Supporting Information

Dioxasampsones A and B, two polycyclic polyprenylated acylphloroglucinols with unusual epoxy ring fused skeleton from *Hypericum sampsonii*

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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) and octadecylsilanized (ODS) silica gel (YMC Ltd., Japan) were used for open column chromatography (CC).

Plant Material

The aerial parts of *Hypericum sampsonii* were collected from Wuming, Guangxi Province, China, in June, 2012, and authenticated by Professor Songji Wei (Guangxi University of Traditional Chinese Medicine, Guangxi, China). A voucher specimen (20120618) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, China.

Extraction and Isolation

The air-dried aerial parts of *H.sampsonii* (15 Kg) were refluxed twice with 60% EtOH for 2 hours each time. The crude extract (1.4kg) was column chromatographed over a macroporous resin HP-20 eluted with EtOH-H_2O in gradient. The 90% EtOH-H_2O eluent (198.2 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. 2 (7.2 g, $CHCl_3$/CH_3OH 100:0) was then chromatographed on a silica gel column using cyclohexane-EtOAc gradient elution to yield 9 subfractions (Fr. 2.1-2.9). The subfraction Fr. 2.5 (923 mg, C/E 9:1) was further subjected to ODS column chromatography eluted with MeOH-H_2O (70:30→100:0) and purified by preparative HPLC on ODS column with 75% ACN-H_2O to yield compound **3** (3.5 mg). Another Fr. 5 (43.1 g, $CHCl_3$/CH_3OH 98:2) was then chromatographed on a silica gel column

using cyclohexane-EtOAc gradient elution to yield 11 subfractions (Fr. 5.1-5.11). The subfraction Fr. 5.4 (7.1 g, C/E 99:1) was further subjected to ODS column chromatography which yield 8 subfractions (Fr. 5.4.1-5.4.8). Then the subfraction Fr. 5.4.5 (1.2 g, MeOH/H_2O 90:10) was subjected to a silica gel column using cyclohexane-EtOAc-acetone gradient elution to yield 9 subfractions (Fr. 5.4.5.1-5.4.5.9). Fr. 5.4.5.3 was purified by preparative HPLC on ODS column with 75% MeOH-H_2O to yield compound **2** (21.5 mg), while Fr. 5.4.5.4 was purified by preparative HPLC on ODS column with 70% ACN-H_2O to yield compound **1** (4.3 mg).

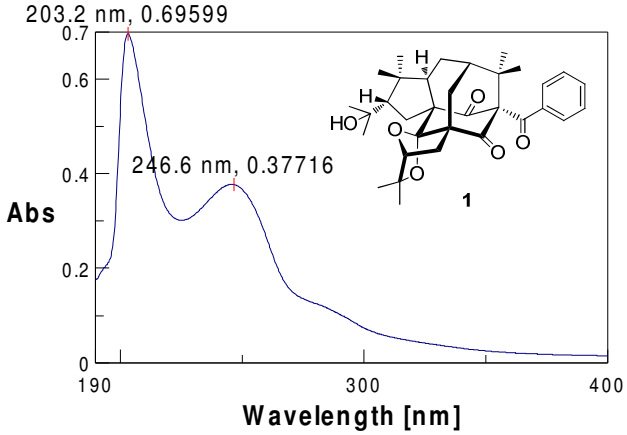
Physico-chemical constants of 1-3

Dioxasampsone A (**1**): colorless crystals; $[\alpha]_D^{23}$ +16.8 (*c* 0.50, $CHCl_3$); UV (CH_3OH) λ_{max} (logε) 203 (4.35), 246 (4.08) nm; IR (KBr) λ_{max} 3434, 2973, 2926, 1730, 1697, 1448, 1385, 1225, 1089, 768, 690 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 204 (+1.81), 239 (-0.91), 281 (+0.49) , 313 (-0.24) nm; HR-ESI-MS m/z 535.3061 $[M+H]^+$ (calcd for $C_{33}H_{43}O_6$, 535.3060).

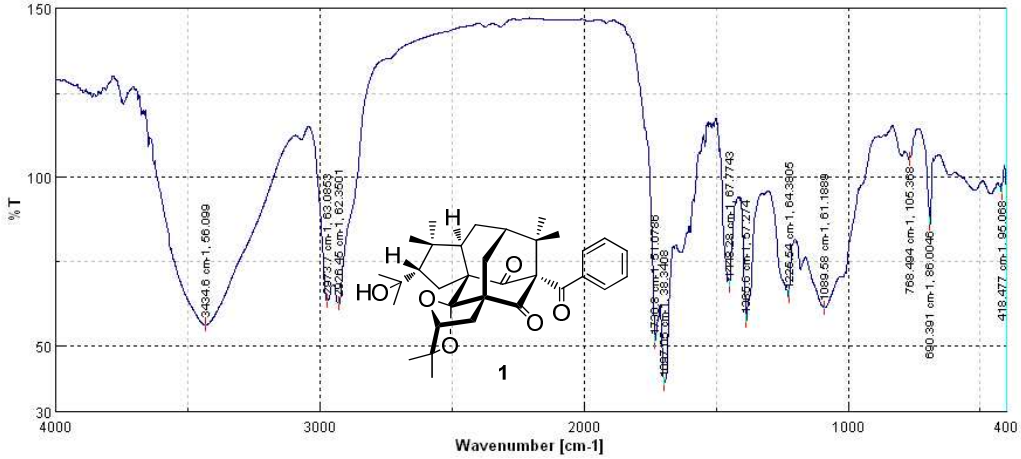
Dioxasampsone B (**2**): colorless oil; $[\alpha]_D^{23}$ +77.0 (*c* 0.50, $CHCl_3$); UV (CH_3OH) λ_{max} (logε) 208 (4.35), 248 (4.20) nm; IR (KBr) λ_{max} 3436, 2969, 2928, 1723, 1693, 1632, 1607, 1448, 1334, 1256, 1227, 1128, 1024, 758, 692 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 210 (+1.19), 252 (-0.79), 290 (+0.37) nm; HR-ESI-MS m/z 551.3008 $[M+H]^+$ (calcd for $C_{33}H_{43}O_7$, 551.3009).

Hypersampson R (**3**): colorless oil; $[\alpha]_D^{23}$ +22.0 (*c* 0.30, $CHCl_3$); UV (CH_3OH) λ_{max} (logε) 203 (4.11), 248 (3.94), 277 (3.78)nm; IR (KBr) λ_{max} 3410, 2957, 2925, 2857, 1727, 1625, 1464, 1378, 1220, 1093, 1024 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 209 (-0.18), 222 (+0.03), 245 (-1.14), 269 (+2.83), 310 (-0.46)nm; HR-ESI-MS m/z 461.2691 $[M+H]^+$ (calcd for $C_{30}H_{37}O_4$, 461.2692).

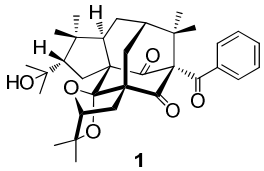
UV spectrum of dioxasampsone A (1) in CH_3OH.



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



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



Elemental Composition Report

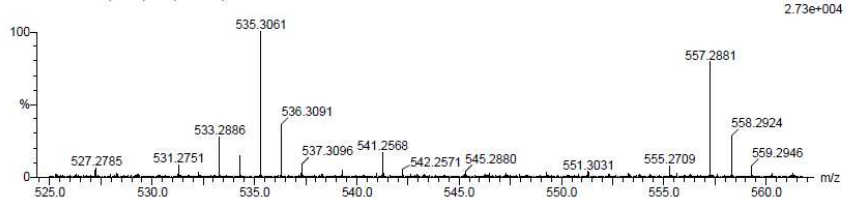
Page 1

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
147 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200

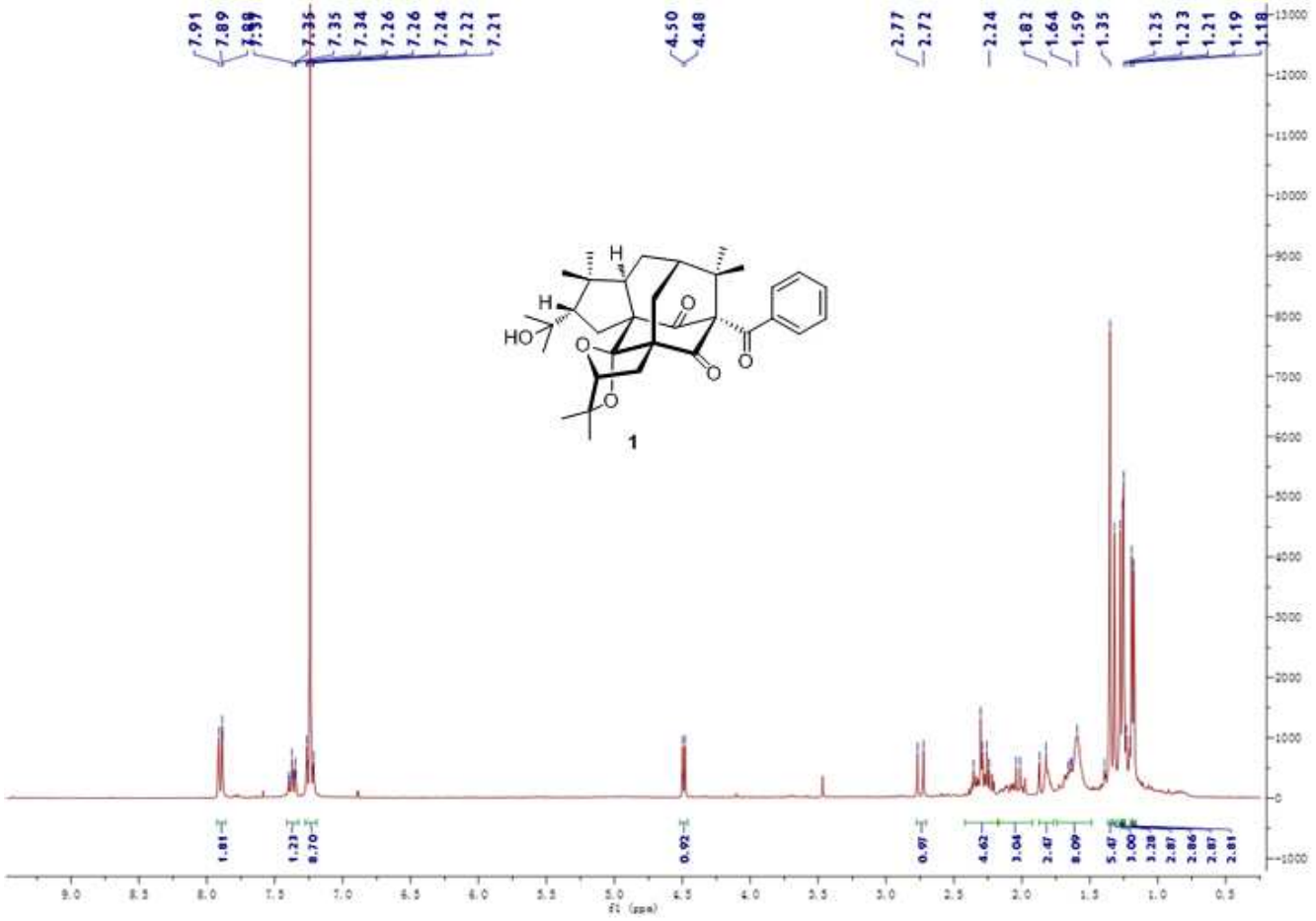
HSD5D5D6-8
20131220-06 237 (1.907) Cm (228:242)

1: TOF MS ES+
2.73e+004

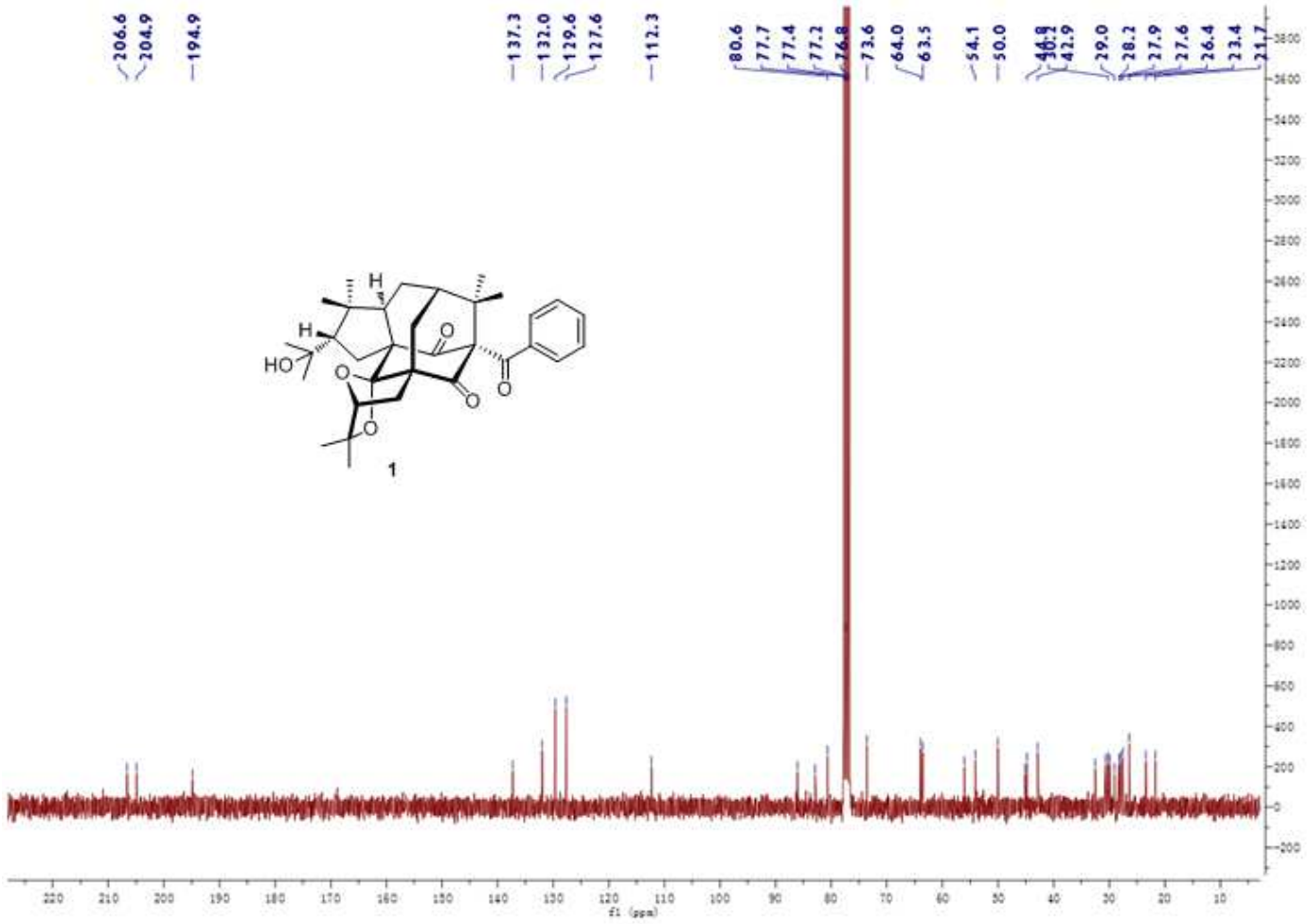


Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
535.3061	535.3060	0.1	0.2	12.5	658.1	n/a	n/a	C33 H43 O6

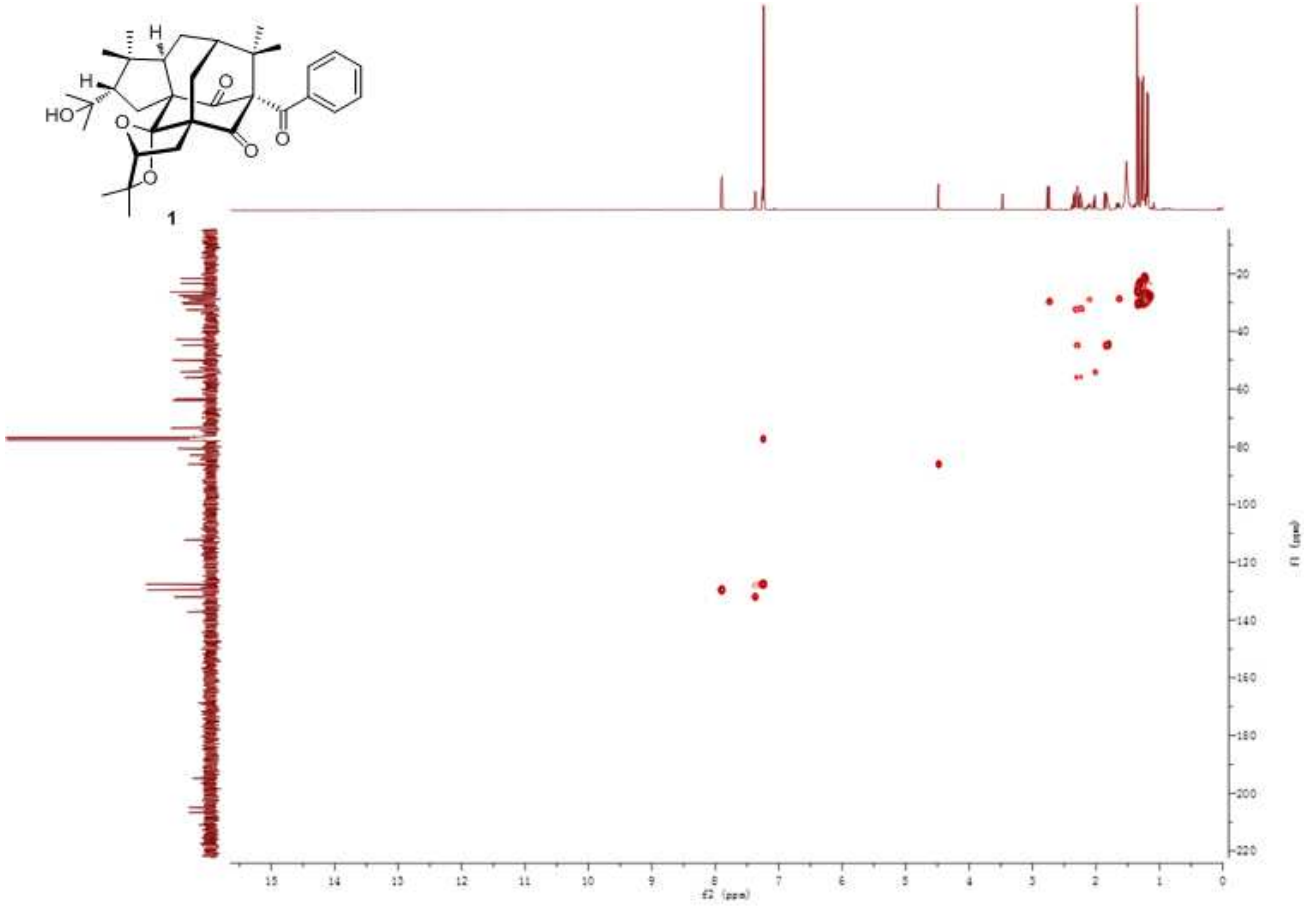
^{1}H NMR (AV-300, 300 MHz) spectrum of dioxasampsone A (1) in $CDCl_3$



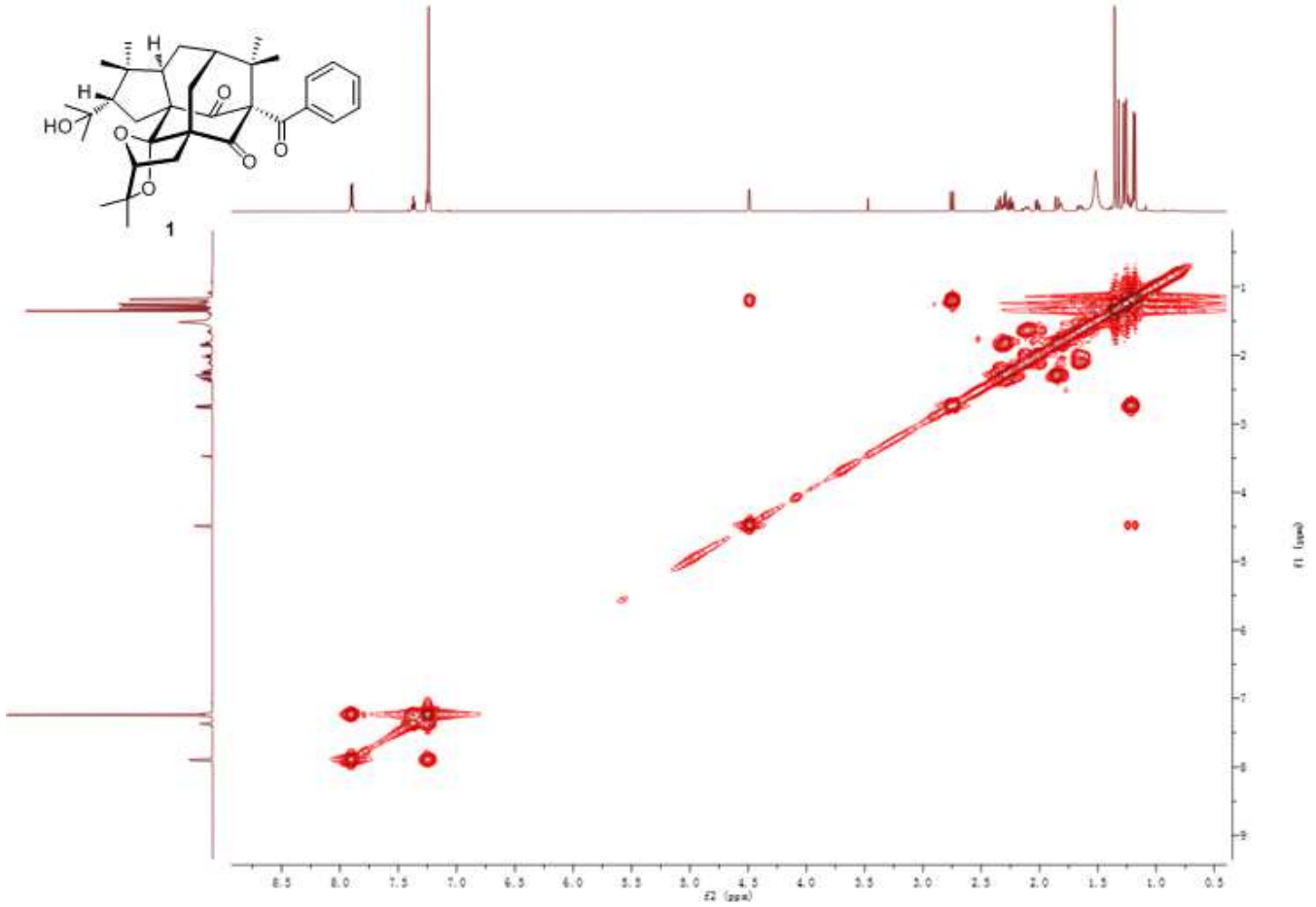
^{13}C NMR spectrum (AV-300, 75 MHz) of dioxasampsone A (1) in $CDCl_3$



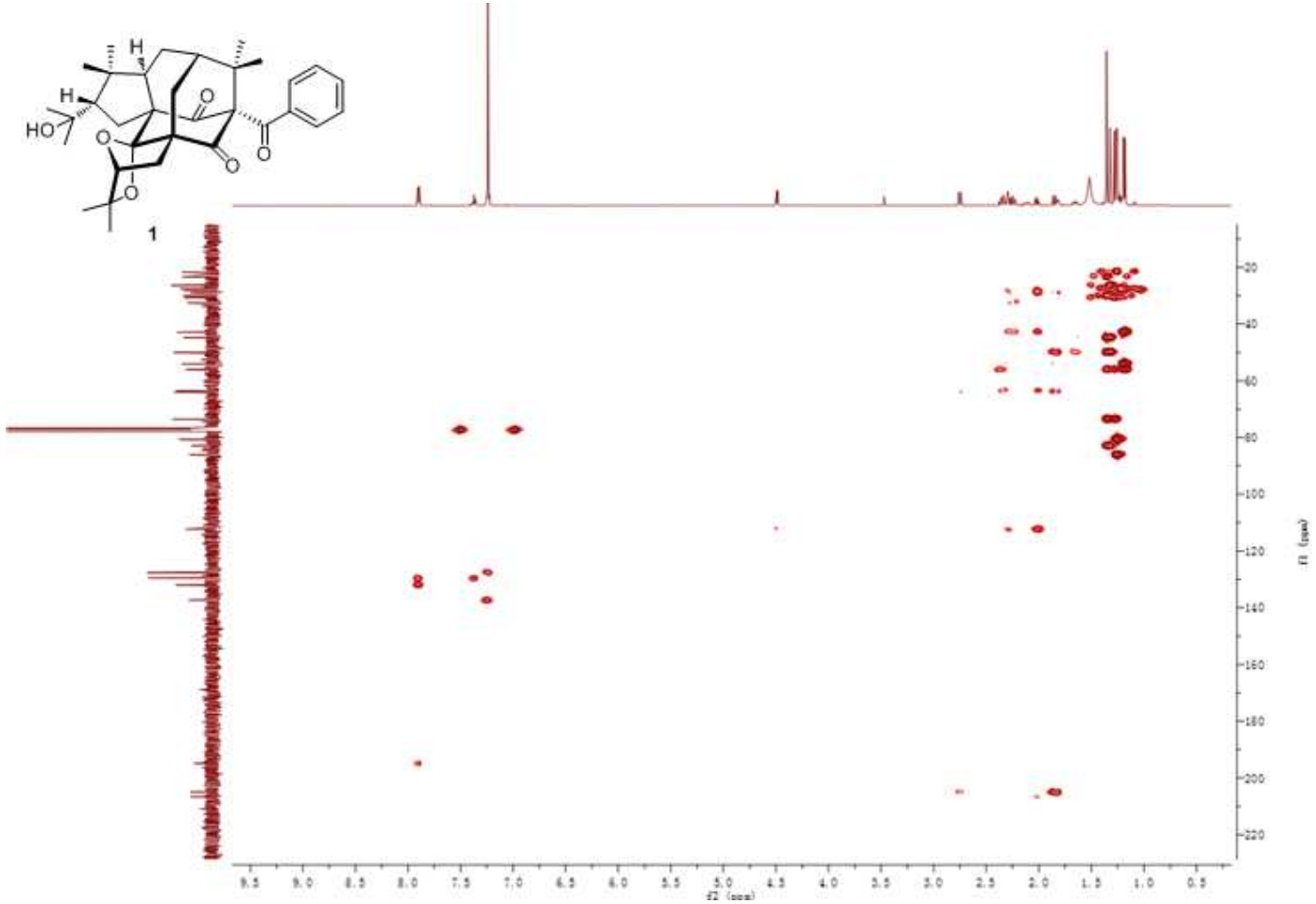
HSQC spectrum (AV-400) of dioxasampsone A (1)1 in $CDCl_3$



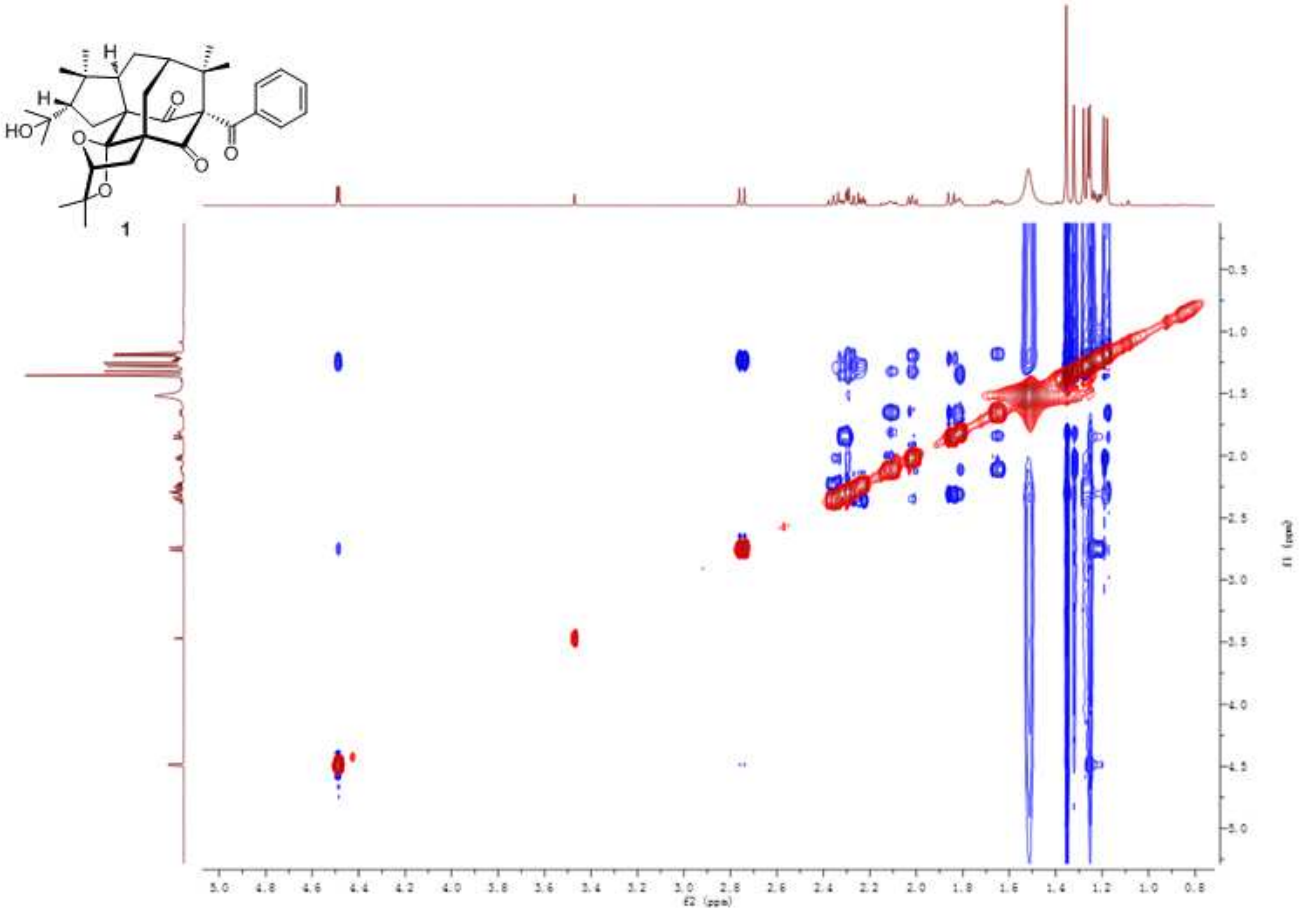
$^{1}H–^{1}H$ COSY spectrum (AV-400) of dioxasampsone A (1) in $CDCl_3$



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



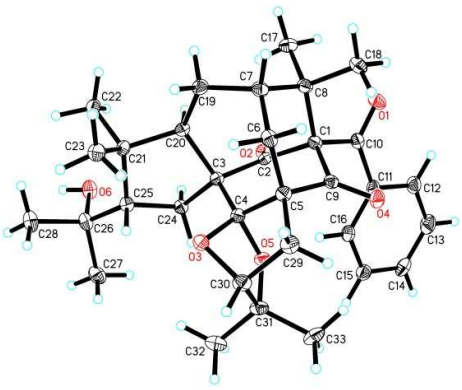
NOESY spectrum (AV-600) of dioxasampsone A (1) in $CDCl_3$



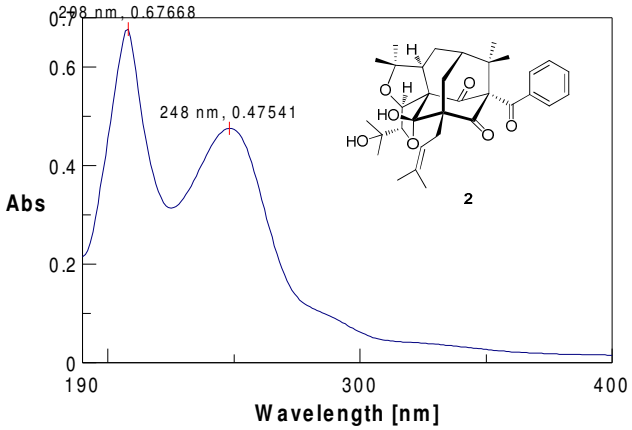
X-ray Crystallographic Analysis of dioxasampsone A (1)

Crystal Data for $C_{33}H_{42}O_6$ (M =534.66): monoclinic, space group $P2_1$, a = 7.6950(2) Å, b = 20.3926(4) Å, c = 9.0759(2) Å, β = 103.862(2)°, V = 1382.72(5) Å^3, Z = 6, T = 150(2) K, μ(Cu Kα) = 0.697 mm^{-1}, $Dcalc$ = 1.284 g/mm^3, 8144 measured reflections, 4311 independent reflections [R_{int} = 0.0239]. The final R_1 was 0.0306 and wR_2 was 0.0928 [I>2σ(I)]. The goodness of fit on F^2 was 0.797. Flack parameter = 0.04(13).

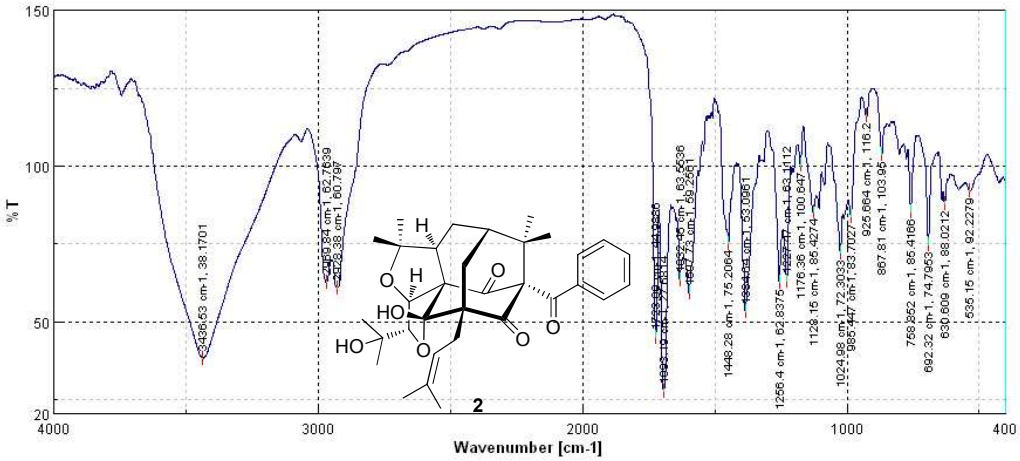
X-ray Crystallographic structure of dioxasampsone A (1)



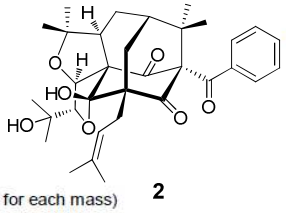
UV spectrum of dioxasampsone B (2) in CH_3OH.



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



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



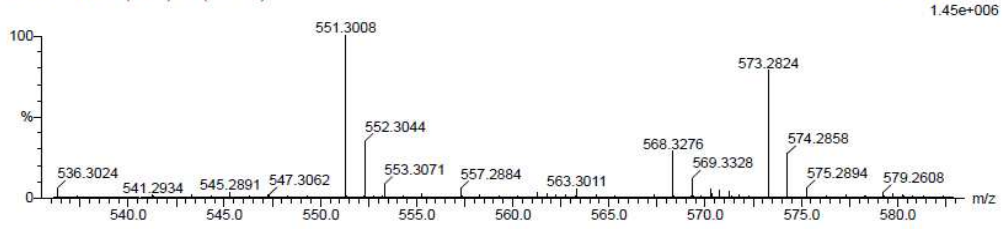
Elemental Composition Report Page 1

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
155 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200

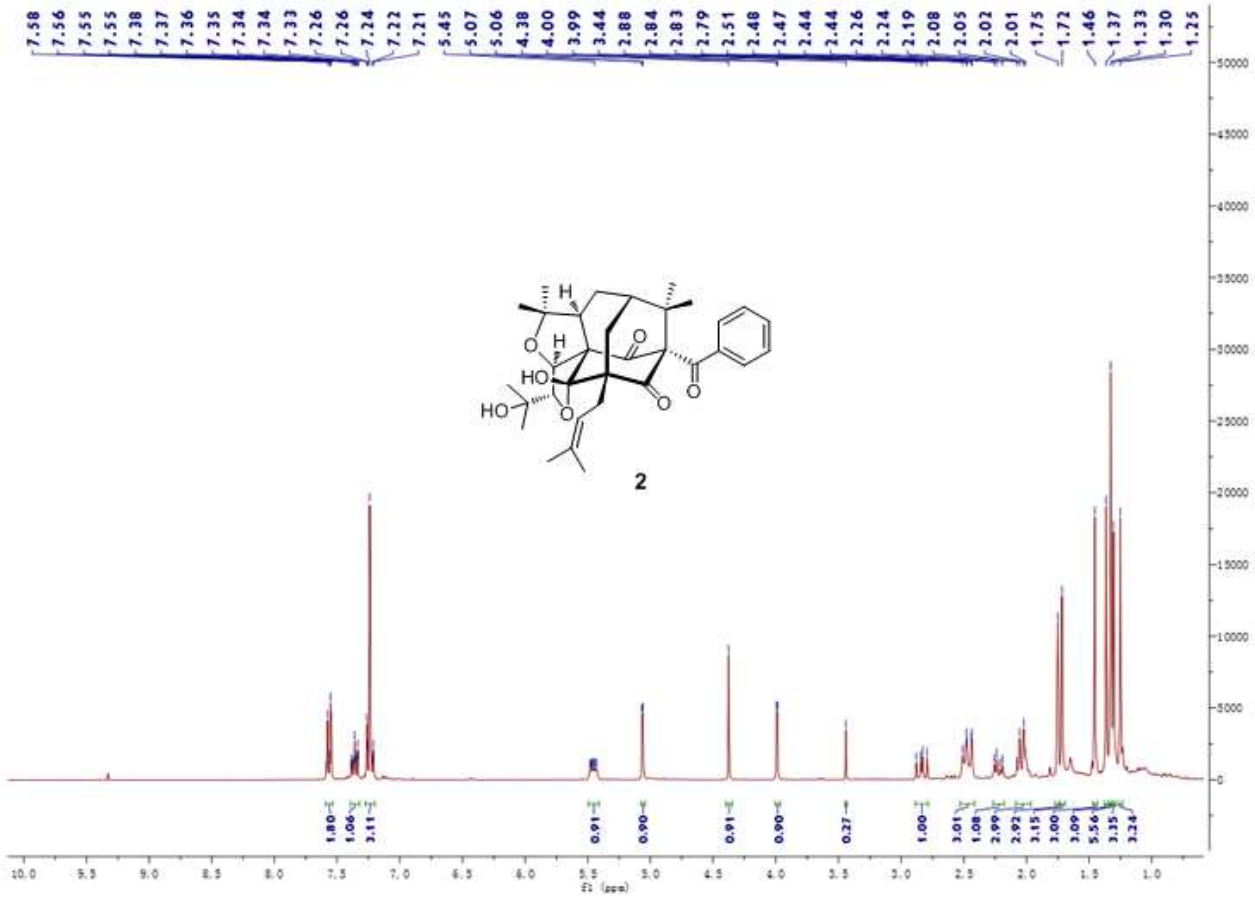
HSD5D5C-1
20130527-02 242 (1.956) Cm (227:251)

1: TOF MS ES+
1.45e+006

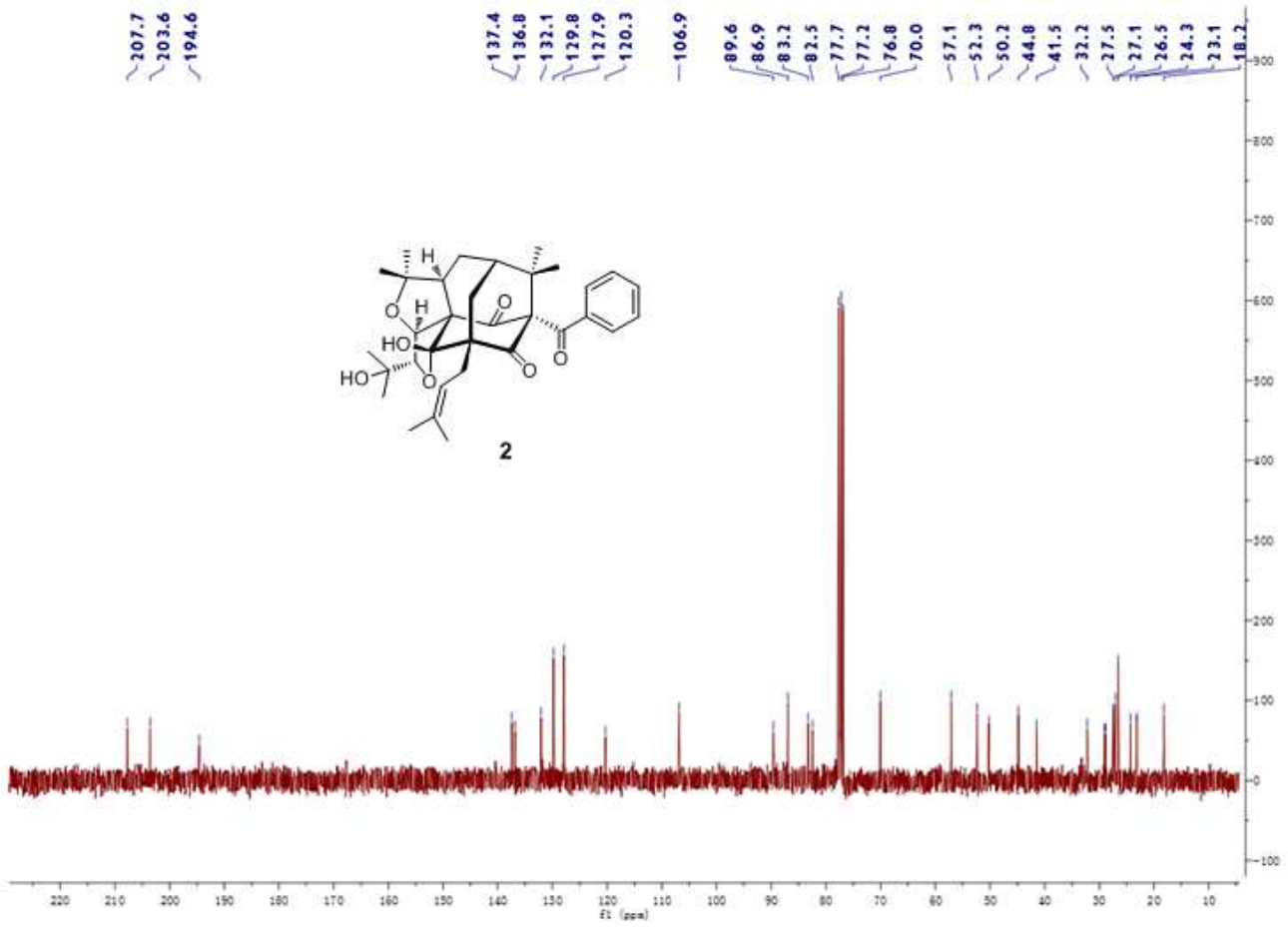


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
551.3008	551.3009	-0.1	-0.2	12.5	1445.0	n/a	n/a	C33 H43 O7

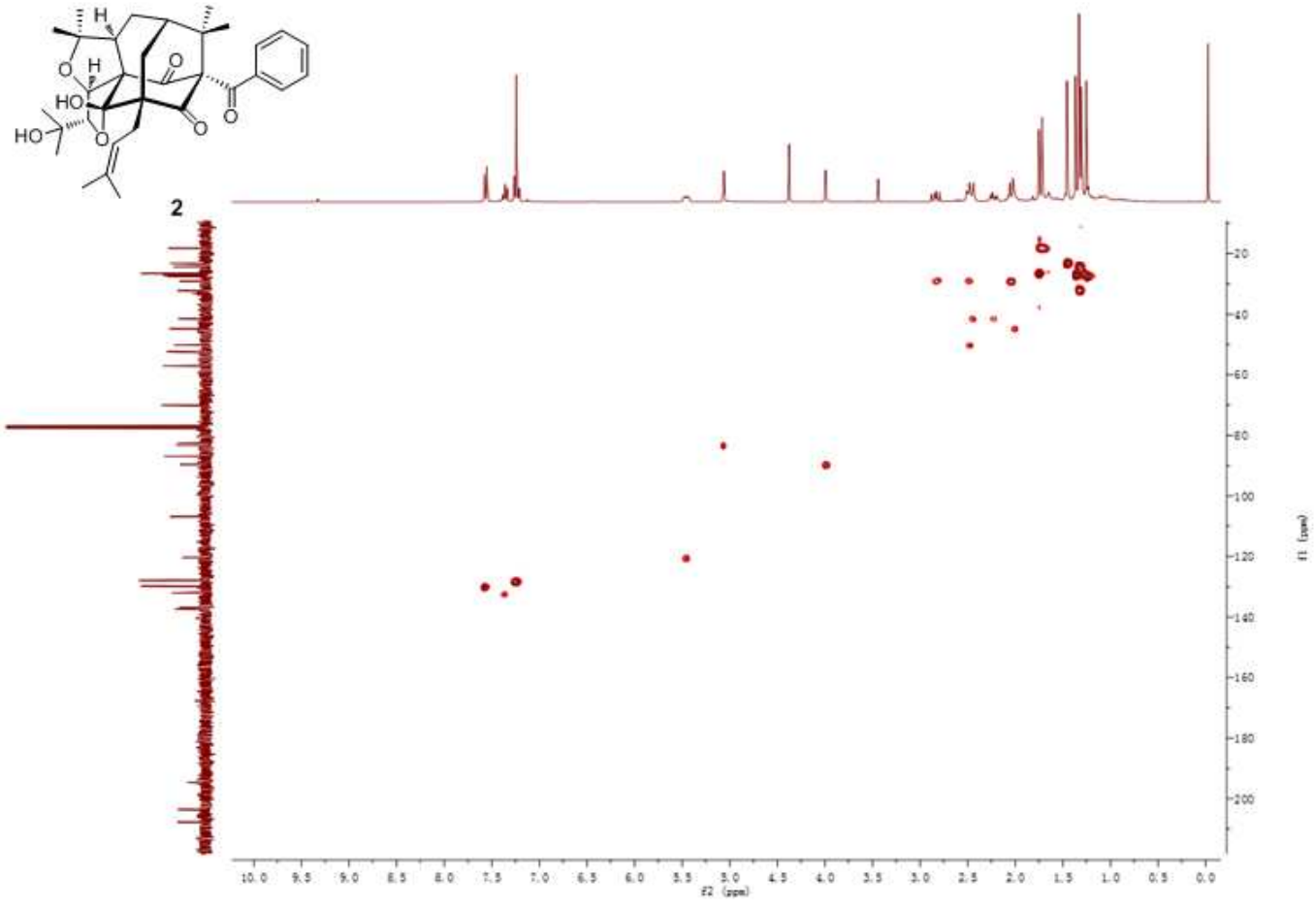
^{1}H NMR (AV-300, 300 MHz) spectrum of dioxasampsone B (2) in $CDCl_3$



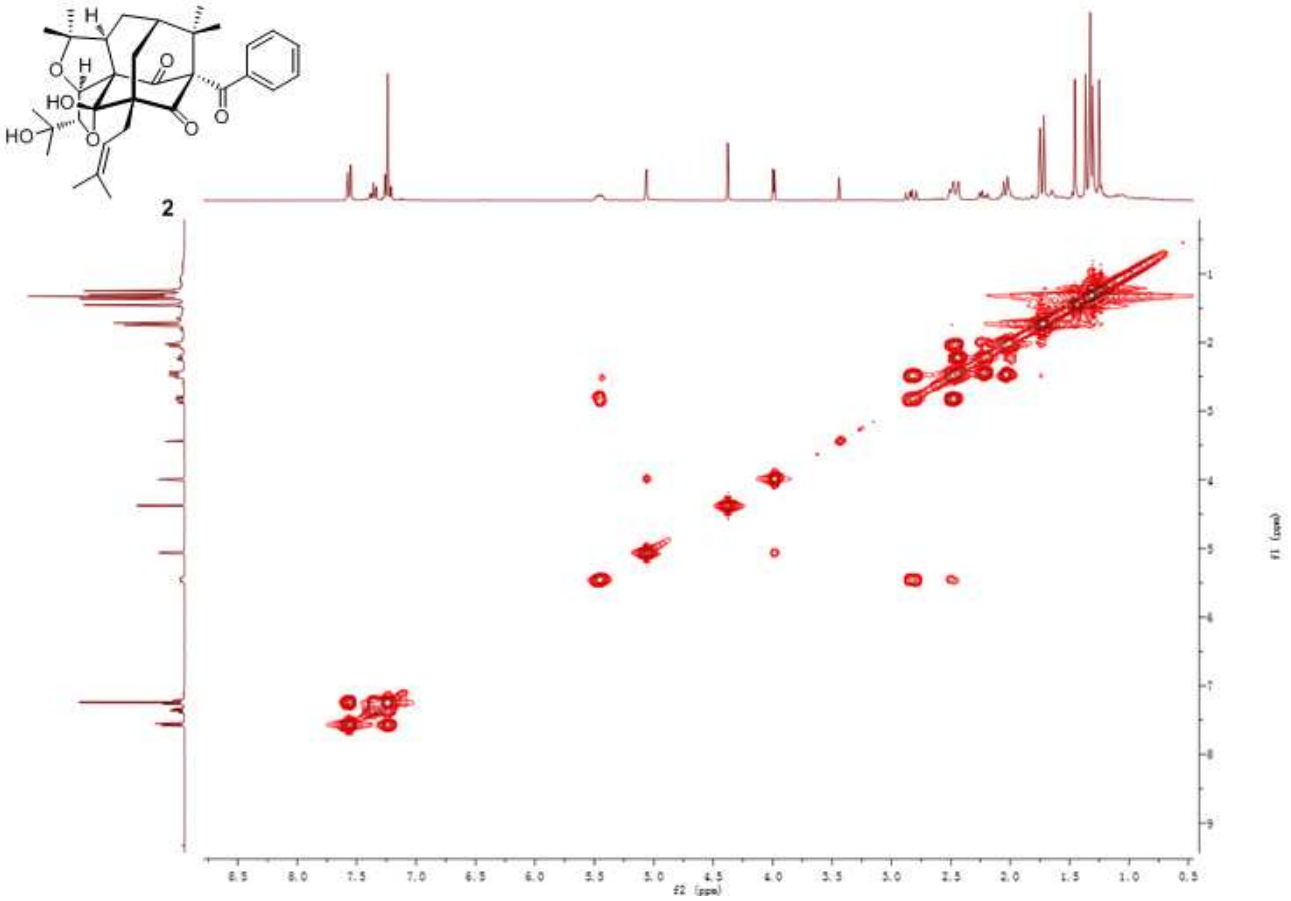
^{13}C NMR spectrum (AV-300, 75 MHz) of dioxasampsone B (2) in $CDCl_3$



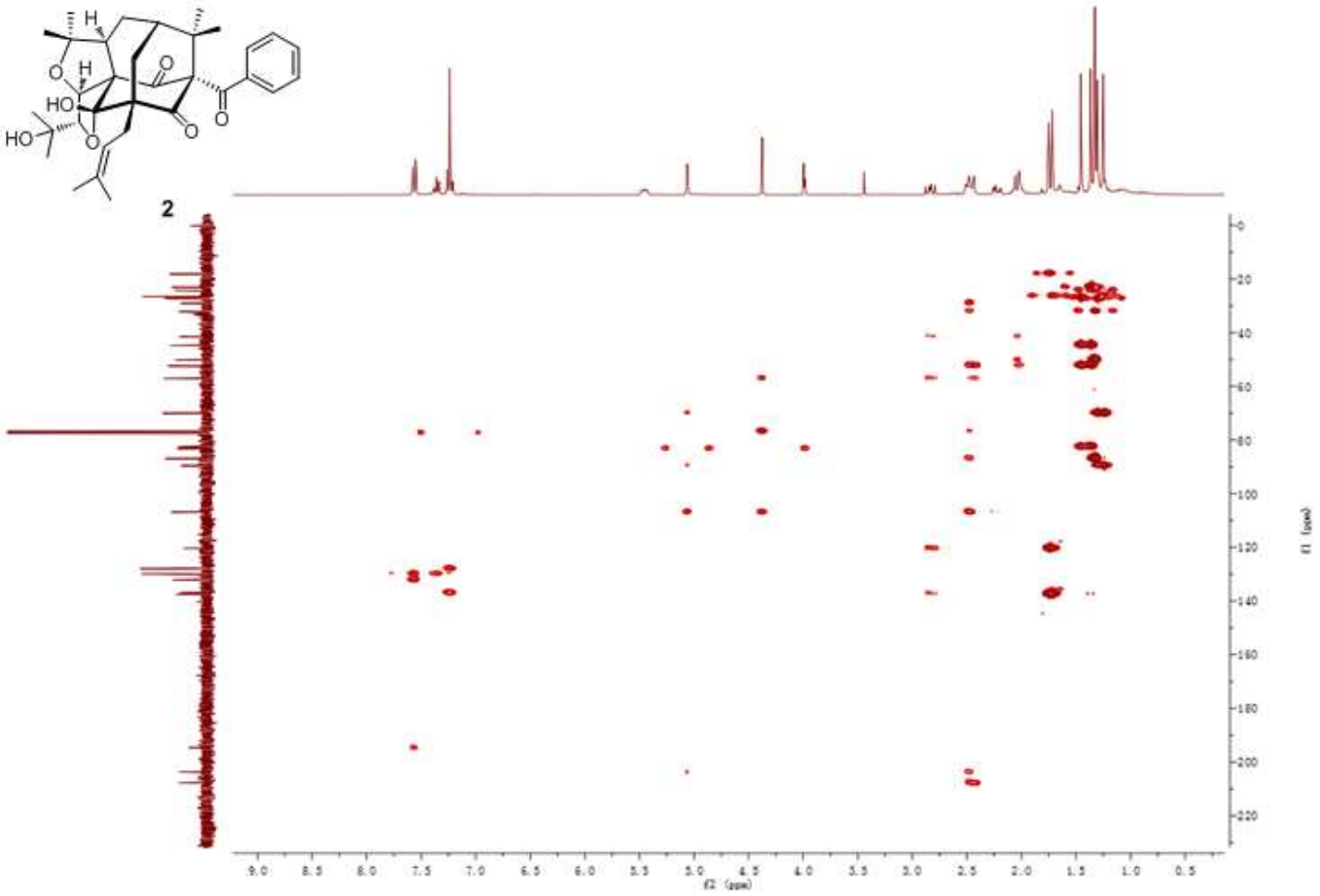
HSQC spectrum (AV-400) of dioxasampsone B (2) in $CDCl_3$



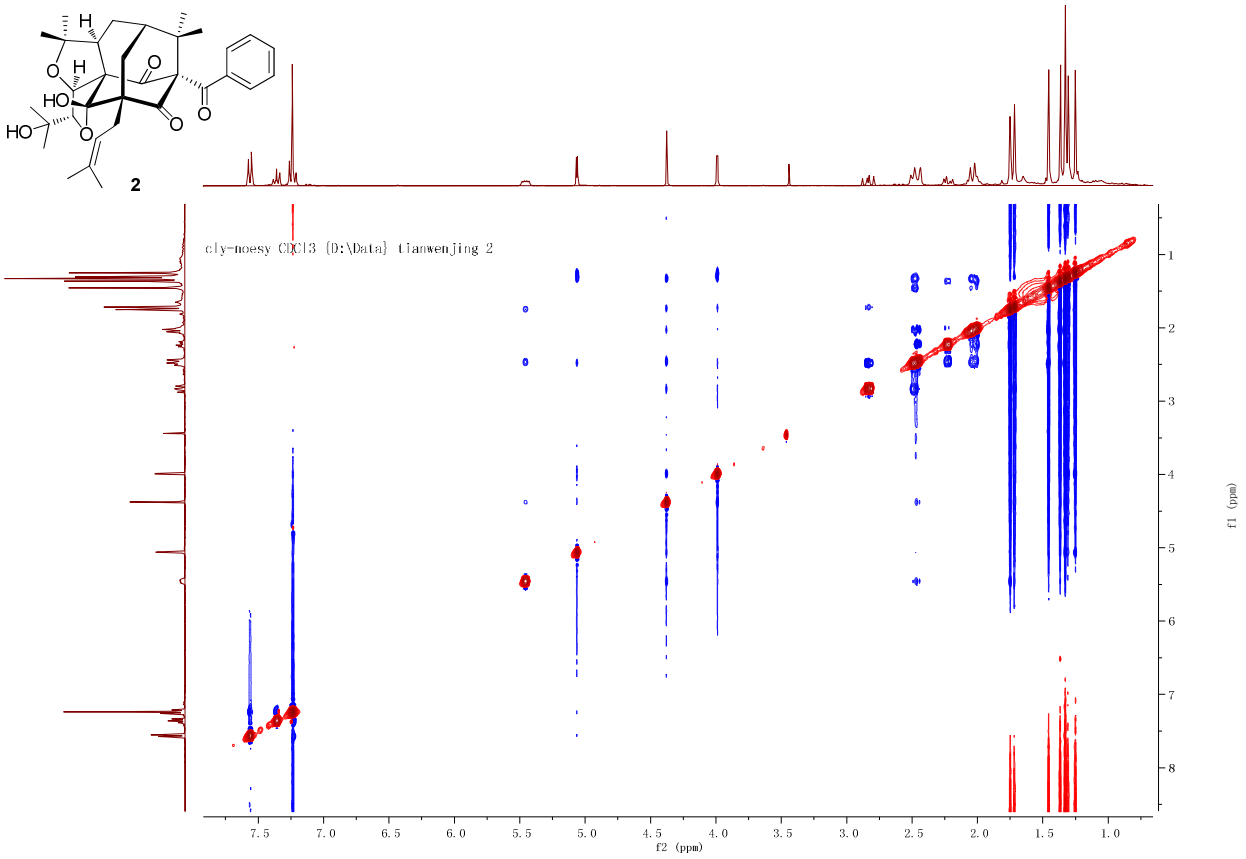
$^{1}H–^{1}H$ COSY spectrum (AV-400) of dioxasampsone B (2) in $CDCl_3$



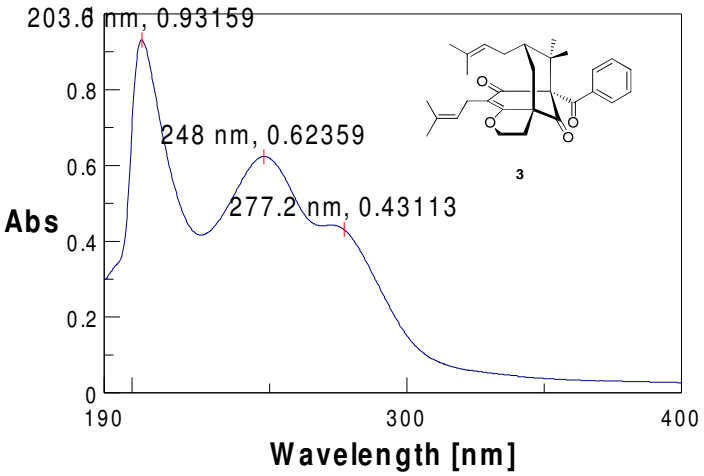
HMBC spectrum (AV-400) of dioxasampsone B (2) in $CDCl_3$



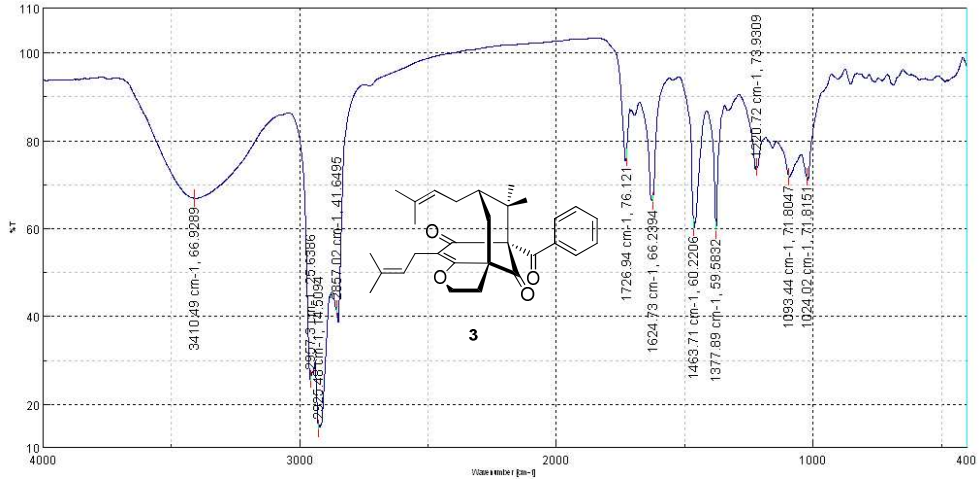
NOESY spectrum (AV-600) of dioxasampsone B (2) in $CDCl_3$



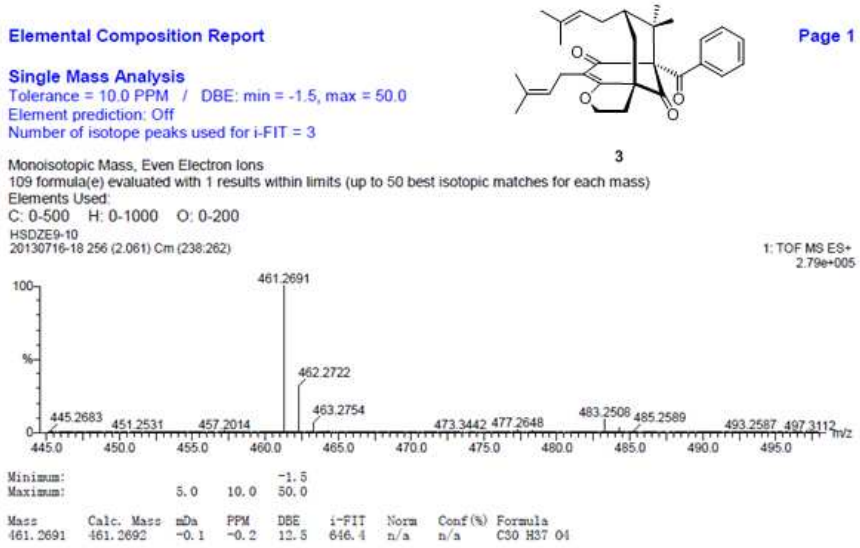
UV spectrum of hypersampsone R (3) in CH_3OH.



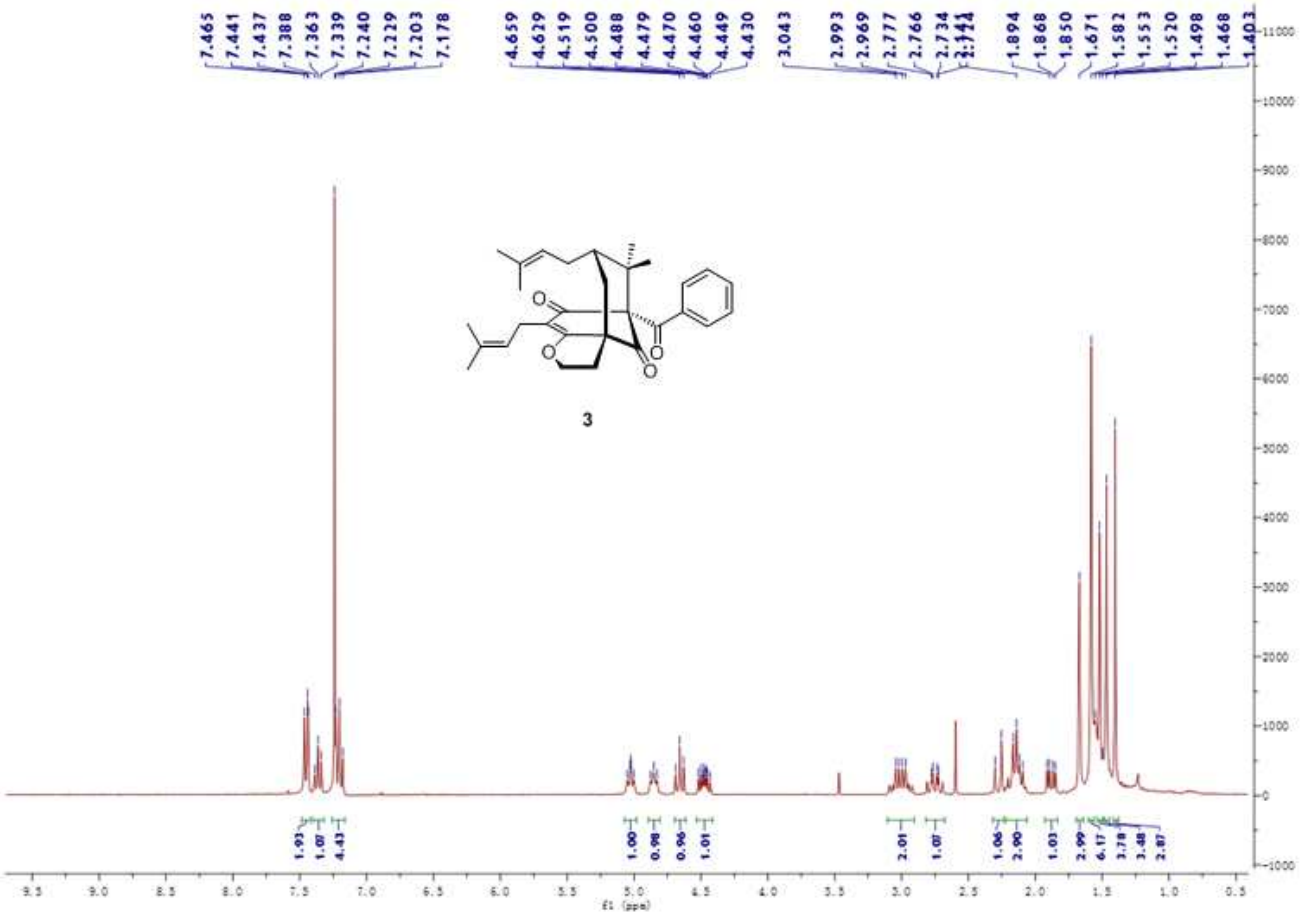
IR (KBr disc) spectrum of hypersampsone R (3).



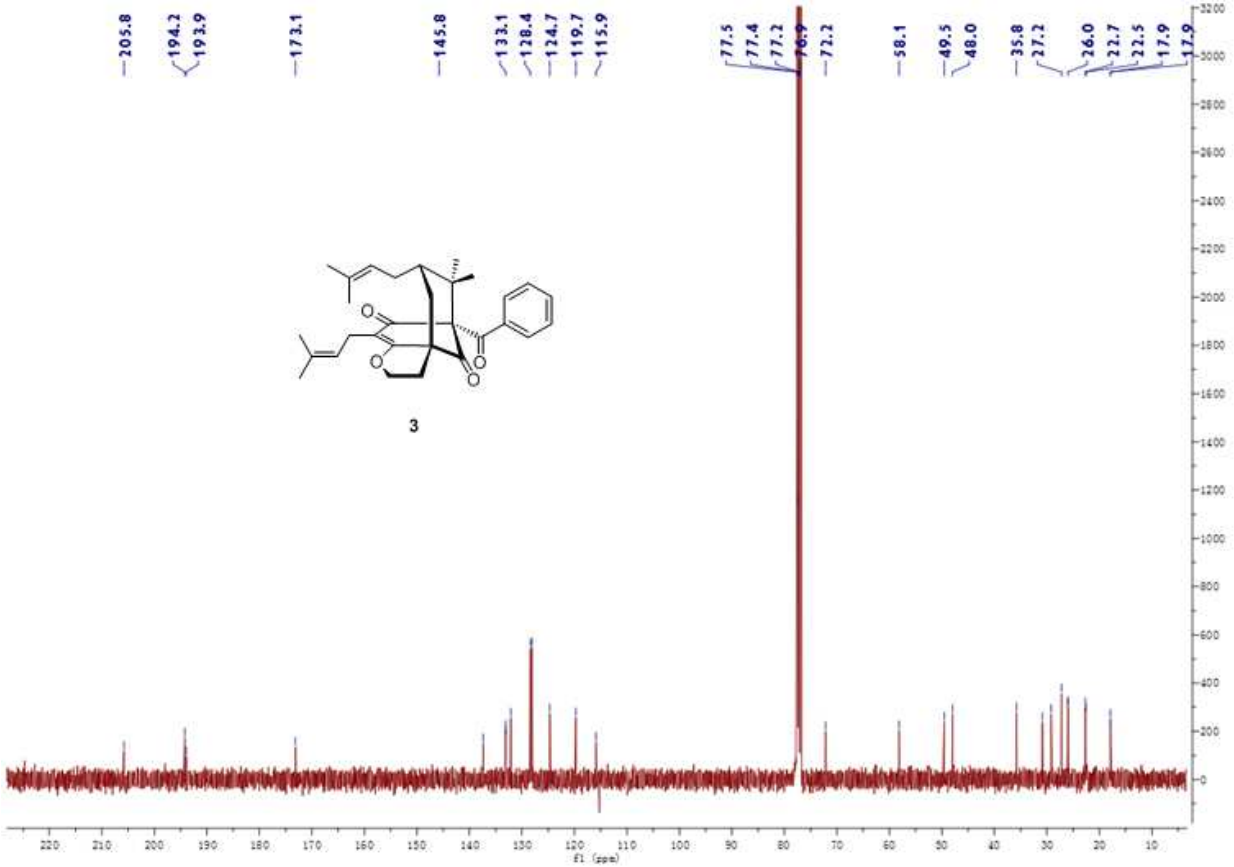
HR-ESI-MS spectrum of hypersampsone R (3).



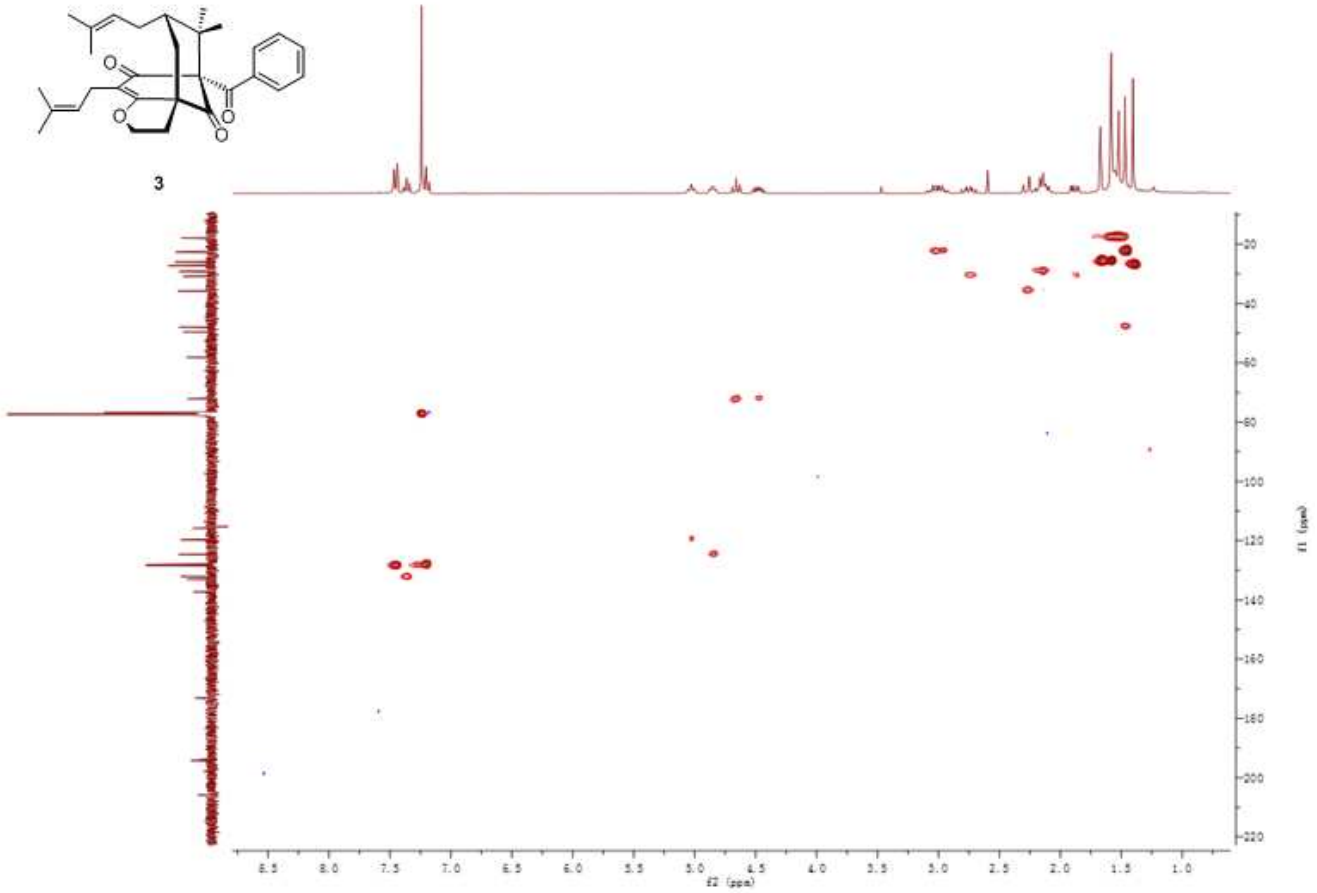
^{1}H NMR (AV-300, 300 MHz) spectrum of hypersampsone R (3) in $CDCl_3$



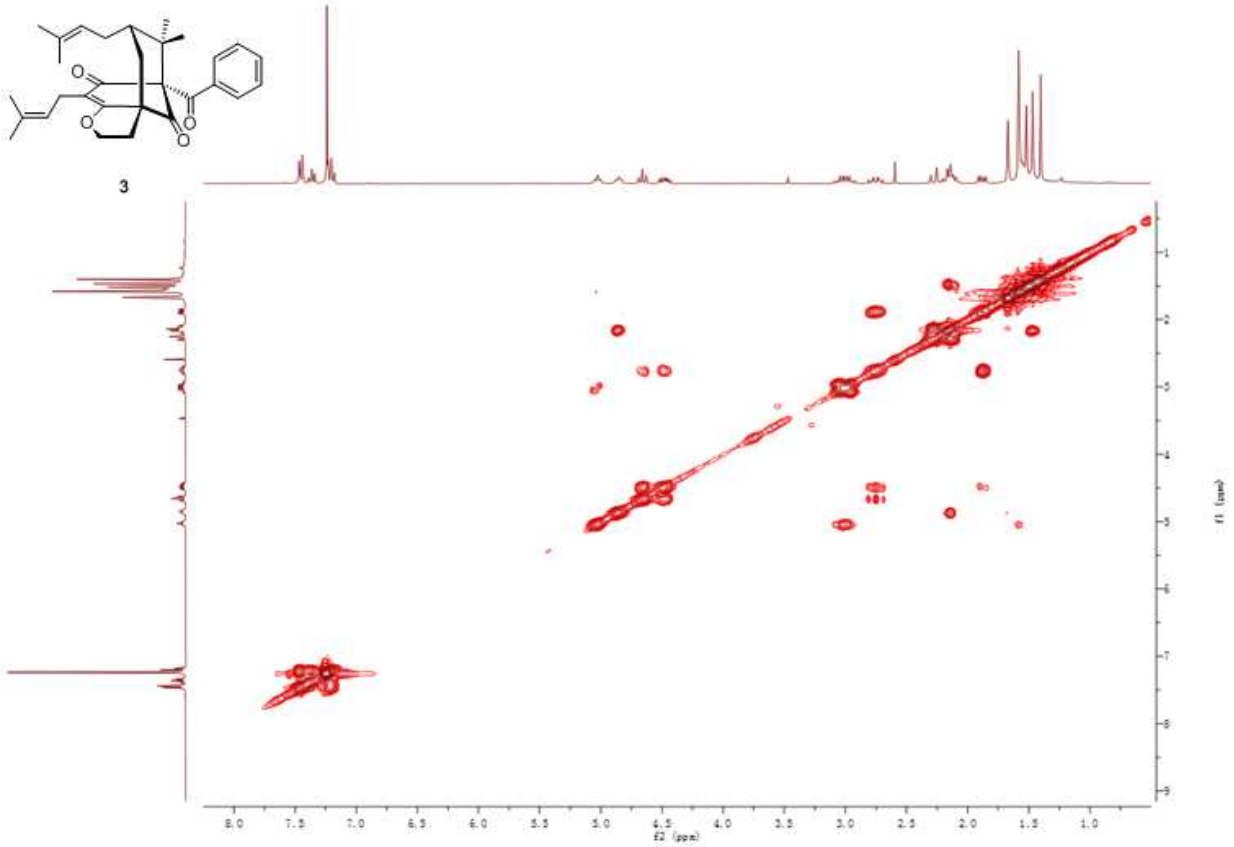
^{13}C NMR spectrum (AV-400, 100 MHz) of hypersampsone R (3) in $CDCl_3$



HSQC spectrum (AV-400) of hypersampsone R (3) in $CDCl_3$



$^{1}H–^{1}H$ COSY spectrum (AV-400) of hypersampsone R (3) in $CDCl_3$



HMBC spectrum (AV-400) of hypersampsone R (3) in $CDCl_3$

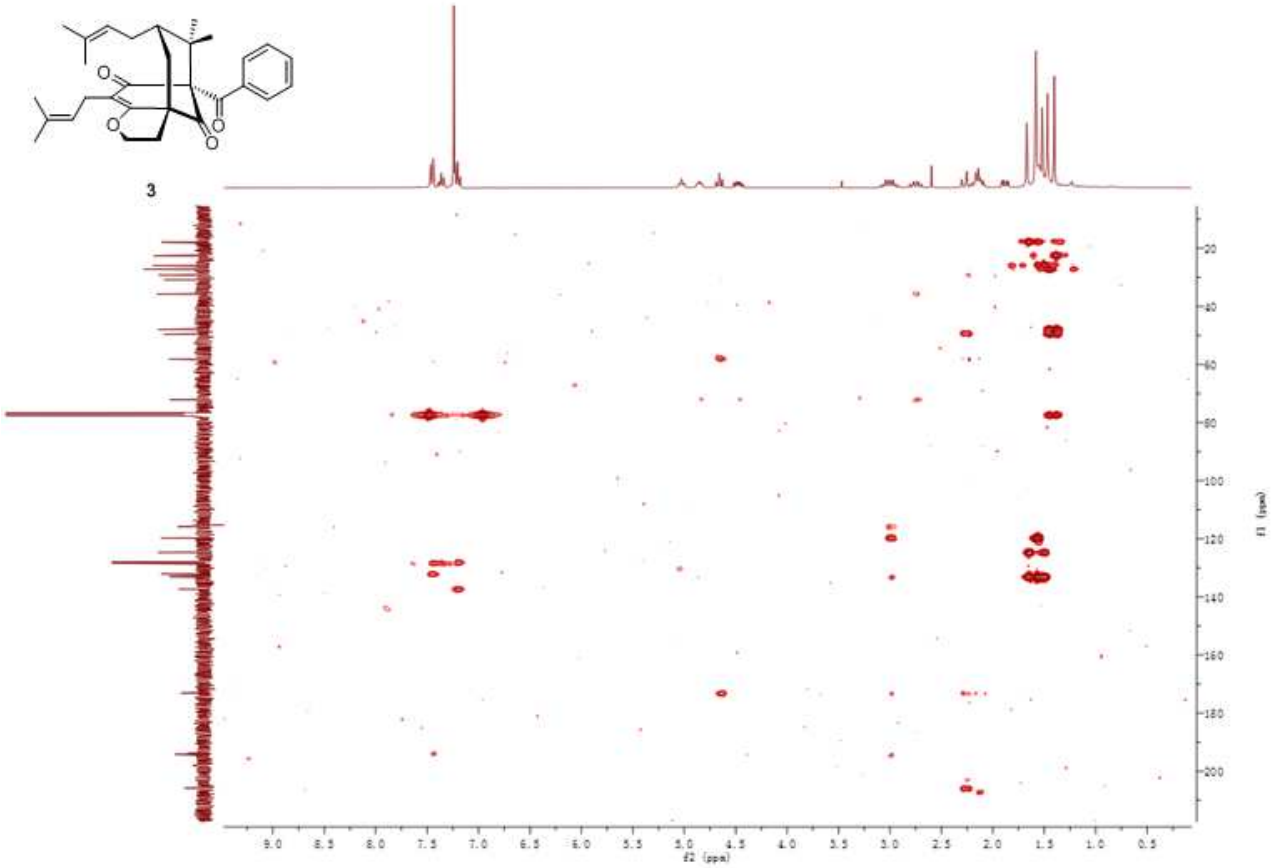
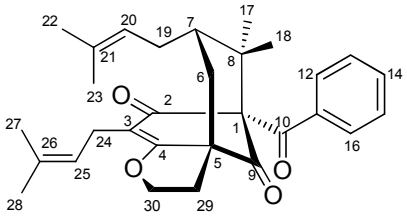


Table 1. ^{1}H (300 MHz) and ^{13}C (100 MHz) NMR Data of **3** in $CDCl_3$ (δ in ppm, J in Hz)

no.	**3**	
	δ_H	δ_C
1		77.4
2		194.2
3		115.9
4		173.1
5		58.1
6α	2.27 (br.d, 14.4Hz)	35.8
6β	2.13 (m)	
7	1.47 (m)	48.0
8		49.5
9		205.8
10		193.9
11		137.3
12,16	7.45 (br.d, 7.5)	128.4
13,15	7.20 (br.t, 7.5)	128.1
14	7.36 (br.t, 7.5)	132.1
17	1.47 (s)	22.5
18	1.40 (s)	27.2
19	2.14 (m)	29.2
20	4.85 (br.t, 7.2)	124.7
21		133.1
22	1.52 (s)	17.9
23	1.67 (s)	26.0
24	3.05 (dd, 14.1, 7.5) 2.96 (dd, 14.1, 7.5)	22.7
25	5.03 (tt, 7.5, 1.2)	119.7
26		133.1
27	1.58 (s)	17.9
28	1.58 (s)	26.0
29	1.88 (dd, 13.2, 5.4) 2.75 (ddd, 13.2, 12.0, 9.3)	30.9
30	4.48 (ddd, 12.0, 9.3, 5.4) 4.66 (br.t, 9.3)	72.2



3

Quantum chemical ECD calculation method

In theoretical calculations, the geometry of the molecules was optimized with Gaussian 09 package[1] at B3LYP/6-31G(d) computational level. The minimum nature of the structure was confirmed by frequency calculations at the same computational level. Then ECD calculations were carried out in the methanol solvent medium using time-dependent density functional theory (TDDFT) with B3LYP functional and DGDZVP basis set.

Bioassays

RXR*α* transcriptional activity assay

Cell Culture. The human renal epithelial cells (293T) (ATCC) were cultured in 37 ºC in DMEM (Hyclone) containing 10% fetal bovine serum (FBS, Hyclone) for 24 h. **Experimental Methods**. The previous dual-luciferase reporter gene assay with some modification was used in the present study [2,3]. In brief, approximately 4×10^4 cells / well were seeded in 48-well plates. The two target plasmids, 20 ng pBind RXR*α* LBD (provided by Dr. Xiao-kun Zhang from the Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA.) and 60 ng PG5 LUC (provided by Dr. Xiao-kun Zhang from the Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA.), were transfected by Liposome 2000 (Invitrogen) in the cell. After 24 h, the cells were exposed to the test compound for 12 h. Then the cells were rinsed with PBS and lysed by buffered solution (1 × PLB) on the oscillating platform for 15 minutes. According to the introduction of the Dual-Luciferase Reporter Assay System kit(promega), the activities of Firefly luciferase (FL) and Rellina luciferase (RL) were checked.

Relative luciferase activity (%) = FL / RL × 100%

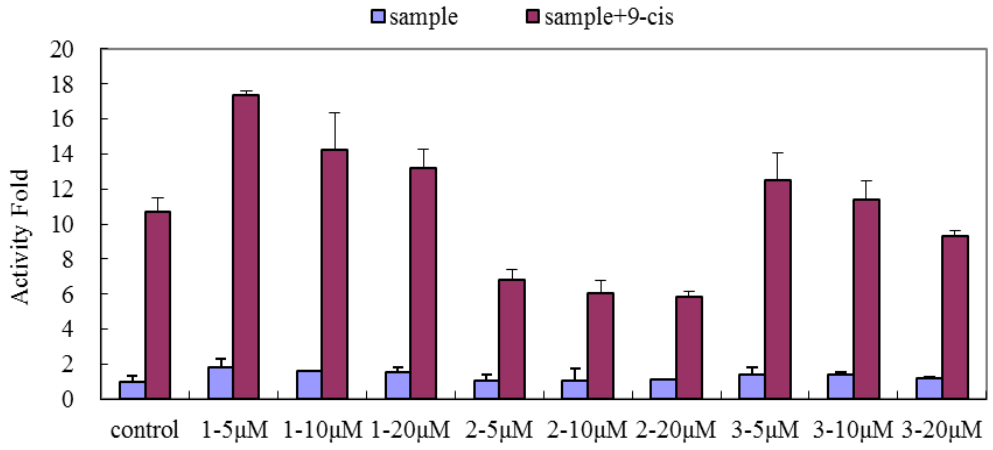


Chart 1. Effects of compounds 1-3 (5, 10, and 20μM) on the transcriptional activities of RXR*α*

Cytotoxicity assay

Cell Culture. Human cervical carcinoma HeLa cells were obtained from the {American Type Culture Collection (ATCC, Manassas, VA, USA)} and were cultured in {DMEM (Hyclone)} supplemented with 10% FBS (Fetal Bovine Serum, Hyclone, USA), 100U/mL penicillin (Hyclone), and 100 μg/mL streptomycin (Hyclone) at 37 °C with 5% CO_2 in a humidified atmosphere. The cells in the exponential phase of growth were used in the experiments.

MTT assay. All test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and further diluted in culture medium upon assay. HeLa cells were incubated in 96-well cell culture clusters (JET) at a density of 0.5×10^4 cells per well and cultured for 12 h. Thereafter, the cells were treated with 5, 10 and 20μM concentrations of **1**, **2** and **3** respectively. After cultured for 48 h, 20 μL of MTT (Solarbio) solution was added and the cells were incubated for an additional 4 h at 37 °C. Then the supernatant was discarded, and the deposited formazan formed in the cells was dissolved with 100 μL of DMSO. All optical densities were measured in the MTT assay using a microplate reader (Thermo Multiskan MK3, Thermo Scientific, Helsinki, Finland). The percentage of cell growth rate was calculated as follows：

$$\text{Growth Rate } (\%) = (OD_{sample}-OD_{blank})/(OD_{control}-OD_{blank})\times 100$$

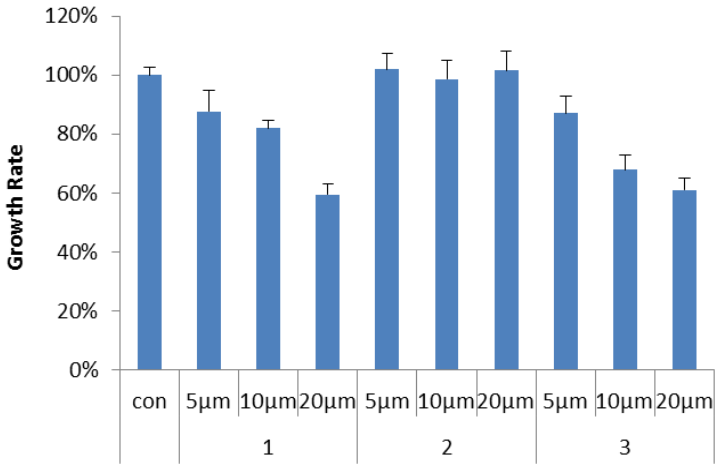


Chart 2. Effects of compounds 1-3 (5, 10, and 20μM) on the cytotoxic effects

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