Supplementary materials

Two New Bioactive Lignans From Leaves and Twigs of *Cleistanthus concinnus* croizat

Shi-rui Fan [a,b], Jing-jing Guo [a,] , Yi-ting Wang [b], Bi-juan Yang [a,], Duo-zhi Chen [a*] and Xiao-jiang Hao[a*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, PR China

[b] Department of Chemical Science and Engineering, Yunnan University, Kunming 650091, Yunnan, PR China

ABSTRACT

Two new lignans (**1**-**2**), along with five known compounds (**3**-**7**) with different structures were isolated from leaves and twigs of *Cleistanthus concinnus* croizat. The new lignans were elucidated as (7'R,8'S)-3,3',5'-trimethoxy-4,4'-dihydroxy-7-en-7',9-epoxy-8,8'-lignan (**1**) and (7'R,8'S)-3,3'-dimethoxy-4,4'-dihydroxy-7-en-7',9-epoxy-8,8'-lignan (**2**) by comprehensive spectroscopic analysis including 1D and 2D NMR as well as HREIMS and comparing their NMR data with known compounds in the literature. Among these isolated compounds, compound **1**, **2**, **3**, and **6** were tested for anti- inflammatory effects by inhibiting NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.

*Corresponding authors.

E-mail addresses: Xiao-Jiang Hao, haoxj@mail.kib.ac.cn; Duo-Zhi Chen, chenduozhi@mail.kib.ac.cn,

Contents

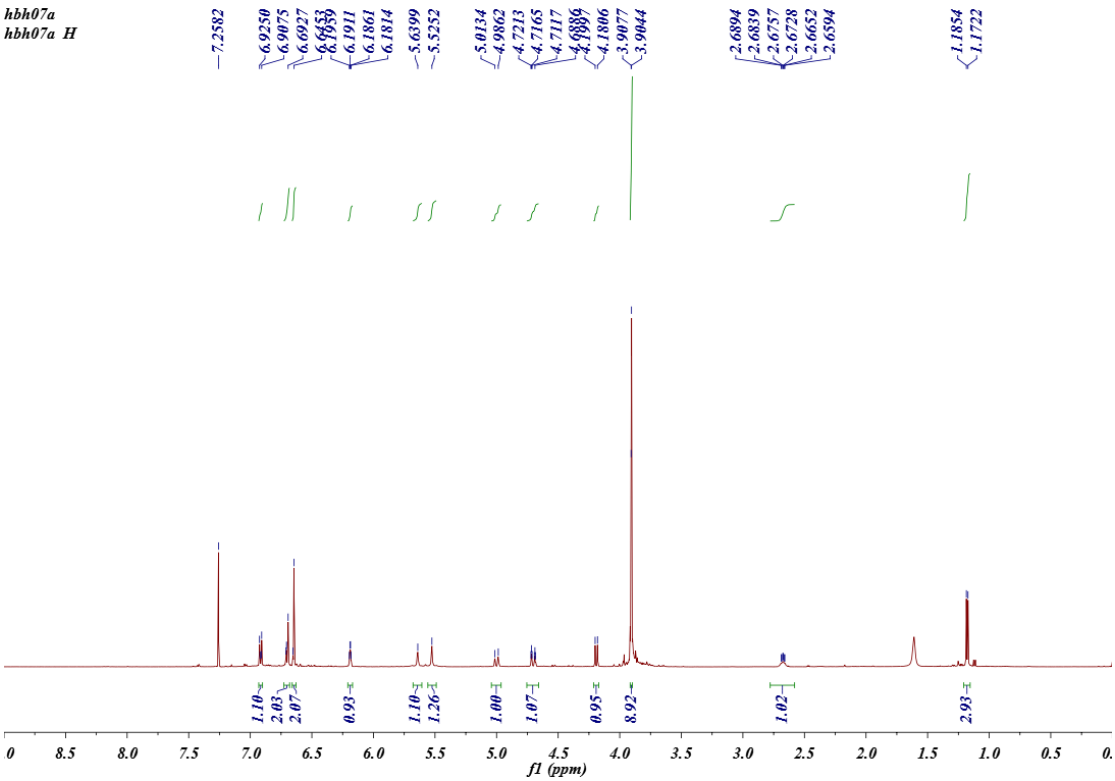


Figure S1. ^{1}H NMR (500 MHz) spectrum of compound **1** in $CDCl_3$.

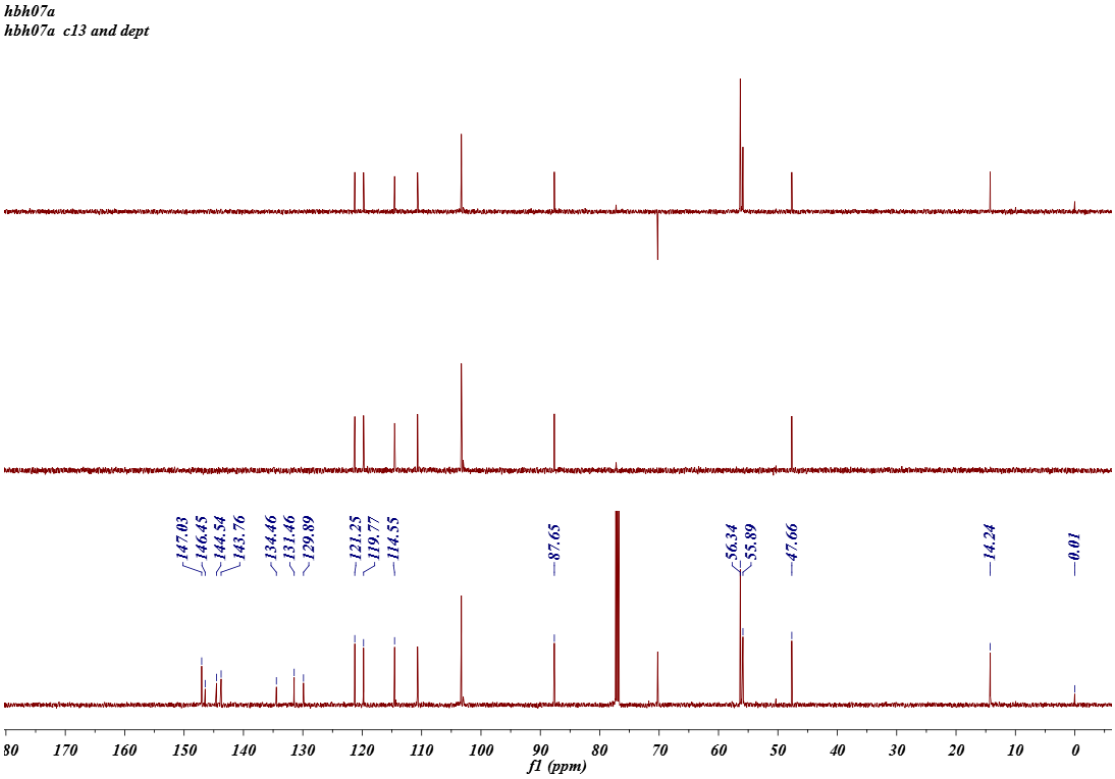


Figure S2. ^{13}C NMR and DEPT (125 MHz) spectrum of compound **1** in $CDCl_3$.

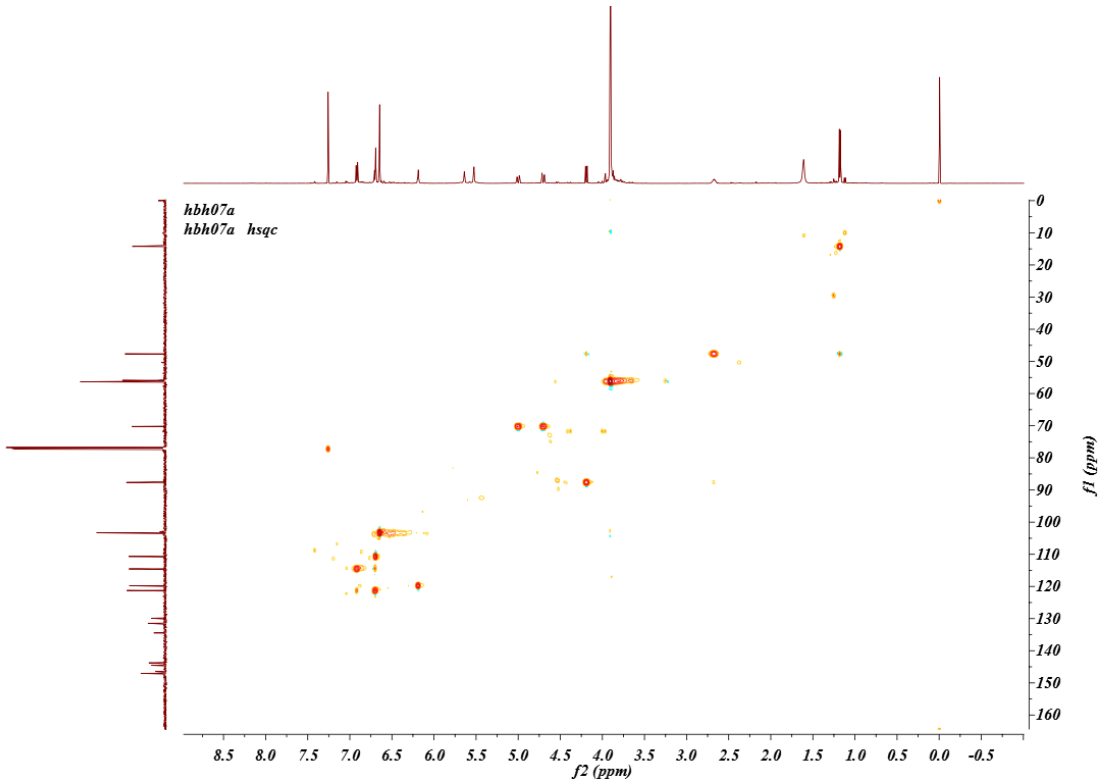


Figure S3. HSQC spectrum of compound **1** in $CDCl_3$.

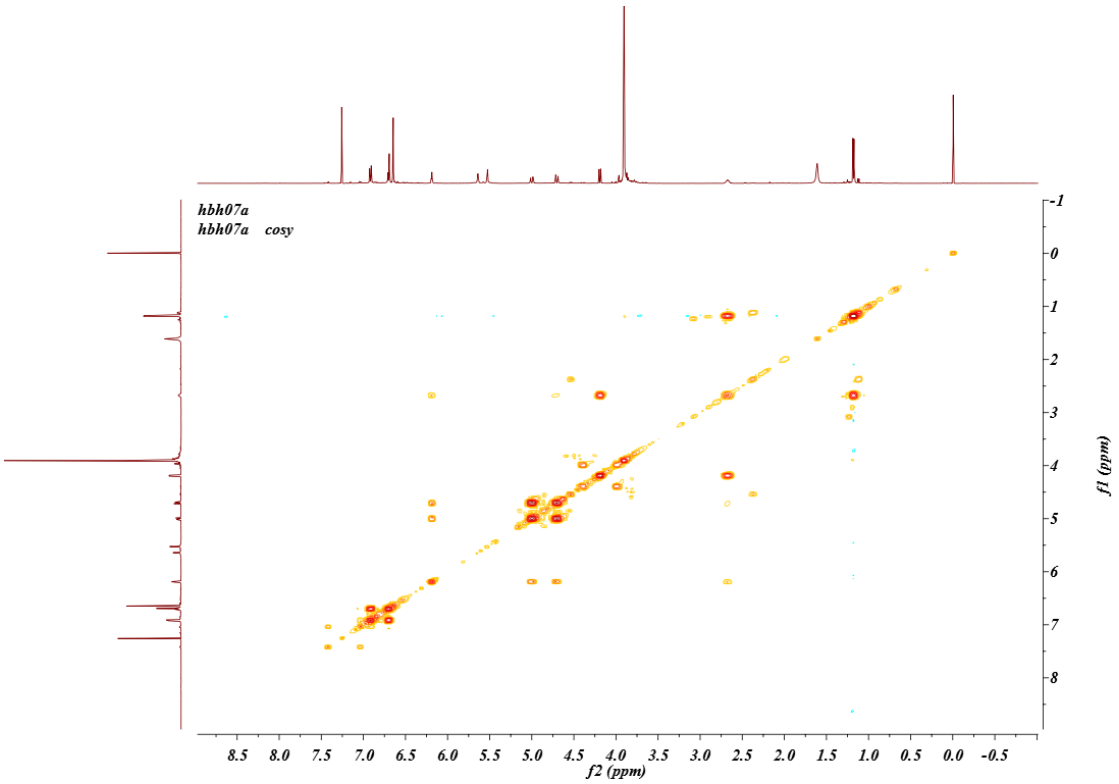


Figure S4. ^{1}H-^{1}H COSY spectrum of compound **1** in $CDCl_3$.

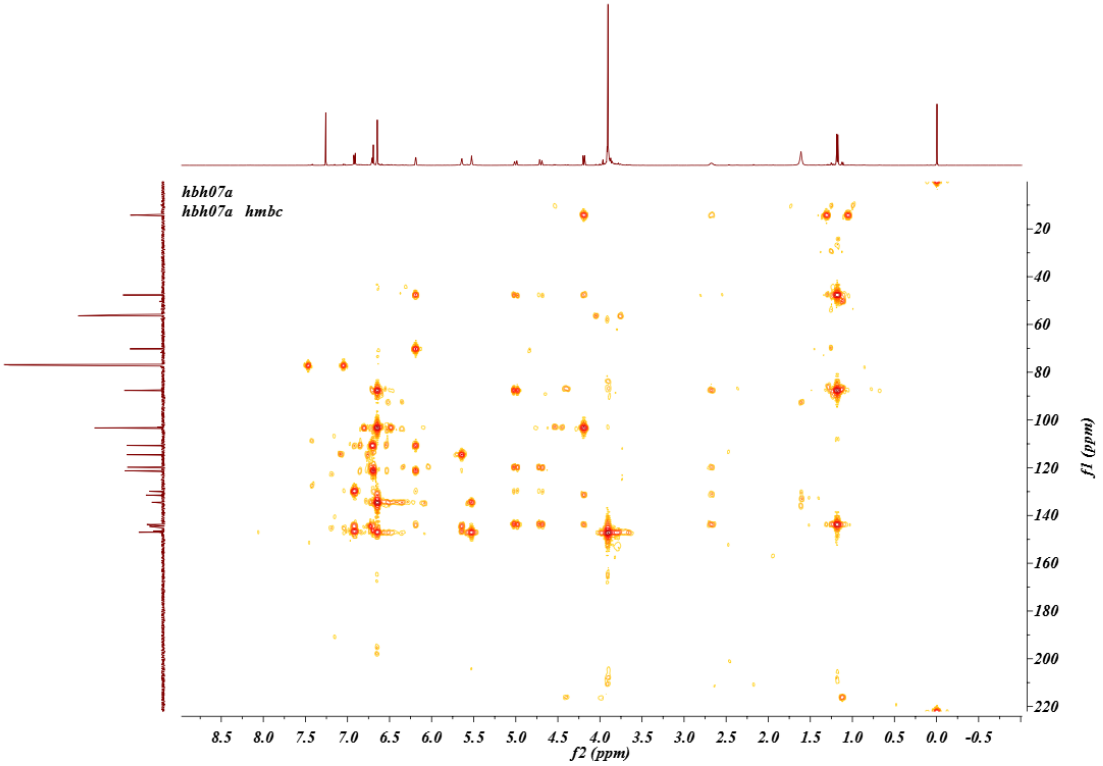


Figure S5. HMBC spectrum of compound **1** in $CDCl_3$.

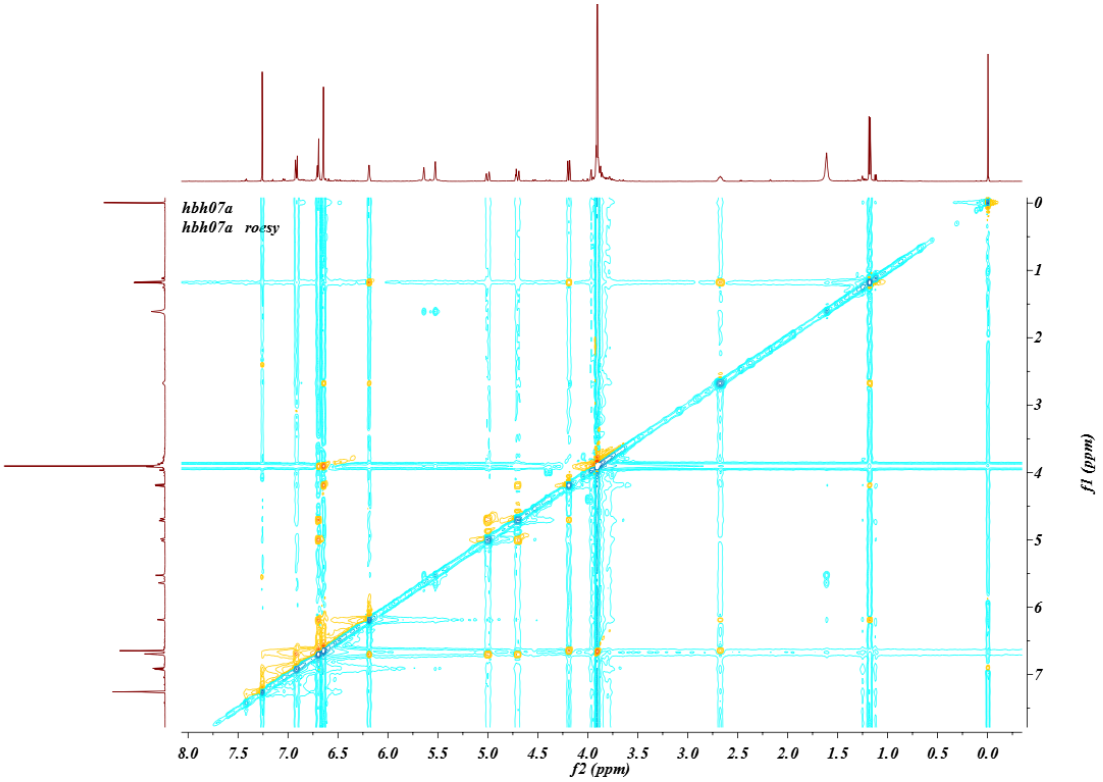
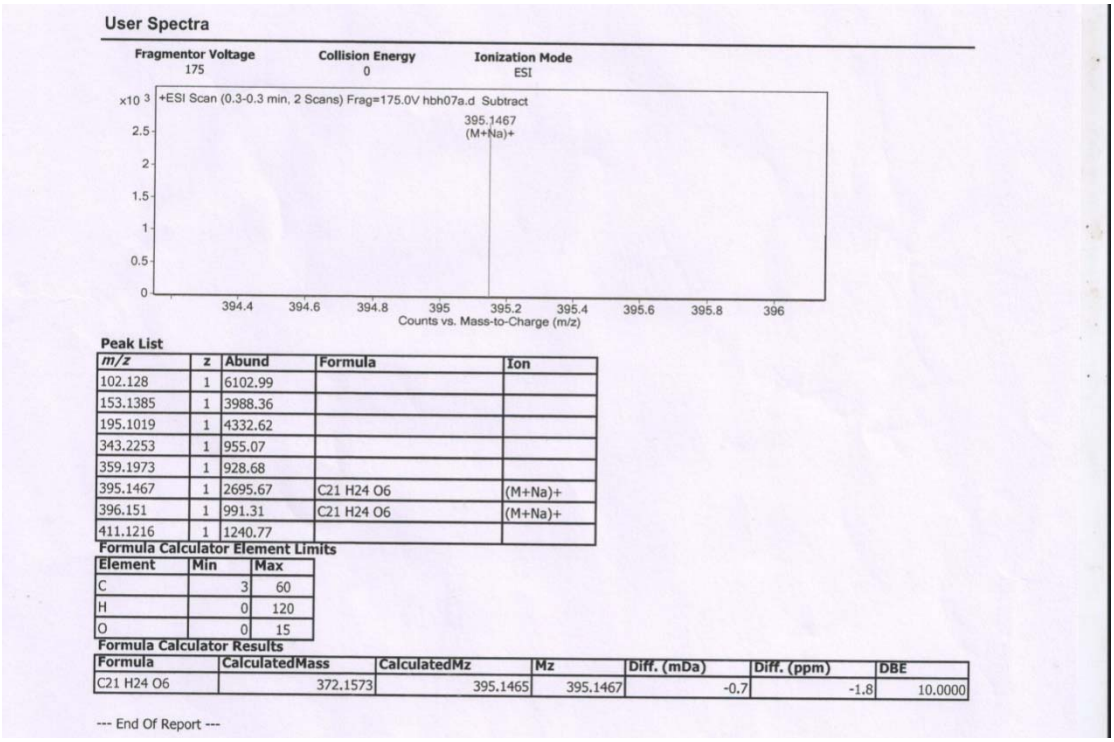


Figure S6. ROESY spectrum of compound **1** in $CDCl_3$.



Peak List

m/z	z	Abund	Formula	Ion
102.128	1	6102.99		
153.1385	1	3988.36		
195.1019	1	4332.62		
343.2253	1	955.07		
359.1973	1	928.68		
395.1467	1	2695.67	C21 H24 O6	(M+Na)+
396.151	1	991.31	C21 H24 O6	(M+Na)+
411.1216	1	1240.77		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	15

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C21 H24 O6	372.1573	395.1465	395.1467	-0.7	-1.8	10.0000

--- End Of Report ---

Figure S7. HRESI(+)MS spectrum of compound **1**.

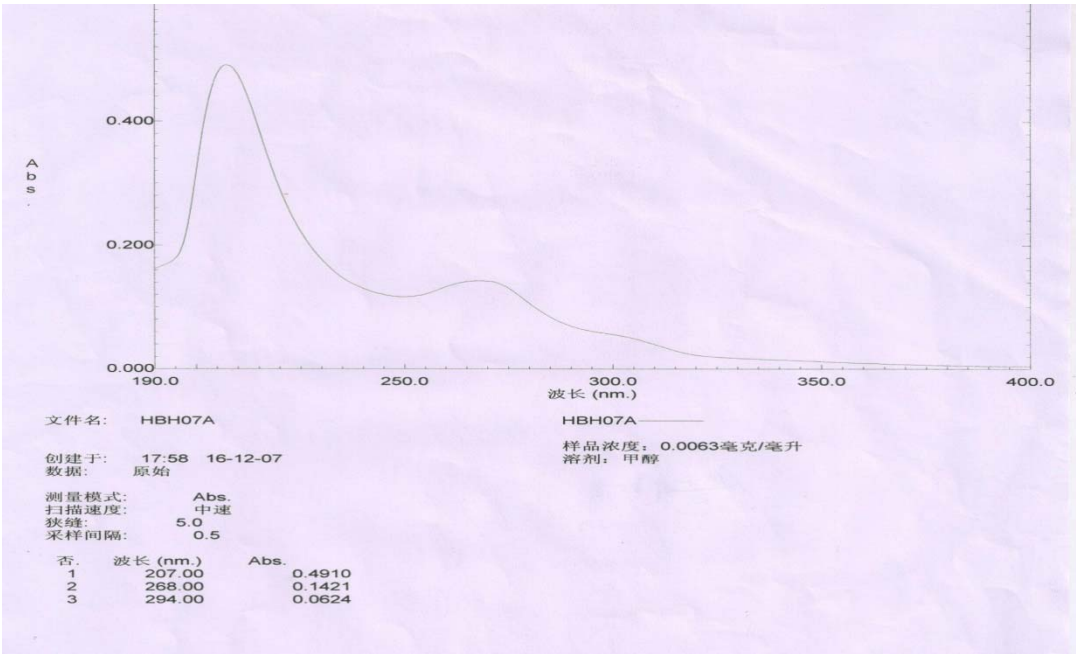


Figure S8. UV spectrum of compound **1**.

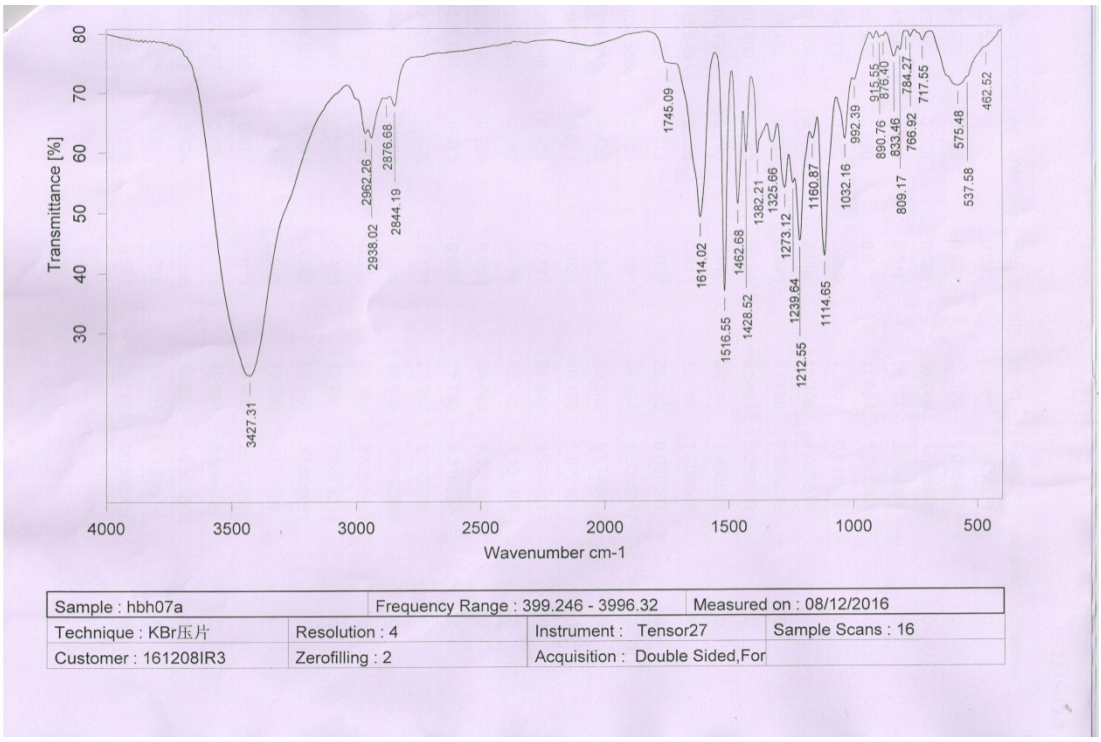


Figure S9.IR spectrum of compound **1**.

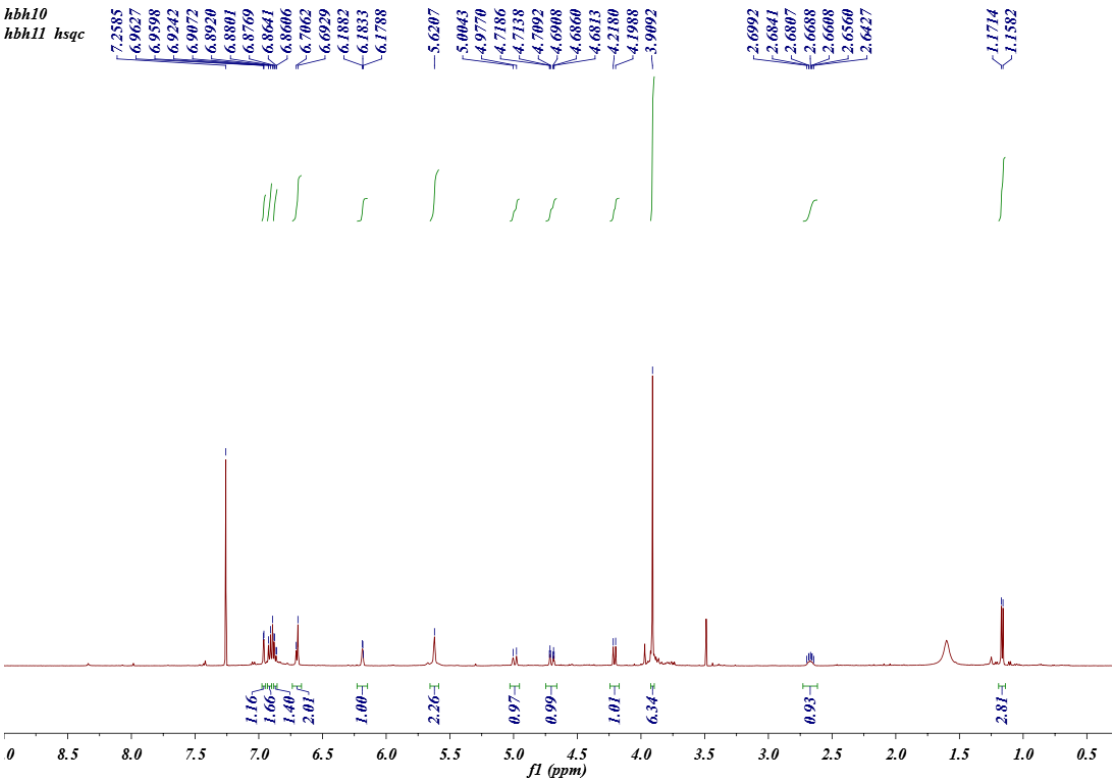


Figure S10. ^{1}H NMR (500 MHz) spectrum of compound **2** in $CDCl_3$.

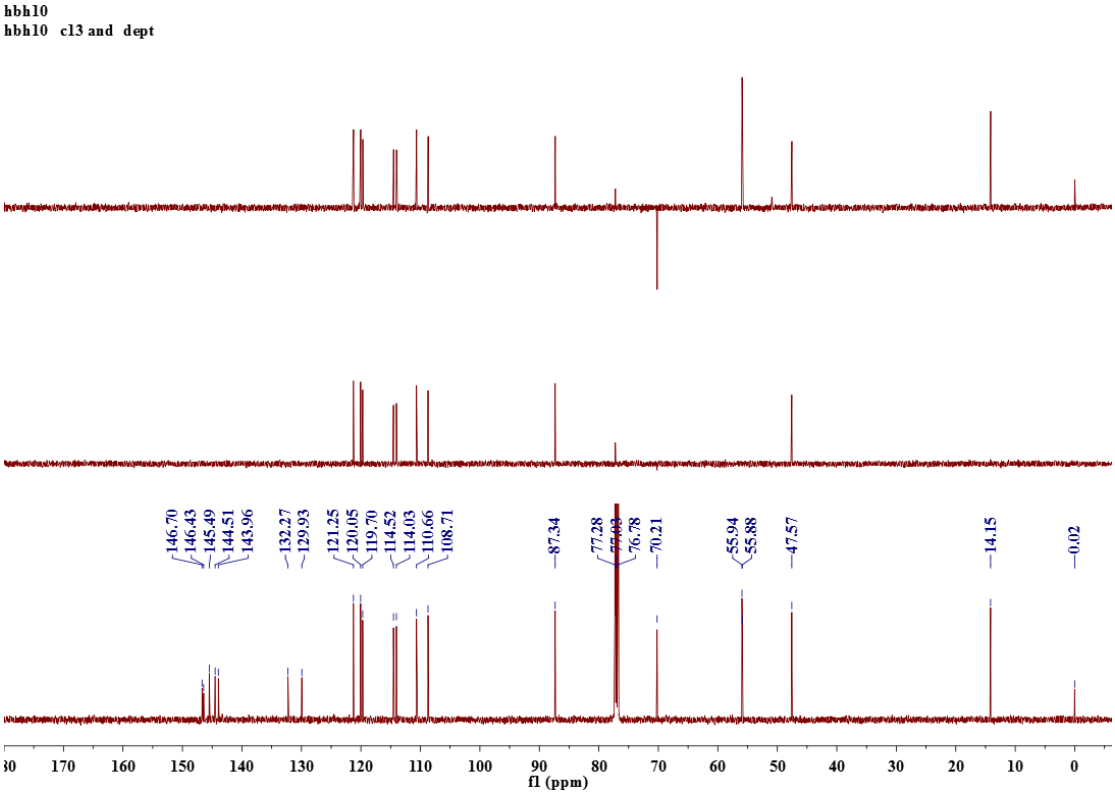


Figure S11. ^{13}C NMR and DEPT (125 MHz) spectrum of compound **2** in $CDCl_3$.

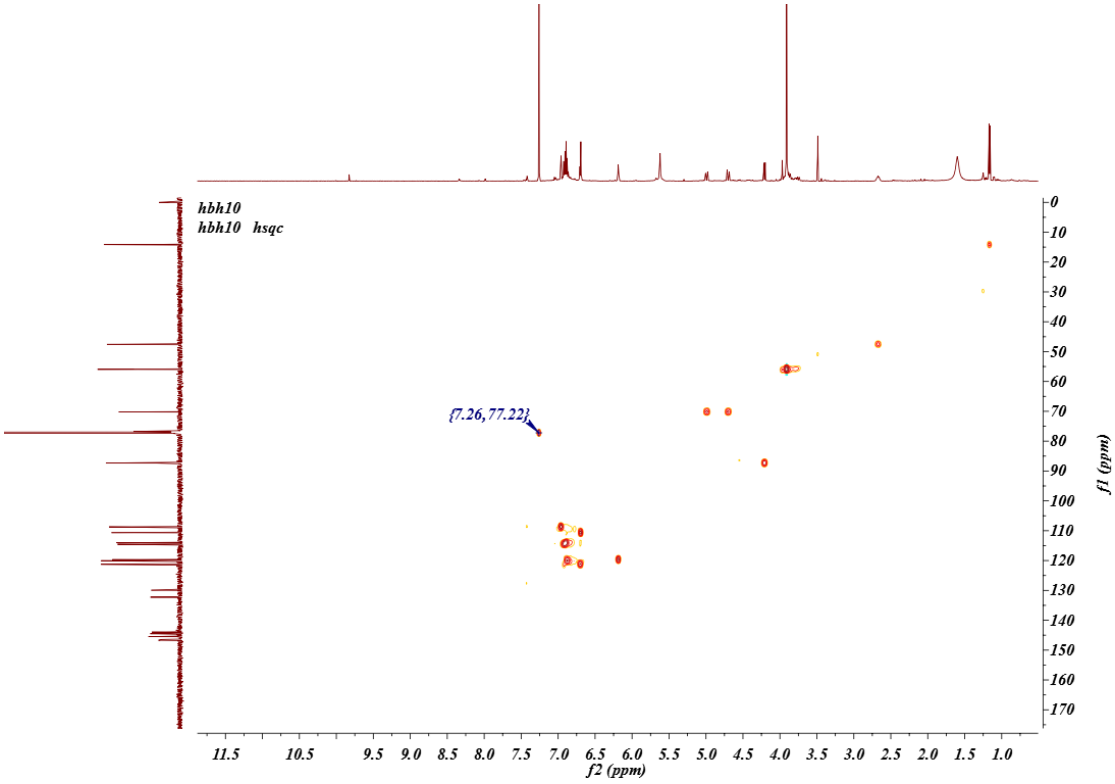


Figure S12. HSQC spectrum of compound **2** in $CDCl_3$.

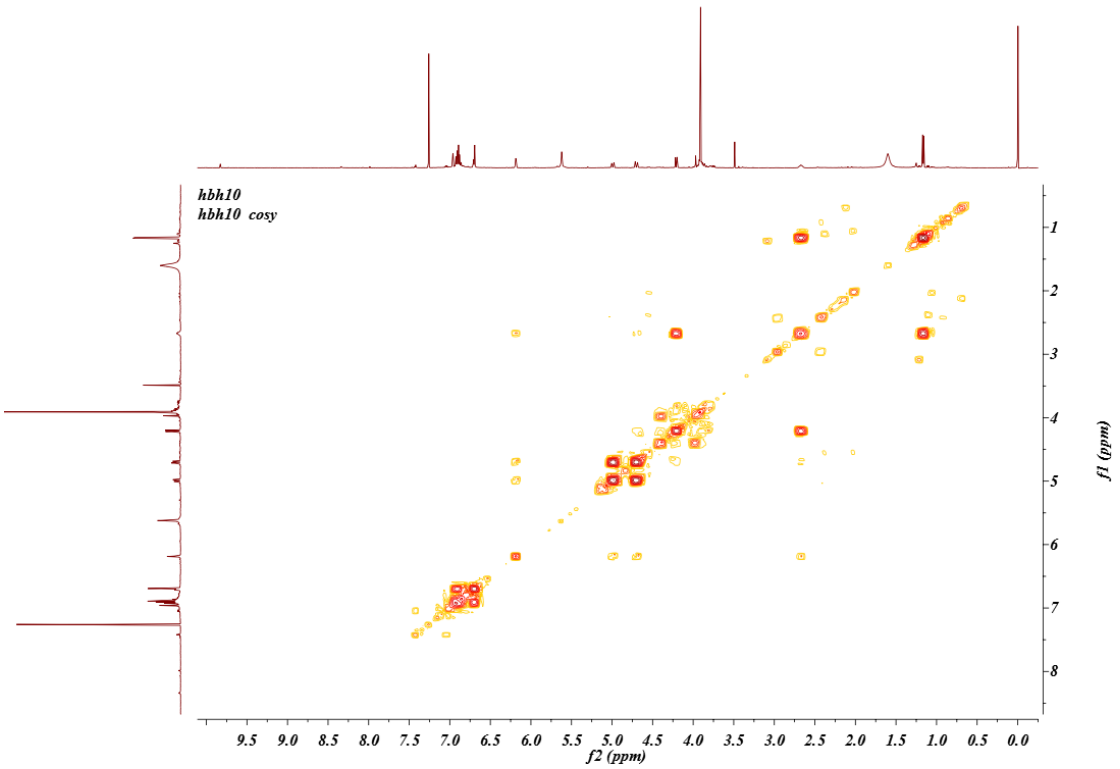


Figure S13. ^{1}H-^{1}H COSY spectrum of compound **2** in $CDCl_3$.

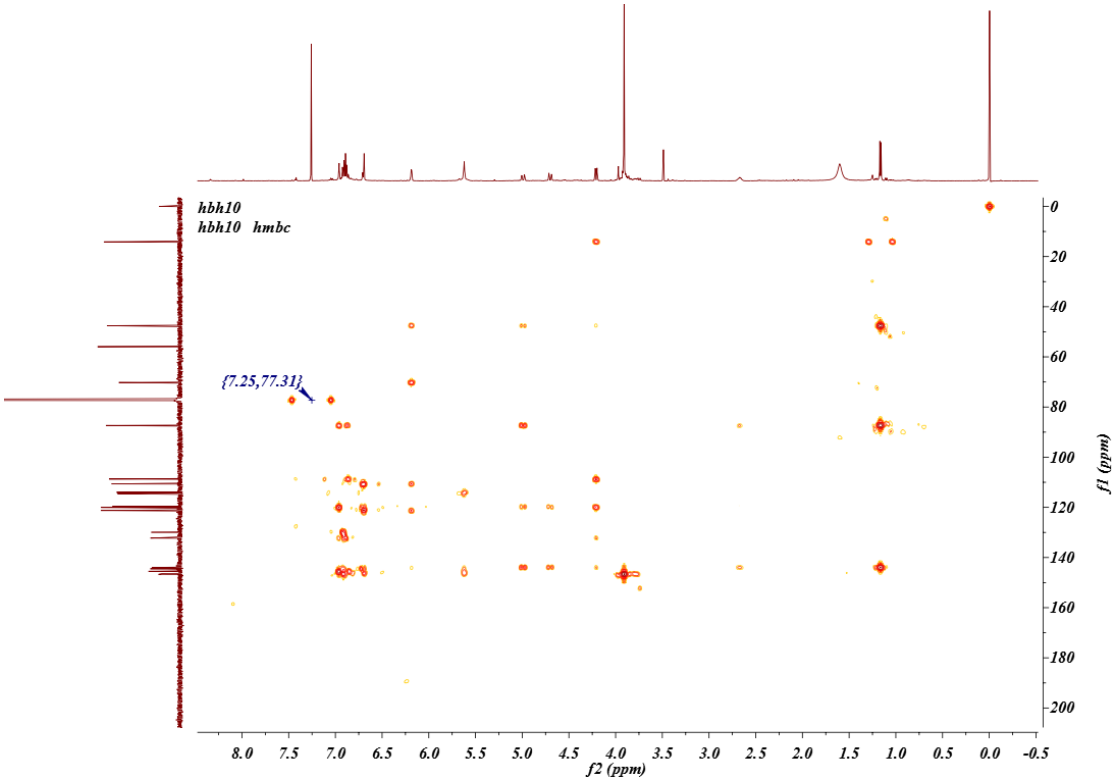


Figure S14. HMBC spectrum of compound **2** in $CDCl_3$.

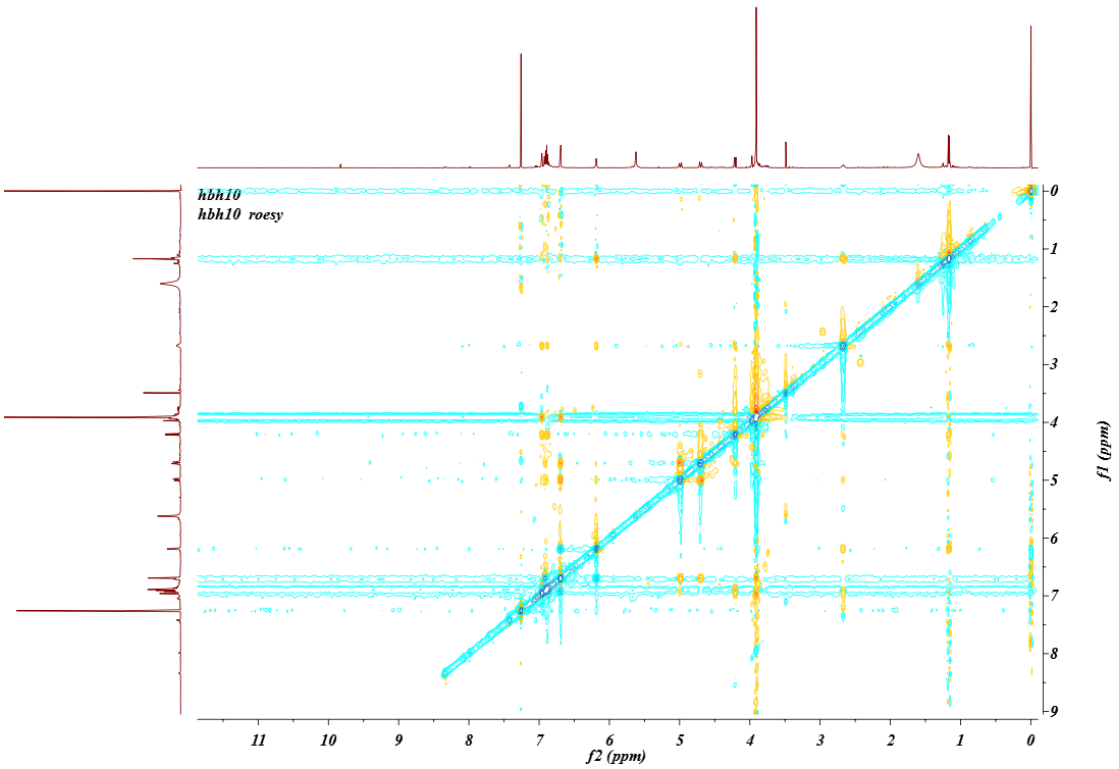
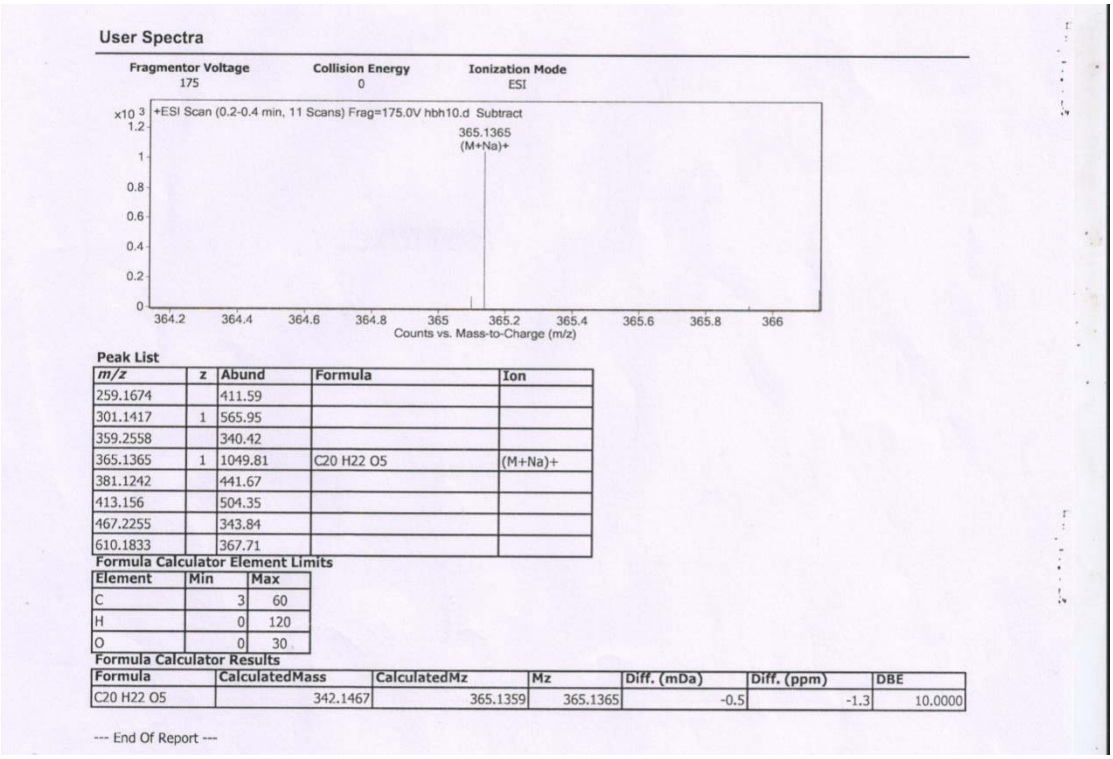


Figure S15. ROESY spectrum of compound **2** in $CDCl_3$.



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
175	0	ESI

Peak List

m/z	z	Abund	Formula	Ion
259.1674		411.59		
301.1417	1	565.95		
359.2558		340.42		
365.1365	1	1049.81	C20 H22 O5	(M+Na)+
381.1242		441.67		
413.156		504.35		
467.2255		343.84		
610.1833		367.71		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C20 H22 O5	342.1467	365.1359	365.1365	-0.5	-1.3	10.0000

--- End Of Report ---

Figure S16. HRESI(+)MS spectrum of compound **2**.

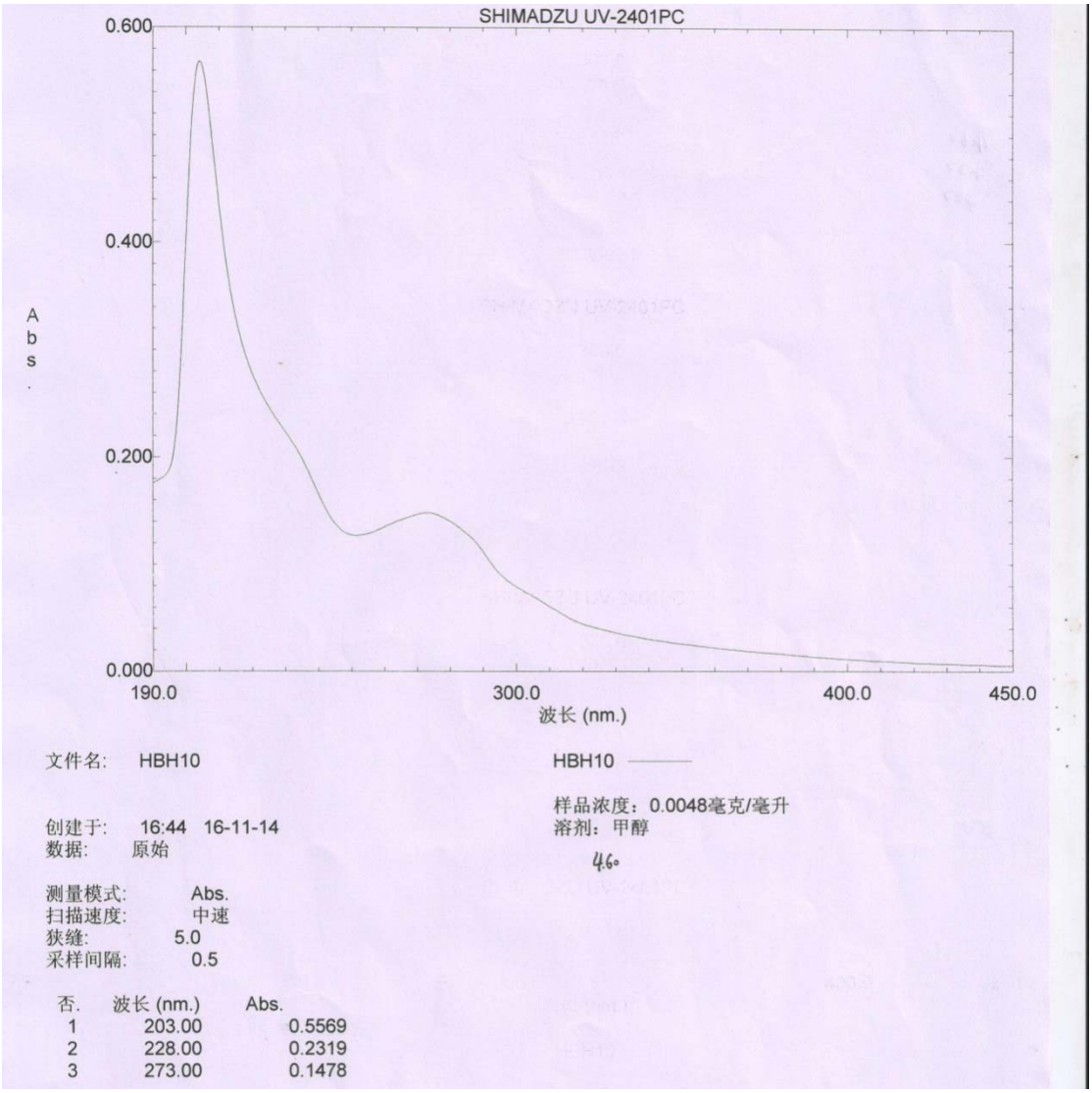


Figure S17. UV spectrum of compound**2**.

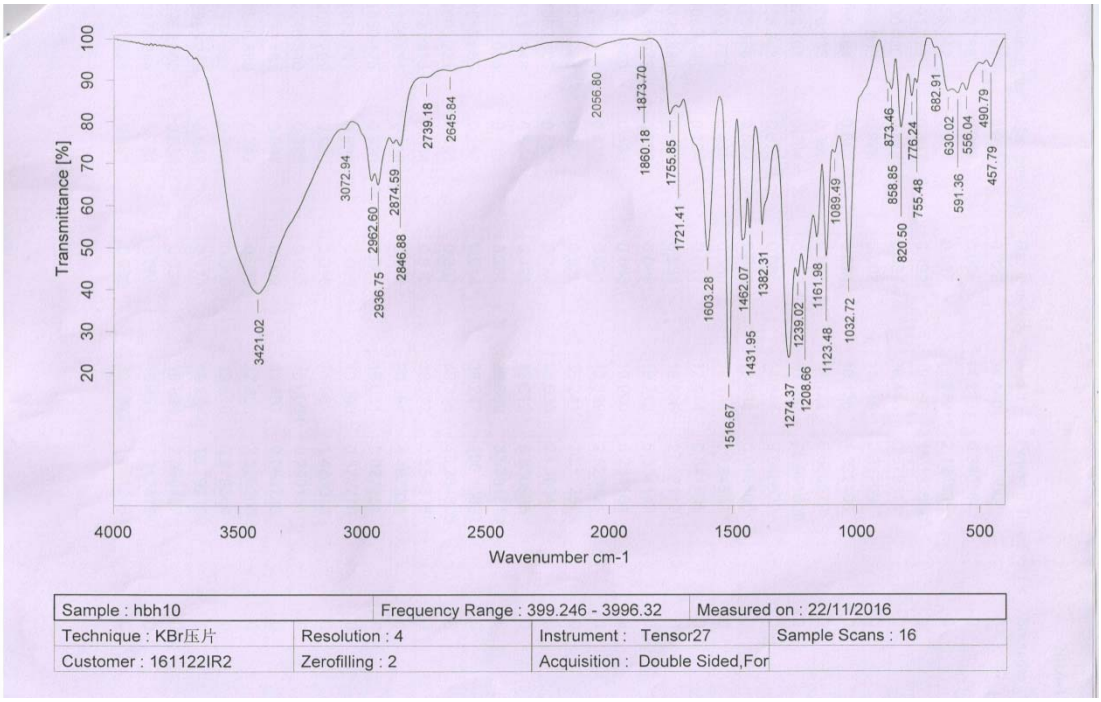


Figure S18.IR spectrum of compound **2**.

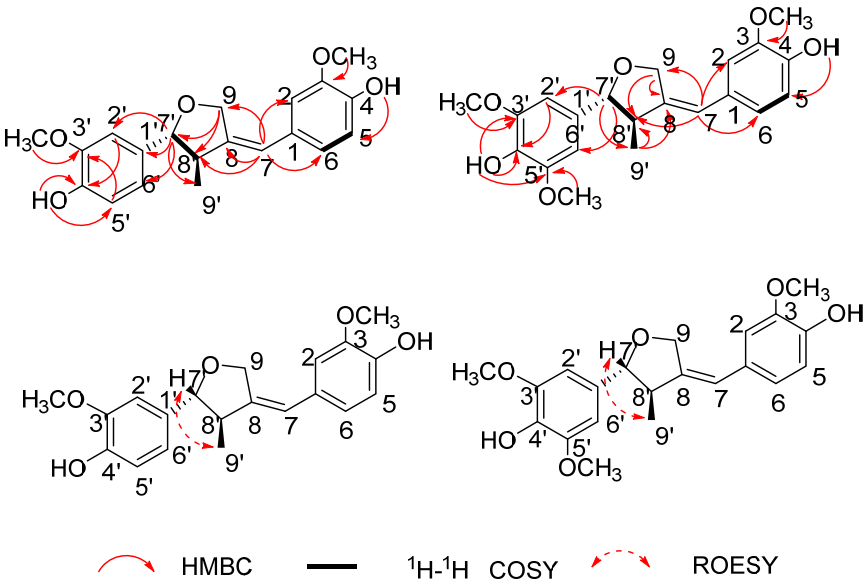


Figure S19. Key ^{1}H-^{1}H COSY, HMBC and ROSEY correlations of compound **1** and compound 2.

Table S1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1-2** in $CDCl_3$.

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1		129.9		129.9
2	6.7 (d, 1.8)	110.7	6.69 (d, 1.8)	110.7
3		144.5		144.5
4		146.5		146.4
5	6.92 (d, 8.8)	114.6	6.90 (d, 8.4)	114.0
6	6.7[a]	121.3	6.7[a]	121.3
7	6.19 (m)	119.8	6.19 (m)	119.7
8		143.8		144.0
9	5.00 (m),4.7 (m)	70.2	4.99 (m), 4.7 (m)	70.2
1'		131.5		132.3
2'	6.65 (s)	103.3	6.96 (d, 1.5)	108.7
3'		147.0		146.7
4'		134.5		145.5
5'		147.0	6.90 (d, 8.4)	114.5
6'	6.65 (s)	103.3	6.87 (dd, 8.4, 1.5)	120.0
7'	4.19 (d, 9.6)	87.6	4.21 (d, 9.6)	87.3
8'	2.68 (m)	47.7	2.67 (m)	47.6
9'	1.18 (d, 6.6)	14.2	1.17 (d, 6.6)	14.2
4-OH	5.64(brs)		5.62 (brs)	
4'-OH	5.53 (brs)		5.62 (brs)	
3-OCH_3	3.91 (s)	55.9	3.91 (s)	55.9
3'-OCH_3	3.90 (s)	56.3	3.91 (s)	55.9
5'-OCH_3	3.90 (s)	56.3		

[a]Overlapped signals are reported without designating multiplicity.

Table S2. Inhibitory effects on NO production in LPS-stimulated RAW 264.7 cells.

Compound	Concentration (μM)	NO inhibition rate(%)
L-NMMA[a]	50	54.02±0.91
1	25	39.85±1.34
2	25	25.18±4.46
3	25	-0.47±0.82
6	25	28.65±1.93

[a]L-NMMA (NG-monomethyl-L-arginine): positive control.

Table S3. IC_{50} of Inhibitory effects on NO production of **1**, **2**, and **6**

Compounds	IC_{50} (μM)	
	Mean	SD
1	45.1	8.1
2	53.6	9.0
6	62.3	7.8
L-NMMA	38.6	7.6