Supplementary Information

Structurally Novel C_{17}-Sesquiterpene Lactones from *Ainsliaea pertyoides*

Zhi-Ran Shi,[ab] Yun-Heng Shen,*[b] Xian-Yuan Zhang,[c] Xin Fang,[b] Ren-Tao Zeng,[b] Qing-Xin Liu,[ab] Zhi-Guo Zhuo,[b] Feng Feng*[a] and Wei-Dong Zhang*[abc]

[a] *Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, P. R. China*

[b] *Department of Phytochemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China*

[c] *Shanghai Institute of Pharmaceutical Industry, Shanghai 200040, P. R. China*

Correspondence E-mail:** ***shenyunheng@hotmail.com;

fengsunlight@163.com

wdzhangy@hotmail.com

Contents

Experimental section

General Experimental Procedures

General. Optical rotations: Autopol VI (serial No. 90079, manufactured by Rudolph Research Analytical, Hackettstown, NJ). IR spectra were recorded on a Bruker Vector 22 spectrometer using KBr disks. UV spectra were recorded with a Varian CARY 50. NMR spectra were obtained using Bruker Ascend-500 spectrometer (500 MHz). The chemical shift (δ) values are given in ppm with TMS as internal standard, and coupling constants (*J*) in Hz. MS were measured with Agilent *MSD-Trap-XCT* (for ESI) and *Q-Tof* micro mass spectrometer (for HR-ESI). Column chromatography (CC): silica gel H (10–40 μm; *Marine Chemical Factory*, Qingdao, P. R. China); Sephadex LH-20 (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA); RP-C18 gel (40−63 μm; Daiso, Co., Japan) were used for column chromatography. Preparative TLC (0.4-0.5 mm, 20×20 cm) was conducted with glass precoated silica gel GF254 (Huiyou Silica Gel Development Co., Ltd.). Spots were detected on TLC under UV light or by heating after spraying with 10 % H_2SO_4 in EtOH and followed by heating.

Plant Material. The whole plants of *Ainsliaea* pertyoides was collected in August 2014, from Gongshan county, Yunnan province, China, and authenticated by Prof. Yuanchuan Zhou in the Nujiang Institute of Medicinal Plants. A voucher specimen (no. 2014108014) is deposited in School of Pharmacy, Second Military Medical University.

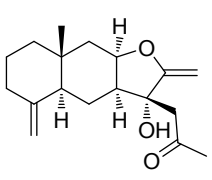
Extraction and Isolation. The air-dried plant material of *A.* pertyoides (10.0 kg) was percolated with 95% EtOH at room temperature, and the extract (0.6 kg) was further partitioned partitioned between EtOAc and H_2O. The EtOAc-soluble partition (80 g) was fractionated on a column of macroporous resin eluted with 30, 80, and 100% MeOH/H_2O, and the 80% MeOH elution (60 g) was separated by an MCI gel column (MeOH/H_2O, 4:6 to 9:1) to afford seven fractions (A−G), the fourth fraction (E, 10 g)

of which was subjected to CC eluted with petroleum ether–acetone (100:1 to 1:2) to yield 6 subfractions (E1–E6). Fraction E4 was separated over a column of RP-18 silica gel (MeOH–H_2O, 5:5 to 9:1) to furnish five fractions (E4a–E4e), and the first fraction (E4a) was purified by semi-preparative HPLC to return compounds 5 (28 mg), 7 (21 mg) and 16 (7 mg). E4b was purified by silica gel CC ($CHCl_3$–MeOH, 500:1 to 150:1) and HPLC to yield 12 (3 mg), 13 (19 mg), 15 (9 mg) and 14 (4 mg). Fraction E5 was sequentially fractionated by RP-18 silica gel (MeOH–H_2O, 5:5 to 4:1) and silica gel (petroleum ether–$CHCl_3$, 5:1 to 1:4) CC, and was finally purified by semi-preparative HPLC to afford 1 (12 mg), 2 (15 mg), 3 (49 mg), 4 (33 mg) and 8 (4 mg). Fraction F was extensively separated by columns of RP-18 silica gel (MeOH–H_2O, 5:5 to 4:1) and silica gel ($CHCl_3$–MeOH, 500:1 to 100:1), and was finally purified by HPLC to give 10 (100 mg), 6 (15 mg), 11 (12 mg) and 9 (16 mg). Fraction G was fractionated in sequence by RP-18 silica gel (MeOH/H_2O, 55% to 70%) CC, silica gel CC (CH_3Cl/MeOH, 500:1 to 150:1), and finally semi-preparative HPLC to afford compounds 17 (2 mg) and 18 (9 mg).

Cytotoxicity Assay

The cytotoxicity of compound **1** was determined by MTT assay (Sigma, St. Louis, MO). Briefly, A549, HCT116, MGC803 and CCRF-CEM cells were inoculated at a density of 1×10^4 cells/well in 96-well microplates and after 24 h incubation were treated with 0.001, 0.01, 0.1, 1, 10 and 100 μM of 1, and doxorubicin for 24, 48 and 72 h. At the end of the incubation, 10 μL of MTT (5 mg/mL) was added to each well, and the plates were incubated for 4 h at 37 °C. The supernatants were aspirated carefully and 150 μL of DMSO were added to each well to dissolve the precipitate. Absorbance was read at 570 nm by a BioTek Synergy 2 plate reader (BioTek Instruments, Inc., Winooski, Vt, USA). Each experiment was performed in triplicate. Consequently, compounds **2**-**18** were tested as that of **1**. Results of three independent experiments were used for statistical analysis. IC_{50} value was calculated by the Logit method.

Figure S1. The structure of compounds **1**−**18**



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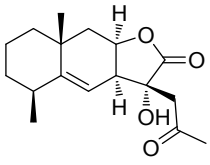

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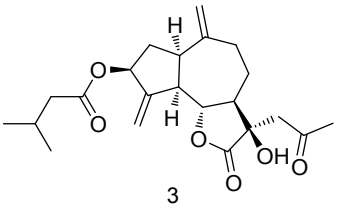

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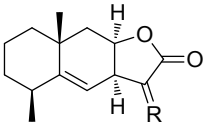
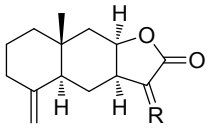
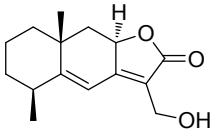


4 R = α-OH, β-CH_2OH
5 R = CH_2



6 R = CH_2
7 R = H, β-Me



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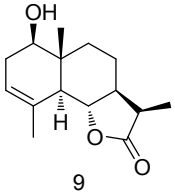

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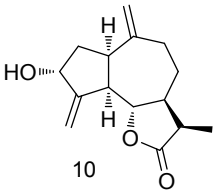
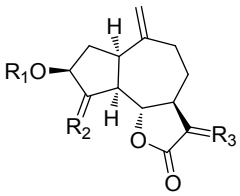
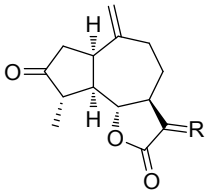


10

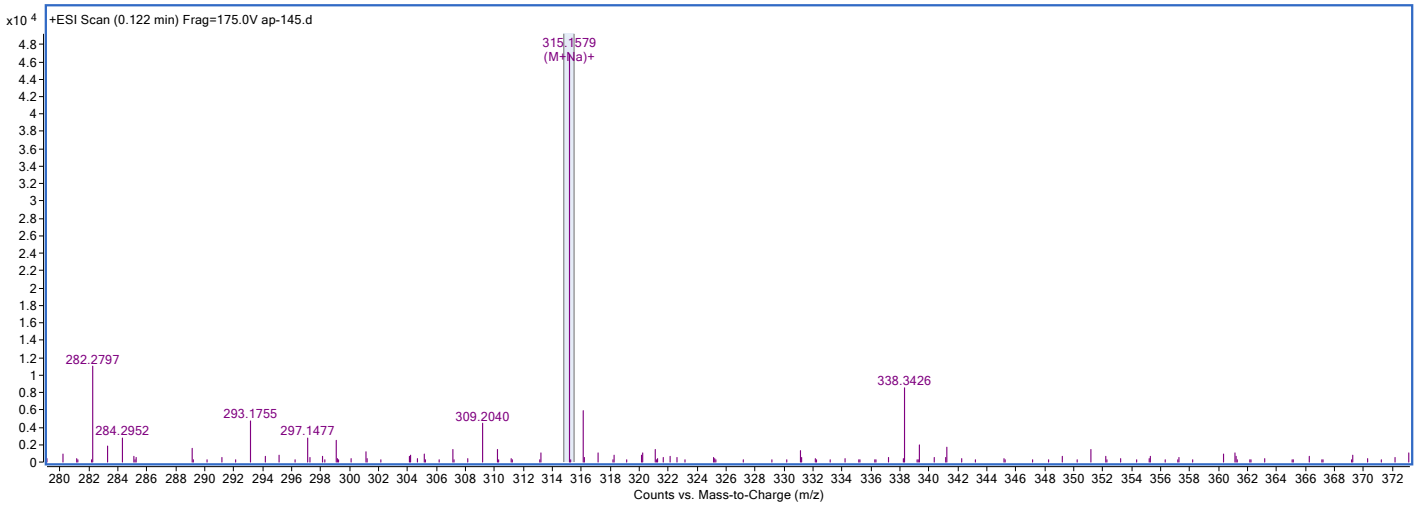
	R_1	R_2	R_3
11	$OCCH_2CH(CH_3)_2$	CH_2	CH_2
12	$OCCH_2CH(CH_3)_2$	CH_2	H, α-CH_3
13	H	H, α-CH_3	H, α-CH_3
14	H	H, α-CH_3	CH_2
15	H	CH_2	H, β-CH_3
16	H	CH_2	CH_2





17 R = CH_2
18 R = H, α-CH_3

Figure S2. HR-ESIMS spectrum of Pertyolide A (**1**)



m/z	Ion	Formula	Abundance				
315.1579	(M+Na)+	C17 H24 Na O4	46876.6				
Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass
TRUE	C17 H24 O4	C17 H24 Na O4	315.1567	86.39		292.1686	292.1675

Figure S3. IR spectrum of Pertyolide A (**1**)

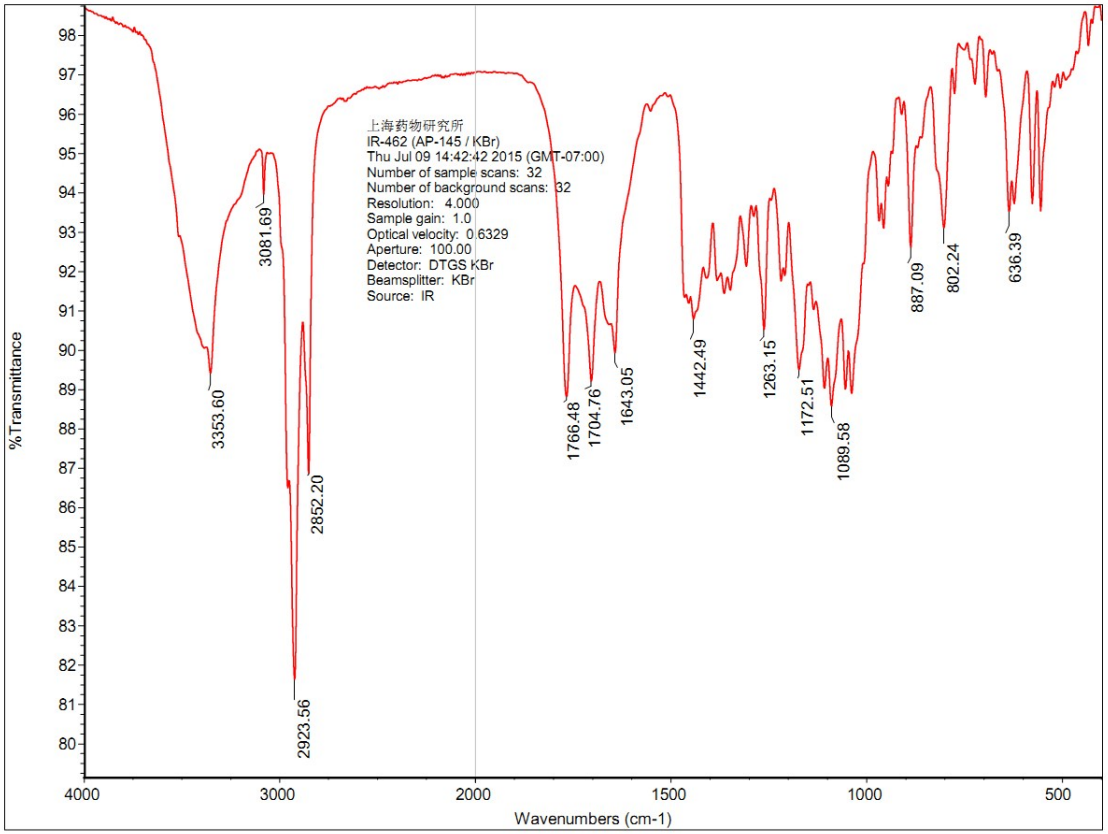


Figure S4. OR Value of Pertyolide A (**1**) in CH_3OH

Rudolph Research Analytical

Tuesday, 03/24/2015

This sample was measured on an Autopol VI, serial number 90079,
manufactured by Rudolph Research Analytical,Hackettstown,NJ.

LotID : 084/MeOH
Set Temperature : 20.0
Temp Corr : OFF

n	Average	Std.Dev.	Maximum	Minimum
6	42.000	0.0000	42.000	42.000

S.No	Sample ID	Time	Result	Scale	OR ° Arc	WLG	Lg.mm	Conc.	Temp.	Comment
1	AP-145	02:42:49 PM	42.000	SR	0.042	589	100.00	0.100	20.2	
2	AP-145	02:42:55 PM	42.000	SR	0.042	589	100.00	0.100	20.2	
3	AP-145	02:43:01 PM	42.000	SR	0.042	589	100.00	0.100	20.2	
4	AP-145	02:43:07 PM	42.000	SR	0.042	589	100.00	0.100	20.2	
5	AP-145	02:43:13 PM	42.000	SR	0.042	589	100.00	0.100	20.2	
6	AP-145	02:43:19 PM	42.000	SR	0.042	589	100.00	0.100	20.1	

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Signature

Figure S5. UV spectrum of Pertyolide A (**1**) in CH_3OH

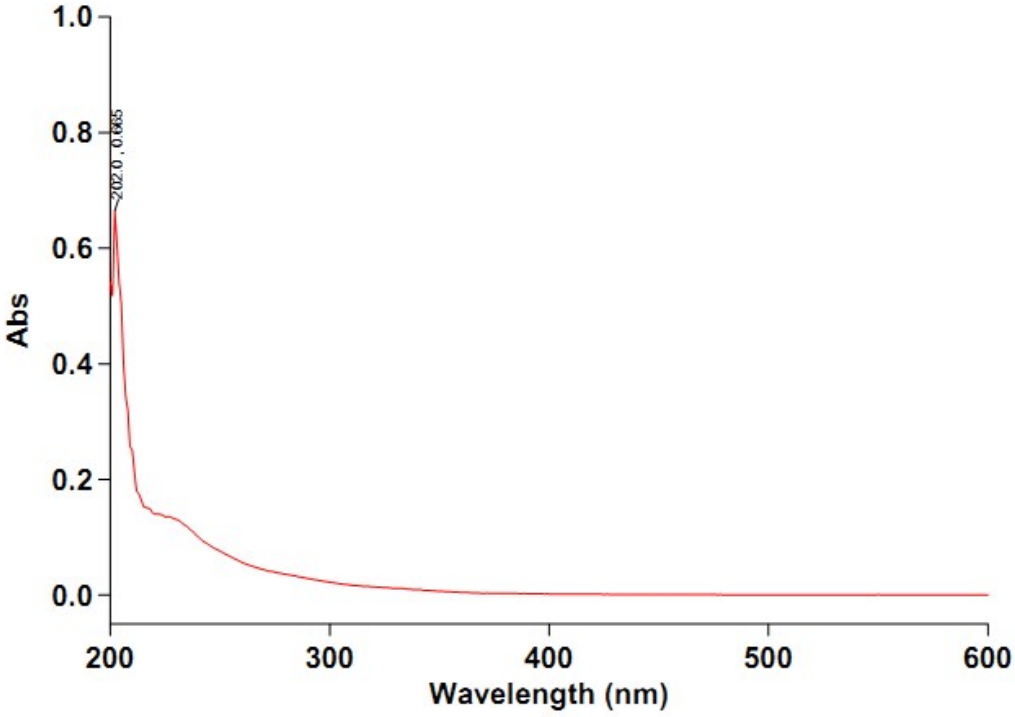
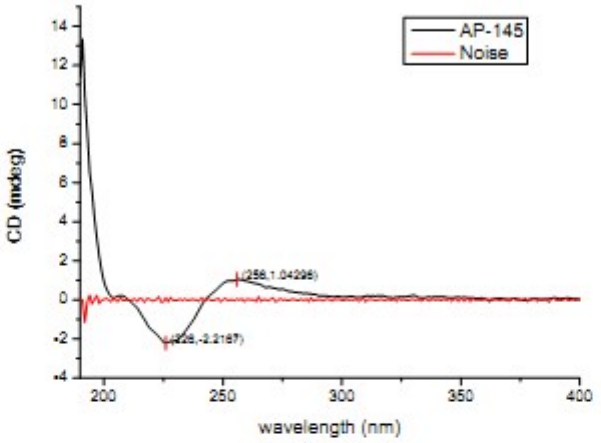


Figure S6. CD spectrum of Pertyolide A (**1**) in CH_3OH



Date	2015/3/25
Instrument	Chirascan
Serial No.	CS 30049
Detector	Photomultiplier Tube (PMT)
Lamp	150 watt xenon arc
Bandwidth(nm)	1.0
Wavelength range(nm)	400 to 190
Time per step (seconds)	0.5
Wavelength step (nm)	1.0
Cell pathlength(mm)	0.5
Concentration (mg/mL)	0.5
Data manipulation	MeOH subtraction
Temperature (℃)	Room temperature
Number of spectra averaged	Sample 3
Smoothing	3 point Savitsky-Golay
Sample Name	AP-145
Operator	Lin
Comment	

Signature

Figure S7. ^{1}H- NMR spectrum of Pertyolide A (**1**) in $CDCl_3$

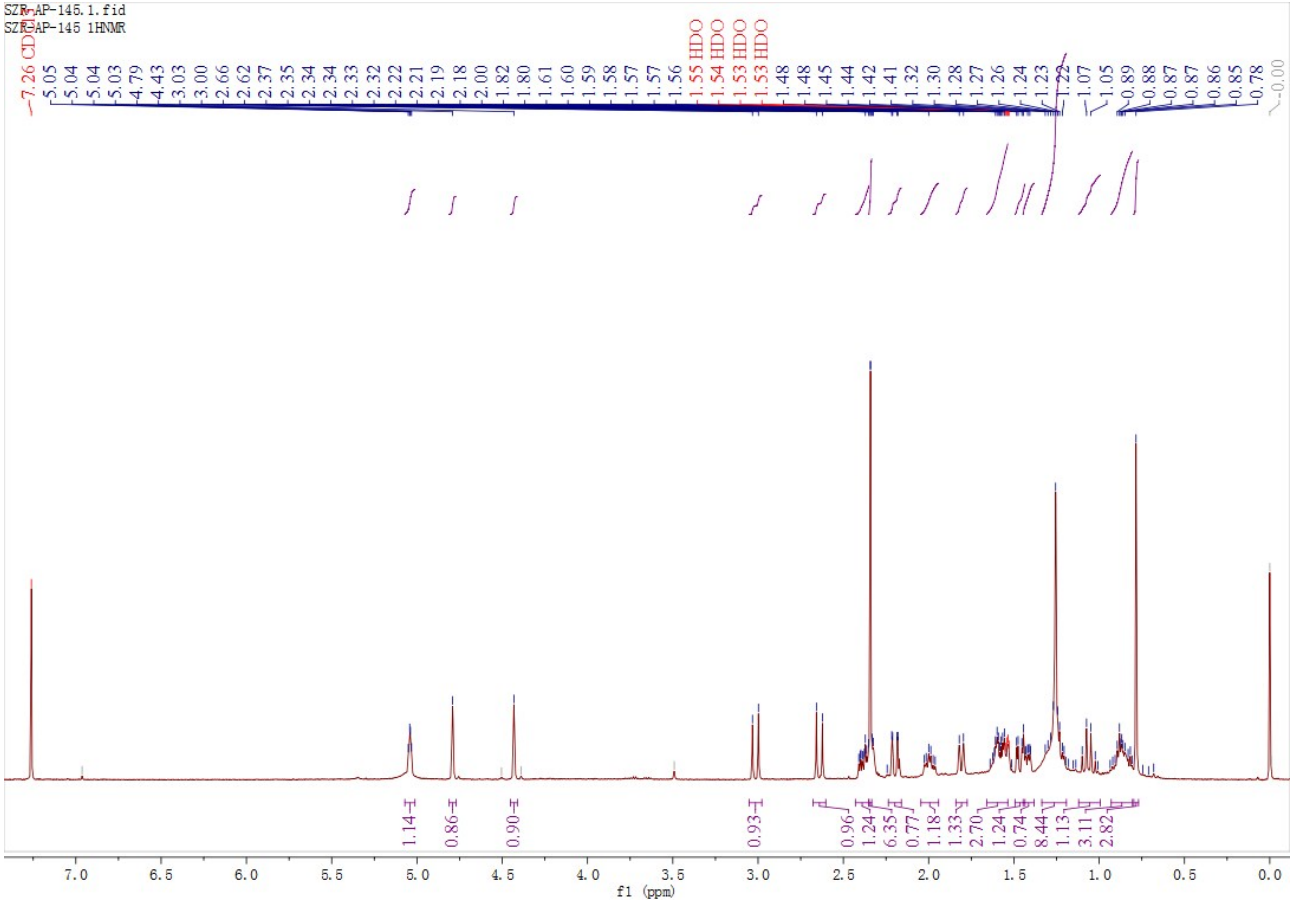


Figure S8. ^{13}C and DEPT-135 NMR spectrum of Pertyolide A (**1**) in $CDCl_3$

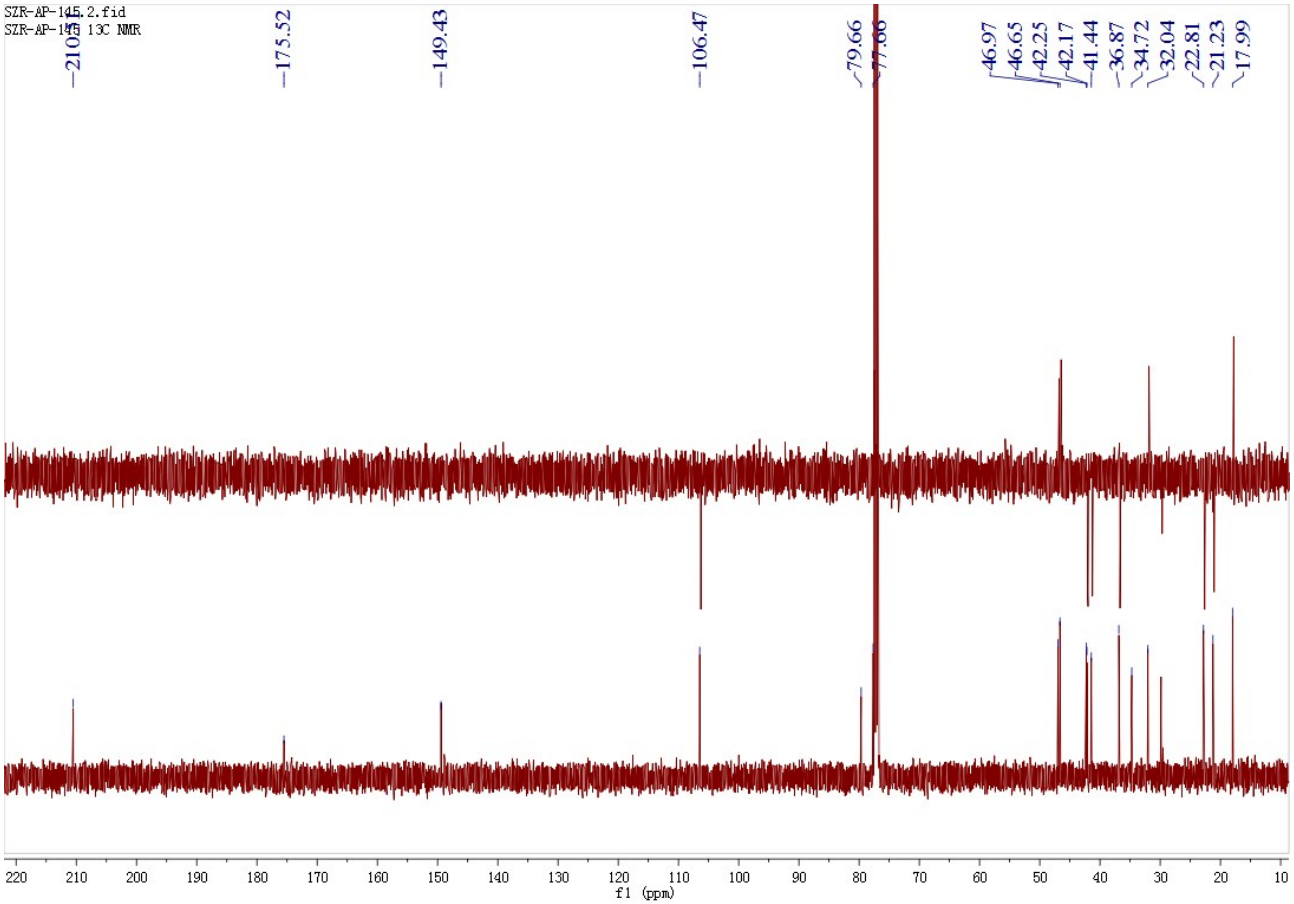


Figure S9. ^{1}H-^{1}H COSY spectrum of Pertyolide A (**1**) in $CDCl_3$

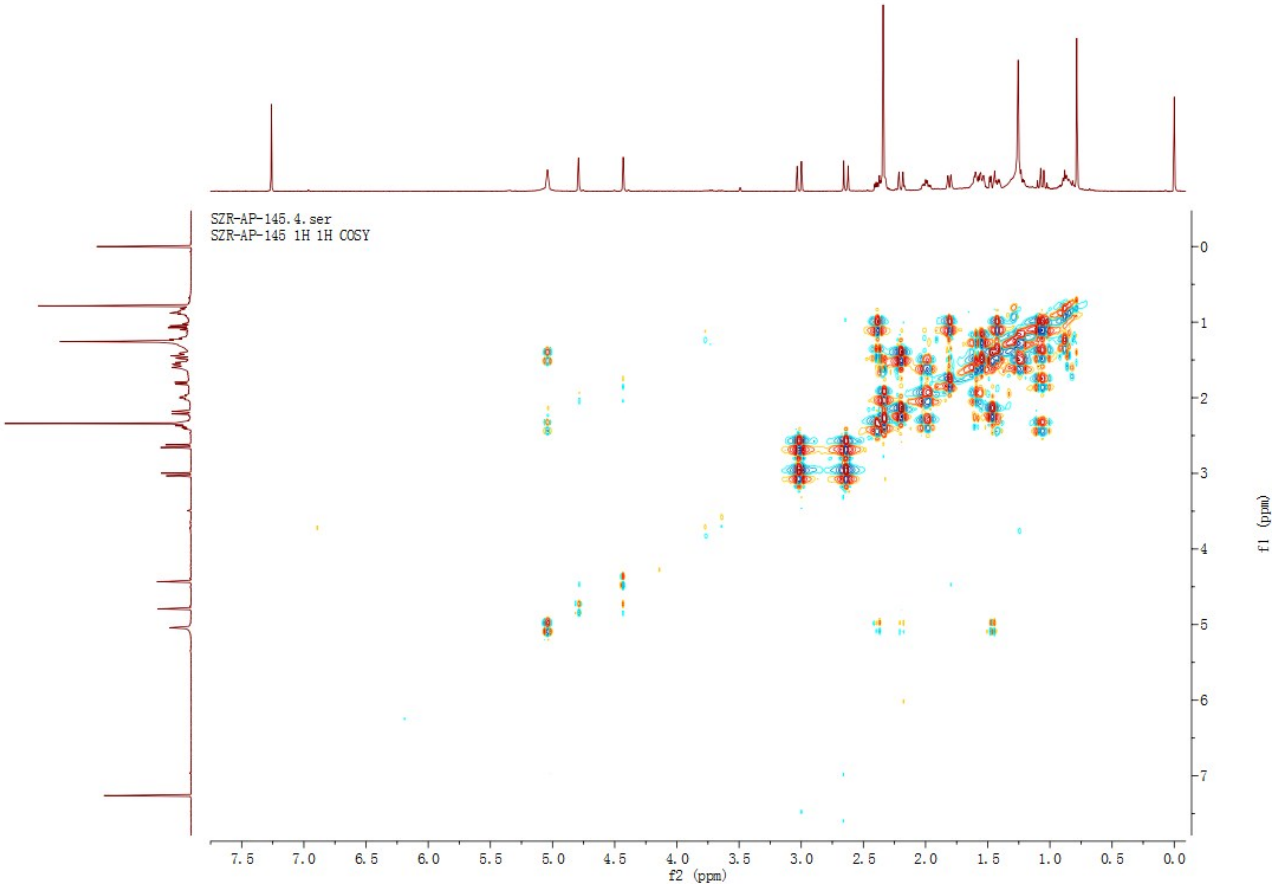


Figure S10. HSQC spectrum of Pertyolide A (**1**) in $CDCl_3$

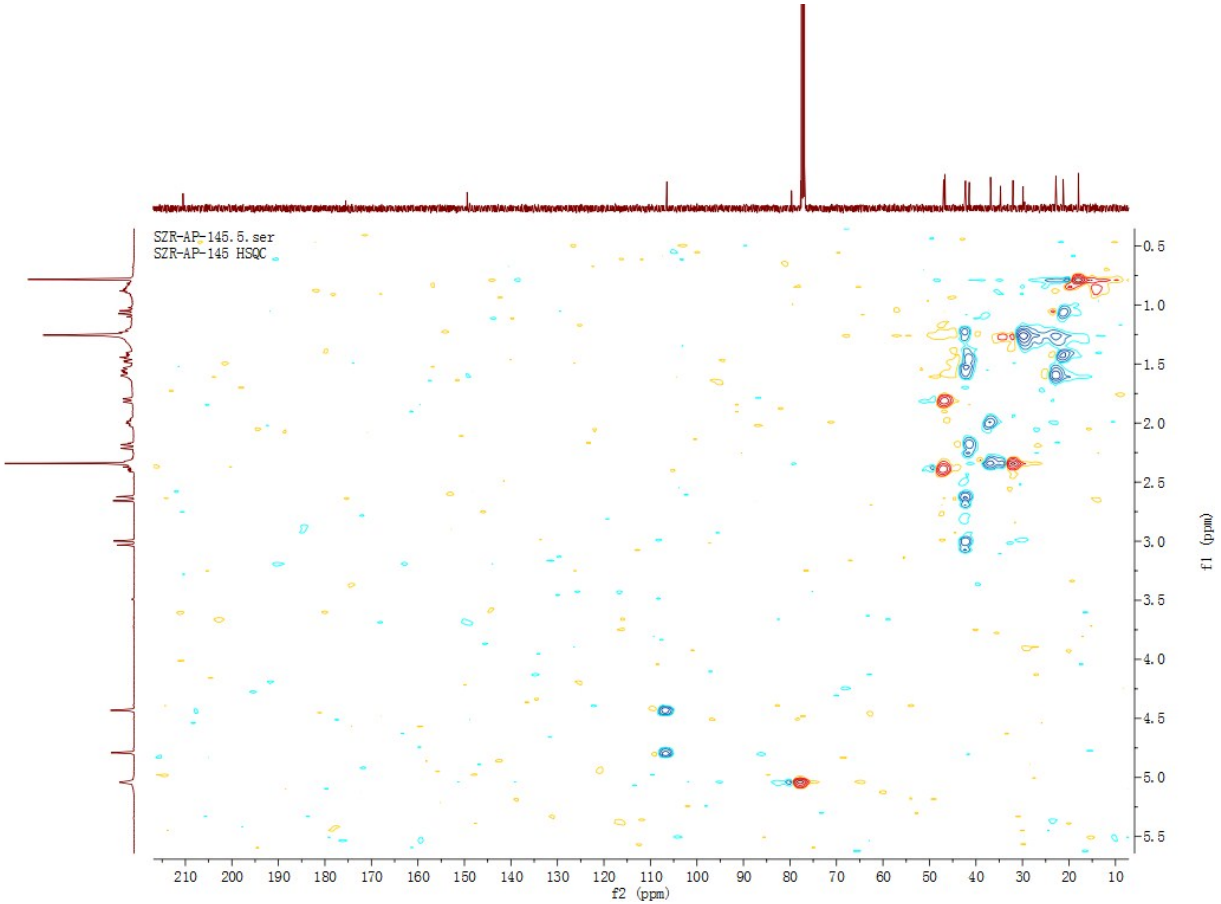


Figure S11. HMBC spectrum of Pertyolide A (**1**) in $CDCl_3$

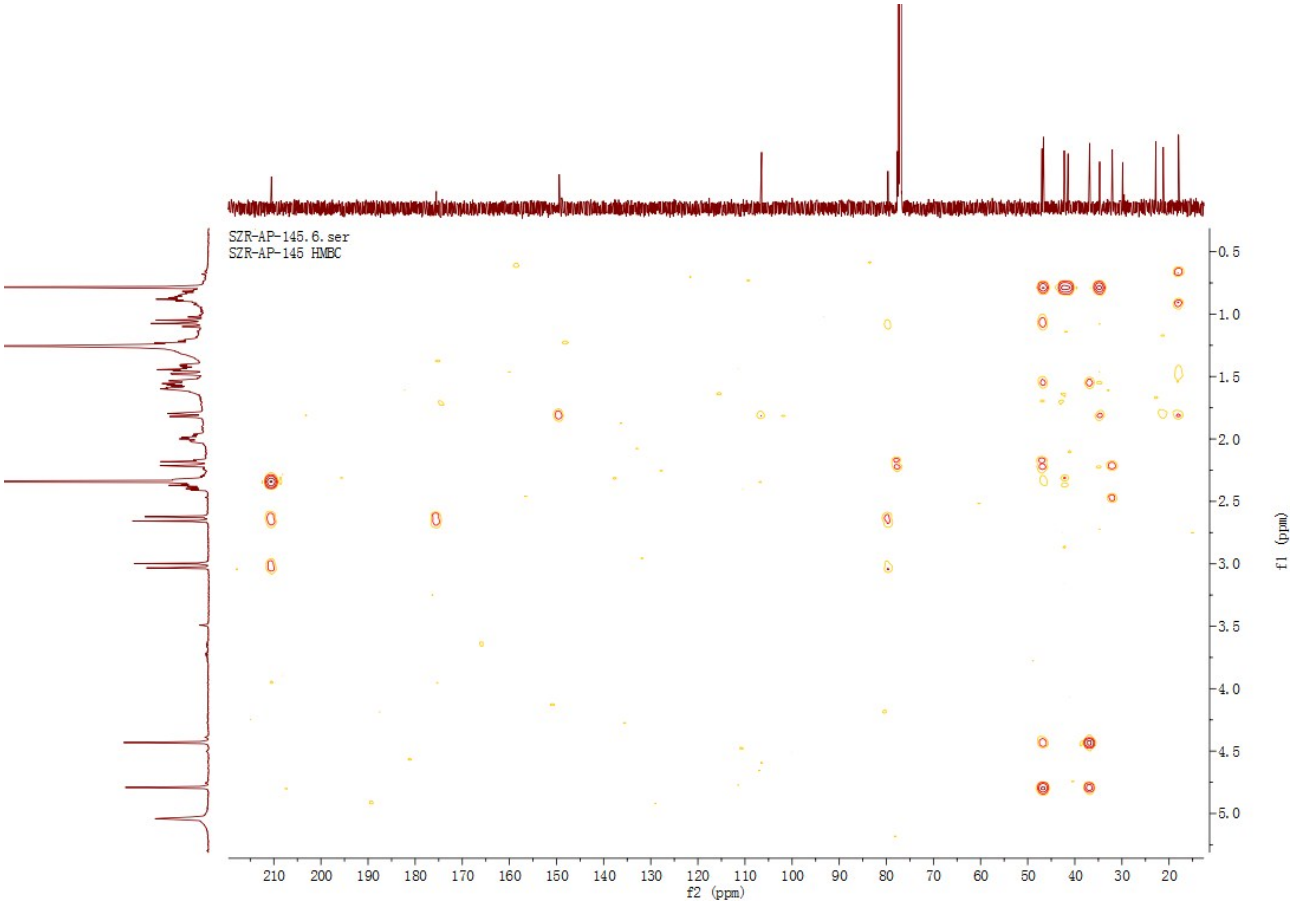


Figure S12. NOESY spectrum of Pertyolide A (**1**) in $CDCl_3$

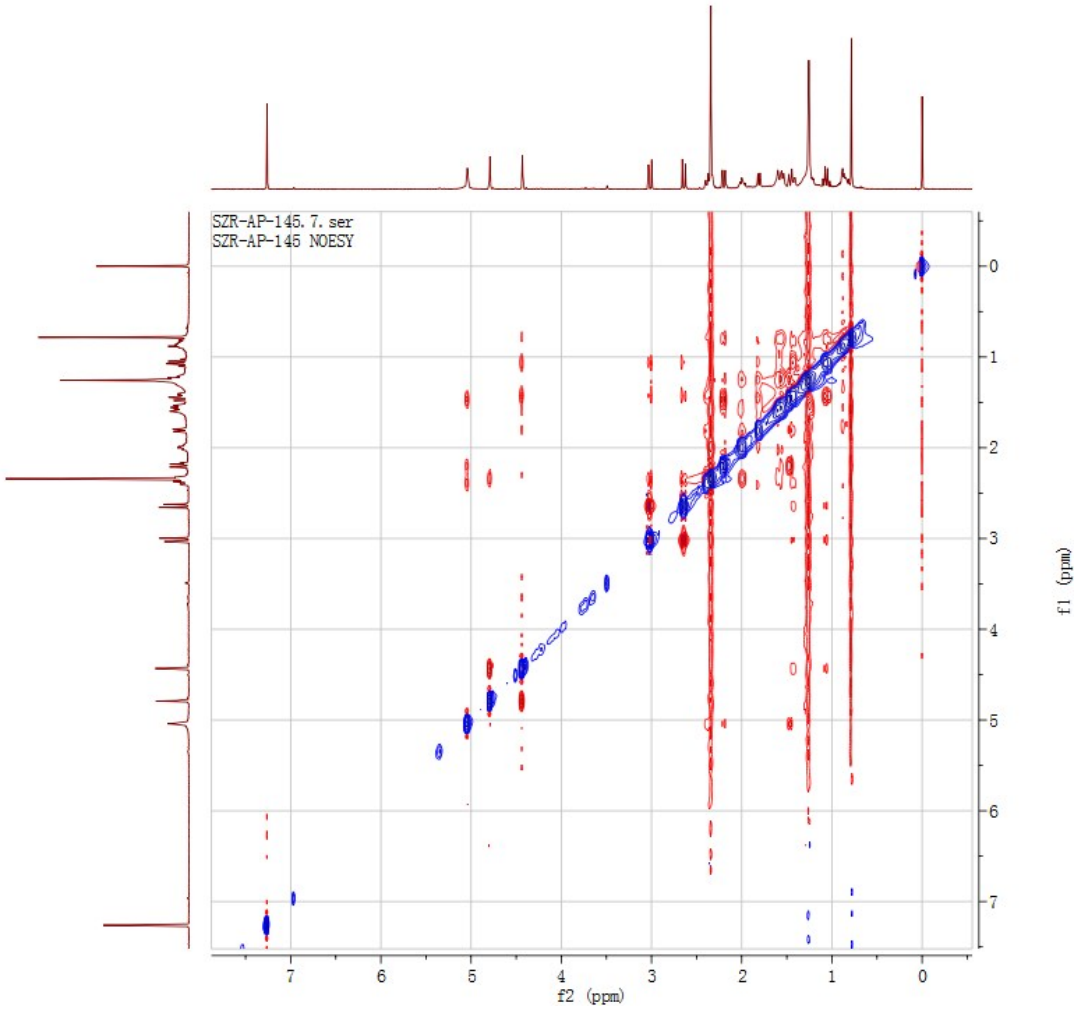
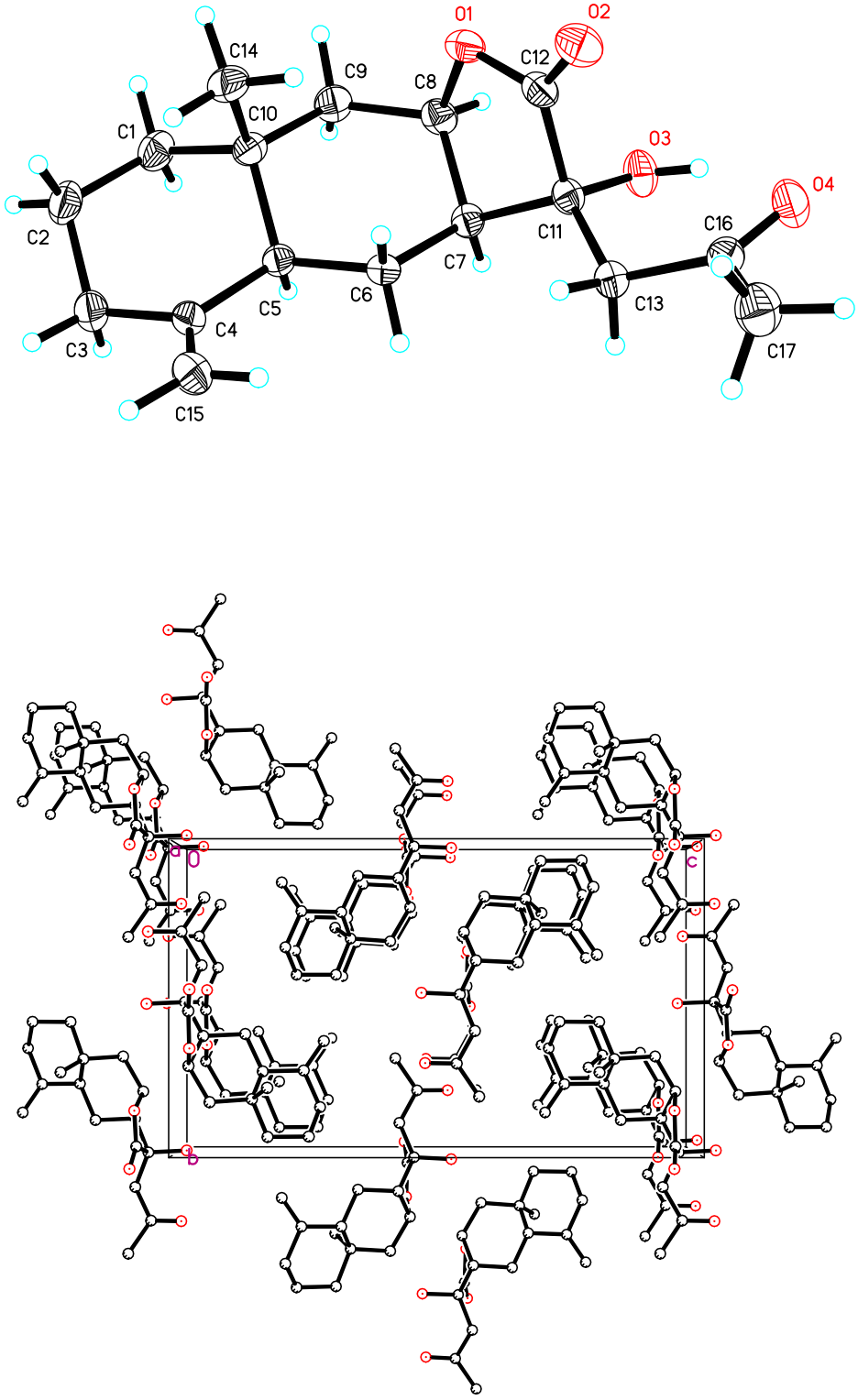


Figure S13. Single X-ray crystal structure and Packing diagram of **1**

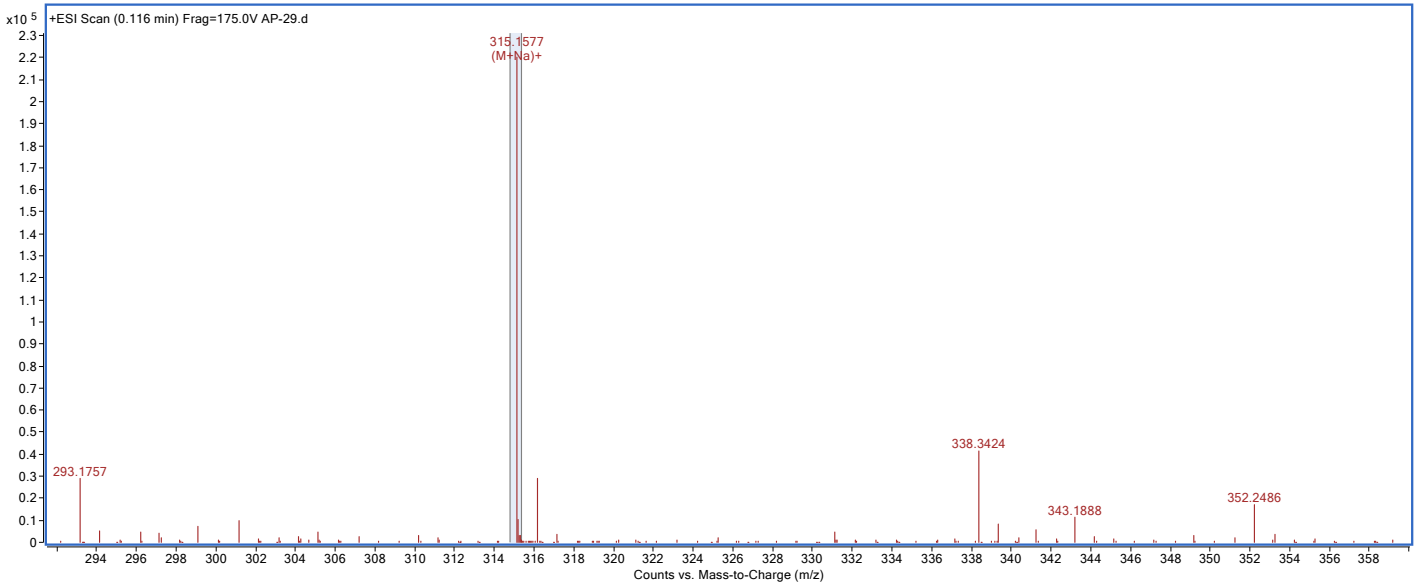


Crystallographic data of Pertyolide A (1)

Table 1. Crystal data and structure refinement for cu_dm15471_0m.

Identification code	cu_dm15471_0m	
Empirical formula	C17 H24 O4	
Formula weight	292.36	
Temperature	296.15 K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 6.38940(10) Å	α= 90°.
	b = 11.8924(2) Å	β= 90°.
	c = 19.9718(4) Å	γ = 90°.
Volume	1517.56(5) $Å^3$	
Z	4	
Density (calculated)	1.280 Mg/m^3	
Absorption coefficient	0.727 mm^{-1}	
F(000)	632	
Crystal size	0.2 x 0.08 x 0.05 mm^3	
Theta range for data collection	4.327 to 70.024°.	
Index ranges	-7<=h<=6, -14<=k<=14, -23<=l<=24	
Reflections collected	9899	
Independent reflections	2731 [R(int) = 0.0285]	
Completeness to theta = 67.679°	97.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7533 and 0.6447	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2731 / 0 / 193	
Goodness-of-fit on F^2	1.060	
Final R indices [I>2sigma(I)]	R1 = 0.0332, wR2 = 0.0881	
R indices (all data)	R1 = 0.0341, wR2 = 0.0896	
Absolute structure parameter	0.02(7)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.147 and -0.174 $e.Å^{-3}$	

Figure S14. HR-ESIMS spectrum of Pertyolide B (**2**)



m/z	Ion	Formula	Abundance
315.1577	(M+Na)+	C17 H24 Na O4	220045.8

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass
TRUE	C17 H24 O4	C17 H24 Na O4	315.1567	89.79		292.1684	292.1675

Figure S15. IR spectrum of Pertyolide B (**2**)

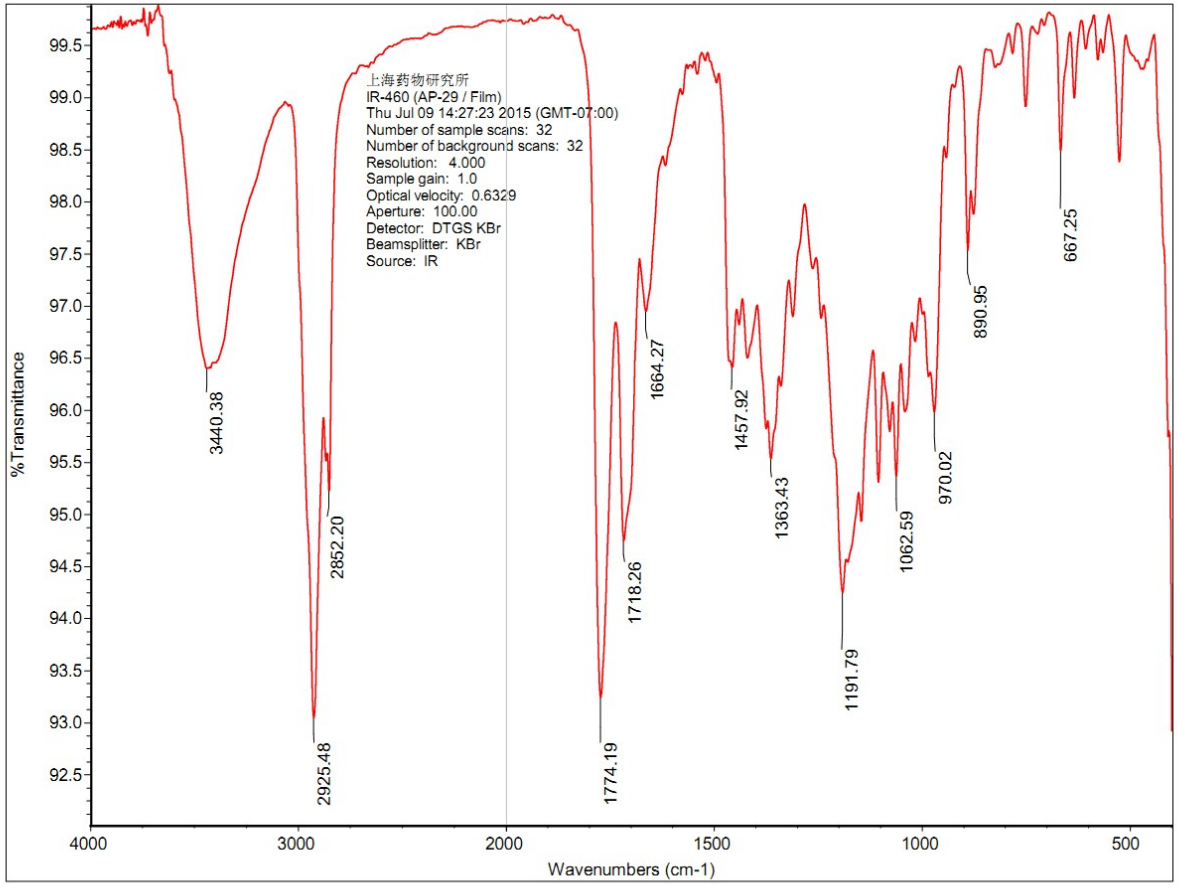


Figure S16. OR Value of Pertyolide B (**2**) in CH_3OH

Rudolph Research Analytical

Tuesday, 03/24/2015

This sample was measured on an Autopol VI, serial number 90079,
manufactured by Rudolph Research Analytical,Hackettstown,NJ.

LotID : 085/MeOH
Set Temperature : 20.0
Temp Corr : OFF

n	Average	Std.Dev.	Maximum	Minimum
6	1.167	0.4082	2.000	1.000

S.No	Sample ID	Time	Result	Scale	OR ° Arc	WLG	Lg.mm	Conc.	Temp.	Comment
1	AP-29	02:48:22 PM	1.000	SR	0.001	589	100.00	0.100	20.0	
2	AP-29	02:48:28 PM	1.000	SR	0.001	589	100.00	0.100	20.0	
3	AP-29	02:48:34 PM	1.000	SR	0.001	589	100.00	0.100	20.0	
4	AP-29	02:48:40 PM	2.000	SR	0.002	589	100.00	0.100	20.0	
5	AP-29	02:48:46 PM	1.000	SR	0.001	589	100.00	0.100	20.0	
6	AP-29	02:48:52 PM	1.000	SR	0.001	589	100.00	0.100	20.0	

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Signature

Figure S17. UV spectrum of Pertyolide B (**2**) in CH_3OH

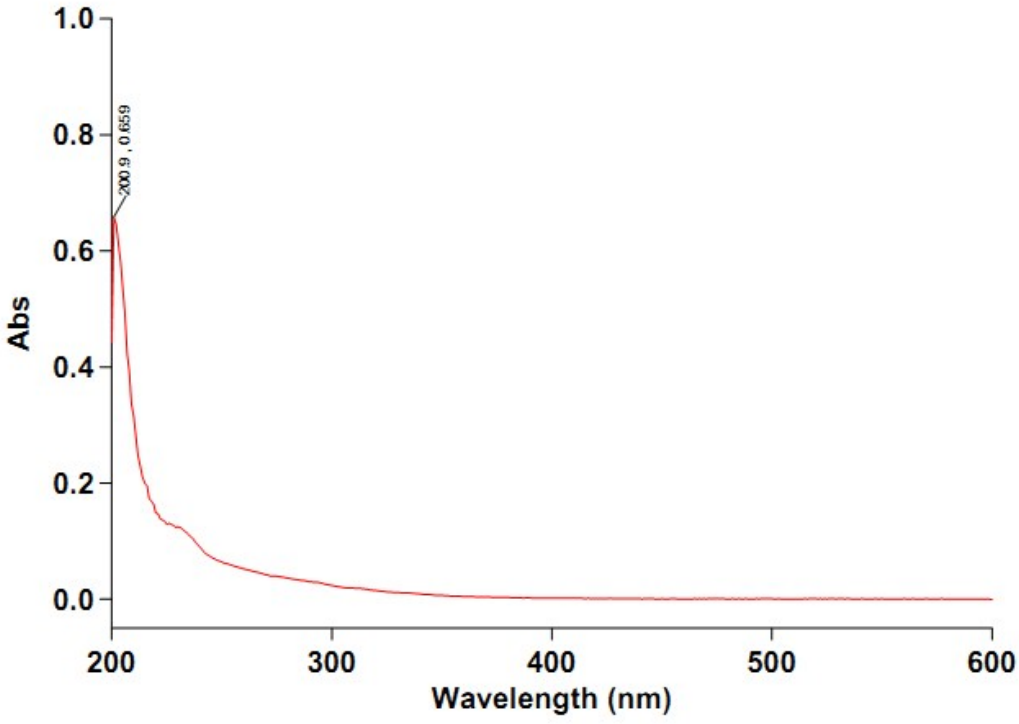
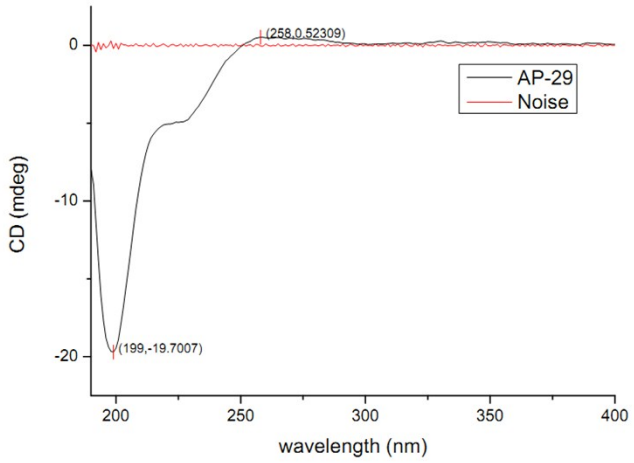


Figure S18. CD spectrum of Pertyolide B (**2**) in CH_3OH

中国科学院上海药物研究所药物质量控制与固体化学研究中心



Date	2015/3/25
Instrument	Chirascan
Serial No.	CS 30049
Detector	Photomultiplier Tube (PMT)
Lamp	150 watt xenon arc
Bandwidth(nm)	1.0
Wavelength range(nm)	400 to 190
Time per step (seconds)	0.5
Wavelength step (nm)	1.0
Cell pathlength(mm)	0.5
Concentration (mg/mL)	0.5
Data manipulation	MeOH subtraction
Temperature (℃)	Room temperature
Number of spectra averaged	Sample 3
Smoothing	3 point Savitsky-Golay
Sample Name	AP-29
Operator	Lin
Comment	

Signature

Figure S19. ^{1}H- NMR spectrum of Pertyolide B (**2**) in $CDCl_3$

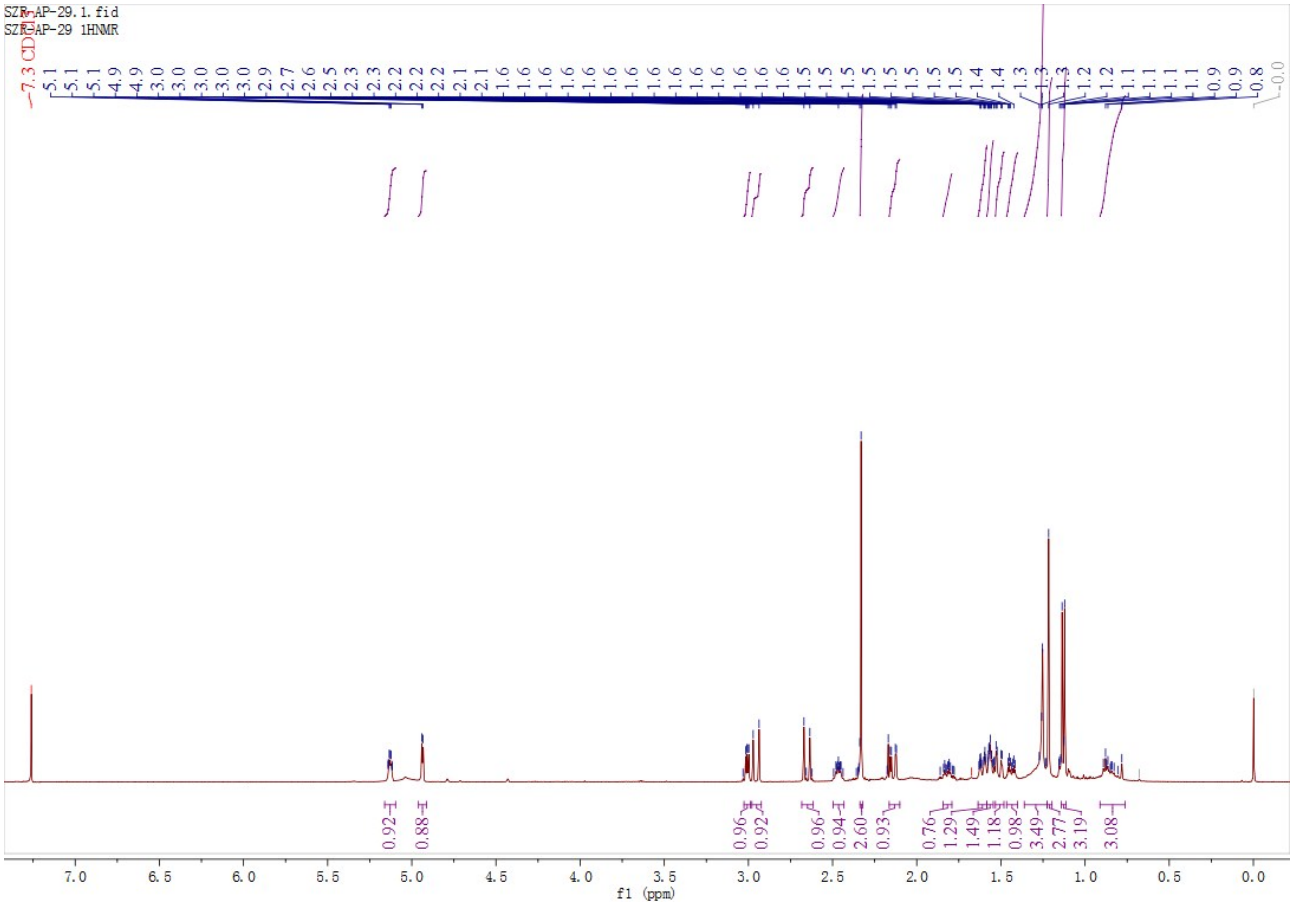


Figure S20. ^{13}C and DEPT-135 NMR spectrum of Pertyolide B (**2**) in $CDCl_3$

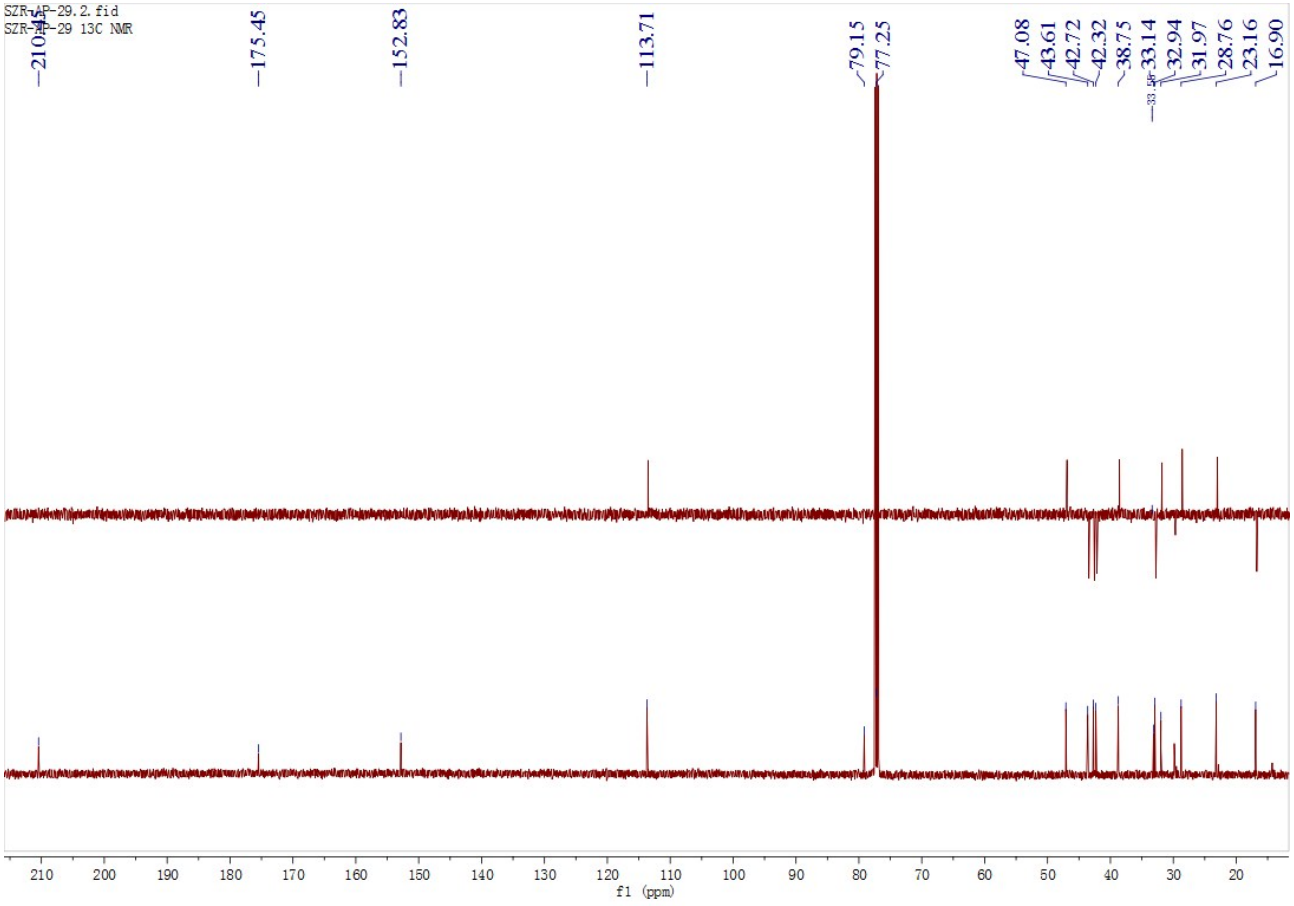


Figure S21. ^{1}H-^{1}H COSY spectrum of Pertyolide B (**2**) in $CDCl_3$

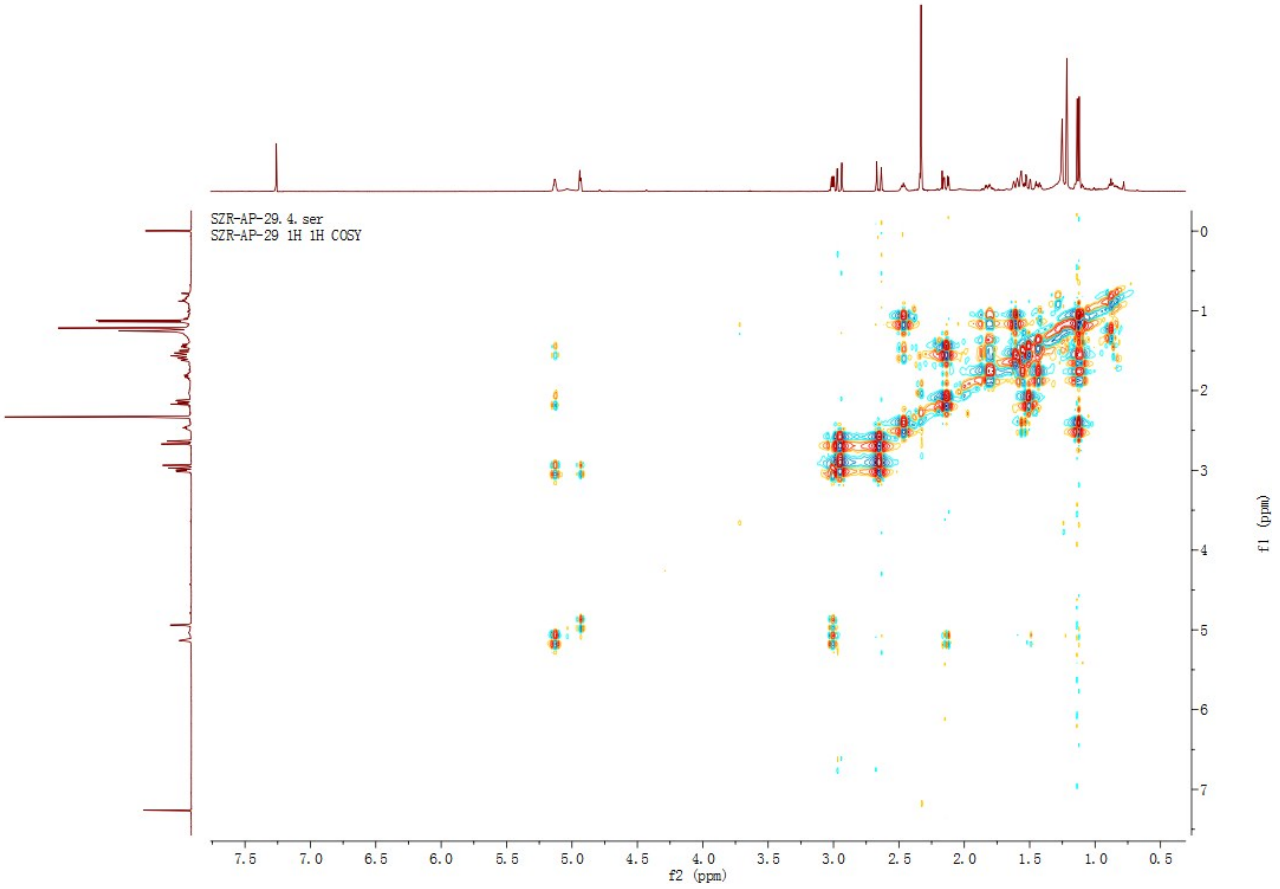


Figure S22. HSQC spectrum of Pertyolide B (**2**) in $CDCl_3$

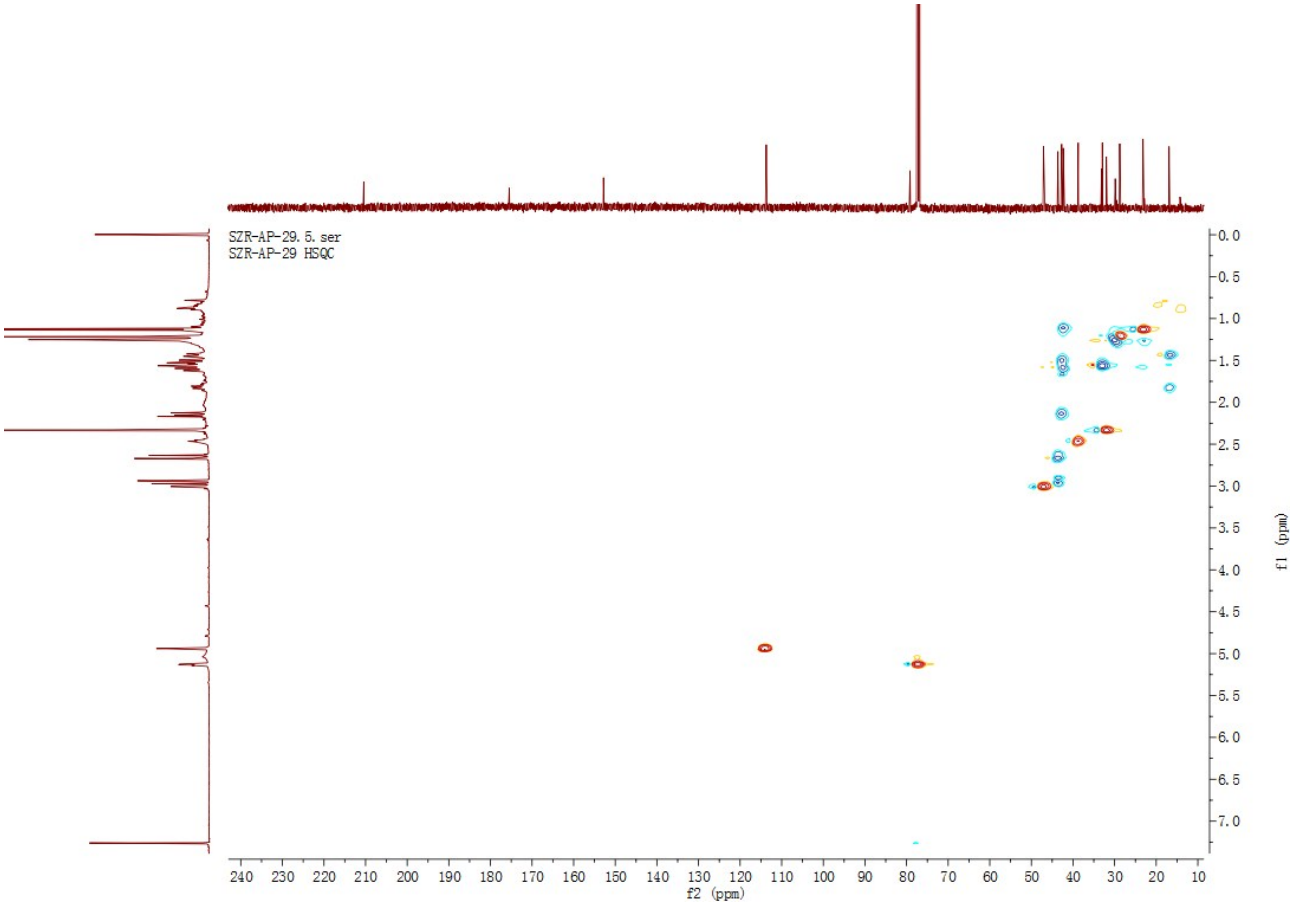


Figure S23. HMBC spectrum of Pertyolide B (**2**) in $CDCl_3$

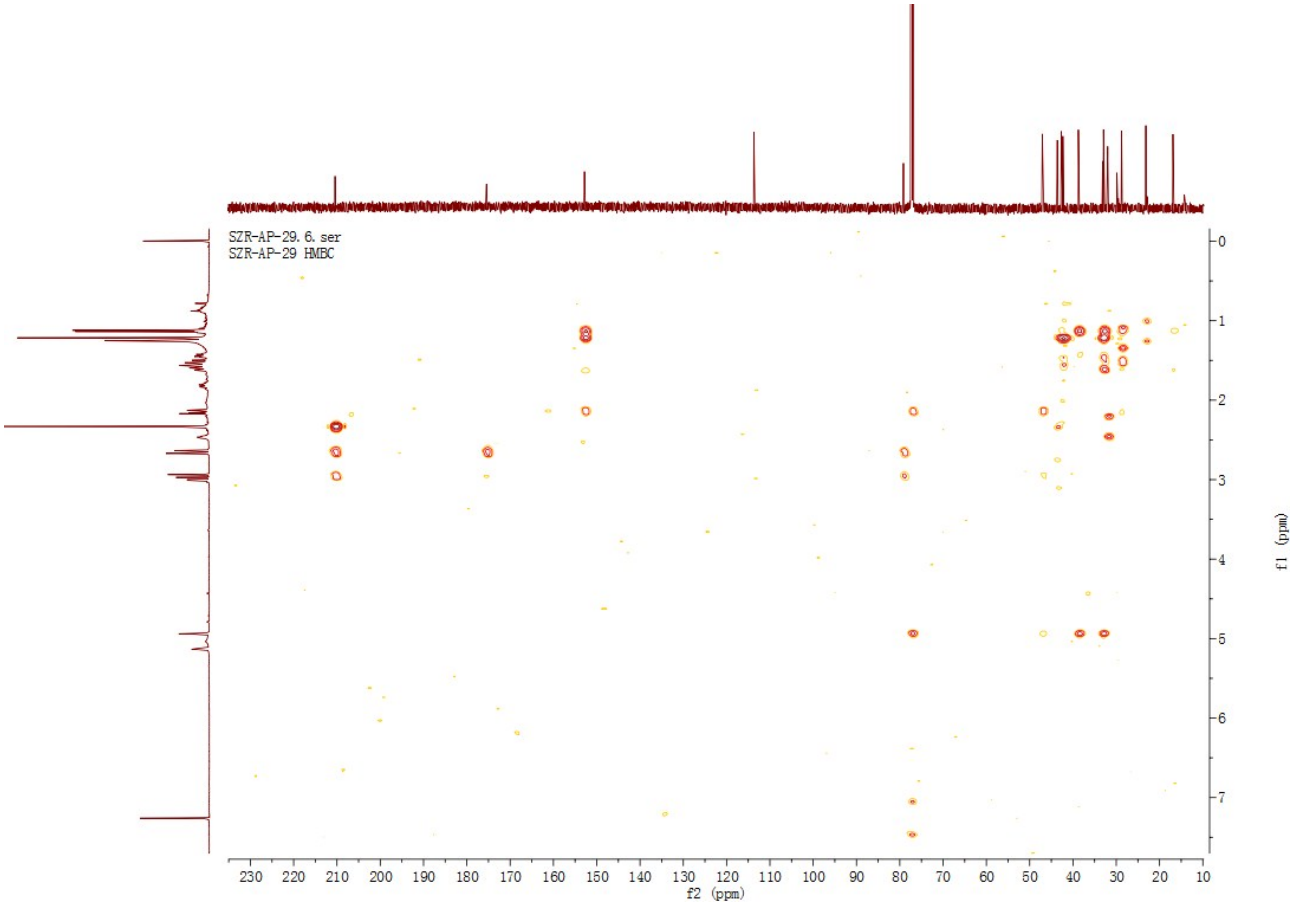


Figure S24. NOESY spectrum of Pertyolide B (**2**) in $CDCl_3$

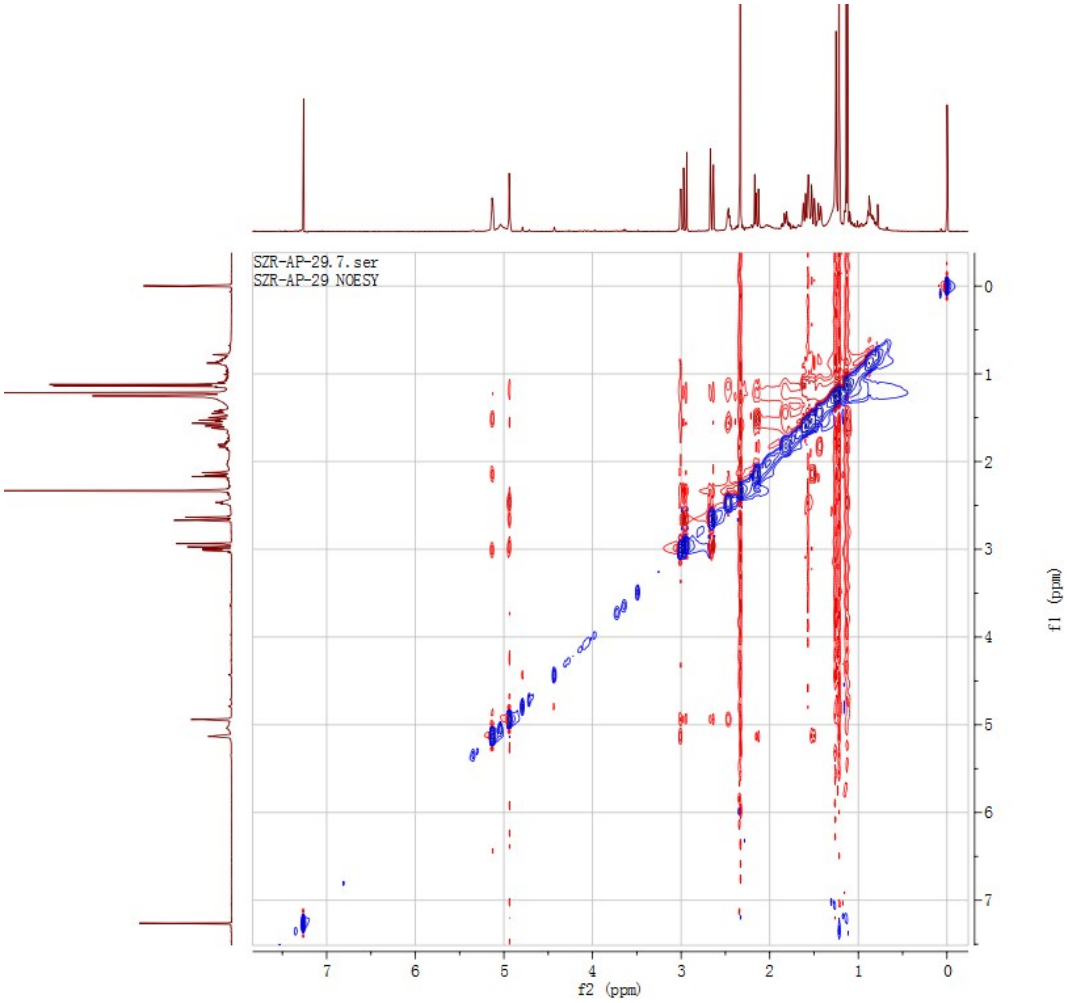
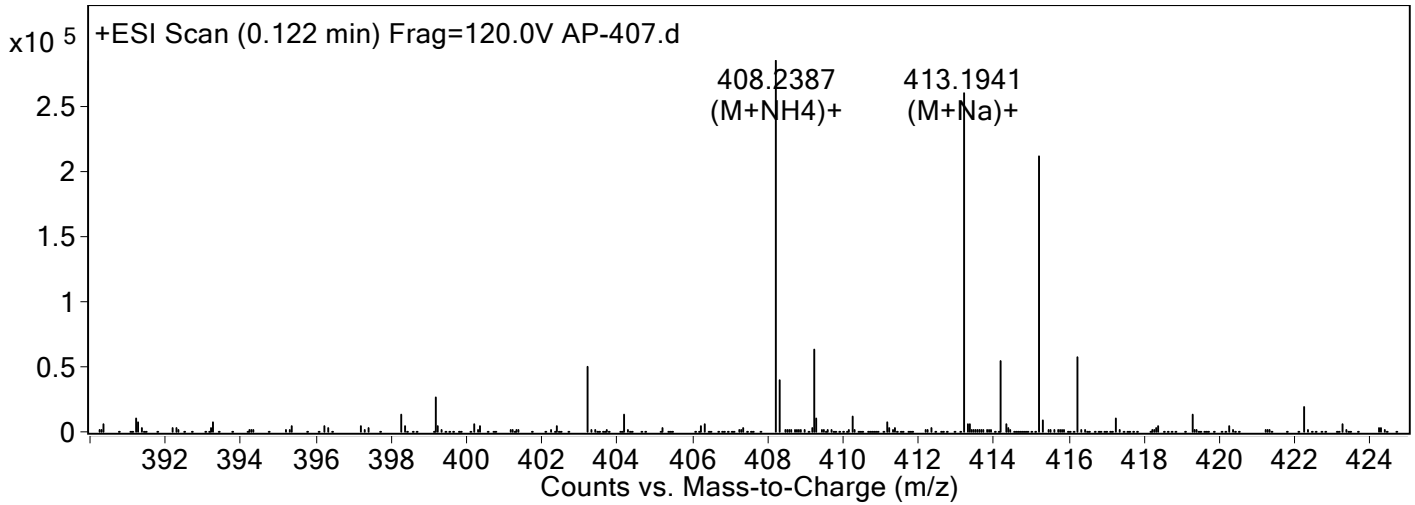


Figure S25. HR-ESIMS spectrum of Pertyolide C (**3**)



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C22 H30 O6	TRUE	390.2048	390.2042	-1.54	C22 H34 N O6	97.46
C22 H30 O6	TRUE	390.2049	390.2042	-1.74	C22 H30 Na O6	80.87

Figure S26. IR spectrum of Pertyolide C (**3**)

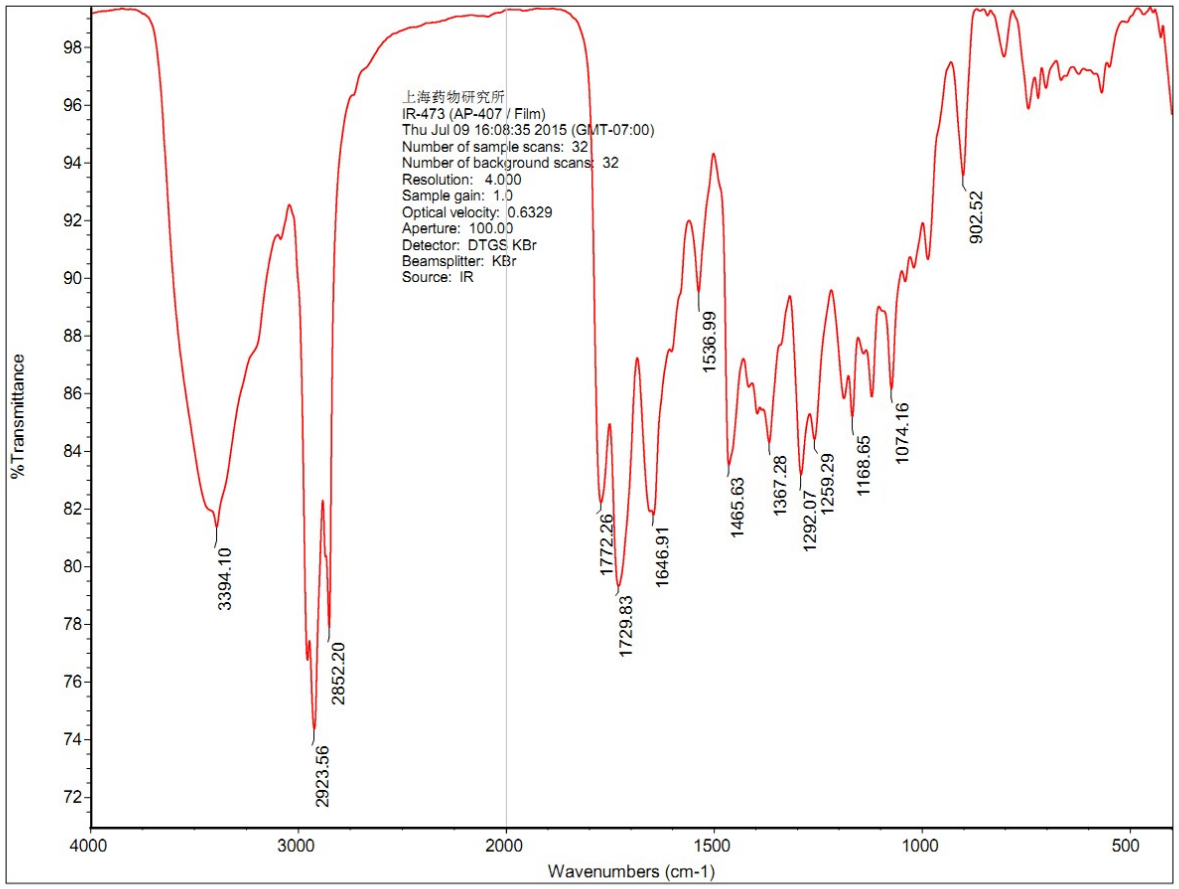


Figure S27. OR Value of Pertyolide C (**3**) in CH_3OH

Rudolph Research Analytical

Thursday, 05/14/2015

This sample was measured on an Autopol VI, serial number 90079,
manufactured by Rudolph Research Analytical,Hackettstown,NJ.

LotID : 157/MeOH
Set Temperature : 20.0
Temp Corr : OFF

n	Average	Std.Dev.	Maximum	Minimum
6	17.000	0.0000	17.000	17.000

S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG	Lg.mm	Conc.	Temp.	Comment
1	AP-407	10:15:31 AM	17.000	SR	0.017	589	100.00	0.100	20.3	
2	AP-407	10:15:37 AM	17.000	SR	0.017	589	100.00	0.100	20.2	
3	AP-407	10:15:43 AM	17.000	SR	0.017	589	100.00	0.100	20.2	
4	AP-407	10:15:49 AM	17.000	SR	0.017	589	100.00	0.100	20.2	
5	AP-407	10:15:55 AM	17.000	SR	0.017	589	100.00	0.100	20.2	
6	AP-407	10:16:01 AM	17.000	SR	0.017	589	100.00	0.100	20.2	

_____
Signature

Figure S28. UV spectrum of Pertyolide C (**3**) in CH_3OH

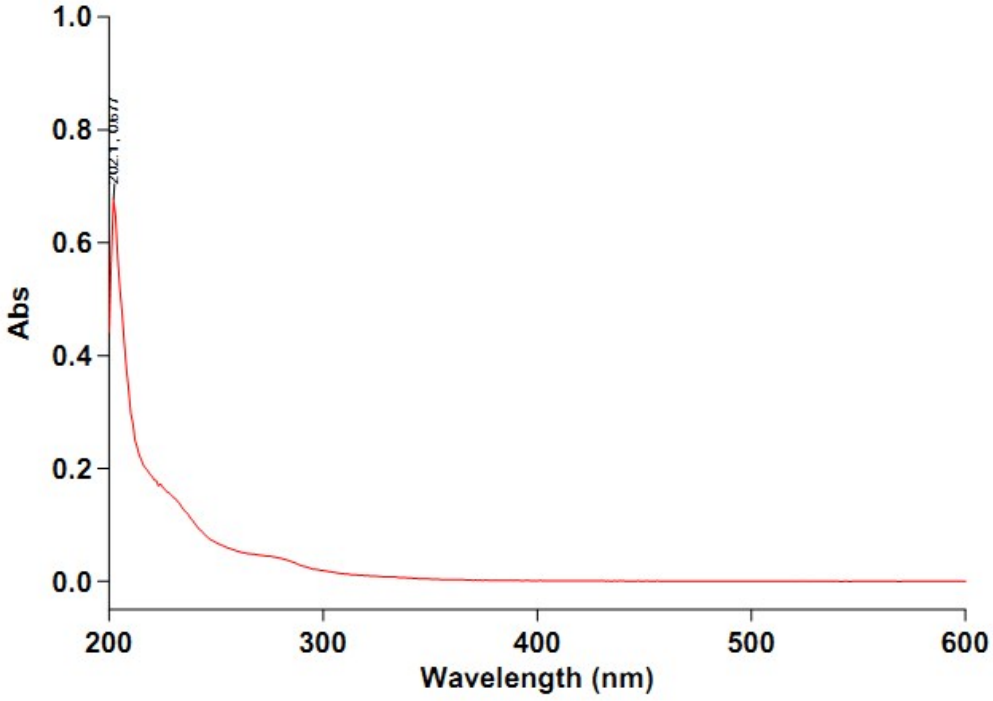


Figure S29. ^{1}H- NMR spectrum of Pertyolide C (**3**) in $CDCl_3$

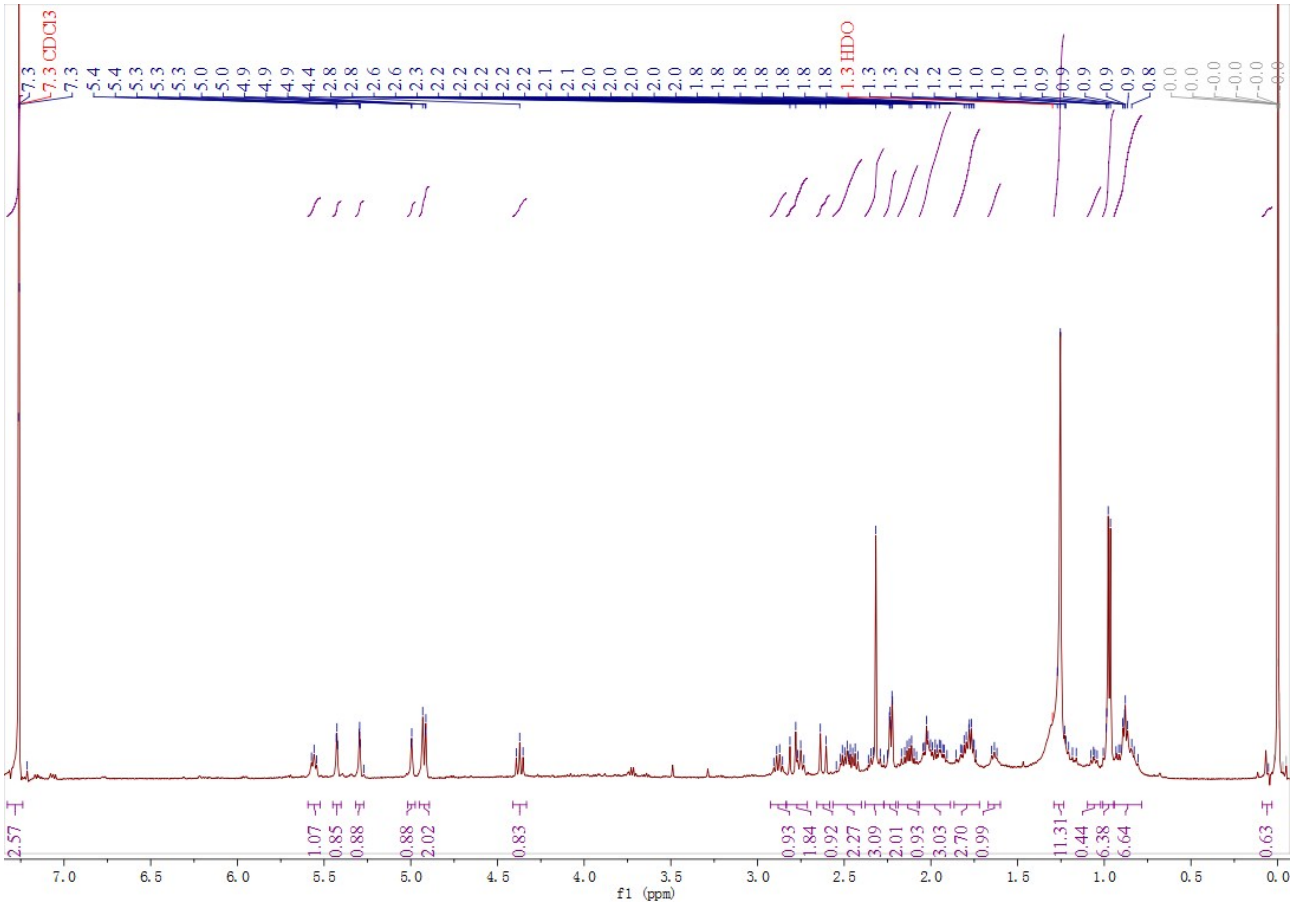


Figure S30. ^{13}C and DEPT-135 NMR spectrum of Pertyolide C (**3**) in $CDCl_3$

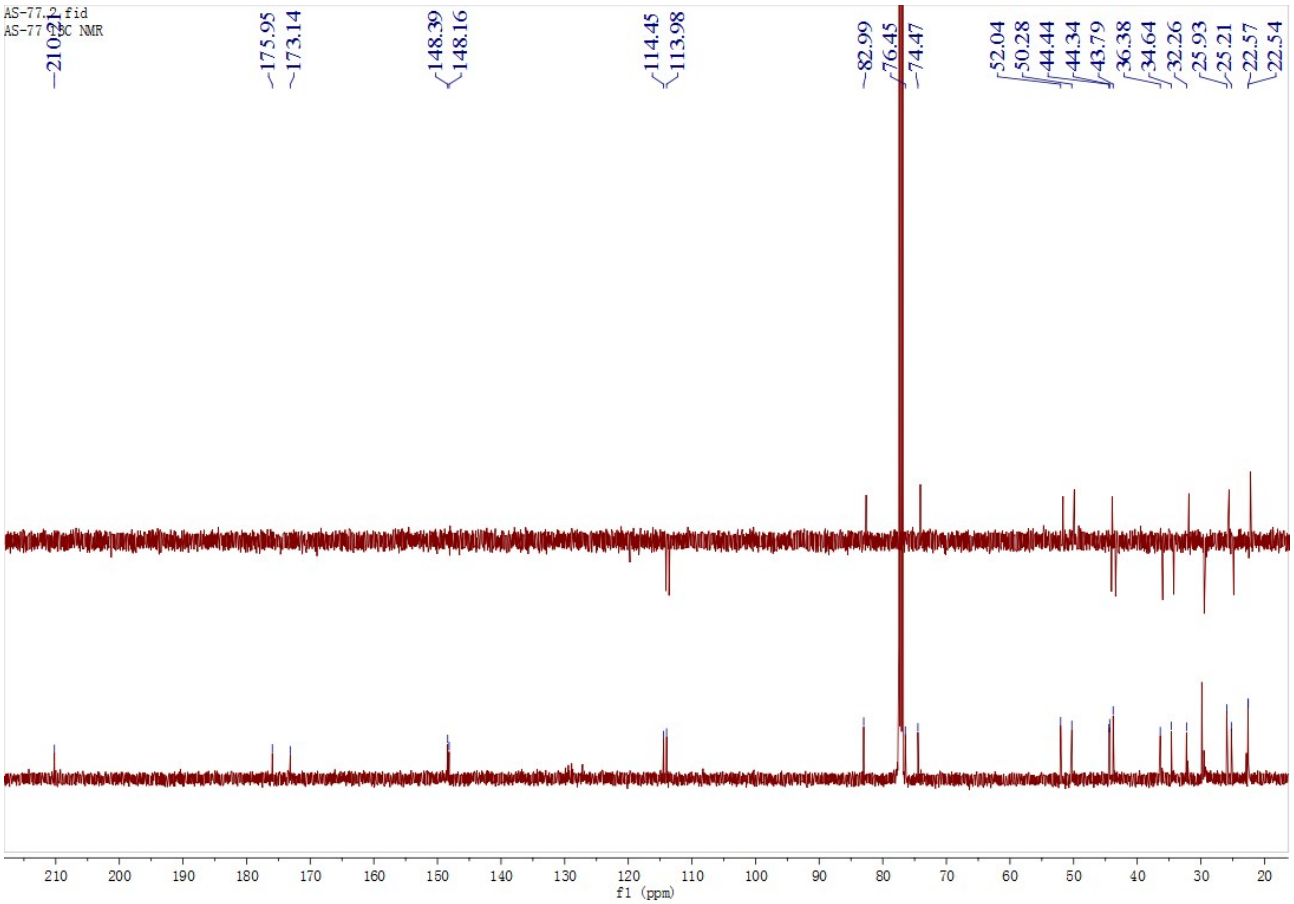


Figure S31. ^{1}H-^{1}H COSY spectrum of Pertyolide C (**3**) in $CDCl_3$

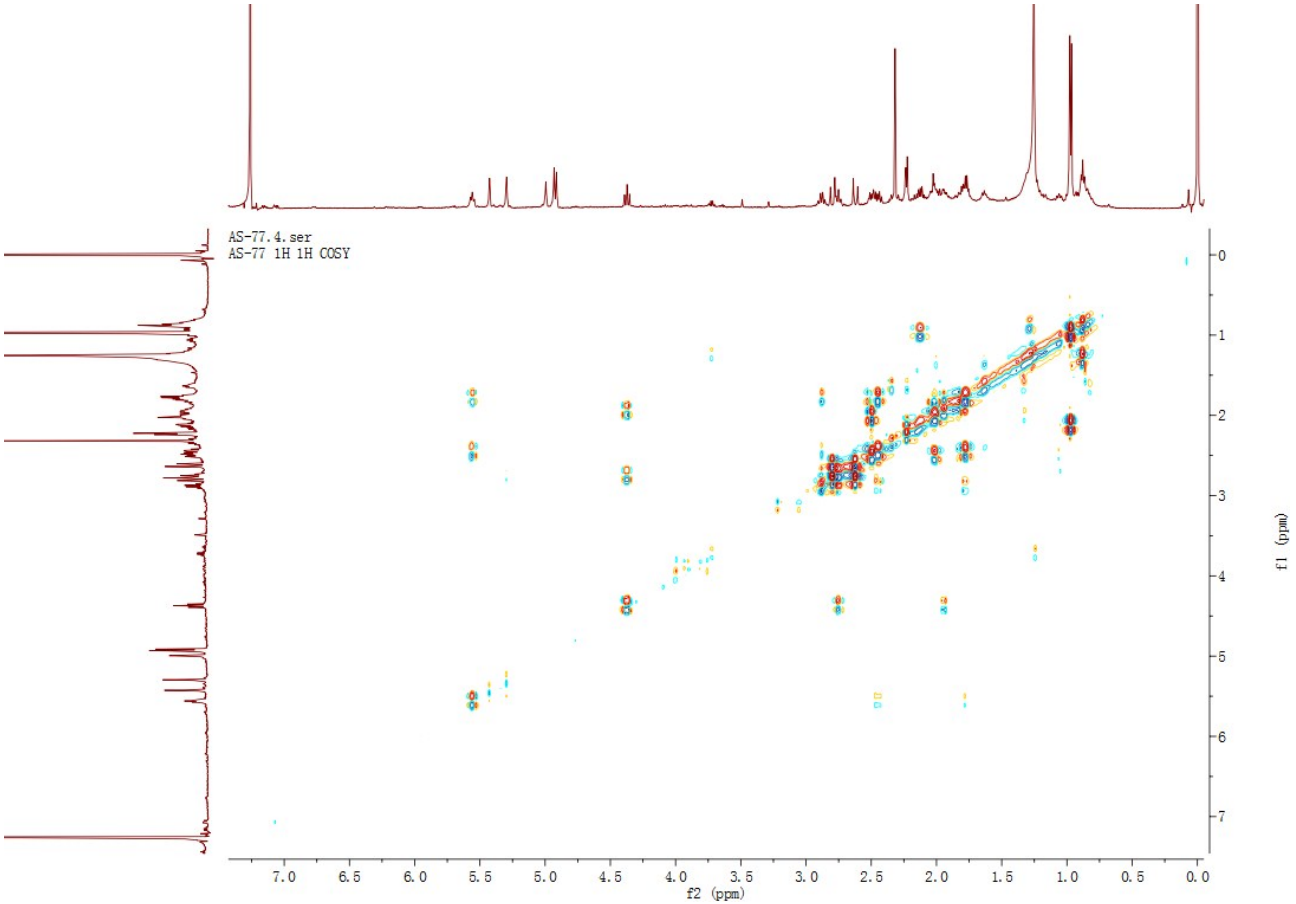


Figure S32. HSQC spectrum of Pertyolide C (**3**) in $CDCl_3$

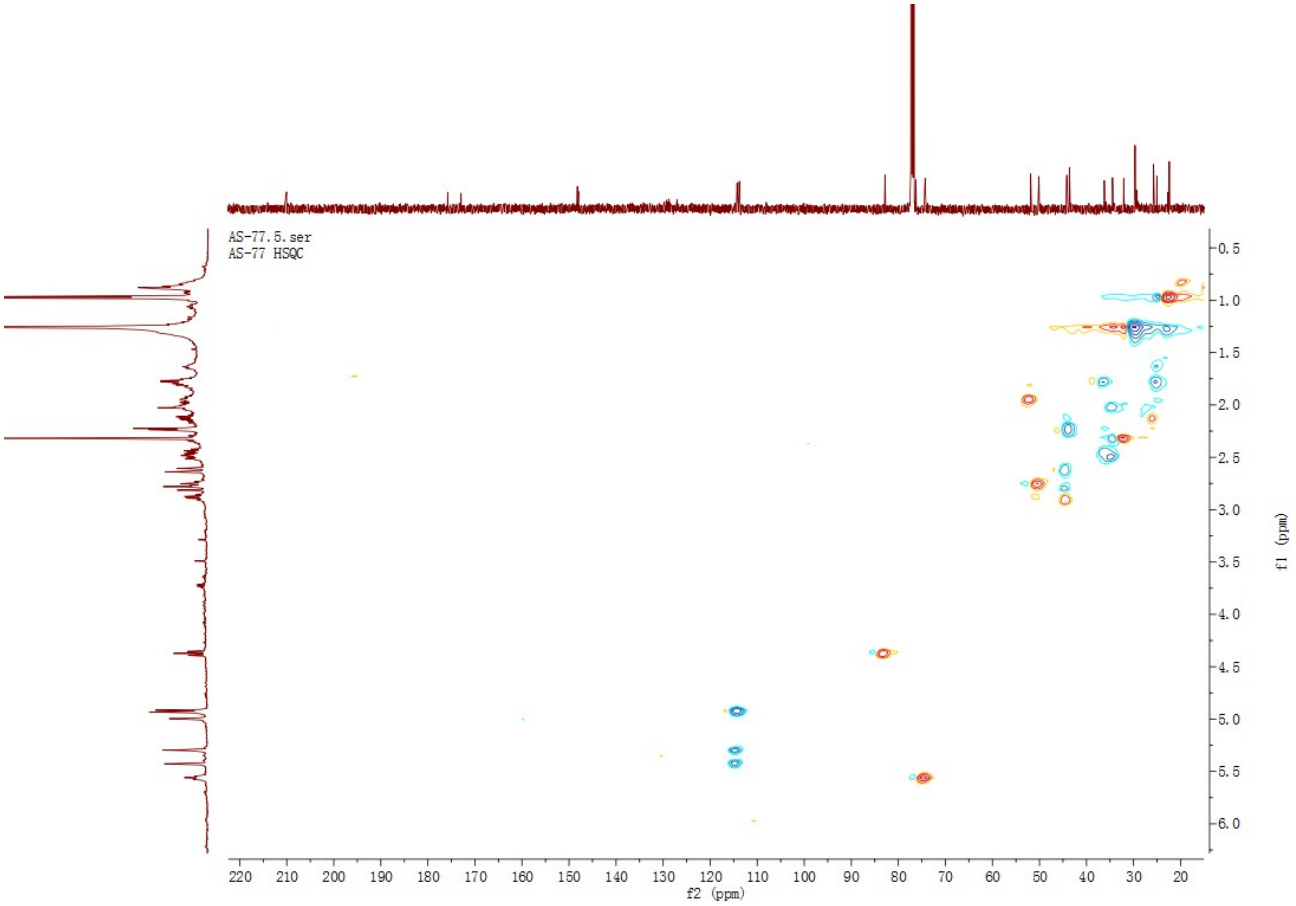


Figure S33. HMBC spectrum of Pertyolide C (**3**) in $CDCl_3$

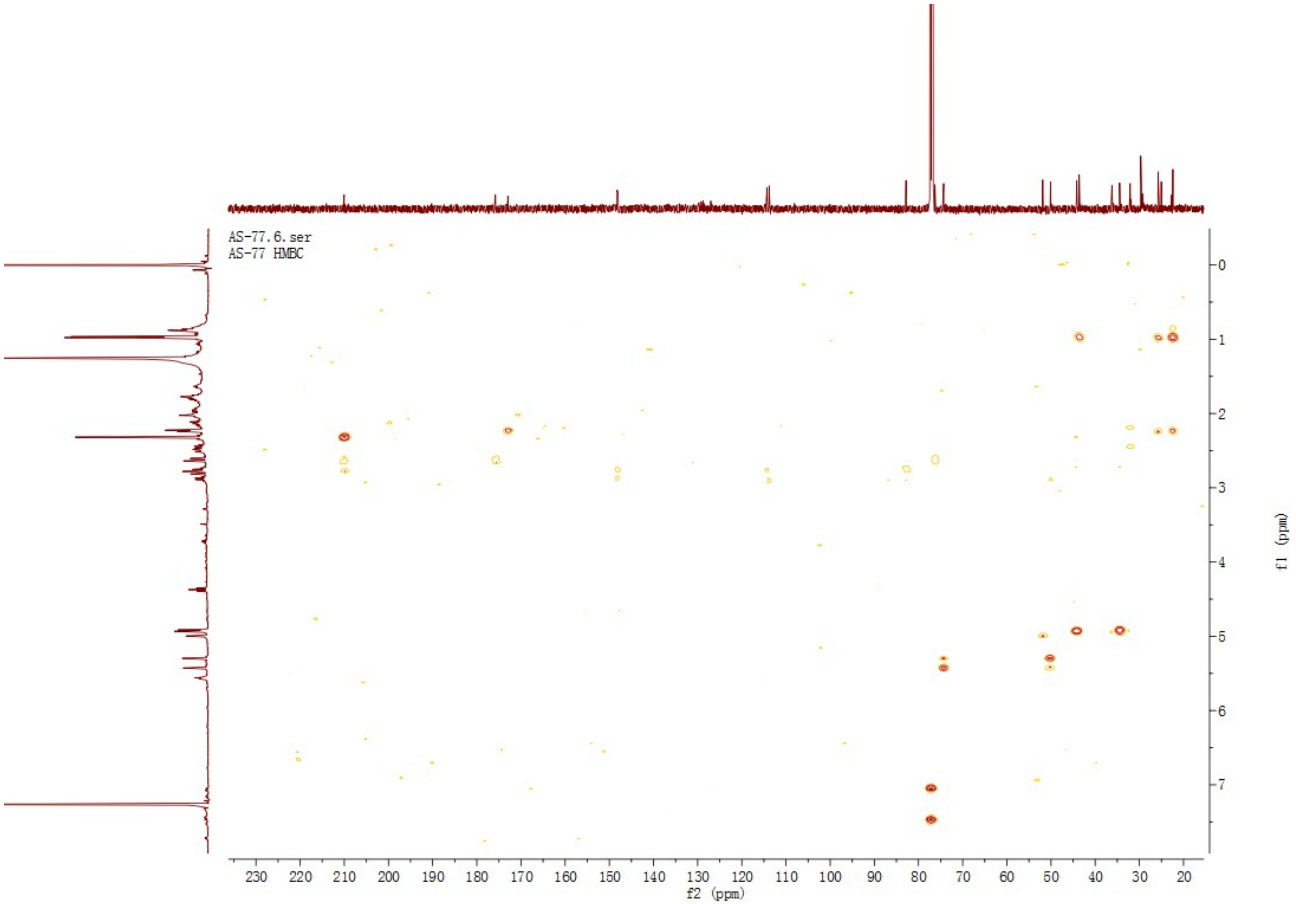


Figure S34. NOESY spectrum of Pertyolide C (**3**) in $CDCl_3$

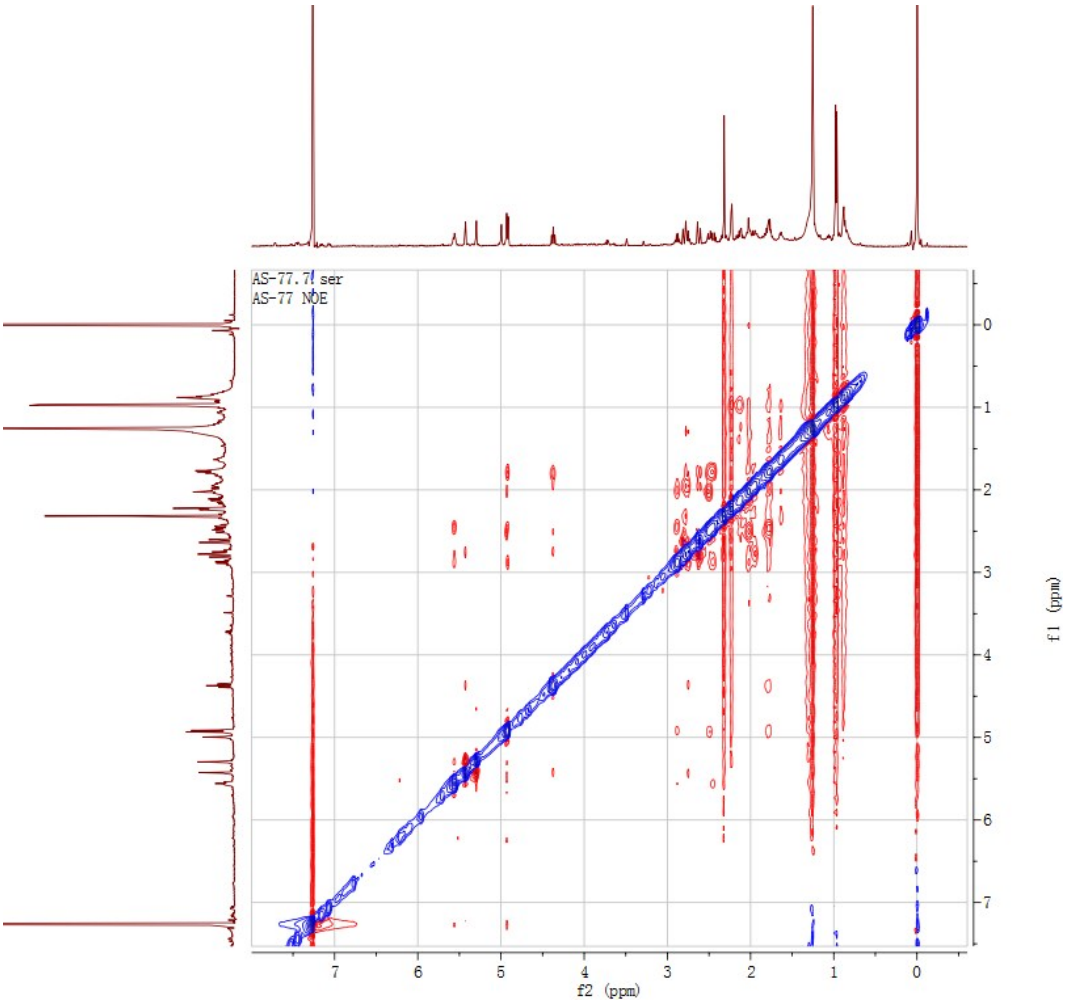
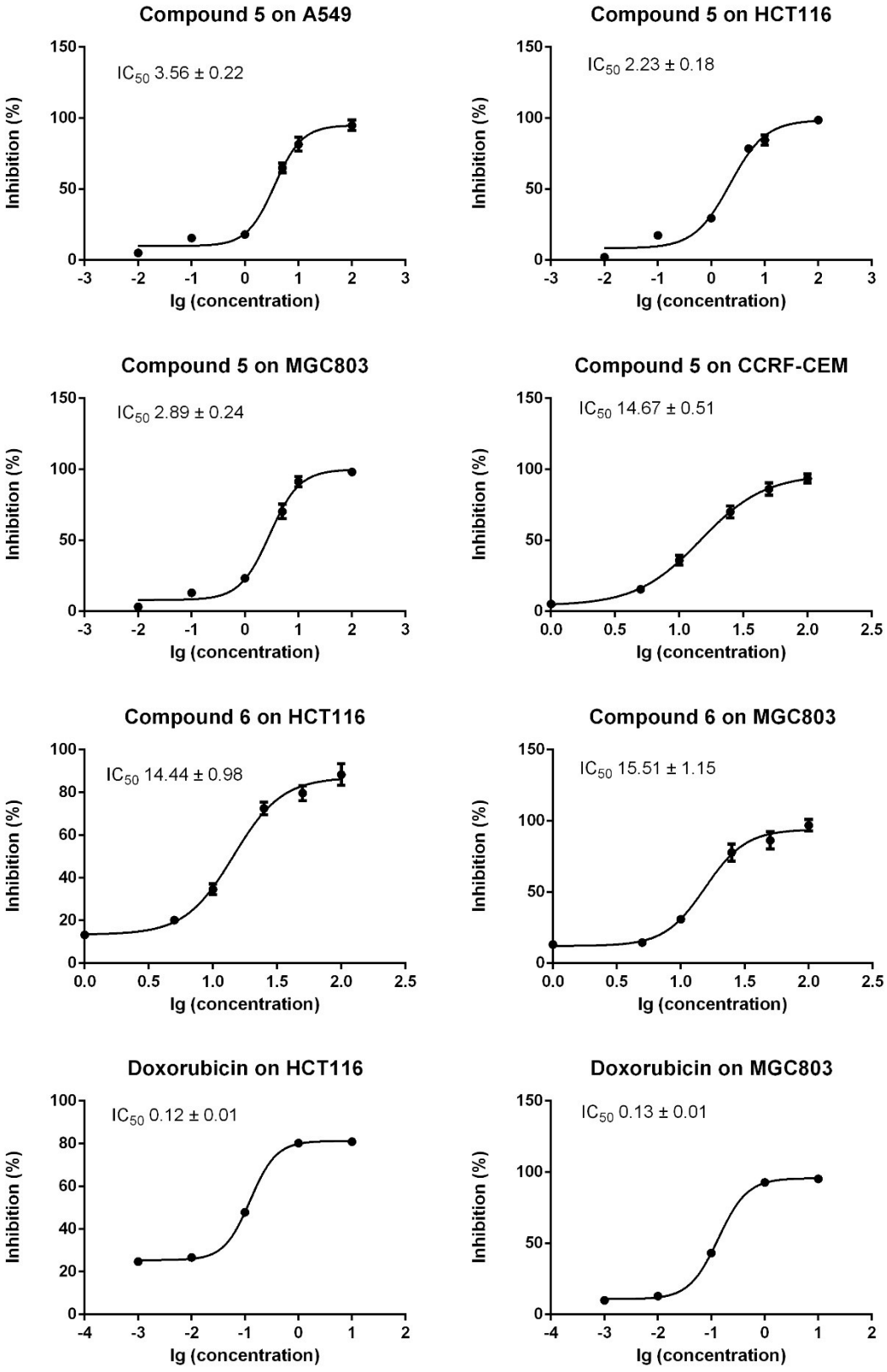


Figure S35. The inhibition rate curves



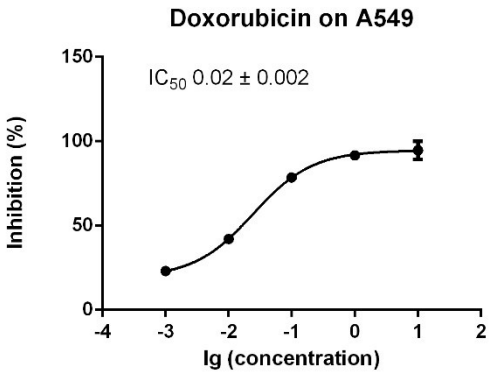

Doxorubicin on A549
IC50 0.02 ± 0.002
Inhibition (%)
150
100
50
0
-4
-3
-2
-1
0
1
2
lg (concentration)

Table S1−3 Inhibition rate (%) of compounds (**5, 6, Doxorubicin**) against cell lines (Mean ± SD, n = 3)

	Compound **5**		
(μM)	A549	HCT116	MGC803
100.00	94.95 ± 3.78	98.71 ±1.91	98.20 ± 1.73
10.00	81.56 ±4.84	84.57 ±3.50	91.41 ± 3.69
5.00	64.98 ±3.36	78.50 ±0.92	70.36 ± 5.14
1.00	18.10 ± 1.11	29.61 ±1.87	23.35 ± 2.13
0.10	15.53 ± 1.42	17.43 ± 1.69	13.00 ± 1.29
0.01	5.00 ± 0.39	2.00 ± 0.20	3.18 ± 0.24

	Compound **5**	Compound **6**	Compound **6**
(μM)	CCRF-CEM	HCT116	MGC803
100.00	93.58 ± 3.17	88.40 ± 4.15	96.97 ± 4.04
50.00	86.20 ± 4.41	79.68 ± 3.46	86.32 ± 6.13
25.00	70.10 ±4.25	72.53 ± 2.95	77.82 ± 5.97
10.00	35.96 ± 3.42	34.61 ± 2.50	30.99 ± 1.13
5.00	15.61 ± 1.40	20.17 ± 1.74	14.56 ± 1.07
1.00	5.21 ± 0.38	13.25 ± 1.06	13.20 ± 0.93

	Doxorubicin			
(μM)	A549	HCT116	MGC803	CCRF-CEM
10	94.66 ± 5.41	80.94 ± 1.13	95.32 ± 1.56	99.34 ± 0.38
1	91.67 ± 2.71	80.25 ± 0.94	92.81 ±1.10	98.63 ± 1.79
0.1	78.61 ±1.85	47.91 ± 0.44	43.16 ± 2.05	98.31 ±2.69
0.01	42.10 ± 0.94	26.75 ± 0.89	12.94 ± 1.21	93.26 ± 1.26
0.001	23.02 ±1.85	24.80 ± 0.71	9.88 ± 0.36	75.58 ± 1.79