SUPPLEMENTAL DATA

Bruceadysentoside A, a new pregnane glycoside and others secondary metabolites with cytotoxic activity from *Brucea antidysenterica* J. F. Mill. (Simaroubaceae)

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Abstract

The chemical investigation of the root barks, leaves and stem barks of *Brucea antidysenterica* J. F. Mill. (Simaroubaceae) led to the isolation of a new pregnane glycoside, named Bruceadysentoside A or 3-O-*β*-L-arabinopyranosyl-pregn-5-en-20-one (**1)** together with seventeen known compounds. Their structures were established from spectral data, mainly HRESIMS, 1D and 2D NMR and by comparison with literature data. Compounds **1**, **2**, **5**, **6**, **8**, **10**, **12** and **13** were tested *in vitro* for their effects on the viability of two different human cancer cell lines, namely prostate PC-3 adenocarcinoma cells and colorectal HT-29 adenocarcinoma cells. No substantial activities were recorded for **2**, **10**, **12** and **13** (up to 10 µM concentration). **1**, **5** and **8** did not show strong anti-proliferative effects up to 100 µM, however **6** exhibited a stronger anti-proliferative effect with IC50 values of ~ 100 µM against PC-3 and ~ 200 µM against HT-29.

Keywords : *Brucea antidysenterica*; Simaroubaceae; pregnane glycoside; bruceadysentoside; cytotoxic activities; hydnocarpin.

3. Experimental

3.1. General

The high resolution ESI mass spectra and the corresponding higher collision dissociation (HCD) measurements (normalized collision energy 50%) were obtained on an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an HESI electrospray ion source (spray voltage 4 kV; capillary temperature 275 °C, source heater temperature 40 °C; FTMS resolution 30.000). Nitrogen was used as the sheath gas. 1D and 2D NMR spectra were recorded on either with a Bruker DRX 400, 500 NMR spectrometers or with an Agilent DD2 NMR (600 MHz) spectrometer. $CDCl_3$, DMSO-d6 and pyridine-d5 were used as NMR solvents. Chemical shifts (δ) are quoted in parts per million (ppm) with tetramethylsilane (TMS) as internal standard and the coupling constants (J) are given in Hz. Column chromatography were carried out on silica gel Merck 60 F254 [(0.2-0.5 mm) and (0.063-0.2mm)] 70-230 and 230-400 mesh (Darmstadt, Germany). Thin layer chromatography plates were performed on Merck precoated silica gel 60 F254 aluminium foil. Spots were detected on TLC under UV lamp (254 and 365 nm) or by heating to 200 °C after spraying with 20% H_2SO_4 (v/v) solution. Different mixtures of n-hexane, EtOAc and MeOH were used as eluting solvents. Solvents were distilled prior to use.

3.2. Plant material

The stem, leaves and roots of *B. antidysenterica* were collected on May 2016 and September 2017 in Bazou in the West region of Cameroon and were authenticated by Mr Nana Victor of the National Herbarium of Cameroon where a voucher specimen were deposited under the registration number 54605 HNC.

3.2.1. Extraction and isolation from leaves

Air-dried and powdered leaves of *B. antidysenterica* (2.2 kg) were macerated in MeOH at room temperature for 72h. After evaporation under reduced pressure, the obtained crude extract (60.5g) was suspended in Hexane/ Ethyl acetate 0.5% then to 1%. The extract was evaporated to dryness and the residue to give 36g was subjected to the column chromatography over silica gel (40-63 μm, 6 x 50 cm) using n-hexane-AcOEt and AcOEt-MeOH gradients as eluents. 321 fractions of 100 ml each were collected as follows: [(1-15), n-hexane-AcOEt 5%], [(16-32), n-hexane-AcOEt 7.5%], [(33-49), n-hexane-AcOEt 10%], [(50-66), n-hexane-AcOEt 12.5%],

[(67-83), n-hexane-AcOEt 15%], [(84-100), n-hexane-AcOEt 17.5%], [(101-117), n-hexane-AcOEt 20%], [(118-134), n-hexane-AcOEt 25%], [(135-151), n-hexane-AcOEt 30%], [(152-168), n-hexane-AcOEt 40%], [(169-185), n-hexane-AcOEt 50%], [(186-202), n-hexane-AcOEt 60%], [(203-219), n-hexane-AcOEt 70%], [(220-236), n-hexane-AcOEt 80%], [(237-253), AcOEt 100%], [(254-270), AcOEt-MeOH 2.5%], [(271-287), AcOEt-MeOH 5%], [(288-304), AcOEt-MeOH 7.5%], [(305-321), AcOEt-MeOH 10%]. These fractions were combined on the basis of their TLC profiles to give 19 sub-fractions (F_1'-F_{19}') as follows: Compounds **3** (44.1 mg) and **4** (35.9 mg) were obtained respectively as white powders directly by simple washing and filtration of the precipitates of the sub-fraction F_3'. **7** (23.2 mg), **5** (14.1 mg)**, 6** (25.9 mg)**, 8** (13.2 mg) and **9** (6.11 g) were obtained respectively as yellow, yellow, beige, white and beige powders directly by simple washing and filtration from F_5', F_8', F_{10}', F_{14}' and F_{17}'. Compounds **2** (2.65 g) and **1** (10.2 mg) were obtained respectively as yellow and beige powders directly by simple washing and filtration from F_6' and F_{12}'.

3.2.2. Extraction and isolation from the stem.

Air-dried, finely powdered stem of *B. antidysenterica* (2.3 kg) was extracted with the mixture CH_2Cl_2/MeOH for 72 hours at room temperature. The solution was evaporated in vacuum to afford a brown residue of 24.10 g. The crude extract (22.10 g) was adsorbed on an equivalent mass of silica and chromatographed over a silica gel column chromatography (40-63 μm, 4.5 x 50 cm) using n-hexane-AcOEt and $CHCl_3$-MeOH gradients as eluents. Fractions of 100 mL each were collected as follows: [(1-15), n-hexane 100%], [(16-32), n-hexane-AcOEt 2.5%], [(1-15), n-hexane-AcOEt 5%], [(16-32), n-hexane-AcOEt 7.5%], [(33-49), n-hexane-AcOEt 10%], [(50-66), n-hexane-AcOEt 12.5%], [(67-83), n-hexane-AcOEt 15%], [(84-100), n-hexane-AcOEt 17.5%], [(101-117), n-hexane-AcOEt 20%], [(118-134), n-hexane-AcOEt 25%], [(135-151), n-hexane-AcOEt 30%], [(152-168), n-hexane-AcOEt 40%], [(169-185), n-hexane-AcOEt 50%], [(186-202), n-hexane-AcOEt 60%], [(203-219), n-hexane-AcOEt 70%], [(220-236), n-hexane-AcOEt 80%], [(237-253), AcOEt 100%], [(254-270), AcOEt-MeOH 2.5%], [(271-287), AcOEt-MeOH 5%]. These fractions were combined on the basis of their TLC profiles to give 18 sub-fractions (F_1-F_{18}) as follows: Compounds **10** (13.9 mg) and **11** (22.1 mg) were isolated as beige powder by filtration of the precipitates of the sub-from F_6 and F_7 respectively. From F_8 and F_{14}, compounds **14** (4.9 mg) and **12** (10.5 mg) were obtained respectively as yellow powder. From F_{17}, compound **13** (26 mg) was isolated as a white powder.

3.2.3. Extraction and isolation from the roots.

Air-dried, finely powdered stem of *B. antidysenterica* (5.00 kg) was extracted with the mixture CH_2Cl_2/MeOH for 72 hours at room temperature. The solution was evaporated in vacuum to afford a brown residue of 150.45 g. The crude extract (65.70 g) was adsorbed on an equivalent mass of silica and chromatographed over a silica gel column chromatography (40-63 μm, 4.5 x 50 cm) using n-hexane-AcOEt and $CHCl_3$-MeOH gradients as eluents. Fractions of 100 mL each were collected as follows: [(1-17), n-hexane 100%], [(18-35), n-hexane-AcOEt 2.5%], [(36-53), n-hexane-AcOEt 5%], [(54-71), n-hexane-AcOEt 7.5%], [(72-89), n-hexane-AcOEt 10%], [(90-107), n-hexane-AcOEt 12.5%], [(108-125), n-hexane-AcOEt 15%], [(126-143), n-hexane-AcOEt 17.5%], [(144-161), n-hexane-AcOEt 20%], [(162-179), n-hexane-AcOEt 25%], [(180-197), n-hexane-AcOEt 30%], [(198-215), n-hexane-AcOEt 40%], [(216-233), n-hexane-AcOEt 50%], [(234-251), n-hexane-AcOEt 60%], [(252-269), n-hexane-AcOEt 75%], [(270-287), n-hexane-AcOEt 80%], [(288-305), AcOEt 100%]. These fractions were combined on the basis of their TLC profiles to give 18 sub-fractions (F_1-F_{18}) as follows: Compounds **15** (19.3 mg) and **16** (20.2 mg) were isolated as white powder by filtration of the precipitates of the sub-from F_3 and F_4 respectively. From F_6, compound **17** (9.4 mg) was obtained as beige powder. From F_8, compound **18** (26 mg) was isolated as a yellow powder.

3.2.4. Acid hydrolysis of bruceadysentoside and identification of the sugar

3 mg of compound 1 was hydrolyzed by 3 mL of 2.0 M HCl at 85 °C during 2 h of reflux. After cooling, the solvent was removed under reduced pressure. The sugar was extracted from the aqueous phase (20 mL) and washed with CH_2Cl_2 (3x10 mL). The CH_2Cl_2 fractions were washed with water and afforded after evaporation the aglycone moiety. The sugar L-arabinose was then identified by co-TLC with authentic samples using the mixture ($CHCl_3$–MeOH–H_2O, 8:5:1) as eluent.

3.3. Cell culture

The prostate cancer cell line PC-3 and the colon cancer cell line HT-29 were cultured in RPMI 1640 completed medium (RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% heat-inactivated FCS) in a humidified atmosphere with 5% CO_2 at 37°C. The cells were routinely grown to subconfluency (~ 70-80%) prior to subsequent usage or subculturing. The adherent cells were rinsed with PBS and detached by using trypsin/EDTA (0.05% in PBS) prior to cell passaging and seeding. RPMI 1640 basal medium, FCS, L-glutamine, PBS and trypsin/EDTA for cell culturing were purchased from Capricorn Scientific GmbH (Ebsdorfergrund, Germany). The culture flasks, multi-well plates and further cell culture

plastics were purchased from TPP (Trasadingen, Switzerland) and Greiner Bio-One GmbH (Frickenhausen, Germany), respectively.

3.4. Cytotoxic activity – *in vitro* cell proliferation assay

Anti-proliferative and cytotoxic effects, respectively, of the compounds were investigated by performing a colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-based cell viability assay (Sigma-Aldrich, Taufkirchen, Germany). For that purpose, prostate (PC-3) and colorectal (HT-29) cancer cells were seeded in low densities into 96-well plates (4,000 – 6,000 cells per well; seeding confluency ~ 10%), and were allowed to adhere for 24 h. Subsequently, the cells were treated for 48 h with compound concentrations up to 200 µM that were prepared in RPMI 1640 complete medium. For control measures, cells were treated in parallel with 1% DMSO (negative control, representing the DMSO content of the highest concentrated test compound concentration) and 100 µM digitonin (positive control, for data normalization set to 0% cell viability), both in standard growth medium. Each data point was determined in triplicates. As soon as the 48 h incubation was finished, the incubation medium was discarded, cell were rinsed once with PBS, and 50 µL/well of MTT solution was added. The MTT solution, i.e. 0.5 mg/mL MTT in RPMI 1640 without phenol red and other supplements, was prepared freshly prior to use. After 30 min incubation under standard growth conditions (37°C, 5% CO_2), the MTT solution was discarded and cells were treated with 50 µL/well DMSO (Duchefa Biochemie, Haarlem, The Netherlands) to solubilize formazan crystals. Finally, the conversion of MTT to formazan by the remaining portion of viable, metabolically active cells was measured at 570 nm absorption with background (670 nm absorption) subtraction by using a SpectraMax M5 multiwell plate reader (Molecular Devices, San Jose, USA). For data analyses GraphPad Rism version 8.0.2 and Microsoft Excel 2013 were used.

(20R)-O-(3)-α-L-arabinopyranosylpregn-5-ene-3β,20-diol (8)

^{1}H NMR (600 MHz, DMSO-*d6*) δ: 0.69 (3H, s, 18-CH_3), 0.88 (1H, m, H-9), 0.96 (3H, s, 19-CH_3, H-14), 1.00 (3H, d, *J* = 6.0 Hz, 21-CH_3), 1.11 (2H, m, H-1, H-12α), 1.32 (1H, m, H-8), 1.39 (1H, m, H-11), 1.46 (1H, m, H-7), 1.51 (1H, m, H-17), 1.52 (1H, m, H-15), 1.81 (2H, m, H-2α, H-12β), 1.89 (1H, m, H-16), 2.12 (1H, m, H-4α, H-2β), 2.35 (1H, m, H-4β), 3.35 (1H, dd, *J* = 2.9 and 12.1 Hz, H-5'α), 3.40 (1H, m, H-3), 3.29 (1H, dd, J = 4.4 and 6.5 Hz, H-3'), 3.52 (1H, m, H-20), 3.59 (1H, d, m, H-4'), 3.64 (1H, dd, *J* = 2.9 and 12.1 Hz, H-5'β), 3.28 (1H, t, *J* = 5.3 Hz, H-2'), 4.17 (1H, d, *J* = 6.6 Hz, H-1'), 5.32 (1H, brs, H-6) ; ^{13}C NMR (150 MHz,

DMSO-*d6*) δ: 12.4 (C-18), 19.5 (C-19), 20.9(C-11), 24.7 (C-16), 26.8 (C-15), 29.9 (C-2), 24.3 (C-21), 31.8 (C-8), 32.0 (C-7), 36.8 (C-10), 37.4 (C-12), 38.8 (C-1), 39.6 (C-4), 42.3 (C-13), 50.3 (C-9), 56.3 (C-14), 58.1 (C-17), 65.8 (C-5'), 68.1 (C-4'), 68.8 (C-20), 71.1 (C-2'), 73.2 (C-3'), 77.4 (C-3), 101.9 (C-1'), 121.6 (C-6), 141.0 (C-5),

(20R)-O-(3)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-pregn-5-ene-3β,20-diol (9)

^{1}H NMR (600 MHz, DMSO-*d6*) δ: 0.68 (3H, s, 18-CH_3), 0.89 (1H, m, H-9), 0.98 (3H, s, 19-CH_3, H-14), 1.00 (3H, d, *J* = 6.0 Hz , 21-CH_3), 1.09 (2H, m, H-1, H-12α), 1.24 (1H, m, H-8), 1.31 (1H, m, H-8), 1.39 (1H, m, H-11), 1.40 (1H, m, H-7), 1.52 (1H, m, H-15),1.56 (1H, m, H-17), 1.81 (2H, m, H-2α, H-12β), 1.90 (1H, m, H-16), 2.17 (1H, m, H-4α, H-2β), 2.51 (1H, m, H-4β), 3.00 (1H, t, J = 8.4 Hz, H-2"), 3.12 (1H, m, H-3), 3.22 (1H, m, H-5"), 3.38 (1H, m, H-3"), 3.48 (1H, m, H-6"α), 3.49 (1H, m, H-20), 3.66 (1H, m, H-6"β), 3.68 (1H, m, H-5'), 3.60 (1H, t, *J* = 8.7 Hz, H-3'), 3.67 (1H, d, m, H-4'), 3.57 (1H, t, *J* = 7.8 Hz, H-2'), 3.60 (1H, m, H-4"), 4.28 (1H, d, *J* = 7.8 Hz, H-1"), 4.52 (1H, d, *J* = 7.1 Hz, H-1'), 5.31 (1H, brs, H-6); ^{13}C NMR (150 MHz, DMSO-*d6*) δ: 12.4 (C-18), 19.6 (C-19), 20.9 (C-11), 24.3 (C-16), 25.8 (C-15), 29.9 (C-2), 24.8 (C-21), 31.8 (C-8), 32.0 (C-7), 36.8 (C-10), 37.3 (C-1), 38.8 (C-12), 40.2 (C-4), 42.4 (C-13), 50.3 (C-9), 56.3 (C-14), 58.1 (C-17), 66.7 (C-5'), 63.4 (C-4'), 68.8 (C-20), 80.0 (C-2'), 73.2 (C-3'), 77.4 (C-3), 99.6 (C-1'), 121.5 (C-6), 141.0 (C-5), 104.5 (C-1"), 74.8 (C-2"), 77.7 (C-3"), 71.2 (C-4"), 76.8 (C-5"), 61.4 (C-6").

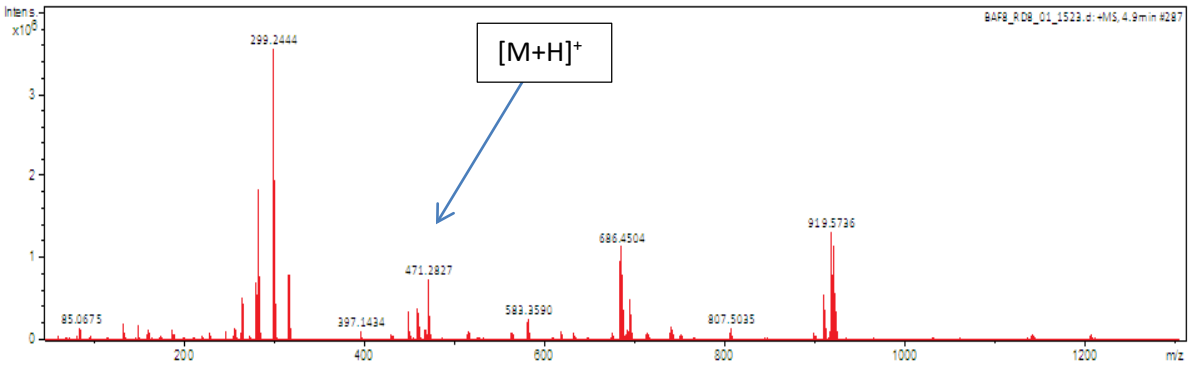


Figure S1: HRESIMS spectrum of compound **1**

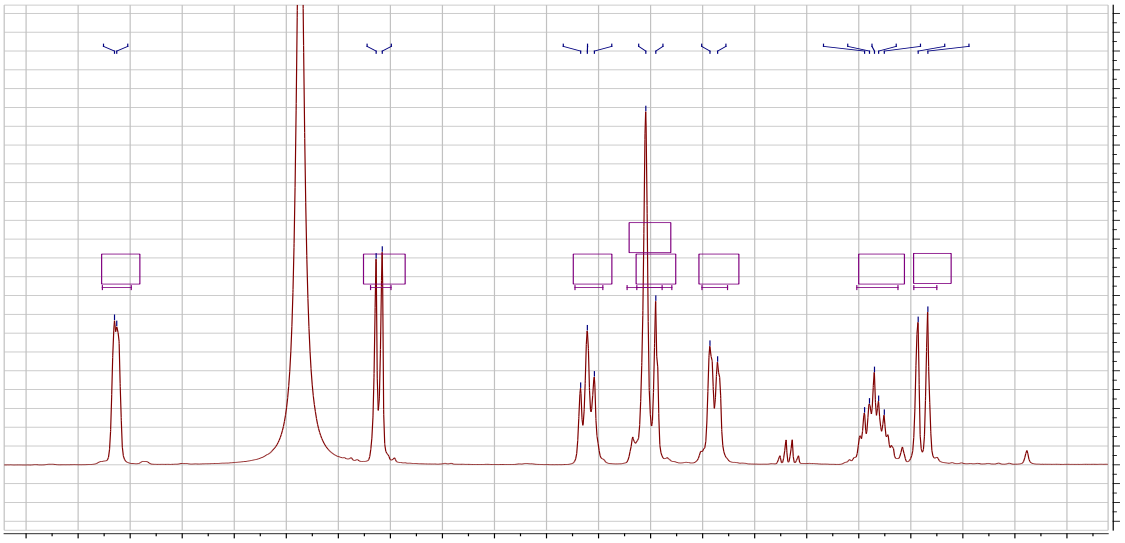


Figure S2: ^{1}H spectrum of compound **1** (sugar region)

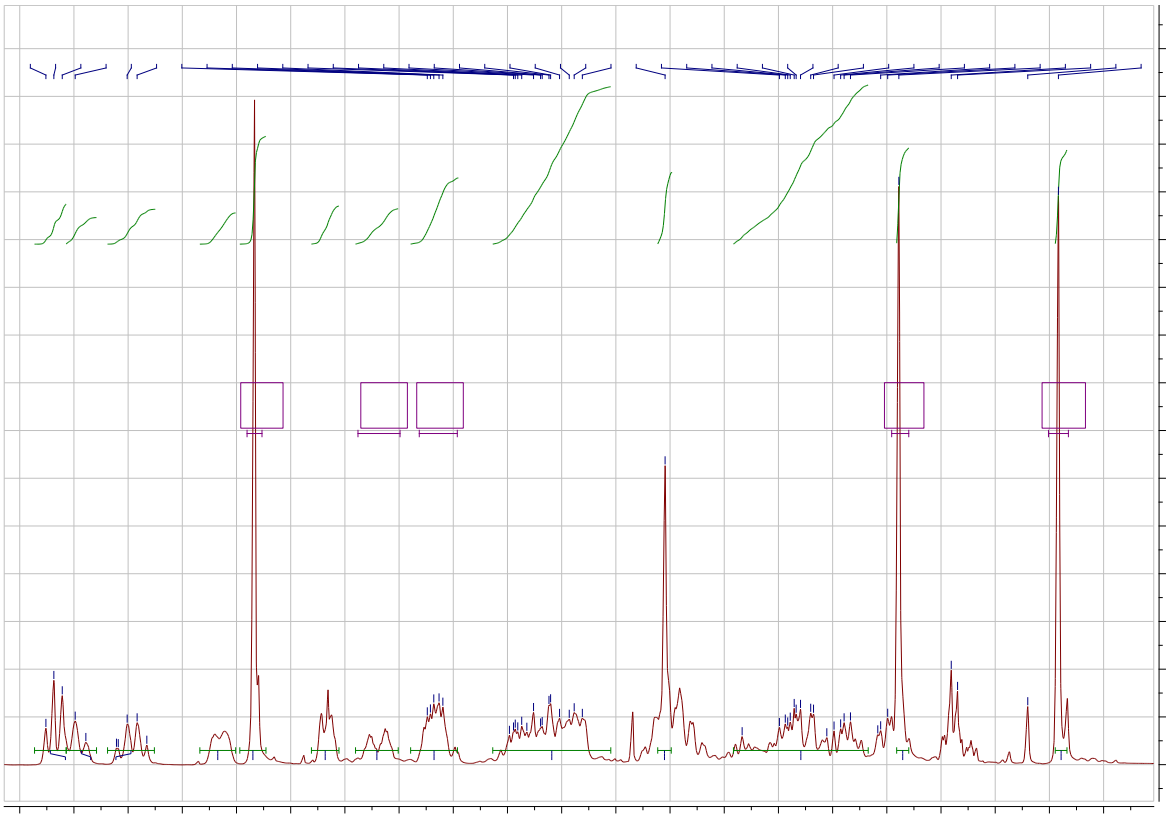


Figure S3: ^{1}H spectrum of compound **1** (aliphatic region)

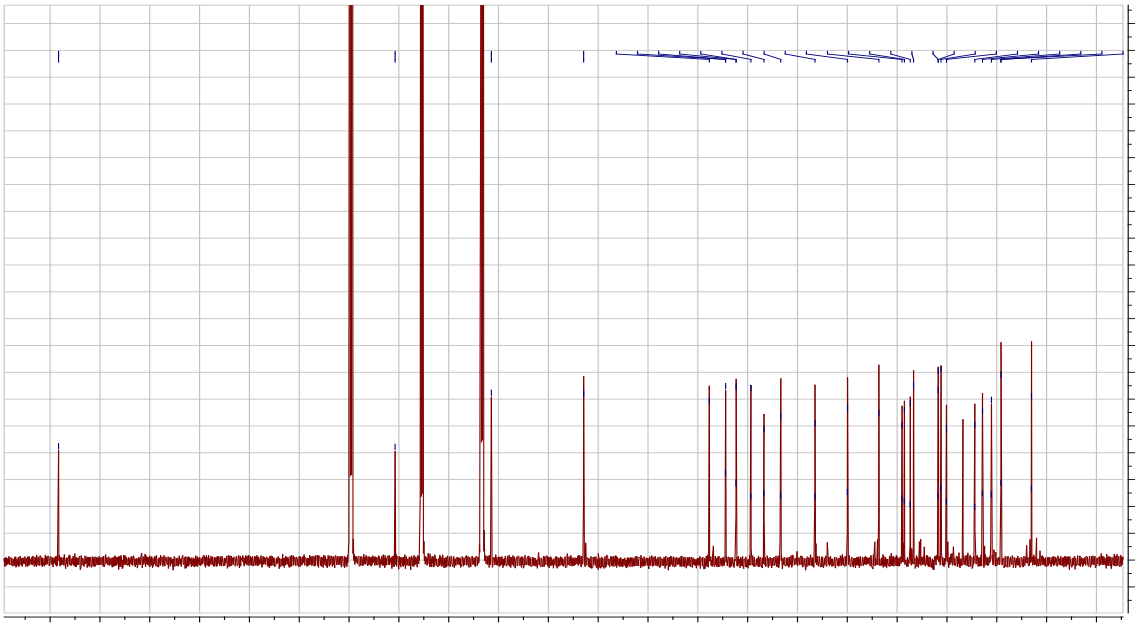


Figure S4: ^{13}C spectrum of compound **1**

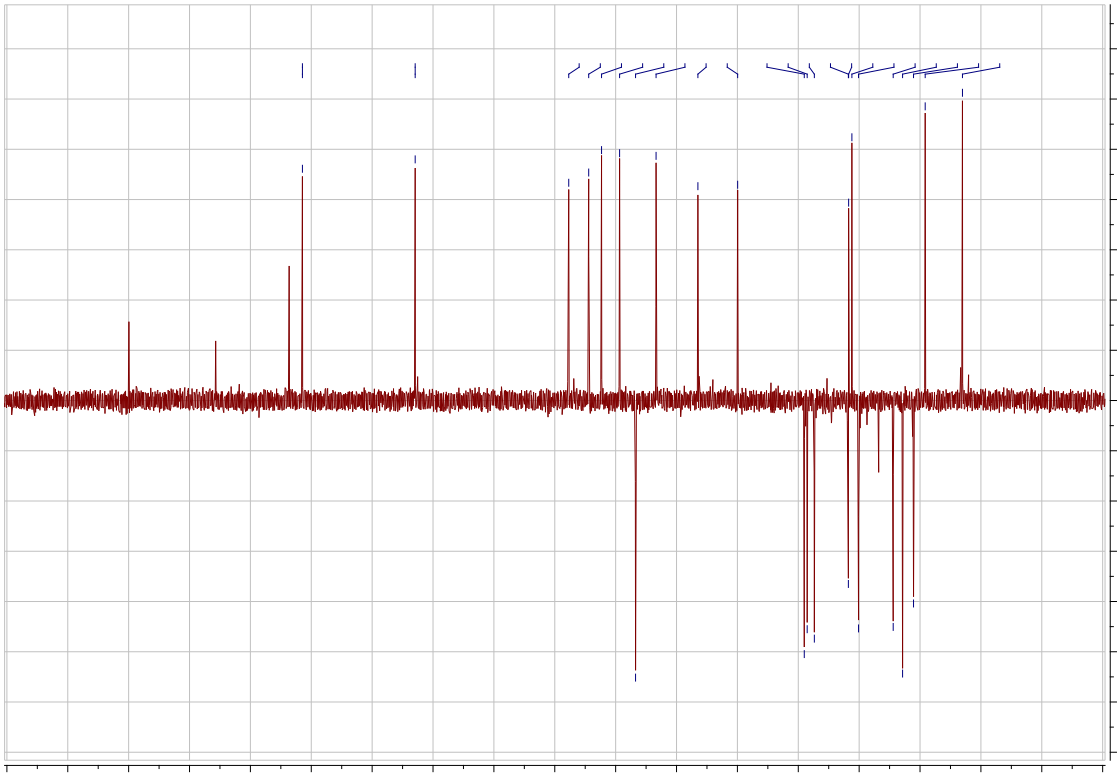


Figure S5: DEPT 135 spectrum of compound **1**

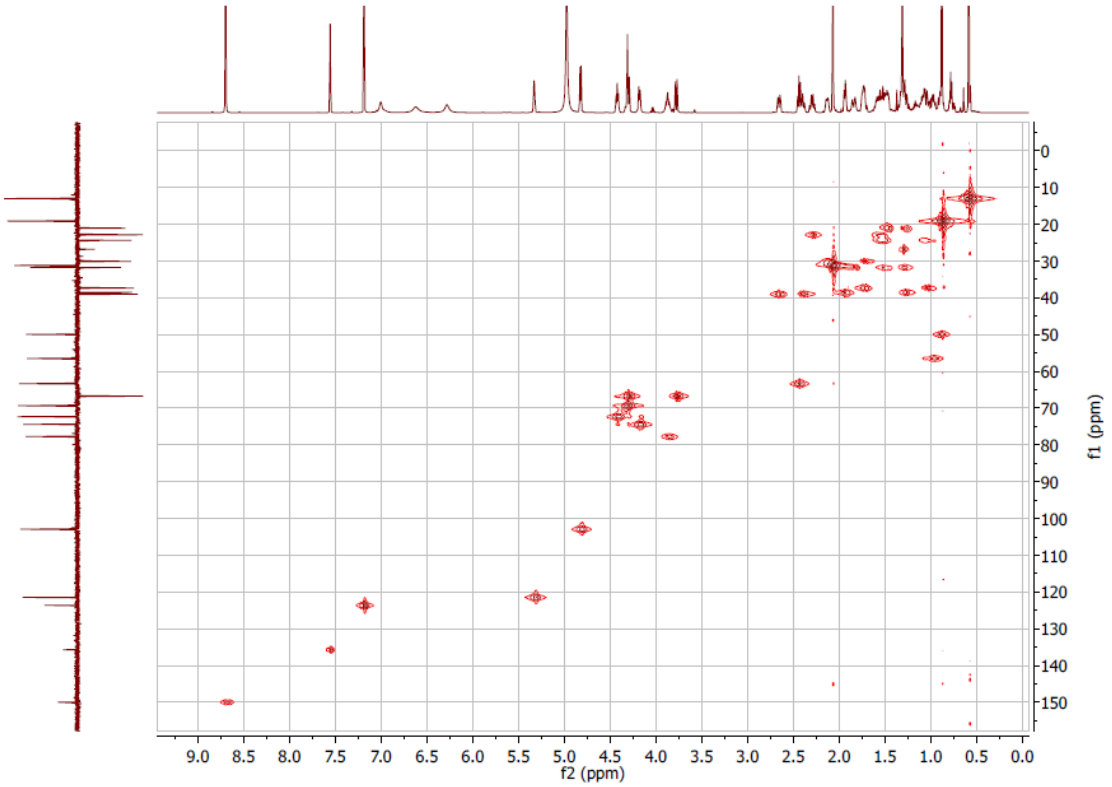


Figure S6: HMQC spectrum of compound **1**

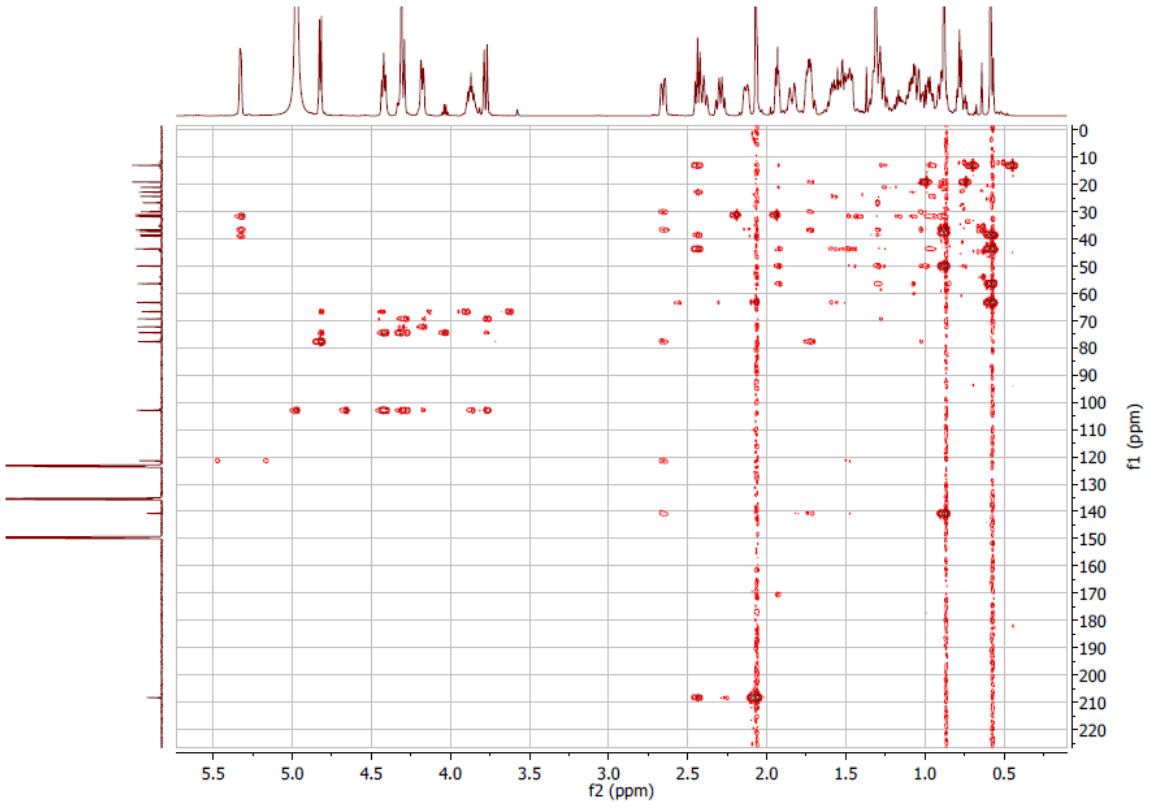


Figure S7: Full HMBC spectrum of compound **1**

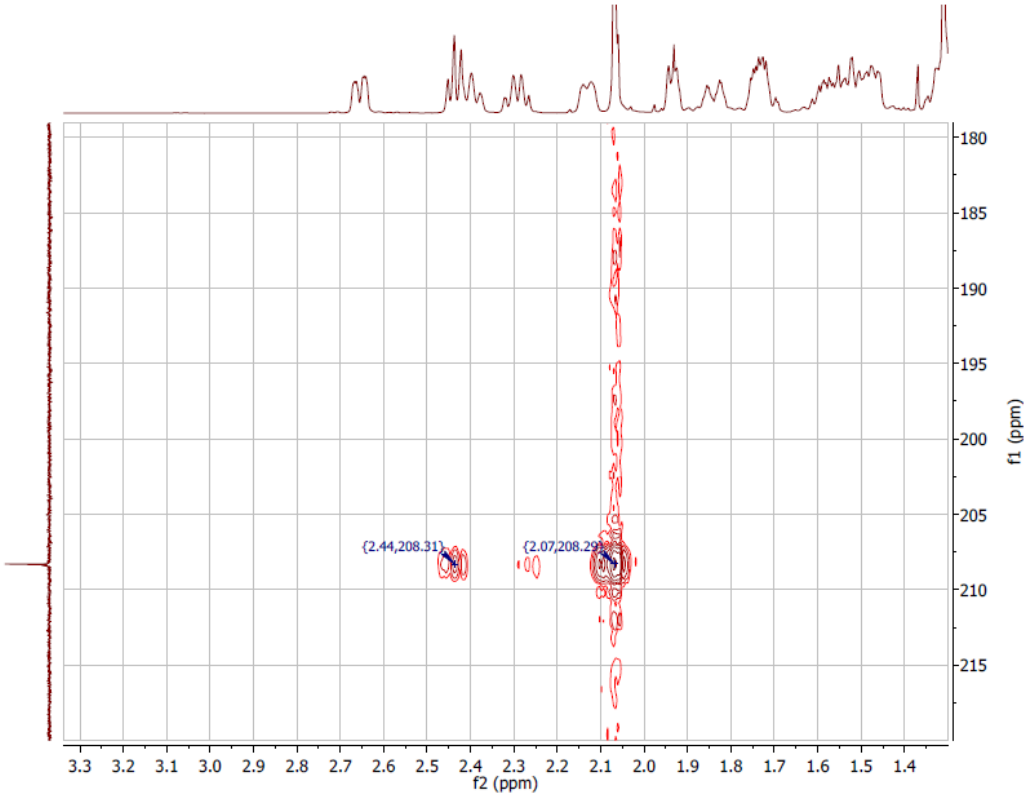


Figure S8: HMBC spectrum of compound **1** showing correlations with carbonyl group

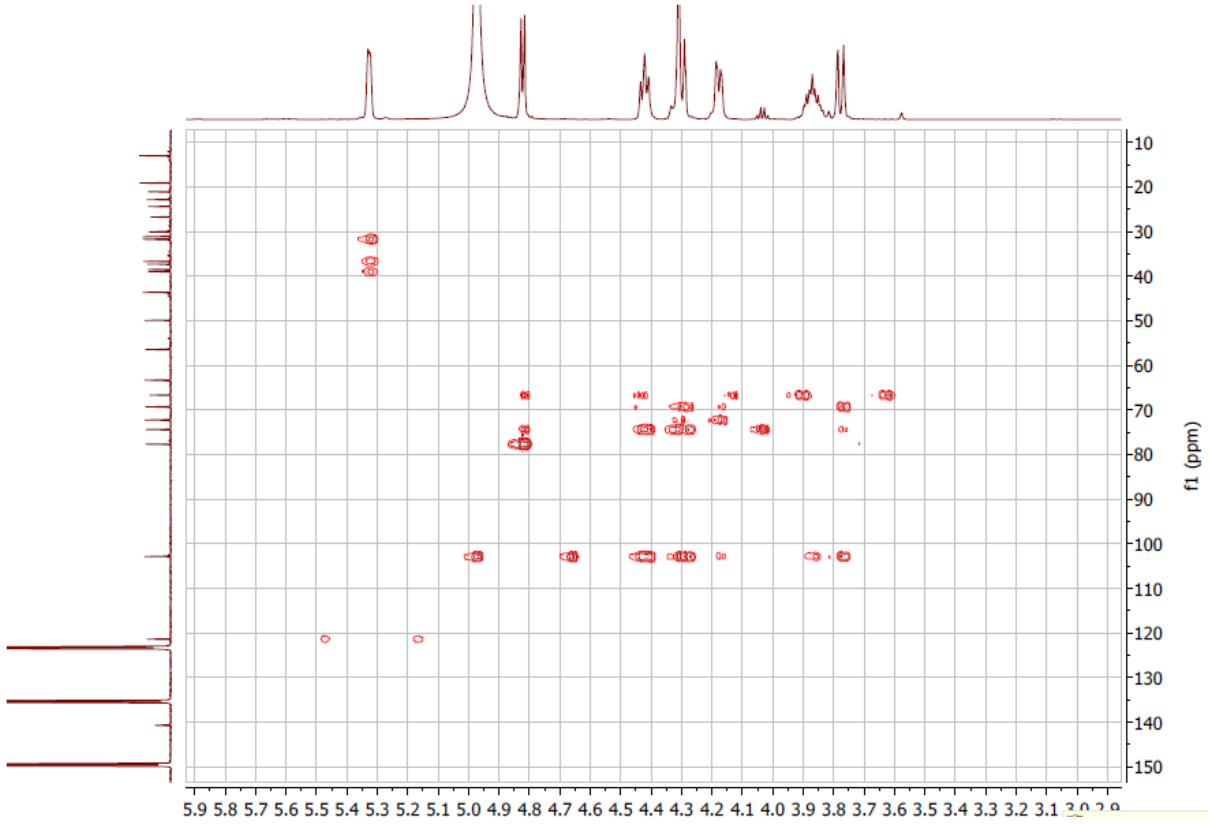
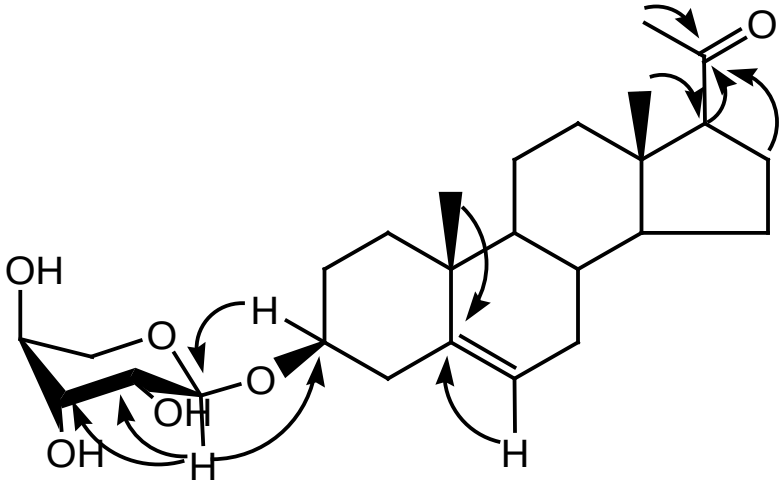


Figure S9: HMBC spectrum of compound **1** (aromatic region)



1

Figure S10. Important COSY and HMBC correlations of compound **1**

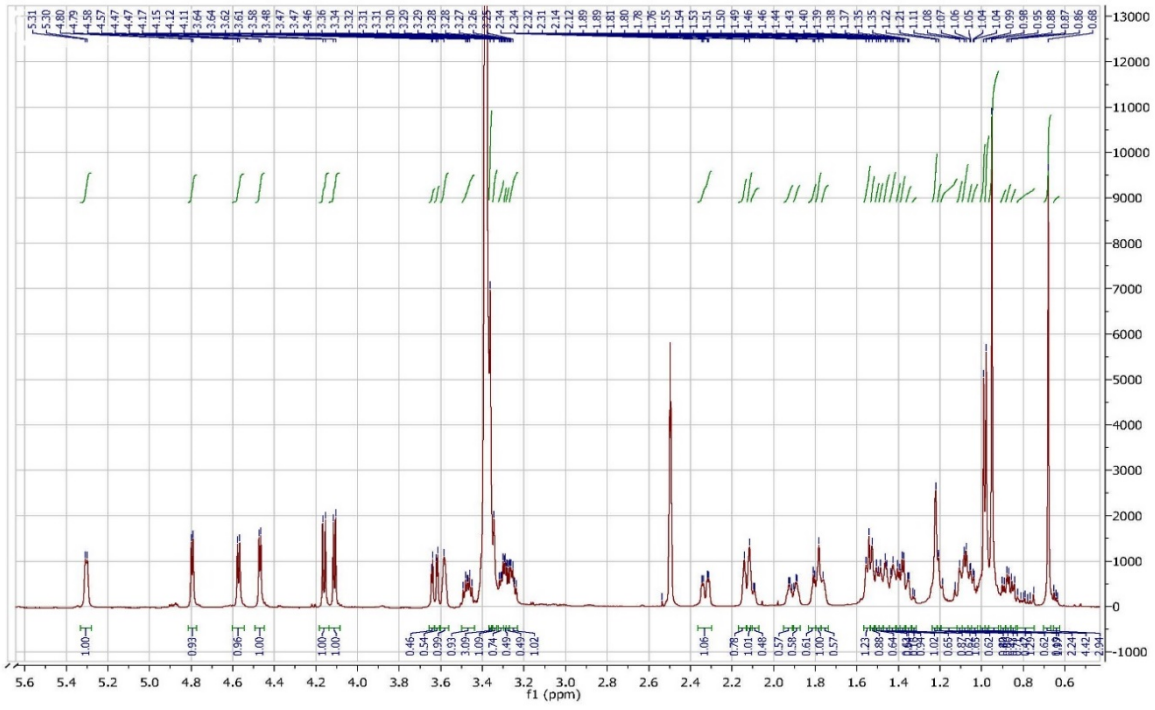


Figure S11: ^{1}H NMR spectrum of compound **8**

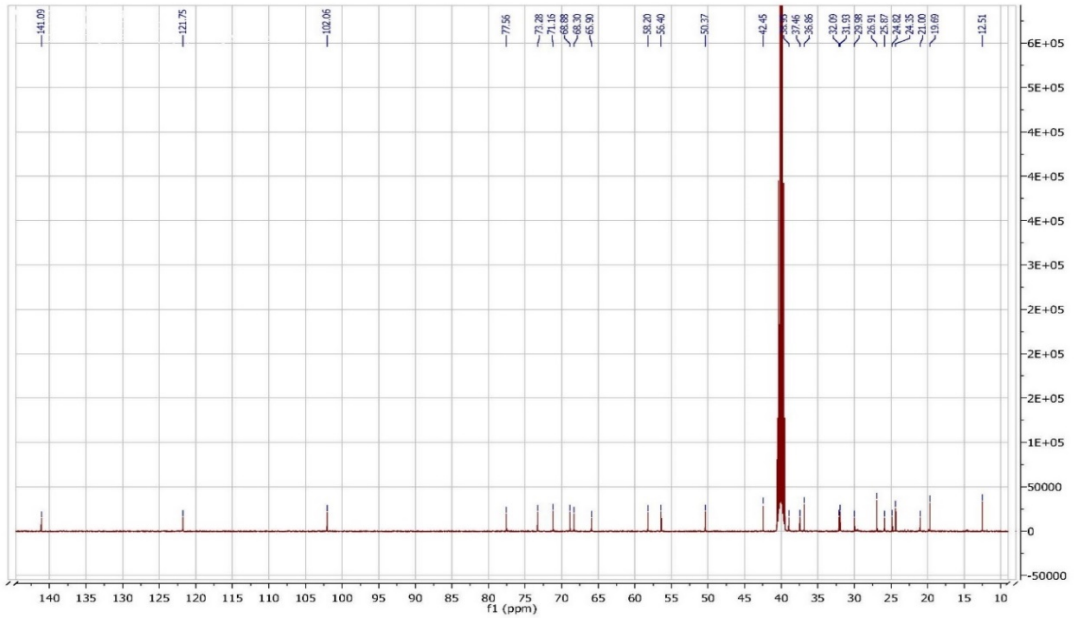


Figure S12: ^{13}C NMR spectrum of compound **8**

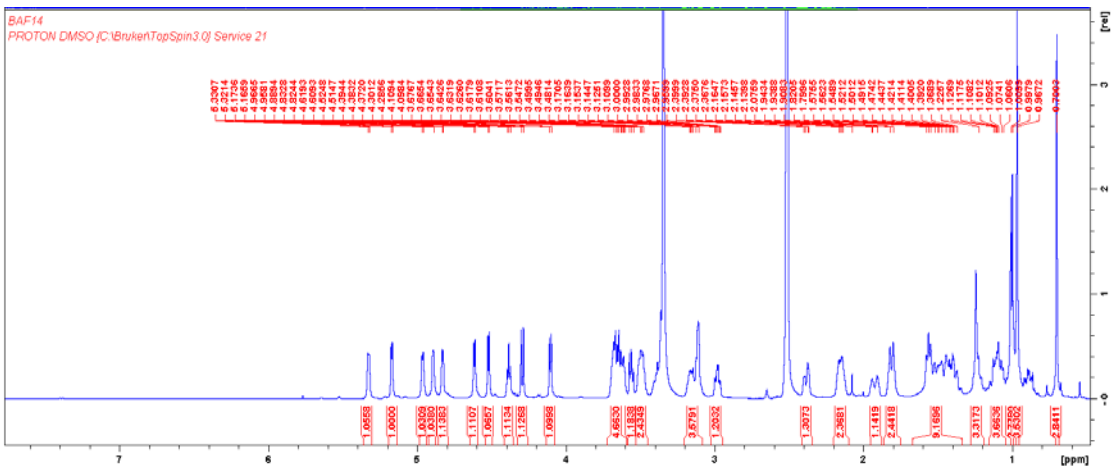


Figure S13: ^{1}H NMR spectrum of compound **9**

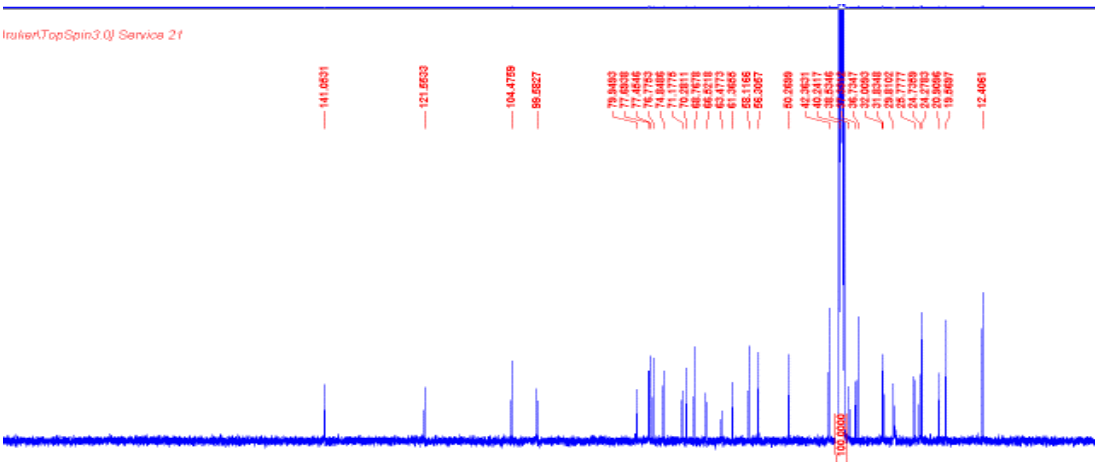


Figure S14: ^{13}C NMR spectrum of compound **9**

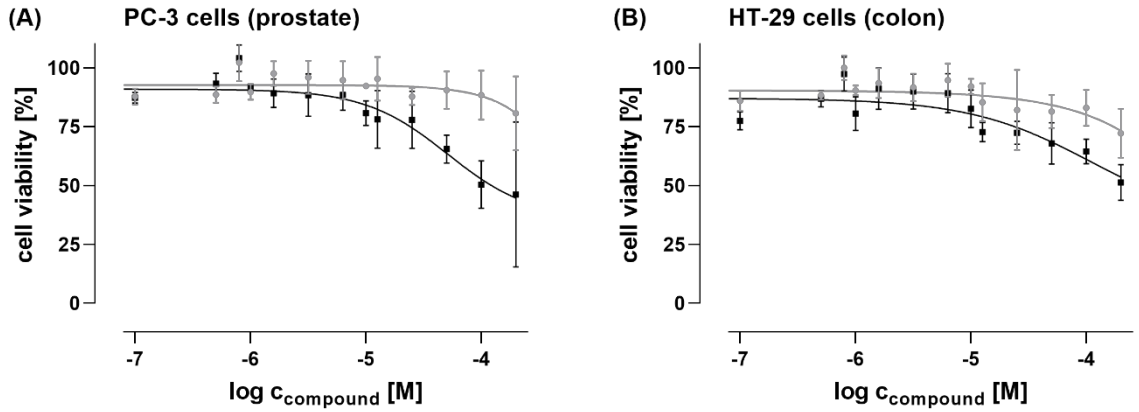


Fig. S15: Cell viability of (A) prostate cancer PC-3 cells and (B) colon cancer HT-29 cells treated for 48 h with FOG062 (1,11-dimethoxycanthin-6-one, **5**; ●) and FOG065 (hydnocarpin, **6**; ■), respectively, as determined by MTT assay.