SUPPLEMENTARY MATERIAL

Novel dammarane-type saponins from *Gynostemma pentaphyllum* and their neuroprotective effect

Shao-Fang Xing[a,b], Man Lin[a,b], Yu-Rong Wang[a,b], Tuo Chang[a,b], Wei-Ye Cui[a,b], Xiang-Lan Piao [a,b,*]

Abstract

Three novel dammarane-type saponins, 2*α*,3*β*,12*β*,20(*S*),24(*S*)-pentahydroxydammar-25-ene-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-20-*O*-*β*-D-glucopyranoside (**1**, namely gypenoside J1), 2*α*,3*β*,12*β*,20(*S*),25-pentahydroxydammar-23-ene-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-20-*O*-*β*-D-glucopyranoside (**2**, namely gypenoside J2) and 2*α*,3*β*,12*β*,20(S)-tetrahydroxydammar-25-en-24-one-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-20-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**3**, namely gypenoside J3) along with one known gypenoside (gypenoside LVII) were isolated from the aerial parts of *G. pentaphyllum* using various chromatographic methods. Their structures were elucidated on the basis of IR, 1D- (^{1}H and ^{13}C), 2D-NMR spectroscopy (HSQC, HMBC and COSY), and mass spectrometry (ESI-MS/MS). Their activity was tested using CCK-8 assay. These four compounds showed little anti-cancer activity with IC_{50} values more than 100 μM against four types of human cancer lines. The effects of them against H_2O_2-induced oxidative stress in human neuroblastoma SH-SY5Y cells were evaluated and they all showed potential neuroprotective effects with 3.64%-18.16% higher cell viability than the H_2O_2-induced model group.

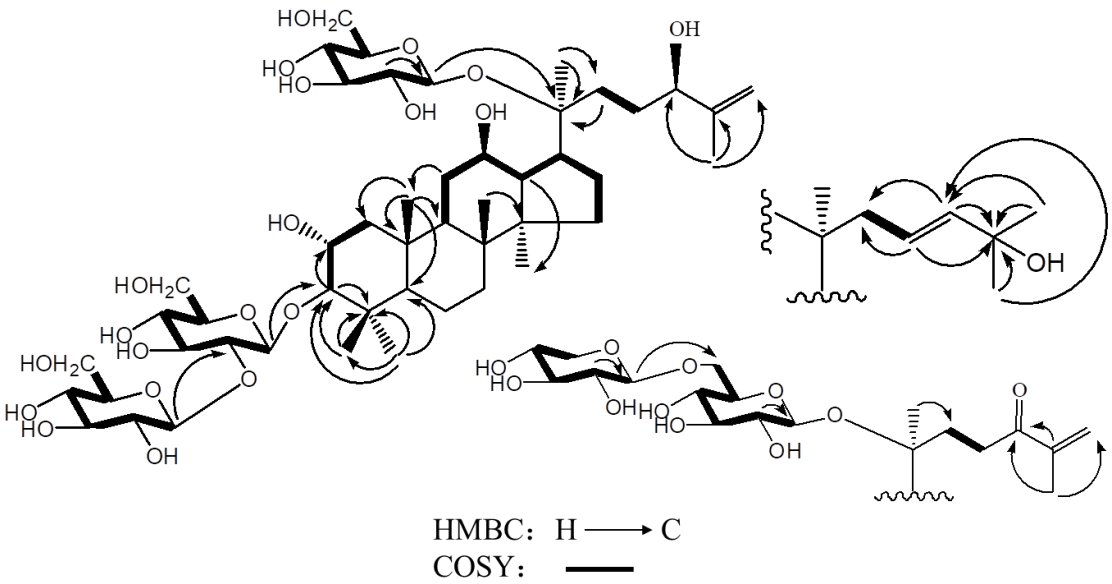

Fig. S**1** Key correlations in HMBC and COSY.

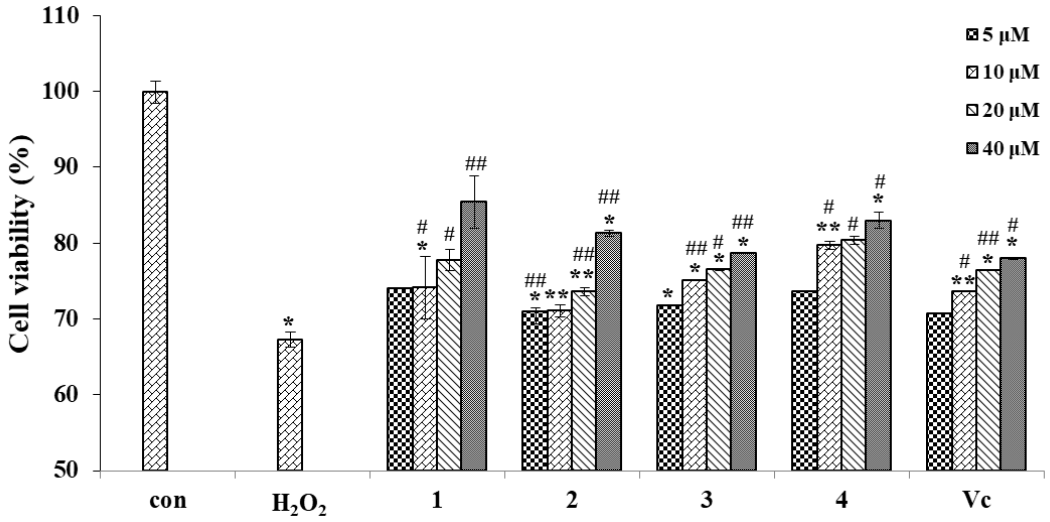

Fig. S**2** Neuroprotective effects of dammarane-type saponins against H_2O_2-induced injury of SH-SY5Y cells. Data were shown as mean ± SD of three separate experiments and expressed as percentage (%) of control. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with H_2O_2-induced model group.

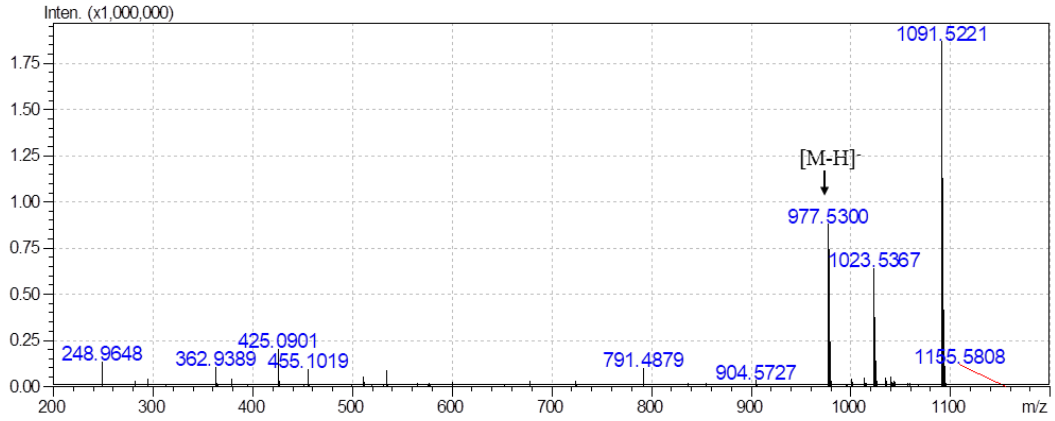

Fig. S**3** HRESIMS of compound **1**

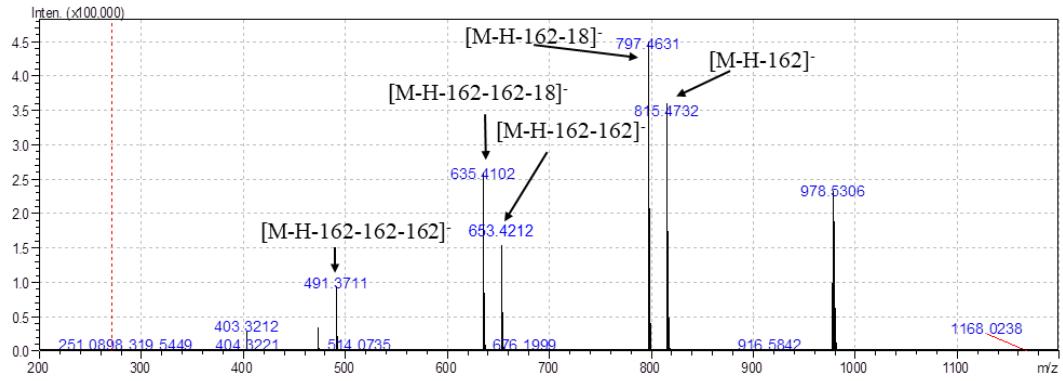

Fig. S**4** MS/MS data of compound **1**

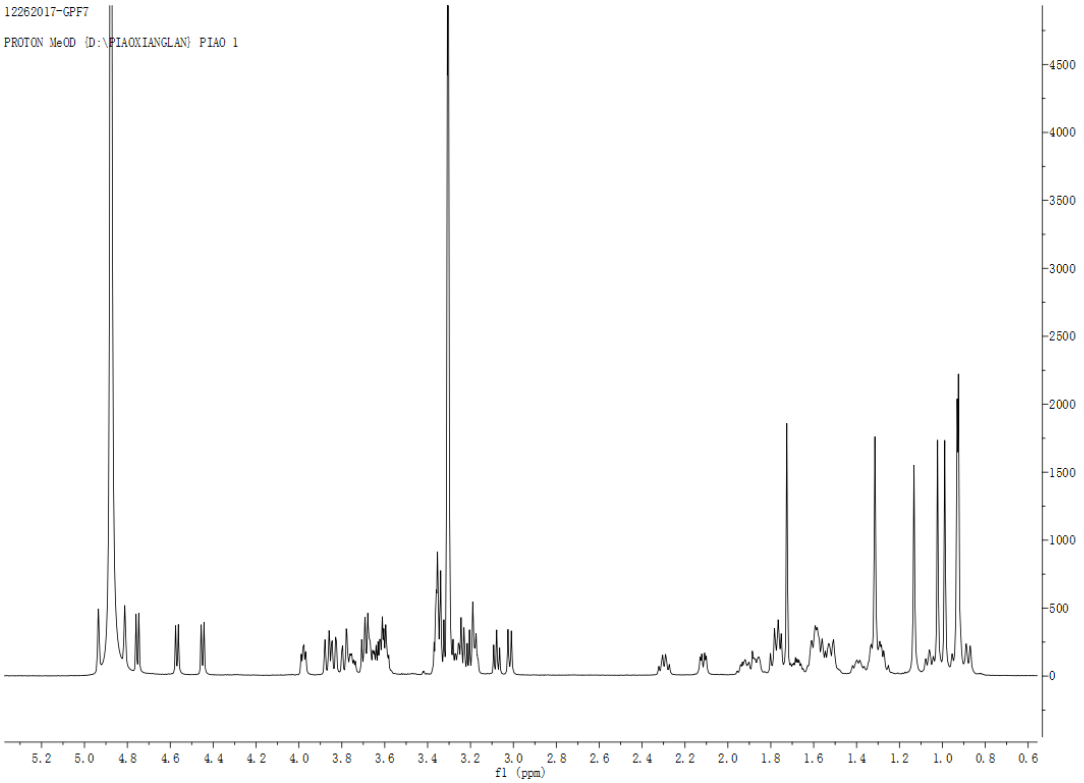

Fig. S**5** ^{1}H NMR spectrum of compound **1** (CD_3OD, 600 MHz)

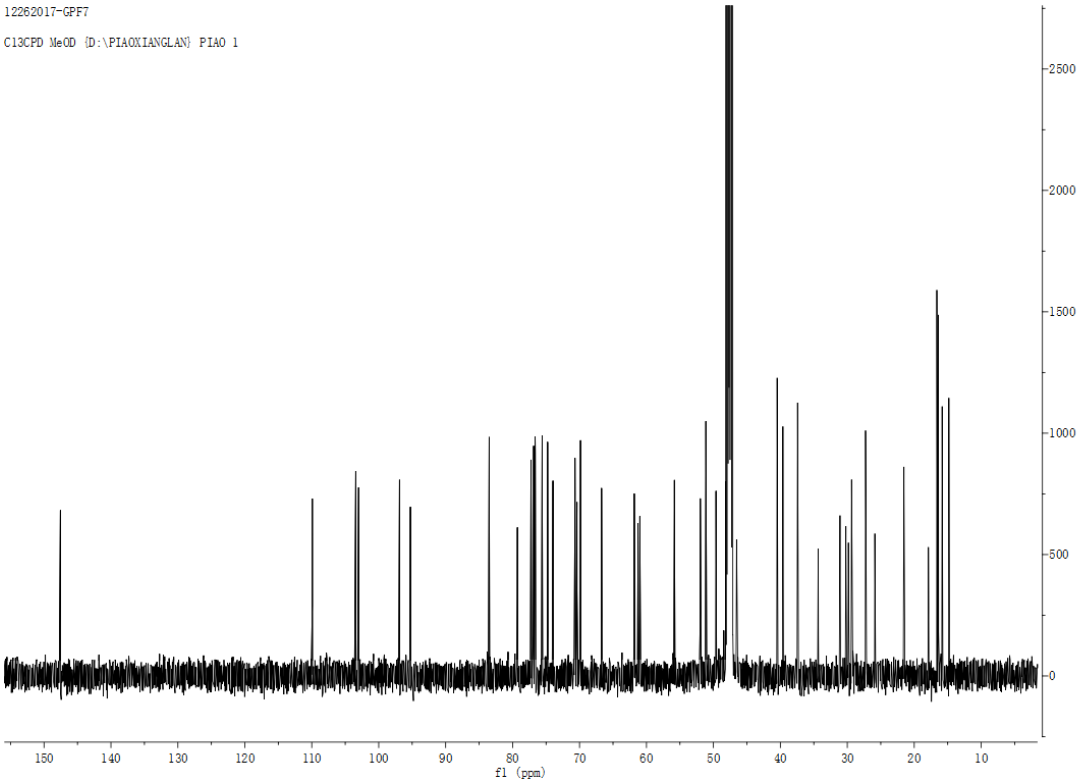

Fig. S**6** ^{13}C NMR of compound **1** (CD_3OD, 150 MHz)

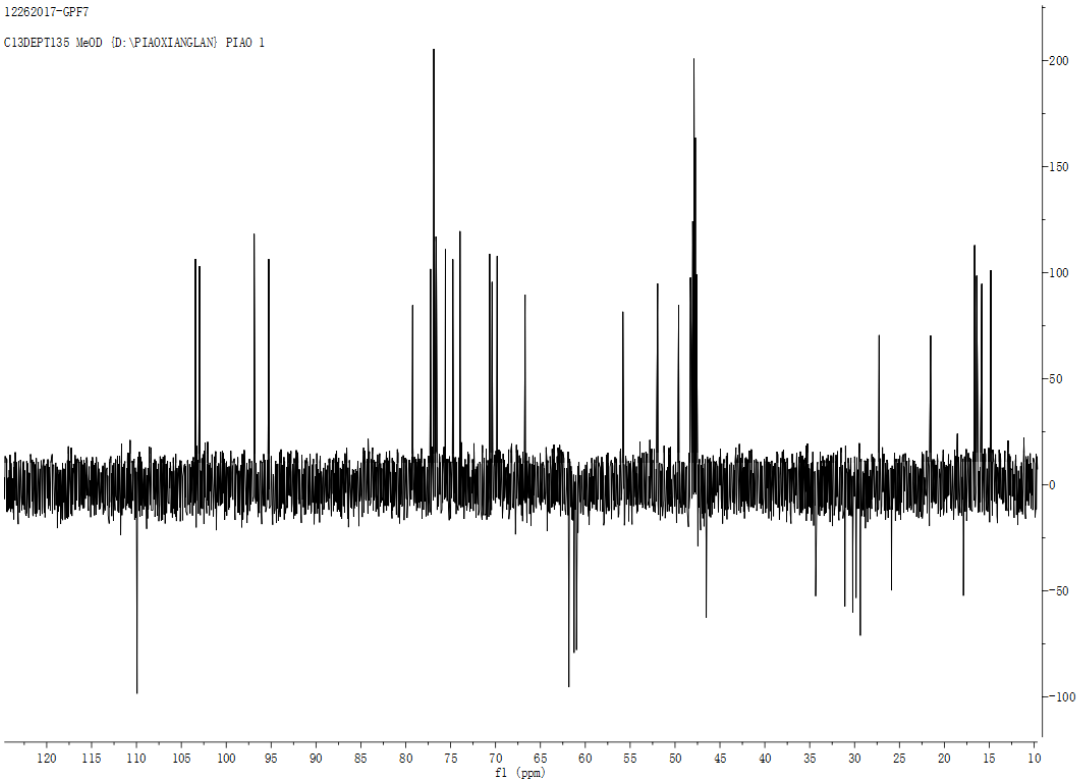

Fig. S**7** DEPT 135 of compound **1** (CD_3OD, 150 MHz)

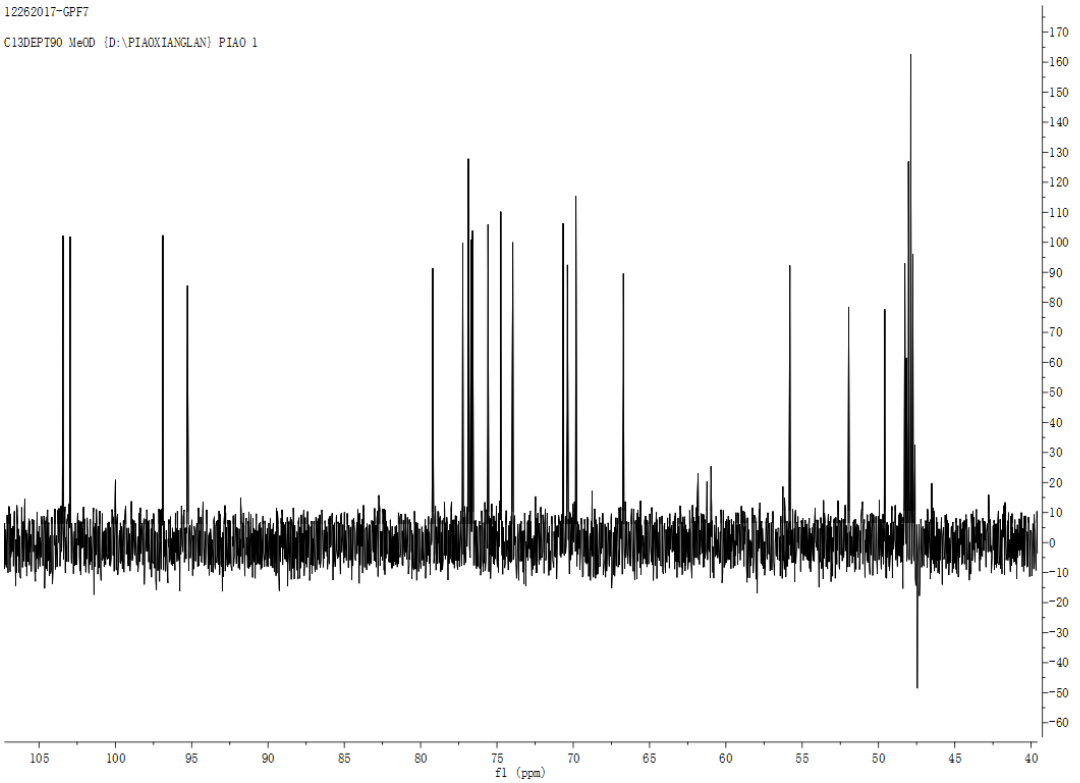

Fig. S**8** DEPT 90 of compound **1** (CD_3OD, 150 MHz)

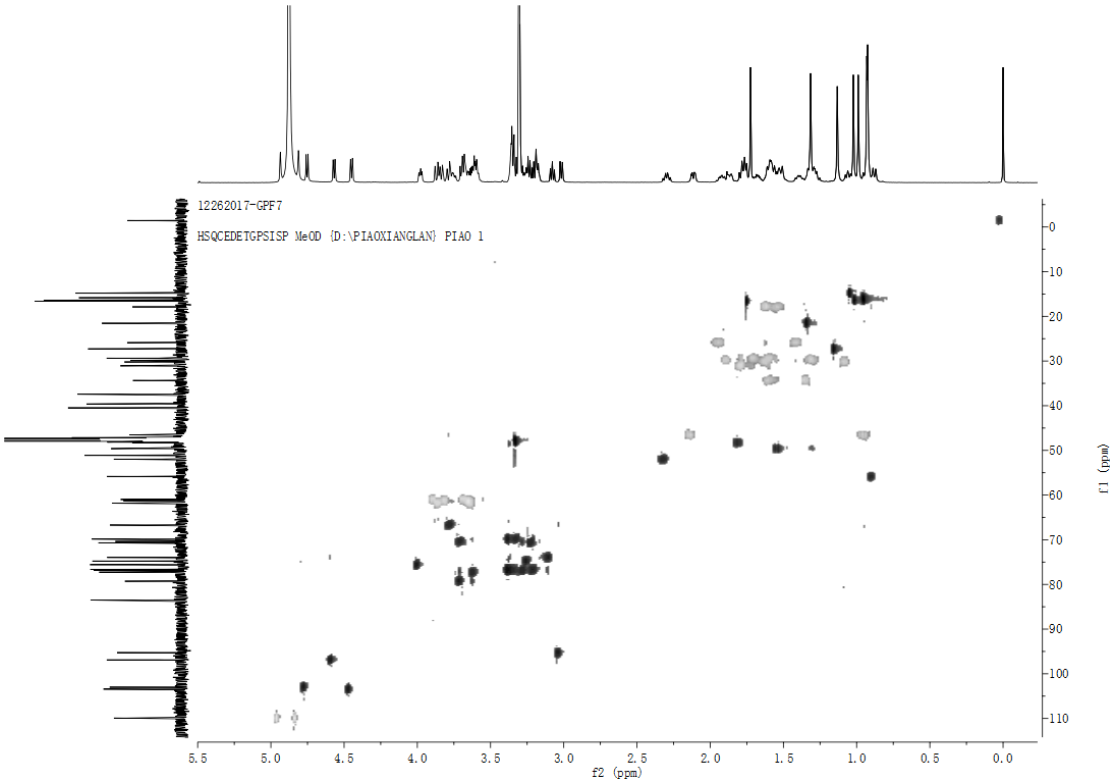

Fig. S**9** HSQC spectrum of compound **1** (CD_3OD, 600 MHz)

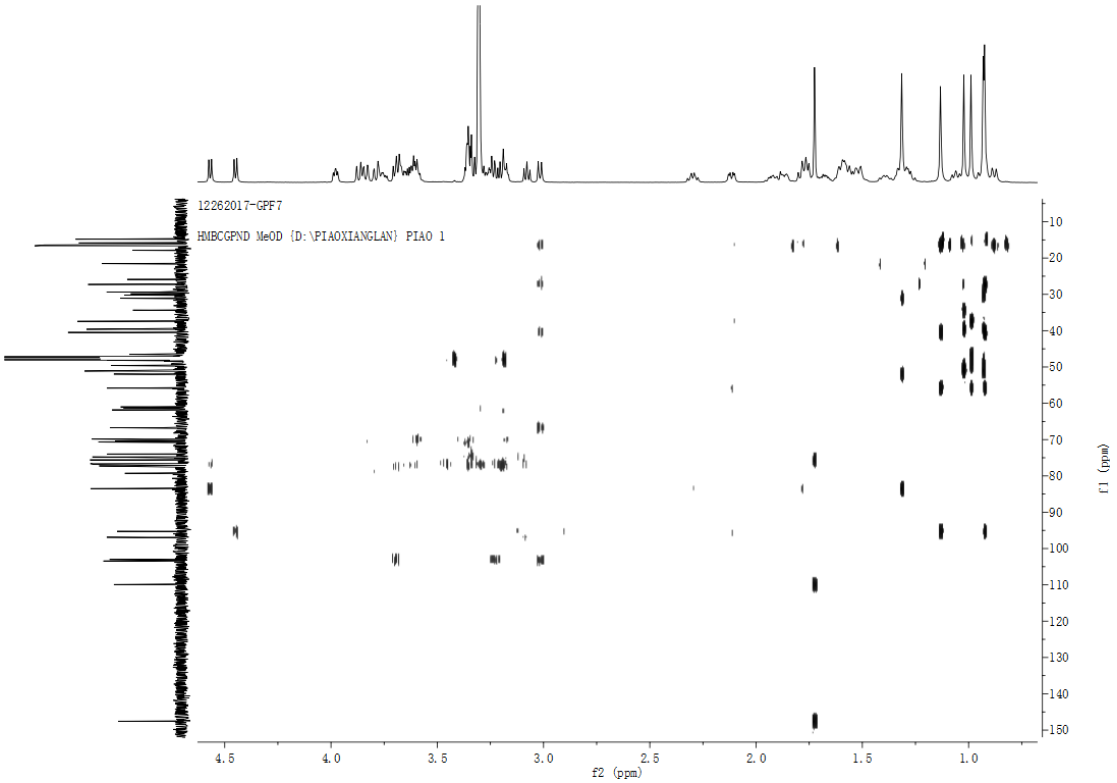

Fig. S**10** HMBC spectrum of compound **1** (CD_3OD, 600 MHz)

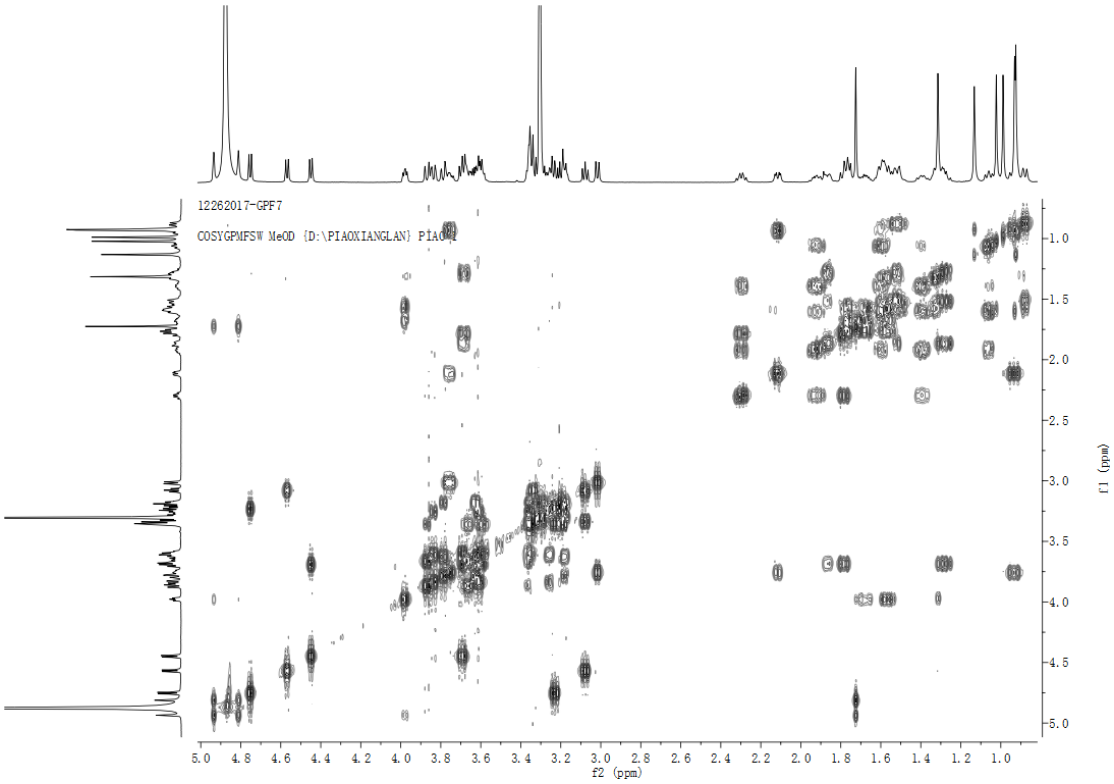

Fig. S**11** ^{1}H-^{1}H COSY of compound **1** (CD_3OD, 600 MHz)

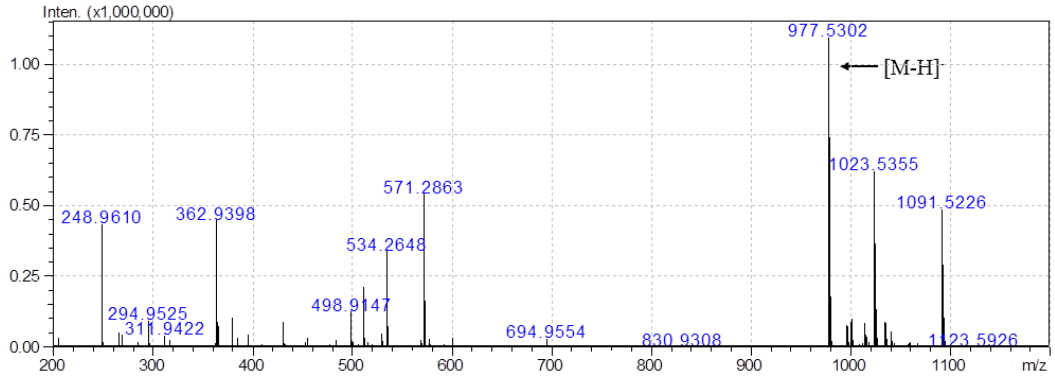

Fig. S**12** HRESIMS of compound **2**

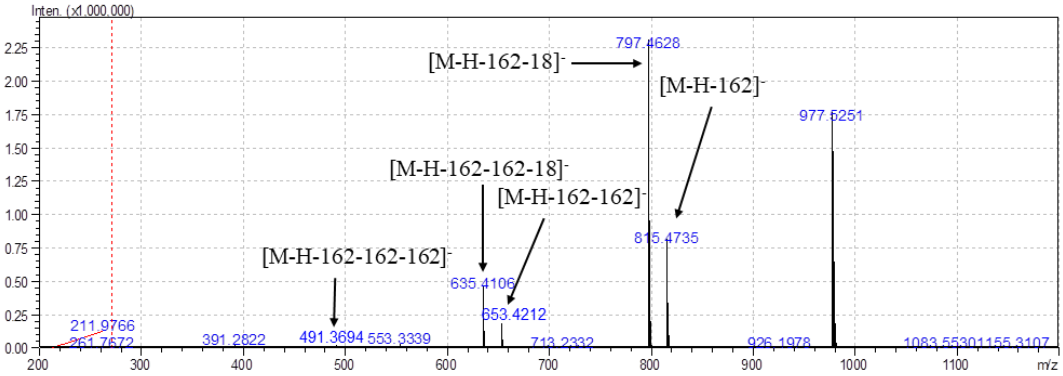

Fig. S**13** MS/MS data of compound **2**

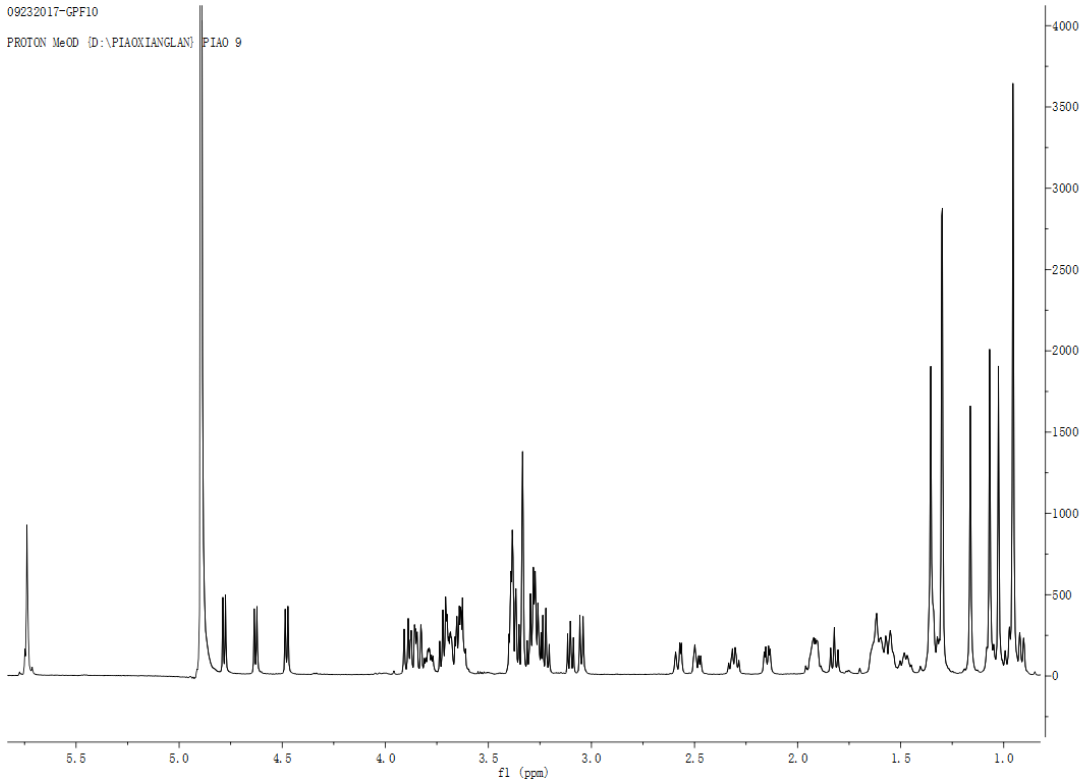

Fig. S**14** ^{1}H NMR spectrum of compound **2** (CD_3OD, 600 MHz)

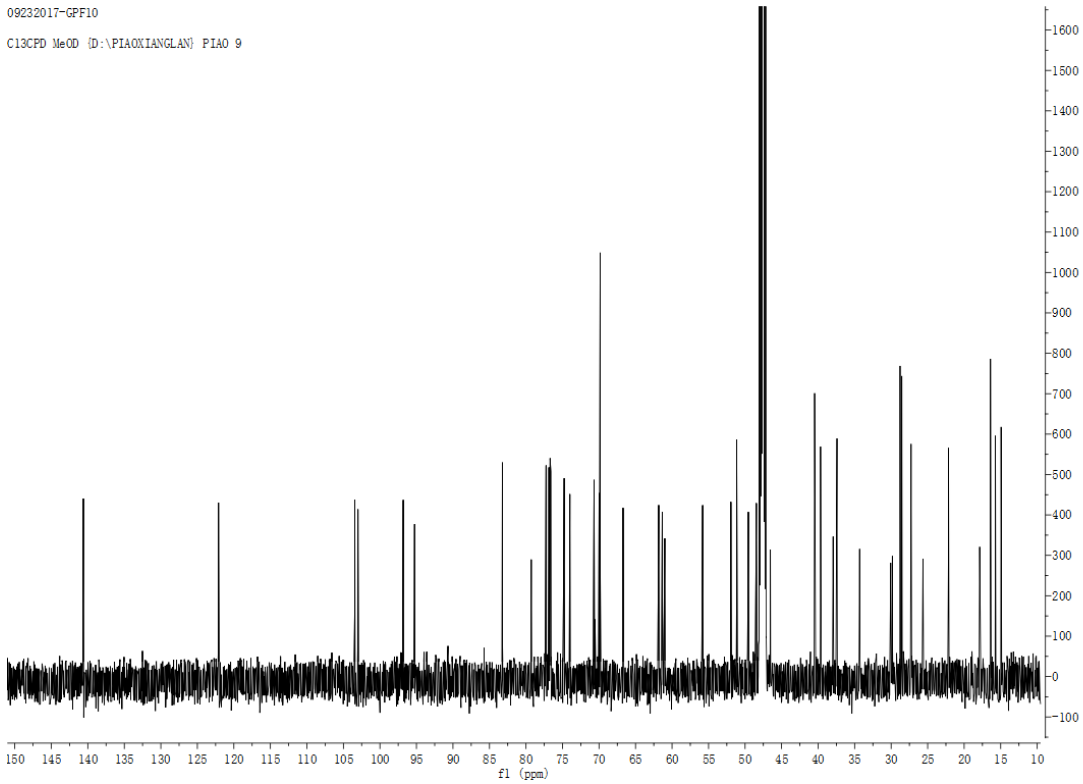

Fig. S**15** ^{13}C NMR of compound **2** (CD_3OD, 150 MHz)

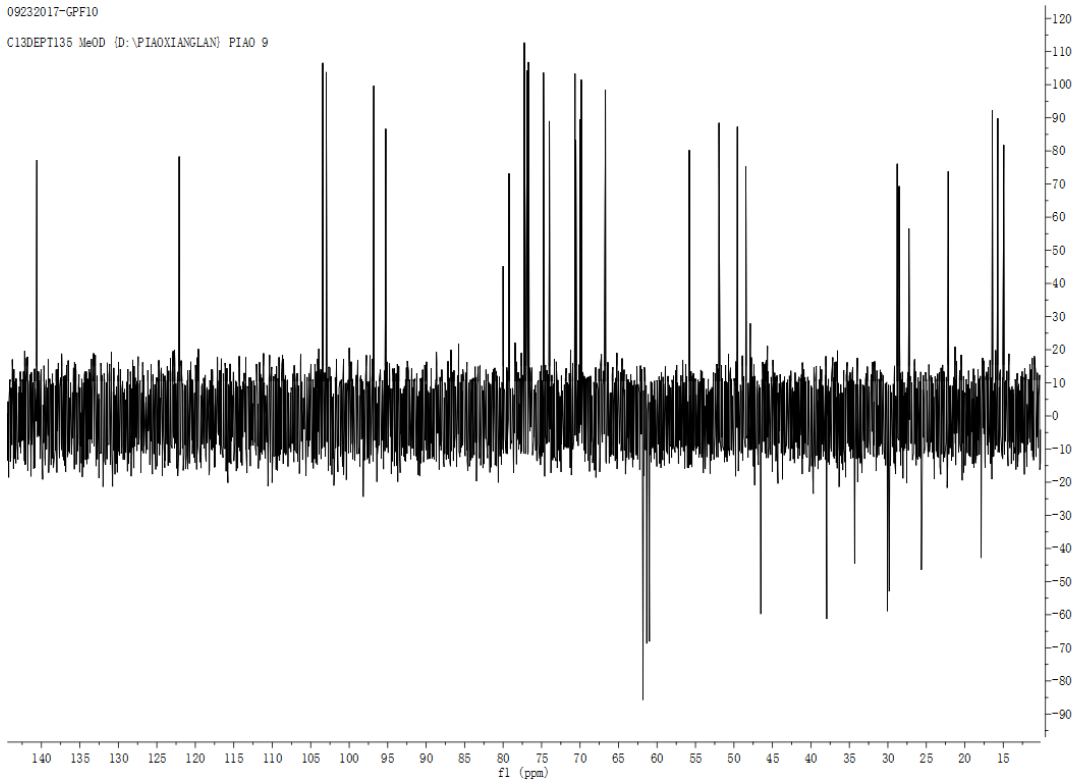

Fig. S**16** DEPT 135 of compound **2** (CD_3OD, 150 MHz)

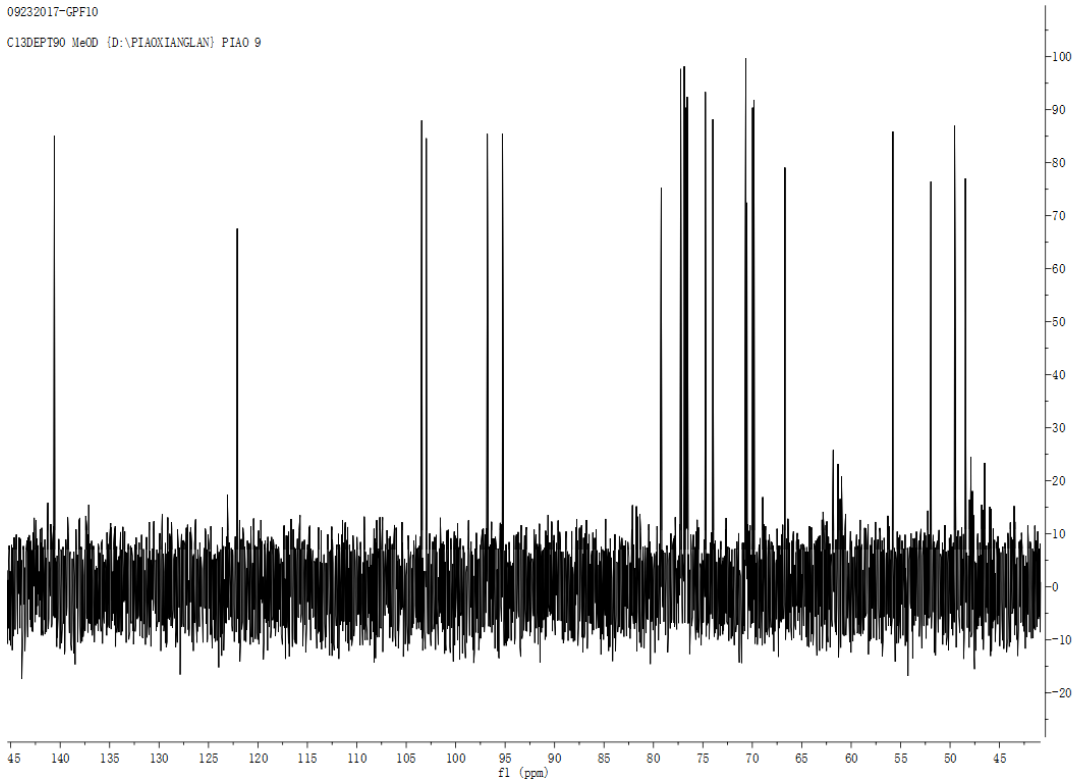

Fig. S**17** DEPT 90 of compound **2** (CD_3OD, 150 MHz)

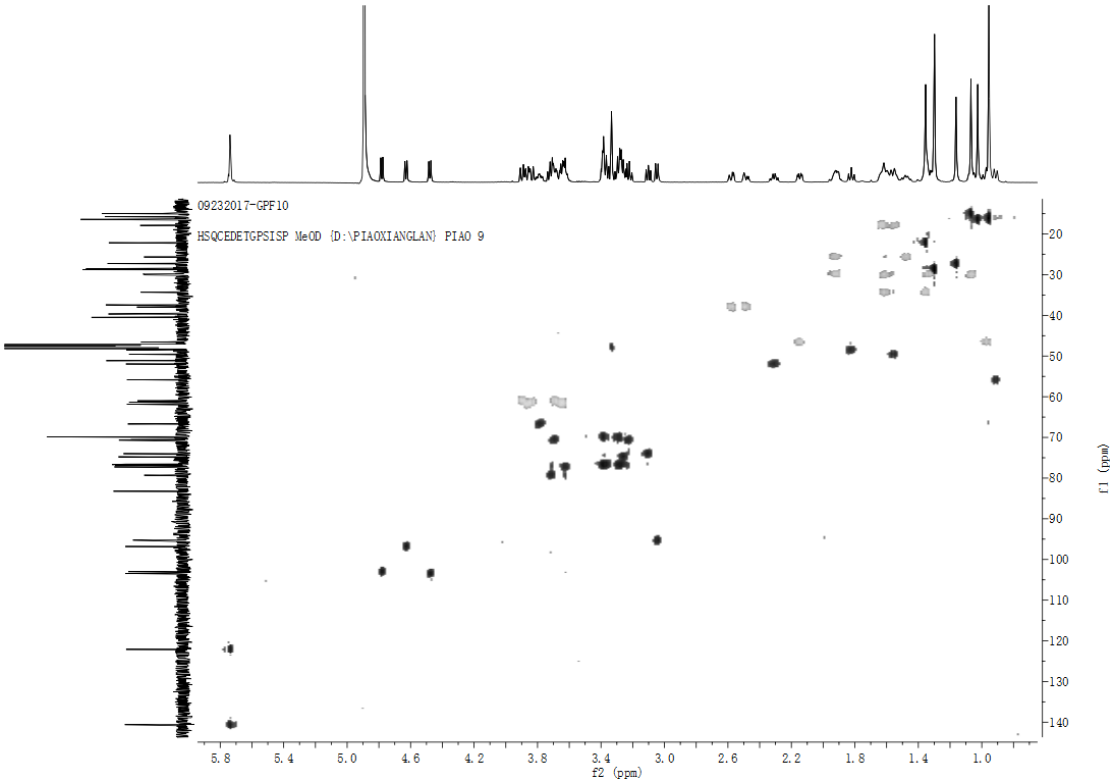

Fig. S**18** HSQC spectrum of compound **2** (CD_3OD, 600 MHz)

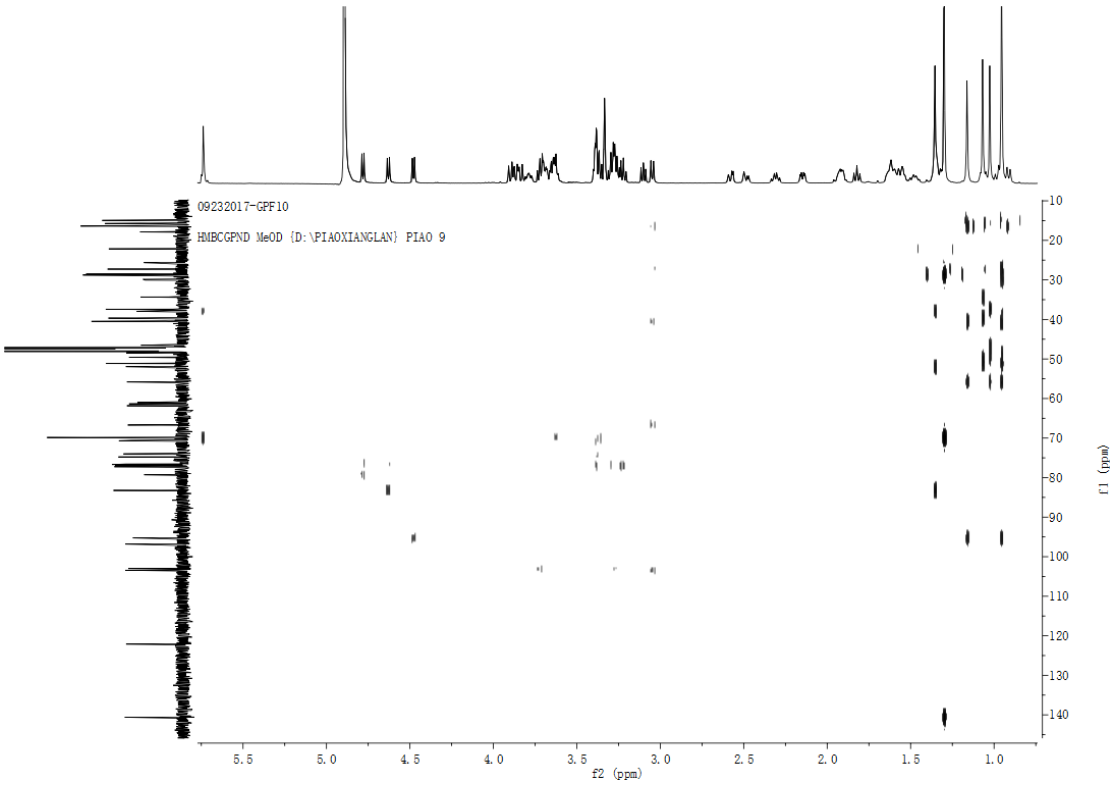

Fig. S**19** HMBC spectrum of compound **2** (CD_3OD, 600 MHz)

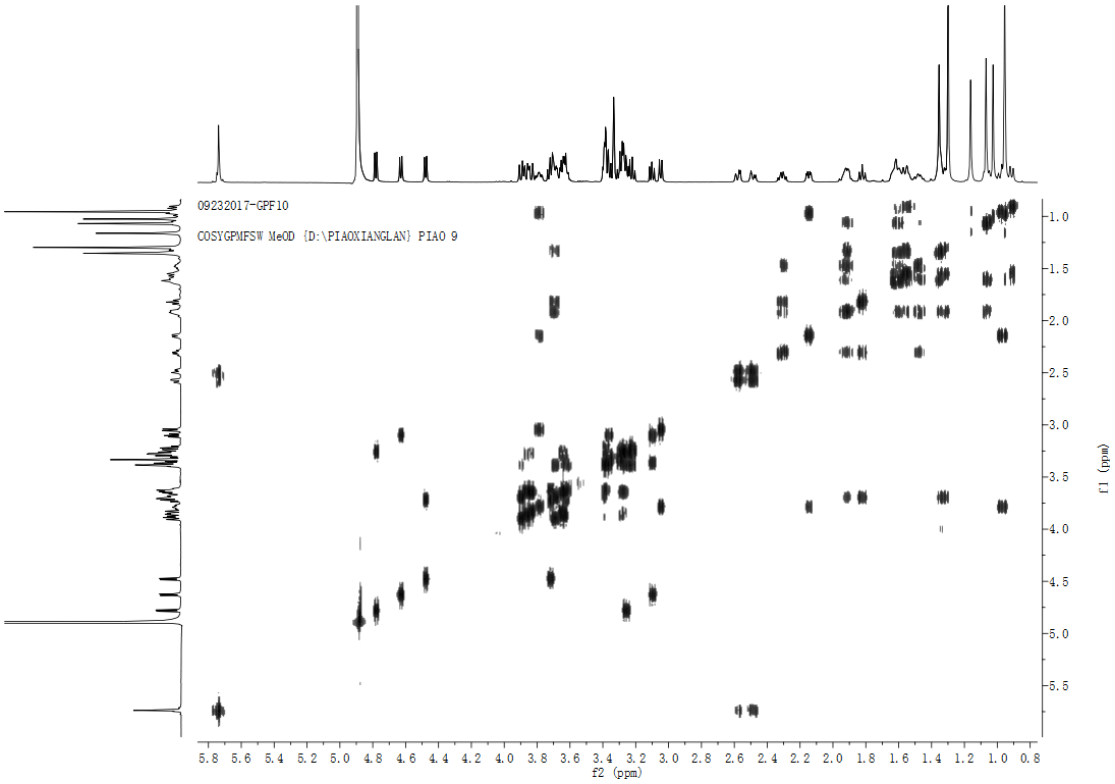

Fig. S**20** ^{1}H-^{1}H COSY of compound **2** (CD_3OD, 600 MHz)

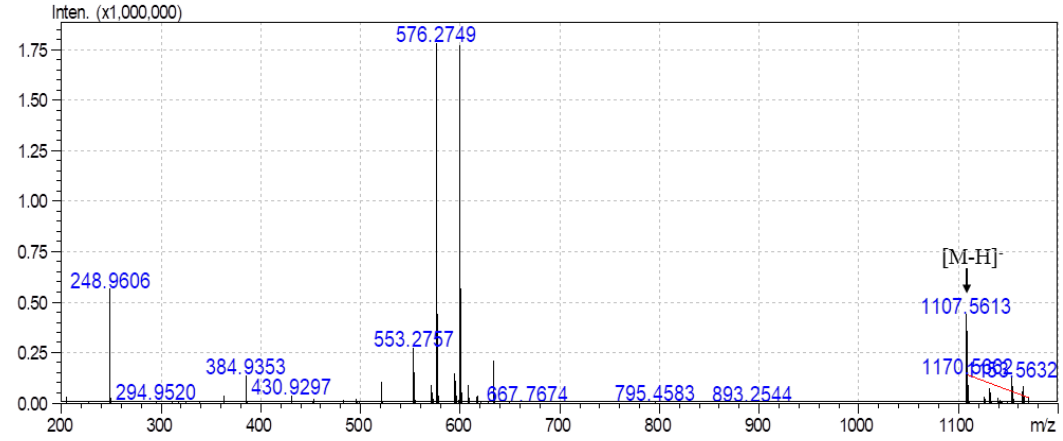

Fig. S**21** HRESIMS of compound **3**

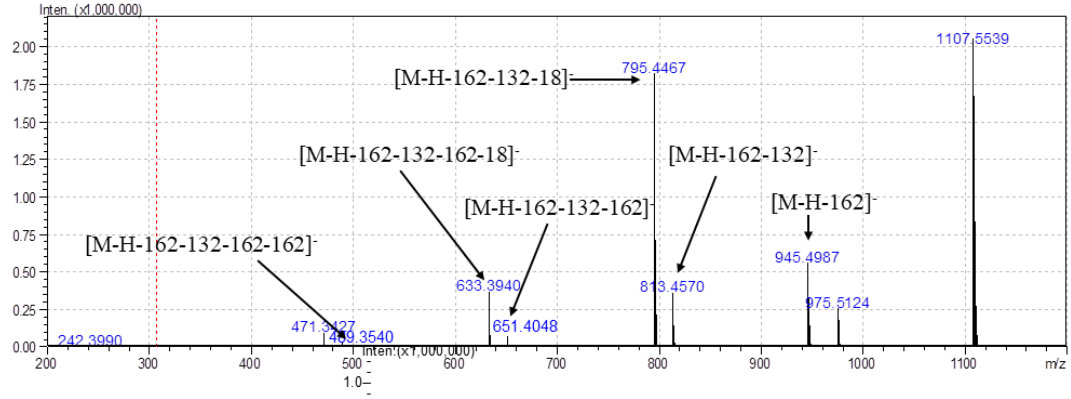

Fig. S**22** MS/MS of compound **3**

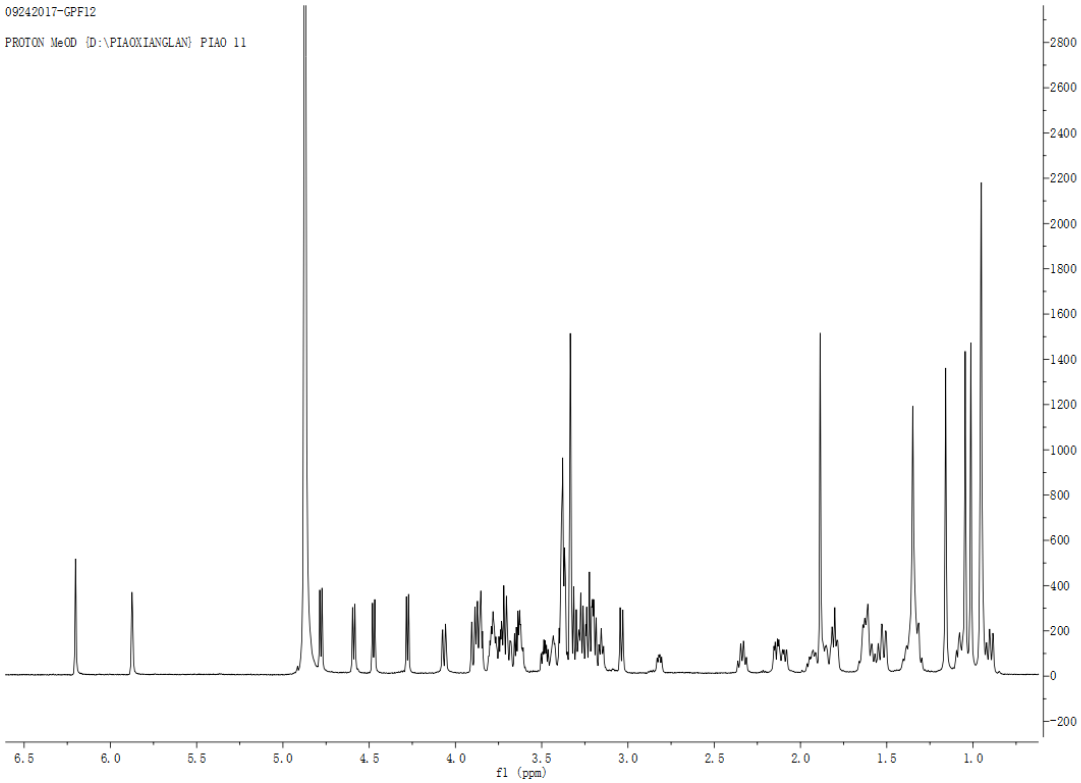

Fig. S**23** ^{1}H NMR spectrum of compound **3** (CD_3OD, 600 MHz)

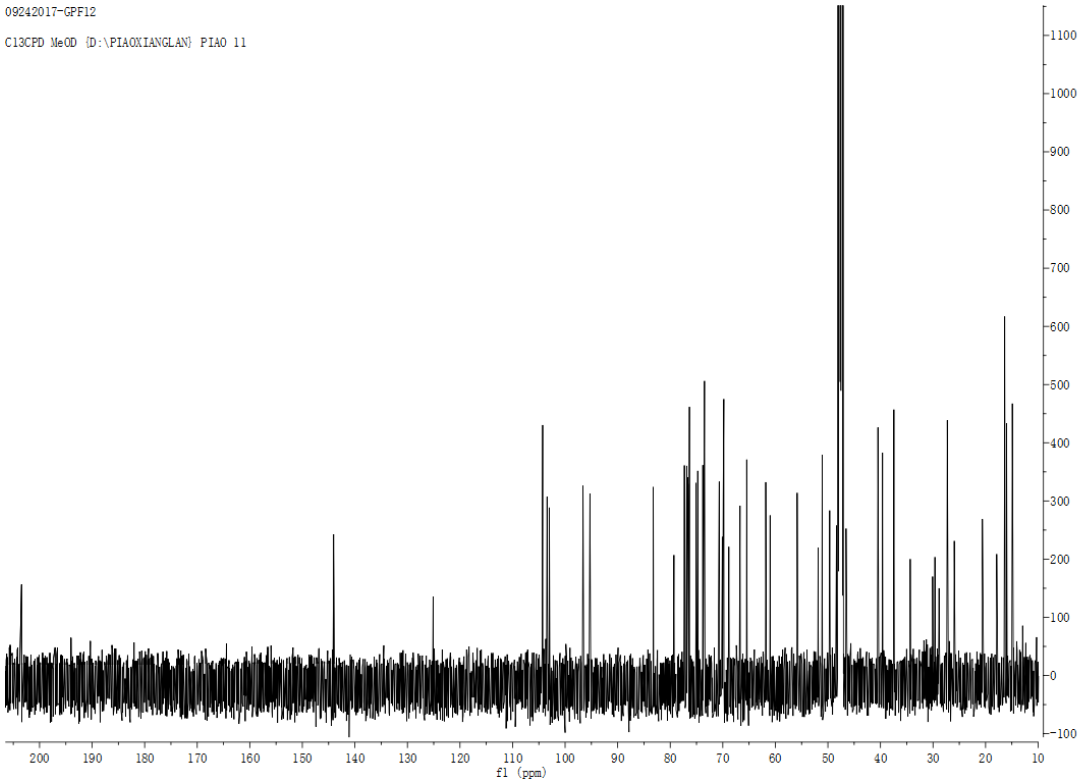

Fig. S**24** ^{13}C NMR of compound **3** (CD_3OD, 150 MHz)

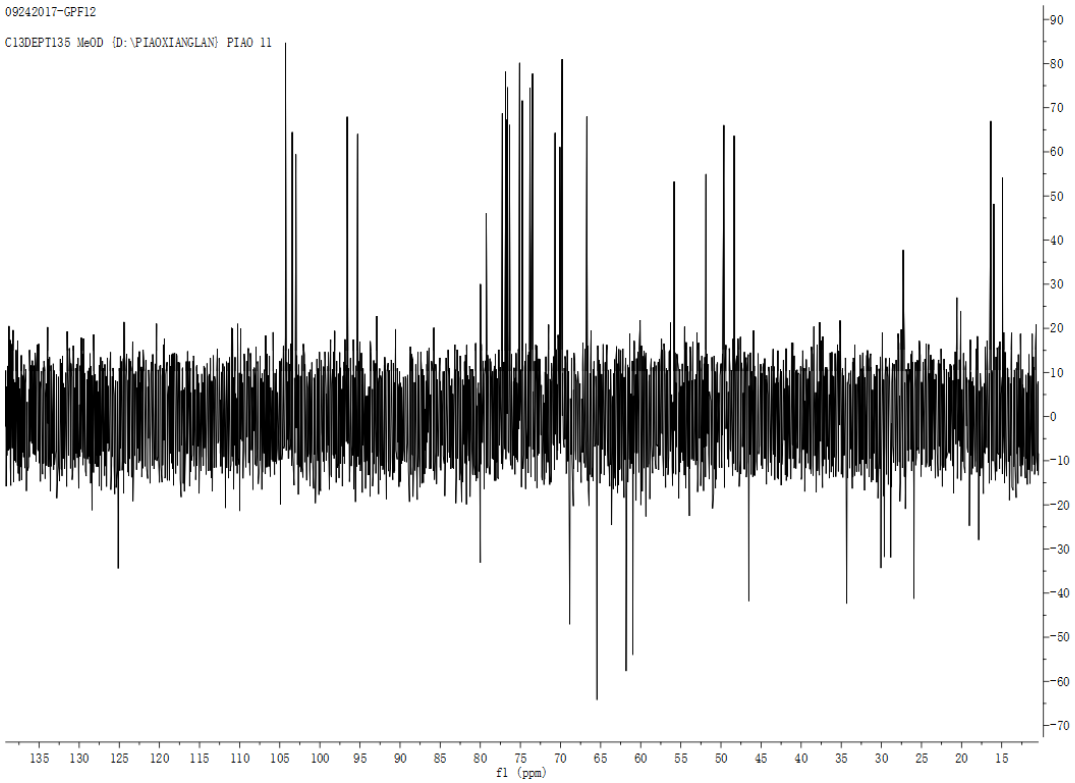

Fig. S**25** DEPT135 of compound **3** (CD_3OD, 150 MHz)

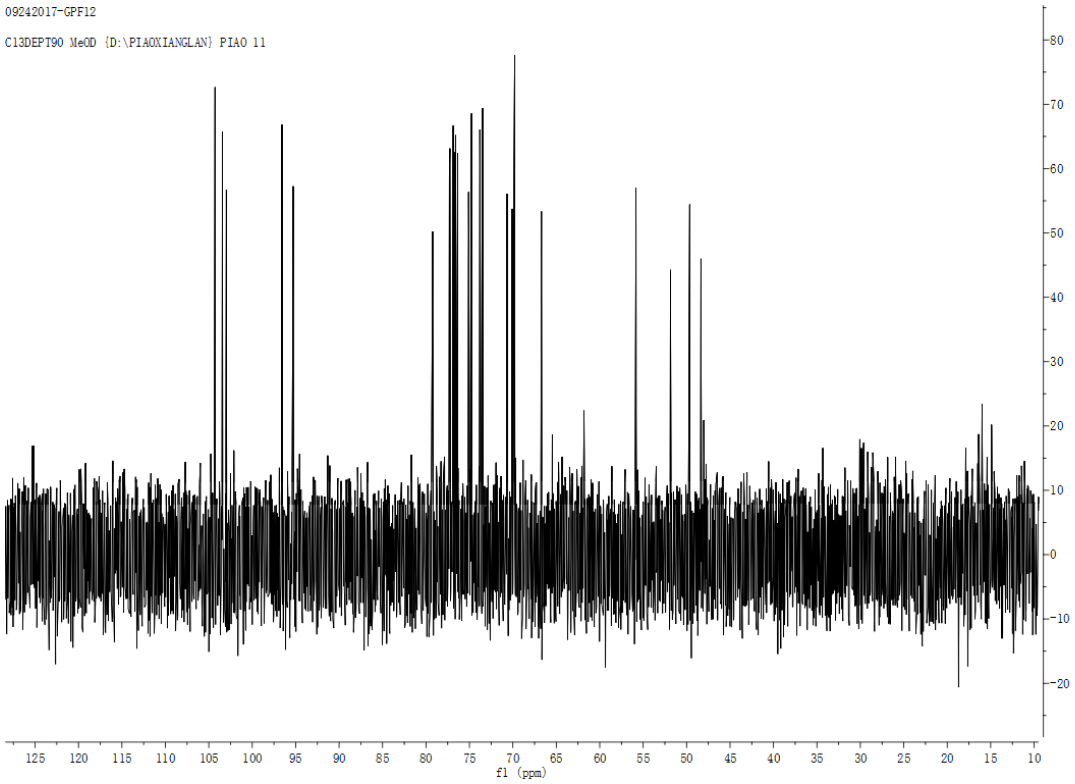

Fig. S**26** DEPT90 of compound **3** (CD_3OD, 150 MHz)

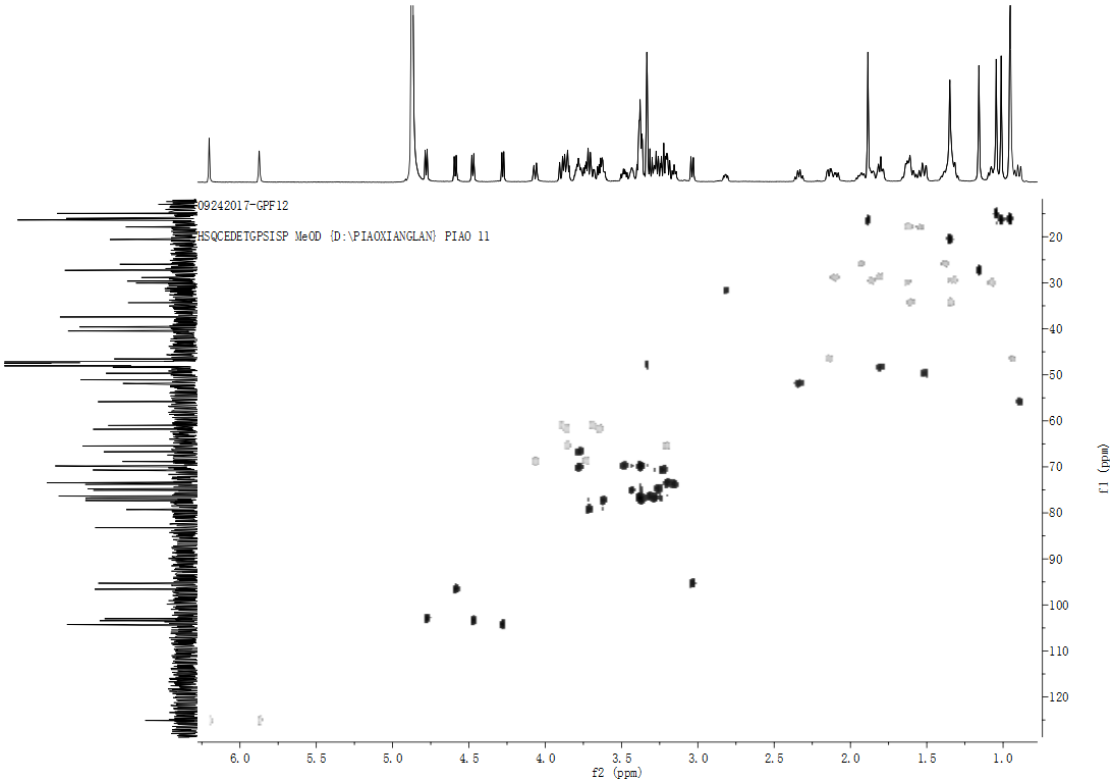

Fig. S**27** HSQC spectrum of compound **3** (CD_3OD, 600 MHz)

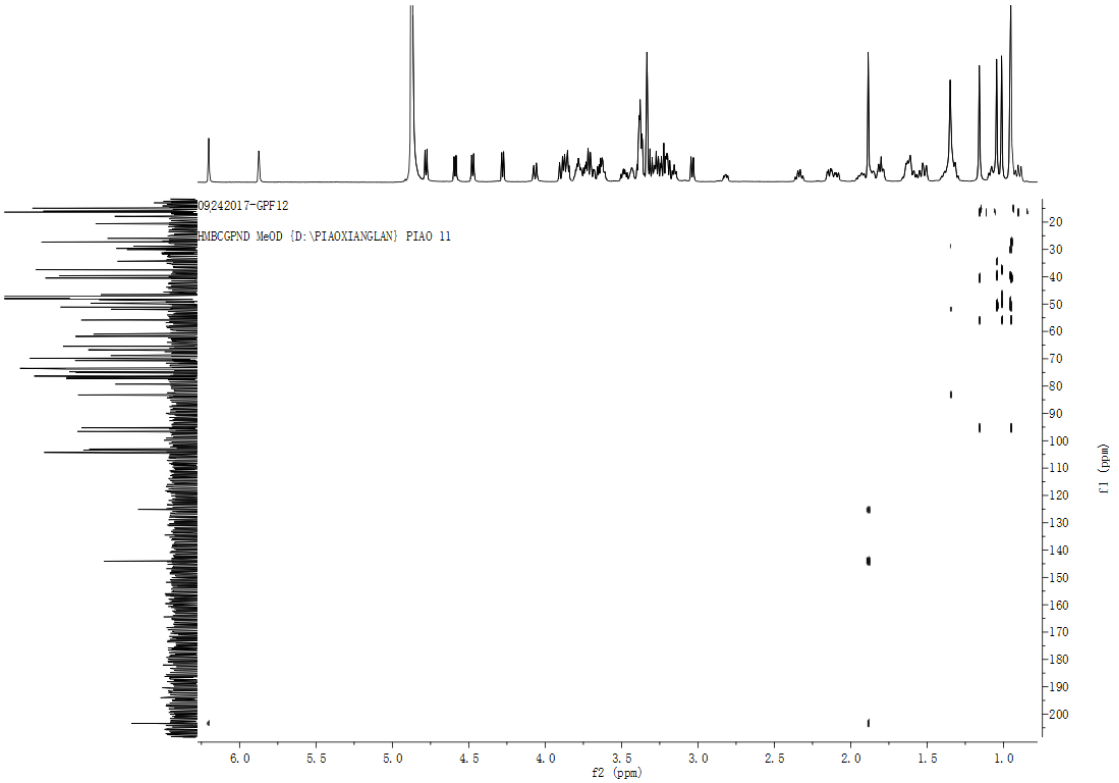

Fig. S**28** HMBC spectrum of compound **3** (CD_3OD, 600 MHz)

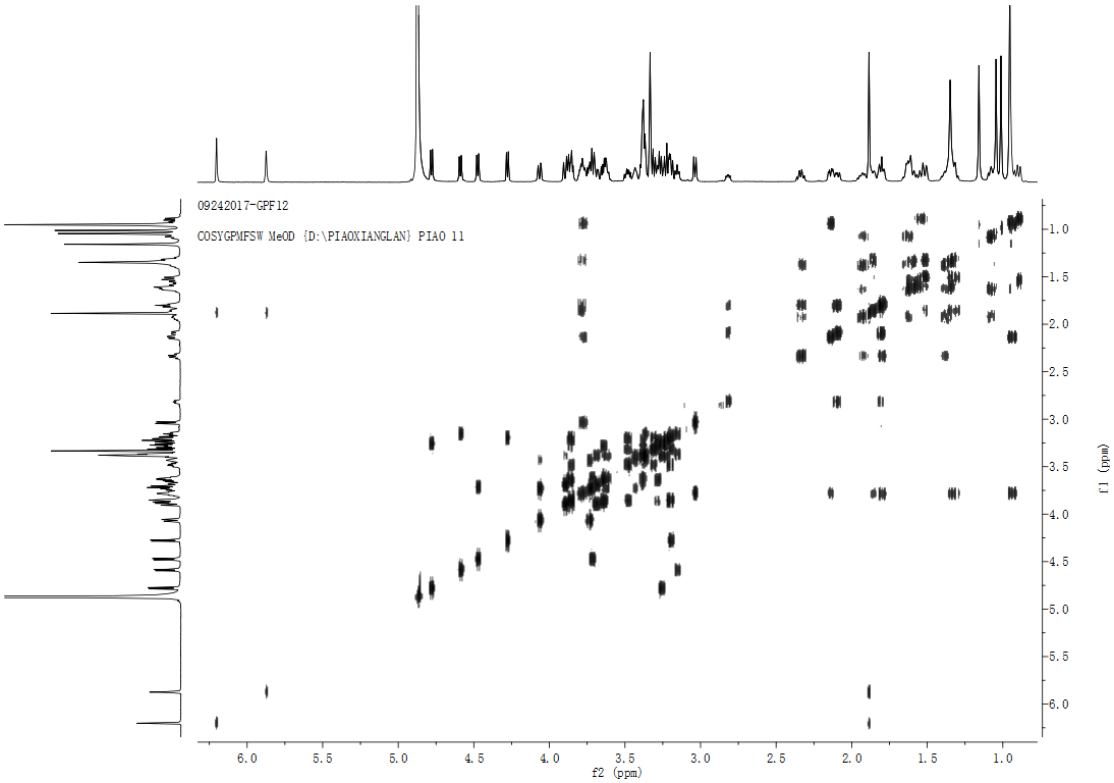

Fig. S**29** ^{1}H-^{1}H COSY of compound **3** (CD_3OD, 600 MHz)

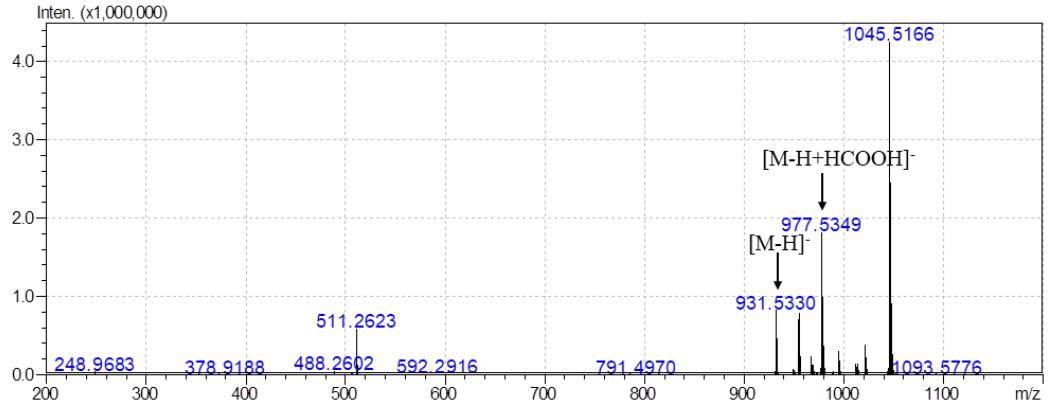

Fig. S**30** HRESIMS of compound **4**

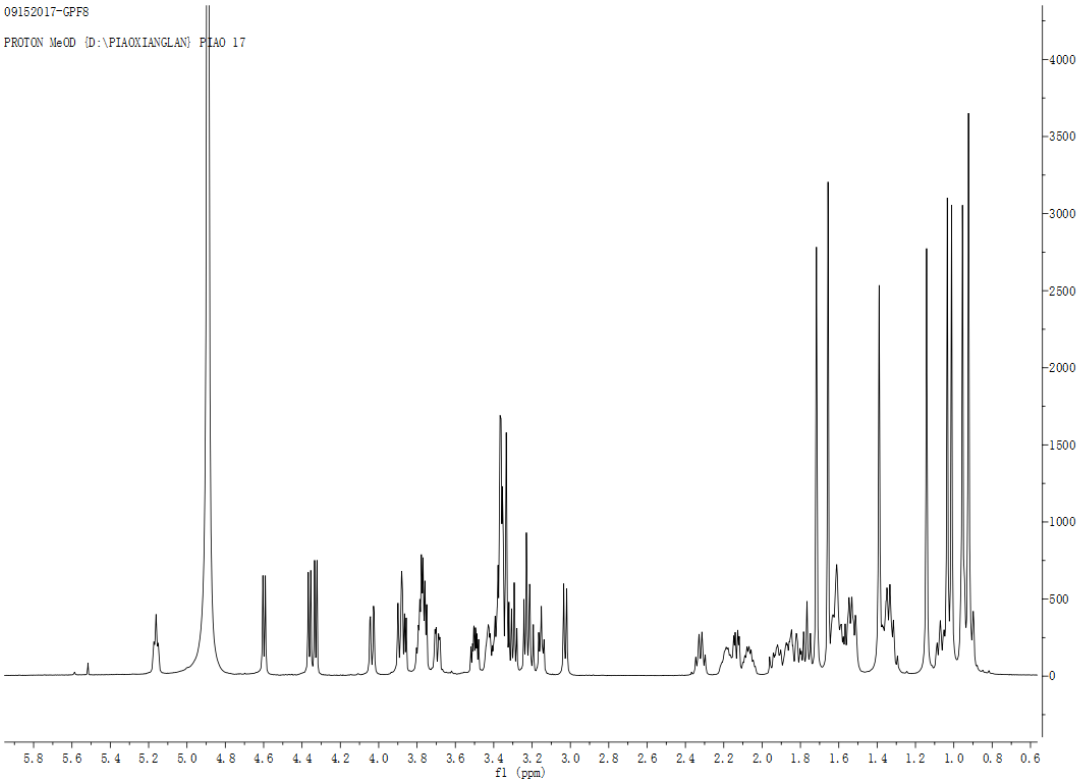

Fig. S**31** ^{1}H NMR spectrum of compound **4** (CD_3OD, 600 MHz)

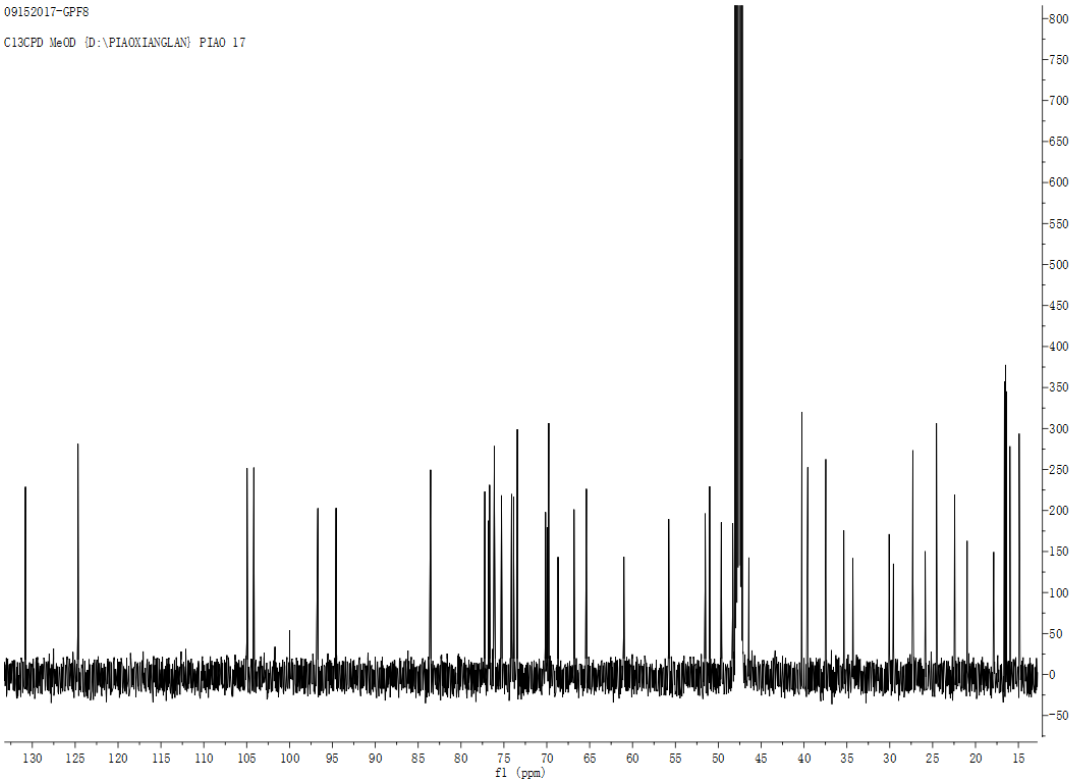

Fig. S**32** ^{13}C NMR spectrum of compound **4** (CD_3OD, 600 MHz)

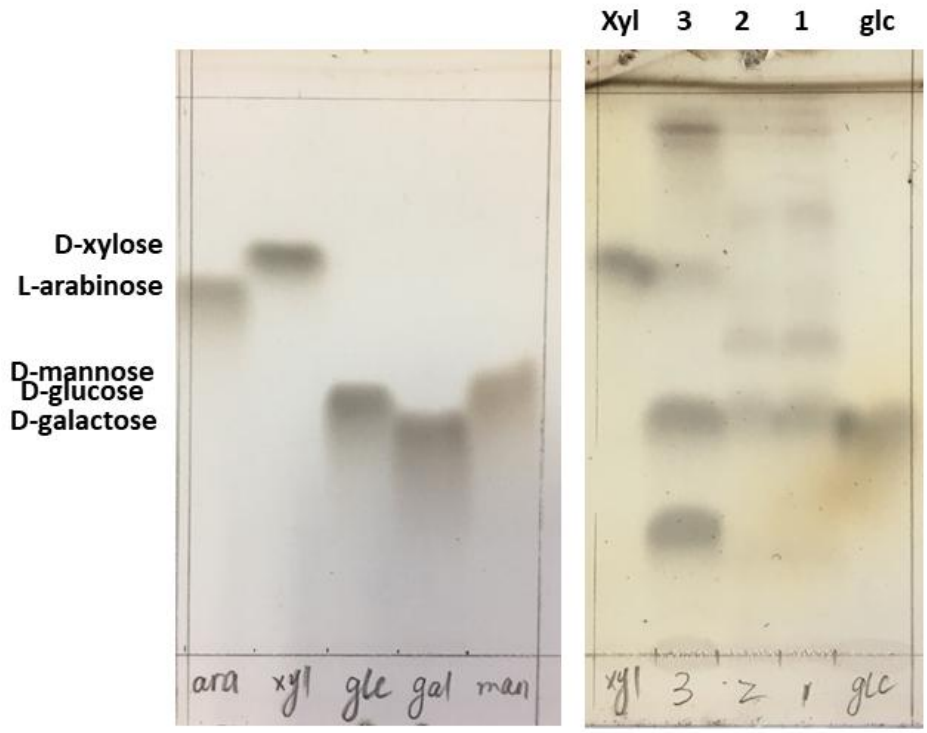

Fig. S**33** Sugar identification of compounds **1**-**3** through TLC

Table S1. ^{1}H NMR (CD_3OD, 600 MHz) Spectroscopic Data for gypenosides J1-J3.

	no.	gypenoside J1	gypenoside J2	gypenoside J3
aglycone	1	0.95 m, 2.12 dd	0.97 m, 2.15 dd	0.94 m, 2.14, dd
		(*J* = 12.6, 4.6)	(*J* = 12.6, 4.6)	(*J* = 12.6, 4.6)
	2	3.78 m	3.79 m	3.78 m
	3	3.04 d (*J* = 9.0)	3.04 d (*J* = 9.0)	3.03 d (*J* = 9.0)
	4			
	5	0.91 d (*J* = 10.2)	0.90 d (*J* = 10.2)	0.90 d (*J* = 11.4)
	6	1.54 m, 1.62 m	1.56 m, 1.63 m	1.55 m, 1.62 m
	7	1.35 m, 1.61 m	1.35 m, 1.62 m	1.34 m, 1.61 m
	8			
	9	1.55 m	1.56 m	1.50 m
	10			
	11	1.09 m, 1.02 m	1.06 m, 1.61 m	1.07 m, 1.64 m
	12	3.71 m	3.69 m	3.78 m
	13	1.82 m	1.83 m	1.81 m
	14			
	15	1.31 m, 1.89 m	1.32 m, 1.91 m	1.31 m, 1.87 m
	16	1.42 m, 1.94 m	1.48 m, 1.92 m	1.37 m, 1.93 m
	17	2.32 q (*J* = 9.3)	2.31 m	2.34 q (*J* = 9.6)
	18	1.02 s	1.07 s	1.05 s
	19	0.99 s	1.03 s	1.01 s
	20			
	21	1.31 s	1.35 s	1.35 s
	22	1.71 m, 1.79 m	2.50 m, 2.57 m	1.82 m, 2.10 m
	23	1.71 m, 1.79 m	5.74 m	2.82 m
	24	4.01 bt	5.74 m	
	25			
	26	4.81 s, 4.94 s	1.30 s	5.87 s, 6.20 s
	27	1.72 s	1.30 s	1.89 s
	28	1.13 s	1.16 s	1.16 s
	29	0.93 s	0.96 s	0.95 s
	30	0.92 s	0.96 s	0.95 s
3-*O*-inner	1'	4.46 d (*J* = 7.7)	4.48 d (*J* = 7.8)	4.48 d (*J* = 7.8)
	2'	3.72 m	3.72 m	3.71 m
	3'	3.62 m	3.63 m	3.62 m
	4'	3.39 m	3.39 m	3.38 m
	5'	3.28 m	3.28 m	3.29 m
	6'	3.65 m, 3.86 dd	3.64 m, 3.87 dd	3.65 m, 3.87 m
		(*J* = 11.8, 2.2)	(*J* = 11.8, 2.2)	
3-*O*-term	1"	4.76 d (*J* = 7.7)	4.78 d (*J* = 7.7)	4.79 d (*J* = 7.7)
	2"	3.26 m	3.26 m	3.26 m
	3"	3.39 m	3.39 m	3.30 m

	4"	3.23 m	3.23 m	3.23 m
	5"	3.39 m	3.39 m	3.38 m
	6"	3.70 m, 3.90	3.70 m, 3.90	3.69 m, 3.88
		d (*J* = 11.5)	d (*J* = 11.5)	d (*J* = 11.5)
20-*O*-inner	1‴	4.58 d (*J* = 7.8)	4.63 d (*J* = 7.8)	4.60 d (*J* = 7.8)
	2‴	3.11 m	3.11 m	3.16 m
	3‴	3.34 m	3.37 m	3.37 m
	4‴	3.33 m	3.29 m	3.38 m
	5‴	3.21 m	3.28 m	3.43 m
	6‴	3.65 m, 3.81	3.65 m, 3.84	3.73 m, 4.06
		d (*J* = 12.2)	dd (*J* = 12.7, 1.9)	d (*J* = 9.8)
20-*O*-term	1⁗			4.28 d (*J* = 7.5)
	2⁗			3.20 m
	3⁗			3.32 m
	4⁗			3.48 m
	5⁗			3.20 m, 3.85 m

Table S2. ^{13}C NMR (CD_3OD, 150 MHz) and HMBC for gypenosides J1-J3.

		gypenoside J1			gypenoside J2			gypenoside J3		
	no.	δ_C	type	HMBC	δ_C	type	HMBC	δ_C	type	HMBC
aglycone	1	46.5	CH_2	H-19	46.5	CH_2	H-19	46.5	CH_2	H-19
	2	66.7	CH	H-3	66.7	CH	H-3	66.7	CH	H-3
	3	95.3	CH	H-1', 28, 29	95.3	CH	H-1',	95.3	CH	H-1',
	4	40.5	C	H-3, 28, 29	40.5	C	H-3, 28	40.5	C	H-3, 28, 29
	5	55.8	CH	H-19, 28, 29	55.8	CH	H-19, 28, 29	55.8	CH	H-19, 28, 29
	6	17.9	CH_2		17.9	CH_2		17.9	CH_2	
	7	34.4	CH_2		34.3	CH_2	H-18	34.3	CH_2	H-18
	8	39.6	C		39.7	C		39.6	C	H-18
	9	49.6	CH	H-19	49.6	CH	H-19	49.7	CH	H-19
	10	37.4	C	H-19	37.4	C	H-19	37.4	C	H-19
	11	30.2	CH_2		30.1	CH_2		30.1	CH_2	
	12	70.4	CH		70.6	CH		70.1	CH	
	13	48.3	CH		48.5	CH		48.4	CH	
	14	51.1	C	H-18, 30	51.2	C	H-30	51.1	C	H-18
	15	29.8	CH_2		29.8	CH_2	H-30	29.6	CH_2	
	16	25.9	CH_2		25.6	CH_2		26	CH_2	
	17	52	CH	H-21	51.9	CH	H-21	51.9	CH	H-21, 30
	18	14.8	CH_3	H-30	14.9	CH_3	H-30	14.9	CH_3	H-30
	19	16.4	CH_3	H-6, 11	16.4	CH_3		16.4	CH_3	
	20	83.5	C	H-1''', 21, 22	83.2	C	H-1''', 13, 17, 21	83.2	C	H-1'''
	21	21.5	CH_3		22.2	CH_3		20.6	CH_3	
	22	31.1	CH_2	H-21	38	CH_2	H-23, 24	28.9	CH_2	H-21
	23	29.4	CH_2		122.1	CH	H-22	31.7	CH_2	
	24	75.6	CH	H-27	140.6	CH	H-26, 27	203.5	C	H-25, 27
	25	147.6	C	H-27	69.9	C	H-23, 24, 26, 27	144.1	C	H-27
	26	109.9	CH_2	H-27	28.8	CH_3	H-27	125.1	CH_2	H-27
	27	16.6	CH_3	H-26	28.5	CH_3		16.5	CH_3	H-26
	28	27.3	CH_3	H-3, 29	27.3	CH_3	H-29	27.3	CH_3	H-3, 18, 29
	29	15.8	CH_3	H-28	15.7	CH_3		16.0	CH_3	
	30	16.4	CH_3	H-13	16.4	CH_3		16.4	CH_3	H-18
3-*O*-inner	1'	103.4	CH		103.4	CH	H-2', 3	103.4	CH	H-3
	2'	79.3	CH	H-1"	79.3	CH	H-1"	79.3	CH	H-1"
	3'	77.3	CH		77.3	CH		77.3	CH	
	4'	69.9	CH		69.9	CH		69.9	CH	
	5'	76.9	CH		76.9	CH		76.9	CH	
	6'	61.8	CH_2		61.8	CH_2		61.8	CH_2	

3-*O*-term	1"	103.0	CH	103.0	CH	H-2'	103.0	CH	H-2'
	2"	74.8	CH	74.8	CH		74.8	CH	
	3"	76.6	CH	76.6	CH		76.6	CH	
	4"	70.7	CH	70.7	CH		70.7	CH	
	5"	76.7	CH	76.7	CH		76.7	CH	
	6"	61.0	CH_2	61.0	CH_2		61.0	CH_2	
20-*O*-inner	1‴	96.9	CH	96.8	CH	H-2‴	96.6	CH	
	2‴	74.0	CH	74.0	CH		73.8	CH	
	3‴	76.9	CH	76.7	CH		77.3	CH	
	4‴	69.9	CH	70.0	CH		69.9	CH	
	5‴	76.6	CH	76.6	CH		75.1	CH	
	6‴	61.3	CH_2	61.3	CH_2		68.9	CH_2	H-1""
20-*O*-term	1""						104.3	CH	H-2"", 6‴
	2""						73.5	CH	
	3""						76.4	CH	
	4""						69.8	CH	
	5""						65.5	CH_2	