Discovery of Neolignan Glycosides with Acetylcolinesterase Inhibitory Activity from Huangjinya Green Tea Guided by UPLC-MS^2 data and GNPS Molecular Networking

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List of supplementary materials

Table S2. The inhibitory activities of the compounds against AChE in vitro .

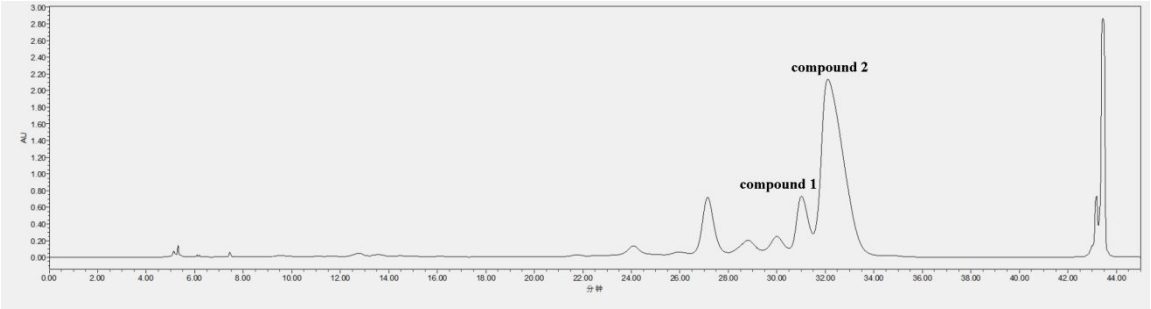

Figure Supporting 1. HPLC preparation of compound **1** and **2** with UV detection at the wavelength 280 nm.

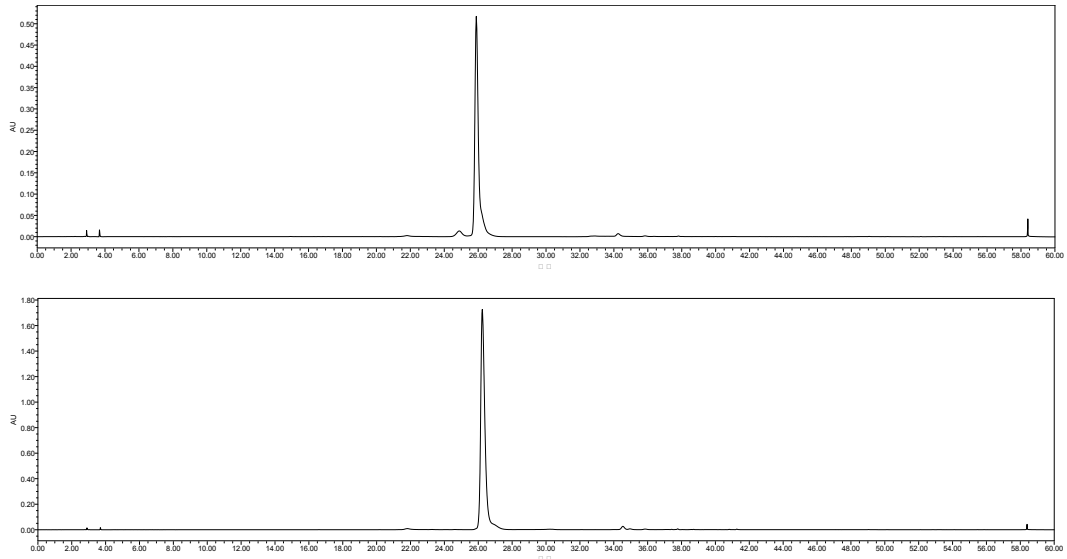

Figure Supporting 2. HPLC pure test of compound **1** and **2** with UV detection at the wavelength 280 nm.

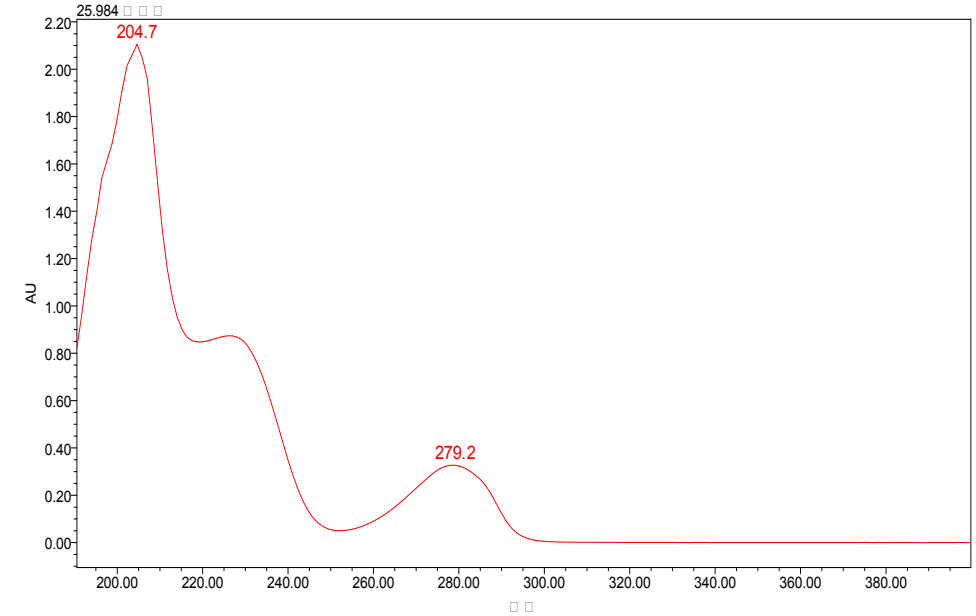

Figure Supporting 3. UV of compound **1**.

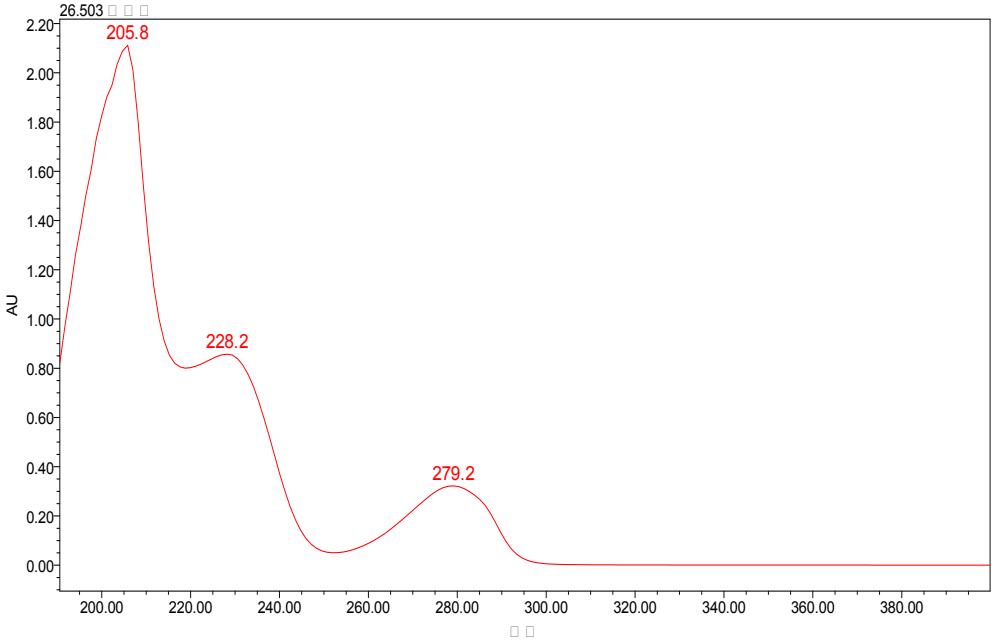

Figure Supporting 4. UV of compound **2**.

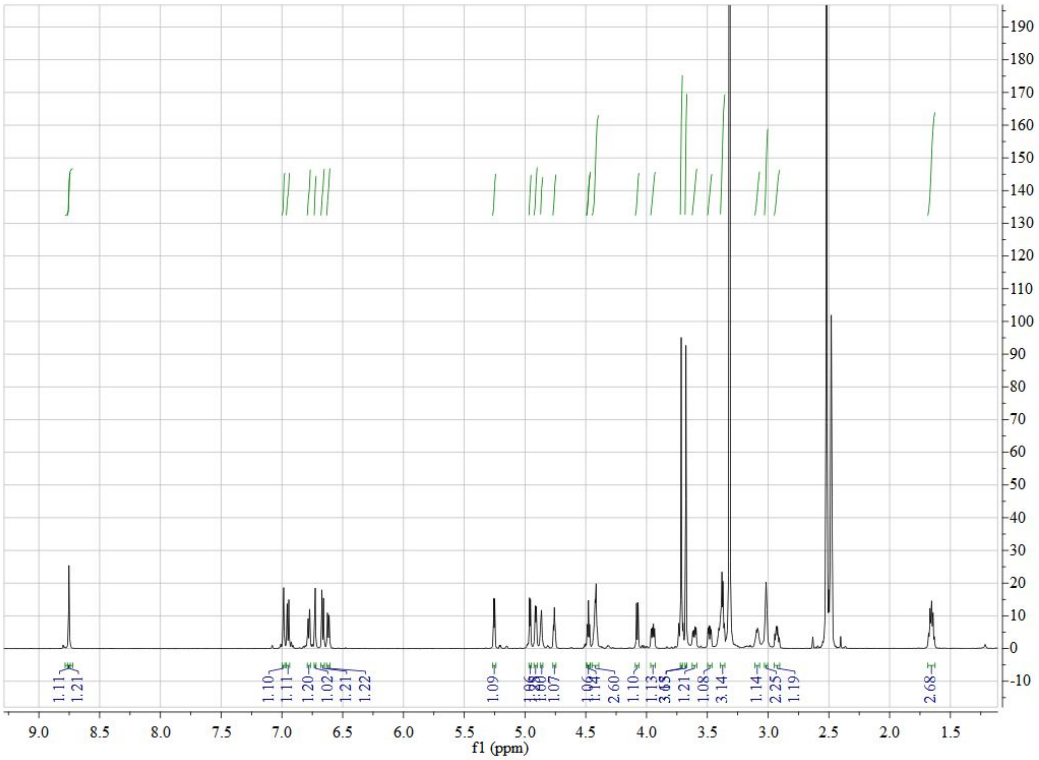

Figure Supporting 5. ^{1}H NMR data of compound **1** in DMSO-d_6.

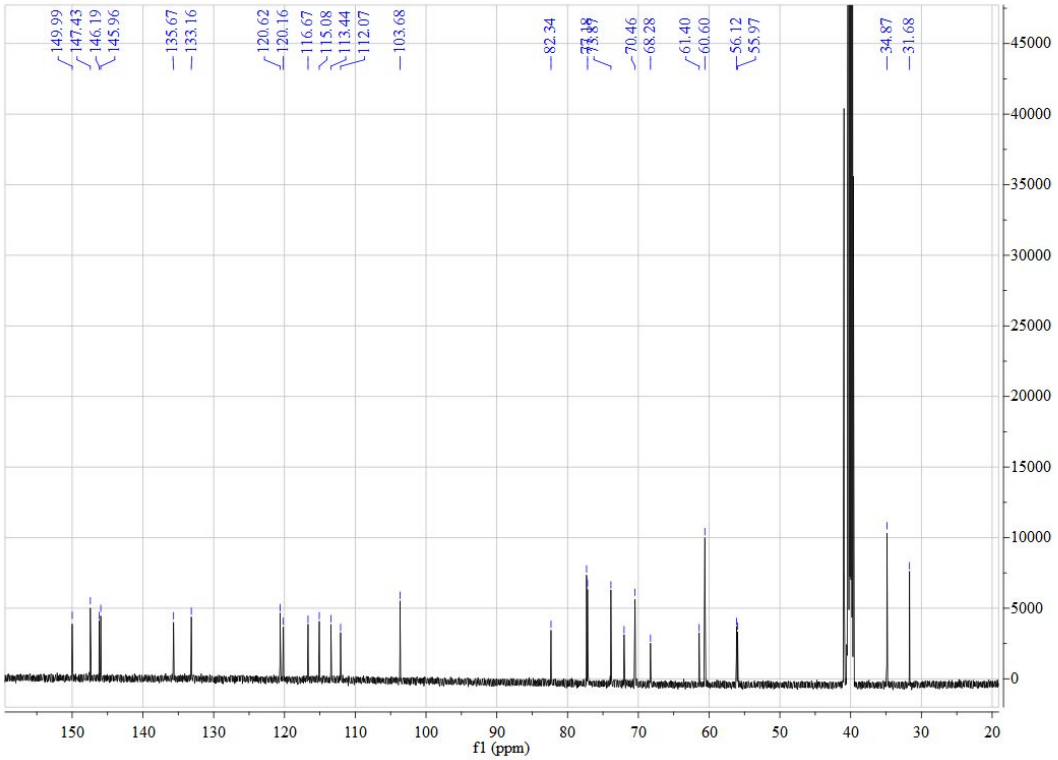

Figure Supporting 6. ^{13}C NMR data of compound **1** in DMSO-d_6.

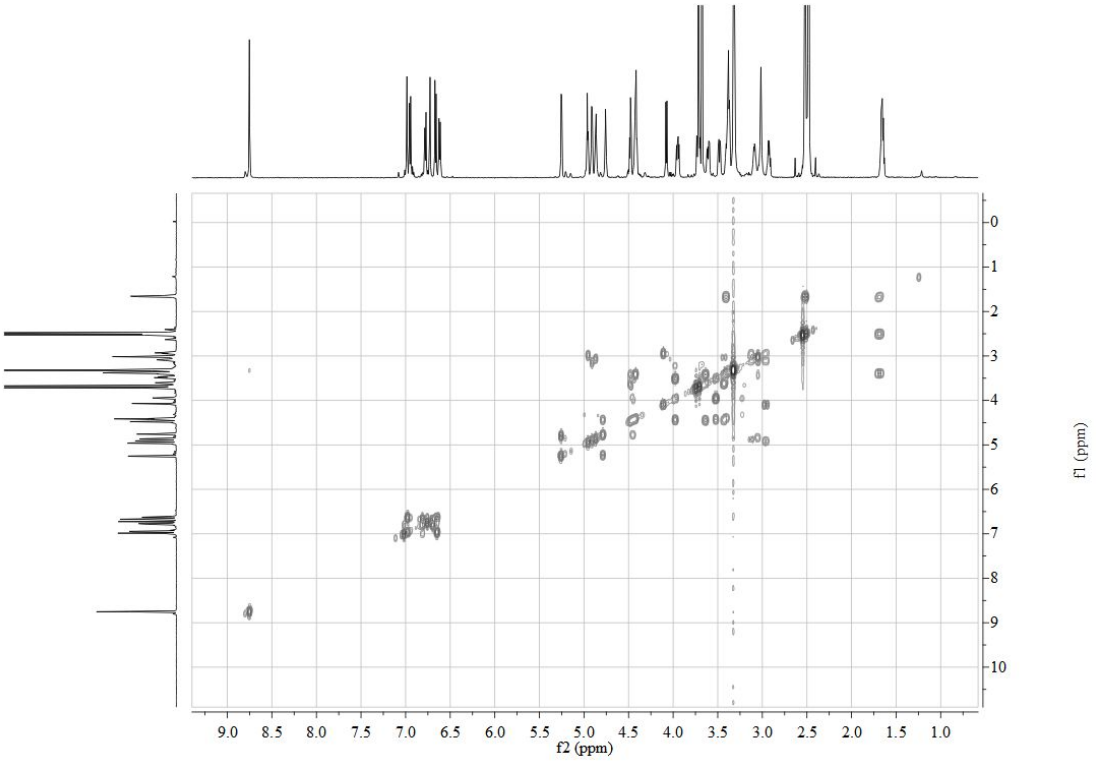

Figure Supporting 7. COSY data of compound **1** in DMSO-d_6.

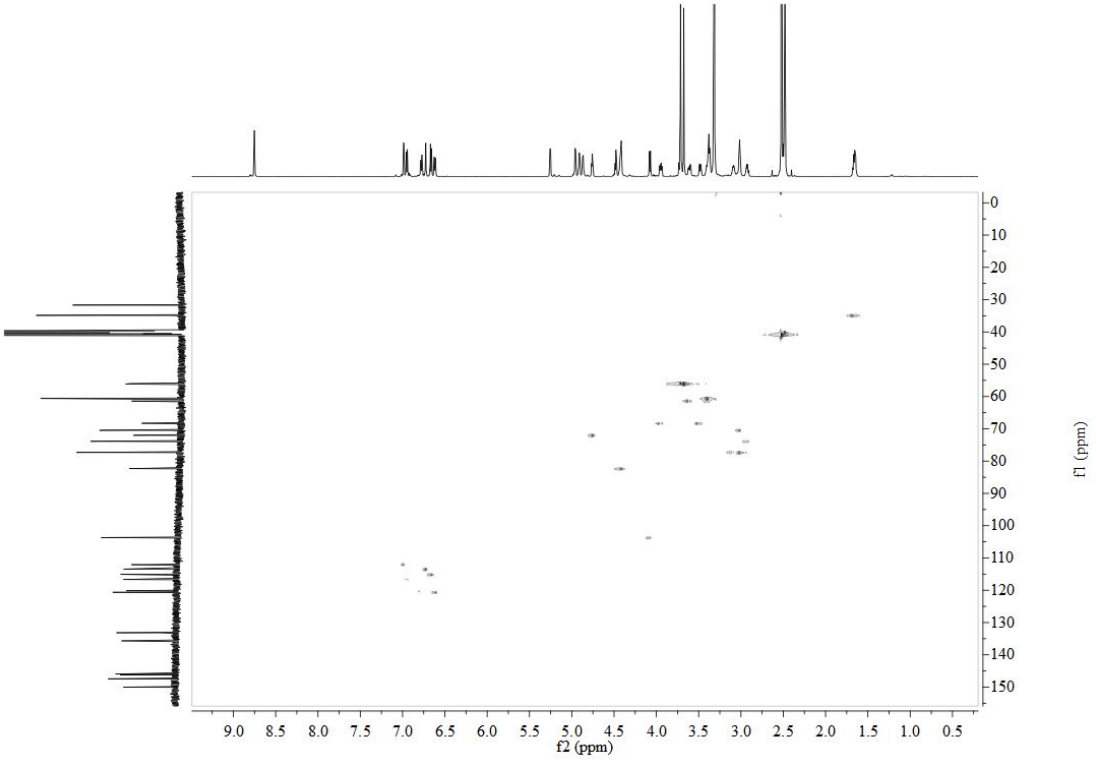

Figure Supporting 8. HSQC data of compound **1** in DMSO-d_6.

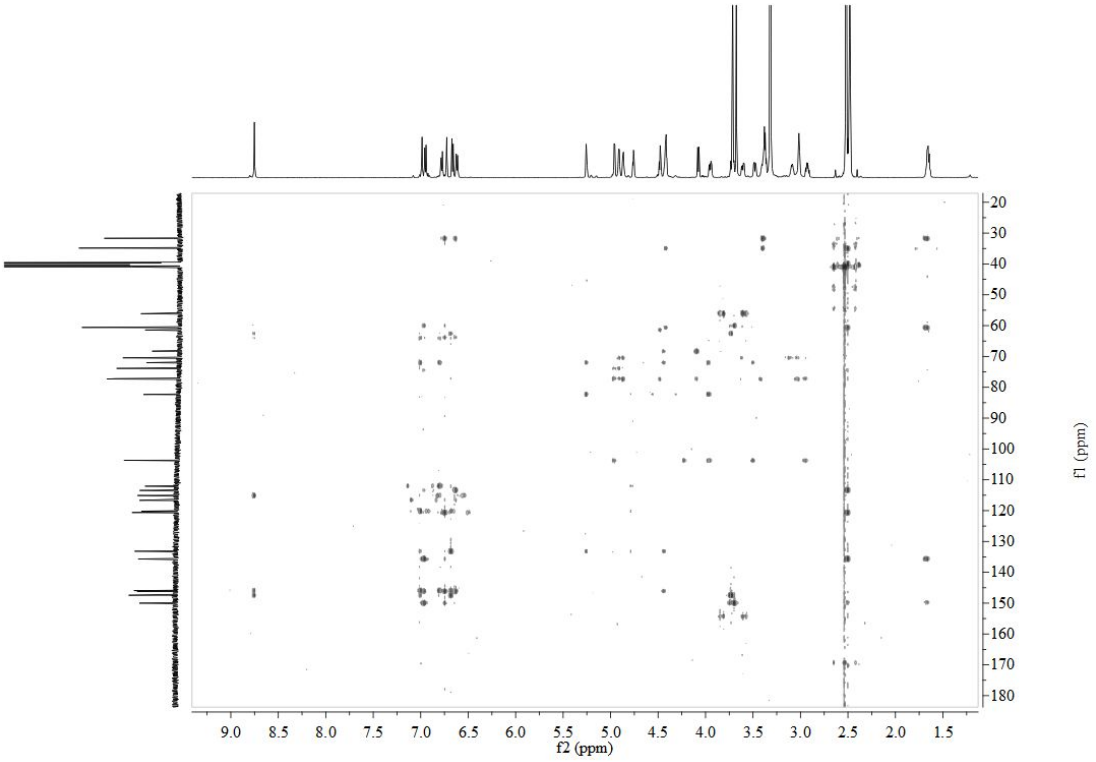

Figure Supporting 9. HMBC data of compound **1** in DMSO-d_6.

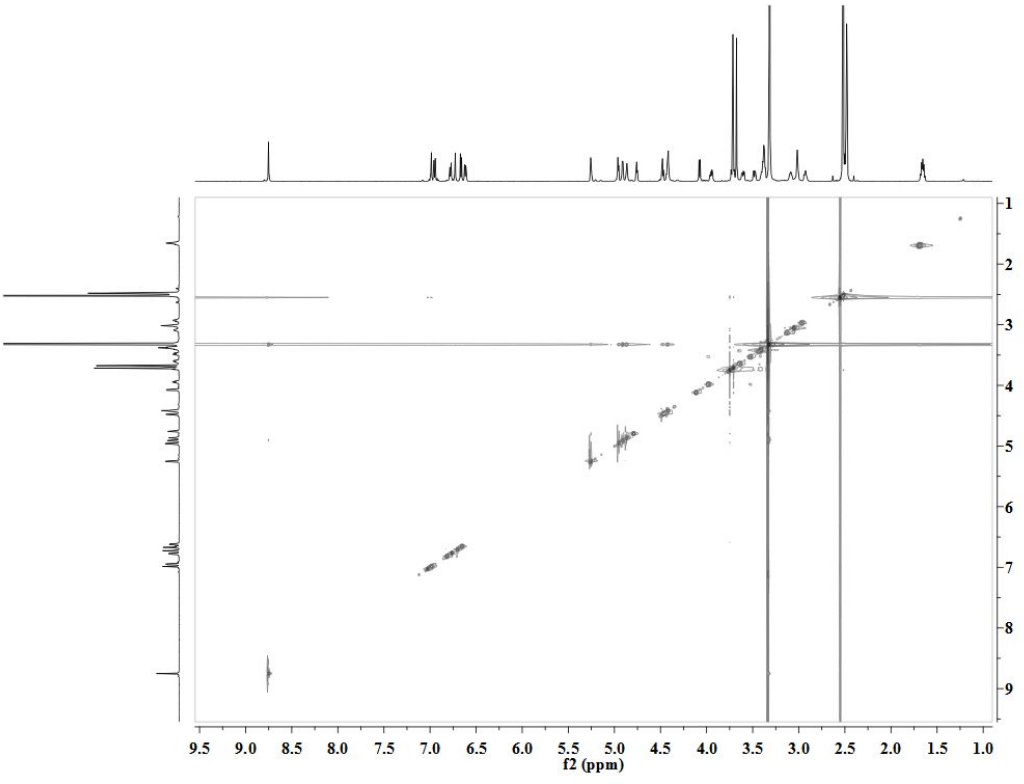

Figure Supporting 10. ROESY data of compound **1** in DMSO-d_6.

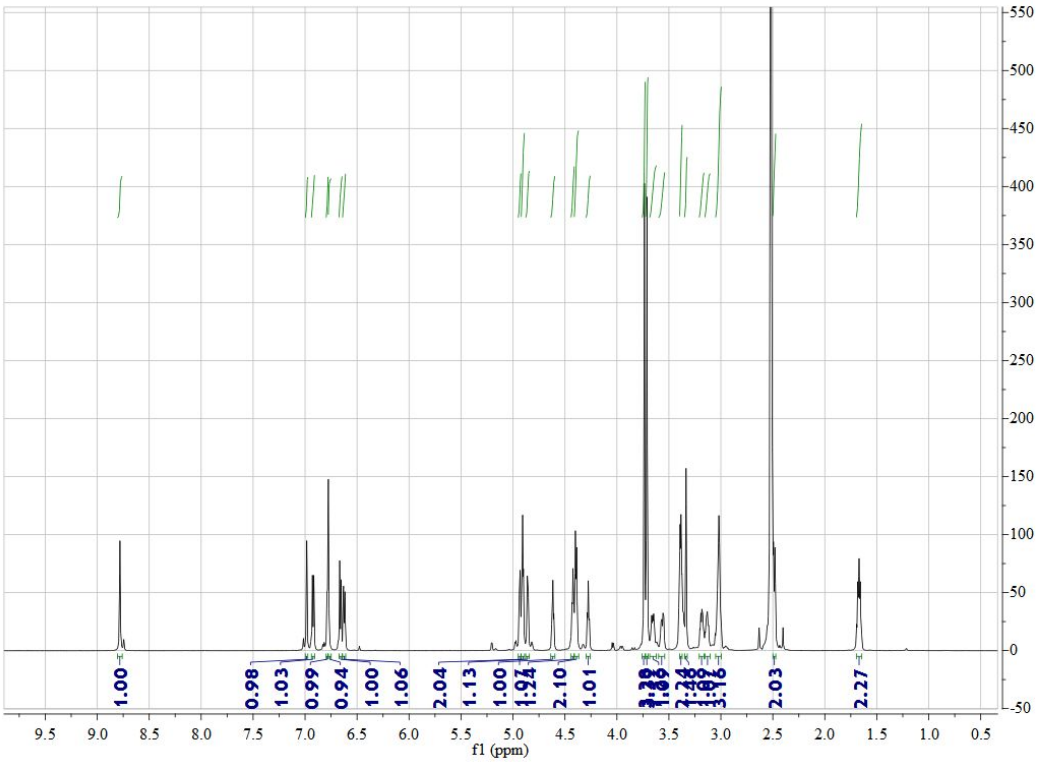

Figure Supporting 11. ^{1}H NMR data of compound **2** in DMSO-d_6.

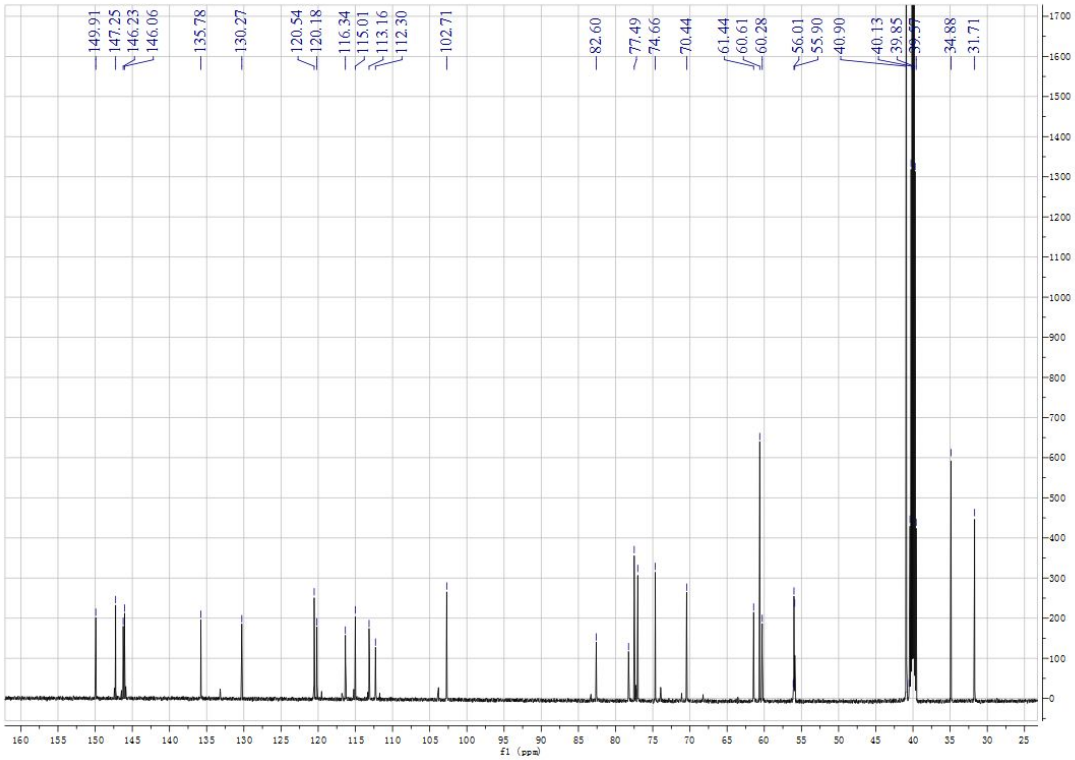

Figure Supporting 12. ^{13}C NMR data of compound **2** in DMSO-d_6.

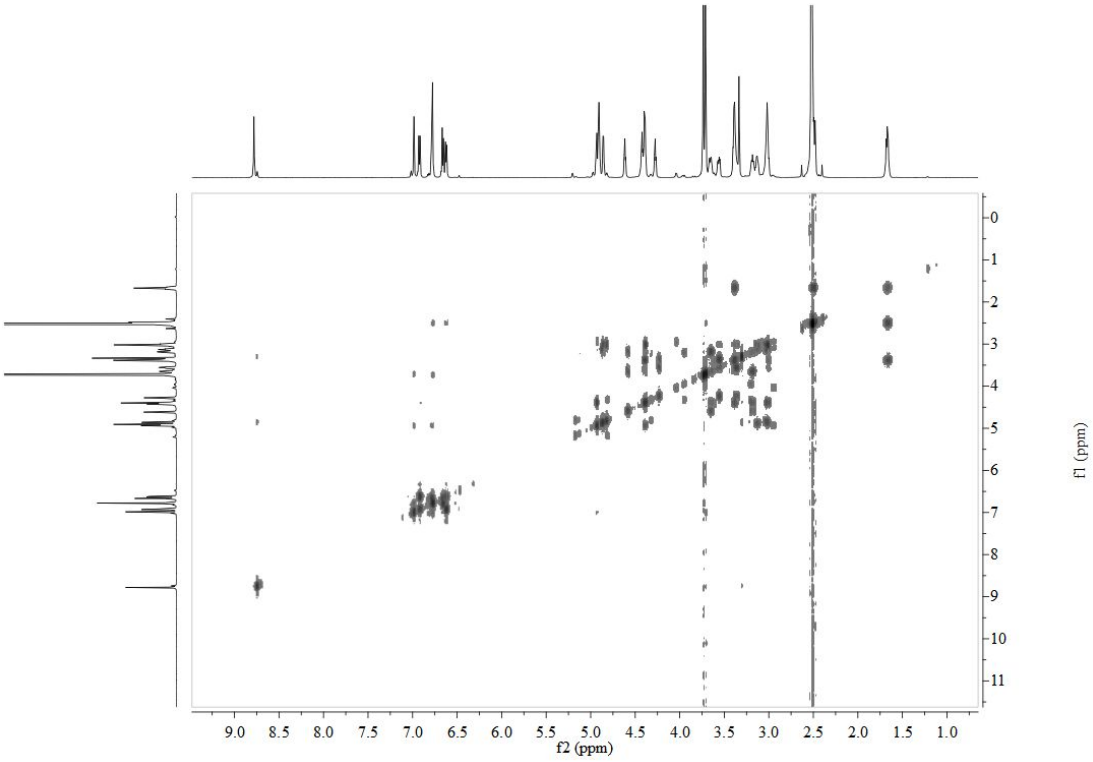

Figure Supporting 13. COSY data of compound **2** in DMSO-d_6.

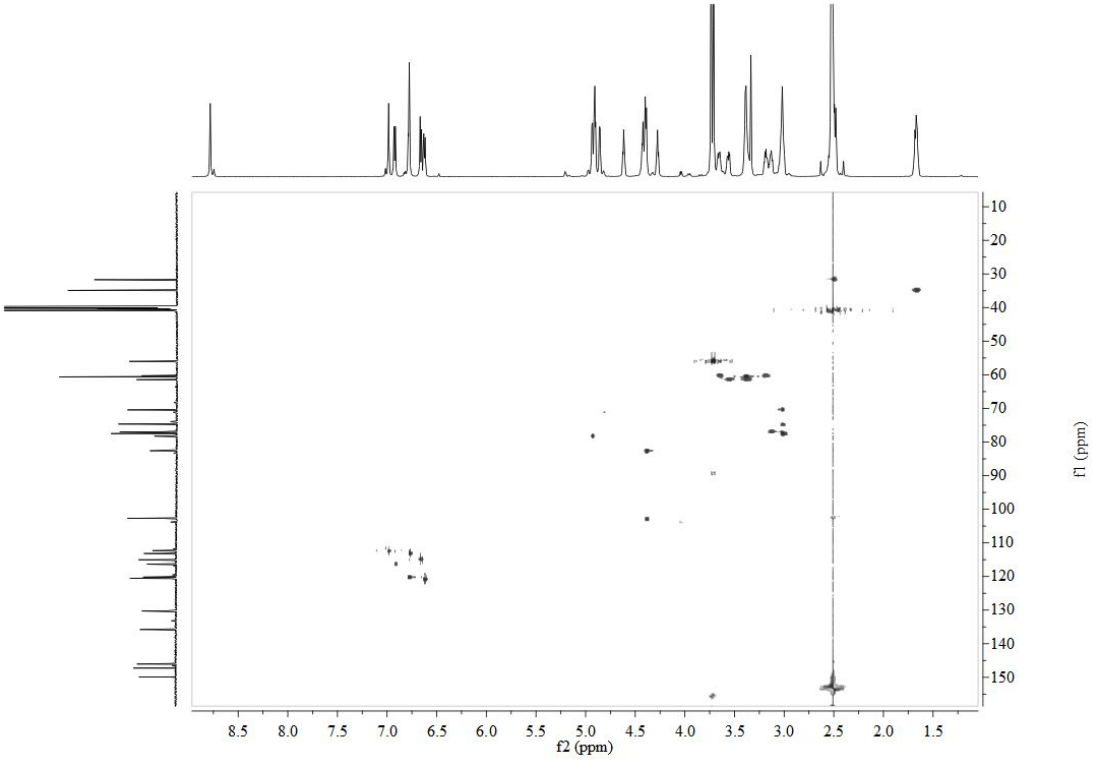

Figure Supporting 14. HSQC data of compound **2** in DMSO-d_6.

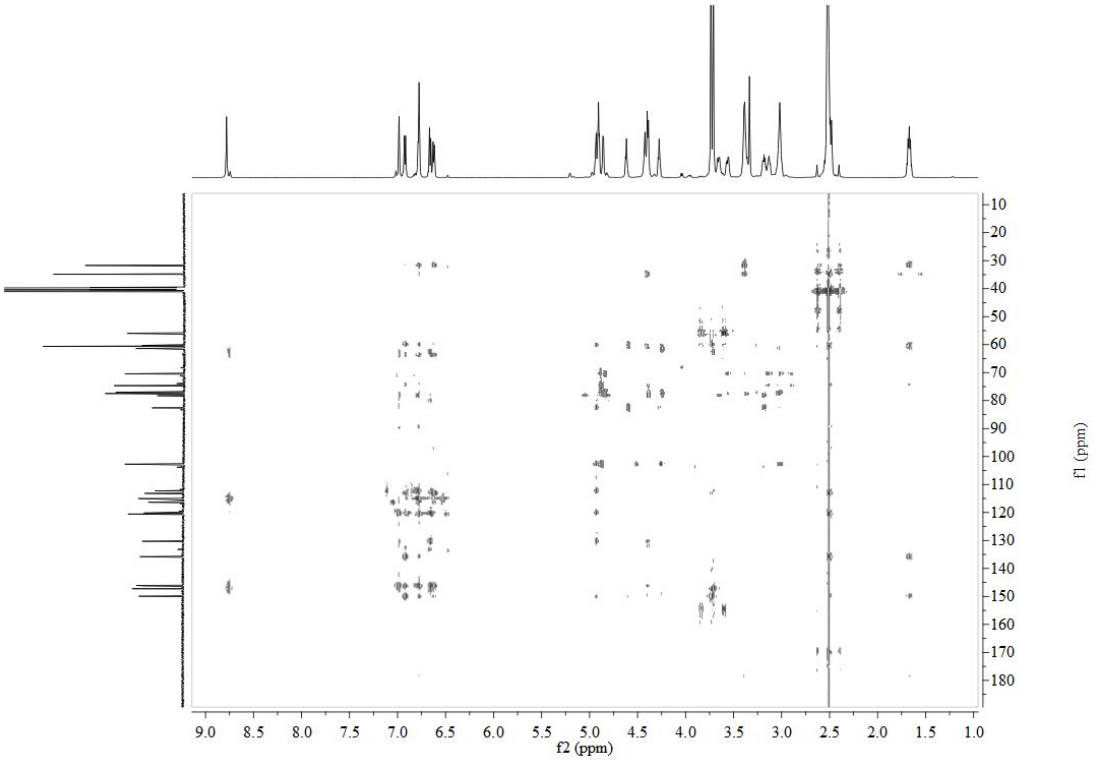

Figure Supporting 15. HMBC data of compound **2** in DMSO-d_6.

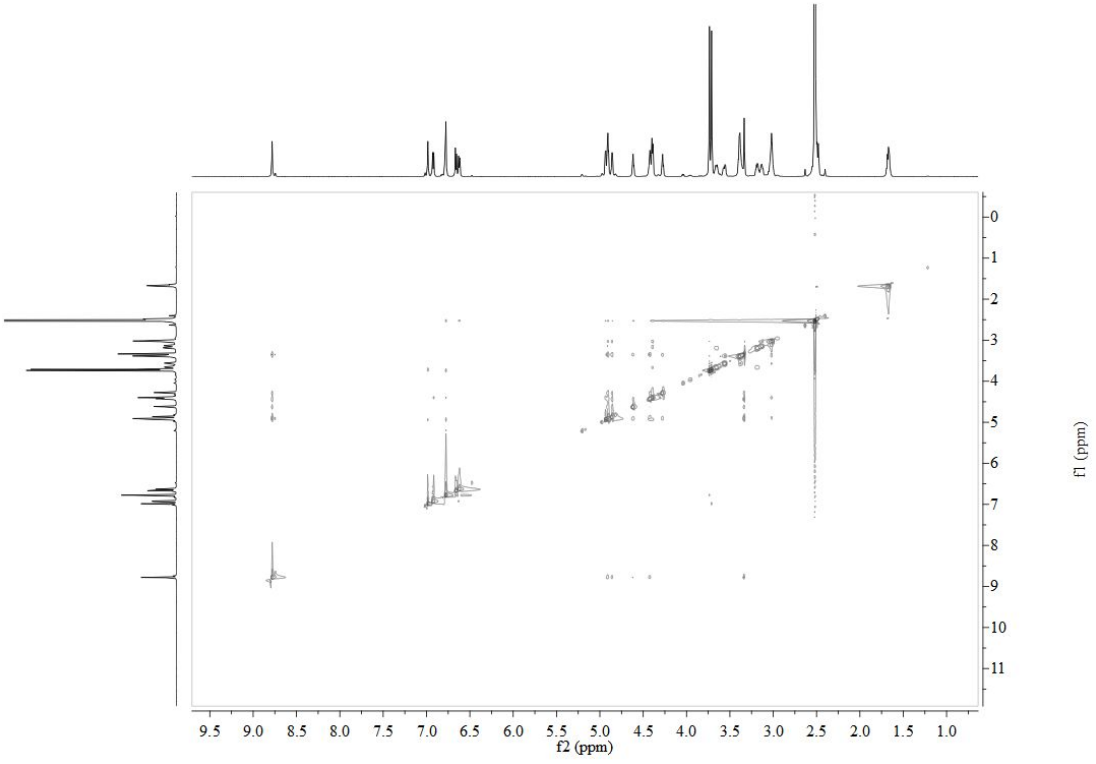

Figure Supporting 16. NOESY data of compound **2** in DMSO-d_6.

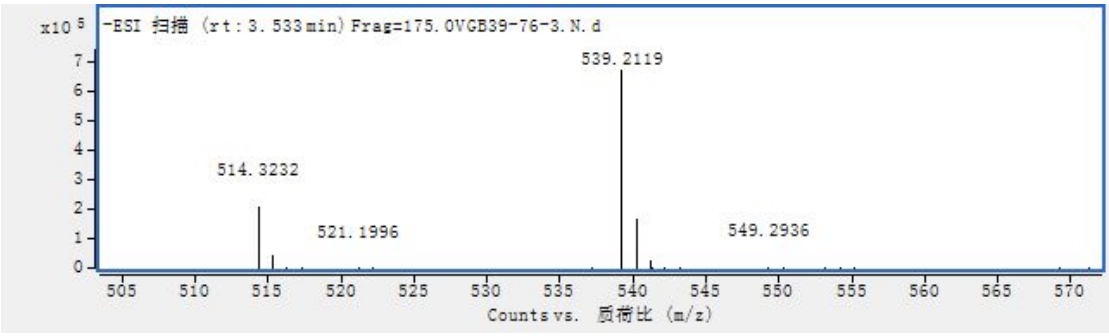

Figure Supporting 17. HR-ESI-MS spectrum of **1**.

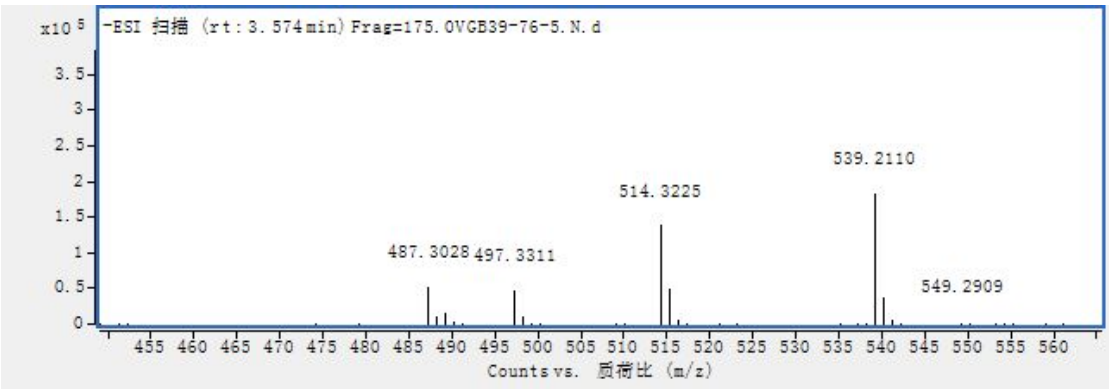

Figure Supporting 18. HR-ESI-MS spectrum of **2**.

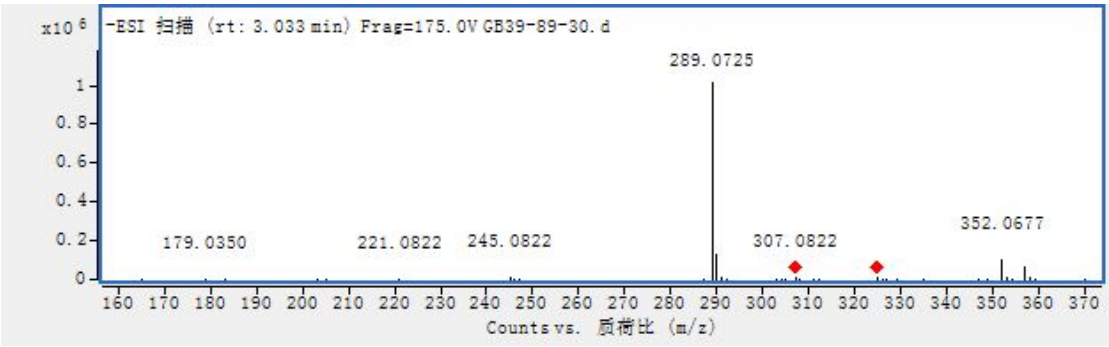

Figure Supporting 19. HR-ESI-MS spectrum of **3**.

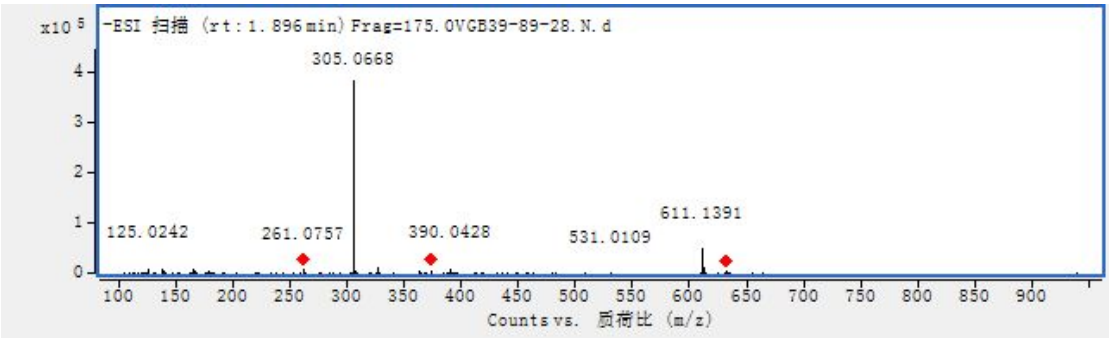

Figure Supporting 20. HR-ESI-MS spectrum of **4**.

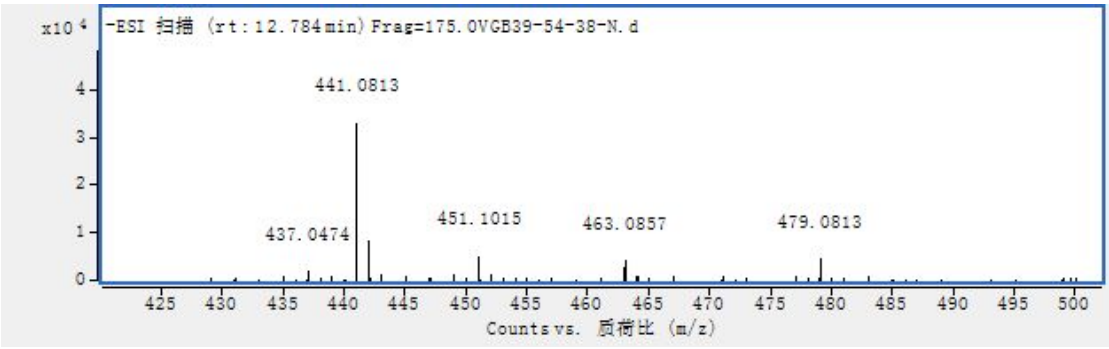

Figure Supporting 21. HR-ESI-MS spectrum of **5**.

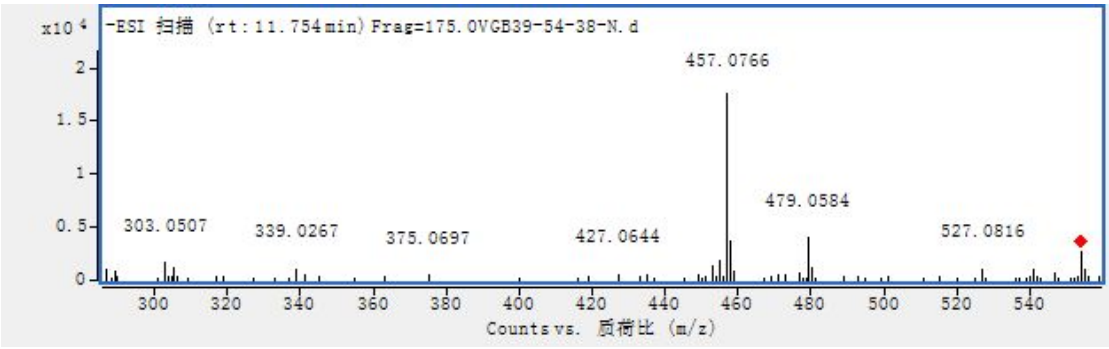

Figure Supporting 22. HR-ESI-MS spectrum of **6**.

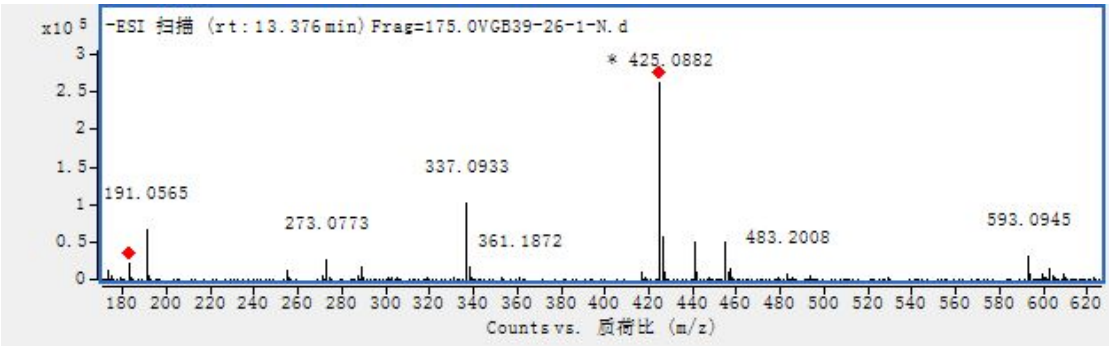

Figure Supporting 23. HR-ESI-MS spectrum of **7**.

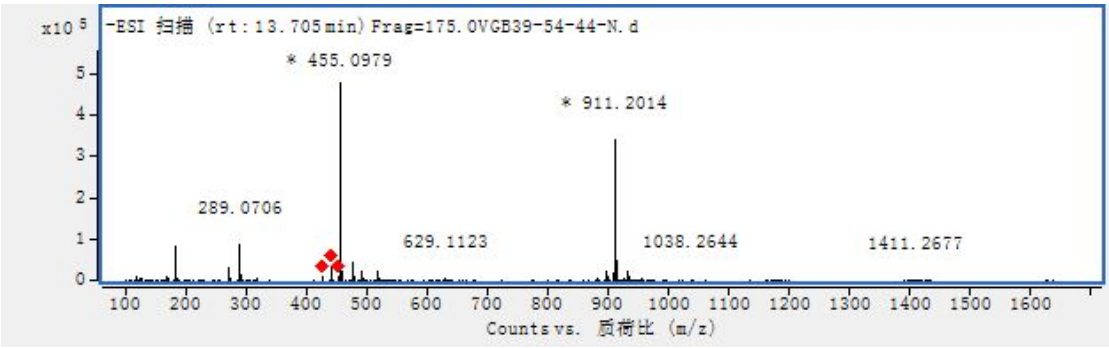

Figure Supporting 24. HR-ESI-MS spectrum of **8**.

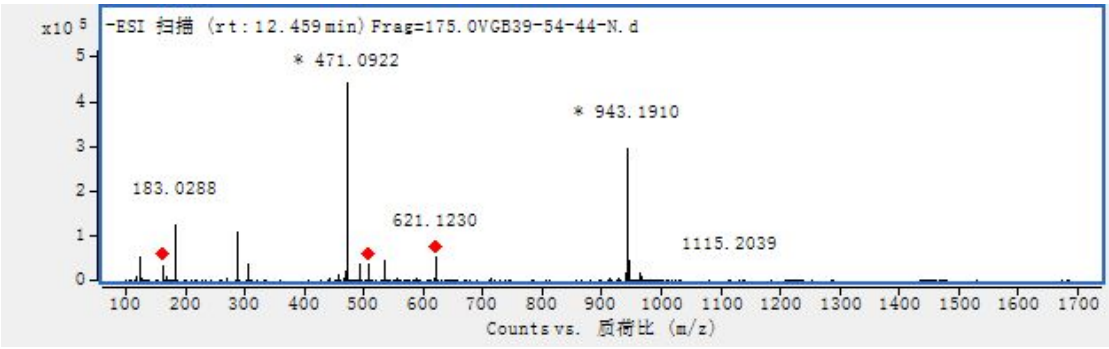

Figure Supporting 25. HR-ESI-MS spectrum of **9**.

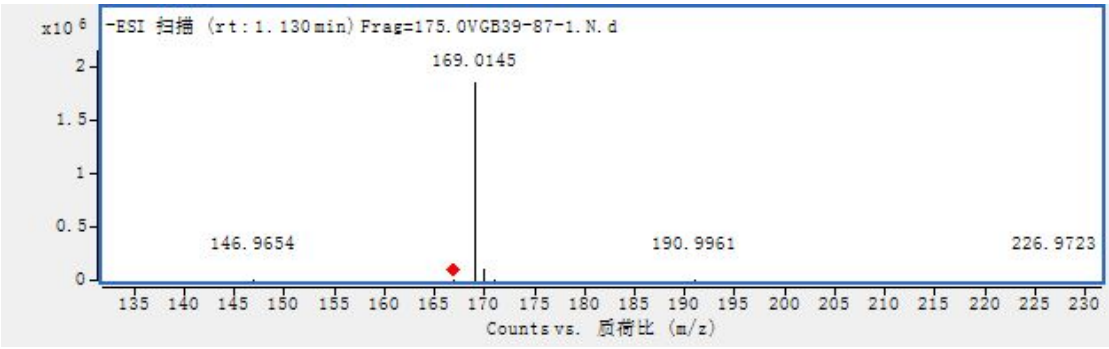

Figure Supporting 26. HR-ESI-MS spectrum of **10**.

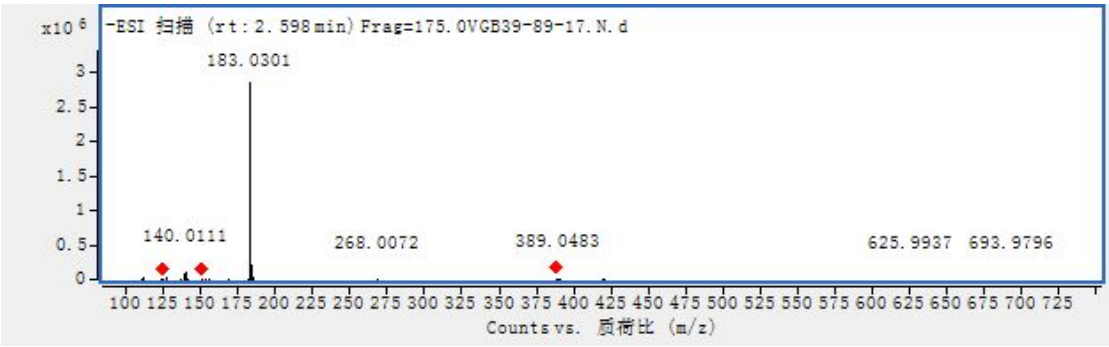

Figure Supporting 27. HR-ESI-MS spectrum of **11**.

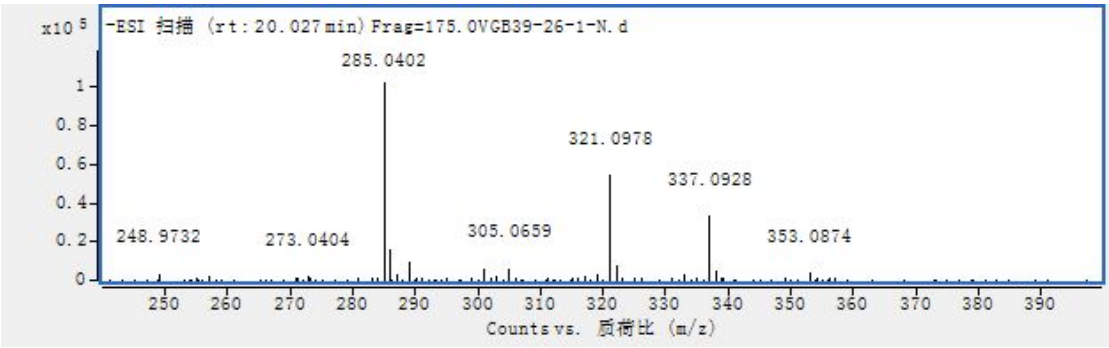

Figure Supporting 28. HR-ESI-MS spectrum of **12**.

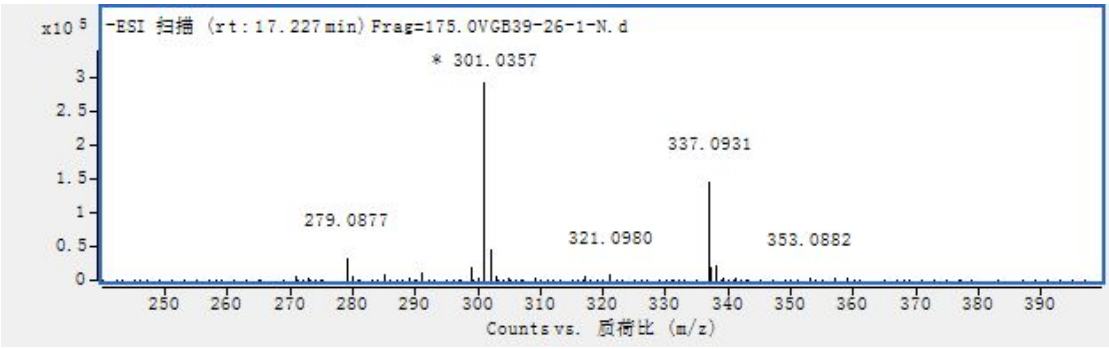

Figure Supporting 29. HR-ESI-MS spectrum of **13**.

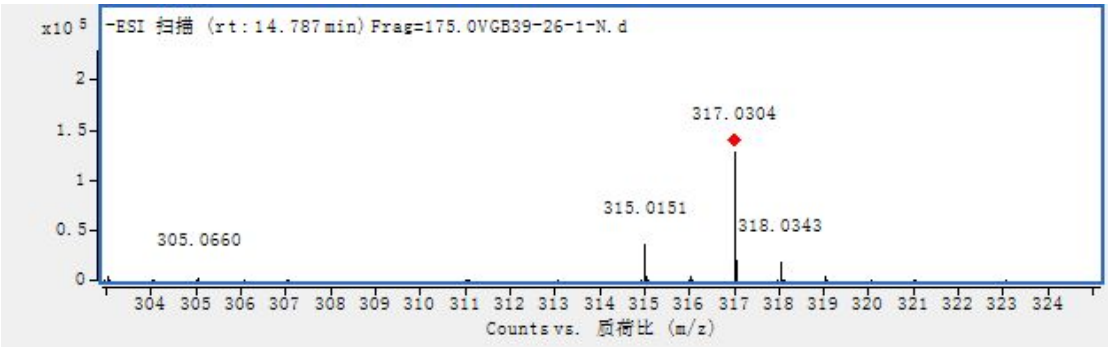

Figure Supporting 30. HR-ESI-MS spectrum of **14**.

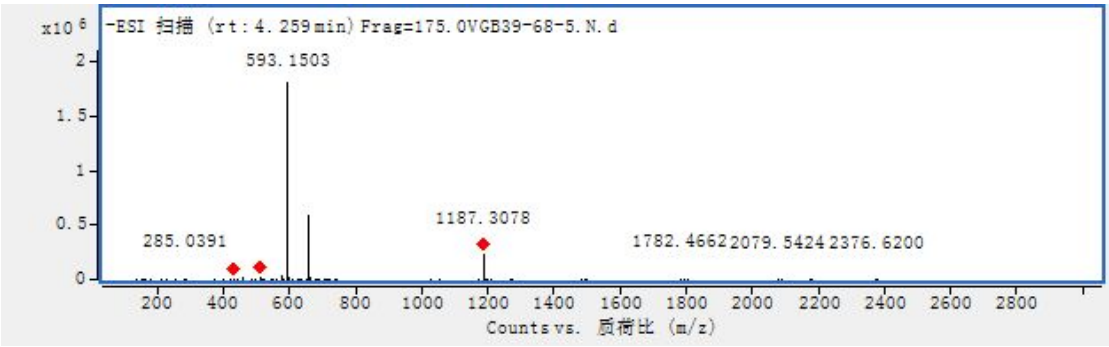

Figure Supporting 31. HR-ESI-MS spectrum of **15**.

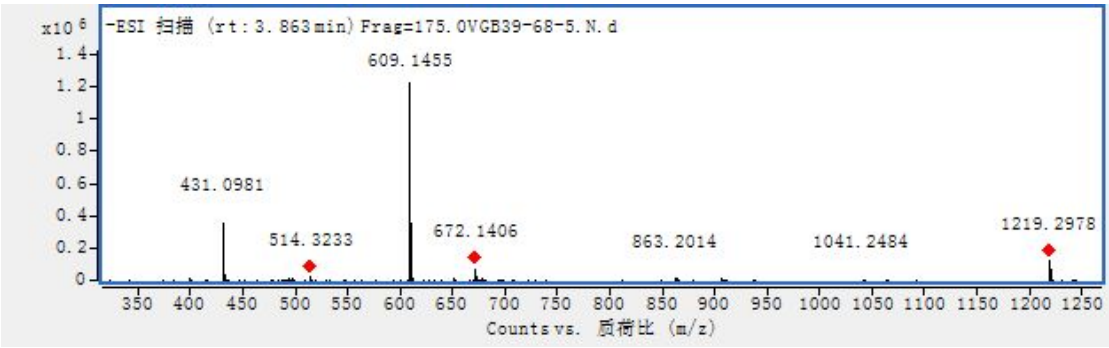

Figure Supporting 32. HR-ESI-MS spectrum of **16**.

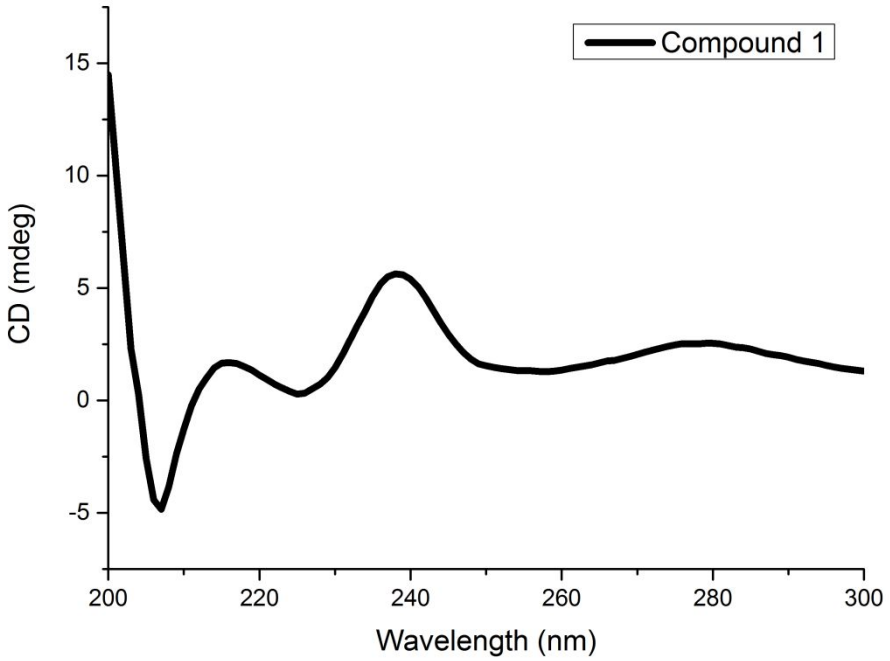

Figure Supporting 33. Circular dichroism spectra of compound **1**.

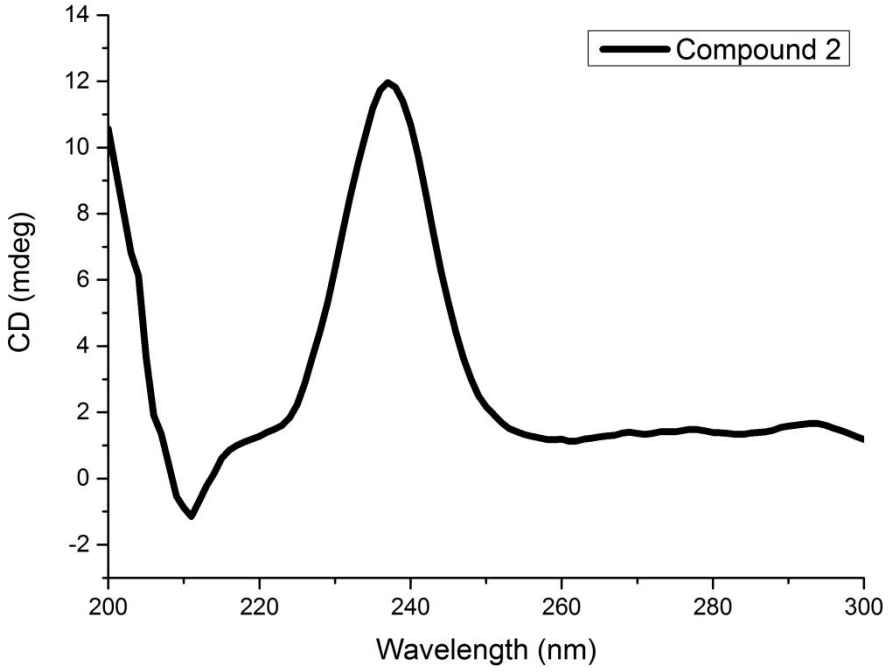

Figure Supporting 34. Circular dichroism spectra of compound **2**.

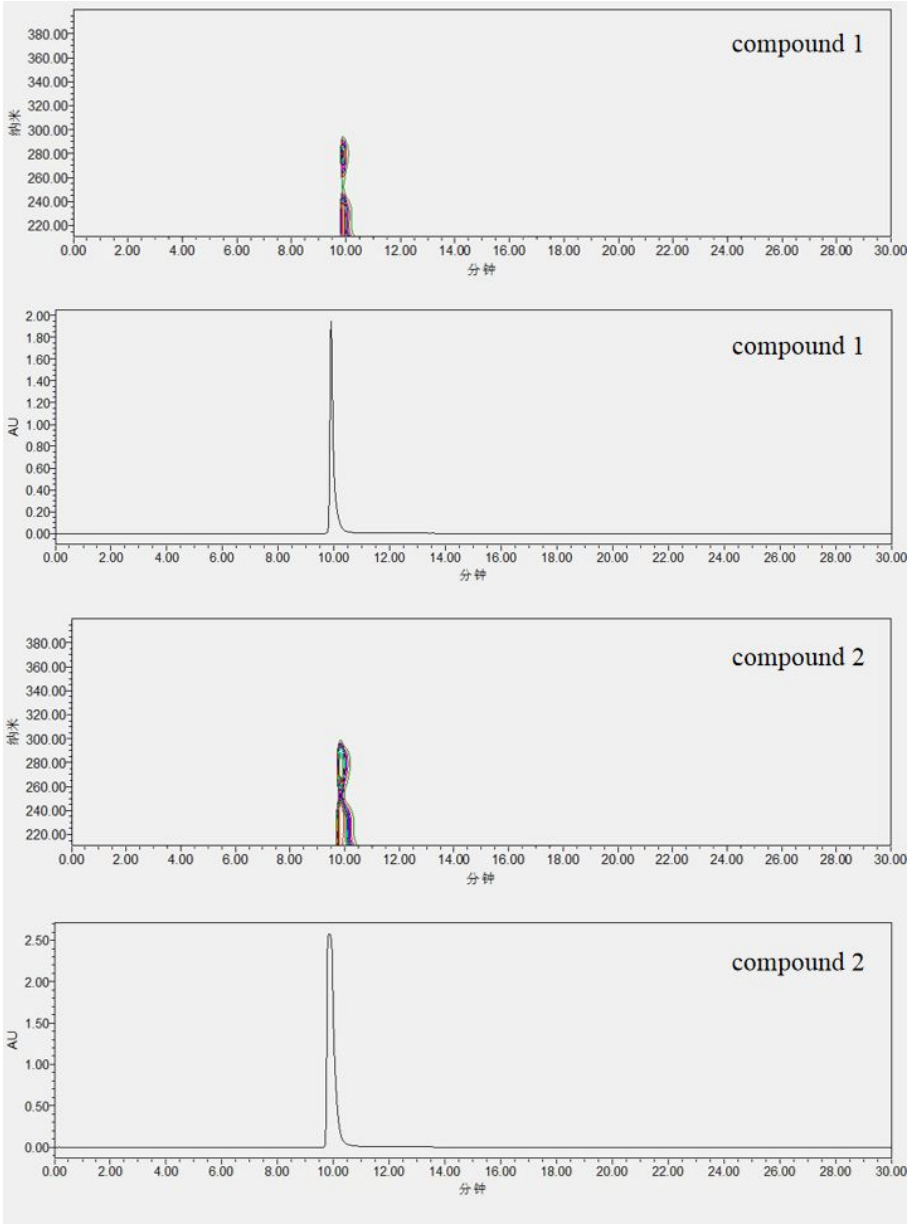

Figure Supporting 35. Purity checks of **1** and **2** by HPLC with a chiral column.

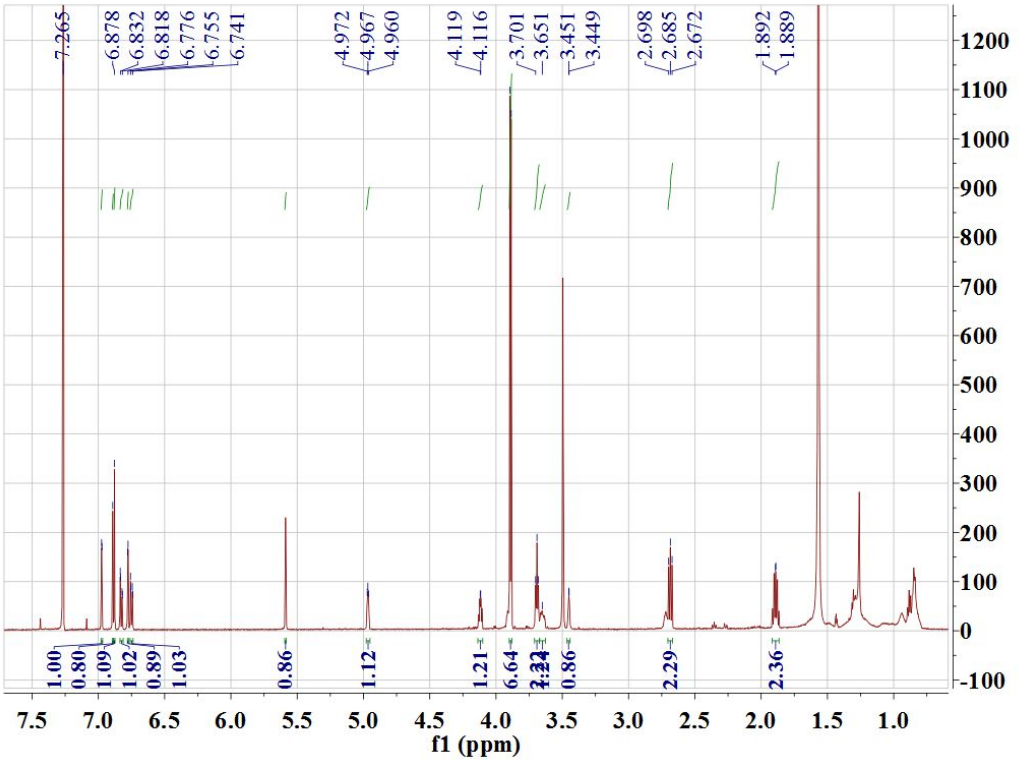

Figure Supporting 36. ^{1}H NMR data of compound **1a** in $CDCl_3$-*d*

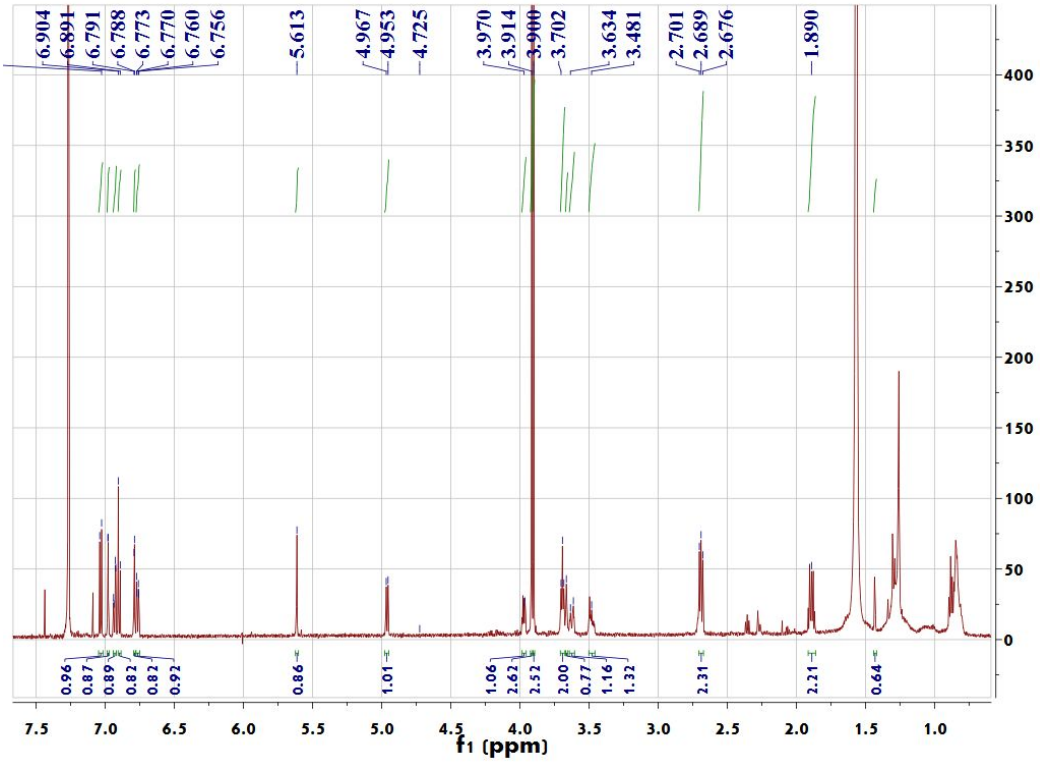

Figure Supporting 37. ^{1}H NMR data of compound **2a** in $CDCl_3$-*d*

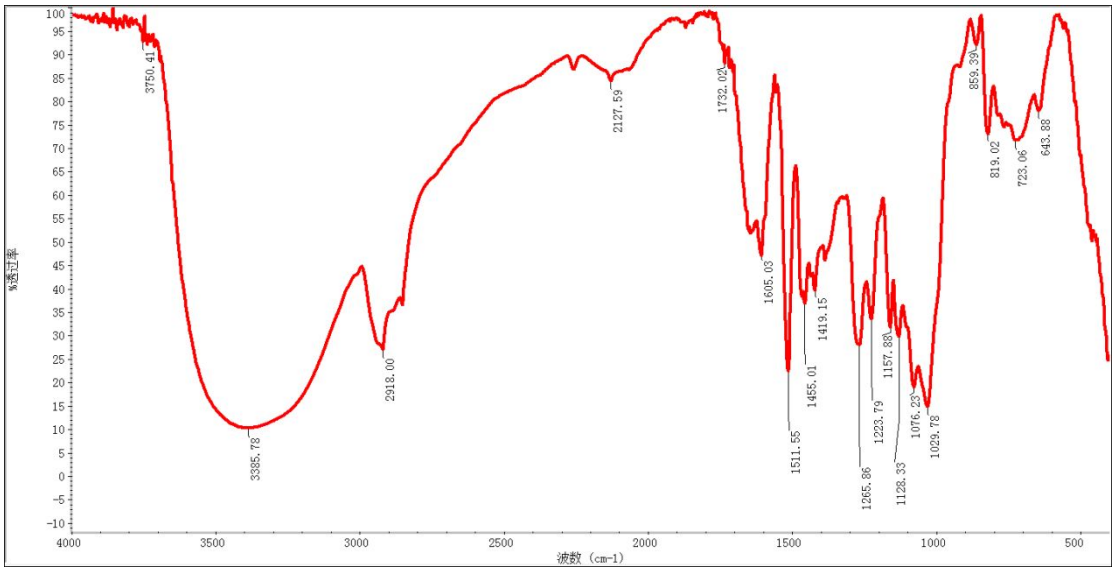

Figure Supporting 38. The IR spectrum of compound **1**.

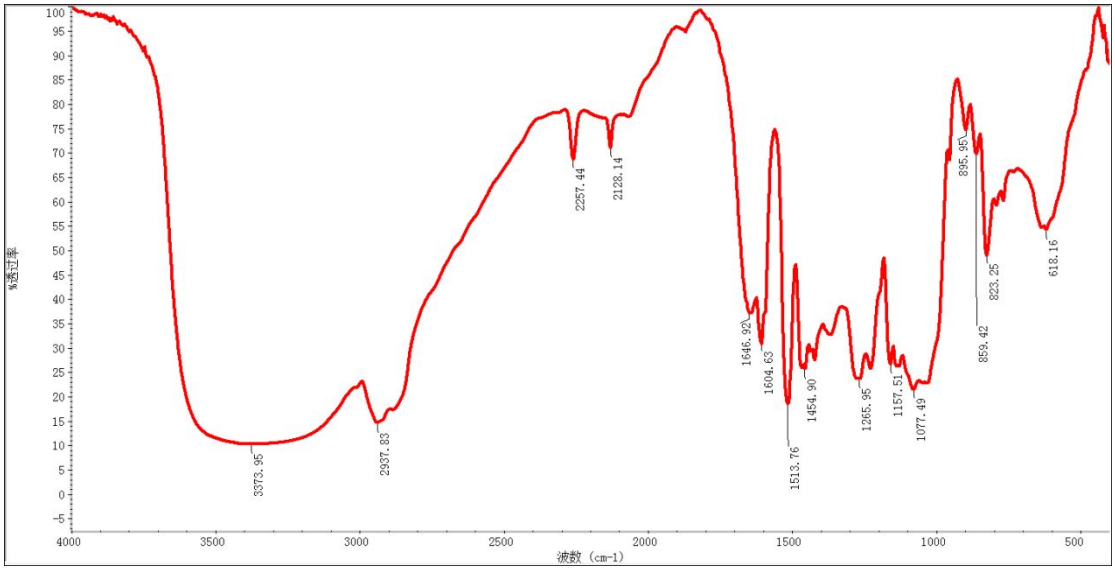

Figure Supporting 39. The IR spectrum of compound **2**.

Acid Hydrolysis and Sugar Analysis of Compound 1. The method was modified according to reference with some modification.[1] Briefly, compound **1** (0.5 mg) was melted in 2 M HCl (0.8 mL), and heated in water bath for 4 h at 80 °C. The reactants were extracted using chloroform to get the supernatant and vacuum freeze-dried. The sample was melted in 2 mL of pyridine mixed with 22 mg/mL L-cysteine methyl ester hydrochloride at 60 °C for 2 h. The solution was then dried by vacuum freeze-drying,

following with the addition of 0.2 mL trimethylsilylimidazole to the solid. The mixture was then heated for 1.5 h at 70 °C, and then partitioned between n-hexane and water. GC-MS was carried out to analyze the n-hexane fraction with injector temperature at 280 °C. The oven temperature firstly started at 160 °C for 1 min, and then rose up to 200 °C at a speed of 6 °C/min , and then kept warming-up until a temperature of 280 °C at a speed of 3 °C/min and held for 5 min. The glucose standard (0.3 mg) was analyzed under the same condition. A comparison between the sugar standard and the sugar units of compound **1** was made with peak and retention time (RT). As a result, D-glucose (RT 22.23 min) was confirmed in compound **1**.

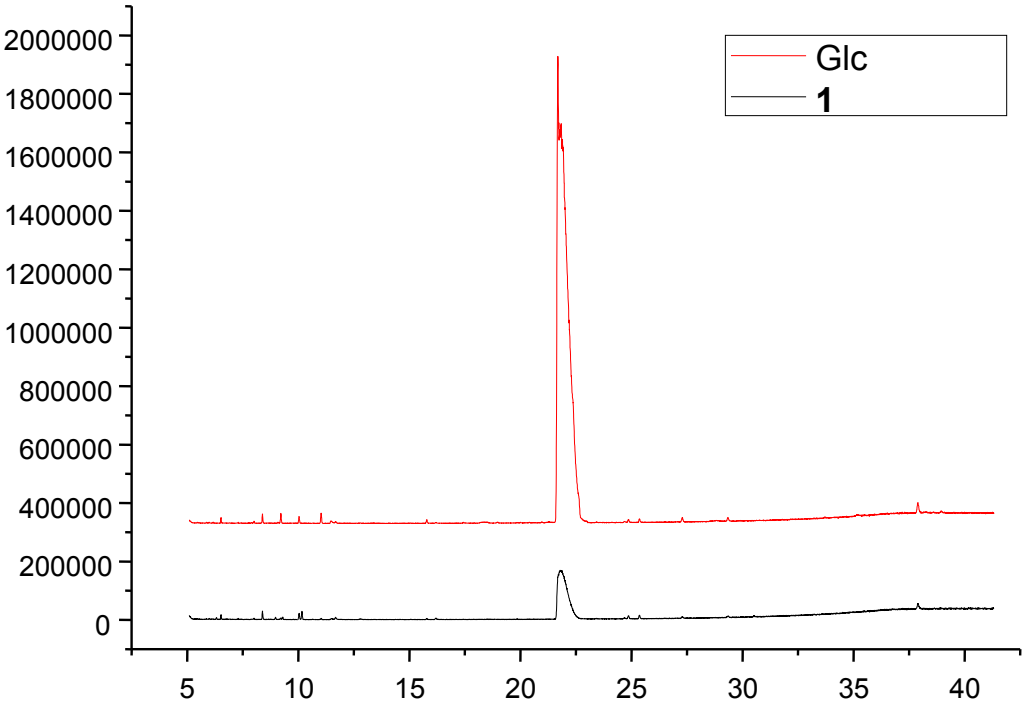

Figure Supporting 40. GC-MS identification of the glucose moiety in compound **1**.

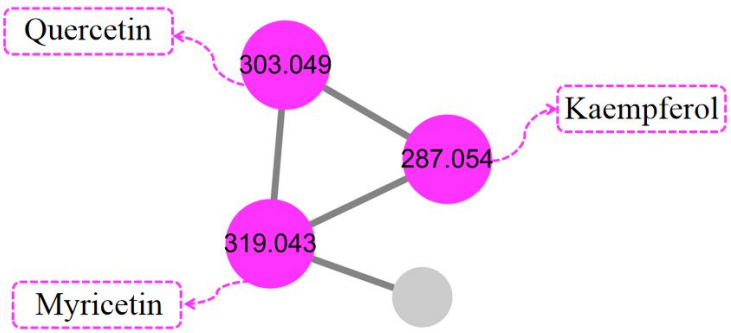

Figure Supporting 41. Molecular networking of flavonols in Huangjinya green tea.

Table Supporting 1. HPLC preparation method for compound **1** and **2** .

Time	Flow（ml/min)	A% (water）	B%（acetonitrile）
0	2	87	13
35	2	87	13
36	2	70	30
38	2	70	30
39	2	87	13
45	2	87	13

Table Supporting 2. The inhibitory activities of the compounds against AChE in vitro .

Compounds	IC_{50} (μM)
1	0.75 ± 0.04
2	0.19 ± 0.02
Huperzine-A	0.29 ± 0.05