Antibacterial activity of phytochemicals from *Bauhinia thonningii* signifiquantly increased in the presence of the efflux pump inhibitor, phenylalanine-arginine-*β*-naphthylamide towards multidrug-resistant phenotypes

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SF1. Experimental

SF1.1. General

The proton and carbon (1D & 2D)-NMR (600/500 MHz) spectra were measured on Bruker AMX machine. The chemical shifts of proton and carbon were recorded base on the internal reference TMS (Tetramethylsilane) in δ (ppm). Moreover, coupling constants (J) were measured in Hz. High resolution mass spectra were obtained on QTOF Spectrometer equipped with a HESI source. The spectrometer was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.40 ppm deviation using Na formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200 °C. Nitrogen was used as sheath gas (10 l/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) UHPLC system consisting of LC-pump, Diode Array Detector (DAD) (λ: 190-600 nm), auto sampler (injection volume 5 µl) and column oven (40 °C). The separations were performed using a Synergi MAX-RP 100A (50 X 2 mm, 2.5µ particle size) with a H_2O (+0.1 % HCOOH) (A)/acetonitrile (+0.1 % HCOOH) (B) gradient (flow rate 500 µL/min, injection volume 5 µL). Samples were analyzed using a gradient program as follows: 95 % A isocratic for 1.5 min, linear gradient to 100 % B over 6 min, after 100 % B isocratic for 2 min, the system returned to its initial condition (90 % A) within 1 min, and was equilibrated for 1 min. UV/Visible spectroscopic data of compounds were recorded on an Evolution 300 spectrophotometer (Thermo Scientific). The purity of compounds and the monitoring of fractions were based on pre-coated silica gel TLC (Thin Layer Chromatography) plates supported on either plastic or aluminum sheets (E. Merck, F_{254}). Spots were visualized on TLC with UV light (254 nm & 365 nm) on CN-6 UV spectrometer then sprayed with ceric sulphate and heated at about 90°C.

SF1.2. Plant material

The leaves of *B. thonningii* were collected in December 2020 in Bandjoun, West Region-Cameroon and identified at the National Herbarium of Cameroon on voucher number 33258/HNC.

SF1.3. Extraction, isolation and purification

2.0 kg of air-dried of *B. thonningii* leaves were crushed and extracted with 15 L of methanol for 72 hours to yield 200.4 g of crude extract. 194.0 g were triturated with EtOAc to afford 96.2 g of EtOAc extract with 78.3 g of residue. 96.0 g of the resulted extract were subjected to

column chromatography (CC) unsing silica gel and eluted with a gradient of *n*-hexane-EtOAc (100:0 to 0:100, v/v) and EtOAc-CH_3OH (100:0 to 0:100, v/v) to afford 168 fractions of 300 mL each collected as follows: (100:0) *n*-hexane-EtOAc, 1-24; (19:1) *n*-hexane-EtOAc, 25-47; (17:3) *n*-hexane-EtOAc, 48-84; (3:1) *n*-hexane-EtOAc, 85-108; (2:3) *n*-hexane-EtOAc, 109-117; (1:3) *n*-hexane-EtOAc, 118-132; (100:0) EtOAc-CH_3OH, 133-144; (9:1) EtOAc-CH_3OH, 145-160 and (0:100) EtOAc-CH_3OH, 161-168. Based on their TLC profiles, these fractions were combined into seven major fractions (A-G): Fractions A(0% *n*-hexane-EtOAc, 10.2 g) or sub-fractions 1-22; B(0%-15% *n*-hexane-EtOAc, 7.5 g) or sub-fractions 23-62; C(15%-25% *n*-hexane-EtOAc, 8.0 g) or sub-fractions 63-85; D(25%-75% *n*-hexane-EtOAc, 25.7 g) or sub-fractions 86-125; E(75%-100% *n*-hexane-EtOAc, 10.5 g) or sub-fractions 126-137; F(0%-10% EtOAc-CH_3OH, 5.7 g) or sub-fractions 138-155 and G(10%-100% EtOAc-CH_3OH, 6.5 g) or sub-fractions 156-168. Fractions A and B were found to contain mainly fatty acids and were not further investigated. Compounds **4** (15.1 mg) and **10** (19.9 mg) precipitated from fraction C (2.1 g), while compound **11** (25.0 mg) precipitated from fraction E (0.2 g), then obtained after simple filtration with EtOAc. Fraction D was subjected to repeated silica gel CC using a gradient elution of *n*-hexane-EtOAc (100:0 to 50:50, v/v) to afford 75 fractions of 75 mL each collected as follows: (19:1) *n*-hexane-EtOAc 1-33; (17:3) *n*-hexane-EtOAc 34-49; (7:3) *n*-hexane-EtOAc 50-63; (1:1) *n*-hexane-EtOAc 64-75. On the based of their TLC profiles, sub-fractions 44-48, 50-51 and 53-54 were grouped separately and identified as a mixture of coumpounds and the rest the fractions were not investigate. The mixture of coumpounds was traited and purified by CC over Sephadex LH-20 eluting with CH_2Cl_2-CH_3OH (1:1), v/v to yield compound **1** (9.9 mg) from sub-fraction 44-48; **2** (12.1 mg) and **8** (6.1 mg) from sub-fraction 50-51; while **9** (15.1 mg) from sub-fraction 53-54.

The residue (77.0 g) from the trituration was subjected to repeated silica gel CC using a gradient elution of *n*-hexane-EtOAc (100:0 to 0:100, v/v) and EtOAc-CH_3OH (100:0 to 1:9, v/v) to afford 67 fractions of 200 mL each collected as follows: (100:0) *n*-hexane-EtOAc, 1-7; (17:3) *n*-hexane-EtOAc, 8-24; (3:1) *n*-hexane-EtOAc, 25-30; (3:2) *n*-hexane-EtOAc, 31-40; (100:0) EtOAc-CH_3OH, 41-55 and (9:1) EtOAc-CH_3OH, 56-67. Based on their TLC profiles, these fractions were grouped in the five major fractions(I-V). Fractions I (0%-15% *n*-hexane-EtOAc, 8.0 g) or sub-fractions 1-23; II (15%-25% *n*-hexane-EtOAc, 7.5 g) or sub-fractions 24-28; III (25%-40% *n*-hexane-EtOAc, 12.0 g) or sub-fractions 29-40; IV (100% EtOAc, 30.7 g) or sub-fractions 41-47 and V(0%-10% EtOAc-CH_3OH, 10.5 g) or sub-fractions 48-67.

A precipitate on powder form was observed from fractions II-IV. After simple filtration and purification with ethyl acetate, compounds **7** (8.1 mg), **3** (11.1 mg) and mixture were obtained

from fractions II, III and IV respectively. The separation of a mixture was possible after CC over sephadex LH-20 eluting with CH_2Cl_2-CH_3OH (1:1), v/v to afford compounds **5** (15.9 mg) and **6** (17.2 mg).

SF2. Structural elucidation

The phytochemical study of *Bauhinia thonningii* led to the isolation and identification of eleven compounds whose structures are show in Figure 1. The spectral properties of these known compounds, including ^{1}H NMR; ^{13}C NMR and MHBC data, were identical to those previously described in the literature.

Compound 1: 6-C-methylquercetin-3, 4'-dimethyl ether; yellow powder, m.p. 195-197°C; *m/z* 345.09 for molecular formula $C_{18}H_{17}O_7$, ^{1}H NMR (600 MHz, CD_3OD) δ 7.70 (d, J = 2.1 Hz, H-2'), 7.63 (dd, J = 8.4, 2.1 Hz, H-6'), 6.96 (d, J = 8.4 Hz, H-5'), 6.45 (s, H-8), 3.95 (*s*, 4'-OCH_3), 3.79 (*s*, 3-OCH_3), 2.09 (*s*, 6-CH_3) and ^{13}C NMR (150 MHz, CD_3OD) δ 178.6 (C-4), 162.5 (C-7), 158.4 (C-5), 156.2 (C-2), 154.8 (C-9), 149.4 (C-4'), 147.5 (C-3'), 138.2 (C-3), 122.3 (C-6'), 121.6 (C-1'), 115.2 (C-5'), 111.5 (C-2'), 107.4 (C-6), 104.4 (C-10), 92.5 (C-8), 59.5 (3-OCH_3), 55.4 (3-CH_3), 6.4 (6-CH_3) [1]**.**

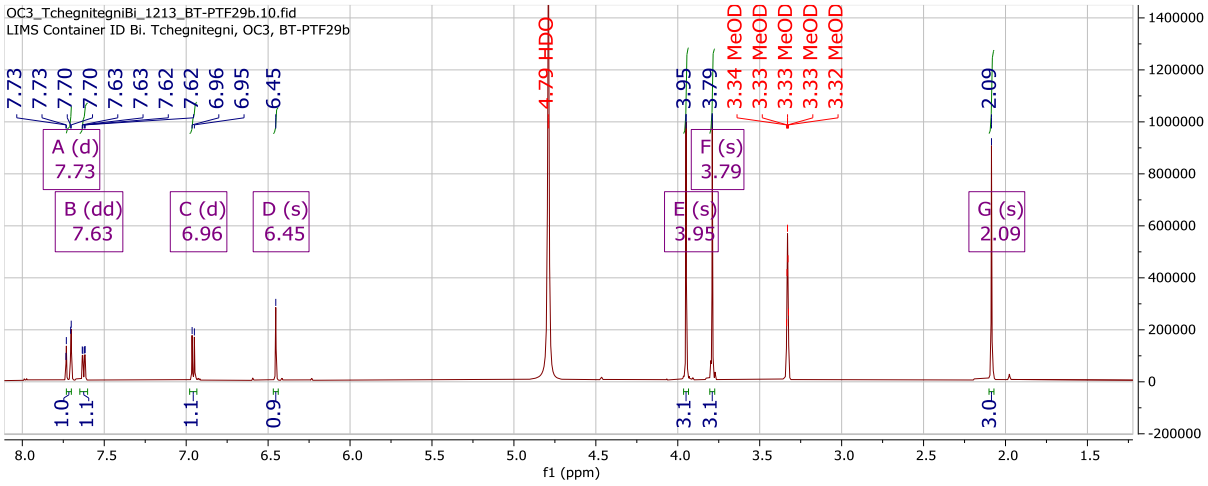

Figure S1: ^{1}H NMR spectrum (600MHz, CD_3OD) of compound (**1**)

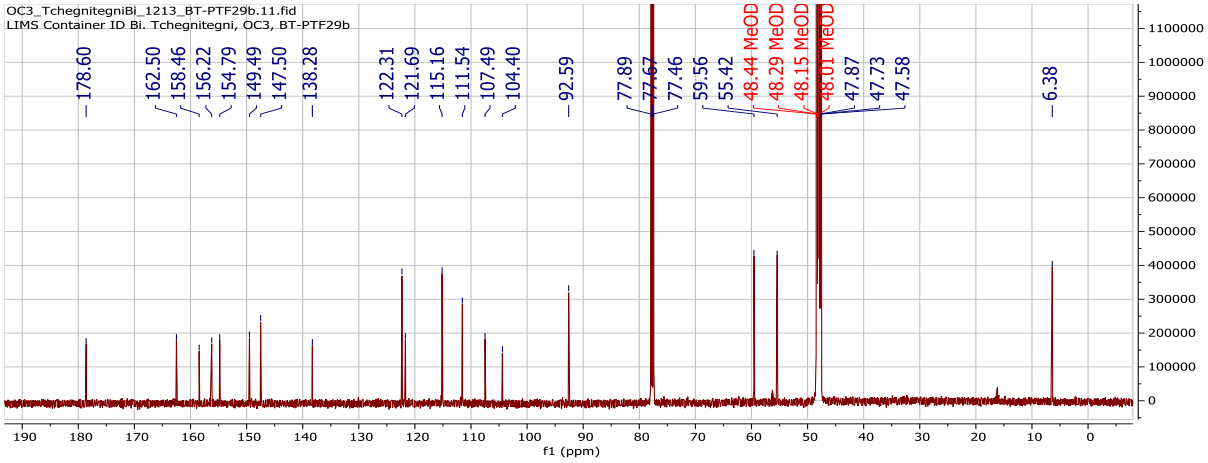

Figure S2: ^{13}C NMR spectrum (150MHz, CD_3OD) of compound (**1**)

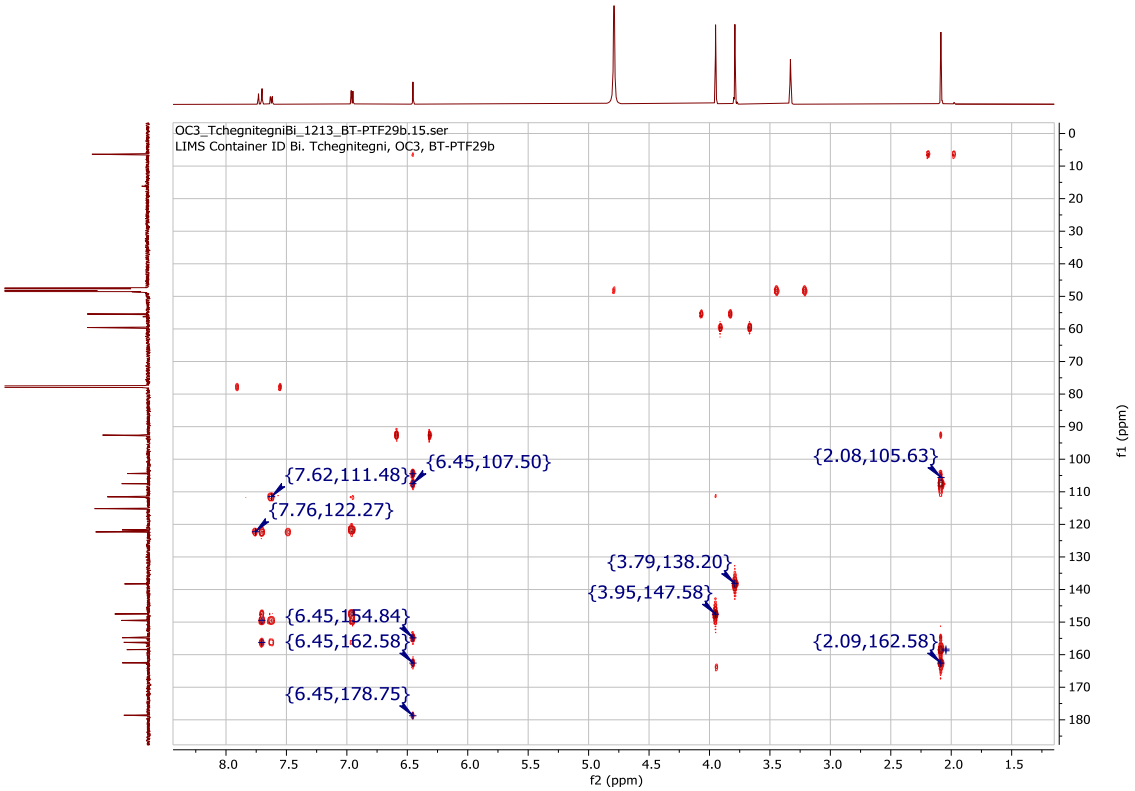

Figure S3: HMBC spectrum (600 MHz, CD_3OD) of compound (**1**)

Compound 2: 6-C-methylquercetin-3,7-dimethyl ether; yellow powder, m.p. 195-197°C; *m/z* 344.32 for molecular formula $C_{18}H_{17}O_7$, ^1H NMR (600MHz, CD_3OD) δ (ppm): 7.66 (d, J = 2.3 Hz, H-2'), 7.56 (dd, J = 8.4, 2.3 Hz, H-6'), 6.95 (d, J = 8.4 Hz, H-5'), 6.45 (s, H-8), 3.93 (s, 7-OCH_3), 3.79 (s, 3-OCH_3), 2.09 (s, 6-CH_3). ^{13}C NMR (600MHz, CD_3OD) δ (ppm) **:** 156.7(C-2), 138.5(C-3), 178.7(C-4), 157.5(C-5), 107.1(C-6), 163.7(C-7), 89.1(C-8), 155.1(C-9), 104.5(C-10), 121.4(C-1'), 121.0(C-2'), 144.9(C-3'), 148.5(C-4'), 115.2(C-5'), 115.2(C-6'), 55.6(3-OCH_3), 59.5(3'-OCH_3), 7.0(6-CH_3) [1,2].

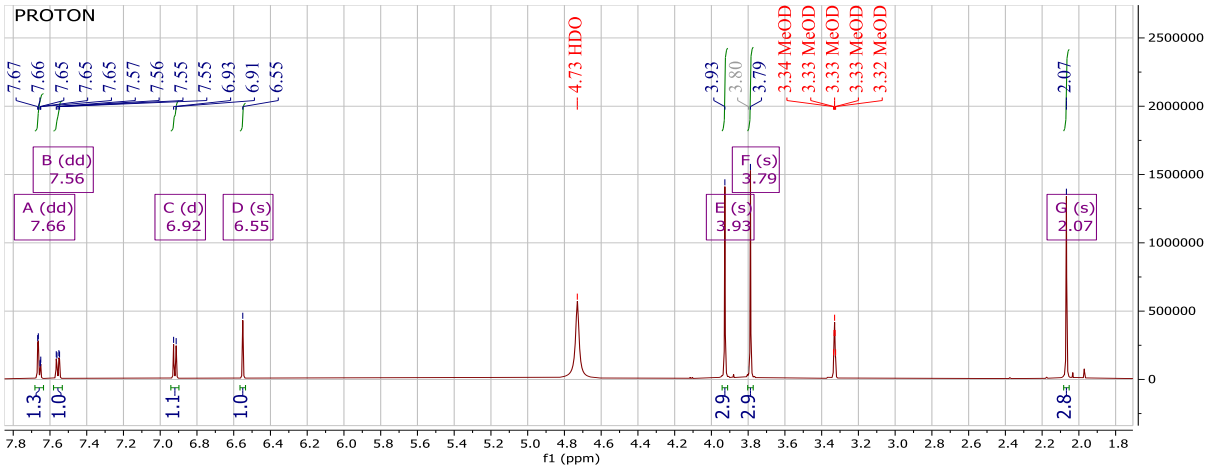

Figure S4: ^1H NMR spectrum (600MHz, CD_3OD) of compound (**2**)

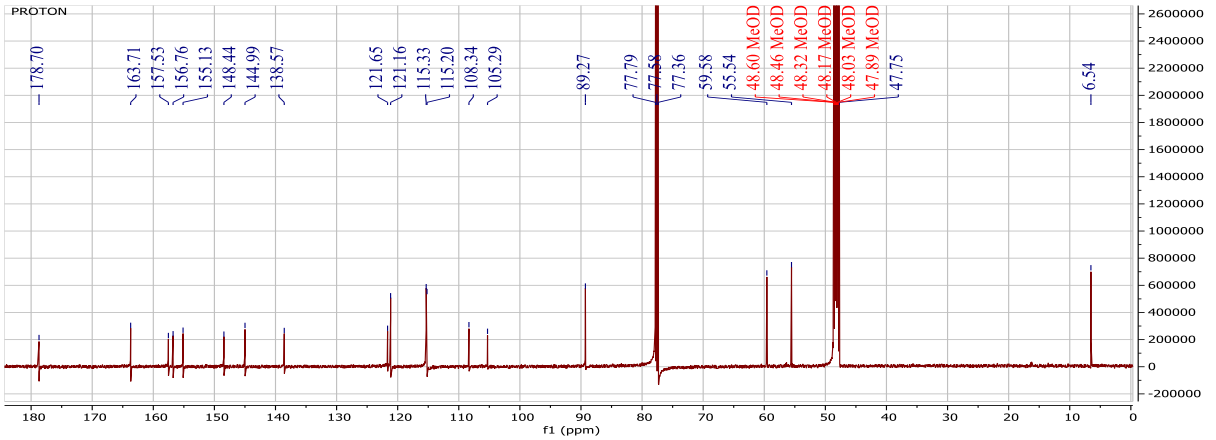

Figure S5: ^{13}C NMR spectrum (150MHz, CD_3OD) of compound (**2**)

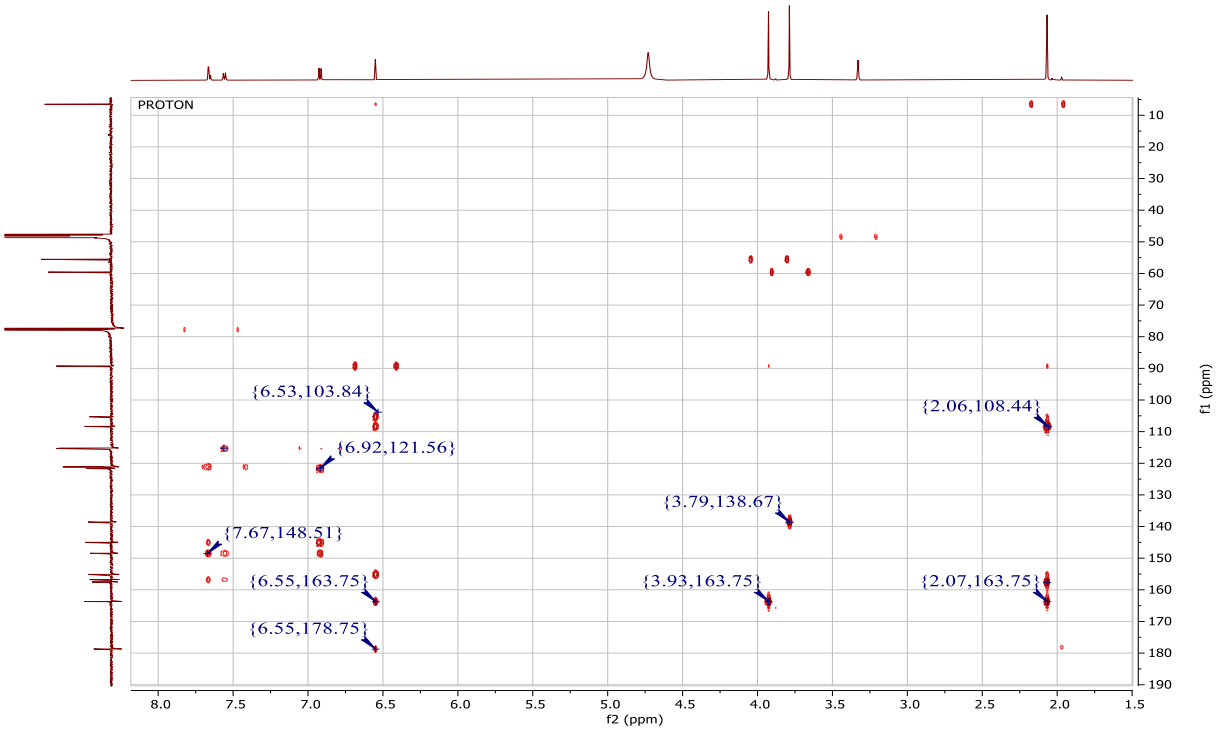

Figure S6: HMBC spectrum (600 MHz, CD_3OD) of compound (**2**)

Compound 3: 6-C-methylquercetin-3,7,3'-trimethyl ether, yellow powder; m.p. 185-187°C; *m/z* 358.35 for molecular formula $C_{19}H_{18}O_7$, ^{1}H NMR (600MHz, Acetone-d_6) δ **(**ppm**):** 6.29 (d, *J* = 2.1 Hz, H-2'), 6.25 (dd, *J* = 8.4, 2.1 Hz, H-6'), 5.59 (d, *J* = 8.4 Hz, H-5'), 5.06 (s, H-8), 2.55 (s, 7- OCH_3), 2.51 (s, 3-OCH_3), 2.40 (s, 3'-OCH_3), 0.69 (s, 6-CH_3). ^{13}C NMR (150MHz, Acetone-d_6) δ (ppm): 156.9(C-1), 139.6(C-2), 179.4(C-3), 158.5(C-4), 109.4(C-5), 164.3(C-6), 90.8(C-7), 155.9(C-8), 106.3(C-9), 122.7(C-10), 112.1(C-1'), 147.8(C-2'), 149.7(C-3'), 115.2(C-5'), 123.5(C-6'), 60.8(7-OCH_3), 56.7(3-OCH_3), 56.6(3'-OCH_3), 7.8(6-CH_3) [1,2**]**.

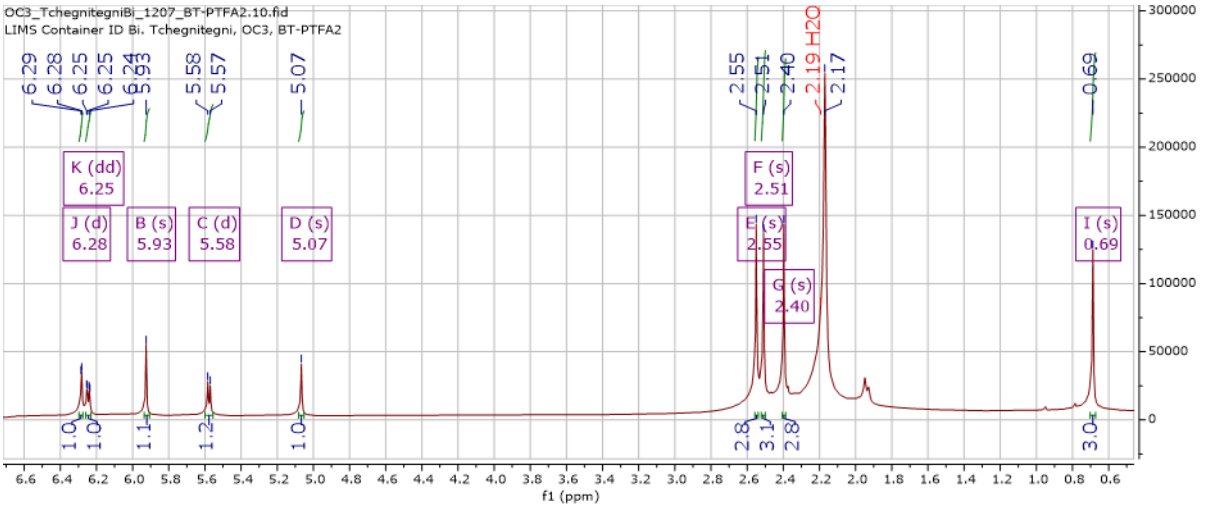

Figure S7: ^{1}H NMR spectrum (600MHz, Acetone-d_6) of compound (**3**)

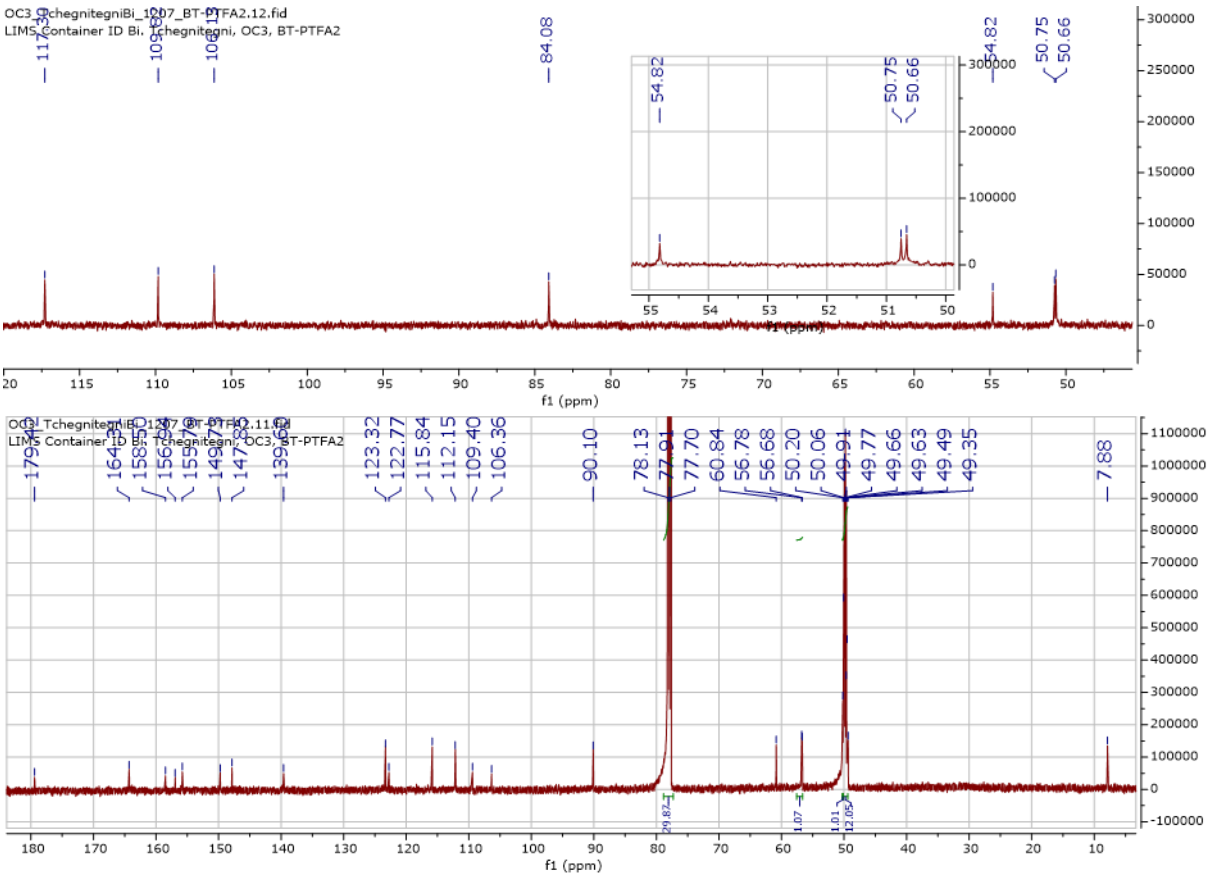

Figure S8: ^{13}C NMR spectrum (150MHz, Acetone-d_6) of compound (**3**)

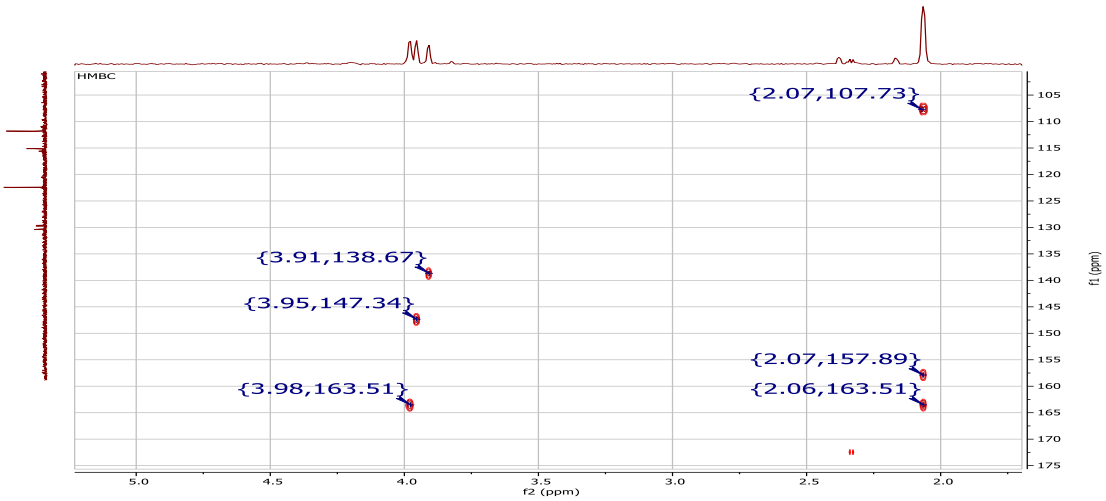

Figure S9: HMBC spectrum (600 MHz, Acetone-d_6) of compound (**3**)

Compound 4: Quercetin; yellow powder, m.p. 314-317°C; *m/z* 302.24 for molecular formula $C_{15}H_{10}O_7$, ^{1}H NMR (500 MHz, Acetone-d_6) δ (ppm) 12.51 (5-OH), 10.81 (7-OH), 9.62 (4'-OH), 9.41 (3-OH), 9.34 (3'-OH), 7.68 (d, *J*=2.1 Hz, H-2'), 7.54 (dd, *J*=8.5, 2.1 Hz, H-6'), 6.89 (dd, *J*= 8.5, 2.1 Hz, H-5'), 6.41 (d, *J*= 2.0 Hz, H-8), 6.19 (d, *J*= 2.0 Hz, H-6); ^{13}C NMR (125 MHz, Acetone-d_6) δ (ppm): 147.2(C-2), 136.1(C-3), 176.2(C-4), 161.1(C-5), 98.6(C-6), 164.3(C-7), 93.7(C-8), 156.5(C-9), 103.4(C-10), 122.3(C-1'), 115.4(C-2'), 145.5(C-3'), 148.1(C-4'), 116.0(C-5'), 120.4(C-6'). [2].

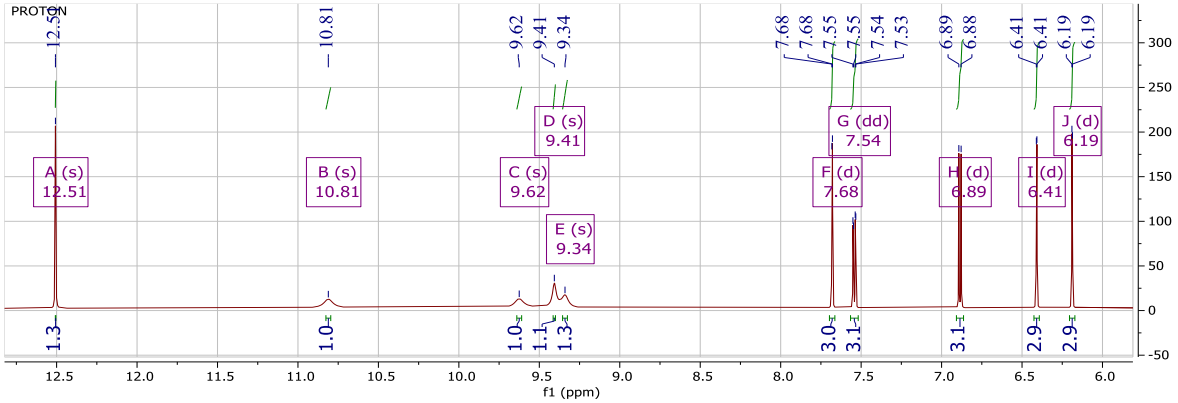

Figure S10: ^{1}H NMR spectrum (500MHz, Acetone-d_6) of compound (**4**)

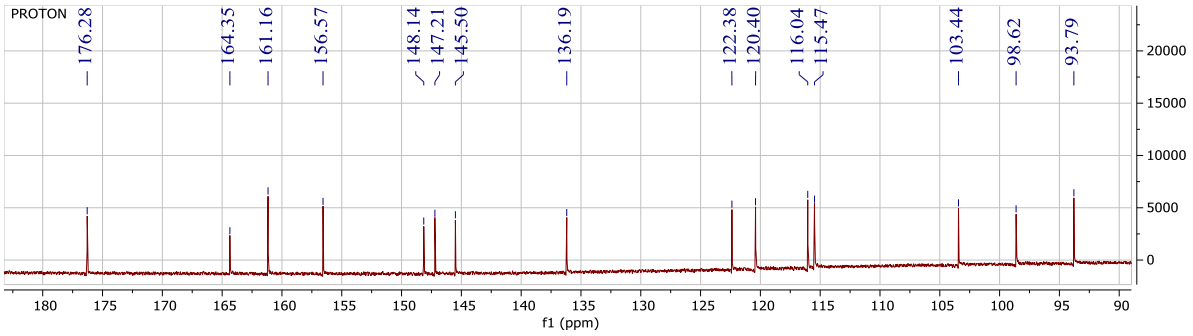

Figure S11: ^{13}C NMR spectrum (125MHz, Acetone-d_6) of compound (**4**)

Compound 5: Quercetine-3-O-L-rhamnopyranoside; yellow powder; m.p. 180-182°C; *m/z* 448.10 for molecular formula $C_{21}H_{20}O_{11}$, ^{1}H NMR (500 MHz, CD_3OD) δ (ppm) 7.35 (d, *J* = 2.1 Hz, H-2'), 7.32 (dd, *J* = 8.3 and 2.2 Hz, H-6') , 6.93 (d , *J* = 8.3 Hz , H-5'), 6.27 (d , *J*=2.1 Hz , H-6), 6.45 (d, *J* = 2.6 Hz, H-8) ; 4.21 (dd, *J* = 3.4, 1.7 Hz, H-2"), 3,74 (dd, *J* = 9.3, 3.4 Hz, H-3"), 3.43 (dq, *J* = 9.4, 6.1 Hz, H-4"), 3.35 (d, *J* = 9.4 Hz, H-5"), 0.97 (d, *J* = 5.8 Hz, H-6"); ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 157.8 (C-2), 134.8 (C-3), 178.3 (C-4), 161.8(C-5), 98.6(C-6), 164.5(C-7), 93.6(C-8), 157.1(C-9), 104.4(C-10), 121.5(C-1'), 115.8(C-2'), 145.6(C-3'), 148.5(C-4'), 115.3(C-5'), 121.7(C-6'), 102.1(C-1"), 70.5(C-2"), 70.8(C-3"), 70.8(C-4"), 71.8(C-5'') and 16.8(C-6'') [1,2].

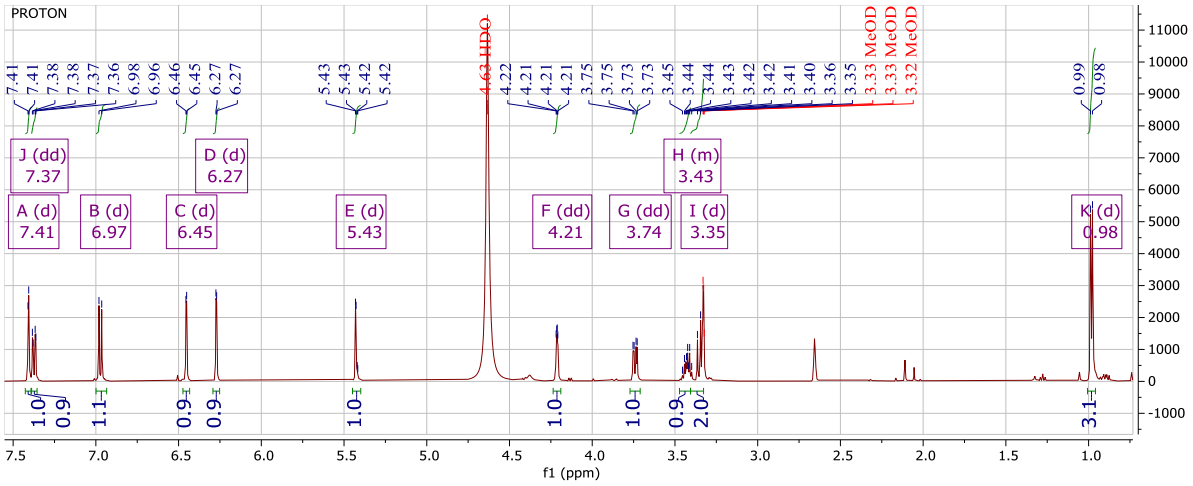

Figure S12: ^{1}H NMR spectrum (500MHz, CD_3OD) of compound (**5**)

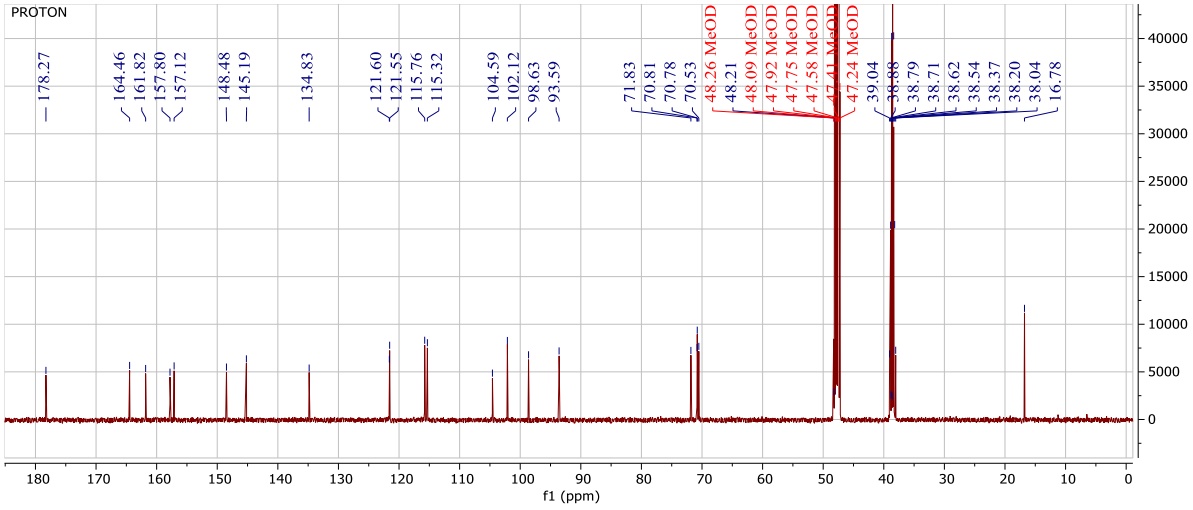

Figure S13: ^{13}C NMR spectrum (125 MHz, CD_3OD) of compound (**5**)

Compound 6: Quercetin-3-*O*-*β*-glucopyranoside**;** yellow powder, m.p. 176-179°C; *m/z* 464.38 for molecular formula $C_{21}H_{20}O_{12}$, ^{1}H RMN (500 MHz, CD_3OD) *δ* (ppm) : 8.50 (d , *J* =

2.1 Hz , H-2'), 8.34 (dd, *J* = 8.3, 2.2 Hz, H-6'), 7.61 (d , *J* = 8.3 Hz ; H-5') ; 7.16 (d ; *J* = 2.6 Hz ; H-8) ; 6.96 (d ; *J*=2.1 Hz ;H-6) ; 6.03(d ; *J* = 7.6 Hz , H-1") ;·4.26 (m, H-2"), 4.33 (q ; H-6a"), 4.21 (q, H-6b"), 4.46 (t, H-3") ; 4.54 (m, H-4"), 4.19 (t , H-5"). ^{13}C RMN (150 MHz, CD_3OD) δ (ppm): 158.0(C-2), 134.9(C-3), 178.8(C-4), 162.3(C-5), 99.1(C-6), 165.3(C-7), 94.3(C-8), 157.9(C-9), 105.0(C-10), 123.3(C-1'), 117.1(C-2'), 145.4(C-3'), 149.3(C-4'), 115.8(C-5'), 122.5(C-6'), 103.9(C-1"), 74.4(C-2"), 72.4(C-3"), 69.3(C-4"), 76.8(C-5"), 61.3(C-6") [1,2].

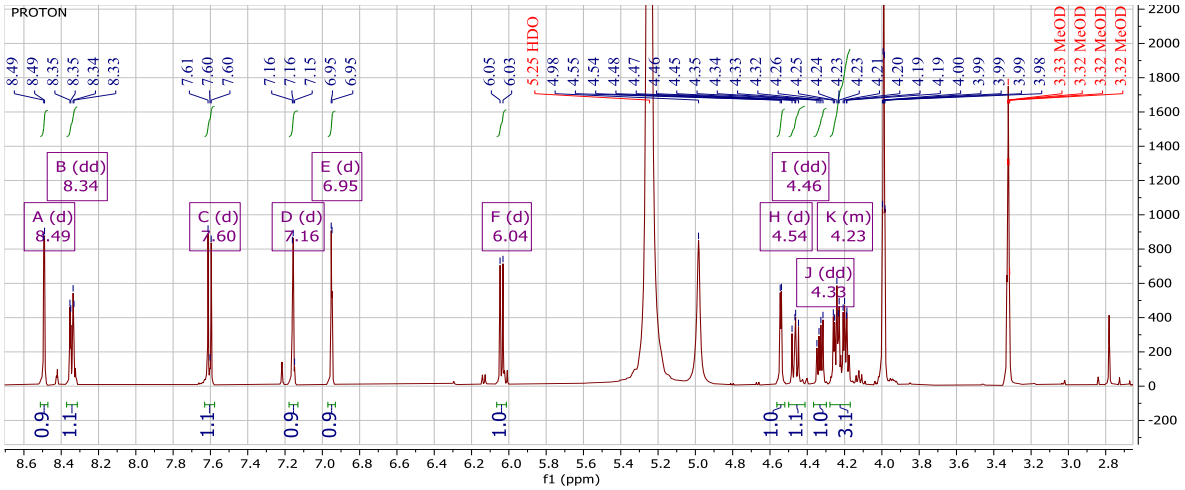

Figure S14: ^{1}H NMR spectrum (500MHz, CD_3OD) of compound (**6**)

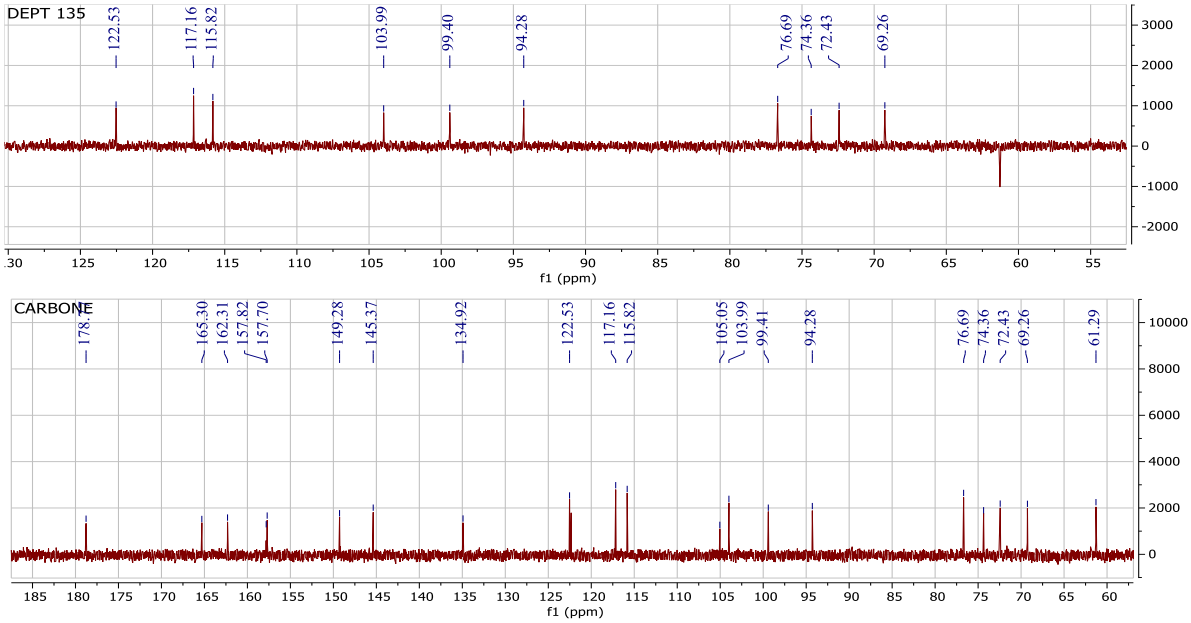

Figure S15: ^{13}C NMR and DEPT 135 (125 MHz, CD_3OD) spectra of compound (**6**)

Compound 7: 6,8-C-dimethylkaempferol 3,7-dimethyléther**;** yellow powder, m.p. 285-287°C; *m/z* 342.35 for molecular formula $C_{19}H_{18}O_6$, ^{1}H NMR (600 MHz, CD_3OD) δ (ppm):

8.03 (d, *J* = 8.3 Hz, H-2'/H-6'), 6.95 (d, *J* = 8.8 Hz, H-3'/H-5'), 3.78 (s, 3-OCH_3), 2.20 (s, 6-CH_3) and 2.02(s, 8-CH_3). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 155.9(C-2), 137.9(C-3), 178.9(C-4), 157.0(C-5), 107.1(C-6), 160.5(C-7), 101.8(C-8), 152.2(C-9), 104.8(C-10), 121.7(C-1'), 130.0(C-2'), 115.2(C-3'), 160.7(C-4'), 115.2(C-5'), 130.0(C-6'), 59.4(3-OCH_3), 7.3(6-CH_3) and 6.7(8-CH_3) [1,2].

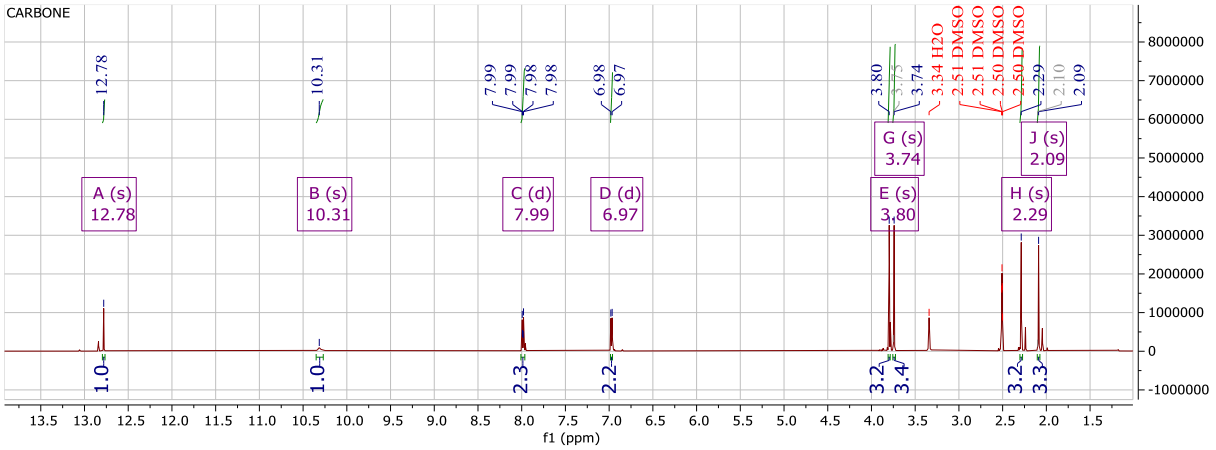

Figure S16: ^{1}H NMR spectrum (600MHz, DMSO-d_6) of compound (**7**)

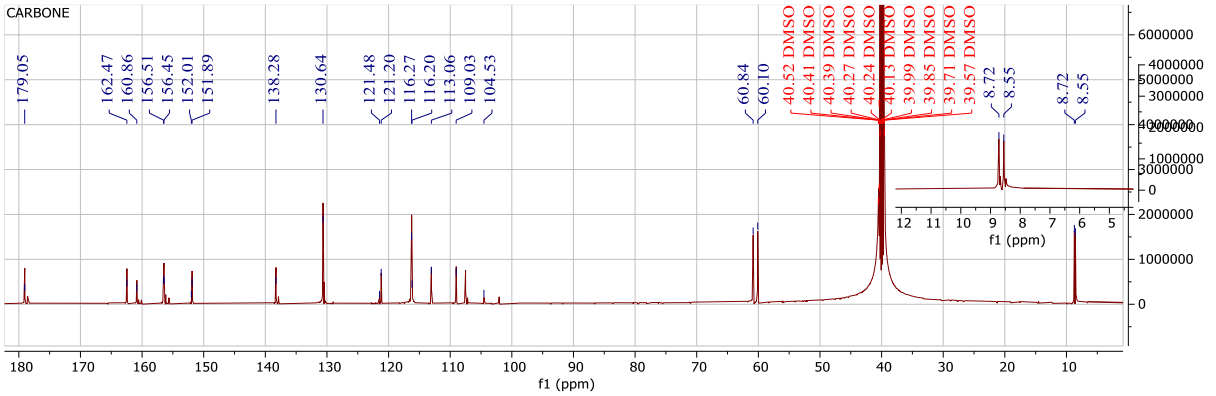

Figure S17: ^{13}C NMR spectrum (150MHz, DMSO-d_6) of compound (**7**)

Compound 8: 6,8-C-dimethylkaempferol-3-methyl ether; yellow powder, m.p. 250-253°C; *m/z* 328.09 for molecular formula $C_{18}H_{16}O_6$, ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 12.78 (5-OH), 7.99 (d, *J* = 8.3 Hz, H-2'/H-6'), 6.97 (d, *J* = 8.5 Hz, H-3'/H-5'), 3.80 (s, 3-OCH_3), 3.74 (s, 7-OCH_3), 2.26 (s, 6-CH_3) and 2.09 (s, 8-CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 156.5(C-2), 138.2(C-3), 179.0(C-4), 156.4(C-5), 113.0(C-6), 162.4(C-7), 109.0(C-8), 152.0(C-9), 107.5(C-10), 121.4(C-1'), 130.4(C-2'), 116.2(C-3'), 160.8(C-4'), 116.2(C-5'), 130.4(C-6'), 60.8(3-OCH_3), 60.1(7-OCH_3), 8.7(6-CH3) and 8.6(8-CH_3) [1,2].

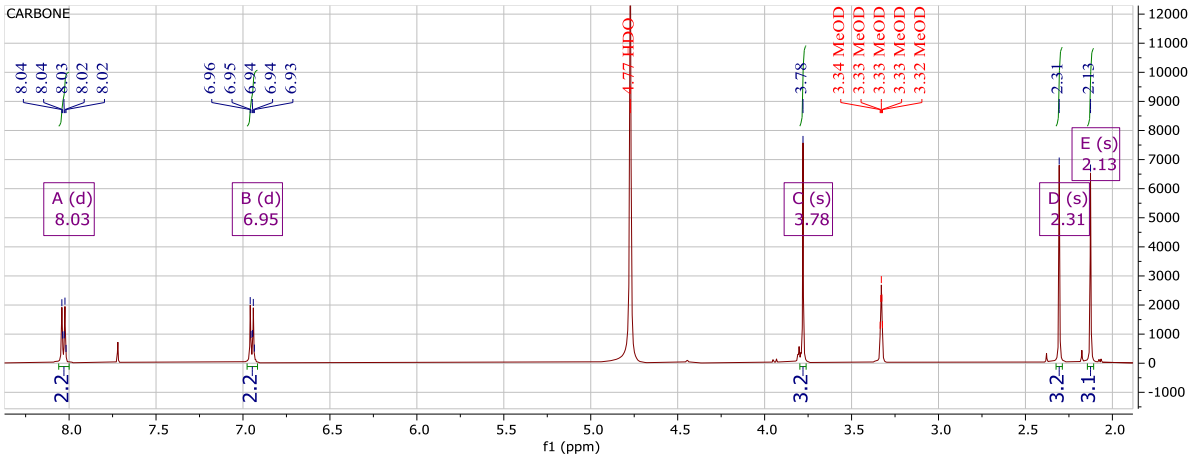

Figure S18: ^{1}H NMR spectrum (600MHz, CD_3OD) of compound (8)

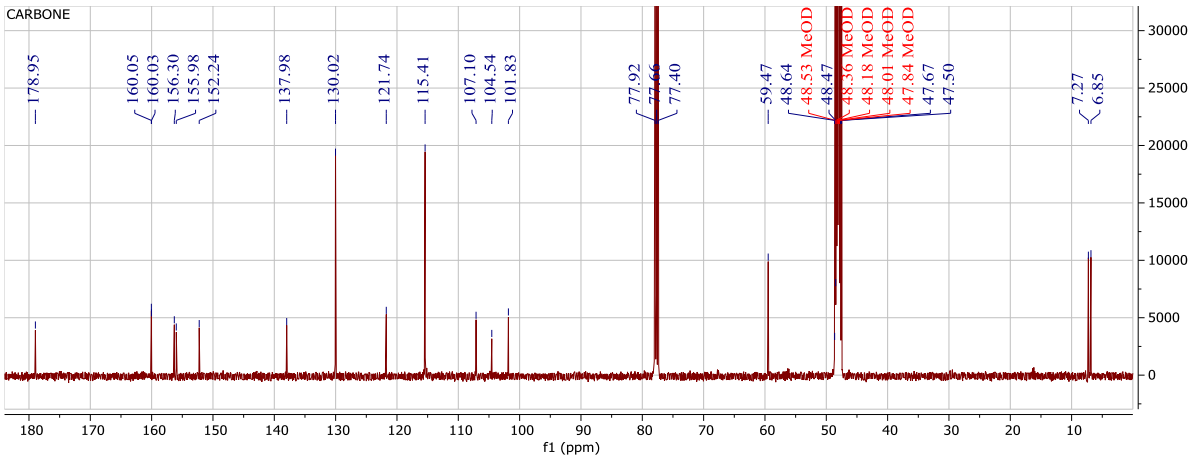

Figure S19: ^{13}C NMR spectrum (150MHz, CD_3OD) of compound (**8**)

Compound 9: **6,8-C-dimethylquercetin-3-methyl ether**; yellow powder, *m/z* 344.09 for molecular formula $C_{18}H_{16}O_7$, ^{1}H NMR (500MHz, Acetone-d_6) δ (ppm) : 12.87(s, 5-OH), 7.65 (d, J = 2.1 Hz, H-2') , 7.54 (dd, J = 8.4, 2.1 Hz, H-6'), 8.89 (d, J = 8.4 Hz, H-5'), 3.55(s, H-8), 2.55 (s, 3- OCH_3), 2.71 (s, 6-OCH_3) and 2.21 (s, 8-OCH_3). ^{13}C NMR (125MHz, Acetone-d_6) δ (ppm): 155.0(C-2), 137.4(C-3), 178.0(C-4), 155.5(C-5), 106.6(C-6), 159.7(C-7), 101.5(C-8), 151.4(C-8), 103.9(C-9), 121.1(C-10), 121.1(C-1'), 115.1(C-2'), 145.2(C-3'),148.5(C-5), 115.8(C-5'), 120.5(C-6'), 59.5(7-OCH_3), 8.2(8-CH_3) 8.0(6-CH_3) [1,2].

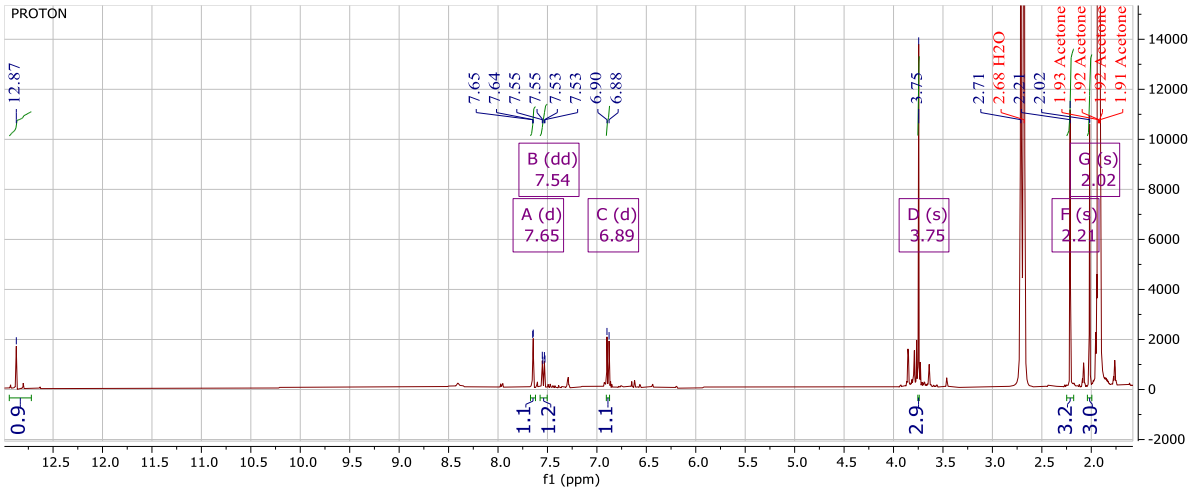

Figure S20: ^{1}H NMR spectrum (500MHz, Acetone-d_6) of compound (**9**)

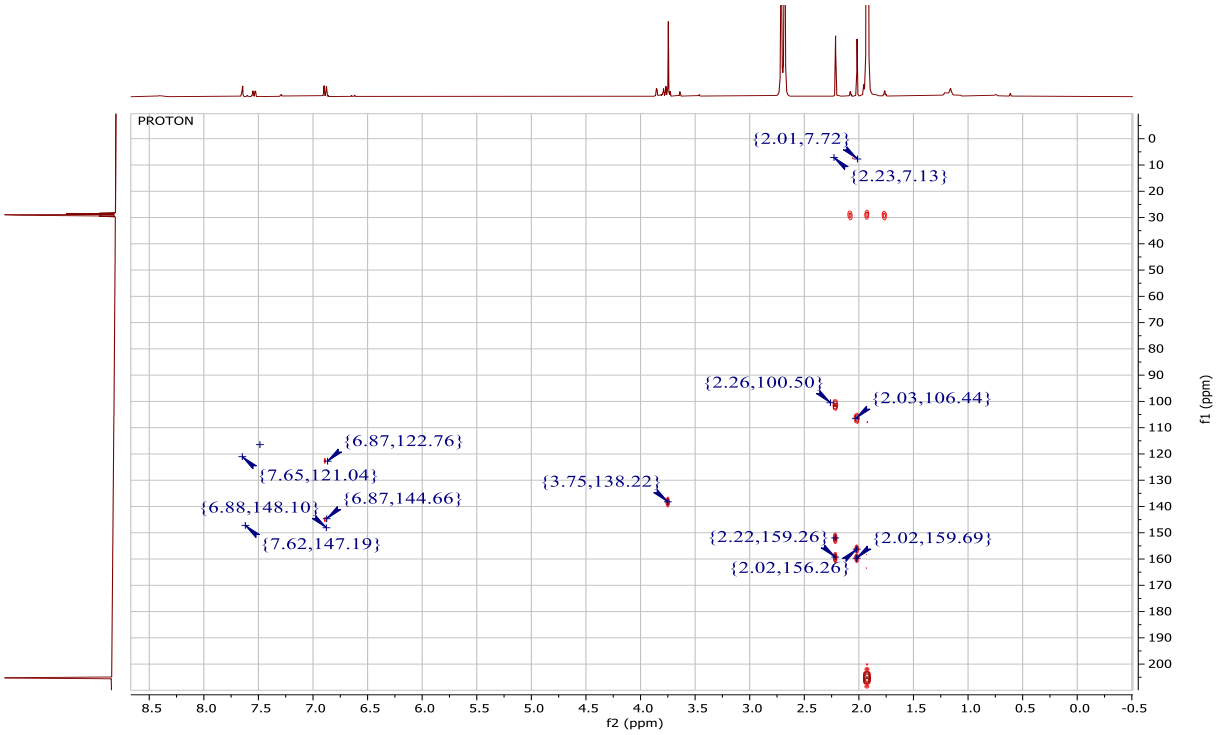

Figure S21: HMBC spectrum (500 MHz, Acetone-d_6) of compound (**9**)

Compound 10: Ursolic Acid; white powder, m.p. 283-285°C; *m/z* 456.71 for molecular formula $C_{30}H_{48}O_3$; ^{1}H NMR (500 MHz, C_5D_5N) δ (ppm) 5.40 (sl, H-12), 3.41 (dd, J = 11.0, 6 Hz, H-3), 2.60 (d, J = 12.0 Hz, H-18), 1.17 (s, H-23), 0.80 (s, H-24), 0.98 (s, H-25), 0.99(s, H-26), 1.19 (s, H-27), 0.96 (d, J = 6.0 Hz, H-29) and 0.88(d, J = 7, 1 Hz, H-30); ^{13}C NMR (125 MHz, C_5D_5N) δ (ppm): 38.5(C-1), 27.4(C-2), 78.9(C-3), 39.1(C-4), 54.7(C-5), 17.9(C-6), 32.6(C-7), 38.9(C-8), 46.9(C-9), 38.2(C-10), 23.8(C-11), 125.5(C-12), 138.1(C-13), 41.6(C-14), 28.9(C-15), 23.8(C-16), 46.9(C-17), 52.3(C-18), 39.1(C-19), 39.8(C-20), 30.1(C-21),

36.5(C-22), 28.2(C-23), 16.8(C-24), 15.1(C-25), 16.1(C-26), 23.2(C-27), 180.7(C-28), 17.0(C-29) and 21.0(C-30) [1,3]..

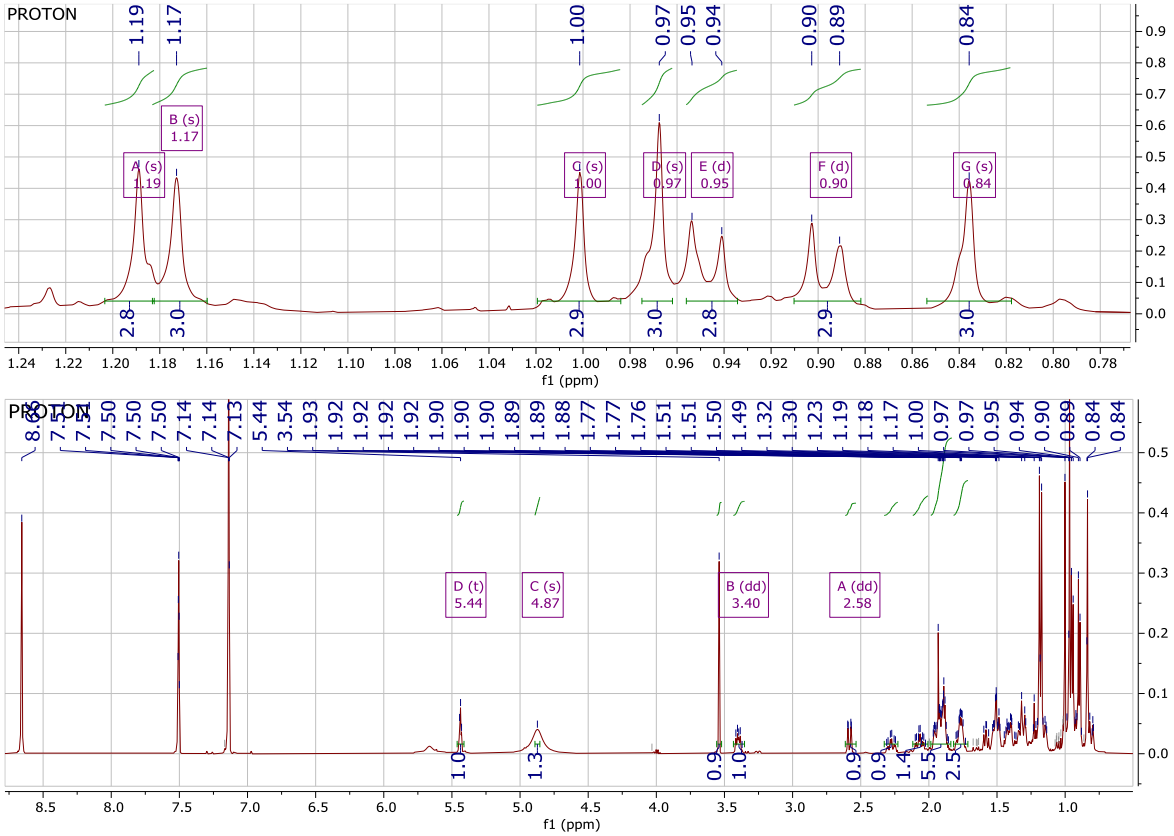

Figure S22: ^{1}H NMR spectrum (500MHz, Pyridine-d_5) of compound **(10)**

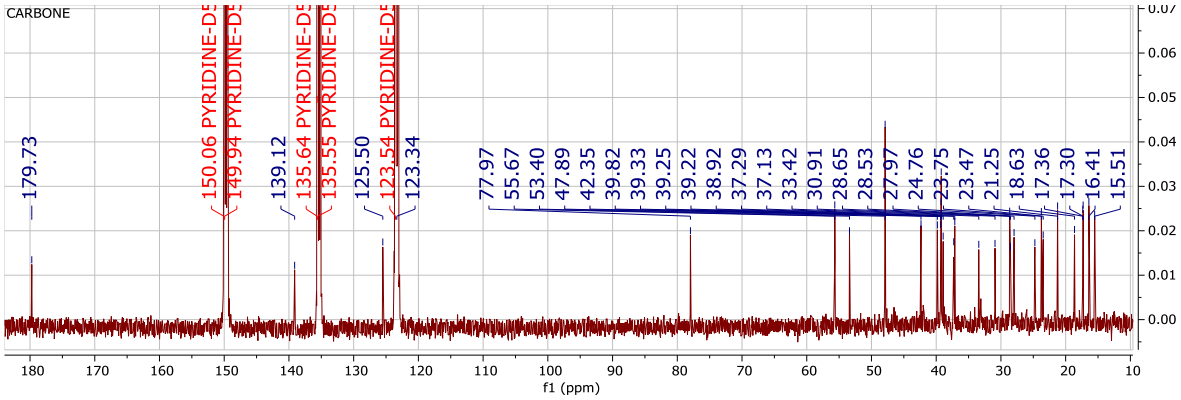

Figure S23: ^{13}C NMR spectrum (125MHz, Pyridine-d_5) of compound **(10)**

Compound 11: 3-*O*-ß-D-glucopyranoside of ß-sitosterol; White powder, m.p. >212°C; *m/z* 456.71 for molecular formula $C_{35}H_{60}O_6$, ^{1}H NMR (500 MHz, C_5D_5N) δ (ppm) 5.31(sl, H-6), 4.21 (d, 8.0Hz, H-1'), 3.12 (m, H-12), 2.10 - 3.10 (m, H-2'- 6'), 0.98 (s, H-19), 0.89 (d, 6.5 Hz, H-21), 0.82 (d, 7.0 Hz, H-29), 0.81 (d, 7,0 Hz, H-26), 0.79 (d, 7,5 Hz, H-27), 0.63 (t, H-18); ^{13}C NMR (125 MHz, C_5D_5N) δ (ppm): 36.6(C-1), 29.1(C-2), 77.2(C-3), 38.7(C-4), 140.8(C-5), 121.6(C-6), 31.8(C-7), 29.7(C-8), 50.0(C-9), 35.9(C-10), 20.1(C-11), 37.2(C-12), 42.3(C-13), 56.6(C-14), 23.0(C-15), 25.8(C-16), 55.8(C-17), 12.1(C-18), 19.5(C-19), 34.5(C-20), 19.3(C-

21), 33.7(C-22), 24.3(C-23), 45.5(C-24), 28.2(C-25), 19.6(C-26), 19.0(C-27), 21.0(C-28), 12.1(C-29), 101.2(C-1'), 73.8(C-2'), 77.3(C-3'), 70.5(C-4'), 77.2(C-5') and 61.5(C-6'[1,4]..

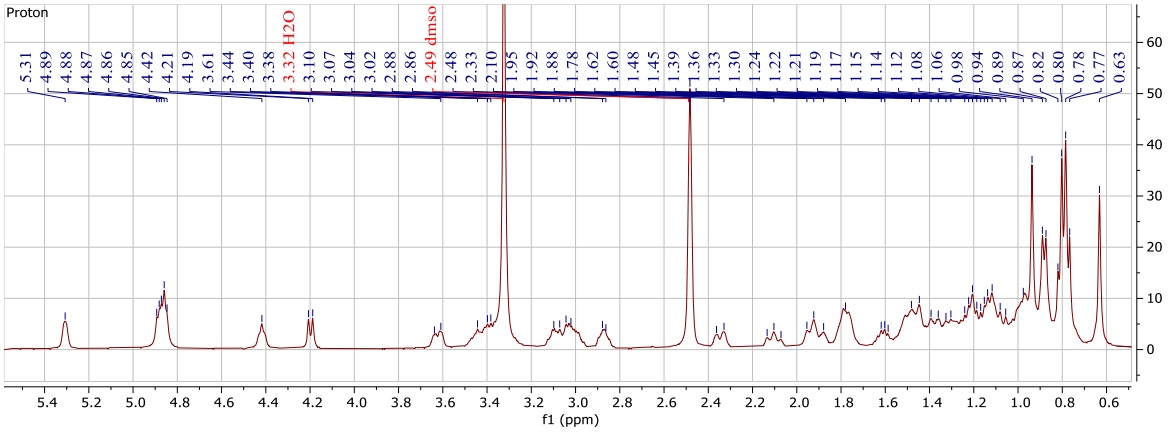

Figure S24: ^{1}H NMR spectrum (400 MHz, DMSO-d_5) compound **(11)**

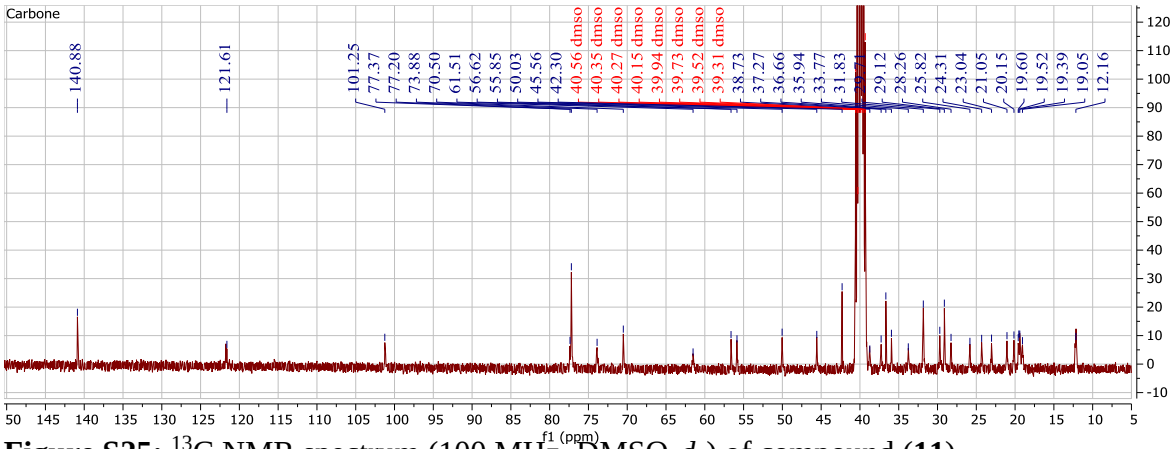

Figure S25: ^{13}C NMR spectrum (100 MHz, DMSO-d_5) of compound (**11**)

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