Supporting Information

Rec. Nat. Prod. **X:X (2020) XX-XX.**

Identification and Structural Characterization of Anti-Endoplasmic Reticulum Stress Compounds from *Ageratum conyzoides* (L.)

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Abbreviations

-OMe methoxy group (-OCH_3)

Table S1: List of primers and primer sequences.

No.	Gene name	Primer probes	Sequence
1	*atf4*	Forward	5′- GGACAGATTGGATGTTGGAGAAAATG-3′
		Reverse	5′-GGAGATGGCCAATTGGGTTCAC-3′
2	*chop*	Forward	5′-GCGACAGAGCCAGAATAACA-3′
		Reverse	5′-GATGCACTTCCTTCTGGAACA-3′
3	*xbp-1*	Forward	5′-TGGGCATCTCAAACCTGCTT-3′
		Reverse	5′-GCGTCCAGCAGGCAAGA-3′
4	*β-actin*	Forward	5′-CCTGAGCGCAAGTACTCTGTGT-3′
		Reverse	5′-GCTGATCCACATCTGCTGGAA-3′

atf4; activating transcription factor 6, *chop;* C/EBP homologous protein, *xbp-1;* X-box-binding protein-1

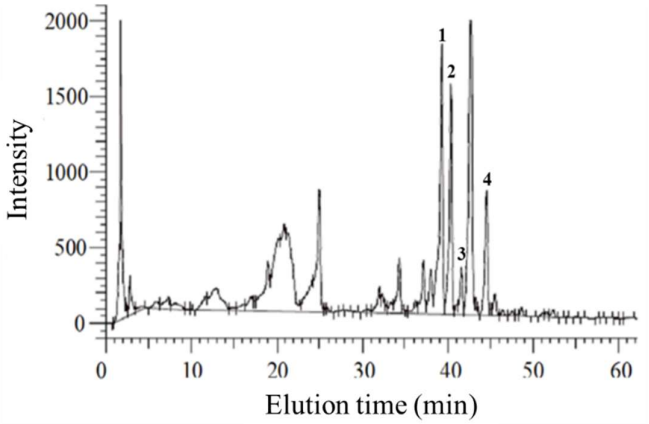
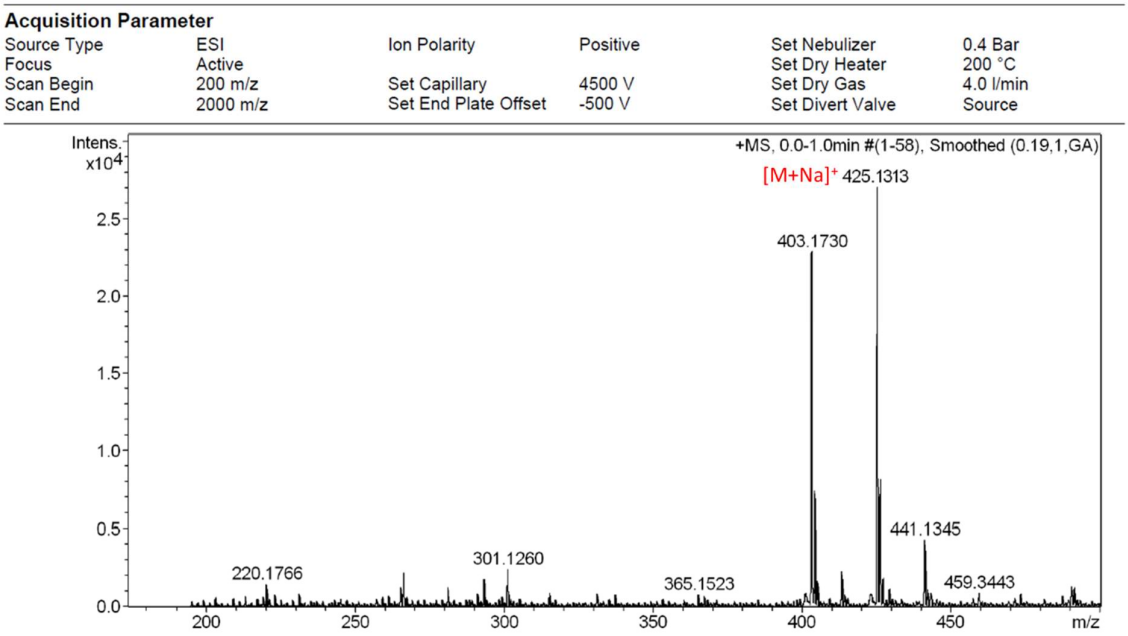


Figure S1: Elution pattern of reverse-phase HPLC from AC-EtOAc fraction

1. Supplementary spectroscopic data of compound 1

Table S2: The comparison of NMR data of compound **1** with similar compound (5,6,7,3′,4′,5′-hexamethoxyflavone).

Position	Compound **1** (CD_3OD)			5,6,7,3′,4′,5′-hexamethoxyflavone ($CDCl_3$) [19]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**HMBC** ($^{1}H \rightarrow {}^{13}C$)	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm
2	163.39		C-2′, 3, 6′	160.1	
3	108.14	6.69 (H, *s*)	1′, 2, 4, 10	107.6	6.73 (H, *s*)
4	179.57		C-3, 8	176.2	
5	153.31		C-(5-OMe)	151.5	
6	141.86		C-(6-OMe), 8	139.5	
7	160.11		C-(7-OMe), 8	157.1	
8	98.09	7.15 (H, *s*)	C-4,6,7,9,10	95.8	6.84 (H, *s*)
9	156.18		C-8	153.6	
10	113.14		C-3, 8	111.8	
1′	127.91		C-2′, 3, 6′	125.8	
2′	104.99	7.25 (2H, *s*)	C-6′	102.7	7.11 (2H, *s*)
3′	155.03		C-2′, (3′-OMe), 6′	125.7	
4′	142.47		C-2′, (4′-OMe), 6′	140.2	
5′	155.03		C-2′, (5′-OMe), 6′	125.7	
6′	104.99	7.25 (2H, *s*)	C-2’	102.7	7.11 (2H, *s*)
5 - OMe	62.61	3.92(3H, *s*)	C-5	61.3	4.00 (3H, *s*)
6 - OMe	61.23	3.85 (3H, *s*)	C-6	60.6	3.91 (3H, *s*)
7 - OMe	57.09	4.02(3H, *s*)	C-7	55.6	3.99 (3H, *s*)
3′ - OMe	56.95	3.95 (3H, *s*)	C-3′	55.6	3.95 (3H, *s*)
4′ - OMe	61.81	3.86 (3H, *s*)	C-4′	60.1	3.92 (3H, *s*)
5′ - OMe	56.95	3.95 (3H, *s*)	C-5′	55.6	3.95 (3H, *s*)



Formular (M)	Ion formula	m/z	Calcd m/z
$C_{21}H_{22}O_8$	$C_{21}H_{22}O_8Na$	425.1315	425.1313

Figure S2: (+)-HR-ESI-MS data of compound **1**

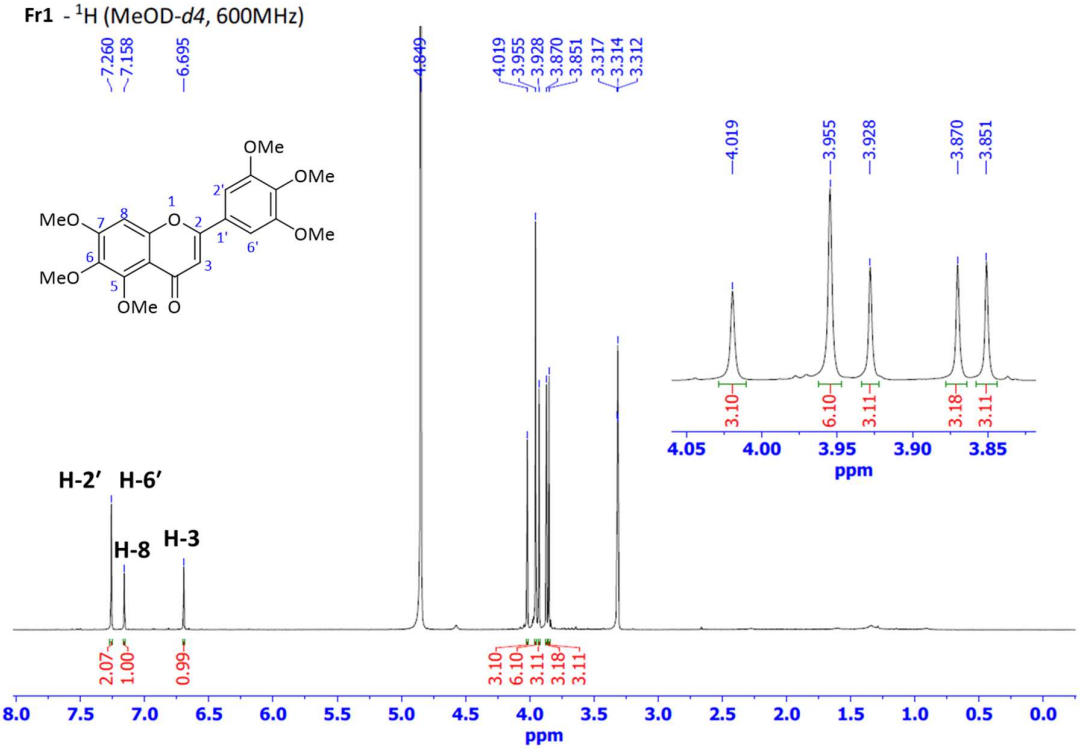


Figure S3: Complete assignment ^{1}H-NMR spectrum of compound **1**

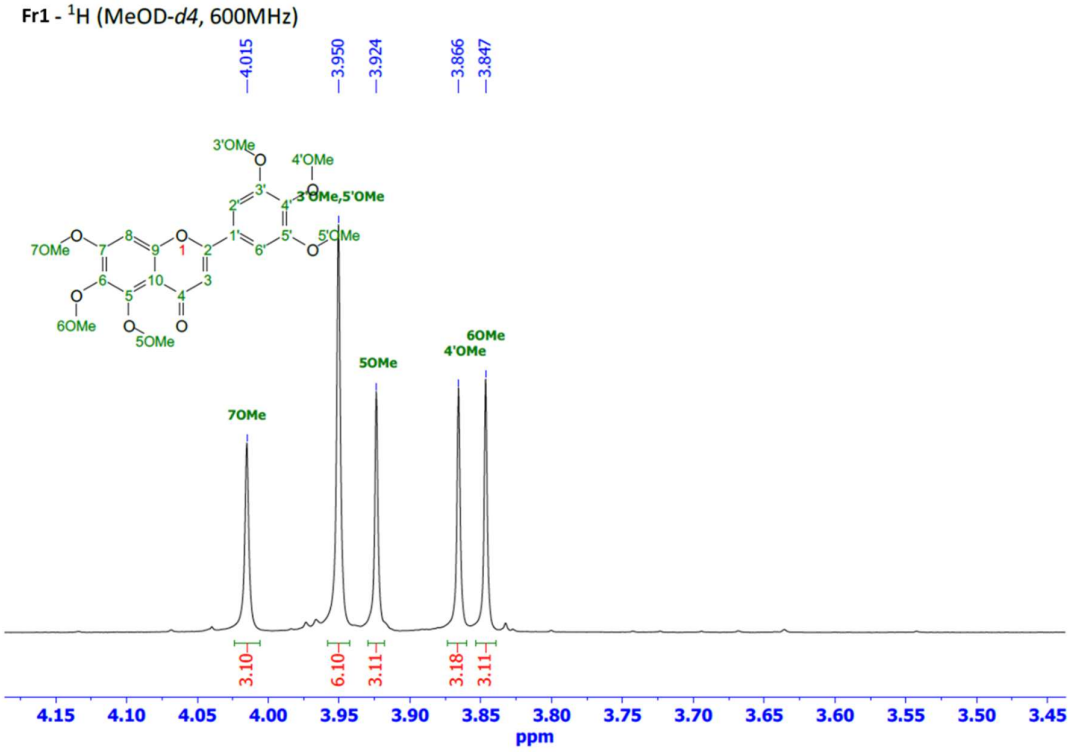


Figure S4: ^{1}H NMR spectrum of compound **1** – expansion

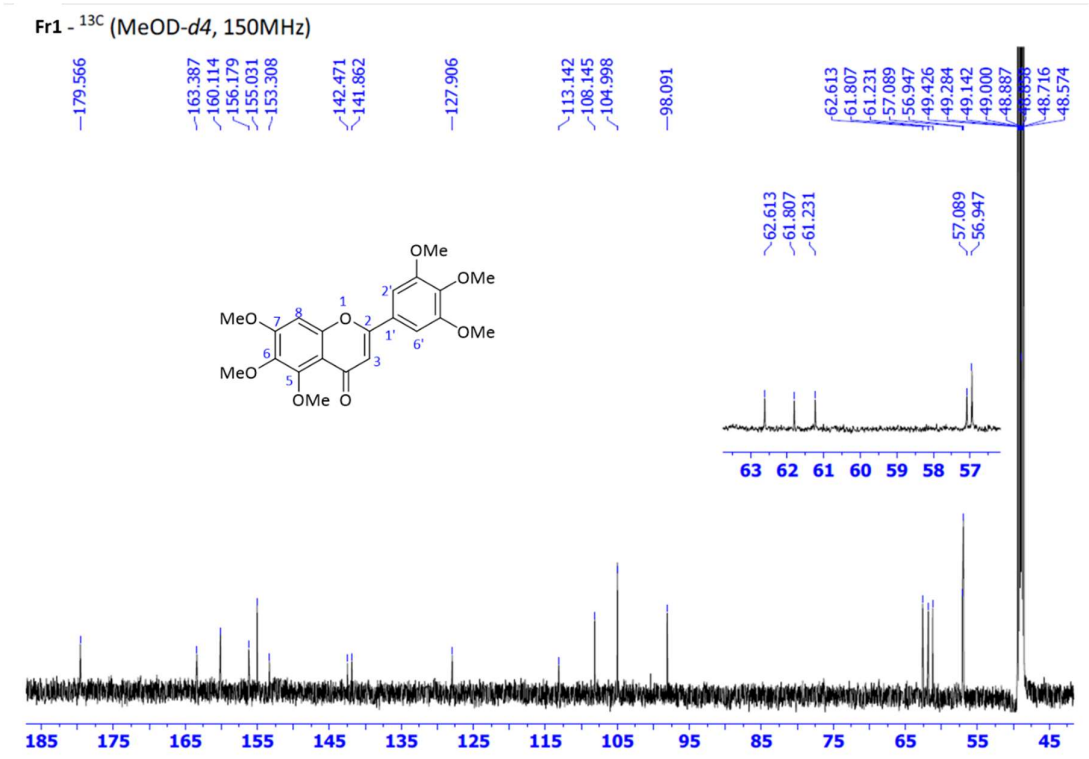


Figure S5: Complete assignment ^{13}C-NMR spectrum of compound **1**

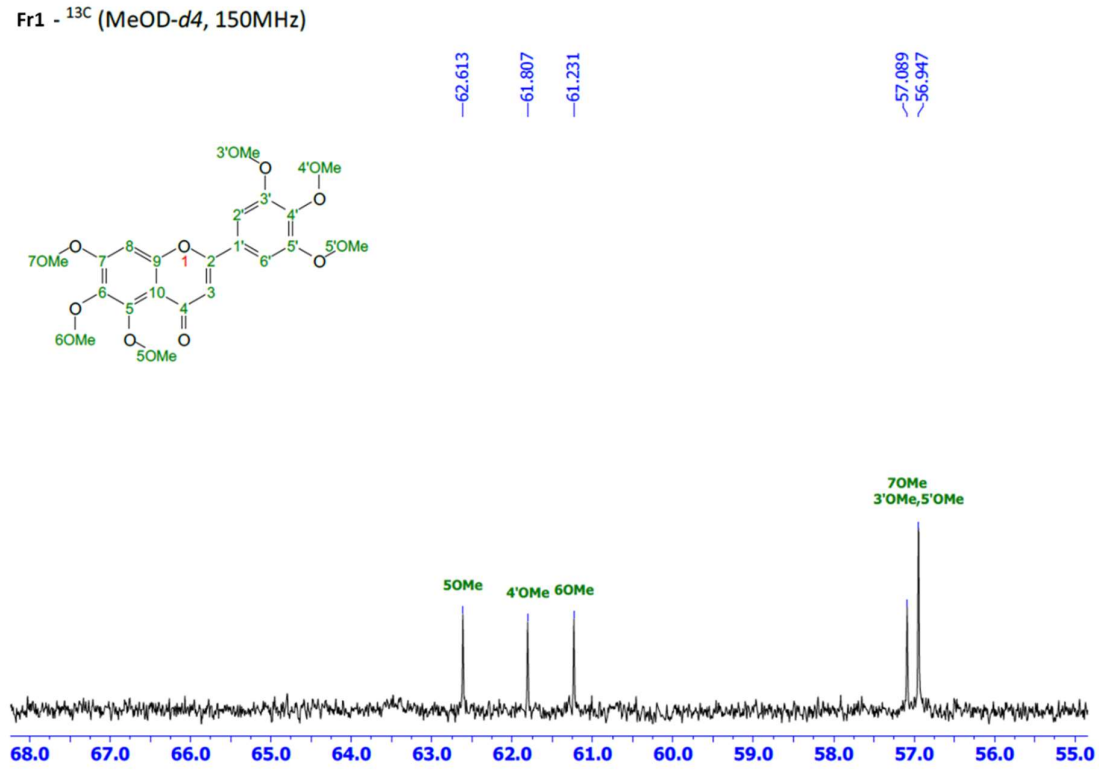


Figure S6: ^{13}C-NMR spectrum of compound **1** – expansion

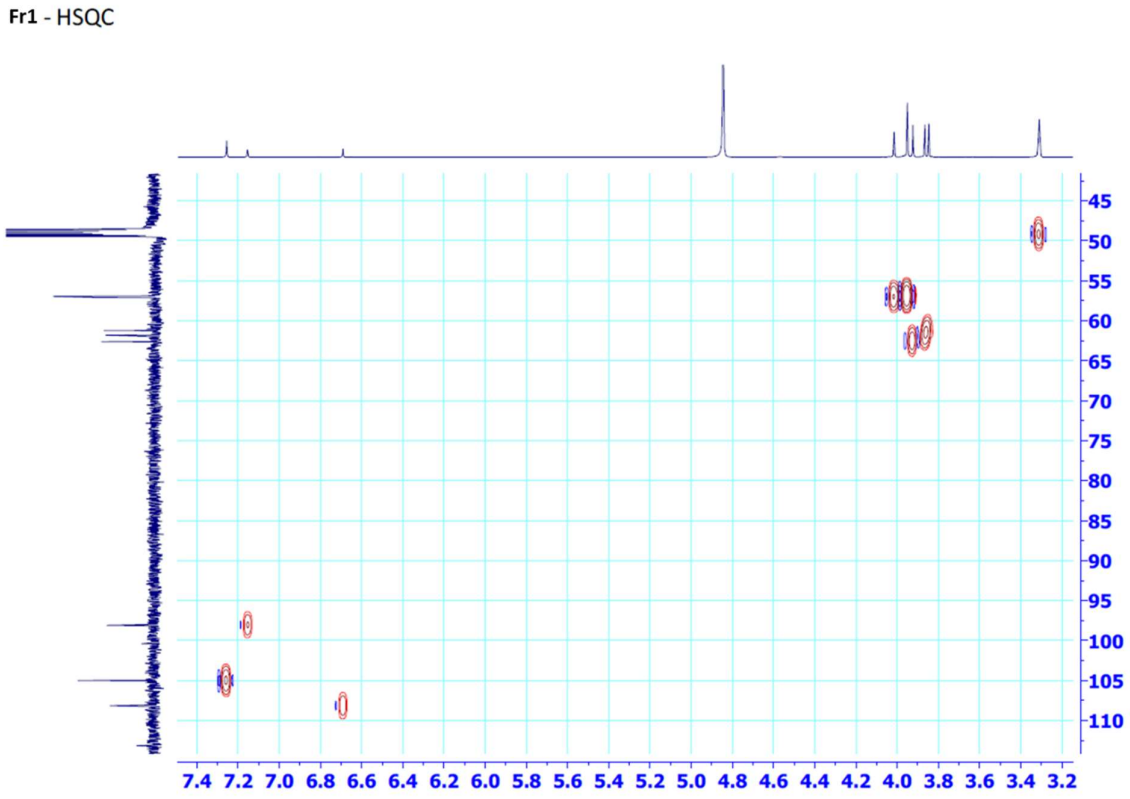


Figure S7: Complete assignment of correlation in 2D NMR-HSQC spectrum of compound **1**

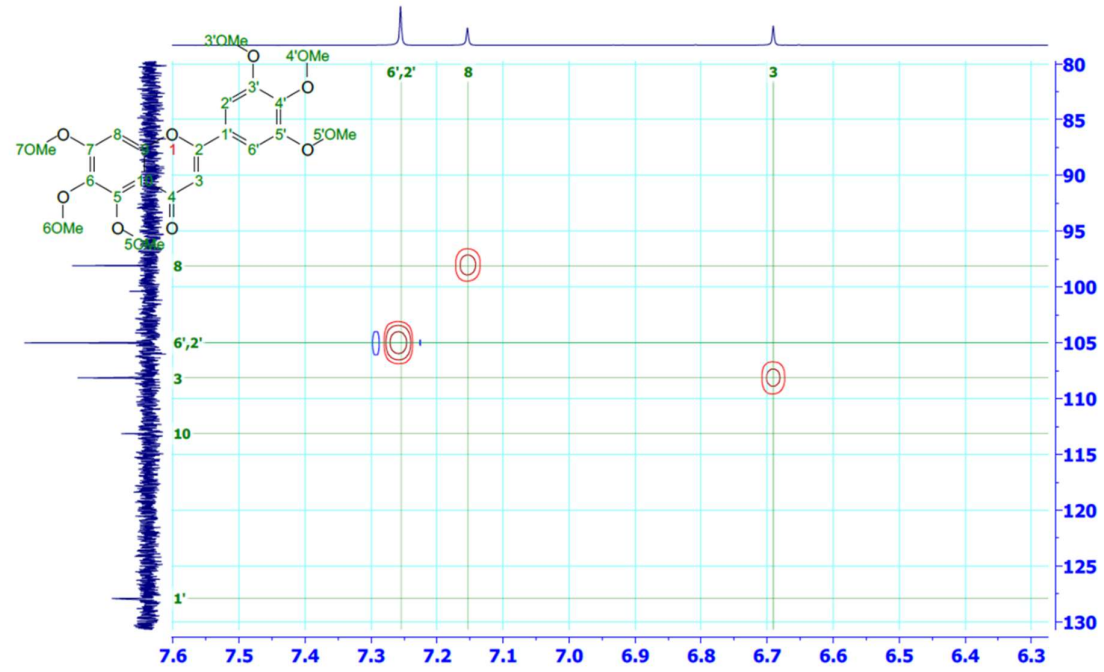


Figure S8: Correlation in 2D NMR-HSQC spectrum of compound **1** – expansion

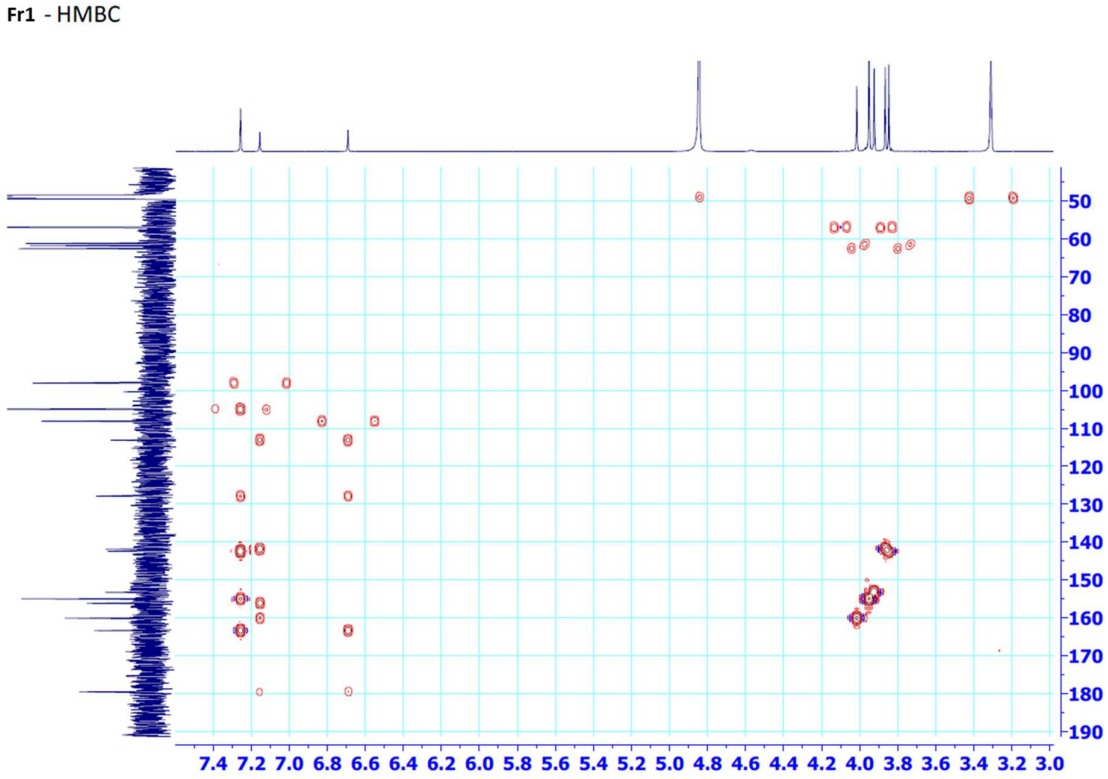


Figure S9: Complete assignment of correlation in 2D NMR-HMBC spectrum of compound **1**

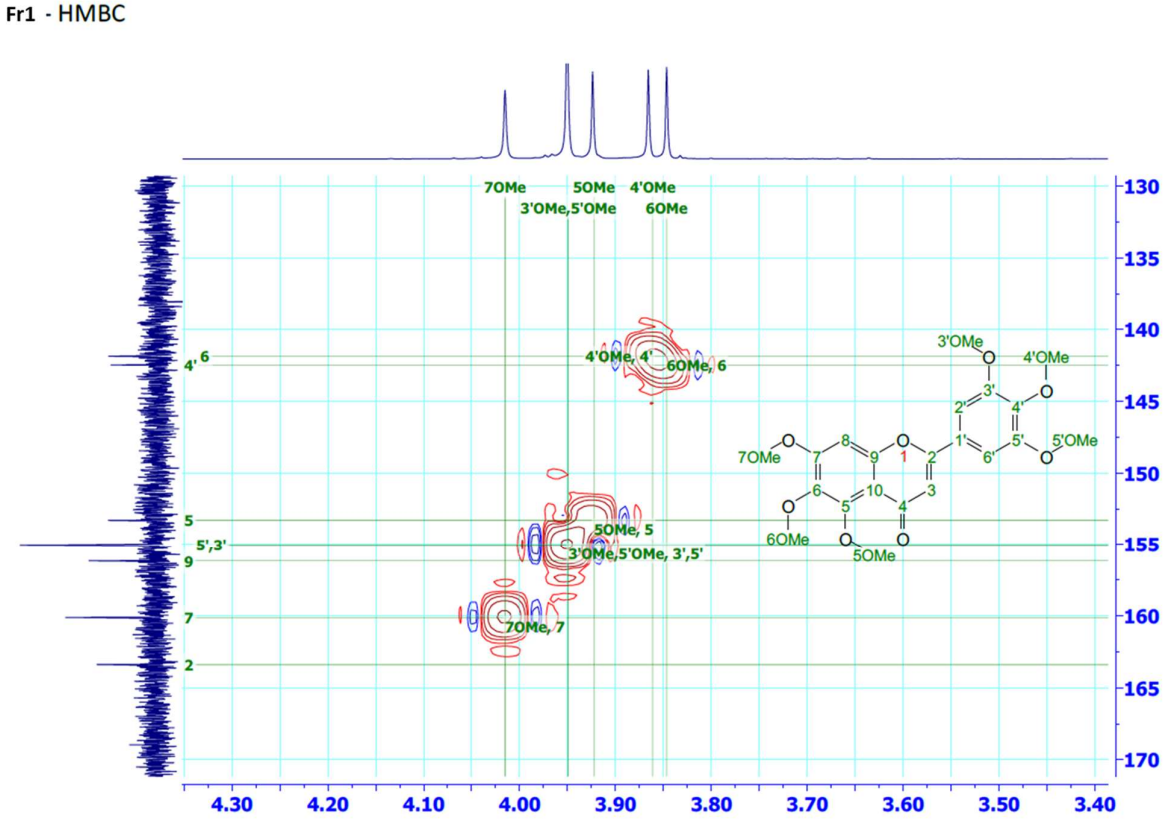


Figure S10: Correlation in 2D NMR-HMBC spectrum of compound **1** – expansion

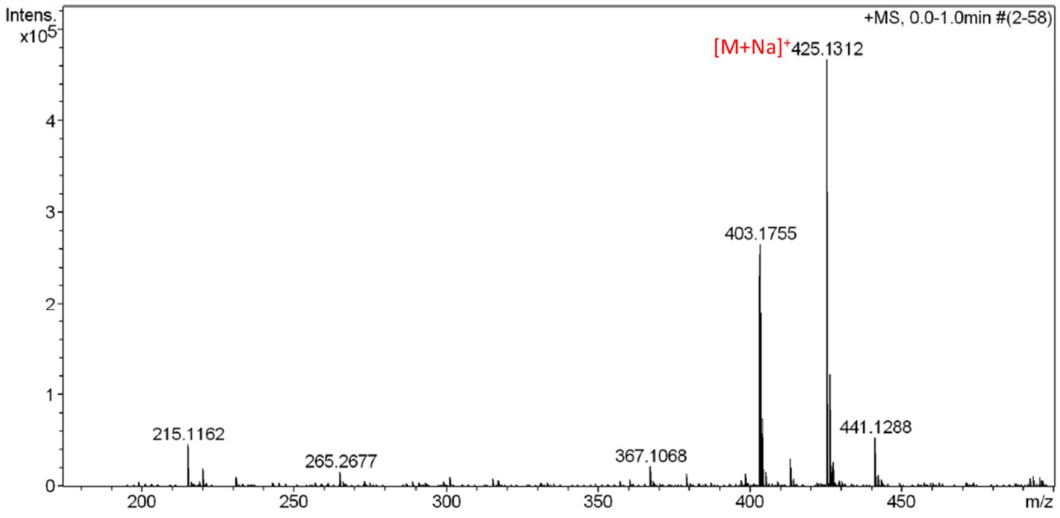
2. Supplementary spectroscopic data of compound 2.

Table S3: The comparison of NMR data of compound **2** with similar compound (5,6,7,8,3′,4′-hexamethoxyflavone).

Position	Compound **2** (CD_3OD)			5,6,7,8,3′,4′-hexamethoxyflavone (Nobiletin – $CDCl_3$) [20]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**HMBC** ($^{1}H \rightarrow {}^{13}C$)	**^{13}C-NMR** δ_C ppm	**^{1}H-NMR** δ_H ppm
2	163.67		C-3, 5′, 6′	161.0	
3	106.97	6.7 (H, *s*)	1′, 2, 4, 10	106.7	6.61 (H, *s*)
4	179.63		C-3	177.4	
5	149.41		C-(5-OMe)	144.0	
6	145.63		C-(6-OMe)	138.0	
7	153.39		C-(7-OMe)	151.4	
8	139.62		C-(8-OMe)	138.0	
9	149.18			147.7	
10	115.38		C-3	114.8	
1′	124.80		C-2′, 3	124.0	
2′	112.86	7.55 (H, *d*, *J* = 1.8 Hz)	C-2, 3′,4′, 6′	108.7	7.4 (H, *d*, *J* = 2.0 Hz)
3′	150.92		C-2′, (3′ - OMe), 5′	149.3	
4′	154.00		C-2′, (4′-OMe), 5′, 6′	151.9	
5′	110.32	7.13 (H, *d, J* = 8.4 Hz)	C-1′, 3′, 4′	111.0	7.0 (H, *d, J* = 8.0 Hz)
6′	121.25	7.66 (1H, *dd*, *J* = 8.4; 1.8 Hz)	C-5′	119.6	7.57 (1H, *dd*, *J* = 8.0; 2.0 Hz)
5 - OMe	62.67	3.89 (3H, *s*)	C-5	62.3	3.97 (3H, *s*)
6 - OMe		3.93 (3H , *s*)	C-6	62.0	3.96 (3H , *s*)
7 - OMe	62.23	4.11(3H, *s*)	C-7	61.8	4.10(3H, *s*)
8 - OMe	62.67	4.03 (3H, *s*)	C-8	61.7	4.04 (3H, *s*)
3′ - OMe	56.61	3.94 (3H, *s*)	C-3′	56.1	3.98 (3H, *s*)
4′ - OMe	62.23	3.93 (3H, *s*)	C-4′	56.0	3.96 (3H, *s*)

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	200 °C
Scan Begin	200 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Formular (M)	Ion formula	m/z	Calcd m/z
$C_{21}H_{22}O_8$	$C_{21}H_{22}O_8Na$	425.1315	425.1312

Figure S11: (+)-HR-ESI-MS data of compound **2**

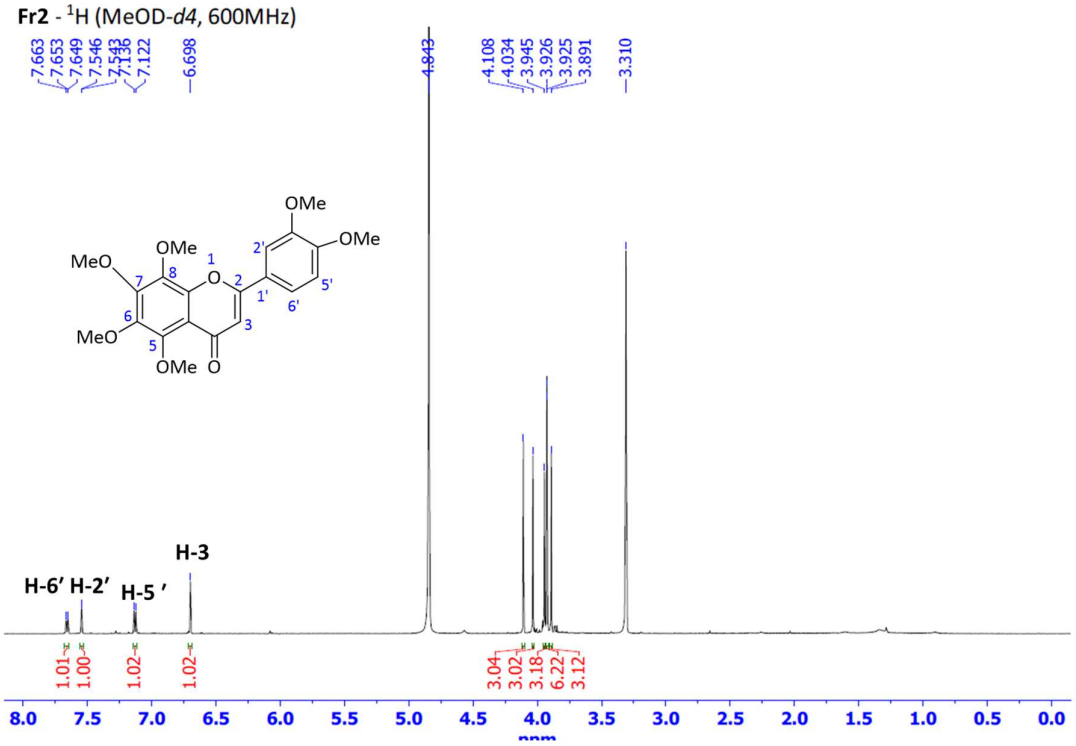


Figure S12: Complete assignment ^{1}H-NMR spectrum of compound **2**

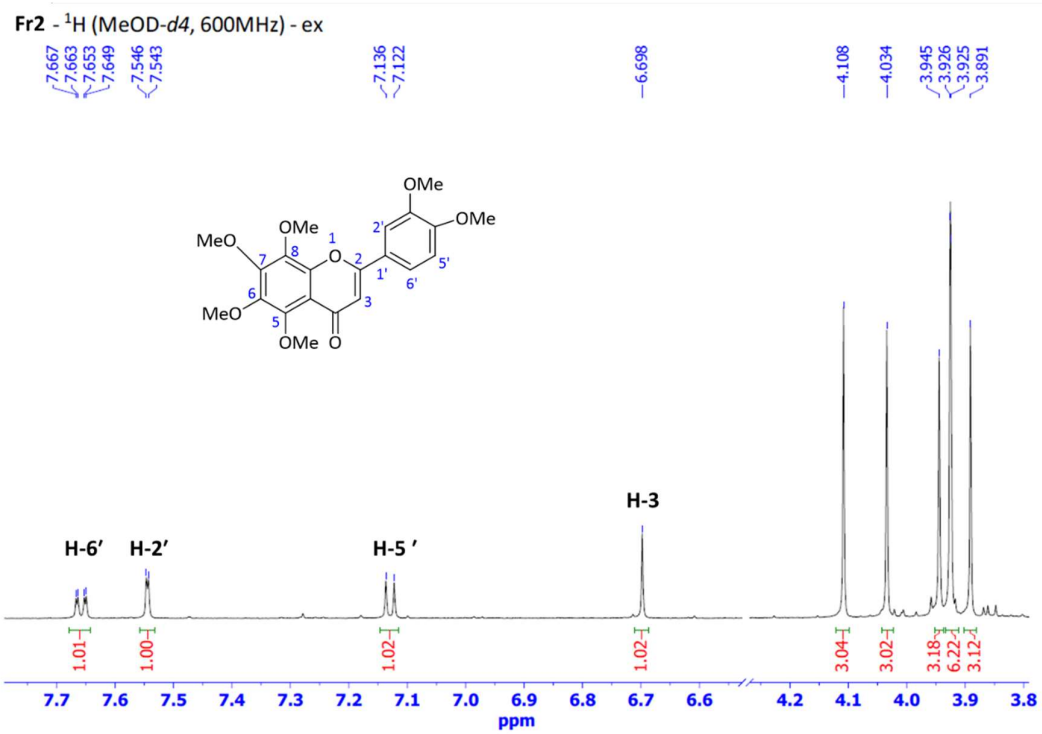


Figure S13: [1]H-NMR spectrum of compound **2** - expansion

Figure S12:

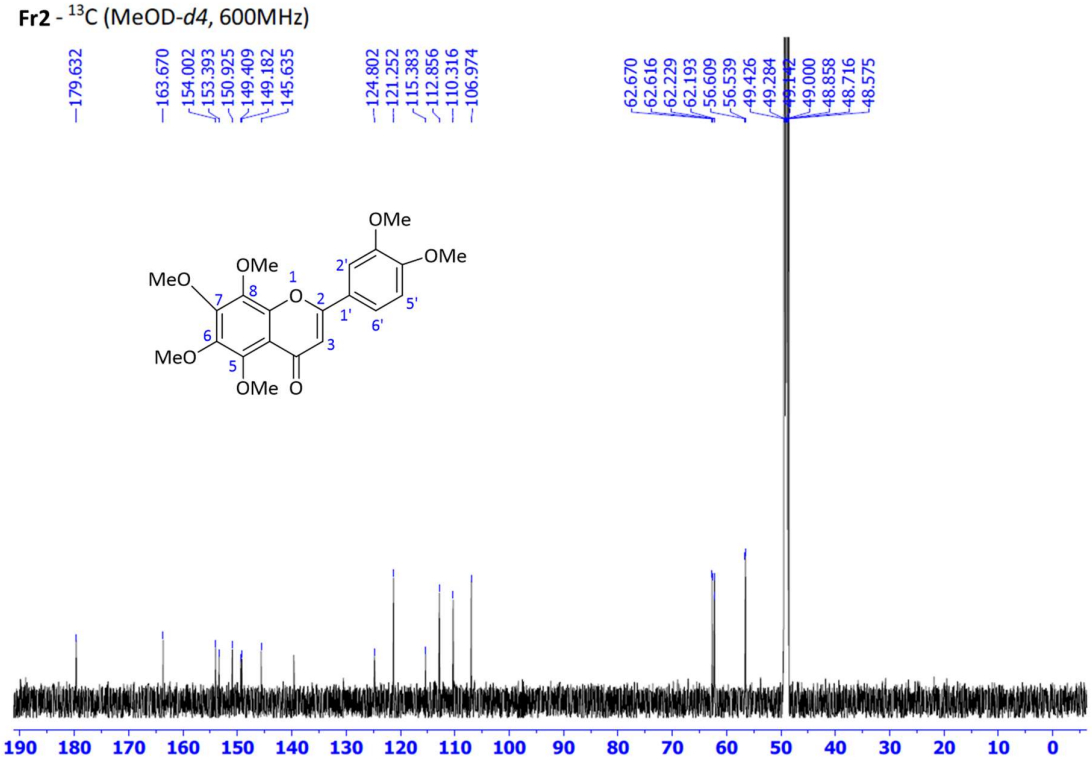


Figure S14: Complete assignment [13]C-NMR spectrum of compound **2**

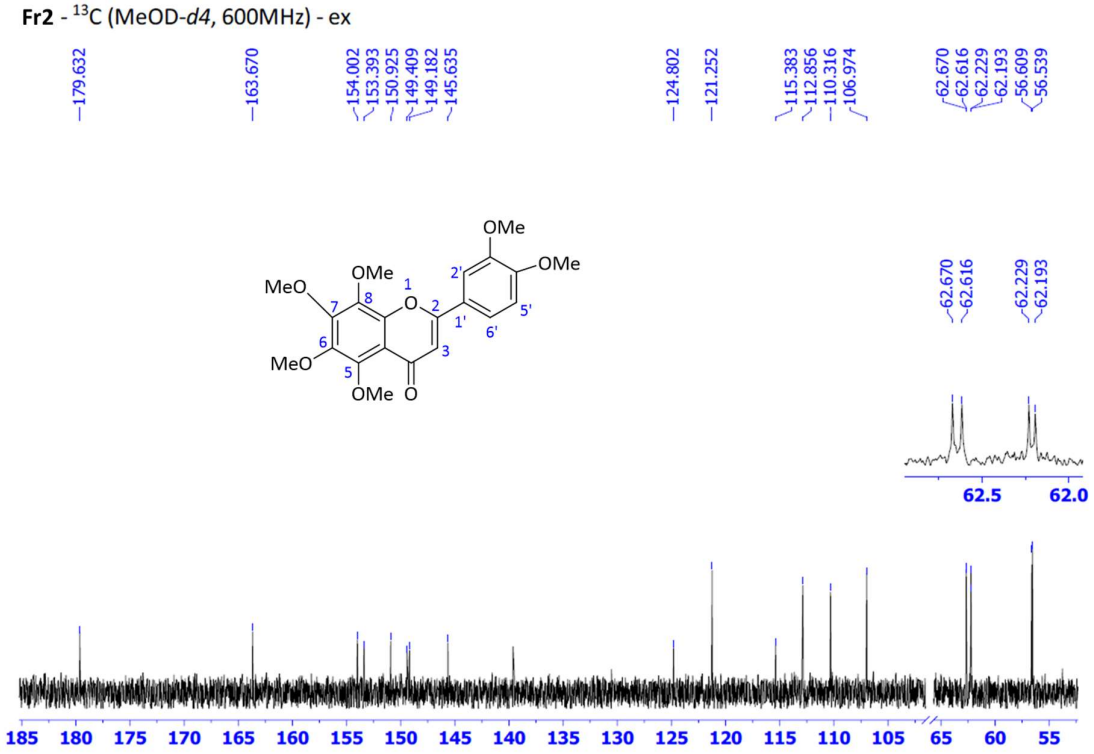


Figure S15: ^{13}C-NMR spectrum of compound **2** – expansion A

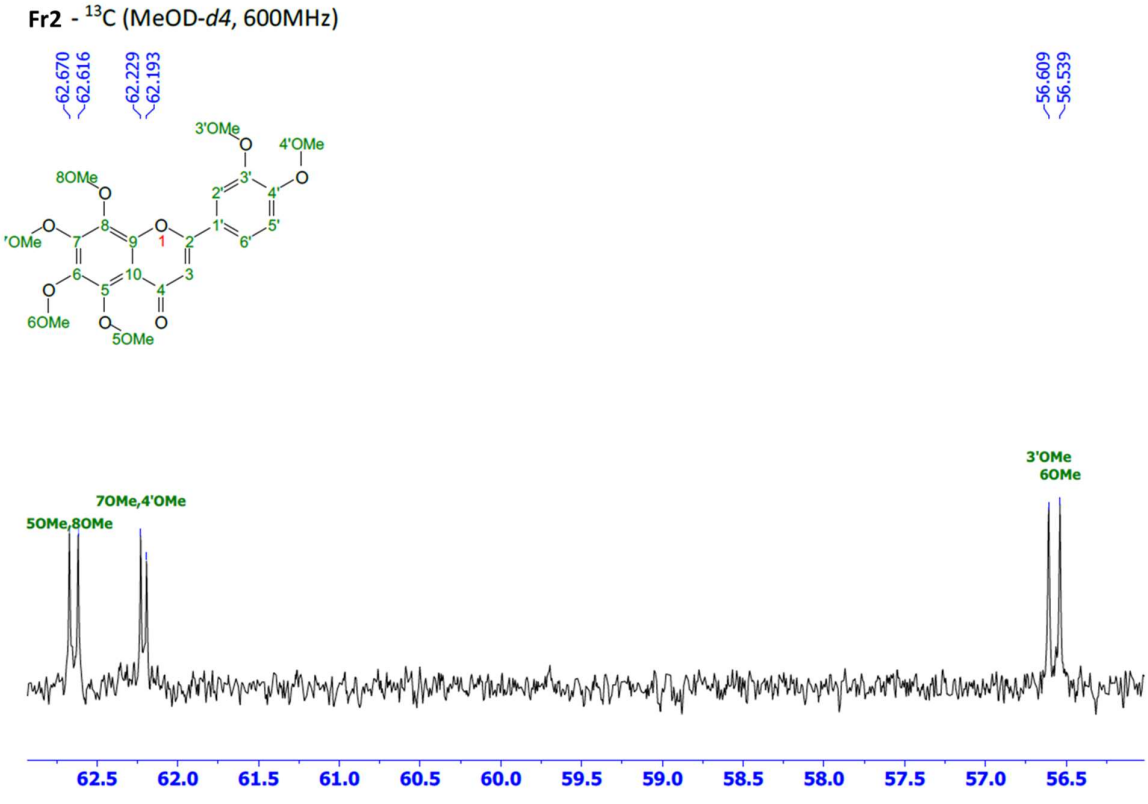


Figure S16: ^{13}C-NMR spectrum of compound **2** – expansion B

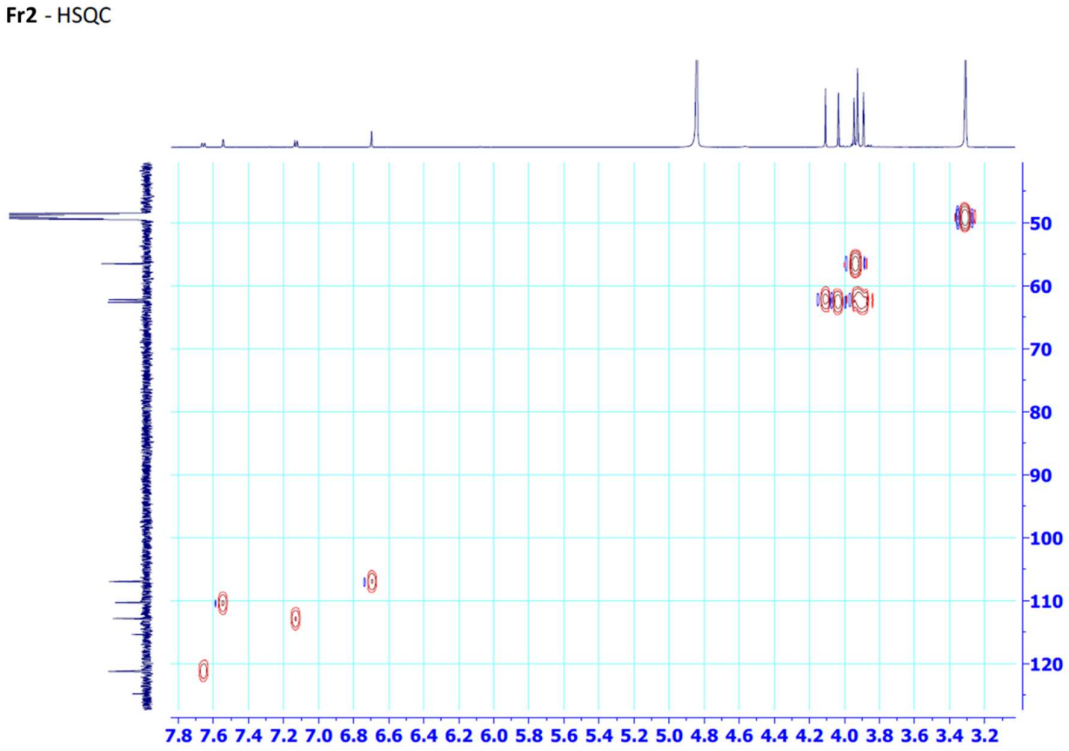


Figure S17: Full assignment of correlation in 2D NMR-HSQC spectrum of compound **2**

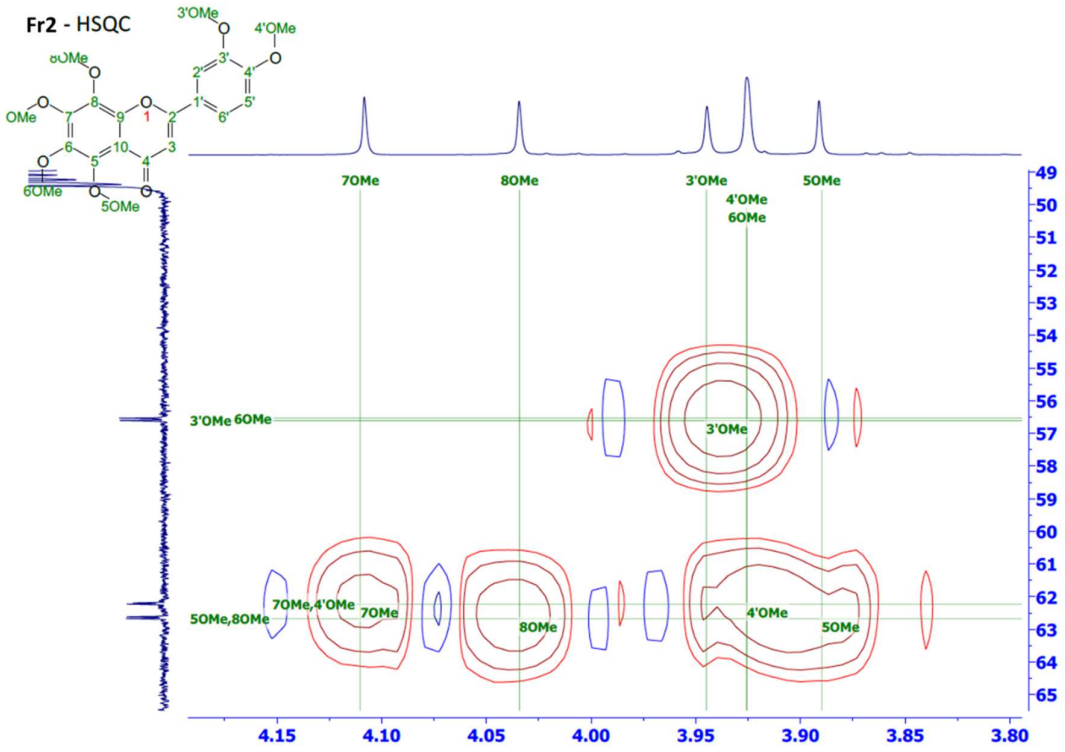


Figure S18: Correlation in 2D NMR-HSQC spectrum of compound (**2**)

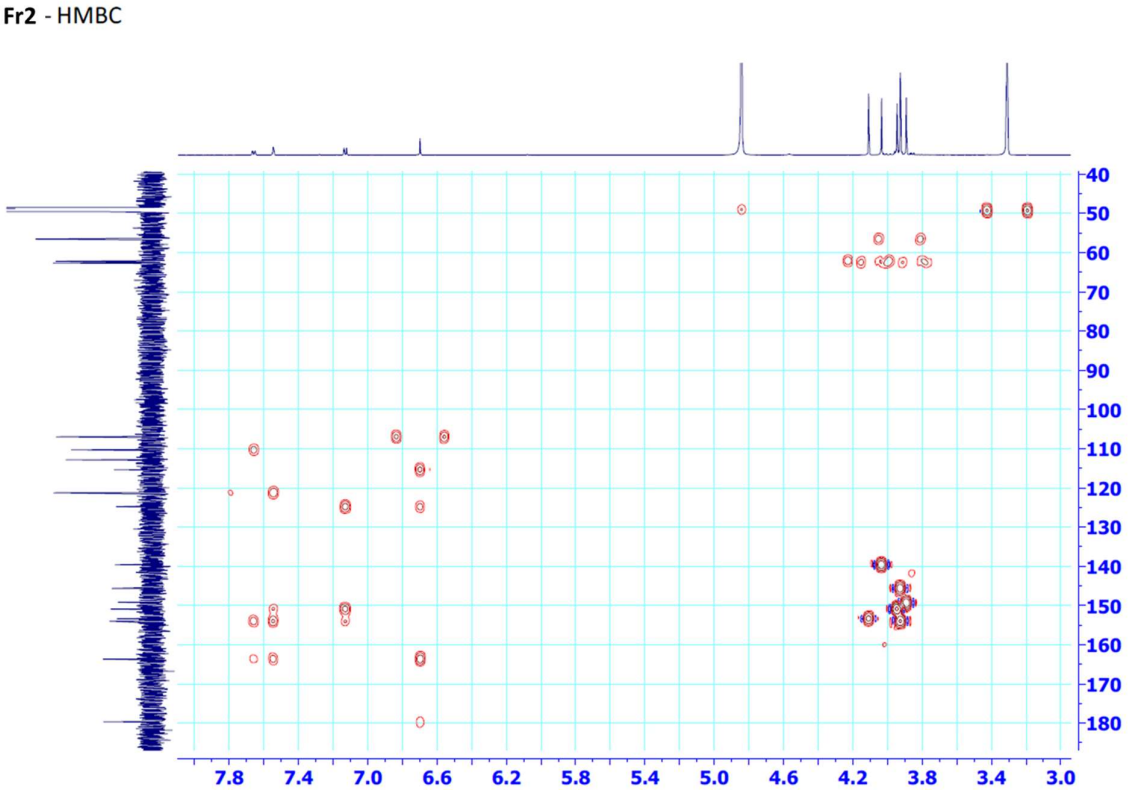


Figure S19: Full assignment of correlation in 2D NMR-HMBC spectrum of compound **2**

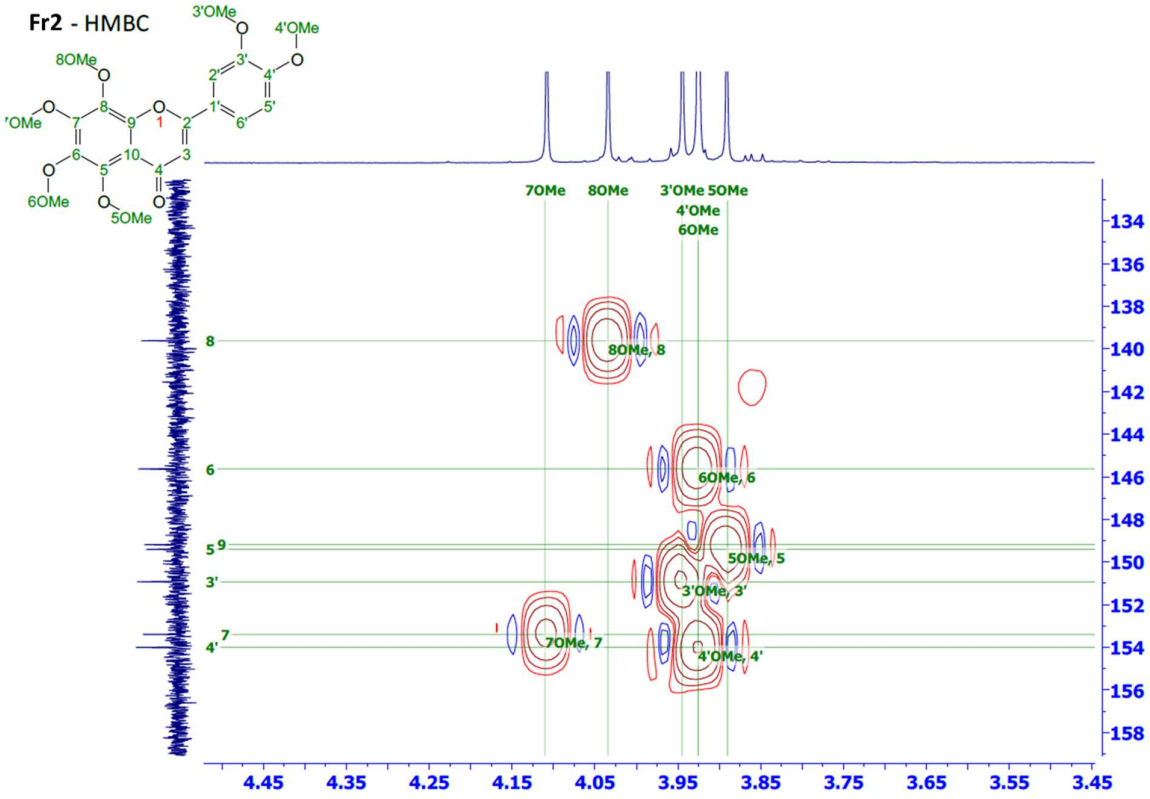


Figure S20: Correlation in 2D NMR-HMBC spectrum of compound **2**

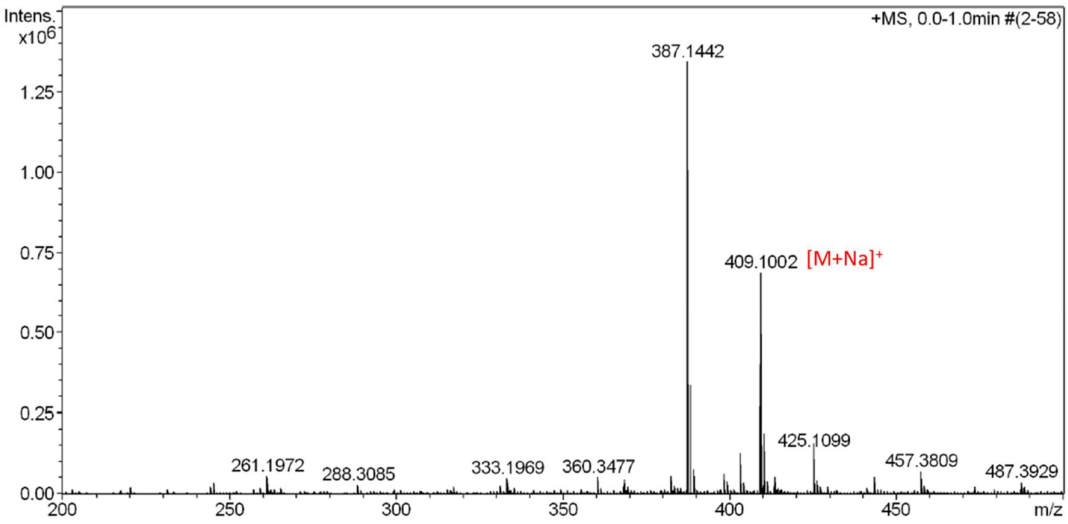
3. Supplementary spectroscopic data of compound 3.

Table S4: The comparison of NMR data of compound **3** with similar compound (3′,4′-Methylenedioxy-5′,5,6,7-tetramethoxyflavone)

Position	Compound **3** (CD_3OD)				3′,4′-Methylenedioxy-5′,5,6,7-tetramethoxyflavone ($CDCl_3$) [21]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**HSQC** ($^1H \rightarrow {}^{13}C$)	**HMBC** ($^1H \rightarrow {}^{13}C$)	**^{13}C-NMR** (100 MHz) δ_C ppm	**^{1}H-NMR** (400 MHz) δ_H ppm
2	163.37			C-2′, 3, 6′	160.7	
3	107.61	6.64 (H, *s*)	C-3	1′, 2, 4, 10	107.8	6.65 (H, *s*)
4	179.61			C-3, 8	177.1	
5	153.33			C-(5-OMe)	152.6	
6	141.87			C-(6-OMe), 8	140.4	
7	160.11			C-(7-OMe), 8	157.7	
8	98.06	7.14 (H, *s*)	C-8	C-4,6,7,9,10	96.2	6.78 (H, *s*)
9	156.15			C-8	154.4	
10	113.11			C-3, 8	112.9	
1′	126.72			C-3, 6′	126.0	
2′	101.47	7.2 (H, *d, J* = 1.2 Hz)	C-2′	C-6′	100.4	7.06 (H, *d, J* = 1.6 Hz)
3′	151.21			C - 2′, 7′	149.5	
4′	140.06			C - 2′, 6′, 7′	138.1	
5′	145.44			C-(5′-OMe), 6′	143.8	
6′	108.59	7.28 (H, *d*, *J* = 1.2 Hz)	C-6′	C-2′	106.6	7.07 (H, *d*, *J* = 1.6 Hz)
7′	103.72	6.07 (2H, *s*)	C-7′	C-3′, 4′	102.2	6.08 (2H, *s*)
5 - OMe	62.62	3.92(3H, *s*)	C-(5-OMe)	C-5	61.5	3.98 (3H, *s*)
6 - OMe	61.81	3.86 (3H, *s*)	C-(6-OMe)	C-6	61.2	3.92 (3H, *s*)
7 - OMe	57.09	4.01(3H, *s*)	C-(7-OMe)	C-7	56.3	3.98 (3H, *s*)
5′ - OMe	57.57	3.99 (3H, *s*)	C-(5′-OMe)	C-5′	56.9	3.99 (3H, *s*)

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	200 °C
Scan Begin	200 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Formular (M)	Ion formula	m/z	Calcd m/z
$C_{20}H_{18}O_8$	$C_{20}H_{18}O_8Na^+$	409.1002	409.1002

Figure S21: (+)-HR-ESI-MS data of compound **3**

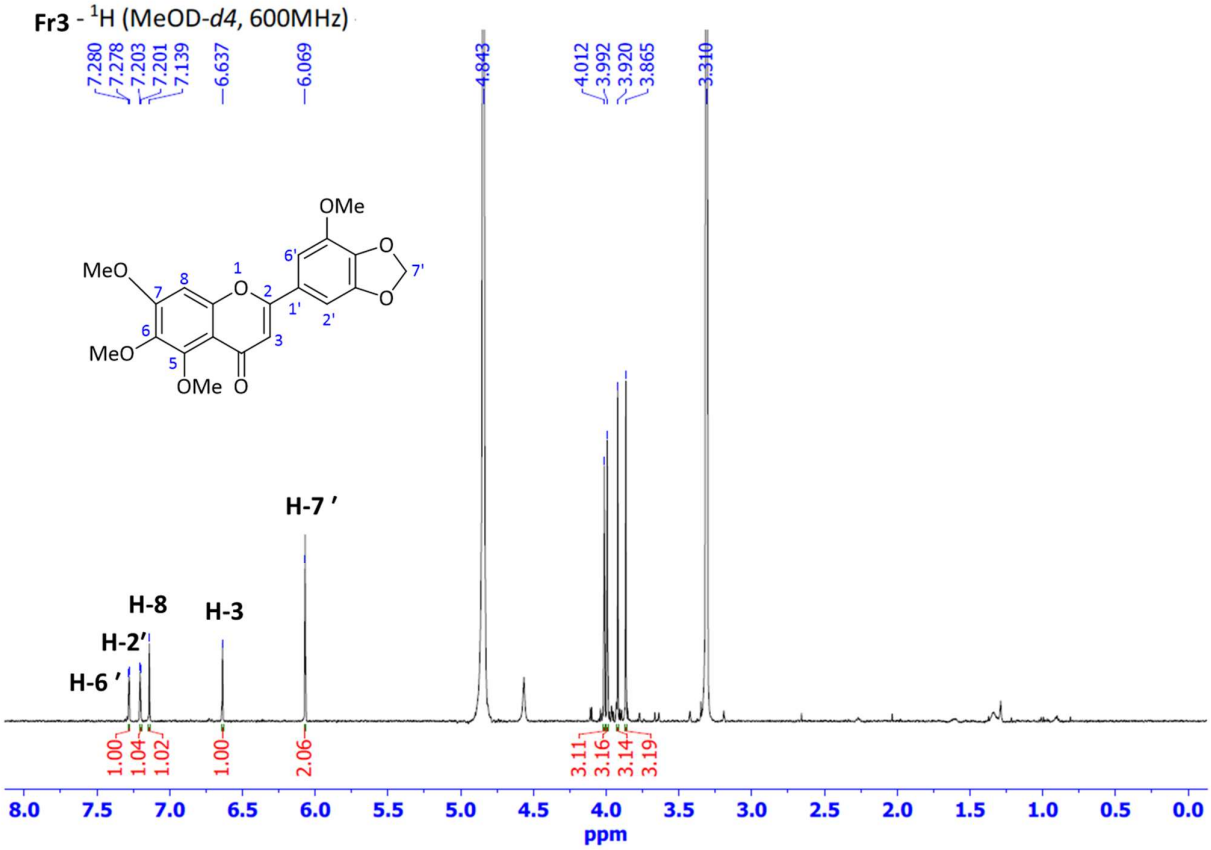


Figure S22: Complete assignment ^{1}H-NMR spectrum of compound **3**

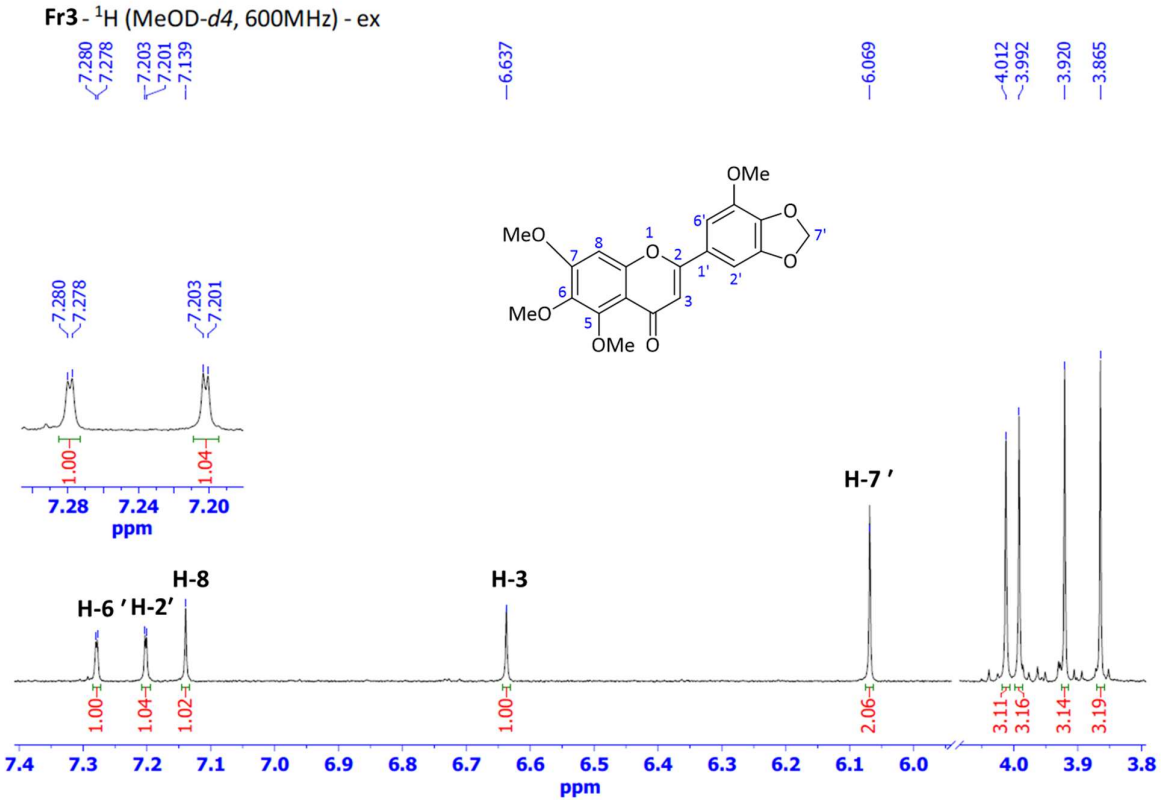


Figure S23: ^{1}H-NMR spectrum of compound **3** – expansion

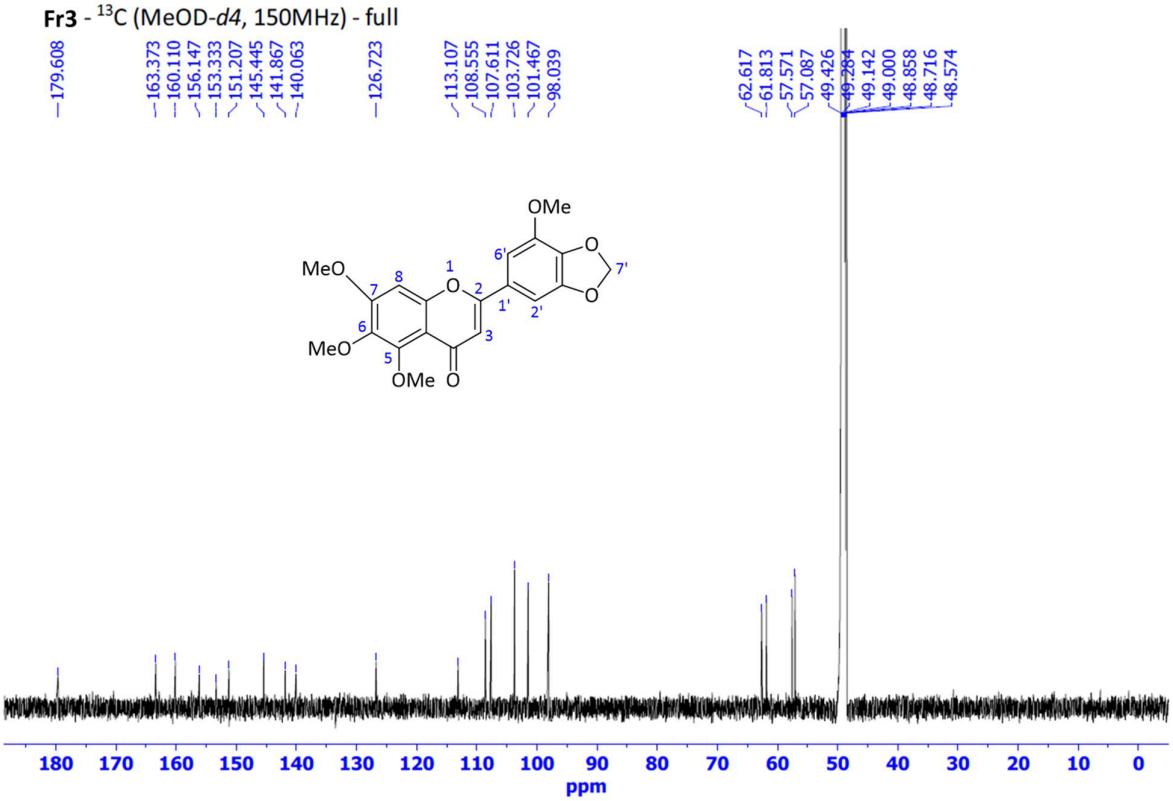


Figure S24: Complete assignment ^{13}C-NMR spectrum of compound **3**

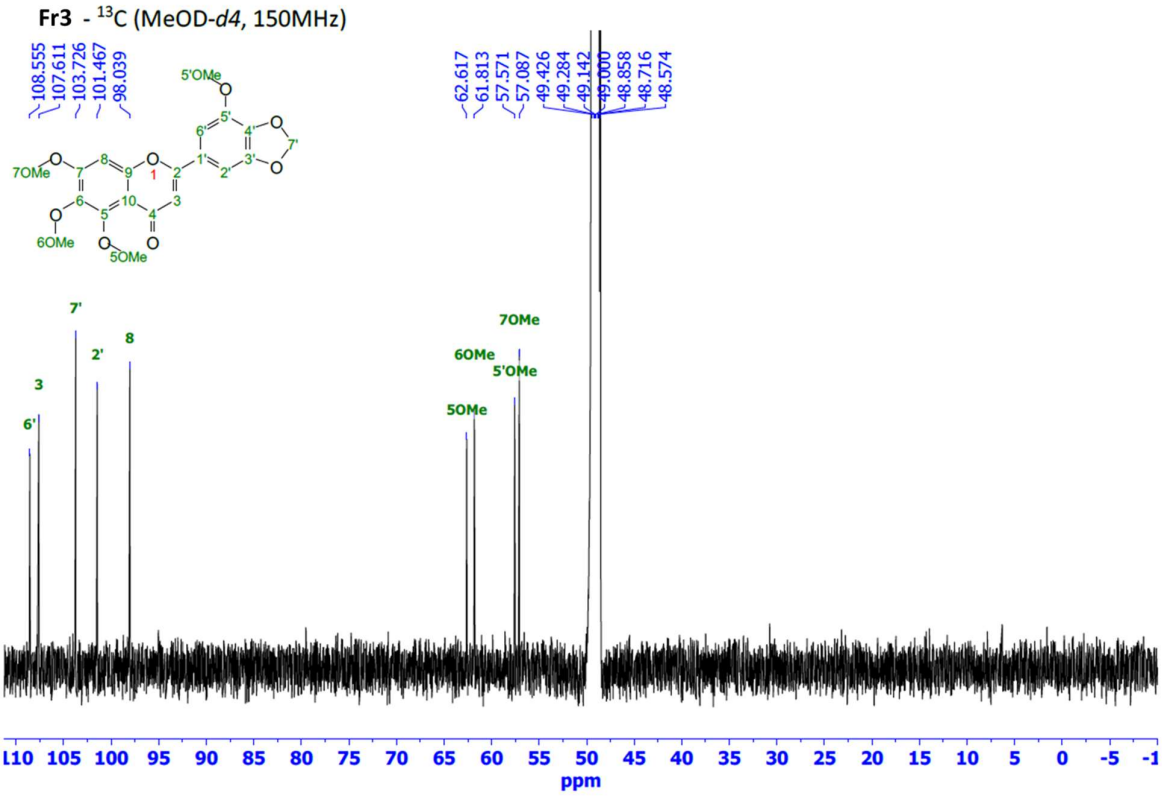


Figure S25: ^{13}C-NMR spectrum of compound **3** – expansion

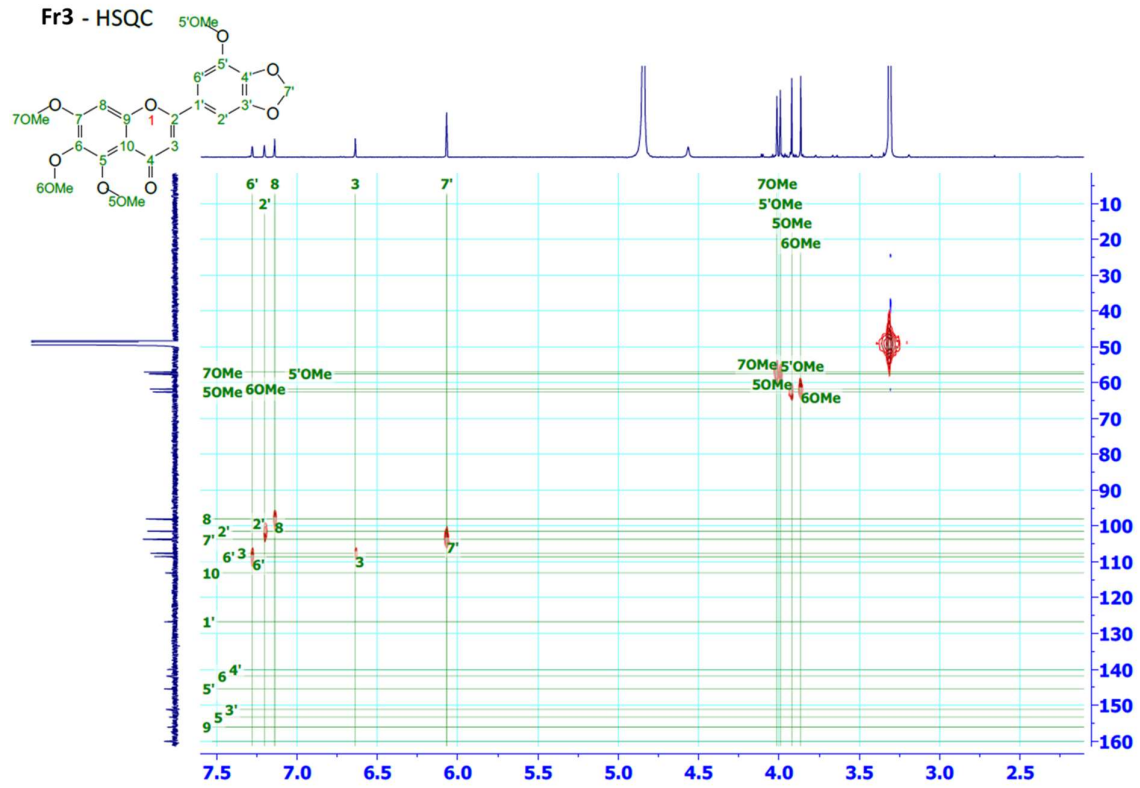


Figure S26: Complete assignment of correlation in 2D NMR-HSQC spectrum of compound **3**

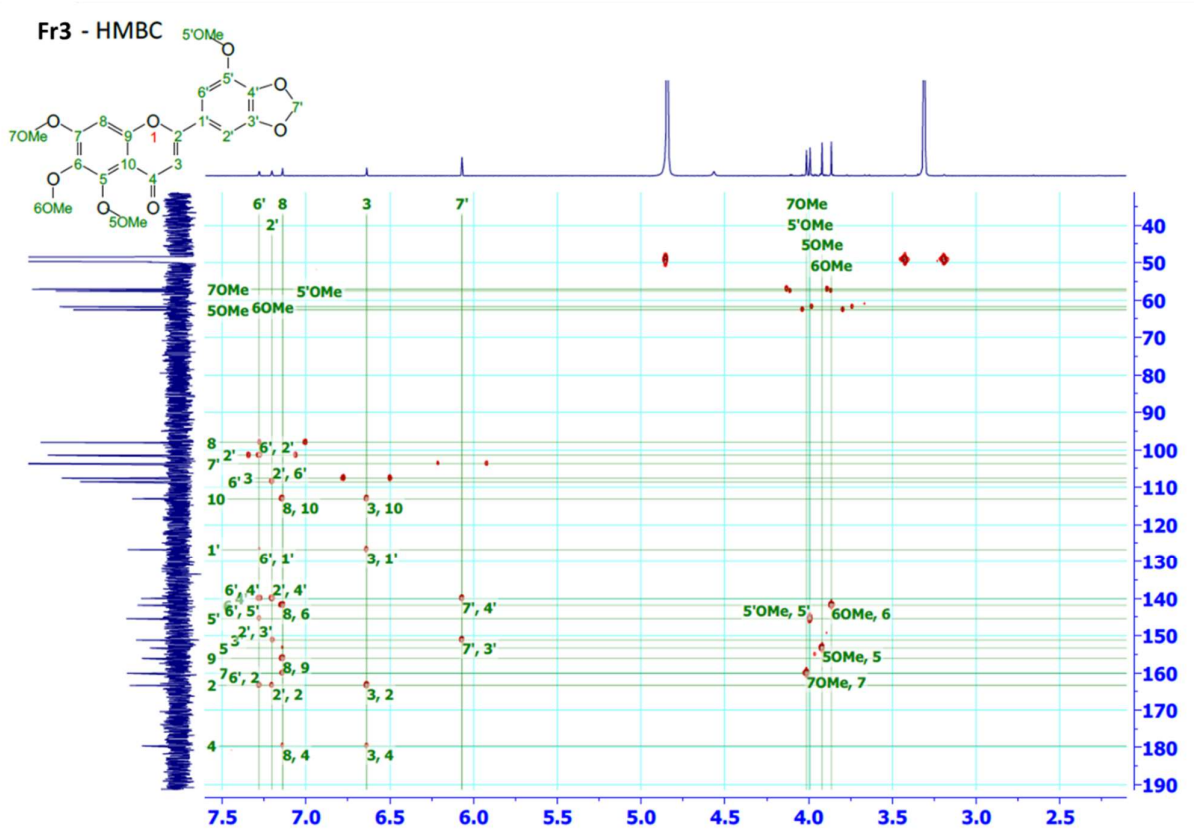


Figure S27: Complete assignment of correlation in 2D NMR-HMBC spectrum of compound **3**

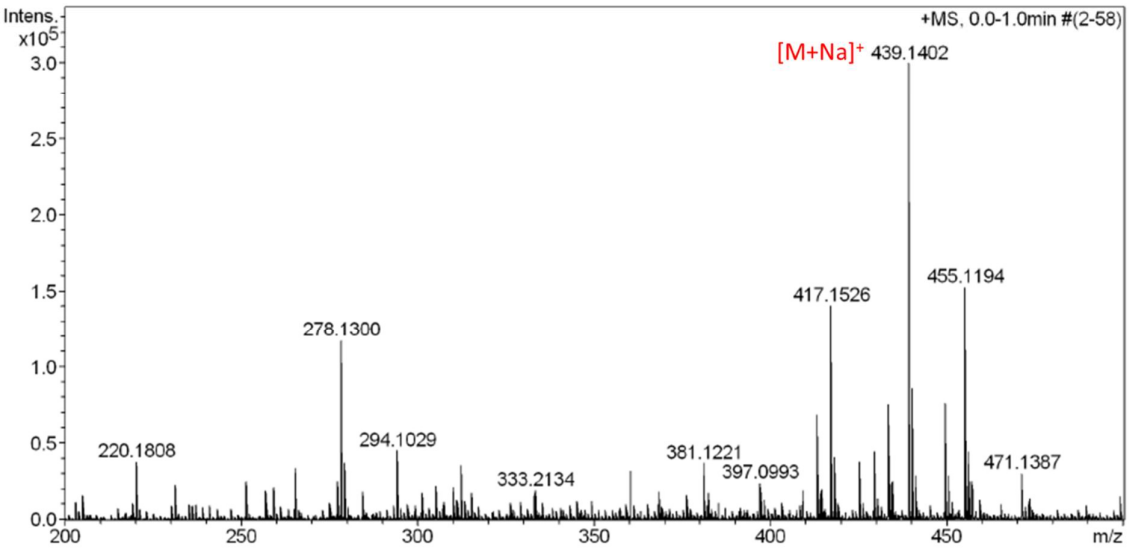
4. Supplementary spectroscopic data of compound 4.

Table S5: The comparison of NMR data of compound **4** with similar compound (5,6,7,8,3′-pentamethoxy-4′,5′-methylenedioxyflavone).

Position	Compound **4** (CD_3OD)				5,6,7,8,3′-pentamethoxy-4′,5′-methylenedioxyflavone ($CDCl_3$) [22]	
	^{13}C-NMR (150 MHz) δ_C ppm	**^{1}H-NMR** (600 MHz) δ_H ppm	**HSQC** ($^1H \rightarrow {}^{13}C$)	**HMBC** ($^1H \rightarrow {}^{13}C$)	**^{13}C-NMR** (125 MHz) δ_C ppm	**^{1}H-NMR** (400 MHz) δ_H ppm
2	163.20			C-2′, 3, 6′	160.76	
3	107.49	6.67 (H, *s*)	C-3	1′, 2, 4, 10	107.41	6.53 (H, *s*)
4	179.59			C-3	177.37	
5	149.40			C-(5-OMe)	148.49	
6	145.68			C-(6-OMe)	144.25	
7	153.46			C-(7-OMe)	151.61	
8	139.61			C-(8-OMe)	138.12	
9	149.12				147.75	
10	115.37			C-3	114.93	
1′	126.65			C-3	125.98	
2′	101.34	7.18 (H, *d, J = 1.2 Hz*)	C-2′	C-2, 3′, 4′, 6′	100.51	7.06 (H, *d, J = 1.7 Hz*)
3′	151.26			C - 2′, 7′	149.66	
4′	140.19			C-2′, 6′, 7′	138.42	
5′	145.45			C-(5′-OMe), 6′	143.99	
6′	108.48	7.30 (H, *d*, *J = 1.8 Hz*)	C-6′	C-2, 2′, 4′, 5′	106.61	7.11 (H, *d*, *J = 1.7 Hz*)
7′	103.79	6.08 (2H, *s*)	C-7′	C-3′, 4′	102.45	6.05 (2H, *s*)
5 - OMe	62.67	3.89 (3H, *s*)	C-(5-OMe)	C-5	62.38	3.91(3H, *s*)
6 - OMe	62.23	3.92 (3H, *s*)	C-(6-OMe)	C-6	62.11	3.92 (3H, *s*)
7 - OMe	62.19	4.11(3H, *s*)	C-(7-OMe)	C-7	61.78	4.07(3H, *s*)
8 - OMe	62.61	4.02(3H, *s*)	C-(8-OMe)	C-8	61.95	3.98(3H, *s*)
5′ - OMe	57.46	3.99 (3H, *s*)	C-(5′-OMe)	C-5′	56.82	3.95 (3H, *s*)

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	200 °C
Scan Begin	200 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Formular (M)	Ion formula	m/z	Calcd m/z
$C_{21}H_{20}O_9$	$C_{21}H_{20}O_9Na^+$	439.1402	409.1107

Figure S28: (+)-HR-ESI-MS data of compound **4**

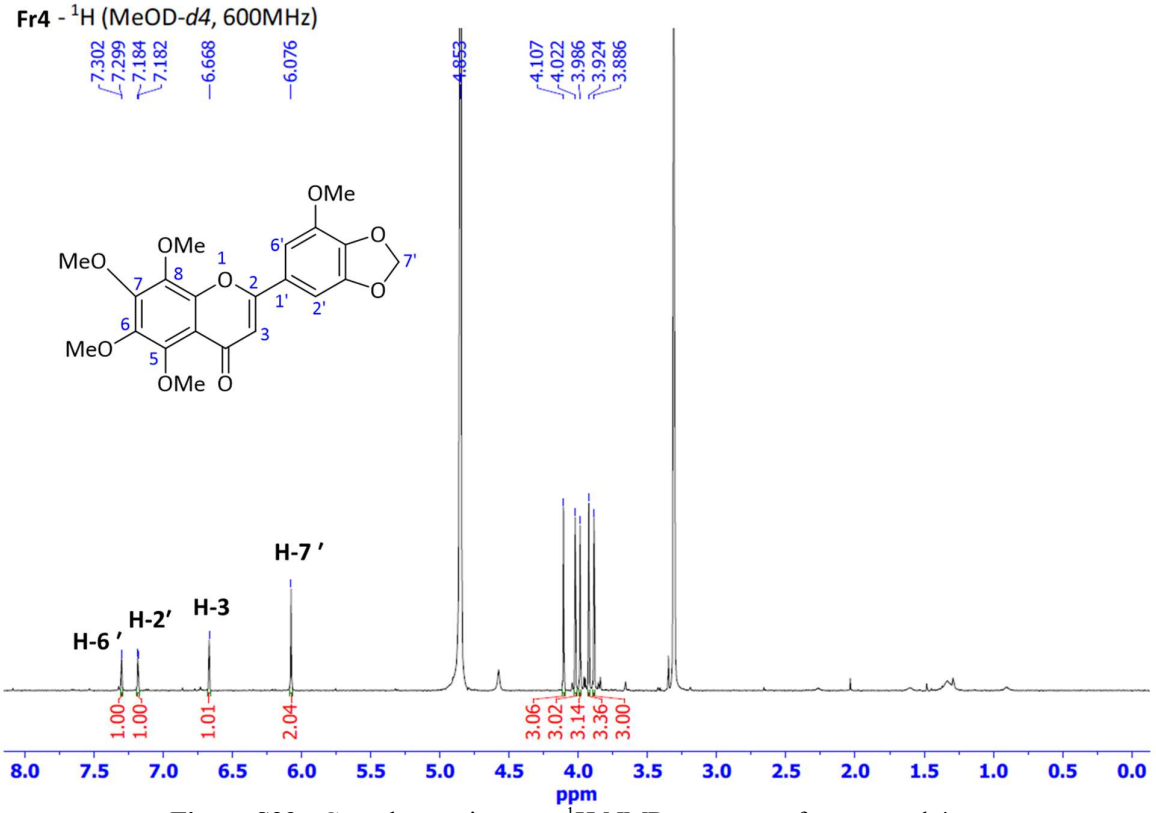


Figure S29: Complete assignment ^{1}H-NMR spectrum of compound **4**

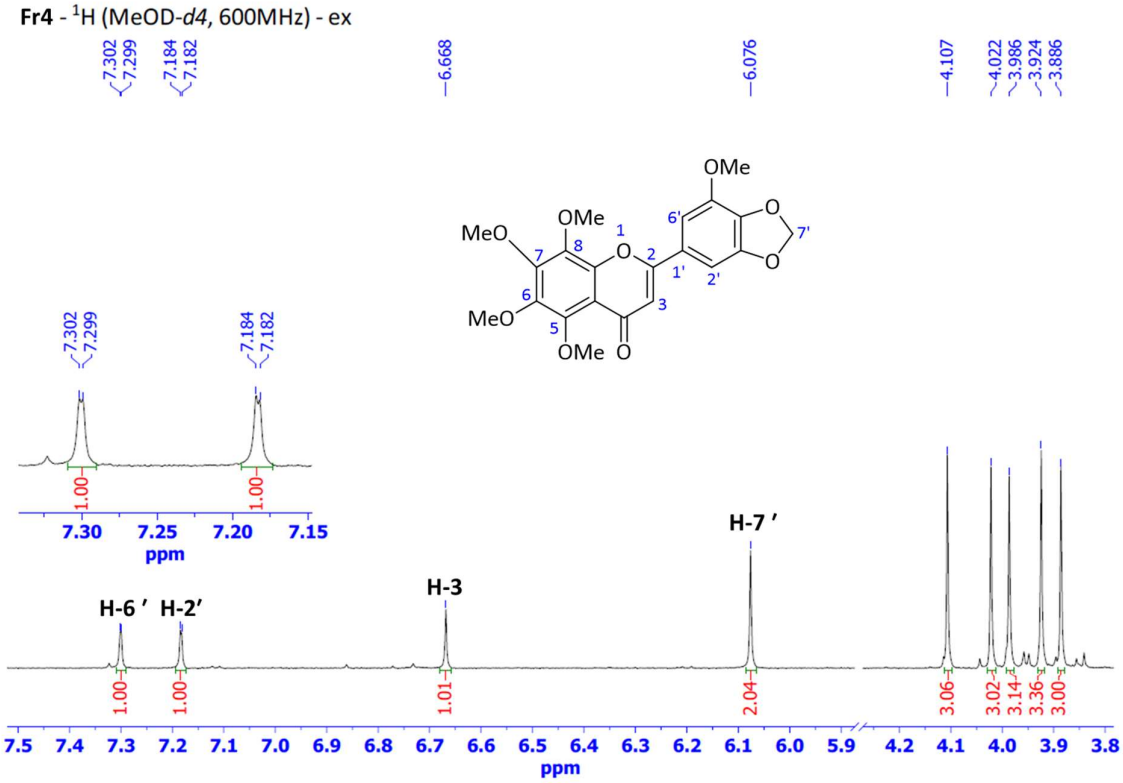


Figure S30: [1]H-NMR spectrum of compound **4** – expansion

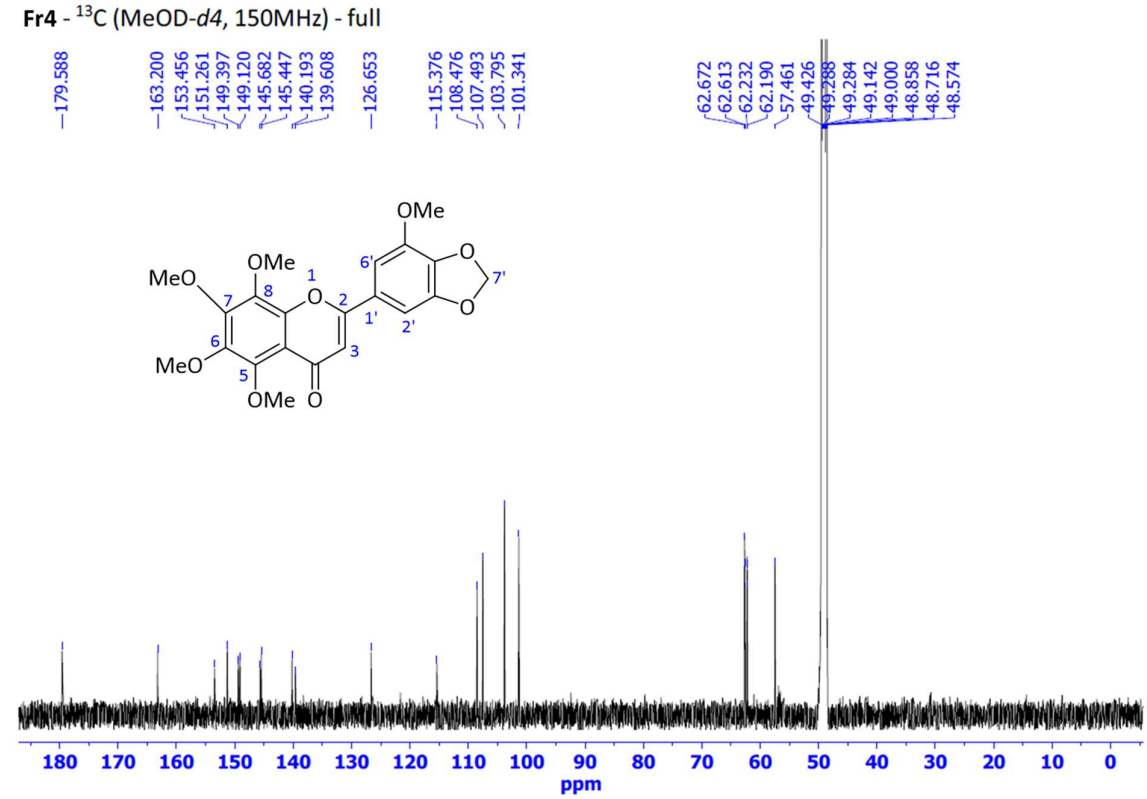


Figure S31: Complete assignment [13]C-NMR spectrum of compound **4**

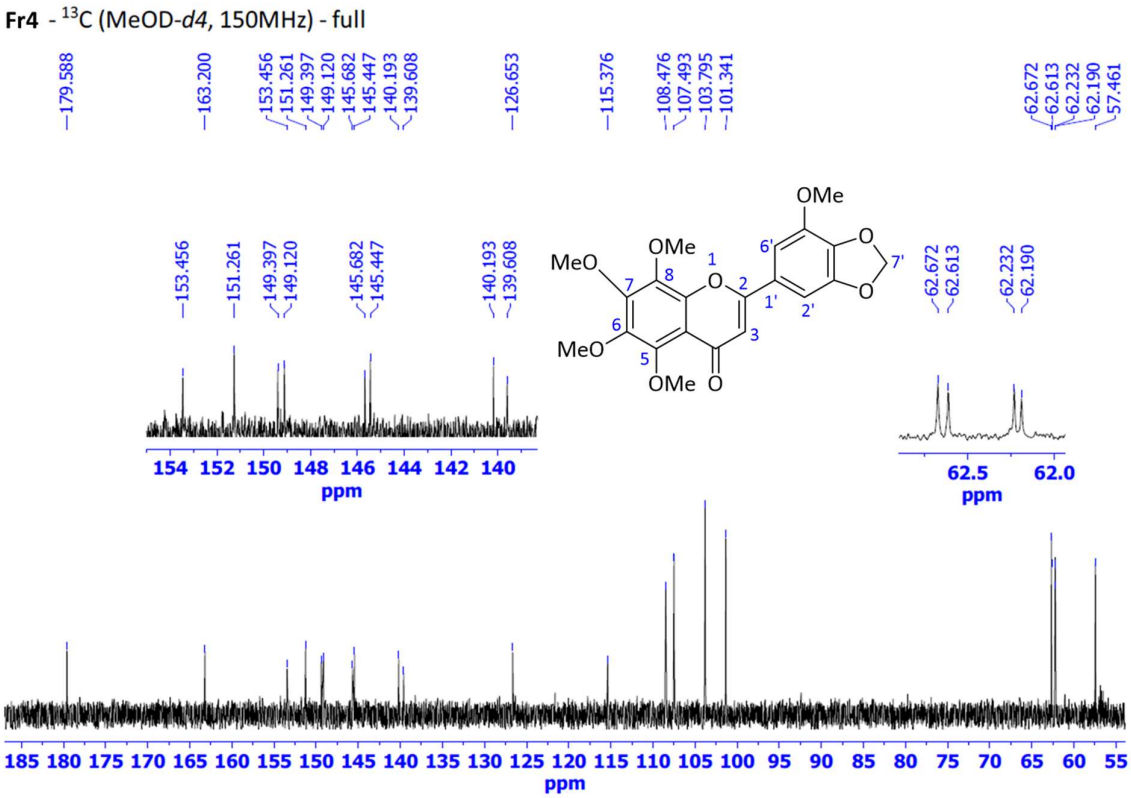


Figure S32: ^{13}C-NMR spectrum of compound **4** – expansion

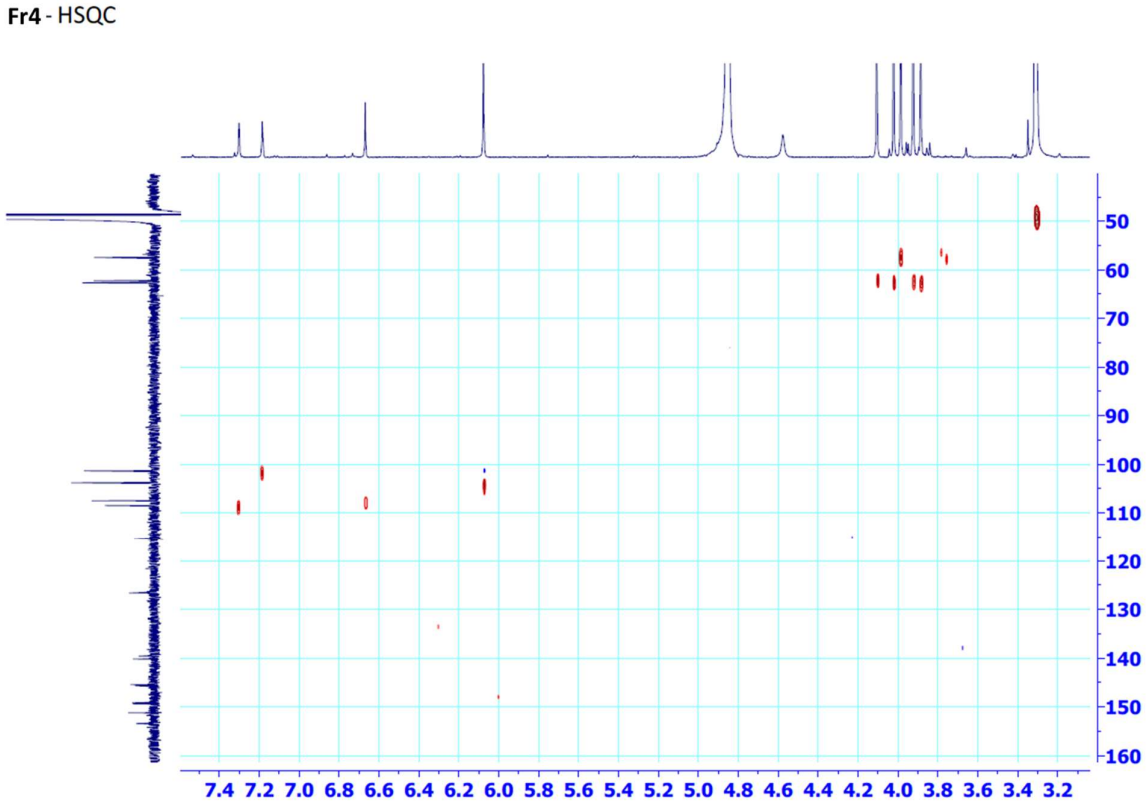


Figure S33: Complete assignment of correlation in 2D NMR-HSQC spectrum of compound **4**

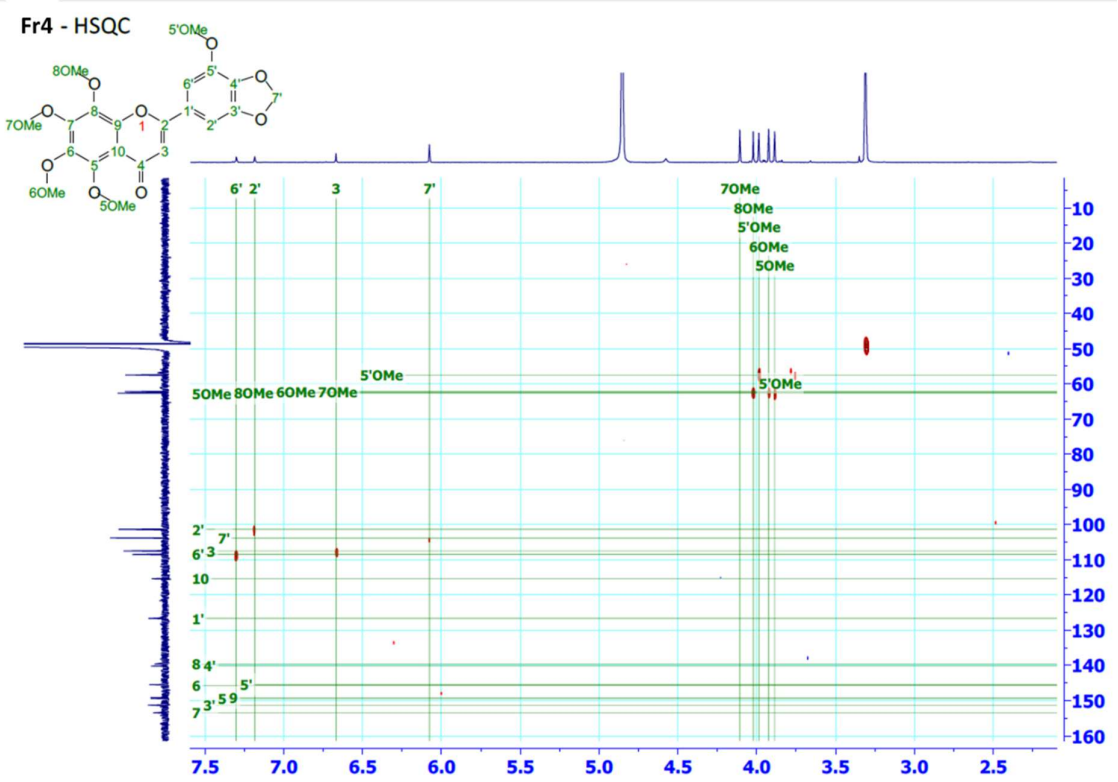


Figure S34: Correlation in 2D NMR-HSQC spectrum of compound **4**

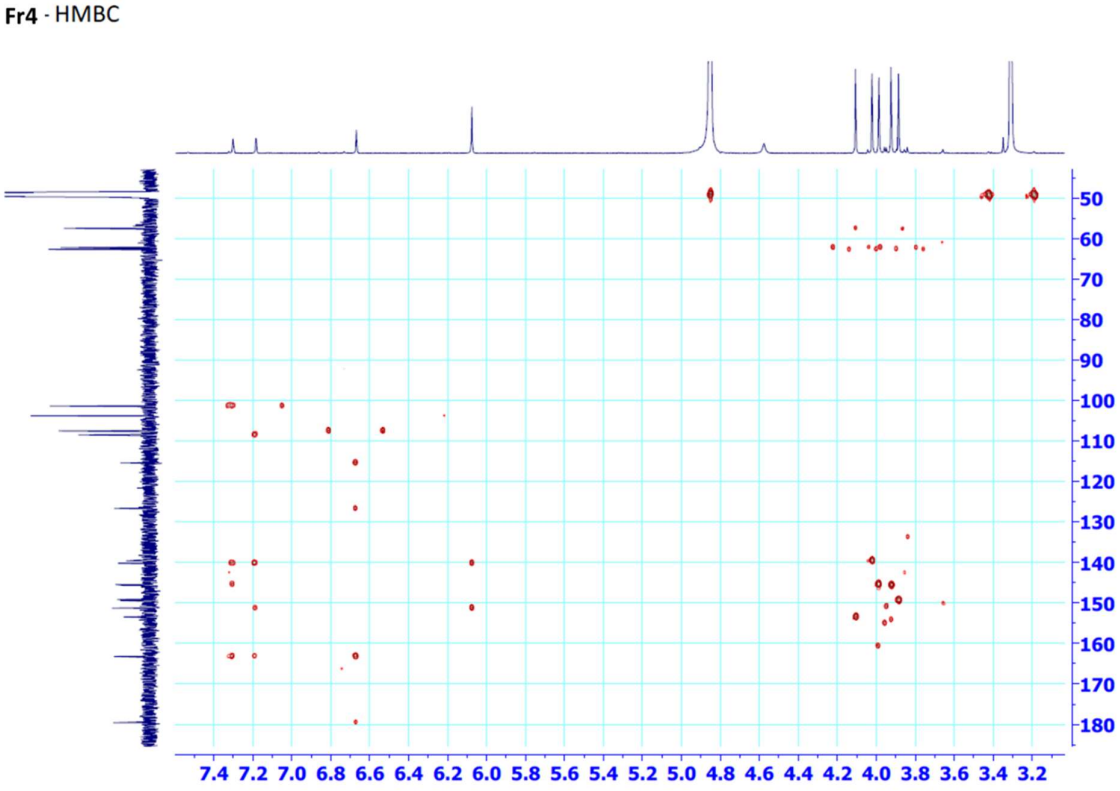


Figure S35: Complete assignment of correlation in 2D NMR-HMBC spectrum of compound **4**

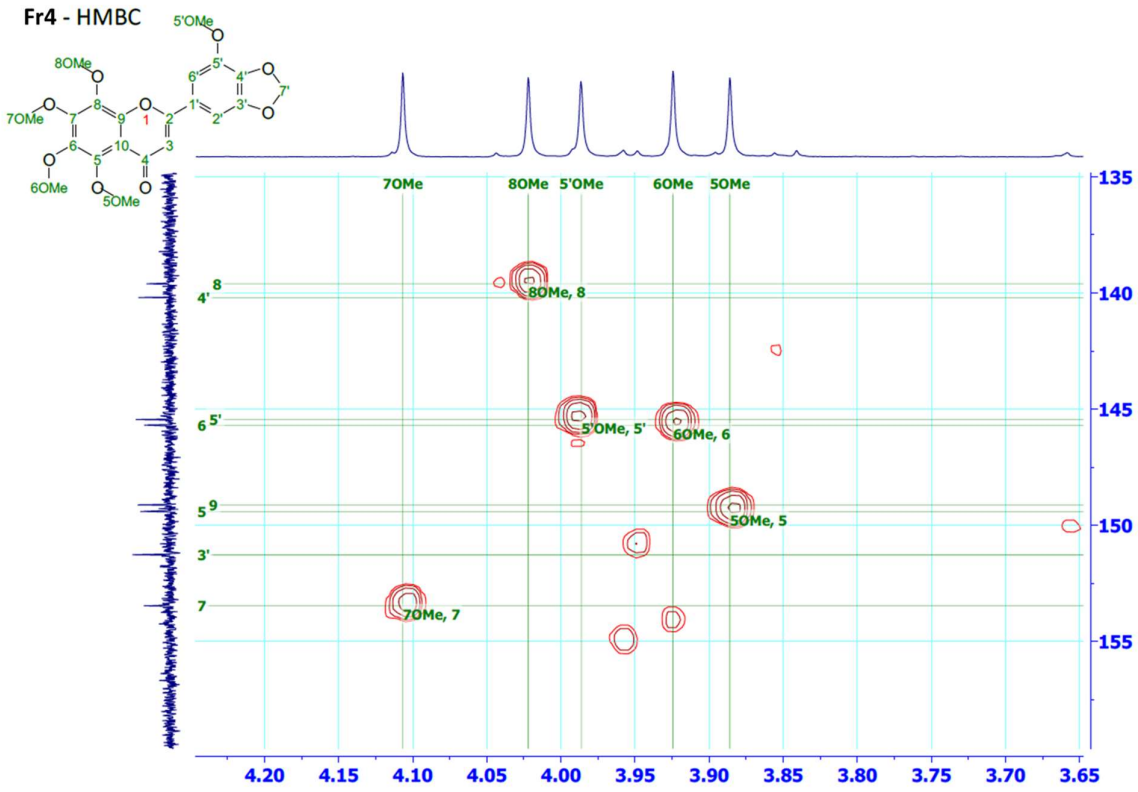


Figure S36: Correlation in 2D NMR-HMBC spectrum of compound **4**