Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Lecanicillones A–C, Three Dimeric Isomers of Spiciferone A with Cyclobutane Ring from an Entomopathogenic Fungus *Lecanicillium* sp. PR-M-3

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Contents

1. General experimental procedures

Optical rotation was measured on Rudolph Autopol-V digital polarimeter and Jasco P-2000 polarimeter. UV spectrum was recorded using a Shimadzu UV-2201 spectrometer. IR spectra were recorded on a Bruker IFS-55 spectrometer (using a KBr disk method). CD spectra were measured on Bio-logic MOS 450 spectropolarimeter. 1D and 2D NMR spectra were acquired with Bruker ARX-300 and AV-600 NMR spectrometers using solvent signals ($CDCl_3$: δ_H 7.26/δ_C 77.16), with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained using Varian QFT-ESI and Bruker micro-TOFQ-Q mass spectrometer (for HRESIMS). Single crystal X-ray crystallography was determined on Gemini E X-ray single crystal diffractometer.

Column chromatography (CC) was performed with silica (100-200 and 200-300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) and Sephadex LH-20 (GE Healthcare, Sweden).

2. Fungal material and fermentation

The fungal strain PR-M-3 was isolated from the soil of a garden of puer tea at Puer in Yunnan, P.R. China in September 2011. It was identified as *Lecanicillium* sp. (GenBank accession no. KP260559) and has been deposited in the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

The fungus PR-M-3 was cultured on a rotary shaker (180 rpm) at 28 °C for 7 days in liquid medium (containing mannitol 2%, D-glucose 2%, yeast extract 0.5%, peptone 0.5%, KH_2PO_4 0.05%, $MgSO_4$ 0.03%, corn syrup 0.1%).

3. Extraction and isolation

The fermentation broth (120 L) of the strain was concentrated and extracted with ethyl acetate and *n*-butanol, successively. The EtOAc crude extracts (35.0 g) were applied on a silica gel column and eluted with Petroleum-Acetone gradient (from 100:0 to 0:100) to afford 14 fractions. Fr. 4 (0.5133 g) was further purified using silica gel column chromatography eluting with Petroleum-ethyl acetate (from 100:0 to 0:100) to give 10 subfractions. Fr. 3 (0.8762 g) was further purified using silica gel column

chromatography eluting with Petroleum-Acetone (from 100:0 to 0:100) to give 8 subfractions. Fr.3-2 was further purified using preparative thin layer chromatography with Petroleum-Acetone (5:1) to give 4 subfractions. Fr.3-2-2 purified by semi-preparative HPLC on ODS column eluted with 53% MeOH-H_2O to yield compound **4** (56.2 mg). Fr.3-2-4 purified by semi-preparative HPLC on ODS column eluted with 62% MeOH-H_2O to yield compounds **3** (7.2 mg), **1** (106.2 mg), and **2** (3.2 mg).

Physicochemical data

Lecanicillone A (**1**): a colorless block crystal (EtOAc); $[\alpha]_D^{20}$ (*c* 1.08, MeOH) +170.3; UV (MeOH) λ_{max} (log ε): 260 (4.1), 214 (4.0), 204 (3.9) nm; IR (KBr) ν_{max}: 1717, 1662, 1625, 1434, 1174 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) : 194 (+2.6), 202 (−14.4), 227 (+3.8), 262 (+28.6) 293 (−3.6) nm; ^{13}C and ^{1}H NMR data, see Table 1; (+)-HRESIMS *m/z* 465.2276 $[M+H]^+$ (calcd for $C_{28}H_{32}O_6$, 465.2272);

Lecanicillone B (**2**): a white amphorous powder; $[\alpha]_D^{20}$ (*c* 0.15, MeOH) −107.0; UV (MeOH) λ_{max} (log ε): 260 (4.2), 214 (4.1), 204 (4.0) nm; IR (KBr) ν_{max}: 1716, 1663, 1625, 1432, 1176cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) : 194 (−5.2), 207 (+14.2), 221 (−1.8), 260 (−21.3) 290 (+4.4) nm; ^{13}C and ^{1}H NMR data, see Table 1; (+)-HRESIMS *m/z* 465.2257 $[M+H]^+$ (calcd for $C_{28}H_{32}O_6$, 465.2272);

Lecanicillone C (**3**): a white amphorous powder; $[\alpha]_D^{20}$ (*c* 0.11, MeOH) +28; UV (MeOH) λ_{max} (log ε): 256 (4.1), 207(4.0) nm; IR (KBr) ν_{max}: 1713, 1659, 1619, 1429, 1184cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) : 200 (+4.1), 219 (+12.4), 254 (+0.3), 273 (+1.3) nm; ^{13}C and ^{1}H NMR data, see Table 1; (+)-HRESIMS *m/z* 465.2262 $[M+H]^+$ (calcd for $C_{28}H_{32}O_6$, 465.2272);

4. Bioassays

(1) Cytotoxic assay

Cytotoxic activities of isolated compounds **1**−**4** and the positive control 5-fluorouracil were evaluated by the trypan blue method[1, 2] against the human leukaemia cell lines (HL-60), and the MTT assay[3, 4] against the human colon cancer cells lines (HCT-116),

and the human pancreatic cancer cell lines (ASPC1). The cell lines were purchased from America Type Culture Collection, ATCC (Rockville, MD, USA) and cultured in RPMI-1640 medium (Gibco, New York, NY, USA) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM glutamine and 10% heat-inactivated foetal bovine serum (Gibco) at 37 °C in humidified atmosphere with 5% CO_2.

Human leukemia HL-60 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in the above medium at a density of 5×10^4 cells/mL at 37 under an atmosphere of 5% CO_2. Cell growth inhibition assay was performed as reported previously. The compounds were dissolved in DMSO, and the amount of DMSO was controlled lower than 0.1% in the final concentration. Cells were incubated with various drug concentrations for 3 days. The number of cells was determined by hemocytometer, and its viability was determined using trypan blue staining. The growth inhibitory ability of the compound was calculated and expressed using the IC_{50} value (half-inhibitory concentration). 5-Fluorouracil (5-FU) and 0.1% DMSO were used as a positive control and a negative control, respectively.

In the MTT assay, briefly, cells suspensions, 200 μl, at a density of 2.5×104 cells/mL, were plated in 96-well microtiter plates and incubated for 24 h at 37 °C under 5% CO_2 and 95% air. Then the test compounds with different concentrations in DMSO were placed into each microtiter plates and further incubated for 72 h. Finally, 50 μl of a 0.4% MTT solution was added to each well and incubated for 4 h. Then, the MTT was removed from the wells and the fromazan crystals were dissolved in DMSO (200 μL) for 10 min with shaking. Then the plate was read immediately on a microtiter plate reader (Bio-RAD) at a wavelength of 570 nm to record the optical density (OD). The IC50 value was defined as the concentration of the control in the MTT assay. 5-Fluorouracil (5-Fu) was used as a positive control.

References:

[1] F. Wang, H. M. Hua, Y. H. Pei, D. Chen, and Y. K. Jing, *J. Nat. Prod.*, 2006, 69, 807-810.

[2] J. Hu, X. D. Shi, J. G. Chen, X. Mao, L. Zhu, L. YU, and J. Y. Shi, *Food Chem*., 2014, 148, 437–444.

[3] K. B. Wang, Y. T. Di, Y. Bao, C.M. Yuan, G. Chen, D. H. Li, J. Bai, H. P. He, X. J. Hao, Y. H. Pei, Y. K. Jing, Z. L. Li, and H. M. Hua, *Org. Lett*., 2014, 16, 4028−4031.

[4] K. B. Wang, C. M. Yuan, C. M. Xue, D. H, Li, Y. K. Jing, H. P. He, X. J. Hao, Y. T. Di, Z. L. Li, and H. M. Hua, *RSC Adv*., 2014, 4, 53725–53729.

Table S1. The *in vitro* cytotoxic activities against HL-60, HCT-116 and ASPC1 cancer cell lines

Compounds	HL-60 IC_{50} (μM)	HCT-116 IC_{50} (μM)	ASPC1 IC_{50} (μM)
1	47.8	>100	>100
2	89.0	>100	>100
3	53.0	>100	>100
4	>100	31.8	32.3
5-Fu	2.80	15.6	2.7

(2) Insecticidal assay

a. Test against diamondback moth (*Plutella xylostella*)

The leaves of cabbage grown in greenhouse were chosen, removed the surface waxy layer and perforate to get 2cm diameter leaf discs by the hole puncher. The solution of the test compound (600 mg/L) was sprayed using Airbrush, placed in 9cm diameter Petri dish and dried at room temperature. Seven regular health insects (third instar) were introduced on each treatment. Each treatment was repeated for 4 times. The pure water was set as CK. The treated insects were placed in a chamber of 25±1°C, 60%-70% relative humidity and day light. After 72h, the number of surviving insects was investigated and the mortality was calculated.

b. Test against army worm (*Mythimna separate*)

The middle part of fresh corn leaves were chosen and cut into 5cm sects. The solution of the test compound (600 mg/L) was sprayed using Airbrush, placed in 9cm diameter Petri dish and dried at room temperature. Seven regular health insects (third instar) were introduced on each treatment. Each treatment was repeated for 4 times. The pure water was set as CK. The treated insects were placed in a chamber of 25±1°C, 60%-70% relative humidity and day light. After 72h, the number of surviving insects was investigated and the mortality was calculated.

c. Test against peach aphid (*Myzus persicae*)

The cabbage leaves with 30-50 peach aphids were taken, the solution of the test

compound (600 mg/L) was sprayed using Airbrush, placed in 9cm diameter Petri dish and dried at room temperature. Each treatment was repeated for 4 times. The pure water was set as CK. The treated insects were placed in a chamber of 25±1°C, 60%-70% relative humidity and day light. After 48h, the survival peach aphids were observed and the mortality was determined.

d. Test against spider mite (*Tetranychus cinnabarinus*)

The adult spider mites were treated into two true leaves of bean plants. After investigated the number of mites, the solution of the test compound (600 mg/L) was sprayed using Airbrush and repeated for 4 times. The pure water was set as CK. The treated bean plants were placed in a chamber of 25±1°C, 60%-70% relative humidity and day light. After 72h, the number of surviving mites was investigated and the mortality was calculated.

5. The spectra of lecanicillone A (1)

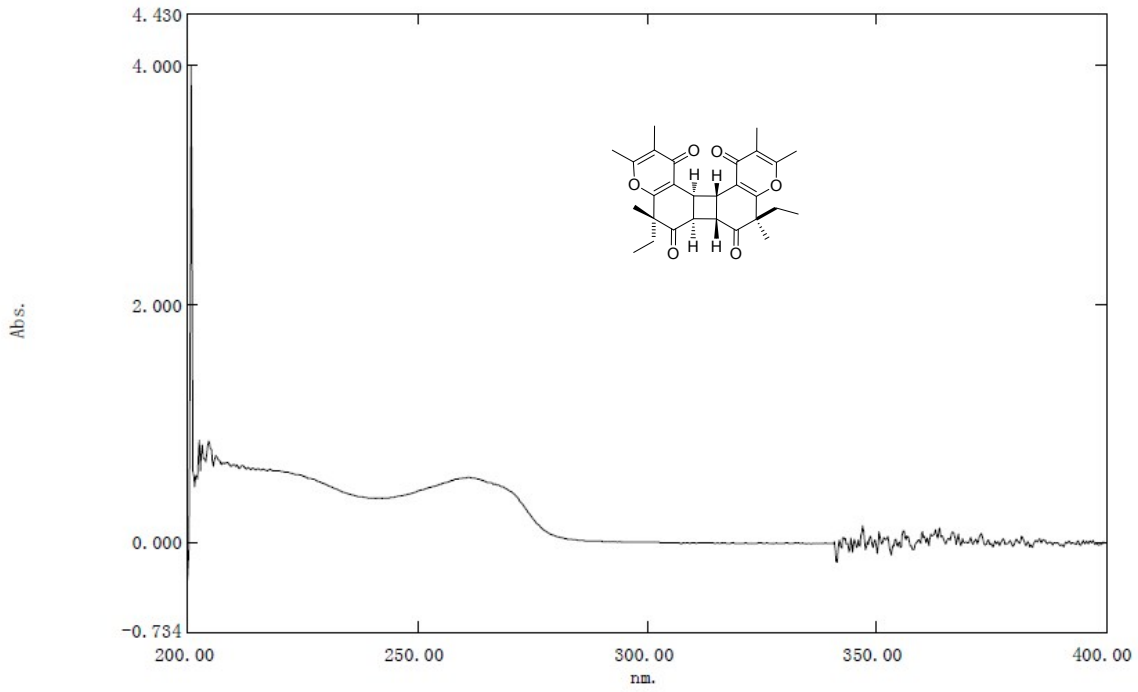
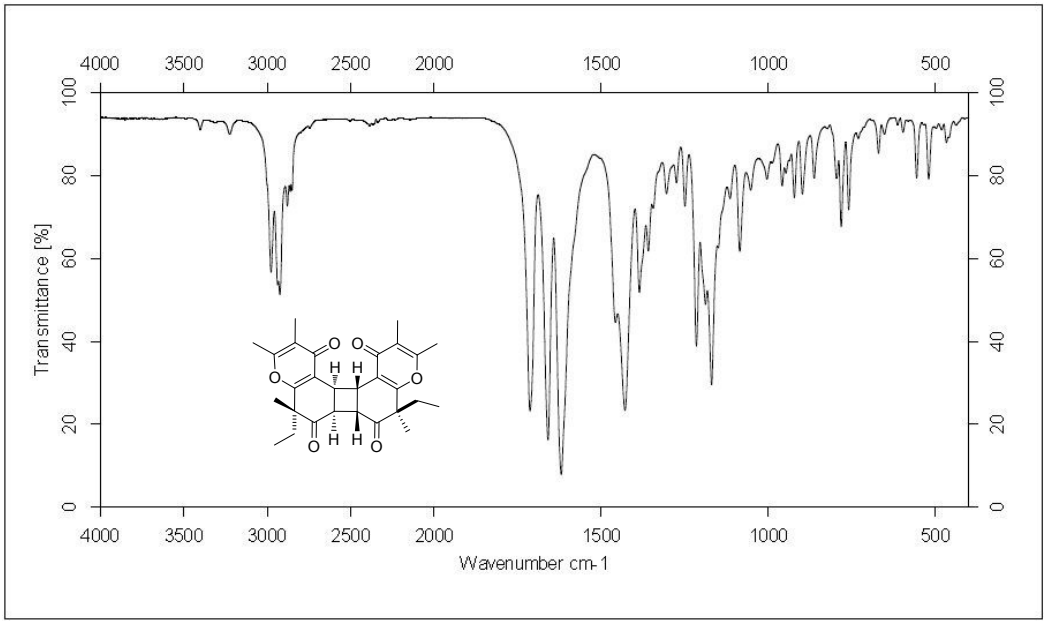


Figure S1. The UV spectrum of compound **1**



Sample : WJ-17-2	Frequency Range : 3999.64 - 400.157	Measured on : 2014-3-13	
Technique : Sample form	Resolution : 2	Instrument : EQUINOX55	Sample Scans : 16
Customer : Default	Zerofilling : 2	Acquisition : Double Sided,For	

Figure S2. The IR spectrum of compound **1**

Mass Spectrum Molecular Formula Report

Analysis Info

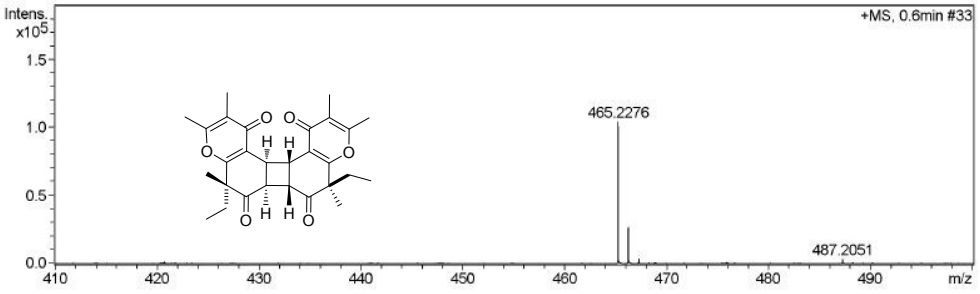
Analysis Name	D:\Data\20131031-CEYANG\WJ-17_1-c,7_01_1993.d	Acquisition Date	10/31/2013 4:18:20 PM
Method	ldj_bga_jh.m	Operator	Bruker Customer
Sample Name	WJ-17	Instrument / Ser#	micrOTOF-Q 125
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C28H32O6H						
Formula, max.							
Measured m/z	465.228	Tolerance	5	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nirogen Rule	no	Electron Configuration	both				
Filter H/C Ratio	no	Minimum	0		Maximum	3	
Estimate Carbon	yes						



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 28 H 33 O 6	0.029	465.2272	-0.88	2.45	-0.41	12.50	ok	even

Figure S3. The HR-ESI-MS spectrum of compound **1**

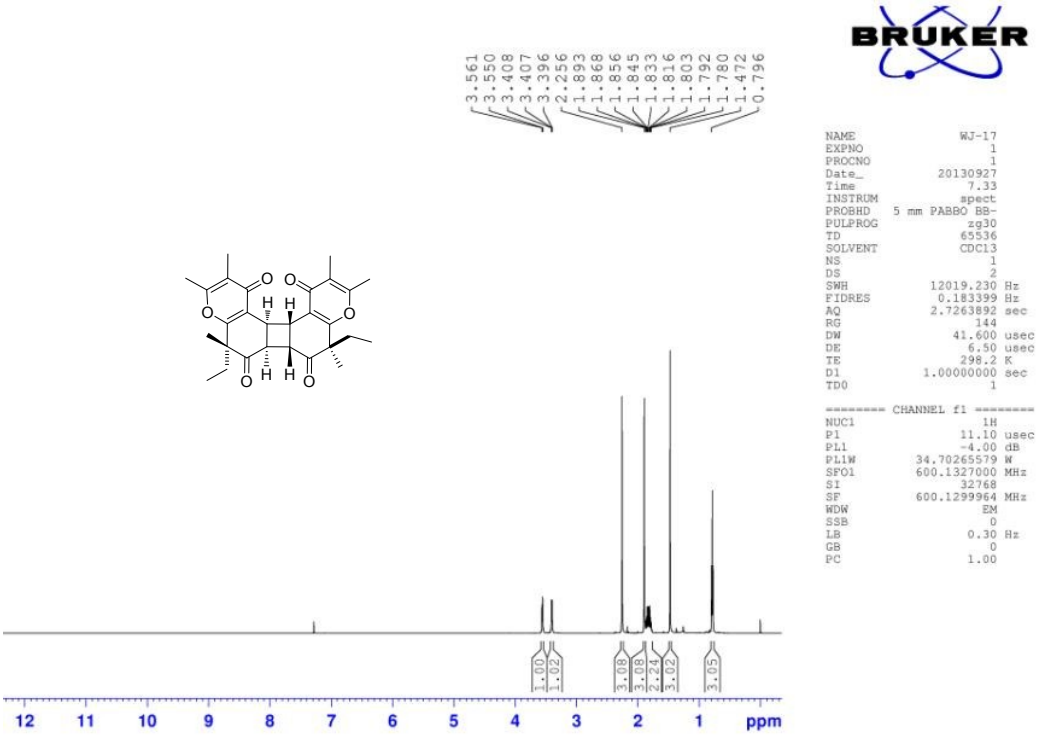


Figure S4. The ^{1}H-NMR spectrum of compound **1**

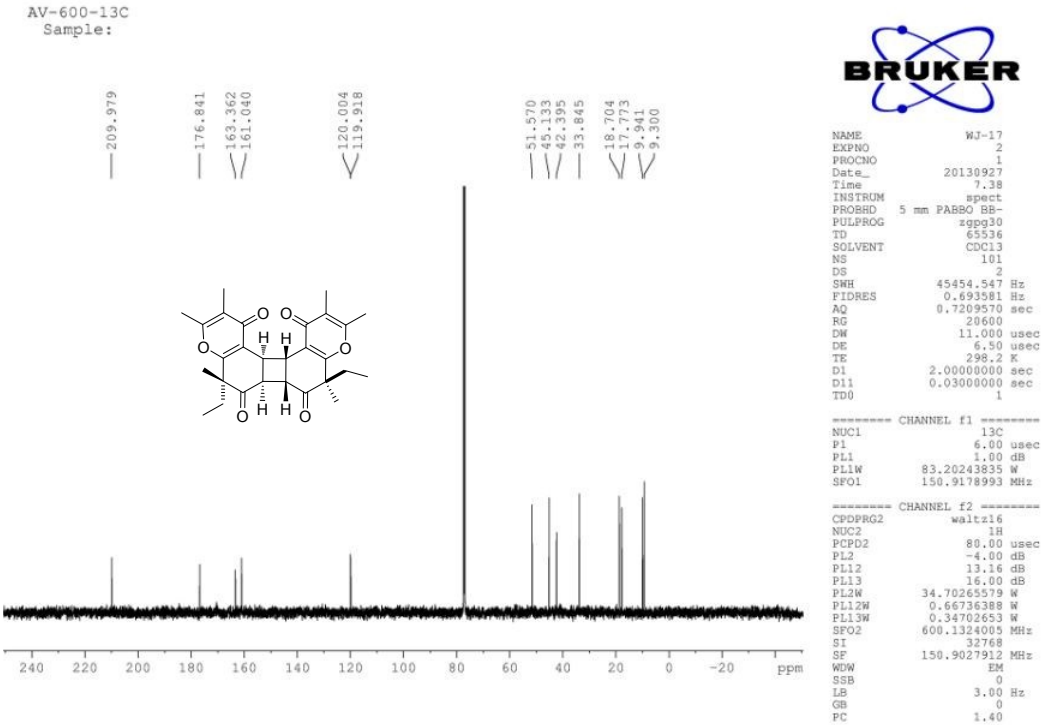


Figure S5. The ^{13}C-NMR spectrum of compound **1**

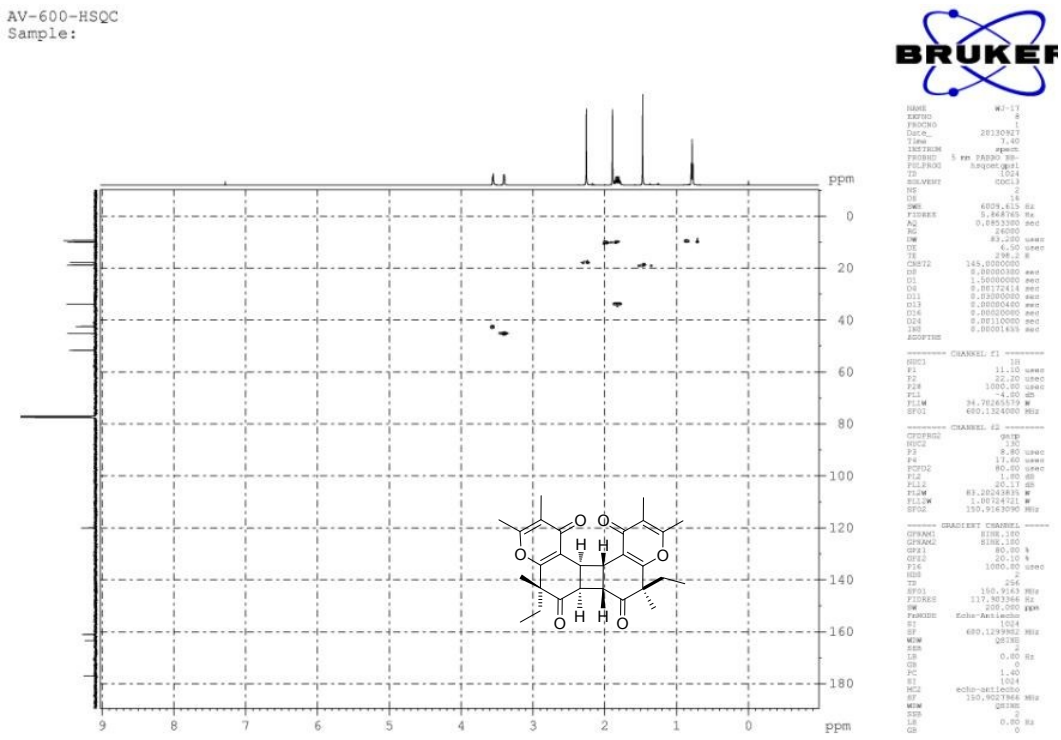


Figure S6. The HSQC spectrum of compound **1**

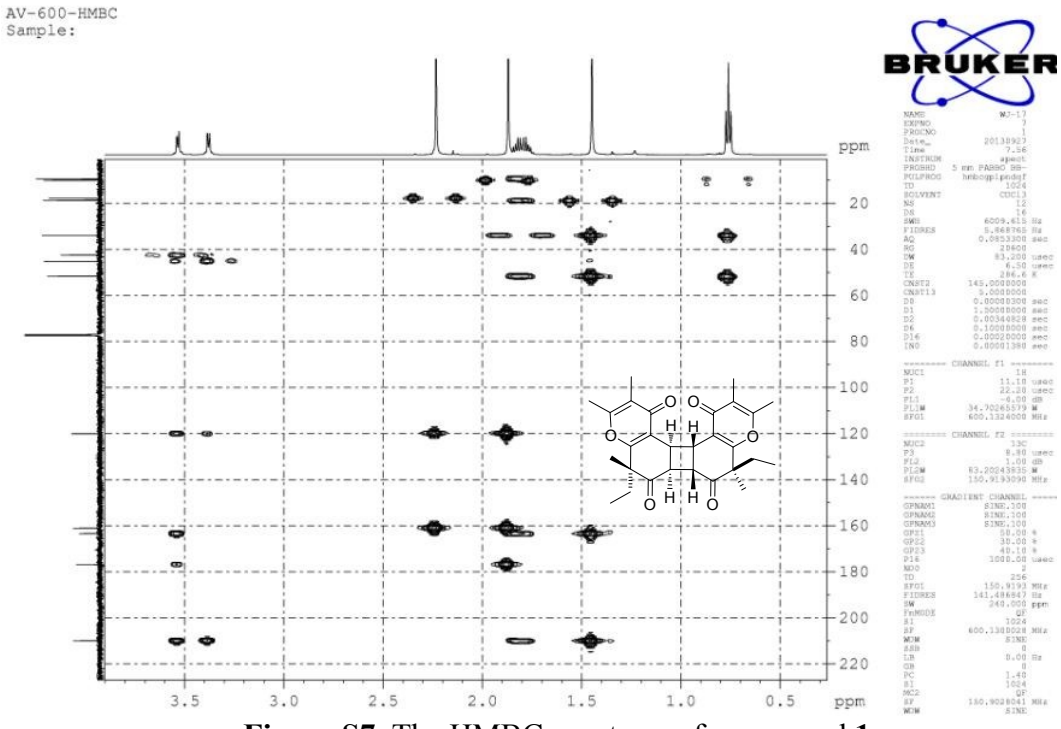


Figure S7. The HMBC spectrum of compound **1**

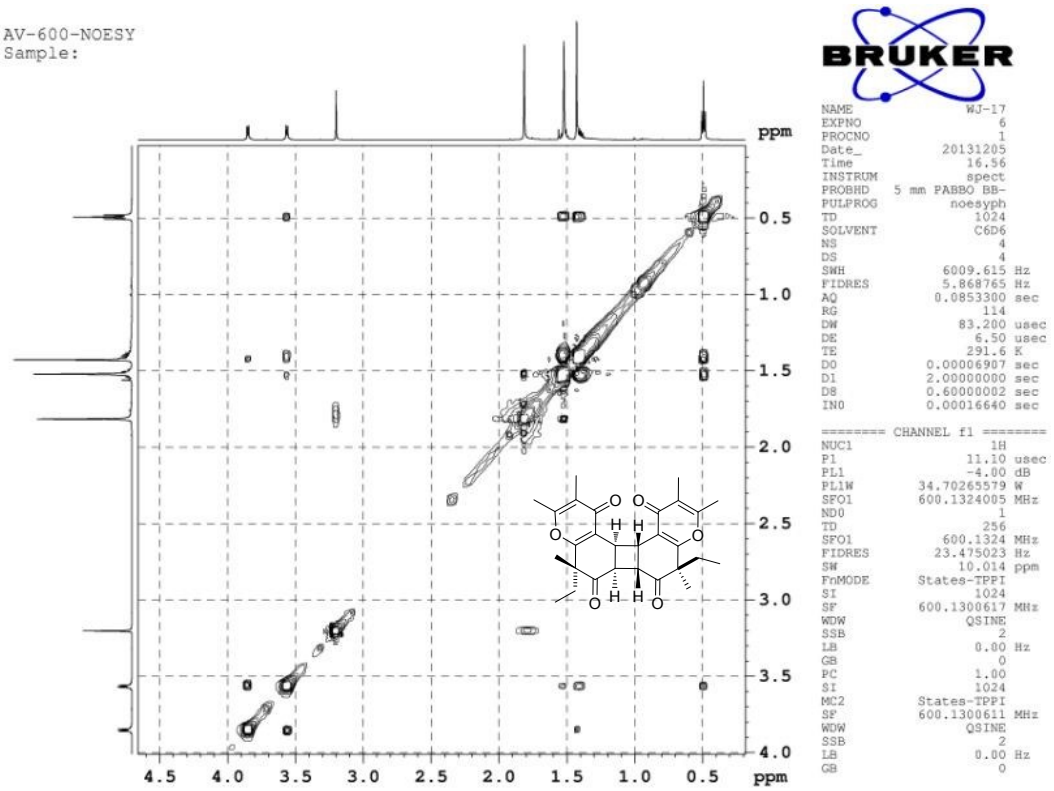


Figure S8. The NOESY spectrum of compound **1**

Computational details for ECD of compound **1**

Computational method

The Spartan 14.0 (Wavefunction Inc., Irvine, CA, USA) searches using molecular mechanics MMFF were performed for **compound 1**, which gave 9 conformers, The low-energy conformers of **compound 1** accounting for more than 5% Boltzmann distribution were further optimized successively in the gas phase by semi-empirical method and the Hartree-Fork (HF) method at the 6-31G (d) level in Gaussian 09 program package,[1] which was reoptimized and analysed frequency, orderly, using the density functional theory (DFT) at the B3LYP/6-31G (d, p) level and the same way in the methanol, resulted in no imaginary frequencies. Solvent effects were taken into consideration by using the conductor polarizable continuum model (CPCM). The conformers of **compound 1** were calculated electronic circular dichroism (ECD) by the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G (d, p) level with the CPCM model in methanol solution. The overall calculated ECD curves of the **compound**

1 were generated severally by Boltzmann weighting of their selected low-energy conformers using SpecDis 1.51 [2-3] with σ = 0.2eV at -10 nm shift.

Table S2. Energy analysis of compound **1**

Label	MMFF	
	rel. E(Kal/mol)	Boltzmann Dist.
1-1	0.00	0.711
1-2	4.96	0.096
1-3	4.96	0.096

Table S3. Computational details for ECD of compound **1**

1-1

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	0.381174	1.811977	0.413886
2	6	0	0.342678	0.699395	-1.924218
3	6	0	-0.763810	3.086430	-1.517703
4	6	0	-0.576407	1.819672	-2.379861
5	6	0	-0.197932	2.917341	-0.119226
6	6	0	0.550466	0.570839	-0.396913
7	6	0	-0.550466	-0.570839	-0.396913
8	6	0	-0.342678	-0.699395	-1.924218
9	6	0	0.576407	-1.819672	-2.379861
10	6	0	0.763810	-3.086430	-1.517703
11	6	0	0.197932	-2.917341	-0.119226
12	6	0	-0.381174	-1.811977	0.413886
13	8	0	-0.342678	4.055447	0.606081
14	6	0	0.081920	4.148328	1.903713
15	6	0	0.702851	3.106182	2.521368
16	6	0	0.917676	1.863515	1.775131
17	6	0	-0.917676	-1.863515	1.775131
18	6	0	-0.702851	-3.106182	2.521368
19	6	0	-0.081920	-4.148328	1.903713
20	8	0	0.342678	-4.055447	0.606081
21	8	0	1.537312	0.899898	2.270520
22	8	0	-1.537312	-0.899898	2.270520
23	6	0	1.212189	3.144527	3.937211
24	6	0	-0.237879	5.507163	2.441747
25	6	0	-1.212189	-3.144527	3.937211

26	6	0	0.237879	-5.507163	2.441747
27	8	0	1.144763	-1.739987	-3.458721
28	8	0	-1.144763	1.739987	-3.458721
29	1	0	1.524840	0.129186	-0.171605
30	1	0	-1.524840	-0.129186	-0.171605
31	1	0	1.252879	0.772918	-2.530731
32	1	0	-1.252879	-0.772918	-2.530731
33	6	0	-2.284816	3.387333	-1.424127
34	6	0	-0.065181	4.279801	-2.253640
35	6	0	1.453068	4.165207	-2.425049
36	6	0	0.065181	-4.279801	-2.253640
37	6	0	2.284816	-3.387333	-1.424127
38	6	0	-1.453068	-4.165207	-2.425049
39	1	0	2.302588	3.037419	3.953392
40	1	0	0.806381	2.300967	4.505051
41	1	0	0.951235	4.066020	4.456904
42	1	0	0.240075	6.275606	1.824569
43	1	0	-1.318993	5.680057	2.404183
44	1	0	0.098355	5.629615	3.469253
45	1	0	-0.951235	-4.066020	4.456904
46	1	0	-0.806381	-2.300967	4.505051
47	1	0	-2.302588	-3.037419	3.953392
48	1	0	-0.240075	-6.275606	1.824569
49	1	0	-0.098355	-5.629615	3.469253
50	1	0	1.318993	-5.680057	2.404183
51	1	0	-2.694518	3.512269	-2.428186
52	1	0	-2.446713	4.307208	-0.857998
53	1	0	-2.822097	2.574299	-0.926613
54	1	0	-0.545966	4.368256	-3.233130
55	1	0	-0.306475	5.189646	-1.695332
56	1	0	1.837496	5.058831	-2.926458
57	1	0	1.968618	4.078066	-1.462748
58	1	0	1.735442	3.301882	-3.037504
59	1	0	0.306475	-5.189646	-1.695332
60	1	0	0.545966	-4.368256	-3.233130
61	1	0	2.694518	-3.512269	-2.428186
62	1	0	2.446713	-4.307208	-0.857998
63	1	0	2.822097	-2.574299	-0.926613
64	1	0	-1.968618	-4.078066	-1.462748
65	1	0	-1.735442	-3.301882	-3.037504
66	1	0	-1.837496	-5.058831	-2.926458

1-2

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z

1	6	0	1.837388	0.362641	0.380185
2	6	0	0.605940	-1.914933	0.370369
3	6	0	3.018033	-1.647373	-0.716916
4	6	0	1.695453	-2.428683	-0.554205
5	6	0	2.916710	-0.231711	-0.182730
6	6	0	0.557588	-0.380074	0.556897
7	6	0	-0.575143	-0.335974	-0.551831
8	6	0	-0.786368	-1.853050	-0.302573
9	6	0	-1.923773	-2.145903	0.662227
10	6	0	-3.249093	-1.390828	0.443036
11	6	0	-2.946419	0.053150	0.067240
12	6	0	-1.770094	0.544054	-0.396352
13	8	0	4.091222	0.435031	-0.336078
14	6	0	4.237662	1.736921	0.063871
15	6	0	3.223847	2.411938	0.665586
16	6	0	1.944053	1.730544	0.894100
17	6	0	-1.685521	1.940268	-0.828147
18	6	0	-2.891037	2.755200	-0.633597
19	6	0	-4.012671	2.173837	-0.134699
20	8	0	-4.042673	0.843097	0.191712
21	8	0	1.009773	2.276948	1.502601
22	8	0	-0.656228	2.404591	-1.344990
23	6	0	3.315403	3.833883	1.147570
24	6	0	5.619553	2.208767	-0.262208
25	6	0	-2.785432	4.201590	-1.031810
26	6	0	-5.349315	2.786304	0.141633
27	8	0	-1.802653	-2.966329	1.555310
28	8	0	1.548230	-3.485516	-1.145454
29	1	0	0.118572	-0.111253	1.521422
30	1	0	-0.113534	-0.151188	-1.526253
31	1	0	0.642116	-2.515073	1.286875
32	1	0	-0.920382	-2.478786	-1.192356
33	6	0	3.371259	-1.606808	-2.226203
34	6	0	4.139461	-2.439081	0.035427
35	6	0	3.960641	-2.574393	1.549942
36	6	0	-4.000984	-2.049331	-0.771150
37	6	0	-4.101672	-1.454197	1.725113
38	6	0	-4.398738	-3.515809	-0.580842
39	1	0	2.505318	4.428016	0.712637
40	1	0	4.263847	4.309563	0.899998
41	1	0	3.180837	3.875038	2.233775
42	1	0	6.359488	1.562585	0.221518
43	1	0	5.790828	2.150823	-1.342401
44	1	0	5.787935	3.233479	0.062425
45	1	0	-2.539659	4.281963	-2.095898
46	1	0	-3.698657	4.765773	-0.845052

47	1	0	-1.964648	4.680446	-0.487840
48	1	0	-6.126764	2.256848	-0.418932
49	1	0	-5.379965	3.839227	-0.130891
50	1	0	-5.591914	2.692973	1.205554
51	1	0	3.418018	-2.625217	-2.615028
52	1	0	4.338931	-1.122741	-2.372247
53	1	0	2.618383	-1.052815	-2.794548
54	1	0	4.185207	-3.431089	-0.425436
55	1	0	5.089628	-1.940840	-0.180761
56	1	0	4.807863	-3.117815	1.978664
57	1	0	3.909217	-1.598428	2.043084
58	1	0	3.053136	-3.129696	1.809569
59	1	0	-3.373818	-1.950102	-1.664027
60	1	0	-4.895679	-1.444113	-0.954033
61	1	0	-4.209272	-2.488068	2.052022
62	1	0	-5.091492	-1.029849	1.548098
63	1	0	-3.625737	-0.895712	2.535947
64	1	0	-3.531873	-4.154246	-0.384306
65	1	0	-5.104722	-3.643532	0.244124
66	1	0	-4.882968	-3.884450	-1.490104

1-3

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-1.770092	0.544063	-0.396359
2	6	0	-0.786362	-1.853038	-0.302624
3	6	0	-3.249071	-1.390817	0.443068
4	6	0	-1.923760	-2.145925	0.662179
5	6	0	-2.946409	0.053152	0.067243
6	6	0	-0.575137	-0.335960	-0.551847
7	6	0	0.557597	-0.380083	0.556879
8	6	0	0.605949	-1.914938	0.370315
9	6	0	1.695456	-2.428663	-0.554281
10	6	0	3.018054	-1.647372	-0.716930
11	6	0	2.916725	-0.231715	-0.182731
12	6	0	1.837399	0.362633	0.380178
13	8	0	-4.042672	0.843090	0.191717
14	6	0	-4.012683	2.173830	-0.134697
15	6	0	-2.891060	2.755198	-0.633611
16	6	0	-1.685536	1.940275	-0.828167
17	6	0	1.944054	1.730537	0.894095
18	6	0	3.223855	2.411928	0.665606
19	6	0	4.237681	1.736910	0.063913
20	8	0	4.091243	0.435024	-0.336051

21	8	0	-0.656253	2.404607	-1.345022
22	8	0	1.009764	2.276941	1.502578
23	6	0	-2.785472	4.201584	-1.031843
24	6	0	-5.349336	2.786281	0.141626
25	6	0	3.315409	3.833872	1.147595
26	6	0	5.619581	2.208754	-0.262132
27	8	0	1.548212	-3.485458	-1.145591
28	8	0	-1.802638	-2.966421	1.555198
29	1	0	-0.113524	-0.151150	-1.526262
30	1	0	0.118585	-0.111286	1.521413
31	1	0	-0.920379	-2.478760	-1.192416
32	1	0	0.642136	-2.515100	1.286805
33	6	0	-4.101580	-1.454164	1.725195
34	6	0	-4.001044	-2.049323	-0.771069
35	6	0	-4.398833	-3.515784	-0.580719
36	6	0	4.139439	-2.439100	0.035456
37	6	0	3.371347	-1.606794	-2.226199
38	6	0	3.960595	-2.574360	1.549973
39	1	0	-1.964718	4.680471	-0.487855
40	1	0	-3.698715	4.765752	-0.845126
41	1	0	-2.539671	4.281943	-2.095925
42	1	0	-6.126749	2.256958	-0.419116
43	1	0	-5.592034	2.692747	1.205505
44	1	0	-5.379930	3.839257	-0.130697
45	1	0	3.181011	3.875016	2.233822
46	1	0	4.263789	4.309606	0.899880
47	1	0	2.505226	4.427961	0.712786
48	1	0	6.359499	1.562512	0.221539
49	1	0	5.787987	3.233434	0.062593
50	1	0	5.790854	2.150901	-1.342330
51	1	0	-4.209180	-2.488032	2.052113
52	1	0	-5.091403	-1.029800	1.548233
53	1	0	-3.625588	-0.895680	2.535996
54	1	0	-4.895730	-1.444081	-0.953915
55	1	0	-3.373920	-1.950126	-1.663980
56	1	0	-4.883126	-3.884418	-1.489950
57	1	0	-3.531979	-4.154248	-0.384224
58	1	0	-5.104777	-3.643473	0.244287
59	1	0	5.089626	-1.940898	-0.180731
60	1	0	4.185161	-3.431123	-0.425377
61	1	0	3.418092	-2.625198	-2.615038
62	1	0	4.339039	-1.122755	-2.372194
63	1	0	2.618510	-1.052771	-2.794566
64	1	0	3.909183	-1.598377	2.043081
65	1	0	3.053077	-3.129638	1.809608
66	1	0	4.807802	-3.117784	1.978722

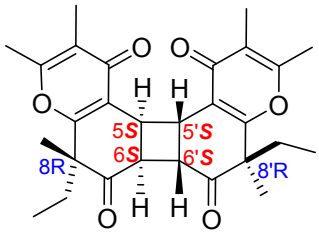
References

[1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma , K . ; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision C1; Gaussian, Inc.: Wallingford, CT, 2010.

[2] Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *Spec Dis*, version 1.51, University of Würzburg, Germany, 2010.

[3] Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. Quantifying the Comparison of Calculated and Experimental Electronic Circular Dichroism Spectra, Chirality 2013, 25, 243–249.

Table S4. 2D Structures of **1**

label	structure
1	

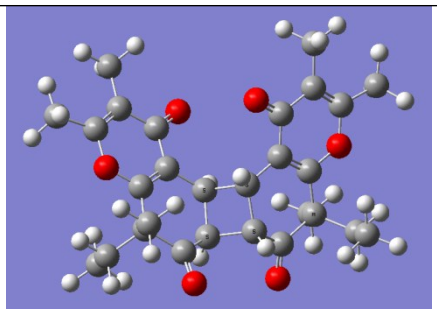
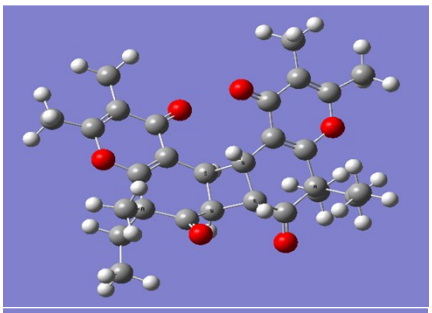
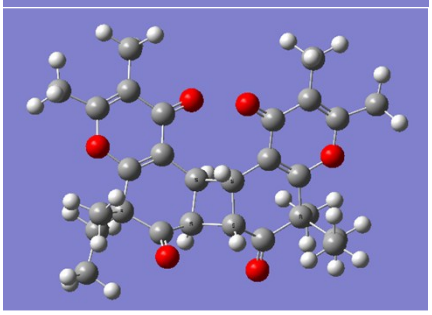
label	conformer	Boltzmann weighting factors
1-1		100.0
1-2		0
1-3		0

Figure S9. B3LYP/6-31 G* optimized lowest energy 3D conformers of **1**.

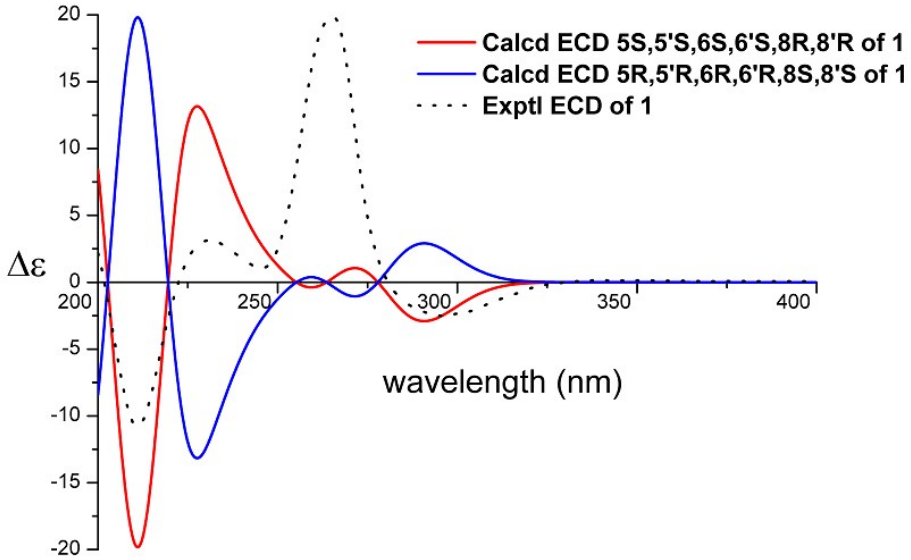


Figure S10. Experimental and calculated ECD spectra of **1**

X-ray Crystallographic Analysis of lecanicillone A (**1**)

Crystal data of **1**: $C_{28}H_{32}O_6$, M = 464.54; monoclinic system, space group P2(1), a = 12.0422(2) Å, b = 6.75793(12) Å, c = 15.1547(3) Å, V = 1199.49(4) Å^3, Z = 2, d = 1.286 g/cm^3, F(000) = 496. A crystal of dimensions 0.35 × 0.35 × 0.30 mm^3 was used for measurement with monochromator graphite, μ(Cu Kα) = 0.727 mm^{-1}. A total of 8390 reflections were collected in the range $6 \leq 2\theta \leq 141.92$, of which 4358 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The structure was refined by full-matrix least squares on F^2 using SHELXL-97 package software. The final reliability factors are: R = 0.0345, wR_2 = 0.0897, and the goodness of fit on F^2 was equal to 0.992. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1037052. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail:deposit@ccdc.cam.ac.Uk).

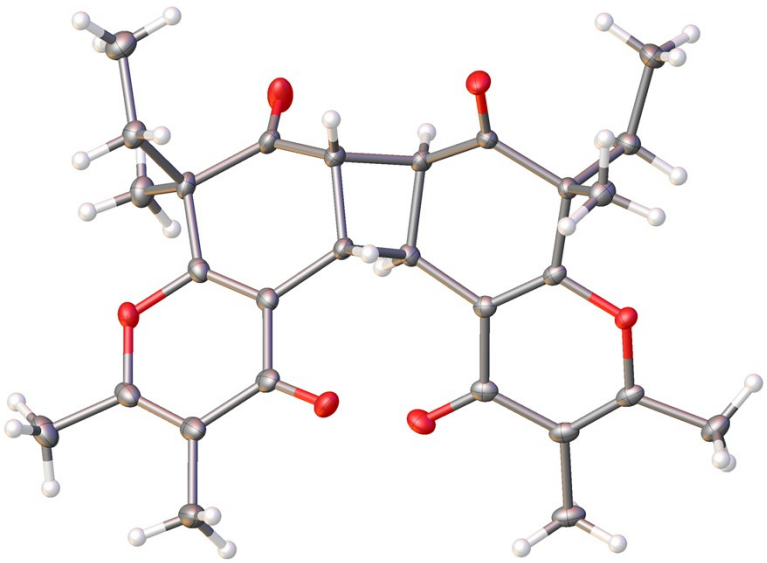


Figure S11. X-ray structure of lecanicillone A (**1**)

6. The spectra of lecanicillone B (2)

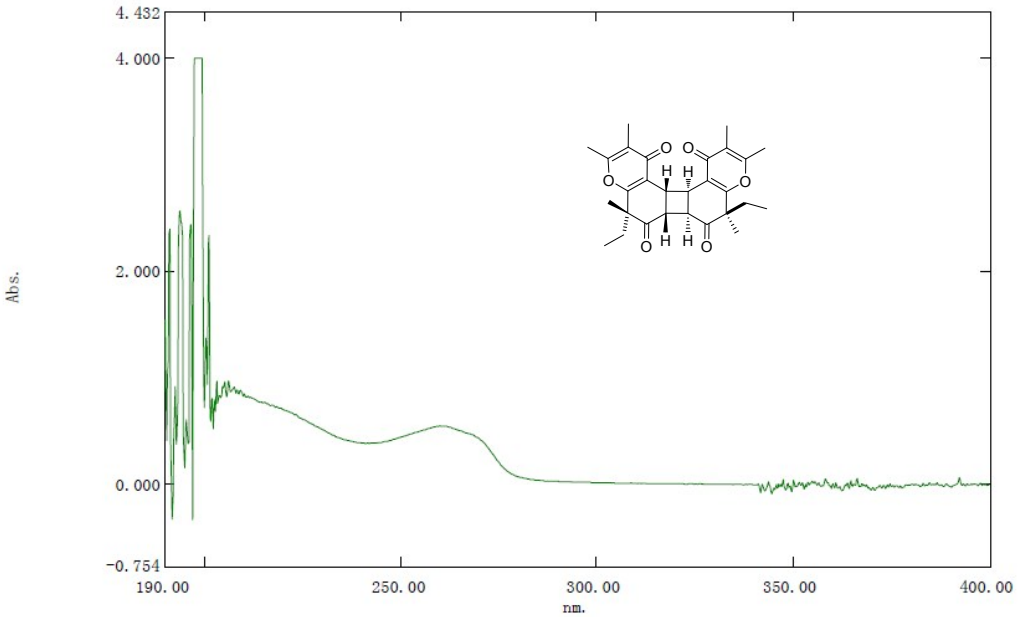
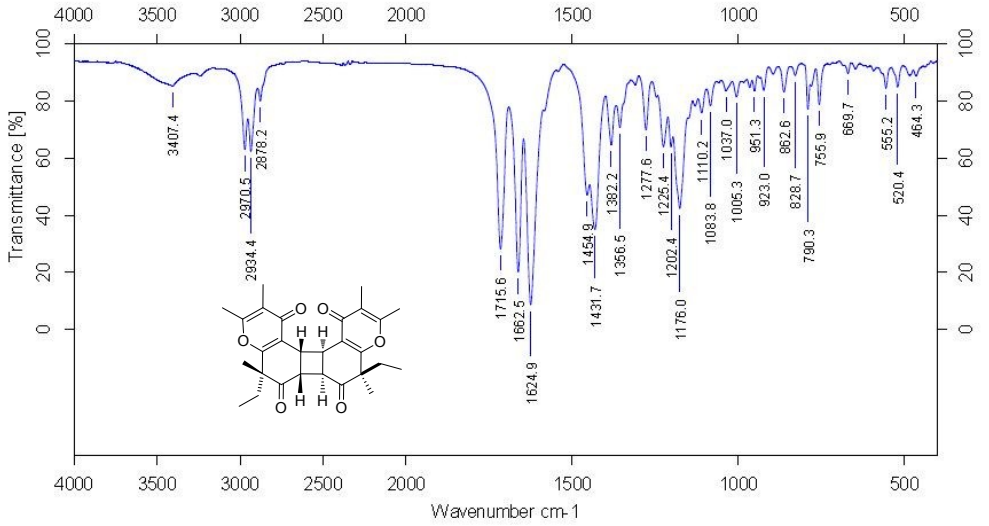


Figure S12. The UV spectrum of compound **2**



Sample : WZY-5	Frequency Range : 3999.64 - 400.157	Measured on : 2014-3-13	
Technique : Sample form	Resolution : 2	Instrument : EQUINOX55	Sample Scans : 16
Customer : Default	Zerofilling : 2	Acquisition : Double Sided,For	

Figure S13. The IR spectrum of compound **2**

Mass Spectrum Molecular Formula Report

Analysis Info

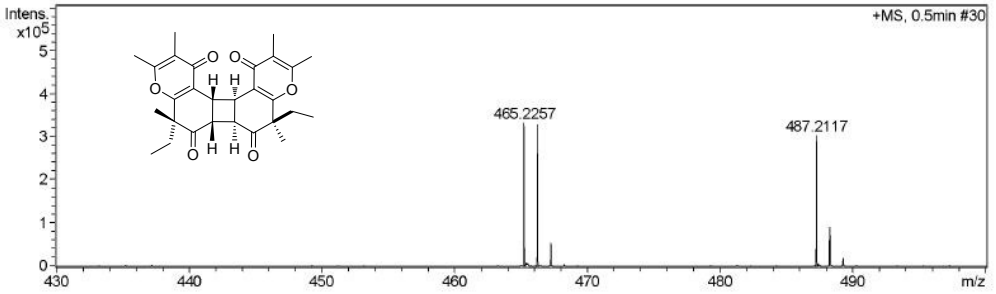
Analysis Name	D:\Data\20131111ceyang\WZY-5_1-b,6_01_2139.d	Acquisition Date	11/11/2013 3:51:06 PM
Method	20131026_ceyang.m	Operator	Bruker Customer
Sample Name	WZY-5	Instrument / Ser#	micrOTOF-Q 125
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.2 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C28H32O6H				
Formula, max.					
Measured m/z	465.226	Tolerance	5 ppm	Charge	1
Check Valence	no	Minimum	0	Maximum	0
Nirogen Rule	no	Electron Configuration	both		
Filter H/C Ratio	no	Minimum	0	Maximum	3
Estimate Carbon	yes				



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 28 H 33 O 6	0.343	465.2272	3.15	-0.10	1.46	12.50	ok	even

Figure S14. The HR-ESI-MS spectrum of compound **2**

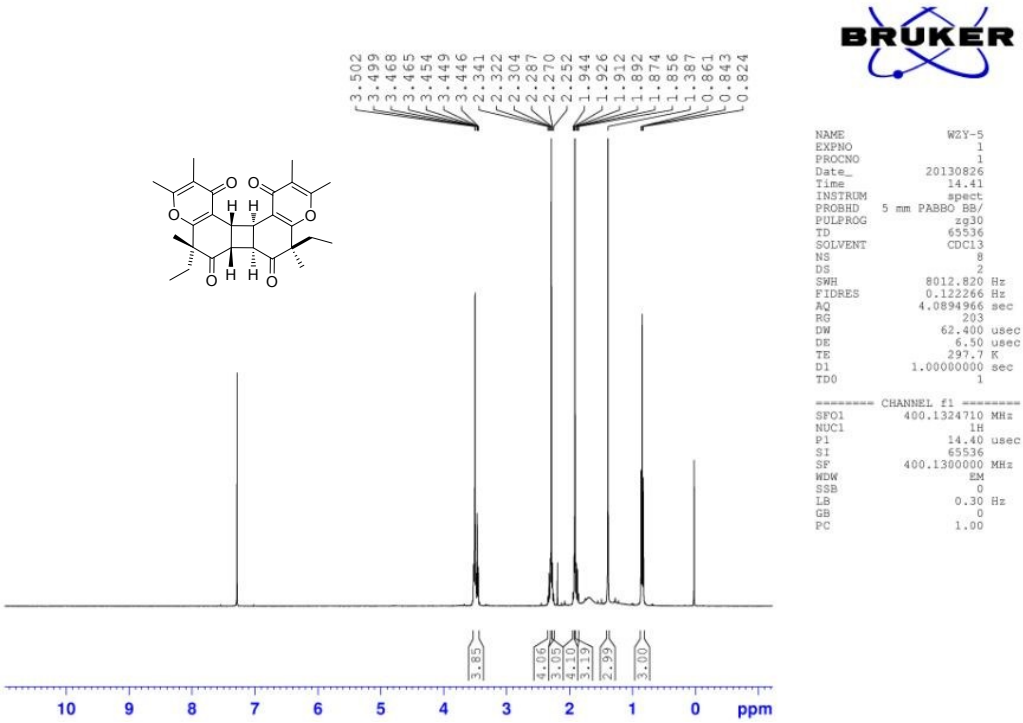


Figure S15. The ^{1}H-NMR spectrum of compound **2**

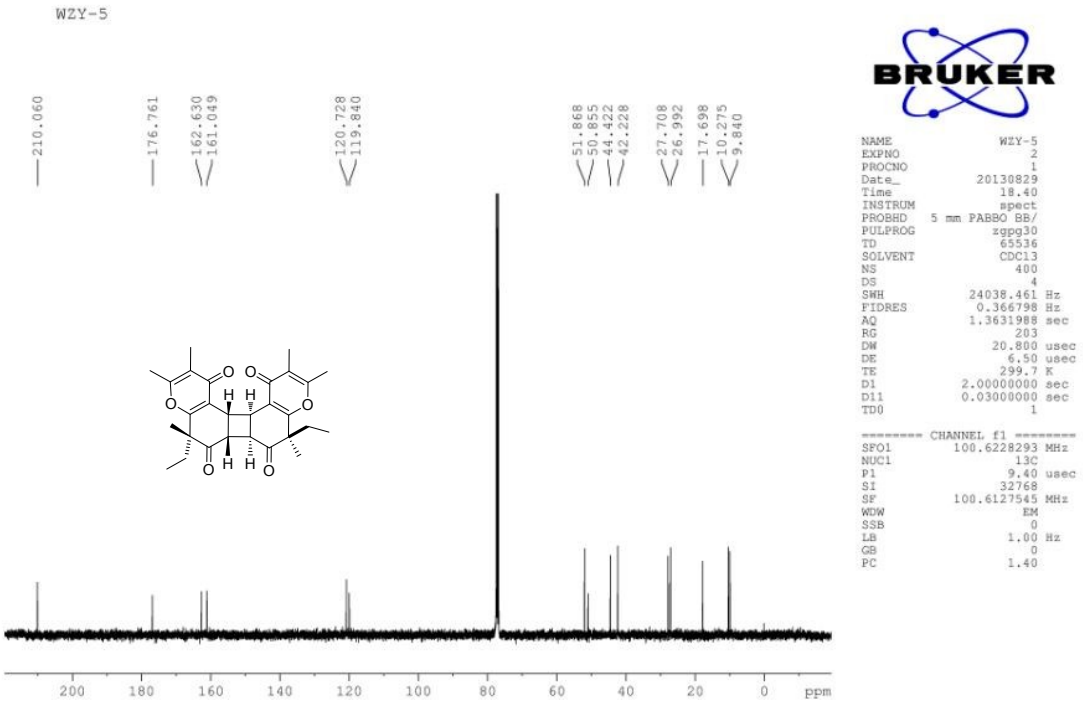


Figure S16. The ^{13}C-NMR spectrum of compound **2**

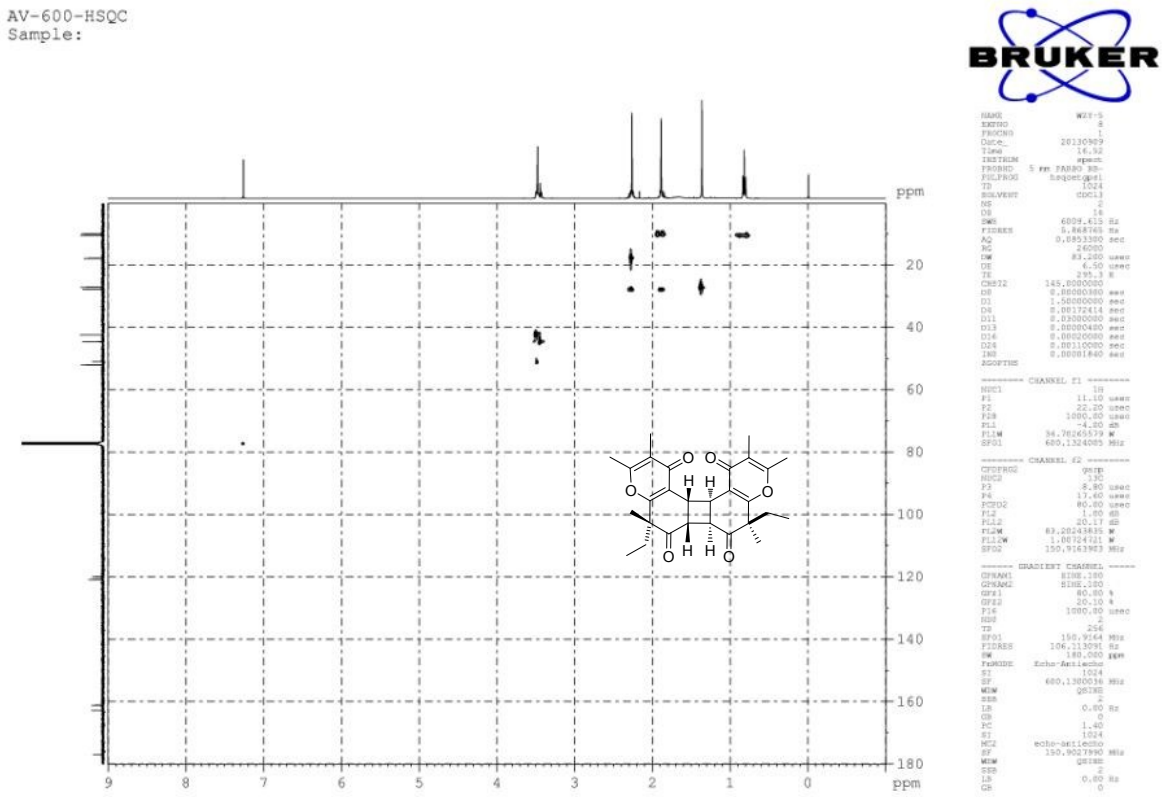


Figure S17. The HSQC spectrum of compound **2**

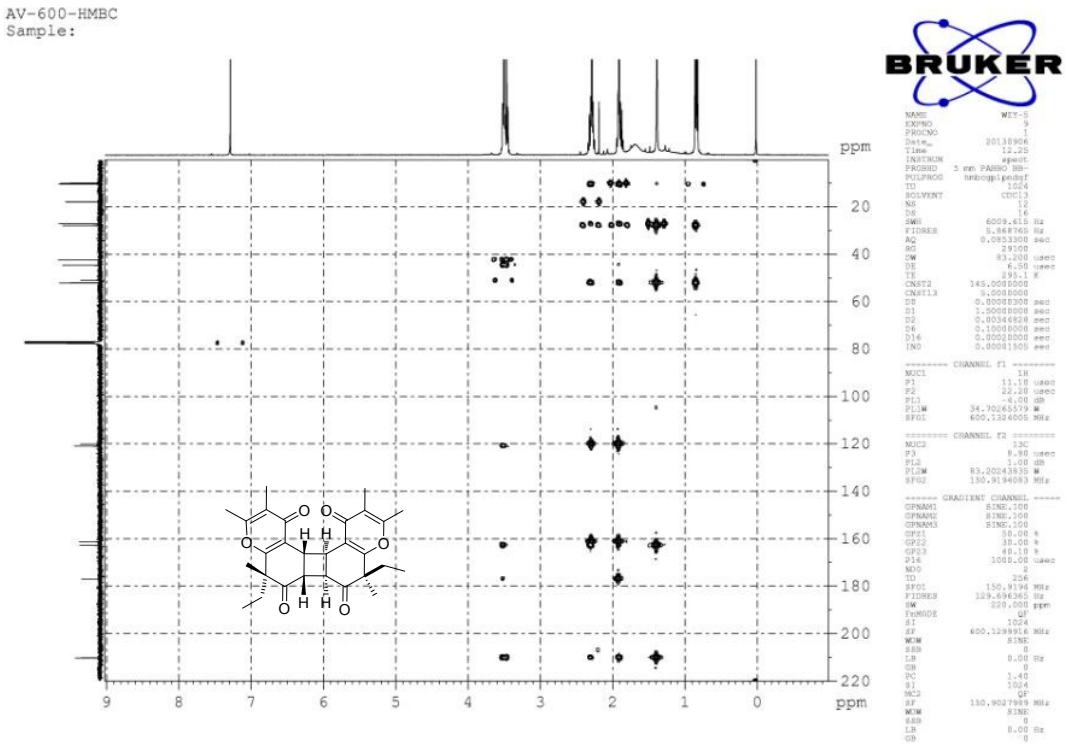


Figure S18. The HMBC spectrum of compound **2**

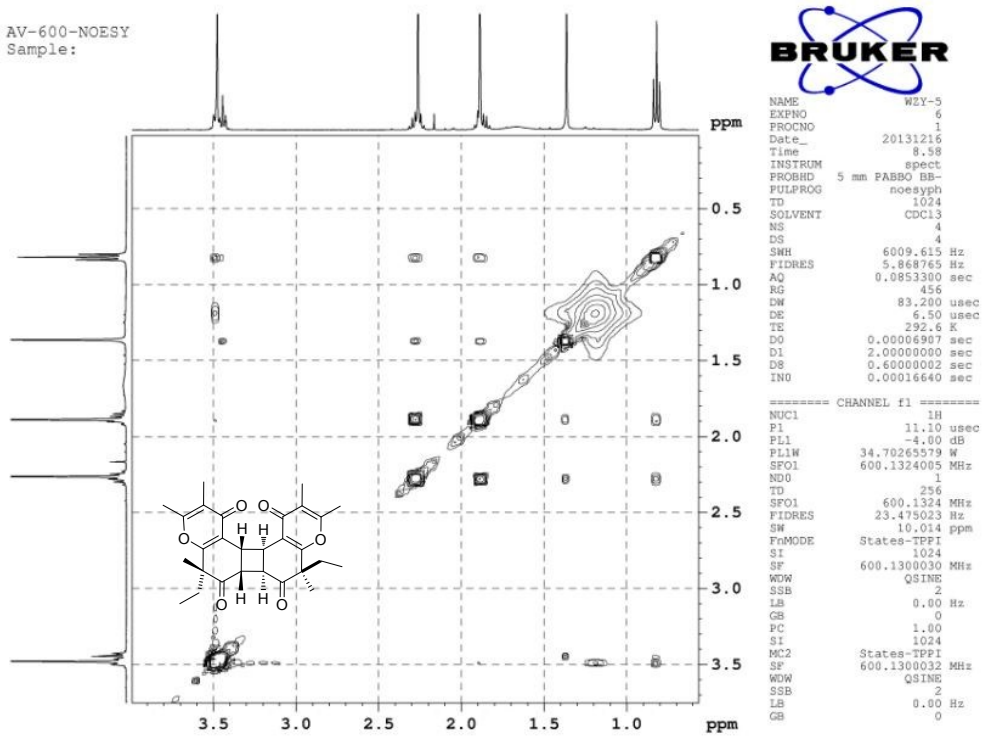


Figure S19. The NOESY spectrum of compound **2**

Computational details for ECD of compound **2**

Computational method

The Spartan 14.0 (Wavefunction Inc., Irvine, CA, USA) searches using molecular mechanics MMFF were performed for **compound 2**, which gave 11 conformers, The low-energy conformers of **compound 2** accounting for more than 5% Boltzmann distribution were further optimized successively in the gas phase by semi-empirical method and the Hartree-Fork (HF) method at the 6-31G (d) level in Gaussian 09 program package,[1] which was reoptimized and analysed frequency, orderly, using the density functional theory (DFT) at the B3LYP/6-31G (d, p) level and the same way in the methanol, resulted in no imaginary frequencies. Solvent effects were taken into consideration by using the conductor polarizable continuum model (CPCM). The conformers of **compound 2** were calculated electronic circular dichroism (ECD) by the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G (d, p) level with the CPCM model in methanol solution. The overall calculated ECD curves of the **compound 2** were generated severally by Boltzmann weighting of their selected low-energy conformers using SpecDis 1.62 [2] with σ = 0.2eV at 28 nm shift.

Table S5. Energy analysis of compound **2**

Label	MMFF	
	rel. E(Kal/mol)	Boltzmann Dist.
2-1	0.00	0.986
2-2	13.89	0.004
2-3	13.89	0.004
2-4	14.13	0.003

Table S6. Computational details for ECD of compound **2**

2-1

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z

1	6	0	-0.993517	2.978401	2.437522
2	6	0	-0.550842	4.099764	1.811667
3	8	0	-0.166691	4.079040	0.496327
4	6	0	-0.175327	2.932713	-0.232177
5	6	0	-0.572108	1.755446	0.308907
6	6	0	-1.060562	1.717340	1.688989
7	6	0	0.250802	3.188174	-1.667929
8	6	0	0.433805	1.864664	-2.439661
9	6	0	-0.401920	0.661444	-2.035295
10	6	0	-0.607808	0.508126	-0.507508
11	6	0	-0.886944	3.966798	-2.395452
12	6	0	2.759869	3.412544	-1.007931
13	6	0	1.556821	4.025435	-1.730809
14	6	0	0.607808	-0.508126	-0.507508
15	6	0	0.401920	-0.661444	-2.035295
16	6	0	-0.433805	-1.864664	-2.439661
17	6	0	-0.250802	-3.188174	-1.667929
18	6	0	0.175327	-2.932713	-0.232177
19	6	0	0.572108	-1.755446	0.308907
20	8	0	0.166691	-4.079040	0.496327
21	6	0	0.550842	-4.099764	1.811667
22	6	0	0.993517	-2.978401	2.437522
23	6	0	1.060562	-1.717340	1.688989
24	8	0	1.190591	1.805011	-3.393583
25	8	0	-1.522405	0.680117	2.192071
26	8	0	-1.190591	-1.805011	-3.393583
27	6	0	-1.556821	-4.025435	-1.730809
28	6	0	0.886944	-3.966798	-2.395452
29	8	0	1.522405	-0.680117	2.192071
30	6	0	-1.452541	2.925119	3.868685
31	6	0	-0.401920	5.486062	2.353883
32	6	0	1.452541	-2.925119	3.868685
33	1	0	-1.527649	-0.039513	-0.282696
34	1	0	1.527649	0.039513	-0.282696
35	1	0	1.308075	-0.660197	-2.652153
36	1	0	-1.308075	0.660197	-2.652153
37	6	0	0.401920	-5.486062	2.353883
38	6	0	-2.759869	-3.412544	-1.007931
39	1	0	-1.822895	3.401202	-2.403450
40	1	0	-1.065146	4.917322	-1.886436
41	1	0	-0.592056	4.171600	-3.428047
42	1	0	2.567195	3.277107	0.061135
43	1	0	3.631619	4.066836	-1.105246
44	1	0	3.028286	2.440012	-1.431076
45	1	0	1.340041	5.014554	-1.317129
46	1	0	1.795142	4.163790	-2.788688
47	1	0	-1.340041	-5.014554	-1.317129

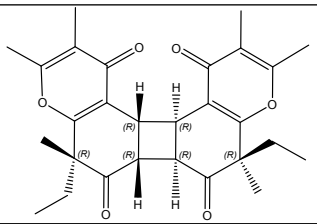
48	1	0	−1.795142	−4.163790	−2.788688
49	1	0	0.592056	−4.171600	−3.428047
50	1	0	1.065146	−4.917322	−1.886436
51	1	0	1.822895	−3.401202	−2.403450
52	1	0	−0.856399	2.196334	4.427944
53	1	0	−2.489757	2.577685	3.916162
54	1	0	−1.386834	3.886706	4.376948
55	1	0	−1.015484	6.183584	1.773982
56	1	0	−0.696768	5.549583	3.399309
57	1	0	0.639265	5.813132	2.263565
58	1	0	2.489757	−2.577685	3.916162
59	1	0	0.856399	−2.196334	4.427944
60	1	0	1.386834	−3.886706	4.376948
61	1	0	0.696768	−5.549583	3.399309
62	1	0	1.015484	−6.183584	1.773982
63	1	0	−0.639265	−5.813132	2.263565
64	1	0	−3.631619	−4.066836	−1.105246
65	1	0	−3.028286	−2.440012	−1.431076
66	1	0	−2.567195	−3.277107	0.061135

References

[1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma , K . ; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision C1; Gaussian, Inc.: Wallingford, CT, 2010.

[2] Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. Quantifying the Comparison of Calculated and Experimental Electronic Circular Dichroism Spectra, Chirality 2013, 25, 243–249.

Table S7. 2D Structure of **2**

label	structure
2-1	

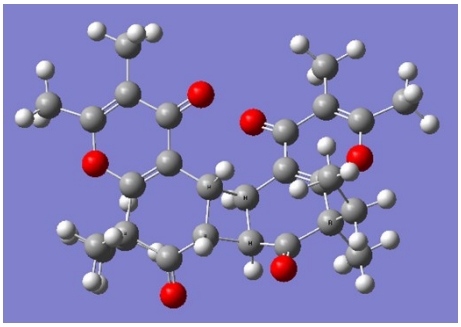
label	conformer	Boltzmann weighting factors
2-1		100

Figure S20. B3LYP/6-31 G* optimized lowest energy 3D conformer of **2**

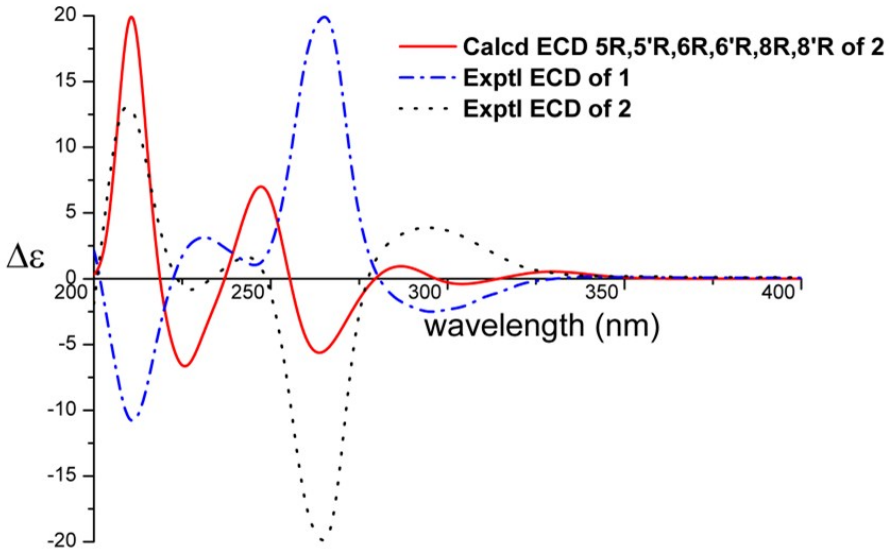


Figure S21. Experimental and suitable calculated ECD spectra of **2**

8. The spectra of lecanicillone C (3)

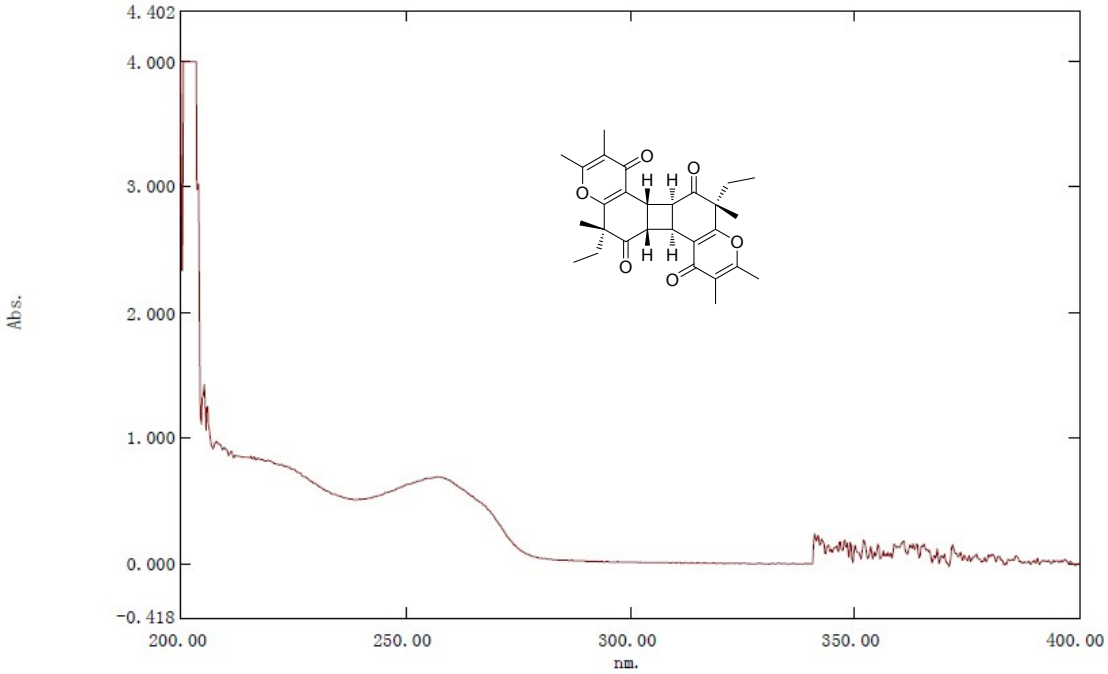
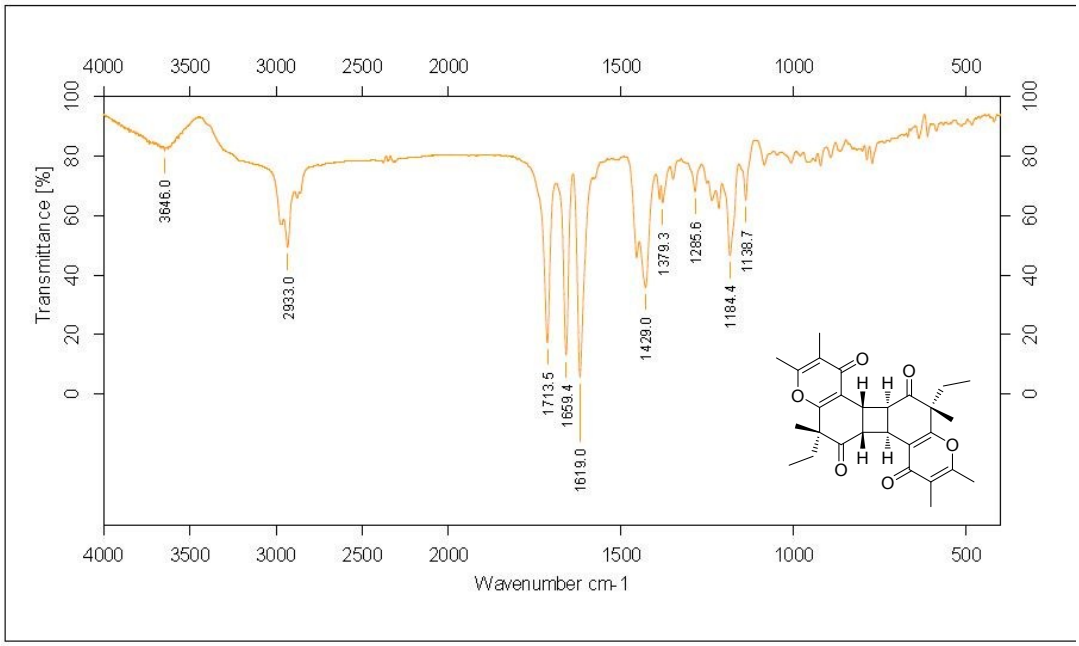


Figure S22. The UV spectrum of compound **3**



Sample : WZY-4	Frequency Range : 3999.64 - 400.157	Measured on : 2014-3-11	
Technique : Sample form	Resolution : 2	Instrument : EQUINOX55	Sample Scans : 16
Customer : Default	Zerofilling : 2	Acquisition : Double Sided,For	

Figure S23. The IR spectrum of compound **3**

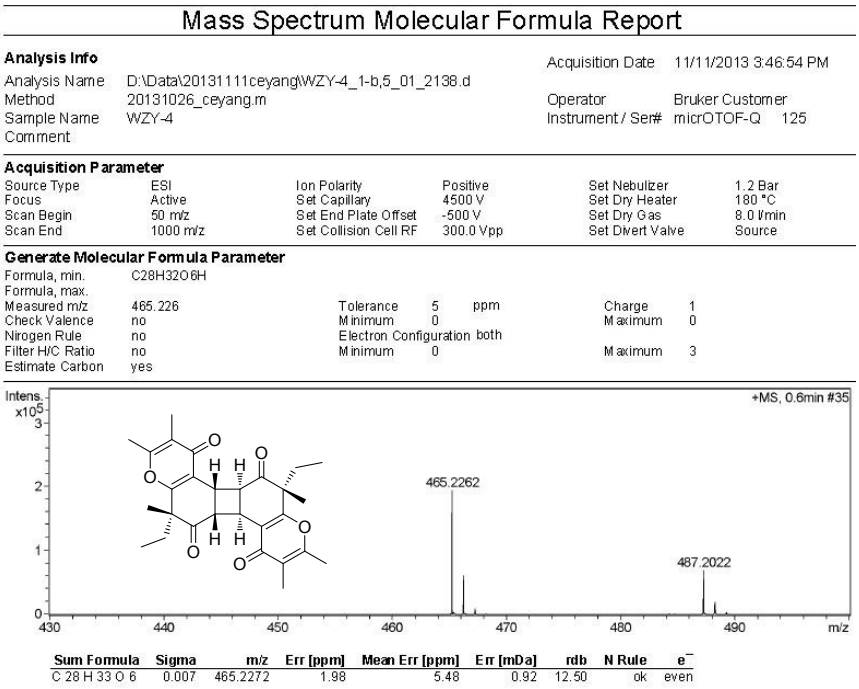


Figure S24. The HR-ESI-MS spectrum of compound **3**

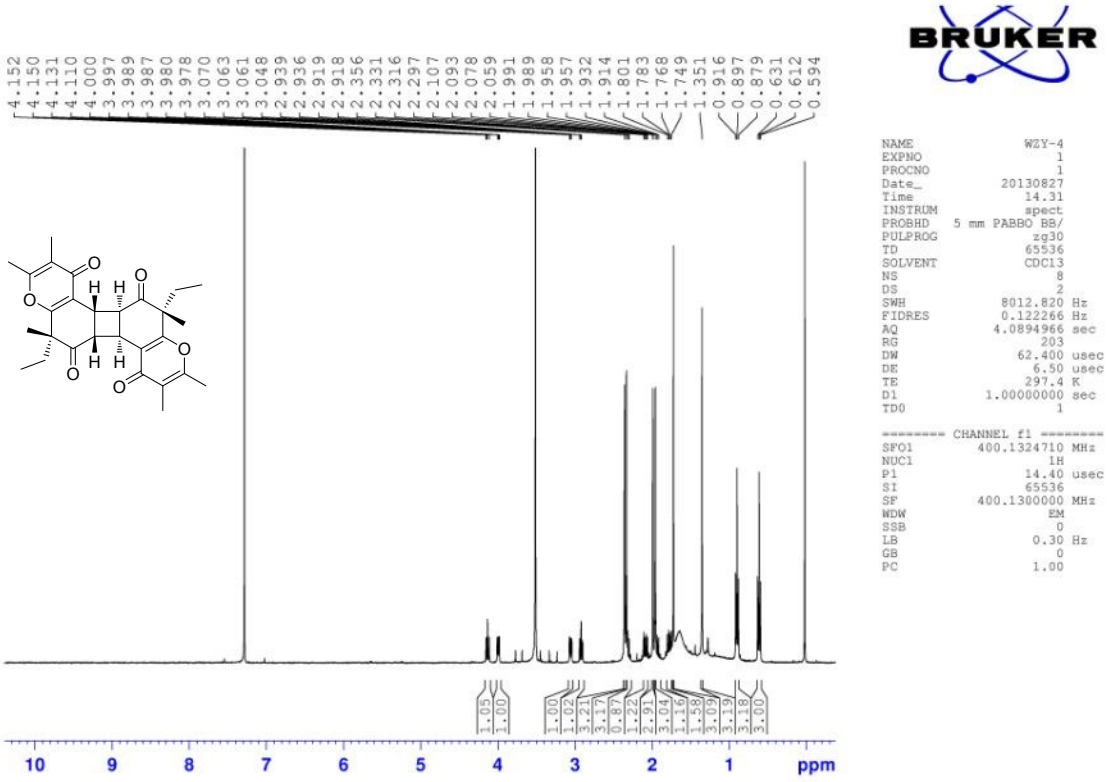


Figure S25. The ^{1}H-NMR spectrum of compound **3**

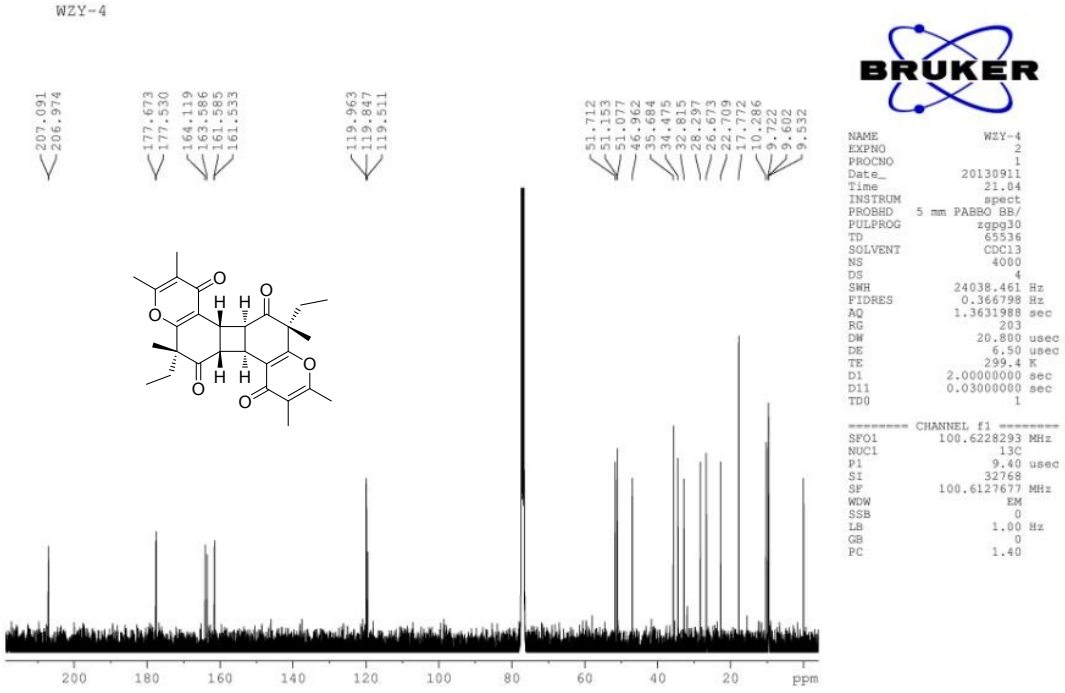


Figure S26. The ^{13}C-NMR spectrum of compound **3**

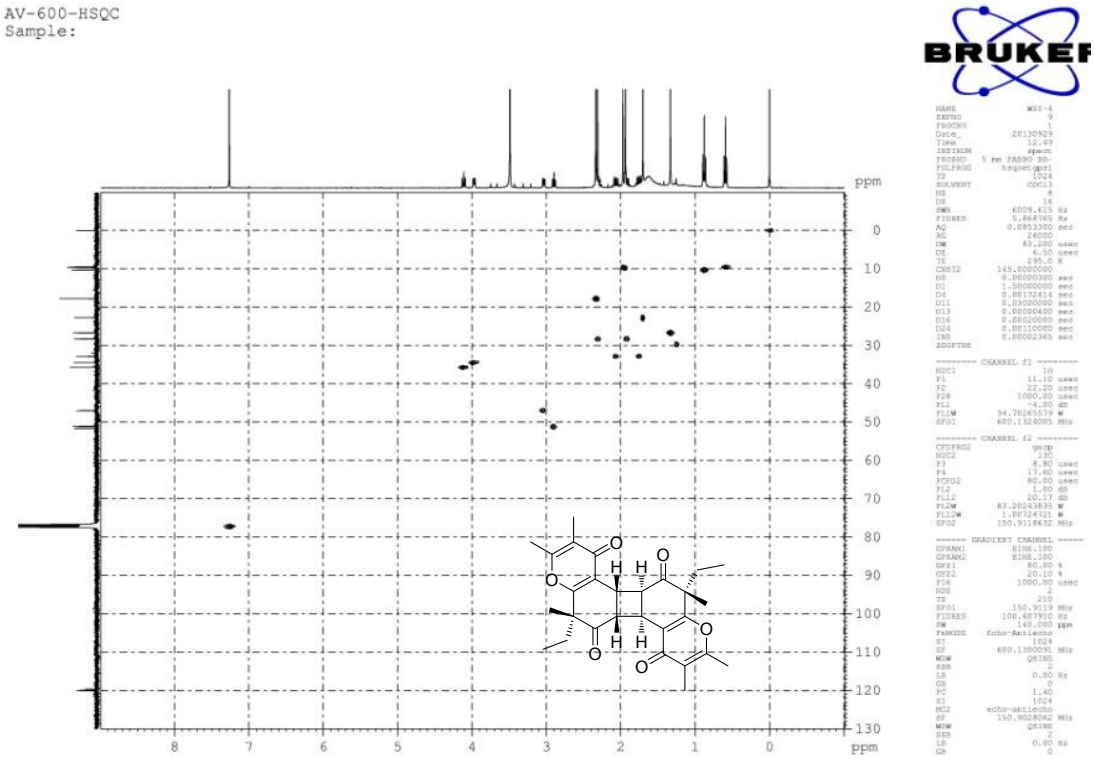


Figure S27. The HSQC spectrum of compound **3**

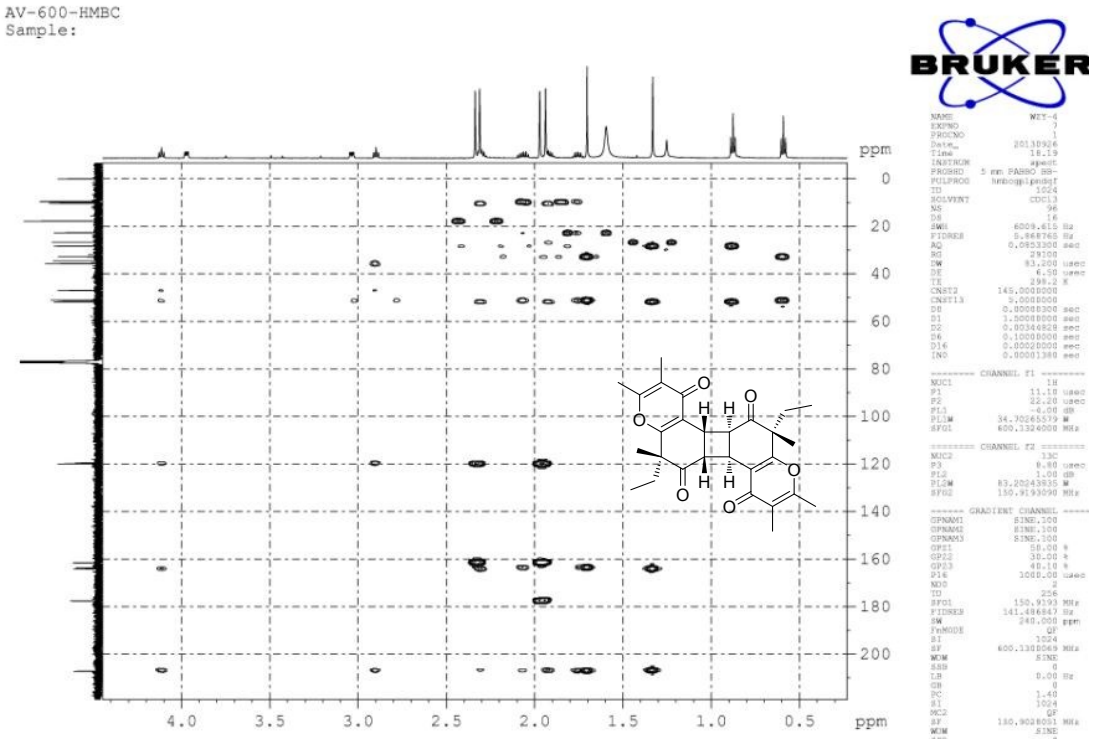


Figure S28. The HMBC spectrum of compound **3**

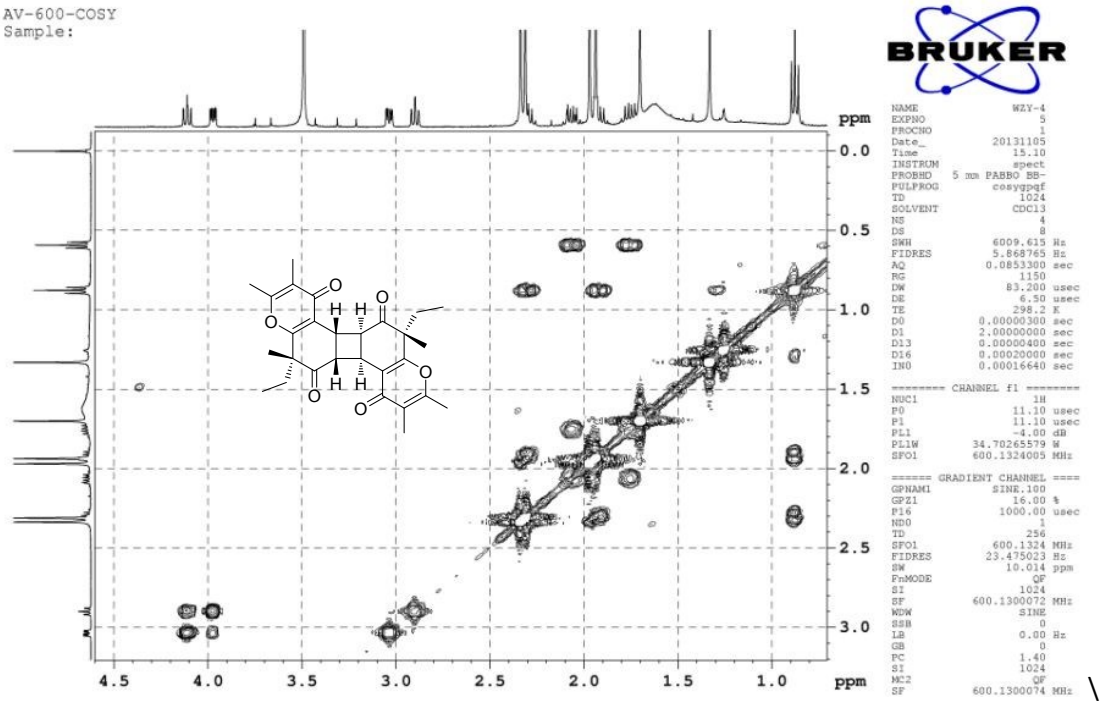


Figure S29. The ^{1}H-^{1}H COSY spectrum of compound **3**

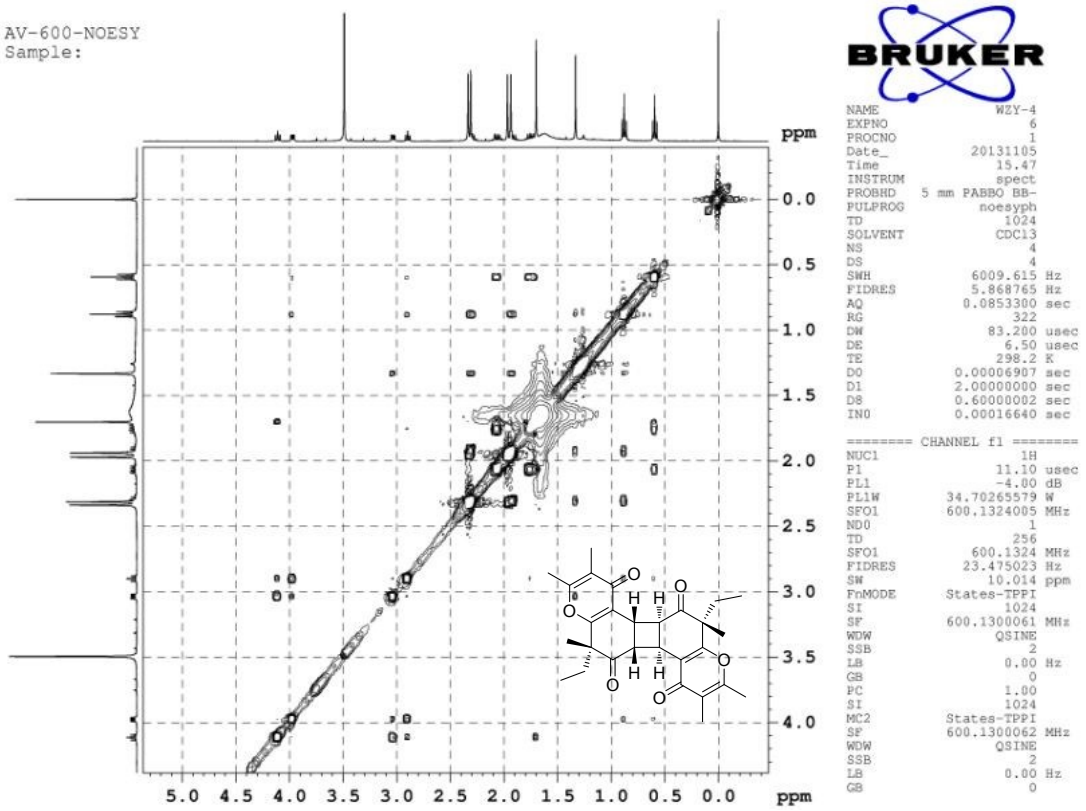


Figure S30. The NOESY spectrum of compound **3**

Computational details for ECD of compound **3**

Computational methods

The Spartan 14.0 (Wavefunction Inc., Irvine, CA, USA) searches using molecular mechanics MMFF were performed for 5*S*,5′*R*,6*R*,6′*S*,8*R*,8′*R* of **3** (**3a**) and 5*R*,5′*S*,6*S*,6′*R*,8*R*,8′*R* of **3** (**3b**), which gave respectively 9 and 10 conformers. The low-energy conformers of **3a** and **3b** separately accounting for more than 7% Boltzmann distribution was further optimized successively in the gas phase by semi-empirical method and the Hartree-Fork (HF) method at the 6-31G (d) level in Gaussian 09 program package,[1] which was reoptimized and analysed frequency, orderly, using the density functional theory (DFT) at the B3LYP/6-31G (d, p) level and the same way in the methanol, resulted in no imaginary frequencies. Solvent effects were taken into consideration by using the conductor polarizable continuum model (CPCM). The conformers of **3a** and **3b** were calculated electronic circular dichroism (ECD) by the time-dependent density functional

theory (TD-DFT) method at the B3LYP/6-31G (d, p) level with the CPCM model in methanol solution. The overall calculated ECD curves of the **3a** and **3b** were generated severally by Boltzmann weighting of their selected low-energy conformers using SpecDis 1.62 [2] with σ = 0.3eV at 16nm shift, together with σ = 0.28eV at -5nm shift.

Table S8. Energy analysis of compounds **3**a and **3**b

Label	MMFF	
	rel. E(Kal/mol)	Boltzmann Dist.
3a-1	0.00	0.671
3a-2	3.64	0.154
3b-1	0.00	0.970

Table S9. Computational details for ECD of compound **3a**

3a-1

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-6.099504	-0.278183	-1.366331
2	6	0	-4.816780	0.236465	-0.793955
3	6	0	-2.331607	0.615805	0.411323
4	6	0	-4.337351	1.507005	-0.773622
5	6	0	-3.038714	1.771629	-0.145301
6	6	0	-2.899293	-0.613586	0.342974
7	6	0	-5.051038	2.697494	-1.352646
8	8	0	-4.111454	-0.798704	-0.242674
9	8	0	-2.561572	2.918399	-0.088262
10	6	0	-2.380579	-1.906716	0.941539
11	6	0	-1.001404	0.839685	1.072598
12	6	0	-0.190397	-0.468908	1.270509
13	6	0	-0.904554	-1.791636	1.378721
14	6	0	0.115414	1.327124	0.094795
15	6	0	0.578412	-0.135281	-0.077745
16	6	0	1.128476	2.246282	0.759306
17	6	0	2.643820	2.000171	0.575867
18	6	0	2.937514	0.596978	0.084100
19	6	0	2.044235	-0.384858	-0.190486
20	6	0	2.516539	-1.687939	-0.660193
21	8	0	4.272684	0.392285	-0.048192

22	6	0	4.782404	-0.811244	-0.457849
23	6	0	3.969769	-1.854969	-0.767949
24	6	0	4.451265	-3.201006	-1.235011
25	6	0	6.275941	-0.737087	-0.491921
26	8	0	1.730687	-2.603470	-0.961028
27	8	0	0.757572	3.208757	1.409038
28	6	0	3.192132	3.065284	-0.431752
29	6	0	2.620347	3.007677	-1.850773
30	6	0	3.337134	2.217055	1.945695
31	1	0	-1.117910	1.474699	1.955346
32	1	0	-0.235374	1.836707	-0.808164
33	1	0	0.505202	-0.395620	2.110739
34	1	0	0.067906	-0.611603	-0.917051
35	8	0	-0.339935	-2.769460	1.841868
36	6	0	-3.198471	-2.205280	2.232724
37	6	0	-2.538948	-3.106489	-0.036613
38	6	0	-1.837467	-2.951353	-1.389057
39	1	0	-6.697439	0.516798	-1.807112
40	1	0	-5.891979	-1.028724	-2.136304
41	1	0	-6.689197	-0.766491	-0.583450
42	1	0	-5.262141	3.430790	-0.567059
43	1	0	-5.989478	2.437628	-1.841575
44	1	0	-4.407670	3.201051	-2.081333
45	1	0	4.113241	-3.982215	-0.546056
46	1	0	5.535686	-3.262002	-1.320320
47	1	0	4.012553	-3.436886	-2.209869
48	1	0	6.596885	0.046810	-1.186031
49	1	0	6.659493	-0.473646	0.499311
50	1	0	6.724437	-1.679494	-0.799080
51	1	0	4.279427	2.945172	-0.468046
52	1	0	2.993024	4.046528	0.011161
53	1	0	2.791854	2.034165	-2.321321
54	1	0	3.100569	3.766160	-2.476081
55	1	0	1.543584	3.205482	-1.870149
56	1	0	3.095093	3.213198	2.318637
57	1	0	4.419755	2.126622	1.838286
58	1	0	2.999934	1.480665	2.681022
59	1	0	-2.842852	-3.136677	2.678685
60	1	0	-4.257789	-2.309498	1.985427
61	1	0	-3.093256	-1.402612	2.968523
62	1	0	-3.608794	-3.277006	-0.188055
63	1	0	-2.144739	-3.983080	0.485753
64	1	0	-2.213020	-2.083779	-1.942218
65	1	0	-2.020618	-3.835686	-2.007860
66	1	0	-0.754006	-2.844622	-1.275437

3a-2

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-6.092907	-0.161602	-1.376236
2	6	0	-4.804674	0.296596	-0.769132
3	6	0	-2.314476	0.560500	0.452901
4	6	0	-4.310609	1.557321	-0.660126
5	6	0	-3.004758	1.760316	-0.024676
6	6	0	-2.897567	-0.653874	0.303784
7	6	0	-5.010555	2.794144	-1.152547
8	8	0	-4.112071	-0.783275	-0.292744
9	8	0	-2.506745	2.892787	0.104312
10	6	0	-2.394927	-1.988663	0.817744
11	6	0	-0.977211	0.727355	1.113793
12	6	0	-0.184188	-0.601449	1.241707
13	6	0	-0.926556	-1.913589	1.291357
14	6	0	0.129587	1.248283	0.153547
15	6	0	0.591013	-0.211218	-0.087004
16	6	0	1.183049	2.096096	0.844775
17	6	0	2.621184	2.013571	0.292787
18	6	0	2.947523	0.558710	-0.010705
19	6	0	2.060807	-0.452461	-0.191932
20	6	0	2.542446	-1.783512	-0.558165
21	8	0	4.281883	0.358144	-0.139249
22	6	0	4.801124	-0.871911	-0.448346
23	6	0	3.996839	-1.946015	-0.660426
24	6	0	4.488040	-3.323074	-1.012454
25	6	0	6.293588	-0.786697	-0.499404
26	8	0	1.762352	-2.726086	-0.779830
27	8	0	0.904262	2.832370	1.774730
28	6	0	2.686270	2.811038	-1.060652
29	6	0	2.382785	4.308148	-0.952963
30	6	0	3.609498	2.590355	1.324282
31	1	0	-1.070975	1.319289	2.028367
32	1	0	-0.214229	1.795641	-0.730003
33	1	0	0.512264	-0.580309	2.083683
34	1	0	0.085551	-0.646946	-0.951389
35	8	0	-0.395312	-2.916420	1.739003
36	6	0	-3.241568	-2.375862	2.065424
37	6	0	-2.538303	-3.115534	-0.246993
38	6	0	-1.802554	-2.877078	-1.568906
39	1	0	-6.674321	0.667146	-1.775085
40	1	0	-5.892663	-0.869659	-2.187243
41	1	0	-6.695901	-0.684579	-0.626488
42	1	0	-5.102399	3.519149	-0.337754

43	1	0	-6.004537	2.593491	-1.551207
44	1	0	-4.416633	3.280393	-1.934108
45	1	0	4.154464	-4.044938	-0.259573
46	1	0	5.572959	-3.383850	-1.091001
47	1	0	4.051806	-3.644274	-1.963815
48	1	0	6.600146	-0.028017	-1.226905
49	1	0	6.685441	-0.481717	0.476765
50	1	0	6.747149	-1.736697	-0.774324
51	1	0	1.997354	2.345264	-1.773828
52	1	0	3.693954	2.666074	-1.465670
53	1	0	1.386803	4.497087	-0.540284
54	1	0	2.420781	4.762112	-1.947754
55	1	0	3.111139	4.828089	-0.324883
56	1	0	3.290225	3.586267	1.630228
57	1	0	4.613878	2.650563	0.901520
58	1	0	3.647863	1.963571	2.219473
59	1	0	-2.895465	-3.336280	2.453259
60	1	0	-4.295314	-2.460439	1.788798
61	1	0	-3.151611	-1.626380	2.857279
62	1	0	-3.605950	-3.261432	-0.435837
63	1	0	-2.167362	-4.031478	0.222539
64	1	0	-2.138277	-1.956413	-2.058259
65	1	0	-1.999468	-3.703175	-2.259703
66	1	0	-0.719001	-2.817090	-1.425181

Table S10. Computational details for ECD of compound **3b**

3b-1

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	2.822216	-0.633339	0.027673
2	6	0	2.245413	-1.951580	0.507106
3	6	0	0.878336	-1.760159	1.195114
4	6	0	0.156544	-0.447458	1.050988
5	6	0	0.980029	0.854327	0.844157
6	6	0	2.305982	0.610741	0.182455
7	6	0	3.049635	1.771372	-0.314275
8	6	0	4.331992	1.498967	-0.970689
9	6	0	4.757690	0.213908	-1.082865
10	8	0	4.017208	-0.826361	-0.591364
11	6	0	-0.589060	-0.124150	-0.310299
12	6	0	-0.122593	1.339957	-0.146004
13	6	0	-2.053130	-0.369596	-0.437181
14	6	0	-2.945071	0.637465	-0.274584

15	6	0	-2.637292	2.085659	0.064180
16	6	0	-1.162109	2.243563	0.498683
17	6	0	-2.516196	-1.705910	-0.806996
18	6	0	-3.965211	-1.871719	-0.961875
19	6	0	-4.778903	-0.799544	-0.773997
20	8	0	-4.275428	0.432008	-0.442460
21	8	0	2.611387	2.927667	-0.184614
22	8	0	0.393216	-2.677849	1.837846
23	6	0	2.025454	-2.913046	-0.695006
24	6	0	3.226545	-2.629329	1.512086
25	6	0	3.574582	-1.797365	2.749476
26	8	0	-0.840019	3.110840	1.291402
27	6	0	-2.820654	2.954482	-1.216392
28	6	0	-3.580721	2.616897	1.176305
29	6	0	-3.573166	1.814672	2.481224
30	8	0	-1.720378	-2.643959	-0.987496
31	1	0	1.098007	1.497313	1.720158
32	1	0	0.229749	1.841109	-1.053897
33	1	0	-0.547143	-0.357405	1.882234
34	1	0	-0.070743	-0.610388	-1.139239
35	6	0	6.012258	-0.312478	-1.704990
36	6	0	5.087080	2.696102	-1.479082
37	6	0	-4.440732	-3.250025	-1.330567
38	6	0	-6.269255	-0.720067	-0.873598
39	1	0	1.294847	-2.518203	-1.405475
40	1	0	2.969459	-3.080126	-1.218139
41	1	0	1.652629	-3.869821	-0.322366
42	1	0	2.758047	-3.568568	1.818696
43	1	0	4.139480	-2.884712	0.965492
44	1	0	4.258870	-2.354231	3.396695
45	1	0	4.063582	-0.854401	2.485014
46	1	0	2.683307	-1.560257	3.339596
47	1	0	-3.850128	2.869586	-1.573731
48	1	0	-2.617118	4.003269	-0.983868
49	1	0	-2.151397	2.637509	-2.020820
50	1	0	-3.281924	3.648900	1.378433
51	1	0	-4.595075	2.649816	0.768147
52	1	0	-4.252193	2.272207	3.207311
53	1	0	-3.904118	0.782679	2.327493
54	1	0	-2.576526	1.789265	2.931358
55	1	0	6.622372	0.482279	-2.129217
56	1	0	6.606012	-0.847556	-0.956324
57	1	0	5.765288	-1.025988	-2.498001
58	1	0	6.023657	2.434337	-1.970460
59	1	0	4.468665	3.257132	-2.187347
60	1	0	5.309692	3.379840	-0.653173
61	1	0	-5.518171	-3.305906	-1.481791

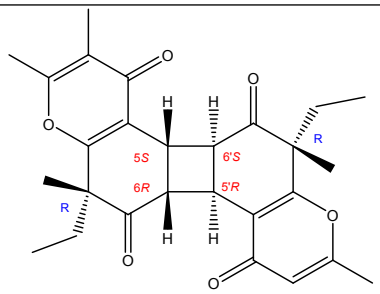
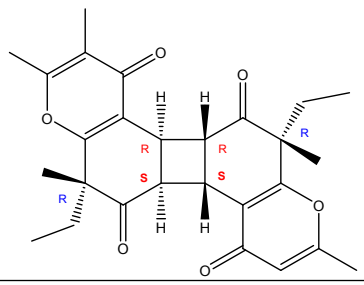
62	1	0	−3.942524	−3.583259	−2.246517
63	1	0	−4.163701	−3.966039	−0.549407
64	1	0	−6.709769	−1.671229	−1.165174
65	1	0	−6.693764	−0.419388	0.090149
66	1	0	−6.555226	0.039373	−1.608634

References

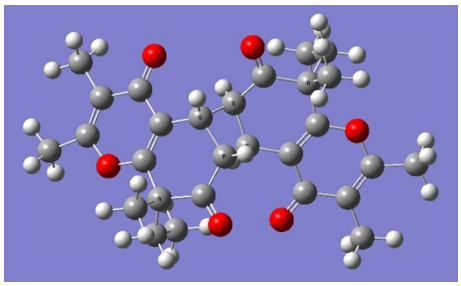
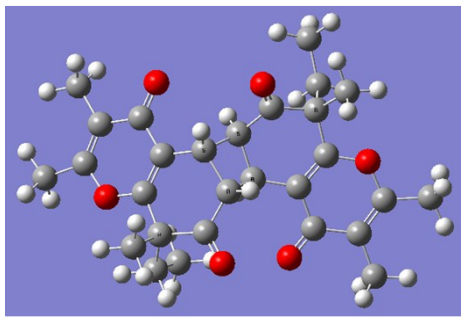
[1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma , K . ; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision C1; Gaussian, Inc.: Wallingford, CT, 2010.

[2] Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. Quantifying the Comparison of Calculated and Experimental Electronic Circular Dichroism Spectra, Chirality 2013, 25, 243–249.

Table S11. 2D Structures of **3a** and **3b**

label	structure
3a	
3b	

B3LYP/6-31G (d, p) optimized lowest energy 3D conformers of **3a**

label	conformer	Boltzmann weighting factors
3a-1		37.39
3a-2		62.61

B3LYP/6-31G (d, p) optimized lowest energy 3D conformers of **3b**

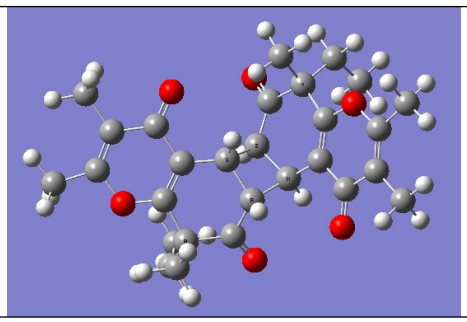
label	conformer	Boltzmann weighting factors
3b-1		100

Figure S31. B3LYP/6-31 G (d, p) optimized lowest energy 3D conformer of **3**

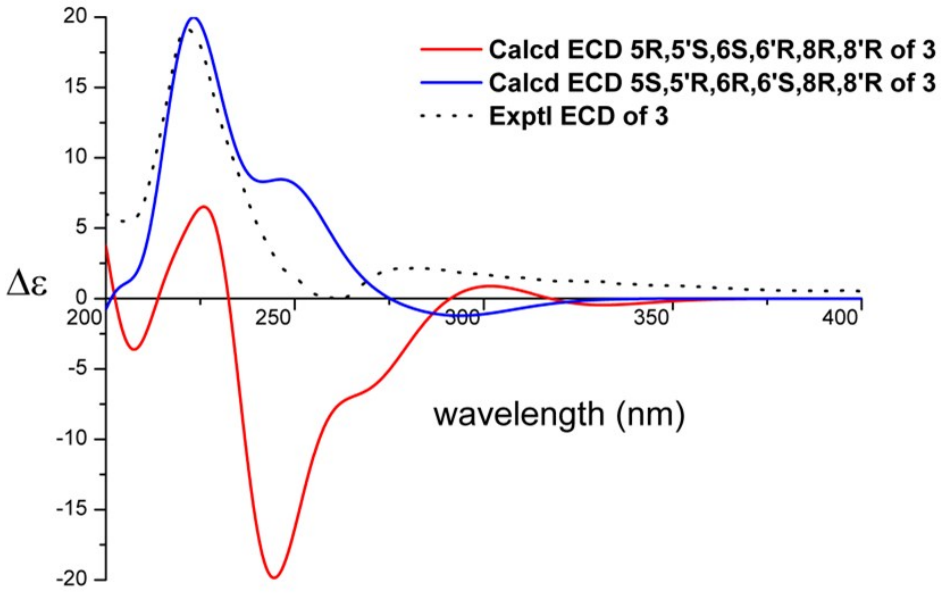


Figure S32. Experimental and calculated ECD spectra of **3**

9. The spectra of spiciferone A (4)

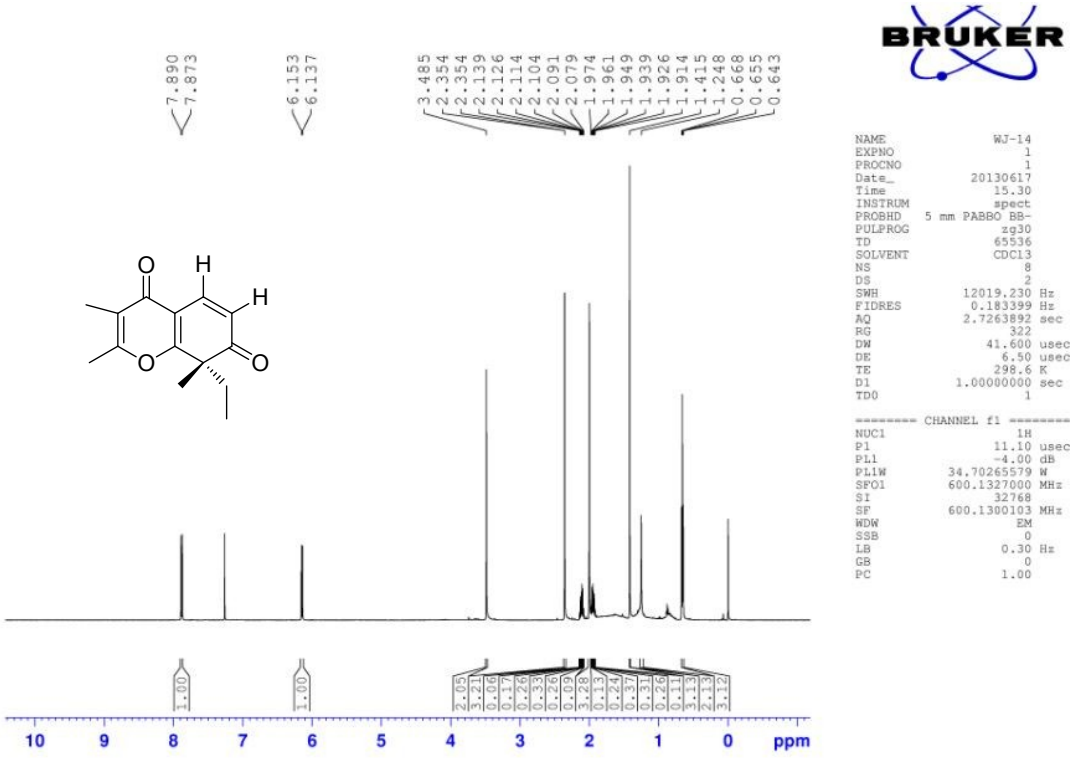


Figure S33. The ^{1}H-NMR spectrum of compound **4**

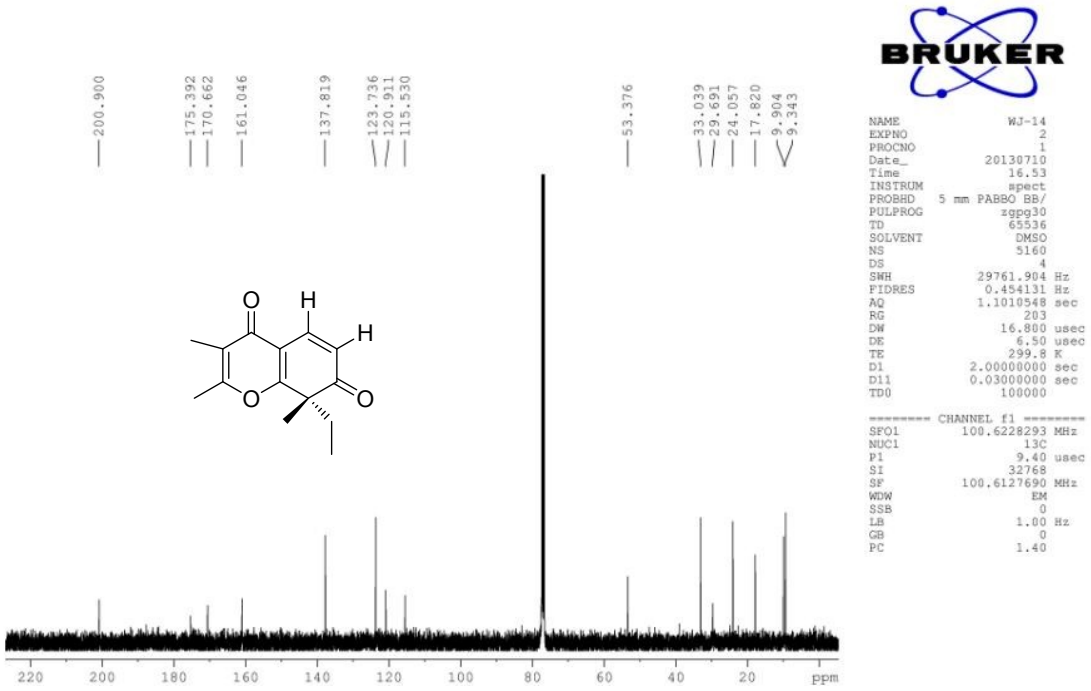


Figure S34. The ^{13}C-NMR spectrum of compound **4**

10. Photochemical synthsis of spiciferone A dimers

The photochemical synthesis was carried out in solution and solid states, respectively. A saturated solution of spiciferone A (**4**) (2.0 mg, 0.086 mmol) in 2.0 mL of EtOAc, kept in a vial of 10 mL, was exposed to normal environment of the laboratory where the extraction and isolation were performed. The reaction was monitored by silica gel TLC and HPLC/DAD (Fig. S35). Since it reached a balance, the reaction was stopped after 5 days (Fig. S36). Other different solvents, including MeOH and acetone, were also investigated. In the solid state, compound **4** was kept in a vial and exposed for 5 days.

11. HPLC/DAD analyses of photoreaction mixtures and crude EtOAc extract of strain PR-M-3

Fermentation of the strain PR-M-3 was carried out in dark. Then the fermentation broth was filtered and extracted by EtOAc, and extreme care was taken to avoid light-irradiation during the procedure of filtration and extraction. The EtOAc crude extract was detected by HPLC/DAD (Fig. S37).

Chromatographic conditions: Analytic HPLC was carried out on a Shimazu LC-20AB

high performance liquid chromatography system with a SPD-M20A DAD detector. All the separations were carried out at 25 ºC on a Phenomenex ODS column (4.6 mm × 250 mm, i.d. 5 μm). Detection wavelength was set at 254 nm.

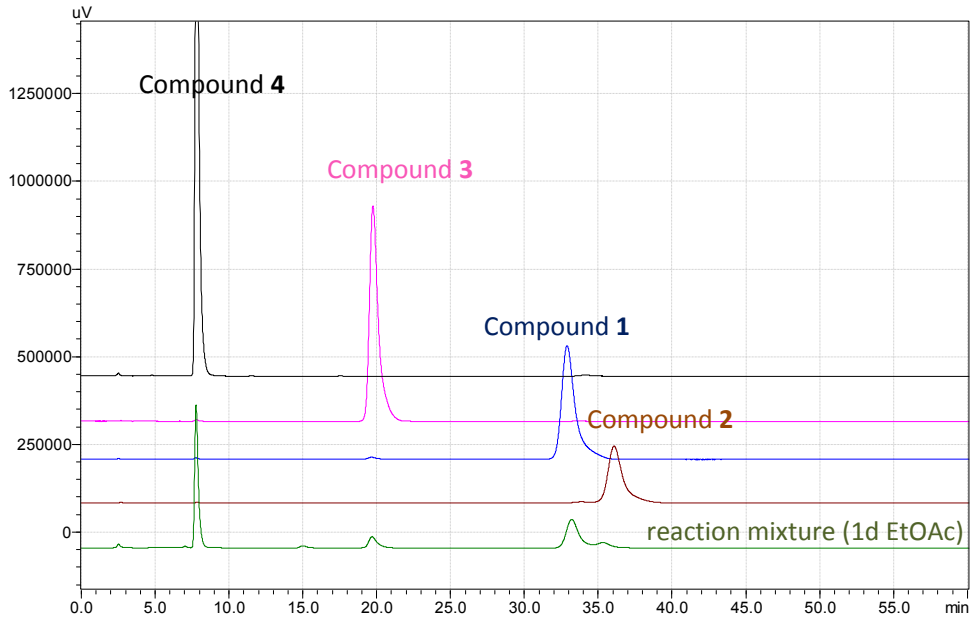


Figure S35. HPLC analysis of the reaction mixture using MeOH-H_2O (70:30, 1 mL/min) as eluent

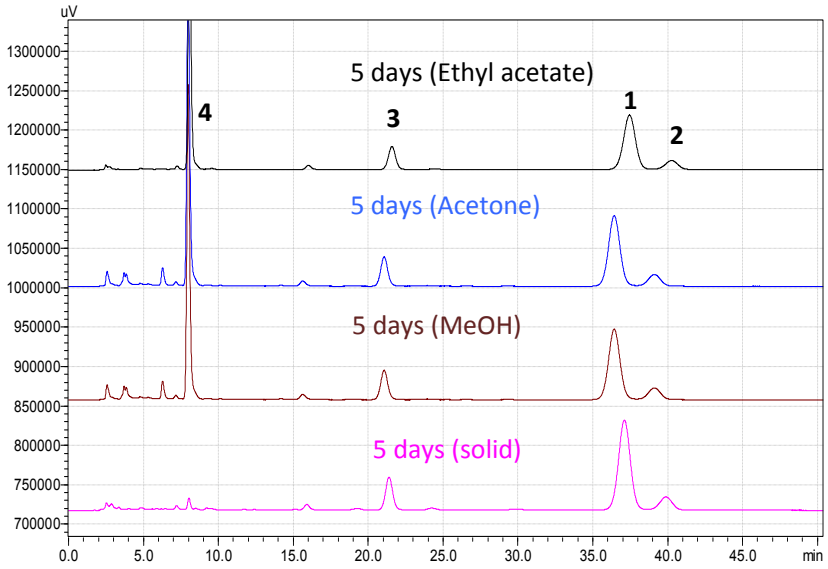


Figure S36. HPLC analysis of photoreaction mixtures in different solvents at 5th day using MeOH-H_2O (70:30, 1 mL/min) as eluent

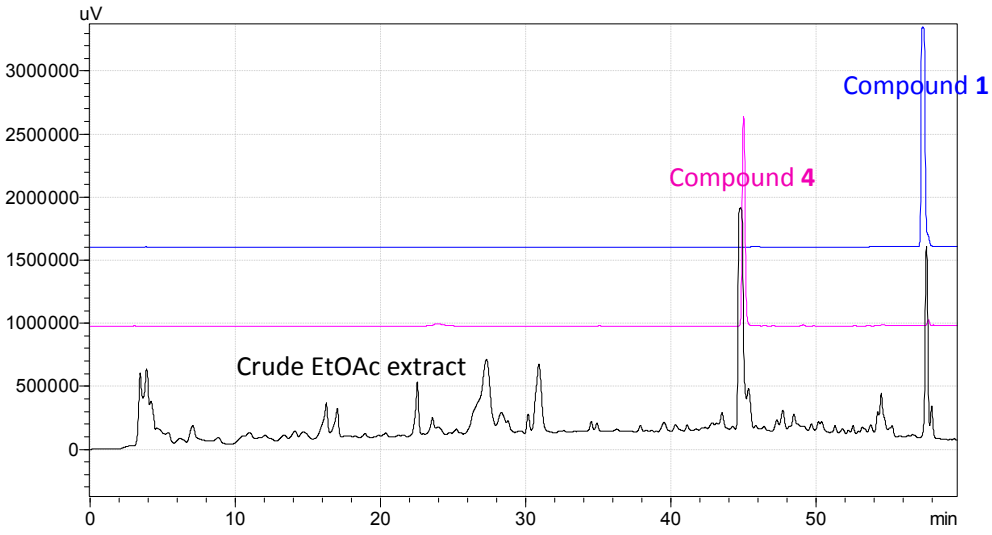


Figure S37. HPLC analysis of crude EtOAc extract of strain PR-M-3 using MeOH-H_2O (5:95-100:0, 1 mL/min) gradient elution