Cytotoxic prenylated flavonoids from the leaves of Macaranga indica

Doan Thi Mai Huong, Le Tran Nguyen Vu, Luu The Anh, Nguyen Thi Cuc, Nguyen Xuan Nhiem, Bui Huu Tai, Phan van Kiem, Marc Litaudon, Tran Dang Thach, Chau van Minh, et al.

Manuscript Details

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Abstract

Three new prenylated flavonoids, macarindicins D-F (1-3) together with eight known compounds, macadenathin B (4), glyasperin A (5), kaempferol (6), quercetin (7), quercitrin (8), (+)-isolariciresinol (9), (–)-woonenoside XI (10), and (+)-lyoniresinol 4-O-β-D-glucopyranoside (11) were isolated from the leaves of Macaranga indica Wight. Their structures were determined on the basis of extensive spectroscopic methods, including 1D-, 2D-NMR and MS data. All the isolated compounds were tested for their cytotoxic activities against KB, MCF-7, HepG-2, and LU human cancer cell lines. As results, compound 2 showed significant cytotoxic activity on all human cancer cell lines with IC50 values ranging from 11.0 to 17.0 μM. Compound 3-5, exhibited moderate cytotoxic activity against four cancer cell lines with an IC50 values ranging from 15.0 to 38.2 μM.

Keywords	Macaranga indica; macarindicin D; macarindicin E; macarindicin F; cytotoxic
Taxonomy	Molecular Structure, Natural Product Biochemistry

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No data was used for the research described in the article

Highlights

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- Three new prenylated flavonoids were isolated from *Macaranga indica.*
- The structures were successfully determined by spectroscopic evidence.
- Comp. **2** showed significant activity on tested cancer cells (IC_{50} ranging 11.0~17.0 μM).

Graphical abstract

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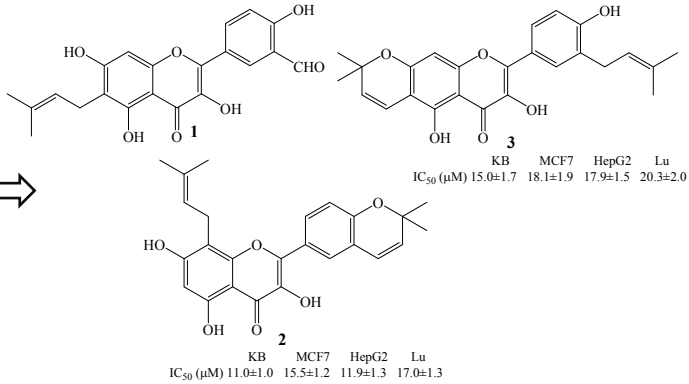
Macaranga indica



Cytotoxic prenylated flavonoids from the leaves of *Macaranga indica*

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ABSTRACT

Three new prenylated flavonoids, macarindicins D-F (**1-3**) together with eight known compounds, macadenathin B (**4**), glyasperin A (**5**), kaempferol (**6**), quercetin (**7**), quercitrin (**8**), (+)-isolariciresinol (**9**), (–)-woonenoside XI (**10**), and (+)-lyoniresinol 4-*O*-β-D-glucopyranoside (**11**) were isolated from the leaves of *Macaranga indica* Wight. Their structures were determined on the basis of extensive spectroscopic methods, including 1D-, 2D-NMR and MS data. All the isolated compounds were tested for their cytotoxic activities against KB, MCF-7, HepG-2, and LU human cancer cell lines. As results, compound **2** showed significant cytotoxic activity on all human cancer cell lines with IC_{50} values ranging from 11.0 to 17.0 μM. Compound **3-5**, exhibited moderate cytotoxic activity against four cancer cell lines with an IC_{50} values ranging from 15.0 to 38.2 μM.

Keywords: *Macaranga indica*; macarindicin D; macarindicin E; macarindicin F; cytotoxic

1. Introduction

Prenylated flavonoids play important roles in the plant's defensive strategy. Recently, the research on prenylated flavonoids have received the attention from the scientists due to their promising and diverse bioactivities on multitarget tissues. Prenylated flavonoids showed potential cytotoxic activity against tumor and cancer cell lines (Chen et al. 2014).

The genus *Macaranga* (Euphorbiaceae) comprises of about 300 species which are mainly distributed in tropics of Africa, Asia, Australia, and the Pacific regions. The *Macaranga* genus have been used in traditional medicine for the treatments of cuts, swellings, boils, bruises, and sores. Phytochemical investigation of this genus confirmed the presence of stilbenes, flavonoids, coumarins, terpenoids, and tannins (Magadula 2014). The extracts and those compounds showed a wide spectrum of pharmacological activities including anti-cancer, anti-inflammatory, anti-oxidant, anti-microbial, and anti-plasmodial activities. Previous phytochemical investigations of *M. indica* led to the isolation and identification of flavonoids and prenylated derivatives (Yang et al. 2015a). As part of our research on anticancer drug candidates from 2,500 Vietnamese medicinal plants, the ethyl acetate extract of *M. indica* was found to inhibit 69% the growth of KB human cancer cell line at the concentration of 1.0 μg/ml. Herein, we report the isolation and structure elucidation of three new prenylated flavonoids together with eight known compounds from *M. indica* and evaluation of their cytotoxic effects.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was defined as $C_{21}H_{18}O_7$ by HR-ESI-MS at *m/z* 383.1125 $[M+H]^+$ (Calcd. for $[C_{21}H_{19}O_7]^+$, 383.1131). The ^{1}H-NMR spectrum of **1** showed the signals of three aromatic protons with ABX system of B ring at δ_H 7.18 (1H, d, J = 9.0 Hz), 8.29 (1H, dd, J = 2.0, 9.0 Hz), and 8.50 (1H, d, J = 2.0 Hz); one aromatic proton of A ring at δ_H 6.50 (1H, s) suggested the presence of flavonol moiety; one

olefinic proton at δ_H 5.19 (1H, t, J = 7.0 Hz) and two methyl groups at δ_H 1.63 (3H, s) and 1.73 (3H, s) suggested the presence of a prenyl group; and one aldehydic proton at δ_H 10.35 (1H, s). The ^{13}C-NMR and HSQC spectra (Table 1) of **1** showed the signals of 21 carbons, including one carbonyl carbon (δ_C 175.9), one aldehydic carbon (δ_C 190.5), eleven non-protonateds (δ_C 102.8, 110.3, 122.3 ×2, 130.6, 136.2, 145.0, 154.0, 157.4, 161.7, and 161.8), five methines (δ_C 92.7, 117.7, 122.1, 128.3, and 134.9) one methylene (δ_C 20.9), and two methyl carbons at (δ_C 17.6 and 25.4). Analysis of the ^{1}H- and ^{13}C-NMR data of **1** suggested the structure **1** was a prenylated flavone, similar to those of glyasperin A (**5**) (Nomura et al. 1992), except for the disappearance of isoprenyl group at C-3′ of B ring. This could be oxidation of prenyl group to form aldehydic group. The positions of the functional groups were confirmed by analysis of the HSQC and HMBC spectra (Figure 2). The HMBC correlations between H-5′ (δ_H 7.18) and C-1′ (δ_C 122.3)/C-3′ (δ_C 122.3); between H-6′ (δ_H 8.29) and C-2′ (δ_C 128.3)/C-4′ (δ_C 161.8)/C-2 (δ_C 145.0); between aldehydic proton (δ_H 10.35) and C-2′ (δ_C 128.3)/C-3′ (δ_C 122.3)/C-4′ (δ_C 161.8) suggested the positions of hydroxy group at C-4′ and the aldehydic group at C-3′. The HMBC correlations between H-8 (δ_H 6.50) and C-6 (δ_C 110.3)/C-7 (δ_C 161.7)/C-9 (δ_C 154.0)/C-10 (δ_C 102.8); between H-1″ (δ_H 3.24) and C-5 (δ_C 157.4)/C-6 (δ_C 110.3)/C-7 (δ_C 161.7) suggested the position of prenyl group at C-6 and the hydroxy groups at C-5 and C-7. Thus, the structure of **1** was determined and named macarindicin D.

Macarindicin E (**2**) possessed a molecular formula of $C_{25}H_{24}O_6$ as deduced from HR-ESI-MS at *m/z* 421.1642 $[M+H]^+$ (Calcd. for $[C_{25}H_{25}O_6]^+$, 421.1651). The ^{1}H-NMR spectrum of **2** showed the following signals: three ABX aromatic protons of B ring at δ_H 6.92 (1H, d, J = 9.0 Hz), 7.85 (1H, d, J = 2.5 Hz), and 7.93 (1H, dd, J = 2.5, 9.0 Hz); one aromatic proton of A ring at δ_H 6.30 (1H, s) assigned the presence of flavonol moiety; three olefinic protons δ_H 5.17 (1H, t, J = 6.5 Hz), 5.85 (1H, t, J = 10.0 Hz), and 6.46 (1H, t, J = 10.0 Hz); four methyl groups at δ_H 1.62 (3H, s), 1.74 (3H, s), 1.42 (6H, s) suggested the appearance of two prenyl groups. The ^{13}C-

NMR and HSQC spectra of **2** showed the signals of 25 carbons, including one carbonyl, twelve non-protonateds, seven methines, one methylene, and four methyl carbons. The anaylsis of ^{1}H- and ^{13}C-NMR data indicated the strucuture of **2** was similar to those of macadenathin B (**4**) (Yang et al. 2015b), excepted for the change of prenyl group at A ring. The HMBC correlations between H-6 (δ_H 6.30) and C-5 (δ_C 158.2)/C-7 (δ_C 161.2)/C-8 (δ_C 105.6)/C-10 (δ_C 103.0); between H-1″ (δ_H 3.43) and C-7 (δ_C 161.2)/C-8 (δ_C 105.6)/C-9 (δ_C 153.4) suggested the position of prenyl group at C-8. The HMBC correlations between H-5′ (δ_H 6.92) and C-1′ (δ_C 123.7)/C-3′ (δ_C 120.6); between H-6′ (δ_H 7.93) and C-2 (δ_C 146.0)/C-2′ (δ_C 125.6)/C-4′ (δ_C 153.9); between H-6″ (δ_H 6.46) and C-2′ (δ_C 125.6)/C-3′ (δ_C 120.6) C-4′ (δ_C 153.9)/C-8″ (δ_C 77.0) suggested the position second prenyl group at C-3′. The cyclization of prenyl group with B ring of flavonol via oxy-bridge at C-4′ and C-8″ was confirmed by the shift to low field of C-8″ (δ_C 77.0) as well as HR-ESI-MS. Based on the above evidence, the structure of **2** was determined and named macarindicin E.

Compounds **3** was also obtained as yellow amorphous powders. HR-ESI-MS experiments resulted in the same molecular formula as that of **2** (see Experimental). The ^{1}H- and ^{13}C-NMR data were almost the same to those of macarindicin E (**2**), suggesting the possibilities exchange of pyran ring and prenyl positions. The HMBC correlations between H-8 (δ_H 6.51) and C-6 (δ_C 103.9)/C-7 (δ_C 158.3)/ C-9 (δ_C 155.1)/C-10 (δ_C 103.9); H-4″ (δ_H 1.43)/ H-5″ (δ_H 1.43) and C-2″ (δ_C 128.8)/C-3″ (δ_C 77.8); and between H-1″ (δ_H 6.61) and C-5 (δ_C 154.6)/C-6 (δ_C 103.9)/C-7 (δ_C 158.3) suggested the cyclization of prenyl group with flavonol (pyran ring) at C-7 and C-3″. In addition, the presnce of prenyl at C-3′ was confirmed by HMBC correlations from H-9″ (δ_H 1.70)/H-10″ (δ_H 1.70) to C-7″ (δ_C 122.3)/C-8″ (δ_C 131.7); from H-6″ (δ_H 3.28) to C-2′ (δ_C 129.2)/C-3′ (δ_C 127.7)/C-4′ (δ_C 157.0). Consequently, the structure of **3** was determined and named macarindicin F.

The known compounds, macadenathin B (**4**) (Yang et al. 2015b), glyasperin A (**5**) (Nomura et

al. 1992), kaempferol (**6**), quercetin (**7**), quercitrin (**8**) (Nhiem et al. 2011), (+)-isolariciresinol (**9**) (Jutiviboonsuk et al. 2005), (–)-woonenoside XI (**10**) (Niwa et al. 2004), and (+)-lyoniresinol 4-*O*-β-D-glucopyranoside (**11**) (Buske et al. 2001) were identified by comparison of their NMR data with those reported in the literature.

Compounds **1**–**11** were evaluated for cytotoxic activity against KB, MCF-7, HepG-2, and LU human cancer cell lines (Table 2). As results, compound **2** showed significant cytotoxic activity on all human cancer cell lines with IC_{50} values ranging from 11.0 to 17.0 μM, compared to those of positive control, mitoxantrol. Compound **3-5**, exhibited moderate cytotoxic activity against four cancer cell lines with IC_{50} values ranging from 15.0 to 38.2 μM. Compounds **6-11** did not show cytotoxic activity (IC_{50}>100 μM). In the structure-activity relationship, prenylated flavonoids with pyran ring at A or B ring of flavonol (**2-4**) of showed stronger cytotoxic activity than prenylated derivatives (**1** and **5**) as well as remaining flavonoids (**6-8**). Recent reports have shown significant cytotoxic effects of prenylated flavonoids (Chen et al. 2014). Flavonoids have the potential of modulating many biological events in cancer such as apoptosis, vascularization, cell differentiation, cell proliferation, etc (Lopez-Lazaro 2002). These results suggest that discovery of prenylated flavonoids may increase the possibility of finding new anticancer agents. The mechanism of action of the **2** from this plant or other sources need to be further studied.

3. Experimental

3.1. General experimental procedures

All NMR spectra were recorded on a Bruker 500 MHz. HR-ESI-MS spectra were obtained using an AGILENT 6550 iFunnel Q-TOF LC/MS system. Column chromatography (CC) was performed on silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd.). For thin layer chromatography (TLC), pre-coated

silica-gel 60 F254 (0.25 mm, Merck) and RP-18 F254S (0.25 mm, Merck) plates were used.

3.2. Plant material

The leaves of *Macaranga indica* Wight were collected at Huong Hoa, Quangtri, Vietnam in August 2010 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (MI1008) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried leaves of *M. indica* (1.92 kg) were sonicated with hot MeOH three times (3×6 L) to yield 155 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with CH_2Cl_2 and EtOAc to obtain the CH_2Cl_2 (MI1, 88.0 g), EtOAc (MI2, 16.0 g), and H_2O (MI3) extracts after removal of the solvents *in vacuo.*

The MI1 fraction was chromatographed on a silica gel column eluting with a gradient solvent of CH_2Cl_2/MeOH (100:0→2.5/1, v/v) to give five fractions, MI1A-MI1E. MI1B was chromatographed on an RP-18 column eluting with acetone/water (2.5/1, v/v) to give four fractions, MI1B1-MI1B4. MI1B1 was chromatographed on a silica gel column eluting with *n*-hexane/EtOAc (5/1, v/v) to give two fractions, MI1B1A-MI1B1B. Compounds **3** (7.0 mg) and **3** (35.0 mg) were obtained from MI1B1B on a silica gel column eluting with *n*-hexane/acetone (3/1, v/v). MI1B2 was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (60/1, v/v) to give three smaller fractions, MI1B2A-MI1B2C. Compound **1** (12.0 mg) was obtained from MI1B2B on a silica gel column, using *n*-hexane/EtOAc (3/1, v/v) as eluent solvents. MI1B2C was chromatographed on a silica gel column eluting with *n*-hexane/EtOAc (3/1, v/v) to yield compound **2** (10.0 mg). MI1B4 was chromatographed on a silica gel column eluting with CH_2Cl_2/EtOAc (20/1, v/v) to give two fractions, MI1B4A-MI1B4B. MI1B4A was chromatographed on a sephadex LH-20 column eluting with MeOH/water (1/1, v/v) to yield compound **5** (70.0 mg). The MI1D was chromatographed on silica gel column eluting with *n*-

hexane/acetone (2/1, v/v) to give four fractions, MI1D1-MI1D4. MI1D3 was chromatographed on a silica gel column eluting with *n*-hexane/EtOAc (1/1, v/v) to give two smaller fractions, MI1D3A-MI1D3B. MI1D3A was chromatographed on a Sephadex LH-20 column eluting with MeOH/water (1/1, v/v) to yield compound **9** (6.0 mg). MI1E was chromatographed on a RP-18 column eluting with acetone/water (2/1, v/v) to give three fractions, MI1E1-MI1E3. MI1E2 was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (5/1, v/v) to give two fractions, MI1E2A-MI1E2B. MI1E2A was chromatographed on a sephadex LH-20 column eluting with MeOH/water (1/1, v/v) to yield compound **8** (9.0 mg).

The MI2 fraction was chromatographed on a silica gel column eluting with a gradient of CH_2Cl_2/ MeOH (40/1→1/1, v/v) to give four fractions, MI2A-MI2D. MI2A was chromatographed on a RP-18 column eluting with MeOH/water (1.5/1, v/v) to give two smaller fractions, MI2A1-MI2A2. MI2A1 was chromatographed on a sephadex LH-20 column eluting with MeOH/water (1/1, v/v) to yield compound **7** (40.0 mg). Compound **6** (35.0 mg) was obtained from MI2A2 on a silica gel column using CH_2Cl_2/MeOH (10/1, v/v) as eluent solvents.

The MI3 was chromatographed on a Diaion HP-20 column first eluting with water to remove sugar components, then increasing concentration of MeOH in water (25, 50, 75, and 100 % of MeOH) to obtain four fractions, MI3A-MI3D. The MI3B fractions were chromatographed on a silica gel column eluting with a gradient of CH_2Cl_2/MeOH (10/1→0/1) to give four fractions, MI3B1-MI3B4. MI3B1 was chromatographed on a RP-18 column eluting with MeOH/water (1/2, v/v) to give three fractions, MI3B1A- MI3B1C. MI3B1A was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (5/1, v/v) to yield compounds **10** (8.0 mg) and 11 (12.0 mg).

*3.3.1. Macarindicin A (**1**)*

Yellow amorphous powder. HR-ESI-MS *m/z*: 383.1125 $[M + H]^+$ (Calcd. for $[C_{21}H_{19}O_7]^+$, 383.1131). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): see Table 1.

*3.3.2. Macarindicin B (**2**)*

Yellow amorphous powder. HR-ESI-MS *m/z*: 421.1642 $[M + H]^+$ (Calcd. for $[C_{25}H_{25}O_6]^+$, 421.1651). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): see Table 1.

*3.3.3. Macarindicin C (**3**)*

Yellow amorphous powder. HR-ESI-MS *m/z*: 421.1642 $[M + H]^+$ (Calcd. for $[C_{25}H_{25}O_6]^+$, 421.1651). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): see Table 1.

3.4. Cytotoxic assays: See reference (Thu et al. 2015)

Acknowledgment

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Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at sciencedirect.com.

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Figure 1. Chemical structures of compounds **1**–**11**.

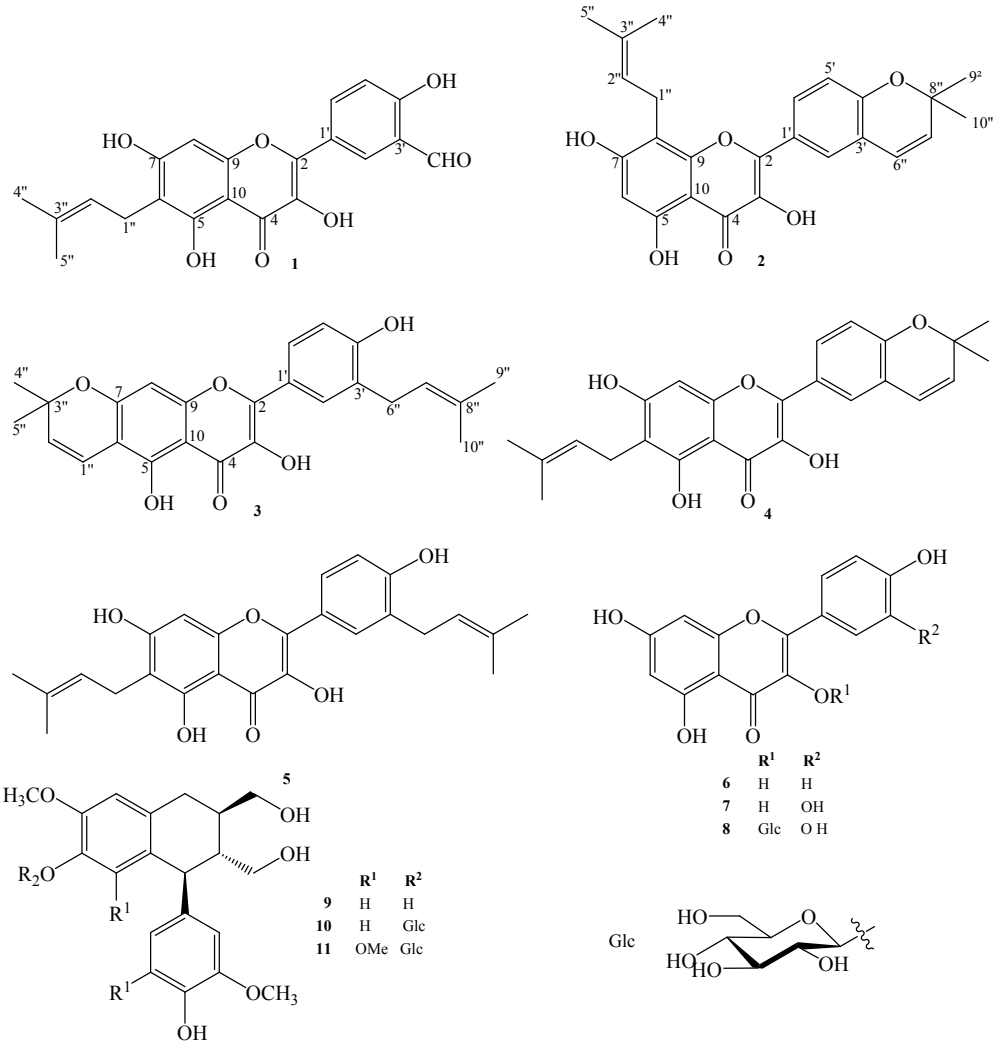

Figure 2. The key HMBC correlations of compounds **1**–**3**.

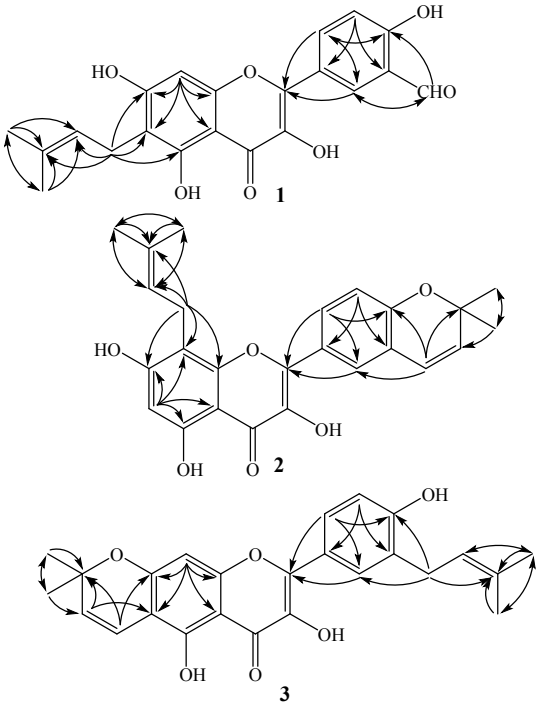

Table 1. ^{1}H- and ^{13}C-NMR spectroscopic data for compounds **1** - **3** in DMSO-d_6.

C	1		2		3	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
2	145.0	-	146.0	-	147.5	-
3	136.2	-	135.9	-	135.7	-
4	175.9	-	176.1	-	175.9	-
5	157.4	-	158.2	-	154.6	-
6	110.3	-	97.8	6.30 (s)	103.9	-
7	161.7	-	161.2	-	158.3	-
8	92.7	6.50 (s)	105.6	-	94.5	6.51 (s)
9	154.0	-	153.4	-	155.1	-
10	102.8	-	103.0	-	103.9	-
1′	122.3	-	123.7	-	121.4	-
2′	128.3	8.50 (d, 2.0)	125.6	7.85 (d, 2.5)	129.2	7.93 (d, 2.0)
3′	122.3	-	120.6	-	127.7	-
4′	161.8	-	153.9	-	157.0	-
5′	117.7	7.18 (d, 9.0)	116.0	6.92 (d, 9.0)	114.8	6.94 (d, 8.5)
6′	134.9	8.29 (dd, 2.0, 9.0)	128.8	7.93 (dd, 2.5, 9.0)	128.8	7.88 (dd, 2.0, 8.5)
1″	20.9	3.24 (d, 7.0)	21.1	3.43 (d, 6.5)	114.5	6.61 (d, 10.0)
2″	122.1	5.19 (t, 7.0)	122.5	5.17 (t, 6.5)	128.8	5.79 (d, 10.0)
3″	130.6	-	130.9	-	77.8	-
4″	25.4	1.63 (s)	25.3	1.62 (s)	27.8	1.43 (s)
5″	17.6	1.73 (s)	17.8	1.74 (s)	27.8	1.43 (s)
6″	190.5	10.35 (s)	121.2	6.46 (d, 10.0)	28.1	3.28 (d, 7.5)
7″			131.8	5.85 (d, 10.0)	122.3	5.30 (t, 7.5)
8″			77.0	-	131.7	-
9″			27.8	1.42 (s)	25.4	1.70 (s)
10″			27.8	1.42 (s)	17.6	1.70 (s)

Table 2. Cytotoxic effects of compounds **1-11**

Compounds	IC_{50} (μM)[a]			
	KB	**MCF7**	**HepG2**	**LU**
1	47.4±2.4	>100	53.1±3.7	>100
2	11.0±1.0	15.5±1.2	11.9±1.3	17.0±1.3
3	15.0±1.7	18.1±1.9	17.9±1.5	20.3±2.0
4	32.9±2.1	33.1±3.2	33.1±1.5	35.0±1.7
5	38.2±2.4	34.4±3.1	30.1±4.0	34.6±3.7
6	>100	>100	>100	>100
7	>100	>100	>100	>100
8	>100	>100	>100	>100
9	>100	>100	>100	>100
10	>100	>100	>100	>100
11	>100	>100	>100	>100
MX[b]	7.8±0.7	10.3±1.1	8.2±0.6	7.7±0.4

[a]The concentration that inhibits 50% of cell growth was calculated (IC_{50}). Compounds were tested at a maximum concentration of 100 μM. Data are means of three experiments.

[b]Mitoxantrone (MX), an anticancer agent, was used as reference compound.

SUPPLEMENTARY MATERIAL

Cytotoxic prenylated flavonoids from the leaves of *Macaranga indica*

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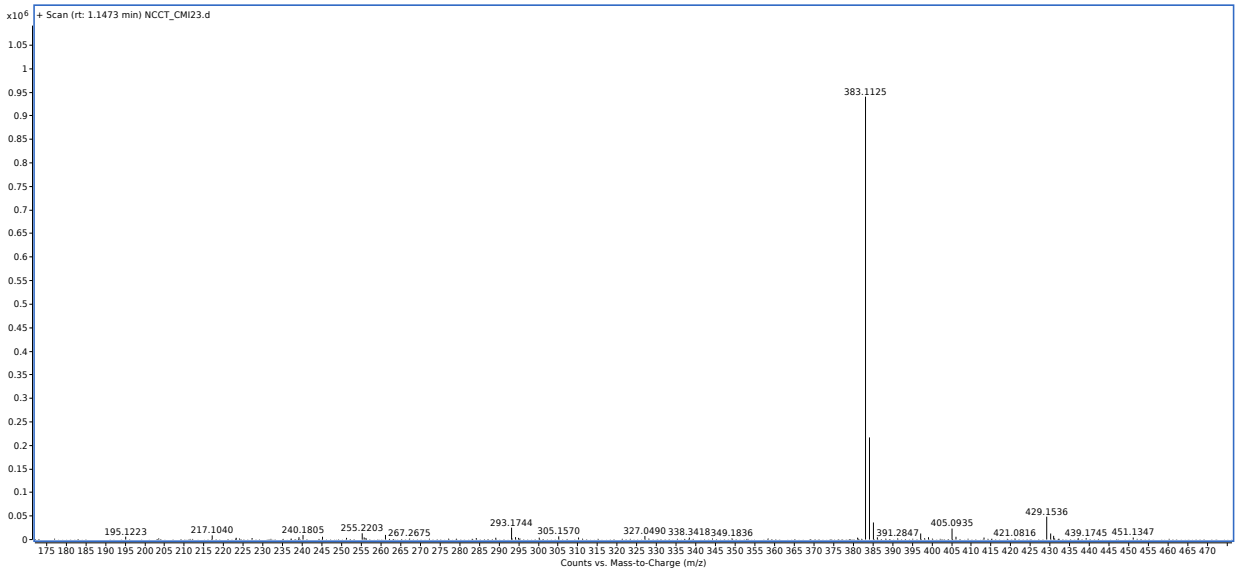
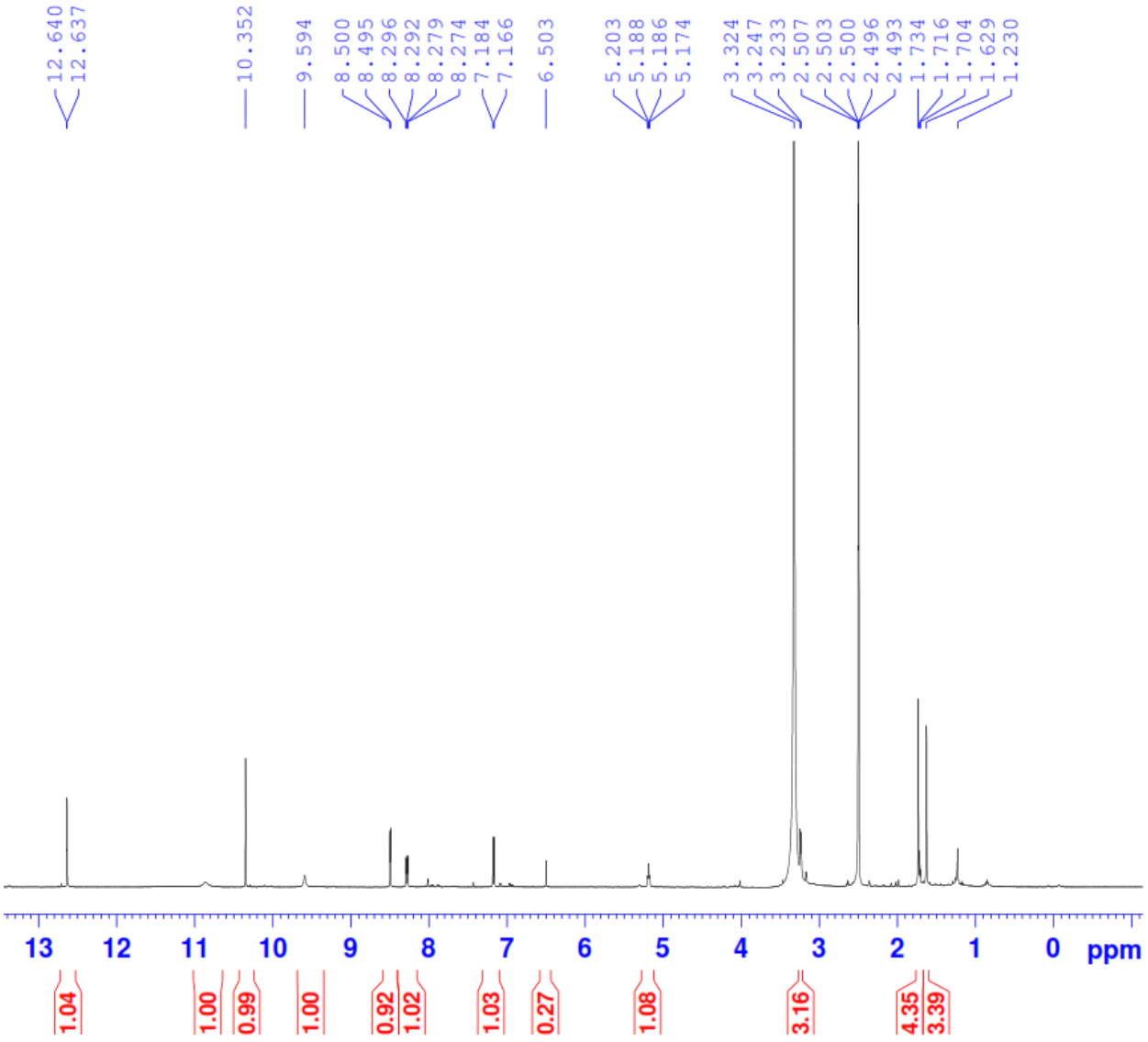
Content

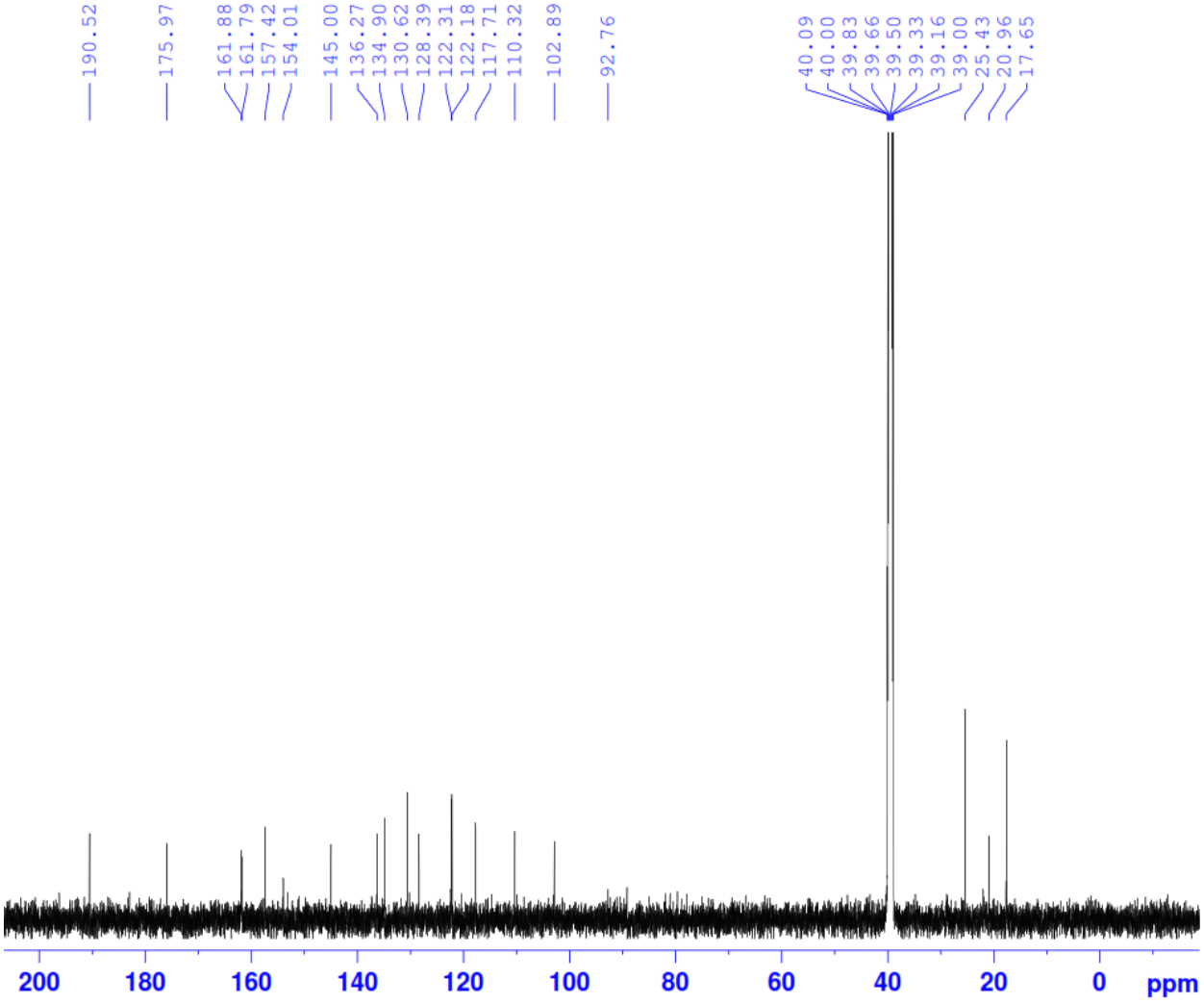
1. HR-ESI-MS of compound **1**



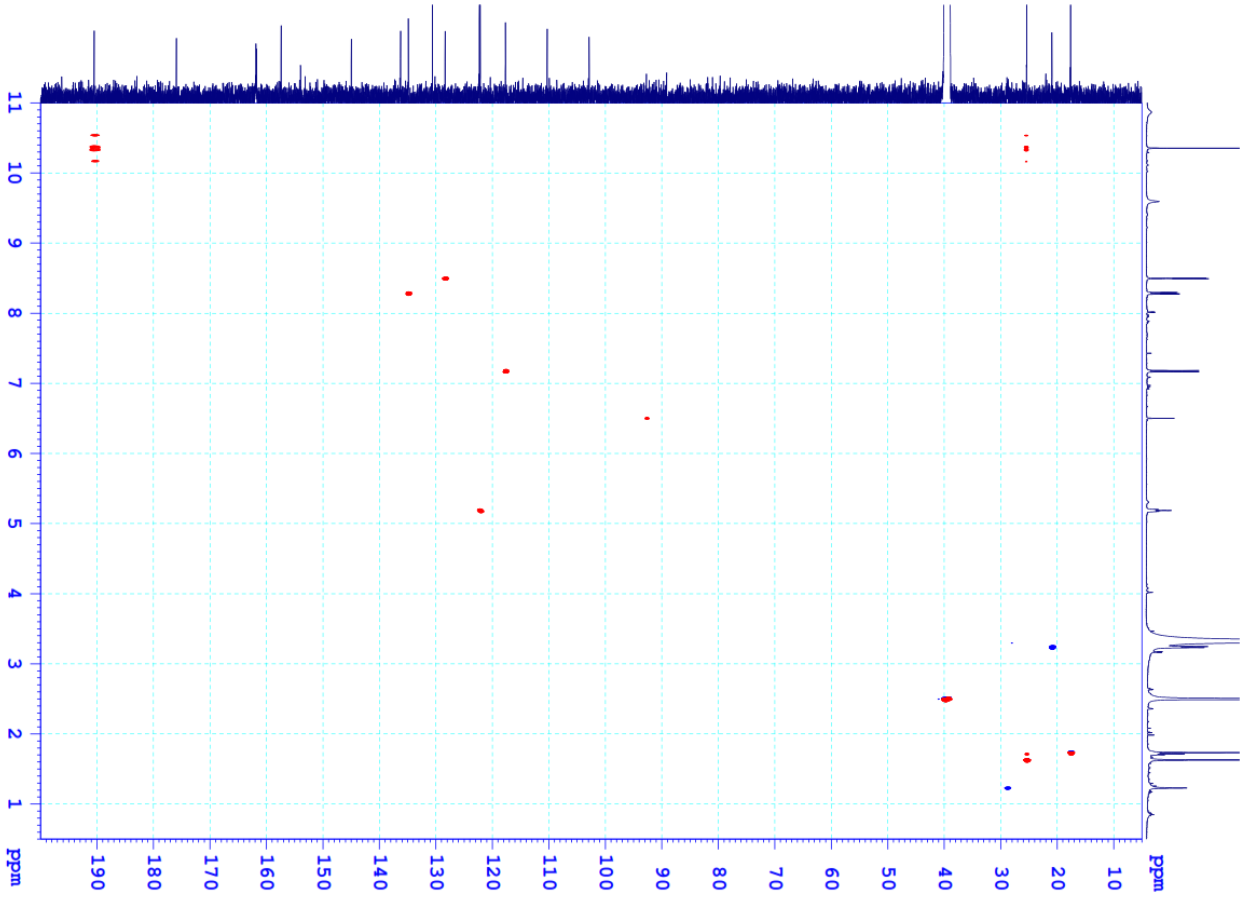
2. ^{1}H-NMR spectrum of compound **1**



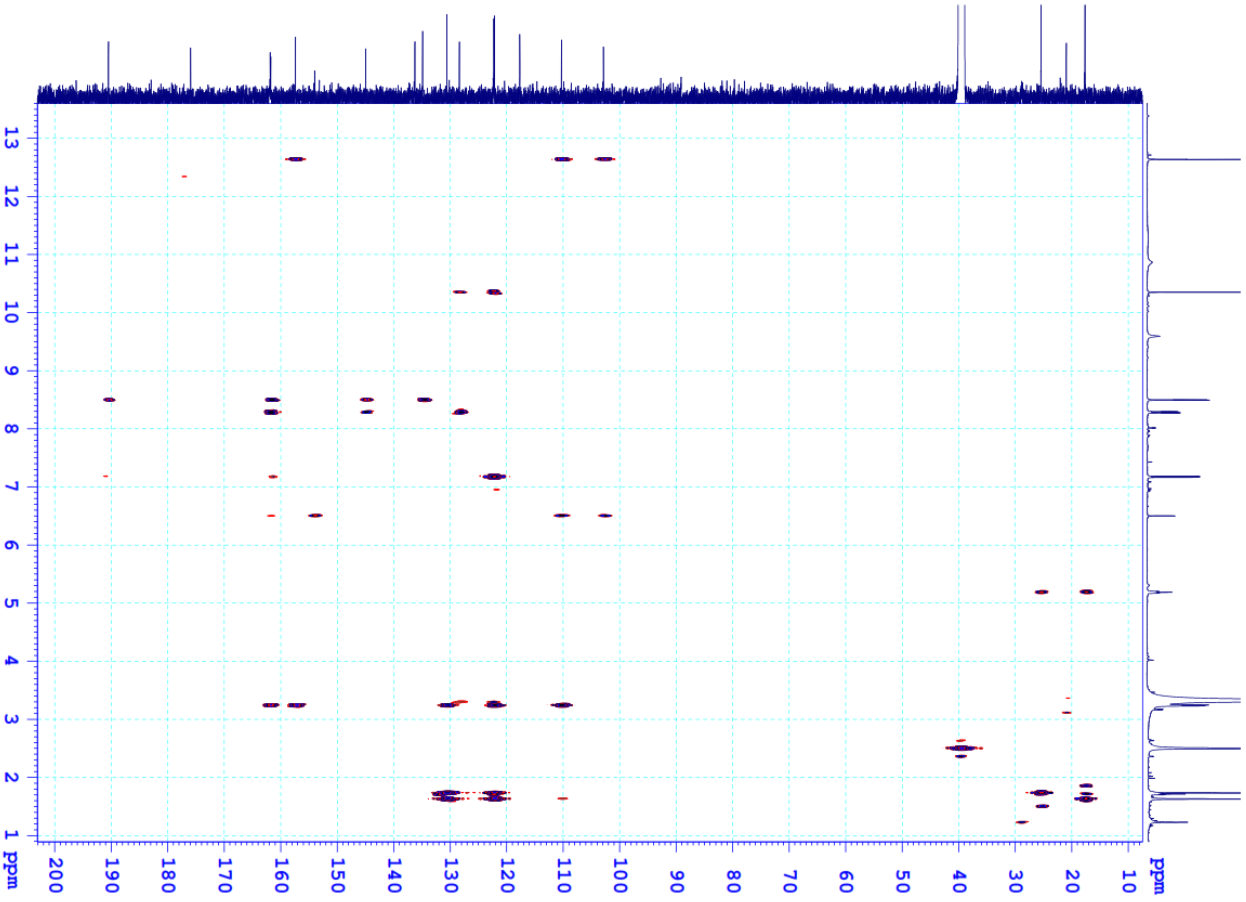
3. ^{13}C-NMR spectrum of compound **1**



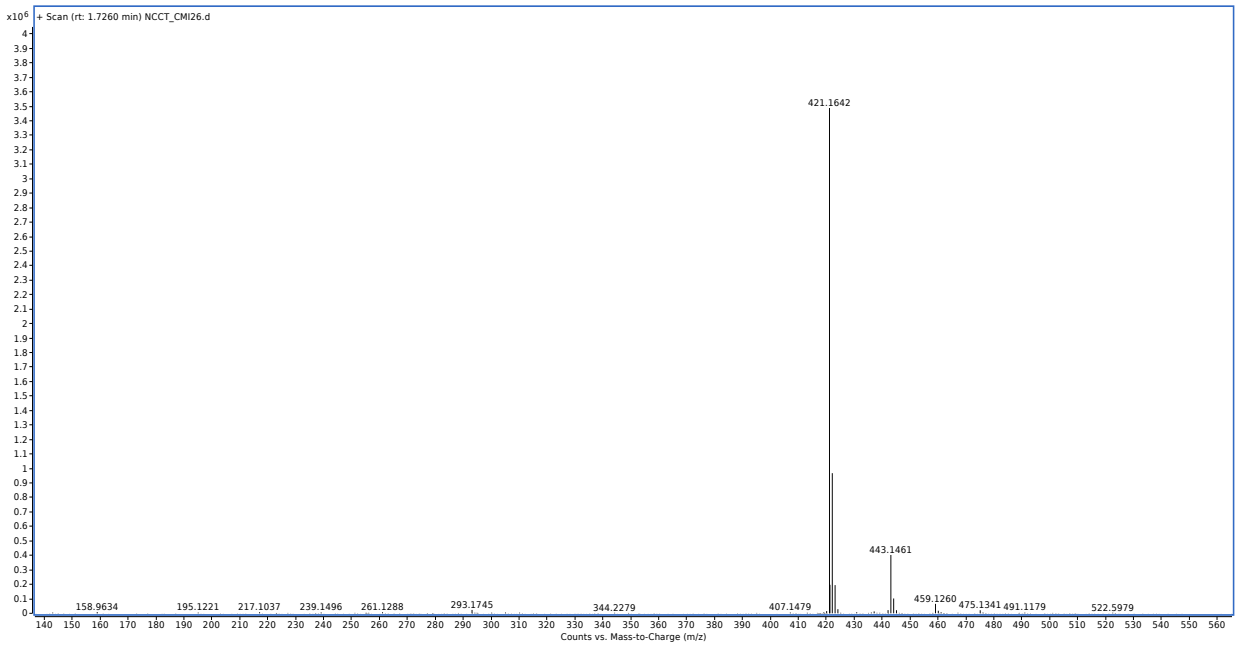
4. HSQC spectrum of compound **1**



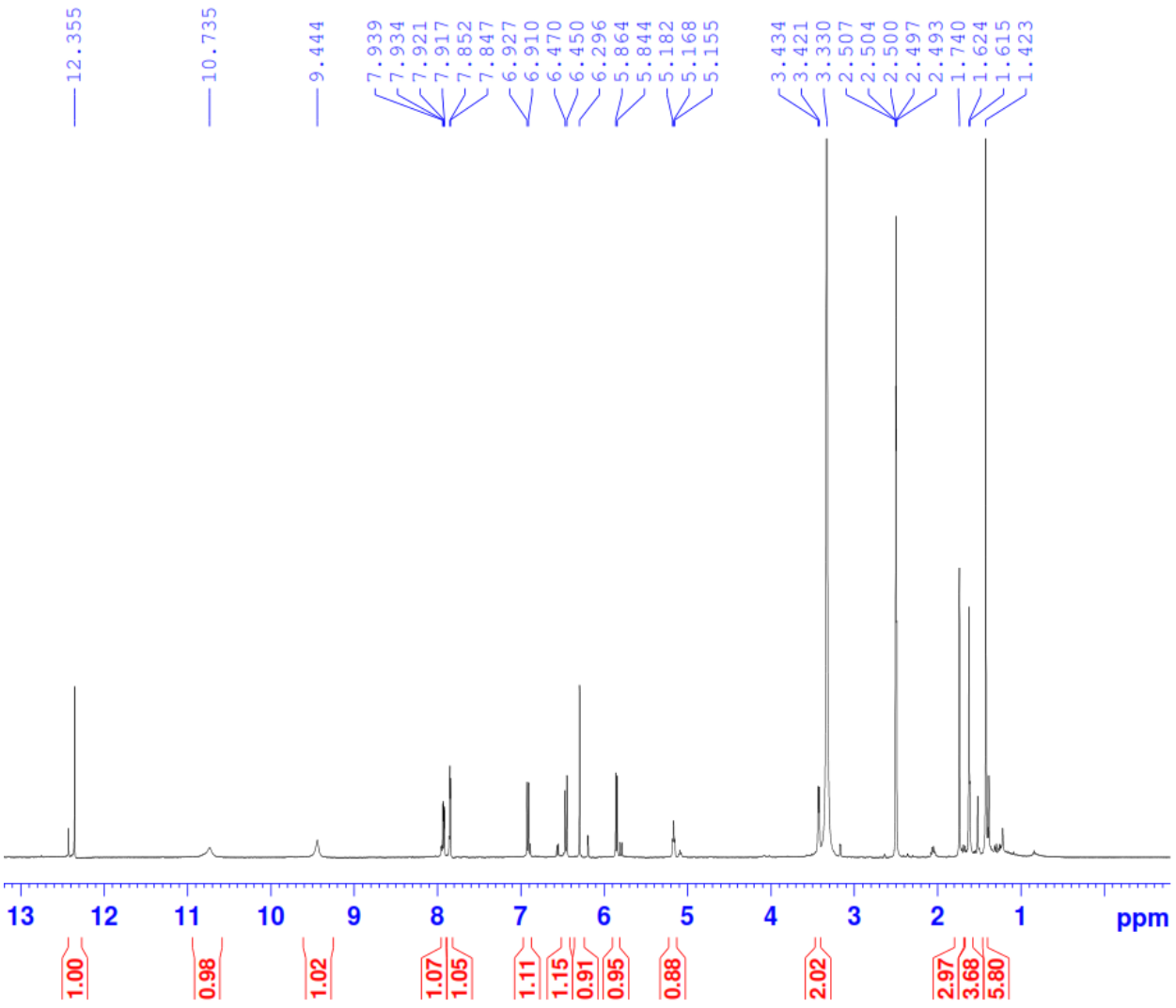
5. HMBC spectrum of compound **1**



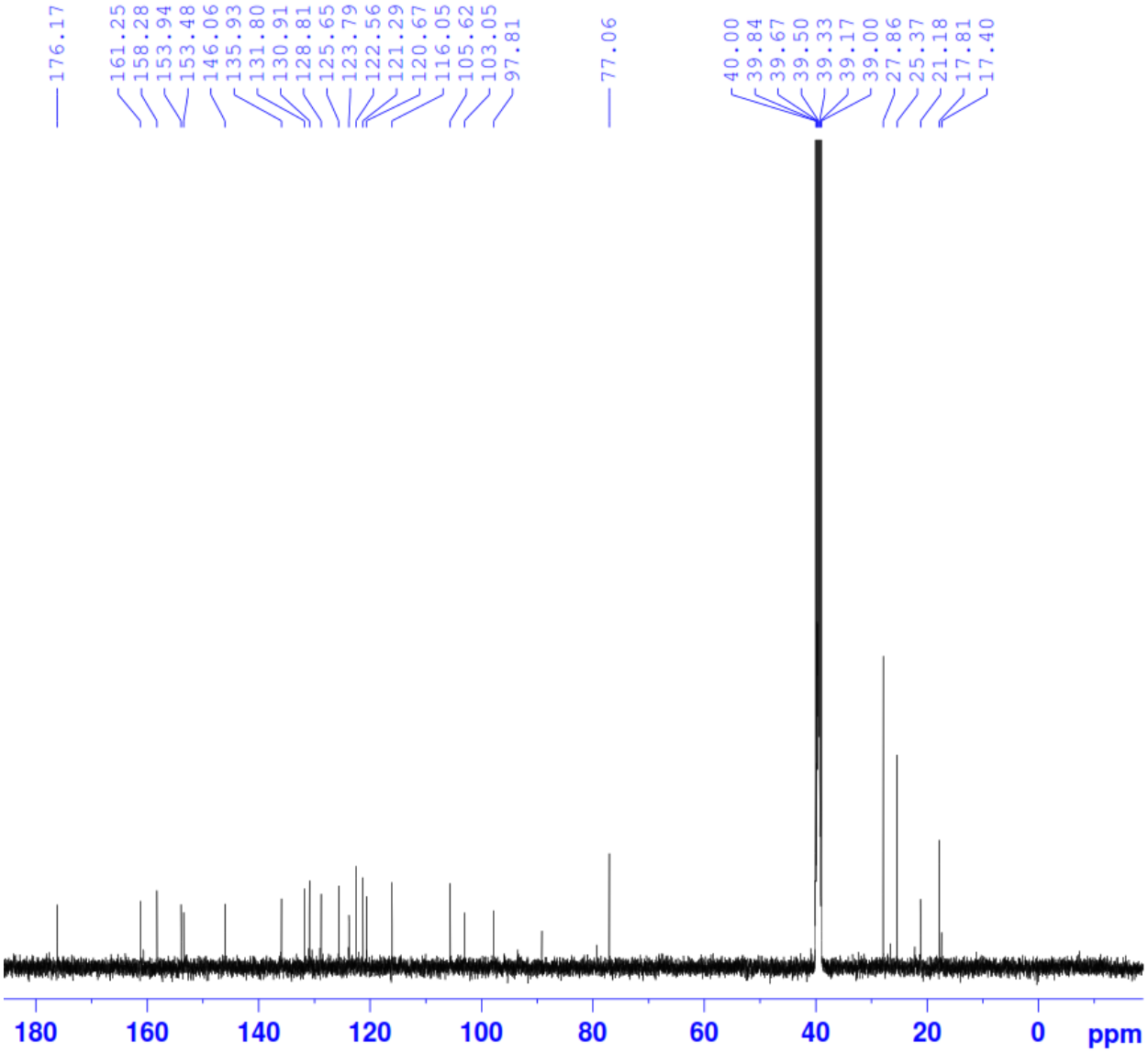
6. HR-ESI-MS of compound **2**



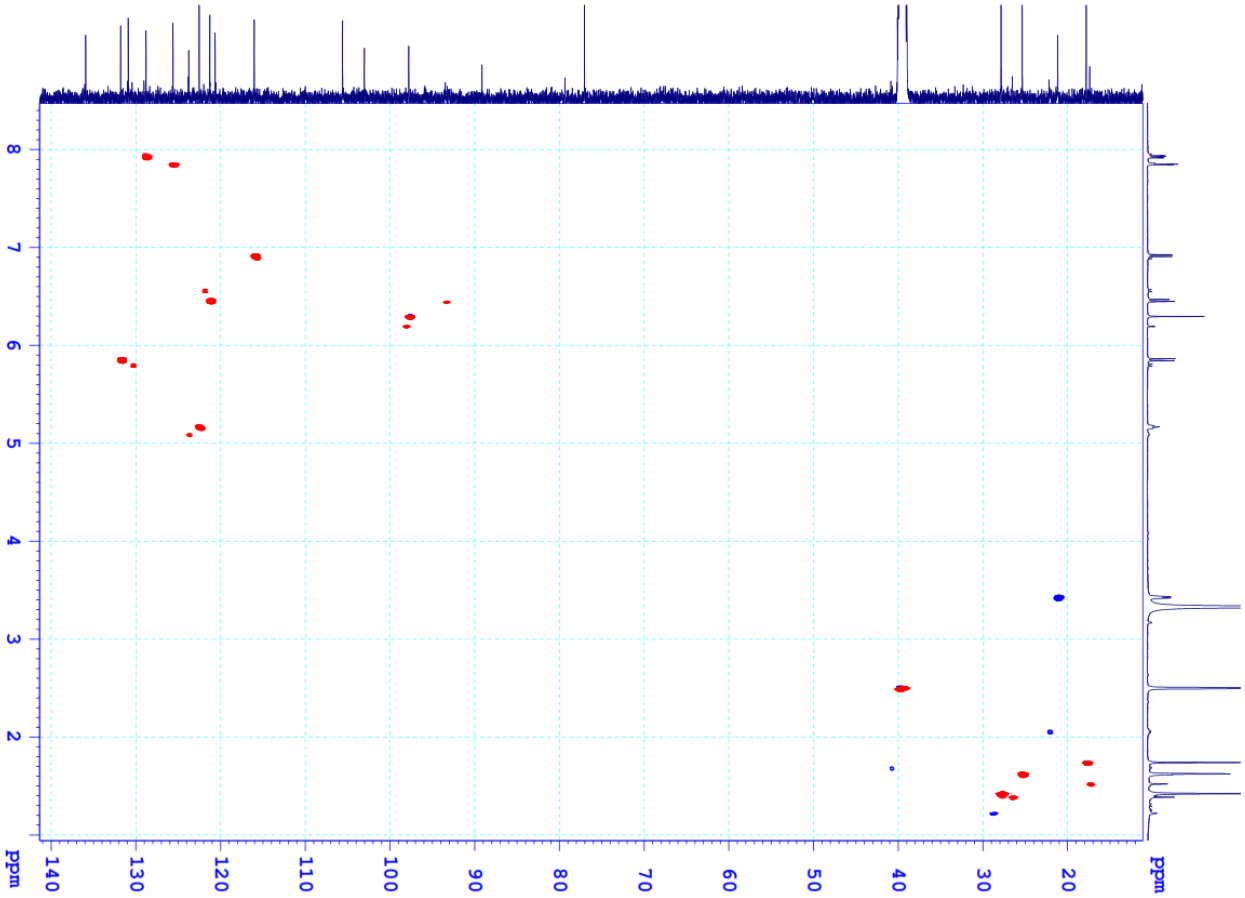
7. ^{1}H-NMR spectrum of compound **2**



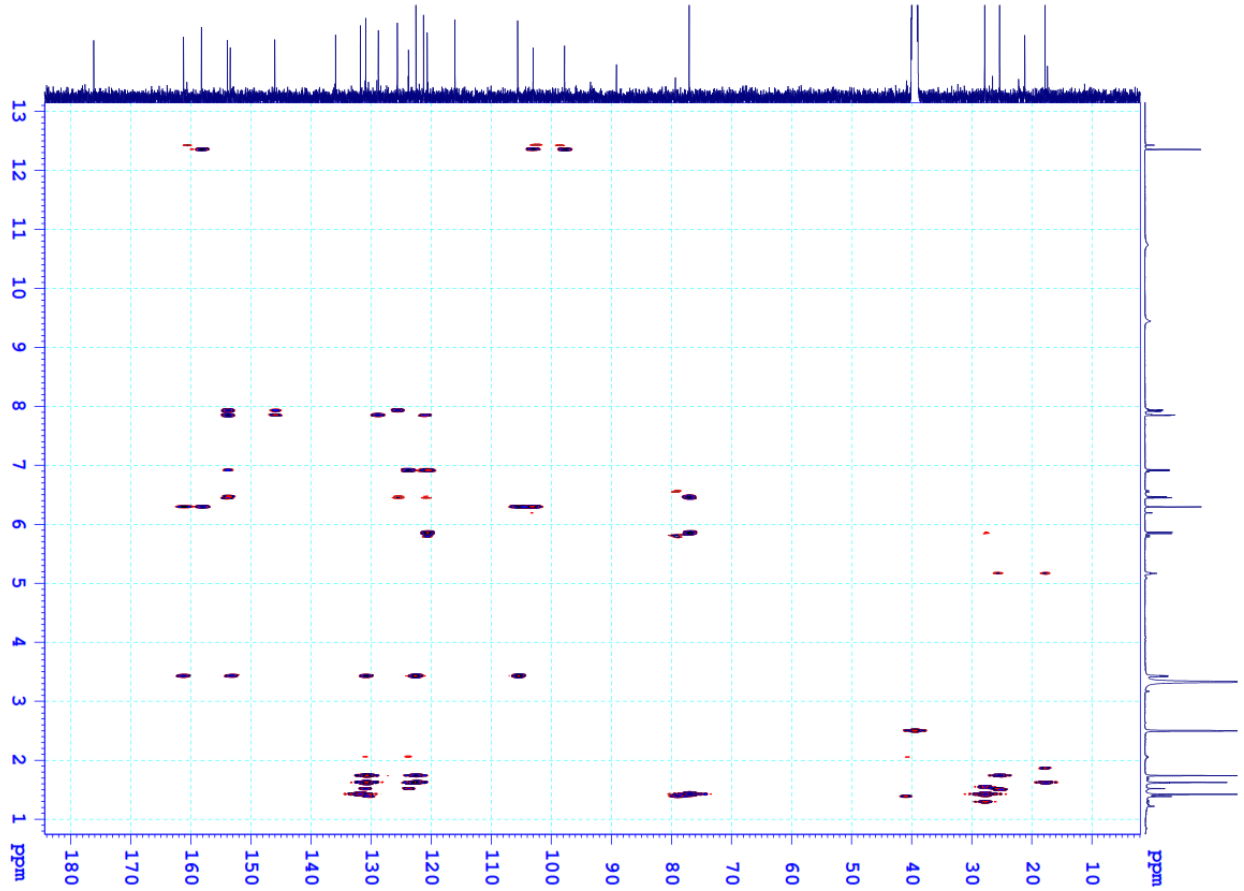
8. ^{13}C-NMR spectrum of compound **2**



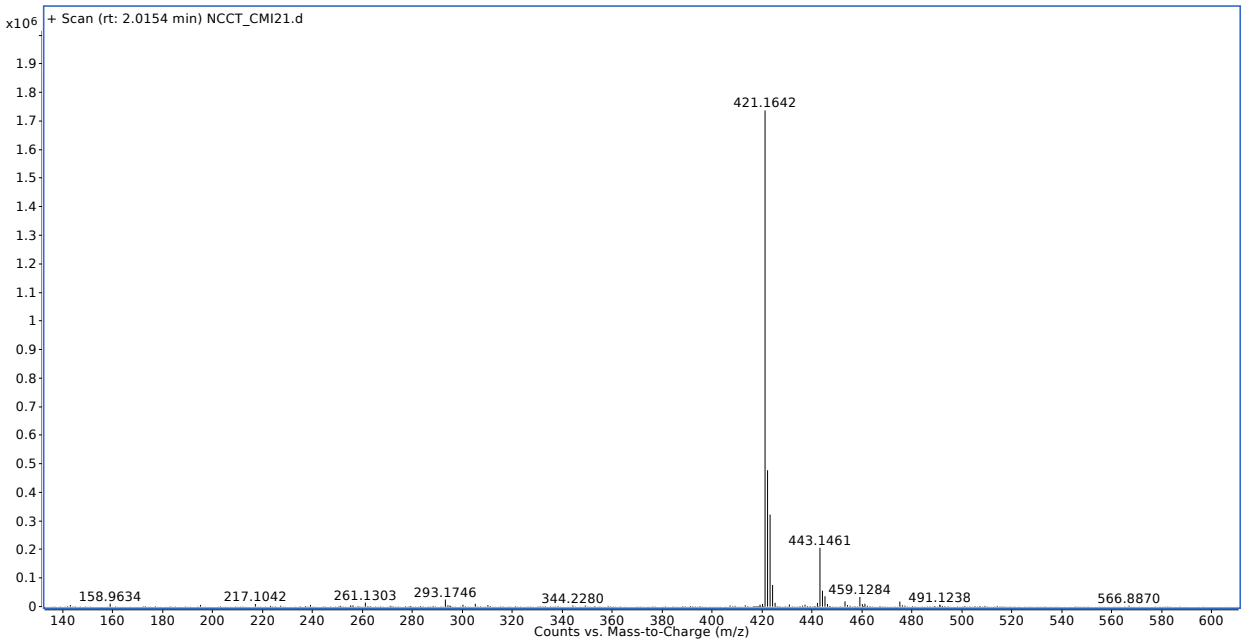
9. HSQC spectrum of compound **2**



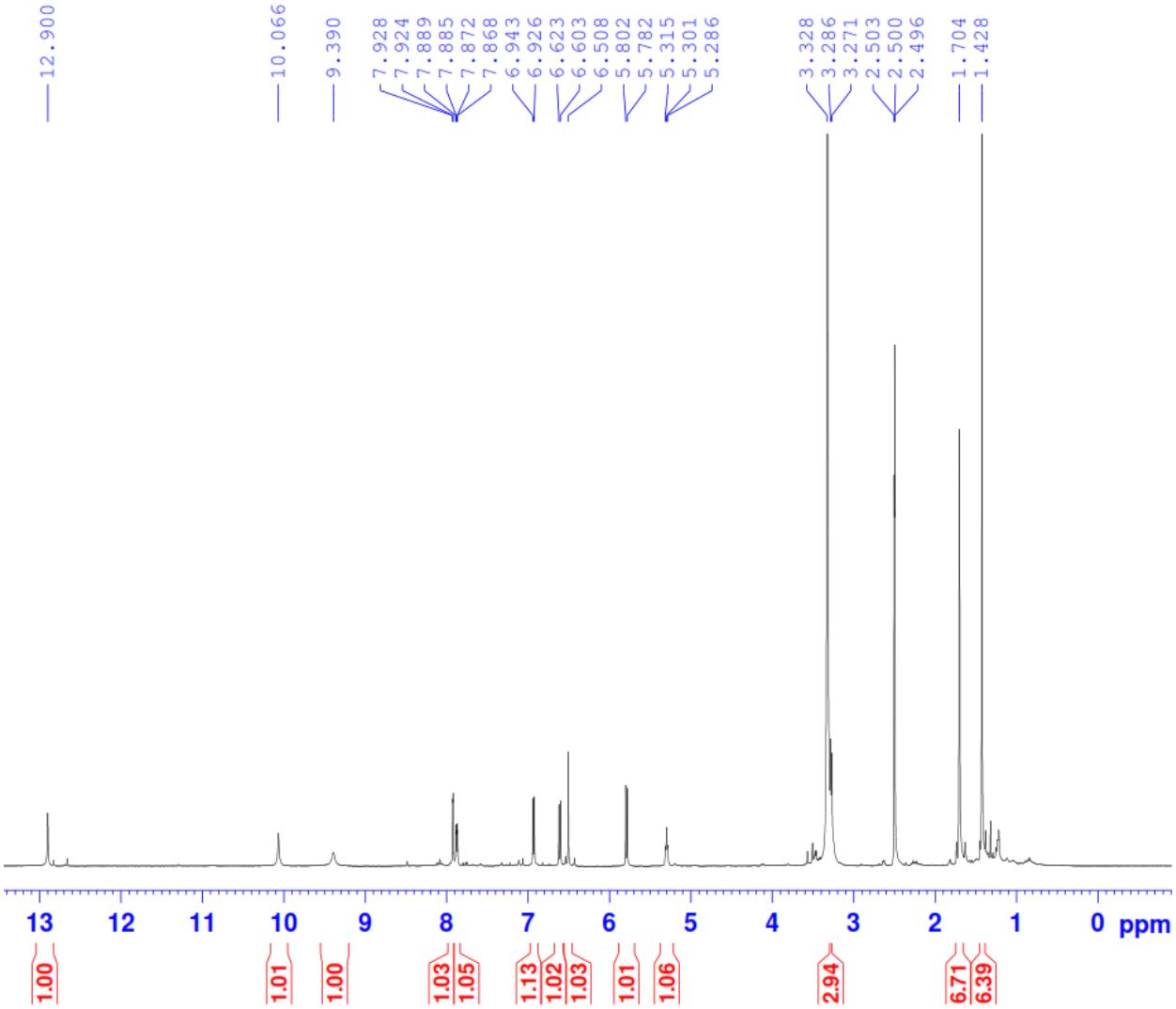
10. HMBC spectrum of compound **2**



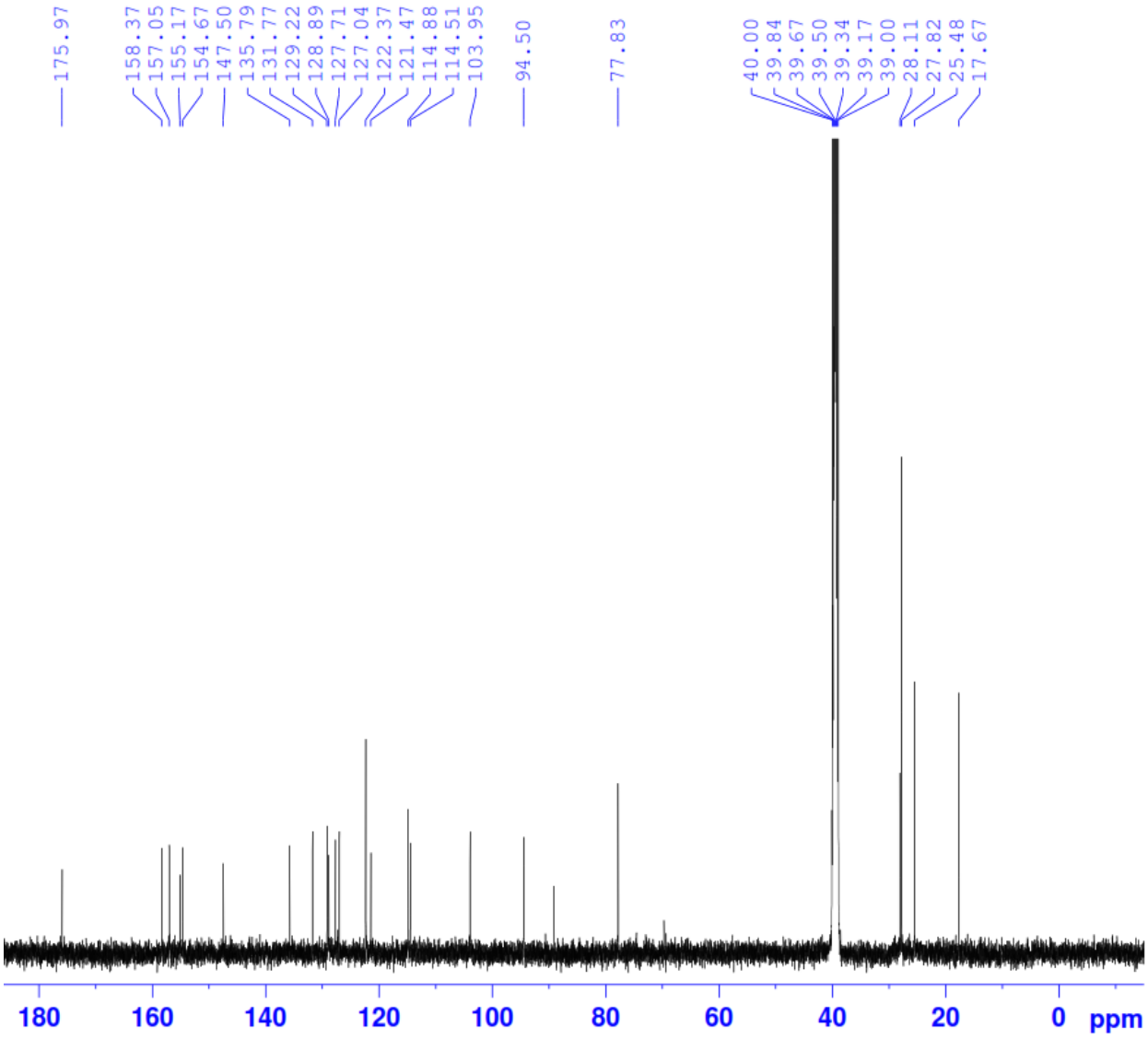
11. HR-ESI-MS of compound **3**



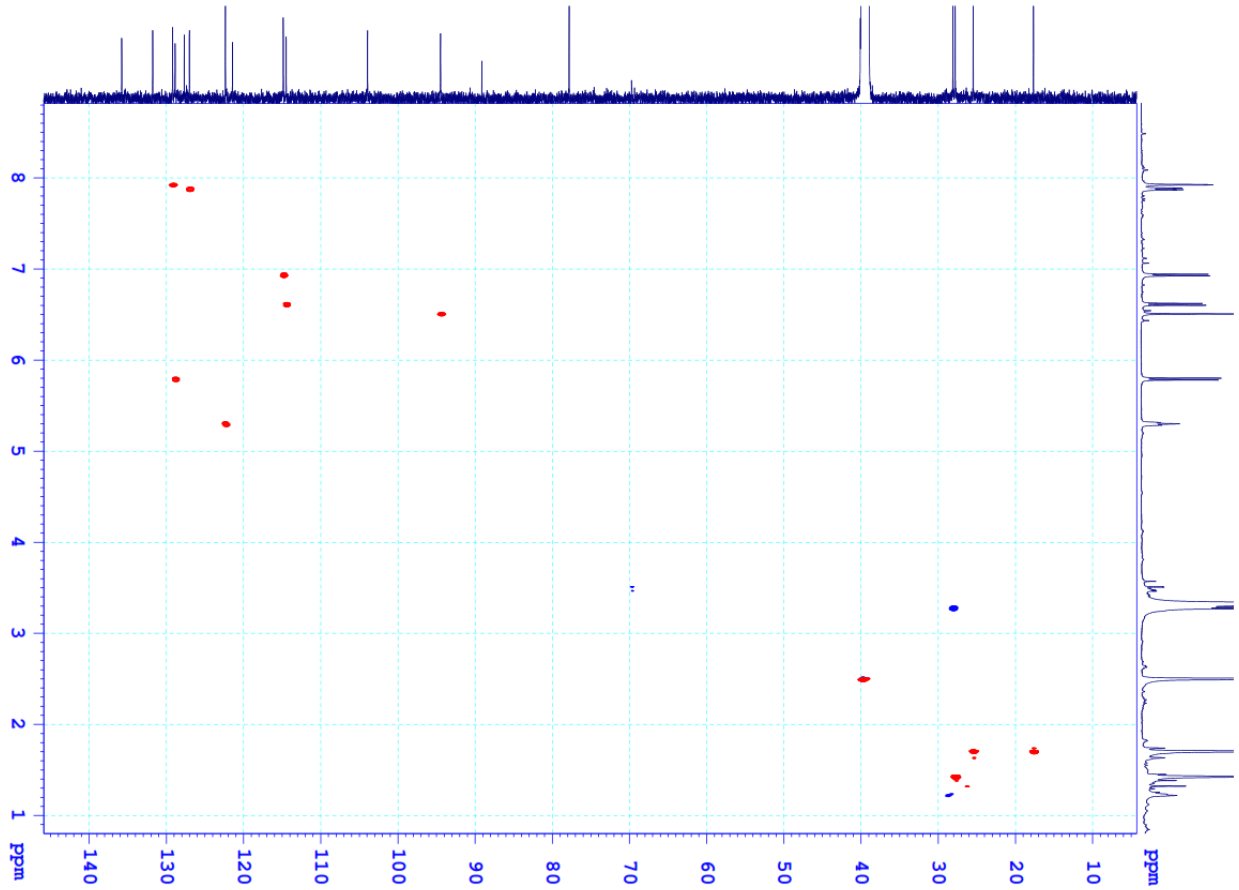
12. ^{1}H-NMR spectrum of compound **3**



13. ^{13}C-NMR spectrum of compound **3**



14. HSQC spectrum of compound **3**



15. HMBC spectrum of compound **3**