Supplementary data

Norsampsone E, an unprecedented decarbonyl polycyclic polyprenylated acylphloroglucinols with homoadamantyl core from *Hypericum sampsonii*

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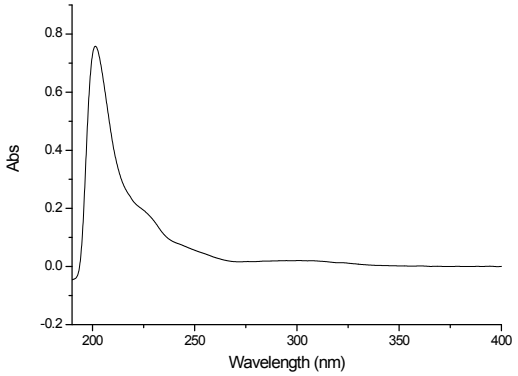
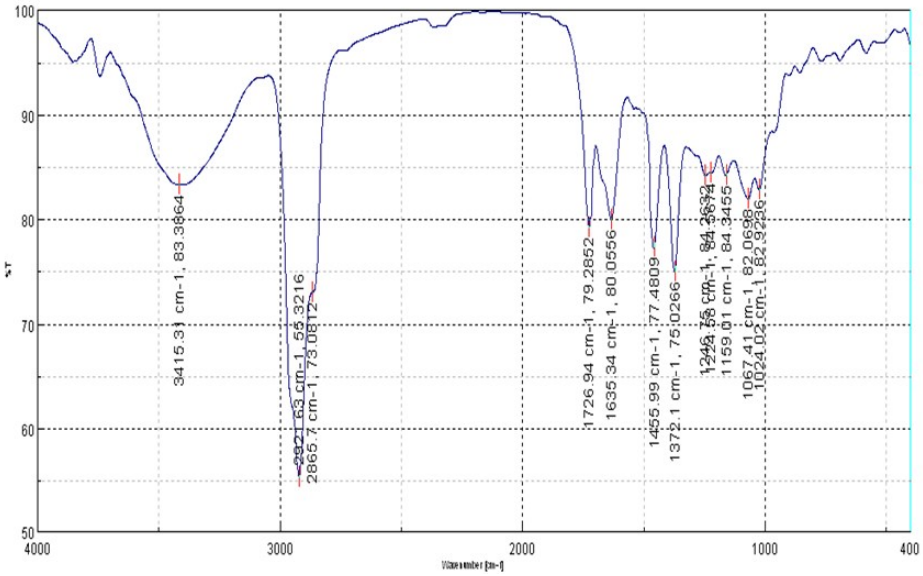
List of Supporting Information

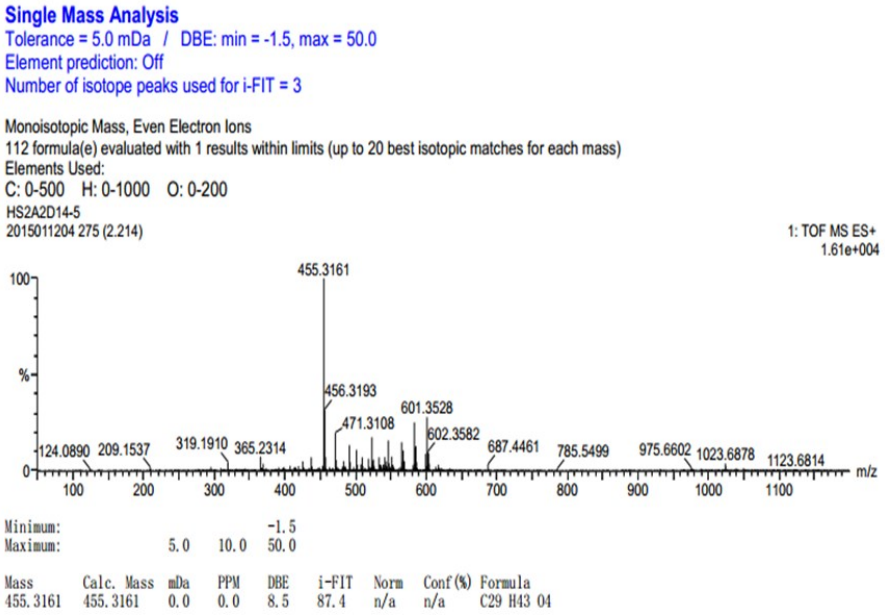
UV spectrum of Norsampsone E (1) in CH_3OH.



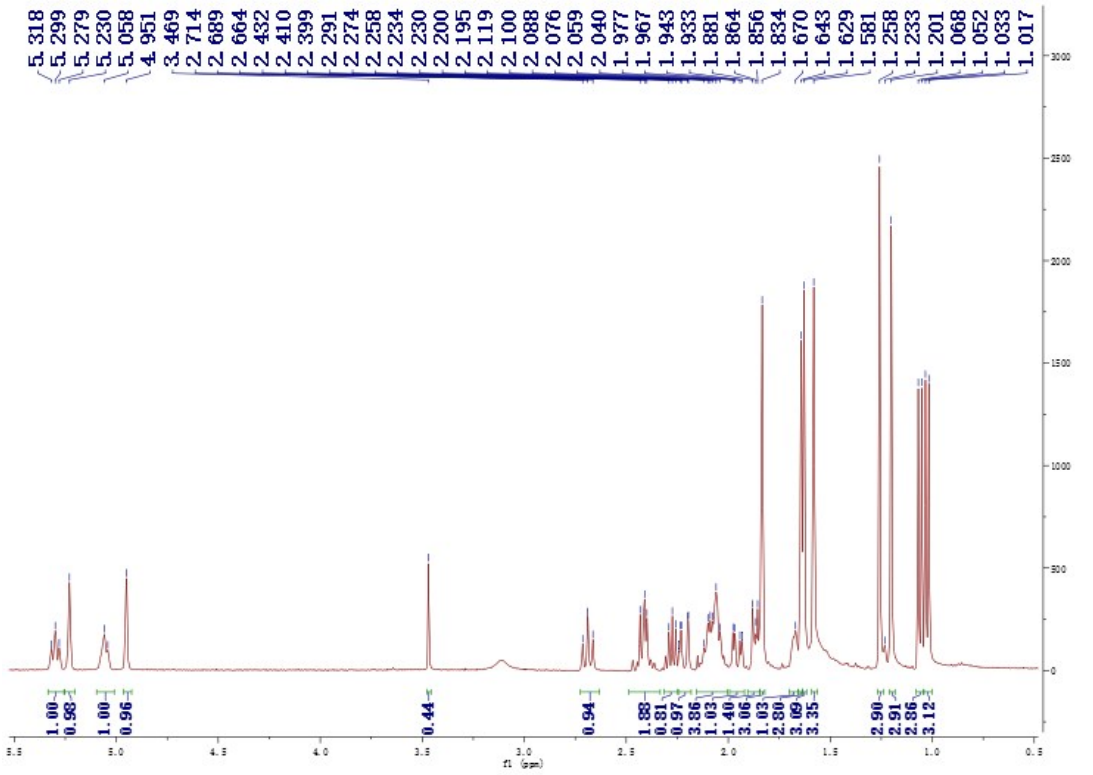
IR (KBr disc) spectrum of Norsampsone E (1).



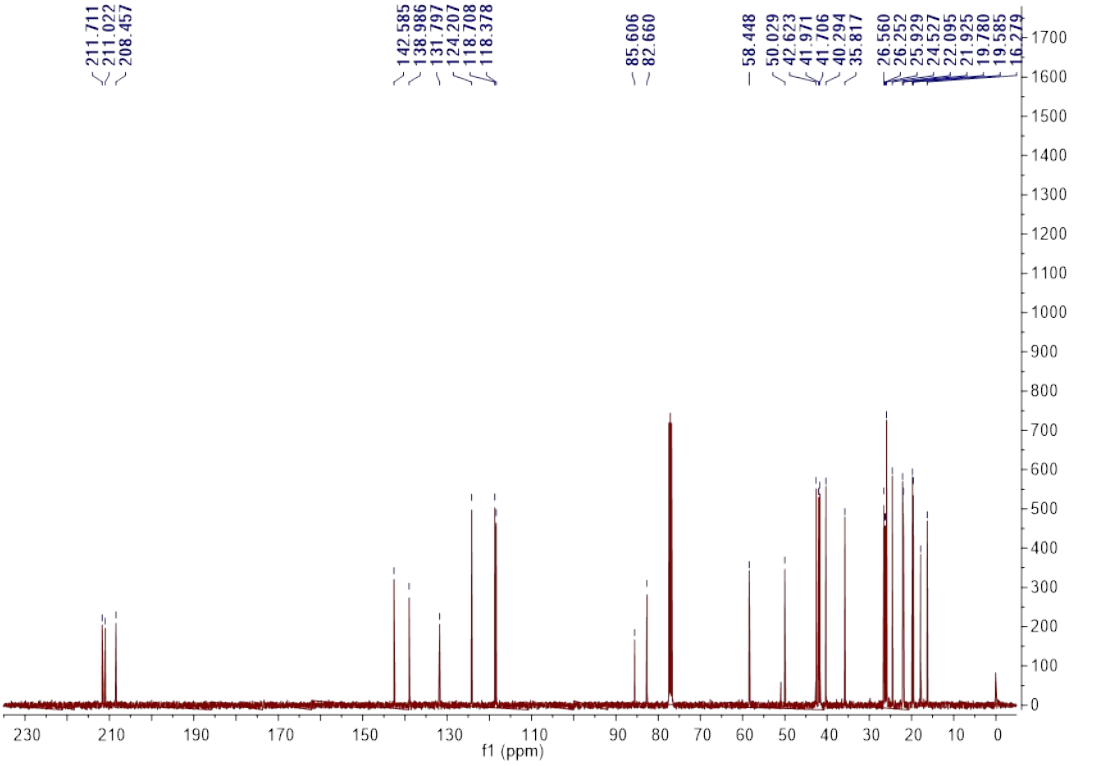
HR-ESI-MS spectrum of Norsampsone E (1).



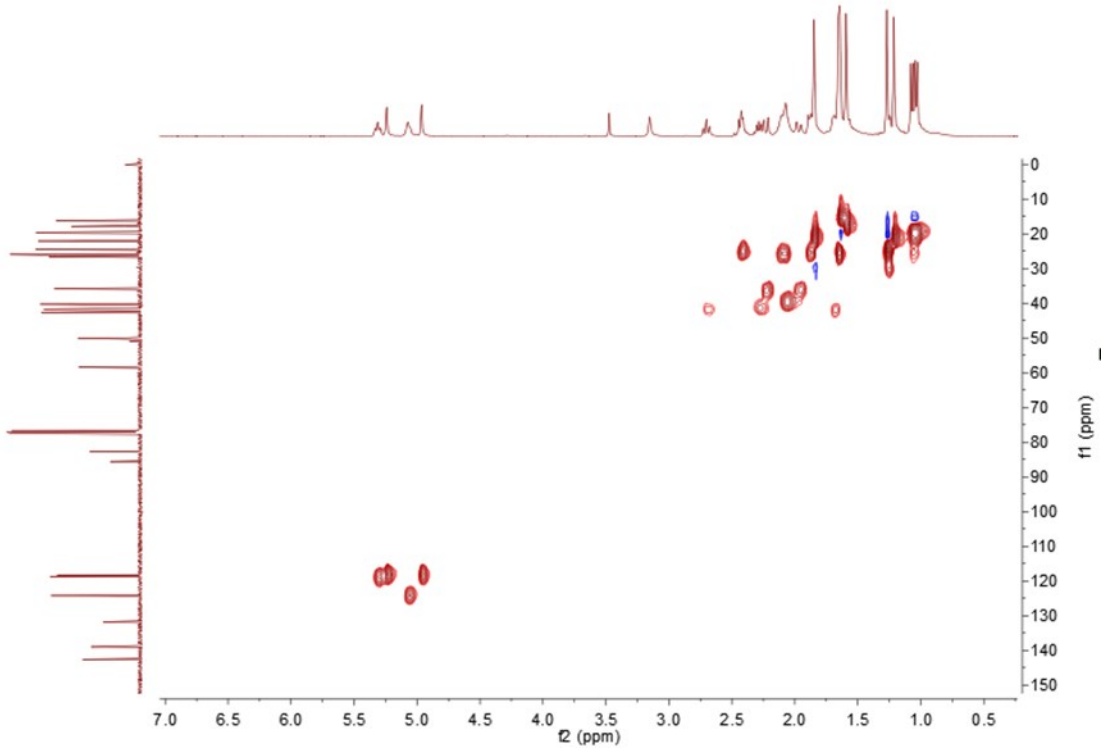
^{1}H NMR (AV-400, 400 MHz) spectrum of Norsampsone E (1) in $CDCl_3$



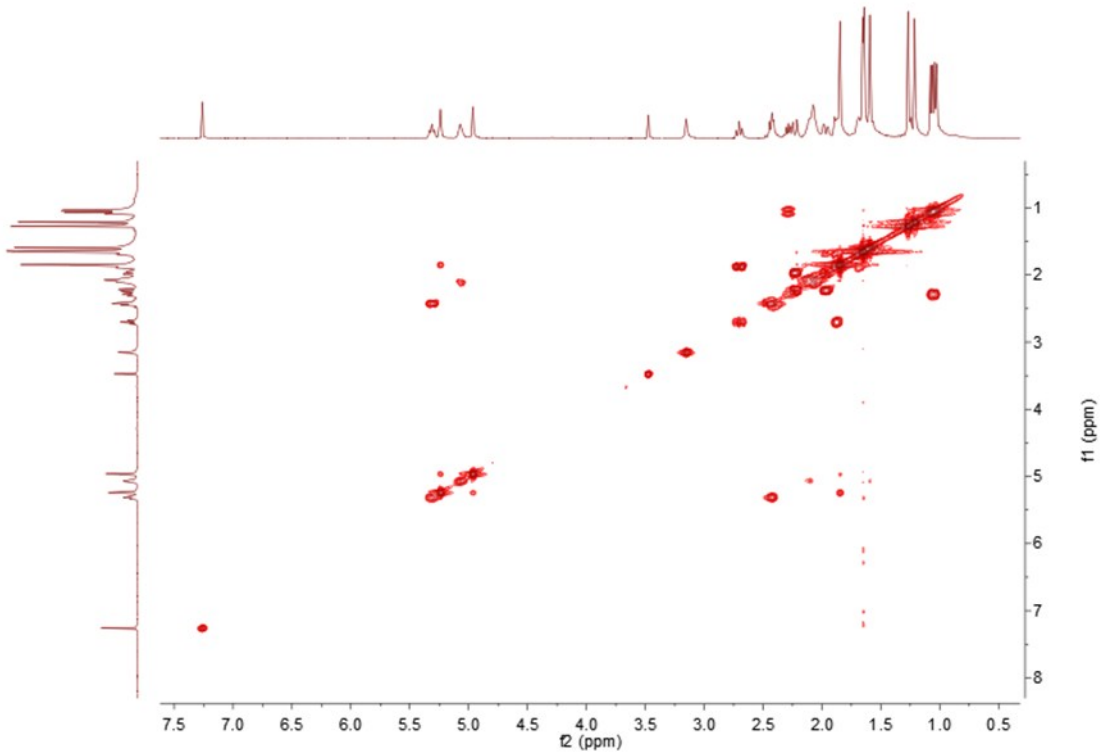
^{13}C NMR spectrum (AV-400, 100 MHz) of Norsampsone E (1) in $CDCl_3$



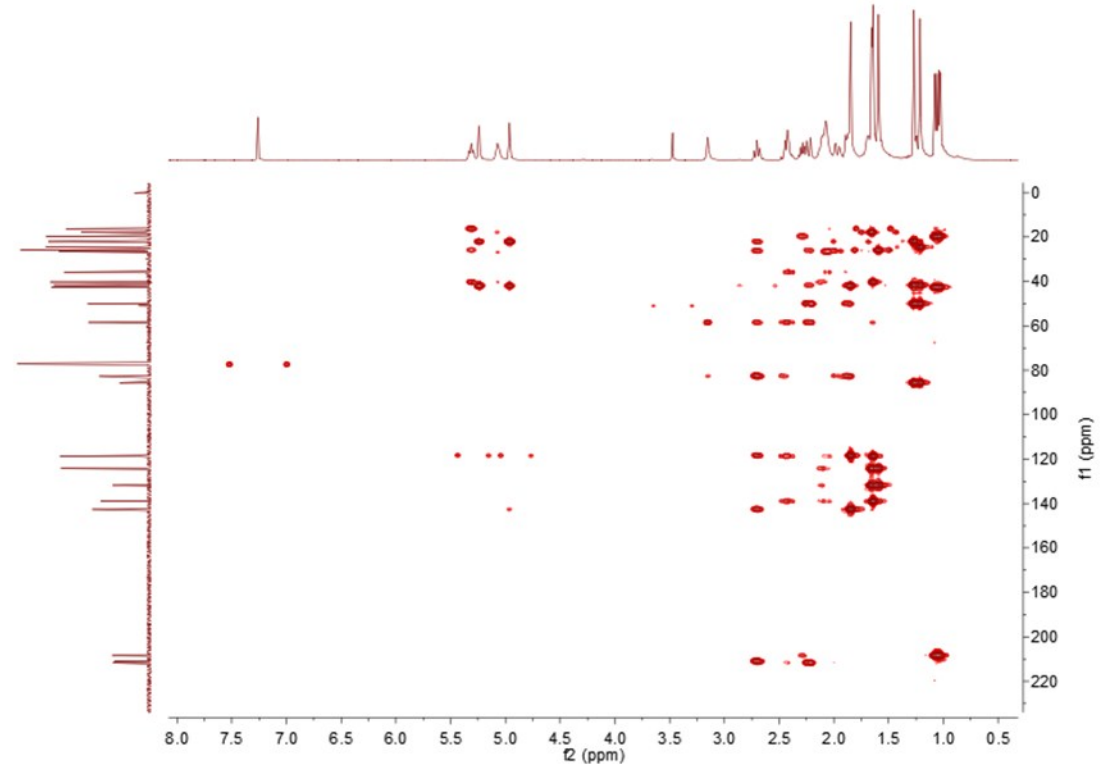
HSQC spectrum (AV-400) of Norsampsone E (1) in $CDCl_3$



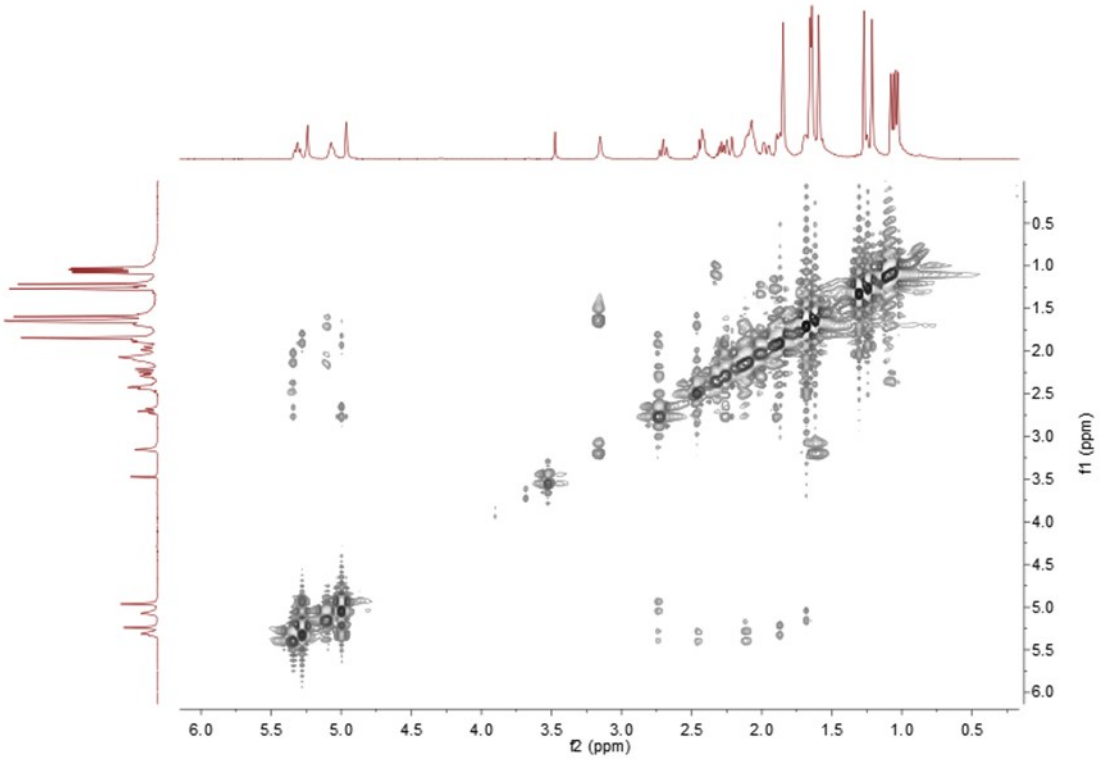
$^1H-^1H$ COSY spectrum (AV-400) of Norsampsone E (1) in $CDCl_3$



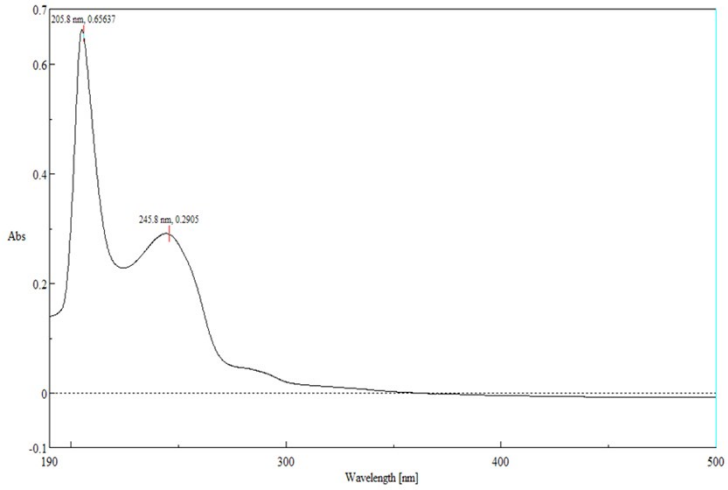
HMBC spectrum (AV-400) of Norsampsone E (1) in $CDCl_3$



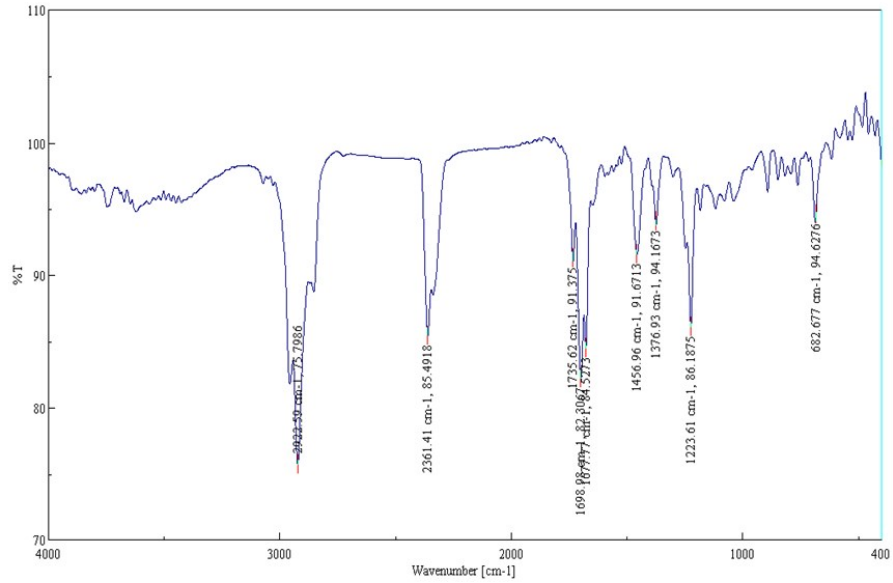
NOESY spectrum (AV-400) of Norsampsone E (1) in $CDCl_3$



UV spectrum of hypersampsone X (2) in CH_3OH.



IR (KBr disc) spectrum of hypersampsone X (2).

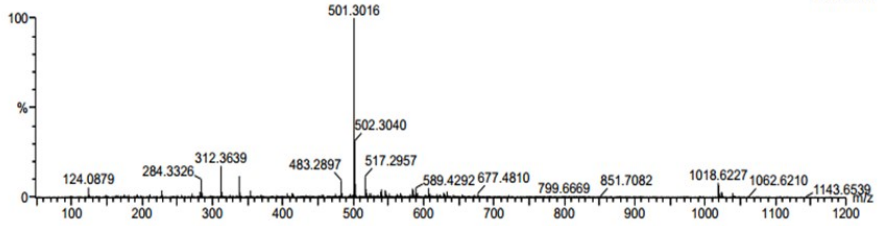


HR-ESI-MS spectrum of hypersampsone X (2).

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

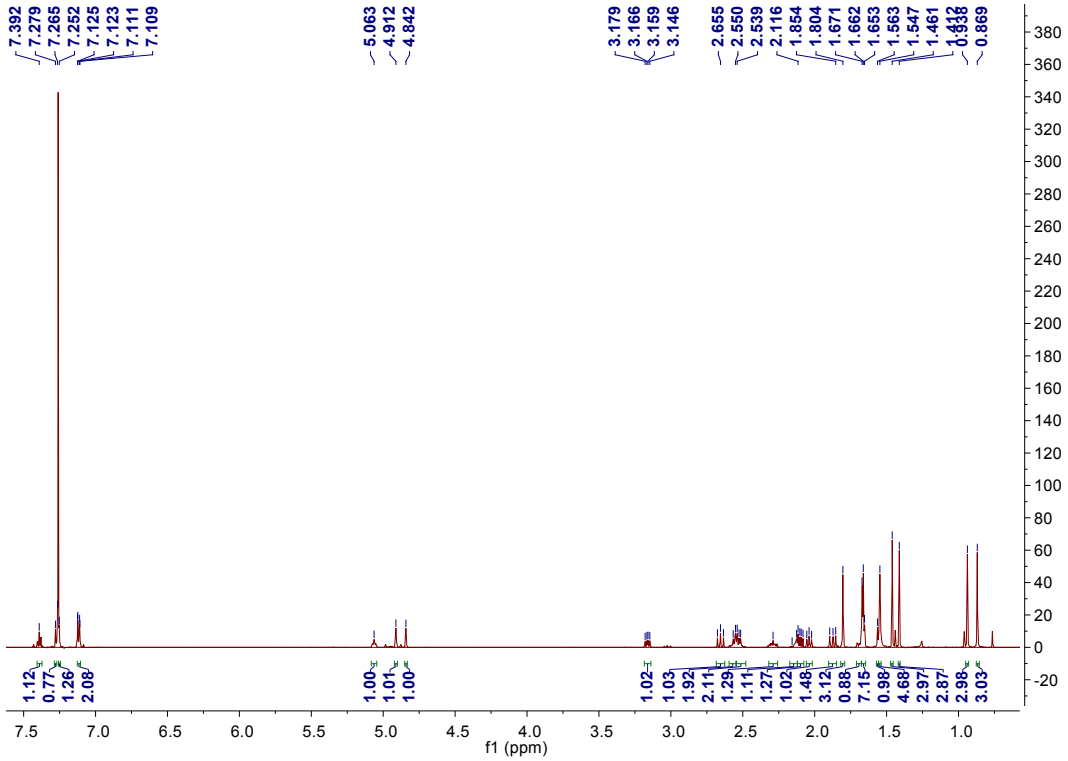
Monoisotopic Mass, Even Electron Ions
677 formula(e) evaluated with 4 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200 Na: 0-1 Br: 0-8

2015011206 266 (2.147) 1: TOF MS ES+ 2.72e+004

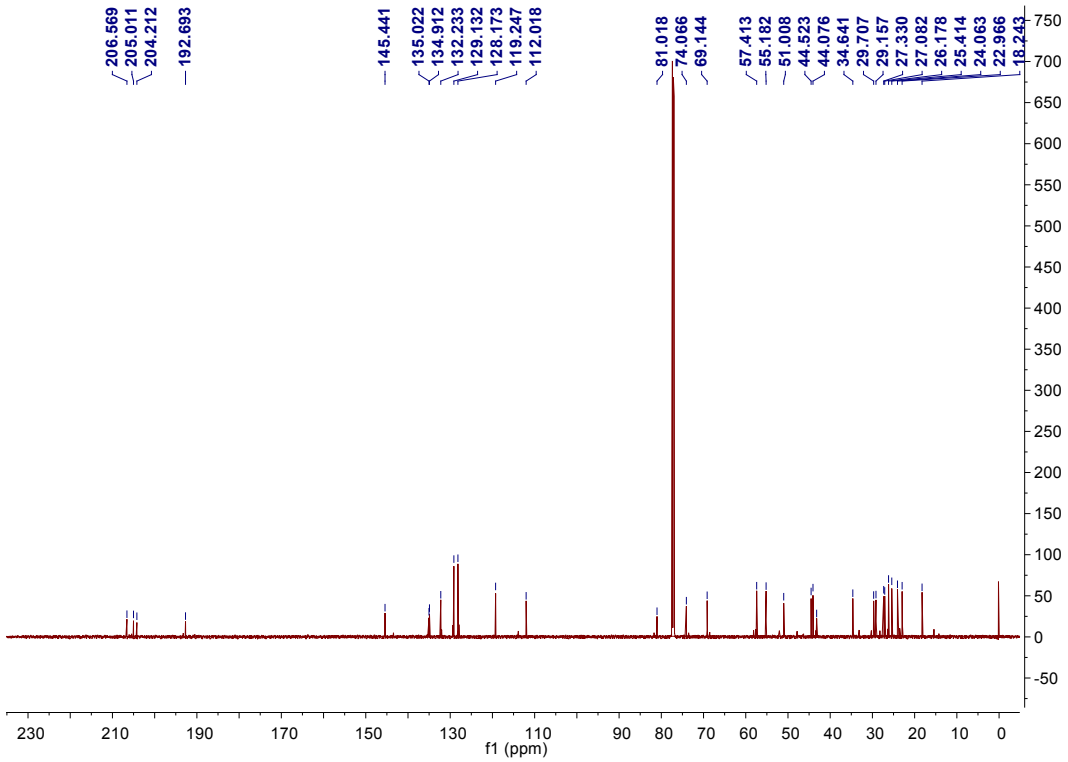


Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
501.3016	501.2981	3.5	7.0	10.5	137.4	0.762	46.66	C31 H42 O4 Na
	501.3064	-4.8	-9.6	4.5	137.9	1.214	29.71	C26 H45 O9
	501.3005	1.1	2.2	13.5	138.5	1.793	16.65	C33 H41 O4
	501.3040	-2.4	-4.8	1.5	139.3	2.663	6.98	C24 H46 O9 Na

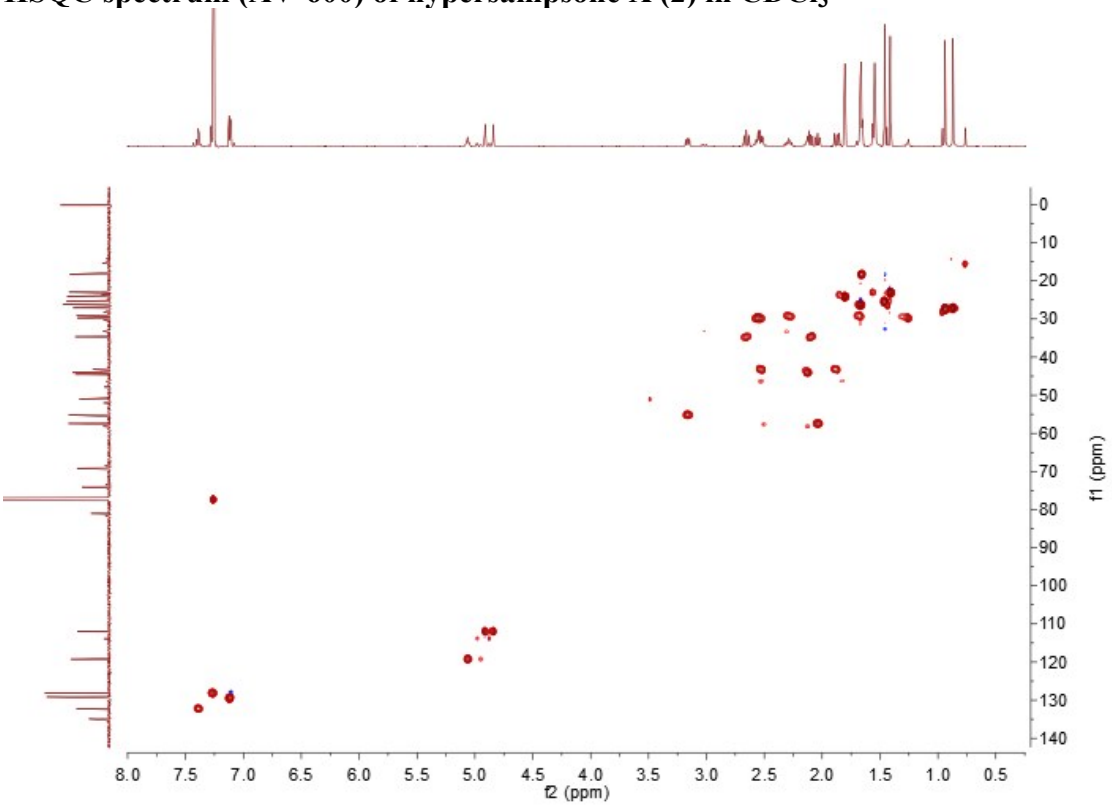
^{1}H NMR (AV-600, 600 MHz) spectrum of hypersampsone X (2) in $CDCl_3$



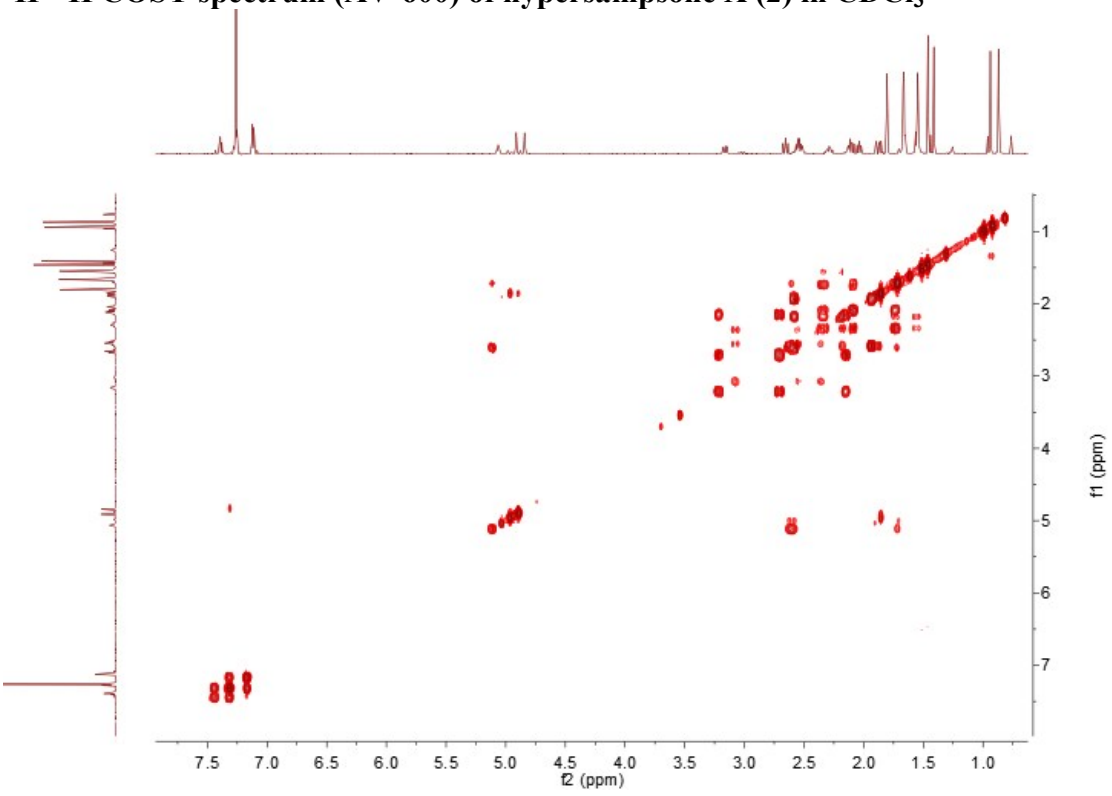
^{13}C NMR spectrum (AV-600, 150 MHz) of hypersampsone X (2) in $CDCl_3$



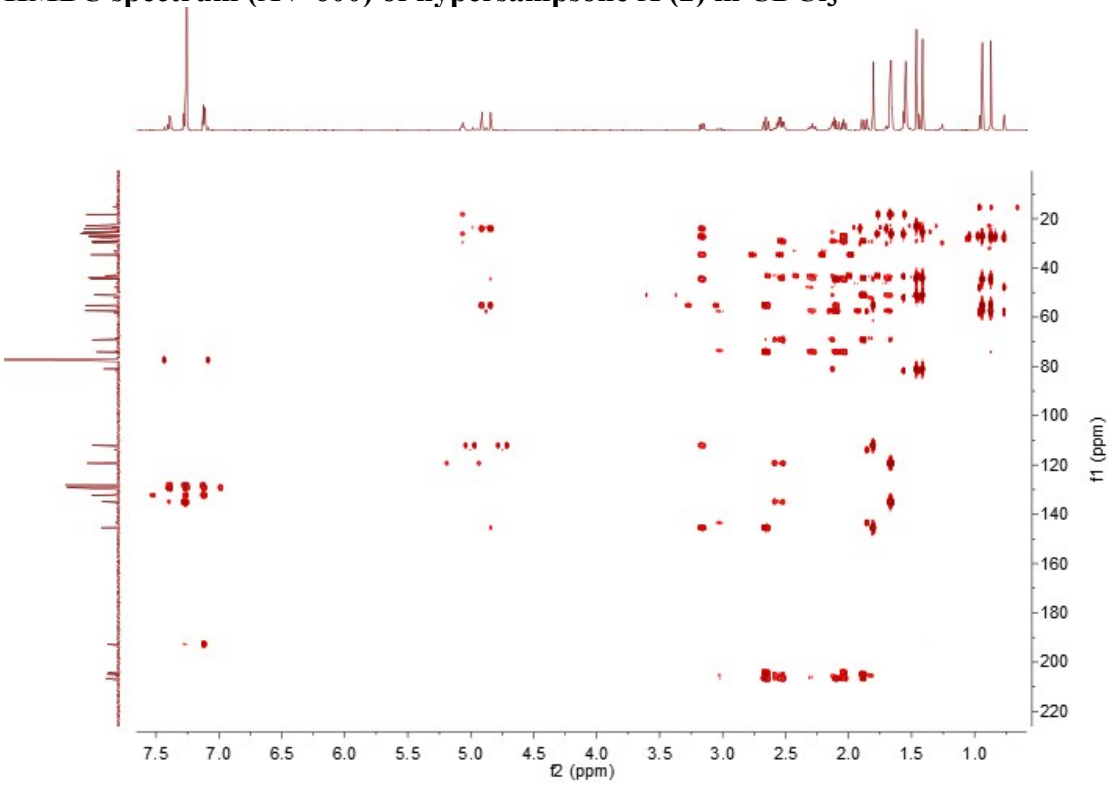
HSQC spectrum (AV-600) of hypersampsone X (2) in $CDCl_3$



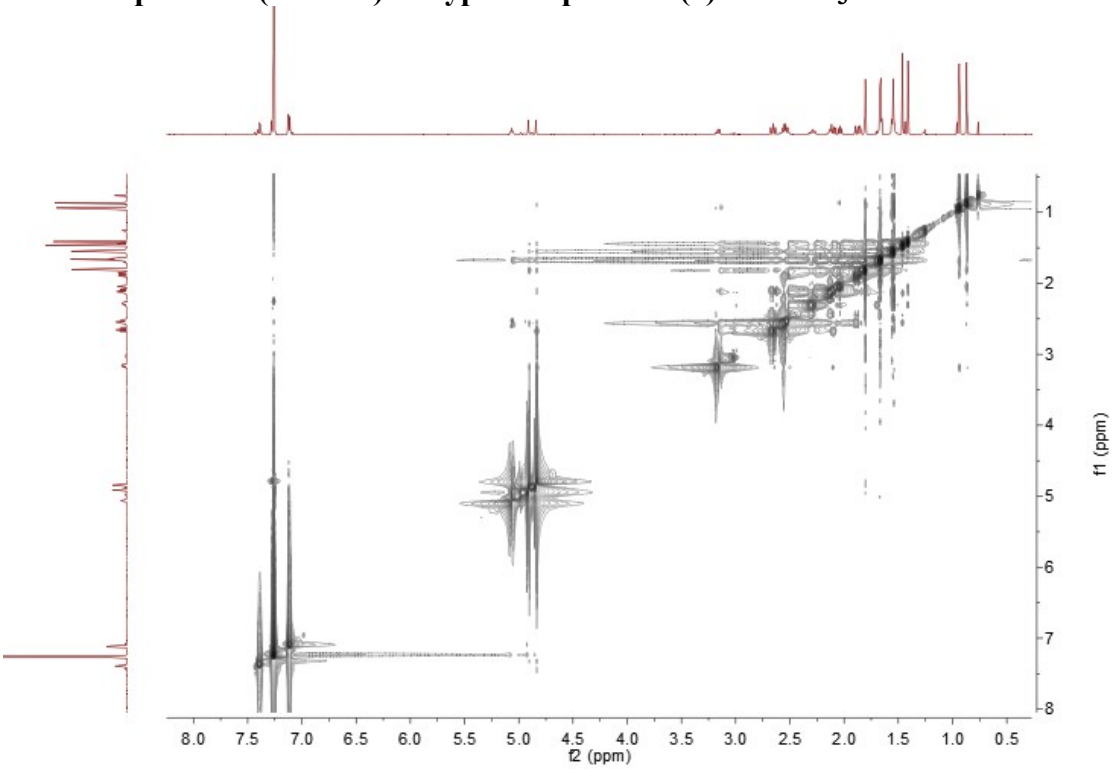
$^{1}H-^{1}H$ COSY spectrum (AV-600) of hypersampsone X (2) in $CDCl_3$



HMBC spectrum (AV-600) of hypersampsone X (2) in $CDCl_3$



NOESY spectrum (AV-600) of hypersampsone X (2) in $CDCl_3$

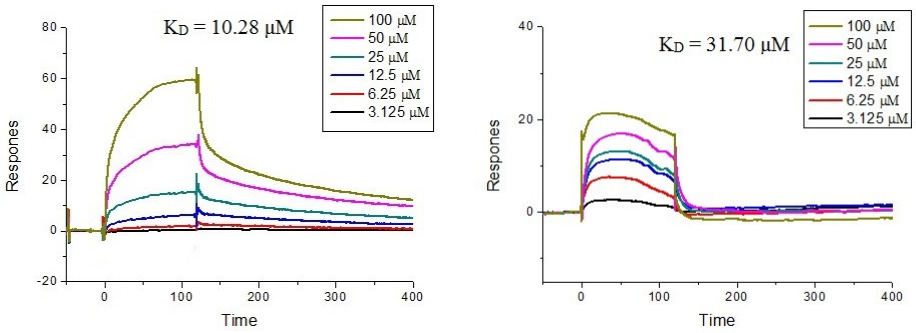


Bioassays

Biacore assay

RXRa-LBD protein purified. The 0.79 kb DNA fragment corresponding to the ligand-binding domain (LBD) of human RXRα (genes 592-1386) was excised from MCF-7 cells and sub-cloned into pET-15b between the BamHI and NheI restriction site. Transformed E. coli BL_{21}(DE3) were grown at 37℃ in LB medium until OD_{600} = 0.6-0.8, Protein expression was initiated by 0.4 mM IPTG, and this procedure sustained at 18 ℃ for 16 h. Following centrifugation, resuspension and sonication processes, the combinated protein was purificated by Ni^{2+}-NTA agarose column at low temperatures.

SPR assay. The measurements were performed on the Biacore T200 (Biacore GE) at 25 ºC in a running buffer comprising PBS (pH 7.5), 150 mM NaCl, 10 mM $MgCl_2$ and 0.1% P20. CM5 chips (Biacore GE) were first treated with EDC-NHS mixture at a flow rate of 10 ml/min. Purified RXRα-LBD protein was immobilized on the sensor chip by the standard amine coupling protocol with resonance unit around 8000 RU. All test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and further diluted in PBS. Gradient concentrations of each compound (100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM) were injected through flow cells immobilized with purified RXRα-LBD protein. The chip is being exposed to ligand solution during 0–120 s, and the ligand is dissociated from the chip by running buffer from 120 to 420 s. The K_D values were calculated from the experimental curve with Biacore T200 evaluation software package. The formation of surface-bound complexes was analyzed according to the interaction type of A+B↔AB.



Compound **1** Compound **2**

Figure 1. SPR results of compound 1-2 binding to RXRα-LBD.

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 [1, 2]. In brief, approximately 1.5 × 10^4 cells / well were seeded in 96-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 50 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%

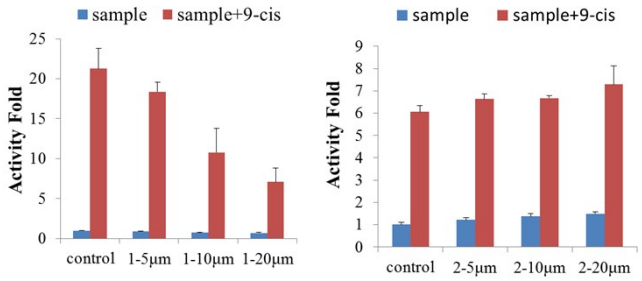


Figure 2. Effects of compounds 1-2 (5, 10, and 20*μ*M) on the transcriptional activities of RXR*α*

Reference

(1) Zhang, X. K.; Lehmann, J.; Hoffmann, B.; Dawson, M. I.; Cameron,J.; Graupner, G.; Hermann, T.; Tran, P.; Pfahl, M. *Nature* **1992**, *358*, 587–591.

(2) Duan, Y. H.; Dai, Y.; Wang, G. H.; Zhang, X.; Chen, H. F.; Chen, J. B.; Yao, X. S.; Zhang, X. K. *J. Nat. Prod.* **2010**, 73, 1283-1287.