Electronic Supplementary Information

Winchinines A and B, two unusual monoterpene indole alkaloids with a third nitrogen atom from *Winchia calophylla*

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1. General experimental procedures

UV spectra were recorded on a Jasco V-550 UV/Vis spectrometer (Jasco, Tokyo, Japan). IR spectra (KBr disks, in cm^{-1}) were obtained using a Jasco FT/IR-480 Plus Fourier Transform spectrometer (Jasco, Tokyo, Japan). Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco, Tokyo, Japan) with a 1 cm cell at room temperature. Melting points were obtained on an X-5 micro-melting point apparatus (Fukai Instrument, Beijing, China) without correction. CD spectra were obtained on a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan) at room temperature. X-ray crystallographic analysis was carried out on an Agilent Gemini S Ultra CCD diffractometer with Cu Kα radiation (λ = 1.54178 Å). HR-ESI-MS spectra were acquired using an Agilent 6210 LC/MSD TOF-MS spectrometer (Agilent Technologies, CA, USA). NMR spectra were measured with a Bruker AV-400 spectrometer. Column chromatographies (CC) were performed on silica gel (200-300 mesh, Qingdao Marine Chemical Plant, China), ODS (Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Uppsala, Sweden). Preparative and semi-preparative HPLC were carried out using Agilent 1260 series instrument equipped with 1260 series multiple wavelength detector and Cosmosil $5C_{18}$-MS-II columns (250×20 mm; 250×10 mm). All solvents used in CC and HPLC were of analytical (Shanghai Chemical Plant, Shanghai, China) grade and chromatographic grade (Fisher Scientific, NJ, USA), respectively.

2. Plant material

The twigs and leaves of *W. calophylla* were collected from Haikou city, Hainan Province of China in November 2013, and authenticated by Prof. Guang-Xiong Zhou (Jinan University, Guangzhou, China). A voucher specimen (No. 2014112302) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, China.

3. Extraction and isolation of 1and 2

Air-dried, powdered twigs and leaves (16.0 kg) of *W. calophylla* were extracted with 95% EtOH under reflux to afford 1.2 kg of crude extract, which was suspended in H_2O and acidified with 5% HCl to pH 3. The solution was further partitioned with $CHCl_3$ to remove the neutral components. The aqueous layer was then basified with

$NH_3 \cdot H_2O$ to pH 9 and re-extracted with $CHCl_3$ to obtain a total alkaloid fraction. The $CHCl_3$ solution was concentrated to give a residue (215 g), which was subjected to silica gel column eluting with $CHCl_3$-CH_3OH to afford 8 fractions (Fr. A-H). Fr. G was further purified using silica gel column chromatography eluting with PE/EtOAc to give 8 subfractions (Fr. G1-G8). Fr. G5 was subjected to Sephadex LH-20 ($CHCl_3$-CH_3OH, 1:1), followed by preparative HPLC (CH_3OH-H_2O, 65:35) to yield **1** (10.8 mg). Fr. D was separated by silica gel column with $CHCl_3$/CH_3OH (100:0 → 90:10) as eluent to yield seven subfractions (Fr. D1-D7). Then, subfraction D3 was subjected to ODS column using MeOH/H_2O (70:30 → 100:0) as eluent and further purified by reversed-phase preparative HPLC (CH_3CN-H_2O, 70:30) to afford **2** (7.4 mg).

4. Physico-chemical constants of 1-2

Compound **1**: Colorless crystals ($CHCl_3$); m.p. 185~186 ℃; [α]25 D+52.7° (*c* 1.0, CH_3OH); UV (CH_3OH) λ_{max} nm: 207, 245, 302; IR (KBr) υ_{max} 3290, 2935, 2784, 1754, 1609, 1488, 1381, 1262, 1179, 1051, 742 cm^{-1}; HR-ESI-MS *m/z* 340.2027 $[M+H]^+$ (calcd for $C_{20}H_{26}N_3O_2$, 340.2020).

Compound **2**: White powder, m.p. 155~158 ℃; [α]25 D+19.2° (*c* 1.0, CH_3OH); UV (CH_3OH) λ_{max} nm: 206, 296; IR (KBr) υ_{max} 3323, 2937, 2790, 2362, 1680, 1540, 1508, 1457, 1383, 1328, 1179, 1141, 747 cm^{-1}; HR-ESI-MS *m/z* 308.2128 $[M+H]^+$ (calcd for $C_{20}H_{26}N_3$, 308.2121).

5. Single crystal X-ray crystallographic analysis of 1

The structure was solved by direct methods and refined by full-matrix least-squares on *F*2 using SHELXL-97 package software. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as deposition number CCDC 1451513.

X-ray crystallographic data of 1: $C_{20}H_{25}N_3O_2$, triclinic, *P*1, *a* = 7.8960(10) Å, *b* = 8.4207(7) Å, *c* = 14.3158(8) Å, β = 81.536(10)°, γ = 89.868(8)°, *V* = 929.27(17) $Å^3$, *T* = 173(2) K, *Z* = 2, *D*c = 1.328 mg/mm^3, *F*(000) = 400. The final refinement gave *R* = 0.0556, *R*w = 0.1540, *S* = 1.116 and Flack parameter = -0.4(3). Crystal data

of **1** were deposited in the Cambridge Crystallographic Data Centre (CCDC 1451513).

6. Quantum chemical ECD calculation method

Calculation details for 1 and 2: The conformational analysis of **1** and **2** were performed in Sybyl 8.0 program by using MMFF94s molecular mechanic force field. All of the obtained conformers were further optimized at B3LYP/6-31+G(d) level in Gaussian 09 package,[1] followed by the CD calculation of each single conformer by means of TDDFT methods at B3LYP/6-31+G(d) level. The overall calculated CD curves were obtained by means of Bolthzmann weighting of single CD data (with a half-bandwidth of 0.3 eV). The calculated ECD spectra of **1** and **2** were subsequently compared with the experimental ones. The ECD spectra were produced by SpecDis 1.60 software. [2]

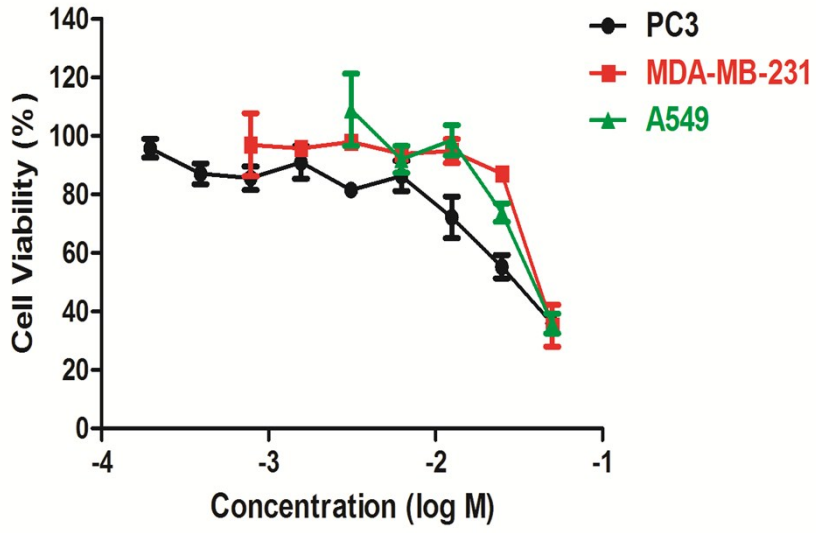
[1] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, **2009**.

[2] T. Bruhn, A. Schaumlöffel, Y. Hemberger, G. Bringmann, SpecDis version 1.60, University of Wuerzburg, Germany, **2012**.

7. Cytotoxicity assay

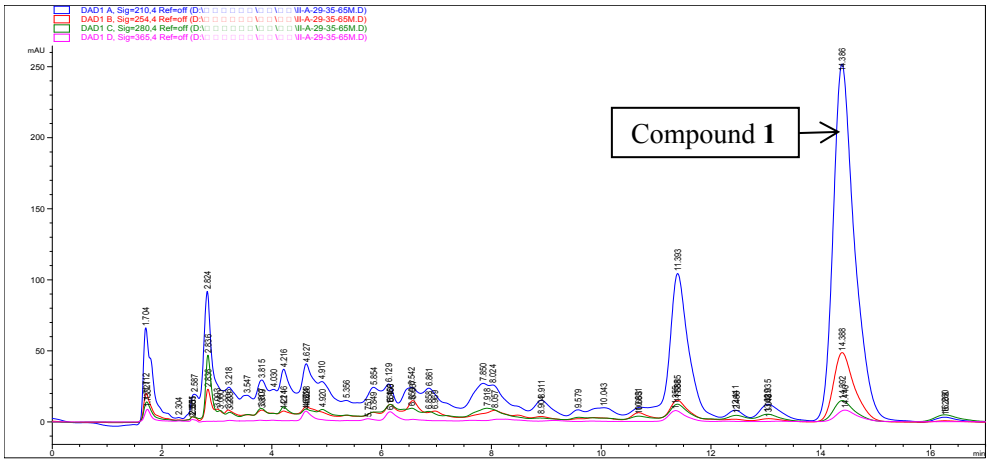
The human hepatocellular carcinoma cell line HepG2, human breast cancer cell line MDA-MB-231, human prostate cancer cell line PC-3, and human lung cancer A-549 cell lines were obtained from the American Type Culture Collection (ATCC). All of these cell lines were cultured in RPMI 1640 medium, supplemented with 10% FBS (v/v) at 37 °C in a humidified atmosphere of 5% CO_2 (v/v). Cells were cultured in 96-well plates for 24 h. Then the cells were treated with compounds **1**−**5** at various concentrations for 72 h. After incubated for another 4 h with 30 μL aliquot of MTT solution (5 mg/ml in PBS), the medium was discarded, and 100 μL of DMSO was added to dissolve the produced formazan. The absorbance was measured at 570 nm using a microplate Reader (Thermo scientific multiskan MK3, USA). Each well was performed in triplicate in 3 independent experiments. The concentration giving 50% inhibition (IC_{50}) was determined from the dose−response curves using Prism software and expressed as the mean ± SD. Doxorubicin (Sigma, USA) was used as the positive control.

Figure S1 The viability of MDA-MB-231, PC3, and A549 cells treated with compound **2** at the indicated concentrations for 72h was determined using an MTT assay.



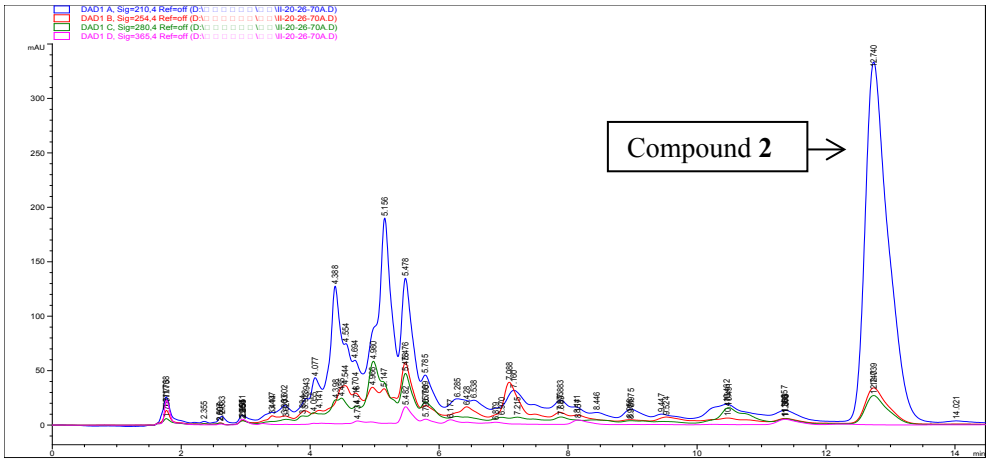
8. HPLC analyse of fractions which compounds 1 and 2 are collected.

Figure S2: Fraction of compound **1**:



CH_3OH-H_2O, 65:35 Rt: 14.4 min

Figure S3: Fraction of compound **2**:



CH_3CN-H_2O, 70:30 Rt: 12.7 min

9. UV, IR, HRESIMS, CD and NMR spectra of 1-2

Figure S4 UV spectrum of **1** (MeOH)

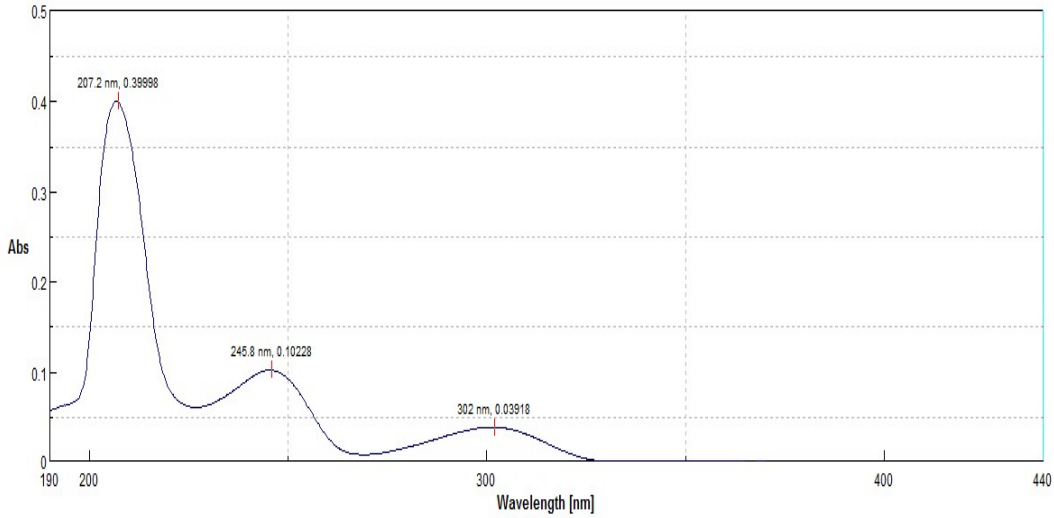
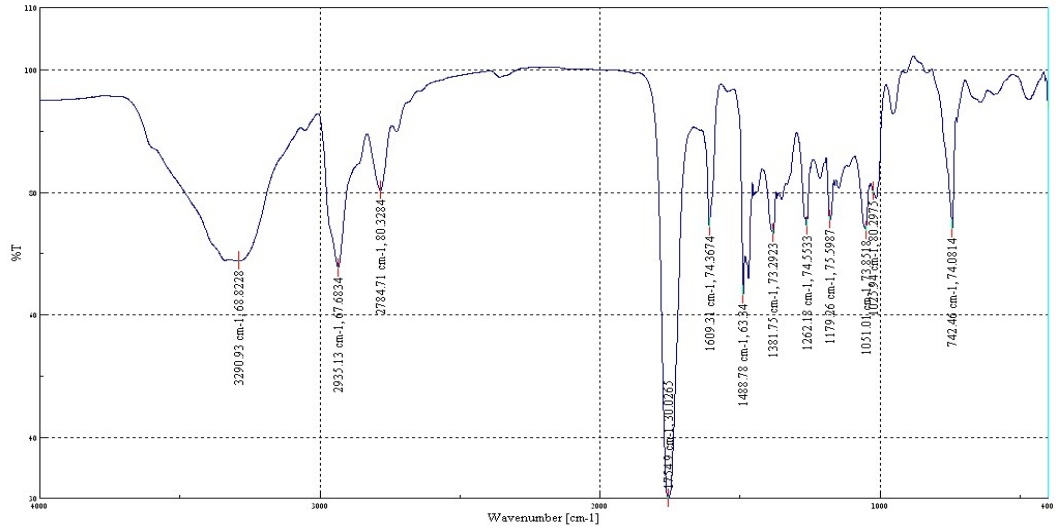


Figure S5 IR spectrum of **1** (KBr)



3290 cm^{-1}	ν -C=O-NH_2-
2935 cm^{-1}	ν –CH_3
2784 cm^{-1}	ν –CH_2
1754 cm^{-1}	ν –C=O
1609, 1488 cm^{-1}	ν –C=C-
1381 cm^{-1}	δ -CH-
1262 cm^{-1}	ν –C-O
1179, 1051, 1025, 742 cm^{-1}	δ –CH-

Figure S6 ^{1}H NMR spectrum of **1** (500 Hz, $CDCl_3$)

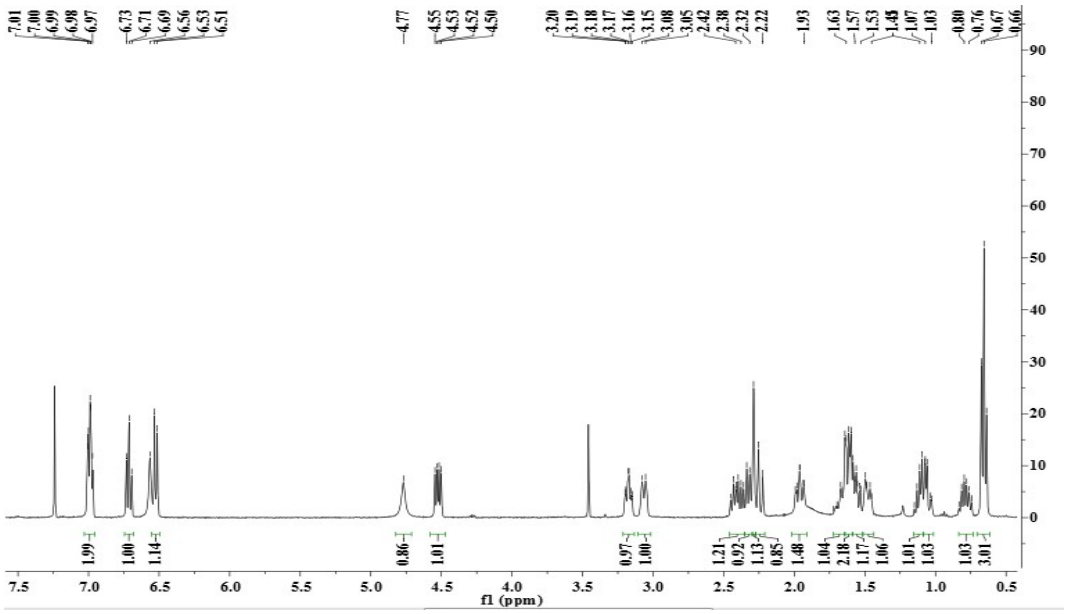


Figure S7 ^{13}C NMR spectrum of **2** (125 Hz, $CDCl_3$)

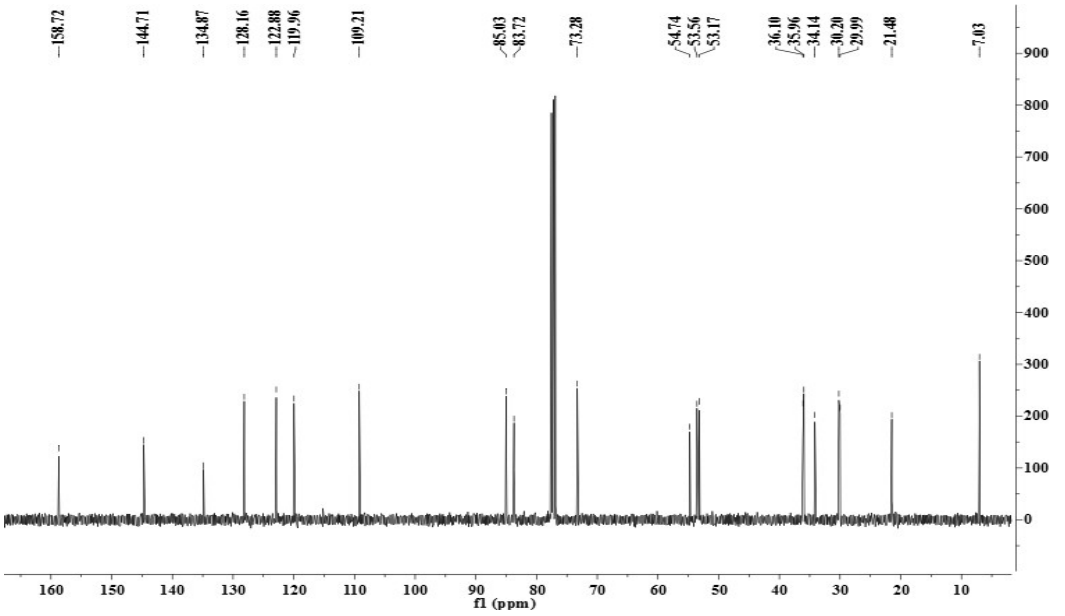


Figure S8 ^{1}H-^{1}H COSY spectrum of **1** ($CDCl_3$)

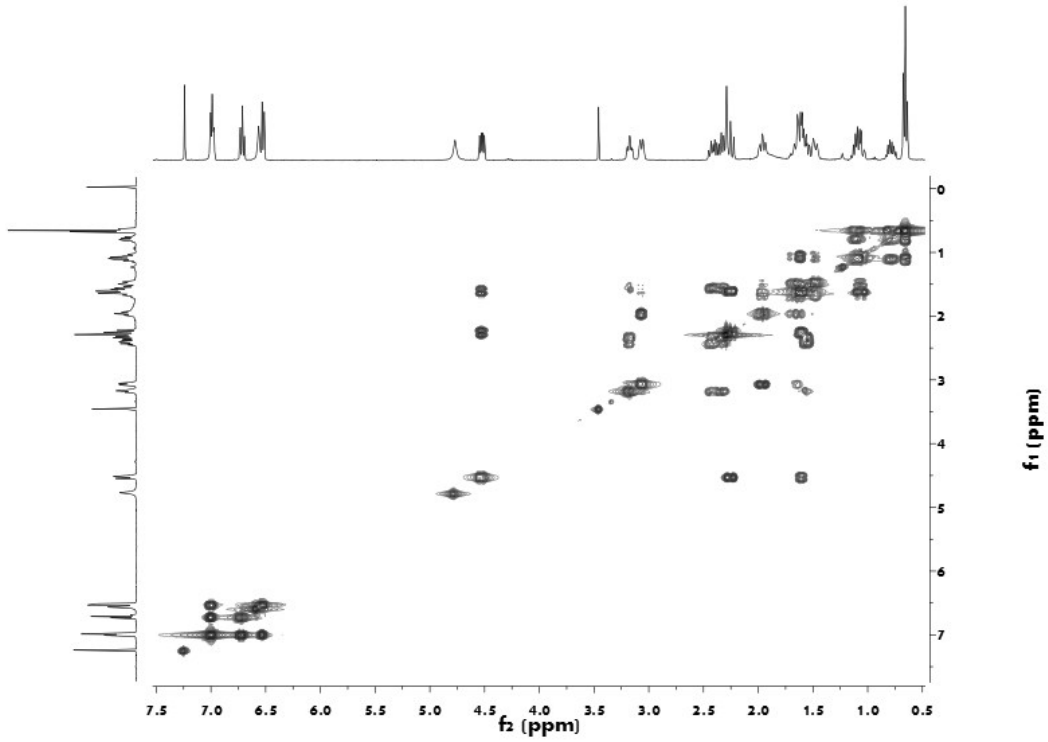


Figure S9 HSQC spectrum of **1** ($CDCl_3$)

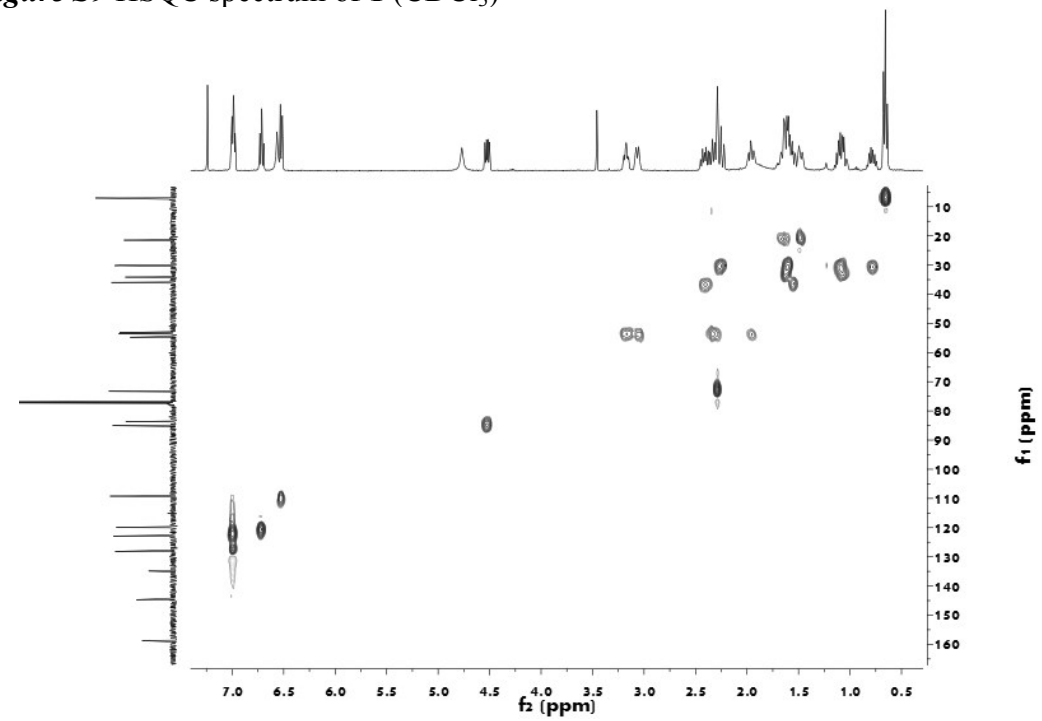


Figure S10 HMBC spectrum of **1** ($CDCl_3$)

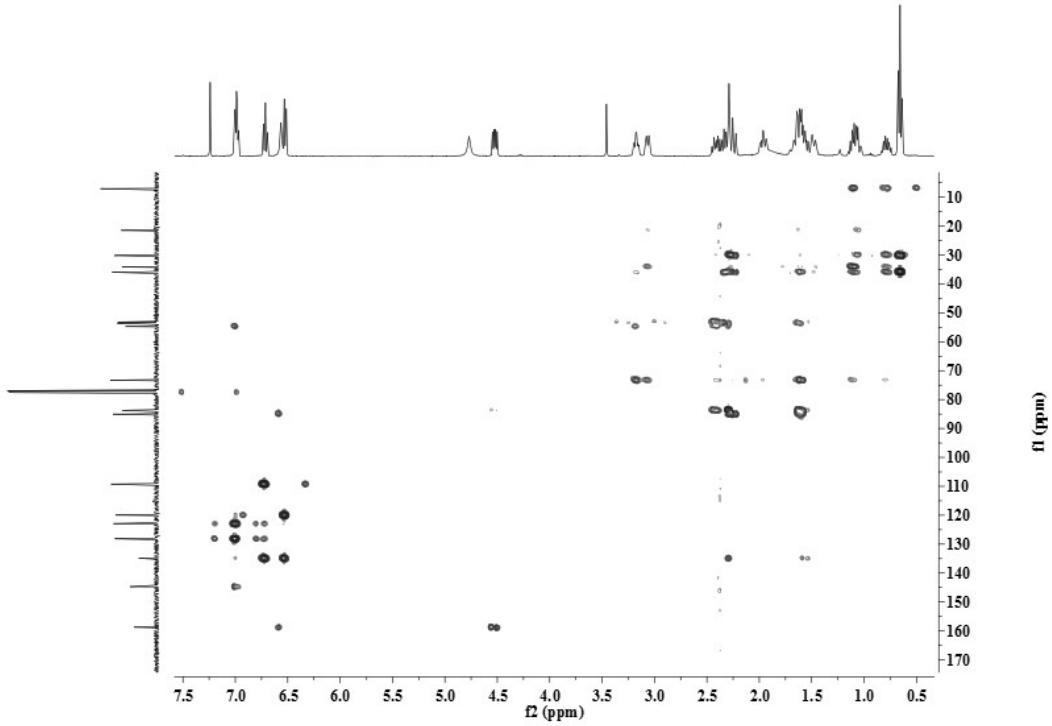


Figure S11 NOESY spectrum of **1** ($CDCl_3$)

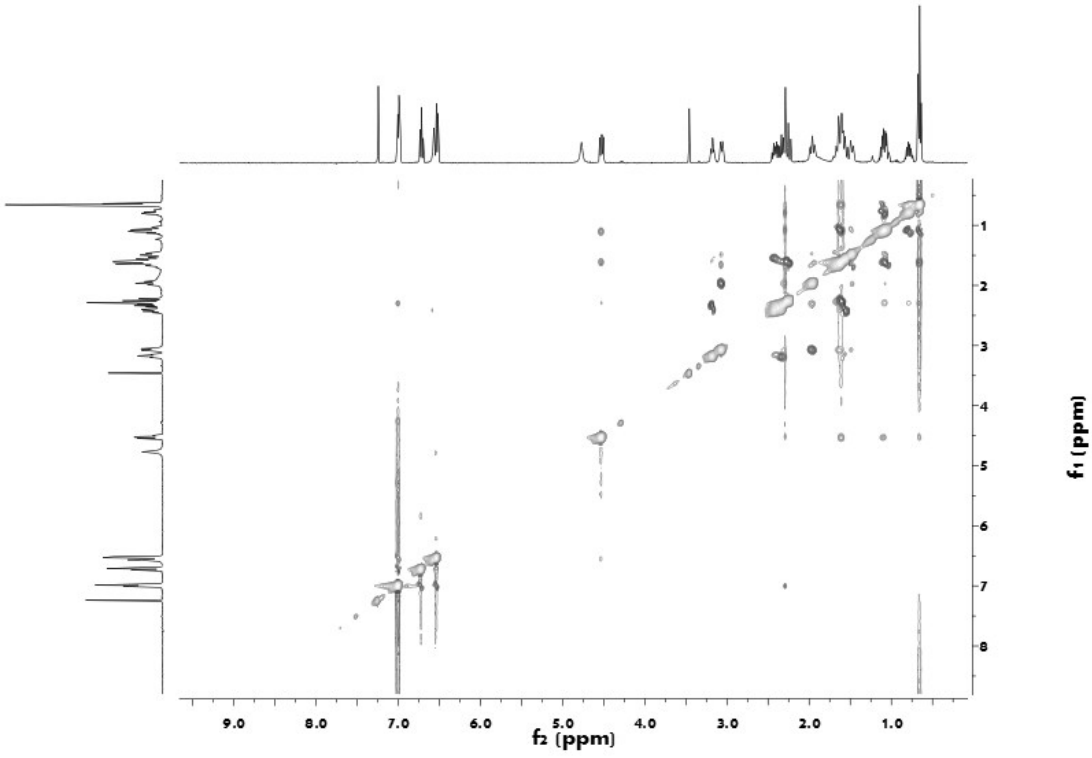


Figure S12 HR-ESI-MS spectrum of **1**

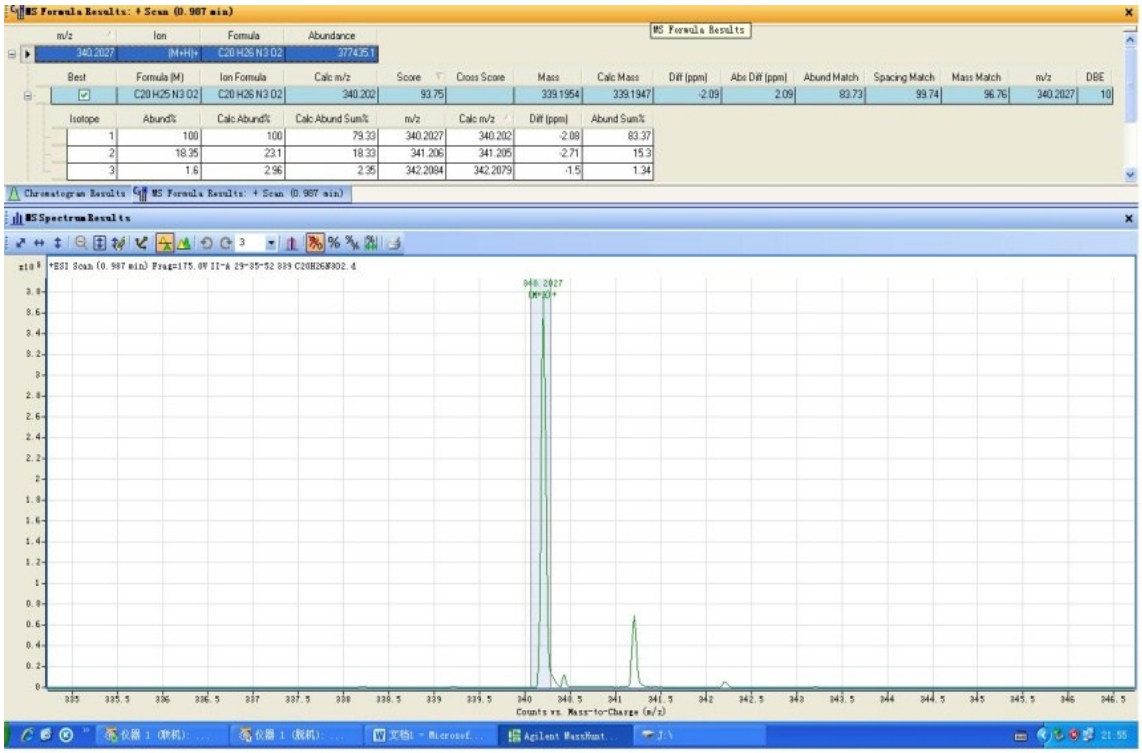


Figure S13 CD spectrum of **1** (MeCN)

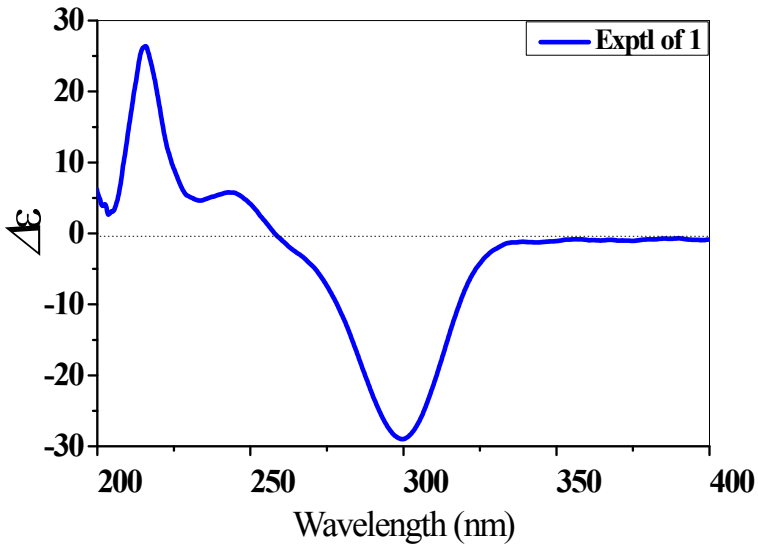


Figure S14 UV spectrum of **2** (MeOH)

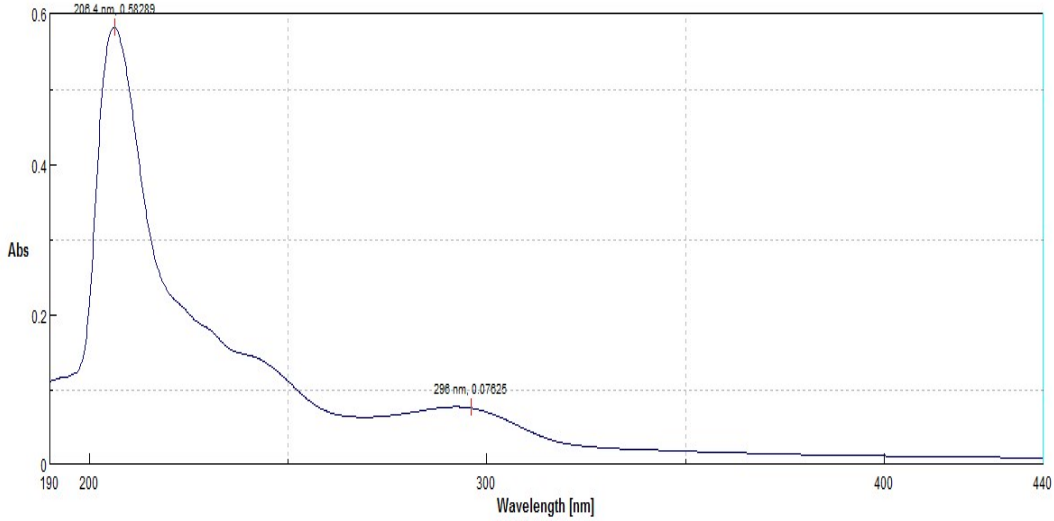
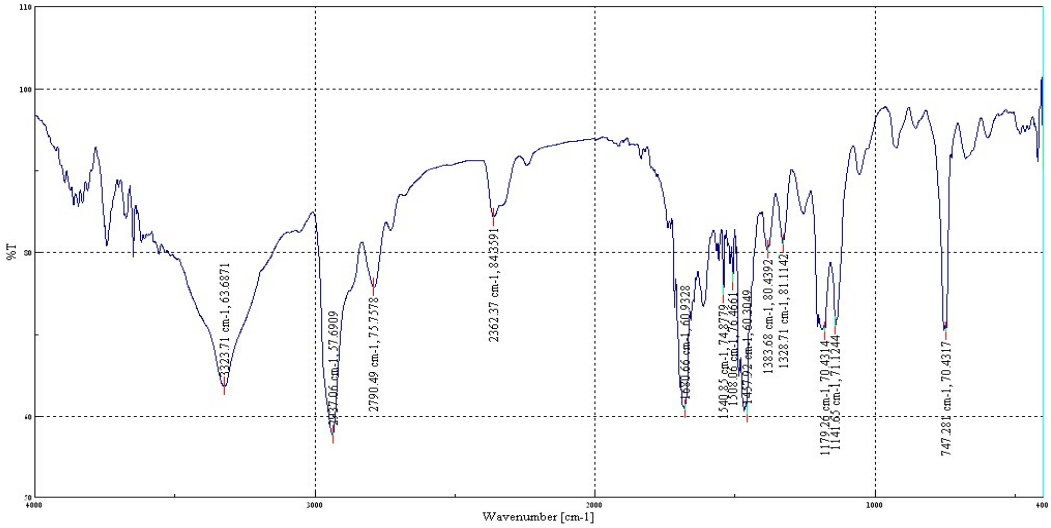


Figure S15 IR spectrum of **2** (KBr)



3323 cm^{-1}	ν –NH
2937 cm^{-1}	ν –CH_3
2790 cm^{-1}	ν –CH_2
2362 cm^{-1}	ν –CN
1680, 1540, 1457 cm^{-1}	ν –C=C-
1383 cm^{-1}	δ -CH-
1179, 1141, 747 cm^{-1}	δ –CH-

Figure S16 ^{1}H NMR spectrum of **2** (400 Hz, $CDCl_3$)

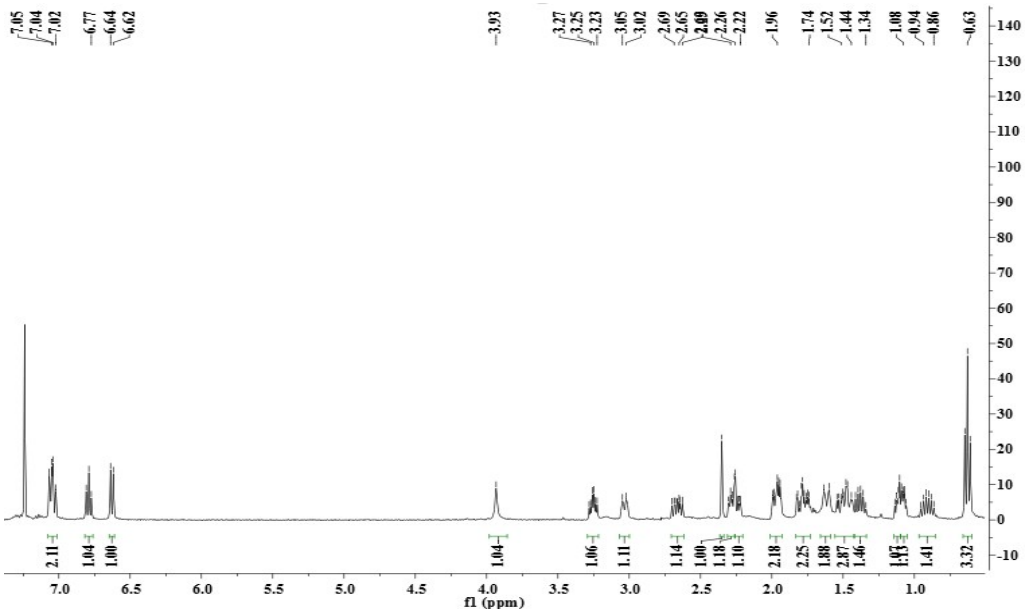


Figure S17 ^{13}C NMR spectrum of **2** (100 Hz, $CDCl_3$)**)**

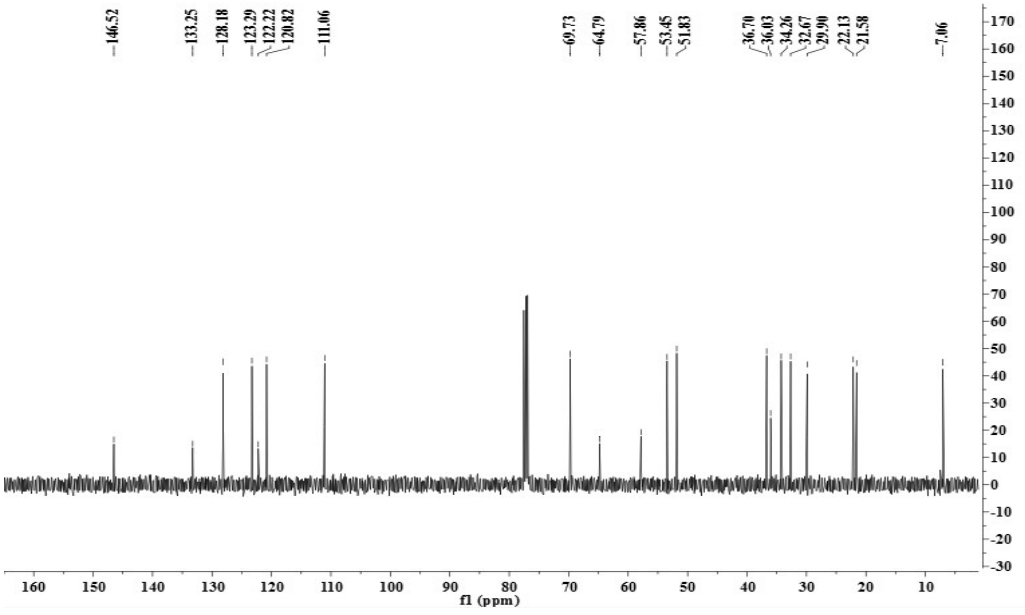


Figure S18 ^{1}H-^{1}H COSY spectrum of **2** ($CDCl_3$)

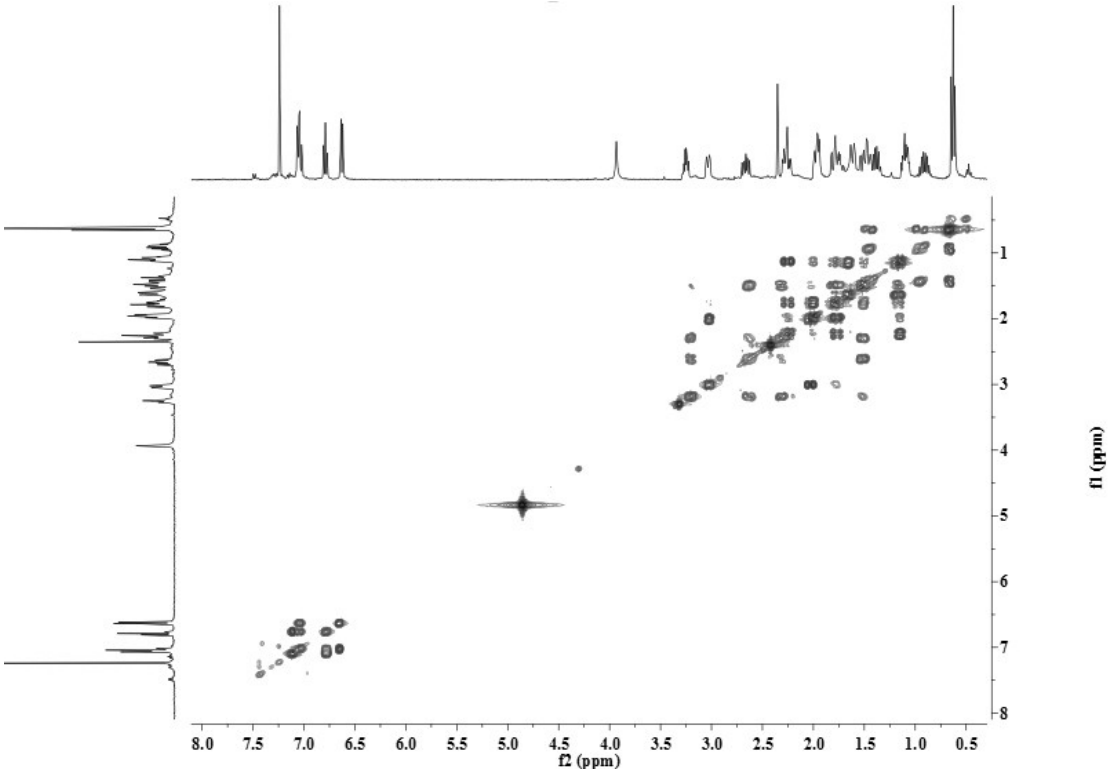


Figure S19 HSQC spectrum of **2** ($CDCl_3$)

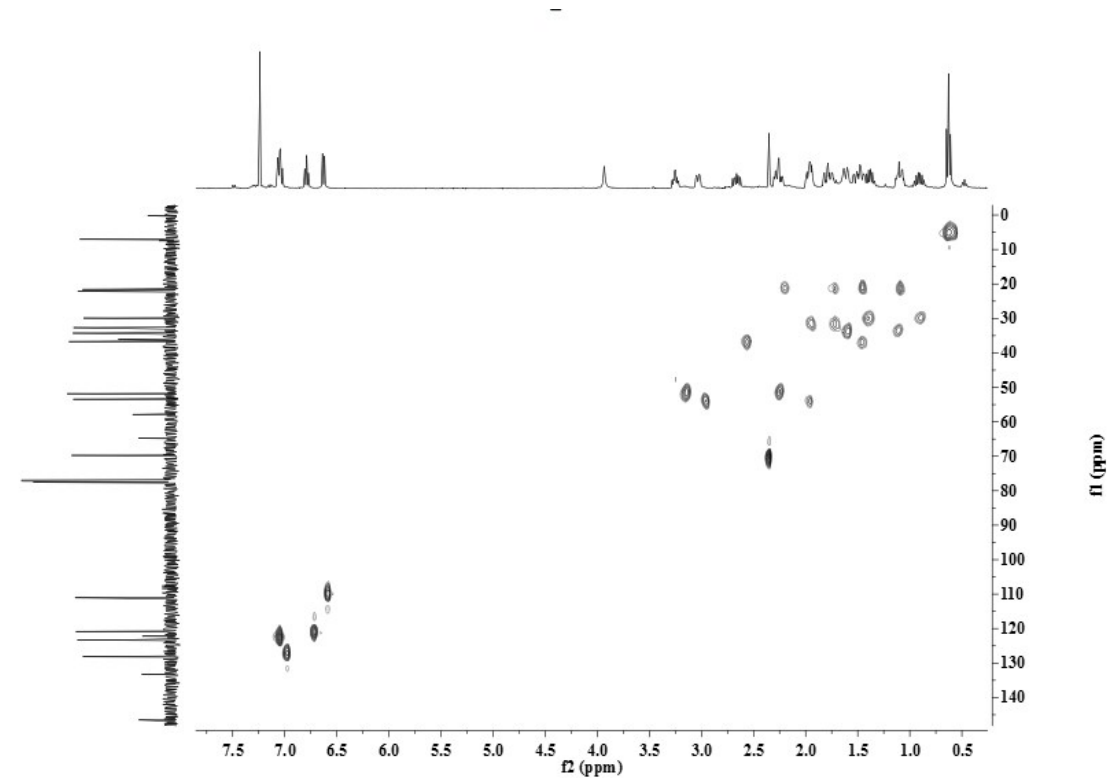


Figure S20 HMBC spectrum of **2** ($CDCl_3$)

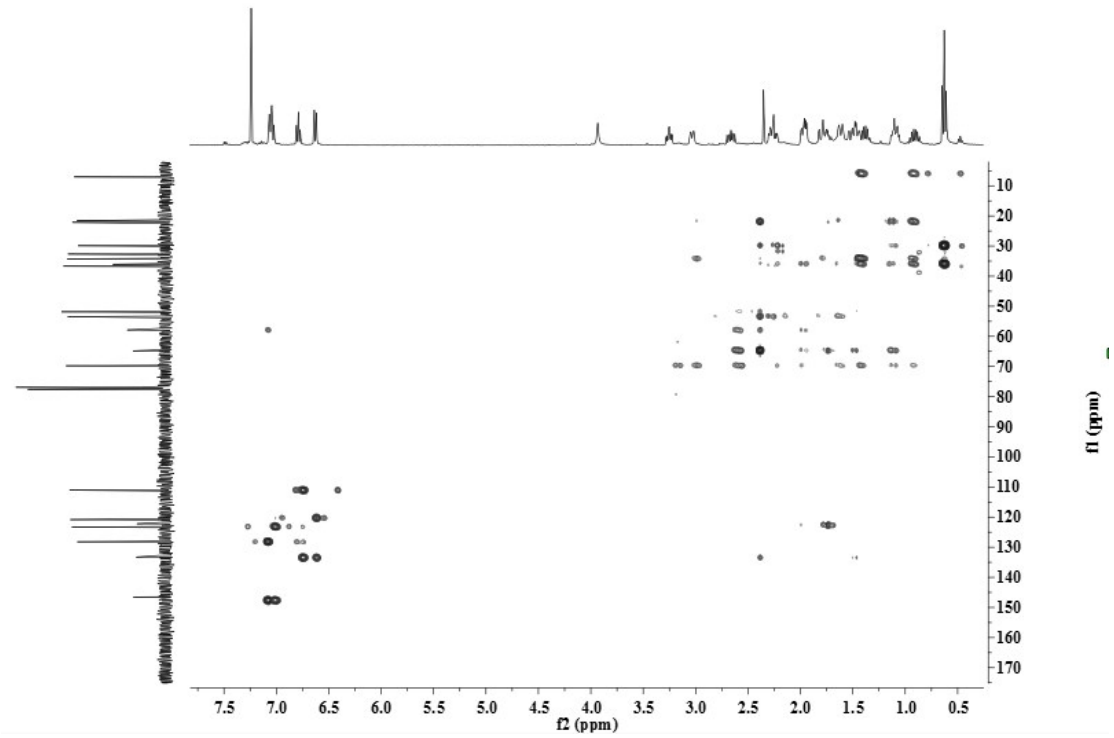


Figure S21 NOESY spectrum of **2** ($CDCl_3$)

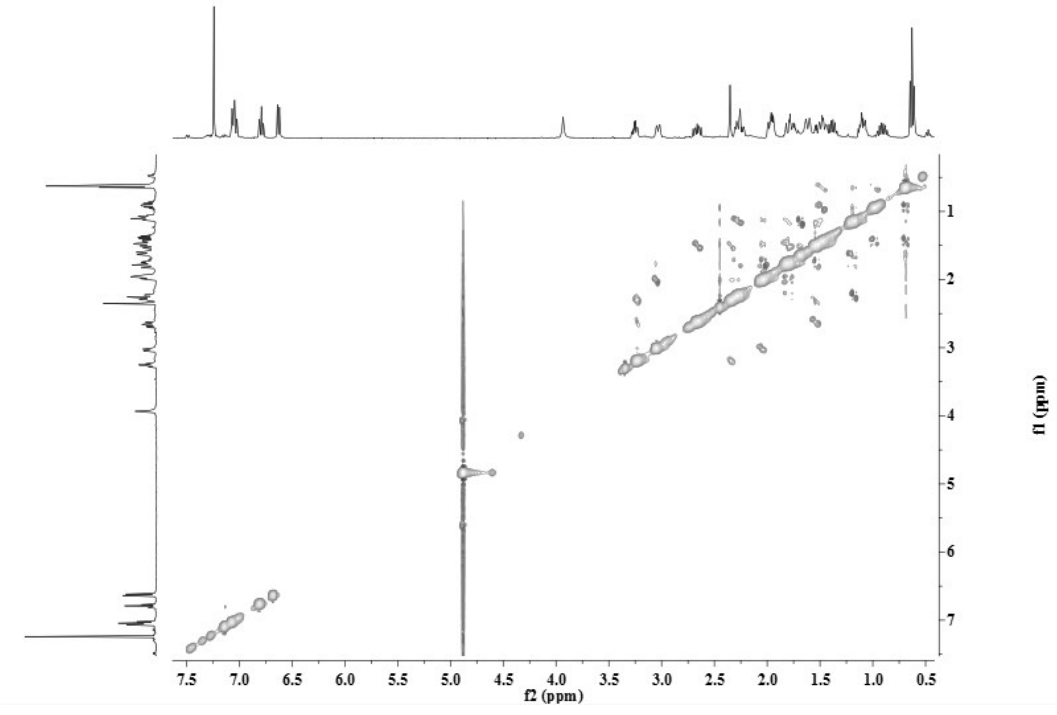


Figure S22 HR-ESI-MS of **2**

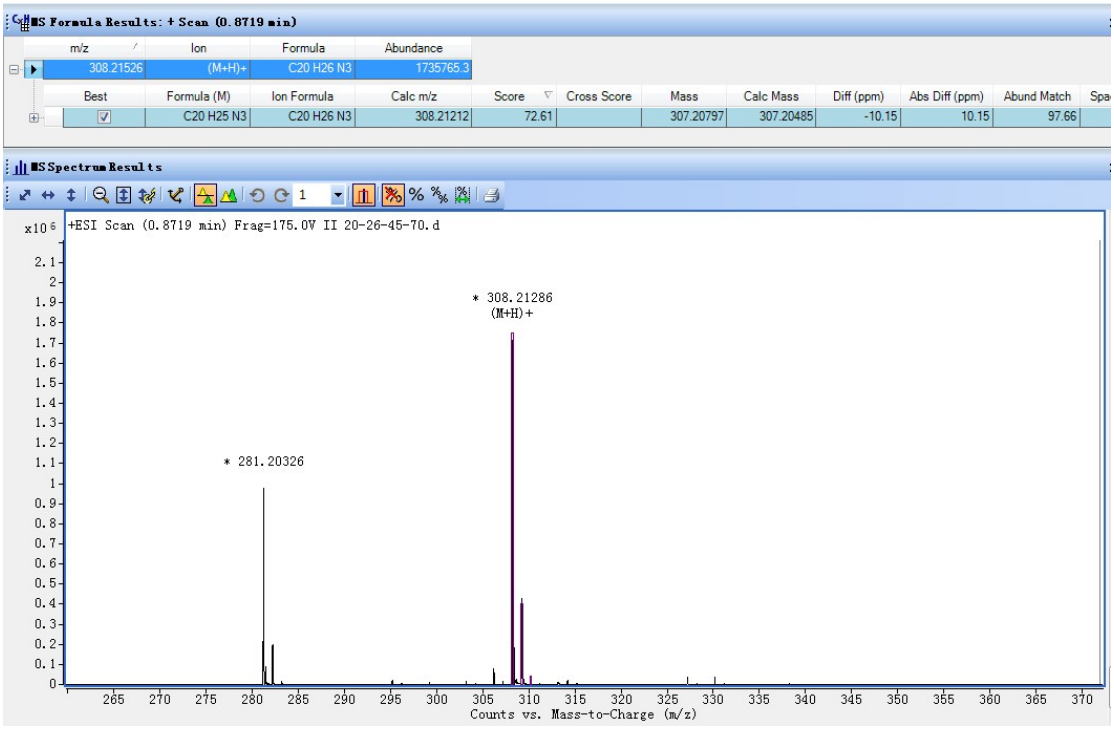


Figure S23 CD spectrum of **2** (MeCN)