Supplementary Material

Synthesis of ursolic acid arylidene-hydrazide hybrid compounds and investigation of their cytotoxic and antimicrobial effects

Halil Şenol[a]*, Rabia Büşra Şahin[b], Berre Mercümek[b], Halil Burak Kapucu[c], Ebru Hacıosmanoğlu[c], Harika Öykü Dinç[d], Pelin Yüksel Mayda[d]

[a]*Bezmialem Vakif University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34093 Fatih, Istanbul, Turkey;* [b]*Bezmialem Vakif University, Faculty of Pharmacy, 34093 Fatih, Istanbul, Turkey;* [c]*Bezmialem Vakif University, Faculty of Medicine, Department of Biophysics, 34093 Fatih, Istanbul, Turkey;* [d]*Bezmialem Vakif University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 34093 Fatih, Istanbul, Turkey*

* Corresponding author's e-mail: hsenol@bezmialem.edu.tr (Halil Şenol)

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Abstract: In this study, 13 new hybrid compounds **(7a-m)** were synthesized starting from ursolic acid, and their cytotoxic activities were investigated on the BEAS-2B and A549 cell lines. In addition, the synthesized compounds were tested against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* to determine their anti-microbial properties. The hybrid compounds that exhibited the lowest cytotoxicity against the BEAS-2B were **7k, 7b,** and **7g**. The cytotoxicity of the compounds against A549 was evaluated, the IC_{50} value of **7k**, **7b,** and **7g** are found as 0.15 μM, 0.31 μM, and 0.26 μM, respectively. The results showed that the selectivity of **7k** was 7 times higher than doxorubicin against the A549 cells. According to the antimicrobial activity studies **7c** is found as the most effective compound against *S. aureus.* Almost all compounds showed a similar inhibition potential against *E. coli and C. albicans.*

Keywords: Ursolic Acid, Arylidene-hydrazide, Hybrid Molecules, Cytotoxicity, Antimicrobial, A549, BEAS-2B

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1. Experimental Section

1.1. General

All solvents, chemicals, and other supplies used in the experiments were purchased from Sigma Aldrich, Merck, TCI Chemicals, and other suppliers. Although commercially available chemicals and solvents have high purity, purification procedures were performed as described in the literature, when necessary (Perrin and Armarego 1993; Senol Halil et al. 2016; Bayrak et al. 2018).

In general, column chromatography was used in the chromatographic separations. Silica gel was used as the stationary phase, and a mixture of ethyl acetate and hexane was used as the mobile phase. The experiments and column chromatographies were monitored by thin-layer chromatography (TLC), and detection of spots was conducted using UV light, cerium(IV)sulfate solution 10% in sulfuric acid, and heating in stove at 100 °C. Nuclear magnetic resonance (NMR) analyses (^{1}H-NMR and ^{13}C-APT NMR) were used for determination of chemical structures. HRMS analyses were performed for determination of molecular weight. HPLC analysis were used for determination of purity.

Melting points were determined by Stuart SMP30 melting point apparatus. ^{1}H-NMR and ^{13}CAPT NMR spectra were recorded by Bruker Avance NEO NMR Spectrometer at 500 and 125 MHz, respectively. Coupling constant values were given in Hertz (Hz). Chemical shifts were reported in δ (parts per million) units relative to the internal standard tetramethylsilane (δ = 0.00 ppm) and the peak splits were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), dd (doublet of doublet) and dt (doublet of triplet). HRMS spectra were recorded using ESI technique by Thermo Fischer Scientific Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. HPLC chromatograms were recorded using the Waters preparative HPLC and PDA detector.

1.2. Syntheses

1.2.1. Synthesis of methyl derivatives of ursolic acid (4a and 4b)

A round-bottomed flask was charged with freshly distilled tetrahydrofuran (THF) and sodium hydride (NaH) (5.25 g, 130 mmol, 3 equiv.). Ursolic acid (20 g, 44 mmol, 1 equiv.) was added and stirred for 30

minutes at room temperature. Methyl iodide (MeI) (3.5 mL, 55 mmol, 1.25 equiv.) was added and the resulting mixture was stirred overnight under an inert atmosphere. According to TLC analysis after completion of the reaction, the excess sodium hydride was carefully quenched with water (15 mL). The reaction solvent was removed under reduced pressure. The residue was washed with water (3x300 mL) and extracted with chloroform (3x300 mL). Organic layers were combined, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and residue was adsorbed on silica gel. The products were purified by silica gel column chromatography using an ethyl acetate and hexane mixture (1:9). While dimethyl derivative of ursolic acid **(4a)** was firstly eluted from the column (white solid, 2.75 g, 13% yield), the 3-methyl-ursolic acid **(4b)** (white solid, 17 g, 80% yield) was secondly eluted.

*Compound **4a**:* ^{1}H NMR (500 MHz, $CDCl_3$), δ 5.18 (t, *J* = 3.61, Hz, 1H), 3.54 (s, 3H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.72, 4.32 Hz, 1H), 2.16 (d, *J* = 11.07 Hz, 1H), 1.93 (dt, *J* = 13.42, 4.54 Hz, 1H), 1.84 (dd, *J* = 8.86, 3.64 Hz, 2H), 1.05 (s, 3H), 0.96 (s, 3H), 0.91 (d, *J* = 6.19 Hz, 3H), 0.89 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$), δ 177.9, 138.0, 125.5, 88.5, 57.4, 55.6, 52.7, 51.4, 47.9, 41.8, 39.4, 38.9, 38.7, 38.5, 38.4, 36.8, 36.5, 32.8, 30.5, 30.2, 28.0, 28.9, 24.1, 23.5, 23.2, 21.9, 21.1, 18.1, 16.9, 16.8, 16.3, 15.3; ESI-HRMS: Formula: $C_{32}H_{52}O_3$; Exact mass: 484.39165; Calculated m/z $[M+H]^+$: 485.39947; Found m/z $[M+H]^+$: 485.39990; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 21.17 min, 96.0%.

*Compound **4b**:* m.p.: 212 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.16 (t, *J* = 3.7 Hz, 1H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 2.11 (dd, *J* = 11.10, 1.80 Hz, 1H), 1.92 (dt, *J* = 13.40, 4.30 Hz, 1H), 1.84 (dd, *J* = 8.90, 3.80 Hz, 2H), 1.00 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.79 (d, *J* = 6.50 Hz, 3H), 0.69 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.1, 136.9, 124.8, 87.8, 56.5, 54.8, 51.5, 46.9, 46.5, 40.9, 38.5, 38.0, 37.8, 37.7, 37.5, 36.0, 35.7, 31.9, 29.6, 27.1, 27.0, 23.0, 22.6, 22.3, 21.0, 20.2, 17.1, 16.1, 16.0, 15.3, 14.4; ESI-HRMS: Formula: $C_{31}H_{50}O_3$; Exact mass: 470.37600; Calculated m/z $[M+H]^+$: 471.38382; Found m/z $[M+H]^+$: 471.38281; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 12.57 min, 97.7%.

1.2.2. Synthesis of acyl chloride of 3-methyl-ursolic acid (5)

A round-bottomed flask was charged with DCM (250 mL) and **4b** (10 g, 21 mmol, 1 equiv.) Oxalyl chloride (3.64 mL, 43 mmol, 2 equiv.) was added in an inert atmosphere and stirred at room temperature

overnight. According to the ^{13}C NMR analysis of the reaction mixture, the carboxylic acid group was completely converted into acyl chloride. The reaction solvent and excess oxalyl chloride were removed under reduced pressure. The desired product **(5)** was obtained as pure (white solid, quantitative yield).

*Compound **5**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.23 (t, *J* = 3.7 Hz, 1H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 2.15 (dd, *J* = 11.30, 1.90 Hz, 1H), 2.04 (d, *J* = 4.40 Hz, 0H), 1.91 – 1.85 (m, 2H), 1.03 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H), 0.81 (d, *J* = 6.50 Hz, 3H), 0.75 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.5, 136.6, 127.2, 88.7, 58.9, 57.6, 55.8, 54.1, 47.6, 42.1, 39.8, 39.6, 38.7, 38.6, 38.6, 37.0, 35.4, 33.0, 30.5, 28.2, 27.8, 25.4, 23.5, 23.4, 22.1, 21.0, 18.2, 17.0, 16.4, 15.5.

1.2.3. Synthesis of hydrazide of 3-methyl ursolic acid (6)

A round-bottomed flask was charged with DCM (250 mL) and compound **5** (6 g, 12 mmol, 1 equiv.). Hydrazine hydrate (1 mL, 24 mmol, 2 equiv.) was added and stirred for overnight at room temperature. After completion of the reaction, mixture was washed with water (3x100 mL) and extracted with DCM (3x150 mL). Organic layers were combined, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and residue adsorbed on silica gel. The products were purified by silica gel column chromatography using an ethyl acetate and hexane mixture (1:9). The hydrazide of 3-methylursolic acid **(6)** was obtained as white solid (8 g, 80% yield).

*Compound **6**:* m.p.: 120 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (s, 1H), 5.28 (t, *J* = 3.70 Hz, 1H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.03 (s, 3H), 0.89 (d, *J* = 9.00 Hz, 6H), 0.85 (s, 3H), 0.80 (d, *J* = 6.40 Hz, 3H), 0.70 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.2, 139.8, 126.3, 88.6, 57.6, 55.7, 52.9, 47.5, 47.3, 42.3, 39.6, 39.1, 38.7, 38.5, 37.0, 36.9, 32.5, 30.7, 28.1, 27.8, 24.9, 23.4, 23.4, 22.0, 21.2, 18.1, 17.2, 16.6, 16.3, 15.4; ESI-HRMS: Formula: $C_{31}H_{52}N_2O_2$; Exact mass: 484.40288; Calculated m/z $[M+H]^+$: 485.41070; found m/z $[M+H]^+$: 485.40878; calculated m/z $[M+Na]^+$: 507.39265; found m/z $[M+Na]^+$: 507.39075; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 6.53 min, 99.8%.

1.2.4. General synthesis of triterpene arylidene-hydrazide hybrid compounds (7a-m)

A round-bottomed flask was charged with acetonitrile-chloroform (1:1) mixture (50 mL) and compound **6** (500 mg, 10 mmol, 1 equiv.) was added and stirred until dissolved. Corresponding benzaldehyde derivative (12 mmol, 1.25 equiv.) was added and stirred at reflux temperature in the presence of acetic acid (0.5 mL)

in an inert atmosphere for overnight. The reaction was terminated according to TLC analysis. The reaction solvent was removed under reduced pressure and residue adsorbed on silica gel. The hybrid compounds (**7a-m**) were purified by silica gel column chromatography using ethyl acetate hexane (1:9) mixture as mobile phase.

*Compound **7a:*** This compound was synthesized using benzaldehyde (70 mg) according to the general synthesis procedure of hybrid compounds (white solid, 250 mg, 84% yield). m.p.: 270 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.03 (s, 1H), 7.62 (dd, *J* = 6.70, 2.90 Hz, 2H), 7.34 – 7.22 (m, 3H), 5.43 (t, *J* = 3.50 Hz, 1H), 3.27 (s, 3H), 2.58 (dd, *J* = 11.60, 4.40 Hz, 1H), 1.05 (s, 3H), 0.89 (d, *J* = 3.5 Hz, 6H), 0.83 (d, *J* = 6.30 Hz, 3H), 0.80 (s, 3H), 0.66 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 147.4, 140.3, 133.9, 130.2, 128.6, 128.5, 127.7, 126.3, 88.6, 57.6, 55.7, 53.4, 47.8, 47.5, 42.6, 39.7, 39.0, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.9, 25.1, 23.6, 23.4, 22.0, 21.2, 18.1, 17.3, 17.0, 16.4, 15.5; ESI-HRMS: Formula: $C_{38}H_{56}N_2O_2$; Exact mass: 572.43418; calculated m/z $[M+H]^+$: 573.44200; found m/z $[M+H]^+$: 573.43958; HPLC-PDA: λ 254 nm, MeCN:MeOH (1:1), Rt: 10.98 min, 99.7%.

*Compound **7b:*** This compound was synthesized using 4-methylbenzaldehyde (78 mg) according to the general synthesis procedure of hybrid compounds (white solid, 280 mg, 92% yield). m.p: 219 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.96 (s, 1H), 7.53 (d, *J* = 7.80 Hz, 2H), 7.09 (d, *J* = 7.90 Hz, 2H), 5.42 (t, *J* = 3.60 Hz, 1H), 3.27 (s, 3H), 2.58 (dd, *J* = 11.60, 4.40 Hz, 1H), 2.27 (s, 3H), 1.05 (s, 3H), 0.89 (d, *J* = 3.3 Hz, 3H), 0.83 (d, *J* = 6.4 Hz, 3H), 0.80 (s, 3H), 0.66 (s, 3H), 0.66 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 147.4, 140.4, 131.1, 129.3, 129.3, 127.7, 126.3, 88.6, 57.6, 55.7, 53.5, 47.8, 47.5, 42.6, 39.8, 39.7, 39.0, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.9, 25.1, 23.6, 23.3, 22.0, 21.5, 21.2, 18.1, 17.3, 17.0, 16.3, 15.5; ESI-HRMS: Formula: $C_{39}H_{58}N_2O_2$; Exact mass: 586.44983; calculated m/z $[M+H]^+$: 587.45765; found m/z $[M+H]^+$: 587.45471; calculated m/z $[M+Na]^+$: 609.43960; found m/z $[M+Na]^+$: 609.43683; HPLC-PDA: λ 254 nm, MeCN:MeOH (1:1), Rt: 14.97 min, 99.9%.

*Compound **7c:*** This compound was synthesized using 4-chlorobenzaldehyde (90 mg) according to the general synthesis procedure of hybrid compounds (white solid, 275 mg, 8%7 yield). m.p.: 175 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.02 (s, 1H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.29 – 7.23 (m, 2H), 7.20 (s, 1H), 5.42 (t, *J* = 3.50 Hz, 1H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.60, 4.30 Hz, 1H), 1.06 (s, 3H), 0.89 (d, *J*

= 4.5 Hz, 6H), 0.83 (d, *J* = 6.4 Hz, 3H), 0.81 (s, 3H), 0.67 (s, 3H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8, 146.1, 140.4, 136.2, 132.4, 128.9, 128.8, 126.4, 88.6, 77.3, 77.1, 76.8, 57.6, 55.6, 53.5, 48.0, 47.5, 42.6, 39.7, 39.7, 39.0, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.8, 25.1, 23.6, 23.3, 22.0, 21.2, 18.1, 17.3, 17.0, 16.3, 15.5; ESI-HRMS**:** Formula: $C_{38}H_{55}{}^{35}ClN_2O_2$; Exact mass: 606.39521; calculated m/z $[M+H]^+$: 607.40303; found m/z $[M+H]^+$: 607.40057; HPLC-PDA: λ 300 nm, MeCN:MeOH (1:1), Rt: 6.11 min, 99.2%.

*Compound **7d:*** This compound was synthesized using 4-bromobenzaldehyde (120 mg) according to the general synthesis procedure of hybrid compounds (white solid, 295 mg, 87% yield). m.p: 190 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.00 (s, 1H), 7.50 (d, *J* = 8.20 Hz, 2H), 7.42 (d, *J* = 8.60 Hz, 2H), 5.42 (t, *J* = 3.60 Hz, 1H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.06 (s, 3H), 0.89 (s, 6H), 0.84 (d, *J* = 6.50 Hz, 3H), 0.82 (s, 3H), 0.67 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 146.1, 140.5, 132.9, 131.9, 129.0, 126.4, 124.5, 88.6, 57.6, 55.7, 53.5, 48.0, 47.5, 42.6, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.8, 25.1, 23.6, 23.3, 22.0, 21.2, 18.1, 17.3, 17.0, 16.3, 15.5; ESI-HRMS: Formula: $C_{38}H_{55}{}^{81}BrN_2O_2$; Exact mass: 650.34469; calculated m/z $[M+H]^+$: 653.34660; found m/z $[M+H]^+$: 653.34821; HPLC-PDA: λ 254, MeCN:MeOH (1:1), Rt: 17.27 min, 98.8%.

*Compound **7e:*** This compound was synthesized using 4-fluorobenzaldehit (80 mg) according to the general synthesis procedure of hybrid compounds (white solid, 250 mg, 82% yield). m.p.: 260 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.01 – 6.92 (m, 2H), 5.42 (t, *J* = 3.60 Hz, 1H), 3.28 (s, 3H), 2.58 (dd, *J* = 11.70, 4.20 Hz, 1H), 1.06 (s, 3H), 0.89 (s, 6H), 0.83 (d, *J* = 6.50 Hz, 3H), 0.81 (s, 3H), 0.67 (d, *J* = 2.10 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 165.0, 163.0, 146.2, 140.4, 140.4, 130.2, 130.1, 129.5, 129.5, 126.3, 115.8, 115.7, 88.6, 57.6, 55.6, 53.5, 47.9, 47.5, 42.6, 39.7, 39.7, 39.0, 38.7, 38.6, 36.9, 36.9, 32.6, 30.8, 29.7, 28.2, 27.8, 25.1, 23.6, 23.3, 22.0, 21.2, 18.1, 17.3, 17.0, 16.3, 15.5; ^{19}F NMR (471 MHz, $CDCl_3$) δ -109.6; ESI-HRMS: Formula: $C_{38}H_{55}FN_2O_2$; Exact mass: 590.42476; calculated m/z $[M+H]^+$: 591.43258; found m/z $[M+H]^+$: 591.43048; calculated m/z $[M+Na]^+$: 613.41453; found m/z $[M+Na]^+$: 613.41241; HPLC-PDA: λ 254 nm, MeCN:MeOH (1:1), Rt: 11.21 min, 99.6 %.

*Compound **7f:*** This compound was synthesized using 3-nitrobenzaldehyde (155 mg) according to the general synthesis procedure of hybrid compounds (white solid, 310 mg, 97% yield). m.p.: 305 ºC; ^{1}H

NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.38 (t, *J* = 1.90 Hz, 1H), 8.28 (s, 1H), 8.14 (dd, *J* = 8.30, 2.30 Hz, 1H), 8.05 (d, *J* = 7.80 Hz, 1H), 7.49 (t, *J* = 8.00 Hz, 1H), 5.45 (t, *J* = 3.7 Hz, 1H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.8, 4.2 Hz, 1H), 1.07 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.85 (d, *J* = 6.4 Hz, 3H), 0.83 (s, 3H), 0.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.2, 148.6, 144.9, 140.4, 136.0, 132.8, 129.7, 126.5, 124.6, 122.5, 88.6, 57.6, 55.6, 53.5, 48.2, 47.5, 42.6, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.8, 25.2, 23.6, 23.4, 22.0, 21.2, 18.1, 17.3, 17.0, 16.3, 15.5; ESI-HRMS: Formula: $C_{38}H_{55}N_3O_4$; Exact mass: 617.41926; calculated m/z $[M+H]^+$: 618.42708; found m/z $[M+H]^+$: 618.42426; calculated m/z $[M+Na]^+$: 640.40903; found m/z $[M+Na]^+$: 640.40662; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 4.94 min, 99.8%.

*Compound **7g**:* This compound was synthesized using 4-nitrobenzaldehyde (97 mg) according to the general synthesis procedure of hybrid compounds (white solid, 270 mg, 84% yield). m.p.: 165 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.24 (s, 1H), 8.13 (d, *J* = 8.60 Hz, 2H), 7.78 (d, *J* = 8.50 Hz, 2H), 5.44 (t, *J* = 3.50 Hz, 1H), 3.28 (s, 3H), 2.58 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.06 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.84 (d, *J* = 6.50 Hz, 3H), 0.81 (s, 3H), 0.66 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 148.5, 144.6, 140.2, 128.1, 126.5, 123.9, 88.5, 57.6, 55.6, 53.4, 48.2, 47.5, 42.5, 39.7, 39.6, 39.0, 38.7, 38.5, 36.9, 32.6, 30.8, 28.1, 27.8, 25.1, 23.6, 23.4, 22.0, 21.2, 18.1, 17.2, 17.0, 16.3, 15.5; HRMS: Formula: $C_{38}H_{55}N_3O_4$; Exact mass: 617.41926; calculated m/z $[M+H]^+$: 618.42708; found m/z $[M+H]^+$: 618.42487; HPLC-PDA: λ 366 nm, MeCN:MeOH (1:1), Rt: 5.08 min, 99.9%.

*Compound **7h:*** This compound was synthesized using 4-dimethylamino benzaldehyde (150 mg) according to the general synthesis procedure of hybrid compounds (yellow solid, 298 mg, 93% yield). m.p.: 290 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (s, 1H), 7.80 (s, 1H), 7.52 (d, *J* = 8.50 Hz, 2H), 6.58 (d, *J* = 8.50 Hz, 2H), 5.42 (t, *J* = 3.50 Hz, 1H), 3.28 (s, 3H), 2.92 (s, 6H), 2.58 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.05 (s, 3H), 0.89 (s, 3H), 0.83 (d, *J* = 6.50 Hz, 3H), 0.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 151.8, 147.9, 140.6, 129.2, 126.2, 121.4, 111.6, 88.6, 57.6, 55.7, 53.6, 47.7, 47.6, 40.2, 39.8, 39.7, 39.1, 38.7, 38.6, 37.0, 32.6, 30.9, 28.2, 27.8, 25.1, 23.6, 23.3, 22.0, 21.3, 18.1, 17.3, 17.1, 16.4, 15.5; HRMS: Formula: $C_{40}H_{61}N_3O_2$; Exact mass: 615.47638; calculated m/z $[M+H]^+$: 615.47638; found m/z $[M+H]^+$:

616.48236; calculated m/z $[M+Na]^+$: 638.46615; found m/z $[M+Na]^+$: 638.46405; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 5.43 min, 99.9%.

*Compound **7i**:* This compound was synthesized using 2-methoxybenzaldehyde (88 mg) according to the general synthesis procedure of hybrid compounds (white solid, 245 mg, 78% yield). m.p.: 220 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.28 (s, 1H), 7.97 (dd, *J* = 7.80, 1.8 Hz, 1H), 6.88 (t, *J* = 7.50 Hz, 1H), 6.80 (d, *J* = 8.30 Hz, 1H), 5.46 (t, *J* = 3.60 Hz, 1H), 3.78 (s, 3H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.06 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.83 (d, *J* = 1.70 Hz, 6H), 0.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 157.9, 143.0, 140.7, 131.5, 127.3, 126.4, 122.2, 120.9, 110.8, 88.6, 57.6, 55.7, 55.6, 53.6, 47.7, 47.5, 42.6, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 36.8, 32.6, 30.8, 28.2, 27.8, 25.2, 23.6, 23.3, 22.0, 21.2, 18.1, 17.3, 17.0, 16.3, 15.4; ESI-HRMS: Formula: $C_{39}H_{58}N_2O_3$; Exact mass: 602.44474; calculated m/z $[M+H]^+$: 603.45257; found m/z $[M+H]^+$: 603.45026; calculated m/z $[M+Na]^+$: 625.43451; found m/z $[M+Na]^+$: 625.43219; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 5.29 min, 99.9%.

*Compound **7j**:* This compound was synthesized using 4-methoxybenzaldehyde (88 mg) according to the general synthesis procedure of hybrid compounds (white solid, 265 mg, 85% yield). m.p.: 180 ^oC; ^1H NMR (500 MHz, $CDCl_3CDCl_3$) δ 8.98 (s, 1H), 7.92 (s, 1H), 7.58 (d, *J* = 8.40 Hz, 2H), 6.87 – 6.72 (m, 2H), 5.42 (t, *J* = 3.50 Hz, 1H), 3.74 (s, 3H), 3.28 (s, 3H), 2.58 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.05 (s, 3H), 0.89 (s, 3H), 0.83 (d, *J* = 6.40 Hz, 3H), 0.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 161.4, 147.1, 140.5, 129.3, 126.5, 126.3, 114.1, 88.6, 57.6, 55.7, 55.4, 53.5, 47.8, 47.5, 42.6, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.8, 25.1, 23.6, 23.3, 22.0, 21.2, 18.1, 17.3, 17.0, 16.3, 15.5; HRMS: Formula: $C_{39}H_{58}N_2O_3$; Exact mass: 602.44474; calculated m/z $[M+H]^+$: 603.45257; found m/z $[M+H]^+$: 603.45038; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 4.95 min, 98.5 %.

*Compound **7k**:* This compound was synthesized using 2,4-dimethoxy benzaldehyde (107 mg) according to the general synthesis procedure of hybrid compounds (white solid, 280 mg, 85% yield). m.p.: 235 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.16 (s, 1H), 7.93 (d, *J* = 8.70 Hz, 1H), 7.19 (s, 1H), 6.44 (dd, *J* = 8.70, 2.30 Hz, 1H), 6.34 (d, *J* = 2.30 Hz, 1H), 5.45 (d, *J* = 3.80 Hz, 1H), 3.76 (d, *J* = 3.80 Hz, 6H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.06 (s, 3H), 0.90 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.68 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 162.9, 159.3, 143.0, 140.7, 128.6, 126.4, 115.2, 105.4,

98.1, 88.6, 57.6, 55.7, 55.6, 55.5, 53.7, 47.7, 47.6, 42.6, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 36.9, 32.7, 30.9, 28.2, 27.8, 25.2, 23.6, 23.3, 22.1, 21.2, 18.1, 17.3, 17.1, 16.3, 15.4; ESI-HRMS: Formula: $C_{40}H_{60}N_2O_4$; Exact mass: 632.45531; calculated m/z $[M+H]^+$: 633.46313; found m/z $[M+H]^+$: 633.46051; calculated m/z $[M+Na]^+$: 655.44508; found m/z $[M+Na]^+$: 655.44275; HPLC-PDA: λ 300 nm, MeCN:MeOH (1:1), Rt: 5.05 min, 99.7%.

*Compound **7l**:* This compound was synthesized using 3,5-dimethoxy benzaldehyde (107 mg) according to the general synthesis procedure of hybrid compounds (white solid, 290 mg, 88% yield). m.p.: 295 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (s, 1H), 7.89 (s, 1H), 6.78 (d, *J* = 2.40 Hz, 2H), 6.41 (t, *J* = 2.30 Hz, 1H), 5.43 (t, *J* = 3.50 Hz, 1H), 3.73 (s, 6H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.70, 4.30 Hz, 1H), 1.06 (s, 3H), 0.89 (s, 3H), 0.84 (d, *J* = 6.40 Hz, 3H), 0.82 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 160.9, 147.3, 140.5, 135.8, 126.4, 105.4, 103.2, 88.6, 60.4, 57.5, 55.7, 55.5, 53.6, 47.9, 47.5, 42.6, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 32.6, 31.6, 30.8, 28.1, 27.8, 25.1, 23.6, 23.3, 22.7, 22.0, 21.2, 21.0, 18.1, 17.3, 17.0, 16.3, 15,5, 14.2, 14.1; ESI-HRMS: Formula: $C_{40}H_{60}N_2O_4$; Exact mass: 632.45531; calculated m/z $[M-H]^+$: 631.44748; found m/z $[M-H]^+$: 631.44946; HPLC-PDA: λ max, MeCN:MeOH (1:1), Rt: 5.68 min, 99.7%.

*Compound **7m**:* This compound was synthesized using 4-hydroxy-3-methoxy benzaldehyde (100 mg) according to the general synthesis procedure of hybrid compounds (white solid, 282 mg, 88% yield). m.p.: 274 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.80 (s, 1H), 7.45 (s, 1H), 6.97 – 6.75 (m, 2H), 5.87 (s, 1H), 5.44 (d, *J* = 3.50 Hz, 1H), 3.86 (s, 3H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.70, 4.20 Hz, 1H), 1.06 (s, 3H), 0.90 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 148.2, 147.3, 147.1, 140.7, 126.3, 126.3, 123.7, 113.9, 107.8, 88.6, 57.6, 56.2, 55.7, 53.7, 47.9, 47.5, 42.7, 39.8, 39.7, 39.1, 38.7, 38.6, 36.9, 32.6, 30.8, 28.2, 27.8, 25.2, 23.6, 23.3, 22.0, 21.2, 18.1, 17.3, 17.1, 16.3, 15.5; ESI-HRMS: Formula: $C_{39}H_{58}N_2O_4$; Exact mass: 618.43966; calculated m/z $[M+H]^+$: 619.44748; found m/z $[M+H]^+$: 619.44513; HPLC-PDA: λ 300 nm, MeCN:MeOH (1:1), Rt: 4.05 min, 99.9%.

1.3. Cell culture

BEAS-2B human healthy bronchial epithelial cells and A549 lung cancer cells lines were used in this study. BEAS-2B and A549 were grown in DMEM/F12 and DMEM, respectively, both supplemented with

10% FBS and 100 U/mL of penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2. After reaching 80% confluency, the cells were detached using 0.25% trypsin-EDTA. For further experiments, cells were re-suspended in the growth medium after collection and centrifugation (Meran et al. 2018).

1.4. MTT assay

An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess cell viability. Briefly, 5x103 cells were seeded into flat-bottom 96-well plate with growth medium. After 24 h incubation, it was treated with increasing doses of samples for 24 hours and the assay was conducted. The absorbance values were recorded at 540 nm using microplate reader. All the experiments were carried out in triplicates, and the results were presented as a mean ± standard deviation. The concentration-dependent graph was drawn by comparing the data for each substance whose measurement was repeated at least 3 times, and the relative % cell viability was determined (Haselsberger et al. 1996; Kumar et al. 2018; Meran et al. 2018; Senol H. et al. 2020; Osmaniye et al. 2021).

1.5. Antimicrobial activity studies

1.5.1. Materials

Microorganisms used in the study were obtained from Bezmialem Vakif University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology. In the research; Gram (+) bacteria *Staphylococcus aureus* (ATCC 25923), gram (-) bacteria *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028) yeast were used. Vancomycin and ciprofloxacin were used as antibiotics and amphotericin B was used as antifungals for the control (Chuah et al. 2014).

1.5.2. Resazurin Microplate Test (REMA)

Resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide) microplate method was used to determine the antibacterial and antifungal activities and minimum inhibitory concentrations (MIC) of the compounds. Activities was determined as two replicates. Stock solutions of the tested compounds at a concentration of 1000 µg/mL were sterilized by passing through a 0.22 µm diameter membrane filter. First, 50 µL of Mueller Hinton Broth medium was added to each well. Diluted samples were added to the first well of 96-

well microplates at 1000 µg/mL. 50 µL of control antibiotic and antifungal compound was added to the first well and diluted as a series. Only DMSO was placed on one column of the plate as negative control and 50 µL of standard bacteria and yeast were placed on one column of the plate as positive control and diluted as a series. McFarland suspension with a turbidity value of 0.5 was prepared from the microorganism colonies and then diluted 1:100. 10 µL of the prepared final suspension was added to the plate wells. The plates were covered with parafilm and the bacteria were incubated at 37 °C for 24 hours and yeast for 48 hours. After incubation, 10 µL of 33.75 mg of resazurin dissolved in 5 mL of distilled water and 10 µL of 20% Tween 80 were added to all wells, and the results were evaluated visually after the plates were left to incubate for 2-4 hours. MIC value was determined as the lowest concentration value that prevented colour change from purple to pink .

Tables

Table S1. Cytotoxic effect of hybrid compounds on relative viability of BEAS-2B cells

Conc.	0.1 µM	0.5 µM	1 µM	5 µM	10 µM	IC_{50} µM
Control	100	100	100	100	100	
6	93.96	92.11	84.27	32.16	13.51	2.23
7a	87.72	79.84	65.07	35.05	25.42	1.04
7b	93.54	91.36	90.65	33.31	20.14	2.29
7c	96.30	42.52	39.72	36.55	22.12	0.34
7d	91.47	91.33	89.37	80.80	69.17	1.46
7e	84.01	66.40	66.26	65.35	53.10	0.22
7f	75.50	28.33	26.98	24.28	20.19	0.16
7g	97.07	89.49	80.09	21.14	13.98	1.67
7h	86.11	47.92	44.22	33.69	19.11	0.38
7i	99.23	83.87	69.22	67.60	33.46	1.84
7j	81.14	75.64	74.29	67.73	54.52	0.35
7k	82.08	80.48	78.96	57.63	16.44	2.35
7l	69.29	62.26	55.05	21.94	13.81	0.49
7m	97.68	32.55	30.35	28.68	18.70	0.29
DOX						0.16

Table S2. Cytotoxic effect of hybrid compounds on the relative viability of A549 cells

Conc.	0.1 µM	0.5 µM	1 µM	5 µM	10 µM	IC_{50} µM
Cont.	100	100	100	100	100	
6	93.77	42.94	37.84	26.73	23.95	0.31
7a	80.02	34.63	34.16	32.32	23.65	0.19
7b	97.48	38.40	33.86	33.72	21.11	0.31
7c	99.92	39.57	30.83	30.17	28.36	0.32
7d	94.74	62.12	61.63	58.47	36.58	0.60
7e	82.35	67.74	66.13	64.31	38.69	0.58
7f	65.79	34.55	31.98	29.15	22.87	0.12
7g	91.75	38.25	34.76	33.17	25.00	0.26
7h	73.48	47.92	31.54	31.18	20.65	0.16
7i	79.24	67.24	62.86	61.60	37.95	0.45
7j	82.57	55.09	52.83	52.37	40.17	0.22
7k	82.47	30.10	29.53	29.09	27.59	0.15
7l	69.06	31.38	31.16	31.16	19.71	0.14
7m	90.26	43.32	41.56	35.53	33.58	0.23
DOX*						0.07

Table S3. Antimicrobial effects of synthesized compounds

	MIC values		
Compounds	***C. albicans***	***E.coli***	***S. aureus***
1	125	125	62,5
4b	125	125	62,5
6	125	250	125
7a	125	125	125
7b	125	125	125
7c	125	125	62,5
7d	125	125	125
7e	125	125	250
7f	250	250	250
7g	125	125	125
7h	125	125	125
7i	250	125	125
7j	250	125	125
7k	250	250	125
7l	250	125	250
7m	125	125	125
Amfoterisin B	31.25	-	-
Siprofloksasin	-	31.25	-
Vankomisin	-	-	15.625

Figures

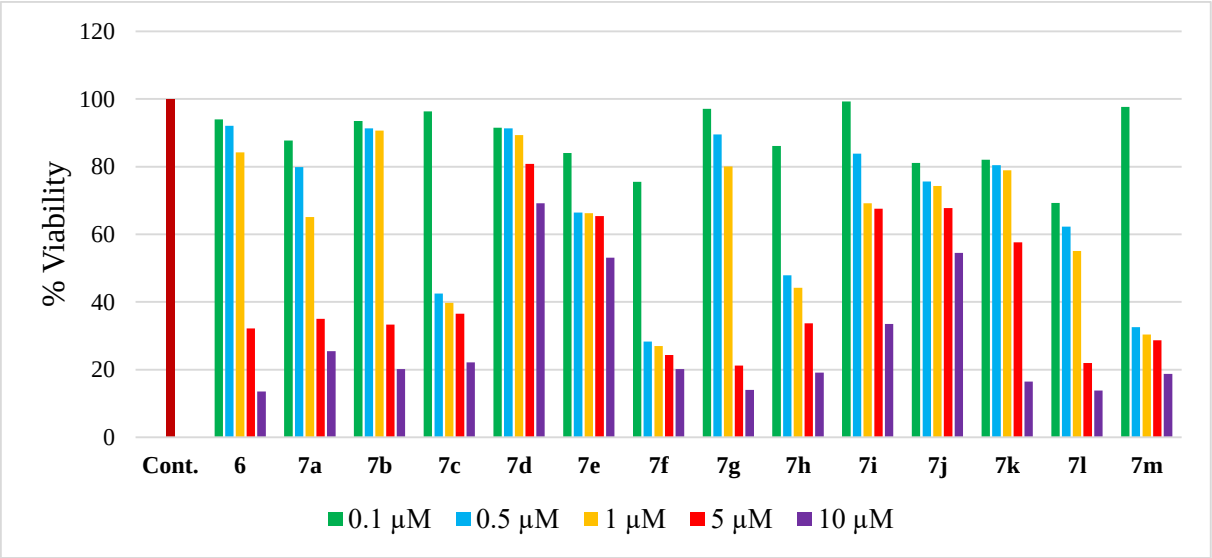


Figure S1: Cytotoxic effects of the hybrid compounds on relative viability of BEAS-2B cells

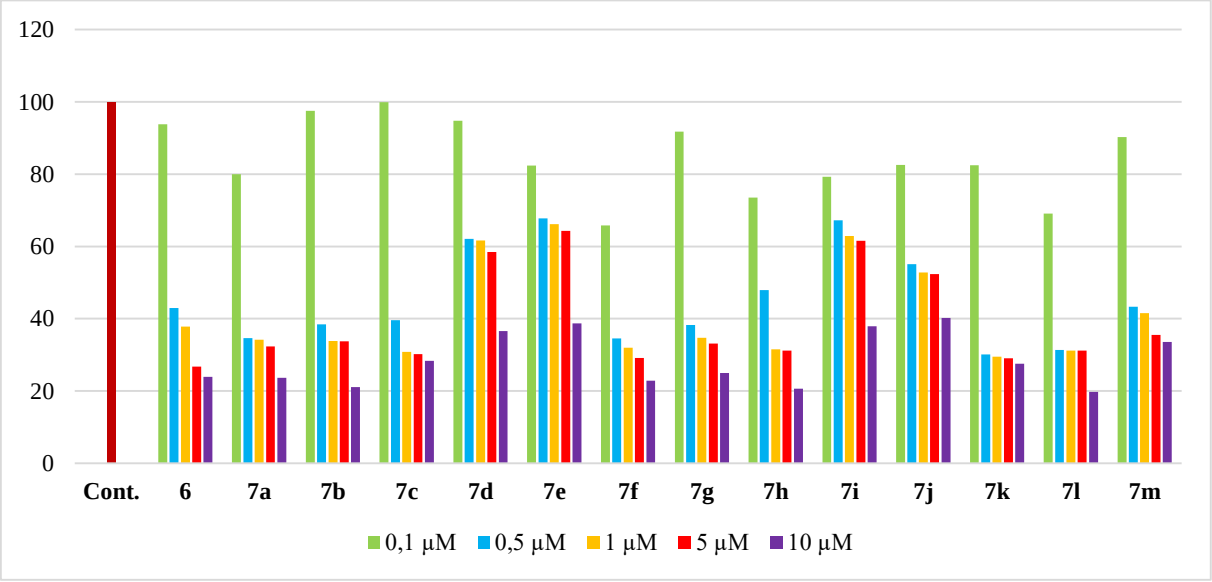


Figure S2: Cytotoxic effects of the hybrid compounds on relative viability of A549 cells

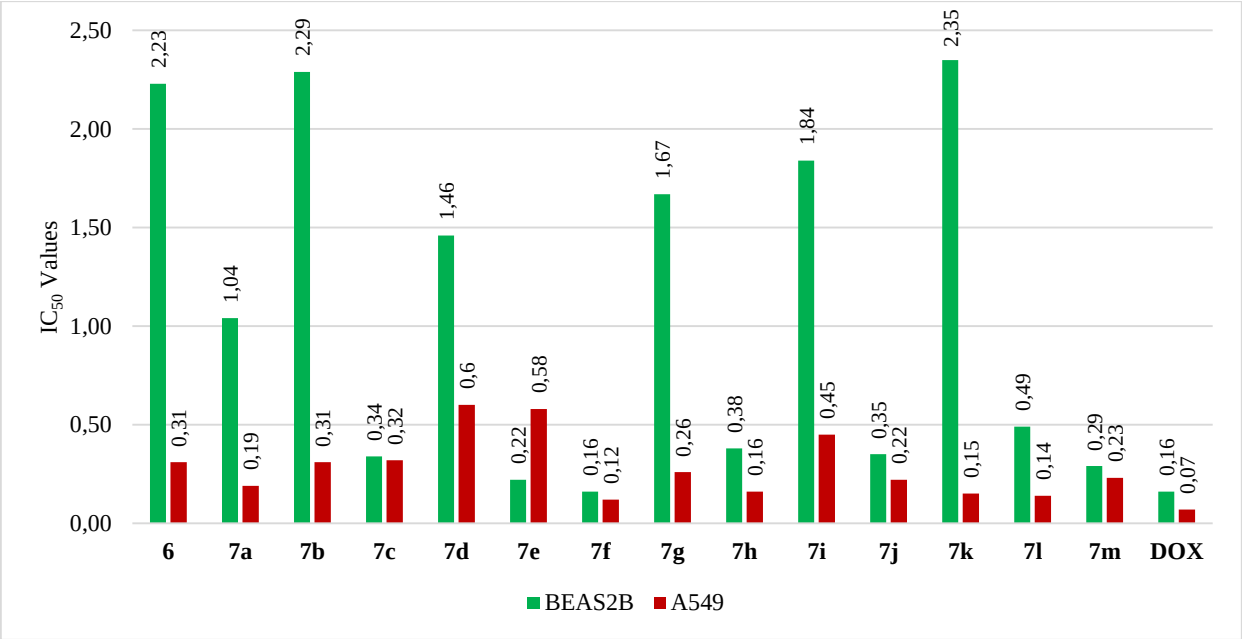


Figure S3: IC_{50} values of hybrid compounds on the BEAS-2B and A549 cell lines

NMR, HRMS, and HPLC Spectra

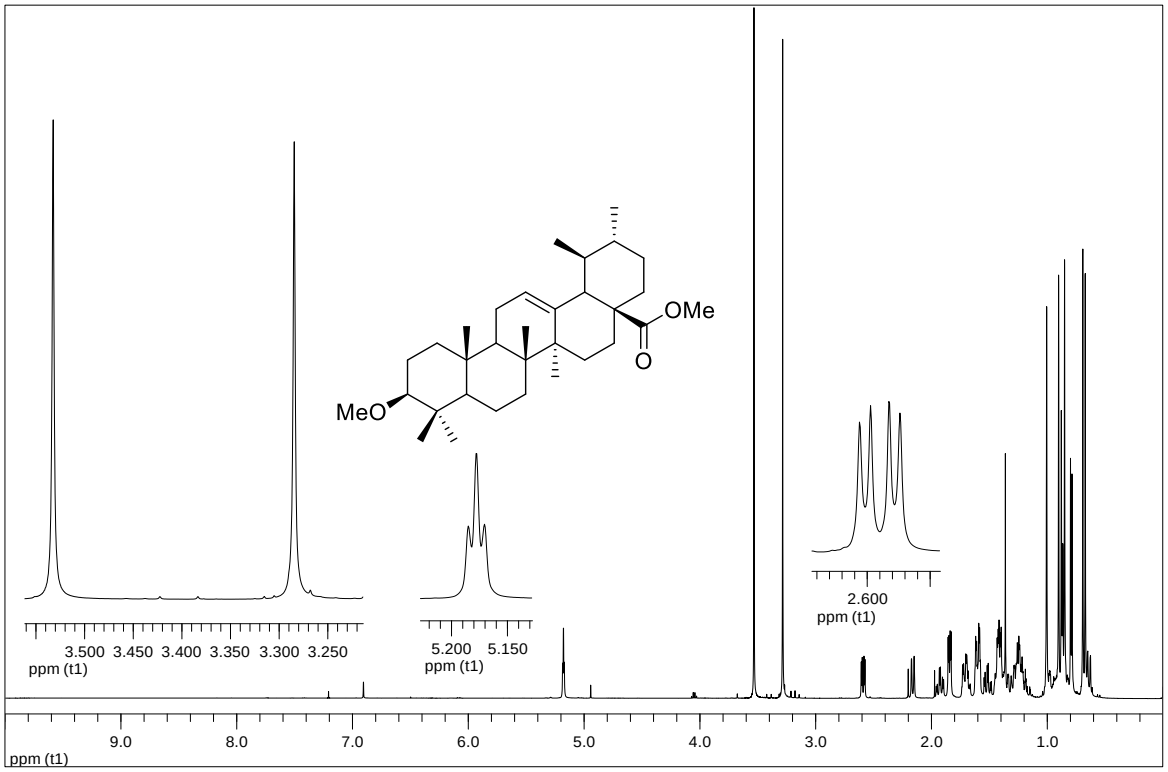


Figure S4: ^{1}H NMR spectrum of **4a** ($CDCl_3$, 500 MHz)

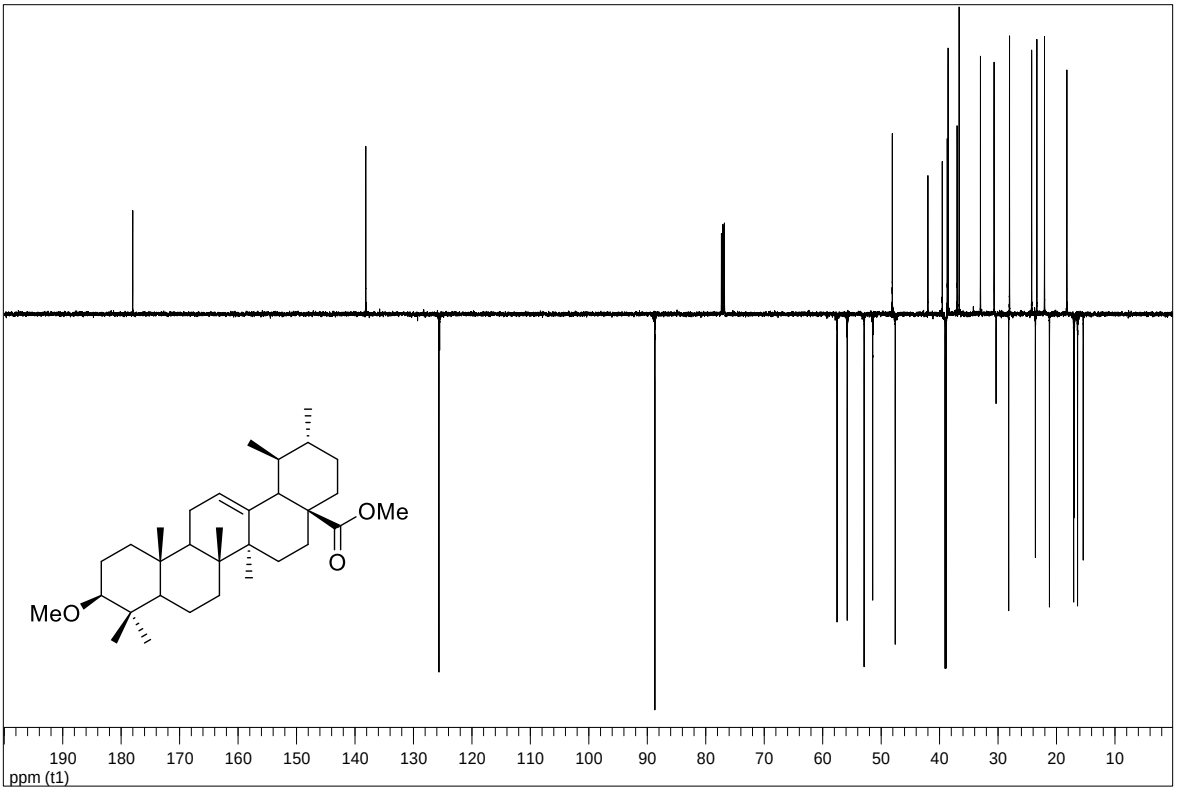


Figure S5: ^{13}C APT NMR spectrum of **4a** ($CDCl_3$, 125 MHz)

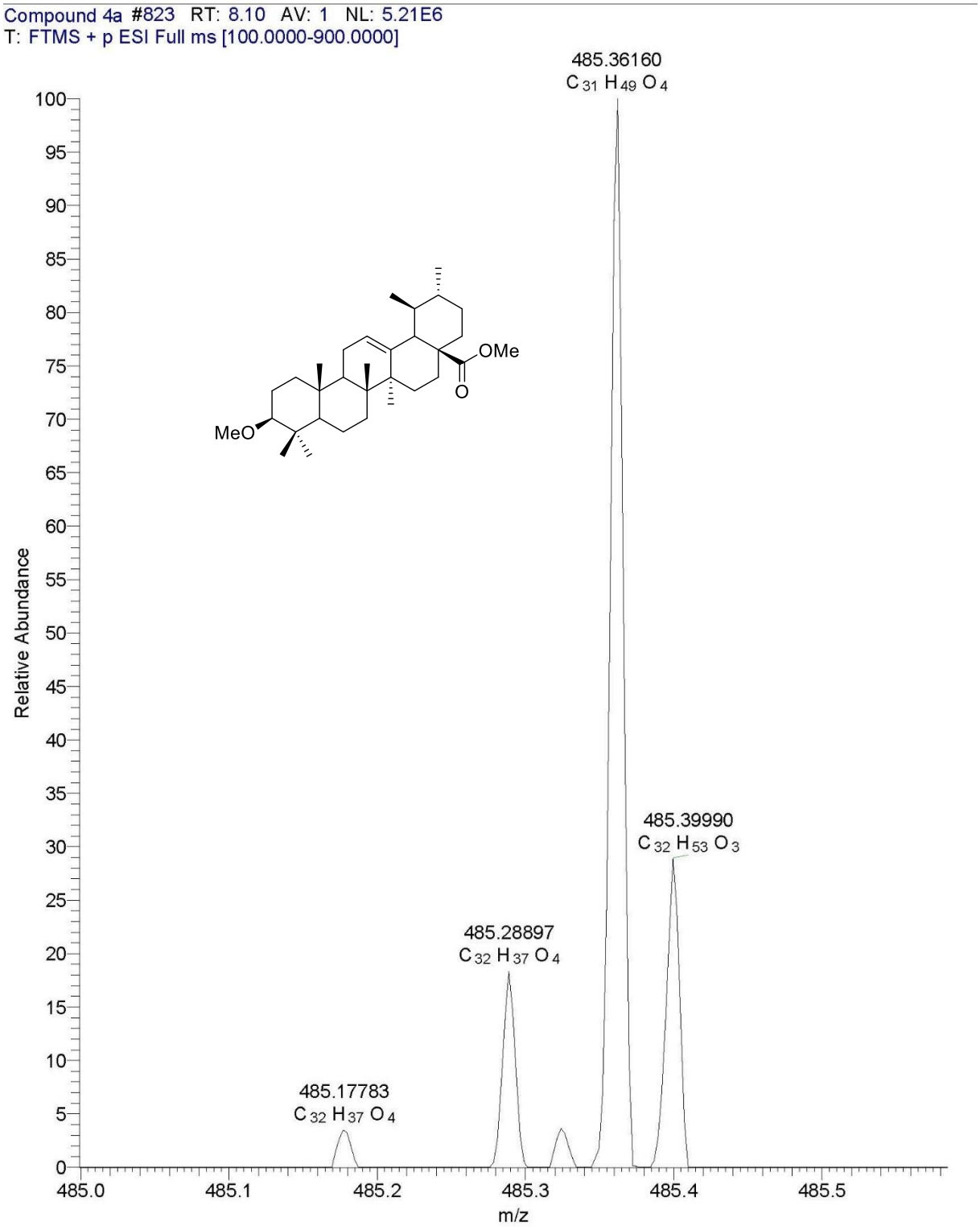
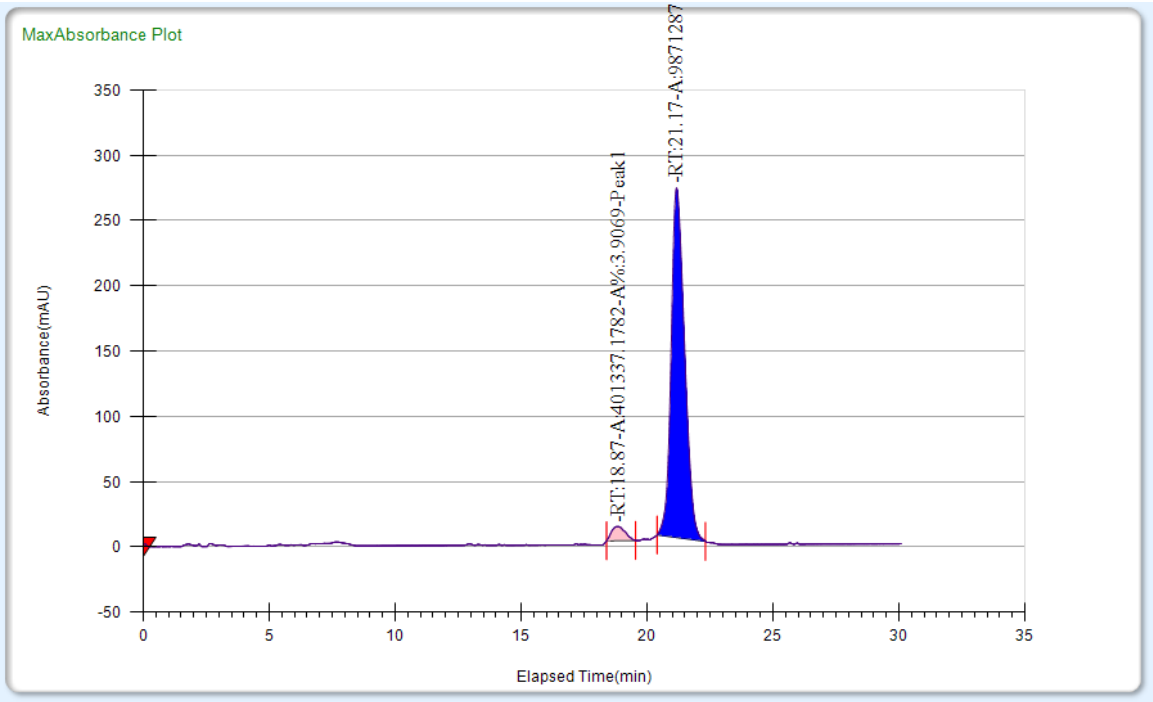


Figure S6: ESI-HRMS spectrum of compound **4a**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	3,9069		18.87 min	10,6844
4a	Peak2	2	96,0931	10272624,2	21.17 min	267,8206

Figure S7: HPLC chromatogram and purity analysis of compound **4a**

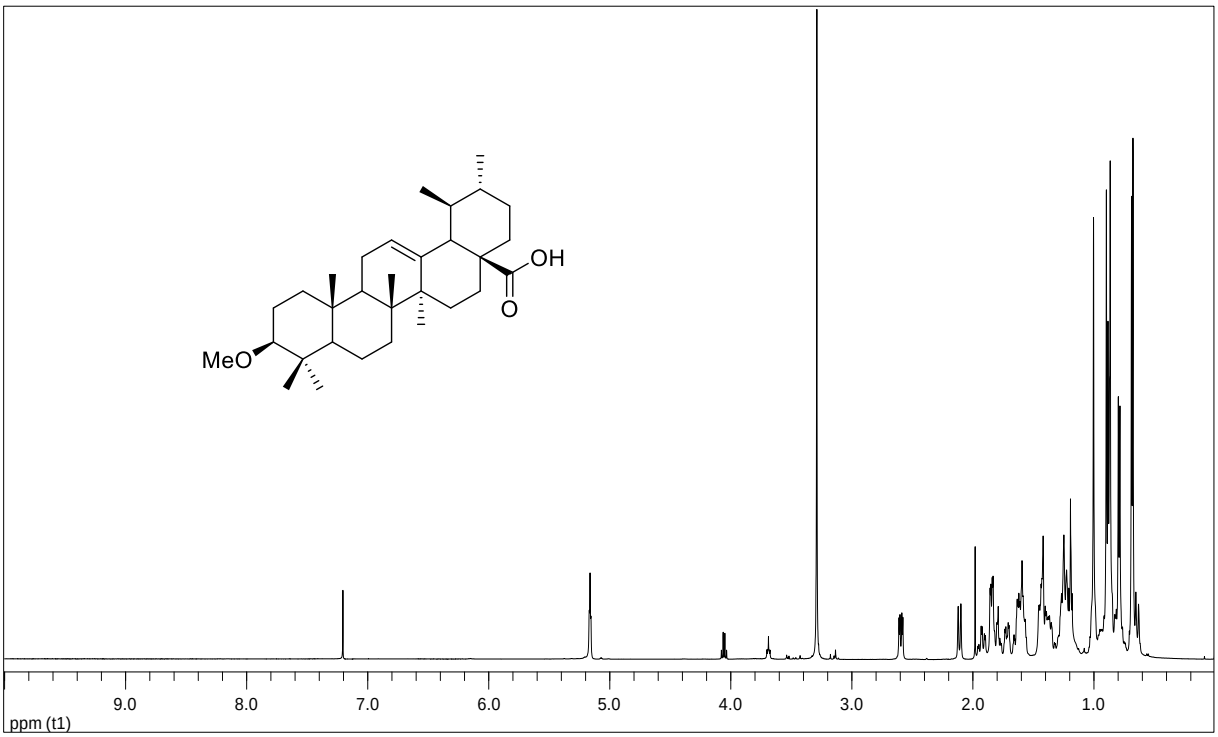


Figure S8: ^{1}H NMR spectrum of **4b** ($CDCl_3$, 500 MHz)

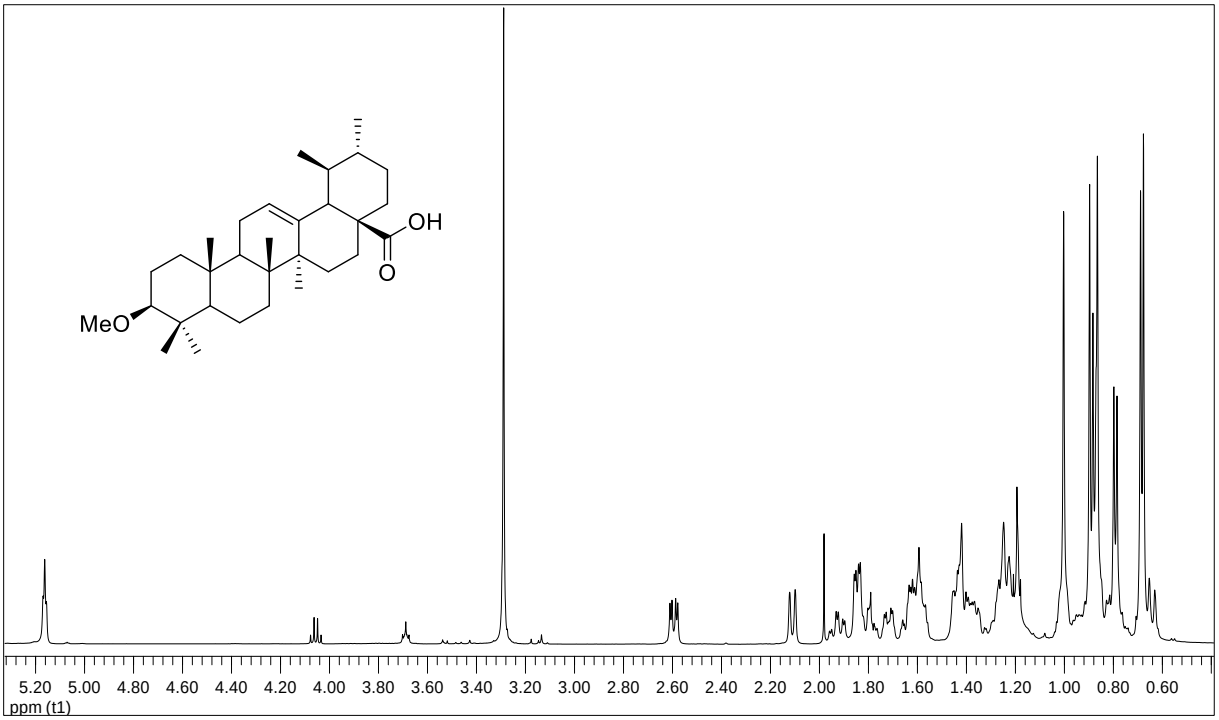


Figure S9: Extended ^{1}H NMR spectrum of **4b (** ($CDCl_3$, 500 MHz)

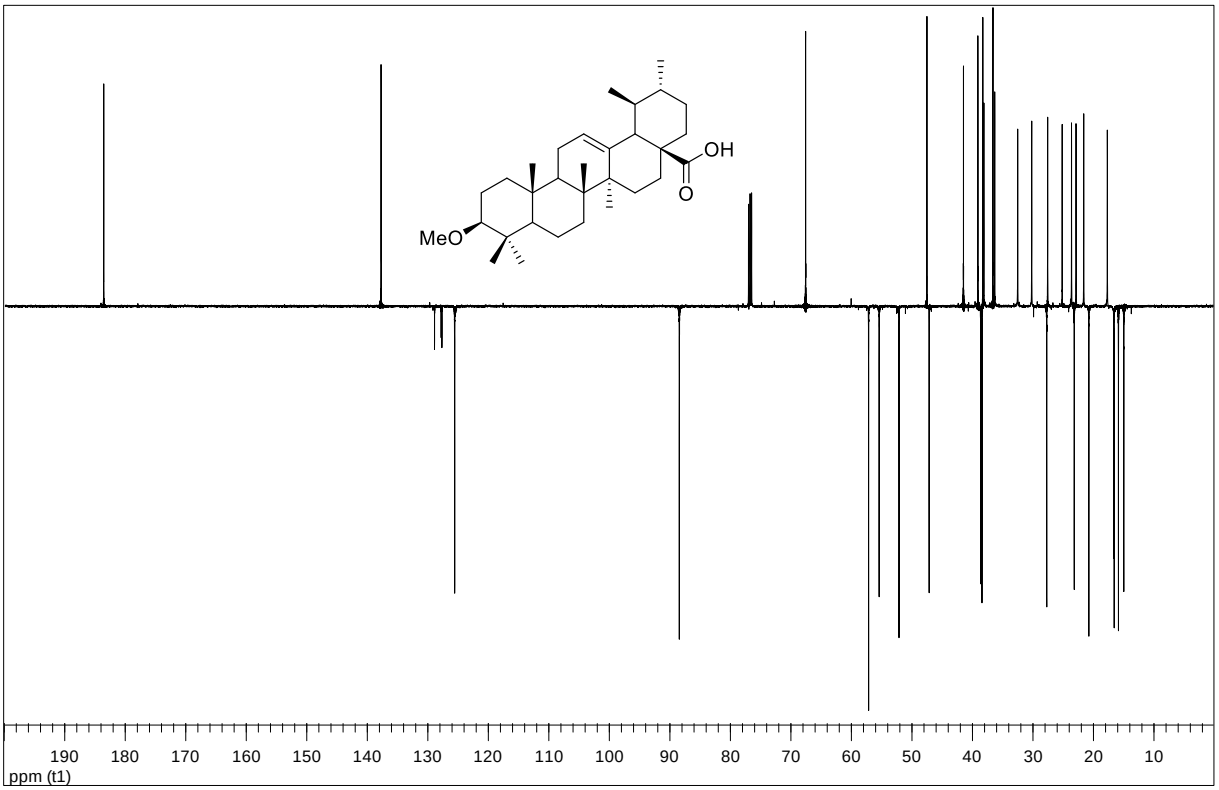


Figure S10:^{13}C APT NMR spectrum of **4b** ($CDCl_3$, 125 MHz)

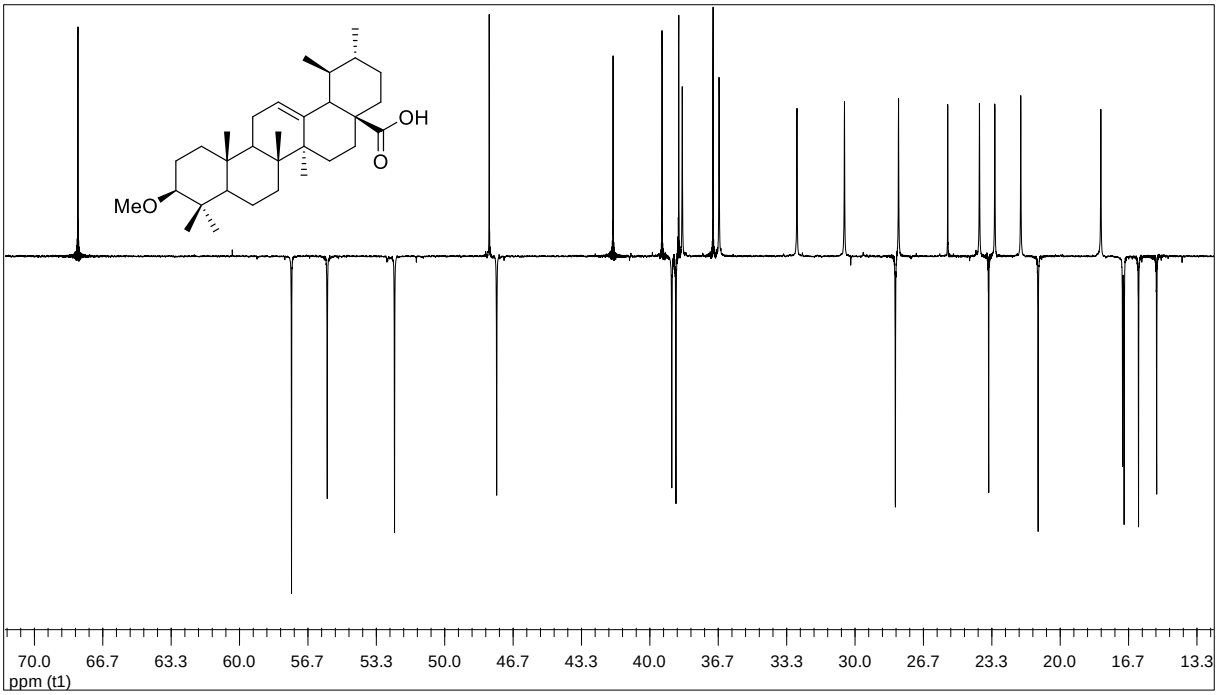


Figure S11: Extended ^{13}C APT NMR spectrum of **4b** ($CDCl_3$, 125 MHz)

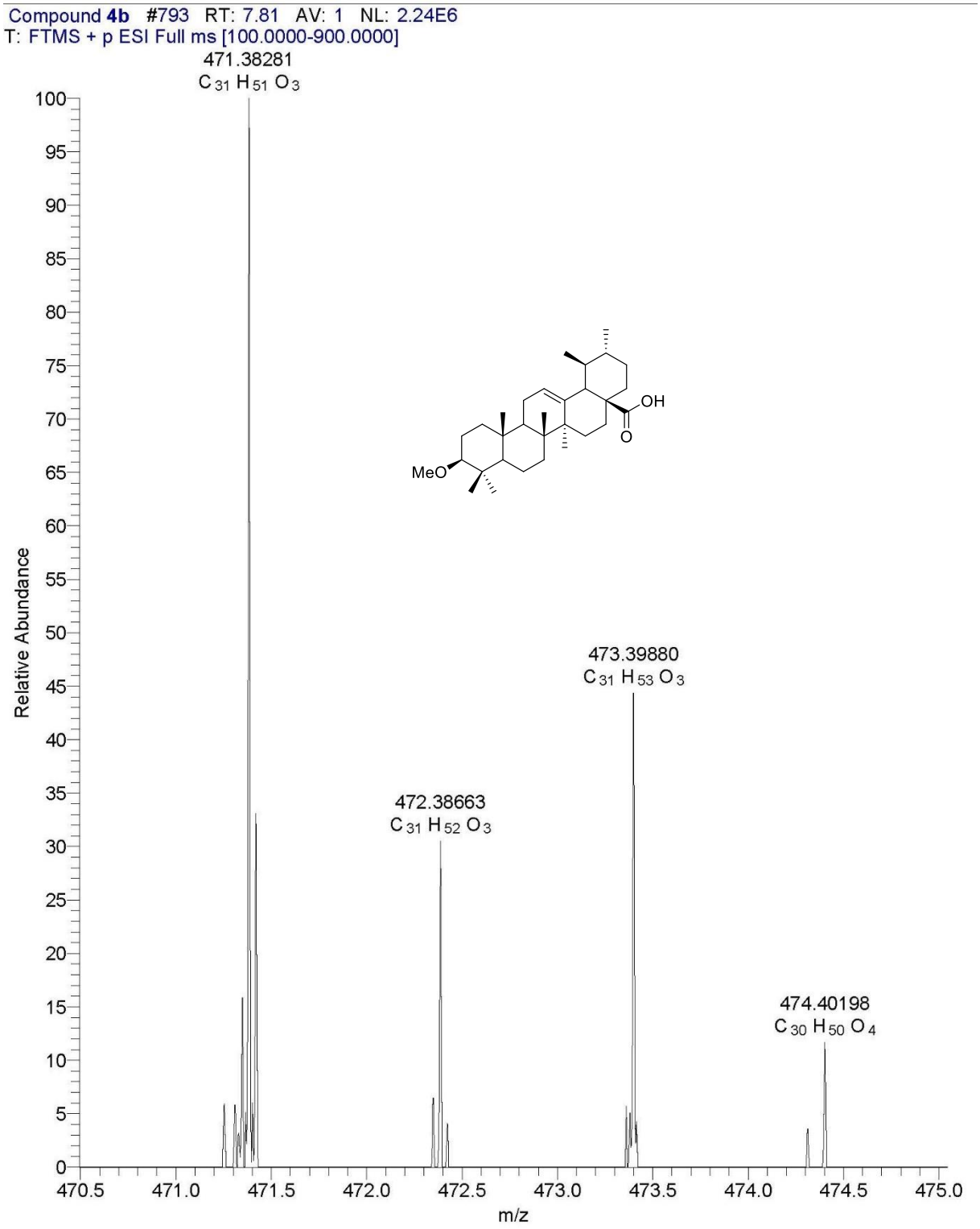
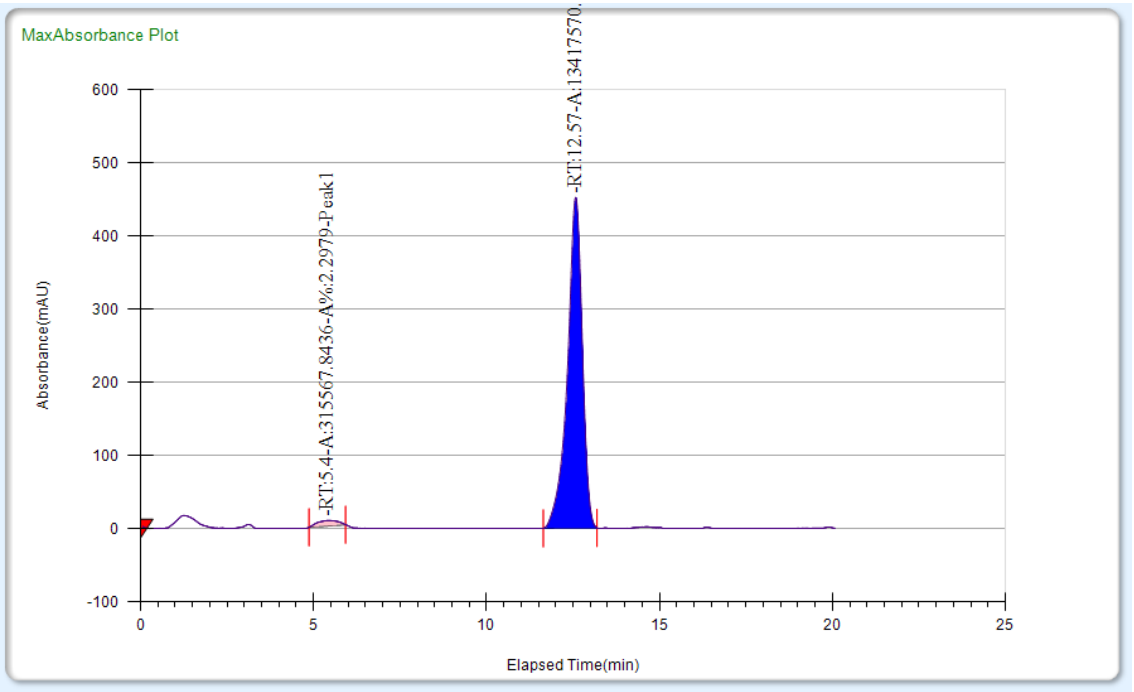


Figure S12: ESI-HRMS spectrum of compound **4b**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	2,2979		5.4 min	7,4914
4b	Peak2	2	97,7021	13733138,6	12.57 min	451,8462

Figure S13: HPLC chromatogram and purity analysis of compound **4b**

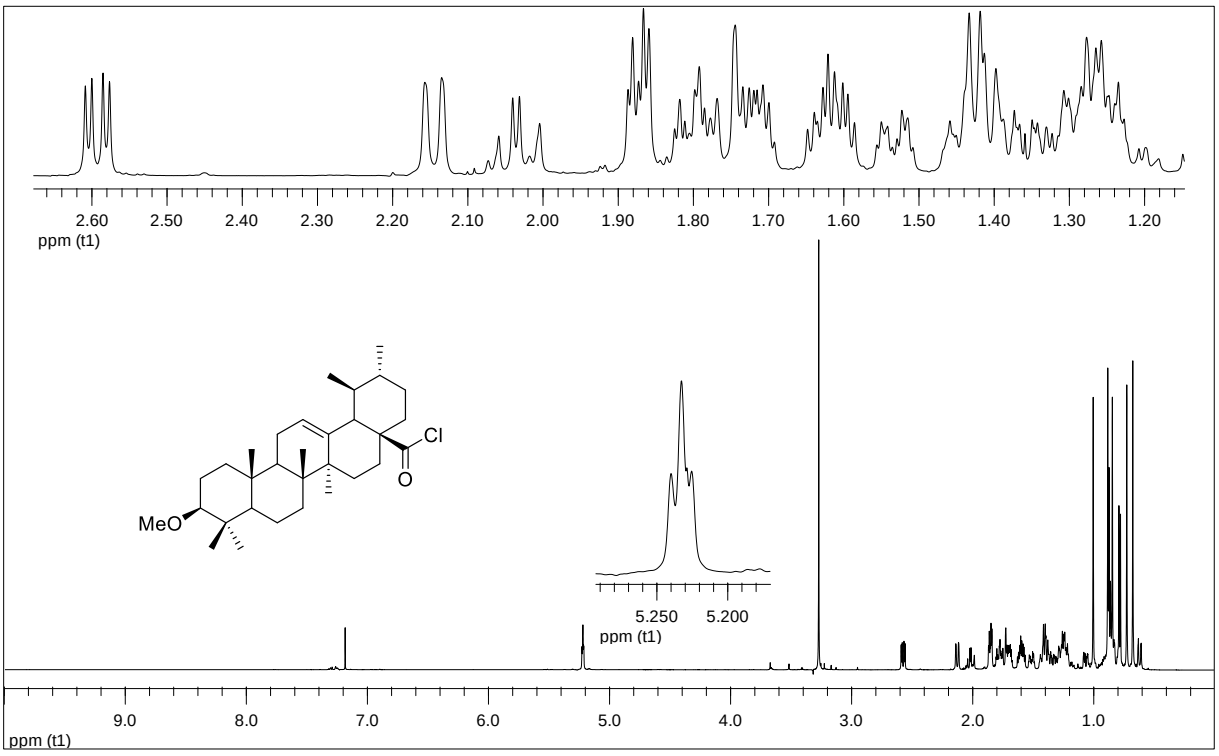


Figure S14: ^{1}H NMR spectrum of **5** ($CDCl_3$, 500 MHz)

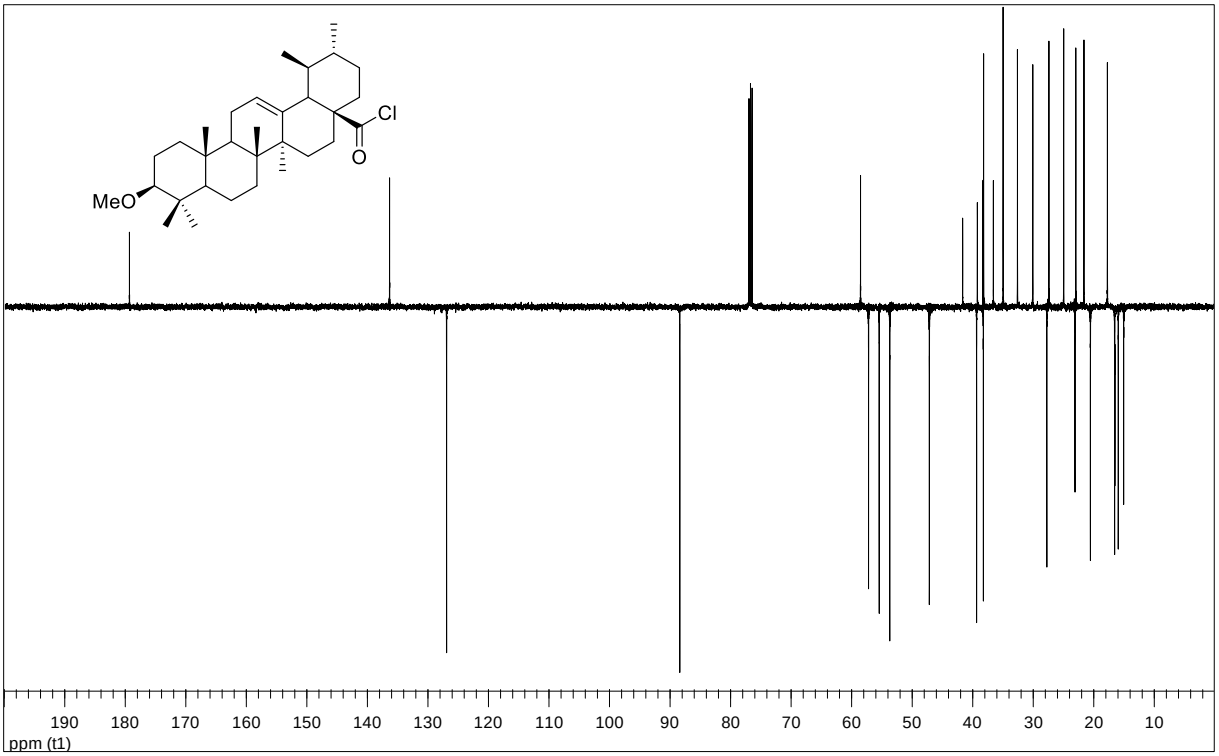


Figure S15: ^{13}C APT NMR spectrum of **5** ($CDCl_3$, 125 MHz)

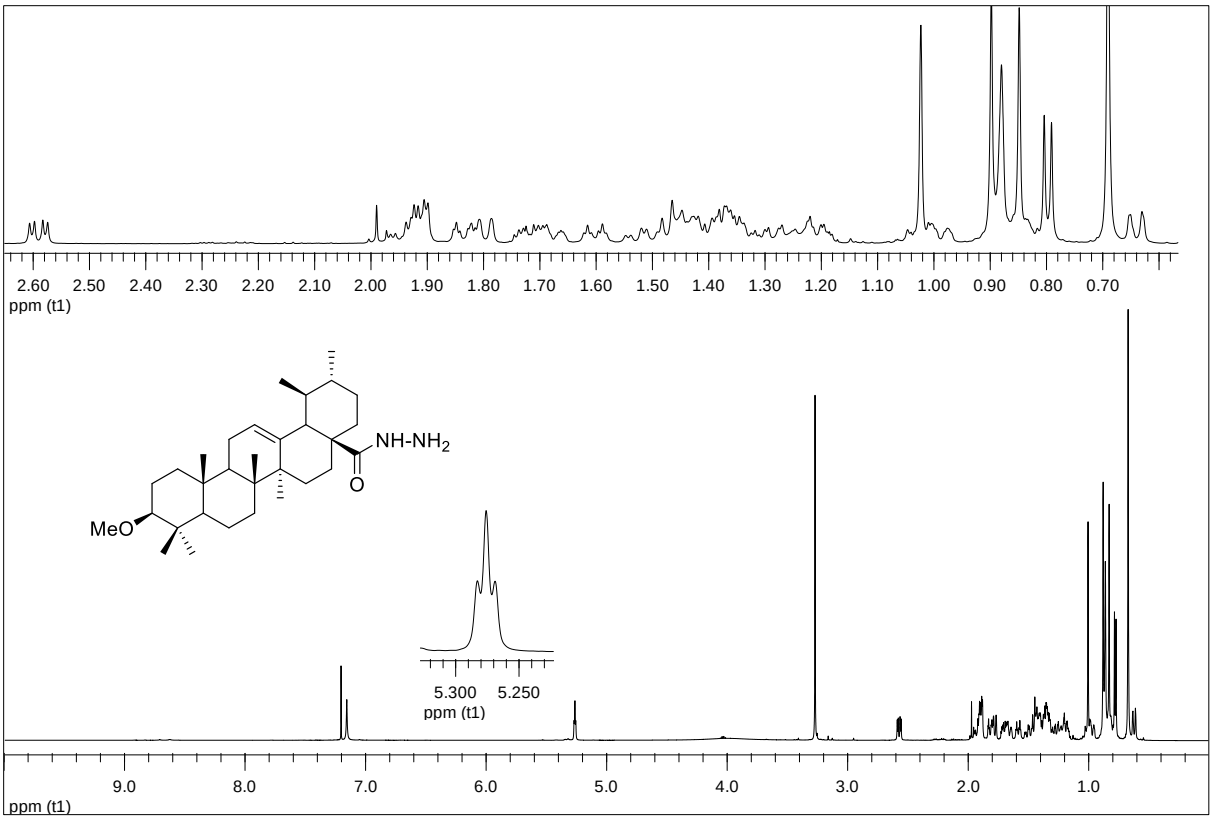


Figure S16: ^{1}H NMR spectrum of **6** ($CDCl_3$, 500 MHz)

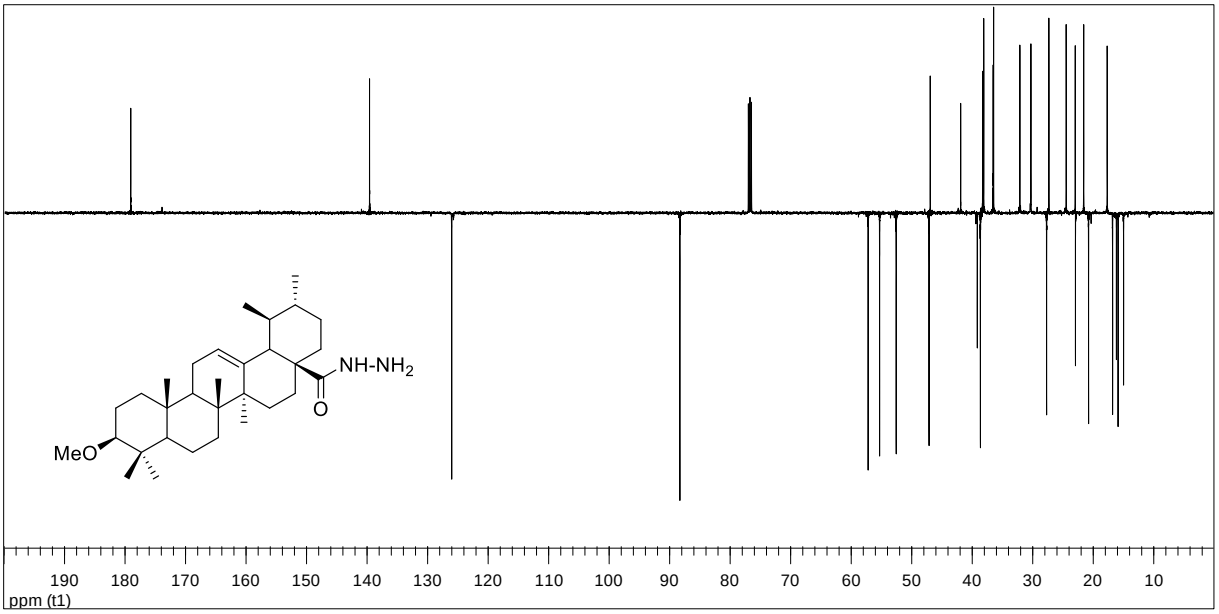


Figure S17: ^{13}C APT NMR spectrum of **6** ($CDCl_3$, 125 MHz)

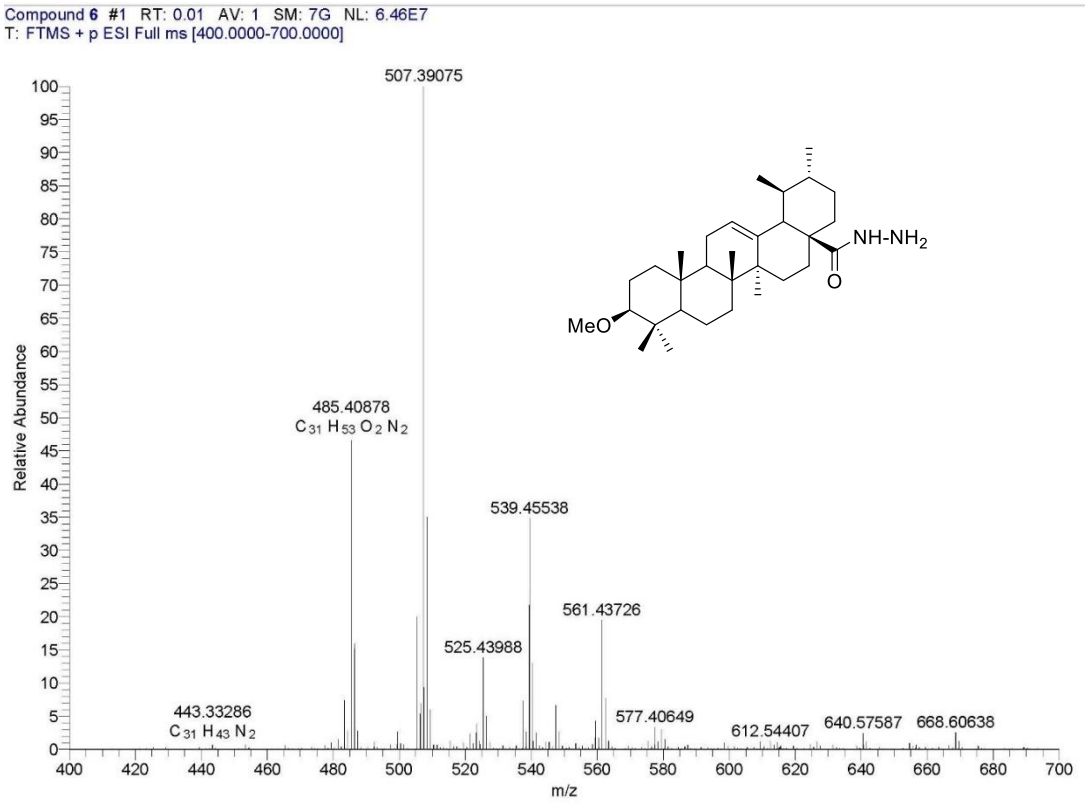
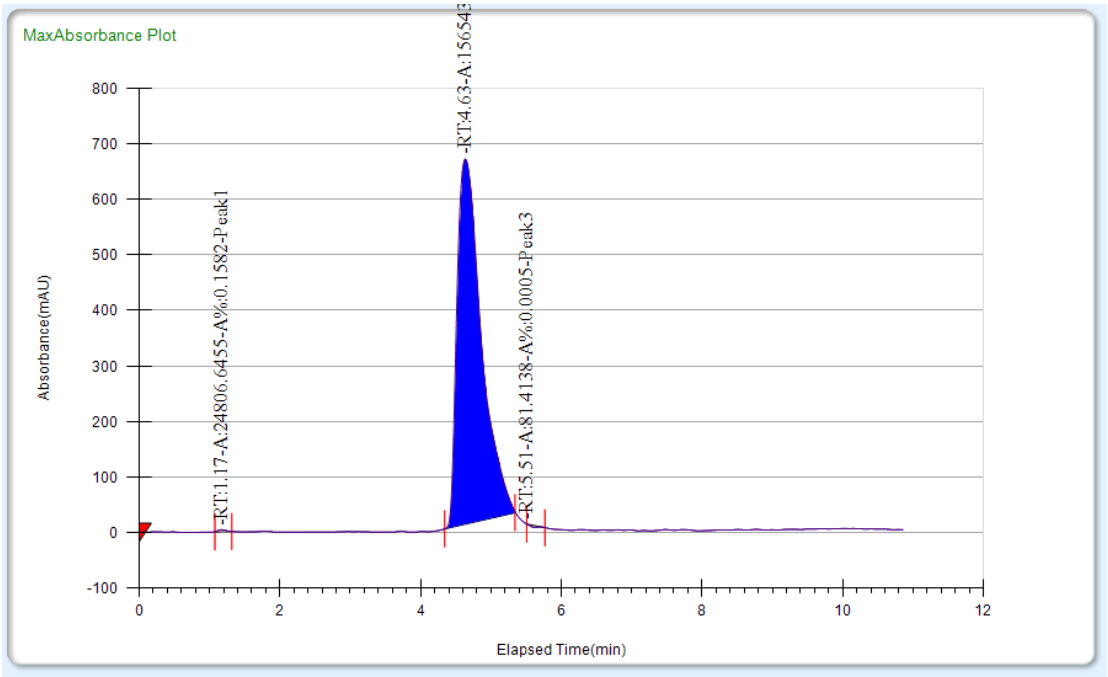


Figure S18: ESI-HRMS spectrum of compound **6**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,1582		1.17 min	3,4323
6	Peak2	2	99,8413		4.63 min	657,8985
	Peak3	3	0,0005	15679204,13	5.51 min	0,0003

Figure S19: HPLC chromatogram and purity analysis of compound **6**

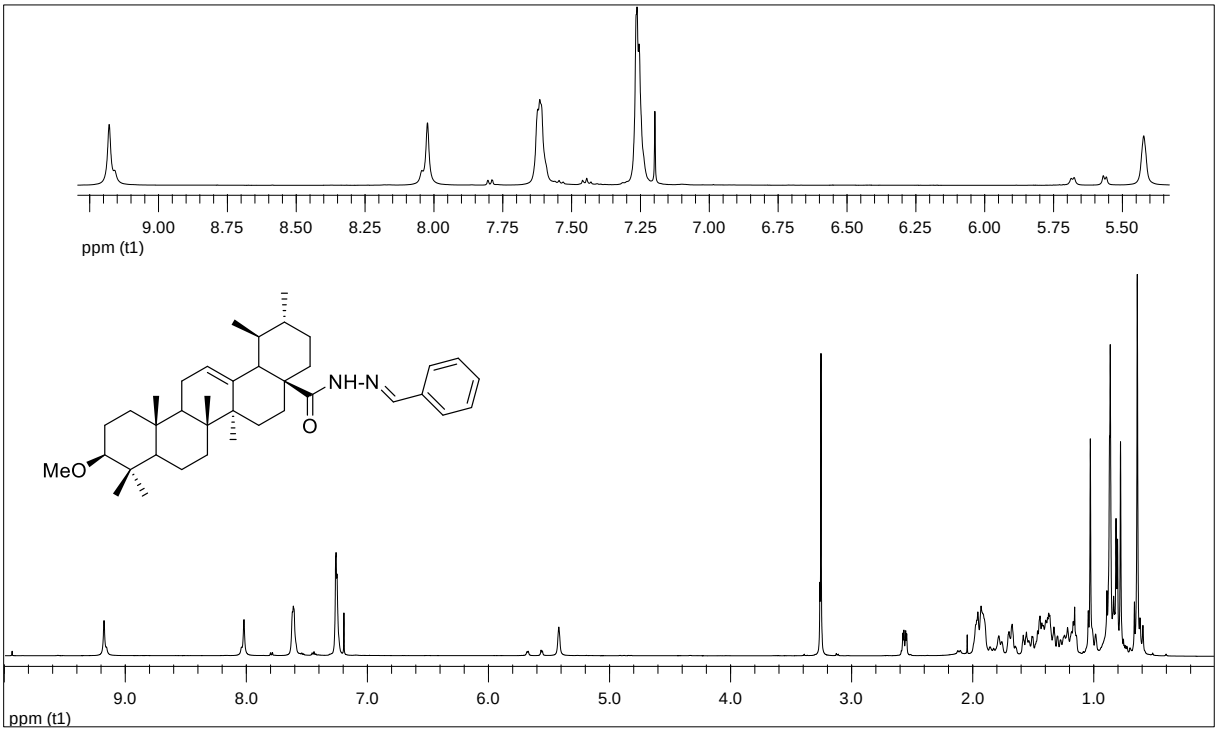


Figure S20: ^{1}H NMR spectrum of **7a** ($CDCl_3$, 500 MHz)

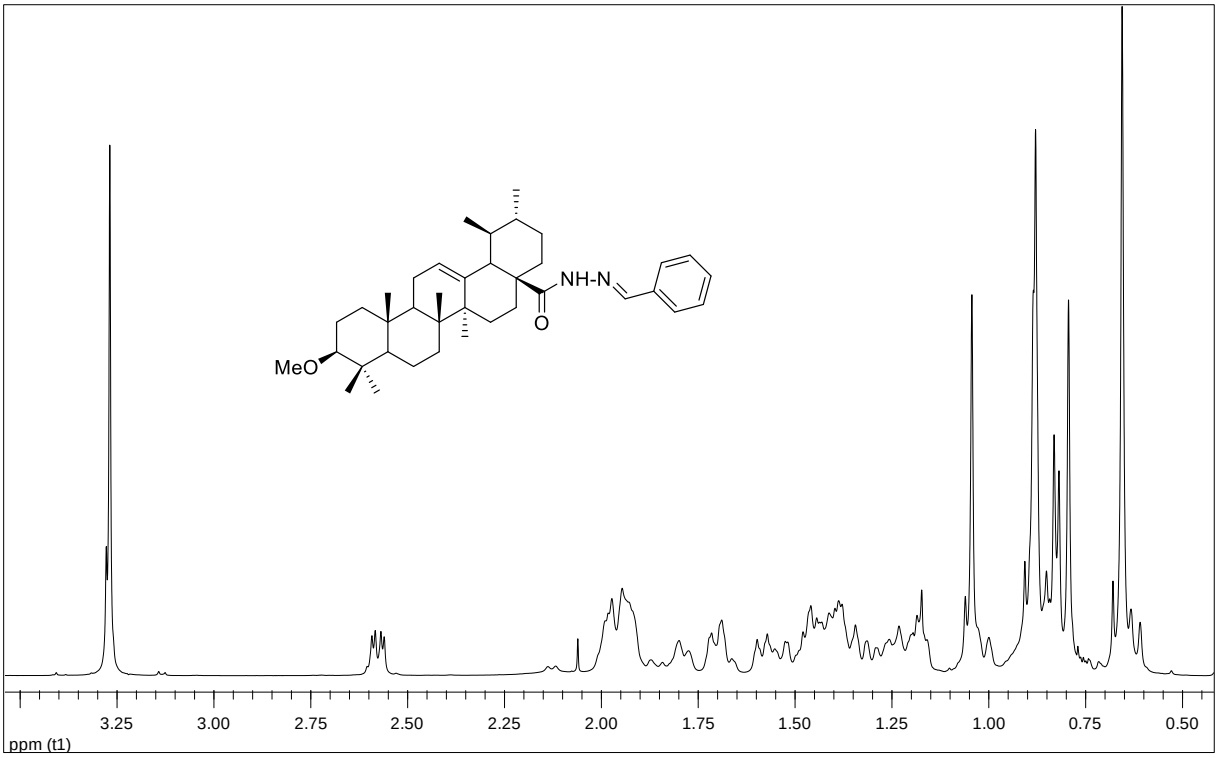


Figure S21: Extended ^{1}H NMR spectrum of **7a (** ($CDCl_3$, 500 MHz)

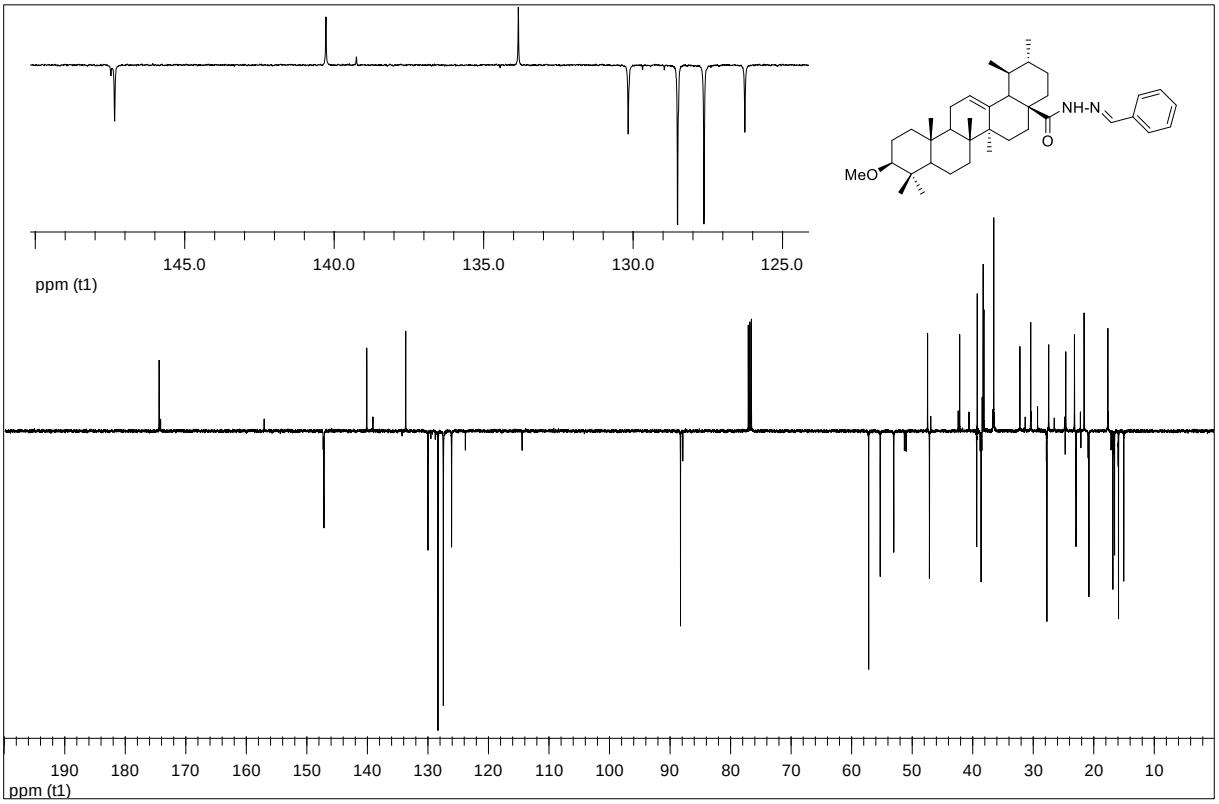


Figure S22: ^{13}C APT NMR spectrum of **7a** ($CDCl_3$, 125 MHz)

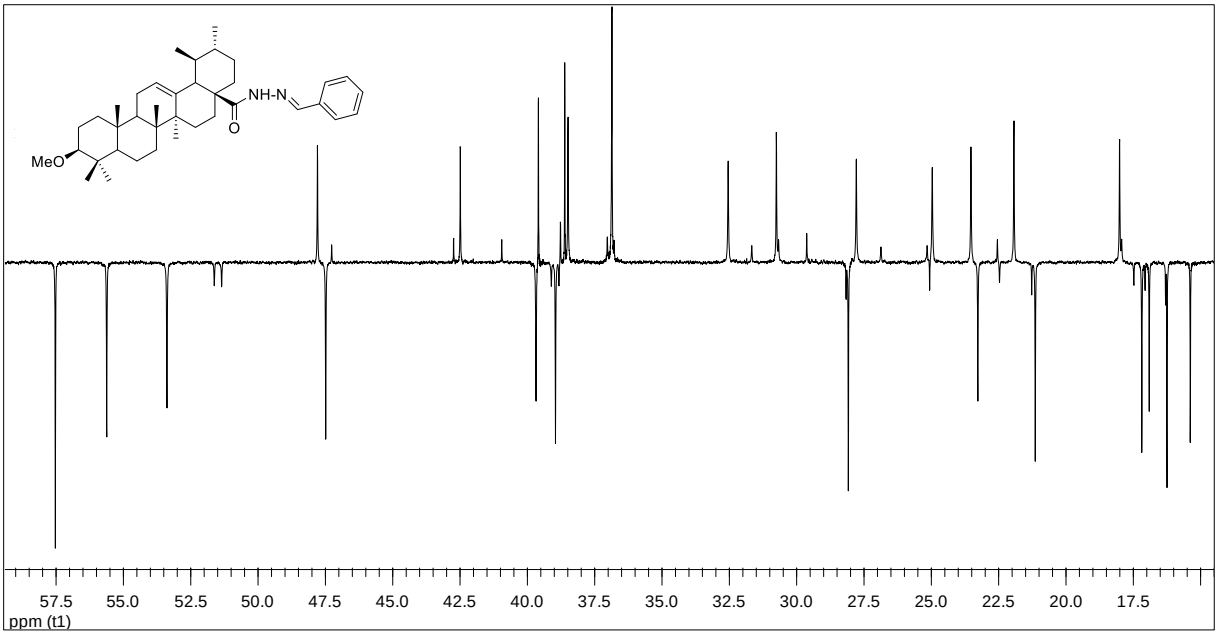


Figure S23: Extended ^{13}C APT NMR spectrum of **7a** ($CDCl_3$, 125 MHz)

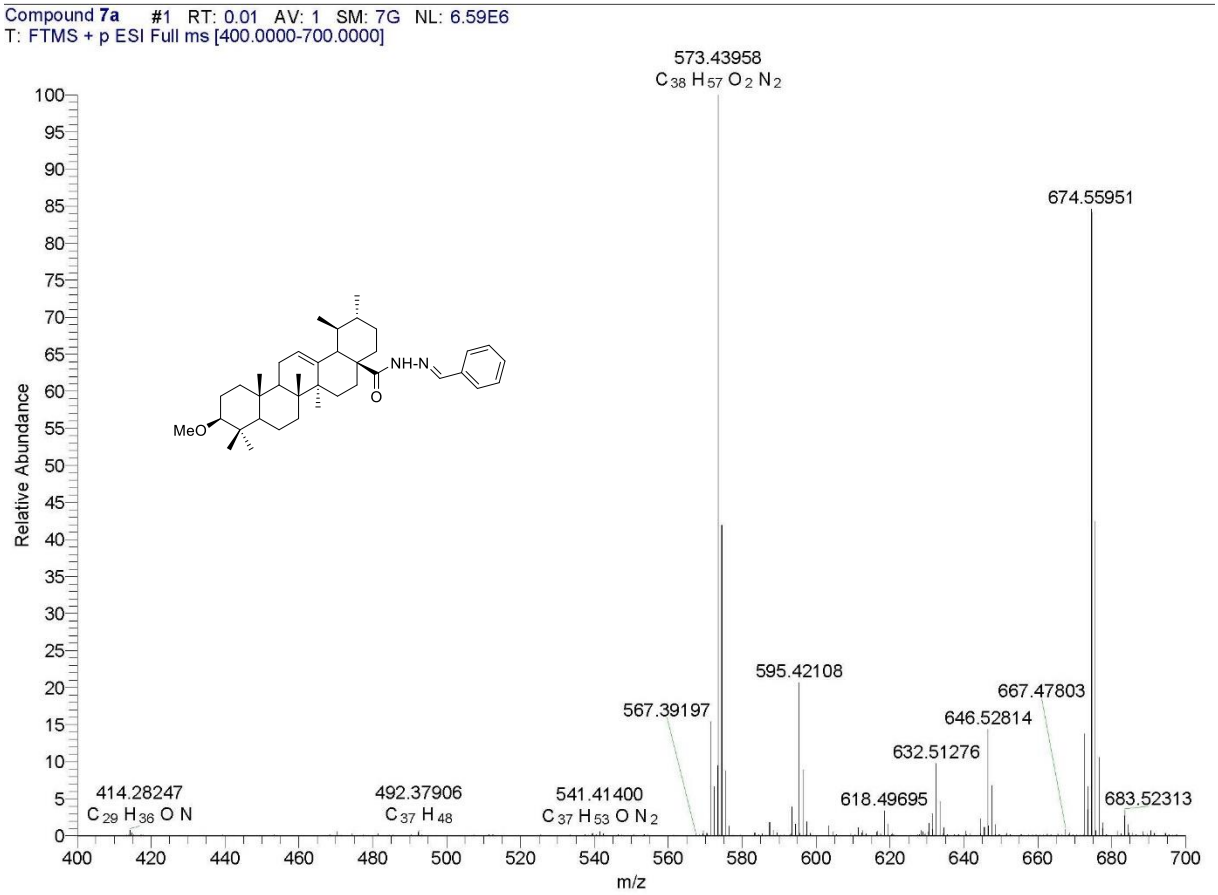
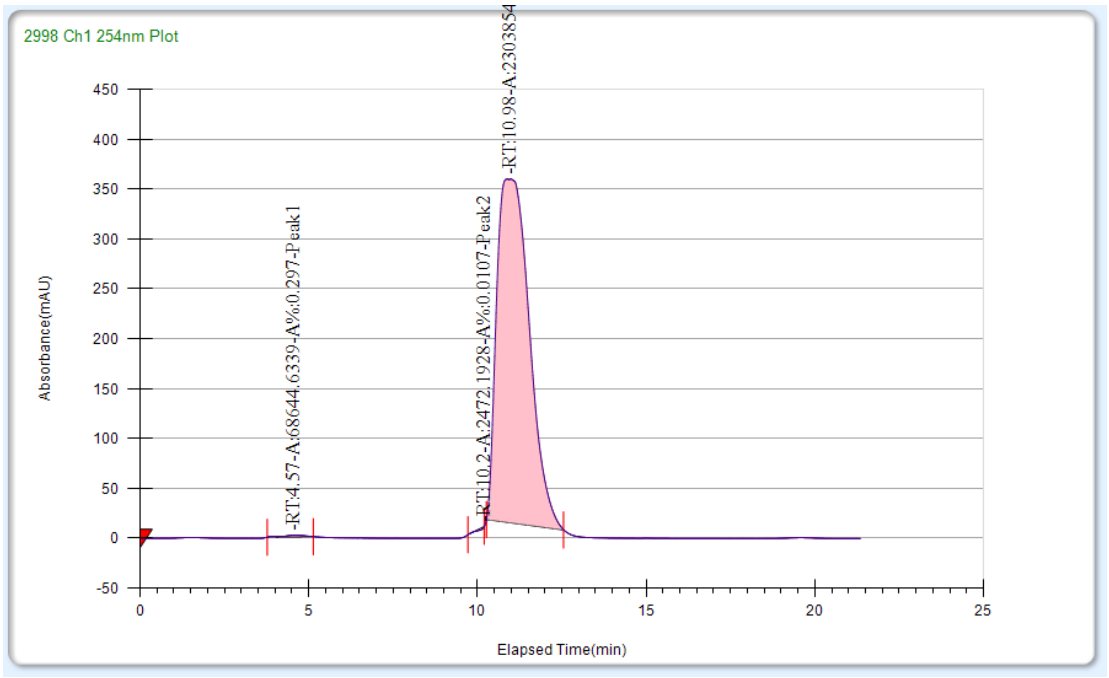


Figure S24: ESI-HRMS spectrum of compound **7a**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,297		4.57 min	1,5077
	Peak2	2	0,0107		10.2 min	0,0264
7a	Peak3	3	99,6923	23109666,66	10.98 min	344,7872

Figure S25: HPLC chromatogram and purity analysis of compound **7a**

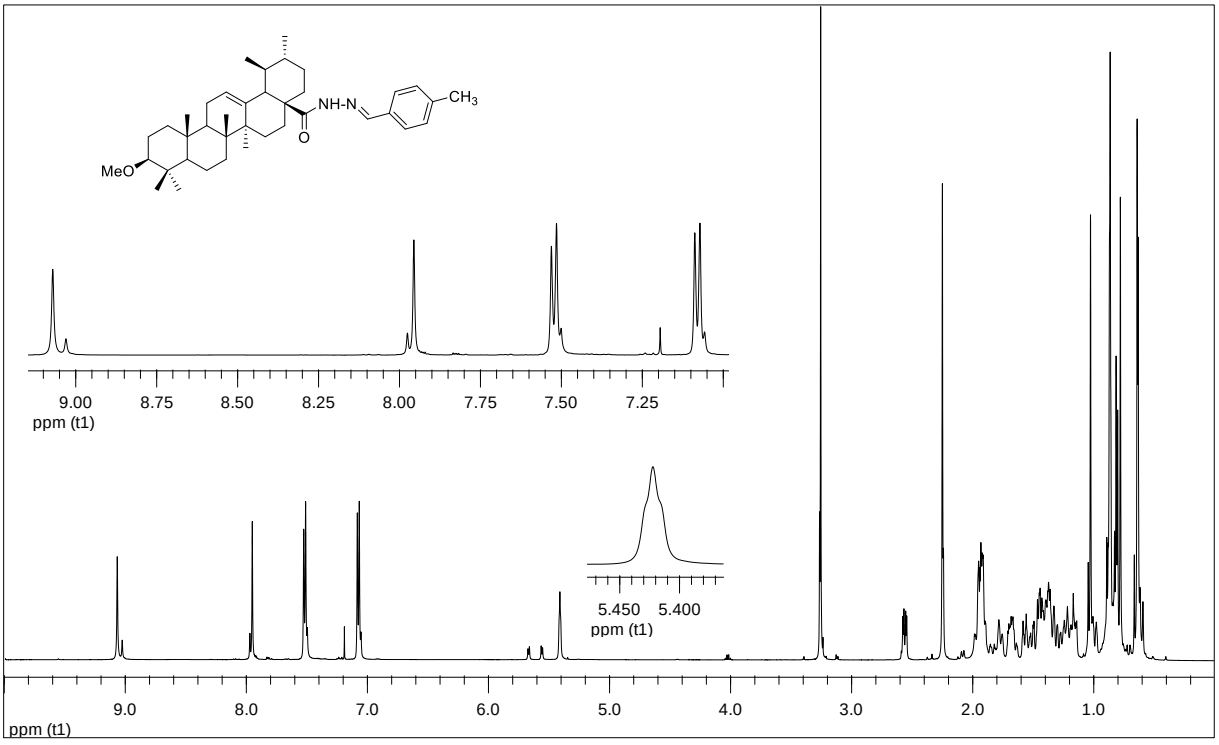


Figure S26: ^{1}H NMR spectrum of **7b** ($CDCl_3$, 500 MHz)

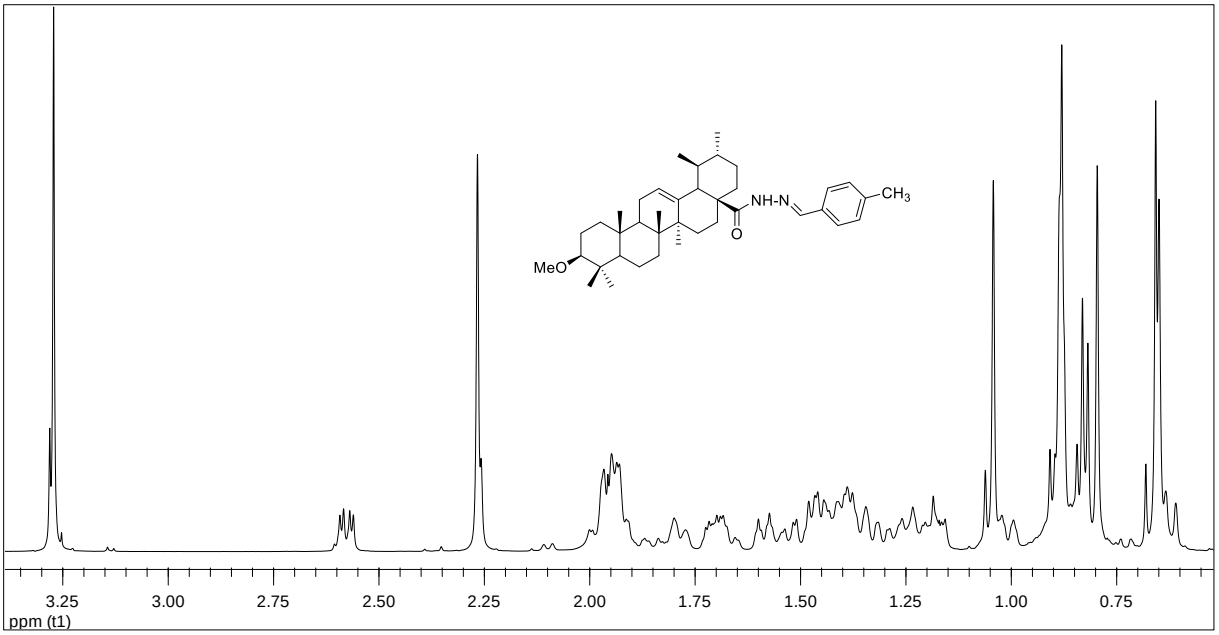


Figure S27: Extended ^{1}H NMR spectrum of **7b** ($CDCl_3$, 500 MHz)

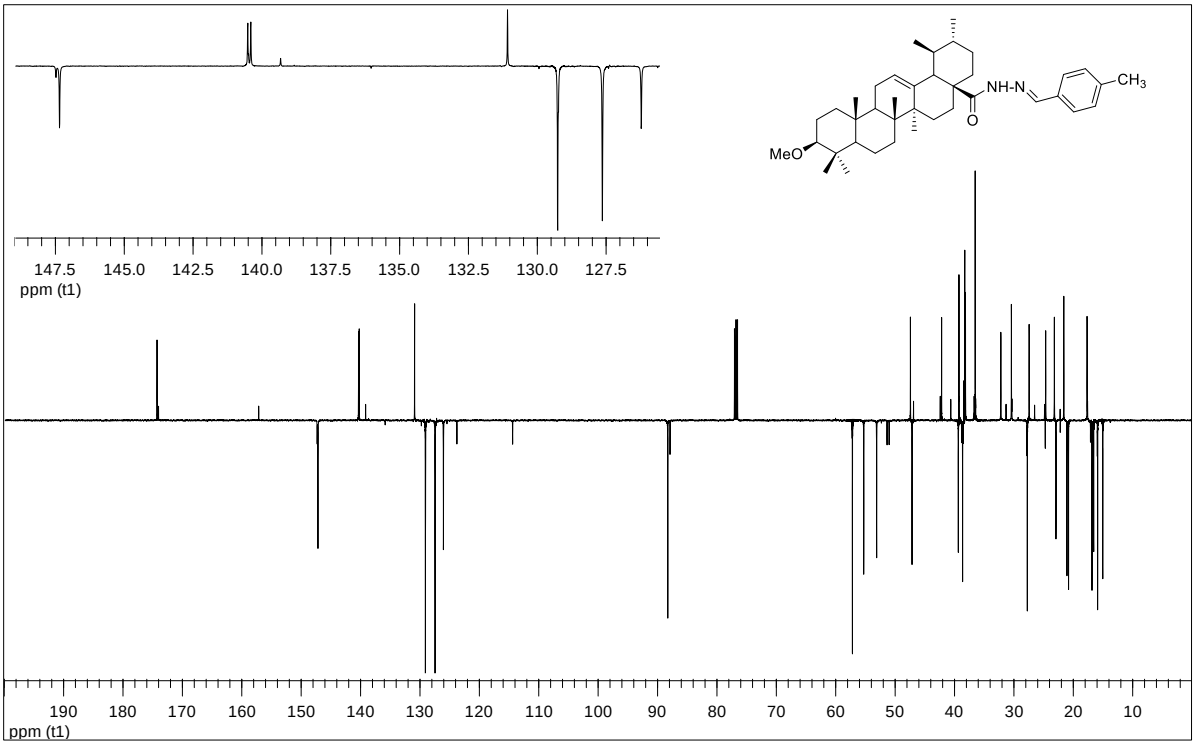


Figure S28: ^{13}C APT NMR spectrum of **7b** ($CDCl_3$, 125 MHz)

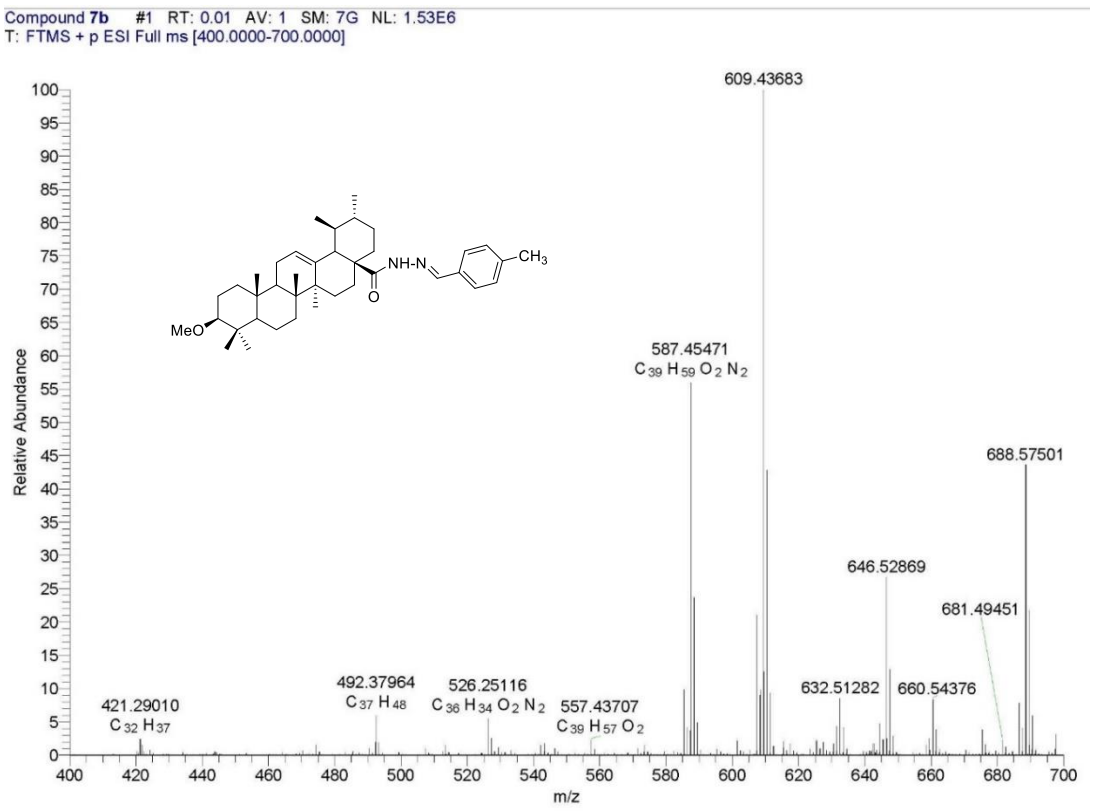
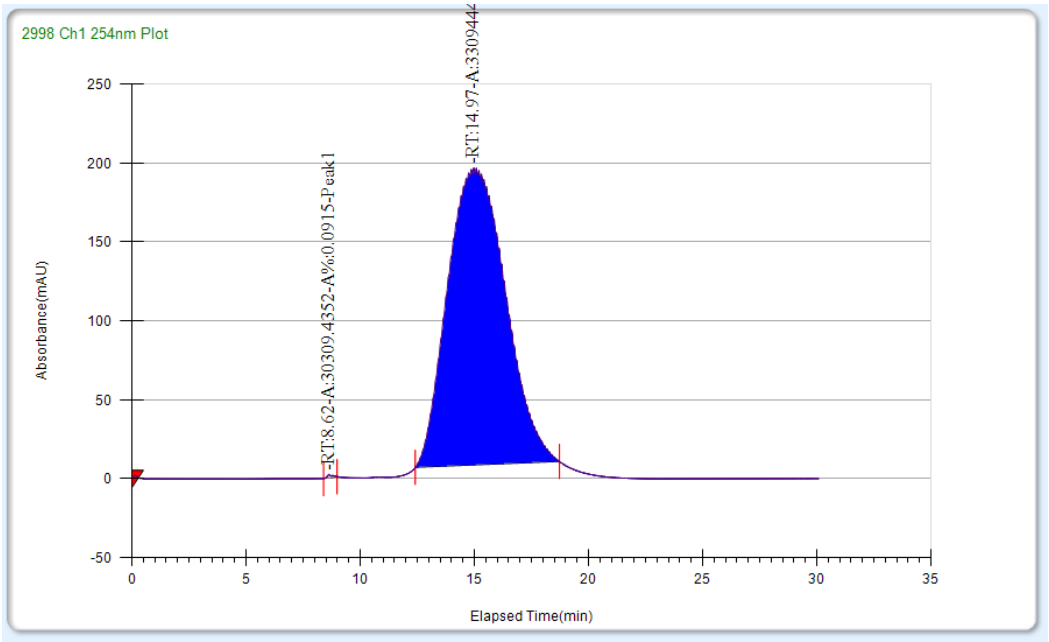


Figure S29: ESI-HRMS spectrum of compound **7b**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,0915		8.62 min	2,2086
7b	Peak2	2	99,9085	33124751,97	14.97 min	188,0107

Figure S30: HPLC chromatogram and purity analysis of compound **7b**

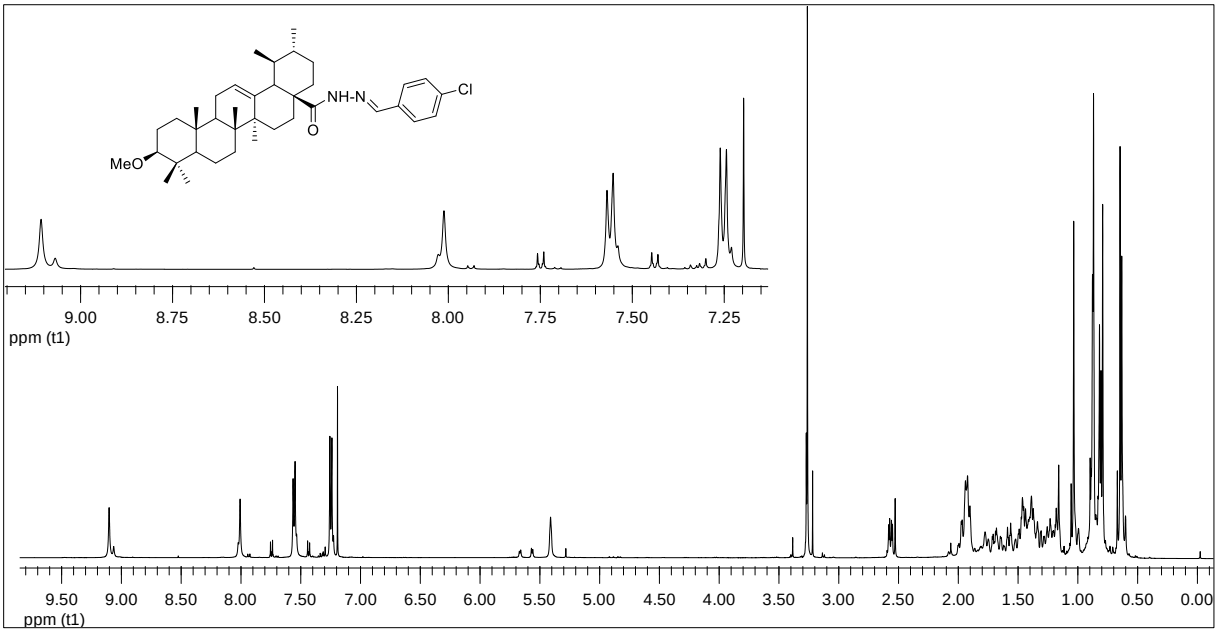


Figure S31: ^{1}H NMR spectrum of **7c** ($CDCl_3$, 500 MHz)

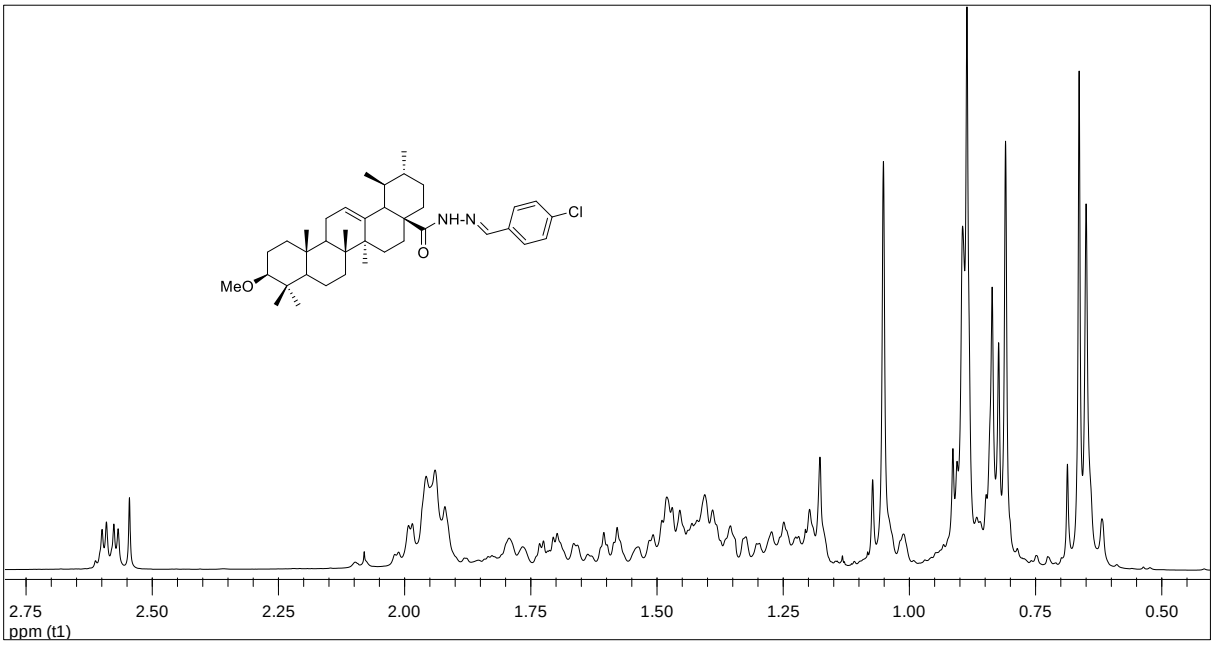


Figure S32: Extended ^{1}H NMR spectrum of **7c** ($CDCl_3$, 500 MHz)

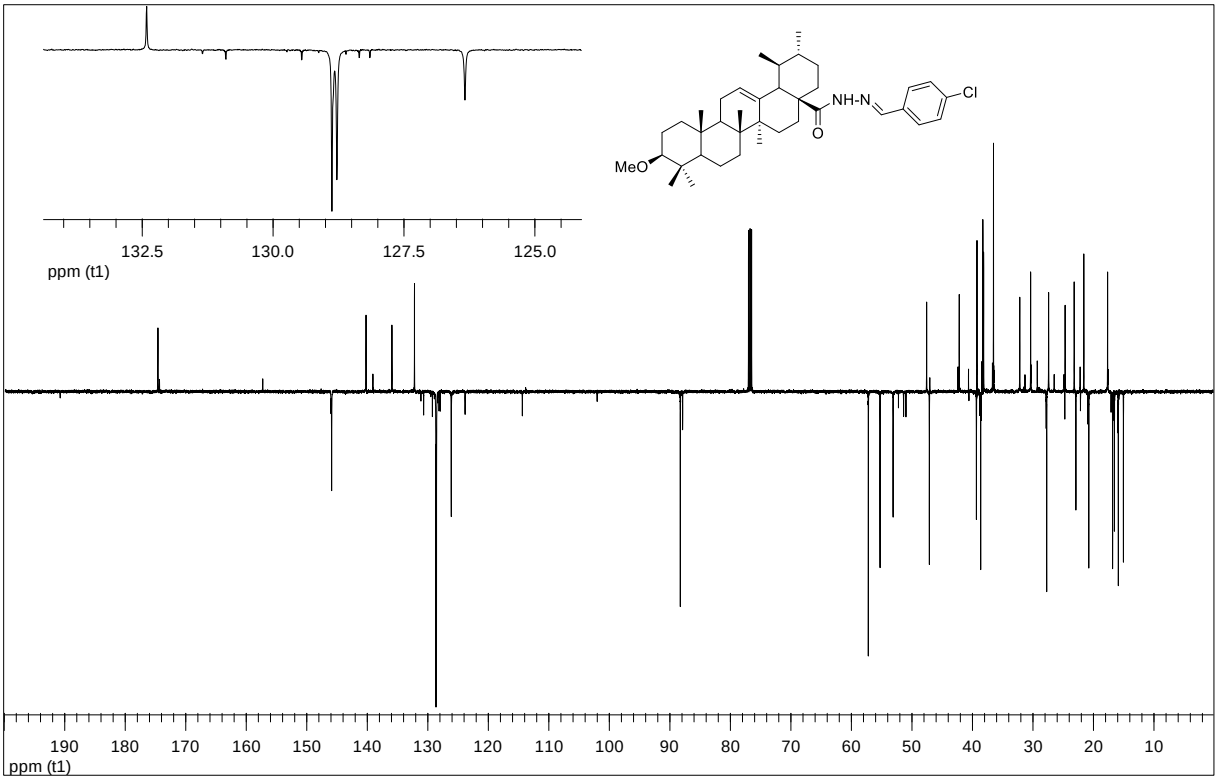


Figure S33: ^{13}C APT NMR spectrum of **7c** ($CDCl_3$, 125 MHz)

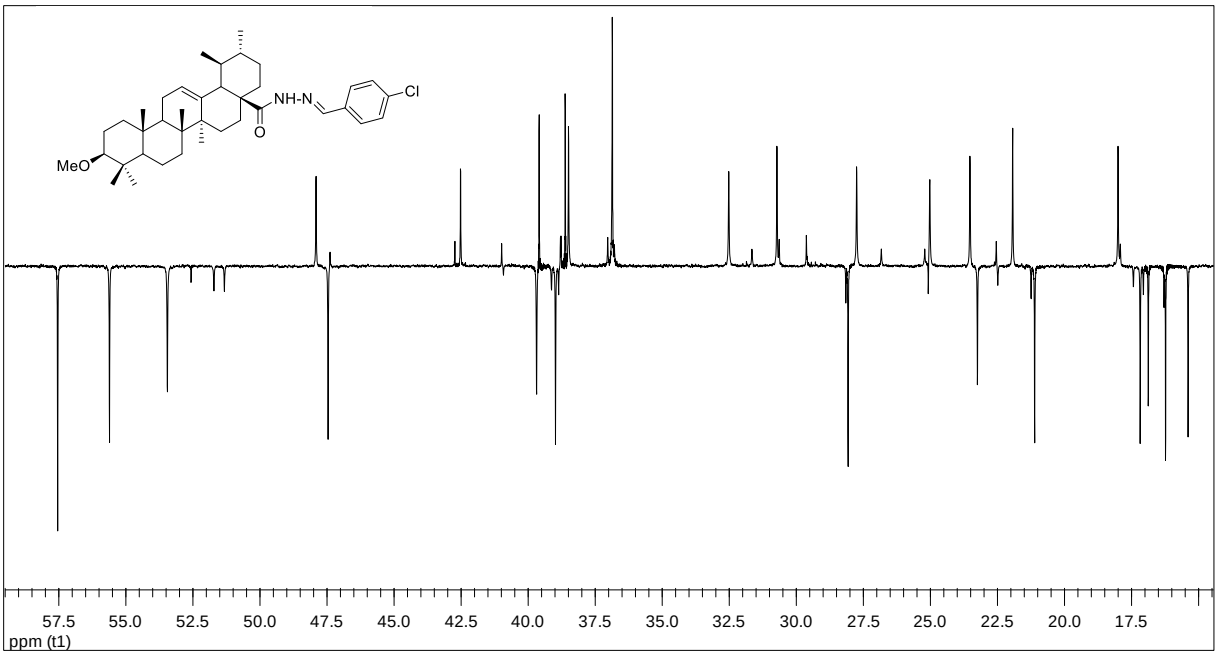


Figure S34: Extended ^{13}C APT NMR spectrum of **7c** ($CDCl_3$, 125 MHz)

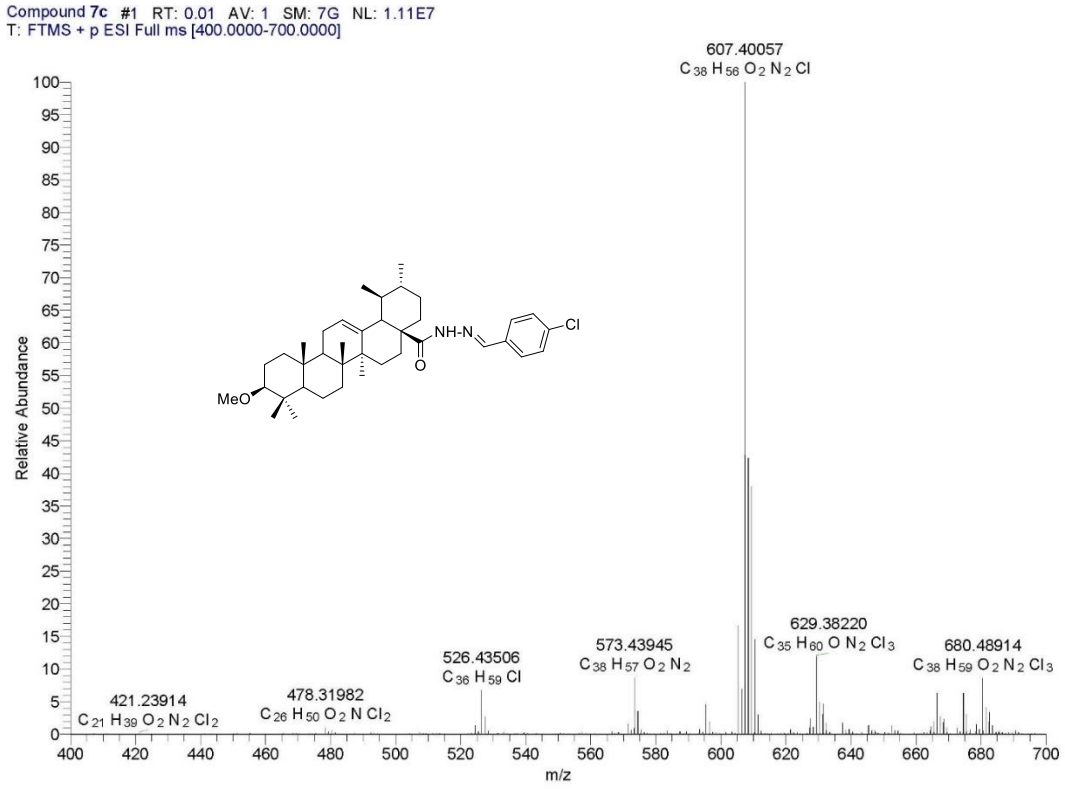
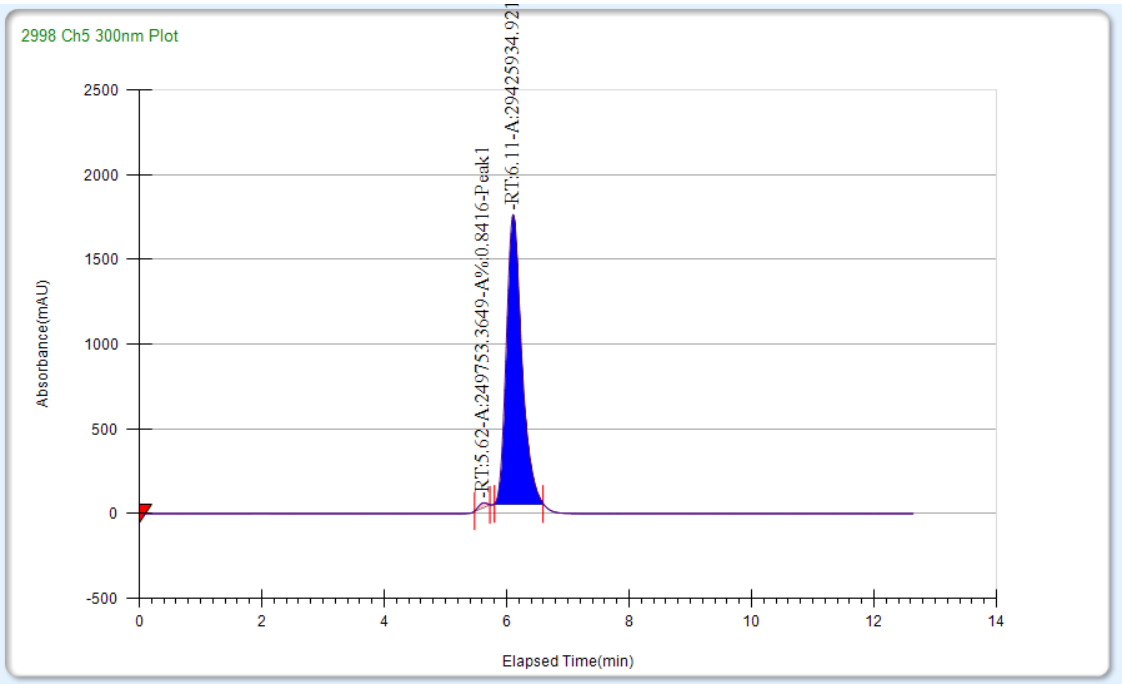


Figure S35: ESI-HRMS spectrum of compound **7c**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,8416		5.62 min	27,1493
7c	Peak2	2	99,1584	29675688,29	6.11 min	1707,1024

Figure S36: HPLC chromatogram and purity analysis of compound **7c**

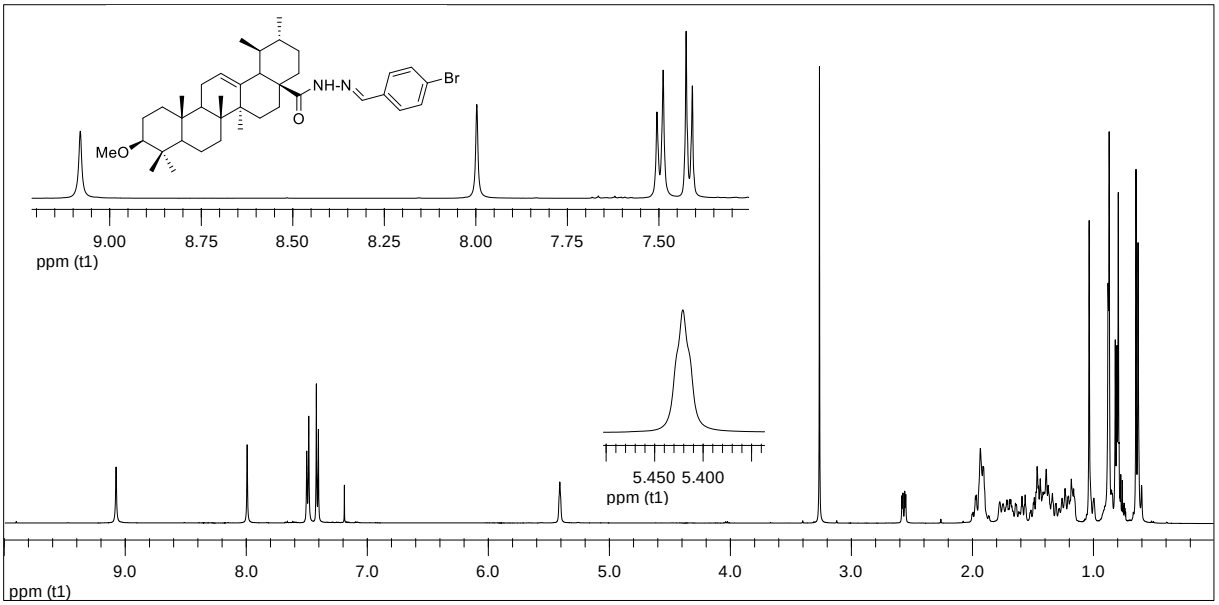


Figure S37: ^{1}H NMR spectrum of **7d** ($CDCl_3$, 500 MHz)

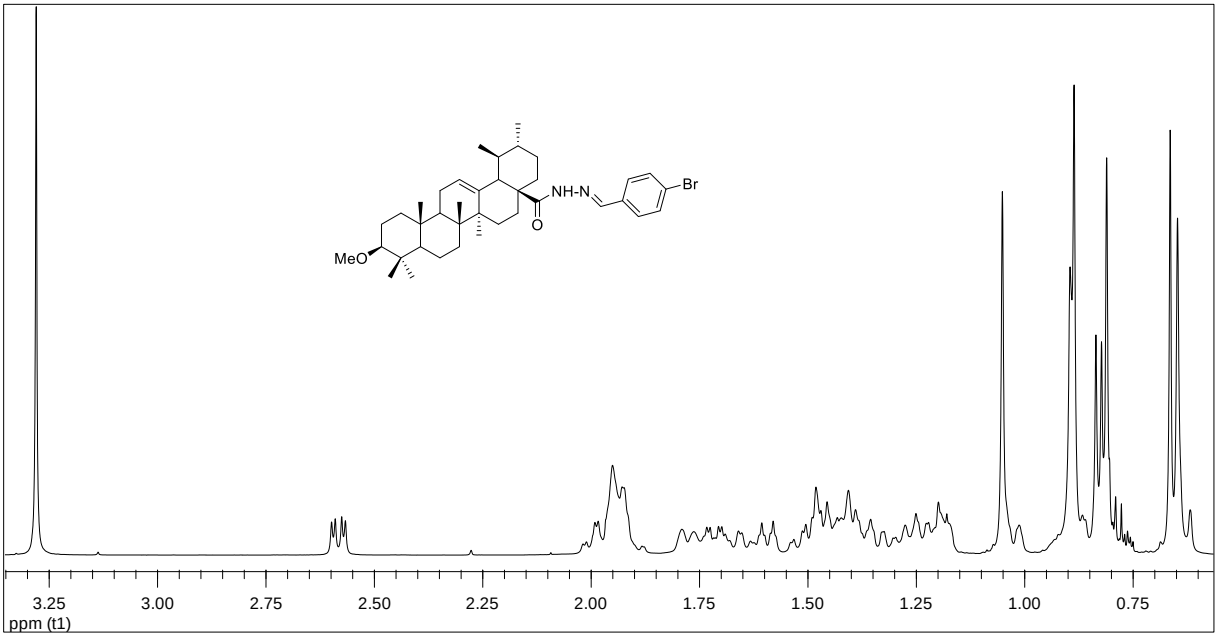


Figure S38: Extended ^{1}H NMR spectrum of **7d** ($CDCl_3$, 500 MHz)

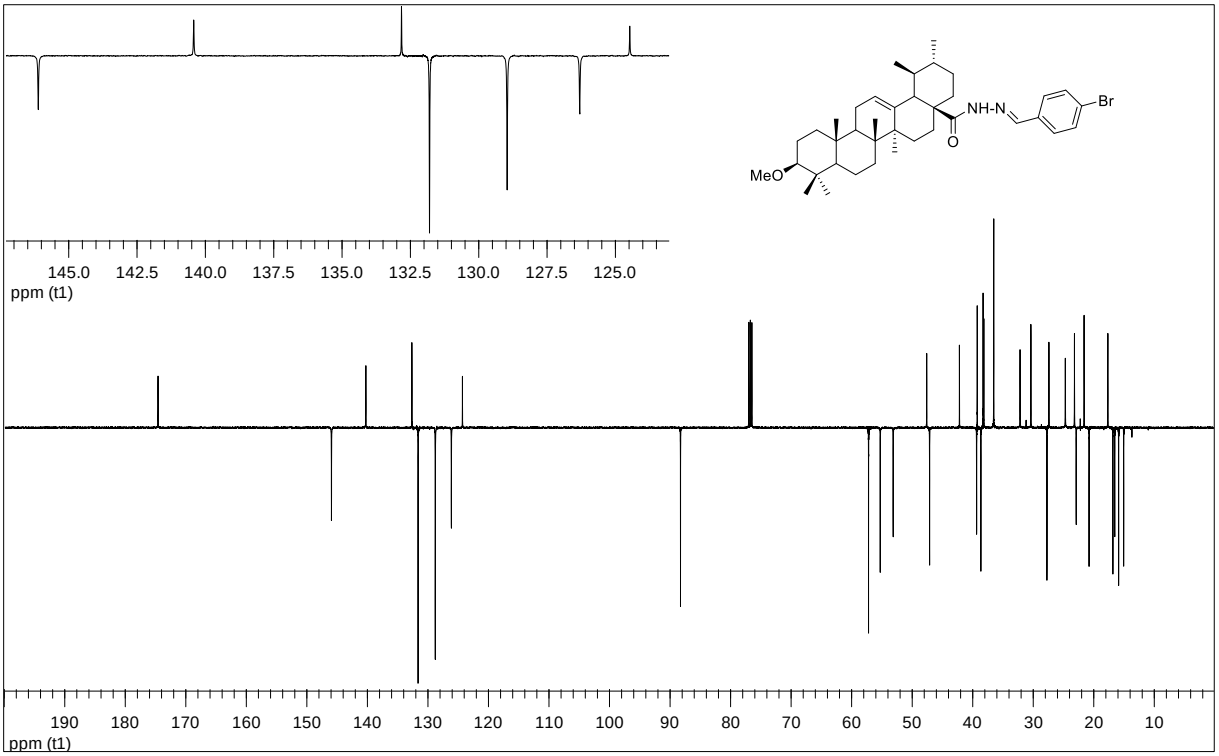


Figure S39: ^{13}C APT NMR spectrum of **7d** ($CDCl_3$, 125 MHz)

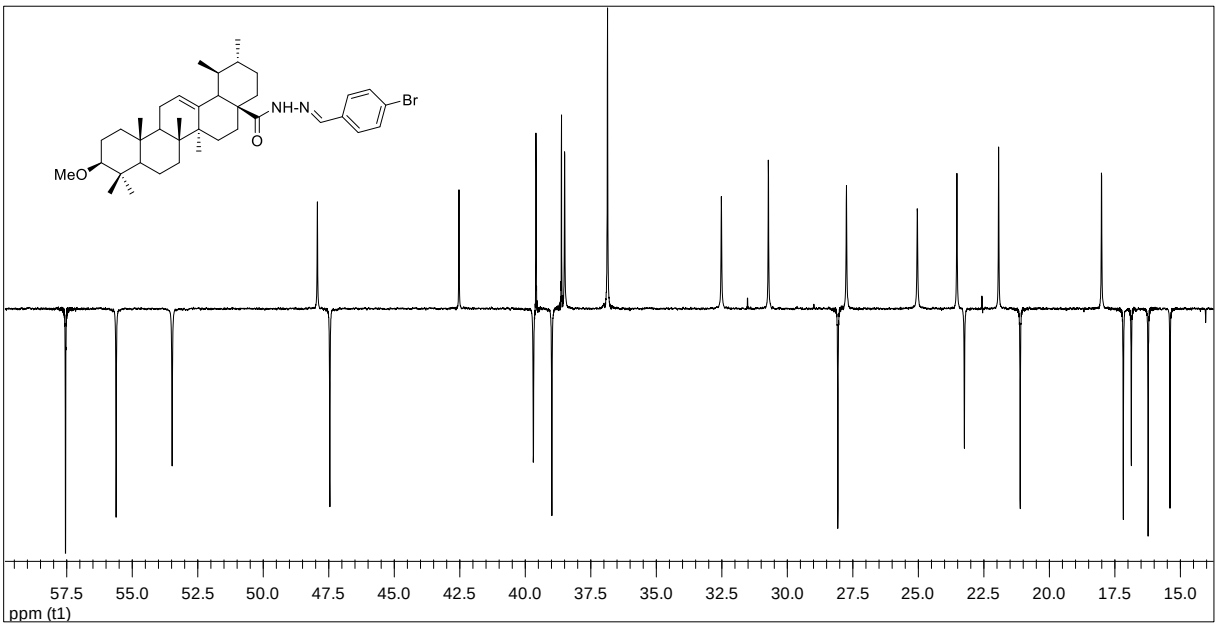


Figure S40: Extended ^{13}C APT NMR spectrum of **7d** ($CDCl_3$, 125 MHz)

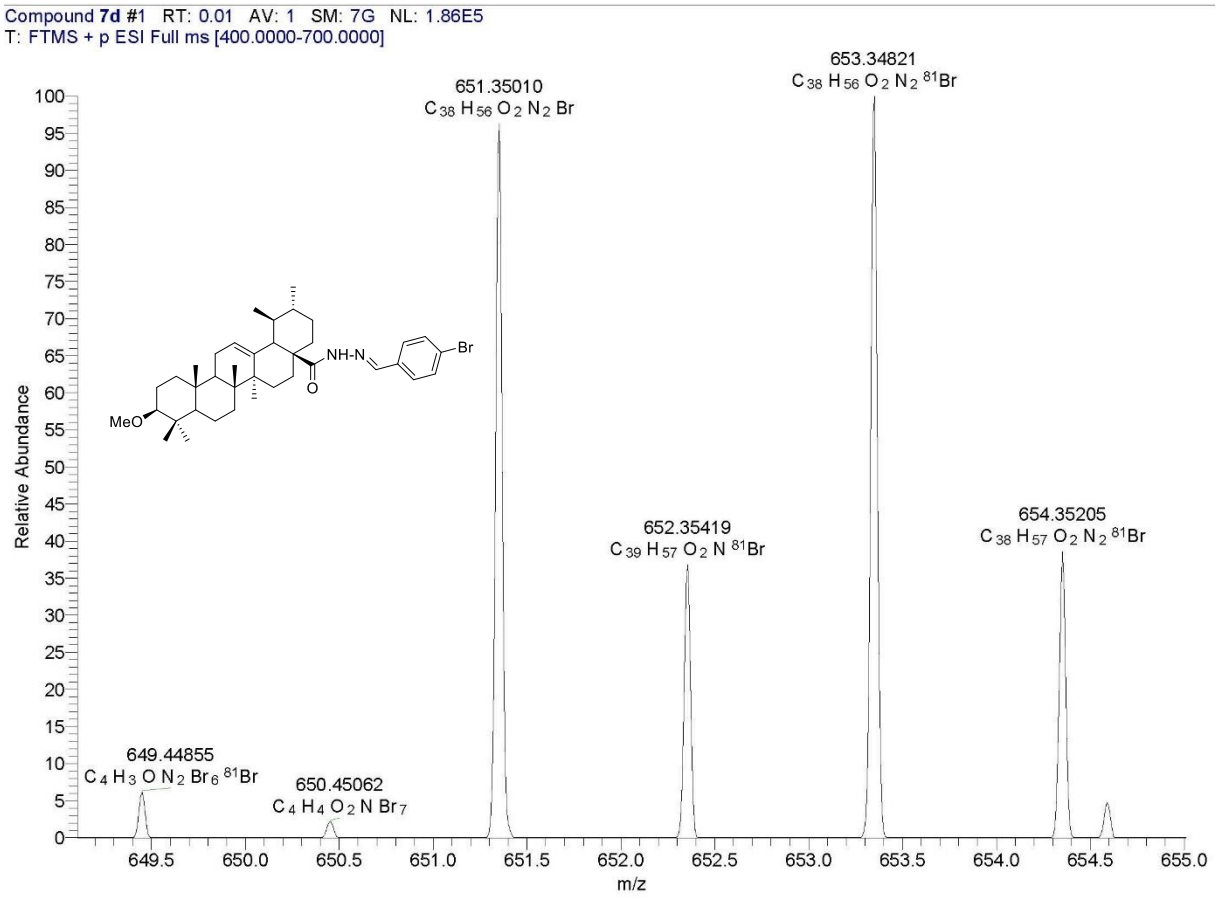
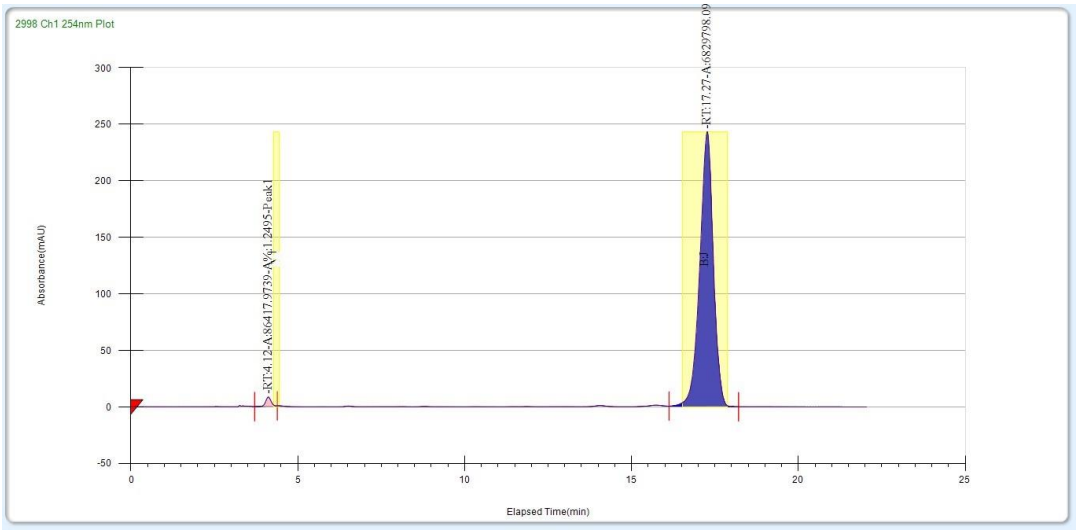


Figure S41: ESI-HRMS spectrum of compound **7d**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	1,2495		4.12 min	8,0354
7d	Peak2	2	98,7505	6916216,072	17.27 min	243,1984

Figure S42: HPLC chromatogram and purity analysis of compound **7d**

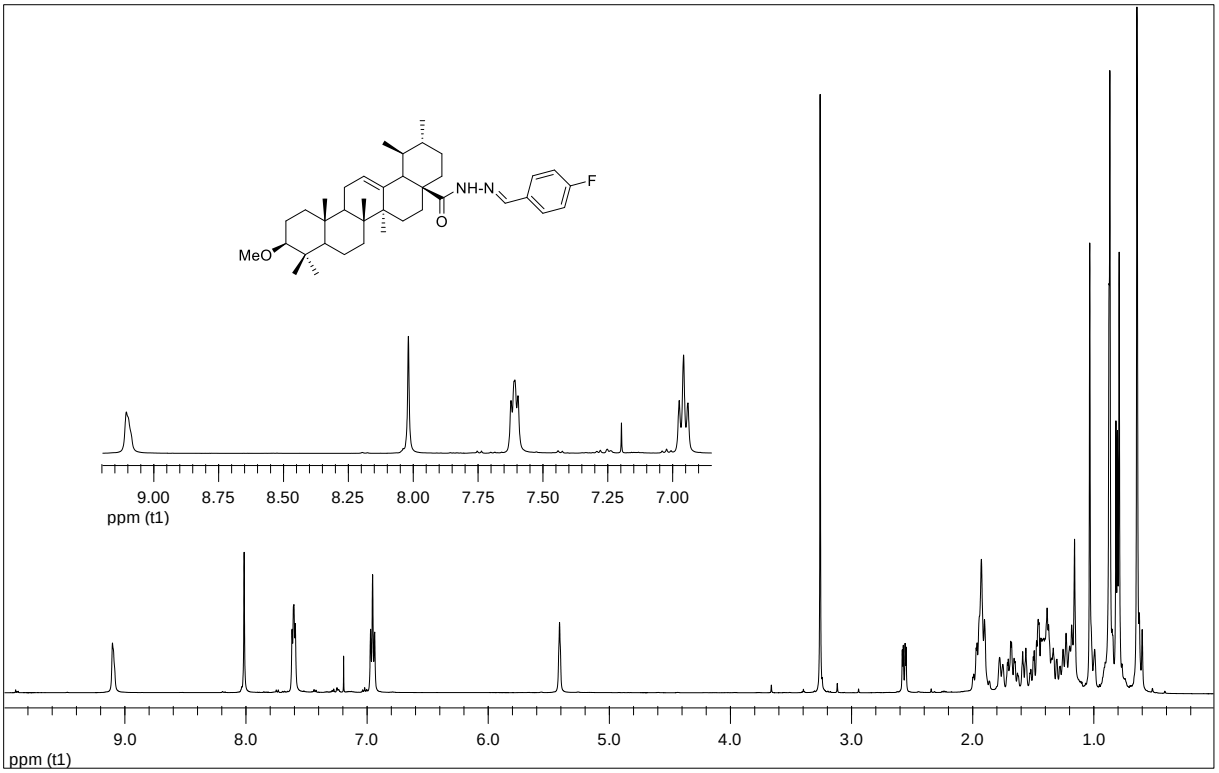


Figure S43: ^{1}H NMR spectrum of **7e** ($CDCl_3$, 500 MHz)

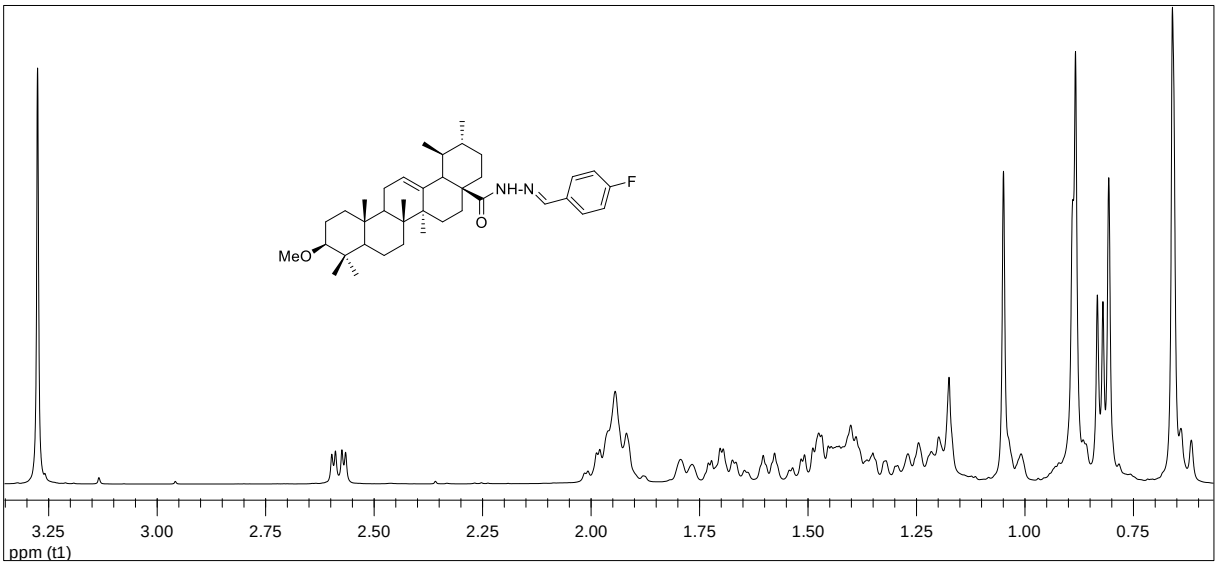


Figure S44: Extended ^{1}H NMR spectrum of **7e** ($CDCl_3$, 500 MHz)

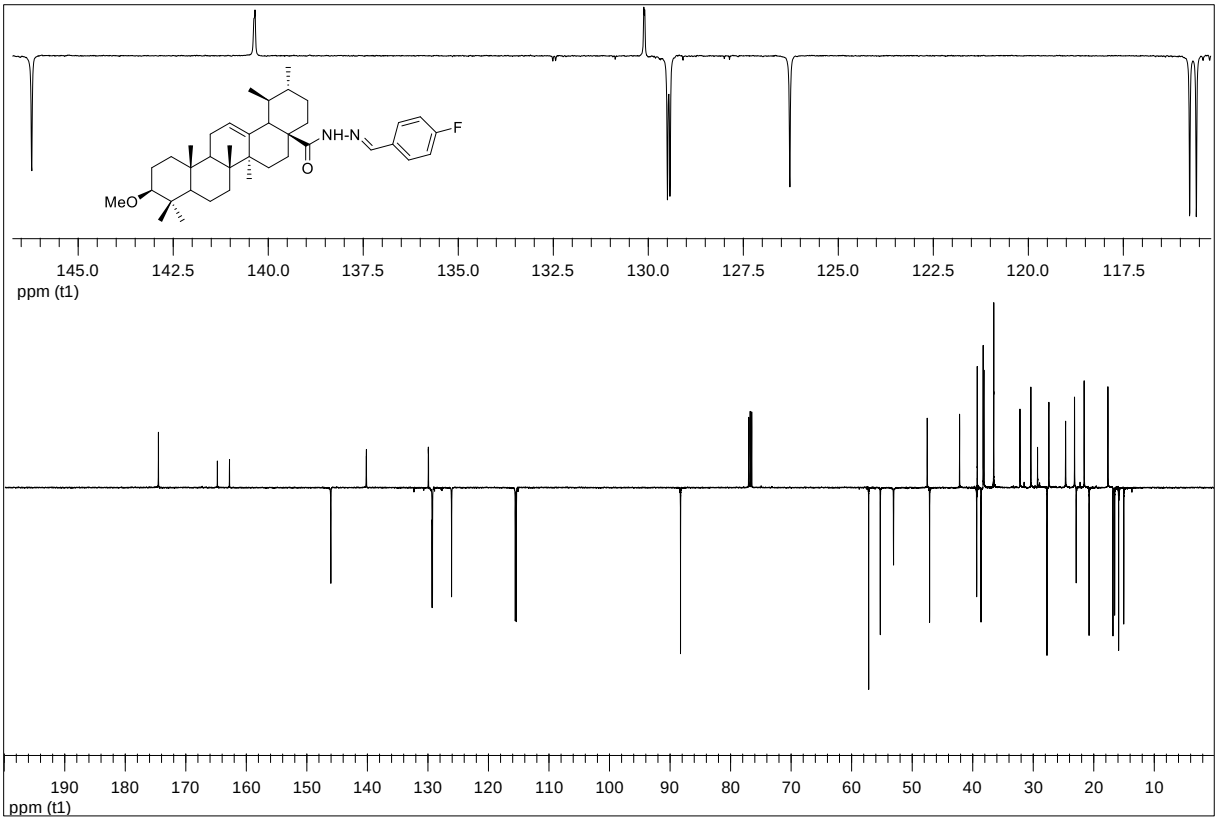


Figure S45: ^{13}C APT NMR spectrum of **7e** ($CDCl_3$, 125 MHz)

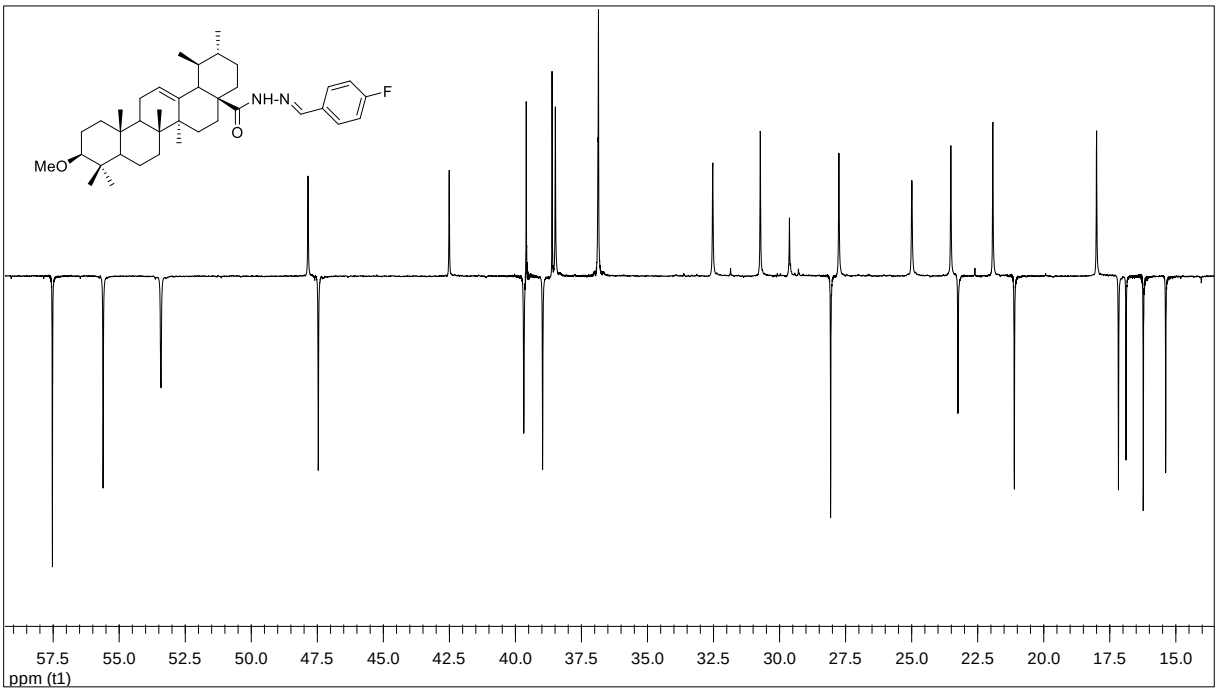


Figure S46: Extended ^{13}C APT NMR spectrum of **7e** ($CDCl_3$, 125 MHz)

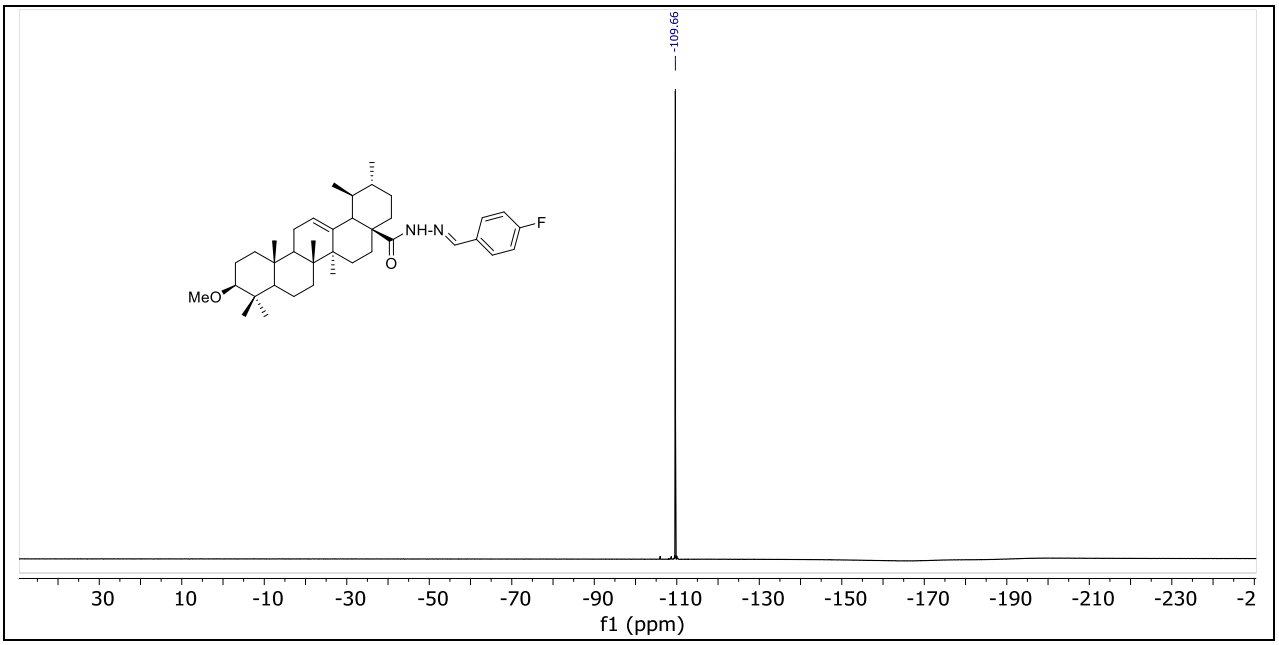


Figure S47: ^{19}F NMR spectrum of **7e** (471 MHz, $CDCl_3$)

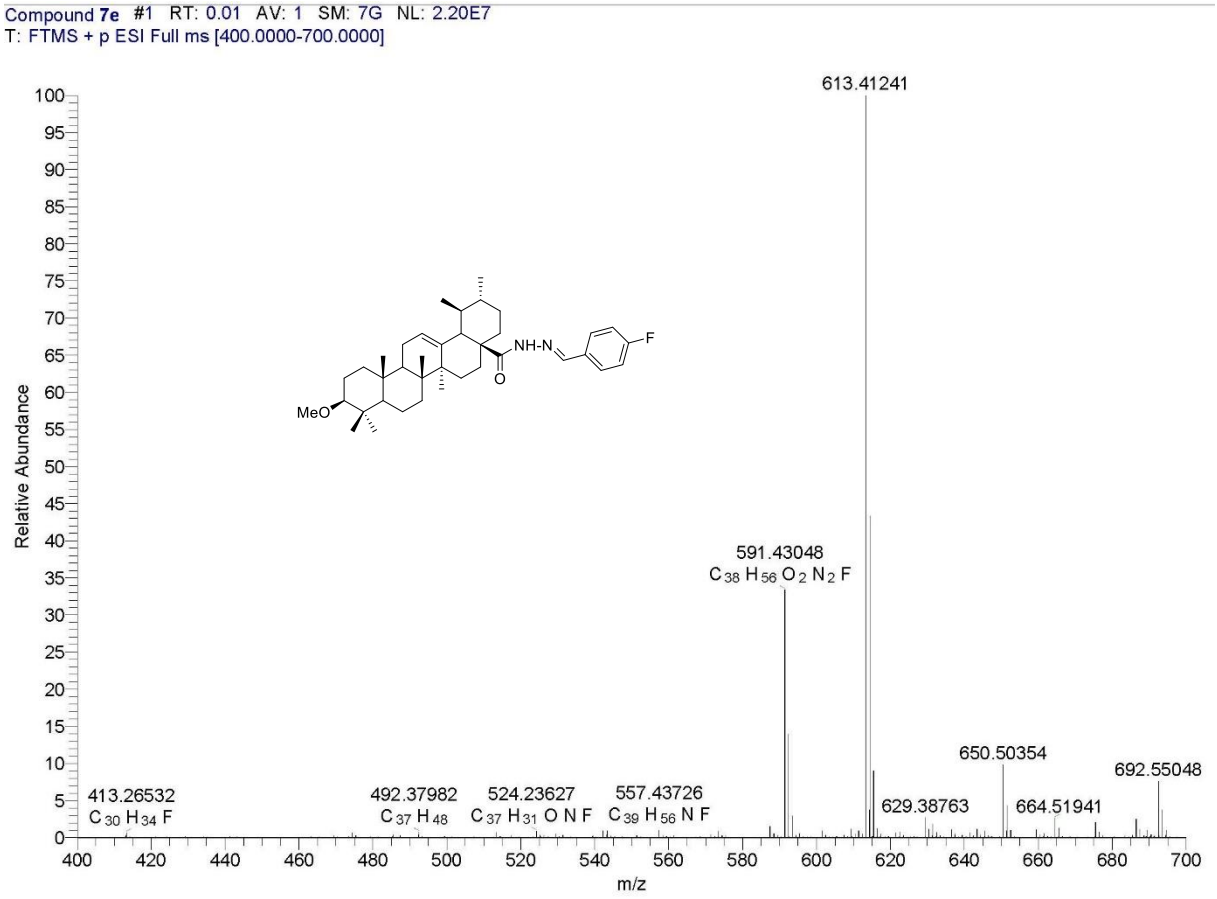
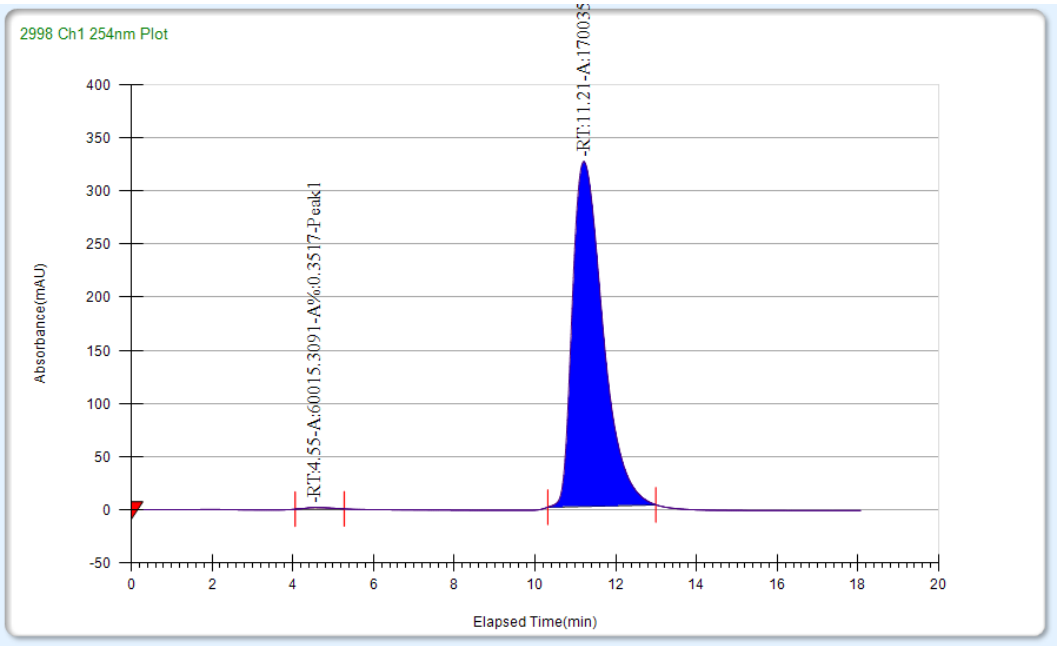


Figure S48: ESI-HRMS spectrum of compound **7e**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,3517		4.55 min	1,4863
7e	Peak2	2	99,6483	17063599,92	11.21 min	324,6118

Figure S49: HPLC chromatogram and purity analysis of compound **7e**

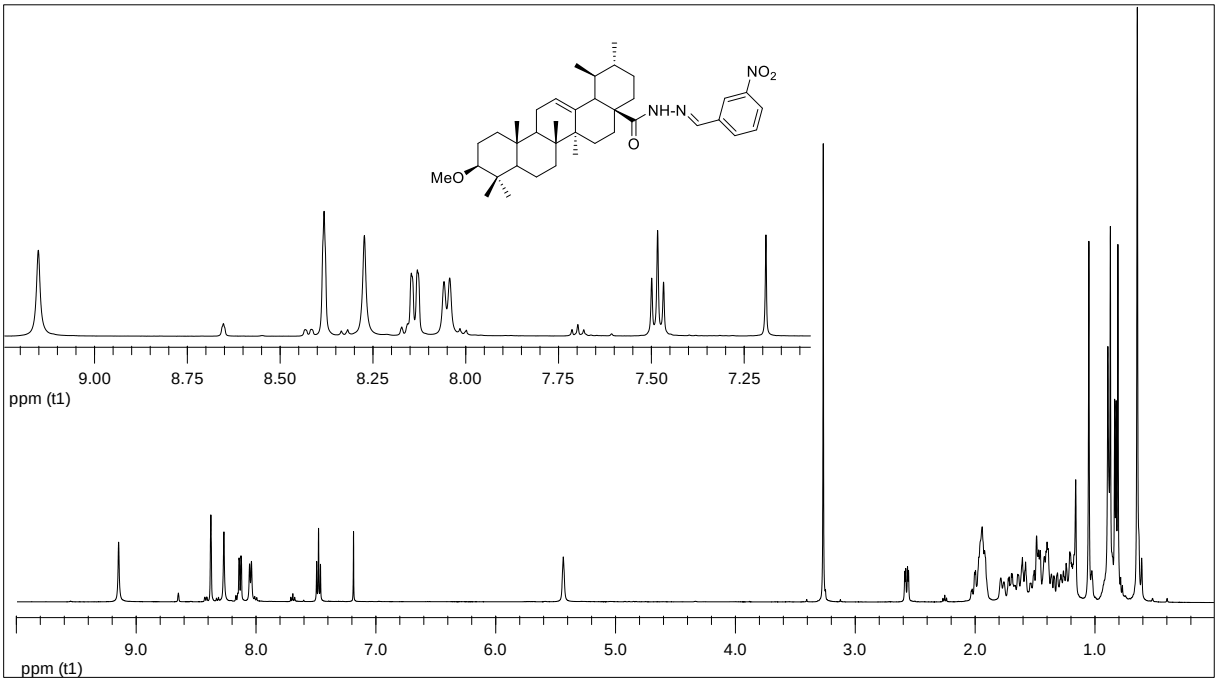


Figure S50: ^{1}H NMR spectrum of **7f** ($CDCl_3$, 500 MHz)

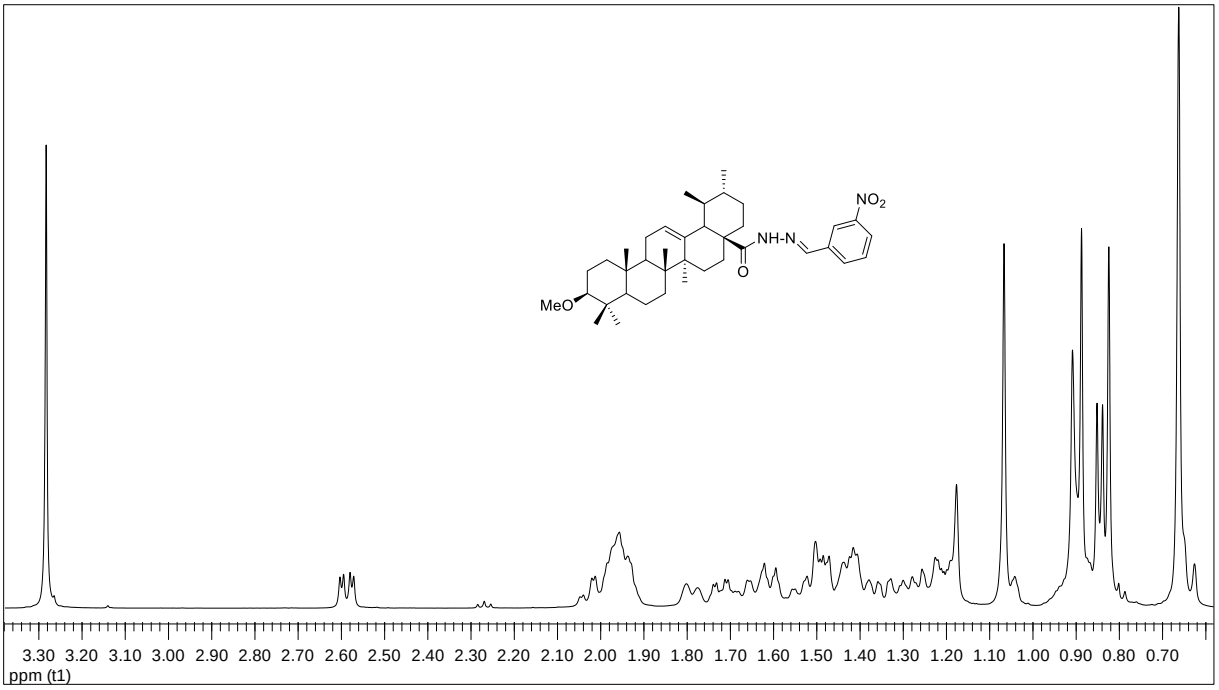


Figure S51: Extended ^{1}H NMR spectrum of **7f** ($CDCl_3$, 500 MHz)

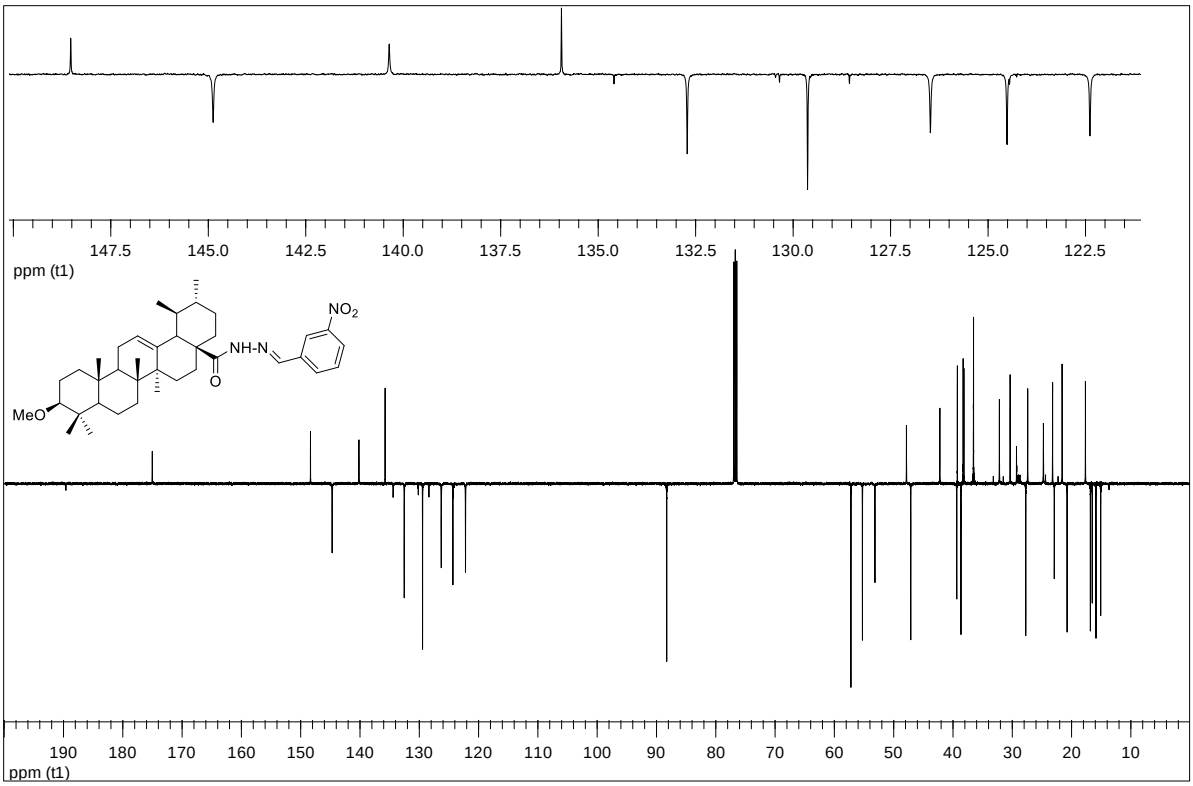


Figure S52: 13C APT NMR spectrum of 7f (CDCl3, 125 MHz)

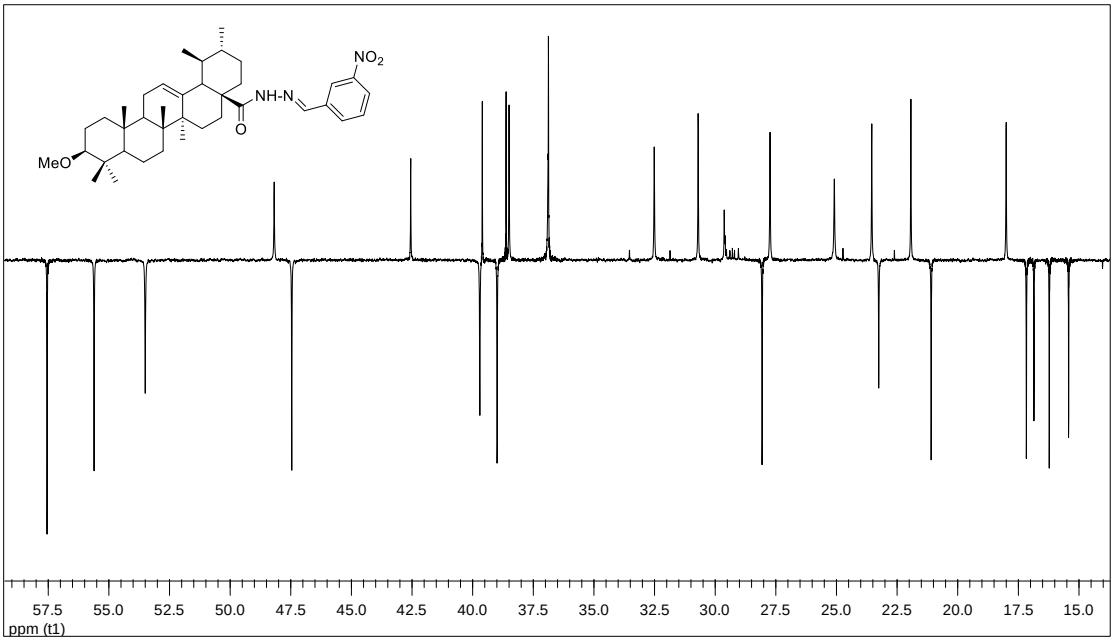


Figure S53: Extended ^{13}C APT NMR spectrum of **7f** ($CDCl_3$, 125 MHz)

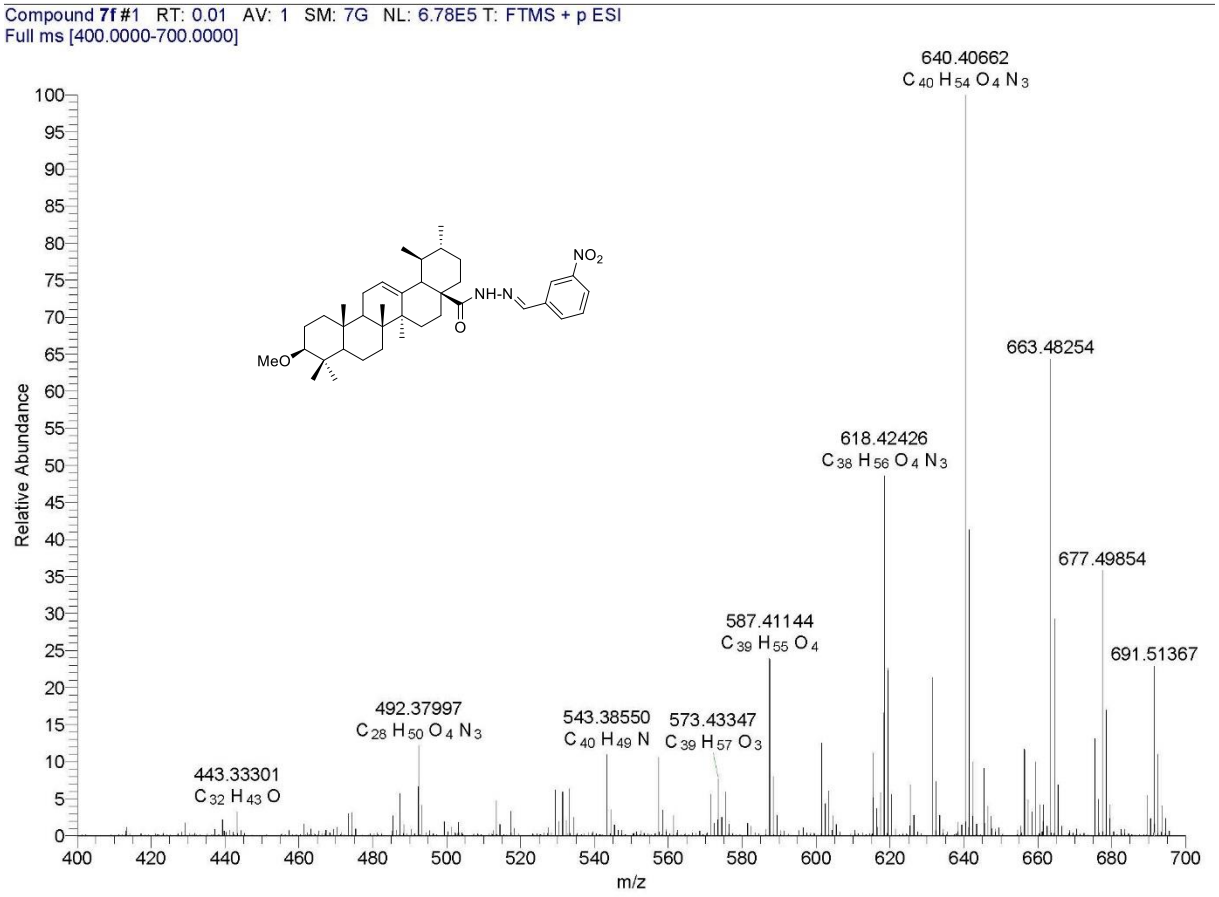
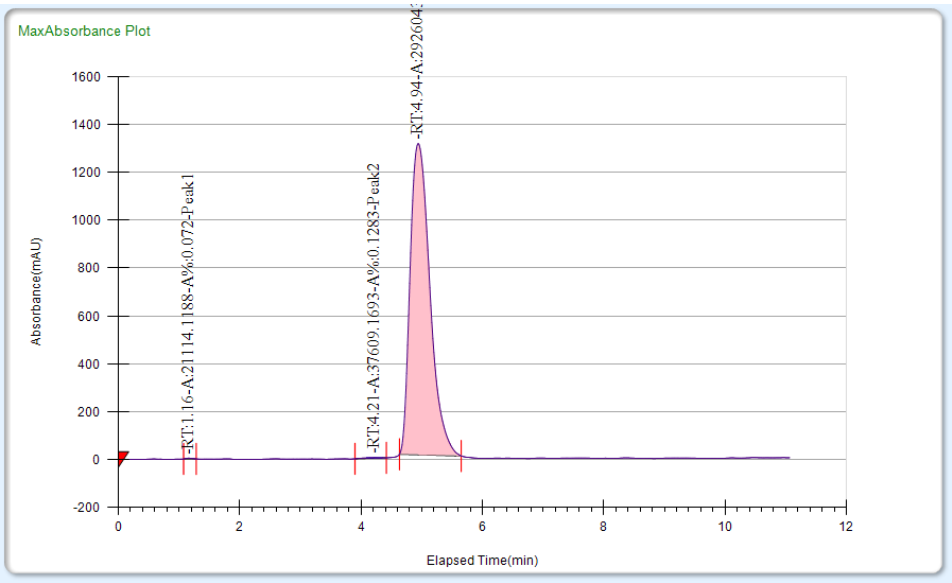


Figure S54: ESI-HRMS spectrum of compound **7f**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,072		1.16 min	3,0282
	Peak2	2	0,1283		4.21 min	1,9787
7f	Peak3	3	99,7997	29319154,4	4.94 min	1301,3202

Figure S55: HPLC chromatogram and purity analysis of compound **7f**

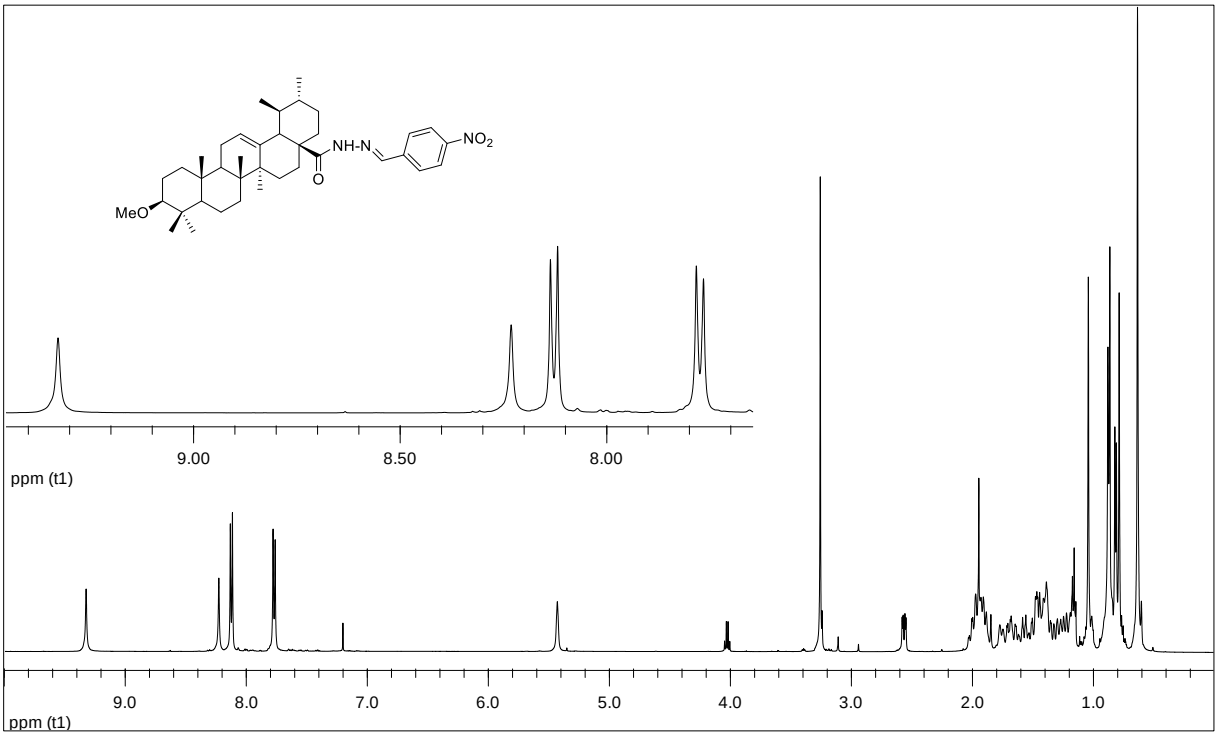


Figure S56: ^{1}H NMR spectrum of **7g** ($CDCl_3$, 500 MHz)

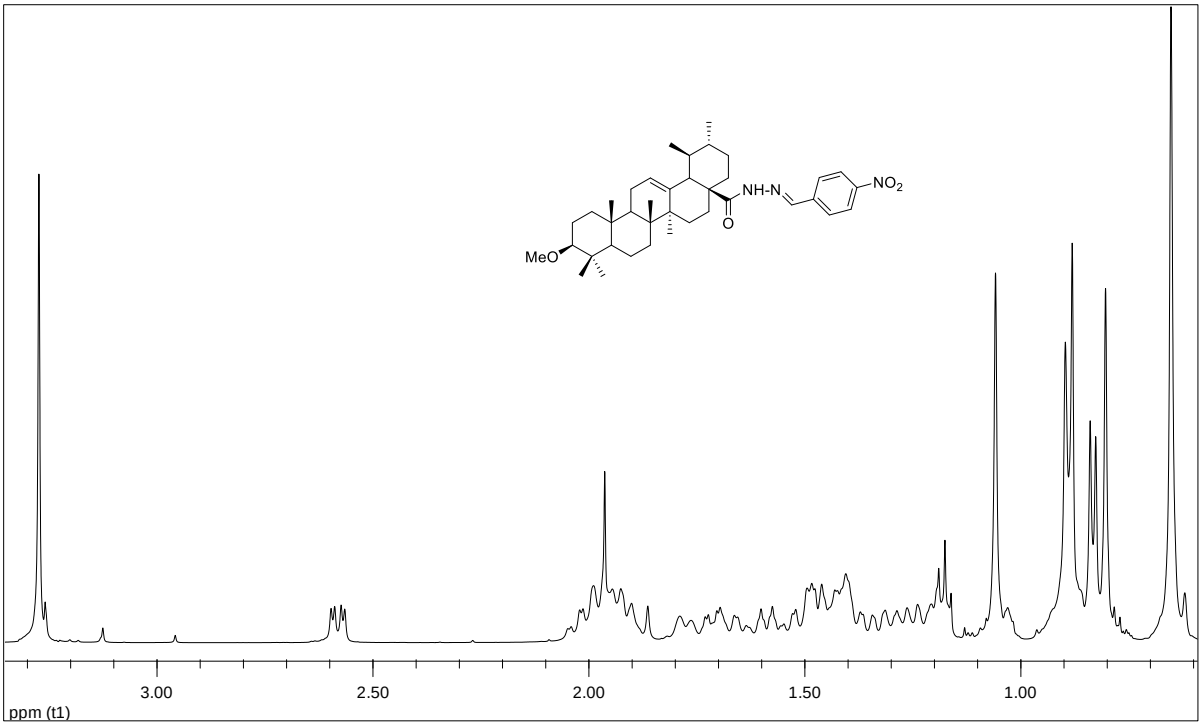


Figure S57: Extended ^{1}H NMR spectrum of **7g** ($CDCl_3$, 500 MHz)

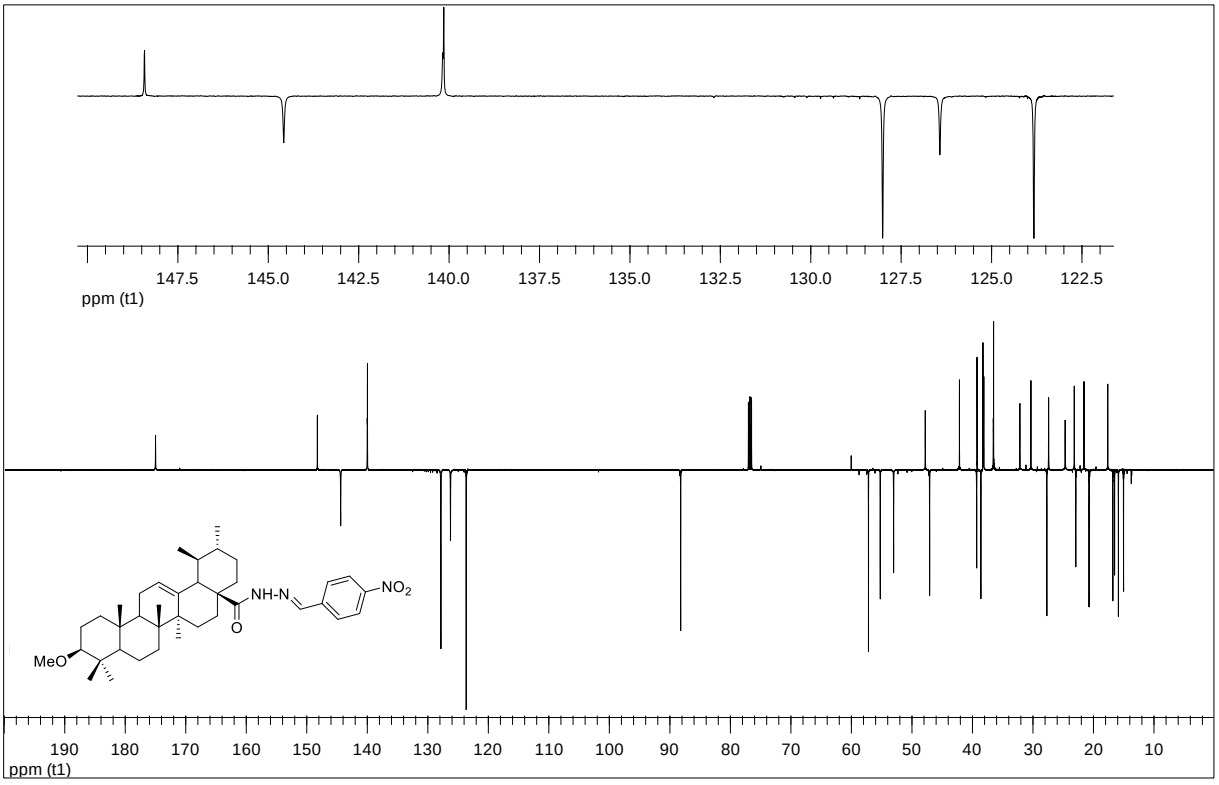


Figure S58: ^{13}C APT NMR spectrum of **7g** ($CDCl_3$, 125 MHz)

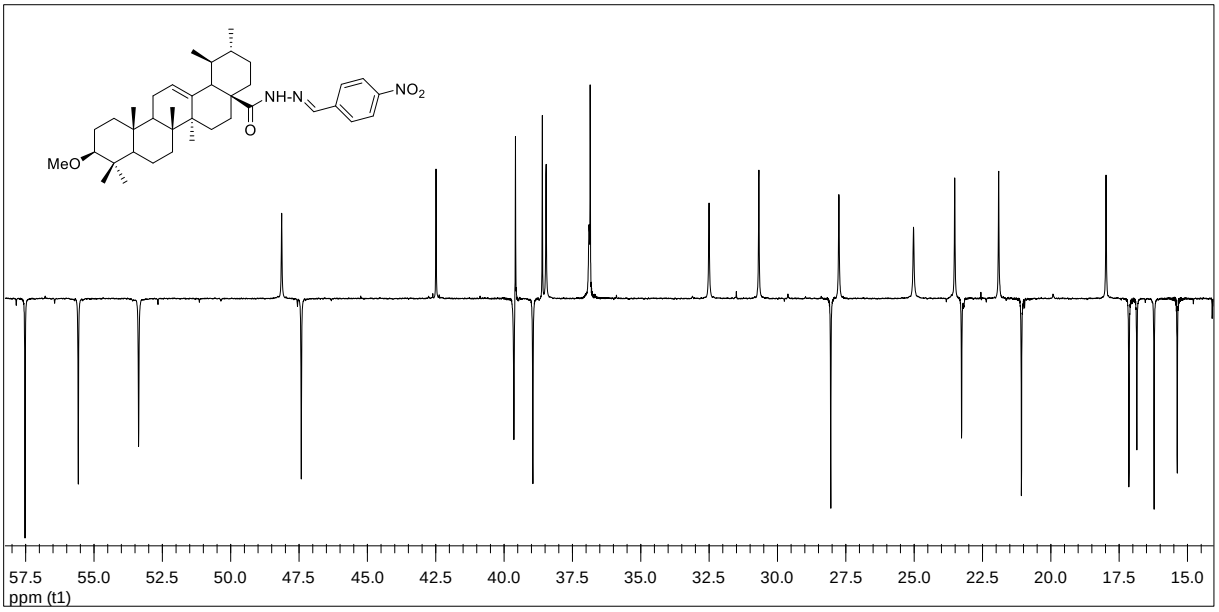


Figure S59: Extended ^{13}C APT NMR spectrum of **7g** ($CDCl_3$, 125 MHz)

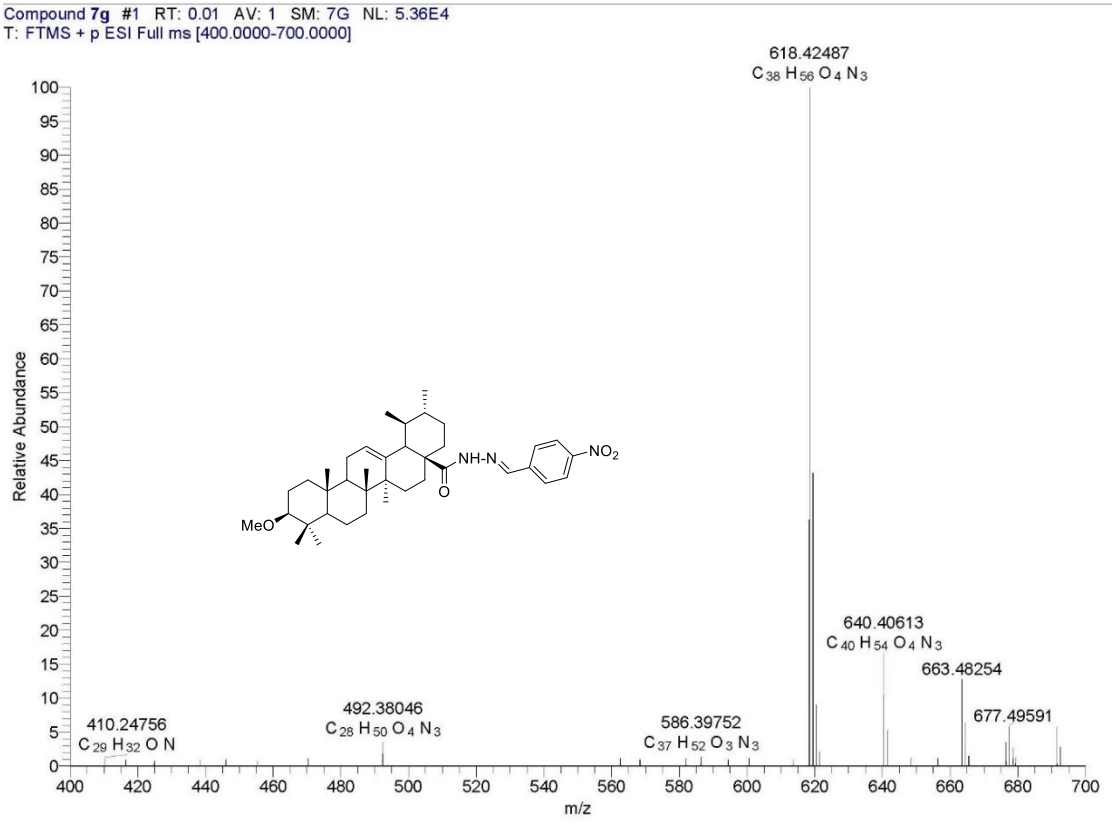
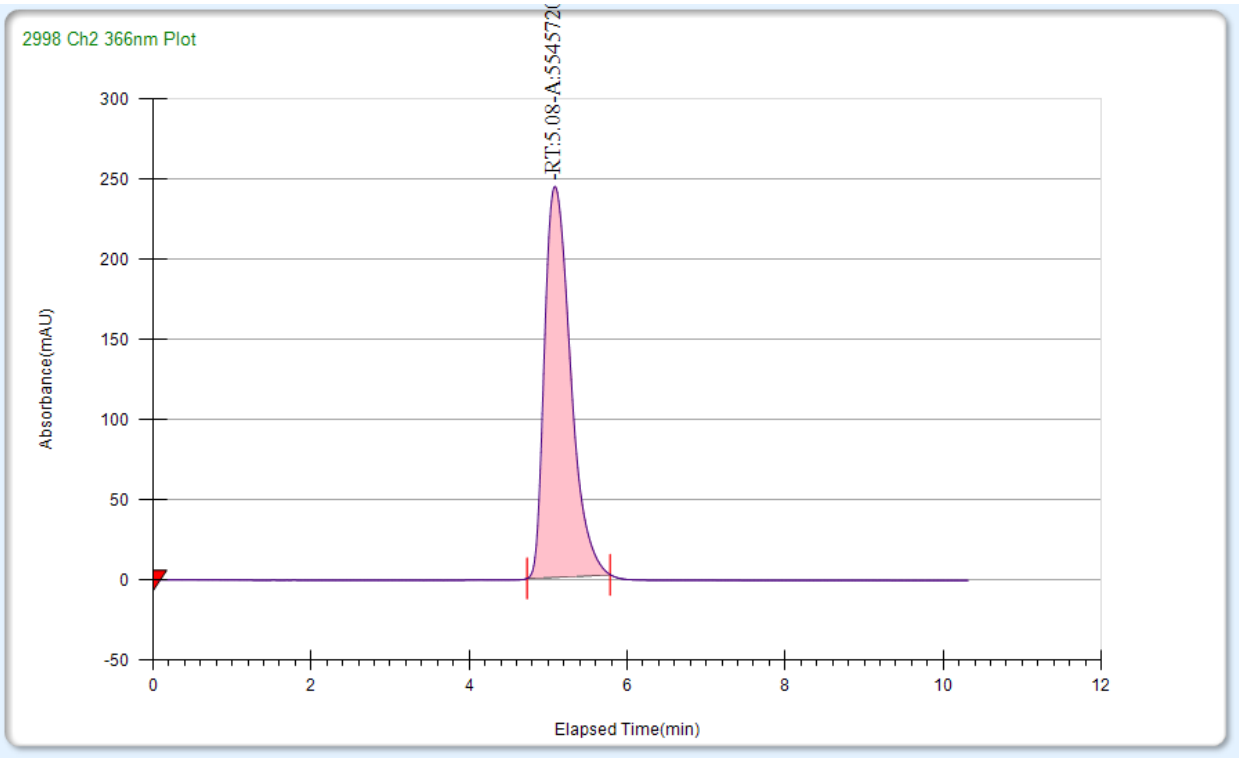


Figure S60: ESI-HRMS spectrum of compound **7g**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
7g	Peak1	1	99,999	5545720,806	5.08 min	243,7175

Figure S61: HPLC chromatogram and purity analysis of compound **7g**

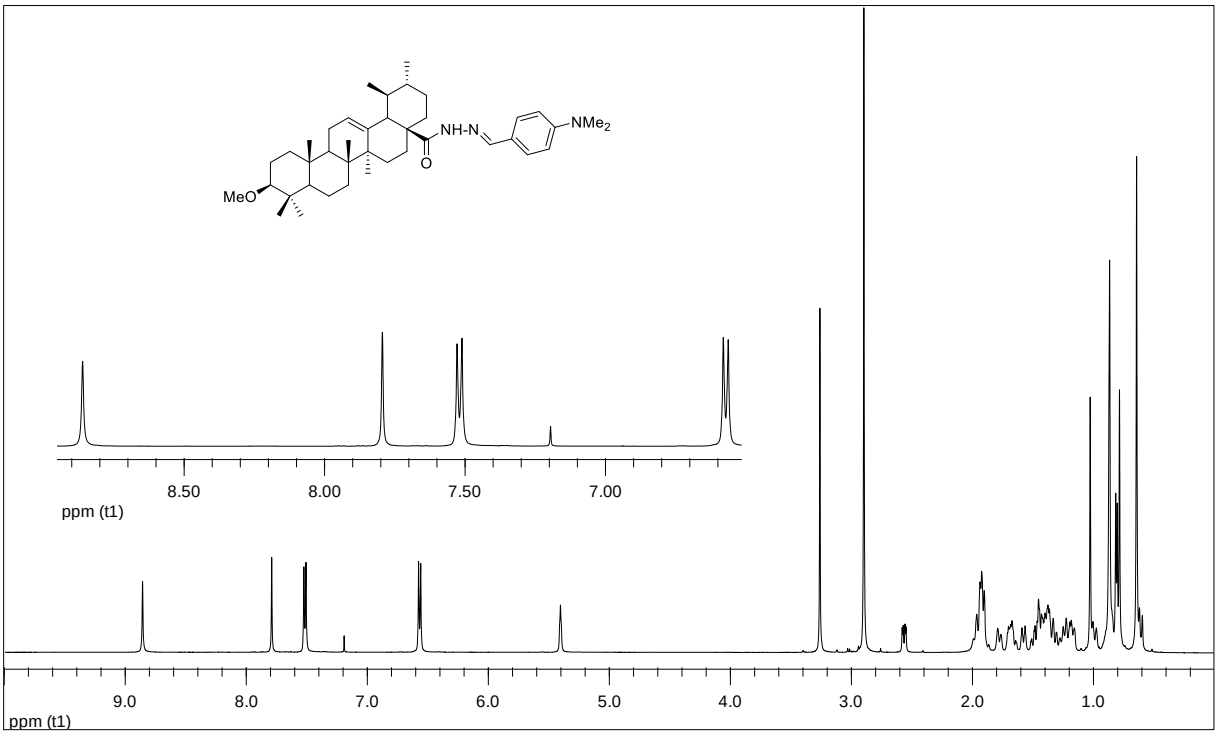


Figure S62: ^{1}H NMR spectrum of **7h** ($CDCl_3$, 500 MHz)

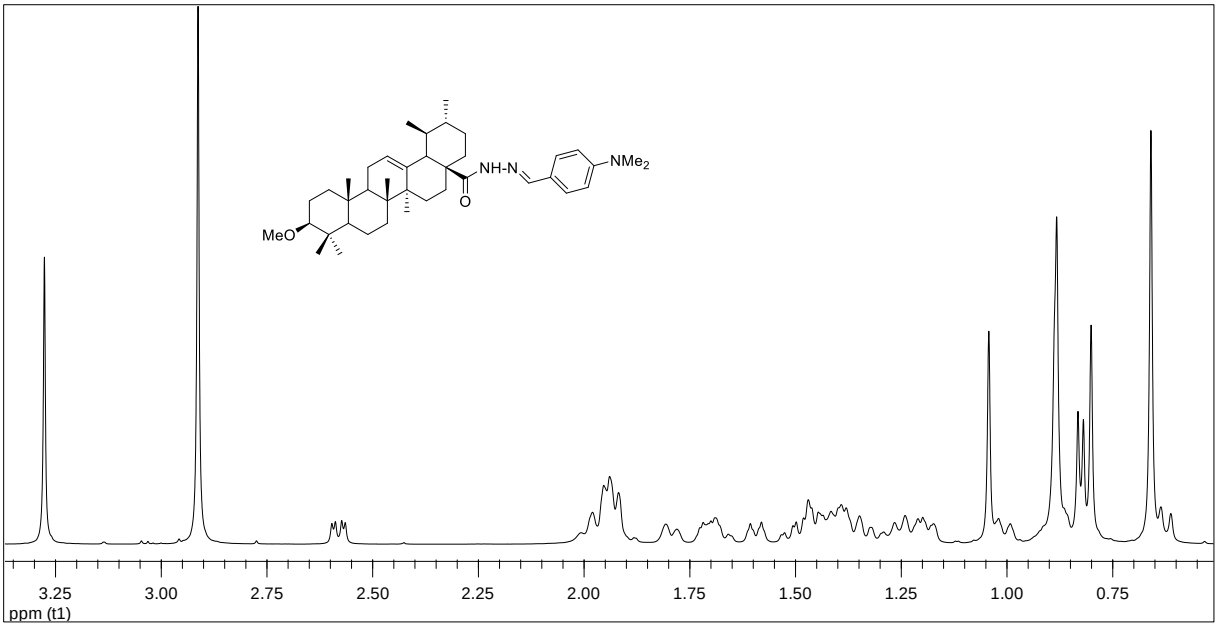


Figure S63: Extended ^{1}H NMR spectrum of **7h** ($CDCl_3$, 500 MHz)

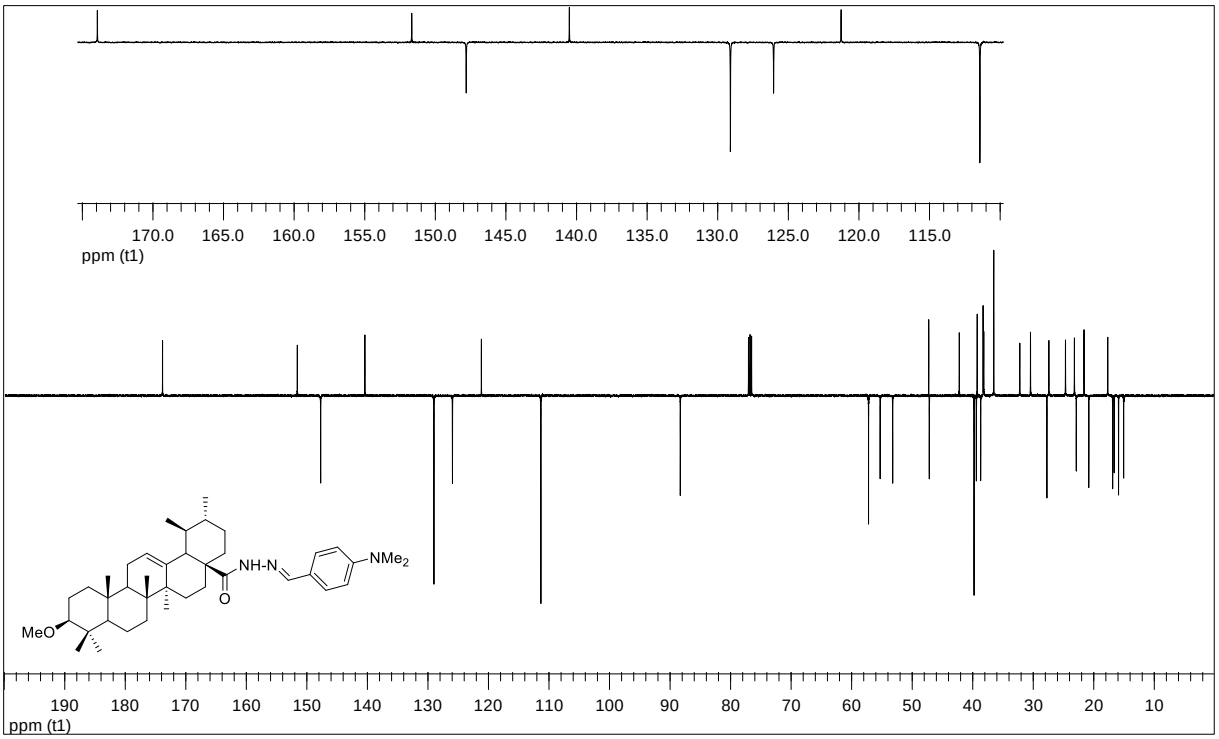


Figure S64: ^{13}C APT NMR spectrum of **7h** ($CDCl_3$, 125 MHz)

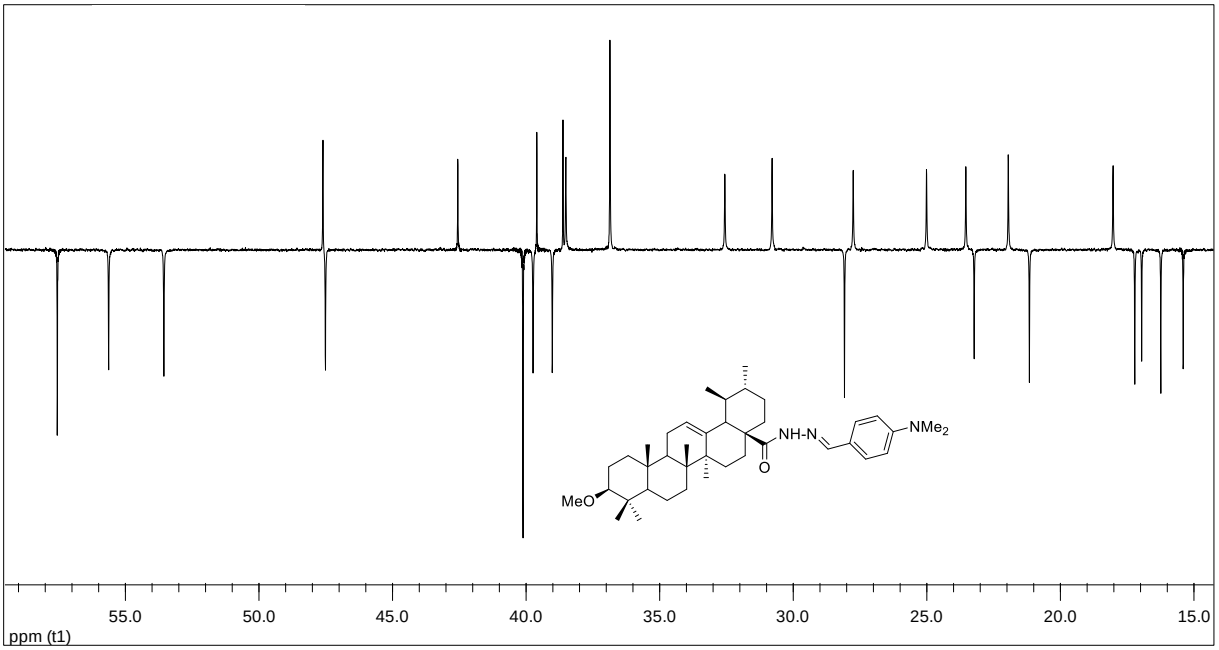


Figure S65: Extended ^{13}C APT NMR spectrum of **7h** ($CDCl_3$, 125 MHz)

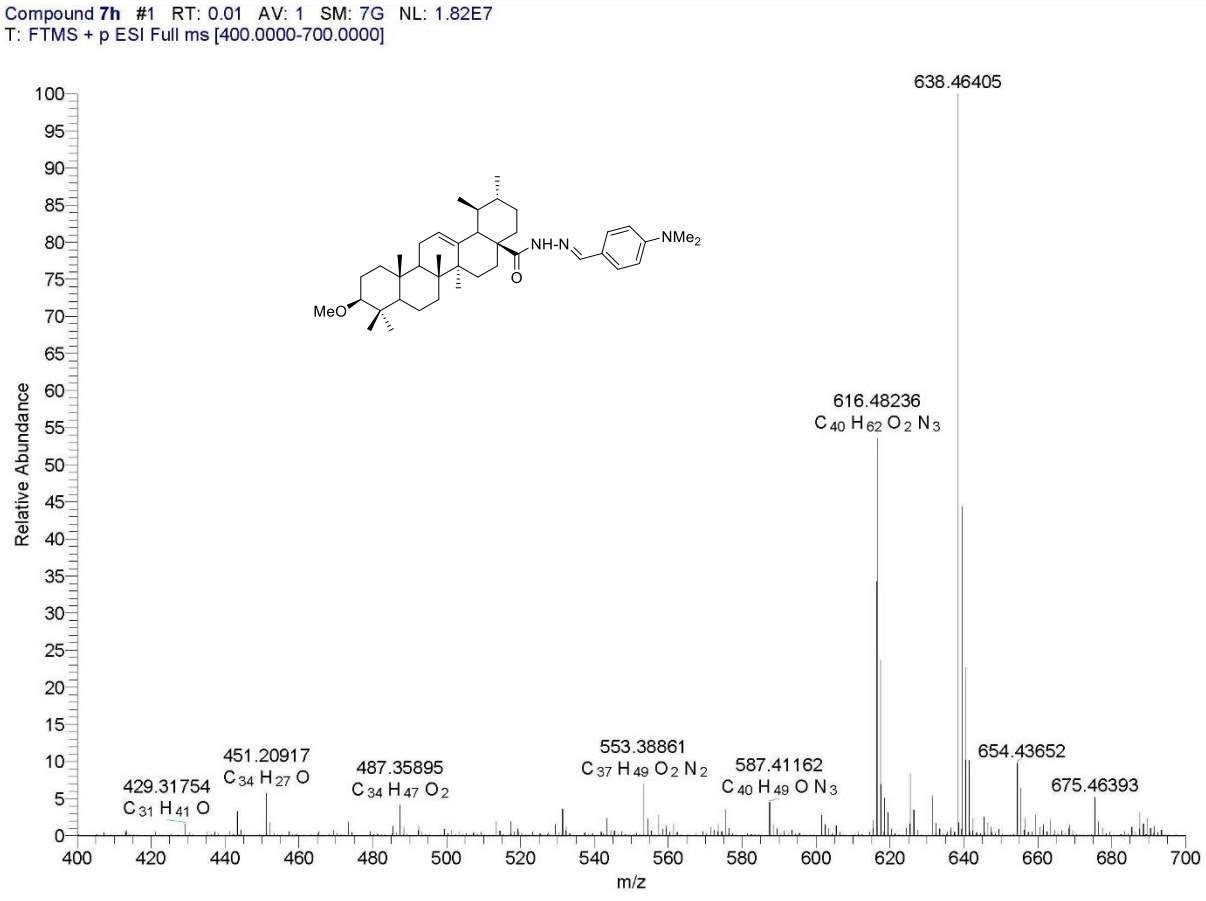
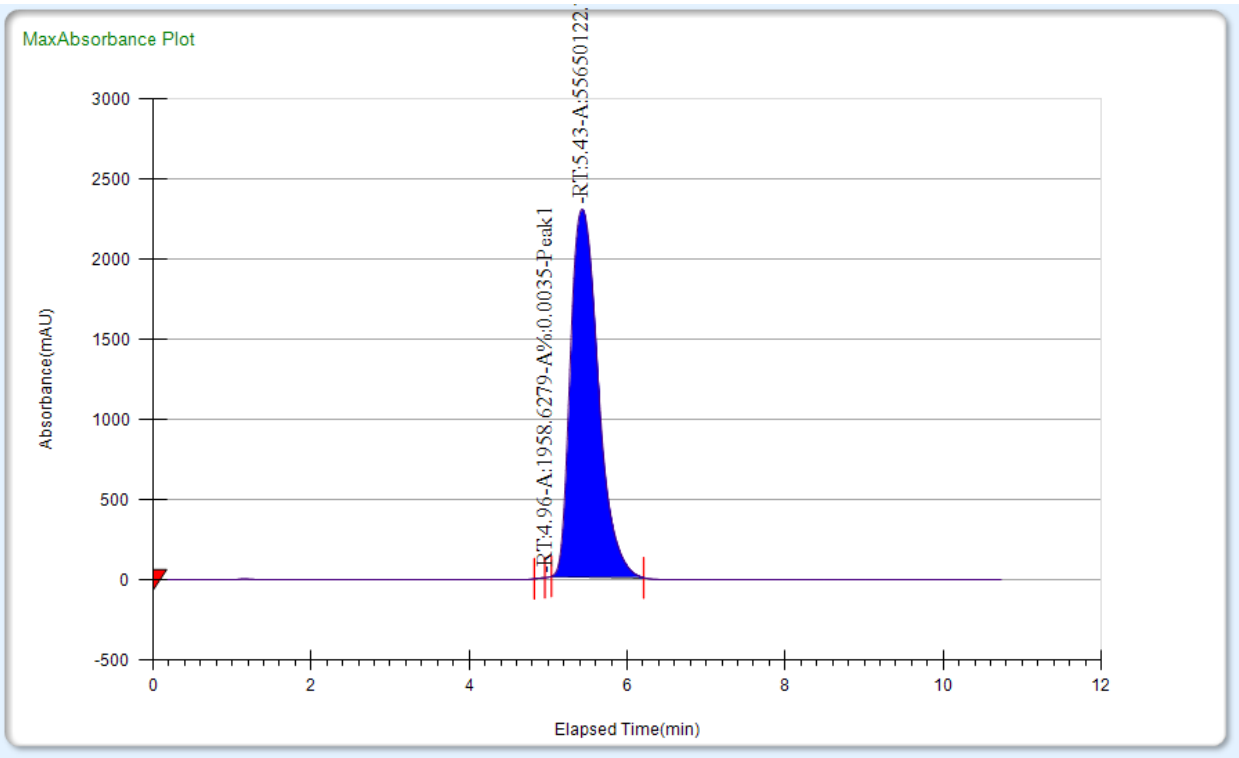


Figure S66: ESI-HRMS spectrum of compound **7h**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,0035		4.96 min	0,05
7h	Peak2	2	99,9965	55652081	5.43 min	2291,861

Figure S67: HPLC chromatogram and purity analysis of compound **7h**

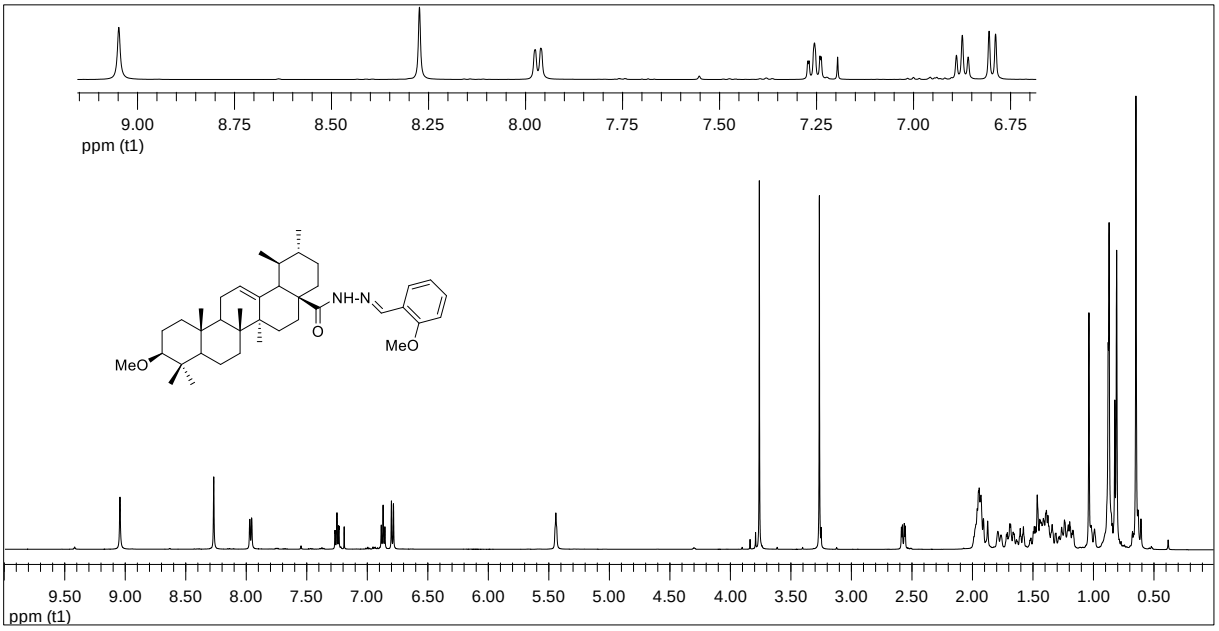


Figure S68: ^{1}H NMR spectrum of **7i** ($CDCl_3$, 500 MHz)

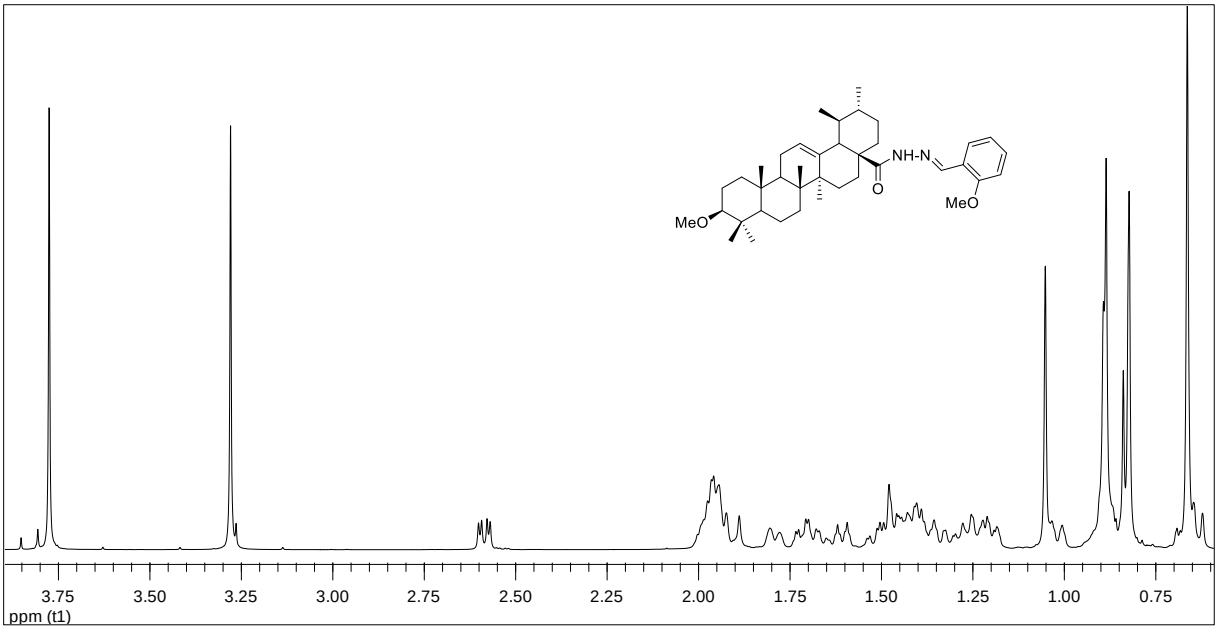


Figure S69: Extended ^{1}H NMR spectrum of **7i** ($CDCl_3$, 500 MHz)

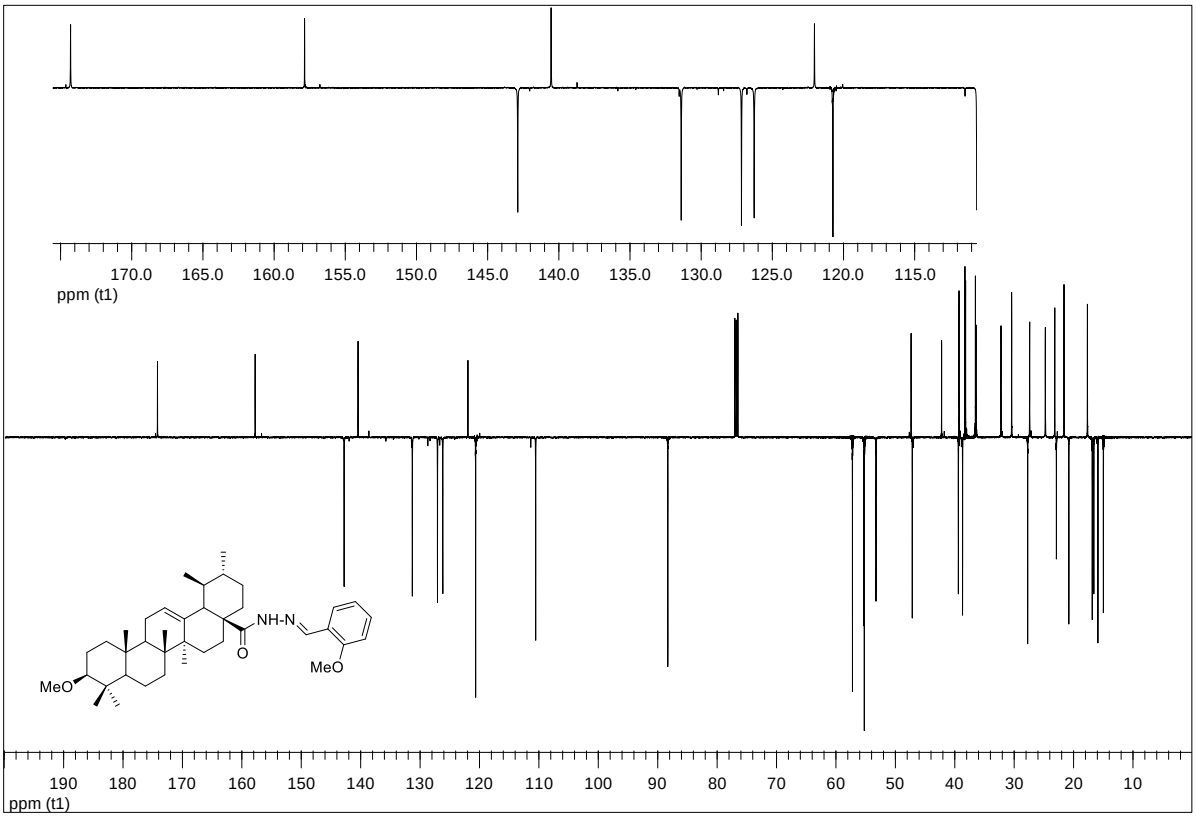


Figure S70: ^{13}C APT NMR spectrum of **7i** ($CDCl_3$, 125 MHz)

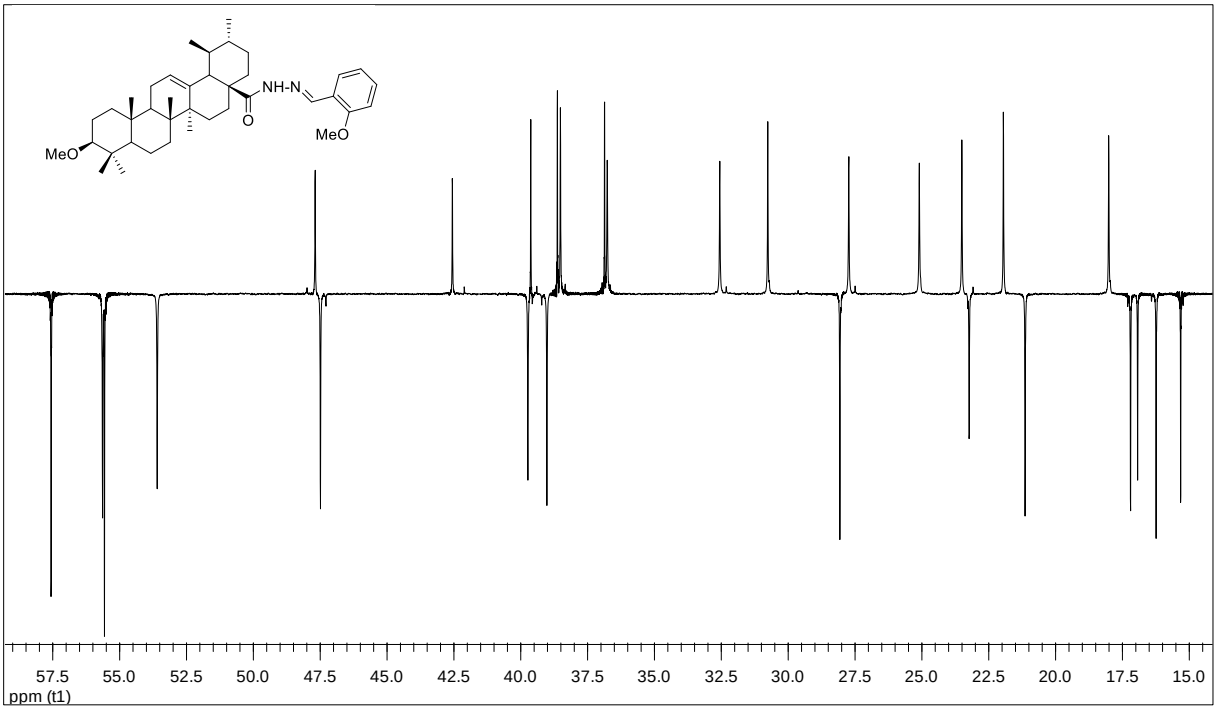


Figure S71: Extended ^{13}C APT NMR spectrum of **7i** ($CDCl_3$, 125 MHz)

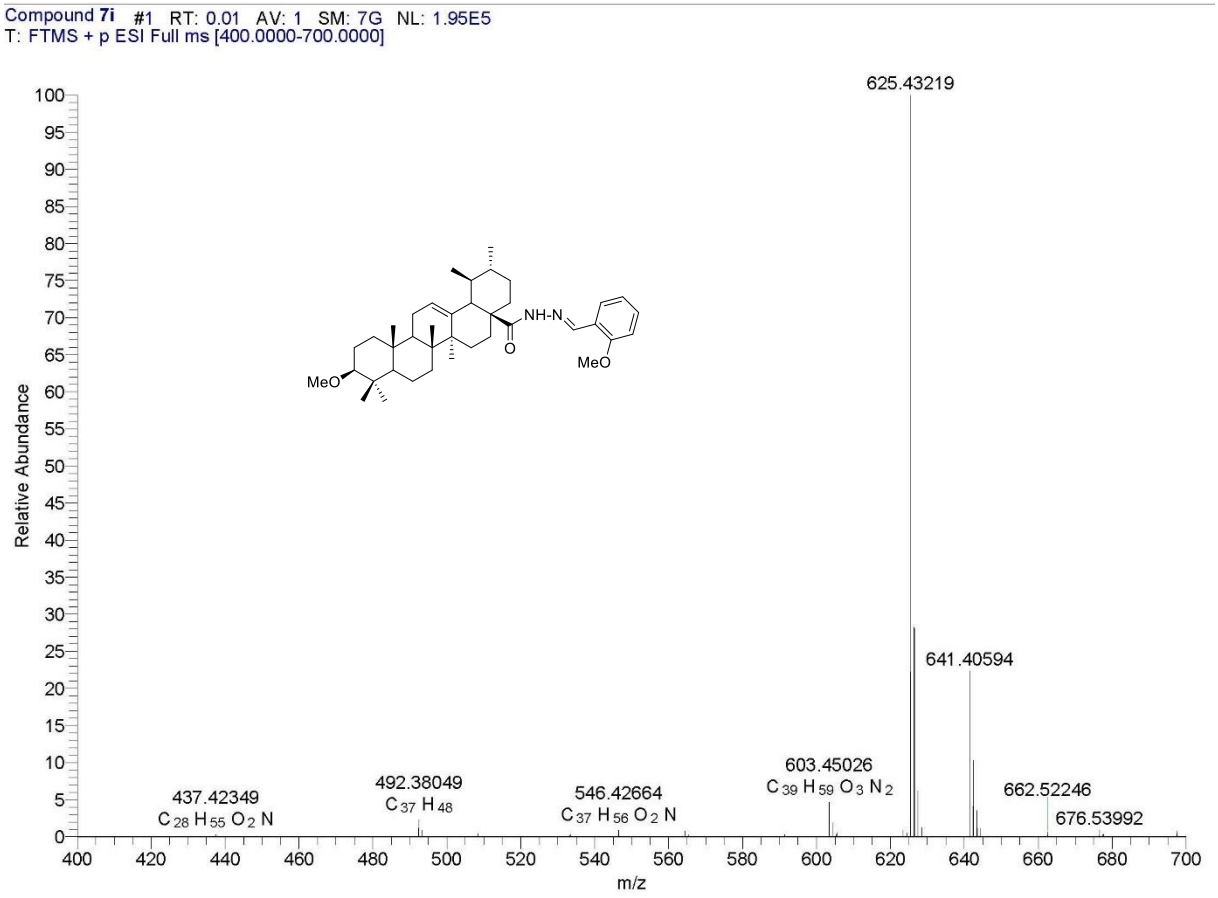
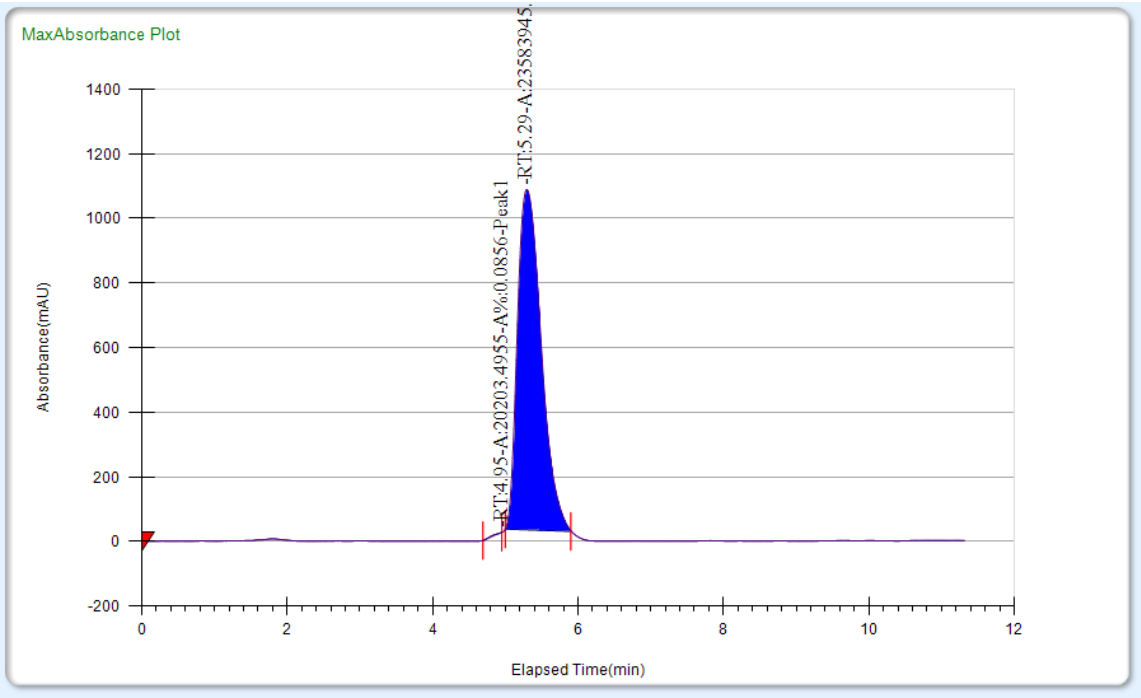


Figure S72: ESI-HRMS spectrum of compound **7i**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,0856		4.95 min	0
7i	Peak2	2	99,9144	23604149	5.29 min	1053,779

Figure S73: HPLC chromatogram and purity analysis of compound **7i**

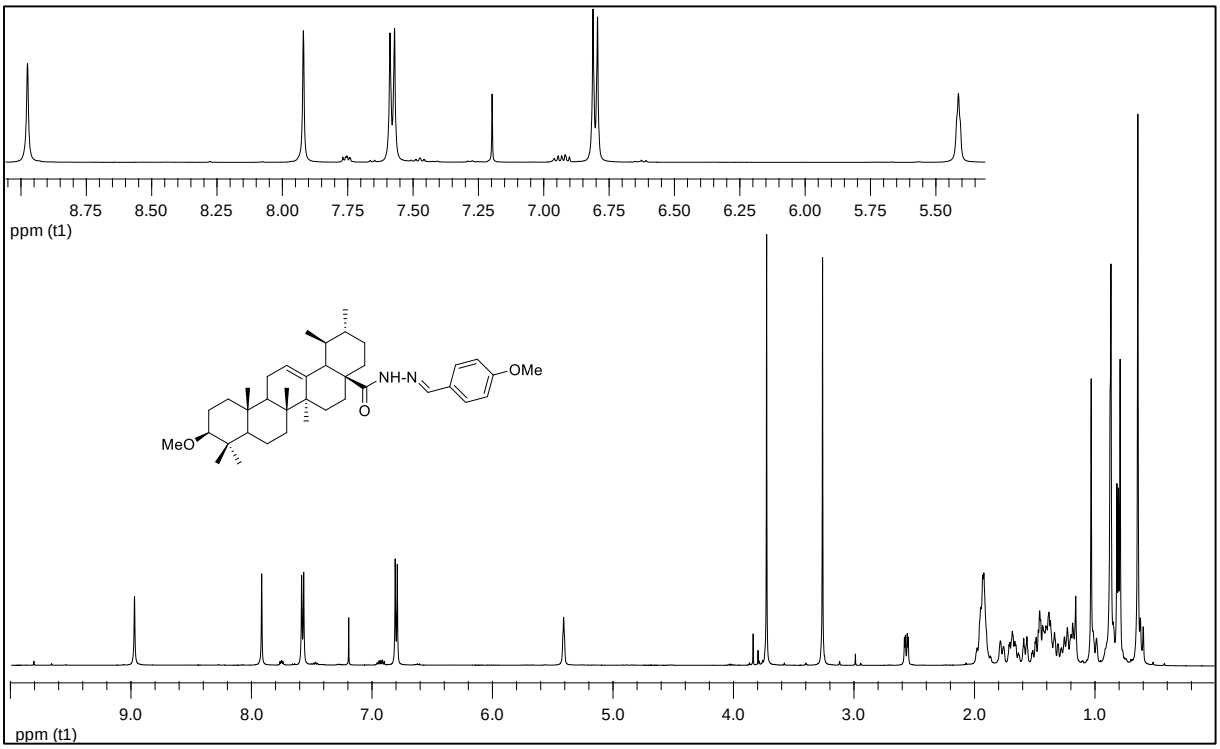


Figure S74: ^{1}H NMR spectrum of **7j** ($CDCl_3$, 500 MHz)

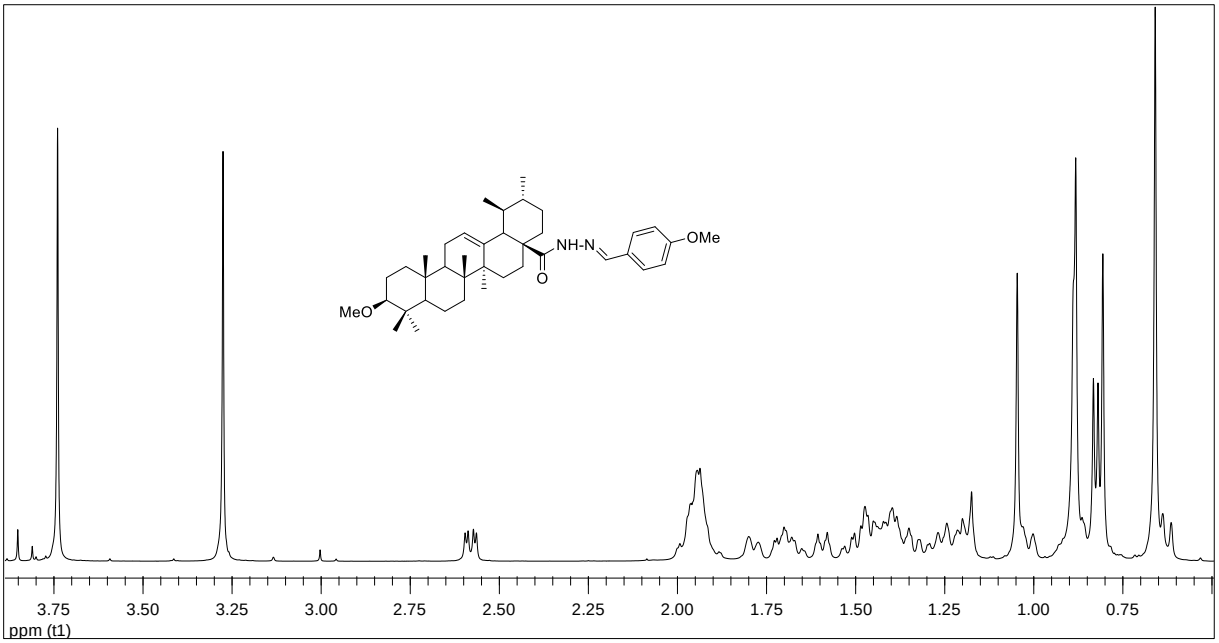


Figure S75: Extended ^{1}H NMR spectrum of **7j** ($CDCl_3$, 500 MHz)

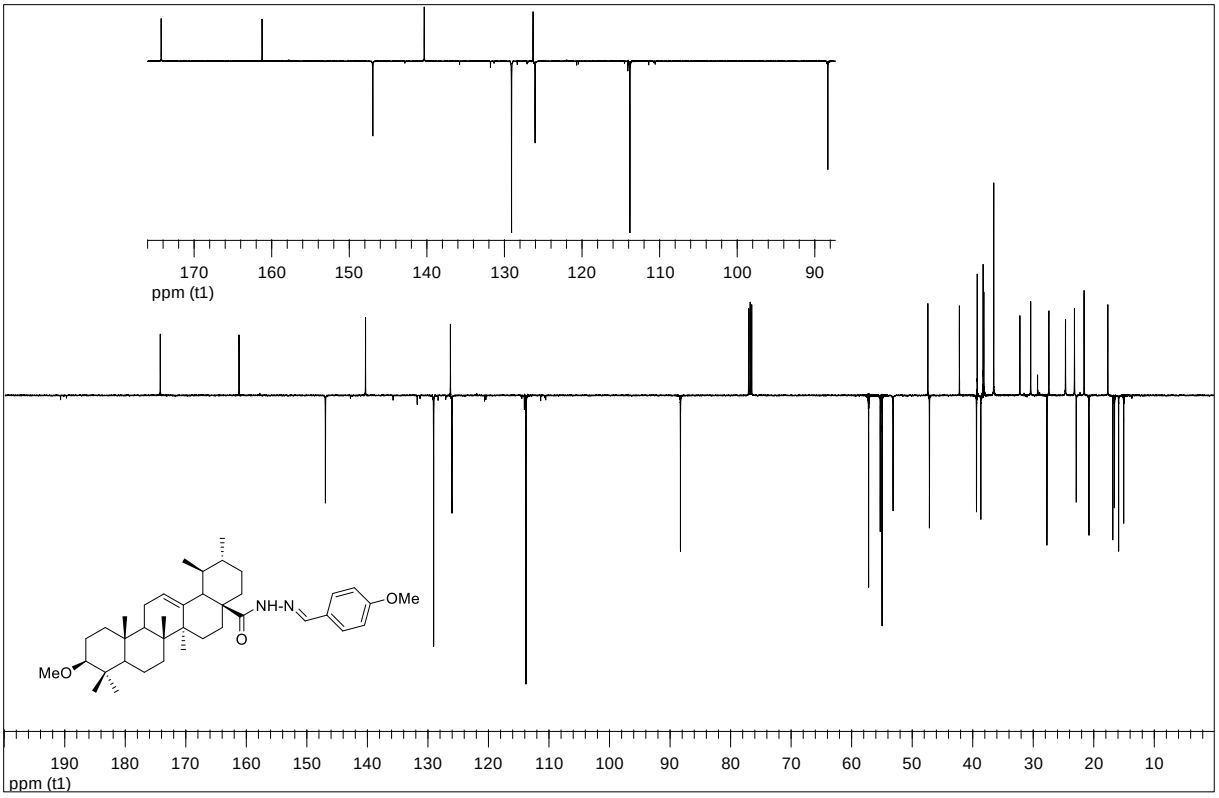


Figure S76: ^{13}C APT NMR spectrum of **7j** ($CDCl_3$, 125 MHz)

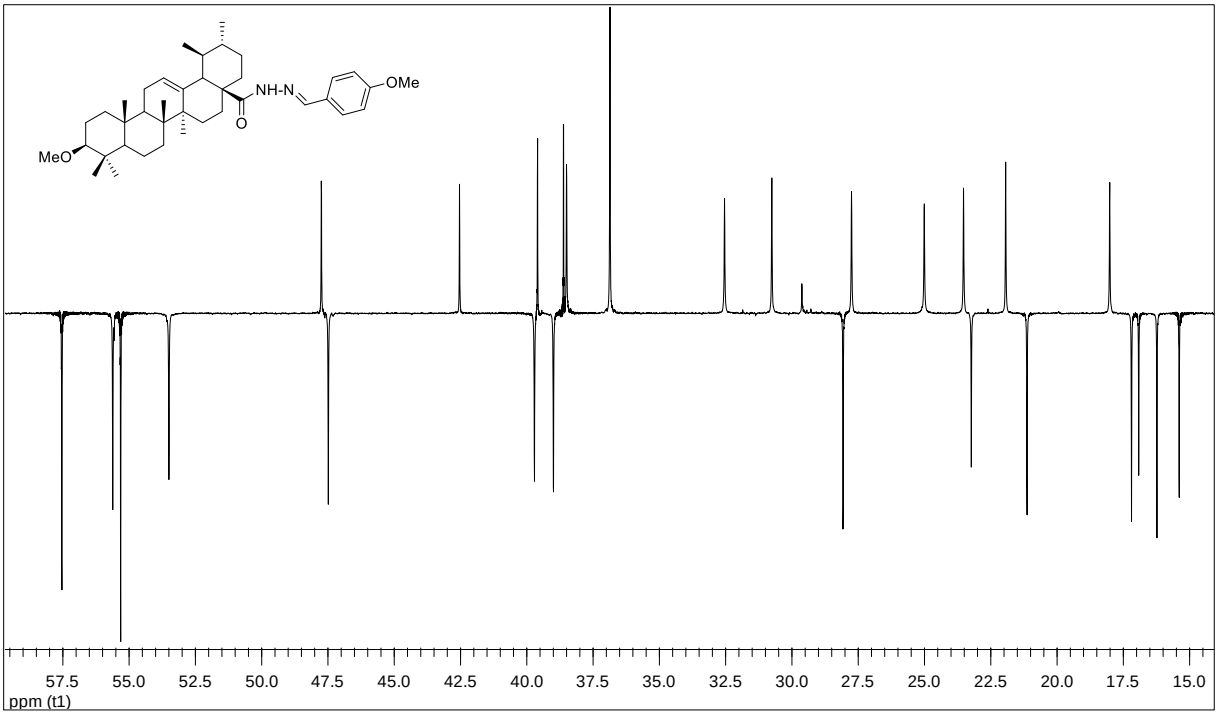


Figure S77: Extended ^{13}C APT NMR spectrum of **7j** ($CDCl_3$, 125 MHz)

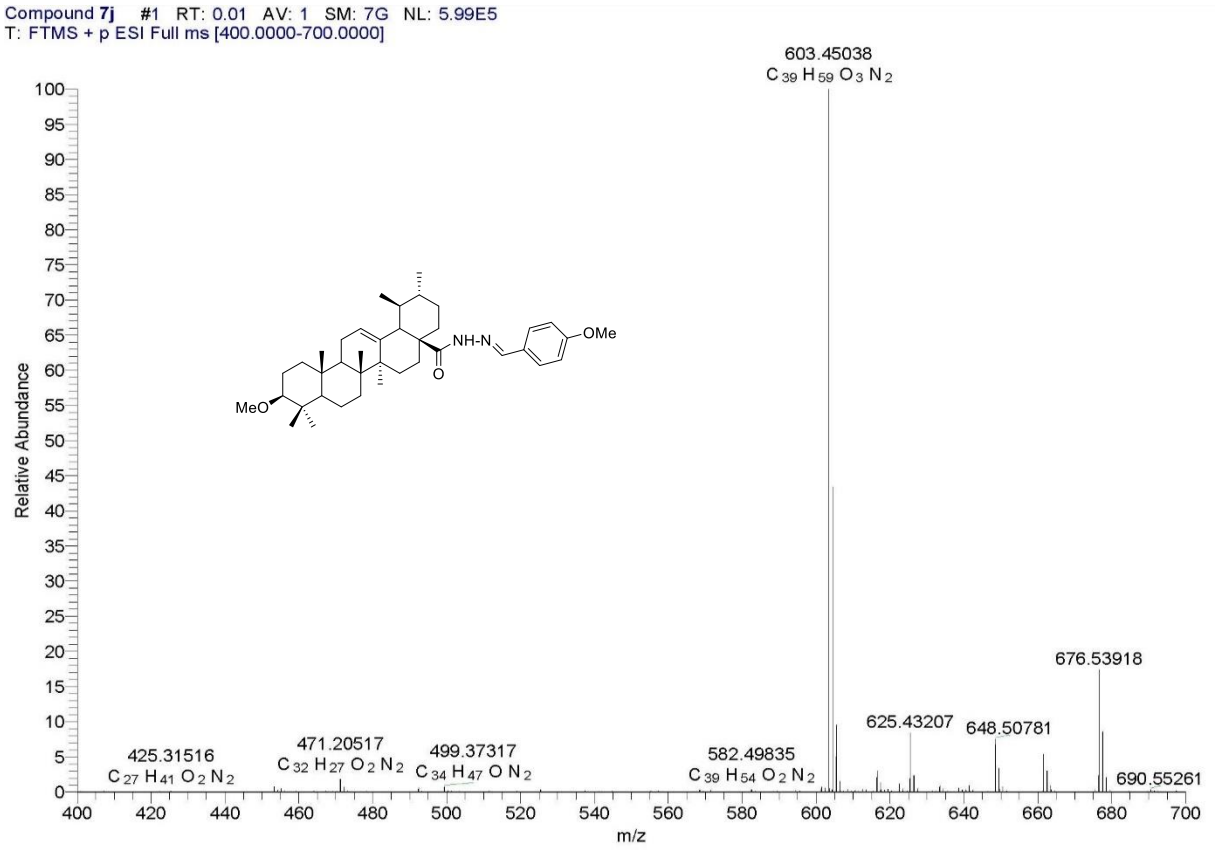
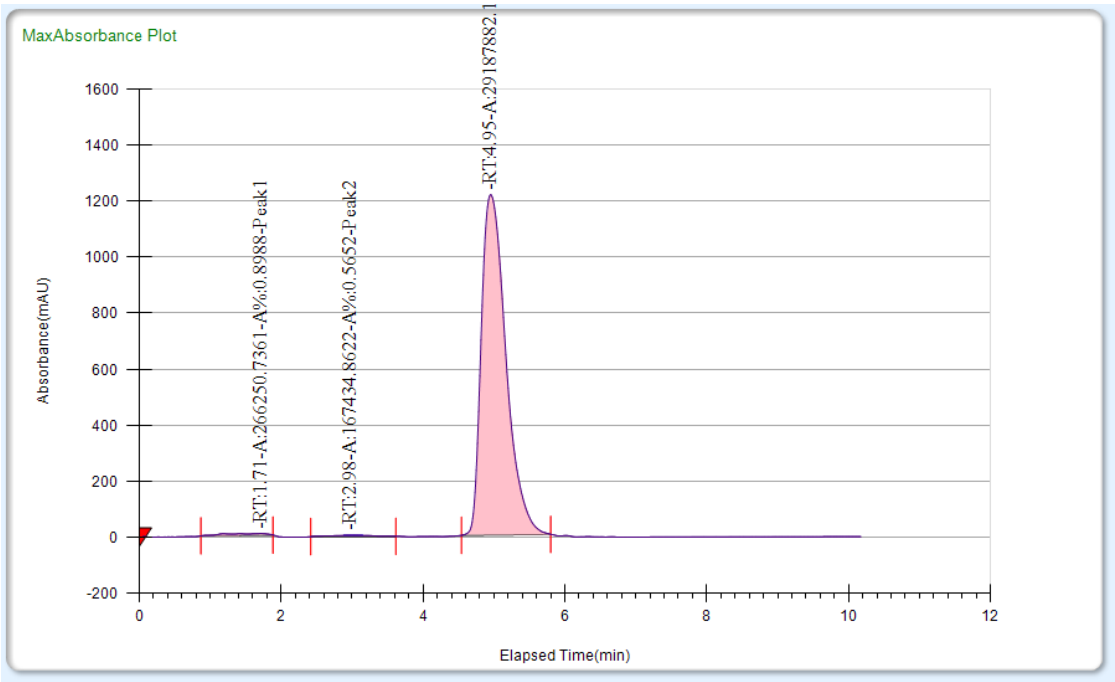


Figure S78: ESI-HRMS spectrum of compound **7j**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,8988		1.71 min	6,0774
	Peak2	2	0,5652		2.98 min	4,2079
7j	Peak3	3	98,5359	29621568	4.95 min	1215,683

Figure S79: HPLC chromatogram and purity analysis of compound **7j**

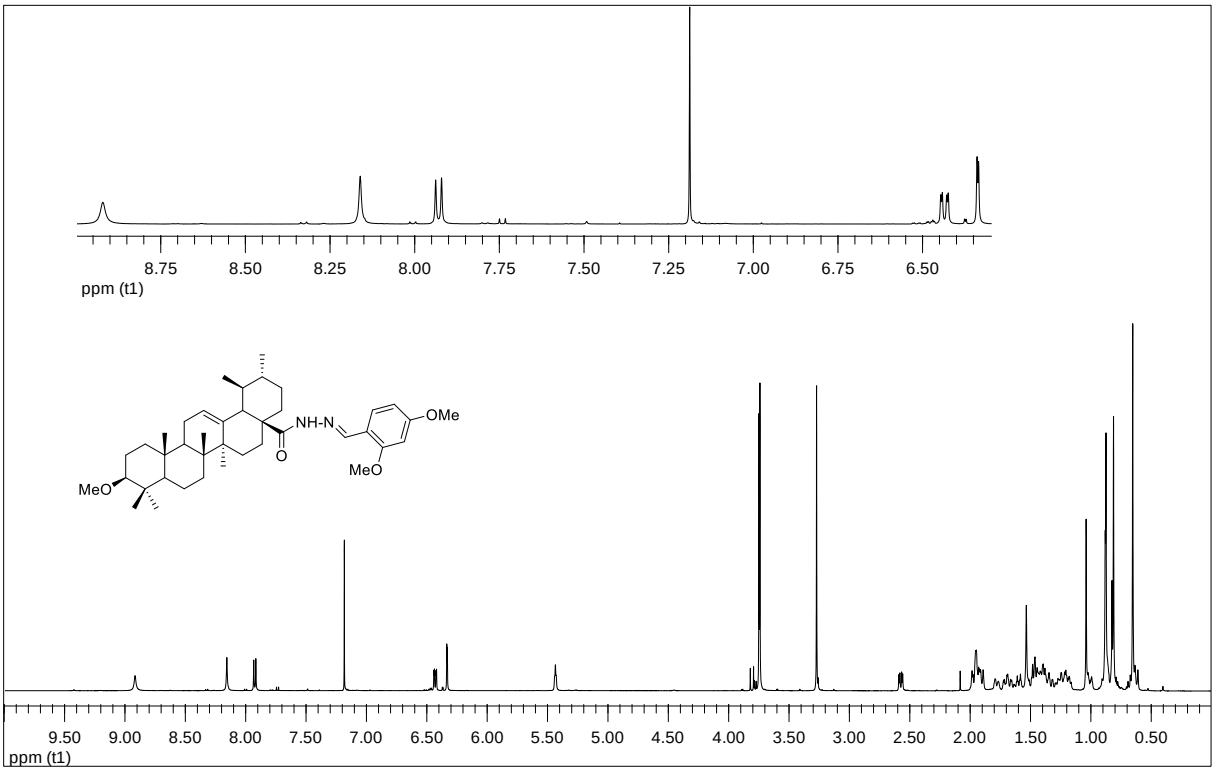


Figure S80: ^{1}H NMR spectrum of **7k** ($CDCl_3$, 500 MHz)

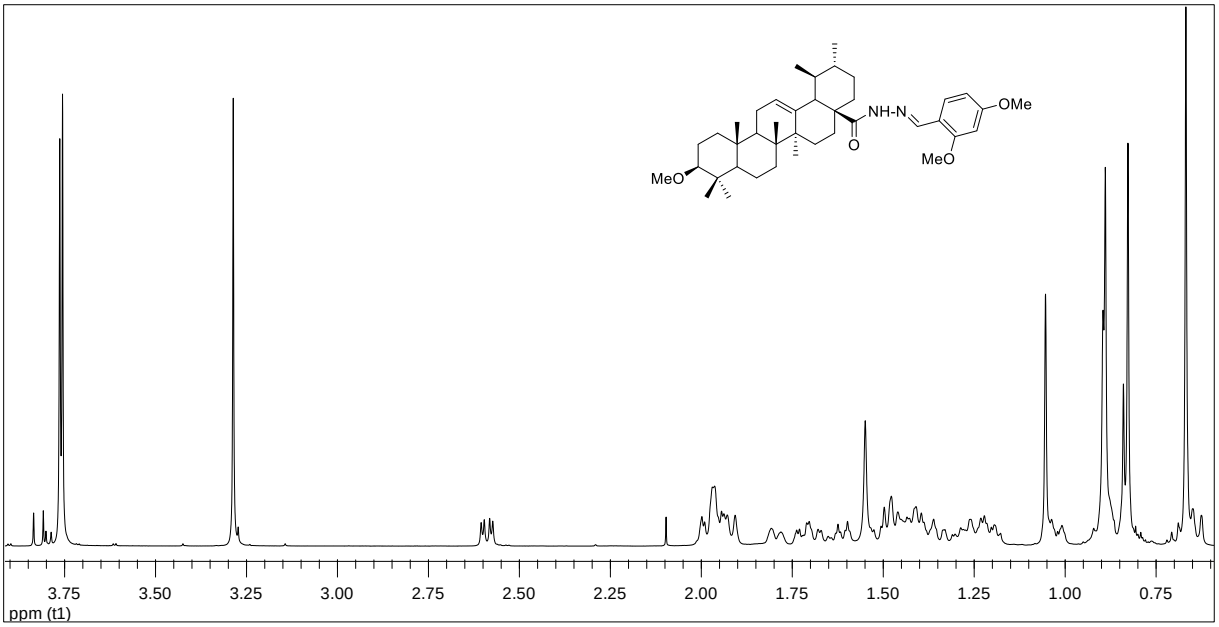


Figure S81: Extended ^{1}H NMR spectrum of **7k** ($CDCl_3$, 500 MHz)

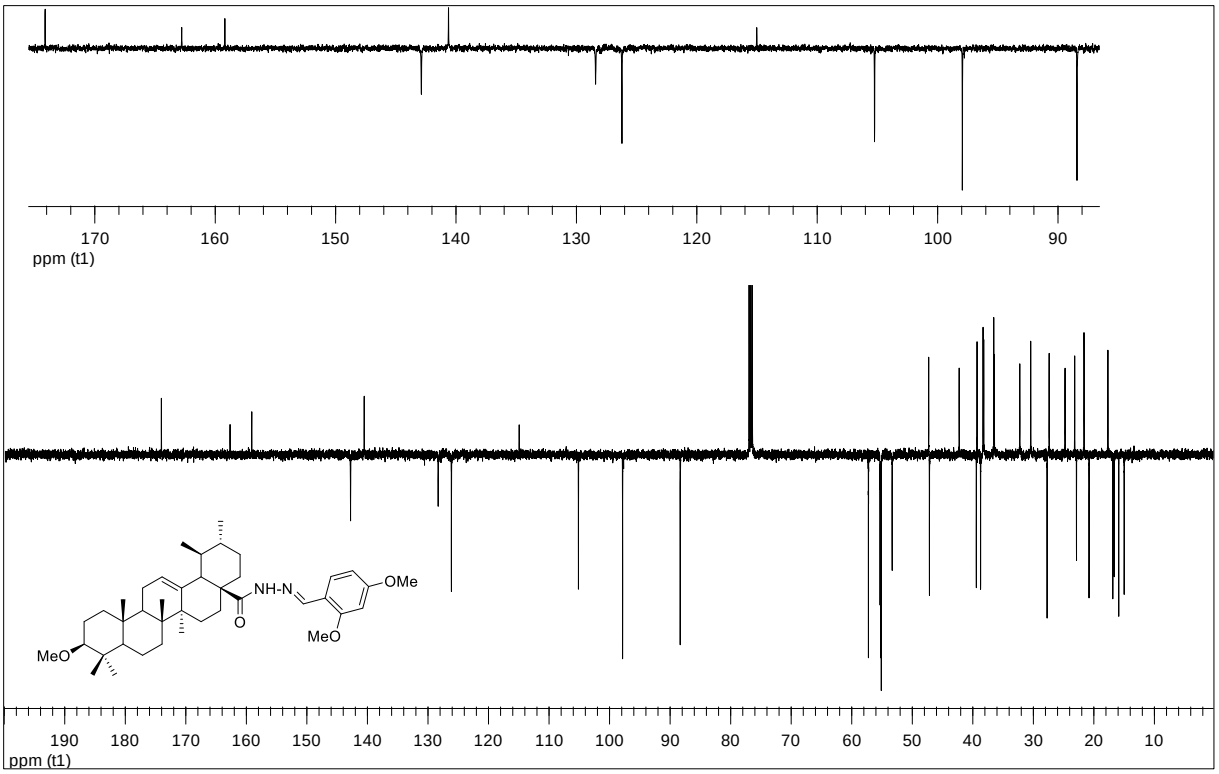


Figure S82: ^{13}C APT NMR spectrum of **7k** ($CDCl_3$, 125 MHz)

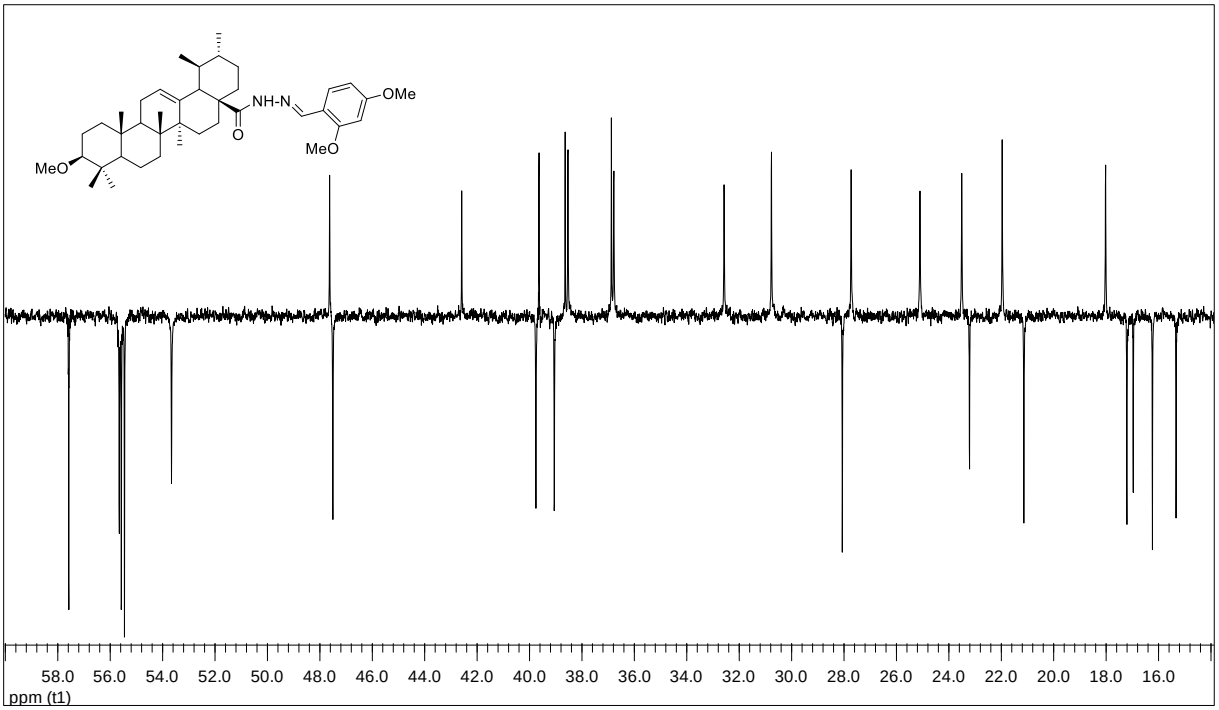


Figure S83: Extended ^{13}C APT NMR spectrum of **7k** ($CDCl_3$, 125 MHz)

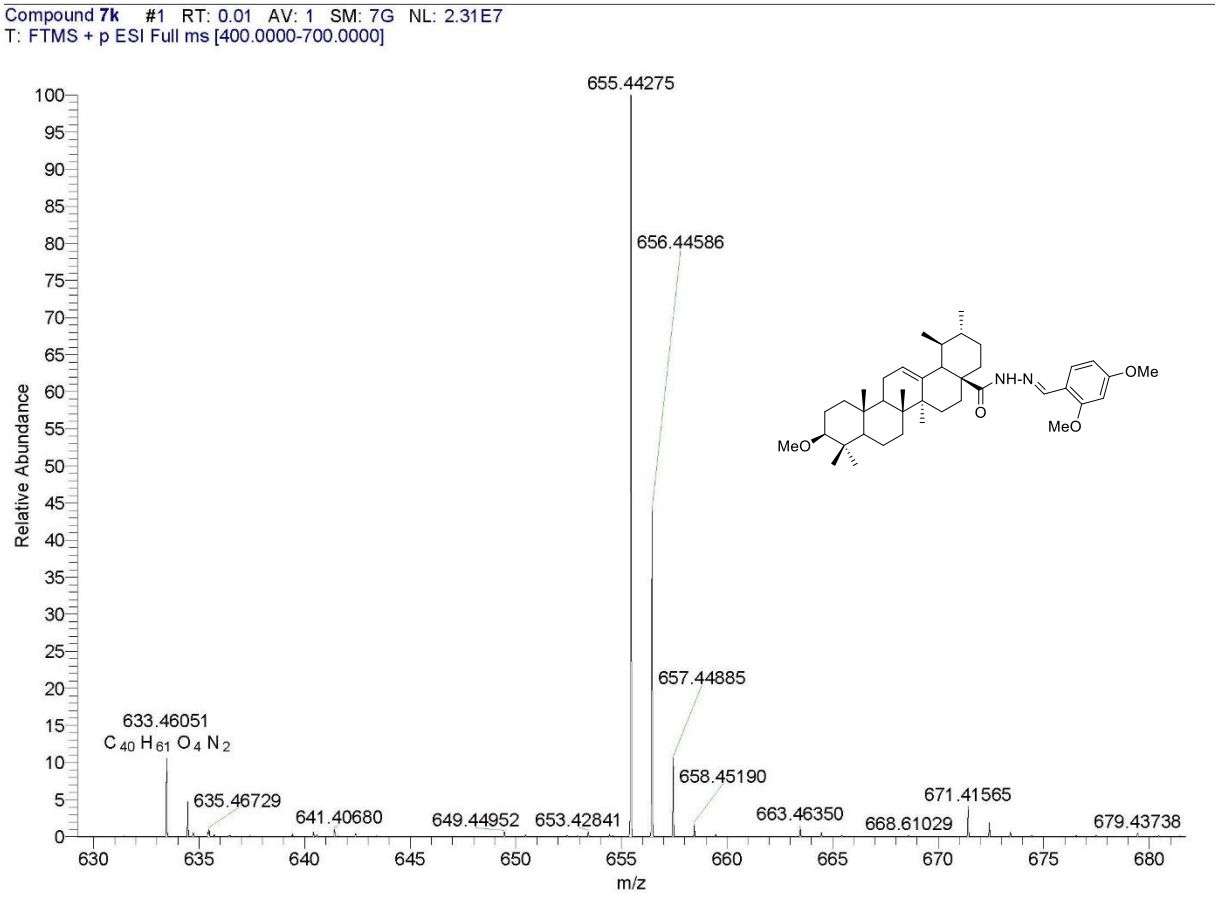
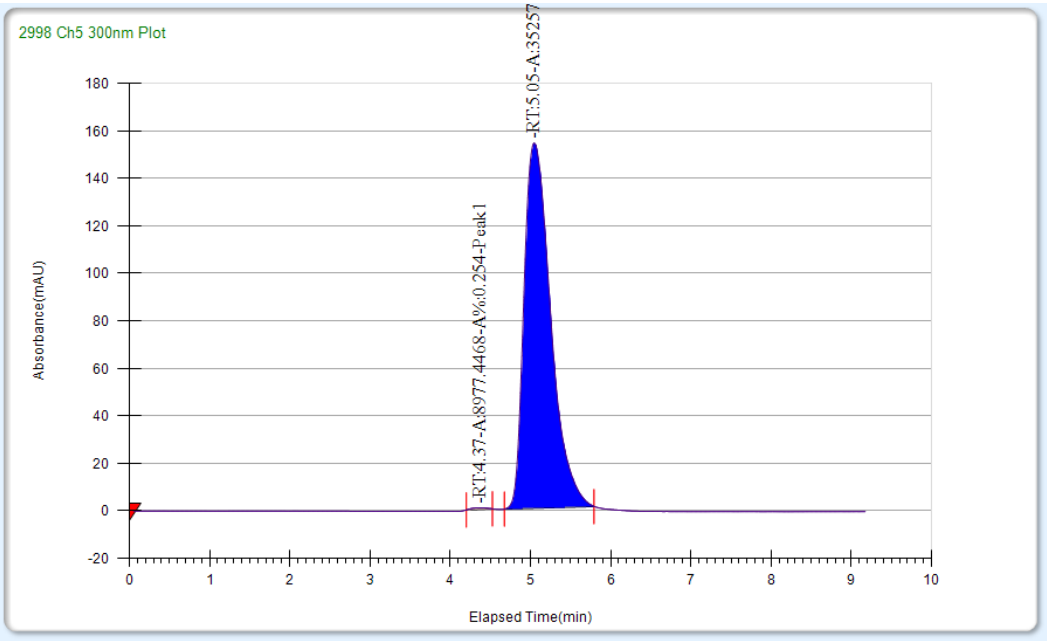


Figure S84: ESI-HRMS spectrum of compound **7k**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,254		4.37 min	0,694
7k	Peak2	2	99,746	3534743	5.05 min	153,7251

Figure S85: HPLC chromatogram and purity analysis of compound **7k**

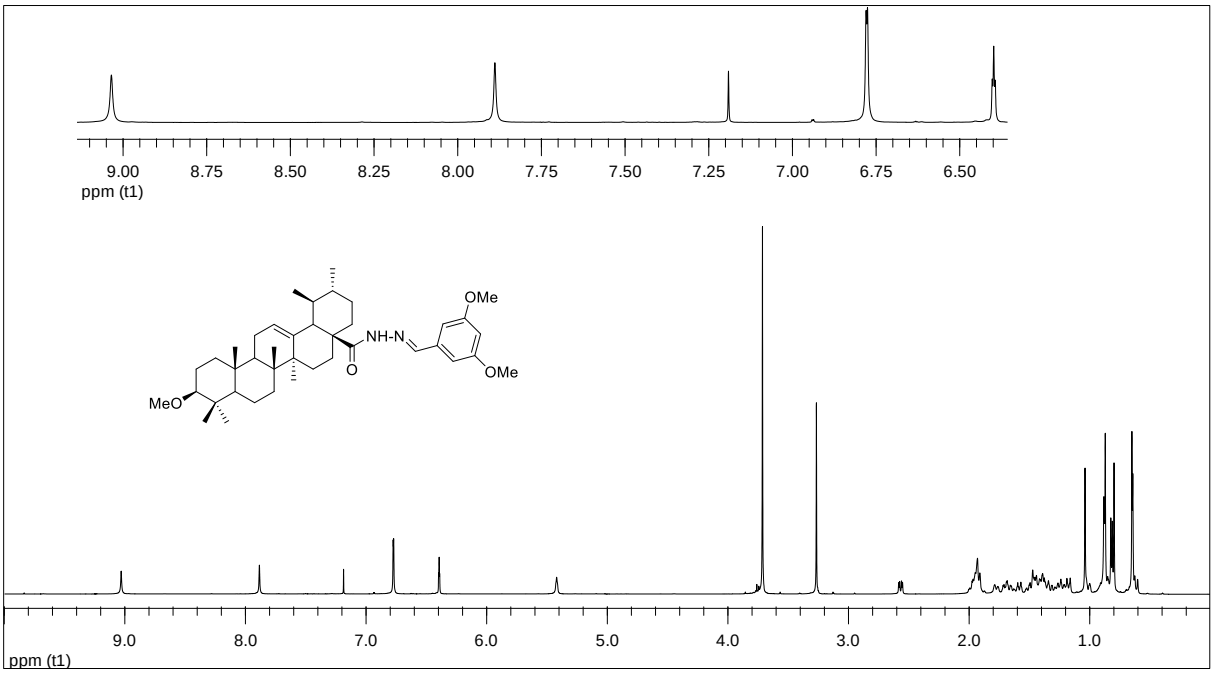


Figure S86: ^{1}H NMR spectrum of **7l** ($CDCl_3$, 500 MHz)

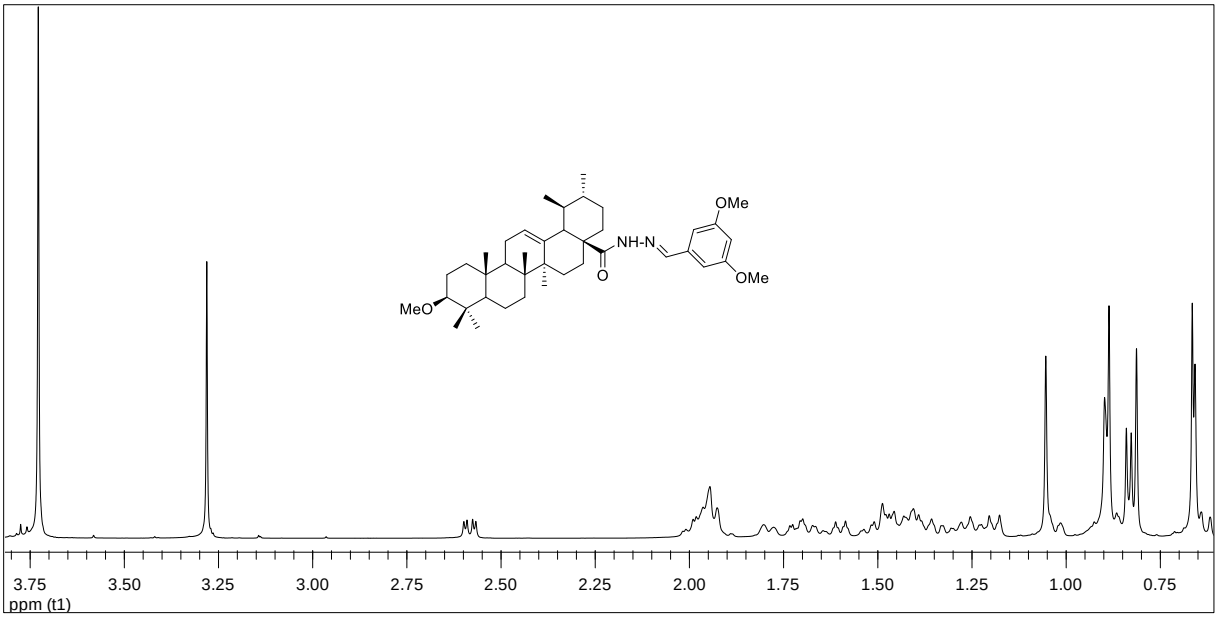


Figure S87: Extended ^{1}H NMR spectrum of **7l** ($CDCl_3$, 500 MHz)

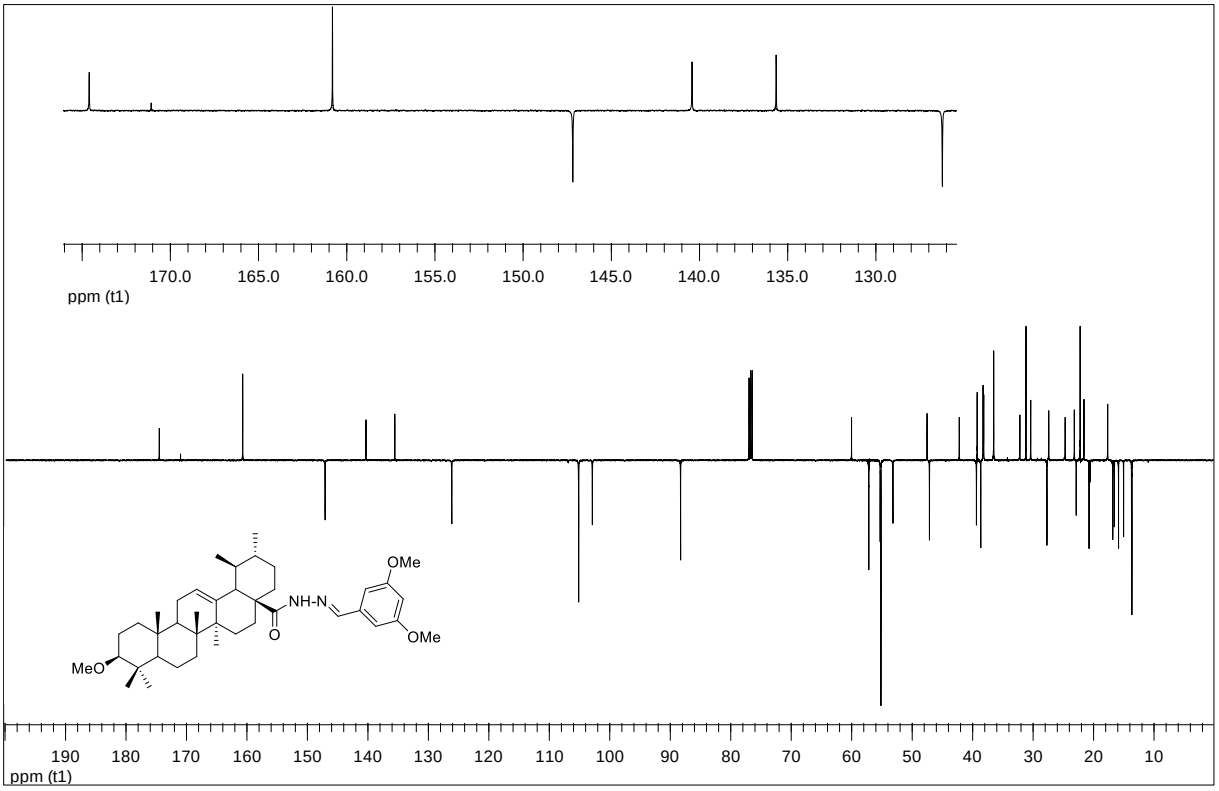


Figure S88: ^{13}C APT NMR spectrum of **7l** ($CDCl_3$, 125 MHz)

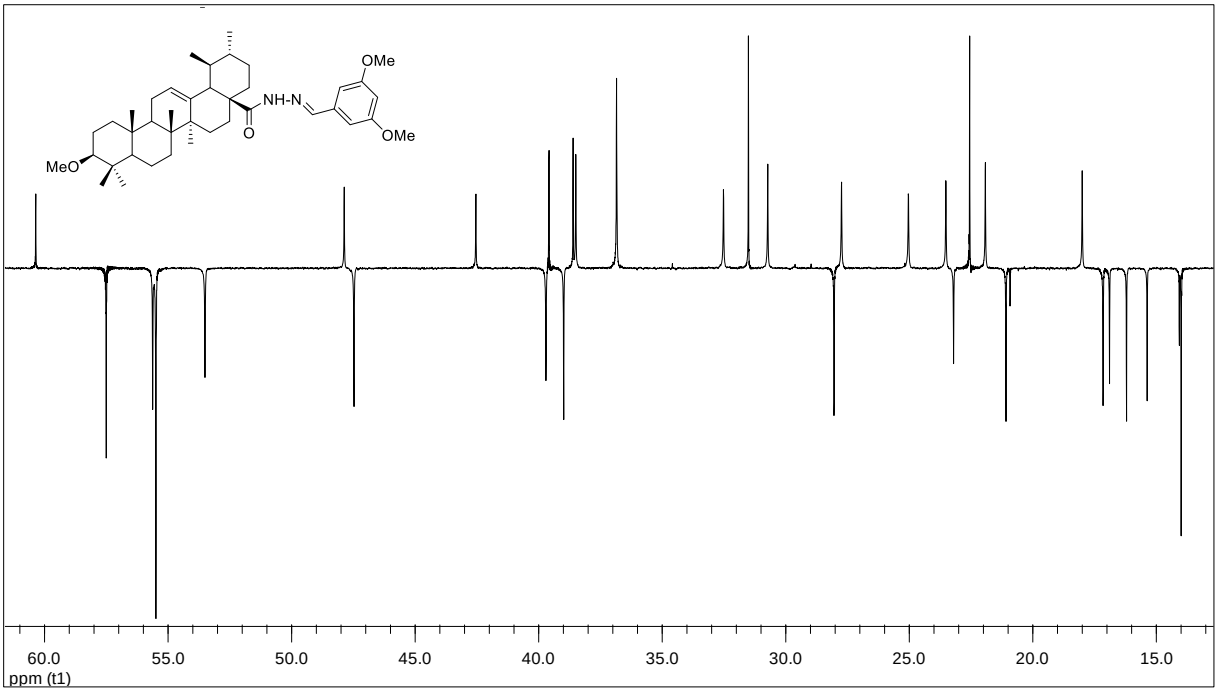


Figure S89: Extended ^{13}C APT NMR spectrum of **7l** ($CDCl_3$, 125 MHz)

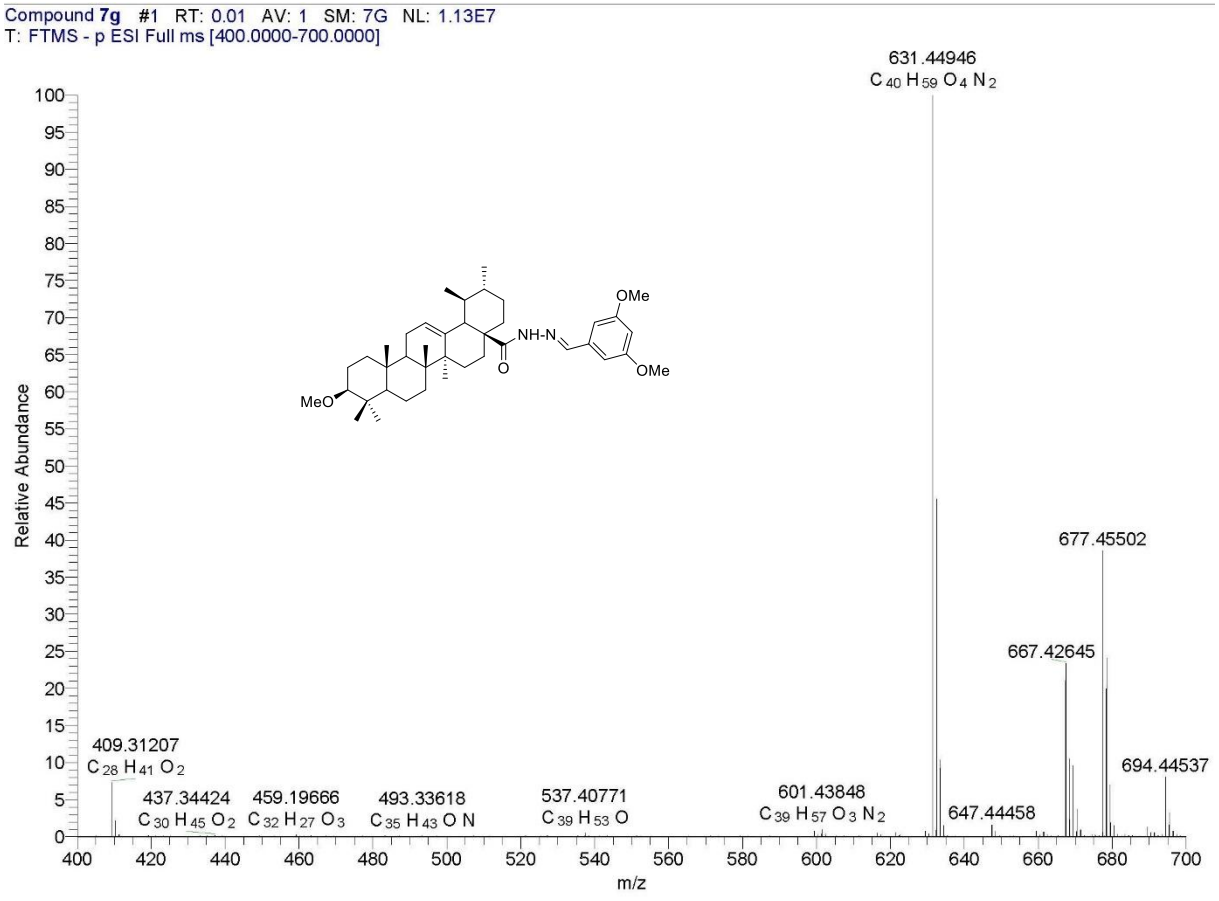
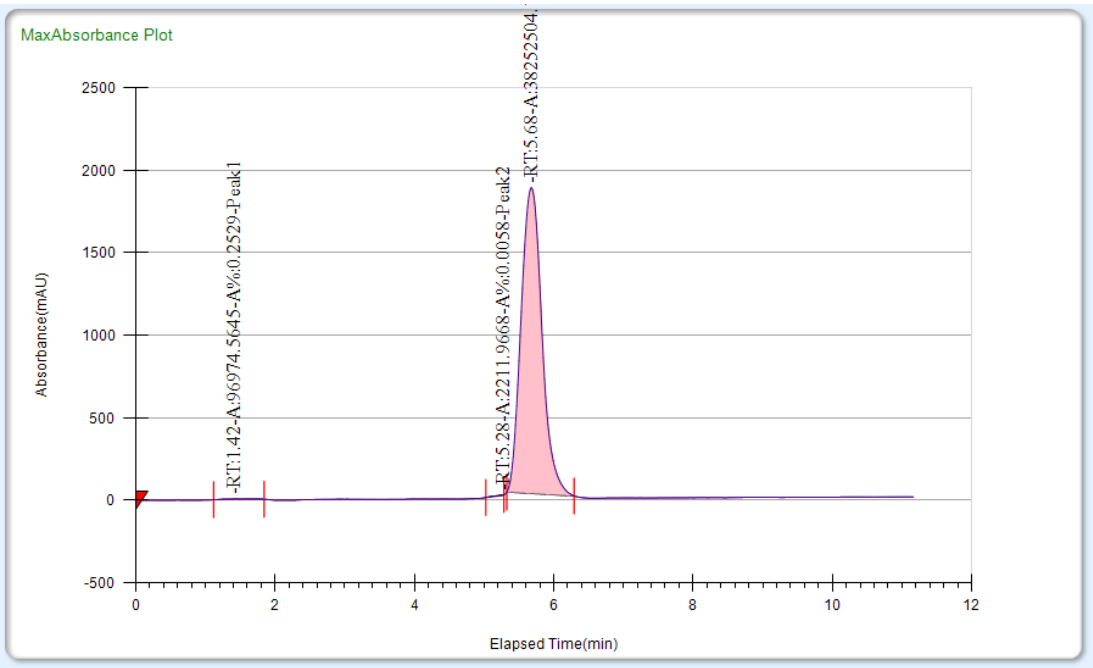


Figure S90: ESI-HRMS spectrum of compound **7l**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,2529		1.42 min	3,5765
	Peak2	2	0,0058		5.28 min	0
7l	Peak3	3	99,7414	38351691	5.68 min	1852,483

Figure S91: HPLC chromatogram and purity analysis of compound **7l**

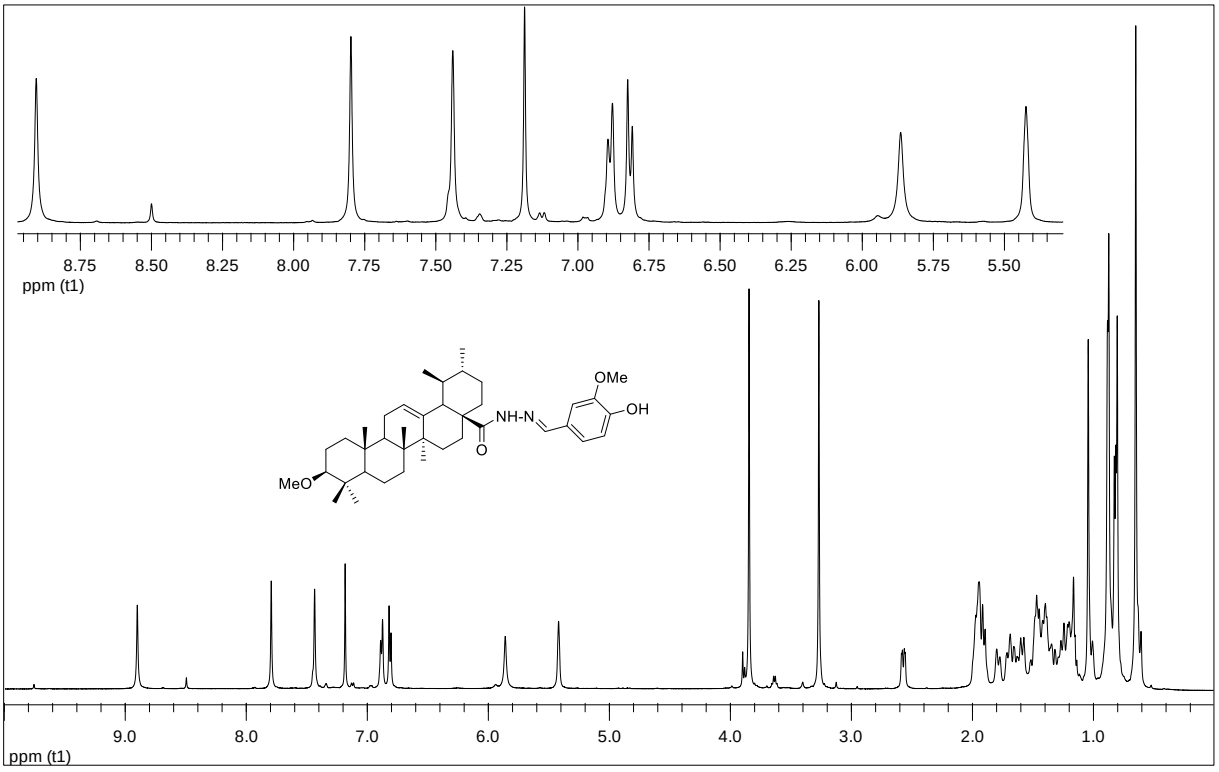


Figure S92: ^{1}H NMR spectrum of **7m** ($CDCl_3$, 500 MHz)

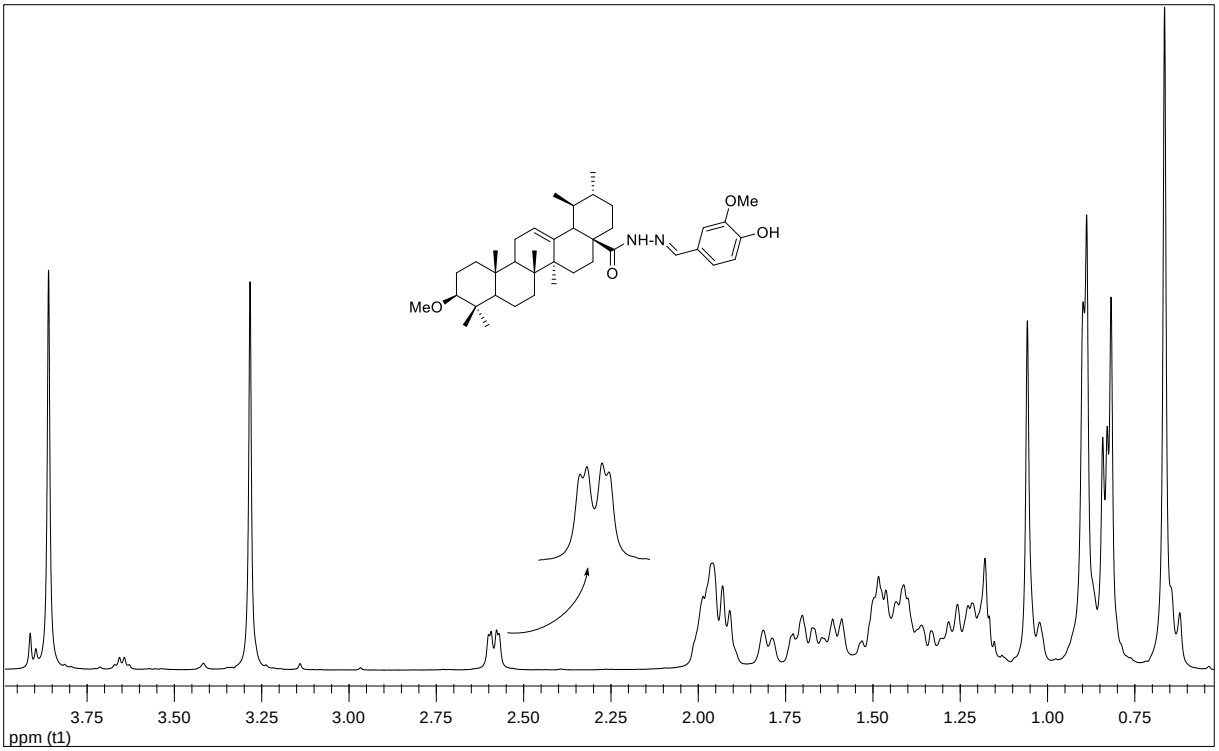


Figure S93: Extended ^{1}H NMR spectrum of **7m** ($CDCl_3$, 500 MHz)

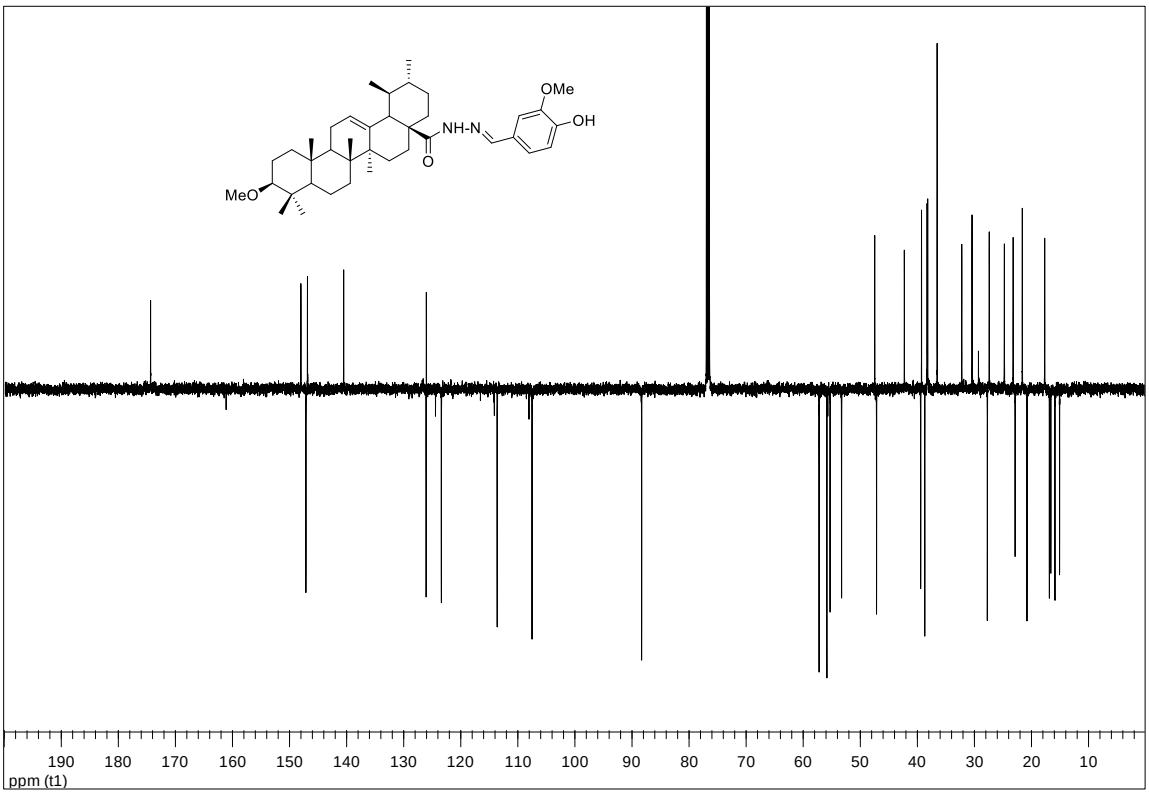


Figure S94: ^{13}C APT NMR spectrum of **7m** ($CDCl_3$, 125 MHz)

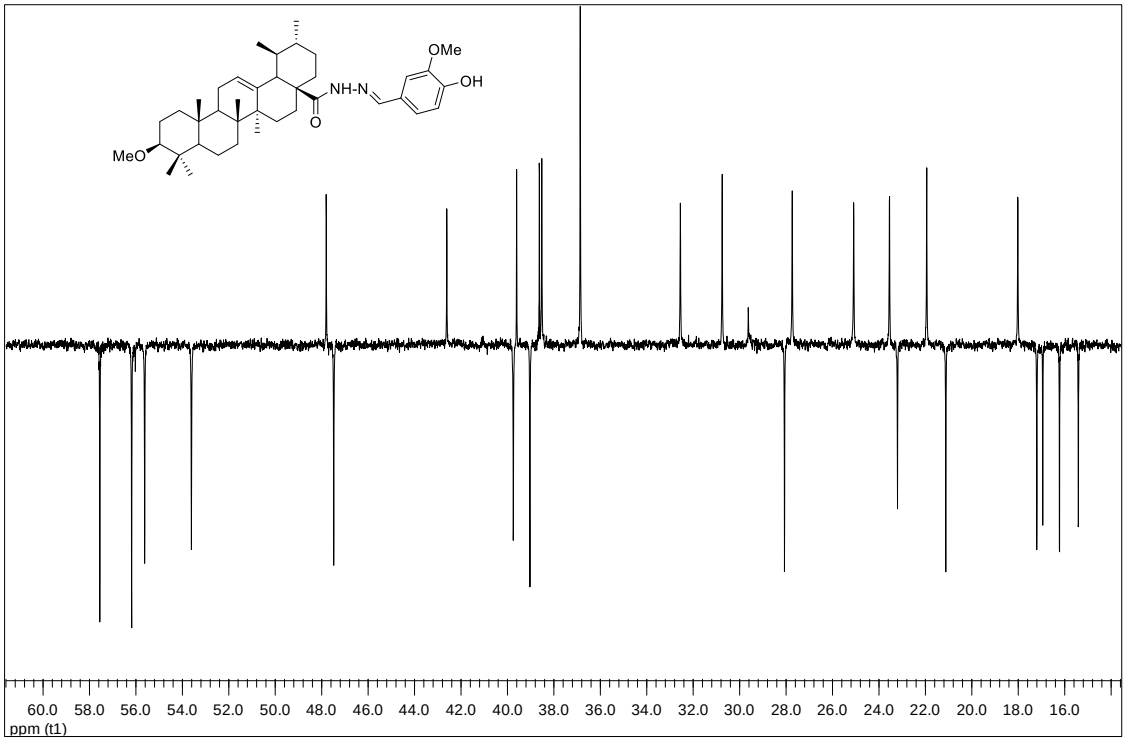


Figure S95: Extended ^{13}C APT NMR spectrum of **7m** ($CDCl_3$, 125 MHz)

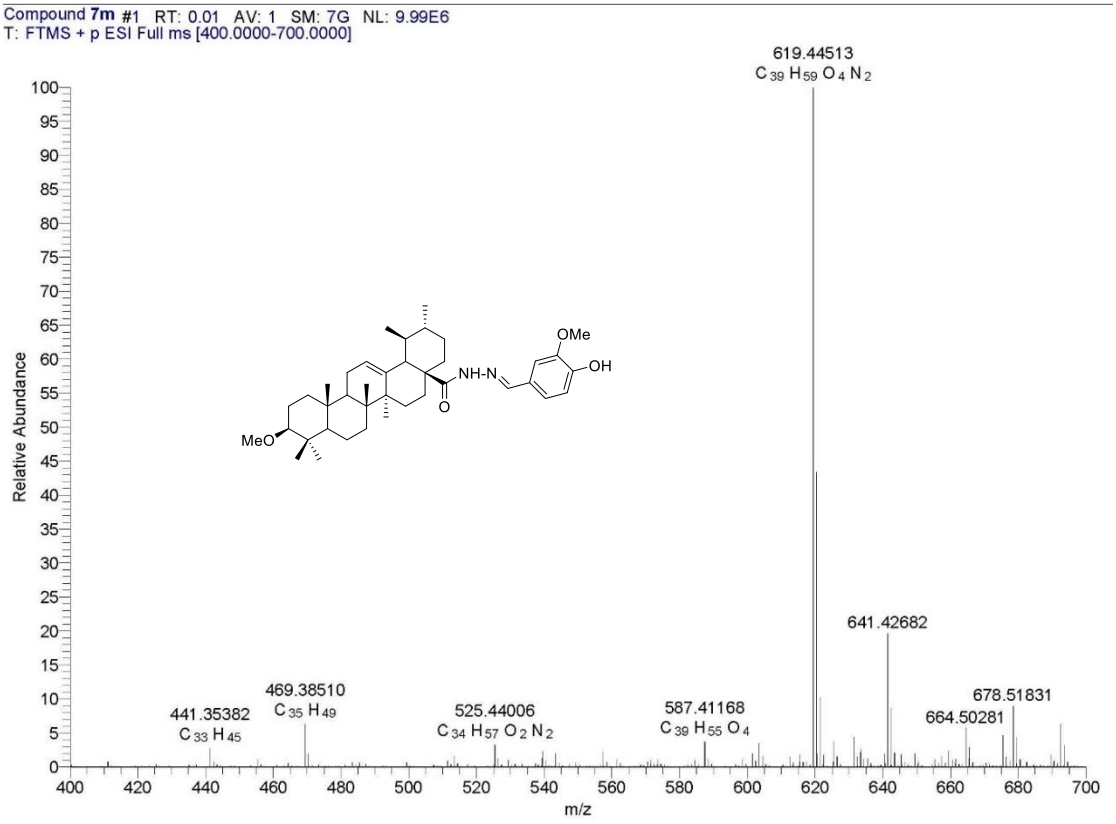
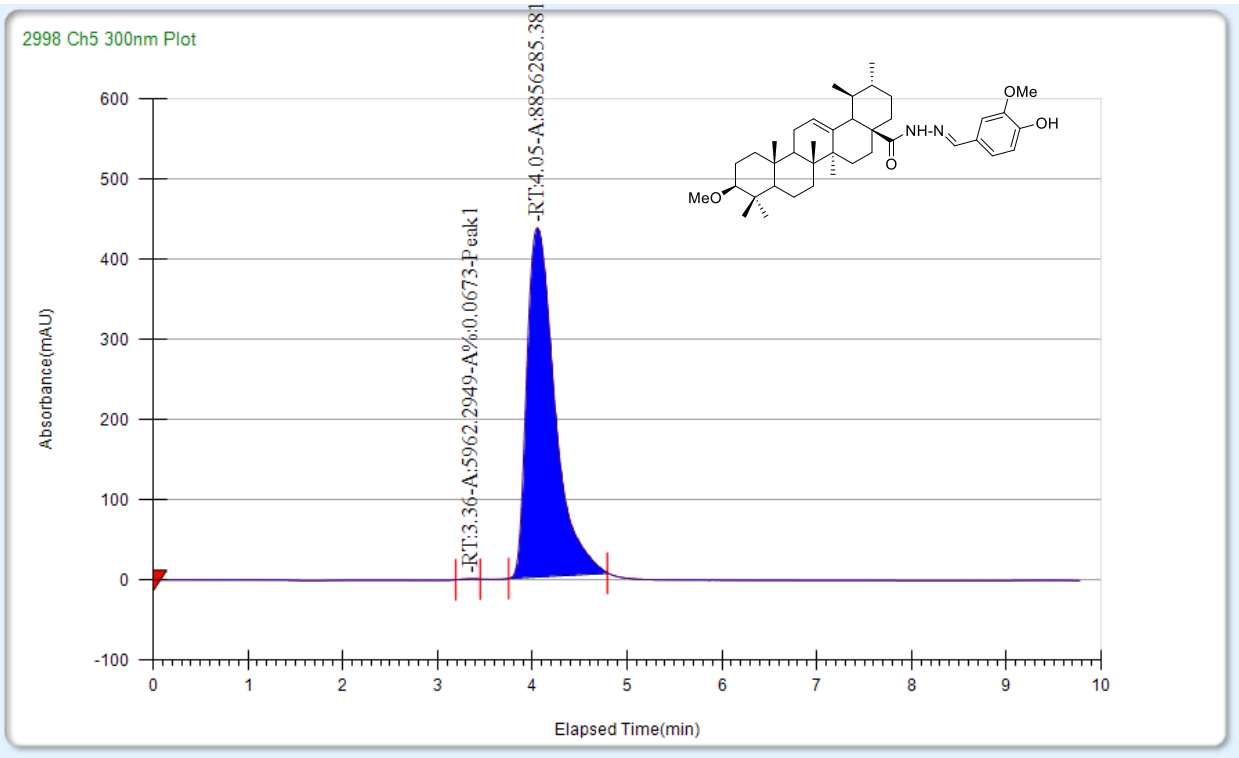


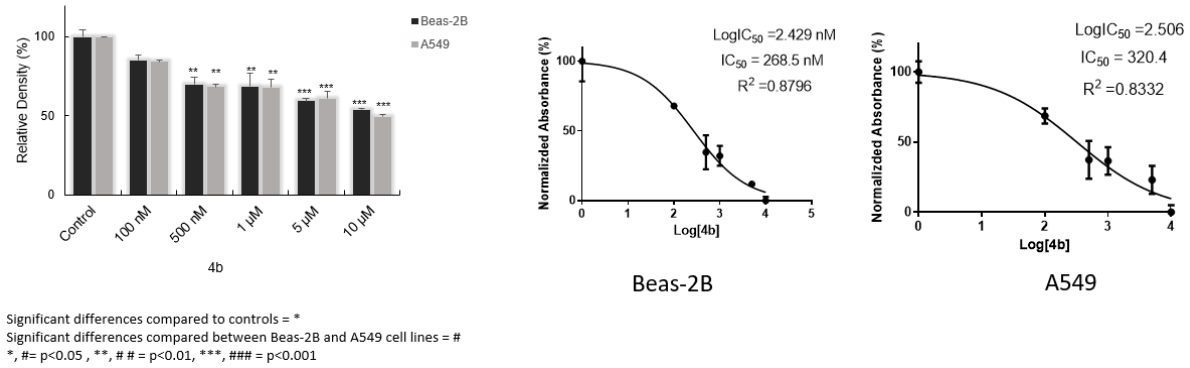
Figure S96: ESI-HRMS spectrum of compound **7m**



No	Peak Name	Peak Number	Area Percent	Area Sum	Retention Time	Height
	Peak1	1	0,0673		3.36 min	0,5202
7m	Peak2	2	99,9327	8862248	4.05 min	435,4713

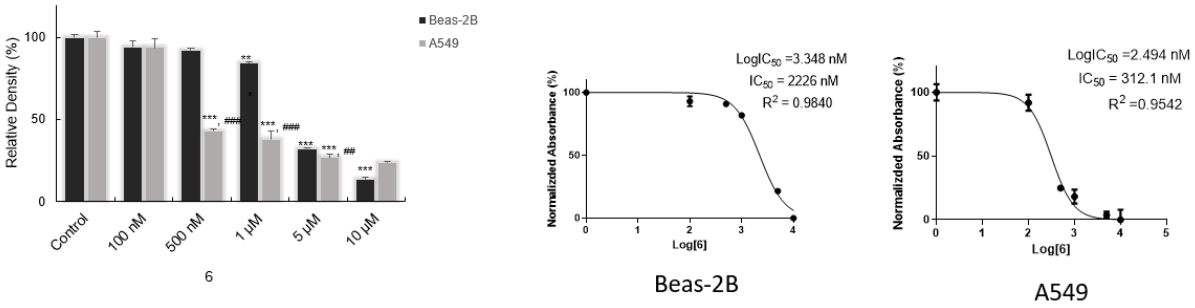
Figure S97: HPLC chromatogram and purity analysis of compound **7m**

Biological Activity Assays Inhibition Curves with IC_{50} Values



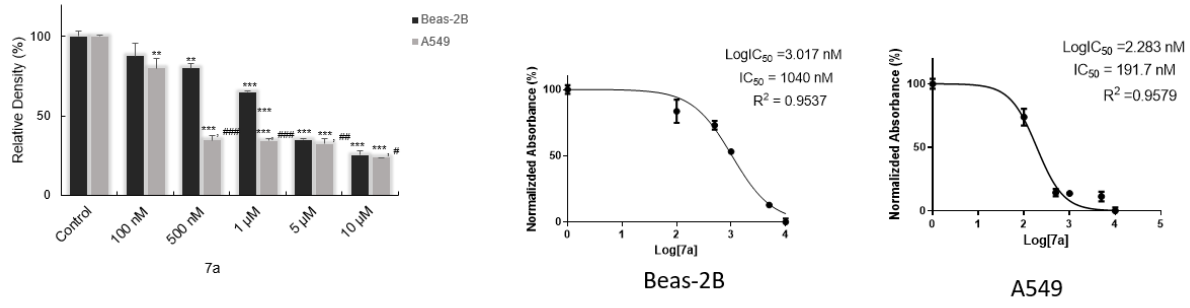
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S98: The cytotoxic effect on relative cell viability and inhibition curves of compound **4b**



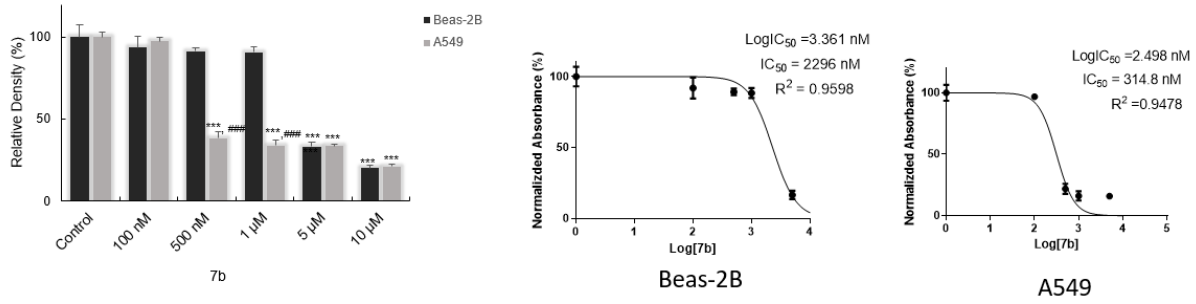
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S99: The cytotoxic effect on relative cell viability and inhibition curves of compound **6**



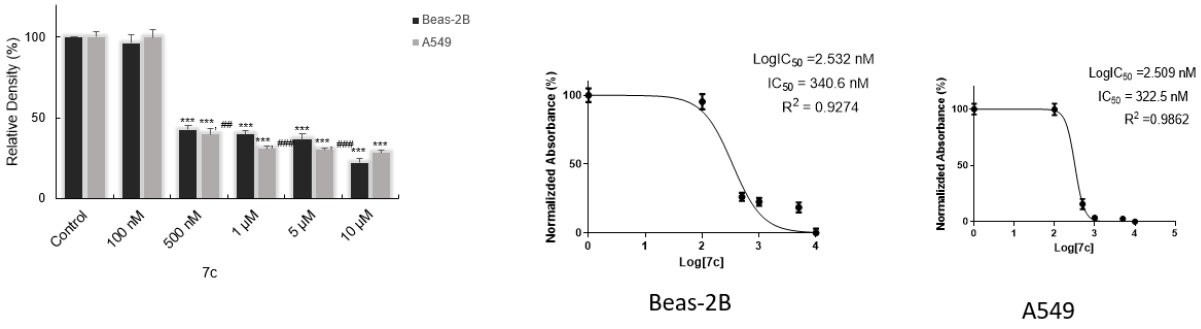
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S100: The cytotoxic effect on relative cell viability and inhibition curves of compound **7a**



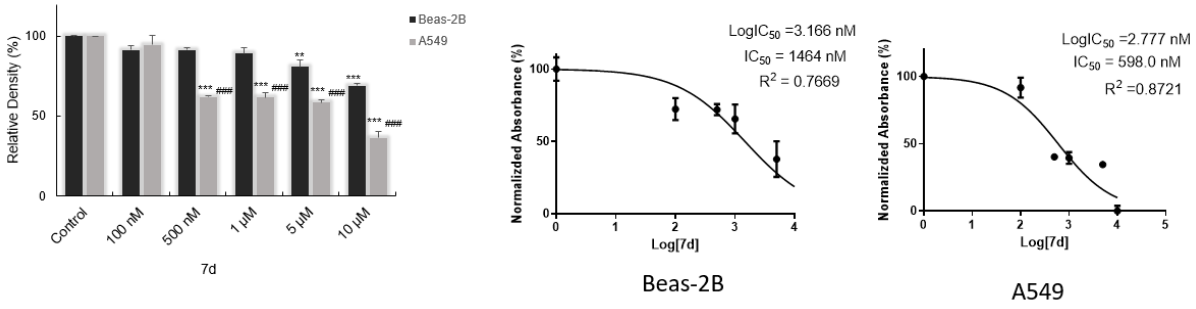
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S101: The cytotoxic effect on relative cell viability and inhibition curves of compound **7b**



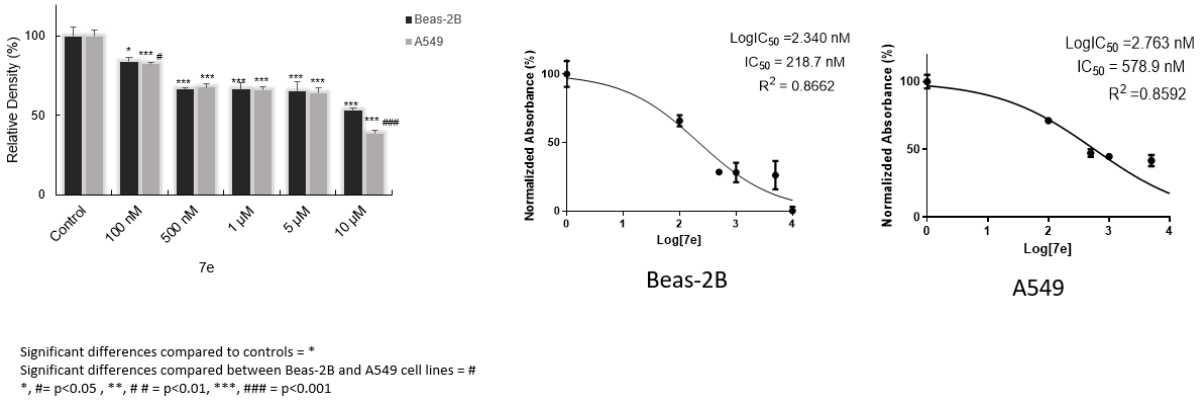
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S102: The cytotoxic effect on relative cell viability and inhibition curves of compound **7c**



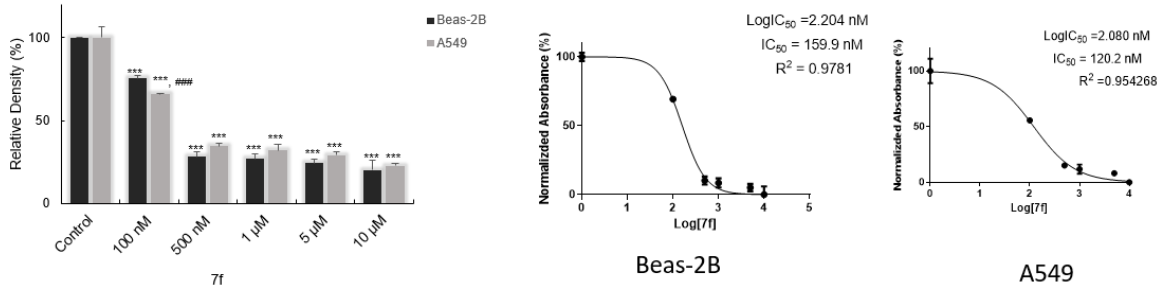
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S103: The cytotoxic effect on relative cell viability and inhibition curves of compound **7d**



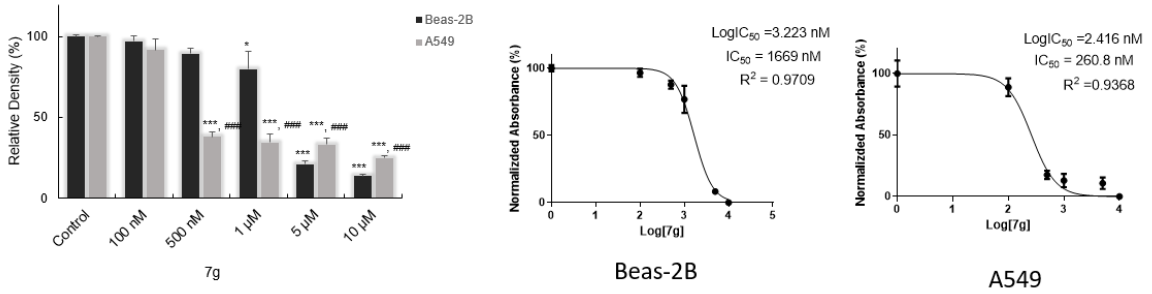
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S104: The cytotoxic effect on relative cell viability and inhibition curves of compound **7e**



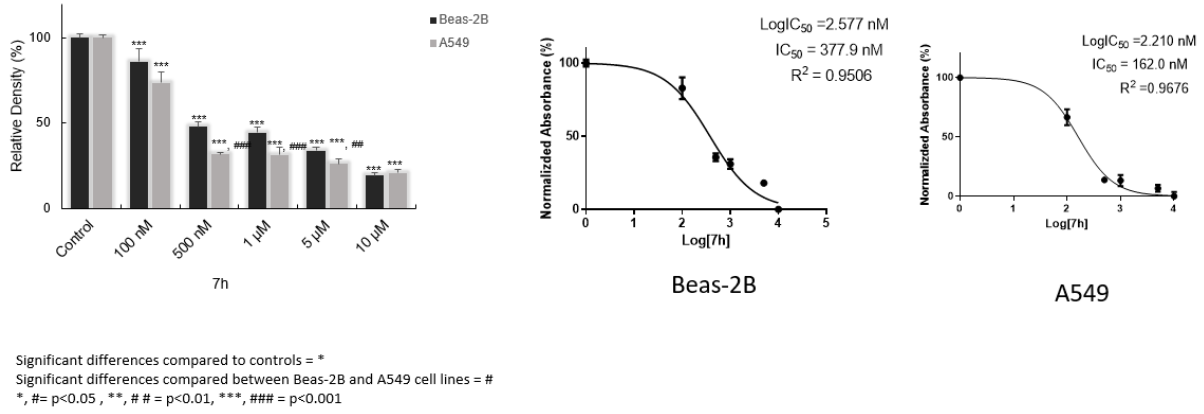
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S105: The cytotoxic effect on relative cell viability and inhibition curves of compound **7f**



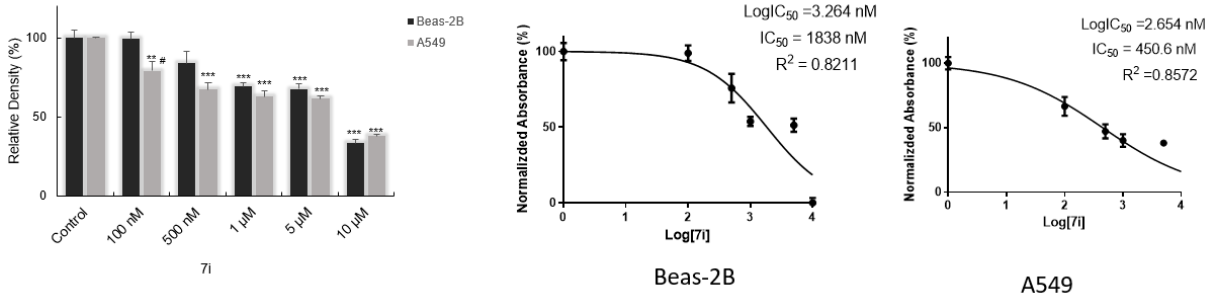
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S106: The cytotoxic effect on relative cell viability and inhibition curves of compound **7g**



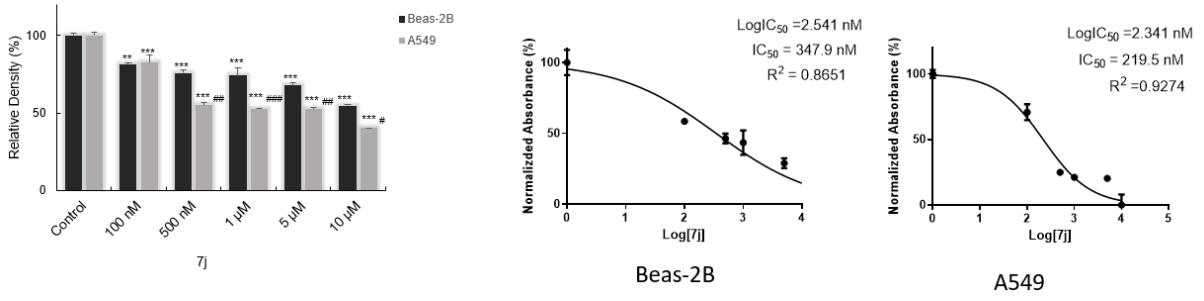
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S107: The cytotoxic effect on relative cell viability and inhibition curves of compound **7h**



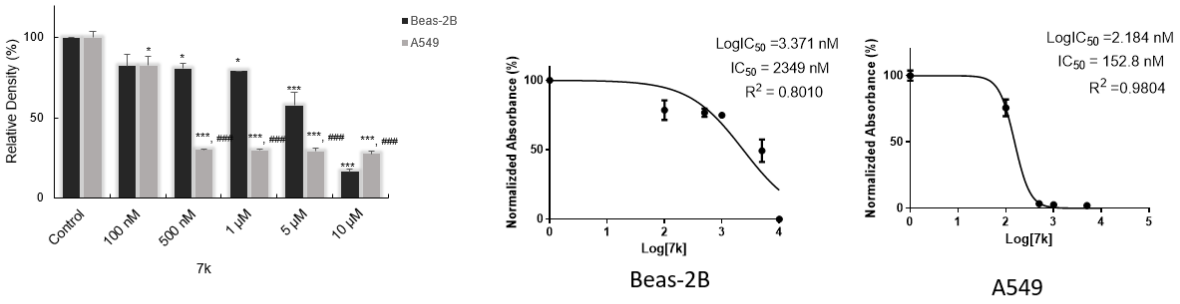
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S108: The cytotoxic effect on relative cell viability and inhibition curves of compound **7i**



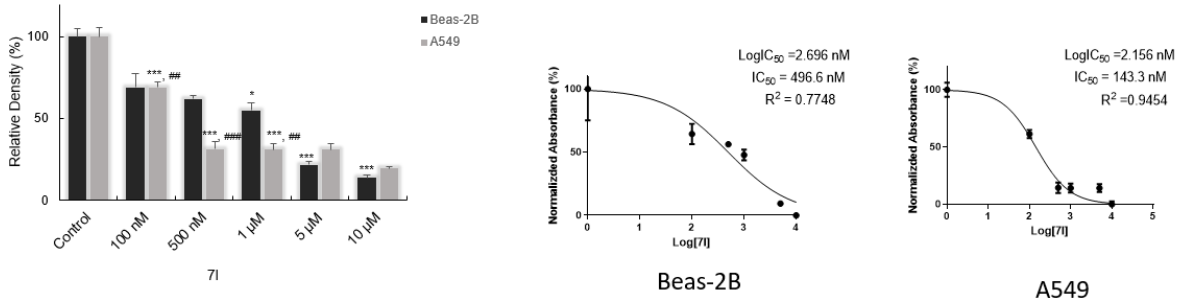
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S109: The cytotoxic effect on relative cell viability and inhibition curves of compound **7j**



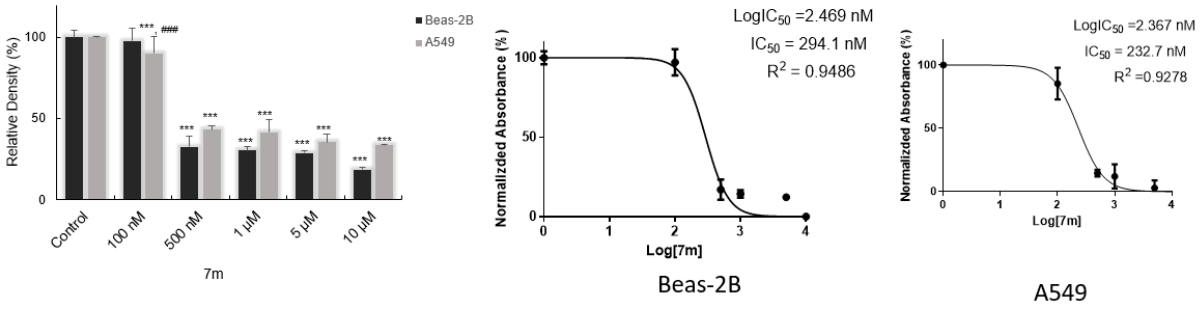
Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S110: The cytotoxic effect on relative cell viability and inhibition curves of compound **7k**



Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S111: The cytotoxic effect on relative cell viability and inhibition curves of compound **7l**



Significant differences compared to controls = *
Significant differences compared between Beas-2B and A549 cell lines = #
*, #= p<0.05 , **, # # = p<0.01, ***, ### = p<0.001

Figure S112: The cytotoxic effect on relative cell viability and inhibition curves of compound **7m**

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