

Supplementary material

New Phenolic Acids from *Elephantopus scaber* Linn. and their Anti- inflammatory Activity

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ABSTRACT

Two new phenolic acids, ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**), 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**), together with three known compounds (**3–5**) were isolated from the whole plant of *Elephantopus scaber* Linn.. The structures of the new compounds were elucidated using detailed spectroscopic analysis. Compound **3** was obtained and given its NMR data for the first time. All isolates were evaluated for their anti-inflammatory activity *via* inhibiting the production of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated murine macrophage RAW 264.7 cells, and **1**, **4** and **5** showed a moderate inhibition with IC₅₀ values ranging from 11.85 to 20.62 μ M.

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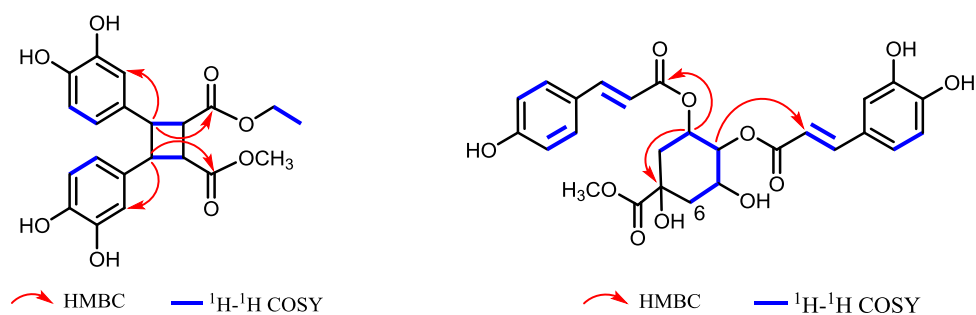


Figure S1. Key ^1H - ^1H COSY and HMBC correlations of compounds **1** and **2**.

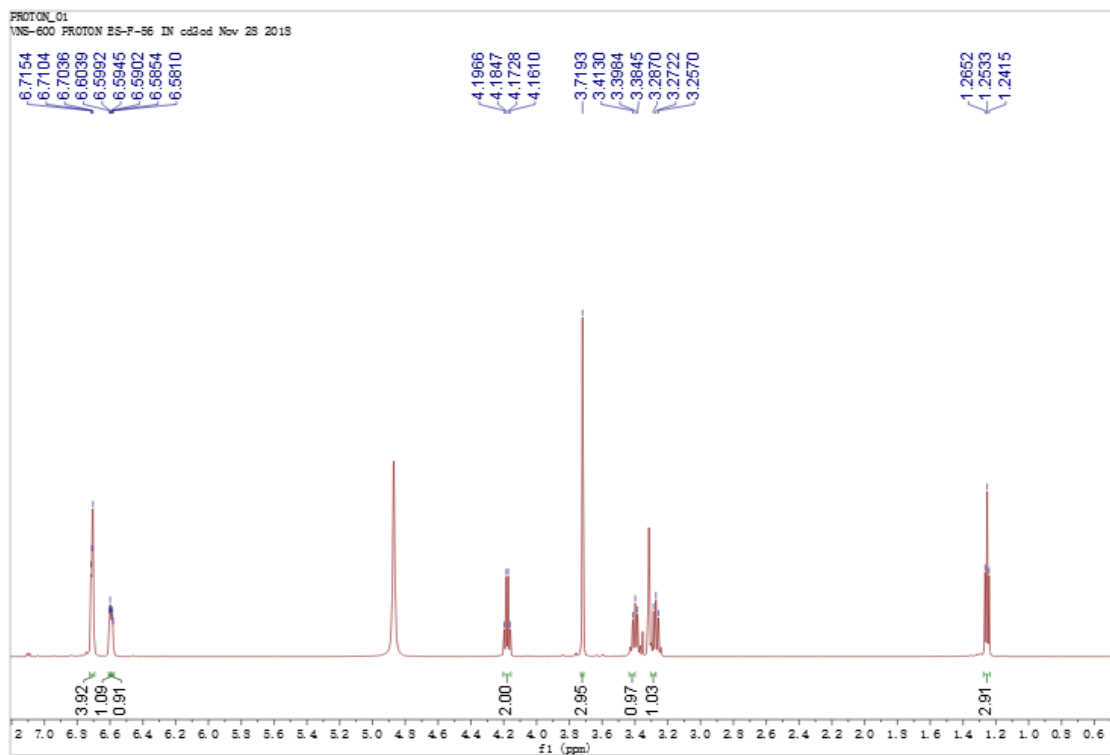


Figure S2. ^1H NMR spectrum (600 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD_3OD .

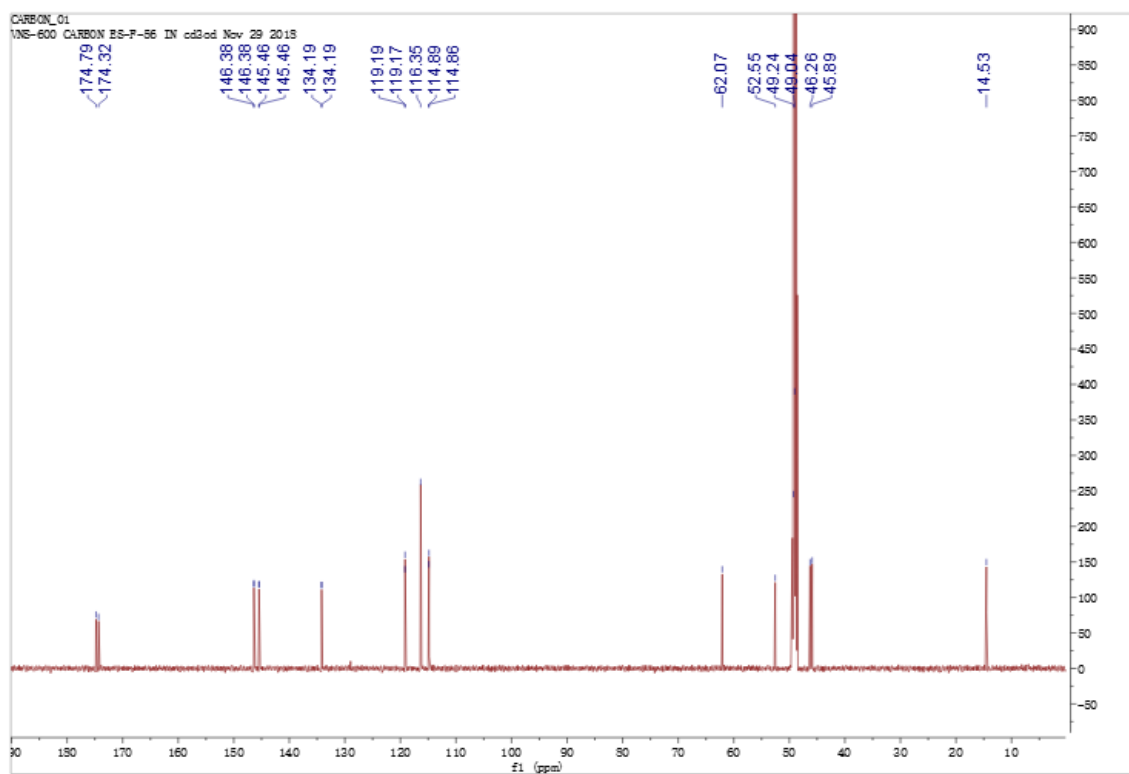


Figure S3. ^{13}C NMR spectrum (150 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD_3OD .

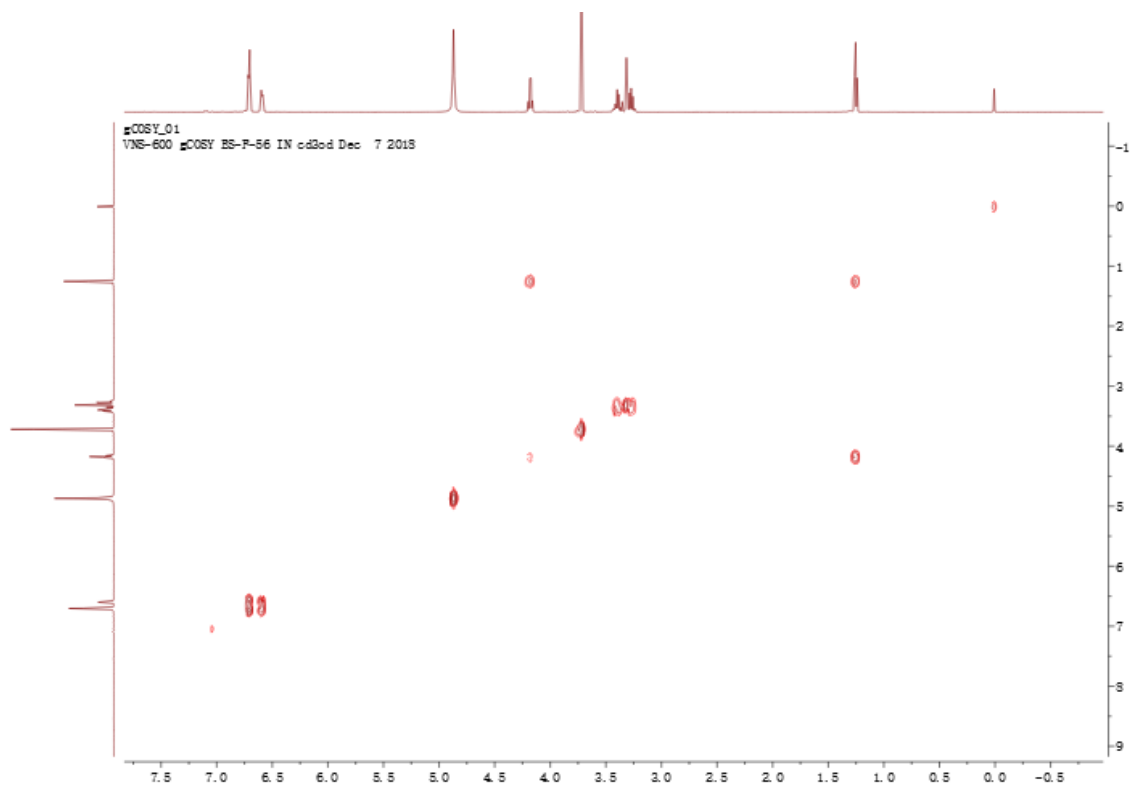


Figure S4. ^1H - ^1H COSY spectrum (600 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD_3OD .

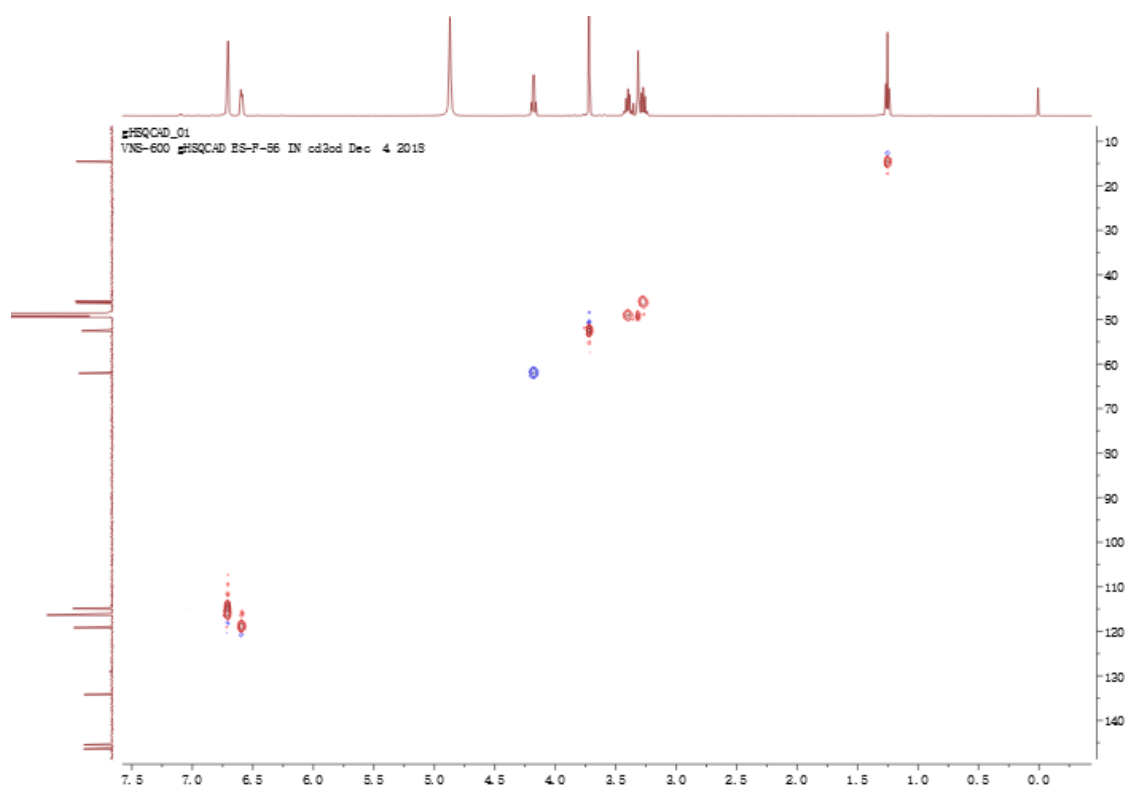


Figure S5. HSQC spectrum (600 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD₃OD.

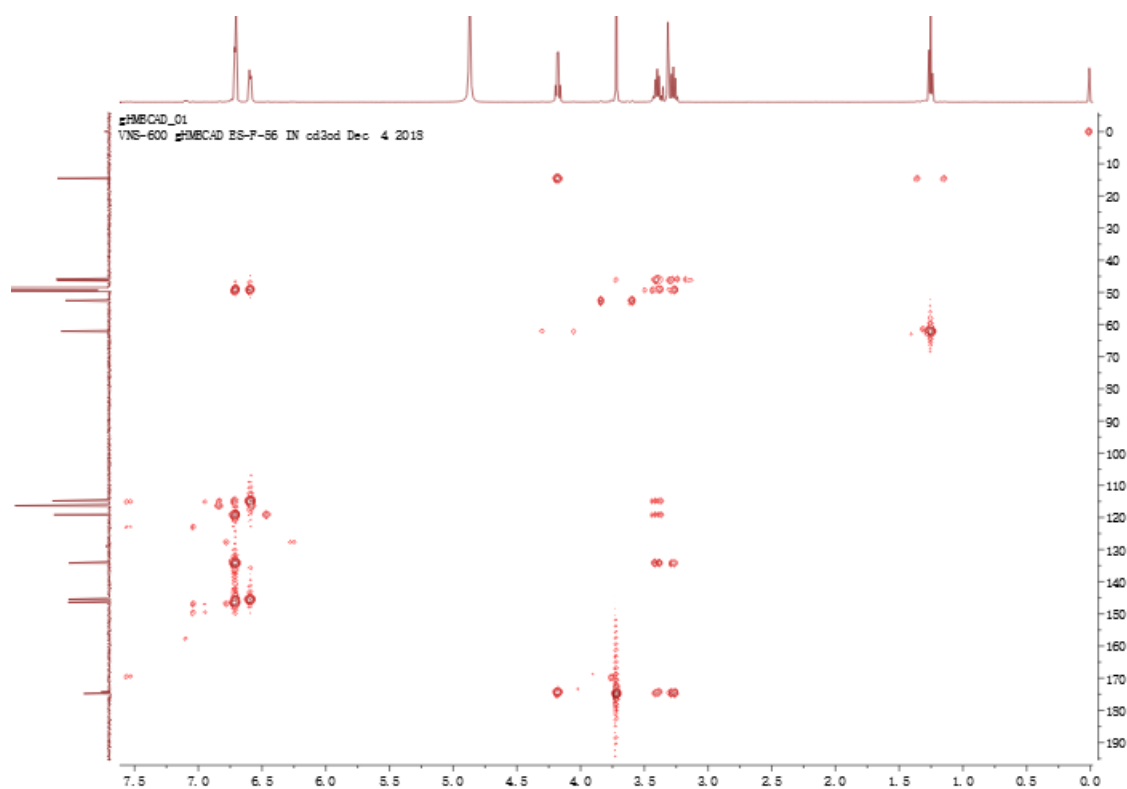


Figure S6. HMBC spectrum (600 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD₃OD.

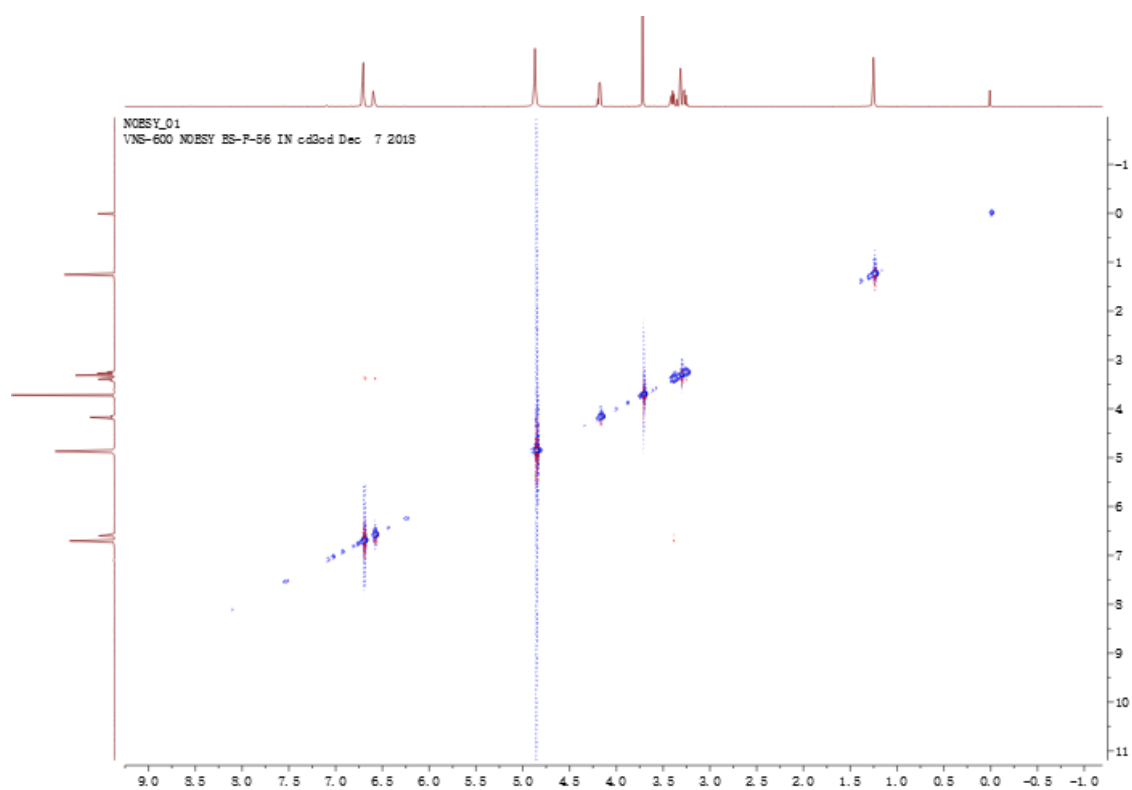


Figure S7. NOESY spectrum (600 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD₃OD.

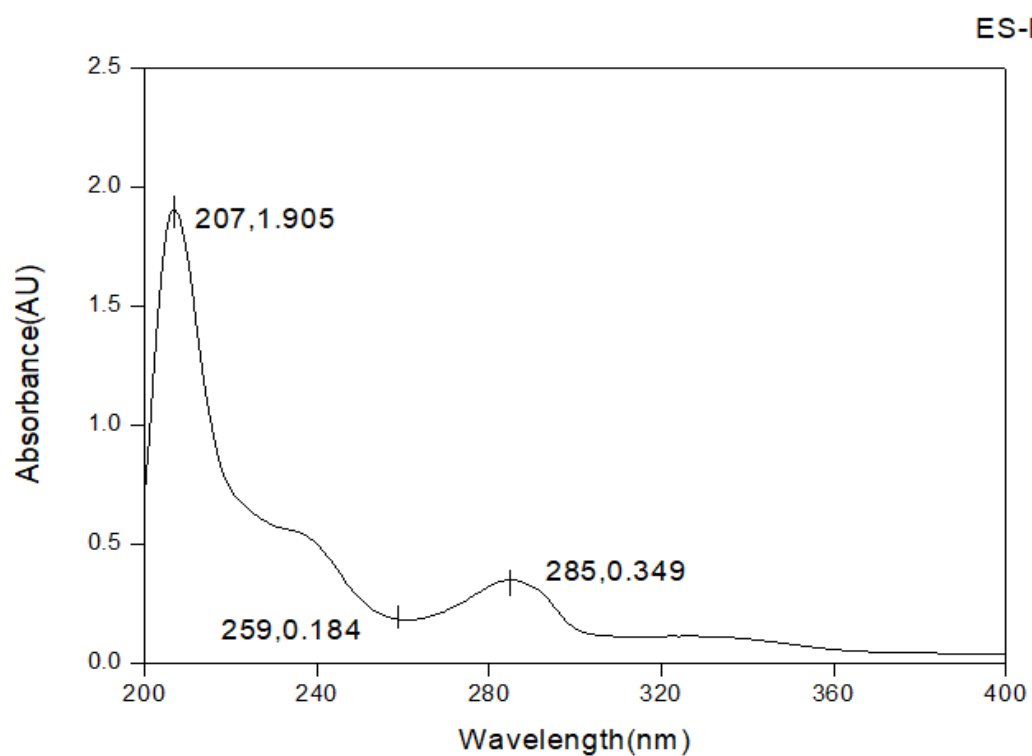


Figure S8. UV spectrum of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**).

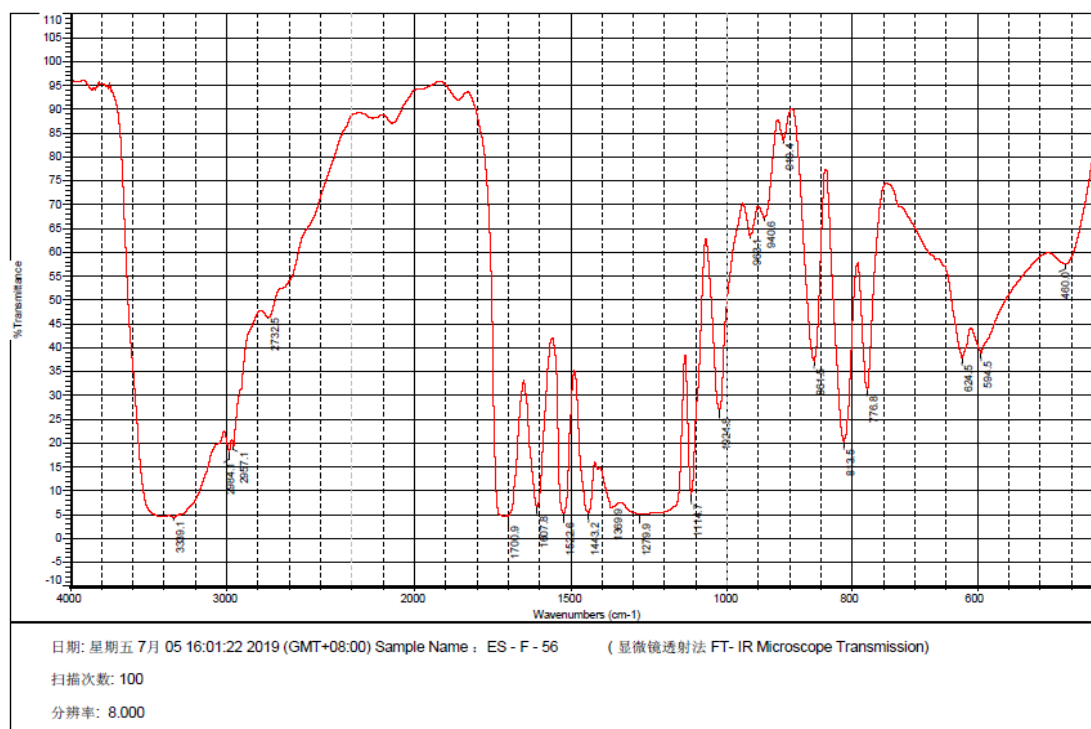


Figure S9. IR spectrum of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**).

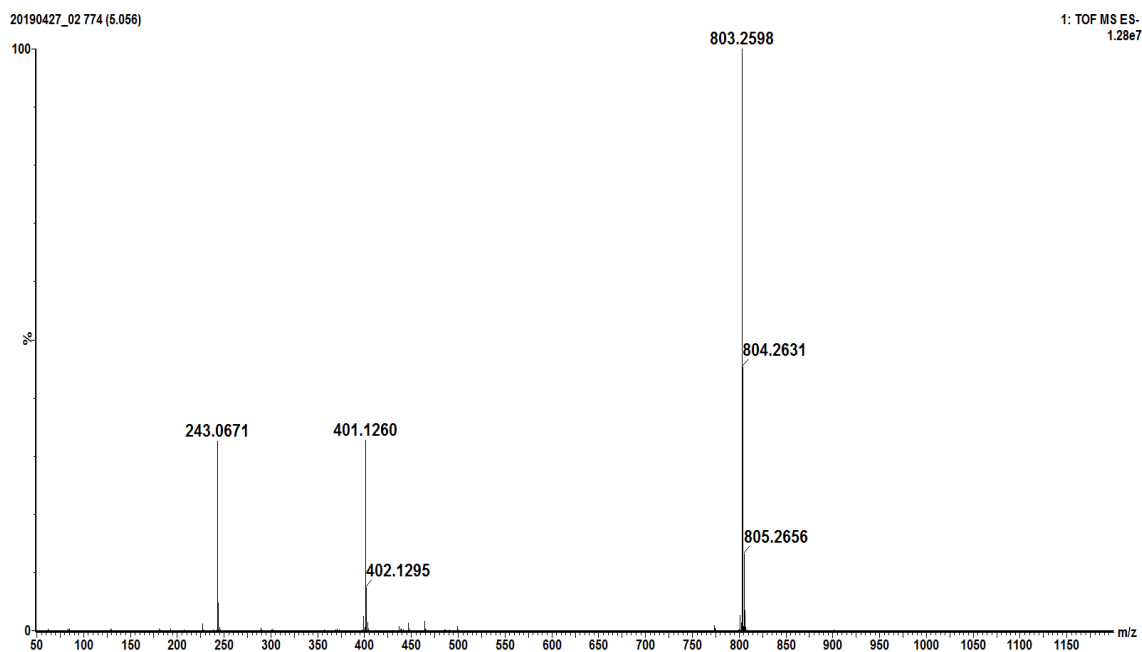


Figure S10. HRESIMS spectrum of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**).

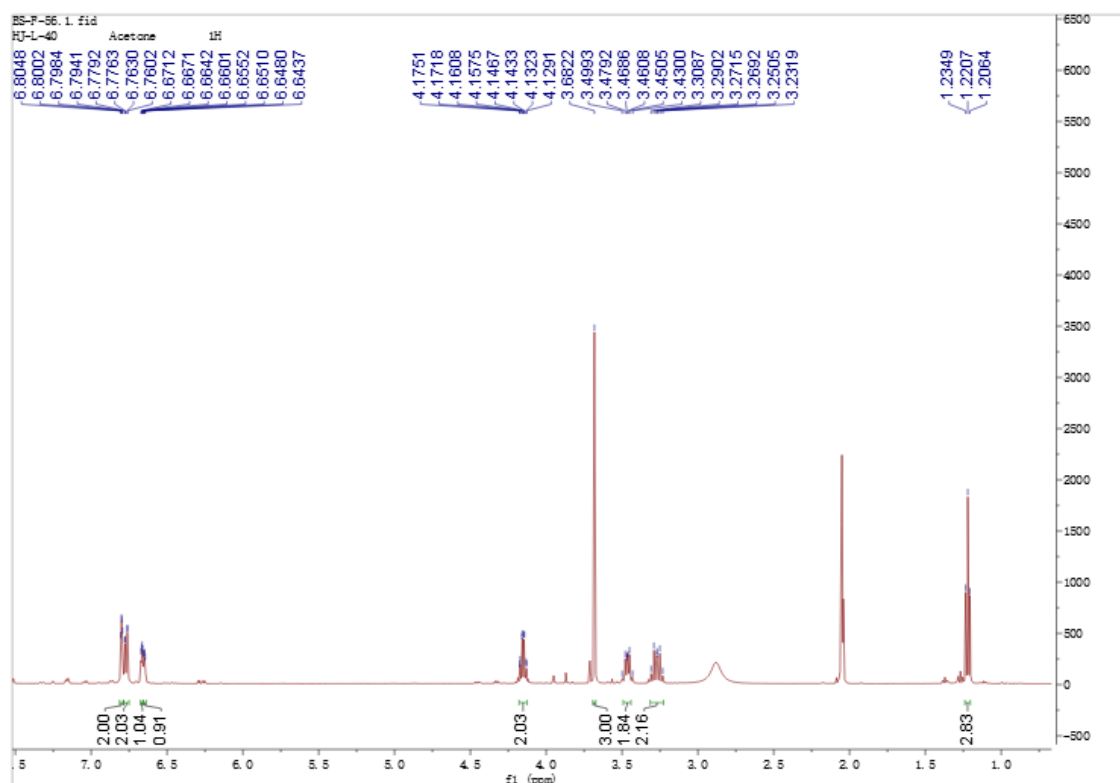


Figure S11. ^1H NMR spectrum (500 MHz) of ethyl 3,3',4,4'-tetrahydroxy- δ -truxinate (**1**) in CD_3COCD_3

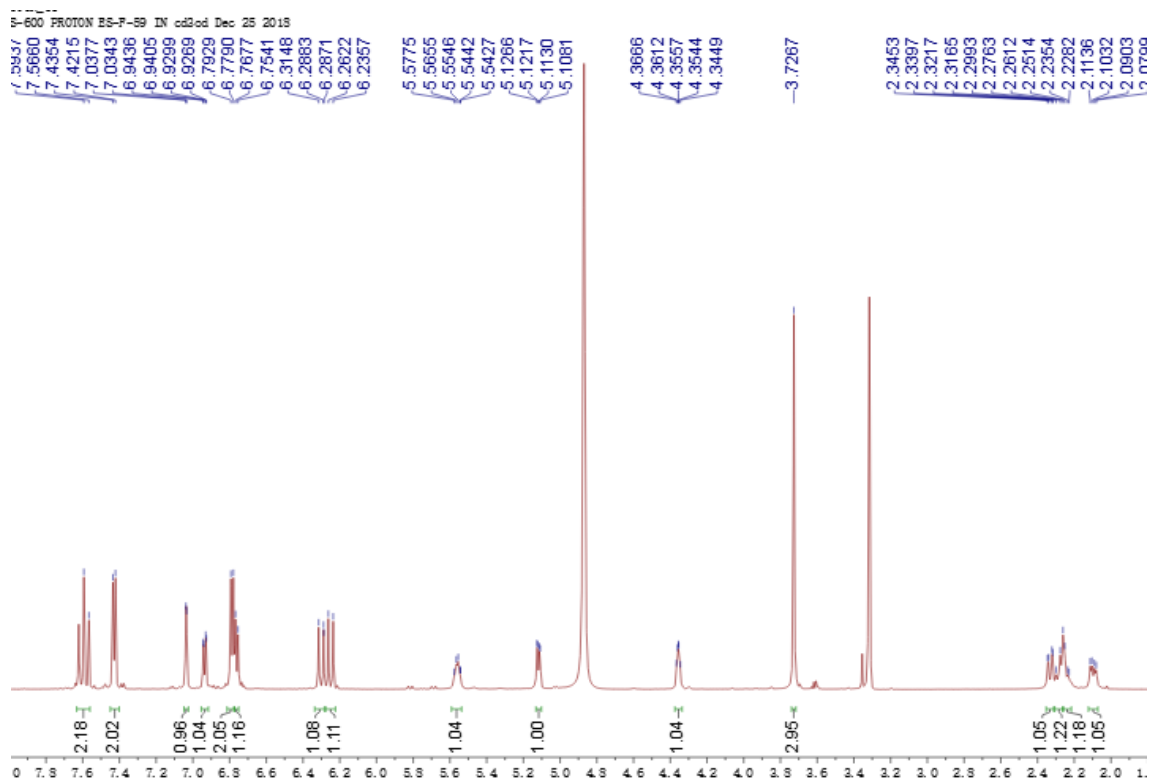


Figure S12. ^1H NMR spectrum (600 MHz) of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**) in CD_3OD .

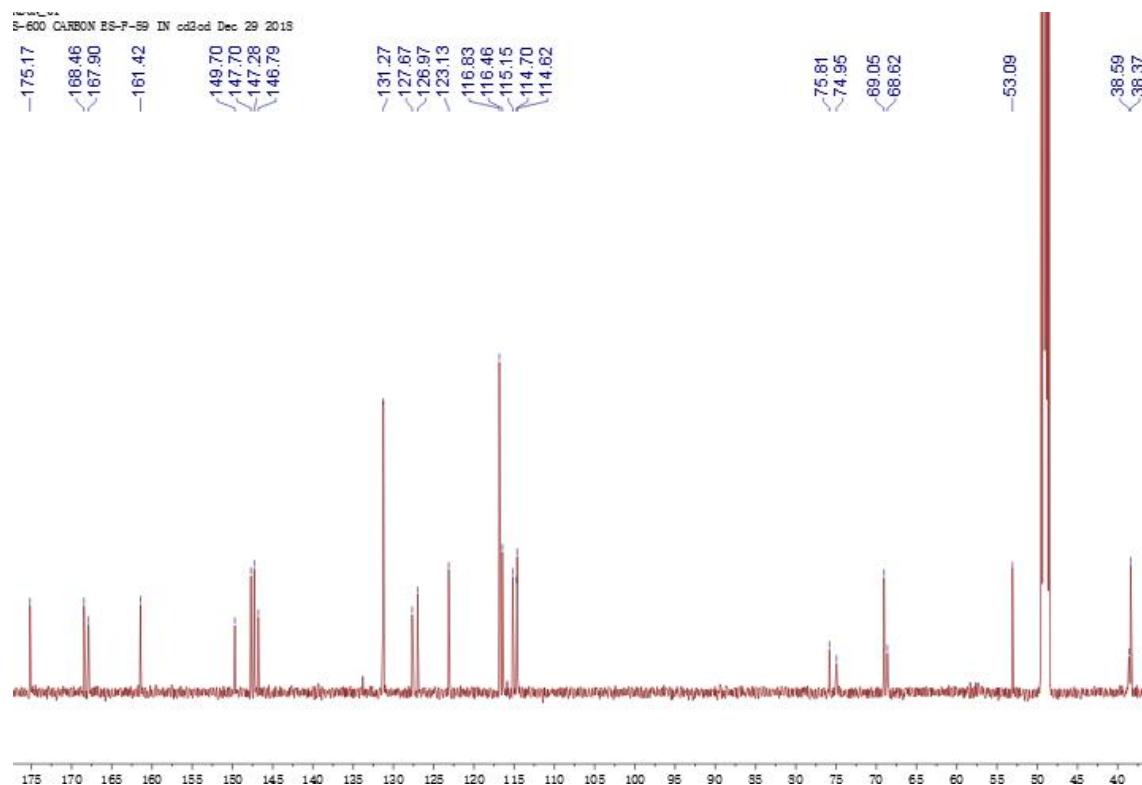


Figure S13. ^{13}C NMR spectrum (150 MHz) of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**) in CD_3OD .

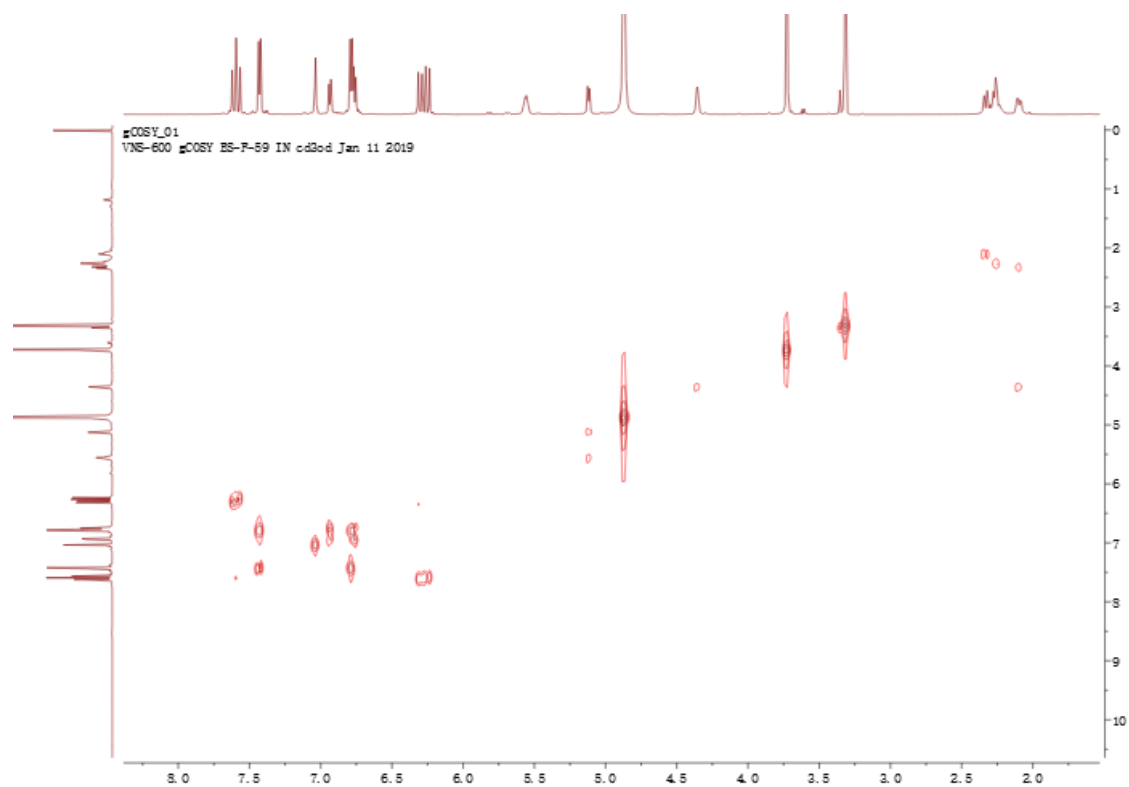


Figure S14. ^1H - ^1H COSY spectrum (600 MHz) of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**) in CD_3OD .

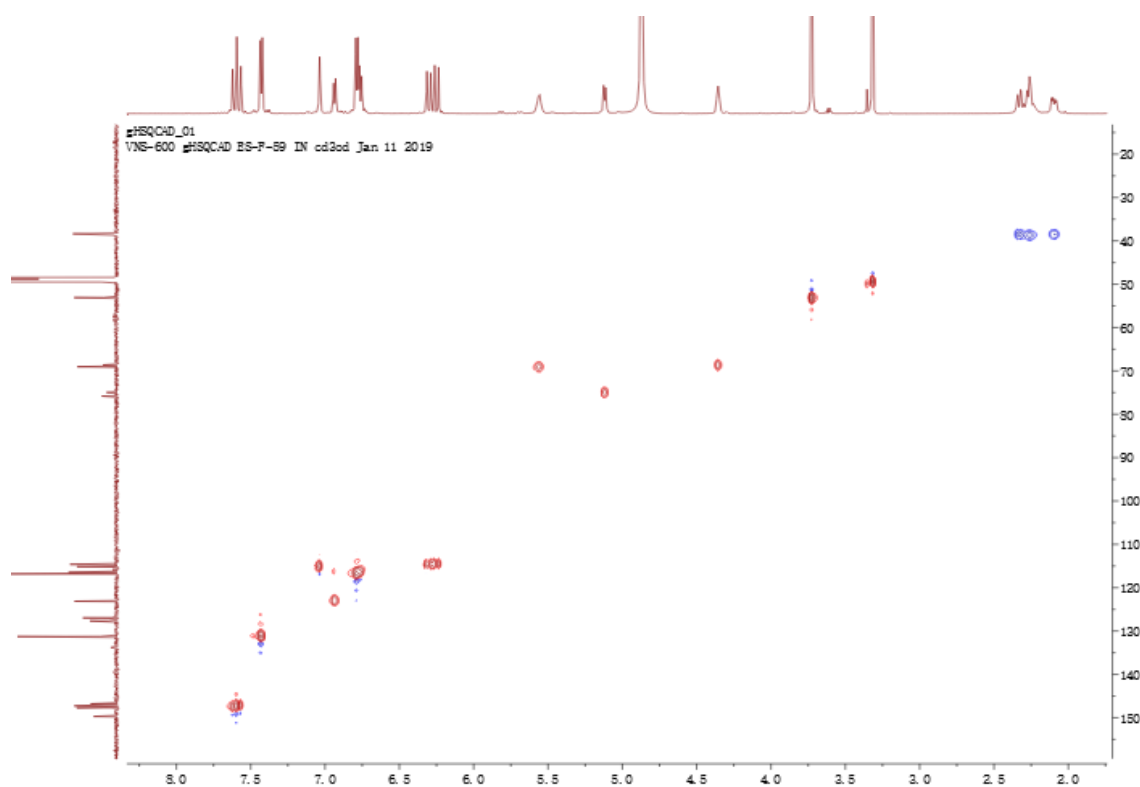


Figure S15. HSQC spectrum (600 MHz) of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**) in CD₃OD.

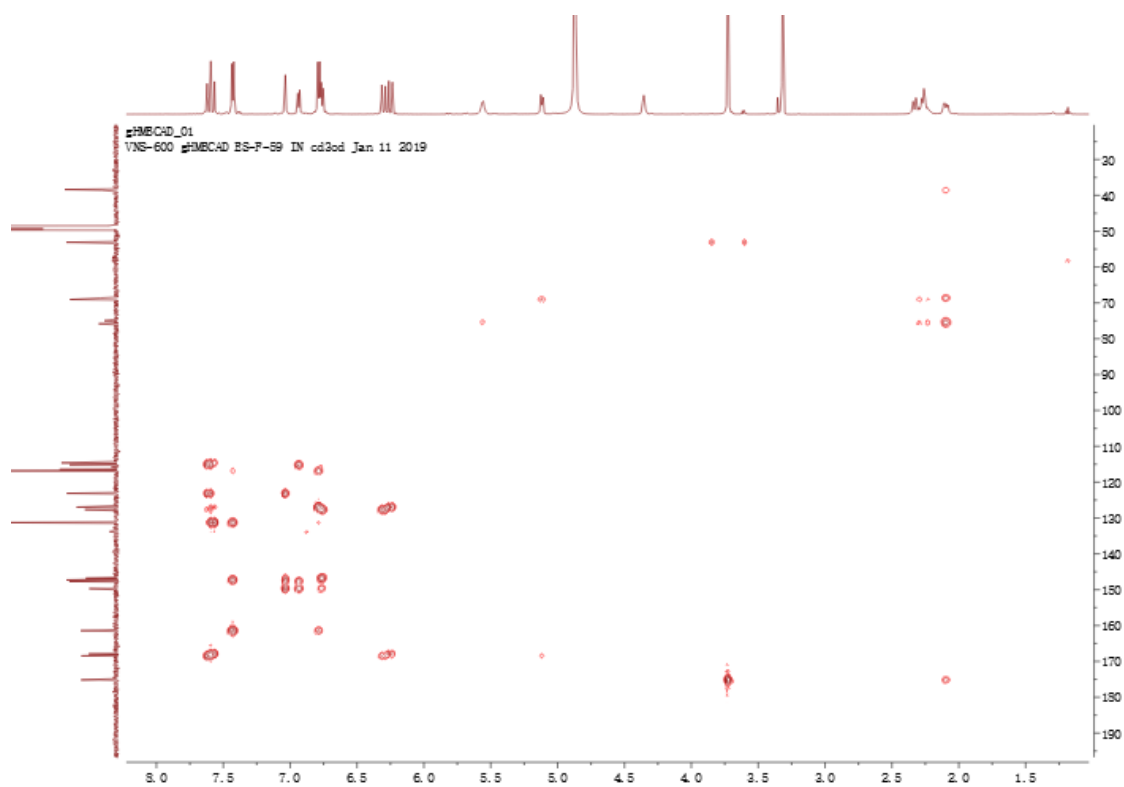


Figure S16. HMBC spectrum (600 MHz) of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**) in CD₃OD.

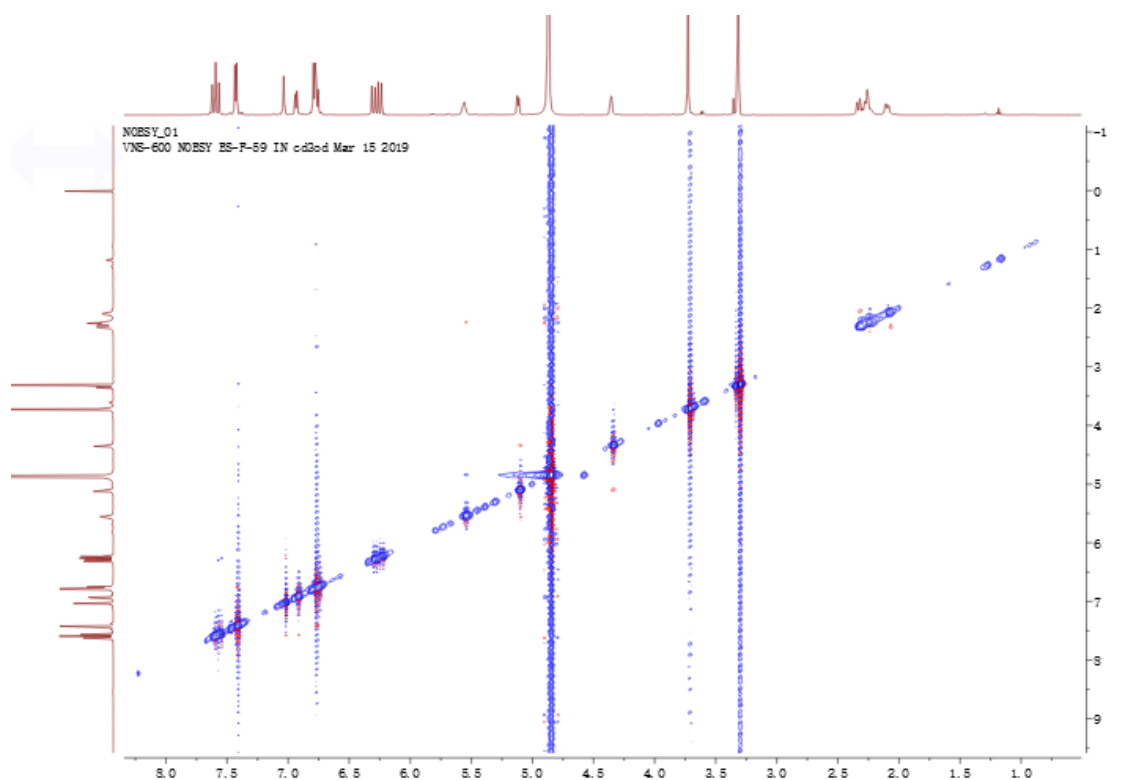


Figure S17. NOESY spectrum (600 MHz) of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**) in CD₃OD.

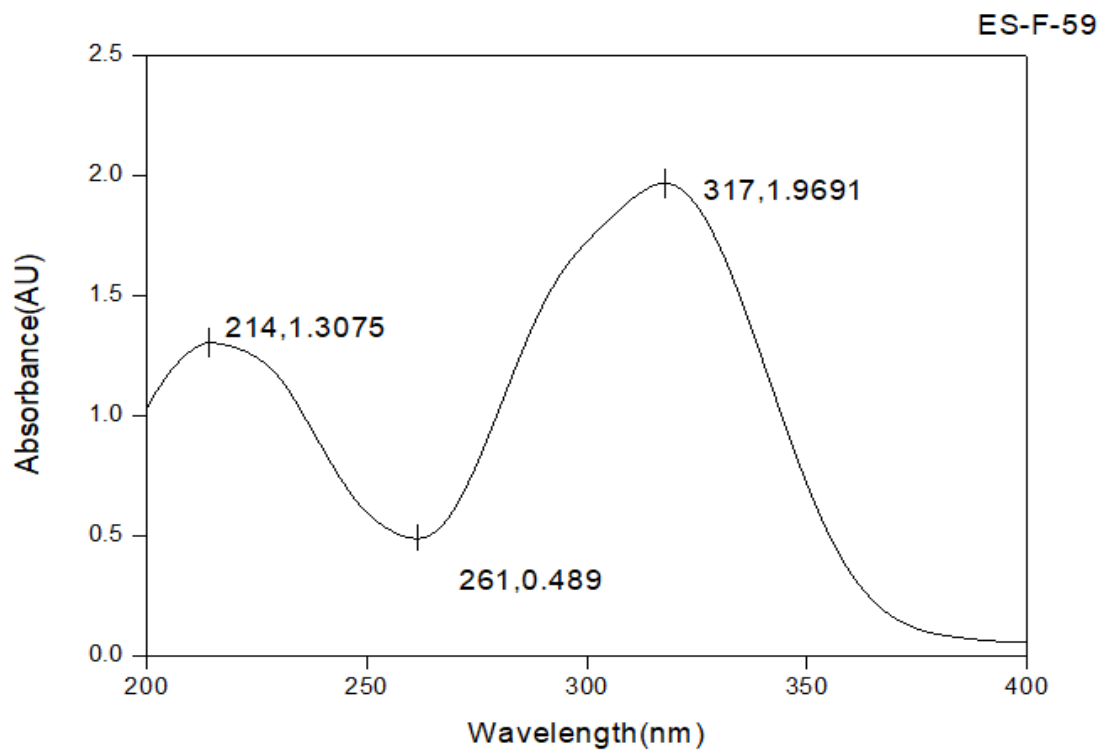


Figure S18. UV spectrum of 3-*O*-*p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**).

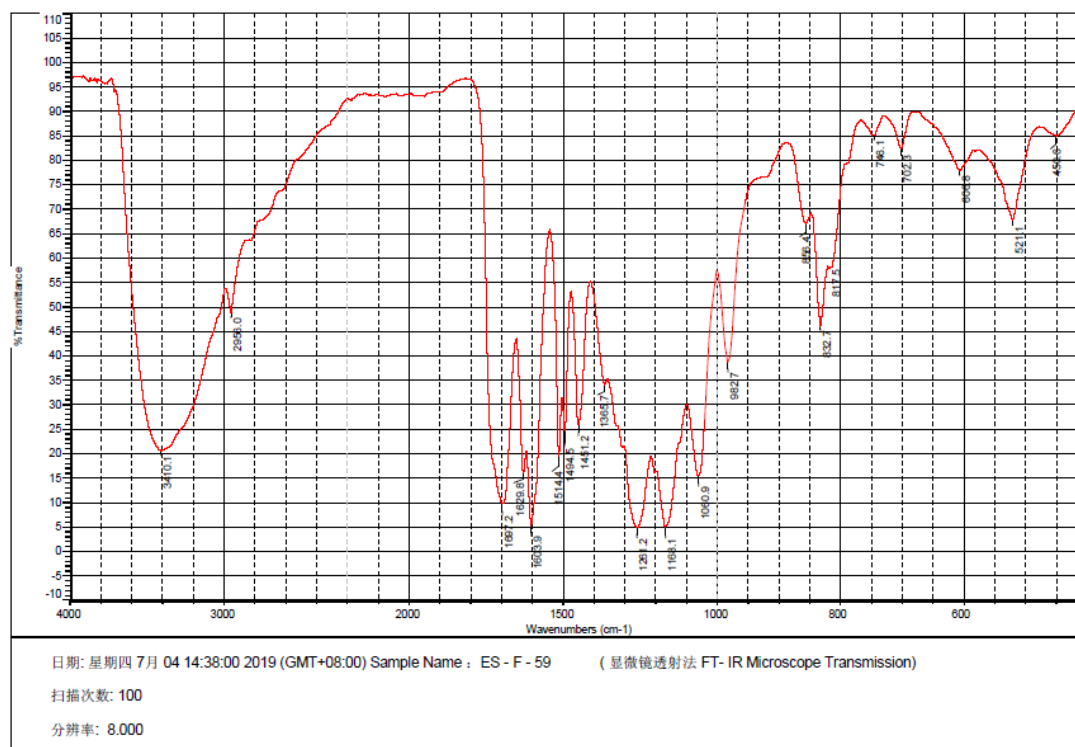


Figure S19. IR spectrum of 3-*O-p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**).

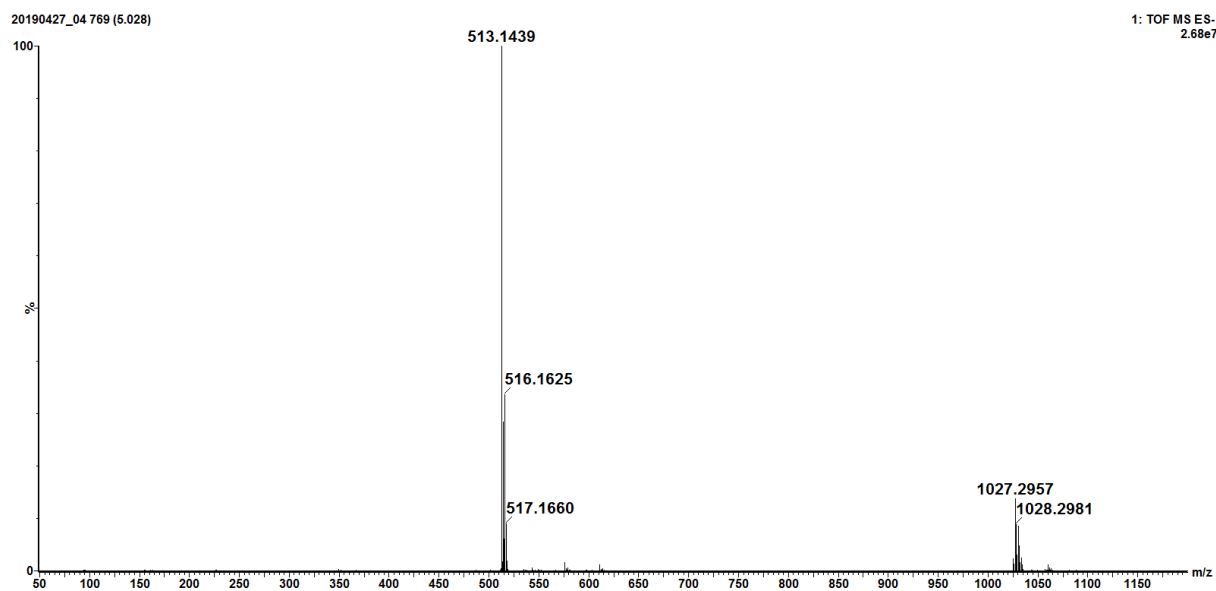


Figure S20. HRESIMS spectrum of 3-*O-p*-coumaroyl-4-*O*-caffeoyl quinic acid methyl ester (**2**).

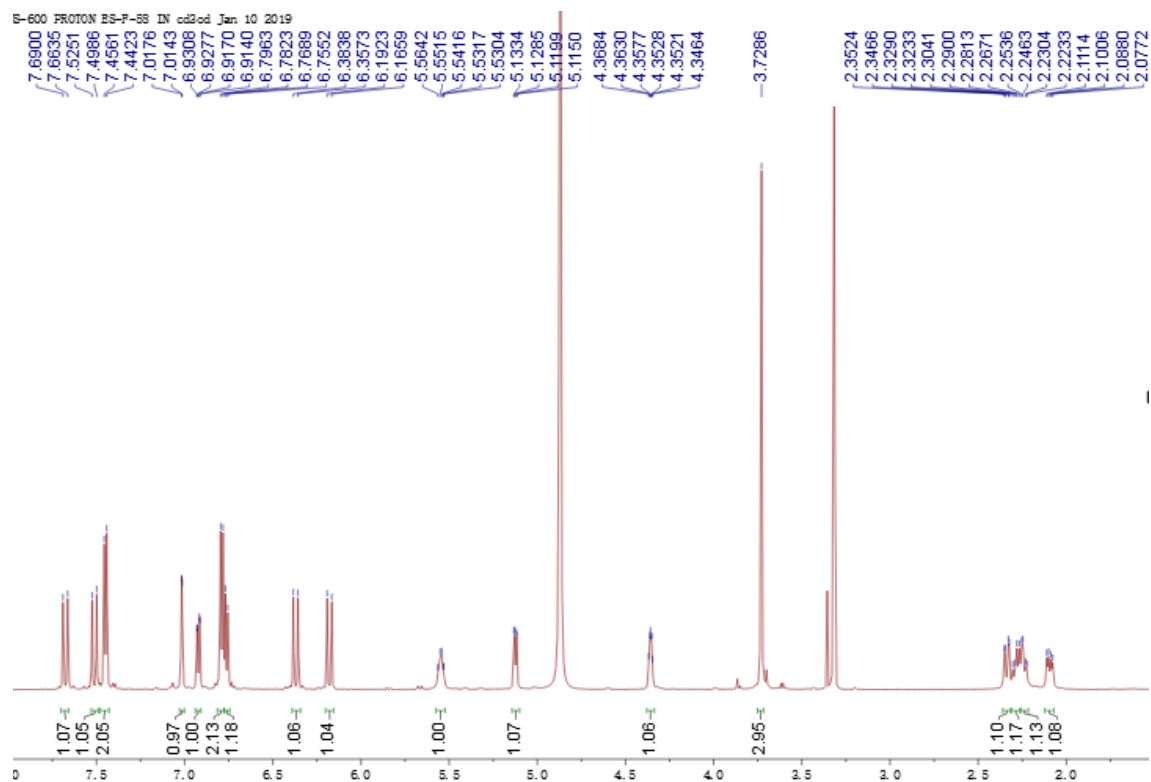


Figure S21. ^1H NMR spectrum (600 MHz) of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**) in CD_3OD .

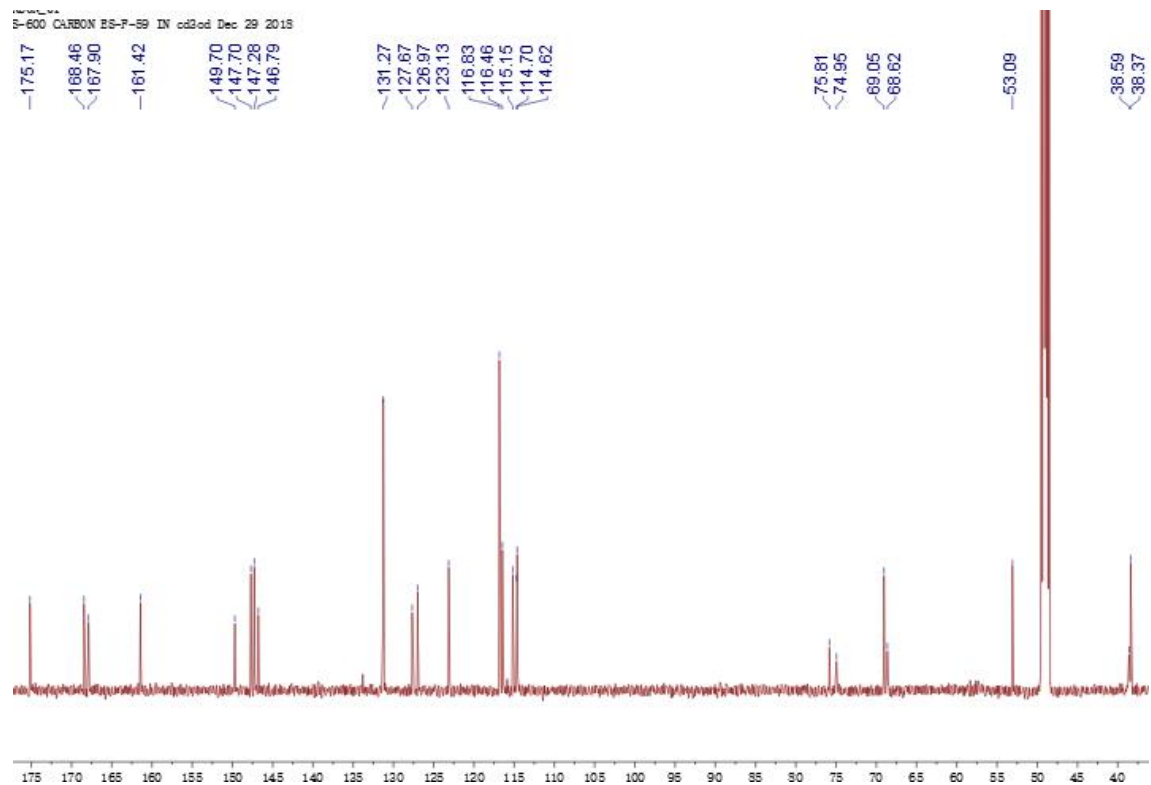


Figure S22. ^{13}C NMR spectrum (150 MHz) of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**) in CD_3OD .

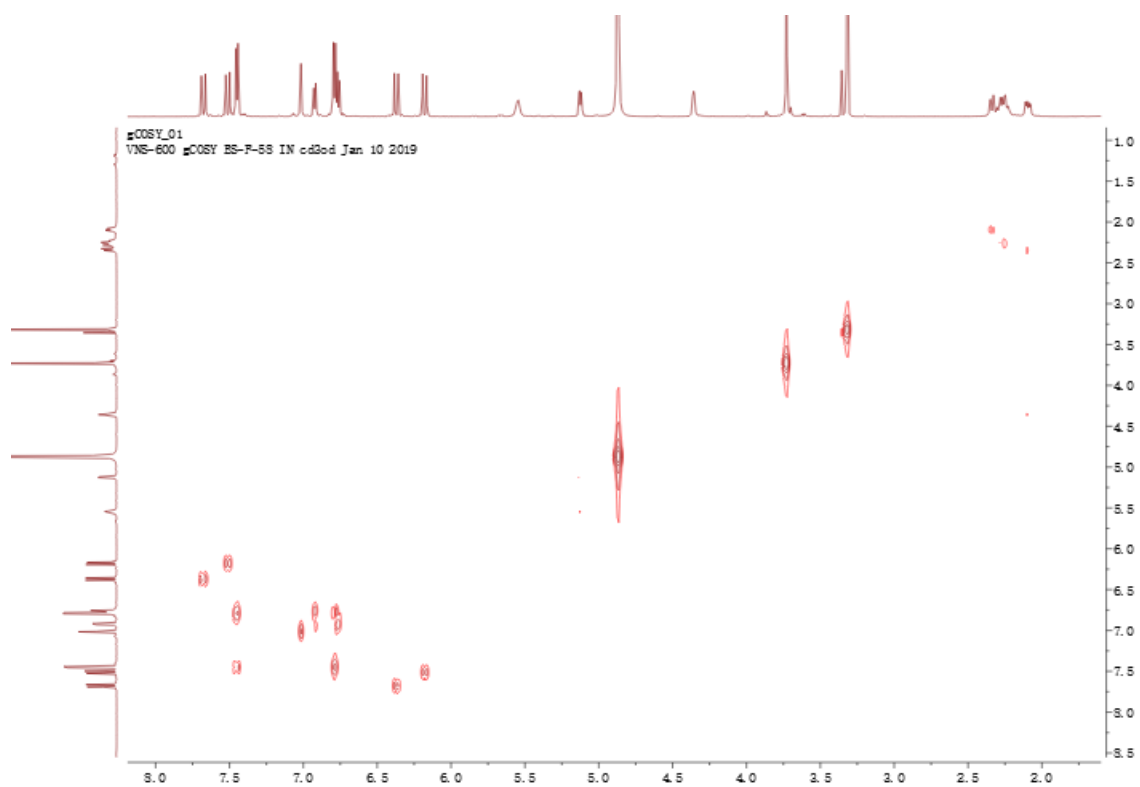


Figure S23. ^1H - ^1H COSY spectrum (600 MHz) of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**) in CD_3OD .

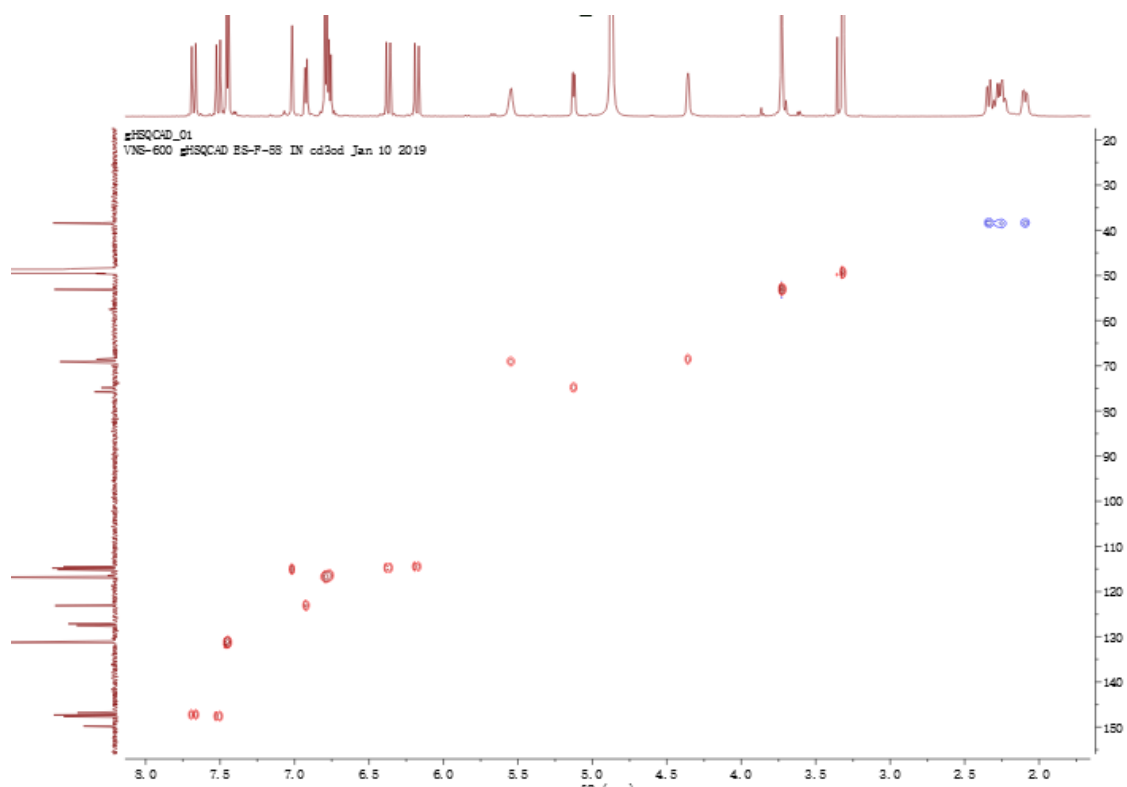


Figure S24. HSQC spectrum (600 MHz) of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**) in CD_3OD .

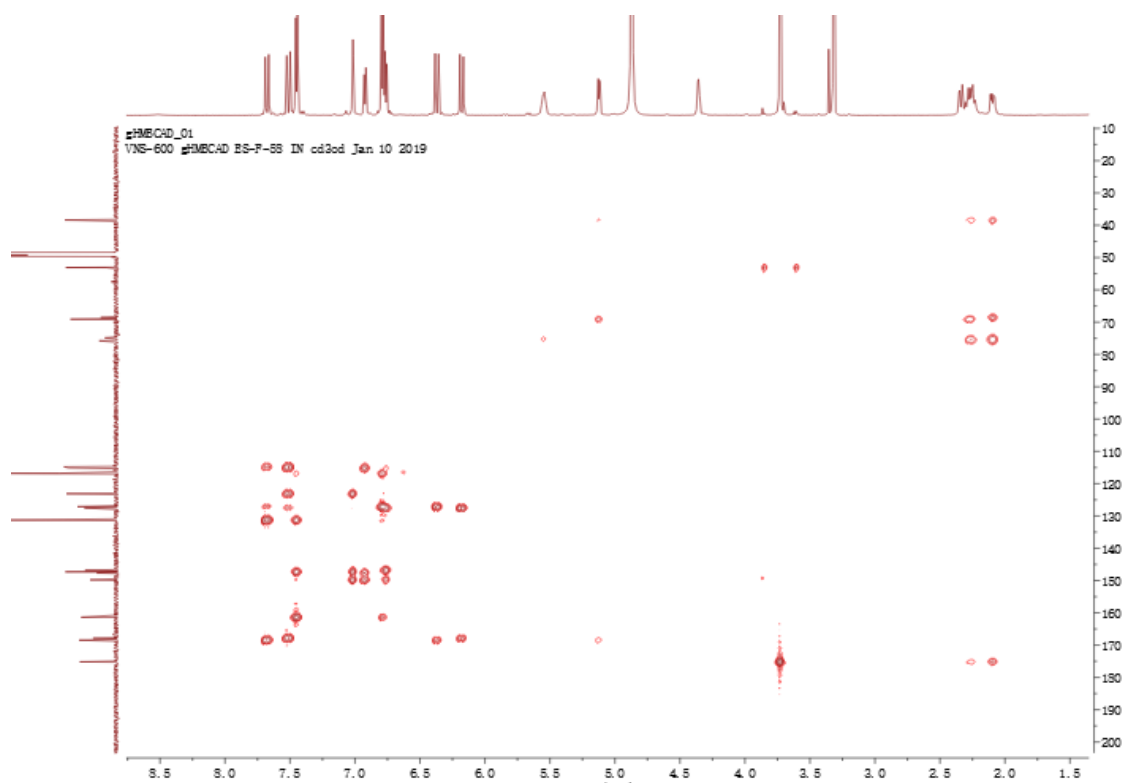


Figure S25. HMBC spectrum (600 MHz) of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**) in CD₃OD.

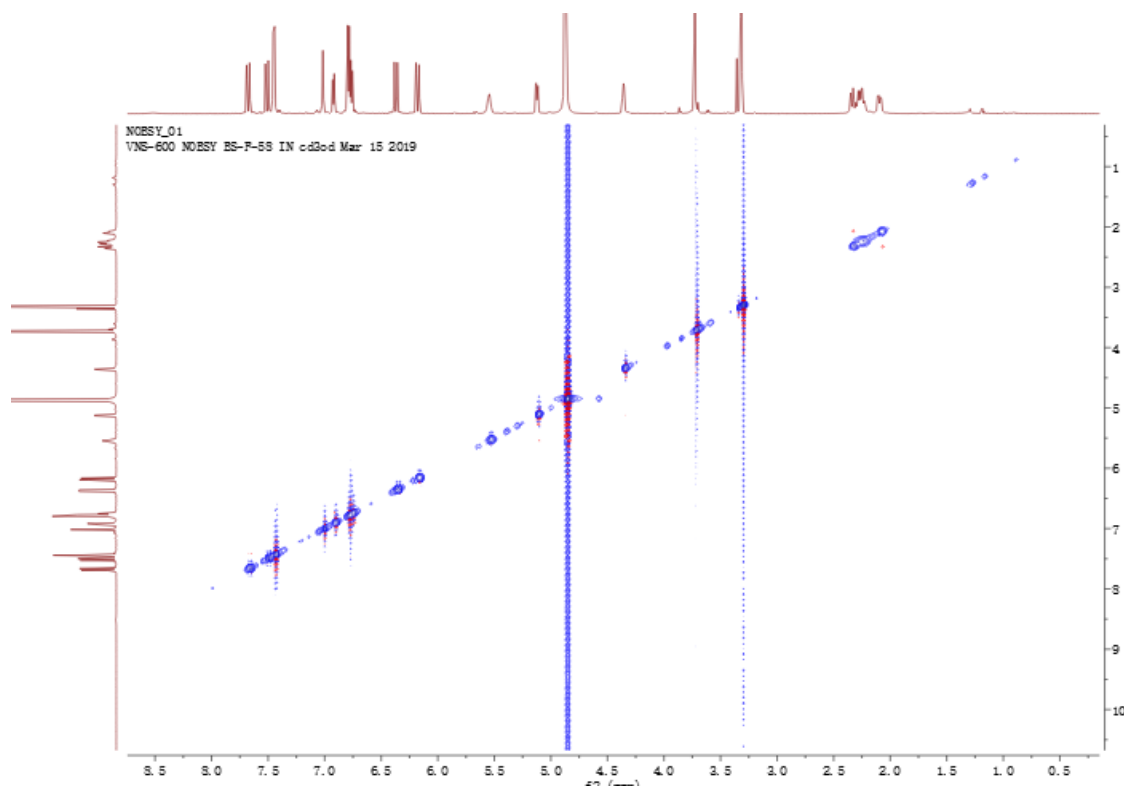


Figure S26. ROESY spectrum (600 MHz) of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**) in CD₃OD.

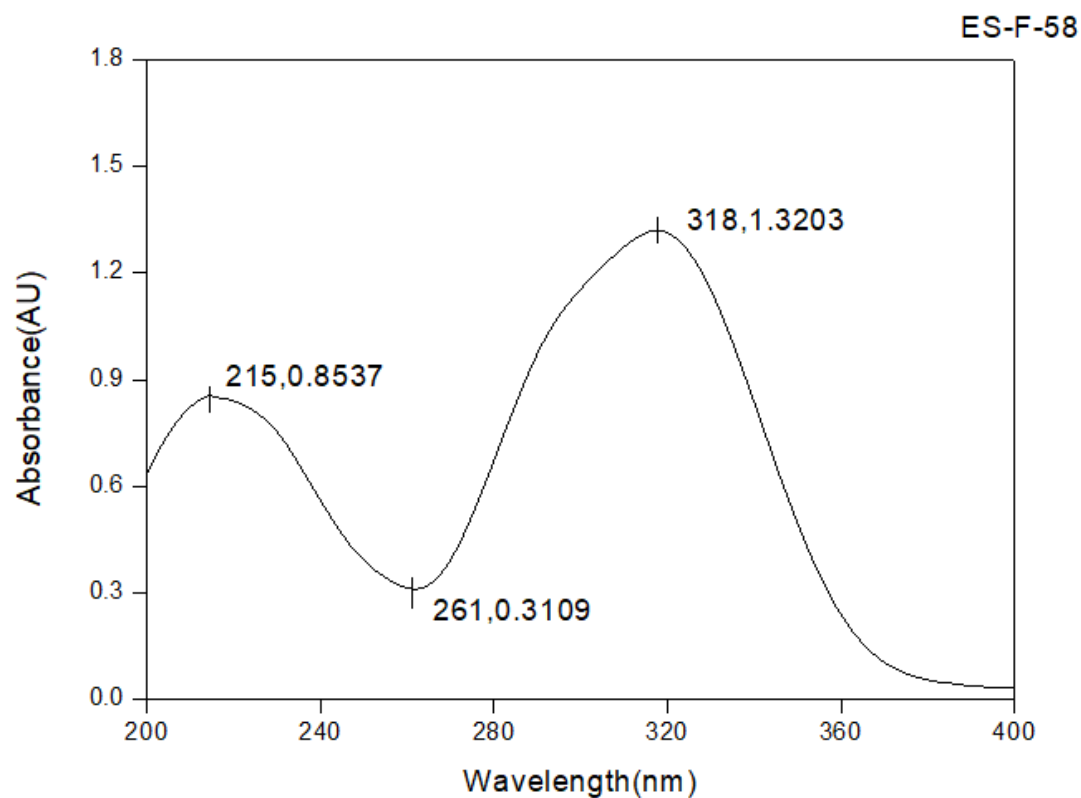


Figure S27. UV spectrum of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**).

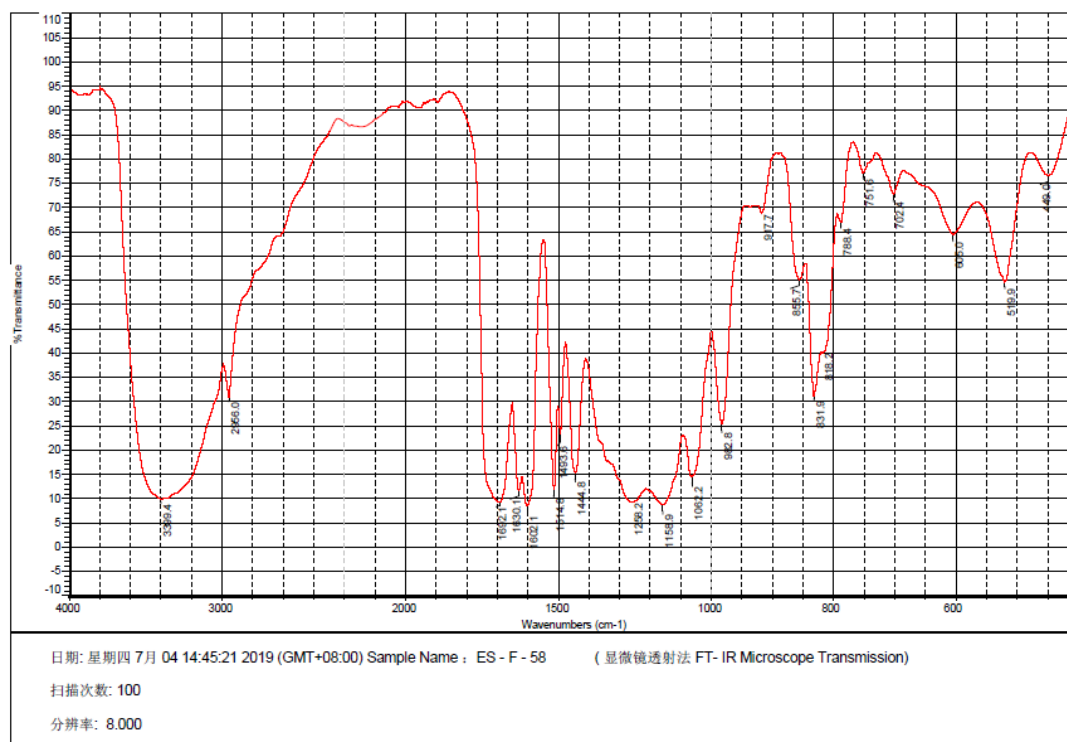


Figure S28. IR spectrum of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**).

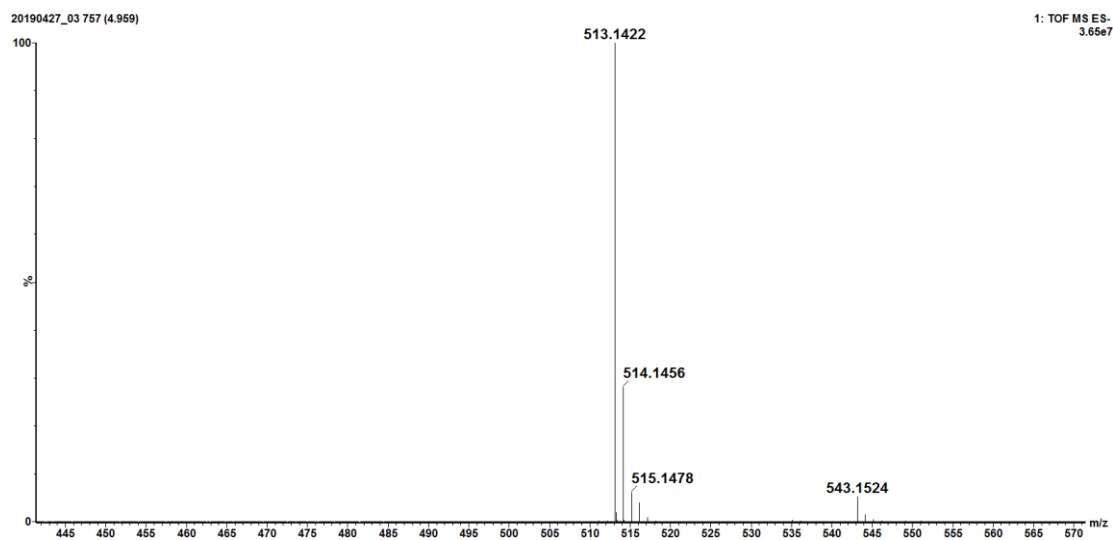


Figure S29. HRESIMS spectrum of 3-*O*-caffeoyl-4-*O*-*p*-coumaroyl quinic acid methyl ester (**3**).

Table S1. ¹H (600 MHz) and ¹³C NMR (150 MHz) data of compounds **1**, **2** and **3** in CD₃OD.

NO.	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		134.2		75.8		75.8
2 α	6.71 d (2.1)	119.2	2.27 m	38.6	2.24 dd (4.3, 13.9)	38.5
2 β					2.29 dd (8.5, 13.9)	
3		146.4	5.55 m	69.1	5.54 m	69.1
4		145.5	5.12 dd (2.9, 8.2)	75.0	5.12 dd (3.0, 8.1)	74.8
5	6.70 d (8.2)	116.4	4.36 m	68.6	4.36 m	68.6
6 α	6.58 dd (2.1, 8.2)	114.9	2.10 dd (6.3, 14.0)	38.4	2.09 dd (6.5, 14.0)	38.4
6 β			2.33 dd (3.2, 14.0)		2.34 dd (3.4, 14.0)	
7	3.40 t (8.6)	49.0		175.2		175.2
7-OCH ₃			3.73 s	53.1	3.73 s	53.1
8	3.27 t (8.6)	45.9				
9		174.3				
10	4.18 m	62.1				
11	1.25 t (7.1)	14.5				
1'		134.2		127.7		127.1
2'	6.71 d (2.1)	119.2	7.43 d (8.2)	131.3	7.02 d (2.0)	115.1
3'		146.4	6.79 d (8.2)	116.8		146.8
4'		145.5		161.4		149.8
5'	6.70 d (8.2)	116.4	6.79 d (8.2)	116.8	6.76 d (8.2)	116.5
6'	6.59 dd (2.1, 8.2)	114.9	7.43 d (8.2)	131.3	6.92 d d (2.0, 8.2)	123.1
7'	3.40 t (8.6)	49.2	7.58 d (15.9)	147.3	7.51 d (15.9)	147.7
8'	3.27 t (8.6)	46.2	6.25 d (15.9)	114.6	6.18 d (15.9)	114.5
9'		174.8		167.9		167.9
9'-OCH ₃	3.71 s	52.6				
1''				127.0		127.5
2''			7.04 d (2.0)	115.2	7.45 d (8.2)	131.3
3''				146.8	6.79 d (8.2)	116.8
4''				149.7		161.4
5''			6.76 d (8.2)	116.5	6.79 d (8.2)	116.8
6''			6.94 dd (2.0, 8.2)	123.1	7.45 d (8.2)	131.3
7''			7.61 d (15.9)	147.7	7.68 d (15.9)	147.3
8''			6.30 d (15.9)	114.7	6.37 d (15.9)	114.8
9''				168.5		168.4

Table S2. ¹H (500 MHz) data of compound **1** in CD₃COCD₃, and ¹H (600 MHz) and ¹³C NMR (150 MHz) data of compounds **4** and **5** in CD₃OD.

NO.	1	4		5	
	δ_{H}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1			131.9		132.3
2	6.80 d (2.2)	6.64 d (2.1)	114.1	6.65 d (2.2)	114.1
3			144.4		145.2
4			145.3		144.3
5	6.76 d (8.1)	6.66 d (8.1)	115.6	6.64 d (8.2)	115.5
6	6.65 dd (2.2, 8.1)	6.51 dd (2.1, 8.1)	117.6	6.50 dd (2.2, 8.2)	117.5
7	3.46 t (9.5)	3.30 dd like	47.2	3.28 dd like	47.0
8	3.25 t (9.5)	3.19 dd like	44.2	3.06 dd like	44.4
9			172.5		172.7
9-OCH ₃		3.64 s	52.0		
10	4.15 m				
11	1.22 t (7.1)				
1'			131.9		132.2
2'	6.81 d (2.2)	6.64 d (2.1)	114.1	6.66 d(2.2)	114.1
3'			144.4		145.3
4'			145.3		144.4
5'	6.77 d (8.1)	6.66 d (8.1)	115.6	6.64 d (8.2)	115.6
6'	6.66 dd (2.2, 8.1)	6.51 dd (2.1, 8.1)	117.6	6.51 dd (2.2, 8.2)	117.6
7'	3.47 t (9.5)	3.30 dd like	47.2	3.28 dd like	47.2
8'	3.26 t (9.5)	3.19 dd like	44.2	3.06 dd like	44.8
9'			172.5		173.7
9'-OCH	3.68 s	3.64 s	52.0	3.64 s	51.9

Table S3. Inhibition of compounds **1–5** on the NO production in LPS-activated RAW264.7 cells.

Compounds	IC ₅₀ (μ M) ^a
1	12.93 \pm 0.27
2	77.80 \pm 2.32
3	71.28 \pm 1.71
4	11.85 \pm 0.31
5	20.62 \pm 0.43
<i>L</i> -NAME ^b	35.96 \pm 0.90

^a All data are present as the mean of IC₅₀ values with the standard deviation from the triplicate measurement.

^b N-Nitro-L-arginine methyl ester hydrochloride (*L*-NAME) is an A medicine to diminish NO.

Table S4. Effects of compounds **1–5** (100 μ M) on RAW264.7 cells viability.

Compounds	cell viability vs control (%)
1	88.39 \pm 2.64
2	92.74 \pm 3.55
3	95.36 \pm 2.31
4	90.58 \pm 3.27
5	91.18 \pm 1.28