SUPPLEMENTARY MATERIAL

New compounds from the stems of *Fissistigma acuminatissimum* Merr. and their anti-inflammatory activity in vitro

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Abstract

Three new compounds, 4,5,6,7-tetramethoxy-3-benzoylbenzofuran (**1**), 4-hydroxy-3,5,6-trimethoxydihydrochalcone-2-O-β-D-glucopyranoside (**2**) and 2-hydroxy-3,4,5,6-tetramethoxyphenylethyl benzoate (**3**) along with five known flavonoids were isolated from the dichloromethane fraction of the stems of *Fissistigma acuminatissimum* Merr.'s ethanol extracts. The compounds were obtained by various classical column chromatographic methods, and the structure elucidation was completed primarily on the basis of spectroscopic analyses including UV, 1D, 2D-NMR and HR-ESI-MS. All of these compounds were isolated from *F. acuminatissimum* for the first time. All the fractions and compounds were evaluated for their anti-inflammatory activity against lipopolysaccharide (LPS)-stimulated tumor necrosis factor α (TNF-α) production in RAW264.7 cells in vitro. The dichloromethane fraction showed the most potent inhibition(38.2%) at 60 μg/mL, compound **1** (70.2%) and **3** (65.2%) showed significant inhibition at 10 μM.

Keywords: *Fissistigma acuminatissimum* Merr.; 3-benzoylbenzofuran; dihydrochalcone; phenylethyl benzoate; anti-inflammatory activity

List of Supplementary Material

Figure S37 HMBC spectrum for Compound **3** (DMSO-d_6)
Figure S38 HMBC spectrum for Compound **3**-a (DMSO-d_6)
Figure S39 HMBC spectrum for Compound **3**-b (DMSO-d_6)
Figure S40 NOESY spectrum for Compound **3** (DMSO-d_6)
Figure S41 MS spectrum for Compound **3**
Figure S42 UV spectrum for Compound **3**

Table S1 ^{1}H and ^{13}C NMR spectroscopic data for compounds **1** and **3**

Position	**1**			Position	**3**		
	δ_H	δ_C	HMBC		δ_H	δ_C	HMBC
2	8.39(1,s)	149.6	3,3a,9	1		112.7	
3		120.8		2		144.6	
3a		115.5		3		136.9	
4		141.1		4		145.4	
5		144.3		5		138.6	
6		143.6		6		147.9	
7		134.7		7	2.97(2,t, J=7.1)	23.2	1,2,6
7a		143.4		8	4.35(2, t, J=7.1)	63.6	1,7’
1’		137.9		1’		129.9	
2’	7.88(1,m)	129.3	3’,4’,7’	2’	7.93(1,m)	129.1	1’,3’,4’, 7’
3’	7.57(1,m)	128.7	1’,2’	3’	7.49(1,m)	128.6	2’,4’
4’	7.70(1,m)	133.4	2’,3’,5’,6’	4’	7.62(1,m)	133.2	2’,3’,5’,6’
5’	7.57(1,m)	128.7	1’, 6’	5’	7.49(1,m)	128.6	4’,6’
6’	7.88(1,m)	129.3	4’,5’,7’	6’	7.93(1,m)	129.1	1’, 4’,5’,7’
7’		188.8		7’		165.9	
4-OCH_3	3.59(3, s)	60.7	4	3-OCH_3	3.69(3, s)	60.8	3
5-OCH_3	3.79(3, s)	61.2	5	4-OCH_3	3.82(3, s)	60.8	4
6-OCH_3	3.89(3, s)	61.5	6	5-OCH_3	3.71(3, s)	60.8	5
7-OCH_3	4.03(3, s)	61.1	7	6-OCH_3	3.74(3, s)	60.9	6
				2-OH	8.83(1, s)		

Values in ppm (δ)

^{1}H NMR (400 MHz, in DMSO-d_6), ^{13}C NMR (100 MHz, in DMSO-d_6)

Table S2 ^{1}H and ^{13}C NMR spectroscopic data for compounds **2**

Position	**2**		
	δ_H	δ_C	HMBC
1		117.9	
2		143.3	
3		137.2	
4		142.7	
5		137.9	
6		146.9	
7	2.84(2H, m)	19.4	8,1’,2’,6’
8	3.02(2H, m)	39.6	
9		200.2	
1’		136.3	
2’	8.03(1H, m)	128.0	3’,4’,9
3’	7.52(1H, m)	128.5	1’,2’,4’
4’	7.63(1H, m)	132.9	3’,5’
5’	7.52(1H, m)	128.5	1’,4’,6’
6’	8.03(1H, m)	128.0	4’,5’,9
1”	4.93(1H, d, *J*=7.36)	102.8	2
2”	3.18(1H, m)	73.9	
3”	3.20(1H, m)	76.3	
4”	3.06(1H, m)	70.0	
5”	3.07(1H, m)	77.2	
6”	3.62(2H, dd, *J*=11.56,3.92)	60.9	
3-OCH_3	3.70(3H, s)	60.1	3
5-OCH_3	3.72(3H, s)	60.5	5
6-OCH_3	3.73(3H, s)	60.6	6
4-OH	8.88(1H, s)		

Values in ppm (δ)

^{1}H NMR (400 MHz, in DMSO-d_6), ^{13}C NMR (100 MHz, in DMSO-d_6)

Table S3 ^{1}H and ^{13}C NMR spectroscopic data for compounds **4** and **5**

Position	**4**		Position	**5**	
	δ_H	δ_C		δ_H	δ_C
1		116.2	1		106.1
2		144.1	2	7.30(1H, m)	128.3
3		136.4	3	7.23(1H, m)	128.3
4		138.7	4	7.18(1H, m)	125.9
5		145.0	5	7.23(1H, m)	128.3
6		147.3	6	7.30(1H, m)	128.3
7	2.82(2H, t, *J*=8.0 Hz)	19.1	7	2.89(2H, t, *J*=7.6 Hz)	30.0
8	3.12(2H, t, *J*=8.0 Hz)	38.4	8	3.25(2H, t, *J*=7.6 Hz)	45.4
9		199.7	9		204.6
1’		137.1	1’		141.3
2’	7.98(1H, m)	127.9	2’		156.6
3’	7.52(1H, m)	128.7	3’		129.7
4’	7.63(1H, m)	133.1	4’		158.1
5’	7.52(1H, m)	128.7	5’	6.27(1H, s)	88.0
6’	7.98(1H, m)	127.9	6’		158.1
3-OCH_3	3.70(3H, s)	60.8	3’-OCH_3	3.61(3H, s)	59.9
4-OCH_3	3.73(3H, s)	60.9	4’-OCH_3	3.88(3H, s)	56.2
5-OCH_3	3.81(3H, s)	60.8	6’-OCH_3	3.90(3H, s)	56.0
6-OCH_3	3.73(3H, s)	60.8	2’-OH	13.14(1H, s)	
2-OH	8.73(1H, s)				

Values in ppm (δ)

^{1}H NMR (400 MHz, in DMSO-d_6), ^{13}C NMR (100 MHz, in DMSO-d_6)

Table S4 ^{1}H and ^{13}C NMR spectroscopic data for compounds **6**, **7** and **8**

Position	**6**		Position	**7**		Position	**8**	
	δ_H	δ_C		δ_H	δ_C		δ_H	δ_C
2		160.0	2		163.6	2		159.4
3	6.87(1H, s)	107.3	3	7.09(1H, s)	104.9	3	6.79(1H, s)	108.0
4		175.7	4		182.8	4		175.9
5		147.0	5		145.4	5		151.1
6		143.4	6		135.9	6	6.70(1H, s)	93.7
7		150.9	7		152.7	7		156.5
8		137.5	8		132.7	8		129.9
9		147.3	9		148.5	9		155.7
10		114.1	10		106.4	10		107.8
1’		131.4	1’		130.7	1’		131.0
2’	8.05(1H, m)	125.7	2’	8.09(1H, m)	126.4	2’	8.03(1H, m)	125.8
3’	7.60(1H, m)	129.0	3’	7.62(1H, m)	128.7	3’	7.58(1H, m)	129.2
4’	7.60 (1H, m)	130.7	4’	7.62(1H, m)	132.3	4’	7.58(1H, m)	131.5
5’	7.60 (1H, m)	129.0	5’	7.62(1H, m)	128.7	5’	7.58(1H, m)	129.2
6’	8.05 (1H, m)	125.7	6’	8.09(1H, m)	126.4	6’	8.03(1H, m)	125.8
5-OCH_3	3.70(3H, s)	62.8	6-OCH_3	3.83(3H, s)	60.6	5-OCH_3	3.99(3H, s)	56.3
6-OCH_3	3.73(3H, s)	61.7	7-OCH_3	4.94(3H, s)	62.0	7-OCH_3	3.89(3H, s)	56.4
7-OCH_3	3.81(3H, s)	61.3	8-OCH_3	4.04(3H, s)	61.5	8-OCH_3	3.85(3H, s)	61.0
8-OCH_3	3.73(3H, s)	61.2	5-OH	12.62(1H, s)				

Values in ppm (δ)

^{1}H NMR (400 MHz, in DMSO-d_6), ^{13}C NMR (100 MHz, in DMSO-d_6)

Table S5 The cell survival rate under the influence of tested fractions (n=3)

Concentration (μg/mL)	total extract (100%)	petroleum ether fraction (100%)	dichloromethane fraction (100%)	ethyl acetate fraction (100%)	N-butanol fraction (100%)
0	100±1.1	100±1.1	100±1.1	100±1.1	100±1.1
40	105.5±2.1	96.5±8.5	102.2±1.2	100.1±1.5	107.7±6.1
60	102.0±1.5	92.9±3.7	99.8±2.1	98.1±4.2	105.5±4.2
80	68.0±8.0	87.1±6.8	96.8±4.1	100.3±2.4	79.4±17.7
100	59.7±13.4	76.8±9.5	95.5±6.6	98.1±3.4	69.7±21.3
160	51.3±11.3	63.8±7.8	61.9±2.7	91.0±4.5	50.5±13.7

Table S6 Inhibitory activity against TNF-α of tested fractions and compounds (n=3)

Samples	Inhibition rate (%)
total extract (40 μg/mL)	16.2
total extract (60 μg/mL)	27.7
petroleum ether fraction (40 μg/mL)	2.5
petroleum ether fraction (60 μg/mL)	2.4
dichloromethane fraction (40 μg/mL)	33.5
dichloromethane fraction (60 μg/mL)	38.2
ethyl acetate fraction (80 μg/mL)	16.1
ethyl acetate fraction (100 μg/mL)	25.4
N-butanol fraction (40 μg/mL)	14.1
N-butanol fraction (60 μg/mL)	23.2
Compound 1(10 μM)	70.2
Compound 2(10 μM)	20.7
Compound 3(10 μM)	65.2
Compound 4(10 μM)	42.2
Compound 5(10 μM)	21.3
Compound 6(10 μM)	19.0
Compound 7(10 μM)	17.9
Compound 8(10 μM)	18.0

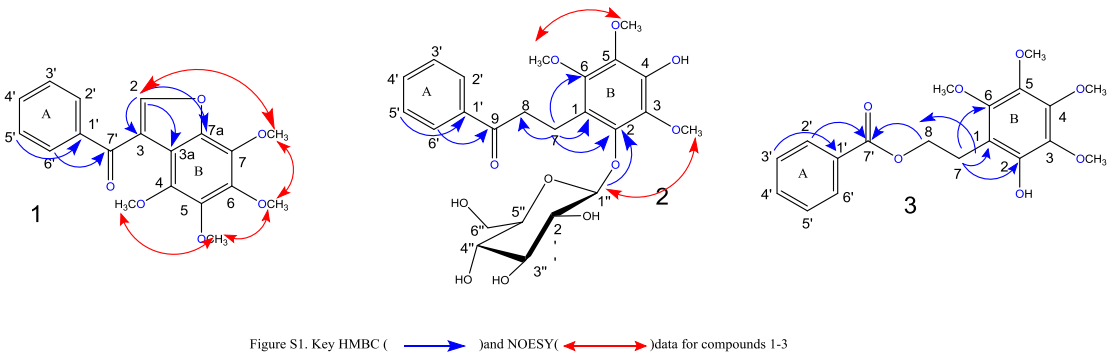

Figure S1 Key HMBC and NOESY correlations of compounds **1-3**

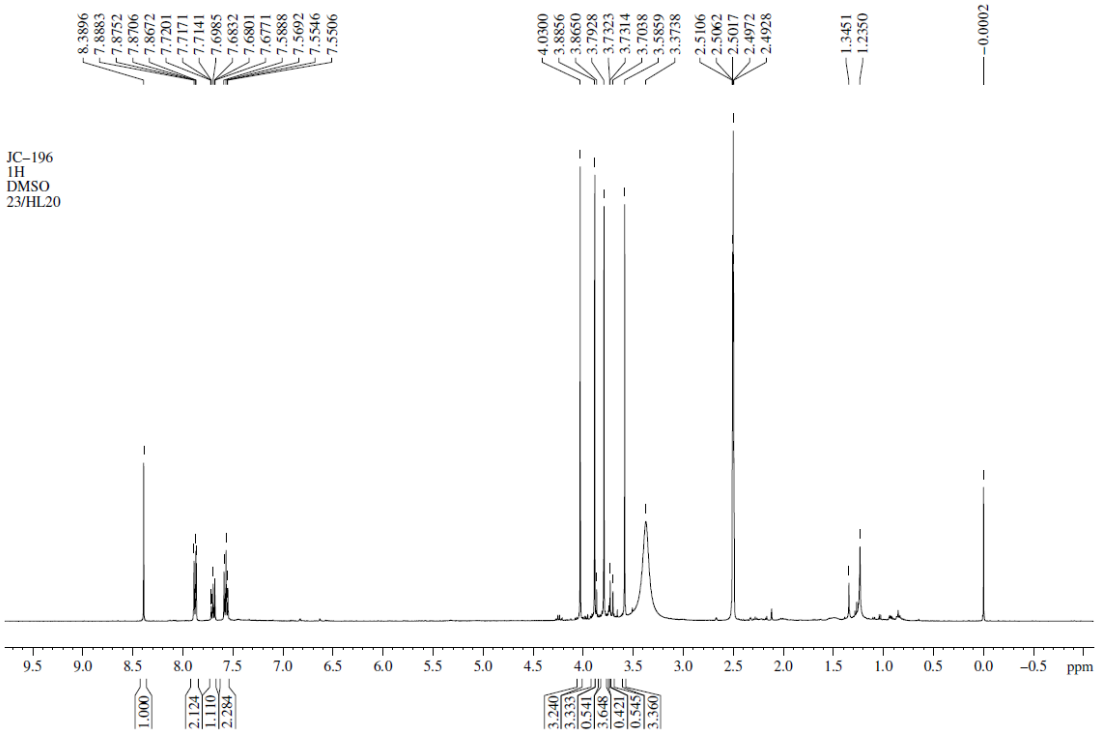

Figure S2 ^{1}H-NMR spectrum for Compound **1**

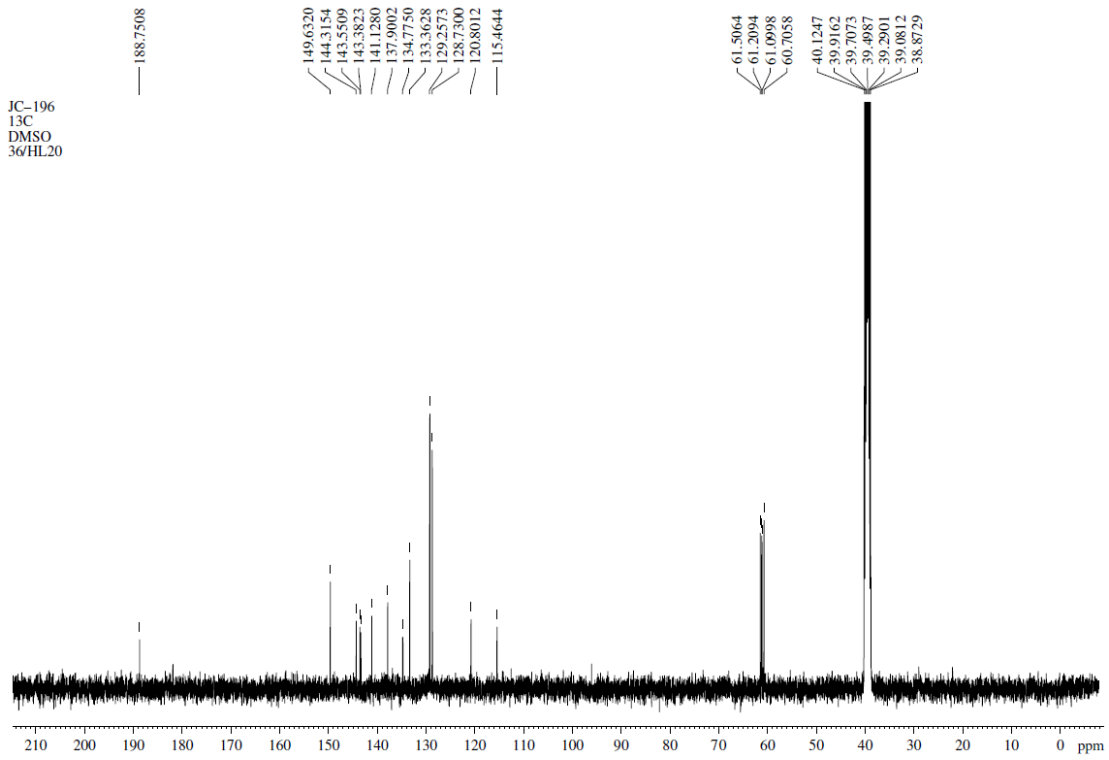

Figure S3 ^{13}C-NMR spectrum for Compound **1**

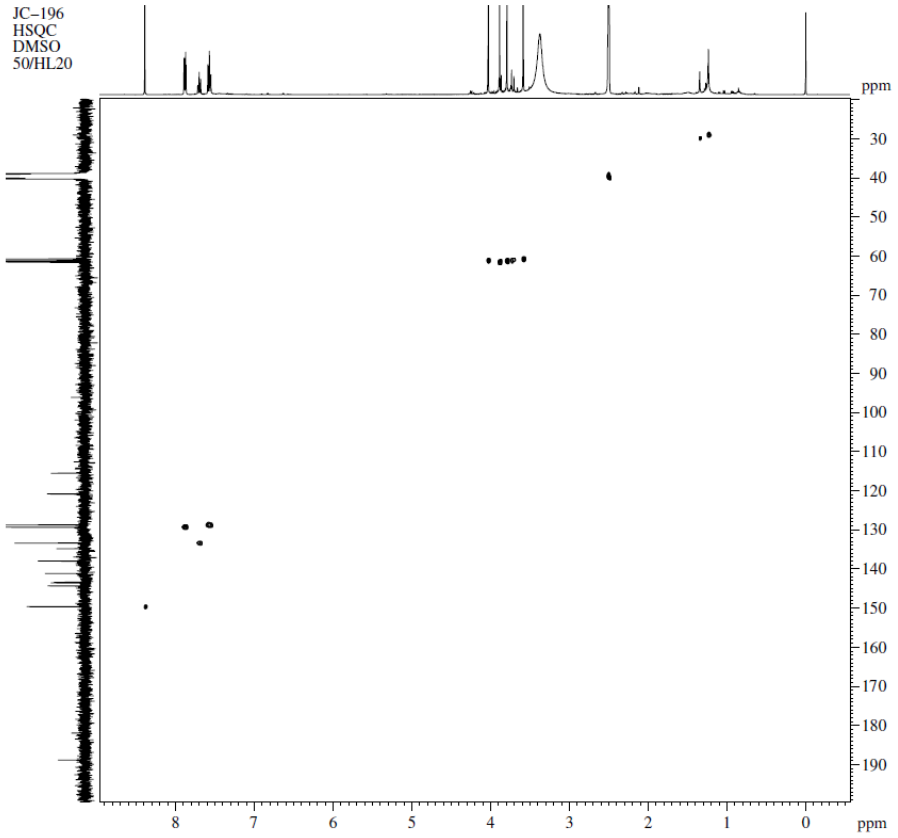

Figure S4 HSQC spectrum for Compound **1**

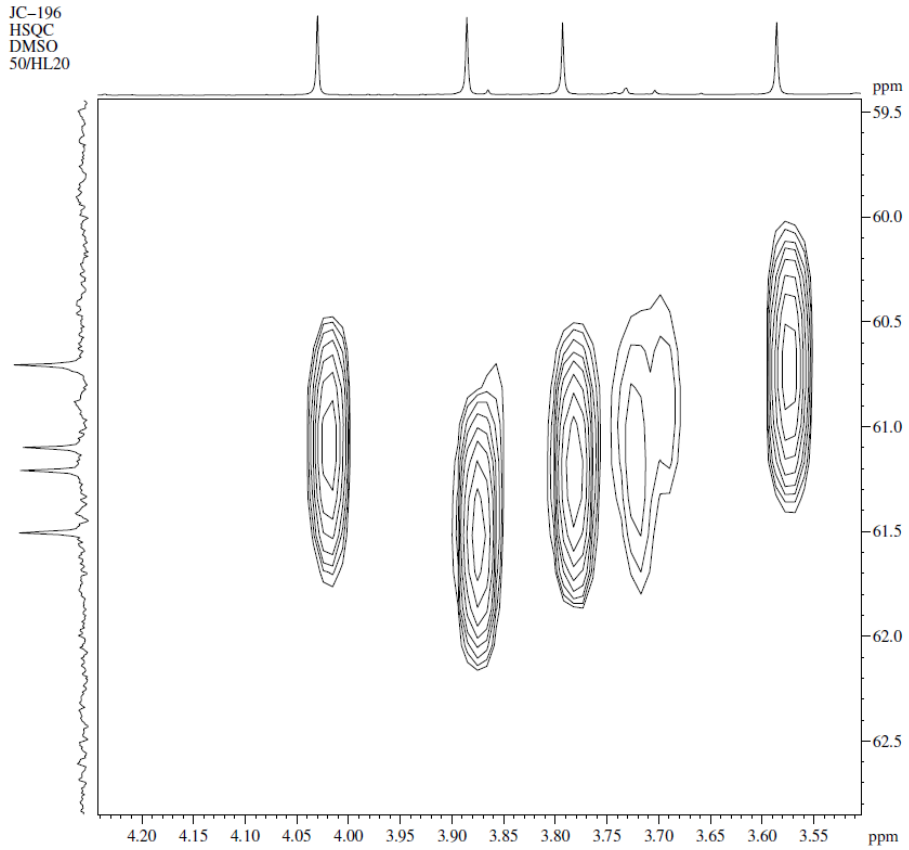

Figure S5 HSQC spectrum for Compound **1**-a

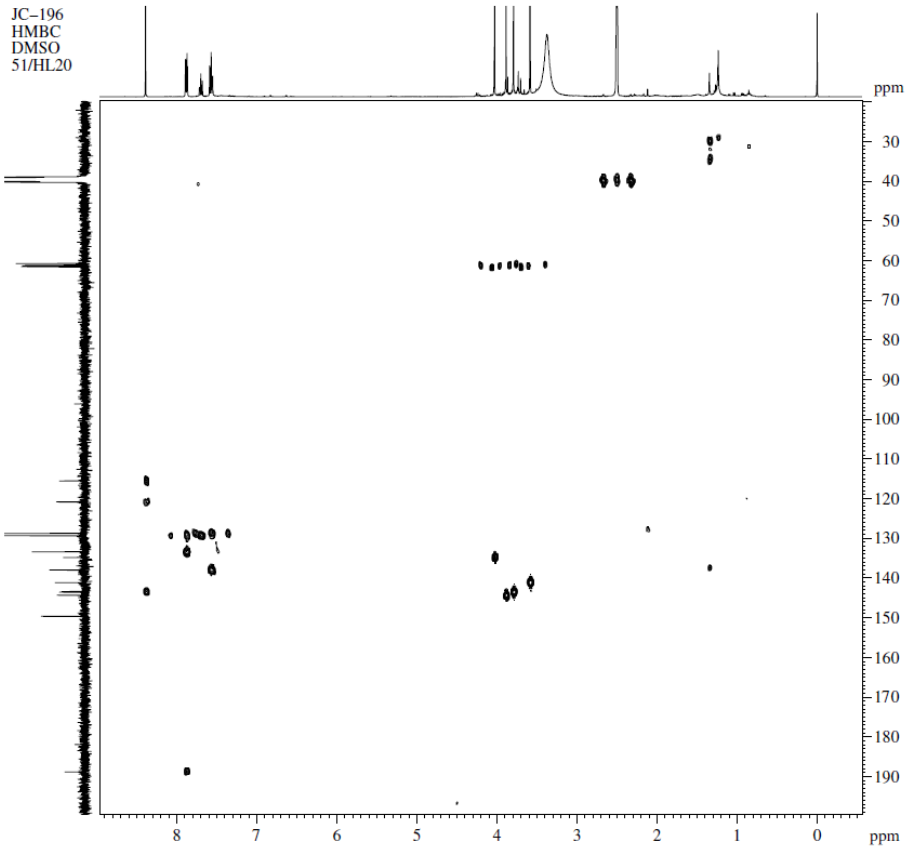

Figure S6 HMBC spectrum for Compound **1**

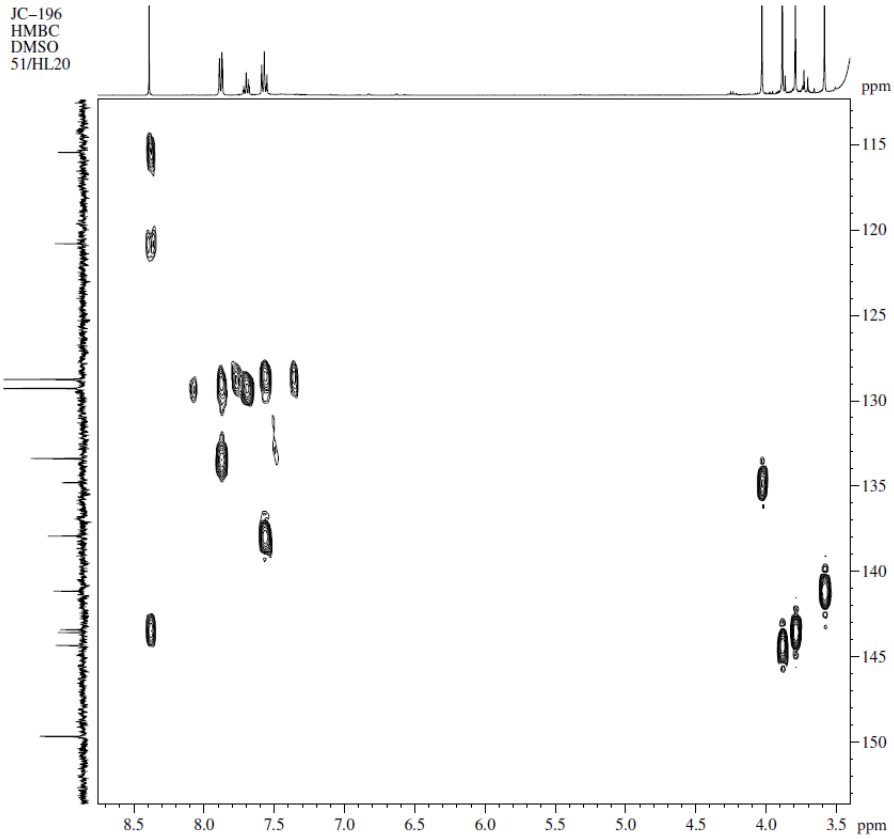

Figure S7 HMBC spectrum for Compound **1**-a

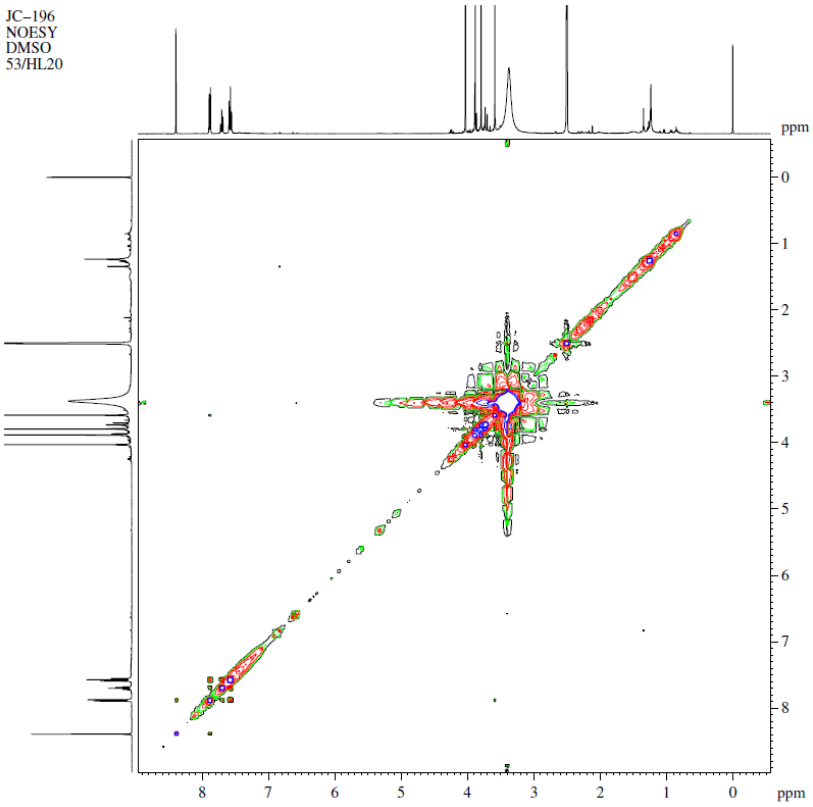

Figure S8 NOESY spectrum for Compound **1**

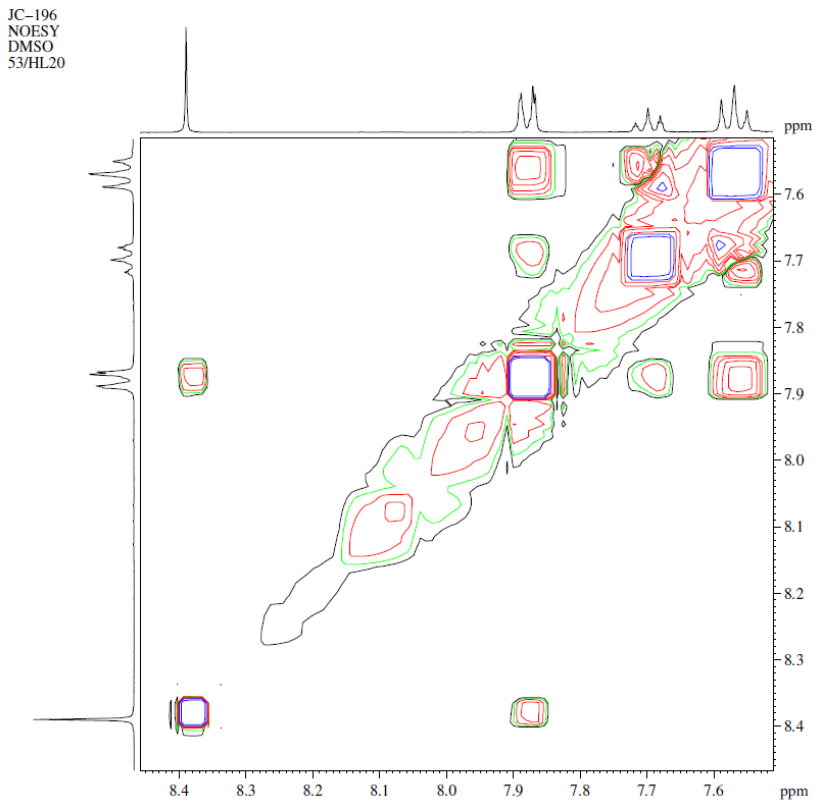

Figure S9 NOESY spectrum for Compound **1**-a

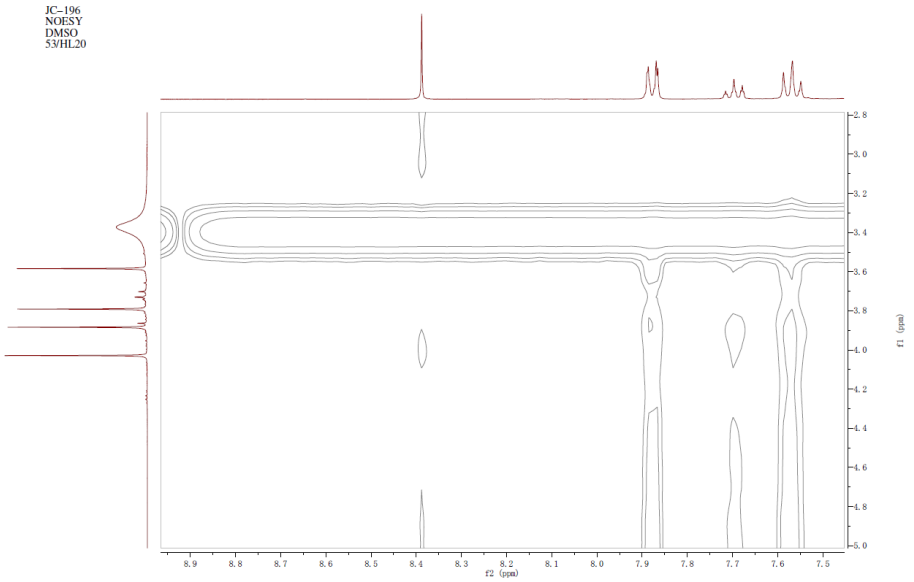

Figure S10 NOESY spectrum for Compound **1**-b

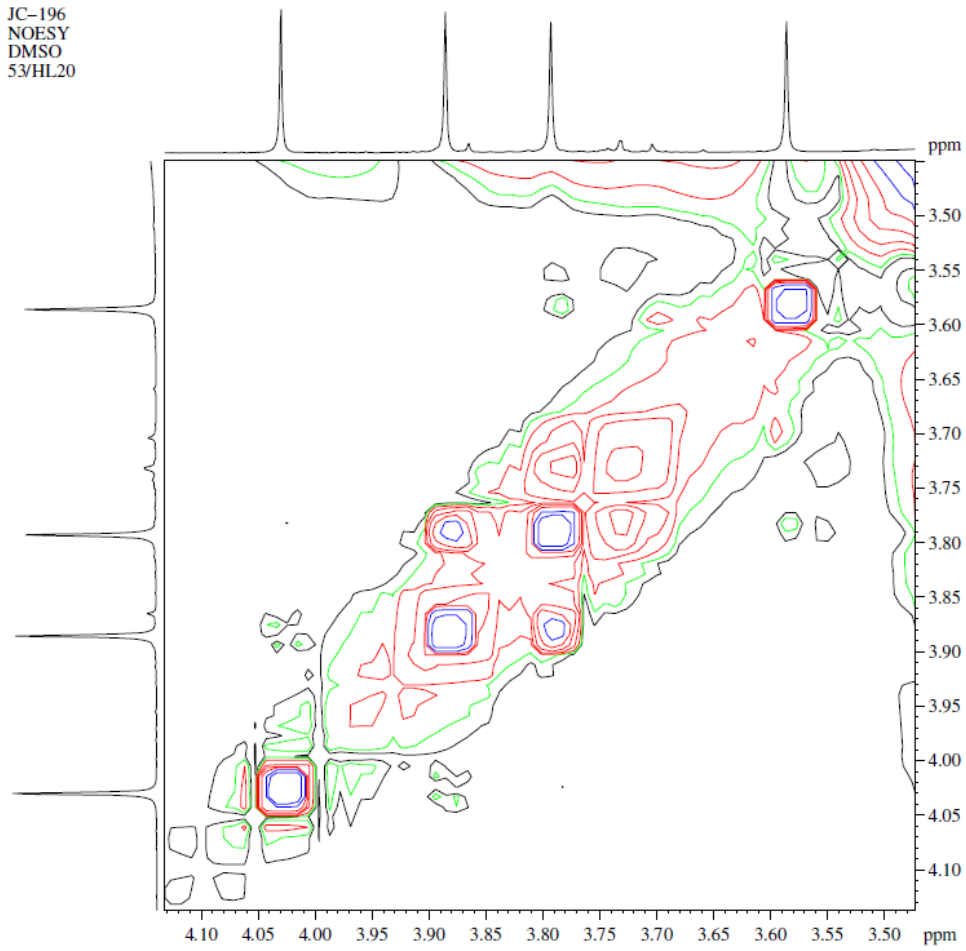

Figure S11 NOESY spectrum for Compound **1**-c

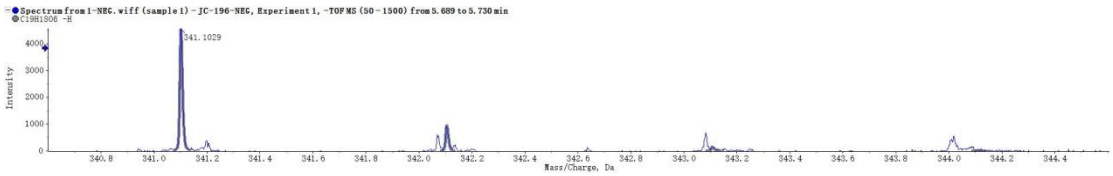

Figure S12 MS spectrum for Compound **1**

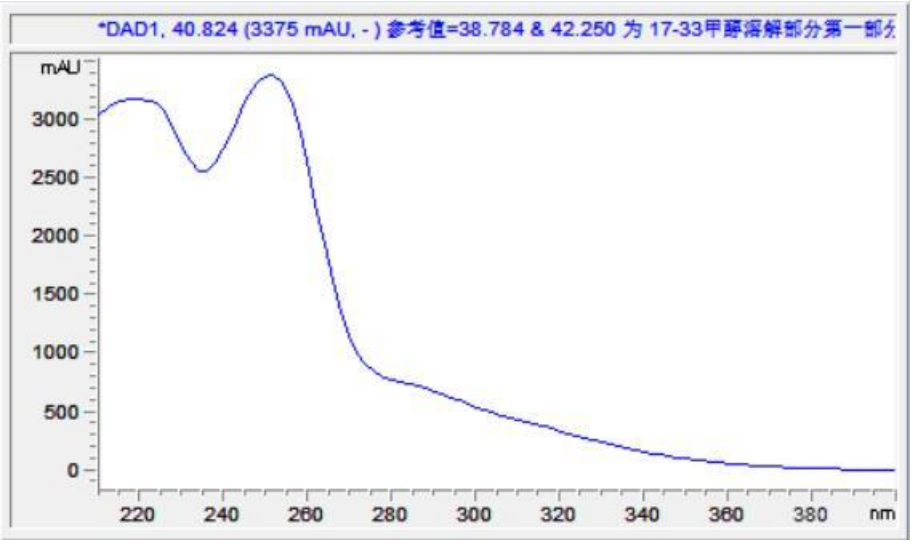

Figure S13 UV spectrum for Compound **1**

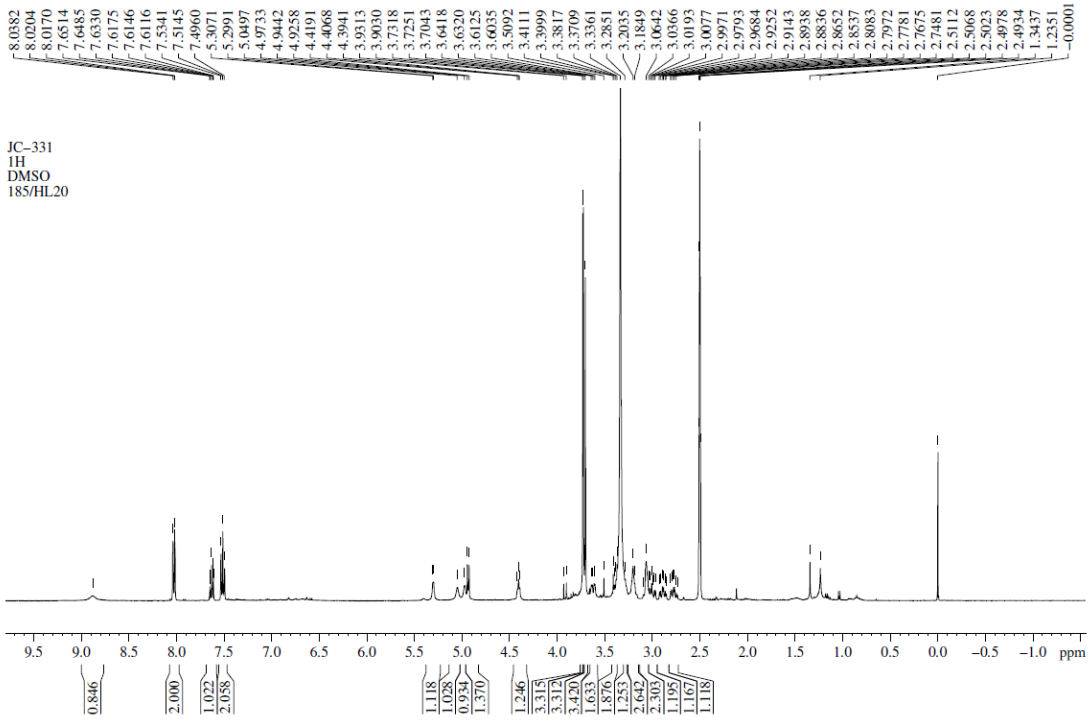

Figure S14 ^{1}H-NMR spectrum for Compound **2**

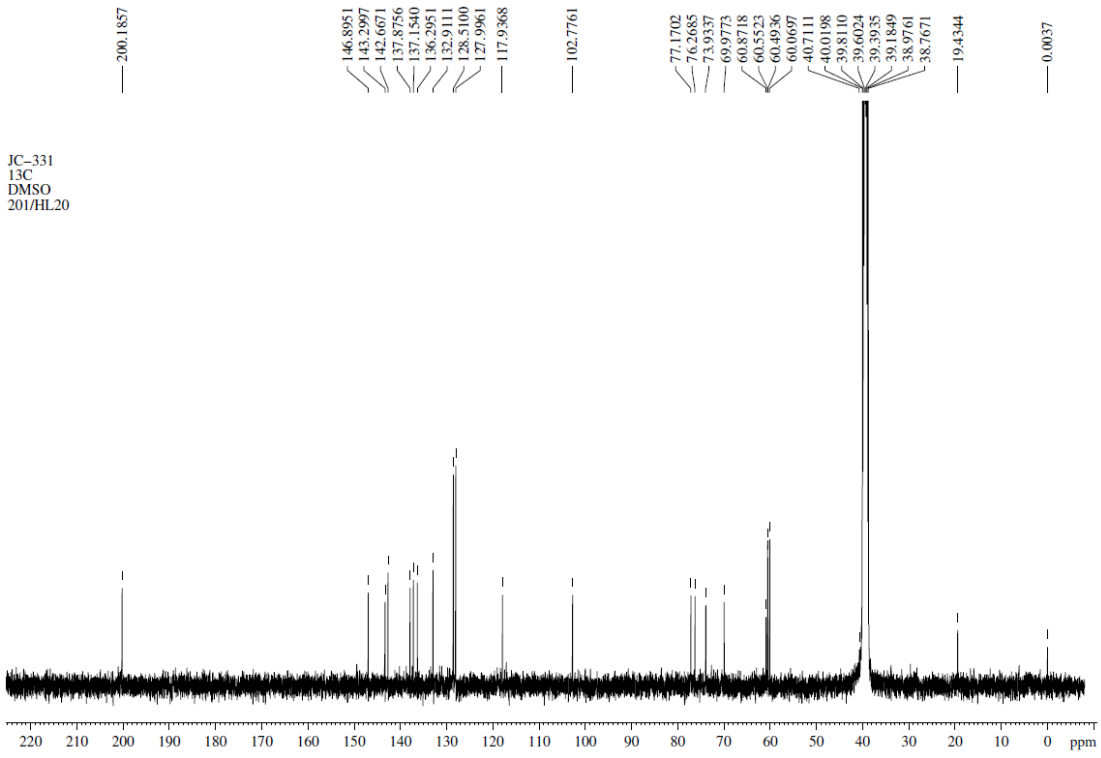

Figure S15 ^{13}C-NMR spectrum for Compound **2**

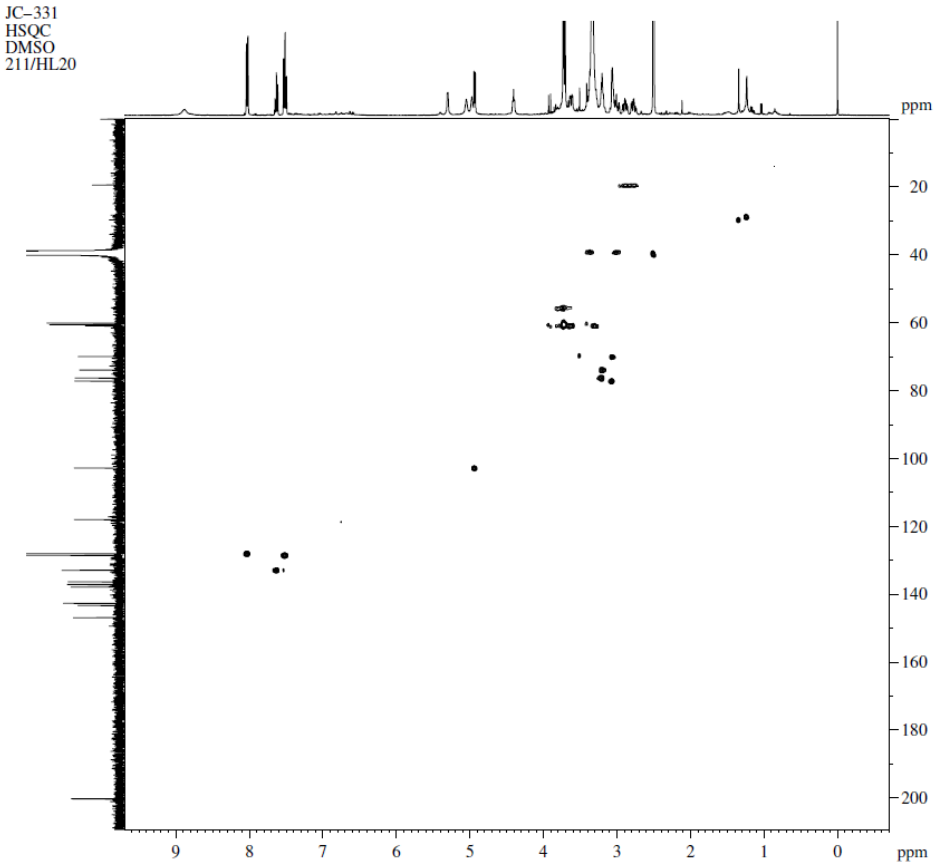

Figure S16 HSQC spectrum for Compound **2**

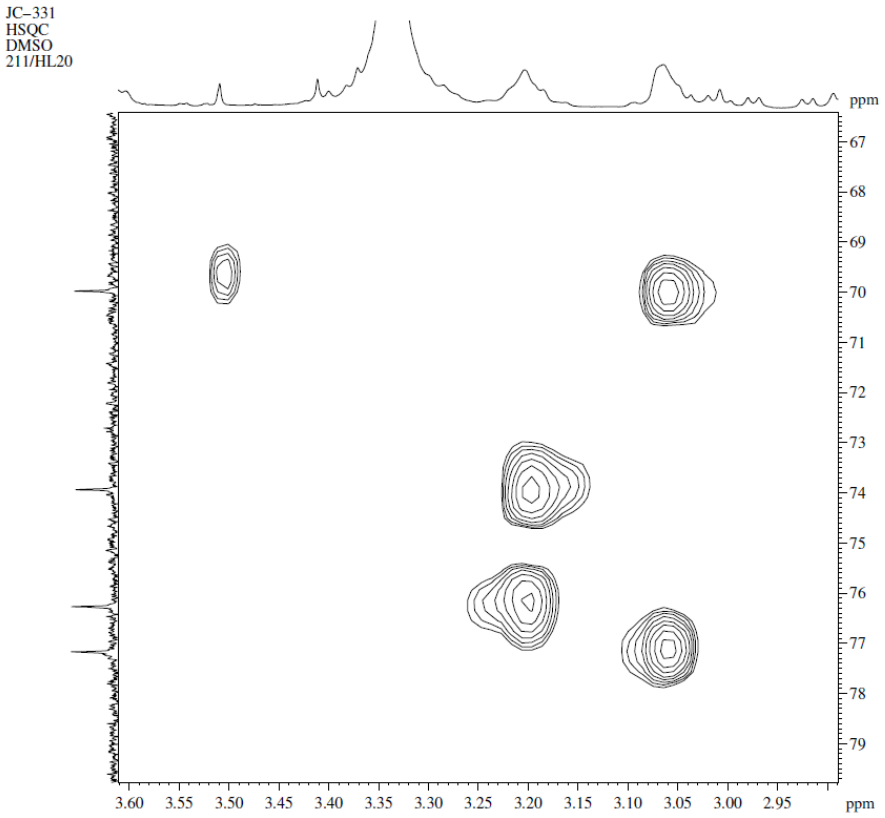

Figure S17 HSQC spectrum for Compound **2**-a

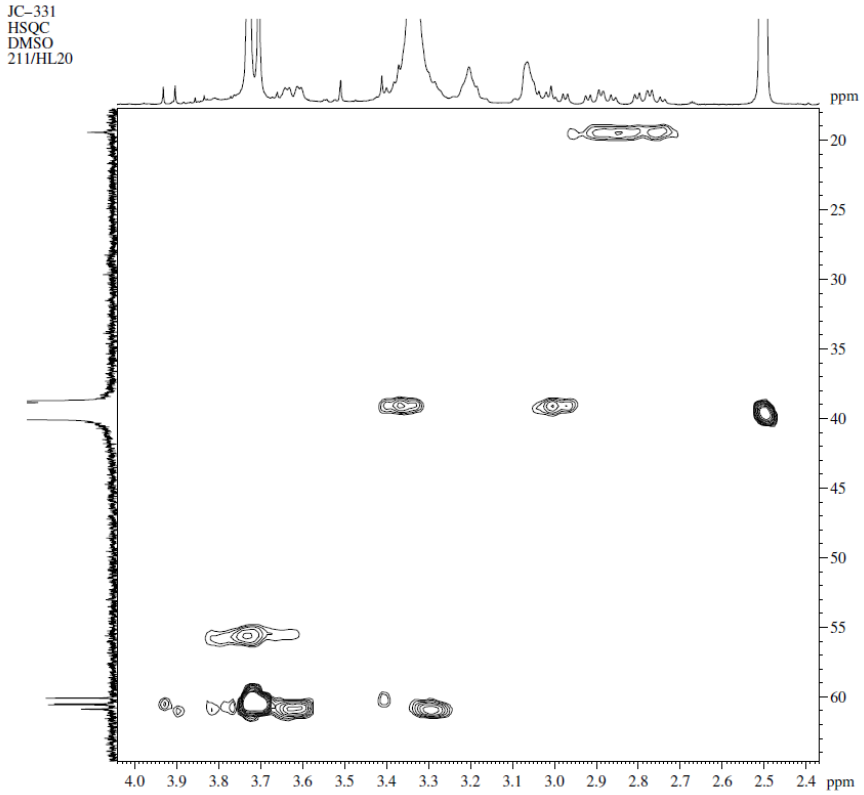

Figure S18 HSQC spectrum for Compound **2**-b

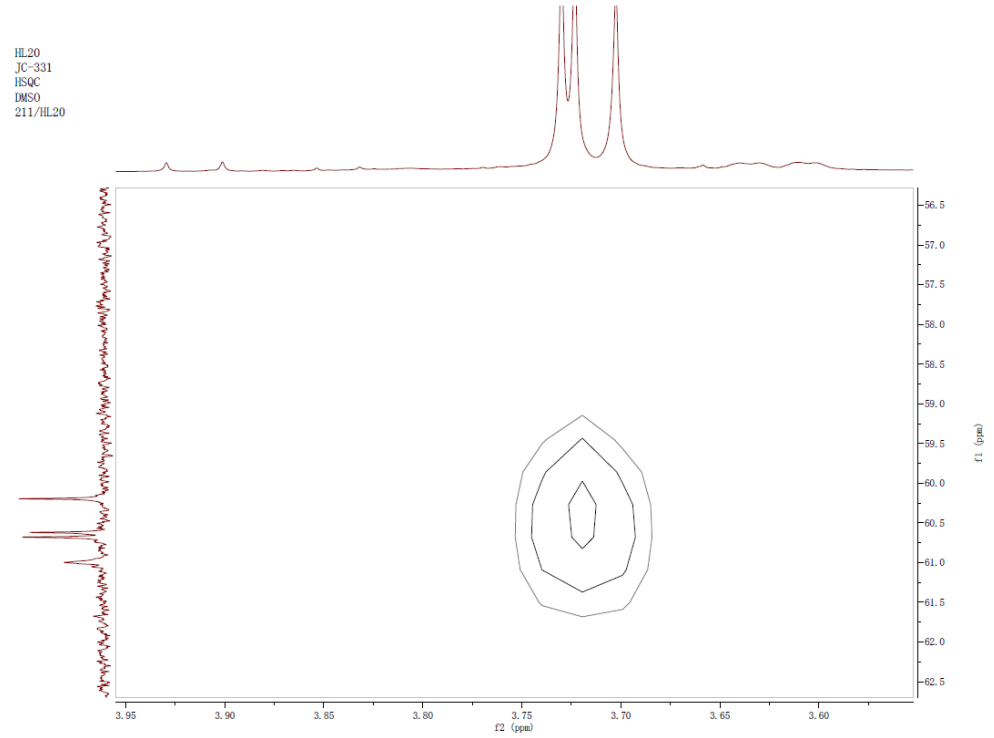

Figure S19 HSQC spectrum for Compound **2**-c

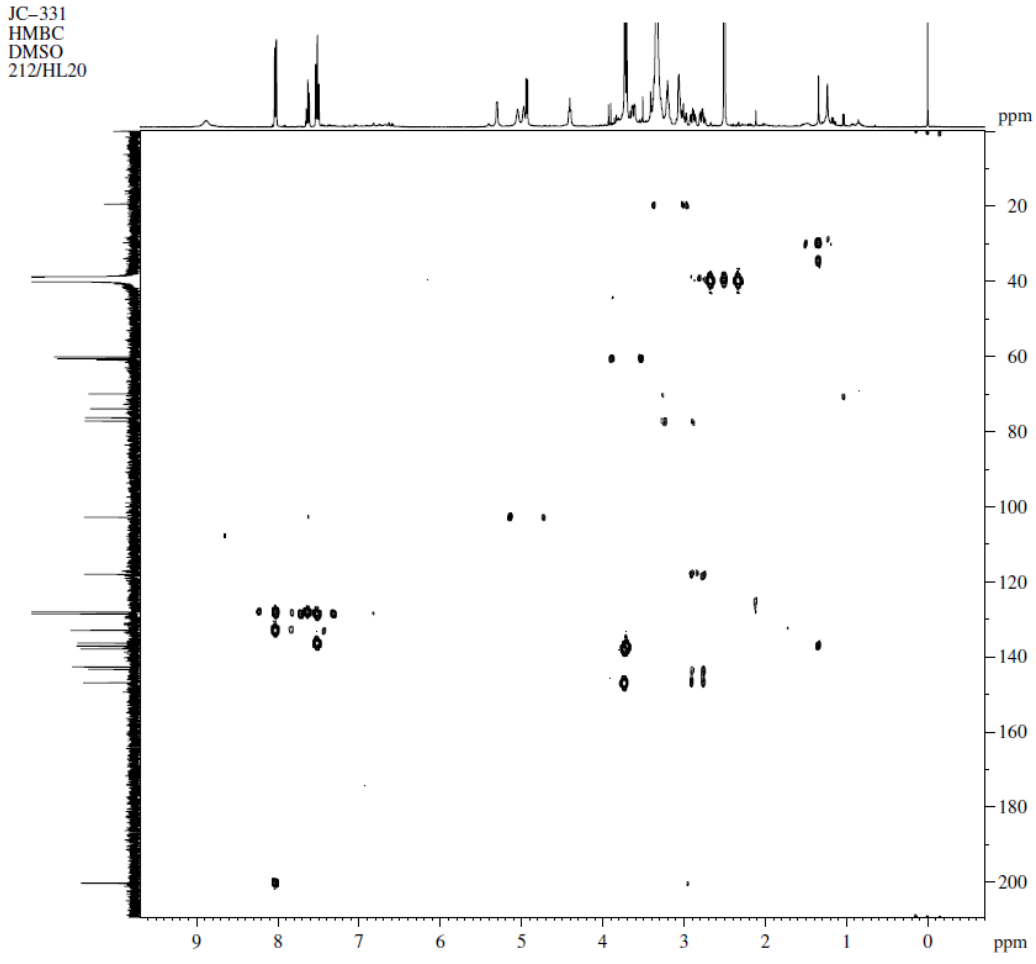

Figure S20 HMBC spectrum for Compound **2**

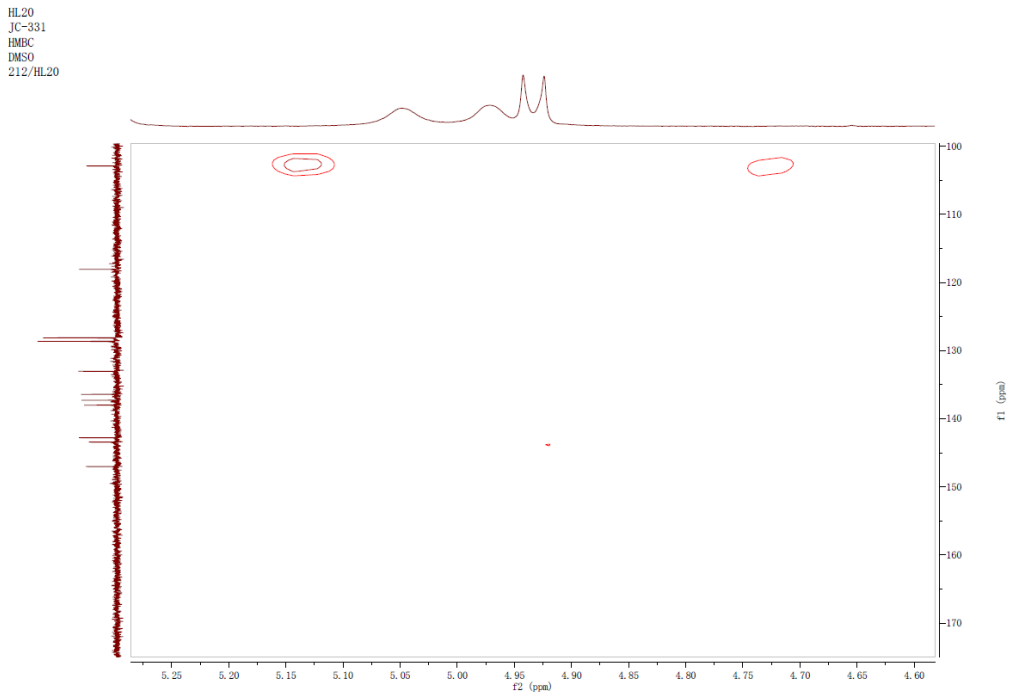

Figure S21 HMBC spectrum for Compound **2**-a

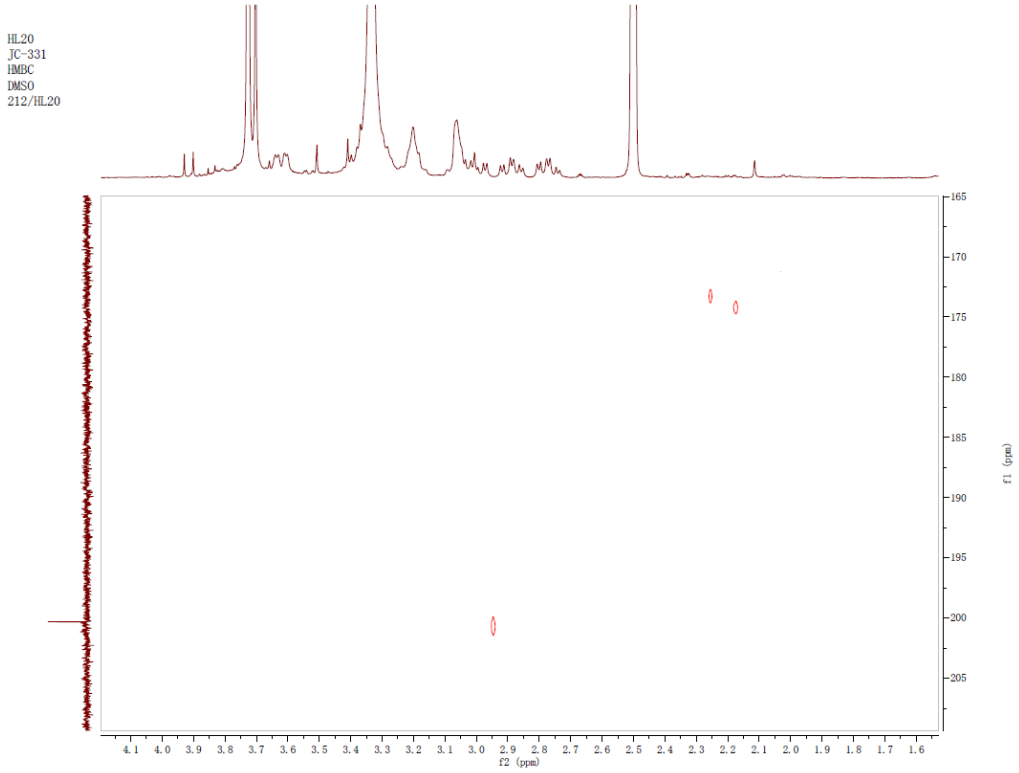

Figure S22 HMBC spectrum for Compound **2**-b

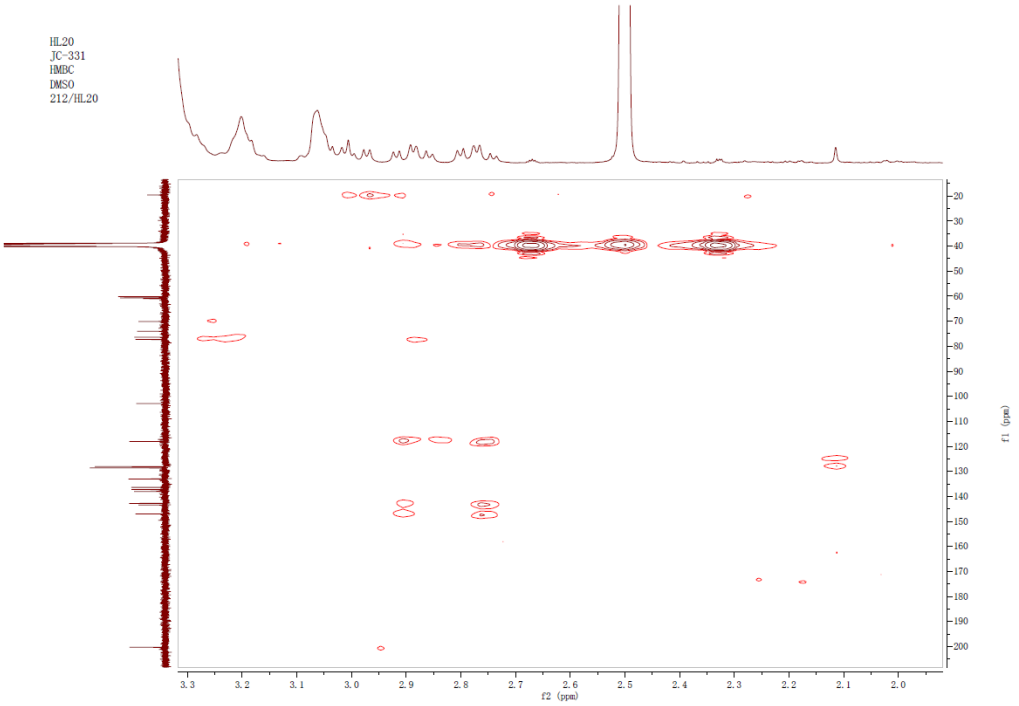

Figure S23 HMBC spectrum for Compound **2**-c

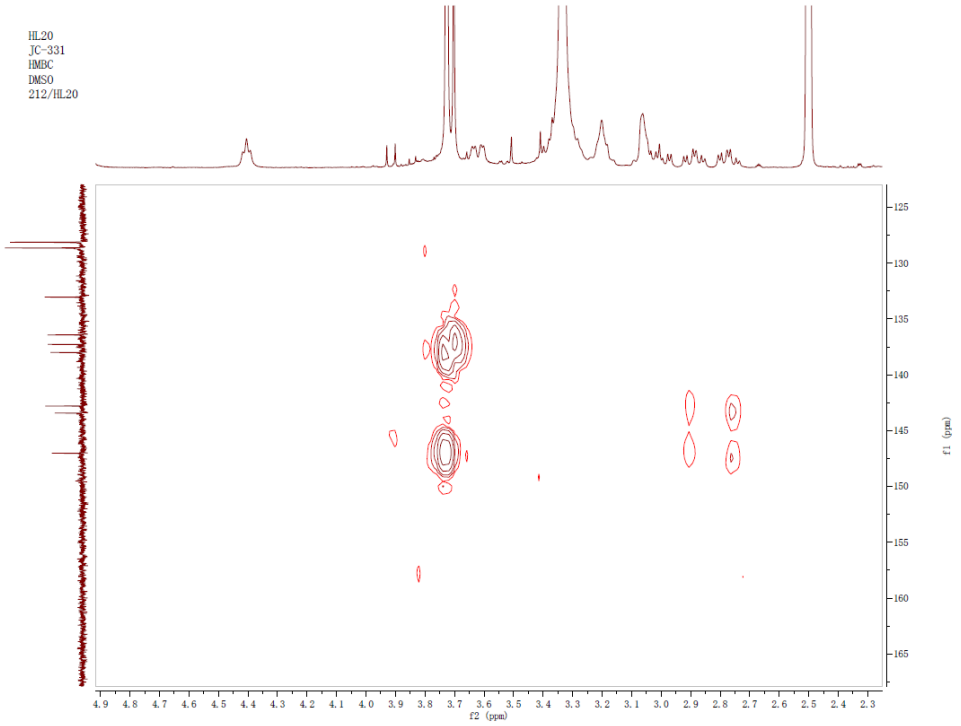

Figure S24 HMBC spectrum for Compound **2**-d

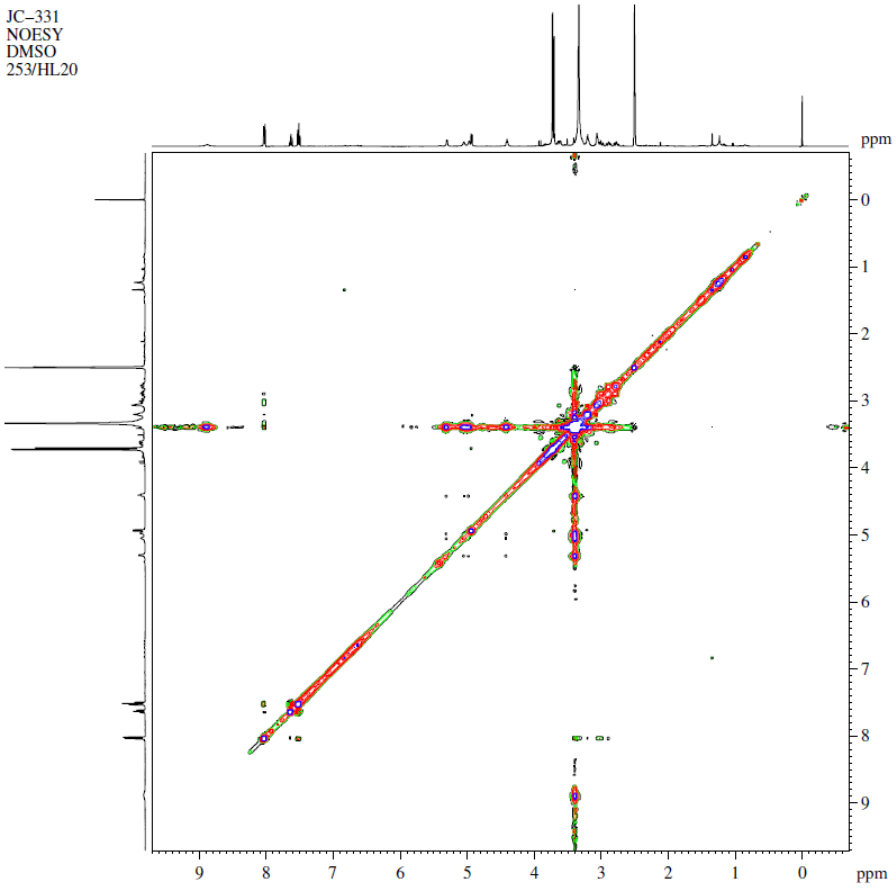

Figure S25 NOESY spectrum for Compound **2**

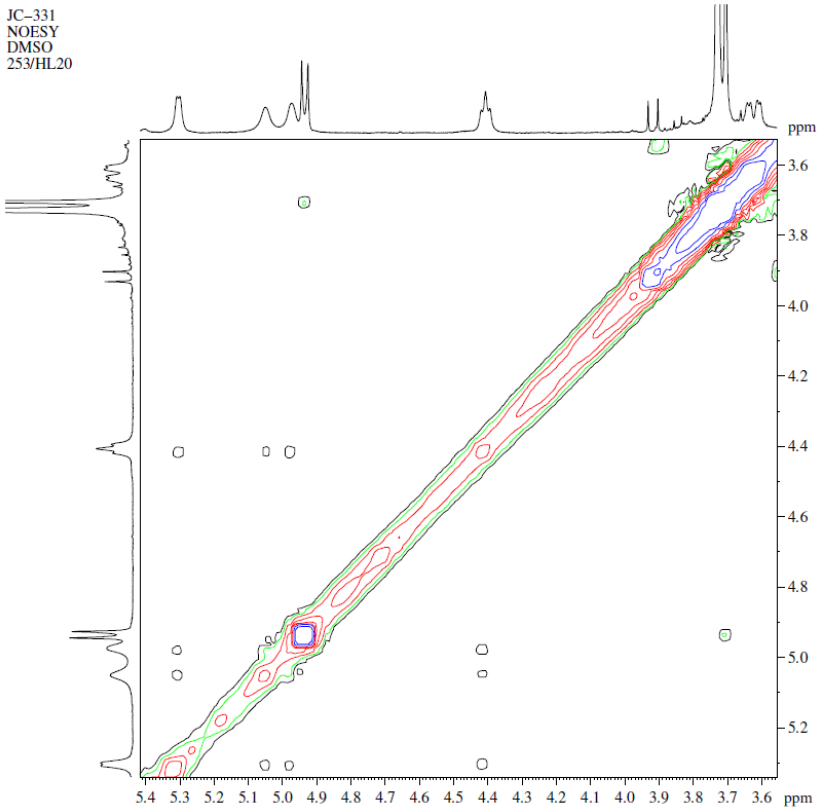

Figure S26 NOESY spectrum for Compound **2**-a

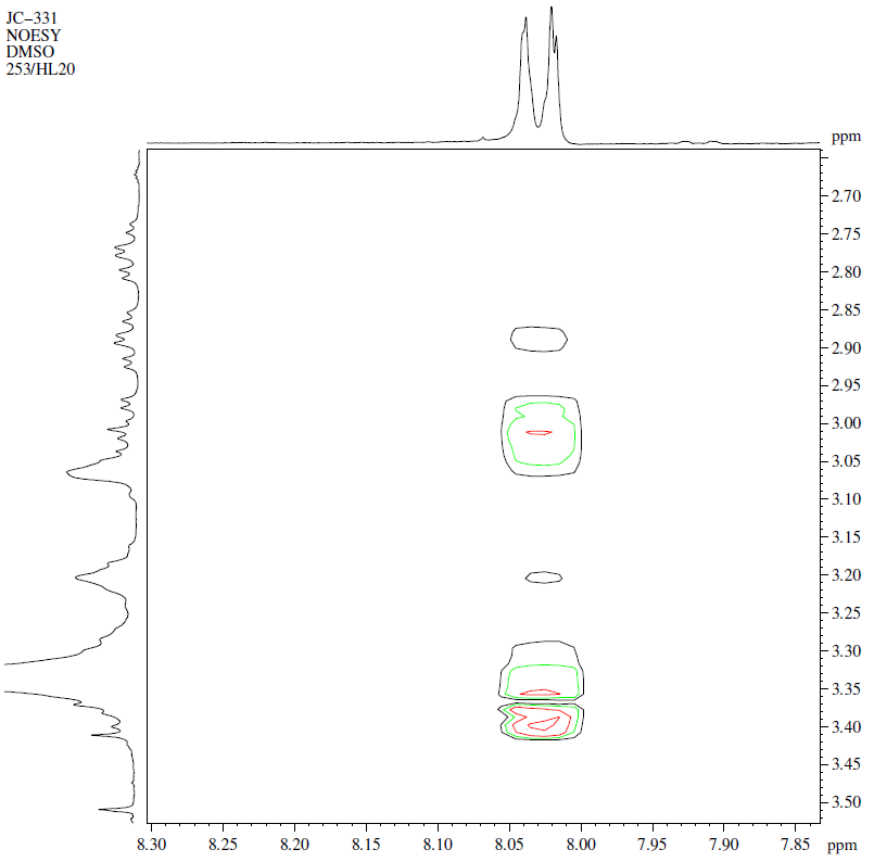

Figure S27 NOESY spectrum for Compound **2**-b

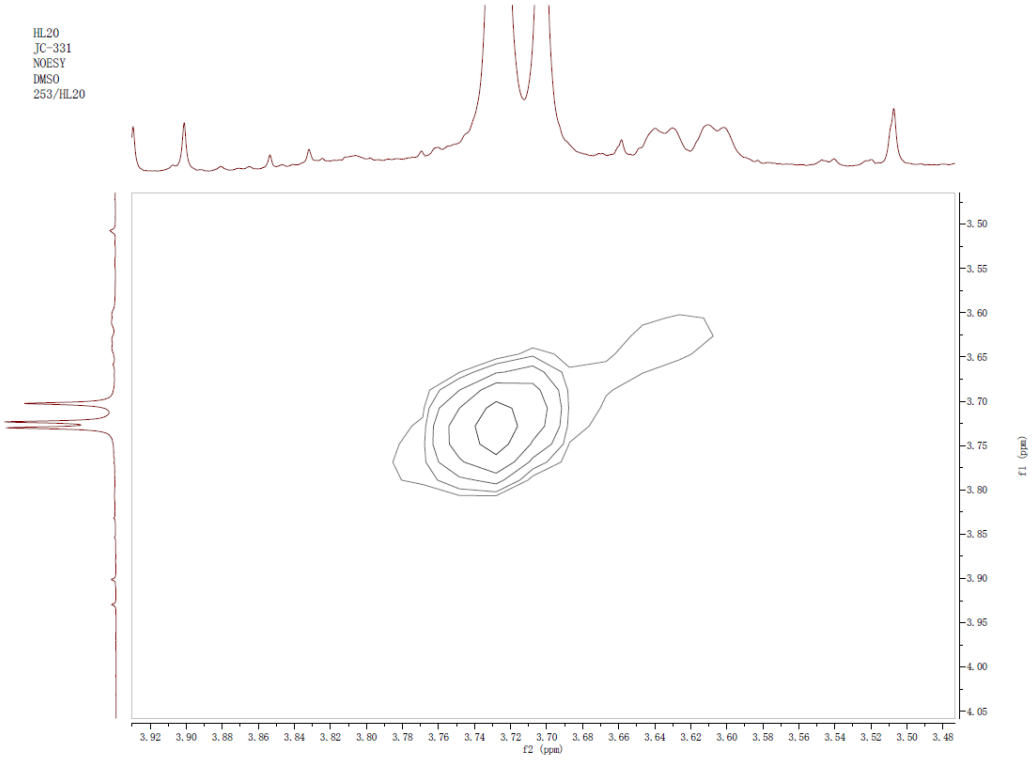

Figure S28 NOESY spectrum for Compound **2**-c

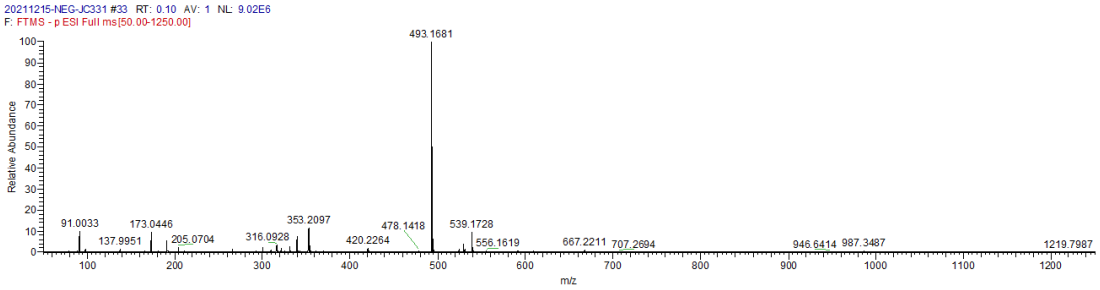

Figure S29 MS spectrum for of Compound **2**

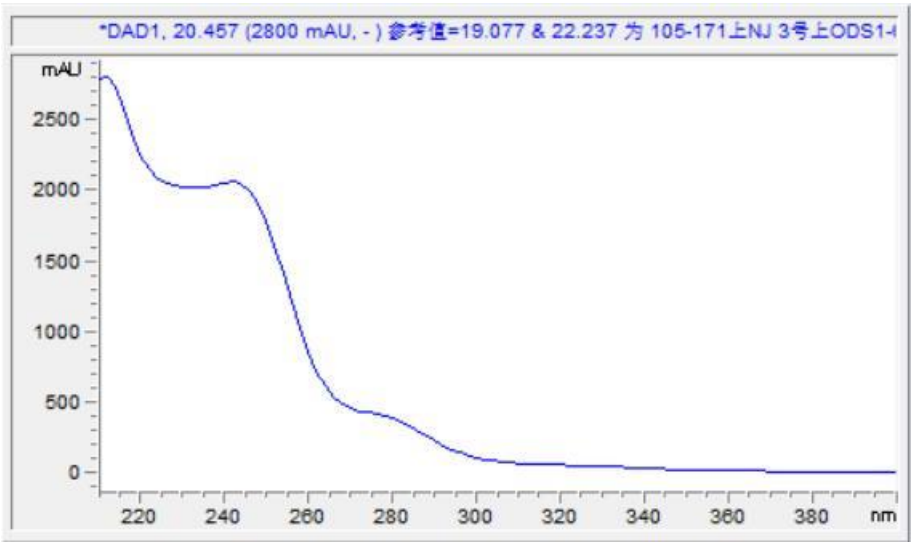

Figure S30 UV spectrum for of Compound **2**

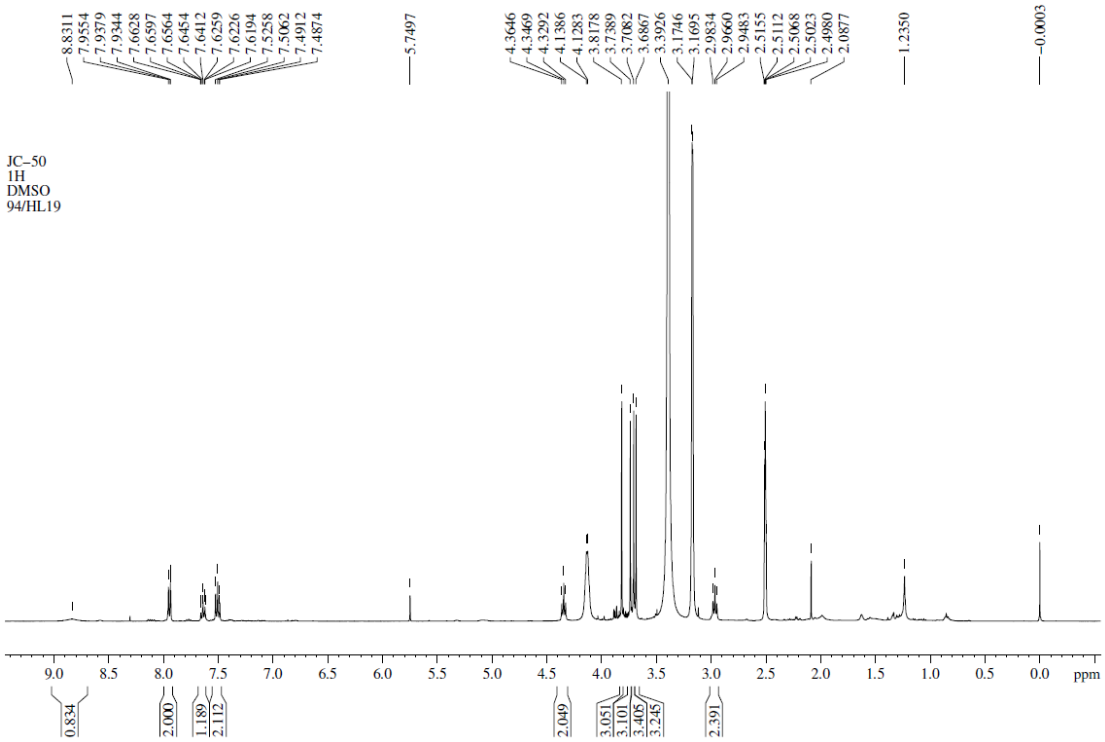

Figure S31 ^{1}H-NMR spectrum for Compound **3** (DMSO-d_6)

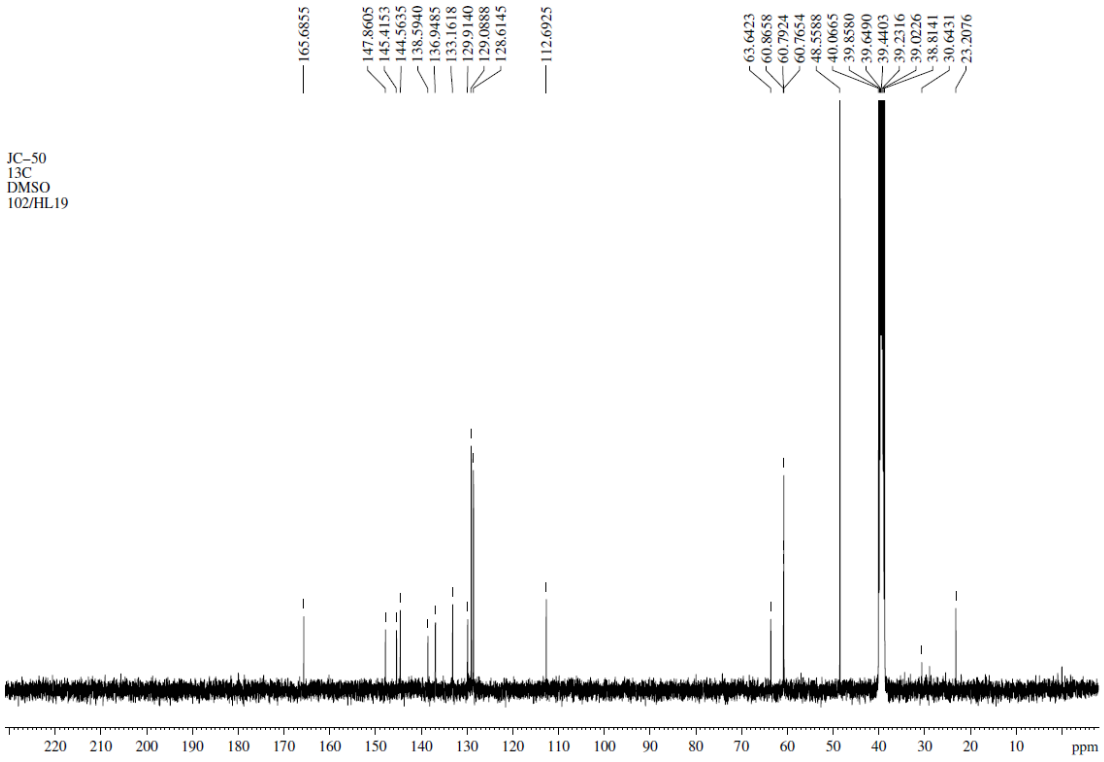

Figure S32 ^{13}C-NMR spectrum for Compound **3**

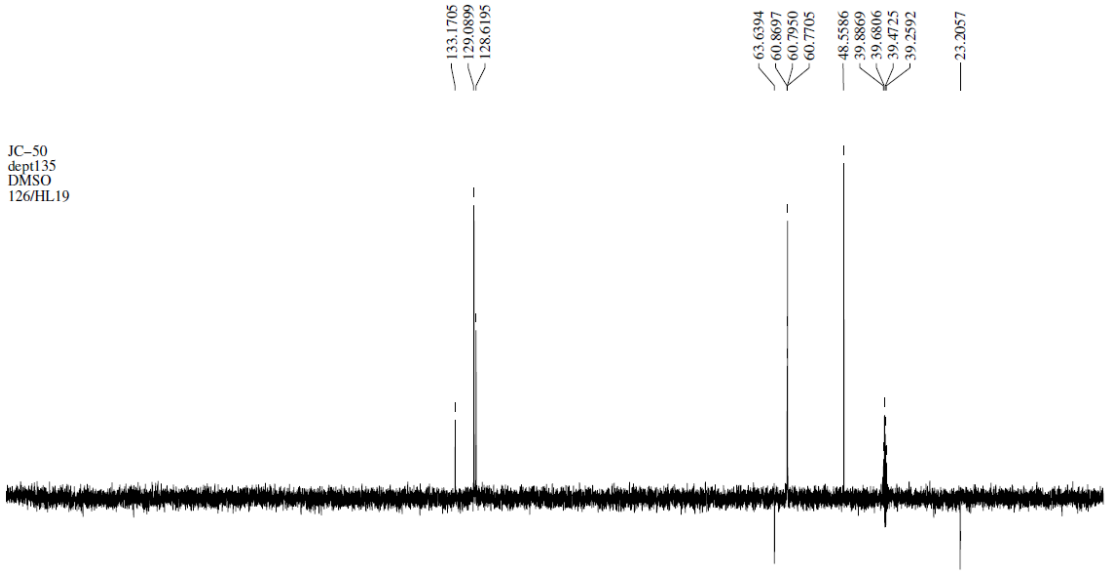

Figure S33 DEPT135 spectrum for Compound **3**

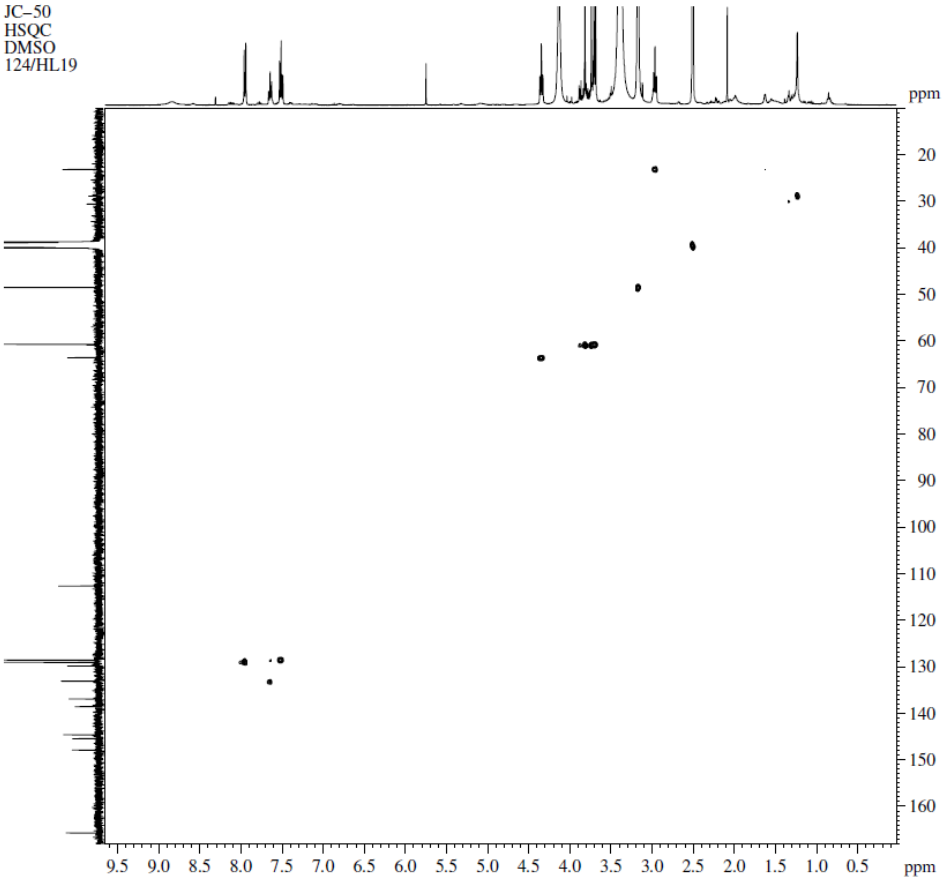

Figure S34 HSQC spectrum for Compound **3**

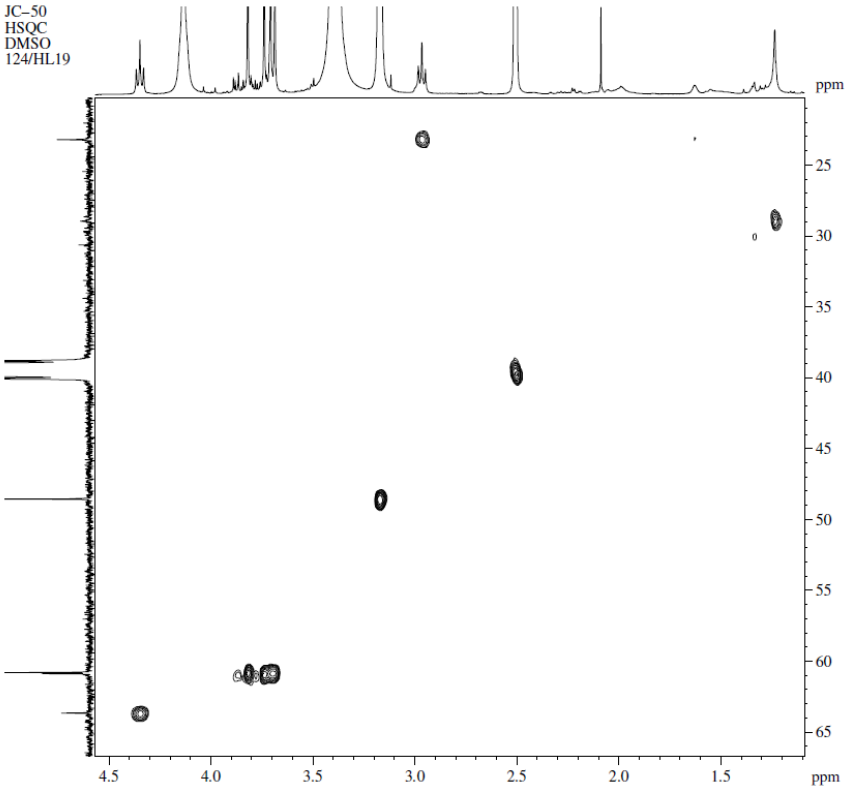

Figure S35 HSQC spectrum for Compound **3**-a

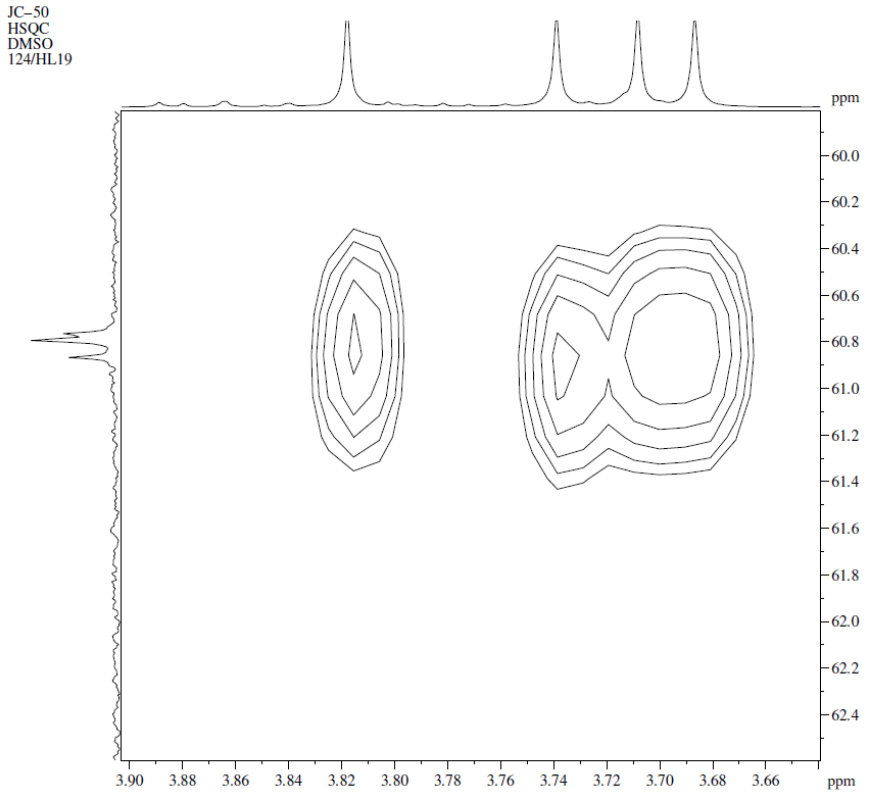

Figure S36 HSQC spectrum for Compound **3**-b

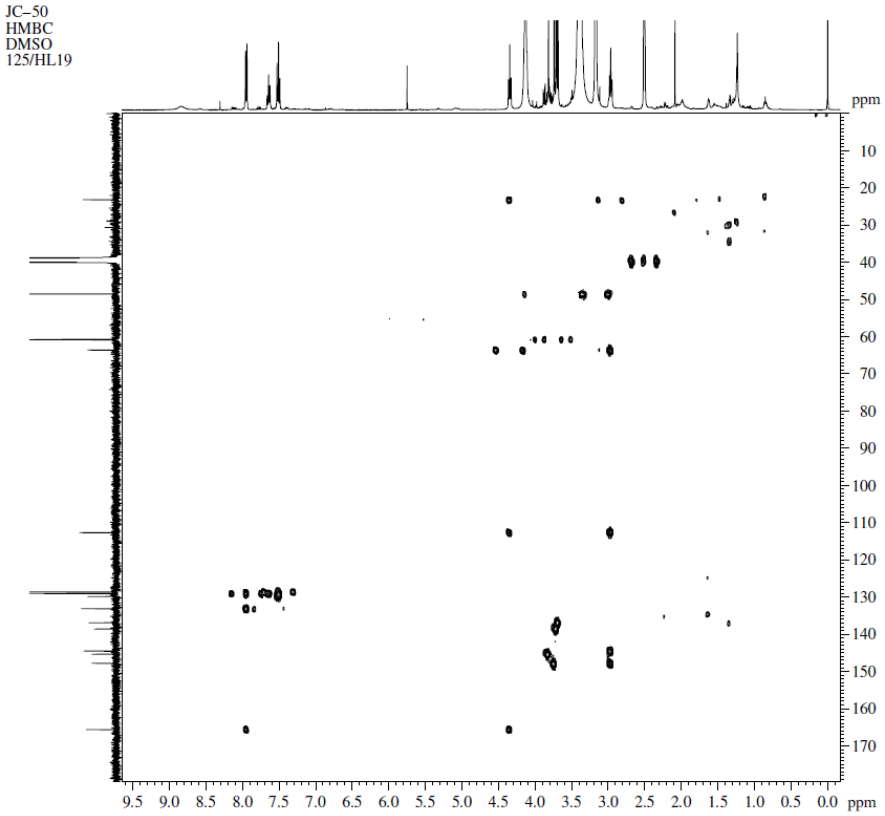

Figure S37 HMBC spectrum for Compound **3**

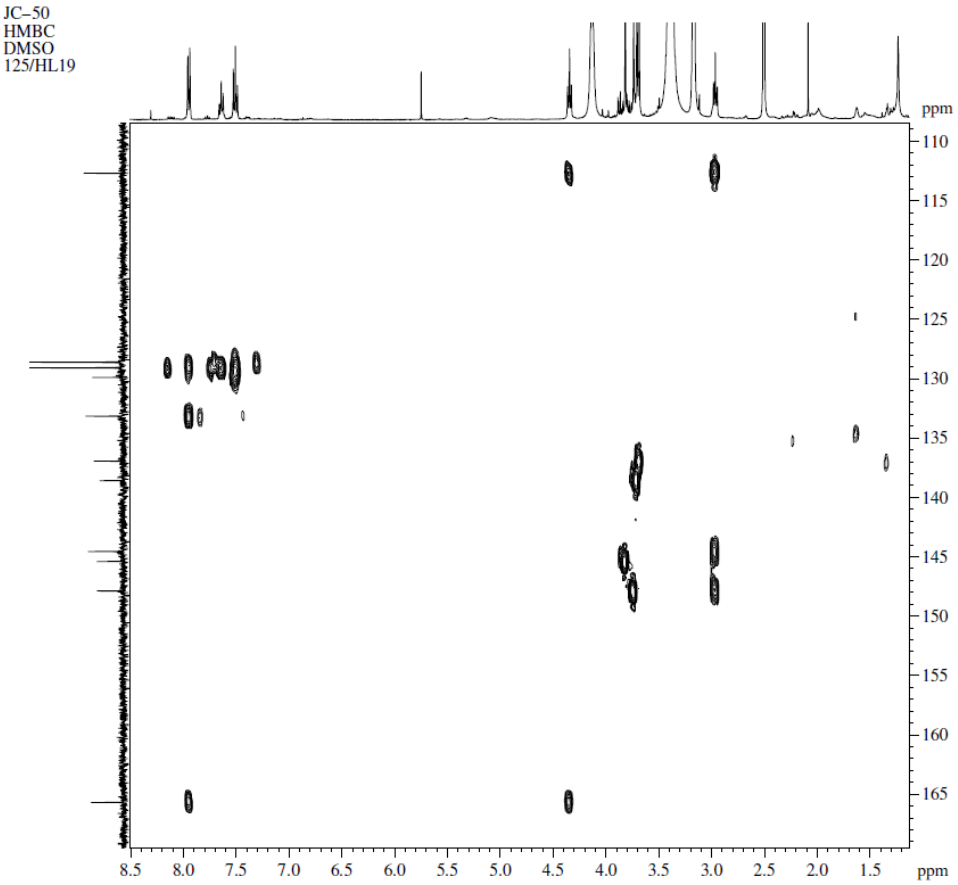

Figure S38 HMBC spectrum for Compound **3**-a

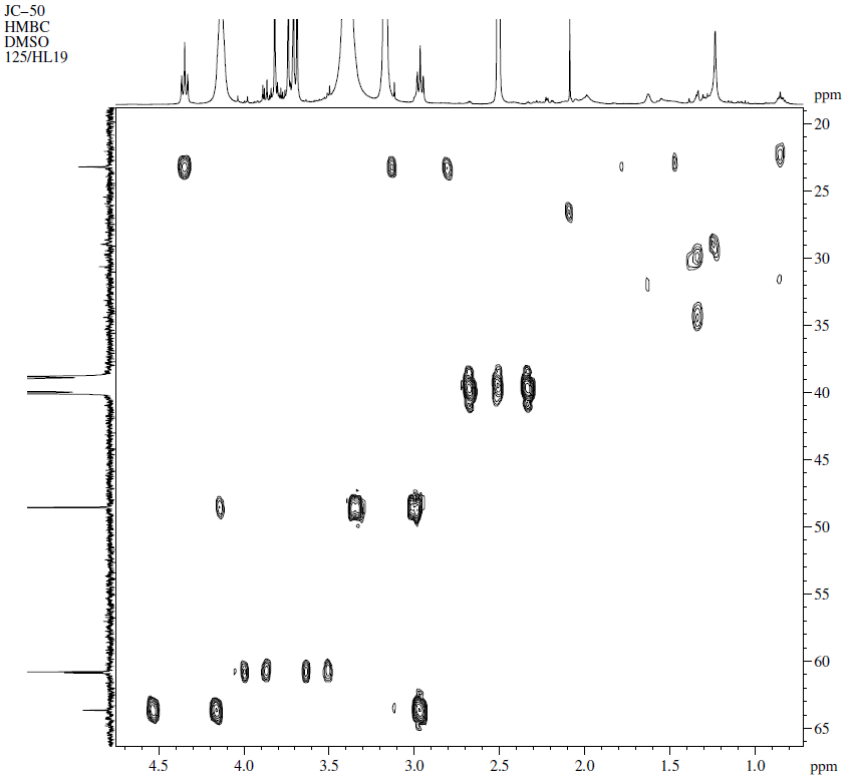

Figure S39 HMBC spectrum for Compound **3**-b

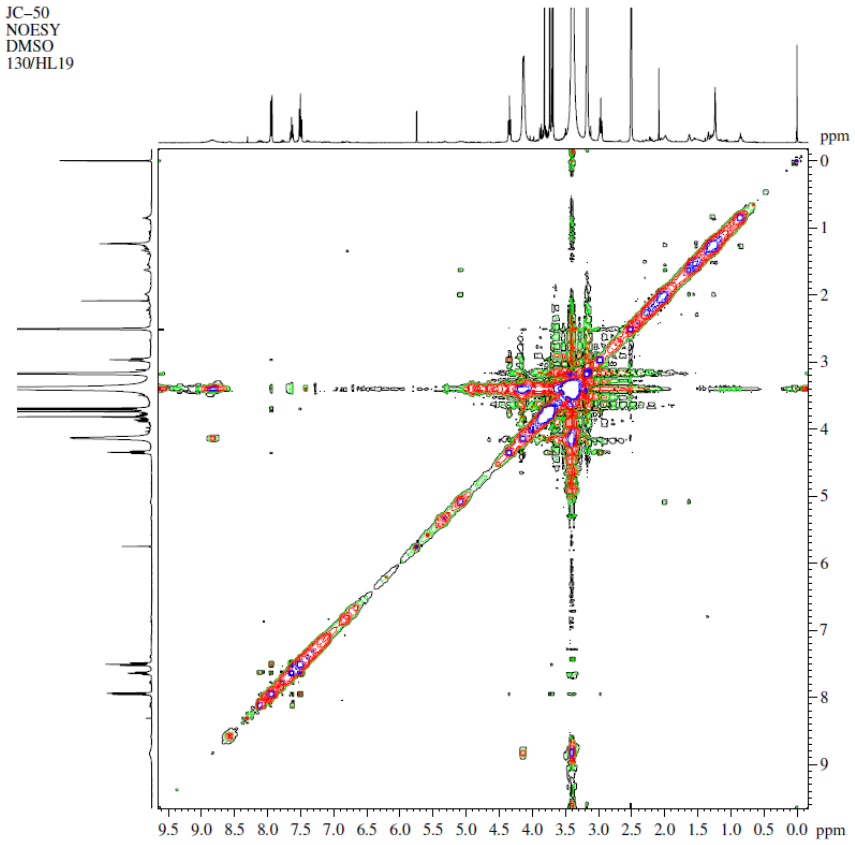

Figure S40 NOESY spectrum for Compound **3** (DMSO-d_6)

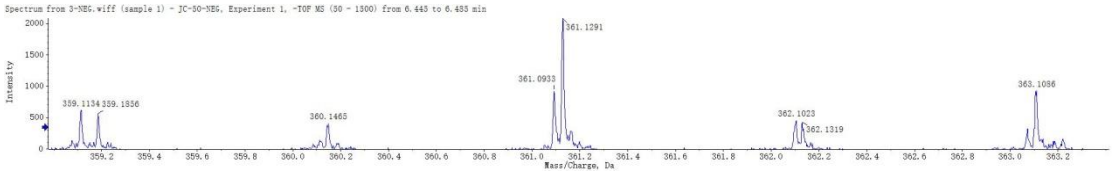

Figure S41 MS spectrum for Compound **3**

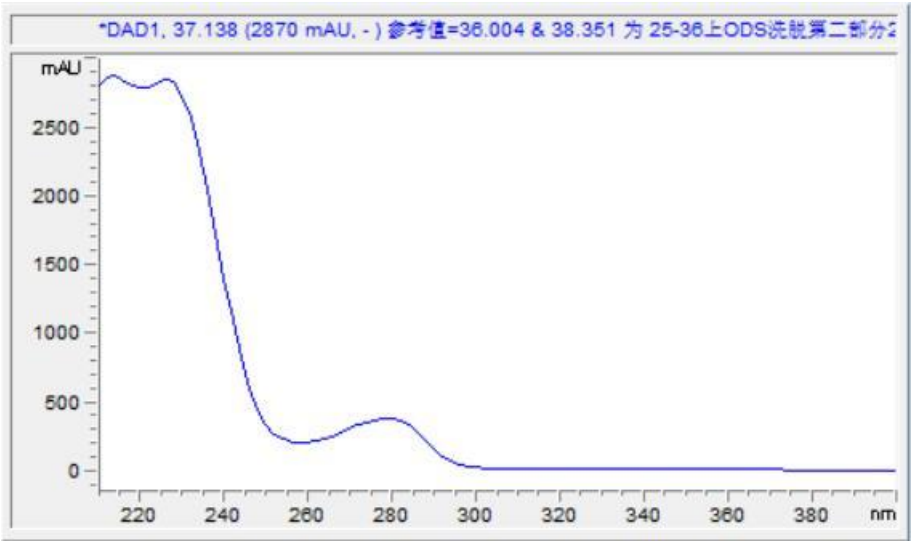

Figure S42 UV spectrum for Compound **3**