SUPPLAMENTARY MATERIAL

Chemical constituents isolated from the aerial parts of *Helleborus cyclophyllus* (A. Braun) Boiss. (Ranunculaceae), evaluation of their antioxidant and anti-inflammatory activity *in vitro* and virtual screening of molecular properties and bioactivity score

Olga St. Tsiftsoglou[1], Michalis K. Stefanakis[2], Eirini N. Kalpourtzi[1], Dimitra I. Hadjipavlou-Litina[3], Diamanto M. Lazari[1*]

[1]Laboratory of Pharmacognosy, Faculty of Health sciences, School of Pharmacy, Aristotle University of Thessaloniki 54124, Thessaloniki, Greece; [2]Laboratory of Organic Chemistry, School of Sciences and Engineering, Department of Chemistry, University of Crete, Heraklion, Greece; [3]Department of Pharmaceutical Chemistry, Faculty of Health Sciences, School of Pharmacy, Aristotle University of Thessaloniki, Greece

Chemical investigation of ethyl acetate extract from the aerial parts of *Helleborus cyclophyllus* (A. Braun) Boiss. led to the isolation of ten natural products, and their structures were identified to be: 2-deoxy-D-ribono-1,4-lactone (**1**), 2-O-feruloyl-L-malate (**2**), three flavonoids: quercetin 3-O-*β*-D-galactopyranoside (**3**), quercetin 3-O-6″-(3-hydroxy-3-methyl-gloutaryl)-*β*-D-glucopyranoside (**4**) and quercetin 3-O-(2‴-caffeoylsophoroside) (**5**), 6-O-caffeoyl-1-O-*p*-coumaroyl-*β*-D-glucopyranoside (**6**), two ecdysteroids: 20-hydroxyecdysone (**7**) and polypodine B (**8**) and two bufadienolides: deglucohellebrin (**9**) and hellebrin (**10**), on the basis of MS and NMR spectra. This is the first report on the occurrence of compounds **(2)-(6)** in the genus *Helleborus*. The chemotaxonomic significance of these compounds was summarised. Bioactivity score, molecular and pharmacokinetic properties of the isolated compounds were calculated by online computer software program Molinspiration. Compounds showed promising bioactivity scores for drug targets. Moreover, some of the isolated phenolic compounds were tested for their antioxidant and antiinflammatory activities. Tested compounds present antioxidant ability correlated to the presence of the phenolic hydroxyl groups.

Key word: *Helleborus cyclophyllus***,** Ranunculaceae, triterpenoids, flavonoids

Corresponding author. e-mail:**dlazari@pharm.auth.gr**

1. Experimental

1.1. General experimental procedures

Column chromatography (C.C.) was carried out on silica gel 60 (Merck Art. 9385), gradient elution with the solvents mixtures indicated in each case. T.L.C.: silica gel (Kieselgel F_{254}, Merck, Art. 5554); Detection on T.L.C. plates: UV light (absorbance: 254 and 366 nm), vanillin-H_2SO_4 spray reagent on silica gel.

Spectroscopic data. NMR: The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in CD_3OD using: Bruker AMX 500 and AGILENT DD2 500 (500.1 MHz for ^{1}H-NMR and 125.5 MHz for ^{13}C-NMR) spectrometers. Chemical shifts are reported in δ (ppm) values relative to TMS (3.31 ppm for ^{1}H-NMR and 49.05 ppm for ^{13}C-NMR for CD_3OD). MS: LCQ Advantage (ion trap), Electrospray, Thermo, Source Voltage (kV): 4.00, Sheath Gas Flow Rate: 60, Aux Gas Flow Rate: 10, Capillary Temp. (C): 290^{o}C, Tube Lens Voltage (V): -95 V, (-) ESI Full Scan 110-1000.

1.2. Plant material

The whole plant *Helleborus cyclophyllus* (A. Braun) Boiss. [synonym: *H. odorus Willd.* subsp. *cyclophyllus* (A. Braun) Maire & Petitm.] was collected in February 2007 from the Hortiatis Mountain, Thessaloniki (Macedonia, Northern Greece). The plant was taxonomically identified by Dr. Nikos Krigas (Researcher in National Agricultural Research Foundation, Laboratory for the Conservation and Evaluation of Native and Floricultural Species, Thermi, Thessaloniki Greece). A voucher specimen has been deposited at the herbarium of TAU under No. Krigas N. & Lazari D. 8001.

1.3. Extraction and isolation

The air-dried aerial parts of the plant (364.62g) were finely ground and extracted repeatedly at room temperature with petroleum ether (40^{o}-60^{o}C), dichloromethane, methanol and methanol-water mixture (7:3). The methanol extract was concentrated, and the residue redissolved in boiling water (H_2O). The water-soluble fraction was filtered and extracted successively with diethyl-ether (Et_2O), ethyl acetate (EtOAc) and n-butanol (n-BuOH). The ethyl acetate residue (2.58g) was subjected to column chromatography on Sephadex LH-20 using MeOH (100%) to give several fractions

A-R. Fraction C (856.0mg) was submitted to C.C. on silica gel using CH_2Cl_2-MeOH-H_2O mixtures of increasing polarity as eluents to give fifteen fractions (CA-CP). Fraction CH (eluted with CH_2Cl_2-MeOH-H_2O 80:20:2.0, 73.1mg) was submitted to C.C. on Sephadex LH-20 with as eluent MeOH, to yield twelve fractions (CHA-CHM). Two of these fractions, CHC (11.3mg) and CHD (27.3mg) were identified as compound (**8**) (11.3mg and 27.3mg). Fraction CI (eluted with CH_2Cl_2-MeOH-H_2O 75:25:2.5, 197.3mg) was submitted to C.C. on silica gel using CH_2Cl_2-MeOH-H_2O mixtures of increasing polarity as eluents to give twenty three fractions (CIA-CIX). Fraction CIN (eluted with CH_2Cl_2-MeOH-H_2O 80:20:2.0, 53.3mg) was further fractionated by semipreparative HPLC (MeOH:H_2O, 1:1, 1.50ml/min) and allowed the isolation of compound (**7**) (t_R=13.76 min, 11.9mg). Also, fraction CIO (eluted with CH_2Cl_2-MeOH-H_2O 80:20:2.0, 53.8mg) was further fractionated by semipreparative HPLC (CH_3CN:H_2O, 1:1, 1.00ml/min) and allowed the isolation of compound (**7**) (t_R=11.40min, 6.3mg). Fraction CK (eluted with CH_2Cl_2-MeOH-H_2O 75:25:2.5, 109.2mg) was submitted to Sephadex LH-20 using MeOH (100%) as eluent to give seven fractions (CKA-CKG). Fraction CKC (49.9mg) was further fractionated by semipreparative HPLC (MeOH:H_2O, 1:1, 1.20ml/min) and allowed the isolation of compounds (**9**) (t_R=32.94 min, 0.6mg) and (**10**) (t_R=24.51 min, 1.7mg). Fraction E (582.8mg) was submitted to C.C. on silica gel using EtOAc-MeOH-H_2O mixtures of increasing polarity as eluents to give twenty fractions (EA-EU). Fraction EF (eluted with EtOAc-MeOH-H_2O 90:10:1.0, 36.4mg) was further fractionated by semipreparative HPLC (MeOH:H_2O, 1:1, 1.20ml/min) and allowed the isolation of compound (**1**) (tR=10.52 min, 5.4mg). Fraction G (131.3mg) was submitted to C.C. on silica gel using EtOAc-MeOH-H_2O mixtures of increasing polarity as eluents to give fifteen fractions (GA-GP). From these, fractions GN (eluted with EtOAc-MeOH-H_2O 50:50:5, 16.9mg) and GO (eluted with EtOAc-MeOH-H_2O 50:50:5, 15.7mg) was identified as compound (**2**). Fraction H (35.5mg) was further fractionated by semipreparative HPLC (MeOH-H_2O, 1:1, 1.00ml/min) and allowed the isolation of compounds (**4**) (t_R=29.29min, 4.3mg) and (**5**) (t_R=13.86min, 1.7mg). Fraction I (18.7mg) was further fractionated by semipreparative HPLC (MeOH-H_2O, 1:1, 1.00ml/min) and allowed the isolation of compounds (**4**) (t_R=30.20min, 4.4mg) and (**6**) (t_R=36.30min, 1.1mg). Fraction K (18.1mg) was further fractionated by semipreparative HPLC (MeOH-H_2O, 1:1,

1.00ml/min) and allowed the isolation of compounds (**3**) (t_R=28.46min, 1.1mg) and (**6**) (t_R=35.76min, 3.9mg).

1.4 In vitro experiments

1.4.1. Chemicals

1,1-Diphenyl 2-picryl hydrazyl (DPPH), Lipoxygenase (1.13.11.12) type I-B (Soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis. MO, USA). Nordihydroguaiaretic acid (NDGA) and trolox were purchased from Merck. All other chemicals were of analytical grade. A Perkin Elmer Lambda 20 UV-Vis spectrophotometer has been used for the radical scavenging activity experiments.

1.4.2 Inhibition of linoleic acid lipid peroxidation

Production of conjugated dienehydroperoxide by oxidation of sodium linoleate in an aqueous solution was monitored at 234 nm in the presence of 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) of 50 μl of 40 mM AAPH solution as a free radical initiator in 0.05 M phosphate buffer, pH 7.4. Oxidation was carried out in the presence of the tested samples (100μM). The rate of oxidation at room temperature was monitored by recording the increase in absorption at 234 nm caused by conjugated dienehydroperoxides. Trolox was used as a reference drug (Peperidou *et al*., 2014).

1.4.3 Soybean lipoxygenase inhibition study in vitro

The tested compounds dissolved in DMSO (10mM stock solution) were incubated (final concentration 100μM) at room temperature with sodium linoleate (0.1 mM) and 0.2 ml of enzyme solution (1/9x10-4 w/v in saline) in tris buffer pH 9. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm compared with the appropriate standard inhibitor Nordihydroguaiaretic acid (NDGA) (Peperidou *et al*., 2014).

1.4.4 Interaction with DPPH

To a solution of DPPH (0.1mM in methanol) the tested samples (stock solution 10mM) dissolved in DMSO were added (100μM). After 20/60 min the antioxidant activity was recorded at 517nm and the percentage of reducing activity (RA) was

calculated and compared to the reference compound NDGA (nordihydroguaiaretic acid) (Peperidou *et al*., 2014).

1.5. In silico study

Smiles notations of all the isolated compounds were fed in the online molinspiration software version 2011.06 (www.molinspiration.com) for calculation of molecular properties (Log P, Total polar surface area, number of hydrogen bond donors and acceptors, molecular weight, number of atoms, number of rotatable bonds etc.) and prediction of bioactivity score for drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors).

Table S1. ^{1}H and ^{13}C NMR of compound **1** (CD_3OD, 500 MHz)

No	Compound **1**	
	δ_C	δ_H
1	177.35	-
2_α	37.79	2.92 dd (J=12.5, 3.5)
2_β		2.38 dd (J=12.5, 3.5)
3	68.33	4.43 dt (J=7.0, 2.5)
4	88.80	4.37 dd (J=5.5, 3.5)
5_α	61.15	3.77 dd (J=12.5, 3.5)
5_β		3.69 dd (J=12.5, 3.5)

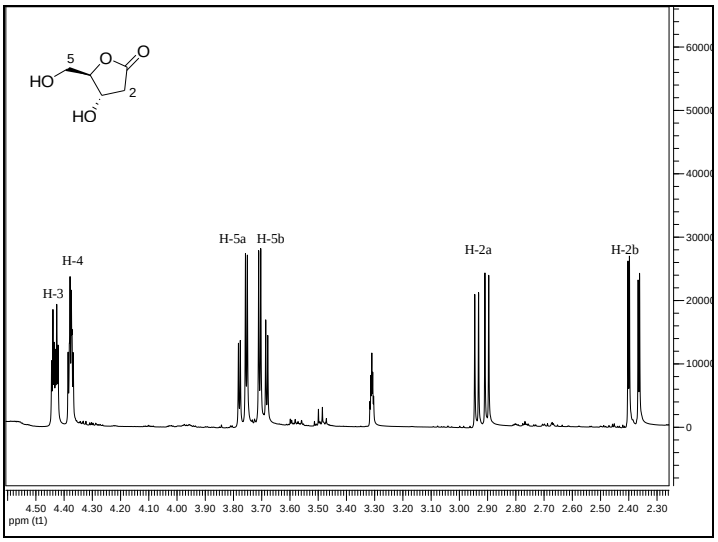


Figure S1.^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **1**

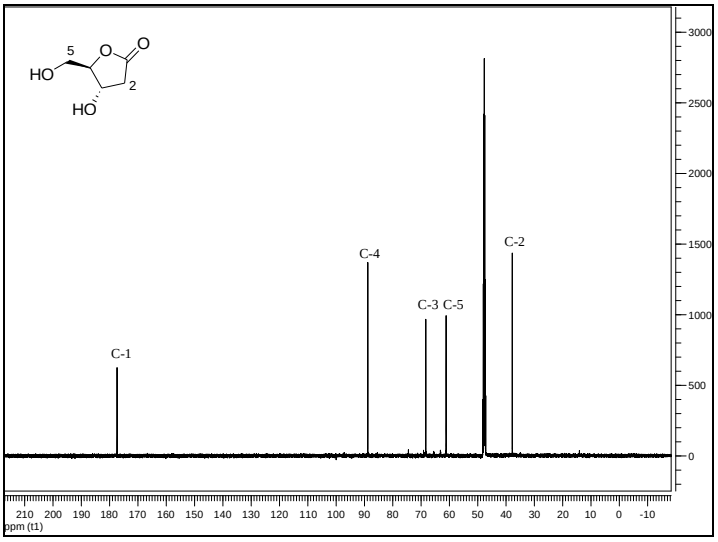


Figure S2.^{13}C-NMR Spectrum (CD_3OD, 125MHz) of Compound **1**

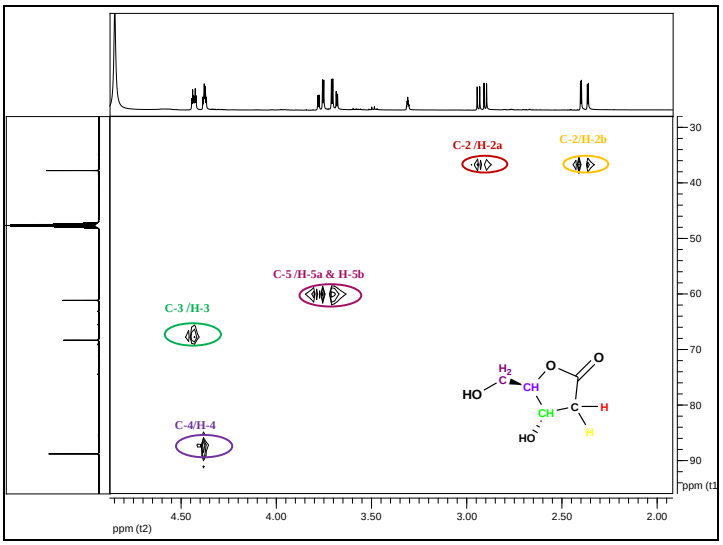


Figure S3. HSQC Spectrum (CD_3OD, 500 MHz) of Compound **1**

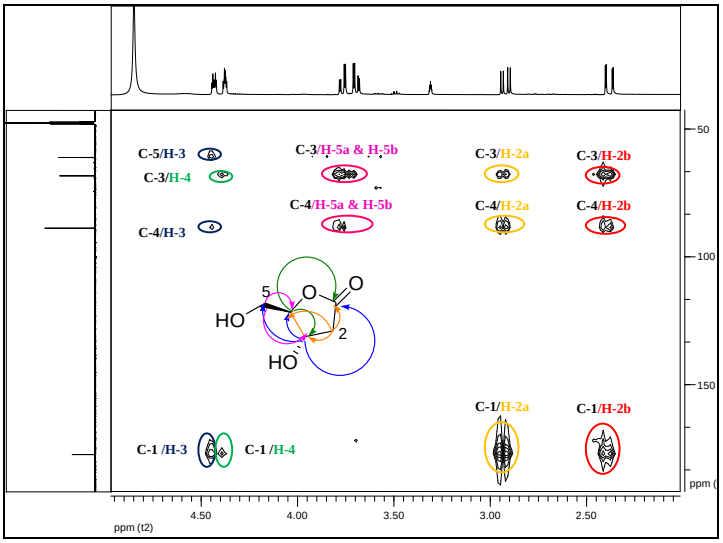


Figure S4. HMBC Spectrum (CD_3OD, 500 MHz) of Compound **1**

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

No	Compound **2**	
	δ_C	**δ_H**
1	176.30	-
2	72.81	5.36dd (J=9.5, 3.5)
3a	39.22	2.87dd (J=16.0, 4.0)
3b		2.73dd (J=16.0, 9.5)
4	175.22	-
1′	127.48	-
2′	110.61	7.18d (J=1.5)
3′	148.52	-
4′	149.00	-
5′	115.00	6.80 d (J=8.0)
6′	122.02	705dd (J=8.0, 1.5)
7′	144.70	7.61 d (J=16.0)
8′	114.20	6.41 d (J=16.0)
9′	168.73	-
-OCH_3	54.90	3.88 s

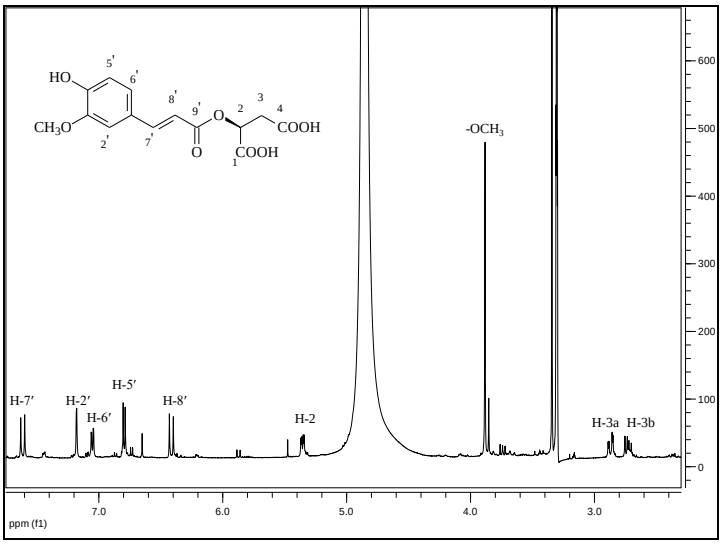


Figure S5. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **2**

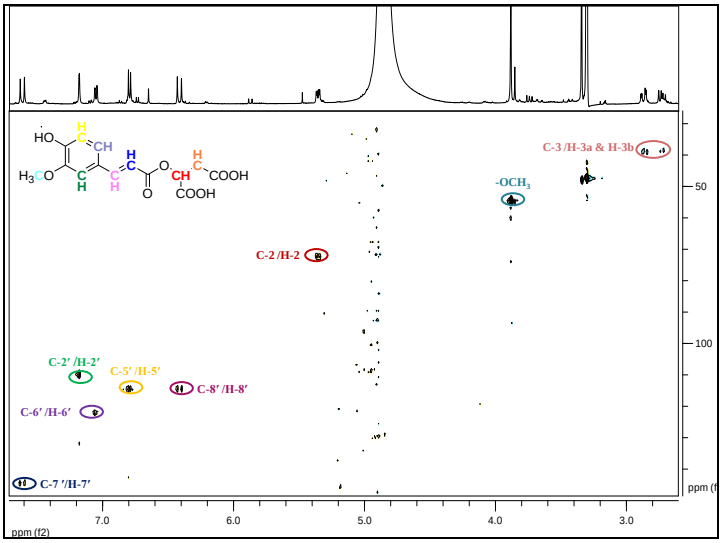


Figure S6. HSQC Spectrum (CD_3OD, 500 MHz) of Compound **2**

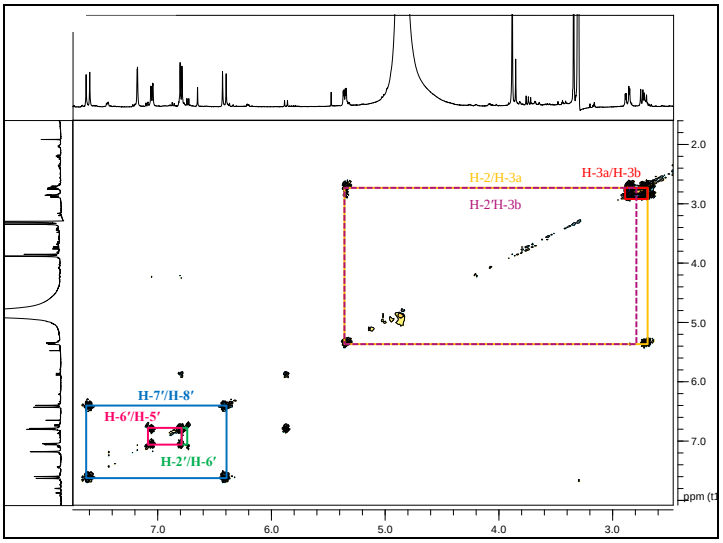


Figure S7. COSY-NMR Spectrum (CD_3OD, 500 MHz) of Compound **2**

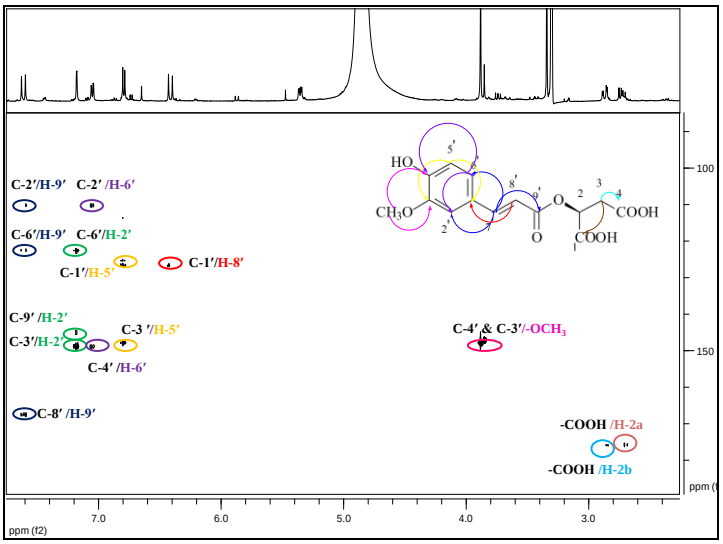
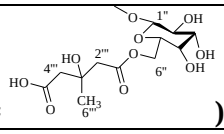
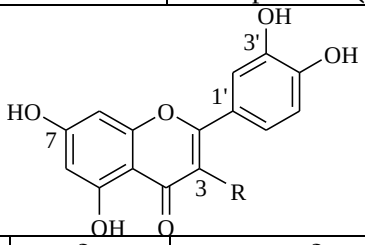


Figure S8. HMBC Spectrum (CD_3OD, 500 MHz) of Compound **2**

Table S3. ^{1}H and ^{13}C NMR of compounds **3** and **4** (CD_3OD, 500 MHz)

No	Compound **3 (**R= -O-*β*-D-galactopyranoside**)**		Compound **4 (R=**  **)**	
				
	δ_H	**δ_C**	**δ_H**	**δ_C**
2	-	o.s	-	157.51
3	-	134.51	-	134.22
4	-	o.s	-	178.07
5	-	o.s	-	162.39
6	6.20d (J=2.0)	98.42	6.21d (J=2.0)	98.71
7	-	164.68	-	164.53
8	6.40d (J=2.0)	93.12	6.40d (J=2.0)	94.43
9	-	156.54	-	156.92
10	-	104.05	-	104.78
1′	-	o.s	-	121.12
2′	7.84d (J=2.0)	116.24	7.81d (J=2.0)	116.32
3′	-	144.36	-	144.39
4′	-	148.57	-	148.56
5′	6.86 d (J=8.5)	114.20	6.86 d (J=8.5)	114.78
6′	7.58dd (J=8.5, 2.0)	121.86	7.61dd (J=8.5, 2.0)	121.61
1″	5.16 d (J=8.0)	103.27	5.10 d (J=8.0)	103.83
2″	3.81 dd (J=9.5, 8.0)	72.81	3.81o.s	71.51
3″	3.52 dd (J=9.5,32.0)	74.37	3.56o.s	73.05
4″	3.84 dd (J=3.0, 1.0)	68.19	3.80o.s	68.81
5″	3.47br d (J=6.0)	76.78	3.69o.s	73.35
6″a	3.64dd (J=11.0, 6.0)	60.31	4.13 d (J=6.0)	63.18
6″b	3.56dd (J=11.0, 6.0)		4.13 d (J=6.0)	
1‴	-	-	-	170.88
2a‴	-	-	2.50d (J=14.5)	44.88
2b‴	-	-	2.43d (J=14.5)	
3‴	-	-	-	69.22
4a‴	-	-	2.48 d (J=15.5)	44.61
4b‴	-	-	2.43 d (J=15.0)	
5‴	-	-	-	173.35
6‴	-	-	1.19 s	26.22

o.s : overlapping signal

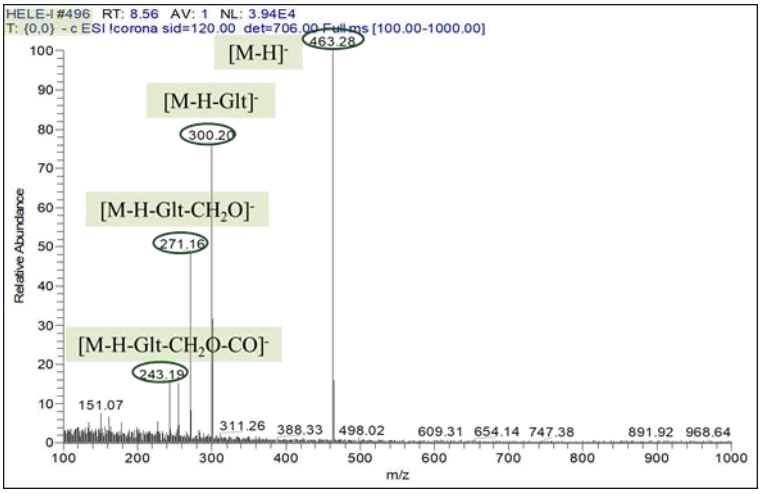


Figure S9. ESI-MS Spectrum (negative ion mode) of Compound **3**

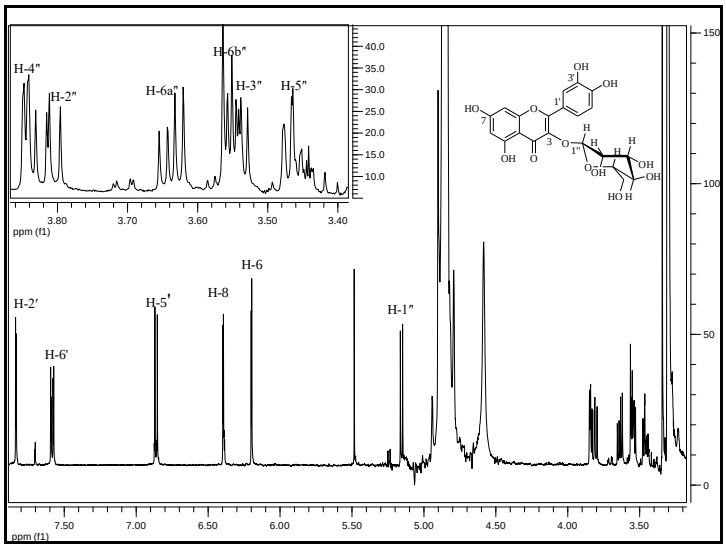


Figure S10. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **3**

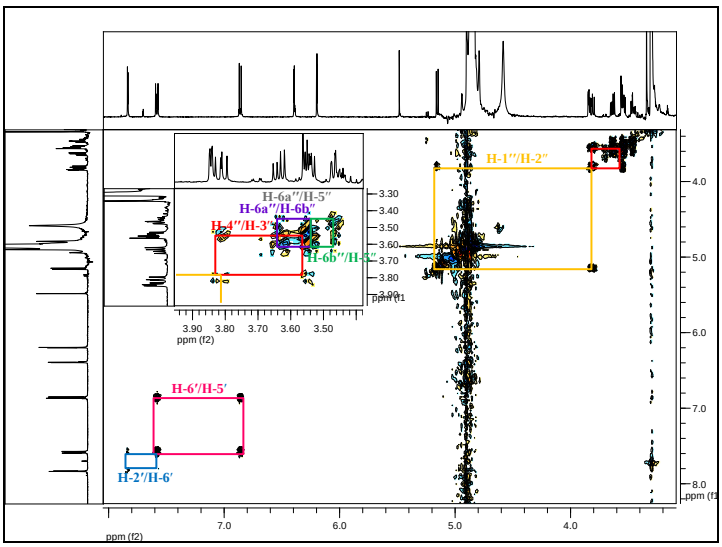


Figure S11. ^{13}C-NMR Spectrum (CD_3OD, 500MHz) of Compound **3**

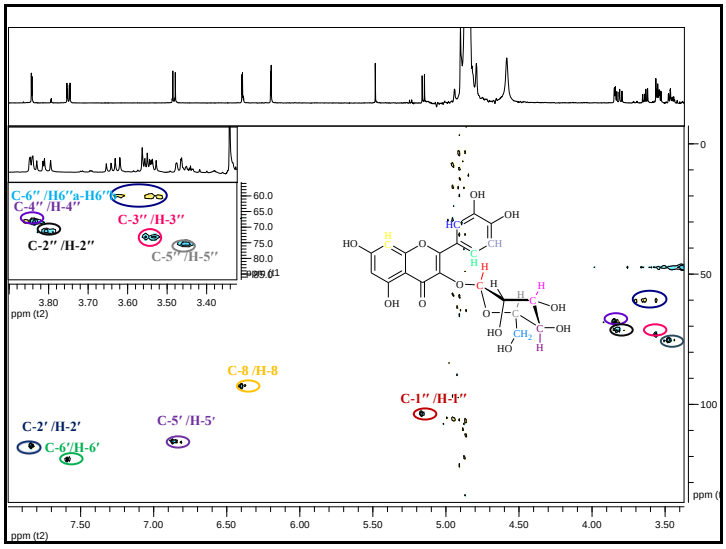


Figure S12. HSQC Spectrum (CD_3OD, 500 MHz) of Compound **3**

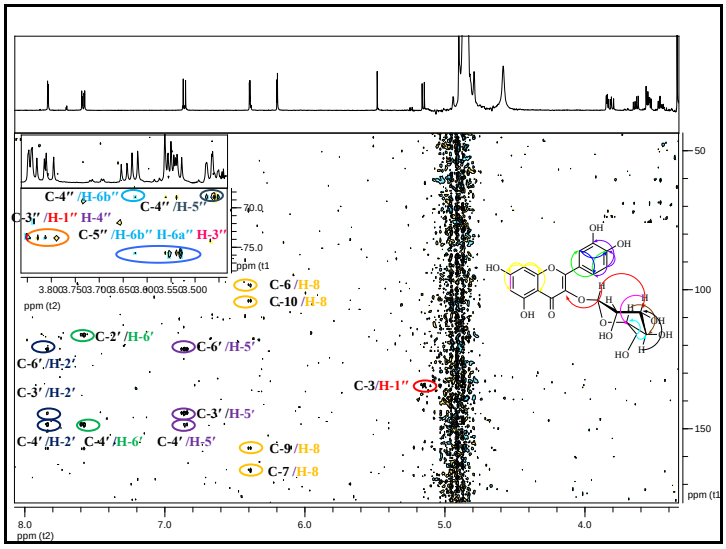


Figure S13. HMBC Spectrum (CD_3OD, 500 MHz) of Compound **3**

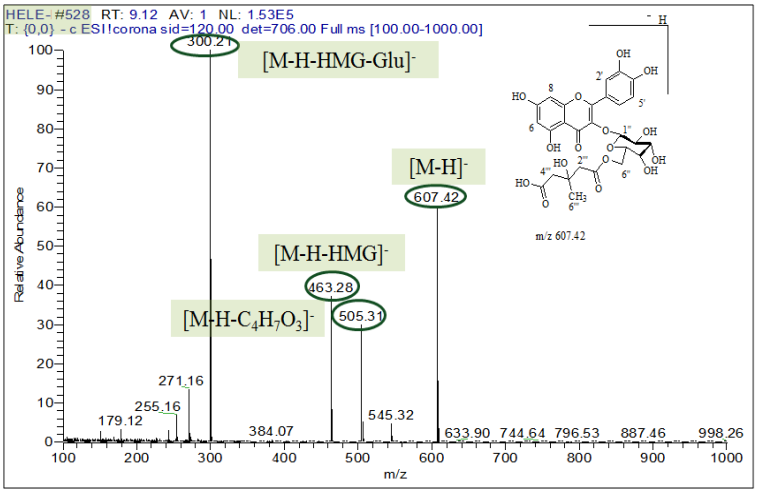


Figure S14. ESI-MS Spectrum (negative ion mode) of Compound **4**

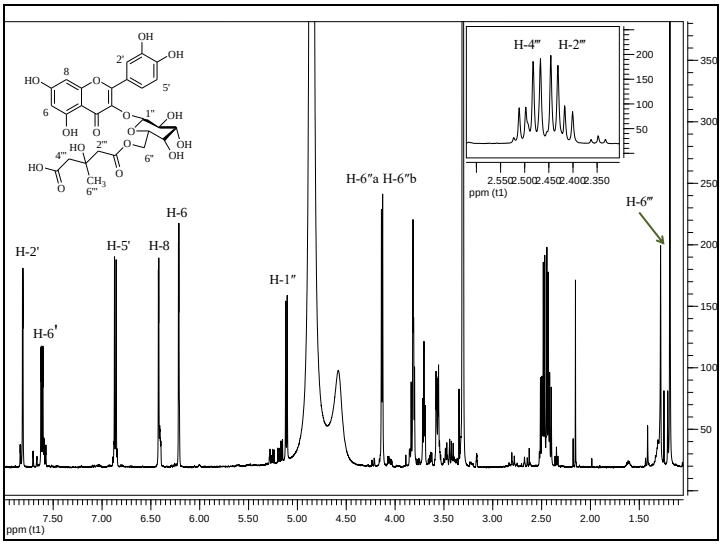


Figure S15. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **4**

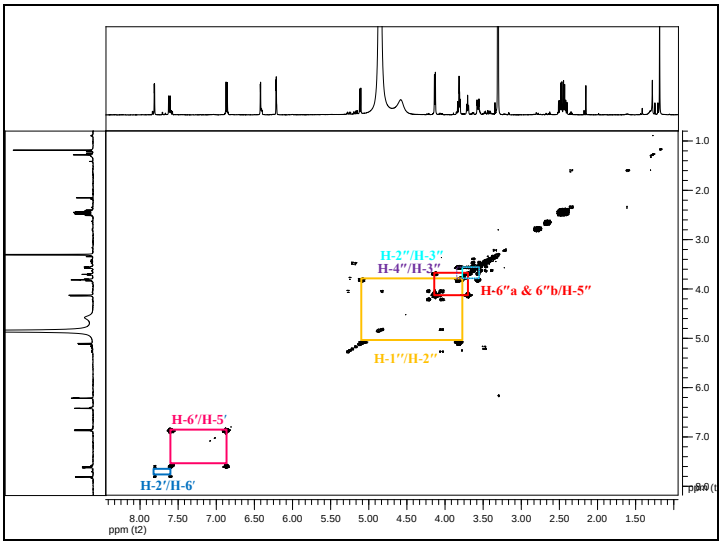


Figure S16. COSY Spectrum of compound **4** (CD_3OD, 500 MHz)

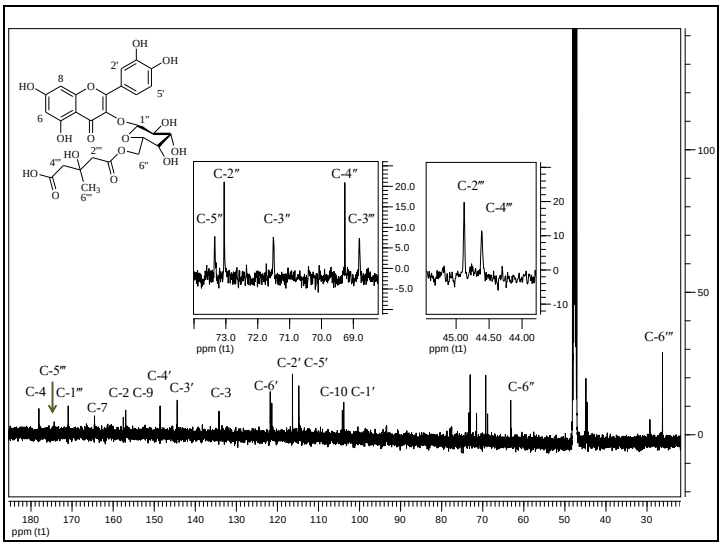


Figure S17. ^{13}C-NMR Spectrum (CD_3OD, 500MHz) of Compound **4**

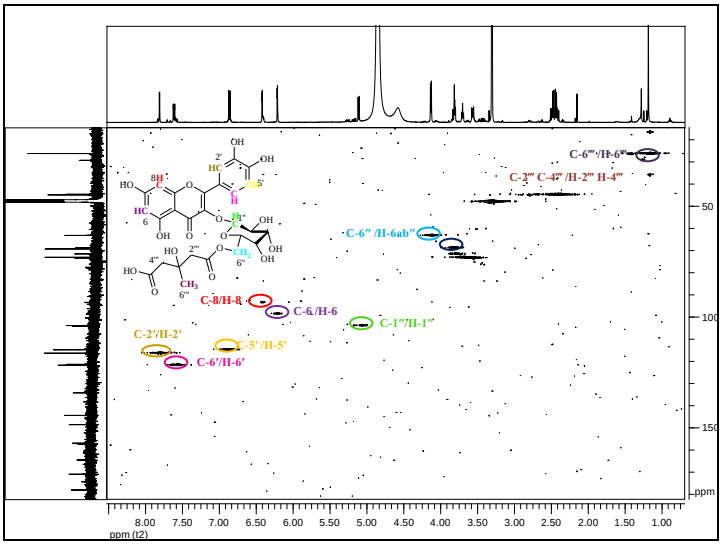


Figure S18. HSQC Spectrum (CD_3OD, 500 MHz) of Compound **3**

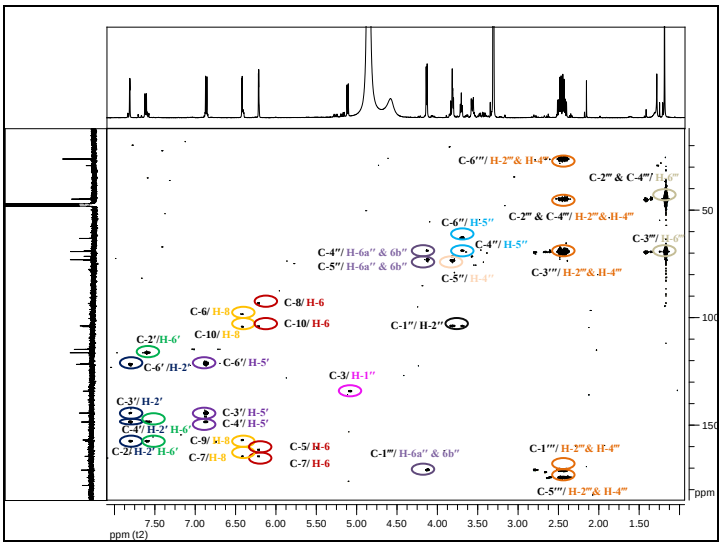


Figure S19. HMBC Spectrum (CD_3OD, 500 MHz) of Compound **4**

Table S4. ^{1}H and ^{13}C NMR of compound **5** (CD_3OD, 500 MHz)

No	Compound **5**	
	δ_C	**δ_H**
1	156.78	-
2	133.43	-
3	178.03	-
4	161.53	-
5	98.25	-
6	164.16	6.16 s
7	93.21	-
8	156.47	6.23 s
9	104.46	-
10	120.94	-
1′	115.91	-
2′	144.35	7.56 d (J=1.0)
3′	148.16	-
4′	114.78	-
5′	121.77	6.86d (J=8.5)
6′	97.88	7.39d (J=8.5)
1″	78.03	5.77 d (J=7.5)
2″	144.35	3.96o.s
3″	75.69	3.78o.s
4″	69.02	3.80-2.50o.s
5″	76.55	3.80-2.50o.s
6″a	61.02	3.80-2.50o.s
6″b		3.80-2.50o.s
1‴	98.50	5.19d (J=8.0)
2‴	71.89	4.90o.s
3‴	73.80	3.66o.s
4‴	70.04	3.44o.s
5‴	74.79	3.80-2.50o.s
6a‴	60.53	3.80-2.50o.s
6b‴		3.80-2.50o.s
C=O	167.31	-
1⁗	126.06	-
2⁗	114.74	6.75 s
3⁗	145.06	-
4⁗	147.84	-
5⁗	113.74	6.56o.s
6⁗	120.94	6.56o.s
α	113.56	6.11d (J=15.5)
β	145.30	7.35d (J=15.5)

o.s. : overlapping signal

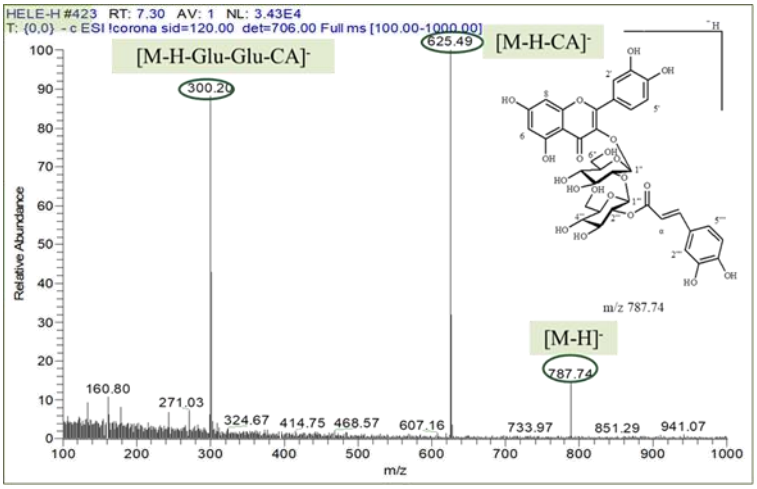


Figure S20. ESI-MS Spectrum (negative ion mode) of Compound **5**

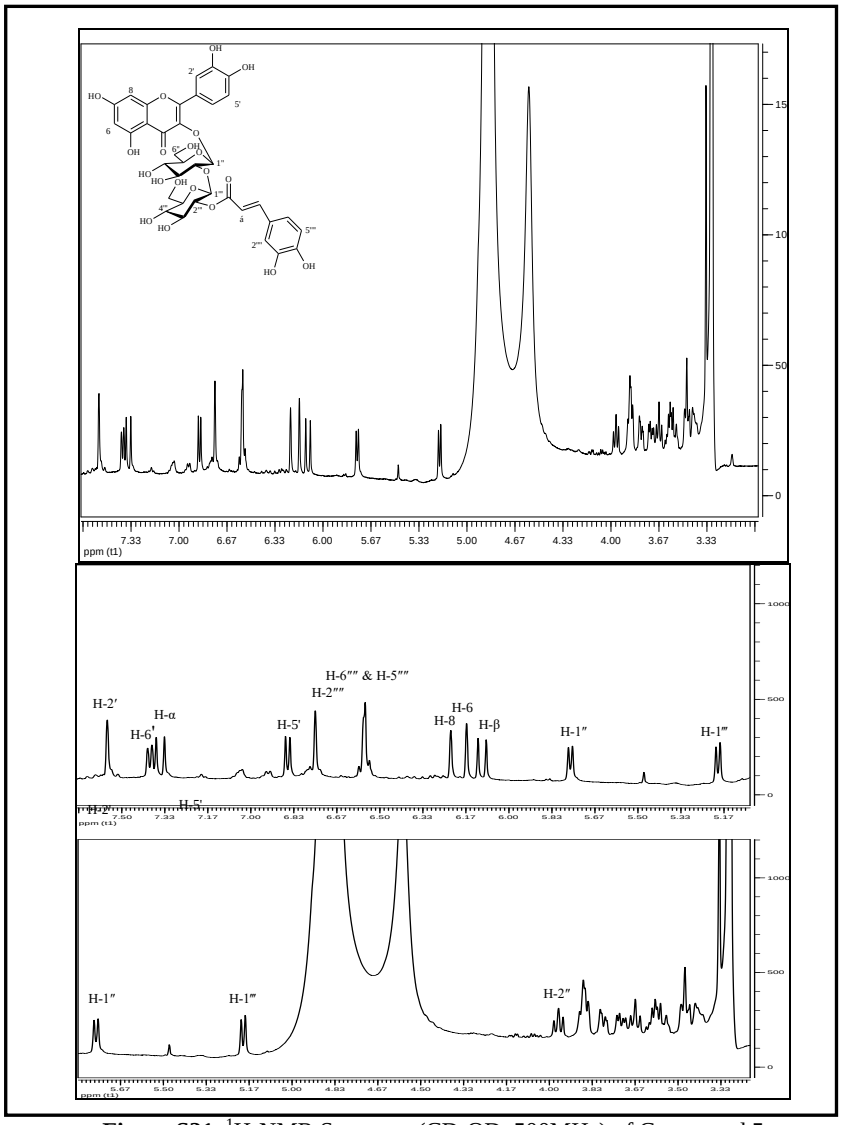


Figure S21. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **5**

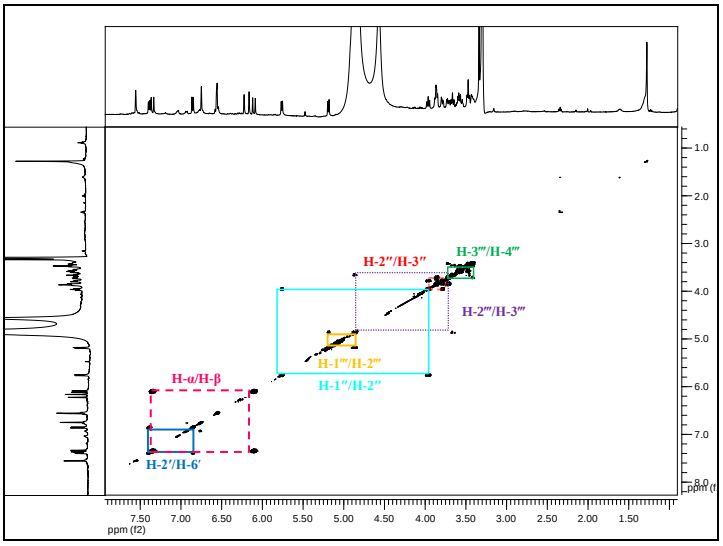


Figure S22. COSY Spectrum (CD_3OD, 500MHz) of Compound **5**

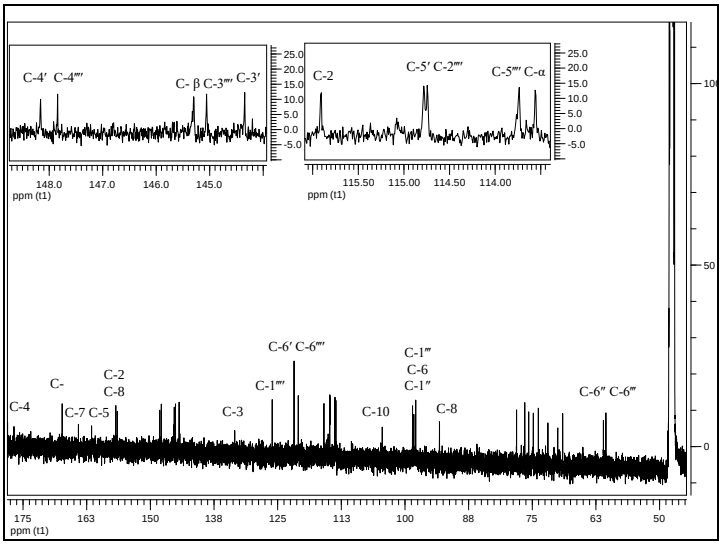


Figure S23. ^{13}C-NMR Spectrum (CD_3OD, 500MHz) of Compound **5**

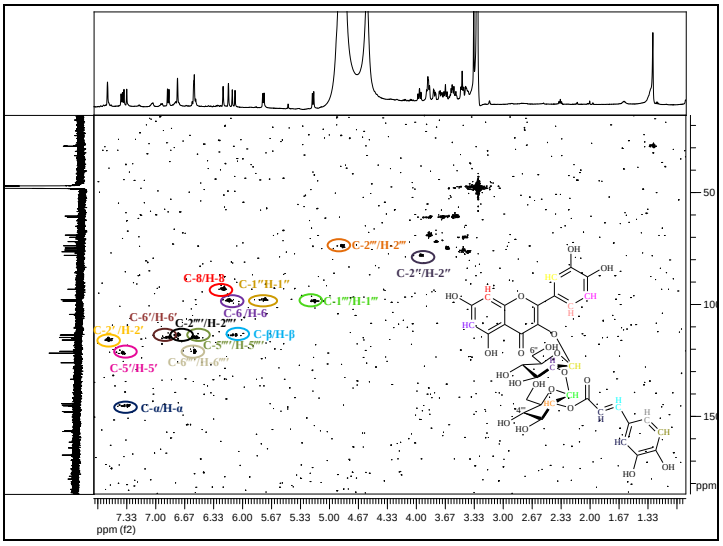


Figure S24. HSQC Spectrum (CD_3OD, 500MHz) of Compound **5**

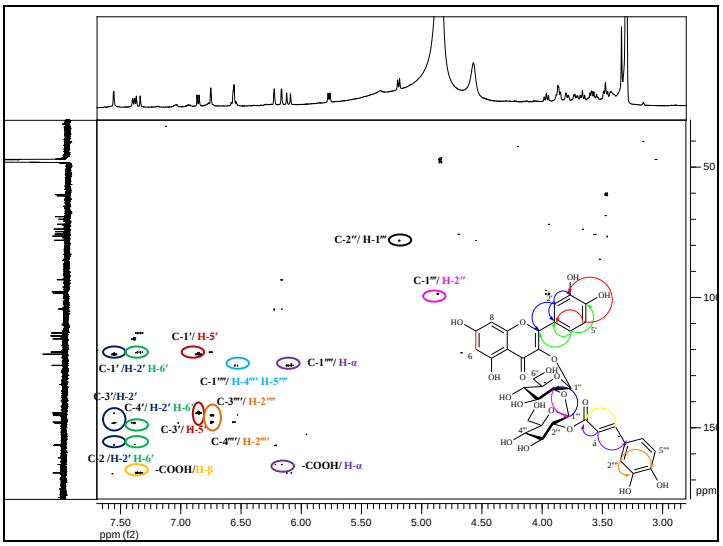


Figure S25. HMBC Spectrum (CD_3OD, 500MHz) of Compound **5**

Table S5. ^{1}H-NMR of compound **6** (CD_3OD, 500 MHz)

No	Compound **6**
	δ_H
1	5.59d (J=7.5)
2	3.20-3.80o.s
3	3.20-3.80o.s
4	3.20-3.80o.s
5	3.20-3.80o.s
6a	4.50dd (J=12.0, 6.0)
6b	4.31 dd (J=12.0, 1.5)
1′	-
2′	7.05d (J=1.5)
3′	-
4′	-
5′	6.77 d (J=8.5)
6′	6.95dd (J=8.0, 2.0)
7′	7.73d (J=16.0)
8′	6.38d (J=16.0)
9′	-
1″	-
2″	7.48d (J=8.5)
3″	6.81d (J=8.5)
4″	-
5″	6.81d (J=8.5)
6″	7.48d (J=8.5)
7″	7.56d (J=16.0)
8″	6.29d (J=15.5)
9″	-

o.s: overlapping signal

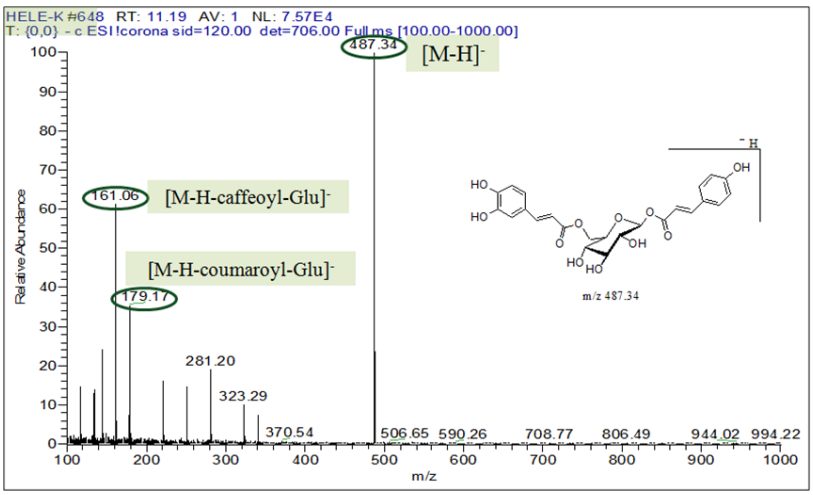


Figure S26. ESI-MS Spectrum (negative ion mode) of Compound **6**

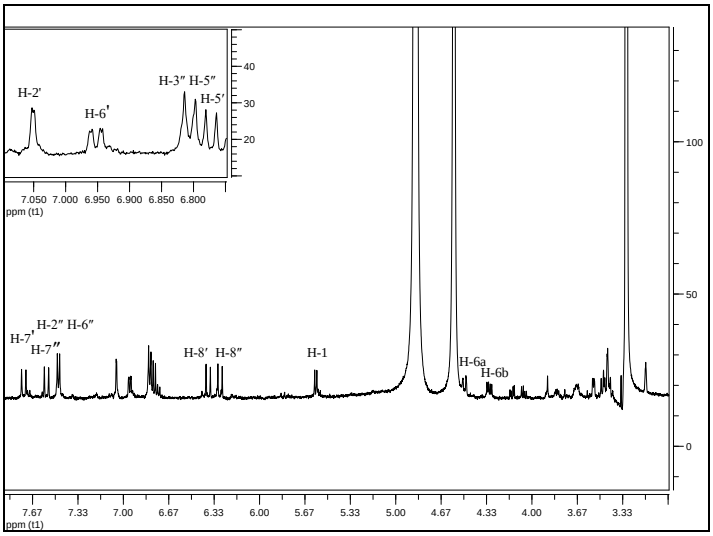
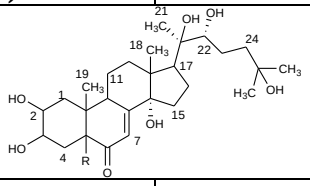


Figure S27. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **6**

Table S6. ^{1}H and ^{13}C NMR of compounds **7** and **8** (CD_3OD, 500 MHz)

No	Compound **7 (R=H)**		Compound **8 (R=OH)**	
				
	δ_H	**δ_C**	**δ_H**	**δ_C**
1ax	1.39 o.s	37.40	1.65-1.75 o.s	31.82
1eq	1.77 o.s			
2ax	3.84 m	68.74	3.94 m	67.00
3eq	3.94 m	68.55	3.99 m	68.45
4	1.65-1.75	32.91	1.75-2.10 o.s	34.77
5	2.38 m	51.83	-	78.91
6	-	206.49	-	200.95
7	5.81 d (J=2.5)	122.17	5.85 d (J=2.5)	119.17
8	-	168.02	-	166.17
9	3.14 m	35.14	3.19 m	37.61
10	-	39.32	-	44.04
11	1.65-1.80o.s	21.45	1.70-1.80 o.s	21.13
12ax	2.13 m	32.56	1.85-2.15o.s	31.21
12eq				
13	-	49.00	-	47.23
14	-	85.27	-	83.64
15	1.50-2.00o.s	31.83	1.90-2.00 o.s	30.35
16	1.75-1.95 o.s	21.57	1.80-2.00 o.s	20.07
17	2.39 m	50.57	2.39 m	49.05
18	0.89 s	18.10	0.89 s	15.58
19	0.96 s	24.46	0.92 s	16.67
20	-	77.96	-	76.48
21	1.19 s	21.10	1.20 s	19.67
22	3.33 m	78.46	3.33 o.s	77.03

23	1.30-1.65 o.s	27.39	1.25-1.65 o.s	25.97
24a	1.45o.s	42.44	1.45 o.s	41.01
24b	1.79o.s		1.82 o.s	
25	-	71.34	-	69.91
26	1.19 s	29.00	1.19 s	27.57
27	1.19 s	29.76	1.19 s	28.36

o.s : overlapping signal

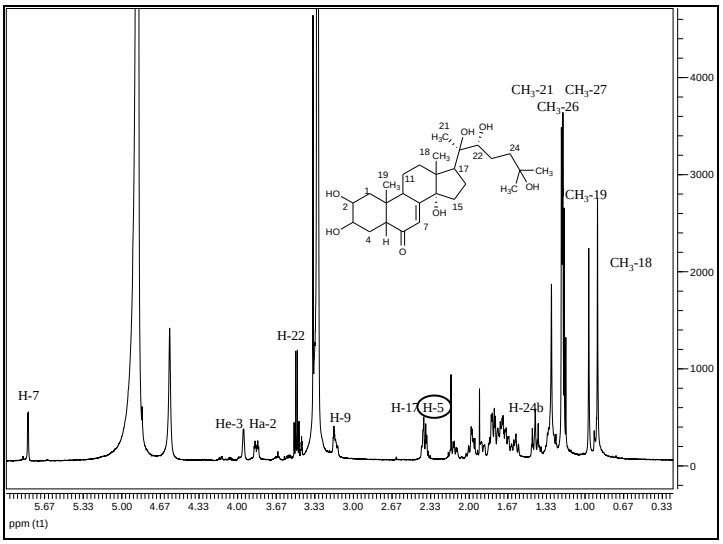


Figure S28. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **7**

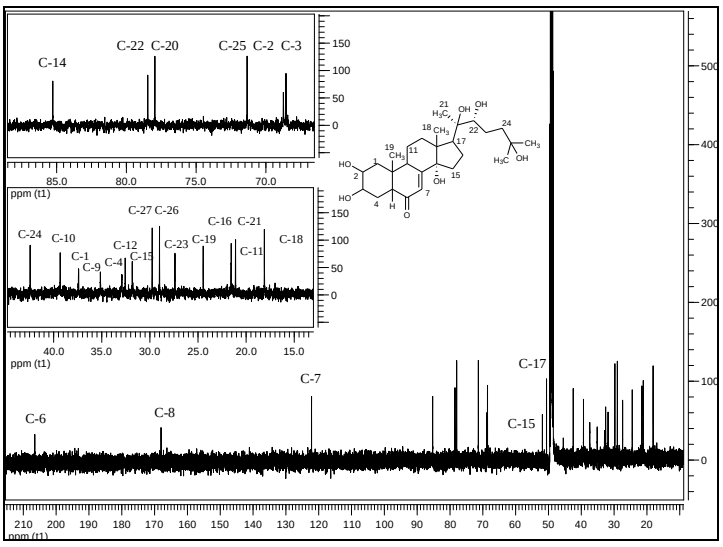


Figure S29. ^{13}C-NMR Spectrum (CD_3OD, 125MHz) of Compound **7**

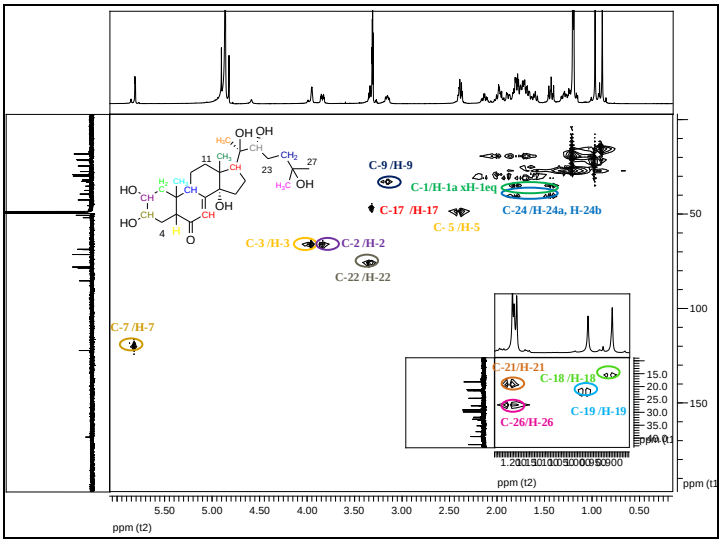


Figure S30. HSQC Spectrum (CD_3OD, 500 MHz) of Compound **7**

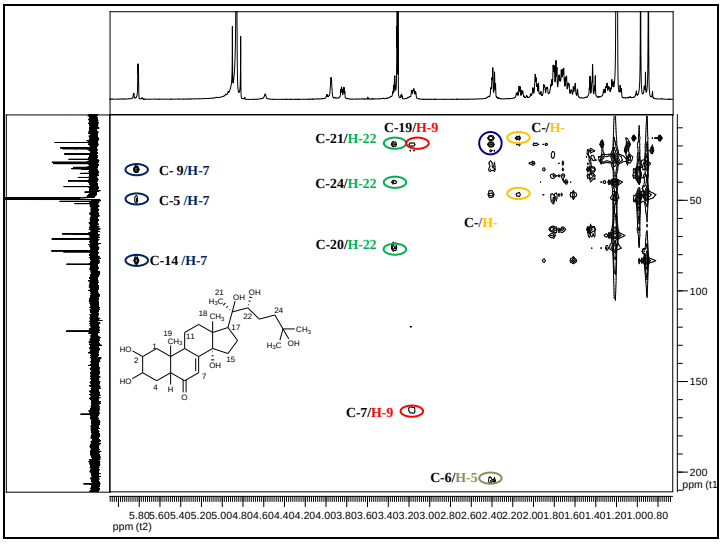


Figure S31. HMBC Spectrum (CD_3OD, 500 MHz) of Compound **7**

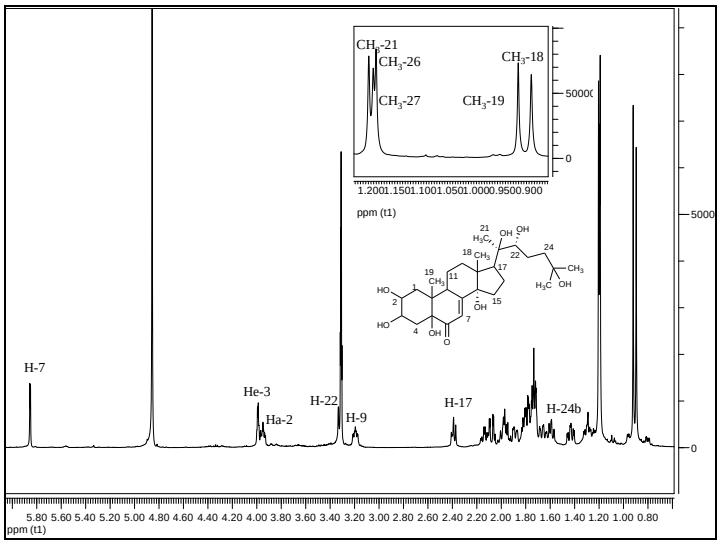


Figure S32. ^{1}H-NMR Spectrum (CD_3OD, 500MHz) of Compound **8**

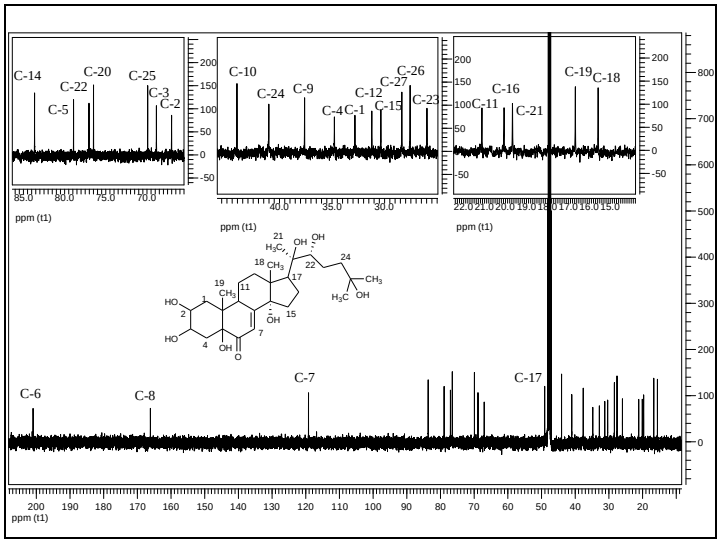


Figure S33. ^{13}C-NMR Spectrum (CD_3OD, 125MHz) of Compound **8**

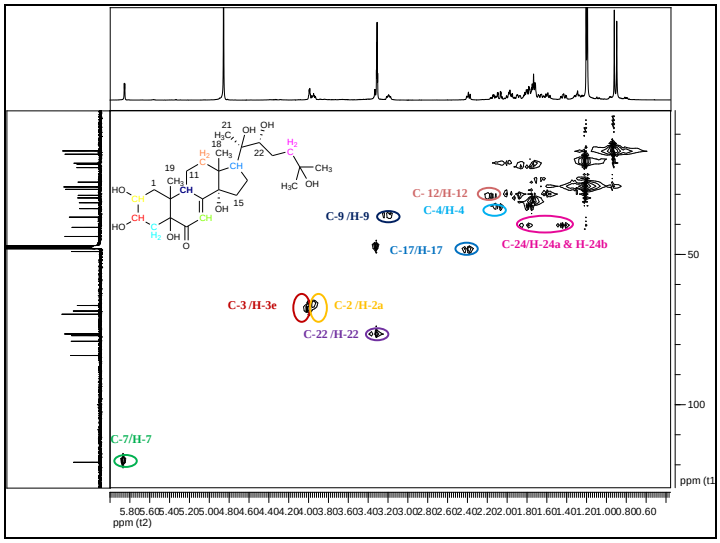
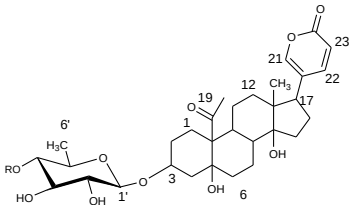


Figure S34. HSQC Spectrum (CD_3OD, 500 MHz) of Compound **8**

Table S7. ^{1}H and ^{13}C NMR of compounds **9** and **10** (CD_3OD, 500 MHz)

No	Compound **9 (R=H)**		Compound **10 (R=Glc)**	
				
	δ_H	**δ_C**	**δ_H**	**δ_C**
1	1.70-2.05	17.60	o.s	18.29
2	1.83 o.s	24.63	o.s	o.s
3	4.14br	73.85	4.14br	72.65
4	1.65-2.15 o.s	34.84	o.s	o.s
5	-	73.51	-	o.s
6	1.65-2.15 o.s	35.84	o.s	o.s
7	1.35-2.15 o.s	23.90	o.s	o.s
8	1.94 o.s	41.63	o.s	o.s
9	1.66 o.s	39.17	o.s	o.s
10	-	54.78	-	54.73
11	1.45-1.55o.s	22.22	o.s	39.11
12	1.45-1.55o.s	40.07	o.s	47.79
13	-	47.03	-	o.s
14	-	84.23	-	o.s
15	1.65-2.05o.s	30.91	o.s	o.s
16	1.75-2.20 o.s	28.30	o.s	27.92
17	2.55 dd (J=9.5, 6.5)	50.56	2.55 dd (J=9.5, 7.0)	49.35
18	0.67 s	15.69	0.68 s	14.41
19	-	208.32	-	209.12
20	-	123.44	-	122.40
21	6.28 d (J=9.5)	149.14	6.28 d (J=9.5)	148.40
22	7.98 dd (J=10.0, 2.5)	147.86	7.99 dd (J=10.0, 3.0)	146.35
23	7.42 brs	114.05	7.43 d (J=2.5)	113.41
24	-	163.36	-	164.04
1′	4.84 o.s	99.53	4.84 o.s	99.82
2′	3.77 o.s	72.36	o.s	74.59
3′	3.62 o.s	71.14	o.s	o.s
4′	3.56 o.s	71.04	o.s	o.s
5′	3.63 o.s	69.34	o.s	66.82
6′	1.26 d (J=6.0)	16.58	1.34 d (J=6.0)	16.35
1″	-	-	4.59 d (J=8.0)	103.71
2″	-	-	3.21 dd (J=9.0, 7.5)	74.59
3″	-	-	o.s	o.s
4″	-	-	o.s	o.s
5″	-	-	o.s	o.s
6a″	-	-	3.84 o.s	60.99
6b″	-		3.67 o.s	

o.s : overlapping signal

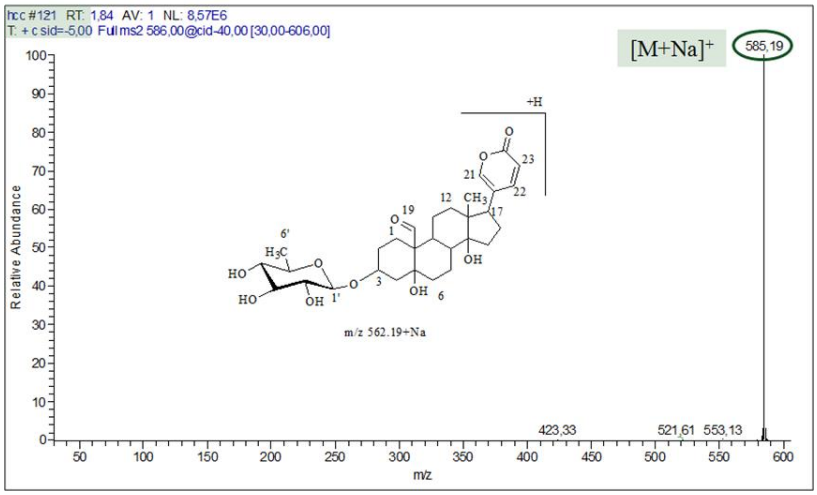


Figure S35. ESI-MS Spectrum (negative ion mode) of compound **9**

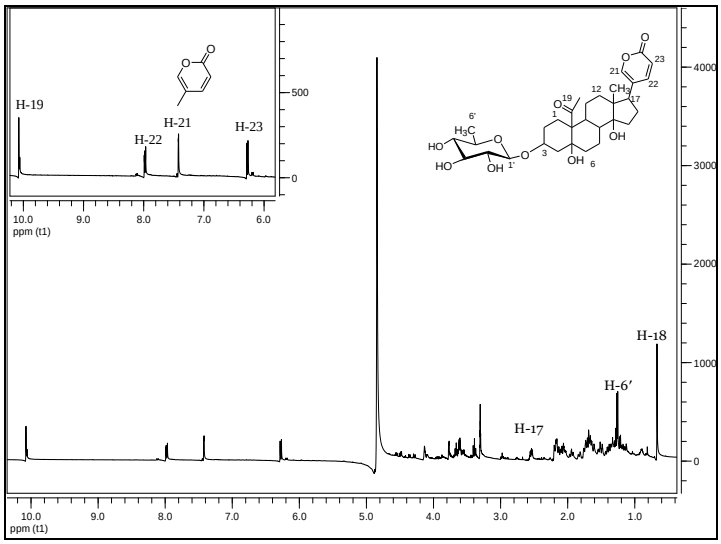


Figure S36. ^{1}H-NMR Spectrum of compound **9** (CD_3OD, 500MHz)

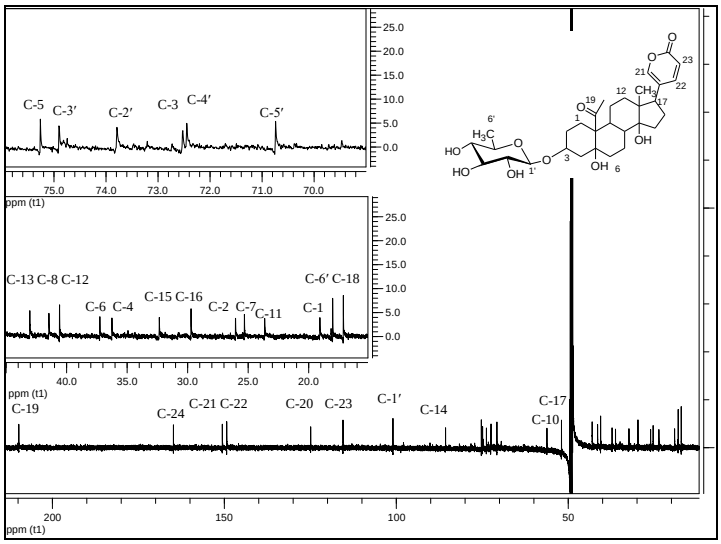


Figure S37. ^{13}C-NMR Spectrum of compound **9** (CD_3OD, 125MHz)

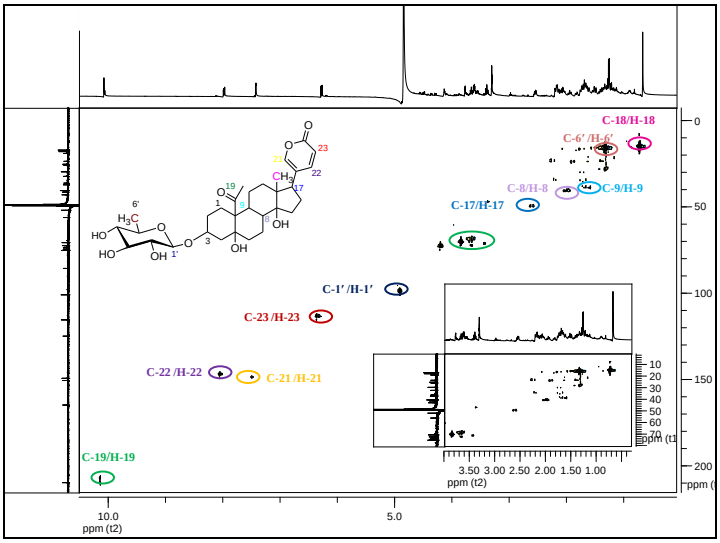


Figure S38. HSQC Spectrum of compound **9** (CD_3OD, 500 MHz)

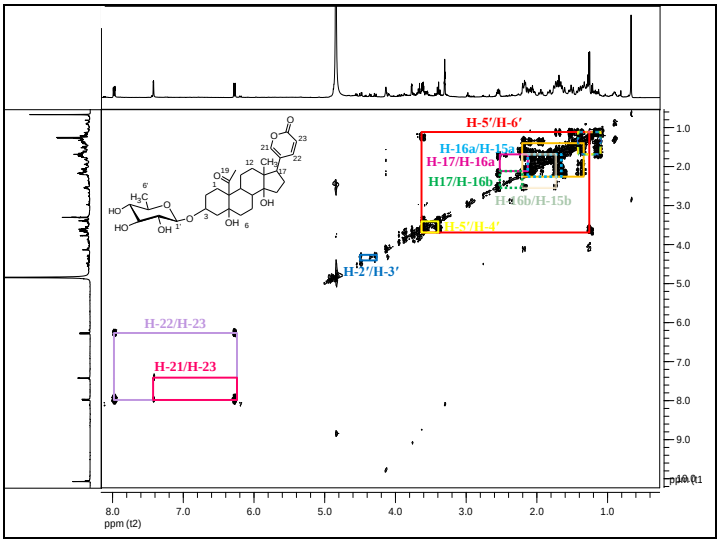


Figure S39. COSY Spectrum of compound **9** (CD_3OD, 500 MHz)

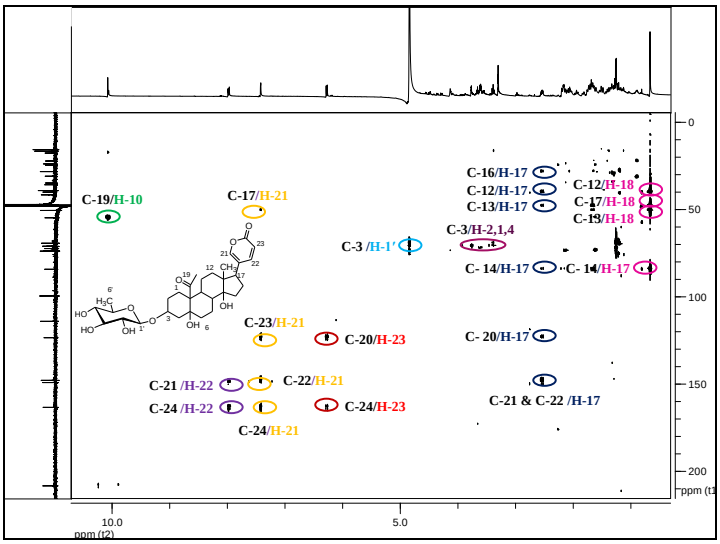


Figure S40. HMBC Spectrum of compound **9** (CD_3OD, 500 MHz)

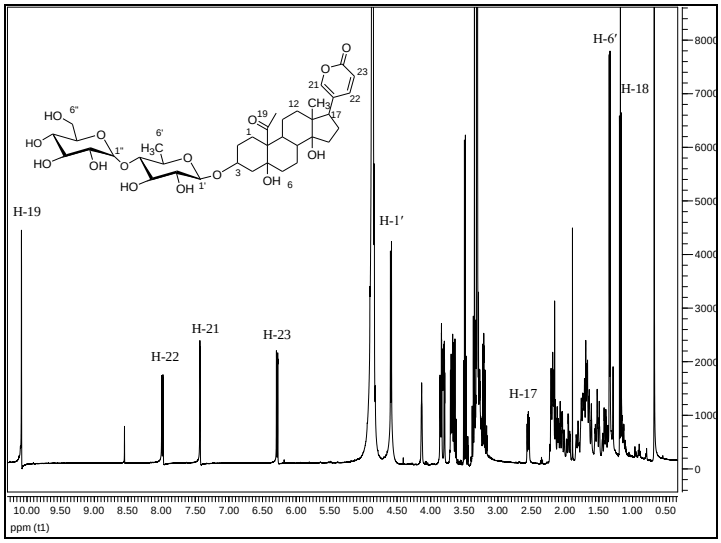


Figure S41. ^{1}H-NMR Spectrum of compound **10** (CD_3OD, 500MHz)

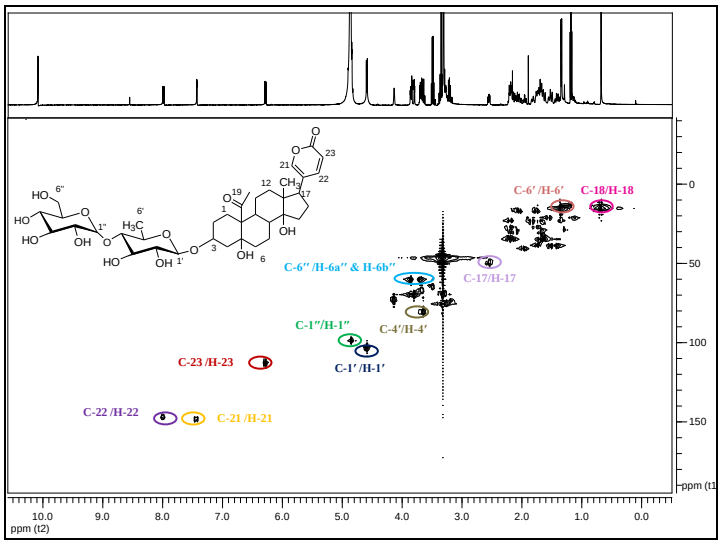


Figure S42. HSQC Spectrum of compound **10** (CD_3OD, 500 MHz)

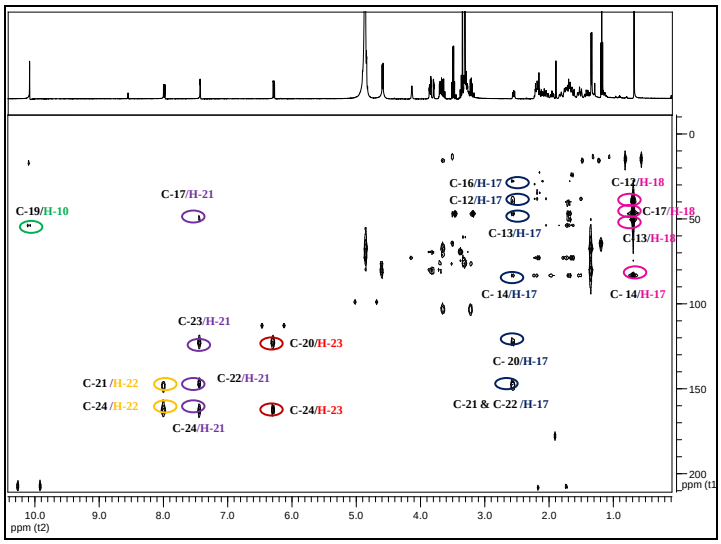
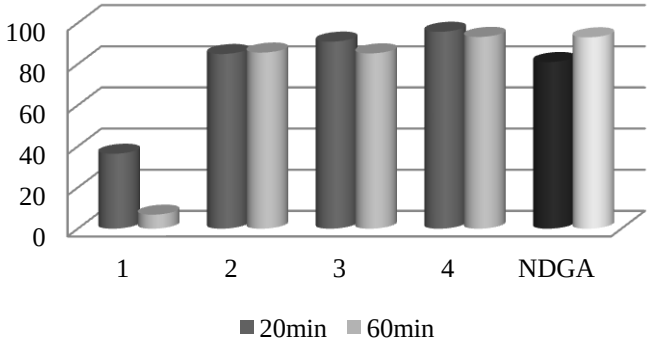
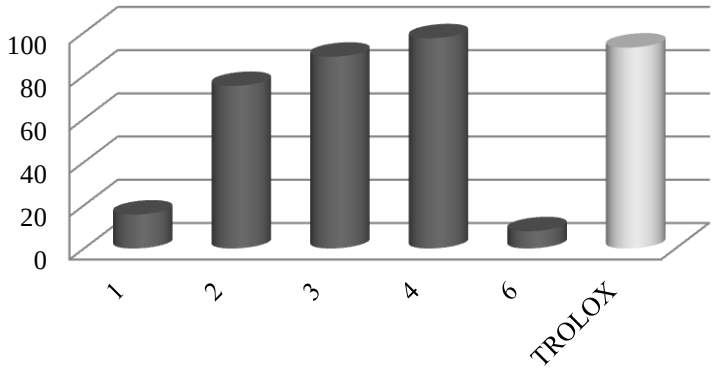


Figure S43. HMBC Spectrum of compound **10** (CD_3OD, 500 MHz)

Table S8: Percentage (%) interaction of compounds **1, 2, 3, 4, 6** with DPPH, their% soybean LOX inhibitory activity and their % inhibition of lipid peroxidation, na= no active

Parameters and samples	% Interaction with the stable free radical of DPPH		% Inhibitionof LOX	% Inhibition of lipid peroxidation
Time	20min	60min		
Compound	20μl	20μl	10μl	10μl
(**1**)	36.4	6.8	n.a	15.7
(**2**)	84.9	85.6	n.a	75.2
(**3**)	90.9	85.2	60.6	88.8
(**4**)	95.7	93.2	23.3	97.3
(**6**)	-	-	20.2	8.0
NDGA	81.0	93.0	96.0	
TROLOX				93.0





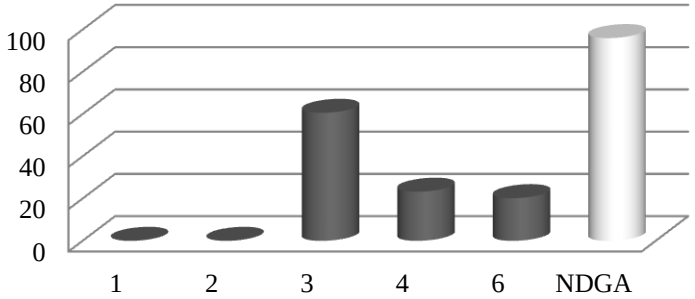


Table S9. Drug likeness score for isolated compounds

Compound	Drug likeness score for compounds									
	miLogP	**TPSA**	**N**	**N (O/N)**	**N(OH/NH)**	**N (violationLipinsky)**	**Nrotb.**	**M.V**	**M.W**	**logBB**
Compound 1	-2.38	66.76	9	4	2	0	1	113.06	132.12	-0.87
Compound 2	0.61	130.37	22	8	3	0	8	260.62	310.26	-1.05
Compound 3	-0.36	210.50	33	12	8	2	4	372.21	464.38	-2.00
Compound 4	-0.59	274.11	43	16	9	3	10	493.84	608.50	-2.67
Compound 5	-0.19	336.19	56	20	12	3	11	639.14	788.66	-3.53
Compound 6	1.60	183.21	35	11	6	2	9	413.41	488.44	-1.42
Compound 7	1.36	138.44	34	7	6	1	5	464.48	480.64	-1.01
Compound 8	0.41	158.67	35	8	7	1	5	478.38	496.64	-1.36
Compound 9	1.24	166.89	40	10	5	1	4	509.23	562.66	-1.31
Compound 10	-0.69	246.04	51	15	8	3	7	641.35	724.80	-2.40

Table S10. Bioactivity scores for drug targets by Molinspiration software

Compound	GPCR receptor	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor
Compound 1	-1.66	-1.52	-1.95	-2.34	-1.98
Compound 2	0.15	-0.09	-0.15	0.45	0.10
Compound 3	0.06	-0.04	0.13	0.20	-0.06
Compound 4	-0.01	-0.48	-0.24	-0.05	0.01
Compound 5	-1.54	-2.79	-2.21	-2.19	-1.14
Compound 6	0.07	-0.04	-0.08	0.09	0.07
Compound 7	0.16	0.17	-0.32	0.92	0.32
Compound 8	0.11	0.12	-0.32	0.90	0.26
Compound 9	0.05	-0.18	-0.40	0.14	0.04
Compound 10	-0.72	-1.60	-1.45	-1.11	-0.45
Adenosine	1.10	0.54	0.87	-1.74	-0.01
Capsazepine	0.02	-0.30	-0.33	-0.31	-0.12
K-252a	0.26	0.08	1.27	-0.08	0.16
Corticosterone	0.02	-0.05	-0.94	1.02	0.05
Z-VAD-(OMe)-FMK	0.35	0.12	-0.18	-0.17	1.03