Supporting information

Ecdysterols and triterpene glycoside from *Achyranthes aspera* L. and their NO production inhibitory activity

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Methods

Equipment

The CD spectra were recorded on a ChiraScan spectrometer. NMR spectra were measured on a Bruker Avance III 600 MHz (150 MHz for ^{13}C NMR and 600 MHz for ^{1}H NMR). Column chromatography (CC) was performed using silica gel (40 - 63 µm) or reversed phase C-18 (RP18, 150 µm) as adsorbents. Thin layer chromatography was performed on pre-coated plates. Semi-preparative HPLC was performed on an Agilent 1260 infinity II system including binary pump, autosampler, DAD detector, fraction collector, and equipped with YMC J'sphere ODS-H80 (20 × 250 mm, 4µm) column. Mobile phase was an isocratic system of acetonitrile/water at flow rate of 3 mL/min.

Nitric oxide assay

Nitric oxide assay was performed as previously described [1, 2] In brief, RAW 264.7 cells were cultured in DMEM containing L-glutamine (2 mM), HEPES (10 mM), sodium pyruvate (1 mM), and fetal bovine serum (10 %). The cells (2×10^5 cells/well) were incubated in humidified atmosphere (95 % air and 5 % CO_2) at 37 °C. After 24h incubation, each well was added by compounds (0.4 - 100 µM) or vehicle and followed by LPS (1µg/mL) in the next 2h. The cells were then incubated for an additional 24h. After that, cell viability was then measured by MTT assay and amount of NO production in cell medium was determined by Griess reaction. Cultural medium (100 µL) was mixed with equal volume of Griess reagent and incubated in room temperature for 10 minutes. Absorbance was measured at 540 nm on a microplate reader. Nitrite concentration as an indicator of NO production was determined using a standard curve which was built by $NaNO_2$ serial diluted solutions. Experiments were performed in triplicate. IC_{50} values were generated by TableCurve 2Dv4 software.

References

[1] B. H. Tai, P. H. Yen, N. H. Hoang, P. T. T. Huong, N. V. Dung, B. V. Thanh, N. T. Cuong, N. A. Bang, N. X. Nhiem, P. V. Kiem, *RSC Adv*. **2022**, *12*, 25433

[2] S. Cheenpracha, E. J. Park, B. Rostama, J. M. Pezzuto, L. C. Chang, *Mar. Drugs* **2010**, *8*, 429–437.

Table S1. NO production inhibition effects of compounds **1-6.**

Con. (μM)	**1**		**2**		**3**	
	Inhibition (%)	**Survival cells (%)**	**Inhibition (%)**	**Survival cells (%)**	**Inhibition (%)**	**Survival cells (%)**
100	13.58	87.04	59.76	84.12	64.79	91.29
20	8.95	88.82	42.31	88.78	47.04	96.40
4	4.94		29.59		32.54	
0.8	0.93		15.98		21.60	
IC_{50}	>100	-	40.47±4.90	-	27.21±1.23	-
Con. (μM)	**4**		**5**		**6**	
	Inhibition (%)	**Survival cells (%)**	**Inhibition (%)**	**Survival cells (%)**	**Inhibition (%)**	**Survival cells (%)**
100	66.10	87.67	60.36	93.16	7.10	95.75
20	43.15	91.44	46.75	95.71	2.78	96.17
4	27.40		35.80		1.23	
0.8	14.38		22.19		-1.85	
IC_{50}	33.44±1.53	-	27.89±2.11	-	>100	-
Con. (μg/mL)			L-NMMA*			
			Inhibition (%)	**Survival cells (%)**		
100			100.67	94.09		
20			79.36	98.54		
4			24.71			
0.8			10.03			
IC_{50}			7.99 ± 0.58 (μg/mL) 32.24 ± 2.35 (μM)	-		

*positive control compound

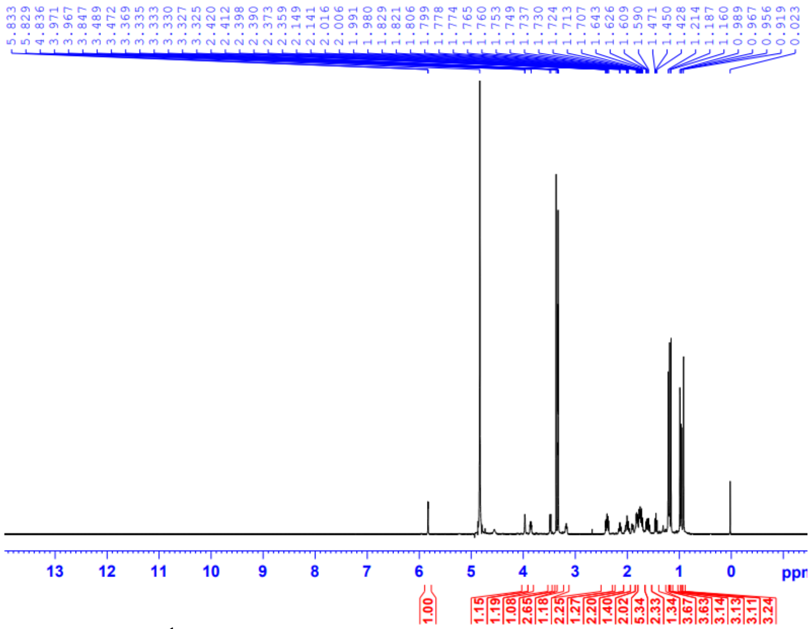

Figure S1. ^{1}H-NMR spectrum of compound **1** in CD_3OD.

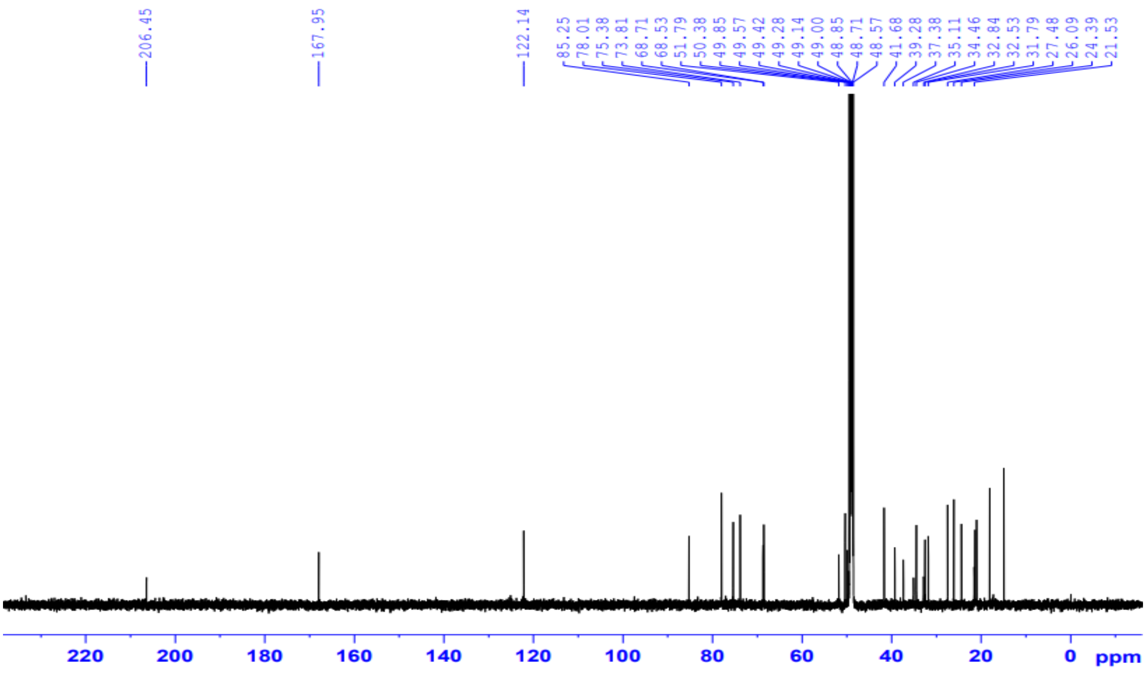

Figure S2. ^{13}C-NMR spectrum of compound **1** in $CDCl_3$.

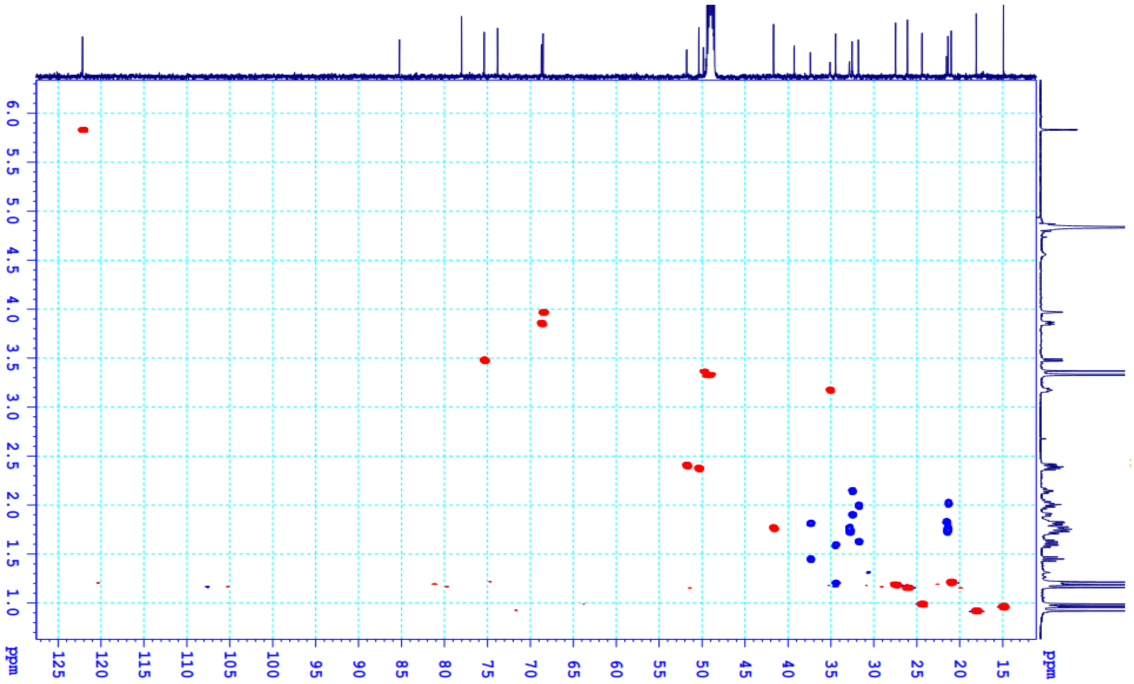

Figure S3. HSQC spectrum of compound **1.**

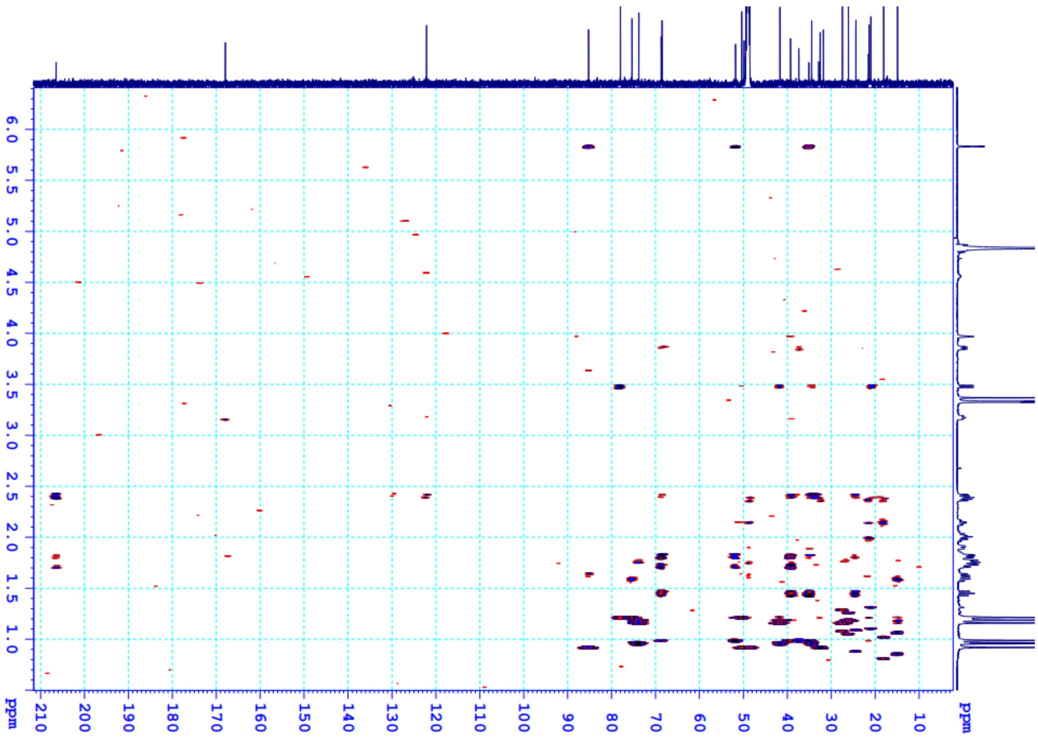

Figure S4. HMBC spectrum of compound **1.**

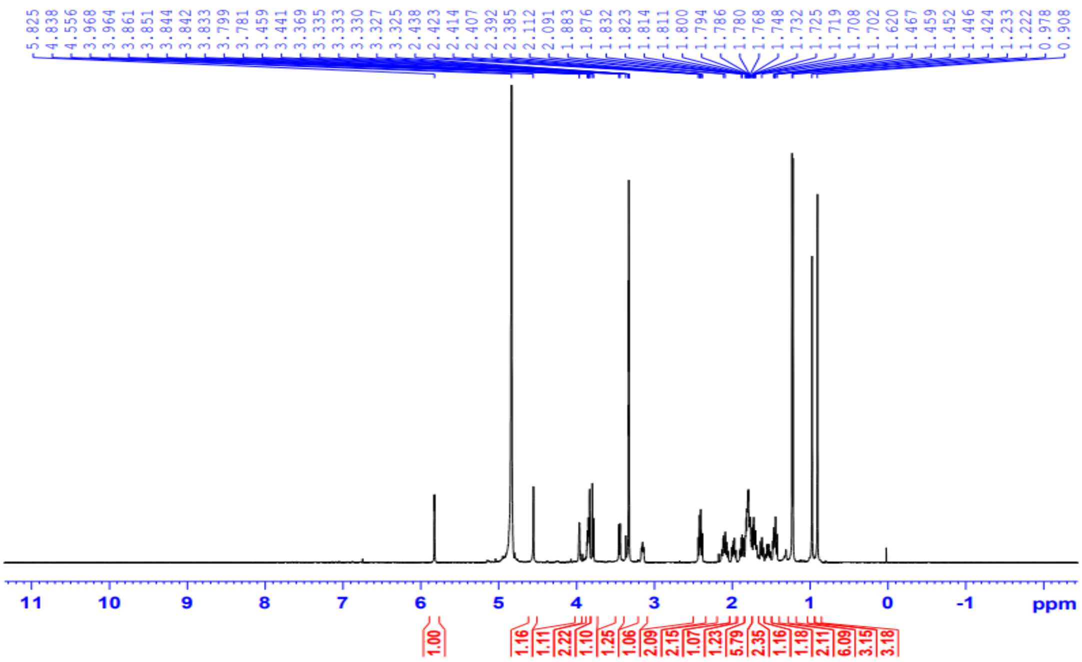

Figure S5. ^{1}H-NMR spectrum of compound **2** in CD_3OD.

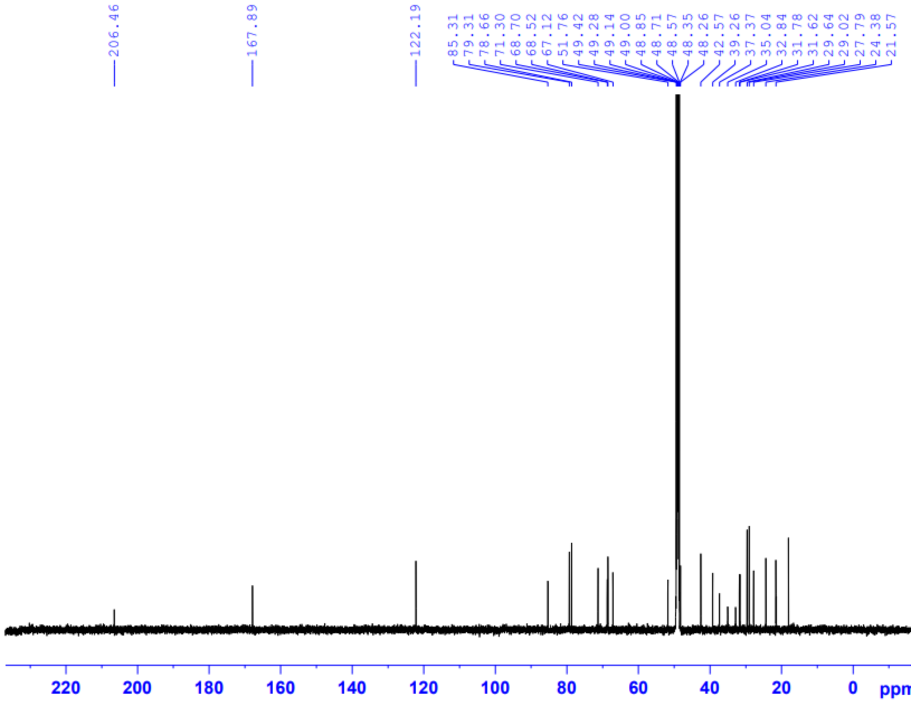

Figure S6. [13]C-NMR spectrum of compound **2** in CD_3OD.

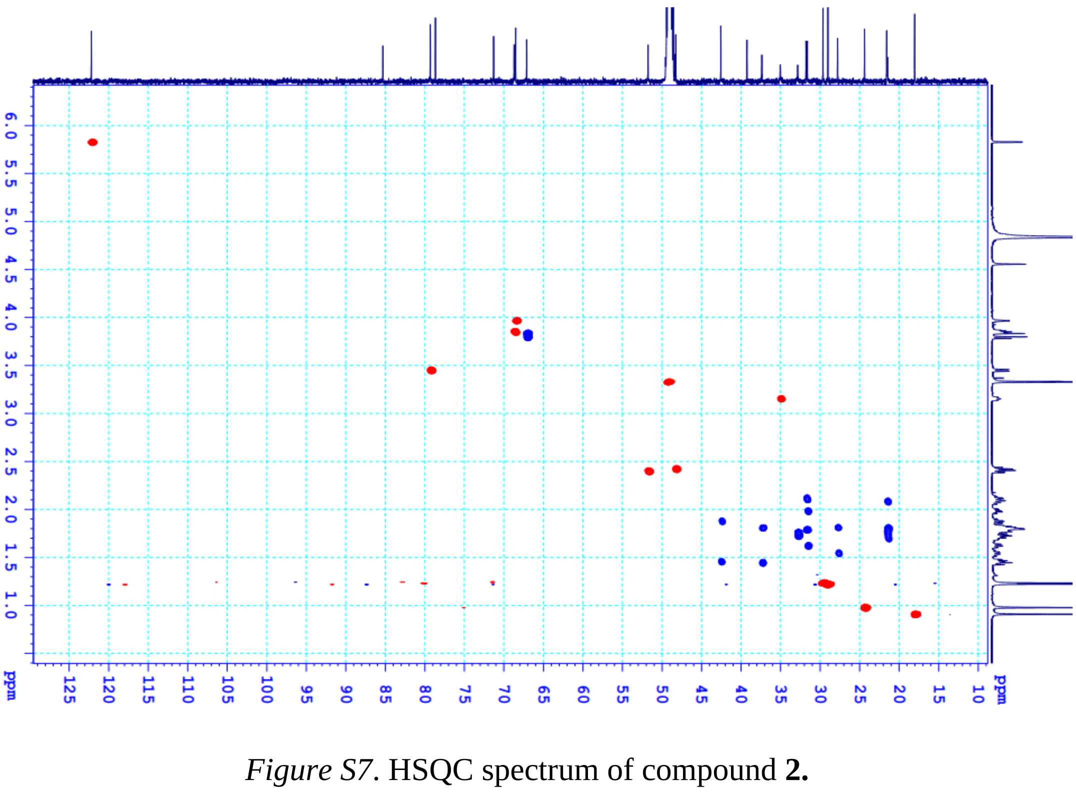

Figure S7. HSQC spectrum of compound **2.**

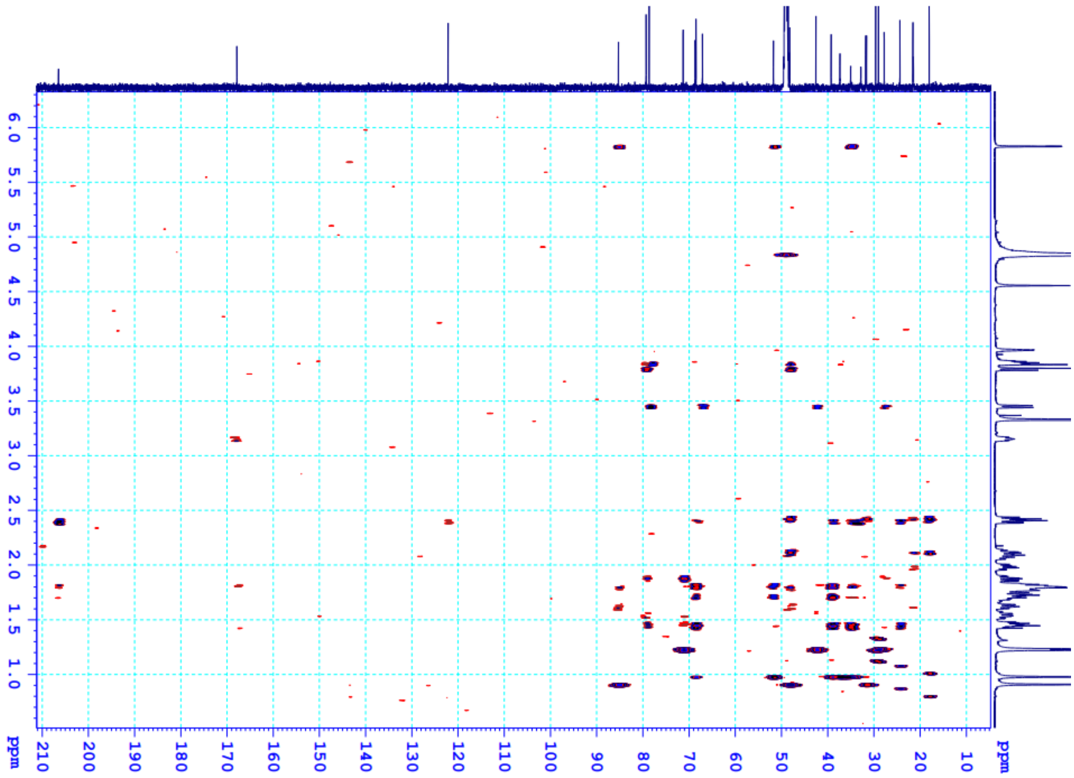

Figure S8. HMBC spectrum of compound **2.**

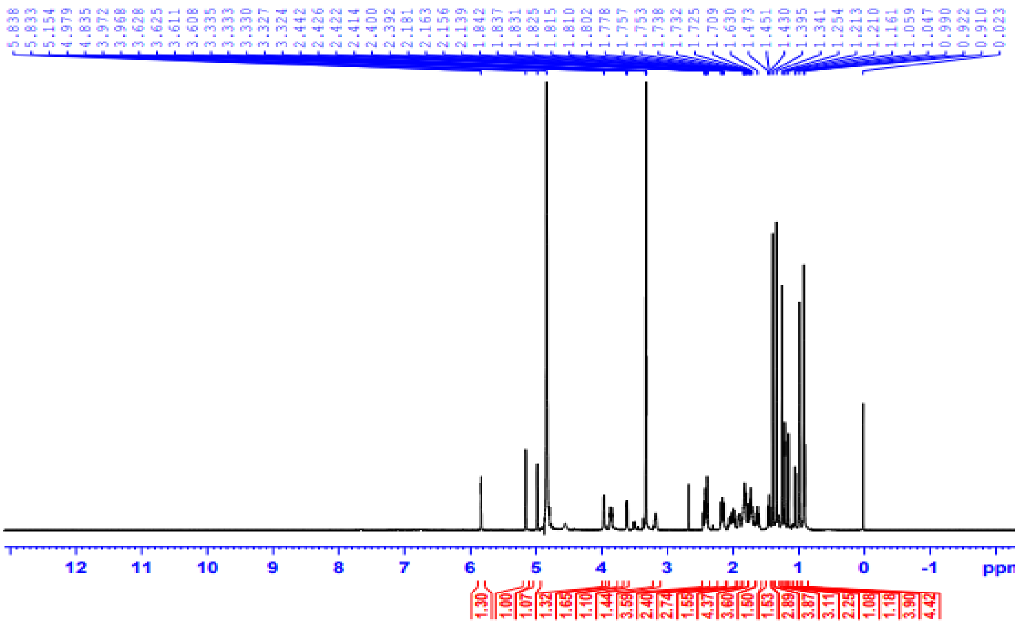

Figure S9. ^{1}H-NMR spectrum of compound **3** in CD_3OD.

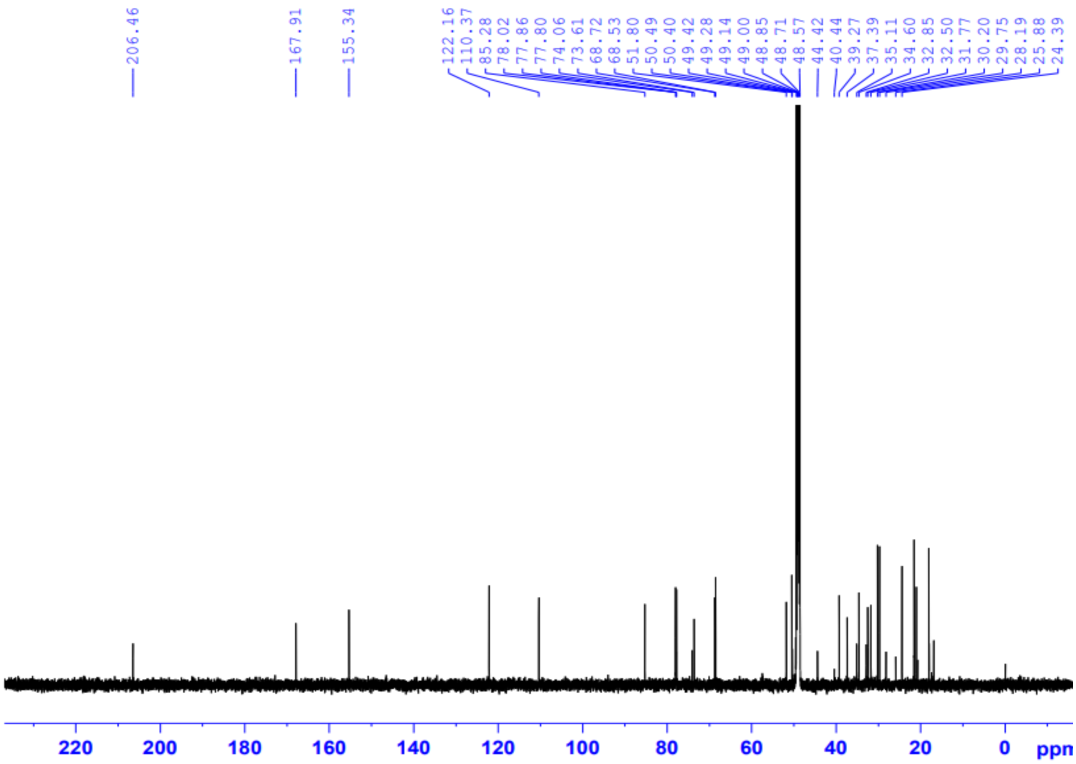

Figure S10. [13]C-NMR spectrum of compound **3** in CD_3OD.

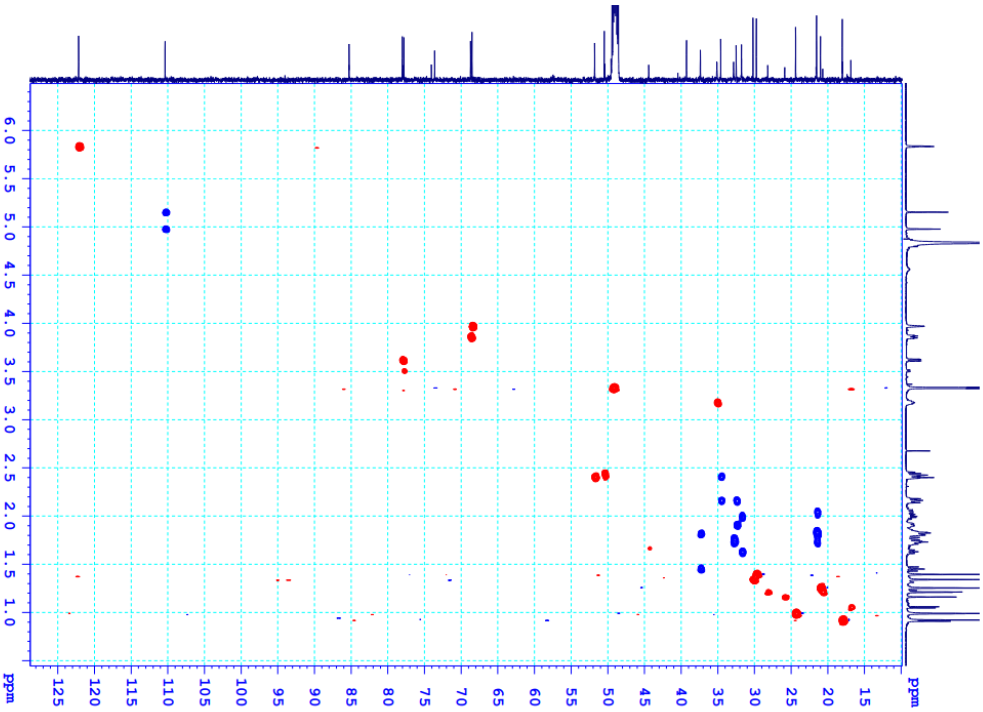

Figure S11. HSQC spectrum of compound **3.**

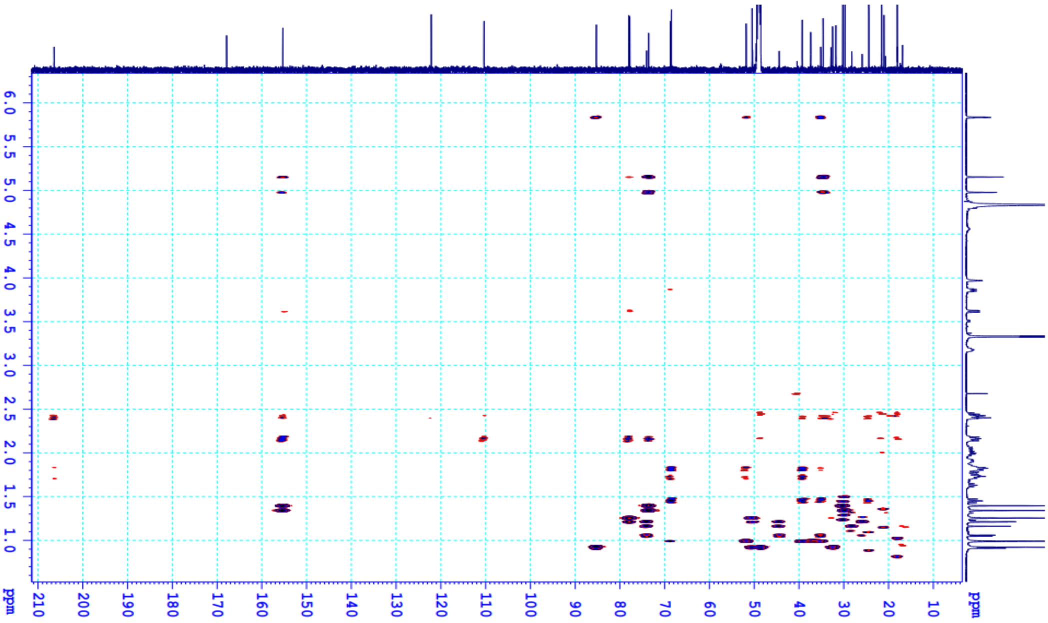

Figure S12. HMBC spectrum of compound **3.**

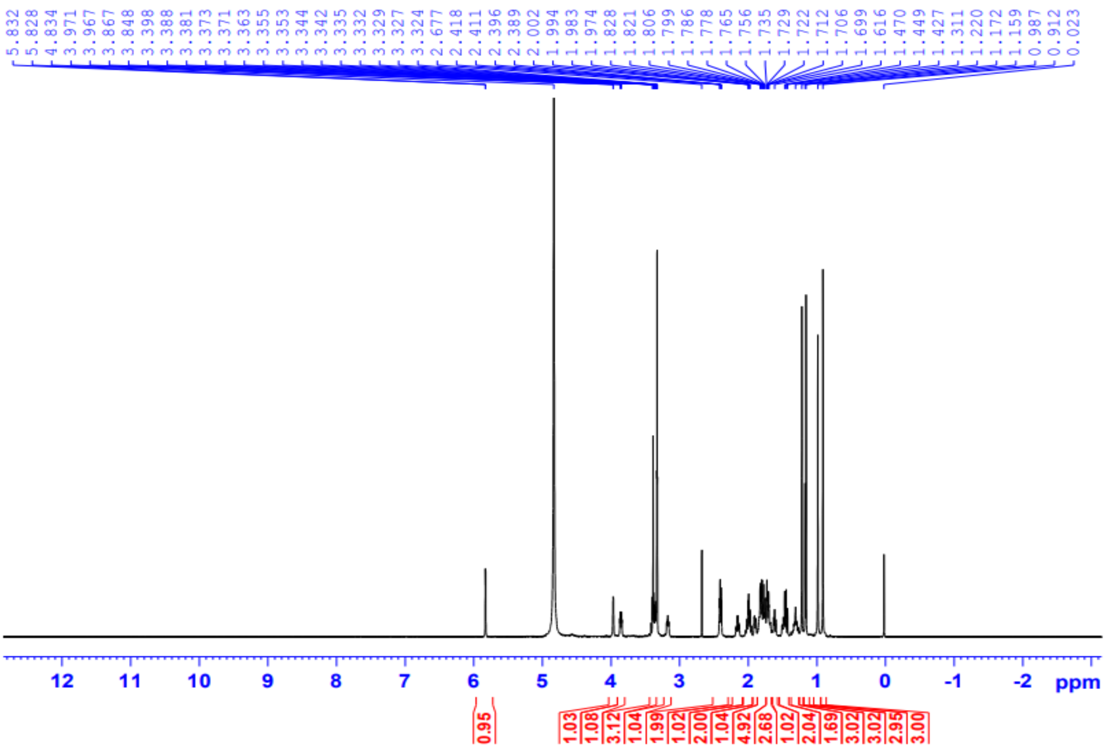

Figure S13. ^{1}H NMR spectrum of compound **4** in CD_3OD.

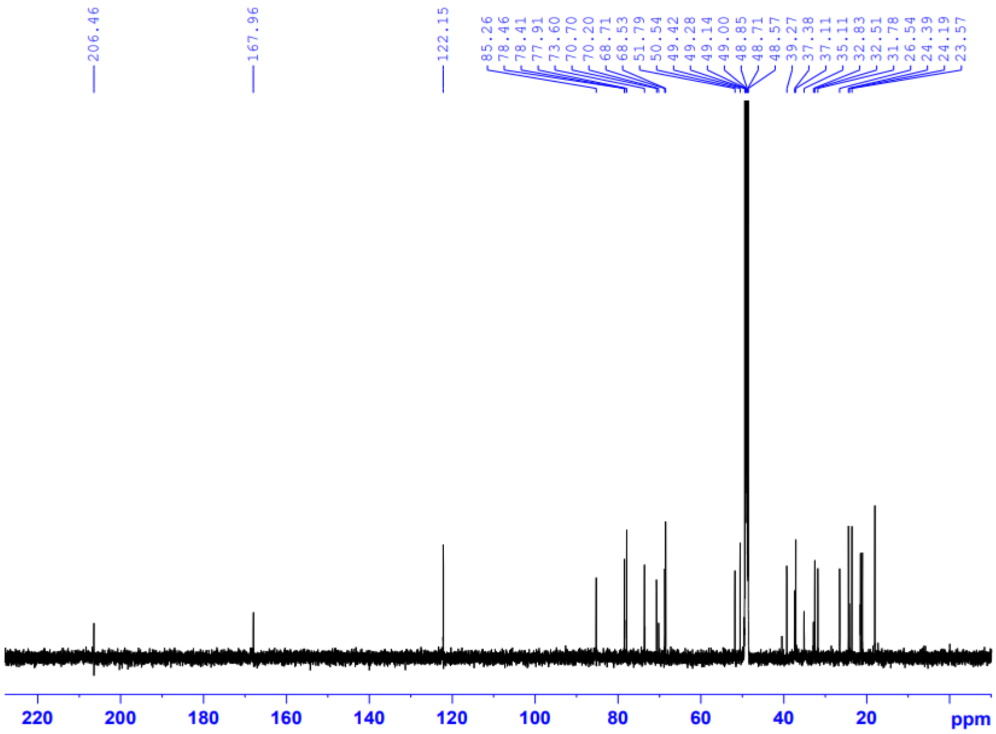

Figure S14. [1]H NMR spectrum of compound **4** in CD_3OD.

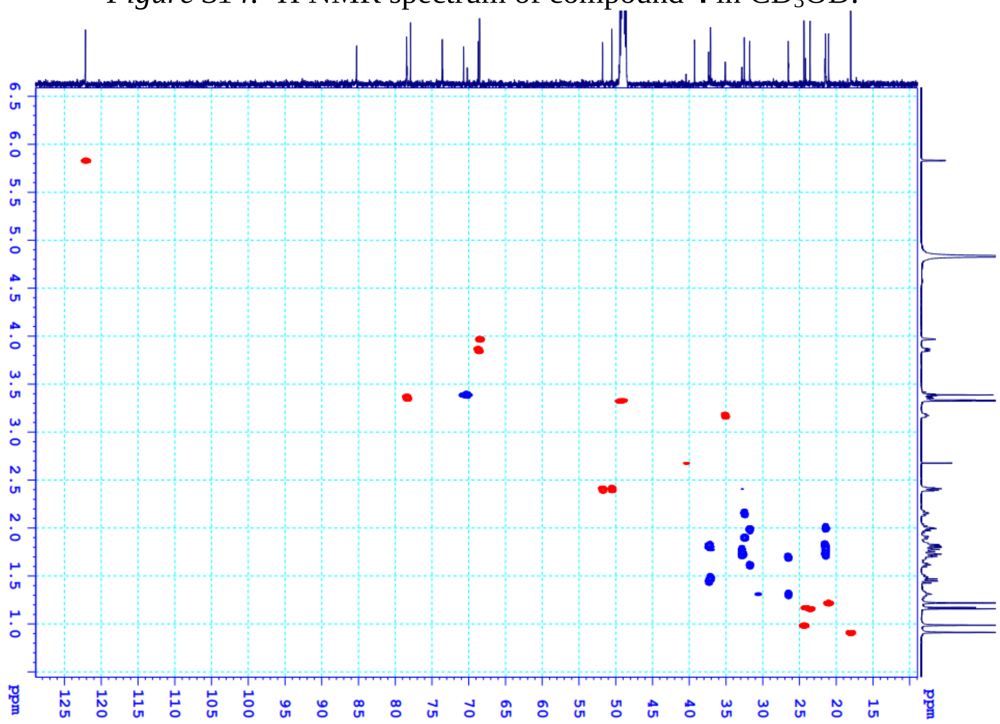

Figure S15. HSQC spectrum of compound **4** in CD_3OD.

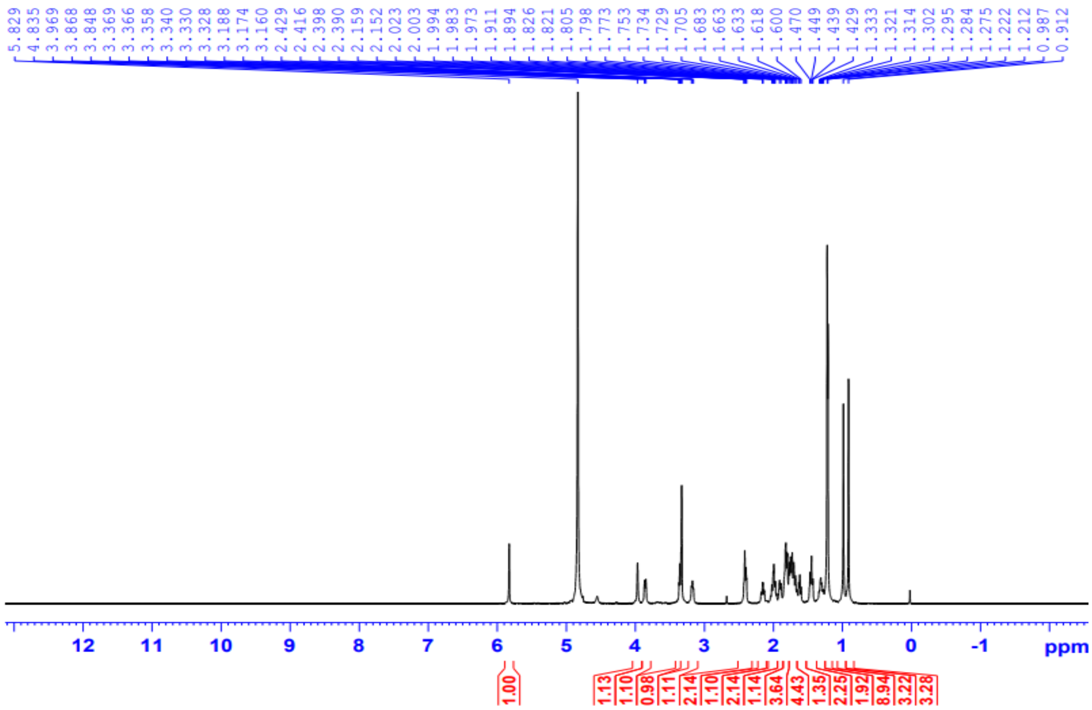

Figure S16. ^{1}H NMR spectrum of compound **5** in CD_3OD.

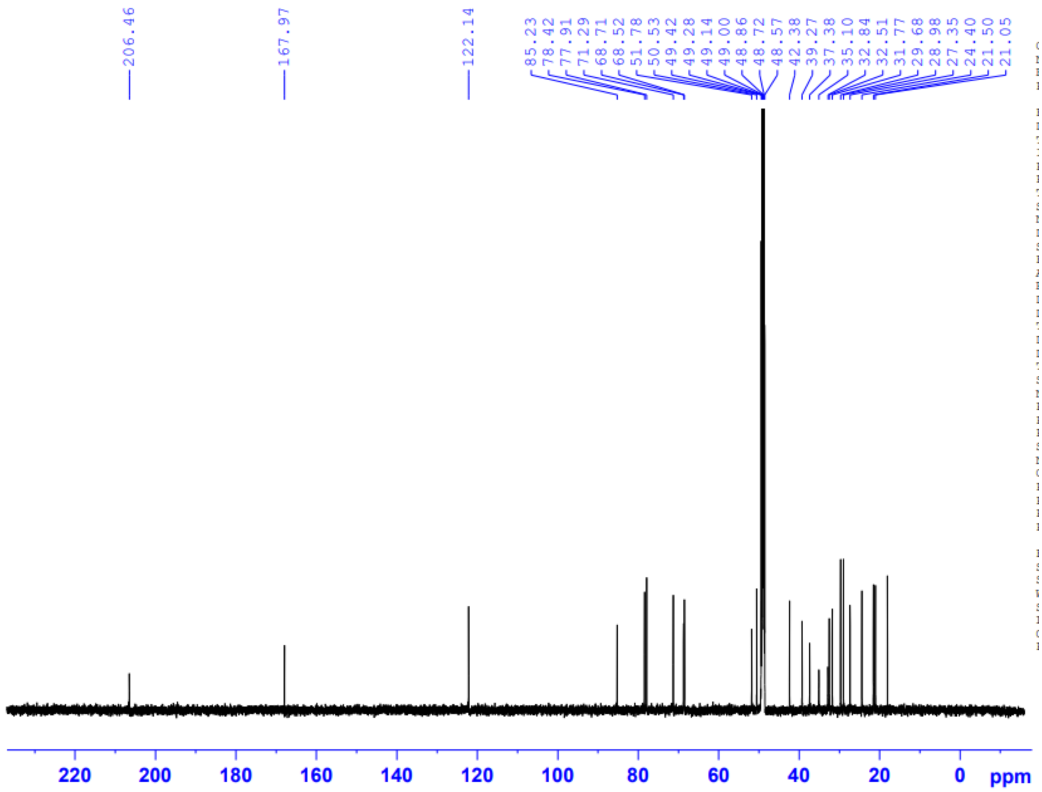

Figure S17. ^{13}C NMR spectrum of compound **5** in CD_3OD.

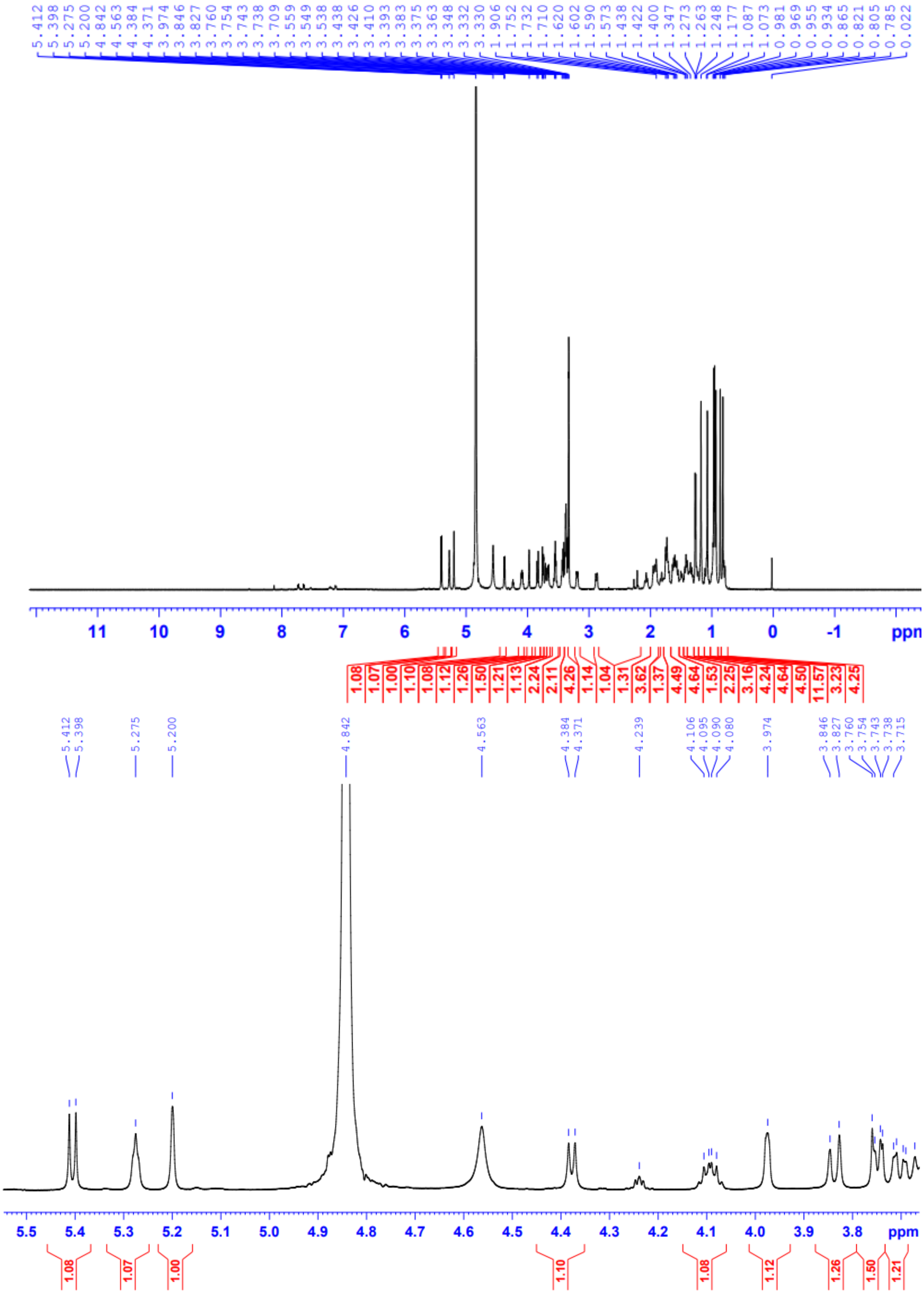

Figure S18. ^{1}H NMR spectrum of compound **6** in CD_3OD.

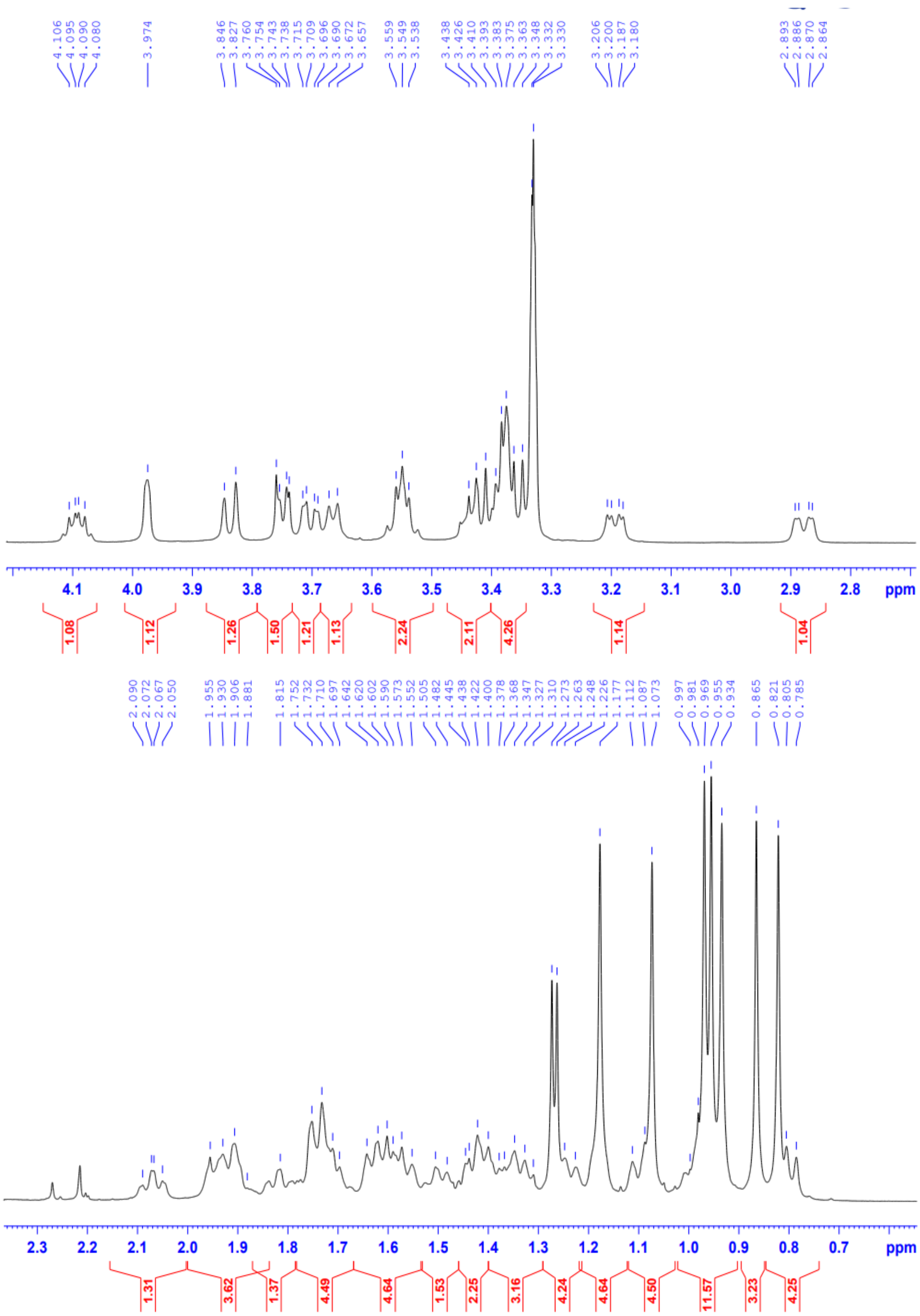

Figure S19. Extended ^{1}H NMR spectrum of compound **6** in CD_3OD.

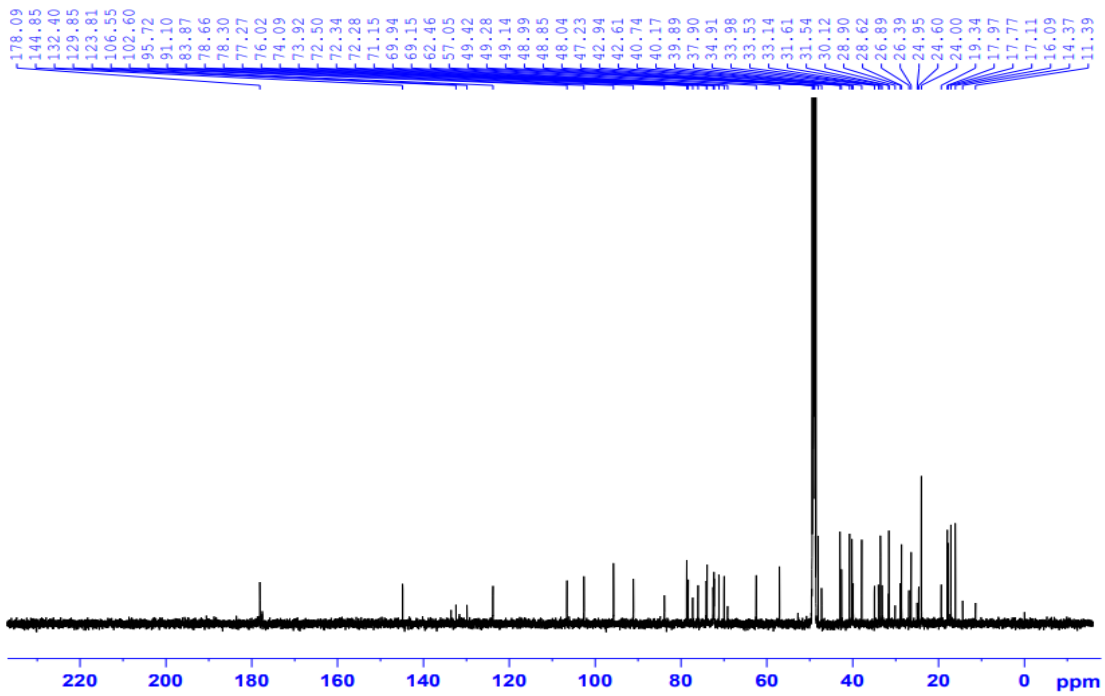

Figure S20. ^{13}C NMR spectrum of compound **6** in CD_3OD.

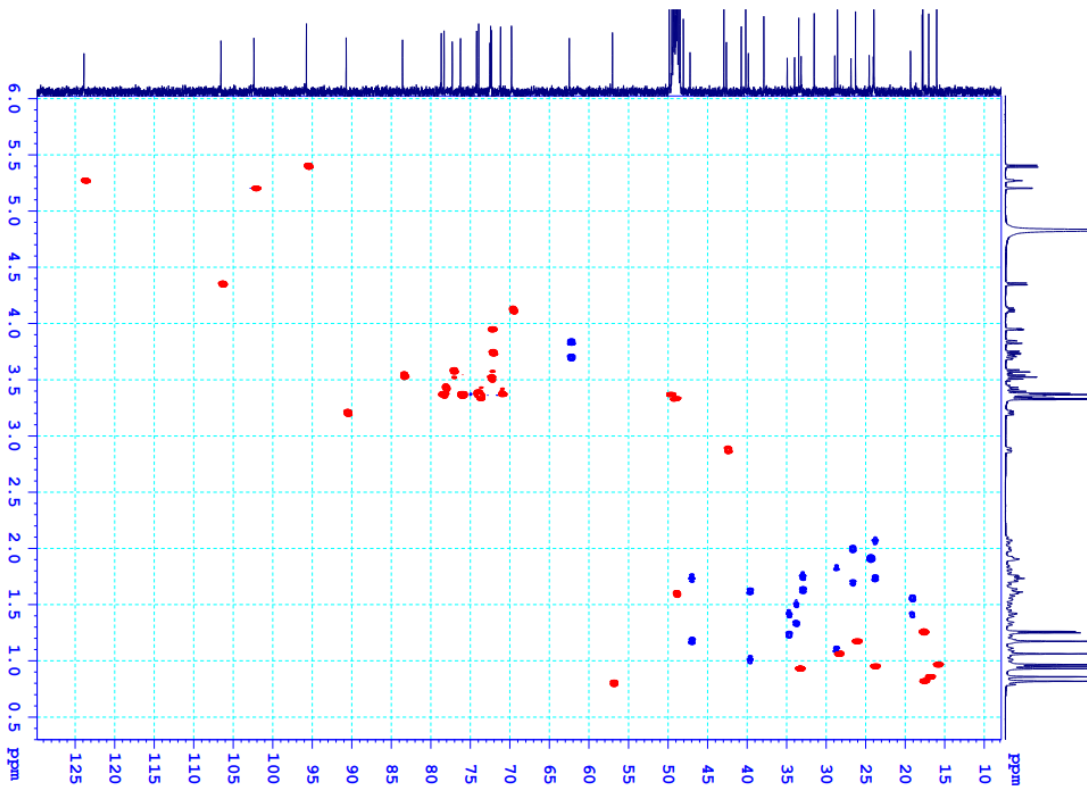

Figure S21. HSQC spectrum of compound **6** in CD_3OD.