Phytochemical Analysis, Biological Evaluation and Docking Study of Root Extracts of *Brucea antidysenterica*

By

Tewabech Alemu

Thesis Submitted to the Department of Applied Chemistry School of Applied Natural Science in Partial Fulfillment of the Requirements for Master of Science Degree in Applied Chemistry (Medicinal Chemistry)

Office of Graduate Studies

Adama Science and Technology University

August, 2019

Adama, Ethiopia

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Co- Advisor: Dr. Yadessa Melaku

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We, the undersigned, members of the Board of Examiners of the final open defense by Tewabech Alemu Yetna have read and evaluated his thesis entitled "**Phytochemical Analysis, Biological Evaluation and Docking Study of Root Extracts of *Brucea antidysentrica*"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Masters in Medicinal Chemistry

_____	_____	_____
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DECLARATION

I declare that this thesis is my original work and has not been presented for a degree or diploma in this or other University and that all sources of materials used for this thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as a University advisor.

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APPROVAL SHEET

This is to certify that the thesis entitled "**Phytochemical Analysis, Biological Evaluation and Docking Study of Root Extracts of *Brucea antidysentrica*"** submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Chemistry with specialization in medicinal chemistry of School of Applied Natural Science, M.Sc program of Department of Applied Chemistry, Adama Science and Technology University and is a record of original research carried out by Tewabech Alemu (ID. No. A/PR15407/10), under my supervision, and no part of the thesis has been submitted for any other degree or diploma in any other University.

_____	_____	_____
Name of major advisor	Signature	Date
_____	_____	_____
Name of co-advisor	Signature	Date

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I praise the almighty God and His mother St. Virgin Marry for providing me this chance and capability to accomplish this work successfully.

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List of Acronyms and Abbreviations

1D	One dimension
2D	Two dimensions
COSY	Correlation Spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
HMBC	Heteronuclear Multi Bond Correlation
HPLC	High-performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	Half maximal inhibitory concentration
IR	Infrared
MS	Mass Spectroscopy
NMR	Nuclear magnetic resonance
TLC	Thin layer chromatography

Abstract

Natural products have been a source of medicinal agents since thousands of years and remarkable numbers of modern drugs have been derived from natural sources. *Brucea antidysentrica,* commonly known as Aballo (Amharic) and Qomonyo (in Afan Oromo), was used to treat various diseases such as leprosy, wound, diarrhea, fever, eye disease, rabies and cancer. The roots of *Brucea antidysentrica* were extracted by dichloromethane/methanol (1:1), methanol (100%) and acid-base extraction protocol to give 12.77g, 9.4g and 5.1g (3.65 %, 2.68 % and 1.46 %) yields, respectively. Phytochemical screening analysis conducted on roots extracts of *B. antidysentrica* showed the presence of alkaloid, tannins, flavonoids, steroids and saponins, terpenoids, and phytosterols. Silica gel column chromatographic separation of the crude extract (mixture of CH_2Cl_2: MeOH (1:1) and MeOH, acid-base) afforded a known β-carboline alkaloid, flazin methyl ether derivative (**1**)**,** and indole alkaloids, canthin-6-one (**2**), and 1,11-dimethoxycanthin-6-one (**3**) of which the first two compounds are isolated for the first time from the species. The structures of compounds were characterized using spectroscopic methods (IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, HSQC and HMBC). The extracts and isolated compounds were also evaluated *in vitro* for their antibacterial activities using disc diffusion method against *E. coli*, *S. aureus*, *B. subtilis* and *S. thphimurium.* Strong inhibition zone values (12.6±0.60 and 12.5±0.87 mm for alkaloid **2** and **3**) were observed against *E. coli* and *S. thphimurium* compared to standard ciprofloxacin (27.3±2.52 and 29±1.00 mm). The radical scavenging activity of dichloromethane/methanol (1:1), acid-base extract, and alkaloids **1**-**3** were 83.3 %, 80%, 85.3 %, 87.5 % and 78.4 % at 100 µg/mL, respectively. Suggesting that compound **1** and **2** displayed slightly highest radical scavenging activity indicating the potential of the plant as herbal remedies. The molecular docking data showed flazin methyl ether derivative (**1**) and 1,11-dimethoxycanthin-6-one (**3**) were found to show hydrogen bond interaction with active site amino acid residues PHE196 and ALA 51 at a distance of 1.5 A^{o}. Hydrophobic interactions were observed between **2** and **3** with VAL201, LYS57, GLY200, ASN198, ALA51, LEU52, and ASN198, LEU52, VAL201, LYS57, respectively, suggesting the compounds may act as potential inhibitors of DNA gyrase enzyme.

Key words: antibacterial activity, phytochemical screening, *Brucea antidysentrica*, alkaloids, antioxidant activity, molecular docking

1. Introduction

1.1. Background

Plants have nutritional value, in the eye of local people, medicinal and ritual or magical values (Abbink, 1995). Traditional medicine has not only played the vital role in providing healing but has also contributed to discovery of pharmaceutically active substances in plants (Pearce *et al*., 1992). Natural products are important sources of biologically active drugs. There has been an increasing interest in the medicinal plants as natural products in different parts of the world (Saiprasana *et al*., 2012). The medicinal value of these plants depends on bioactive phytochemical activities in the human body. Phytochemicals are bioactive compounds of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body tissue including bark, root, leaves, stem, flower, fruits and seeds; hence any part of the plant body may contain active components (Ugochukwu *et al*., 2013). Herbs are used in many domains, including medicine, nutrition, flavoring, beverage, dyeing, repellents, fragrance, and cosmetics (Djeridane *et al*., 2006). Many species have been recognized to have medicinal properties and beneficial impacts in health, including, antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, anticacinogenic potential, hypolipidemic, and antimutagenic effects (Aaby *et al*., 2004).

It has been estimated that up to 90% of the population in the developing countries rely on the use of medicinal plants in one way or another to meet their primary healthcare needs (WHO, 2002). According to Schippman *et al*. (2002), more than 50,000 plant species are used for medicinal purpose world wide of which almost 13% are flowering plants, containing active chemical constituents such as (alkaloid, glycoside, saponnins, essential oil, bitter principle tannins and mucilage's). In plant parts for example root, stem, bark, fruit and seeds which produce definite curing physiological response in the treatments of various ailments in humans and other animals (Adhikari *et al*., 2010). Traditional medicines used to affect singularly or in combination to treat, diagnose and prevent illness and maintain well-being (Mazid *et al*., 2012).

Majority of Ethiopians still use traditional medicine for treating various health problems since medicinal plants have lower cost compared to modern medical attention (Addis *et al*., 2001). Ethnomedicinal survey in Ethiopia and other parts of the world indicated that the genus *Brucea* (Simaroubaceae) has been used traditionally for many therapeutic purposes. *B. antidysentrica* is a species of flowering shrub/tree growing up to 7 meters tall in the family Simaroubaceae (Cragg and Newman, 2005). It grows widely in tropical Africa including Guinea, Nigeria, Ethiopia, Angola, Malawi and Zambia, usually at the edge of semi-humid forests at relatively high altitudes (Jansen, 1981). In Ethiopia, *B. antidysentrica* is commonly known as Aballo (Amharic). Preparations made from different parts of the plant are used to treat various conditions in the traditional Ethiopian medicine to treat: leprosy, wound, diarrhea, fever, eye disease, rabies and tumor/cancer, among others (Fullas, 2001).

1.2. Statement of problem

The increase in resistance to many commercially produced synthetic antimicrobial agents by microorganisms has been increasing with time. Suggesting the need of searching for alternative antimicrobial agents (Ramesh and Okigbo, 2008). Most of the bacterial and fungal pathogens have shown the capability of developing resistance to some of commercially available antimicrobial agents. This has led to the search for new, cheap and effective antimicrobial agents that can combat the increasing resistance by the pathogenic microbes. Medicinal plants play a role in the treatment of various human and livestock ailments in rural Ethiopia; it needs further scientific evidence of their traditional medication. To the best of our knowledge, a comprehensive biological study, isolation of compounds and docking study of roots extracts of *B. antidysentrica* had not been reported so far particularly in Ethiopia. However, the local people in the country use this plant for various ethnomedicinal purposes without having proper scientific evidence with regard to the chemical composition and biological uses of the plant. The plant was selected for this study on the basis of ethnomedical information by which local communities use it and their genera are rich source of variety classes of secondary metabolites with different pharmacological properties. Therefore, the study is aimed at phytochemical analysis, biological evaluation and docking study of roots extracts and isolated compounds of *B. antidysentrica.*

1.3. Objectives

1.3.1. General objective

The overall objective of this study is to extract, isolate and characterize the chemical constituents present in the root extracts of *B. antidysenterica*, evaluate their biological activities and docking study.

1.3.2. Specific objectives

- To extract compounds from roots of *B. antidysentrica* using solvent extraction (dichloromethane/methanol (1:1), methanol (100%) and acid-base extraction protocol).
- To isolate the chemical constituents from the crude extracts of using silica gel column chromatographic techniques.
- To elucidate the structure of isolated compounds based on spectroscopic methods (IR, UV-Vis, NMR (both 1D and 2D) and comparison with literature data.
- To test *in vitro* antibacterial activity of isolated chemical constituents from root extracts of *B. antidysenterica* by disc diffusion method against two Gram positive bacteria *Staphylococcus aureus* (*S. aureus*), *Bacillus subtlis* (*B. subtilis)* and two Gram negative bacteria *Escherichia coli* (*E. coli), Salmonella thphimurium (S. thphimurium)* strains.
- To test antioxidant activity of crude extracts and isolated pure compounds from root extracts of *B. antidysenterica*.
- To examine molecular docking study of isolated compounds that showed strong *in vitro* antibacterial activity using Autodoc version 4.2 soft ware on selected proteins.

1.4. Significance of the study

Increasing the knowledge of ethnomedicinal, phytochemical and biological activities of plant constituents and determination of the structure of the active chemical constituents may help in sustaining use of natural products in drug discovery and development research. Therefore, this study is expected to:

- Provide documentary information about ethnomedicinal uses, chemical constituents and biological activity of *B. antidysentrica.*

- Provides scientific information of the plant in the primary health care of traditional systems of medicine.
- Provide scientific investigation of the plant as a source of antimicrobial, and antioxidant compounds and substantiate the observed promising activity with molecular docking analysis on target protein of selected strain of microorganism.

2. Literature review

2.1. Background

2.1.1. Family Simaroubaceae

The Simaroubaceae family includes 32 genera and more than 170 species of tree and brushes of pan tropical distribution. It is characterized by its content of bitter substances, mostly responsible for its pharmaceutical properties. The principal geographical distribution center is located at tropical America, extending to the West Africa, Madagascar, Asia (Malaysia) and regions of Australia bathed by the Pacific (Muhammad *et al*., 2004). There are several reports on Simaroubaceae family has been isolation of compounds for Simaroubaceae family. Quassinoids, alkaloids, triterpenes, steroids, coumarins, anthraquinones, and flavonoids are some of the compounds reported for this family (Barbosa *et al*., 2011). The reports also found that quassinoid identified from this family have a wide spectrum of biological activities such as antitumor, antimalaria, antiviral, feeding deterrent, amebicide, antiparasitic and herbicidal (Alves *et al*., 2014) and antioxidant (Viswanad *et al*., 2011).

2.1.2. Genus *Brucea*

The genus *Brucea* is widely distributed in tropical Africa and Asia (Table 1). It is a very bitter or dioecius shrub small tree. The genus comprises about ten species of which *B. javanica, B. mollis, B. antidysentrica,* and *B. quineeniss* are the most common species (Roberts, 1994). In Ethiopia, *B. ferruginea* and *B. antidysentrica* are found of which *B. antidysentrica* is well known.

Table 1. *Brucea* species and their occurrence

Species of the genus *Brucea*	**Occurrence**	**Reference**
B.antidysentrica	Ethiopia, Asia, Angola, Cameroon, Congo, Malawi, Tanzania	Hedenberg *et.al*., 1989
B. erythraea	Eritrea	Brian *et al*., 2000
B. tenuifolla	Uganda and Tanzania	Hedenberg *et.al*., 1989
B. macrocarpa	Kenya	Hedenberg *et.al*., 1989
B. saltutaris	Guinea	Hedenberg *et.al*., 1989
B. ferruginea	Ethiopia	Hedenberg *et.al*., 1989
B. amarissima	Asia	Keng *et al*., 1968
B. sumatrana	Asia	Keng *et al*., 1968
B. javanica	Asia	Keng *et al*., 1968
B. mollis	Asia	Keng *et al*., 1968

2.2. Ethnobotanical uses of genus *Brucea*

Different species of the genus *Brucea* are used as a traditional medicine to cure many diseases worldwide. The seed of *B. javanica* have been used in traditional medicine to treat dysentery and malaria (Sakaki *et al*., 1986). They have also been employed topically for the treatment of wart and corns (Li Pana *et al*., 2009). The root barks of *B. antidysentrica* mixed with milk were used by people living in northern Ethiopia for treating dysentery. In the early 16th century, an European traveler called Francisco Alvarez reported the use of herbs as purgatives in Ethiopia. Another British traveler, James Bruce, who stayed in Ethiopia from 1969 to 1971, also reported the wide use of a plant locally known as Abalo in Amharic, Waginos in Geeze or Qomonyo in Afan Oromo that was later named *B. antidysentrica* (Simaroubaceae), as a remedy against dysentery. The use of plants in religious ceremonies as well as for magic and medicinal purposes is very common and widely distributed in Ethiopia (Endashaw, 2007).

The fruit powder mixed with honey and fermented for seven days is taken orally to treat bullad (weight loss, fever, itching, diarrhea), fruit powder mixed with milk is taken orally for three days for hemorrhoids, root powder mixed with honey is taken orally for mushuro (weight loss, dysentery and fever), juice of leaves is taken orally in the morning for dysentery and dressing with inner bark paste mixed with butter or oil for *chiffea* (Eczema) (Tilahun *et al*., 2007). Furthermore the dry root is smoked and inhaled and the flower of *B. antidysentrica* is grounded, boiled and fumigated to treat evil eye (Misha *et al*., 2014). *B. antidysentrica* has a number of other therapeutic applications like treating scabies and external parasites (Bekele and Reddy, 2015), as an antidysentric agent (Limenih *et al*., 2015), wound healing effect (d'Avigdor *et al*., 2014), treatment of Gonorrhea (Lulekal *et al*., 2008), eczema and hookworm (Gebeyehu *et al*., 2014), as anticancer and ant-malaria (Abera *et al*., 2003) and treatment of Trypanosomosis (Tamiru *et al*., 2013) among others. The fruits, leaves and roots of *B. antidysenterica* (Figure 1) are mixed with small amount of water and applied to treat wound (Mesfin, 1986). According to Keng (1968), *B. sumartana, B. amarissima, B. antidysentica,* and *B. javanica* are species found in Asia and known by local names *ko-sam, ya-tan-tzu, k'u-shen-tzu and lao-ya-tan,* respectively. They are used as herbal remedies against human amoebiasis.

Figure 1. Picture of *B. antidysentrica* (Picture taken by Tewabech Alemu on 28/02/2019)

2.3. Phytochemical and pharmacological study

Different classes of secondary metabolites have been isolated from *Brucea* genus among which quassinoids, alkaloids, triterpenoids, and flavonoids are mostly cited (Fiaschetti *et al*., 2011). Recently, extensive attention has been paid to the *Brucea* genus with emphasis on its chemical constituents because of their multifaceted biological activities observed. Its core components are quassinoids, which possess various biological activities including anti-tumor (Fiaschetti *et al*., 2011), anti-malarial, anthelmintic and anti-babesial (Narkao *et al*., 2009), anti-viral (Chen *et al*., 2009), anti-bacterial (Sawangjaroen, 2005) and hypoglycemic activities (NoorShahida *et al*., 2009).

From the chloroform-soluble sub-fraction of a methanol extract of the combined twigs, leaves, and inflorescence of *B. javanica* isolated new triterpenoids (**1–5**), together with two known quassinoids, bruceantin (**6**) and bruceine A (**7**), and a known flavonolignan, (-) hydnocarpin (**8**) (Lin pan *et al*., 2009). Bruceantin (**6**) and bruceine A (**7**) (Figure 2) demonstrated significant inhibitory activities for human cancer cell lines, of which the former displayed potent cytotoxic activity. Bruceantin (**6**) was formerly of interest for development as an anticancer agent by the US National Cancer Institute (Cragg *et al*., 2005), treating human hematological malignancie (Cuendet and Pezzuto, 2004). Seung *et al*. (2017) reported five quassinoids, including brusatol (**9**), bruceantin (**10**), and brucein A (**11**), bruceantinol (**12**) and brucein B (**13**) (Figure 2), from the CH_3OH extract of fruit *B. javanica*. All isolated compounds exhibited inactivation effects in systemic host plants, and compounds **11** and **12** were potent, with a minimum inhibitory concentration of 10 μM. Furthermore, compound **11** was found to have a protective effect at the tested concentration of 40 μM.

According to Qing-Mei *et al*. (2015) reported a new quassinoid, bruceene A (**14**) along with seventeen known quassinoids (**15–31**) (Figure 2) from the fruits of *B. javanica*. Compounds **18, 20, 23** and **25** with 3-hydroxy-3-en-2-one moiety showed potent inhibitory activity against the MCF-7 and MDA-MB-231 cells with IC_{50} values in the ranges of 0.063–0.182 μM and 0.081–0.238 μM, respectively, while glycosidation at 3-OH significantly decreased the cytotoxicity. It was also found that the most potent compound **20** induced apoptosis in MCF-7 cells via the

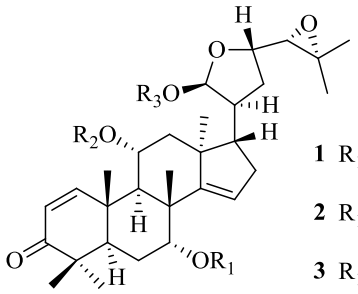
intrinsic mitochondrial apoptotic pathway. Quassinoids in genus *Brucea* often have a methyl carboxylatemoiety at C-13; In contrast, similar compounds in *Ailanthus* genus bear a methyl or exomethylene group at this position (Wang *et al*., 2013). According to Shi-Hui *et al*. (2013) apotirucallane-type triterpenoids (**32-45**), named brujavanones A–N, were isolated from the twigs of *B. javanica*, along with (**46-49**) and seven known lignans (Figure 2) from the seeds of *B. javanica*. The cytotoxic activities of triterpenoids and quassinoids against two human tumor cell lines, HL-60 and A-549, were evaluated, but all the compounds were found to be inactive (IC_{50} > 10 μM).

From the seeds of *B. amarissima* isolated four new quassinoid glucosides, javanicosides I-L (**50-53**), along with two known quassinoids, bruceins D(**16**) and E(**55**) and seven known quassinoid glucosides, yadanziosides B (**24**), C (**27**), E (**17**) ,I **(51**) and K (**54**) bruceoside B (**57**) and yadanzigan (**56**) (Figure 2) (Ik Hwi *et al*., 2004). From the bark of *B. antidysenterica* Bruceantin (**6**) and its analogue compounds were isolated. They are capable of inducing an array of biological responses including anti-inflammatory effect with murine models (Kupchan *et al*., 1973). Bruceantin shows marked amoebicidal activity *in vitro* in the colony assay. At concentration of 0.2 μg/mL and above, all of the parasites *Entamoeba histolytica* (the dysentery amoeba) were killed. At 0.1 μg/mL few parasites survive at 48 hr, and all of the parasites were killed after 72 hr to 0.1 μg/mL (Frances *et al*., 1982).

Compounds such as Bruceantarin (**10**), Bruceantinol (**12**), Dehydrobruceantin (**58**), Dehydrobruceantarin (**59**), Dehydrobrucein (**60**), Dehydrobruceantol (**61**) (Kupchan *et al*., 1975), (Toyota *et al*., 1990), Bruceanols A (**62**), Bruceanols B (**63**) (Masayoshi *et al*., 1985), Bruceanols C(**64**) (Narihiko *et al., 1*988), Bruceanols D (**65**), Bruceanols E (**66**), Bruceanols F (**67**) (Imamura *et al*., 1993), Bruceanols G (**68**) Bruceanols H (**69**) (Imamura *et al*., 1995) Brucantinoside A (**70**), Brucantinoside B (**71**) (Masayoshi *et al*., 1981), Brucantinoside C(**72**) (Narihiko *et al.*, 1986), Yadanzioside G (Toshiro *et al*., 1985) Bruceanic acid A (**73**) Methyl ester of 12 (**74**), Bruceanic acid B (**75**), Bruceanic acid C (**76**) and Bruceanic acid D (**77**) isolated from different parts of this plant. Bruceantin (**6**) and analogues compounds are triterpenes based of the quassinoid type isolated from the bark of the Ethiopian tree *B. antidysenterica*. Two

cytotoxic antileukemic alkaloids such as, 1,11-dimethoxycanthin-6-one (**82**) and the known 11-hydroxycanthin-6-one (**80**) as well as the known canthin-6-one (**78-86**) isolated from stem *B. antidysentrica* (Narihiko *et al*., 1986). Recently, 3-(3-hydroxybutyl) phenol (**87**) and glycoside compound B6: ethyl 4-(3-(6-(tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2 yloxy)hexyl)phenyl) butanoate (**88**) were isolated for berries of *B. antidysentrica* (Tariku, 2019).

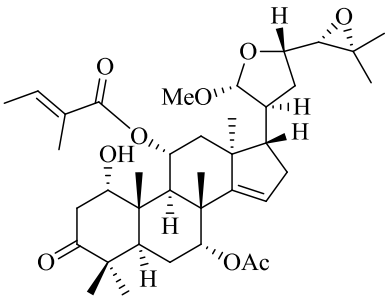
Isolation of Indole alkaloids such as, bruceollines H-N (**89-95**), quassinoids, yadanziolides T-V (**98-100**) and known analogues bruceolline E (**96**), bruceolline F (**97**), bruceine D (**101**) and yadanziolides B (**102**) were reported from ethanol extract of the stems of *B. mollis* (Hui Chen *et al*., 2011). Mai *et al*., (2013) reported isolation of soulameanone (**103**), isobruceine B (**104**), 9-methoxy-canthin-6-one (**105**), bruceolline F (**106**), niloticine (**107**), octatriacontan-1-ol (**108**), bombiprenone (**109**), α-tocopherol (**110**), inosine (**111**), and apigenin 7-*O*-β-D-glucopyranoside (**112**) (Figure) from leaves, stems and roots of *B. mollis*.

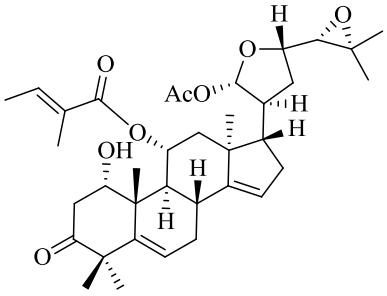


1 R_1 = H, R_2 = Hexanoyl, R_3 = Ac

2 R_1 = AC, R_2 = Hexanoyl, R_3 = Ac

3 R_1 = AC, R_2 = Tigloyl, R_3 = Ac





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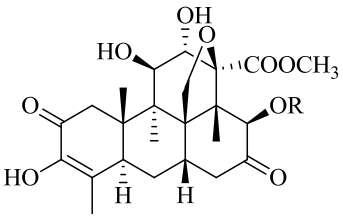
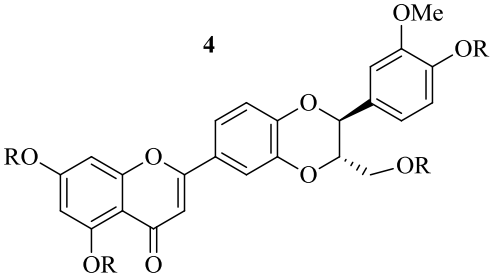


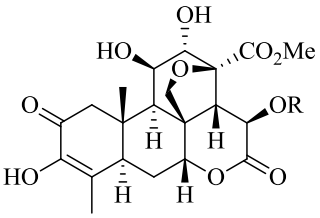
6 R = (E)-3,4- Dimethyl-2-pentenoyl

7 R = Isopentanoyl



4

8 R = H
8a R = Ac



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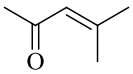

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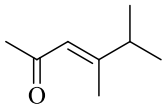

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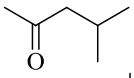

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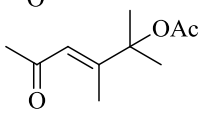
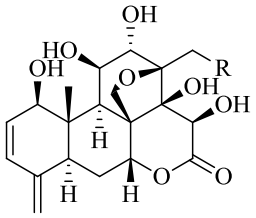
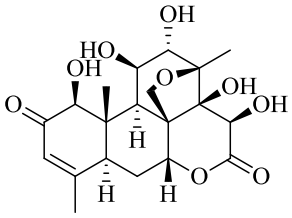


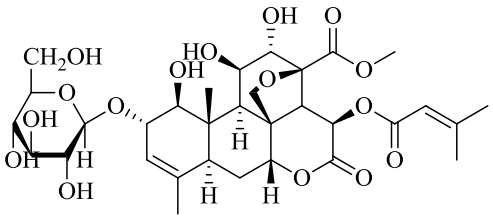
13 R =



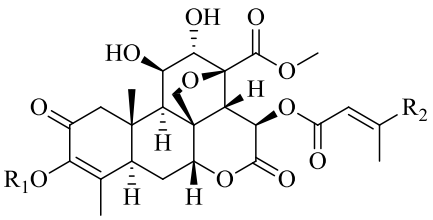
14 R = OH
15 R = H



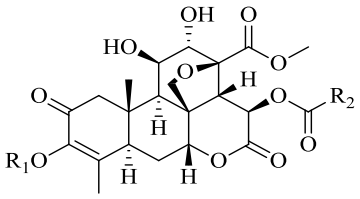
16



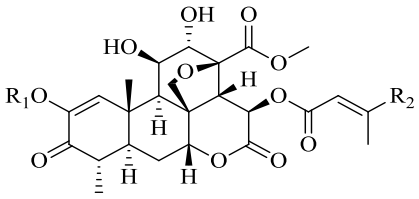
17



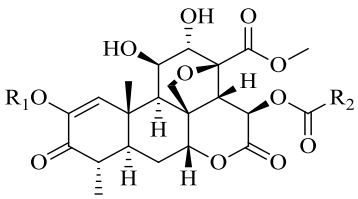
	R_1	R_2
18	H	CH_3
19	Glu	CH_3
20	H	$C(CH_3)_2OOCCH_3$
21	Glu	$C(CH_3)_2OOCCH_3$
22	Glu	$CH(CH_3)_2$



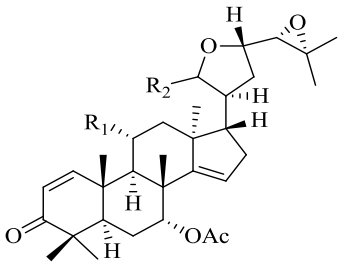
	R1	R_2
23	H	$CH_2CH(CH_3)_2$
24	Glu	$CH_2CH(CH_3)_2$
25	H	C_6H_5



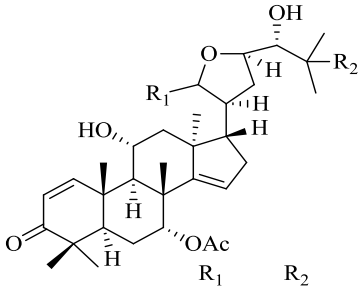
	R_1	R_2
26	Glu	CH_3
27	Glu	$C(CH_3)_2OH$
28	Glu	$C(CH_3)_2OOCCH_3$



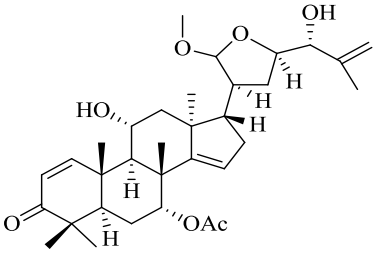
	R_1	R_2
29	Glu	CH_3
30	Glu	$CH_2CH(CH_3)_2$
31	Glu	C_6H_5



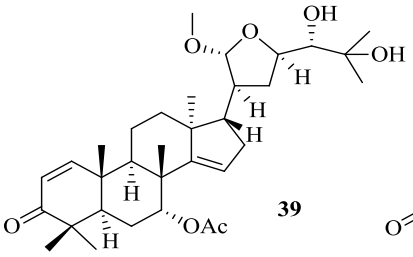
	R_1	R_2
32	OH	β-OAc
33	OH	α-OMe
34	H	OH



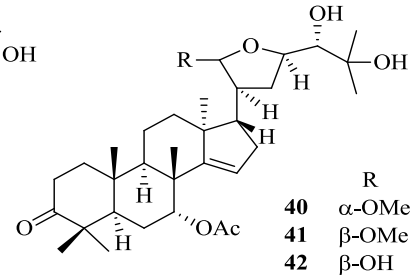
	R_1	R_2
35	β-OMe	OH
35a	α-OMe	OH
36	β-OH	OH
37	α-OMe	OMe



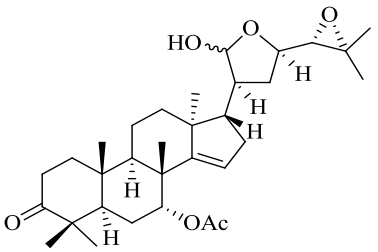
38



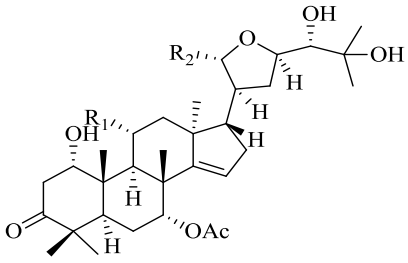
39



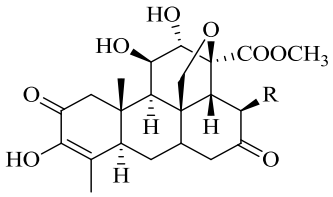
	R
40	α-OMe
41	β-OMe
42	β-OH

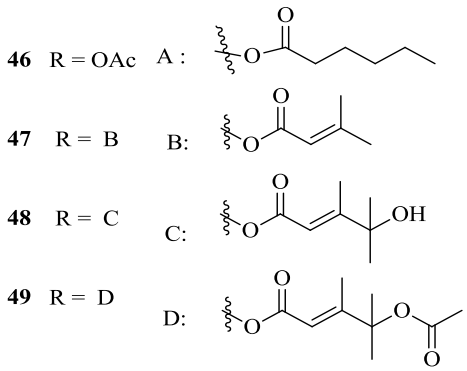


43

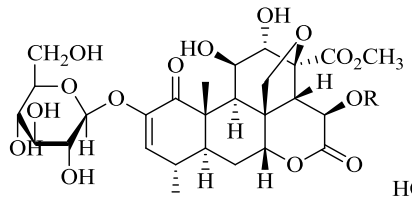


	R_1	R_2
44	Ac	OMe
45	H	OEt

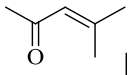




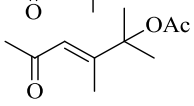
46	R = OAc	A:
47	R = B	B:
48	R = C	C:
49	R = D	D:

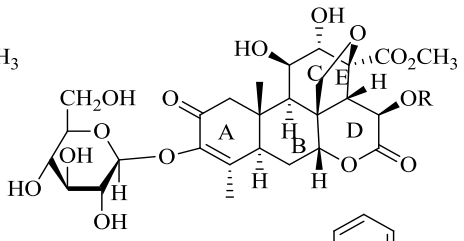


50 R =

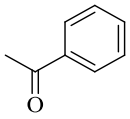


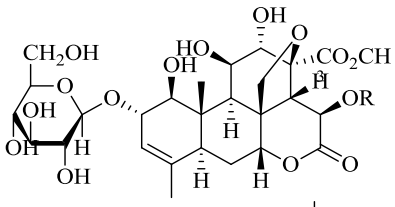
54 R =



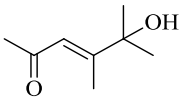


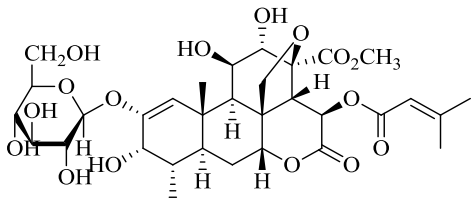
51 R =



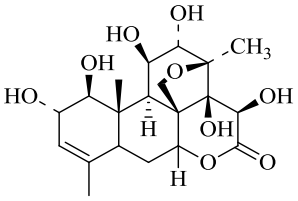


52 R =

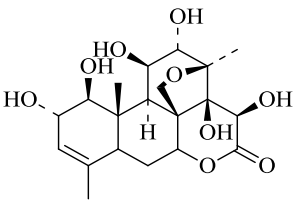




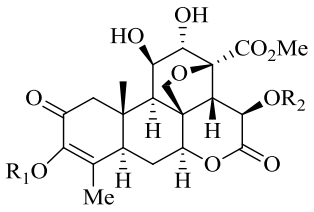
53



55

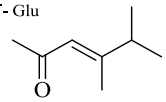


56

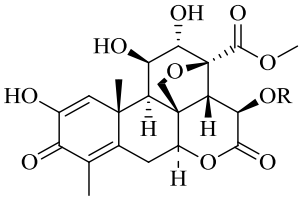


R_1 = β-Glu

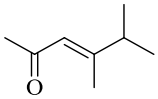
R_2 =



57



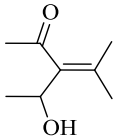
58 R =

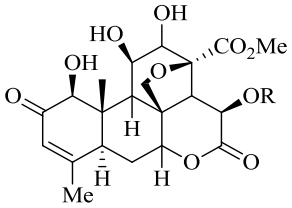


59 R = COC_6H_5

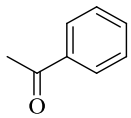
60 R $COCH_3$

61 R =

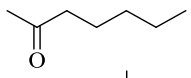




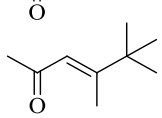
62 R =



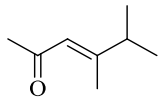
63 R =

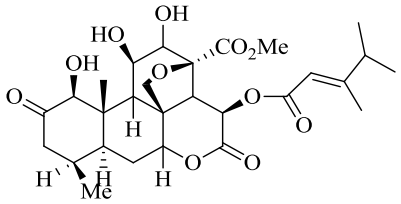


64 R =

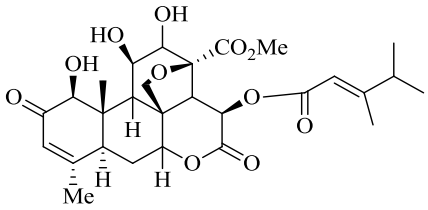


65 R =

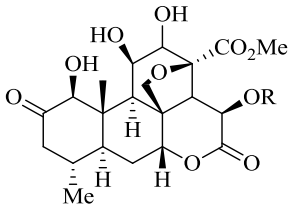




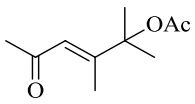
66



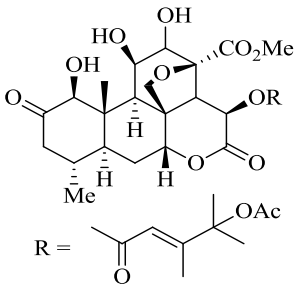
67



R =

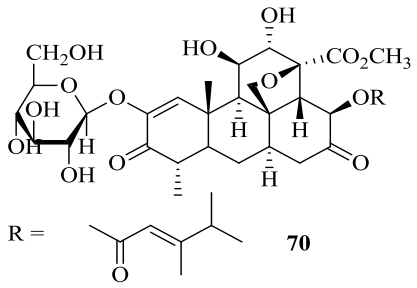


68



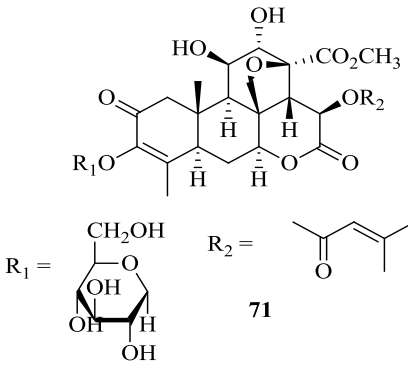
R =

69



R =

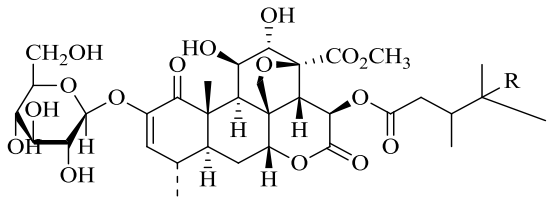
70



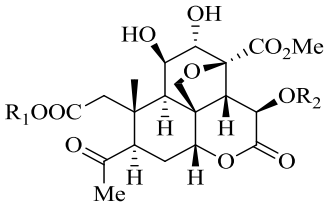
R_1 =

R_2 =

71

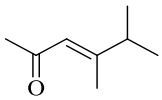


72 R = $OCOCH_3$



R_1 = H

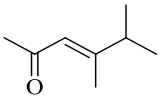
R_2 =



73

R_1 = Me

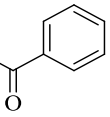
R_2 =



74

R_1 = H

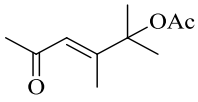
R_2 =



75

R_1 = H

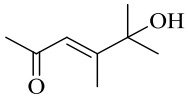
R_2 =



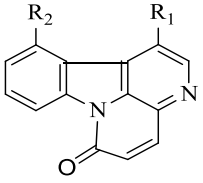
76

R_1 = H

R_2 =



77



78. R1=H, R2= OH

79. R1=R2= H

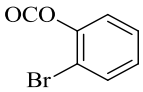
80. R1=R2= OMe

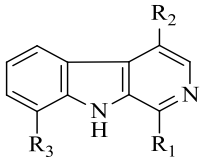
81. R1=OMe, R2= H

82. R1=H, R2= OMe

85. R1=H, R2=OCOMe

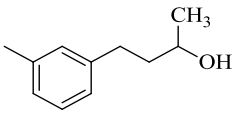
86. R1=H, R2=



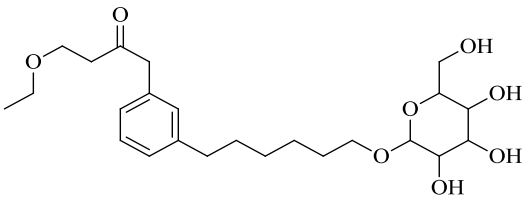


83. R1= CO2Me, R2=R3=H

84. R1= CH2CH2OMe, R2=R3=OMe



87



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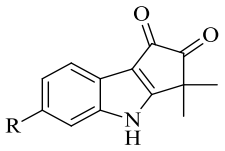
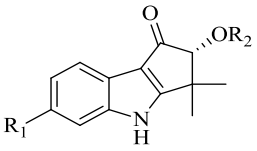
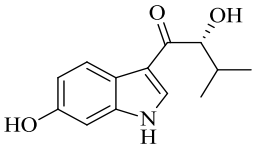


89 R = OH
90 R = H



	R1	R2
91	OH	H
92	H	H
93	H	Glu



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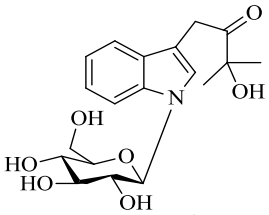

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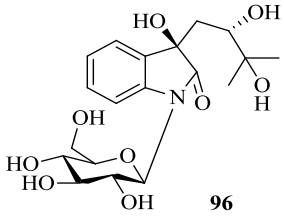

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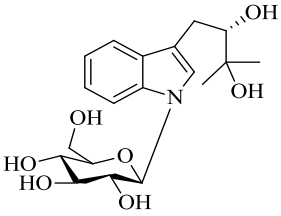

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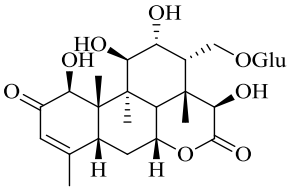
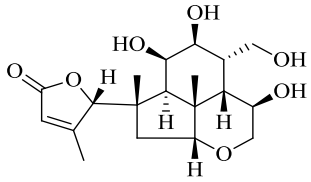


98 R = H
99 R = Glu



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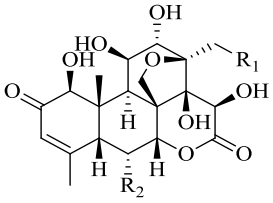
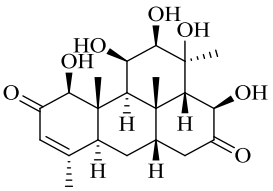


	R_1	R_2
101	CH_3	H
102	CH_2OH	OH



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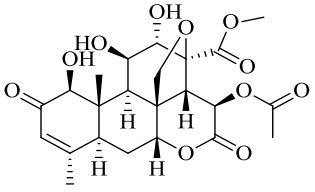

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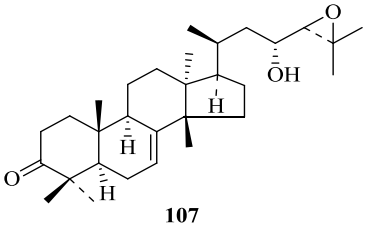

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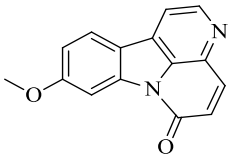

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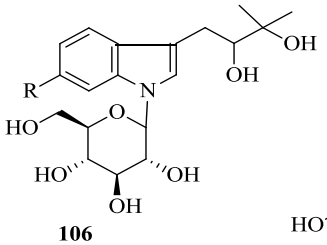

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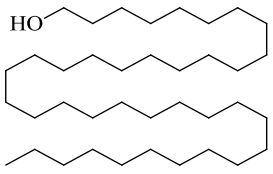

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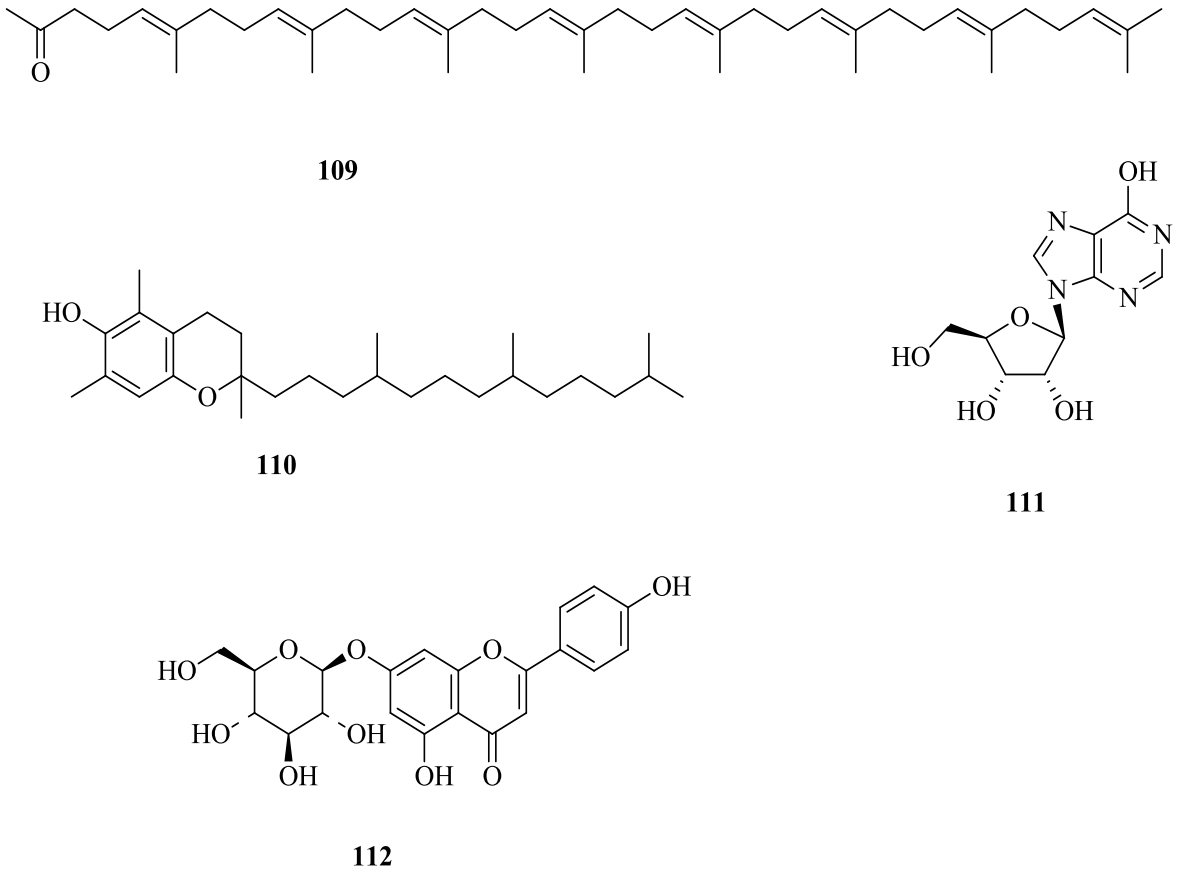

Figure 2. The chemical structures of isolated compounds (**1-112**) from genus *Brucea*

3. Materials and Methods

3.1. Plant material collection and identification

The plant material was collected from Dejen (Lomami), East Gojam, Amhara region, Ethiopia, which is 236 kilometers far from Addis Ababa. The geographical coordinates of the area were 10^{o} 10' 0" North, 38^{o}8'0" East. The plant material was identified with the help of Botanist Shambel Alemu and voucher specimen was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

3.2. Chemicals and reagents

The chemicals and reagents used in this investigation were silica gel, sulfuric acid, hydrochloric acid, ferric chloride, mayer reagent, ammonia solution. Solvents used in this study were ethanol, methanol, dichloromethane, chloroform, hexane, distilled water and ethyl acetate. All the chemicals and reagents used in this study were analytical grade and are of pure Aldrich grade.

3.3. Instrumentation

TLC was performed using precoated aluminum-backed supported silica gel 60 F254 (0.3 mm thickness). TLC visualization was done using UV-light at λ 354 and 365 nm. Column chromatography was carried out using silica gel 60-120 mesh ASTM. The ultraviolet and visible (UV-Vis) spectrum was run using Spectroscopic Genesys™ 2PC UV-Vis scanning spectrometer. Melting point was determined using Cambridge microscopy instruments. IR spectrums were recorded using Perkin-Elmer FT-IR spectrometer in the range of 4000-400 cm^{-1} using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker advance 300, 400 and 500 MHz spectrometer and $CDCl_3$ solvent and Tetramethylsilane (TMS) was used as an internal standard.

3.4. Extraction

3.4.1. Extraction

The collected roots of *B. antidysentrica* were air dried at room temperature and grounded in to powder form using grinding mills. The grounded roots (350 g) of *B. antidysentrica* were extracted with 2 L dichloromethane/methanol (1:1) for 72 hr with occasional shaking at room temperature. The solution was filtrated and concentrated by a vacuum rotary evaporator at 50 ^{o}C, to afford 12.77 g (3.65 %) brownish crude extract. The marc left was further extracted with methanol for 72 hr with occasional shaking at room temperature. The solution was filtered and concentrated by a vacuum rotary evaporator at a temperature of about 50 ^{o}C to afford 9.4 g (2.68 %) brown crude extract. The TLC profile of dried crude extracts were checked and finally the extracts were collected in labeled sterile containers and put in deep freezer until needed for further experiment.

3.4.2. Acid-Base extraction of alkaloid constituents

Alkaloids constituents were selectively extracted in acid-base extraction approach as per a standard procedure previously described by Alexander (2005). Roots (350 g) of *B. antidysentrica* were extracted in *n*-hexane separately, the solution was filtered, and the residue was extracted in ethanol at room temperature for 72 hr. Then solvent was evaporated using a rotary evaporator at a temperature of about 50 ^{o}C to yield crude extracts. The defatted ethanolic crude extract was suspended in 5 % HCl to pH 5. After pre-saturation with water and partition by adding chloroform, the extract was separated in to aqueous layer and organic layer. Then, the aqueous and organic layers were separated using separatory funnel and repeated for three times. The acidic aqueous phase was basified by adding 5 % NH_3 solution to pH 11, and then partitioned by chloroform. Then the aqueous and organic layers were separated using separatory funnel and the process was repeated three times. The chloroform extracts were combined, and concentrated under vacuum rotary evaporator to obtain 5.1 g (1.46 %) of brown crude alkaloid extract. Finally, the crude extracts were checked by TLC profile (*n*-hexane/EtOAc solvent system) and chemical test of alkaloids by Mayer reagent then the crude extract was collected in labeled sterile containers and put in deep freezer until needed for further experiment (for isolation compound).

3.4.3. Phytochemical screening tests

3.4.3.1 Test for flavonoids: To 0.5 g portion of crude extract (DCM: MeOH (1:1), MeOH (100 %) extract), 10 mL of ethyl acetate was added and heated for 3 min using steam bath. The mixture was filtered, and the filtrate was mixed with 1mL of dilute ammonia solution. Formation of intense yellow color ratifies the presence of flavanoids (Sofowora and Debiyi, 1978).

3.4.3.2 Test for saponins: To 0.5 g of crude extract (DCM: MeOH (1:1), MeOH (100 %) extract), 5 mL of distilled water was added and shaken while heating to boil. Frothing showed the presence of saponnins (Evans and Trace, 1989).

3.4.3.3 Test for phenols: To 0.5 g of crude extract (DCM: MeOH (1:1), MeOH (100 %) extract), few drops of 2 % of $FeCl_3$ was added and the formation of bluish green to black color indicates the presence of phenols (Roopashree *et al*., 2008).

3.4.3.4 Test for tannins: The crude extract (DCM: MeOH (1:1), MeOH (100 %) extract) (0.5 g) was boiled in 10 mL of water in a test tube and filtered. To the filtrate, few drops of 0.1 % $FeCl_3$ was added to give a brownish green or a blue-black color which confirms the presence of tannins (Ayoola *et al*., 2008).

3.4.3.5 Test for terpenoids (Salkowski test): 5 mL of each extract (DCM: MeOH (1:1), MeOH (100 %) extract) will be mixed with 2 mL of chloroform, and 3 mL concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids (Ugochukwu *et al.*, 2013).

3.4.3.6 Test for steroids: Steroids was conducted following the methods of Alhadi *et al.* (2015) one ml of the extracts was dissolved in 10 mL of chloroform and equal volume of concentrated H_2SO_4 was added by sides of the test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids.

3.4.3.7 Detection of phytosterols (Salkowski's test): Crude extract (DCM: MeOH (1:1), MeOH (100 %)) (0.5 g) was treated with a few drops of chloroform and filtered. To the filtrate, few drops of concentrated H_2SO_4 was added, shaken, and allowed to stand for few min. Appearance of the golden yellow color indicates the presence of triterpenes (Roopashree *et al*., 2008).

3.4.3.8 Test for glycosides (modified Borntrager's test): Crude extract (DCM: MeOH (1:1), MeOH (100 %)) (0.5 g) was treated with $FeCl_3$ solution and immersed in boiling water for 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution Formation of the rose-pink color in the ammonical layer confirms the presence of anthranol glycosides (Roopashree *et al*., 2008).

3.4.3.9 Test for Alkaloid: 0.5 mg of crude extract (DCM: MeOH (1:1), MeOH (100 %)) was separately stirred with 1 % HCl (0.5 mL) on a water bath for 5 min and filtered. These filtrates were divided into three equal parts. To one portion of filtrate, Mayer's reagent (Potassium mercuric iodide solution) (1 mL) was added. Formation of orange colored precipitate gives an indication of the presence of alkaloids (Joshi *et al*., 2013; Abdullah *et al*., 2013).

3.5. Isolation of compounds

3.5.1. Column Chromatography Technique

The crude extracts of dichloromethane/methanol (1: 1) and Methanol showed very close TLC profile and hence mixed together and dried. The crude extract (10 g) was adsorbed on 10 g of silica gel (mesh size 60-120), subjected to silica gel chromatography (170 g of silica gel) using *n*-hexane for packing and elusion was carried out with increasing gradient of ethyl acetate in *n*-hexane and methanol in dichloromethane as eluent. A total of 145 fractions each 100 mL (Table 2) were collected and concentrated at 40 ^{o}C under reduced pressure. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fractions from 29-31 afforded compound **1** with single spot on TLC (EtOAc/*n*-hexane, 1:1, R_f value of (0.44, 30 mg).

Table 2. Summary of fraction of Mixed Extracts (CH_2Cl_2: MeOH (1:1) and methanol Extracts)

Solvent system	Ratio	Fractions	Solvent system	Ratio	Fractions
n-hexane/EtOAc	9.9:0.1	F_1-F_2	CH_2Cl_2/MeOH	9.5:0.5	F_{81}-F_{107}
	9.8:0.2	F3-F_4		9:1	F_{108}-F_{128}
"	9.7:0.3	F_5-F_6	"	7:3	F_{129}-F_{131}
"	9.6:0.4	F_7-F8	"	5:5	F_{132}-F_{134}
"	9.5:0.5	F_9-F_{10}	"	3:7	F_{135}-F_{140}
"	9:1	F_{11}-$F1_2$	MeOH	100	F_{141}-F_{145}
"	8.5:1.5	F_{13}-F_{15}			
"	8:2	F_{16}- F_{23}			
"	7:3	F_{24}-F_{27}			
"	5:5	F_{28}-F_{33}			
"	2.5:7.5	F_{34}-F_{45}			
EtOAc	100	F_{46}-F_{80}			

Similarly, acid-base crude extract (5 g) was adsorbed with 5 g of silica gel mesh size (60-120) and subjected to silica gel column chromatography (150 g of silica gel used *n*- hexane for packing) using increasing gradient of ethyl acetate in *n*-hexane and methanol in dichloromethane as eluent. A total of 221 fractions each 100 mL (Table 3) were collected and concentrated at 40 ^oC under reduced pressure. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fractions from 52-54 afforded single spot with similar R_f values (compound **2**) on TLC (EtOAc/*n*-hexane, 9:1, R_f value of 0.57, 25 mg). Fraction 84-87 afforded single spot with similar R_f values (compound **3**) on TLC (EtOAc/*n*-hexane, 8:2, R_f value of 0.41, 30 mg).

Table 3. Summary of fractions of acid-base extracts

Solvent system	Ratio	Fractions	Solvent system	Ratio	Fractions
n-hexane/EtOAc	9.9:0.1	F_1-F_6	CH_2Cl_2/MeOH	9.5:0.5	F_{129}-F_{139}
	9.8:0.2	F_7-F_{10}		9:1	F_{140}-F_{1150}
“	9.7:0.3	F_{11}-F_{14}	“	8.5:1.5	F_{151}-F_{165}
“	9.6:0.4	F_{15}-F_{21}	“	8:2	F_{166}-F_{180}
“	9.5:0.5	F_{22}-F_{26}	”	7:3	F_{181}-F_{208}
“	9:1	F_{27}-F_{32}	“	5:5	F_{209}-F_{216}
“	8.5:1.5	F_{33}-F_{38}	Methanol	100	F_{217}-F_{221}
“	8:2	F_{39}- F_{46}			
“	7:3	F_{47}-F_{59}			
“	5:5	F_{60}-F_{74}			
“	2.5:7.5	F_{75}-F_{94}			
EtOAc	100	F_{95}-F_{128}			

3.5.2. Spectral data of isolated compounds

The spectroscopic techniques (UV, IR, NMR (both 1D and 2D) were employed to elucidate the structure of isolated compounds. The IR spectrum was recorded on a PerkinElmer model FTIR spectrometer using KBr pellets. The ^{1}H-NMR and ^{13}C-NMR were obtained in $CDCl_3$ on Bruker Avance 400 MHz spectrometer. Assignments and correlation were done on the basis of COSY, gHSQC and gHMBC experiments.

3.6. Biological Evaluation

The antibacterial activity s of crude extracts and isolated compounds were done in collaboration with the Department of Applied Biology, Adama Science and Technology University.

3.6.1. Antibacterial activity

In vitro antibacterial activity of crude extracts and isolated compounds were done against the bacterial species *S. aureus*, *E. coil*, *S. thphimurium* and *B. subtilis.* The bacterial cultures were inoculated into the nutrient broth (inoculation medium) and incubated overnight at 37 ^{o}C.

Inoculated medium containing 24 hr grown culture were added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. The solution was poured approximately 20 mL of sterile MHA in sterile culture plates and allowed to attain room temperature. Sterile agar-disc diffusion previously soaked in a known concentration (0.01 mg/mL and 0.5 mg/mL per disc) extract or pure compound of *B. antidysentrica* and standard drugs was prepared in DMSO using nutrient agar tubes and carefully placed at the centre of the labeled seeded plate. Mueller–Hinton sterile agar plates were seeded with indicator bacterial strains ($1.3 * 10^8$ cfu/mL) and allowed to stay at 37 °C for 3 hr. Control experiments were carried out under similar conditions using ciprofloxacin for antibacterial activity as a standard drug. The zones of growth inhibition around the disks were measured after 24 hr of incubation at 37 °C for bacteria. The inhibition zones were measured with a ruler and compared with the positive control disk (disk containing Ciprofloxacin) of the same concentration. DMSO used as negative control during the whole test on bacteria. The results were expressed as mean value ± standard deviation (Murai *et al*., 1995).

3.6.2. Antioxidant activity

DPPH assay: The free radical scavenging activity of the dichloromethane/methanol (1:1) extract, Methanol extract and isolated compounds were measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method (Egwaikhide, 2007). With this method, it is possible to determine the radical scavenging power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. As a result of the color changing from purple to yellow, the absorbance was decreased when the DPPH radical is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule (Nishibe *et al*., 1995). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity (Andrzejewska-Goleck *et al*., 1986).

The dichloromethane/methanol (1:1) extract was dissolved in four vials containing methanol to give 500, 250, 125 and 62 µg/mL. To each, 1 mL of the above extracts were added each 4 mL of 0.04 % DPPH which was given 100, 50, 25 and 12 µg/mL. The resulting solution was placed in an oven at 37 ^{o}C for 30 min and subjected to UV-Vis spectrophotometer to record absorbance at 517 nm. This was repeated for the acid-base extracts and isolated compounds. The percentage

DPPH inhibition was calculated according to the following formula (Maksyutina, 1971).

$$\% \text{ of radical scavenging activity} = \frac{Ab_{standard} - Ab_{analyte}}{Ab_{standard}} \times 100$$

3.7. Molecular docking study of isolated compounds for antibacterial activity

Molecular docking studies were performed in order to predict the interaction of synthesized compounds with the binding sites of DNA-gyrase. Topoisomerases are interesting antibacterial drug targets, since bacteria express two is forms, DNA gyrase and topoisomerase IV, which were both required for maintenance of proper DNA topology during transcription and replication (Duraipandiyan *et al.* 2014).

In this study, isolated compounds were subjected to molecular docking studies using the ADT version 1.5.2 and Auto Dock version 4.2 docking program to investigate the potential binding mode. Crystal structure of the enzyme (PHE196 and ALA51) with resolution 2.3 Å was chosen as the protein model. The structures of ligands were optimized using the HyperChem 7.0 software. Auto Dock version 4.2 was used to prepare the molecules and parameters before submitting it for docking analysis with Auto Dock. Polar hydrogen atoms were added while non-polar hydrogen atoms were merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, were allowed to rotate during the automated docking process and then prepared protein and ligand structures were saved in the PDBQT format suitable for calculating energy grid maps. A grid box size of 46×46×46 Å points with a grid spacing of 0.375 Å was considered. Lamarckian genetic algorithm (LGA) program with an adaptive whole method search in the Auto Dock was chosen to calculate the different ligand conformers. After 200 independent docking runs for each ligand, a cluster analysis was done. In according to the root mean square deviation (RMSD) tolerance of 2.0 Å conformations were clustered and ranked by energy of which the conformation with the best scored pose and the lowest binding energy was selected for the ligands (Mansourian *et al*., 2015; Morris *et al*., 1998).

4. Results and Discussion

4.1. Phytochemical screening

Phytochemical screening tests were done to determine the class of compounds present in the crude extracts following standard experimental protocols. The results suggested that alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, steroids, phytosterols and glycosides are present within the crude root extracts (Table 4). The results obtained in this study suggest the identified phytochemical compounds may be the bioactive constituents of this plant suggesting the plant as a valuable reservoir of bioactive compounds of substantial medicinal importance.

Table 4. Phytochemical screening results on root extracts of *Brucea antidysentrica*

Phytochemical screening	Test	DCM: MeOH (1:1)	MeOH 100%
Flavonoids	Alkaline test	+	+
Saponnins	Froth test	+	+
Phenols	Ferric chloride test	+	+
Tannins	Gelatin test	+	+
Terpenoids	Salkowski's test	+	+
Steroids	Salkowski's test	+	+
Phytosterols	Salkowski's test	+	+
Glycosides	Modified Borntrager's test	+	+
Alkaloids	Mayer test	+	+

+ indicates presence,

4.2. Characterization of compounds

Compound **1** was obtained as brown crystals with melting point of 155 ^{o}C. Its R_f value was 0.44 (50 % EtOAc in *n*-hexane as eluent). The spot turned orange when sprayed with Mayer reagent, which is an indication of an alkaloid. Its IR spectrum (Appendix 1) showed a broad absorption band at 3441.92 cm^{-1} suggesting the presence of hydroxyl (O-H) group. The absorption bands at 1744 cm^{-1}, 1637 cm^{-1}, 1462 cm^{-1} and 1230.05 cm^{-1} suggested the presence of carbonyl group, aromatic system (C=C), C=N and C-O stretching vibrations, respectively. Furthermore, the IR

absorptions at 2854.95 and 2927.92 cm^{-1} suggest sp^3 C-H stretching and sp^2 C-H stretching vibrations respectively.

The ^{1}H NMR spectrum (Appendix 2 and 3) revealed the existence of aromatic protons at δ 8.82 (*d*, H-13, *J*= 5), 8.67 (*d*, H-4, *J*= 8.2), 7.98 (*d*, H-1, *J*=5), 7.00 (*d*, H-4', *J*=9.9), 8.09 (*dd*, H-3', *J*=8.8, 12.7) allylic and ortho coupled with protons at δ 7.98 (*d*, H-1, *J*=4) and 7.00 (*d*, H-4', *J*=8), respectively, 7.55 (H-6, *t*, *J*=7.8) and 7.72 (H-7, *t*, *J*=7.8) (Table 3). The ^{13}C NMR and DEPT-135 spectra (Appendix 4 and 5) revealed a total of 18 well resolved carbon peaks. The presence of eleven aromatic carbon groups, one methine group (C-6'), one methyl and six quaternary carbon atoms (of which four of them were aromatic carbon) were all evident from the ^{13}C NMR spectrum. Furthermore, the ^{13}C NMR spectrum supported the existence of carboxylic acid carbonyl carbon at δ173.5 (C-9) and sp^2 oxygenated quaternary carbon at δ 159.5 (C-2' and C-5') (Table 5), respectively. The peaks at δ 67.8 and 62.3 suggest oxygenated methylene and methyl forming CH_2-O-CH_3 moiety which was also supported by DEPT-135 spectrum where the former peak is pointing down whereas the later pointing up. Peaks at δ 139.2 and 145.5 suggest olefinic methine near to heteroatom preferably next to nitrogen, supported by DEPT-135 spectrum. From NMR data peaks at δ 14-37 and 178.6 belong to fatty acid contamination which might co-elute with the compound. From the above NMR data and comparison with literature the compound was found to be derivative of flazin methyl ether (**1**). Flazin methyl ether previously isolated from juice of black currants (*Ribes nigrum* L.) (Blech and Budzikiewicz, 1994). However, the derivative of flazin methyl ether (**1**) is the first report from root of *B. antidysentrica.*

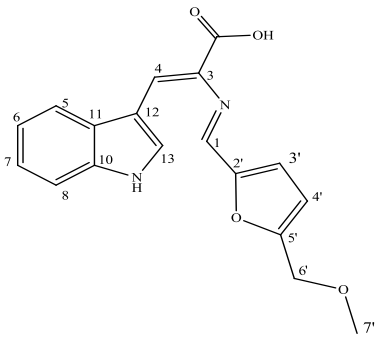

Figure 3. Structure of flazin methyl ether derivative (**1**)

Table 5. ^{1}H, ^{13}C and DEPT-135 NMR spectral data of flazin methyl ether derivative (**1**)

Position	**1**			**Flazin methyl ether** (Blech and Budzikiewicz, 1994).	
	^{1}H	**^{13}C**	**DEPT-135**	**^{1}H**	**^{13}C**
1	7.98(1H, *d*, J=5)	145.5	145.5	-	137.1
3	-	130.7	-	-	132.2
4	8.67 (1H, *d*, J= 8,2)	117.3	117.3	8.85(1H, *s*)	115.9
5	7.98 (1H, *d, J*=5)	127.9	127.9	8.41(1H, *d, J*=7.8)	122.0
6	7.55(*t, J=7.8*)	128.1	128.1	7.34(1H,*dd,J*=7.8/8.2)	120.5
7	7.72 (*t, J=7.8*)	129.1	129.1	7.65(1H,*dd,J*=7.8/8.2)	128.9
8	7.00(1H,*d, J*=9.9)	116.5	116.5	7.82(1H, *d, J*=8.2)	112.9
10		139.6	-	-	141.5
11		124.3	-	-	120.9
12		127.1	-	-	129.9
13	8.82(1H, *d, J*=5)	139.2	139.2	-	132.0
2’		159.5	-	-	151.7
3’	8.09(1H,*dd,J*=8.8/12.7)	116.5	116.5	7.45(1H, *d, J*=3.4)	111.1
4’	7.00(1H, *d, J*=9.9)	116.5	116.5	6.77(1H,*dd, J*=3.4)	111.9
5’	-	159.5	-		153.2
CO	-	173.5	-		166.5
CH_2	3.96(2H, *s*)	62.3	62.3	4.64(2H, *s*)	65.5
CH_3	3.31(3H,*s*)	68.7	68.7	3.34(3H, *s*)	57.2

Compound **2** was isolated as yellowish amorphous solid with melting point of 149 ^{o}C (literature value 155- 156 ^{o}C, Ohmto *et al*., 1976) and R_f value of 0.57 (80 % EtOAc in *n*-hexane as eluent). The IR (KBr) spectrum (Appendix 6) of the compound showed sharp absorption bands at 2924 cm^{-1} and 2854.54cm^{-1} were assigned to sp^2 C-H. The imine (C=N) and C=C stretching bands occurred at 1637 cm^{-1} and 1400 cm^{-1},respectively.

The ^{1}H NMR spectrum (Appendix 7) showed peaks at δ 8.07 (H-4, *d, J*=8.8), 7.54 (H-10, *t, J*=7.6), 7.72 (H-9, *t, J*=7.6) and 8.82 (H-2, *d, J*=5), 7.98 (H-1, *d, J*=5), 7.00 (H-5, *d, J*=9.8) and

8.11 (1H-11, *d, J*=7.8) that could be attributed to benzoyl moiety. The ^{13}C NMR and DEPT-135 spectrum (Appendix 8 and 9) of showed the presence of fourteen carbon signals and two nitrogens which were characteristic of canthin-6-one. The presence of aromatic peaks were observed between δ 116.4-159.5 of which six of them are quaternary sp^2 at δ 159.5, 139.5, 136.1, , 130.5, 132.1 and 124.4 and additional C=N, C-N and *n*--hexane contamination peaks are also observed. Upfeild chemical shift of carbonyl carbon at δ 159.5 (C-6) suggest that the carbonyl group is directly linked to nitrogen of indole moiety and it is also α, β-conjugated (δ 139.4 (C-4) and 129.0, C-5) suggesting that the compound have β-carboline alkaloid canthine-6-one skeleton.

The COSY spectrum (Appendix 10) supported correlations between H1↔ H2, H4↔H5, H8↔H9, H9↔ H10 and H10 ↔ H11(Table 4). The HSQC spectrum (Appendix11) suggested significant direct connectivity between C-1 and H-1, C-2 and H-2, C-4 and H-4, C-5 and H-5, H-8 and C-8, H-9 and C-9, H-10 and C-10 and H-11 and C-11 (Table 6). The HMBC spectrum (Appendix 12) showed correlations between H-1 and C-2, 12, correlations between H-2 with C-1,15,16, correlation between H-4 and C-6,9. From the HMBC correlations of H-5 with C-6,16, H-8 with C-12, H-9 with C-11,13, H-10 with C-8,12 and H-11 with C-13,15 (Table 6). Thus, based on the above spectral data and comparison with literature, compound **2** was suggested to be identical with canthin-6-one previously isolated from bark of *Picrolemma huberi* (Kazuo and Taichi, 1985) and roots of *Brucea mollis* (Ouyang *et al*., 1994).

Table 6. ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC NMR spectral data of canthin-6-one (**2**)

Position	**2**						Canthin-6-one (Ouyang et al., 1994).	
	^{1}H	^{13}C	DEPT-135	COSY	HSQC	HMBC	^{1}H	^{13}C
1	7.98	116.4	116.5	$H_1 \leftrightarrow H_2$	$H_1 \rightarrow C_1$	$H1 \rightarrow C_{2,12}$	7.59(1H, *d*, *J*=5)	115.4
2	8.82	145.7	145. 7		$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,\ 15,16}$	8.58(1H, *d*, *J*=5)	144.8
4	8.07	139.4	139.4	$H_4 \leftrightarrow H_5$	$H_4 \rightarrow C_4$	$H_4 \rightarrow C_{6,9}$	7.77(1H, *d*, *J*=9.7)	138.6
5	7.00	129.0	129.0		$H_5 \rightarrow C_5$	$H_5 \rightarrow C_{6,16}$	6.75(1H, *d*, *J*=9.7)	127.9
6	-	159.5						158.2
8	8.69	117.3	117.3	$H_8 \leftrightarrow H_9$	$H_8 \rightarrow C_8$	$H_8 \rightarrow C_{12}$	8.28(1H, *d*, *J*=7.7)	116.3
9	7.72	130.9		$H_9 \leftrightarrow H_{10}$	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{11,13}$	7.45(1H, *t*, *J=7.7*)	129.8
10	7.54	125.7	125.7	$H_{10} \leftrightarrow H_{11}$	$H_{10} \rightarrow C_{10}$	$H_{10} \rightarrow C_{8,12}$	7.28(lH, *t*, *J=7.7*)	124.7
11	8.11	122.7	122.7		$H_{11} \rightarrow C_{11}$	$H_{11} \rightarrow C_{13,15}$	7.73(1H, *d*, *J*=7.7)	121.6
12	-	124.4						123.3
13	-	139.5						138.2
14	-	130.5						128.9
15	-	132.1						130.9
16	-	136.1						135.2

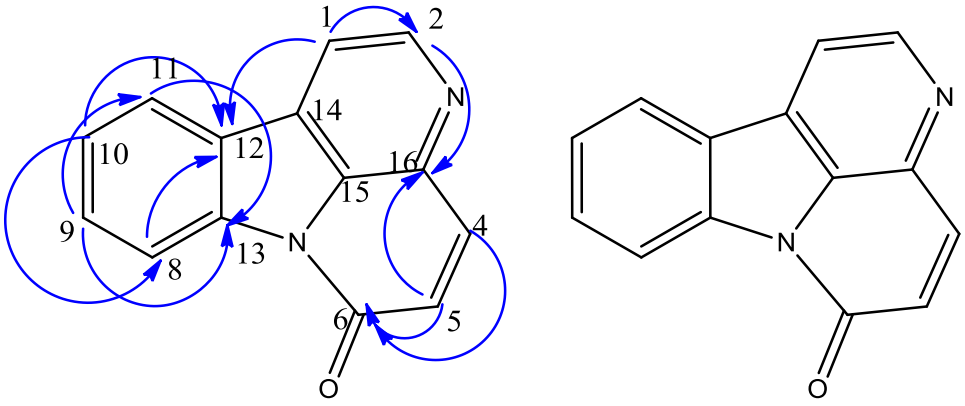

Figure 4. Proposed structure of canthin-6-one **(2)**

Compound **3** was isolated as yellowish amorphous solid with melting point of 180 ^{o}C and R_f value of 0.41 (20 % ethyl acetate in *n*-hexane as eluent). The IR spectrum of the compound

showed the presence of SP^3 C-H and SP^2 C-H band at 2924 and 2872 cm^{-1}, C=O band at 1744 cm^{-1}, C=C and C=N at 1644 cm^{-1} and C-O at 1080 and C-N at 1398 cm^{-1}.

The ^{1}H NMR spectrum (Appendix 14) revealed the presence of two three-proton singlet at δ 4.08 (3H) and 4.23 (3H) attributed to methoxy protons, the presence of aromatic protons were observed at δ 8.5 (*s*, H-1), 8.38 (*d*, *J*=8.2, H-8), 7.97 (*d*, *J*=9.8, H-4), 7.65 (*t*, *J*=8.2, H-9), 7.01 (*d*, *J*=8.3, H-5) and 6.86 (*d*, *J*=9.8, H-10). The ^{13}C NMR and DEPT-135 spectra (Appendix 15-16) showed two methoxy carbon atom signals appearing at δ 57.7 and 56.2. The presences of aromatic peaks were observed at δ 160.4, 155.9, 151.3, 140.1, 139.2, 132.1, 132.9, 131.6, 130.5, 113.1, 109.8 and 107.9. In agreement with the spectral data of **3**, upfeild chemical shift of carbonyl carbon at δ 160.4 (C-6) suggest that the carbonyl group is directly linked to nitrogen of indole moiety and it is also α,β-conjugated (δ 139.15 (C-4) and 125.1 C-5) suggesting that the compound have β-carboline alkaloid canthine-6-one skeleton, supported by DEPT-135 spectrum.

Close inspection of the 2D-NMR spectra (Appendix 17-19) showed the following correlations. COSY (Appendix 17) spectrum showed correlations between aromatic protons appearing at δ 7.97 (H-4) and δ 6.86 (H-5), δ 8.38 (H-8) and δ7.65 (H-9), δ 7.65 (H-9) and δ 7.01 (H-10). HSQC spectrum (Appendix 18) showed ^{1}J correlations between aromatic and methoxy protons appearing at δ 8.5, 7.97, 6.86, 8.4, 7.65, 7.01, 4.23 and 4.08 with δ 132.07, 139.2, 125.1, 109.8, 131.6, 107.9, 57.7 and 56.2, respectively (Table 7). HMBC spectrum (Appendix 19) showed correlations between H-2 with C-1, 11, 14, H-5 with C-6,9, H-8 with C-10,14, H-9 with C-1,16 H-10 with C-1,8,14. The HMBC spectrum also indicated correlations of the methoxy protons at δ 4.23 (H-17) and 4.08 (H-18) with C-11 and C-1, respectively. Thus, based on the above spectral data (Table 7) and extensive comparison with literature, compound **3** was identified as 1,11-dimethoxycanthin-6-one (**3**), previously reported from stem of *B. antidysentrica* (Narihiko *et al*., 1986).

Table 7. ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC NMR spectral data of compound **3**

Position	compound **3**			COSY data	HSQC data	HMBC data	**1,11-dimethoxychantin-6-one** (Narihiko *et al*., 1986)	
	^{1}H NMR	^{13}C NMR	DEPT-135				Reported ^{1}H NMR	Reported ^{13}C NMR
1	-	155.9	-				-	155.6
2	8.5 (1H, *s*)	132.1	132.1		$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,9,11,14}$	8.41 (1H, *s*)	131.1
4	7.97(1H,*d*,*J*=9.8)	139.2	139.2	$H_4 \leftrightarrow H_5$	$H_4 \rightarrow C_4$	$H_4 \rightarrow C_{6,13}$	7.9(1H,*d*,*J*=10)	139.0
5	6.86(1H,*d*,*J*=9.8	125.1	125.1		$H_5 \rightarrow C_5$	$H_5 \rightarrow C_{6,9}$	6.79(1H,*d*,*J*=10	131.8
6	-	160.4	-				-	160.2
8	8.38(1H,*d*,*J*=8.2)	109.8	109.788	$H_8 \leftrightarrow H_9$	$H_8 \rightarrow C_8$	$H_8 \rightarrow C_{10,14}$	8.29(1H,*d*,*J*=8)	109.5
9	7.65(1H,*t*,*J*=8.2)	131.6	131.6	$H_9 \leftrightarrow 10$	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{1,16}$	7.84(1H,*t*, *J*=8)	124.8
10	7.01(1H,*d*,*J*=8.3)	107.9	107.9		$H_{10} \rightarrow C_{10}$	$H_{10} \rightarrow 1,8,14$	6.86(1H,*d*,*J*=8)	107.5
11	-	151.3	-				-	151.0
12	-	113.1	-				-	116.65
13	-	132.9					-	132.6
14	-	113.1	-				-	112.8
15	-	130.5	-				-	130.2
16	-	140.1	-				-	139.8
OCH_3	4.23(3H,s)	57.7	57.7			$H_{17} \rightarrow C_{11}$	4.21(3H,s)	57.4
OCH_3	4.08(3H,s)	56.2	56.2			$H_{18} \rightarrow C_1$	4.06(3H,s)	56.22

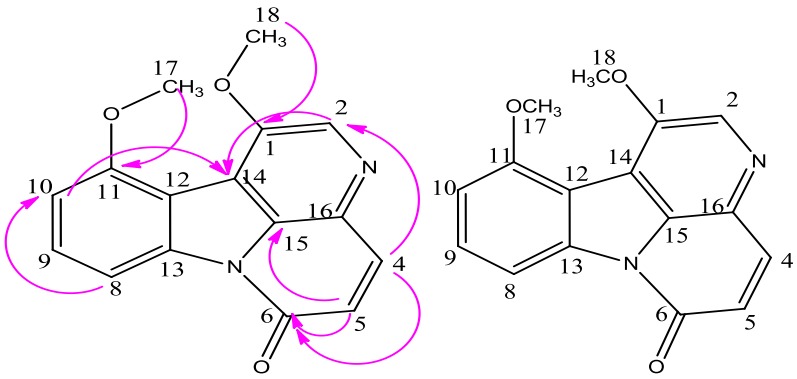

Figure 5. Structure of 1, 11-dimethoxycanthin-6-one (**3)**

4.3. Biological activity of crude extract and isolated compounds of *Brucea antidysentrica*

4.3.1. Antibacterial activity

The crude dichloromethane: methanol (1:1), acid-base crude extracts and isolated compounds were tested for their antibacterial activity *in-vitro* against *S. aureus, E. coli, B. subtilis* and *S. thphimurium* at different concentration (0.01 and 0.5 mg/mL). Ciprofloxacin was used as positive control. The zone of inhibition values indicated that all the compounds (0.01 and 0.5 mg) exhibited a varied range of 5.3-12.66 mm of antibacterial activities (Table 8) against all the tested bacterial strains. The antibacterial activity of crude extracts and compounds at 0.01 mg weaker than at 0.5 mg. This indicated that the antibacterial activities were increased with increased concentration of crude extracts and compounds. Compound **2** (0.5 mg) showed strong activity (12.66±0.6 and 12.33±2.31 mm) against *E. coli* and *S. thphimurium* and weak zone of inhibition (antibacterial activity) against *S. aureus* compared to ciprofloxacin (27.7±2.52 and 29±1.00 mm). Compound **3** (0.5 mg) showed strong antibacterial activity (12.5±0.87 and 12.3±1.65 mm) against *S. thphimurium* and *B. subtilis* comparable activity against *E. coli* and *S. aureus* compared to ciprofloxacin (29±1.00 and 33.3±3.22 mm). The results of the antibacterial activity showed that the dichloromethane: methanol (1:1), acid-base crude extracts and compound displayed good antibacterial activity against *E. coli, S. aurous, S. thphimurium* and *B. subtilis***.**

Table 8. Zone of bacterial growth inhibition diameter (mm)

Sample (0.01mg/mL)	**Inhibition diameter (mm)** ±SD			
	E. coli	***S. thphimurium***	***B. subtilis***	***S. aureus***
Methanol/dichloromethane (1:1) extract	8.66±1.53	10.33±1.53	11.33±0.58	7.66±0.58
Acid-base extraction	9±1.00	10.33±1.15	9±1.00	5.3±2.85
Compound **1**	11.6±2.30	10.33±1.41	8.3±0.58	9±1.00
Compound **2**	11.33±2.52	9±1.22	9±1.00	10±1.00
Compound **3**	9±2.00	10±0.00	11.6±3.79	9.3±2.08
Ciprofloxacin	25±1.00	27.3±0.58	29±1.00	20.6±1.16
Sample (0.5 mg/mL)	**Inhibition diameter (mm)** ±SD			
	E. coli	***S. thphimurium***	***B. subtilis***	***S. aureus***
Methanol/dichloromethane (1:1) extract	11.6±1.53	10.66± 1.15	11.33±1.15	9.33±0.58
Acid-base extraction	11.33±2.08	11.0±1.73	12.0±3.74	11.33±0.58
Compound **1**	11.33±0.58	11.66±3.06	11.33±1.53	11.0±1.00
Compound **2**	12.66±0.60	12.33±2.31	11.0±0.707	9.6±1.16
Compound **3**	11.3±0.58	12.5±0.87	12.3±1.65	10±1.00
Ciprofloxacin	27.3±2.52	29±1.00	33.6±3.222	23.5±0.58

SD = standard deviation

4.3.2. Antioxidant activity

DPPH is a simple method; it is possible to determine the radical scavenging power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. In the DPPH scavenging assay, crude extracts and pure compounds of *B. antidysentrica* were investigated for their free radical scavenging activity via their reaction with the stable DPPH radicals. The reduction of the DPPH was followed via the decrease in absorbance at 517 nm. The various crude extracts and pure compounds of *B. antidysentrica* significantly reduced the DPPH (Table 9). The DPPH radical scavenging activities (%) of extracts and isolated compounds were found to be 87.5 (compound **2**), 85.3 (compound **1**), 83.3 (DCM: methanol (1:1) extract), 80.00 (Acid-base extract**)** and 78.4 (compound **3**) respectively at 100 µg/ml (Table 9). It was observed that the DPPH scavenging activity increased with increasing concentration of the samples in the assay. For the various concentrations, compound **2** exhibited slightly highest percent inhibition of the DPPH as compared to the other extracts and isolated compounds. The positive control,

ascorbic acid showed maximum scavenging effect at very low and high concentration (75, 85, 95 and 97 % at 12, 25, 50 and 100 µg/ml) (Figure 6).

Table 9. % radical scavenging activity of crude extracts and compounds isolated from roots of *B. antidysentrica*

Conc (µg/ml)	Samples									
	DCM:MeOH(1:1)extract		Acid-base extract		compound **1**		compound **2**		Compound **3**	
	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity
100	0.2	83.3	0.26	80	0.15	85.3	0.1	87.5	0.28	78.4
50	0.3	75	0.27	79.2	0.23	77.4	0.2	83.3	0.31	76.1
25	0.35	70.8	0.3	76.9	0.3	70.5	0.3	75	0.34	73.8
12	0.4	66.6	0.4	69.2	0.4	60.7	0.38	68.3	0.45	65.4

Conc-Concentration, A- Absorbance

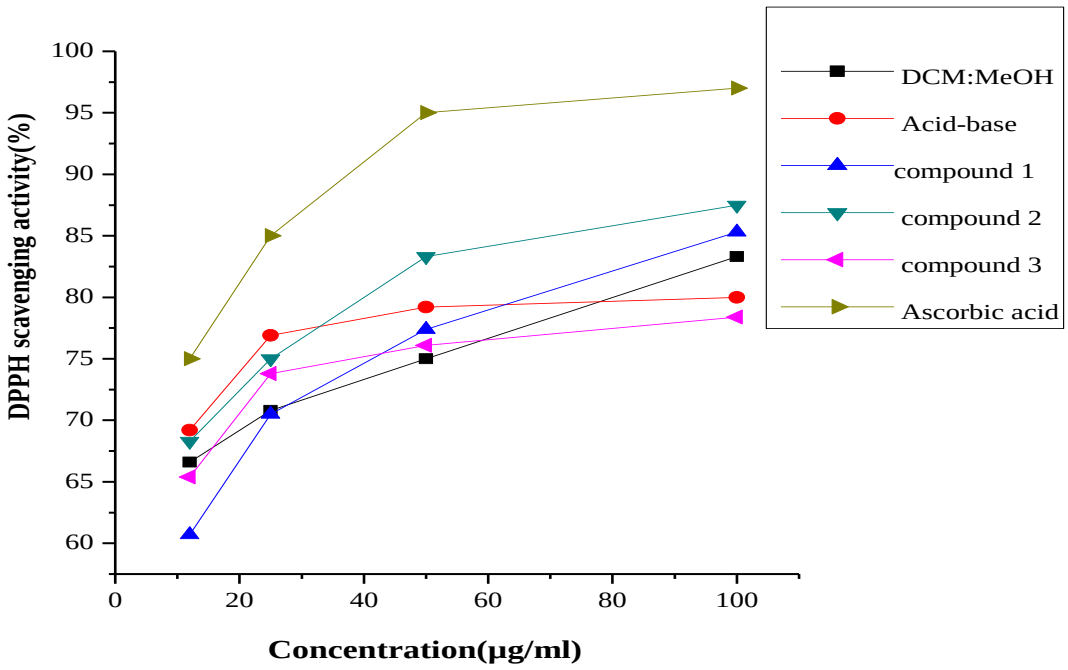

Figure 6. DPPH scavenging activity (%) of *B. antidysentrica* extracts, isolated compounds and positive reference (L-Ascorbic acid)

4.5. Docking study

Molecular docking studies were performed for three alkaloids in order to predict the interaction of ligand molecules with the binding sites of DNA-gyrase. In docking study, all the compounds were found to have minimum binding energy ranging from -5.8 to -4.8 KJ/mol. On comparative basis compounds **1, 2** and **3** revealed minimum binding energy of -5.8, -5.7 and -5.7 kcal/mol, respectively (Table 10). These molecules were unwrapped by active site amino acid residues at the active site pocket region (Figure 7). The protein comprises of fourteen active site amino acid residues, which are promiscuous to the ligands. Out of which only two (PHE196 and ALA 51) were found to interact directly with the ligands. The other residues were in close proximity to the inhibitor and hydrophobic in nature. Compound **1** was found to show hydrogen bond interaction with active site amino acid residue PHE196 at a distance of 1.5A^{o} and compound **3** was found to show hydrogen bond interaction with active site amino acid residue ALA 51 at a distance of 1.5A^{o}. From the figure yellow dashed line indicates a possible hydrogen bond formed between the connections residues (Figure 7) and red color also indicated the hydrogen bond interaction (Figure 8). Compound **2** had no hydrogen bond interaction with the protein. Compound **1** showed two hydrophobic interactions with TRY218 and GLU219, compound **2** showed six hydrophobic interactions with VAL201, LEU52 LYS57, GLY200 ASN198 and ALA52; compound **3** with ASN 198, LEU52, VAL201 and LYS57. All ligands displayed no pi to pi and pi to cation interactions with the protein. From this study, it can be concluded that compounds **1-3** may act as potential inhibitors of DNA gyrase enzyme.

Table 10. Docking score of DNA gyrase protein and isolated compounds (**1-3**)

Mode	Derivative of flazin methyl ether (**1**)			Canthin-6-one (**2**)			1,11-dimethoxycanthin-6-one (**3**)		
	Affinity (Kcal/mol)	dist from best mode		Affinity (Kcal/mol)	dist from best mode		Affinity (Kcal/mol)	dist from best mode	
		rmsd i.b	rmsd u.b		rmsd i.b	rmsd u.b		rmsd i.b	rmsd u.b
1	-5.8	0.000	0.000	-5.7	0.000	0.000	-5.7	0.000	0.000
2	-5.5	8.225	10.733	-5.7	22.422	25.040	-5.3	1.873	4.233
3	-5.5	1.802	5.669	-5.6	1.130	3.936	5.2	2.391	4.508
4	-5.4	8.135	11.181	-5.5	12.175	13.368	-5.0	15.021	16.977
5	-5.3	2.110	3.485	-5.3	22.536	25.215	-5.0	15.786	17.759
6	-5.3	7.920	11.682	-5.2	14.292	15.937	-4.9	16.498	18.132
7	-5.3	23.777	26.168	-5.2	14.977	16.780	-4.8	16.396	18.263
8	-5.1	7.742	10.427	-5.2	14.559	0.000	-4.8	13.336	15.474
9	-5.1	8.762	11.879	-5.1	14.289	25.040	-4.8	14.126	16.244

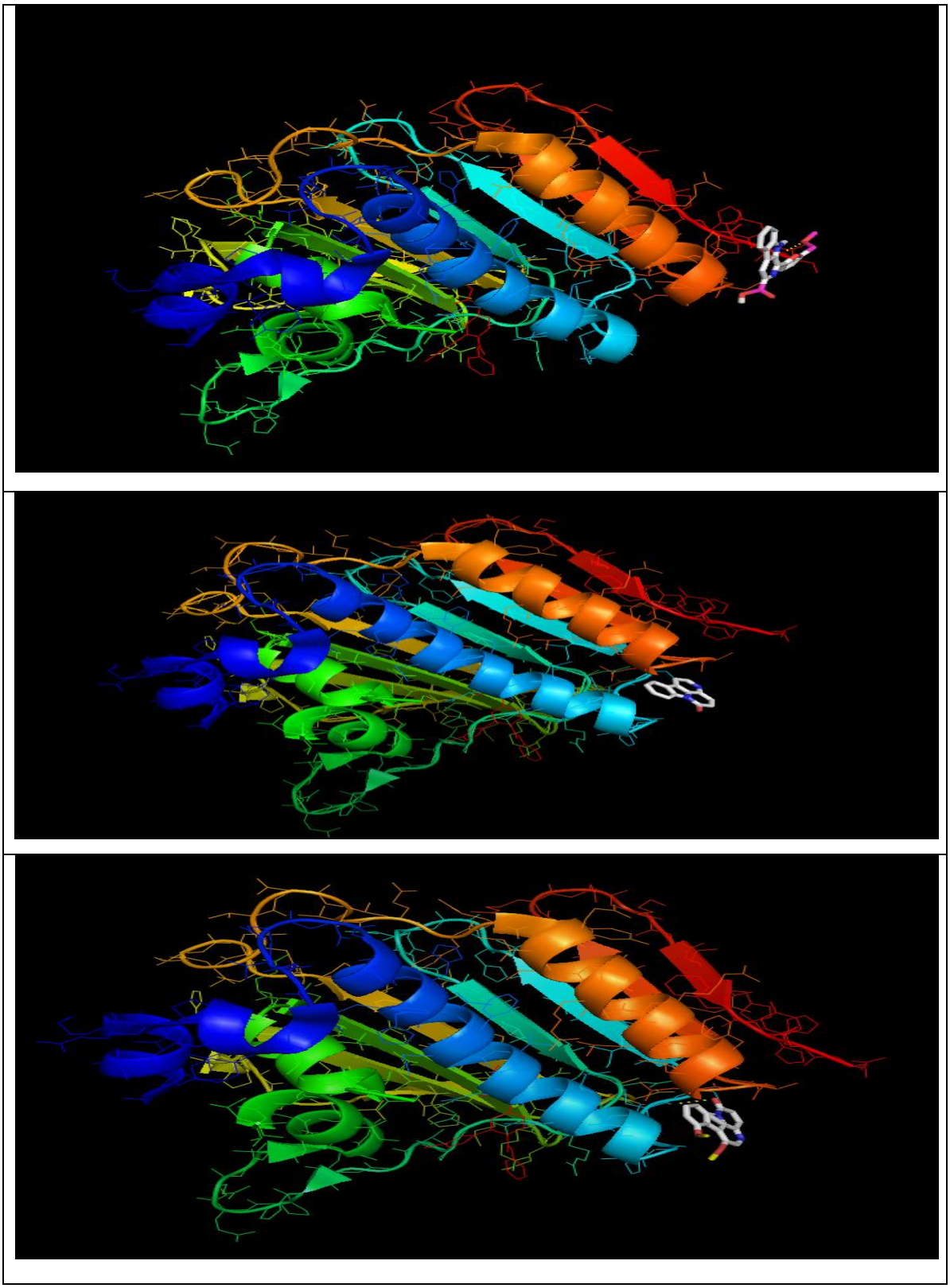

Figure 7. Molecular docking of ligand molecules (compounds **1**-**3**) with DNA gyrase receptor

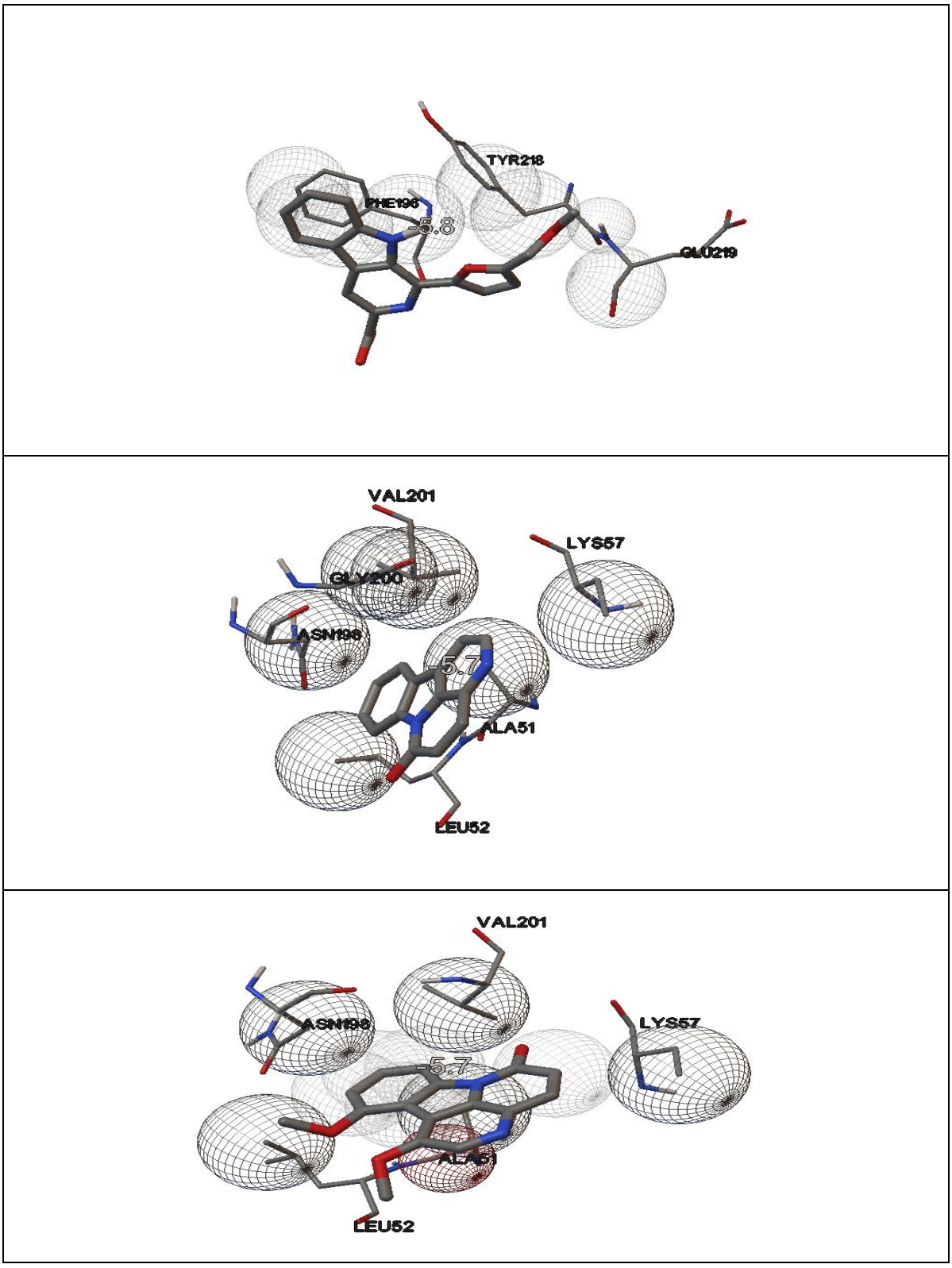

Figure 8. H-bond and hydrophobic interaction of ligand molecules (**1-3**) with DNA gyrase protein

5. Conclusion and Recommendation

5.1. Conclusion

For decades traditional medicines have been used and continue to be used as an alternative approach on treatment for various diseases caused by protozoa, bacteria, fungi, viruses and helminthes. Currently, the growing interest of consumers in substances of natural origin in association with the increasing concern surrounding potentially harmful infections disease has directed to a rising interest in the use of plant extracts as functional ingredients in many pharmaceutical products. *B. antidysentrica* is one of these medicinal plants used traditionally to heal various infectious diseases. In this study, the phytochemical screening tests showed that crude extracts of root *B. antidysentrica* plants are rich in alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, steroids, phytosterols and glycosides. Chromatographic separation of the crude extracts furnished one novel derivative of flazin methyl ether derivative (**1**), canthine-6-one (**2**) and 1,11-dimethoxycanthin-6-one (**3**). The extracts and isolated compounds were also evaluated *in vitro* for antibacterial activities using the disc diffusion method against *E coli*, *S. aureus, S. thphimurium* and *B. subtilis***.** Strong antibacterial activity (12.66±0.60 mm) was observed for compound **2** against *E coli*, and 12.5±0.87 mm inhibition zone was observed for 1,11-dimethoxycanthin-6-one (**3**) against *S. thphimurium* compared to standard ciprofloxacin (27.3±2.52 and 29±1.00 mm). Compound (**1**) and (**2)** had slightly highest scavenging of DPPH free radical than that of the positive control. Molecular docking study was done using Autodock and it was found that among isolated compounds shows the highest and best scoring pose (lowest energy) which was -5.8 Kcal/mole of compound **1**, -5.7 Kcal/mol of compound **2** and -5.7 Kcal/mol of compound **3**. The finding of these pharmacologically important secondary metabolites, biological evaluation and molecular docking from root extracts brings the attention of experts to look more on the medicinal importance of the plant.

5.2. Recommendation

These observations promote further research work with biological activity (antifungal, anticancer and cytotoxicity) on crude extracts as well as isolated compounds. Additional research efforts are also recommended to conduct structure-activity relationships, 2D NMR and MS studies to have a better understanding of structural and chemical parameters required to develop related drug lead compounds.

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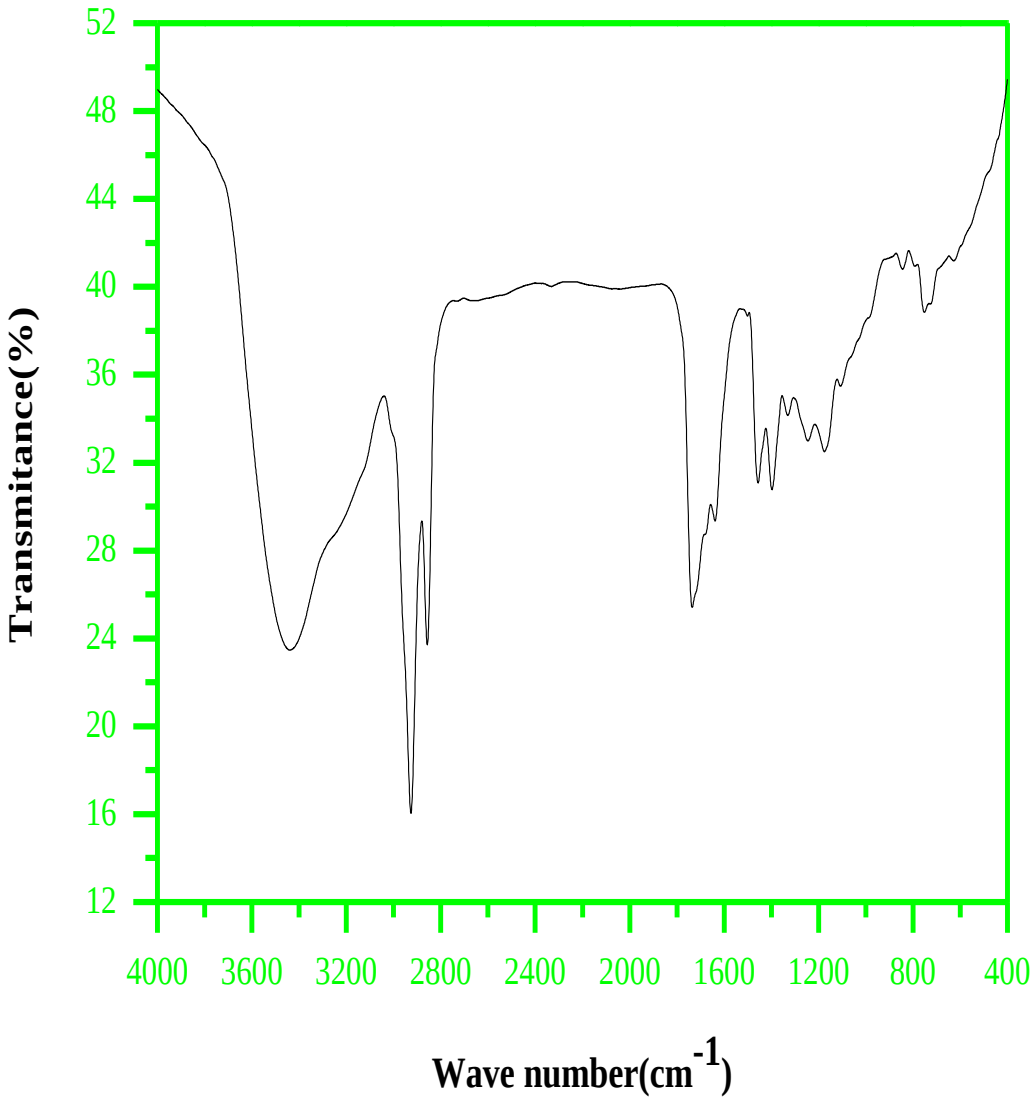
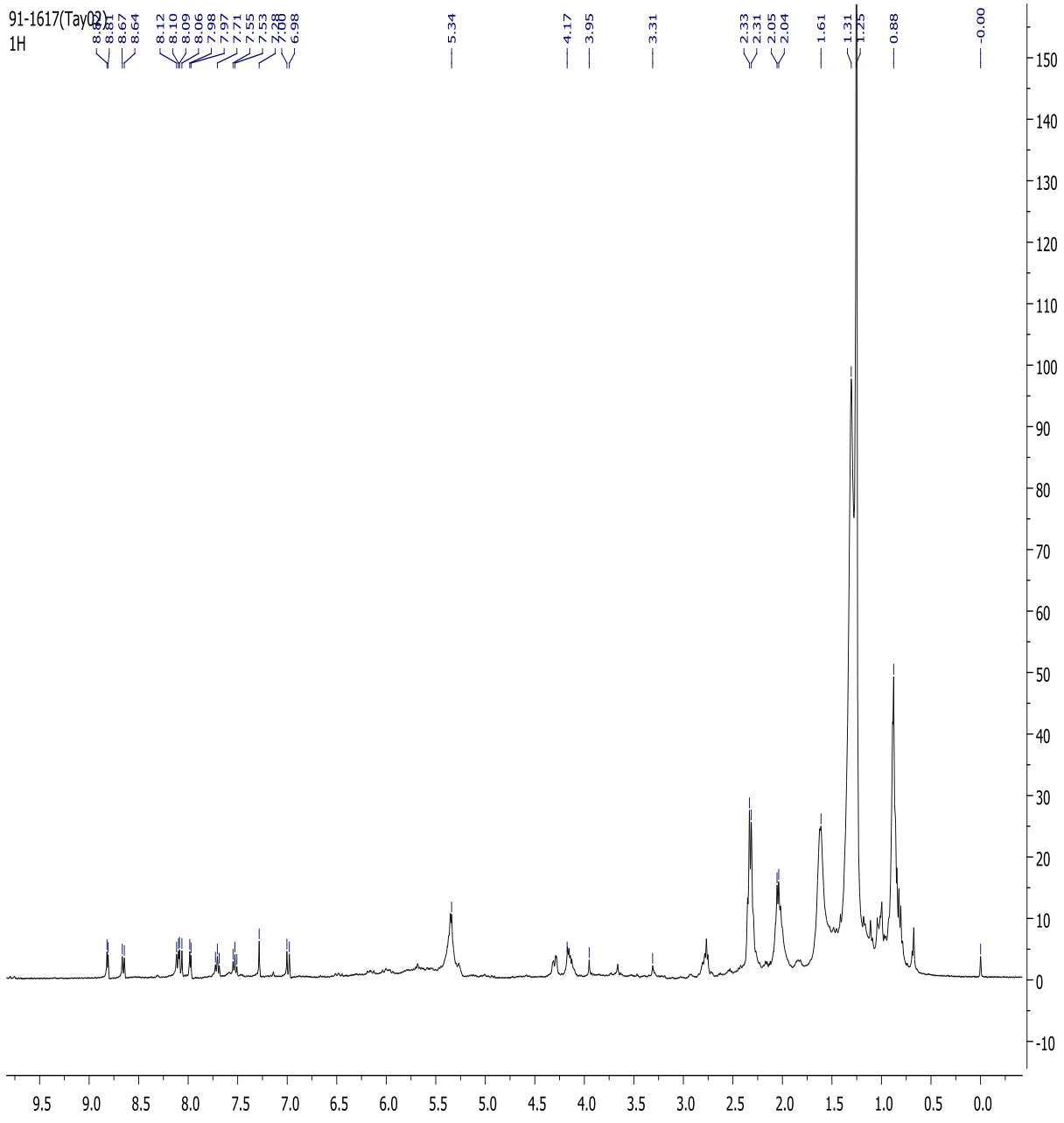
Appendixes

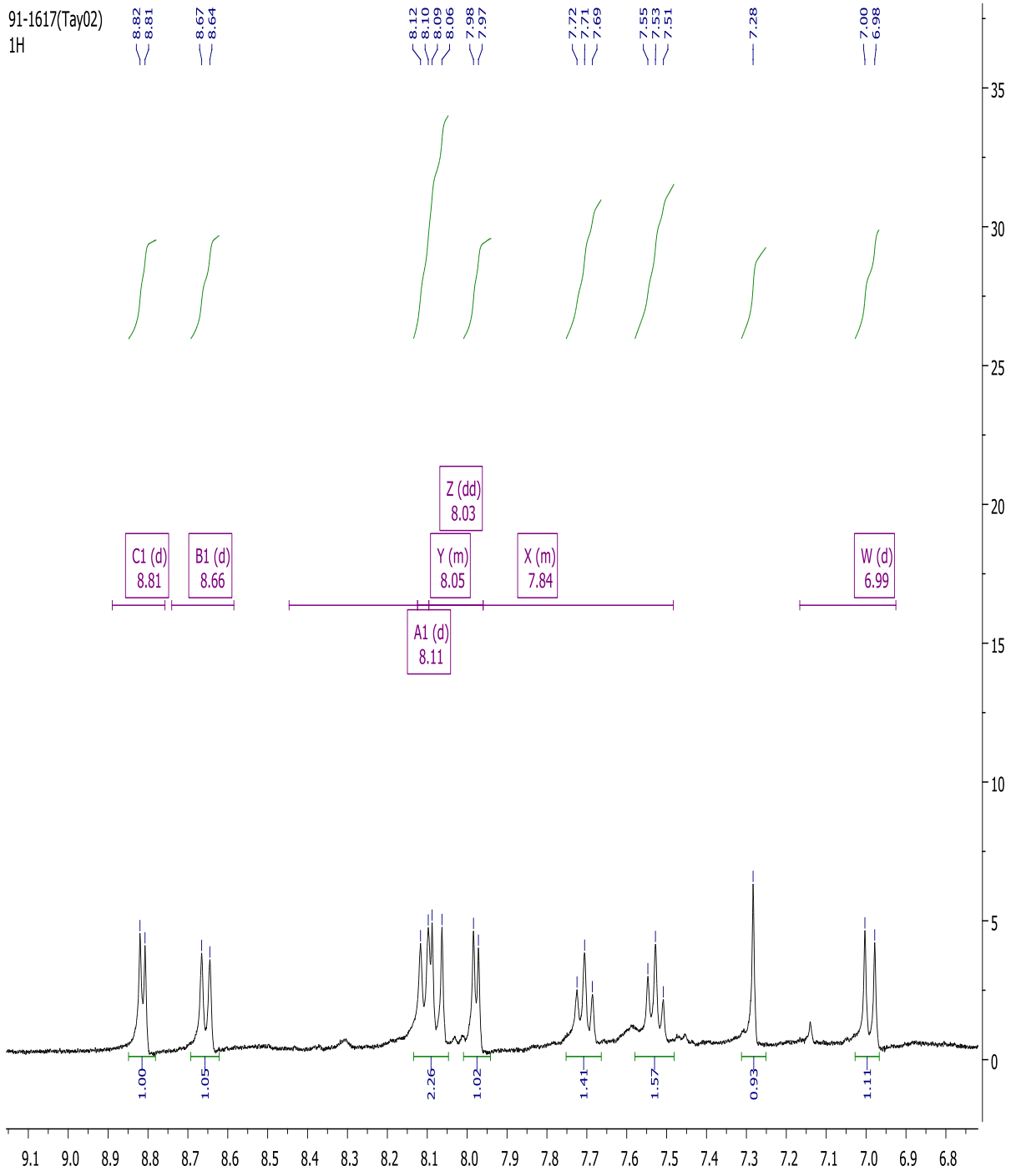
Appendix 1: IR spectrum of compound **1**



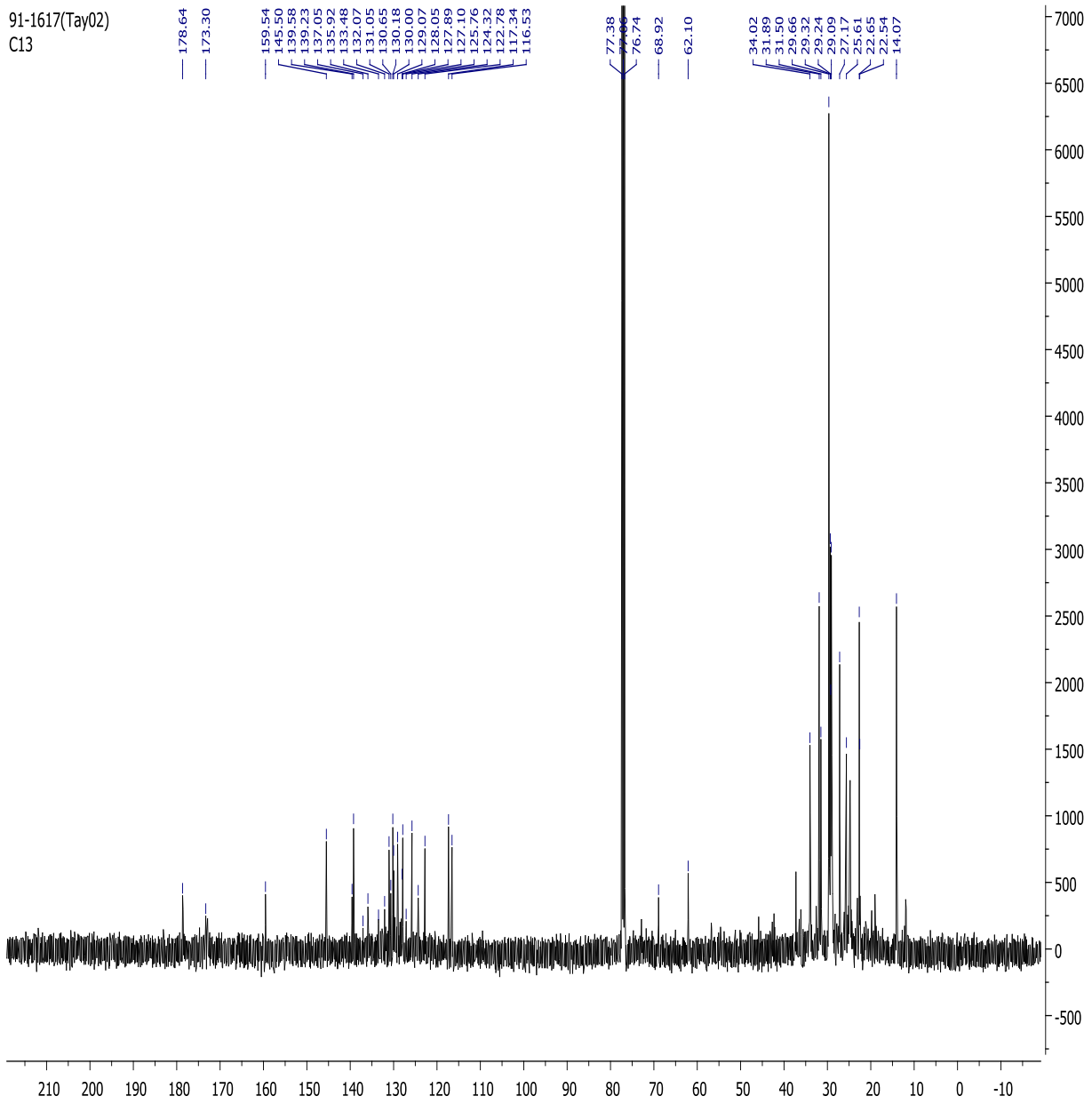
Appendix 2: ^{1}H NMR spectrum of compound **1**



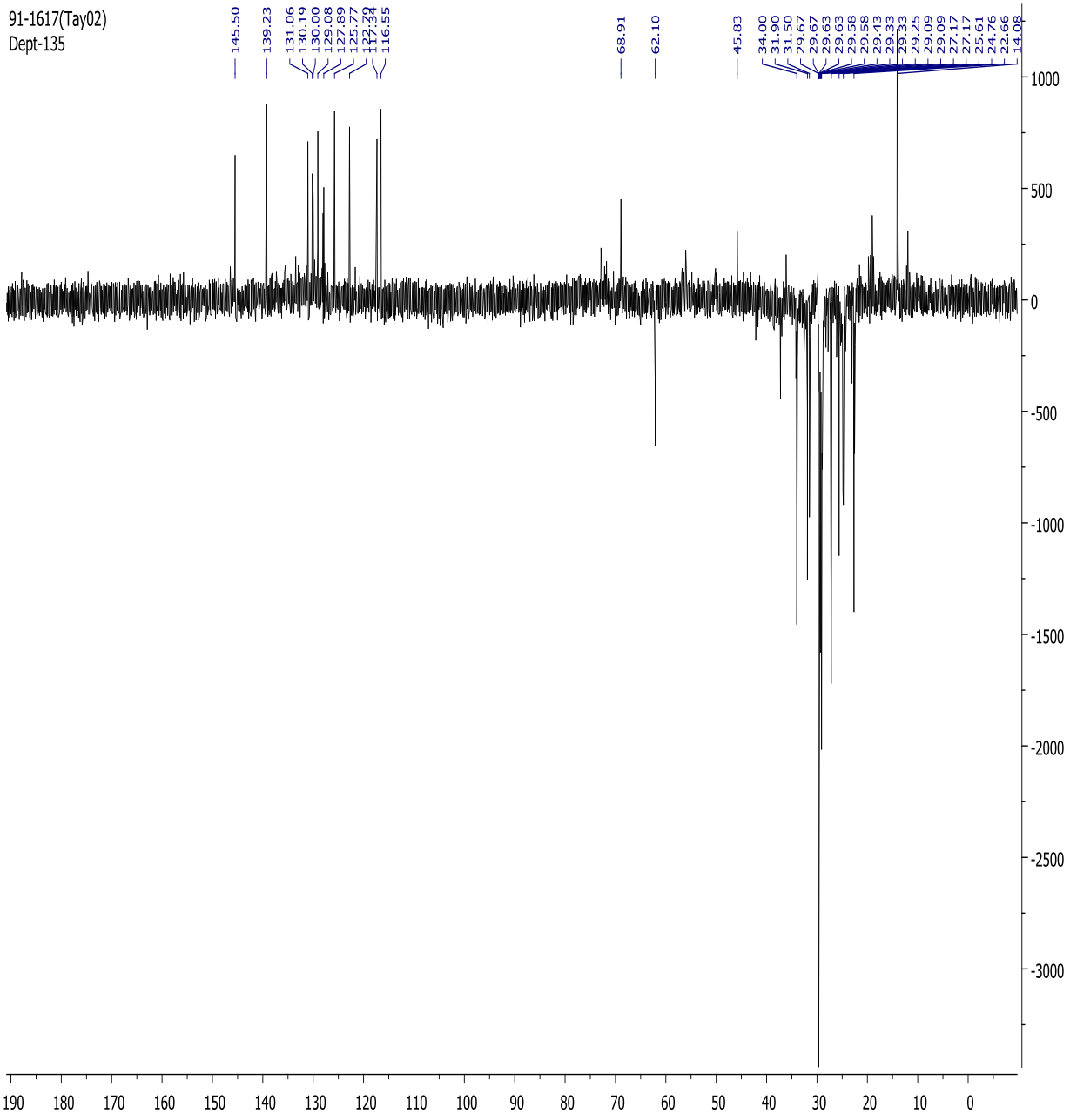
Appendix 3: ^{1}H NMR (from 6-9 ppm) spectrum of compound **1**



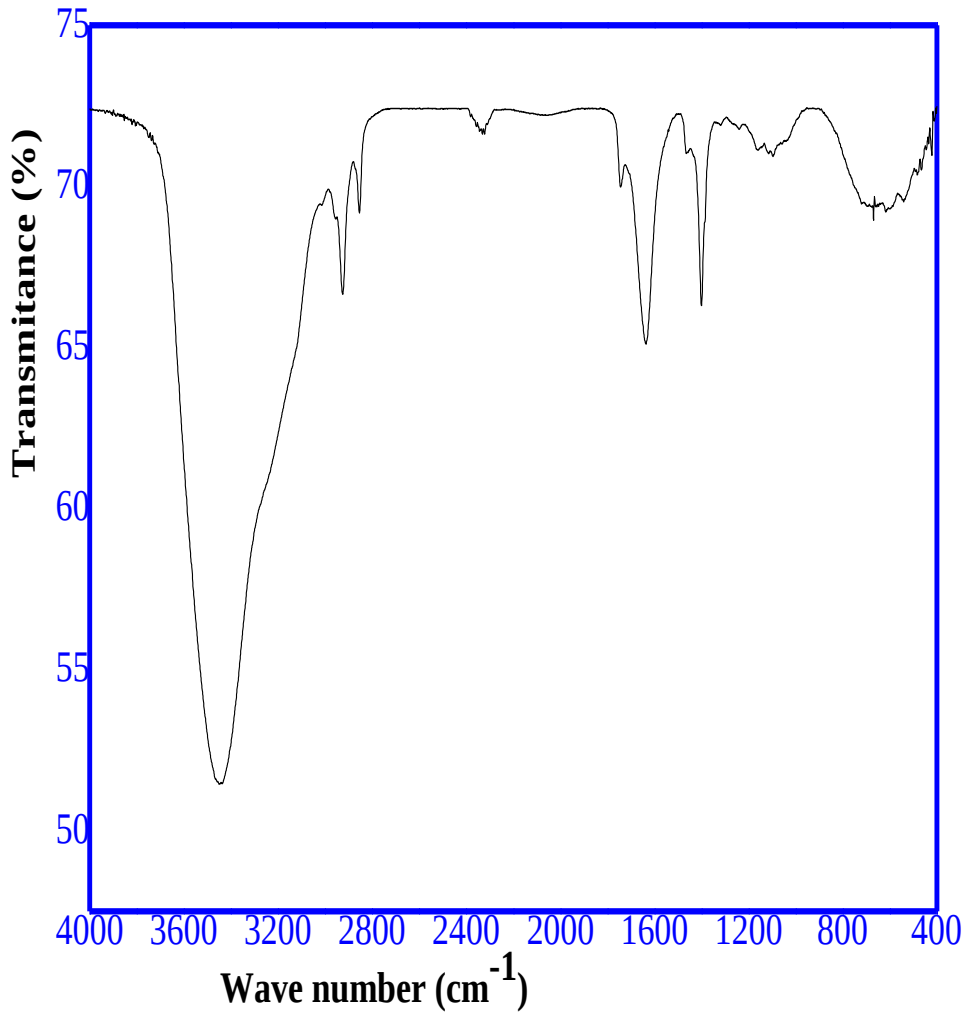
Appendix 4: ^{13}C NMR spectrum of compound **1**



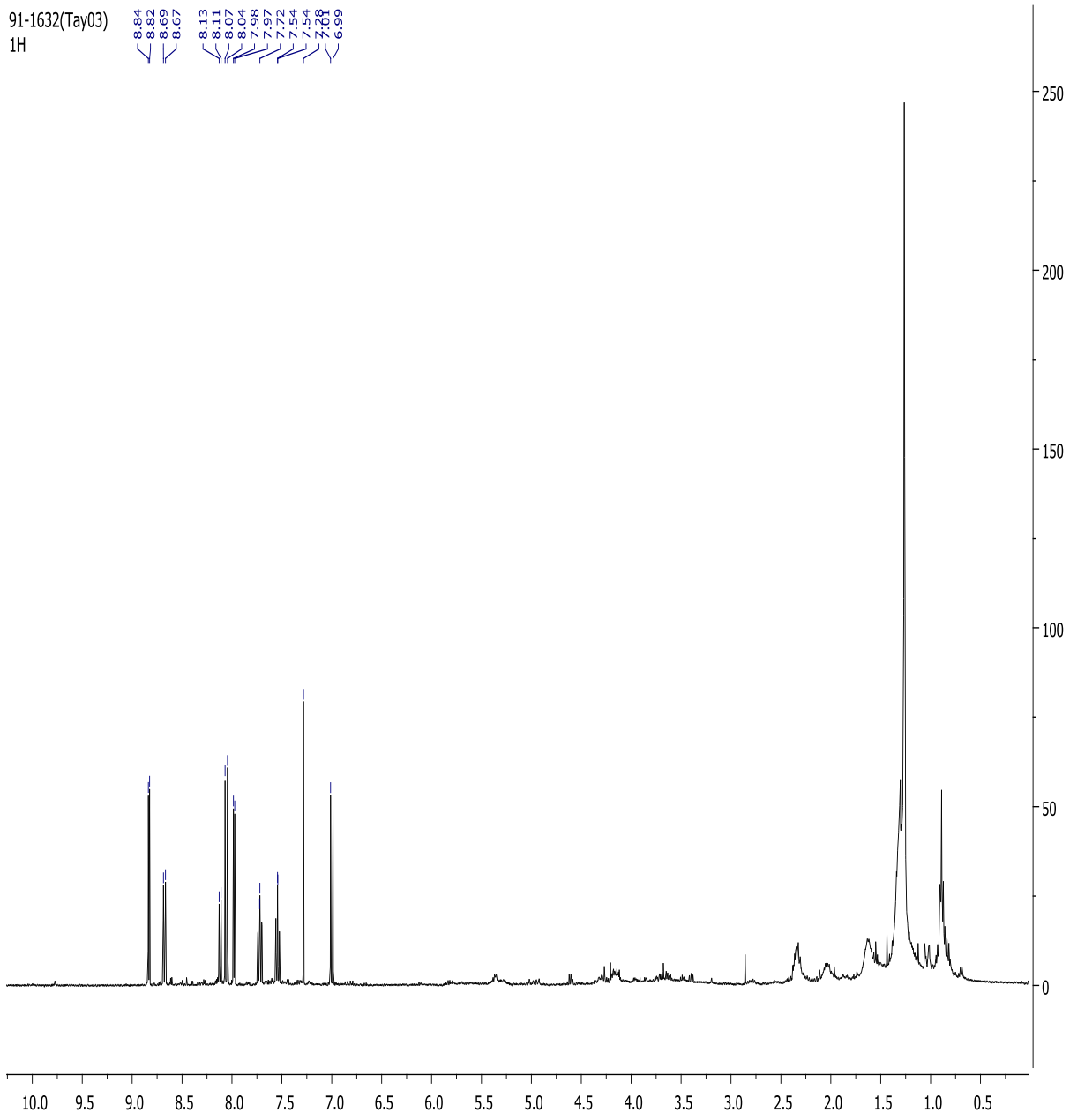
Appendix 5: DEPT-135 spectrum of compound **1**



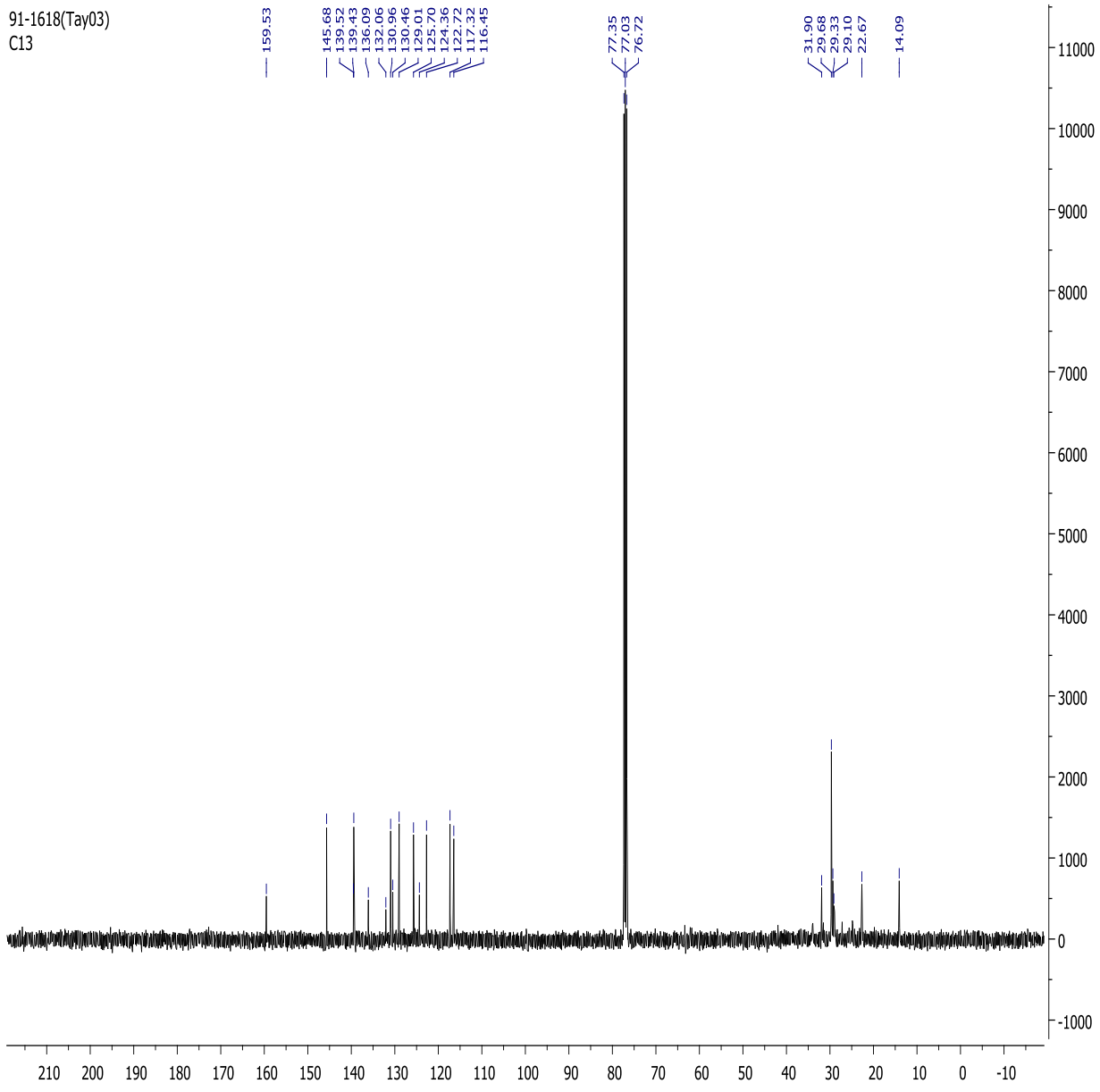
Appendix 6: IR spectrum of compound **2**



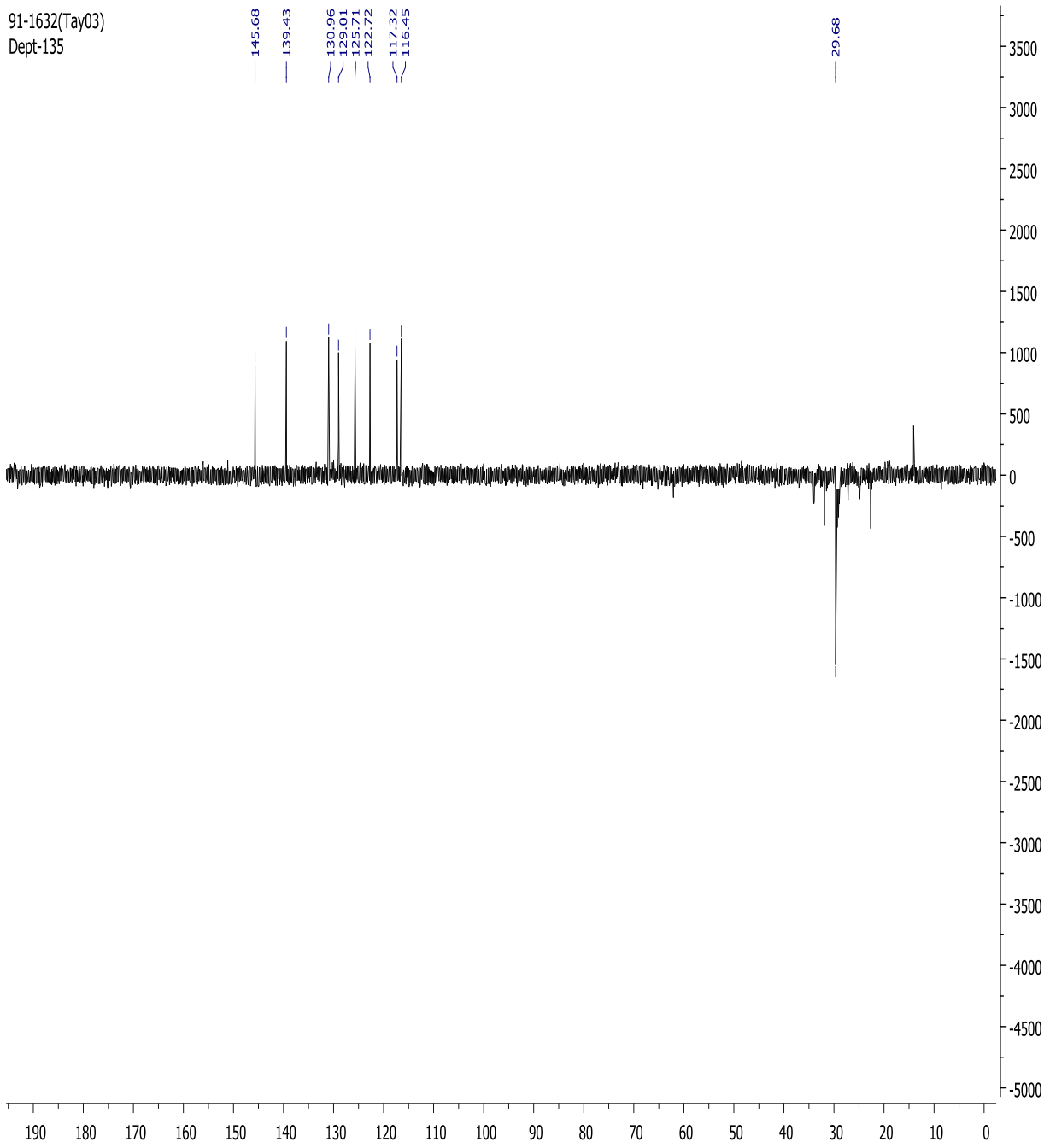
Appendix 7: ^{1}H NMR spectrum of compound **2**



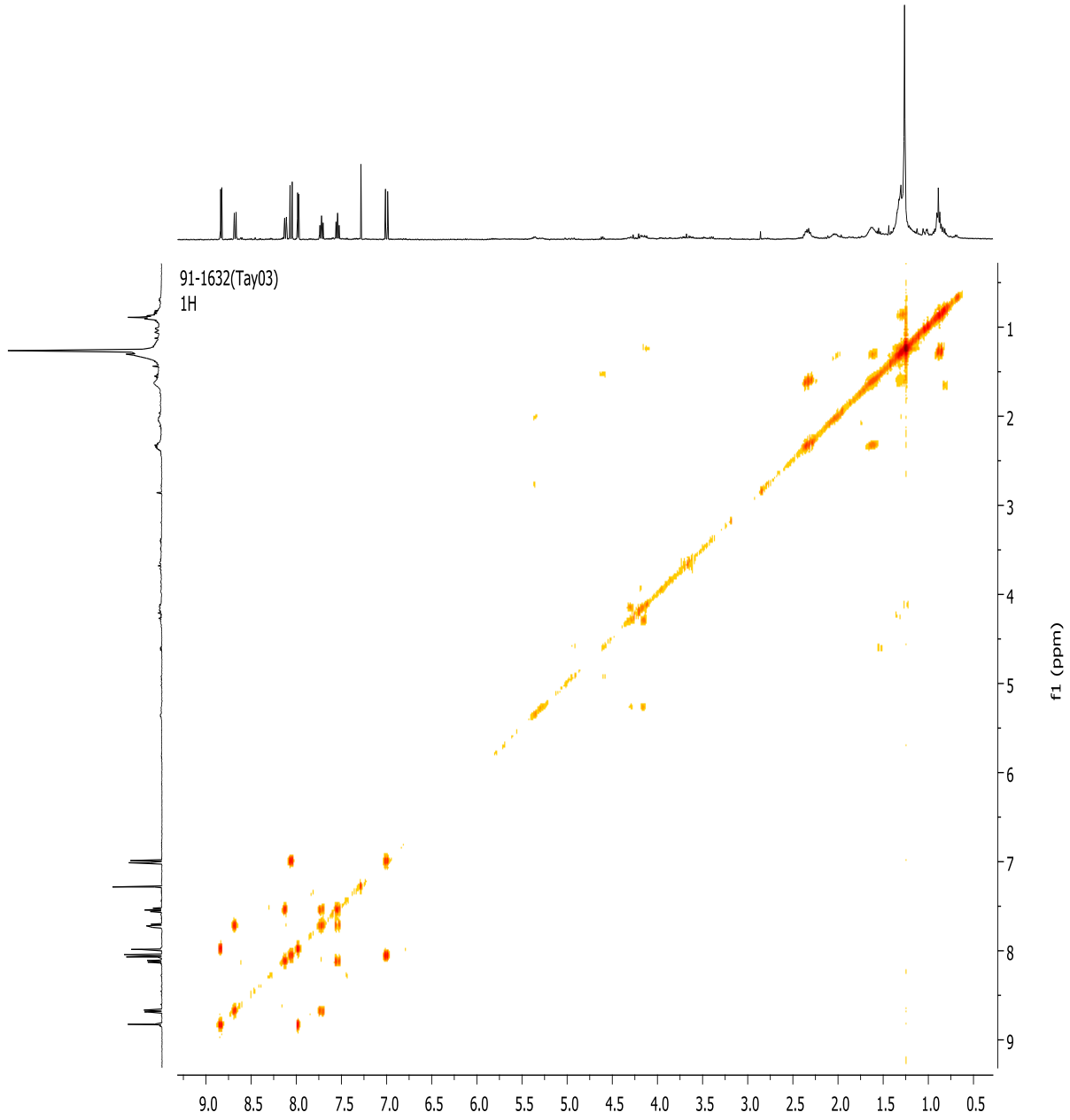
Appendix 8: ^{13}C NMR spectrum of compound **2**



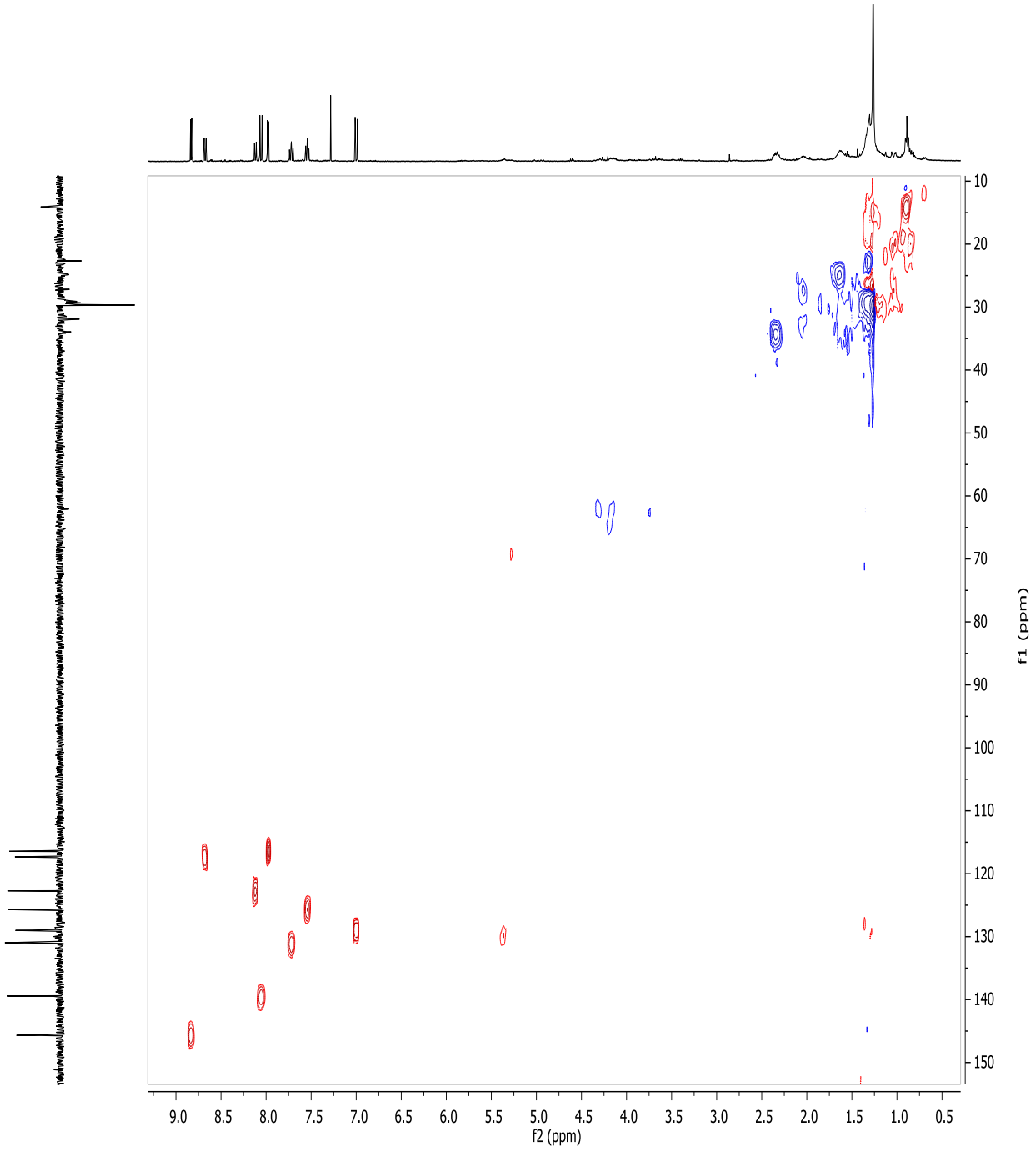
Appendix 9: DEPT-135 spectrum of compound **2**



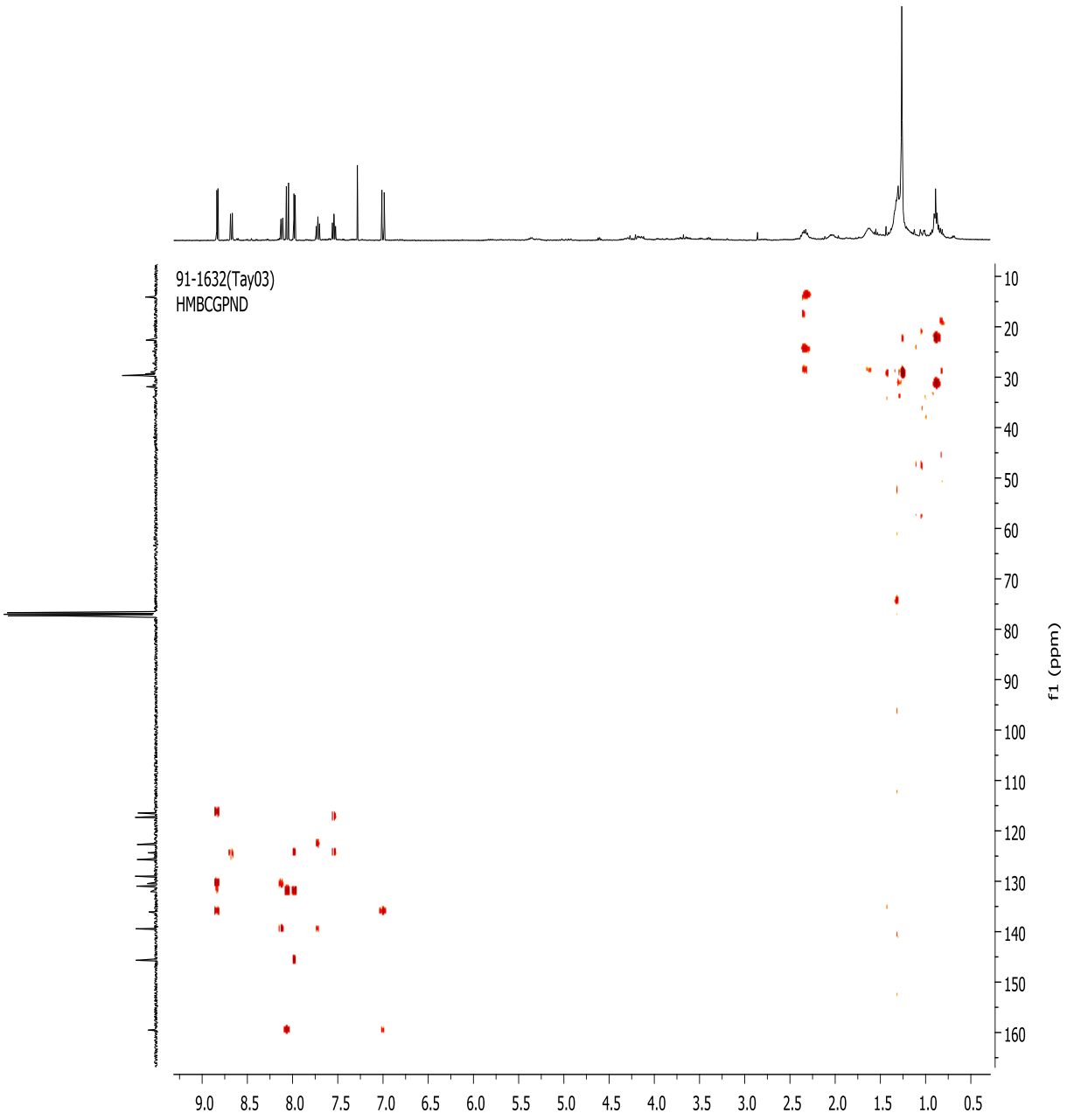
Appendix 10: COSY spectrum of compound **2**



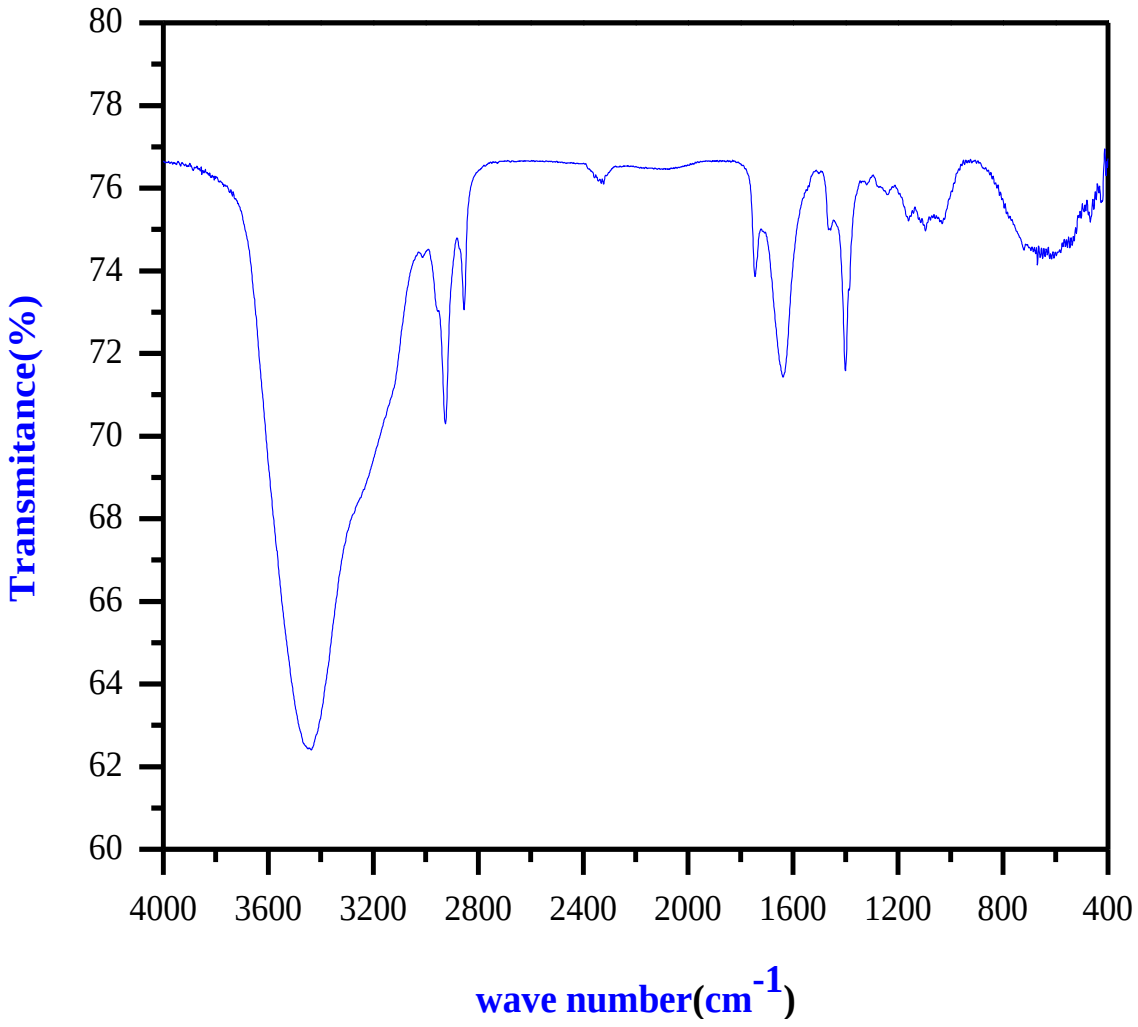
Appendix 11: HSQC spectrum of compound **2**



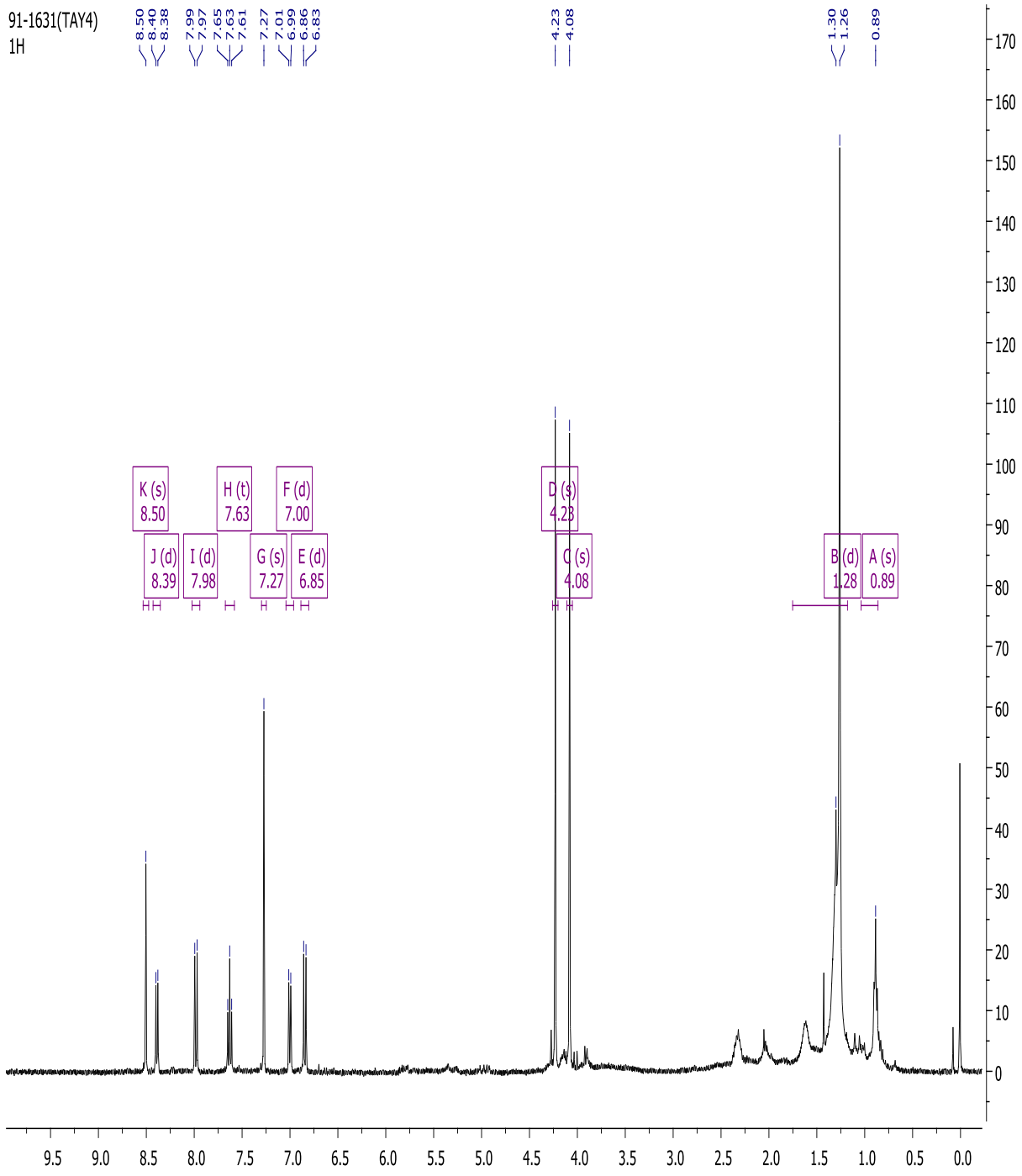
Appendix 12: HMBC spectrum of compound **2**



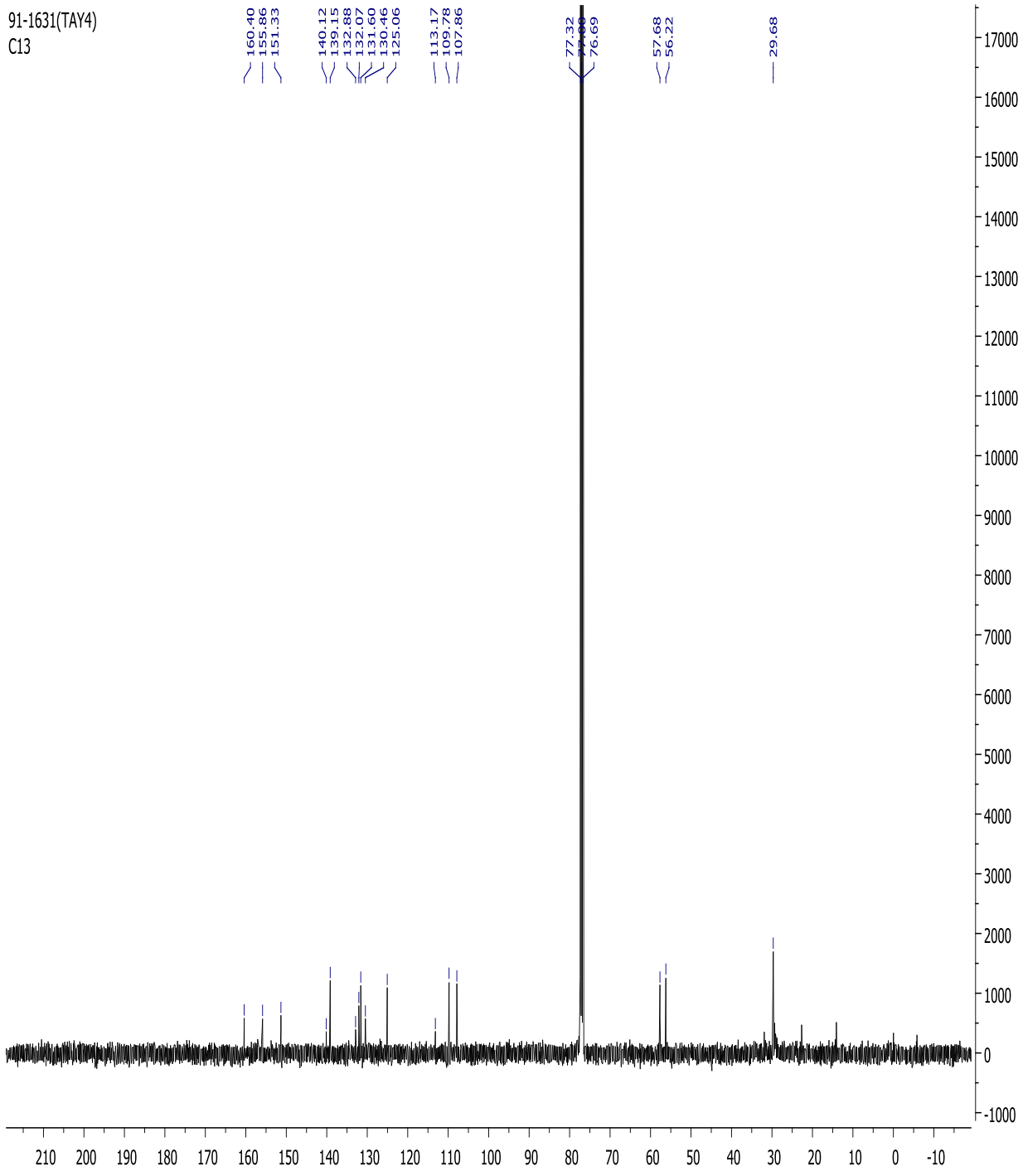
Appendix 13: IR spectrum of compound **3**



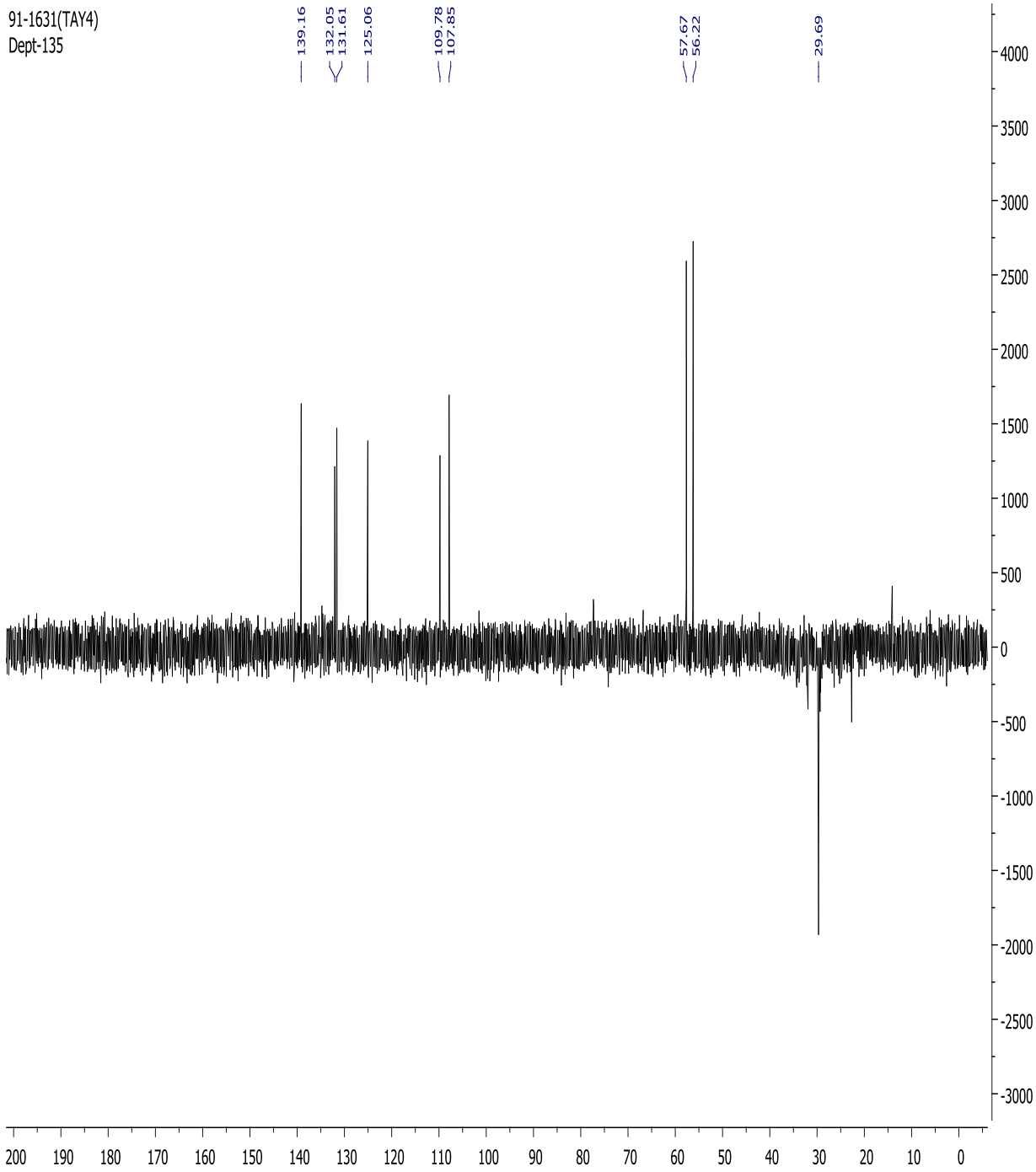
Appendix 14: ^{1}H NMR spectrum of compound **3**



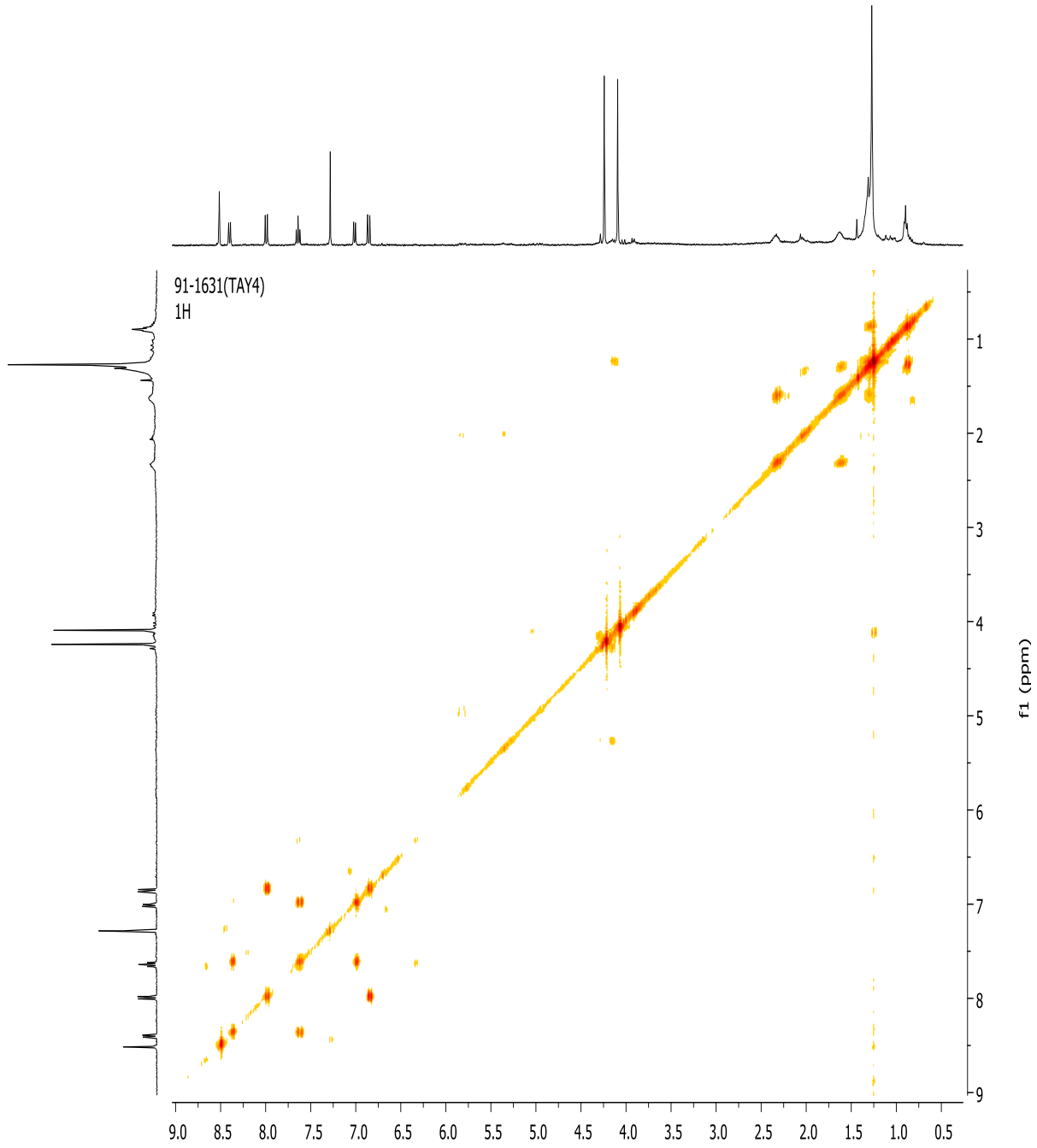
Appendix 15: ^{13} C NMR spectrum of compound **3**



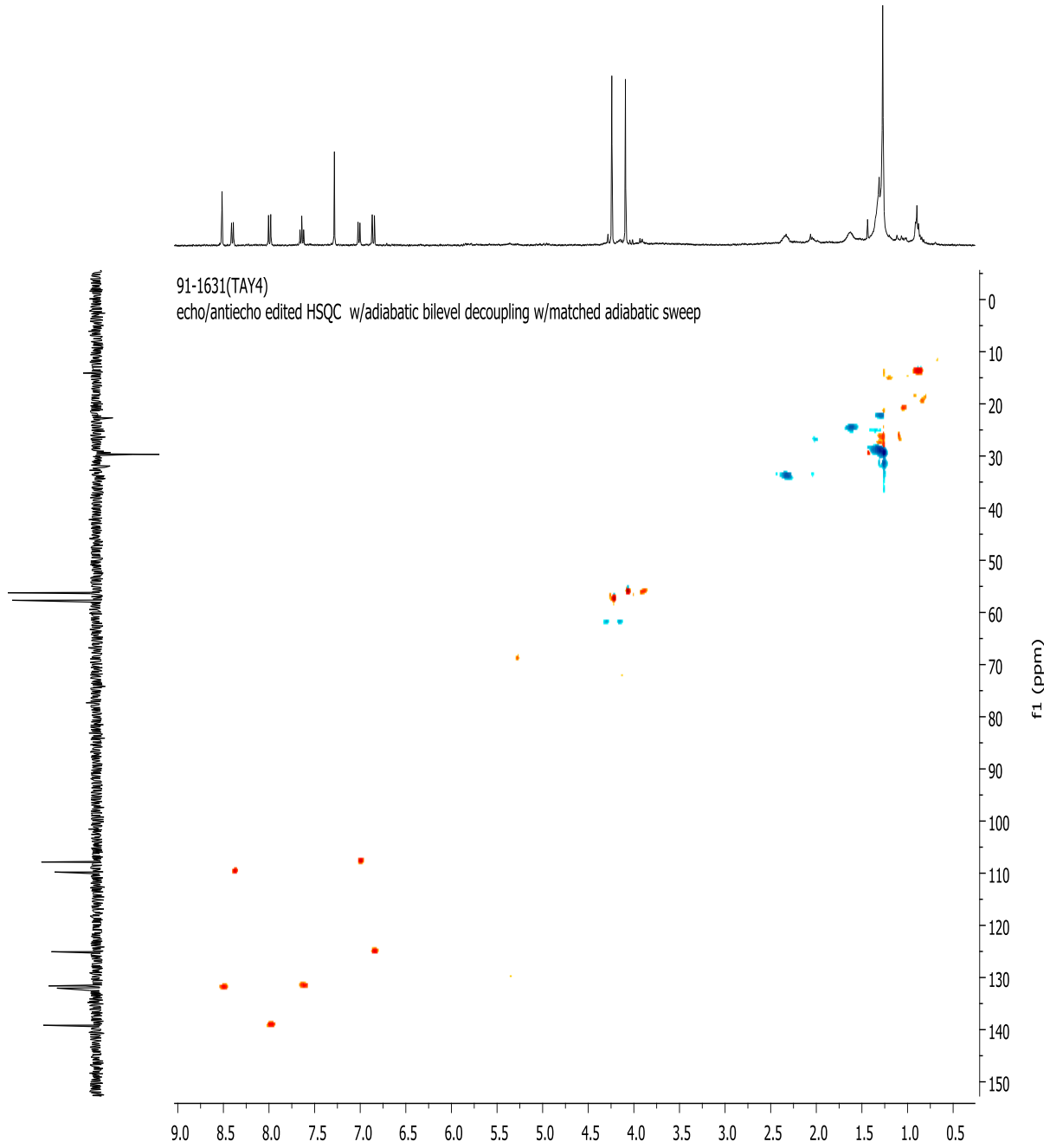
Appendix 16: DEPT-135 spectrum of compound **3**



Appendix 17: COSY spectrum of compound **3**



Appendix 18: HSQC spectrum of compound 3



Appendix 19: HMBC spectrum of compound **3**