

Supporting Information

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

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Abbreviations

-OMe methoxy group (-OCH₃)

Table S1: List of primers and primer sequences.

No.	Gene name	Primer probes	Sequence
1	<i>atf4</i>	Forward	5'- GGACAGATTGGATGTTGGAGAAAATG-3'
		Reverse	5'-GGAGATGGCCAATTGGGTTTCAC-3'
2	<i>chop</i>	Forward	5'-GCGACAGAGCCAGAATAACA-3'
		Reverse	5'-GATGCACTTCCTTCTGGAACA-3'
3	<i>xbp-1</i>	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

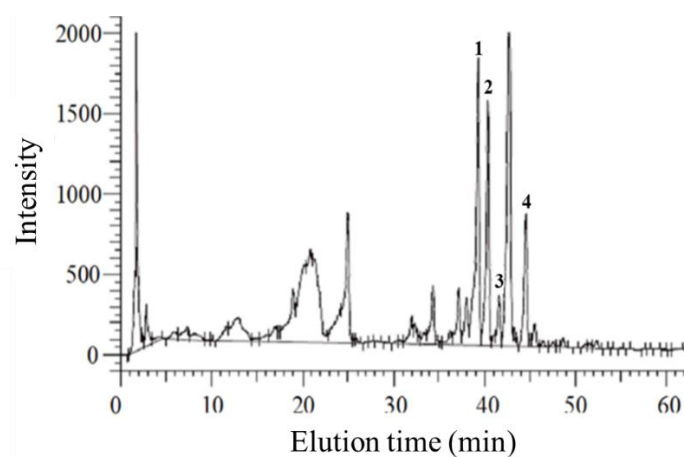


Figure S1: Elution pattern of reverse-phase HPLC from AC-EtOAc fraction

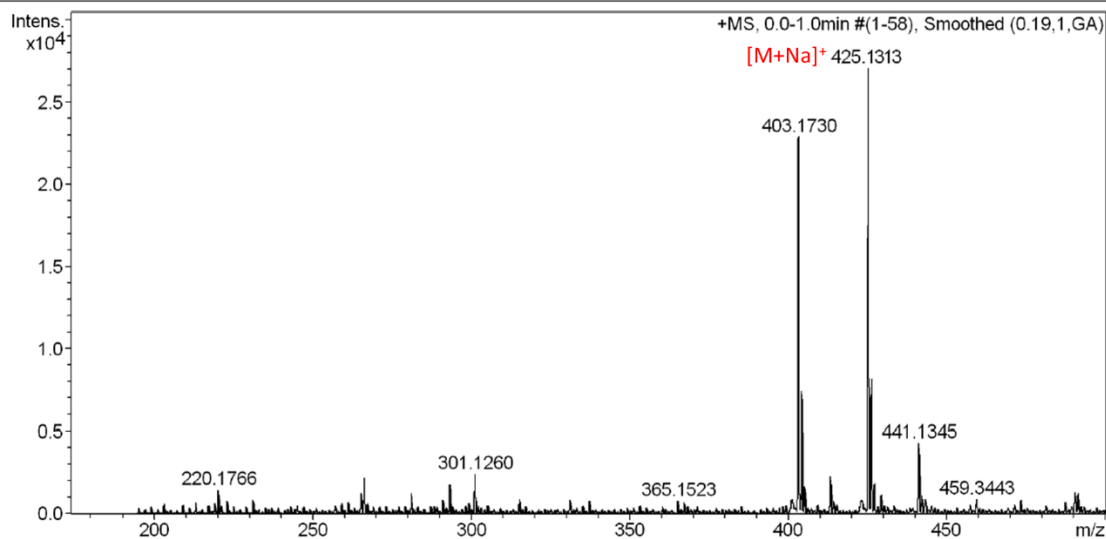
1. Supplementary spectroscopic data of compound 1

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

Position	Compound 1 (CD ₃ OD)			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 → 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)

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 ₈ Na	425.1315	425.1313

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

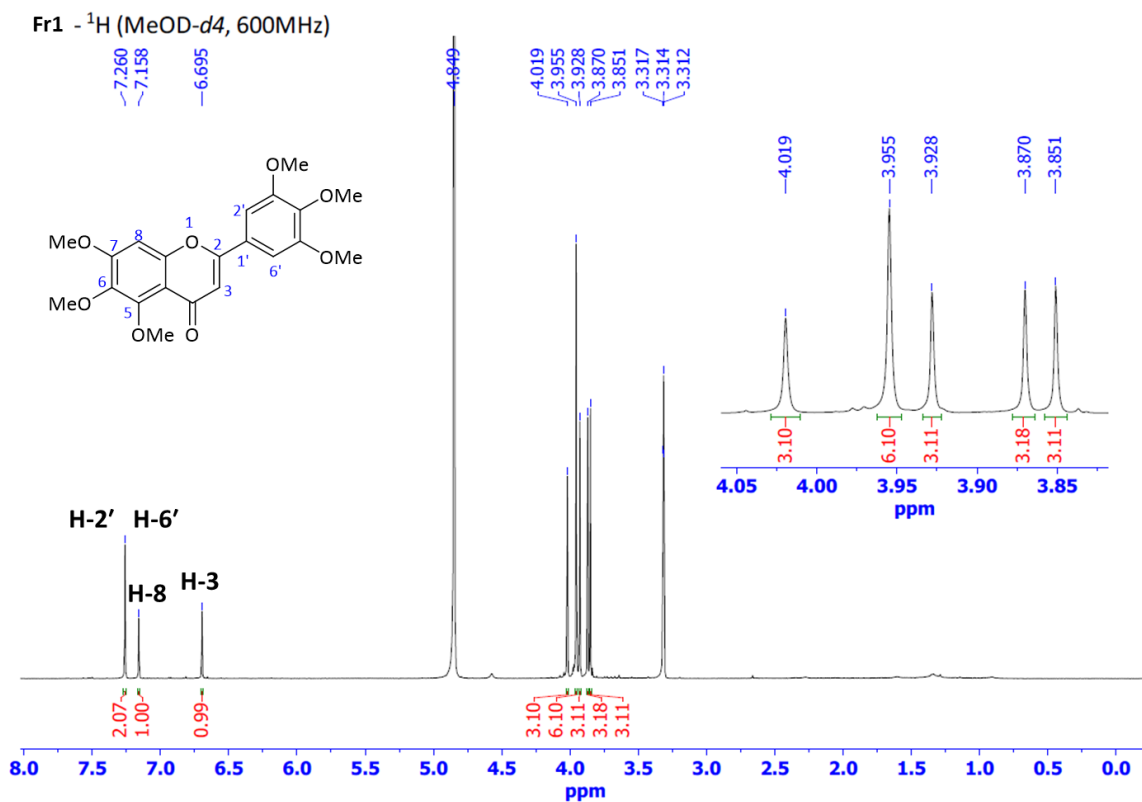


Figure S3: Complete assignment ¹H-NMR spectrum of compound **1**

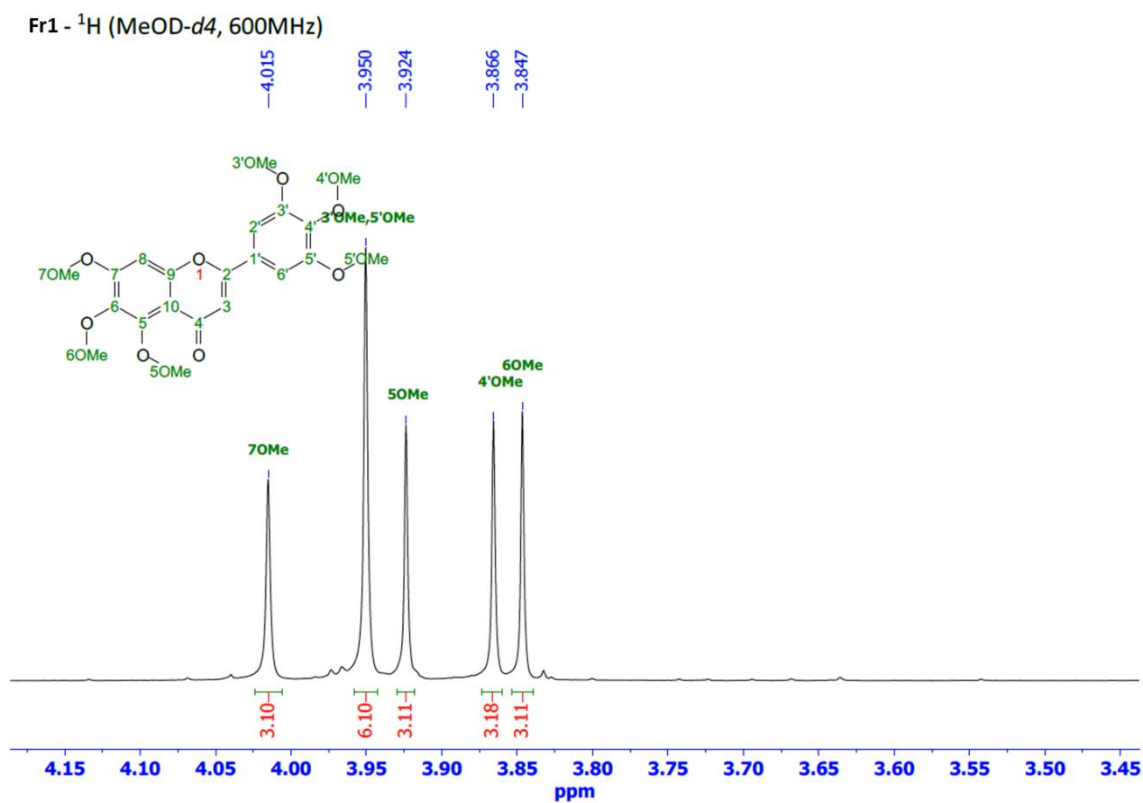


Figure S4: ^1H NMR spectrum of compound 1 – expansion

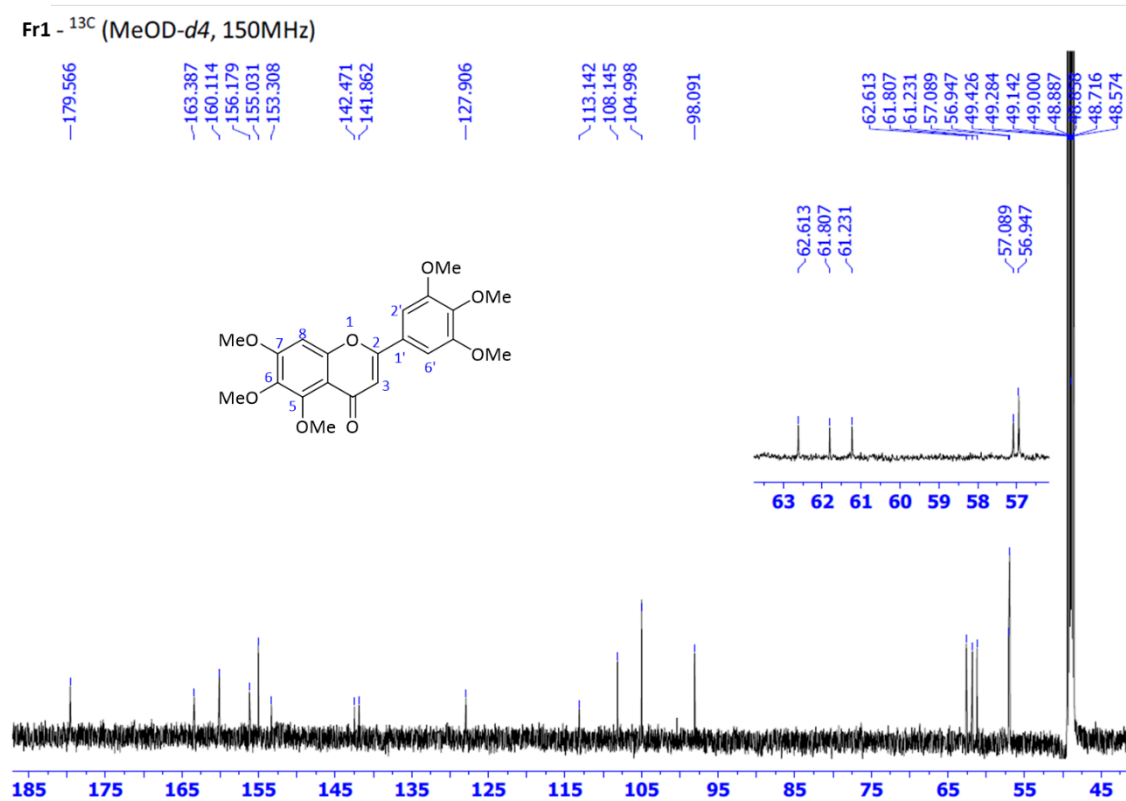


Figure S5: Complete assignment ^{13}C -NMR spectrum of compound 1

Fr1 - ^{13}C (MeOD- d_4 , 150MHz)

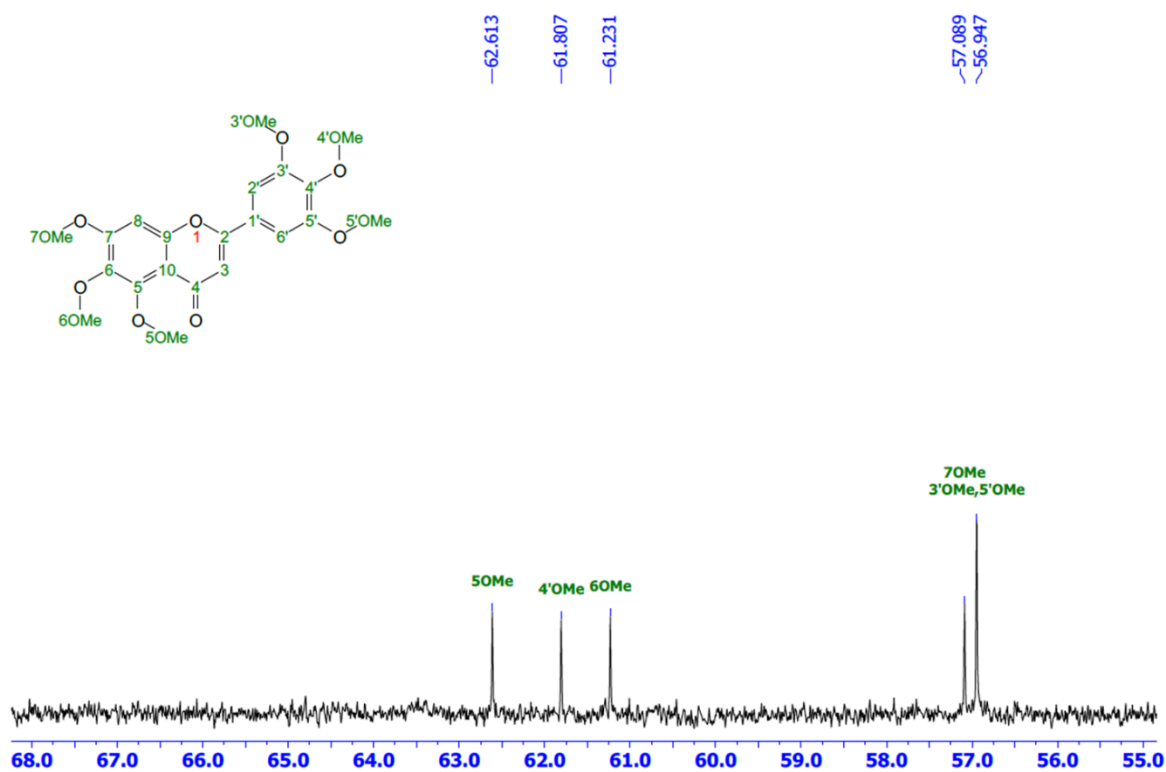


Figure S6: ^{13}C -NMR spectrum of compound 1 – expansion

Fr1 - HSQC

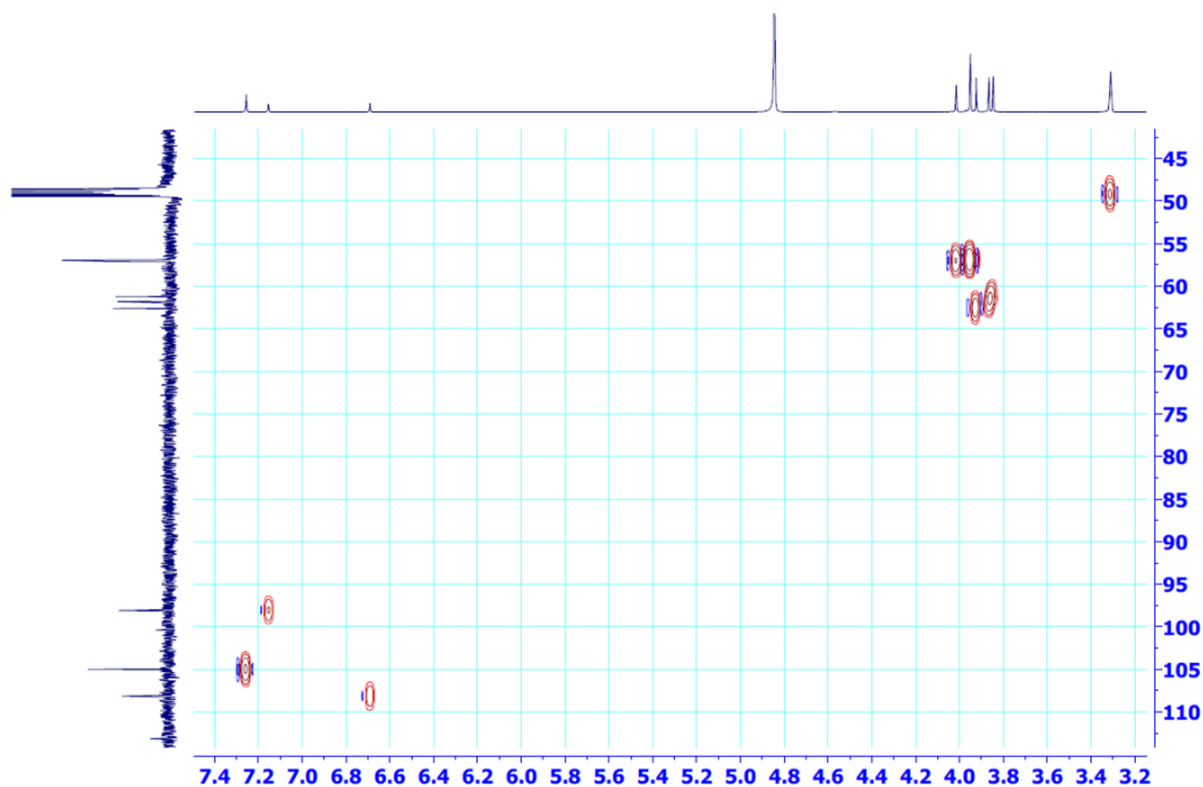


Figure S7: Complete assignment of correlation in 2D NMR-HSQC spectrum of compound 1

Fr1 - HSQC

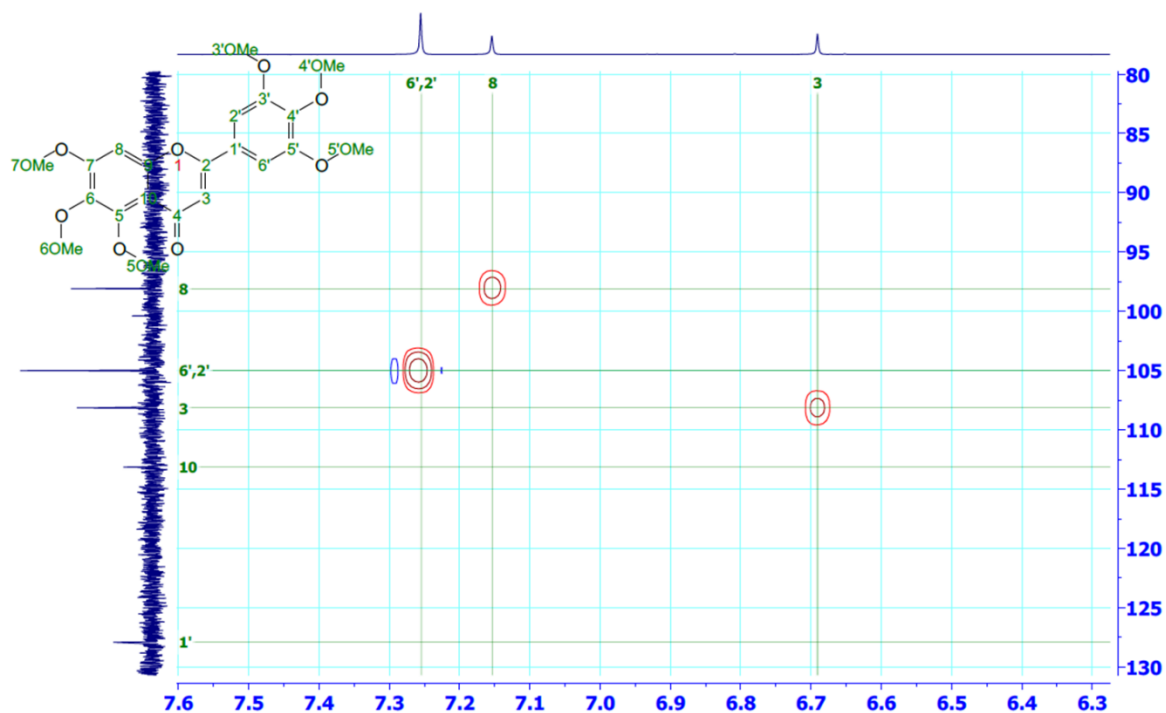


Figure S8: Correlation in 2D NMR-HSQC spectrum of compound **1** – expansion

Fr1 - HMBC

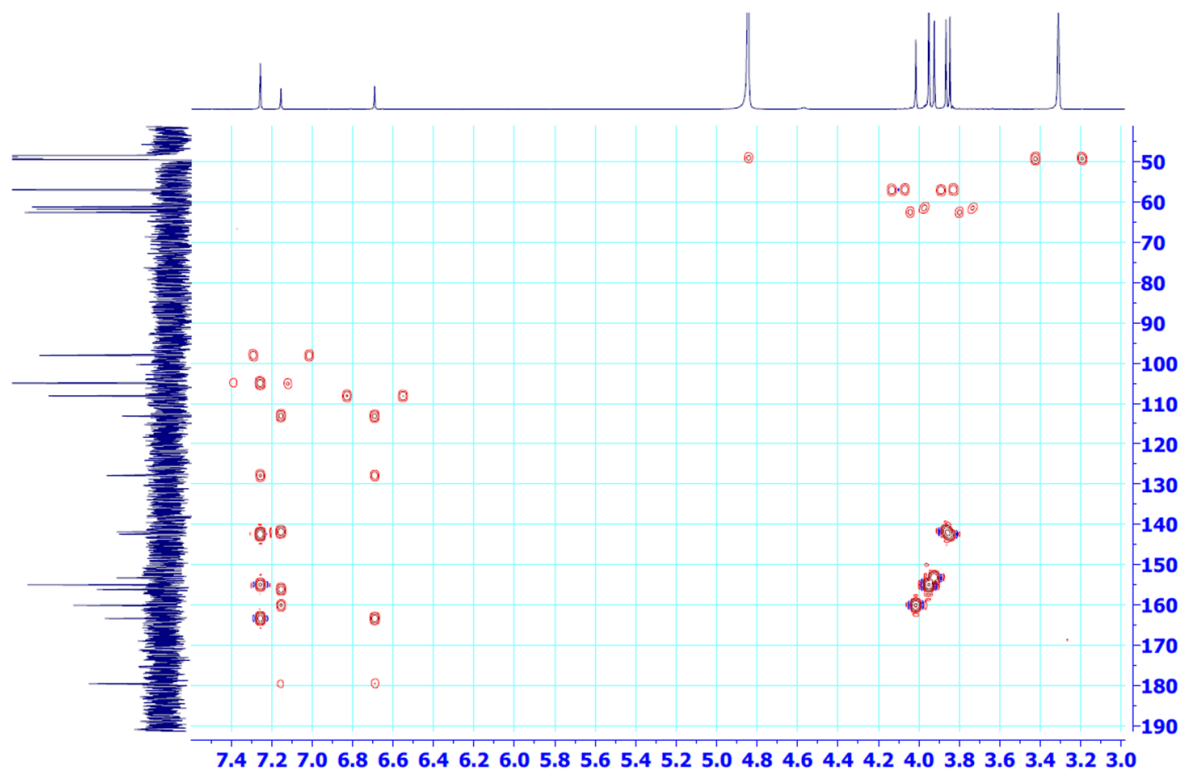


Figure S9: Complete assignment of correlation in 2D NMR-HMBC spectrum of compound **1**

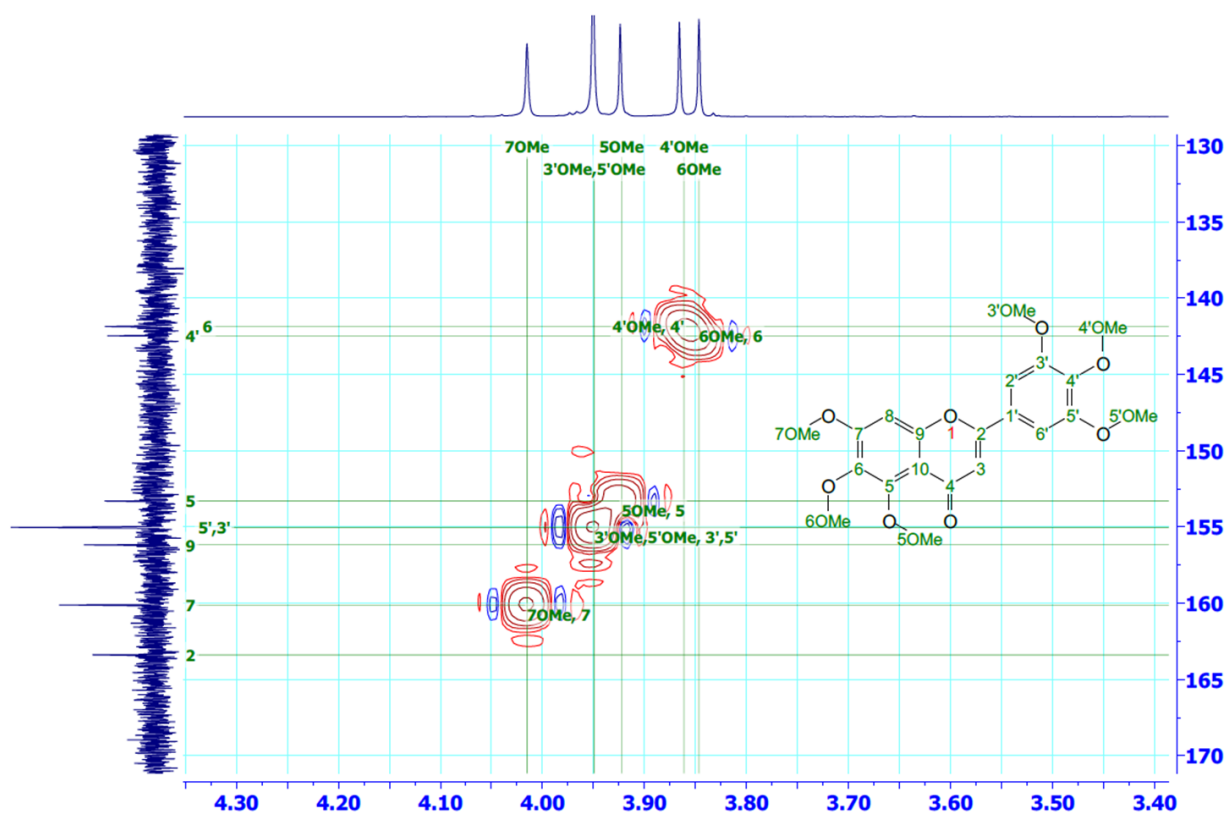


Figure S10: Correlation in 2D NMR-HMBC spectrum of compound **1** – expansion

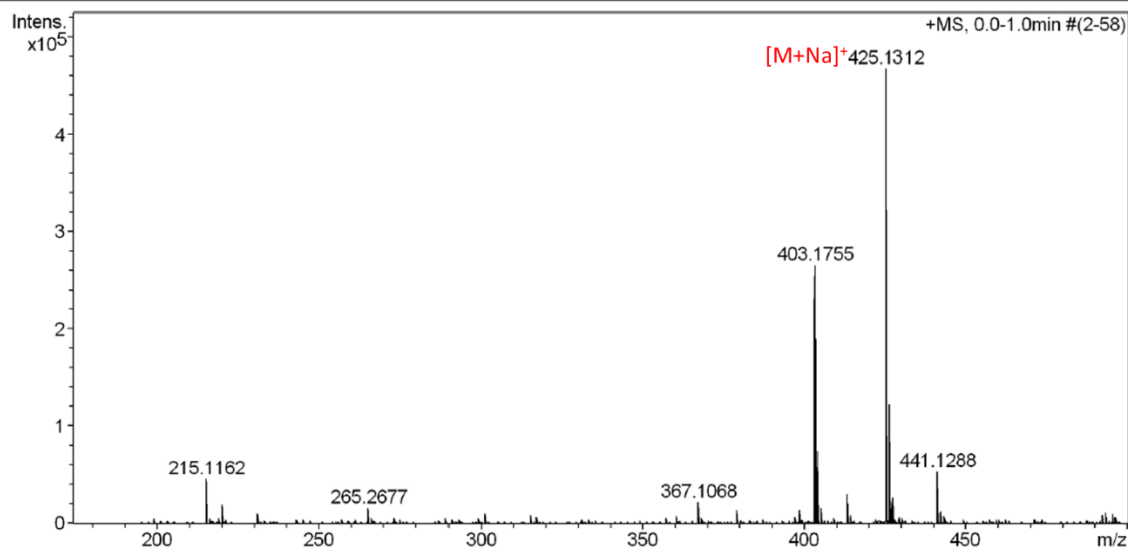
2. Supplementary spectroscopic data of compound 2.

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

Position	Compound 2 (CD ₃ OD)			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 → 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 ₈ Na	425.1315	425.1312

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

Fr2 - ¹H (MeOD-*d*₄, 600MHz)

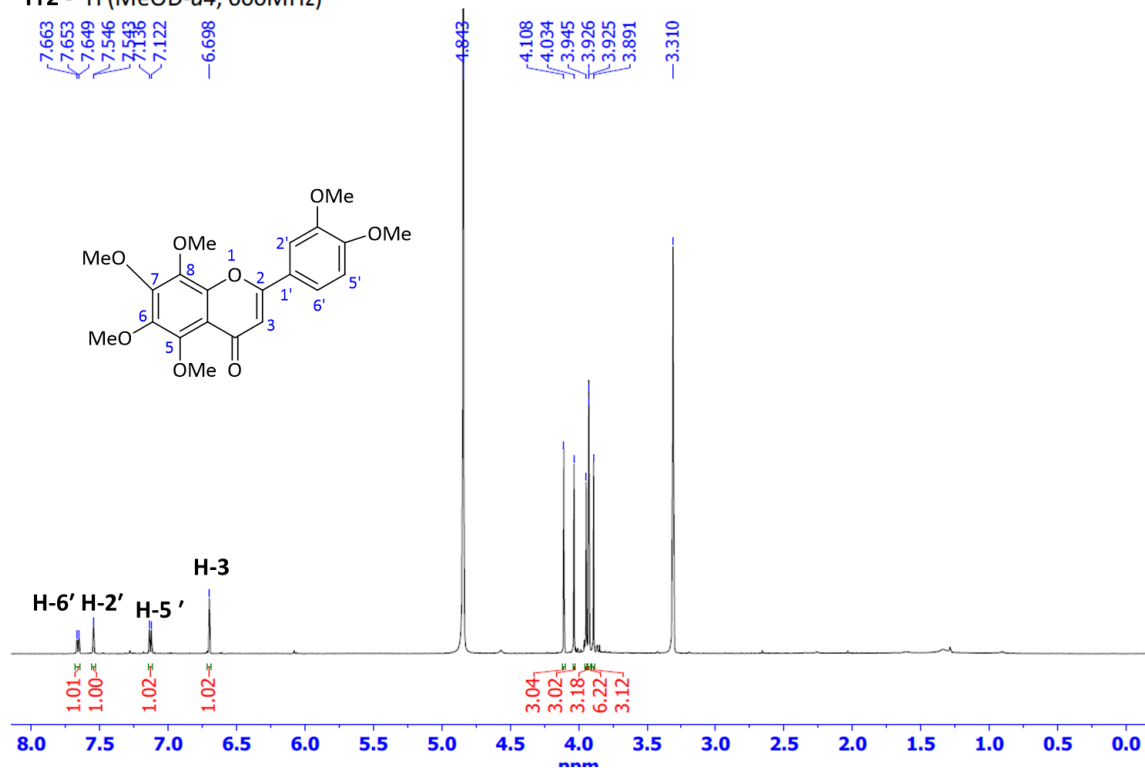


Figure S12: Complete assignment ¹H-NMR spectrum of compound 2

Fr2 - ^1H (MeOD- d_4 , 600MHz) - ex

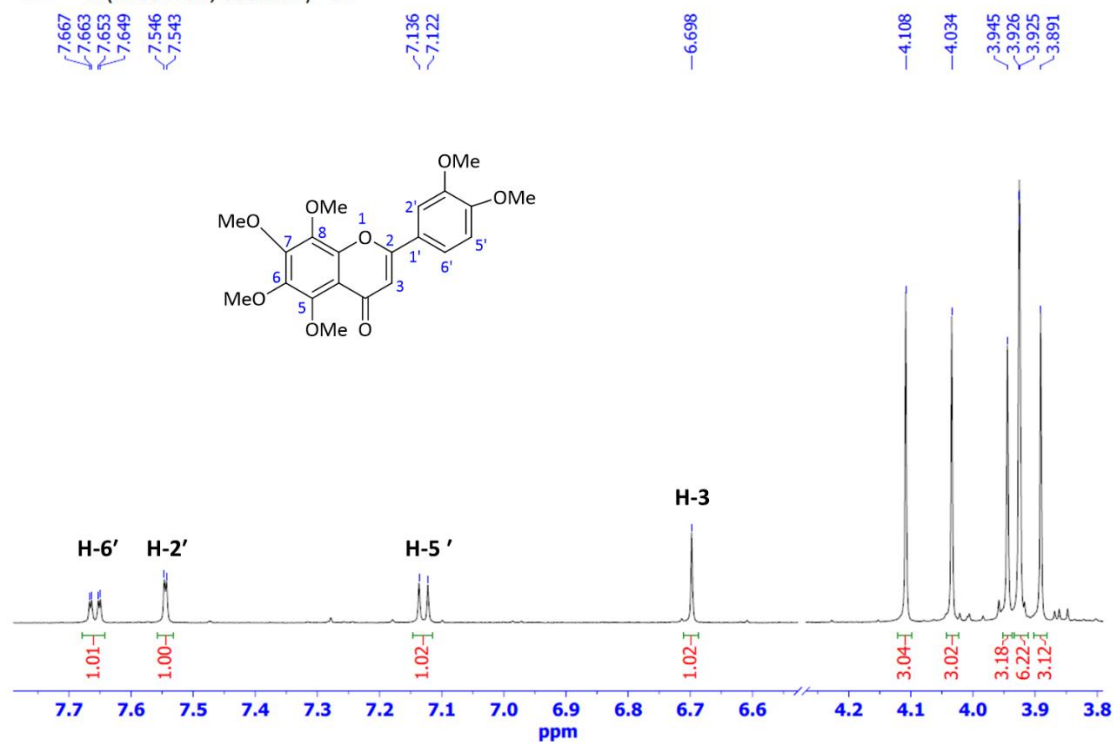


Figure S13: ^1H -NMR spectrum of compound 2 - expansion

Figure S12:

Fr2 - ^{13}C (MeOD- d_4 , 600MHz)

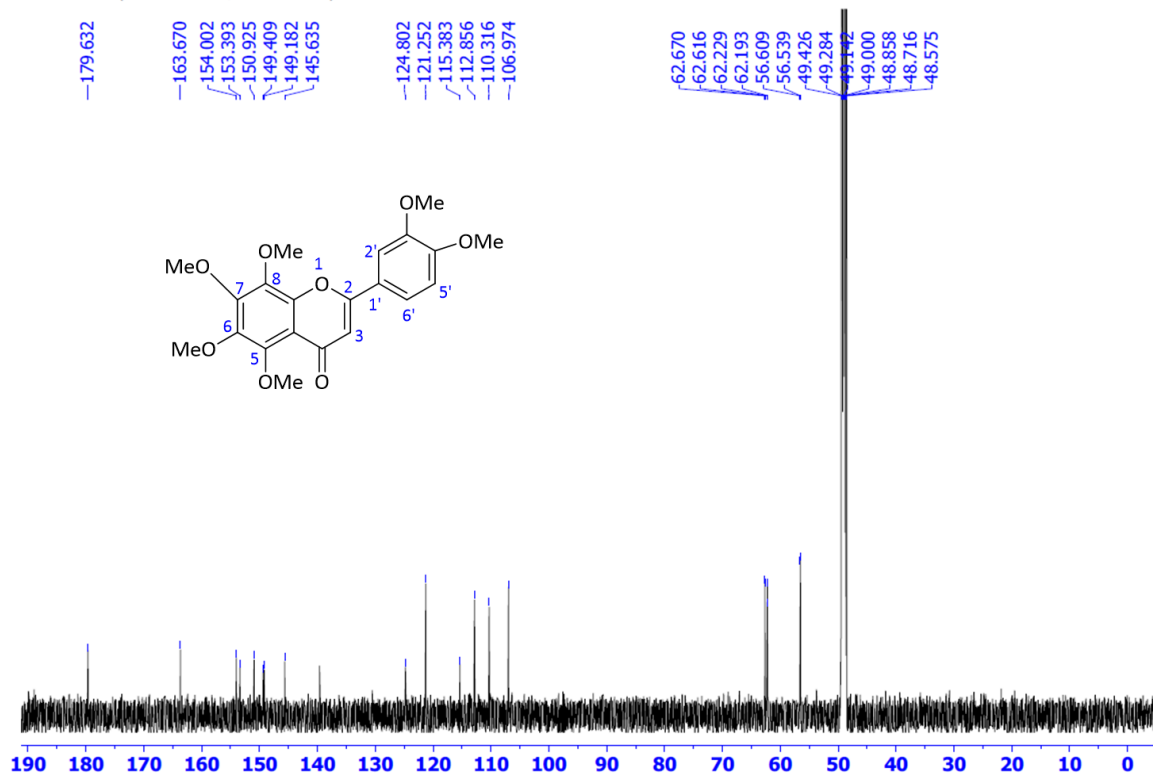


Figure S14: Complete assignment ^{13}C -NMR spectrum of compound 2

Fr2 - ^{13}C (MeOD- d_4 , 600MHz) - ex

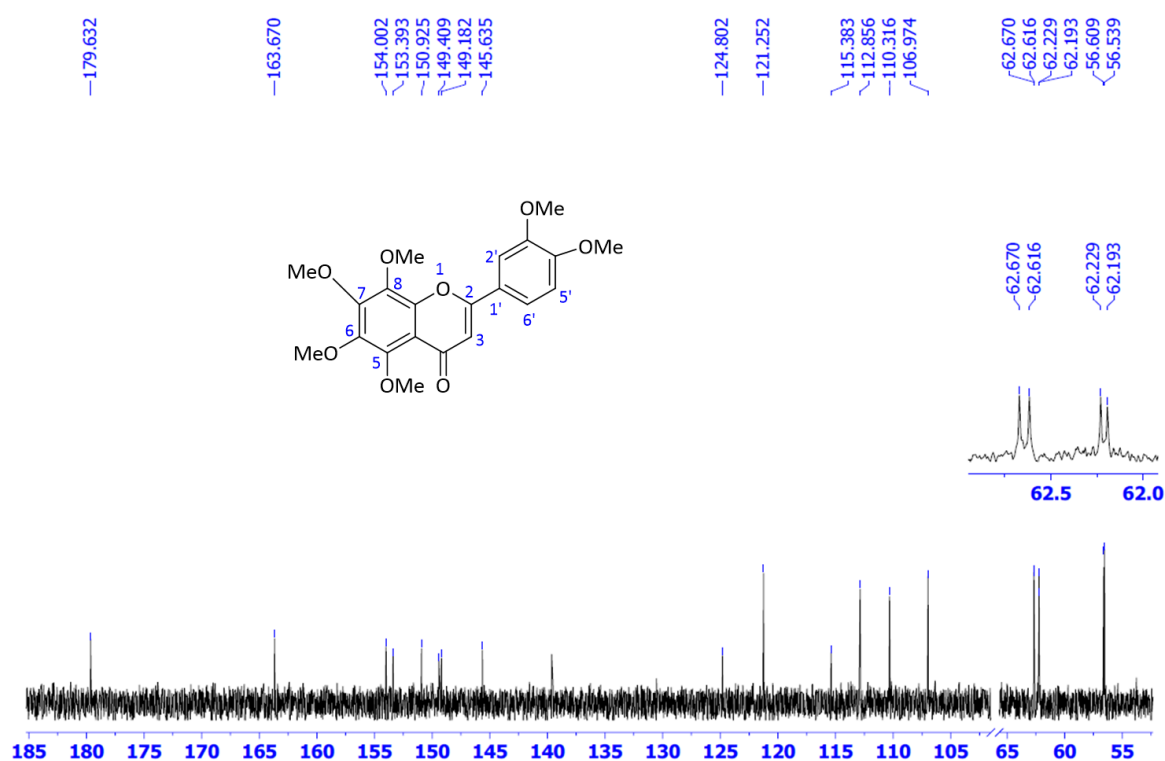


Figure S15: ^{13}C -NMR spectrum of compound 2 – expansion A

Fr2 - ^{13}C (MeOD- d_4 , 600MHz)

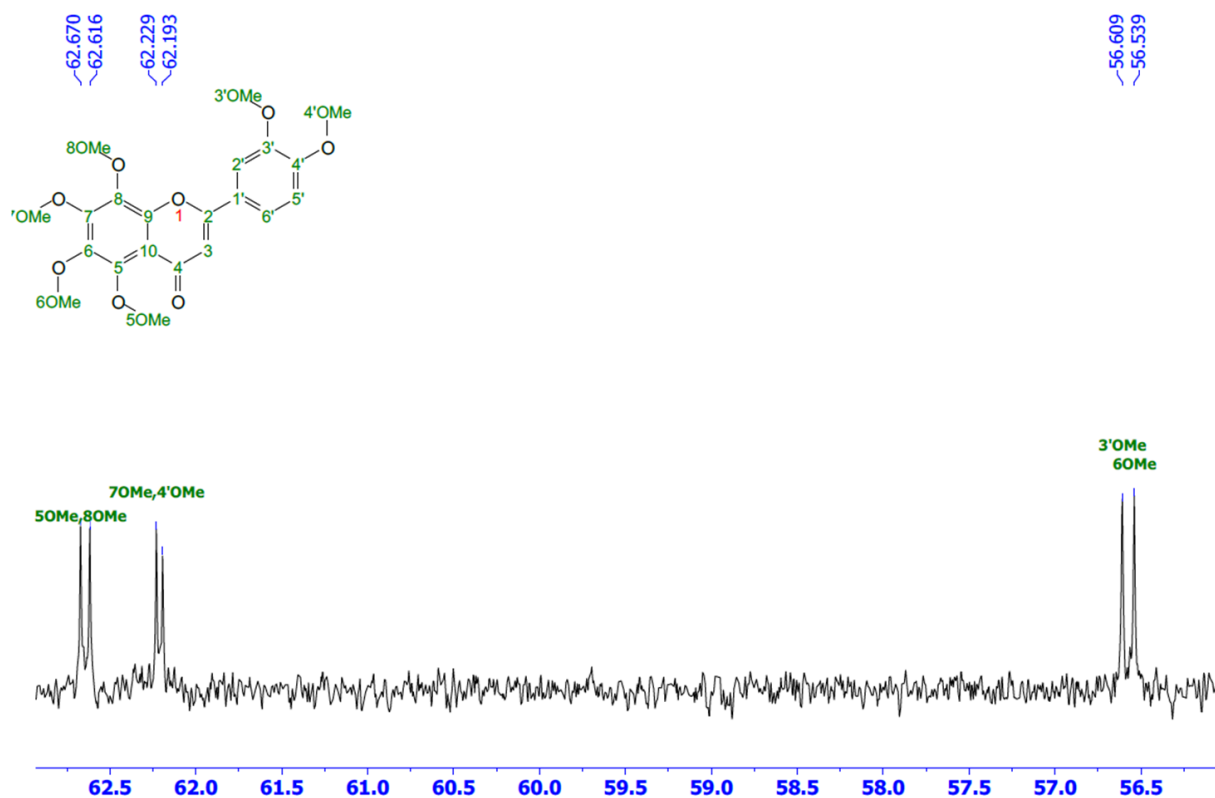


Figure S16: ^{13}C -NMR spectrum of compound 2 – expansion B

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Fr2 - HMBC

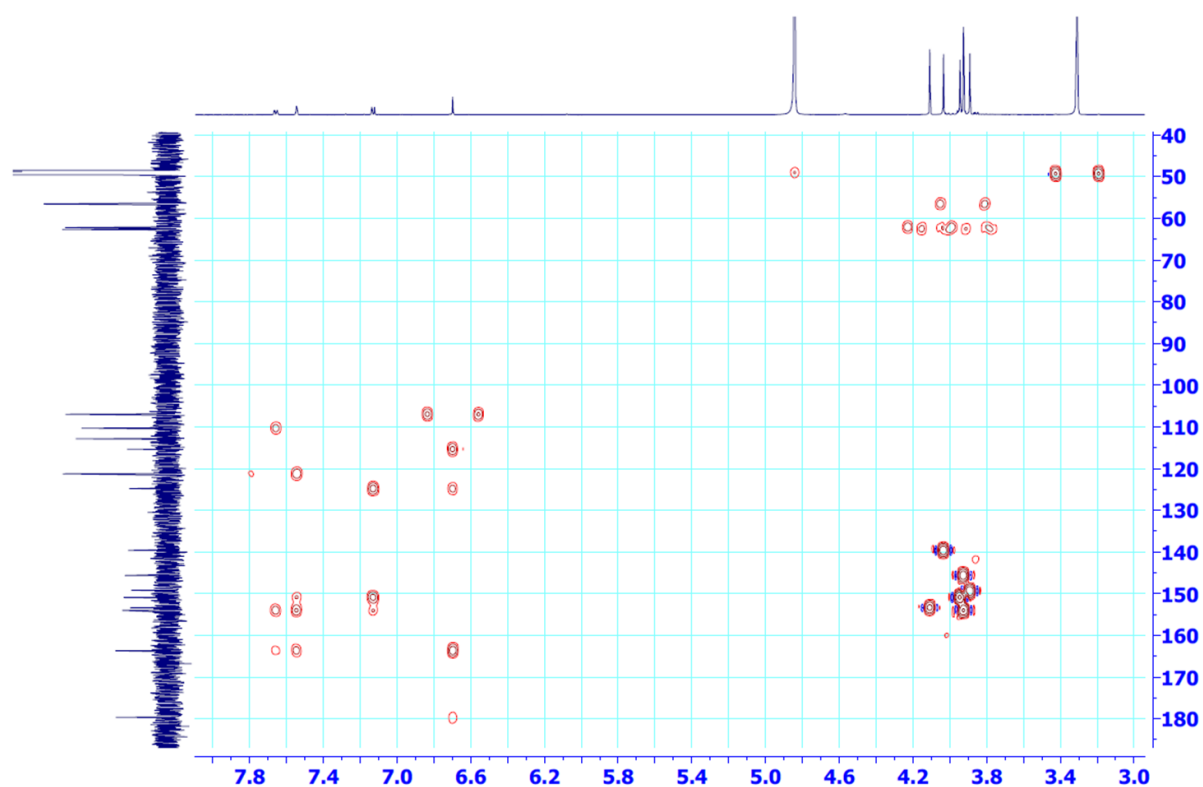


Figure S19: Full assignment of correlation in 2D NMR-HMBC spectrum of compound 2

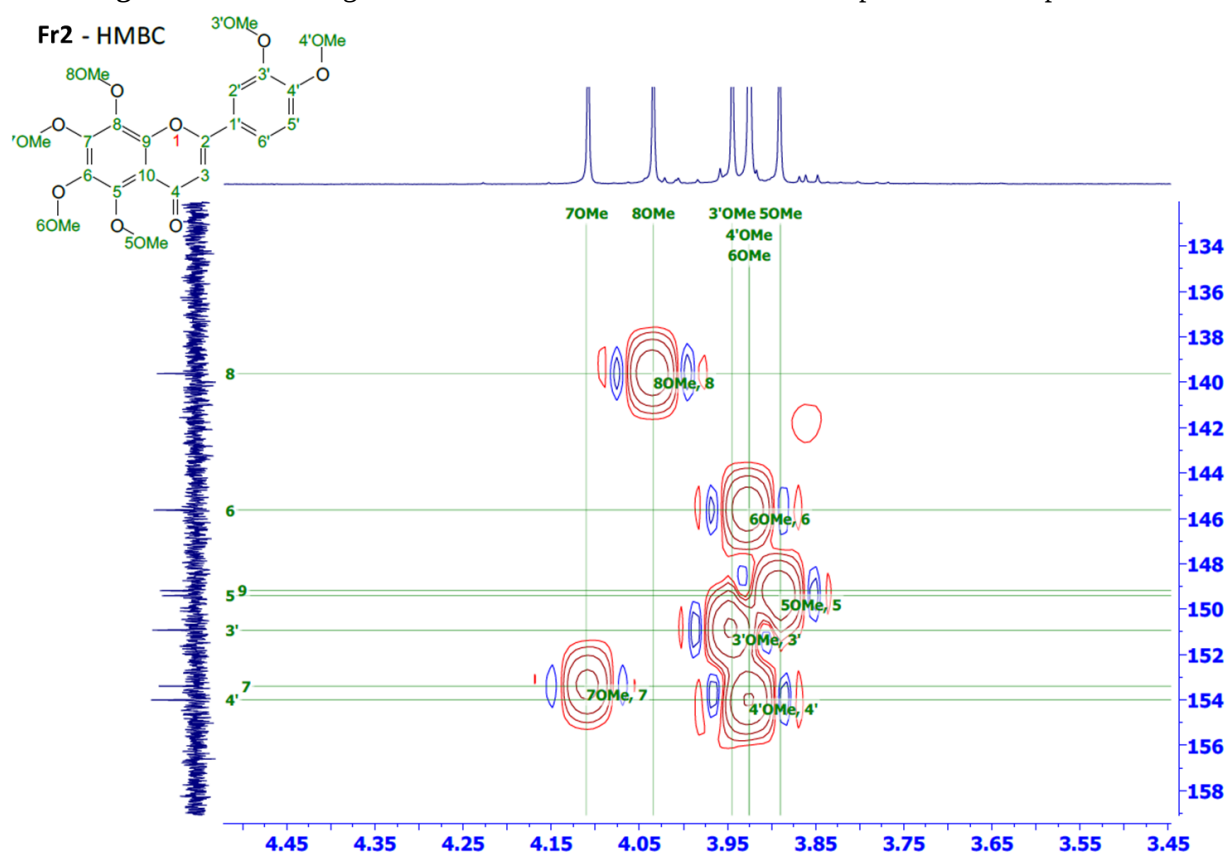


Figure S20: Correlation in 2D NMR-HMBC spectrum of compound 2

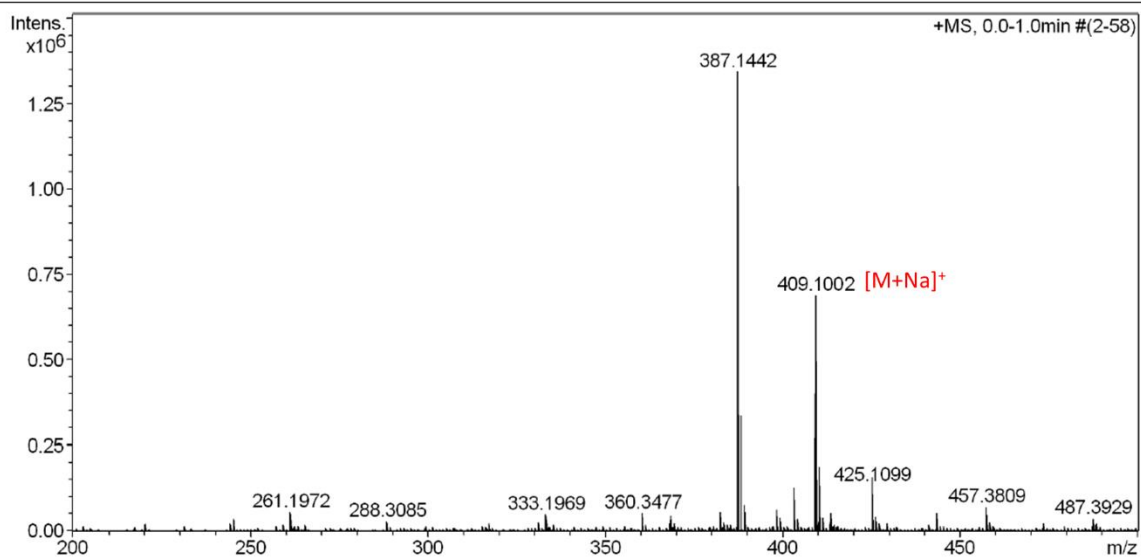
3. Supplementary spectroscopic data of compound 3.

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 ₃ OD)				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 (¹ H → ¹³ C)	HMBC (¹ H → 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 ₈ Na ⁺	409.1002	409.1002

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

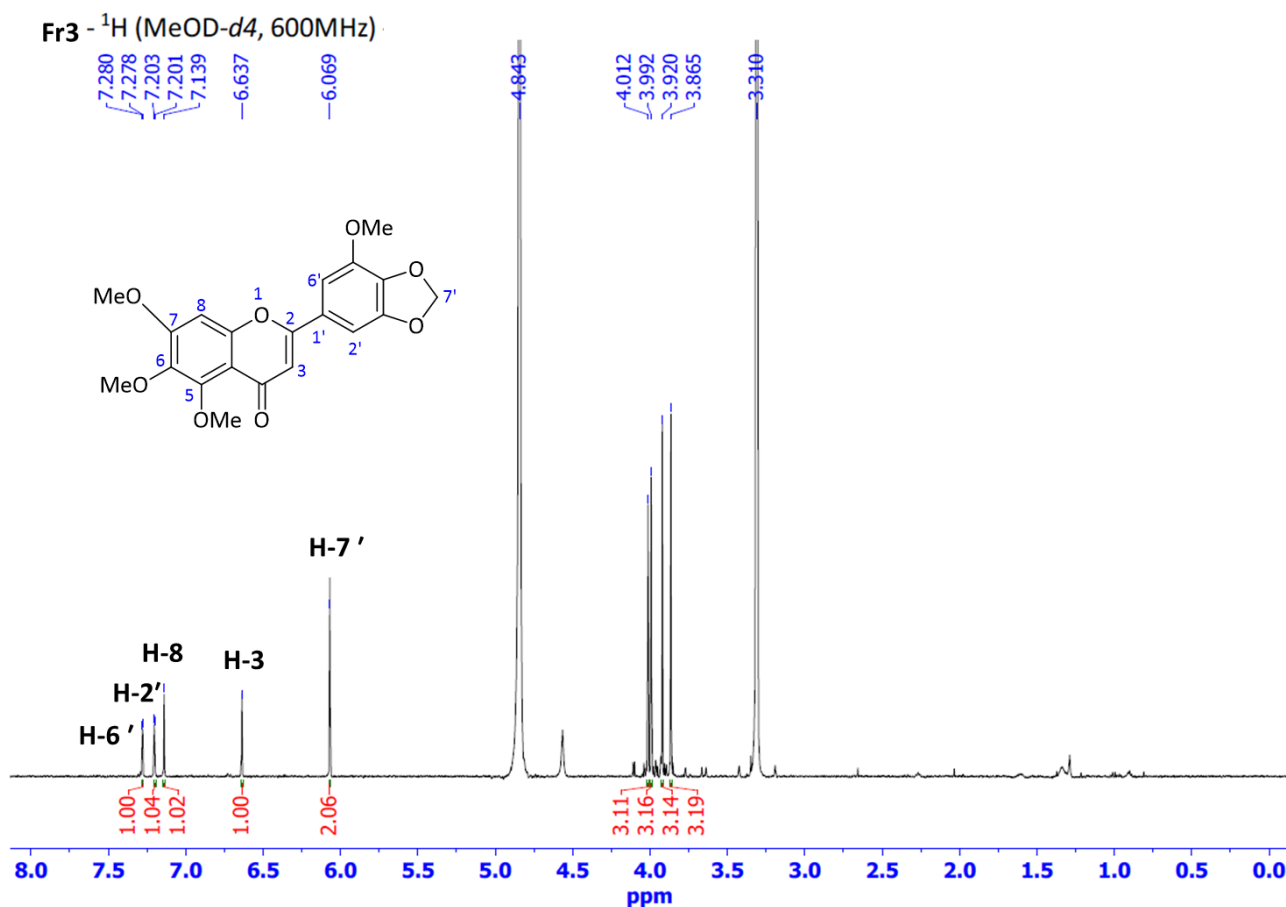


Figure S22: Complete assignment ¹H-NMR spectrum of compound **3**

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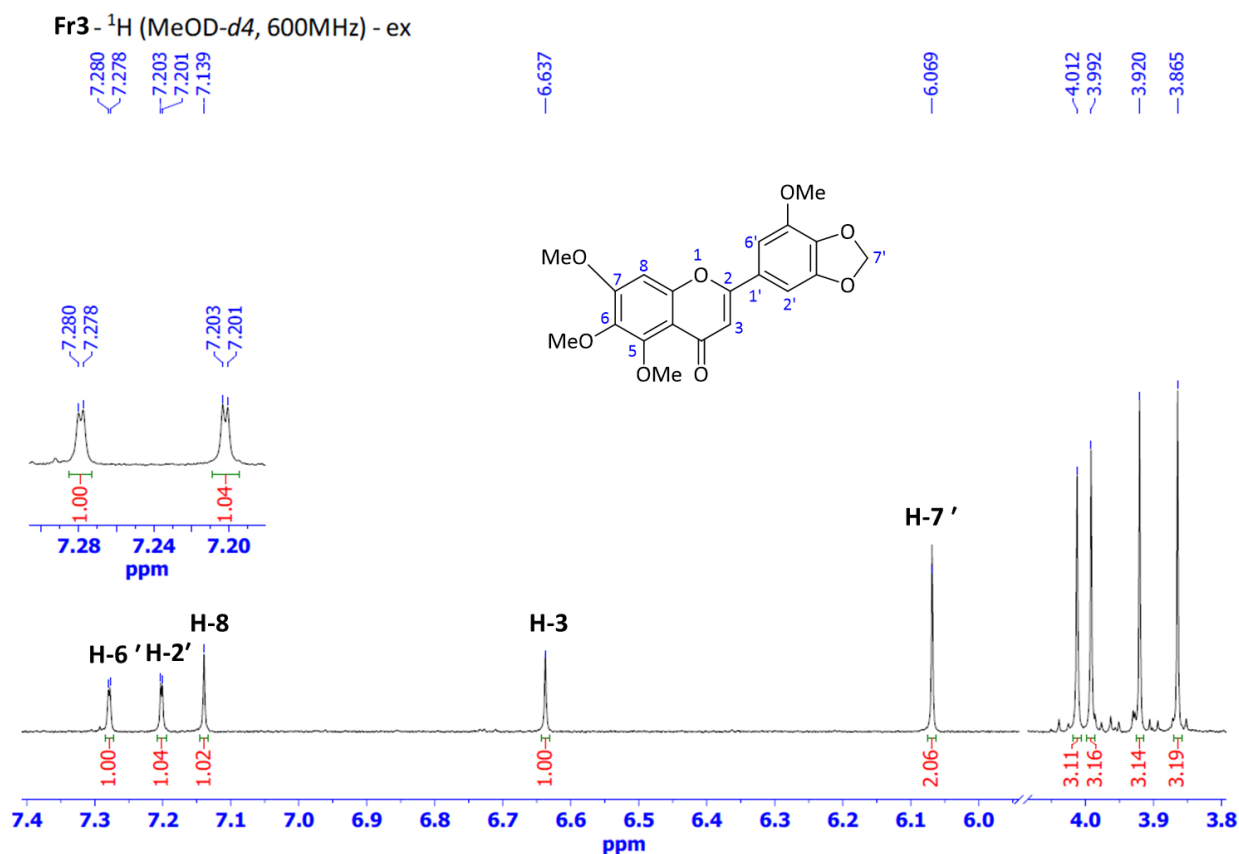


Figure S23: ^1H -NMR spectrum of compound 3 – expansion

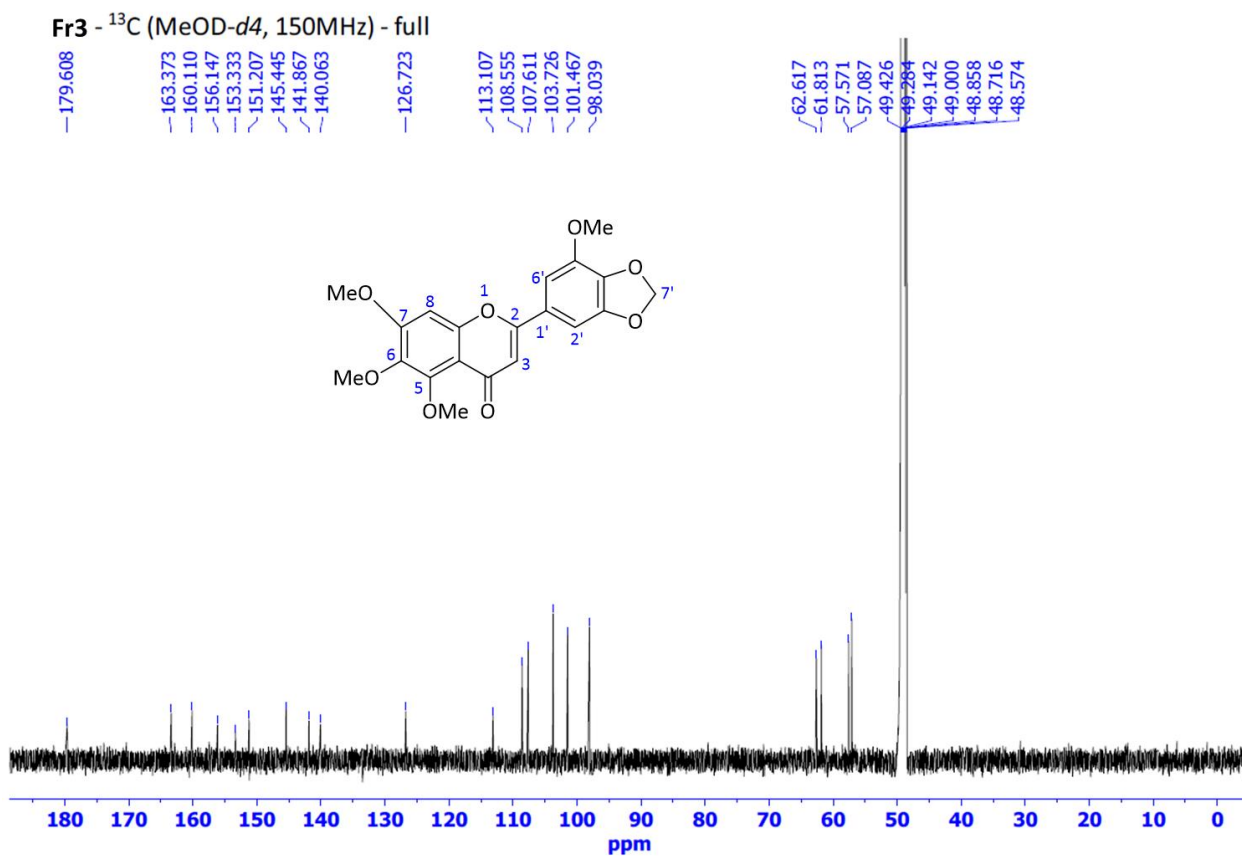


Figure S24: Complete assignment ^{13}C -NMR spectrum of compound 3

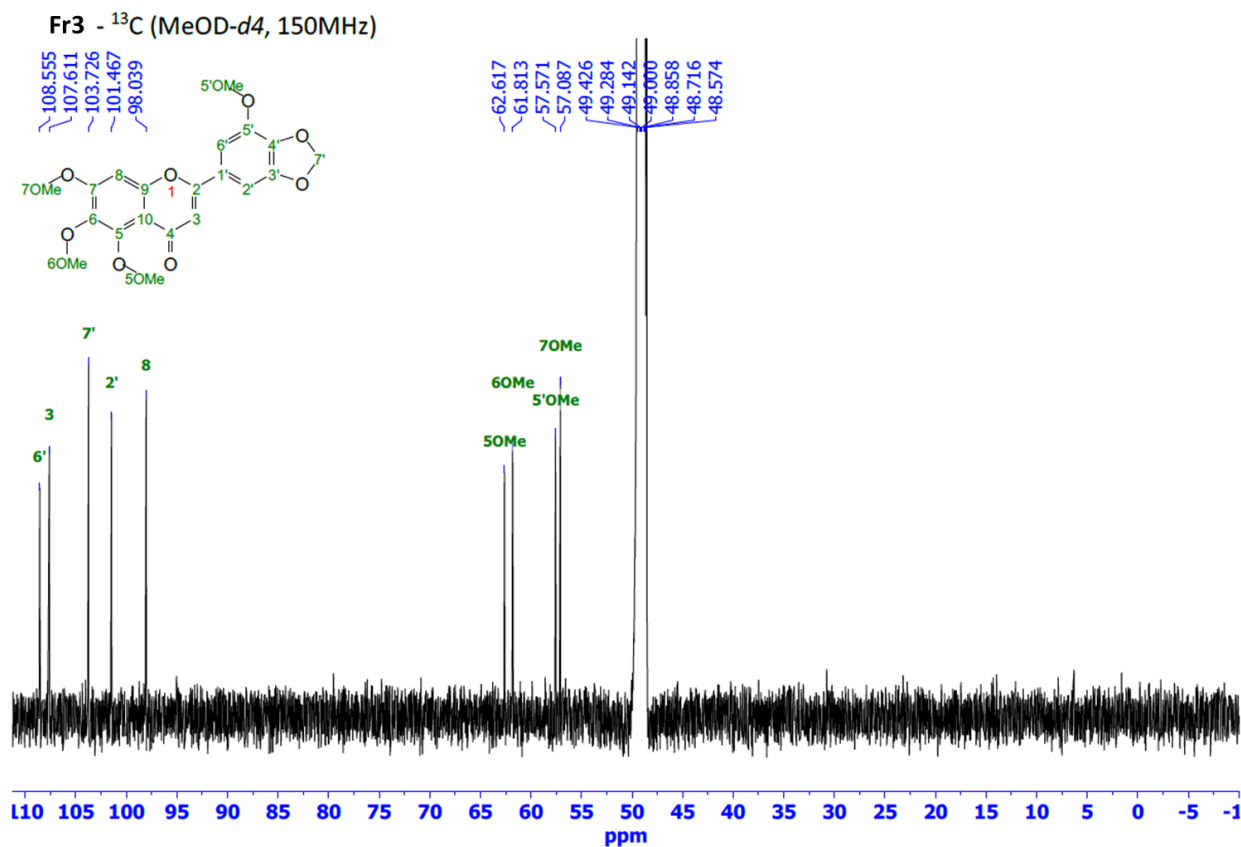


Figure S25: ^{13}C -NMR spectrum of compound 3 – expansion

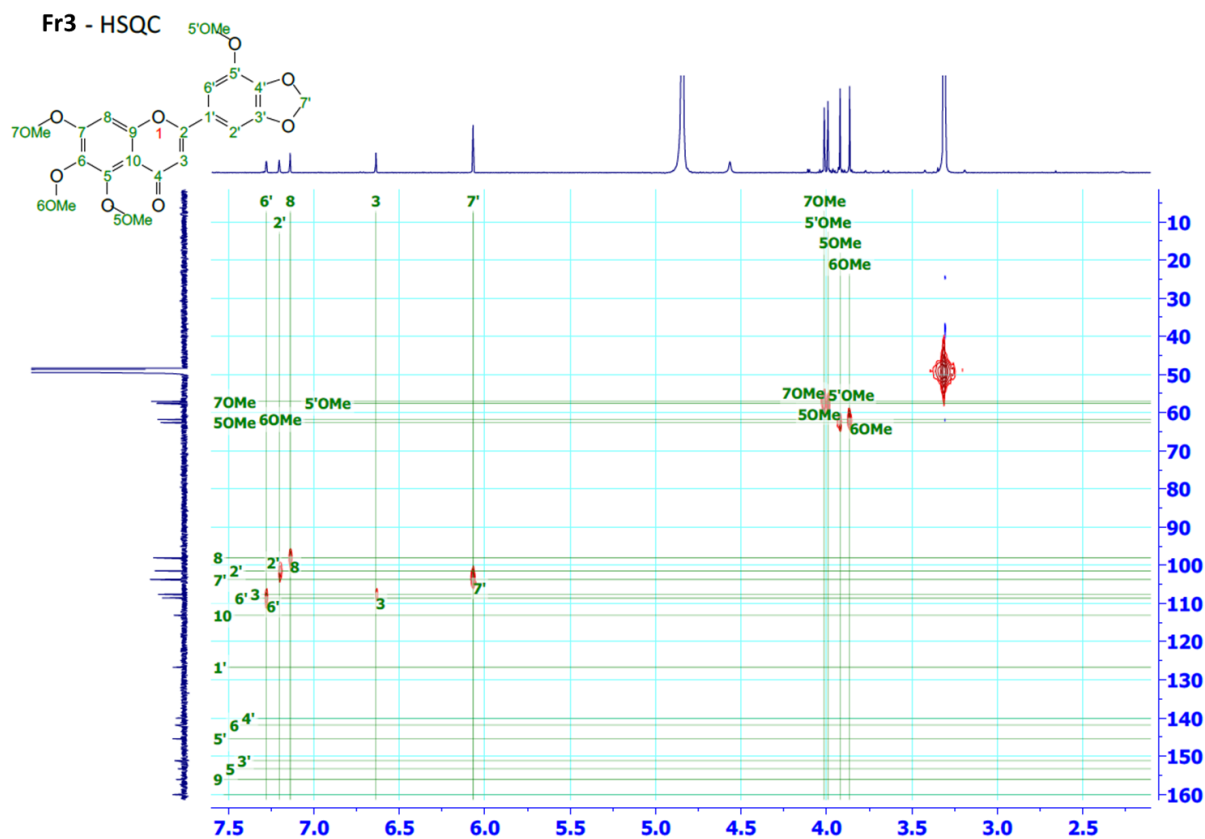


Figure S26: Complete assignment of correlation in 2D NMR-HSQC spectrum of compound 3

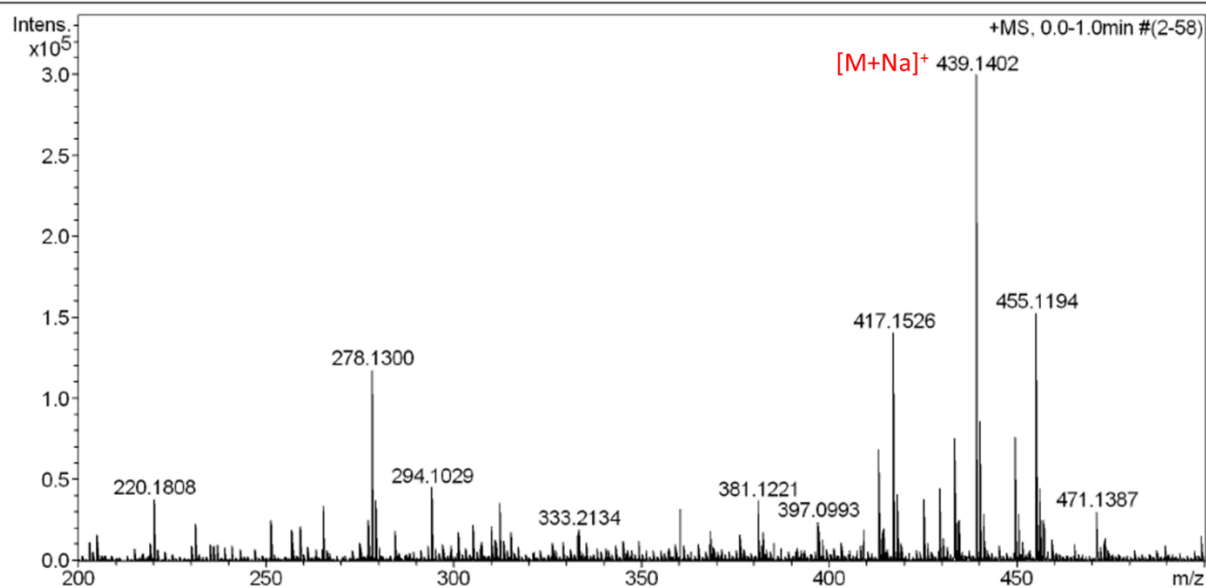
4. Supplementary spectroscopic data of compound 4.

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

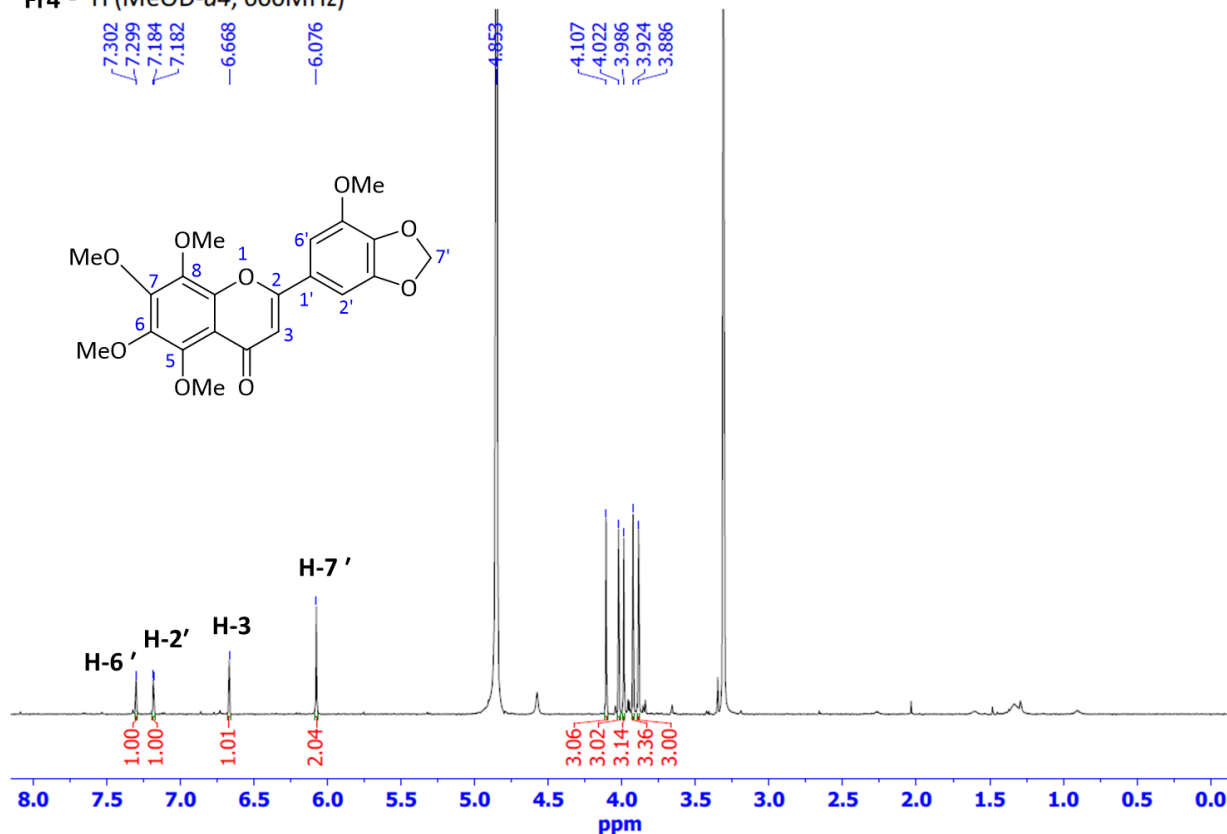
Position	Compound 4 (CD ₃ OD)				5,6,7,8,3'-pentamethoxy-4',5'-methylenedioxyflavone (CDCl ₃) [22]	
	¹³ C-NMR (150 MHz) δ _C ppm	¹ H-NMR (600 MHz) δ _H ppm	HSQC (¹ H → ¹³ C)	HMBC (¹ H → ¹³ C)	¹³ C-NMR (125 MHz) δ _C ppm	¹ H-NMR (400 MHz) δ _H ppm
2	163.20			C-2', 3, 6'	160.76	
3	107.49	6.67 (H, s)	C-3	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, <i>d</i> , <i>J</i> = 1.2 Hz)	C-2'	C-2, 3', 4', 6'	100.51	7.06 (H, <i>d</i> , <i>J</i> = 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, <i>d</i> , <i>J</i> = 1.8 Hz)	C-6'	C-2, 2', 4', 5'	106.61	7.11 (H, <i>d</i> , <i>J</i> = 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 ₉ Na ⁺	439.1402	409.1107

Figure S28: (+)-HR-ESI-MS data of compound **4****Fr4** - ¹H (MeOD-*d*₄, 600MHz)**Figure S29:** Complete assignment ¹H-NMR spectrum of compound **4**

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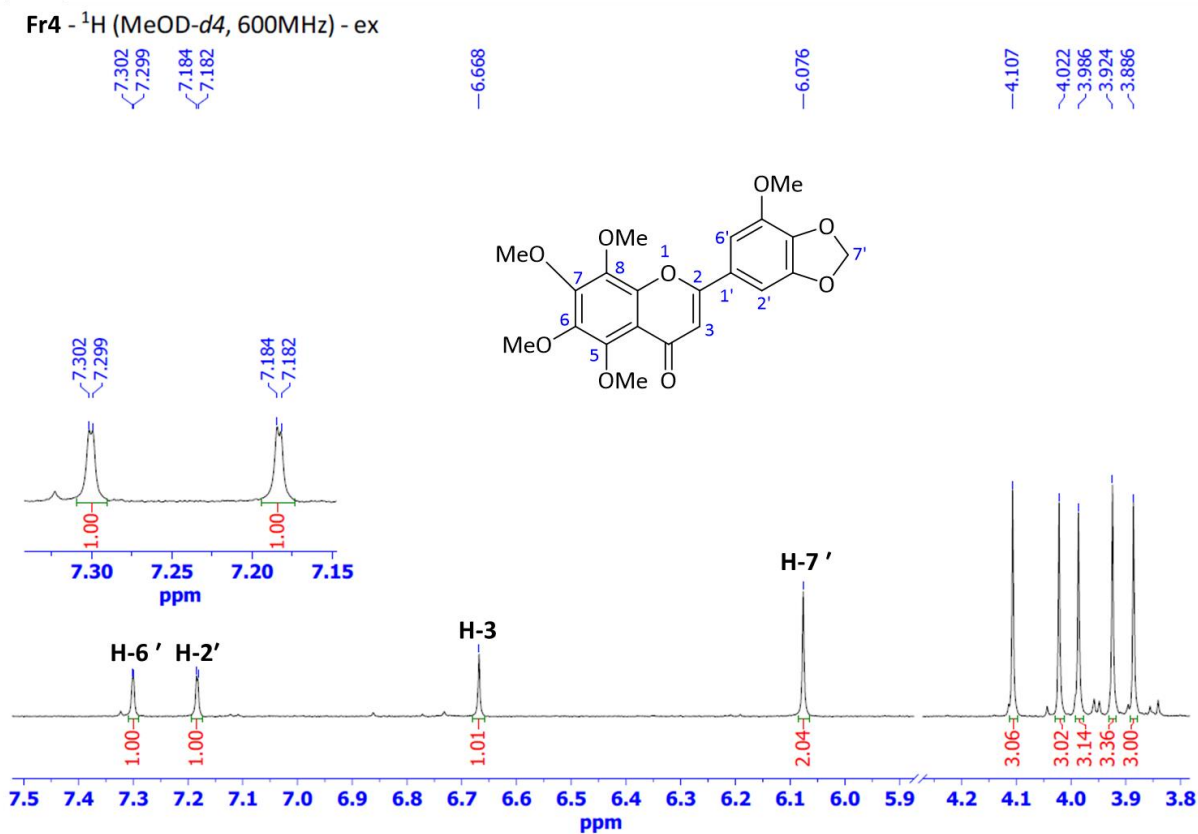


Figure S30: ^1H -NMR spectrum of compound 4 – expansion

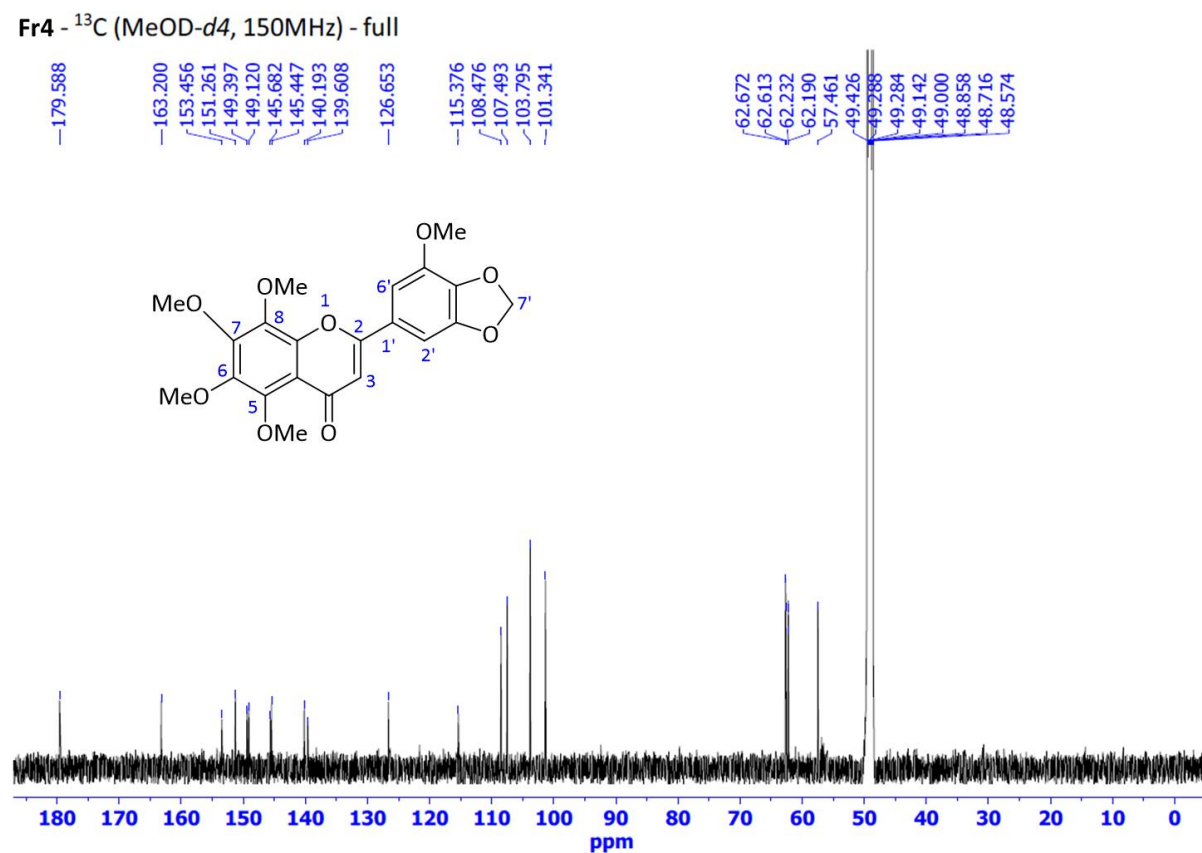


Figure S31: Complete assignment ^{13}C -NMR spectrum of compound 4

Fr4 - ^{13}C (MeOD- d_4 , 150MHz) - full

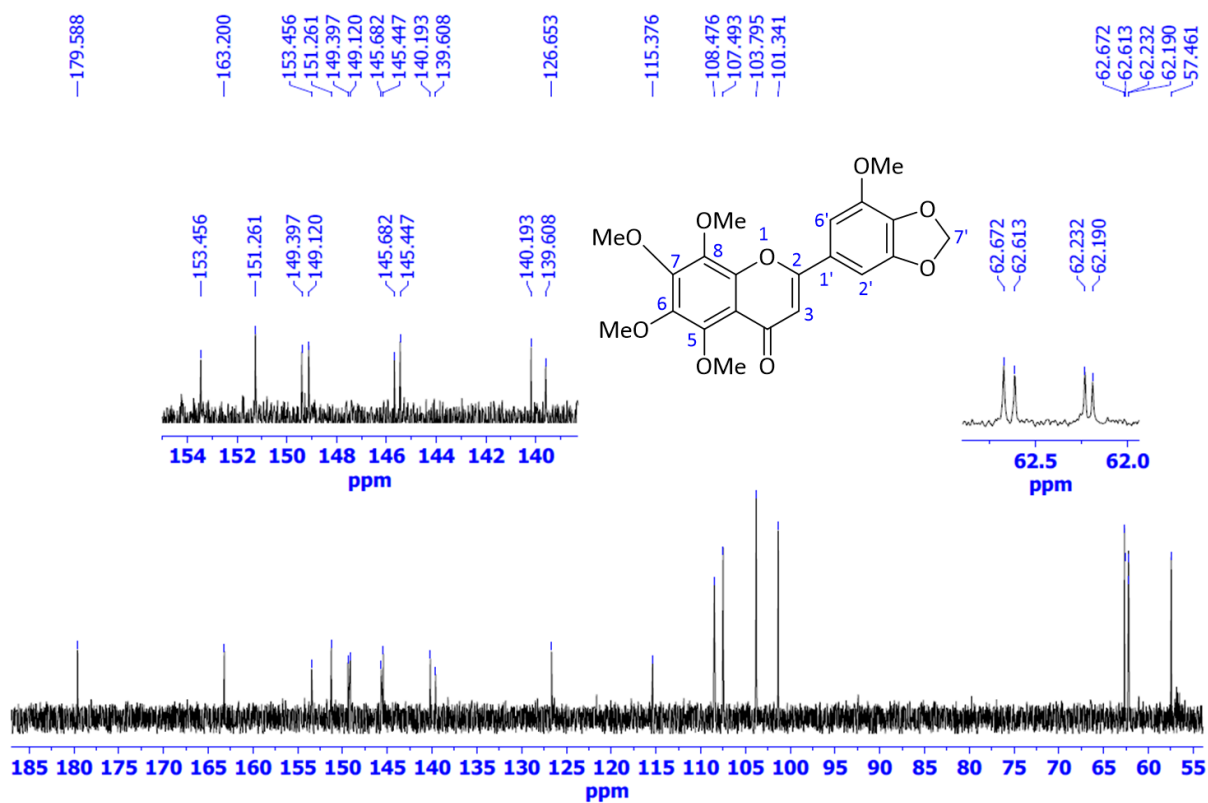


Figure S32: ^{13}C -NMR spectrum of compound 4 – expansion

Fr4 - HSQC

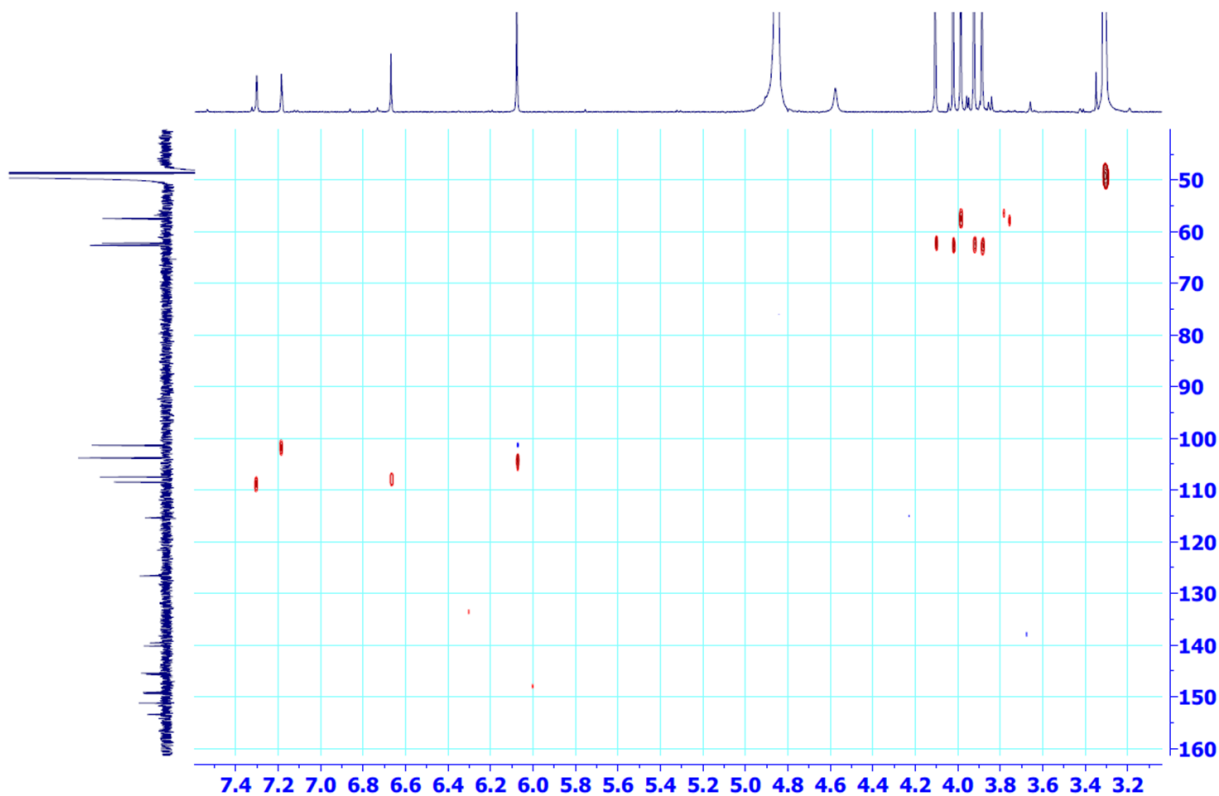


Figure S33: Complete assignment of correlation in 2D NMR-HSQC spectrum of compound 4

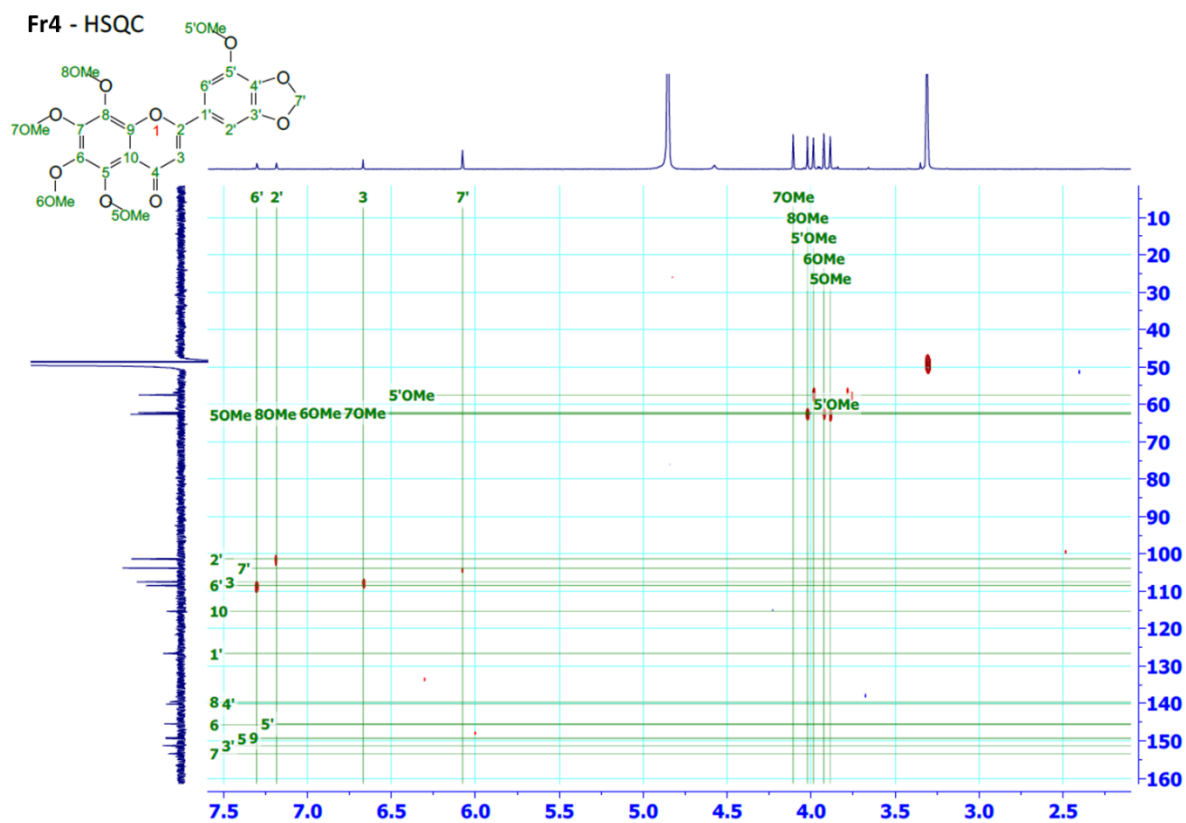


Figure S34: Correlation in 2D NMR-HSQC spectrum of compound **4**

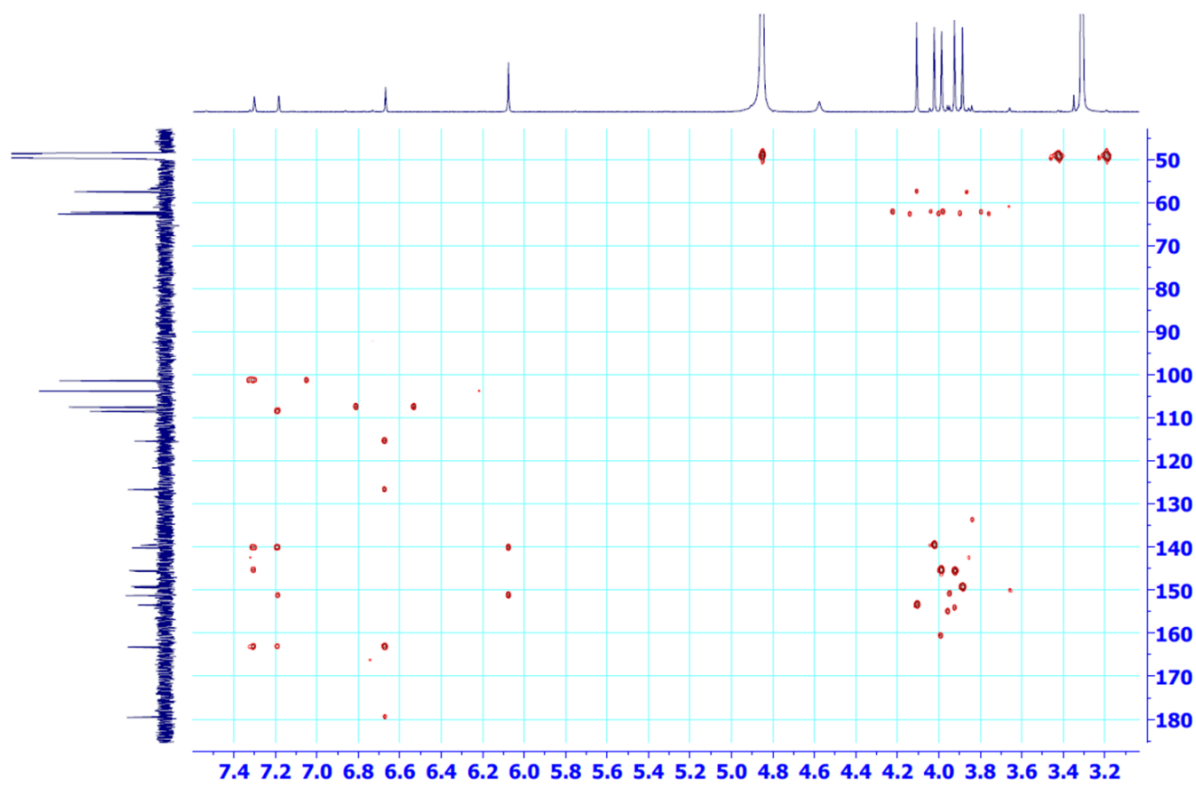
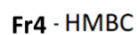


Figure S35: Complete assignment of correlation in 2D NMR-HMBC spectrum of compound **4**

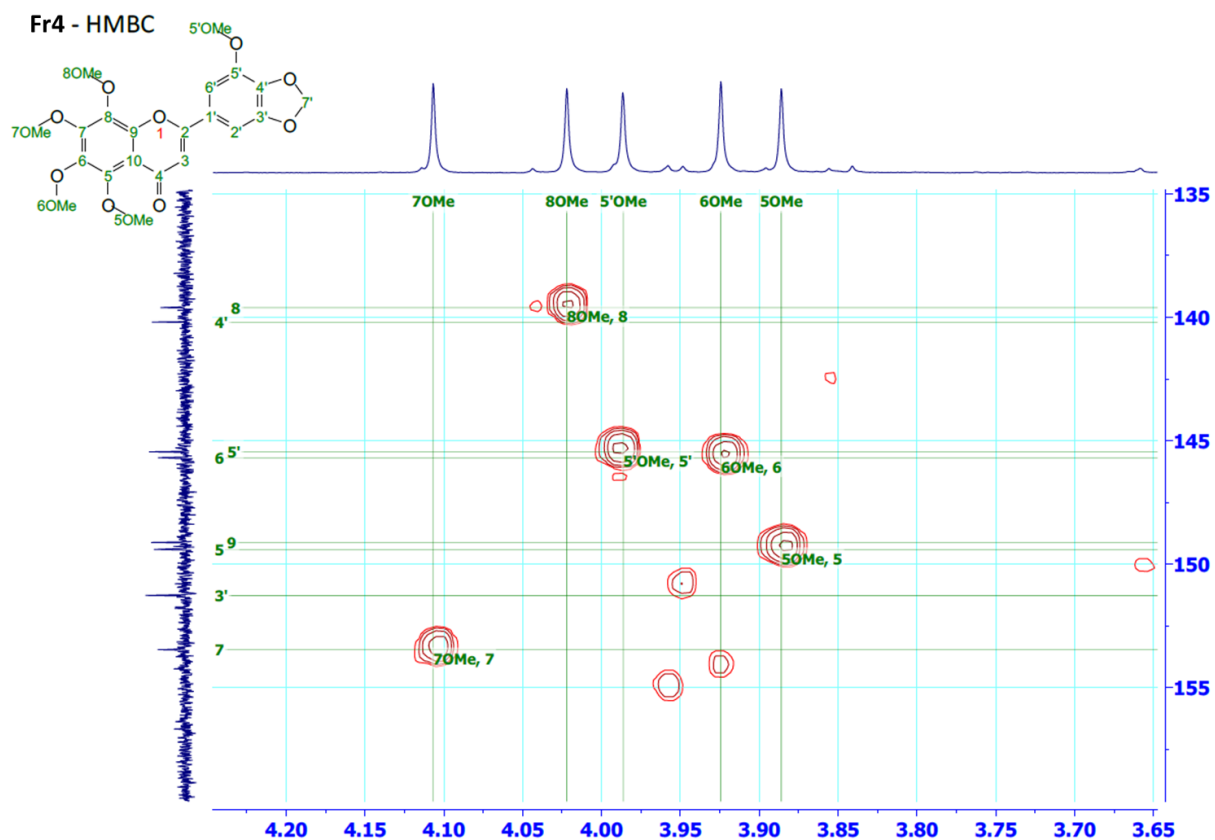


Figure S36: Correlation in 2D NMR-HMBC spectrum of compound 4