

SUPPLEMENTARY MATERIAL

Phytochemical, antimicrobial and antiquorum-sensing studies of *Pulicaria undulata* L.: A revision on the structure of 1 β ,2 α ,3 β ,19 α ,23-pentahydroxy-urs-12-en-28-oic acid

Fatma M. Abdel Bar^{a,b*}, Marwa Elsbaey^b, Noha Taha^c, Abdelaziz Elgaml^d, Gamal M. Abdel-Fattah^c

Affiliation:

^a Department of Pharmacognosy, Faculty of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia.

^b Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

^c Department of Microbiology, Botany Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt.

^d Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

Abstract

Phytochemical study of the aerial part of *Pulicaria undulata* L. led to the isolation of nine compounds. The structure of 1 β ,2 α ,3 β ,19 α ,23-pentahydroxy-urs-12-en-28-oic acid (**4**) was revised and confirmation of the stereochemical configuration of the hydroxyl groups was established using NOESY and selective decoupling experiments. The other compounds were identified as 1,2-dehydro-1,10 α -dihydropseudoivalin (**1**), axillarin (**2**), grandifloric acid-15- α -glucoside (**3**), myrianthic acid (**5**), caffeic acid (**6**), quercetin (**7**), paniculose IV (**8**) and caffeic anhydride (**9**). The structures were characterized by 1D, 2D NMR spectroscopy and confirmed with HRMS. Antimicrobial and antiquorum-sensing activities of the different extracts and isolated compounds of the plant were investigated. Generally, the phenolic rather than the terpenoidal compounds exhibited remarkable antimicrobial and antiquorum-sensing activity.

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1. Experimental

1.1. General experimental

TLC is performed on silica gel 60 GF254 plates (Merck, Germany). Silica gel G 60-230 mesh (Merck, Germany) or RPC18 40 μm (BAKERBOND, Phillipsburg, NJ, USA) were used for column chromatography. Organic solvents were of reagent grade (ElNasr Co., Cairo, Egypt). 1D and 2D NMR spectra were obtained using Jeol 500 MHz TM spectrometer (Jeol, Tokyo, Japan) in chloroform-*d* or methanol-*d*₄. Selective decoupling experiment was performed on a 400 MHz for BRUKER Avance III spectrometer (Bruker AG, Switzerland) in pyridine-*d*₅. HRMS was determined using BRUKER MicroTOF-Q (Bruker, Germany).

1.2. Plant materials

The aerial parts of *Pulicaria undulata* were collected in September 2015 from Wadi Hagol, Egypt, shade-dried, powdered and kept for further investigation. The plant was identified by Prof. Dr. Ibrahim Mashaly, Faculty of Science, Mansoura University, Egypt.

1.3. Phytochemical studies

Two kilograms of the dried powdered plant (*Pulicaria undulata*) were extracted by rinsing with MeOH (5 x 4 L). The combined methanol extracts were concentrated using rotary evaporator at 50 °C then allowed drying at room temperature. The total dried methanol extract (271.4 g) was suspended in MeOH-H₂O (50:50 v/v, 1L), then partitioned successively with petroleum ether (3 x 1L), CH₂Cl₂ (4 x 1L) and EtOAc (4 x 1L). The extracts, in each case, were evaporated to dryness under reduced pressure to yield petroleum ether extract (34 g), CH₂Cl₂ extract (37.37 g) and ethyl acetate extract (42 g). The CH₂Cl₂ and ethyl acetate extracts were selected for further phytochemical investigation on the basis of preliminary phytochemical and antimicrobial screening. Detailed chromatographic isolation procedures of the CH₂Cl₂ and ethyl acetate extracts are outlined in Figures 4 & 5.

1.4. Antimicrobial assay (Abdel-Rahman et al. 2017)

Microorganisms were provided from the laboratory of Microbiology and Immunology department at Faculty of Pharmacy, Mansoura University, Egypt. The primary screening was carried out using agar disc-diffusion method. For the antibacterial assay, Mueller Hinton agar medium was used and for the antifungal assay, YPD agar medium was used. Concisely, the overnight cultures of the different strains were diluted to OD_{600nm} of 0.1 (equivalent to 8×10^7 cells/mL), except for *C. albicans* that was diluted to OD_{600nm} of 0.5 (equiv. to 8×10^7 cells/mL). The diluted cultures were spread on the agar plates using sterile swab followed by the application of sterile cellulose papers discs (6 mm each). Stock solutions of compounds or extracts in DMSO (10 mg/300 µL) were used. In each case, 100 µg from the stock solution (3 µL) was adsorbed into the paper discs. DMSO was used as a negative control; Ampicillin (100 µg/disc) and gentamicin (100 µg/disc) were used as standard antibacterial compounds; while fluconazole (40 µg/disc) was used as a standard antifungal compound. The plates were incubated at 37°C for 24 h. Diameters of the growth inhibition zones were measured to the nearest millimeter using a caliber.

1.5. Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) for the active compounds and/or extracts was determined using broth microdilution method in 96-well plates as described previously (Abdel-Rahman et al. 2017). Briefly, the overnight cultures of the bacterial strains were diluted to OD_{600nm} of 0.01 (equiv. to 8×10^6 cells/mL), while that of *C. albicans* was diluted to OD_{600nm} of 0.5 (equiv. to 8×10^7 cells/mL). The active compounds and/or extracts, as well as standard antimicrobial drugs were diluted by two-fold serial dilutions in DMSO (5000, 2500, 1250, 625, 312.5, 156.25, 78.125, 39.062, and 19.531 µg/mL). Subsequently, dilutions were applied to the diluted cultures in the microtiter plates as 5 % of the final volume in each well. The plates were incubated at 37°C for 24 h. The MIC values were determined as the lowest concentration that completely inhibits the visible growth of the microorganism after overnight incubation.

1.6. Antiquorum-sensing activity (El-Gohary and Shaaban 2017)

Firstly, *Chromobacterium violaceum* were cultured in LB broth and incubated for 16–18 h in an orbital incubator at 28°C and 150 rpm, and then the culture was adjusted to 0.5 McFarland standard (equiv. to 1×10^6 cells/mL). Aliquot of *Ch. violaceum* (50 µL) was inoculated into LB agar (50 mL), poured into plates, left to solidify, then wells were made using cork borer. Aliquots of 50 µL of stock solutions of compounds or extracts in DMSO (5 mg/mL) were applied into the wells. Similarly, the positive control (catechin) and negative control (DMSO) were added to each plate. Inhibition of pigment production around the wells was then checked after incubation at 30°C for 48 h. A clear halo around the disk indicated bacterial growth inhibition (r1), while turbid halo-harboring pigmentless bacterial cells indicated a positive quorum-sensing inhibition (r2). Thus, the pigment inhibition (QS inhibition) was measured by subtracting bacterial growth inhibition (r1) from the total radius (r2); thus, QS inhibition = (r2 - r1) in mm.

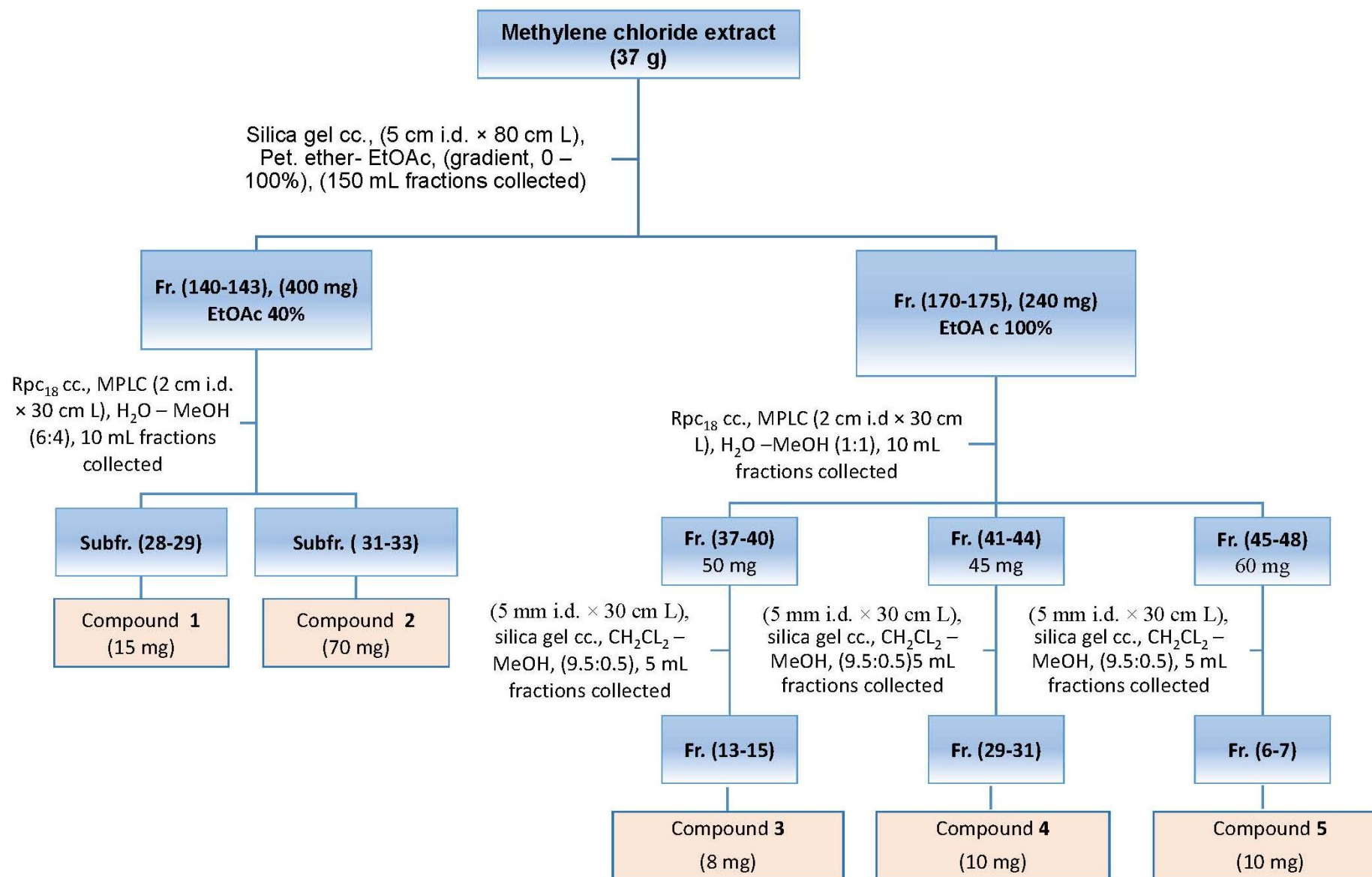


Figure S1. Fractionation procedure of the methylene chloride extract of *Pulicaria undulata*.

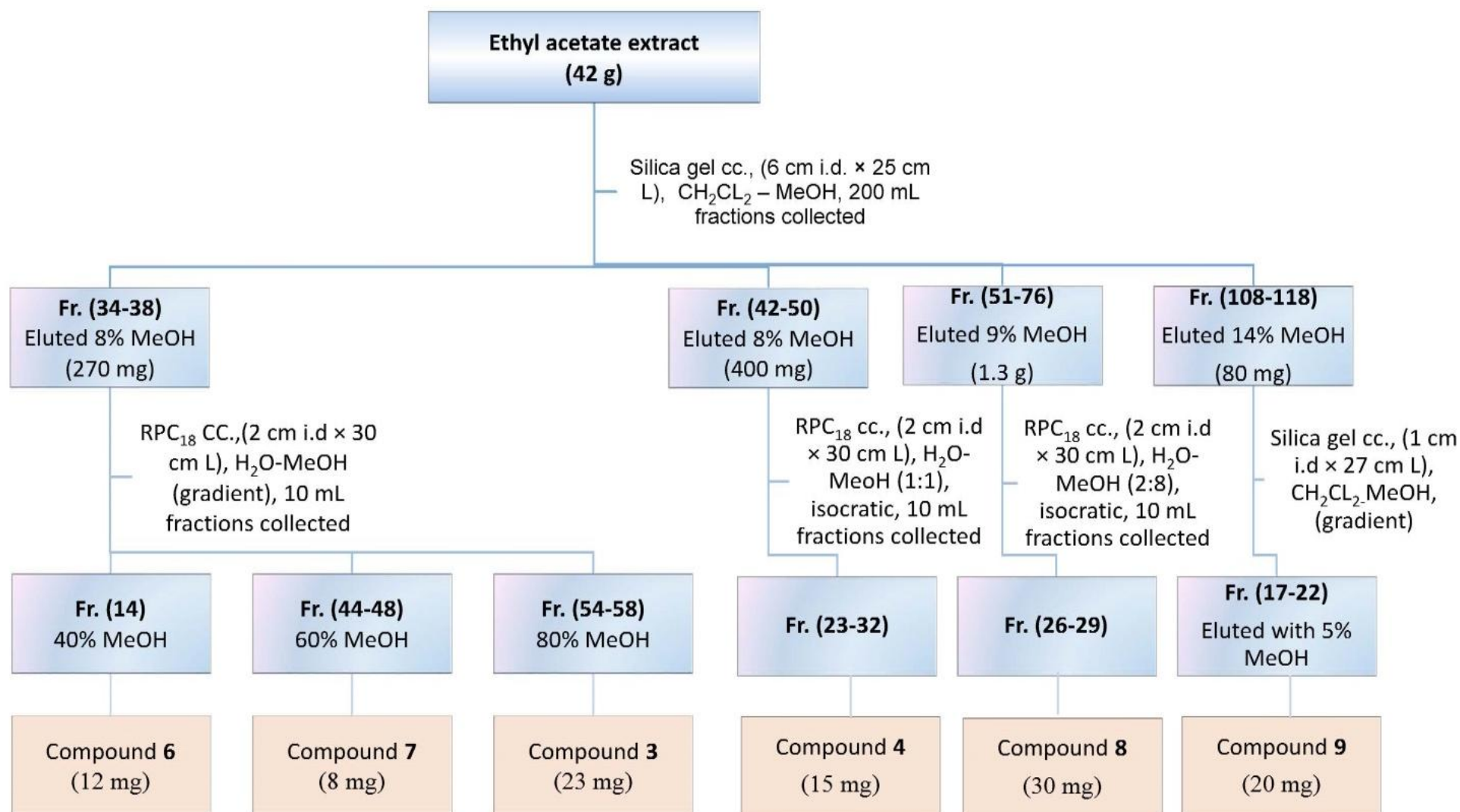
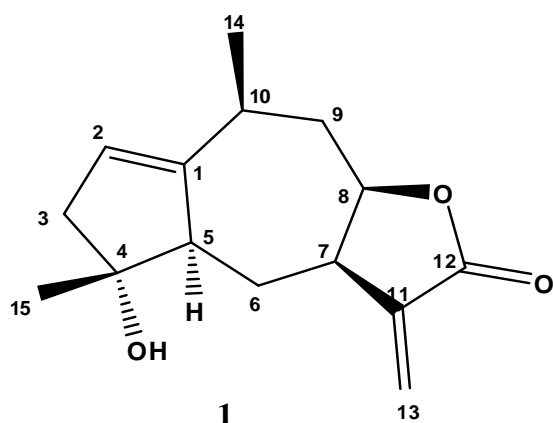


Figure S2. Fractionation procedure of the ethyl acetate extract of *Pulicaria undulata*.

Compound 1:

**Table S1.** ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **1** in $\text{CDCl}_3\text{-CD}_3\text{OD}$ (1:1):

H/C no.	Type	APT (\square_{C})	^{13}C NMR (Starvi et al., 2008)	\square_{H} (J in Hz)
1	C	149.2	151.1	---
2	CH	118.8	120.3	5.33, t (2.0)
3	CH_2	46.2	47.5	2.36, brd (12.5)
4	C	80.8	82.1	---
5	CH	58.1	59.7	2.35, m
6	CH_2	31.2	32.7	H_a : 1.96, m H_b : 1.32, ddd (12.0, 12.0, 1.0)
7	CH	41.8	43.3	3.26, m
8	CH	81.1	82.6	4.82, m
9	CH_2	37.7	39.1	H_a : 1.92, m H_b : 1.56, ddd (12.0, 11.5, 1.5)
10	CH	31.1	32.4	2.18, brs
11	C	139.9	142.4	---
12	C	170.5	172.1	---
13	CH_2	122.5	122.9	H_a : 6.22, d (3.0) H_b : 5.58, d (3.0)
14	CH_3	19.8	20.6	1.17, d (6.0)
15	CH_3	23.8	24.7	1.27, s

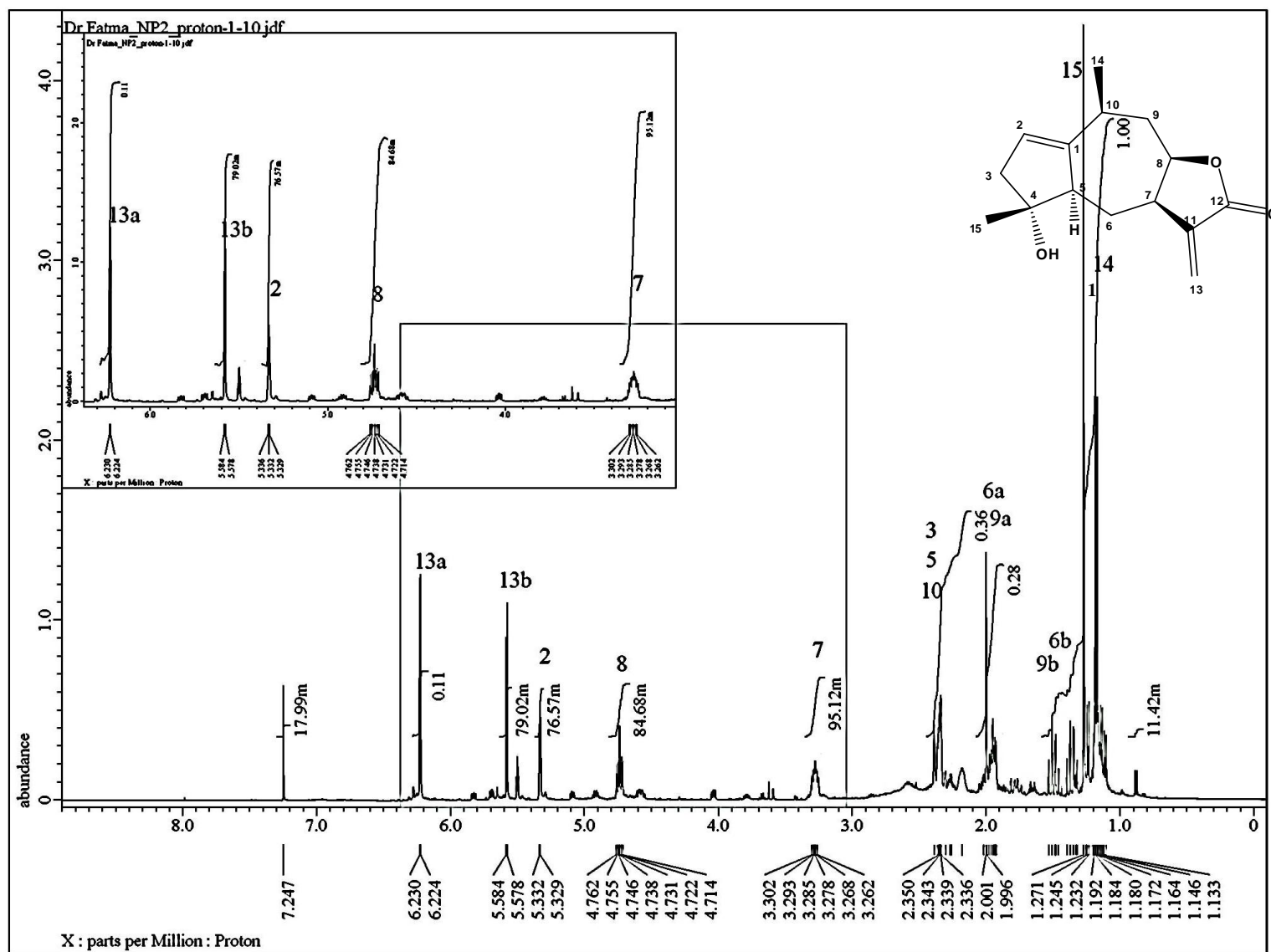


Figure S3. ^1H NMR (500 MHz) spectrum of compound **1** in $\text{CDCl}_3\text{-CD}_3\text{OD}$ (1:1).

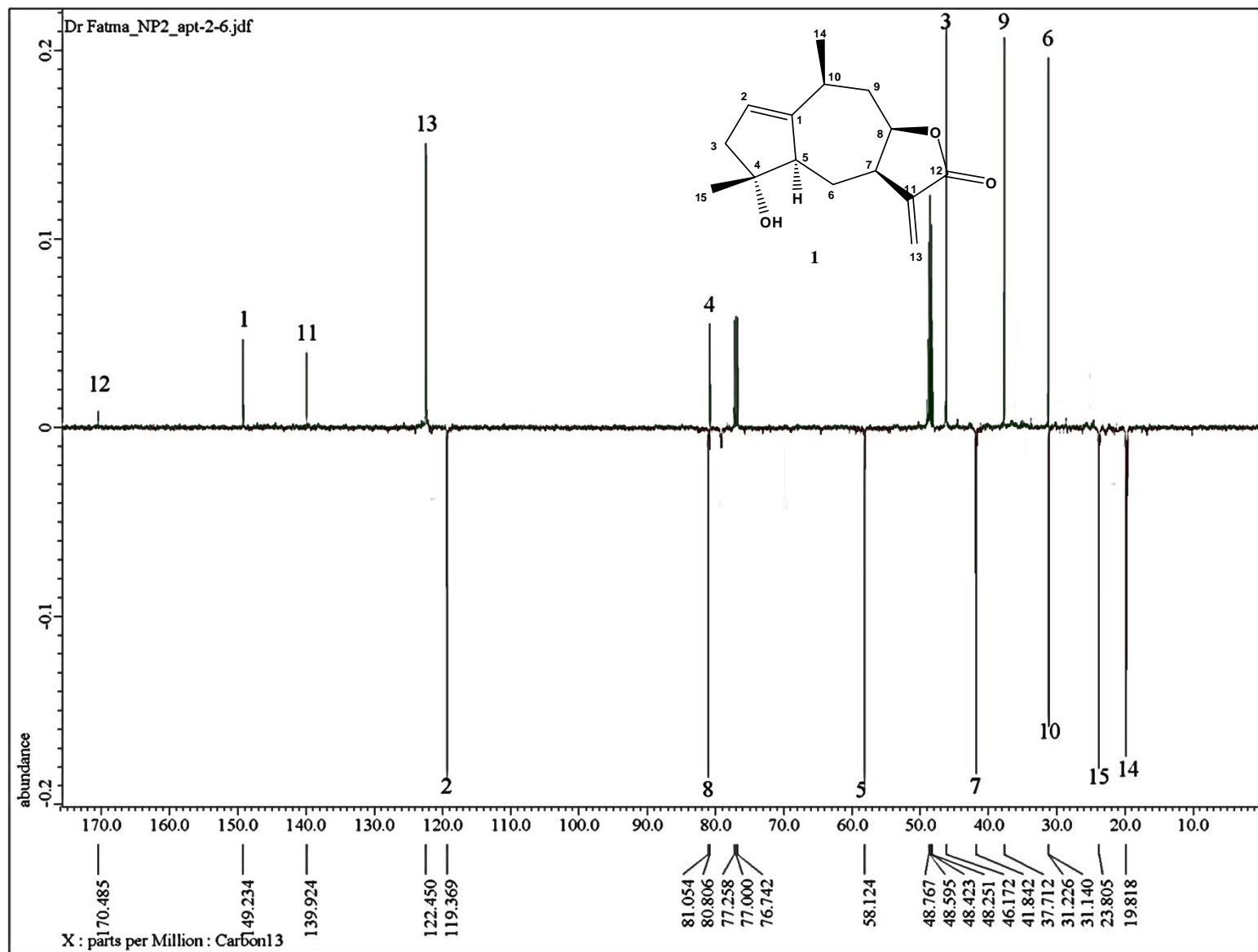


Figure S4. ^{13}C NMR (APT, 125 MHz) spectrum of compound **1** in $\text{CDCl}_3\text{-CD}_3\text{OD}$ (1:1).

Compound 2:

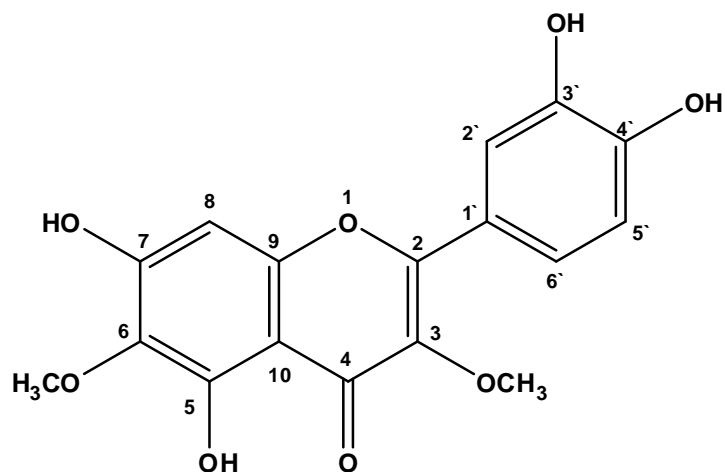


Table S2. ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **2** in CD_3OD :

H/C no.	Type	APT (\square_{C})	\square_{H} (J in Hz)
2	C	154.5	---
3	C	140.0	---
4	C	181.1	---
5	C	154.6	---
6	C	133.4	---
7	C	158.9	---
8	CH	95.8	6.49, s
9	C	159.7	---
10	C	107.1	---
1'	C	123.7	---
2'	CH	117.2	7.61, d ($J= 2.5$ Hz)
3'	C	147.3	---
4'	C	150.8	---
5'	CH	123.1	6.88, d ($J= 8.5$ Hz)
6'	CH	117.2	7.51, dd ($J= 8.5, 2.5$ Hz)
3-OCH ₃	OCH ₃	61.3	3.78, s
6-OCH ₃	OCH ₃	61.7	3.87, s

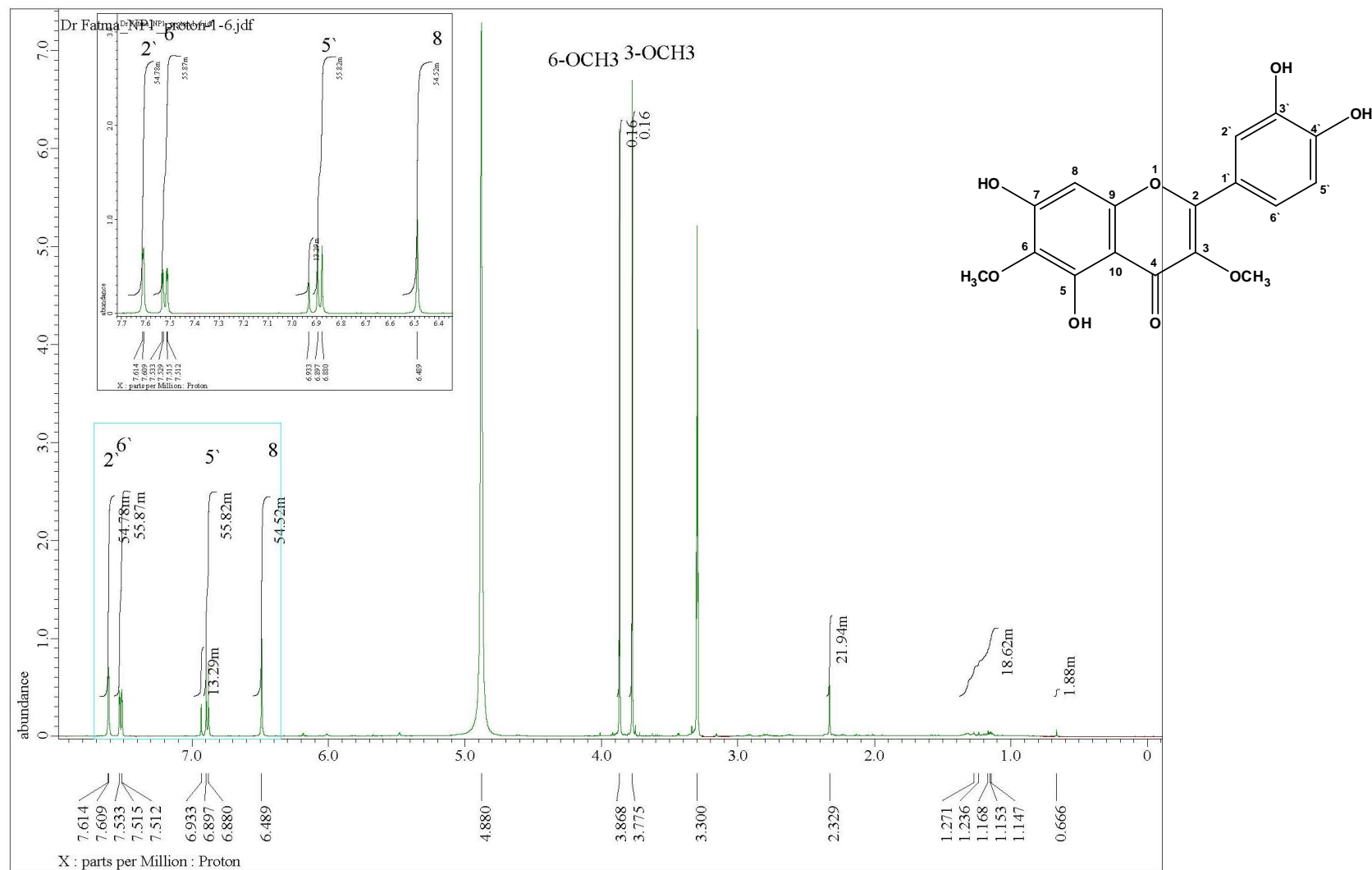


Figure S5. ^1H NMR (500 MHz) spectrum of compound **2** in CD_3OD .

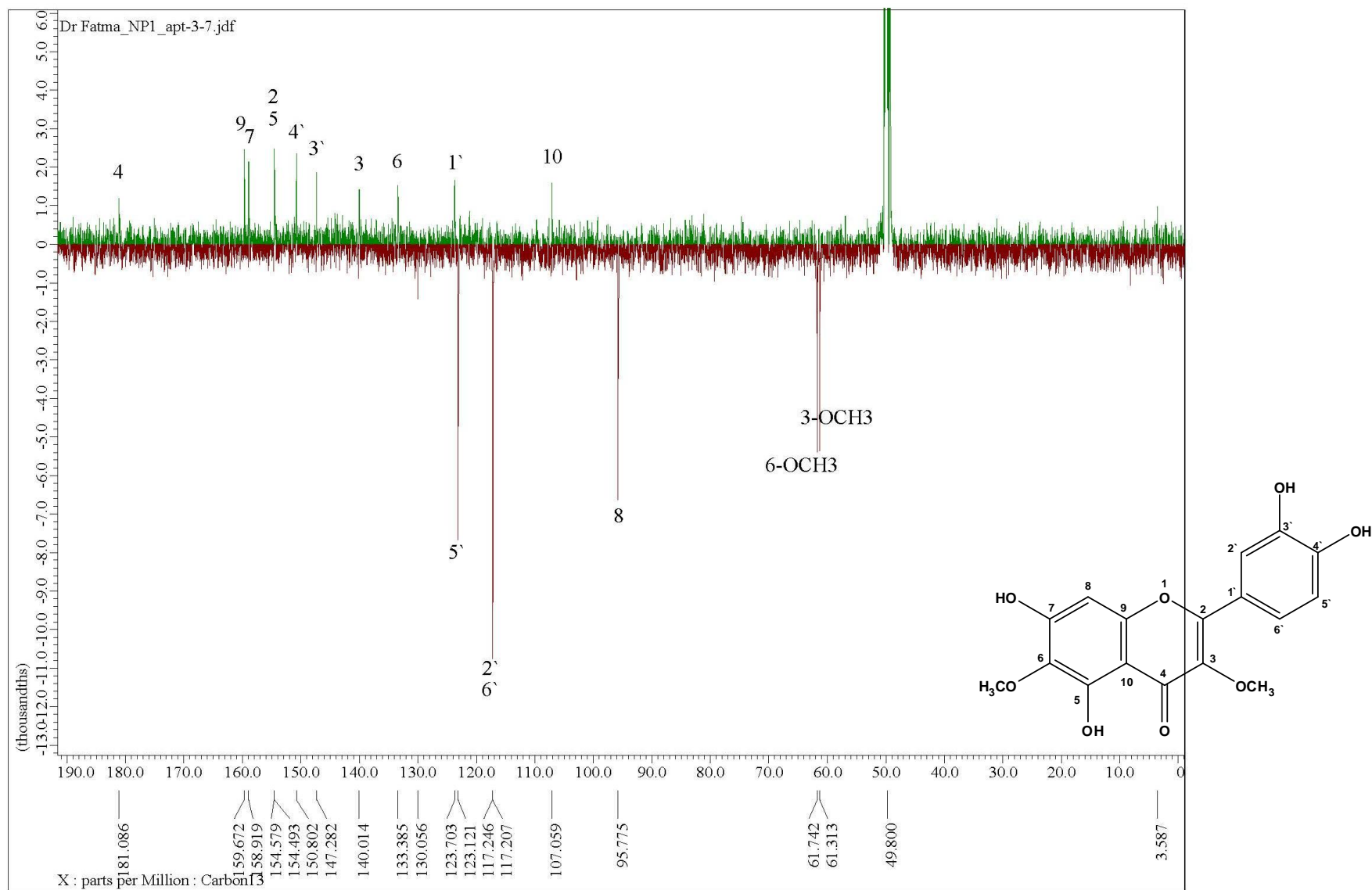


Figure S6. ^{13}C NMR (APT, 125 MHz) spectrum of compound **2** in CD_3OD .

Compound 3:

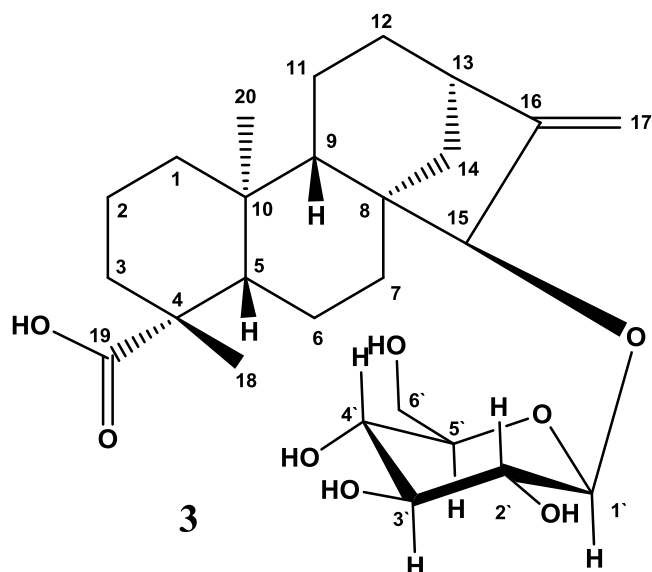


Table S3. ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **3** in CD_3OD :

H/C no.	Type	APT (\square_{C})	\square_{H} (J in Hz)
1	CH_2	40.0	H_a : 1.96, td (13.0, 4.0); H_b : 1.01 (2H), td (13.0, 3.5)
2	CH_2	19.8	H_a : 1.72, m; H_b : 1.45 (2H), dd (14.0, 4.0)
3	CH_2	38.4	H_a : 2.09, brd (13.5); H_b : 0.92, td (14.5, 3.5)
4	C	45.5	---
5	CH	58.0	1.01 (2H), td (13.0, 3.5)
6	CH_2	23.6	H_a : 1.61, td (14.5, 4.0); H_b : 1.56, m
7	CH_2	40.0	H_a : 1.87, brd (12.0); H_b : 1.25, dt (13.0, 1.8)
8	C	48.0	---
9	CH	48.2	1.45 (2H), dd (14.0, 4.0)
10	C	42.7	---
11	CH_2	21.2	H_a : 1.38, m; H_b : 1.77, m
12	CH_2	35.4	H_a : 1.83, m; H_b : 1.56, brd (8.0)
13	CH	42.5	2.56, brs
14	CH_2	41.3	H_a : 2.02, d (12.0); H_b : 1.15, dd (12.0, 2.4)
15	CH	92.4	3.84, brs
16	C	158.1	---
17	CH_2	107.2	H_a : 5.30, s; H_b : 4.92, s
18	CH_3	30.3	1.18, s
19	C	182.8	---
20	CH_3	17.4	0.98, s
1'	CH	107.1	4.42, d (7.5)
2'	CH	76.3	3.25, m
3'	CH	78.4	3.36, m
4'	CH	72.3	3.27, m
5'	CH	79.1	3.39, m
6'	CH_2	63.6	H_a : 3.85, dd (11.5, 2.5); H_b : 3.73, dd (12.0, 5.0)

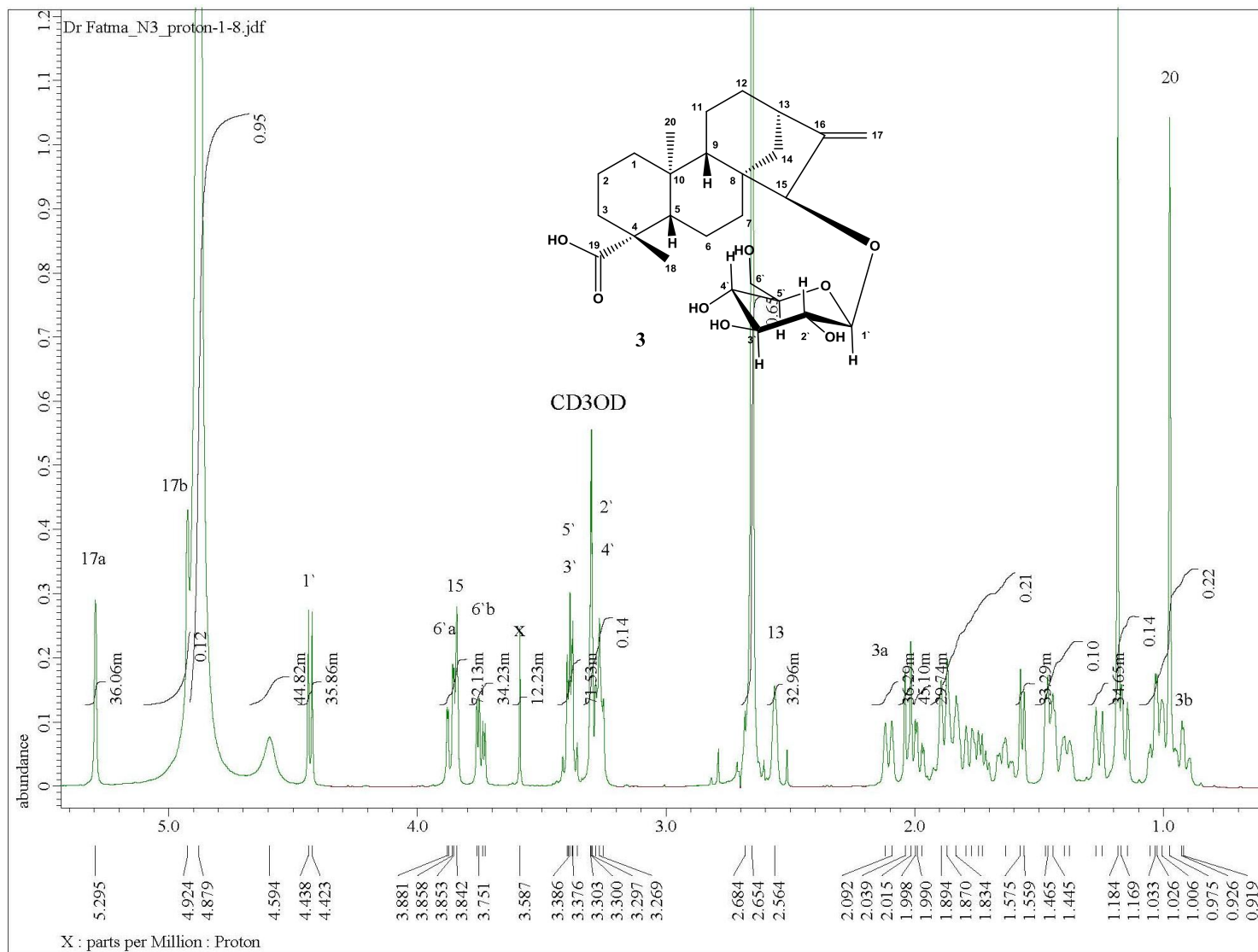


Figure S7. ^1H NMR (500 MHz) spectrum of compound **3** in CD_3OD .

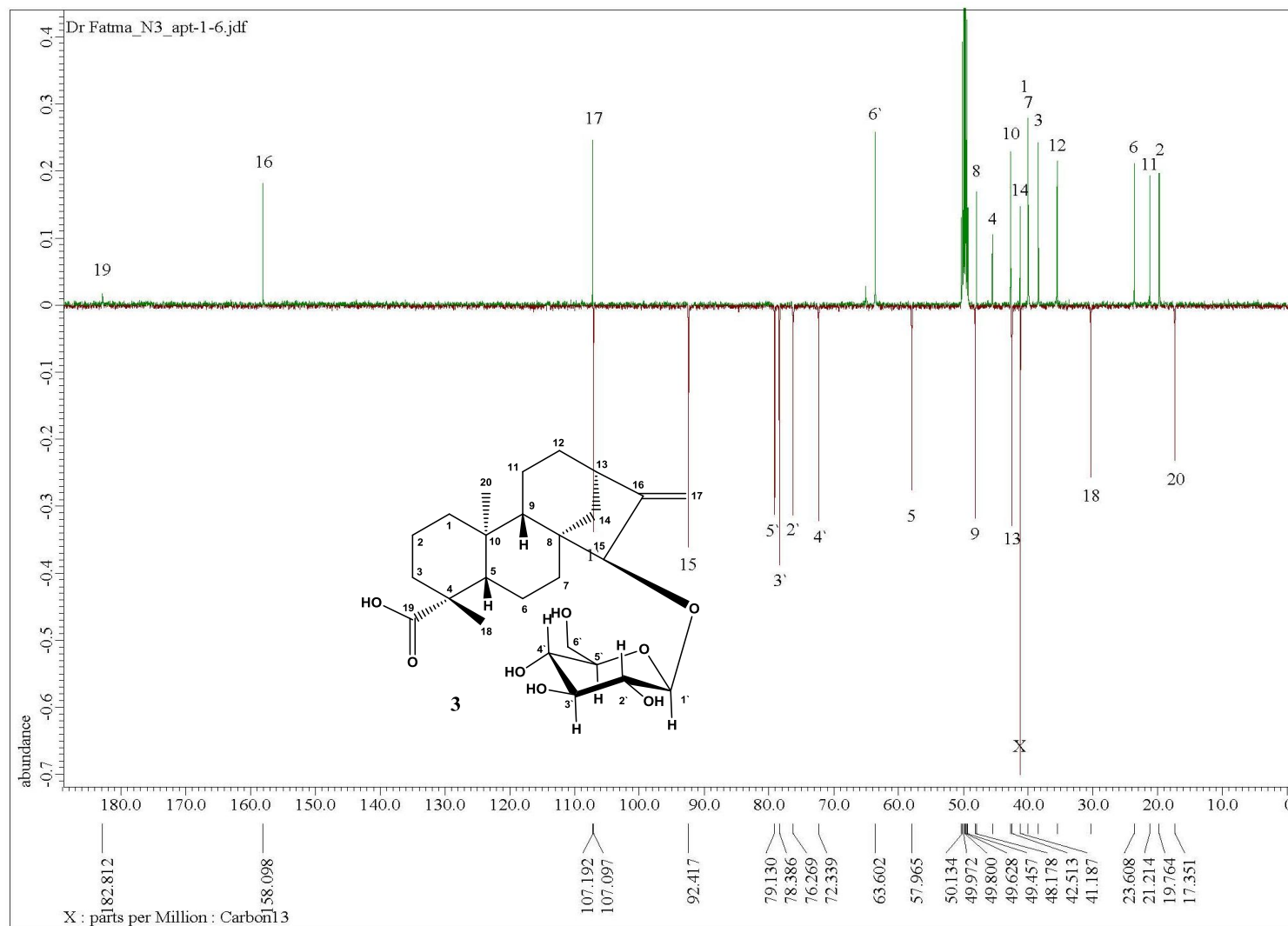


Figure S8. ^{13}C NMR (APT, 125 MHz) spectrum of compound **3** in CD_3OD .

Table S4. ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **4** in CD_3OD :

H/C no.	Type	APT (δ_{C})	δ_{H} (J in Hz)	^{13}C NMR (Gupta and Singh 1989)
1	CH	85.8	3.04, d (8.5)	74.9
2	CH	75.6	3.40, dd (9.5, 8.5)	74.6
3	CH	75.8	3.38, d (10.0)	79.9
4	C	44.7	---	42.6
5	CH	46.4	1.22, m	53.5
6	CH_2	19.5	1.49, 2H, m	17.9
7	CH_2	34.4	1.25, 2H, m	33.0
8	C	42.4	---	41.2
9	CH	49.8	1.92, dd (11.0, 7.0)	48.0
10	C	43.3	---	37.7
11	CH_2	29.1	2.23, m	24.3
12	CH	131.4	5.28, brs	129.1
13	C	139.7	---	139.7
14	C	44.5	---	42.2
15	CH_2	30.5	0.98, m	29.5
16	CH_2	25.7	1.20, 2H, m	26.4
17	C	49.8	--	48.5
18	CH	55.8	2.48, brs	53.0
19	C	74.4	---	72.5
20	CH	43.9	1.28, m	42.3
21	CH_2	27.4	H_a : 2.55, dd (13.5, 10.0) H_b : 1.49, m	28.1
22	CH_2	39.8	H_a : 1.76, m H_b : 1.61, m	38.1
23	CH_2	66.8	H_a : 3.47, d (11.0) H_b : 3.25, d (11.5)	66.3
24	CH_3	14.6	0.68, s	16.2
25	CH_3	14.6	0.77, s	11.8
26	CH_3	18.6	1.01, s	16.9
27	CH_3	25.7	1.17, s	25.6
28	C	183.2	---	180.5
29	CH_3	27.9	1.32, s	27.5
30	CH_3	17.4	0.90, d (6.5)	16.5

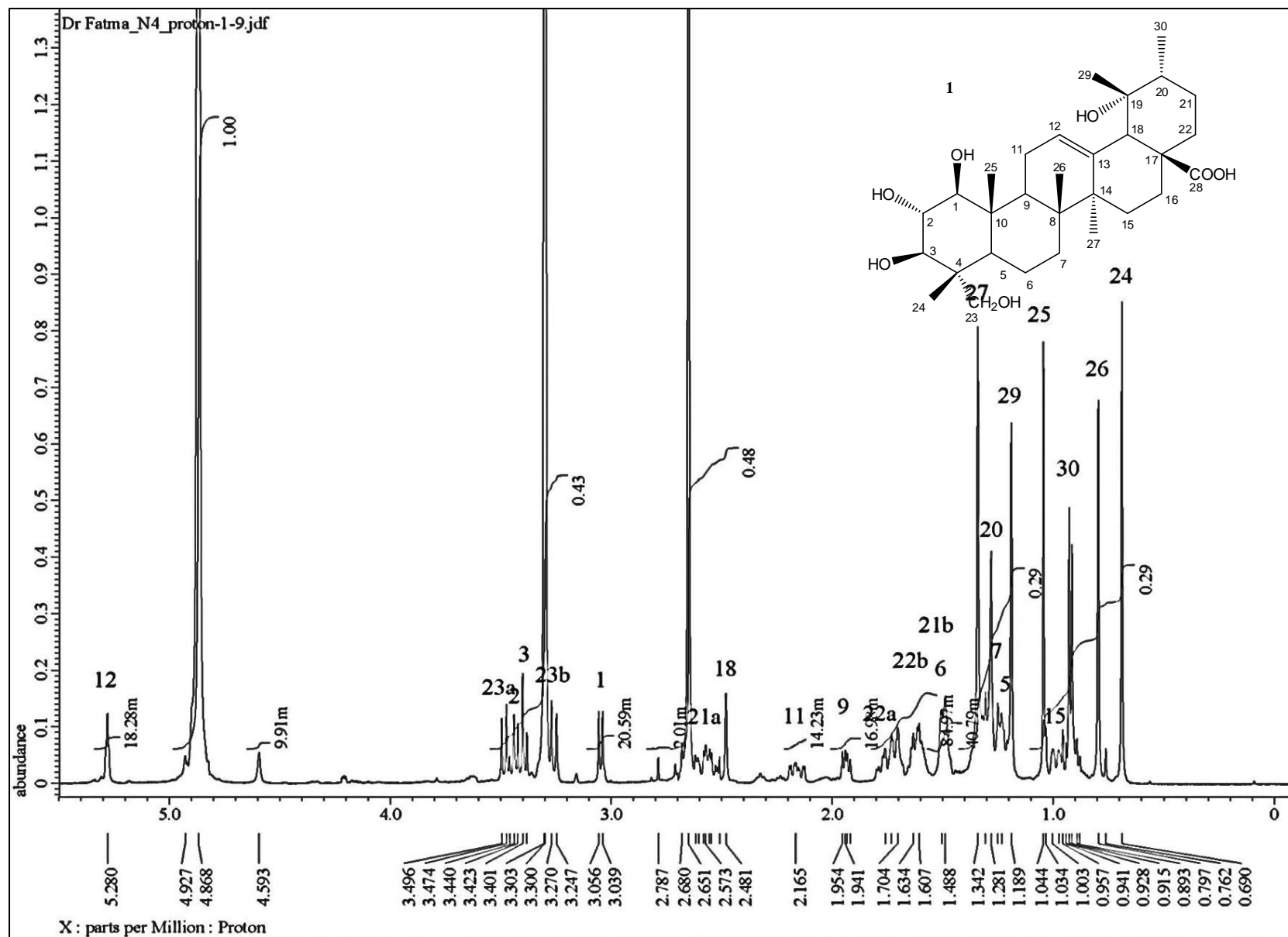


Figure S9. ^1H NMR (500 MHz) spectrum of compound **4** in CD_3OD .

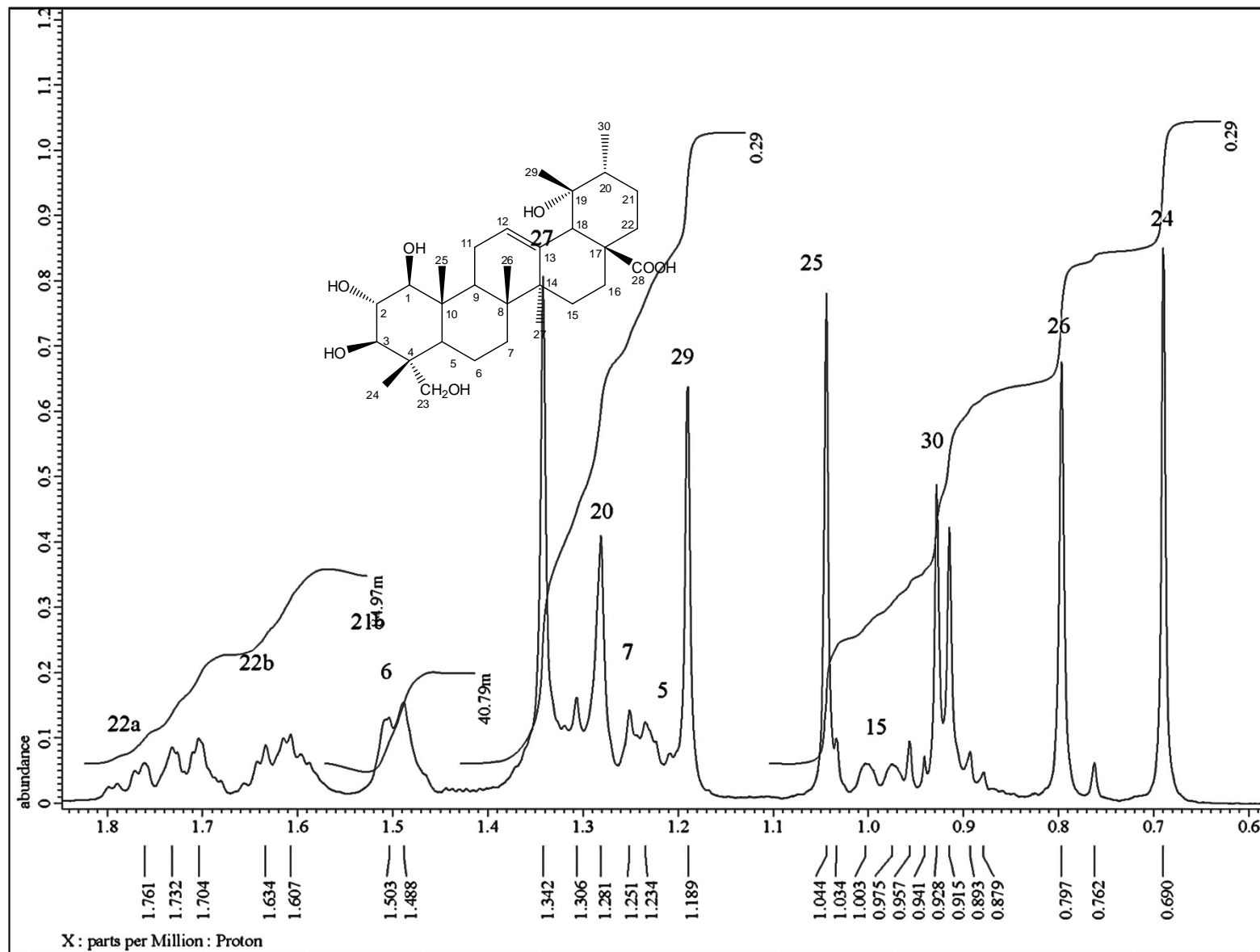


Figure S10. Expansion of ^1H NMR spectrum (0.58-1.84 ppm) of compound **4** in CD_3OD .

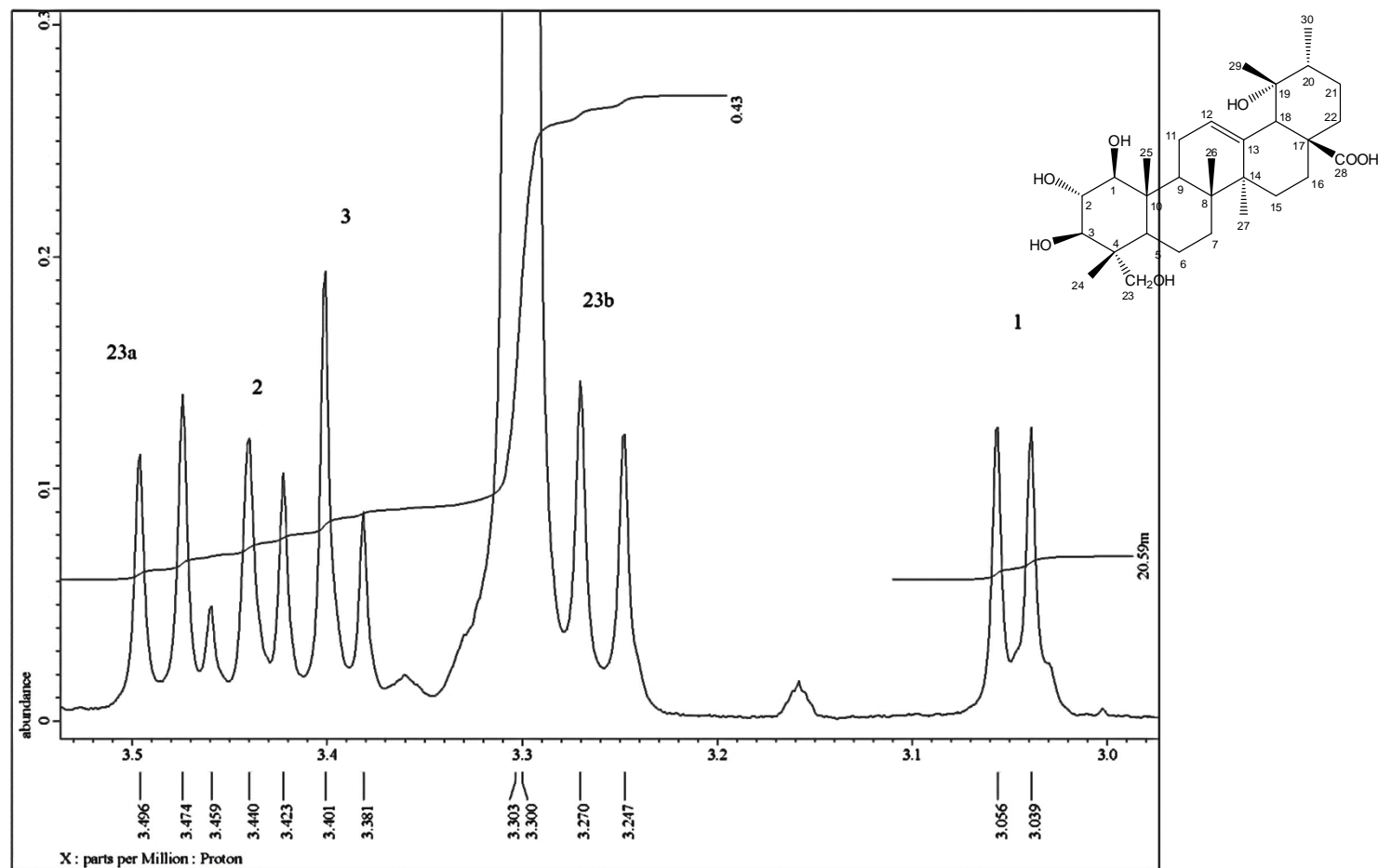


Figure S11. Expansion of ^1H NMR spectrum (2.70–3.54 ppm) of compound **4** in CD_3OD .

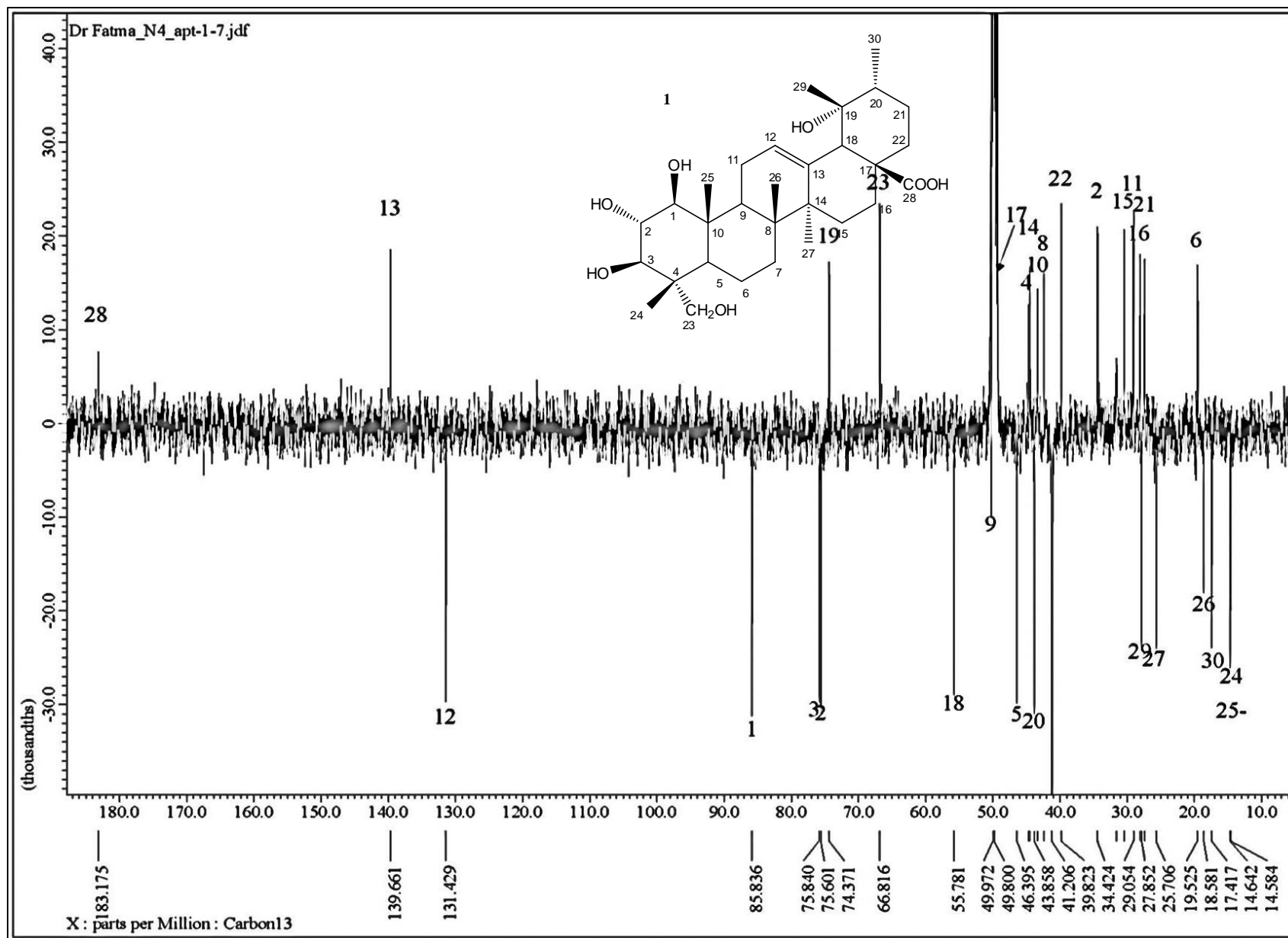


Figure S12. ^{13}C NMR (APT, 125 MHz) spectrum of compound **4** in CD_3OD .

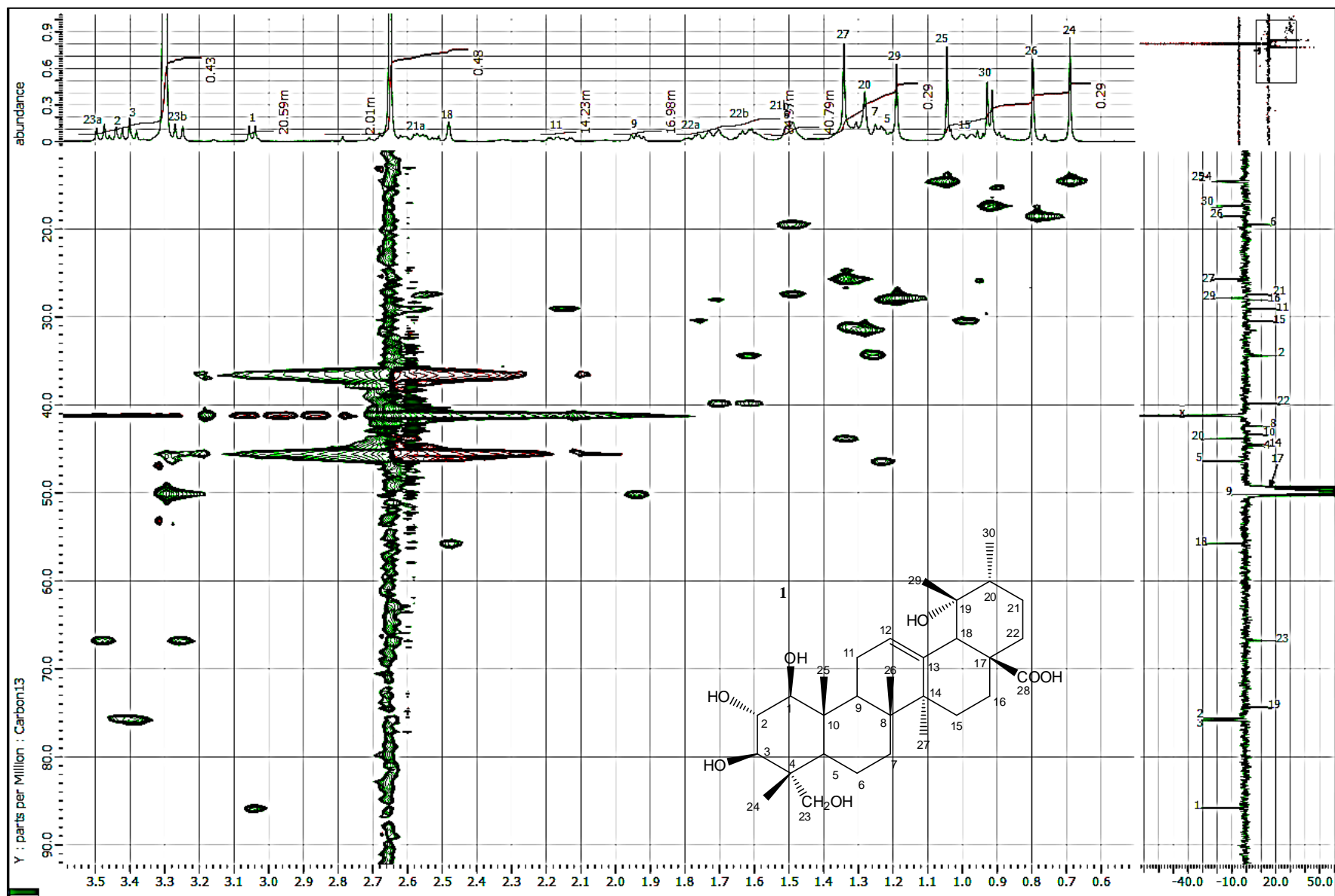


Figure S13. HSQC spectrum of compound **4** in CD_3OD .

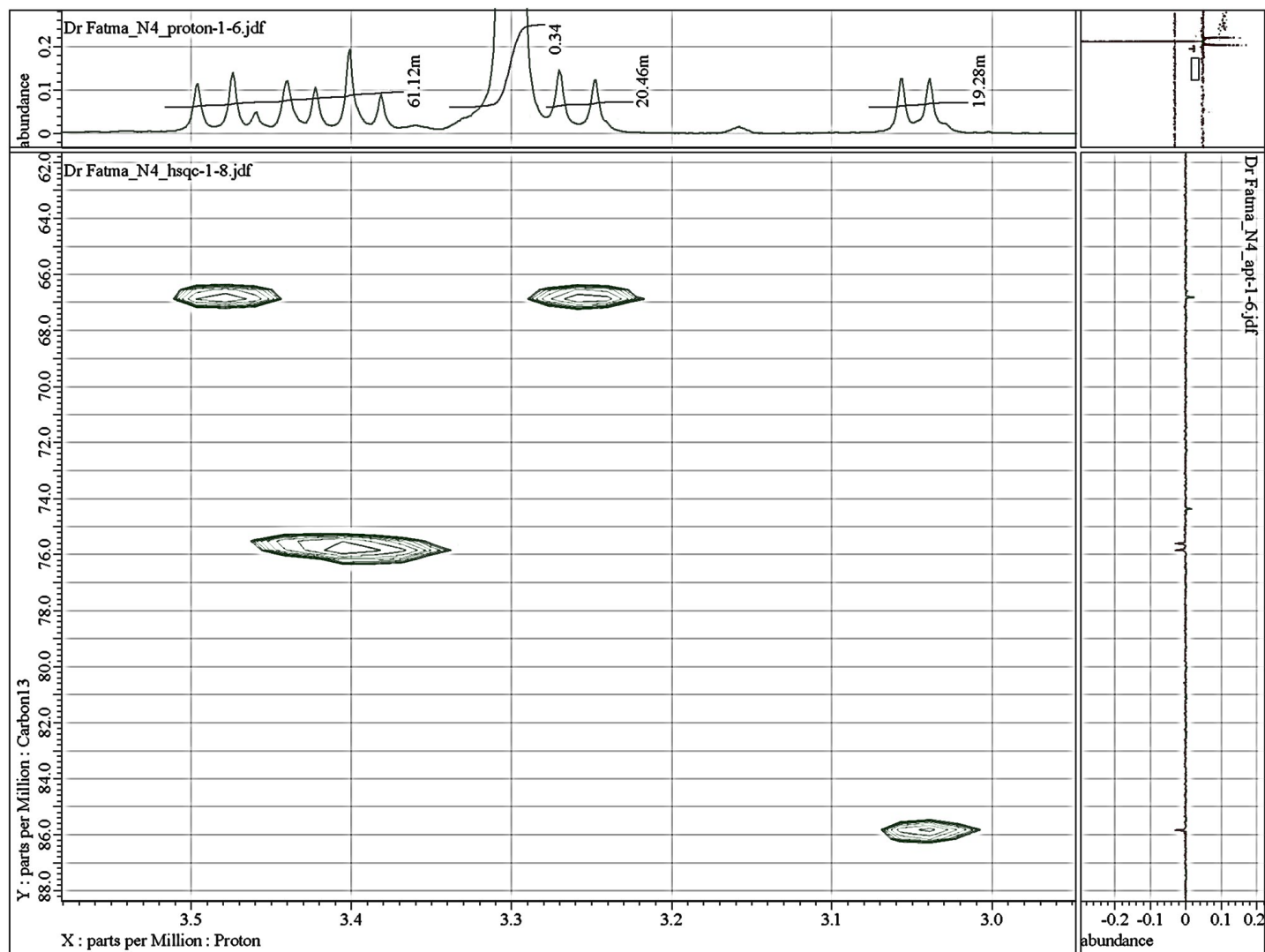


Figure S14. Expansion (3.05-3.58 ppm) of HSQC spectrum of compound **4** in CD₃OD.

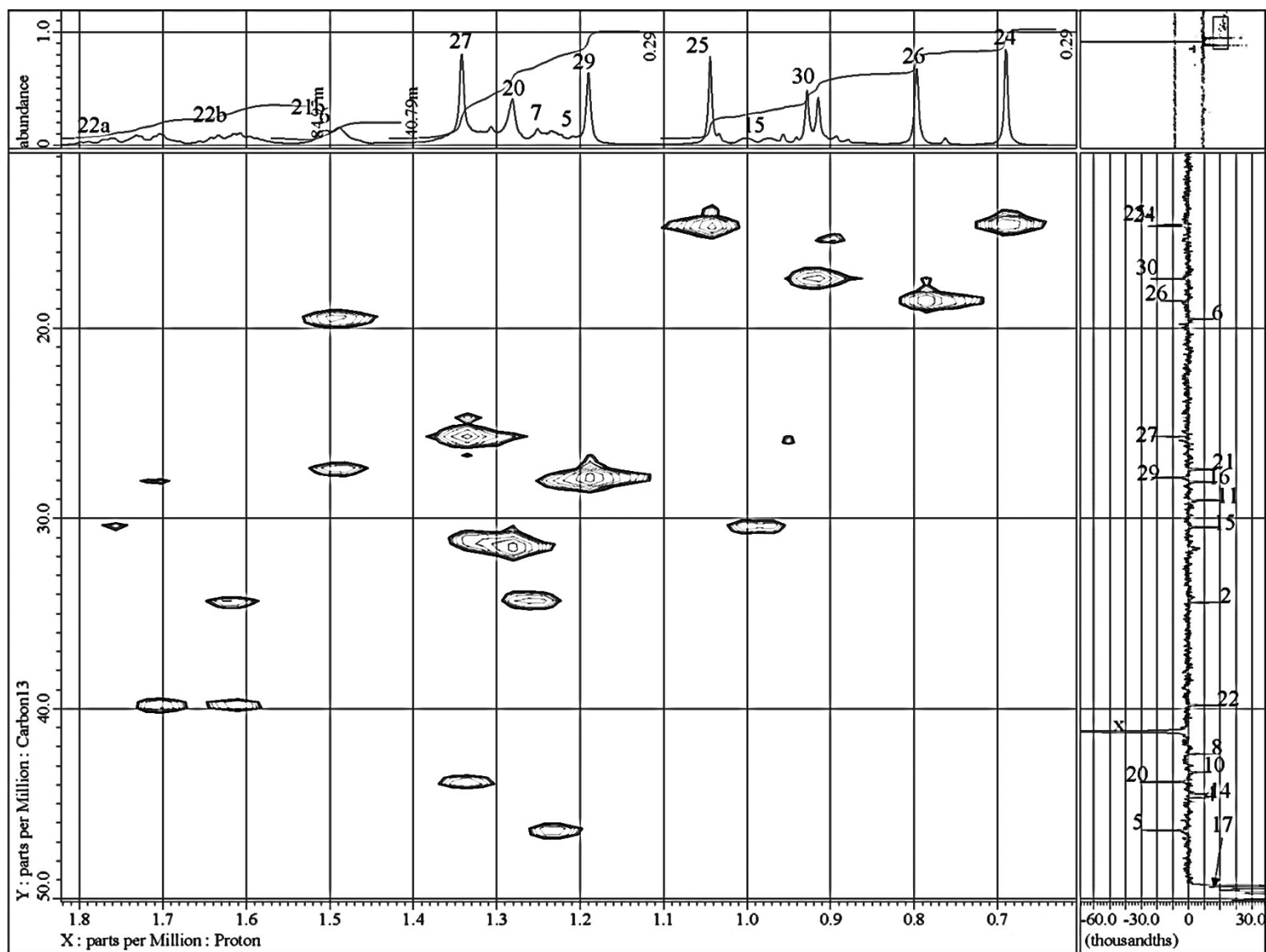


Figure S15. Expansion (0.62-1.82 ppm) of HSQC spectrum of compound 4 in CD₃OD.

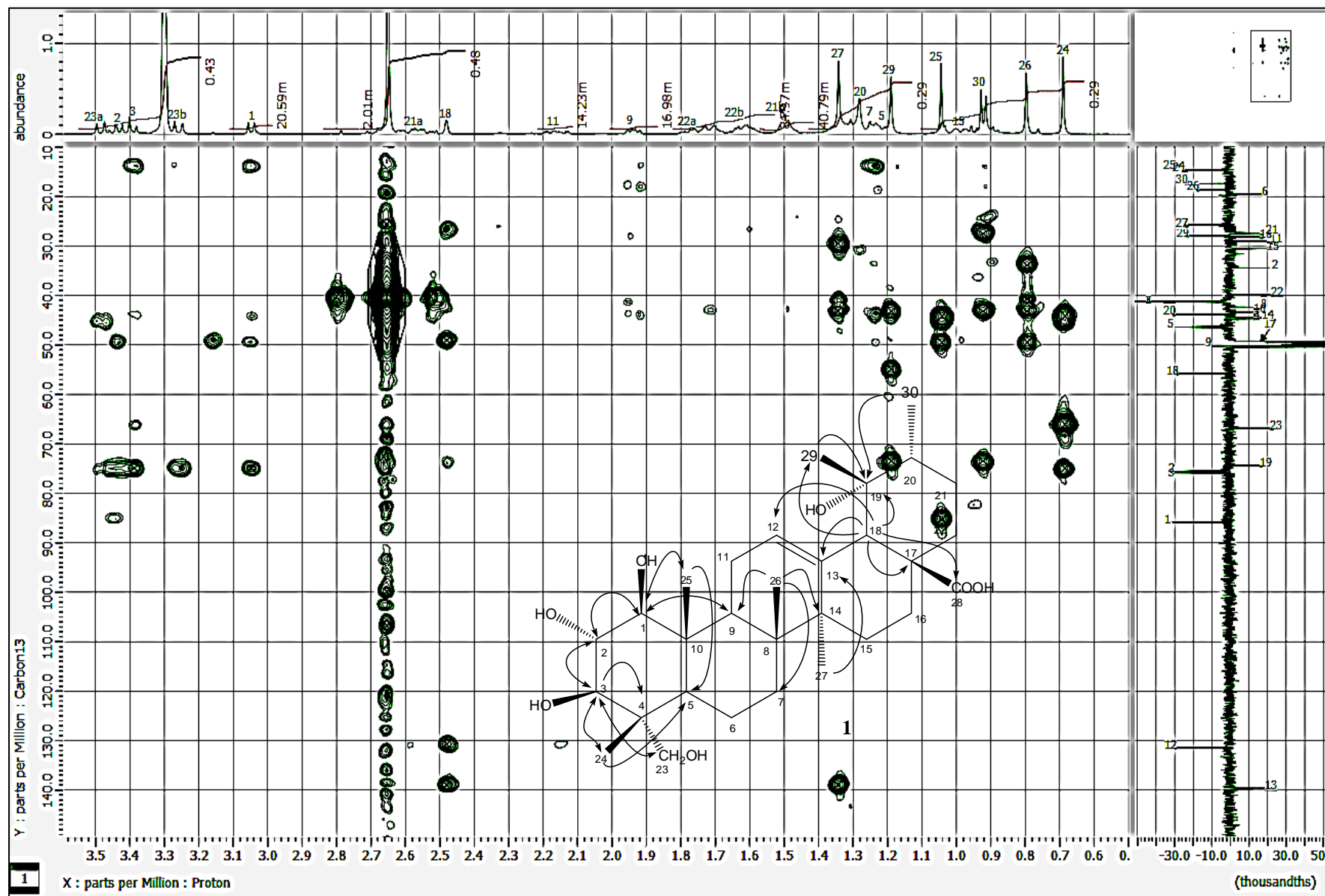


Figure S16. HMBC spectrum of compound 4 in CD₃OD.

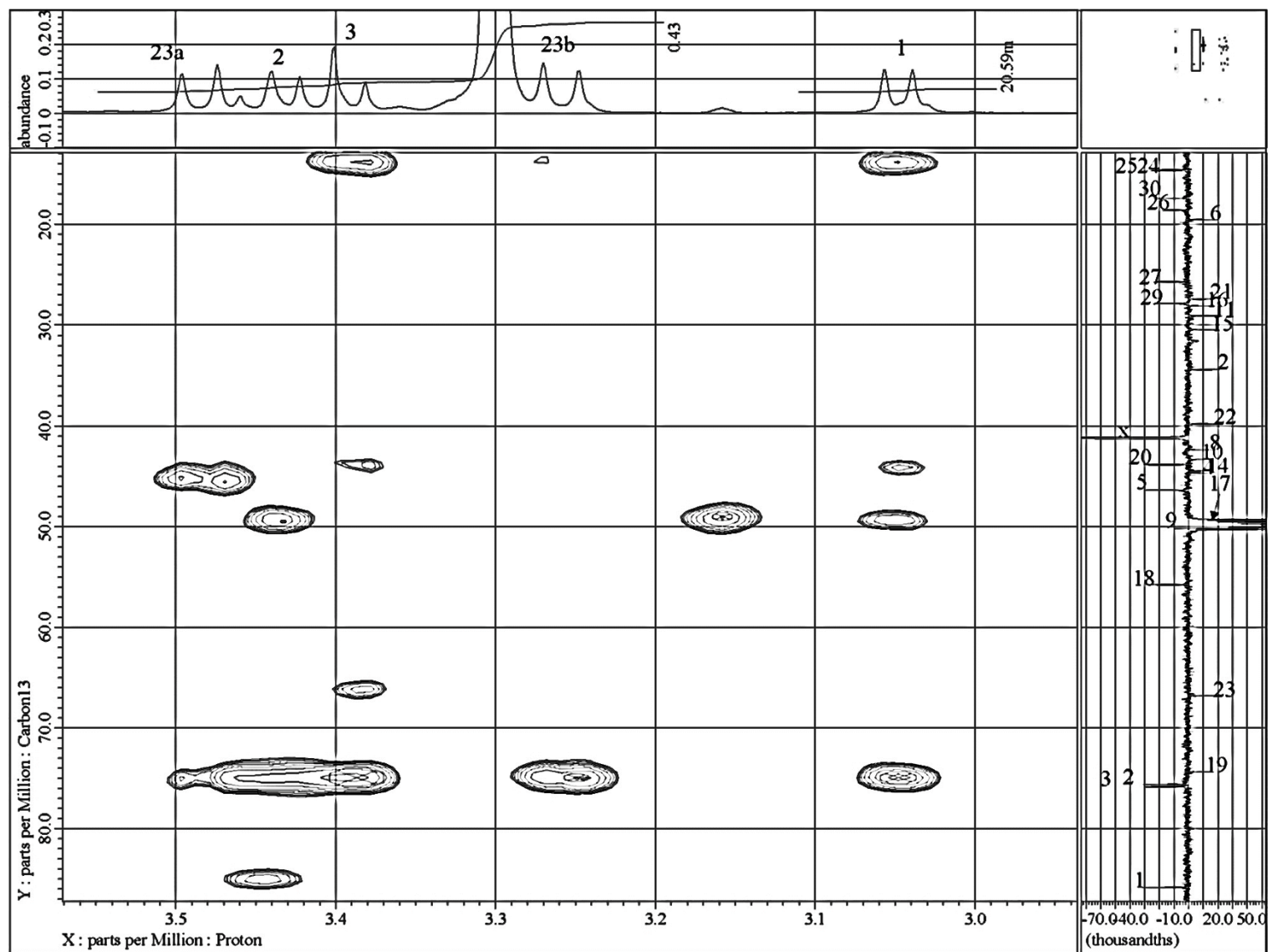


Figure S17. Expansion of HMBC spectrum (2.5-3.6 ppm) of compound **4** in CD₃OD.

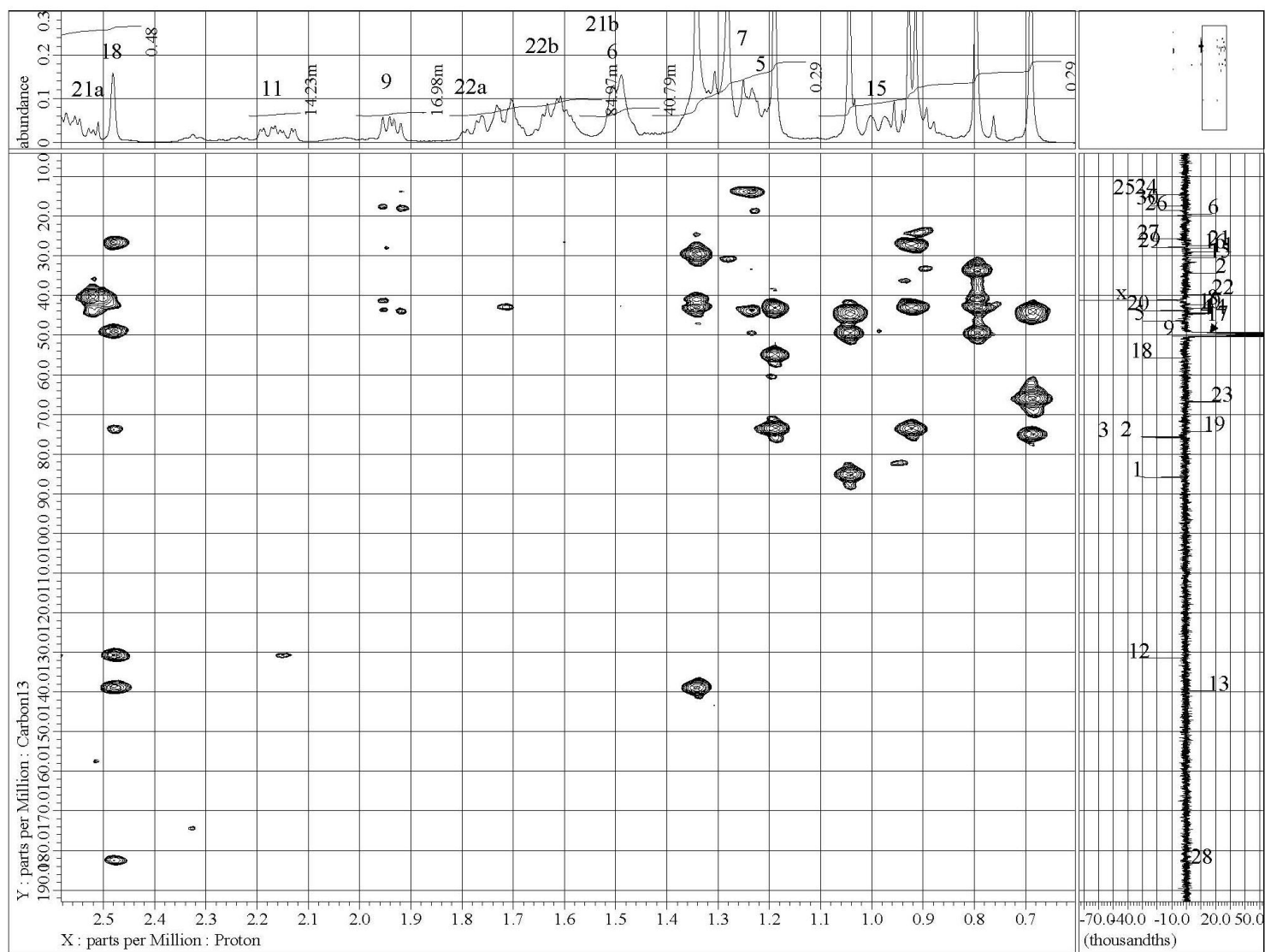


Figure S18. Expansion of HMBC spectrum (0.6-2.6 ppm) of compound 4 in CD₃OD.

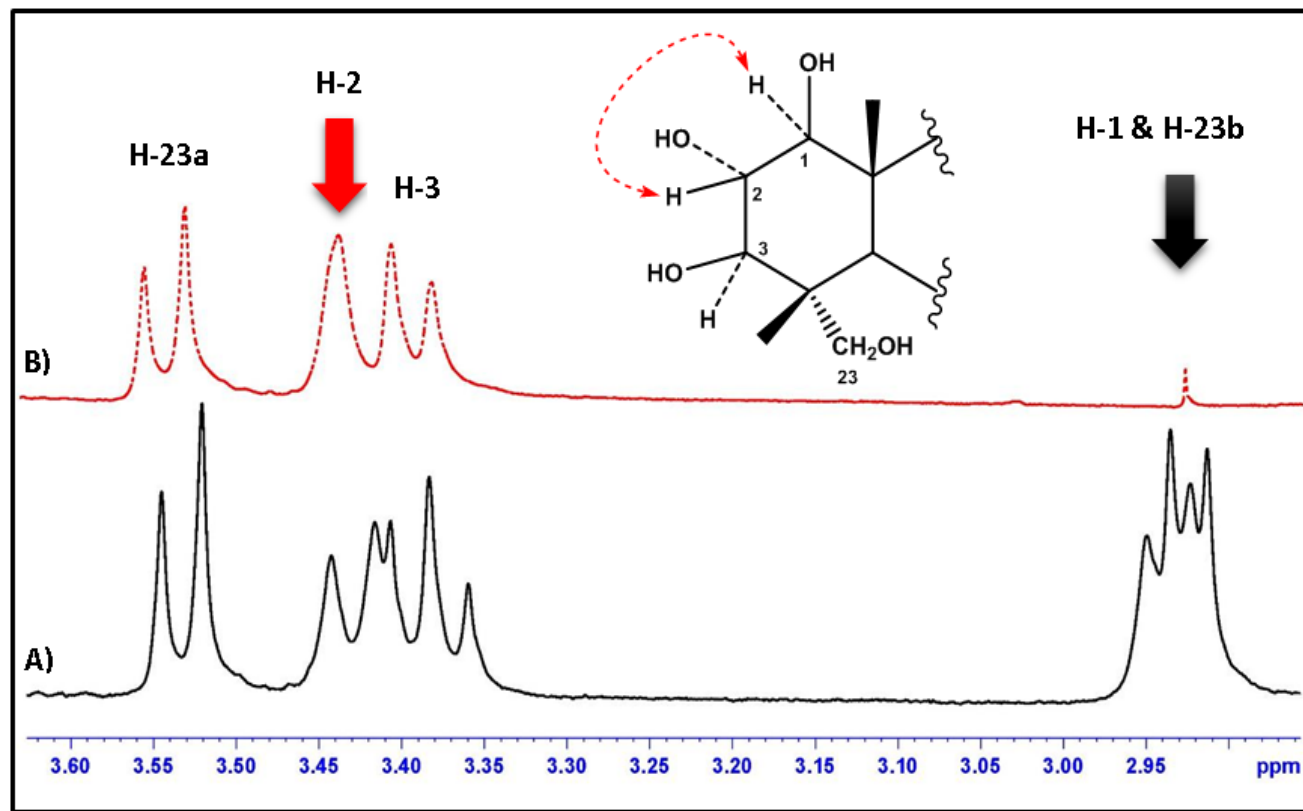


Figure S19. Expansion of ^1H NMR spectrum (2.90-3.60 ppm, 400 MHz, pyridine- d_6) of compound **4** showed **A**) Normal spectrum; **B**) Selective decoupling experiment of H-1 and consequent decoupling of H-2.

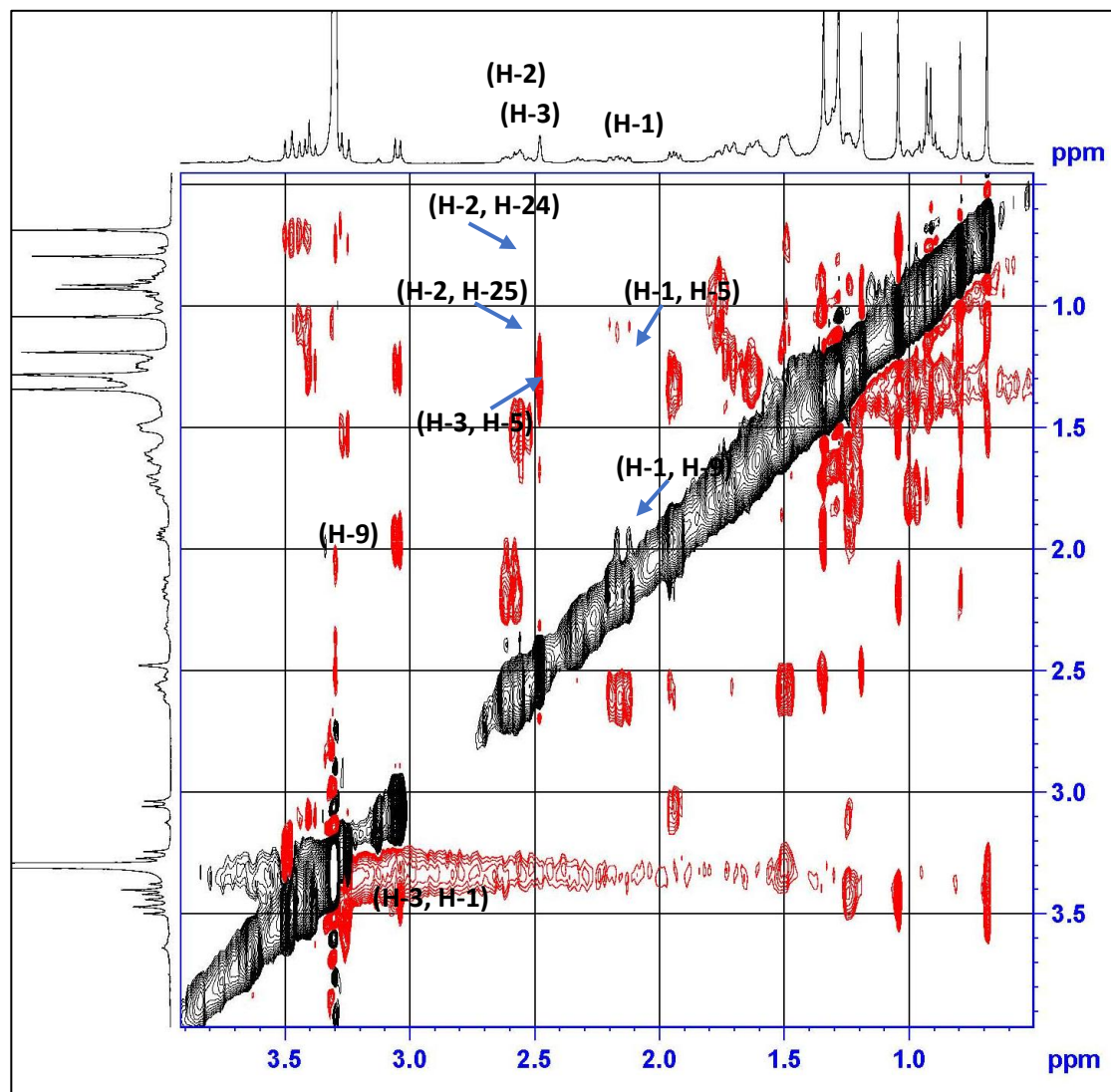
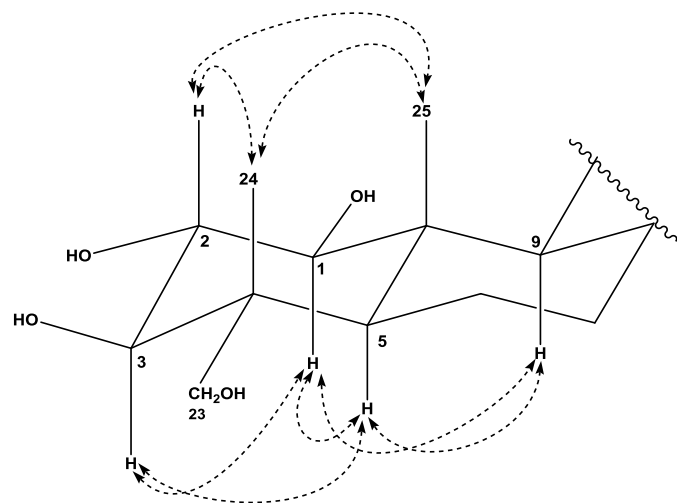


Figure S20. Selected key NOESY correlations of compound **4**.

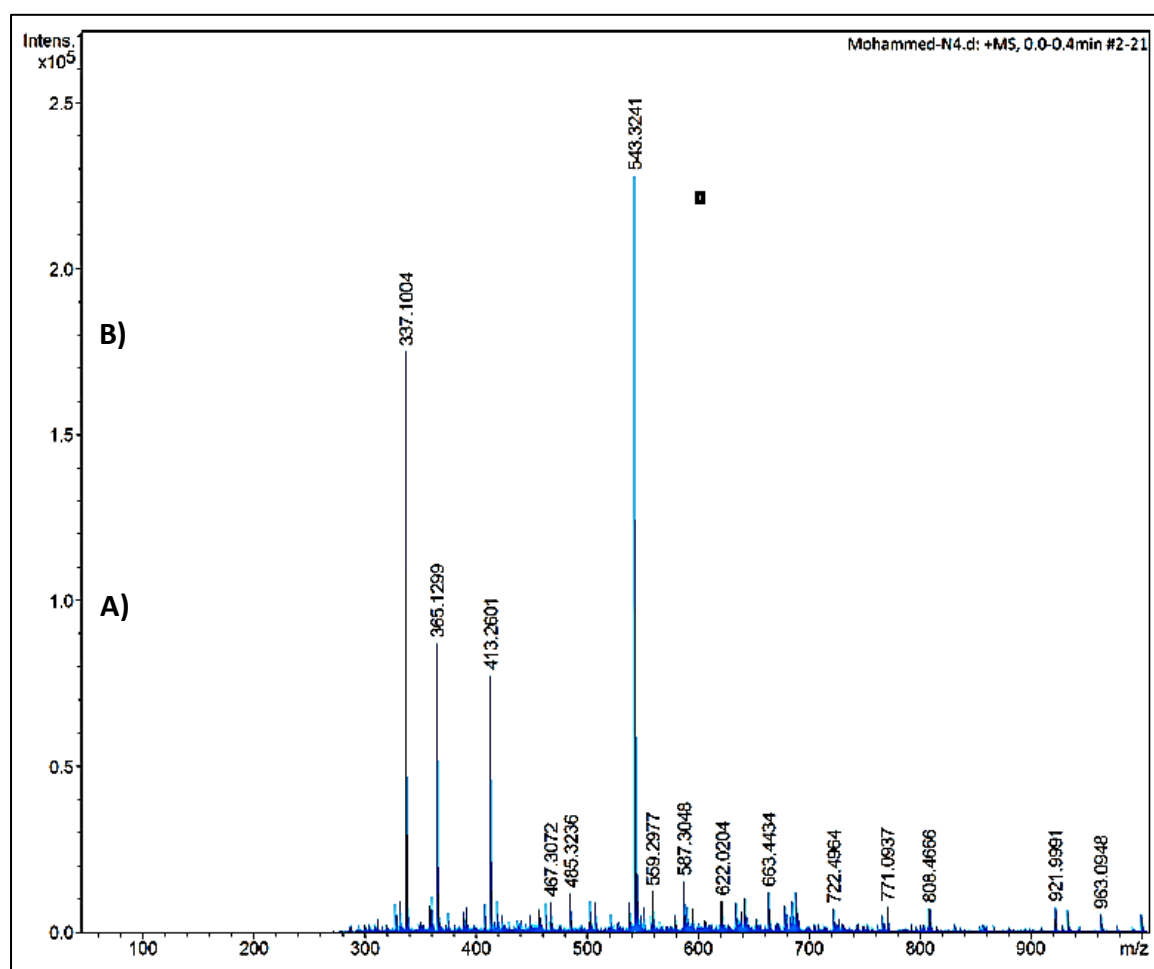


Figure S21. High resolution mass (HRMS) spectrum of compound **4**.

Table S5. Comparison of ^{13}C NMR chemical shift values (ppm) for C-1, C-2 and C-3 of compound **4** with those published for 1,2,3,23,19-pentahydroxy-urs-12-en-28-oic acids.

Configuration A-ring C #	\square_{C} (ppm)			4 ***
	$\square\square\square\square\square\square\square\square(\text{OH})^*$ (Wandji et al. 2003)	$\square\square\square\square\square\square\square\square(\text{OH})^{**}$ (Chen et al. 2015)	$\square\square\square\square\square\square\square\square(\text{OH})^{**}$ (Gupta and Singh 1989)	
C-1	77.5	81.0	74.9	85.8
C-2	71.0	71.8	74.6	75.6
C-3	80.1	79.4	79.9	75.8

*

Measured in $\text{DMSO}-d_6$.

** Measured in pyridine- d_5 .

*** Measured in methanol- d_4 .

Compound 5:

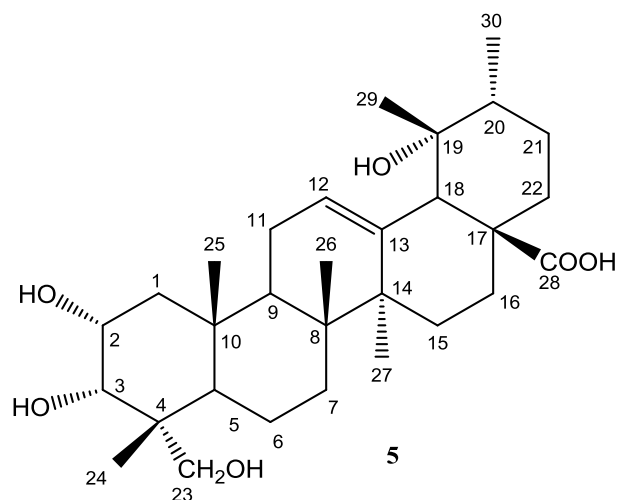


Table S6. ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **5** in CD_3OD :

H/C no.	Type	APT (\square_{C})	\square_{H} (J in Hz)
1	CH_2	41.9	
2	CH	70.5	3.65, ddd (11.5, 4.5, 2.0)
3	CH	79.0	3.33, d (9.5)
4	C	44.9	---
5	CH	43.9	
6	CH_2	20.0	
7	CH_2	34.3	
8	C	39.9	---
9	CH	48.8	
10	C	39.8	---
11	CH_2	25.6	
12	CH	130.0	5.27, brs
13	C	141.0	---
14	C	43.5	---
15	CH_2	30.4	
16	CH_2	27.4	
17	C	48.6	--
18	CH	55.9	2.47, s
19	C	74.4	---
20	CH	41.0	
21	CH_2	28.1	
22	CH_2	39.8	
23	CH_2	67.1	H _a : 3.47, d (11.5) H _b : 3.24, d (11.5)
24	CH_3	14.7	0.68, s
25	CH_3	18.3	0.77, s
26	CH_3	18.3	1.01, s
27	CH_3	25.7	1.17, s
28	C	181.4	---
29	CH_3	27.9	1.32, s
30	CH_3	17.4	0.90, d (6.5)

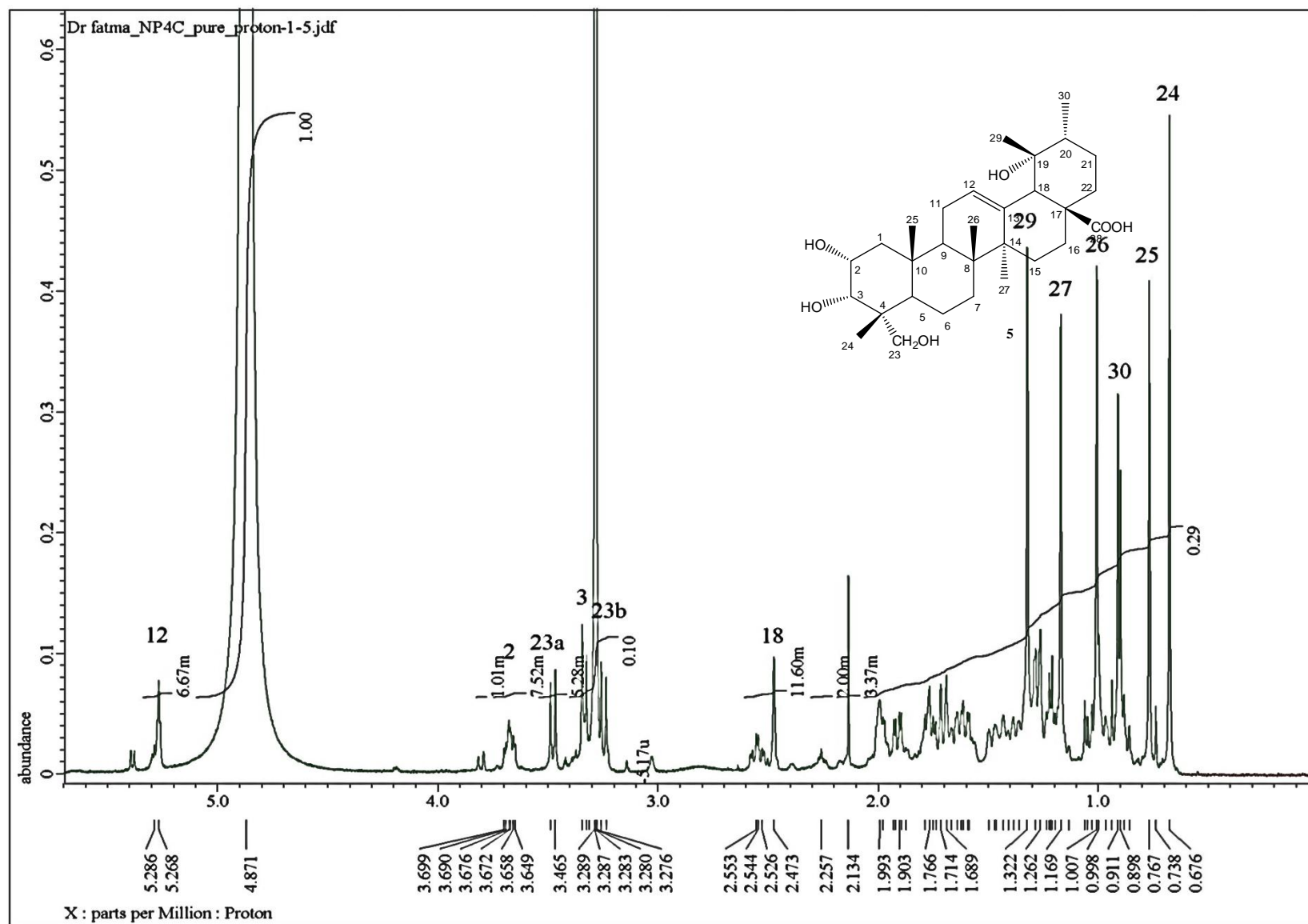


Figure S22. ^1H NMR (500 MHz) spectrum of compound **5** in CD_3OD .

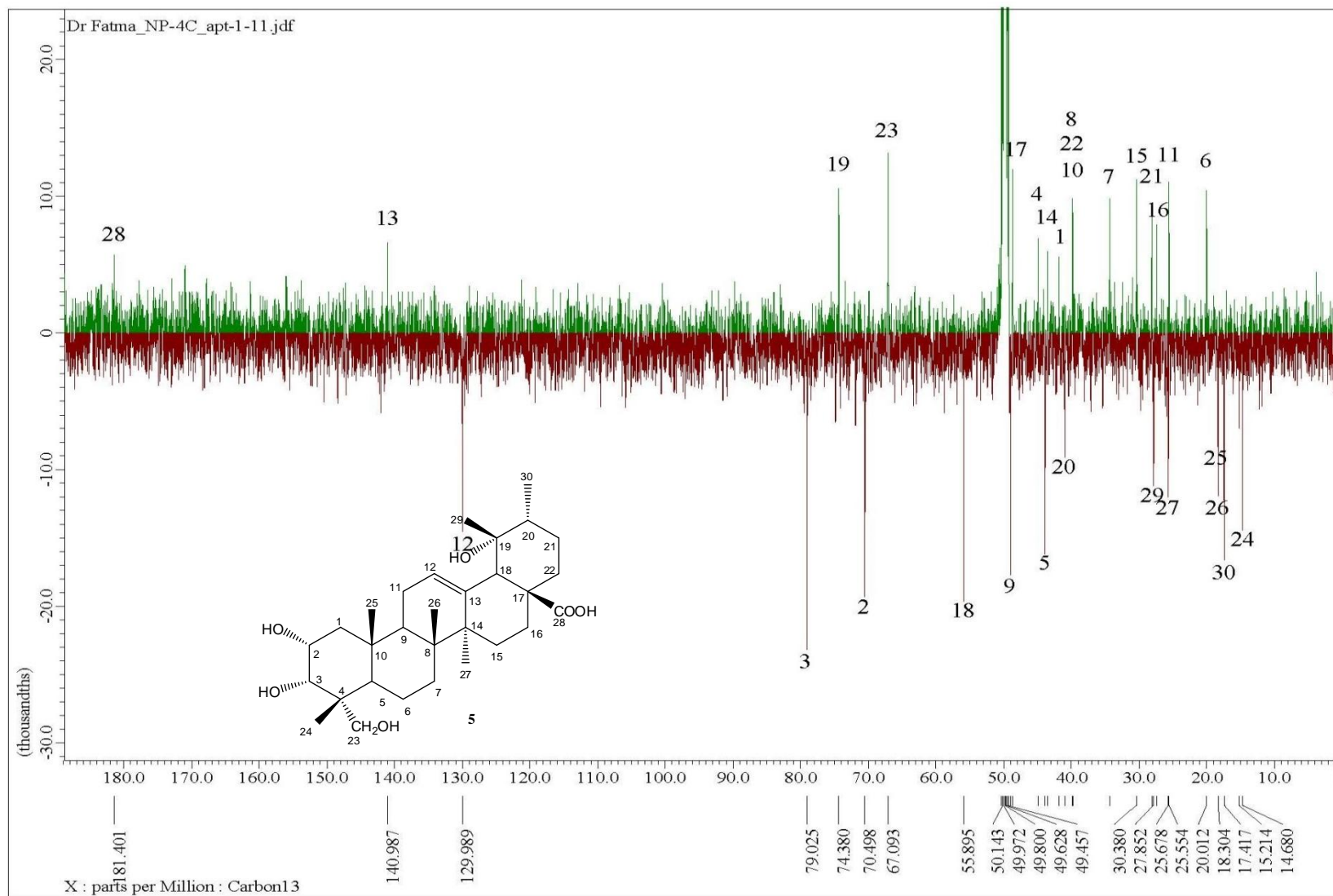


Figure S23. ^{13}C NMR (APT, 125 MHz) spectrum of compound **5** in CD_3OD .

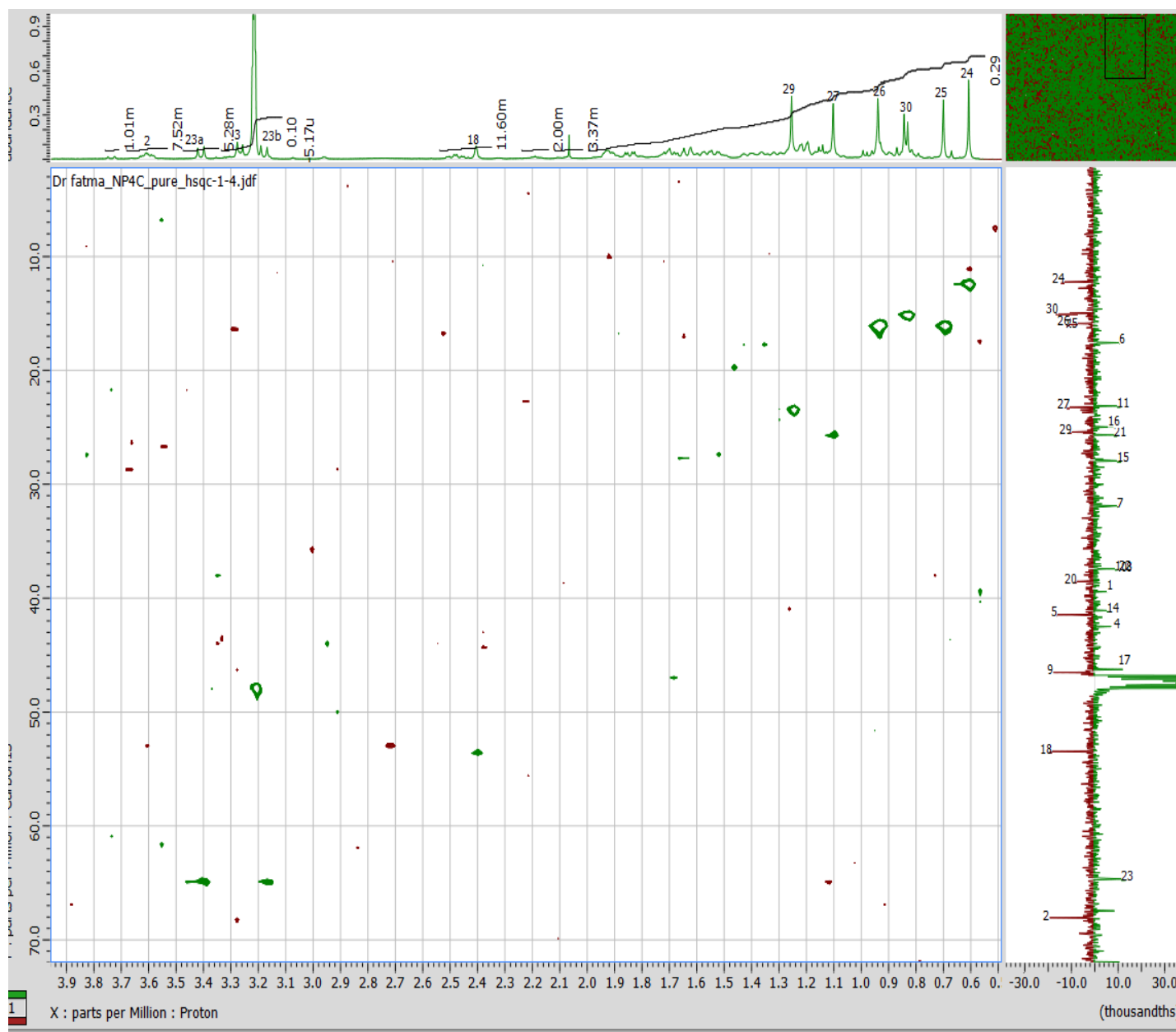
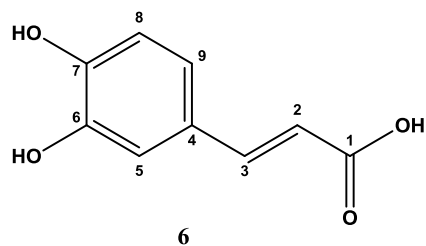


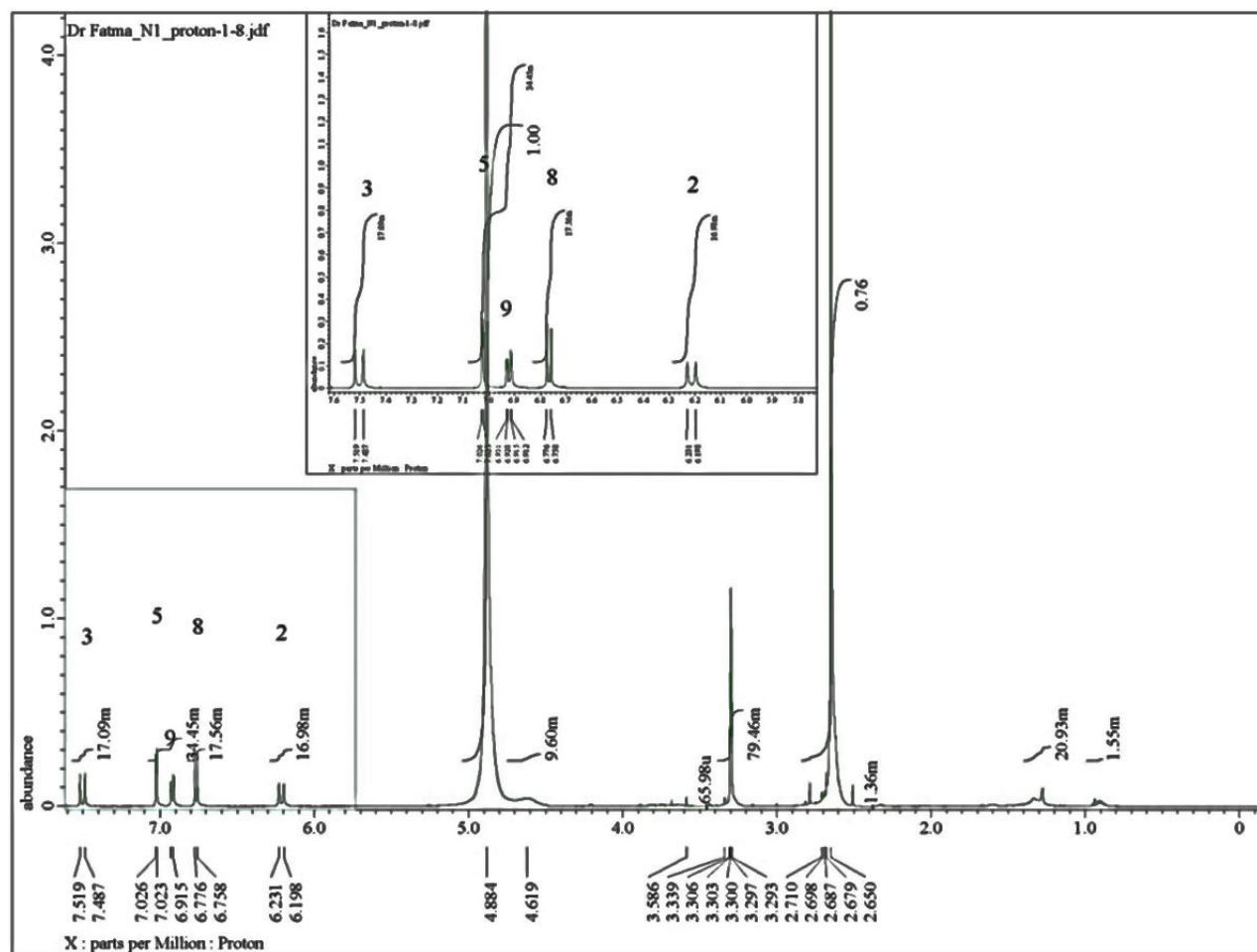
Figure S24. HSQC spectrum of compound **5** in CD₃OD.

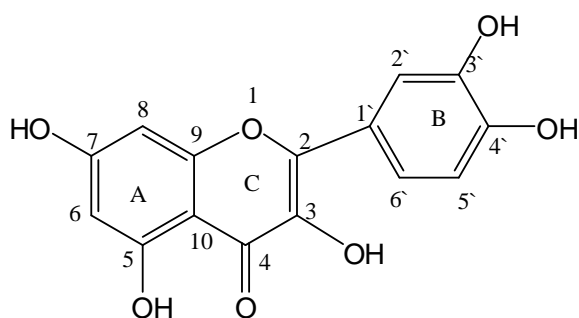
Figure S25. HMBC spectrum of compound **5** in CD₃OD.

Compound 6:

Table S7. ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **6** in CD_3OD :

H/C no.	\square_{H} (J in Hz)
2	6.20 ($J=16.5$ Hz)
3	7.49 ($J=16.0$ Hz)
5	7.02 ($J=1.5$ Hz)
8	6.76 ($J=9.0$ Hz)
9	6.91 ($J=9.0, 1.5$ Hz)

Figure S26. ^1H NMR (500 MHz) spectrum of compound **6** in CD_3OD .

Compound 7:**Table S8.** ^1H (500 MHz) and ^{13}C NMR (125 MHz) data of compound 7 in CD_3OD :

Position	Compound N ₂	
	^{13}C NMR (δ_{C})	δ_{H} (J in Hz)
2	149.6	---
3	138.1	---
4	178.2	---
5	163.3	---
6	100.0	6.17, 1H, brs
7	166.4	-----
8	95.2	6.39, 1H, brs
9	159.0	---
10	105.3	---
1'	124.9	---
2'	116.8	7.73
3'	147.0	---
4'	148.8	---
5'	117.0	6.87, 1H, d, (8.5)
6'	122.5	7.62, 1H, d, (8.5)

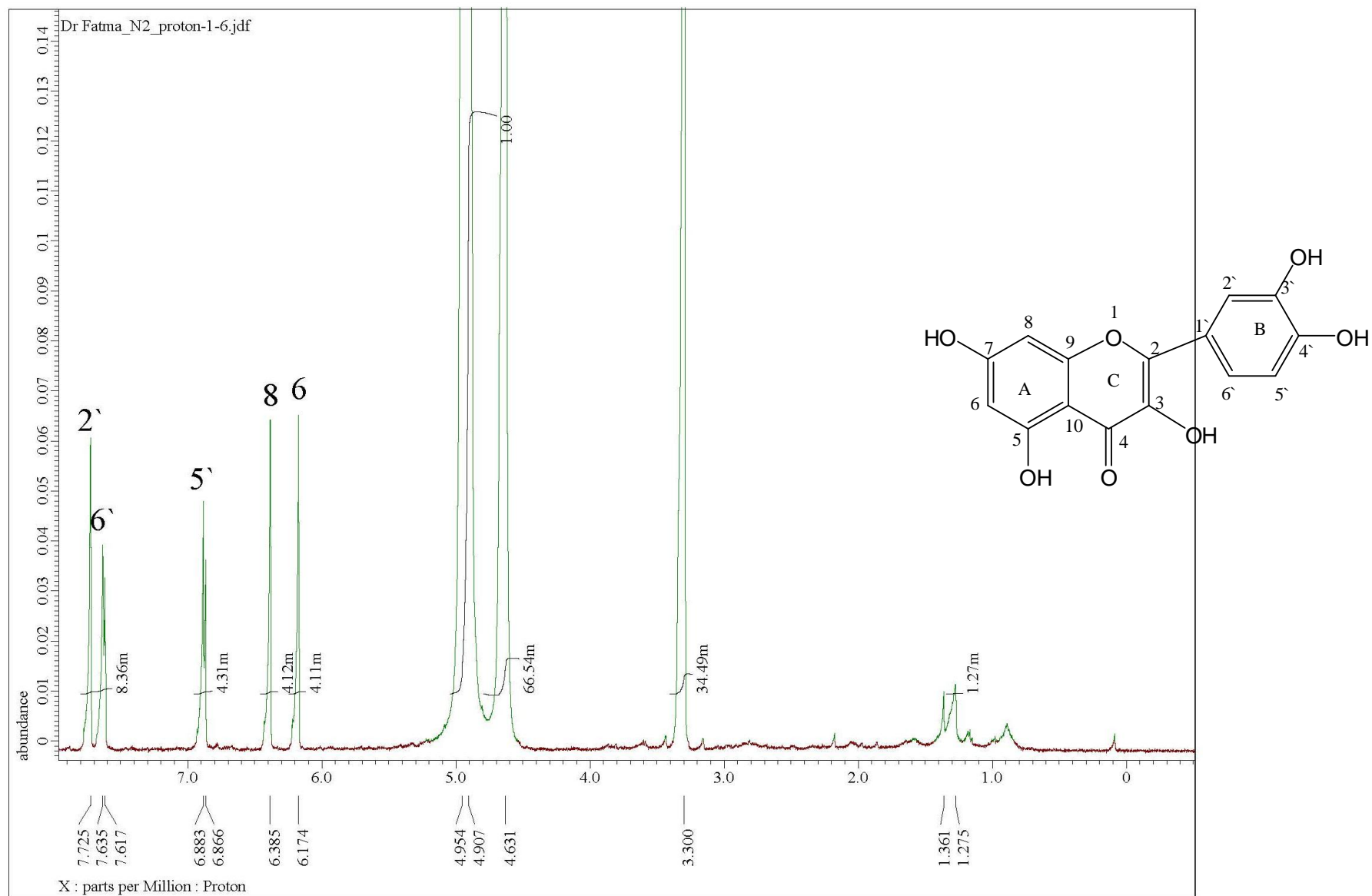


Figure S27. ^1H NMR (500 MHz) spectrum of compound **7** in CD_3OD .

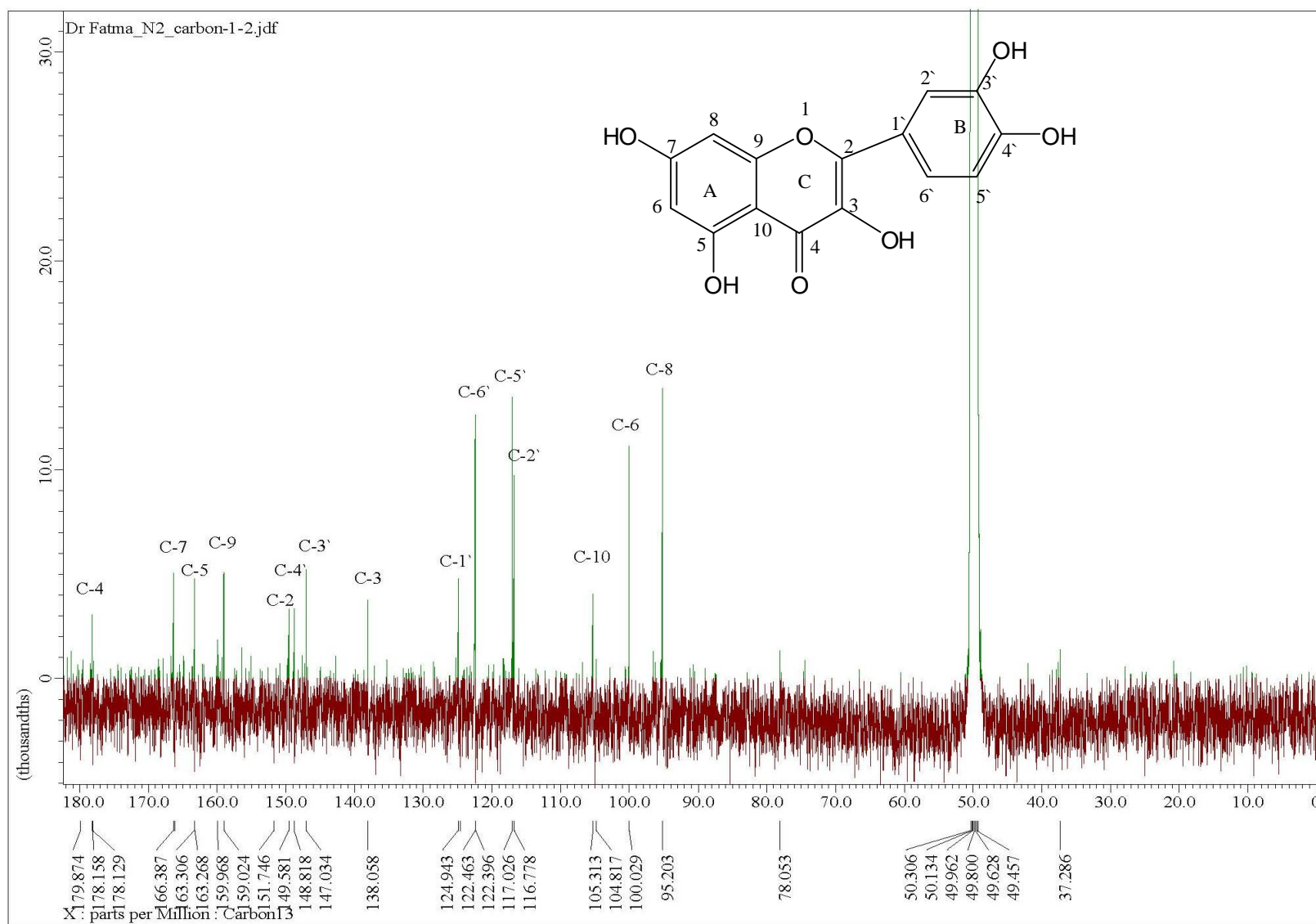
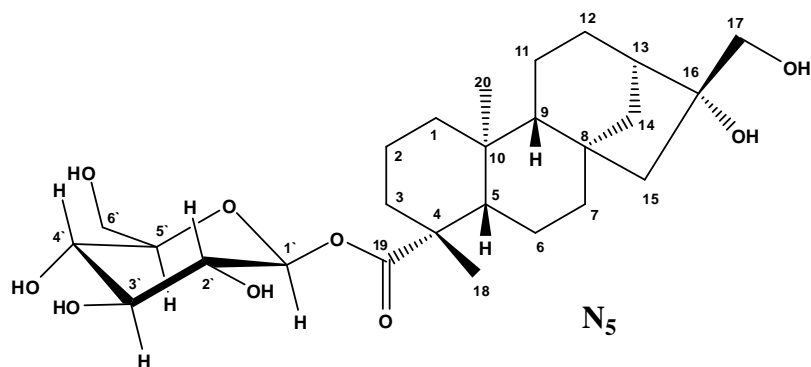


Figure S28. ^{13}C NMR (125 MHz) spectrum of compound **7** in CD_3OD .

Compound 8:

Table S9. ^1H (500 MHz) and ^{13}C NMR (125 MHz) data of compound **8** in CD_3OD :

H/C no.	Type	APT (Δ_C)	Δ_H (J in Hz)
1	CH_2	42.6	0.97-2.18 (Not assigned)
2	CH_2	20.9	
3	CH_2	41.7	
4	C	45.9	---
5	CH	59.3	0.80 (2H), td (12.5, 3.5)
6	CH_2	24.0	0.97-2.18 (Not assigned)
7	CH_2	44.1	
8	C	46.6	
9	CH	58.1	
10	C	39.9	
11	CH_2	20.5	
12	CH_2	28.0	
13	CH	47.0	
14	CH_2	38.9	
15	CH_2	54.5	
16	C	83.8	---
17	CH_2	67.7	H_a : 3.80, d (12.0); H_b : 3.57, d (12.0)
18	CH_3	29.8	1.20, s
19	C	179.0	---
20	CH_3	17.2	0.97, s
1'	CH	96.4	5.39, d (8.0)
2'	CH	74.8	3.30-3.40
3'	CH	79.5	
4'	CH	71.9	
5'	CH	79.5	
6'	CH_2	63.2	H_a : 3.69, d (5.5) ; H_b : 3.67, d (4.5)

Figure S29. ^1H NMR (500 MHz) spectrum of compound **8** in CD_3OD .

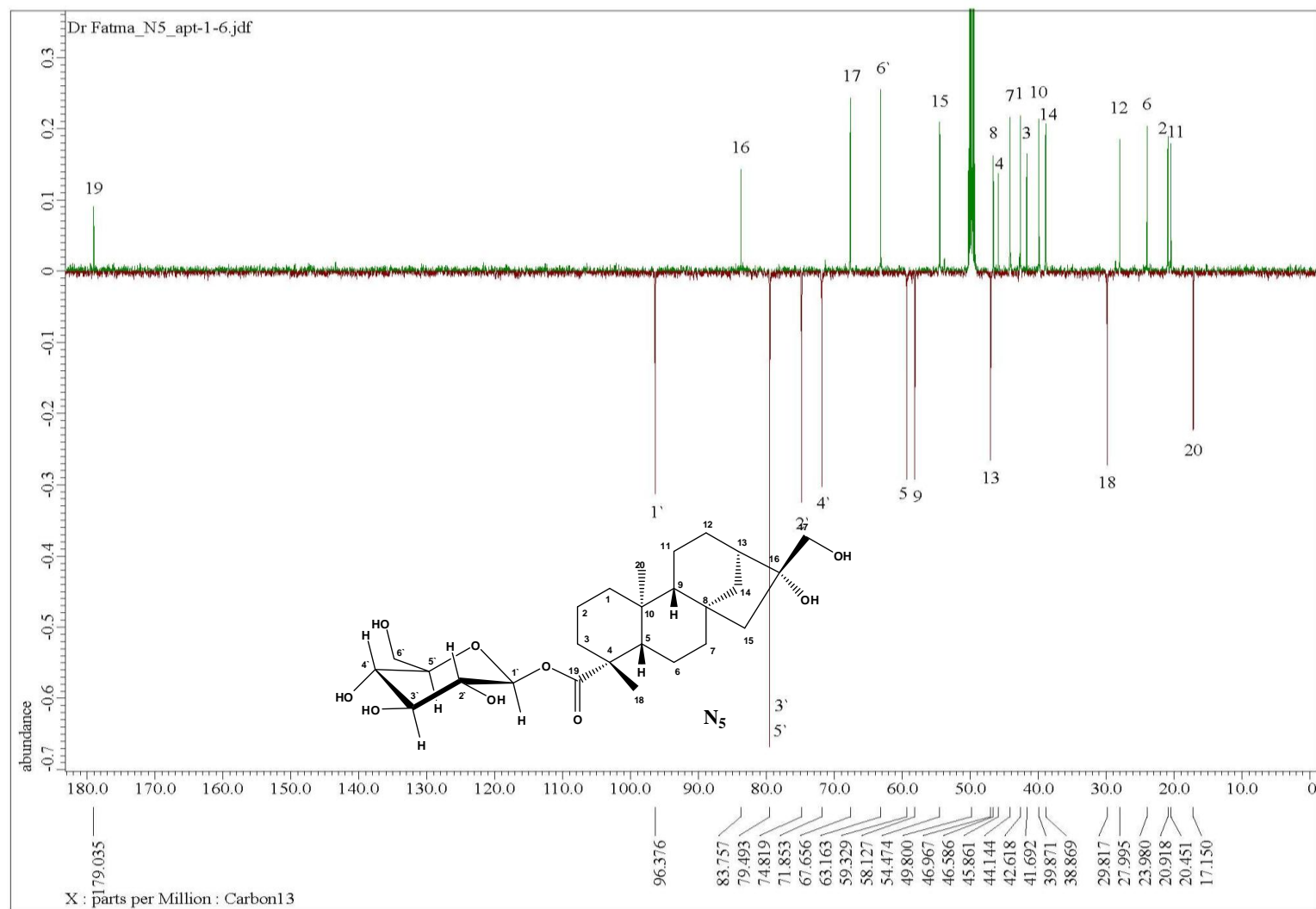
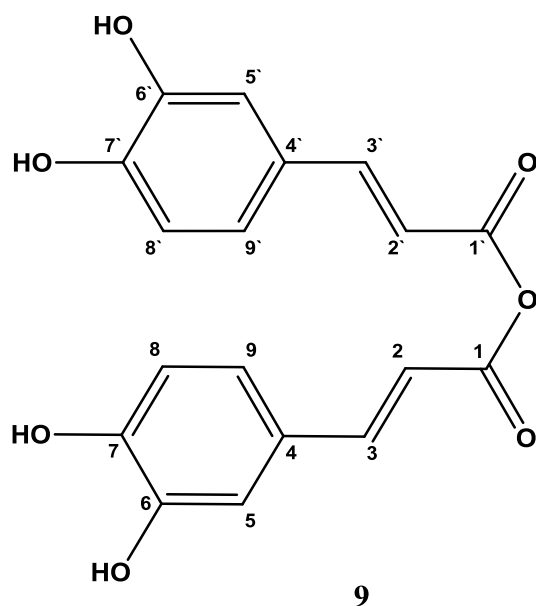


Figure S30. ^{13}C NMR (APT, 125 MHz) data of compound **8** in CD_3OD .

Compound 9:

Table S10. ^1H (500 MHz) and ^{13}C NMR (APT, 125 MHz) data of compound **9** in CD_3OD :

H/C no.	Type	APT (ΔC)	ΔH (J in Hz)
1	C	168.8	--
2	CH	117.2	6.25, d (16.0)
3	CH	148.1	7.56, d (16.0)
4	C	128.5	--
5	CH	115.9	7.05, d (2.0)
6	C	147.5	--
7	C	150.4	--
8	CH	116.0	6.77, d (8.5)
9	CH	123.8	6.92, d (8.5, 2)
1'	C	168.8	--
2'	CH	117.2	6.25, d (16.0)
3'	CH	148.1	7.56, d (16.0)
4'	C	128.5	--
5'	CH	115.9	7.05, d (2.0)
6'	C	147.5	--
7'	C	150.4	--
8'	CH	116.0	6.77, d (8.5)
9'	CH	123.8	6.92, d (8.5, 2)

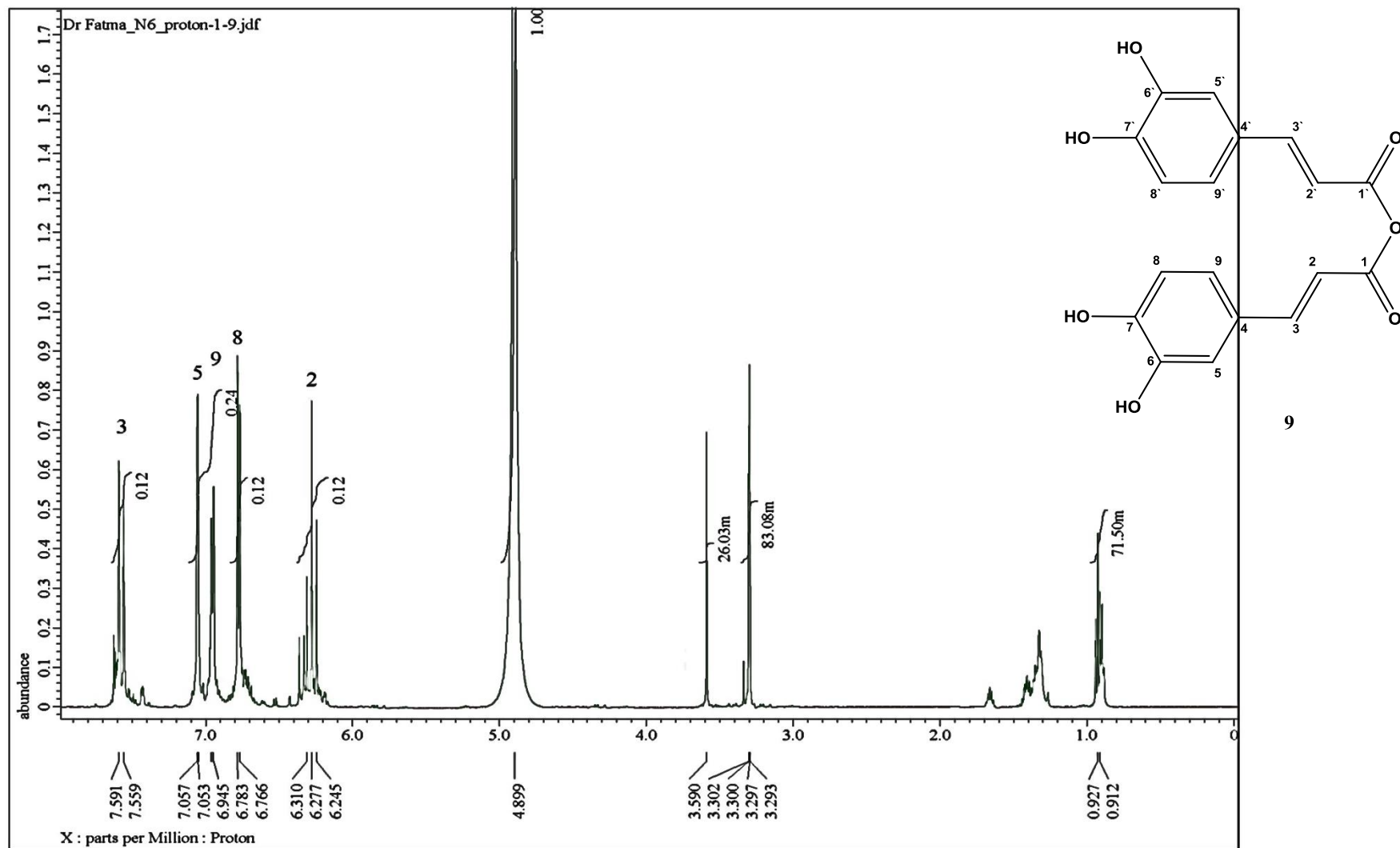


Figure S31. ^1H NMR (500 MHz) spectrum of compound **9** in CD_3OD .

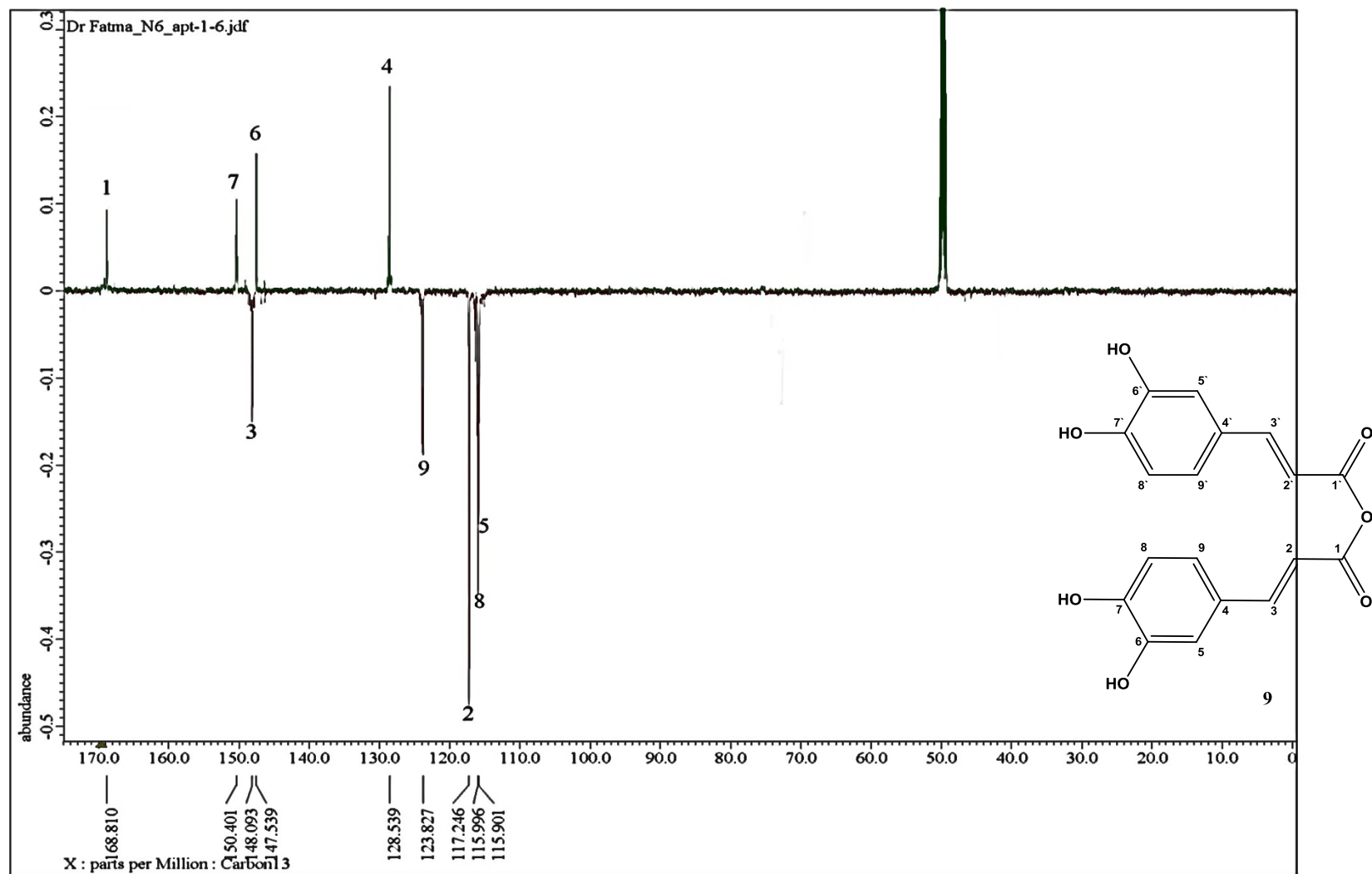


Figure S32. ^{13}C NMR (APT, 125 MHz) spectrum of compound **9** in CD_3OD .

Table S11. Susceptibility testing and MIC values ($\mu\text{g/mL}$) of compounds or extracts of *Pulicaria undulata* L.

Organism	<i>E. coli</i>		<i>K. pneumoniae</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>		<i>C. albicans</i>	
	IZD ^{a,b}	MIC	IZD ^{a,b}	MIC	IZD ^{a,b}	MIC	IZD ^{a,b}	MIC	IZD ^{a,b}	MIC
1	-	ND	8 \pm 1.0	2500	8 \pm 0.0	2500	7 \pm 0.0	2500	8 \pm 1.0	2500
2	14 \pm 0.5	625	12 \pm 1.0	1250	11 \pm 0.0	1250	18 \pm 2.0	312.5	16 \pm 1.0	625
3	8 \pm 1.0	2500	-	ND	12 \pm 0.5	1250	-	ND	10 \pm 1.0	2500
4	-	ND	-	ND	-	ND	-	ND	-	ND
5	-	ND	-	ND	-	ND	-	ND	-	ND
6	8 \pm 1.0	2500	10 \pm 0.5	1250	11 \pm 1.0	1250	18 \pm 1.0	312.5	15 \pm 0.0	625
7	7 \pm 0.0	2500	8 \pm 0.5	2500	12 \pm 1.0	1250	17 \pm 1.0	312.5	14 \pm 2.0	625
8	9 \pm 0.0	1250	-	ND	13 \pm 2.0	625	-	ND	8 \pm 1.0	2500
9	9 \pm 2.0	1250	8 \pm 0.0	2500	10 \pm 2.0	1250	15 \pm 1.0	1250	12 \pm 2.0	1250
MeOH extract	13 \pm 1.0	625	9 \pm 1.0	2500	11 \pm 1.0	1250	12 \pm 0.0	625	14 \pm 1.0	625
EtOAc extract	-	ND	8 \pm 0.0	2500	8 \pm 1.0	2500	-	ND	-	ND
CH₂Cl₂ extract	14 \pm 1.0	625	10 \pm 0.0	1250	13 \pm 2.0	625	18 \pm 1.0	312.5	15 \pm 2.0	625
Ampicillin	25 \pm 0.0	39.062	18.8 \pm 0.2	78.125	18.1 \pm 0.1	78.125	21 \pm 0.0	156.25	-	ND
Gentamicin	26 \pm 0.0	19.531	25 \pm 0.0	78.125	20.4 \pm 0.2	39.062	31 \pm 0.0	19.531	-	ND
Fluconazole	-	ND	-	ND	-	ND	-	ND	24.7 \pm 0.0	78.125
DMSO	-	ND	-	ND	-	ND	-	ND	-	ND

E. coli: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus* and *C. albicans*: *Candida albicans*. ^a Results are expressed as the mean of triplicates \pm SD. ^b Not active (-); weak activity (2-9 mm); moderate activity (10-15 mm); strong activity (> 15 mm). ND: not determined.

Table S12. Anti-quorum sensing activity of the compounds and extracts of *Pulicaria undulata*.

Compound/ extract	Quorum-sensing inhibition against <i>Ch. violaceum</i> ^a	Compound/ extract	Quorum-sensing inhibition against <i>Ch. violaceum</i> ^a
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1	-	8	-
2	4.5 ± 0.1	9	-
3	-	MeOH extract	3.3 ± 0.1
4	-	EtOAc extract	-
5	-	CH₂Cl₂ extract	3.5 ± 0.2
6	-	Catechin	4.8 ± 0.0
7	4.0 ± 0.2	DMSO	-

^a QS inhibition (radius of pigment inhibition in mm) = radius of growth and pigment inhibition (r2) - radius of bacterial growth inhibition (r1).

References:

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