Supporting Information

Synthesis, Molecular Docking, and Biological Evaluation of [3, 2-b] Indole Fused 18β-Glycyrrhetinic Acid Derivatives against Skin Melanoma

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1. Experimental section

1.1. Chemistry

All the required chemicals, reagents and solvents for modification were purchased from Sigma-Aldrich. Reactions were monitored through TLC on silica gel 60 F254 plates (E. Merck) by using ceric ammonium sulphate solution as spraying reagent for detection of spots. Purification of all synthesized derivatives was done through column chromatography using silica gel 60-120 mesh as stationary phase.

1.1.1. Isolation of 18β-Glycyrrhetinic acid (A01)

GA. was isolated in bulk quantity from MeOH extract of stem bark of *Glycyrrhiza glabra* and characterized by spectroscopic techniques.

GA-O. To a solution of compound **GA** (5 g, 11 mmol) in DCM was added PCC (3.54 g, 16 mmol) dissolved in DCM dropwise till dark colour appears and kept it at RT for 2 h. After completion, reaction mixture was passed through celite and filtrate was concentrated at rota vapour. Purification was done through column chromatography with EtoAc: Hexane (1: 13) as the eluent to afford product **7** colourless solid (3.5 g, 70% yield).

GPD-1^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.10 (dd, *J* = 11.0, 4.0 Hz, 1H),7.06 (t, *J* = 6.9 Hz, 1H), 5.82 (s, 1H), 3.96 (d, *J* = 15.6 Hz, 1H), 2.68 (s, 1H),2.26 (d, *J* = 15.4 Hz, 2H), 2.13 – 2.05 (m, 2H),2.03 (d, *J* = 14.3 Hz, 2H), 1.95 (d, *J* = 16.4 Hz, 1H), 1.87 (dd, *J* = 21.4, 11.6 Hz, 1H), 1.78 (d, *J* = 9.2 Hz, 1H), 1.69 (d, *J* = 13.5 Hz, 2H), 1.62 (d, *J* = 15.0 Hz, 1H),1.53 (d, *J* = 12.4 Hz, 2H),1.46 (d, *J* = 12.4 Hz, 2H),1.43 (s, 3H), 1.32 (s, 3H), 1.25 (s, 6H), 1.23 (s, 3H), 1.19 (s, 3H), 1.07 (d, *J* = 13.5 Hz, 2H), 0.89 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$)*δ* 199.35, 181.31, 169.12, 140.04, 136.22, 128.86, 128.30, 121.01, 118.93, 118.59, 110.16, 107.26, 60.55, 53.04, 48.21, 45.44, 43.82, 43.37, 41.02, 38.06, 37.77, 37.59, 34.05, 32.13, 31.97, 31.07, 30.97, 28.62, 28.45, 26.60, 26.52, 23.50, 23.34, 18.56, 18.37, 16.04.HRMS (ESI+): $C_{36}H_{48}NO_3$ m/z calcd for (M + H) +542.3634, found (M + H) +542.3634.

GPD-2

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.35 – 7.28 (m, 1H), 7.23 – 7.16 (m, 1H), 7.16 – 7.11 (m, 1H), 6.90 (d, *J* = 7.9 Hz, 1H), 5.78 (s, 1H), 3.89 (d, *J* = 15.6 Hz, 1H),2.67 (d, *J* = 7.4

Hz, 1H),2.23 (d, *J*= 15.8 Hz, 2H),2.03 (d, *J* = 14.2 Hz, 2H),1.95 (d, *J* = 18.1 Hz, 2H), 1.76 (d, *J* = 12.6 Hz, 2H), 1.68 – 1.59 (m, 4H), 1.53 (d, *J* = 14.3 Hz, 1H),1.42 (s, 3H), 1.39 – 1.36 (m, 2H), 1.31 (s, 3H), 1.26 (s, 3H), 1.24 (s, 3H), 1.21 (s, 3H), 1.16 (s, 3H), 1.07 (d, *J* = 9.3 Hz, 2H), 0.86 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 200.37, 179.68, 170.19, 136.99, 128.54, 127.06, 122.93, 121.80, 119.52, 114.12, 113.16, 106.97, 60.47, 52.89, 49.75, 49.54, 49.32, 48.29, 45.42, 43.72, 43.38, 41.16, 37.99, 37.73, 37.40, 34.07, 31.89, 31.01, 30.81, 28.58, 28.43, 26.57, 23.25, 18.30, 15.95, 14.09. HRMS (ESI+): $C_{36}H_{46}BrNO_3$ m/z calcd for (M - H) 618.2587, found (M –H) 618.2583.

GPD-3 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.16 (ddd, *J* = 12.2, 9.2, 3.4 Hz, 2H), 6.84 (td, *J* = 9.2, 2.5 Hz, 1H), 5.82 (s, 1H), 3.88 (d, *J* = 15.5 Hz, 1H), 2.23 (d, *J* = 15.6 Hz, 2H), 2.04 (dd, *J* = 18.1, 9.2 Hz, 3H), 1.97 – 1.89 (m, 2H), 1.84 – 1.75 (m, 2H), 1.68 (d, *J* = 13.5 Hz, 2H), 1.63 (s, 1H), 1.53 (d, *J* = 9.8 Hz, 2H), 1.42 (s, 3H), 1.39 – 1.35 (m, 2H), 1.32 (s, 3H), 1.25 (d, *J* = 2.0 Hz, 6H), 1.22 (s, 3H), 1.18 (s, 3H), 1.08 – 1.02 (m, 2H), 0.88 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 199.95, 181.83, 181.41, 169.39, 142.16, 132.63, 128.80, 110.60, 110.51, 109.05, 108.78, 107.60, 60.45, 52.92, 48.19, 45.42, 43.83, 43.36, 40.98, 38.01, 37.75, 37.50, 34.16, 32.05, 31.93, 31.05, 30.96, 28.62, 28.47, 26.58, 26.48, 23.53, 23.33, 18.53, 18.35, 16.02. HRMS (ESI+): $C_{36}H_{47}FNO_3$ m/z calcd for (M + H) +560.3540, found (M + H) +560.3547.

GPD-4 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 4.4 Hz, 2H), 7.21 (d, *J* = 8.7 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 5.73 (s, 1H), 3.84 (d, *J* = 15.5 Hz, 1H), 2.64 (s, 1H), 2.21 (d, *J* = 15.3 Hz, 2H), 2.03 (td, *J* = 13.4, 3.9 Hz, 1H), 1.98 – 1.84 (m, 3H), 1.75 (t, *J* = 12.8 Hz, 2H), 1.62 (d, *J* = 13.6 Hz, 3H), 1.54 – 1.44 (m, 2H), 1.39 (s, 3H), 1.37 – 1.32 (m, 1H), 1.29 (s, 3H), 1.25 (s, 1H), 1.22 (s, 3H), 1.18 (s, 3H), 1.16 (s, 3H), 1.14 (s, 3H), 1.05 (dd, *J* = 23.8, 11.6 Hz, 2H), 0.82 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 200.66, 179.34, 170.69, 142.62, 142.30, 134.55, 128.34, 128.16, 122.09, 114.38, 110.66, 110.58, 107.02, 60.45, 52.92, 48.32, 45.42, 43.68, 43.38, 41.19, 37.95, 37.69, 37.40, 34.14, 31.97, 31.84, 30.98, 30.71, 28.52, 28.37, 26.53, 26.38, 23.17, 18.42, 18.24, 15.91. HRMS (ESI+):$C_{37}H_{46}F_3NO_4$m/z calcd for (M + H) +626.3464, found (M + H) +626.3457.

GPD-5 ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (dd, *J* = 20.7, 8.4 Hz, 1H), 6.57 (t, *J* = 10.3 Hz, 1H), 5.70 (d, *J* = 28.3 Hz, 1H), 3.79 (d, *J* = 15.5 Hz, 1H), 2.58 (s, 1H), 2.21 (t, *J* = 52.7 Hz, 4H), 1.93 (dd, *J* = 29.3, 11.7 Hz, 4H), 1.56 (dd, *J* = 25.5, 12.7 Hz, 5H), 1.35 (s, 3H), 1.31 (s, 3H), 1.26 (s, 4H), 1.18 (s, 9H), 1.14 (s, 3H), 1.09 (s, 3H), 0.78 (d, *J* = 9.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.00, 176.27, 150.68, 128.34, 122.37, 114.10, 104.46, 103.27, 77.36,

77.05, 76.73, 60.38, 55.37, 52.87, 48.26, 48.21, 47.45, 45.29, 43.37, 41.08, 39.74, 36.69, 34.23, 31.94, 30.98, 29.72, 28.53, 26.39, 23.50, 23.32, 21.43, 18.52, 18.32, 16.00, 14.16, 13.65.

HRMS (ESI+):$C_{36}H_{45}F_2NO_3$ m/z calcd for (M + H) +578.346, found (M + H) +578.3447.

GPD-6 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 12.4 Hz, 1H), 7.23 (d, *J* = 7.5 Hz, 1H), 6.94 (dd, *J* = 7.6, 4.7 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 5.76 (s, 1H), 3.90 (d, *J* = 15.3 Hz, 1H), 3.40 (d, *J* = 13.3 Hz, 1H), 2.69 (s, 1H), 2.26 (d, *J* = 14.9 Hz, 2H), 2.17 – 1.87 (m, 4H), 1.84 – 1.60 (m, 5H), 1.55 (d, *J* = 11.7 Hz, 2H), 1.44 (s, 6H), 1.36 (s, 3H), 1.28 (s, 3H), 1.23 (s, 3H), 1.20 (s, 3H), 1.18 (s, 2H), 1.13 – 1.02 (m, 2H), 0.87 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 199.90, 180.86, 169.31, 148.07, 140.97, 128.82, 123.98, 119.15, 119.09, 114.36, 108.15, 106.20, 60.48, 52.98, 48.21, 45.42, 43.80, 43.37, 41.03, 38.05, 37.76, 37.68, 34.14, 32.07, 31.93, 31.04, 30.98, 29.26, 28.61, 28.43, 26.59, 26.49, 23.49, 23.33, 18.53, 18.35, 16.01. HRMS (ESI+): $C_{36}H_{46}FNO_3$ m/z calcd for 559.3462 found (M - H) 558.3383.

GPD-7 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 1.8 Hz, 1H), 7.04 – 6.94 (m, 1H), 5.78 (s, 1H), 3.90 (d, *J* = 15.6 Hz, 1H), 2.66 (s, 2H), 2.24 (dd, *J* = 14.5, 6.7 Hz, 4H), 2.08 – 1.99 (m, 2H), 1.99 – 1.91 (m, 2H), 1.87 (dd, *J* = 13.5, 4.2 Hz, 1H), 1.80 – 1.74 (m, 1H), 1.66 (d, *J* = 13.4 Hz, 2H), 1.63 – 1.53 (m, 3H), 1.49 (d, *J* = 17.1 Hz, 1H), 1.42 (s, 3H), 1.31 (s, 3H), 1.25 (s, 3H), 1.24 (s, 2H), 1.21 (d, *J* = 1.8 Hz, 6H), 1.16 (s, 3H), 0.86 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 200.32, 179.66, 170.09, 141.00, 136.62, 128.58, 126.57, 119.34, 119.18, 110.21, 107.09, 107.04, 60.46, 52.87, 48.28, 45.42, 43.74, 43.38, 41.15, 37.99, 37.75, 37.41, 34.09, 32.03, 31.91, 31.02, 30.89, 29.71, 28.60, 28.46, 26.58, 26.45, 23.27, 18.48, 18.32, 16.00. HRMS (ESI+):$C_{36}H_{47}F_3ClNO_3$ m/z calcd for (M + H) +576.3244, found (M + H) +576.3246.

GPD-8^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.04 – 6.90 (m, 3H), 5.81 (s, 1H), 3.94 (d, *J* = 15.6 Hz, 1H), 3.38 (s, 1H), 2.67 (s, 2H), 2.48 (s, 3H), 2.24 (d, *J* = 3.6 Hz, 2H), 2.18 (s, 1H), 2.10 (d, *J* = 6.8 Hz, 1H), 2.05 (s, 2H), 1.96 (s, 2H), 1.88 (d, *J* = 13.2 Hz, 3H), 1.77 (d, *J* = 11.7 Hz, 2H), 1.66 (s, 2H), 1.52 (d, *J* = 5.8 Hz, 2H), 1.43 (s, 7H), 1.34 (s, 4H), 1.27 (s, 4H), 1.24 (s, 4H), 1.22 (s, 5H), 1.19 (s, 5H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.02, 181.04, 169.24, 149.01, 148.91, 146.42, 135.61, 128.85, 127.78, 119.26, 119.17, 116.36, 60.54, 53.07, 48.17, 45.43, 43.80, 43.36, 38.03, 37.72, 34.09,

32.11, 31.93, 31.11, 30.98, 28.62, 28.47, 26.58, 26.49, 23.55, 23.35, 23.23, 20.55, 18.56, 18.36, 16.78, 16.04. HRMS (ESI+):$C_{36}H_{47}F_3ClNO_3$ m/z calcd for (M + H) +556.3791, found (M + H) +556.3785.

GPD-9 ^{1}H NMR (400 MHz, MeOD) δ 8.38 (s, 1H), 8.20 (d, *J* = 8.9 Hz, 1H), 7.98 (d, *J* = 8.9 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.34 (d, *J* = 9.0 Hz, 1H), 3.94 (d, *J* = 15.7 Hz, 1H), 3.34 (d, *J* = 1.5 Hz, 1H), 2.94 – 2.85 (m, 1H), 2.75 (s, 1H), 2.62 (dd, *J* = 15.9, 9.6 Hz, 1H), 2.55 (s, 1H), 2.42 (dd, *J* = 13.2, 8.4 Hz, 1H), 2.26 (s, 1H), 2.20 (s, 1H), 2.10 (t, *J* = 12.6 Hz, 1H), 1.91 (d, *J* = 13.2 Hz, 2H), 1.68 (d, *J* = 14.0 Hz, 1H), 1.61 (d, *J* = 13.0 Hz, 2H), 1.48 (s, 2H), 1.43 (s, 4H), 1.26 (s, 4H), 1.19 (s, 5H), 1.12 (s, 3H), 1.09 (s, 3H), 0.86 (s, 3H).^{13}C NMR (101 MHz, MeOD) δ 200.01, 178.81, 171.40, 144.15, 139.76, 139.65, 127.21, 126.87, 124.11, 60.44, 59.85, 54.43, 52.40, 44.88, 44.72, 43.10, 42.86, 40.58, 39.04, 37.34, 37.10, 36.03, 33.66, 33.44, 31.29, 31.18, 30.29, 27.75, 27.44, 25.85, 25.62, 22.42, 20.33, 18.11, 17.58, 14.92. HRMS (ESI+):$C_{36}H_{46}N_2O_5$ m/z calcd for (M + H) +587.3485, found (M + H) +587.3484

GPD-10 ^{1}H NMR (400 MHz, $CDCl_3$:MEOD) δ 7.53 (s, 1H), 7.30 (s, 1H), 7.05 (s, 1H), 5.76 (s, 1H), 3.82 (d, *J* = 15.3 Hz, 1H), 3.35 (s, 2H), 2.70 (s, 1H), 2.25 (t, *J* = 15.3 Hz, 3H), 2.11 (t, *J* = 11.8 Hz, 3H), 2.03 – 1.91 (m, 3H), 1.77 (dd, *J* = 27.9, 15.3 Hz, 3H), 1.63 (dd, *J* = 25.5, 12.3 Hz, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.39 (s, 1H), 1.31 (s, 3H), 1.27 (s, 3H), 1.23 (s, 3H), 1.21 (s, 3H), 1.16 (s, 3H), 1.08 (d, *J* = 14.2 Hz, 1H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$: MEOD) δ 204.94, 183.26, 175.42, 147.57, 136.07, 135.04, 134.13, 132.08, 127.67, 123.76, 120.05, 111.17, 64.39, 57.10, 49.37, 47.61, 47.39, 45.17, 41.82, 41.63, 41.40, 38.27, 35.86, 35.74, 34.87, 34.06, 33.47, 32.34, 32.10, 30.43, 30.25, 27.01, 26.46, 22.32, 22.04, 19.62.
HRMS (ESI+):$C_{36}H_{45}Cl_2NO_3$m/z calcd for $(M - H)^+$ 608.2698, found $(M - H)^+$ -608.2701

GPD-11 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.11 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.00 (t, *J* = 7.7 Hz, 1H), 5.82 (s, 1H), 3.94 (d, *J* = 15.6 Hz, 1H), 2.67 (s, 1H), 2.26 (d, *J* = 15.7 Hz, 2H), 2.14 – 1.98 (m, 2H), 1.98 – 1.85 (m, 2H), 1.84 – 1.72 (m, 2H), 1.69 (d, *J* = 13.5 Hz, 3H), 1.62 (d, *J* = 12.9 Hz, 2H), 1.54 (d, *J* = 12.6 Hz, 2H), 1.46 (d, *J* = 9.1 Hz, 2H), 1.43 (s, 3H), 1.42 – 1.37 (m, 1H), 1.35 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H), 1.07 (d, *J* = 13.4 Hz, 1H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

200.03, 181.50, 169.50, 141.00, 133.29, 129.79, 128.78, 120.44, 119.76, 117.21, 115.74, 108.46, 60.46, 52.95, 48.20, 45.43, 43.84, 43.37, 40.98, 38.00, 37.76, 37.63, 34.15, 32.04, 31.93, 31.03, 30.94, 28.63, 28.49, 26.58, 26.46, 23.48, 23.35, 18.52, 18.34, 16.02. HRMS (ESI+):$C_{36}H_{46}ClNO_3$m/z calcd for (M + H) +576.3244, found (M + H) +576.7248.

GPD-12 ^{1}H NMR (400 MHz, MEOD: $CDCl_3$) δ 7.81 (d, *J* = 2.8 Hz, 1H), 7.39 (ddd, *J* = 23.4, 15.3, 8.6 Hz, 3H), 5.79 (s, 1H), 3.91 (d, *J* = 15.7 Hz, 1H), 3.37 (s, 1H), 2.73 (s, 1H), 2.28 (d, *J* = 14.0 Hz, 2H), 2.22 (s, 3H), 2.21 (d, *J* = 0.6 Hz, 2H), 2.20 (s, 1H), 2.09 (d, *J* = 19.5 Hz, 1H), 1.97 (d, *J* = 14.0 Hz, 3H), 1.66 (dd, *J* = 27.9, 22.1 Hz, 4H), 1.46 (s, 3H), 1.43 (s, 1H), 1.36 (s, 3H), 1.29 (d, *J* = 7.6 Hz, 6H), 1.23 (s, 2H), 1.18 (s, 3H), 1.13 (d, *J* = 5.0 Hz, 1H), 1.10 (d, *J* = 5.2 Hz, 2H), 0.89 (d, *J* = 5.1 Hz, 3H). ^{13}C NMR (101 MHz, MEOD: $CDCl_3$) δ 204.79, 183.30, 147.44, 132.13, 131.79, 127.43, 127.36, 125.49, 123.42, 115.13, 110.93, 104.02, 64.34, 56.86, 49.38, 47.62, 47.37, 45.14, 41.85, 41.61, 41.14, 38.08, 35.86, 35.77, 34.86, 34.59, 34.27, 33.53, 32.41, 32.21, 30.26, 27.08, 26.69, 22.33, 22.11, 19.73, 17.83. $C_{37}H_{47}N_2O_3$m/z calcd for (M + H) +567.3587, found (M + H) +567.3584.

GPD-13 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.33 (d, *J* = 3.4 Hz, 1H), 7.16 (d, *J* = 1.7 Hz, 2H), 5.76 (s, 1H), 3.85 (d, *J* = 15.5 Hz, 1H), 3.41 – 3.33 (m, 1H), 2.67 (s, 1H), 2.23 (t, *J* = 11.8 Hz, 2H), 2.12 – 1.82 (m, 5H), 1.80 – 1.49 (m, 6H), 1.43 (s, 3H), 1.40 – 1.36 (m, 1H), 1.32 (s, 3H), 1.29 (s, 1H), 1.25 (s, 3H), 1.21 (d, *J* = 5.2 Hz, 6H), 1.15 (s, 3H), 1.06 (d, *J* = 13.8 Hz, 1H), 0.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.03, 181.50, 169.50, 141.00, 133.29, 129.79, 128.78, 120.44, 119.76, 117.21, 115.74, 108.46, 60.46, 52.95, 48.20, 45.43, 43.84, 43.37, 40.98, 38.00, 37.76, 37.63, 34.15, 32.04, 31.93, 31.03, 30.94, 28.63, 28.49, 26.58, 26.46, 23.48, 23.35, 18.52, 18.34, 16.02. $C_{36}H_{47}BrNO_3$ m/z calcd for (M+ H) +620.2739, found (M+ H) +620.2733

GPD-14 ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 – 6.82 (m, 1H), 6.64 (d, *J* = 8.3 Hz, 1H), 6.56 (d, *J* = 7.9 Hz, 1H), 5.31 (s, 1H), 3.73 (d, *J* = 5.3 Hz, 3H), 3.69 (d, *J* = 8.7 Hz, 2H), 3.63 (s, 2H), 2.61 (d, *J* = 15.6 Hz, 2H), 2.34 (d, *J* = 7.7 Hz, 2H), 2.28 – 2.02 (m, 2H), 1.95 (t, *J* = 8.1 Hz, 2H), 1.84 – 1.61 (m, 2H), 1.53 (d, *J* = 10.9 Hz, 2H), 1.42 (d, *J* = 11.6 Hz, 3H), 1.32 (s, 3H), 1.27 (d, *J* = 7.3 Hz, 3H), 1.18 (d, *J* = 7.6 Hz, 6H), 1.13 (d, *J* = 8.3 Hz, 6H), 0.94 (d, *J* = 10.6 Hz, 2H), 0.77 (d, *J* = 15.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.27, 179.93, 152.00, 138.29, 125.14, 118.94, 115.84, 114.08, 113.73, 113.72, 113.50, 113.49, 59.44,

54.65, 51.98, 47.18, 44.40, 42.80, 42.33, 40.11, 37.01, 36.78, 33.11, 30.85, 29.95, 28.66, 27.57, 27.45, 25.42, 22.43, 22.28, 21.66, 20.02, 17.29, 15.03, 13.15. $C_{37}H_{49}NO_4$ m/z calcd for (M+ H) +572.3740, found (M+ H) +572.3743.

1.2. Biology

1.2.1. Chemicals and reagents

Cell culture media DMEM (Dulbecco's Modified Eagle Medium), Dulbecco's phosphate buffer saline (DPBS), Trypsin-EDTA (Ethylenediaminetetraacetic acid), 3-(4,5dimetylthiazolyl)- diphenyl tetrazolium bromide (MTT), DMSO (Dimethyl sulphoxide), Penicillin G, Streptomycin, Sodium bicarbonate Sodium pyruvate, RIPA lysis buffer, 1% protease and phosphatase inhibitor cocktail, Bradford reagent and Beta Actin antibody used as loading control were purchased from Sigma Aldrich. FBS (Fetal Bovine Serum) was purchased from GIBCO USA. Dichlorofluorescein diacetate (H2DCF-DA) was procured from Thermofisher. DAPI (4', 6-diamidino-2-phenylindole hydrochloride) was supplied by Invitrogen, Thermo Fischer Scientific. Antibodies against Bcl2, BAX and Caspase 9 were purchased from Cell Signalling Technology.

1.2.2. Cell culture

Human melanoma cell line, A375 and Murine melanoma cell line, B16F10 were purchased from American Type Culture Collection (Rockville, MD, USA). Primary Human Dermal Fibroblasts, HDF were purchased from HiMedia, India. Cell lines were cultured in DMEM media supplemented with 10%FBS, Penicillin G (120mg/L), Streptomycin (270mg/L), Sodium bicarbonate (1.2g/L) and Sodium pyruvate (220mg/L) and maintained in a humidified chamber (37°C, 5% CO_2).

1.2.3. Cell survival assay

MTT assay was performed for cell survival analysis [16]. Briefly, A375 cells (5×10^3), B16F10 cells (5×10^3) and HDF cells (10^4) were seeded in 96 well plates and incubated for 24 hours. Cells were treated with different concentrations of GPD-12 and were incubated for 24 hours. Cells were again incubated with MTT solution (100µL/well) at a concentration of 250µg/ml PBS for 3 hours. Formazan crystals formed were dissolved in DMSO (100µL/well). The

absorbance was measured at 570nm using plate reader, Multiskan spectrum; Thermo Electron Corporations, USA.

1.2.4. Colony formation assay

A375 cells were seeded at a density of 10^3 cells per well of 6 well plate and kept overnight. The media was replaced with fresh media containing different concentrations of GPD-12. After 24 hours the media containing GPD-12 was removed and cells were further cultured for 6 days in media without any effectors. On day 6, cells were washed with DPBS, fixed with methanol and stained with crystal violet dye. Cells were washed with distilled water and air dried for some time. Stained colonies were captured by EVOS- FL Cell Imaging System (Thermo Fisher).

1.2.5. DAPI Staining

A375 cells were seeded in 6 well plate (10^4 cells per well). After 24 hours, cells were subjected to treatment with different concentrations of GPD-12. Treatment was terminated after 24 hours. Cells were fixed with 4% Para formaldehyde, permeabilized with 0.1% Triton X-100 and stained with DAPI (5µg/ml). Imaging was done by EVOS- FL Cell Imaging System (thermo fisher).

1.2.6. Reactive Oxygen Species measurement using Fluorescence microscopy

A375 cells were seeded in 35mm dishes. After 24 hours, cells were subjected to treatment with different concentrations of GPD-12 and incubated for 6 hours. Cells were washed with DPBS and stained with Dichlorodihydrofluorescein diacetate (H2DCF-DA) dye (5µg/ml) for 30 minutes. Cells were again washed with DPBS and imaging was done under a thin layer of DPBS (EVOS- FL Cell Imaging System, thermo fisher).

1.2.7. Cell lysate preparation and western blotting

After respective treatments, cells were harvested and lysis was performed using RIPA lysis buffer containing 1% protease and phosphatase inhibitor cocktail. Protein estimation was done by Bradford Protein Estimation Method using Bovine Serum Albumin as standard. Protein samples were denatured at 100°C for 3 min in 5x Laemmli buffer and equal amounts of protein samples (40µg) were separated by SDS-PAGE (10-12%) using Miniprotein Tetra

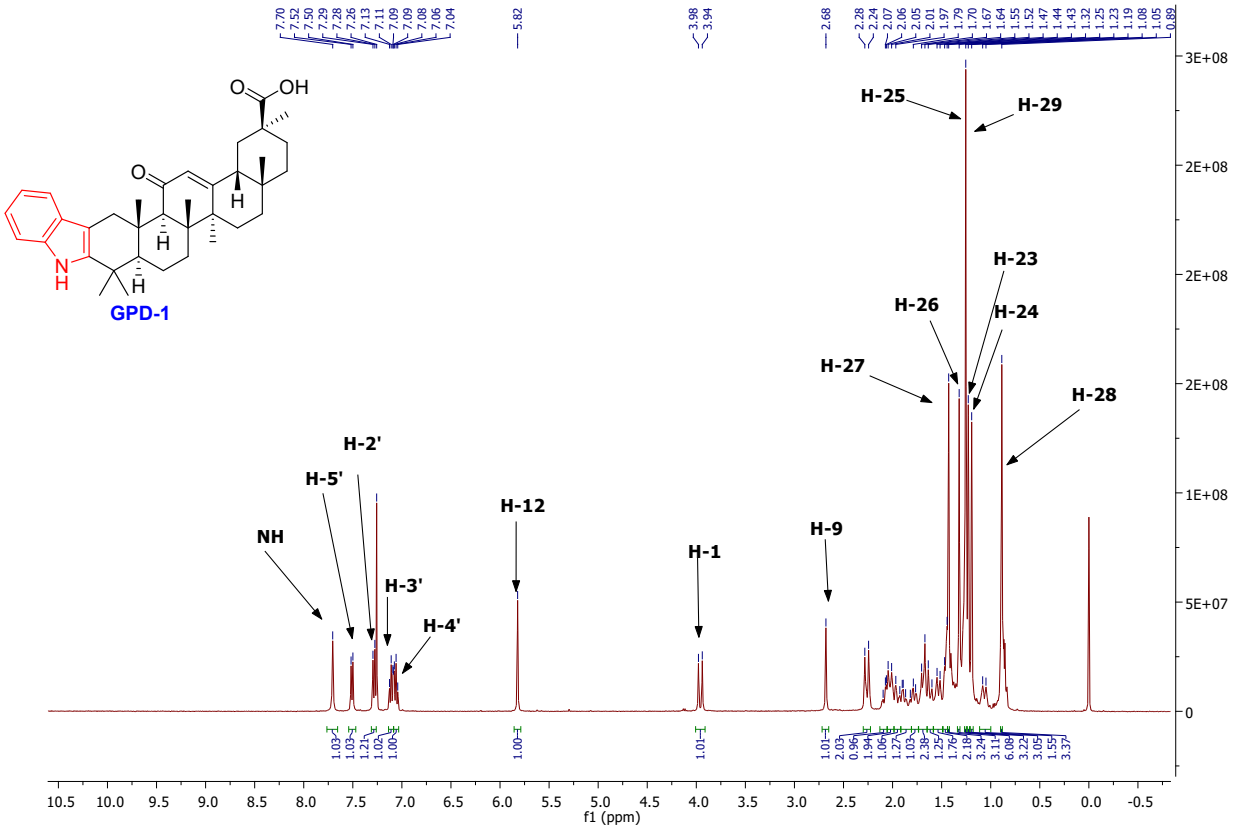
System (Bio-Rad). Proteins were transferred onto PVDF membranes and membranes were blocked using 5% skimmed milk. Membranes were incubated overnight at 4°C with primary antibodies. Membranes were washed with Tris Buffered Saline containing Tween 20 and reincubated with secondary antibody for 2 hours at room temperature. Membranes were developed using immobilon Western Chemiluminescent HRP substrate. Blots were quantified using Image lab™ software (BioRad).

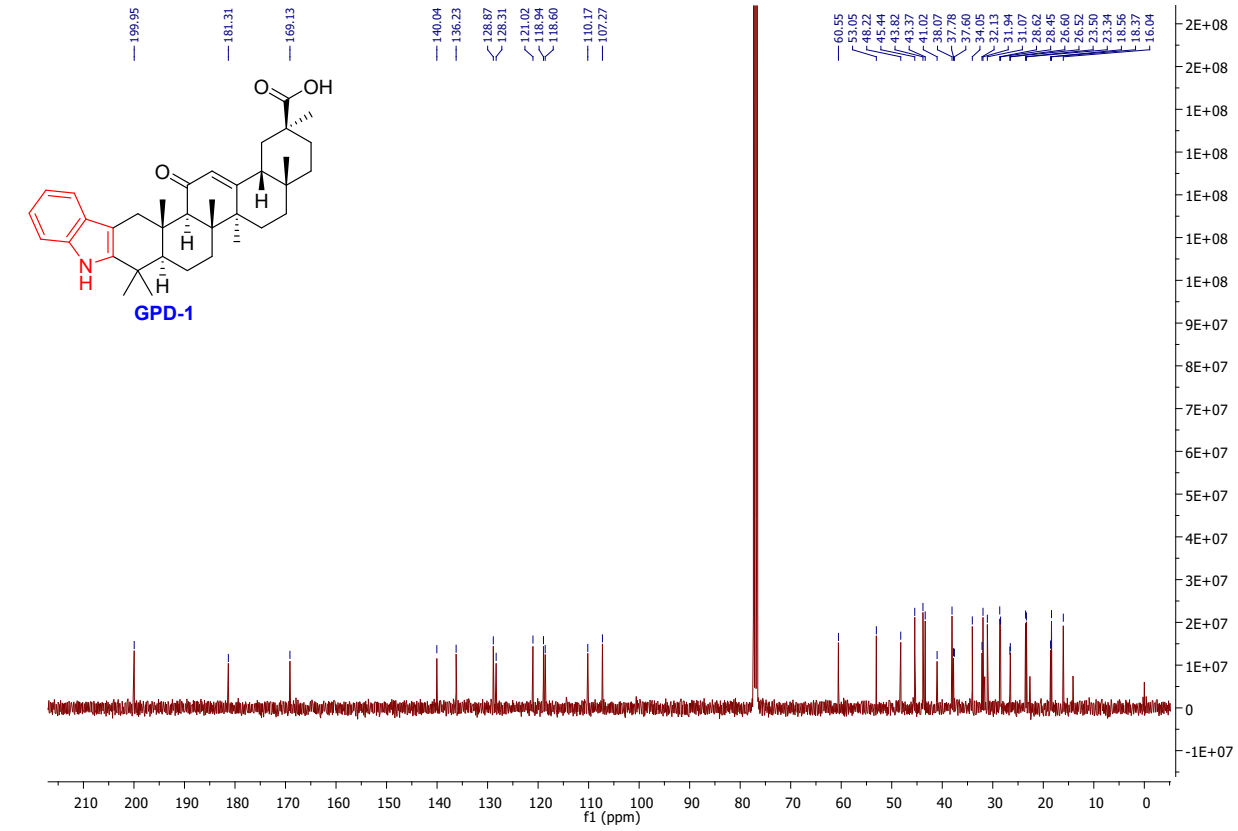
1.2.8. Statistical analysis

Data is expressed as mean ± Standard Deviation (SD) from three independent experiments. Results were considered significant at *$p<0.05$, **$p<0.01$. Data was analyzed by using GraphPad Prism software 9.

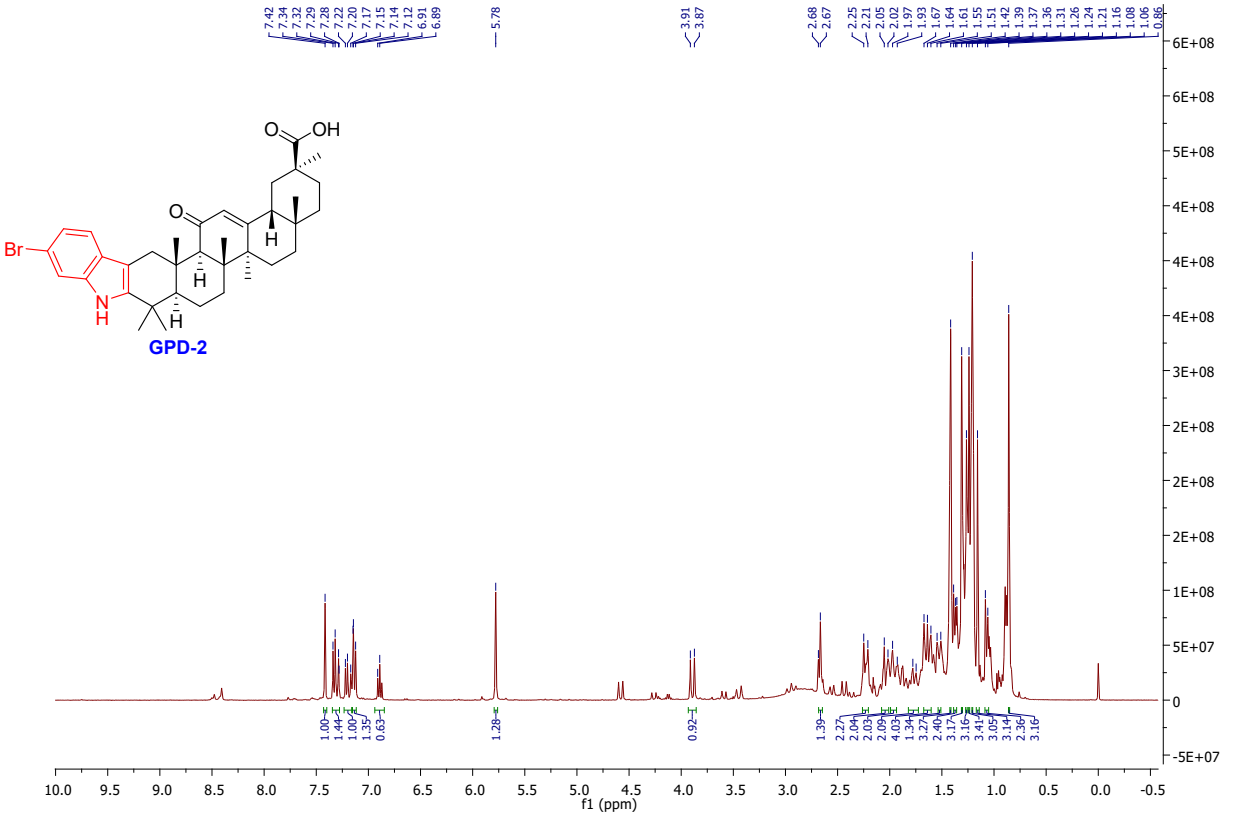
2. NMR (1H, 13CNMR, 2D NMR) Spectra of [3, 2-b] Indole Fused 18β-Glycyrrhetinic Acid (GPD-1 to GPD-14)

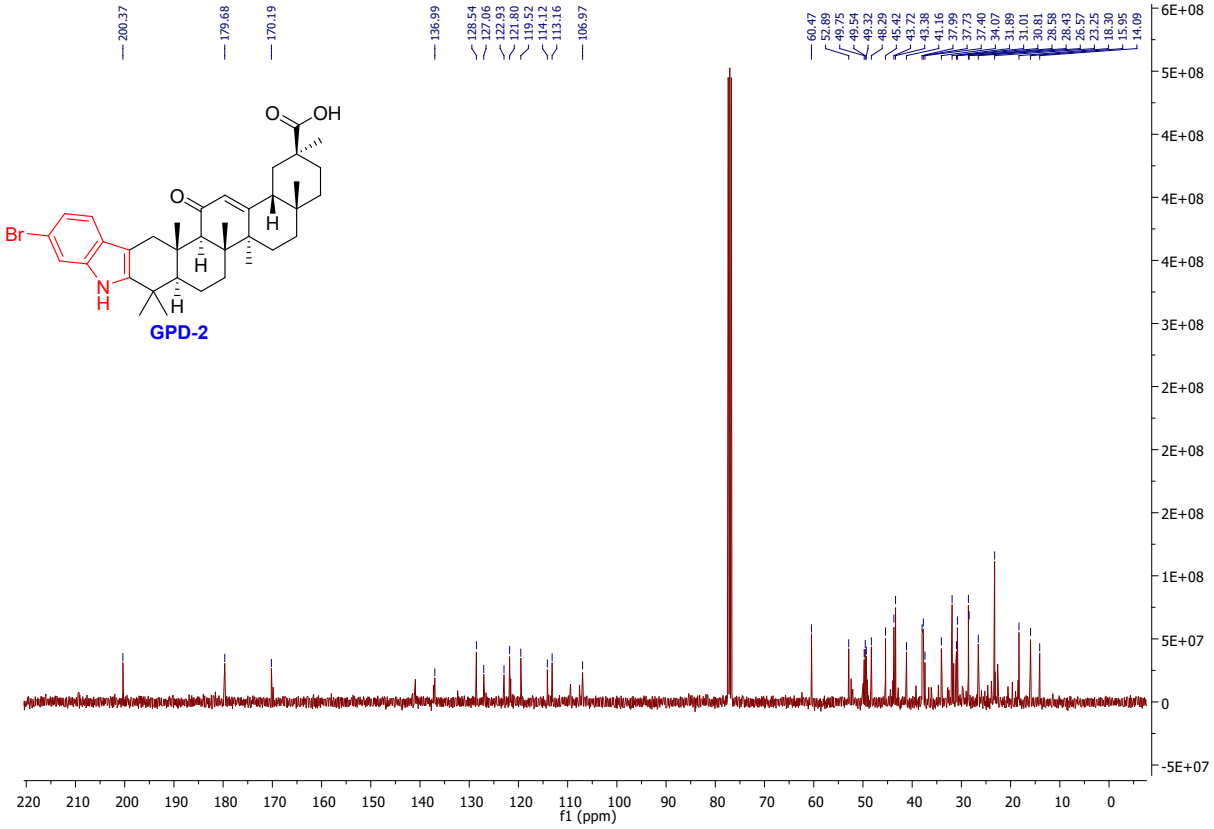
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-1



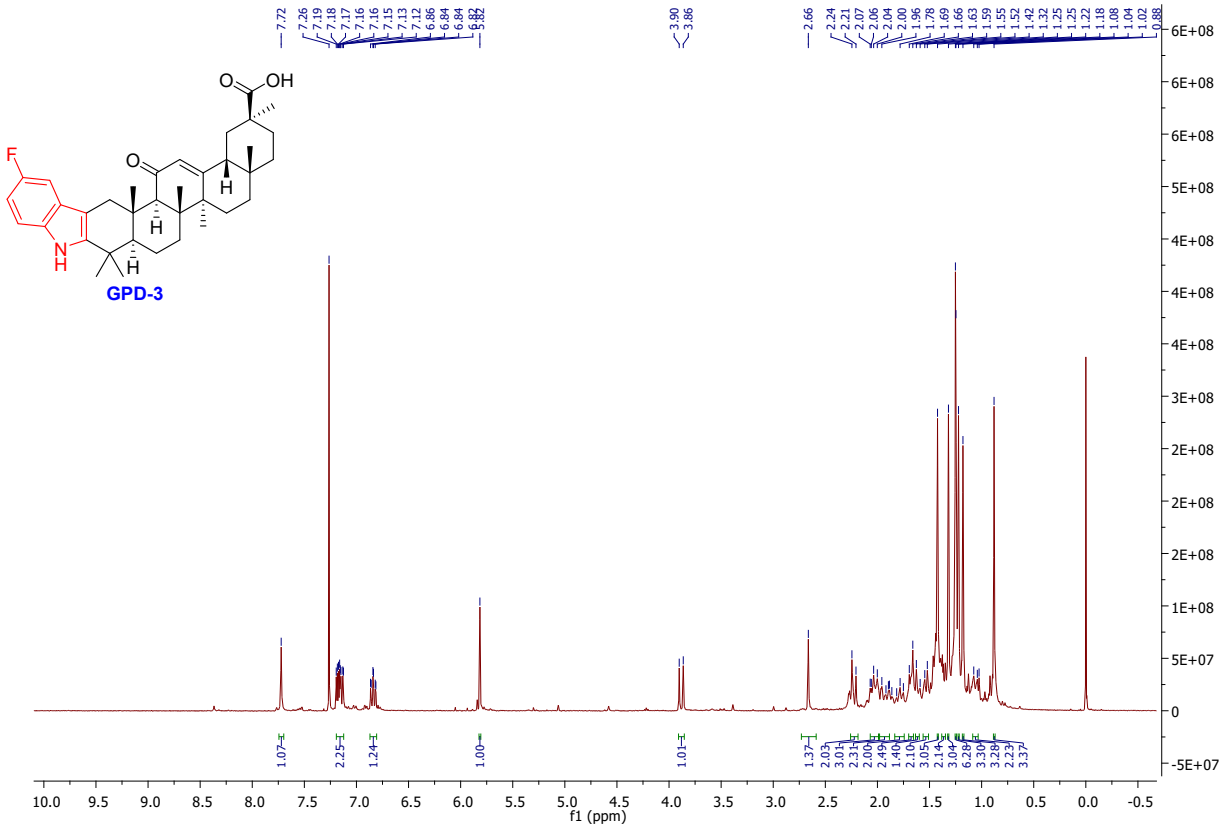


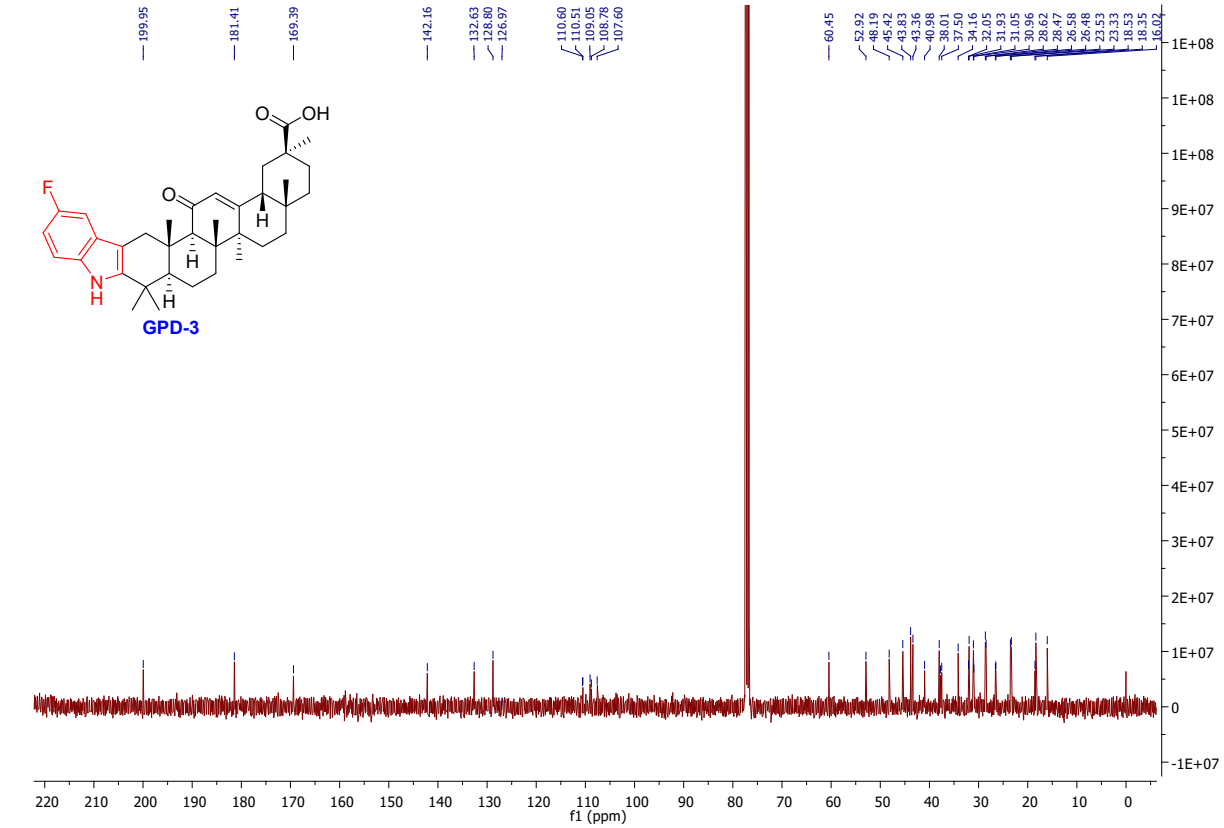
^{1}H NMR (400 Hz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-2



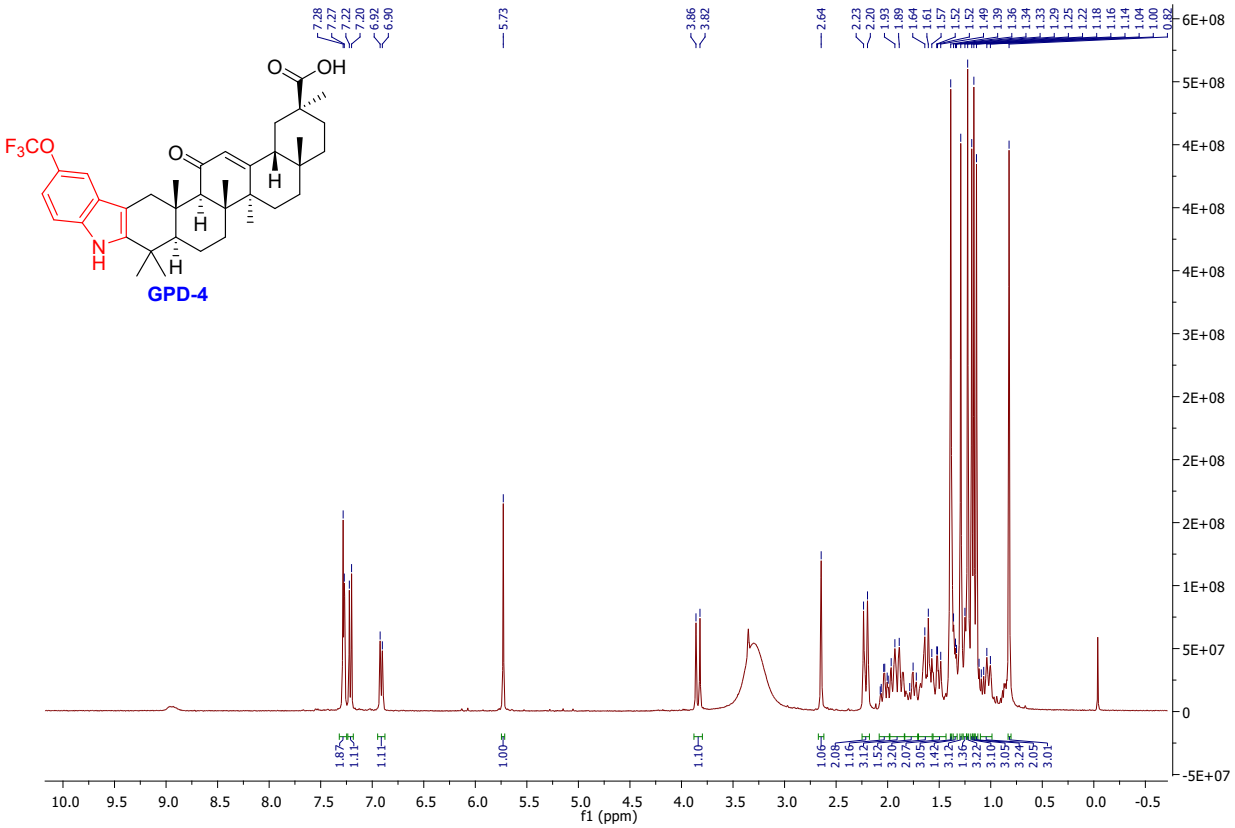


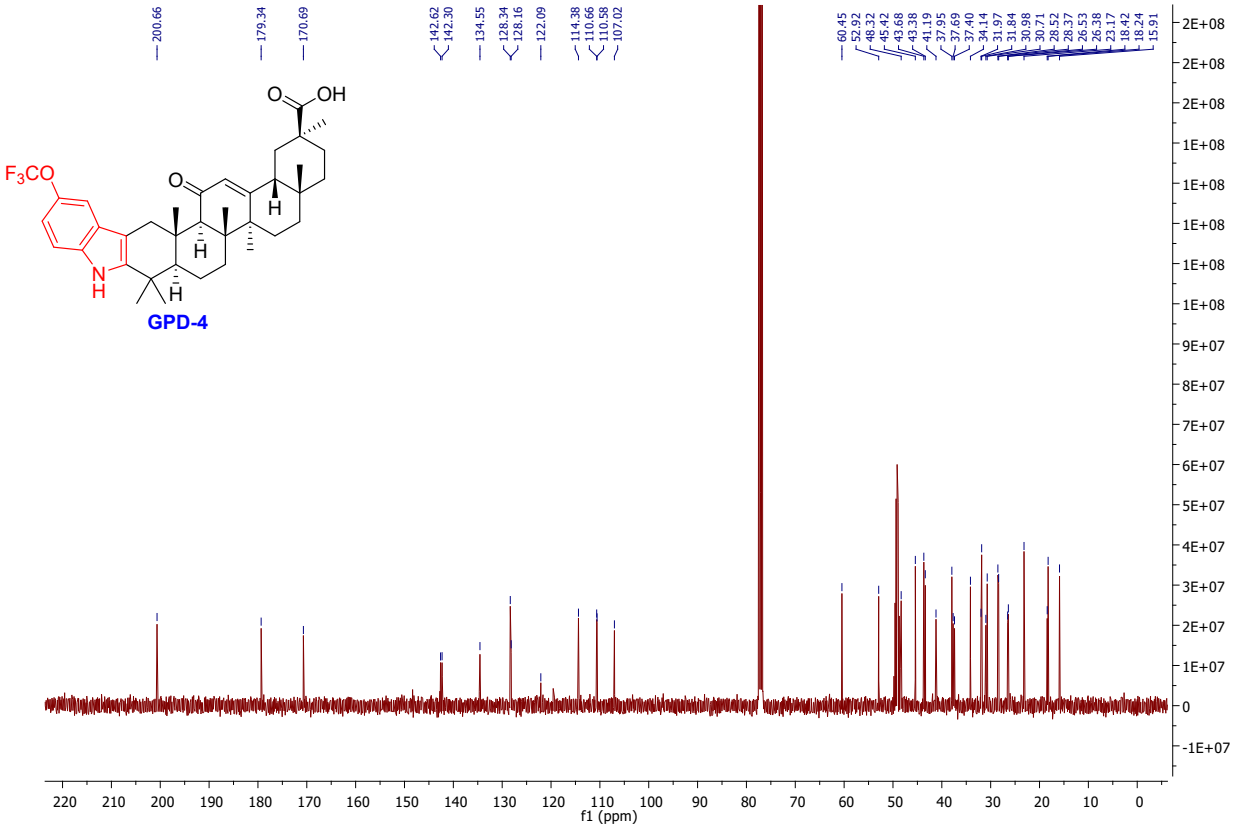
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-3



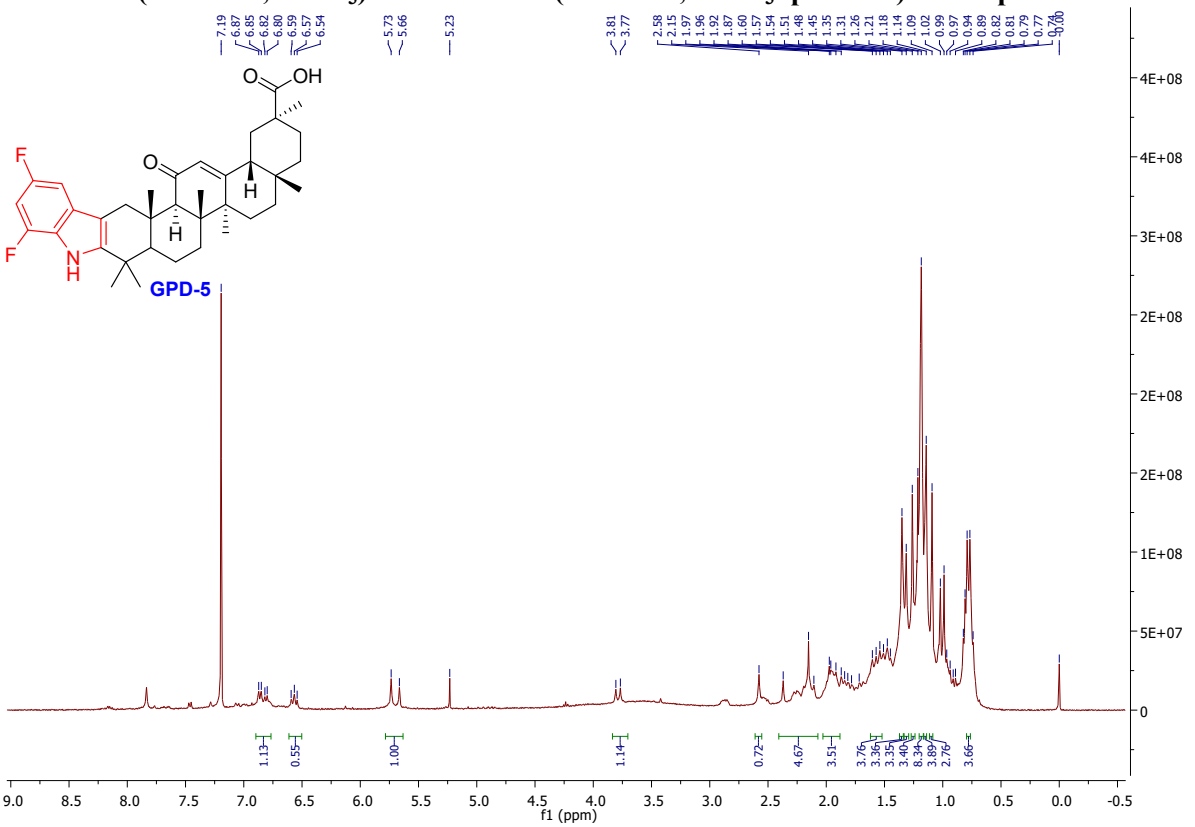


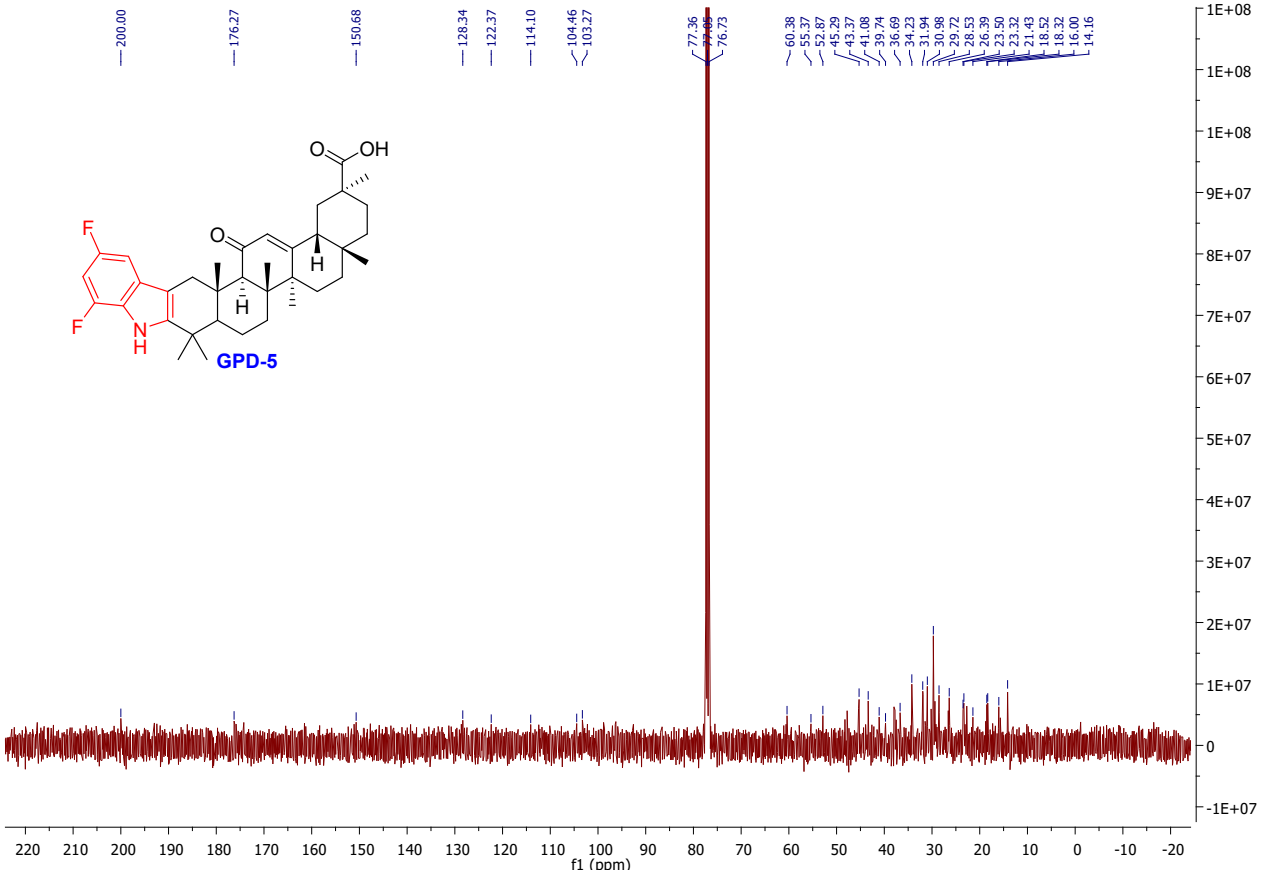
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-4



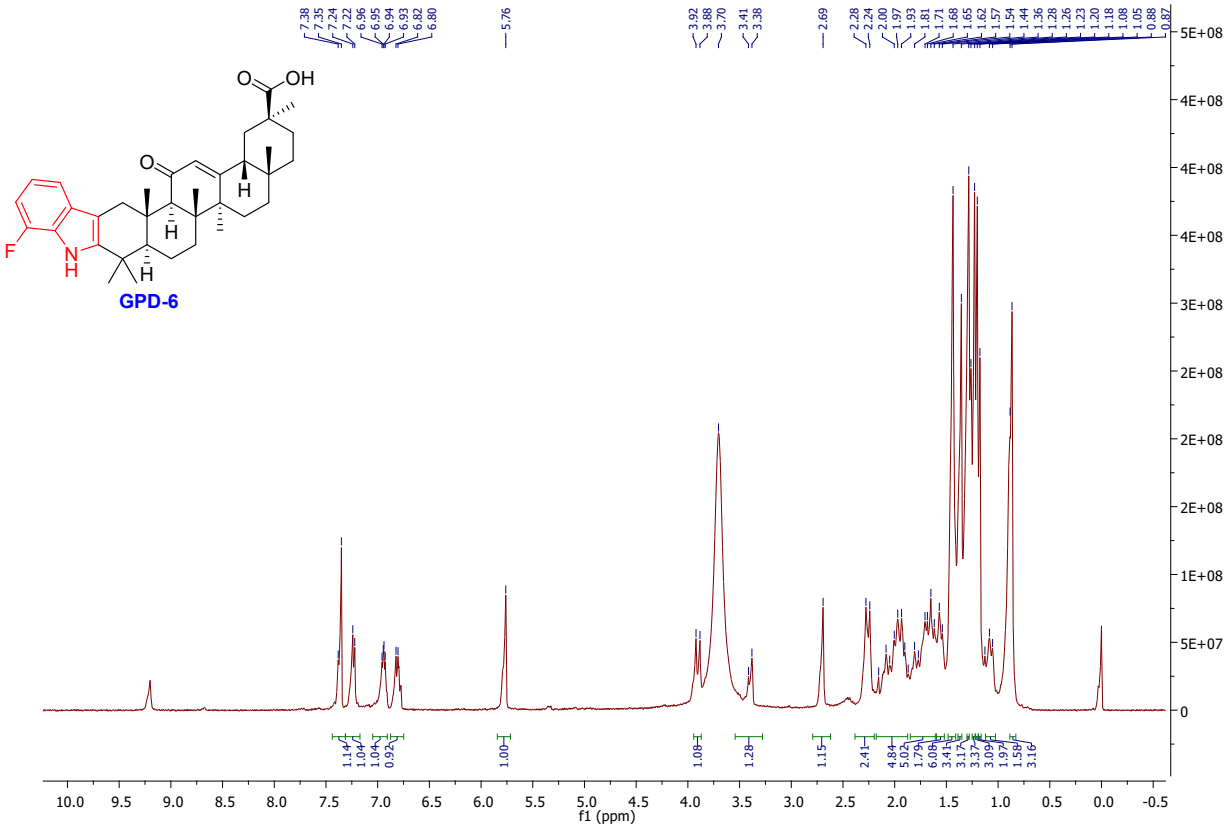


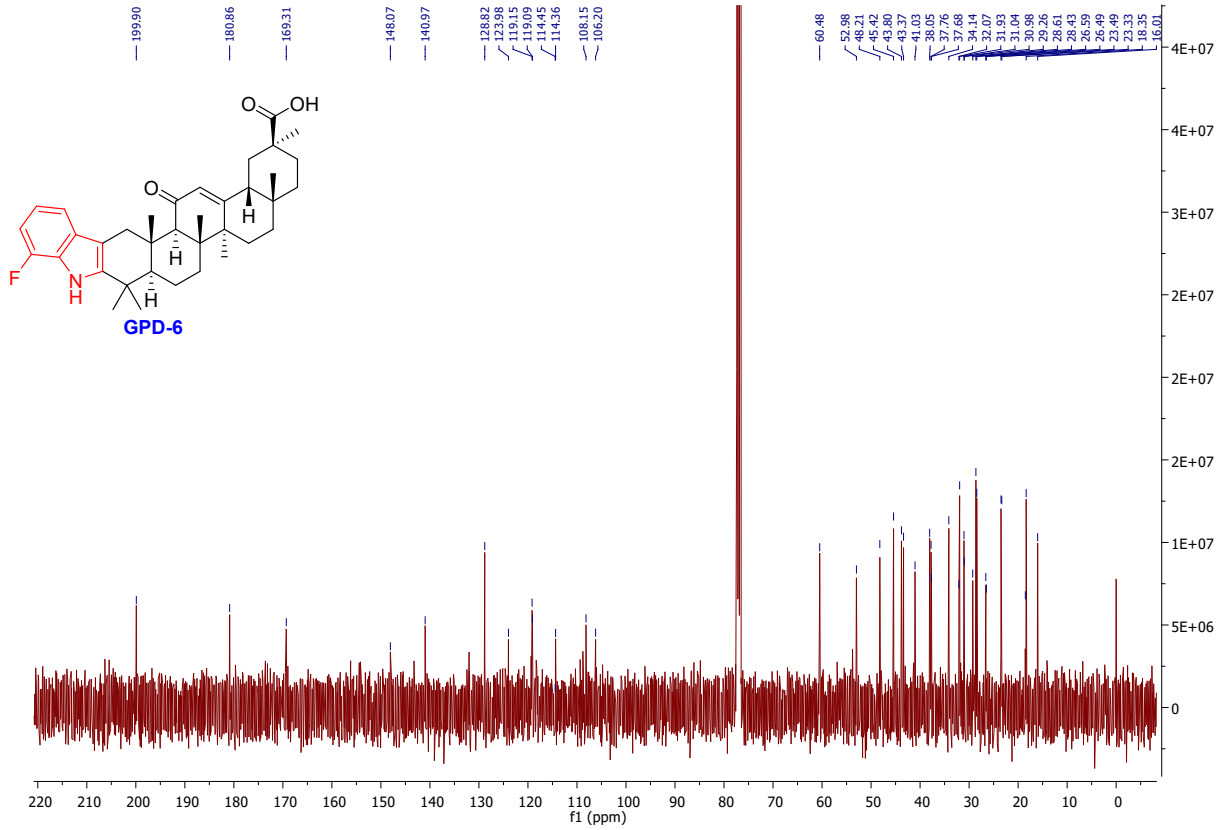
^{1}H NMR (400 MHz, $CDCl_3$) ^{13}C NMR and (101 MHz, $CDCl_3$ spectrum) of compound GPD-5



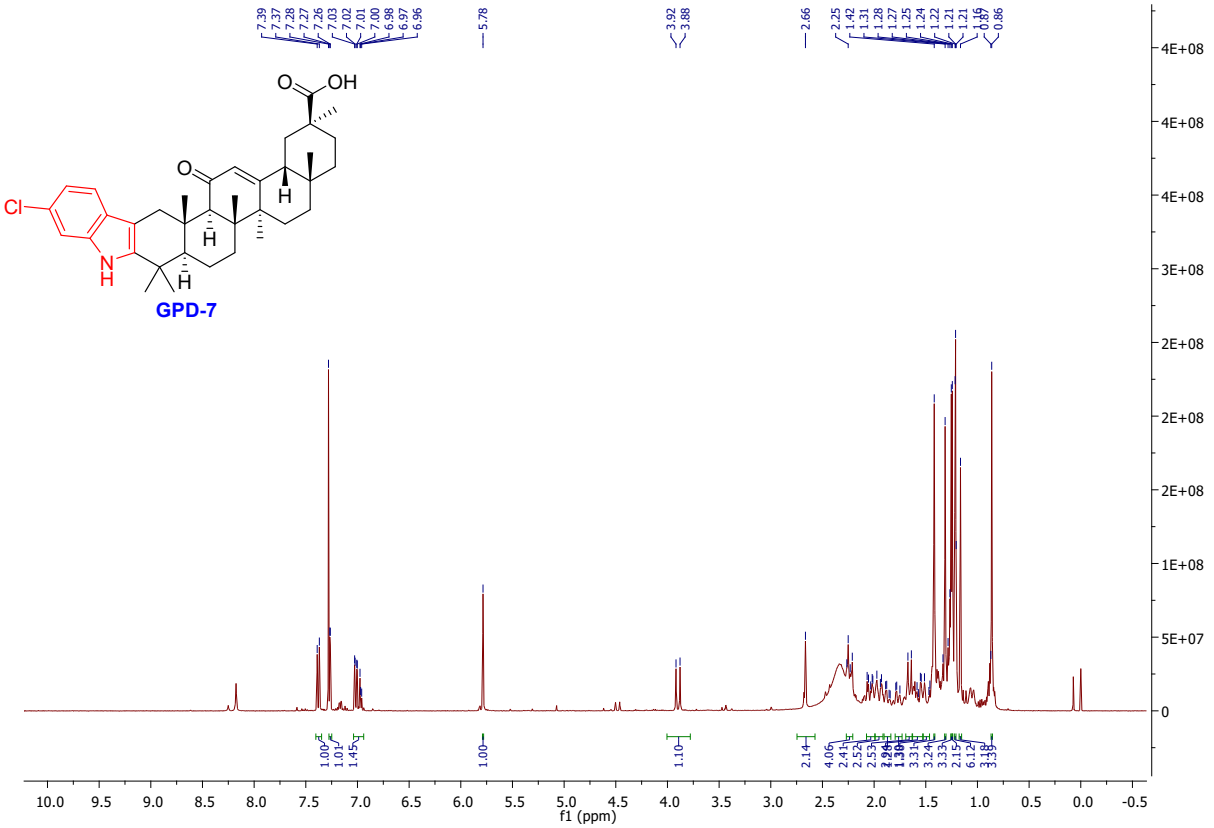


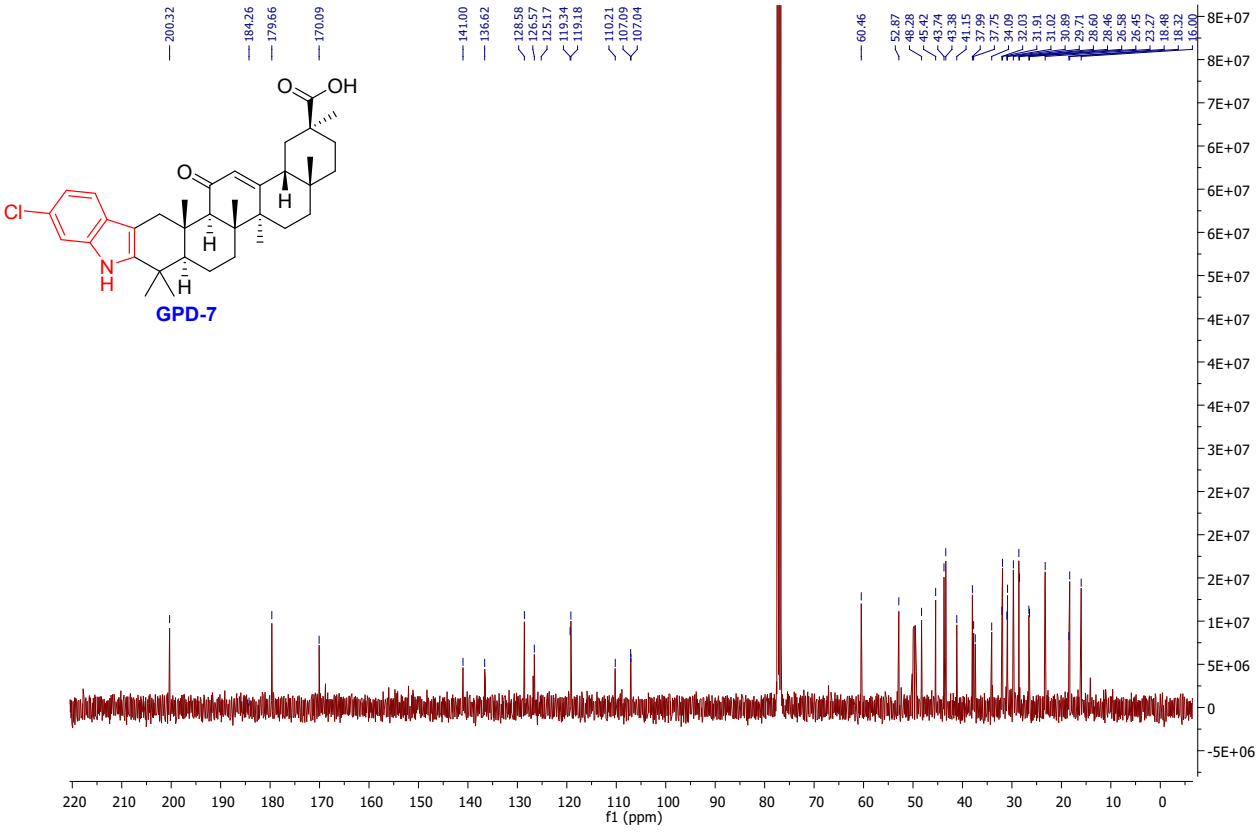
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-6



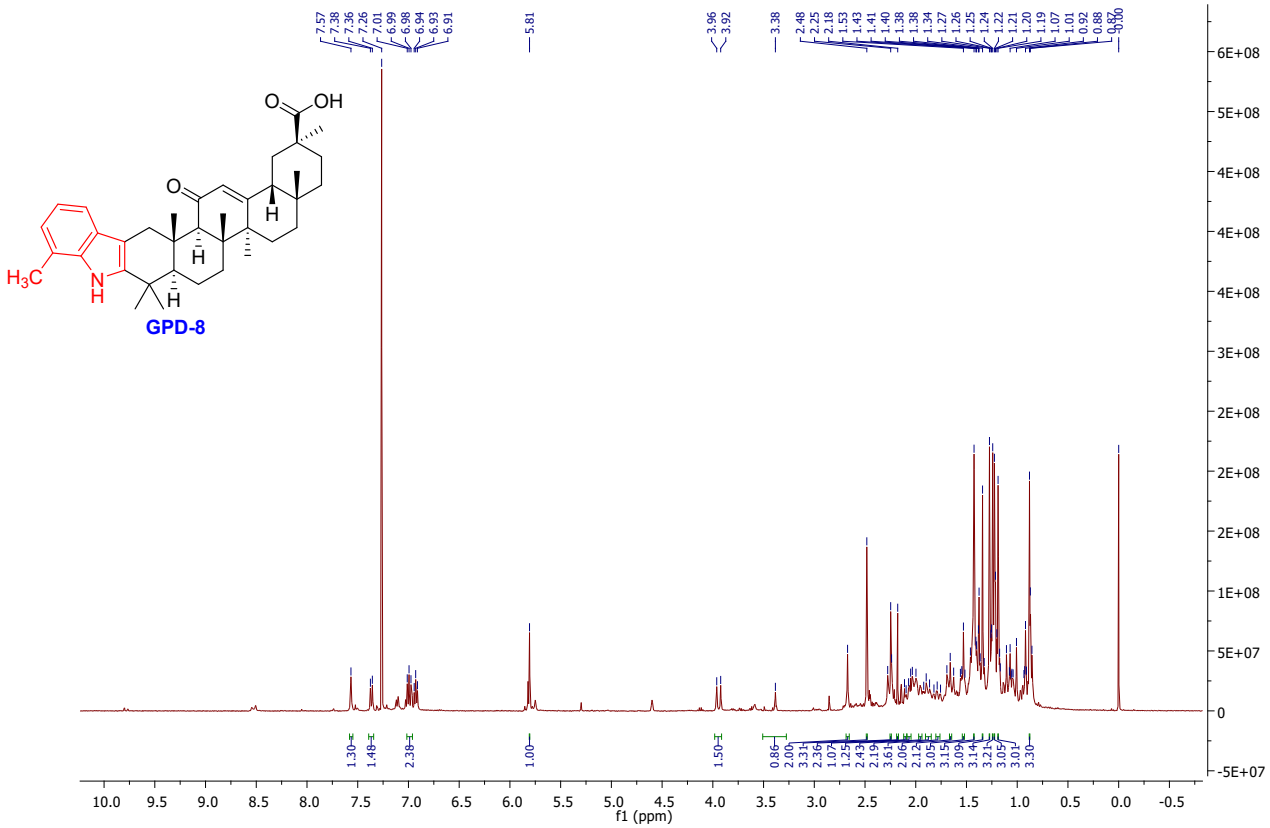


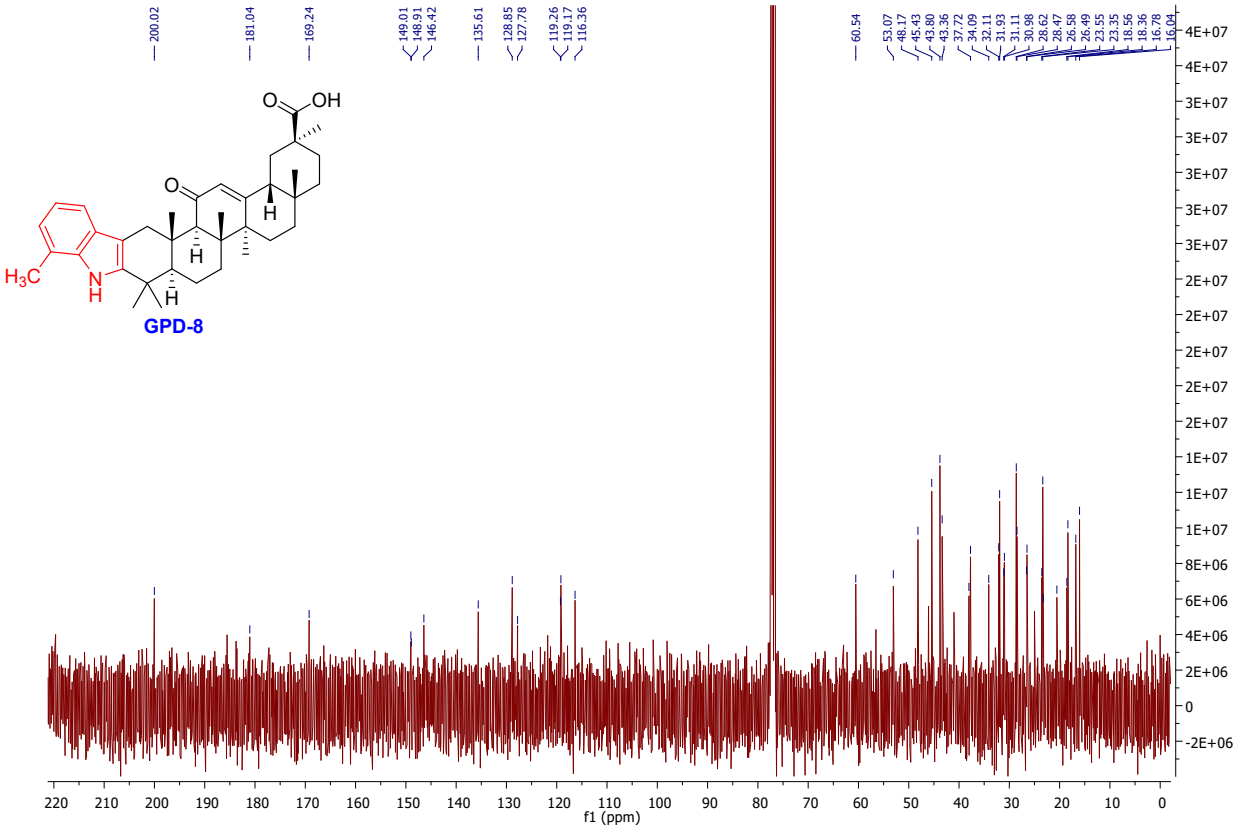
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-7



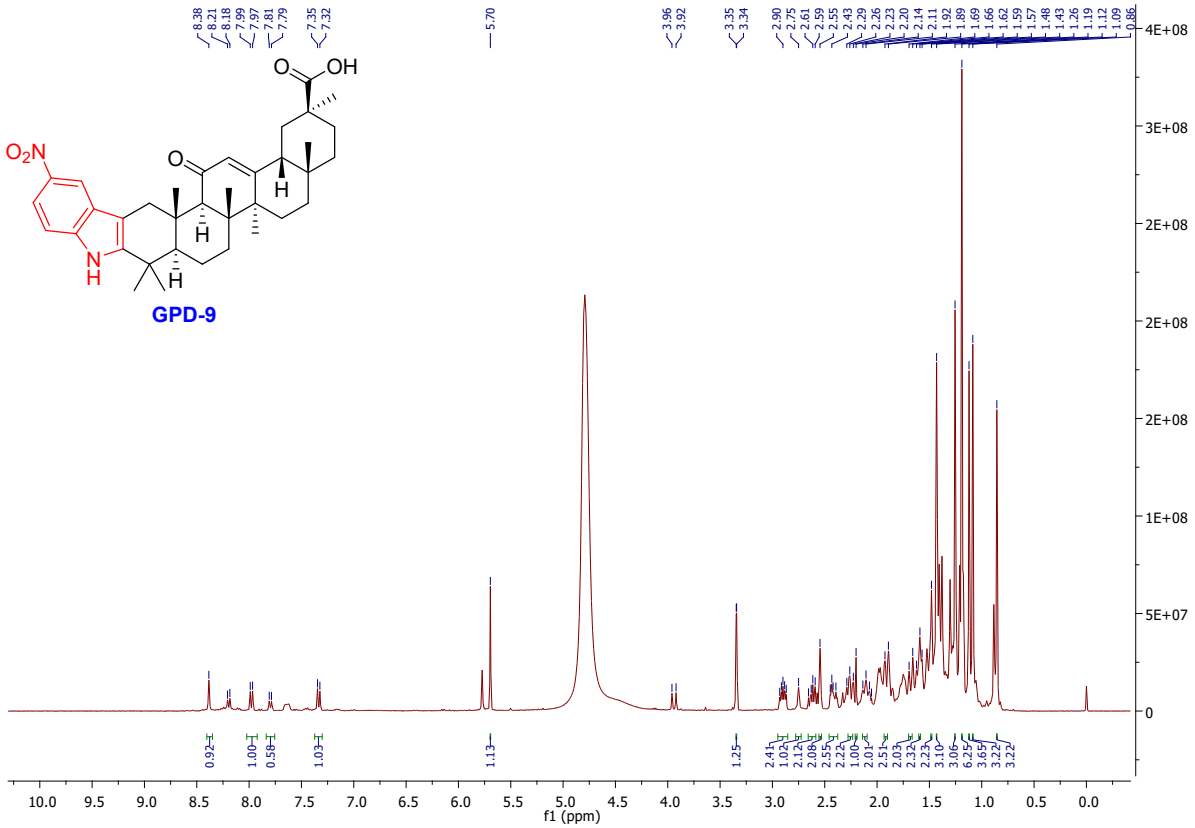


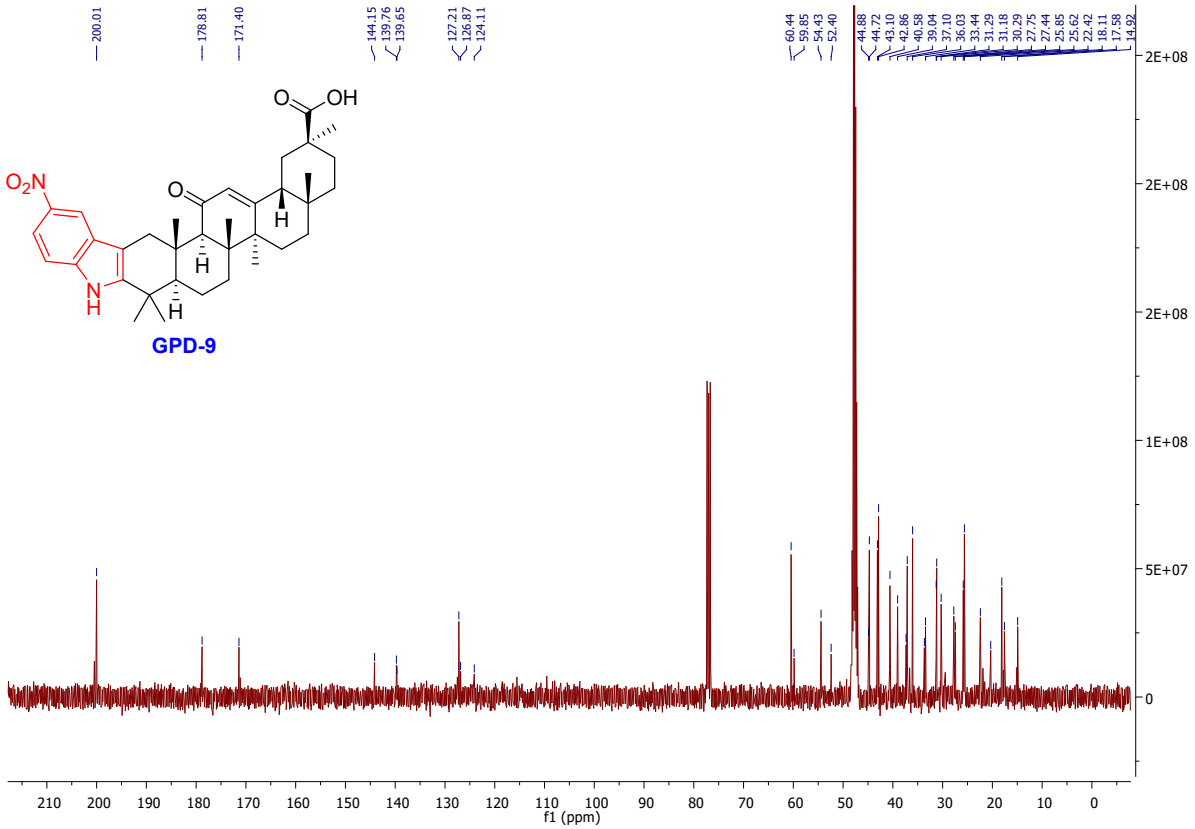
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-8



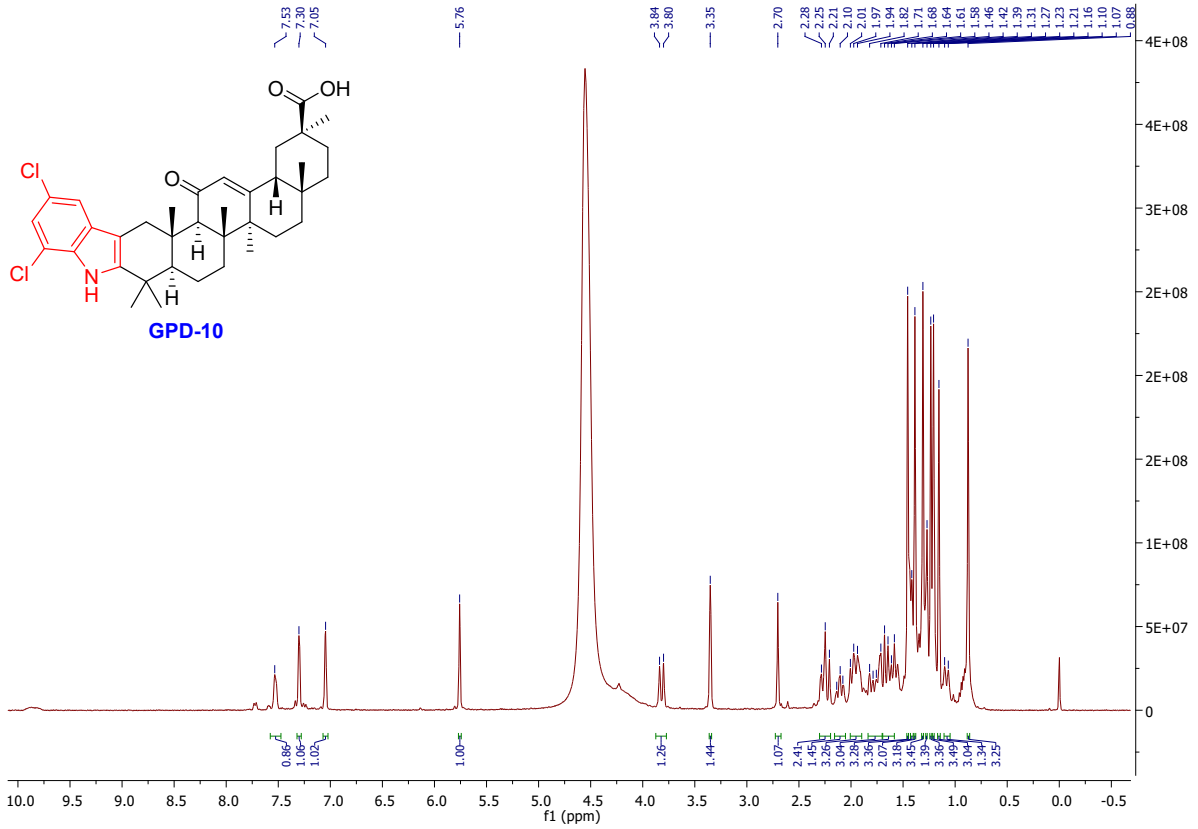


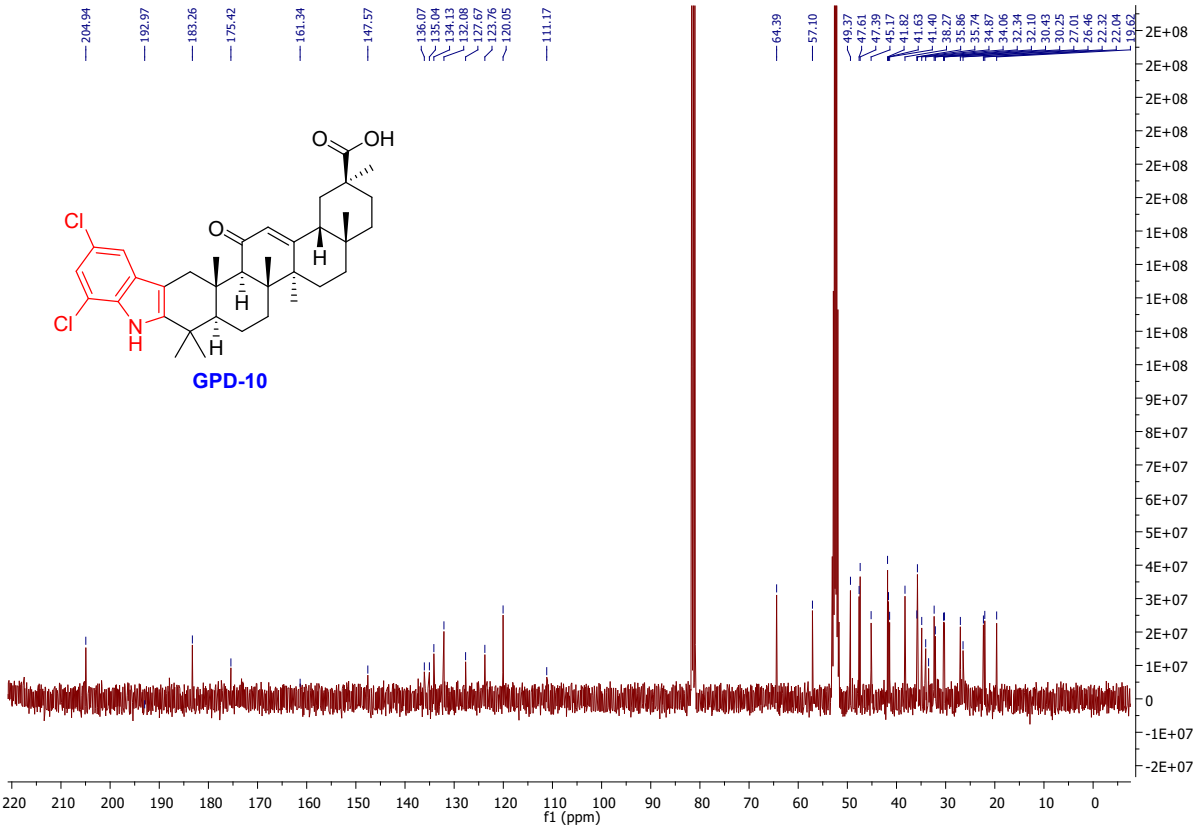
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-9



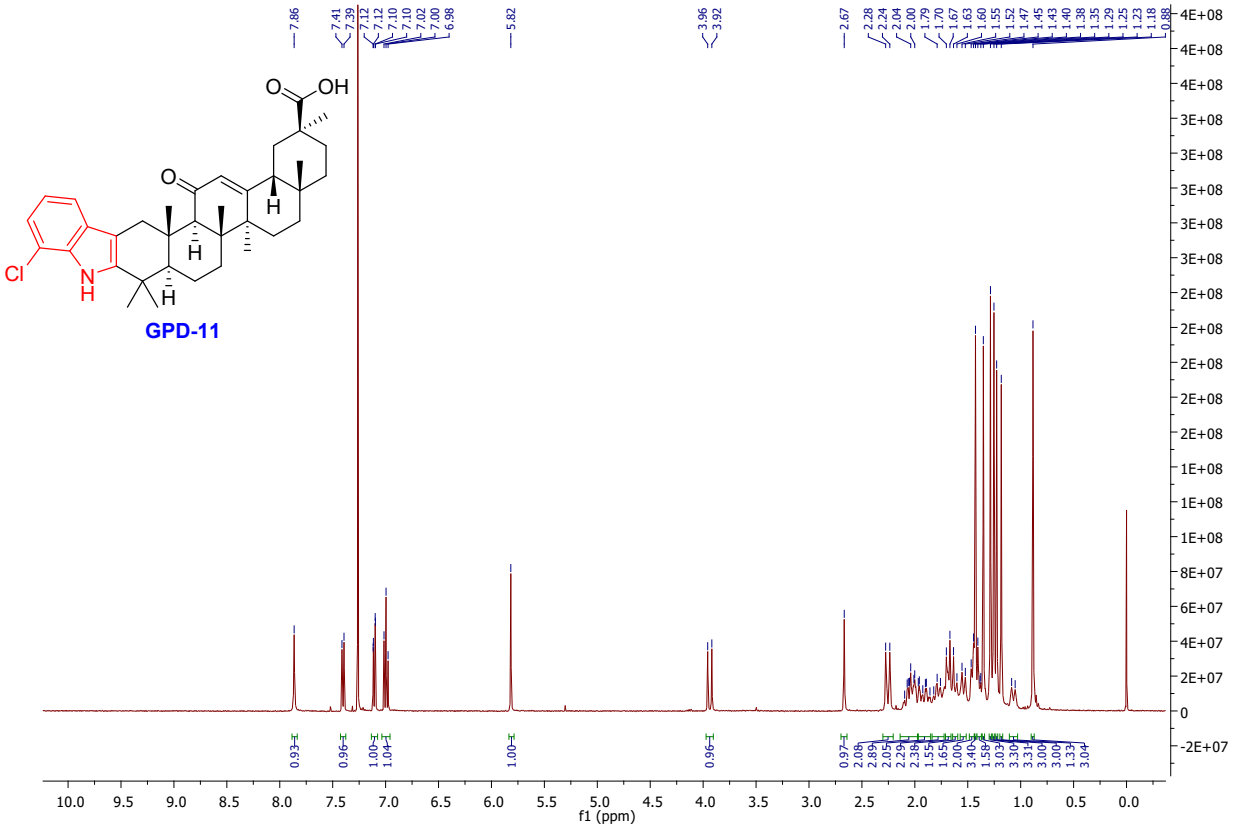


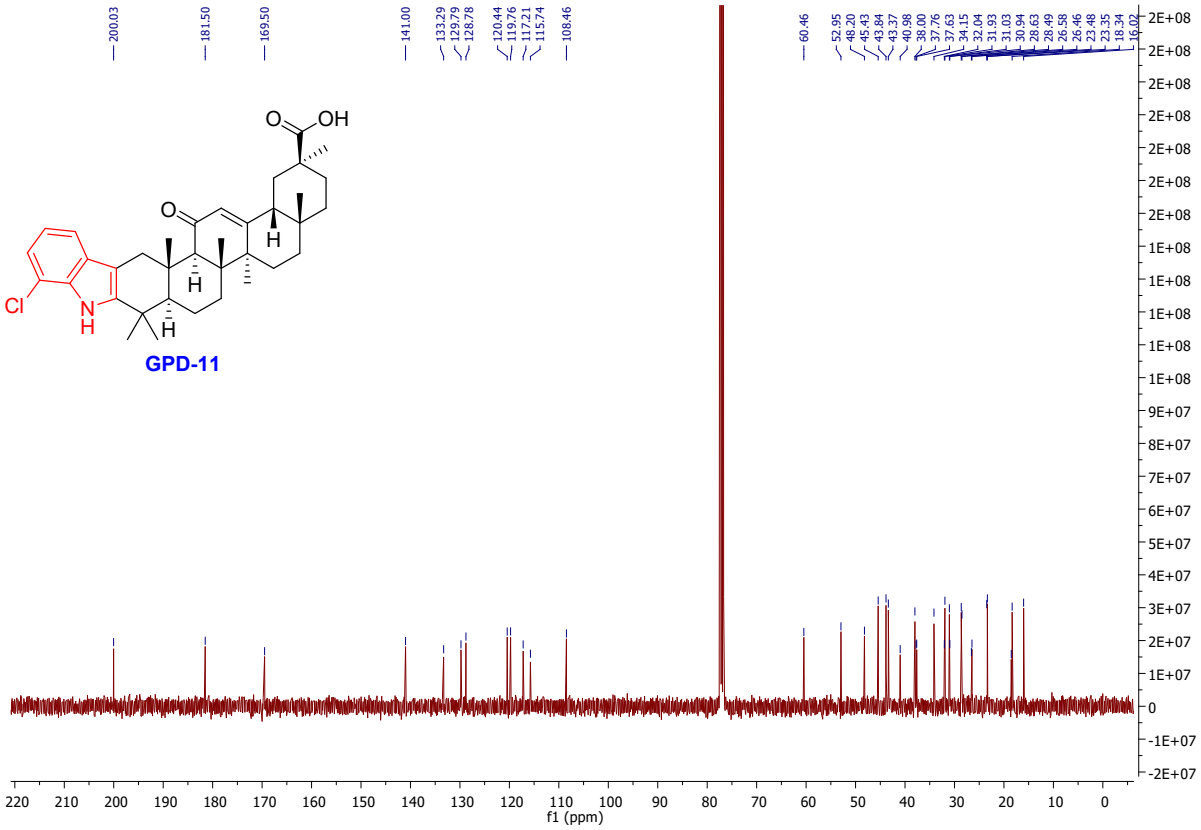
^{1}H NMR (400 MHz, CDCl3: MEOD) and ^{13}C NMR (101 MHz, $CDCl_3$: MEOD) spectrum of compound GPD-10



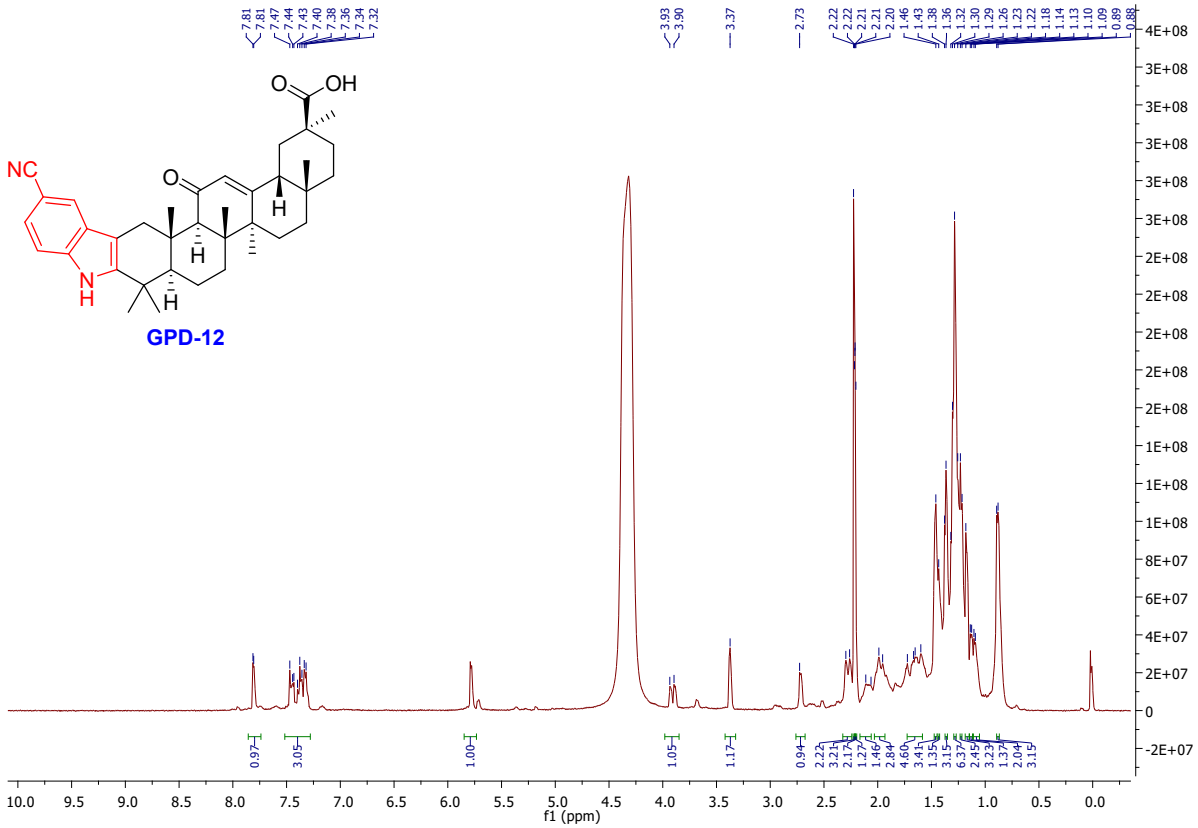


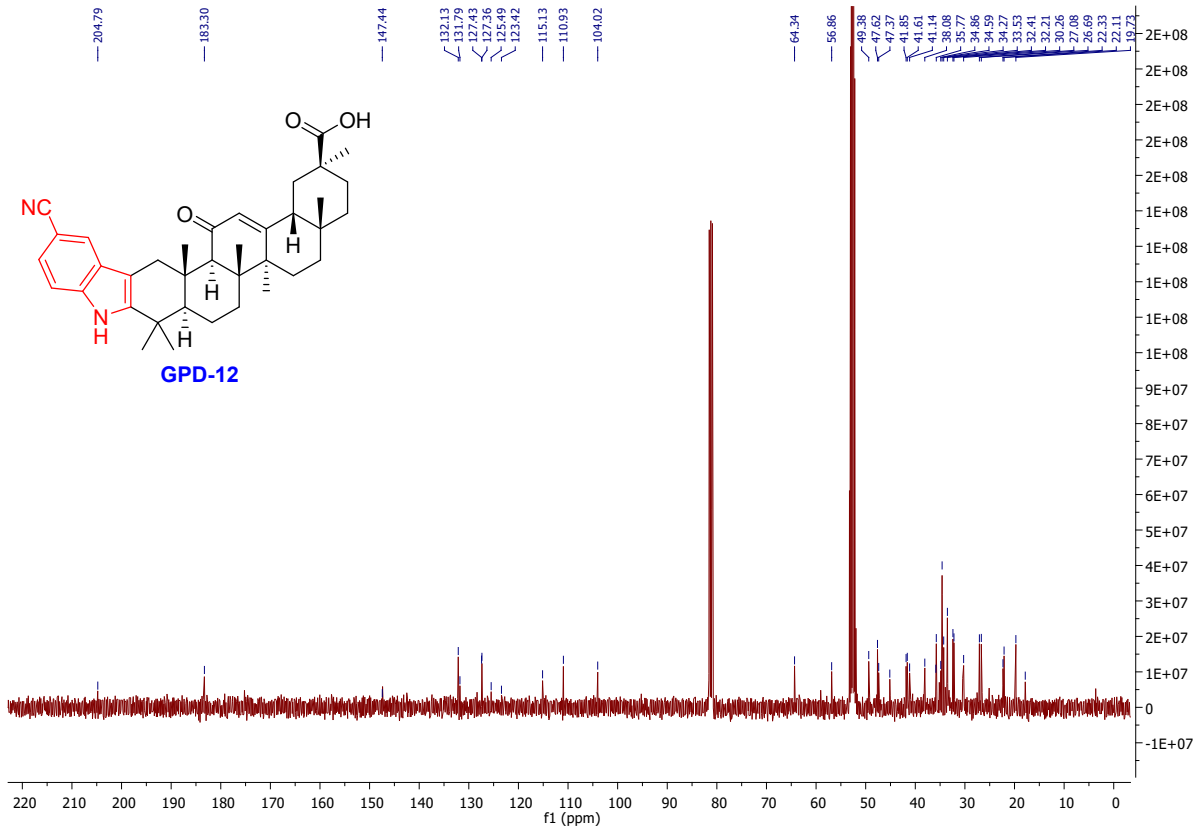
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-11



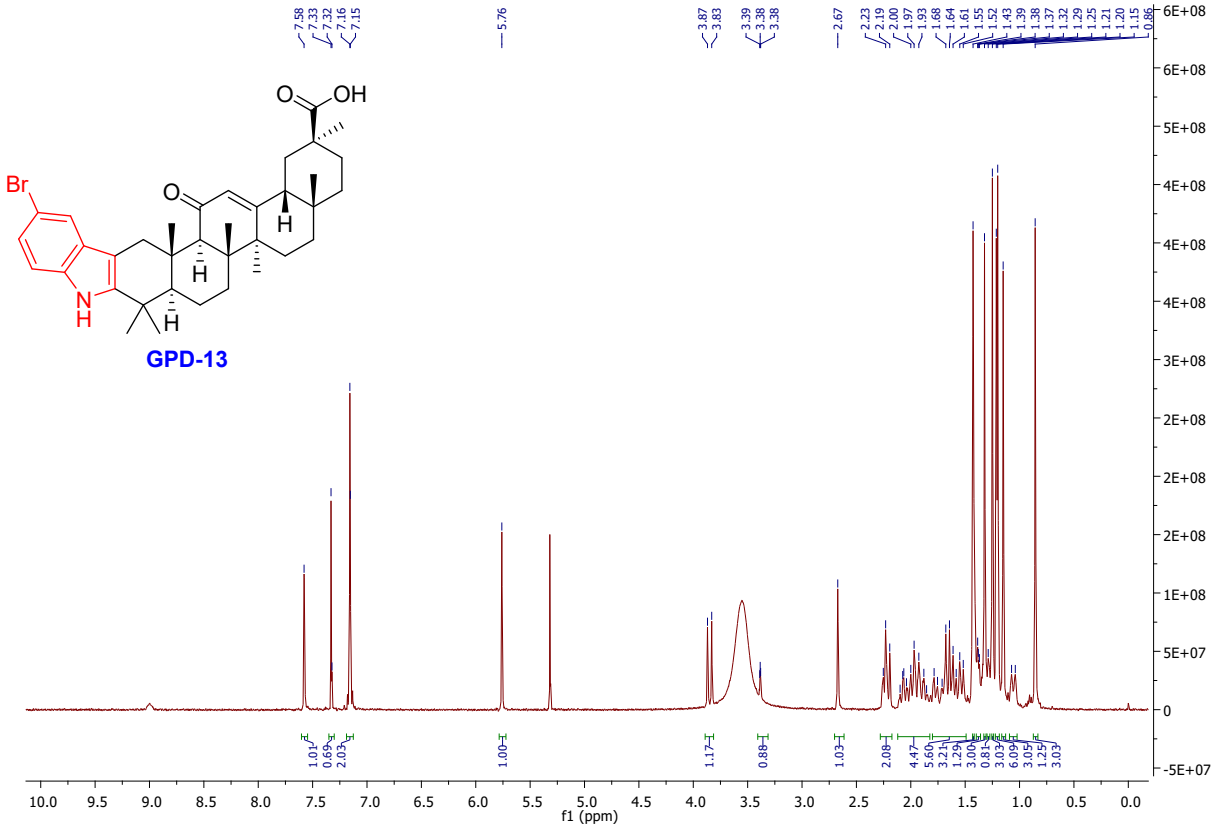


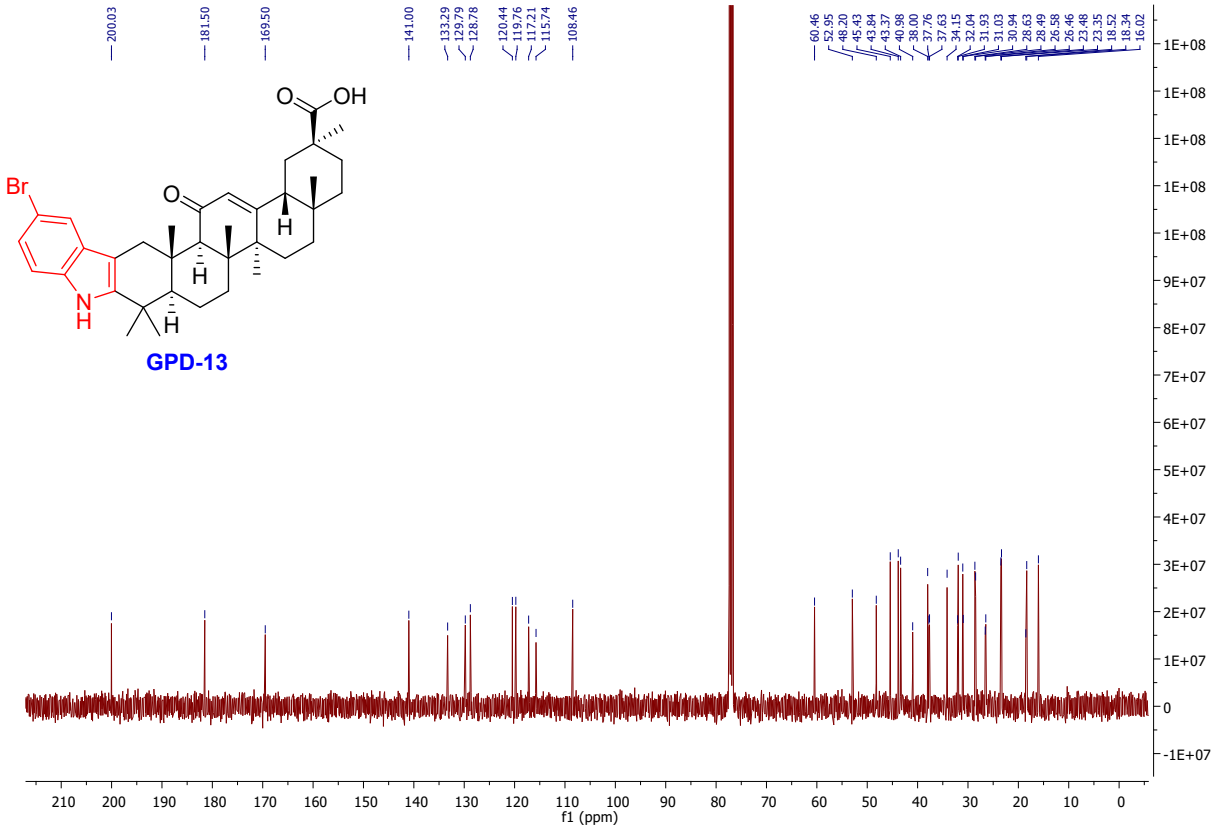
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-12



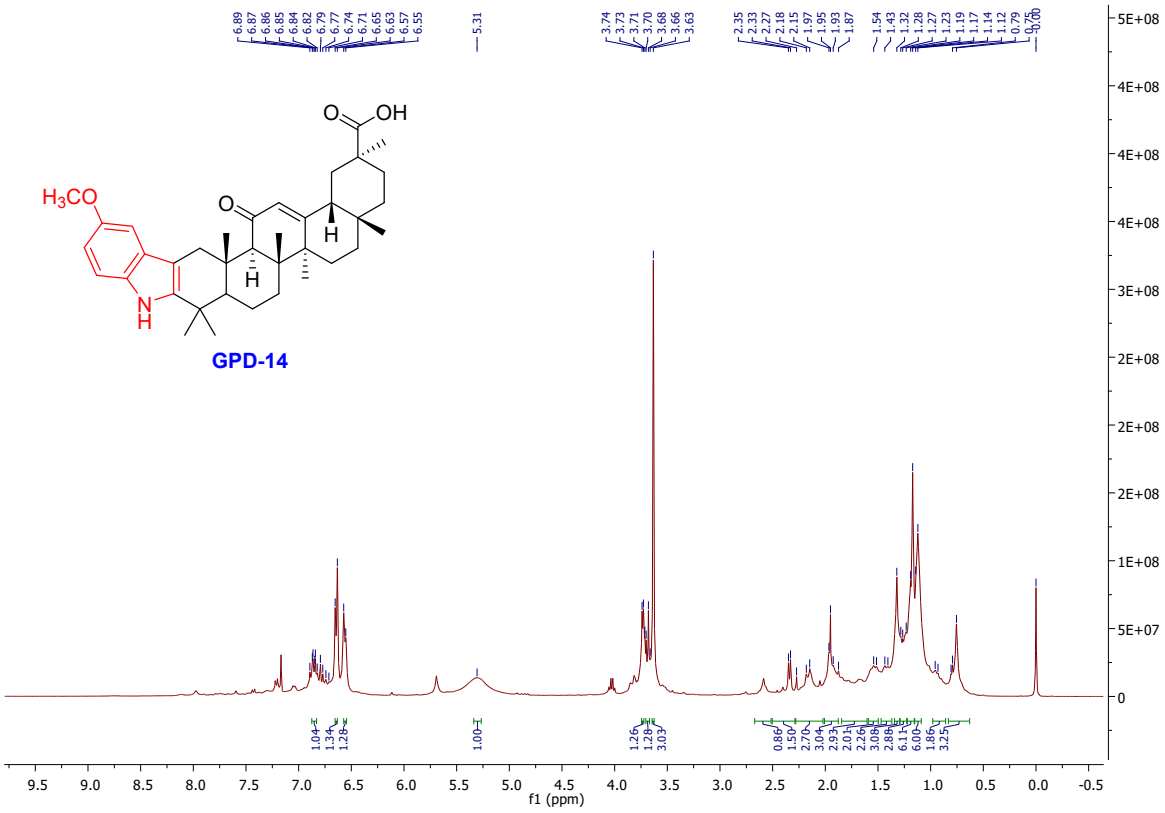


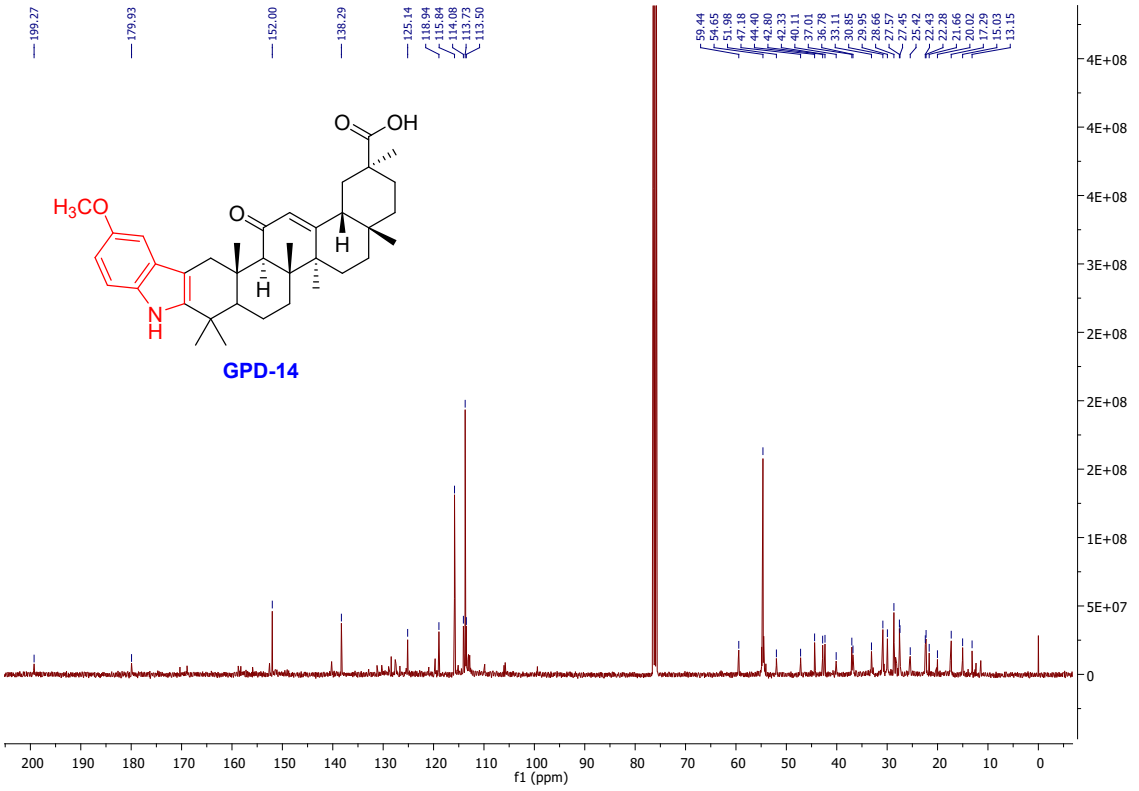
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-13





^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound GPD-14





3. HRMS Spectra of of [3, 2-b] Indole Fused 18β-Glycyrrhetinic Acid (GPD-1 to GPD-14)

Elemental Composition Report Page 1

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
365 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-44 H: 0-100 N: 0-7 O: 0-6

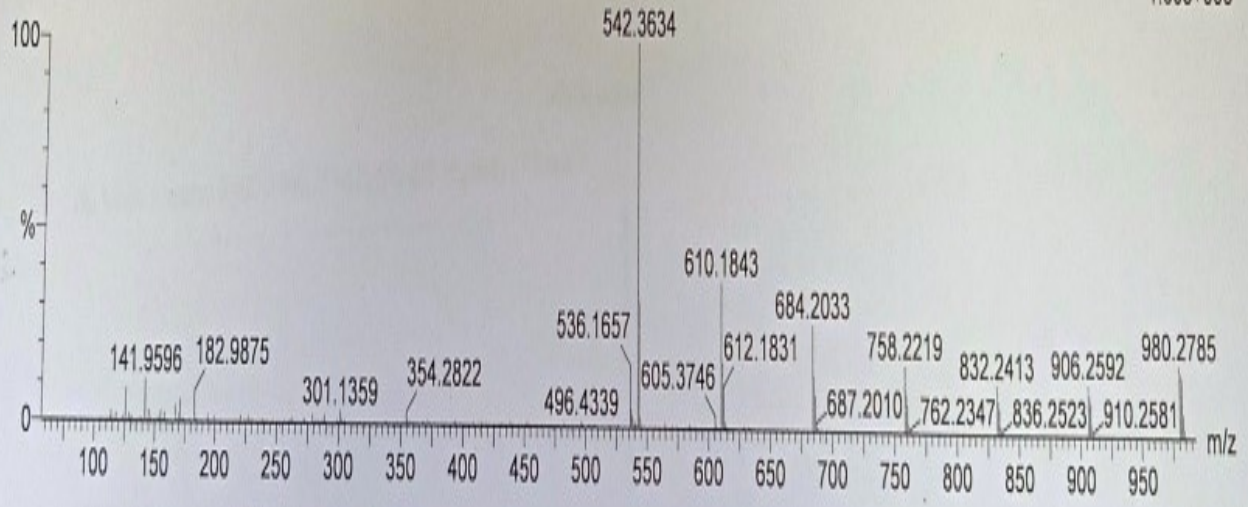
JPD-1

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

22-Jul-2021
14:33:06

220721_58 16 (0.327) Cm (16:17)

1: TOF MS ES+
1.60e+006



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
542.3634	542.3634	0.0	0.0	13.5	49.6	n/a	n/a	C36 H48 N O3

Elemental Composition Report

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

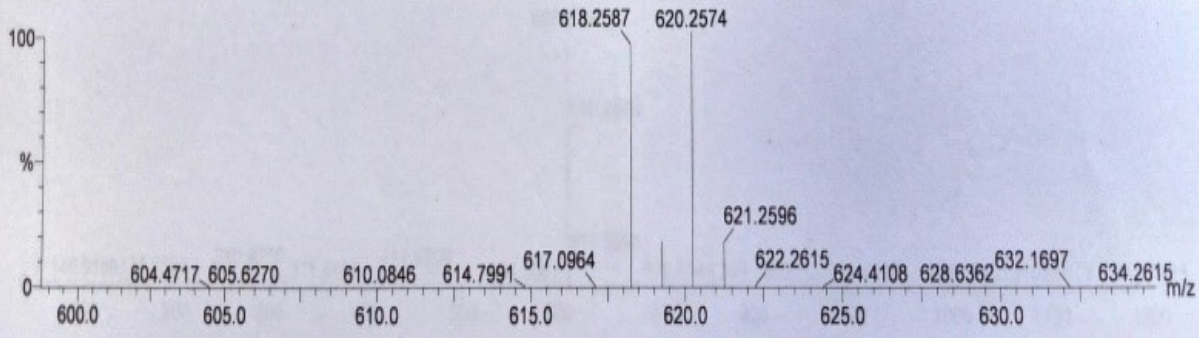
Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-3 Cl: 0-1 Br: 0-1

GPD-2

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

30-Jul-2021
15:00:30
1: TOF MS ES-
7.91e+005

300721_60 13 (0.276) Cm (13:14)



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
618.2587	618.2583	0.4	0.6	14.5	33.4	n/a	n/a	C36 H45 N O3 Br

Elemental Composition Report

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

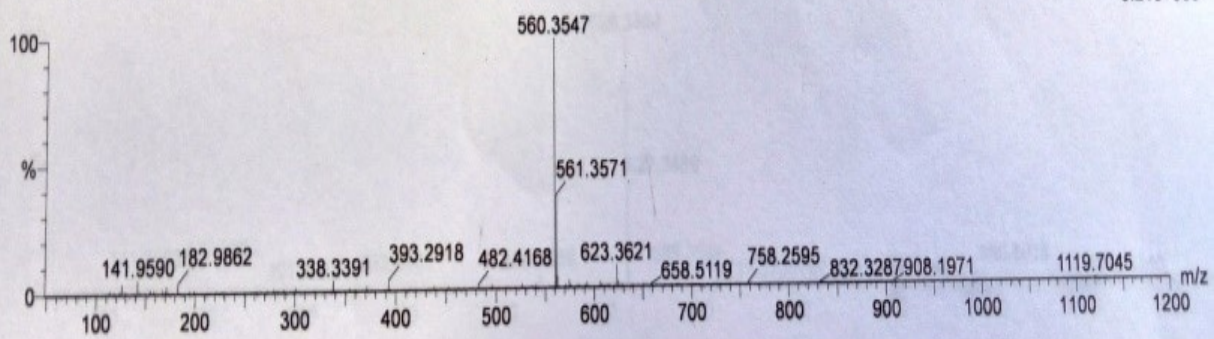
Monoisotopic Mass, Even Electron Ions
118 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-2 O: 0-3 F: 0-3

GPD-3

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

26-Jul-2021
12:22:31
1: TOF MS ES+
5.21e+006

260721_19 12 (0.259) Cm (12:14)



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
560.3547	560.3540	0.7	1.2	13.5	34.5	n/a	n/a	C36 H47 N O3 F

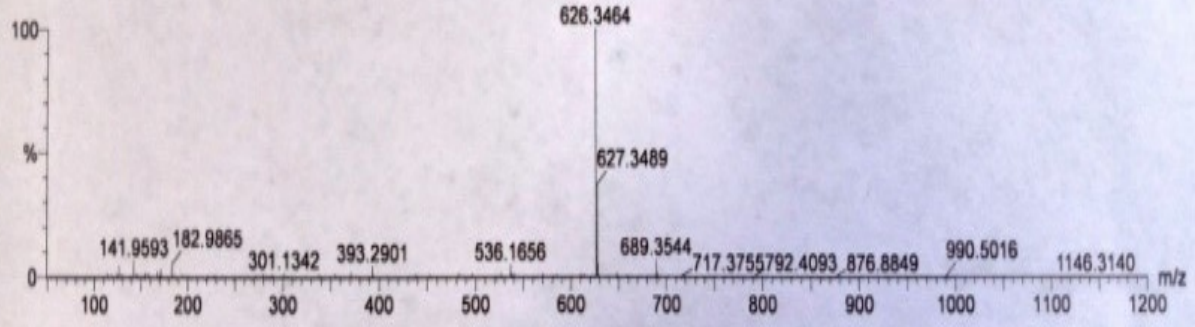
Elemental Composition Report

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
75 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-37 H: 0-100 N: 0-2 O: 0-4 F: 0-3

GPD-4 QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015
26-Jul-2021
12:25:05
260721_20 13 (0.276) Cm (13:16)
1: TOF MS ES+
5.66e+006



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
626.3464	626.3457	0.7	1.1	13.5	57.6	n/a	n/a	C37 H47 N O4 F3

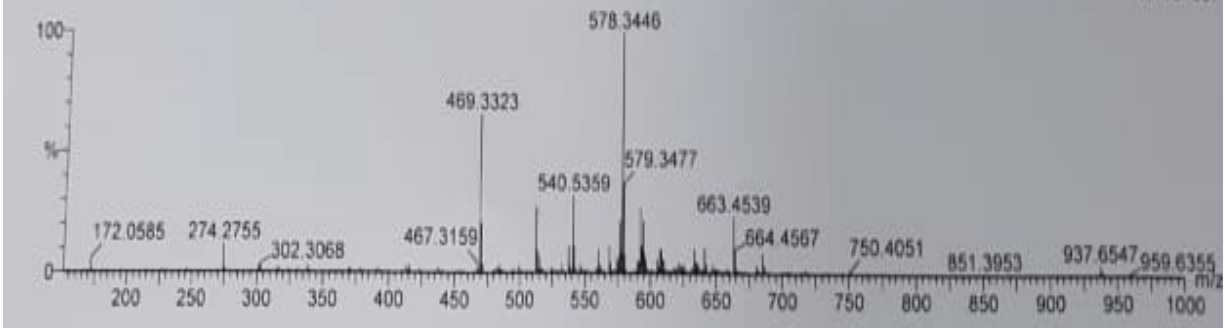
Elemental Composition Report

Single Mass Analysis

Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
29 formula(e) evaluated with 1 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-3 F: 0-2

GPD-5 QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015
09-Jan-2023
15:32:45
090123_02 8 (0.172)
1: TOF MS ES+
1.41e+007



Minimum:				-1.5				
Maximum:		2.0	50.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
578.3446	578.3446	0.0	0.0	13.5	930.1	n/a	n/a	C36 H46 N O3 F2

Elemental Composition Report

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

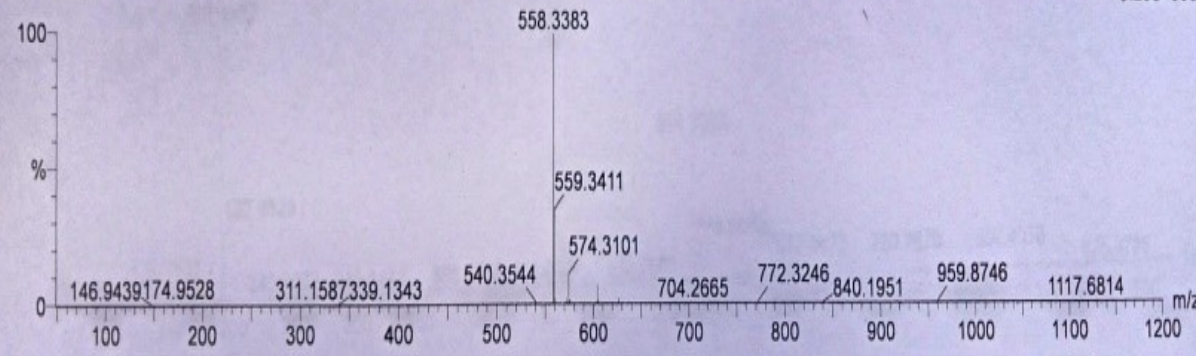
Monoisotopic Mass, Even Electron Ions
109 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-3 F: 0-4

GPD-6

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

30-Jul-2021
14:55:22
1: TOF MS ES-
3.29e+006

300721_58 12 (0.259) Cm (12:14)



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
558.3383	558.3383	0.0	0.0	14.5	37.1	n/a	n/a	C36 H45 N O3 F

Elemental Composition Report

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

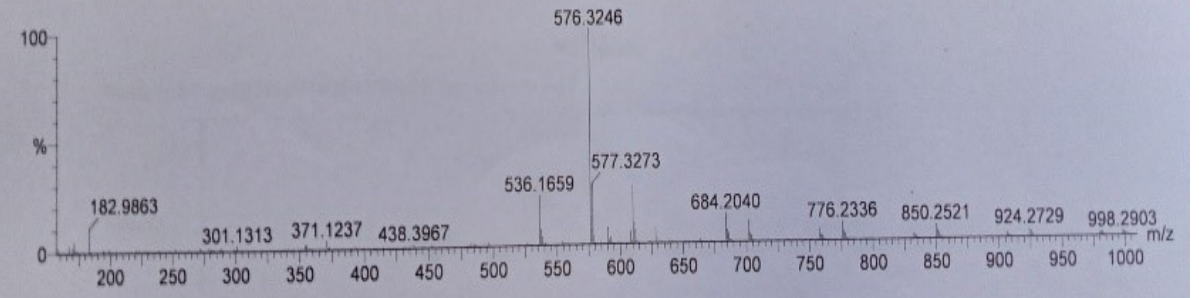
Monoisotopic Mass, Even Electron Ions
166 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-5 Cl: 0-1 Br: 0-1

GPD-7

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

28-Jul-2021
11:45:58
1: TOF MS ES+
1.14e+006

280721_31 15 (0.310) Cm (15:16)



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
576.3246	576.3244	0.2	0.3	13.5	31.3	n/a	n/a	C36 H47 N O3 Cl

Elemental Composition Report

Single Mass Analysis

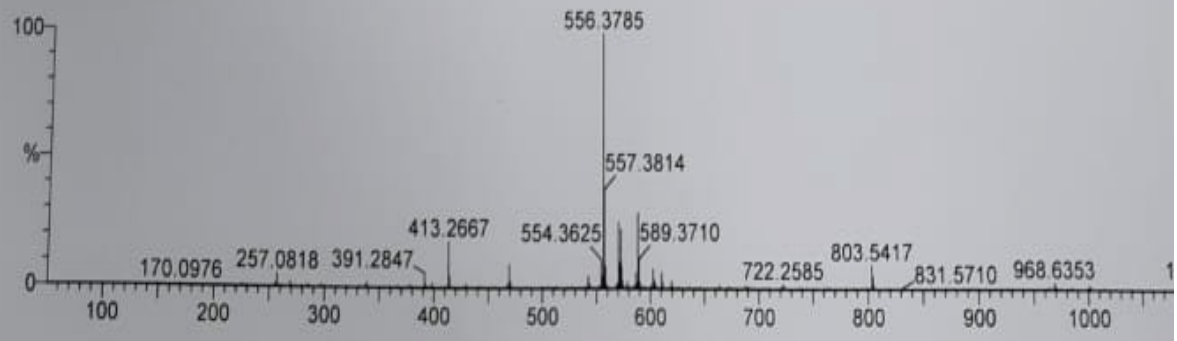
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
12 formula(e) evaluated with 1 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 0-37 H: 0-100 N: 0-1 O: 0-3

GPD-8

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

050123_03 7 (0.155)



Minimum:				-1.5				
Maximum:		2.0	50.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
556.3785	556.3791	-0.6	-1.1	13.5	913.5	n/a	n/a	C37 H50 N O3

gle Mass Analysis

olerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

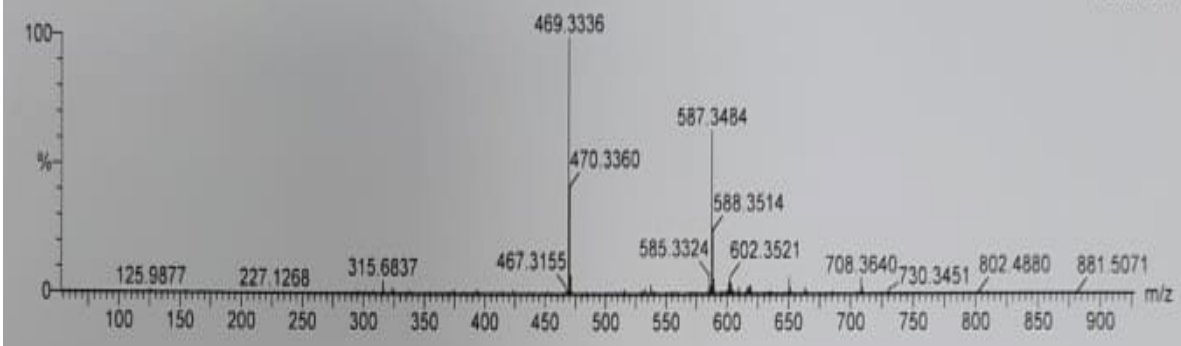
Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-2 O: 0-5

GPD-9

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

05-Jan-2023
12:38:58
1: TOF MS ES+
1.67e+007

050123_05 8 (0.172)



Minimum:				-1.5				
Maximum:		2.0	50.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
587.3484	587.3485	-0.1	-0.2	14.5	812.0	n/a	n/a	C36 H47 N2 O5

Elemental Composition Report

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

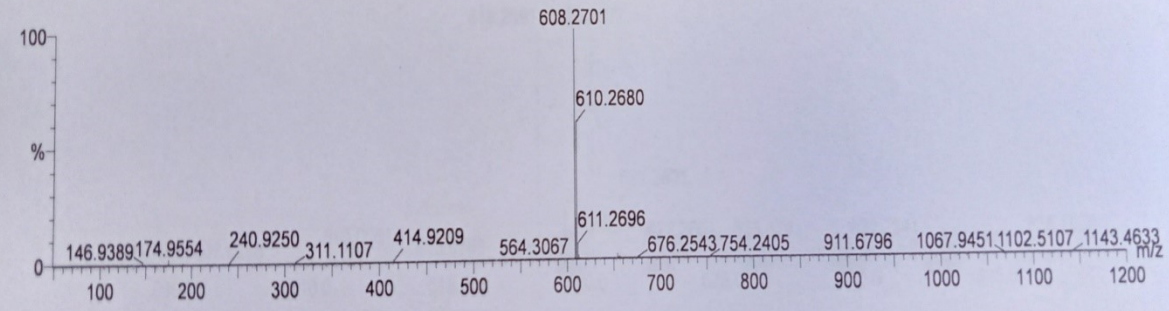
Monoisotopic Mass, Even Electron Ions
122 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-3 Cl: 0-2 Br: 0-1

GPD-10

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

30-Jul-2021
15:03:05
1: TOF MS ES-
9.91e+005

300721_61 14 (0.293) Cm (14:16)



Minimum:				-1.5				
Maximum:		2.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
608.2701	608.2698	0.3	0.5	14.5	32.9	n/a	n/a	C36 H44 N O3 Cl2

Elemental Composition Report

Single Mass Analysis

Tolerance = 4.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

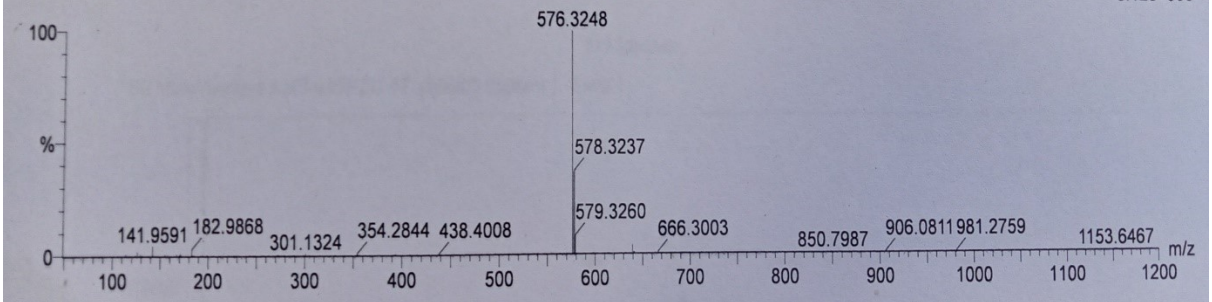
Monoisotopic Mass, Even Electron Ions
167 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-3 Cl: 0-2 Br: 0-1

GPD-11

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

30-Jul-2021
12:05:01
1: TOF MS ES+
3.12e+006

300721_28 14 (0.293) Cm (14:15)



Minimum:				-1.5				
Maximum:		2.0	4.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
576.3248	576.3244	0.4	0.7	13.5	28.4	n/a	n/a	C36 H47 N O3 Cl

gle Mass Analysis
lerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

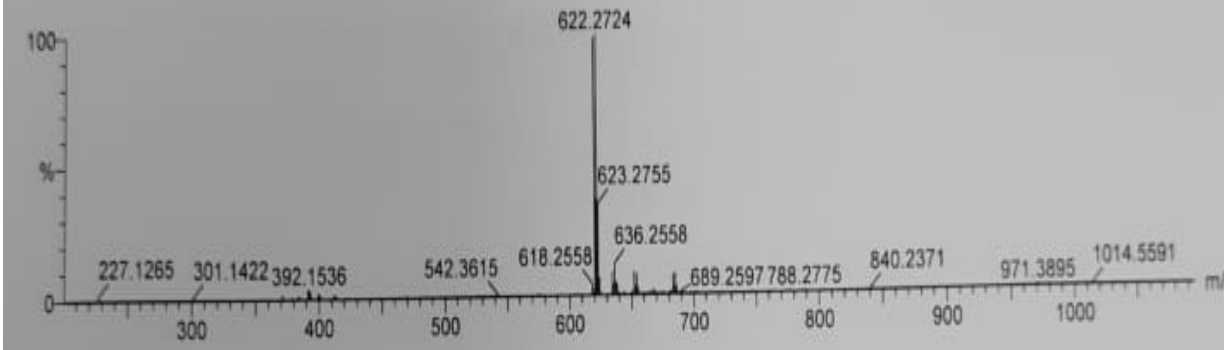
Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 0-36 H: 0-100 N: 0-1 O: 0-3 Br: 0-1

GPD-13

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

05-Jan-2023
12:36:23
1: TOF MS ES+
1.04e+007

050123_04 12 (0.259)



Minimum:				-1.5				
Maximum:		2.0	50.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
620.2733	620.2739	-0.6	-1.0	13.5	688.9	n/a	n/a	C36 H47 N O3 Br

Page 1

Elemental Composition Report

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

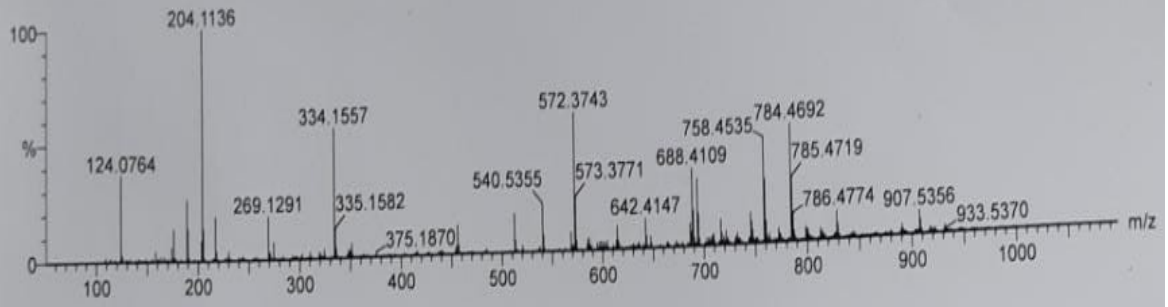
Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 0-37 H: 0-100 N: 0-1 O: 0-4

GPD-14

QMI DIVISION, CSIR-IIIM JAMMU
Xevo G2-XS QTOF YFC2015

27-Jan-2023
12:09:38
1: TOF MS ES+
9.55e+006

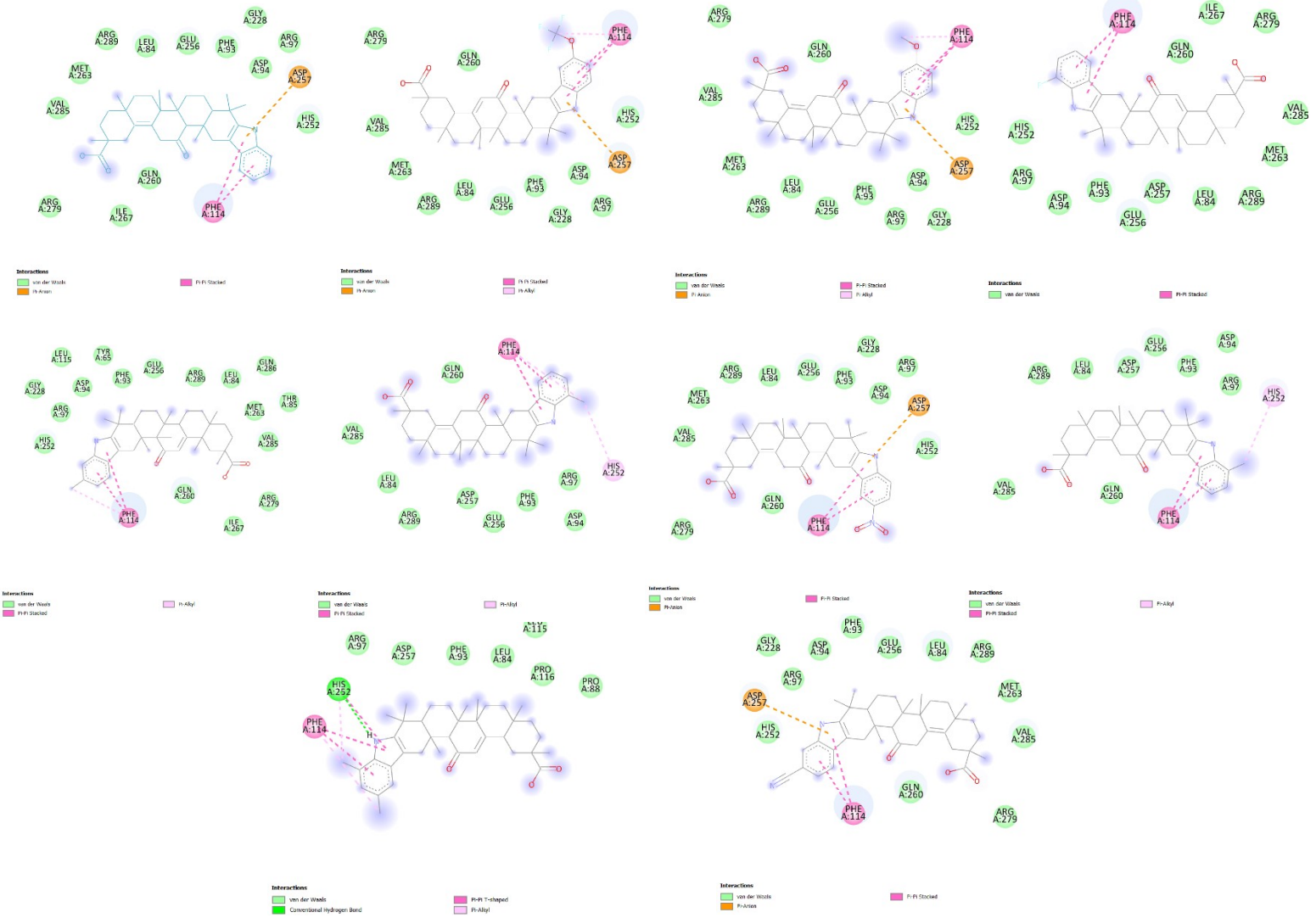
27012023_02 9 (0.208)



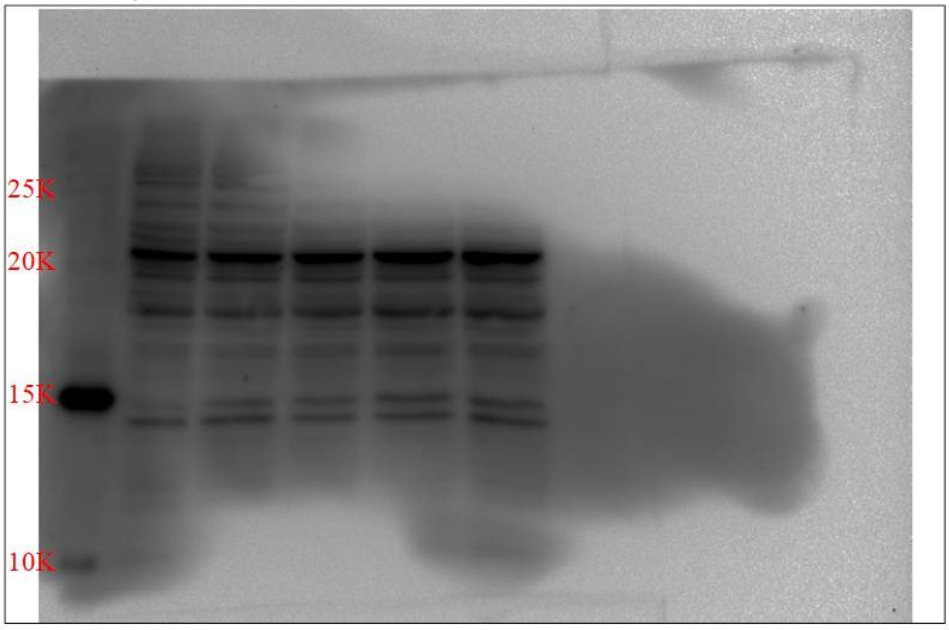
Minimum:				-1.5				
Maximum:		2.0	50.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
572.3743	572.3740	0.3	0.5	13.5	888.9	n/a	n/a	C37 H50 N O4

4. Molecular docking data

2D images of compound

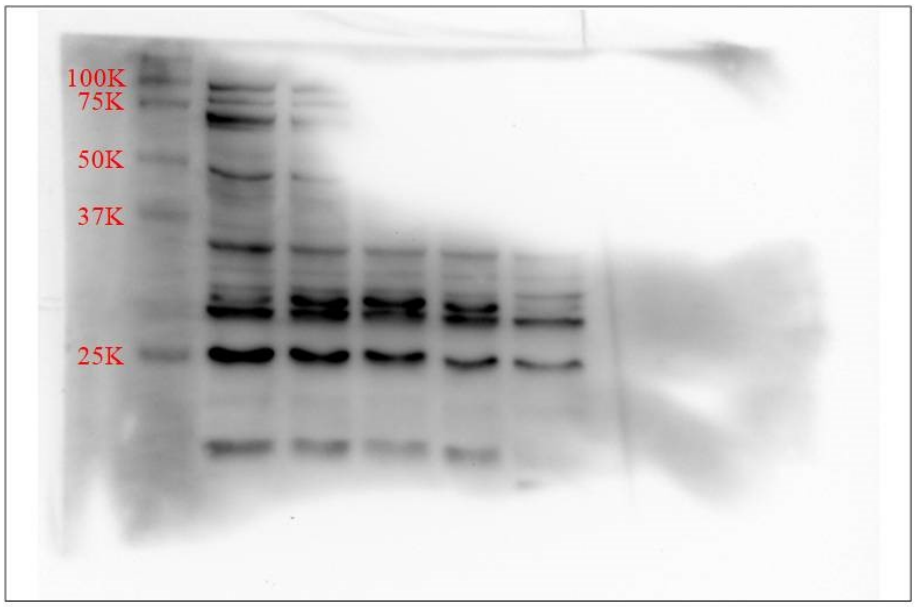


5. Raw images of Western blot



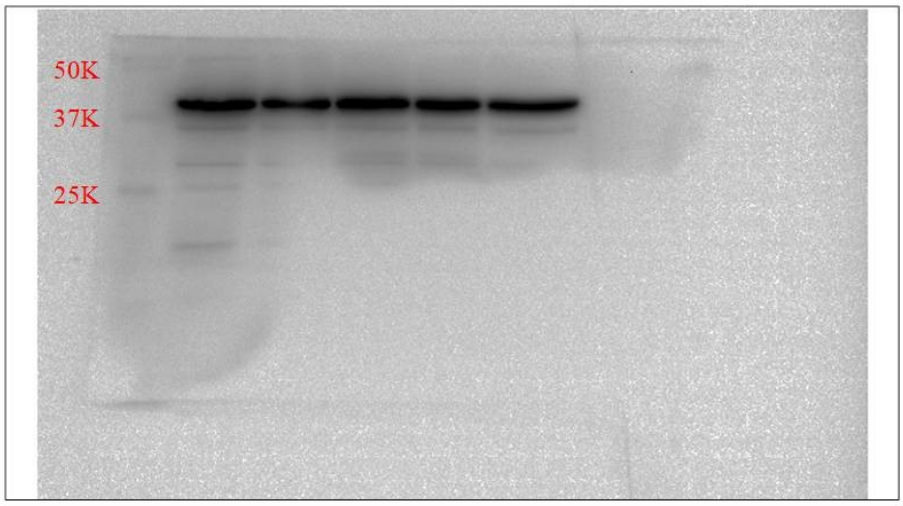
Bax on SDS PAGE gel, Molecular Weight-23KDa

The molecular marker used is Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards by Bio-Rad
Catalog # 161 0375



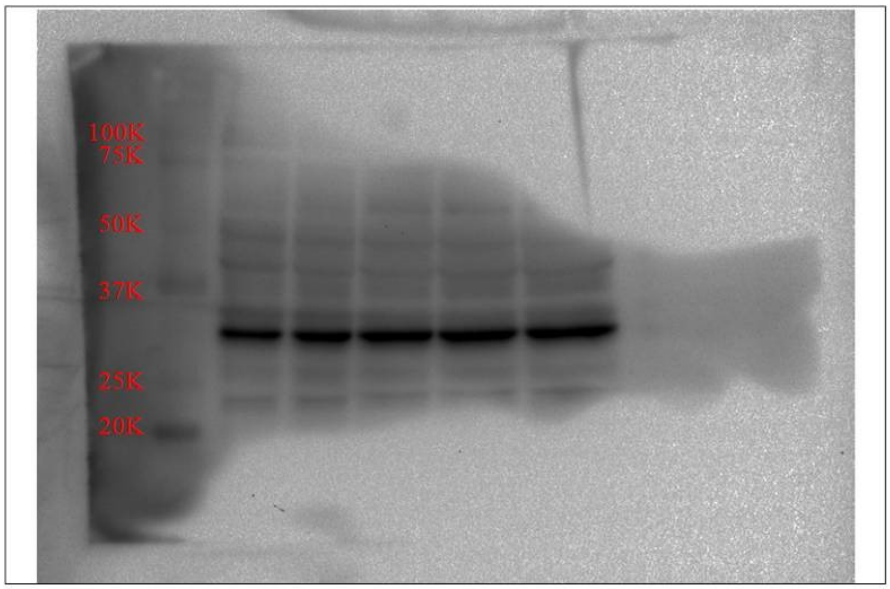
Bcl2 on SDS PAGE gel, Molecular Weight-26KDa

The molecular marker used is Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards by Bio-Rad
Catalog # 161 0375



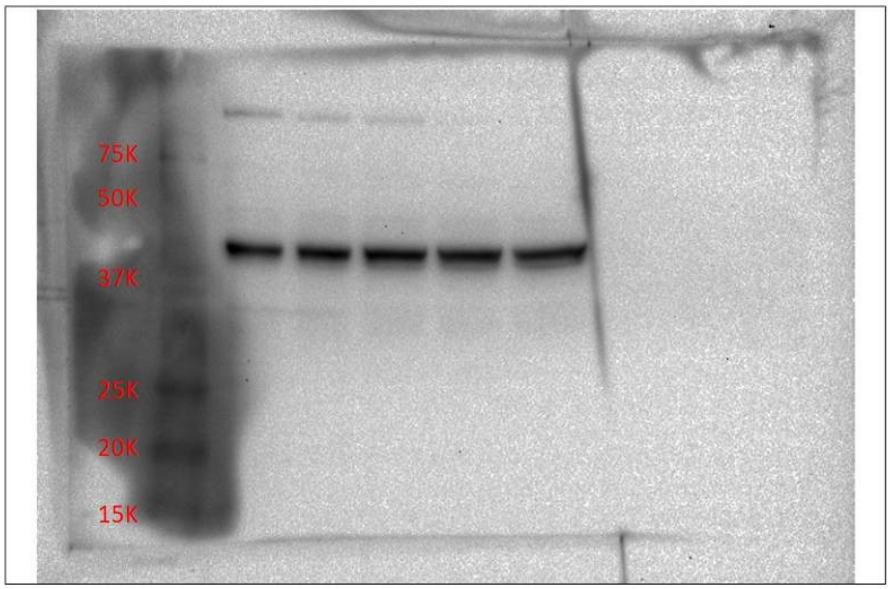
β-Actin (Bax and Bcl-2) on SDS PAGE gel, Molecular Weight-42KDa

The molecular marker used is Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards by Bio-Rad
Catalog # 161 0375



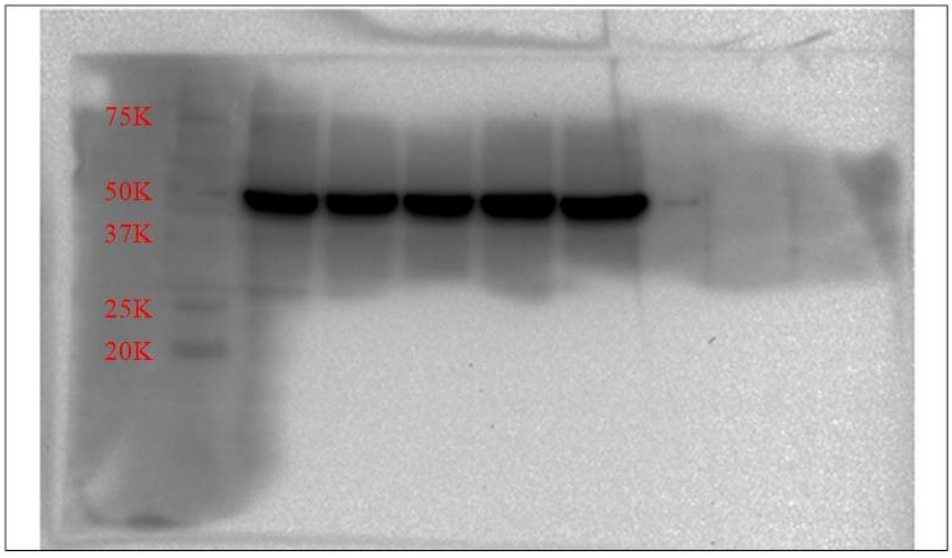
Caspase 3 on SDS PAGE gel, Molecular Weight-32KDa

The molecular marker used is Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards by Bio-Rad
Catalog # 161 0375



Caspase 9 on SDS PAGE gel, Molecular Weight-46KDa

The molecular marker used is Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards by Bio-Rad
Catalog # 161 0375



β-Actin (Caspase-3 and Caspase-9) on SDS PAGE gel, Molecular Weight-42KDa

The molecular marker used is Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards by Bio-Rad
Catalog # 161 0375