Supplementary Materials

Anti-inflammatory constituents isolated from the flowers of *Hosta plantaginea* via suppression of the NF-κB signaling pathway in LPS-stimulated RAW 264.7 macrophages

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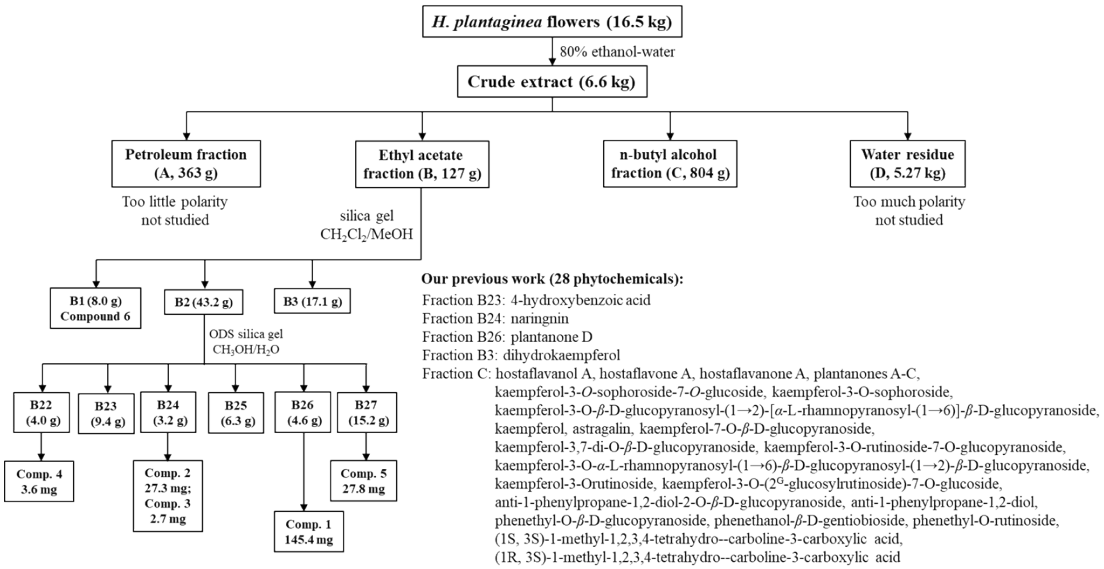
The detailed flow chart of the study design focusing on extraction and isolation of

phytochemicals from an ethanolic crude of the flowers of *Hosta plantaginea* as follows:



Based on this work (compounds **1**-**6**) and previous studies, a total of 34 phytochemicals, including 21 flavonoids, 3 phenethyl alcohols, 4 phenylpropanoids, 3 alkaloids, and 3 benzoic acids were isolated from ethyl acetate and n-butanol fractions that derived from an ethanolic extract of the flowers of *H. plantaginea*. The drawback is that the fractions of petroleum ether and water residue were not studied due to their polarities were too small and too large, respectively.

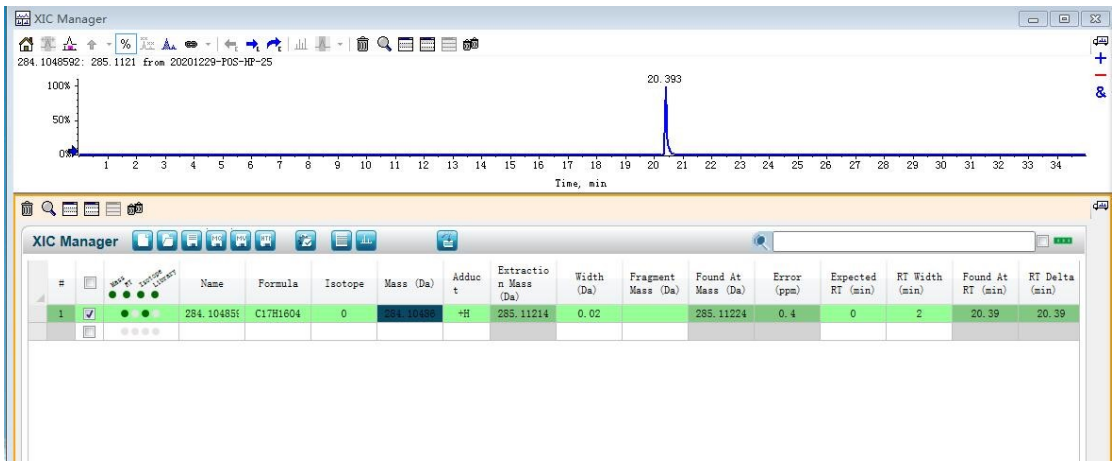


Fig. S1 The HR-ESI-MS of compound **1**.

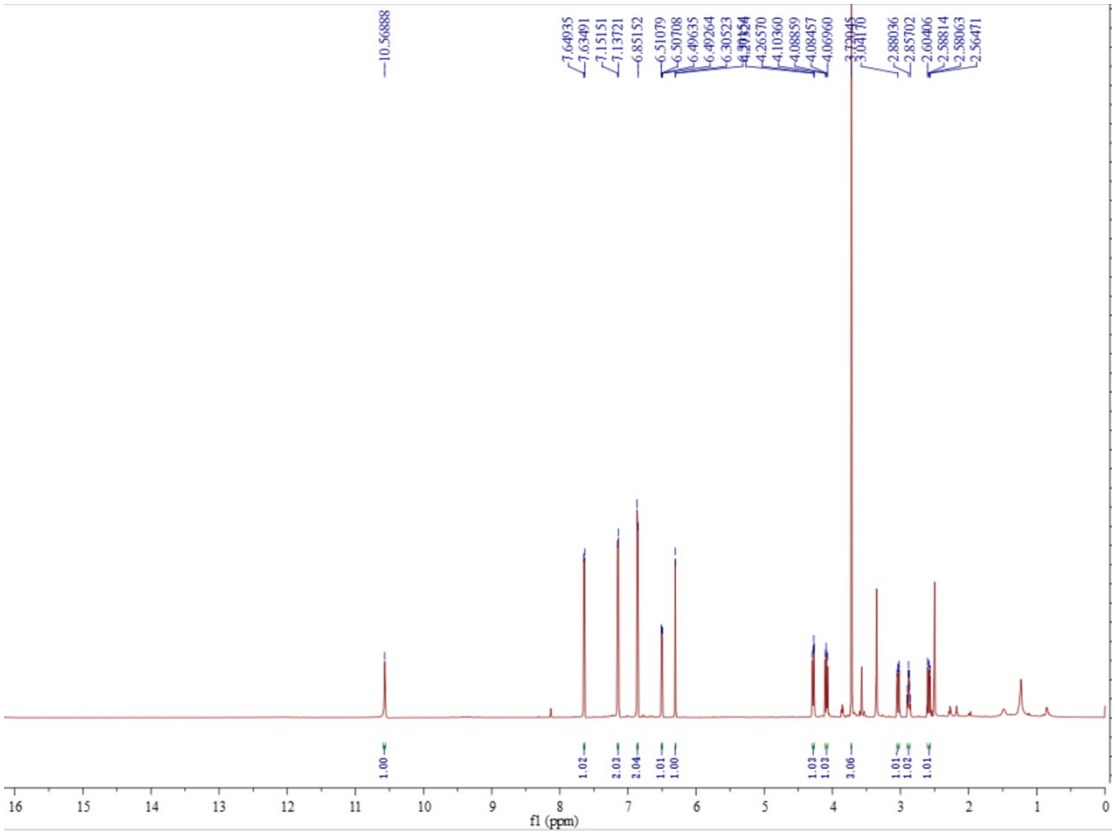


Fig. S2 ^{1}H NMR spectrum (600 MHz, in DMSO-d_6) of **1**.

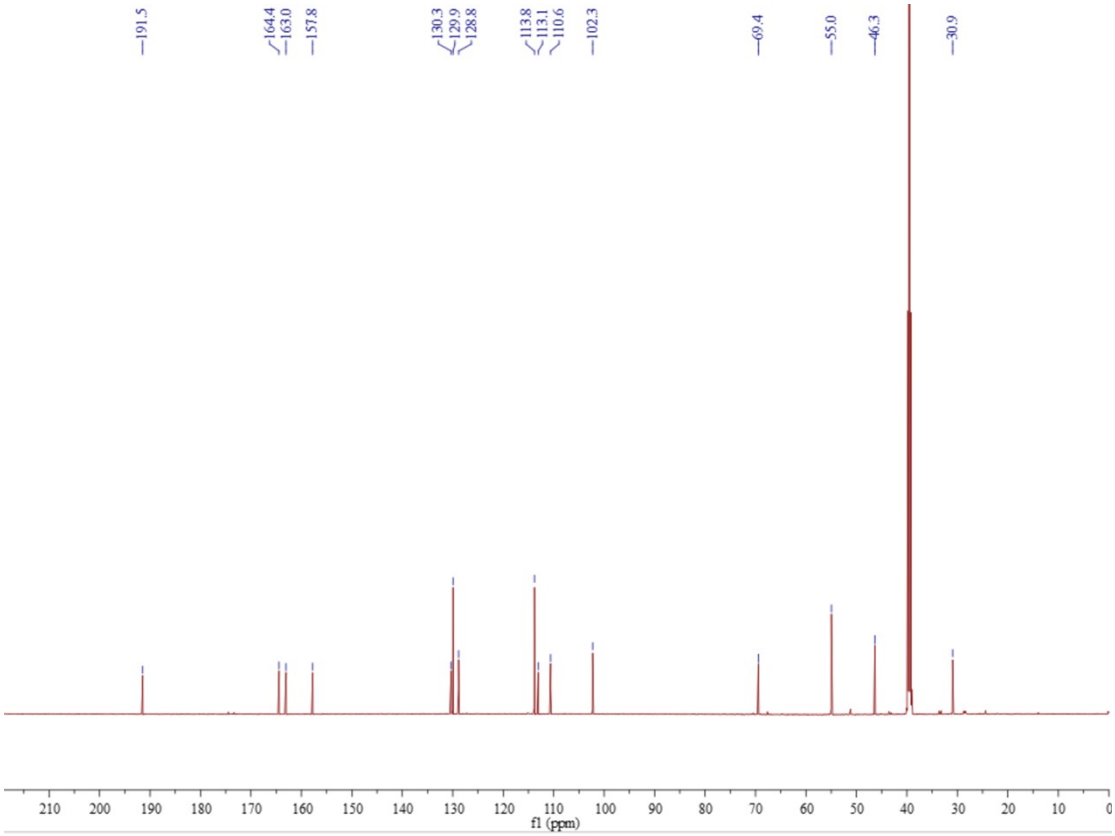


Fig. S3 ^{13}C NMR spectrum (150 MHz, in DMSO-d_6) of **1**.

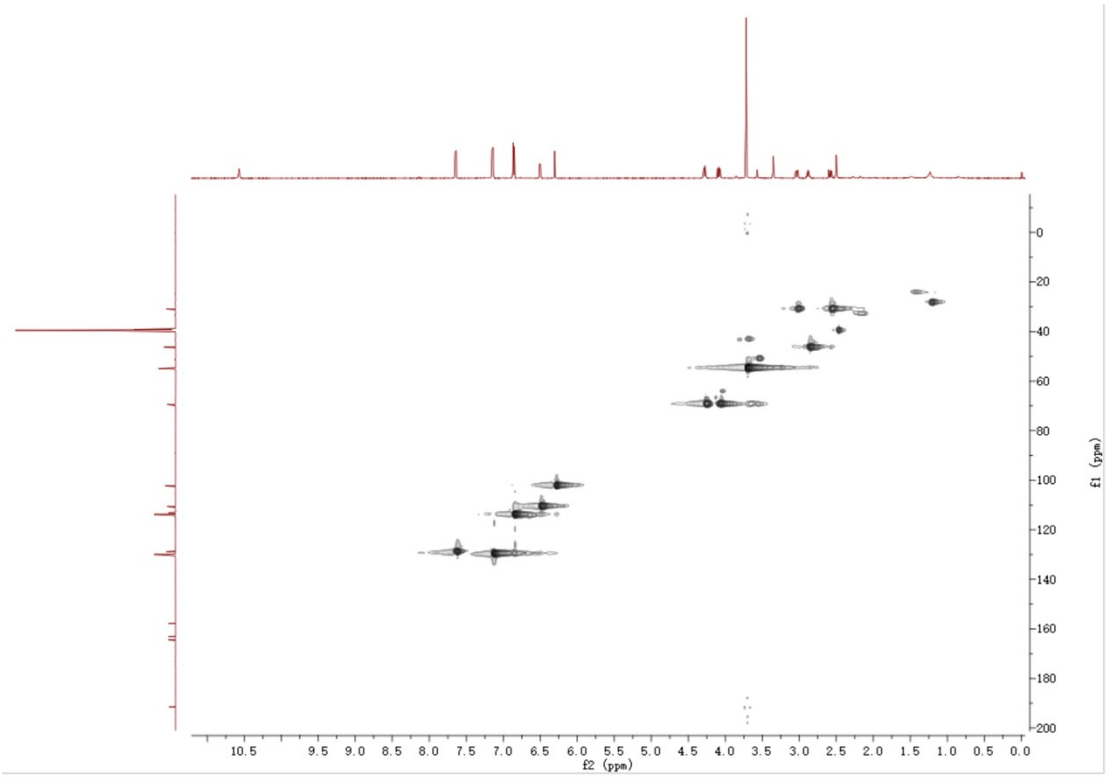


Fig. S4 HSQC spectrum of **1**.

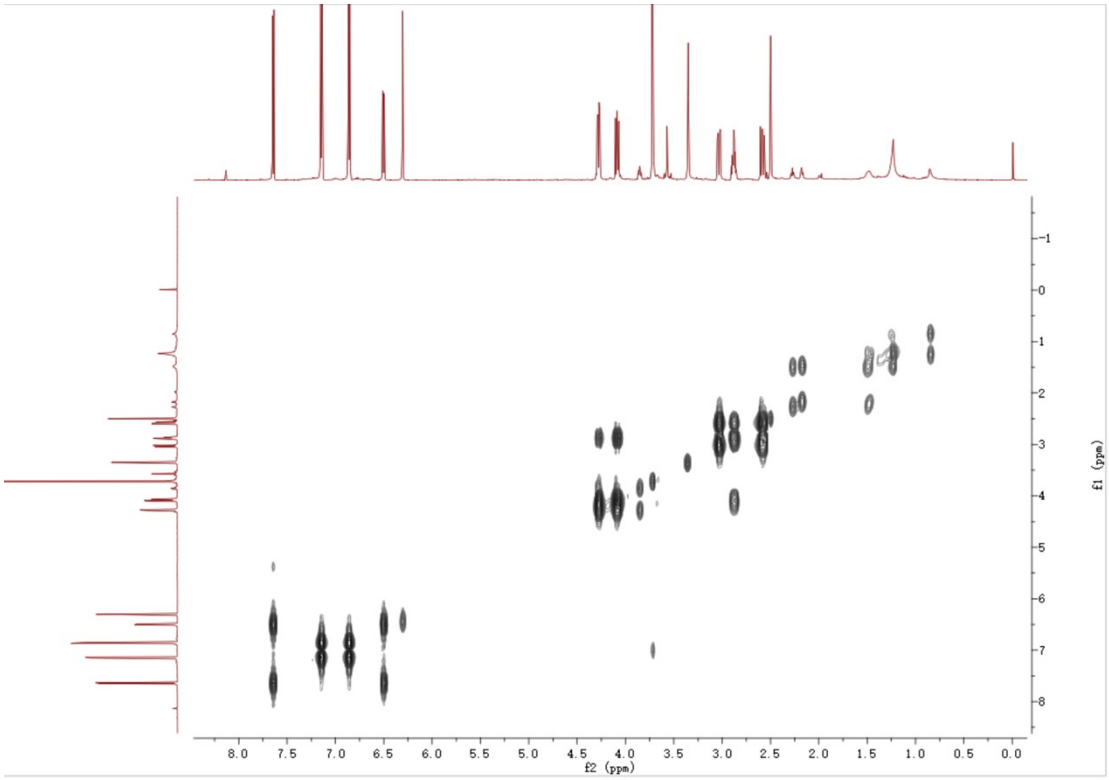


Fig. S5 ^{1}H-^{1}H COSY spectrum of **1**.

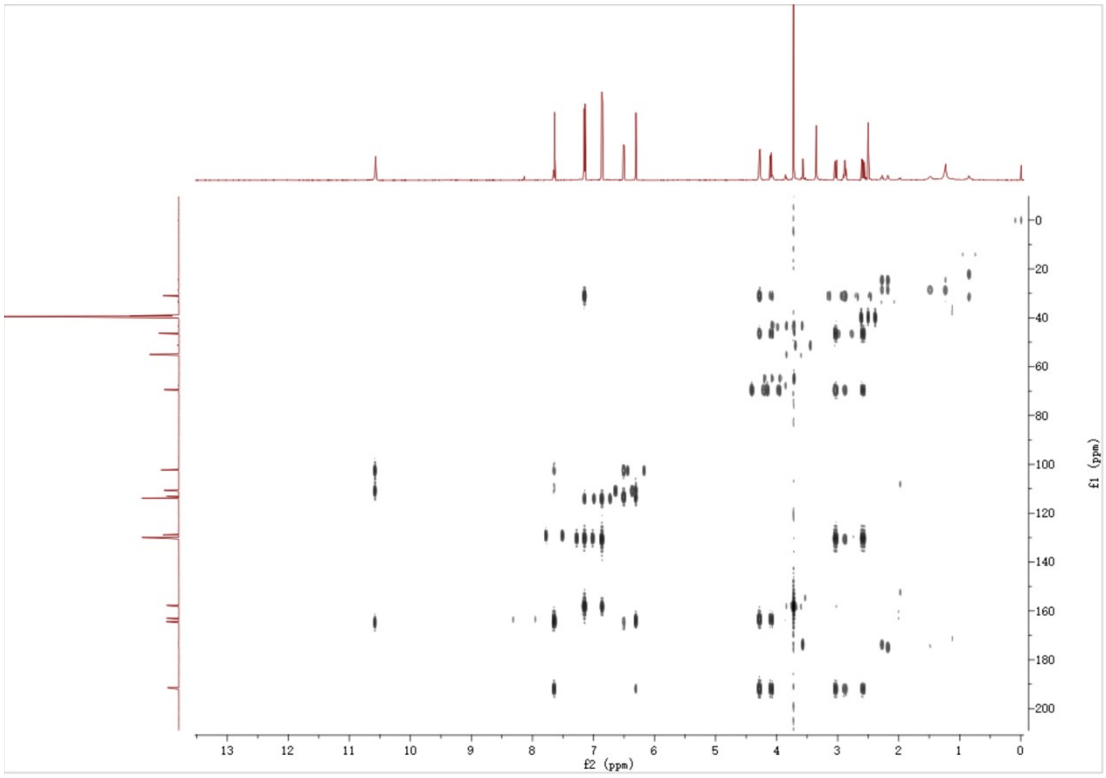


Fig. S6 HMBC spectrum of **1**.

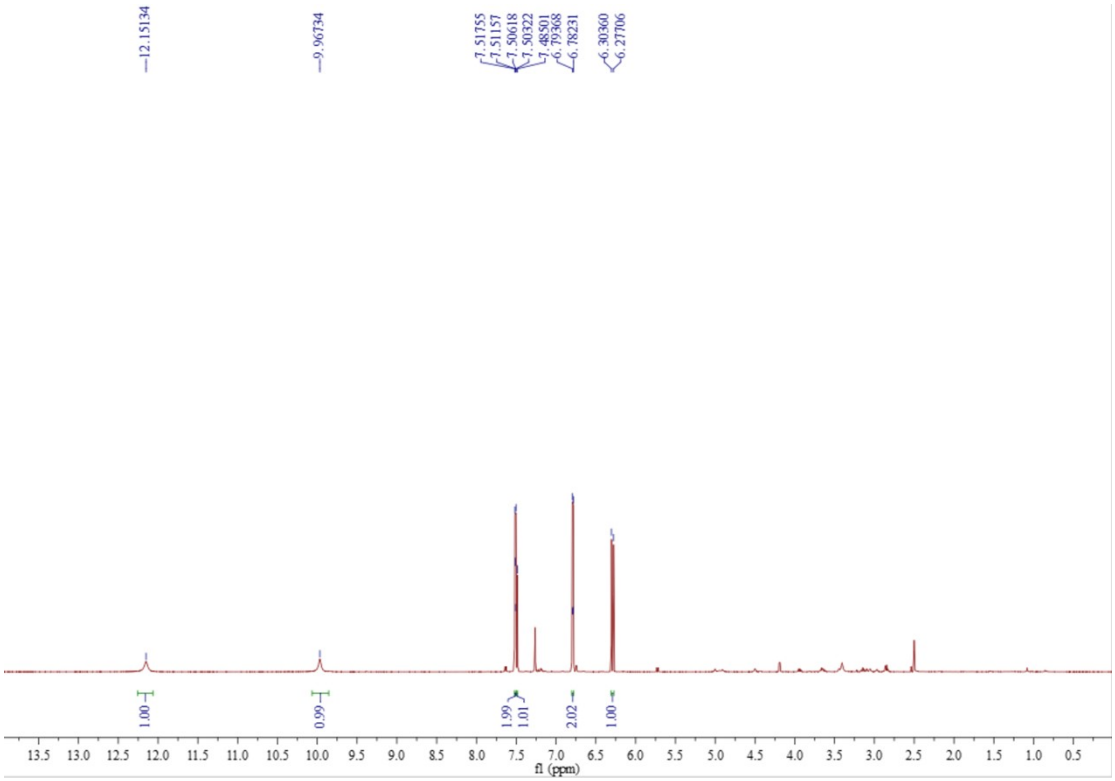


Fig. S7 ^{1}H NMR spectrum (600 MHz, in DMSO-d_6) of **2**.

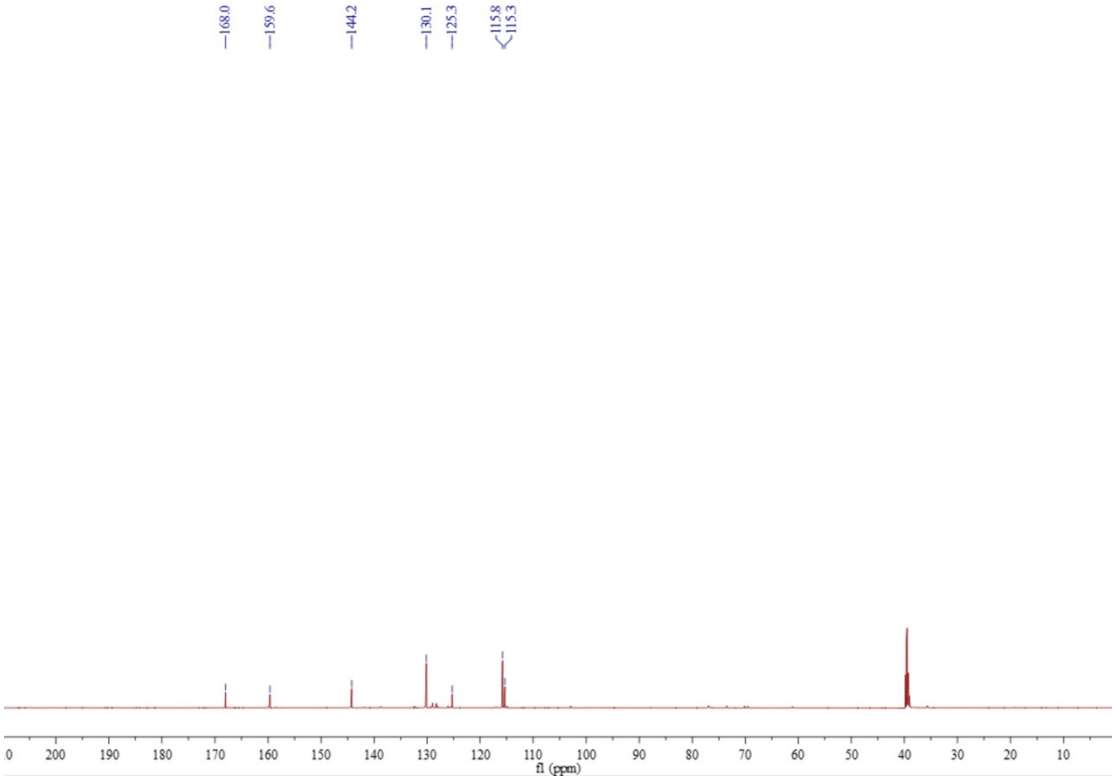


Fig. S8 ^{13}C NMR spectrum (150 MHz, in DMSO-d_6) of **2**.

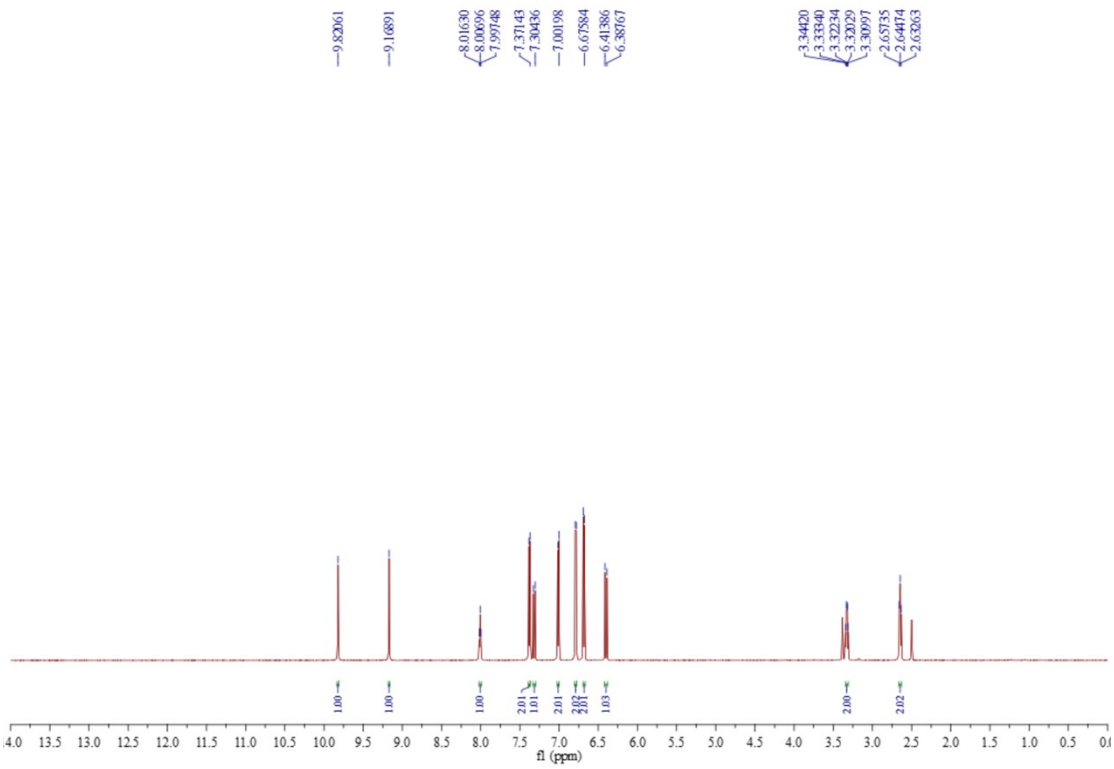


Fig. S9 ^{1}H NMR spectrum (600 MHz, in DMSO-d_6) of **3**.

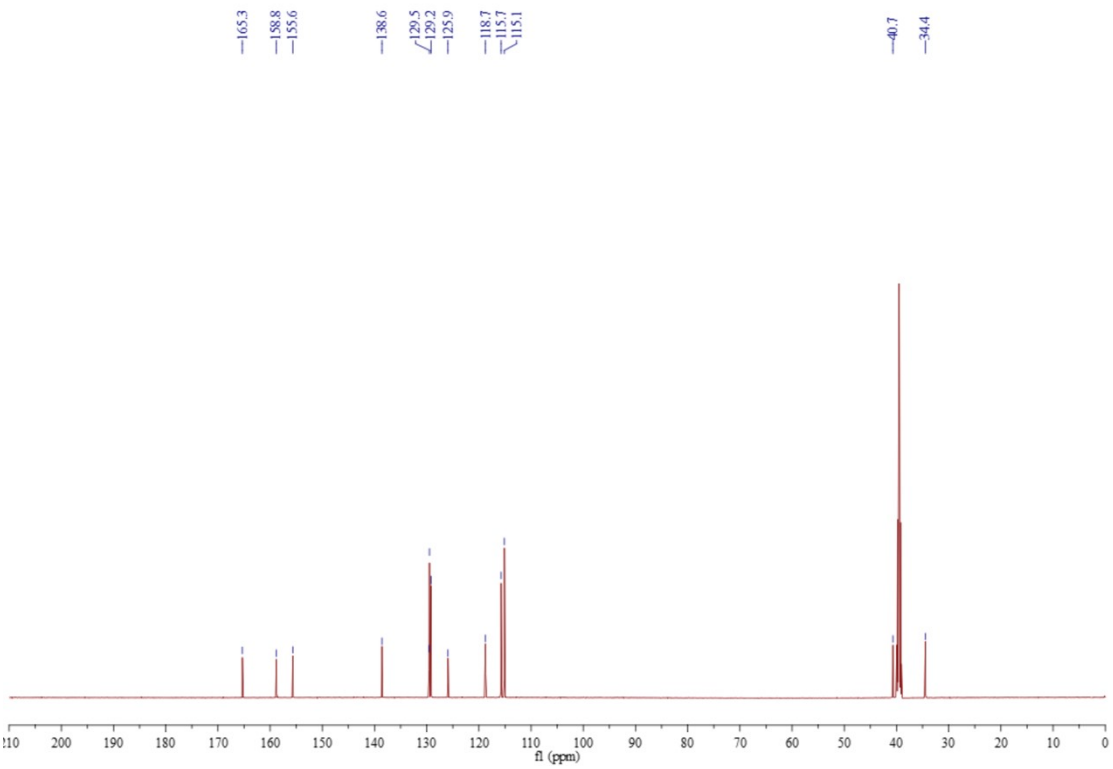


Fig. S10 ^{13}C NMR spectrum (150 MHz, in DMSO-d_6) of **3**.

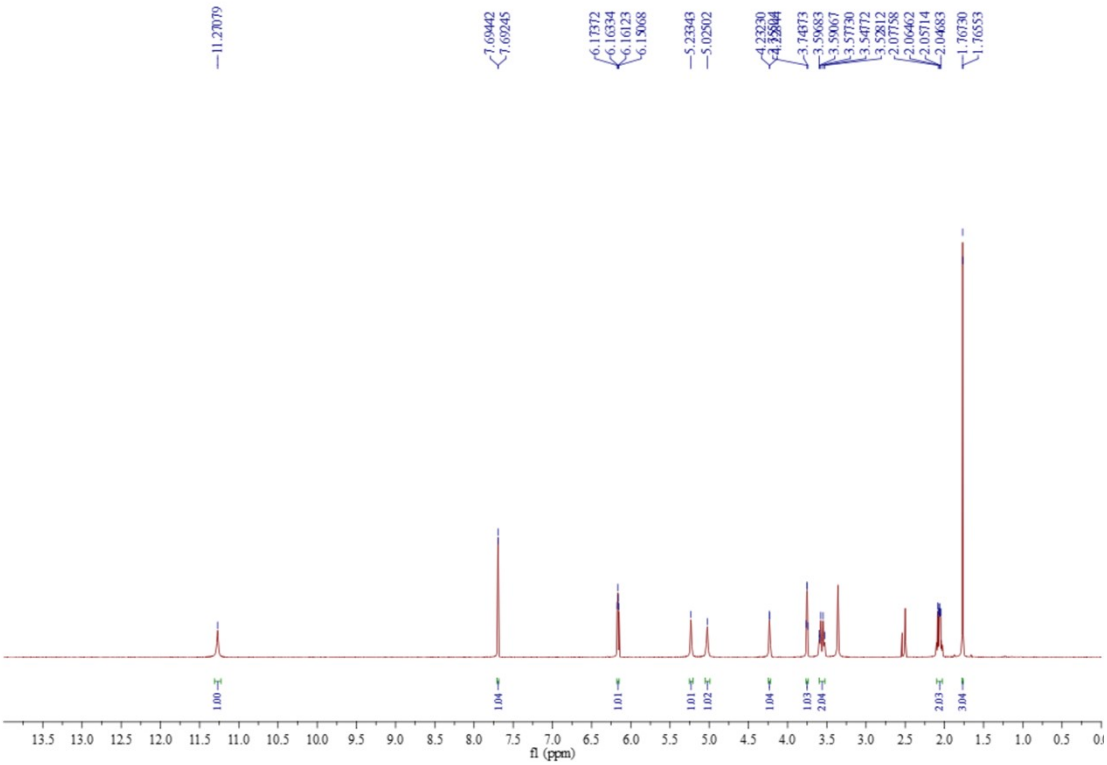


Fig. S11 ^{1}H NMR spectrum (600 MHz, in DMSO-d_6) of **4**.

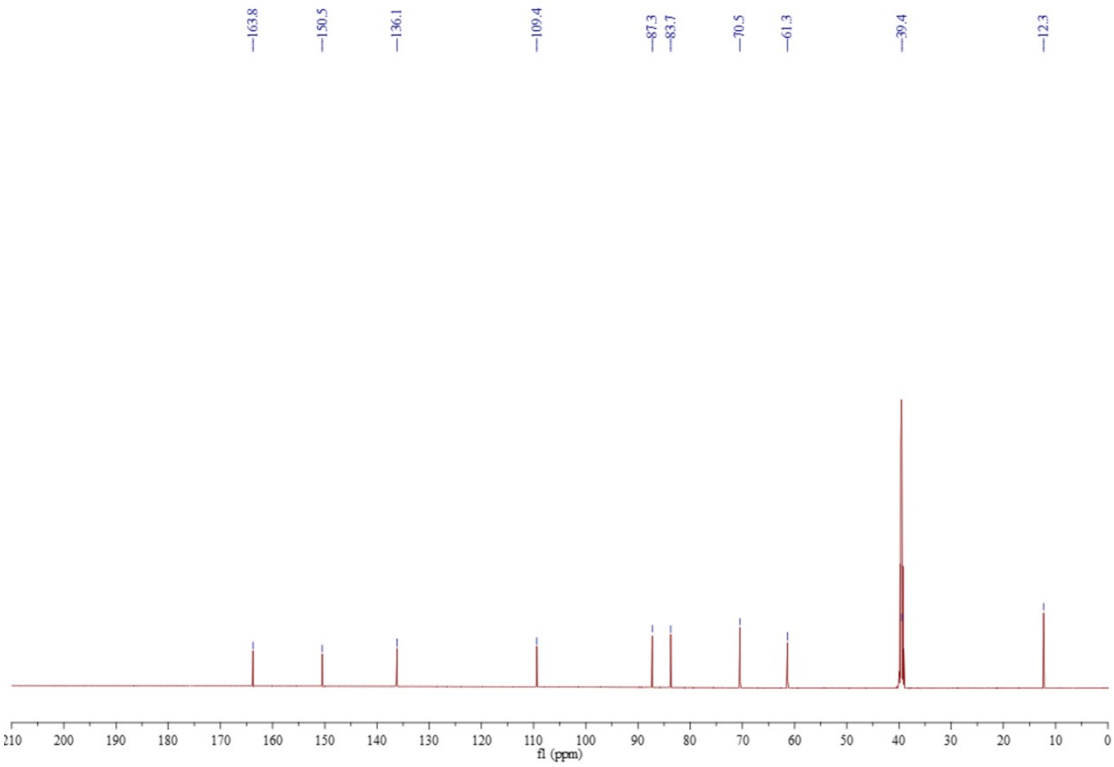


Fig. S12 ^{13}C NMR spectrum (150 MHz, in DMSO-d_6) of **4**.

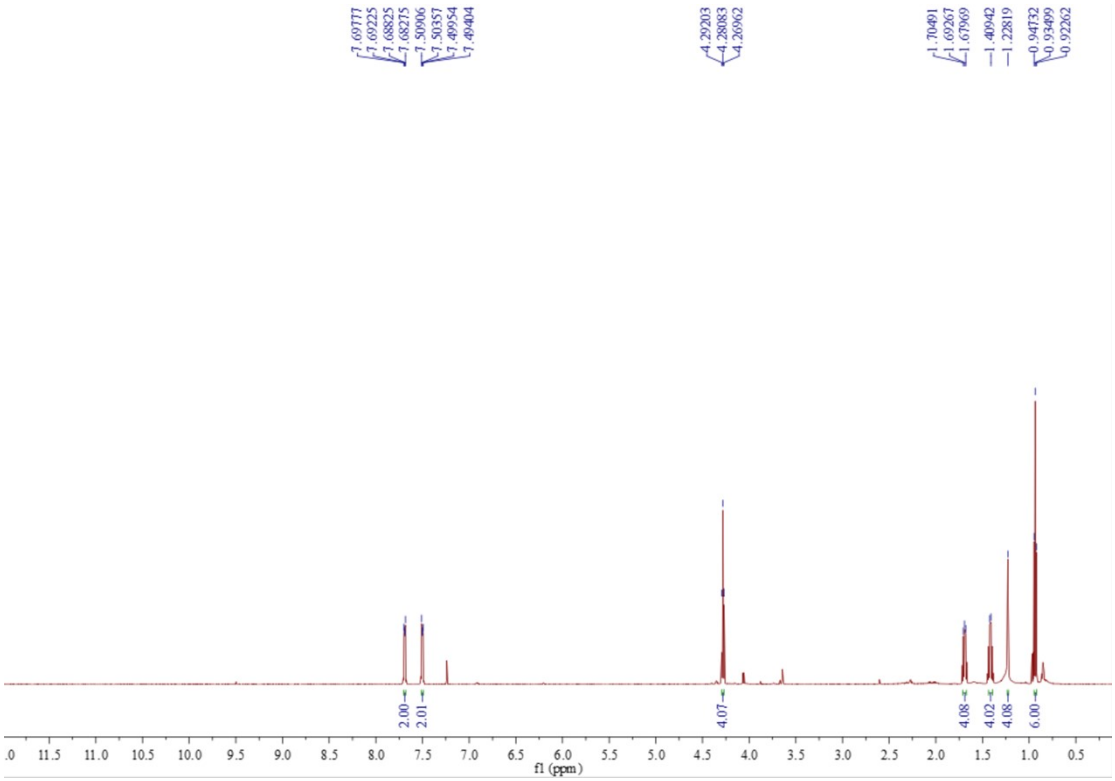


Fig. S13 ^{1}H NMR spectrum (600 MHz, in $CDCl_3$) of **5**.

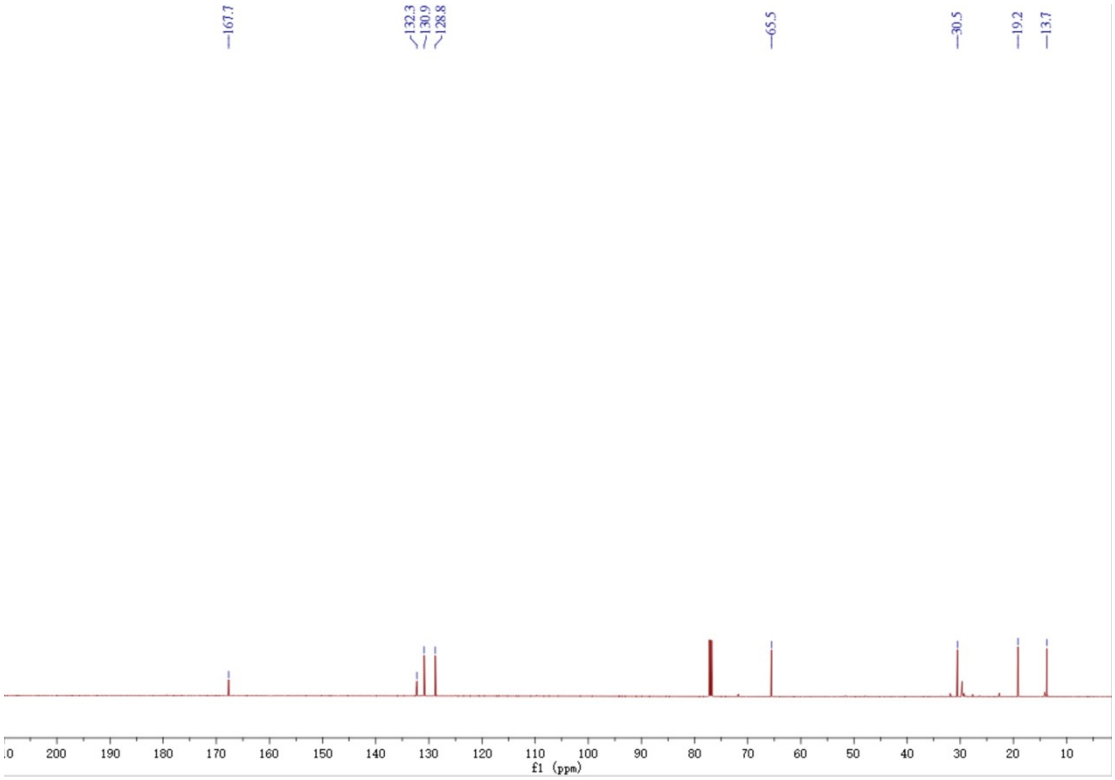


Fig. S14 ^{13}C NMR spectrum (150 MHz, in $CDCl_3$) of **5**.

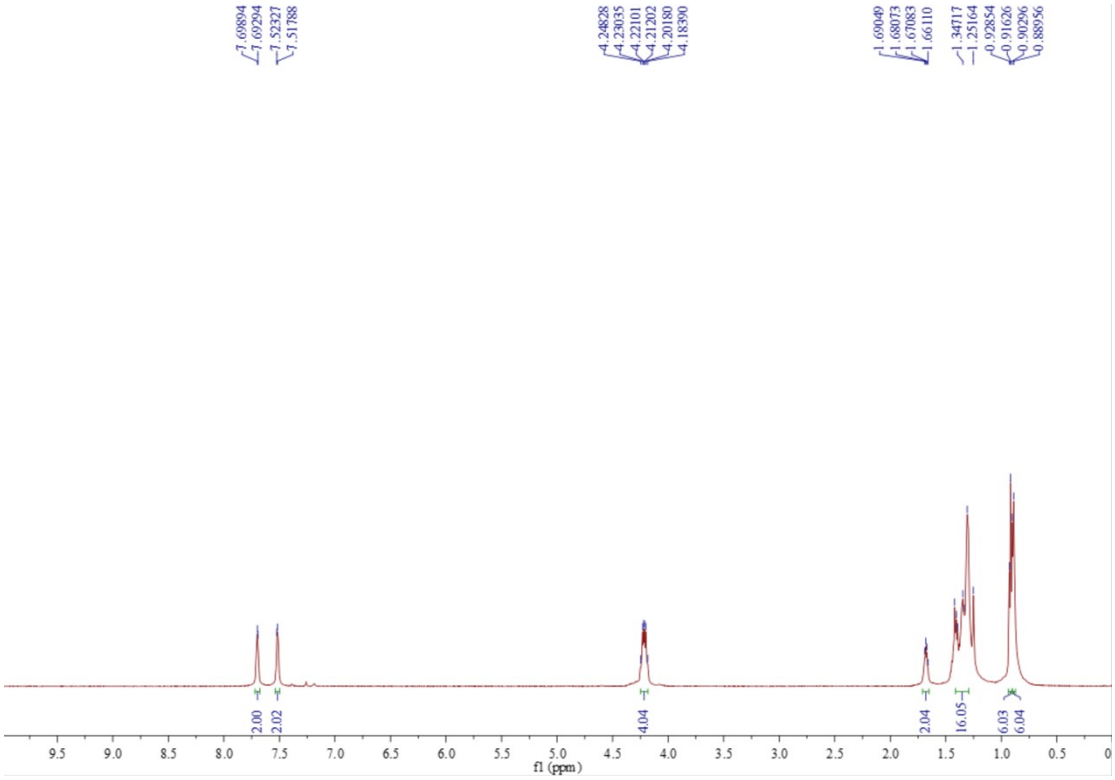


Fig. S15 ^{1}H NMR spectrum (600 MHz, in $CDCl_3$) of **6**.

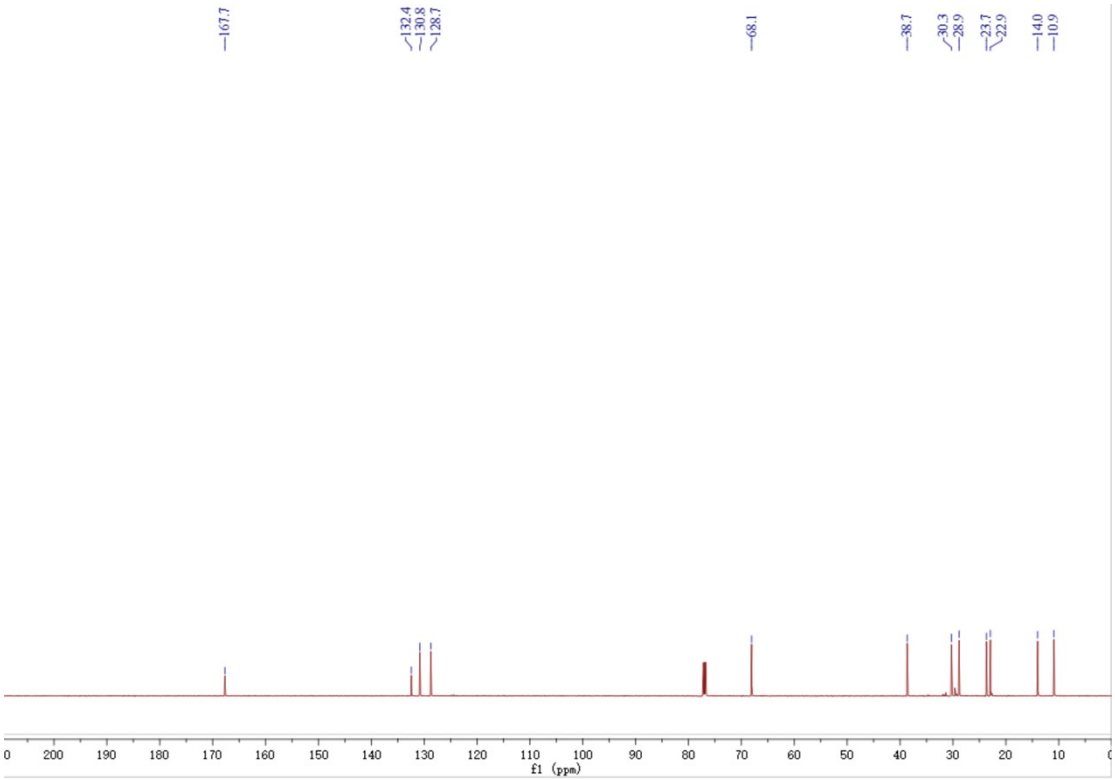


Fig. S16 ^{13}C NMR spectrum (150 MHz, in CDCl3) of **6**.

The ethanolic crude extract of *H. plantaginea* flowers (labeled as YZH) using a Shimadzu System (Kyoto, Japan), equipped with a LC-3AD solvent delivery system, a SIL-30ACXR auto-sampler, a CTO-30AC column oven, a DGU-20A3 degasser and a CBM-20A controller. Chromatographic separation was conducted on a Luna Omega C_{18} (100 mm× 2.1 mm, 1.6 μm) keeping at 35°C. Water (A) and acetonitrile (B) were used as the mobile phase. The gradient elution with the flow rate of 0.3 mL/min was performed as follows: 0-20 min 5%-20% B; 20-40 min 20%-65% B; 40-55 min 65%-95% B; 55-60 min 95%-95% B; 60.1-65 min 5%-5% B. The sample inject volume was 2 μL.

UHPLC-Q-TOF-MS/MS detection was conducted on a Triple TOF™ 5600+ system with a Duo Spray source in both positive and negative electrospray ion mode (AB SCIEX, Foster City, CA, USA). The MS analysis was carried out by the ESI source in both positive- and negative-ion modes. The parameters were set as follows: ion spray voltage, -5500 V; ion source temperature, 500℃; curtain gas, 40 psi; nebulizer gas (GS1), 50 psi; heater gas (GS2), 50 psi; and decluster potential (DP), -100 V. Mass ranges were set at 100-1500 Da for the TOF-MS scan and 100-1500 Da for the TOF MS/MS experiments. In the IDA-MS/MS experiment, the collision energy (CE) was set at 45 eV, and the collision energy spread (CES) was (±) 15 eV for the UHPLC-Q-TOF-MS/MS detection. The most intensive five ions from each TOF-MS scan were selected as MS/MS fragmentation. LC-MS/MS data were analyzed using PeakView®1.2 software (AB SCIEX, Foster City, CA, USA).

By extracting ions at *m/z* 283.09758, 163.04007, 282.22357, 241.08300, 363.25299, and 391.28429 with mass window of 5 ppm, extracted ion chromatograms of compounds **1**–**6** were created (Figs. S17-24).

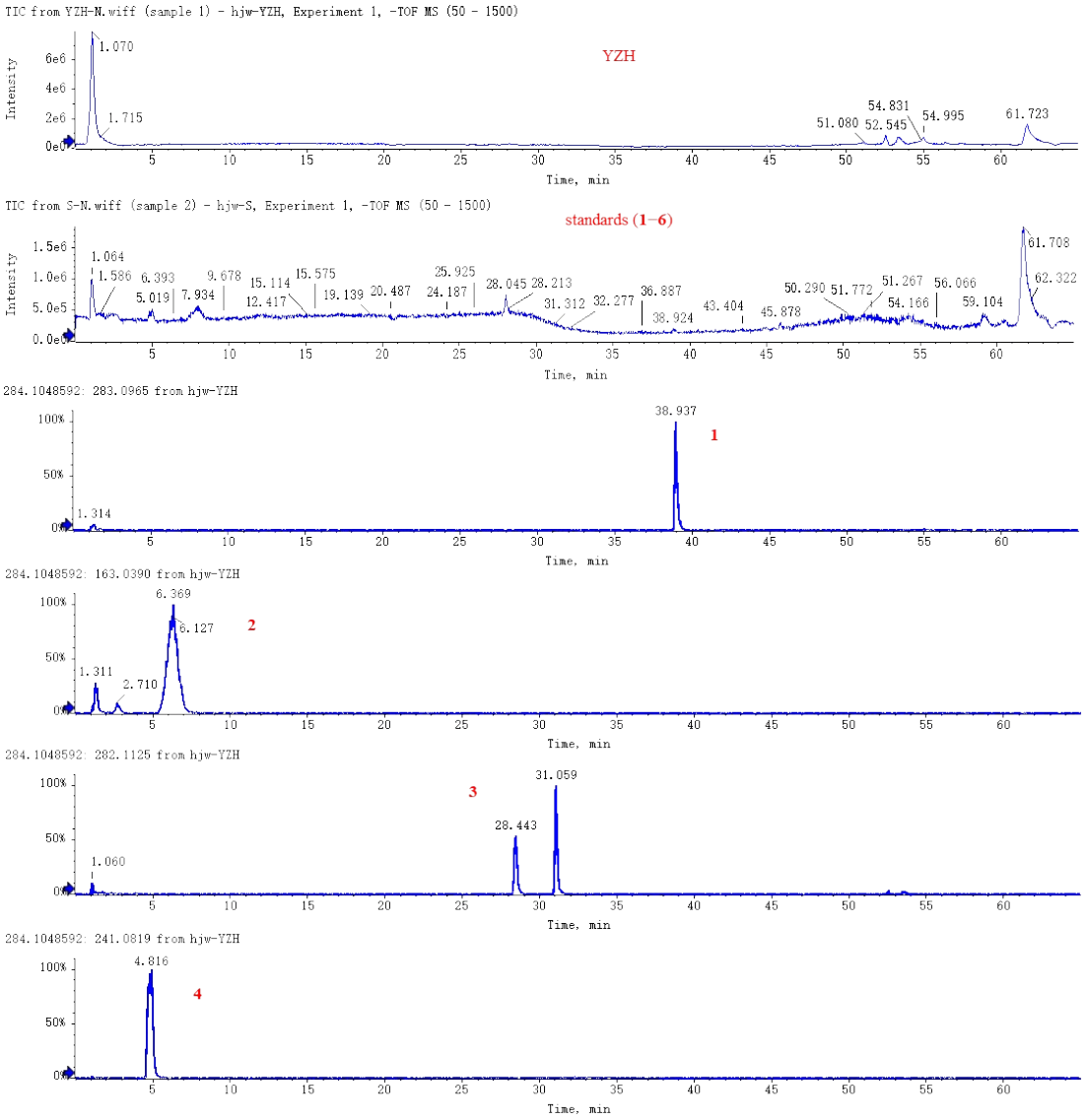


Fig. S17 UHPLC-Q-TOF-MS/MS of the crude extract of *H. plantaginea* flowers, standards (**1−6**) and **1−4** in negative ion mode.

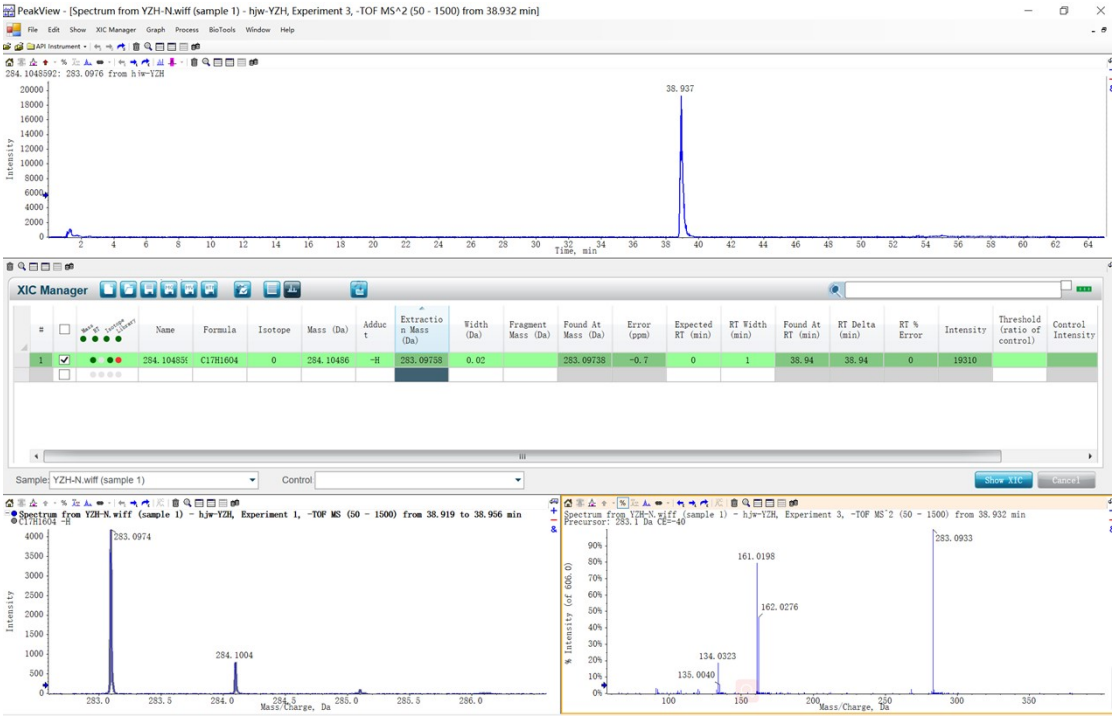


Fig. S18 UHPLC-Q-TOF-MS/MS of **1** in negative ion mode.

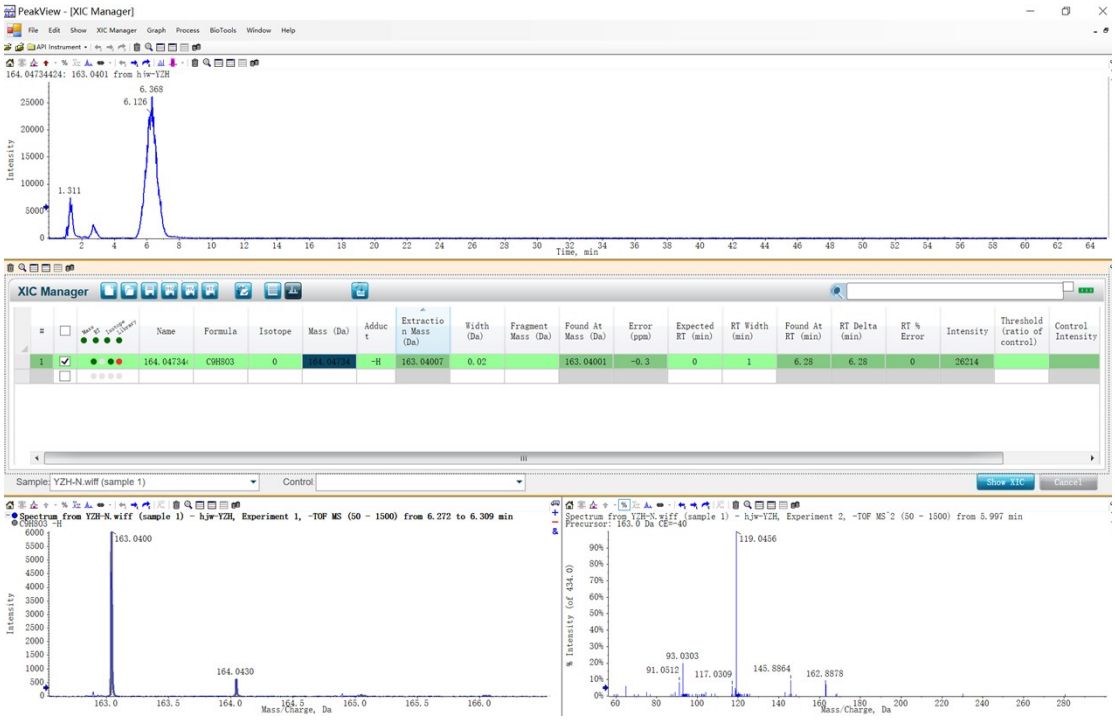


Fig. S19 UHPLC-Q-TOF-MS/MS of **2** in negative ion mode.

Fig. S20 UHPLC-Q-TOF-MS/MS of **3** in negative ion mode.

Fig. S21 UHPLC-Q-TOF-MS/MS of **4** in negative ion mode.

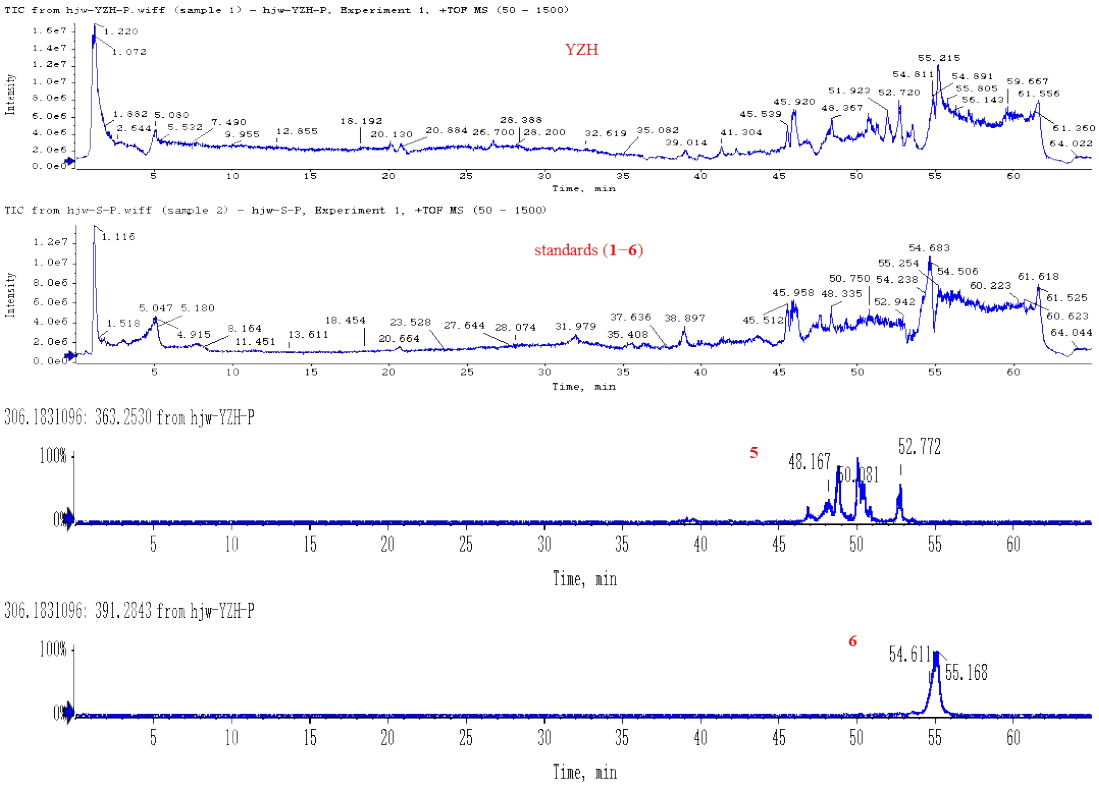


Fig. S22 UHPLC-Q-TOF-MS/MS of the crude extract of *H. plantaginea* flowers, standards (**1**−**6**), **5** and **6** in positive ion mode.

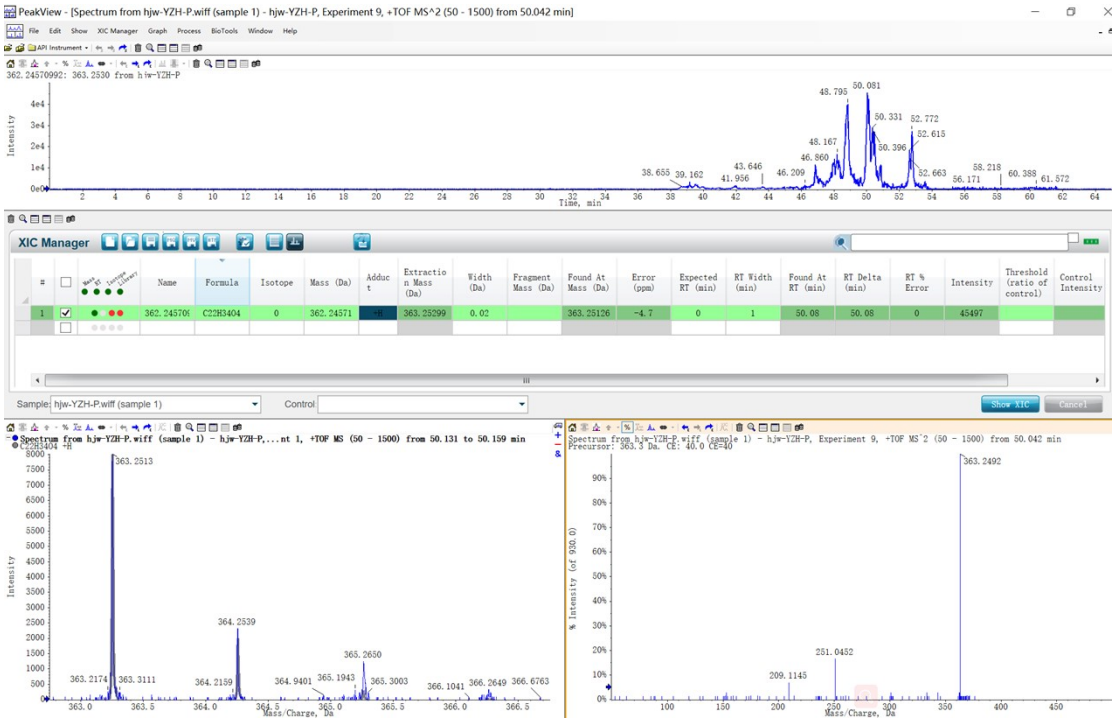


Fig. S23 UHPLC-Q-TOF-MS/MS of **5** in positive ion mode.

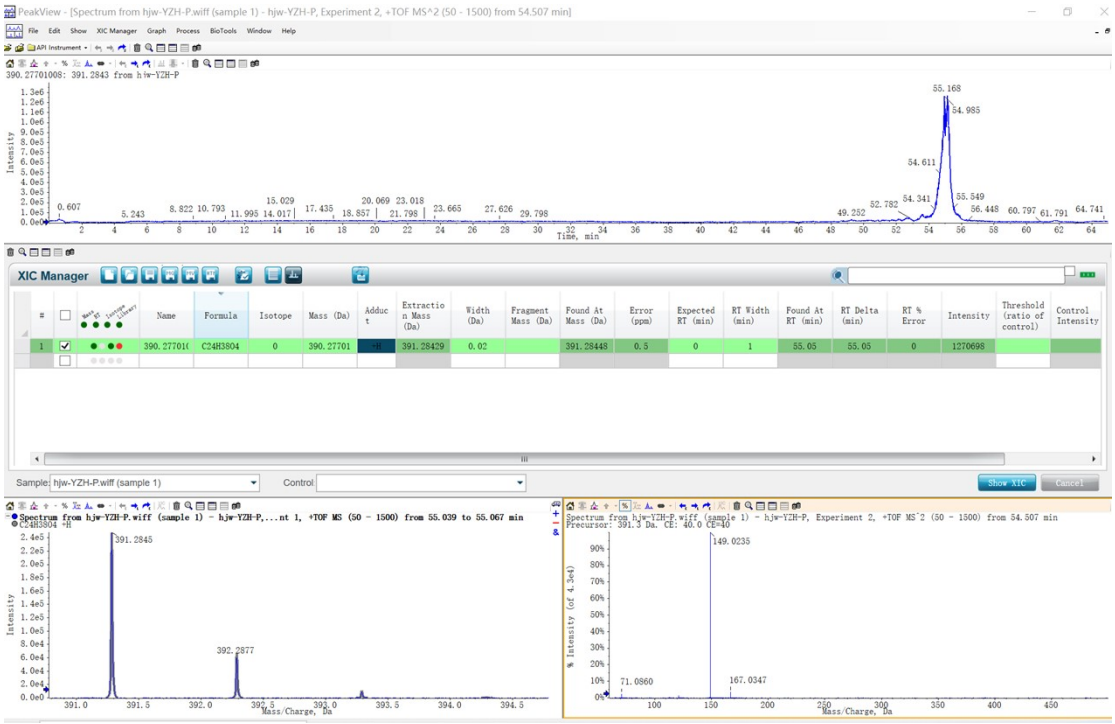
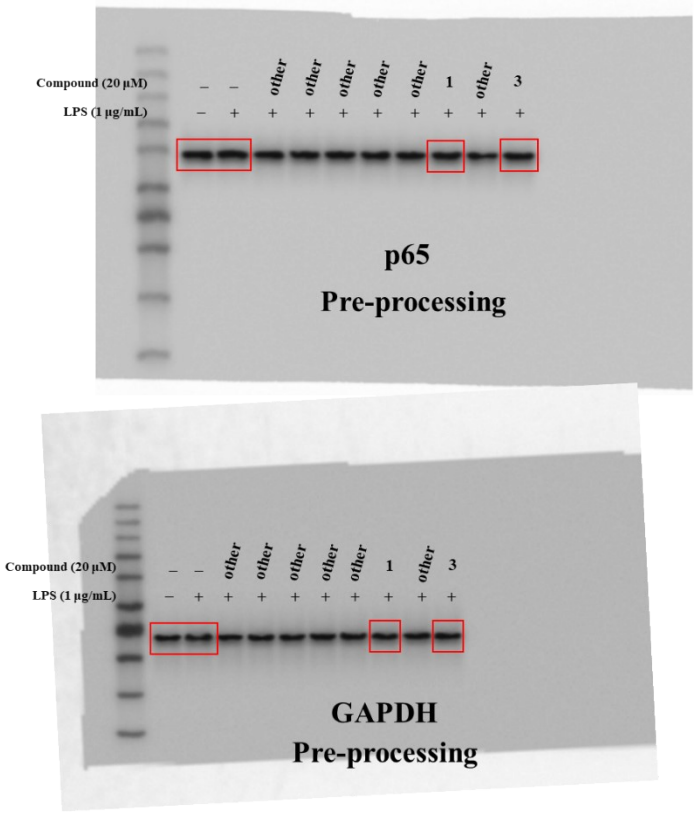
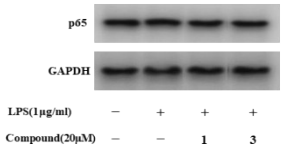


Fig. S24 UHPLC-Q-TOF-MS/MS of **6** in positive ion mode.

Figure 5A

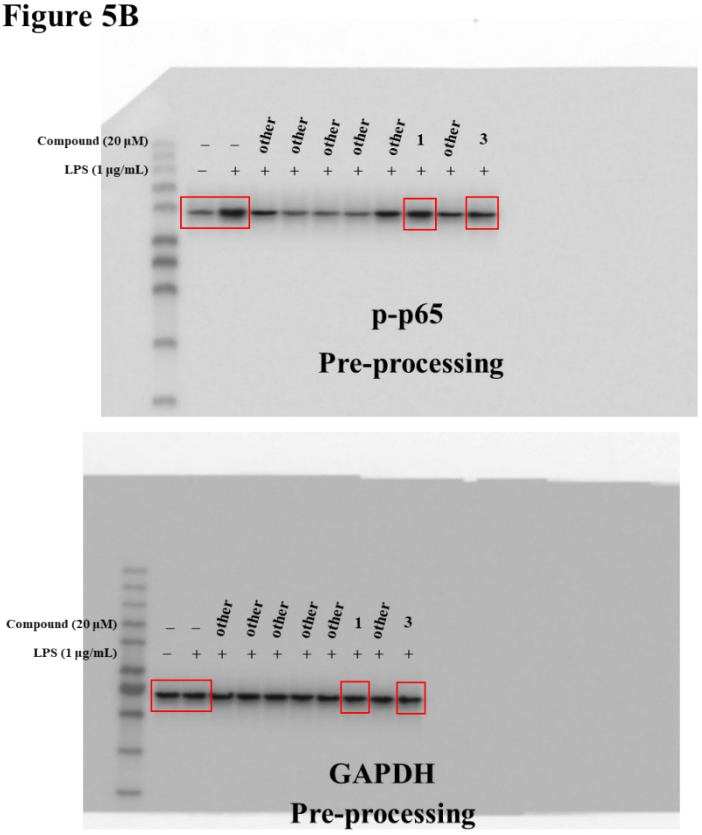


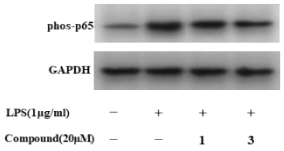


p65 and GAPDH

Post-processing

Figure 5B





p-p65 and GAPDH

Post-processing