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## Flavone C-glycosides from the leaves of *Amesiodendron chinense*

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### Abstract

Three new flavone 6-C-glycosides, amesiflavones A-C (1-3) together with four known compounds, (+)-aptosimon (4), (+)-isolariciresinol (5), cleomiscosin A (6), and cleomiscosin C (7) were isolated from the leaves of Amesiodendron chinense (Merr.) Hu. Their structures were elucidated by spectroscopic methods, including 1D-, 2D-NMR and MS spectra and comparing with those reported in the literature. All compounds were evaluated for cytotoxic activities against five human cancer cell lines, KB, SK-LU-1, MCF-7, HepG-2, and SW-480. As the results, compounds 1, 2, 4, 6, and 7 showed weak activity on all tested human cancer cell lines with IC<sub>50</sub> values ranging from 71.0 to 146.0  $\mu$ M.

<b>Keywords</b>	Amesiodendron chinense; Sapindaceae; amesiflavone A; amesiflavone B; amesiflavone C; cytotoxic
<b>Taxonomy</b>	Flavone, Natural Product Biochemistry
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## Highlights

### **Flavone C-glycosides from the leaves of *Amesiodendron chinense***

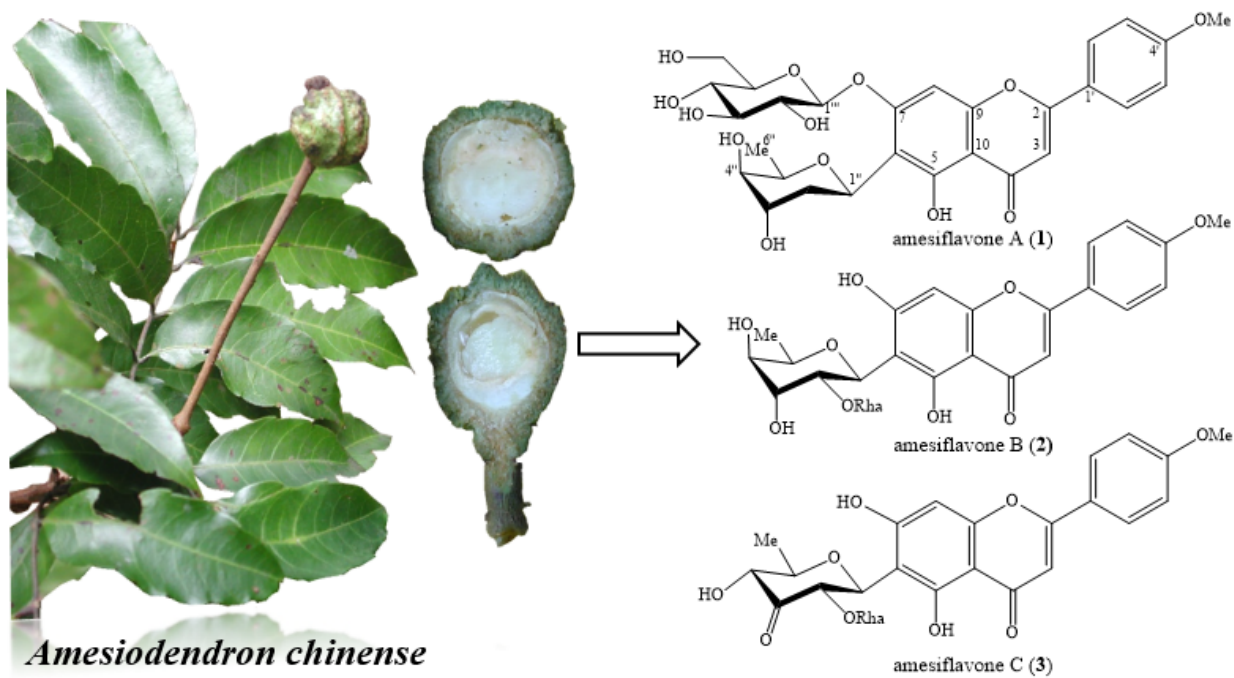
Ho Van Ban, Trinh Thi Thanh Van, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Nguyen Le Tuan, Marc Litaudon, Chau Van Minh, Pham Van Cuong, Nguyen Quoc Vuong, Nguyen Xuan Nhiem

- Three new flavones, amesiflavones A-C were isolated from *Amesiodendron chinense*
- The structures were successfully determined by spectroscopic evidence.
- Compounds showed cytotoxic effects.

## Graphical abstract

### Flavone C-glycosides from the leaves of *Amesiodendron chinense*

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## **Flavone C-glycosides from the leaves of *Amesiodendron chinense***

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## ABSTRACT

Three new flavone 6-*C*-glycosides, amesiflavones A-C (**1-3**) together with four known compounds, (+)-aptosimon (**4**), (+)-isolariciresinol (**5**), cleomiscosin A (**6**), and cleomiscosin C (**7**) were isolated from the leaves of *Amesiodendron chinense* (Merr.) Hu. Their structures were elucidated by spectroscopic methods, including 1D-, 2D-NMR and MS spectra and comparing with those reported in the literature. All compounds were evaluated for cytotoxic activities against five human cancer cell lines, KB, SK-LU-1, MCF-7, HepG-2, and SW-480. As the results, compounds **1**, **2**, **4**, **6**, and **7** showed weak activity on all tested human cancer cell lines with IC<sub>50</sub> values ranging from 71.0 to 146.0 μM.

**Keywords:** *Amesiodendron chinense*; Sapindaceae; amesiflavone A; amesiflavone B; amesiflavone C; cytotoxic

## 1. Introduction

The genus *Amesiodendron* Hu (Sapindaceae Juss.) includes only three species (*A. integrifoliolatum*, *A. tienlinensis*, and *A. chinense*), is distributed in China and South-east Asia. *Amesiodendron chinense* (Merr.) Hu was listed in 2006 IUCN Red List of threatened species. The chemical and biological investigation of this plant has not been studied yet. As part of our research on anticancer reagent candidates from over than 2,500 Vietnamese plants, the ethyl acetate extract of *A. chinense* was found to inhibit the growth of KB human cancer cell line with IC<sub>50</sub> value of of 20 µg/mL. Herein, we report the isolation and structure elucidation of three new flavone C-glycosides together with four known compounds from *A. chinense* and their cytotoxic effects.

## 2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. The HR-ESI-MS analysis revealed the molecular formula to be C<sub>28</sub>H<sub>32</sub>O<sub>13</sub> with a cluster ion peak at *m/z* 577.1910 [M + H]<sup>+</sup> (Calcd. for [C<sub>28</sub>H<sub>33</sub>O<sub>13</sub>]<sup>+</sup>, 577.1916). The <sup>1</sup>H-NMR spectrum of **1** revealed signals of four aromatic protons of B ring at δ<sub>H</sub> 7.10 (2H, d, *J* = 9.0 Hz) and 8.00 (2H, d, *J* = 9.0), two singlet proton of A and C rings at δ<sub>H</sub> 6.74 and 7.06 (each 1H, s) assigned to a flavone aglycone, two anomeric protons at δ<sub>H</sub> 4.96 (d, *J* = 7.5 Hz) and 5.54 (dd, *J* = 3.0, 12.5 Hz) assigned to two monosaccharide moieties. The <sup>13</sup>C-NMR and HSQC spectra of **1** showed signals of one carbonyl carbons at δ<sub>C</sub> 184.2, seven non-protonated carbons at δ<sub>C</sub> 107.0, 114.3, 124.3, 158.5, 160.1, 164.6, and 166.3, fifteen methines at δ<sub>C</sub> 67.1, 69.5, 71.1, 71.6, 72.3, 75.0, 77.1, 78.7, 96.3, 103.7, 104.8, 115.7×2, and 129.5×2, one methylene at δ<sub>C</sub> 62.7, and two methyl carbons at δ<sub>C</sub> 17.5 and 56.1. Analysis of <sup>1</sup>H- and <sup>13</sup>C-NMR spectra indicated the structure of **1** was similar to those of apigenin 6-*C*-β-boivinopyranosyl-7-*O*-β-glucopyranoside (Zhu et al. 2010) except for the addition of a methoxy group at C-4'. The position of methoxy group at C-4' was confirmed by HMBC correlations from H-2'/H-6' (δ<sub>H</sub> 8.00), H-3'/H-5' (δ<sub>H</sub> 7.10), and methoxy

group ( $\delta_{\text{H}}$  3.90) to C-4' ( $\delta_{\text{C}}$  164.6). The HMBC correlations between H-3 ( $\delta_{\text{H}}$  6.74) and C-1' ( $\delta_{\text{C}}$  124.3)/C-2 ( $\delta_{\text{C}}$  166.3)/C-4 ( $\delta_{\text{C}}$  184.2)/C-10 ( $\delta_{\text{C}}$  107.0) and between H-8 ( $\delta_{\text{H}}$  7.06) and C-6 ( $\delta_{\text{C}}$  114.3)/C-7 ( $\delta_{\text{C}}$  164.8)/C-9 ( $\delta_{\text{C}}$  158.5)/C-10 ( $\delta_{\text{C}}$  107.0) confirmed the two protons at C-3 and C-8. Acid hydrolysis of **1** revealed D-glucose (identified as trimethylsilyl (TMS) derivative by a gas chromatography method). In addition, the  $^{13}\text{C}$ -NMR spectral data of **1** showed the presence of one *O*- $\beta$ -D-glucose and one *C*- $\beta$ -boivinose unit (Zhu et al. 2010). The HMBC correlations from Boi H-1'' ( $\delta_{\text{H}}$  5.54) to C-5 ( $\delta_{\text{C}}$  160.1)/C-6 ( $\delta_{\text{C}}$  114.3)/C-7 ( $\delta_{\text{C}}$  164.8) of the aglycone and from Glc H-1''' ( $\delta_{\text{H}}$  4.96) to C-7 ( $\delta_{\text{C}}$  164.8) (Figure 2) indicated the position of *C*- $\beta$ -D-boivinopyranosyl and *O*- $\beta$ -D-glucopyranosyl moieties at C-6 and C-7, respectively. Consequently, the structure of **1** was determined to be acacetin-6-*C*- $\beta$ -D-boivinopyranosyl-7-*O*- $\beta$ -D-glucopyranoside and named as amesiflavone A.

Compound **2** was also obtained as a yellow amorphous powder and its molecular formula was determined to be  $\text{C}_{28}\text{H}_{32}\text{O}_{13}$  by HR-ESI-MS at  $m/z$  577.1915 [ $\text{M} + \text{H}$ ] $^{+}$  (Calcd. for [ $\text{C}_{28}\text{H}_{33}\text{O}_{13}$ ] $^{+}$ , 577.1916). The  $^1\text{H}$ -NMR spectrum of **2** revealed *C*-6-substituted of apigenin protons:  $\delta_{\text{H}}$  7.09 (2H, d,  $J = 7.5$  Hz) and 7.93 (2H, d,  $J = 7.5$  Hz), 6.54 (1H, s), and 6.63 (1H, s). The analysis NMR spectra of **2** indicated the flavone aglycone was identical to those of **1**. The NMR features for the first sugar moiety of **2** allowed to identify to be 6-deoxyhexopyranosyl moiety. Furthermore, this was confirmed by the COSY correlations of Gul H-1'' ( $\delta_{\text{H}}$  5.41)/gul H-2'' ( $\delta_{\text{H}}$  4.45)/gul H-3'' ( $\delta_{\text{H}}$  4.26)/gul H-4'' ( $\delta_{\text{H}}$  3.69)/gul H-5'' ( $\delta_{\text{H}}$  4.22)/gul H-6'' ( $\delta_{\text{H}}$  1.29). The 6-deoxyhexopyranosyl at C-6 was proved by HMBC correlations between Gul H-1'' ( $\delta_{\text{H}}$  5.41) and C-5 ( $\delta_{\text{C}}$  161.1)/C-6 ( $\delta_{\text{C}}$  110.1)/C-7 ( $\delta_{\text{C}}$  164.9). The *axial* orientation of Gul H-1'', Gul H-2'', and Gul H-5'' and *equatorial* orientation of Gul H-3'' and Gul H-4'' were confirmed by the large coupling constant between Gul H-1'' and Gul H-2'' ( $J = 9.8$  Hz) and small coupling constants between Gul H-2'' and Gul H-3'' ( $J = 3.0$  Hz) and between Gul H-3'' and Gul H-4'' ( $J = 3.5$  Hz) as well as NOESY correlations between Gul H-1'' and Gul H-5''. Based



on above evidence, the C-glycoside was proved as C- $\beta$ -6-deoxygulopyranoside (Carnat et al. 1998). Acid hydrolysis of **2** gave L-rhamnose (identified as TMS derivative by a gas chromatography method). The position of  $\alpha$ -L-rhamnopyranosyl at Gul C-2'' was confirmed by HMBC correlations from Rha H-1''' ( $\delta_{\text{H}}$  4.82) to Gul C-2'' ( $\delta_{\text{C}}$  70.8). Consequently, the new structure of **2** was elucidated as acacetin-6-C-(2- $\alpha$ -L-rhamnopyranosyl)- $\beta$ -6-deoxygulopyranoside and named as amesiflavone B.

Amesiflavone C (**3**) possessed a molecular formula of C<sub>28</sub>H<sub>30</sub>O<sub>13</sub> as deduced from HR-ESI-MS at  $m/z$  575.1761 [M + H]<sup>+</sup> (Calcd. for [C<sub>28</sub>H<sub>31</sub>O<sub>13</sub>]<sup>+</sup>, 575.1759). The <sup>1</sup>H-NMR spectrum of compound **3** showed the presence of 6-C-substituted acacetin aglycone with the typical proton signals at  $\delta_{\text{H}}$  8.02 (2H, d,  $J = 9.0$  Hz), 7.12 (2H, d,  $J = 9.0$  Hz), 6.56 (1H, s), 6.80 (1H, s), and 3.87 (3H, s). Two anomeric protons at  $\delta_{\text{H}}$  4.88 (d,  $J = 10.0$  Hz) and 4.69 (br s) together with two secondary methyl group at 0.74 (3H, look like s) and 1.33 (d,  $J = 6.0$  Hz), suggested the presence of two monosaccharide moieties. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data of **3** were almost the same to those of cassiaoccidentalins A (Hatano et al. 1999) except for the addition of methoxy group at C-4'. The position of a methoxy group at C-4' was also confirmed by HMBC correlation from methoxy group ( $\delta_{\text{H}}$  3.87) to C-4' ( $\delta_{\text{C}}$  162.2). The signals in <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of unexpectedly separated (some cases showed the broaden signals) at normal temperature (25°C) but duplication collapsed at high temperature (60°C) (See Supporting information), which indicated the existence of rotational isomers, caused by the restricted rotation around the C (sp<sup>3</sup>)–C (sp<sup>2</sup>) bond between C-glucosyl and flavone (Zhou et al. 2019). The HMBC correlations between Rib H-1'' ( $\delta_{\text{H}}$  4.88) and Rib C-2'' ( $\delta_{\text{C}}$  75.4)/rib C-3'' ( $\delta_{\text{C}}$  205.7)/rib C-5'' ( $\delta_{\text{C}}$  77.8) and between Rib H-6'' ( $\delta_{\text{H}}$  1.33) and Rib C-4'' ( $\delta_{\text{C}}$  77.2)/Rib C-5'' ( $\delta_{\text{C}}$  77.8) and also COSY correlations of Rib H-1''/H-2''; H-4''/H-5''/H-6'' confirmed the presence of 6-deoxy-ribo-hexos-3-ulopyranosyl moiety. The HMBC correlations between Rha H-1''' ( $\delta_{\text{H}}$  4.69) and Rib C-2'' ( $\delta_{\text{C}}$  75.4); Rib H-1'' ( $\delta_{\text{H}}$  4.88) and C-5 ( $\delta_{\text{C}}$  161.5)/C-6 ( $\delta_{\text{C}}$

107.4)/C-7 ( $\delta_C$  161.8) determined the sugar linkages as 6-C-(2 $\square$ - $\alpha$ -L-rhamnopyranosyl)- $\beta$ -6-deoxy-ribo-hexos-3-ulopyranosyl. Thus, the structure of **3** was elucidated and named as amesiflavone C.

The known compounds, (+)-aptosimon (**4**) (Yamauchi and Yamaguchi 2003), (+)-isolariciresinol (**5**) (Jutiviboonsuk et al. 2005), cleomiscosin A (**6**) (Yang et al. 2014), and cleomiscosin C (**7**) (Tanaka et al. 1988) were identified by comparison of their NMR data with those reported in the literature.

All compounds were screened for cytotoxic activity against five human cancer cell lines, KB (human epidemoid carcinoma), SK-LU-1 (human lung cancer), MCF-7 (human breast cancer), HepG2 (human hepatocyte carcinoma), and SW-480 (Human colon adenocarcinoma) at the concentration of 200  $\mu$ M. Compounds **3-5** did not show cytotoxic activity (% cell survival >50%). Thus, compounds **1, 2, 4, 6, and 7** were furthered evaluated at the smaller concentrations, 100, 10, 1.0  $\mu$ M. As the results, compounds **1, 2, 4, 6, and 7** showed weak activity on tested human cancer cell lines with  $IC_{50}$  ranging from 71.0 to 146.0  $\mu$ M.

### **3. Experimental**

#### *3.1. General*

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  $\mu$ m, 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 *Amesiodendron chinense* (Sapindaceae) were collected at Sontra, Danang, Vietnam in June 2018 and identified by Dr. Do Van Hai, Institute of Ecology and Biological

Resources, VAST. A voucher specimen (PTH15032018) was deposited at the Institute of Marine Biochemistry, VAST.

### 3.3. Extraction and isolation

Dried and powdered leaves (7.0 kg) of *A. chinense* were extracted with MeOH (25 L) at room temperature for three times (each time, three days) and removed the solvent in *vacuo* to yield MeOH extract (250 g). The MeOH extract was suspended with H<sub>2</sub>O (2 L) and successively partitioned with *n*-hexane, and ethyl acetate (EtOAc) to yield *n*-hexane (ACH, 70 g), EtOAc (ACE, 50 g) residues, and water layer (ACW).

ACE layer was applied on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>/EtOAc (10/1, v/v) to give ACE1 (6 g), ACE2 (8 g), ACE3 (13 g), ACE4 (11 g), and ACE5 (7 g). The ACE1 fraction was chromatographed on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>/EtOAc (4/1, v/v) to yield compound **4** (30 mg). The ACE2 fraction was applied on a silica gel column eluting with *n*-hexane/EtOAc (1/4, v/v) to give two smaller fractions, ACE2.1 (2.5 g) and ACE2.2 (1.5 g). The ACE2.1 fraction was chromatographed on a silica gel column with solvent eluent of *n*-hexane/EtOAc (1/4, v/v) then purified on a sephadex LH-20 column to give **5** (7.0 mg). The ACE2.2 fraction was subjected on an RP-18 column with solvent eluents of MeOH/H<sub>2</sub>O (1/1, v/v) then purified by a sephadex LH-20 column eluting with MeOH to yield compound **1** (12.0 mg). ACE3 fraction was applied on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>/EtOAc (4/1, v/v) then purified on an RP-18 column eluting with MeOH/H<sub>2</sub>O (1/1, v/v) to yield **2** (8.0 mg). The ACE4 fraction was chromatographed on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>/EtOAc (1/1, v/v) then on an RP-18 column eluting with MeOH/H<sub>2</sub>O (1/1, v/v) to yield compound **3** (50 mg). The ACE5 fraction was applied on a silica gel column eluting with EtOAc/acetone (4/1, v/v) to give two smaller fractions, ACE5.1 (1.7 g) and ACE5.2 (2 g). The ACE5.1 fraction was chromatographed on an RP-18 column eluting with MeOH/H<sub>2</sub>O (1/1, v/v) and then purified on a sephadex LH-20 column eluting with MeOH to give

compound **6** (9.0 mg). The ACE5.2 fraction was applied on a silica column eluting with CH<sub>2</sub>Cl<sub>2</sub>/acetone (6/1 v/v) and then purified on a sephadex LH-20 column to give compound **7** (10 mg).

### 3.3.1. *Amesiflavone A (1)*

Yellow amorphous powder;  $[\alpha]_D^{25} = -76.0$  (*c* 0.1, MeOH); UV  $\lambda_{\max}$  (MeOH, nm): 212, 273, 326; HR-ESI-MS *m/z*: 577.1910 [M + H]<sup>+</sup> (Calcd. for [C<sub>28</sub>H<sub>33</sub>O<sub>13</sub>]<sup>+</sup>, 577.1916); IR max (film) cm<sup>-1</sup>: 3318, 2904, 1653, 1607, 1510, 1255, 1183, 1071; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz) and <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): see Table 1.

### 3.3.2. *Amesiflavone B (2)*

Yellow amorphous powder;  $[\alpha]_D^{25} = -43.0$  (*c* 0.1, MeOH); UV  $\lambda_{\max}$  (MeOH, nm): 215, 273, 323; HR-ESI-MS *m/z*: 577.1915 [M + H]<sup>+</sup> (Calcd. for [C<sub>28</sub>H<sub>33</sub>O<sub>13</sub>]<sup>+</sup>, 577.1916); IR max (film) cm<sup>-1</sup>: 3703, 2904, 1656, 1625, 1248, 1183; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz) and <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): see Table 1.

### 3.3.3. *Amesiflavone C (3)*

Yellow amorphous powder;  $[\alpha]_D^{25} = -25.0$  (*c* 0.1, MeOH); UV  $\lambda_{\max}$  (MeOH, nm): 216, 272, 330; HR-ESI-MS *m/z*: 575.1761 [M + H]<sup>+</sup> (Calcd. for [C<sub>28</sub>H<sub>31</sub>O<sub>13</sub>]<sup>+</sup>, 575.1759); IR max (film) cm<sup>-1</sup>: 3413, 3306, 2940, 1731, 1650, 1623, 1605, 1261, 1136, 1102, 1056, 1027; <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>, 500 MHz) and <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 125 MHz): see Table 1.

### 3.4. *Acid hydrolysis*

Each compound (**1** and **3**, 2.0 mg) was separately dissolved in 1.0 N HCl (dioxane–H<sub>2</sub>O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. The solution was thoroughly driven out under N<sub>2</sub> overnight then partitioned in water : CHCl<sub>3</sub> (1:1), the aqueous layer was concentrated to dryness using N<sub>2</sub>. The residue was dissolved in 0.1 mL of dry pyridine, followed by addition of L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL).

The reaction mixture was heated at 60 °C for 2 h and then added trimethylsilylimidazole solution (0.1 mL) at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and water (0.1 mL each), and the organic layer was analyzed by GC: DB-5 column (0.32 mm ID × 30 m length), detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, and carrier gas He (2 mL/min). Under these conditions, the standard sugars gave peaks at  $t_R$  (min) 14.11 and 14.26 for D- and L-glucose, and 4.50 for L-rhamnose, respectively. Peaks at  $t_R$  (min) 14.11 of D-glucose for **1** and 4.50 of L-rhamnose for **3** were observed.

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

#### **Disclosure statement**

No potential conflict of interest was reported by the authors.

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## REFERENCES

- Carnat A.P., Carnat A., Fraisse D., Lamaison J.L., Heitz A., Wylde R., Teulade J.C., 1998. Violarvensin, a new flavone di-*C*-glycoside from *Viola arvensis*. *J. Nat. Prod.* 61, 272-274.
- Hatano T., Mizuta S., Ito H., Yoshida T., 1999. *C*-glycosidic flavonoids from *Cassia occidentalis*. *Phytochemistry* 52, 1379-1383.
- Jutiviboonsuk A., Zhang H., Tan G.T., Ma C., Hung N.V., Cuong N.M., Bunyapraphatsara N., Soejarto D.D., Fong H.H.S., 2005. Bioactive constituents from roots of *Bursera tonkinensis*. *Phytochemistry* 66, 2745-2751.
- Tanaka H., Ishihara M., Ichino K., Ito K., 1988. Total synthesis of coumarinolignans, aquillochin (cleomiscosin C) and cleomiscosin D. *Chem. Pharm. Bull.* 36, 3833-3837.
- Thu V.K., Thang N.V., Nhiem N.X., Tai B.H., Nam N.H., Kiem P.V., Minh C.V., Anh H.L.T., Kim N., Park S., Kim S.H., 2015. Oleanane-type saponins from *Glochidion glomerulatum* and their cytotoxic activities. *Phytochemistry* 116, 213-220.
- Yamauchi S. Yamaguchi M., 2003. Synthesis of (+)-aptosimon, a 4-oxofurofuran lignan, by *erythro* selective aldol condensation and stereoconvergent cyclization as the key reactions. *Biosci. Biotech. Bioch.* 67, 838-846.
- Yang J., Liu W., Li S., Ye H., Tang H., Chen L., Peng A., 2014. Coumarinolignans isolated from the seeds of *Brucea javanica*. *Helv. Chim. Acta* 97, 278-282.
- Zhou G., Yan R., Wang X., Li S., Lin J., Liu J., Zhao Z., 2019. The overlooked rotational isomerism of *C*-glycosyl flavonoids. *Phytochem. Rev.* 18, 443-461.
- Zhu D., Yang J., Lai M.-X., Wang Q., 2010. A new *C*-glycosylflavone from *Pogonatherum crinitum*. *Chin. J. Nat. Med.* 8, 411-413.

**Table 1.** <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopic data for compounds **1 - 3**.

C	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta_C^a$	$\delta_H^a$ (mult., <i>J</i> in Hz)	$\delta_C^a$	$\delta_H^a$ (mult., <i>J</i> in Hz)	$\delta_C^b$	$\delta_H^b$ (mult., <i>J</i> in Hz)
2	166.3	-	165.9	-	163.0	-
3	104.8	6.74 (s)	104.7	6.63 (s)	103.3	6.80 (s)
4	184.2	-	184.3	-	181.6	-
5	160.1	-	161.1	-	161.5	-
6	114.3	-	110.1	-	107.4	-
7	164.8	-	164.9	-	161.8	-
8	96.3	7.06 (s)	96.0	6.54 (s)	93.3	6.56 (br s)
9	158.5	-	158.9	-	156.5	-
10	107.0	-	105.5	-	103.3	-
1'	124.3	-	124.7	-	122.6	-
2', 6'	129.5	8.00 (d, 9.0)	129.3	7.93 (d, 7.5)	128.0	8.02 (d, 9.0)
3', 5'	115.7	7.10 (d, 9.0)	115.7	7.09 (d, 7.5)	114.4	7.12 (d, 9.0)
4'	164.6	-	164.4	-	162.2	-
4'-OMe	56.1	3.90 (s)	56.1	3.91 (s)	55.3	3.87 (s)
	<b>Boi</b>		<b>Gul</b>		<b>Rib</b>	
1''	67.1	5.54 (dd, 3.0, 12.5)	70.3	5.41 (d, 9.8)	73.0	4.88 d, 10.0)
2''	31.3	3.08 (ddd, 3.0, 12.5, 14.0), ax 1.47 (ddd, 3.0, 3.0, 14.0), eq	70.8	4.45 (br d, 9.8)	75.4	5.29 (like-br s)
3''	69.5	4.06 (brd, 3.0)	67.7	4.26 (m)	205.7	-
4''	71.1	3.39 (brd, 3.5)	73.0	3.69 (br d, 3.5)	77.2	3.92 (m)
5''	72.3	4.12 (q, 6.5)	72.3	4.22 (m)	77.8	3.42 (m)
6''	17.5	1.24 (d, 6.5)	17.8	1.29 (d, 6.5)	18.7	1.33 (d, 6.0)
	<b>Glc</b>		<b>Rha</b>		<b>Rha</b>	
1'''	103.7	4.96 (d, 7.5)	97.5	4.82 (br s)	99.0	4.69 (s)
2'''	75.0	3.64 (dd, 7.5, 9.5)	72.4	3.85 (br s)	69.9	3.74 (br s)
3'''	77.1	3.55 (dd, 9.0, 9.5)	72.2	3.40 (dd, 3.0, 9.5)	70.2	3.09 (m)
4'''	71.6	3.43 (dd, 9.0, 9.5)	73.3	3.14 (dd, 9.0, 9.5)	71.1	3.01 (dd, 3.0, 9.0)
5'''	78.7	3.62 (m)	69.8	2.41 (m)	68.5	2.45 (m)
6'''	62.7	4.04 (dd, 6.5, 12.0) 3.78 (dd, 2.0, 12.0)	16.6	0.87 (d, 6.5)	17.1	0.74 (br s)

<sup>a</sup>)recorded in CD<sub>3</sub>OD; <sup>b</sup>)recorded in DMSO-d<sub>6</sub> at 60°C; Boi, boivinopyranosyl; Glc, glucopyranosyl; Gul, 6-deoxygulopyranosyl; Rha, rhamnopyranosyl; Rib, 6-Deoxy-ribohexos-3-ulose

**Table 2.** Cytotoxic effects of compounds 1-7

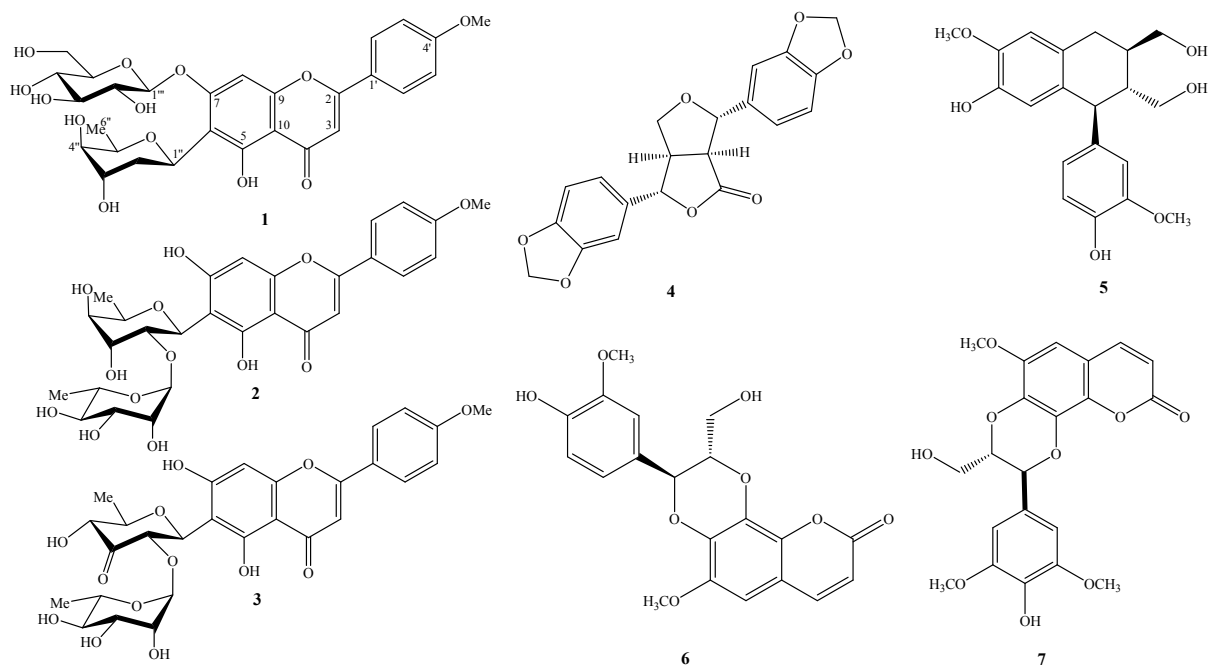
Compound	IC <sub>50</sub> (μM) <sup>a)</sup>				
	KB	SK-LU-1	MCF7	HepG2	SW480
1	89.3±6.6	118.2±9.4	95.6±9.3	71.0±6.2	102.8±8.9
2	94.6 ± 5.0	132.2± 9.1	138.1±8.6	104.5±7.4	109.4±2.0
3	>200	>200	>200	>200	>200
4	88.5 ±8.4	143.5±10.7	140.1±6.3	120.1±6.9	99.0±6.2
5	>200	>200	>200	>200	>200
6	118.0±10.0	141.3±14.8	146.0±8.8	96.8±12.7	113.0±11.9
7	92.6±6.9	139.6±13.5	153.9±13.7	127.9±8.9	124.7±13.8
Ellipticine <sup>b)</sup>	1.6±0.2	1.8±0.1	2.0±0.2	1.8±0.2	1.5±0.2

<sup>a)</sup>The concentration that inhibits 50% of cell growth was calculated (IC<sub>50</sub>). Compounds were tested at a maximum concentration of 200 μM. Data are means of three experiments.

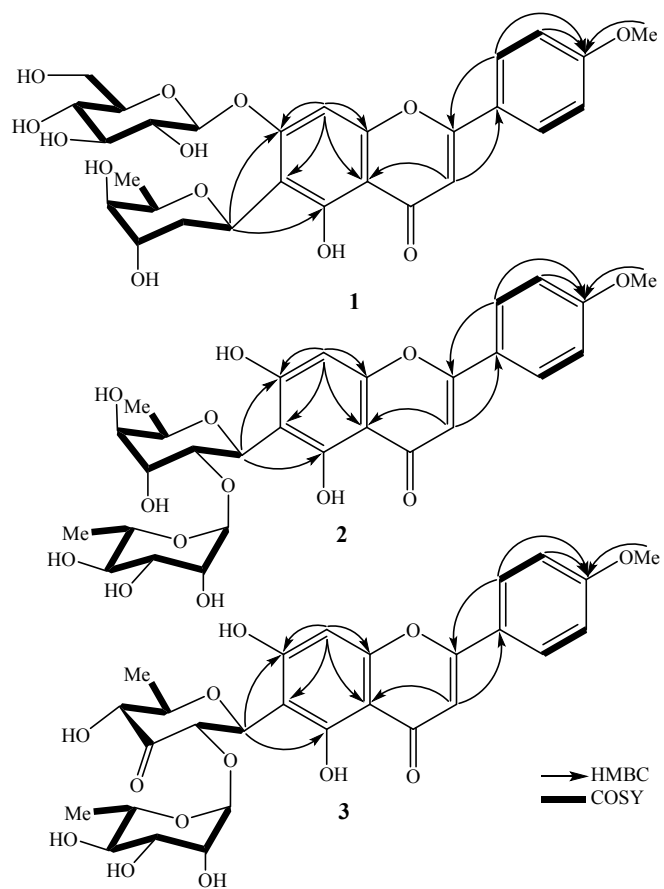
<sup>b)</sup>Ellipticine was used as a positive control.



**Figure 1.** Chemical structures of compounds 1–7.



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



## **Flavone C-glycosides from the leaves of *Amesiodendron chinense***

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The authors declared no conflict of interest

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## SUPPLEMENTARY MATERIAL

### Flavone C-glycosides from the leaves of *Amesiodendron chinense*

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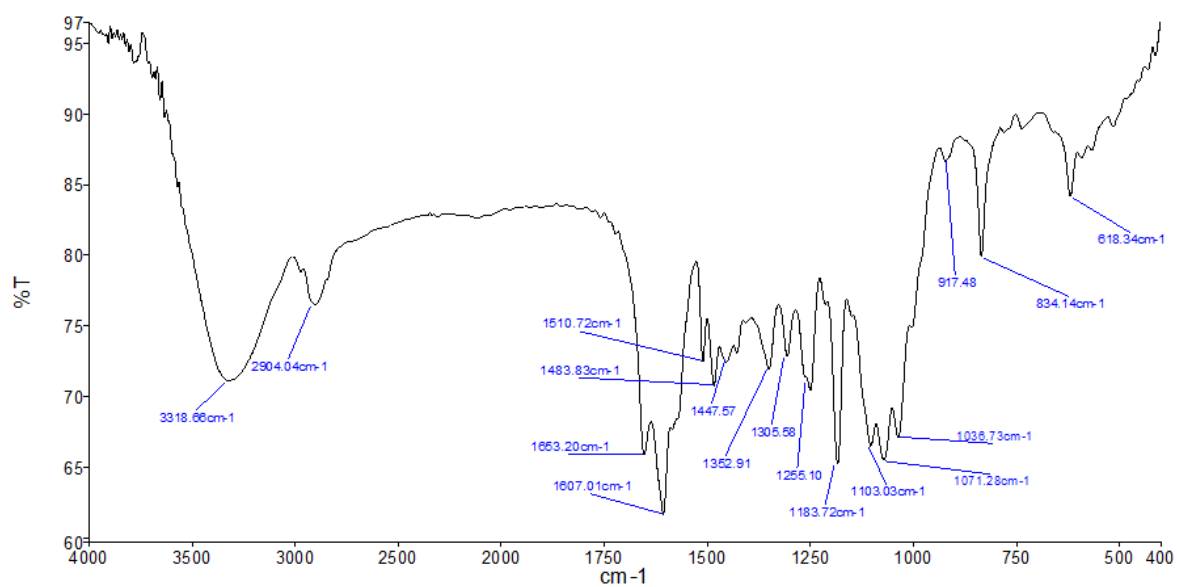
**ABSTRACT:** Three new flavone 6-C-glycosides, amesiflavones A-C (**1-3**) together with four known compounds, (+)-aptosimon (**4**), (+)-isolariciresinol (**5**), cleomiscosin A (**6**), and cleomiscosin C (**7**) were isolated from the leaves of *Amesiodendron chinense* (Merr.) Hu. Their structures were elucidated by spectroscopic methods, including 1D-, 2D-NMR and MS spectra and comparing with those reported in the literature. All compounds were evaluated for cytotoxic activities against five human cancer cell lines, KB, SK-LU-1, MCF-7, HepG-2, and SW-480. As the results, compounds **1**, **2**, **4**, **6**, and **7** showed weak activity on all tested human cancer cell lines with IC<sub>50</sub> values ranging from 71.0 to 146.0  $\mu$ M.

**Keywords:** *Amesiodendron chinense*; Sapindaceae; amesiflavone A; amesiflavone B; amesiflavone C; cytotoxic

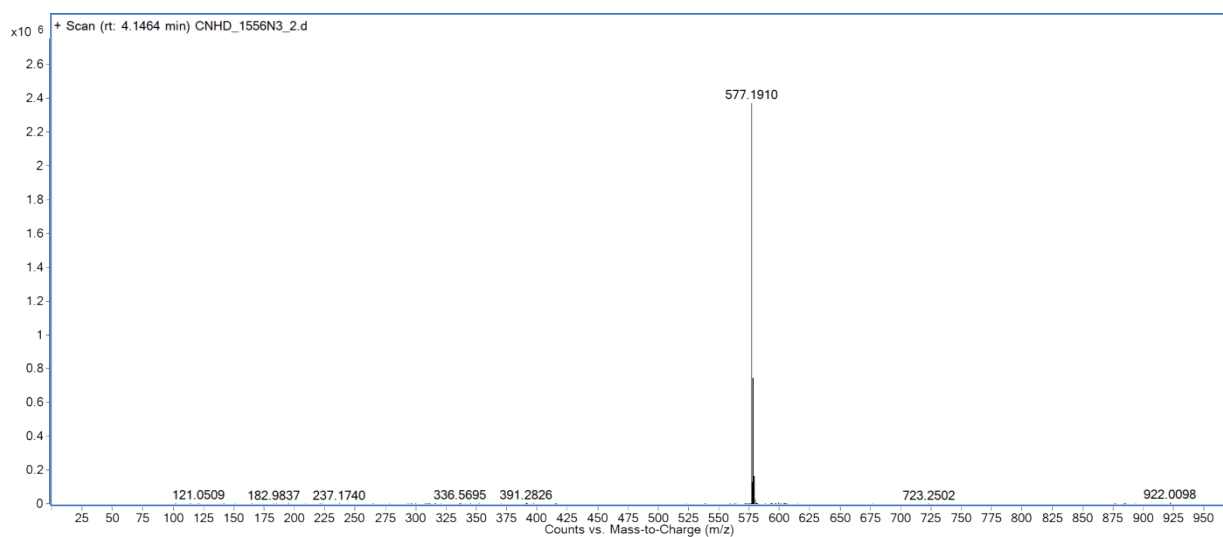
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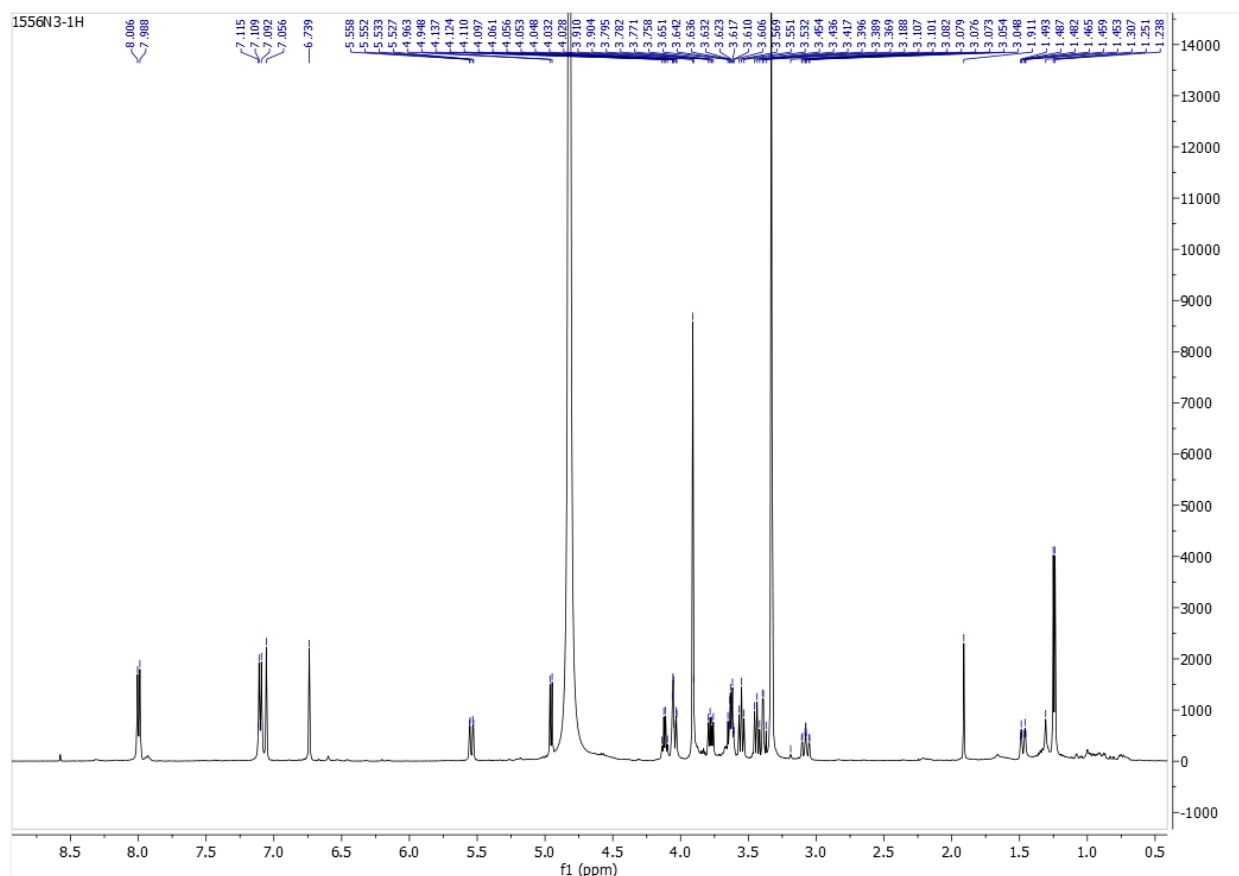
**Figure S1.** IR spectrum of compound **1**



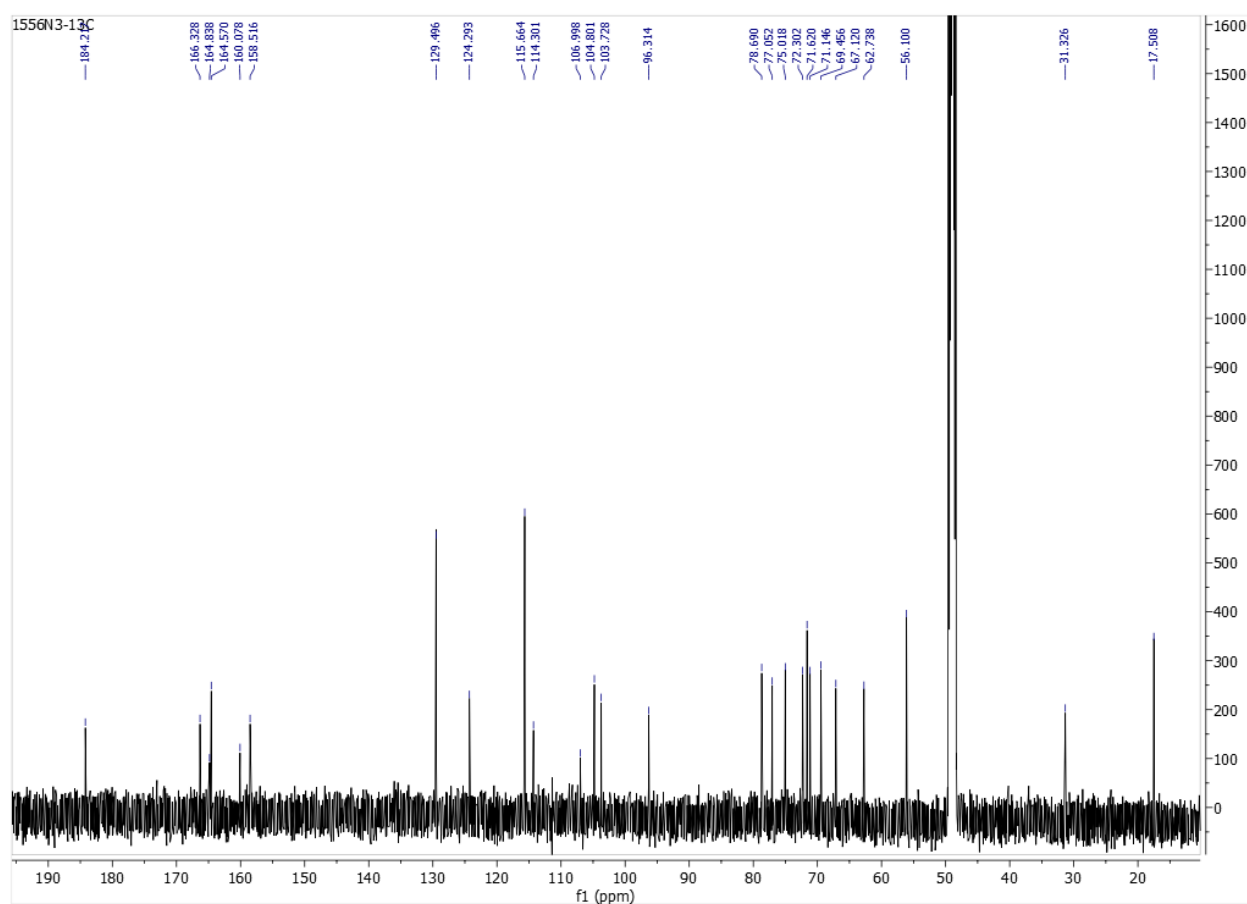
**Figure S2.** HR-ESI-MS of compound **1**



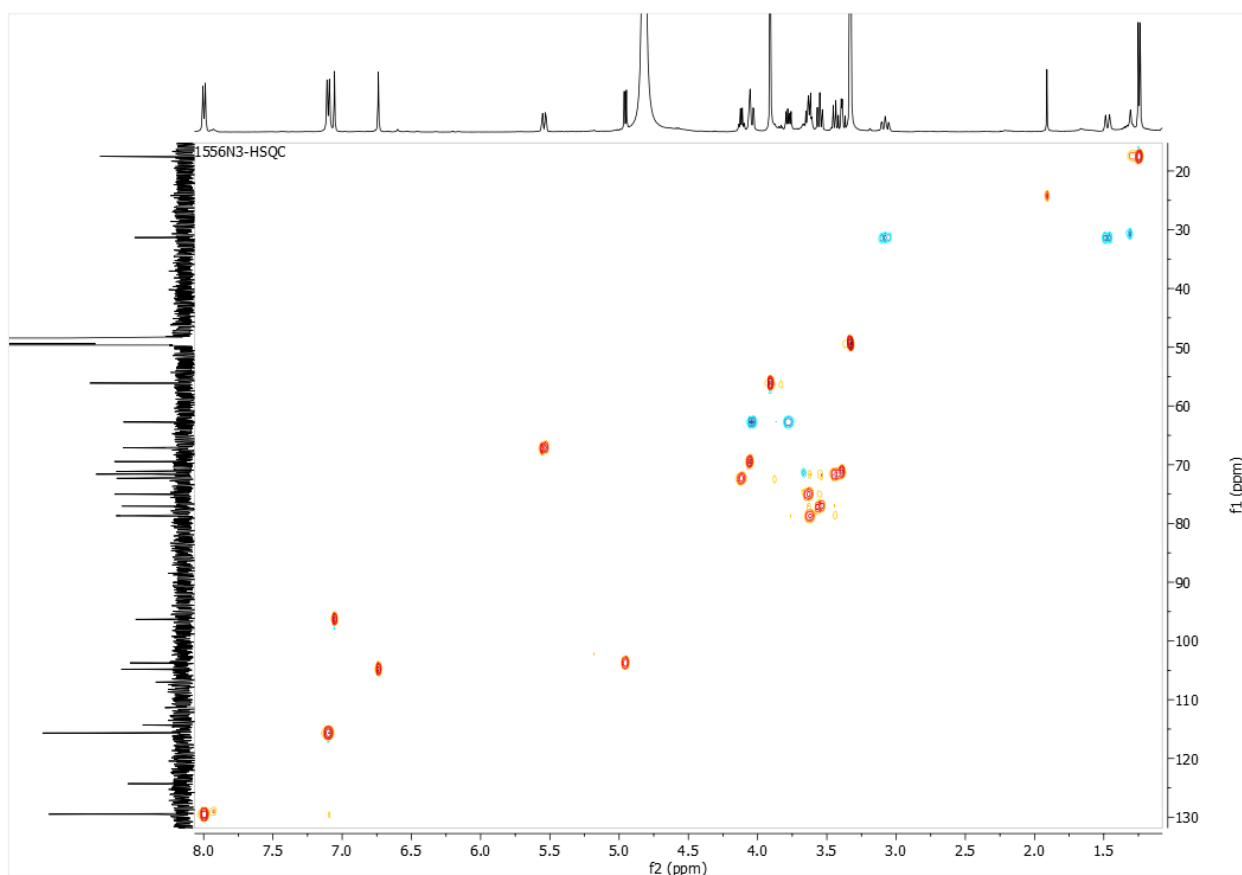
**Figure S3.**  $^1\text{H-NMR}$  spectrum of compound **1**



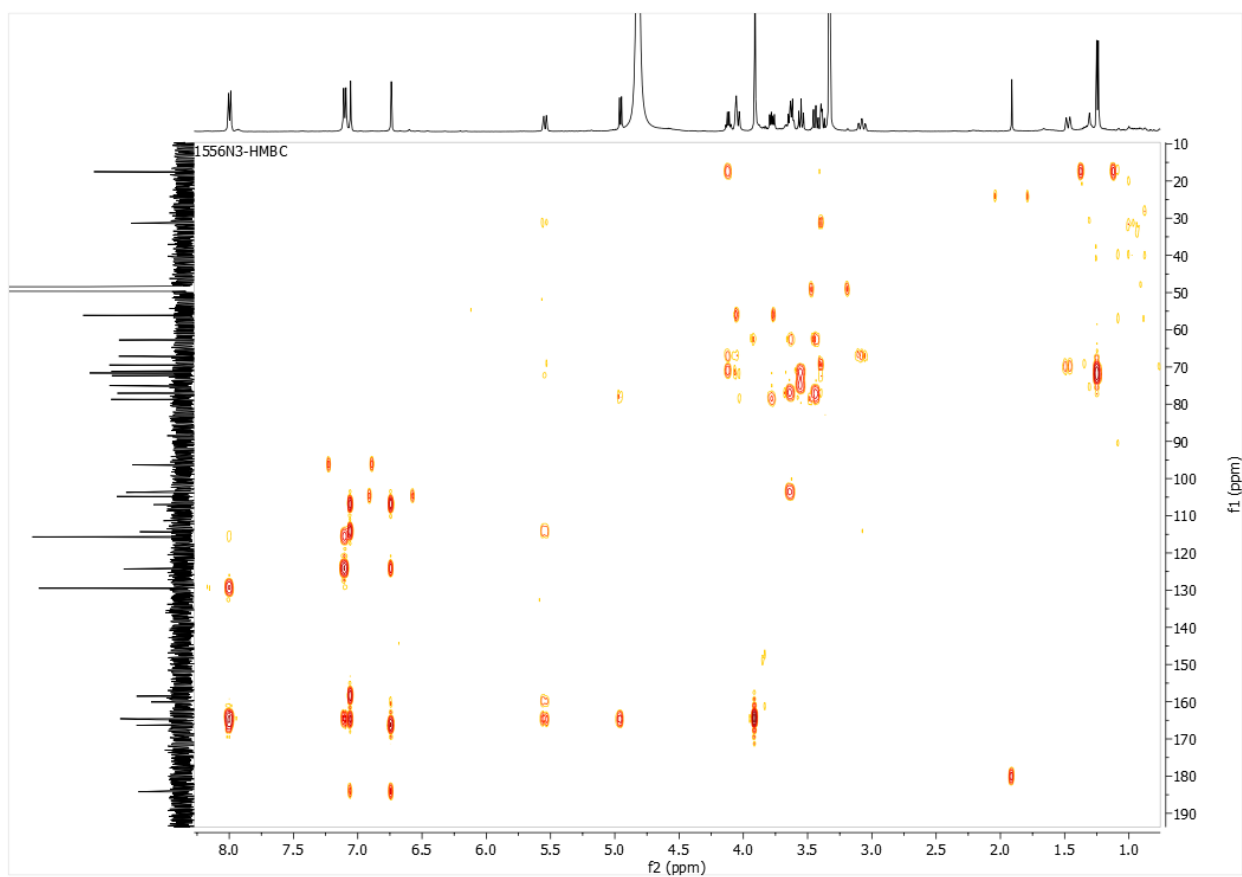
**Figure S4.**  $^{13}\text{C-NMR}$  spectrum of compound **1**



**Figure S5.** HSQC spectrum of compound **1**

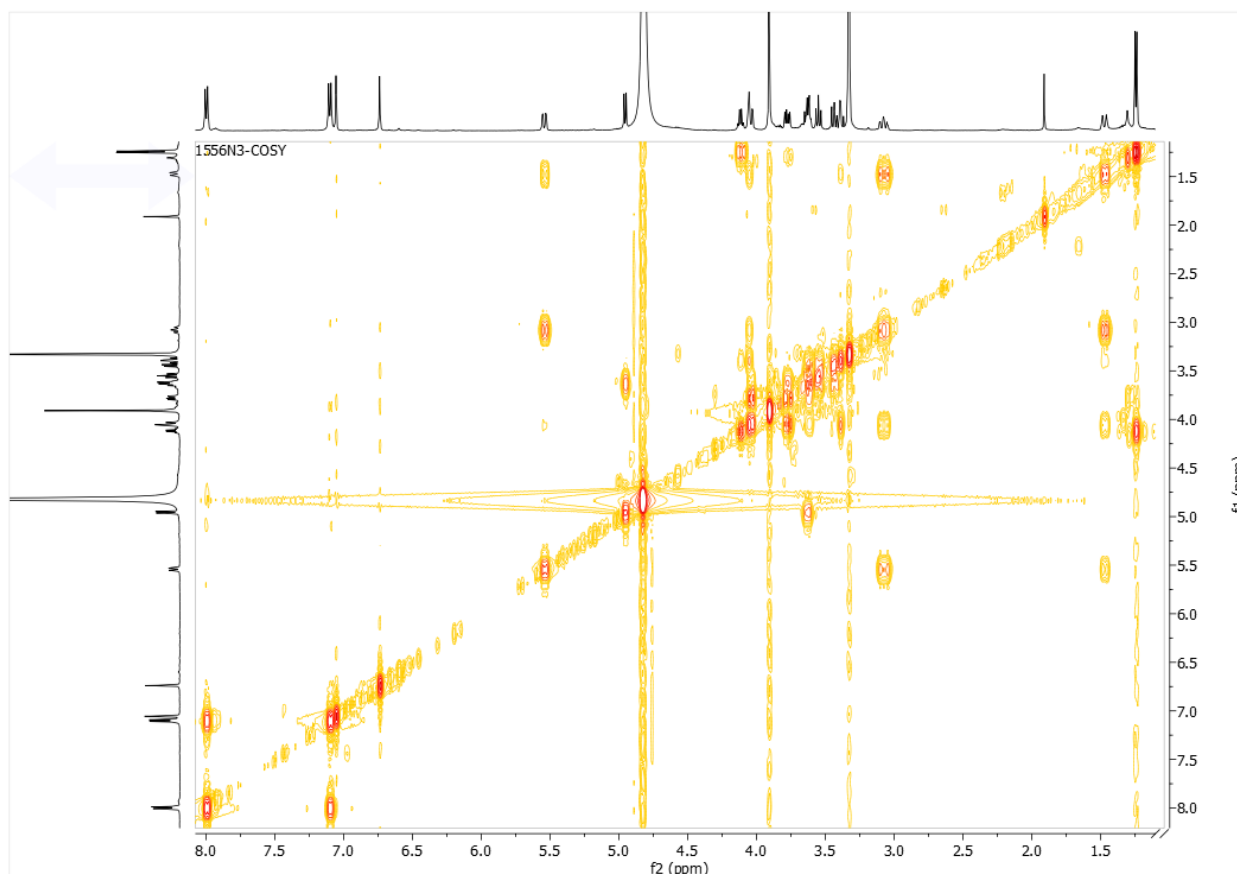


**Figure S6.** HMBC spectrum of compound **1**

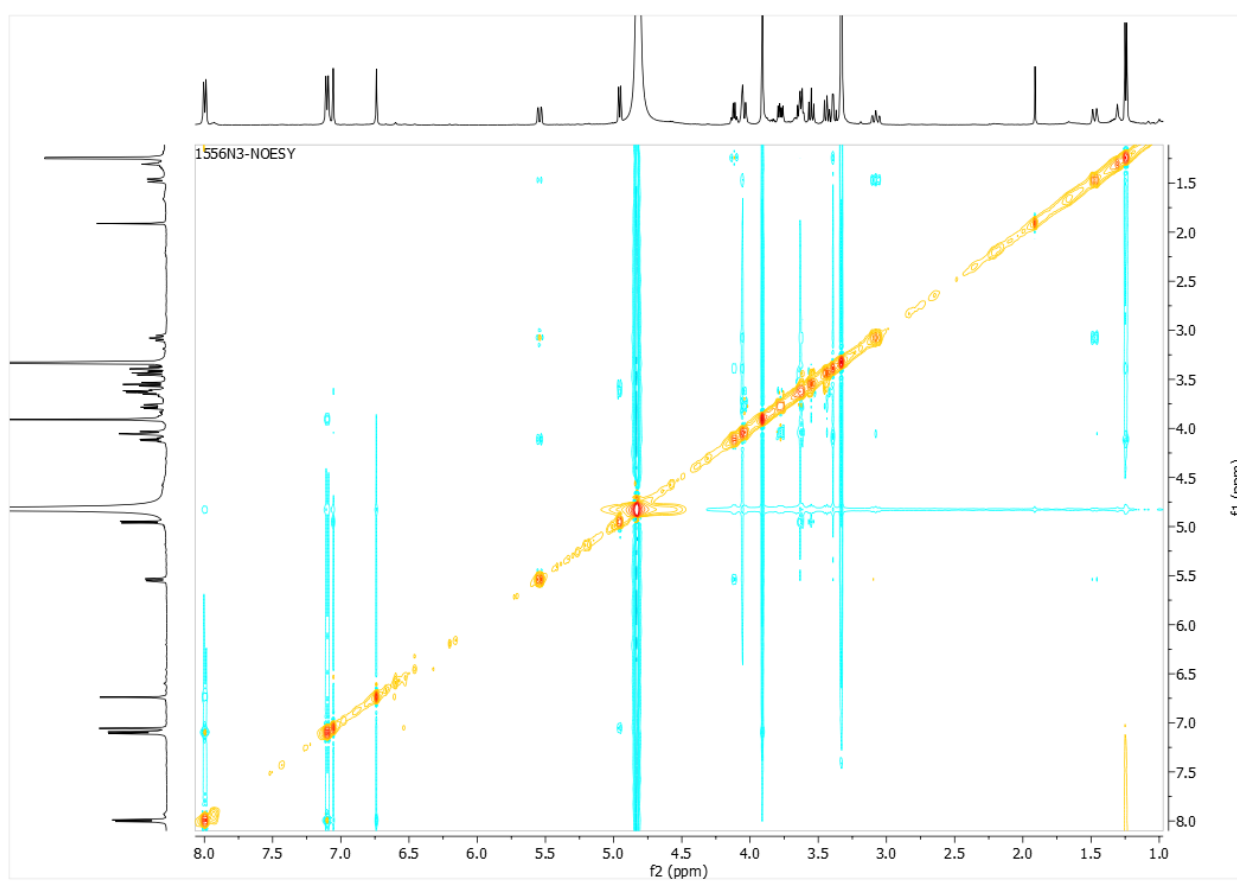




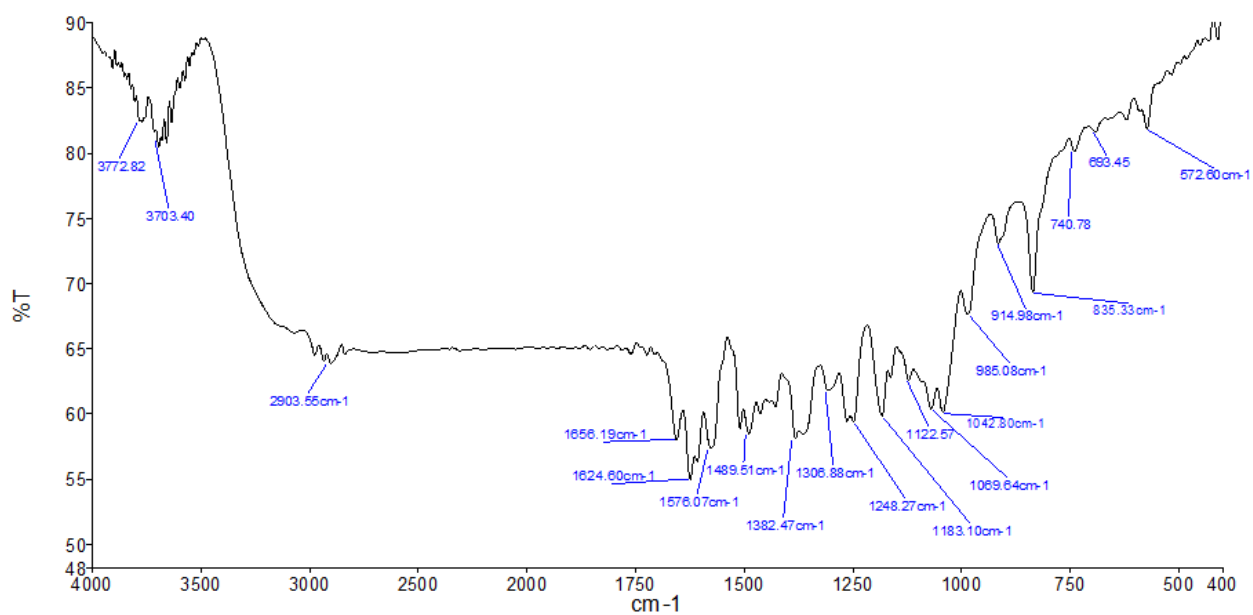
**Figure S7.** COSY spectrum of compound 1



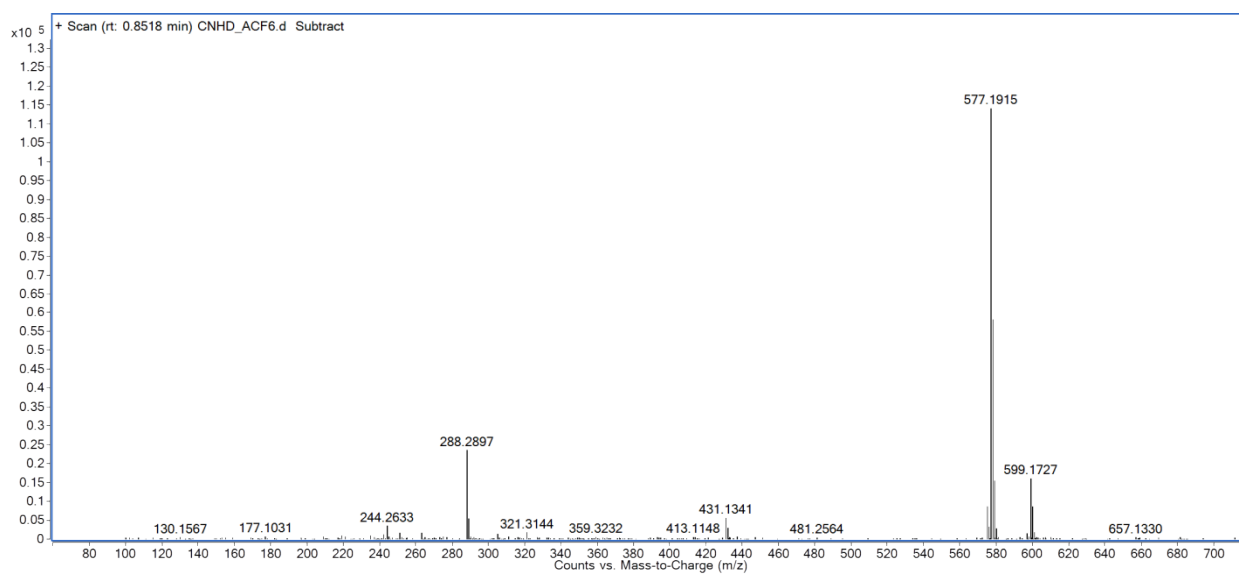
**Figure S8.** NOESY spectrum of compound 1



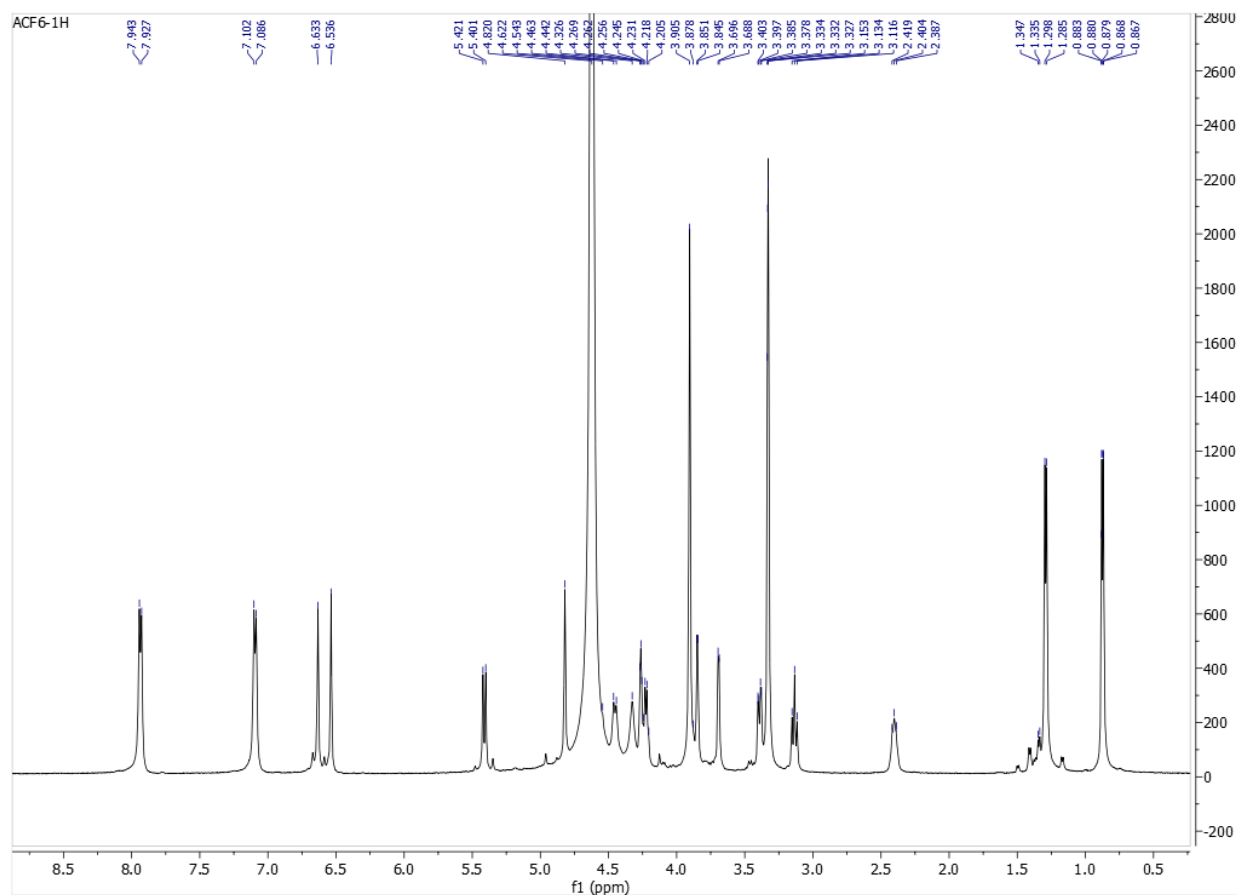
**Figure S9.** IR spectrum of compound **2**



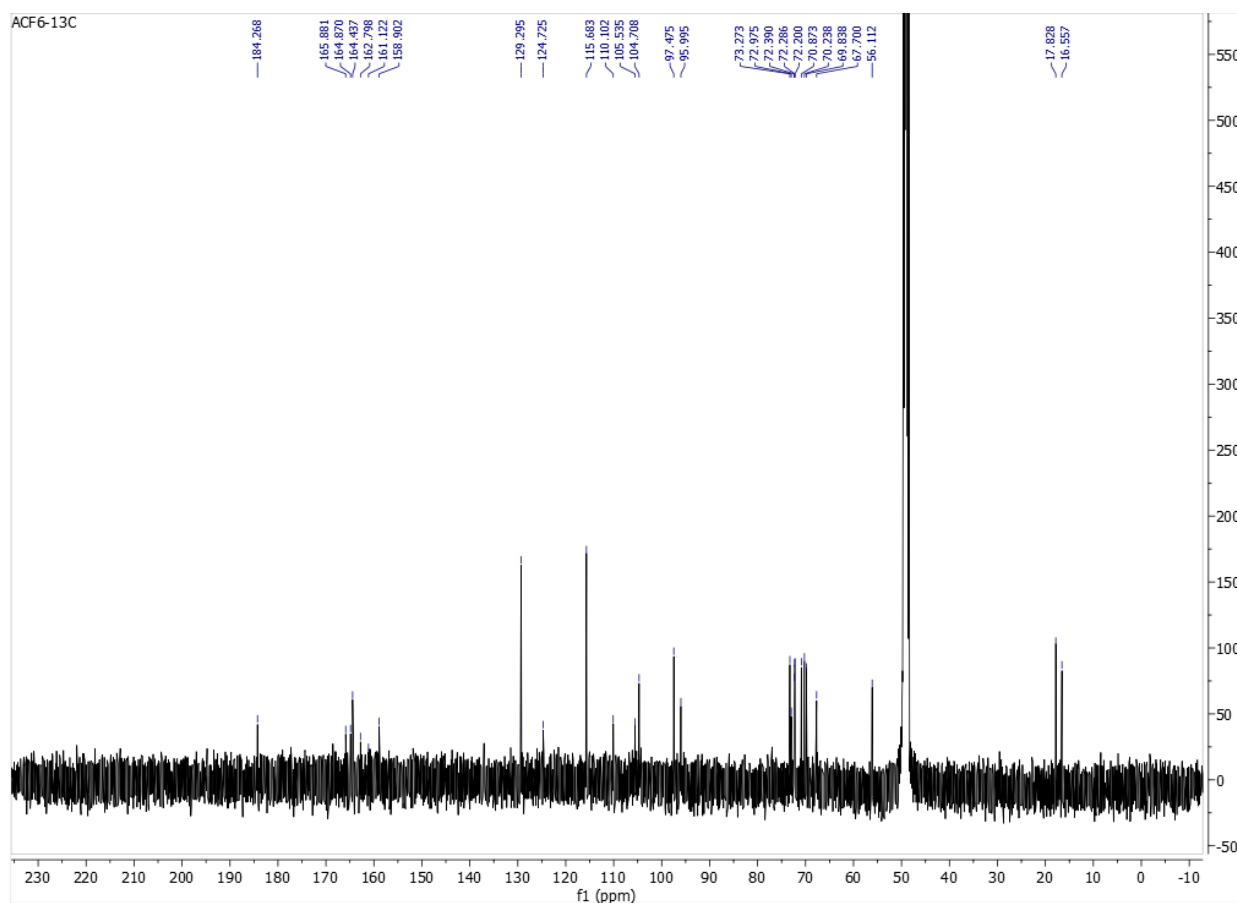
**Figure S10.** HR-ESI-MS of compound **2**



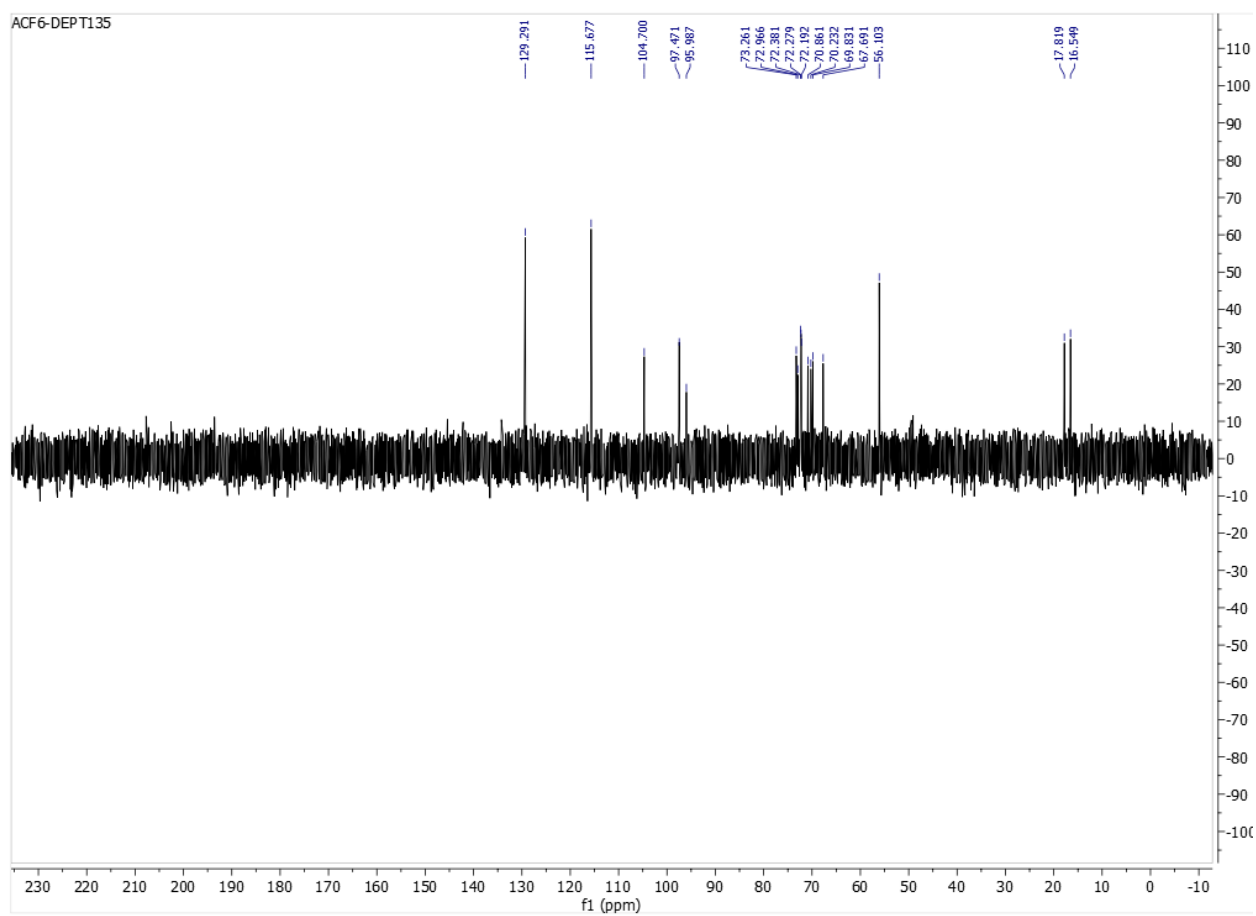
**Figure S11.**  $^1\text{H}$ -NMR spectrum of compound **2**



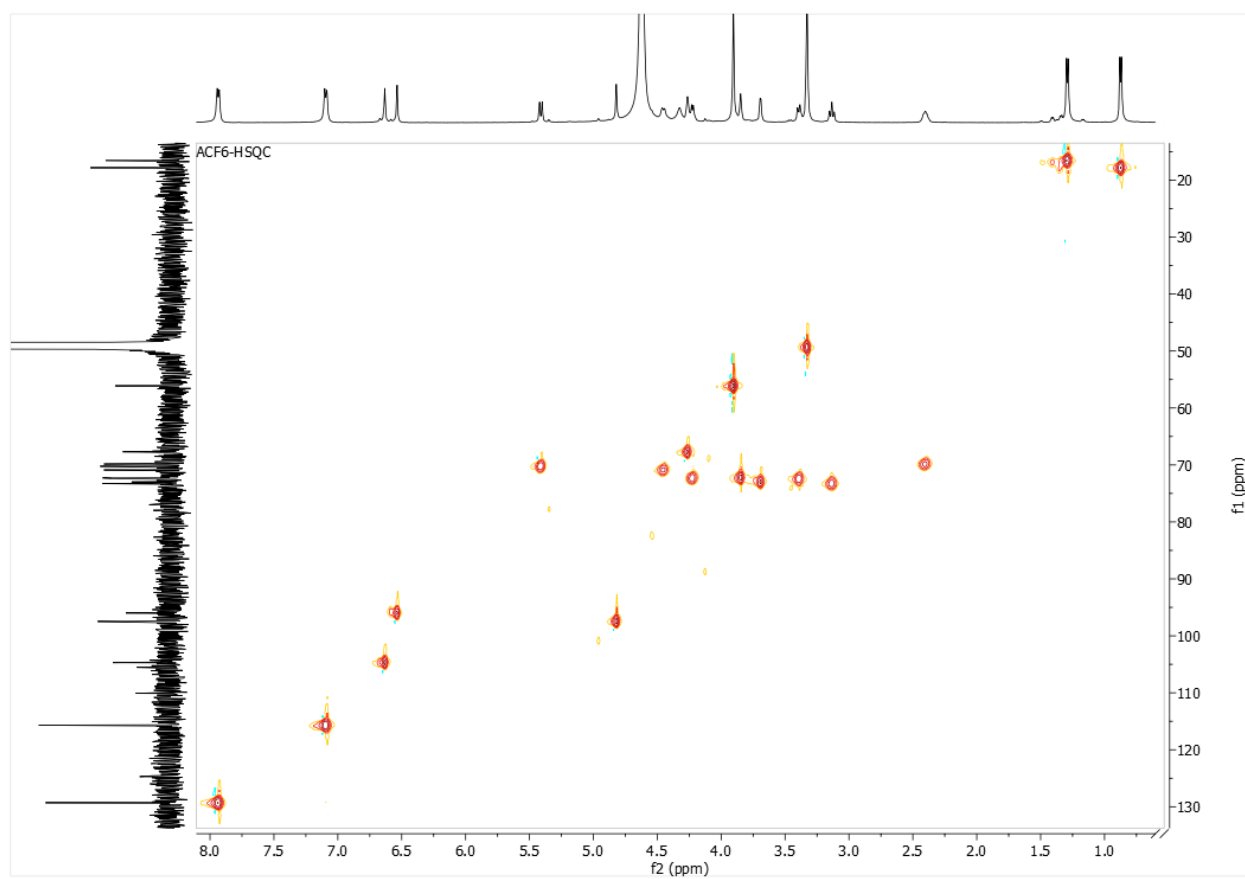
**Figure S12.**  $^{13}\text{C}$ -NMR spectrum of compound **2**



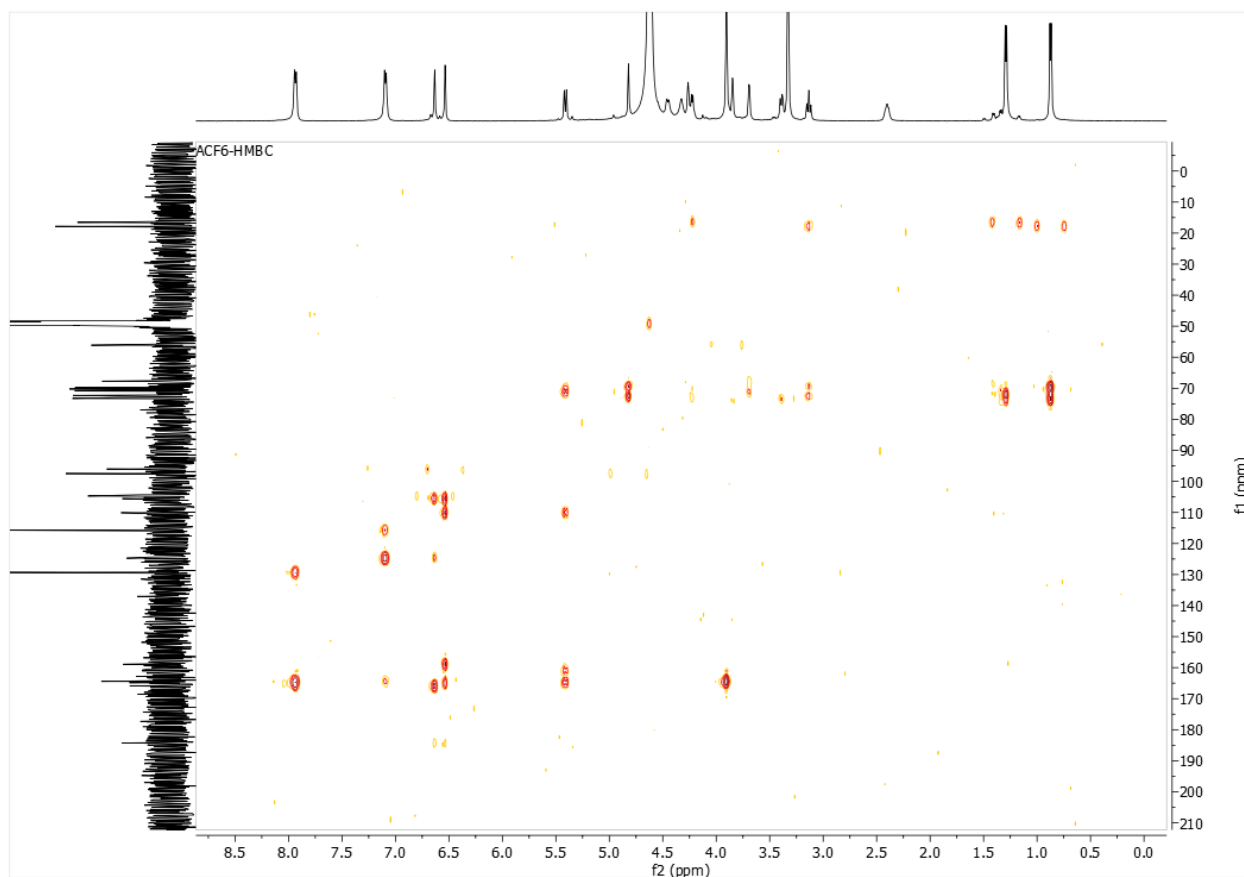
**Figure S13.** DEPT135 spectrum of compound **2**



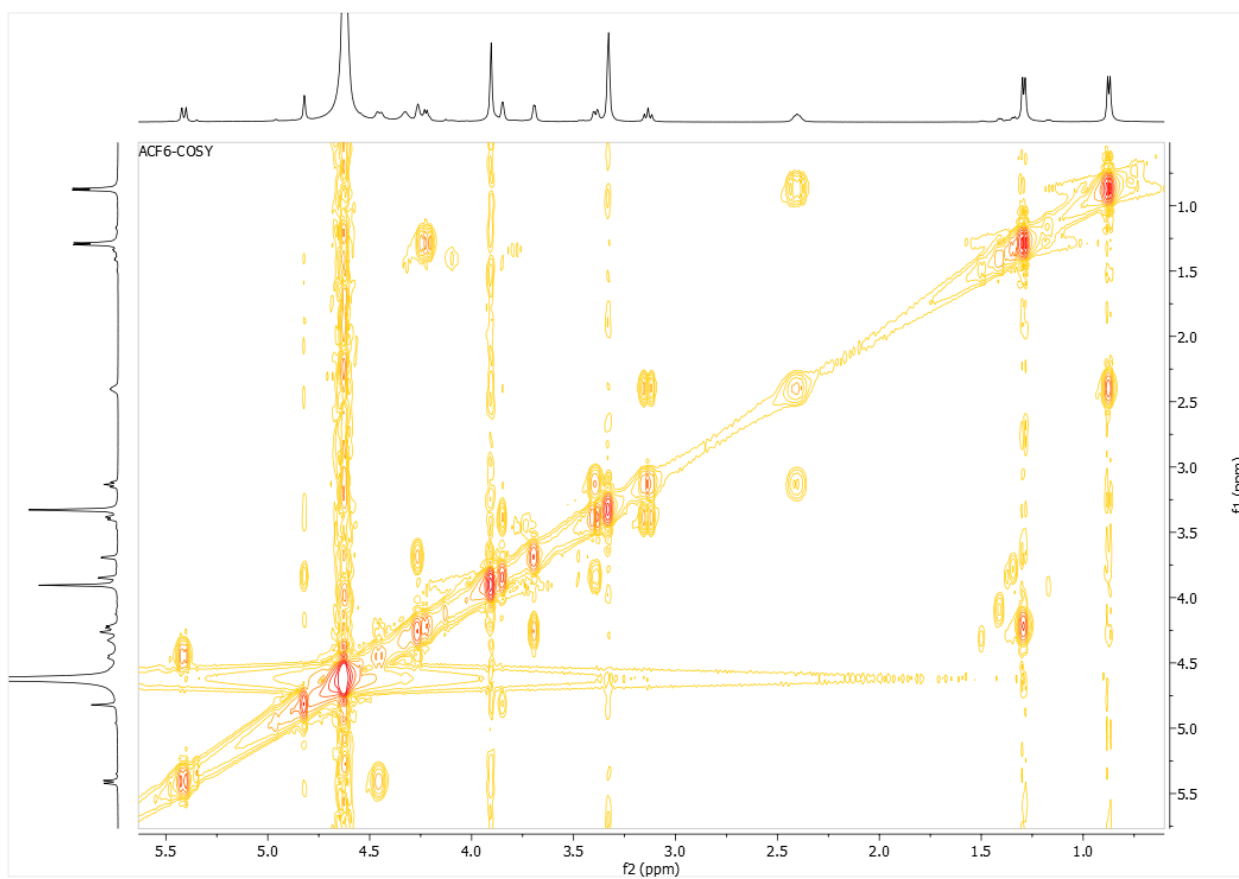
**Figure S14.** HSQC spectrum of compound **2**



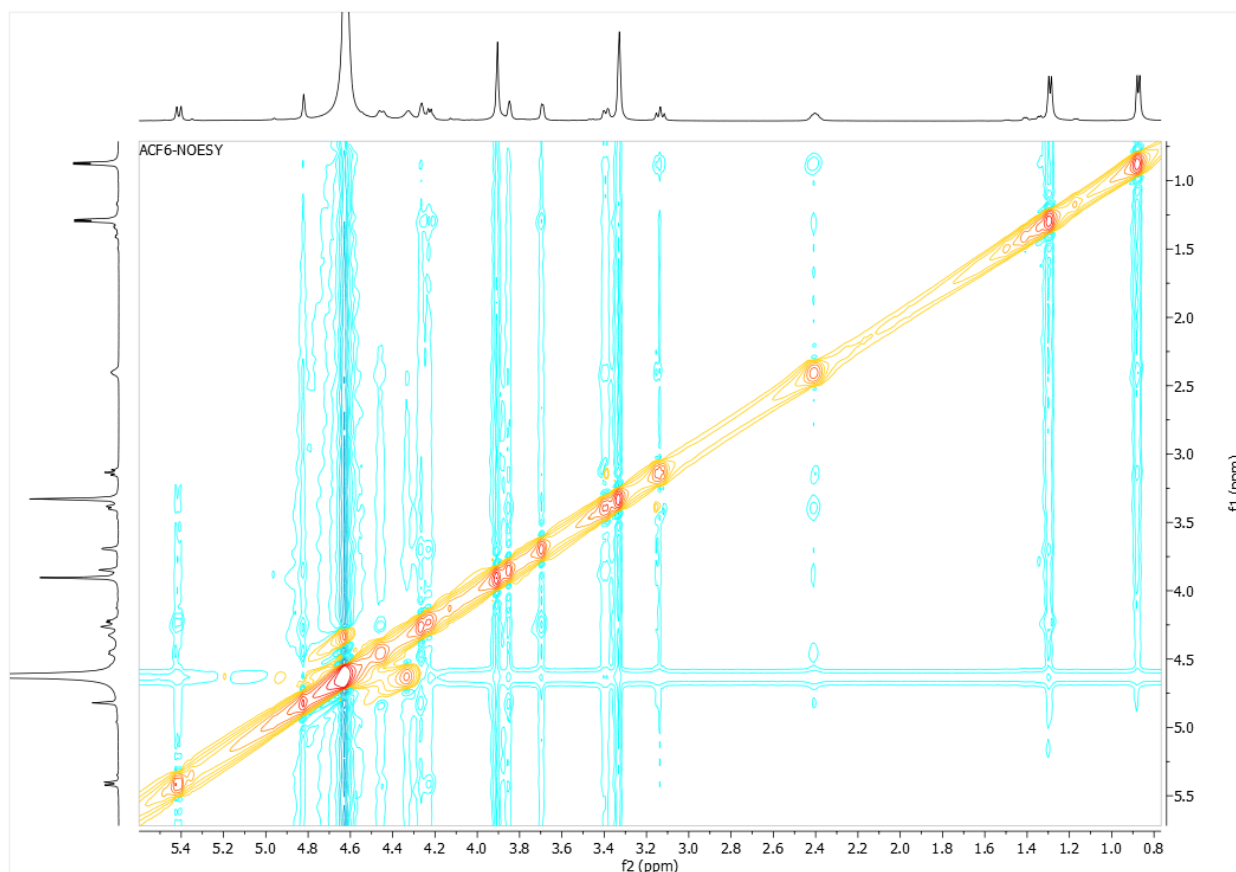
**Figure S15.** HMBC spectrum of compound **2**



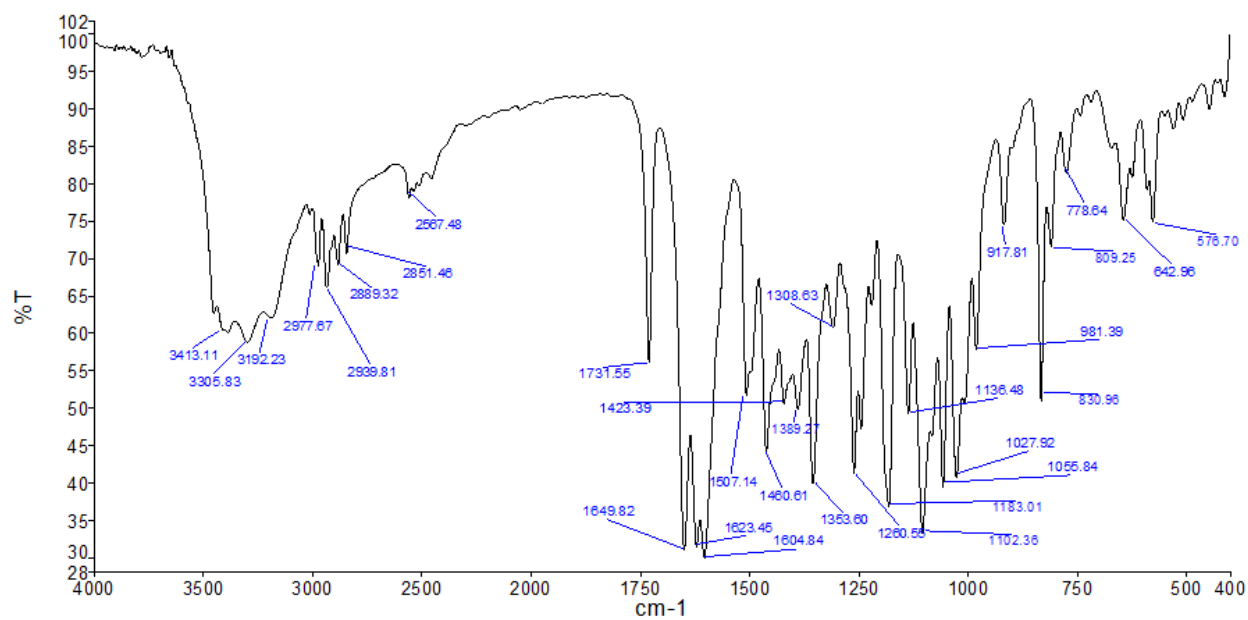
**Figure S16.** COSY spectrum of compound **2**



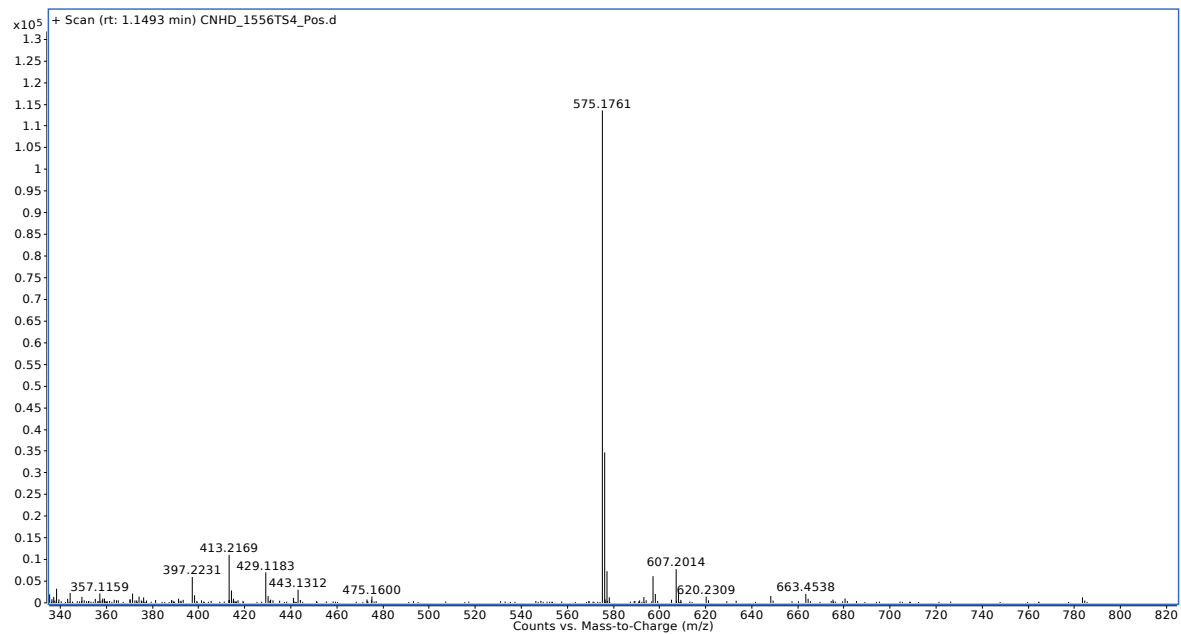
**Figure S17.** NOESY spectrum of compound 2



**Figure S18.** IR spectrum of compound 3



**Figure S19.** HR-ESI-MS of compound **3**



**Figure S20.** <sup>1</sup>H-NMR spectrum of compound **3** at 30°C

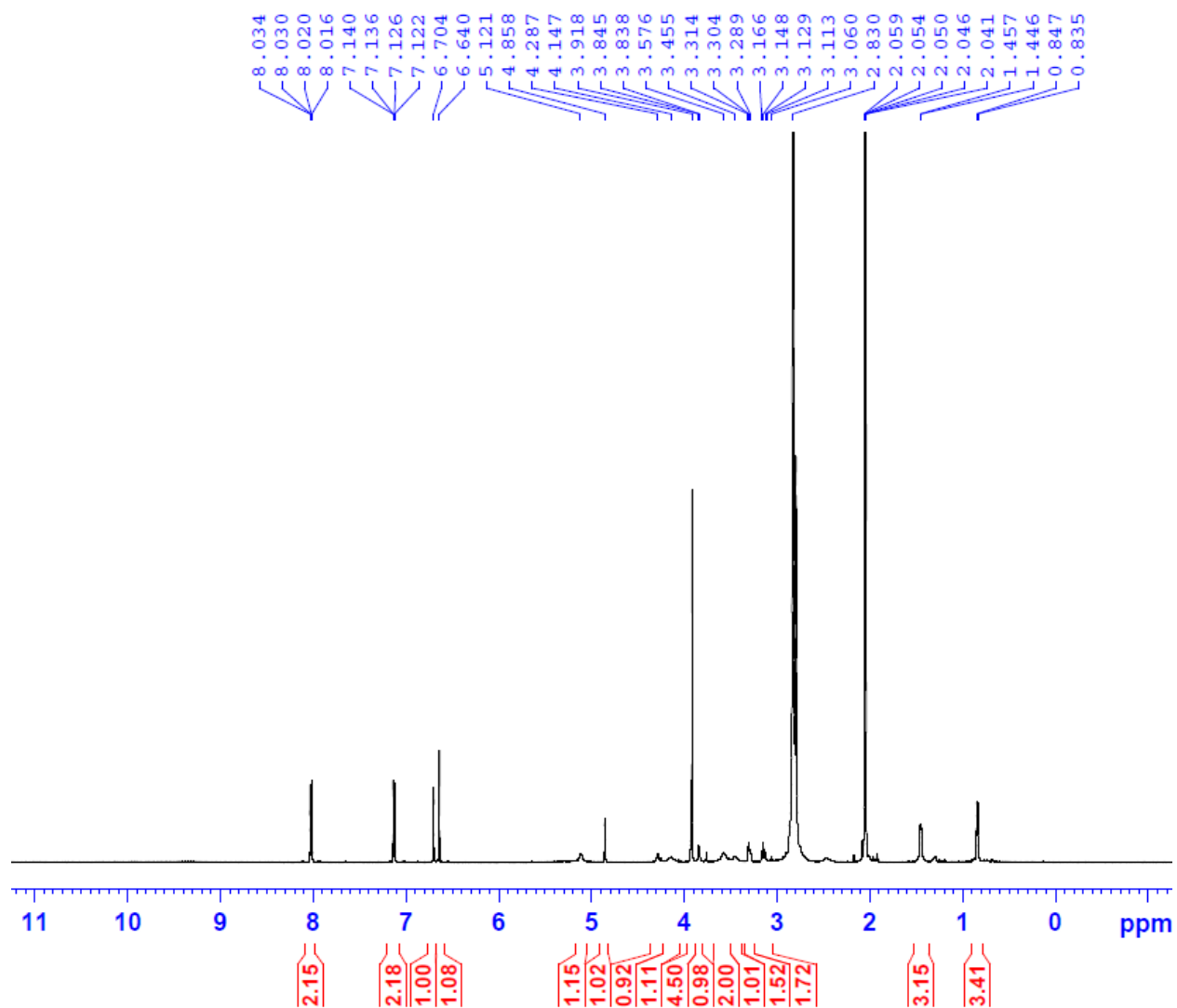


Figure S21.  $^1\text{H-NMR}$  spectrum of compound **3** at 60°C

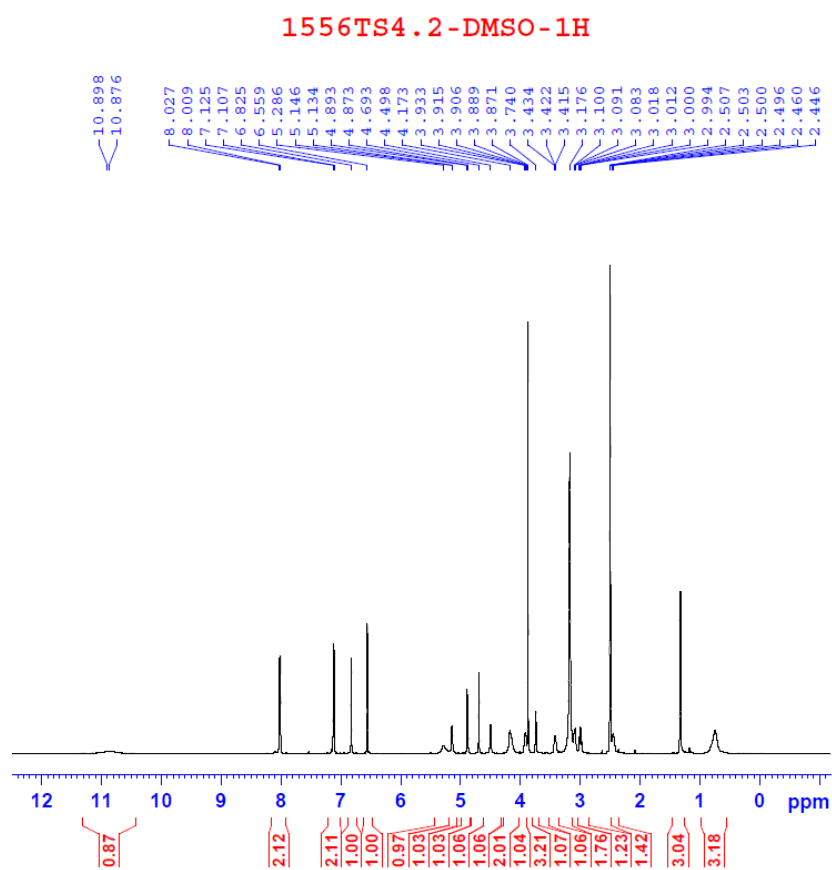


Figure S22.  $^{13}\text{C-NMR}$  spectrum of compound **3** at 30°C

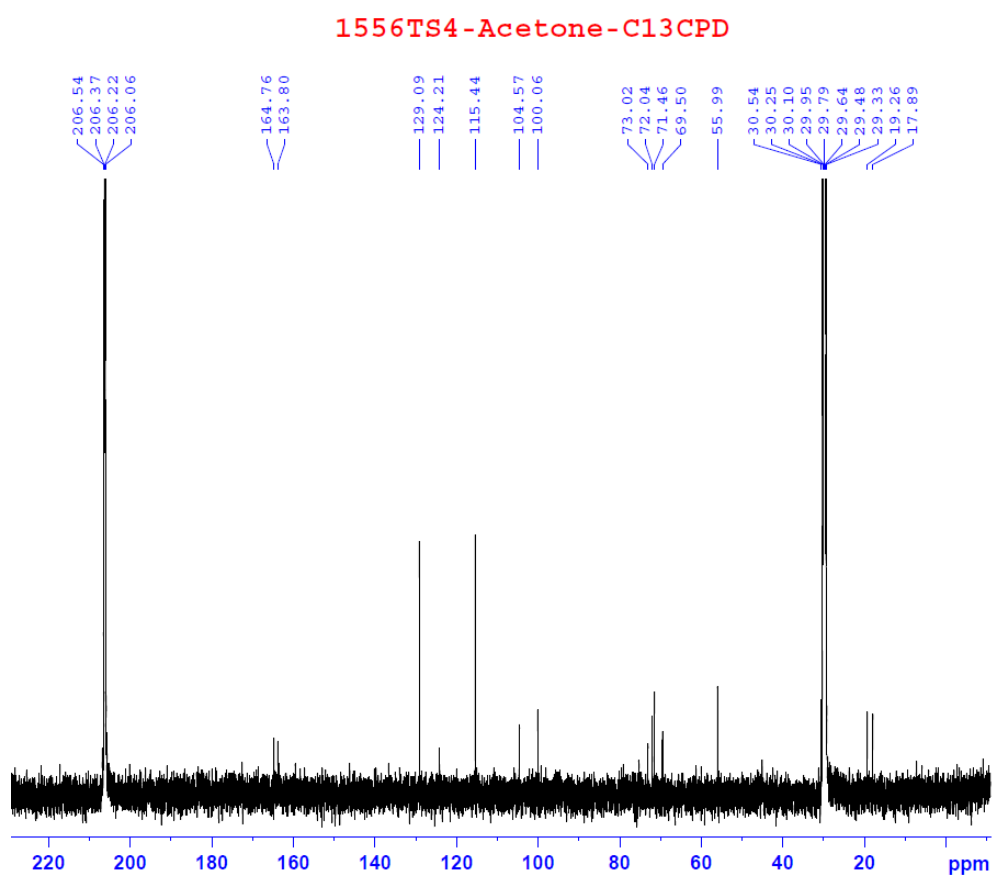






Figure S25. HMBC spectrum of compound 3

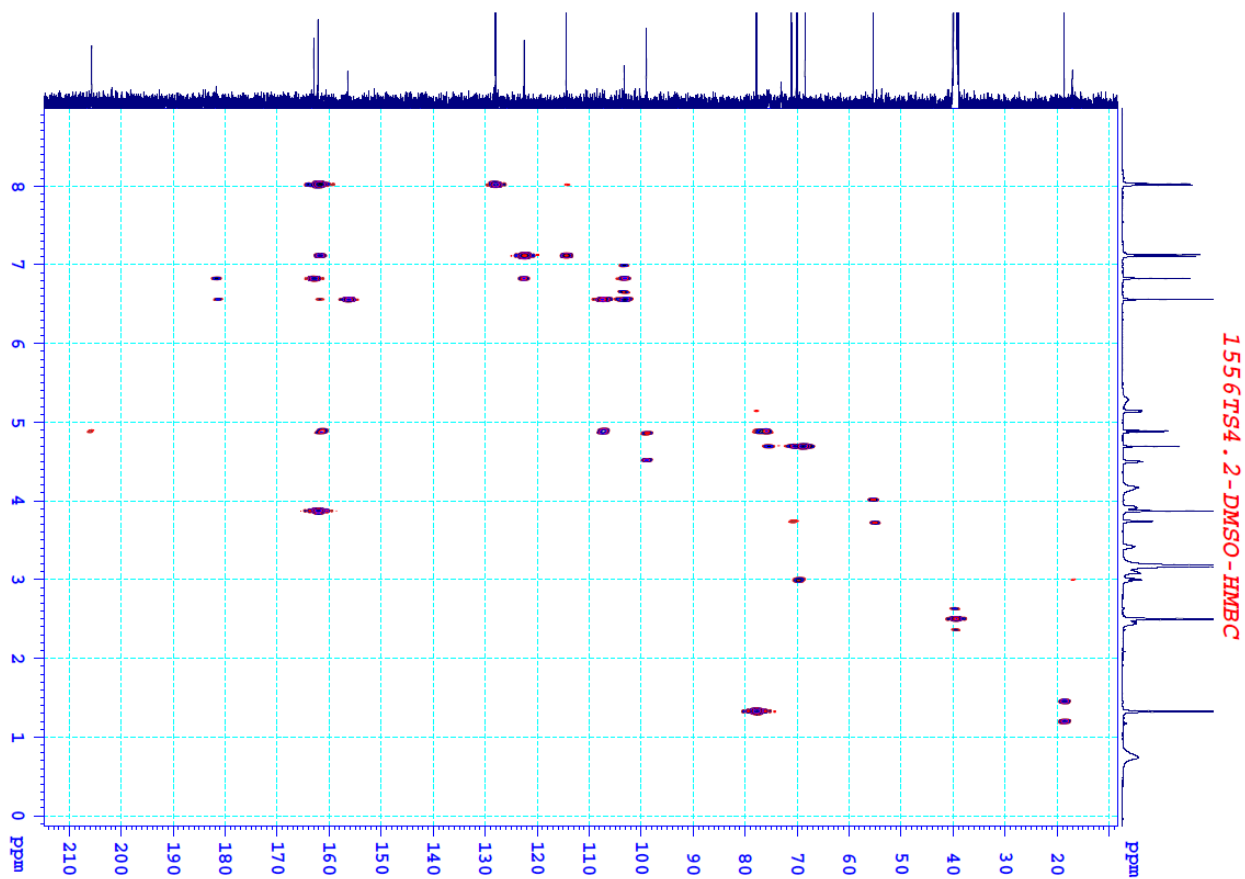
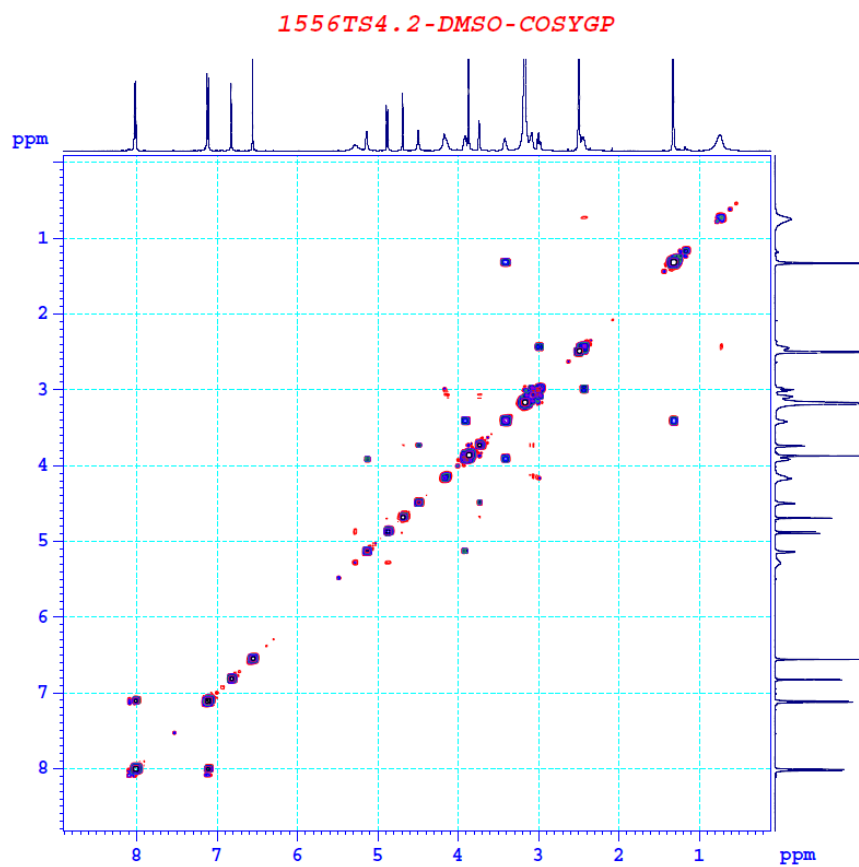


Figure S26. NOESY spectrum of compound 3



**Figure S27.** NOESY spectrum of compound **3**

