Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary materials to:

C-8 Mannich base derivatives of baicalein display improved glucuronidation stability: exploring the mechanism by experimentation and theoretical calculations

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The materials include the following:

Methods:

1. UFLC analysis of BA and its glucuronides.

BA glucuronidation samples were analyzed by a UFLC spectrometry system (Shimadzu, Kyoto, Japan), equipped with two LC-20AD pumps, a DGU-20A3 vacuum degasser, a SIL-20ACHT auto-sampler, a CTO-20AC column oven, an SPD-M 20A DAD, a CBM-20A communications bus module, a mass detector (2010EV) with an ESI interface, and a computer equipped with UFLC-MS solution software (version 3.41; Shimadzu). A Shim-pack VP-ODS (150.0 × 2.0 mm; 3μm; Shimadzu) analytical column with an ODS guard column (5×2.0 mm i.d, 2.2 μm; Shimadzu) was used to separate these flavonoids and their glucuronides. The column temperature was kept at 40°C. The mobile phase was acetonitrile (A) and water containing 0.2% formic acid (B) at a flow rate of 0.4 ml/min. For BA analysis, the following gradient was used: 0 to 10 min, 90% B to 55% B, 10 to 13 min, 5% B, 13 to 17min balance to 90% B.

2. Identification of baicalein, BA-a, BA-j and their mono-glucuronide by mass spectrometry

Mass detection was performed on a Shimadzu LCMS-2010EV instrument with an ESI interface in negative ion mode from *m/z*100 to 800. The detector voltage was set at -1.55 kV for negative ion detection. The curved desolvation line temperature and the block heater temperature were both set at 250°C, whereas the curved desolvation line voltage was set at 40 V. Other MS detection conditions were as follows: interface voltage, 4.5 kV and +4.0 kV for positive and negative ion detection, respectively; nebulizing gas (N_2) flow, 1.5 l/min; and drying gas (N_2) pressure, 0.06 MPa. Data processing was performed using the LC-MS solution software (version 3.41; Shimadzu).

3. Characterization of the major metabolite of BA-a and the metabolite of BA-j

The metabolites were dissolved in DMSO-d_6 (Euriso-Top, Saint-Aubin, France) for NMR analysis. The structures of the metabolites were determined by NMR spectra including ^{1}H NMR, ^{13}C NMR, heteronuclear singular quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC). All experiments were performed on a Bruker 500 MHz NMR Spectrometer. Chemical shifts were reported in parts per million (ppm, δ scale) and referenced to tetramethylsilane at 0 ppm for ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz).

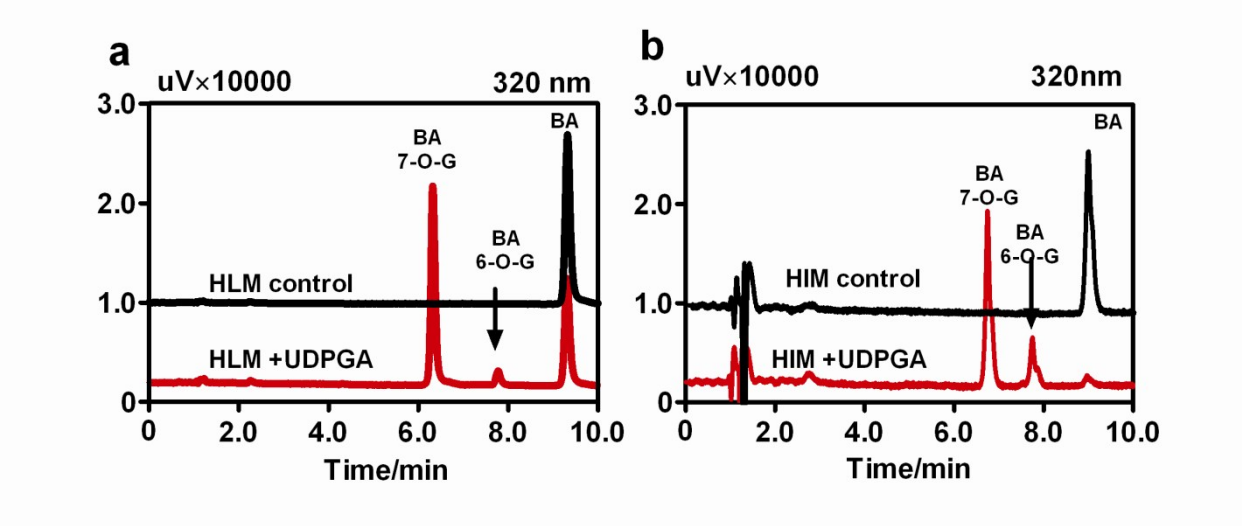

Fig. S1 Baicalein 10uM was incubated in HLM and HIM contained UDPGA co-factor for 10min. Both 7-O-glucuronide and 6-O-glucuronide can be detected in baicalein HLM and HIM incubation.

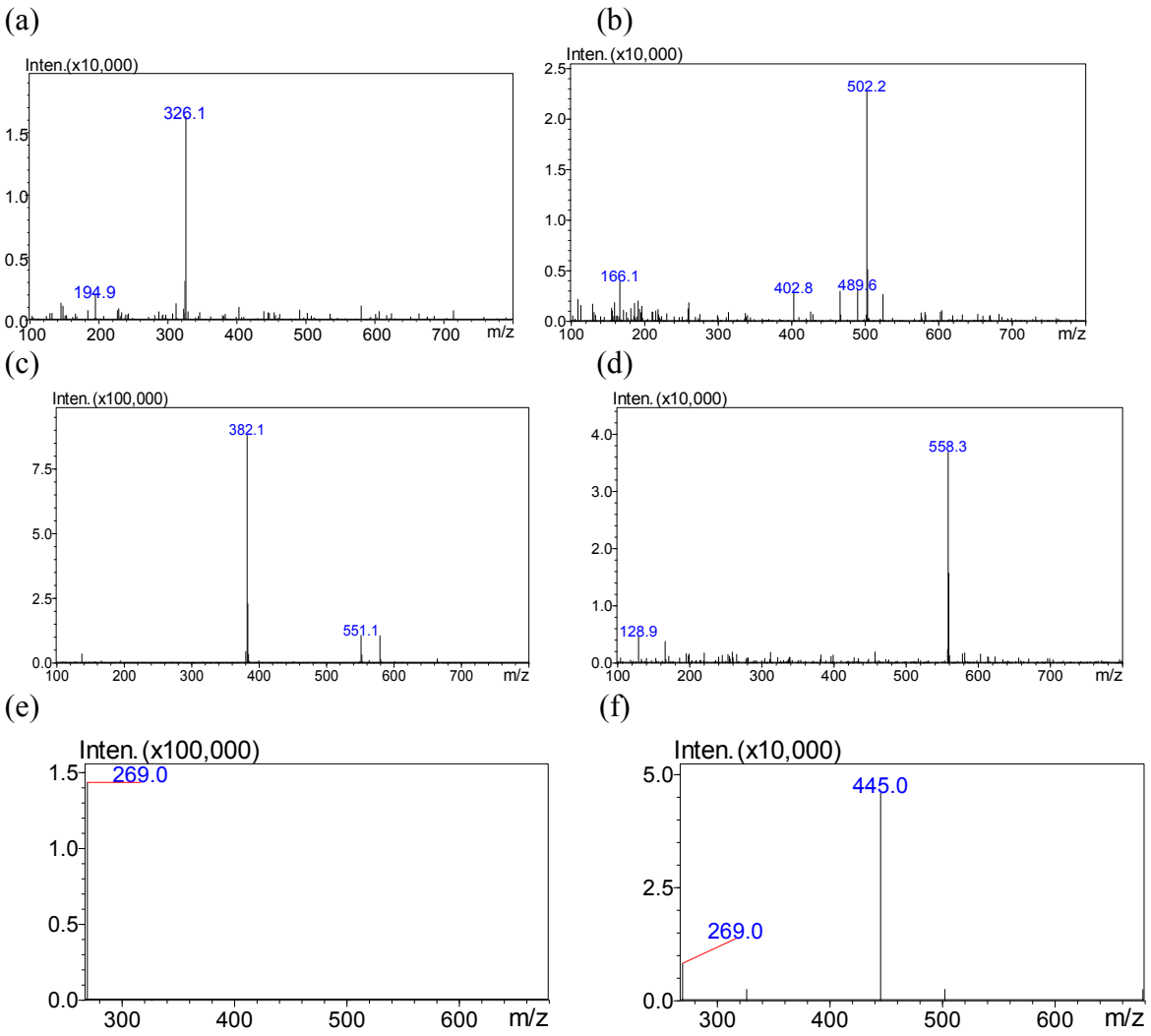

Fig. S2: Mass spectroscopy detection for baicalein, BA-a, BA-j and their corresponding mono-glucuronides. Under the negative ion mode, (a): BA-a displayed a molecular ion [M-1] at m/z326.2; (b)the mono-glucuronide of BA-a displayed a molecular ion [M-1] at m/z502.2 ;(c): BA-j displayed a molecular ion [M-1]at m/z382.1; (d) the mono-glucuronide of BA-j displayed a molecular ion[M-1] at m/z558.3; (e)baicalein displayed a molecular ion [M-1] at m/z269; and (f) the mono-glucuronide of baicalein displayed a molecular ion [M-1] at m/z445

S-table 1. Proton and carbon NMR chemical shift assignments for BA-a and its 6-O-glucuronide

Position	BA-a 6-O-G		BA-a	
	^{13}C (ppm)	^{1}H (ppm) (J in Hz)	^{13}C (ppm)	^{1}H (ppm) (J in Hz)
2	160.79		160.87	
3	104.43	6.80 (1H, s)	104.43	6.81 (1H, s)
4	180.35		180.71	
5	153.18		149.21	
6	131.33		131.38	
7	169.34		161.82	
8	96.89		101.05	
9	152.51		143.78	
10	98.84		96.66	
11	52.07	4.32 (2H, s)	53.09	4.21 (1H, s)
12	42.12	2.77 (3H, s)	42.7	2.61 (3H, s)
13	42.12	2.77 (3H, s)	42.7	2.61 (3H, s)
1’	131.21		131.29	
2’	126.10	8.07-8.08(2H, d, J=6.35)	126.00	8.04-8.06 (2H, m)
3’	129.02	7.56-7.58 (3H, m)	129.04	7.57-7.58 (3H, m)
4’	129.39	7.56-7.58 (3H, m)	129.91	7.57-7.58 (3H, m)
5’	129.02	7.56-7.58 (3H, m)	129.04	7.57-7.59 (3H, m)
6’	126.10	8.07-8.08 (2H, d, J=6.35)	126.00	8.04-8.06 (2H, m)
G1	106.64	4.52-4.53 (1H, d, J=7.50)		
G2	73.21	3.27 (1H, t, J=8.05)		
G3	73.85	3.19 (1H, t, J=8.75)		
G4	71.82	3.41(1H, t, J=8.70)		
G5	76.35	3.57-3.59 (1H, d, J=10.55)		
COOH	171.00	13.07 (1H, s)		

S-table 2. Proton and carbon NMR chemical shift assignments for BA-j and its 6-O-glucuronide

Position	BA-j 6-O-G		BA-j	
	^{13}C (ppm)	^{1}H (ppm) (J in Hz)	^{13}C (ppm)	^{1}H (ppm) (J in Hz)
2	161.07		163.23	
3	104.48	6.83 (1H, s)	104.05	7.09 (1H,s)
4	180.53		182.22	
5	152.29		148.3	
6	131.20		128.81	
7	168.01		153.59	
8	96.84		95.97	
9	153.00		149.26	
10	99.42		104.92	
11	50.71	4.32 (2H,t, J=14.50,13.75)	59.39	4.50-4.52 (2H,d, J=10.0)
12	31.05	1.63-1.86 (2H, brs)	31.3	1.85-1.93 (2H, brs)
13	39.50	2.73-3.05(2H, m)	50.75	3.17-3.19 (2H, brs)
14	48.57	3.27 (1H, t, J=8.3,7.8)	63.64	3.88 (1H, brs)
15	39.50	2.73-3.05 (2H, m)	48.58	3.44-3.46 (2H brs)
16	31.05	1.63-1.86 (2H, brs)	29.36	1.57-1.59 (2H, brs)
1′	130.77		130.81	
2′	126.11	8.07 (2H, m)	126.68	8.07-8.17 (2H, m)
3′	129.04	7.57 (2H, m)	129.25	7.60-7.64 (3H, m)
4′	131.39	7.60 (1H, m)	132.08	7.60-7.64 (3H, m)
5′	129.04	7.57 (2H, m)	129.25	7.60-7.64 (3H, m)
6′	126.11	8.07 (2H, m)	126.68	8.07-8.17 (2H, m)
G1	106.23	4.56(1H, d, J=7.55)		
G2	73.19	3.33 (1H, m)		
G3	76.24	3.20 (1H, m)		
G4	71.31	3.40 (1H, m)		
G5	76.11	3.58 (1H, m)		
COOH	170.00			

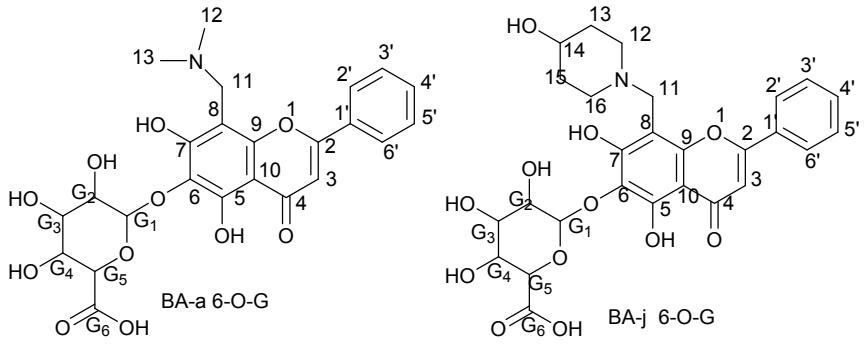

Fig. S3 Molecular structures of BA-a 6-O-glucuronide and BA-j 6-O-glucuronide.

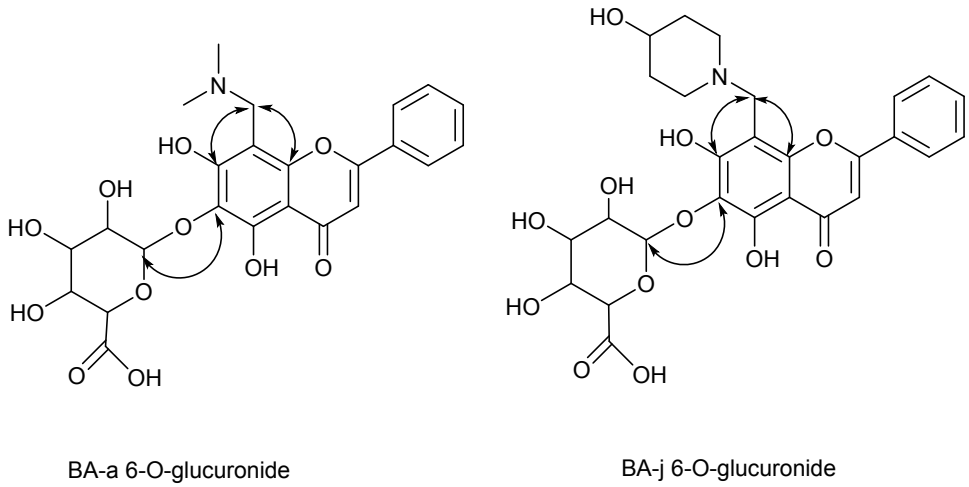

Fig. S4 The key HMBC correlations in BA-a 6-O-glucuronide and BA-j 6-O-glucuronide.

Note: A connectivity between G1-CH (4.52-4.53 ppm) and C-6 (131.33 ppm) in HMBC spectra of BA-a metabolite was detected which could assigned the metabolite as 6-O-glucuronide of BA-a. In addition, the chemical shifts of C-6 (131.33 ppm) and C-1' (131.21 ppm) of BA-a metabolite were almost overlapped. The correlation spot (6.8, 131.21 ppm) should be the connectivity between 3-CH and C-1'. Connectivity between 11-CH_2 (4.32 ppm) and C-12, 13 (42.12 ppm); 11-CH_2 (4.32 ppm) and C-10 (98.84 ppm) were detected which confirmed that the dimethylamino-methyl substituent were at C-8 position. In BA-j-G HMBC spectra, connectivity between G1-CH (4.56 ppm) and C-6 (131.20 ppm) was found, therefore the glucuronide of BA-j in HLM was identified as 6-O-glucuronide as well. The correlation between11-CH_2 (4.32 ppm) and C-7 (168.01 ppm), C-9 (153 ppm) could all be detected, which strongly indicated the 4-hydroxypiperidin-1-yl)-methyl substituent was at C-8 position of BA-j metabolite.

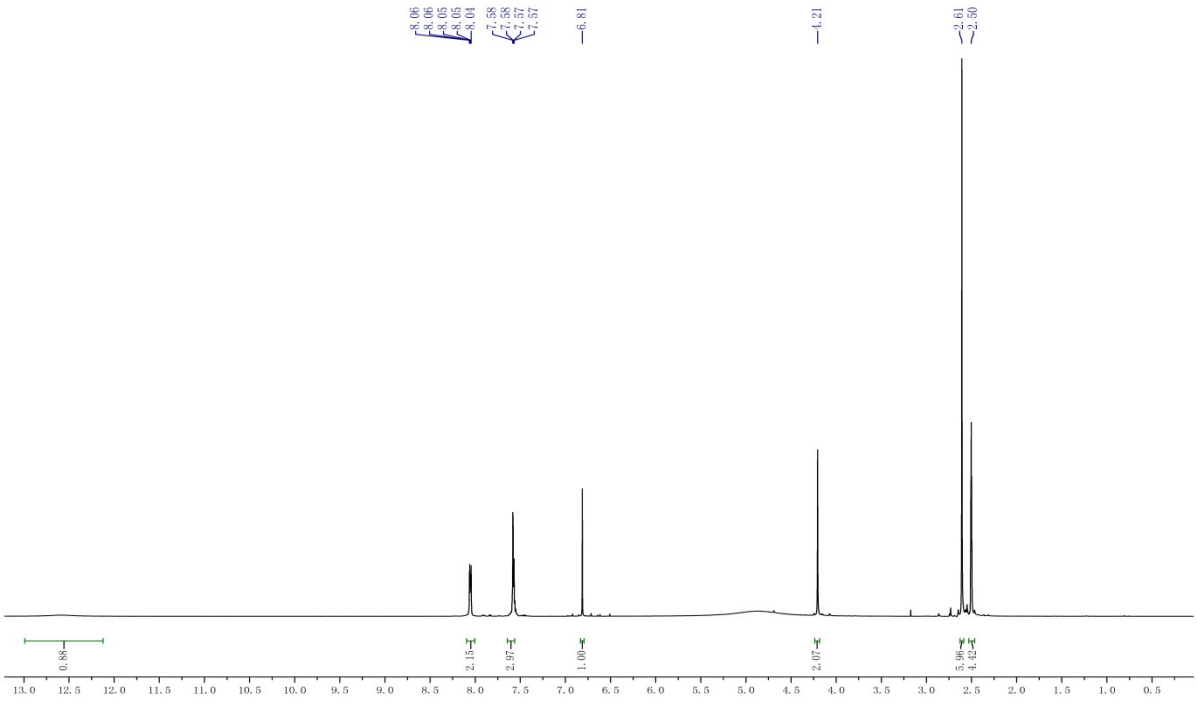

Fig. S5 ^{1}H NMR spectrumfor BA-a

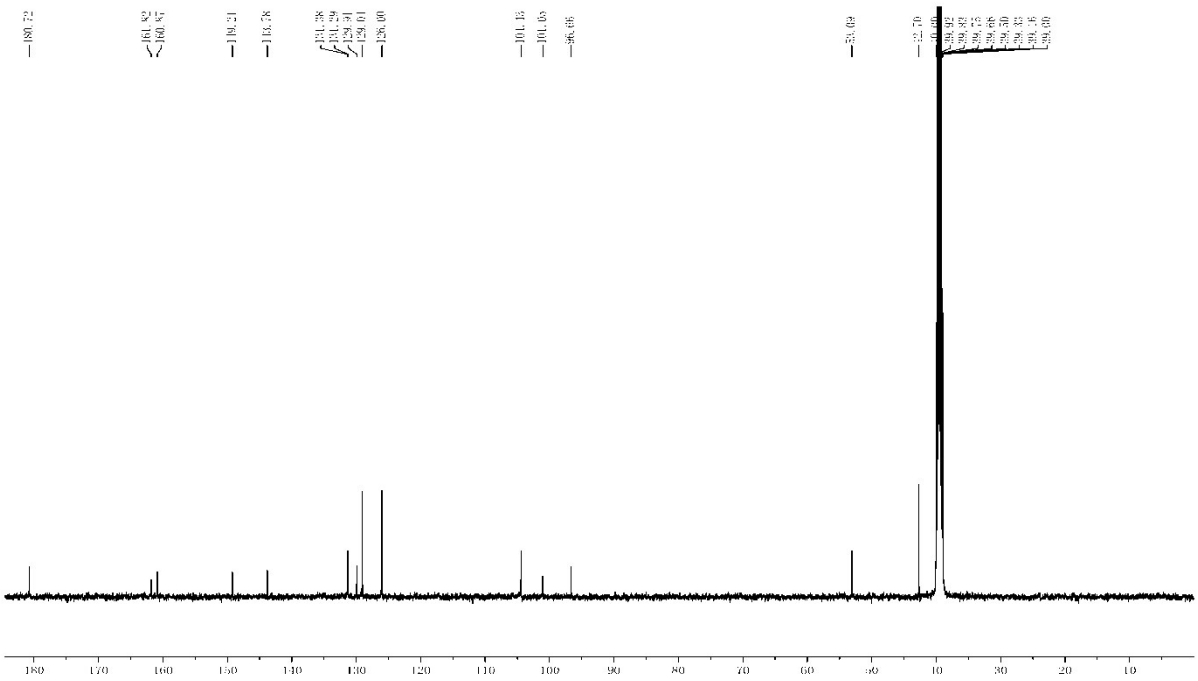

Fig. S6 ^{13}C NMR spectrum for BA-a

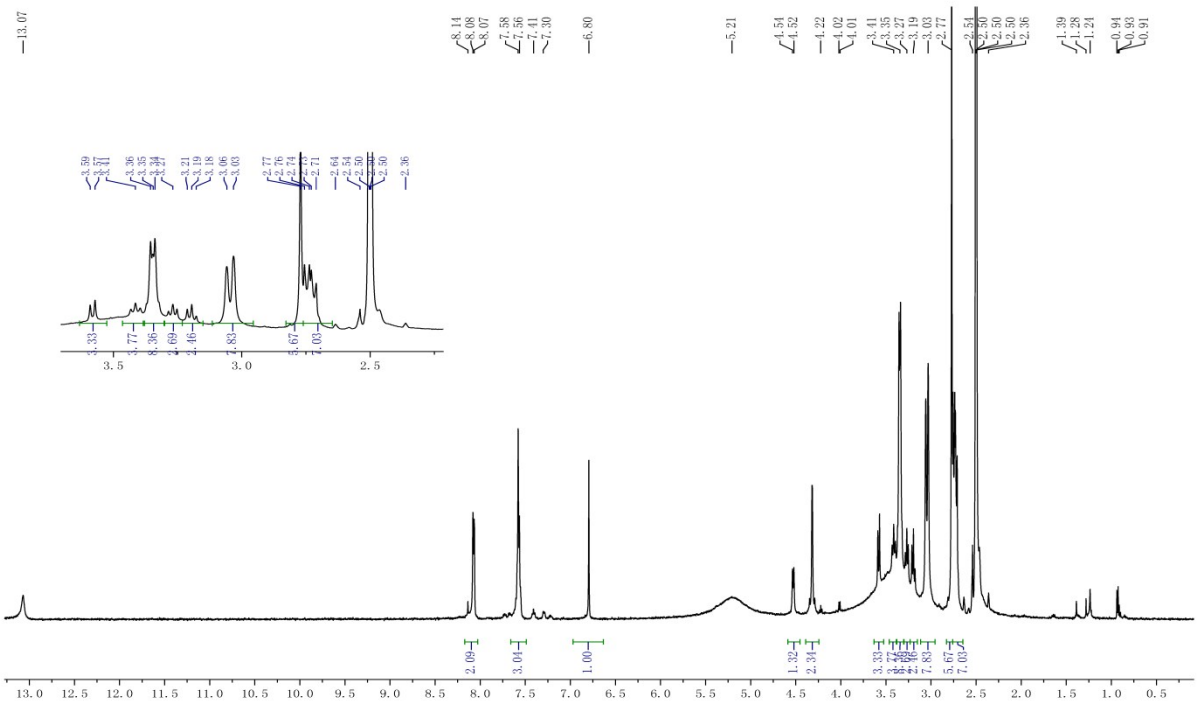

Fig. S7 ^{1}H NMR spectrum for BA-a 6-O-glucuronide

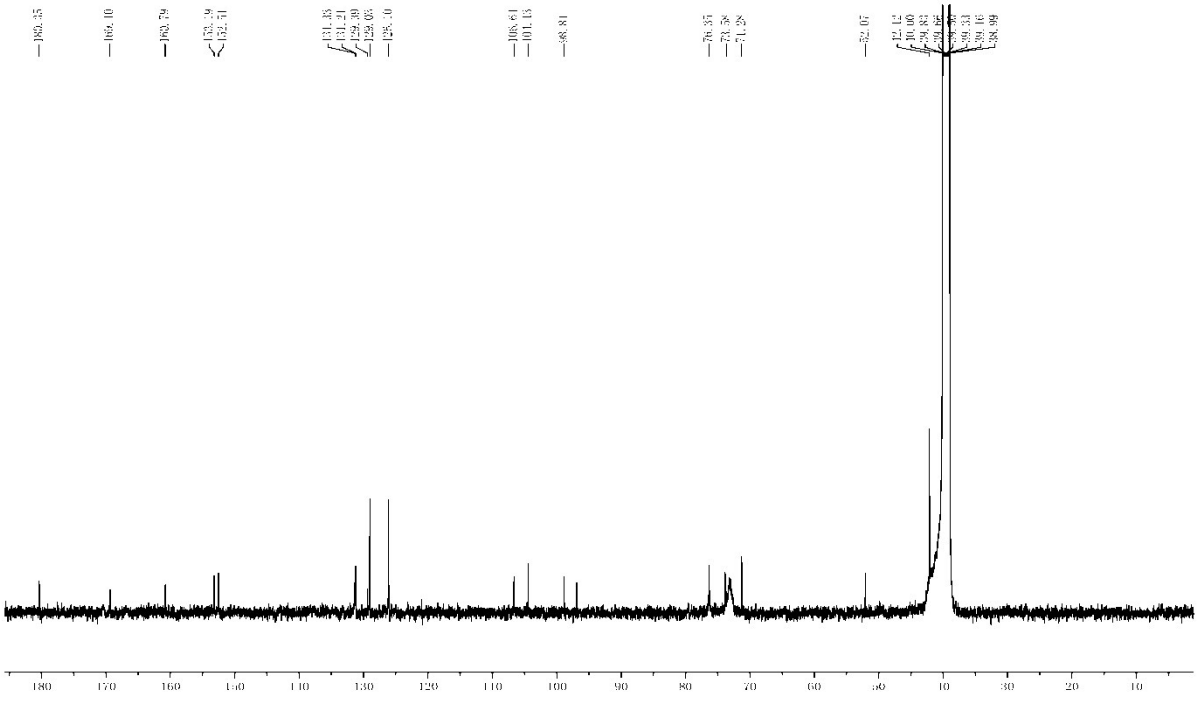

Fig. S8 ^{13}C NMR spectrum for BA-a 6-O-glucuronide

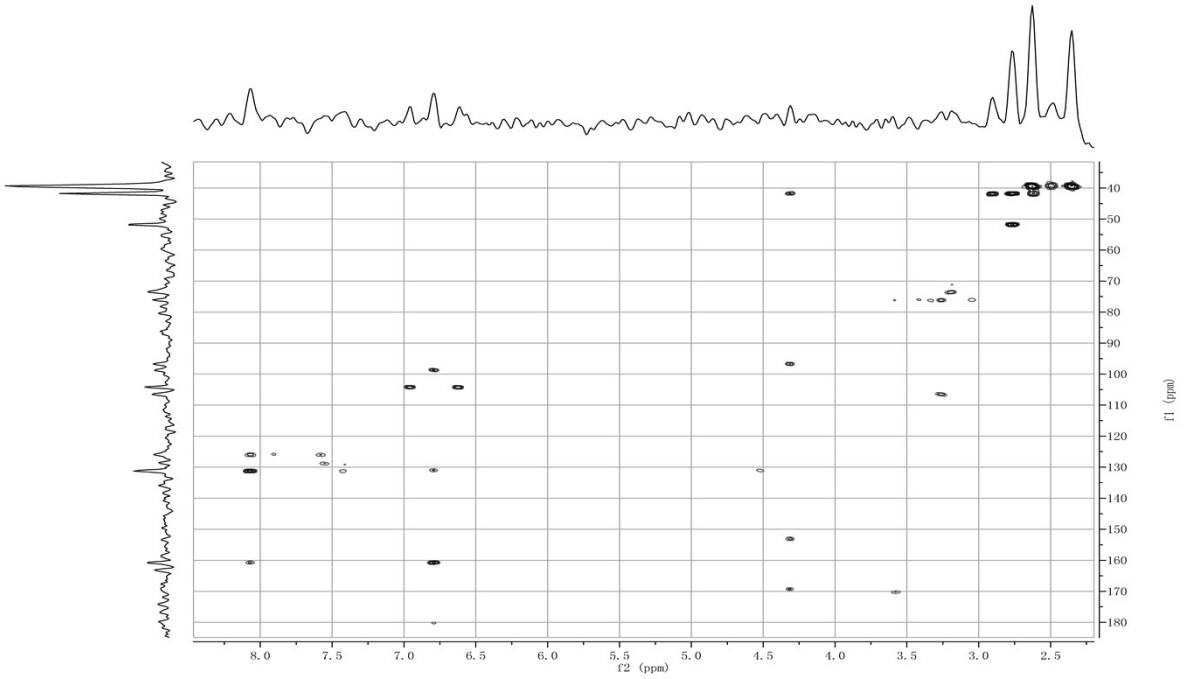

Fig. S9 HMBC spectrum of BA-a 6-O-glucuronide

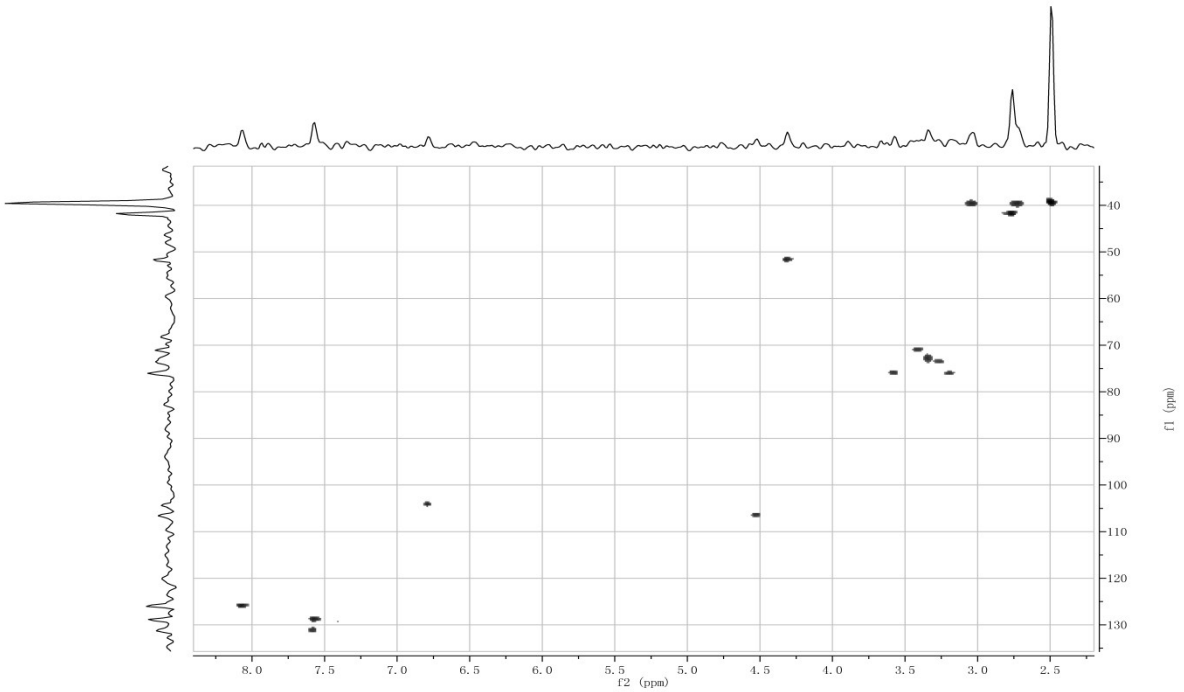

Fig. S10 HSQC spectrum of BA-a 6-O-glucuronide

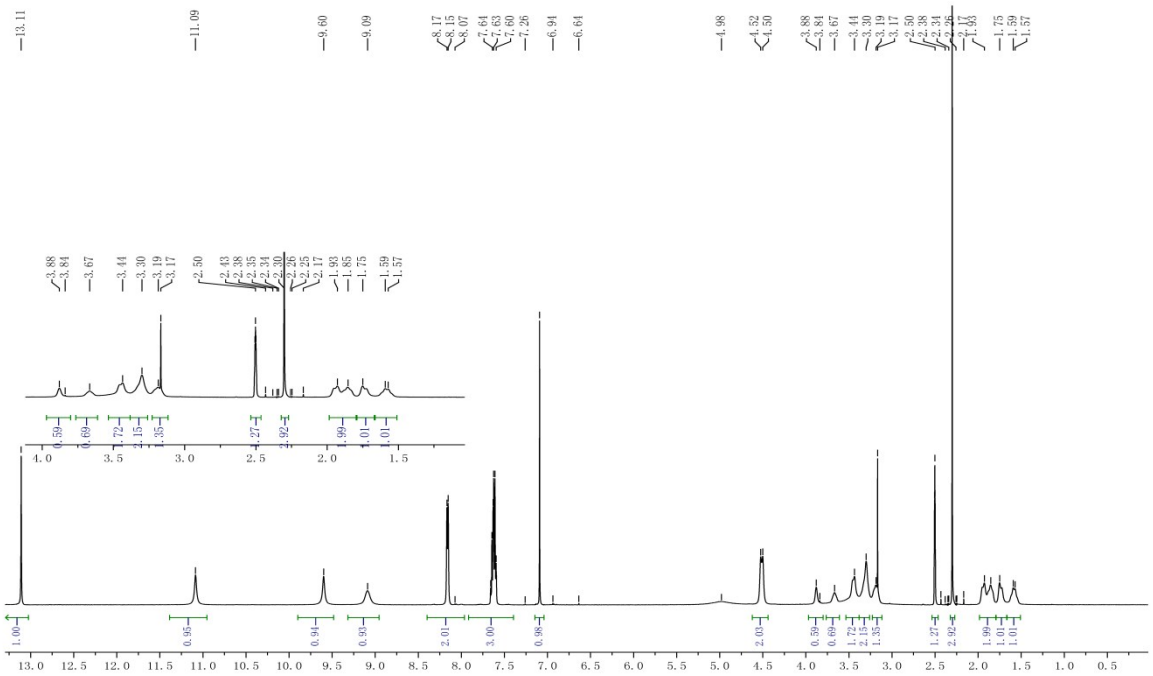

Fig. S11 ^{1}H NMR spectrum of BA-j

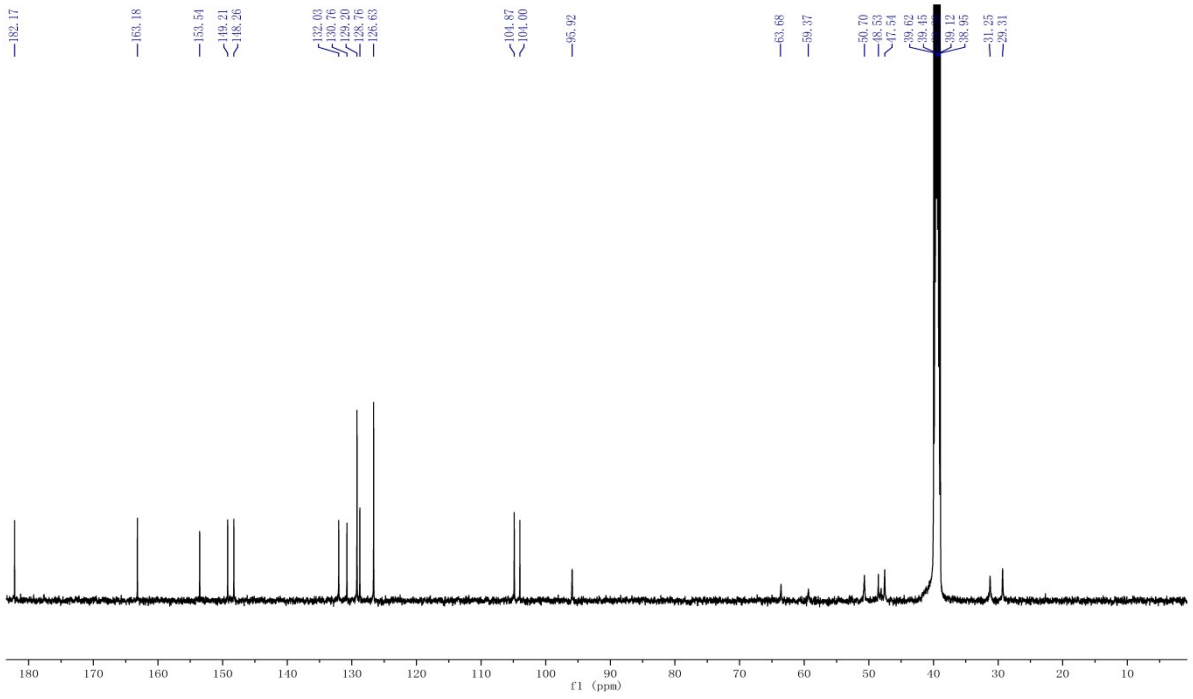

Fig. S12 ^{13}C NMR spectrum of BA-j

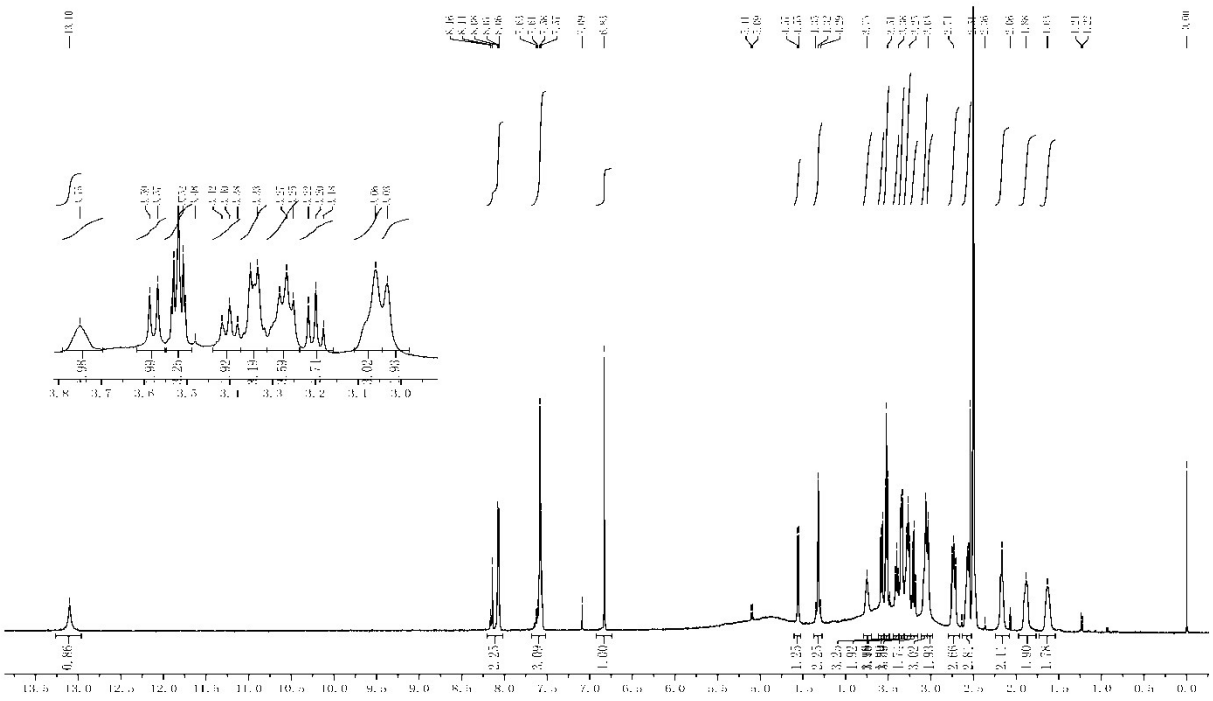

Fig. S13 ^{1}H NMR spectrum of BA-j 6-O-glucuronide

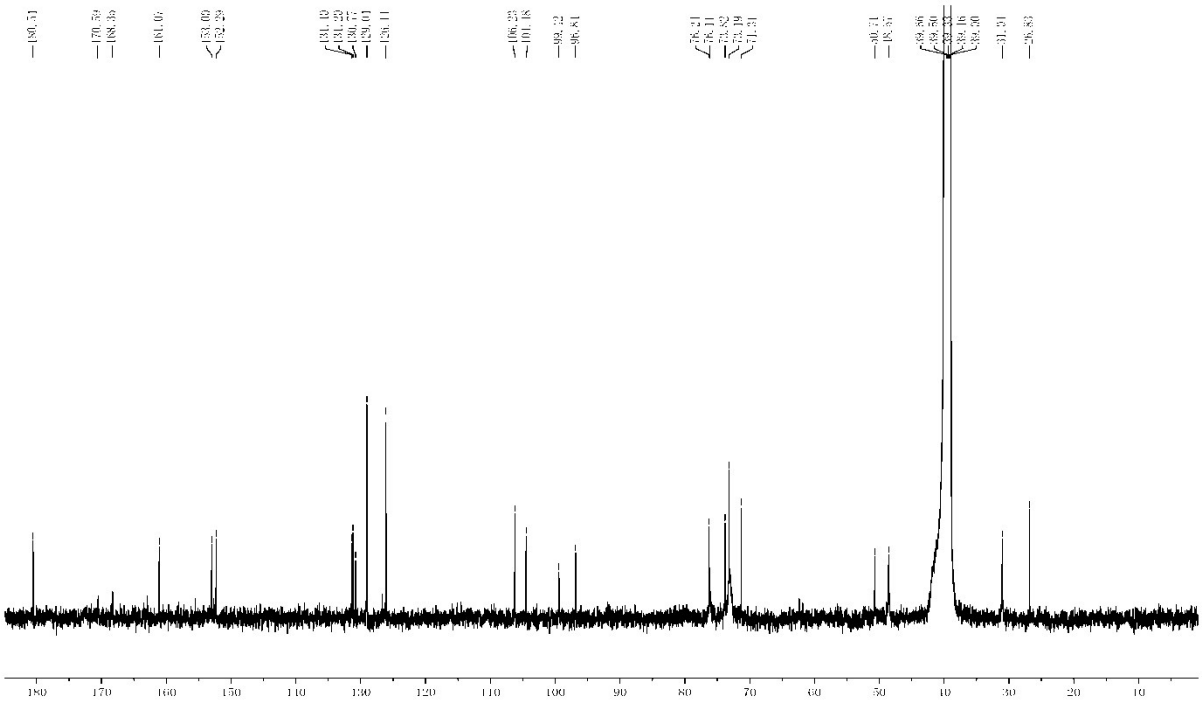

Fig. S14 ^{13}C NMR spectrum of BA-j 6-O-glucuronide.

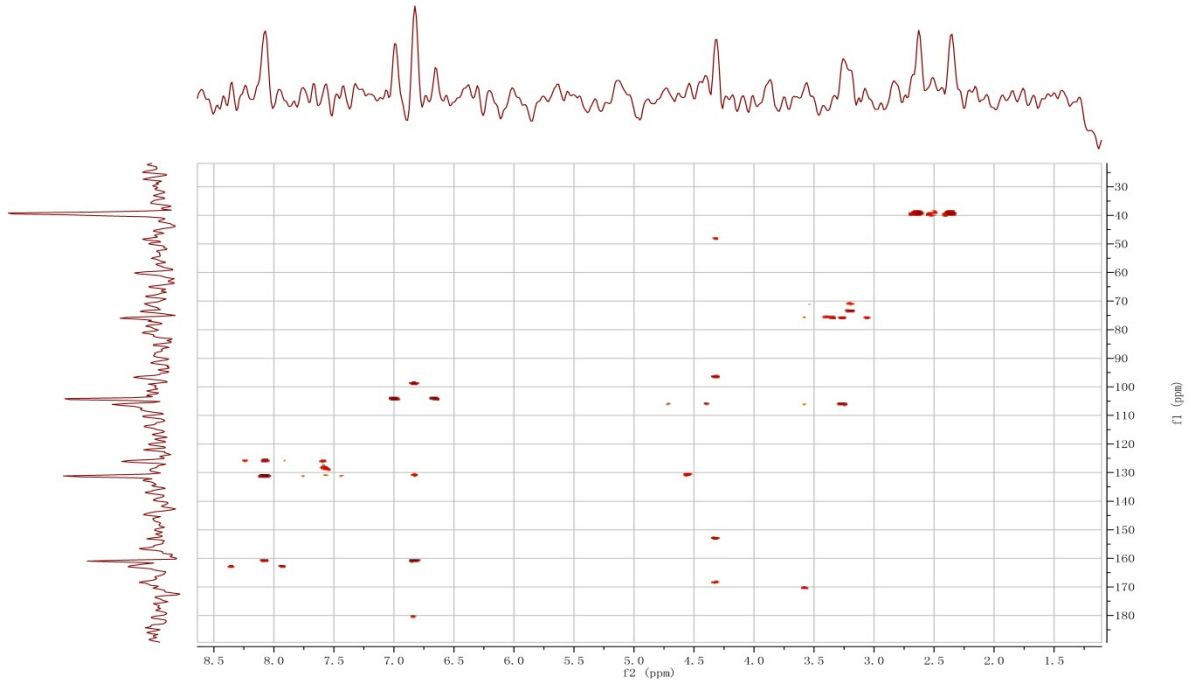

Fig. S15 HMQC spectrum of BA-j 6-O-glucuronide

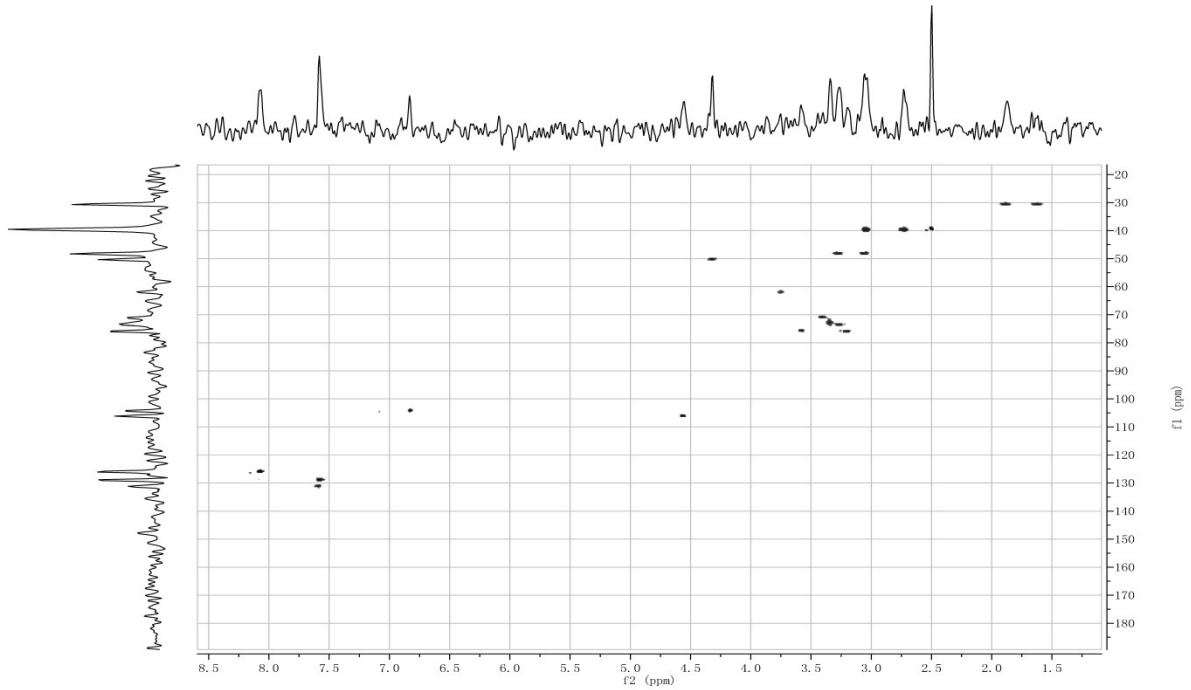

Fig. S16 HSBC spectrum of BA-j 6-O-glucuronide