Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary data

Hypersampsones S-W, new polycyclic polyprenylated acylphloroglucinols from *Hypericum sampsonii*

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[b] *Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou 510632, P. R. China.*
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[d] *College of Chemistry and Chemical Engineering, Lanzhou University, lanzhou 730000, P. R. China.*
[e] *State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Taipa, Macau, P. R. China.*

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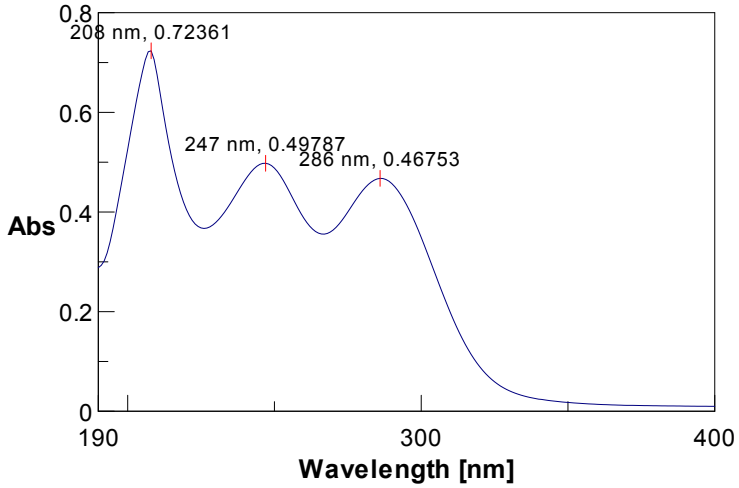
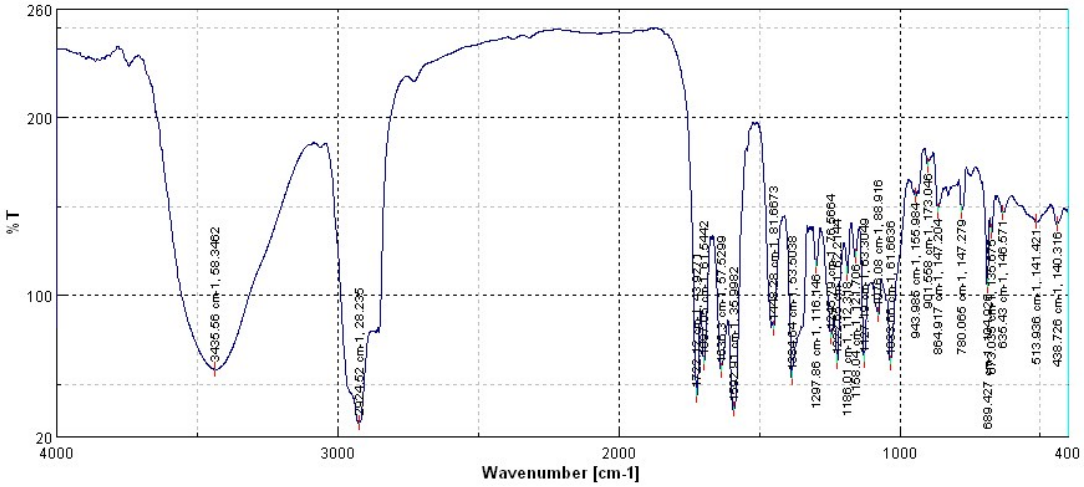
* E-mail address: tyaoxs@jnu.edu.cn; daiyi1004@163.com

List of Supporting Information

UV spectrum of hypersampsone S (1) in CH_3OH.



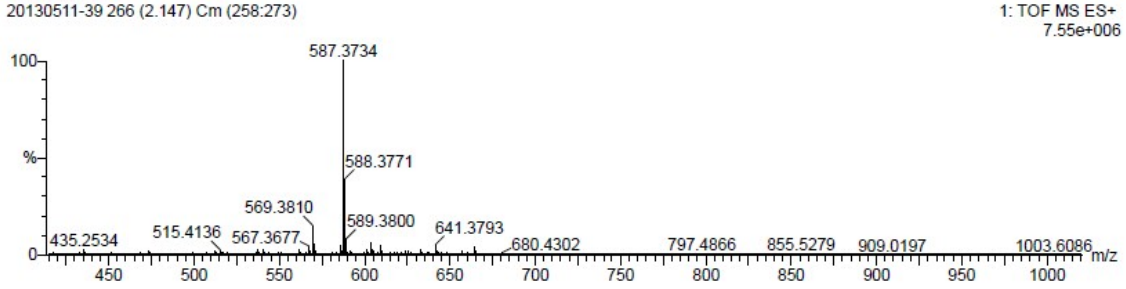
IR (KBr disc) spectrum of hypersampsone S (1).



HR-ESI-MS spectrum of hypersampsone S (1).

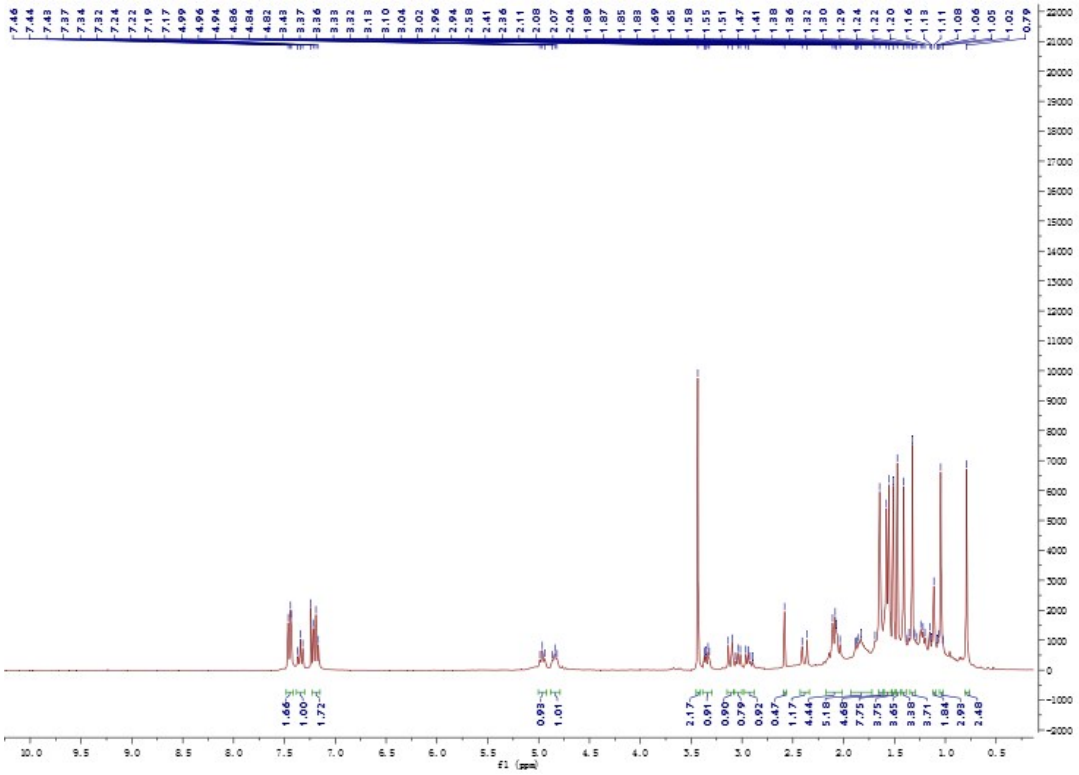
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
173 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
HSD5F8B-3
20130511-39 266 (2.147) Cm (258:273)

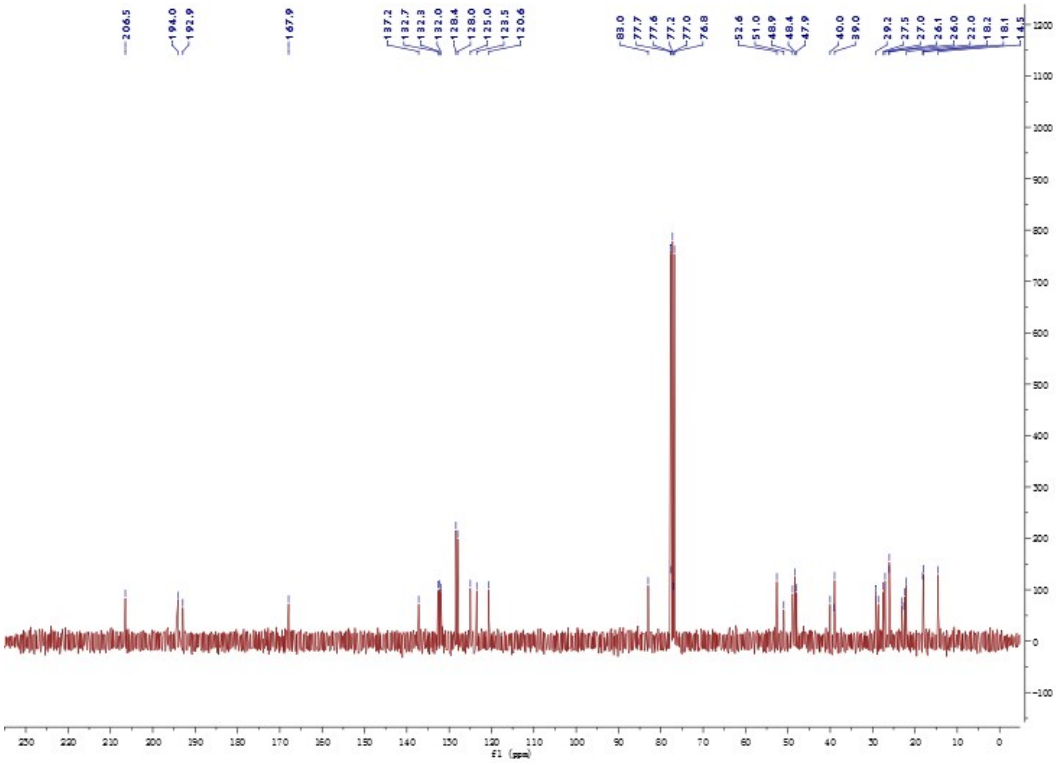


Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
587.3734	587.3736	-0.2	-0.3	13.5	1517.1	n/a	n/a	C38 H51 O5

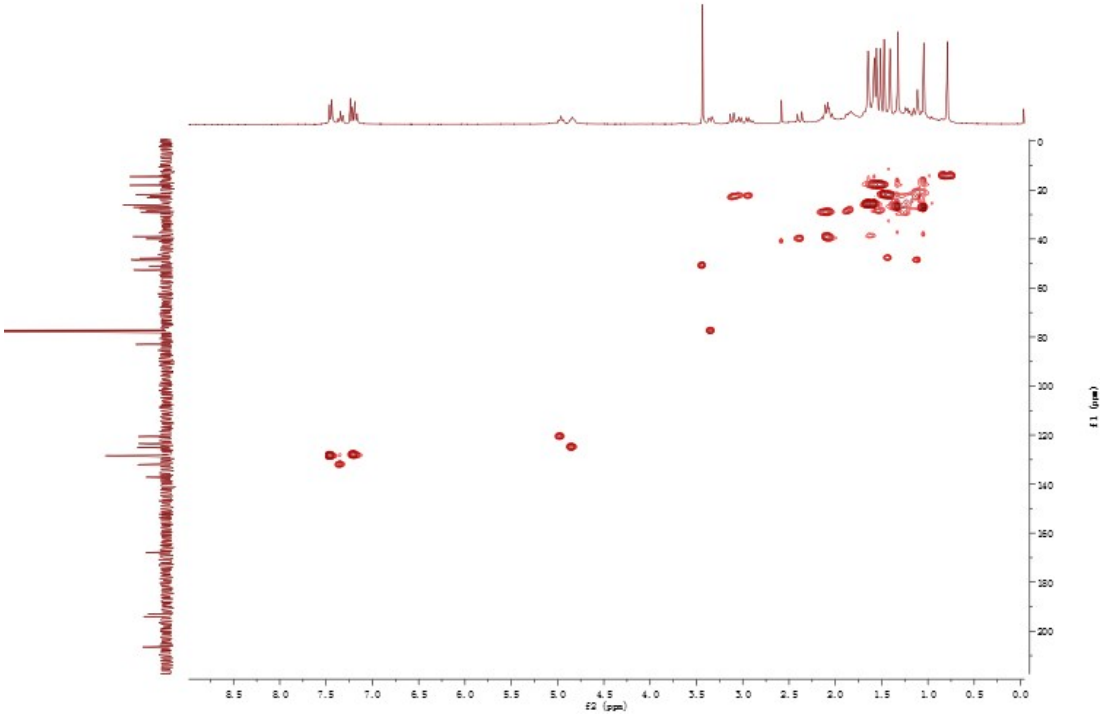
^{1}H NMR (AV-300, 300 MHz) spectrum of hypersampsone S (1) in $CDCl_3$



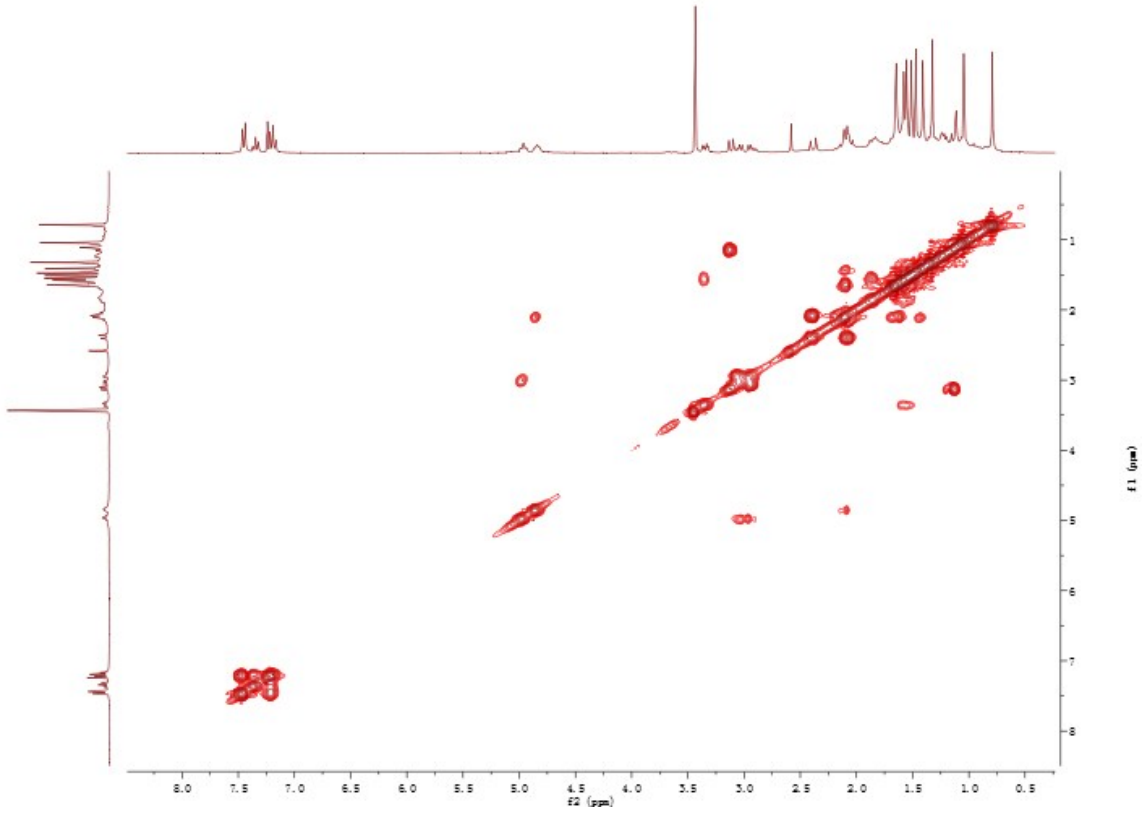
^{13}C NMR spectrum (AV-300, 75 MHz) of hypersampsone S (1) in $CDCl_3$



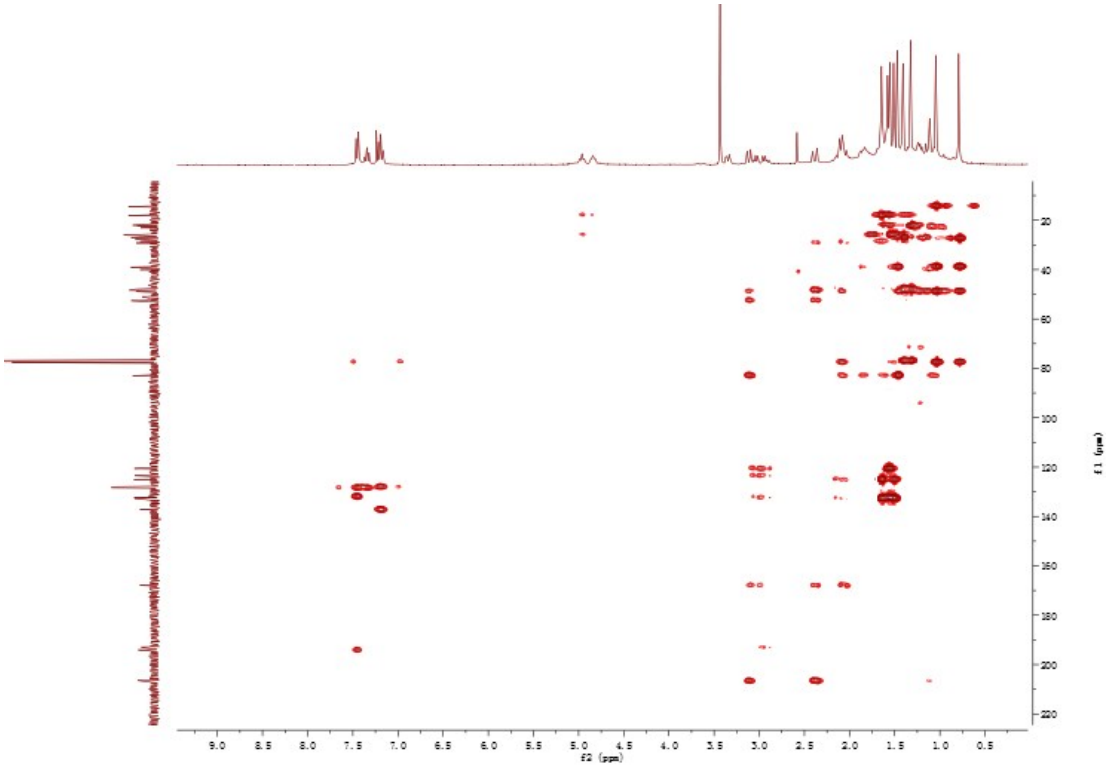
HSQC spectrum (AV-400) of hypersampsone S (1) in $CDCl_3$



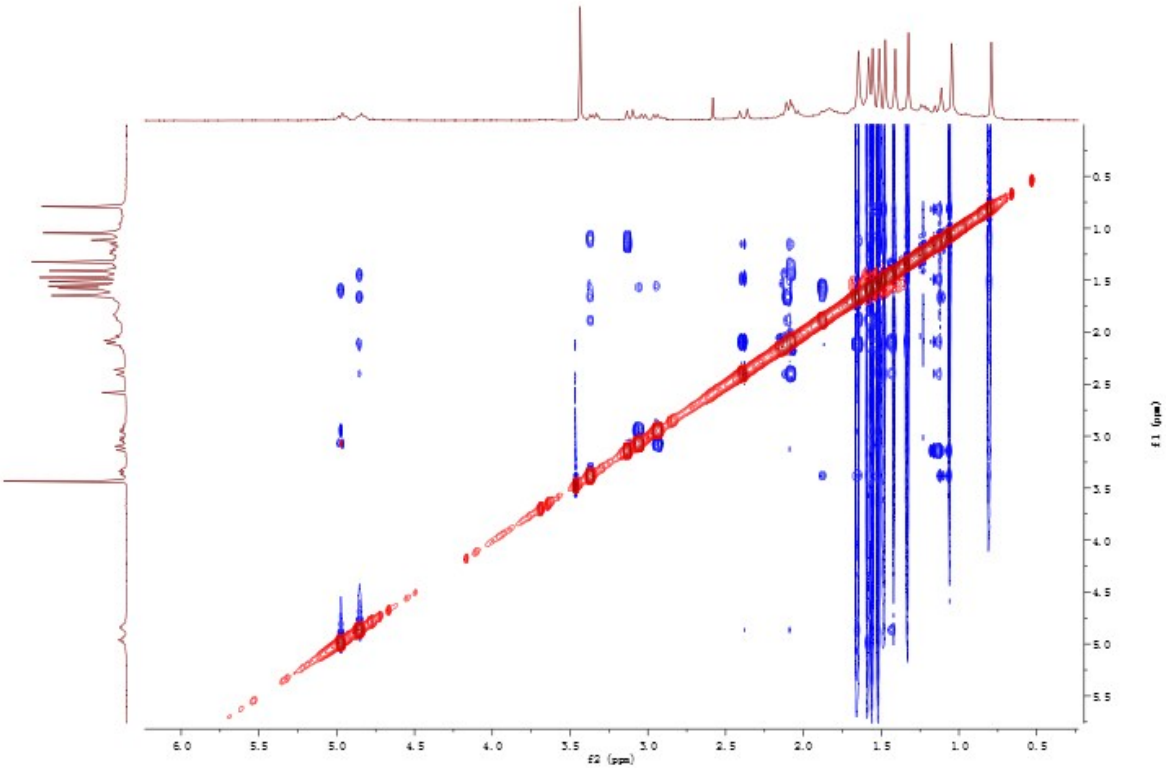
$^1H-^1H$ COSY spectrum (AV-400) of hypersampsone S (1) in $CDCl_3$



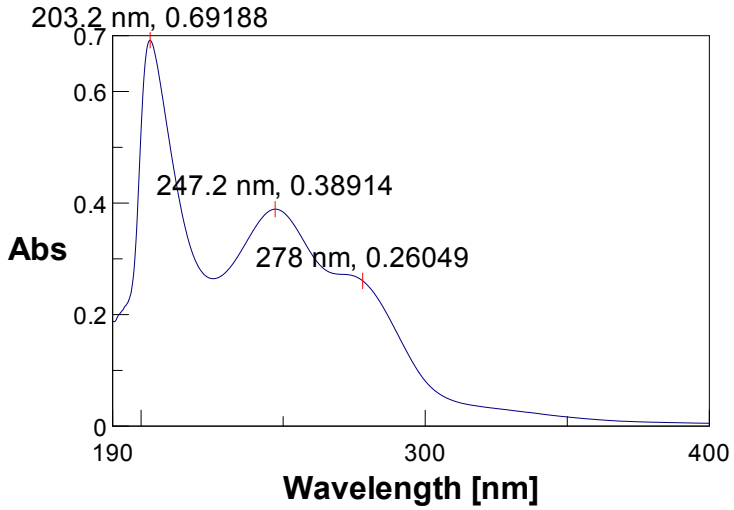
HMBC spectrum (AV-400) of hypersampsone S (1) in $CDCl_3$



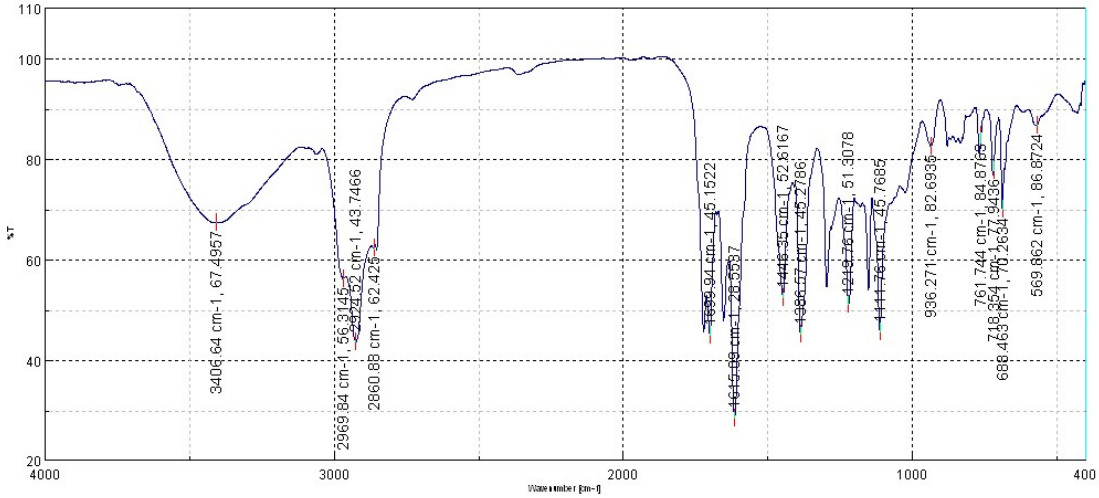
NOESY spectrum (AV-600) of hypersampsone S (1) in $CDCl_3$



UV spectrum of hypersampsone T (2) in CH_3OH.



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

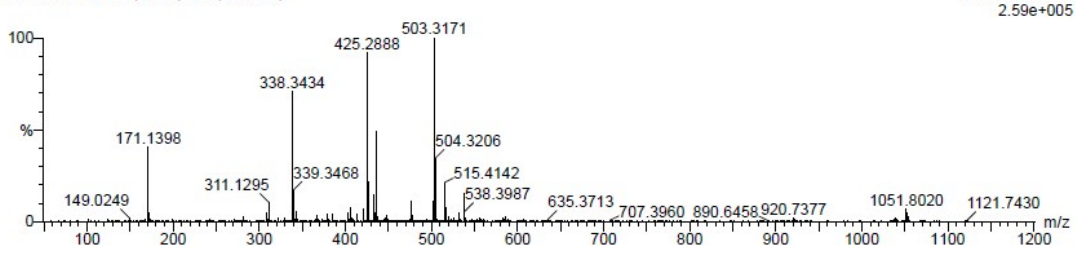


HR-ESI-MS spectrum of hypersampsone T (2).

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

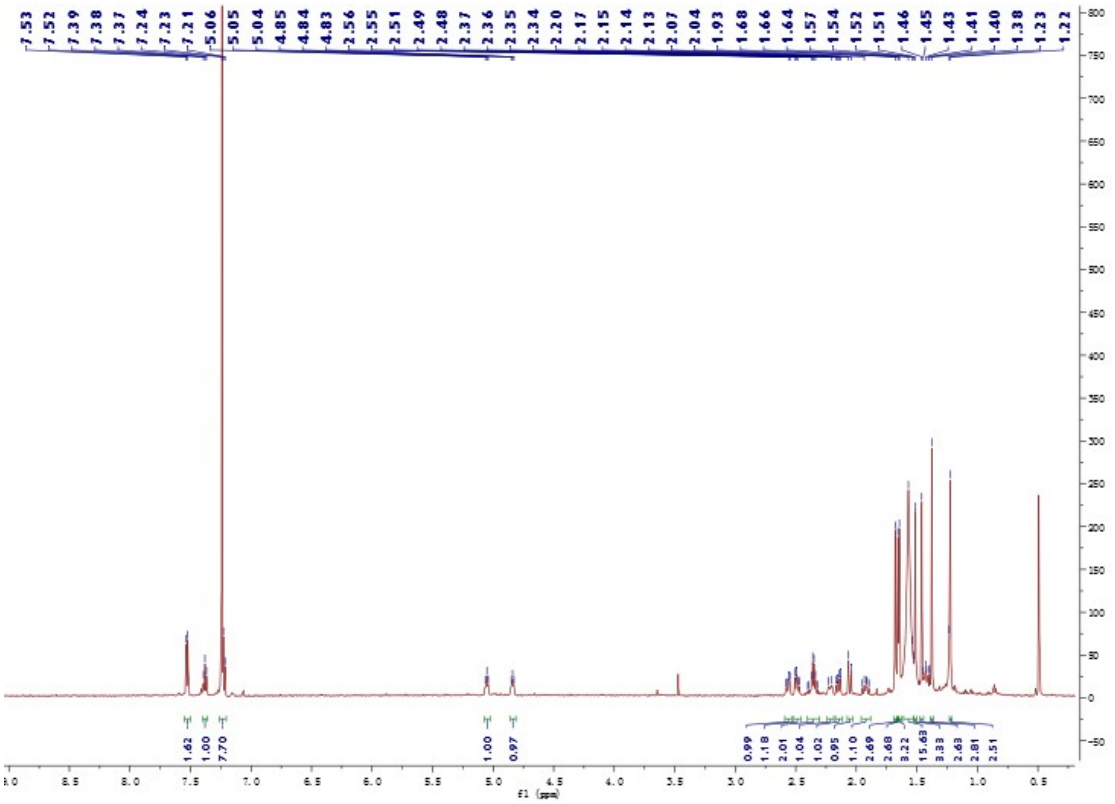
Monoisotopic Mass, Even Electron Ions
133 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200
HSD1E-4
20131016-02 275 (2.214) Cm (274:277)

1: TOF MS ES+
2.59e+005

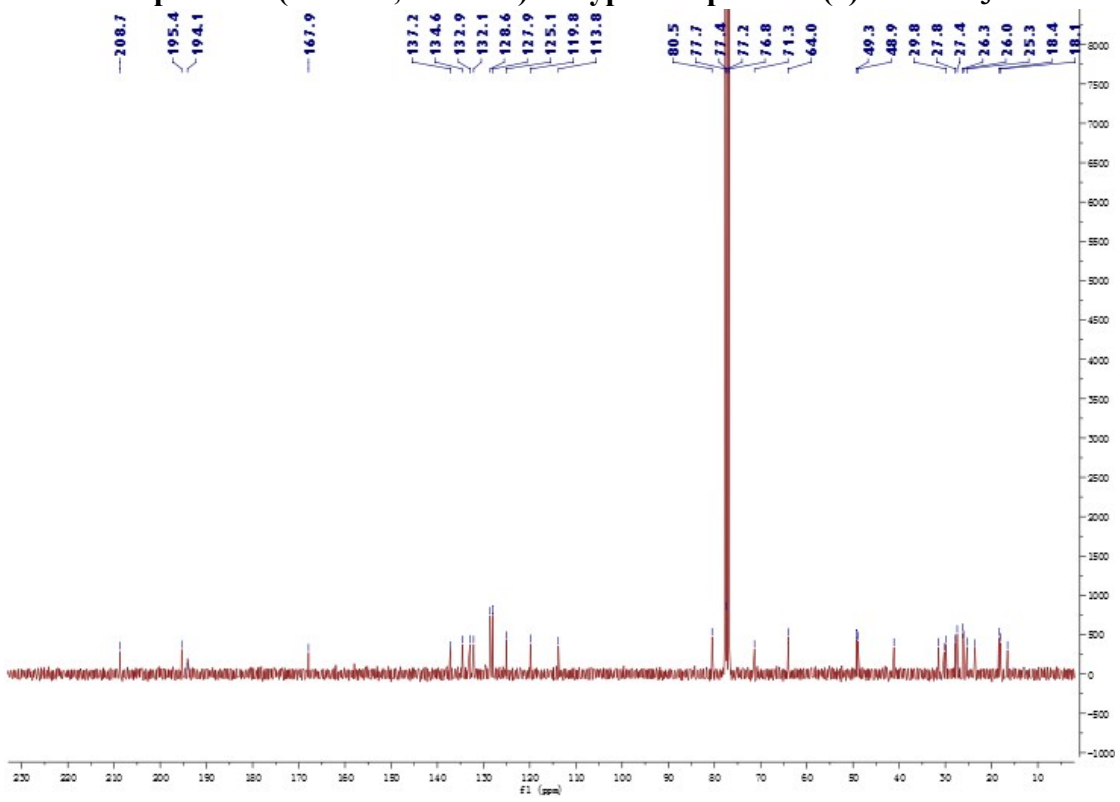


Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
503.3171	503.3161	1.0	2.0	12.5	447.6	0.001	99.88	C33 H43 O4
	503.3220	-4.9	-9.7	3.5	454.3	6.691	0.12	C26 H47 O9

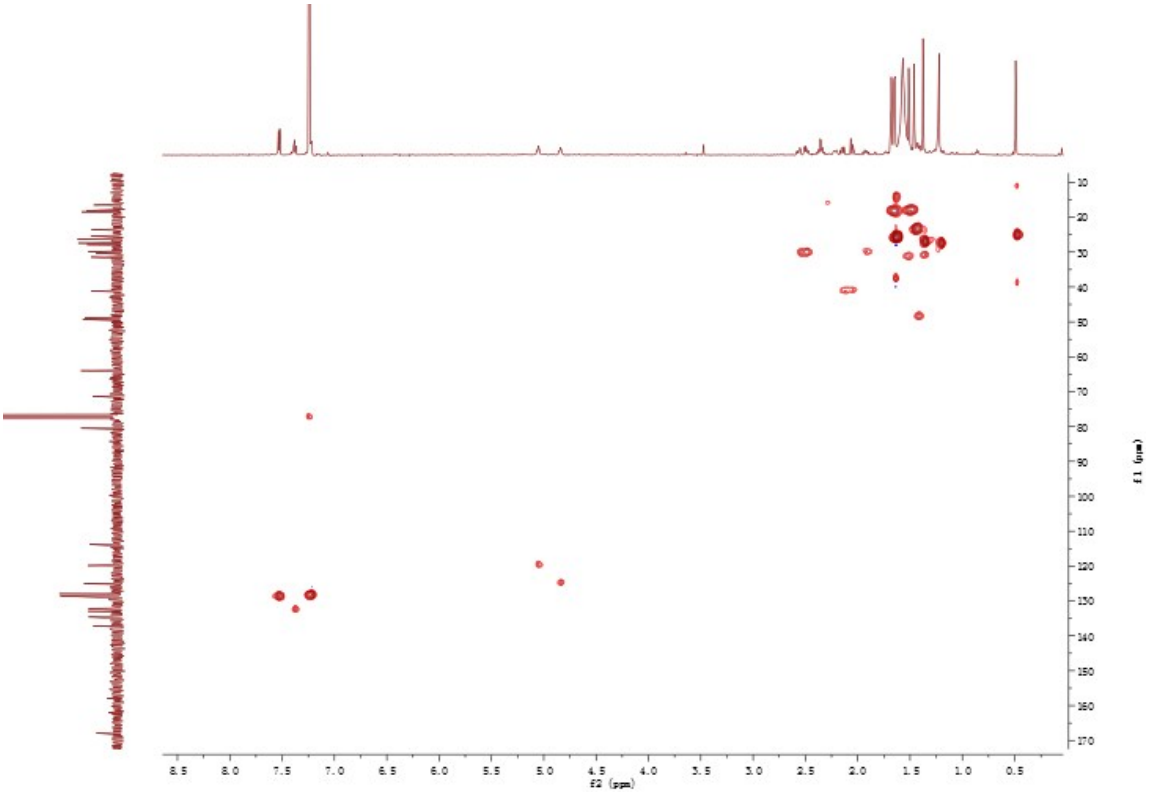
^{1}H NMR (AV-600, 600 MHz) spectrum of hypersampsone T (2) in $CDCl_3$



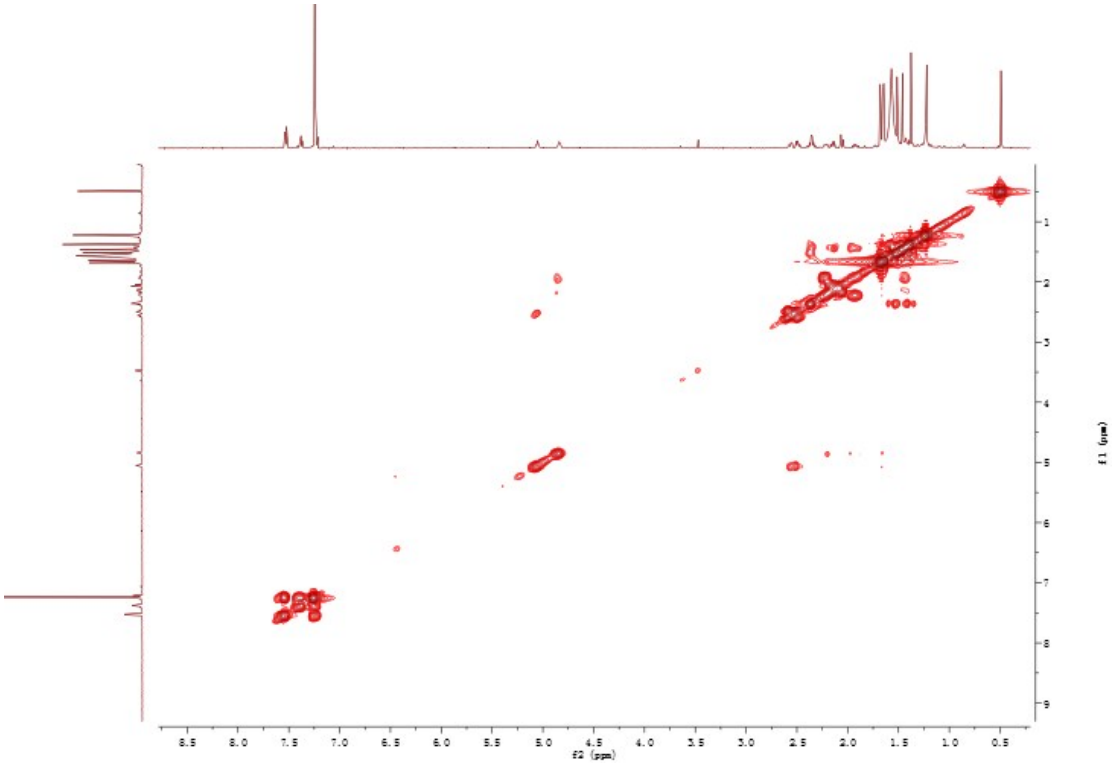
^{13}C NMR spectrum (AV-300, 75 MHz) of hypersampsone T (2) in $CDCl_3$



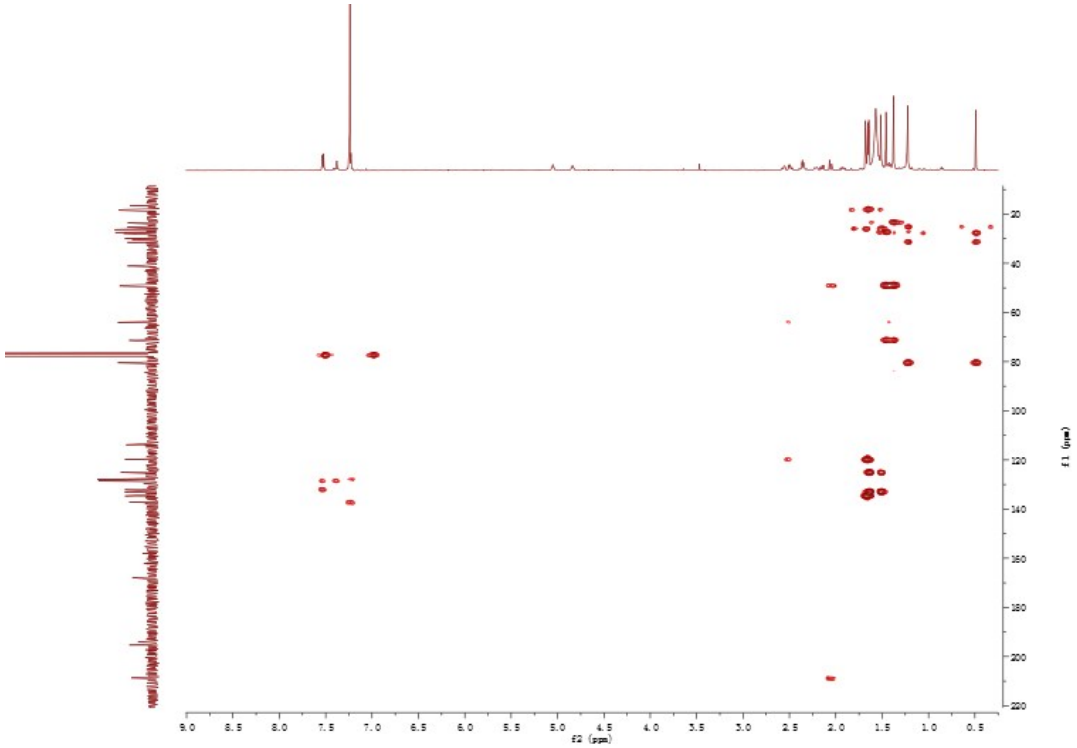
HSQC spectrum (AV-400) of hypersampsone T (2) in $CDCl_3$



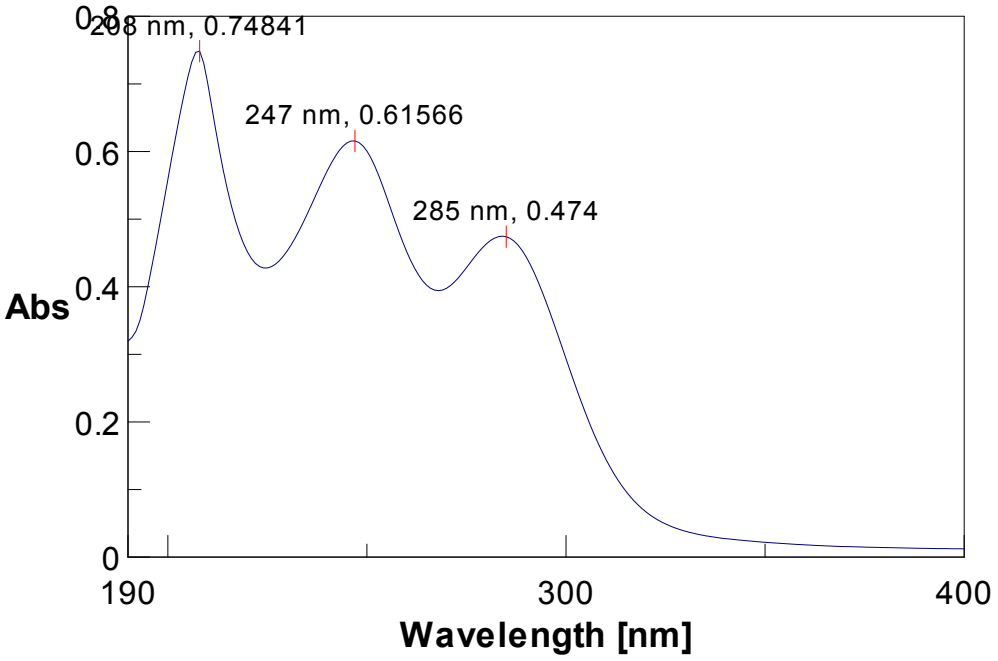
$^{1}H-^{1}H$ COSY spectrum (AV-400) of hypersampsone T (2) in $CDCl_3$



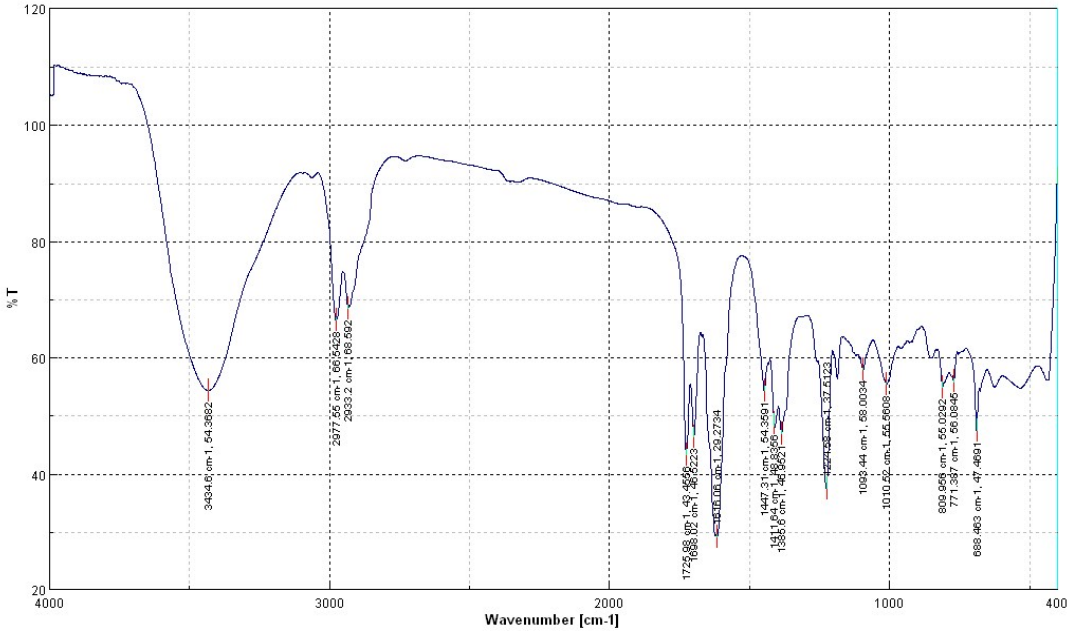
HMBC spectrum (AV-400) of hypersampsone T (2) in $CDCl_3$



UV spectrum of hypersampsone U (3) in CH_3OH.



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



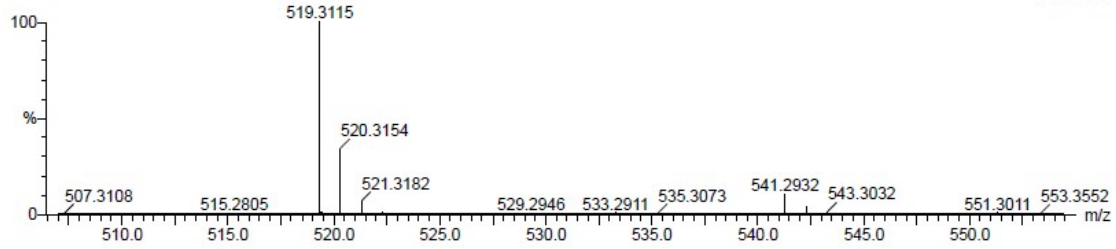
HR-ESI-MS spectrum of hypersampsone U (3).

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
140 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

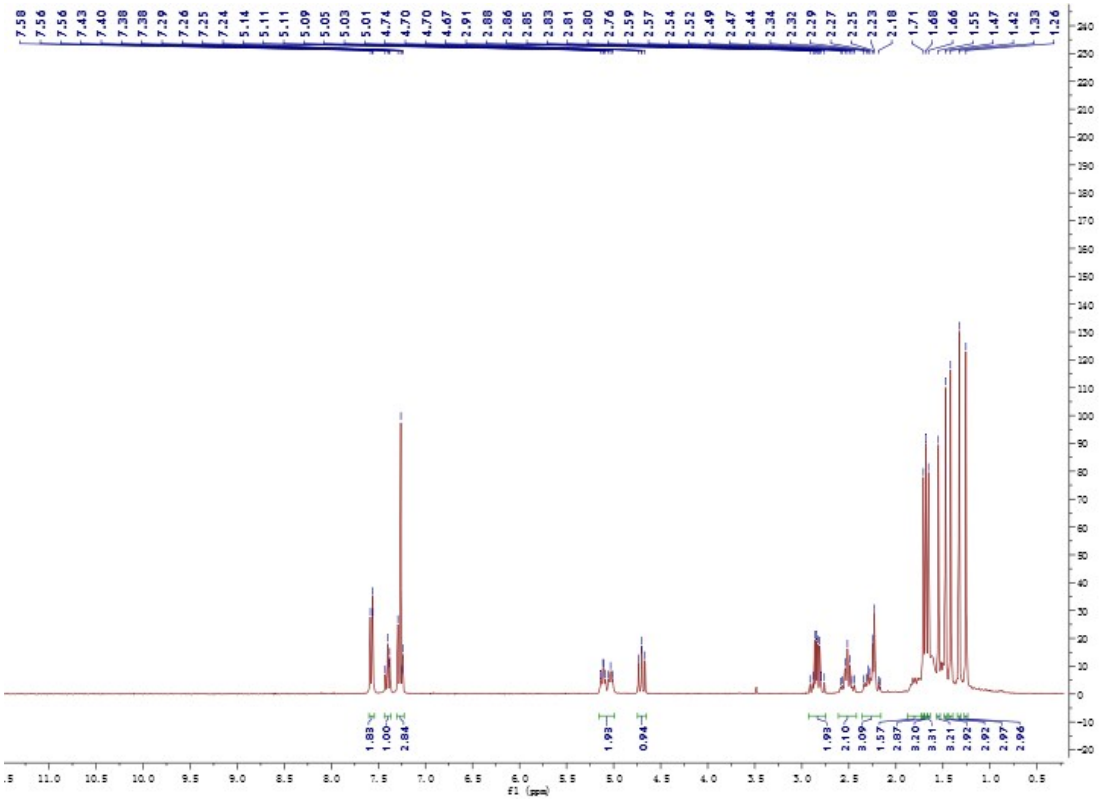
HSD5F6B3-7
20130422-6 245 (1.978) Cm (228:253)

1: TOF MS ES+
1.06e+007

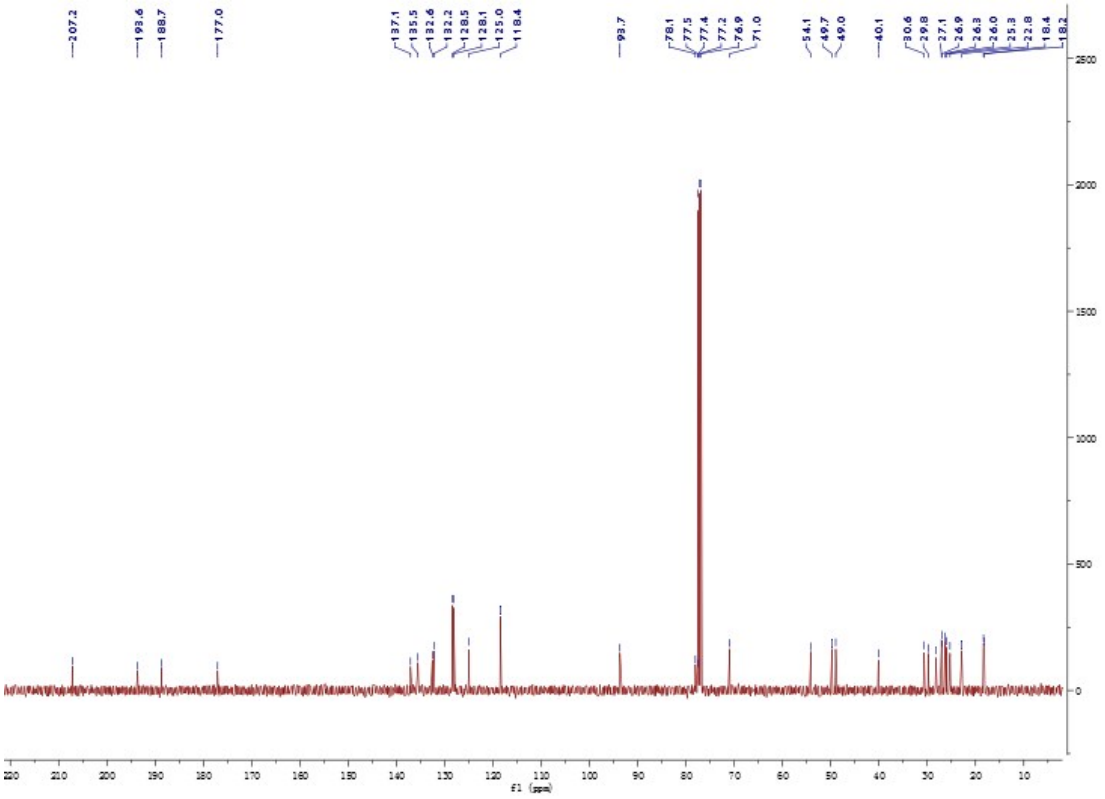


Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
519.3115	519.3110	0.5	1.0	12.5	1674.5	n/a	n/a	C33 H43 O5

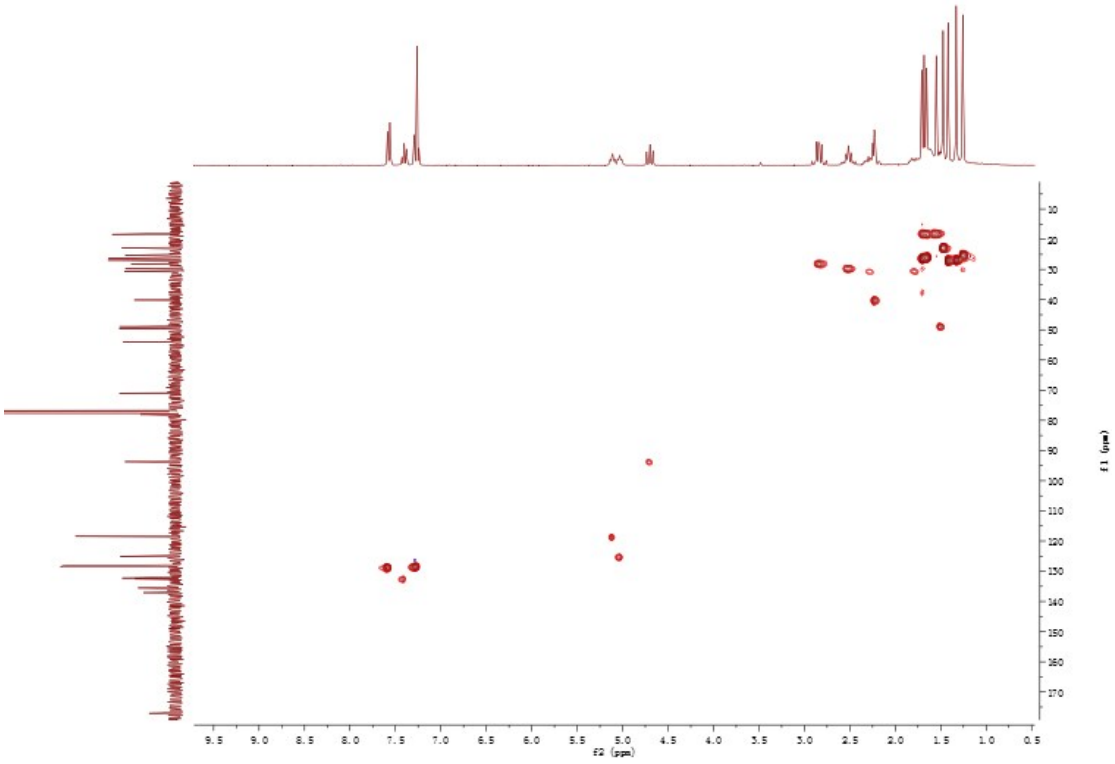
^{1}H NMR (AV-300, 300 MHz) spectrum of hypersampsone U (3) in $CDCl_3$



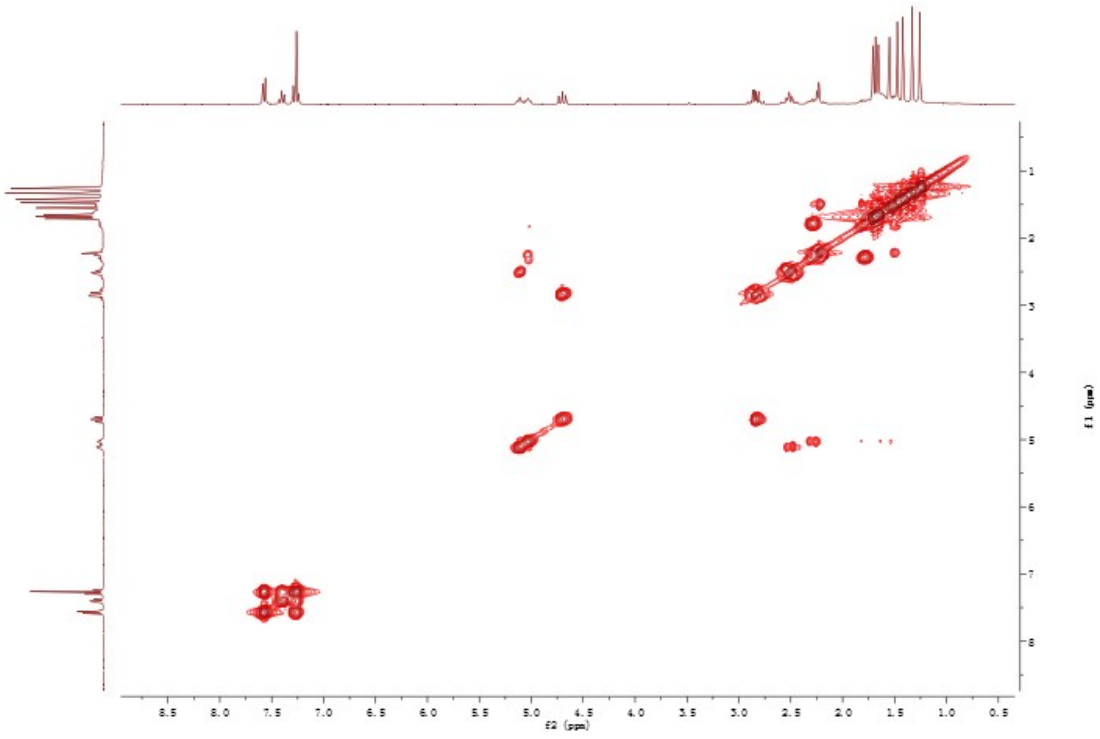
^{13}C NMR spectrum (AV-400, 100 MHz) of hypersampsone U (3) in $CDCl_3$



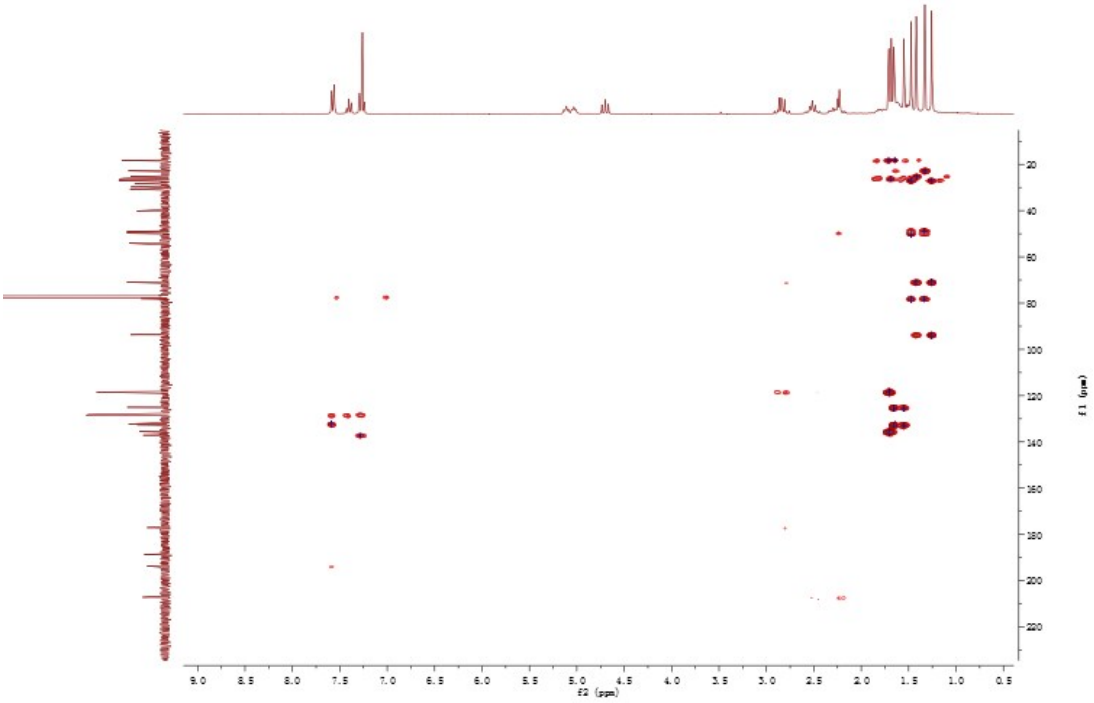
HSQC spectrum (AV-400) of hypersampsone U (3) in $CDCl_3$



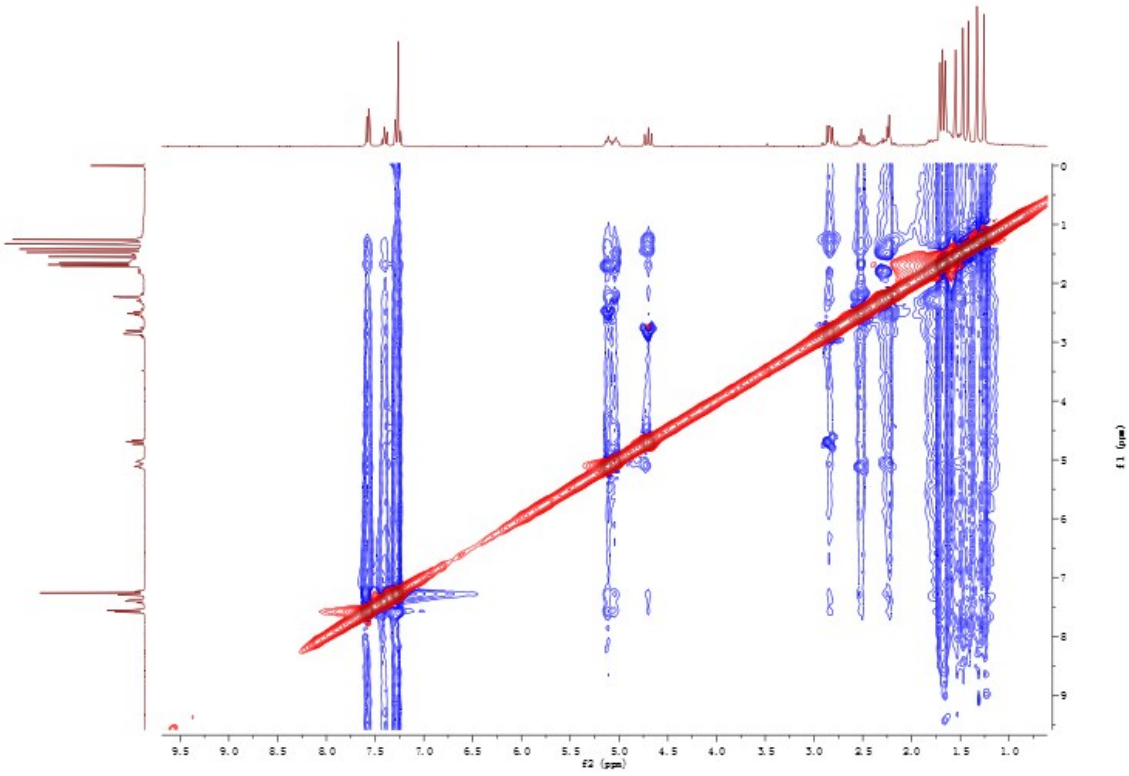
$^{1}H-^{1}H$ COSY spectrum (AV-400) of hypersampsone U (3) in $CDCl_3$



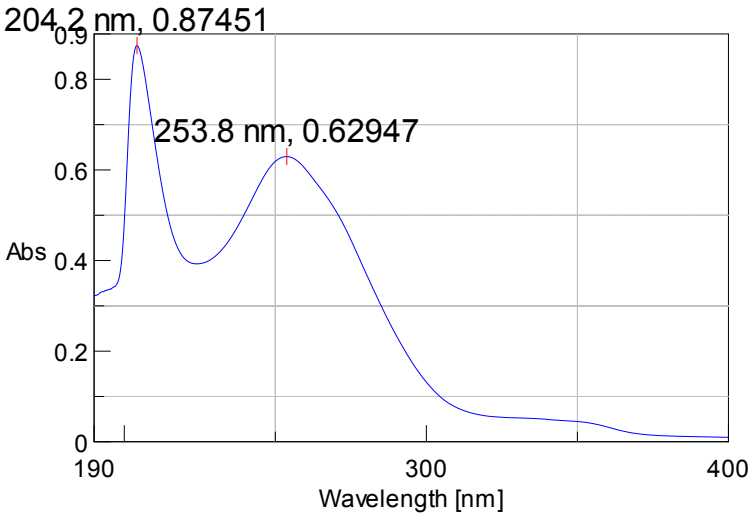
HMBC spectrum (AV-400) of hypersampsone U (3) in $CDCl_3$



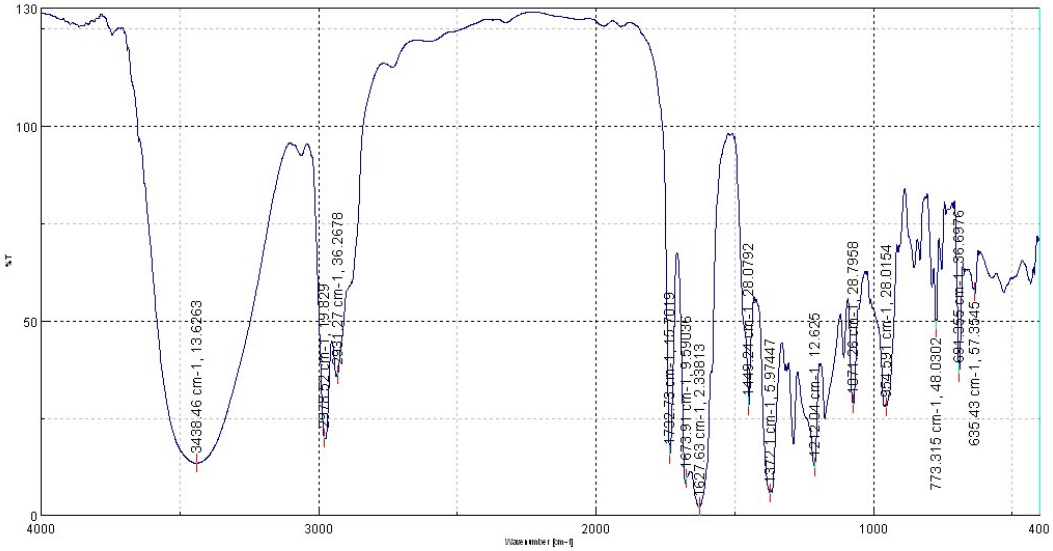
NOESY spectrum (AV-400) of hypersampsone U (3) in $CDCl_3$



UV spectrum of hypersampsone V (4) in CH_3OH.



IR (KBr disc) spectrum of hypersampsone Q (4).



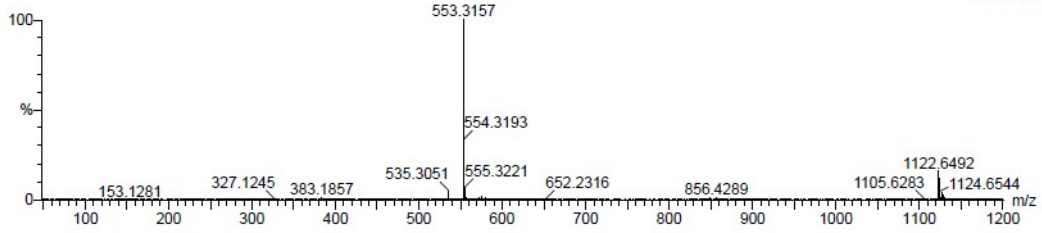
HR-ESI-MS spectrum of hypersampsone Q (4).

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
150 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

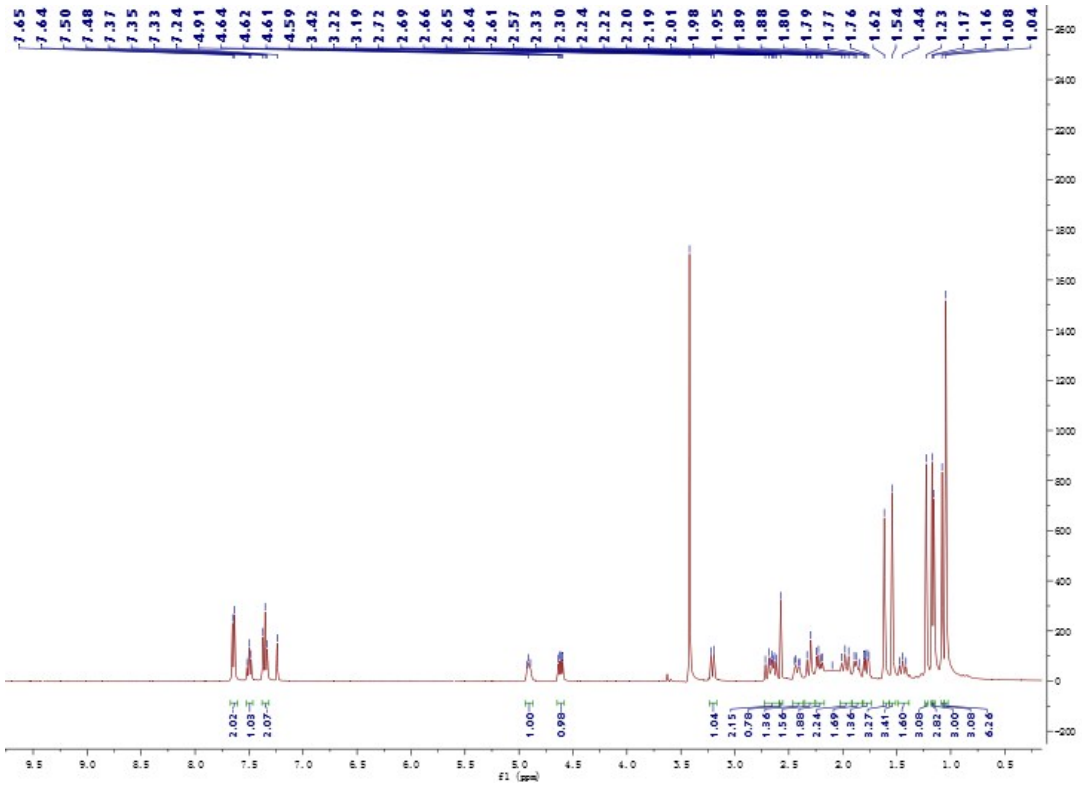
HSD7K2-1-4
20141201-28 685 (5.493)

1: TOF MS ES+
4.29e+005

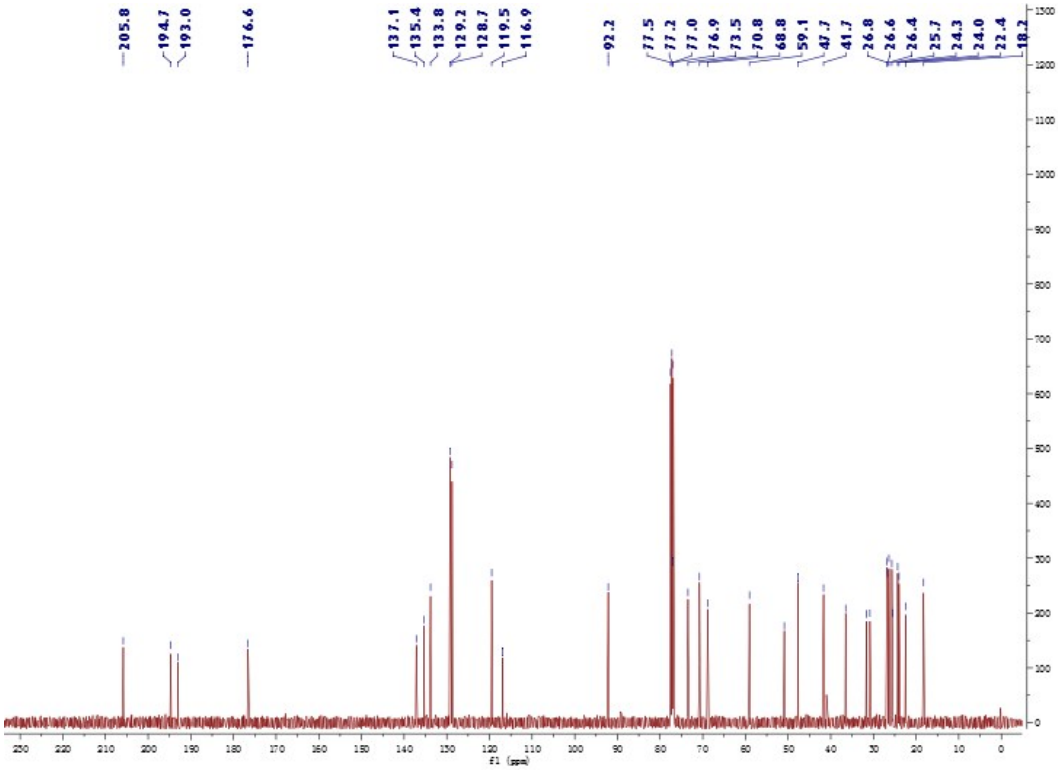


Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
553.3157	553.3165	-0.8	-1.4	11.5	276.9	n/a	n/a	C33 H45 O7

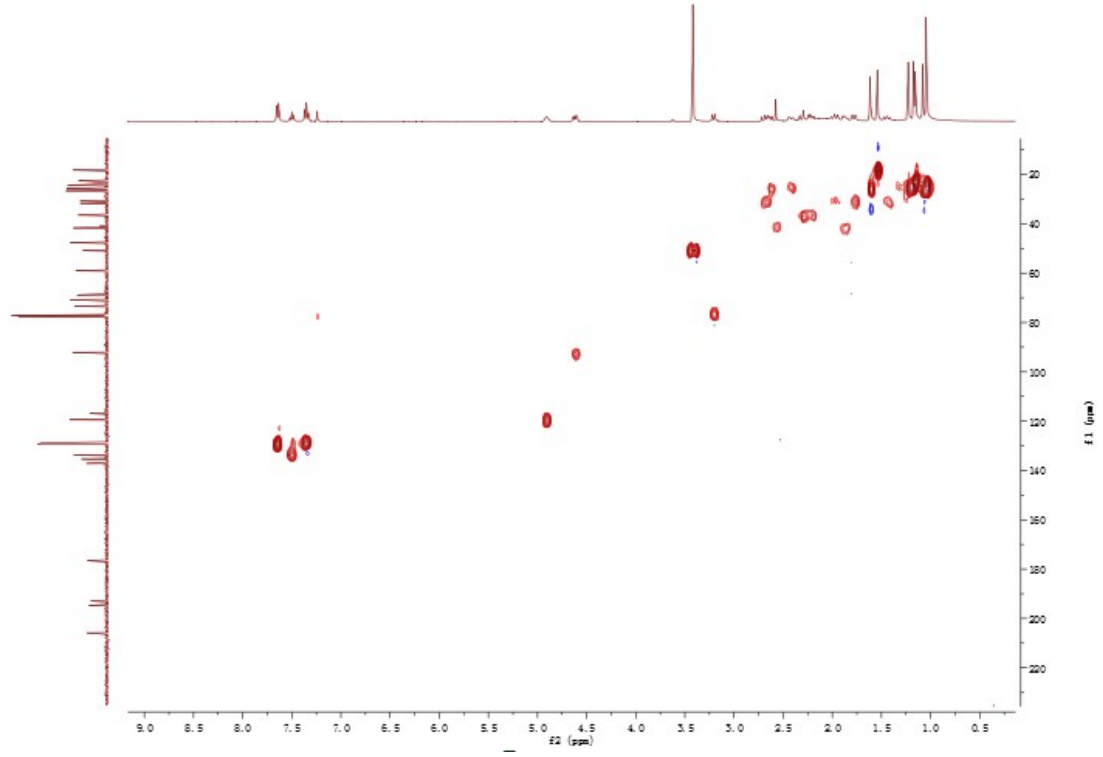
^{1}H NMR (AV-400, 400 MHz) spectrum of hypersampsone V (4) in $CDCl_3$



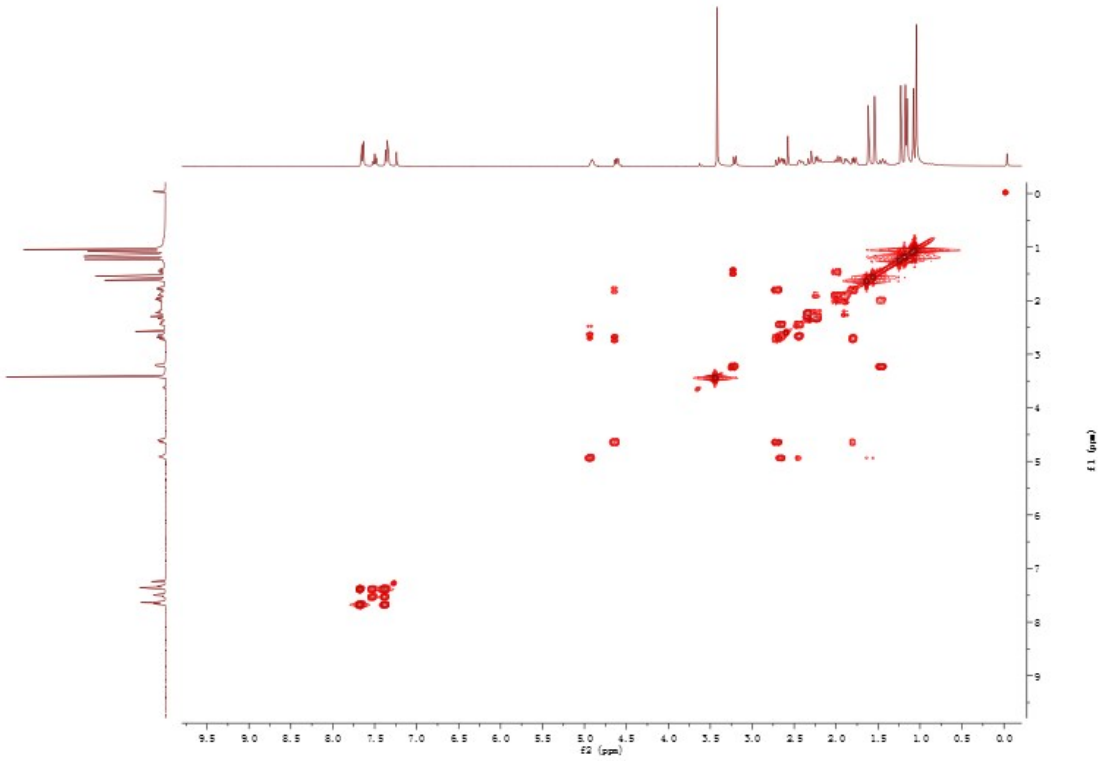
^{13}C NMR spectrum (AV-400, 100 MHz) of hypersampsone V (4) in $CDCl_3$



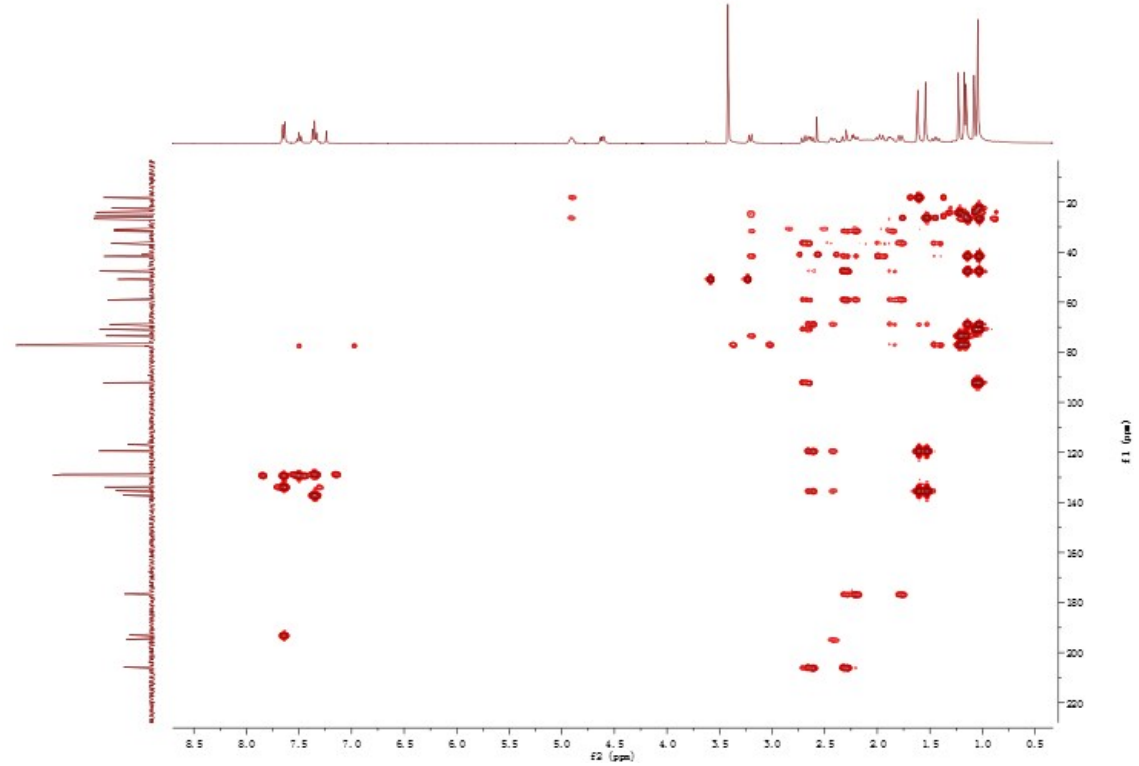
HSQC spectrum (AV-400) of hypersampsone V (4) in $CDCl_3$



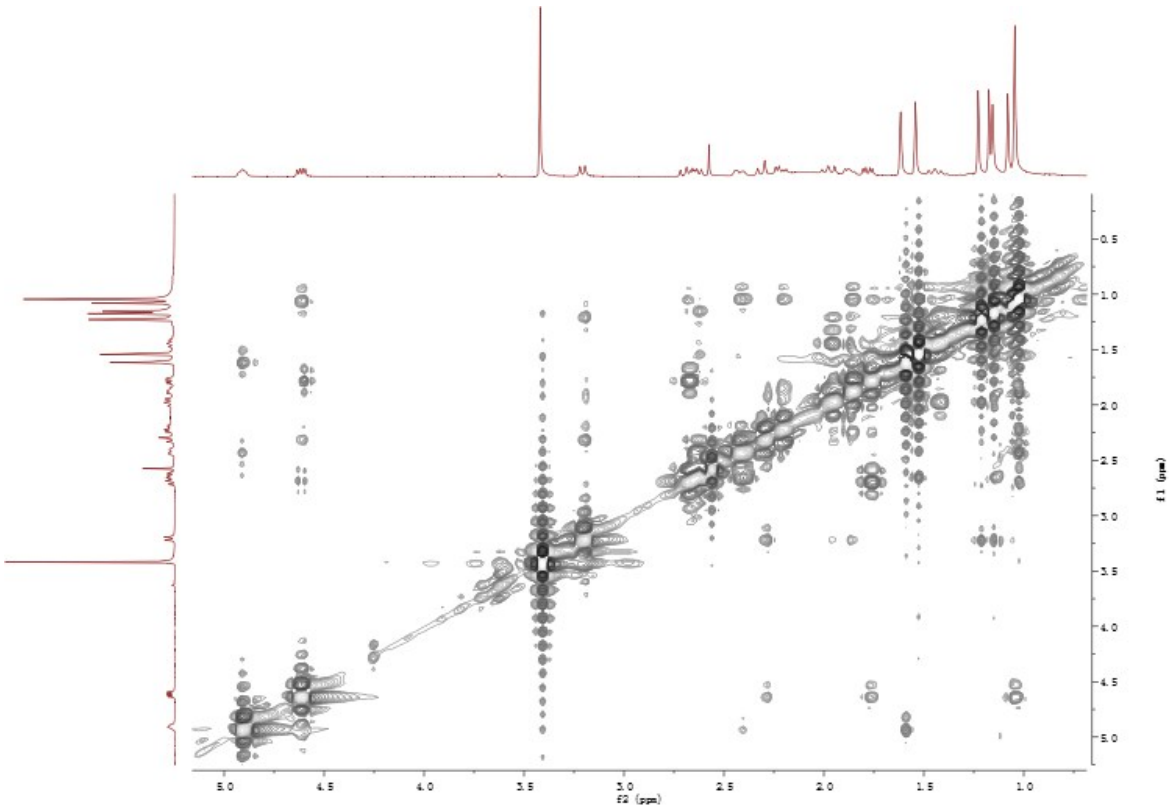
$^1H-^1H$ COSY spectrum (AV-400) of hypersampsone V (4) in $CDCl_3$



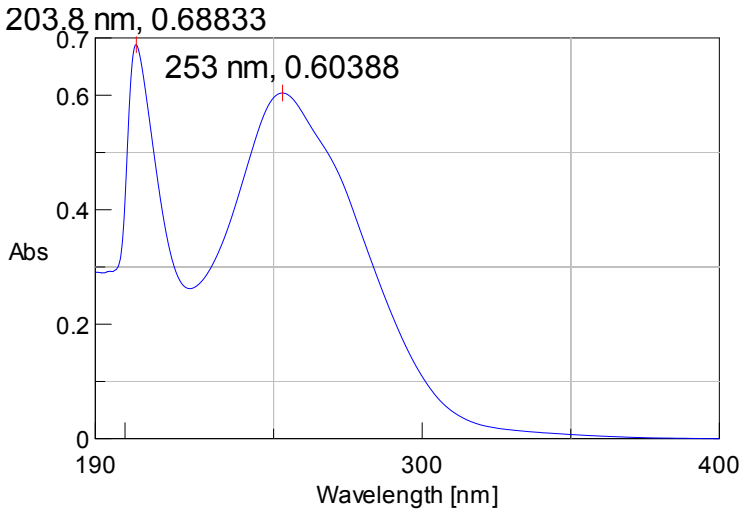
HMBC spectrum (AV-400) of hypersampsone V (4) in $CDCl_3$



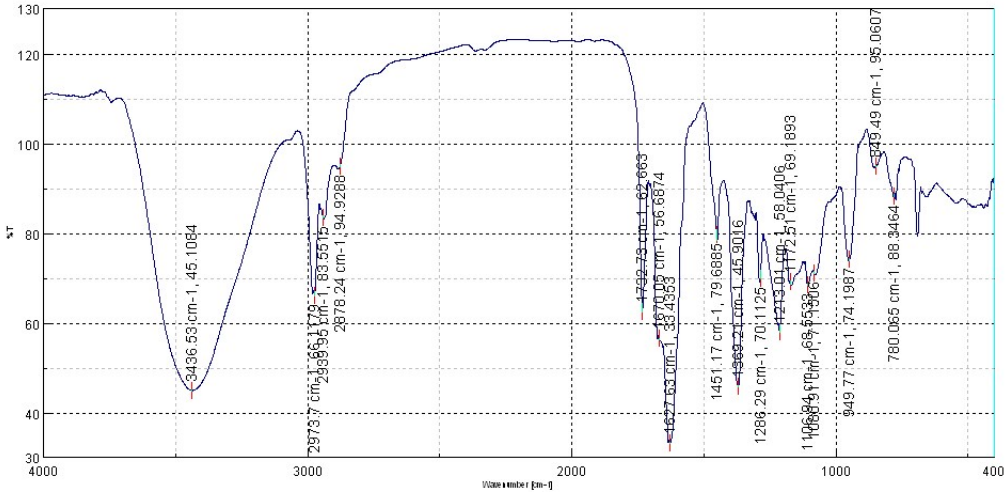
NOESY spectrum (AV-400) of hypersampsone V (4) in $CDCl_3$



UV spectrum of hypersampsone W (5) in CH_3OH.



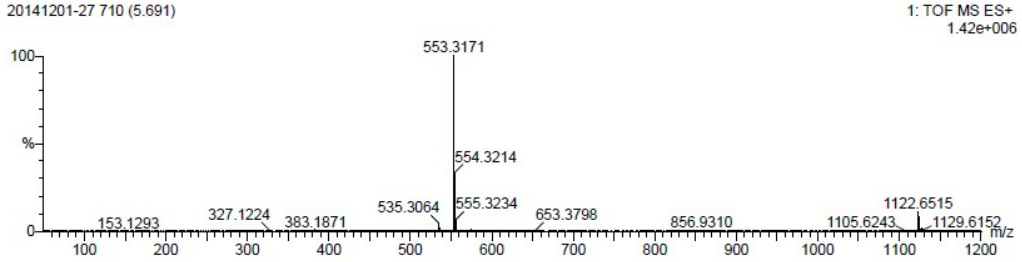
IR (KBr disc) spectrum of hypersampsone W (5).



HR-ESI-MS spectrum of hypersampsone W (5).

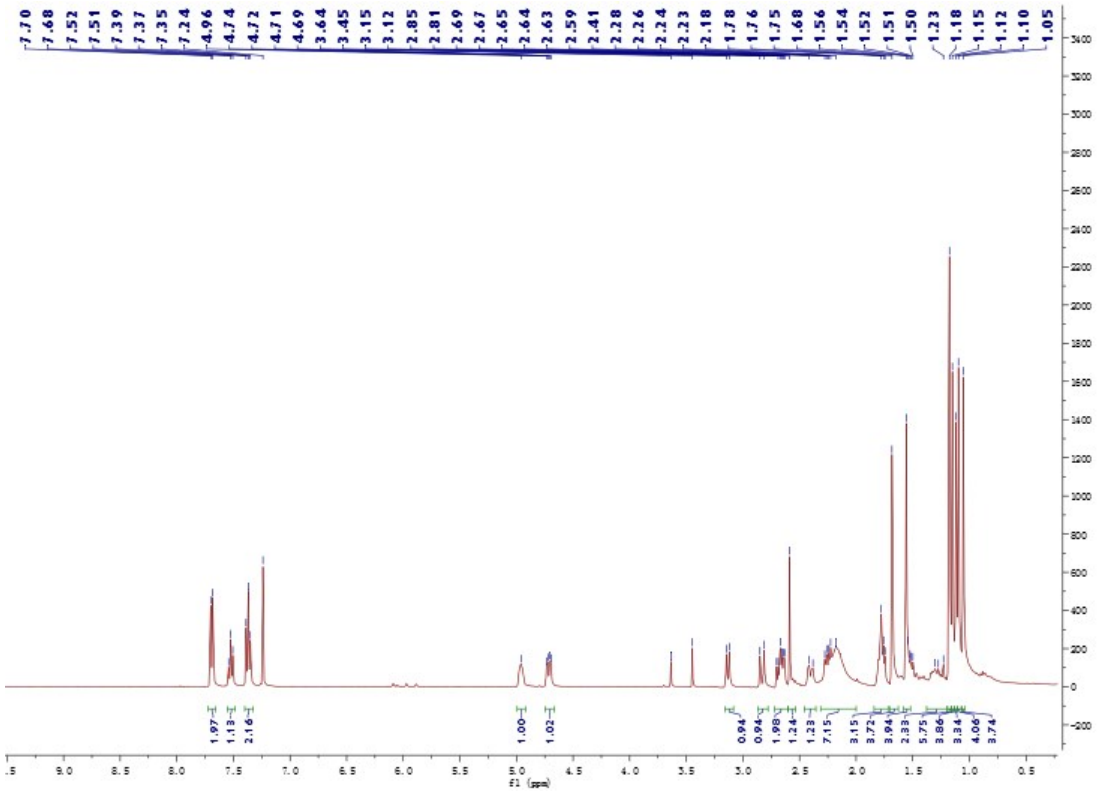
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
150 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
HSD7K2-2
20141201-27 710 (5.691)

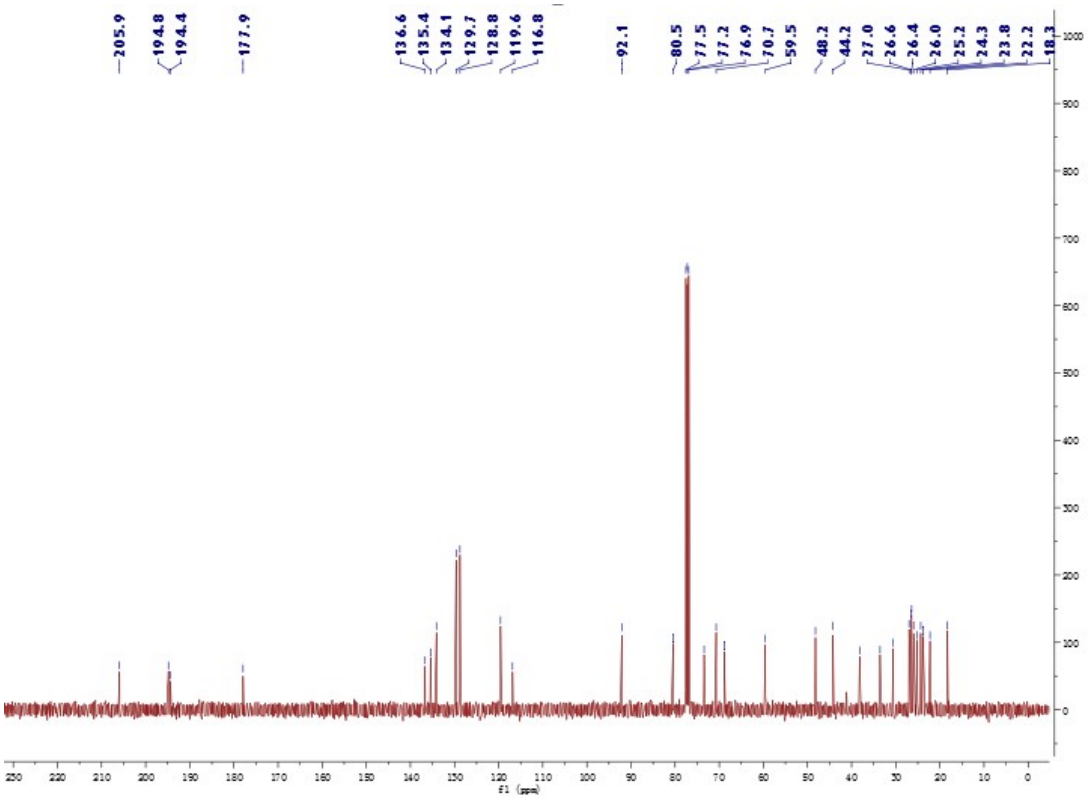


Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
553.3171	553.3165	0.6	1.1	11.5	462.1	n/a	n/a	C33 H45 O7

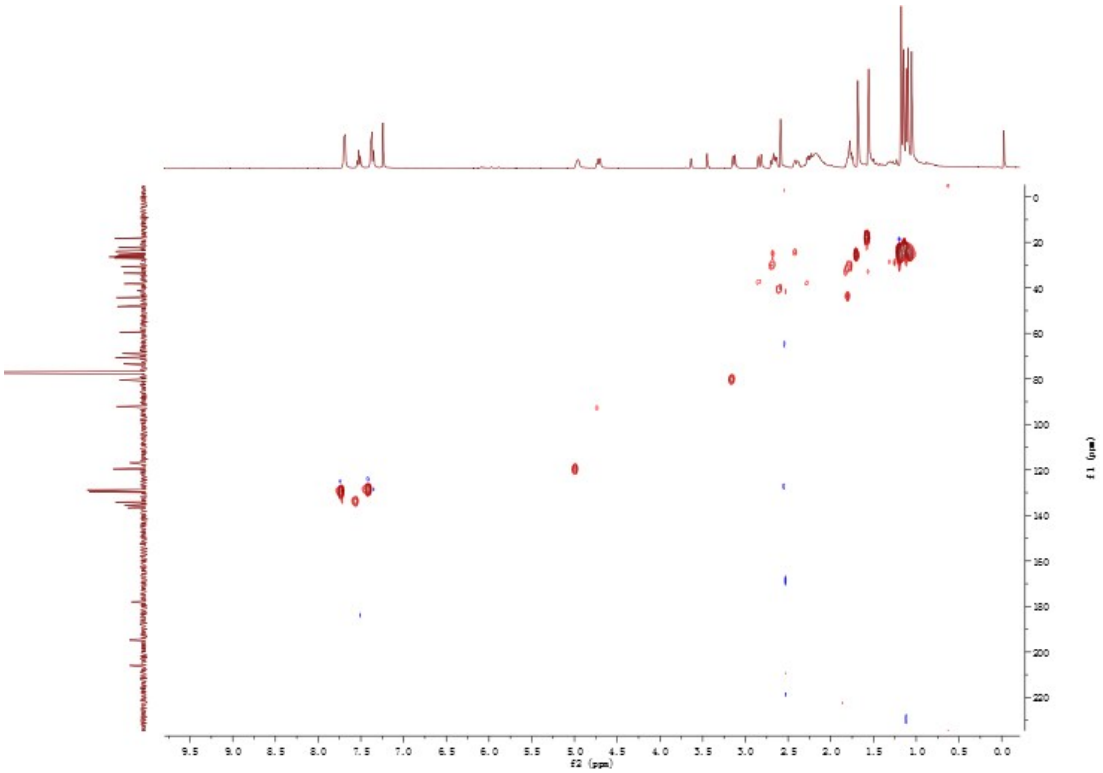
^{1}H NMR (AV-400, 400 MHz) spectrum of hypersampsone W (4) in $CDCl_3$



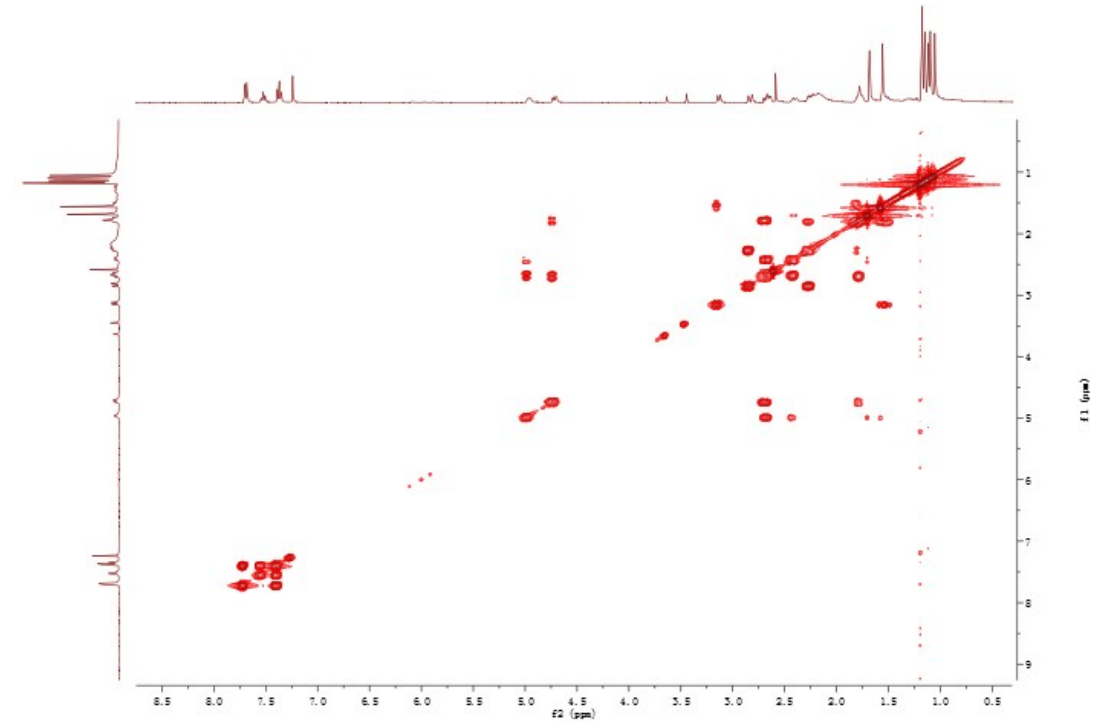
^{13}C NMR spectrum (AV-400, 100 MHz) of hypersampsone W (4) in $CDCl_3$



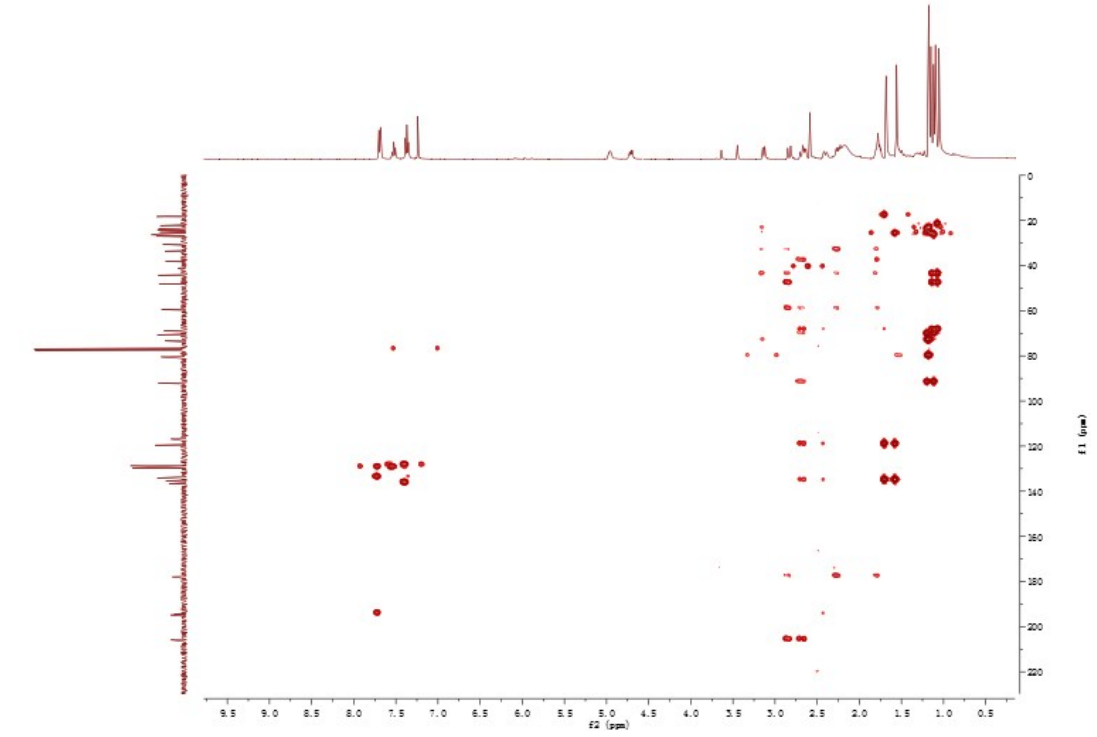
HSQC spectrum (AV-400) of hypersampsone W (5) in $CDCl_3$



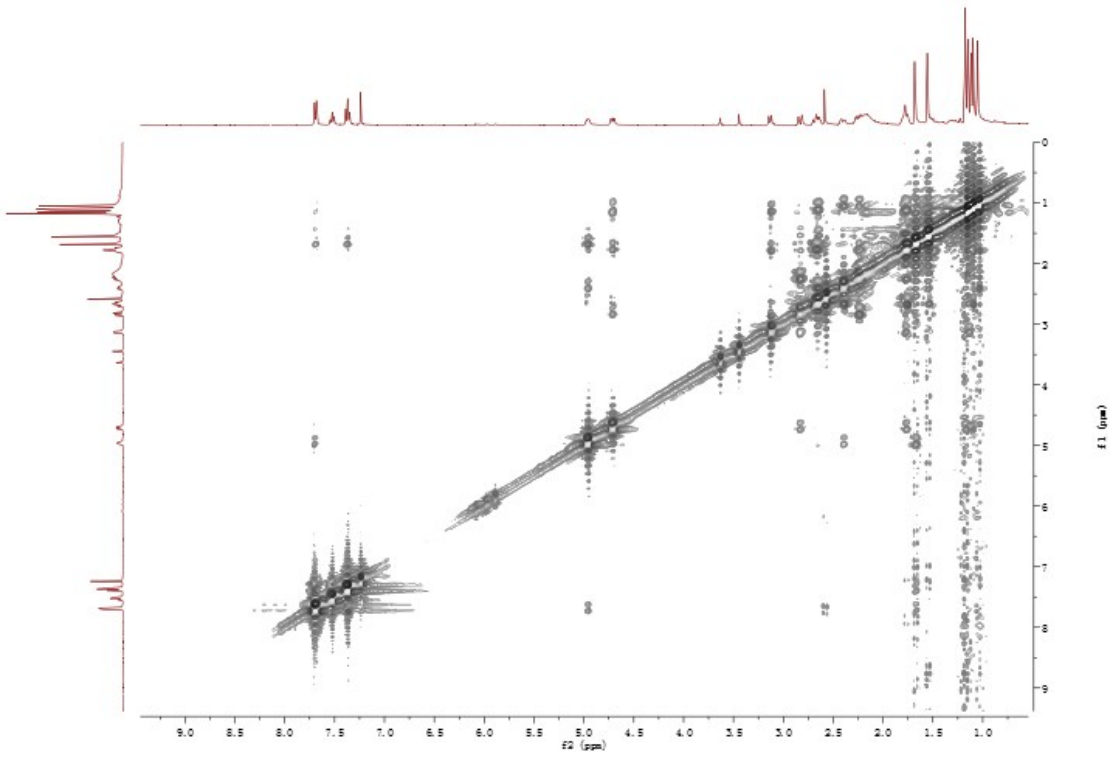
$^1H-^1H$ COSY spectrum (AV-400) of hypersampsone W (5) in $CDCl_3$



HMBC spectrum (AV-400) of hypersampsone W (5) in $CDCl_3$



NOESY spectrum (AV-400) of hypersampsone W (5) in $CDCl_3$



Computational details of 2 and 3

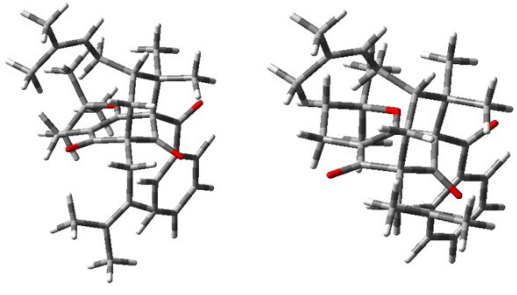
Based on the known relative configuration, two pairs of enantiomers (1R,5S,7S)-**2a**, (1S,5R,7R)-**2b**, (1R,5S,7S,25S)-**3a** and (1S,5R,7R,25R)-**3b** was employed for the conformational random search using the Merck Molecular Force Field method in the Best module of Discovery studio 2.5.5 (Accelrys, San Diego, CA, 2009) software package. The conformations form conformational search results with an energy cut off of 17 kJ/mol (approximately 4 kcal/mol) was selected to further geometry optimization and ECD calculation. As a result, there were 2, 7, 14, 8 low energy conformations with an energy cut off of 4 kcal/mol for **2a**, **2b**, **3a**, **3b**, respectively (Figure 1).

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 quantum chemical theoretical calculations (Diedrich and Grimme, 2003) for ECD were carried out in the solvent medium using time-dependent density functional theory (TDDFT) with B3LYP functional and dgdzvp forcefield basis set with 60 electronic transitions. The energies, oscillator strengths, and rotational strengths of the electronic excitations of all the conformers were calculated using the TD-DFT method at the B3LYP/dgdzvp level.

Based on the relativity energy, boltzmann weighted average of the different low energy conformations was calculated for **2a**, **2b**, **3a** and **3b**. The ECD for each molecule is calculated based on boltzmann weighted average of the conformations search results. Percentage for each conformations are shown in figure 1. As a result, the overall pattern of calculated ECD spectra of **2a**, **3a** were well matched the experimental data of **2** and **3** (Figure 2).

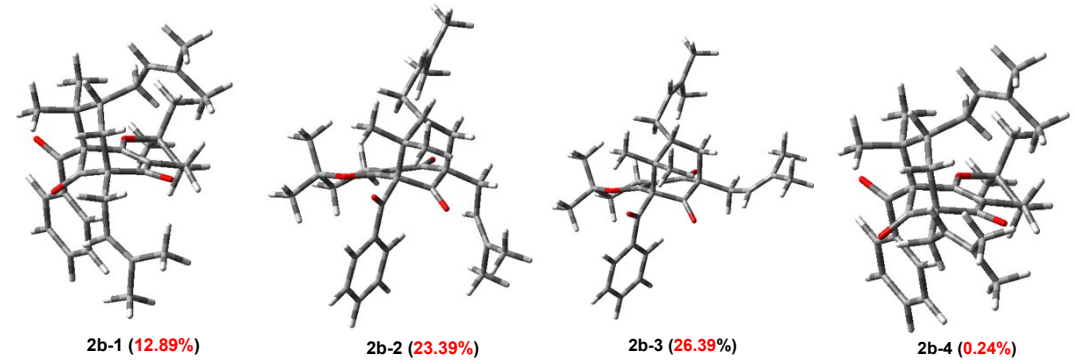
Selected conformations of 2a and their percentage



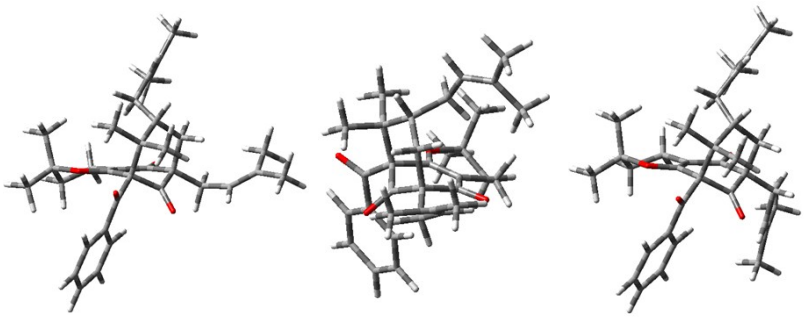
2a-1 (50%)

2a-2 (50%)

Selected conformations of 2b and their percentage

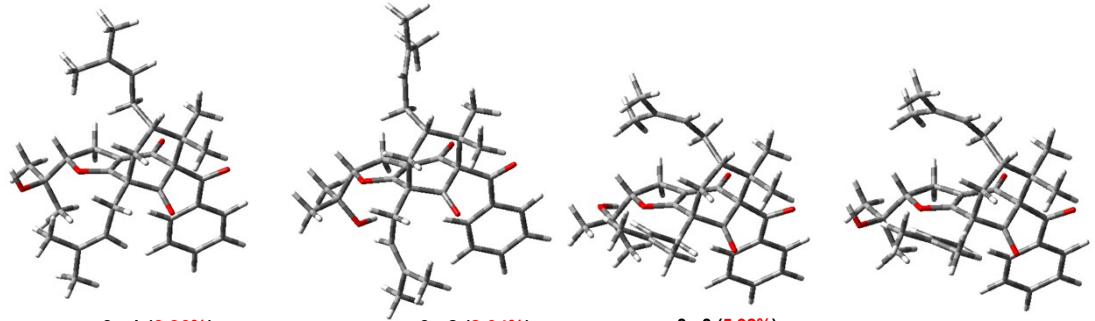


2b-1 (12.89%) 2b-2 (23.39%) 2b-3 (26.39%) 2b-4 (0.24%)

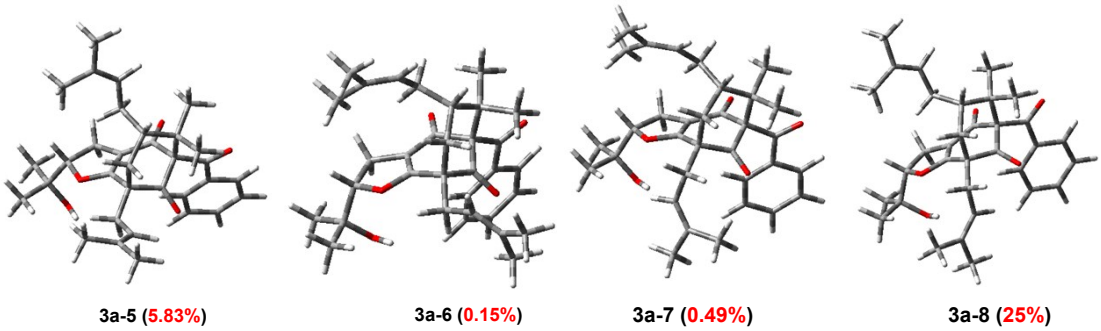


2b-5 (28.8%) 2b-6 (8.72%) 2b-7 (0%)

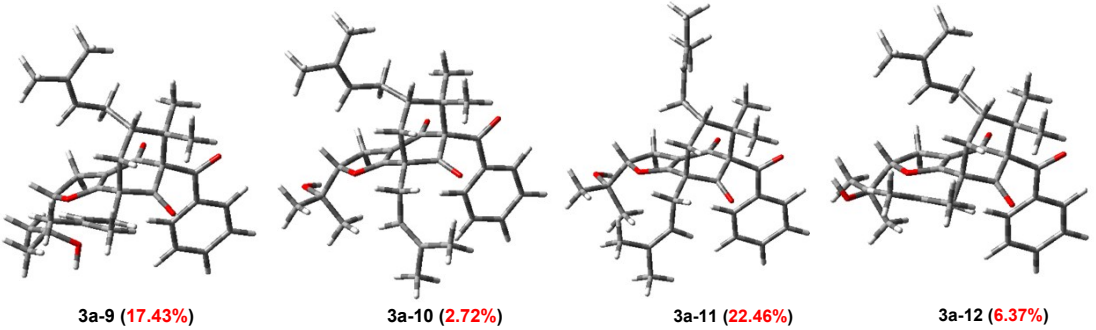
Selected conformations of 3a and their percentage



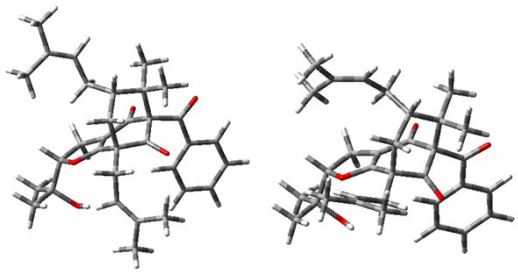
3a-1 (6.36%) 3a-2 (2.04%) 3a-3 (5.92%) 3a-4 (0%)



3a-5 (5.83%) 3a-6 (0.15%) 3a-7 (0.49%) 3a-8 (25%)



3a-9 (17.43%) 3a-10 (2.72%) 3a-11 (22.46%) 3a-12 (6.37%)



Selected conformations of 3b and their percentage

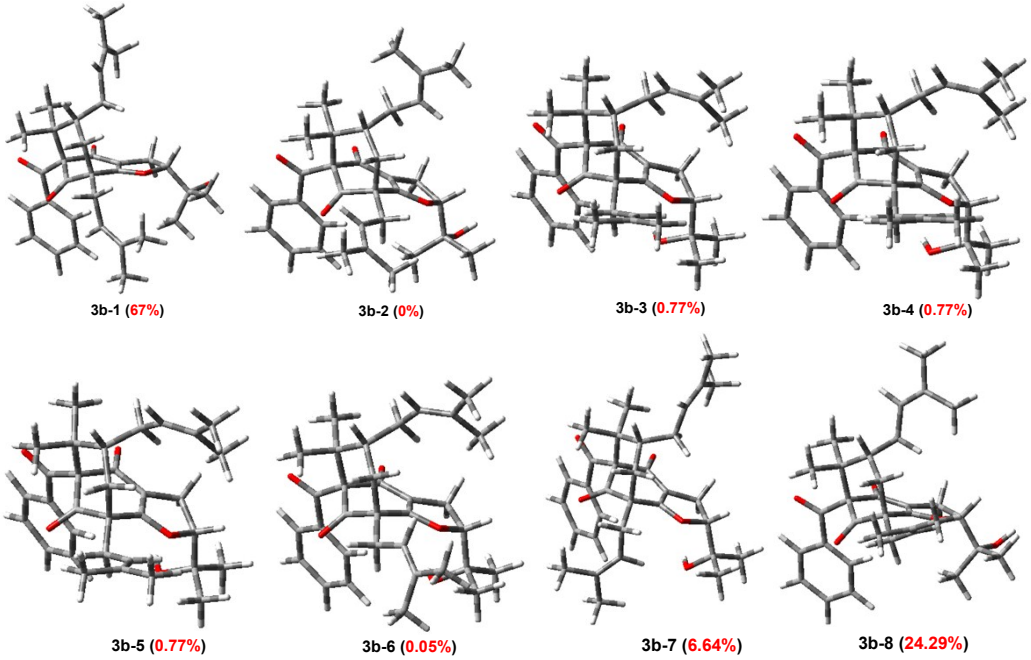


Figure 1. Selected Low energy conformations for 2a, 2b, 3a, 3b and their percentage.

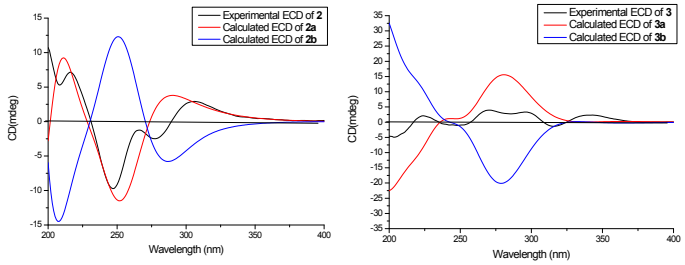


Figure 2. Calculated ECD results of 2 and 3 based on boltzmann weighted average of the selected low energy conformations.

Reference

(1)Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2009.

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 [1,2]. 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%

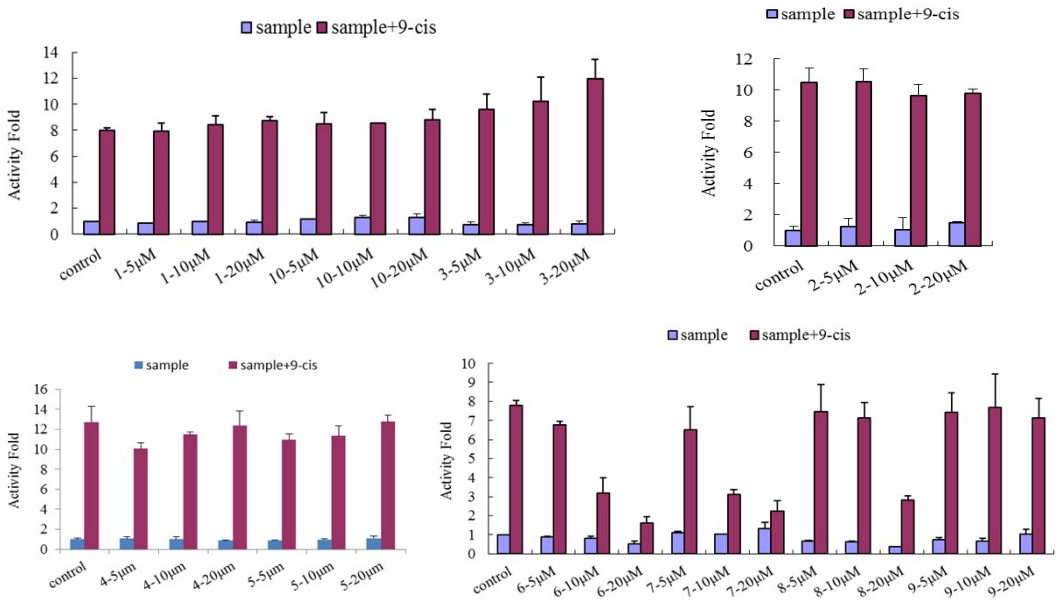


Chart 1. Effects of compounds 1-10 (5, 10, and 20*μ*M) on the transcriptional activities of RXR*α*

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 **1-10** 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：

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

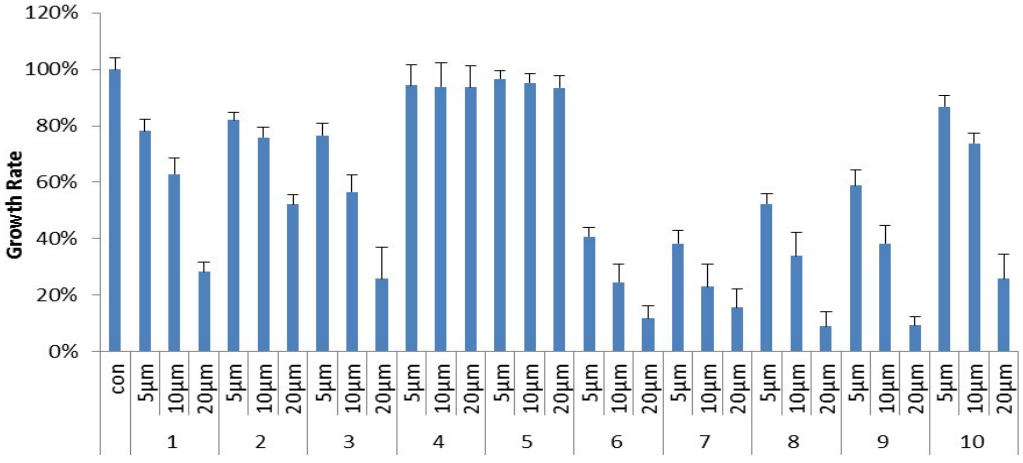


Chart 3. Effects of compounds 1-10 (5, 10, and 20 μM) on the cytotoxic effects

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.