Supporting Information

Rec. Nat. Prod. 16:6 (2023) 1090-1094

5,6-Dihydroxypyranoflavone, a New Flavonoid with an Oxidized Prenyl Group from Dietary plant *Citrus hystrix*

Jin-Rui Zhang[1,3], Fu-Jin-Wen Li[2]* and Long-Teng Cui[1]*

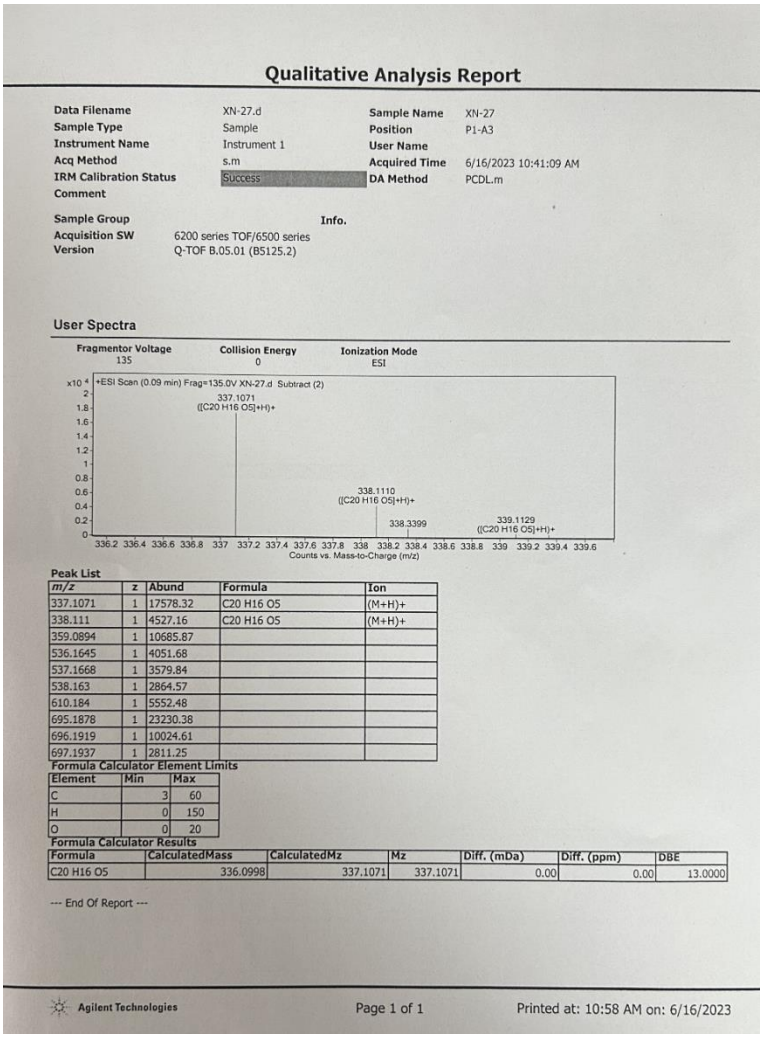
[1] *School of Physical Education, Yunnan Minzu University, Kunming 650031, P.R. China.*

[2] *Yunnan Association of Minority Sports, Kunming 650500, P.R. China.*

[3] *School of Physical Education, Neijiang Normal University, Neijiang 641100, P.R. China.*

Table of Contents	Page
Figure S1: The HR-ESI-MS spectrum of **1**	3
Figure S2: The ESI-MS spectrum of **1**	3
Figure S3: The ^{1}H-NMR (400 MHz, DMSO-*d*6) spectrum of **1**	4
Figure S4: The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **1**	4
Figure S5: The ^{13}C NMR (100 MHz, DMSO-*d*6) spectrum of **1**	5
Figure S6: The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1**	5
Figure S7: The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of synthetic **1**	6
Figure S8: The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of synthetic **1**	6
Figure S9: The ^{13}C NMR (100 MHz, DMSO-*d*6) spectrum of **1** (From δ_C 120 to 150 ppm)	7
Figure S10: The HSQC spectrum of **1**	7
Figure S11: The HSQC spectrum of **1** (From δ_C 100 to140 ppm)	8
Figure S12: The ^{1}H-^{1}H COSY spectrum of **1**	8
Figure S13: The ^{1}H-^{1}H COSY of H-1"/H-2", H-2'(6')/H-3'(5'), and H-2'(6')/H-4' of **1**	9
Figure S14: The HMBC spectrum of **1**	9
Figure S15: The HMBC correlations of 5-O<u>H</u> to C-5, C-6 and C-10 of **1**	10
Figure S16: The HMBC correlations of C<u>H</u>$_3$-4"/5" to C-3" and C-2" of **1**	10
Figure S17: The HMBC correlations of C<u>H</u>-1" to C-7, C-8 and C-9 of **1**	11
Figure S18: The HMBC correlations of C<u>H</u>-3 to C-2, C-4, C-10 and C-1' of **1**	11
Figure S19: The ^{1}H-NMR (400 MHz, CD_3OD) spectrum of **2**	12
Figure S20: The ^{13}C NMR (100 MHz, CD_3OD) spectrum of **2**	12
Figure S21: The ^{1}H-NMR (400 MHz, CD_3OD) spectrum of **3**	13
Figure S22: The ^{13}C NMR (100 MHz, CD_3OD) spectrum of **3**	13
Figure S23: The ^{1}H-NMR (400 MHz, CD_3OD) spectrum of **4**	14
Figure S24: The ^{13}C NMR (100 MHz, CD_3OD) spectrum of **4**	14
Figure S25: The image of the *Citrus hystrix*	15
Figure S26: The exact search report from scifinder of **1**	15
Figure S27: The 95%-98% similarity search report from scifinder of **1**	16

Figure S28: The 94%-95% similarity search report from scifinder of **1**	17
Table S1: ^{1}H and ^{13}C NMR Data of Compound **1** and 5,6,4'-trihydroxypyranoflavone	17
S1: Synthesis of 5,6-dihydroxypyranoflavone (**1**)	18
S2: DPPH Radical scavenging assays	18
S3: ABTS Radical scavenging assay	18



Qualitative Analysis Report

Data Filename	XN-27.d	Sample Name	XN-27
Sample Type	Sample	Position	P1-A3
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	6/16/2023 10:41:09 AM
IRM Calibration Status	Success	DA Method	PCDL.m
Comment			

Sample Group Info.
Acquisition SW 6200 series TOF/6500 series
Version Q-TOF B.05.01 (B5125.2)

User Spectra

Fragmentor Voltage 135 | Collision Energy 0 | Ionization Mode ESI

Peak List

m/z	z	Abund	Formula	Ion
337.1071	1	17578.32	C20 H16 O5	(M+H)+
338.111	1	4527.16	C20 H16 O5	(M+H)+
359.0894	1	10685.87		
536.1645	1	4051.68		
537.1668	1	3579.84		
538.163	1	2864.57		
610.184	1	5552.48		
695.1878	1	23230.38		
696.1919	1	10024.61		
697.1937	1	2811.25		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	150
O	0	20

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C20 H16 O5	336.0998	337.1071	337.1071	0.00	0.00	13.0000

--- End Of Report ---

Agilent Technologies Page 1 of 1 Printed at: 10:58 AM on: 6/16/2023

Figure S1: The HR-ESI-MS spectrum of **1**

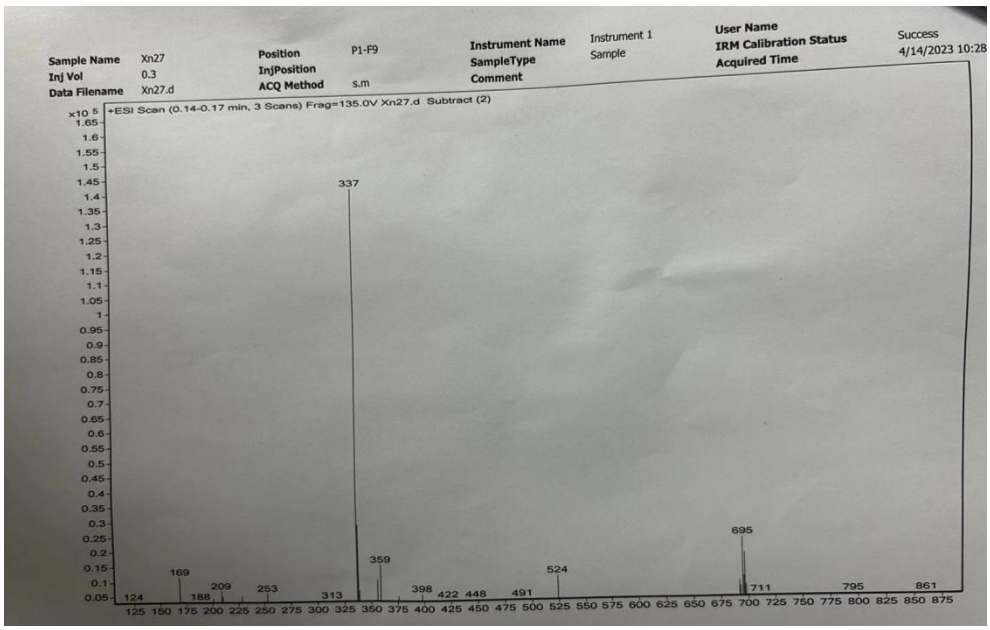


Figure S2: The ESI-MS spectrum of **1**

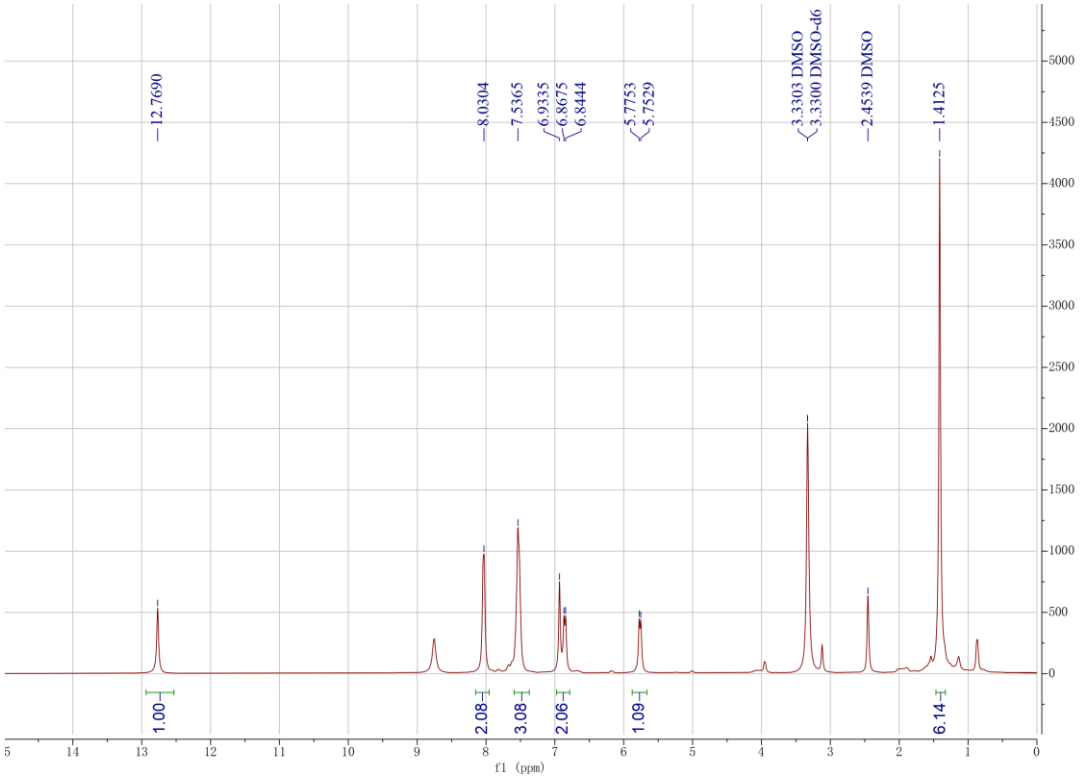


Figure S3: The ^{1}H-NMR (400 MHz, DMSO-*d*6) spectrum of **1**

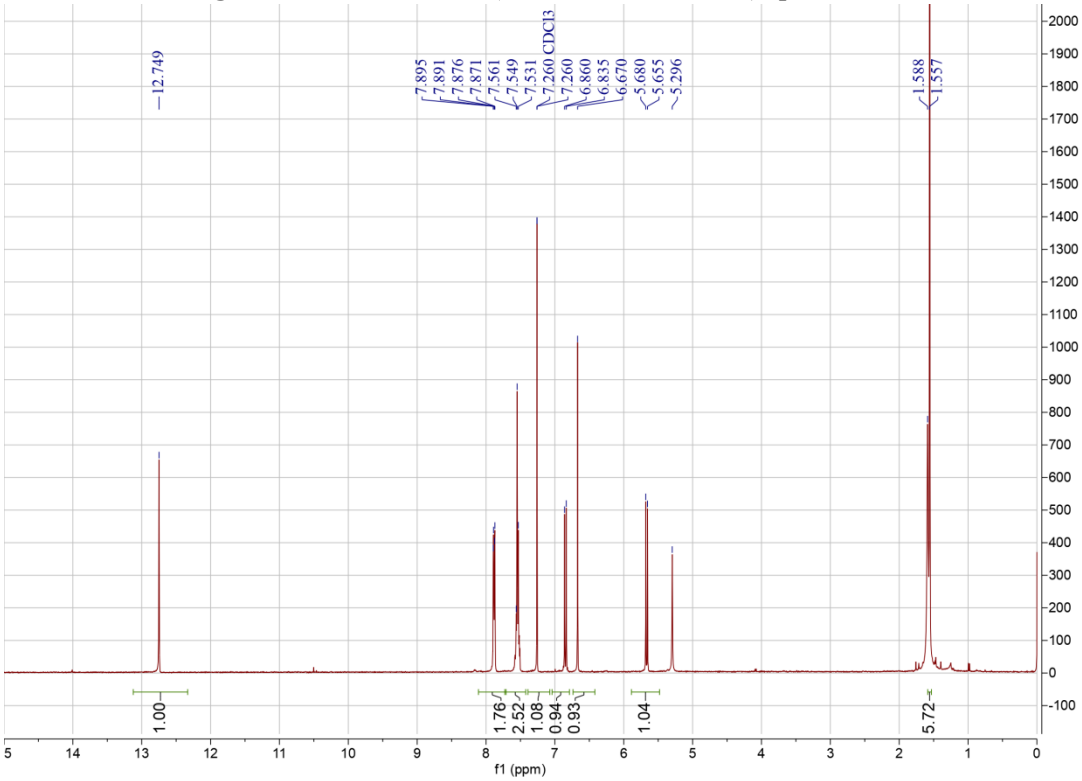


Figure S4: The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **1**

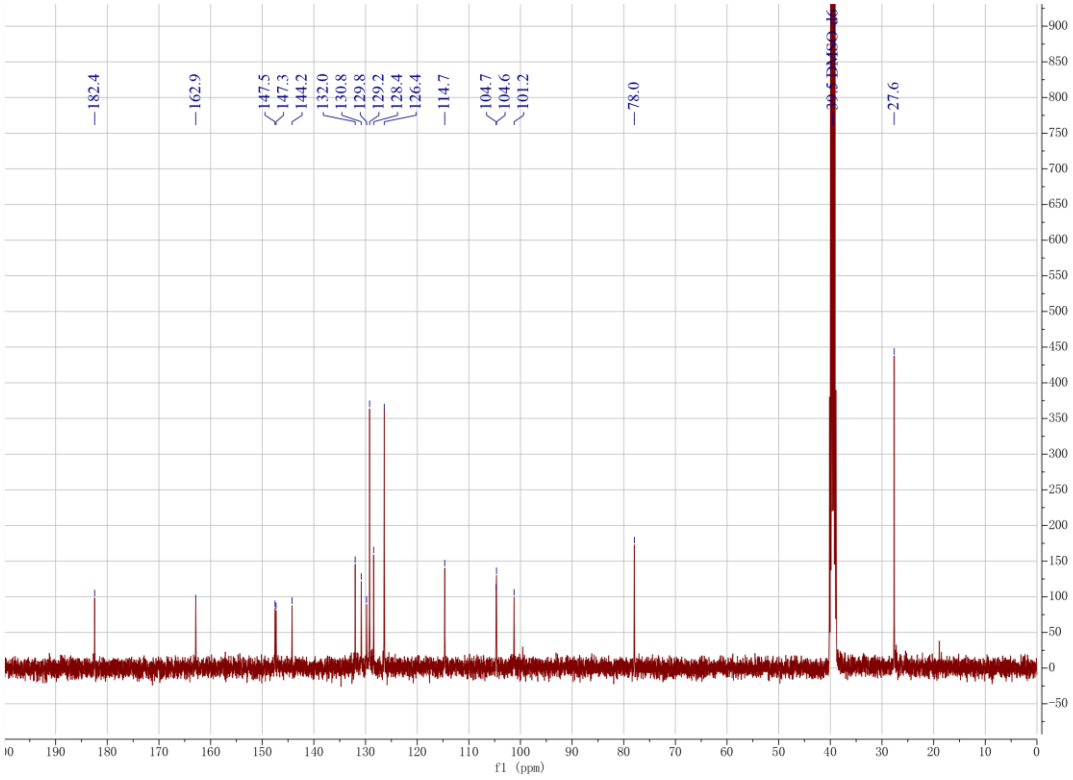


Figure S5: The ^{13}C NMR (100 MHz, DMSO-*d6*) spectrum of **1**

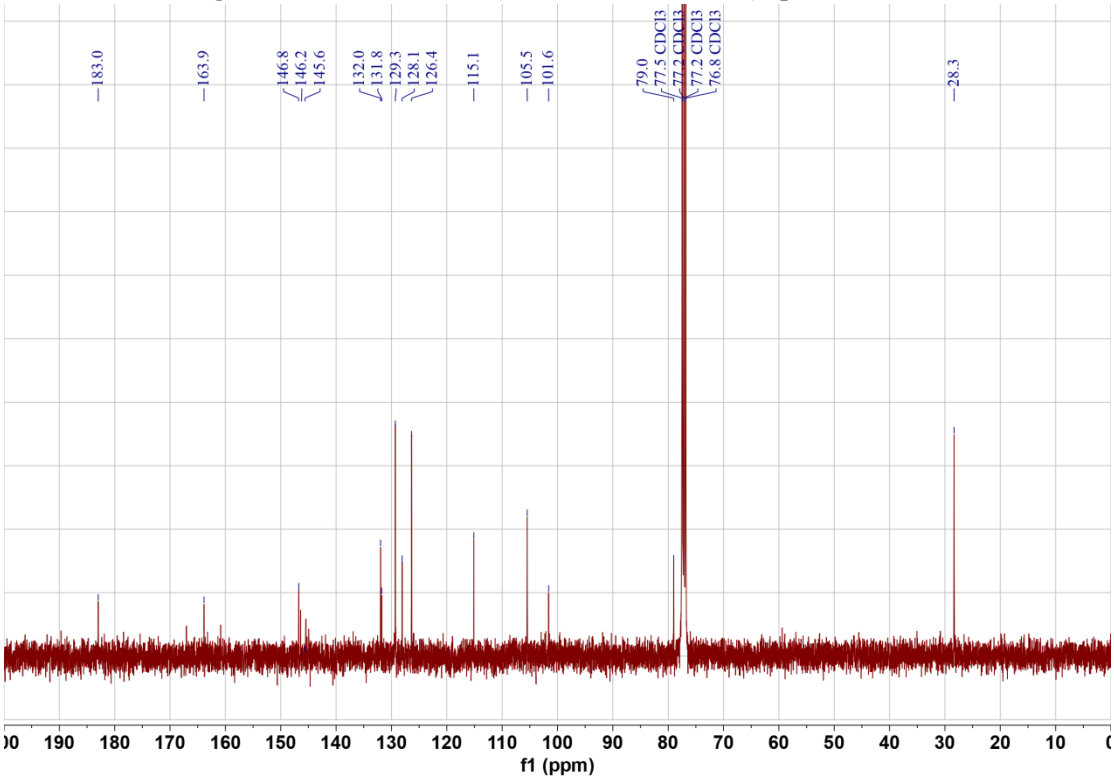


Figure S6: The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1**

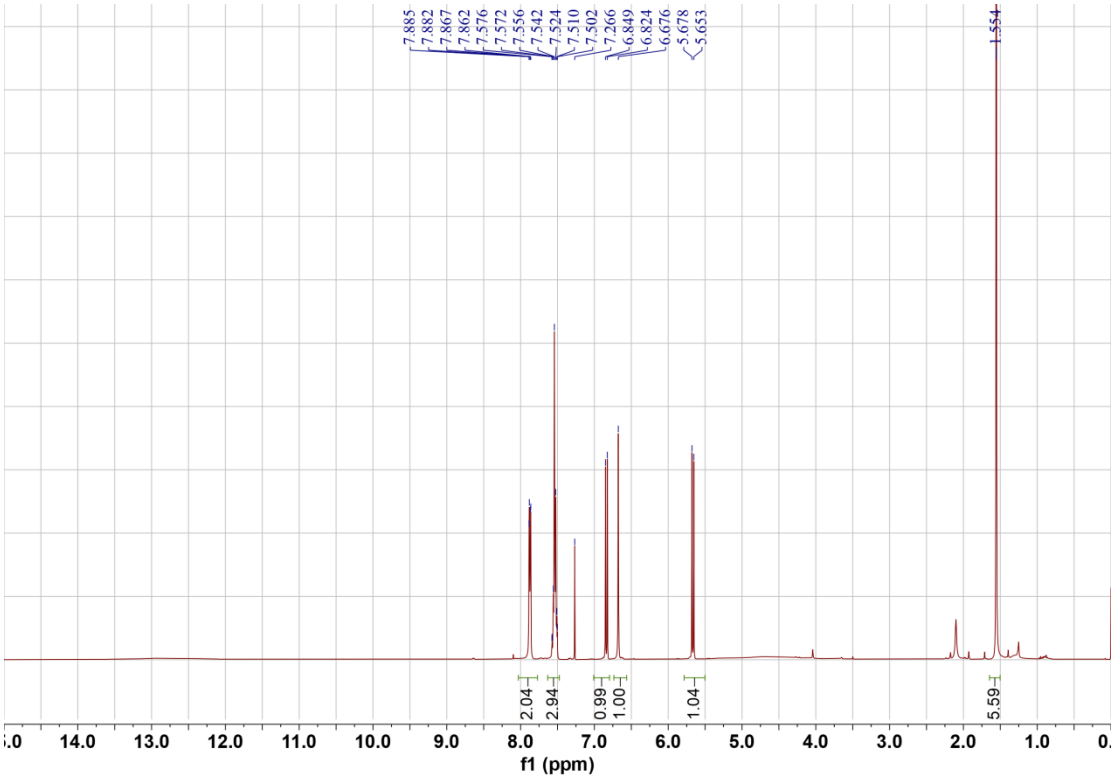


Figure S7: The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of synthetic **1**

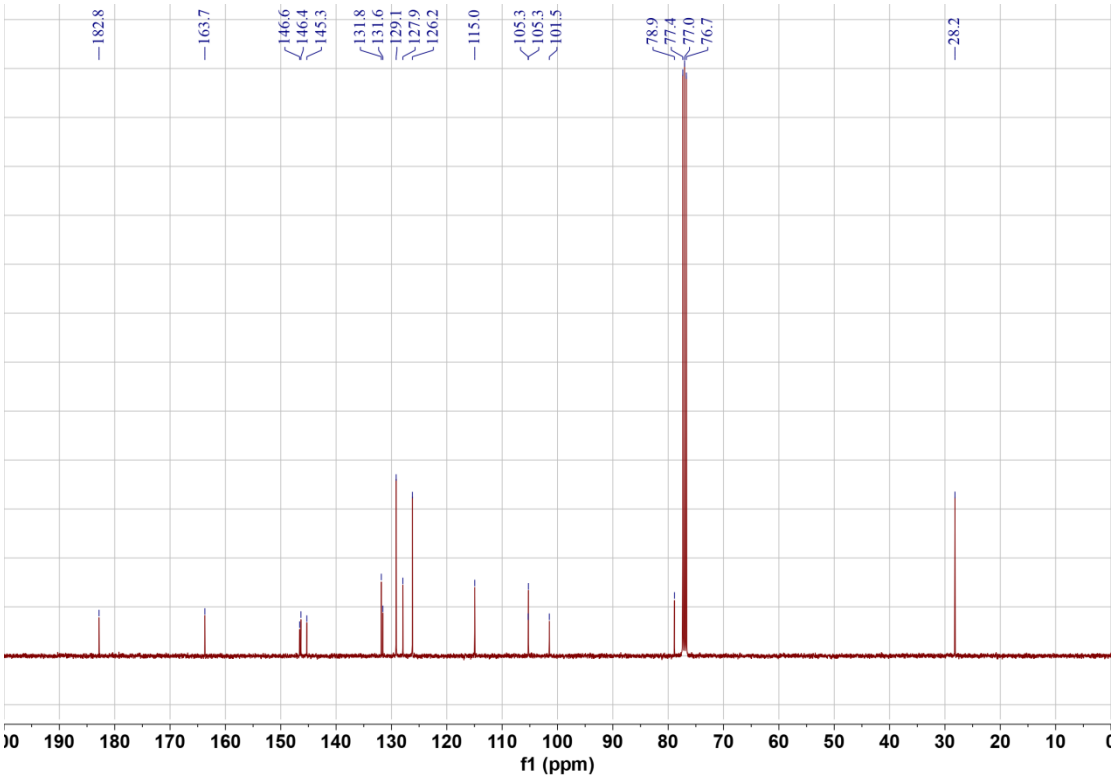


Figure S8: The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of synthetic **1**

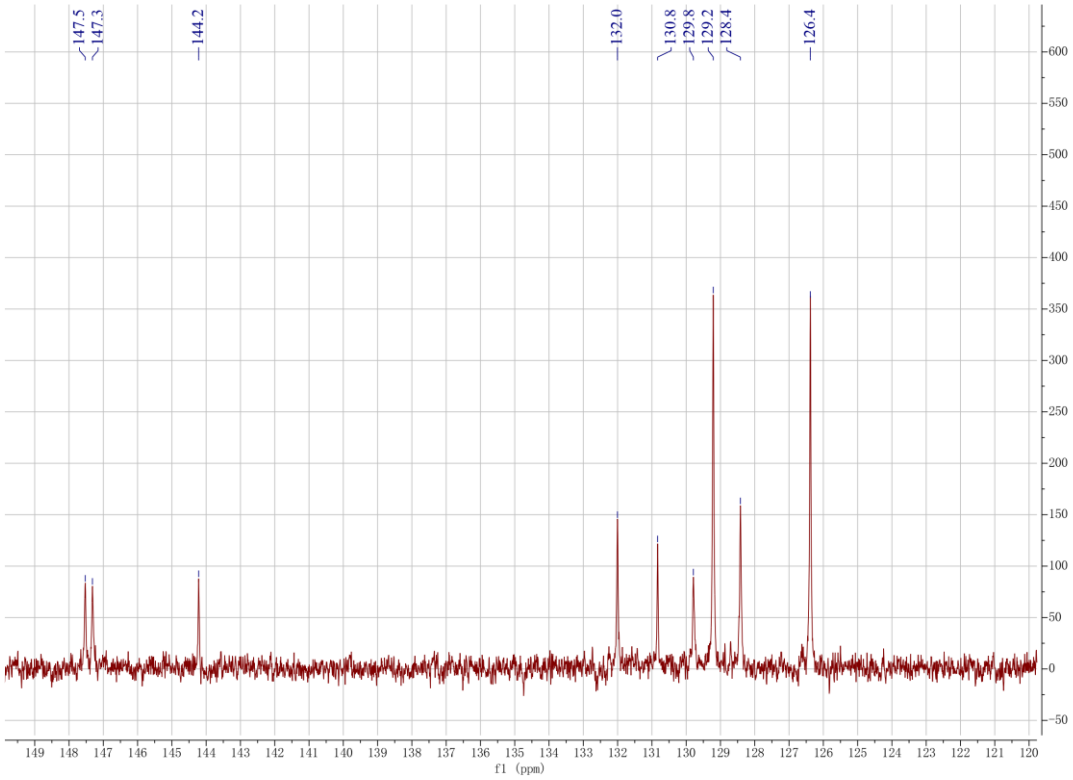


Figure S9: The ^{13}C NMR (100 MHz, DMSO-*d*6) spectrum of **1** (From δ_C 120 to 150 ppm)

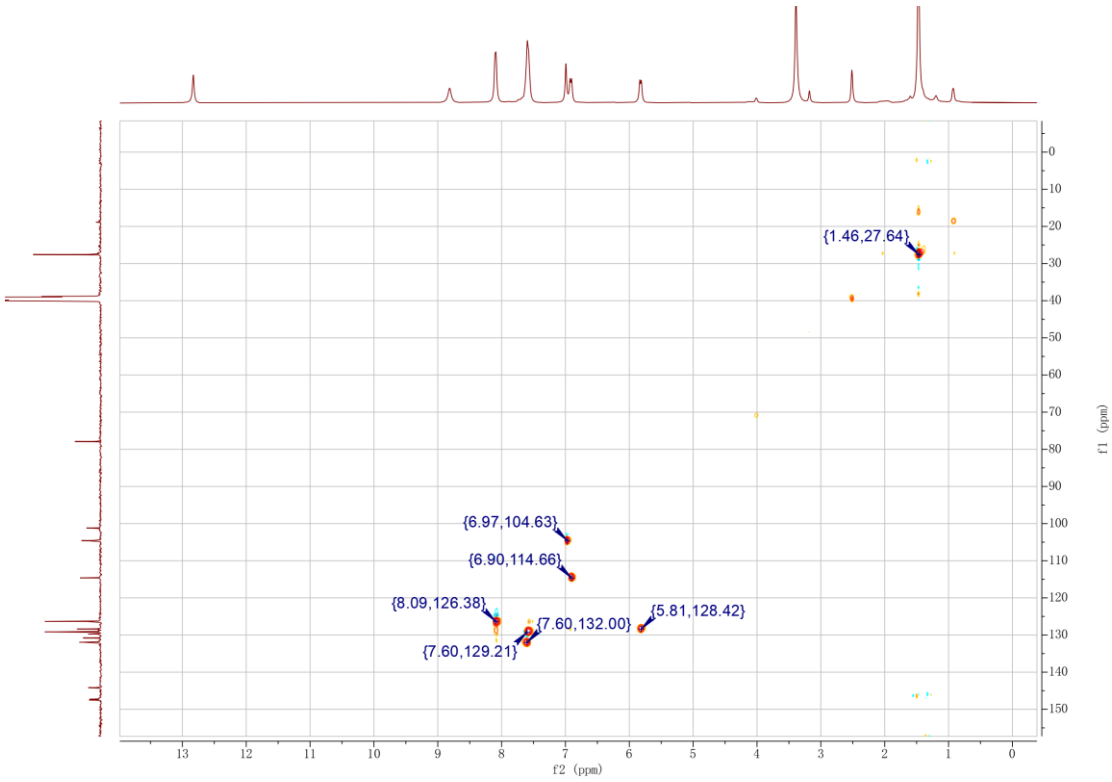


Figure S10: The HSQC spectrum of **1**

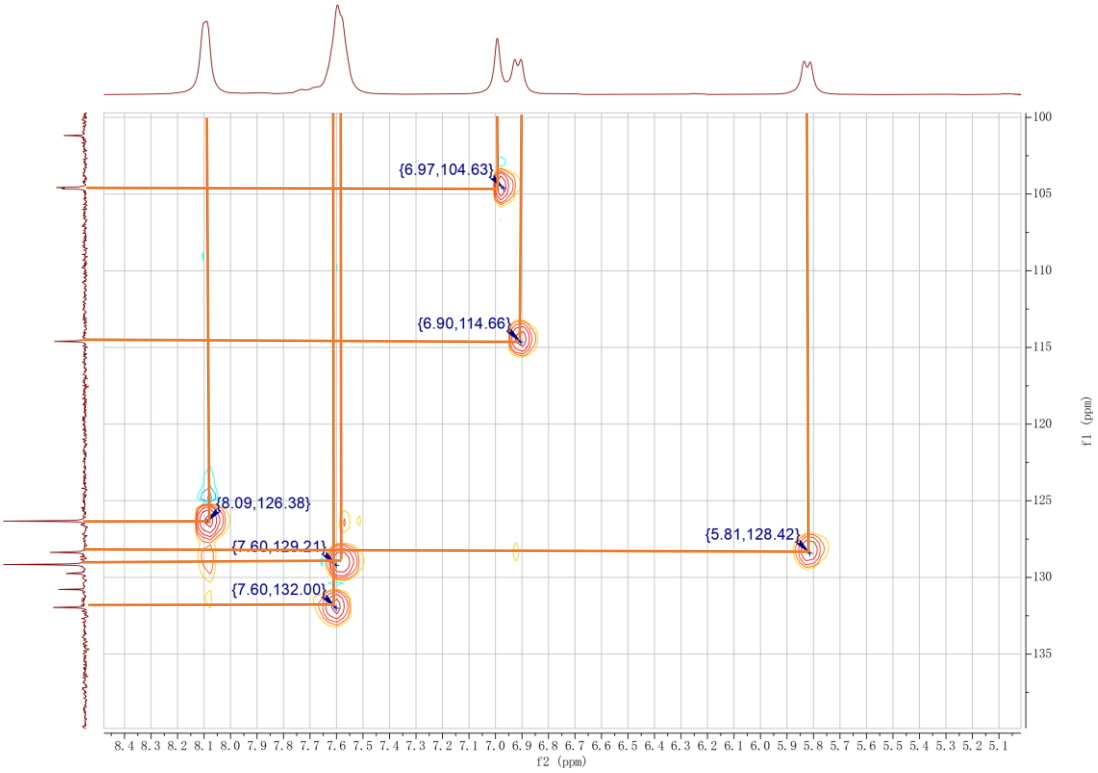


Figure S11: The HSQC spectrum of **1** (From δ_C 100 to140 ppm)

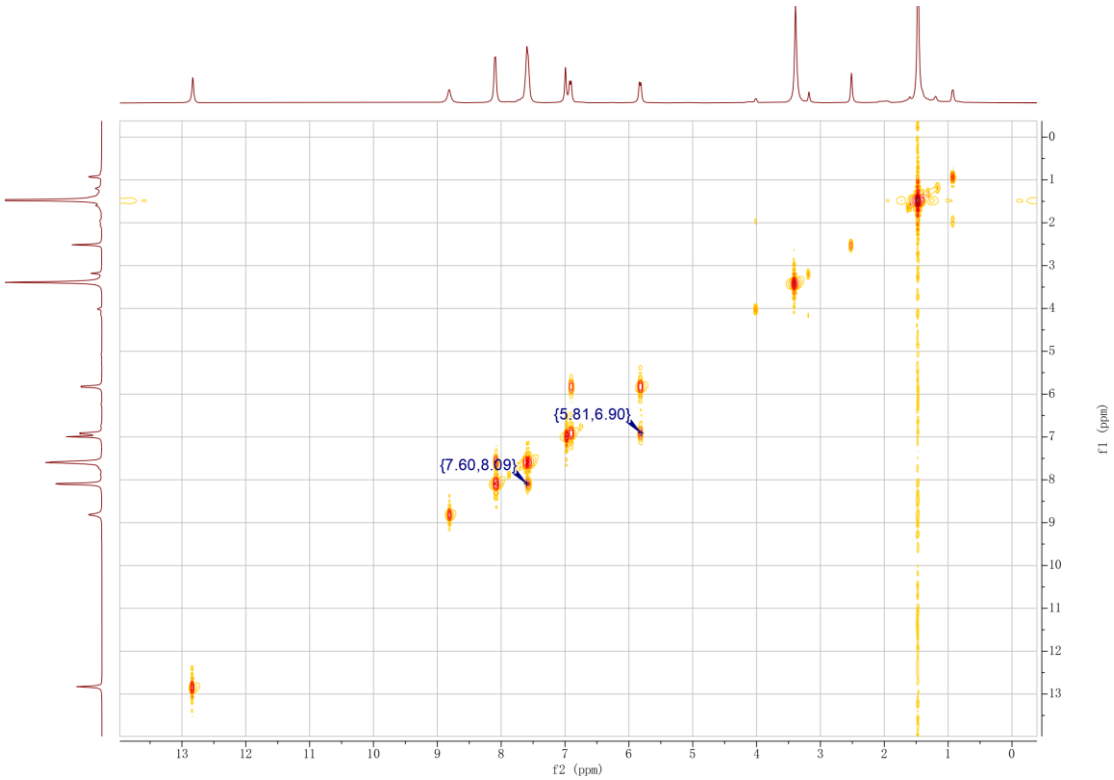


Figure S12: The ^{1}H-^{1}H COSY spectrum of **1**

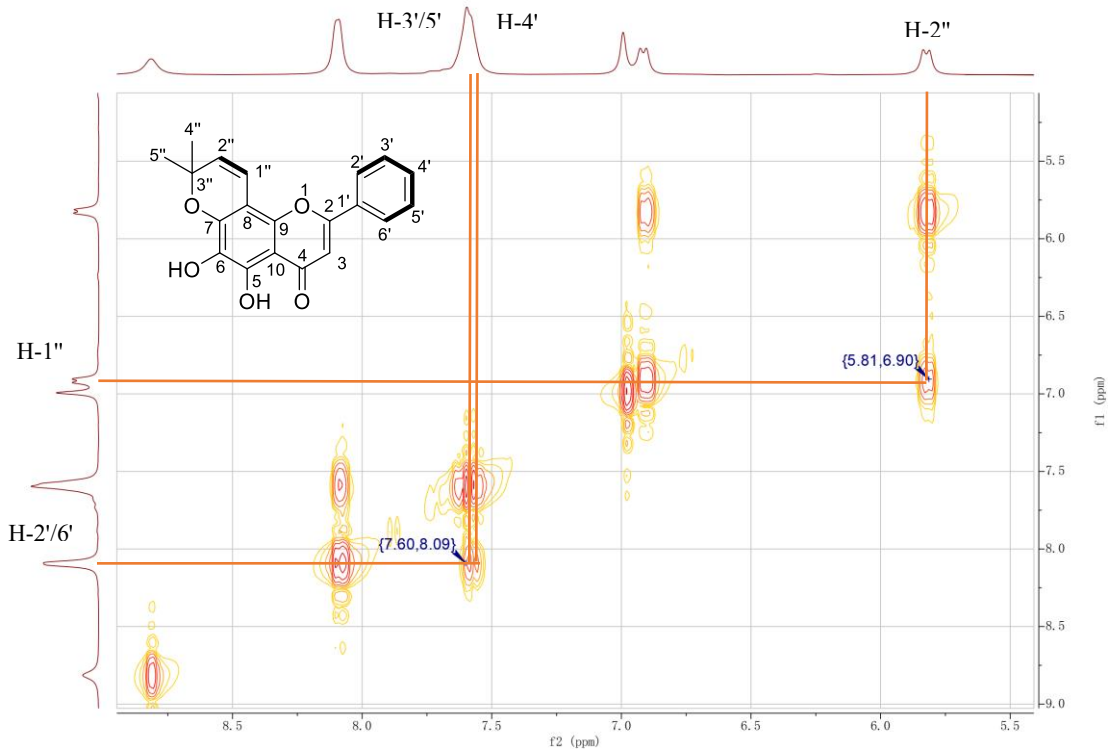


Figure S13: The ^{1}H-^{1}H COSY of H-1"/H-2", H-2'(6')/H-3'(5'), and H-2'(6')/H-4' of **1**

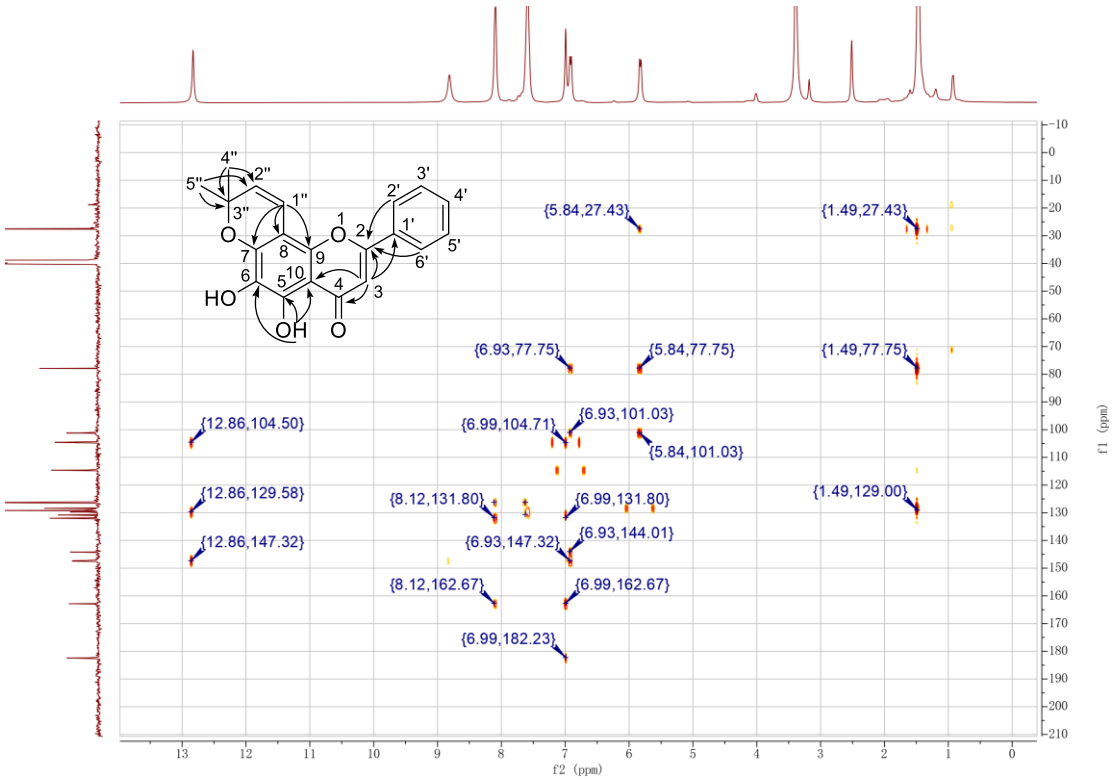


Figure S14: The HMBC spectrum of **1**

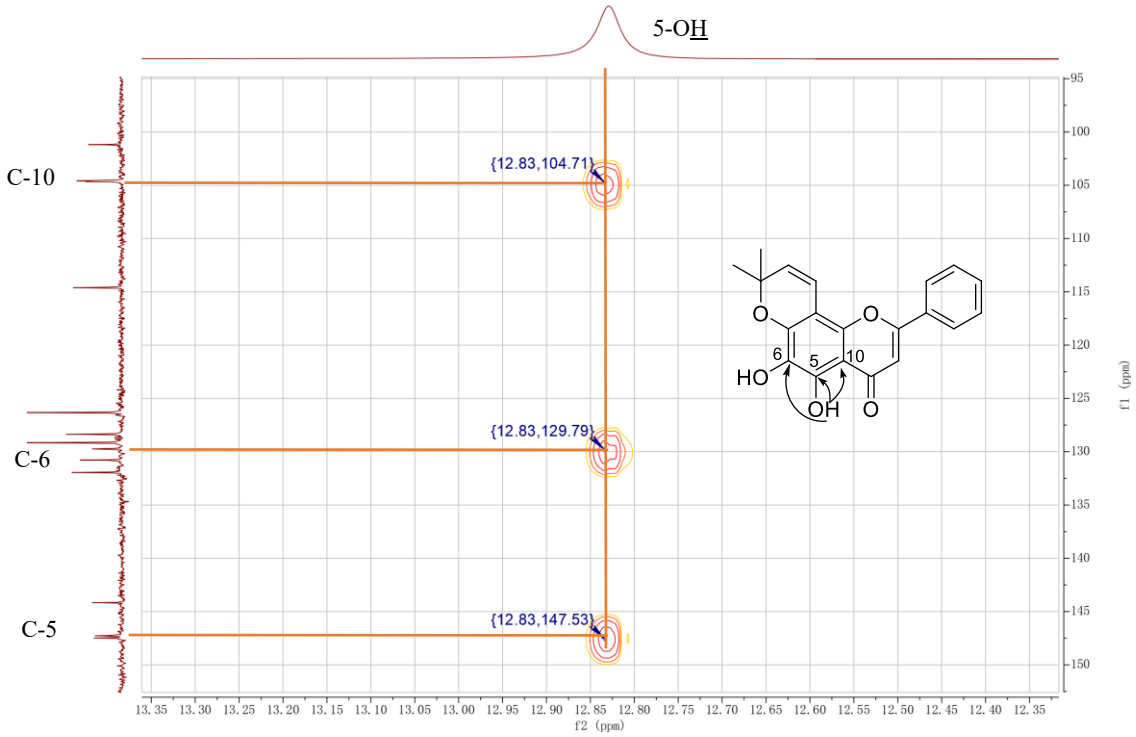


Figure S15: The HMBC correlations of 5-O<u>H</u> to C-5, C-6 and C-10 of **1**

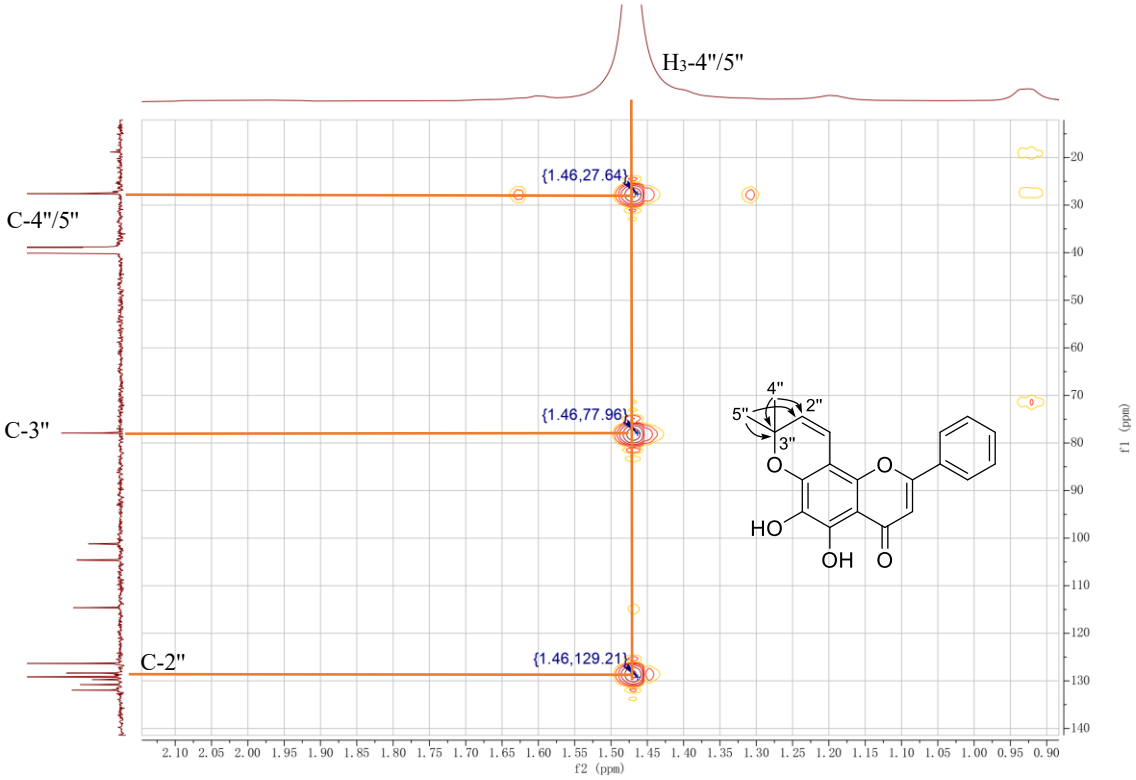


Figure S16: The HMBC correlations of C<u>H</u>$_3$-4"/5" to C-3" and C-2" of **1**

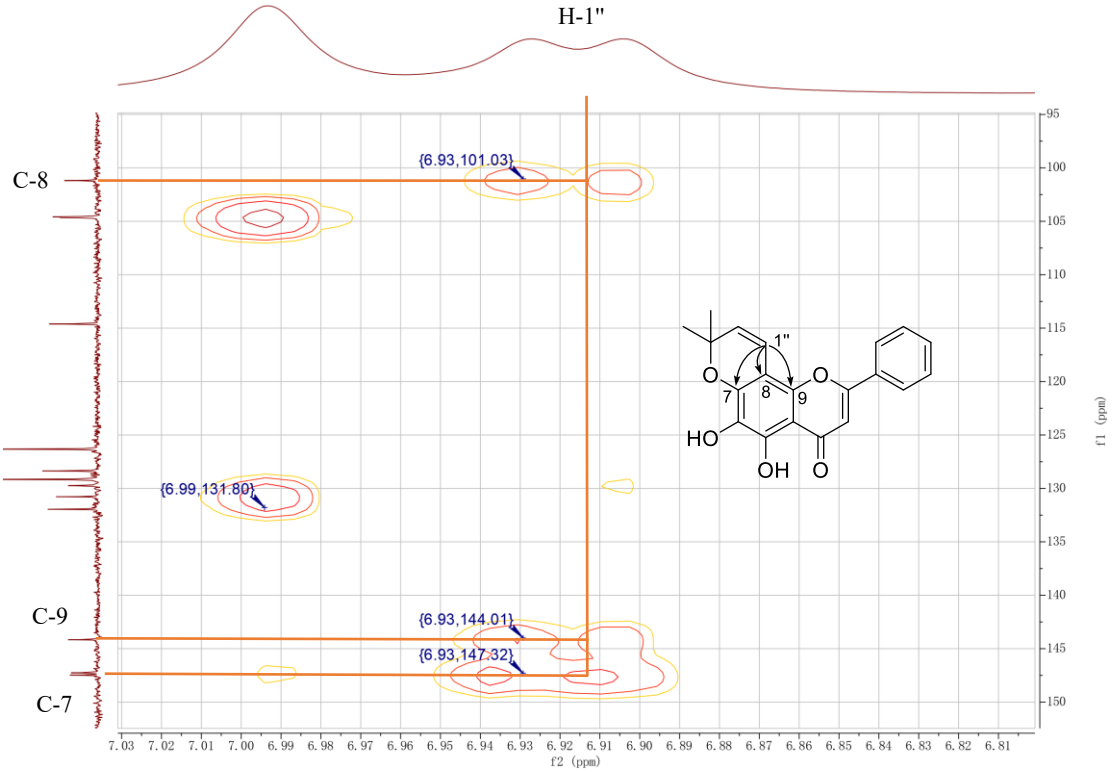


Figure S17: The HMBC correlations of CH-1" to C-7, C-8 and C-9 of **1**

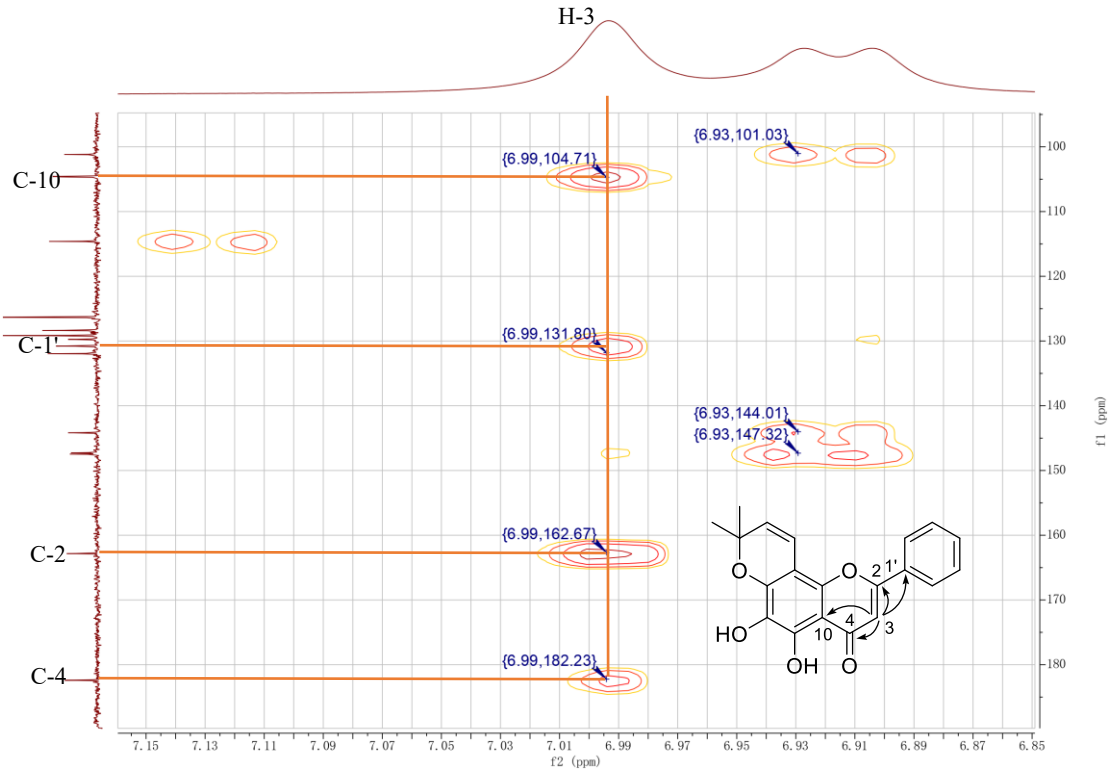


Figure S18: The HMBC correlations of CH-3 to C-2, C-4, C-10 and C-1' of **1**

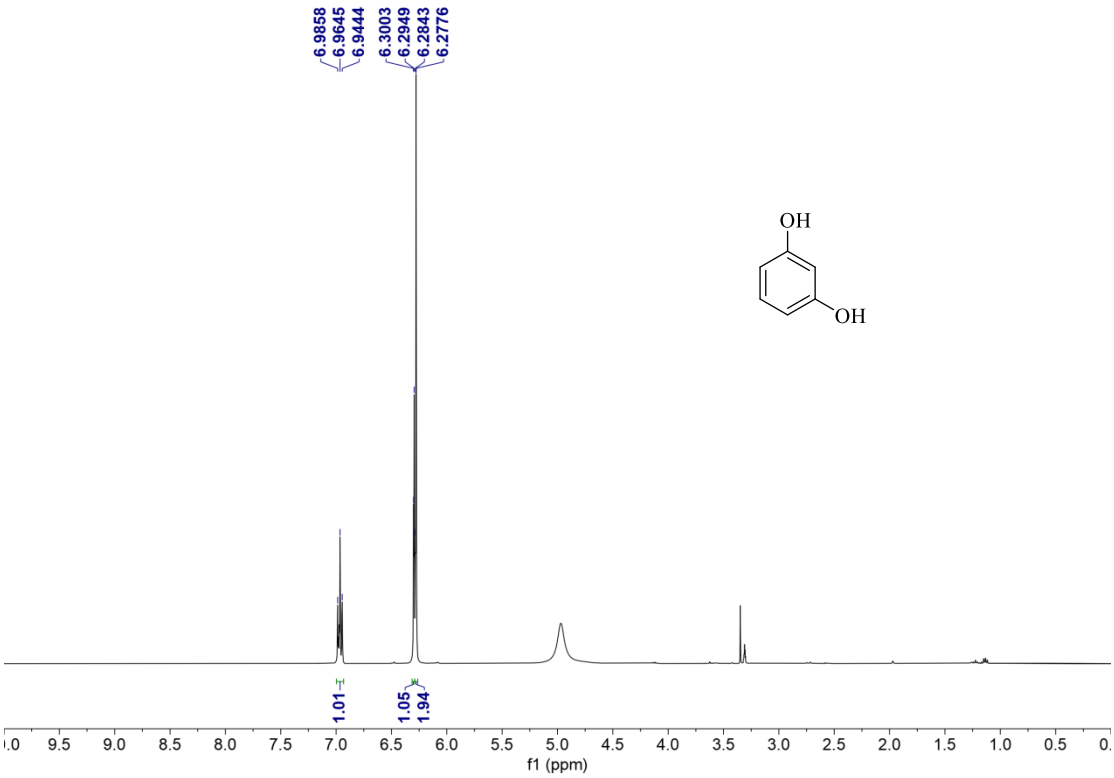


Figure S19: The ^{1}H-NMR (400 MHz, CD_3OD) spectrum of **2**

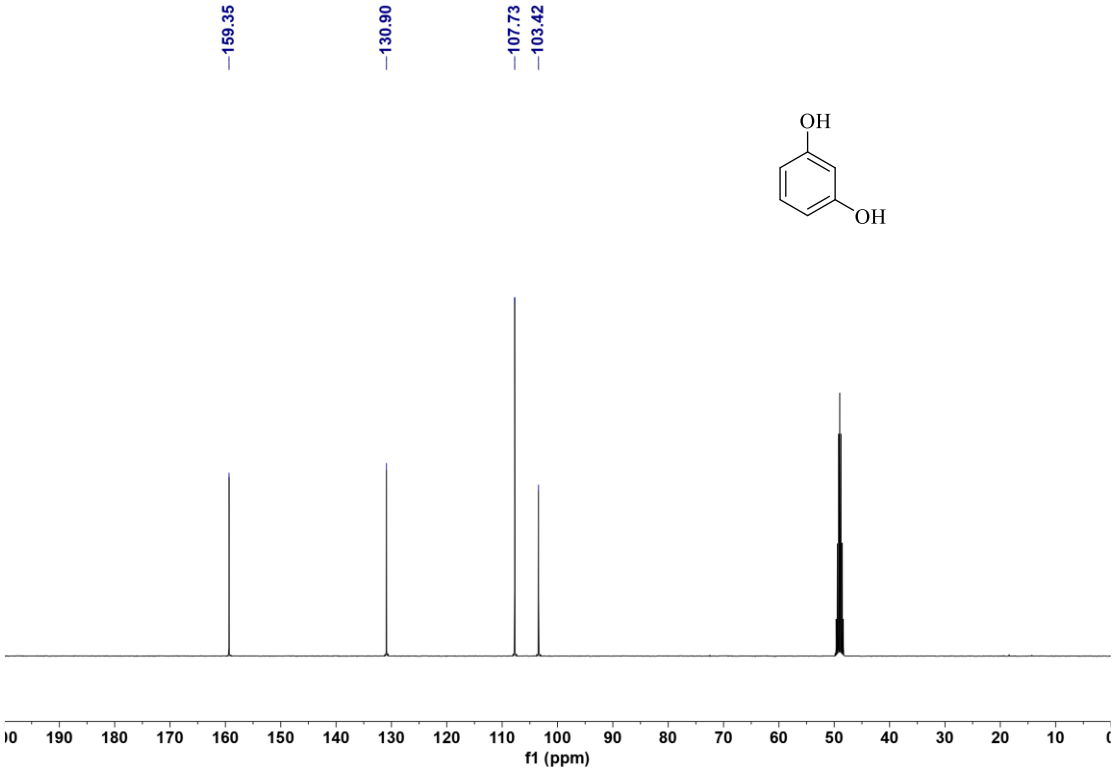


Figure S20: The ^{13}C NMR (100 MHz, CD_3OD) spectrum of **2**

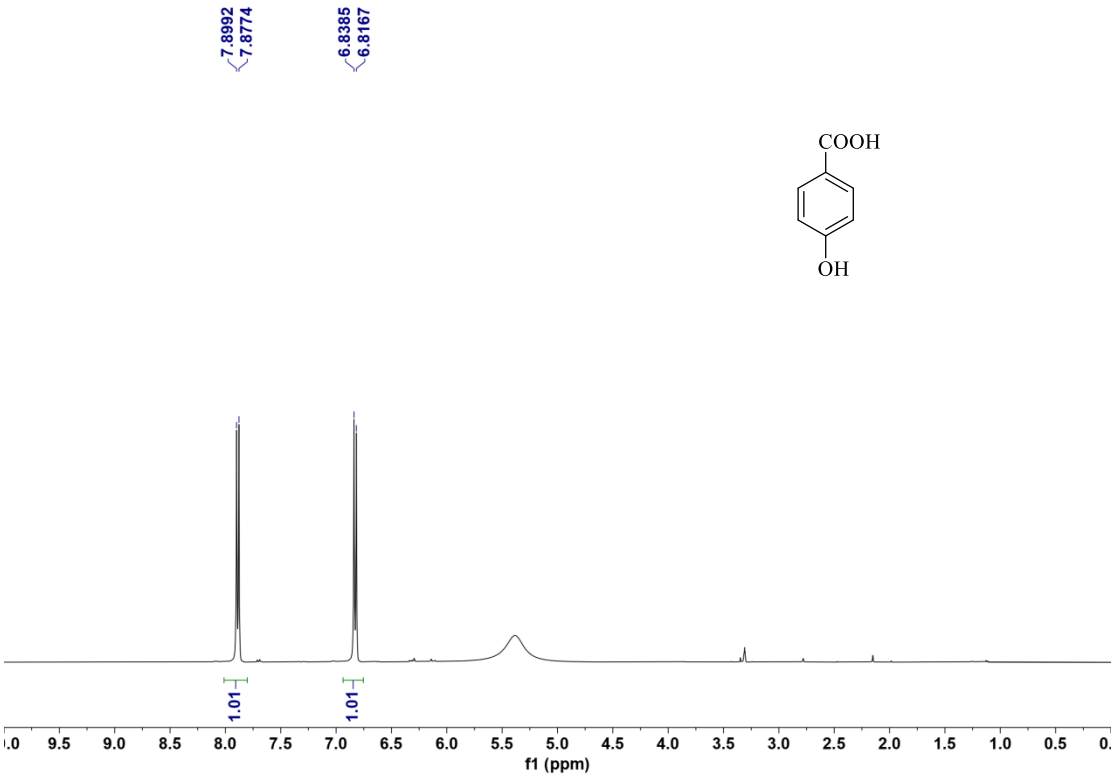


Figure S21: The ^{1}H-NMR (400 MHz, CD_3OD) spectrum of **3**

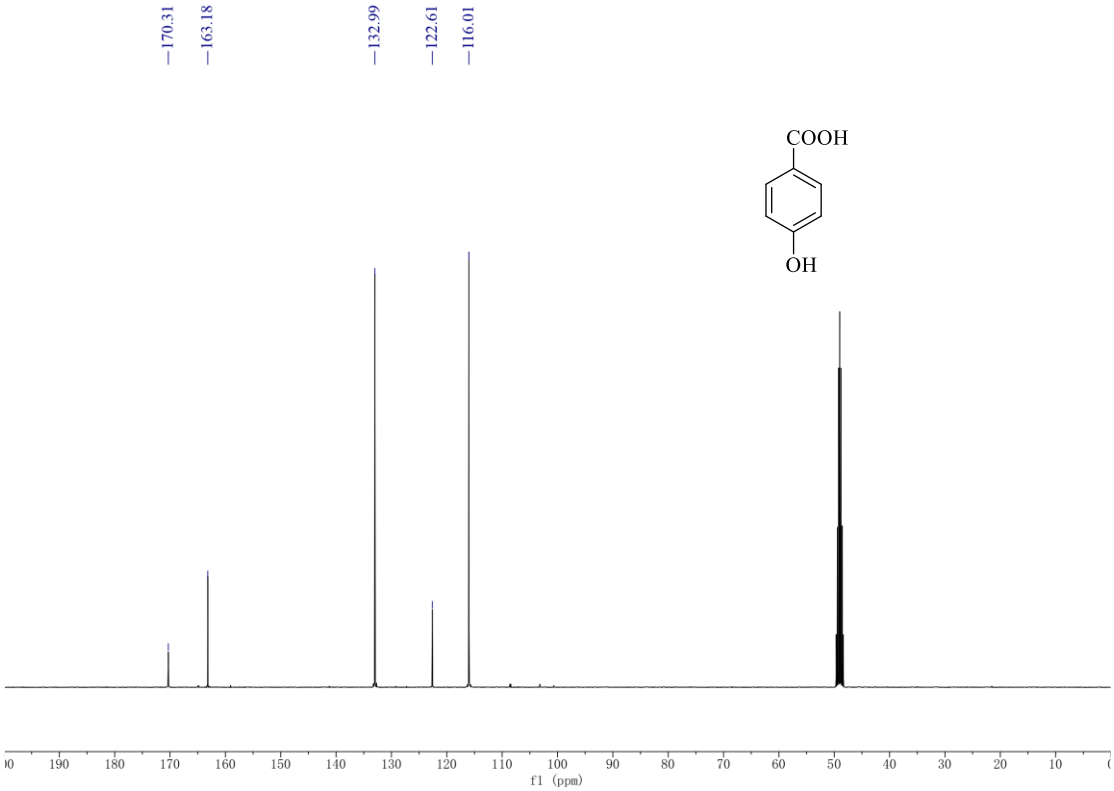


Figure S22: The ^{13}C NMR (100 MHz, CD_3OD) spectrum of **3**

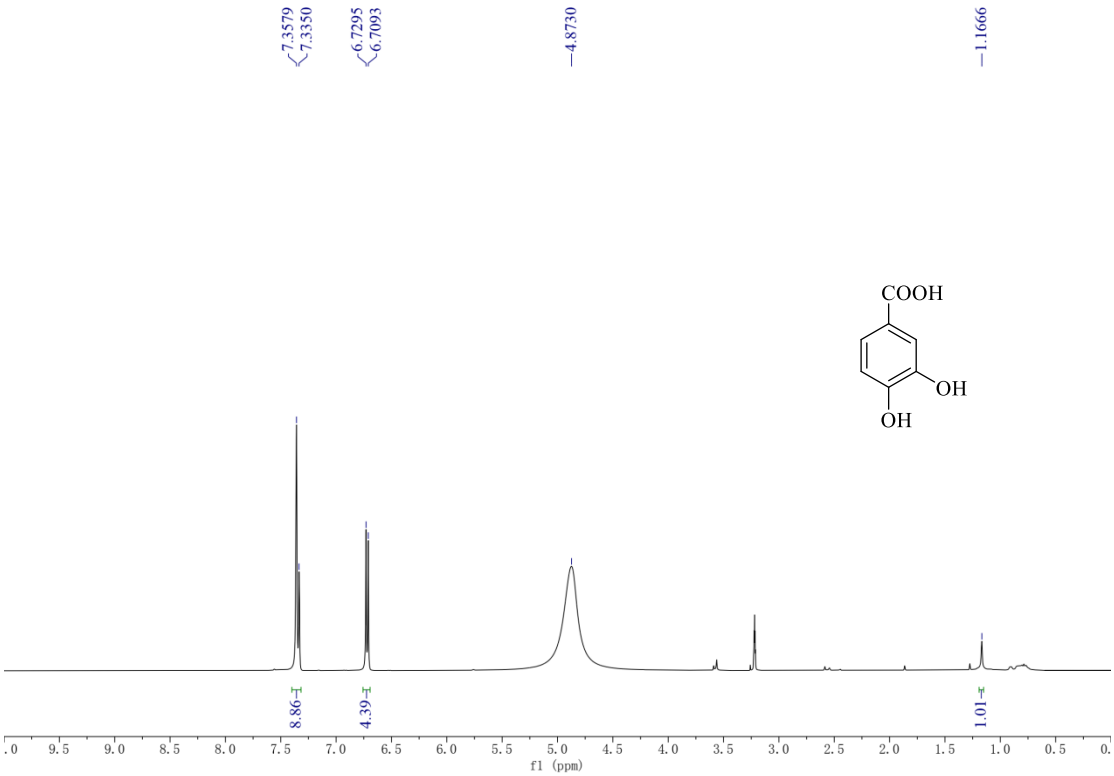


Figure S23: The ^{1}H-NMR (400 MHz, CD_3OD) spectrum of **4**

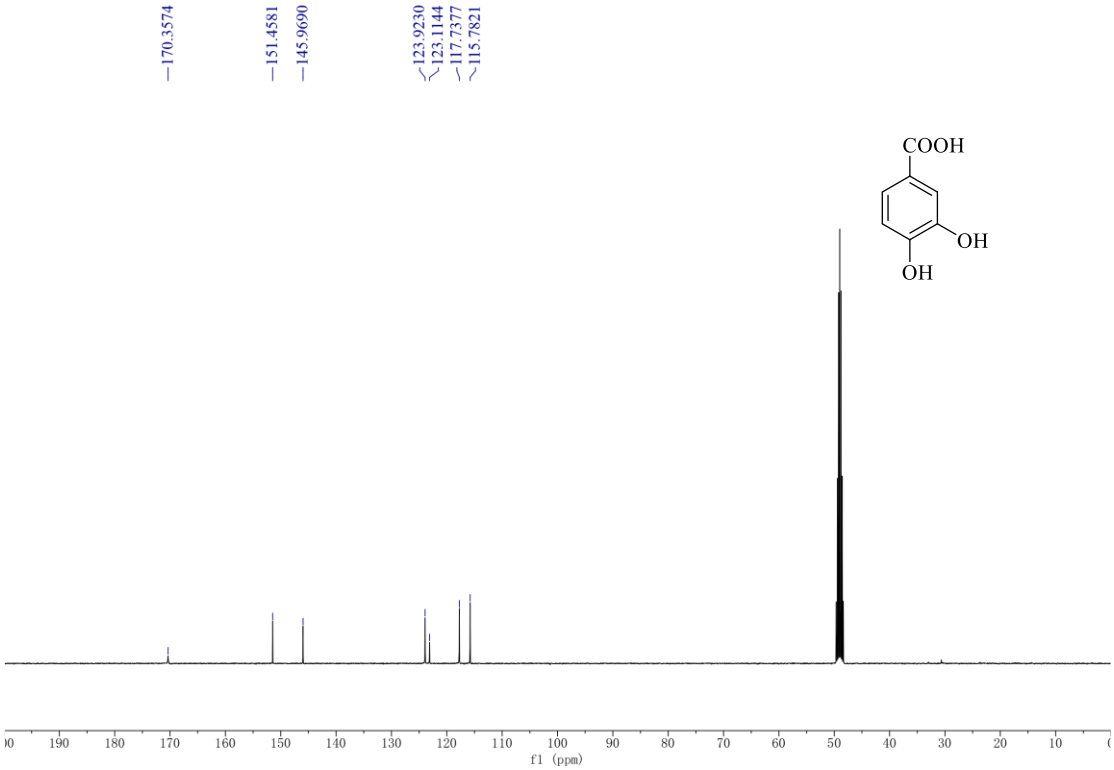


Figure S24: The ^{13}C NMR (100 MHz, CD_3OD) spectrum of **4**

Figure S25: The image of the *Citrus hystrix*

The herbarium number of *Citrus hystrix* registered at https://sweetgum.nybg.org/science/ih/ was 3787305.

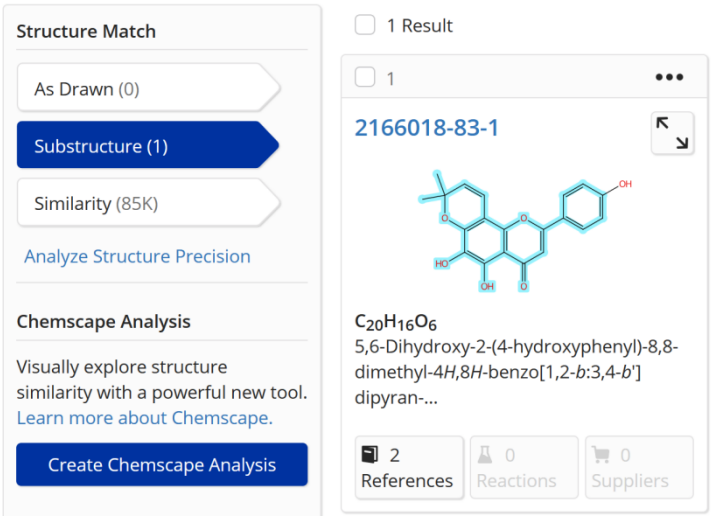


Figure S26: The exact search report from scifinder of **1**

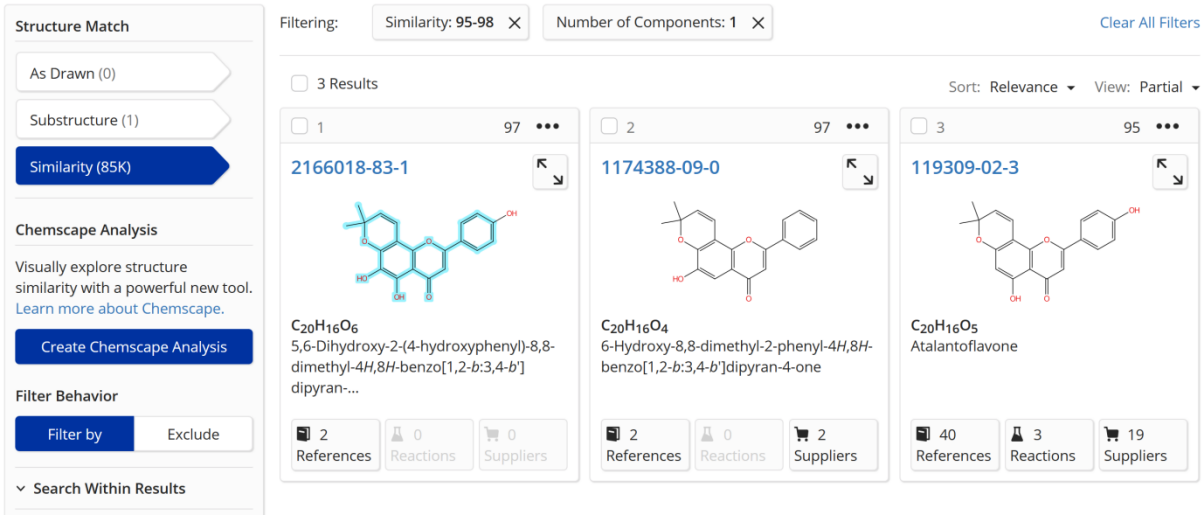


Figure S27: The 95%-98% similarity search report from scifinder of **1**

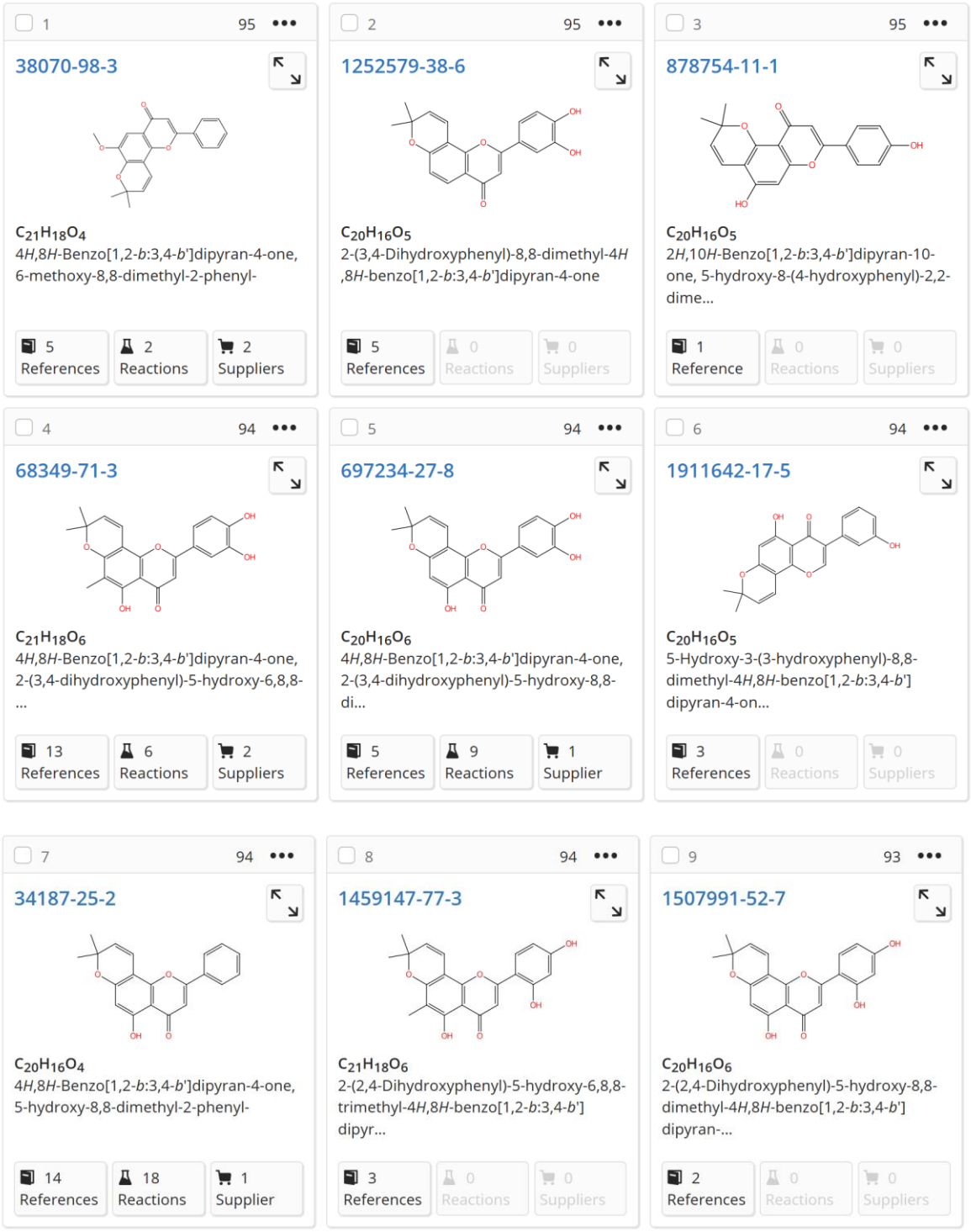
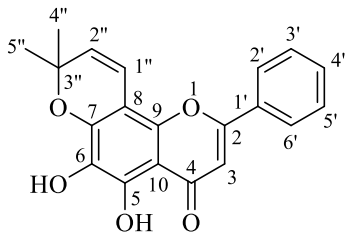
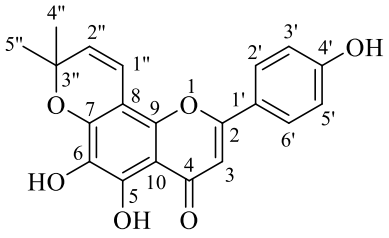


Figure S28: The 94%-95% similarity search report from scifinder of **1**

Table S1: ^{1}H and ^{13}C NMR Data of Compound **1** in DMSO-*d*6 and 5,6,4'-trihydroxypyranoflavone in $CDCl_3$



5,6-dihydroxypyranoflavone (**1**)



5,6,4'-trihydroxypyranoflavone

No.	**1**		5,6,4'-trihydroxypyranoflavone	
	δ_C	δ_H	δ_C	δ_H
2	162.9 s	-	159.4 s	-
3	104.6 d	6.93 s	99.4 d	6.21 s
4	182.4 s	-	175.4 s	-
5	147.3 s	-	160.1 s	-
6	129.8 s	-	135.4 s	-
7	147.5 s	-	150.9 s	-
8	101.2 s	-	101.3 s	-
9	144.2 s	-	146.0 s	-
10	104.7 s	-	103.7 s	-
1'	130.8 s	-	122.4 s	-
2'/6'	127.3 d	8.03 overlapped	129.4 d	8.04 d (8.8)
3'/5'	129.2 d	7.53 overlapped	115.6 d	6.92 d (8.8)
4'	132.0 d	7.53 overlapped	158.8 d	-
1"	114.7 d	6.86 d (8.9)	114.7 d	6.75 d (9.6)
2"	128.4 d	5.76 d (8.9)	127.2 d	5.56 d (10.0)
3"	78.0 s	-	78.1 s	-
4"/5"	27.6 q	1.41 s	28.0 q	1.41 s
5-OH	-	12.77 s		-

S1: Synthesis of 5,6-dihydroxypyranoflavone (1)

Baicalein (1 equiv, 135 mg, 0.5 mmol) and 3-methyl-2-butenal (2 equiv, 84 mg, 1.0 mmol) were dissolved in anhydrous pyridine (2 mL), and the reaction was performed by stirring the mixture under nitrogen at 110 ºC for 10 hours. Then, the solution was reduced under a vacuum. The resulting mixture was directly subjected to silica gel column eluted with petroleum ether/ethyl acetate (ratio 8:2) to afford compound **1** as a yellow solid (59 mg, 0.175 mmol, 35%).

S2: DPPH Radical Scavenging Assay

The DPPH assay was carried out as previously described [1-4]. L-Ascorbic acid was used as positive controls, and reaction mixtures containing 100 μL of 200 μM DPPH solution and 100 μL of 2-fold serial dilutions of the sample with concentrations in the range of 160, 80, 40, 20, 10, 5, and 2.5 μM were placed in a 96-well microplate and incubated at 37 ℃ for 30 min. After incubation, the absorbance was read at 517 nm by an Emax precision microplate reader, and the mean of three readings was obtained. Scavenging activity was calculated by the following equation:

$$\text{Level of inhibition (\%)} = [1 - (A_{control} - A_{sample})/A_{control}] \times 100\%$$

The IC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

S3: ABTS Radical Scavenging Assay

The ABTS assay was carried out as previously described [1-4] The $ABTS^+$ radical was obtained by the reaction of a 6 mM ABTS solution in water with potassium persulfate (2.45 mM) without light at 25 ºC for 16 h before use. The absorbance of the $ABTS^+$ dilution was regulated with ethanol to 0.70 ± 0.02 at 734 nm at 25 ºC. L-Ascorbic acid was used as positive controls, and reaction mixtures containing 100 μL of ABTS solution and 2-fold serial dilutions of the sample with concentrations in the range of 30, 15, 7. 5, 3. 75, 1.875, and 0.9375 μM were placed in a 96-well microplate and incubated at 25 ºC for 30 min. After incubation, the absorbance was read at 734 nm by an Emax precision microplate reader, and the mean of three readings was obtained. Scavenging activity was calculated by the following equation:

$$\text{Level of inhibition (\%)} = [1 - (A_{control} - A_{sample})/A_{control}] \times 100\%$$

The IC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of ABTS radicals.

References

[1] H.M. Zhang, C.F. Wang, S.M. Shen, G.L. Wang, P. Liu, Z.M. Liu, Y.Y. Wang, S.S. Du, Z.L. Liu and Z.W. Deng (2012). Antioxidant phenolic compounds from Pu-erh tea, *Molecules* **17**, 14037-14045.

[2] A. Ertas, H. Cakirca, I. Yener, M. Akdeniz, M. Firat, M, G. Topcu and U. Kolak (2021). Bioguided Isolation of Secondary Metabolites from *Salvia cerino-pruinosa* Rech. f. var. *cerino-pruinosa*, *Rec. Nat. Prod*. 15, 585-592

[3] H. Kiziltas, A.C. Gören, Z. Bingöl, S. H. Alwasel and I. Gulcin (2021). Anticholinergic, antidiabetic

and antioxidant activities of *Ferula orientalis* L. Determination of Its Polyphenol Contents by LC-HRMS, *Rec. Nat. Prod*. **15**, 513-528.

[4] H. Kiziltas, Z. Bingöl, A.C. Gören, S. M. Pinar, S.H. Alwasel, and İ. Gülçin (2021). LC-HRMS profiling of phytochemicals, antidiabetic, anticholinergic and antioxidant activities of evaporated ethanol extract of Astragalus brachycalyx Fischer, *J. Chem. Metrol.***15**, 135-151.