Potential active constituents responsible for treating acute pharyngitis in the flowers of *Hosta plantaginea* (Lam.) Aschers and their pharmacokinetics

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Supporting material

The preparation of the hosta flower extract

Air-dried hosta flowers (1.5 kg) were ground into a powder using a grinder (800C, Yongkang Azela Electric Appliance Co., Ltd). The powder was then placed in a 20 L round-bottom glass flask containing 10 L of 70% aqueous ethanol, and the mixture was subjected to heat reflux extraction at 98 °C for 2 h. After that, the mixture was filtered, and the residue was again extracted with another 10 L of 70% aqueous ethanol under the same conditions and then filtered. The two filtrates were combined, evaporated to a certain volume, and then freeze-dried, yielding dry extract.

HPLC-QTOF/MS parameters

HPLC analysis was performed with an Agilent 1260 HPLC system (Agilent, USA) equipped with an Agilent ZORBAX SB-C18 column (4.6×50 mm, 1.8 μm) maintained at 30°C. The flow rate was set at 0.5 mL/min. The column was eluted with a gradient of acetonitrile-formic acid (100:0.1, v/v. A) and water–formic acid (100:0.1, v/v. B): 0-60 min, 5-100% A; 60-70 min, 100% A. The temperature of the auto-sampler was kept at 4°C.

Mass spectrometry was performed with an Agilent 6530 QTOF/MS mass spectrometer (Agilent, USA) using a negative ion electrospray ionization (ESI) mode with Auto MS/MS scan. The full scan of ions ranged from 100 to 1000 Da. The capillary was set to -3.5 kV, and the drying gas flow and the temperature were kept at 9 mL/min and 350°C, respectively. The nebulizer gas pressure was

set at 45 psi. The sheath gas flow and its temperature were maintained at 12 mL/min and 400°C, respectively. Nitrogen was used as an auxiliary gas as well as a nebulizer gas. The fragment and collision energy (CE) were set -150 and -20 eV, respectively. Data acquisition and processing were performed with Agilent MassHunter Workstation Data Acquisition and Agilent MassHunter Qualitative Analysis (versions B. 06. 00), respectively.

The conditions of UHPLC-QQQ/MS.

The samples were injected into an Agilent 1290 UHPLC system (Agilent, USA) equipped with an Agilent Poroshell 120 EC-C18 column (2.1 × 100 mm, 1.9 μm) maintained at 25 °C. The auto-sampler temperature was kept at 4 °C and the injection volume was set at 5 μL. The column was eluted with a gradient consisting of mobile phase A (water-formic acid at a ratio of 100:0.1) and mobile phase B (methanol-formic acid at a ratio of 100:0.1). The gradient lasted for 11 min and consisted of 10-100% mobile phase B. The flow rate was set at 0.3 mL/min.

Mass spectrometry was performed with an Agilent 6460 QQQ/MS mass spectrometer (Agilent, USA) using a negative ion electrospray ionization (ESI) mode. The capillary was set to 3.5 kV, and the flow of the drying gas was kept at 6 mL/min while the temperature was maintained at 350 °C. The nebulizer gas pressure was set at 45 psi. The sheath gas flow and its temperature were maintained at 12 L/min and 400 °C, respectively. Nitrogen was used as an auxiliary gas as well as a nebulizer gas. Data acquisition was achieved with an Agilent MassHunter Workstation Data Acquisition (versions B. 05. 01) whereas data processing was performed with an Agilent

MassHunter Qualitative Analysis (versions B. 06. 00).

Blood sample preparation.

An aliquot (100 μL) of the internal standard solution was dispensed into a centrifuge tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was mixed with 100 μL of plasma by vortexing for 1 min followed by the addition of 800 μL acetonitrile and 1 min of vortexing to precipitate the proteins. The sample was then centrifuged at 11000 × *g* for 10 min and the supernatant was transferred to a new centrifuge tube and evaporated to dryness as described above. The residue was dissolved in 100 μL acetonitrile-water (90:10, v/v), and 5 μL of the solution was used for UHPLC-QQQ analysis.

Method validation.

The analytical method was conducted according to the FDA guideline for the validation of a bioanalytical method, which includes calibration curve, stability, a lower limit of quantification (LLOQ), matrix effect, recovery, accuracy and precision, and selectivity. The procedure for the validation tests was mainly referenced from our previous work.[40]

Data analysis.

DAS 2.0 pharmacokinetic program (Chinese Pharmacological Society) was used to plot the plasma concentration versus time curves for the compounds under investigation. A non-compartmental model was used to calculate the following parameters: area under the plasma concentration versus time curve (AUC) from zero to the last sampling time ($AUC_{0–72h}$) and infinity ($AUC_{0–\infty}$), the peak plasma concentration (C_{max}), and the time to reach C_{max} (T_{max}) after administering the mangosteen extract.

Table S1 ^{1}H-NMR and ^{13}C-NMR spectra data of compound 4. (δ, 0 = TMS, in DMSO)

No.	^{1}H-NMR (300 MHz，DMSO-*d*)	^{13}C-NMR (150MHz, DMSO-*d*)
C-4		177.7
C-7		162.9
C-5		160.9
C-4′		160.2
C-9		159.9
C-2		156.1
C-3		133.5
C-2′ ,6′	8.06 (2H, d, J=8.8, Hz, H=2’, 6’)	131.0
C-1′		129.8
C-3′ ,5′	6.90 (2H, d, J=8.8 Hz, H=3’, 5’)	115.2
C-10		105.7
C-1′ ′ ′		100.7
C-6	6.44 (1H, d, J=2.16 Hz, H=6)	99.7
C-1′ ′		99.4
C-8	6.80 (1H, d, J=2.16 Hz, H=8)	94.5
C-2′ ′	5.49 (1H, d, J=6.9 Hz, H=2’’)	77.6
C-3′ ′ ′	4.61 (1H, d、J=8.3 Hz, H=3’’’)	77.2
C-3′ ′		76.4
C-5′ ′		76.4
C-5′ ′ ′		74.2
C-2′ ′ ′		73.1
C-4′ ′ ′		69.9
C-4′ ′		69.6
C-6′ ′ ′		60.8
C-6′ ′		60.6

Table S2 ^{1}H-NMR and ^{13}C-NMR spectra data of compound 5. (δ, 0 = TMS, in D2O)

No.	^{1}H-NMR (300 MHz, D_2O)	^{13}C-NMR (150MHz, D_2O)
C-4		178.0
C-7		161.9
C-5		159.9
C-4’		159.1
C-9		131.5
C-2		156.0
C-3		118.3
C-2’,6’	7.86 (2H, d, J=7.2 Hz)	133.0
C-1’		105.9
C-3’,5’	6.74 (2H, d, J=7.5 Hz)	116.8
C-10		102.3
C-1’’’	4.51 (1H, d, J=7.8 Hz)	100.6
C-1’’’’	4.1 (1H, d, J=6.9 Hz)	99.8
C-1’’	4.92 (1H, d, J=8.0 Hz)	95.6
C-6	6.23 (1H, d, J=2.3 Hz)	99.0
C-8	6.53(1H, d, J=2.3 Hz)	95.6
C-2’’		79.2
C-3’’’		76.0
C-5’’’		75.3
C-5’’		75.6
C-2’’’		73.6
C-3’’		75.9
C-4’’’’		71.9
C-3’’’’		74.6
C-2’’’’		71.6
C-4’’’		70.0
C-4’’		69.9
C-5’’’’		69.2
C-6’’		68.5
C-6’’’		60.9
C-6’’’’	0.93 (3H, d, J=6.3Hz)	16.3
C-1’’’’’	5.52 (1H, d, J=6.1Hz	99.4
C-2’’’’’		72.6
C-3’’’’’		75.3
C-4’’’’’		69.6
C-5’’’’’		76.1
C-6’’’’’		60.4

Table S3 ^{1}H-NMR and ^{13}C-NMR spectra data of compound 6. (δ, 0 = TMS, in D2O)

No.	^{1}H-NMR (300 MHz, D_2O)	^{13}C-NMR (150MHz, D_2O)
C-4		177.7
C-7		162.1
C-5		159.7
C-4’		159.7
C-9		158.5
C-2		155.9
C-3		133.9
C-2’,6’	7.7530 (2H, d, J=8.2 Hz)	131.2
C-1’		120.5
C-3’,5’	6.7370 (2H, d, J=8.2 Hz)	115.3
C-10		105.9
C-1’’’	4.7032 (1H, d, J=7.6 Hz)	102.6
C-1’’’’		100.7
C-1’’	4.9647 (1H, d, J=6.9 Hz)	99.5
C-6	6.2888 (1H, d, J=2.0 Hz)	99.4
C-8	6.4539(1H, d, J=2.0 Hz)	95.5
C-2’’		76.1
C-3’’’		75.5
C-5’’’		75.4
C-5’’		74.7
C-2’’’		73.4
C-3’’		72.7
C-4’’’’		71.6
C-3’’’’		70.1
C-2’’’’		69.8
C-4’’’		69.5
C-4’’		69.2
C-5’’’’		68.5
C-6’’		67.5
C-6’’’		60.5
C-6’’’’	0.9898 (3H, d, J=6.0Hz)	16.4

Table S4 Extraction recovery, matrix effect, intra-day and inter-day precisions and accuracies of compound 4-6 from QC samples prepared in rat plasma (n = 6).

Analytes	QC Samples (ng/mL)	Extraction recovery (%)	Matrix Effect (%)	Intra-day		Inter-day	
				Precision (RSD, %)	Precision (RSD, %)	Precision (RSD, %)	Precision (RSD, %)
4	10	94.2±7.1	86.4±9.3	6.1	1.3	4.3	5.5
	100	88.5±6.4	90.6±5.7	1.4	7.9	6.6	4.1
	1000	87.5±6.1	94.7±2.9	2.2	4.3	5.4	6.5
5	10	90.2±7.7	87.5±4.4	4.2	7.1	4.3	9.3
	100	88.4±3.6	89.7±6.4	5.0	8.6	8.4	7.6
	1000	92.3±8.2	92.6±7.2	3.7	7.7	6.7	5.4
6	10	86.6±7.2	88.7±5.8	4.1	2.5	7.9	3.3
	100	91.2±5.6	93.6±5.4	8.3	9.0	5.3	4.5
	1000	85.4±5.4	86.6±5.7	7.2	7.2	2.4	6.0

Table S5 Stability results of compound 4-6 at three QC levels (n = 3).

Analytes	sample conditions	QC samples (ng/mL)	RSD(%)	RE(%)
	8 h at room temperature	10	6.2	4.8
		100	4.5	6.1
		1000	6.8	-7.2
	Storage at −20 °C for 20 days	10	7.2	9.3
		100	-3.6	6.9
4		1000	5.5	-5.8
	Freeze–thaw stability	10	6.9	-6.1
		100	4.1	7.3
		1000	7.4	4.8
	12 h in autosampler vials kept at 4	20	9.2	5.3
	°C	200	6.3	6.2
		500	8.1	-7.3
	8 h at room temperature	10	7.7	8.7
		100	3.8	7.2
		1000	-6.5	5.9
	Storage at −20 °C for 20 days	10	4.8	-6.4
		100	9.3	3.3
5		1000	6.0	5.7
	Freeze–thaw stability	10	-7.3	6.3
		100	5.8	7.4
		1000	4.6	4.9
	12 h in autosampler vials kept at 4	10	5,2	4.6
	°C	100	9.3	9.3
		1000	8.4	6.4
	8 h at room temperature	10	7.8	4.3
		100	9.2	5.3
		1000	4.6	9.1
	Storage at −20 °C for 20 days	10	-5.3	8.5
		100	4.9	9.0
6		1000	9.7	3.7
	Freeze–thaw stability	10	8.1	6.6
		100	5.6	2.3
		1000	4.4	4.8
	12 h in autosampler vials kept at 4	10	3.7	4.5
	°C	100	6.4	4.2
		1000	7.2	7.7
	8 h at room temperature	10	7.8	4.3
		100	9.2	5.3
		1000	4.6	9.1

	Storage at −20 °C for 20 days	10	-5.3	8.5
		100	4.9	9.0
6		1000	9.7	3.7
	Freeze–thaw stability	10	8.1	6.6
		100	5.6	2.3
		1000	4.4	4.8
	12 h in autosampler vials kept at 4	10	3.7	4.5
	°C	100	6.4	4.2
		1000	7.2	7.7

Table S6 Grade of pharyngitis degree

Group	The pharyngitis degree grade score
Blank control group	1
The aspirin-positive drug group	1
Whole extract group	1
Fractions were eluted with 30% ethanol water	1
Fractions were eluted with 70% ethanol water	2
The fractions were eluted by water	3
Spontaneous recovery	4
Model group	4
Fractions were eluted with 95% ethanol water	5

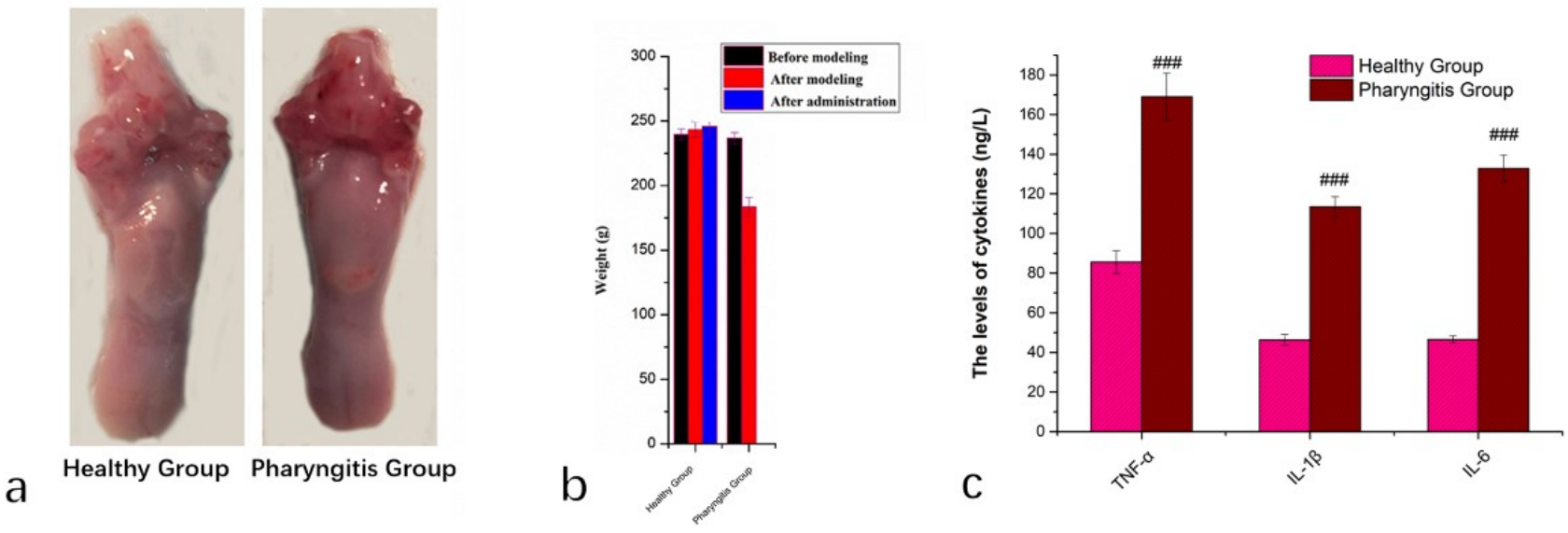

Fig. S1. Effect of ammonia-induced acute pharyngitis on rats. (a) Comparison of pharynx tissues between healthy group and pharyngitis group.

(b) Effect of pharyngitis on weight loss. (c) The effects of the extract of hosta flowers on the cytokine levels of serum.

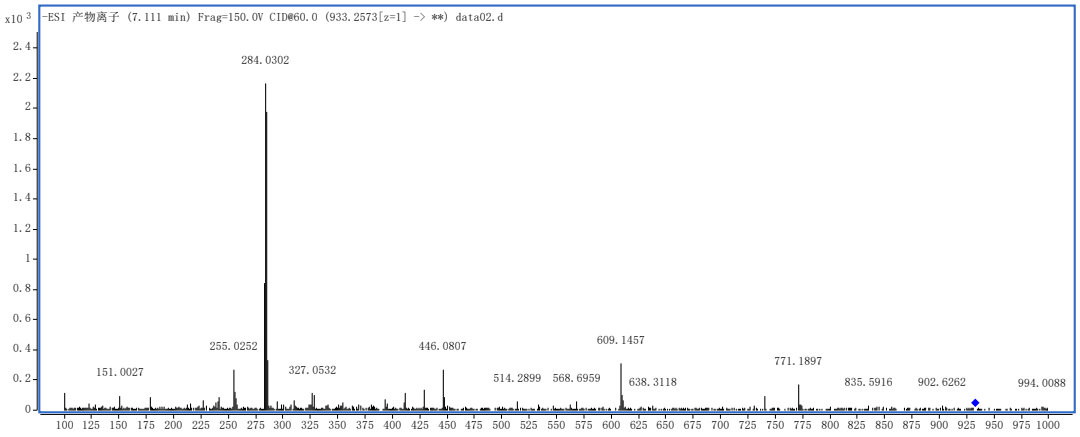

Fig S2 Mass spectrogram of Compound 1

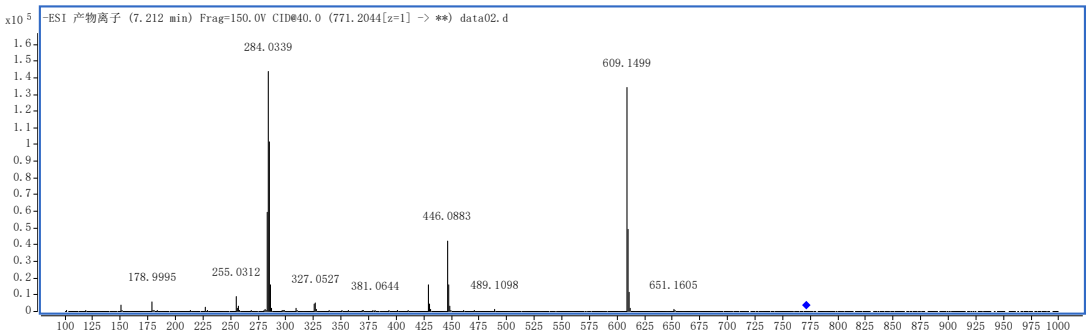

Fig S3 Mass spectrogram of Compound 2

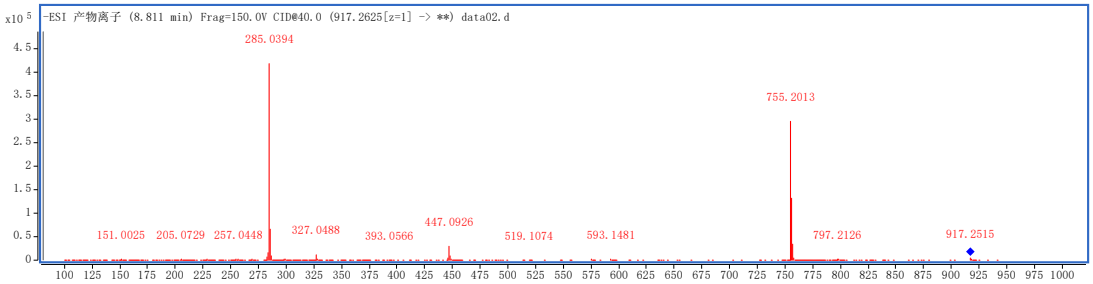

Fig S4 Mass spectrogram of Compound 3

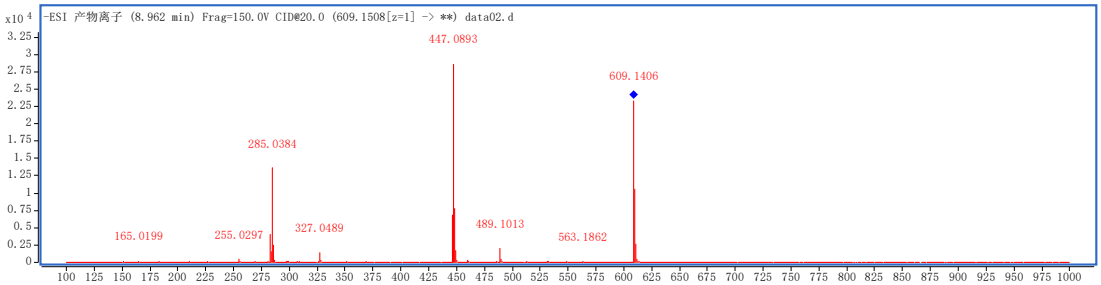

Fig S5 Mass spectrogram of Compound 4

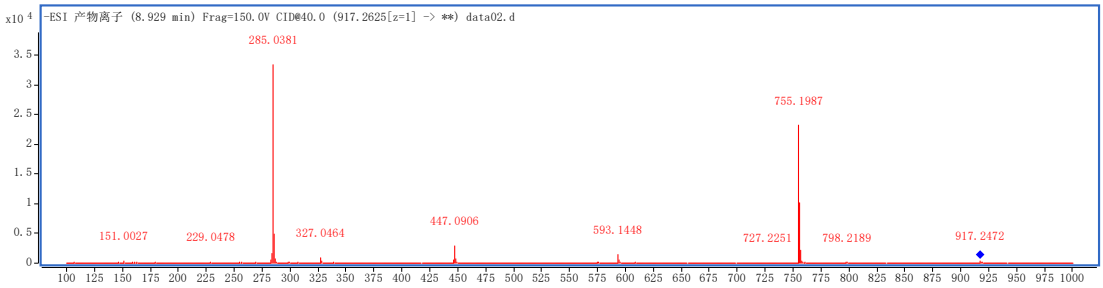

Fig S6 Mass spectrogram of Compound 5

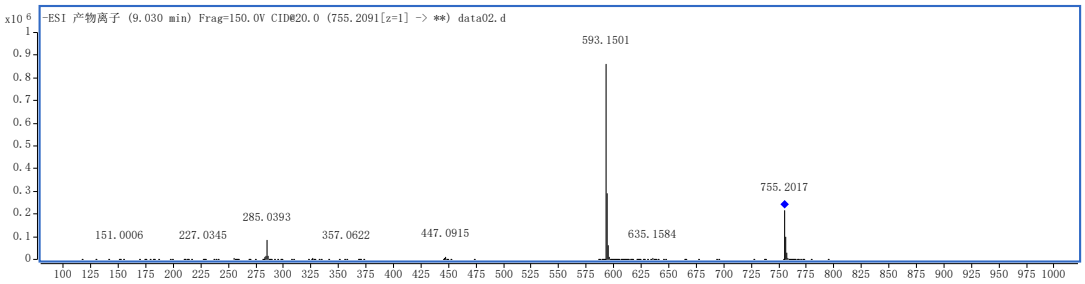

Fig S7 Mass spectrogram of Compound 6

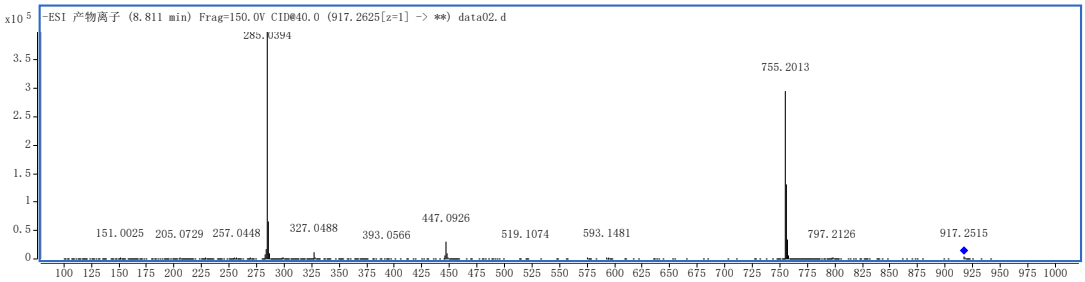

Fig S8 Mass spectrogram of Compound 7

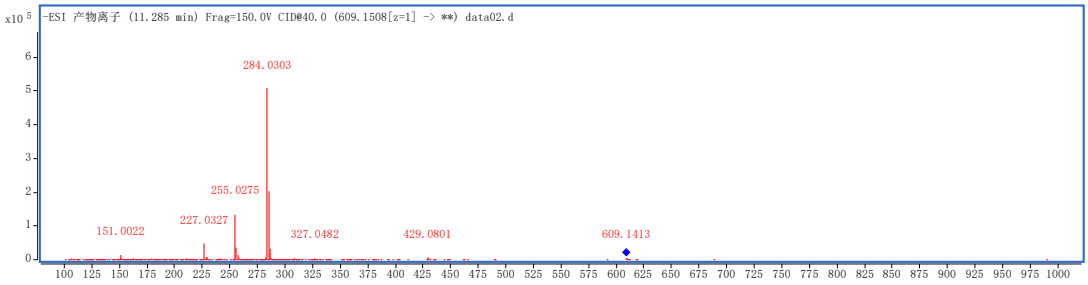

Fig S9 Mass spectrogram of Compound 8

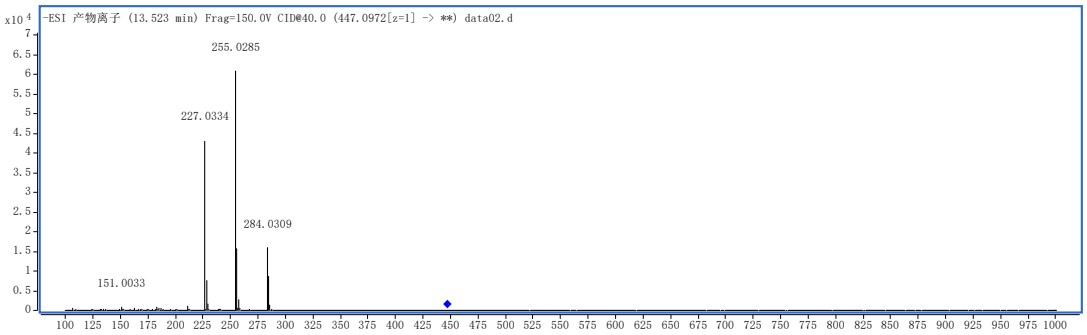

Fig S10 Mass spectrogram of Compound 9

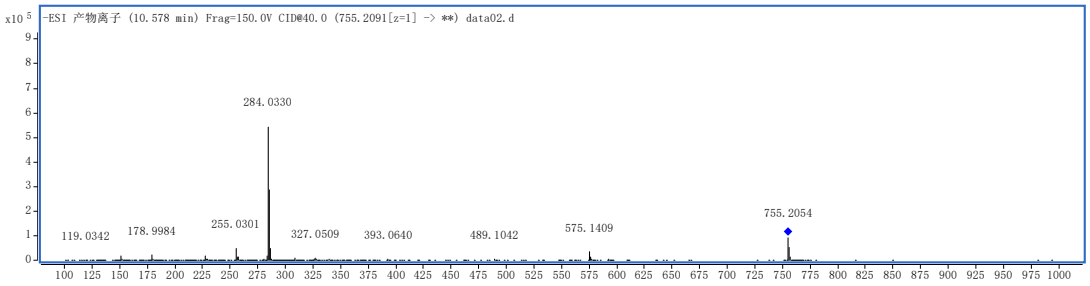

Fig S11 Mass spectrogram of Compound 10

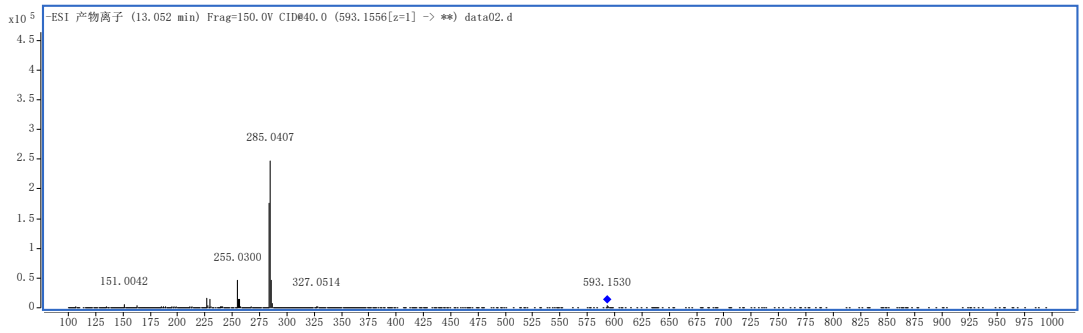

Fig S12 Mass spectrogram of Compound 11

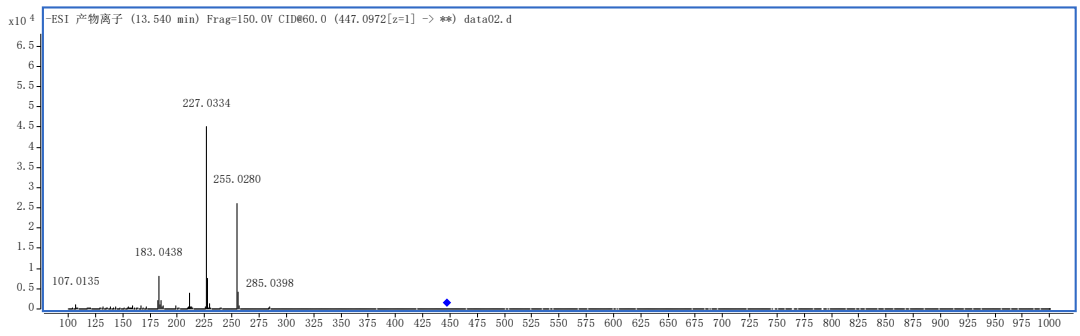

Fig S13 Mass spectrogram of Compound 12

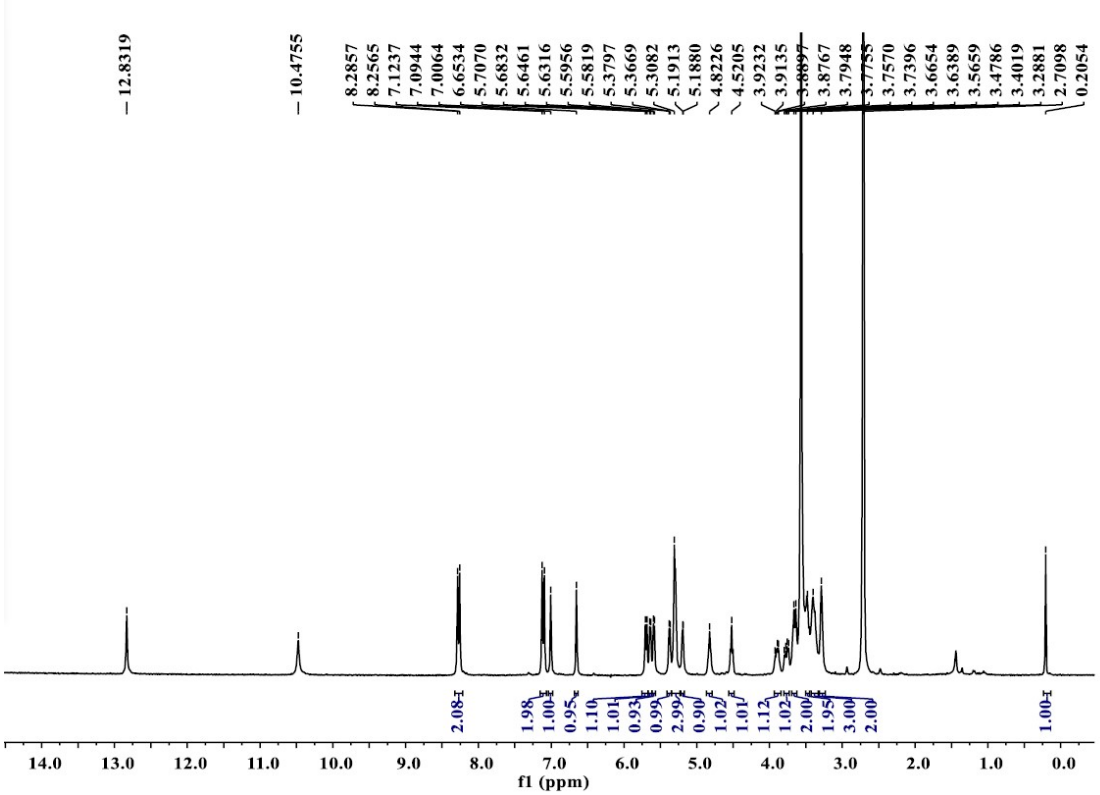

Fig S14 ^{1}H-NMR spectra of Compound 4

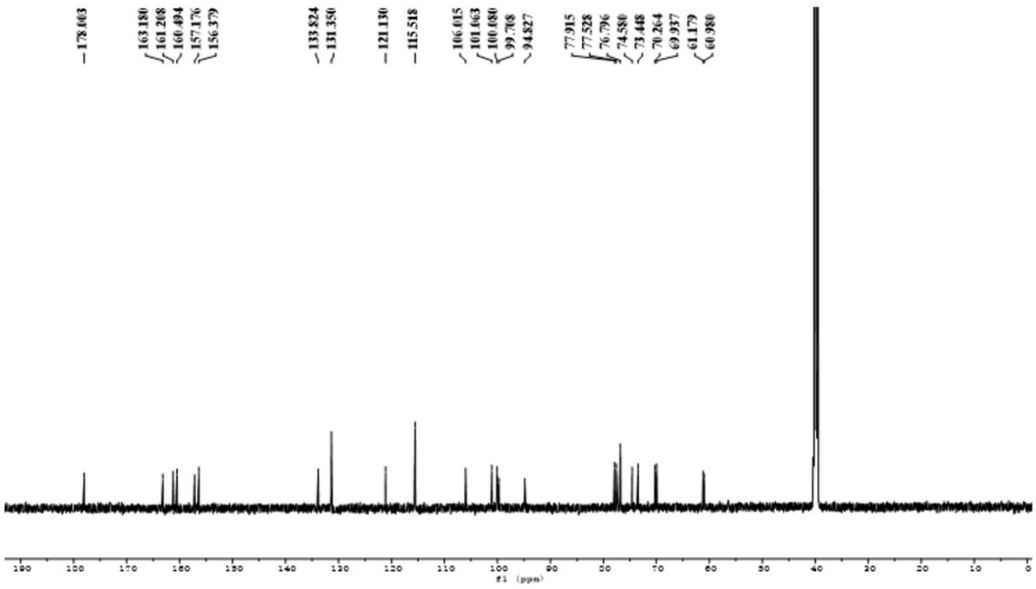

Fig S15 ^{13}C-NMR spectra of Compound 4

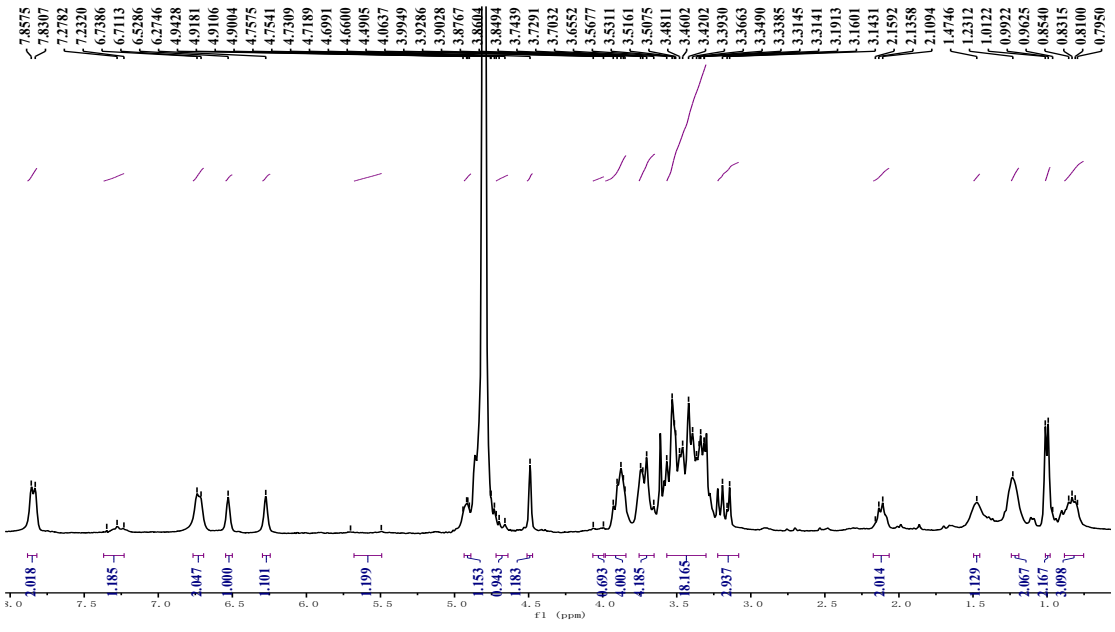

Fig S16 ^{1}H-NMR spectra of Compound 5

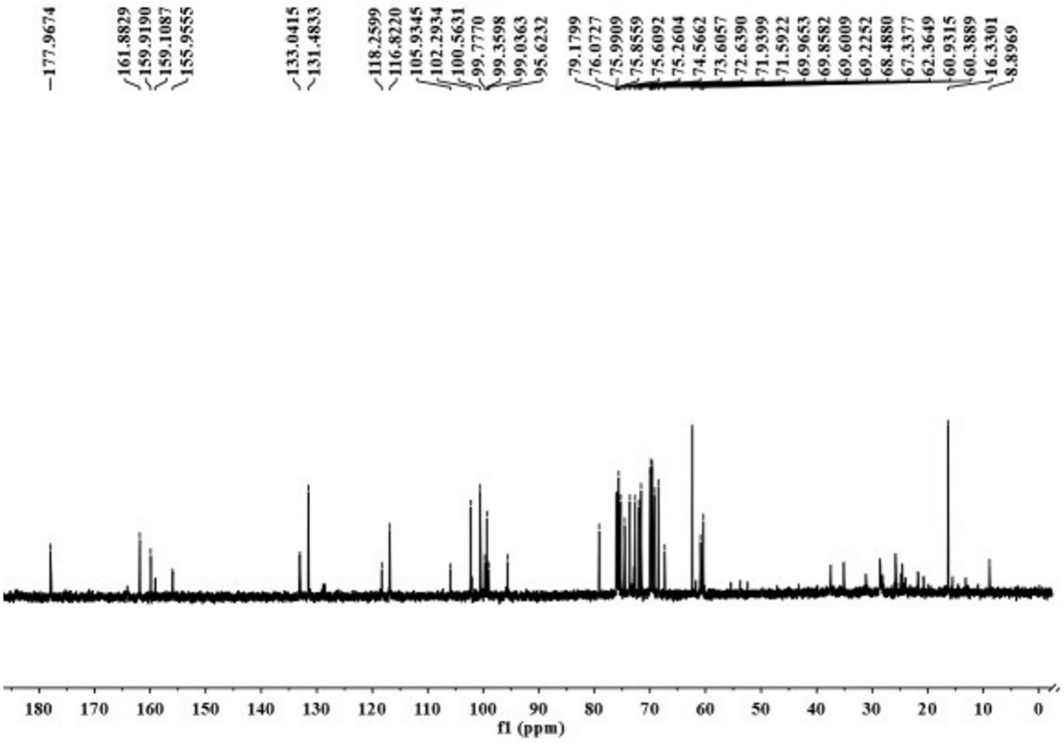

Fig S17 ^{13}C-NMR spectra of Compound 5

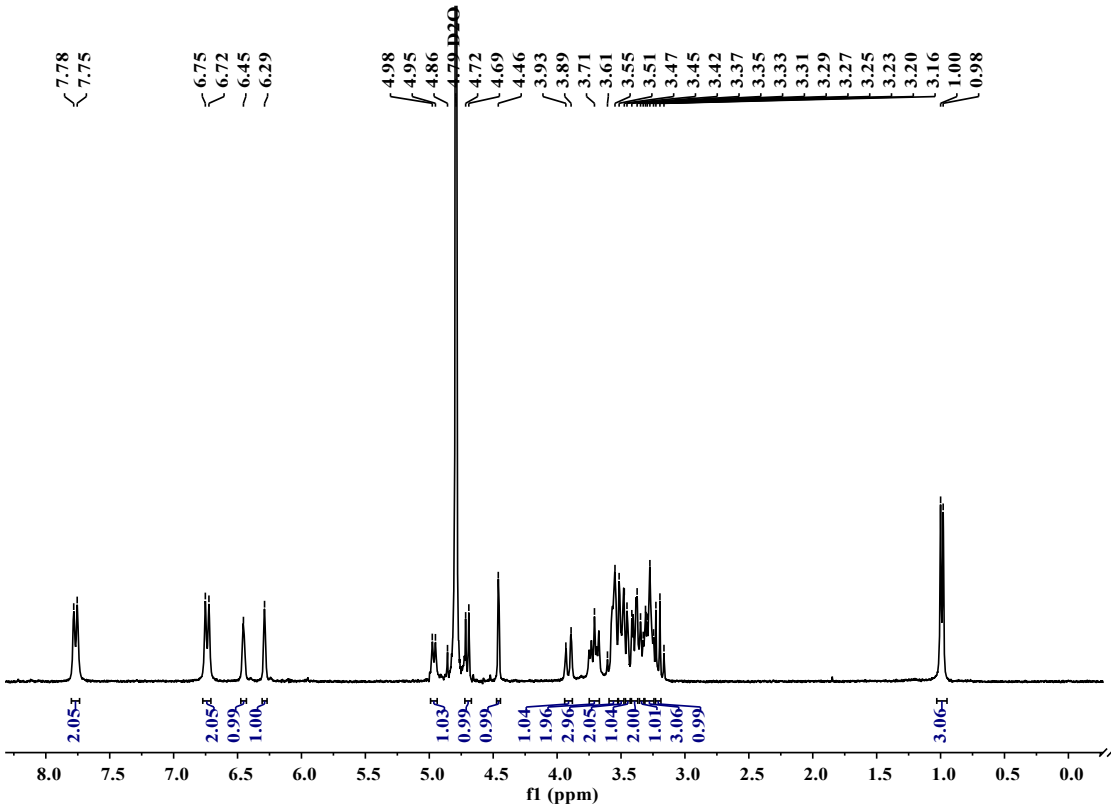

Fig S18 ^{1}H-NMR spectra of Compound 6

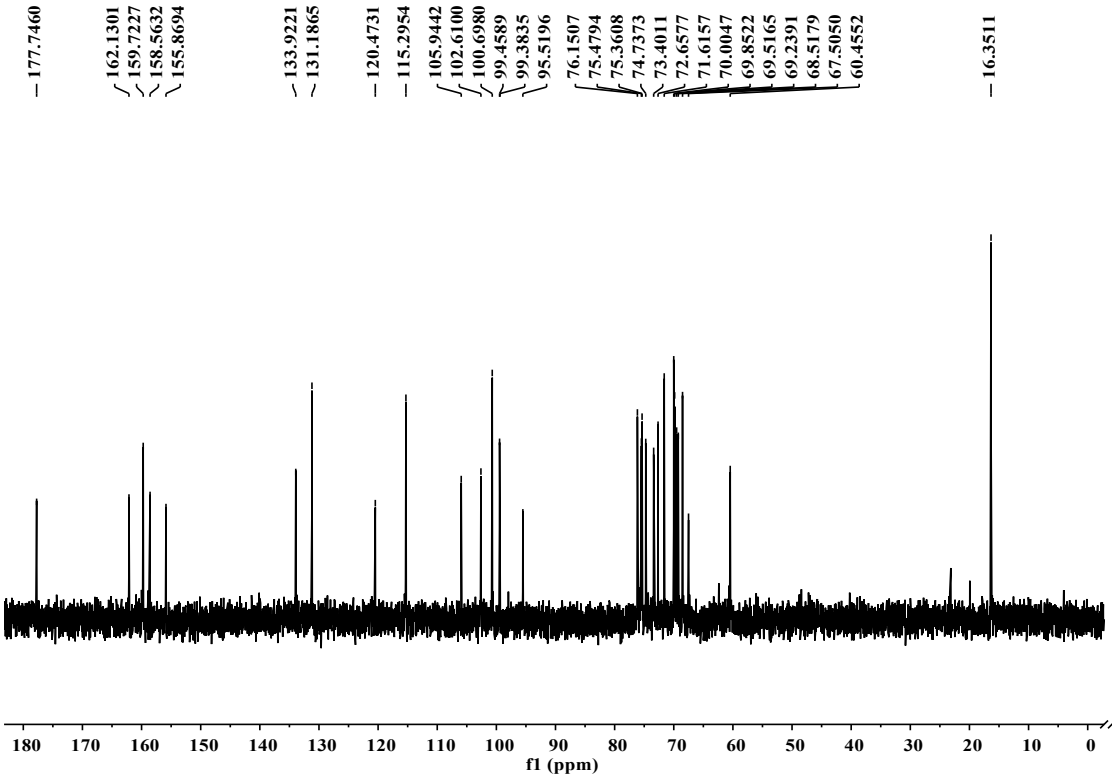

Fig S19 ^{13}C-NMR spectra of Compound 6