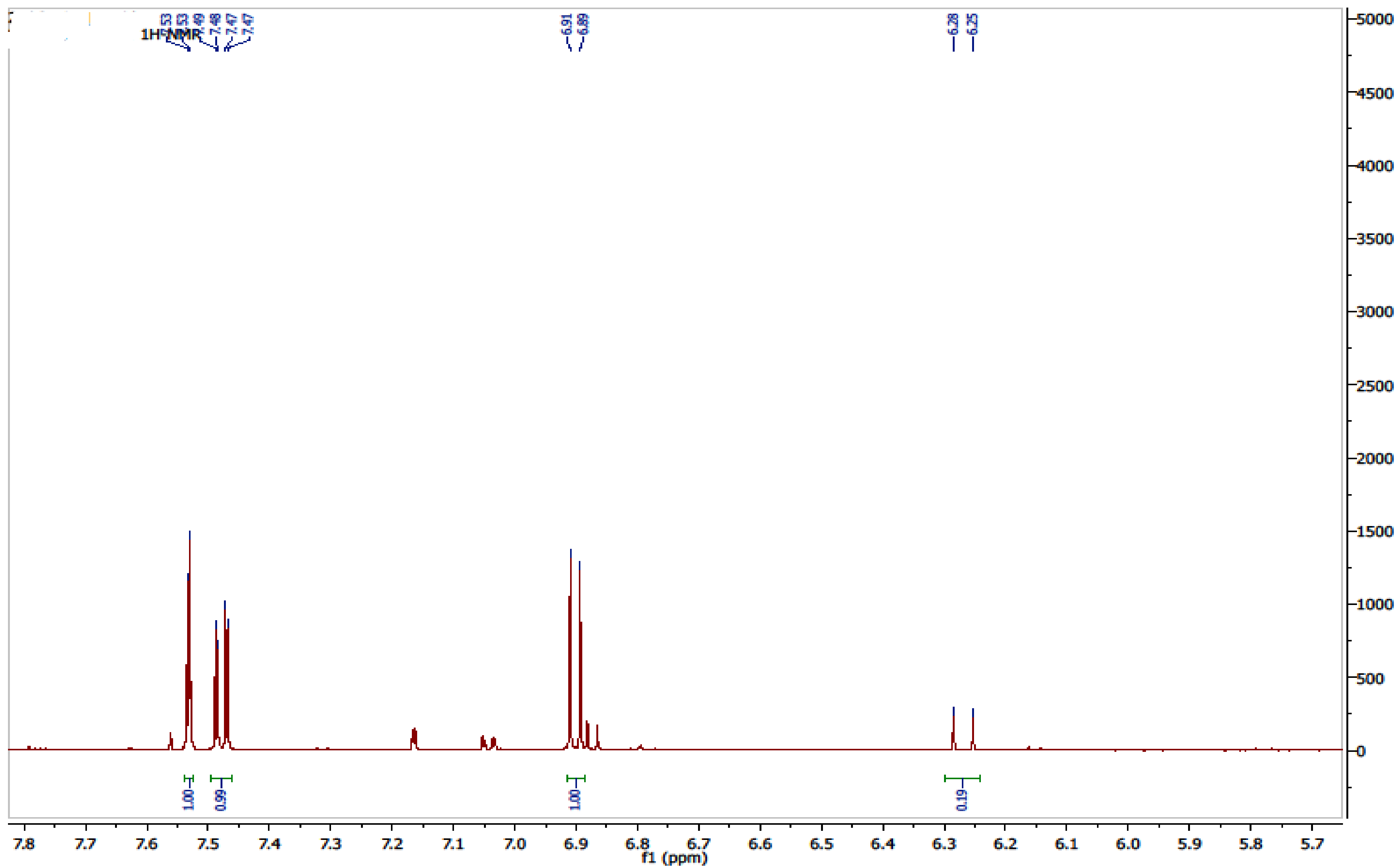
APPENDIX L

SPECTRA FOR COMPOUND 12

^{1}H NMR SPECTRUM FOR COMPOUND 12 ($CDCl_3$, 500 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 12 ($CDCl_3$, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 12 ($CDCl_3$, 125 MHz)