Walrobsins A and B, Two Anti-inflammatory Limonoids from Root Barks of *Walsura robusta*

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Supporting Information

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1. EXPERIMENTAL SECTION

1.1 General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter in MeOH at room temperature. Nuclear magnetic resonance (NMR) spectra were on a Bruker AVIII-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz) (Bruker, Karlsruhe, Germany), with tetramethylsilane (TMS) as an internal standard. Chemical shift values (δ) are given in parts per million (ppm) and coupling constants in Hertz (Hz). The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Electrospray ionization (ESI) and high-resolution electrospray ionization (HRESIMS) were carried out an Agilent 1100 series LC/MSD ion trap mass spectrometer and an Agilent 6529B Q-TOF instrument (Agilent Technologies, Santa Clara, CA, USA), respectively. Preparative high-performance liquid chromatography (*Pre*-HPLC) was performed on a Shimadzu LC-6A system (Shimadzu, Tokyo, Japan) equipped with a Shim-pack RP-C_{18} column (200 mm × 20 mm i.d., 10 μm, Shimadzu, Tokyo, Japan) with flow rate at 10.0 ml/min and column temperature at 25 ºC, detected by a binary channel UV detector at 210 and 230 nm. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao China) and RP-C_{18} silica (40-63 μm, FuJi, Japan) were used for column chromatography. Fractions obtained from column chromatography (CC) were monitored by thin-layer chromatography (TLC) with precoated silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd, China) plates.

1.2 Plant Material. Air-dried root barks of *Walsura robusta* were collected from

Xishuangbanna, Yunnan Province, People's Republic of China, in September 2015, and were authenticated by Professor Shun-Cheng Zhang, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, People's Republic of China. A voucher specimen was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University (accession number 2015-GSS).

1.3 Extraction and isolation.

The dried powder of fruits of *W. robusta* (5.0 kg) was extracted three times (3 × 5L) with 95% EtOH, and the crude (500 g) was suspended in H_2O and extracted with petroleum ether (PE) (3 × 1L) and EtOAc (3 × 1L), successively. The ethyl acetate extract (100.0 g) was subjected to a silica gel column, eluted with a gradient of CH_2Cl_2-MeOH (100:1, 50:1, 25:1, 10:1, 5:1, *v*/*v*) to give six fractions (A1-A6), which were combined based on TLC. Fraction A5 (10.0 g) was chromatographed over a middle chromatogram isolated (MCI) column eluted with a gradient system of MeOH-H_2O (50:5, 75:25, 95:5, *v*/*v*) to give three subfractions (A5A-A5C), respectively. A5B (5.0 g) was sequentially purified by columns of C_{18} silica gel (MeOH-H_2O, 50% to 75%, *v*/*v*) and then further separated over semi-preparative HPLC to knockout the high content of ingredients including cedrelone, 11*β*-acetoxycedrelone and 11*β*-hydroxycedrelone, and then obtain two novel compounds (**1-2**) from the knockouted extracrs under the guidance of strategy in Figure 1 and Figure S1. Walrobsins A-B (**1-2**) was yield as **1** (30 mg), **2** (24 mg), respectively.

1.4 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 × 105 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 540 nm 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.

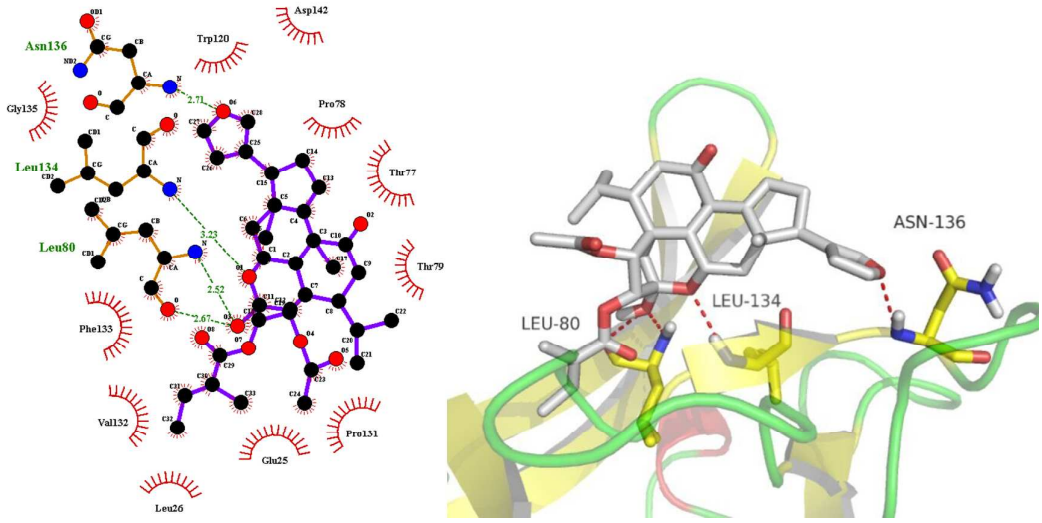


Figure S2. Docking model predicted interaction details between 2MIB and walrobsin A (1)

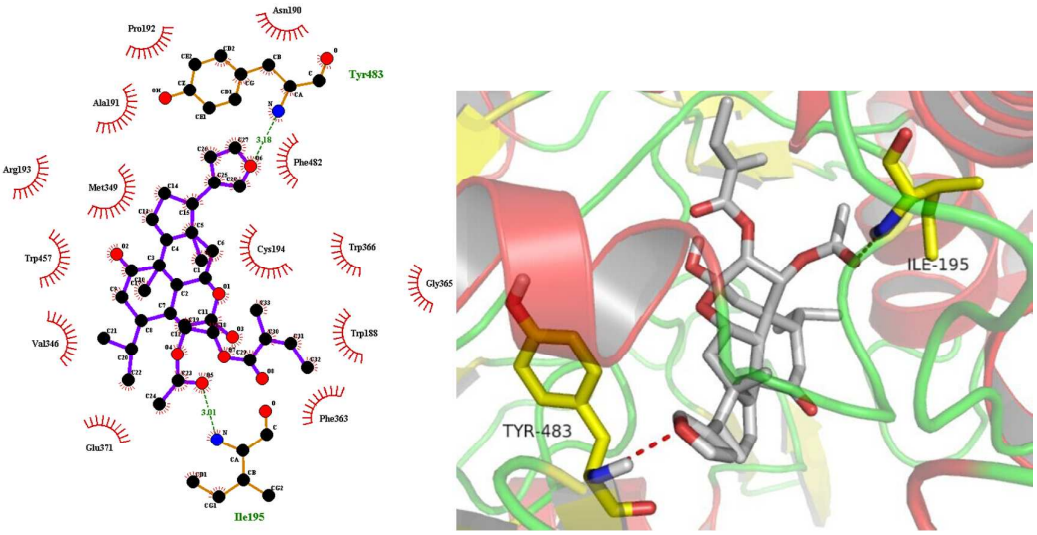


Figure S3. Docking model predicted interaction details between 4UX6 and walrobsin A (1)

1.5 Physical and chemical data

Walrobsin A (1): colorless crystals, $[\alpha]_D^{23}$ – 18.3 (*c*, 0.50, MeOH); UV (MeOH, 3.11 × 10^{-5} mol/L) λ_{max} (log ε) 213 nm (4.86); ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; negative ESIMS *m/z* 601.3 $[M + Cl]^-$; positive ESIMS *m/z* 567.2 $[M + H]^+$; HRESIMS *m/z* 567.2953 $[M + H]^+$ (calcd for $C_{33}H_{43}O_8$, 567.2952).

Walrobsin B (2): colorless crystals, $[\alpha]_D^{23}$ – 9.3 (*c*, 0.70, MeOH); UV (MeOH, 3.02 × 10^{-5} mol/L) λ_{max} (log ε) 208 nm (5.69); ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; negative ESIMS *m/z* 603.3 $[M + Cl]^-$; positive ESIMS *m/z* 569.3 $[M + H]^+$; HRESIMS *m/z* 569.3108 $[M + H]^+$ (calcd for $C_{33}H_{45}O_8$, 569.3109).

1.6 X-ray crystallographic data

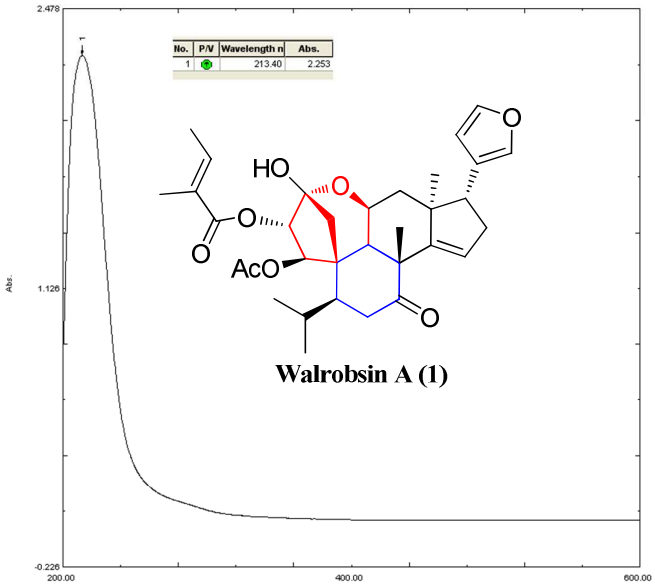
Colorless crystals of **1** and **2** were obtained from a mixture of CH_2Cl_2 and MeOH (1:1, *v/v*). The crystal data were collected using a Bruker Smart 1000 CCD with a graphite monochromator using Cu Kα radiation. The crystals were tested with a diffractometer using Olex2, and the structures were figured out through direct methods with the ShelXS [8] structure solution program and refined with the ShelXL refinement package using least squares minimization. The crystallographic data for **1** and **2** were deposited in the Cambridge Crystallographic Data Centre (CCDC deposition numbers, **1**: 1557716; **2**: 1557185). These data can be obtained freely from

the Cambridge Crystallographic Data Centre by visiting sites of www.ccdc.cam.ac.uk/conts/retrieving.html.

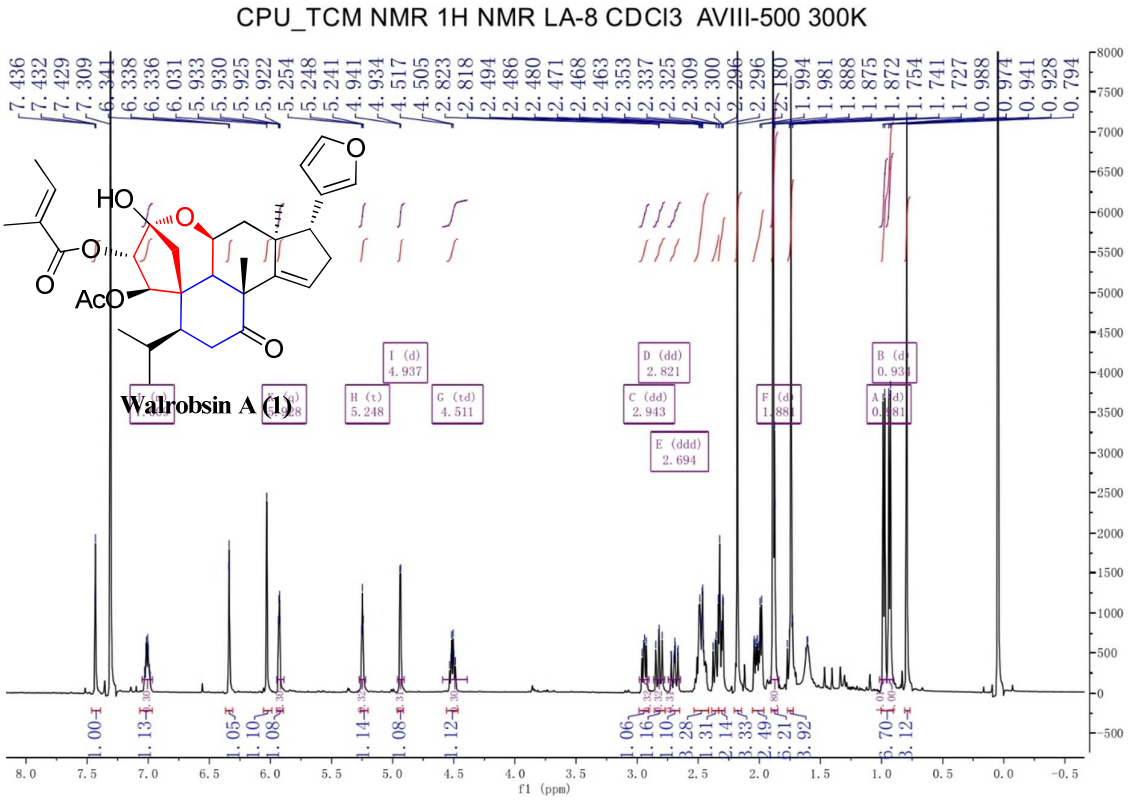
X-ray crystallographic data for 1: $C_{33}H_{42}O_8$ (M = 566.66 g/mol): orthorhombic, space group $P2_12_12_1$ (no.19), a = 9.54080 (10) Å, b = 16.31220 (10) Å, c = 18.8674 (2) Å, V = 2936.36 (5) Å^3, Z = 4, T = 289 (2) K, μ (Cu Kα) = 0.739 mm^{-1}, *Dcalc* = 1.282 g/cm^3, 26402 reflections measured (7.164° ≤ 2θ ≤ 140.158°), 5502 unique (R_{int} = 0.0278, R_{sigma}= 0.0199), which were used in all calculations. The final R_1 was 0.0441 (I > 2σ (I)), and wR_2 was 0.1339 (all data). Flack parameter: 0.04 (5).

X-ray crystallographic data for 2: $C_{33}H_{44}O_8$ (M = 568.68 g/mol): orthorhombic, space group $P2_12_12_1$ (no.19), a = 9.43400 (10) Å, b = 16.40280 (10) Å, c = 19.41280 (10) Å, V = 3004.01 (4) Å^3, Z = 4, T = 291 (2) K, μ (Cu Kα) = 0.723 mm^{-1}, *Dcalc* = 1.257 g/cm^3, 21504 reflections measured (7.056° ≤ 2θ ≤ 142.552°), 5724 unique (R_{int} = 0.0182, R_{sigma}= 0.0124), which were used in all calculations. The final R_1 was 0.0369 (I > 2σ (I)), and wR_2 was 0.1009 (all data). Flack parameter: -0.01 (3).

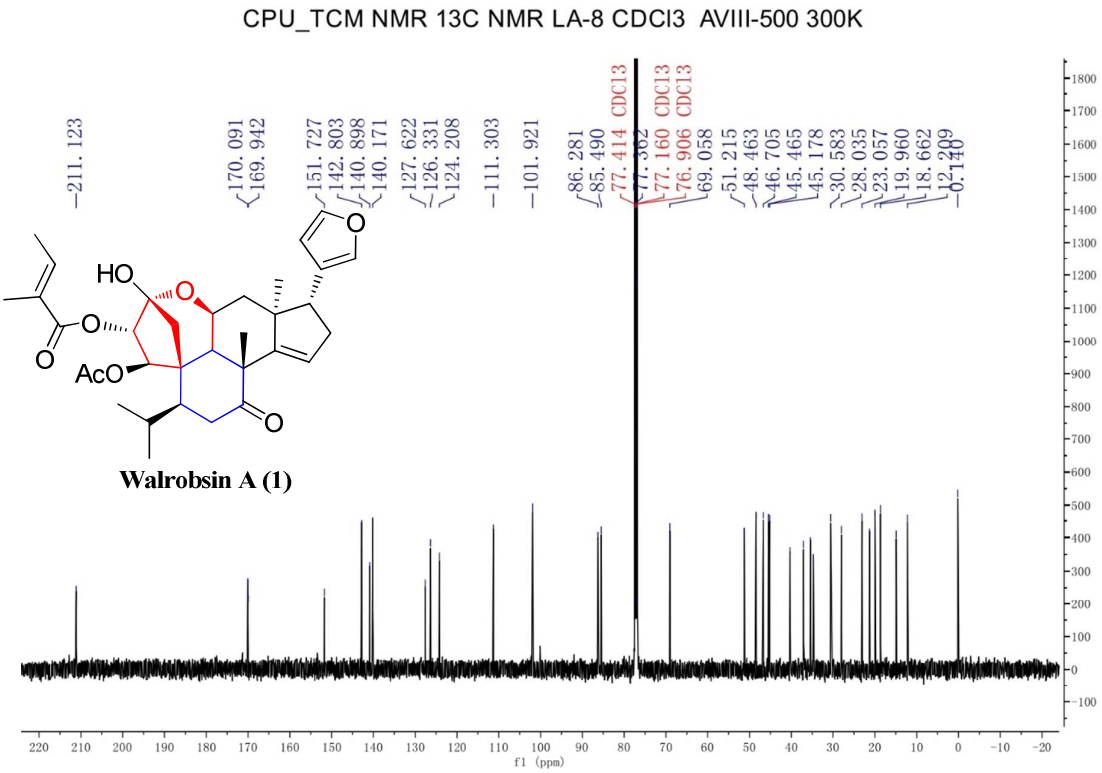
2. NMR, HRESIMS, and UV Spectra



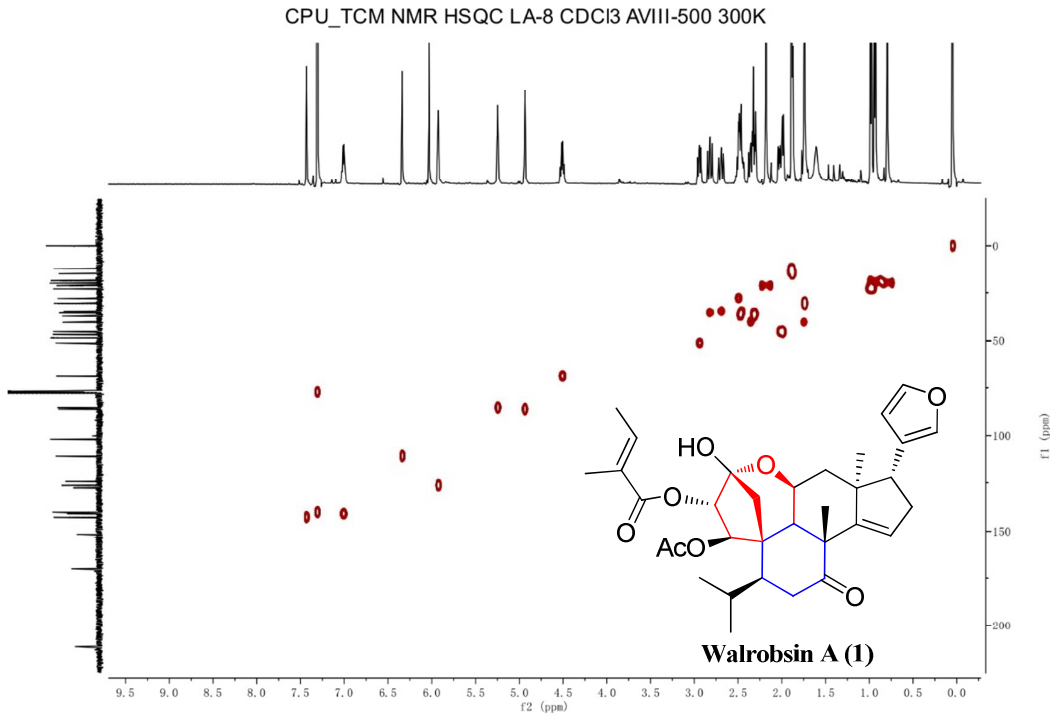
S1-1. UV (MeOH) spectrum of **1**.



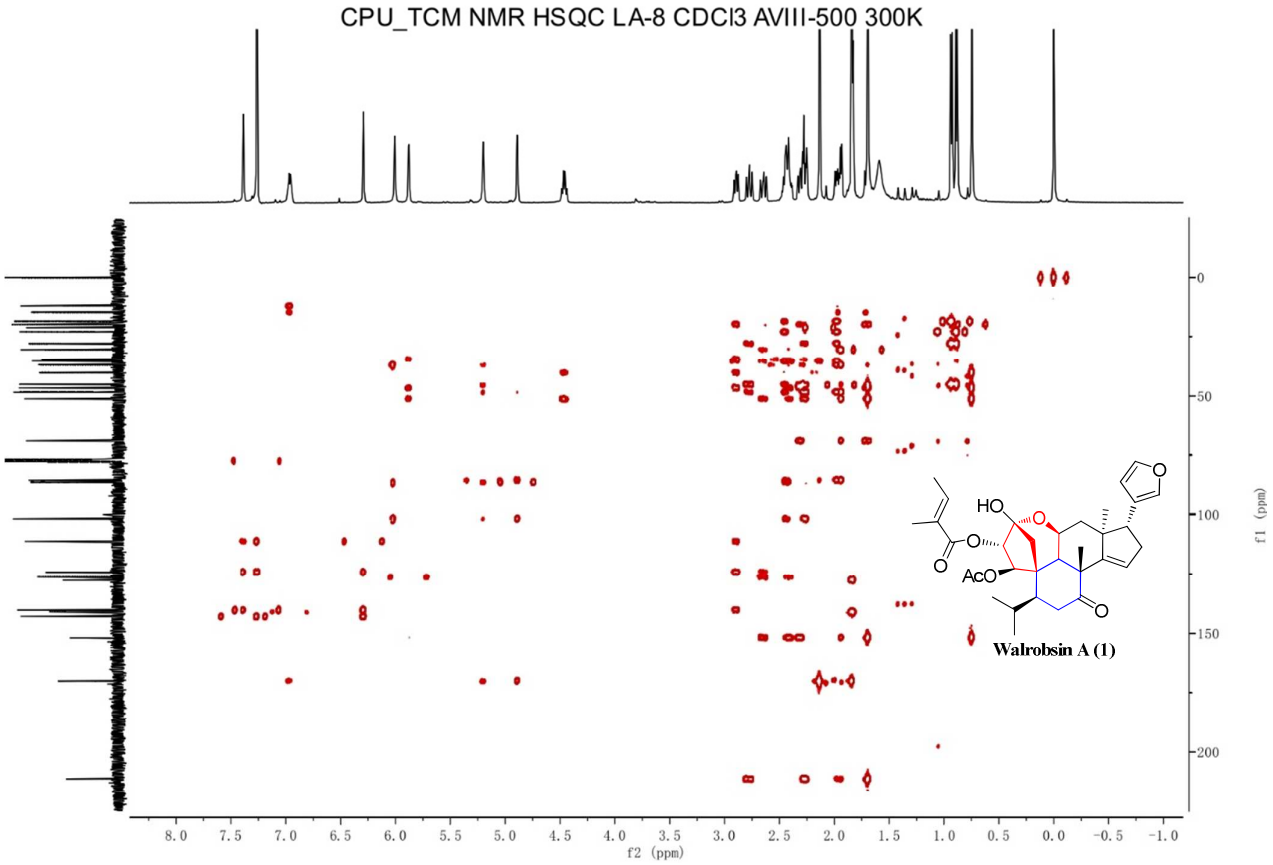
S1-2. ^{1}H NMR (500 MHz; $CDCl_3$) spectrum of **1**.



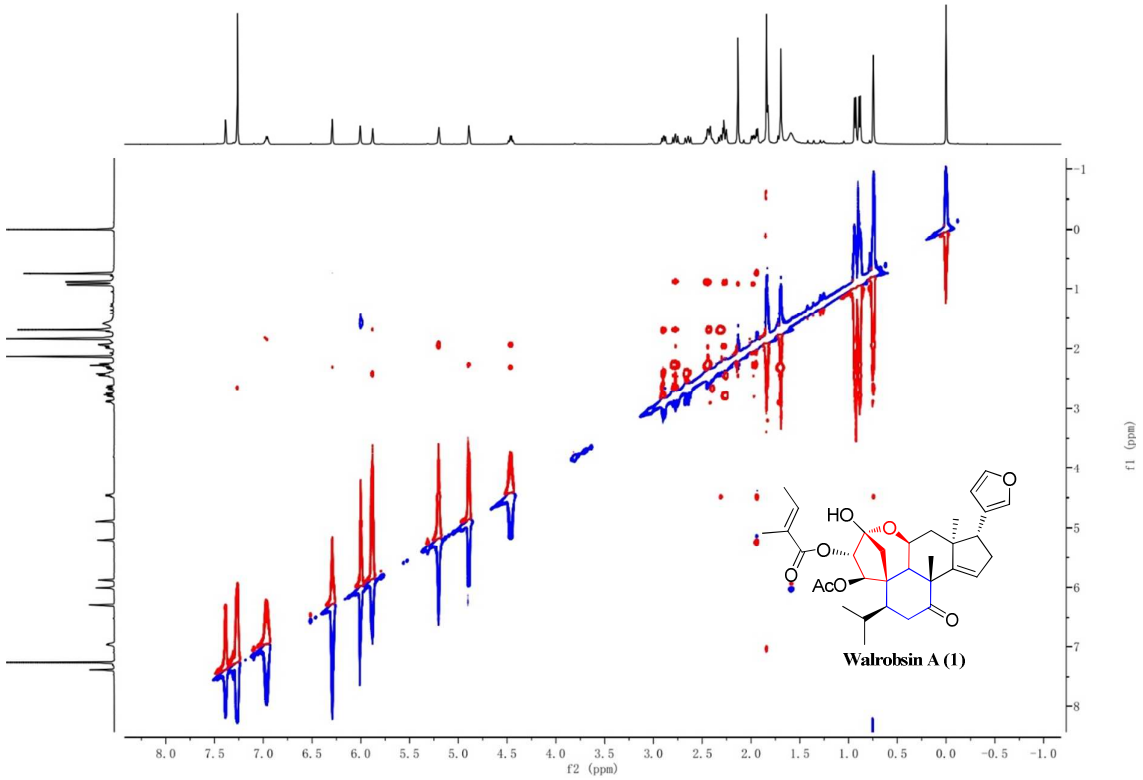
S1-3. ^{13}C NMR (125 MHz; $CDCl_3$) spectrum of **1**.



S1-4. HSQC ($CDCl_3$) spectrum of **1**.



S1-5. HMBC ($CDCl_3$) spectrum of **1**.



S1-6. ROESY ($CDCl_3$) spectrum of **1**.

TCM-CPU HR-ESI-MS Display Report

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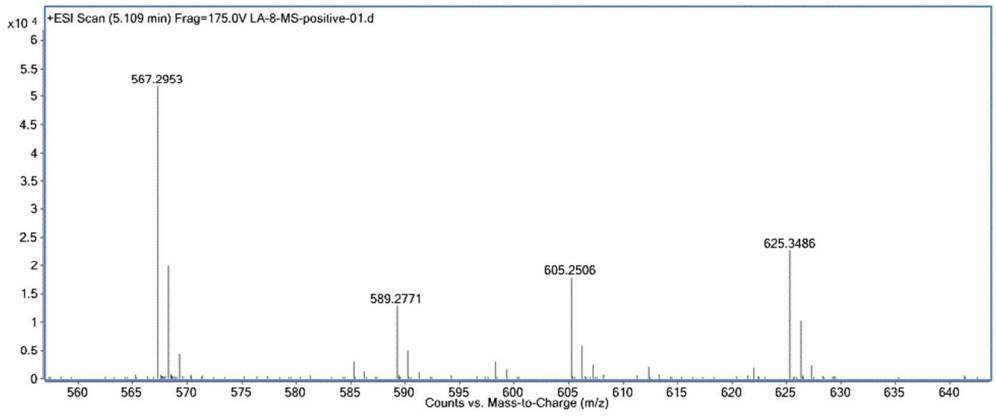
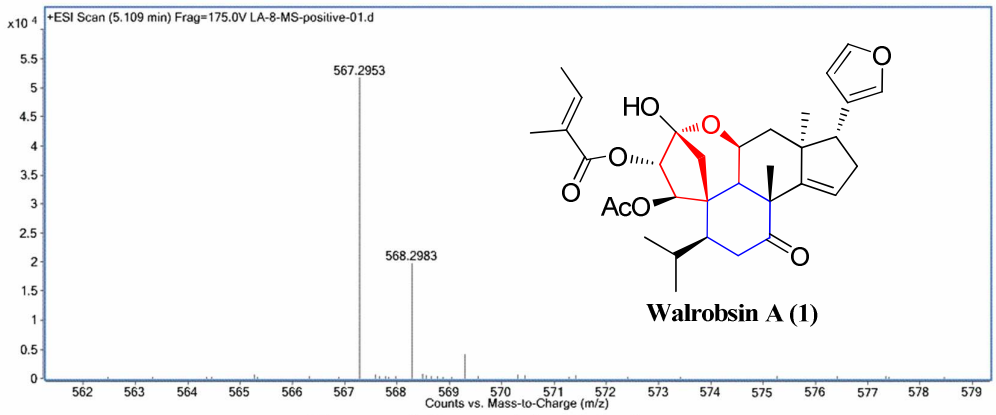
Acq. Date: 07/19 /2016 **Operator:** Administrator





Walrobsin A (1)

Elemental Composition Calculator

Target m/z:	567.2953	**Result type:**	Positive ions	**Species:**	$[M+H]^+$
Elements:		C (0-80); H (0-120); O (0-30);			
Ion Formula		**Calculated m/z**		**PPM Error**	
C33H43O8		567.2952		-0.07	

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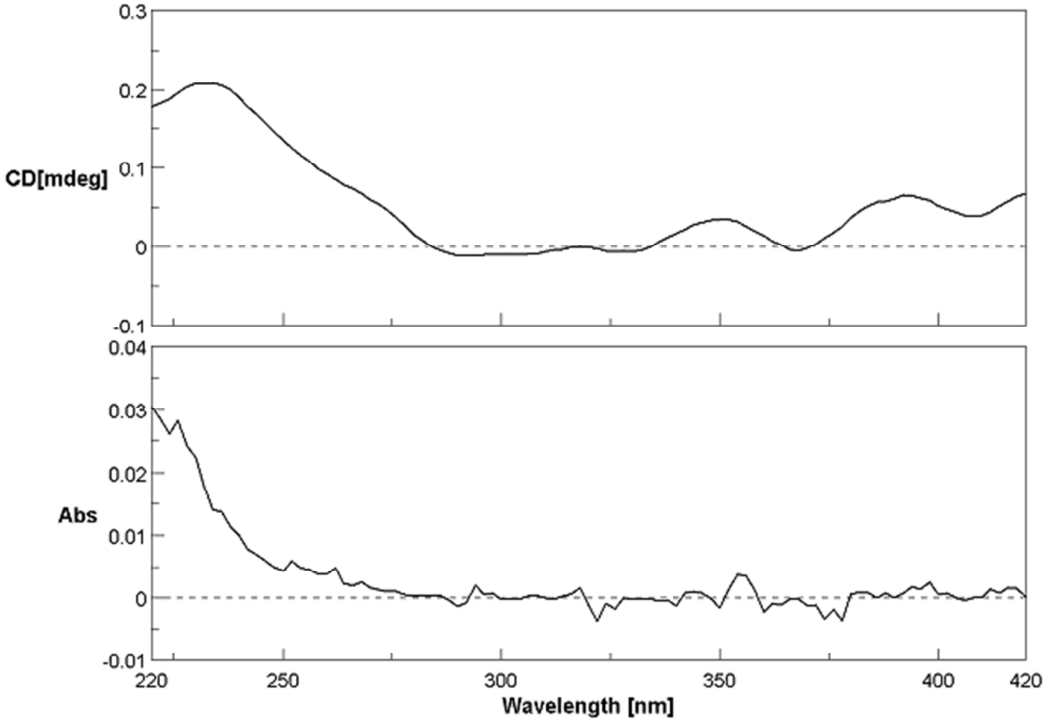
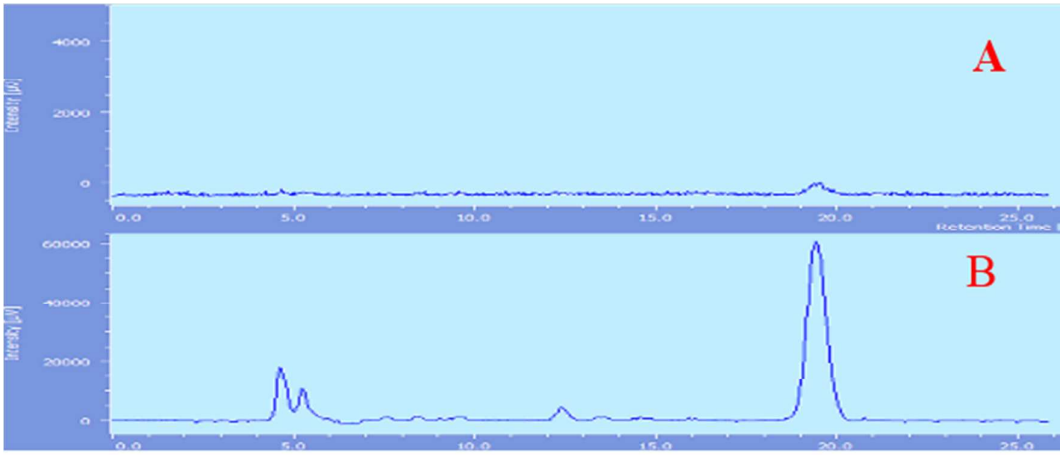
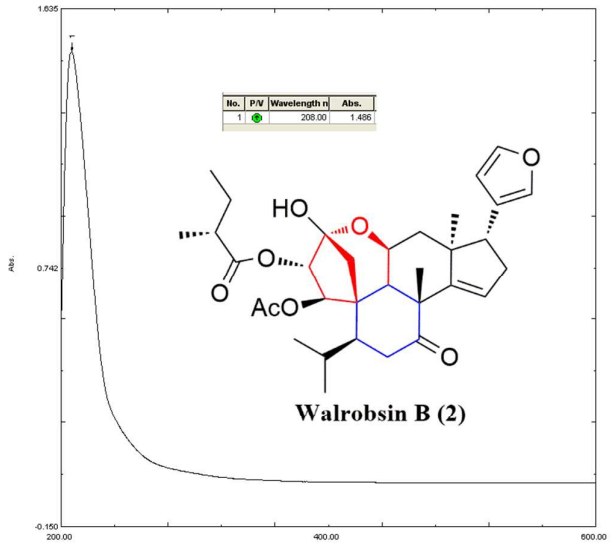
S1-7. HRESIMS spectrum of **1**.



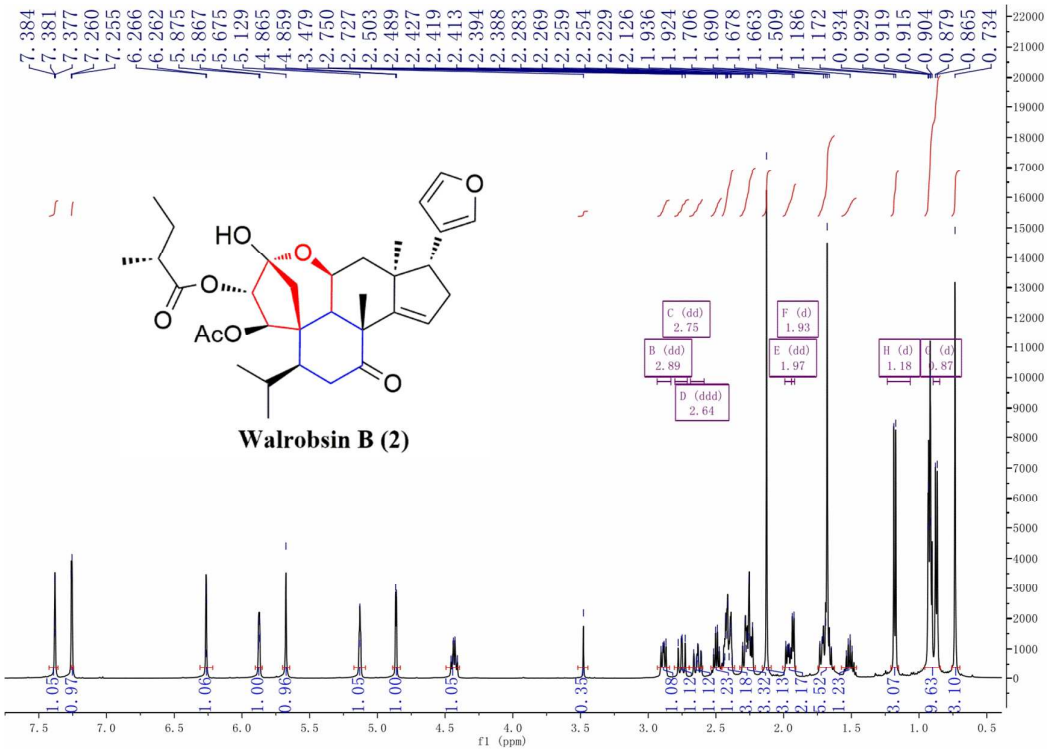
S1-8. CD (MeOH) spectrum of **1**.



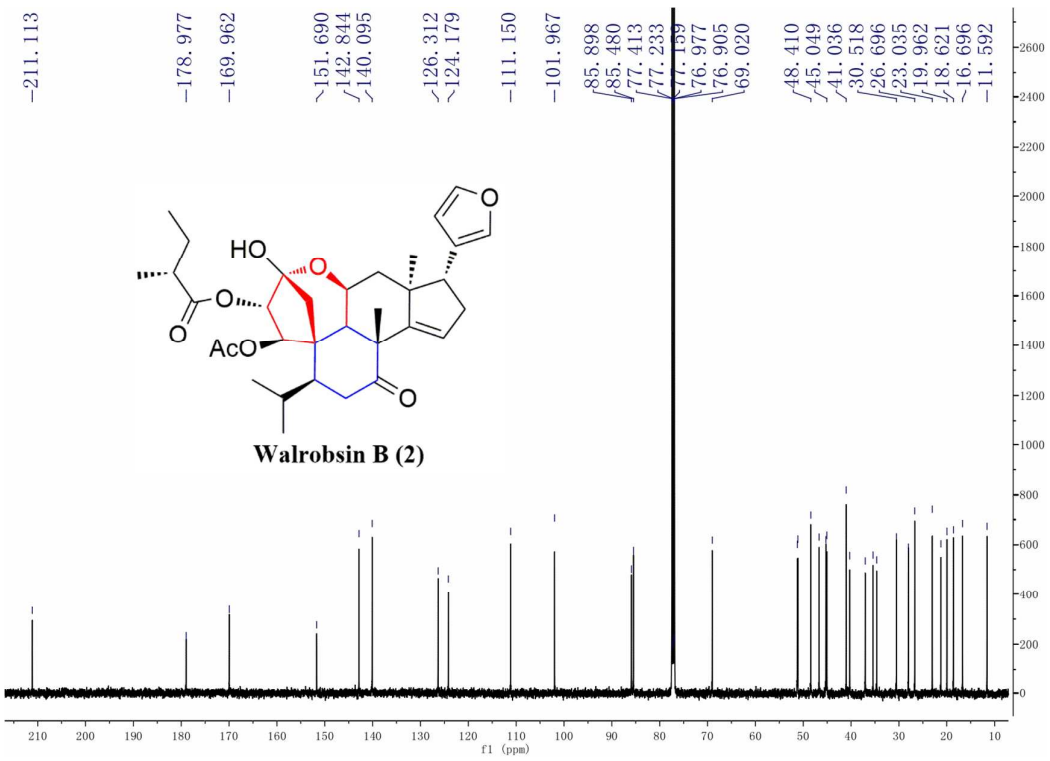
S1-9. The positive chiral HPLC-CD spectra of **1**. A): CD absorption value at 220 nm; B): Retention time of **1**.



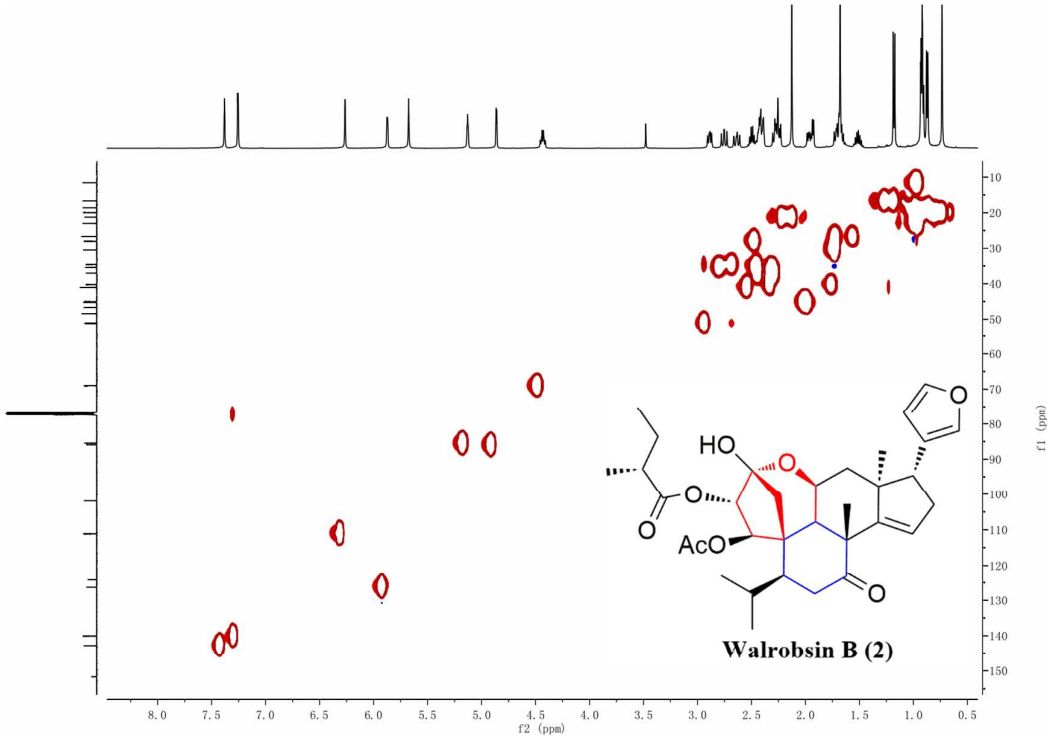
S2-1. UV (MeOH) spectrum of **2**.



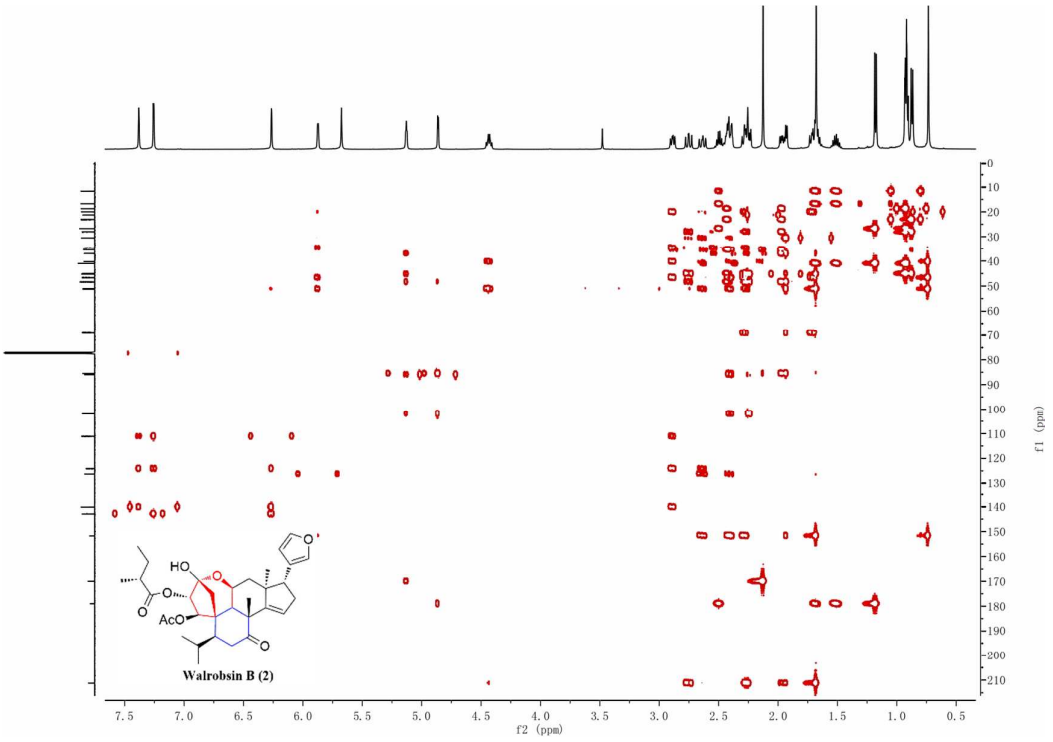
S2-2. ^{1}H NMR (500 MHz; $CDCl_3$) spectrum of **2**.



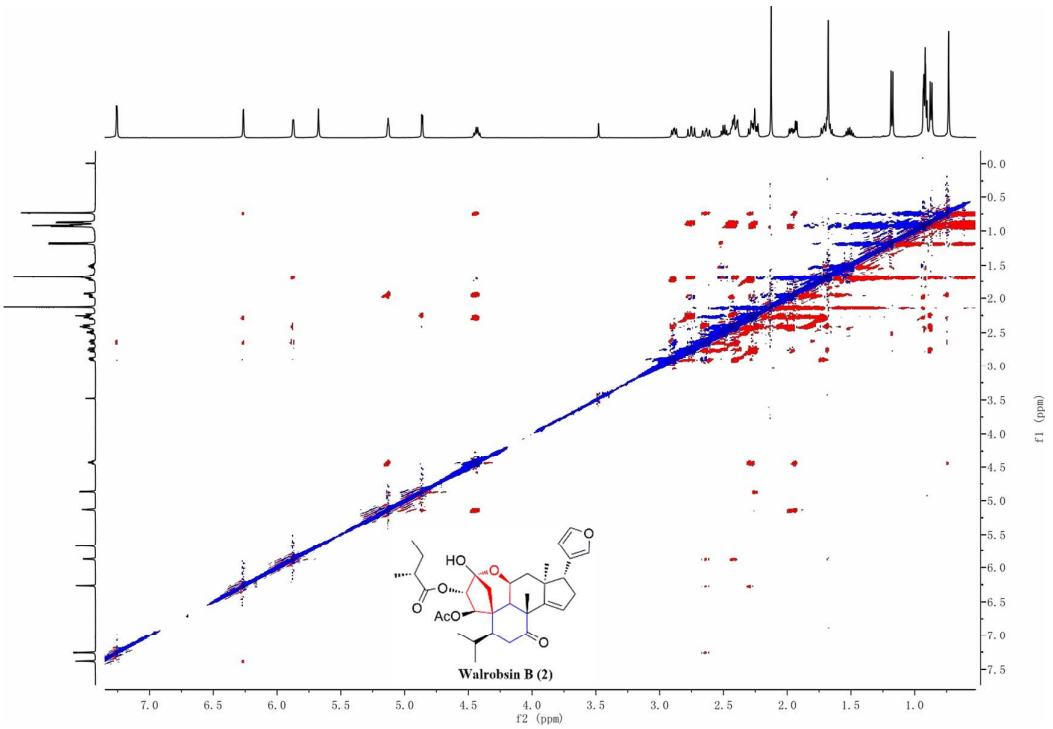
S2-3. ^{13}C NMR (125 MHz; $CDCl_3$) spectrum of **2.**



S2-4. HSQC ($CDCl_3$) spectrum of **2**.



S2-5. HMBC ($CDCl_3$) spectrum of **2**.



S2-6. ROESY ($CDCl_3$) spectrum of **2**.

TCM-CPU HR-ESI-MS Display Report

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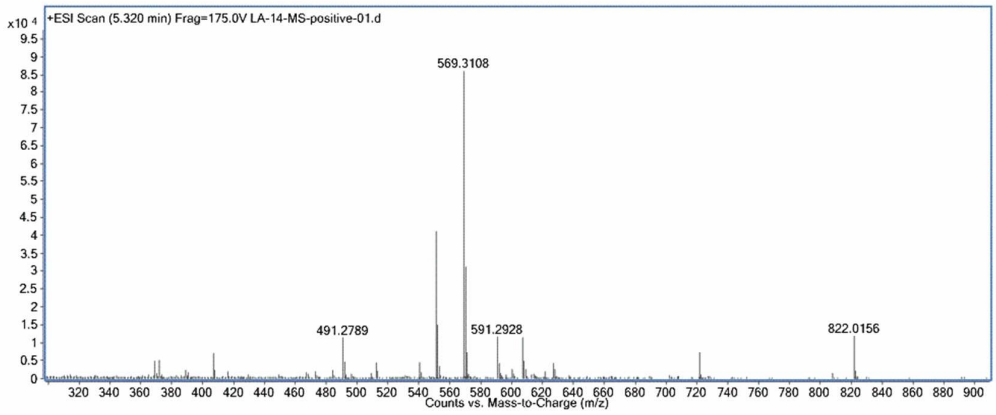
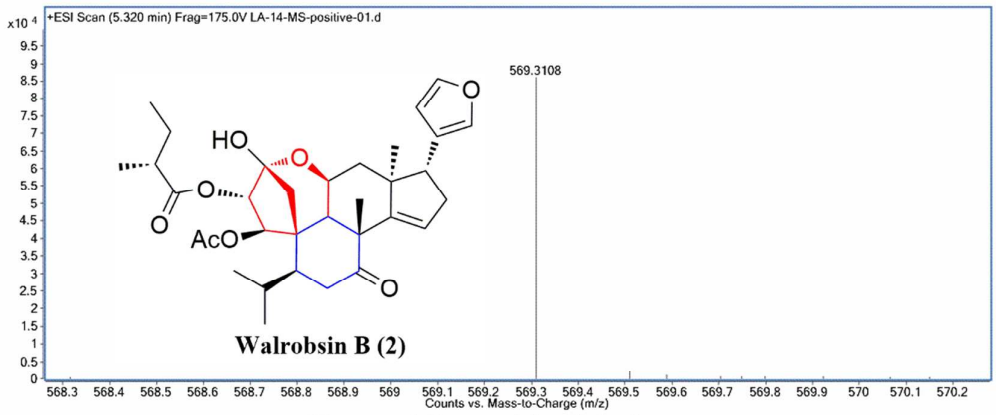
Acq. Date: 07/19 /2016 **Operator:** Administrator





Elemental Composition Calculator

Target m/z:	569.3108	**Result type:**	Positive ions	**Species:**	$[M+H]^+$
Elements:		C (0-80); H (0-120); O (0-30); Na(0-5)			
Ion Formula		**Calculated m/z**		**PPM Error**	
C33H45O8		569.3109		0.15	

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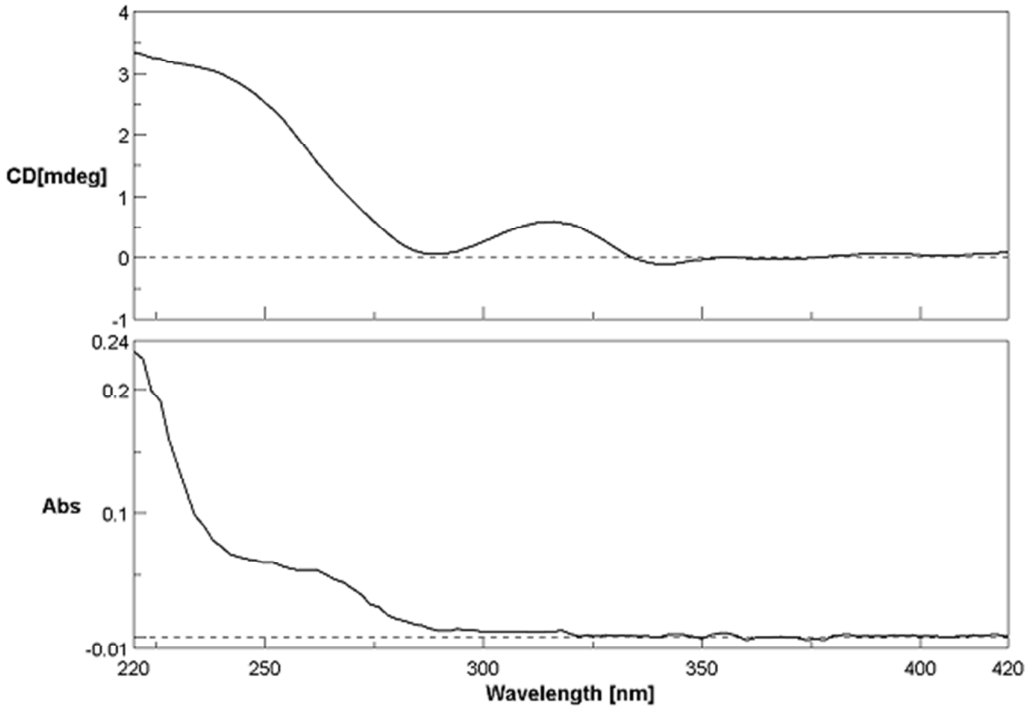
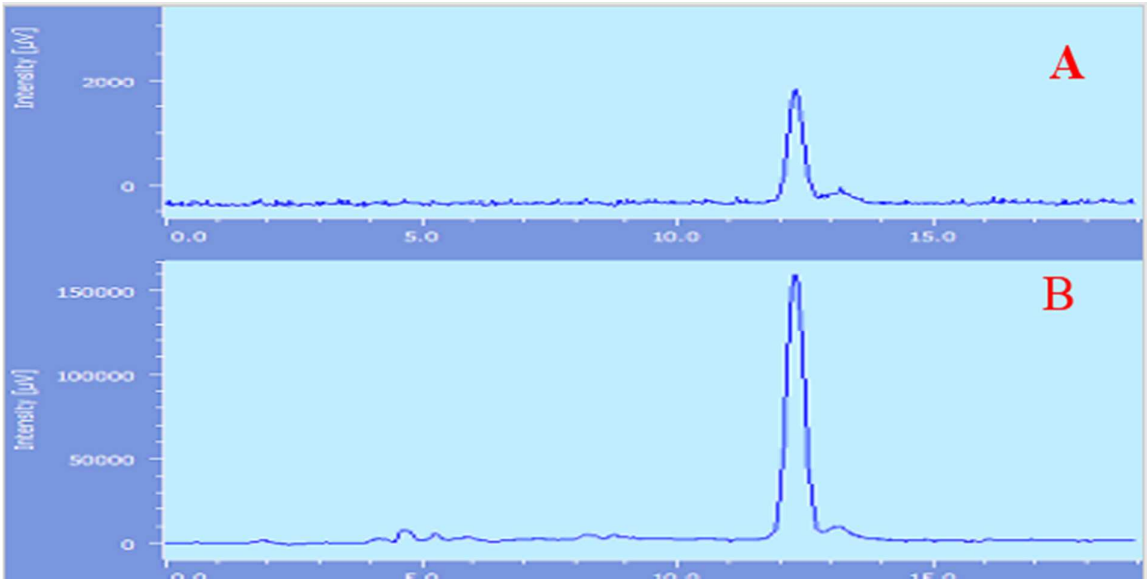
S2-7. HRESIMS spectrum of **2.**



S1-8. CD (MeOH) spectrum of **2**.



S1-9. The positive chiral HPLC-CD spectra of **2**. A): CD absorption value at 220 nm; B): Retention time of **2**.