Supporting Information

Nardoaristolones A and B, Two Terpenoids with Unusual Skeletons from *Nardostachys chinensis* Batal

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List of Supporting Information

General experimental procedures

Optical rotations were measured on a Jasco P-1020 polarimeter with a 1 cm cell at room temperature. UV spectra were recorded on a JASCO V-550 UV/Vis spectrometer. IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. CD spectra were obtained on a Jasco J-810 spectropolarimeter at room temperature. HR-ESI-MS spectra were acquired using a Waters Synapt G2 mass spectrometer. The NMR spectra were measured with a Bruker AV-300/400/600 spectrometer at room temperature. Silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., China), octadecylsilanized (ODS) silica gel (YMC Ltd., Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden) were used for open column chromatography (CC).

Plant Material

The underground parts of *Nardostachys chinensis* were provided by Shijiazhuang Yiling Pharmaceutical Co., Ltd and identified by Dr. Qingcun Tian of the company.

Extraction and Isolation

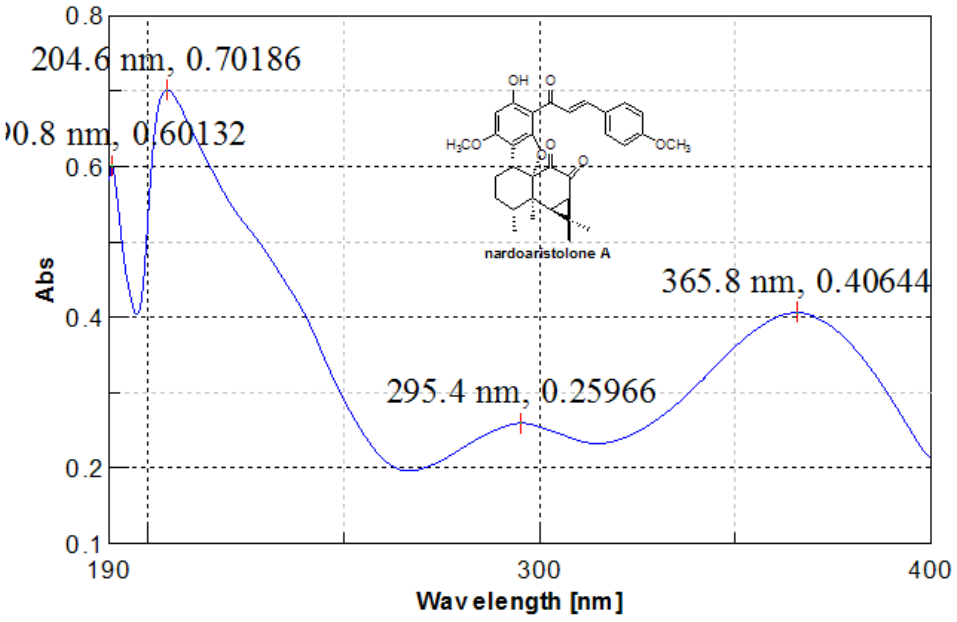
The air-dried medical materials (5 Kg) were refluxed twice with 60% EtOH for 2 hours each time. The crude extract (970.5g) was column chromatographed over a macroporous resin HP-20 eluted with EtOH-H_2O in gradient. The 70% EtOH-H_2O eluent (171.7 g) was fractionated by silica gel column chromatography eluted with $CHCl_3$-CH_3OH (100:0→0:100) to afford twelve fractions (Fr. 1-12). Fr. 3 (53.4 g, $CHCl_3$/CH_3OH 99:1) was then chromatographed on a silica gel column using petroleum ether-EtOAc gradient elution to yield 9 subfractions (Fr. 3.1-3.9). The subfraction Fr. 3.8 (9.1 g, P/E 100:10) was further subjected to ODS column chromatography eluted with MeOH-H_2O (50:50→100:0) and purified by preparative HPLC on ODS column with 45% MeOH-H_2O to yield compound **2** (89.2 mg). Another subfraction Fr. 3.9 (2.1 g, P/E 100:15) was also subjected to ODS column chromatography eluted with MeOH-H_2O (50:50→100:0) and purified by Sephadex LH-20 column chromatography ($CHCl_3$/CH_3OH 7:3) to afford compound **1** (5.6 mg).

Physico-chemical constants of 1-2

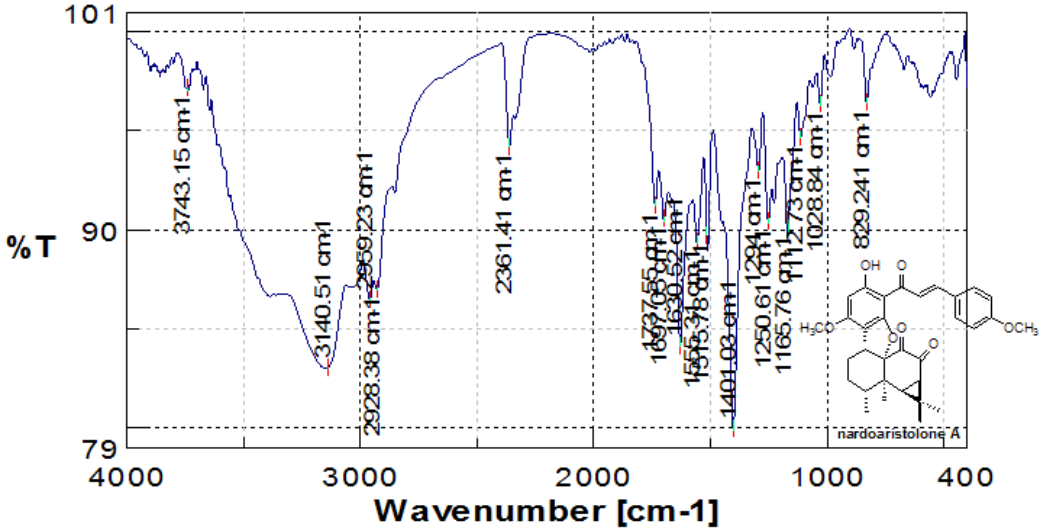
Nardoaristolone A (**1**): Yellow powder; $[\alpha]_D^{26}$ -74.6 (*c* 0.50, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204.6 (3.87), 295.4 (3.44), 365.8 (3.63) nm; IR (KBr) λmax 3140, 2361, 1630, 1515, 1401, 1250, 1165, 829 cm^{-1}; CD (CH_3OH) λ_{max} (Δε) 395 (+2.35), 318 (-2.61), 268 (-1.40), 235 (+0.41), 215 (-1.12) nm; HR-ESI-MS m/z 531.2380 ($[M+H]^+$, calcd for $C_{32}H_{35}O_7$, 531.2383).

Nardoaristolone B (**2**): Light yellow crystals; $[\alpha]_D^{26}$ -19.6 (*c* 0.50, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 254.2 (3.66) nm; IR (KBr) λmax 1704, 1671, 1454, 1370, 1214, 1033, 869 cm^{-1}; CD (CH_3OH) λ_{max} (Δε) 398 (+1.11), 336 (-1.78), 269 (-2.54), 230 (+4.53), 214 (+4.10) nm; HR-ESI-MS m/z 219.1377 ($[M+H]^+$, calcd for $C_{14}H_{19}O_2$, 219.1385).

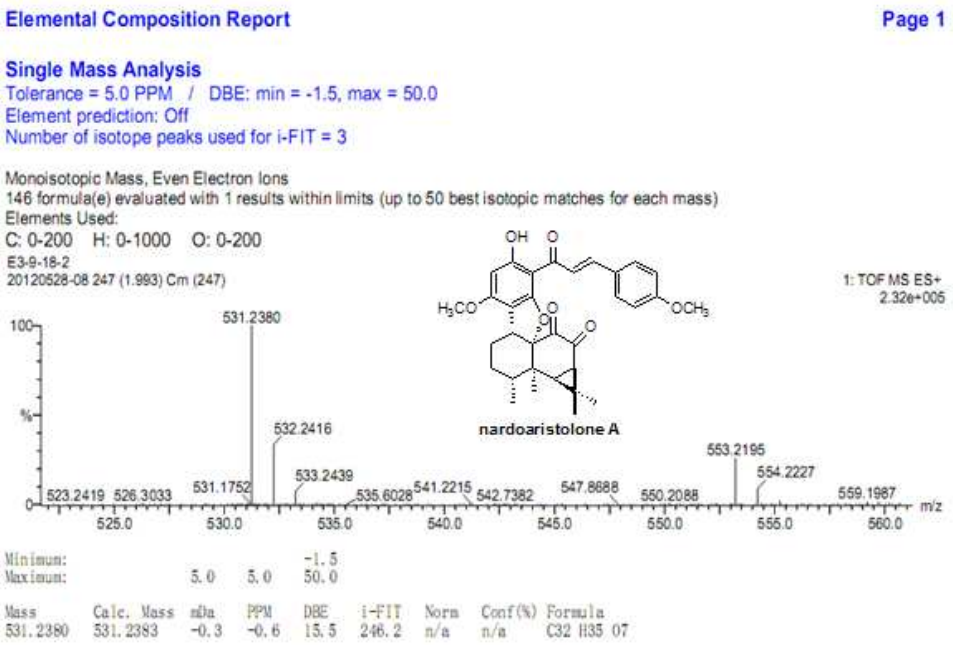
UV spectrum of nardoaristolone A (1) in CH_3OH.



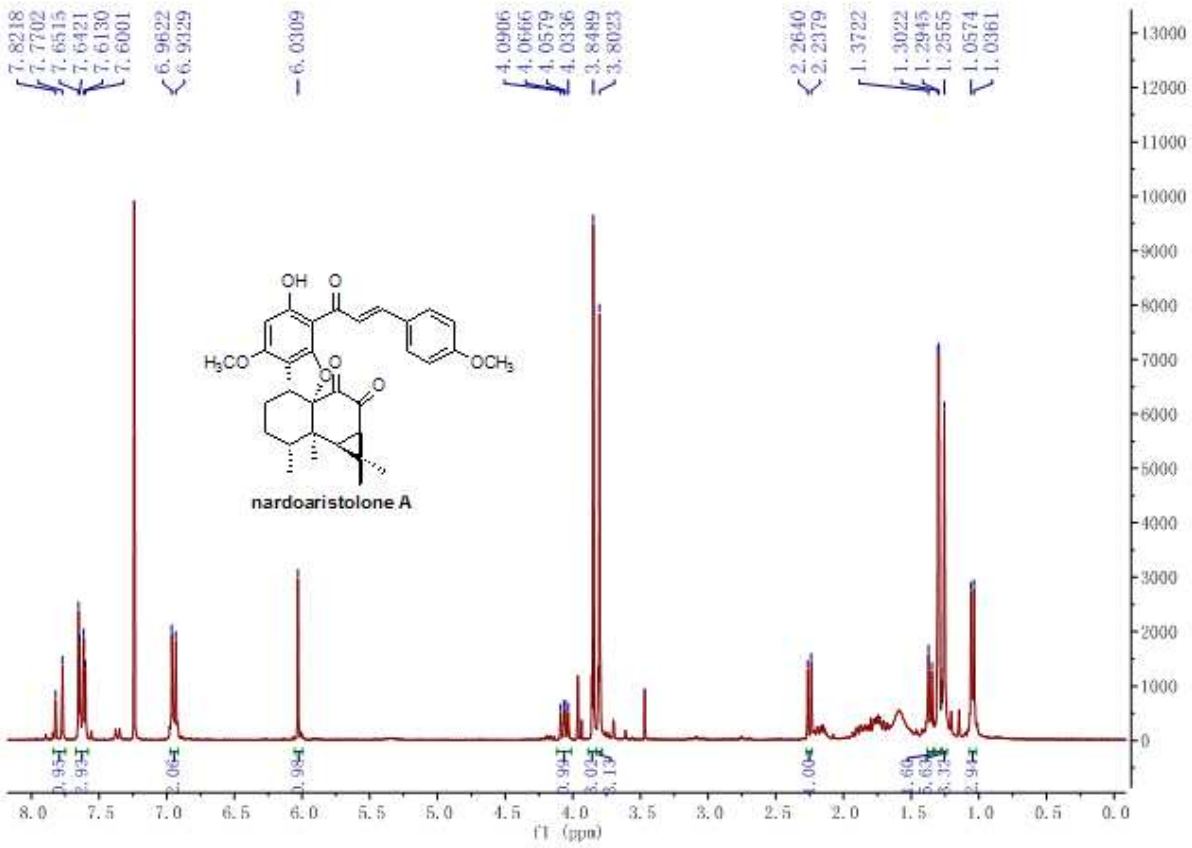
IR (KBr disc) spectrum of nardoaristolone A (1).



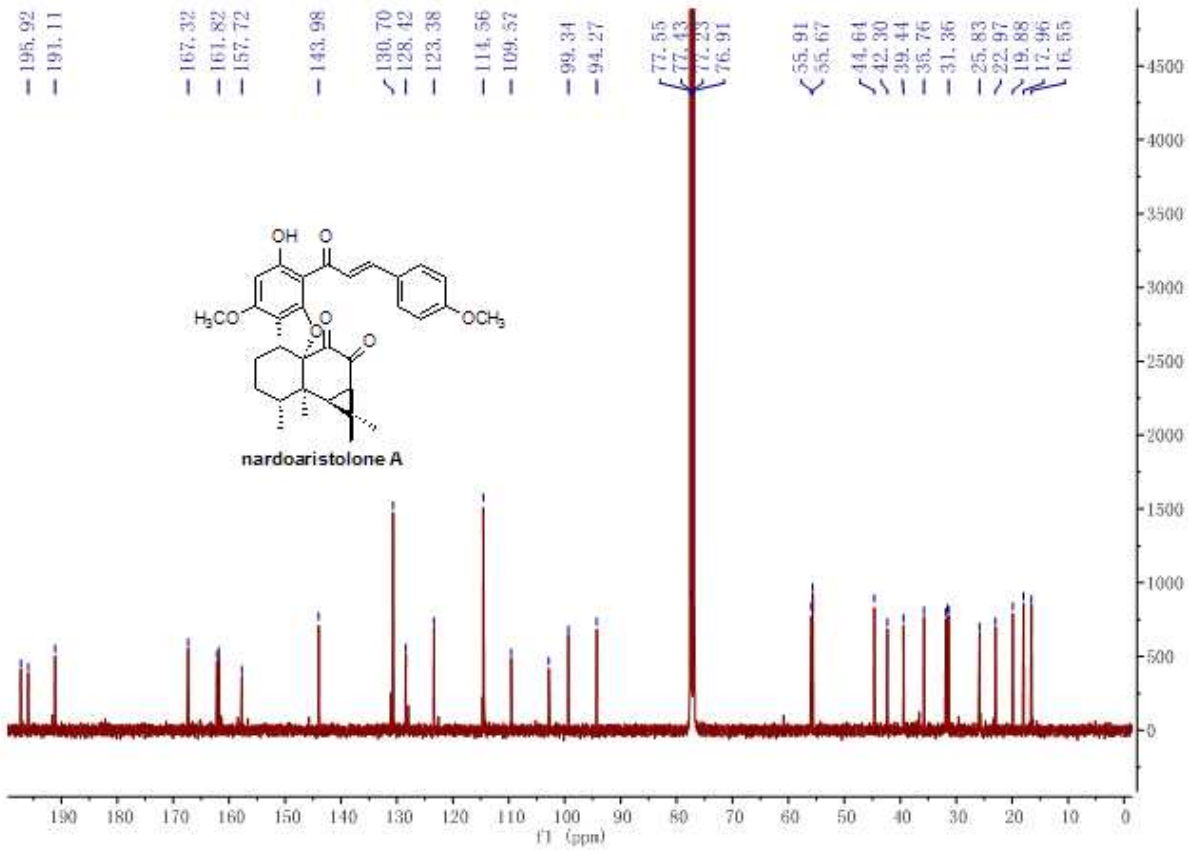
HR-ESI-MS spectrum of nardoaristolone A (1).



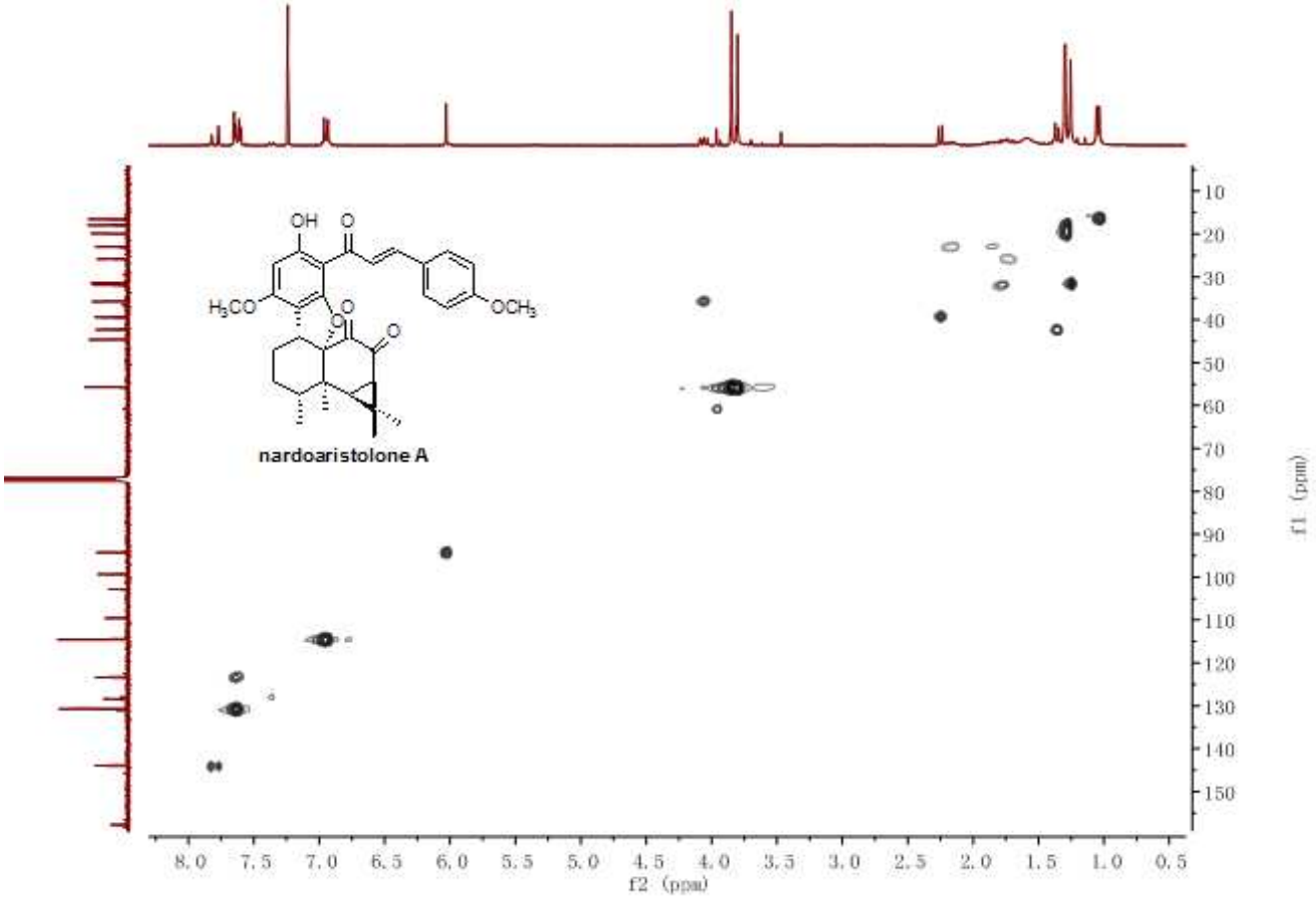
^{1}H NMR (AV-300, 300 MHz) spectrum of nardoaristolone A (1) in $CDCl_3$



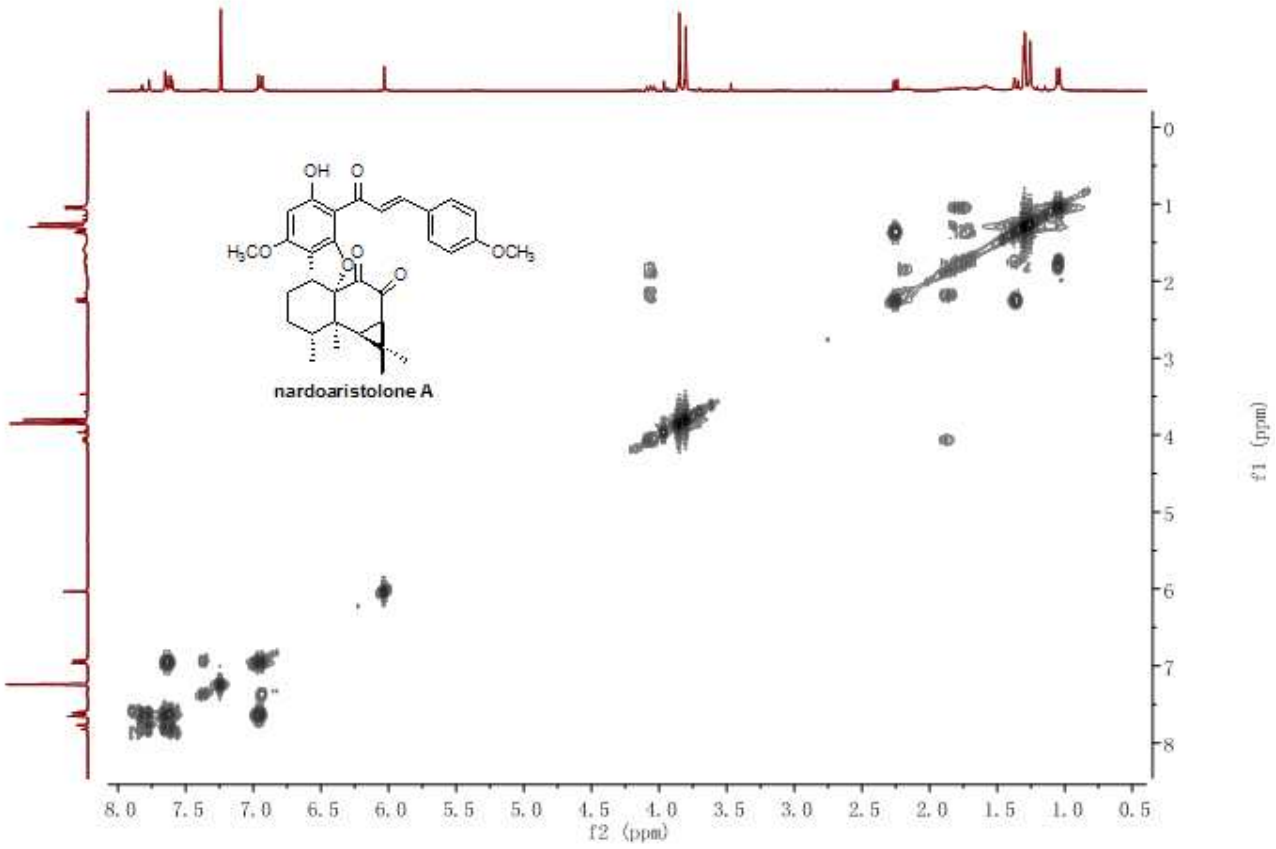
^{13}C NMR spectrum (AV-400, 100 MHz) of nardoaristolone A (1) in $CDCl_3$



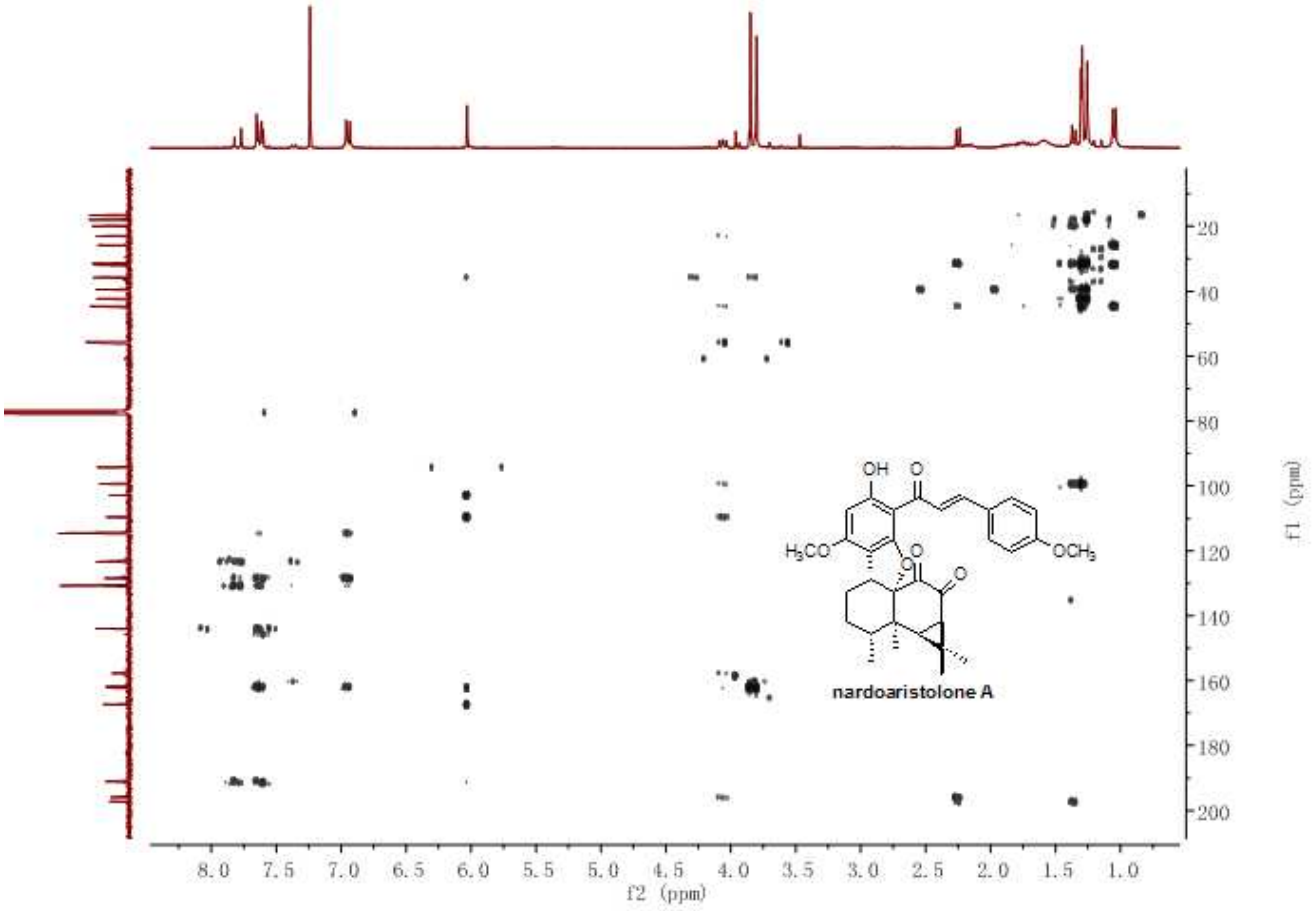
HSQC spectrum (AV-400) of nardoaristolone A (1)1 in $CDCl_3$



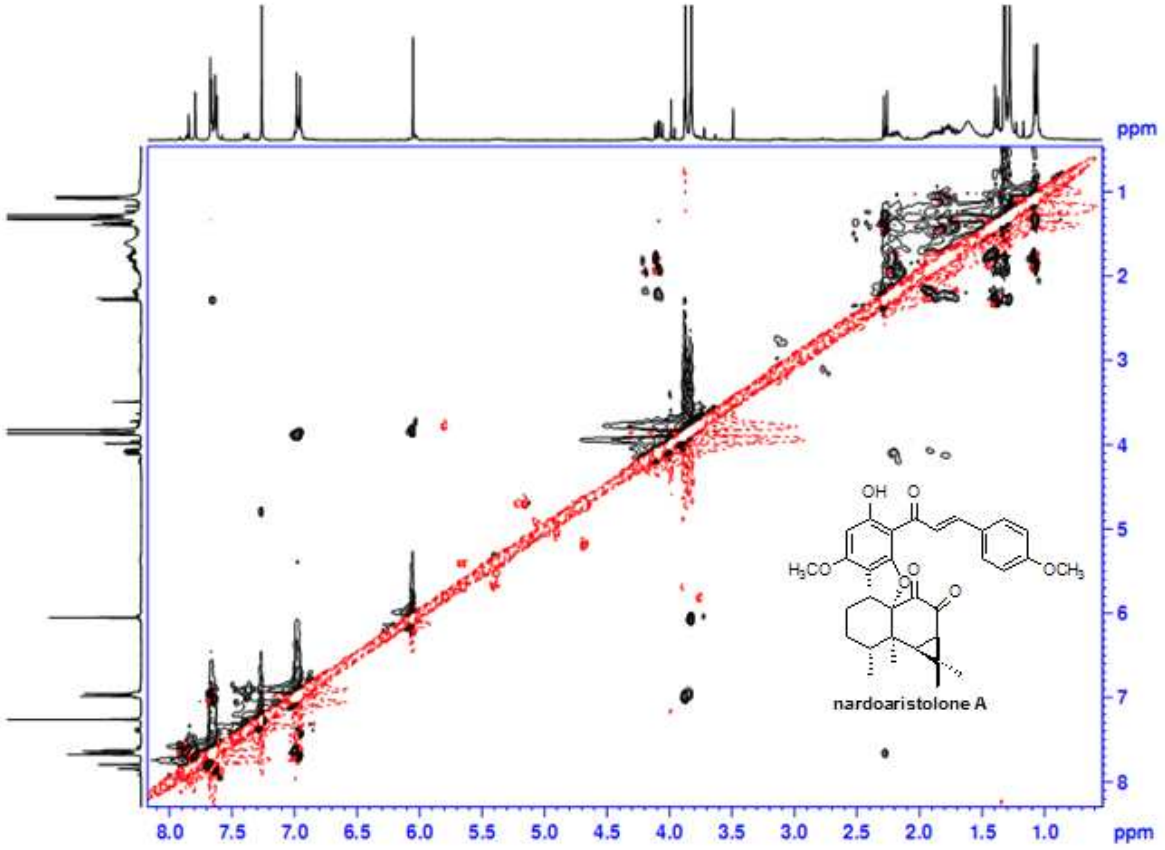
$^{1}H-^{1}H$ COSY spectrum (AV-400) of nardoaristolone A (1) in $CDCl_3$



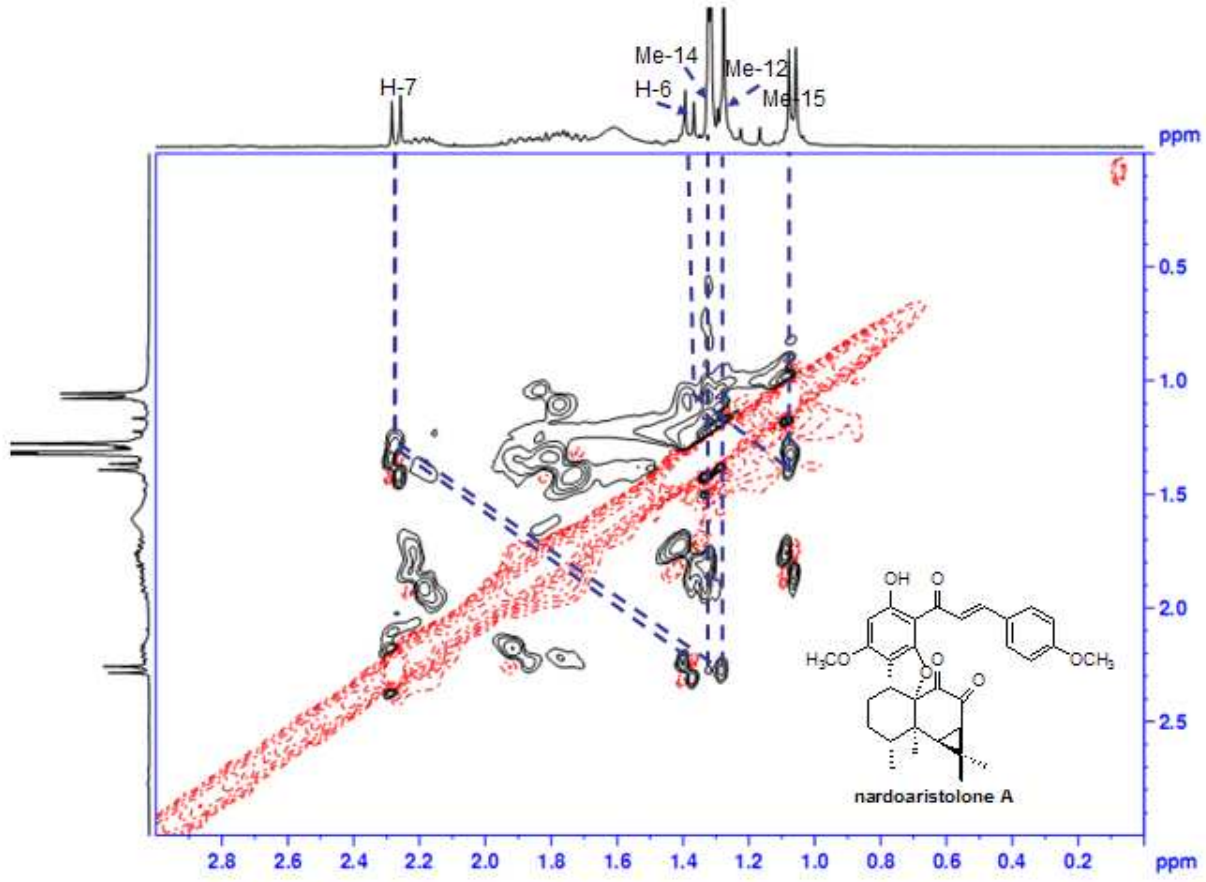
HMBC spectrum (AV-400) of nardoaristolone A (1) in $CDCl_3$



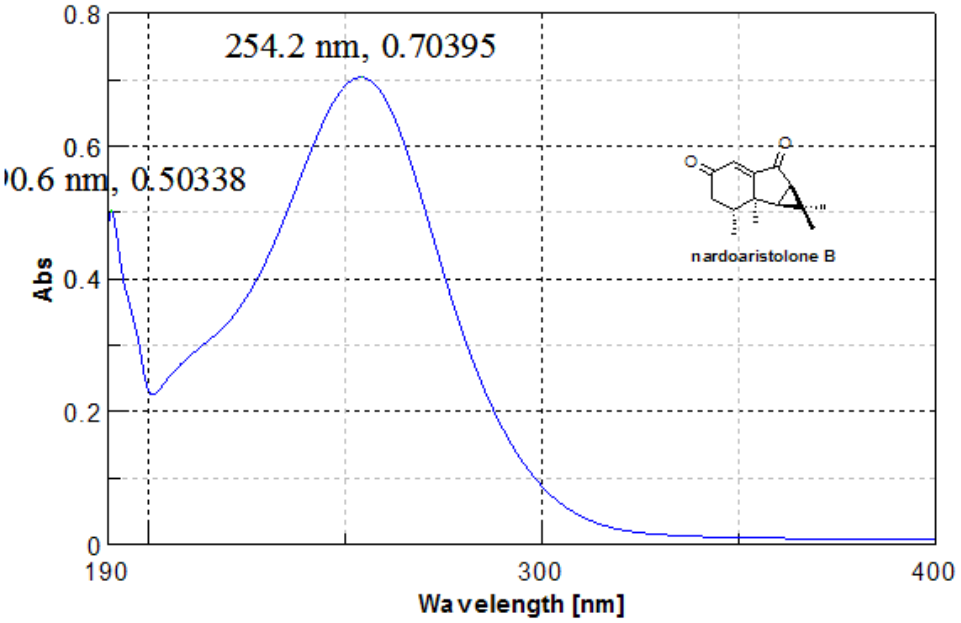
ROESY spectrum (AV-400) of nardoaristolone A (1) in $CDCl_3$



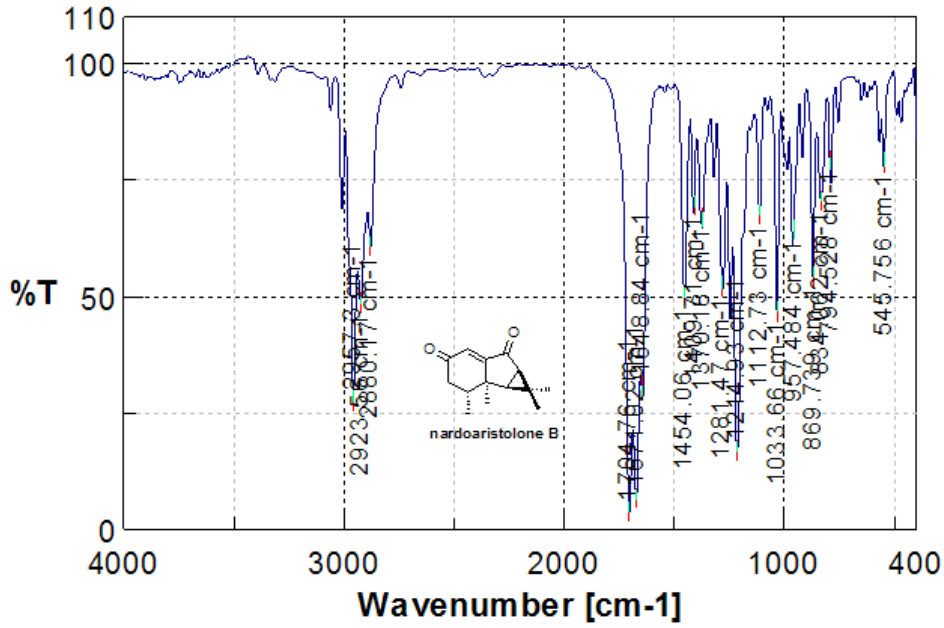
Enlarged ROESY spectrum (AV-400) of nardoaristolone A (1) in $CDCl_3$



UV spectrum of nardoaristolone B (2) in CH_3OH.



IR (KBr disc) spectrum of nardoaristolone B (2).



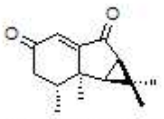
HR-ESI-MS spectrum of nardoaristolone B (2).

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
63 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-200 H: 0-1000 O: 0-200 Na: 0-1

E3-8B-2A4
20120612-20 154 (1.253) Cm (154)

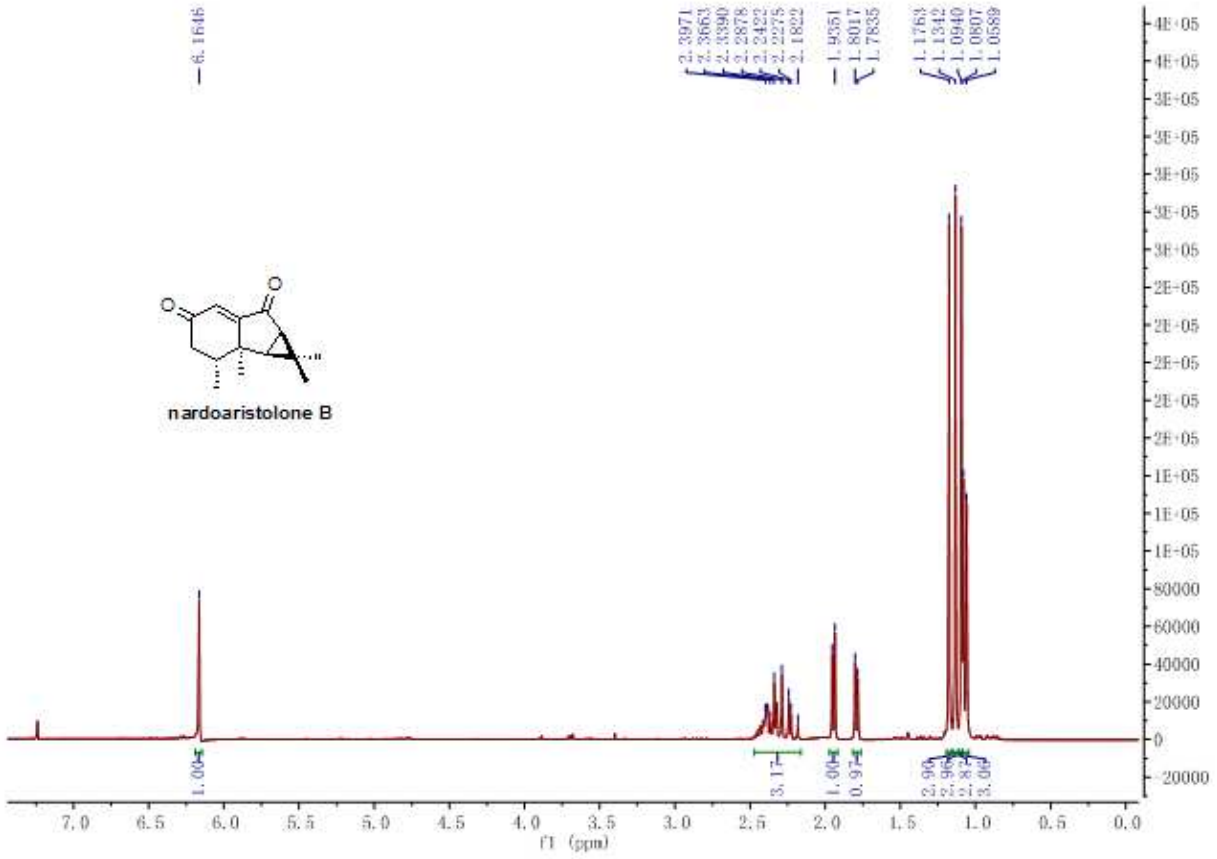


nardoaristolone B

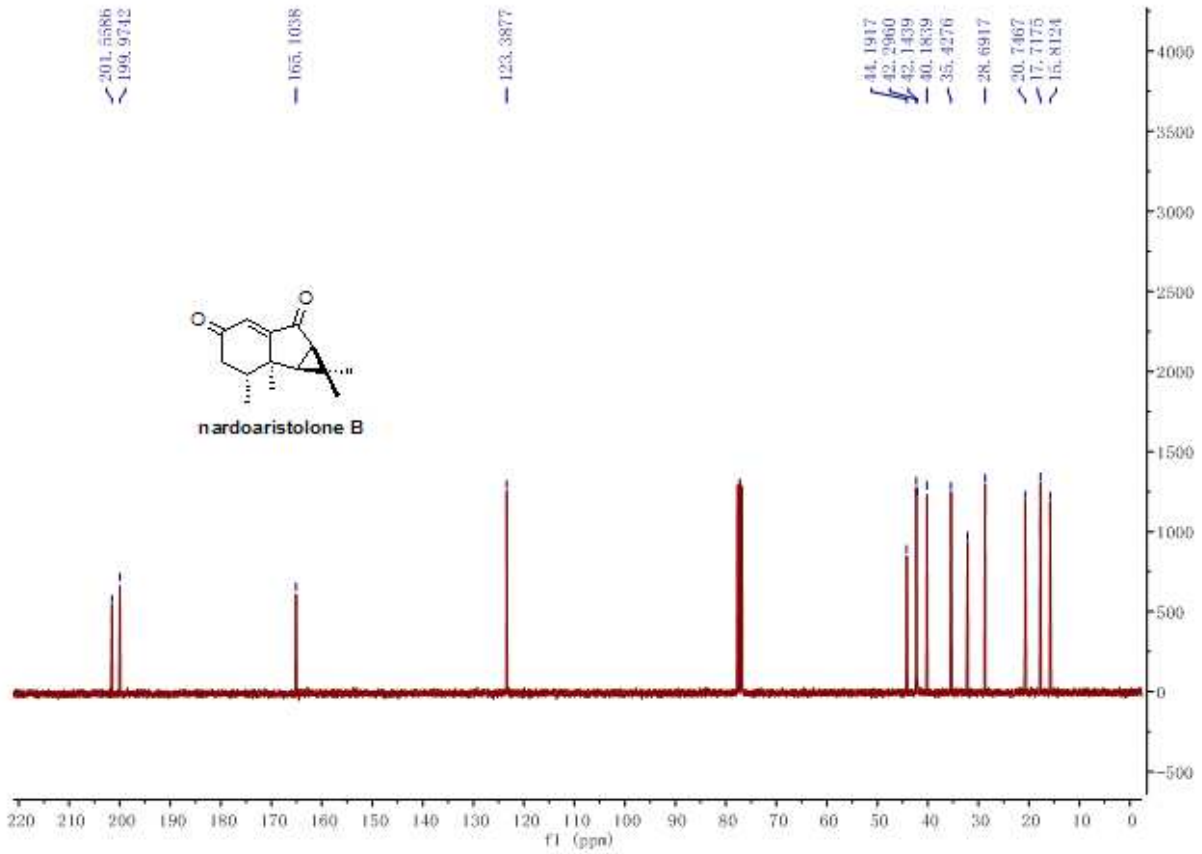
1: TOF MS ES+
1.36e+004

Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
219.1377	219.1385	-0.8	-3.7	5.5	41.8	n/a	n/a	C14 H19 O2

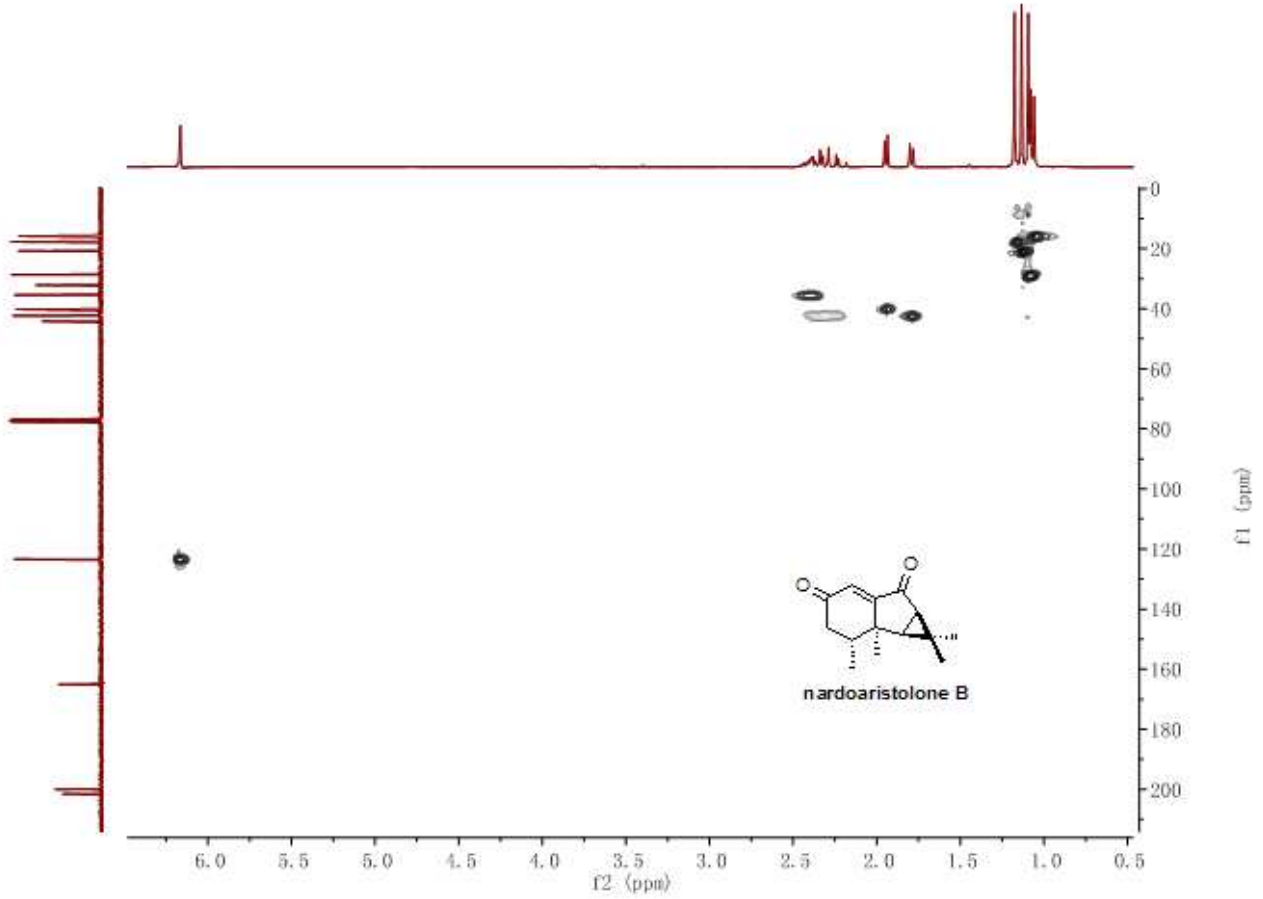
^{1}H NMR (AV-300, 300 MHz) spectrum of nardoaristolone B (2) in $CDCl_3$



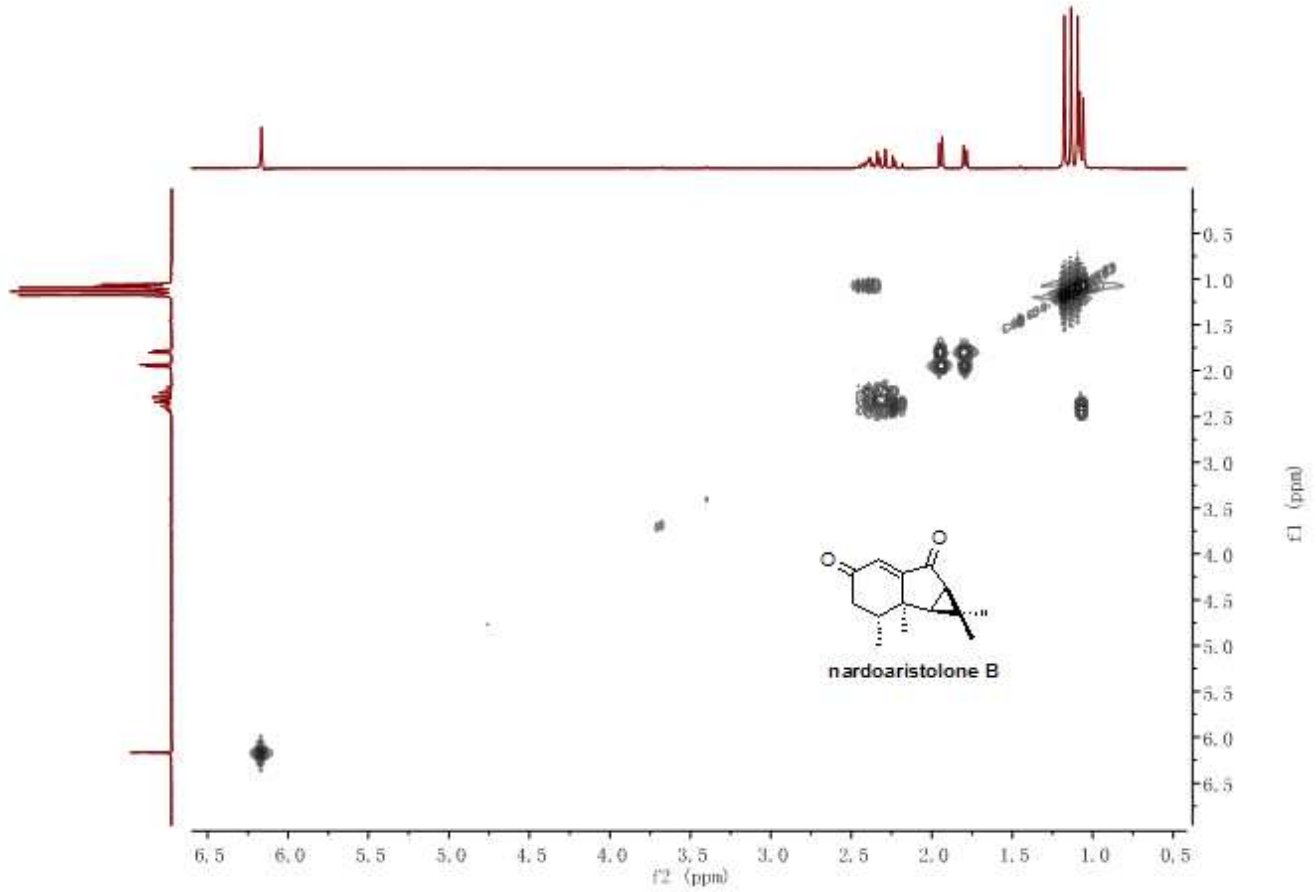
^{13}C NMR spectrum (AV-300, 75 MHz) of nardoaristolone B (2) in $CDCl_3$



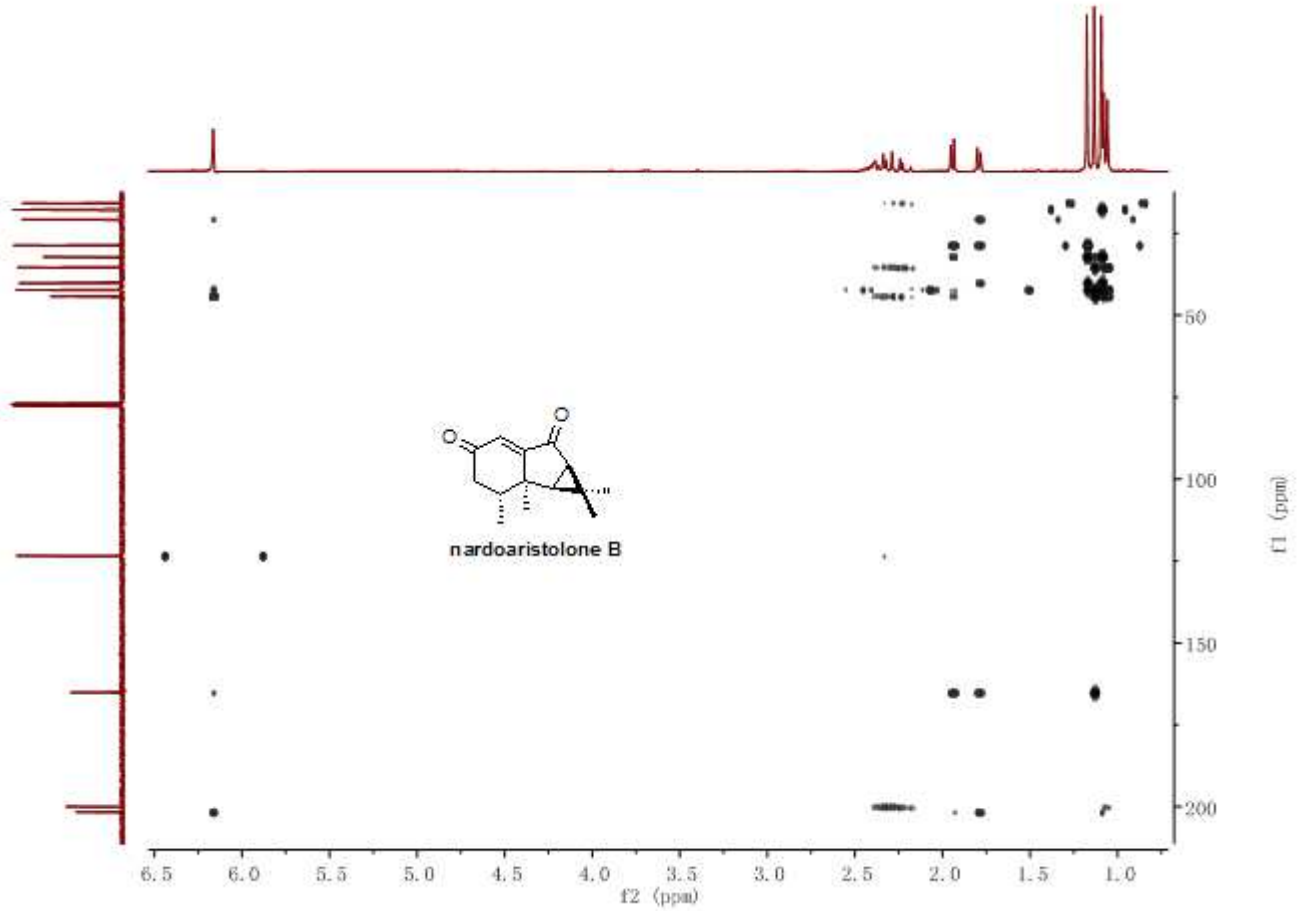
HSQC spectrum (AV-300) of nardoaristolone B (2)1 in $CDCl_3$



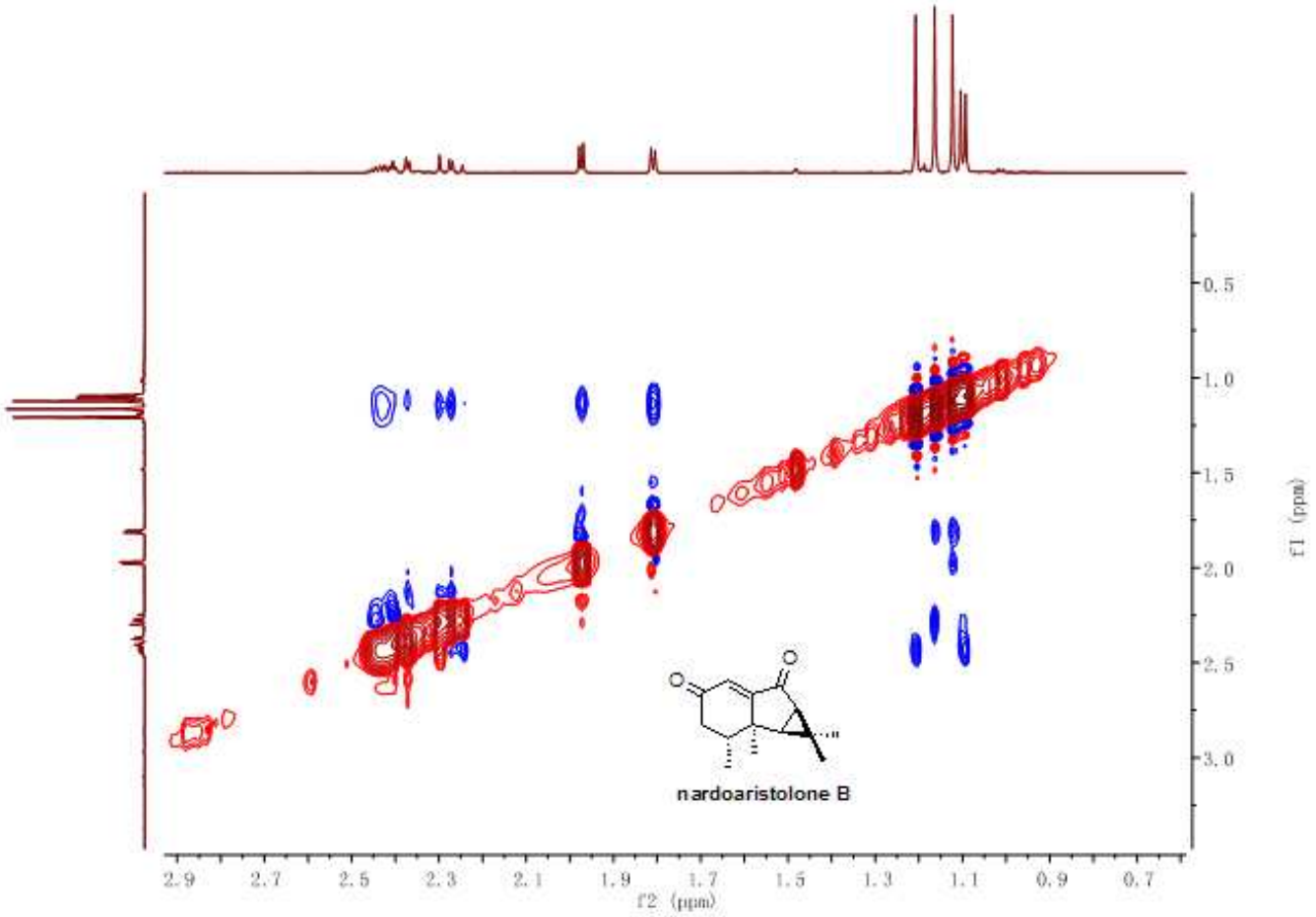
$^{1}H-^{1}H$ COSY spectrum (AV-300) of nardoaristolone B (2) in $CDCl_3$



HMBC spectrum (AV-300) of nardoaristolone B (2) in $CDCl_3$



Enlarged NOESY spectrum (AV-600) of nardoaristolone B (2) in $CDCl_3$



Isolation and culture of cardiomyocytes

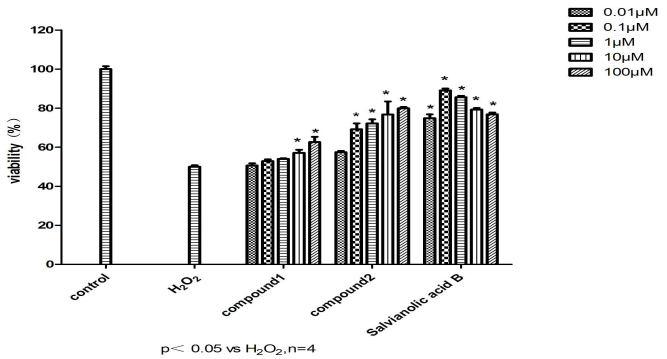
The neonatal rat cardiomyocytes were prepared from one to three-day-old Wistar rats by trypsin as described previously[1,2]. The ventricular myocardium was minced in DMEM (Dulbeco's Modified Eagle Medium, Hyclone, USA), which contains 25 mM D-glucose and 4 mM L-Glutamine. After the successive digestion with 0.25% trypsin, the cells were suspended in DMED containning 10% FBS (Fetal Bovine Serum, Hyclone, USA) and then centrifuged. Pooled cells were planted onto a 50 mL-cell culture bottle and incubated for 1.5-2 h at 37 ℃ in a humidified air with 5% CO_2. The fibroblasts were depleted afterward with differential velocity adherent technique. Finally, the supernatant cells containing non-adherent cells were planted onto the 96-well plates at a density of 10^5 cells·cm^{-2} with 0.1 mM bromodeoxyuridine (Sigma, USA), and incubated for 72 h at the same condition as described above.

Experimental Classification

The cultured cells were divided into control, hydrogen peroxide (H_2O_2), compound **1**, compound **2** and Salvianolic acid B groups. After starved in serum-free DMEM for 12 h, the cells were treated with 0.01, 0.1, 1, 10, 100 μM compound **1** for 12 h, respectively (as well as compound **2** and salvianolic acid B), then all groups were treated with DMEM containing 10% FBS and 100 μM H_2O_2 for 3 h except control group.

MTT assay for cell viability

[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)] (Roche Molecular Biochemicals, Laval, PQ, Canada) was used to quantify the cell survival of each group. After 3 h H_2O_2 injury, the supernatants of each well were replaced with 120 uL serum DMEM containing 20 uL MTT solution (5mg/ml), and the cells were incubated for 4 h at 37 ℃. The cell layers were dissolved with 150 uL DMSO after the supernatants were removed. The optical density of each well was measured at 490 nm using a Model 680 Microplate Reader (Bio-Rad, USA).



Protective effects on H_2O_2-induced myocardial injury of compounds 1 and 2.

References:

(1) Lu, Y.; Zhang, Y.; Wang, N.; Pan, Z.; Gao, X.; Zhang, F.; Shan, H.; Luo, X.; Bai, Y.; Sun, L.; Song, W.; Xu, C.; Wang, Z.; Yang, B. *Circulation*. **2010**, *122*, 2378-2387.

(2) Lu, Y.; Zhang, Y.; Shan, H.; Pan, Z.; Li, X.; Li, B.; Xu, C.; Zhang, B.; Zhang, F.; Dong, D.; Song, W.; Qiao, G.; Yang, B. *Cardiovasc Res* **2009**, *84*, 434-441.