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Chemical Constituents of *Pestalotiopsis microspora* HF 12440

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ABSTRACT

Phytochemical investigation on the liquid media of *Pestalotiopsis microspora* HF 12440, an endophytic fungus isolated from the stem of *Artocarpus heterophyllus*, led to the isolation of three lactones, (+)-acetylpestalotin (**1**), (−)-pestalotin (**2**), (6*S*,7*S*,8*R*)-hydroxypestalotin (**3**) and a lignan, (+)-pinoresinol (**4**). Interestingly, one of them (**1**) is a new compound, while compound **4** is reported for the first time from endophytic fungi. Structures of the isolated compounds were determined based on spectroscopic data, including MS, NMR 1D and 2D. Cytotoxic values of the crude extract and all isolated compounds were evaluated against P388 cells, showing that compound **3** was the most active with IC_{50} 3.34 µg/ml. Overall, this is the first phytochemical report of endophytic fungi isolated from the host plant *Artocarpus*.

INTRODUCTION

Endophytic fungi are defined as microbes that live colonially in various tissues of plants, such as roots, stems, leaves, tubers, fruits, and flowers (Müller, 2015; Stone *et al*., 2004; Wei *et al*., 2016; Zhang *et al*., 2006). In general, the endophytic fungi do not cause any negative effects during life in host plant tissues (Gao *et al*., 2005; Kyekyeku *et al*., 2017). There is a mutually beneficial interaction between endophytic fungi and its host plants. Plants supply nutrients for fungal growth and fungi will produce secondary metabolites to protect the host plants from attacks (Kogel *et al*., 2006). Although the number of endophytic species is estimated to be more than 1 million, nevertheless the phytochemical data of these microbes in the literature are still limited (Guo *et al*., 2008; Jia *et al*., 2016). Therefore, many species of endophytic fungi have not been evaluated chemically.

Artocarpus, the high tree, is an important genus in the mulberry family and fig family, Moraceae. This plant has been widely used as traditional medicine, including anti-inflammation, antimalarial, and remedy to cure fever and diarrhea (Jagtap, 2010; Septama and Panichayupakaranant, 2018). Research on the phytochemical constituents of *Artocarpus* in our laboratory showed that the main secondary metabolites in the plants of this genus are prenylated phenolic compounds such as flavonoids, stilbenes, and 2-arylbenzofurans with a variety of biological activities such as anti-cancer, antimalarial, antimicrobial, cytotoxic, and antioxidant (Achmad *et al*., 1996; Hakim *et al*., 1999; 2002; 2006; Syah *et al*., 2001; 2004; Mustapha *et al*., 2009; 2010). To continue our work on the chemical constituents of *Artocarpus*, we investigated the endophytic fungi from this plant. From this study, we will know the diversity of structures and bioactivity of secondary metabolites from fungi isolated from *Artocarpus*. An endophytic fungus, identified as *P. microspora* HF 12440, was isolated from the stem of *Artocarpus heterophyllus*. This fungal genus has been shown to produce lactones, phenylpropanoids, alkaloids, terpenoids, quinones, peptides, and xanthones with important bioactivities (Kuang *et al*., 2016; Xia *et al*., 2016; Xiao *et al*., 2017; Xu *et al*., 2010). In the present study, we report four compounds (**1**–**4**) from the liquid media of *Pestalotiopsis microspora* HF 12440 and their cytotoxic activities against P388 cells. In fact, this is the first reported study about isolation, structure elucidation, and bioactivity test of the secondary metabolites from *P. microspora* HF 12440 living in *A. heterophyllus*.

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MATERIAL AND METHODS

General experimental procedures

Potato dextrose agar (PDA) and potato dextrose broth (PDB) (Himedia, Mumbai, India) were used for inoculation and cultivation of endophytic fungi. Silica gel 60 G, silica gel Kieselgel 60 (0.063–0.200 mm), silica gel 60 PF254, and silica gel 60 F254 (Merck, Darmstadt, Germany) were used for vacuum liquid chromatography, column chromatography, radial chromatography, and thin layer chromatography, respectively. NMR spectroscopic data were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on Agilent Varian with $CDCl_3$ and $(CD_3)_2CO$ as the solvents and tetramethylsilane (TMS) as the internal standard (Agilent Technologies, Santa Clara, CA). MS data were recorded using a Bruker spectrometer (Bruker Corporation, Billerica, MA). Optical rotation was measured on an Autopol IV polarimeter (Rudolph Research Analytical, New Jersey).

Isolation of endophytic fungus

The stem of *A. heterophyllus* was collected from the garden of School of Pharmacy in Bandung Institute of Technology, Indonesia in December 2016. The stem of *A. heterophyllus* was sterilized with ethanol 70% for 1 minute and NaClO 3.5% for 30 seconds. This sterile stem was cut and then inoculated on the PDA. After 5 days, the fungi were transferred into the other PDA to give a single strain of fungus labeled with BTG-1.

Identification of fungus

The fungus with code BTG-1 was identified using analyses of the internal transcribed spacer (ITS) region of the ribosomal DNA. The fungus was cultivated on the PDB for 72 hours. The DNA was extracted from the mycelial of this fungus using nucleon PhytoPure and then amplified using a primer of ITS-4 and ITS-5 (Raja *et al*., 2017). The PCR (Polymerase Chain Reaction) product was purified with polyethylene glycol precipitation method (Hiraishi *et al*., 1995). The pure PCR product was sequenced with automated DNA sequencer. The sequencing data were trimmed and assembled with BioEdit program and then blasted at National Center for Biotechnology Information (NCBI).

Sequence *ITS* ribosomal DNA gene of *fungus* P. microspora

GAGGTCACCACAAAAAATTGGGGGTTTAGCG-GCTGGGAGTTATAGCACCTAACAAAGCGAGAAAAAAAT-TACTACGCTCAGAGGATACTACAAATCCGCCGTT GTATTTCAGGAACTACAACTAATAAAAGAAGTAGA TTCCCAACACTAAGCTAGGCTTAAGGGTTGAAATGA CGCTCGAACAGGCATACCCACTAGAATACTAATGGGC-GCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCT GCAATTCACATTACTTATCGCATTTCGCTGCG TTCTTCATCGATGCCAGAACCAAGAGATCCGTTGT TGAAAGTTTTGACTTATTAAAATAAGACGCTCAGAT-TACATAAAATAACAAGAGTTTAATGGTCCACC GGCAGCAGCTATAAGAAGACCTATAACTTCTGC-CGAGGCAACAAAAGGTAAGTTCACATGGGTTGG-GAGTTTAGAAAACTCTATAATG

Fermentation and isolation of secondary metabolites

Pestalotiopsis microspora HF 12440 was cultivated on PDB media and incubated at 27°C for 2 weeks. The mycelial and the filtrate were separated with Buchner funnel. The 10 l of the filtrate was extracted three times with ethyl acetate to give 2.2 g of ethyl acetate extract. The crude extract (2.2 g) was fractionated using vacuum liquid chromatography with the gradient polarity solvent system, i.e., dichloromethane, dichloromethane-acetone, acetone, and methanol to afford six fractions, FA, FB, FC, FD, FE, and FF.

A 210 mg of FB was subjected to radial chromatography using silica gel 60 PF254 eluted with hexane and acetone (8:2) to obtain compound **1** (11 mg) and compound **2** (20 mg). FC (314 mg) was fractionated by silica gel 60 PF254 eluted with hexane and ethyl acetate (from 6:4 to 4:6) to give 13 subfractions (FC.1–FC.13). Subfractions FC.11–FC.12 were purified by column chromatography using chloroform and acetone (from 9.5:0.5 to 6:4) to yield compound **3** (13 mg). A 211 mg of FD was submitted to column chromatography eluted with hexane, acetone, and ethyl acetate (7:2:1) to provide 23 subfractions (FD.1–FD.23). FD.9–FD.15 (59 mg) were combined and further separated by column chromatography using silica gel Kieselgel 60 eluted with chloroform and ethyl acetate (from 8:2 to 4:6) to afford compound **4** (6 mg).

Identification of secondary metabolites

The structures of compounds (**1**–**4**) were determined based on the analysis of spectroscopic data, including NMR (1D, 2D) and MS. NMR spectroscopic data were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C with deuterated solvent peaks as reference standards and TMS as the internal standard. Each compound **1** and **3** was diluted with $CDCl_3$ and each compound **2** and **4** was diluted with $(CD_3)_2CO$. The concentration of all compounds was 1.00 mg/ml. FID (Free Induction Decay) data were processed using MestreNova program.

(+)-Acetylpestalotin (1)

Colorless oil; $+81.9^0$ (c.0.0038, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.13 (1H, d, J = 1 Hz, H-3), 5.03 (1H, ddd, J = 4.0, 7.0, and 10.5 Hz, H-7), 4.45 (1H, dt, J = 3.5 and 12.5 Hz, H-6), 3.73 (3H, s, H-1′), 2.58 (1H, ddd, J = 2.0, 12.5, and 17.0 Hz, H-5a), 2.24 (1H, dd, J = 4.0 and 17.0 Hz, H-5b), 2.09 (3H, s, H-3′), 1.73 (2H, m, H-8), 1.30 (4H, m, H-9 and H-10), 0.89 (3H, t, J = 6.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm): δ 172.7 (C-4), 170.7 (C-2′), 166.5 (C-2), 90.4 (C-3), 75.5 (C-6), 73.1 (C-7), 56.3 (C-1′), 29.7 (C-8), 29.4 (C-5), 27.6 (C-9), 22.6 (C-10), 21.0 (C-3′), 14.0 (C-11); MS *m/z* 257.29 $[M + H]^+$ (calcd for $C_{13}H_{20}O_5$, 257.29).

(−)-Pestalotin (2)

Yellow oil; -108.4^0 (c.0.0031, $CHCl_3$); ^{1}H NMR [500 MHz, $(CD_3)_2CO$]: δ 5.09 (1H, d, J = 1.5 Hz, H-3), 4.30 (1H, dt, J = 4.0 and 13.0 Hz, H-6), 3.78 (3H, s, H-1′), 3.61 (1H, m, H-7), 2.78 (1H, ddd, J = 2.0, 13.0, and 17.5 Hz, H-5a), 2.27 (1H, dd, J = 3.5 and 17.0 Hz, H-5b), 1.57 (2H, m, H-8), 1.47 (2H, m, H-9), 1.35 (2H, m, H-10), 0.91 (3H, t, J = 7.0 Hz, H-11); ^{13}C NMR [125 MHz, $(CD_3)_2CO$] (ppm): δ 174.2 (C-4), 166.7 (C-2), 90.5 (C-3), 79.1 (C-6), 72.2 (C-7), 56.6 (C-1′), 33.0 (C-8), 29.8 (C-5), 28.7 (C-9), 23.3 (C-10), 14.3 (C-11); MS *m/z* 215.02 $[M + H]^+$ (calcd for $C_{11}H_{18}O_4$, 215.12).

(6S,7S,8R)-Hidroxypestalotin (3)

Yellow oil; -83.2^0 (c.0.00075, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.12 (1H, d, J = 1.2 Hz, H-3), 4.50 (1H, dt, J= 3.9

and 12.8 Hz, H-6), 3.78 (1H, m, H-8), 3.75 (3H, s, H-1′), 3.48 (1H, br s, H-7), 2.88 (1H, ddd, J = 1.2, 13.0, and 17.0 Hz, H-5a), 2.31 (1H, dd, J = 3.7 and 17.2 Hz, H-5b), 1.60 (1H, m, H-9a), 1.50 (2H, m, H-9b dan H-10a), 1.39 (1H, m, H-10b), 0.93 (3H, t, J = 7.2 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm): δ 173.6 (C-4), 166.9 (C-2), 89.9 (C-3), 78.1 (C-6), 74.0 (C-7), 71.0 (C-8), 56.3 (C-1′), 36.1 (C-9), 29.5 (C-5), 18.9 (C-10), 14.1 (C-11); MS *m/z* 231.10 $[M + H]^+$ (calcd for $C_{11}H_{18}O_5$, 231.12).

(+)-Pinoresinol (4)

Colorless oil; $+45.8^0$ (c.0.0043, $CHCl_3$); ^{1}H NMR (500 MHz, $(CD_3)_2CO$) (ppm): δ 6.95 (d, J = 1.7 Hz, H-2′ and H-2″), 6.79 (dd, J = 1.7 and 8.1 Hz, H-6′ and H-6″), 6.74 (d, J = 8.1 Hz, H-5′ and H-5″), 4.62 (d, J = 4.1 Hz, H-2 and H-6), 4.16 (dd, J = 6.9 and 8.9 Hz, H-4a and H-8a), 3.80 (s, H-7′ and H-7″), 3.76 (dd, J = 3.7 and 9 Hz, H-4b and H-8b), 3.04 (m, H-1 and H-5); ^{13}C NMR [125 MHz, $(CD_3)_2CO$]: 148.3 (C-3′ and C-3″), 146.9 (C-4′ and C-4”), 134.2 (C-1′ and C-1″), 119.6 (C-5′ and C-5″), 115.6 (C-6′ and C-6″), 110.6 (C-2′ and C-2″), 86.6 (C-2 and C-6), 72.2 (C-4 and C-8), 56.2 (C-7′ and C-7″), 55.2 (C-1 and C-5); MS *m/z* 356.91 $[M - H]^+$ (calcd for $C_{20}H_{22}O_6$, 357.14).

Murine leukemia P388 cell assay

The crude extract and all pure compounds (**1**–**4**) were evaluated for their cytotoxicity against murine leukemia P388 cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay as previously reported (Sahidin *et al*., 2005; Yang *et al*., 2008). The cells were seeded in 96-well plates (cell density 3×10^4 cells/cm^3). Each compound (**1**–**4**) was added in various concentrations and incubated for 48 hours, where the crude extract and compounds were dissolved in dimethyl sulfoxide (DMSO). After 48 hours of incubation, 10 µl MTT reagent was added to each sample and then incubated for 4 hours. The MTT-stop solution containing sodium dodecyl sulfate (SDS) was added and the incubation was continued for 24 hours. Optical density was read with a microplate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage of live cells compared with control. The control was made from MTT solution and DMSO (without cells and medium).

RESULTS AND DISCUSSION

Compound **1** (11 mg) was obtained as colorless oil. Its molecular formula was determined as $C_{13}H_{20}O_5$ by EI-MS spectrum (*m/z* 257.29 $[M + H]^+$, calcd for 257.29, implying four degrees of unsaturation. The ^{1}H NMR spectrum (see Supplementary Data) showed a triplet methyl at δ_H 0.89 ppm (J = 6.5 Hz), four methylene signals at δ_H 1.30, 1.73, 2.24, and 2.58 ppm, a singlet methyl (δ_H 2.09 ppm), a methoxyl signal (δ_H 3.73 ppm), two oxygenated methine signals at δ_H 4.45 and 5.03 ppm, and a methine (δ_H 513 ppm, d, 1 Hz). The ^{13}C NMR data showed that this compound consists of 13 carbons, including three methyls, four methylenes, three methines (two oxygenated), and three quaternary carbons. The structure of compound **1** was confirmed by HMBC spectrum. The methoxyl (δ_H 3.73 ppm, H-1′), methine at δ_H 5.13 ppm (H-3), and methylene (δ_H 2.24 and 2.58 ppm, H-5) correlated to quaternary carbon at δ_C 172.7 ppm (C-4). It afforded information on the presence of methoxyl at quaternary carbon, C-4. An oxygenated methine at δ_H 5.03 ppm (H-7) and singlet methyl (δ_H 2.09 ppm, H-3′) correlated to δ_C 170.7 ppm (C-2′) indicated the presence of acetyl at C-7. Based on the correlation (Fig. 2), the structure of compound **1** was suggested as 6-[1-(acetyloxy)pentyl]-5,6-dihydro-4-methoxy-2H-pyran-2-one. The stereocenters of C-6 and C-7 in compound **1** were determined by comparison of the optical rotation data (**1,** $+81.9^0$, c.0.0038, $CHCl_3$; (+)-pestalotin, $+92.5^0$, c.1.30, MeOH; (−)-pestalotin, -94.76^0, c.1.00, MeOH) (Akay *et al*., 2014; Kumar *et al*., 2005; Mayer *et al*., 2002). On the basis of comparison of this data with the literature, the stereochemistry of compound **1** was assigned as C-6(*R*) and C-7(*R*). Compound (**1**) is a new compound.

A 20 mg of compound **2** was isolated as yellow oil. EI-MS showed ion peak at *m/z* 215.02 $[M + H]^+$ indicating that compound **2** had a molecular formula $C_{11}H_{18}O_4$. The spectrum of ^{1}H and ^{13}C NMR of compound **2** were similar with compound **1** (Table 1), except the oxymethine proton (H-7) at δ_H 3.61 ppm arised more shielding than that of compound **1** (δ_H 5.03 ppm). The change in chemical shift of ^{1}H NMR revealed the absence of acetyl group at C-7 in compound **2**. The methylene proton at position 5 and 8 correlated in HMBC spectrum to the two oxymethine carbons at δ_C 72.2 and 79.1 ppm (C-7 and C-6). An olefinic proton (δ_H 5.09 ppm, H-3) correlated to the two quaternary carbons (δ_C 166.7 and 174.2 ppm). The selected HMBC correlations of compound **2** were displayed in Figure 2. The spectroscopic data of compound **2** were similar to literature (Akay *et al*., 2014). Absolute configurations of C-6 and C-7 in compound 2 were assigned as *S* and *S* based on the comparing of the optical rotation and NMR data with the literature (**2**, -108.4^0, c.0.0031, $CHCl_3$; (+)-pestalotin, $+ 92.5^0$, c.1.30, MeOH; (−)-pestalotin, -94.76^0, c.1.00, MeOH) (Akay *et al*., 2014; Kumar *et al*., 2005; Mayer *et al*., 2002). Therefore, compound **2** was elucidated to be (6*S*)-6-[(1*S*)-1-hydroxypentyl]-4-methoxy-5,6-dihydro-2H-pyran-2-one and named (−)-pestalotin.

Compound **3** (13 mg) was acquired as the yellow oil. EI-MS showed mass ion peak at *m/z* 231.10 $[M + H]^+$, establishing the molecular formula $C_{11}H_{18}O_5$ with three degrees of unsaturation. ^{1}H and ^{13}C NMR spectrum of compound **3** were conformable with compound **2** (Table 1), except the proton (δ_H 3.78 ppm) and carbon (δ_C 71.0 ppm) at position 8 appeared more deshielding than that compound **2** (δ_H 1.57 ppm and δ_C 33.0 ppm) because of the presence of hydroxyl at this position. The position of hydroxyl at C-8 was also confirmed by the HMBC spectrum (Fig. 2). Methylene proton at δ_H 1.50 and 1.60 ppm (H-9) correlated to the two oxymethine carbon (δ_C 71.0 and 74.0 ppm, C-8 and C-7). The configurations of chiral carbon at the position C-6, C-7, and C-8 in compound **3** were determined as (*S*), (*S*), and (*R*) based on the comparing of the optical rotation and NMR data with the literature (**3**, -83.2^0, c.0.00075, $CHCl_3$); (6*S*,7*S*,8*R*)-LL-P880β, -59.8^0, c.0.96, MeOH) (Kirihata *et al*., 1990). The structure of compound **3** was displayed in Figure 1.

Compound **4** (6 mg) was obtained as colorless oil. The spectrum of EI-MS showed ion peak at m/z 356.91 $[M - H]^+$. The molecular formula of compound **4** was decided as $C_{20}H_{22}O_6$. There are 10 signals in the spectrum of ^{13}C NMR representing of 20 carbons, consist of four methines (two oxygenated), two methoxyls, two oxymethylenes, and 12 carbons from two benzenes (six quaternary carbons). ^{1}H NMR showed two methines at δ_H 3.04 ppm (2H, q), two oxymethilene protons (δ_H 3.76 and 4.16 ppm, 4H), two methoxyls at δ_H 3.80 ppm (6H, s), two oxygenated methine protons (δ_H 4.62 ppm, 2H), and three aromatic proton signals with ABX system at δ_H 6.74 ppm (2H, d, 8.1 Hz), 6.79

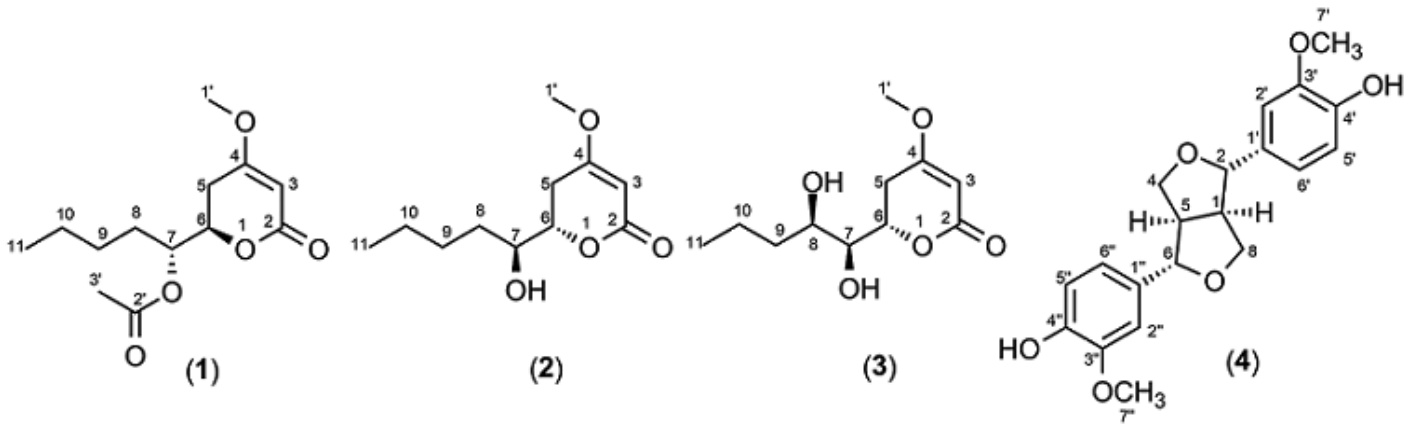

Figure 1. Structures of compounds (**1**–**4**) from ethyl acetate extract of *P. microspora* HF 12440.

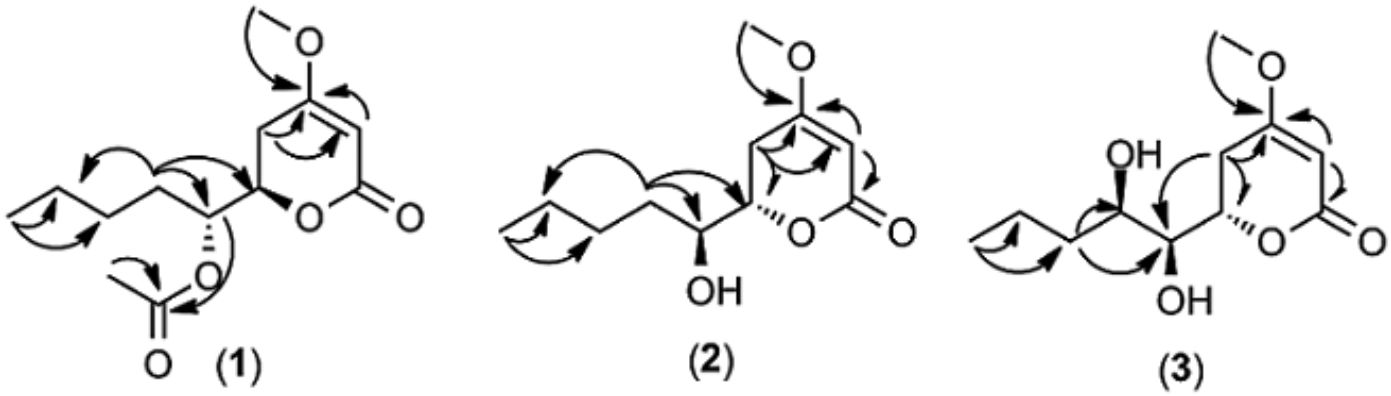

Figure 2. Selected HMBC (H→C) correlation of compounds **1**, **2,** and **3.**

Table 1. ^{1}H and ^{13}C data of compounds **1**, **2**, and **3**.

Position	Compound (1) [a]		Compound (2) [b]		Compound (3) [a]	
	^{1}H(δ, m, *J* (Hz))	^{13}C	^{1}H(δ, m, *J* (Hz))	^{13}C	^{1}H(δ, m, *J* (Hz))	^{13}C
1	-	-	-	-	-	-
2	-	166.5	-	166.7	-	166.9
3	5.13, d, 1	90.4	5.09, d, 1.5	90.5	5.12, d, 1.2	89.9
4	-	172.7	-	174.2	-	173.6
5	2.58, ddd, 2, 12.5, 17 2.24, dd, 4, 17	29.4	2.78, ddd, 2, 13, 17.5 2.27, dd, 3.5, 17	29.8	2.88, ddd, 1.2, 13, 17 2.31, dd, 3.7, 17.2	29.5
6	4.45, dt, 3.5, 12.5	75.5	4.30, dt, 4, 13	79.1	4.50, dt, 3.9, 12.8	78.1
7	5.03, ddd, 4, 7, 10,5	73.1	3.61, m	72.2	3.48, br s	74.0
8	1.73, m	29.7	1.57, m	33.0	3.78, m	71.0
9	1.30, m	27.6	1.47, m	28.7	1.60, m 1.50, m	36.1
10	1.30, m	22.6	1.35, m	23.3	1.50, m 1.39, m	18.9
11	0.89, t, 6.5	14.0	0.91, t, 7	14.3	0.93, t, 7.2	14.1
1′	3.73, s	56.3	3.78, s	56.6	3.75, s	56.3
2′	-	170.7	-	-	-	-
3′	2.09, s	21.0	-	-	-	-

[a]Measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$.

[b]Measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C in $(CD_3)_2CO$.

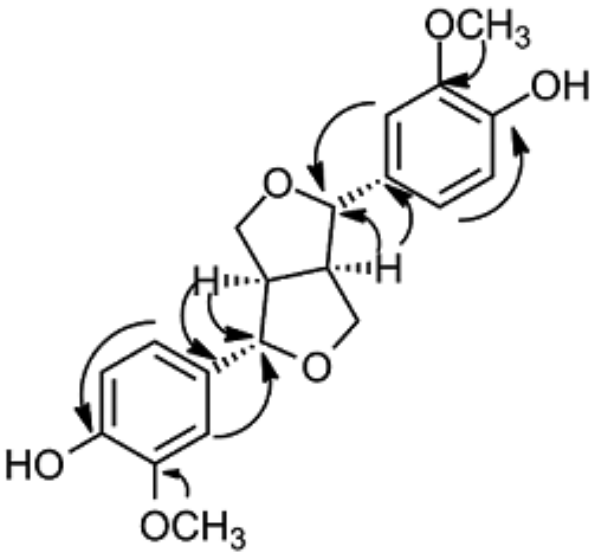

Figure 3. Selected HMBC correlation of compound **4**.

ppm (2H, dd, 1.7 and 8.1 Hz), and 6.95 ppm (2H, d, 1.7 Hz). In the HMBC spectrum, the methoxyl (δ_H 3.80 ppm, H-7′ and H-7″) correlated to the quaternary carbon at δ_C 148.3 ppm (C-3′ and C-3″), indicating the presence of methoxyl at C-3′ and C-3″. The correlation of methine (δ_H 3.04 ppm, H-1 and H-5) and oxygenated methine proton (δ_H 4.62 ppm, H-2 and H-6) with a quaternary carbon at δ_H 134.2 ppm (C-1′ and C-1″) implied the existence of benzyl at the C-2 and C-6 of the furan ring. The selected HMBC correlations of compound (**4**) were shown in Figure 3. The optical rotation and NMR data were used for determining the stereochemistry of compound **4** (**4**, $+45.8^0$, c.0.0043, $CHCl_3$); (+)-pinoresinol, $+ 64.0^0$, c.1.0, $CHCl_3$; (−)-pinoresinol, -34.7^0, c.0.91, CH_3OH) (Brenes *et al*., 2000; Fonseca *et al*., 1979; Lin-gen *et al*., 1982). The result of comparing this data with all literatures showed compound **4** was (+)-pinoresinol. Interestingly, this is the first reported of (+)-pinoresinol (**4**) isolated from endophytic fungi.

The crude extract and all compounds (**1**–**4**) were assayed for their cytotoxicity against murine leukemia P388 cells followed the MTT method. Compound (**3**), (**4**), and the crude extract displayed significant cytotoxicity with IC_{50} 3.34; 3.62; 18.97 µg/ml, respectively. Meanwhile, compound (**1**) and (**2**) showed moderate cytotoxicity with IC_{50} values of 5.60 and 7.1 µg/ml, respectively. The presence of hydroxyl group at C-8 in compound **3** is an important factor for cytotoxic value. Interestingly, compound **1** is much less active because of the absence of a hydroxyl group and the stereochemistry of this compound is different.

CONCLUSION

A new lactone (**1**), (+)-acetylpestalotin, together with three known compounds, (−)-pestalotin (**2**), (6*S*,7*S*,8*R*)-hydroxypestalotin (**3**), and (+)-pinoresinol (**4**) were isolated from the liquid media of *P. microspora* HF 12440, an endophytic fungus from the stem of *A. heterophyllus*. Crude extract and two compounds (**3**–**4**) were active against murine leukemia P388 cells with IC_{50} values of 18.97, 3.34, and 3.62 µg/ml, respectively.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest is associated with this work.

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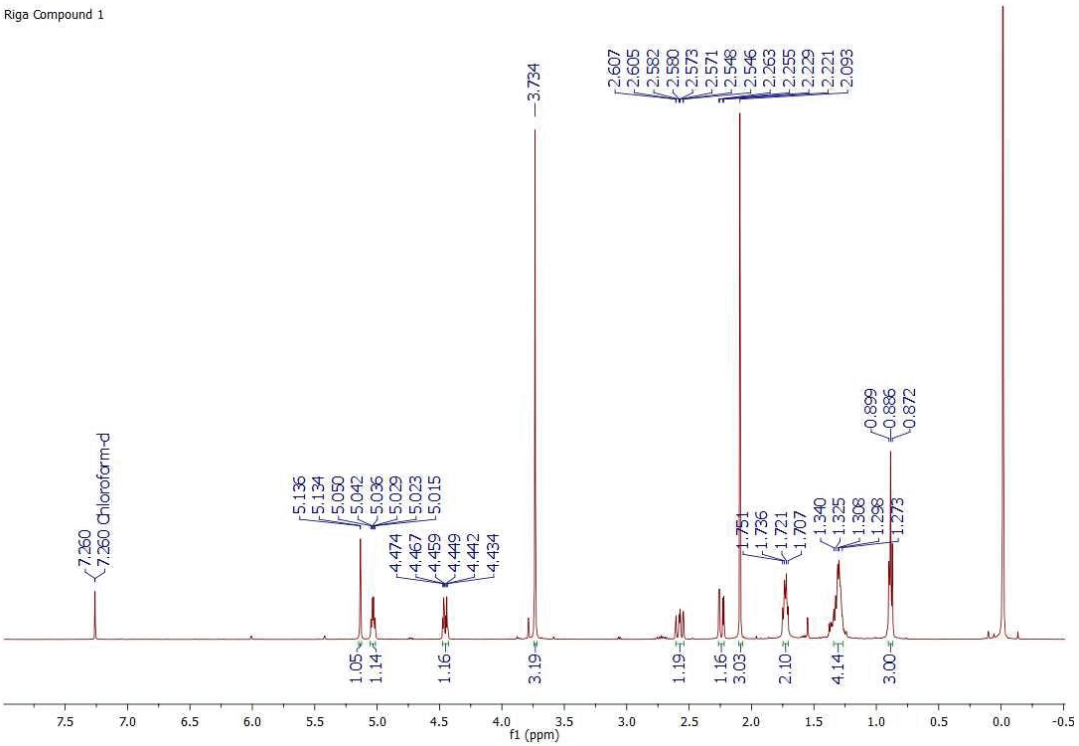
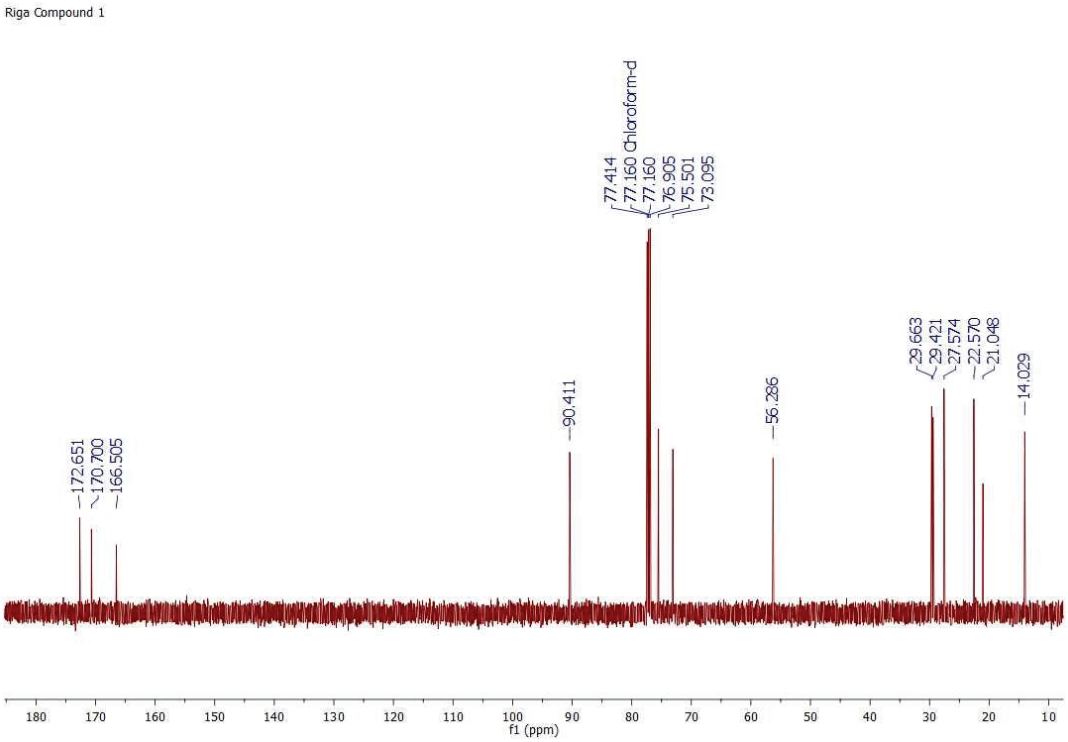
SUPPLEMENTARY DATA

Compound 1



[1]H-NMR spectrum of compound **1**.



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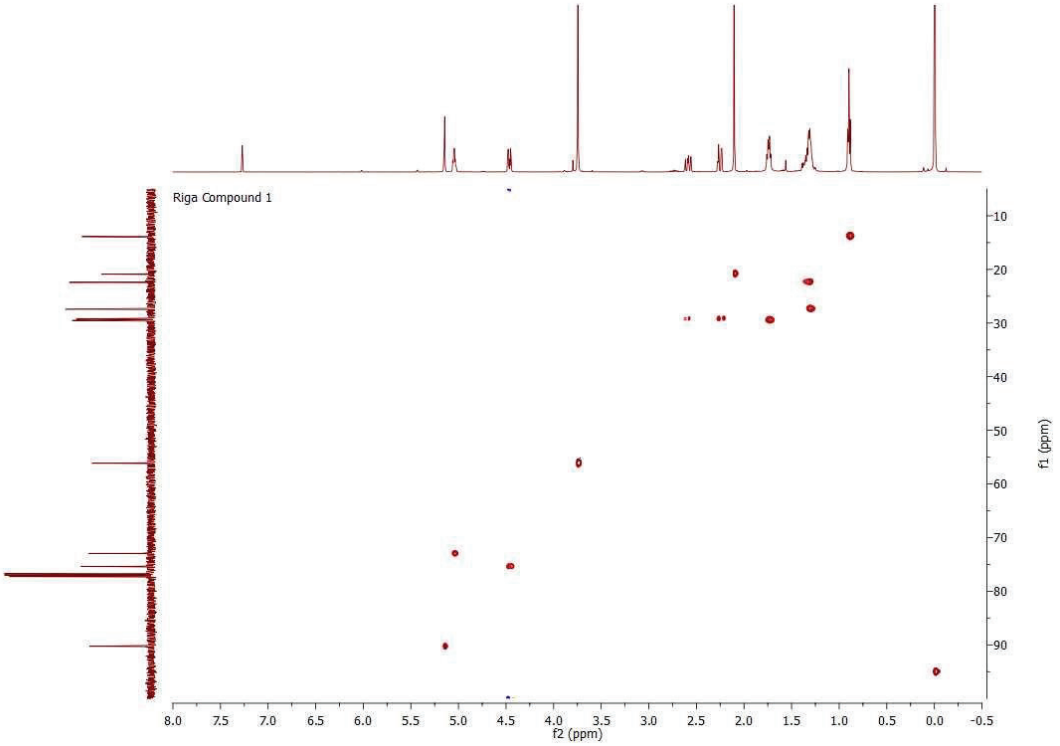

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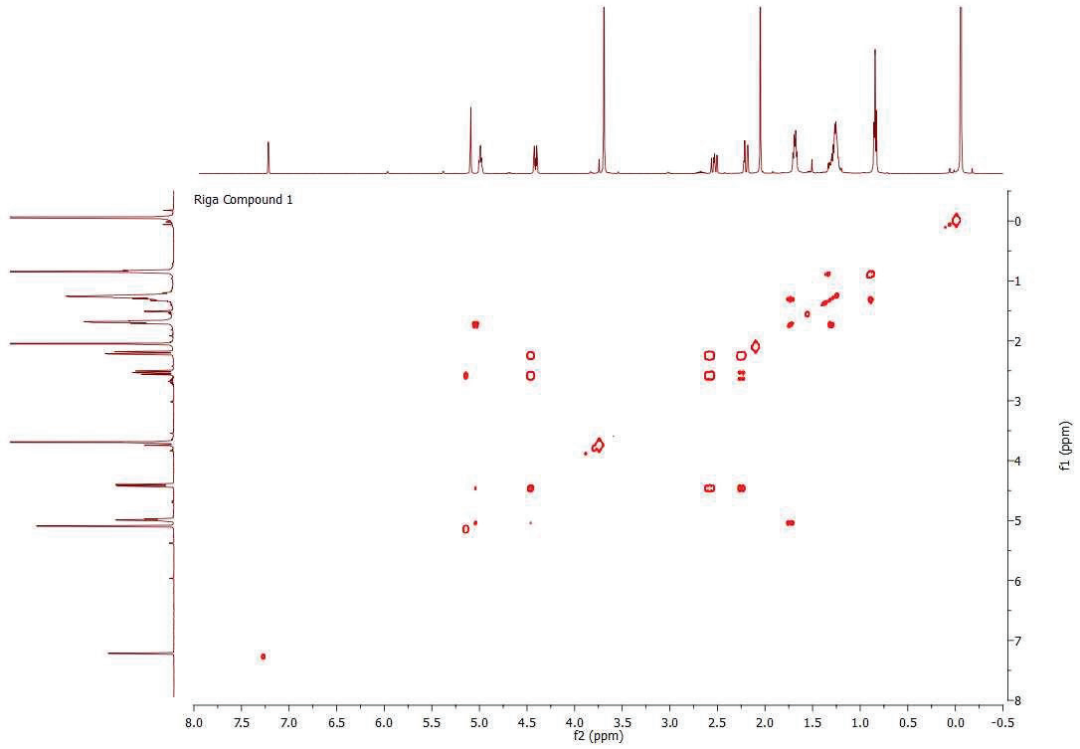

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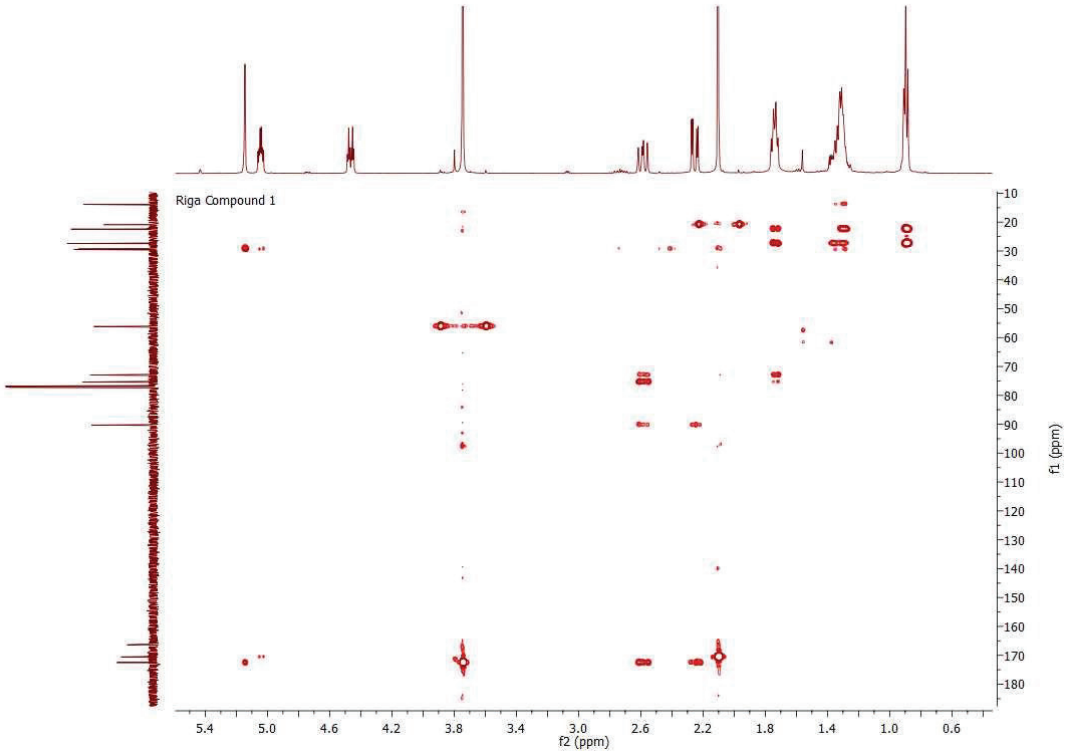
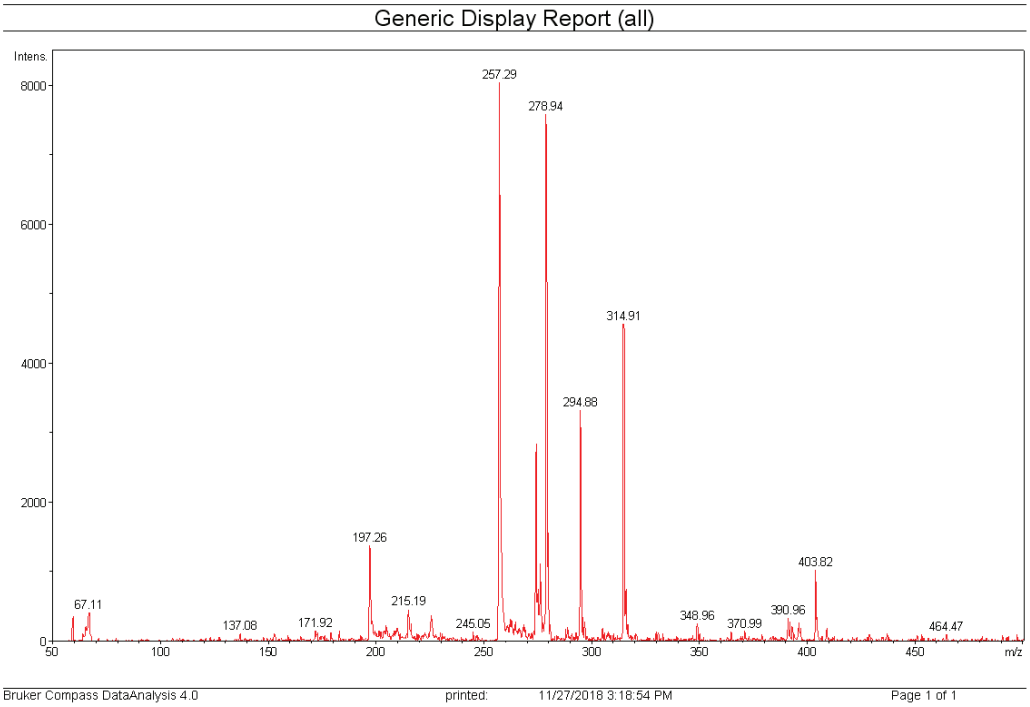
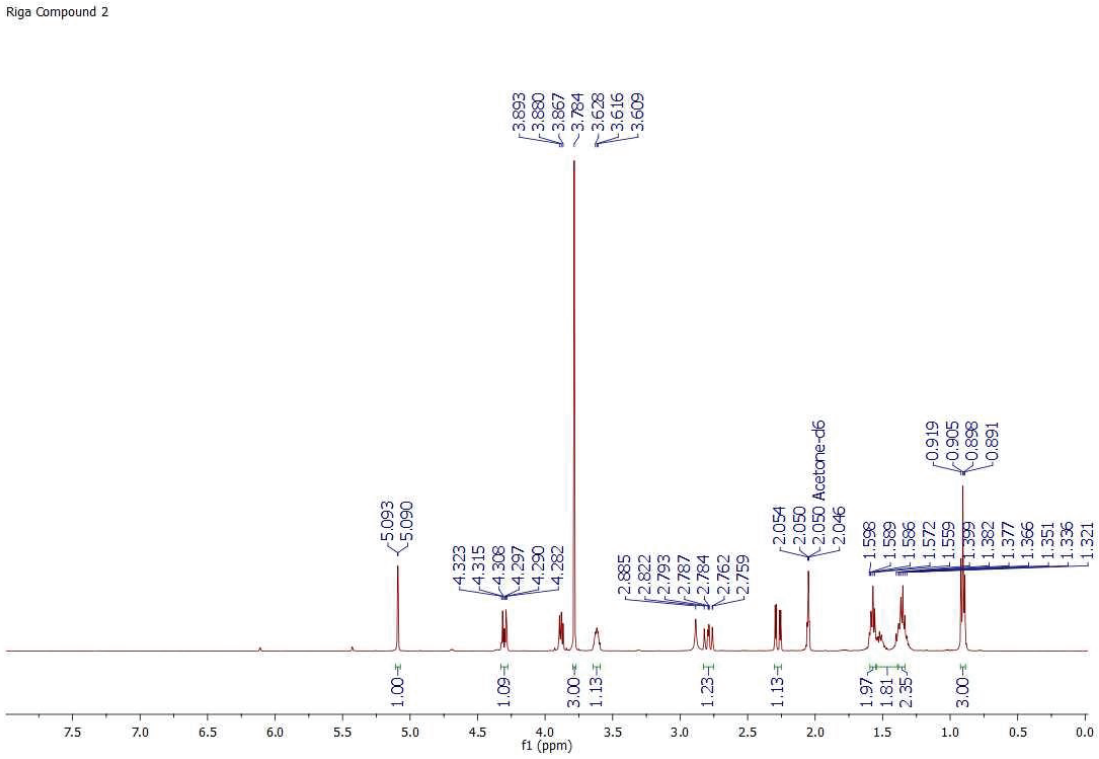


HMBC spectrum of compound **1**.



Mass spectrum of compound **1**.

Compound 2



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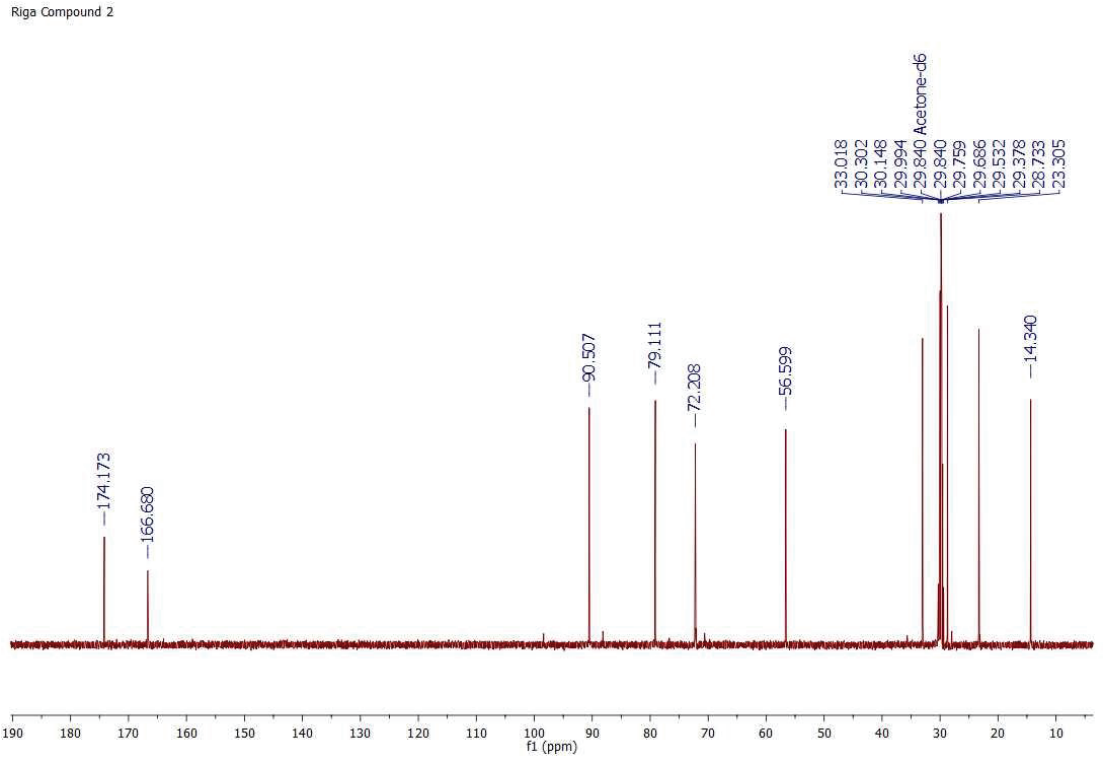

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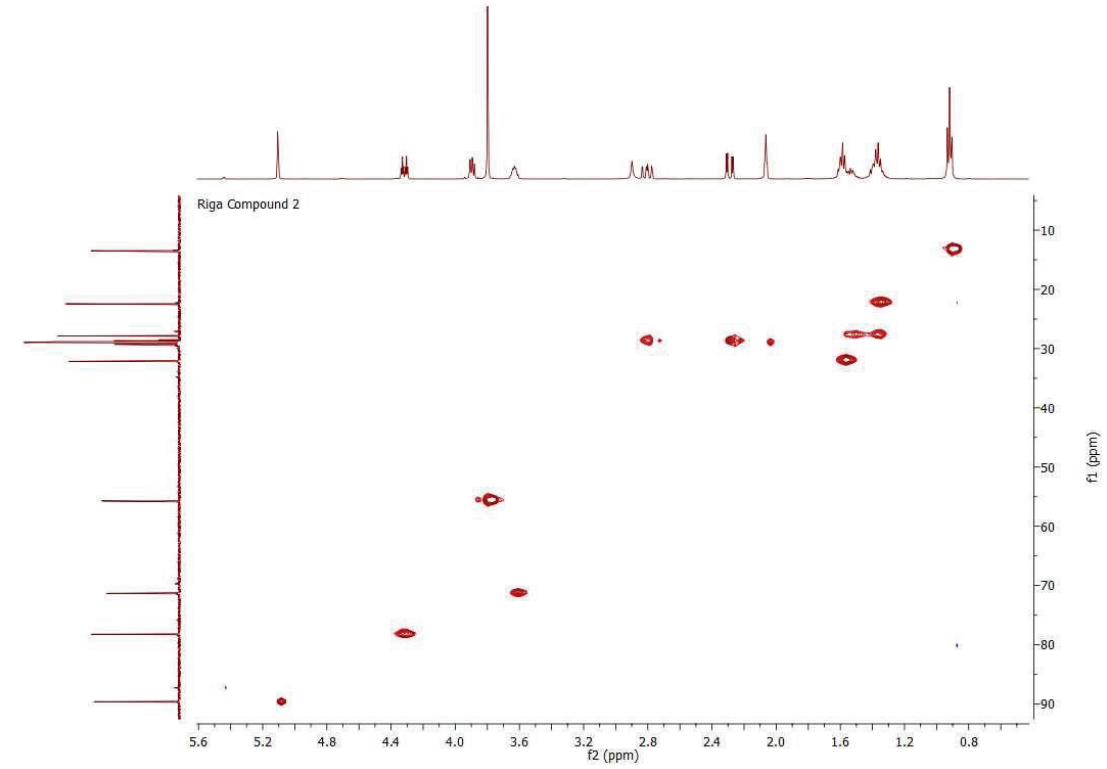

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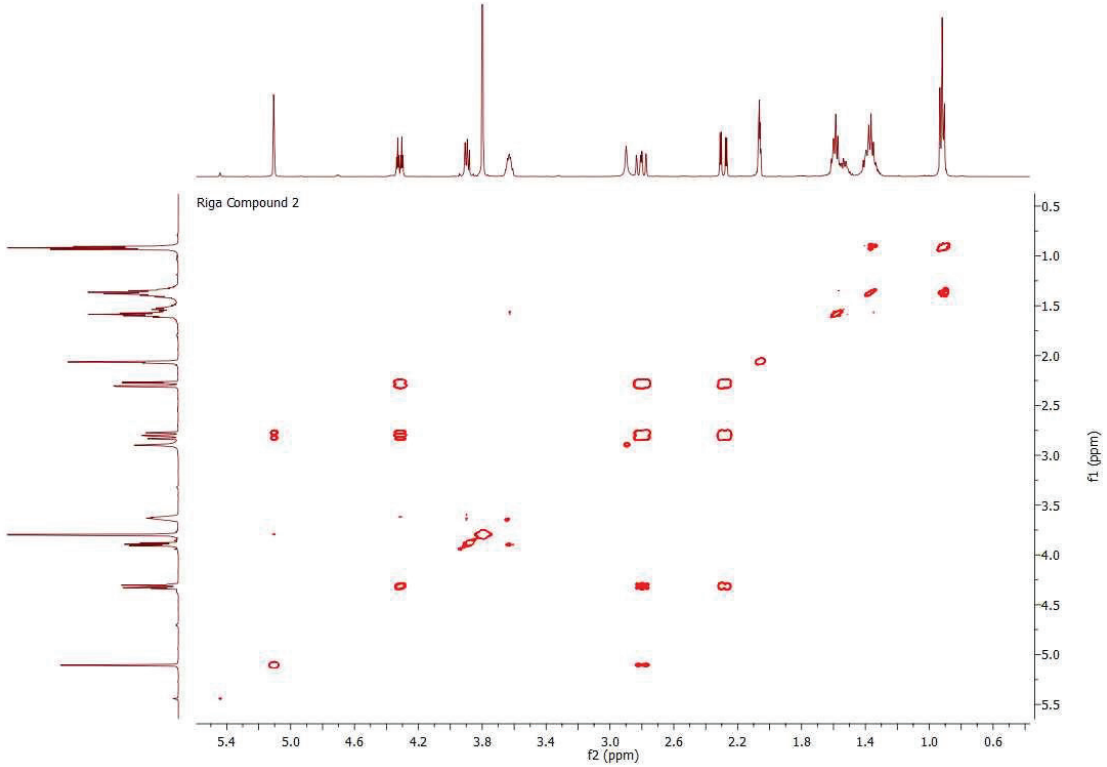

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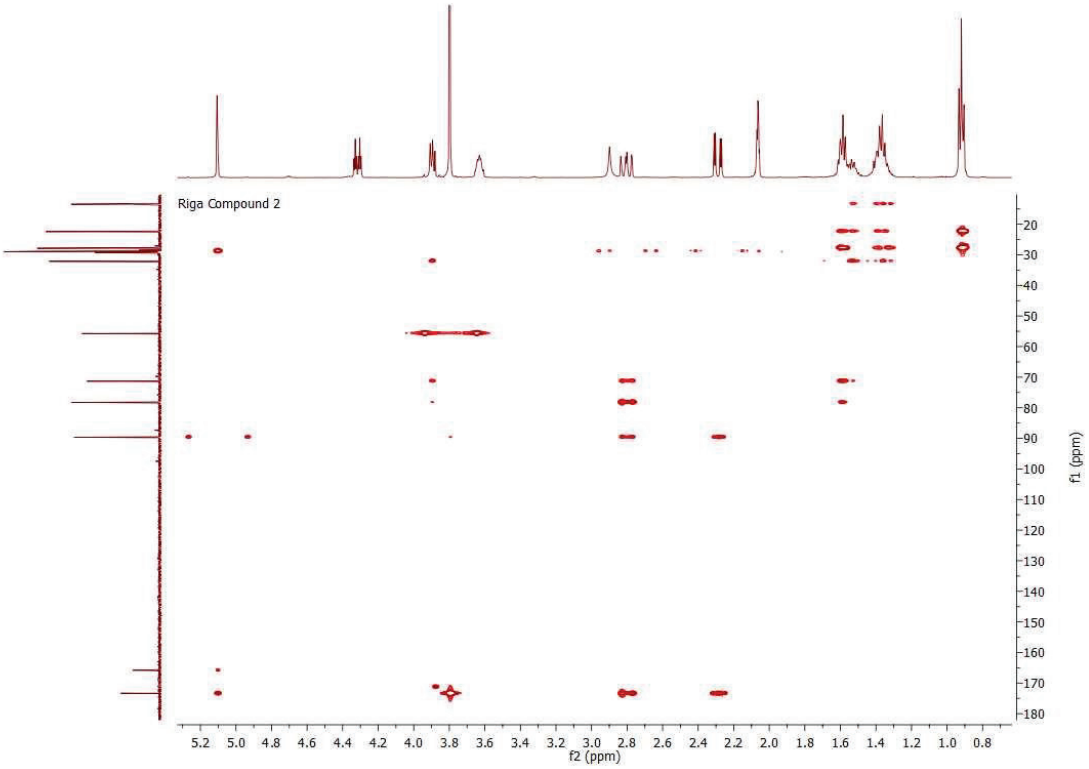
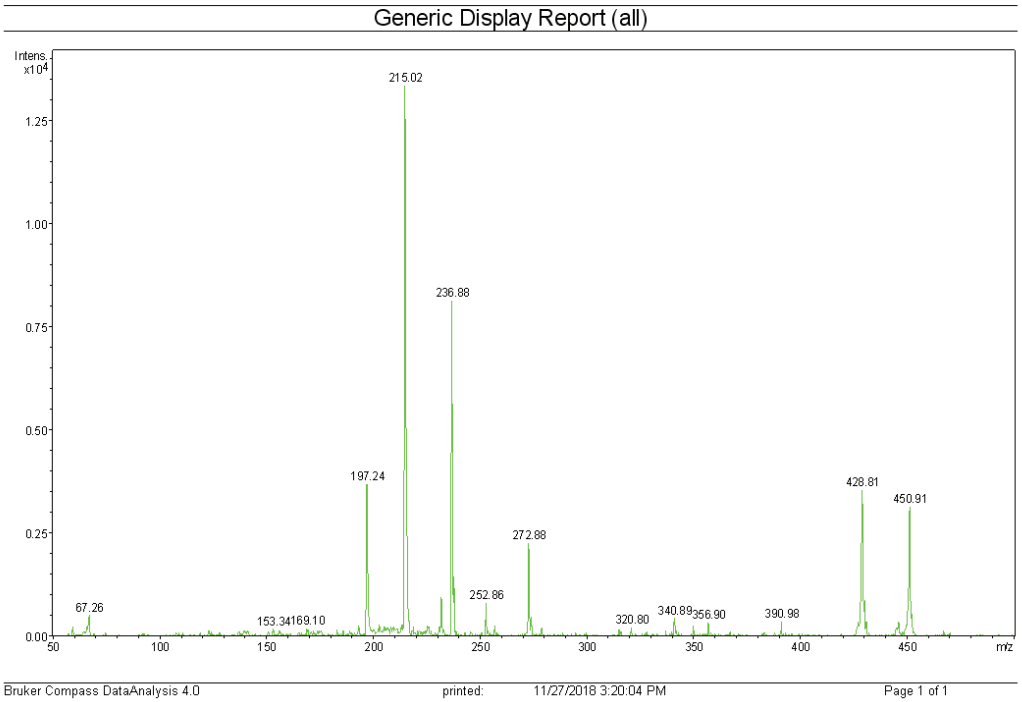
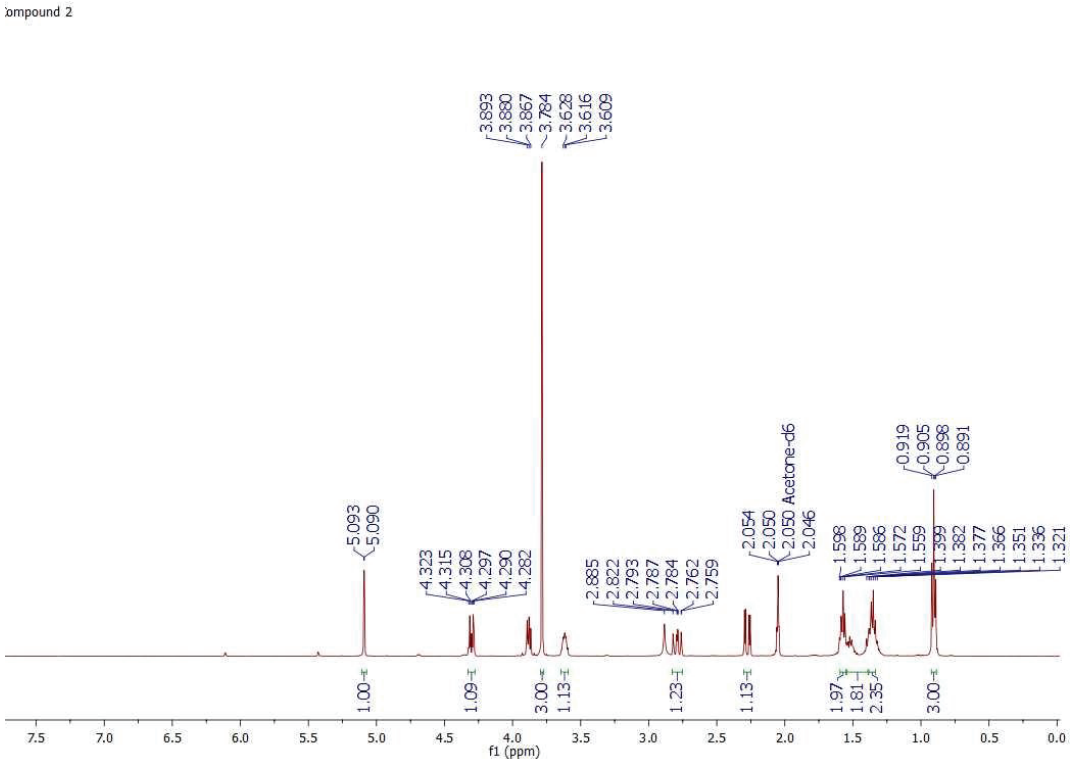


HMBC spectrum of compound **2**.

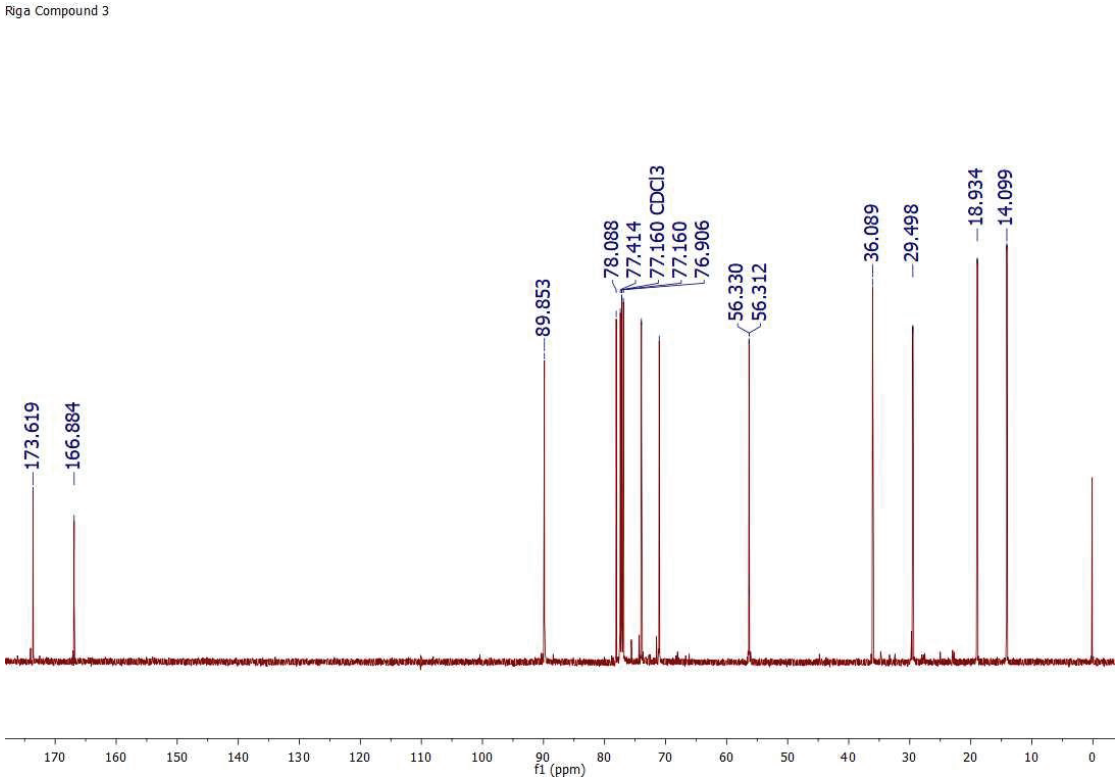


Mass spectrum of compound **2**.

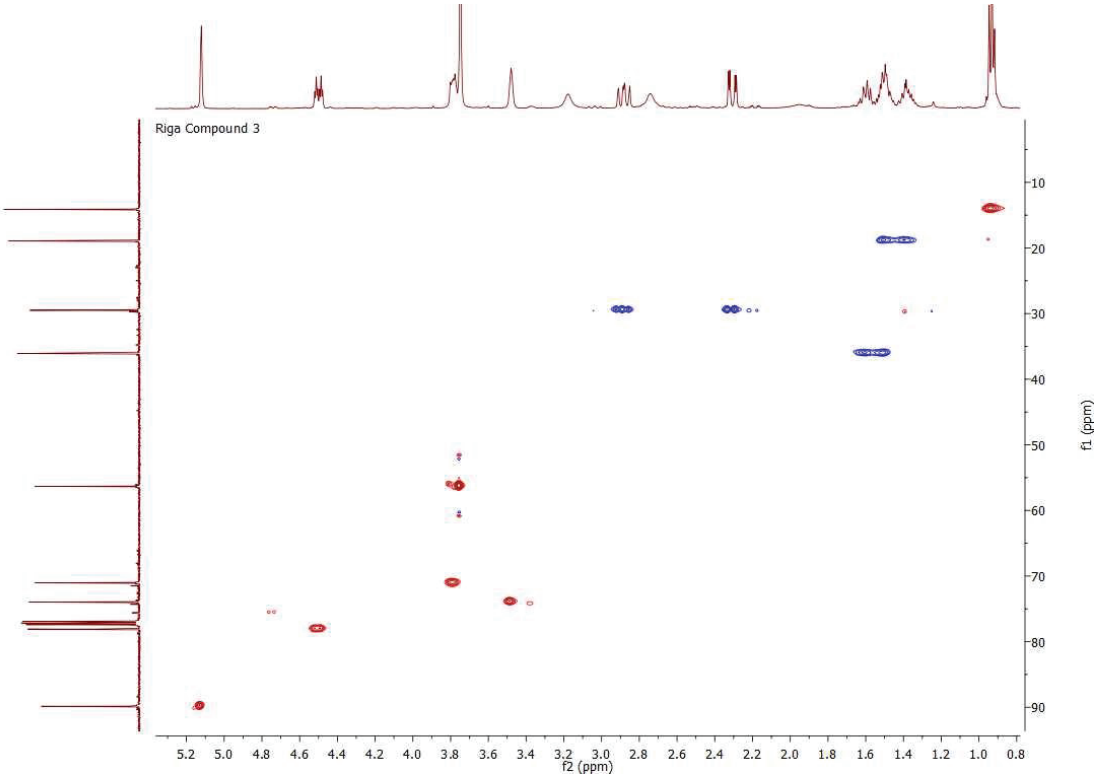
Compound 3



^{1}H-NMR spectrum of compound **3**.



^{13}C-NMR Spectrum of Compound **3**



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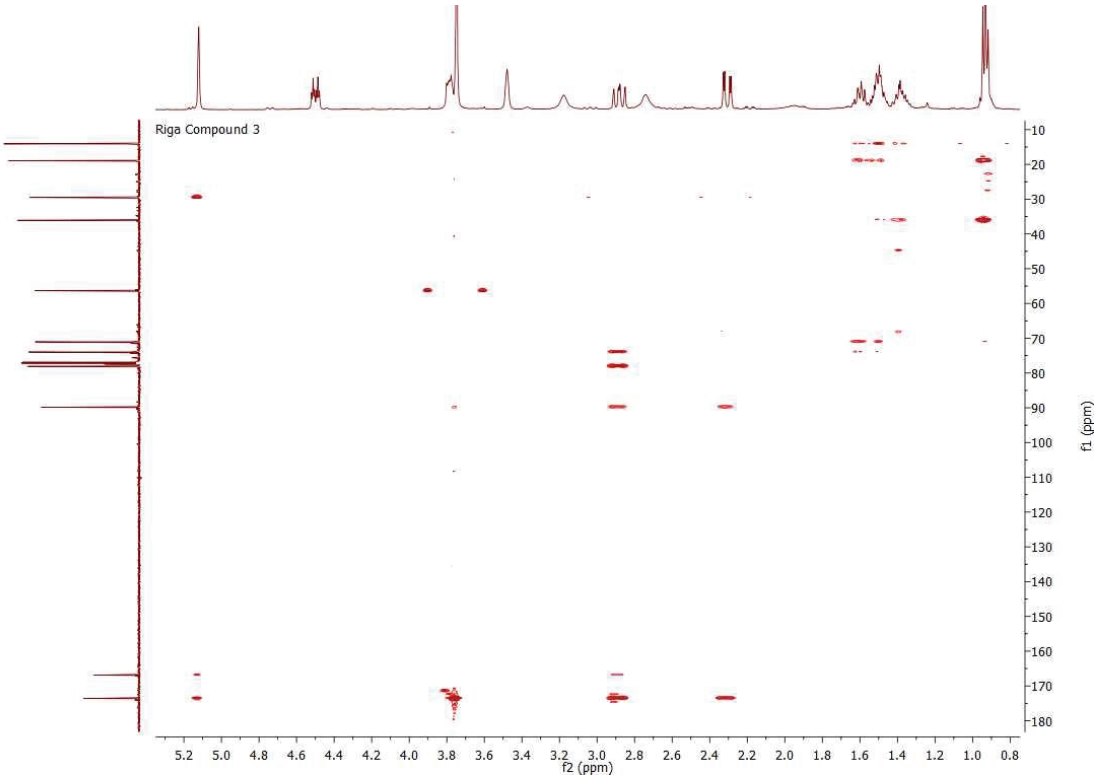
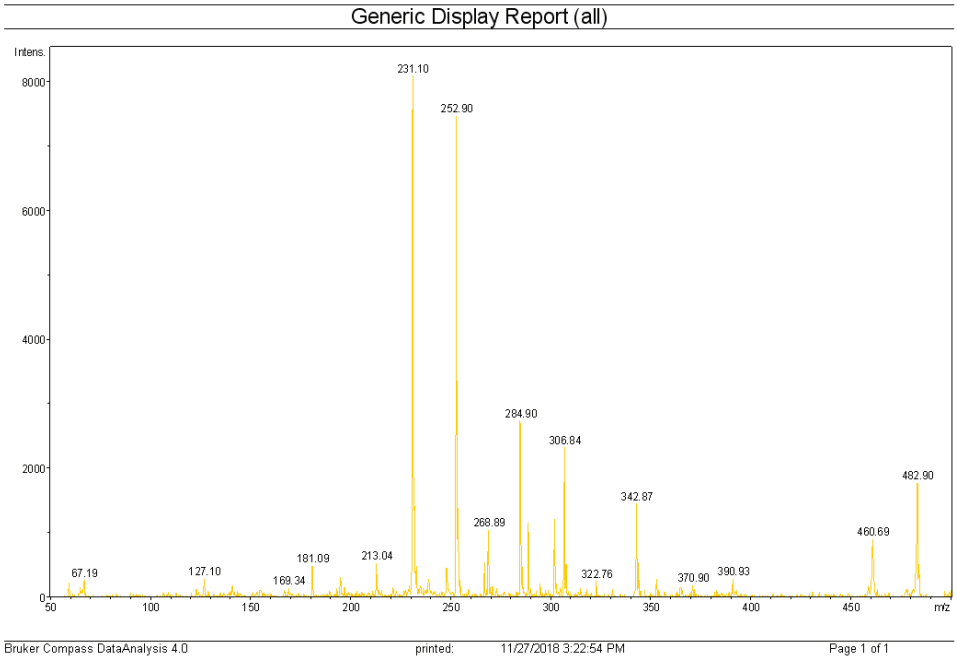
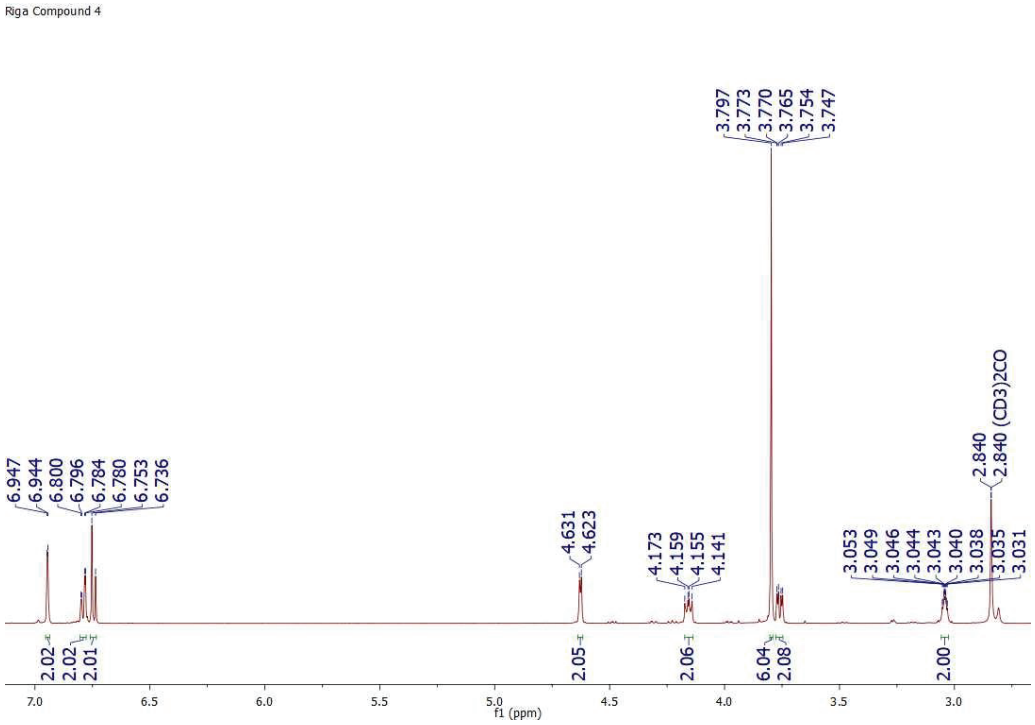


HMBC spectrum of compound **3**.



Mass spectrum of compound **3**.

Compound 4



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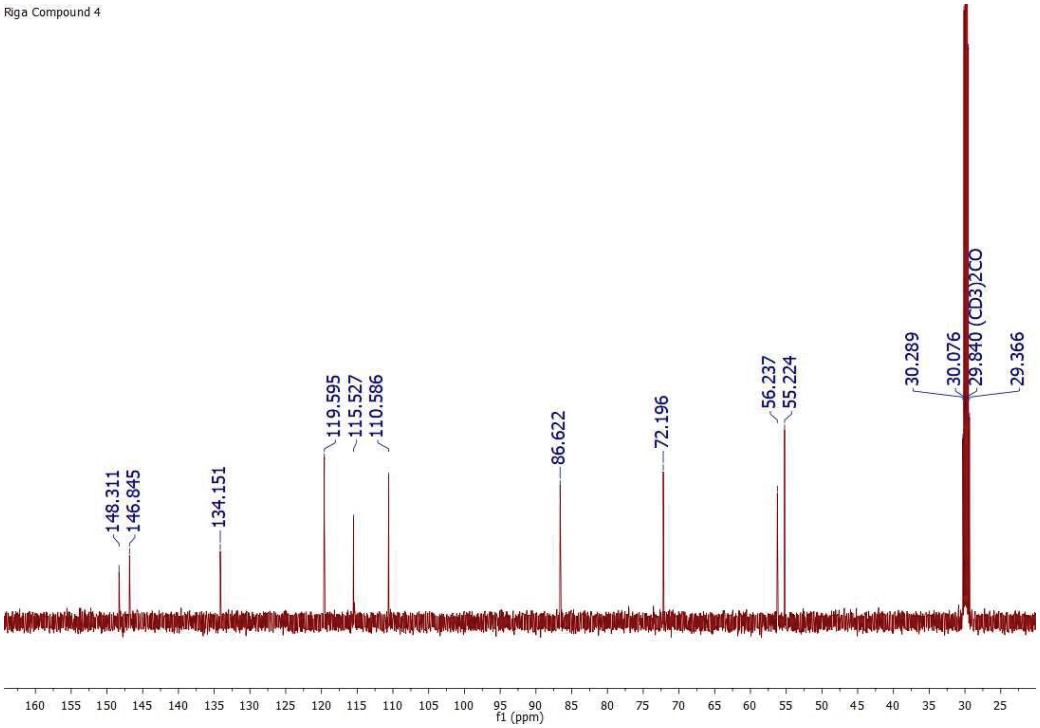

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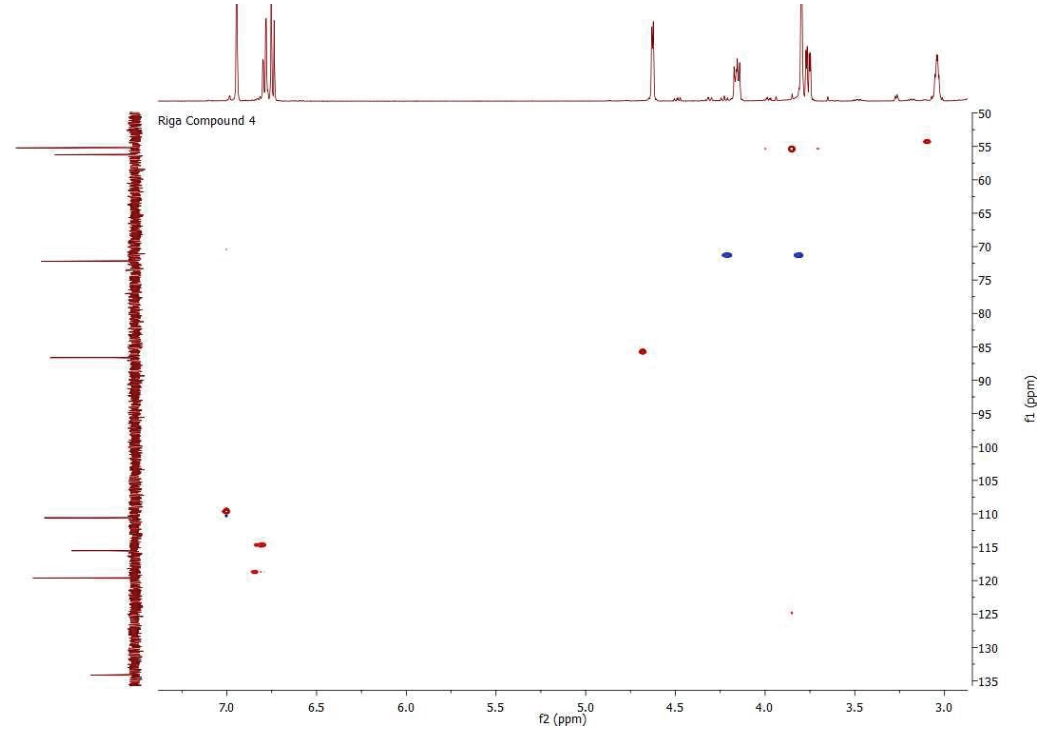

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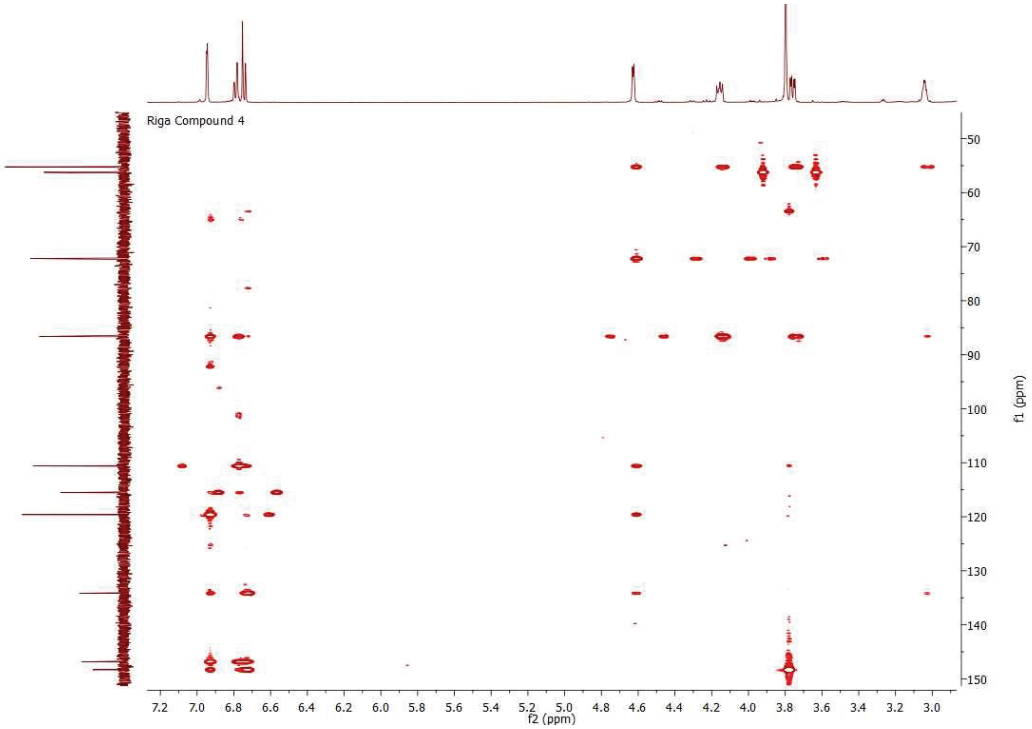

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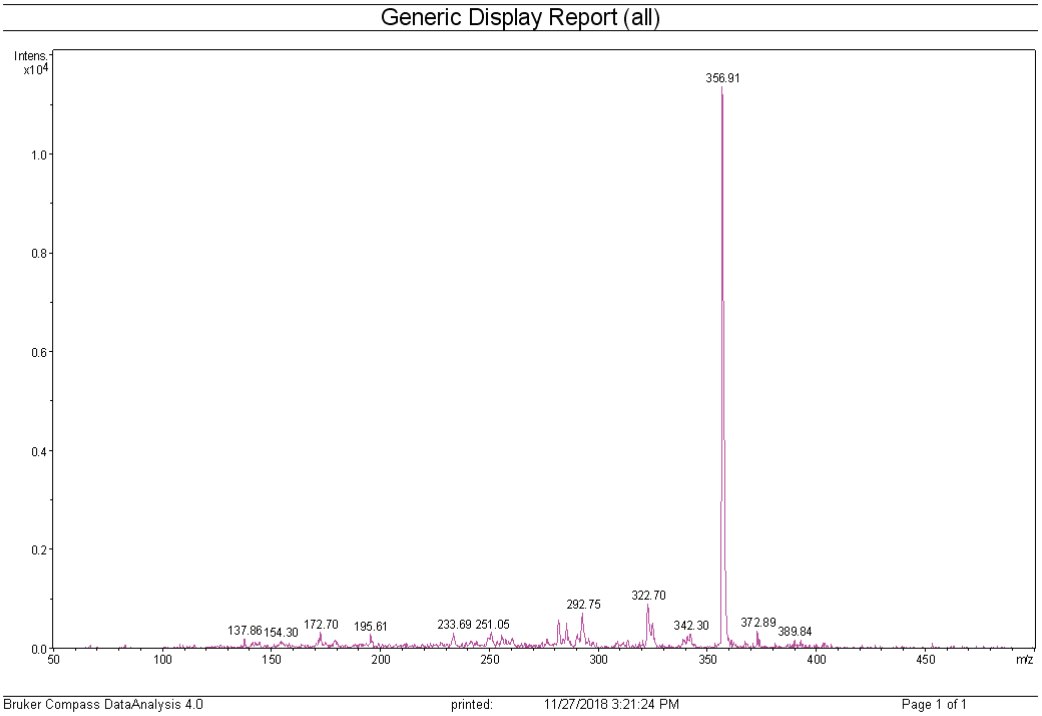

Mass spectrum of compound **4**.