SUPPLEMENTARY MATERIALS

Chemical constituents from *Schisandra sphenanthera* and their cytotoxic activity

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ABSTRACT: Extensive phytochemical investigation of *Schisandra sphenanthera* leaves resulted in the isolation of six highly oxygenated nortriterpenoids (**1**-**6**) and five lignans (**7**-**11**) including a new pre-schisanartane-type, schisandrathera A (**1**), a new dibenzocyclooctadiene glycosides, schisandrathera B (**7**) and two new lignans, schisandrathera C (**8**) and schisandrathera D (**9**). Their chemical structures including absolute configurations were determined extensively by means of HR-ESI-MS, NMR, and ECD spectra. In addition, all isolated compounds were tested for cytotoxic activity against PC3 (prostate cancer) and MCF4 (breast cancer) cell lines. Among these compounds, schirubrisin B (**3**) showed strong cytotoxic effect on both PC3 and MCF7 cell lines with IC_{50} values of 3.21 ± 0.68, 13.30 ± 0.68 μM, respectively, whereas ten remaining compounds were found to be less effective in the investigated models.

Keywords: *Schisandra sphenanthera*, Schisandraceae, schisandrathera A, schisandrathera B, schisandrathera C, schisandrathera D.

Content

Experimental procedure

1. General

NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal Standard. HR-ESI-MS were recorded on an Agilent 6530 accurate mass QTOF LC/MS systems. Column chromatography (CC) was performed using Kieselgel 60, 70-230 mesh and 230-400 mesh (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) used pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany); compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3-5 minutes. Preparative HPLC were carried out on an Agilent 1100 system (Agilent technologies, Santa Clara, CA, USA), using J'sphere ODS-H80 semi-preparative column (20×250 mm, YMC, Kyoto, Japan).

2. Extraction and isolation

The dried powdered plant of *S. sphenanthera* (5kg) was extracted with MeOH for three times (each time 10L) in ultrasonic bath at room temperature. The removal of solvent under vacuum evaporation resulted in 220 g of crude MeOH extract, which was suspended with water and separated in turn with dichloromethane and then ethyl acetate. The water layer was subjected to a Diaion HP20 column and eluted with methanol/water (0/100, 25/75, 50/50, 100/0, v/v) to give four fractions W1-W4, respectively. Fraction W3 and W4 were combined and chromatographed on a silica gel CC eluting with dichloromethane/methanol (25/1, 10/1, 5/1, 5/1, 2/1, 1/1, v/v) to give five fractions W3A-W3F, respectively. Fraction W3C was chromatographed on a RP-18 column eluting with methanol/water (1/1, v/v) to give four fractions, W3C1- W3C4. Fraction W3C3 was first purified on a silica gel CC eluting with dichloromethane/acetone/water (1/2/0.05 v/v/v) to result in fractions W3C3A-W3C3C. W3C3B was further purified by preparative HPLC column eluting with acetonitrile/water (1/3, v/v) to give compounds **7** (3.5 mg) and **10** (8.5 mg). The ethyl acetate part (70 g) was subjected to a silica gel CC and eluted with hexane/ethyl acetate (50/1, 25/1, 10/1, 5/1, 2.5/1, 1/1, v/v) to give six fractions E1-E6. Fraction E1 was first purified by silica gel CC eluting with hexane/ethyl acetate (5/1 v/v) to result in six fractions E1A-E1F. Fraction E1B was further purified by preparative HPLC eluting with methanol/water (4/1 v/v) to give compound **9** (3.5 mg). Fraction E3 was chromatographed on a RP-18 CC eluting with acetone/water (0.8/1 v/v) to give five fractions E3A-E3E. Fraction E3B was repeatedly purified by a silica gel column eluting with hexane/ethyl acetate (1/1 v/v), then further purified by a preparative HPLC column eluting with acetonitrile/water (1.3/1 v/v) to give compounds **2** (10.0 mg) and **6** (3.5 mg). Fraction E3D was subjected to silica gel CC eluting with hexane/acetone (3/1 v/v) to give five fraction E3D1-E3D5. Compound **8** (5.0 mg) was isolated from E3D1 fraction using preparative HPLC column eluting with acetonitrile/water (1/1 v/v).

Compounds **3** (12.0 mg), **4** (8.0 mg), and **5** (4.0 mg) were isolated from E3D5 fraction by using a preparative HPLC column eluted with acetonitrile/water (1/1 v/v). Fraction E3E was chromatographed on a silica gel CC eluting with hexane/acetone (3.7/1 v/v) to result compound **1** (4.5 mg) and five fractions E3E1-E3E5. Fraction E3E3 was continuously purified by preparative HPLC column eluting with methanol/water (3/1 v/v) to give compound **11** (4.2 mg).

*3. TD-DFT calculations ECD of compounds **1-6**, **8**, **9***

Conformational searches carried out on Spartan 14 program (Wavefunction Inc., Irvine, CA, USA). Possible conformations were optimized and subjected to TDDFT calculation on Gaussian 09 program (Frisch et al., 2009). The calculated ECD spectra were composed after correction based on the Boltzmann distribution of the stable conformers using SpecDis v1.64 software (Bruhn et al., 2013). Particularly, stereoisomers **1a-1b**, **2a-2b, 3a-3b, 4a-4b, 5a-5b, 6a-6b, 8a-8d, 9a-9b** were submitted to conformational searches at ground state with semi-empirical AM1 set. The initial stable conformers (Boltzmann distributions over 1.0%) were optimized by DFT calculations at the B3LYP/6-31G(d,p) basic set and polarizable continuum model (PCM) calculation of the solvent methanol. Optimized conformers were subjected to TD-DFT calculation at the B3LYP/6-31G(d,p) level and methanol as a PCM. The ECD spectra at 30 excited states for each conformer were collected and summed to obtain theoretical ECD spectra of each stereoisomer.

References

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Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich AV, Bloino J, Janesko BG, Gomperts R, Mennucci B, Hratchian HP, Ortiz JV, Izmaylov AF, Sonnenberg JL, Williams, Ding F, Lipparini F, Egidi F, Goings J, Peng B, Petrone A, Henderson T, Ranasinghe D, Zakrzewski VG, Gao J, Rega N, Zheng G, Liang W, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Throssell K, Montgomery Jr. JA, Peralta JE, Ogliaro F, Bearpark MJ, Heyd JJ, Brothers EN, Kudin KN, Staroverov VN, Keith TA, Kobayashi R, Normand J, Raghavachari K, Rendell AP, Burant JC, Iyengar SS, Tomasi J, Cossi M, Millam JM, Klene M, Adamo C, Cammi R, Ochterski JW, Martin RL, Morokuma K, Farkas O, Foresman JB, Fox DJ. 2009. Gaussian 09, Rev. D. 01, Gaussian. Inc, Wallingford, CT.

Figure S1. HR-ESI-MS of compound **1**

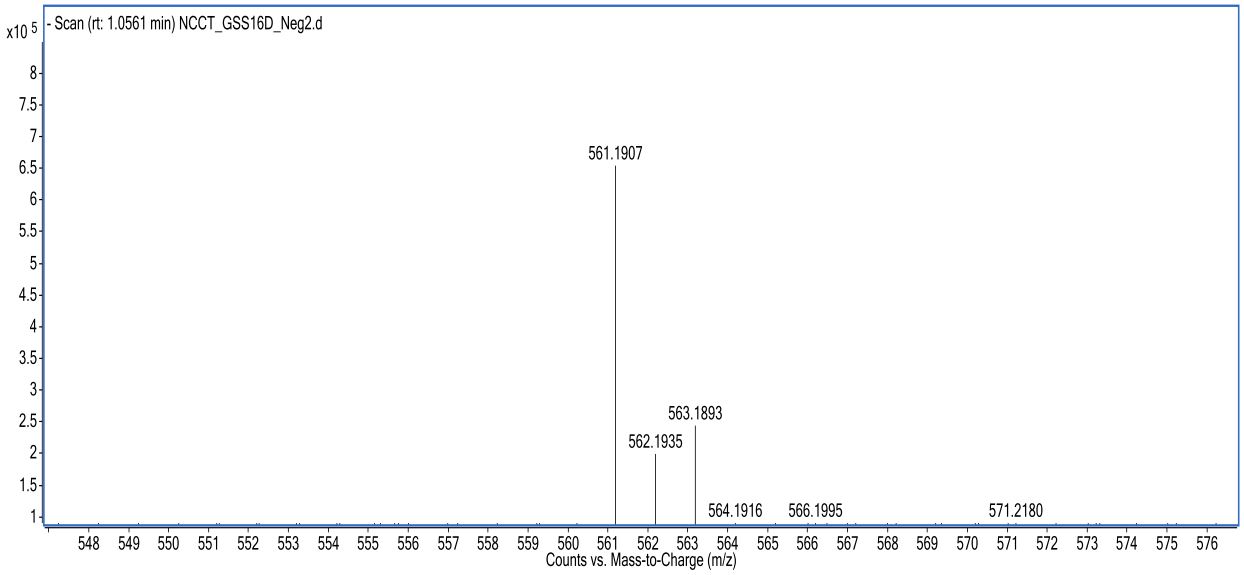

Figure S2. ^{1}H NMR spectrum of compound **1**

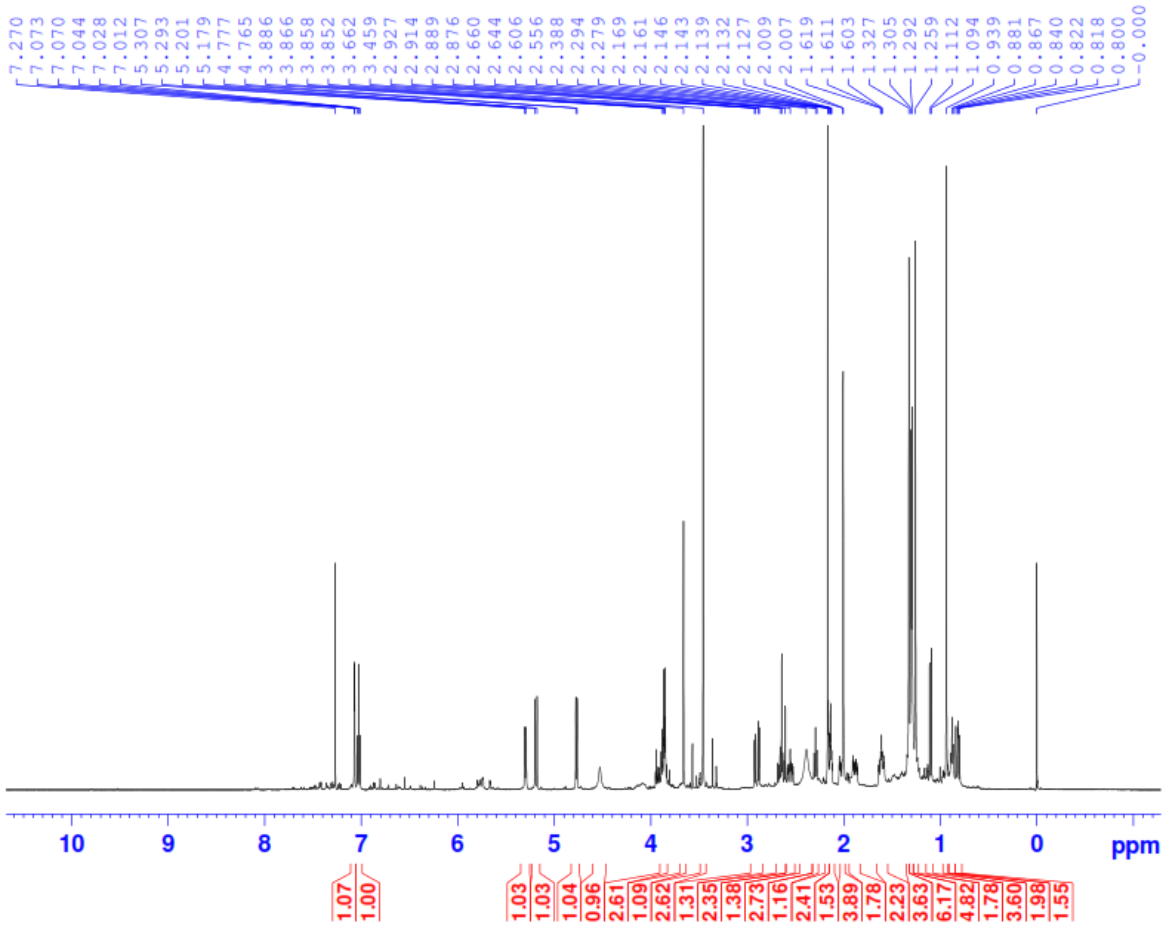

Figure S3. ^{13}C NMR spectrum of compound **1**

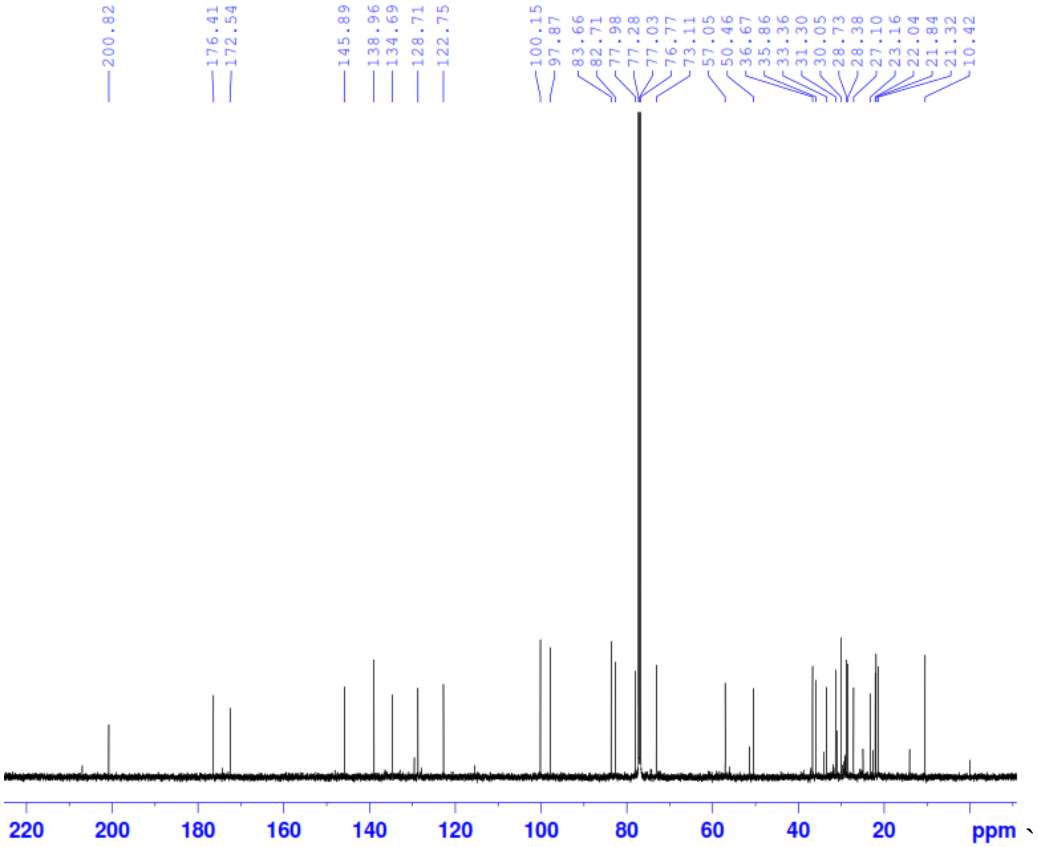

Figure S4. HSQC spectrum of compound **1**

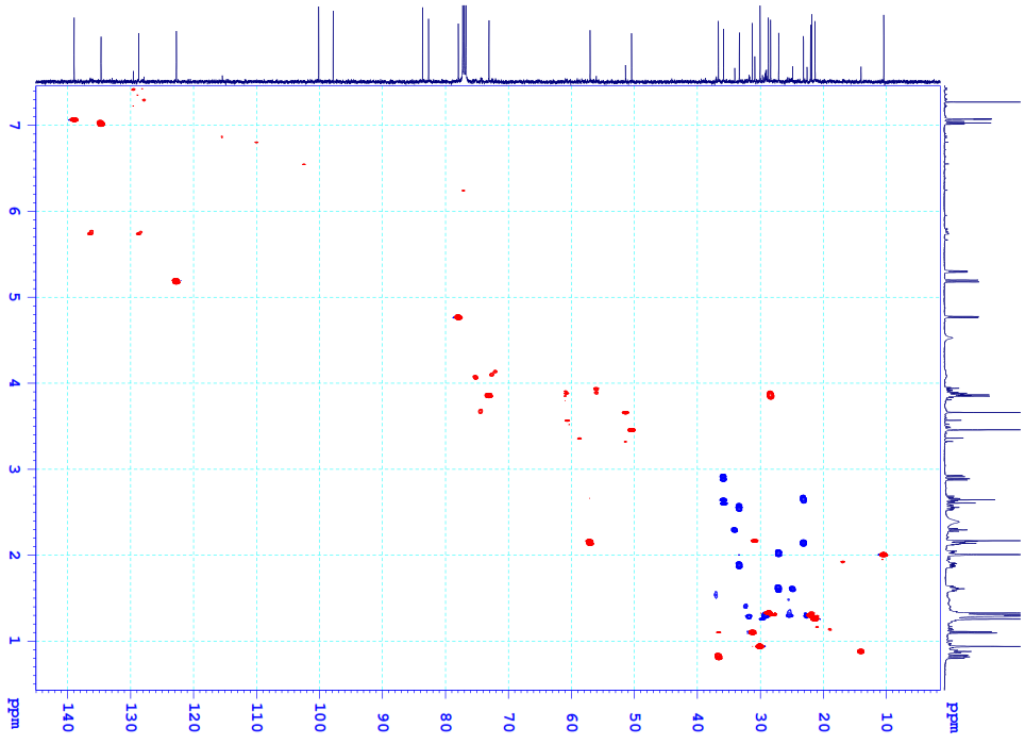

Figure S5. HMBC spectrum of compound **1**

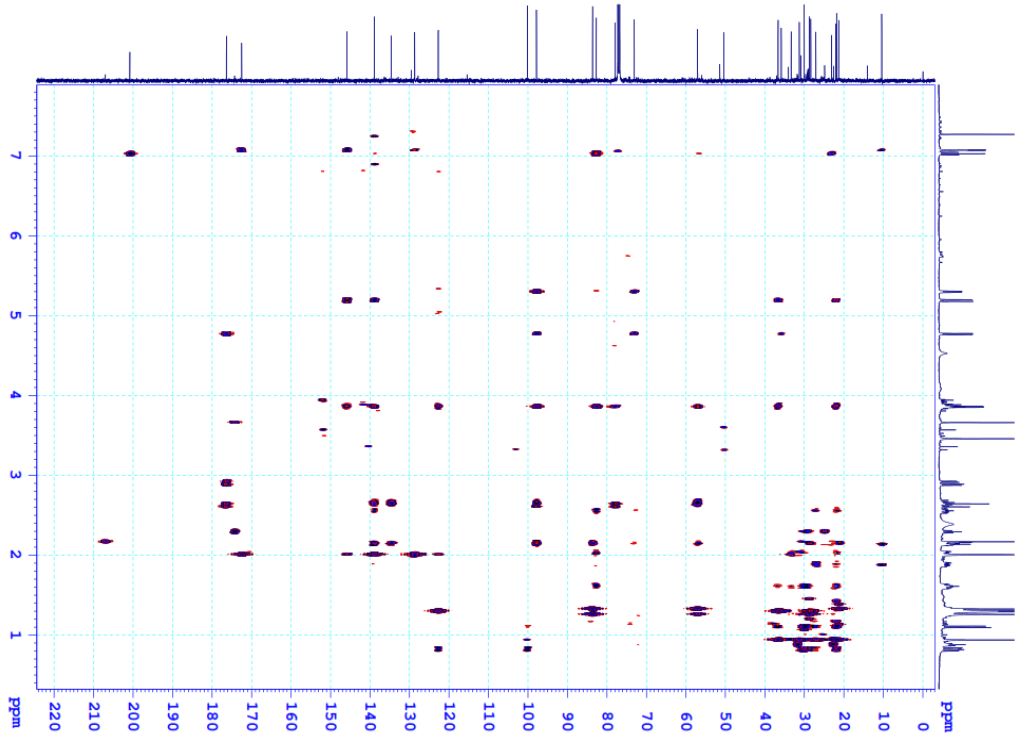

Figure S6. ^{1}H-^{1}H COSY spectrum of compound **1**

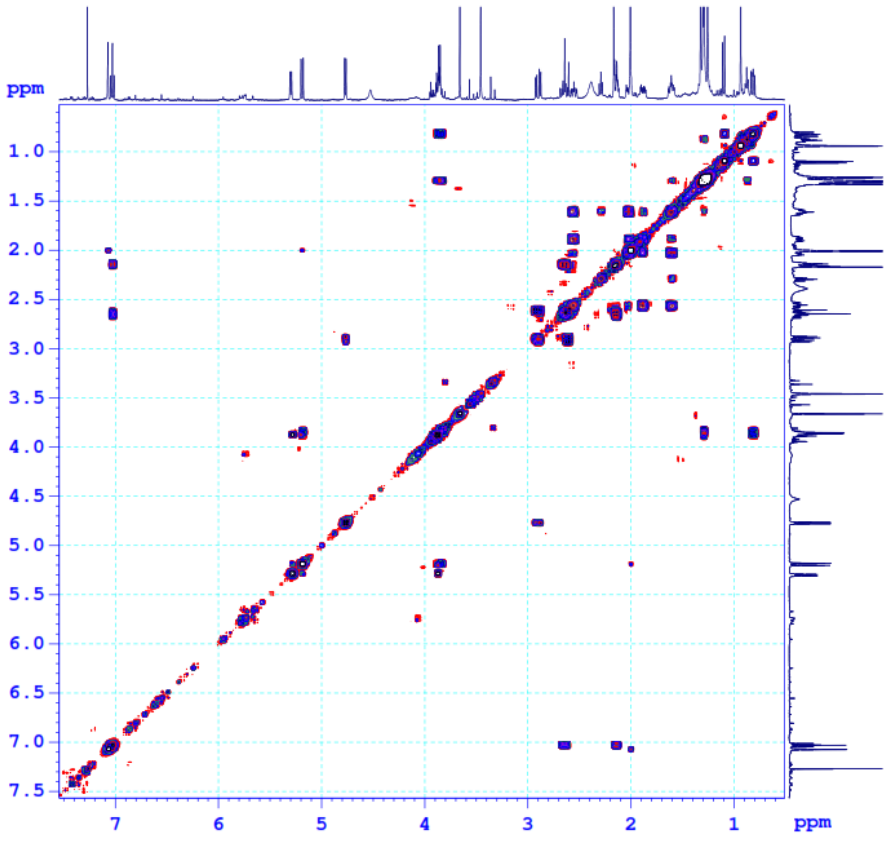

Figure S7. NOESY spectrum of compound **1**

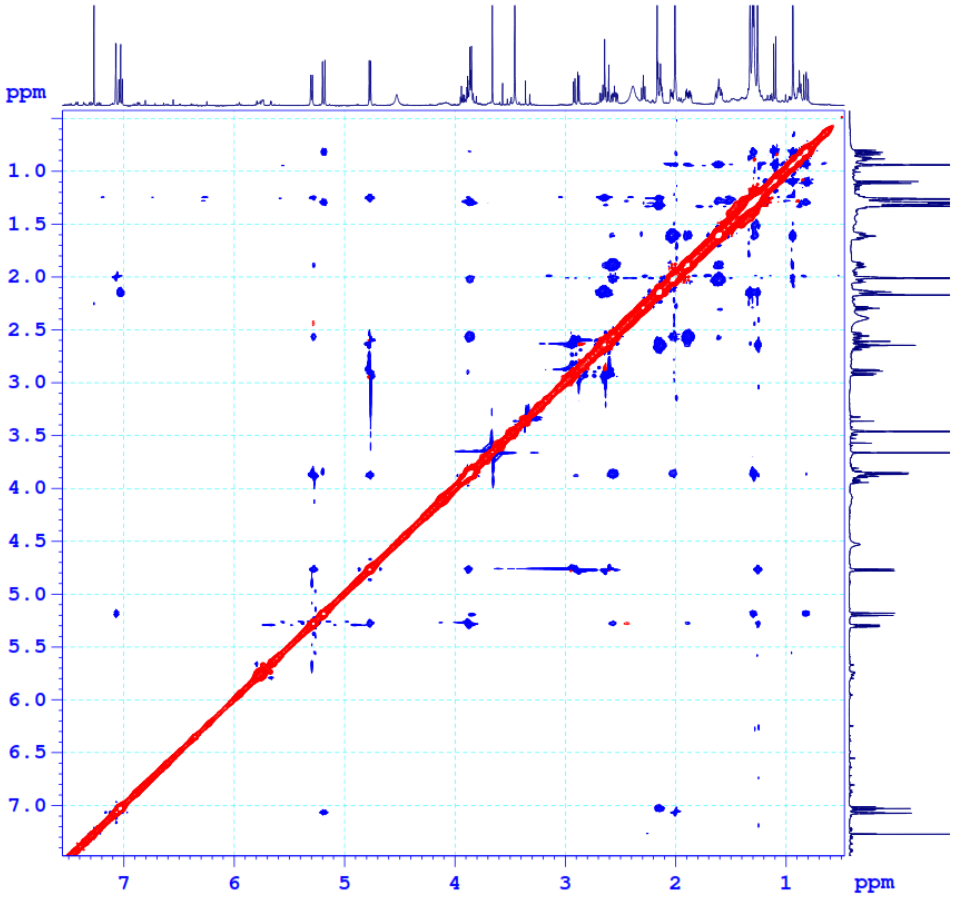

Figure S8. HR-ESI-MS of compound **7**

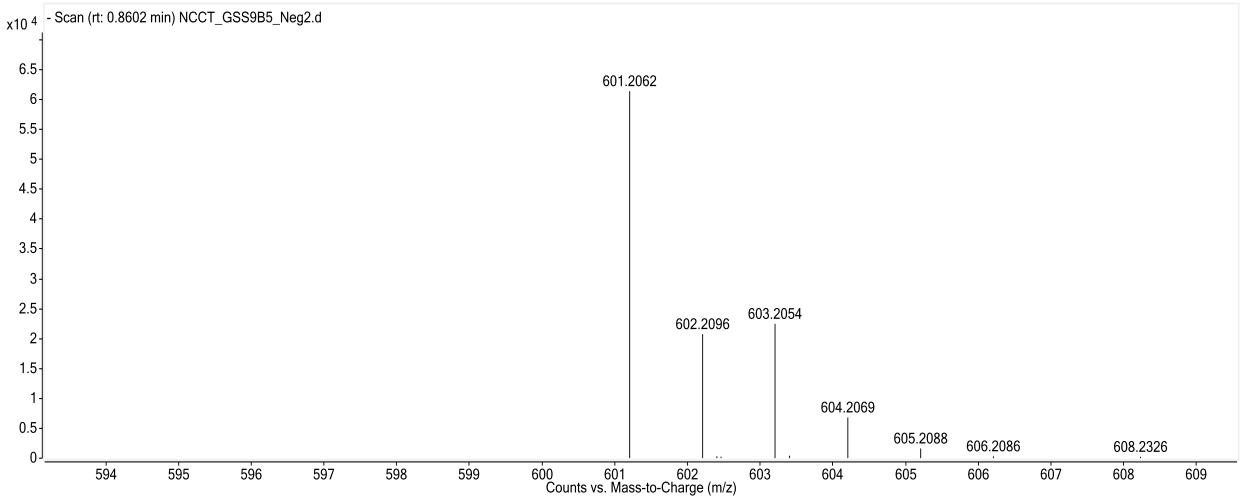

Figure S9: ^{1}H NMR spectrum of compound **7**

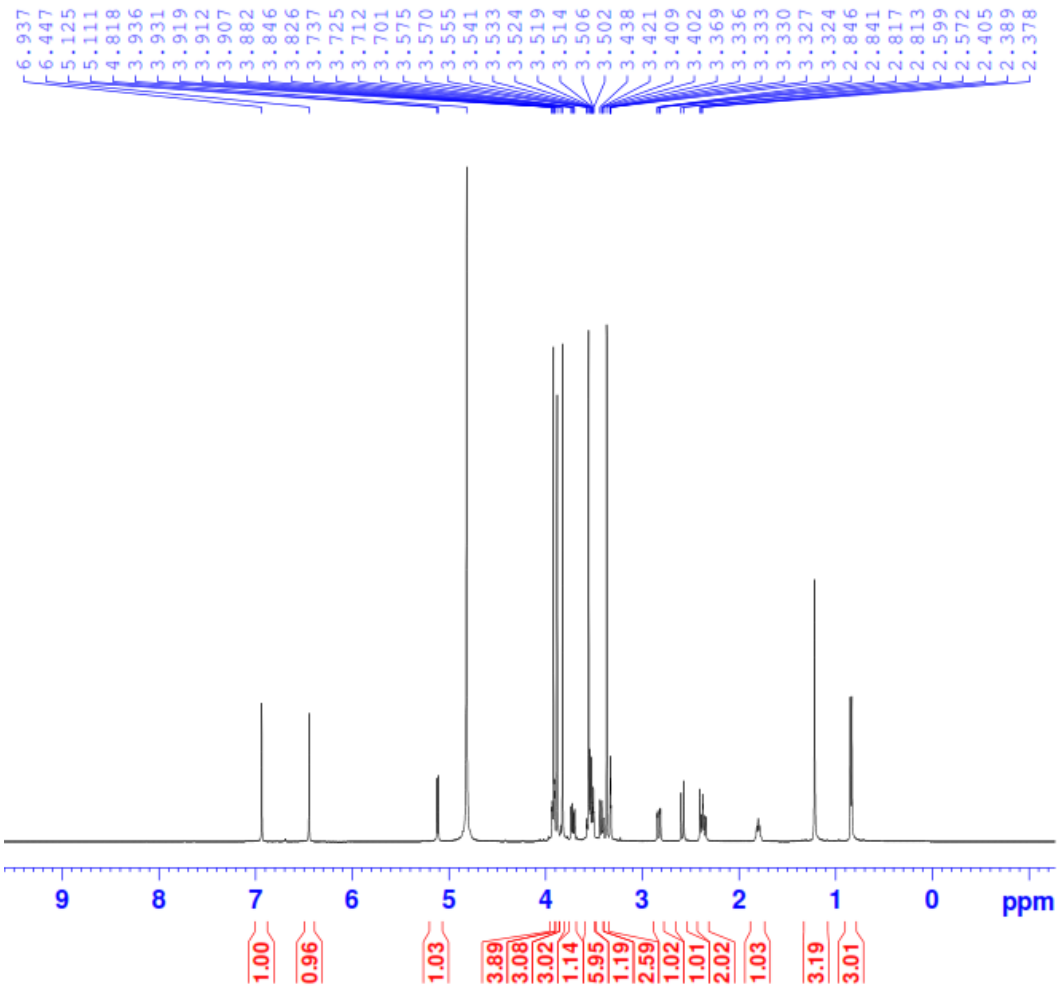

Figure S10. ^{13}C NMR spectrum of compound **7**

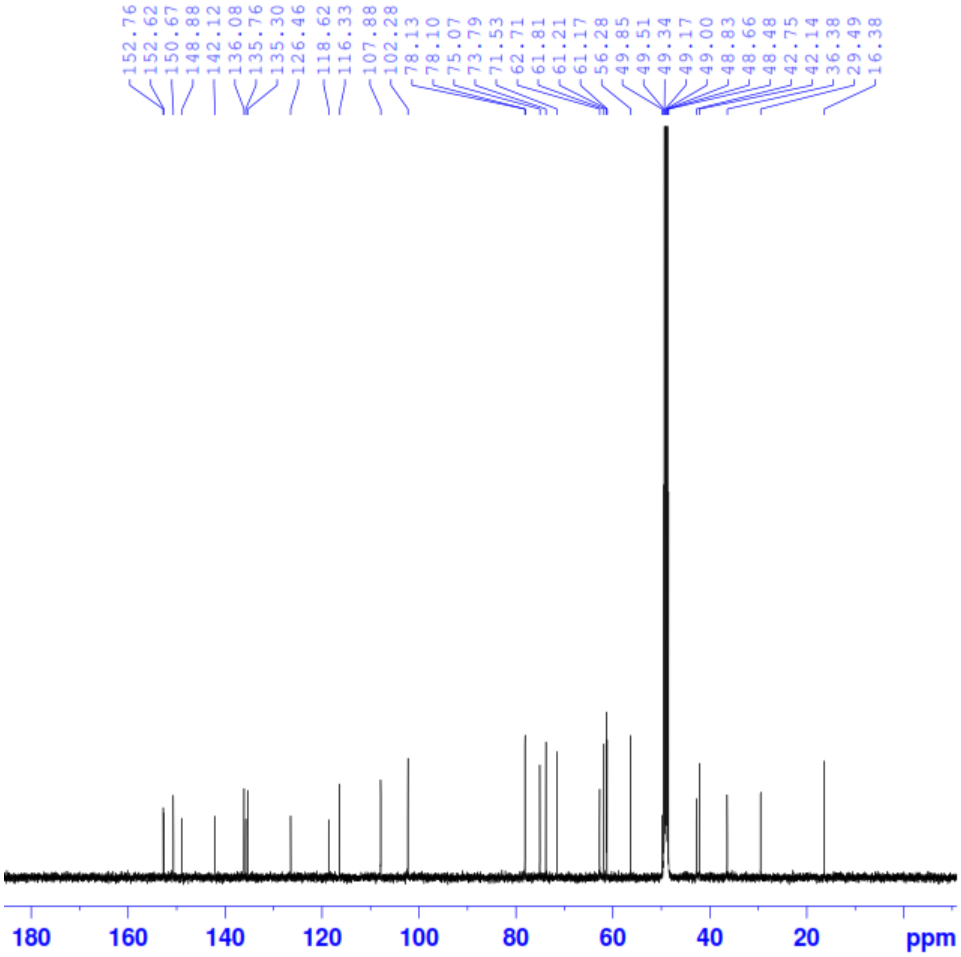

Figure S11. HSQC spectrum of compound **7**

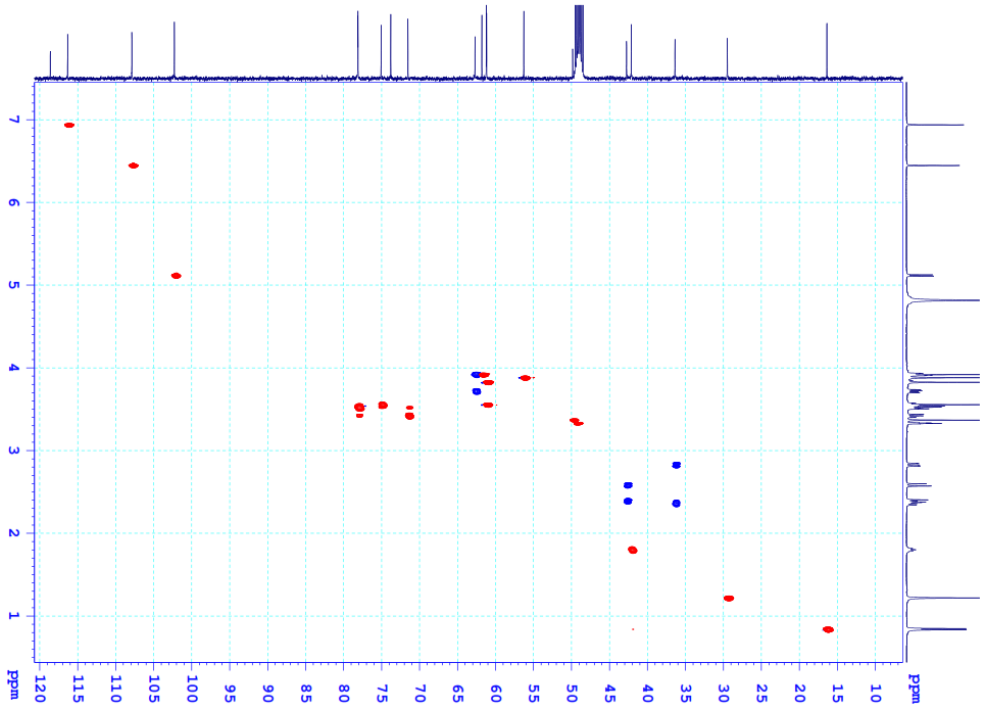

Figure S12. HMBC spectrum of compound **7**

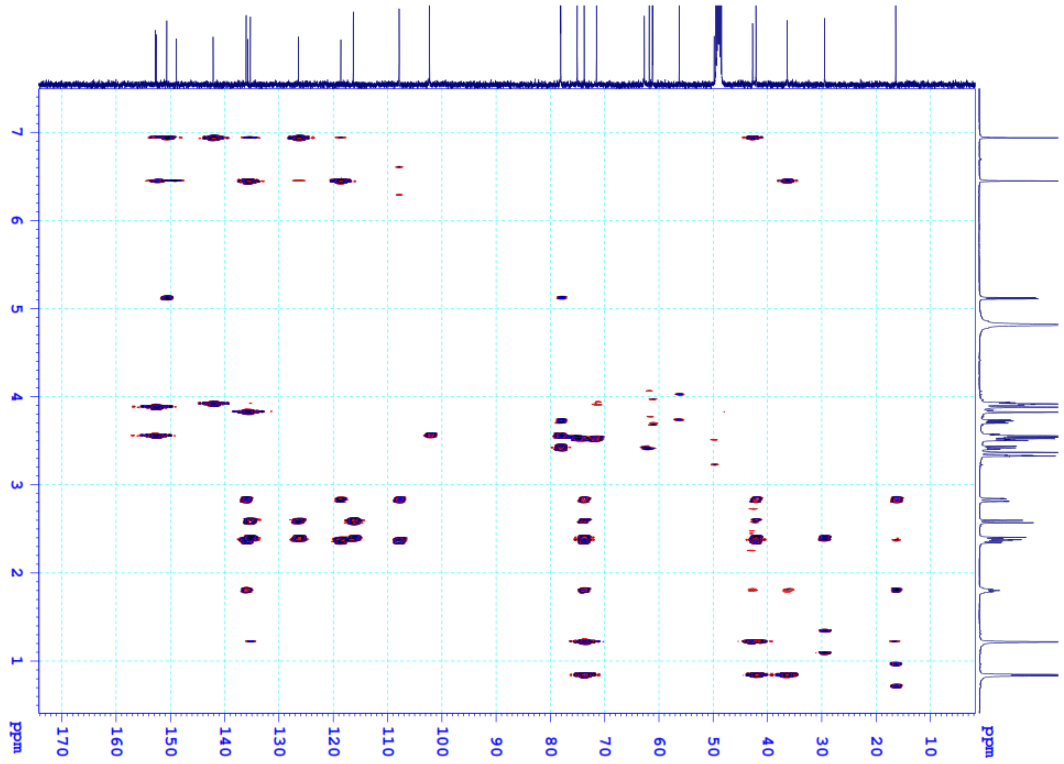

Figure S13. NOESY spectrum of compound **7**

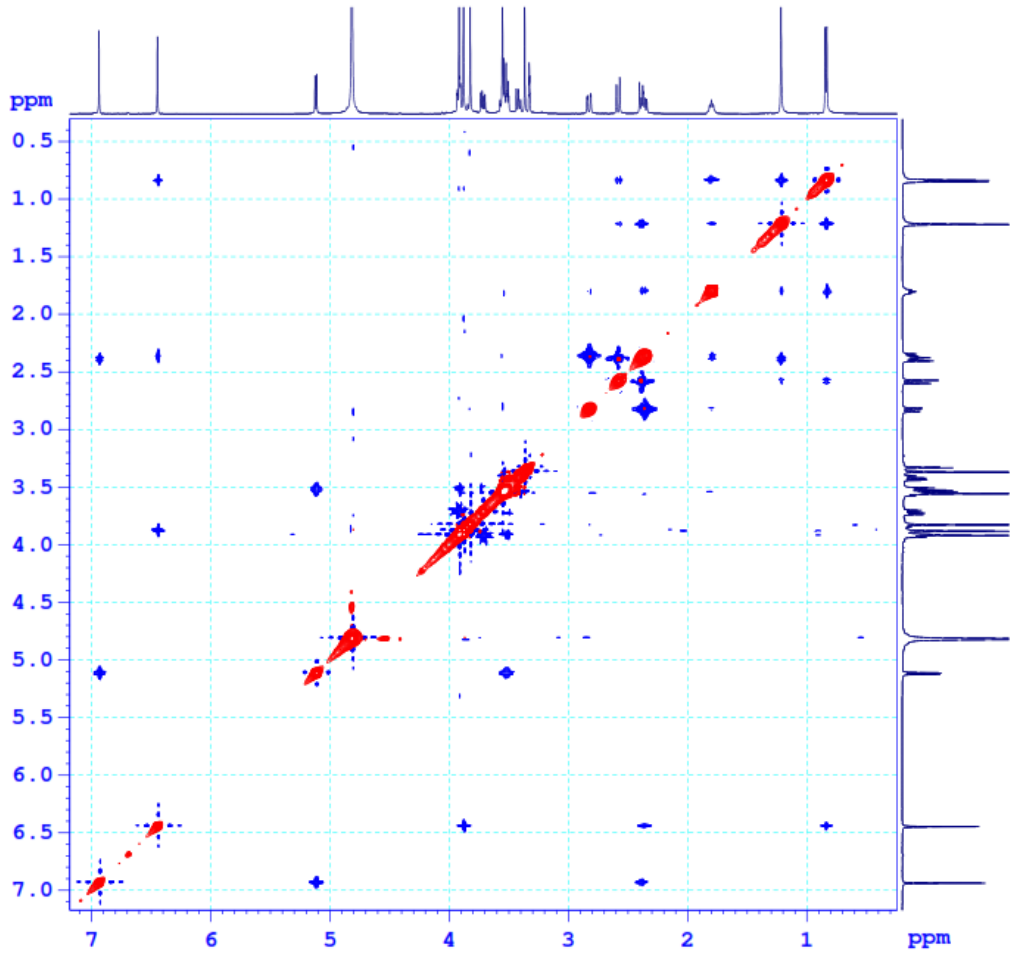

Figure S14. HR-ESI-MS of compound **8**

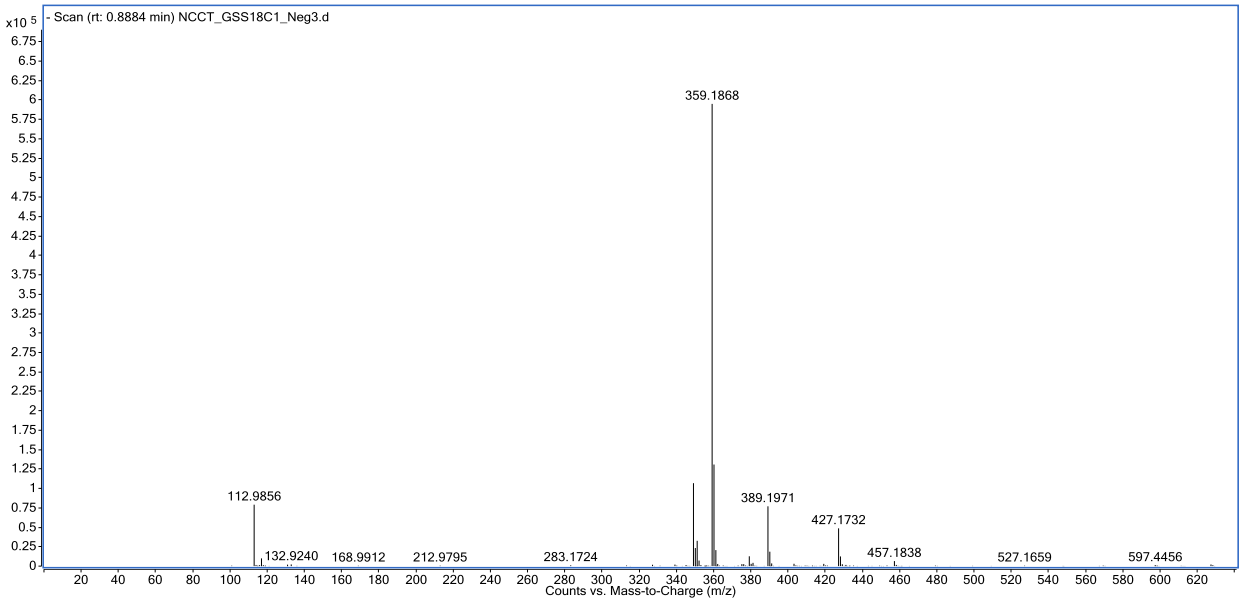

Figure S15. ^{1}H NMR spectrum of compound **8**

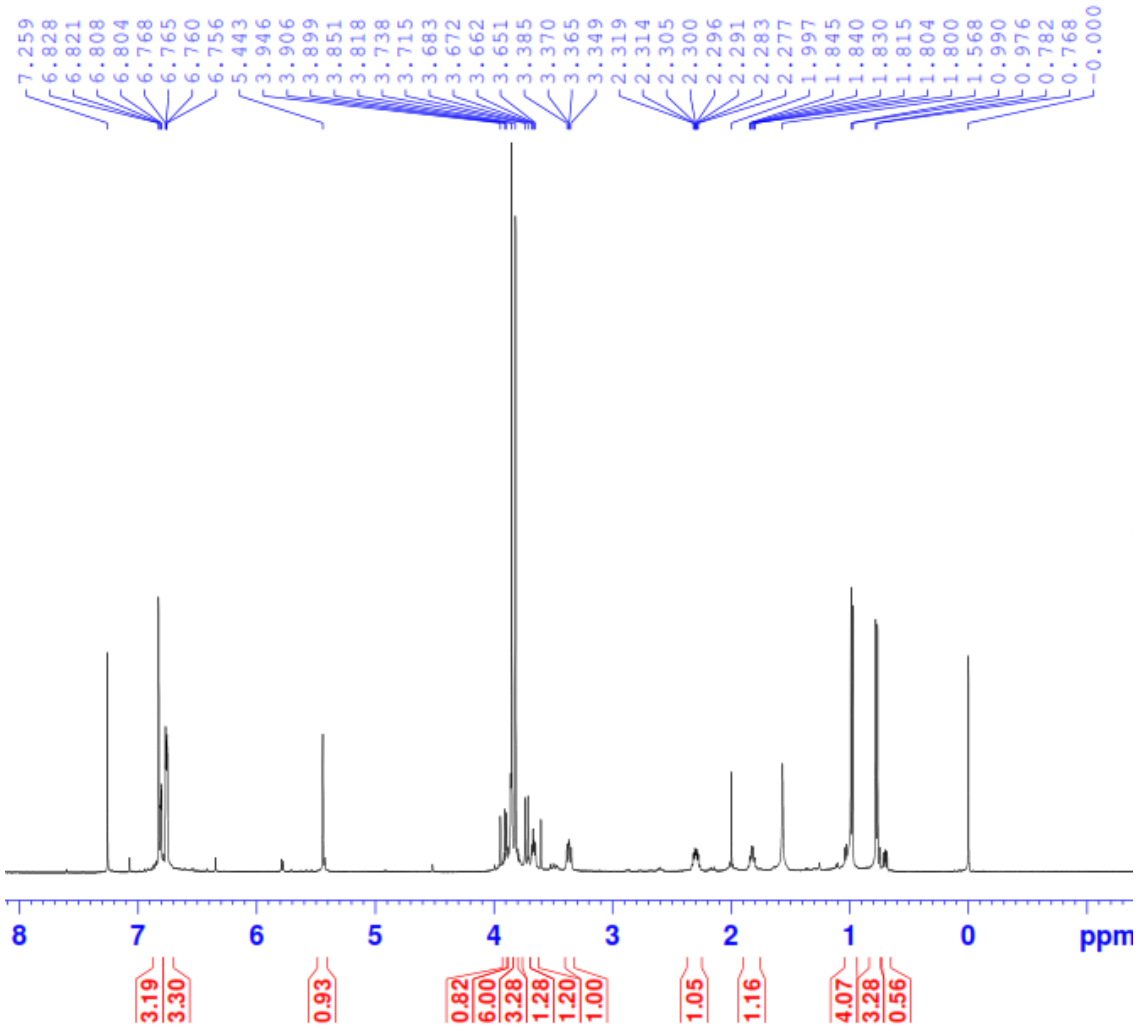

Figure S16. ^{13}C NMR spectrum of compound **8**

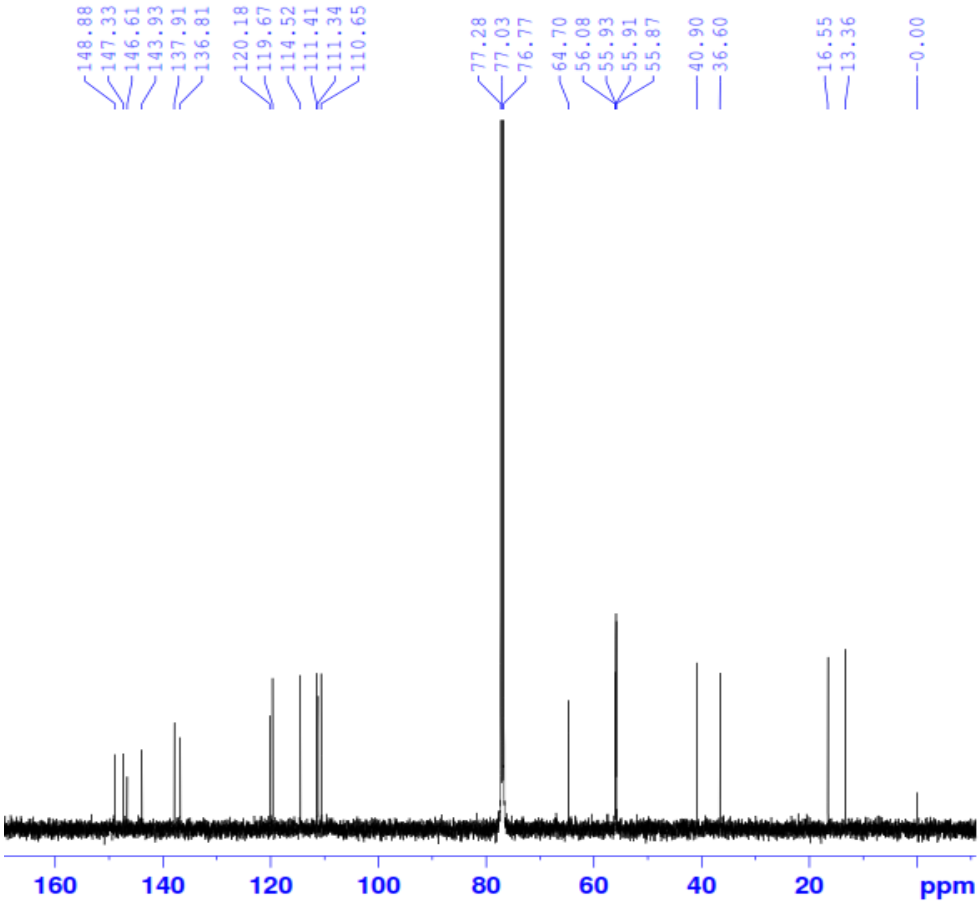

Figure S17. HSQC spectrum of compound **8**

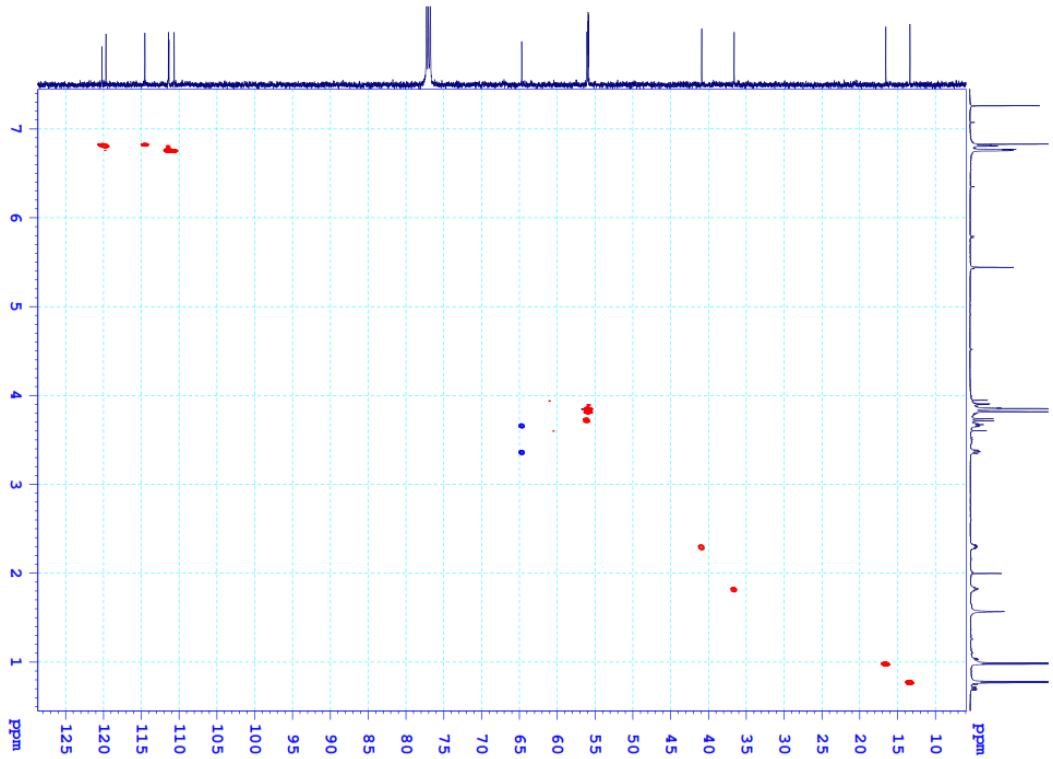

Figure S18. HMBC spectrum of compound **8**

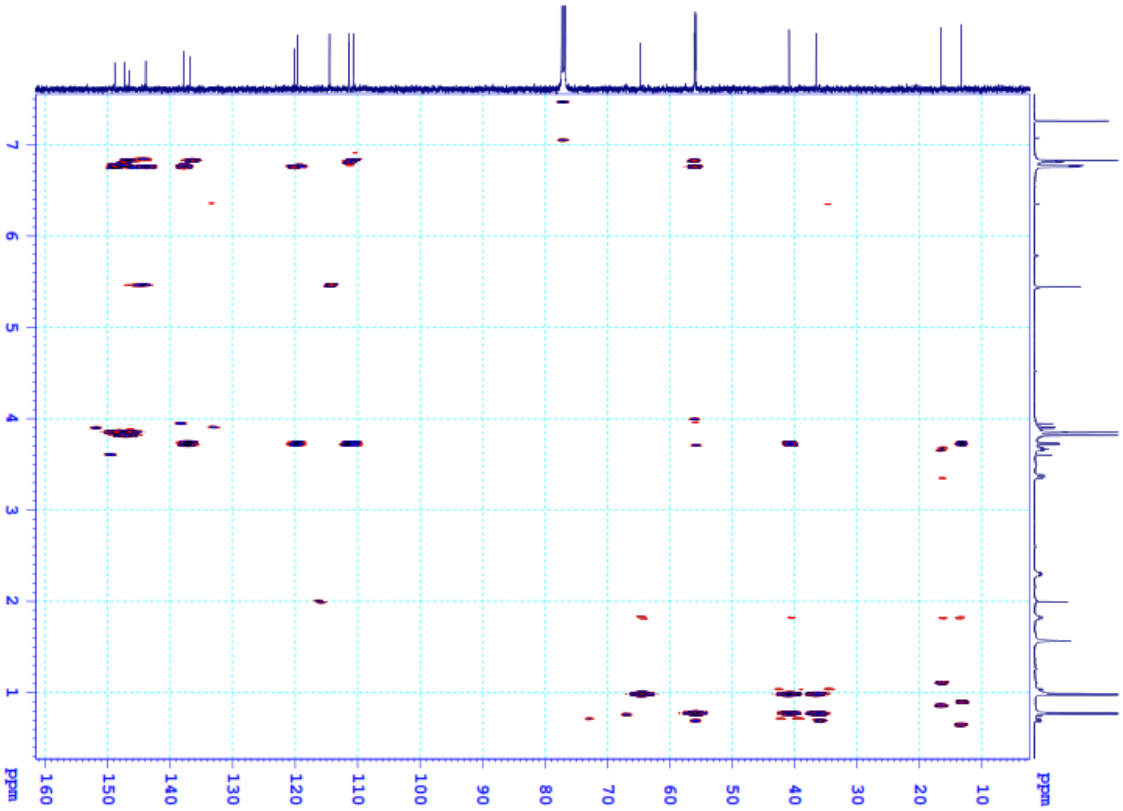

Figure S19. HR-ESI-MS of compound **9**

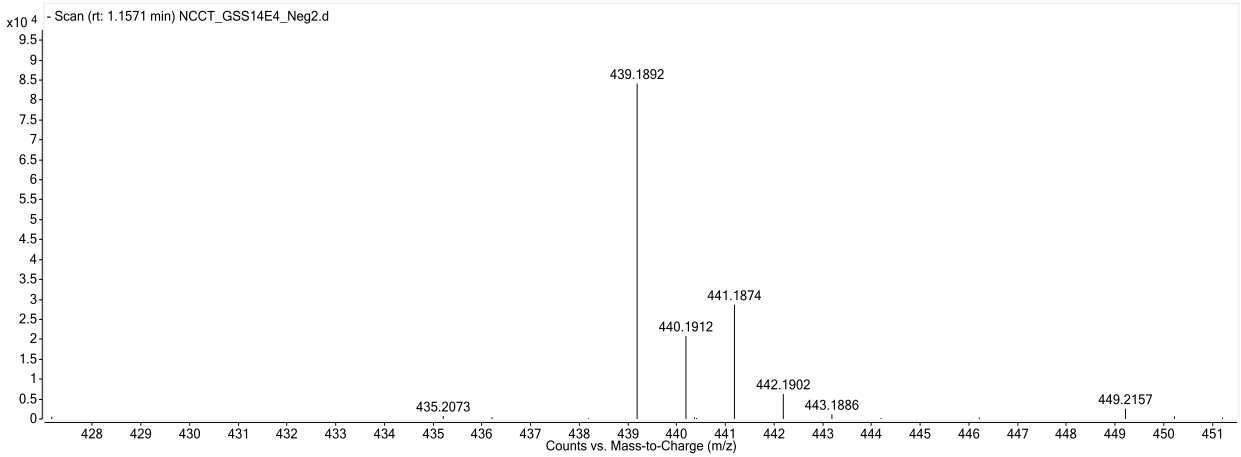

Figure S20. ^{1}H NMR spectrum of compound **9**

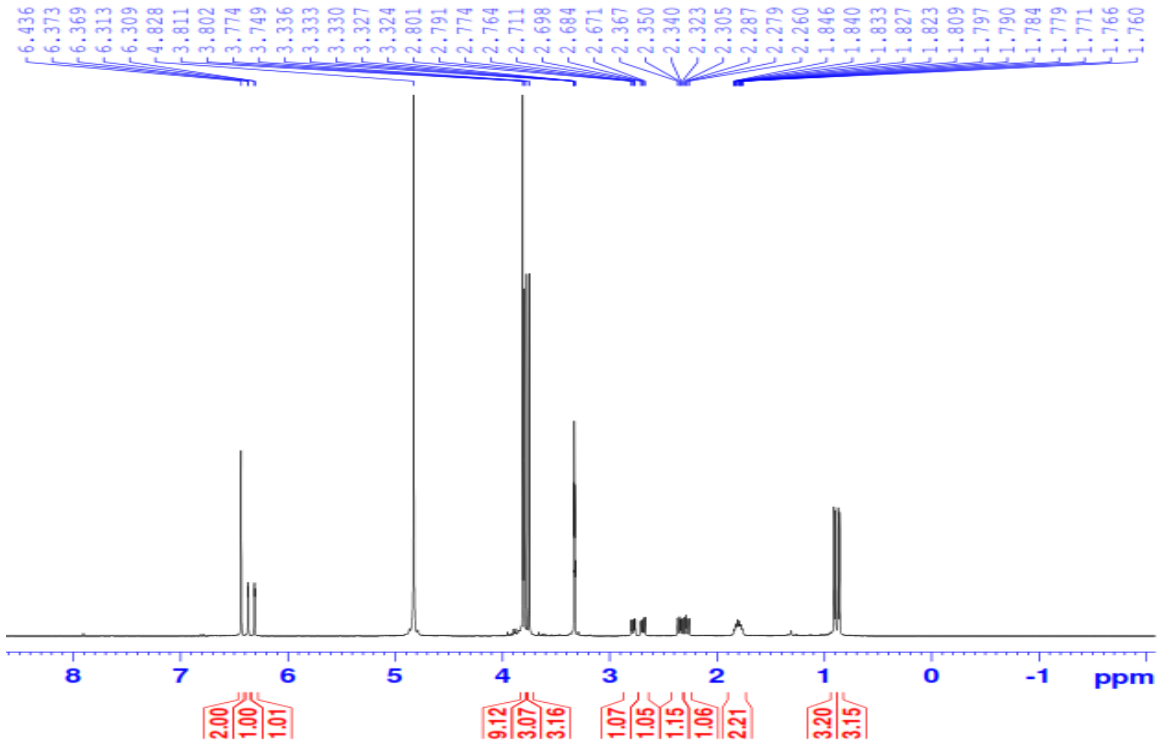

Figure S21. ^{13}C NMR spectrum of compound **9**

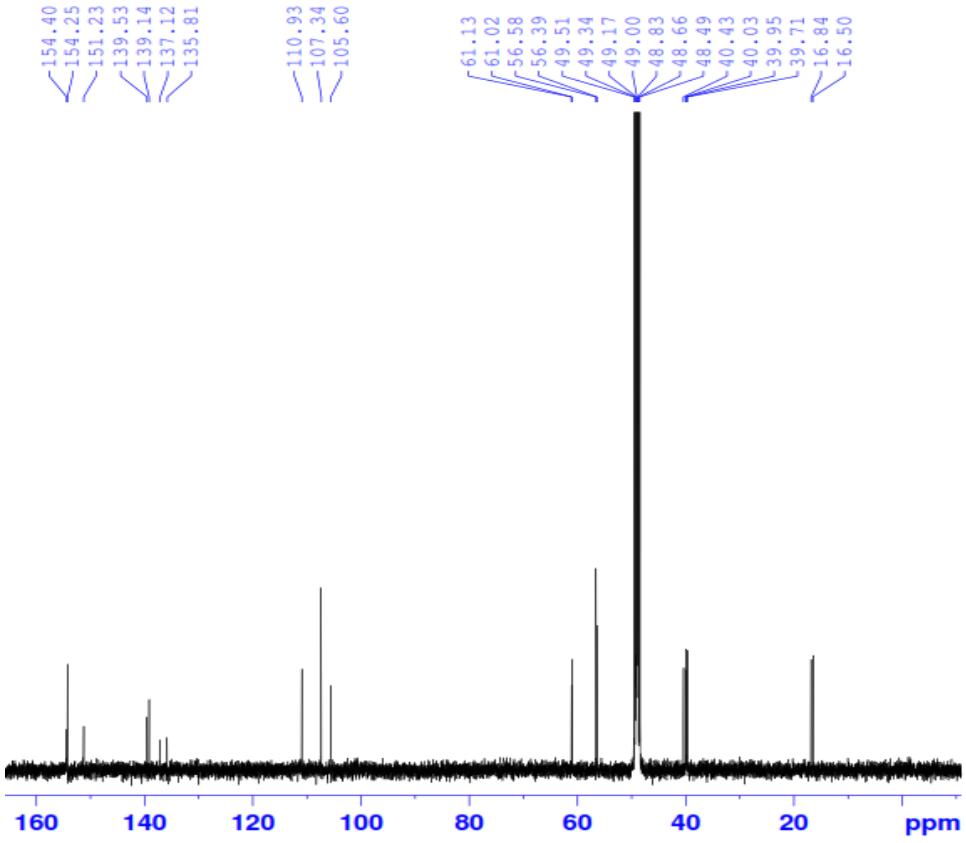

Figure S22. HSQC spectrum of compound **9**

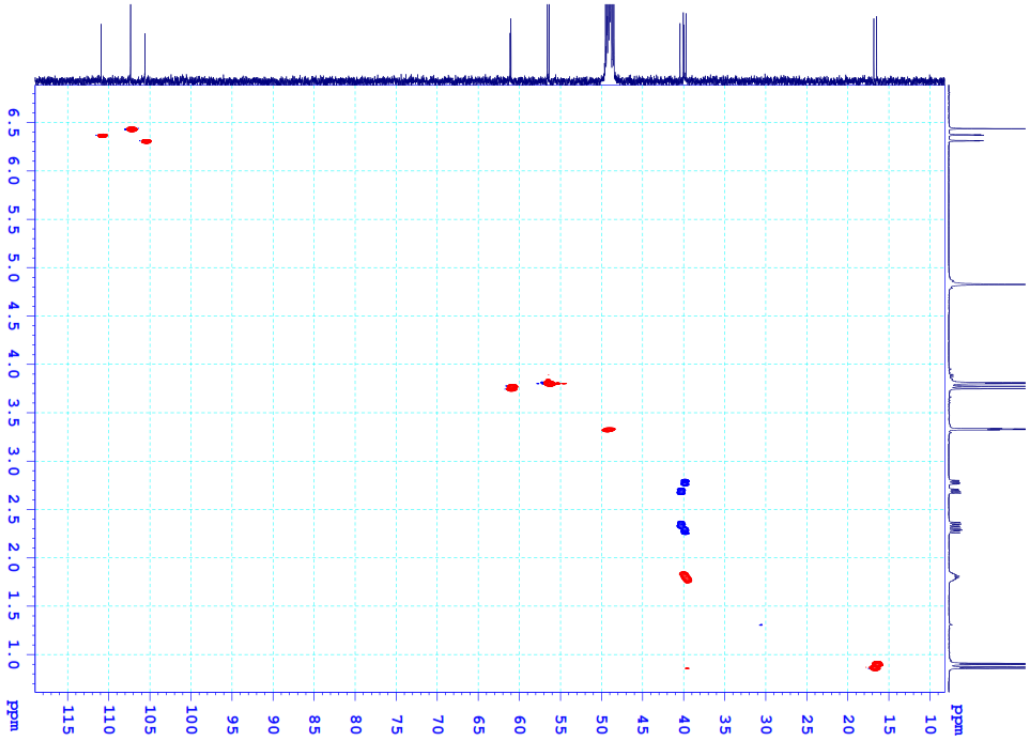

Figure S23. HMBC spectrum of compound **9**

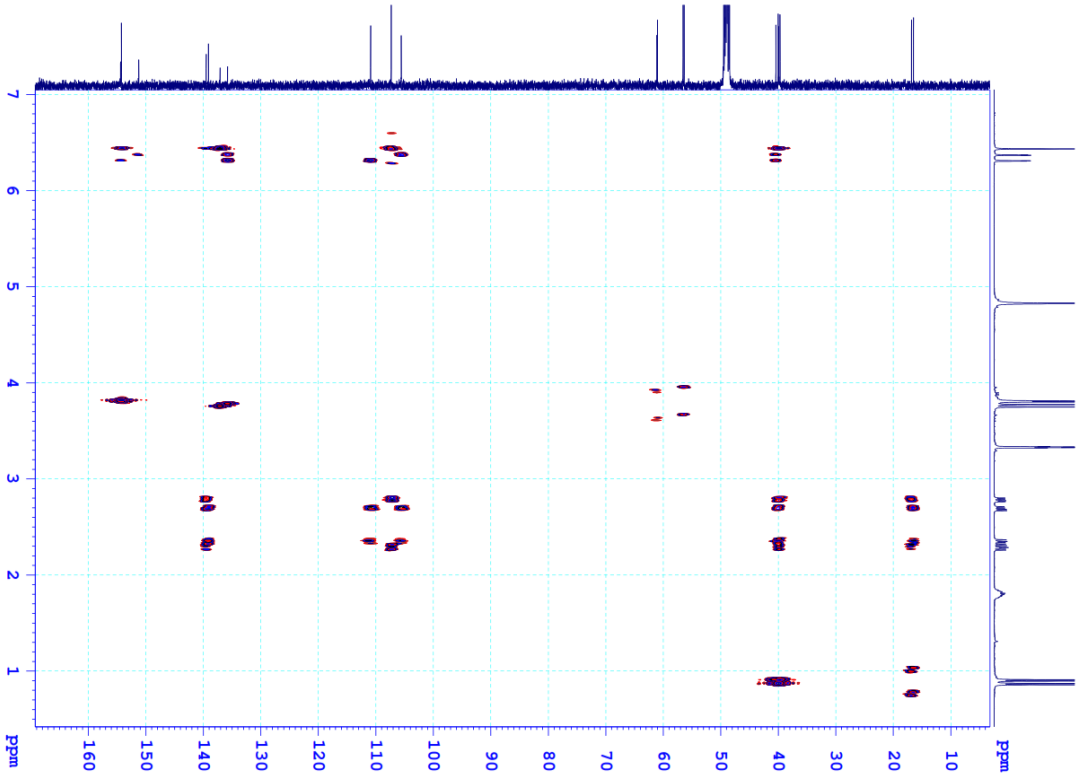

Figure S24. Important HMBC and ^{1}H-^{1}H COSY correlations in compounds **1**, **7**-**9**.

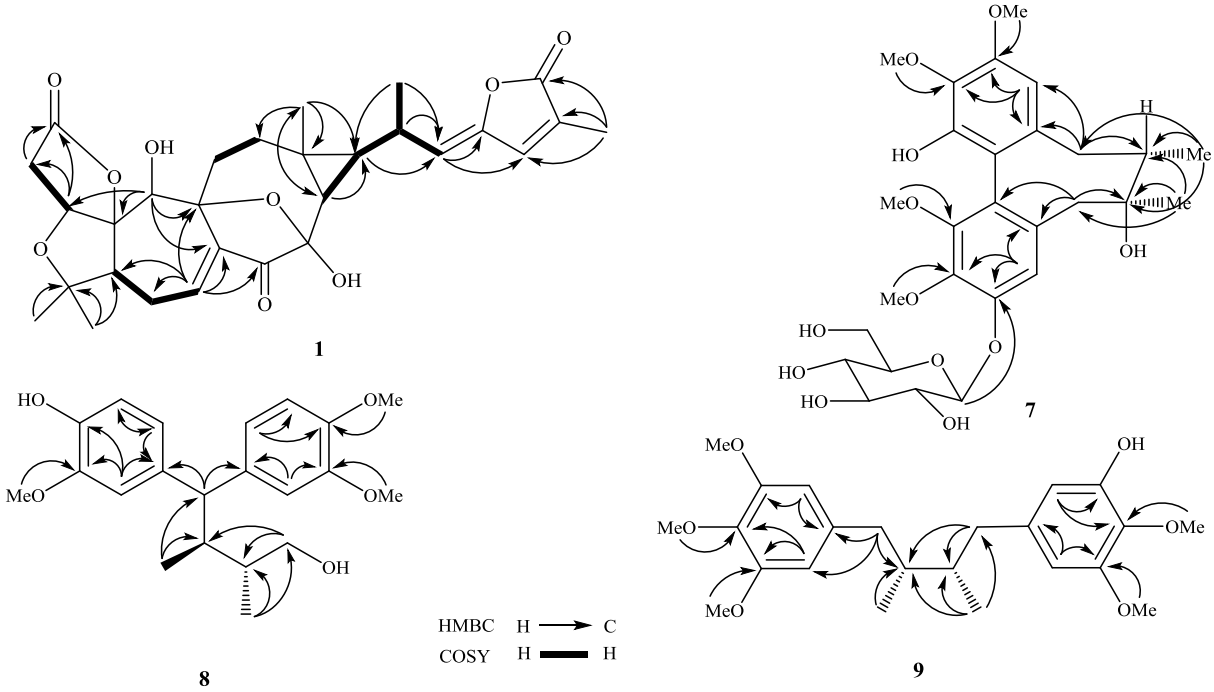

Figure S25. Important NOESY correlations in compounds **1** and **7**.

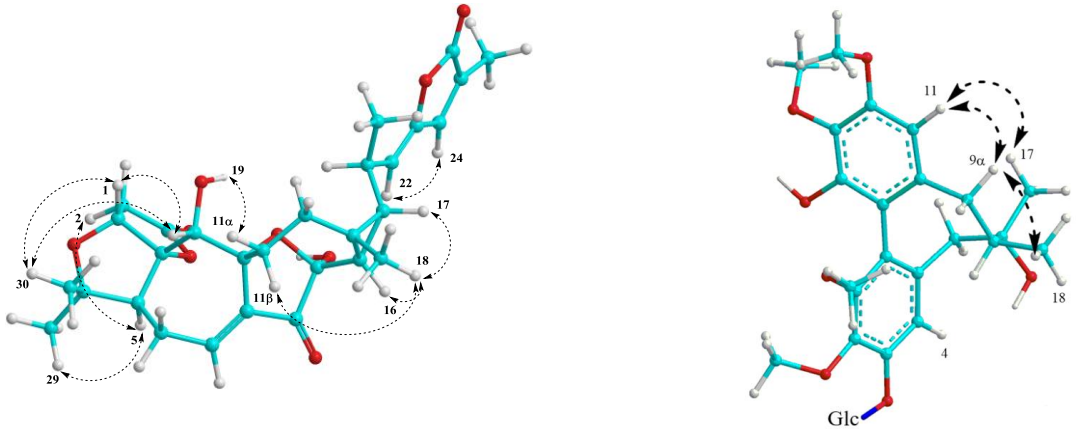

Figure S26. a, b, d, f, g) Experimental and theoretical ECD spectra for possible configurations for compounds **1**, **2**, **6**, **8**, and **9**, respectively. c) Experimental ECD spectra for compounds **3**-**5**. e) Experimental ECD spectrum for compound **7**.

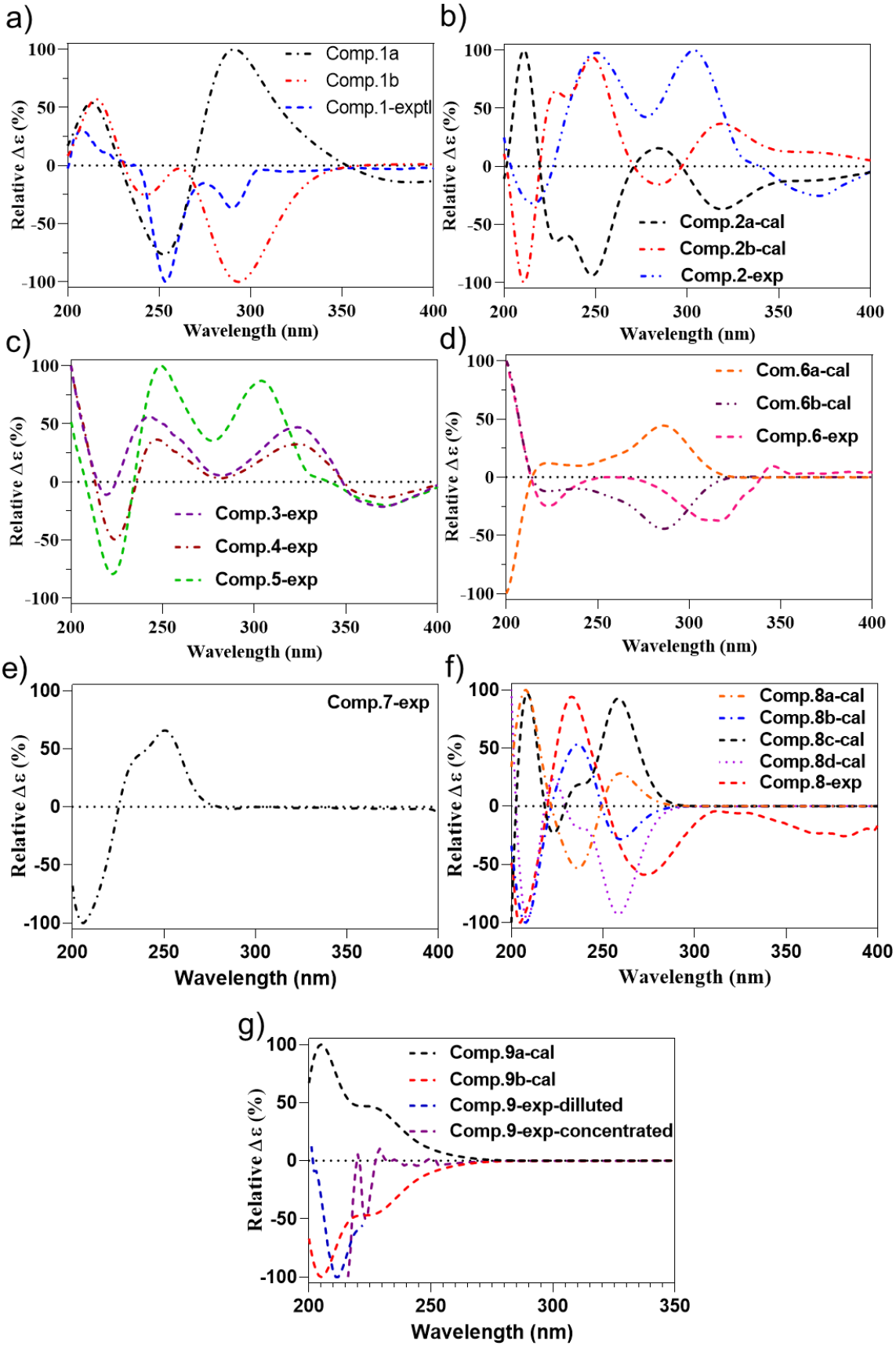

Table S1. ^{1}H and ^{13}C NMR data of compound **1**.

Pos.	1		Pos.	1	
	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., *J* in Hz)		$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., *J* in Hz)
1	78.0	4.77 (d, 6.5)	16	31.3	1.10 (d, 9.0)
2	35.9	2.62 (d, 19.0)	17	36.7	0.82 (dd, 9.0, 11.0)
		2.90 (dd, 19.0, 6.5)			
3	176.4	-	18	30.0	0.94 (s)
4	83.7	-	19	73.1	3.85 (s)
5	57.0	2.15 (m)	20	28.4	3.86 (m)
6	23.2	2.13 (m)	21	21.8	1.30 (d, 6.5)
		2.64 (m)			
7	134.7	7.03 (d, 9.0)	22	122.7	5.19 (d, 11.0)
8	139.0	-	23	145.9	-
9	82.7	-	24	139.0	7.07 (s)
10	97.9	-	25	128.7	-
11	33.4	1.88 (m)	26	172.5	-
		2.56 (m)			
12	27.1	1.61 (m)	27	10.4	2.00 (s)
		2.03 (m)			
13	22.0	-	29	28.7	1.33 (s)
14	200.8	-	30	21.3	1.26 (s)
15	100.1	-			

Measured in [a)]$CDCl_3$, [b)]125 MHz, [c)]500 MHz.

Table S2. ^{13}C NMR data of compounds **2-6**.

Pos.	2	3	4	5	6	Pos.	2	3	4	5	6
	$\delta_C^{a,b}$	$\delta_C^{a,b}$	$\delta_C^{a,b}$	$\delta_C^{a,b}$	$\delta_C^{a,b}$		$\delta_C^{a,b}$	$\delta_C^{a,b}$	$\delta_C^{a,b}$	$\delta_C^{a,b}$	$\delta_C^{a,b}$
1	80.0	79.8	79.8	80.0	81.0	16	197.4	195.9	195.9	197.2	209.8
2	35.2	35.1	35.1	35.3	34.9	17	220.5	219.0	219.0	220.5	219.2
3	174.5	174.2	174.7	174.7	174.1	18	26.3	27.2	27.2	26.3	26.6
4	83.4	83.3	83.2	83.4	84.0	19	42.8	42.8	42.3	42.8	42.4
5	57.2	57.2	57.1	57.3	57.1	20	44.6	74.3	74.4	44.6	74.4
6	23.6	23.6	23.5	23.6	33.6	21	14.8	23.6	23.0	14.9	23.6
7	133.6	134.7	135.5	133.8	68.0	22	40.0	41.7	41.4	39.8	41.5
8	137.9	136.9	136.8	137.9	59.3	23	74.7	72.4	72.1	74.1	72.5
9	81.3	82.8	82.9	81.5	82.0	24	67.6	70.9	74.4	71.3	71.9
10	94.3	93.9	94.1	94.4	94.4	25	42.1	41.6	76.5	77.3	41.6
11	39.1	39.0	38.7	39.0	41.4	26	177.6	177.2	175.4	175.6	177.0
12	30.8	30.6	30.5	30.8	30.2	27	7.6	7.5	17.1	17.6	7.6
13	50.4	49.5	49.3	50.4	49.1	29	27.5	27.5	20.4	27.5	27.7
14	45.0	44.9	44.6	44.8	44.2	30	20.6	20.5	27.4	20.6	20.9
15	98.5	98.0	98.0	98.7	98.0						

Measured in [a)]$CDCl_3$, [b)]125 MHz.

Table S3. ^{1}H and ^{13}C NMR data of compounds **7**, **8** and **9**.

Pos.	7		Pos.	8		9	
	$^{a,c}\delta_C$	$^{a,d}\delta_H$ (*J* in Hz)		$^{a,c}\delta_C$	$^{a,d}\delta_H$ (*J* in Hz)	$^{b,c}\delta_C$	$^{b,d}\delta_H$ (*J* in Hz)
1	152.8	-	1	136.8	-	139.5	-
2	142.1	-	2	110.7	6.75 (d, 1.5)	107.3	6.44 (s)
3	150.7	-	3	146.6	-	154.3	-
4	116.3	6.94 (s)	4	143.9	-	137.1	-
5	135.3	-	5	114.5	6.77 (d, 8.5)	154.2	-
6	42.7	2.59 (d, 13.5) 2.40 (d, 13.5)	6	119.7	6.81 (dd, 8.5, 1.5)	107.3	6.44 (s)
7	73.8	-	7	56.0	3.72 (d, 11.5)	40.0	2.28 (dd, 13.5, 9.5) 2.78 (dd, 13.5, 5.0)
8	42.1	1.80 (m)	8	36.6	1.81 (m)	39.7	1.80 (m)
9	36.4	2.83 (dd, 14.5, 2.5) 2.36 (dd, 14.5, 8.5)	9	13.4	0.77 (d, 7.0)	16.8	0.87 (d, 7.0)
10	135.8	-	1′	137.9	-	139.1	-
11	107.9	6.45 (s)	2′	111.3	6.75 (d, 1.5)	110.9	6.37 (d, 2.0)
12	152.6	-	3′	149.0	-	154.4	-
13	136.1	-	4′	147.3	-	135.8	-
14	148.9	-	5′	111.4	6.76 (d, 8.5)	151.2	-
15	118.6	-	6′	120.2	6.82 (dd, 8.5, 1.5)	105.6	6.31 (d, 2.0)
16	126.5	-	7′	64.7	3.36 (dd, 12.0, 7.0) 3.66 (dd, 12.0, 5.5)	40.4	2.35 (dd, 13.5, 8.5) 2.69 (dd, 13.5, 6.5)
17	16.4	0.82 (d, 7.0)	8′	40.9	2.30 (m)	39.9	1.80 (m)
18	29.5	1.22 (s)	9′	16.5	0.98 (d, 7.0)	16.6	0.90 (d, 7.0)
1-OMe	61.2	3.55 (s)	3-OMe	55.9	3.85 (s)	56.6	3.81 (s)
2-OMe	61.8	3.92 (s)	3′-OMe	55.9	3.85 (s)	56.4	3.80 (s)
12-OMe	56.3	3.88 (s)	4-OMe			61.1	3.75 (s)
13-OMe	61.2	3.83 (s)	4′-OMe	55.8	3.82 (s)	61.0	3.77 (s)
14-OMe	-	-	5-OMe			56.6	3.81 (s)
1′	102.3	5.11 (d, 7.5)					
2′	73.8	3.54 (dd, 7.5, 9.0)					
3′	78.1	3.53 (t, 9.0)					
4′	71.5	3.42 (t, 9.0)					
5′	78.1	3.53 (m)					
6′	62.7	3.93 (dd, 11.5, 2.0) 3.72 (dd, 11.5, 6.0)					

Measured in $^{a)}CDCl_3$, $^{b)}CD_3OD$, $^{c)}$125 MHz, $^{d)}$500 MHz

Table S4. Cytotoxic effect of compounds **1-11** on both PC3 and MCF-7 cancer cells.

	Cell viability (% ± SE) at 30µM	
Compounds	PC3	MCF-7
1	98.50 ± 1.11	92.30 ± 0.83
2	72.40 ± 0.67	69.20 ± 0.62
3	97.00 ± 0.98	98.30 ± 0.99
4	90.00 ± 0.92	92.10 ± 0.83
5	98.00 ± 0.99	78.60 ± 0.71
6	77.70 ± 0.71	81.50 ± 0.73
7	95.00 ± 1.15	80.60 ± 0.72
8	95.50 ± 0.88	79.00 ± 0.71
9	25.60 ± 0.24	14.00 ± 0.13
10	83.90 ± 0.77	82.80 ± 0.74
11	45.80 ± 0.42	35.40 ± 0.32
Capecitabine*	22.00 ± 0.92	17.00 ± 0.90

*Positive control

Table S5. The IC_{50} (µM) of the compounds **9** and **11** on both PC3 and MCF-7 cancer cells.

Compounds	IC_{50} (µM ± SE)	
	PC3	MCF-7
9	19.1 ± 0.29	13.3 ± 0.7
11	3.21 ± 0.68	17.8 ± 1.7
Capecitabine*	11.2 ± 1.44	7.17 ± 3.93

*Positive control