Supplementary Data

Pseudolarenone, an unusual nortriterpenoid lactone with a fused 5/11/5/6/5 ring system featuring an unprecedented bicyclo[8.2.1]tridecane core from *Pseudolarix amabilis*

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Contents

Isolation Procedures

General. Column chromatography (CC): silica gel (200-300 mesh and 100-200 mesh: Huiyou Silica Gel Development Co. Ltd, Yantai, P.R. China); RP-C18 (GHODS AQ 12S50, Japan) and Sephadex LH-20 (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA). TLC: Precoated silica gel 254 plates (Huanghai, 0.15 – 0.20 mm thick for TLC analysis, 0.40 – 0.50 mm thick for preparative TLC), visualization by spraying with 10% H_2SO_4 in EtOH. CD spectra: JASCO-J-810 spectrometer. IR spectra: Bruker Vector-22 Spectrophotometer with KBr discs. Optical rotation: *PerkinElmer* 341 polar meter. UV spectra: *SHIMADAZU UV-2550* spectrophotometer; λ_{max} in nm. NMR Spectra: *Bruker DRX-400* spectrometer (400 MHz); δ in ppm with $SiMe_4$ as internal standard, *J* in Hz. MS: *Agilent MSD-Trap-XCT* (for ESI) and Waters Micromass *Q-Tof* spectrometer (for HR-ESI), in *m/z*.

Plant Material. The cones of *Pseudolarix amabilis* Gord. (Pinaceae) were collected in Jiu Jiang, Jiangxi province, P. R. China, in October 2010, and authenticated by Prof. Han-Ming Zhang of Second Military Medical University. A voucher specimen (No. 20101015) is deposited in School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The air-dried cones (12.0 kg) were powdered and extracted with 80% EtOH for four times at room temperature and then partitioned with petroleum ether, $CHCl_3$, and EtOAc, successively. The $CHCl_3$-soluable extract was subjected to a silica gel column eluting with a gradient petroleum ether (60 – 90 °C)/EtOAc (30:0→0:1) to obtain eight fractions. Fraction 7 was chromatographed over RP-18 CC (MeOH/H_2O, 2:8→10:0) to afford seven subfractions. Subfraction 3 was further chromatogrephed on a silica gel (CH_2Cl_2/MeOH 30:1→0:1) and purified by preparative TLC (cyclohexane/EtOAc 1:1) to afford pseudolarenone (**1**) (20 mg).

Fig. S1 UV spectrum of pseudolarenone (**1**)

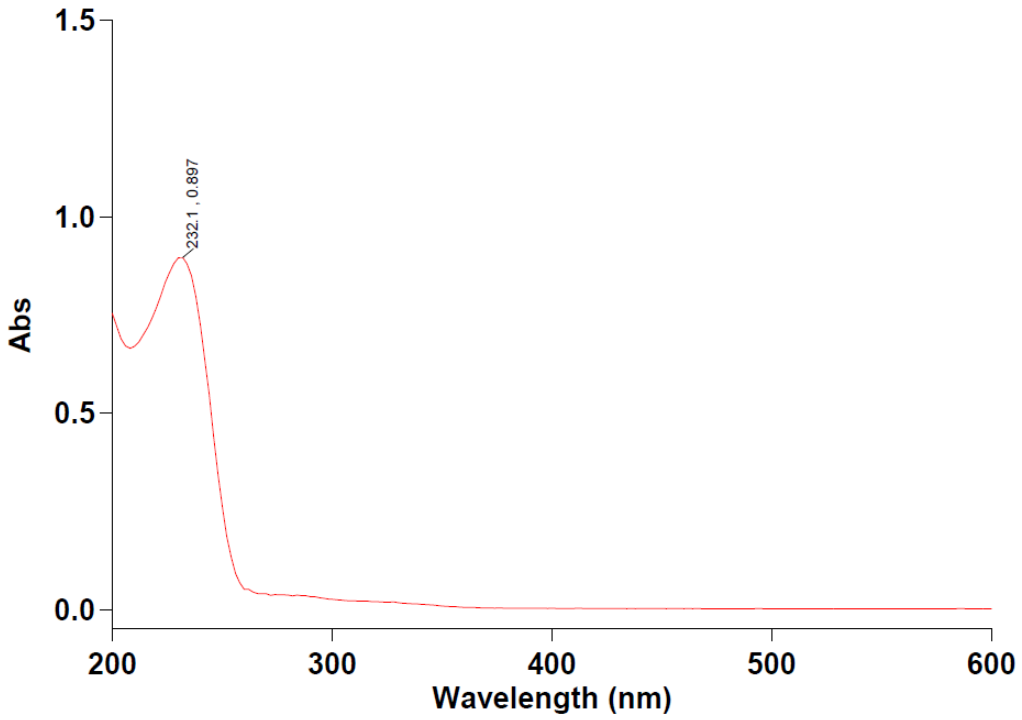


Fig. S2 CD spectrum of pseudolarenone (**1**)

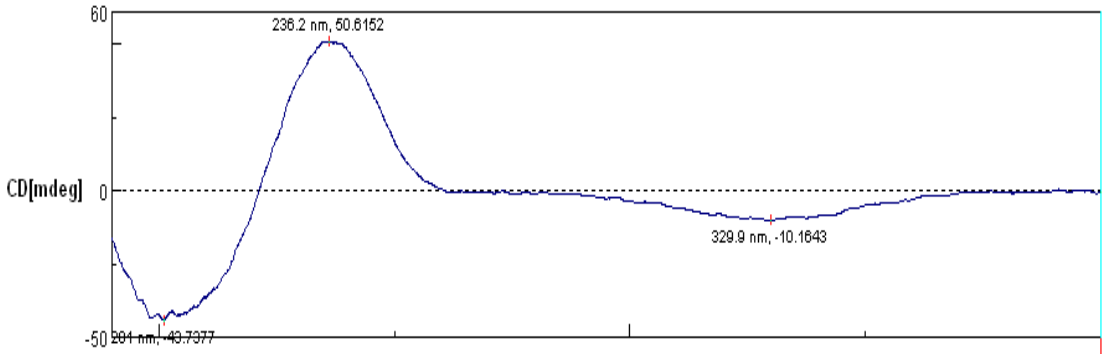


Fig. S3 IR spectrum of pseudolarenone (**1**)

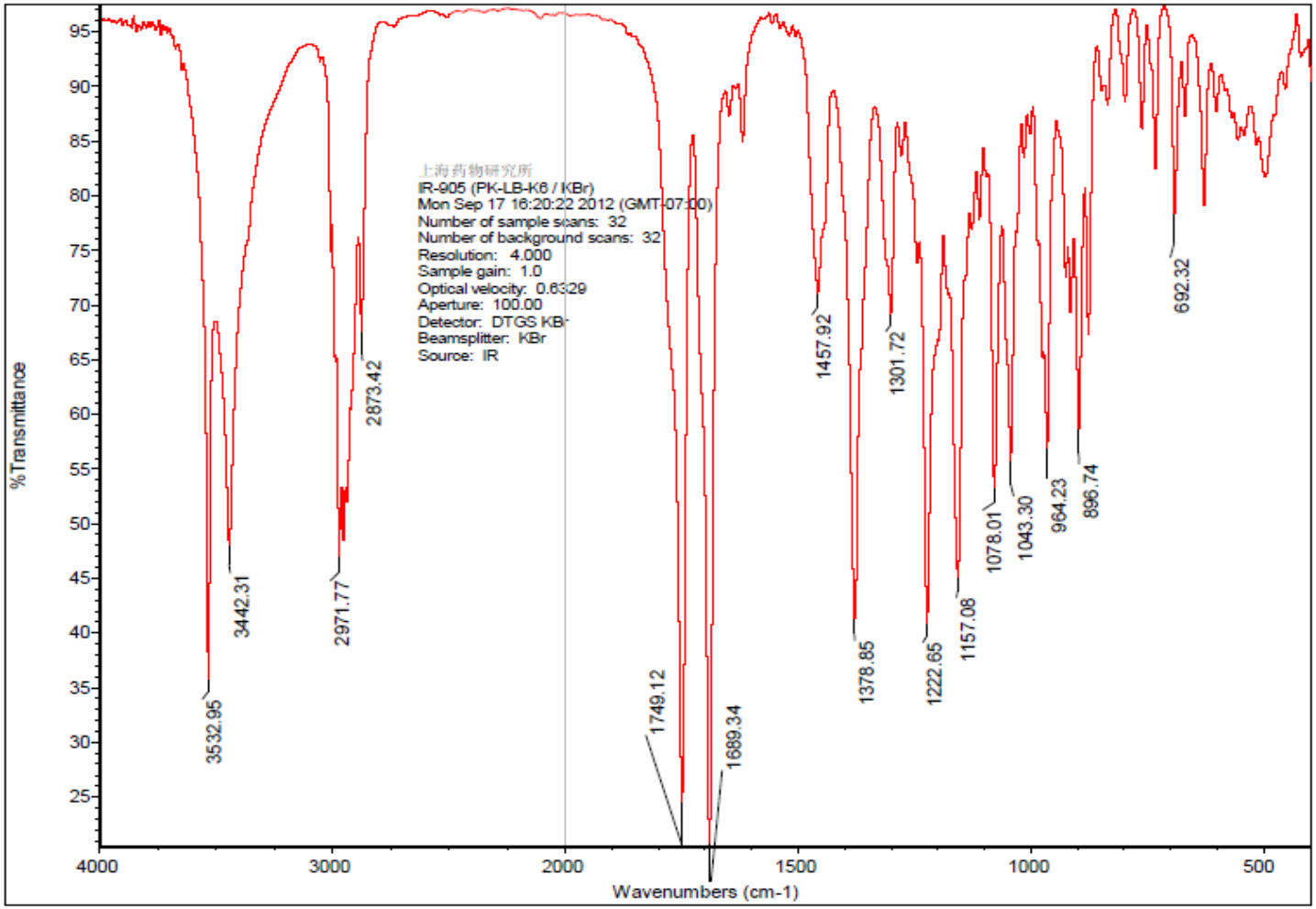


Fig. S4 HRESIMS spectrum of pseudolarenone (**1**)

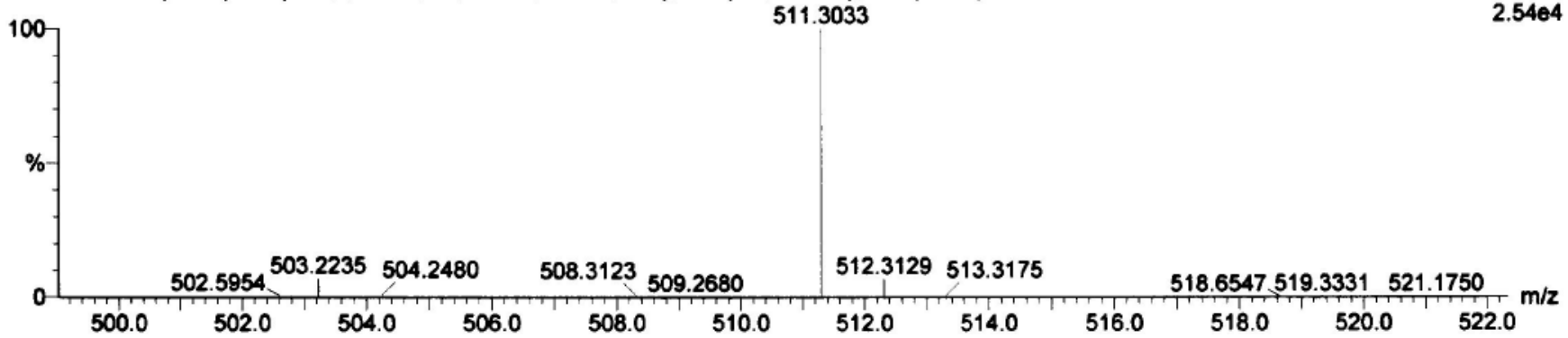
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
13 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 10-30 H: 10-50 O: 1-8 Na: 1-1

SIPI
PK-LB-K6 M.W=488
Q12-1427H 33 (1.163) AM (Cen,4, 80.00, Ar,5000.0,502.21,0.70); Sm (SG, 2x1.00); Cm (33:42)

Q-Tof micro
YA019

30-Aug-2012,13:28:17
TOF MS ES+
2.54e4



Minimum:	70.00				-1.5		
Maximum:	100.00		5.0	10.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
511.3033	100.00	511.3036	-0.3	-0.6	7.5	6630.2	C29 H44 O6 Na

Fig. S5 400 MHz ^{1}H NMR spectrum of pseudolarenone (**1**) in $CDCl_3$

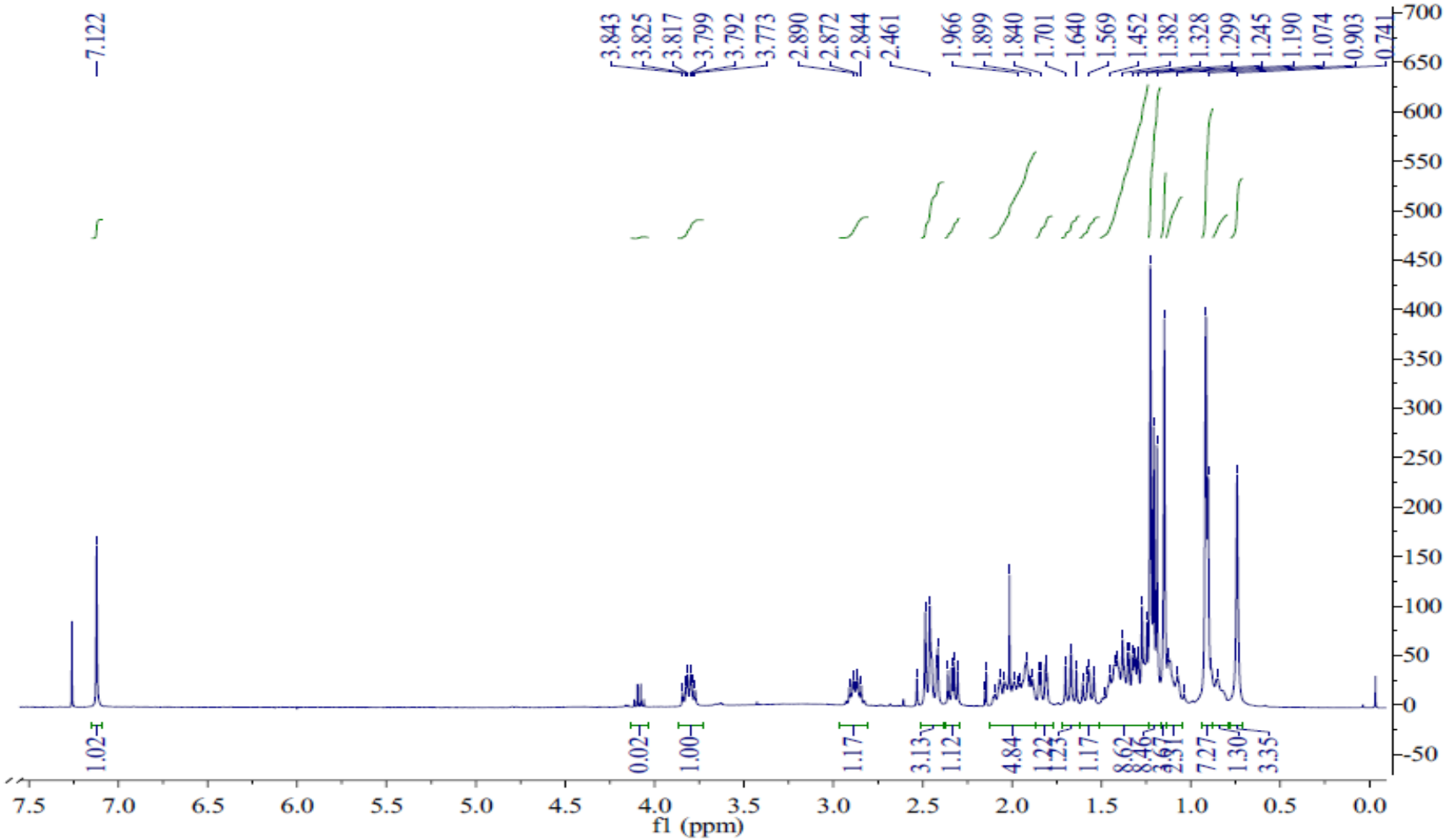


Fig. S6 100 MHz ^{13}C NMR spectrum of pseudolarenone (**1**) in $CDCl_3$

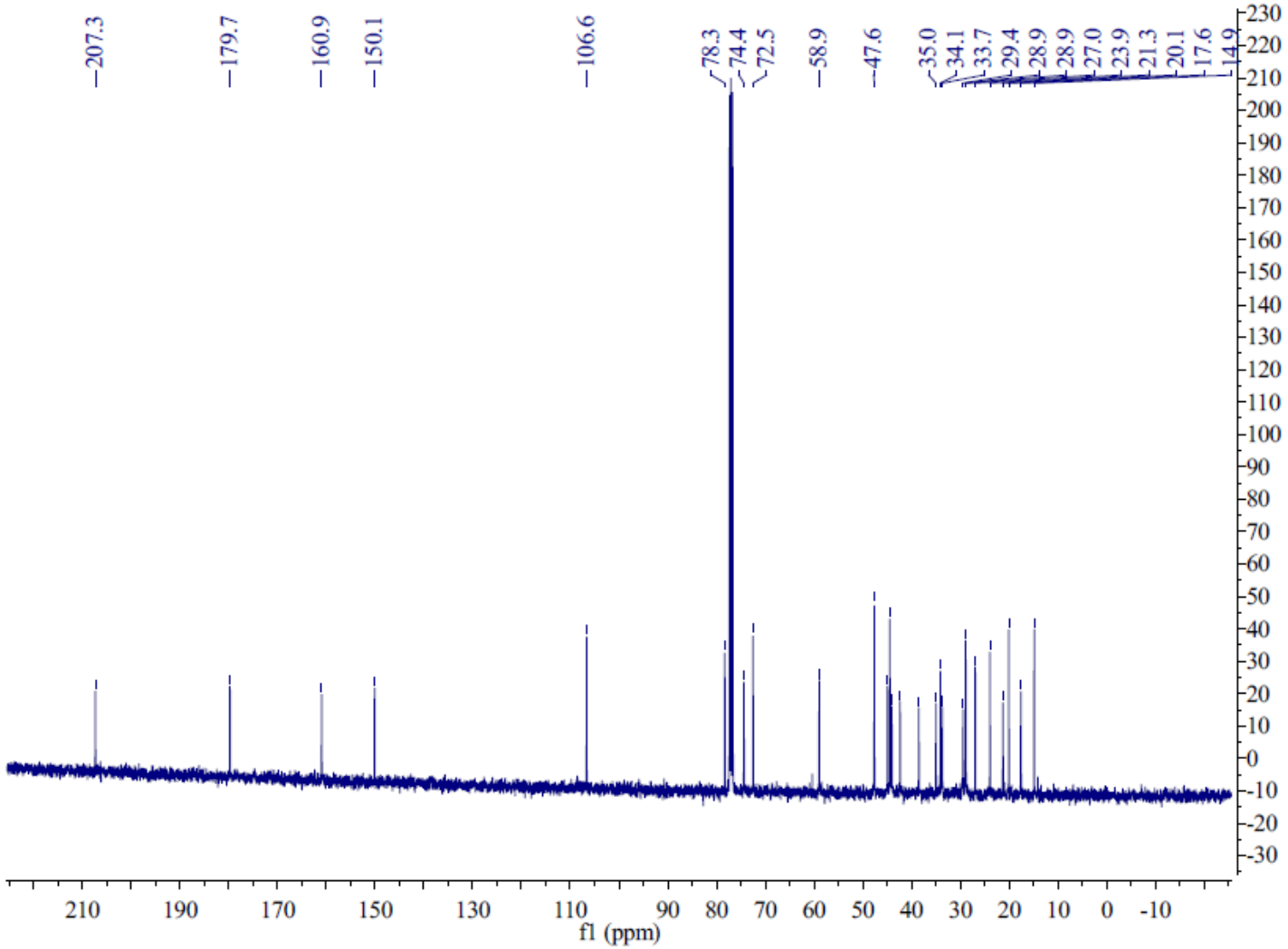


Fig. S7 100 MHz ^{13}C and DEPT NMR spectrum of pseudolarenone (**1**) in $CDCl_3$

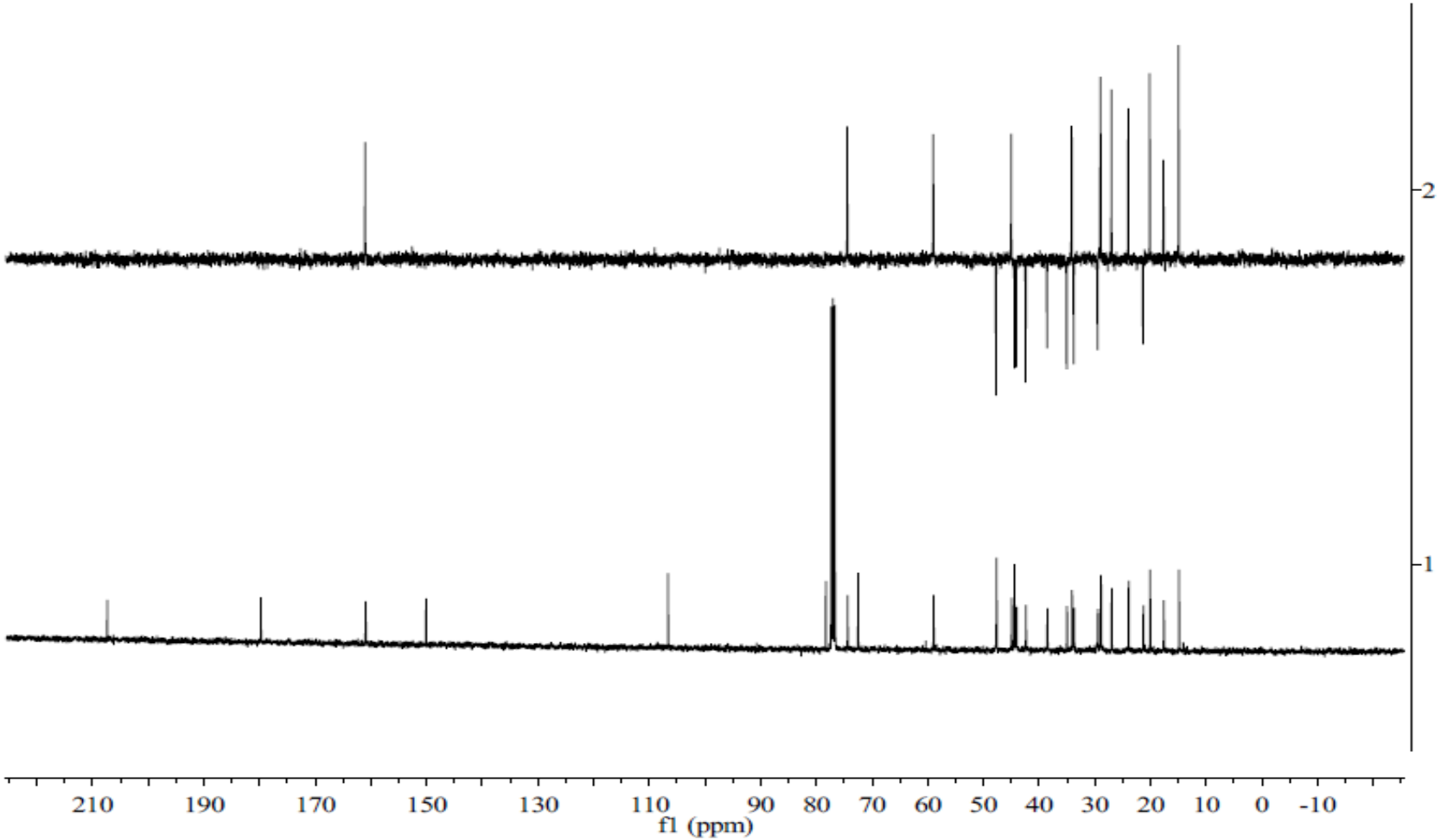


Fig. S8 400 MHz HSQC NMR spectrum of pseudolarenone (**1**) in $CDCl_3$

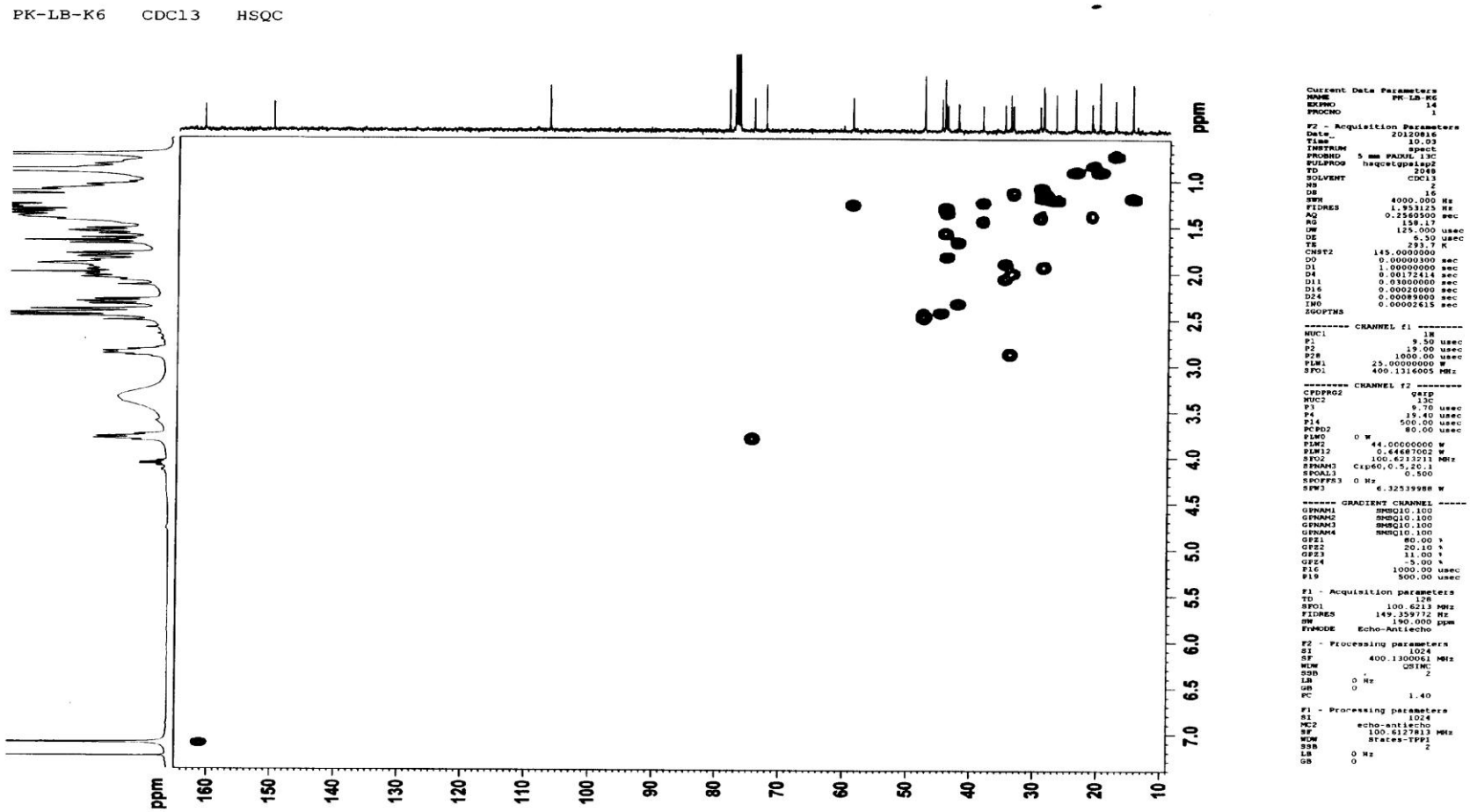


Fig. S9 400 MHz ^{1}H-^{1}H COSY NMR spectrum of pseudolarenone (**1**) in $CDCl_3$

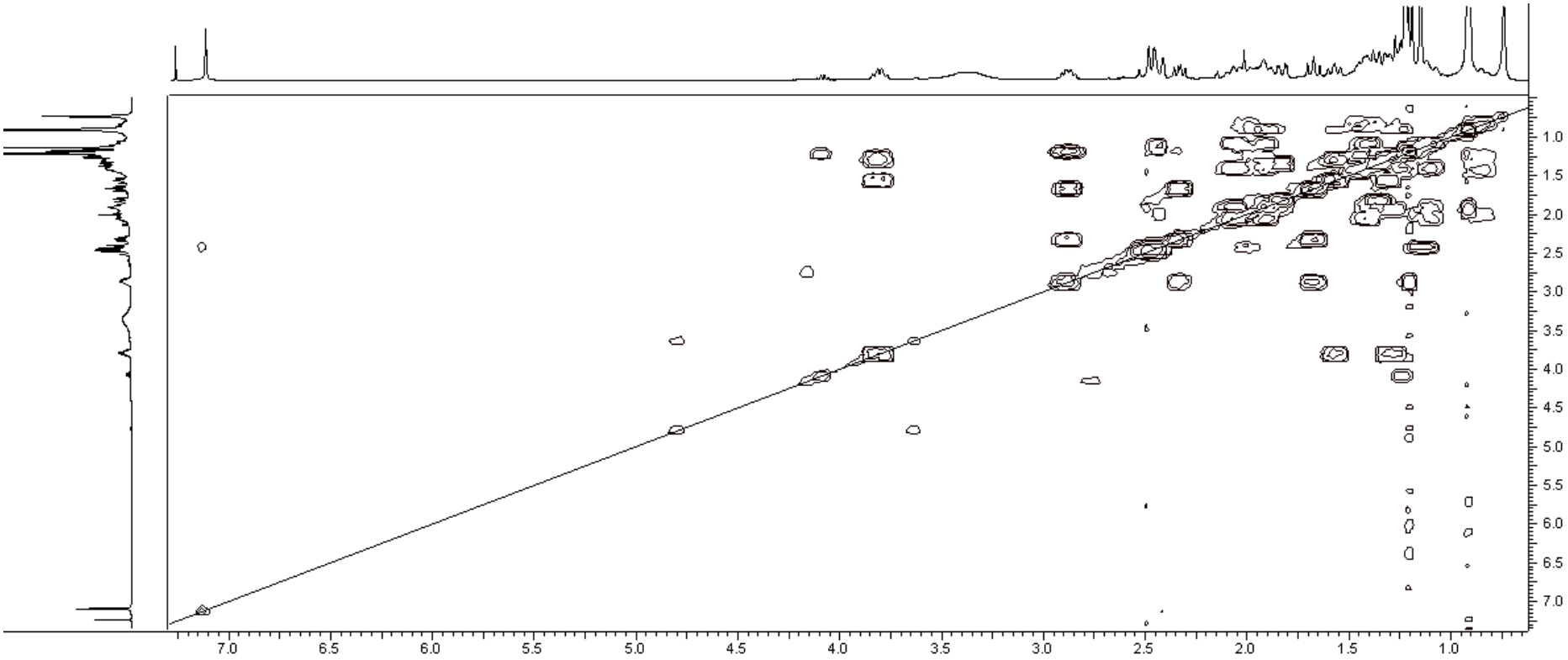
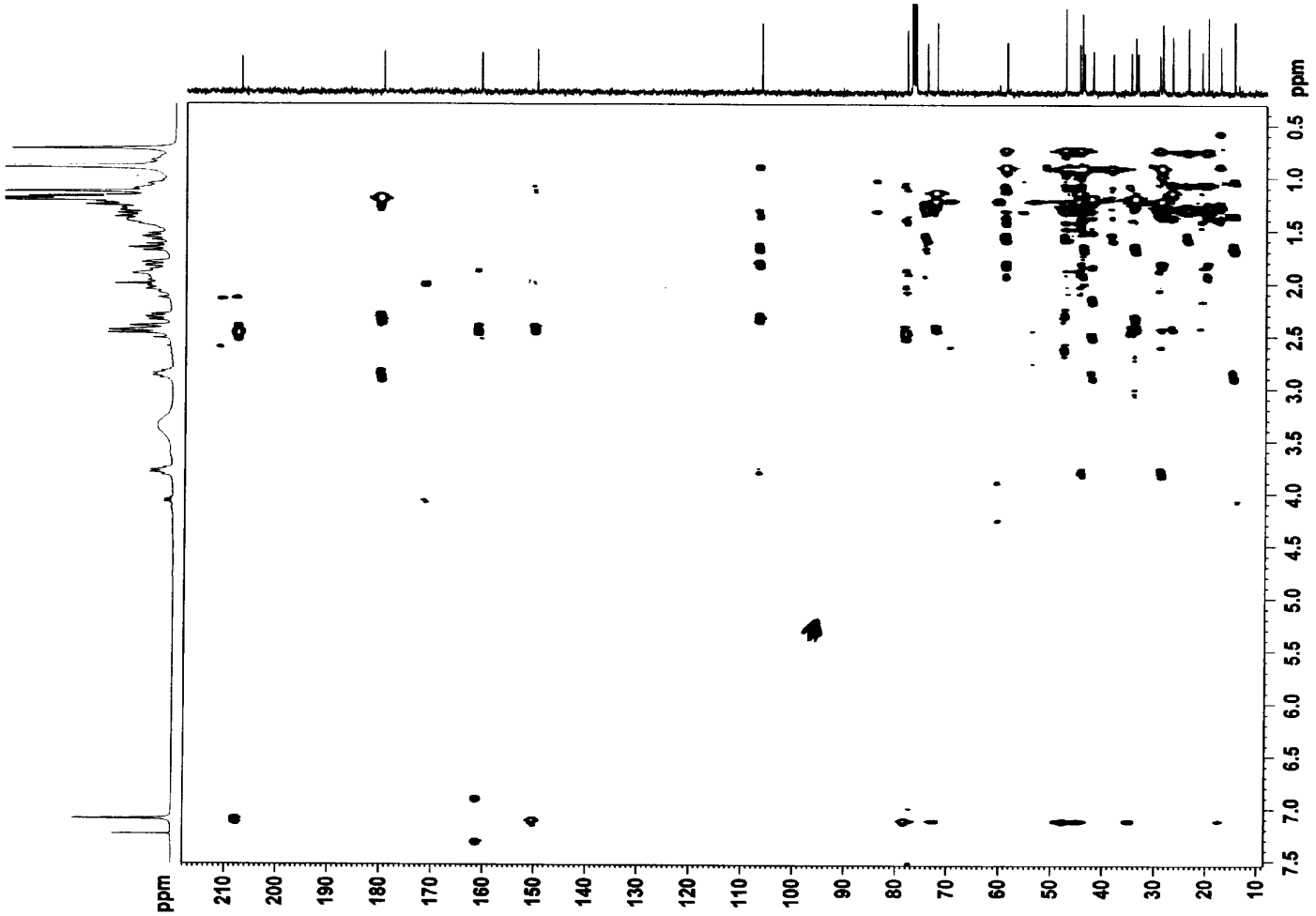


Fig. S10 400 MHz HMBC NMR spectrum of pseudolarenone (**1**) in $CDCl_3$

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PROCNO                       1

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PULPROG          hmbcgpndqf
TD                       2048
SOLVENT                  CDCl3
NS                          8
DS                         16
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FIDRES             1.953125 Hz
AQ                 0.2560500 sec
RG                     158.17
DW                  125.000 usec
DE                    6.50 usec
TE                    293.6 K
CNST13             8.0000000
D0                 0.00000300 sec
D1                 1.00000000 sec
D6                 0.06250000 sec
D16                0.00020000 sec
IN0                0.00002070 sec

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NUC1                       1H
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P2                   19.00 usec
PLW1           25.00000000 W
SFO1           400.1316005 MHz

-------- CHANNEL f2 --------
NUC2                      13C
P3                    9.70 usec
PLW2           44.00000000 W
SFO2           100.6238364 MHz

------ GRADIENT CHANNEL -----
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GPNAM2         SMSQ10.100
GPNAM3         SMSQ10.100
GPZ1                 50.00 %
GPZ2                 30.00 %
GPZ3                 40.10 %
P16                1000.00 usec

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FIDRES         94.334846 Hz
SW                 240.000 ppm
FnMODE                   QF

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WDW                   QSINE
SSB          0
LB           0 Hz
GB           0
PC                     1.40

F1 - Processing parameters
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MC2                     QF
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WDW
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GB           0
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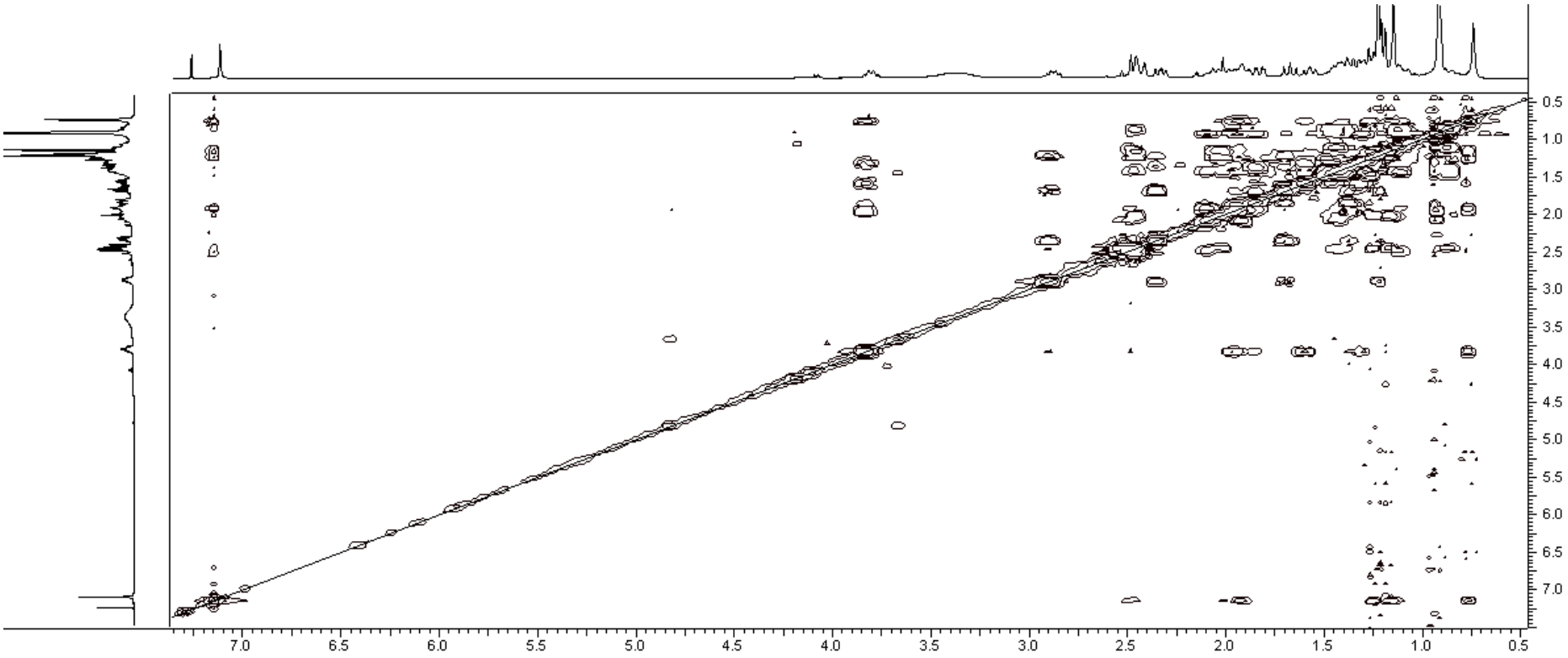
Fig. S11 400 MHz ROESY NMR spectrum of pseudolarenone (**1**) in $CDCl_3$



Biological Activity Assay

CCK-8 assay for cytotoxic activity in RAW264.7 macrophages.

RAW264.7 macrophages obtained from Shanghai Institute for Biological Science (Shanghai, China) were maintained in DMEM (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere containing 5% CO_2 at 37 ºC. Cell viability was also measured by Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Absorbance was read at 450 nm by a BioTek Synergy 2 plate reader (BioTek Instruments, Inc.).

Assay for inhibition activity against nitric oxide (NO) production in LPS-induced RAW 264.7 macrophages

The tested compounds were dissolved in DMSO (Sigma. St. Louis, MO), and diluted with PBS prior to experiment (DMSO final culture concentration < 0.1%). The macrophages were seeded in 48-well plates (5×10^5 cells/well). The cells were co-incubated with drugs and LPS (10 μg/mL) for 18 h, with 50 μM aminoguanidine as positive control. The amount of nitric oxide (NO) was assessed by determining the nitrite concentration in the supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in sequence, with 50 μL 1% sulphanilamide and 50 μL 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbances at 570 nm were read using a BioTek Synergy 2 plate reader.

Statistical Analysis

All quantitative data were expressed as mean ± S.D. as indicated. Multiple comparisons were compared by one-way ANOVA analysis of variance followed by Dunnett's test. Statistical significance was established at $P < 0.05$.

Table 1. Inhibition activity against the production of NO in LPS-induced macrophages

Compounds	Doses (μM)	NO (μM)	Inhibition (%)
Blank		2.21±0.04**	
Control (LPS 10μg/mL)		7.60±0.03	
pseudolarenone	50	2.91±0.04**	45.48
	25	3.66±0.42**	38.29
	10	3.96±0.08**	35.28
	5	4.38±0.00**	31.20
	1	4.83±0.13**	26.82
pseudolarolide I	50	3.09±0.13**	43.73
	25	4.08±0.17**	34.11
	10	4.47±0.04**	30.32
	5	5.28±0.51*	22.45
	1	5.58±0.00**	19.53
Aminoguanidine	50	2.54±0.04**	48.98

** P< 0.01 vs Control, * P< 0.05 vs Control