Trichiconlides A and B: Two Novel Limonoids from the Fruits of *Trichilia connaroides*

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Supporting Information

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2. NMR, IR, HRESIMS, and UV Spectra

1. EXPERIMENTAL SECTION

1.1 General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter at room temperature. IR spectra were recorded on a Bruker Tensor 27 spectrometer using KBr pellets. 1D-and 2D-NMR spectra were measured on a Bruker AVIII-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz) with TMS as an internal standard. HRESIMS was obtained on an Agilent 6529B Q-TOF mass instrument using electrospray ionization. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd, China), MCI (Mitsubishi, Japan) and RP-C18 silica (40-63 μm, FuJi, Japan) were used for column chromatography. Preparative HPLC was carried out using a Shimadzu LC-8A equipped with a Shim-pack RP-C18 column (20 × 200 mm, i.d.) with a flow rate of 10.0 mL/min, detected by a binary channel UV detector. Fractions obtained from CC were monitored by TLC with precoated silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd, China) plates.

1.2 Plant Material. Air-dried fruits of *Trichilia connaroides* were collected from Xishuangbanna, Yunnan Province, People's Republic of China, in June 2014, and were identified by Professor Shun-Cheng Zhang, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, People's Republic of China. A voucher specimen (No. AA201308) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

1.3 Extraction and isolation. The air-dried fruits (5.0 kg) were refluxed with 95% industrial ethanol (3 × 5L). After removal of the solvent under reduced pressure, the

crude extract (500.0 g) was suspended in H_2O (1.5L) and partitioned with petroleum ether (3 × 1 L) and ethyl acetate (3 × 1 L), successively. The ethyl acetate extract (100.0 g) was subjected to a silica gel column, eluted with CH_2Cl_2 and MeOH (100:1, 50:1, 25:1, 10:1, 5:1, v/v) to give five fractions (A1-A5). Fraction A5 (10.5 g) was chromatographed over a MCI column, eluted with a gradient system of MeOH-H_2O (50:5, 75:25, 95:5, v/v) to give three fractions (A5A-A5C). The A5B fraction was chromatographed over a silica gel column and purified by semi-preparative-HPLC with MeOH-H_2O (45:55, v/v) as eluent, respectively, to get **1** (3 mg). Fraction A4 was chromatographed over a MCI to afford three fractions (A4A-A4C) with a gradient elution of MeOH-H_2O (50:5, 75:25, 95:5, v/v). The fraction A4B was chromatographed over an ODS (100 g) column, and purified be semi-preparative-HPLC with MeOH-H_2O (50:50, v/v) to obtain **2** (20 mg), and **3** (10mg).

1.4 Computational Section

The calculation of ECD have been extensively applied in the determination of the absolute configurations of natural chiral molecules. Systematic conformation analyses for compound **1** were performed via Confab using the MMFF94 force field calculation. Conformers with Boltzmann distribution over 1% were chosen as the beginning for ECD calculations. Ground-state geometries were optimized at B3LYP/6-311G** level by the Gaussian09 program package and vibrational analysis was done to confirm these minima. the Self-Consistent Reaction Field method (SCRF) with the C-Polarizable Continuum Model (CPCM) was further employed to perform the conformational analysis and ECD calculation in methanol solution at B3LYP/6-

311G** level. The theoretical ECD spectra was obtained based on the Boltzmann weighting of each conformers. Comparisons of the experimental and calculated spectra were done using SpecDis with UV shift (-18 nm) and a half-bandwidth of 0.3 eV. The absolute configurations of **1** were assigned as1*S*,3*S*,4*R*,5*R*,10*S*,13*S*,8*E*,14*E*,17*R*, and 30*S*, respectively.

1.5 NO production bioassay

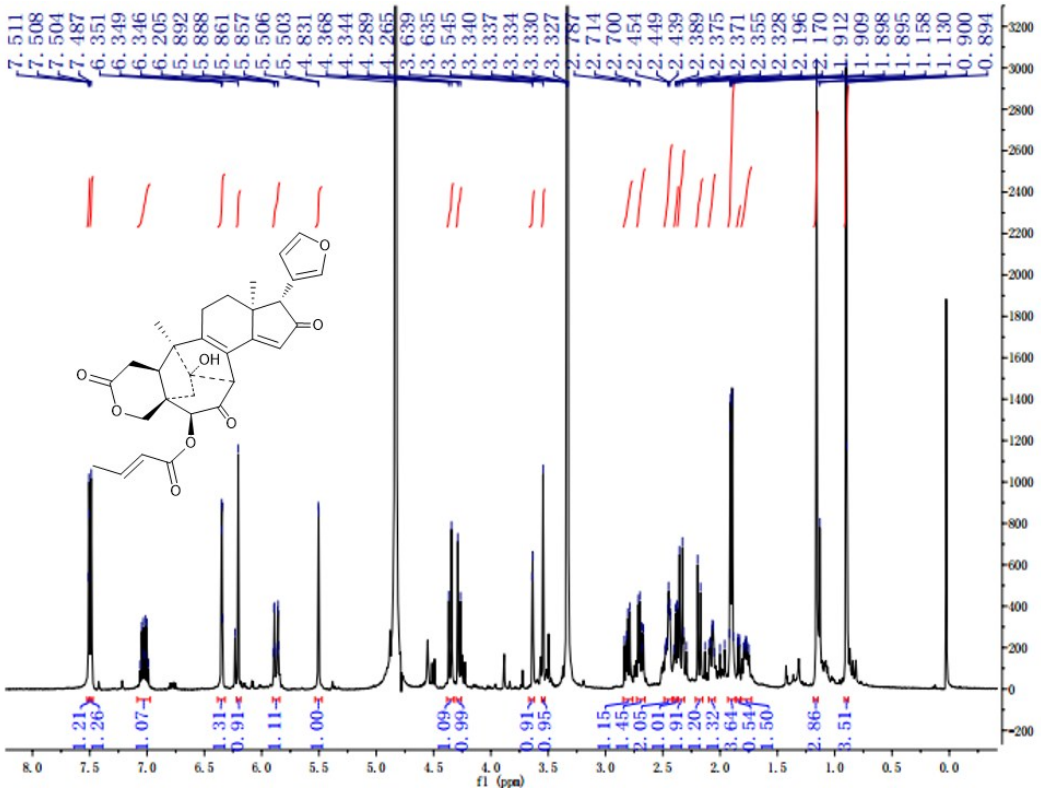
The RAW264.7 cell line was purchased from the Chinese Academic of Sciences. The cells were cultured in DMEM containing 10% FBS with penicillin (100 U/mL) and streptomycin (100 U/mL) at 37 ºC in a humidified atmosphere with 5% CO_2. The cells were allowed to grow in 96-well plates with 1×10^5 cells/ well to treat test compounds. After being incubated for 2 h, the cells were treated with 100 ng/mL of LPS for 18 h. Nitrite in culture media was measured to assess NO production using Griess reagent. The absorbance at 540nm was measured on a microplate reader. N-monomethyl-L-arginine was used as the positive control. Cytotoxicity was determined by the MTT method, after 48 h incubation with test compounds. All the experiments were performed in three independent replicates.

1.6 Single Crystal X-ray Diffraction

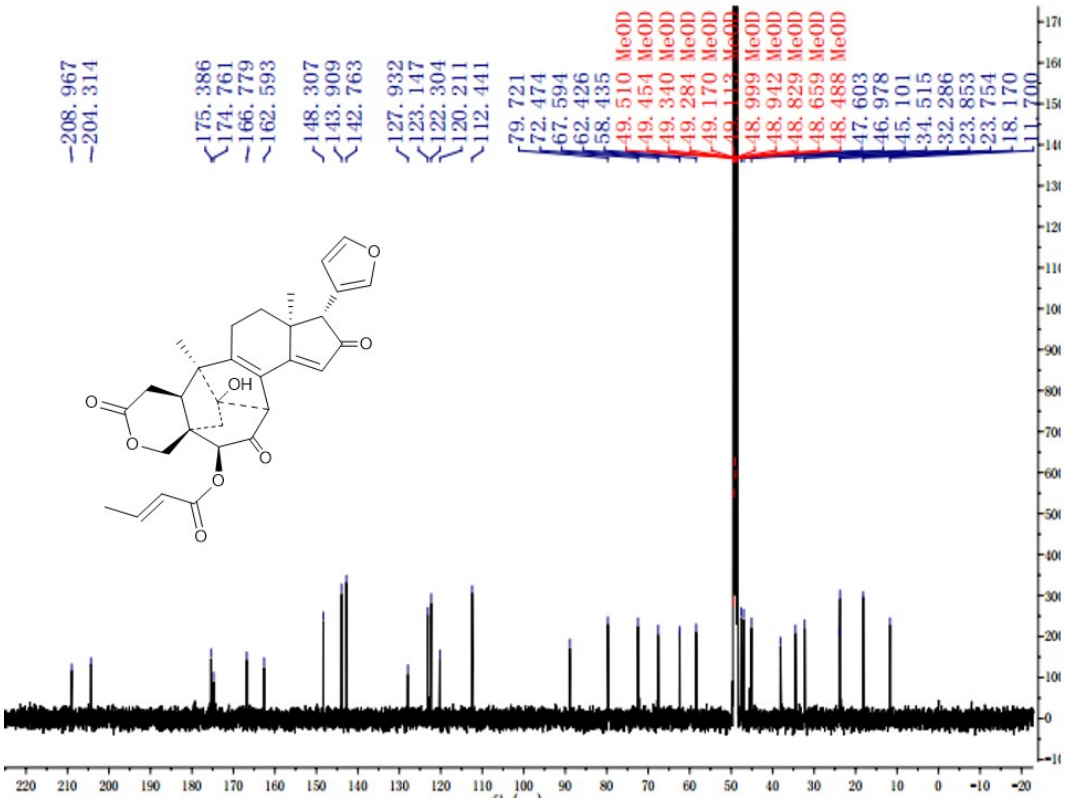
Crystal data of **1**: $C_{27}H_{34}O_{10}$ (MeOH), Crystal Data for $C_{28}H_{38}O_{11}$ (M = 550.58 g/mol): orthorhombic, space group P212121 (no. 19), a = 10.03740(10) Å, b = 12.63980(10) Å, c = 21.0812(2) Å, V = 2674.59(4) Å^3, Z = 4, T = 290(2) K, μ(CuKα) = 0.880 mm^{-1}, Dcalc = 1.367 g/cm^3, 22499 reflections measured ($8.388 \leq 2\theta \leq 139.342$), 4923 unique ($R_{int}$ = 0.0210, R_{sigma} = 0.0138) which were used in all calculations. The final

R1 was 0.0393 [I > 2σ (I)] and wR2 was 0.1114 (all data). The Flack parameter is 0.06 (4). The crystallographic data for **2** was deposited in the Cambridge Crystallographic Data Centre (deposition number **CCDC 1400291**). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK [fax (+44) 1223 336 003; e-mail: deposit@ccdc.cam.ac.uk].

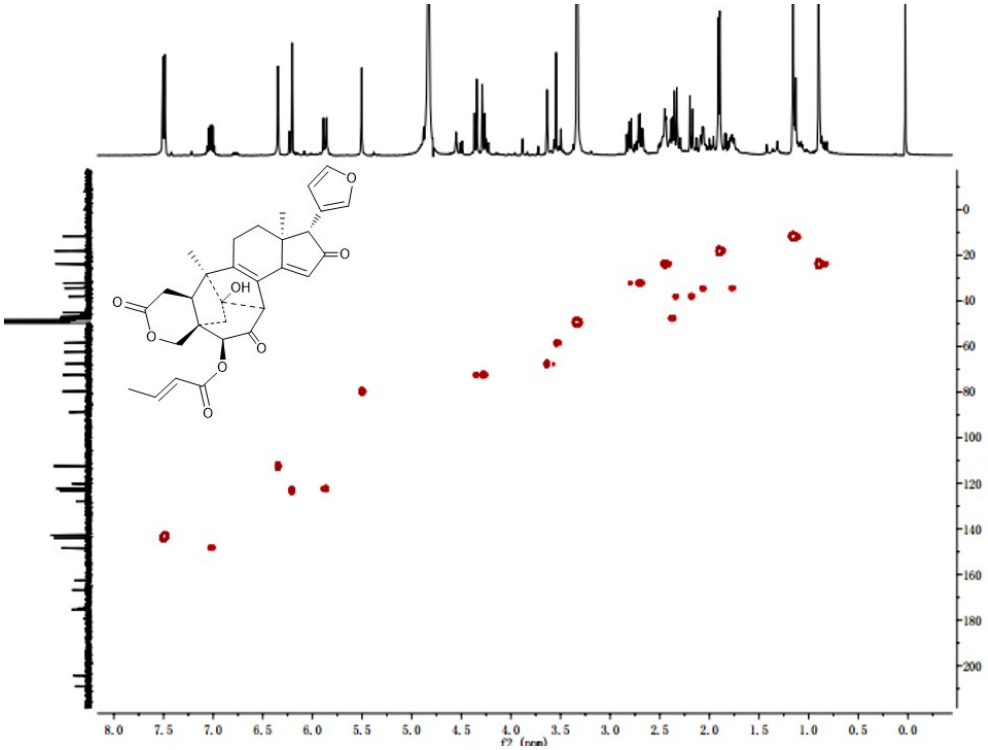
2. NMR, IR, HRESIMS, and UV Spectra



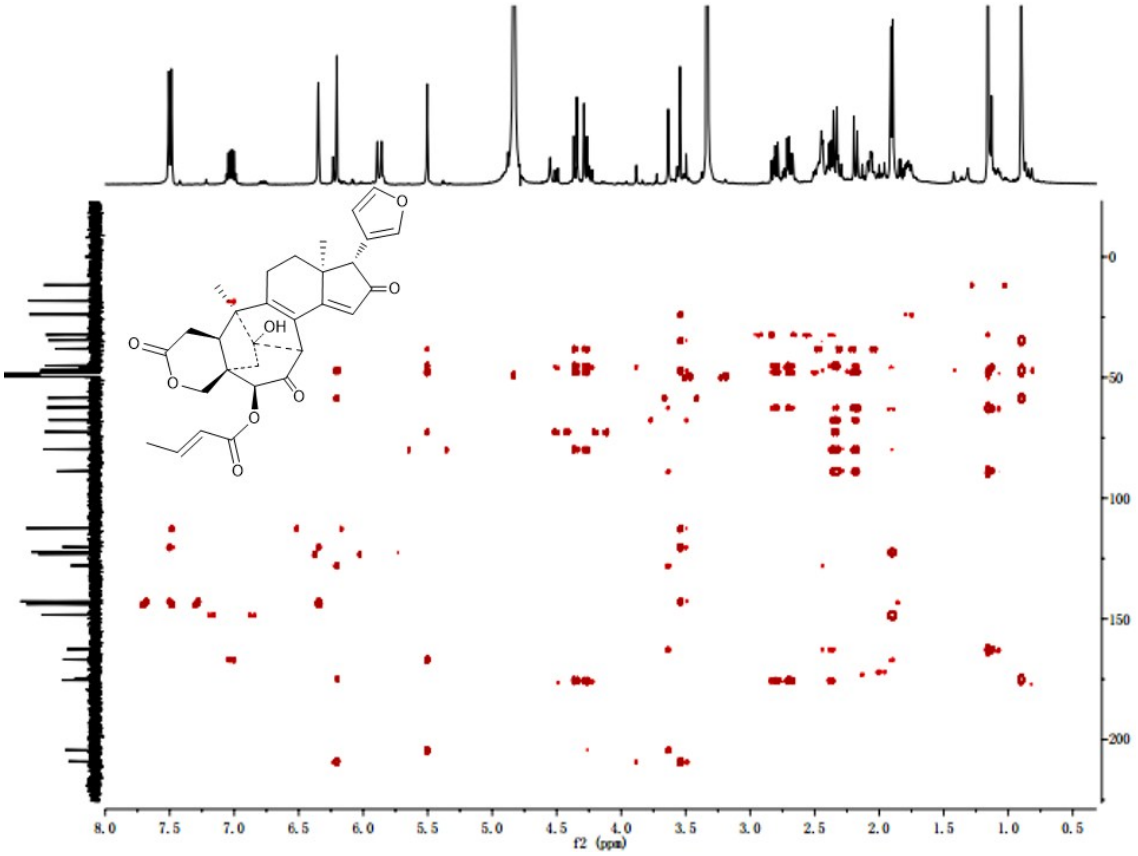
S1. ^{1}H NMR (500 MHz; MeOD-d_4) spectrum of **1**



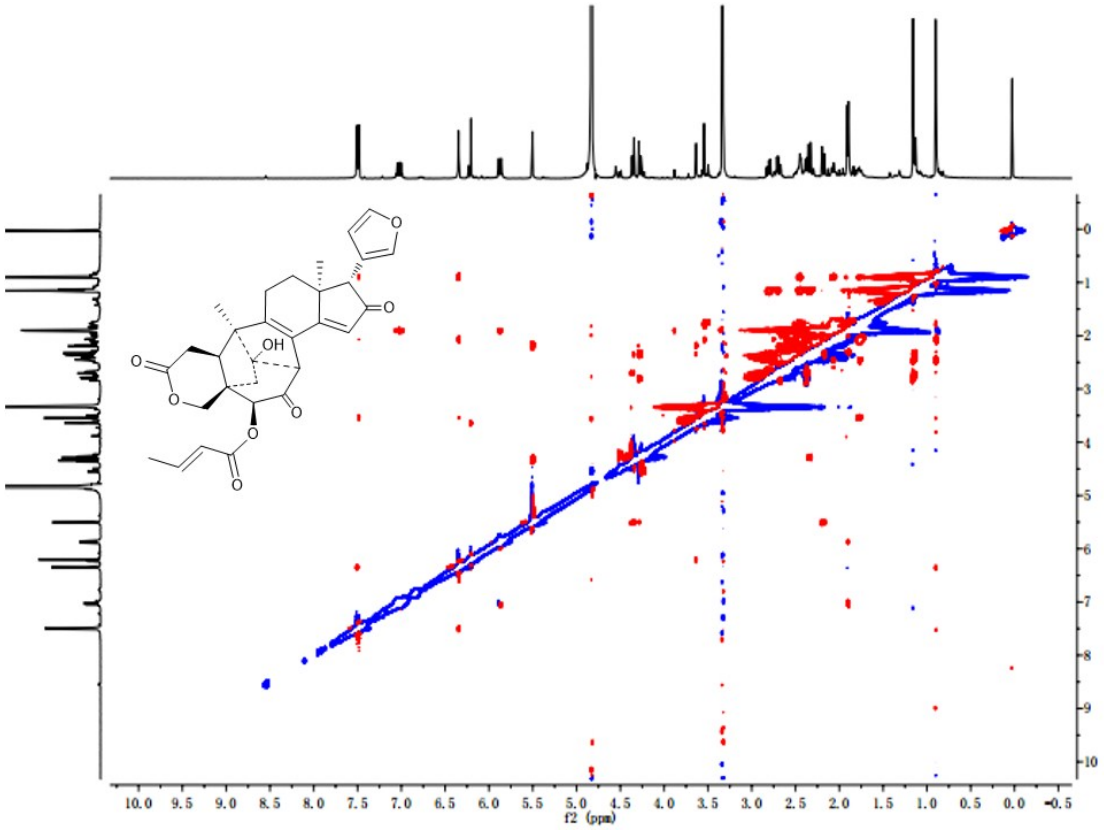
S2. ^{13}C NMR (125 MHz; MeOD-d_4) spectrum of **1**



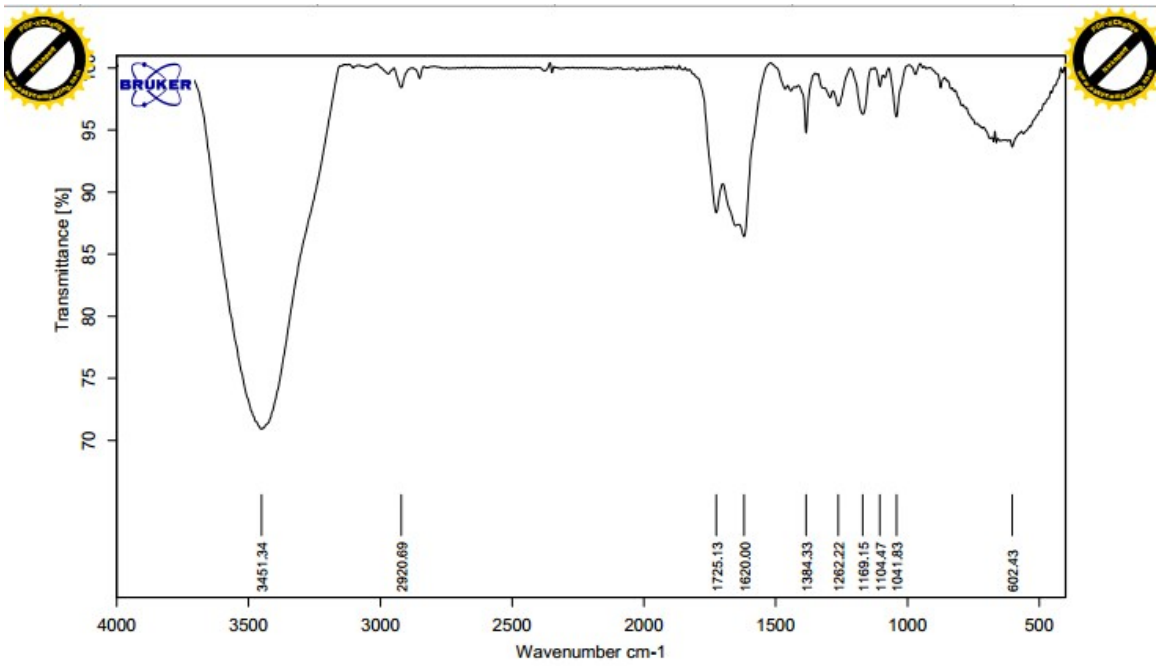
S3. HSQC (MeOD-d_4) spectrum of **1**



S4. HMBC (MeOD-d_4) spectrum of **1**



S5. ROESY (MeOD-d_4) spectrum of **1**

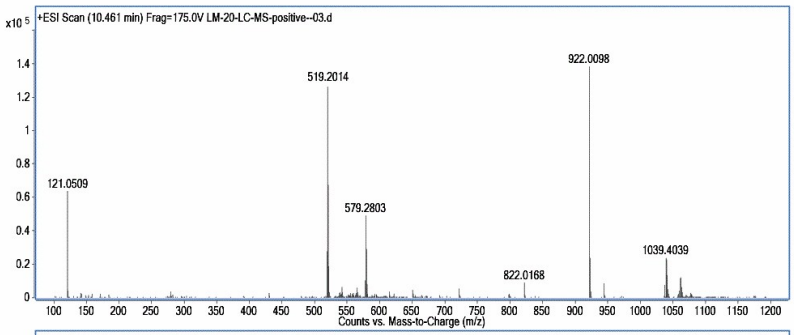


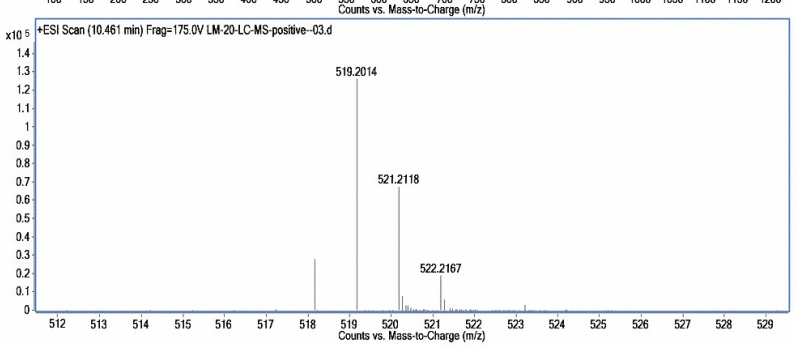
S6. IR spectrum of **1**

TCM-CPU HR-ESI-MS Display Report

Sample Name: LM-20 **Instrument:** Agilent 6520B Q-TOF

Acq. Date: 08/05/2015 **Operator:** Administrator





Elemental Composition Calculator

Target m/z:	519.2014	**Result type:**	Positive ions	**Species:**	$[M+H]^+$
Elements:		C (0-80); H (0-120); O (0-30); N(0-10); Na (0-5)			
Ion Formula		**Calcalated m/z**		**PPM Error**	
C30H31O8		519.2013		-0.11	

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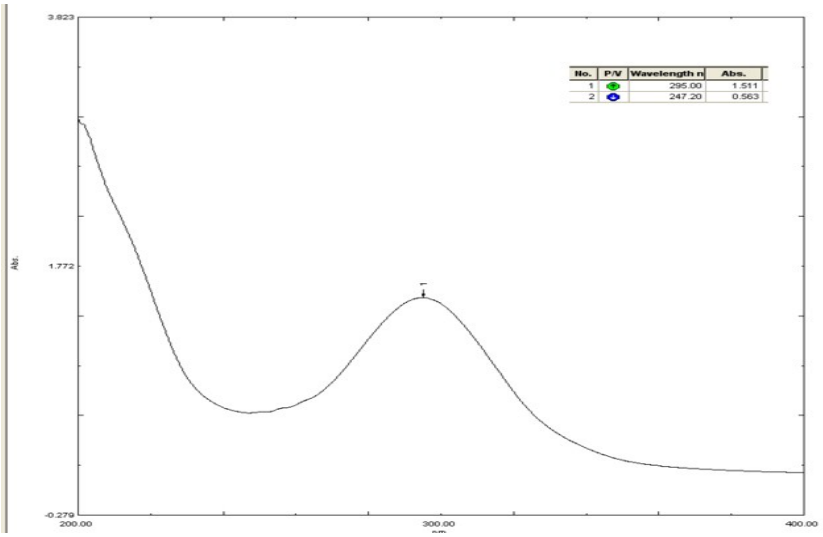
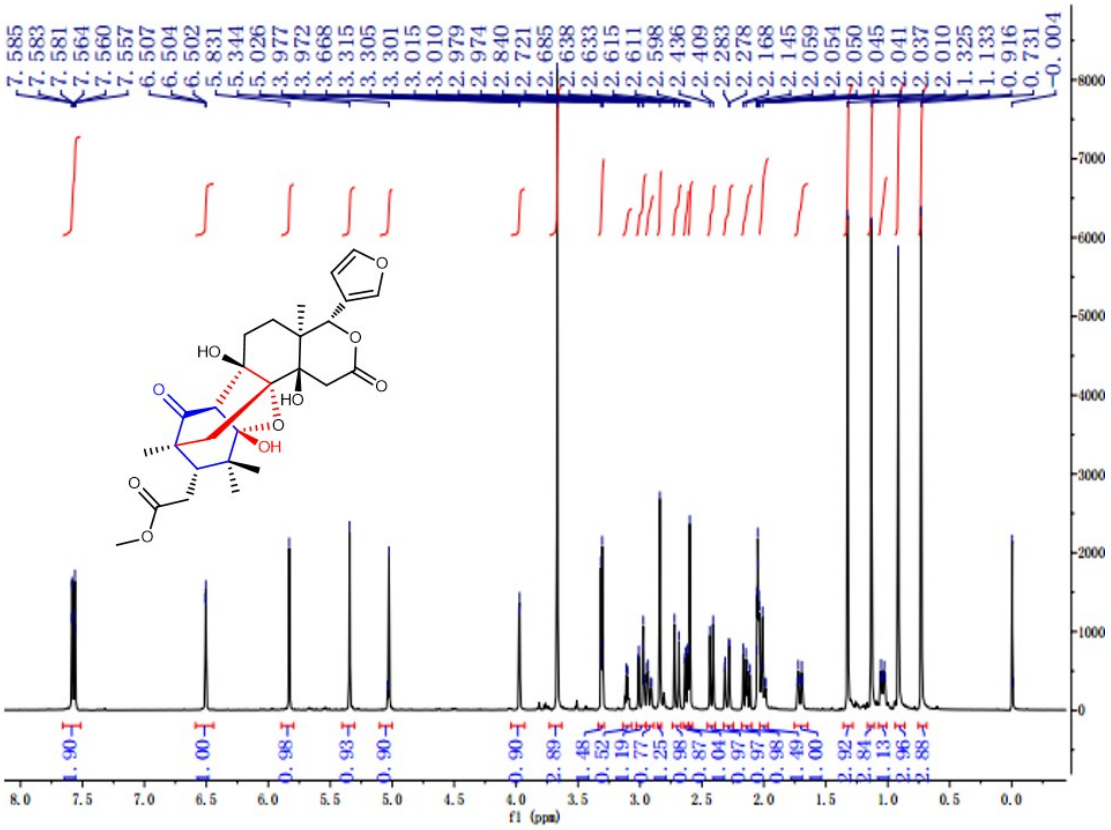
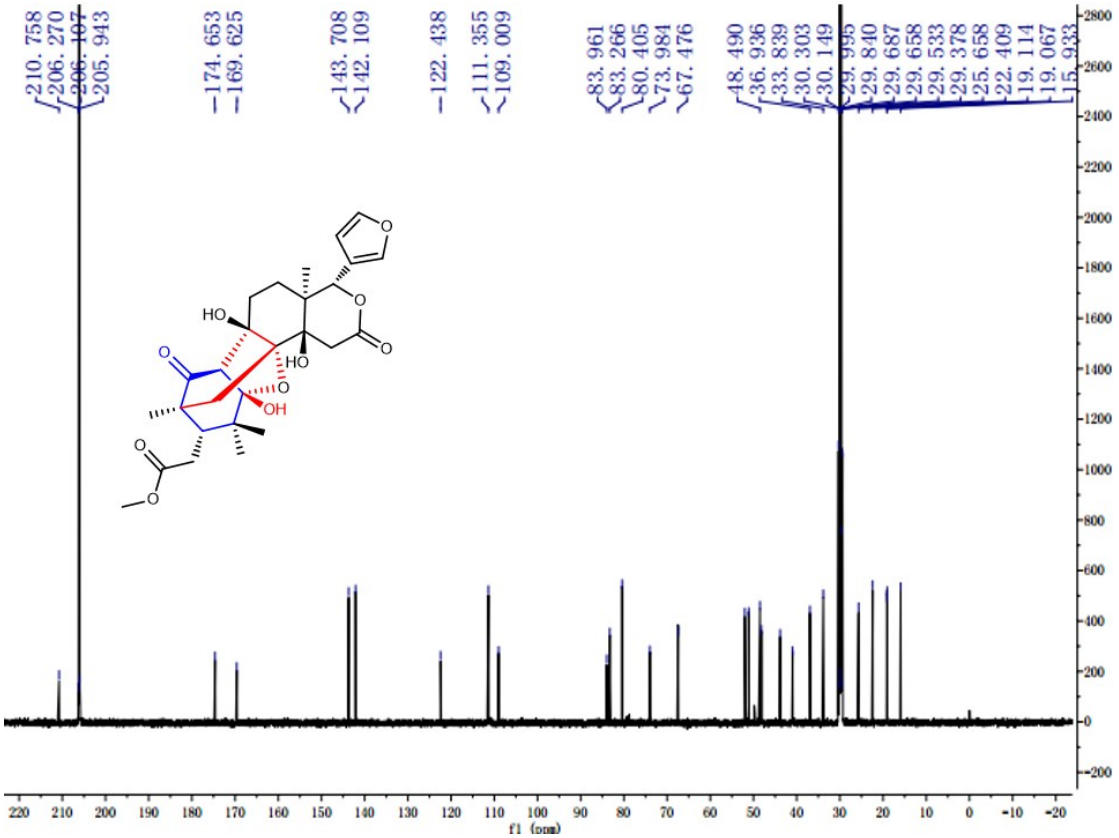
S7. HRESIMS of **1**



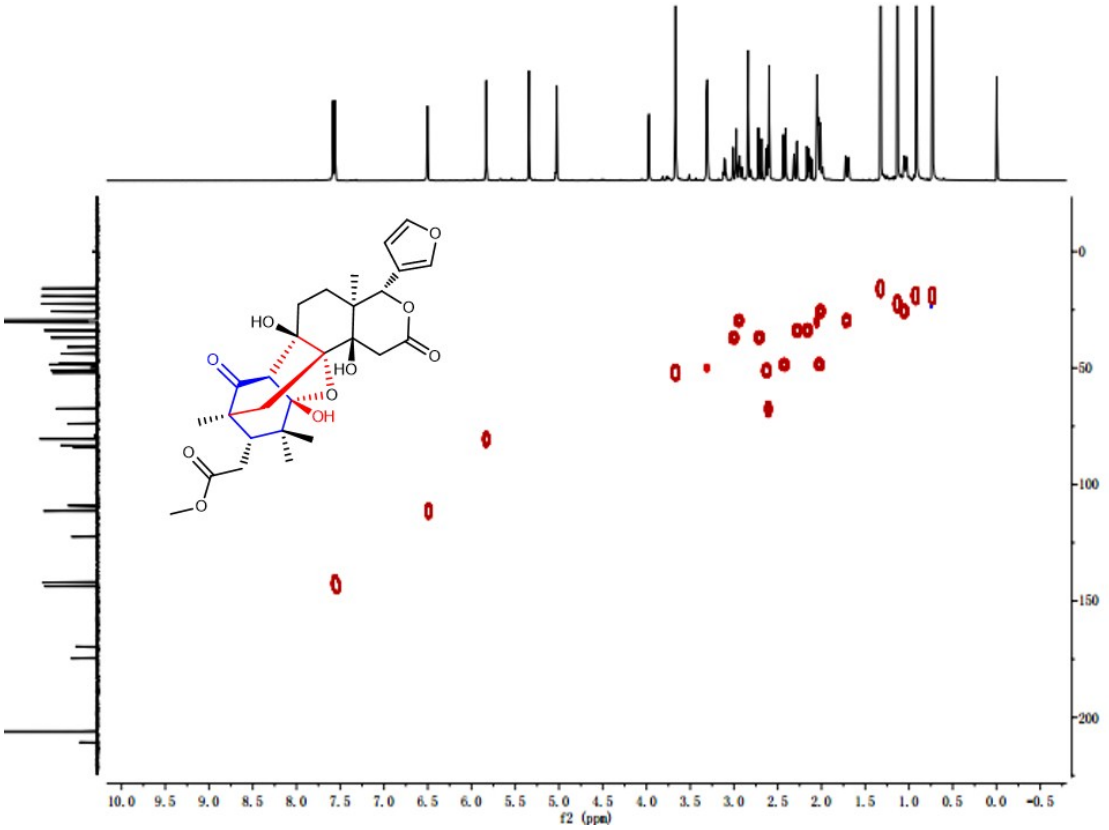
S8. UV spectrum of **1**



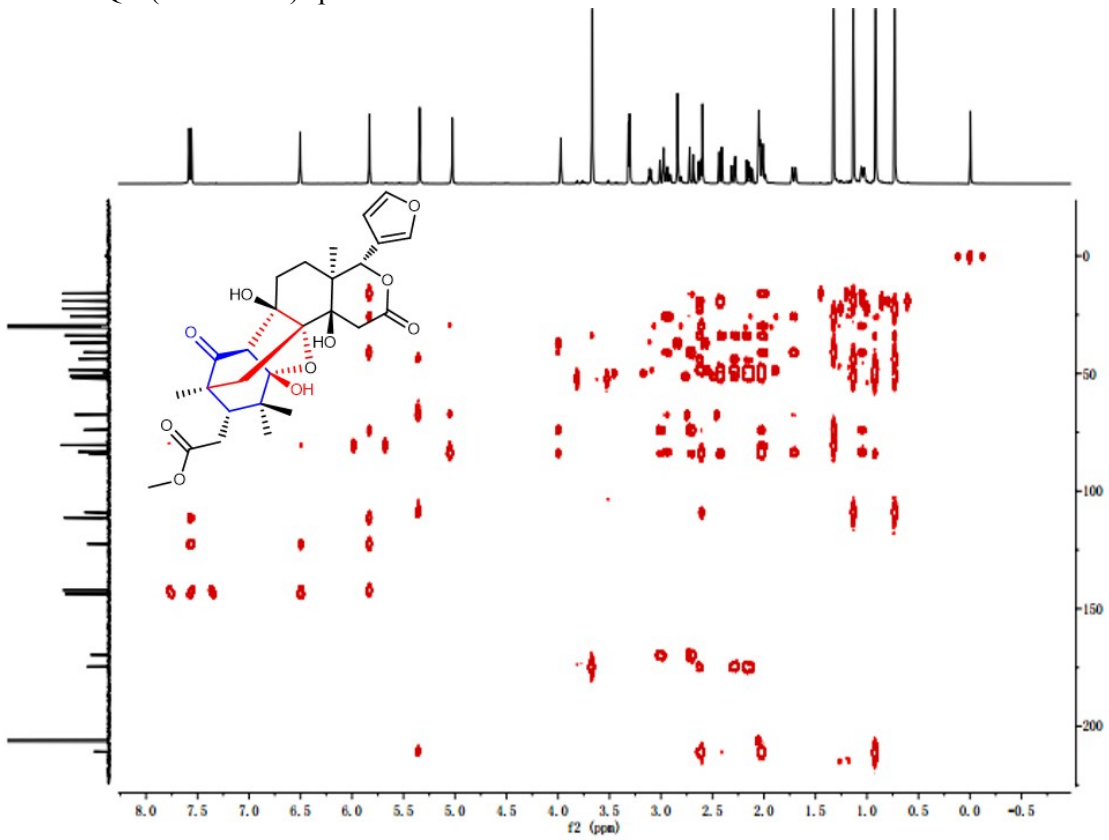
S9. ^{1}H NMR (500 MHz; Acetone-d_6) spectrum of **2**



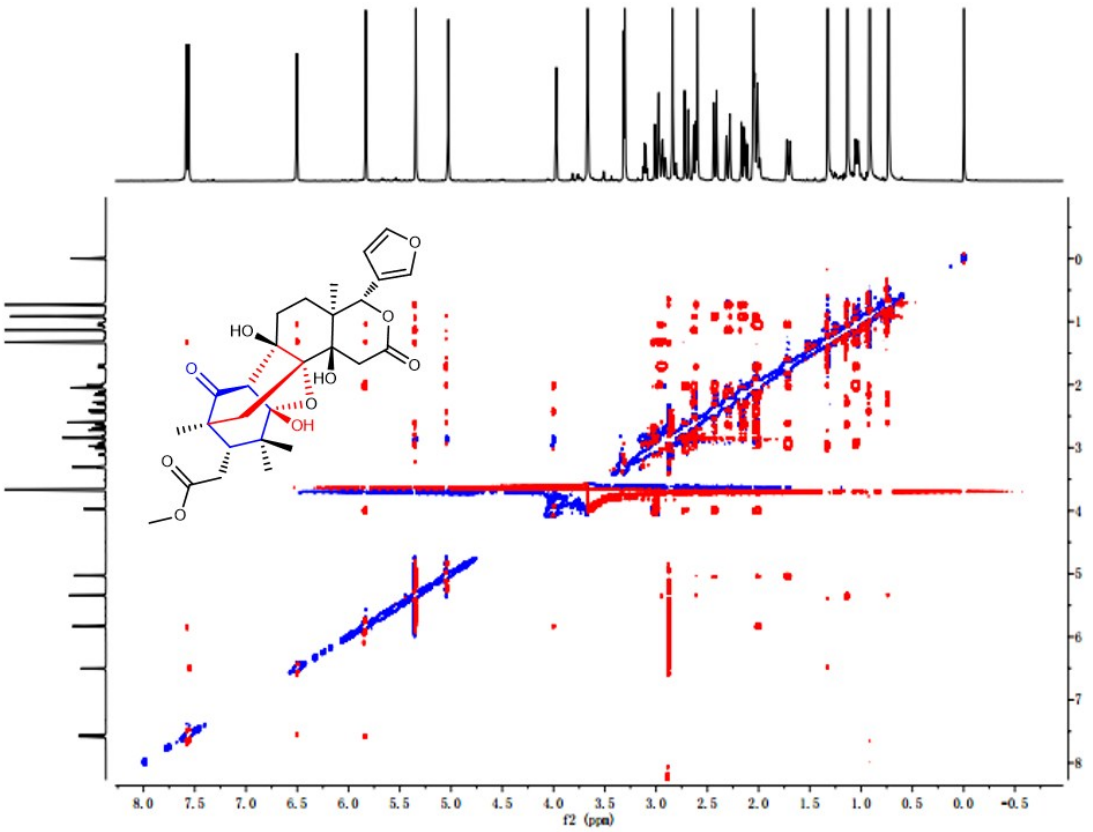
S10. ^{13}C NMR (125 MHz; Acetone-d_6) spectrum of **2**



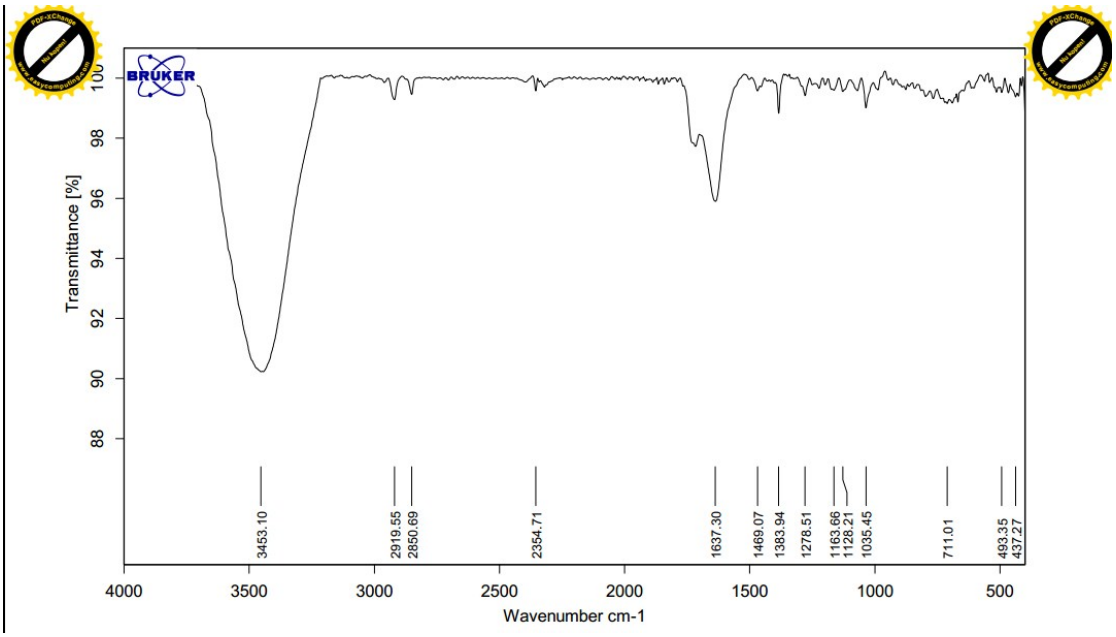
S11. HSQC (Acetone-d_6) spectrum of **2**



S12. HMBC (Acetone-d_6) spectrum of **2**



S13. ROESY (Acetone-d_6) spectrum of **2**



S14. IR spectrum of **2**

TCM-CPU HR-ESI-MS Display Report

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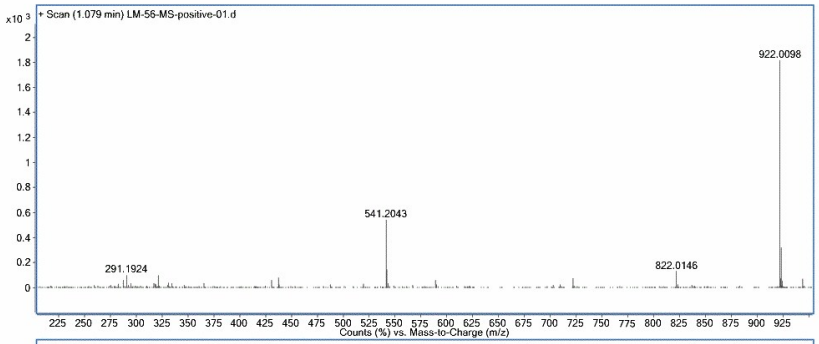
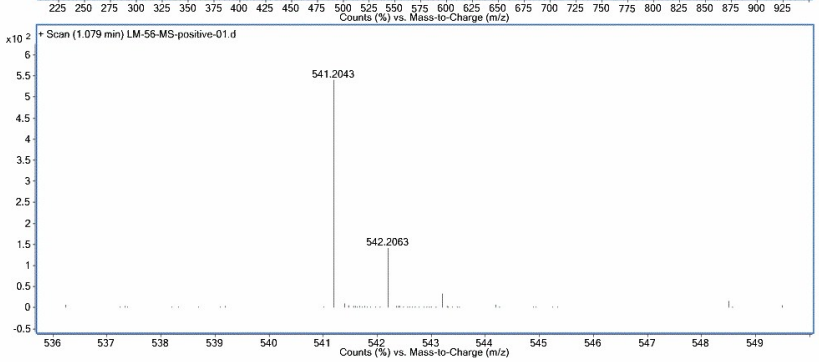
Acq. Date: 05/16/2015 **Operator:** Administrator





Elemental Composition Calculator

Target m/z:	541.2043	**Result type:**	Positive ions	**Species:**	$[M+Na]^+$
Elements:		C (0-80); H (0-120); O (0-30); N(0-10); Na (0-5)			
Ion Formula		**Calcalated m/z**		**PPM Error**	
C27H34NaO10		541.2044		0.26	

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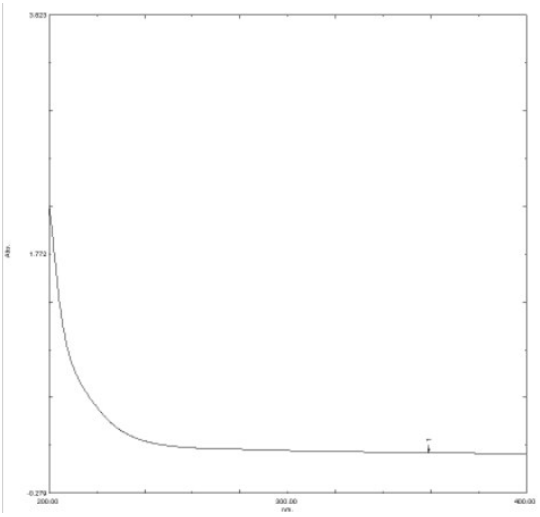
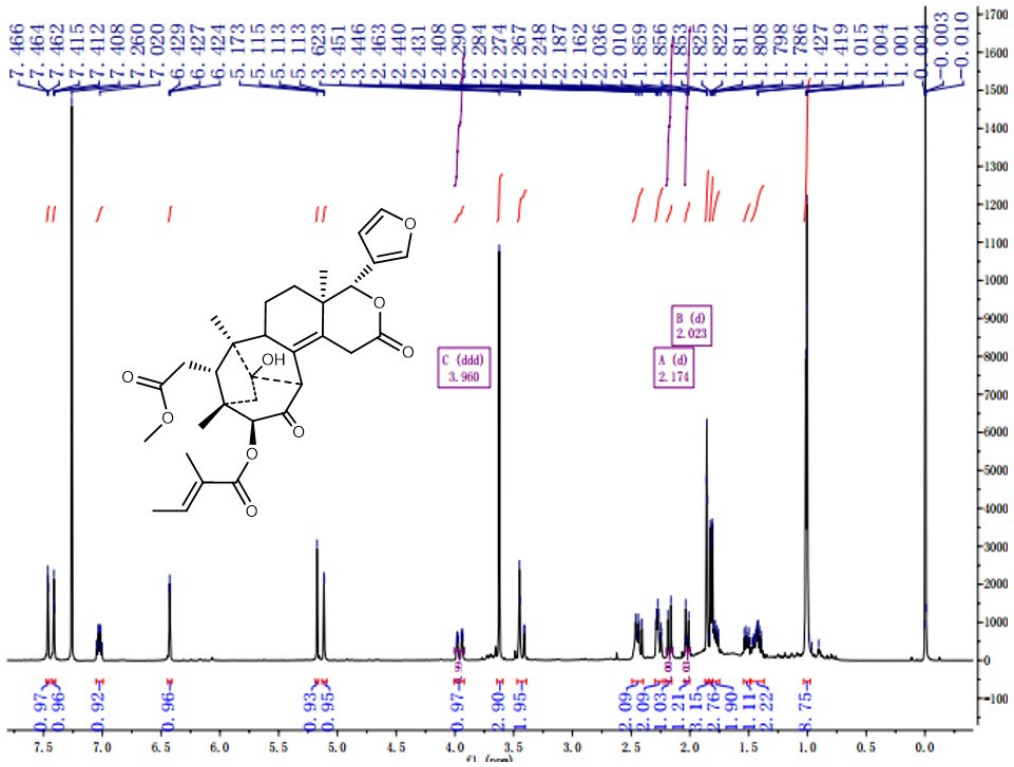
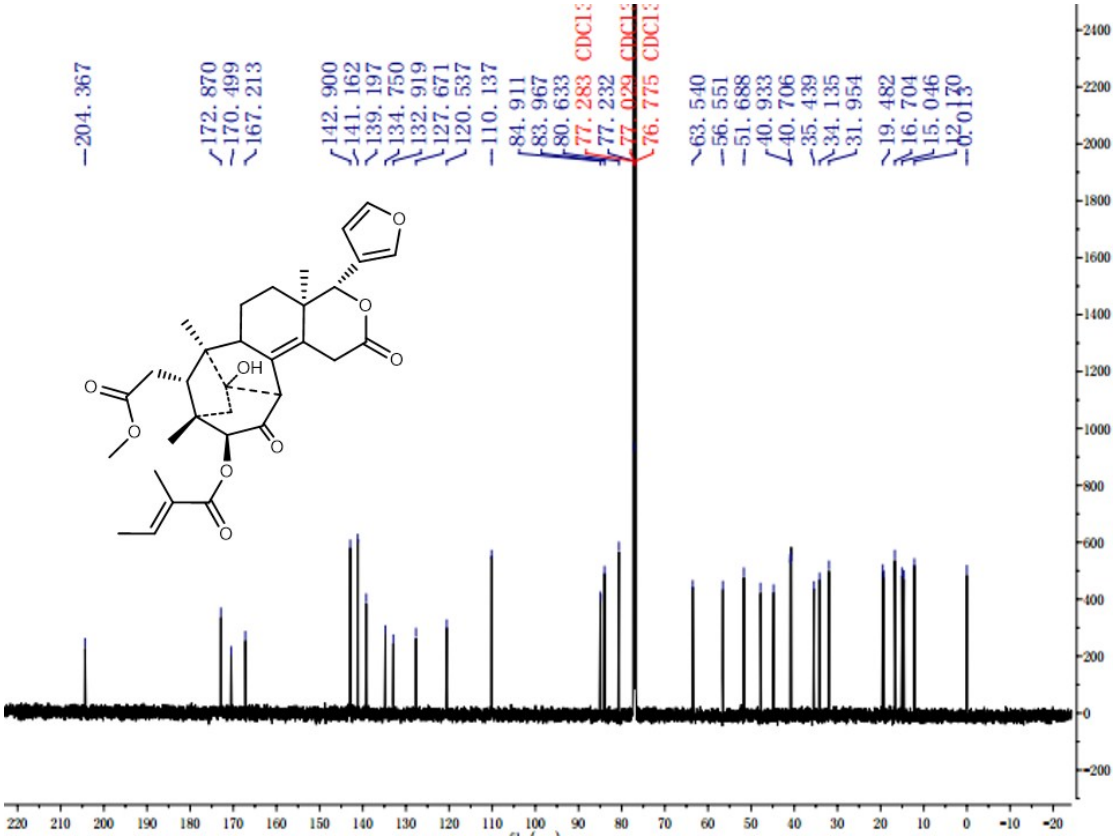
S15. HRESIMS of **2**



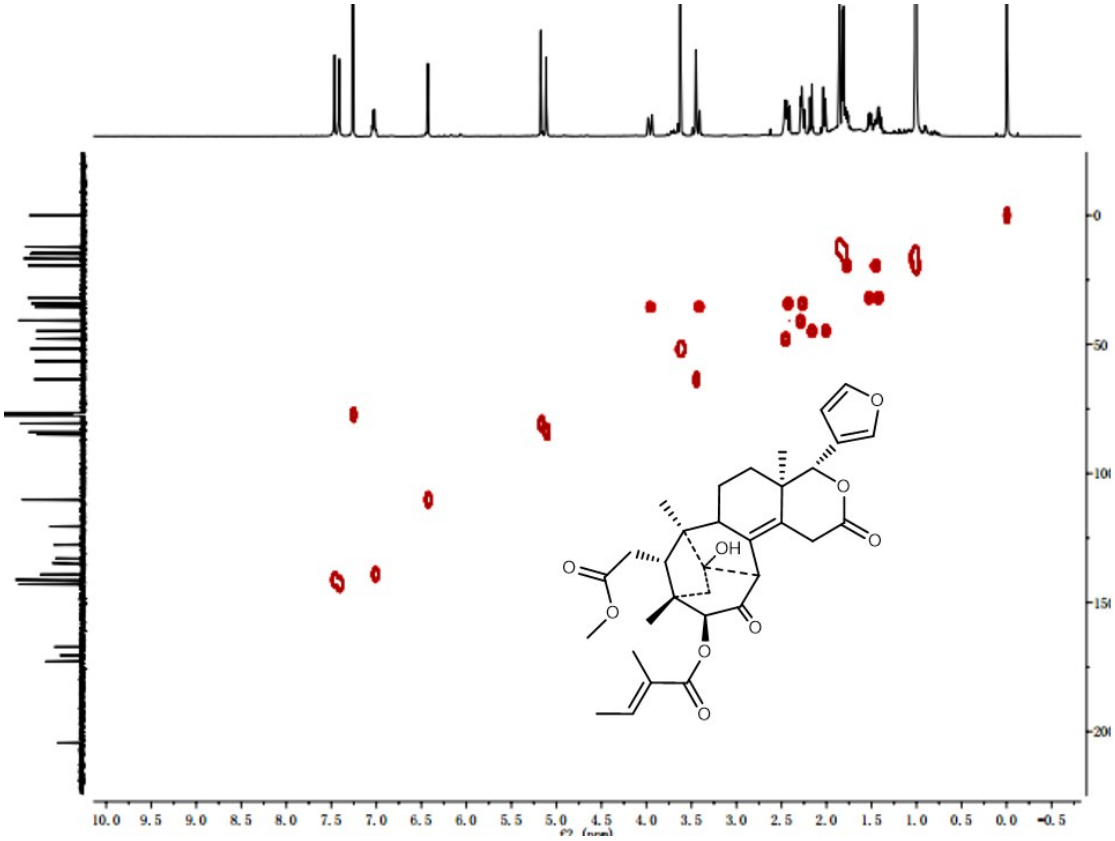
S16. UV spectrum of **2**



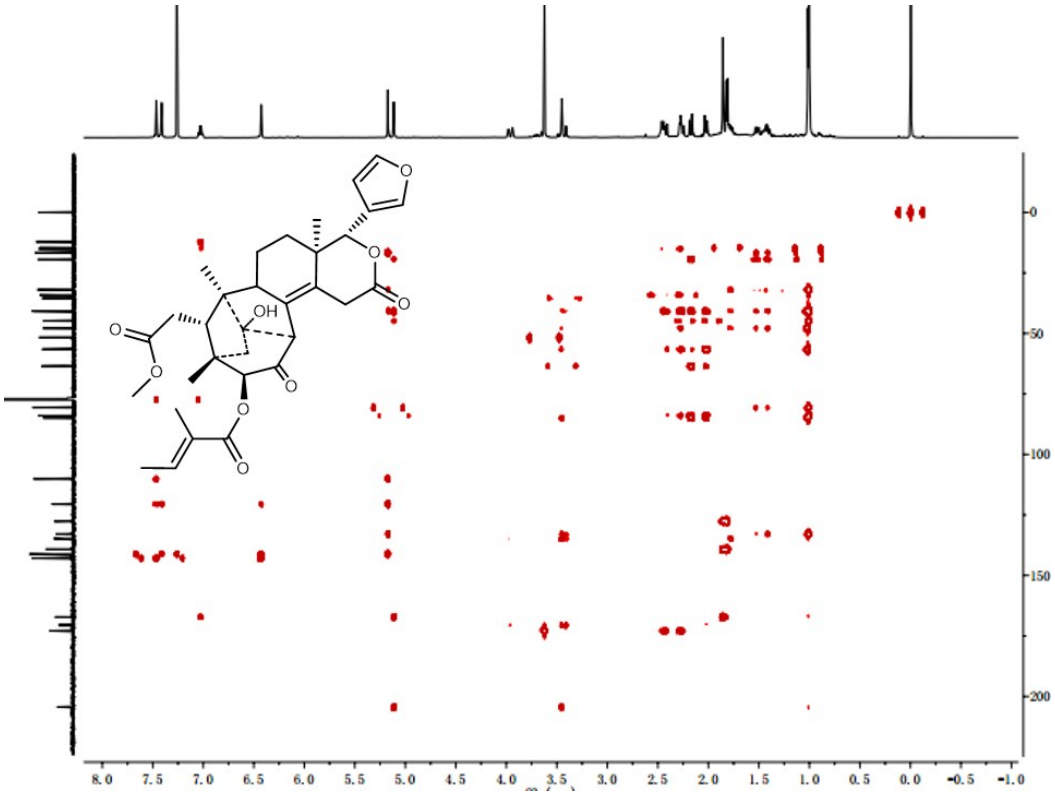
S17. ^{1}H NMR (500 MHz; $CDCl_3$) spectrum of **3**



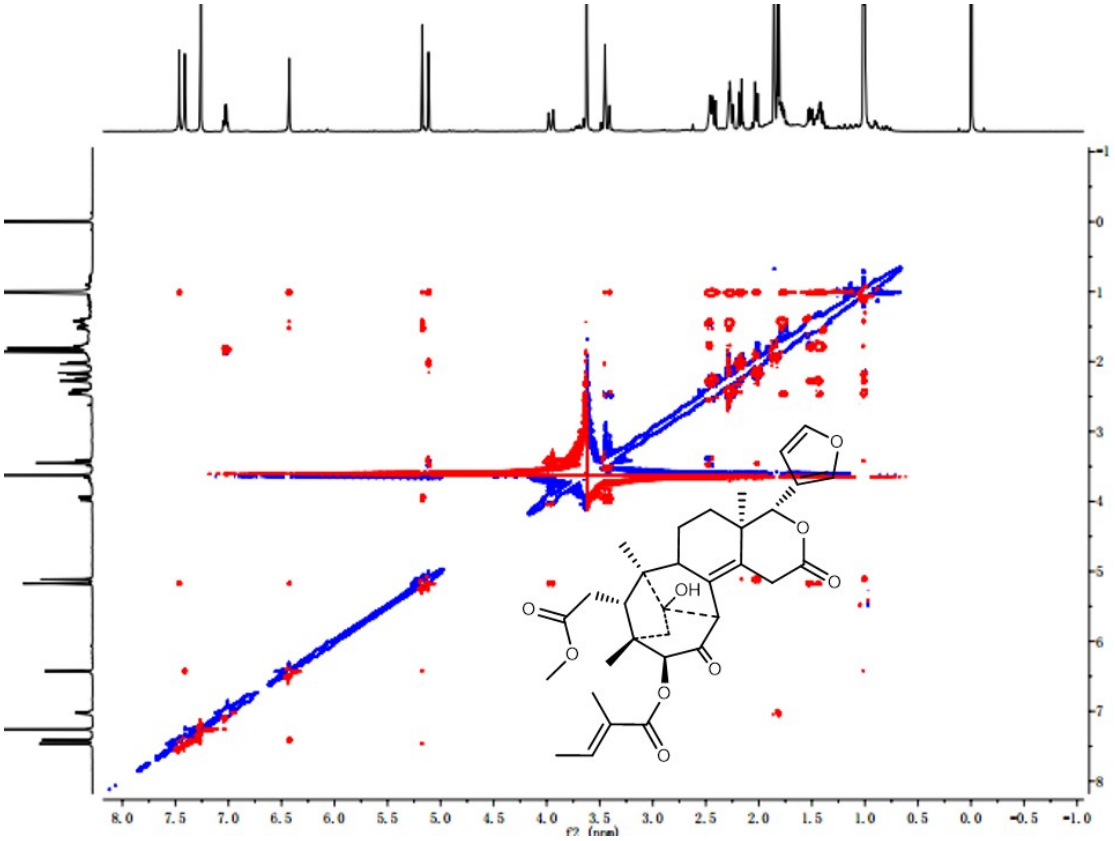
S18. ^{13}C NMR (125 MHz; $CDCl_3$) spectrum of **3**



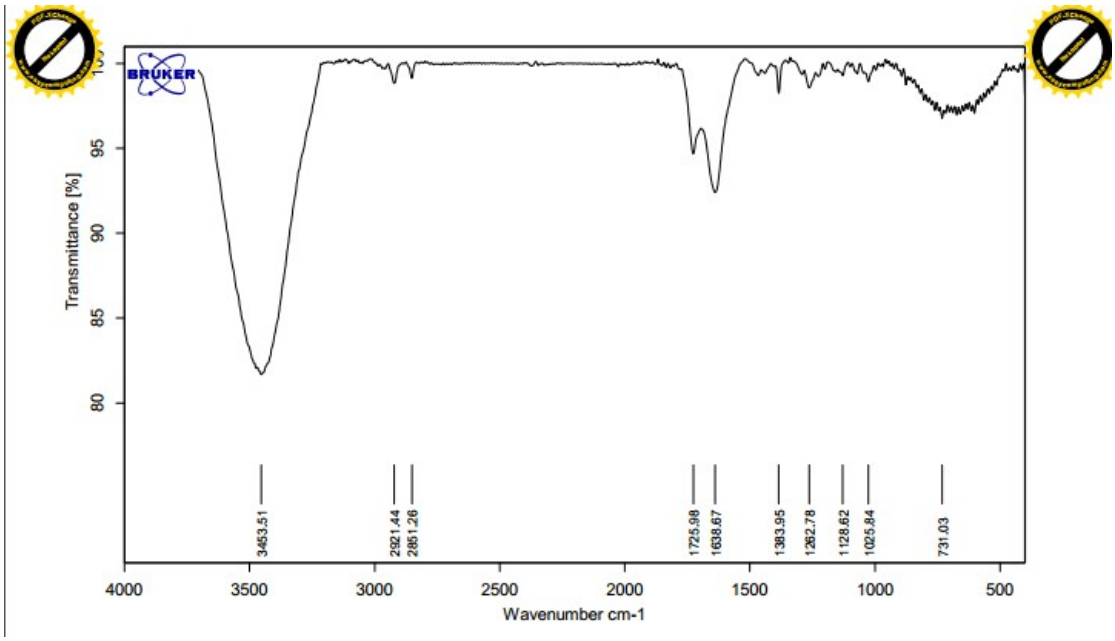
S19. HSQC ($CDCl_3$) spectrum of **3**



S20. HMBC ($CDCl_3$) spectrum of **3**



S21. ROESY ($CDCl_3$) spectrum of **3**

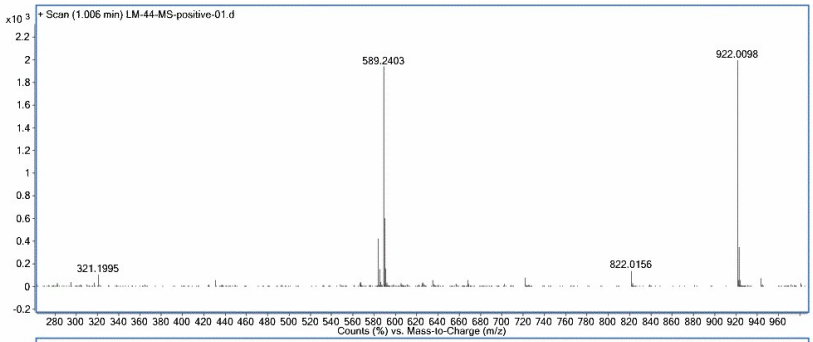


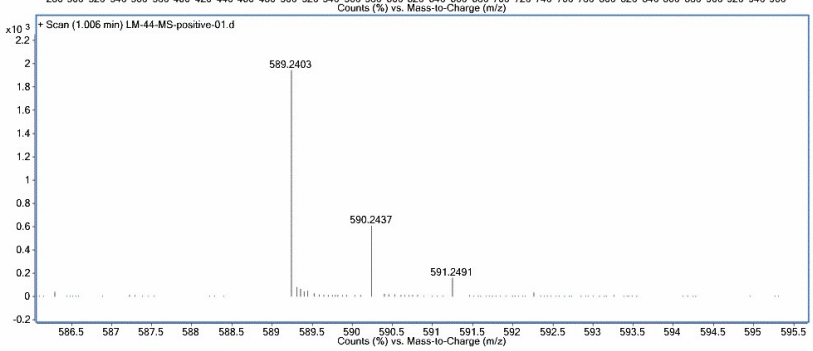
S22. IR spectrum of **3**

TCM-CPU HR-ESI-MS Display Report

Sample Name: LM-44 **Instrument:** Agilent 6520B Q-TOF

Acq. Date: 05/16/2015 **Operator:** Administrator





Elemental Composition Calculator

Target m/z:	589.2403	**Result type:**	Positive ions	**Species:**	$[M+Na]^+$
Elements:		C (0-80); H (0-120); O (0-30); N(0-10); Na (0-5)			
Ion Formula		**Calcalated m/z**		**PPM Error**	
C32H38NaO9		589.2408		0.85	

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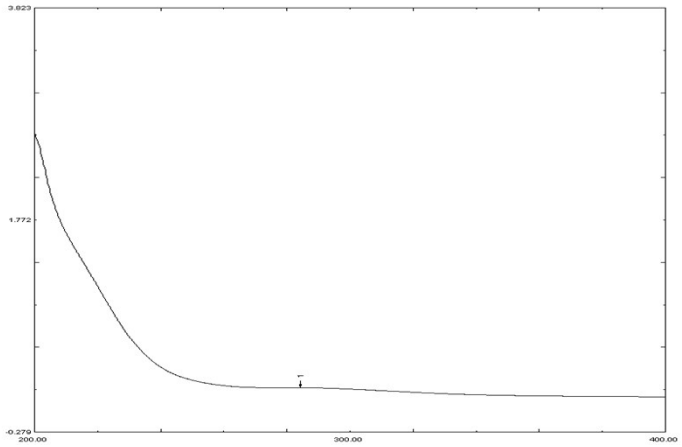
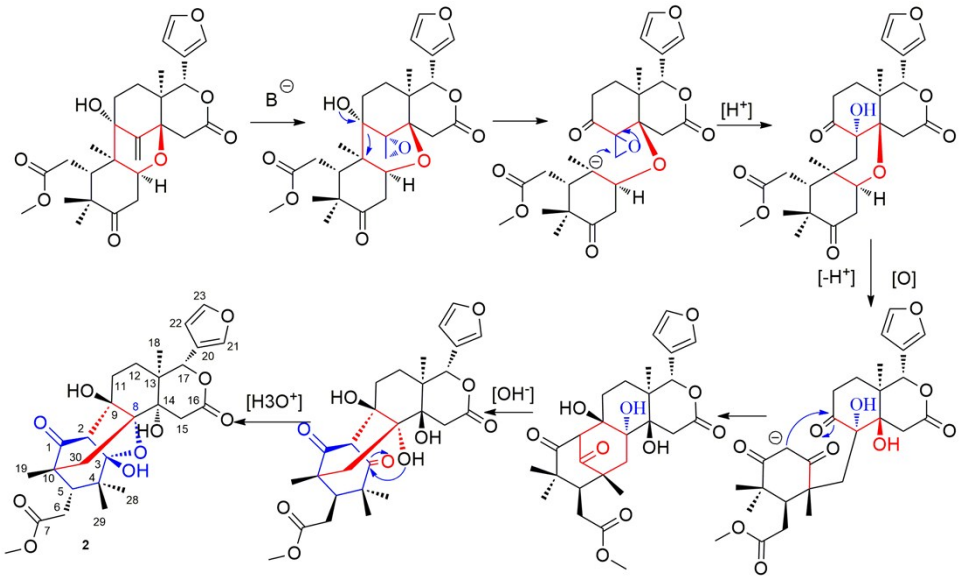
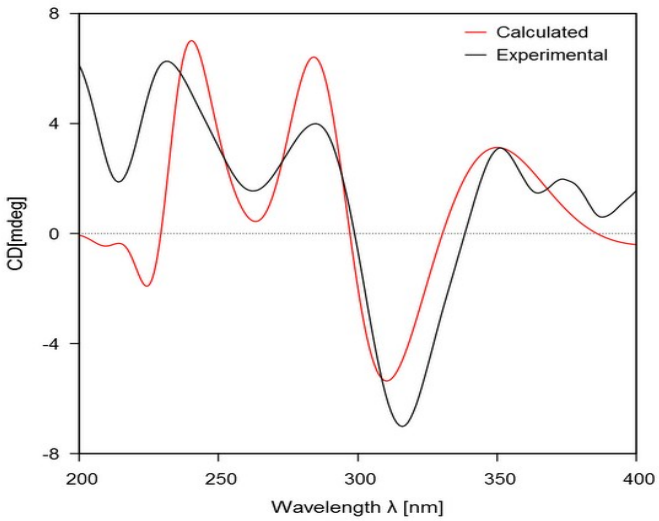
S23. HRESIMS of **3**



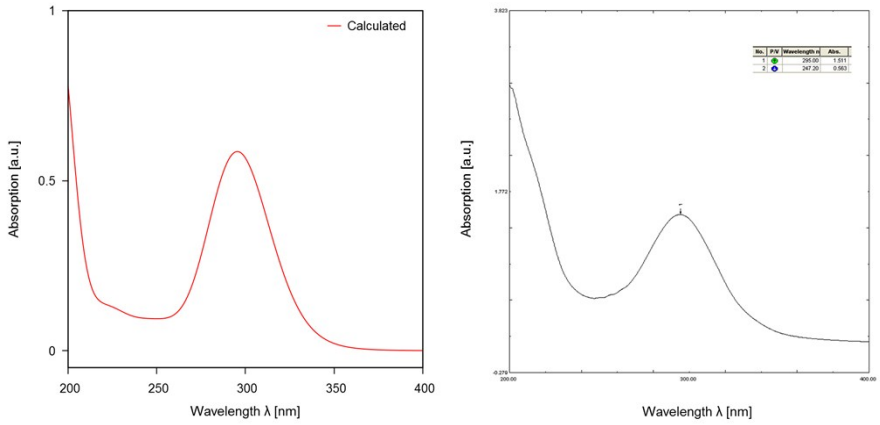
S24. UV spectrum of **3**



S25. **Scheme 2**. Plausible Biogenetic Pathway for **2**.



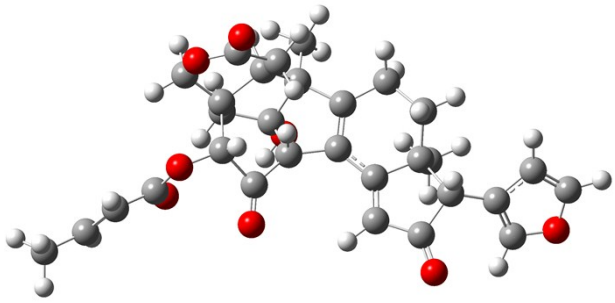
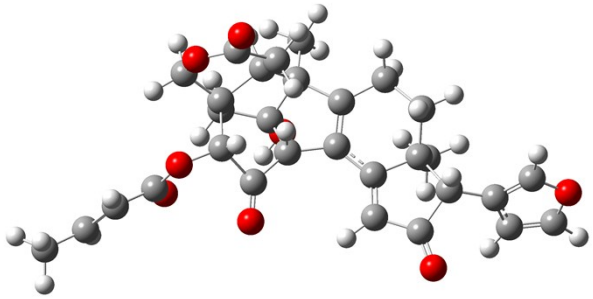
S26. Calculated ECD and experimental ECD spectra of **1**.



S27. Calculated UV and experimental UV spectra of **1**.

Method	Conf	Energy (A.U.)	Energy (kcal/mol)	Percent (%)
MMFF94	1	--	275.82576	75.97
	2	--	276.46871	23.72
B3LYP/6-311G**	1	-1763.382337	-1106539.114	45.74
	2	-1763.382498	-1106539.215	54.26

S28. Energies of the conformers with Boltzmann distribution over 1%.

No.	Conformer	Percent (%)
1		45.74
2		54.26

S29. The conformers with Boltzmann distribution over 1% of **1**.