Supporting Information

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Anti-inflammatory Constituents Isolated From *Launaea sarmentosa* Against Infection by LPS-stimulated Macrophages

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Table S1: List of primers and primer sequences

No.	Gene name	Primer probes	Sequence
1	*iNOS*	Forward	5′- CTTTGCCACGGACGAGAC-3′
		Reverse	5′- TCATTGTACTCTGAGGGCTGAC-3′
2	*IL-6*	Forward	5′- GCTACCAAACTGGATATAATCAGGA-3′
		Reverse	5′- CCAGGTAGCTATGGTACTCCAGAA-3′
3	*TNF-α*	Forward	5′- CTGTAGCCCACGTCGTAGC-3′
		Reverse	5′- TTGAGATCCATGCCGTTG-3′
4	*β-actin*	Forward	5′- GGAGGGGGTTGAGGTGTT-3′
		Reverse	5′- GTGTGCACTTTTATTGGTCTCAA-3′

iNOS; inducible nitric oxide synthase, *COX-2; cyclooxygenase-2*, *HO-1; heme oxygenase-1*

Table S2: Cytotoxicity of LSm and its fractional extract on LPS-stimulated macrophages

Cytotoxicity (cell viability < 95 %); non-treatead with LPS							
Parameter	Control	non-LPS	50	100	200	400	800
LSm	–	n.a	–	–	–	54.00 ± 7.32	15.06 ± 0.87
Hex-ex	–	n.a	–	–	–	89.18 ± 1.57	23.40 ± 0.21
Ea-ex	–	n.a	–	–	–	76.54 ± 5.46	31.06 ± 1.25
Aq-ex	–	n.a	–	–	–	56.29 ± 3.43	25.86 ± 3.27
Cytotoxicity (cell viability < 95 %); treatead with LPS							
	Control	LPS	50	100	200	400	800
LSm	–	94.52 ± 0.58	–	–	–	79.32 ± 1.1	19.99 ± 1.90
Hex-ex	–	93.21 ± 0.19	–	–	–	27.00 ± 0.20	2.46 ± 0.09
Ea-ex	–	94.05 ± 0.82	–	–	–	90.67 ± 3.27	31.77 ± 0.36
Aq-ex	–	91.47 ± 0.65	–	–	–	85.67 ± 1.80	62.94 ± 2.39

" – ": No cytotoxicity; " n.a": Not applicable; $p < 0.05$.

Table S3: Induction efficiency of NO secretion from isolated compounds in LPS-induced inflammation

No	Compound	IC_{50} value of NO inhibition (μM)
1	Succinic acid	>100
2	Quercetin	27.44 ± 0.30
3	2(4-Hydroxylphenyl)acetic acid	>100
4	Luteolin-7-*O*-*β*-glucopyranoside	30.38 ± 0.86
5	Quercetin-3-*O*-rutinoside	>100

1. Supplementary spectroscopic data of compound 1

Table S4: The comparison of NMR data of compound **1** with a similar compound (Succinic acid).

Position	Compound 1 (CD_3OD)		Succinic acid (CD_3OD) [1]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (500 MHz) δ_H ppm
1, 4	176.1	-	174.8	-
2, 3	29.7	2.59	28.4	2.57

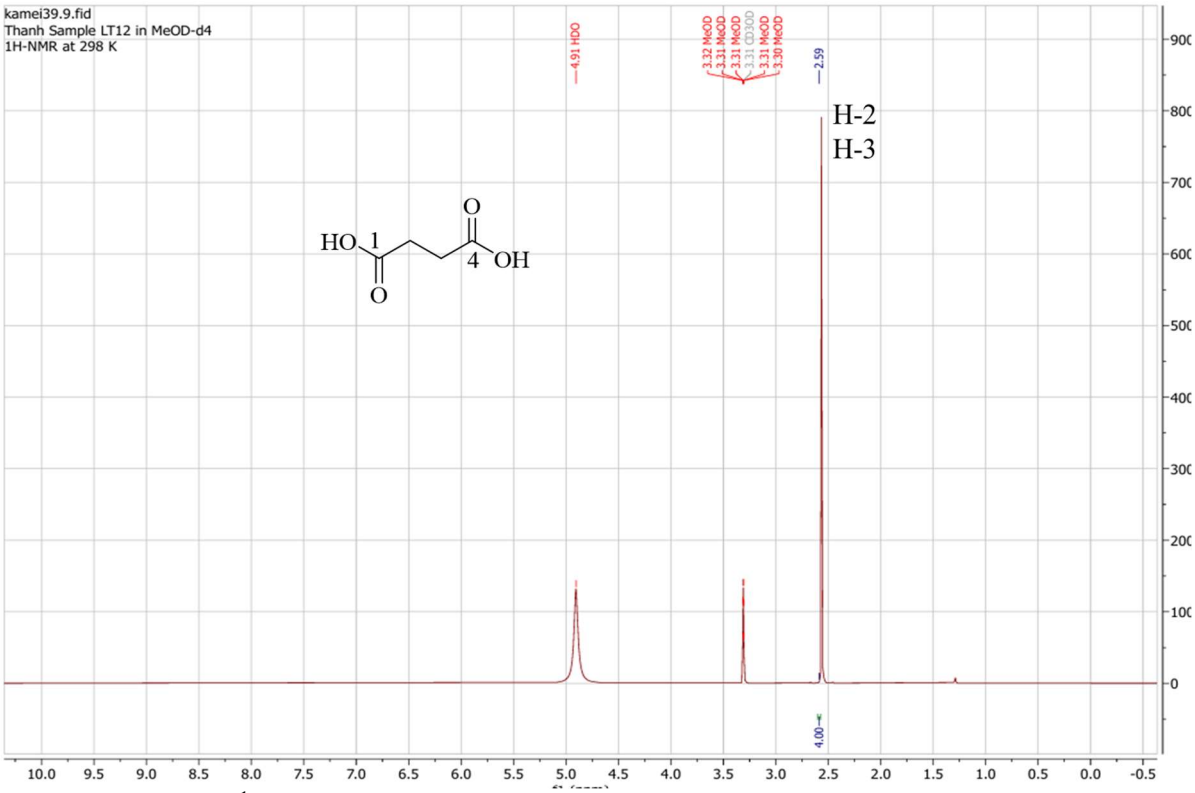

Figure S1: ^{1}H-NMR (600 MHz, CD_3OD) spectrum of compound **1** (succinic acid)

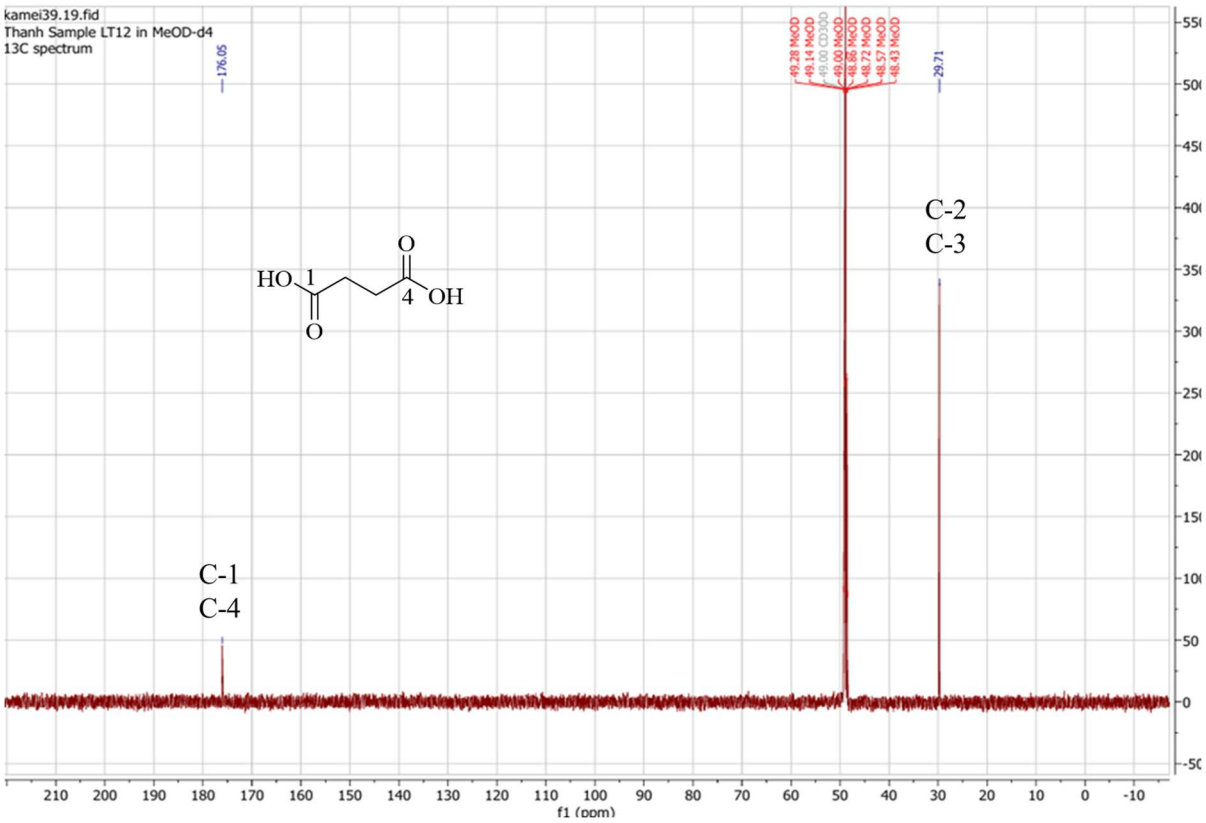

Figure S2: ^{13}C-NMR (150 MHz, CD_3OD) spectrum of compound **1** (succinic acid)

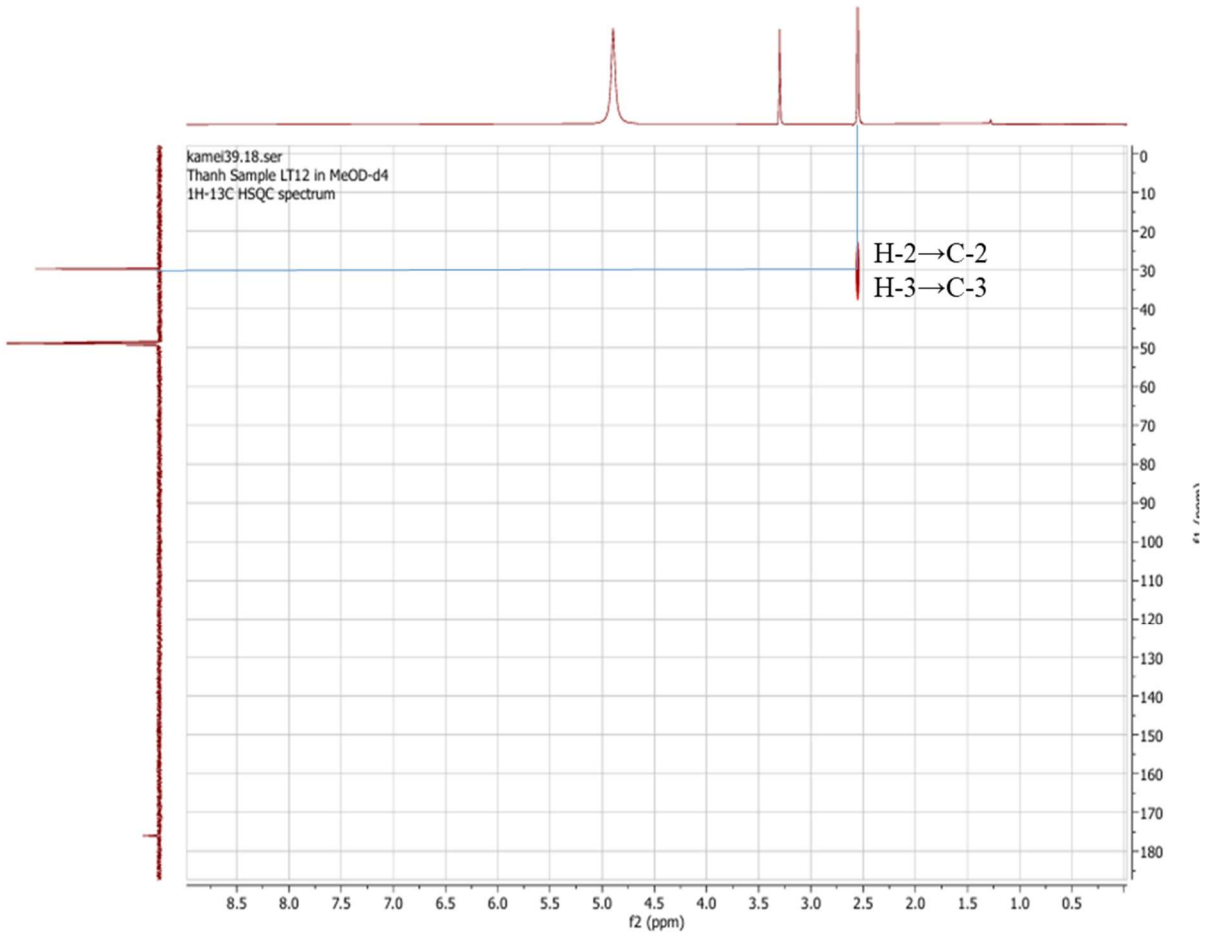

Figure S3: HSQC spectrum of compound **1** (succinic acid)

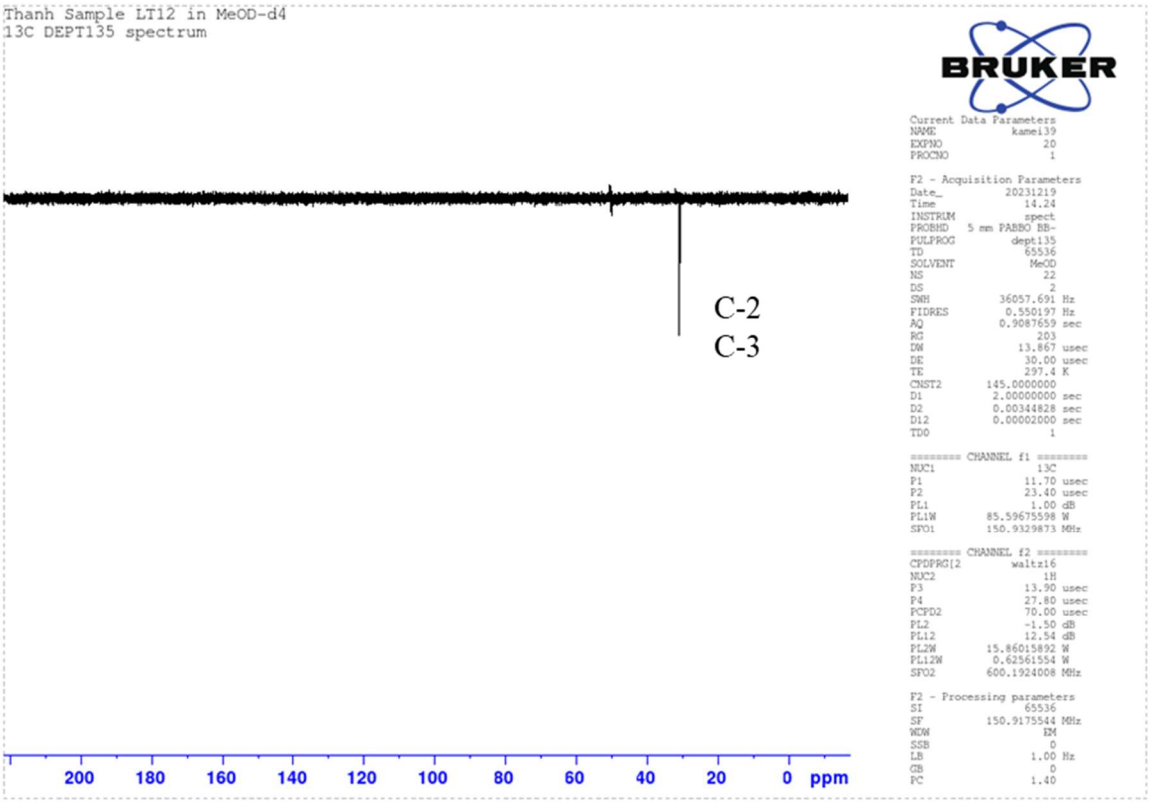

Figure S4: DEPT-135 spectrum of compound **1** (succinic acid)

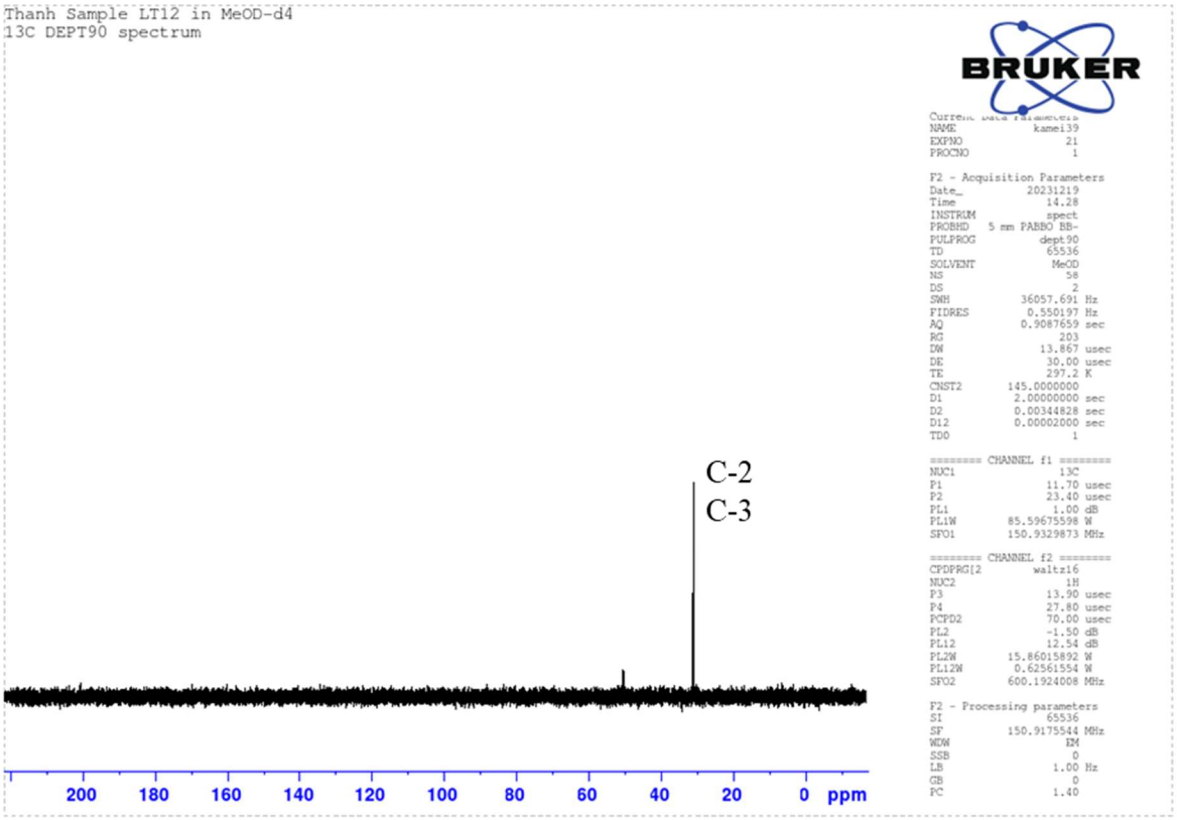

Figure S5: DEPT-90 spectrum of compound **1** (succinic acid)

2. Supplementary spectroscopic data of compound 2.

Table S5: The comparison of NMR data of compound **2** with a similar compound (Quercetin).

Position	Compound 2 (CD_3OD)		Quercetin (CD_3OD) [2]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (500 MHz) δ_H ppm
2	-	148.8	-	148.6
3	-	137.2	-	137.2
4	-	177.3	-	177.3
5	-	162.5	-	162.6
6	6.20 (1H, *d, J* = 1.8 Hz)	99.3	6.20 (1H, *d,* *J* = 2.0 Hz)	99.1
7	-	165.6	-	165.6
8	6.41 (1H, *d, J* = 1.8 Hz)	94.4	6.41 (1H, *d,* *J* = 2.0 Hz)	94.4
9	-	158.3	-	158.2
10	-	104.5	-	104.5
1'	-	124.2	-	124.1
2'	7.75 (1H, *d, J* = 2.4 Hz)	116.0	7.75 (1H, *d,* *J* = 2.2 Hz)	116.0
3'	-	146.2	-	146.2
4'	-	148.0	-	148.0
5'	6.91 (1H, *d, J* = 9 Hz)	116.2	6.99 (1H, *d,* *J* = 8.5 Hz)	116.2
6'	7.66 (1H, *dd, J* = 9.0, 2.4 Hz)	121,7	7.65 (1H, *dd,* *J* = 8.5; 2 Hz)	121,7

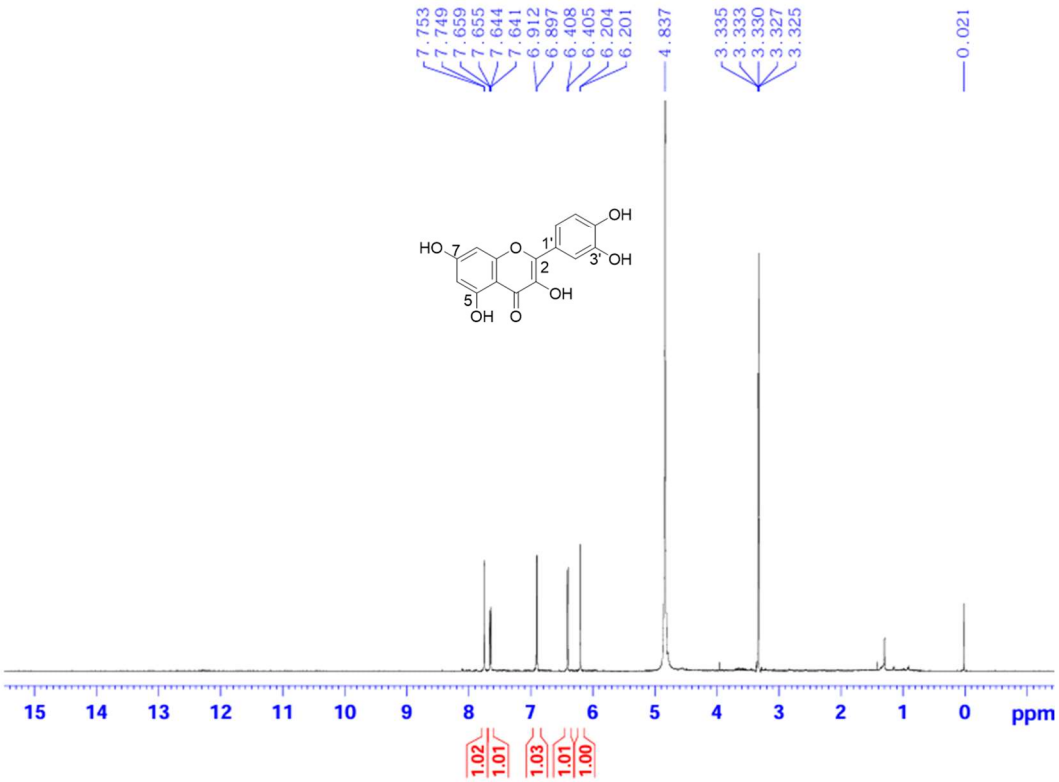

Figure S6: [1]H-NMR (600 MHz, CD_3OD) spectrum of compound **2** (quercetin)

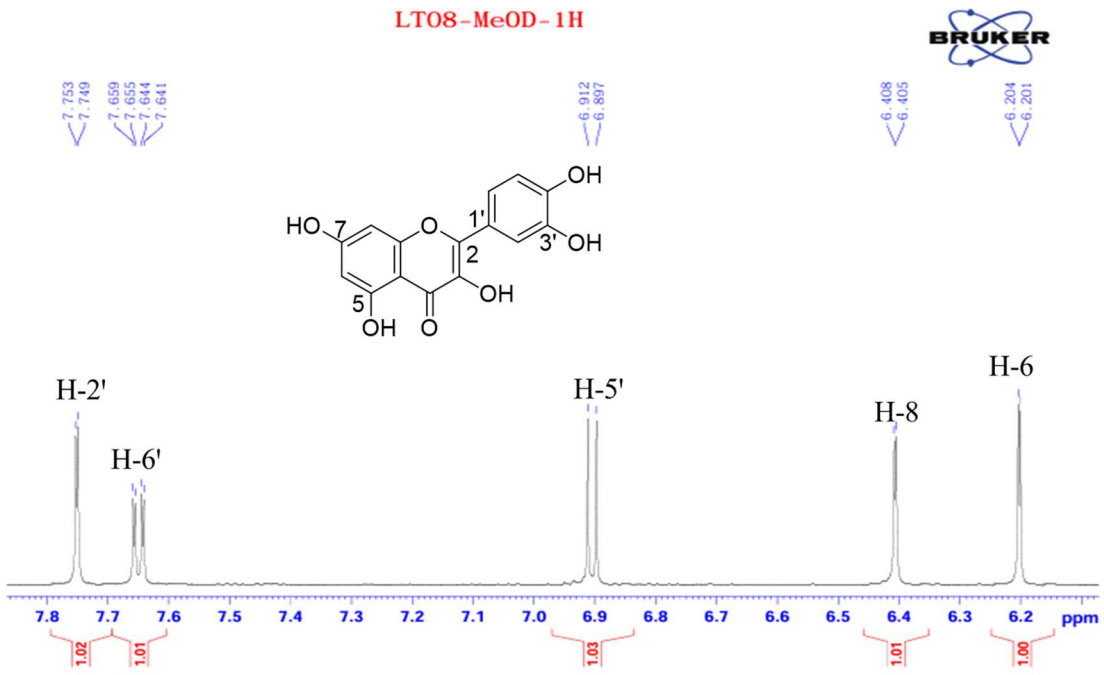

Figure S7: [1]H-NMR (600 MHz, CD_3OD) spectrum of compound **2** (quercetin) (from δ_H 6 ppm to δ_H 8 ppm)

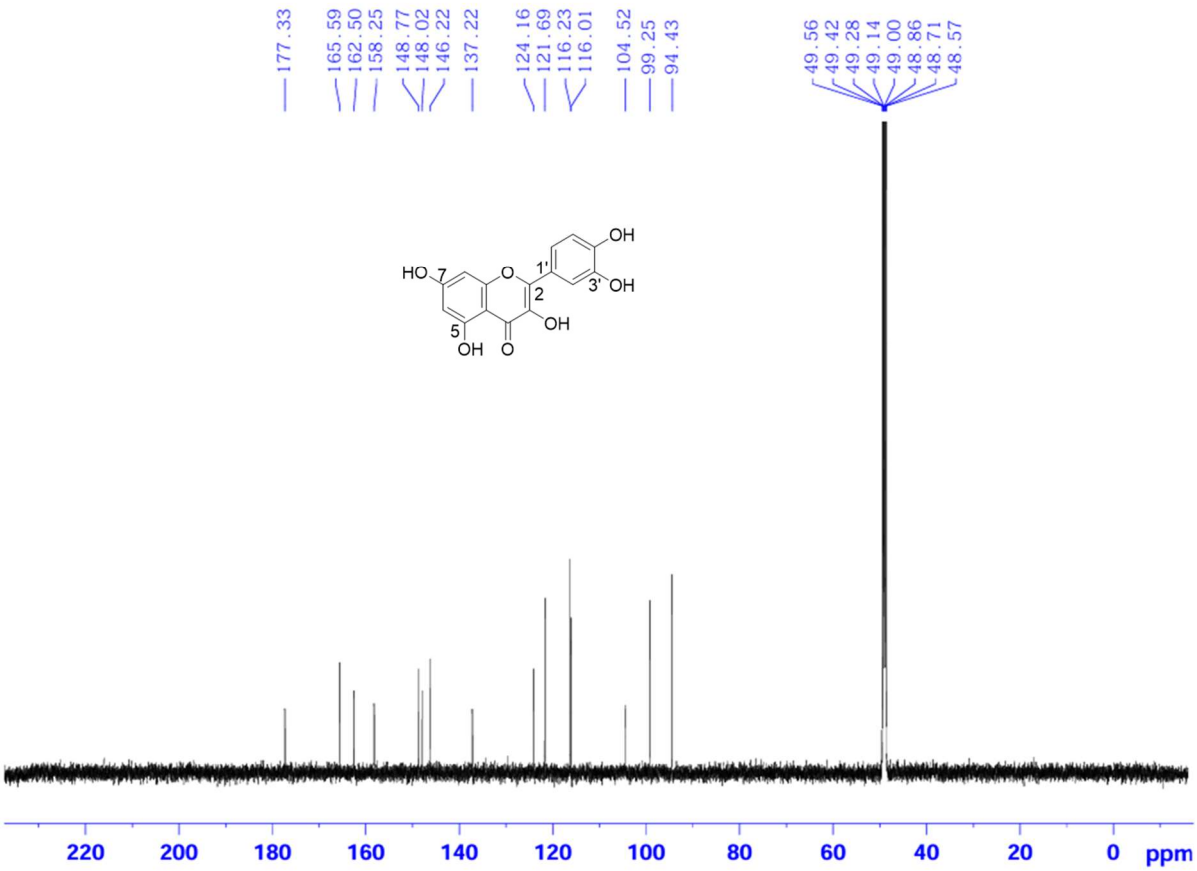

Figure S8: ^{13}C-NMR (150 MHz, CD_3OD) spectrum of compound **2** (quercetin)

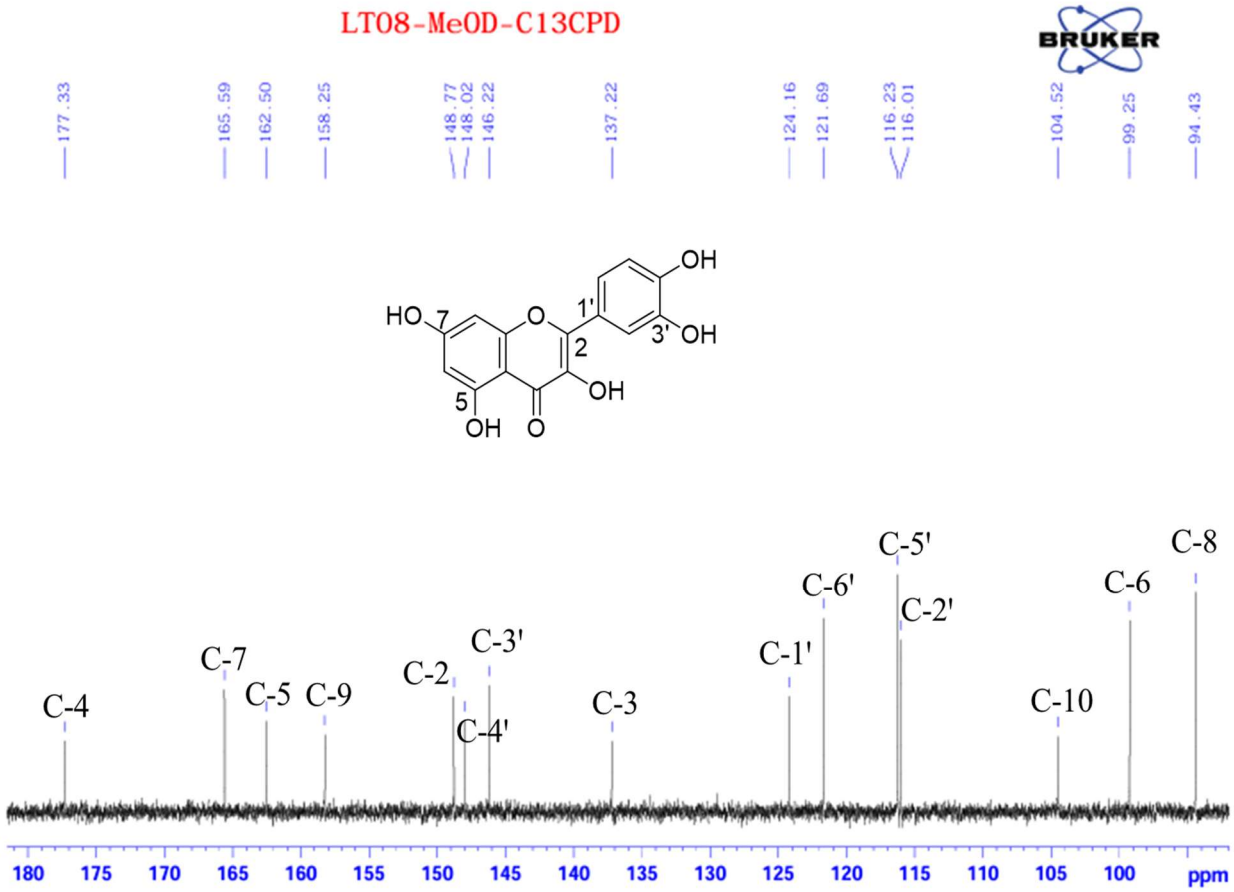

Figure S9: ^{13}C-NMR (150 MHz, CD_3OD) spectrum of compound **2** (quercetin) (from δ_C 95 ppm to δ_C 180 ppm)

4. Supplementary spectroscopic data of compound 3.

Table S6: The comparison of NMR data of compound **3** with a similar compound (Quercetin-3-*O*-rutinoside).

Position	Compound 3 (DMSO-d_6)		Quercetin-3-*O*-rutinoside (DMSO-d_6)[5]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (500 MHz) δ_H ppm
2	-	156.5	-	156.5
3	-	133.3	-	133.3
4	-	177.3	-	177.3
5	-	161.2	-	161.2
6	6.19 (1H, *d*, *J* =1.8 Hz)	98.6	6.19 (1H, *d*, *J* = 2.0 Hz)	98.7
7	-	164.0	-	164.2

8	6.38 (1H, *d*, *J* =1.8 Hz)	93.5	6.38 (1H, *d*, *J* = 2.0 Hz)	93.6
9	-	156.4	-	156.4
10	-	103.9	-	103.9
1'	-	121.1	-	121.1
2'	7.55 (1H, *m*, *J* = 8.4; 2.4 Hz)	115.2	7.53 (1H, *m*, *J* = 8.5; 2.0 Hz)	115.2
3'	-	144.7	-	144.7
4'	-	148.4	-	148.4
5'	6.84 (1H, *d*, *J* = 8.4 Hz)	116.2	6.84 (1H, *d*, *J* = 8.2 Hz)	116.2
6'	7.55 (1H, *m, J* = 8.4; 2.4 Hz)	121.5	7.53 (1H, *m*, *J* = 8.5; 2.0 Hz)	121.7
1"	5.35 (1H, *d*, *J* = 7.2 Hz)	101.1	5.34 (1H, *d*, *J* = 7.3 Hz)	101.2
2"	3.21 – 3.39 (1H, *m*)	74.0	3.15 – 3.41 (*m*)	74.1
3"	3.21 – 3.39 (1H, *m*)	76.4	3.15 – 3.41 (*m*)	76.4
4"	3.21 – 3.39 (1H, *m*)	70.0	3.15 – 3.41 (*m*)	70.0
5"	3.21 – 3.39 (1H, *m*)	75.9	3.15 – 3.41 (*m*)	75.9
6"	3.21 – 3.39 (1H, *m*) 3.72 (1H, *d, J* = 10.2 Hz)	66.9	3.15 – 3.41 (*m*)	66.8
1"'	4.39 (1H, *brd, J* = 0.6 Hz)	100.7	4.38 (1H, *s*)	100.7
2"'	3.04 – 3.09 (1 H, *m*)	70.3	3.05 – 3.10 (*m*)	70.4
3"'	3.04 – 3.09 (1 H, *m*)	70.5	3.05 – 3.10 (*m*)	70.5
4"'	3.04 – 3.09 (1 H, *m*)	71.8	3.05 – 3.10 (*m*)	71.8
5"'	3.04 – 3.09 (1 H, *m*)	68.2	3.05 – 3.10 (*m*)	68.2

6'''	1.00 (3H, *d*, *J* = 6.6 Hz)	17.5	0.99 (3H, *d*, *J* = 6.2 Hz)	17.7
5-OH	12.59 (*s*)		12.61 (s)	

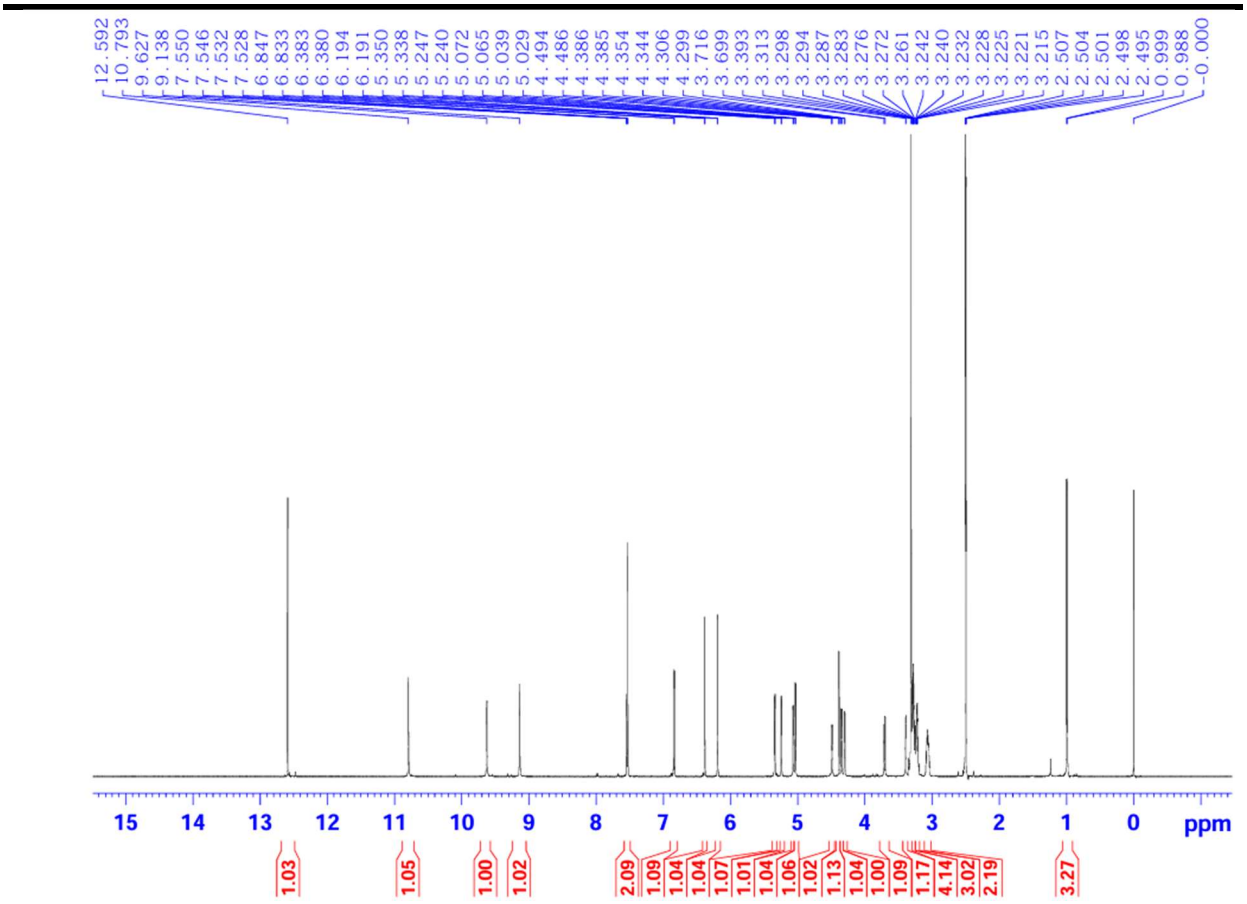

Figure S10: [1]H-NMR (600 MHz, DMSO-d_6) spectrum of compound **3** (quercetin-3-*O*-rutinoside)

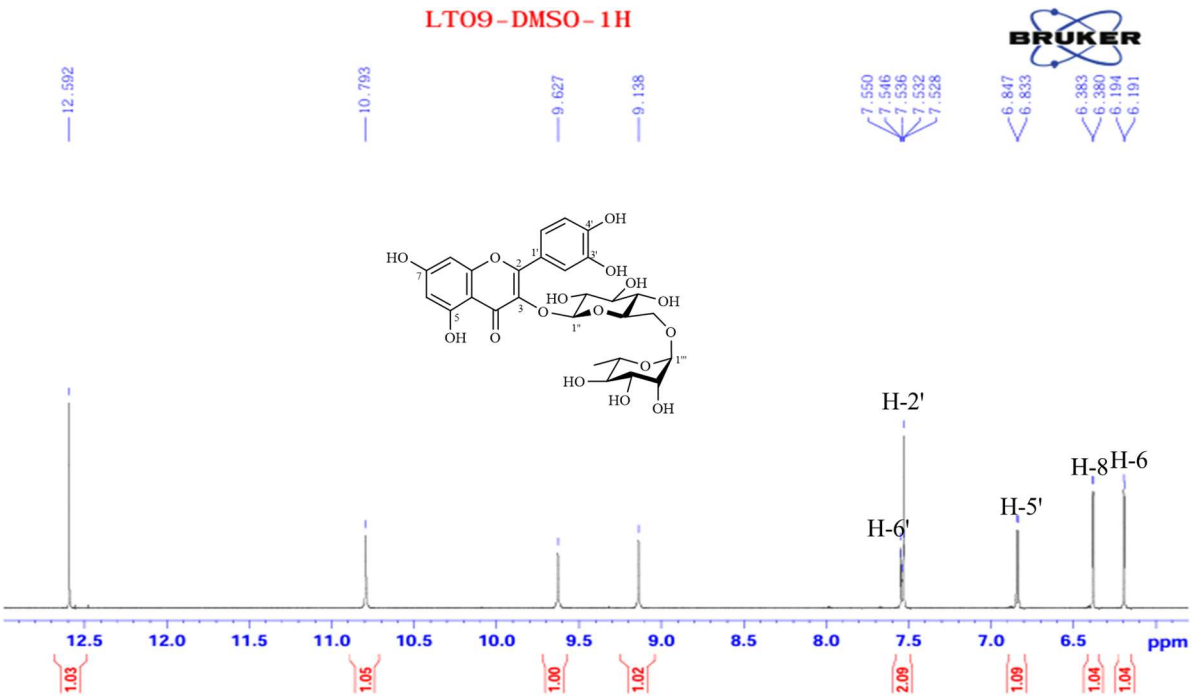

Figure S11: ^{1}H-NMR (600 MHz, DMSO-d_6) spectrum of compound **3** (quercetin-3-*O*-rutinoside) (from δ_H 6 ppm to δ_H 13 ppm)

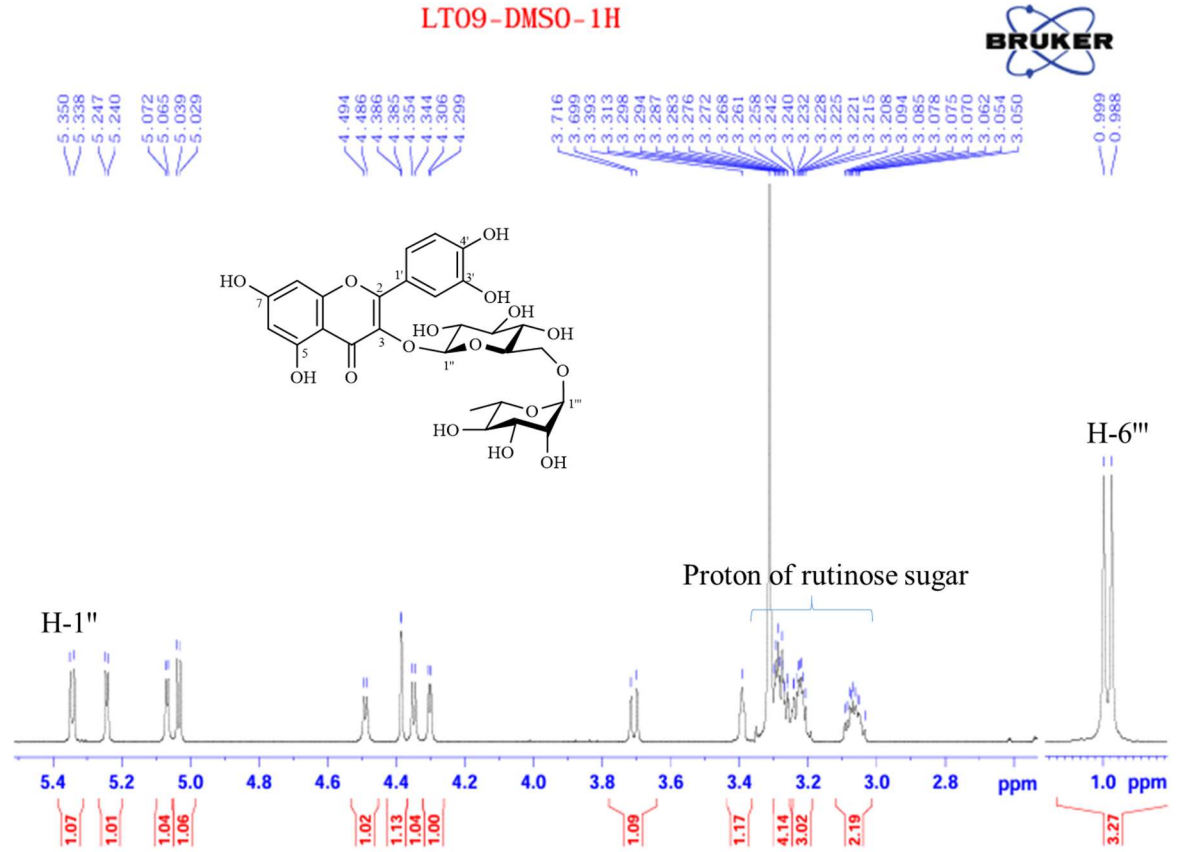

Figure S12: ^{1}H-NMR (600 MHz, DMSO-d_6) spectrum of compound **3** (quercetin-3-*O*-rutinoside) (from δ_H 1.0 ppm to δ_H 5.5 ppm)

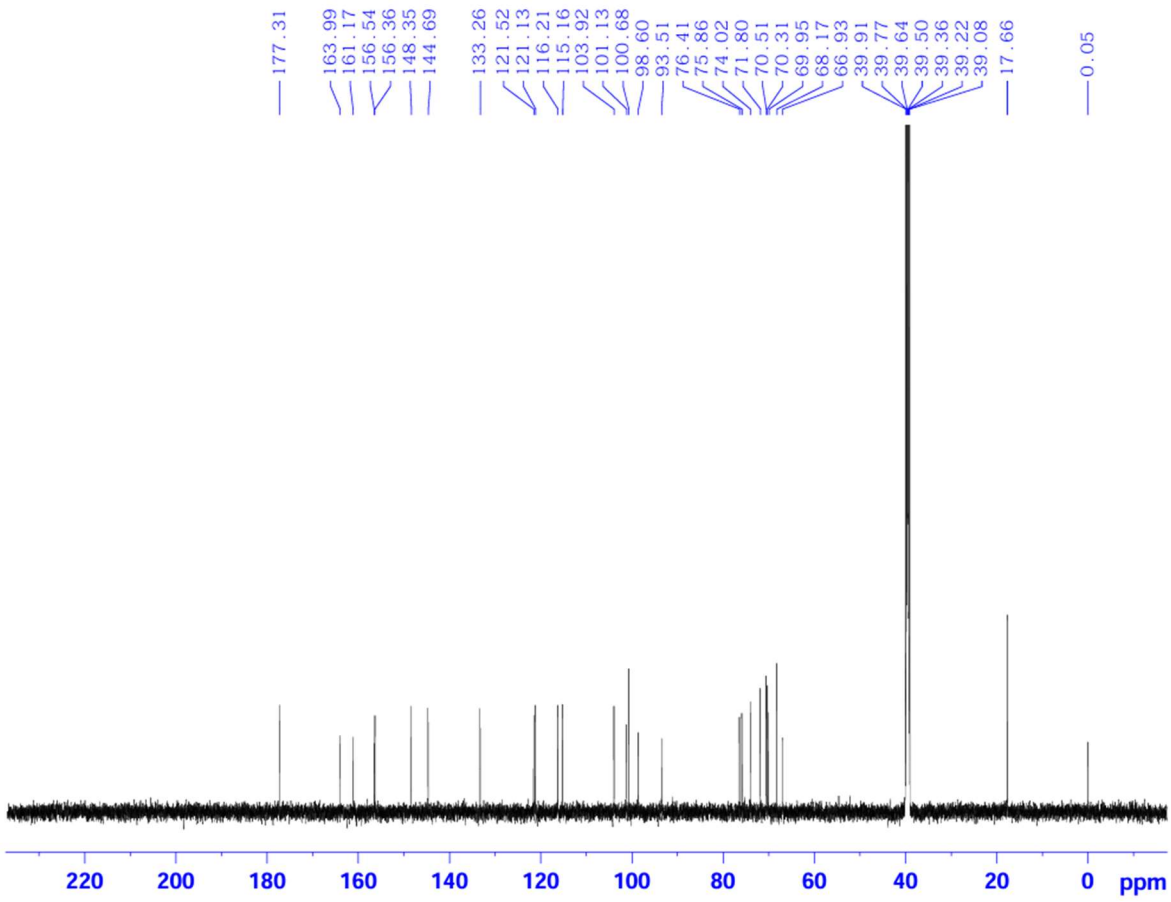

Figure S13: ^{13}C-NMR (150 MHz, DMSO-d_6) spectrum of compound **3** (quercetin-3-*O*-rutinoside)

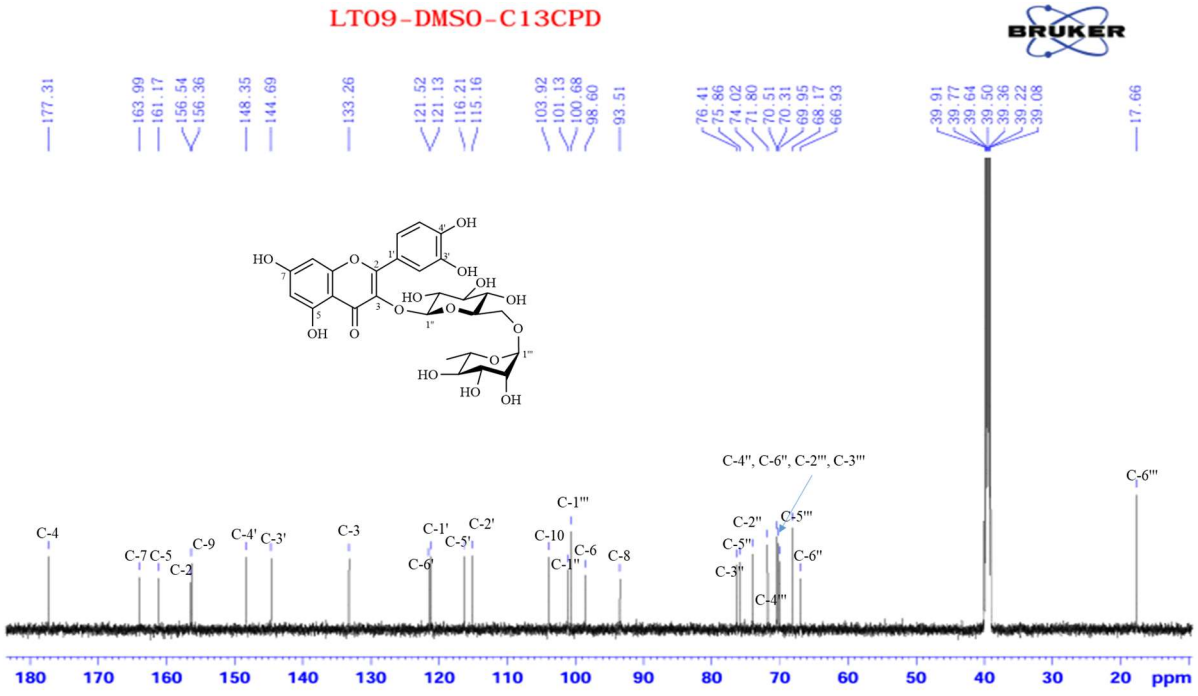

Figure S14: ^{13}C-NMR (150 MHz, DMSO-d_6) spectrum of compound **3** (quercetin-3-*O*-rutinoside) (from δ_C 20 ppm to δ_C 185 ppm)

4. Supplementary spectroscopic data of compound 4.

Table S7: The comparison of NMR data of compound **4** with a similar compound (Luteolin-7-*O*-*β*-glucopyranoside).

Position	Compound 4 (DMSO-d_6)		Luteolin-7-*O*-*β*-D-glucopyranoside (DMSO-d_6) [4]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (500 MHz) δ_H ppm
2	-	164.5	-	164.9
3	6.75 (1H, *s*)	103.2	6.76 (1H, *s*)	103.5
4	-	181.9	-	182.3
5	-	161.1	-	161.6
6	6.45 (1H, *d*, *J* = 2.4 Hz)	99.5	6.45 (1H, *d*, *J* = 1.7 Hz)	99.9
7	-	162.9	-	163.4
8	6.79 (1H, *d*, *J* = 1.8 Hz)	94.7	6.79 (1H, *d*, *J* = 1.7 Hz)	95.1
9	-	156.9	-	157.4
10	-	105.3	-	105.8
1′	-	121.4	-	121.6
2′	7.43 (1H, *d*, *J* = 2.4 Hz)	113.6	7.43 (1H, *d*, *J* = 8.4 Hz)	113.9
3′	-	145.8	-	146.4
4′	-	149.9	-	150.7
5′	6.92 (1H, *d*, *J* = 8.4 Hz)	116.0	6.90 (1H, *d*, *J* = 8.4 Hz)	116.4
6′	7.46 (1H, *dd*, *J* = 8.4; 2.4 Hz)	119.1	7.45 (1H, *dd*, *J* = 8.3; 2.3 Hz)	119.6
1″	5.09 (1H, *d*, *J* = 7.2 Hz)	99.9	5.08 (1H, *d*, *J* = 7.3 Hz)	100.3
2″	3.28 (1H, *m*)	73.1	3.26 (1H, *m*)	73.6
3″	3.45 (1H, *m*)	77.1	3.45 (1H, *m*)	77.6

4″	3.21 (1H, *t*, *J* = 9.0; 9.6 Hz)	69.6	3.17 (1H, *m*)	70.0
5″	3.34 (1H, *m*)	76.4	3.30 (1H, *m*)	76.9
6″	3.50 (1H, *m*) 3.73 (1H, *d*, *J* = 11.4 Hz)	60.6	3.48 (1H, *m*) 3.72 (1H, *d*, *J* = 9.9 Hz)	61.1

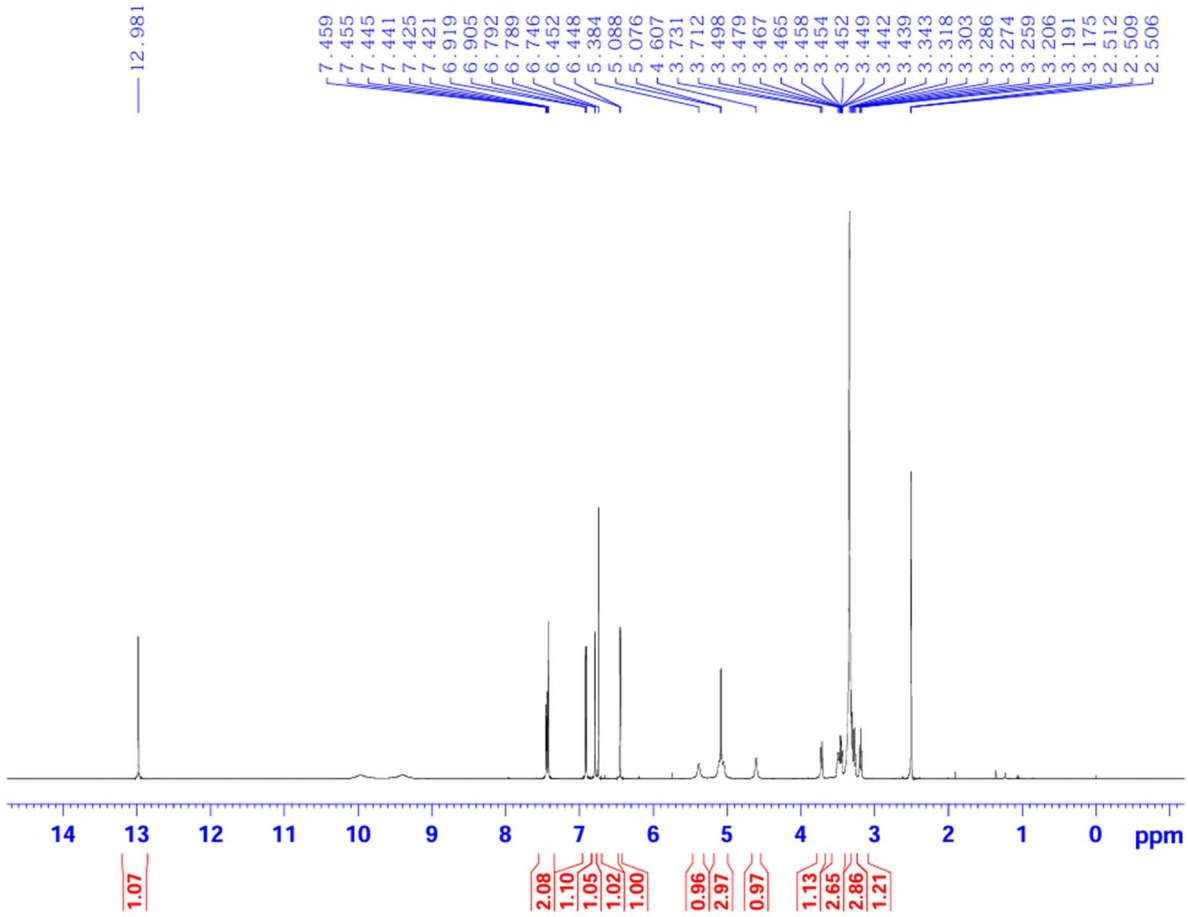

Figure S15: ^{1}H-NMR (600 MHz, DMSO-d_6) spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside)

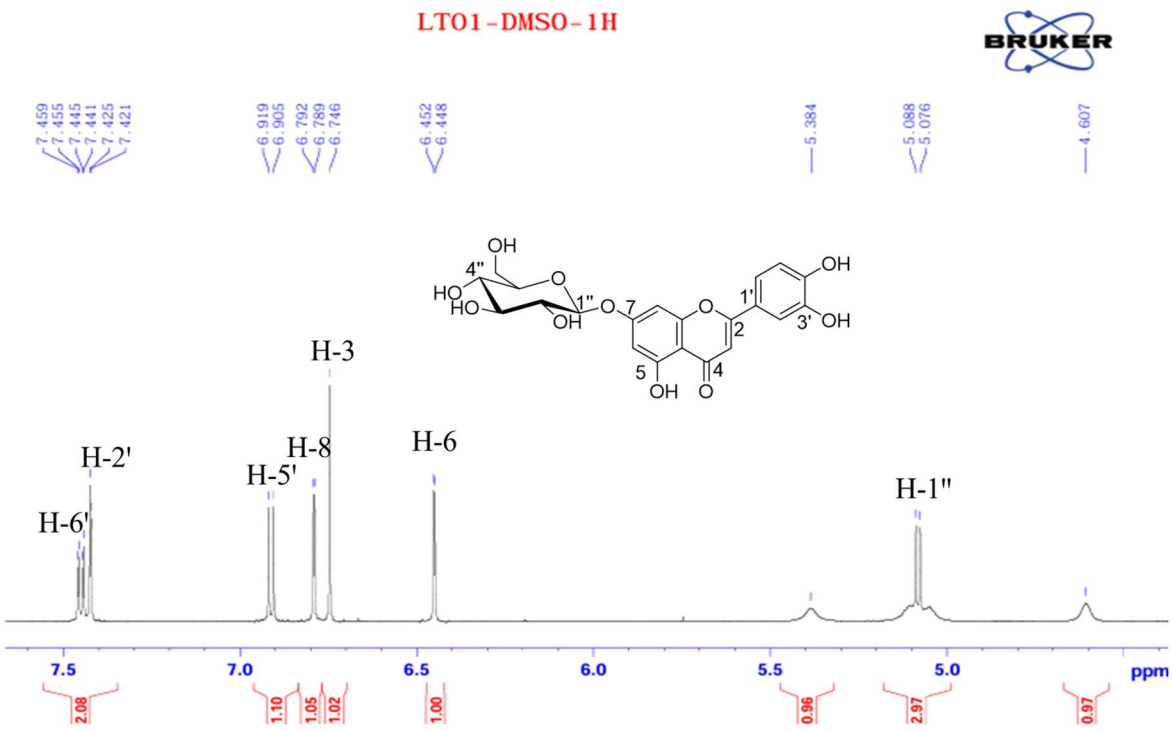

Figure S16: ^{1}H-NMR (600 MHz, DMSO-d_6) spectrum of compound **4** (luteolin-7-*O*-β-D-glucopyranoside) (from δ_H 4 ppm to δ_H 7.5 ppm)

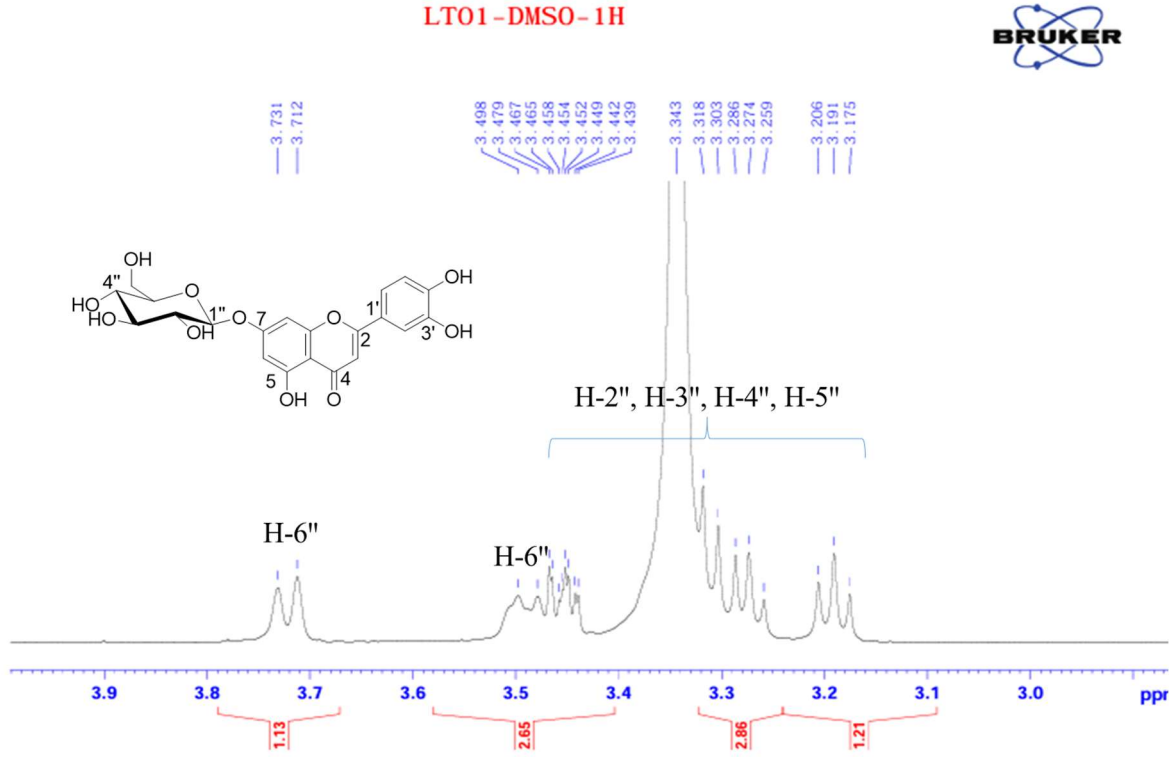

Figure S17: ^{1}H-NMR (600 MHz, DMSO-d_6) spectrum of compound **4** (luteolin-7-*O*-β-D-glucopyranoside) (from δ_H 3 ppm to δ_H 4 ppm)

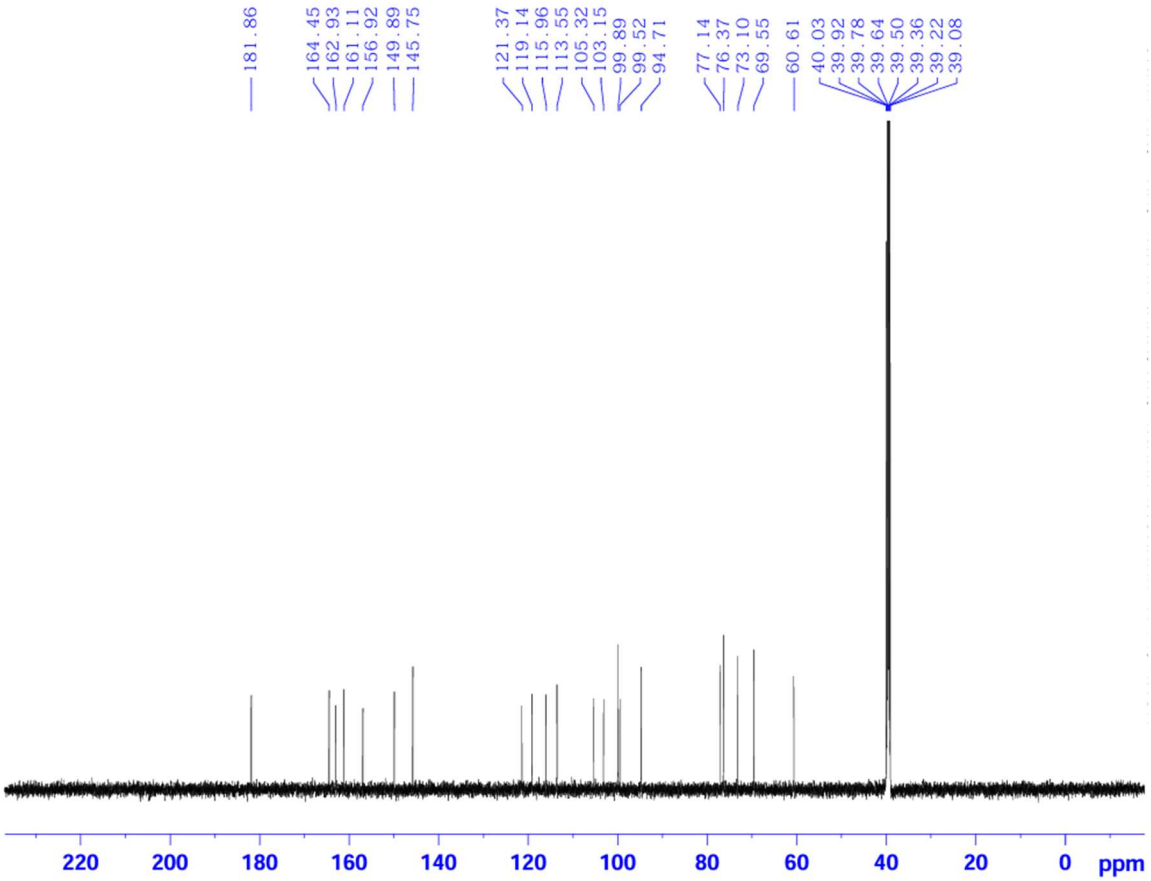

Figure S18: ^{13}C-NMR (150 MHz, DMSO-d_6) spectrum of compound **4** (luteolin-7-*O*-β-D-glucopyranoside)

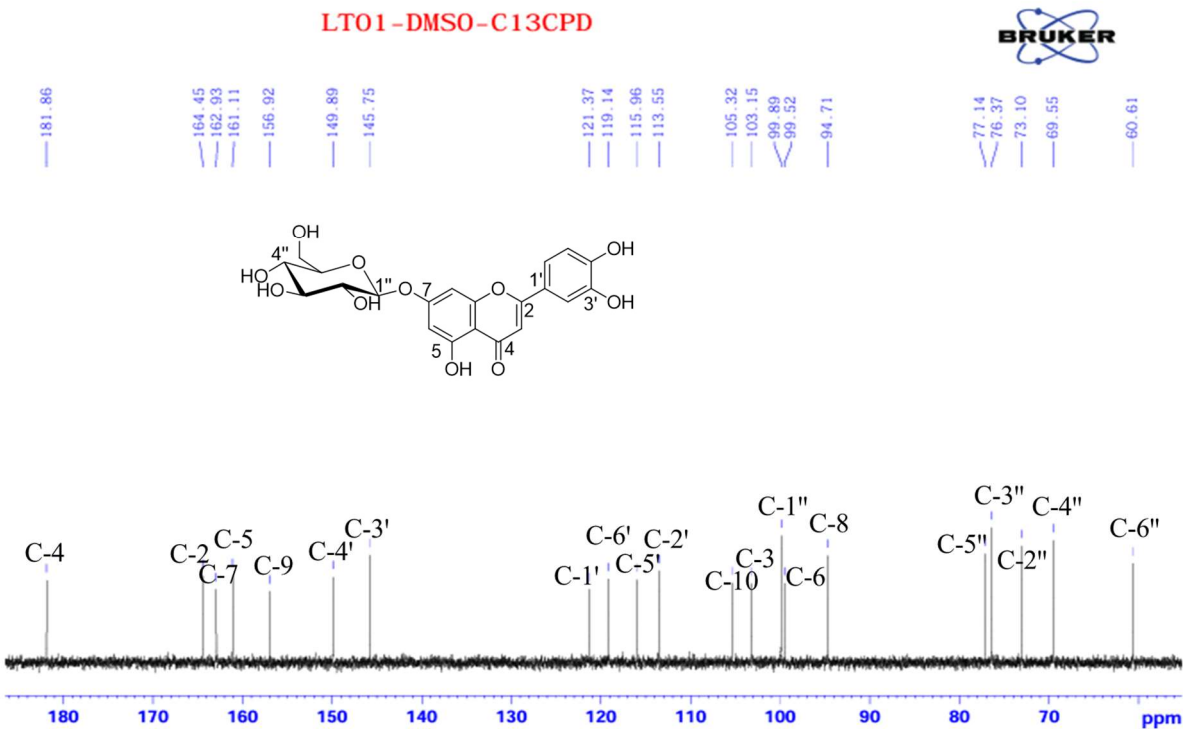

Figure S19: ^{13}C-NMR (150 MHz, DMSO-d_6) spectrum of compound **4** (luteolin-7-*O*-β-D-glucopyranoside) (from δ_C 60 ppm to δ_C 185 ppm)

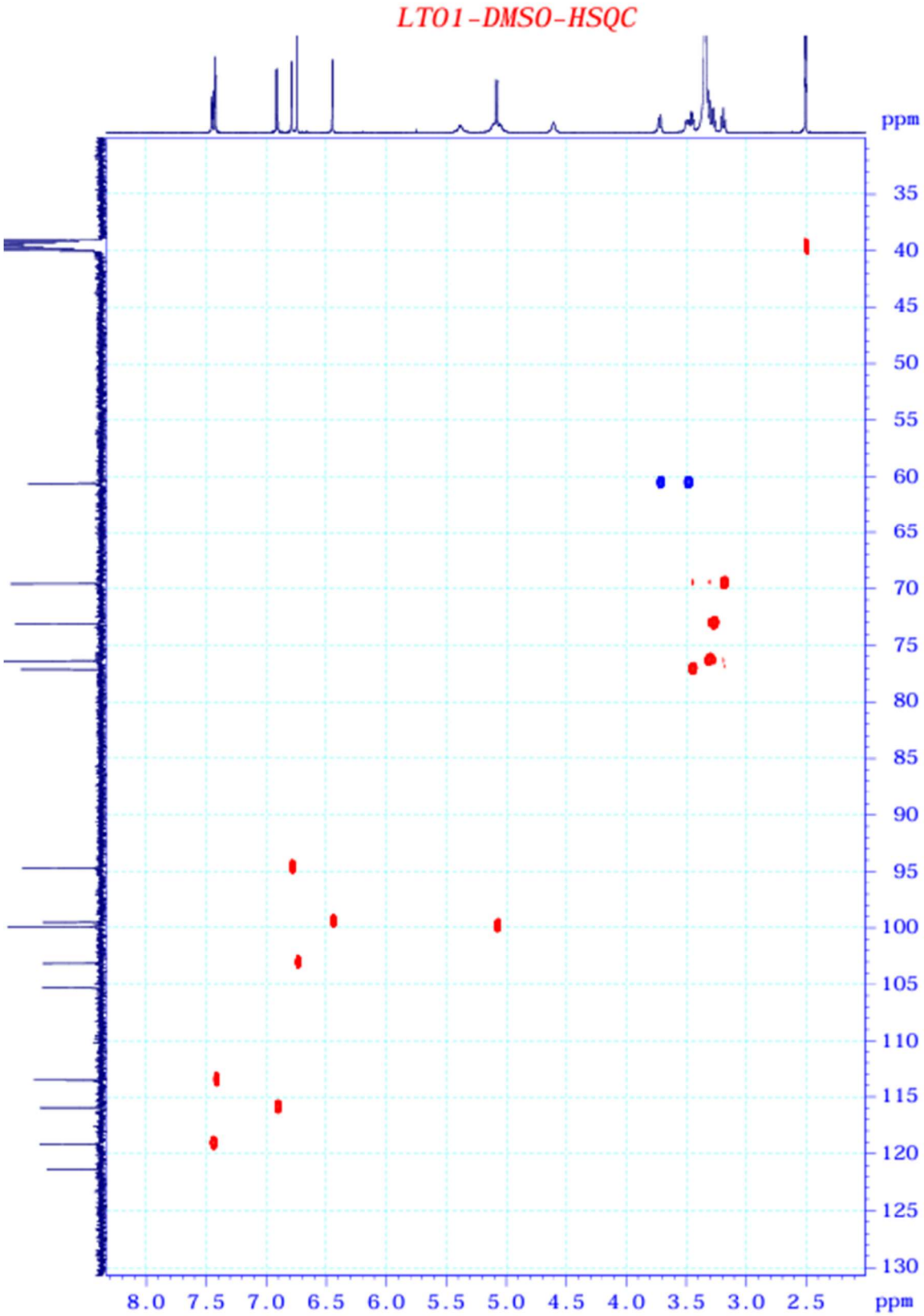

Figure S20: HSQC spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside)

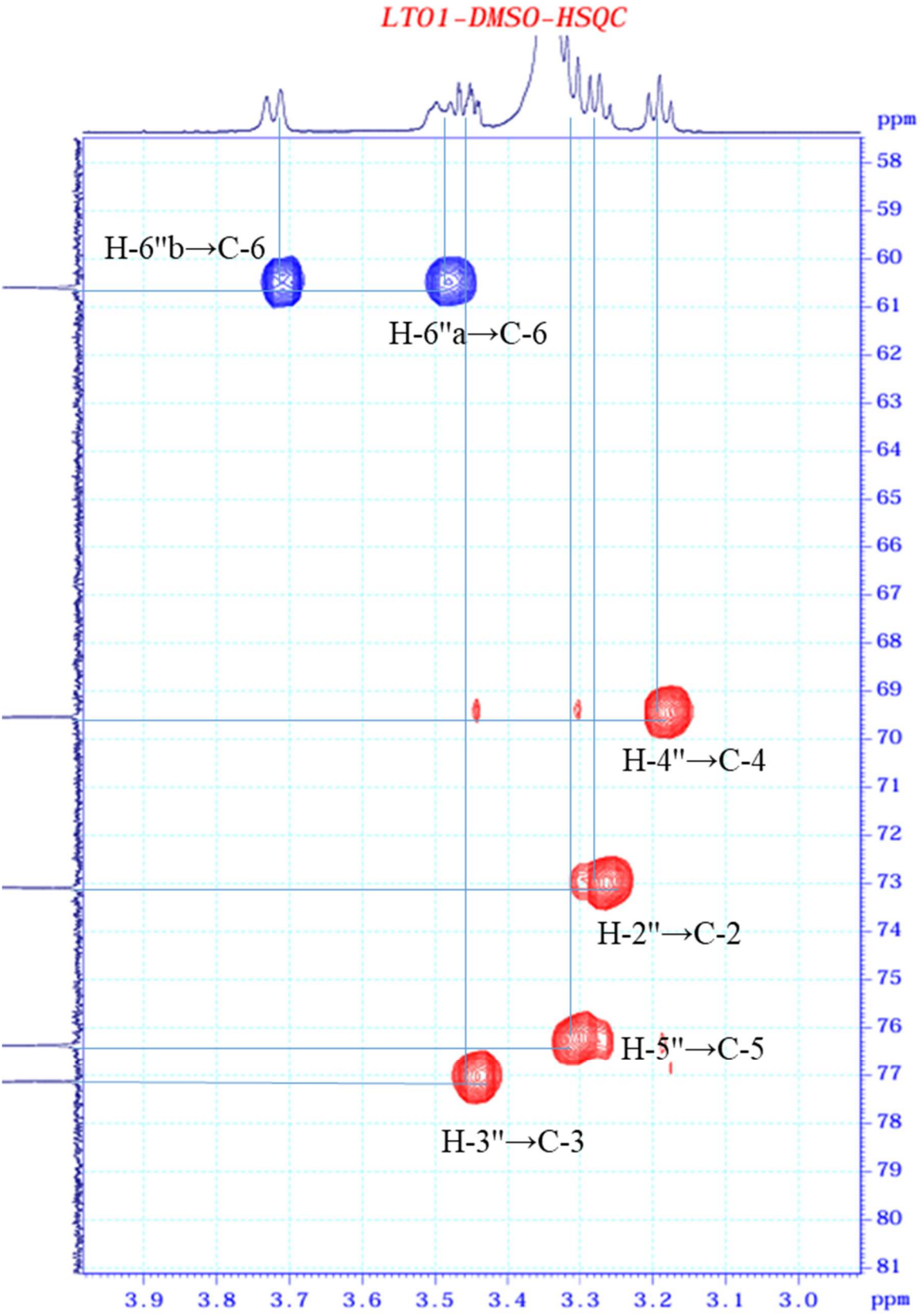

Figure S21: HSQC spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside) (from δ_C 57 ppm to δ_C 8 ppm)

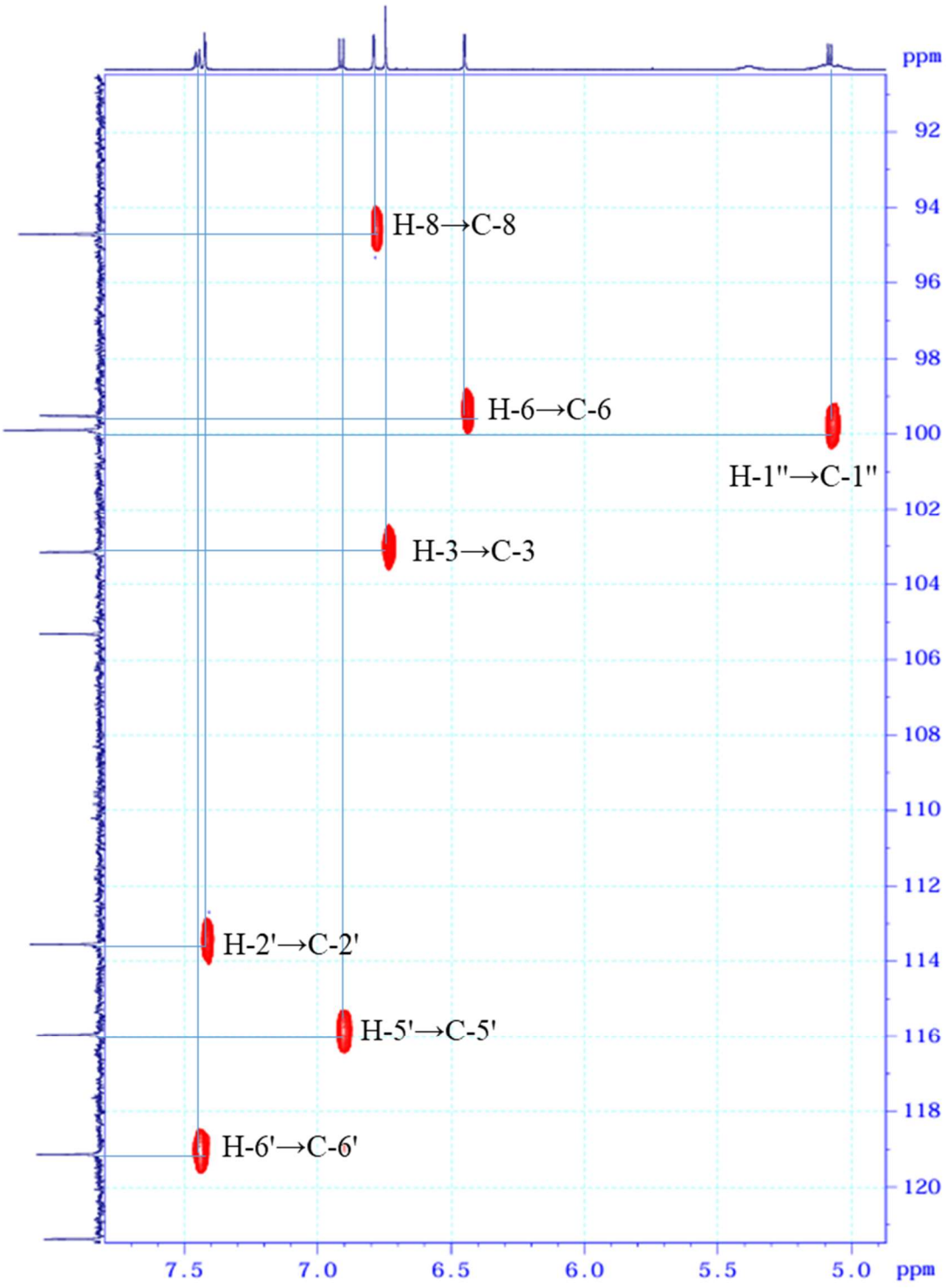

Figure S22: HSQC spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside) (from δ_C 90 ppm to δ_C 120 ppm)

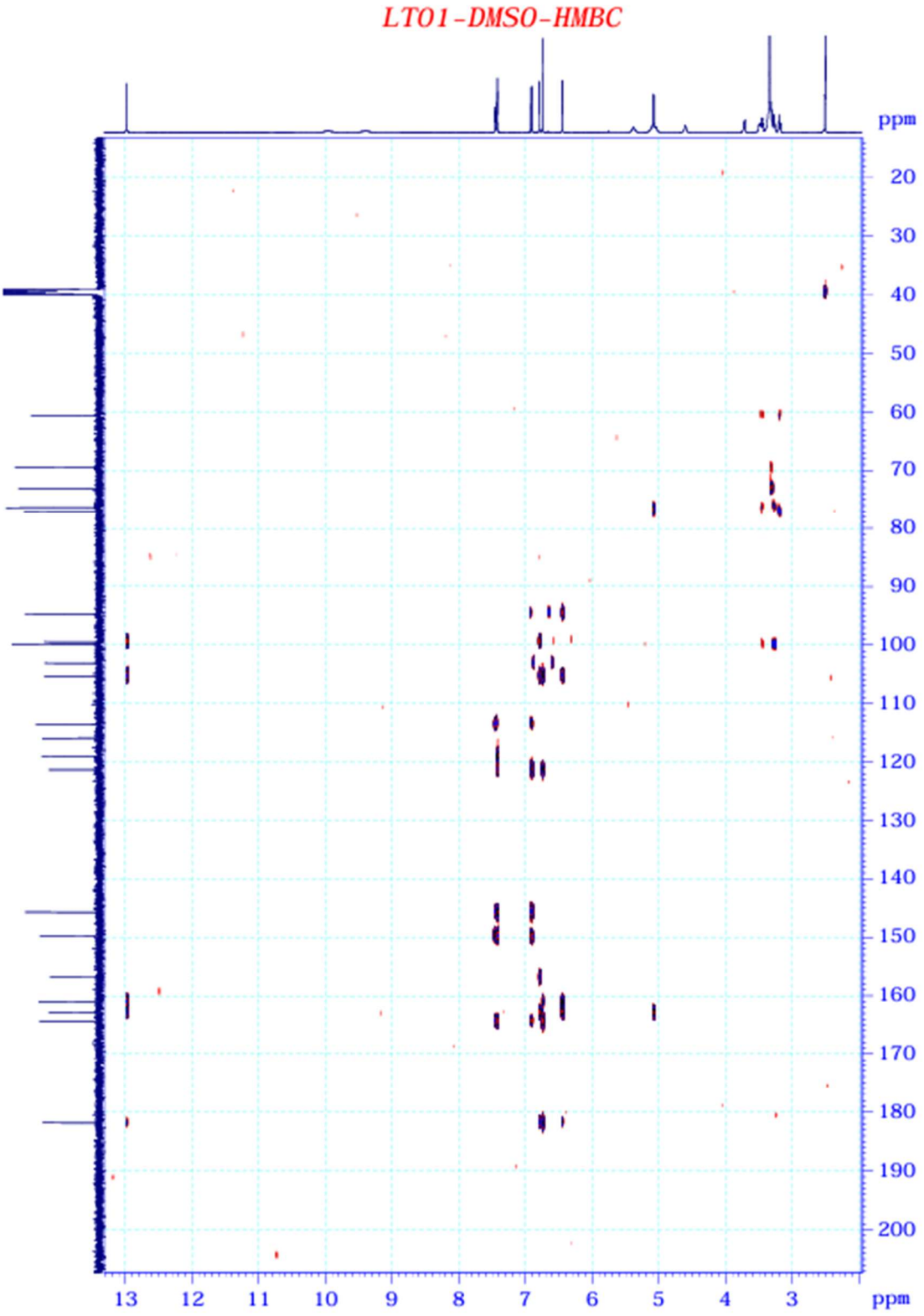

Figure S23: HMBC spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside)

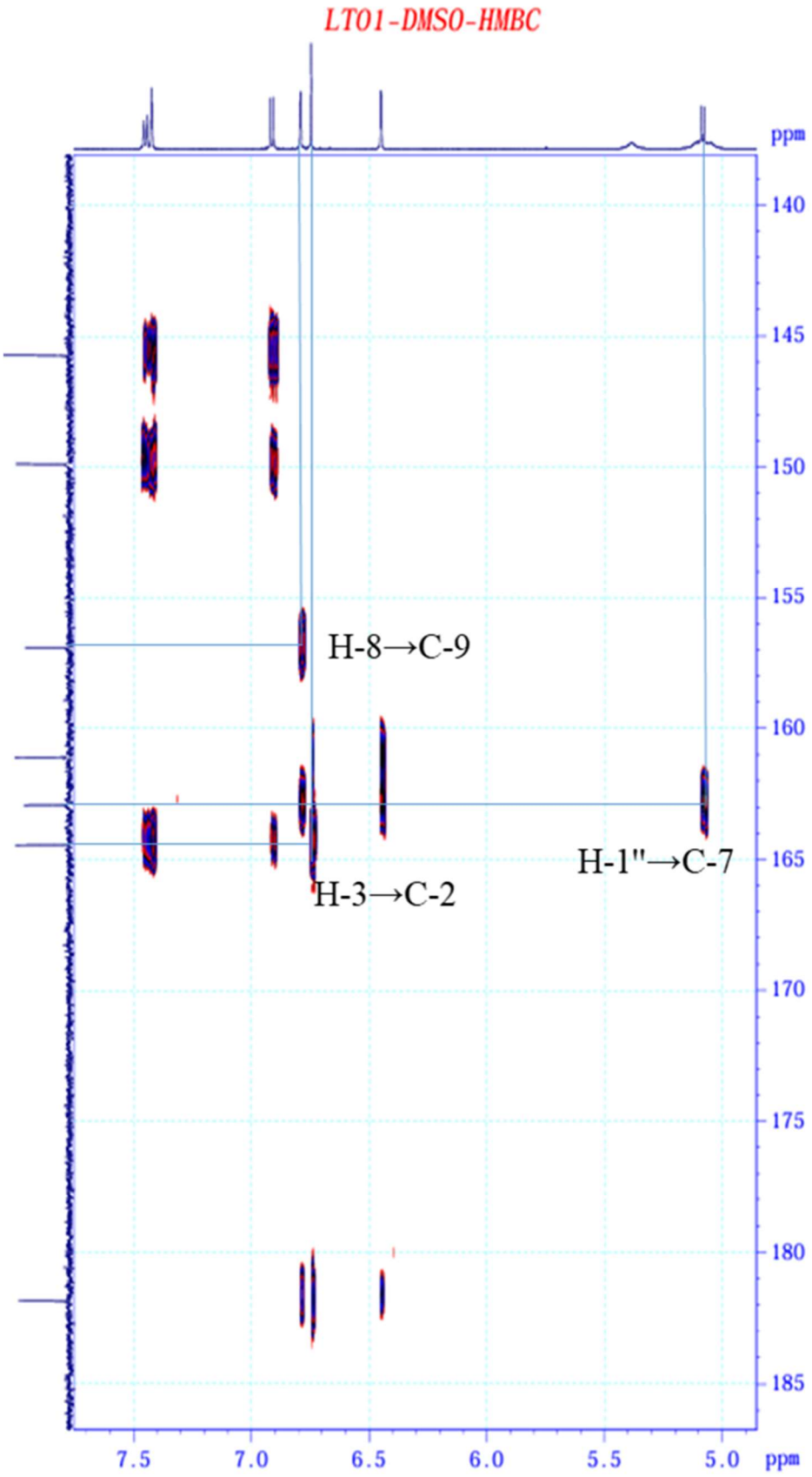

Figure S24: HMBC spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside) (from δ_C 140 ppm to δ_C 185 ppm)

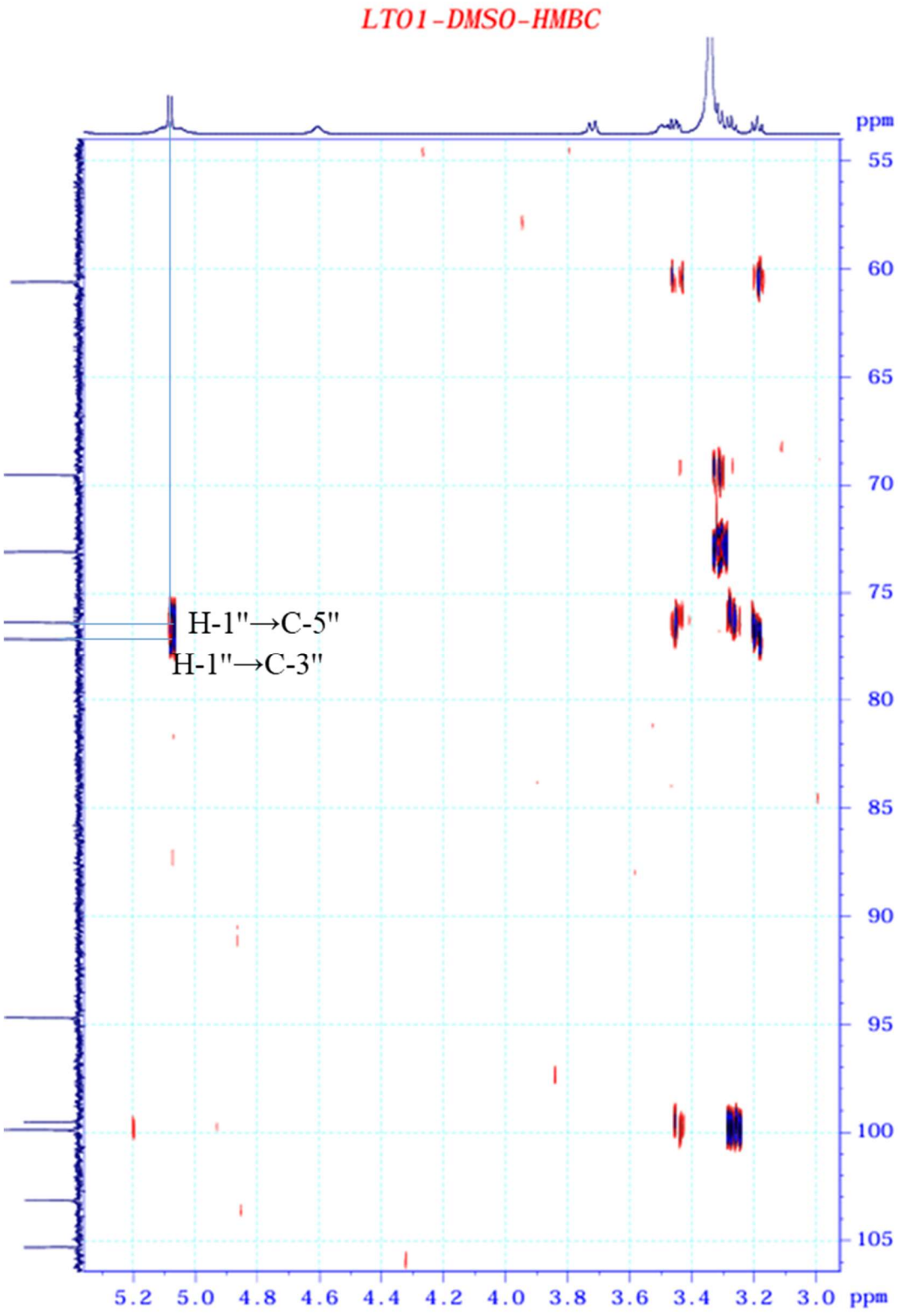

Figure S25: HMBC spectrum of compound **4** (luteolin-7-*O*-*β*-D-glucopyranoside) (from δ_C 55 ppm to δ_C 105 ppm)

5. Supplementary spectroscopic data of compound 5.

Table S8: The comparison of NMR data of compound **5** with a similar compound (2-(4-hydroxyphenyl)acetic acid)

Position	Compound 3 (CD_3OD)		2-(4-Hydroxyphenyl)acetic acid (CD_3OD) [3]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (500 MHz) δ_H ppm
1	-	126.8	-	127.1
2, 6	7.09 (2H, *d, J* = 8.4 Hz)	131.3	7.08 (2H, *d, J* = 8.3 Hz)	131.5
3, 5	6.74 (2H, *d, J* = 8.4 Hz)	116.2	6.72 (2H, *d, J* = 8.5 Hz)	116.3
4	-	157.4	-	157.6
7	3.50 (2H, *s*)	41.1	3.5 (2H, *s*)	41.7
8	-	176.3	-	176.2

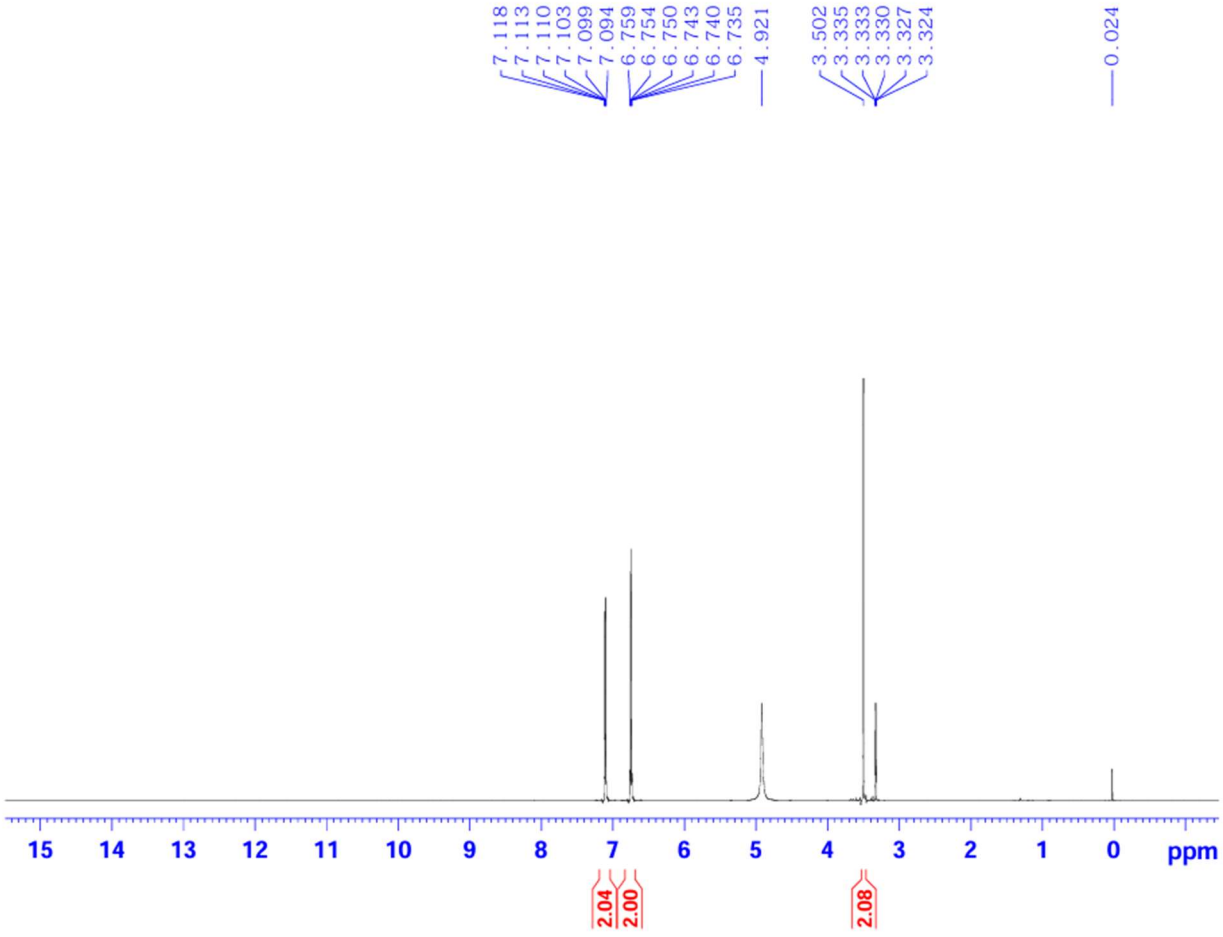

Figure S26: ^{1}H-NMR (600 MHz, CD_3OD) spectrum of compound **5** (2-(4-Hydroxyphenyl)acetic acid)

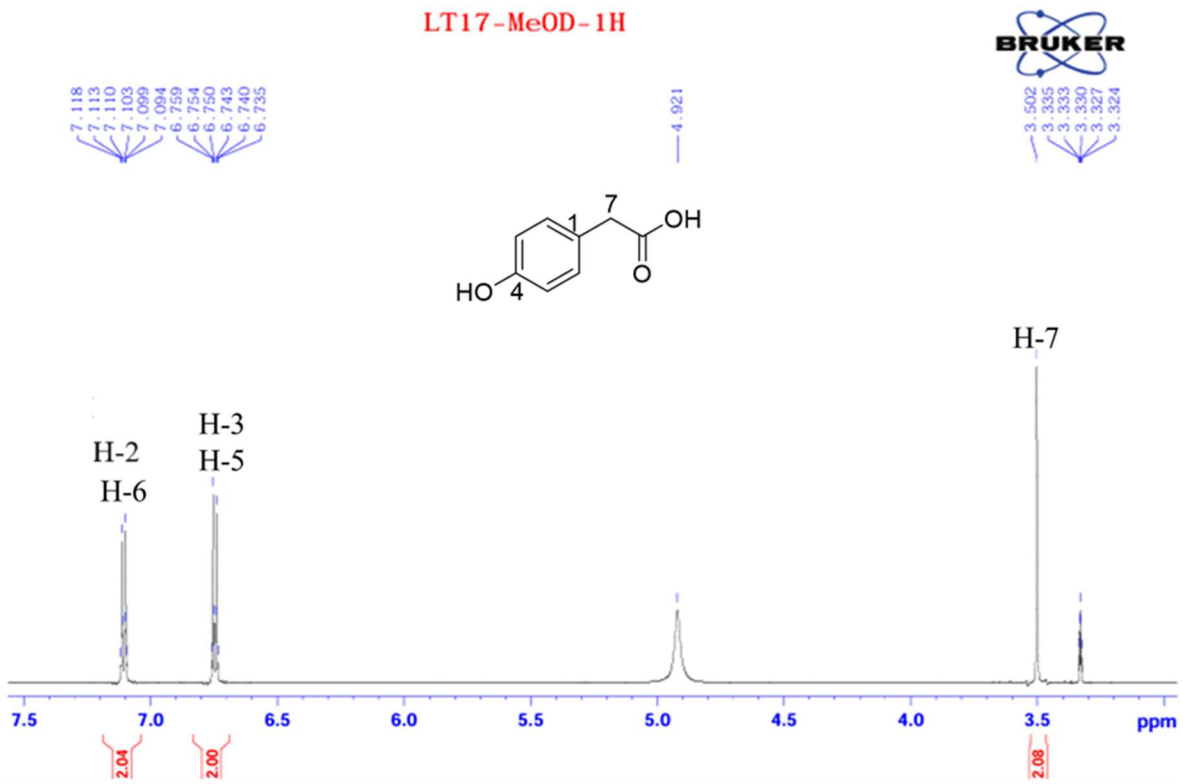

Figure S27: ^{1}H-NMR (600 MHz, CD_3OD) spectrum of compound **5** (2-(4-Hydroxyphenyl)acetic acid) (from δ_H 3 ppm to δ_H 7.5 ppm)

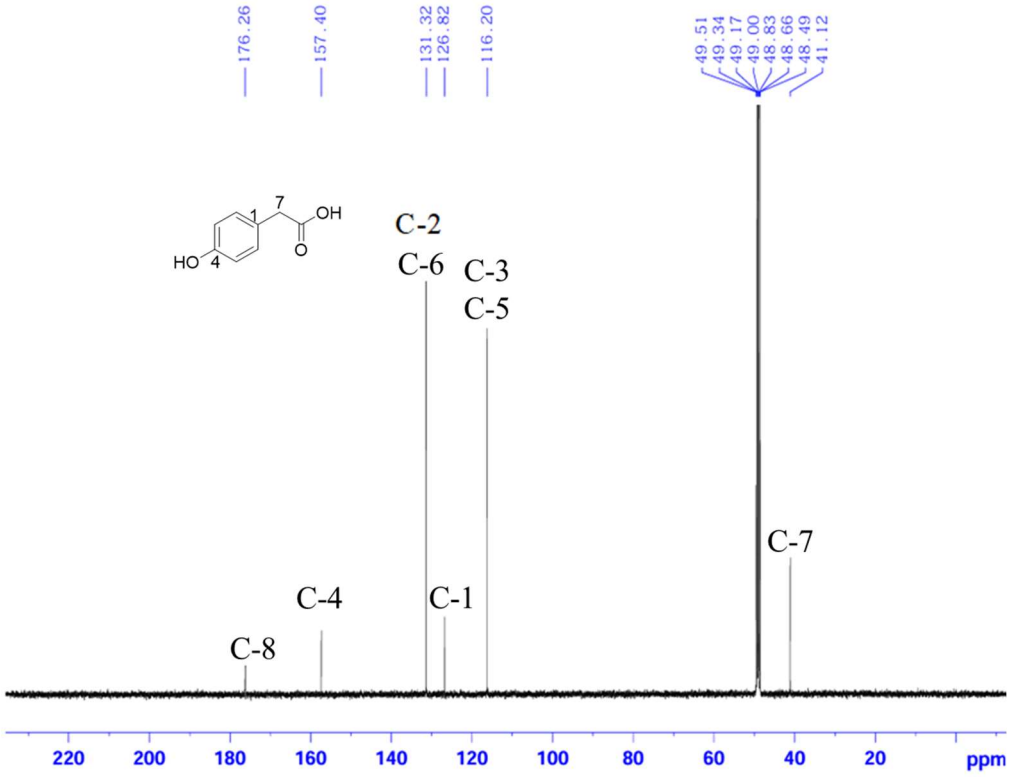

Figure S28: ^{13}C-NMR (150 MHz, CD_3OD) spectrum of compound **5** (2-(4-Hydroxyphenyl)acetic acid)

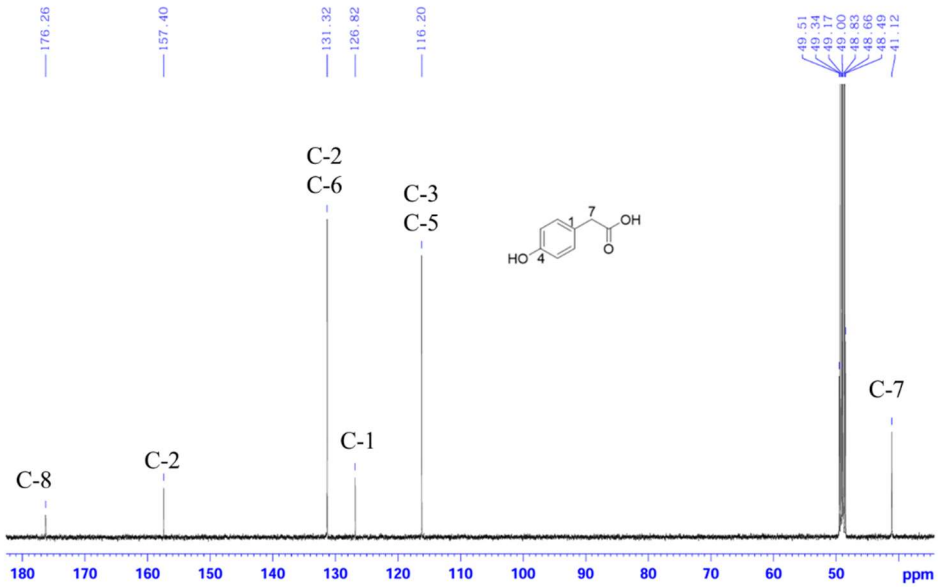

Figure S29: ^{13}C-NMR (150 MHz, CD_3OD) spectrum of compound **5** (2-(4-Hydroxyphenyl)acetic acid) (from δ_C 40 ppm to δ_C 180 ppm)