

SUPPLEMENTARY MATERIALS

Constituents isolated from the leaves of *Glycyrrhiza uralensis* and their anti-inflammatory activities on LPS-induced RAW264.7 cells

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Abstract: Licorice, the root and rhizome of *Glycyrrhiza uralensis* Fisch, is one of the most frequently used Traditional Chinese Medicines in rigorous clinical trials to remove toxins, sputum and relieve coughing. However, the aerial parts are not used so widely at present. It has been reported that the aerial parts have many bioactivities such as anti-microbial and anti-HIV activities. In this study, we aimed to discover the bioactive compounds from the leaves of *G. uralensis*. Four new compounds, licostilbene A-B (**1-2**) and licofuranol A-B (**3-4**), together with eight known flavonoids (**5-12**) were isolated and identified from the leaves of *G. uralensis*. Their structures were elucidated mainly by the interpretation of high-resolution electrospray mass spectrometry (HR-ESI-MS) and nuclear magnetic resonance (NMR) spectroscopic data. Compared with quercetin, which showed a 50% inhibitory concentration (IC₅₀) value of 4.08 µg/ml, compounds **1-9** showed significant anti-inflammatory activities by inhibiting lipopolysaccharide (LPS)-induced nitric oxide (NO) production with IC₅₀ values of 2.60, 2.15, 3.21, 3.25, 2.00, 3.45, 2.53, 3.13 and 3.17 µg/ml, respectively. The discovery of these active compounds is important for the prevention and treatment of inflammation.

Keywords: *Glycyrrhiza uralensis*; licostilbene; licofuranol; anti-inflammatory activity

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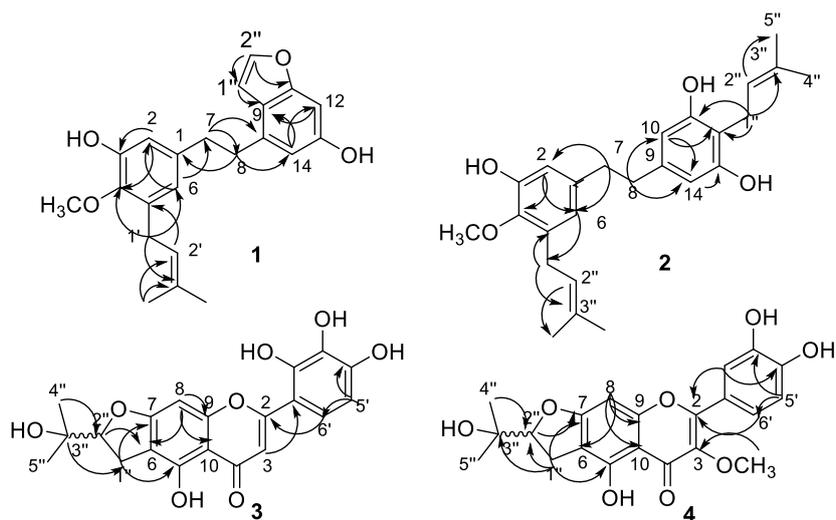


Figure S1: The key HMBC correlations observed for compounds 1-4

Compound 1

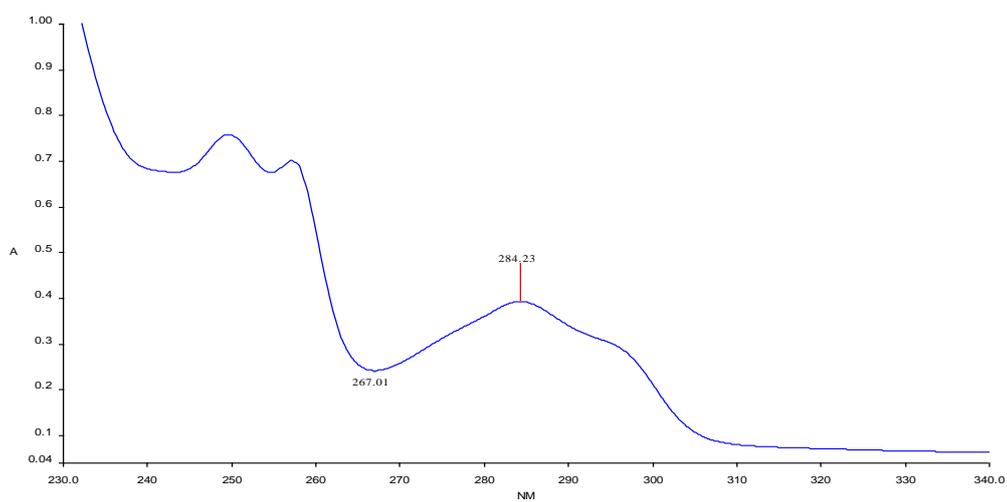


Figure S2: UV spectrum of compound 1 (absorption maxima at 250, 259 and 284 nm)

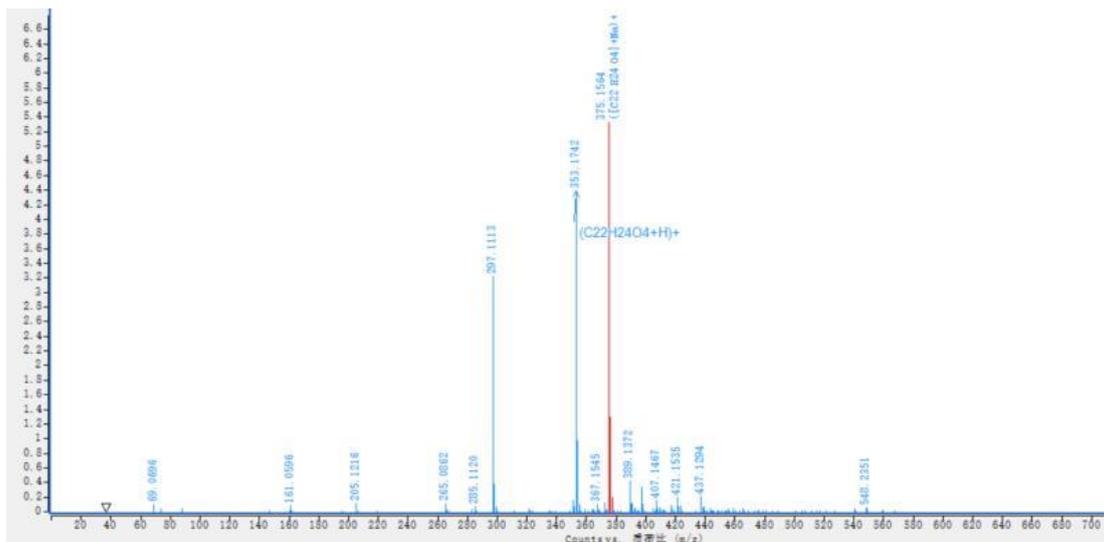


Figure S3: HR-ESI-MS positive mode ion of compound 1

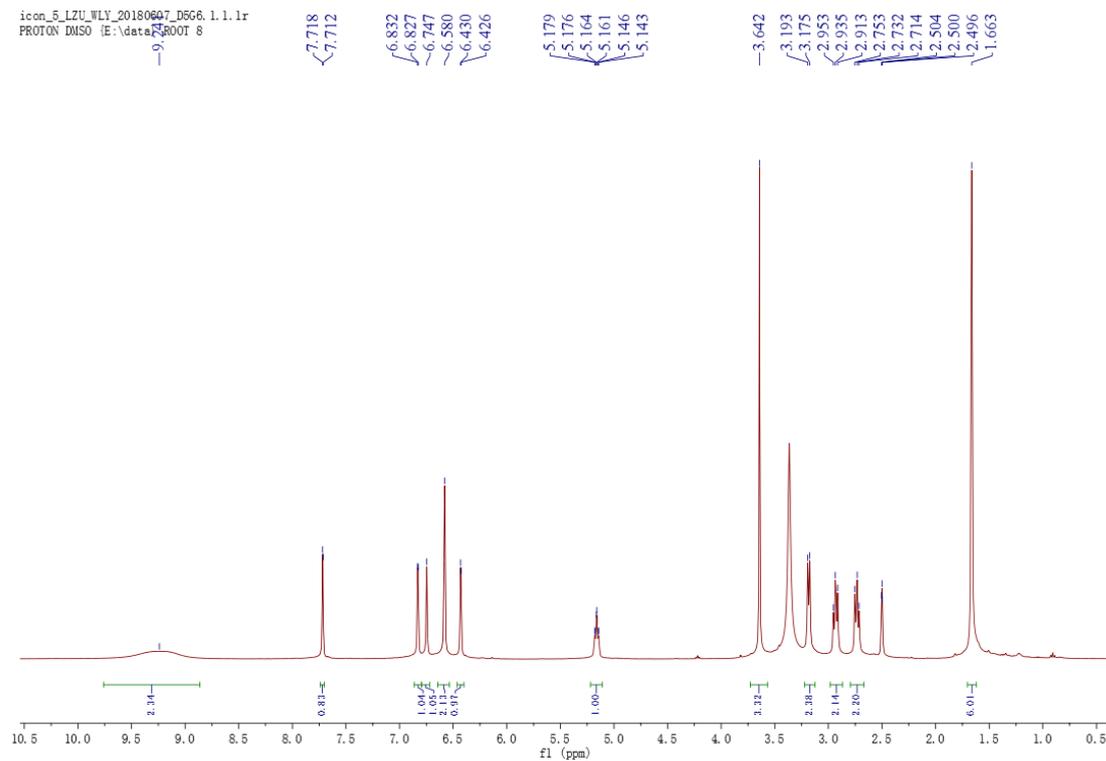


Figure S4: $^1\text{H-NMR}$ spectrum of compound 1

icon_5_LZU_WLY_20180607_D666.2.1.1r
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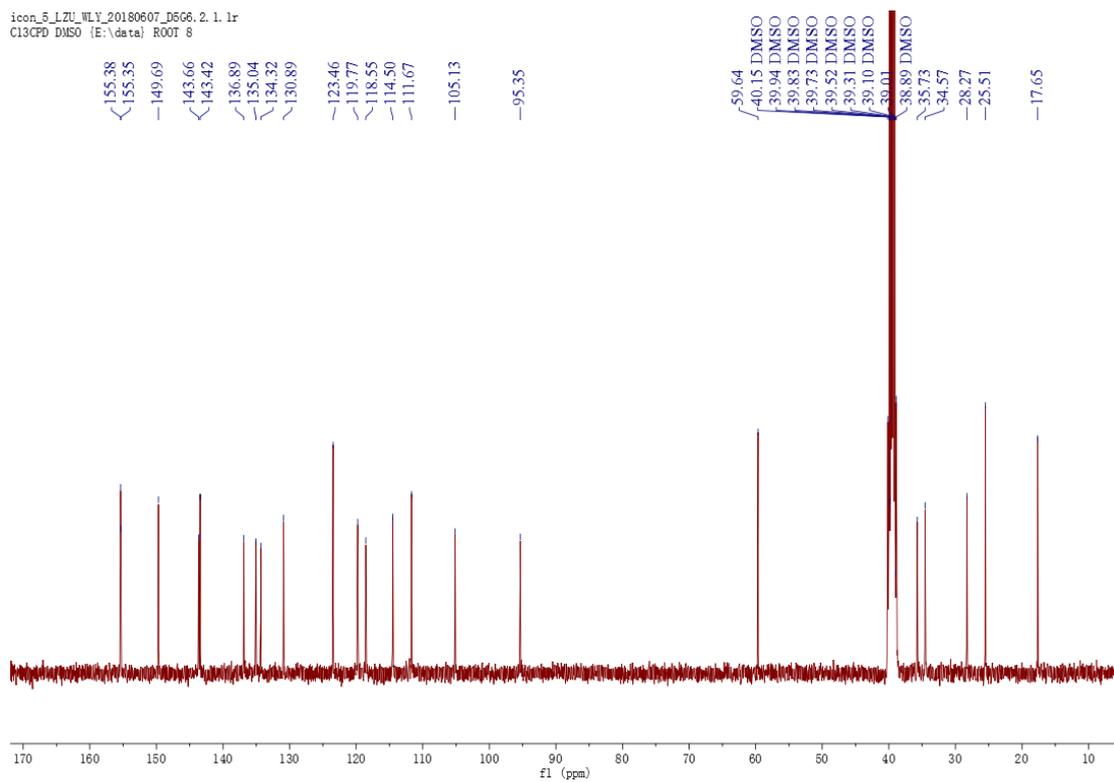


Figure S5: ^{13}C -NMR spectrum of compound 1

icon_5_LZU_WLY_20180917_D666.3.1.1r
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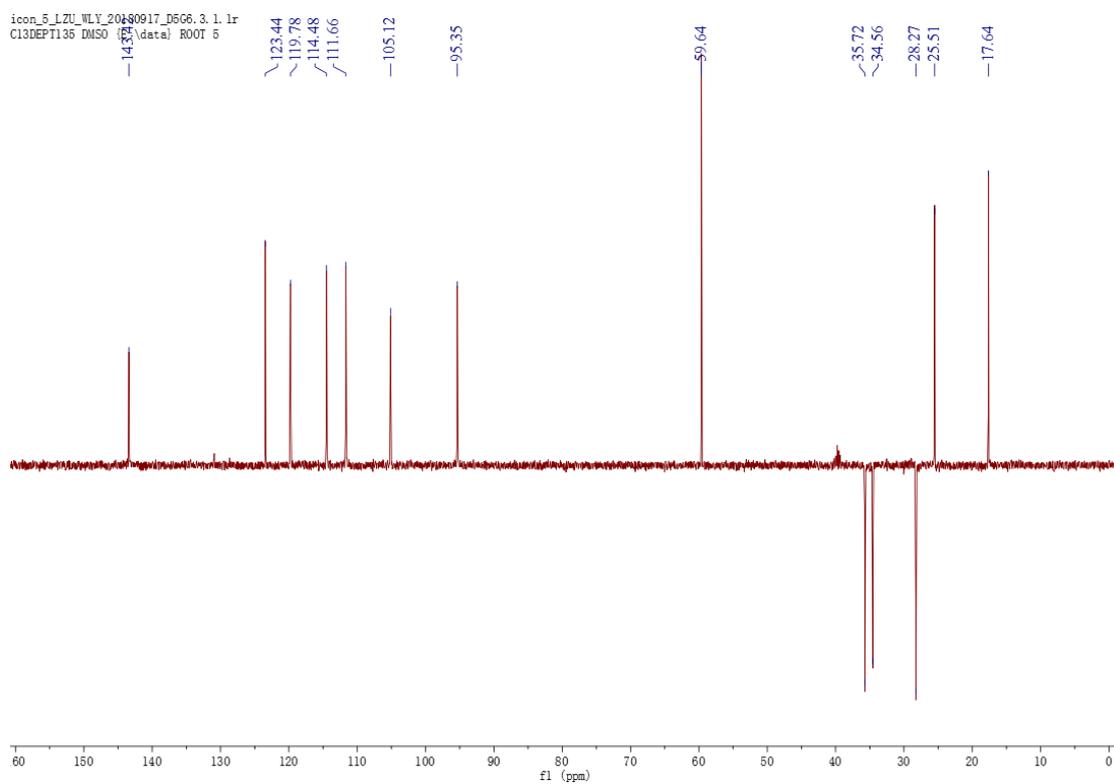


Figure S6: DEPT 135 spectrum of compound 1

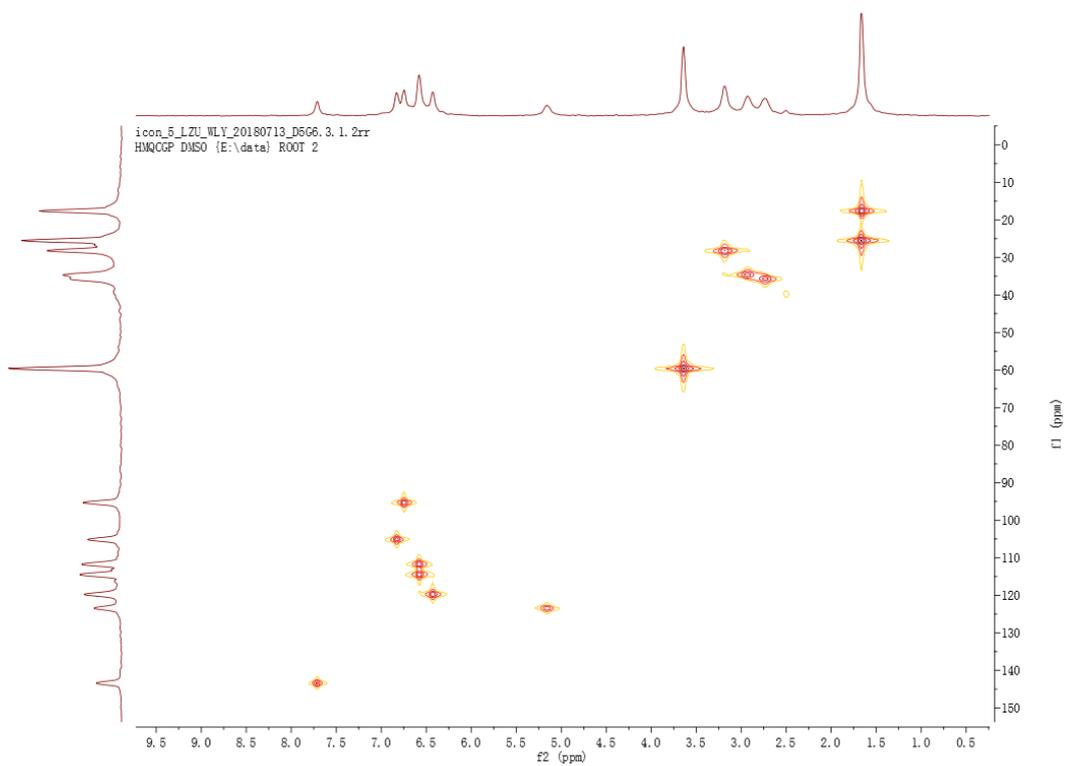


Figure S7: HSQC spectrum of compound 1

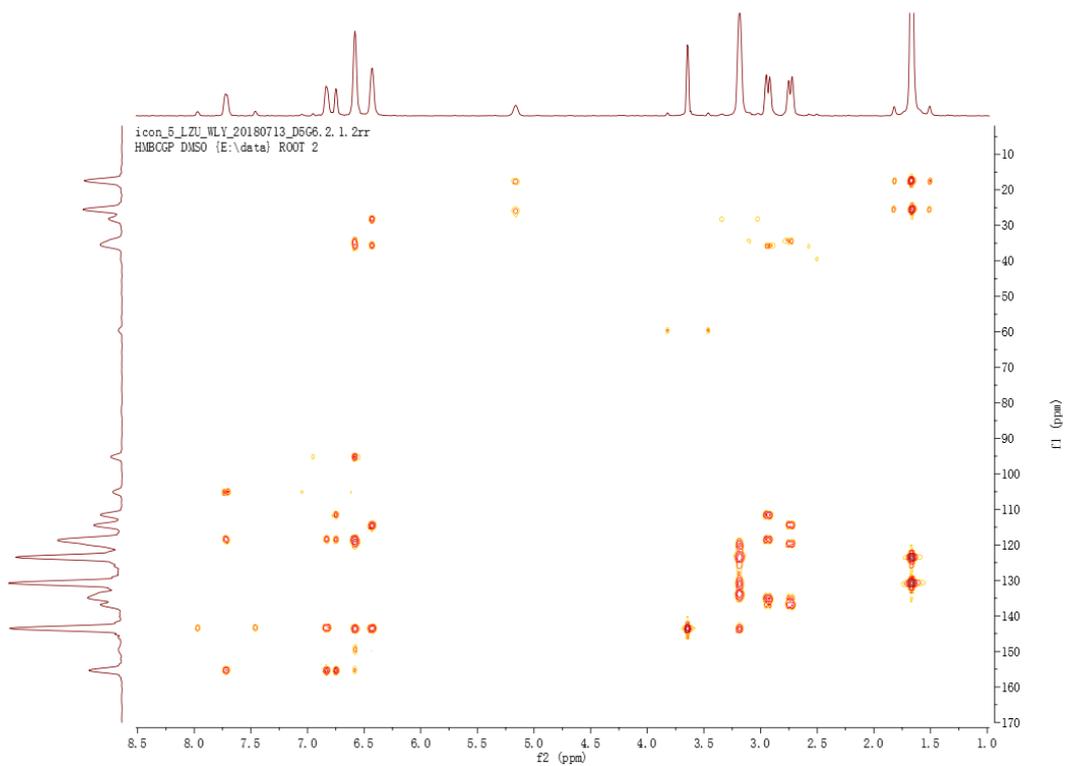


Figure S8: HMBC spectrum of compound 1

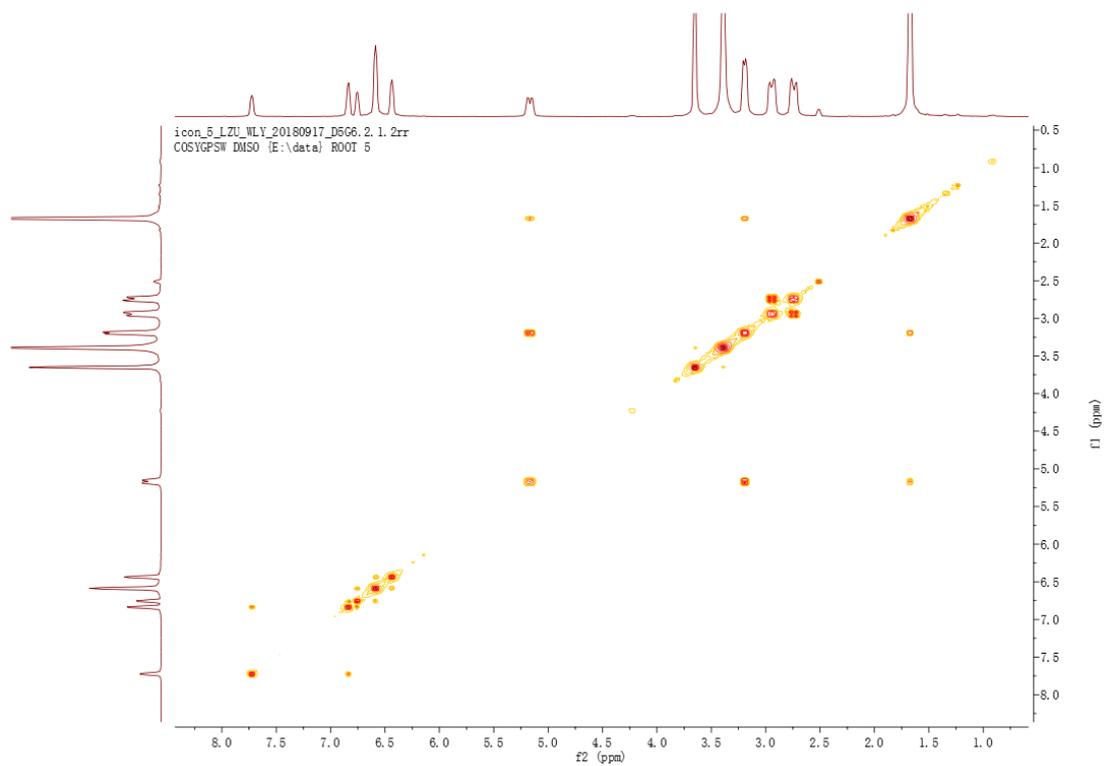


Figure S9: NOESY spectrum of compound 1

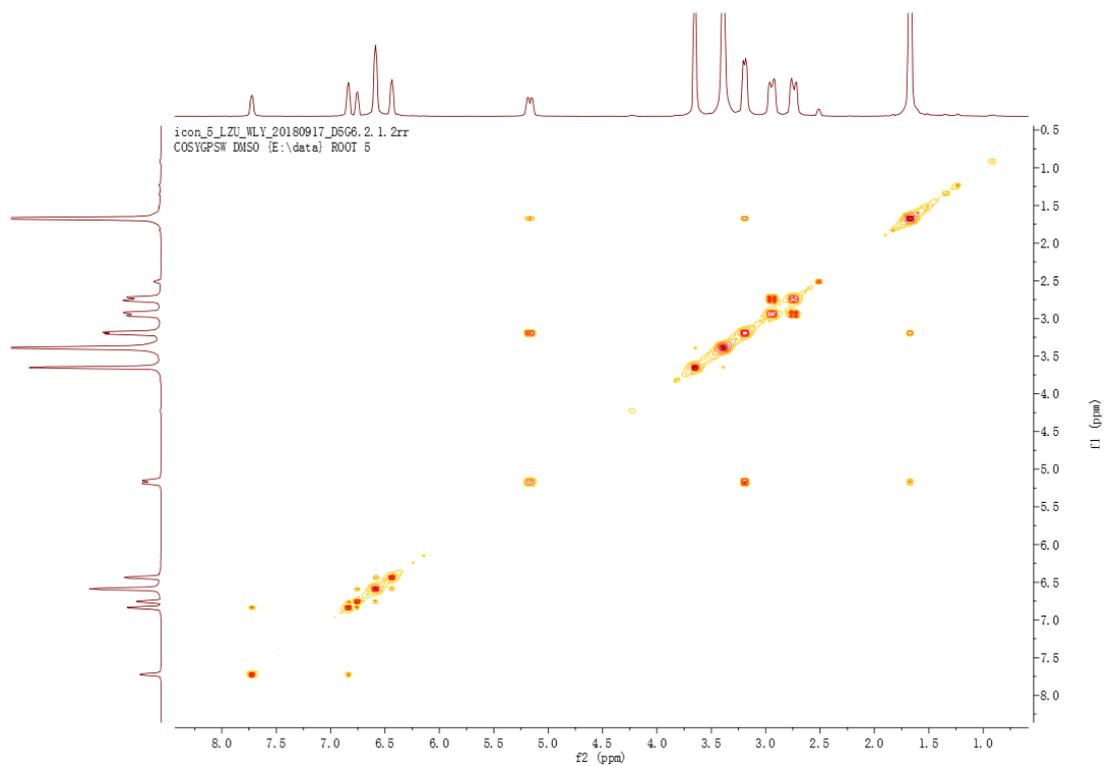


Figure S10: COSY spectrum of compound 1

Compound 2

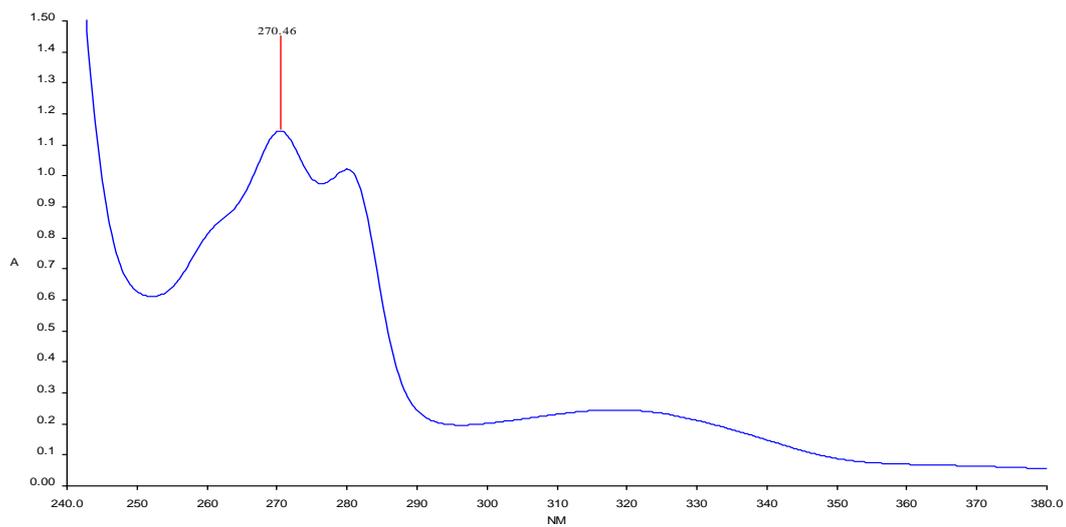


Figure S11: UV spectrum of compound 2 (absorption maxima at 270 and 282 nm)

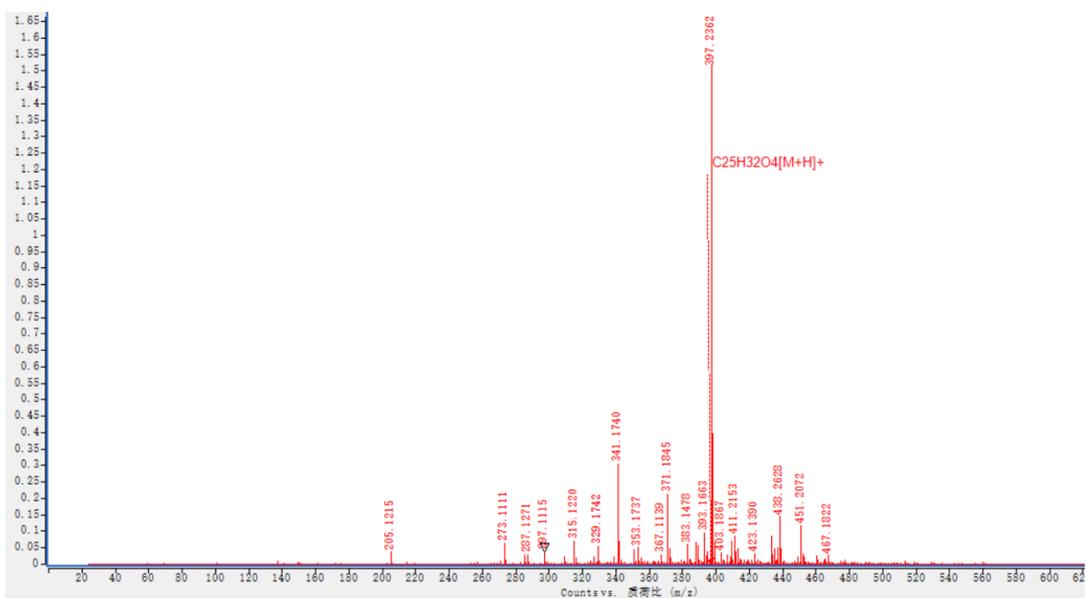


Figure S12: HR-ESI-MS positive mode ion of compound 2

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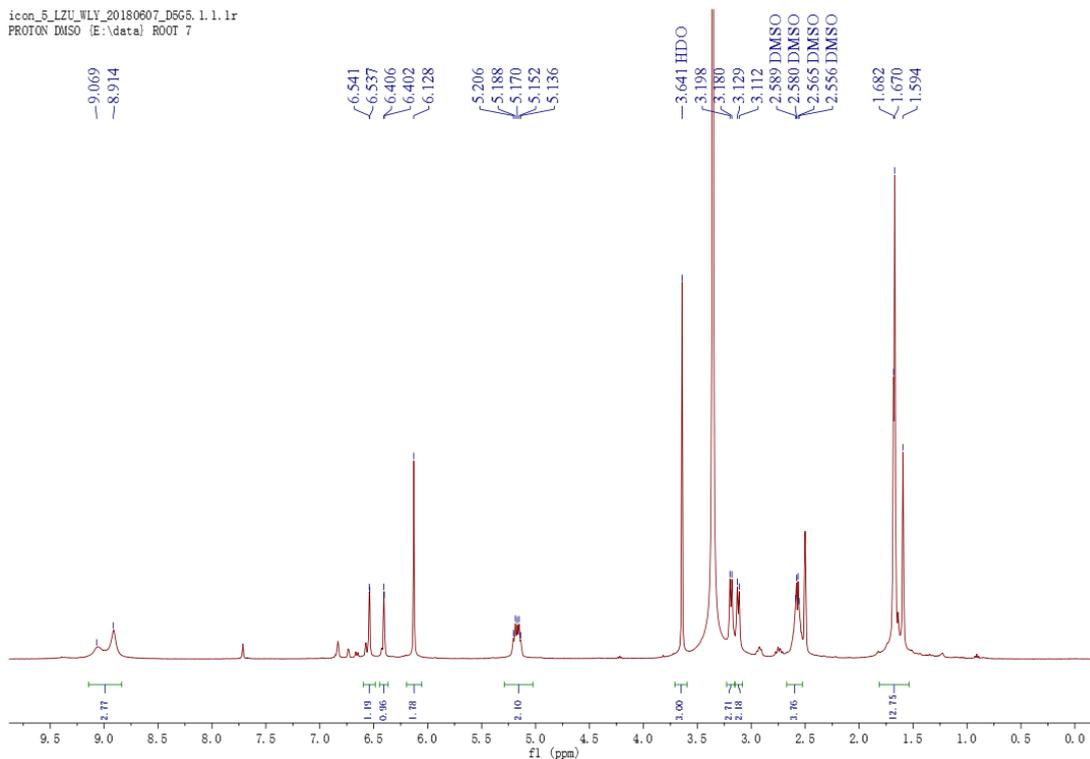


Figure S13: ¹H-NMR spectrum of compound 2

icon_5_LZU_WLY_20180607_D665.2.1.1r
C13CPD DMSO (E:\data) ROOT 7

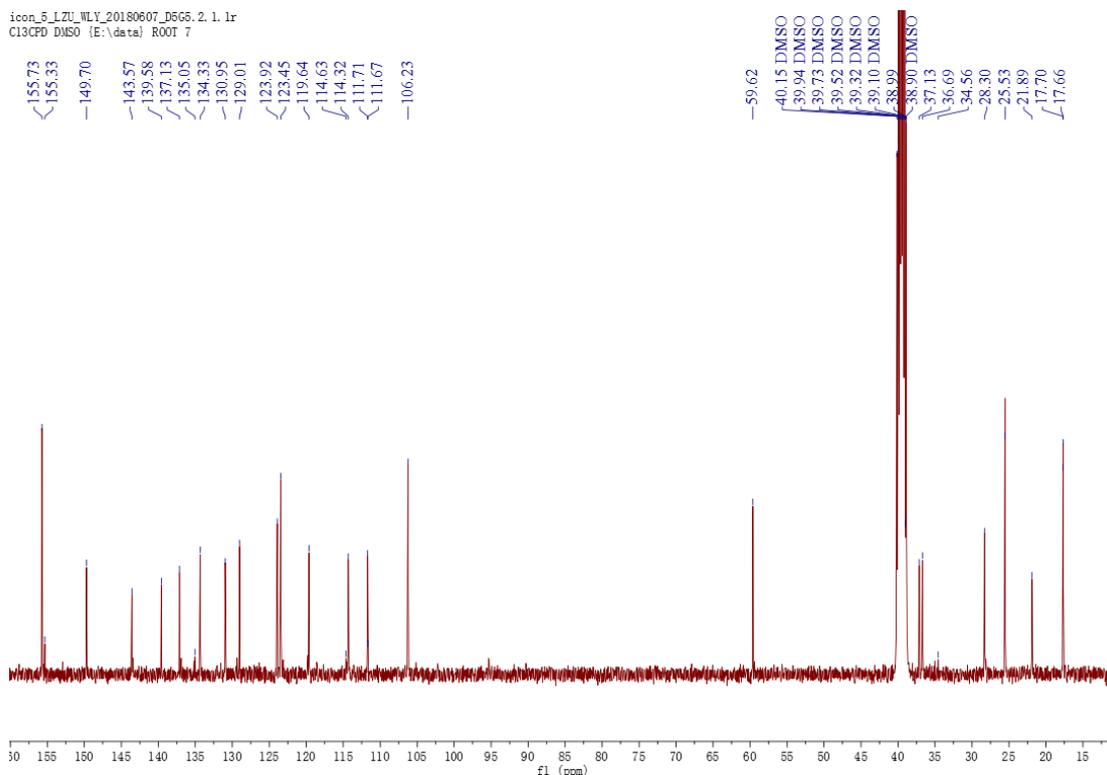


Figure S14: ¹³C-NMR spectrum of compound 2

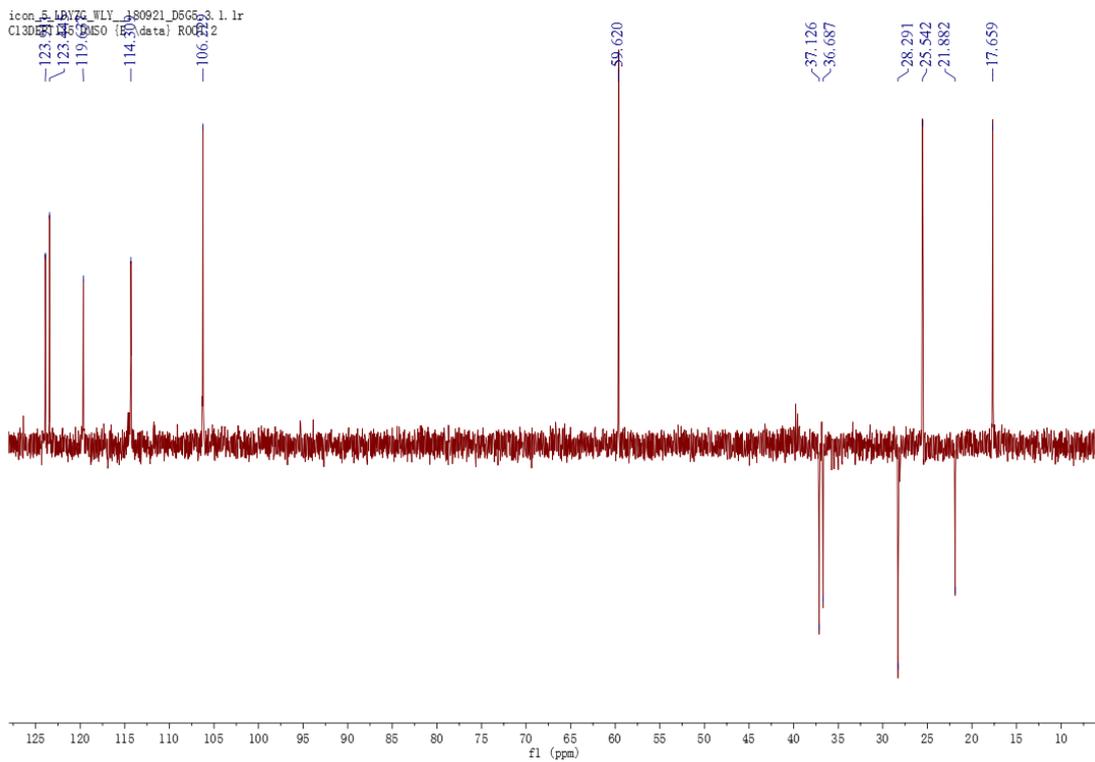


Figure S15: DEPT 135 spectrum of compound 2

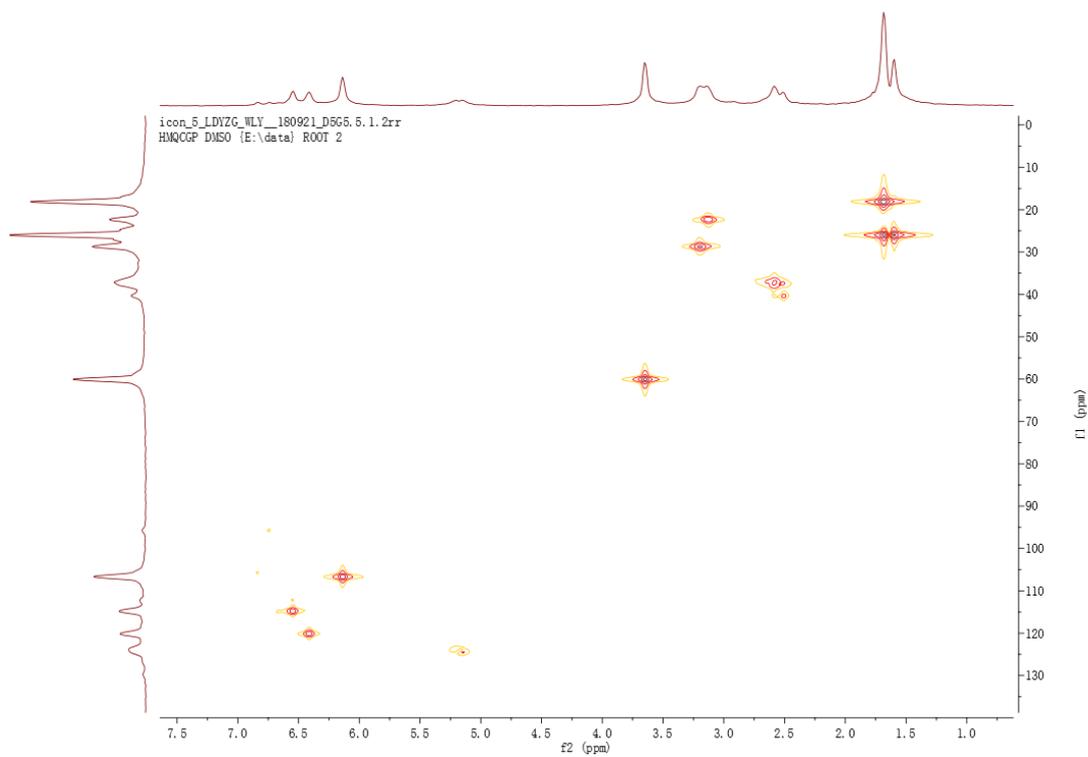


Figure S16: HSQC spectrum of compound 2

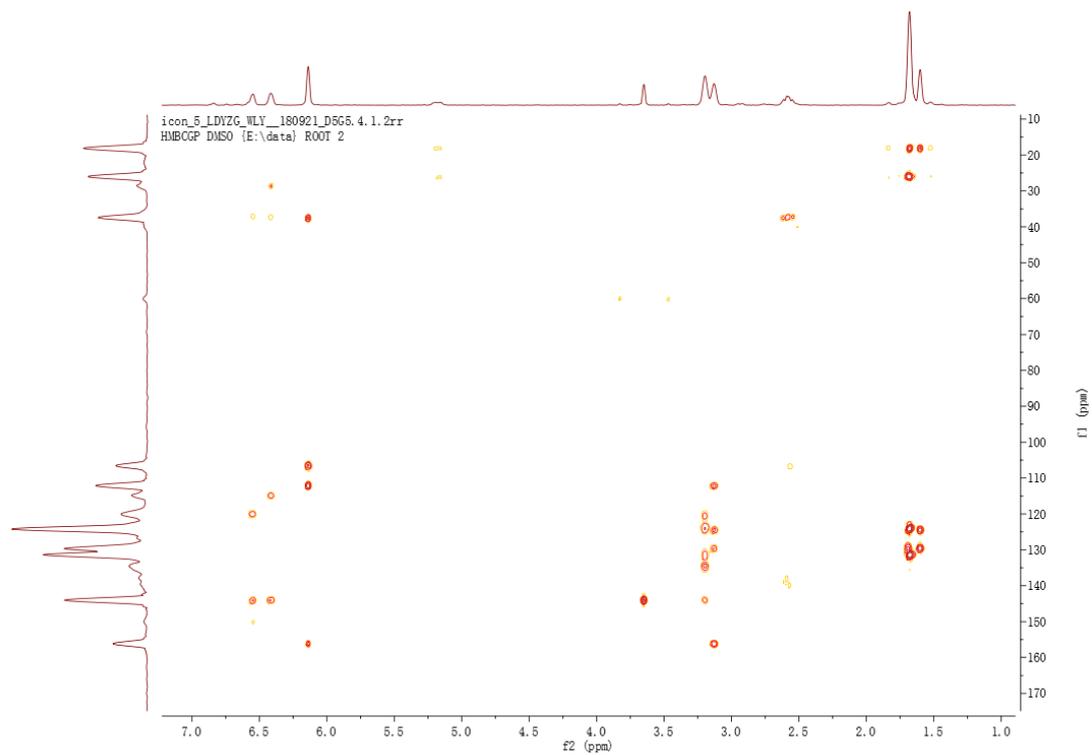


Figure S17: HMBC spectrum of compound 2

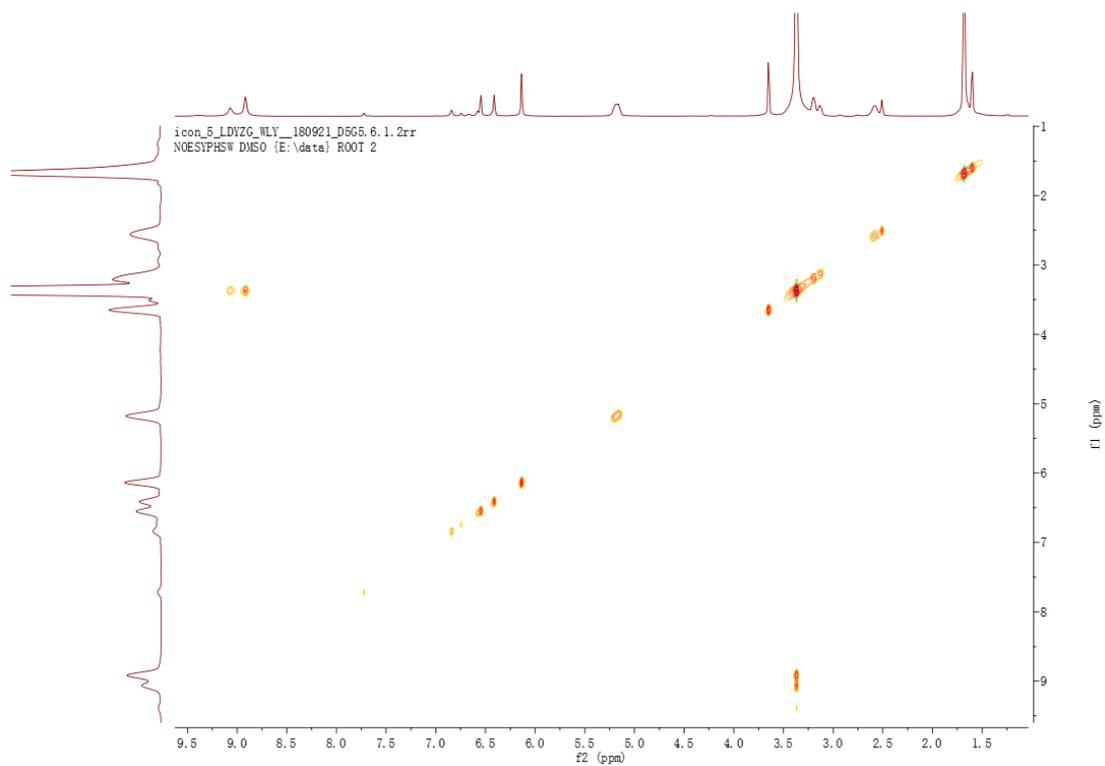


Figure S18: NOESY spectrum of compound 2

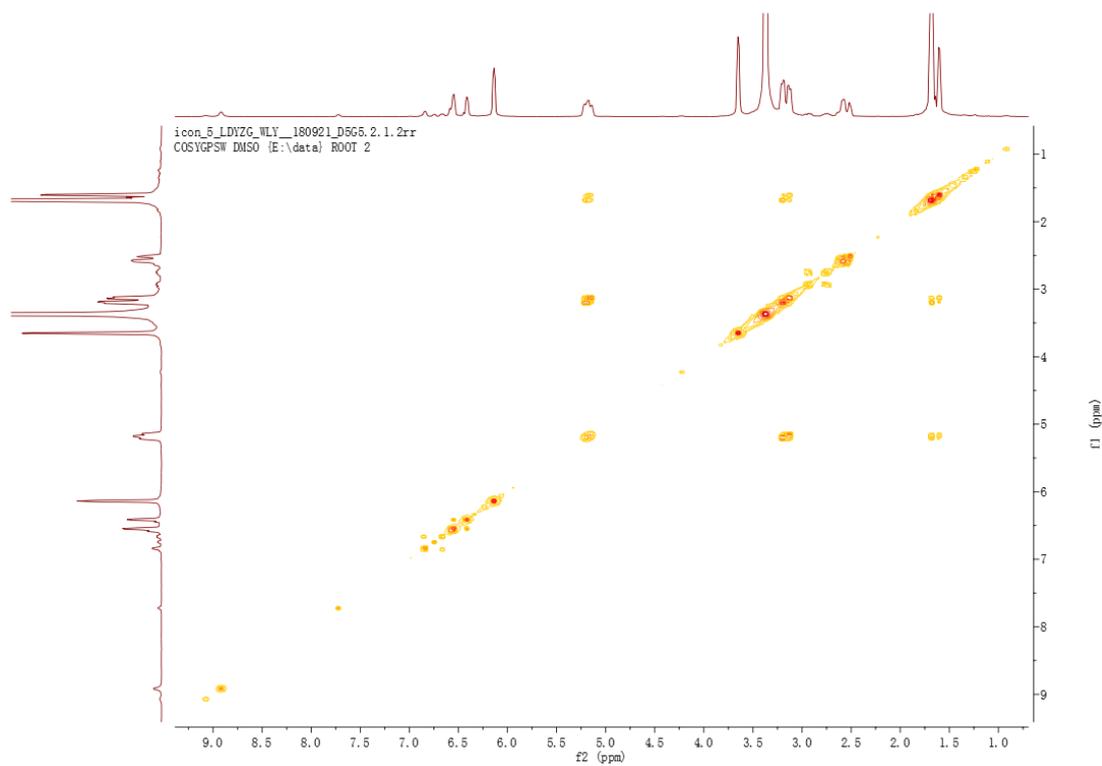


Figure S19: COSY spectrum of compound 2

Compound3

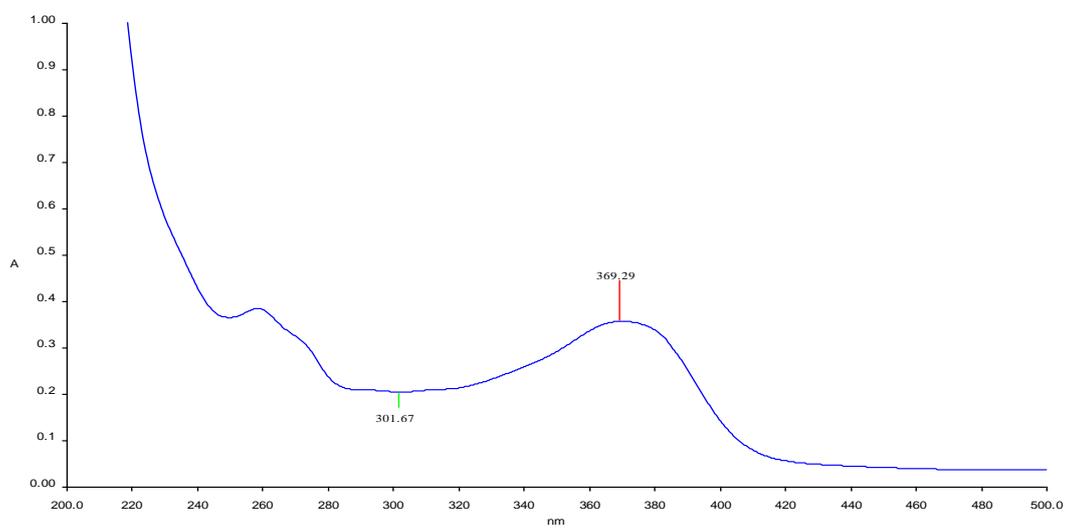


Figure S20: UV spectrum of compound 3 (absorption maxima at 258 and 369 nm)

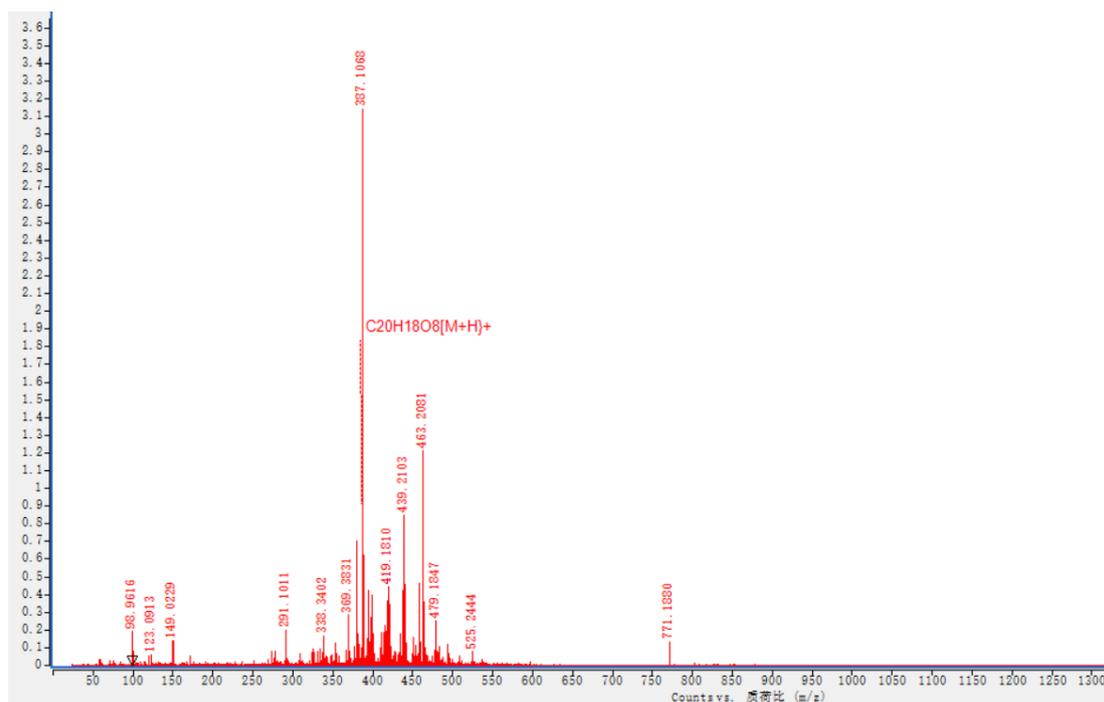


Figure S21:HR-ESI-MS positive mode ion of compound 3

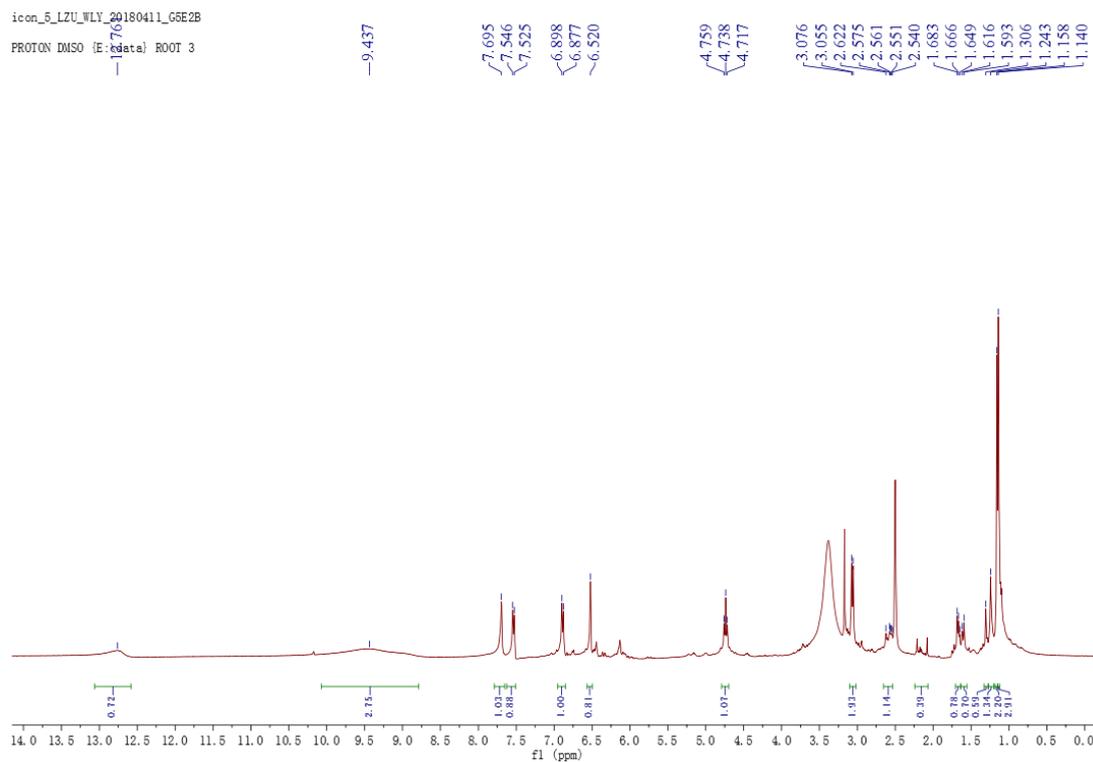


Figure S22: ¹H-NMR spectrum of compound 3 .

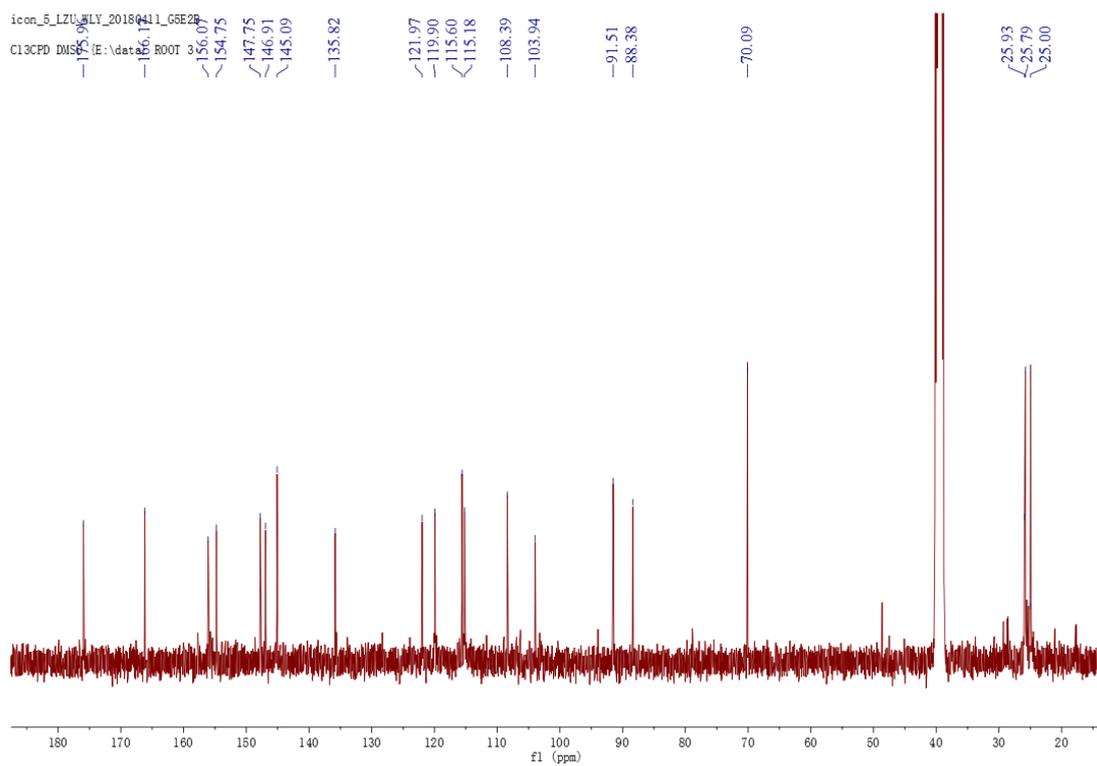


Figure S23: ^{13}C -NMR spectrum of compound **3**

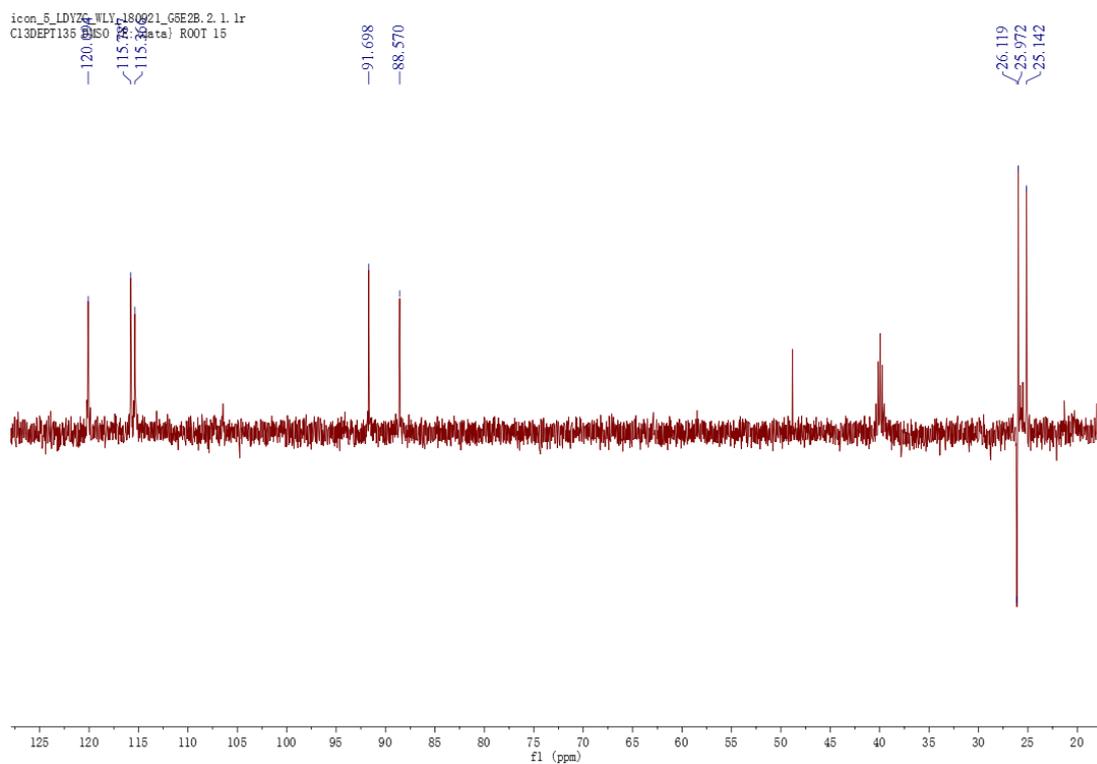


Figure S24: DEPT 135 spectrum of compound **3**

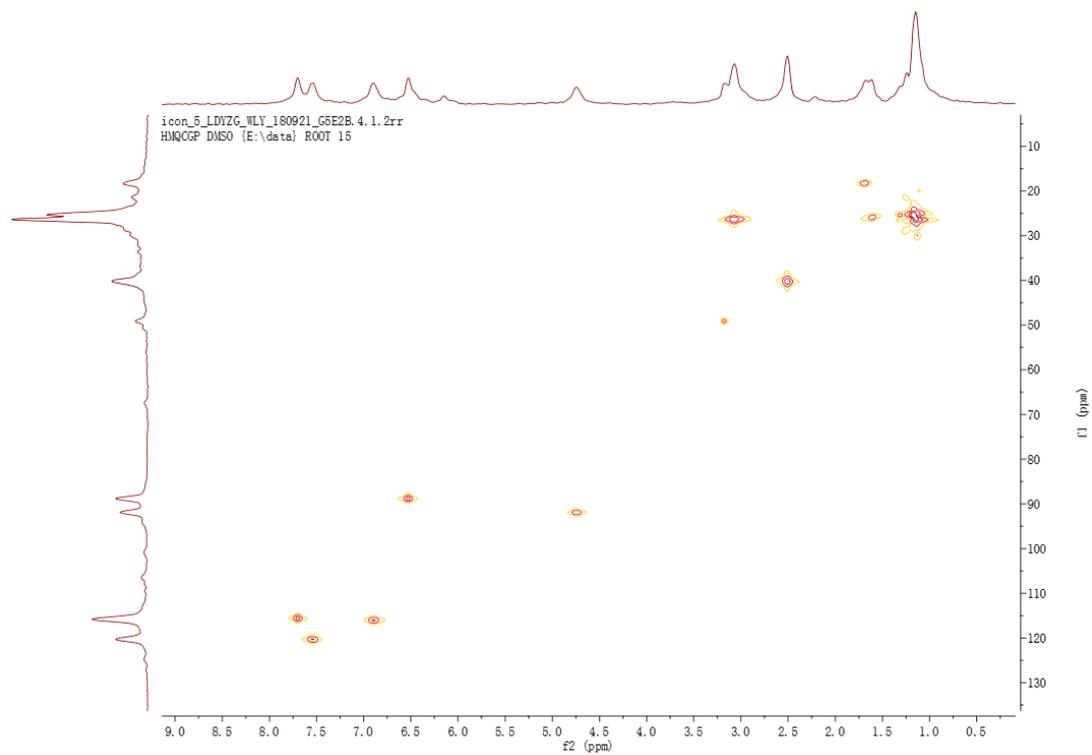


Figure S25: HSQC spectrum of compound 3

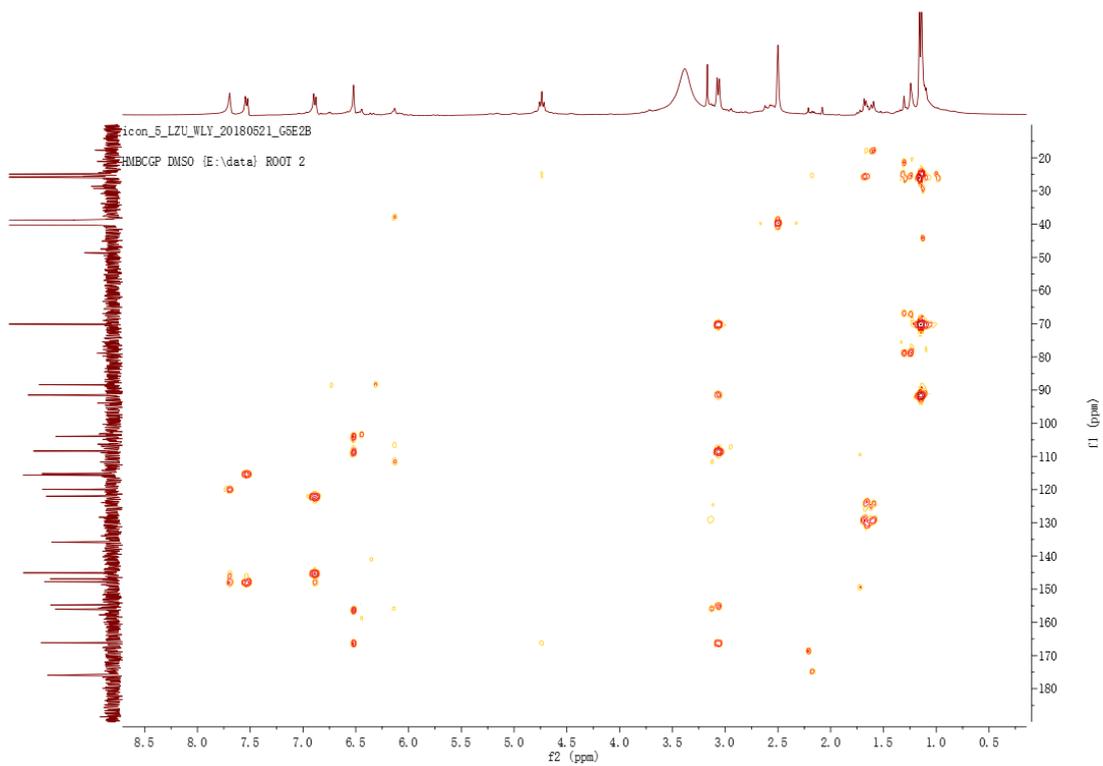


Figure S26: HMBC spectrum of compound 3

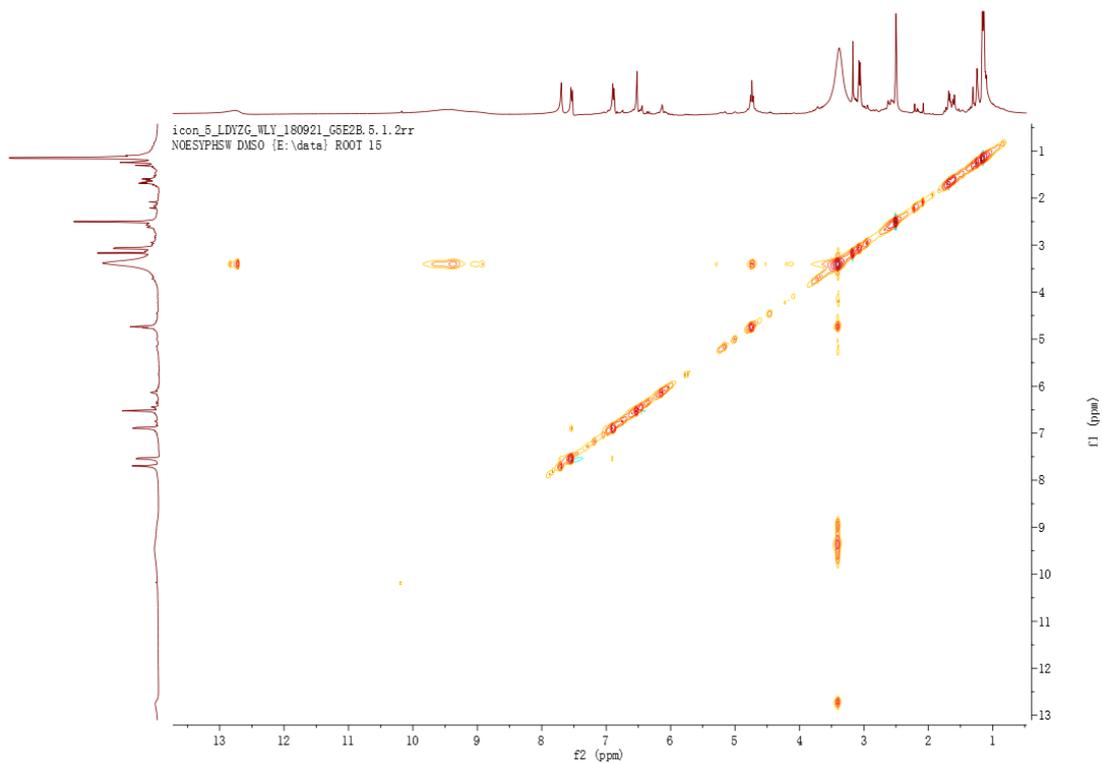


Figure S27: NOESY spectrum of compound 3

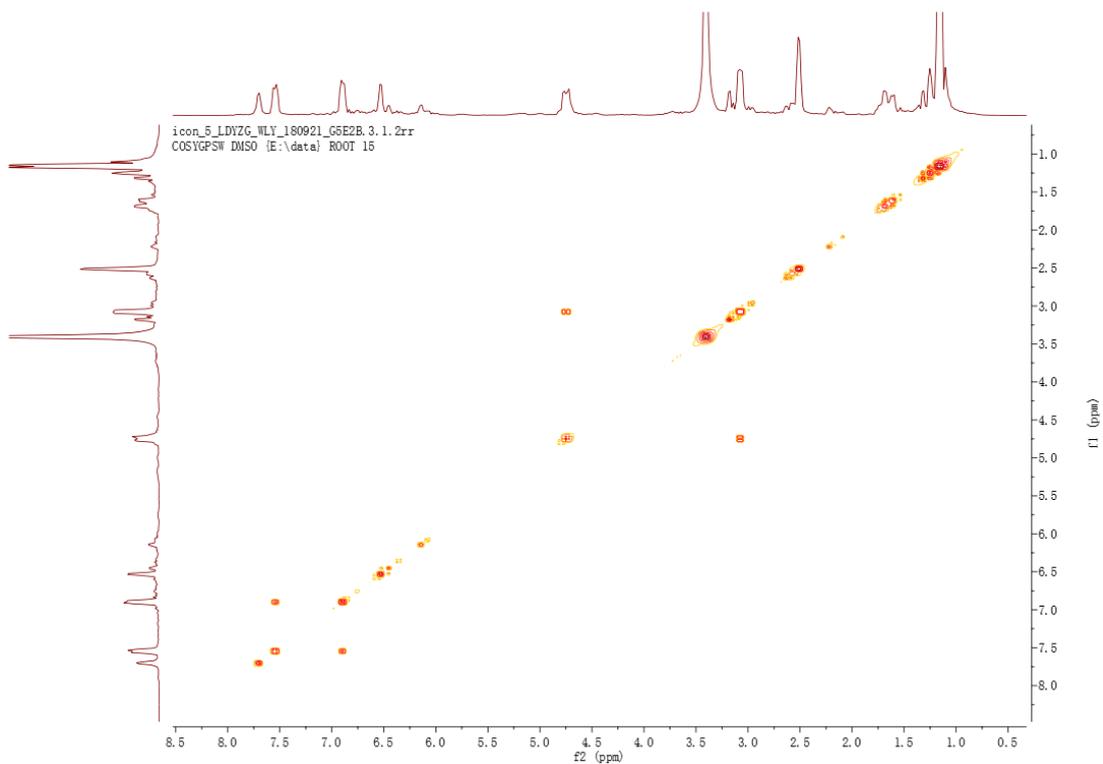


Figure S28: COSY spectrum of compound 3

Compound 4

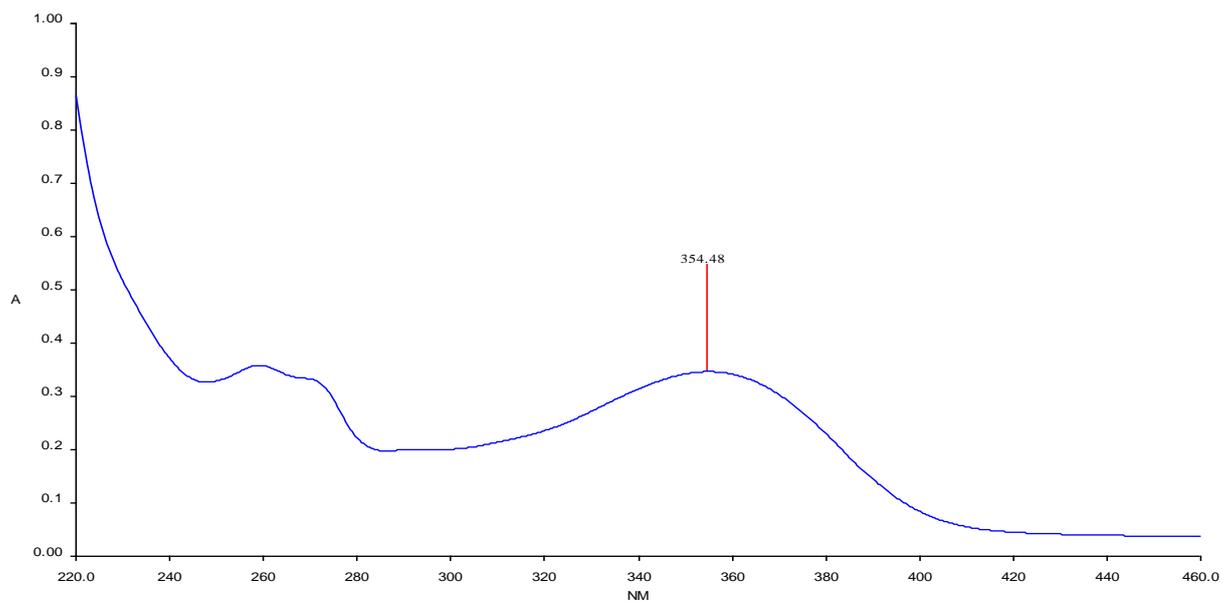


Figure S29: UV spectrum of compound 4 (absorption maxima at 259 and 354 nm)

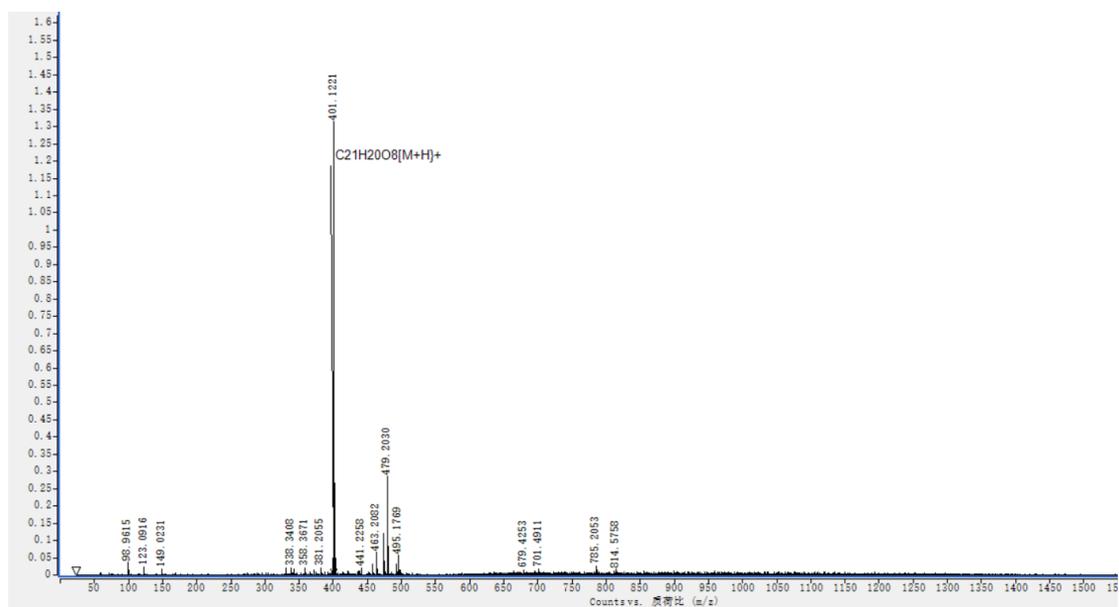


Figure S30: HR-ESI-MS positive mode ion of compound 4

icon_5_LZU_WLY_20180411_G6E2C
PROTON DMSO (E:\data) ROOT 7

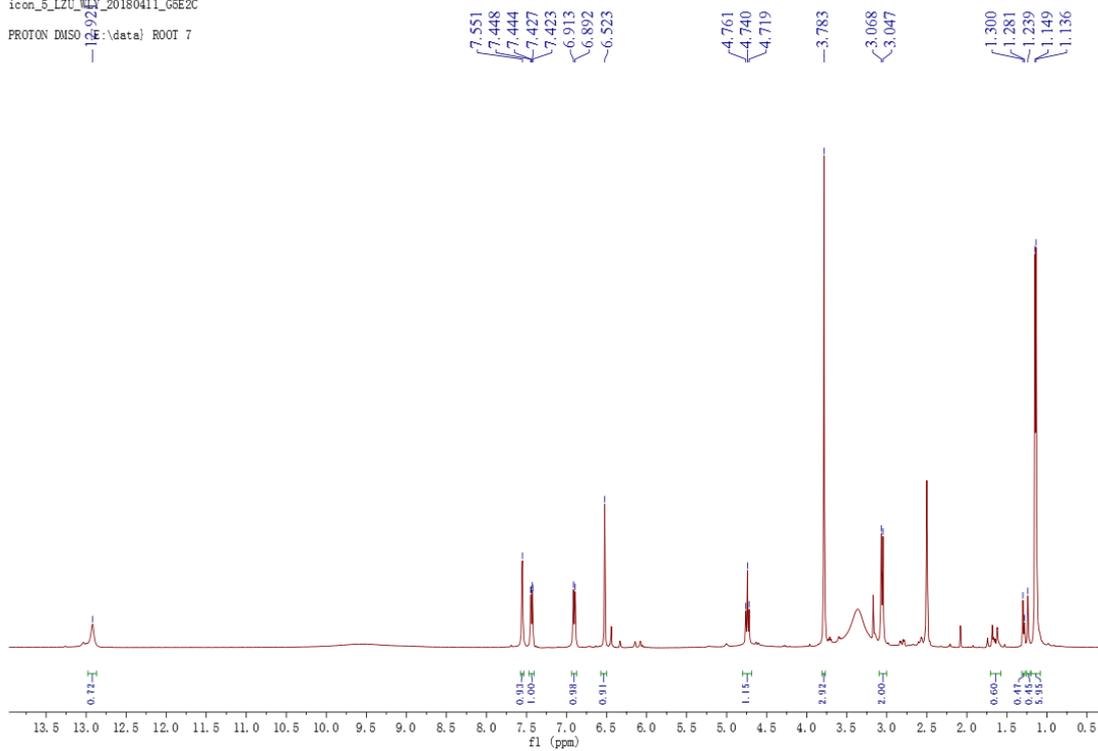


Figure S31: ¹H-NMR spectrum of compound 4

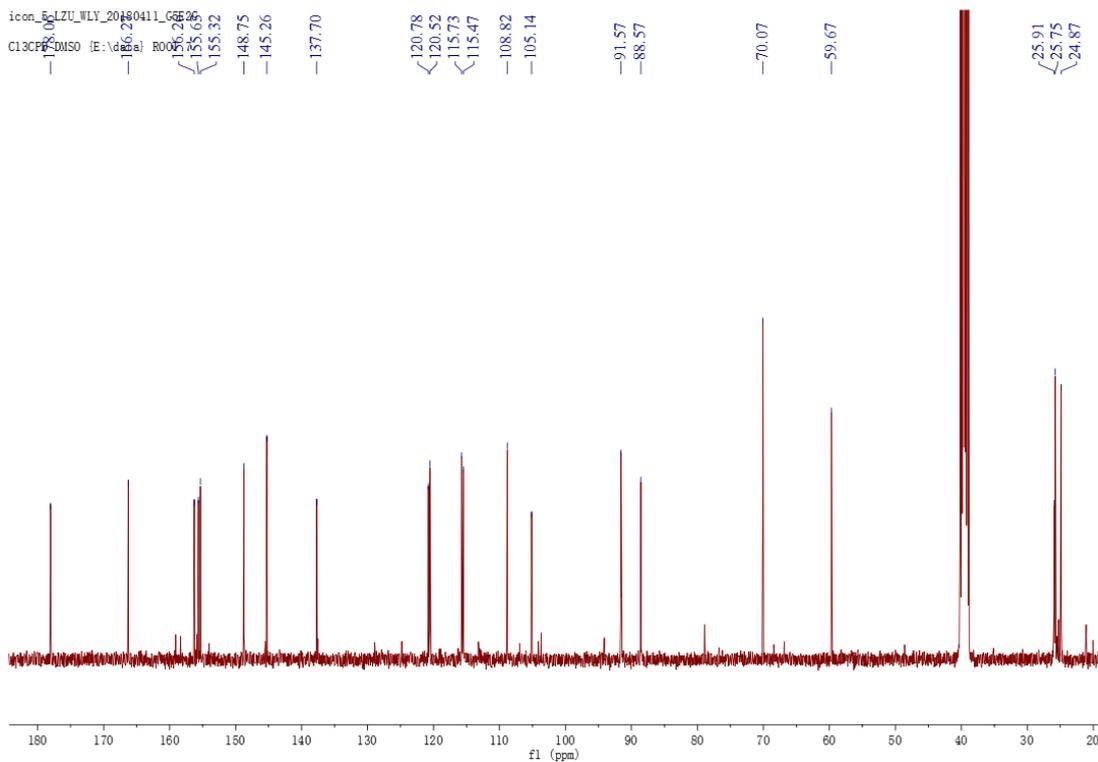


Figure S32: ¹³C-NMR spectrum of compound 4 .

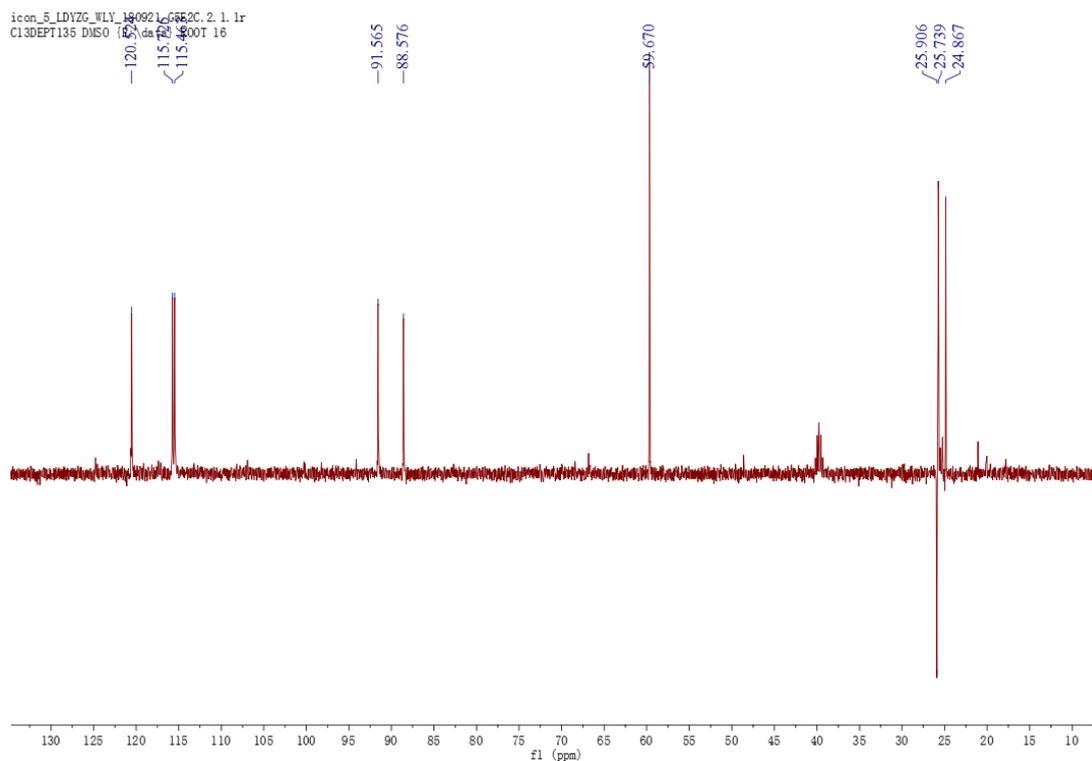


Figure S33: DEPT 135 spectrum of compound 4

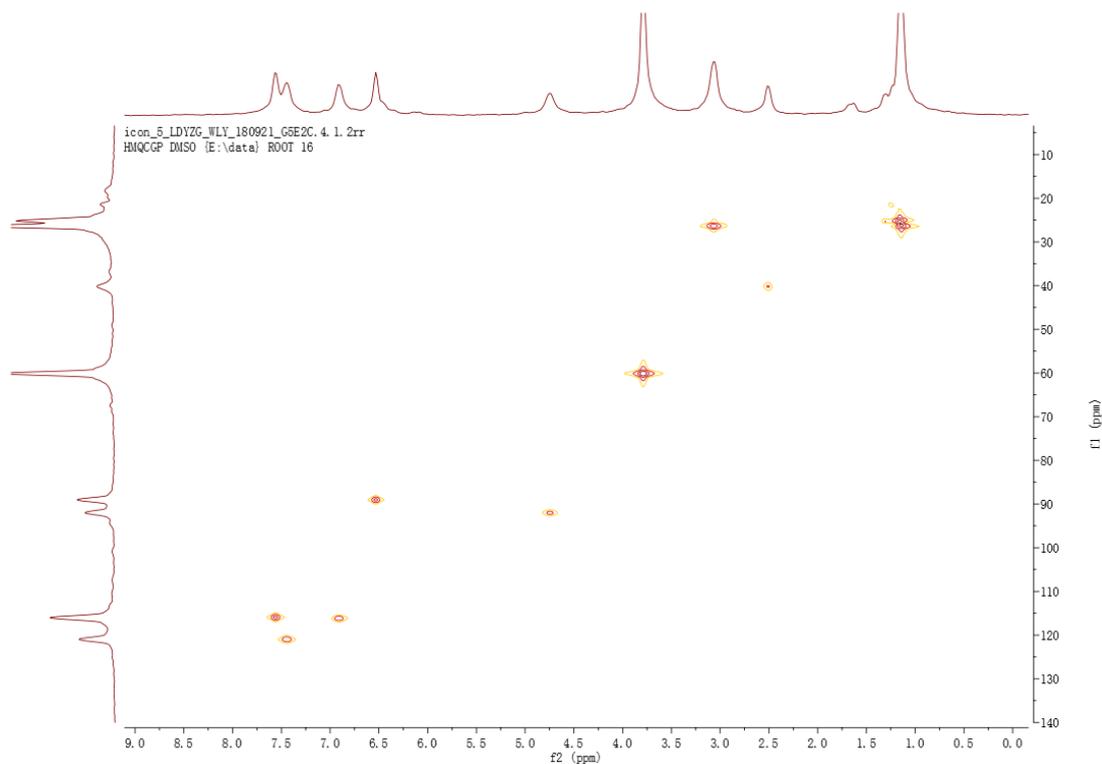


Figure S34: HSQC spectrum of compound 4

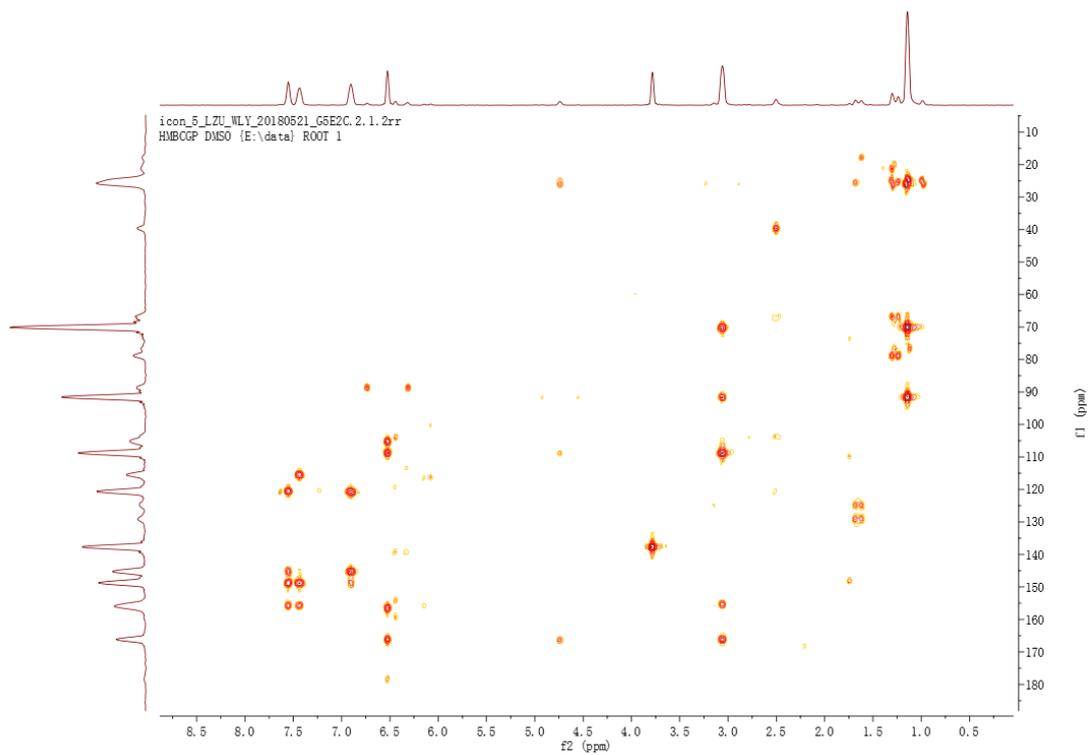


Figure S35: HMBC spectrum of compound 4

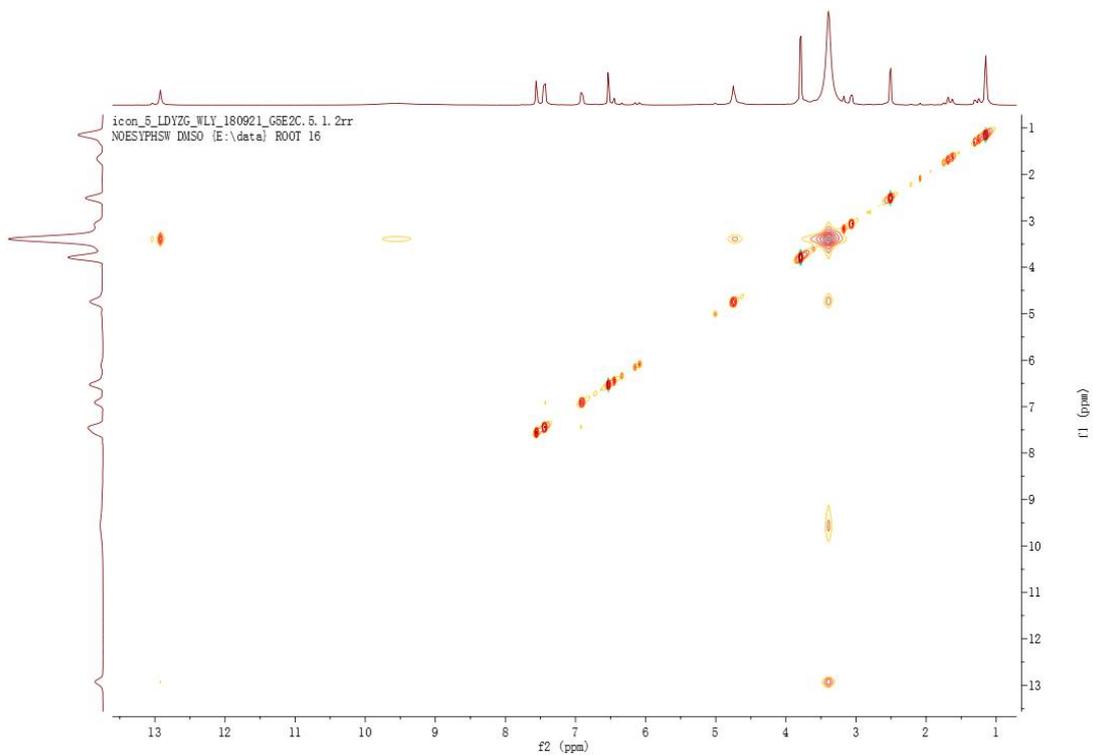


Figure S36: NOESY spectrum of compound 4

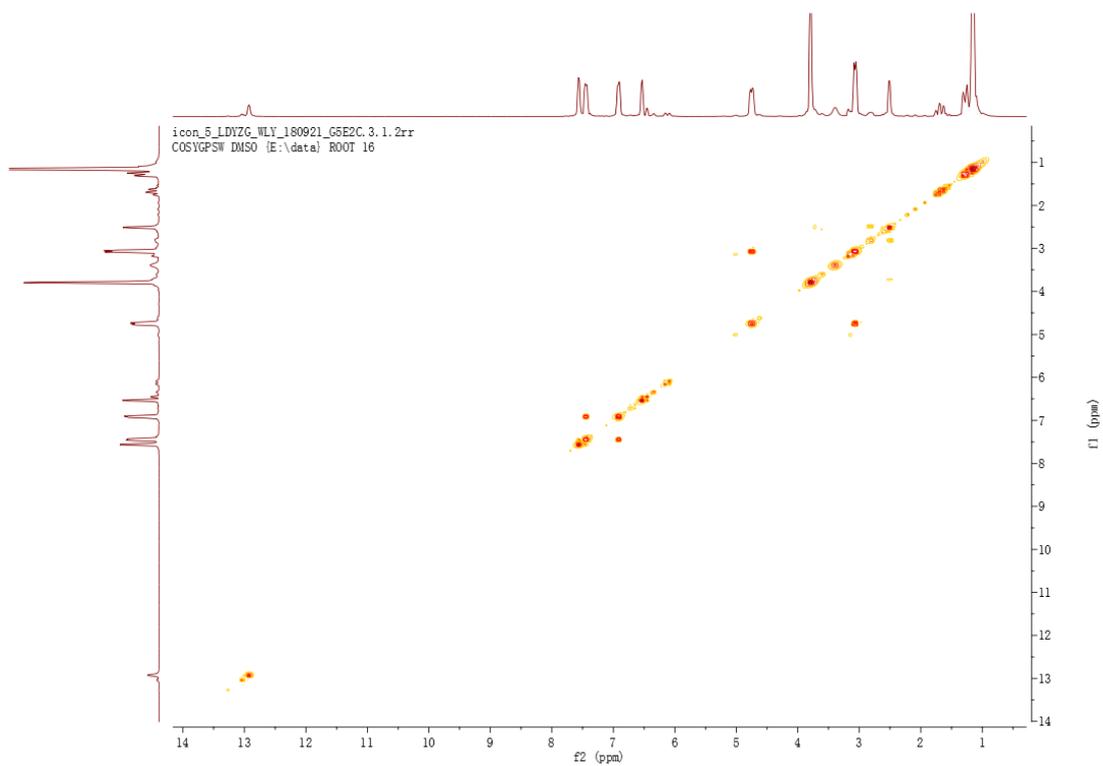


Figure S37: COSY spectrum of compound 4

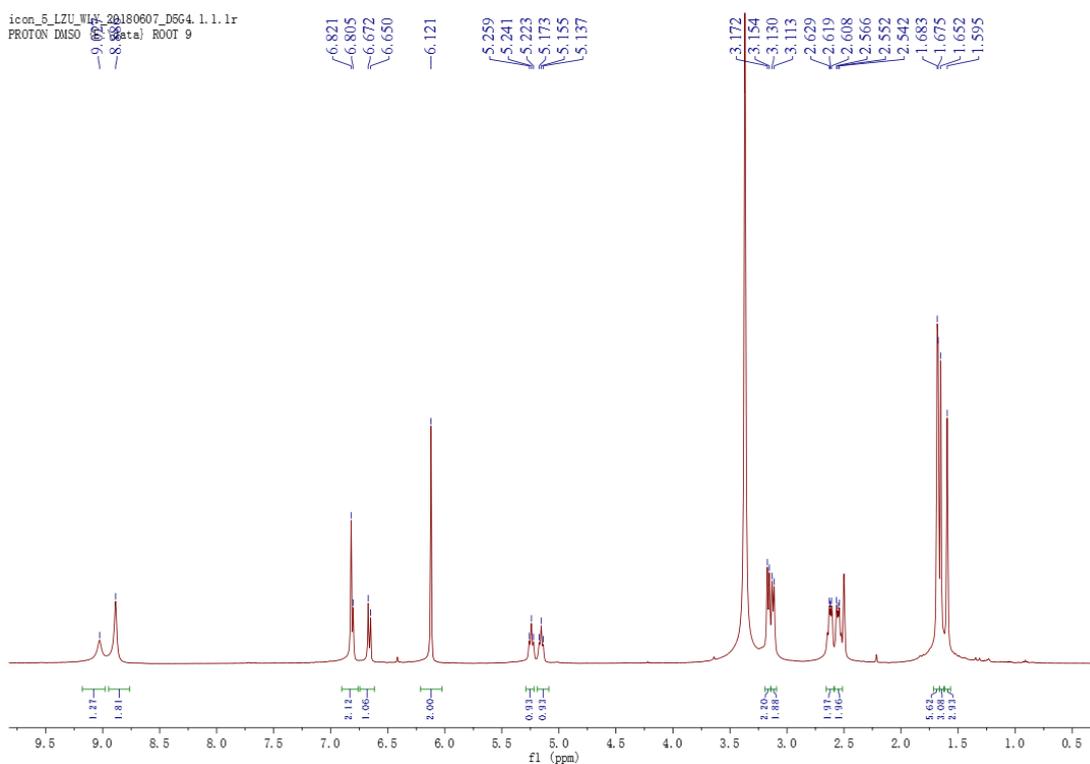


Figure S38: ¹H-NMR spectrum of compound 5

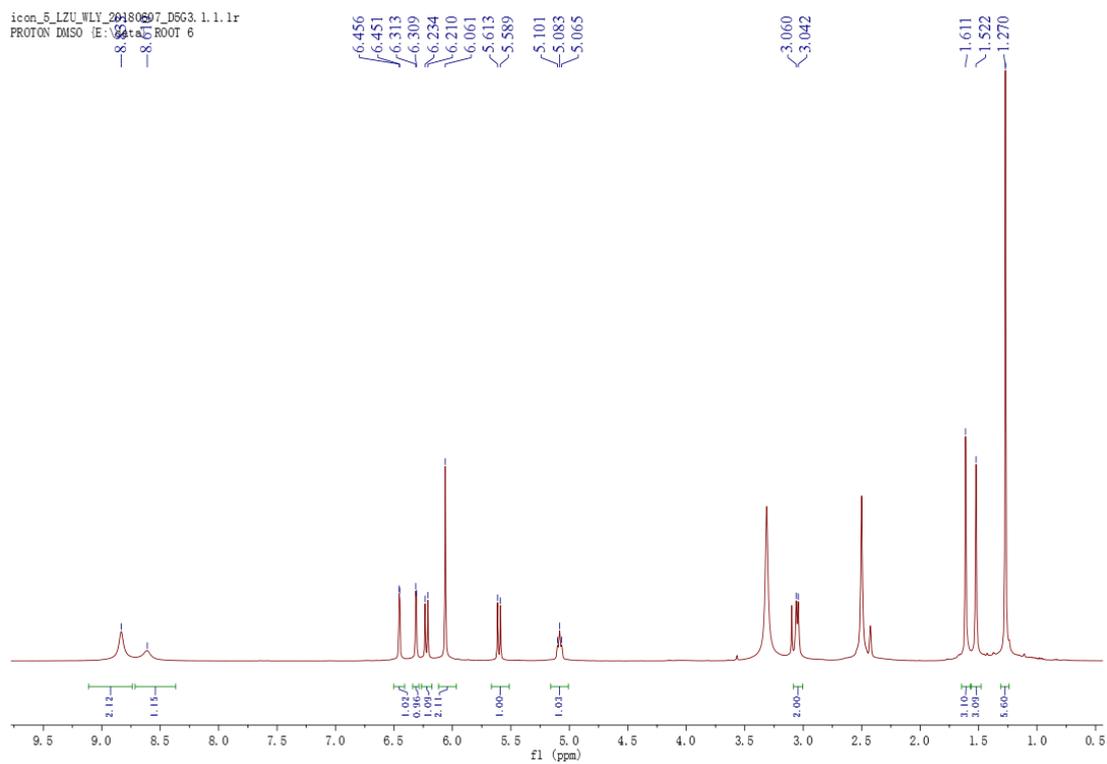


Figure S39: ¹H-NMR spectrum of compound 6

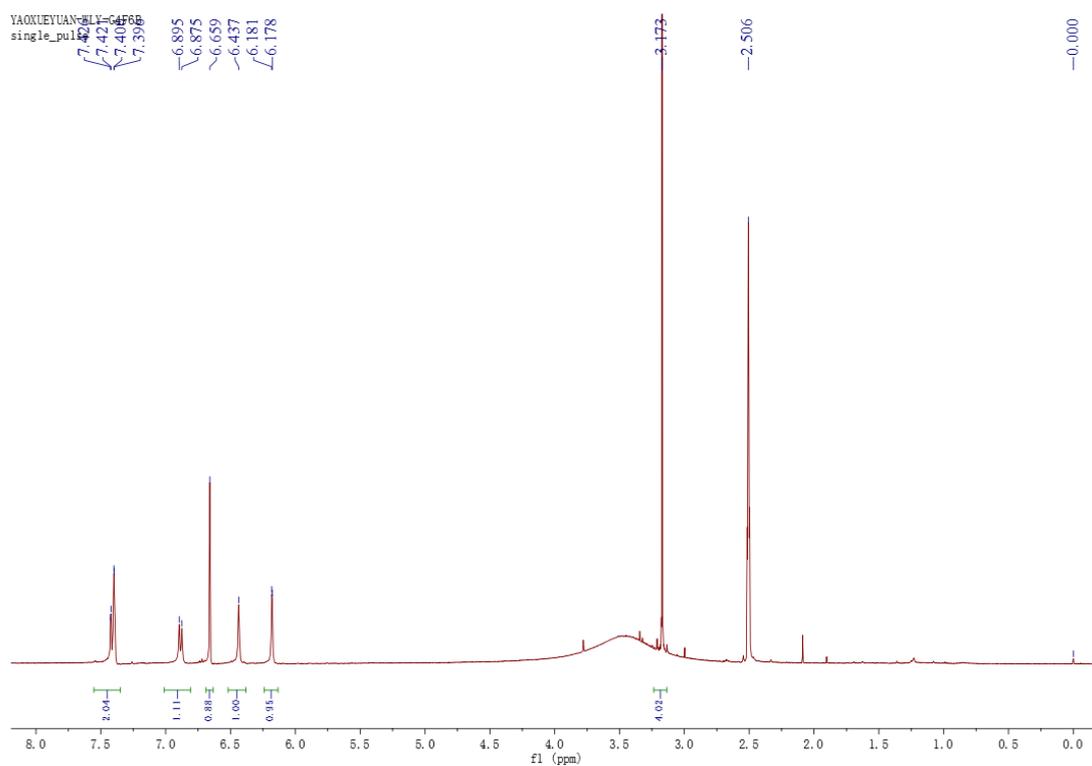


Figure S40: ¹H-NMR spectrum of compound 7

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PROTON DMSO (E:\data\6901-11

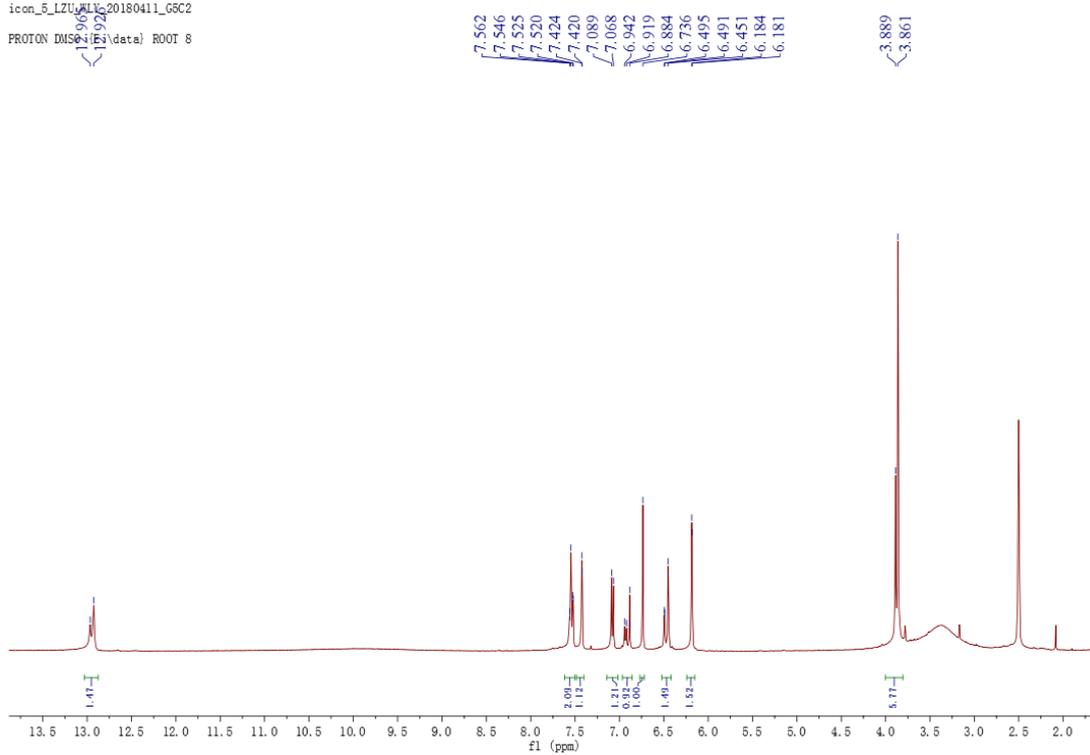


Figure S41: ¹H-NMR spectrum of compound 8

icon_5_LZU_WLY_20180411_G4E2
PROTON DMSO (E:\data\6901-11

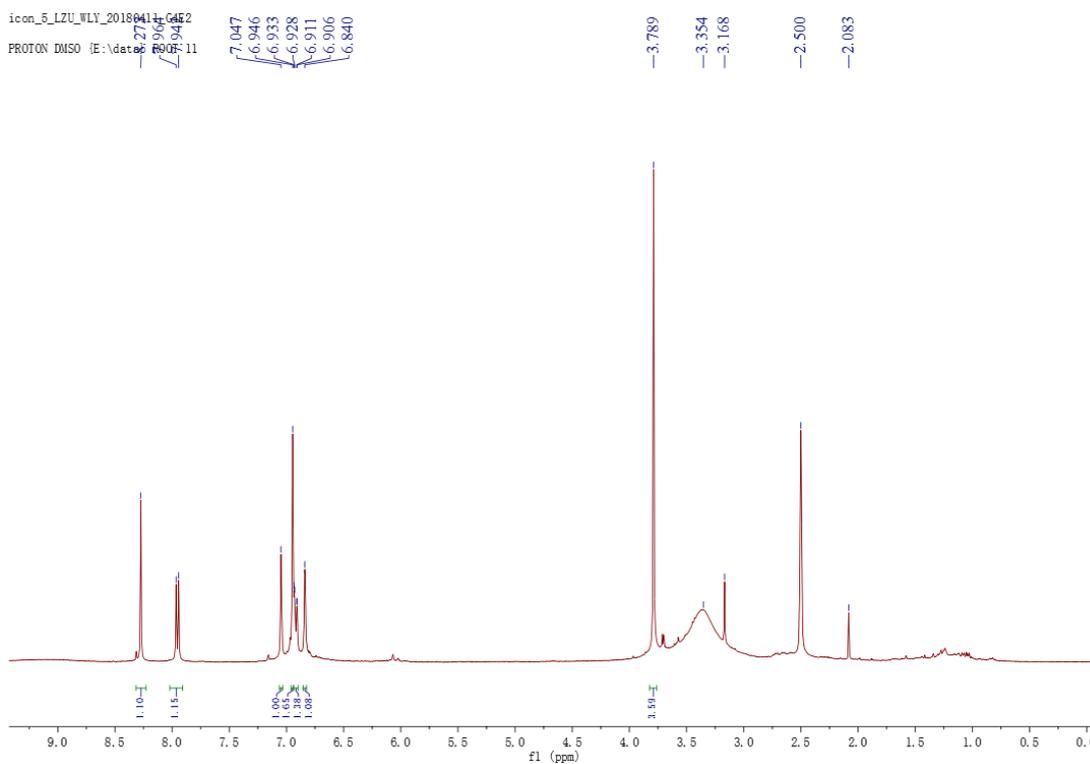


Figure S42: ¹H-NMR spectrum of compound 9

Table S1: The % composition of compounds **1-12** in the leaves of *G. uralensis*

| compound | weight(mg) | total weight of leaves(kg) | composition(%) |
|---|------------|----------------------------|----------------|
| licostilbene A (1) | 18.5 | 8 | 0.0002313 |
| licostilbene B (2) | 8.9 | 8 | 0.0001113 |
| licofuranol A (3) | 26.5 | 8 | 0.0003313 |
| licofuranol B (4) | 15.8 | 8 | 0.0001975 |
| α,α' -dihydro-3,5,4'-trihydroxy-4,5'-d iisopentenylstilbene (5) | 12.6 | 8 | 0.0001575 |
| glycopytilbene B (6) | 28.4 | 8 | 0.0003550 |
| Luteolin (7) | 5.1 | 8 | 0.0000638 |
| quercetin-3,4'-dimethyl ether (8) | 4.2 | 8 | 0.0000525 |
| calycosin (9) | 6.7 | 8 | 0.0000838 |
| scopoletin (10) | 2 | 8 | 0.0000250 |
| diosmetin (11) | 7.9 | 8 | 0.0000988 |
| echinatin (12) | 4.6 | 8 | 0.0000575 |