

SUPPLEMENTARY MATERIAL

Flavonoids from the leaves of *Epimedium koreanum* Nakai and their potential cytotoxic activities

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Abstract

Phytochemical studies on the leaves of *Epimedium koreanum* Nakai have resulted in the discovery of two new flavonol glycosides, koreanoside F (**1**) and koreanoside G (**2**), along with six known flavonoids. Their structures were elucidated on the basis of HRESIMS, UV, IR, 1D NMR and 2D NMR data. Absolute configurations of **1** and **2** was further determined by ¹³C-NMR spectra with gate decoupling (GD). All of the compounds were evaluated for cytotoxic activities by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay. The results indicated that compounds **3**, **5**, **6**, **7** and **8** inhibited the proliferation of A549 and NCI-292 cells with IC₅₀ values of 5.7-23.5 μM. Real-time monitoring in three kinds of lung cancer cells and a kind of human bronchial epithelial cells treated with compound **6** was also assessed.

Key words: *Epimedium koreanum* Nakai; flavonoids; cytotoxicity; flavonol glycoside

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Figure S1 Key HMBC (H→C) and ^1H - ^1H COSY (—) correlations of **1** and **2**

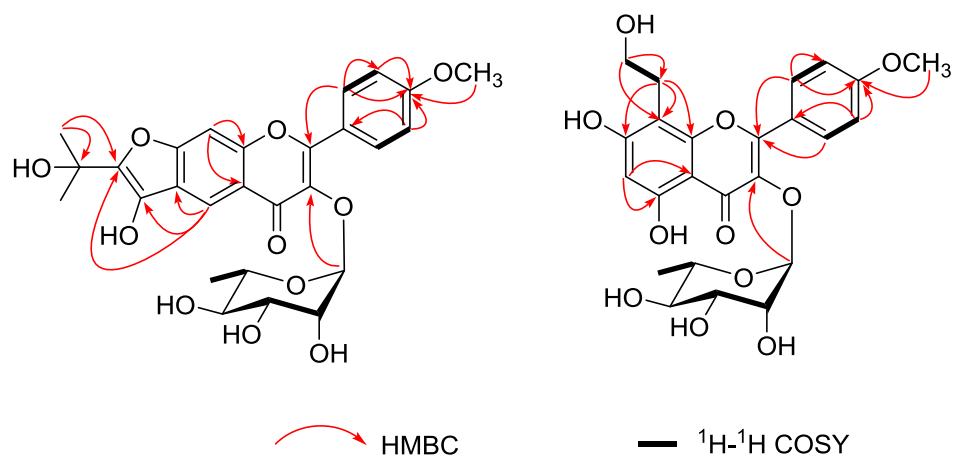


Figure S2 UV spectrum of compound **1** in methanol

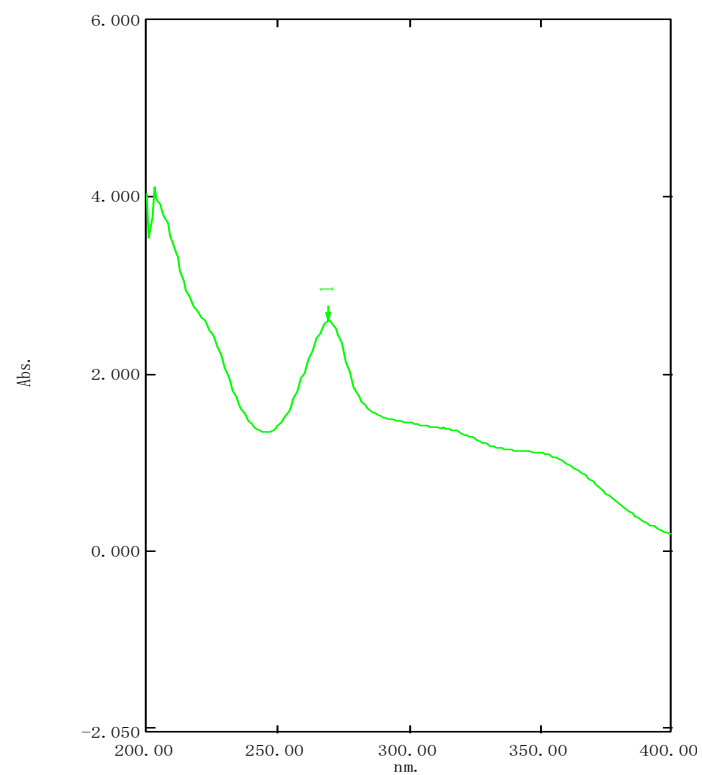


Figure S3 ^1H -NMR (600 MHz, methanol- d_4) spectrum of compound **1**

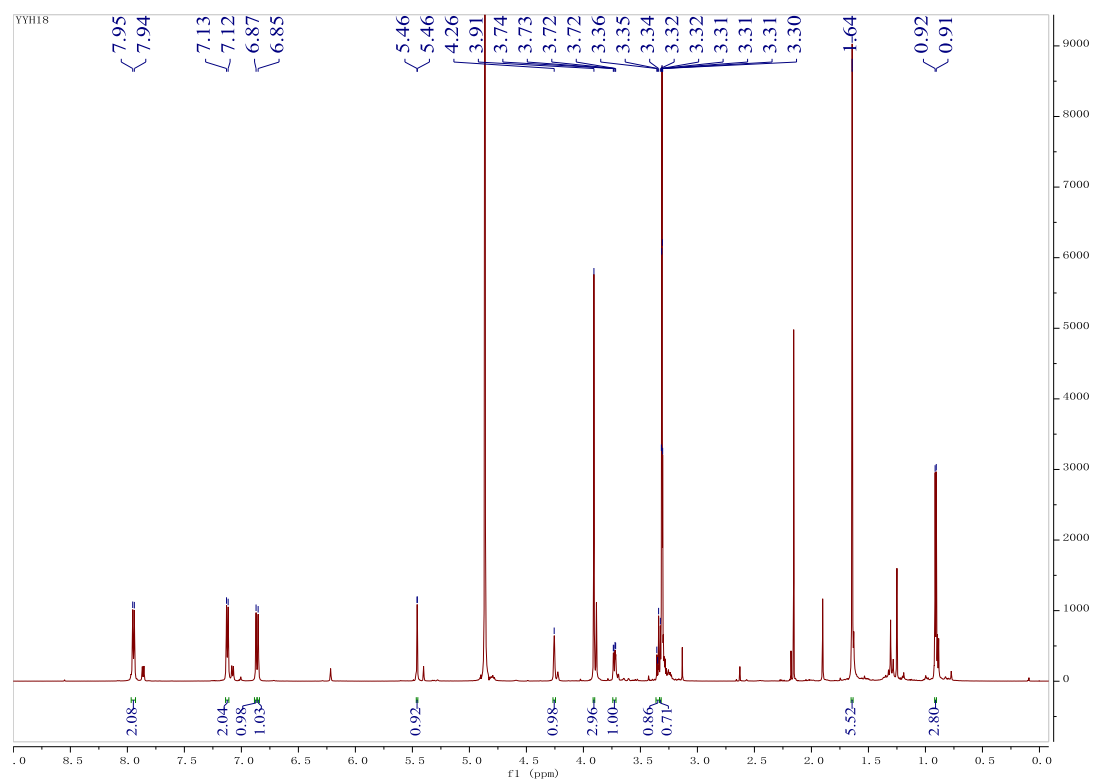


Figure S4 ^{13}C -NMR (150 MHz, methanol- d_4) spectrum of compound **1**

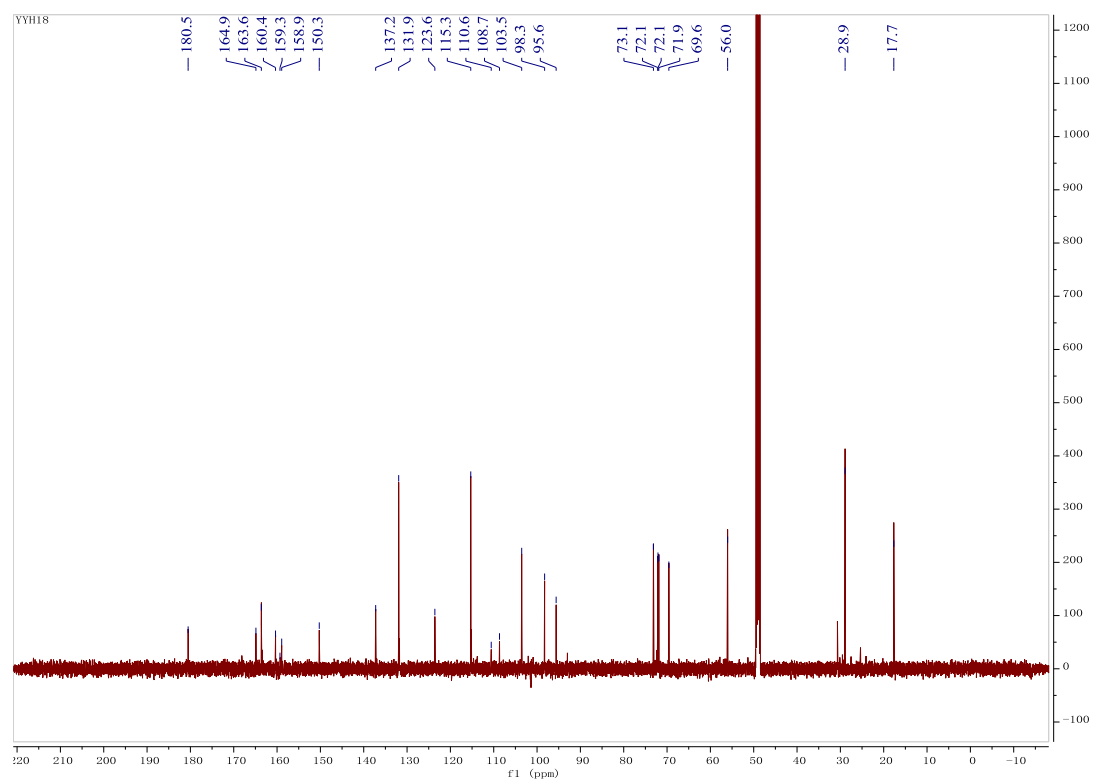


Figure S5 HSQC spectrum of compound **1**

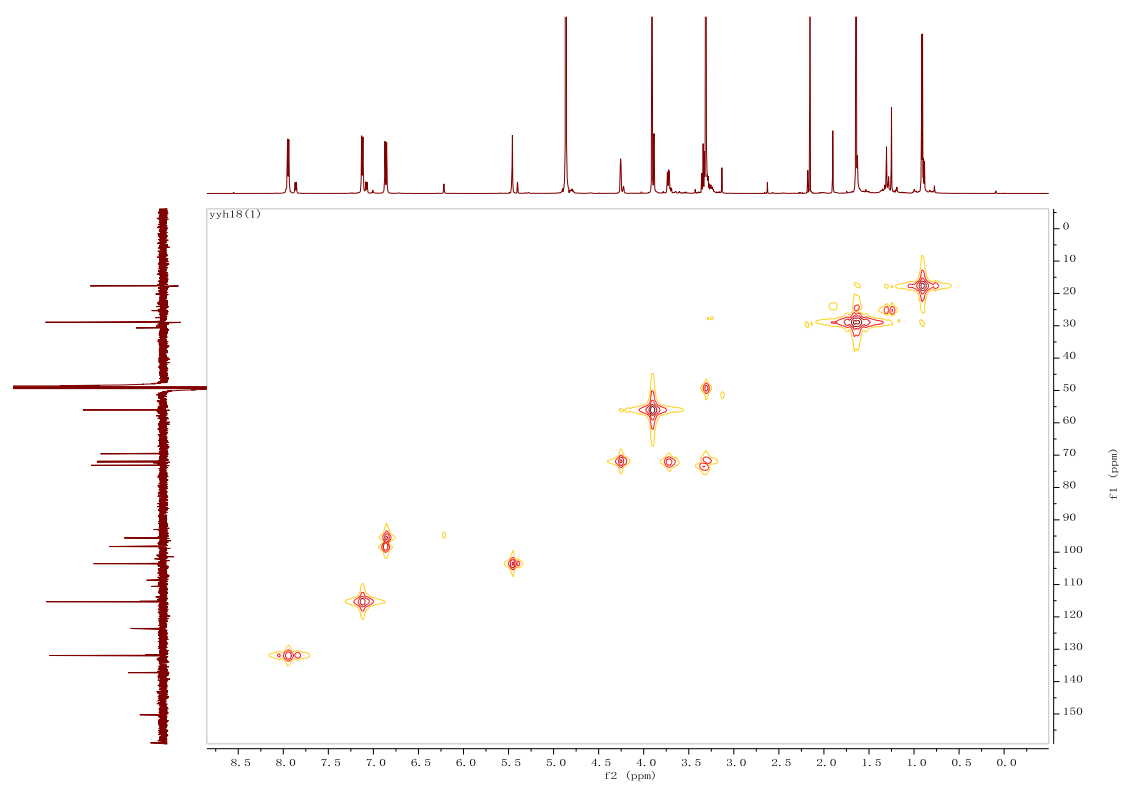


Figure S6 HMBC spectrum of compound **1**

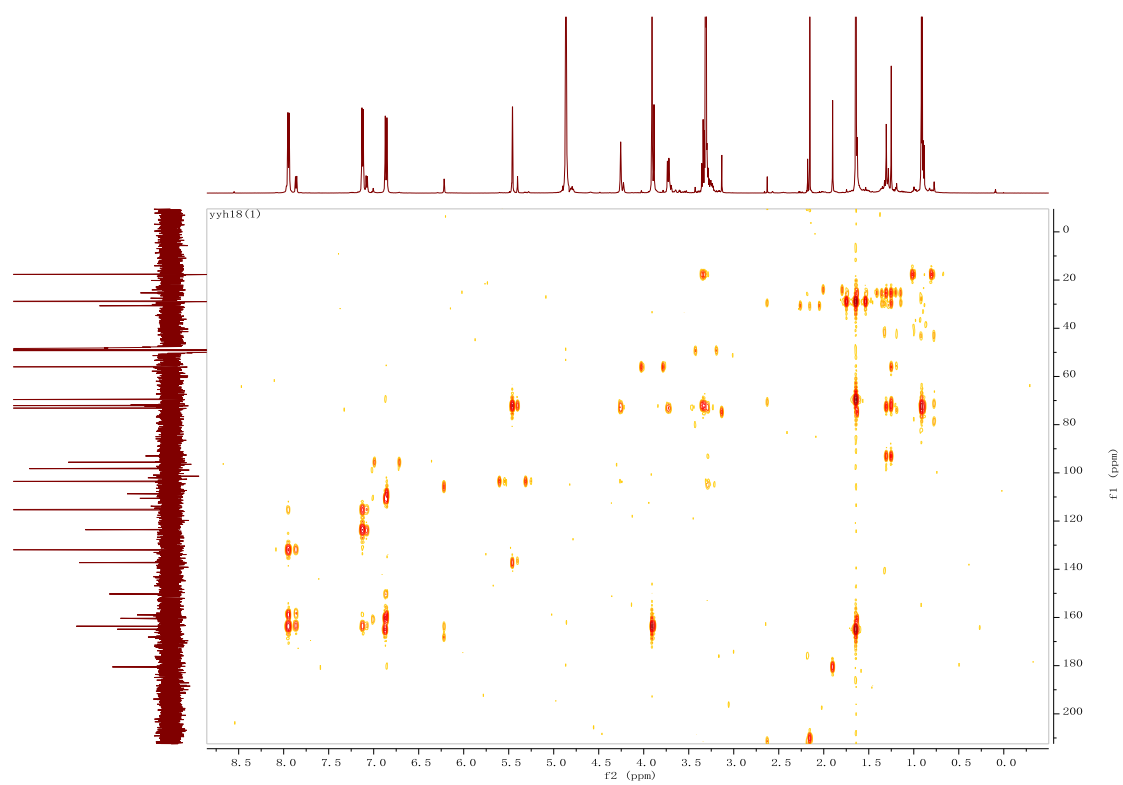


Figure S7 ^1H - ^1H COSY spectrum of compound **1**

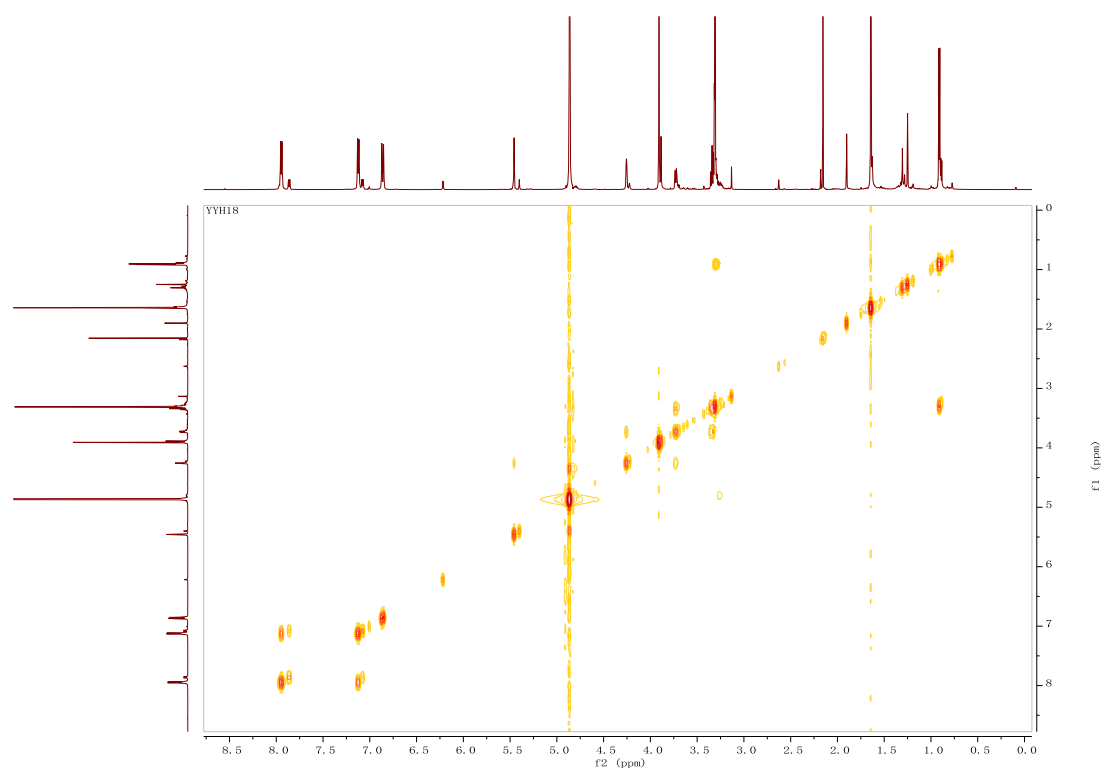


Figure S8 ^{13}C -NMR (150 MHz, pyridine- d_5) spectrum with gate decoupling of compound **1**

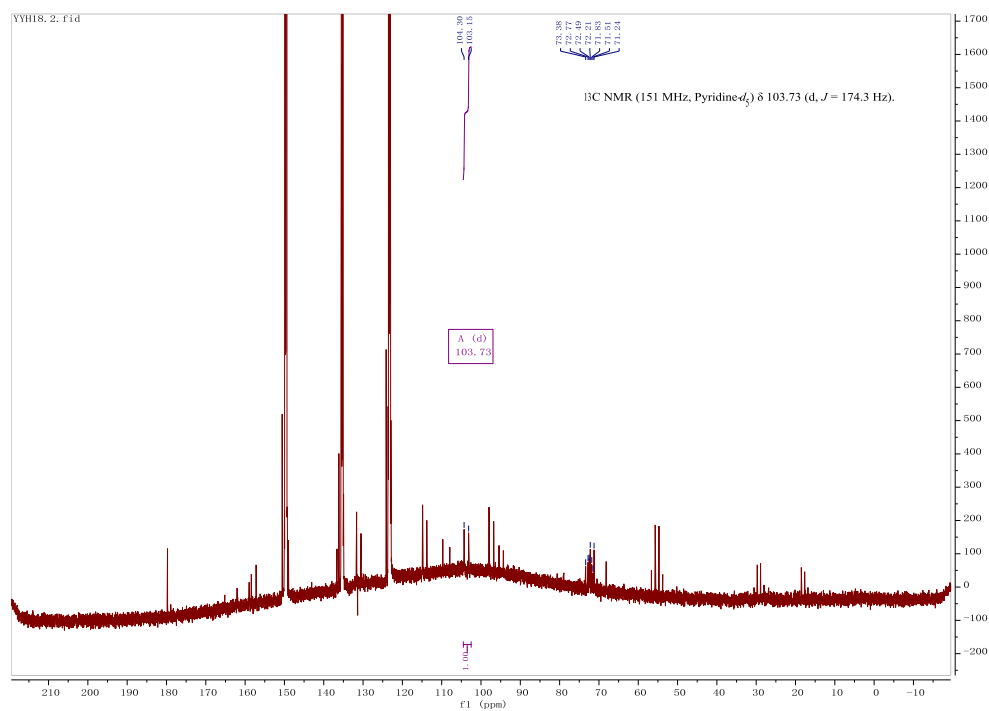


Figure S9 ^{13}C -NMR (150 MHz, pyridine- d_5) spectrum of compound **1**

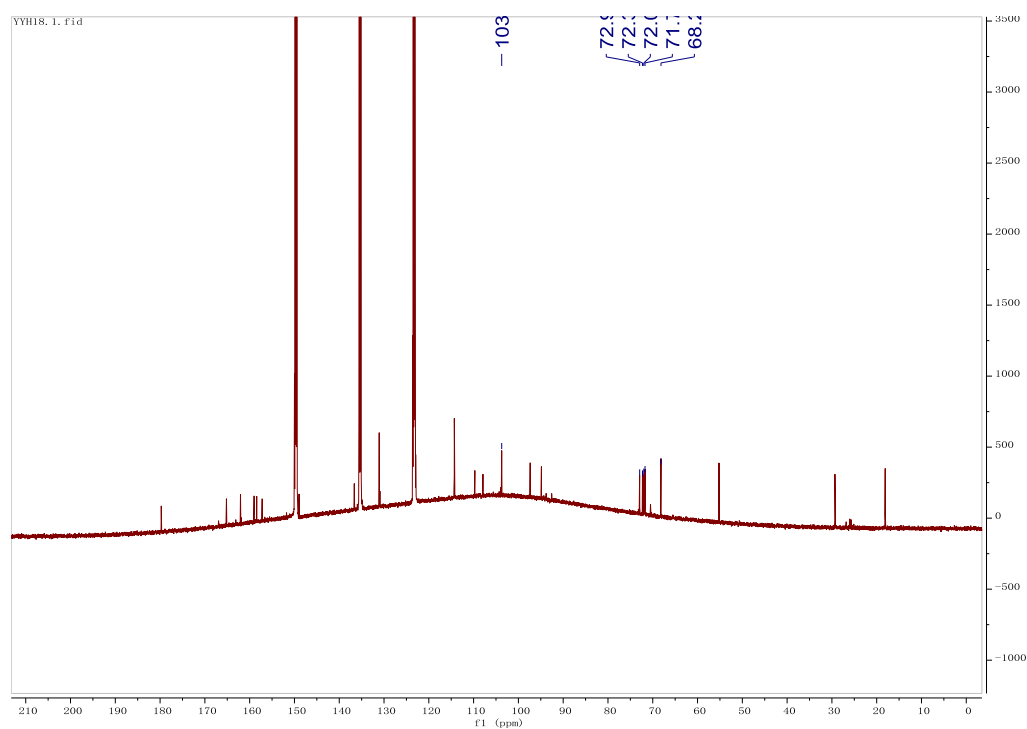


Figure S10 HRESIMS of compound **1**

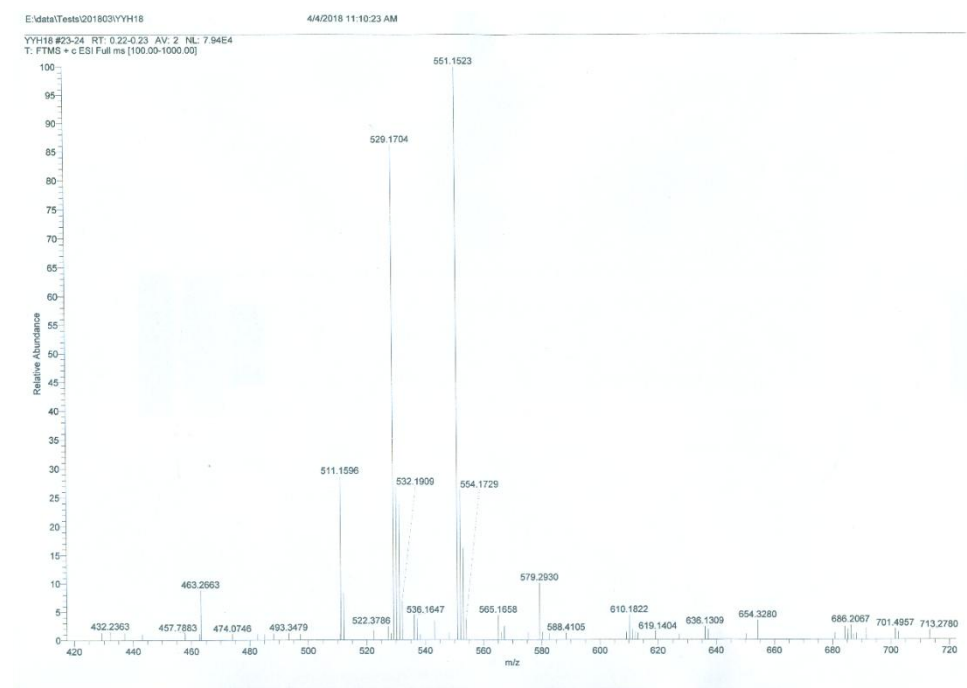


Figure S11 IR of compound **1** (KBr disc)

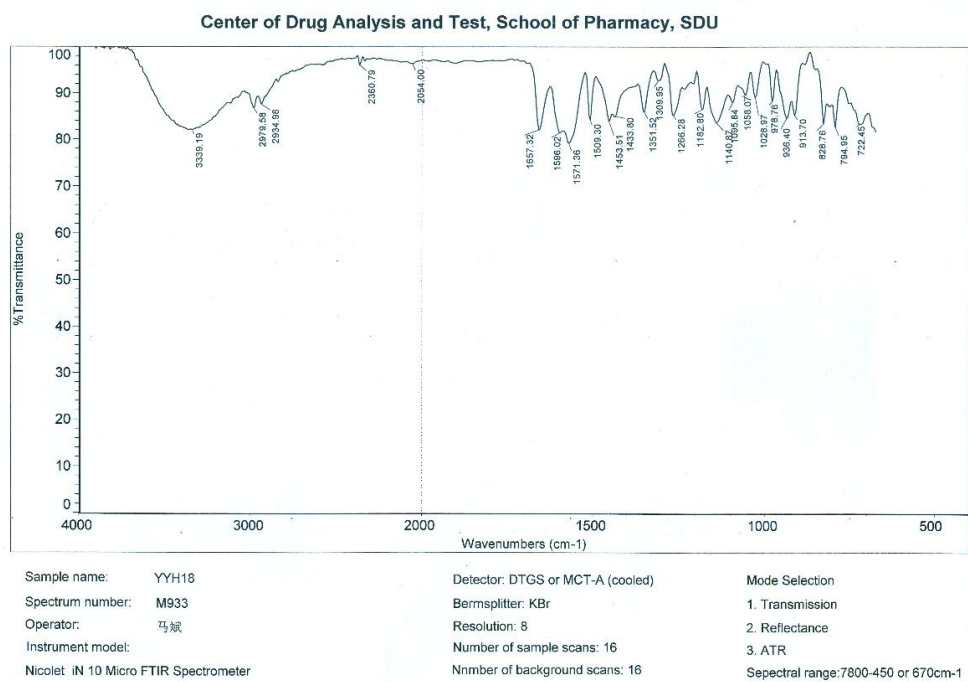


Figure S12 UV spectrum of compound **2** in methanol

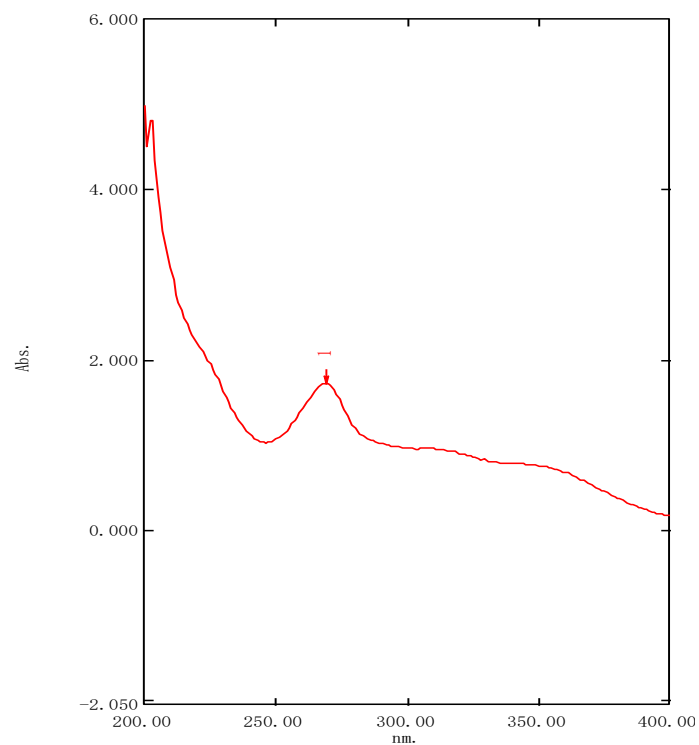


Figure S13 ^1H -NMR (600 MHz, $\text{DMSO-}d_6$) spectrum of compound **2**

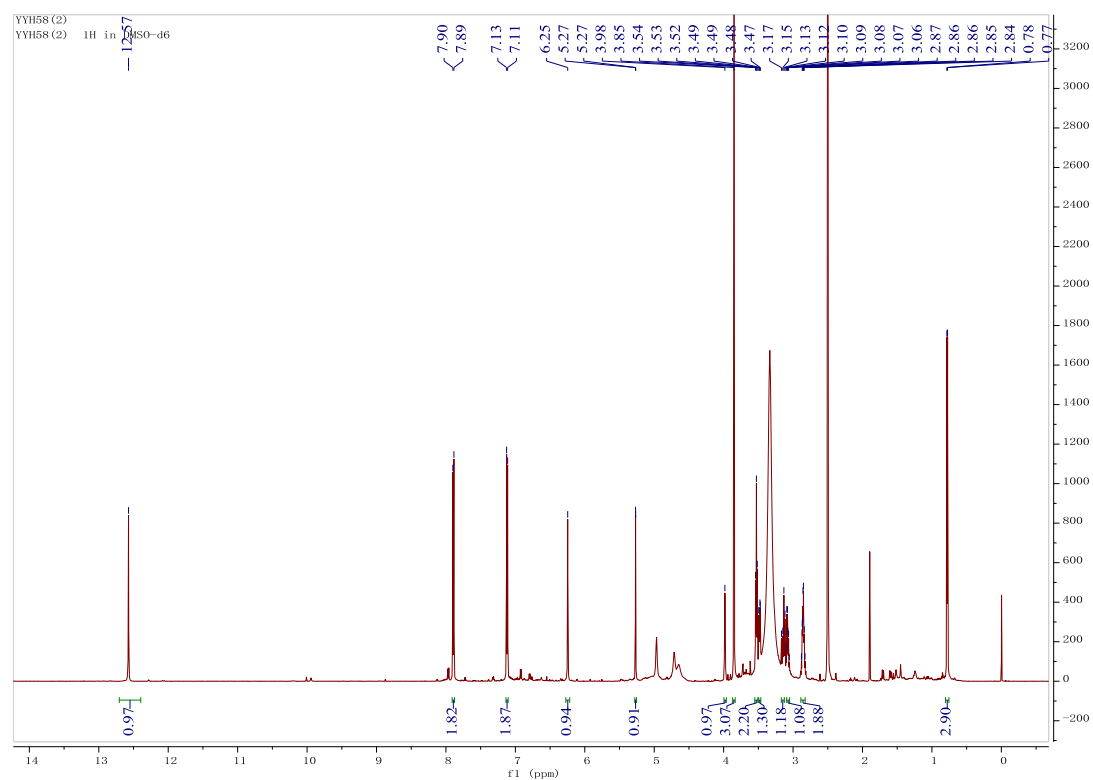


Figure S14 ^{13}C -NMR (150 MHz, $\text{DMSO-}d_6$) spectrum of compound **2**

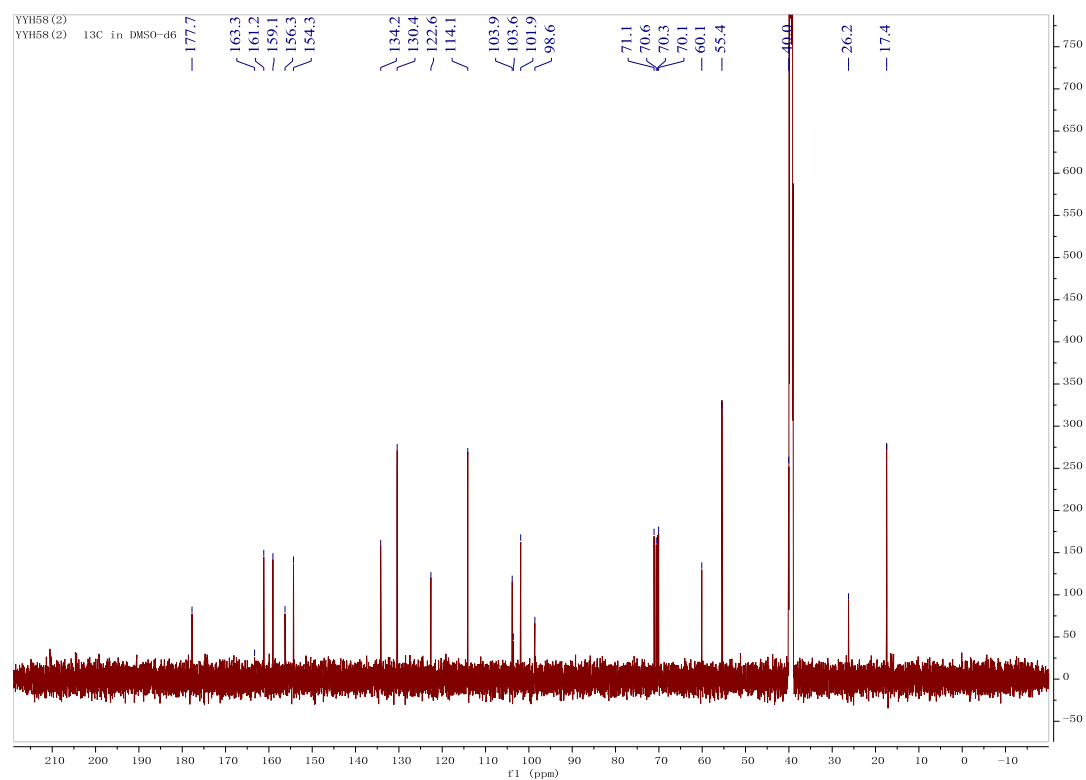


Figure S15 HSQC spectrum of compound **2**

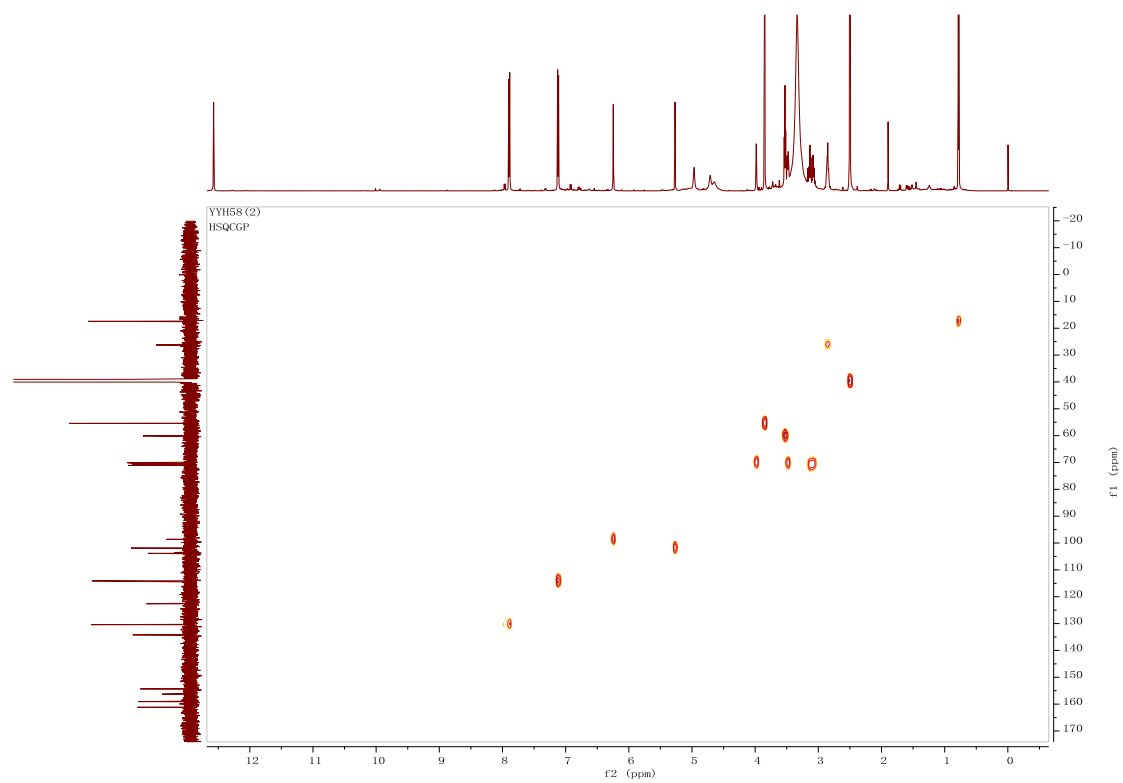


Figure S16 HMBC spectrum of compound **2**

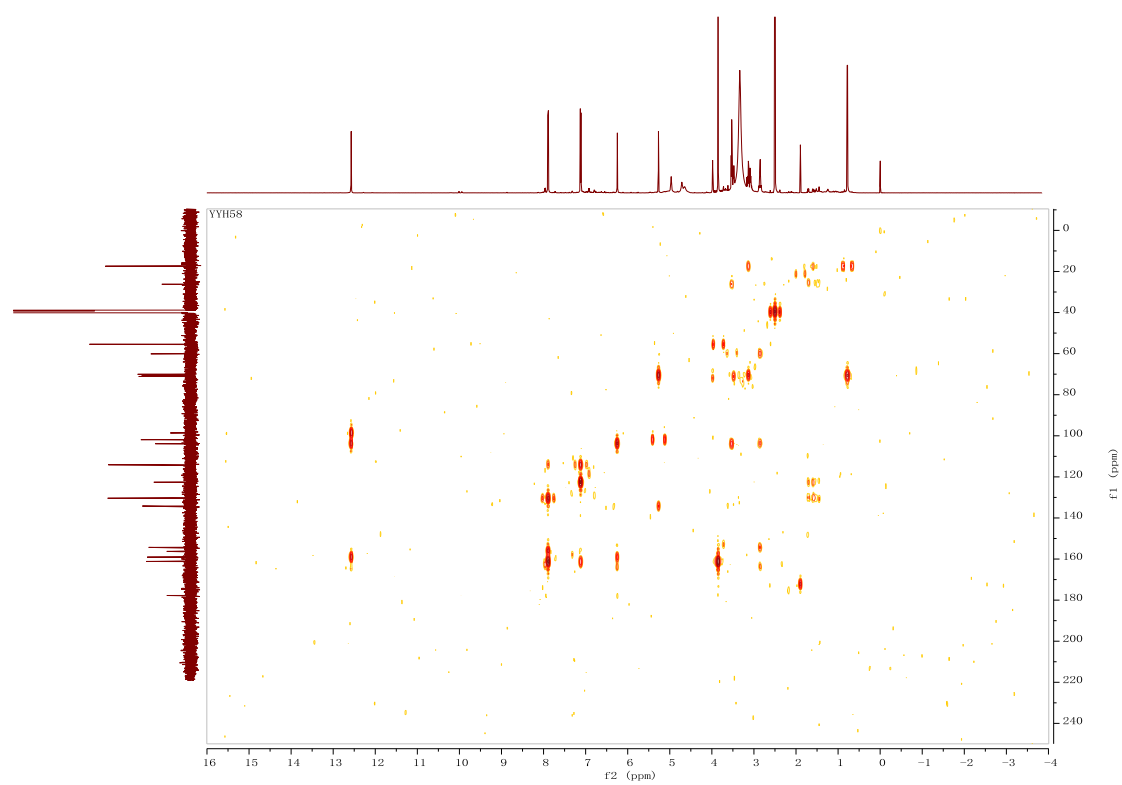


Figure S17 ^1H - ^1H COSY spectrum of compound **2**

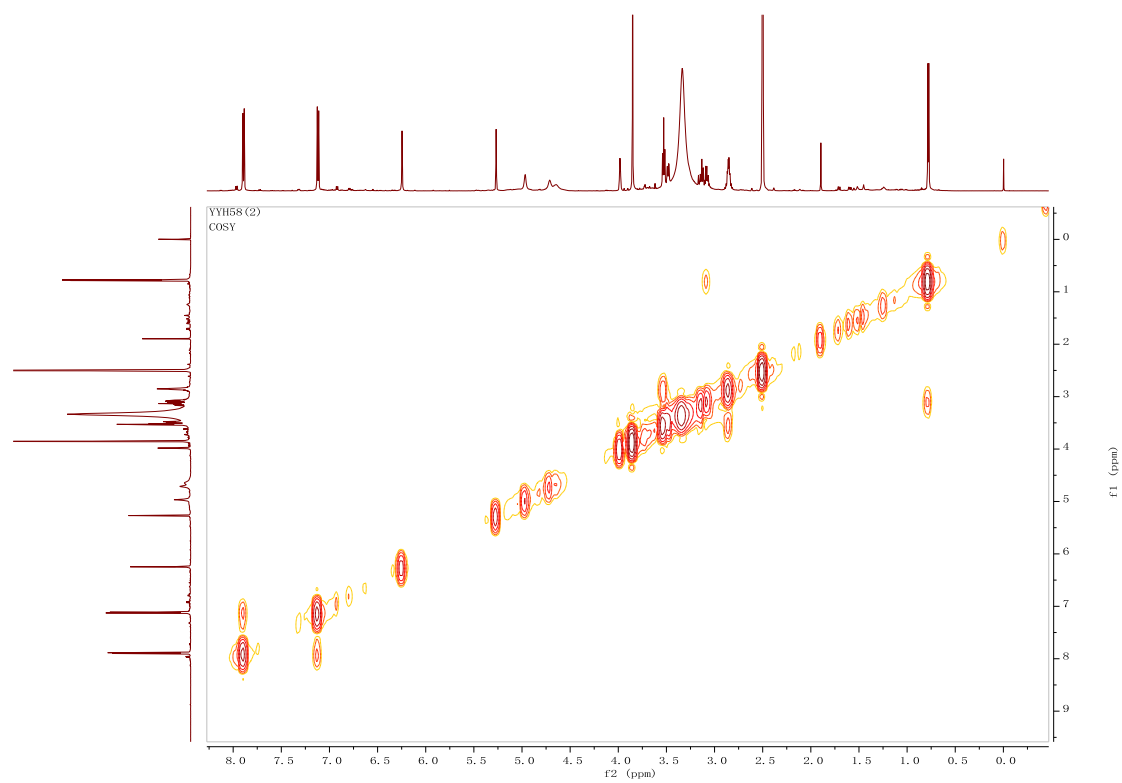


Figure S18 ^{13}C -NMR (150 MHz, pyridine- d_5) spectrum with gate decoupling of compound **2**

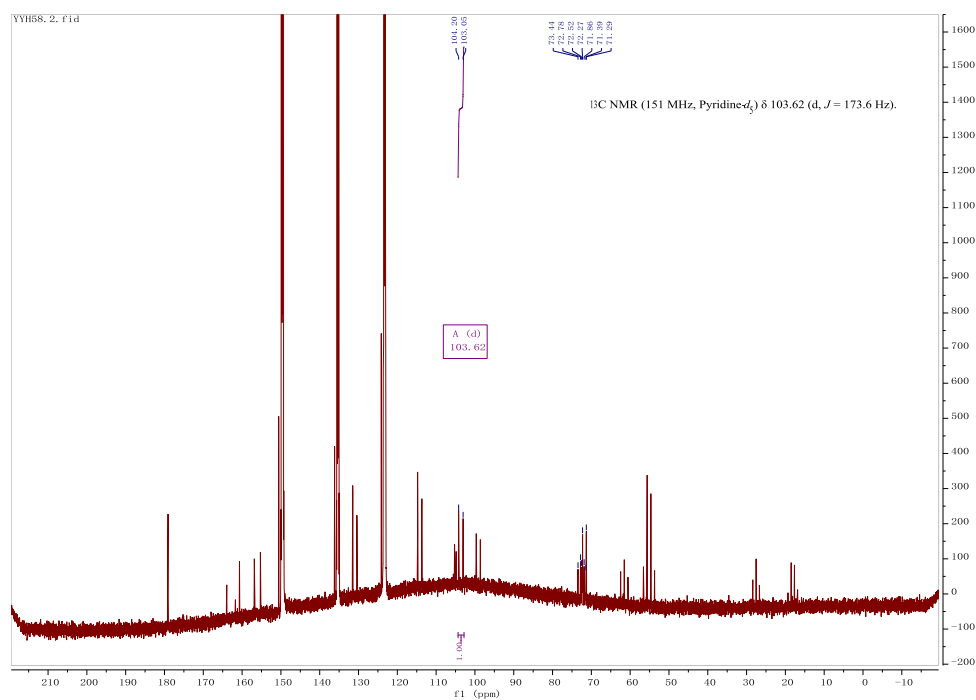


Figure S19 HRESIMS of compound **2**

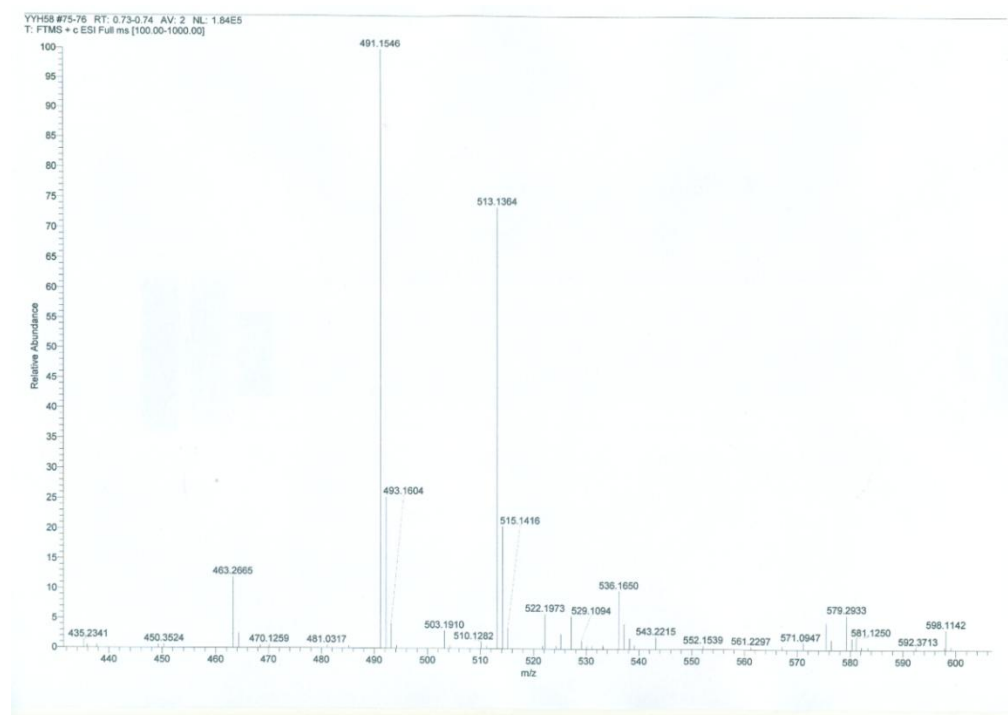


Figure S20 IR of compound **2** (KBr disc)

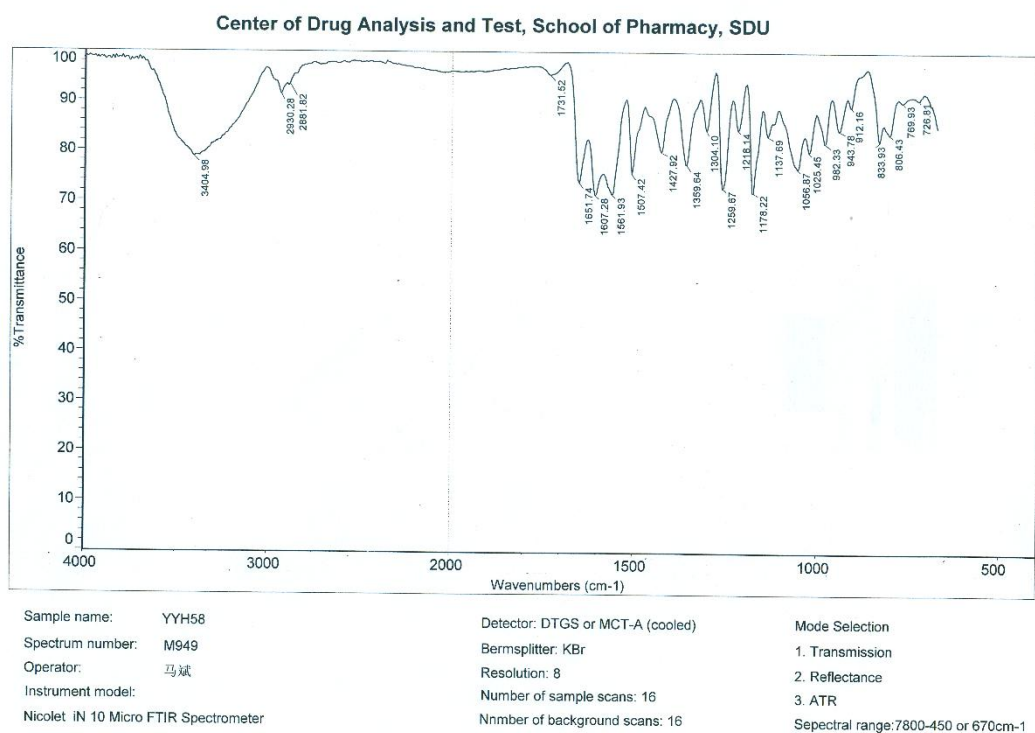


Figure S21 Real-time monitoring of cell viability treated with compound **6**

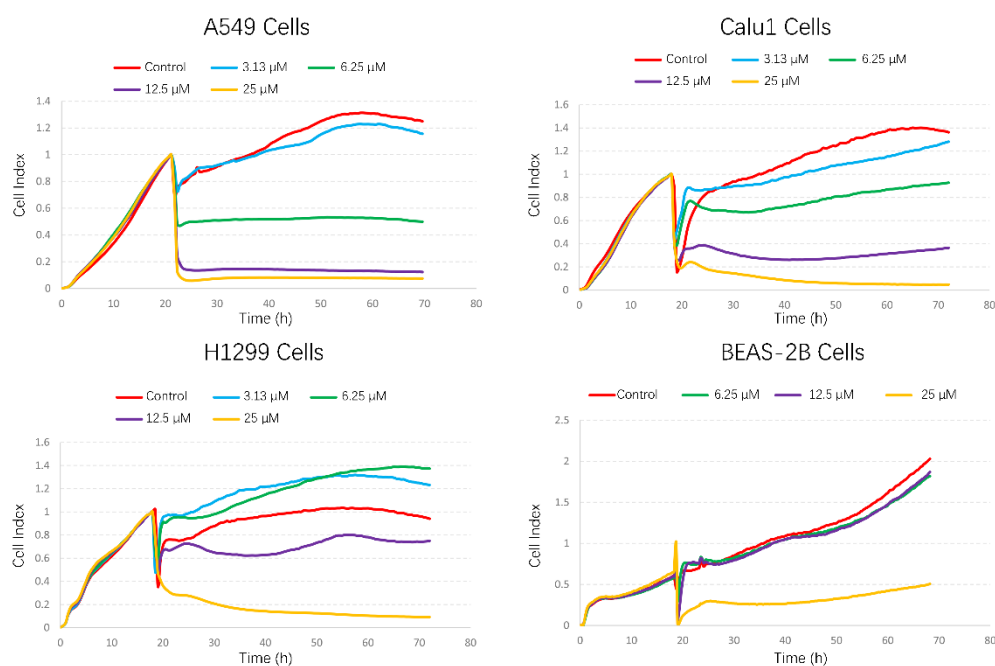


Table S1 ^1H and ^{13}C NMR data of compounds **1** and **2**^a (δ in ppm, J in Hz).

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		158.9		156.3
3		137.2		134.2
4		180.5		177.7
5	6.87, s	98.3		159.1
6		110.6	6.25, s	98.6
7		159.3		163.3
8	6.85, s	95.6		103.9
9		150.3		154.3
10		108.7		103.6
11		160.4	2.88, m	26.2
12		164.9	3.54, m	60.1
13		69.6		
14, 15	1.64, s	28.9		
1'		123.6		122.6
2'	7.95, d (8.5)	131.9	7.90, d (8.9)	130.4
3'	7.13, d (8.5)	115.3	7.13, d (8.9)	114.1
4'		163.6		161.2
5'	7.13, d (8.5)	115.3	7.13, d (8.9)	114.1
6'	7.95, d (8.5)	131.9	7.90, d (8.9)	130.4
4'-OMe	3.91, s	56.0	3.85, s	55.4

5-OH			12.57, s	
Rha				
1''	5.46 d (0.8)	103.5	5.27, d (0.9)	101.9
2''	4.26, s	72.1	3.98, s	70.1
3''	3.74, dd (9.2, 3.2)	72.1	3.48, dd (9.2, 3.1)	70.3
4''	3.36, m	73.1	3.17, m	71.1
5''	3.32, m	71.9	3.08, m	70.6
6''	0.92, d (6.0)	17.7	0.78, d (6.1)	17.4

^a NMR data (δ) were recorded for **1** at 600 MHz for ¹H and 150 MHz for ¹³C in methanol-*d*₄, and for **2** in DMSO-*d*₆.

Table S2 The effects of compounds on the proliferation of A549 and NCI-H292 cells

Compound	IC ₅₀ (μM) for 48 h	
	A549	NCI-H292
1	>50	>50
2	>50	>50
3	18.75	17.17
4	>50	33.96
5	>50	6.439
6	7.901	5.689
7	13.89	14.75
8	18.31	23.53

CDDP	11.43	6.956
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