Supplementary data

Title: Inhibitory Effects of Thirty Compounds from *Potentilla longifolia* on Lipid Accumulation and Their Mechanisms in 3T3-L1 Cells

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ABSTRACT: *Potentilla longifolia* Willd. ex Schlecht, which is a kind of traditional Chinese herb, is often referred to as "Ganyancao" in China, which means "the herb is effective in the treatment of liver inflammation". However, there are little modern studies on its chemical constituents and their pharmacological effects so far. Three new (ganyearmcaoosides A and B and ganyearmcaoic acid A; 1–3) and 27 known compounds (4–30) were isolated from the 95% ethanol extract of the dried aerial parts of this plant, of which 23 were isolated for the first time from this plant. The chemical structures of these compounds were elucidated using 1D and 2D NMR (^{1}H−^{1}H COSY, HMBC, etc.) and HR-ESI-MS analysis. Cytotoxicity assessment was carried out in 3T3-L1 cells exposed to various concentrations (10-80 mM) of 30 compounds for 96 h using MTT assay. The inhibitory effects of the 30 compounds with safe concentrations on the lipid accumulation in 3T3-L1 cells were evaluated using photographic and quantitative assessments of lipid contents by Oil Red O staining, and measurement of the triglyceride levels. Comprehensive analysis from several aspects of these screening experiments showed that compounds 1, 2, 3, 9, 11, 12, and 13 significantly inhibited the differentiation and lipid accumulation in 3T3-L1 cells. Among them, compound 12 (3,8-dimethoxy-5,7,4'- trihydroxyflavone) showed the best inhibitory effect on lipid accumulation such as reducing the accumulation of oil droplets and triglyceride level, and was superior to the reference (ursolic acid) in positive control. Molecular docking analysis showed that compound 12 had greater binding affinities with AMPK than the reference compound. Western blot analysis and RT-PCR results showed that compound 12 enhanced the phosphorylations of AMPK and ACC, and inhibited the expressions of adipogenesis-related proteins and genes including SREBP1c, FAS, SCD1, GPAT, PPARγ and C/EBPα, and thereby

significantly inhibited lipid accumulationin in a concentration-dependent manner. The results from this study revealed that *P. Longifolia* and its bioactive compounds could be promising as potential therapeutic agents for diseases related to lipid accumulation in the future.

Keywords: *Potentilla longifolia*, lipid accumulation, 3,8-dimethoxy-5,7,4'-trihydroxyflavone, SREBP1c, PPARγ, molecular docking, new compound (ganyearmcaooside)

LIST of FIGURES

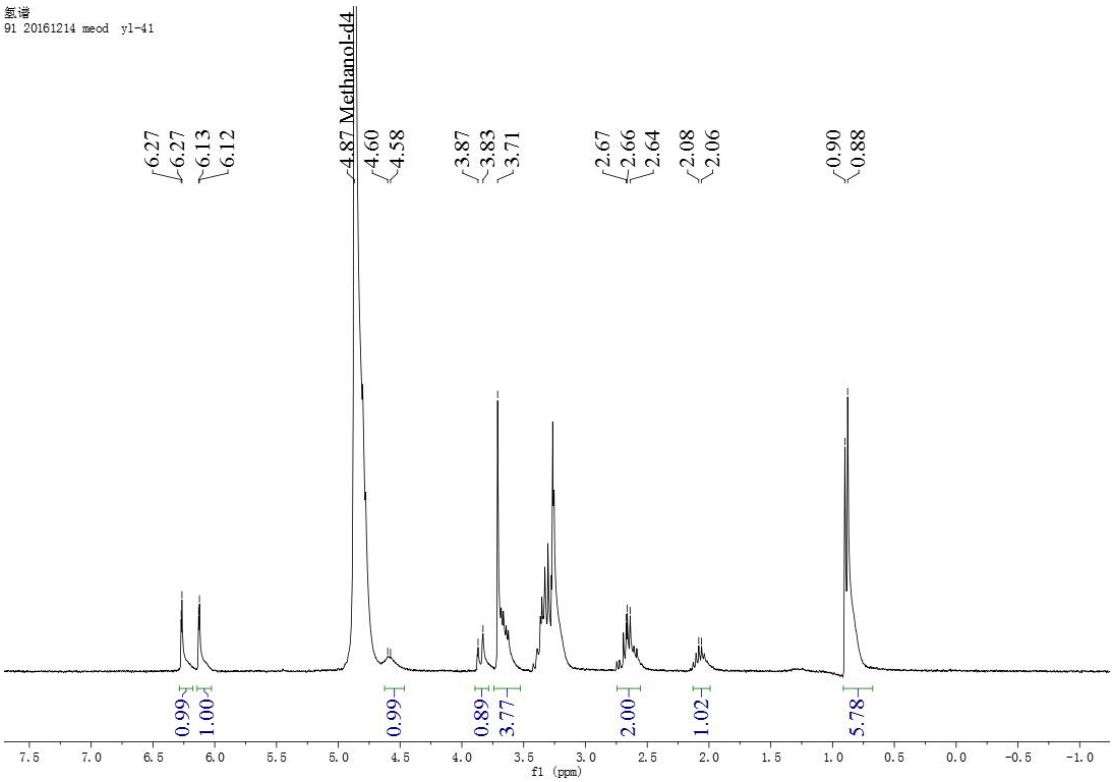

FIGURE S1. ^{1}H NMR spectrum of compound 1 in CD_3OD.

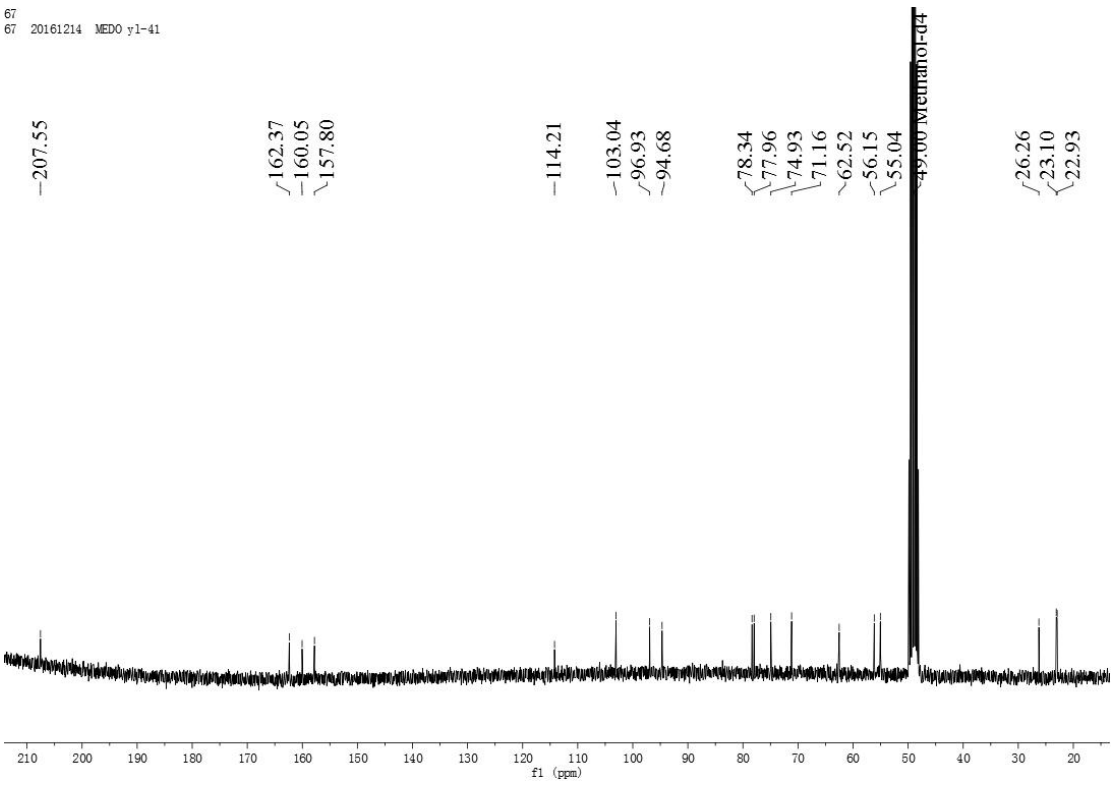

FIGURE S2.^{13}C NMR spectrum of compound 1 in CD_3OD.

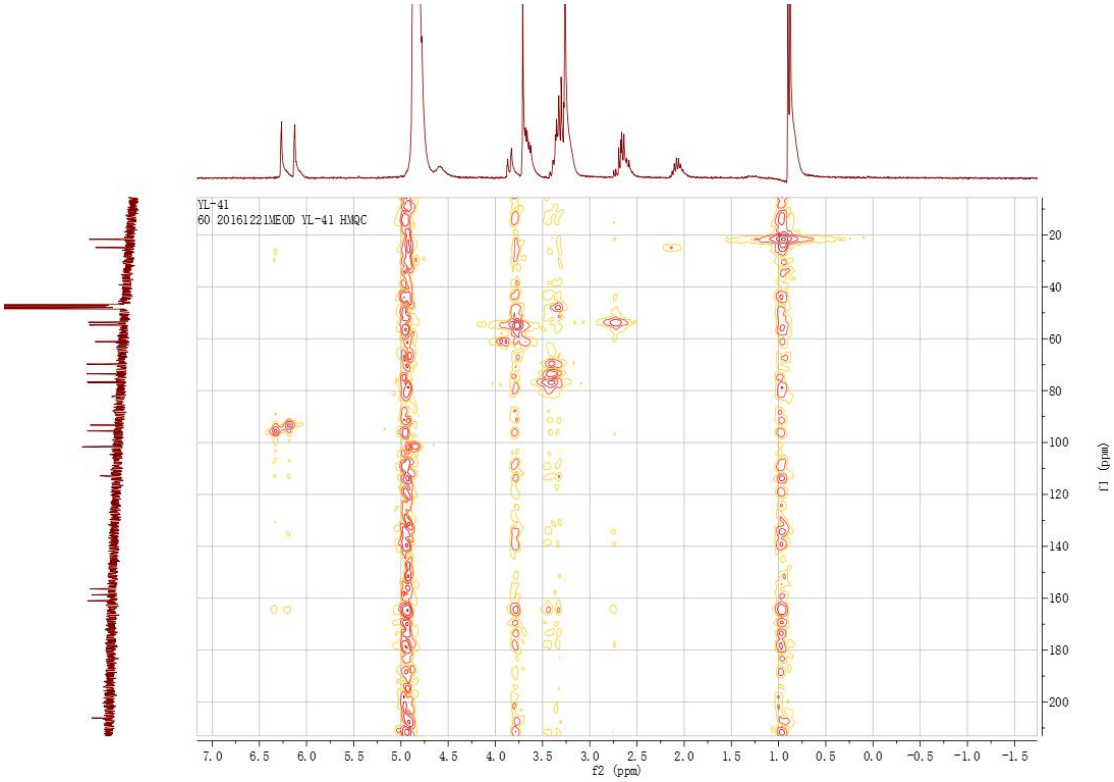

FIGURE S3. HMQC spectrum of compounds 1 in CD_3OD.

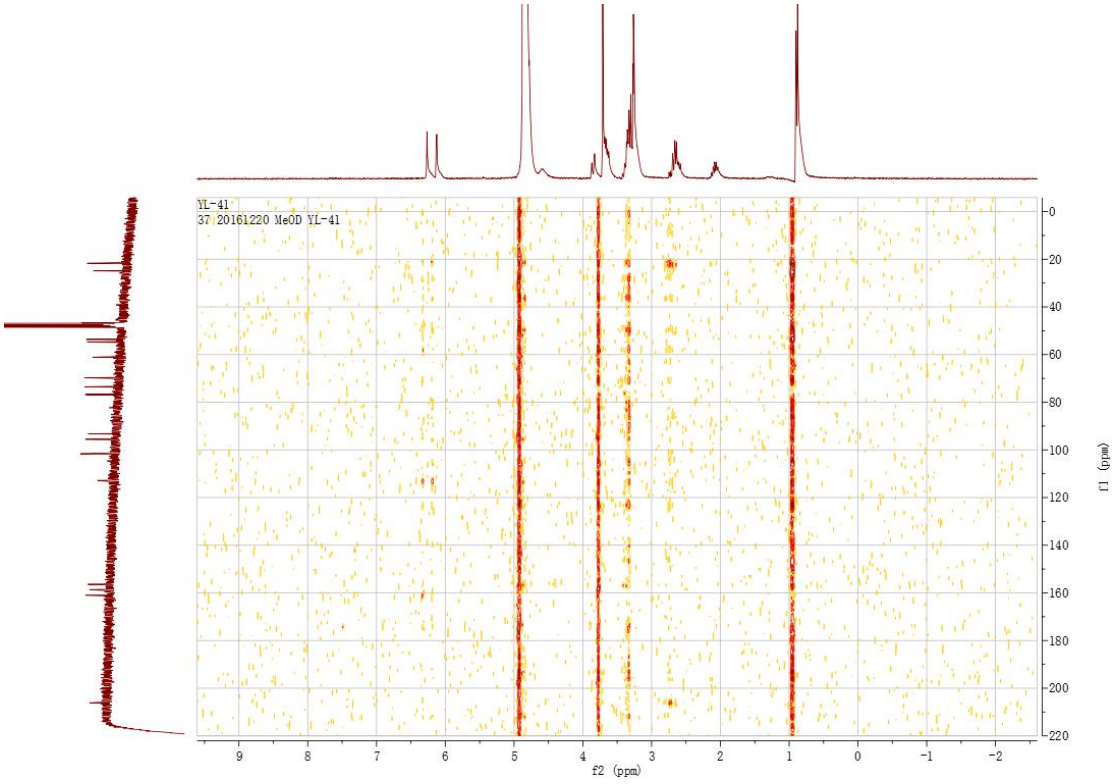

FIGURE S4. HMBC spectrum of compound 1 in CD_3OD.

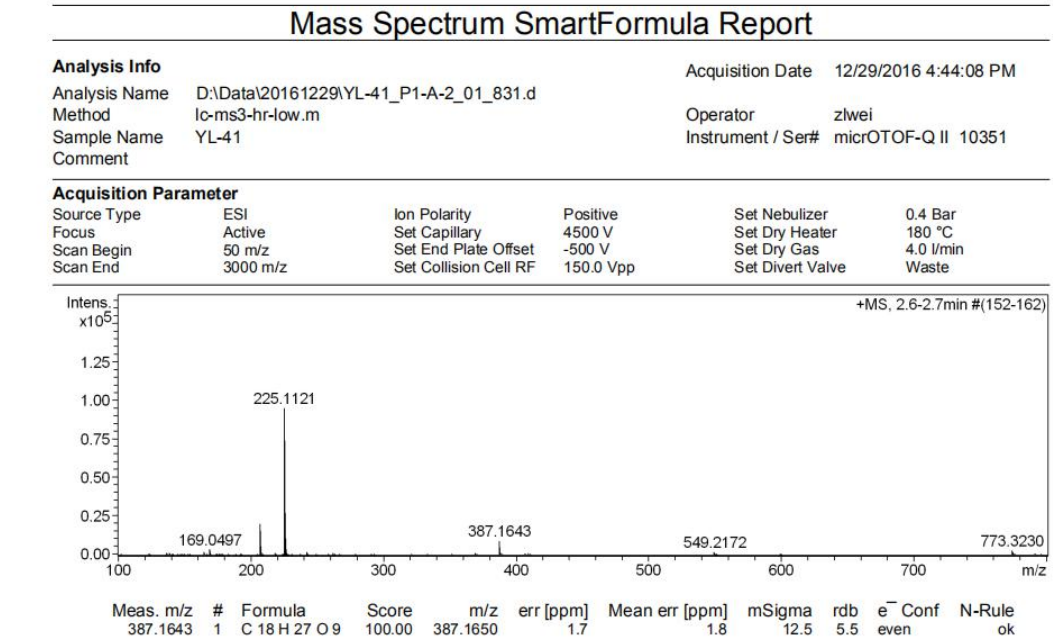

FIGURE S5. HR-ESI-MS spectrum of compound 1.

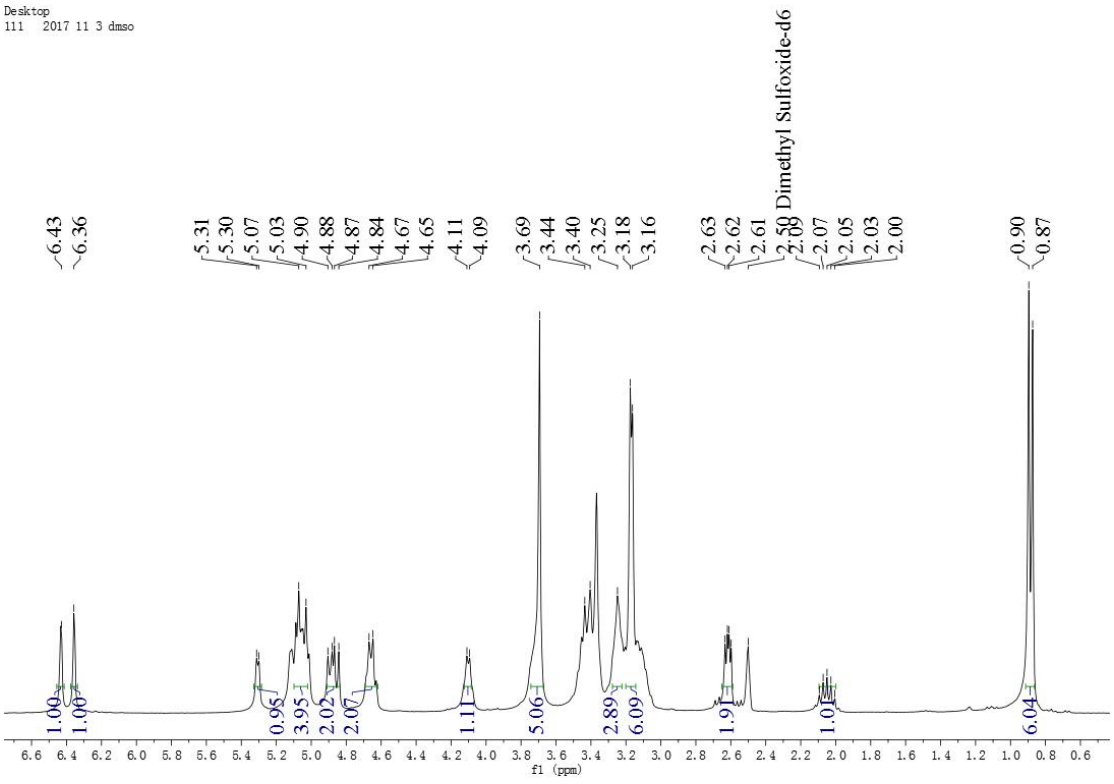

FIGURE S6. ^{1}H NMR spectrum of compound 2 in DMSO.

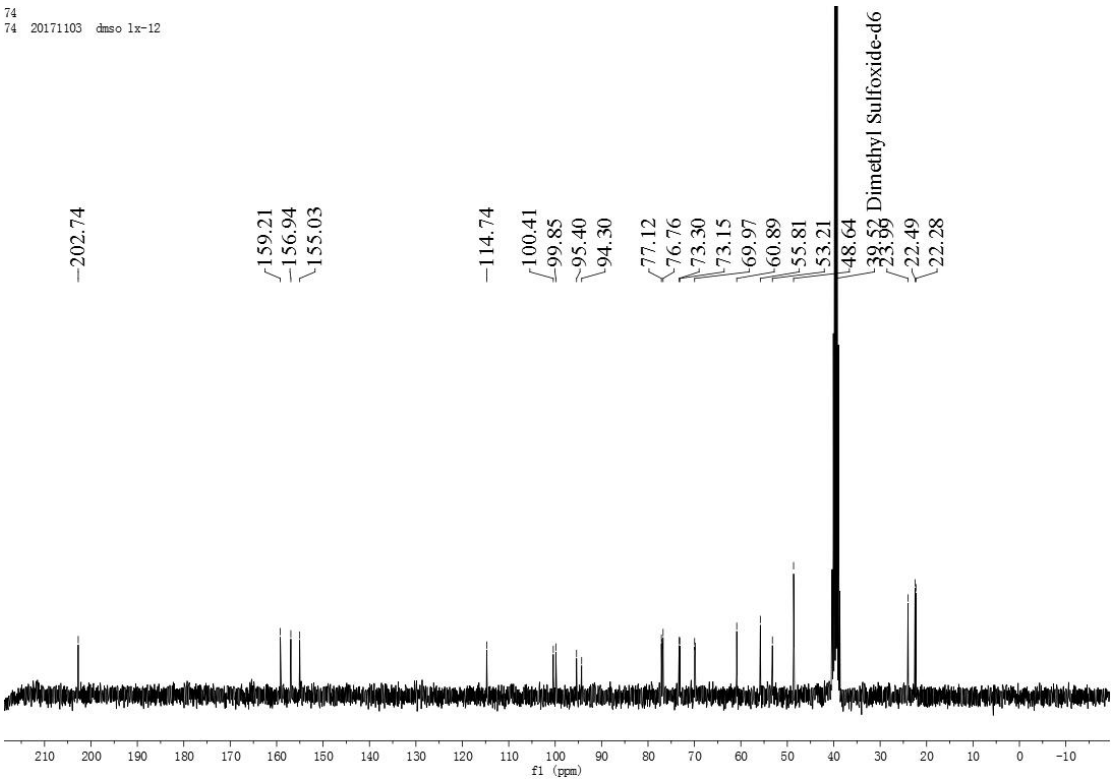

FIGURE S7.[13]C NMR spectrum of compound 2 in DMSO.

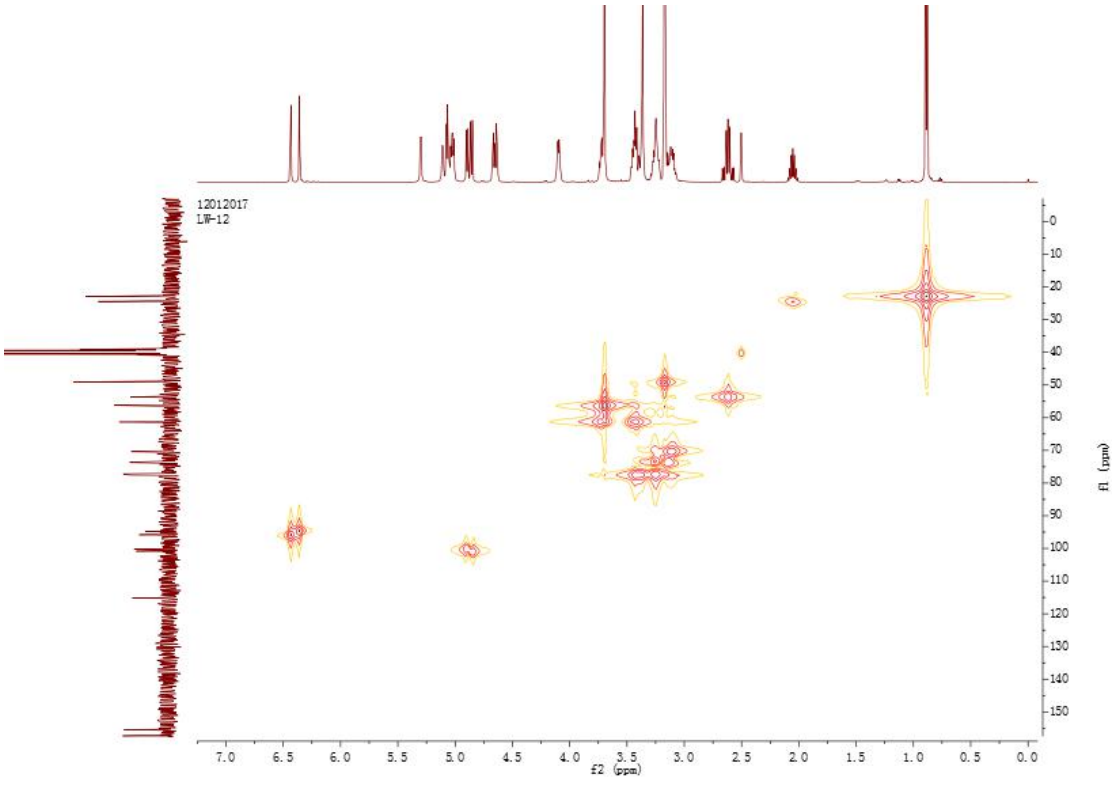

FIGURE S8. HMQC spectrum of compound 2 in DMSO.

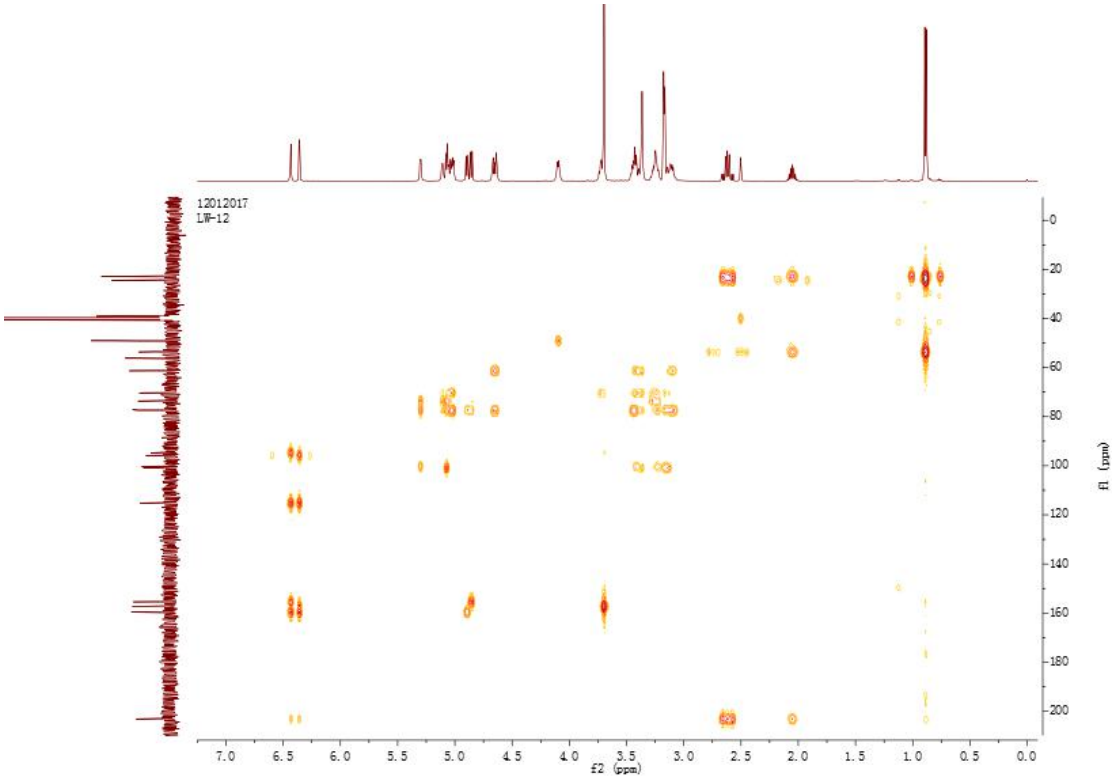

FIGURE S9. HMBC spectrum of compound 2 in DMSO.

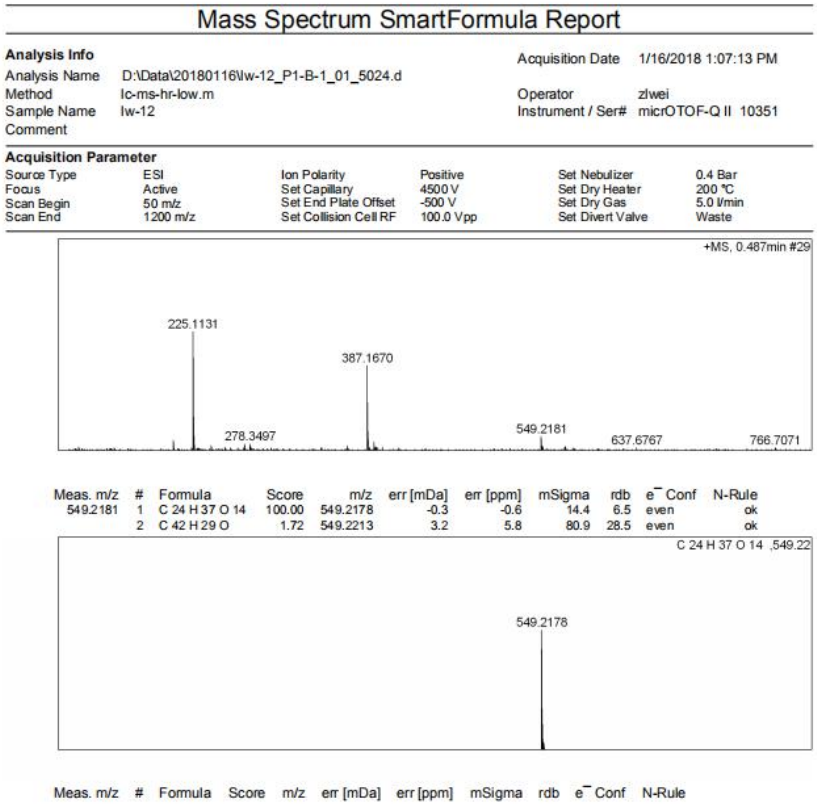

FIGURE S10. HR-ESI-MS spectrum of compound 2.

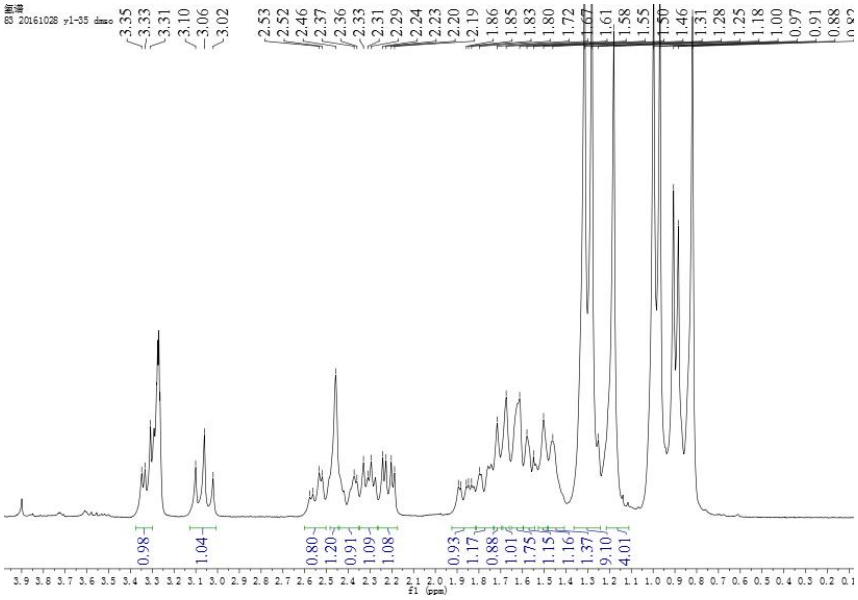

FIGURE S11.[1]H NMR spectrum of compound 3 in CD_3OD.

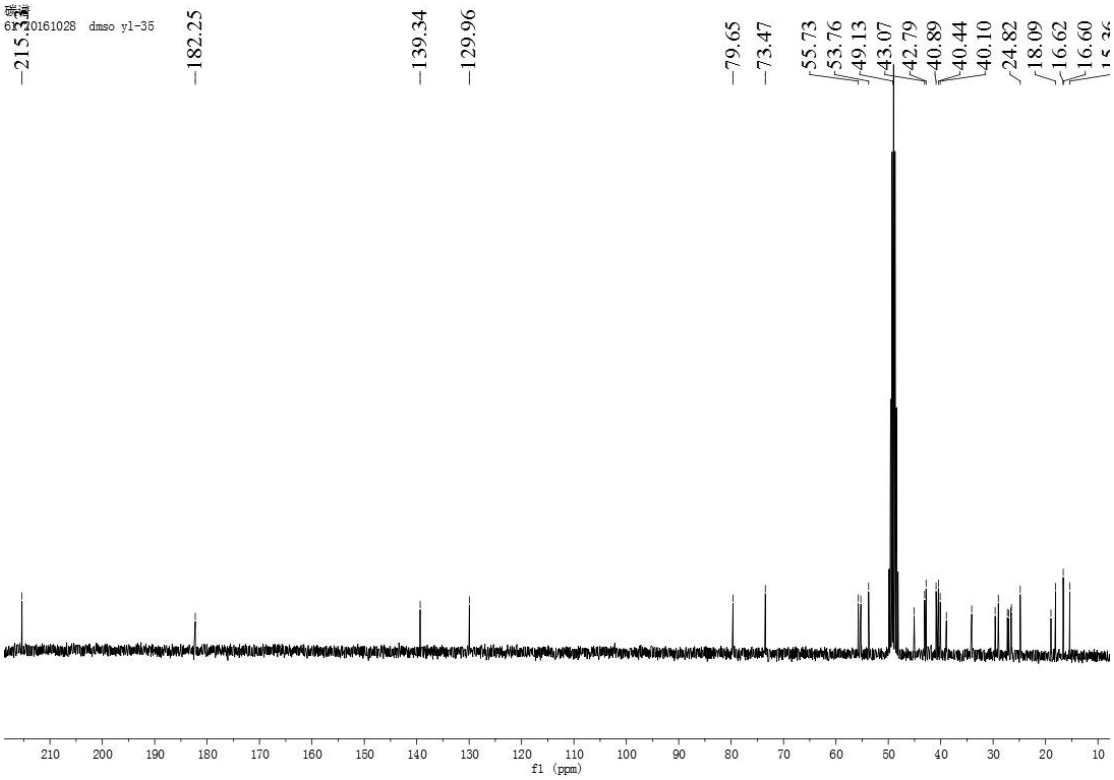

FIGURE S12.[13]C NMR spectrum of compound 3 in CD_3OD.

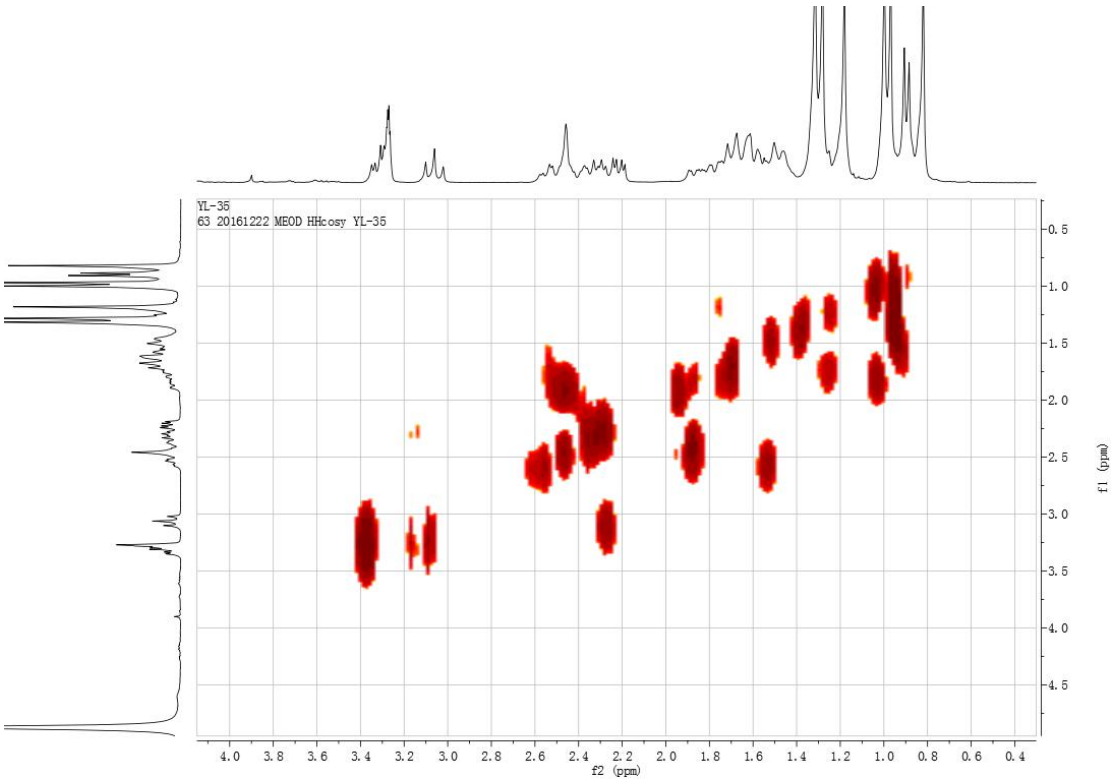

FIGURE S13. $^{1}H-^{1}H$ COSY spectrum of compound 3 in CD_3OD.

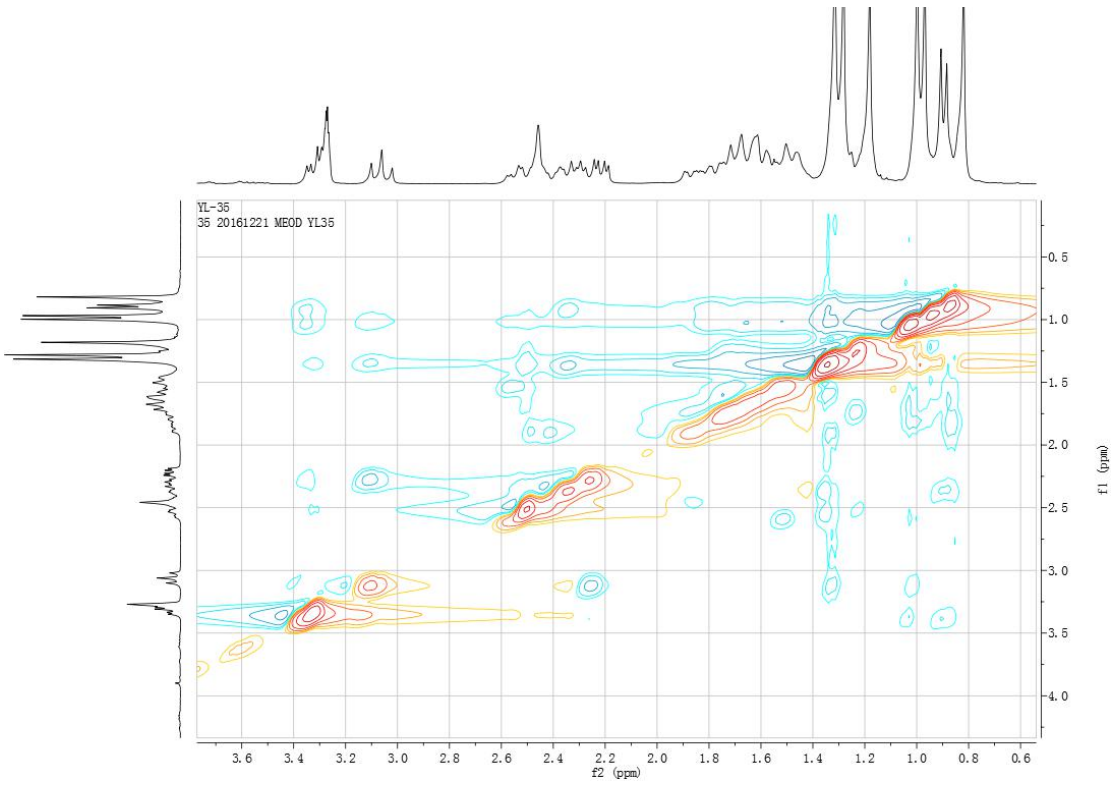

FIGURE S14. NOESY spectrum of compound 3 in CD_3OD.

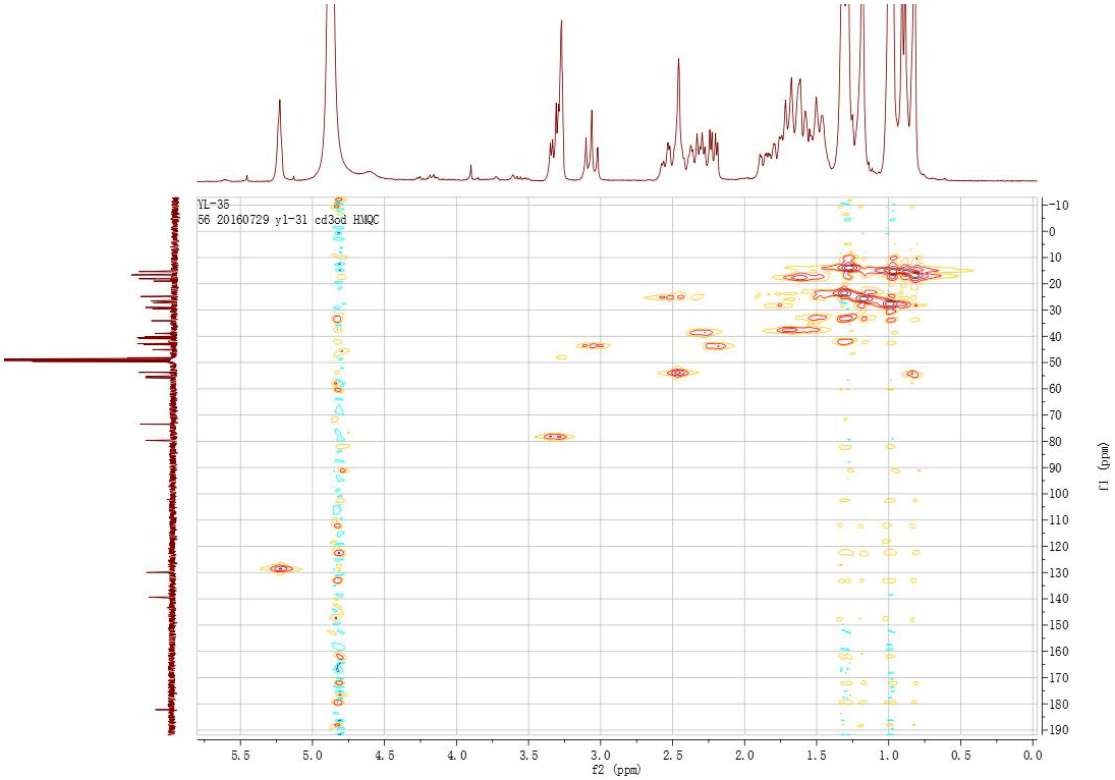

FIGURE S15. HMQC spectrum of compound 3 in CD_3OD.

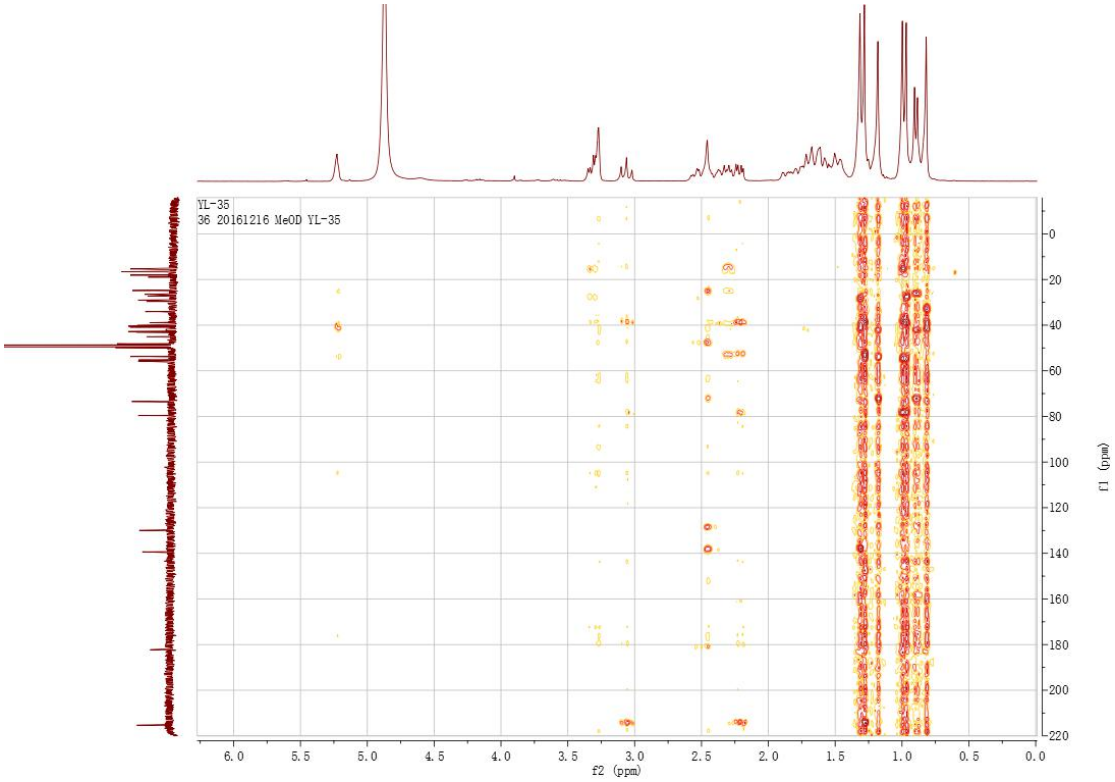

FIGURE S16. HMBC spectrum of compound 3 in CD_3OD.

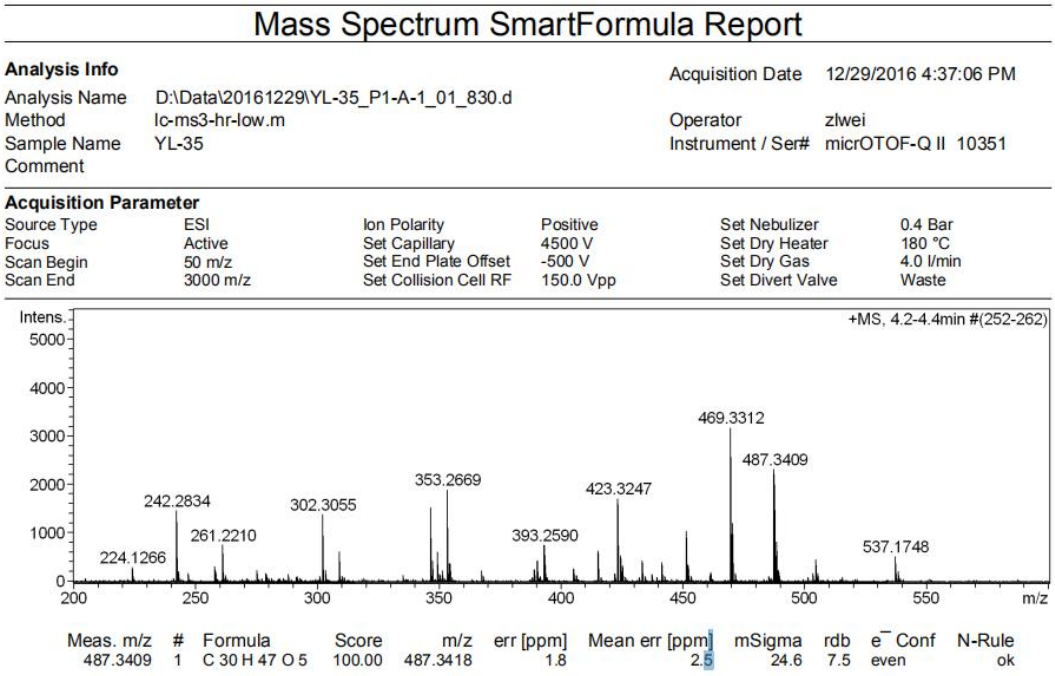

FIGURE S17. HR-ESI-MS spectrum of compound 3.