Discovery of 12-thiazole abietanes as selective inhibitors of the human metabolic serine hydrolase hABHD16A

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Supporting information

1. General methods

Dehydroabietic acid was obtained from Pfaltz and Bauer. The other reagents were obtained from Sigma Aldrich Co., VWR International Oy, and Fluorochem. For thin layer chromatography (TLC) Silica gel 60 F254 was used. Flash column chromatography (FCC) was performed with a Biotage High-Performance Flash Chromatography Sp4-system (Uppsala, Sweden) using a 0.1-mm path length flow cell UV detector/recorder module (fixed wavelength: 254 nm), and 10 g, 25 g or 50 g SNAP cartridges (10–50 mL/min flow rate). IR spectra were obtained using a Vertex 70 (Bruker Optics Inc., MA, USA) FTIR instrument. The FTIR measurements were made with a horizontal attenuated total reflectance (ATR) accessory (MIRacle, Pike Technology, Inc, WI, USA). The transmittance spectra were recorded at a 4 cm^{-1} resolution between 4000 and 600 cm^{-1} using the OPUS 5.5 software (Bruker Optics Inc., MA, USA). NMR spectra were obtained using a Bruker Ascend 400 spectrometer, in $CDCl_3$, or DMSO-d_6. The chemical shifts were reported in parts per million (ppm) and on the δ scale from tetramethylsilane (TMS) as an internal standard. The coupling constants *J* are quoted in Hertz (Hz). If rotamers were observed in the ^{13}C spectrum, the other rotamer peaks were labeled with an asterisk (*). LC-MS analyses were executed with Waters Acquity® UPLC system (Waters, Milford MA, USA) with Acquity PDA detector and Waters Synapt G2 HDMS mass spectrometer (Waters, Milford MA, USA) via an ESI ion source. Samples were analyzed in positive, resolution ion mode. Mass range was set from 100 to 600. Separation was performed in Acquity UPLC® BEH C18 column (1.7 µm, 50 × 2.1 mm, Waters, Ireland) in 40 °C. The mobile phase consisted of 0.1% formic acid both in (A) H2O and (B) acetonitrile (Chromasolv® grade, Sigma-Aldrich, Steinheim, Germany). A linear gradient started at 95% of A and decreased to 10%. Purity of the biologically evaluated compounds was >95%, determined by the UPLC. The synthesis of the intermediates **2**, **3** and **9** has been previously reported.[1]

2. Synthesis

Thioformamide was synthesized according to the literature procedure.[2] ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 9.71 (brs, 1H), 9.41 (brs, 1H), 9.20 (m, 1H).

Methyl 12-bromoacetylabieta-8,11,13-trien-18-oate (4). **3** (1.00 g, 2.81 mmol) was dissolved in MeOH (95 mL). $CuBr_2$ (3.76 g, 16.8 mmol) was added and the mixture was stirred at 65 ºC for 16 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (100 mL). The organic

phase was washed with water (50 mL), half-saturated solution of $NaHCO_3$ in water (3 × 50 mL) and brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a mixture of **4a** and **4b** as brown oil (1.26 g), which was used without further purification.

Methyl 12-(2-aminothiazol-4-yl)abieta-8,11,13-trien-18-oate (5). Crude **4** (282 mg) was partially dissolved in EtOH (13 mL). Triethylamine (0.180 mL, 1.30 mmol) and thiourea (54.0 mg, 0.712 mmol) were added and the resulting mixture was heated at 78 ºC for 1 h 45 min. The reaction mixture was cooled down to room temperature and the solvent was evaporated. The residue was dissolved in ethyl acetate (50 mL) and it was washed with a 1 M solution of NaOH in water (25 mL) and brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give light brown solid. The crude product was purified with automated column chromatography, eluting with *n*-heptane/ethyl acetate 3:1 to give **5** as an amorphous light yellow solid (138 mg, 40% over 2 steps).

FTIR-ATR 3439, 2947, 1718, 1607, 1516, 1246, 1132, 727 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.22 (s, 1H), 6.98 (s, 1H), 6.32 (s, 1H), 5.13 (brs, 2H) 3.67 (s, 3H), 3.29 (hept, J = 6.9 Hz, 1H), 2.90 (m, 2H), 2.30 (m, 1H), 2.24 (dd, J_1 = 12.5 Hz, J_2 = 2.3 Hz, 1H), 1.75 (m, 6H), 1.52 (m, 1H), 1.42 (m, 1H), 1.27 (s, 3H), 1.21 (s, 3H), 1.18 (d, J = 6.9 Hz, 3H), 1.15 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.3, 166.4, 152.1, 146.7, 144.3, 135.2, 132.0, 126.1, 125.9, 105.1, 52.1, 47.8, 45.0, 38.1, 37.1, 36.8, 29.9, 29.0, 25.2, 24.5, 24.2, 21.8, 18.7, 16.7. HRMS calcd for $C_{24}H_{33}N_2O_2S$. $[M+1]^+$ 413.2263 found 413.2260.

Methyl 12-(thiazol-4-yl)abieta-8,11,13-trien-18-oate (6). A mixture of crude **4** (0.100 g) and thioformamide (28.0 mg, 0.459 mmol) in 1,4-dioxane (2.9 mL) was irradiated under microwaves at 100 ºC for 10 min. The reaction mixture was diluted with ethyl acetate (25 mL) and washed with a 1 M solution of NaOH in water (25 mL). The aqueous phase was extracted with ethyl acetate (2 × 15 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown oil. The crude product was purified with automated column chromatography, eluting with an *n*-heptane/ethyl acetate gradient (5 → 40% ethyl acetate) to give **6** as an amorphous light red solid (46.2 mg, 53% over 2 steps).

FTIR-ATR 2935, 1718, 1485, 1244, 1177, 1132, 879, 829 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.87 (d, J = 2.0 Hz, 1H) 7.26 (s, 1H), 7.20 (d, J = 2.0 Hz, 1H), 7.05 (s, 1H), 3.67 (s, 3H), 3.21 (hept, J = 6.9 Hz, 1H), 2.94 (dd, J_1 = 9.0, J_2 = 4.5 Hz, 2H), 2.28 (m, 2H), 1.77 (m, 5H), 1.54 (m, 1H), 1.44 (m, 1H), 1.28 (s, 3H), 1.24 (s, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.15 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.2, 157.3, 151.7, 146.9, 144.3, 135.6, 131.4, 126.3, 126.3, 115.2, 52.1,

47.8, 45.0, 38.1, 37.1, 36.8, 30.0, 29.1, 25.2, 24.3, 24.1, 21.8, 18.7, 16.7. HRMS calcd for $C_{24}H_{32}NO_2S$. $[M+1]^+$ 398.2154 found 398.2153.

12-(2-Aminothiazol-4-yl)abieta-8,11,13-trien-18-ol (7). $LiAlH_4$ (9.70 mg, 0.255 mmol) was suspended in dry tetrahydrofuran (1 mL) and added to a stirred solution of **5** (0.100 g, 0.242 mmol) in tetrahydrofuran (2 mL) at 0 ºC. The mixture was stirred at 0 ºC for 15 min and at room temperature for 5 h. $LiAlH_4$ (9.00 mg, 0.237 mmol) was added and stirring at room temperature was continued for 25 h. The reaction was quenched by slow addition of 2 M hydrochloric acid (3 mL) at 0 ºC. Tetrahydrofuran was evaporated and the residue was diluted with water (20 mL) and neutralized with a 1 M solution of NaOH water. The mixture was extracted with ethyl acetate (30 mL) and the organic phase was washed with water (20 mL) and brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a light brown foam. The crude was purified with automated column chromatography, eluting with an *n*-hexane/ethyl acetate gradient (8 → 66% ethyl acetate) to give **7** as an amorphous slightly yellowish solid (26.1 mg, 28%).

FTIR-ATR 3306, 2926, 1608, 1514, 1315, 1043, 905, 754, 729 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.22 (s, 1H), 6.98 (s, 1H), 6.32 (s, 1H), 5.15 (brs, 2H) 3.45 (d, J = 10.9 Hz, 1H), 3.25 (m, 2H), 2.89 (m, 2H), 2.28 (m, 1H), 1.72 (m, 6H), 1.40 (m, 3H), 1.22 (s, 3H), 1.18 (d, J = 6.9 Hz, 3H), 1.15 (d, J = 6.9 Hz, 3H), 0.88 (s, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 166.5, 151.8, 147.1, 144.1, 135.4, 131.6, 126.0, 126.0, 105.0, 72.3, 44.1, 38.6, 38.0, 37.4, 35.3, 30.0, 29.0, 25.3, 24.4, 24.2, 19.0, 18.7, 17.5. HRMS calcd for $C_{23}H_{33}N_2OS$. $[M+1]^+$ 385.2314 found 385.2312.

12-(Thiazol-4-yl)abieta-8,11,13-trien-18-ol (8). $LiAlH_4$ (8.7 mg, 0.23 mmol) was suspended in dry tetrahydrofuran (1 mL) and added to a stirred solution of **6** (87 mg, 0.22 mmol) in tetrahydrofuran (2 mL) at 0 ºC. The mixture was stirred at 0 ºC for 20 min and at room temperature for 1.5 h. The reaction was quenched by slow addition of 1 M hydrochloric acid (1.5 mL) at 0 ºC. Tetrahydrofuran was evaporated and the residue was diluted with water (20 mL) and extracted with ethyl acetate (30 mL). The organic phase was washed with brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a yellowish foam. The crude product was purified with automated column chromatography, eluting an *n*-hexane/ethyl acetate gradient (5 → 40% ethyl acetate) to give **8** as an amorphous white solid (54.6 mg, 67%).

FTIR-ATR 3346, 2926, 1472, 1379, 1047, 899, 878, 814, 729 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.87 (d, J = 2.0 Hz, 1H) 7.27 (s, 1H), 7.20 (d, J = 2.0 Hz, 1H), 7.05 (s, 1H), 3.47 (d, J = 10.9 Hz, 1H), 3.20 (m, 2H), 2.93 (m, 2H), 2.27 (m, 1H), 1.61 (m, 10H), 1.25 (s, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.15 (d, J = 6.9 Hz, 3H), 0.90 (s, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 157.4, 151.7, 147.3,

144.2, 135.7, 131.2, 126.5, 126.2, 115.2, 72.4, 44.0, 38.6, 38.0, 37.5, 35.2, 30.1, 29.1, 25.4, 24.4, 24.1, 19.0, 18.7, 17.5. HRMS calcd for $C_{23}H_{32}NOS$. $[M+1]^+$ 370.2205 found 370.2205.

12-Bromoacetylabieta-8,11,13-trien-18-amide (10). A mixture of **9** (124 mg, 0.363 mmol) and $CuBr_2$ (487 mg, 2.18 mmol) in methanol (12 mL) was stirred at 65 ºC for 18 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (30 mL). The organic phase was washed with water (25 mL), a half-saturated solution of $NaHCO_3$ in water (3 × 20 mL) and brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a mixture of **10a** and **10b** as a brown oil (146 mg), which was used without further purification.

12-(2-Aminothiazol-4-yl)abieta-8,11,13-trien-18-amide (11). A mixture of crude **10** (145 mg), thiourea (53.0 mg, 0.690 mmol) and triethylamine (96 μL, 0.690 mmol) in EtOH (4.3 mL) was irradiated under microwaves at 120 ºC for 30 min. The reaction mixture was poured into a 1 M solution of NaOH in water (30 mL) and the mixture was extracted with ethyl acetate (3 × 20 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a yellow solid. The crude product was purified with automated column chromatography, eluting with *n*-heptane/ethyl acetate 1:5 to give **11** as an amorphous light yellow solid (38.4 mg, 27% over 2 steps).

FTIR-ATR 3317, 3173, 2932, 1655, 1599, 1514, 1333, 754, 729 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.20 (s, 1H), 6.97 (s, 1H), 6.29 (s, 1H), 5.97 (brs, 1H), 5.80 (brs, 1H), 5.39 (brs, 2H), 3.27 (hept, J = 6.8 Hz, 1H), 2.90 (m), 2.31 (m, 1H), 2.08 (dd, J_1 = 12.4 Hz, J_2 = 2.4 Hz, 1H), 1.67 (m, 8H), 1.27 (s, 3H), 1.22 (s, 3H), 1.17 (d, J = 6.9 Hz, 3H), 1.13 (d, J = 6.8 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 181.7, 166.9, 151.7, 146.7, 144.3, 135.2, 131.9, 126.1, 125.8, 104.8, 47.4, 45.6, 38.1, 37.5, 37.1, 29.8, 29.0, 25.2, 24.4, 24.2, 21.2, 18.8, 16.8. HRMS calcd for $C_{23}H_{32}N_3OS$. $[M+1]^+$ 398.2266 found 398.2266.

12-(Thiazol-4-yl)abieta-8,11,13-trien-18-amide (12). A mixture of crude **10** (0.200 g) and thioformamide (60.0 mg, 1.23 mmol) in dry 1,4-dioxane (6 mL) was irradiated under microwaves at 100 ºC for 10 min. The reaction mixture was diluted with ethyl acetate (40 mL) and washed with a 1 M solution of NaOH in water (40 mL). The aqueous phase was extracted with ethyl acetate (2 × 20 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown oil. The crude product was purified with automated column chromatography, eluting an *n*-hexane/ethyl acetate gradient (12 $\rightarrow$ 100% ethyl acetate) to give **12** as an amorphous yellowish solid (114 mg, 55% over 2 steps).

FTIR-ATR 3333, 2925, 1653, 1599, 1472, 1348, 878, 816, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.92 (d, J = 2.0 Hz, 1H) 7.26 (s, 1H), 7.21 (d, J = 2.0 Hz, 1H), 7.03 (s, 1H), 5.79 (brs, 1H), 5.51 (brs, 1H), 3.19 (hept, J = 6.9 Hz, 1H), 2.94 (dd, J_1 = 9.0, J_2 = 4.7 Hz, 2H), 2.32 (m, 1H), 2.13 (dd, J_1 = 12.5, J_2 = 2.2 Hz, 1H), 1.70 (m, 7H), 1.29 (s, 3H), 1.25 (s, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 181.3, 157.0, 151.9, 146.9, 144.4, 135.7, 131.1, 126.3, 126.3, 115.4, 47.4, 45.6, 38.1, 37.5, 37.2, 30.0, 29.1, 25.2, 24.4, 24.1, 21.2, 18.8, 16.9. HRMS calcd for $C_{23}H_{31}N_2OS$. $[M+1]^+$ 383.2157 found 383.2157.

12-(Thiazol-4-yl)abieta-8,11,13-trien-18-oic acid (13) and 2-hydroxyethyl 12-(thiazol-4-yl)abieta-8,11,13-trien-18-oate (14). 6 (0.140 g, 0.352 mmol) was suspended in ethylene glycol (2 mL), and KOH (0.0700 g, 1.06 mmol) in water (0.2 mL) was added. The mixture was stirred at 130 ºC for 19 h. The reaction mixture was poured into 1 M hydrochloric acid (25 mL) and it was extracted with ethyl acetate (25 + 2 × 15 mL). The combined organic phases were washed with 1 M hydrochloric acid (20 mL), water (20 mL), and brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified with automated column chromatography, eluting an *n*-hexane/ethyl acetate gradient (8 → 66% ethyl acetate) to give **13** as an amorphous colorless solid (8.4 mg, 6%) and **14** as an amorphous colorless solid (64 mg, 42%).

Compound **13**: FTIR-ATR 2932, 1722, 1472, 1248, 1184, 1134, 1092, 827 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.93 (d, J = 2.0 Hz, 1H), 7.24 (s, 1H), 7.21 (d, J = 2.0 Hz, 1H), 7.04 (s, 1H), 3.17 (hept, J = 6.9 Hz, 1H), 2.89 (m, 2H), 2.30 (m, 1H), 2.20 (dd, J_1 = 12.4 Hz, J_2 = 2.1 Hz, 1H), 1.70 (m, 8H), 1.28 (s, 3H), 1.23 (s, 3H), 1.20 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 182.8, 156.9, 152.1, 146.8, 144.3, 135.8, 130.9, 126.4, 126.2, 115.3, 47.5, 44.8, 38.1, 37.0, 36.8, 30.0, 29.1, 25.2, 24.4, 24.2, 21.8, 18.7, 16.5. HRMS calcd for $C_{23}H_{30}NO_2S$. $[M+1]^+$ 384.1997 found 384.2001.

Compound **14**: FTIR-ATR 3385, 2930, 1718, 1468, 1242, 1134, 878, 752 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.89 (d, J = 2.0 Hz, 1H), 7.27 (s, 1H), 7.21 (d, J = 2.0 Hz, 1H), 7.05 (s, 1H), 4.22 (m, 2H), 3.81 (t, J = 4.7 Hz, 2H), 3.20 (hept, J = 6.9 Hz, 1H), 2.93 (m, 2H), 2.30 (m, 2H), 1.79 (m, 6H), 1.51 (m, 2H), 1.30 (s, 3H), 1.24 (s, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.15 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.1, 157.1, 151.9, 146.8, 144.4, 135.6, 131.3, 126.4, 115.3, 66.5, 61.7, 47.9, 45.0, 38.1, 37.1, 36.8, 30.1, 29.1, 25.3, 24.3, 24.1, 21.9, 18.6, 16.7. HRMS calcd for $C_{25}H_{34}NO_3S$. $[M+1]^+$ 428.2259 found 428.2262.

Methyl 12-(2-phenylthiazol-4-yl)abieta-8,11,13-trien-18-oate (15). A mixture of crude **4** (0.100 g) and thiobenzamide (63.0 mg, 0.459 mmol) in EtOH (2.9 mL) was irradiated under microwaves at 120

ºC for 30 min. The reaction mixture was poured into a 1 M solution of NaOH in water (25 mL) and the mixture was extracted with ethyl acetate (25 mL + 2 × 15 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown oil. The crude product was purified with automated column chromatography, eluting with an *n*-heptane/ethyl acetate gradient (2 → 20% ethyl acetate) to give **15** as an amorphous light yellow solid (69.6 mg, 67% over 2 steps).

FTIR-ATR 2939, 1720, 1437, 1246, 1173, 1132, 766, 689 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.02 (m, 2H) 7.44 (m, 3H), 7.31 (s, 1H), 7.15 (s, 1H), 7.07 (s, 1H), 3.68 (s, 3H), 3.30 (hept, J = 6.9 Hz, 1H), 2.95 (dd, J_1 = 9.0, J_2 = 4.6 Hz, 2H), 2.30 (m, 2H), 1.72 (m, 6H), 1.45 (m, 1H), 1.29 (s, 3H), 1.25 (m, 6H), 1.20 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.2, 166.7, 157.7, 146.8, 144.5, 135.7, 134.1, 131.8, 130.0, 129.0, 126.7, 126.4, 126.2, 115.4, 52.1, 47.8, 45.0, 38.1, 37.1, 36.8, 30.0, 29.3, 25.2, 24.4, 24.2, 21.8, 18.7, 16.7. HRMS calcd for $C_{30}H_{36}NO_2S$. $[M+1]^+$ 474.2467 found 474.2467.

Methyl 12-[2-(2-chlorophenyl)thiazol-4-yl]abieta-8,11,13-trien-18-oate (16). A mixture of crude **4** (0.100 g) and 2-chlorothiobenzamide (79.0 mg, 0.459 mmol) in EtOH (2.9 mL) was irradiated under microwaves at 120 ºC for 30 min. The reaction mixture was poured into a 1 M solution of NaOH in water (25 mL) and the mixture was extracted with ethyl acetate (25 mL + 2 × 10 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown-yellow solid. The crude product was purified with automated column chromatography, eluting with an *n*-heptane/ethyl acetate gradient (2 → 20% ethyl acetate) to give **16** as an amorphous light yellow solid (74.0 mg, 66% over 2 steps).

FTIR-ATR 2932, 1722, 1466, 1433, 1242, 1132, 1065, 1040, 758 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.35 (m, 1H) 7.51 (m, 1H), 7.35 (m, 2H), 7.32 (s, 1H), 7.31 (s, 1H), 7.07 (s, 1H), 3.68 (s, 3H), 3.31 (hept, J = 6.8 Hz, 1H), 2.95 (dd, J_1 = 8.9, J_2 = 4.5 Hz, 2H), 2.29 (m, 2H), 1.78 (m, 5H), 1.56 (m, 1H), 1.46 (m, 1H), 1.29 (s, 3H), 1.22 (m, 9H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.2, 162.0, 156.3, 146.9, 144.5, 135.7, 132.2, 132.0, 131.6, 131.1, 130.8, 130.2, 127.2, 126.4, 126.3, 117.5, 52.1, 47.8, 45.0, 38.1, 37.1, 36.8, 30.0, 29.3, 25.3, 24.4, 24.2, 21.8, 18.7, 16.7. HRMS calcd for $C_{30}H_{35}NO_2SCl$. $[M+1]^+$ 508.2077 found 508.2079.

Methyl 12-[2-(4-chlorophenyl)thiazol-4-yl]abieta-8,11,13-trien-18-oate (17). A mixture of crude **4** (0.100 g) and 4-chlorothiobenzamide (79.0 mg, 0.459 mmol) in EtOH (2.9 mL) was irradiated under microwaves at 120 ºC for 1 h. The reaction mixture was poured into a 1 M solution of NaOH in water (25 mL) and the mixture was extracted with ethyl acetate (25 mL + 2 × 10 mL). The organic phases

were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a brown-yellow solid. The crude product was purified with automated column chromatography, eluting an *n*-heptane/ethyl acetate gradient (2 → 20% ethyl acetate) to give **17** as an amorphous light yellow solid (48.9 mg, 43% over 2 steps).

FTIR-ATR 2932, 1722, 1470, 1242, 1132, 1090, 997, 831 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.95 (m, 2H), 7.42 (m, 2H), 7.29 (s, 1H), 7.16 (s, 1H), 7.07 (s, 1H), 3.68 (s, 3H), 3.26 (hept, J = 6.8 Hz, 1H), 2.94 (dd, J_1 = 9.0, J_2 = 4.6 Hz, 2H), 2.28 (m, 2H), 1.78 (m, 5H), 1.56 (m, 1H), 1.46 (m, 1H), 1.29 (s, 3H), 1.22 (m, 9H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.2, 165.4, 157.8, 146.9, 144.5, 135.9, 135.9, 132.5, 131.5, 129.3, 127.9, 126.4, 126.1, 115.7, 52.1, 47.8, 45.0, 38.1, 37.1, 36.8, 30.0, 29.4, 25.2, 24.4, 24.2, 21.8, 18.7, 16.7. HRMS calcd for $C_{30}H_{35}NO_2SCl$. $[M+1]^+$ 508.2077 found 508.2077.

Methyl 12-(2-methylthiazol-4-yl)abieta-8,11,13-trien-18-oate (18). A mixture of crude **4** (0.100 g) and thioacetamide (35.0 mg, 0.459 mmol) in EtOH (2.9 mL) was irradiated under microwaves at 120 ºC for 30 min. The reaction mixture was poured into a 1 M solution of NaOH in water (25 mL) and the mixture was extracted with ethyl acetate (25 mL + 2 × 15 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown oil. The crude product was purified with automated column chromatography, eluting with *n*-heptane/ethyl acetate 9:1 to give **18** as an amorphous white solid (65.6 mg, 72% over 2 steps).

FTIR-ATR 2931, 1717, 1245, 1177, 1132, 1084, 770 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.22 (s, 1H), 7.01 (s, 1H), 6.95 (s, 1H), 3.67 (s, 3H), 3.19 (hept, J = 6.9 Hz, 1H), 2.92 (m, 2H), 2.76 (s, 3H), 2.30 (m, 1H), 2.24 (dd, J_1 = 12.5 Hz, J_2 = 2.3 Hz, 1H), 1.76 (m, 5H), 1.53 (m, 1H), 1.43 (m, 1H), 1.28 (s, 3H), 1.22 (s, 3H), 1.18 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.3, 164.5, 156.0, 146.7, 144.3, 135.4, 131.8, 126.2, 126.1, 114.9, 52.1, 47.8, 45.0, 38.1, 37.1, 36.8, 29.9, 29.1, 25.2, 24.4, 24.1, 21.8, 19.4, 18.7, 16.7. HRMS calcd for $C_{25}H_{33}NO_2S$. $[M+1]^+$ 412.2310 found 412.2309.

Methyl 12-[2-(ethoxycarbonyl)thiazol-4-yl]abieta-8,11,13-trien-18-oate (19). A mixture of crude **4** (0.100 g) and ethyl thiooxamate (61.0 mg, 0.4593 mmol) in EtOH (2.9 mL) was irradiated under microwaves at 120 ºC for 30 min. The reaction mixture was diluted with ethyl acetate (25 mL) and washed with a 1 M solution of NaOH in water (25 mL). The aqueous phase was extracted with ethyl acetate (2 × 10 mL). The organic phases were combined, washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown oil. The crude product was

purified with automated column chromatography, eluting with an *n*-heptane/ethyl acetate gradient (2 → 20% ethyl acetate) to give **19** as an amorphous light yellow solid (23.1 mg, 22% over 2 steps).

FTIR-ATR 2930, 1718, 1433, 1244, 1132, 1082, 754 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.41 (s, 1H), 7.21 (s, 1H), 7.03 (s, 1H), 4.49 (q, J = 7.1 Hz, 2 H), 3.67 (s, 3H), 3.08 (hept, J = 7.2 Hz, 1H), 2.92 (dd, J_1 = 9.1, J_2 = 4.6 Hz, 2H), 2.26 (m, 2H), 1.75 (m, 5H), 1.48 (m, 5H), 1.28 (s, 3H), 1.21 (s, 3H), 1.17 (d, J = 6.8 Hz, 3H), 1.14 (d, J = 6.8 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.2, 160.5, 158.9, 157.3, 146.9, 144.4, 136.1, 130.9, 126.4, 126.2, 121.9, 62.6, 52.1, 47.8, 45.0, 38.1, 37.1, 36.8, 30.0, 29.3, 25.2, 24.3, 24.1, 21.8, 18.6, 16.7, 14.4. HRMS calcd for $C_{27}H_{36}NO_4S$. $[M+1]^+$ 470.2365 found 470.2365.

Methyl 12-(2-cyanothiazol-4-yl)abieta-8,11,13-trien-18-oate (20). 19 (0.0900 g, 0.192 mmol) was dissolved in a 7 M solution of NH_3 in methanol (1.7 mL) and stirred at room temperature for 48 h, and the solvent was evaporated to dryness. The residue was dissolved in THF (1 mL) and triethylamine (0.16 mL, 1.15 mmol) was added. The mixture was cooled down to 0 ºC and trifluoroacetic anhydride (0.080 mL, 0.575 mmol) was added. Stirring was continued at 0 ºC for 10 min and at room temperature for 3 h. The reaction was quenched with a saturated solution of $NaHCO_3$ in water (20 mL), and the aqueous phase was extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified with automated column chromatography, eluting with an *n*-hexane/ethyl acetate gradient (2 → 20% ethyl acetate) to give **20** as an amorphous light yellow solid (37 mg, 46%).

FTIR-ATR 2932, 2223, 1722, 1460, 1244, 1132, 1107, 752 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.49 (s, 1H), 7.22 (s, 1H), 7.07 (s, 1H), 3.67 (s, 3H), 3.09 (hept, J = 6.8 Hz, 1H), 2.94 (dd, J_1 = 9.0, J_2 = 4.6 Hz, 2H), 2.26 (m, 2H), 1.77 (m, 5H), 1.49 (m, 2H), 1.28 (s, 3H), 1.23 (s, 3H), 1.19 (d, J = 6.8 Hz, 3H), 1.16 (d, J = 6.8 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.1, 159.4, 147.2, 144.4, 136.9, 135.4, 129.3, 126.6, 126.3, 121.2, 113.2, 52.1, 47.7, 44.9, 38.1, 37.1, 36.8, 29.9, 29.2, 25.2, 24.3, 24.1, 21.7, 18.6, 16.6. HRMS calcd for $C_{25}H_{31}N_2O_2S$. $[M+1]^+$ 423.2106 found 423.2104.

12-(2-Methylthiazol-4-yl)abieta-8,11,13-trien-18-ol (21). $LiAlH_4$ (9.70 mg, 0.255 mmol) was suspended in dry tetrahydrofuran (1 mL) and added to a stirred solution of **18** (0.100 g, 0.243 mmol) in tetrahydrofuran (2 mL) at 0 ºC. The mixture was stirred at 0 ºC for 15 min and at room temperature for 2 h. The reaction was quenched by slow addition of 2 M hydrochloric acid (1.5 mL) at 0 ºC. Tetrahydrofuran was evaporated and the residue was diluted with water (20 mL) and extracted with ethyl acetate (30 mL). The organic phase was washed with brine (15 mL), dried with anhydrous

Na_2SO_4, filtered and concentrated *in vacuo* to give a yellowish solid. The crude product was purified with automated column chromatography, eluting an *n*-hexane/ethyl acetate gradient (5 → 40% ethyl acetate) to give **21** as an amorphous colorless solid (33.6 mg, 36%).

FTIR-ATR 3354, 2922, 1495, 1443, 1379, 1171, 1053, 754 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.23 (s, 1H), 6.98 (s, 1H), 6.95 (s, 1H), 3.46 (dd, J_1 = 10.9, J_2 = 1.6 Hz, 1H), 3.19 (m, 2H), 2.91 (m, 2H), 2.76 (s, 3H), 2.28 (m, 1H), 1.73 (m, 5H), 1.41 (m, 3H), 1.29 (s, 3H), 1.18 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H), 0.89 (s, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 164.6, 156.0, 147.1, 144.1, 135.5, 131.6, 126.3, 126.0, 114.8, 72.4, 44.1, 38.5, 38.0, 37.4, 35.3, 30.0, 29.1, 25.3, 24.4, 24.1, 19.4, 19.0, 18.7, 17.5. HRMS calcd for $C_{24}H_{33}NOS$. $[M+1]^+$ 384.2361 found 384.2360.

12-(2-Methylthiazol-4-yl)abieta-8,11,13-trien-18-oic acid (22). 18 (143 mg, 0.347 mmol) was suspended in ethylene glycol (2 mL), and KOH (0.0900 g, 1.36 mmol) in water (0.2 mL) was added. The mixture was stirred at 130 ºC for 24 h. The reaction mixture was poured into 1 M hydrochloric acid (20 mL) and it was extracted with ethyl acetate (20 + 2 × 10 mL). The combined organic phases were washed with 1 M hydrochloric acid (17 mL), water (15 mL), and brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **22** as an amorphous colorless solid (64.4 mg, 47%).

FTIR-ATR 2930, 1705, 1502, 1240, 1182, 1132, 756 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.17 (s, 1H), 6.98 (s, 1H), 6.96 (s, 1H), 3.10 (hept, J = 6.8 Hz, 1H), 2.88 (s, 3H), 2.72 (dd, J_1 = 17.1, J_2 = 5.8 Hz, 1H), 2.52 (m, 1H), 2.29 (m, 1H), 1.96 (dd, J_1 = 12.3 Hz, J_2 = 1.6 Hz, 1H), 1.69 (m, 6H), 1.31 (m, 1H), 1.23 (d, J = 6.9 Hz, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 1.13 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 181.8, 166.4, 154.7, 146.5, 143.9, 135.9, 130.4, 126.8, 125.9, 114.8, 52.1, 47.5, 45.2, 38.3, 37.1, 36.6, 30.5, 29.1, 25.4, 24.4, 24.2, 21.7, 18.8, 18.8, 16.4. HRMS calcd for $C_{24}H_{32}NO_2S$. $[M+1]^+$ 398.2154 found 398.2154.

12-(2-Methylthiazol-4-yl)abieta-8,11,13-trien-18-amide (23). 22 (0.120 g, 0.302 mmol) was dissolved in *N*,*N*-dimethylformamide (1.2 mL) and cooled to 0 ºC. 1-Hydroxybenzotriazole hydrate (43.2 mg, 0.320 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (61.3 mg, 0.320 mmol) were added and the stirring was continued at 0 ºC for 20 min, after which the solution was left to come to room temperature. After 10 min, a 25% solution of NH_3 in water (0.070 mL, 0.45 mmol) was added and the mixture was stirred at room temperature for 22 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The organic phases were combined, washed with 1 M hydrochloric acid (15 mL), water (15 mL) and brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a brownish solid. The

crude product was purified with automated column chromatography, eluting an *n*-hexane/ethyl acetate gradient (12 → 100% ethyl acetate) to give **23** as an amorphous colorless solid (91.9 mg, 77%).

FTIR-ATR 3495, 2935, 1653, 1595, 1497, 1348, 1169, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.22 (s, 1H), 7.00 (s, 1H), 6.95 (s, 1H), 5.76 (brs, 1H), 5.43 (brs, 1H), 3.18 (hept, J = 6.9 Hz, 1H), 2.92 (dd, J_1 = 9.0 Hz, J_2 = 4.7 Hz, 2H), 2.76 (s, 3H), 2.32 (m, 1H), 2.12 (dd, J_1 = 12.5, J_2 = 2.3 Hz, 1H), 1.70 (m, 7H), 1.29 (s, 3H), 1.24 (s, 3H), 1.17 (d, J = 6.9 Hz, 3H), 1.13 (d, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 181.3, 164.6, 155.6, 146.7, 144.3, 135.3, 131.8, 126.1, 126.1, 114.9, 47.5, 45.7, 38.1, 37.5, 37.2, 29.9, 29.1, 25.2, 24.4, 24.1, 21.3, 19.4, 18.8, 16.9. HRMS calcd for $C_{24}H_{33}N_2OS$. $[M+1]^+$ 397.2314 found 397.2318.

4-[18-(Methoxycarbonyl)abieta-8,11,13-trien-12-yl]-2,3-dimethylthiazol-3-ium iodide (24). A mixture of **18** (0.100 g, 0.24 mmol) and iodomethane (0.045 mL, 0.73 mmol) in acetone (1 mL) and was heated at 56 ºC for 23 h. The formed precipitate was filtered and washed with ethyl acetate to give **24** as an amorphous light yellow solid (98 mg, 73%).

FTIR-ATR 2924, 1718, 1437, 1246, 1173, 1132, 797 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.75 (s, 0.5H), 7.70 (s, 0.5H), 7.07 (s, 1H), 3.79 (m, 3H), 3.66 (m, 3H), 3.25 (s, 3H), 2.94 (m, 2H), 2.55 (m, 1H), 2.39 (m, 1H), 2.18 (m, 1H), 1.83 (m, 1H), 1.67 (m, 4H), 1.42 (m, 2H), 1.26 (m, 3H), 1.12 (m, 9H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.1, 179.0*, 170.9, 170.9*, 149.0, 148.8*, 148.8, 148.7*, 145.2, 145.1*, 139.6, 128.2, 128.0*, 127.0, 126.9*, 123.0, 122.9*, 120.3, 120.0*, 52.1, 52.1*, 47.6, 44.8, 44.7*, 39.2, 39.0*, 38.3, 38.0*, 37.5, 37.4*, 36.8, 36.8*, 30.5, 30.4*, 30.2, 30.1*, 25.4, 25.1, 25.1*, 25.0*, 23.6, 23.4*, 21.5, 19.7, 19.7*, 18.5, 18.5*, 16.6. HRMS calcd for $C_{26}H_{36}NO_2S$. $[M]^+$ 426.2467 found 426.2467.

3-Ethyl-4-[18-(methoxycarbonyl)abieta-8,11,13-trien-12-yl]-2-methylthiazol-3-ium iodide (25). A mixture of **18** (0.10 g, 0.24 mmol) and iodoethane (0.023 mL, 0.29 mmol) in acetone (1 mL) was heated at 56 ºC for 3 d. The formed precipitate was filtered and washed with ethyl acetate to give **25** as an amorphous light yellow solid (15 mg, 11%).

FTIR-ATR 2947, 1722, 1435, 1246, 1178, 1132, 793 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.75 (m, 0.5H), 7.67 (m, 0.5H), 7.38 (m, 0.5H), 7.32 (m, 0.5H), 7.10 (m, 1H), 4.37 (m, 1H), 4.19 (m, 1H), 3.68 (s, 1.5H), 3.68 (s, 1.5H), 3.32 (s, 1.5H), 3.31 (s, 1.5H), 2.96 (m, 2H), 2.53 (m, 1H), 2.37 (m, 1H), 2.20 (m, 1H), 1.63 (m, 7H), 1.25 (m, 12H), 1.11 (m, 3H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δ ppm 179.1, 179.0*, 170.5, 170.3*, 148.6, 148.5, 148.5*, 148.2*, 145.4, 145.4*, 139.8, 127.7, 127.6*, 127.2,

127.1*, 122.7, 122.7*, 121.0, 120.5*, 52.2, 52.2*, 47.7, 47.2, 47.0*, 44.8, 44.7*, 38.4, 38.0*, 37.4, 37.4*, 36.8, 36.8*, 30.6, 30.6*, 30.2, 25.9, 25.7*, 25.4, 25.1*, 23.1, 22.8*, 21.5, 21.5*, 19.3, 19.2*, 18.5, 16.6, 16.6*, 14.5, 14.4*. HRMS calcd for $C_{27}H_{38}NO_2S$. $[M]^+$ 440.2623 found 440.2628.

4-[18-(Methoxycarbonyl)abieta-8,11,13-trien-12-yl]-3-methylthiazol-3-ium iodide (26). A mixture of **6** (76 mg, 0.19 mmol) and iodomethane (0.036 mL, 0.57 mmol) in acetone (1 mL) was heated at 56 ºC for 24 h. The formed precipitate was filtered and washed with ethyl acetate to give **26** as an amorphous light yellow solid (67 mg, 65%).

FTIR-ATR 2943, 1722, 1470, 1433, 1246, 1132, 760 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δppm 11.36 (m, 1H), 7.83 (d, *J* = 2.3 Hz, 0.5H), 7.77 (d, *J* = 2.3 Hz, 0.5H), 7.23 (s, 0.5H), 7.17 (s, 0.5H), 7.11 (s, 1H), 4.07 (s, 3H), 3.68 (s, 3H), 2.96 (m, 2H), 2.41 (m, 1H), 2.29 (m, 1H), 2.21 (m, 1H), 1.85 (m, 1H), 1.69 (m, 4H), 1.46 (m, 2H), 1.27 (s, 3H), 1.14 (m, 9H). ^{13}C-NMR ($CDCl_3$, 101 MHz): δppm 179.0, 161.0, 149.5, 149.3*, 148.9, 148.8*, 145.2, 140.1, 127.3, 127.2, 127.0*, 122.9, 122.5*, 121.5, 121.5*, 52.2, 47.6, 44.7, 41.6, 41.5*, 38.3, 38.0, 37.4, 36.7*, 30.6, 30.5*, 30.1, 25.5, 25.2, 25.0*, 23.4, 23.2*, 21.4, 18.5, 16.6. HRMS calcd for $C_{25}H_{34}NO_2S$. $[M]^+$ 412.2310 found 412.2313.

3. Routine hydrolase activity assay

Glycerol liberated from the hydrolysis of the preferred MAG substrates (1-AG for hABHD12 and 1-LG for hABHD16A) was determined kinetically using a validated fluorometric assay, as previously described.[3,4] Briefly, glycerol production was coupled via a three-step enzymatic cascade to hydrogen peroxide (H_2O_2) dependent generation of resorufin whose fluorescence (λ_{ex} 530; λ_{em} 590 nm) was kinetically monitored using either a Tecan Infinite M200 plate reader (Tecan Group Ltd., Männedorf, Switzerland) or Perkin Elmer 1420 multilabel counter Victor3 TM (Wallac Oy Turku Finland). The assays routinely contained 0.5 % (w/v) BSA (essentially fatty acid free) as a carrier for lipophilic compounds. Lysates of HEK293 cells transiently overexpressing hABHD12 or hABHD16A were prepared essentially as previously described.[3,4] The amount of lysate protein per well was 0.3 and 0.6 µg for hABHD12-HEK293 and hABHD16A-HEK293 cells, respectively. Final substrate concentrations were 12.5 and 25 µM for 1-AG and 1-LG, respectively. Kinetic readings were taken at 30-min-intervals and time-points 90 or 120 min were chosen to calculate the results.

4. Reversibility assay

Compound reversibility was tested as previously described.[5] Briefly, DMSO/inhibitor (0.5 µL) was incubated together with hABHD16A-HEK293 lysate (4.5 µL, 0.6 µg protein/well) in assay buffer containing 0.5 % w/v BSA for 30 min. After this, a 40-fold dilution of enzyme-inhibitor complex was brought about by addition of the substrate [1-LG, 25 µM final concentration, containing additionally 1 % EtOH (v/v)] in 195 µL of glycerol assay mix and fluorescence was kinetically monitored at 30 min intervals for 120 min at room temperature. Four to five inhibitor concentrations were included to cover the entire dose-response range and the IC_{50} values were determined at time-point 120 min.

5. Activity-based protein profiling (ABPP) of serine hydrolases

Competitive ABPP using rat cerebellar membranes (Rcm) was conducted to visualize the effect of selected inhibitors on ABHD16A activity in native brain membrane proteomes. We used the active site serine-targeting fluorescent fluorophosphonate probe TAMRA-FP as previously described.[3] Briefly, brain membranes (100 µg) were treated for 1 h with DMSO or the selected inhibitors, after which TAMRA-FP labelling was conducted for 1 hour at room temperature (final probe concentration 1 µM). The reaction was quenched by addition of 2× gel loading buffer, after which 20 µg protein (20 µL) was loaded per lane and the proteins were resolved in 10% SDS-PAGE together with molecular weight standards. In gel TAMRA-FP fluorescence was visualized using ChimiDocTM MP Imaging System (BioRad, CA, USA) with Cy3 wavelength settings.

6. Data analysis

The inhibitor dose-response curves and IC_{50} values derived thereof were calculated from nonlinear regressions using Graph-Pad Prism 5.0 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

7.Figure S1.

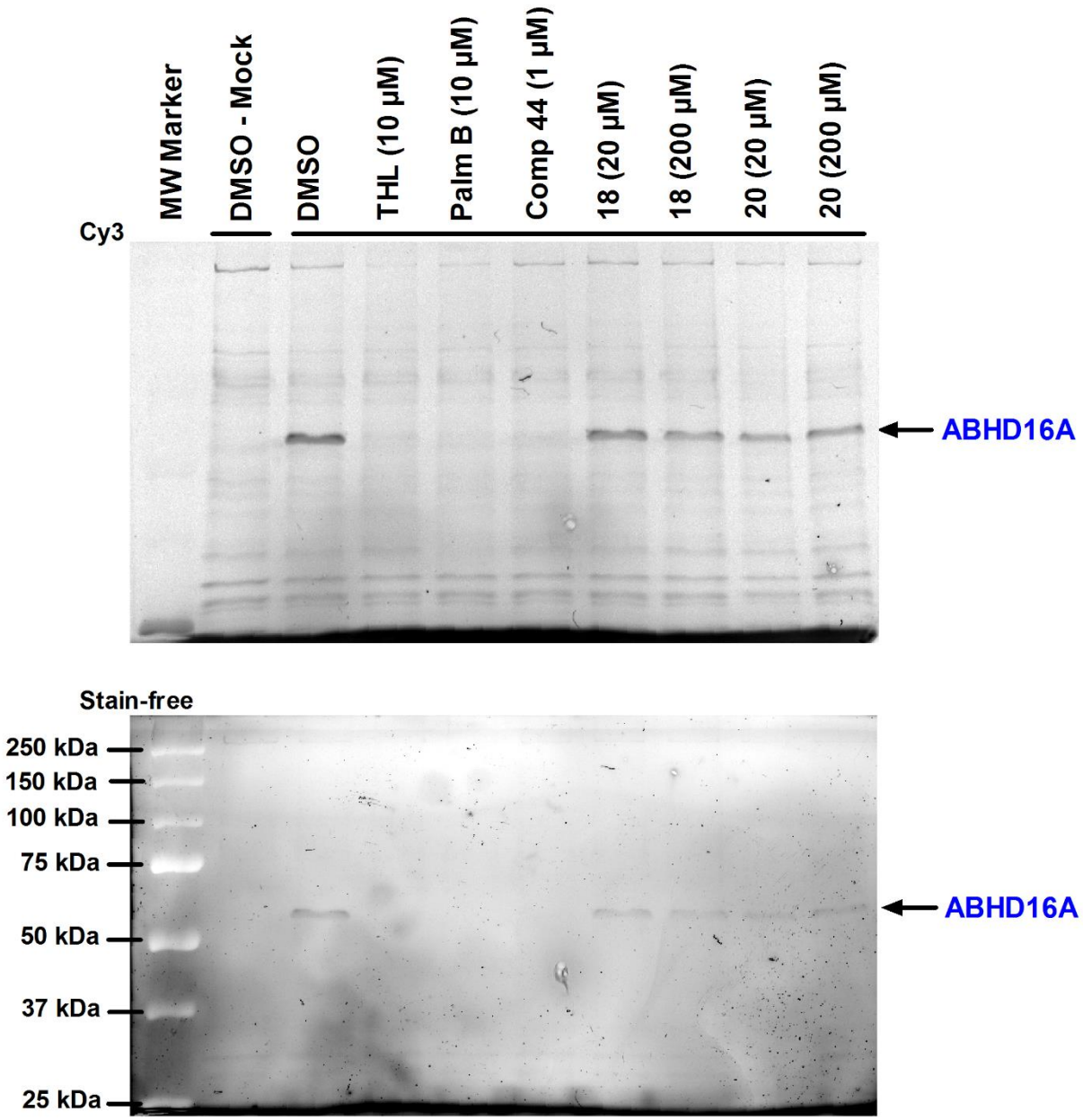
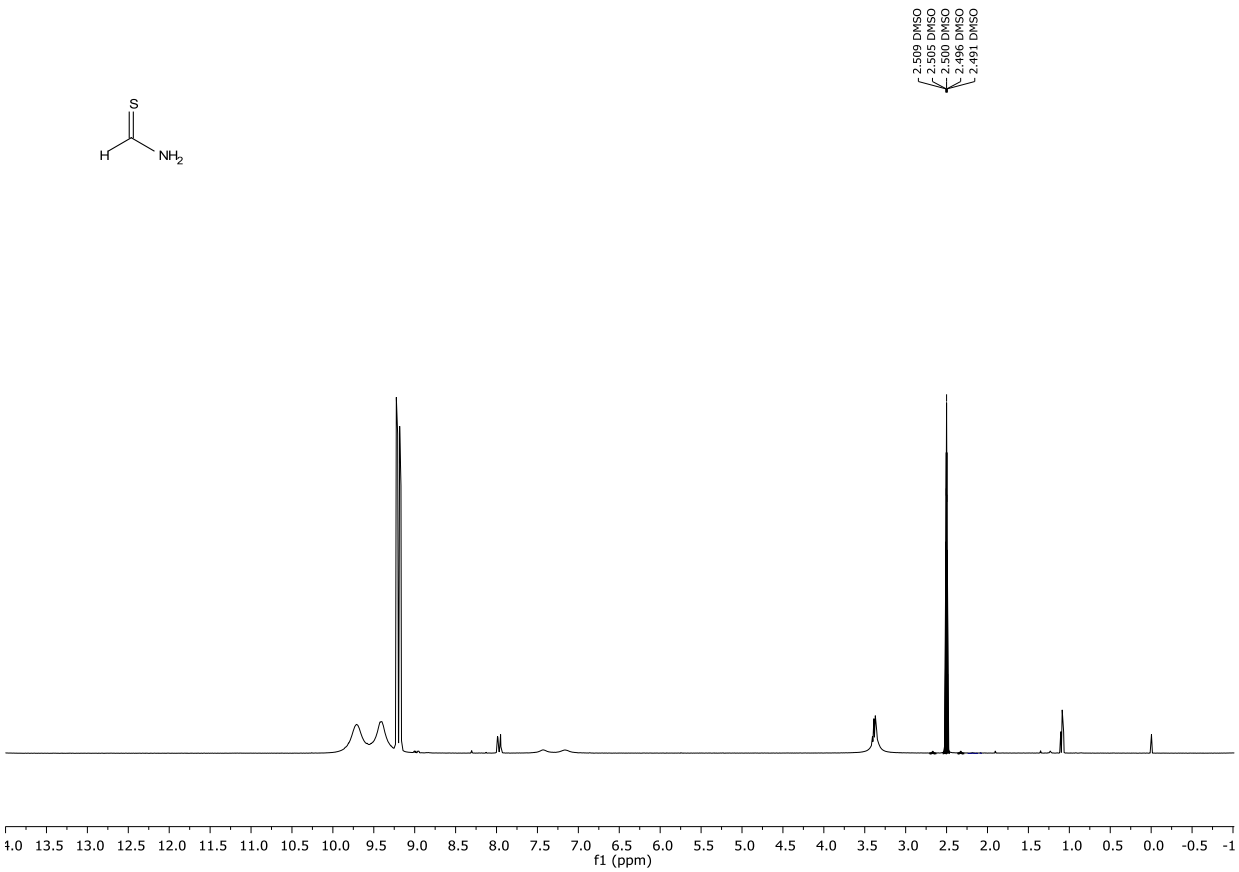
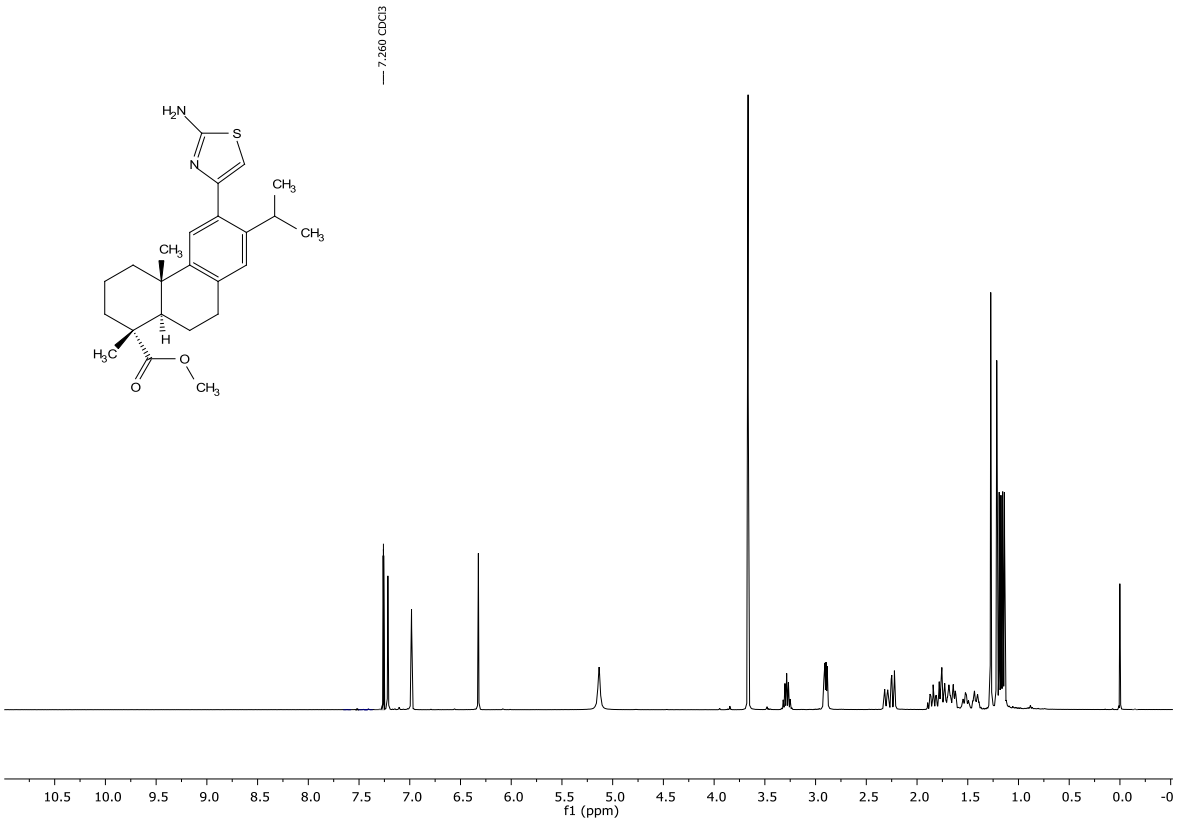


Figure S1. Competitive ABPP using hABHD16A-HEK lysates (0.1 mg/mL; 2.5 µg/reaction; 20 µL per lane) indicates partial inhibition for TAMRA-FP labeling of the enzyme by **18** and **20**. THL (10 µM), palmostatin B (10 µM) and Compound 44 (1 µM) were used as positive controls and in line with our previous results[4], the three inhibitors totally blocked TAMRA-FP labeling of the ~63 kDa serine hydrolase band corresponding to hABHD16A/BAT5. This band is absent from Mock-transfected HEK cells. In line with the outcome of the substrate-based enzyme assays using hABHD16A-HEK lysates (Figure 2C-D), **18** and **20** could only partially inhibit activity probe binding to ABHD16A. The upper gel image was visualized for TAMRA-FP fluorescence using Cy3 wavelength settings whereas stain-free filter was used for the same gel (lower image) to visualize molecular weight (MW) markers. This experiment was repeated once with similar outcome.

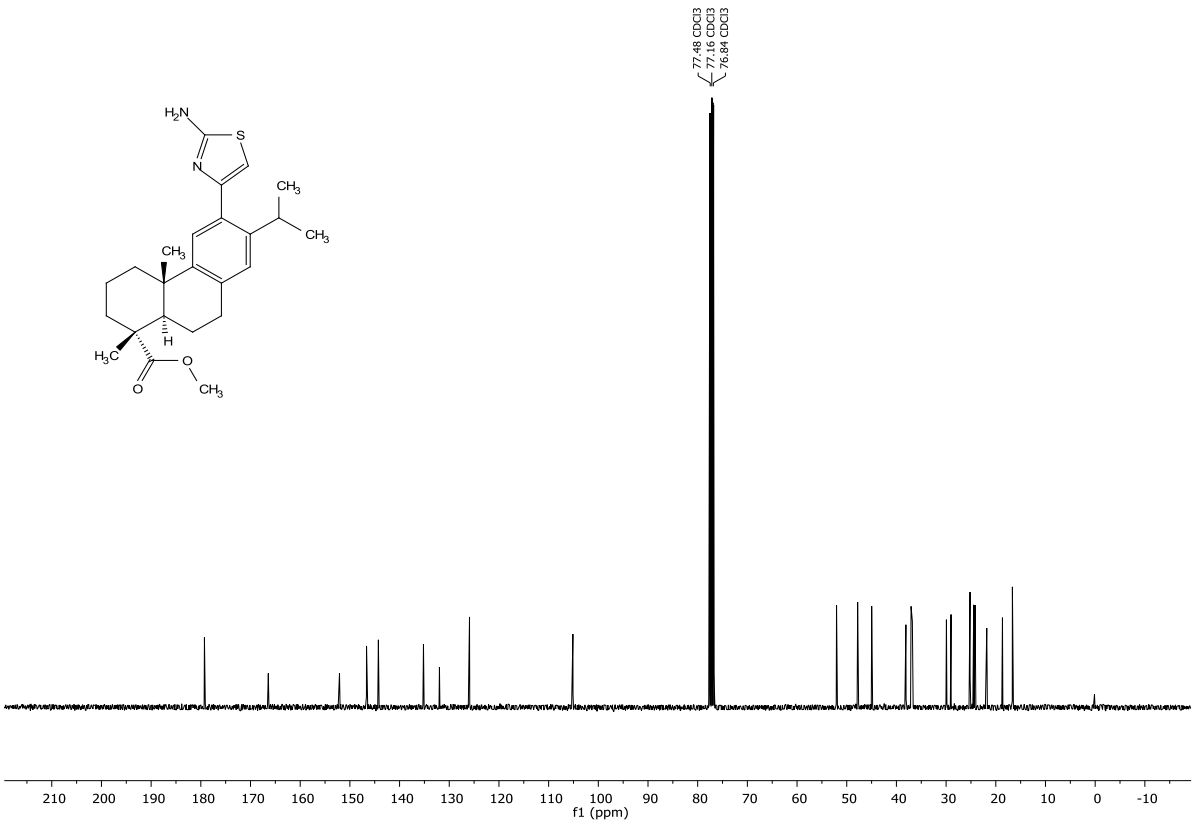
8. NMR Spectra



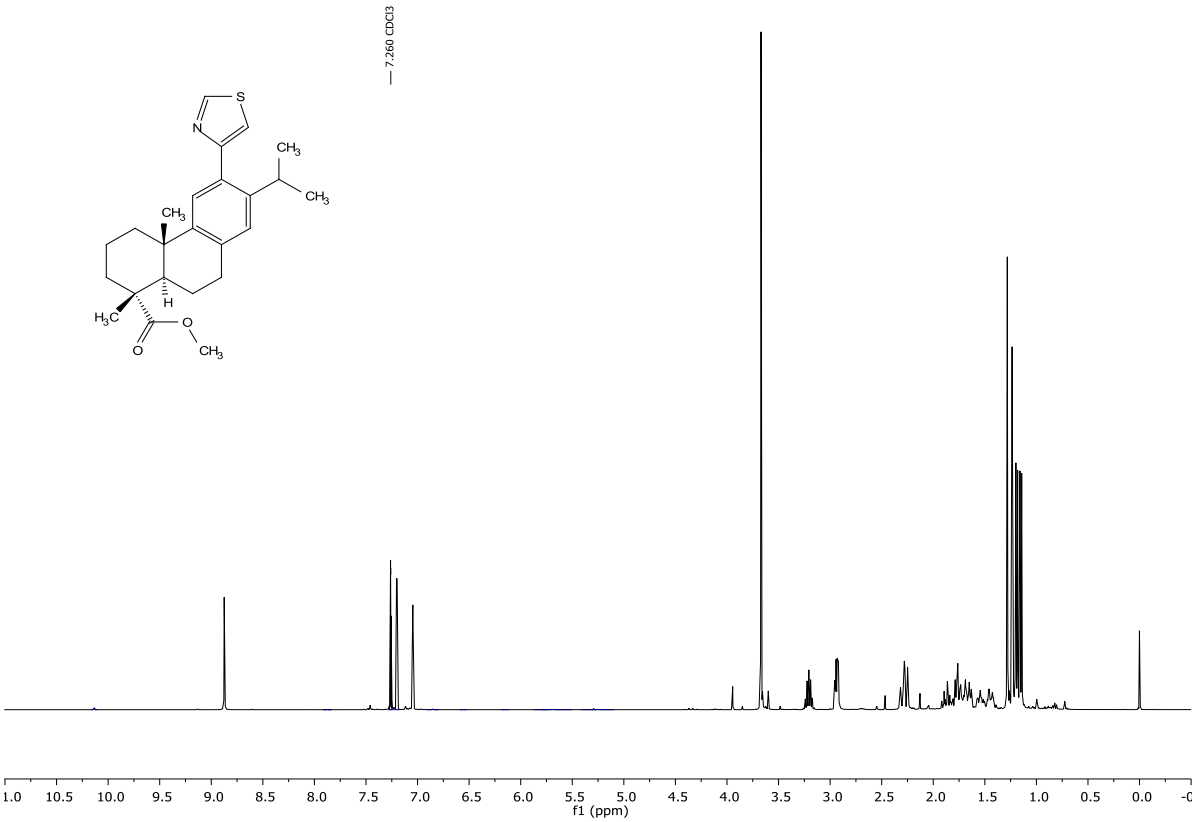
^{1}H NMR of thioformamide in DMSO-d_6



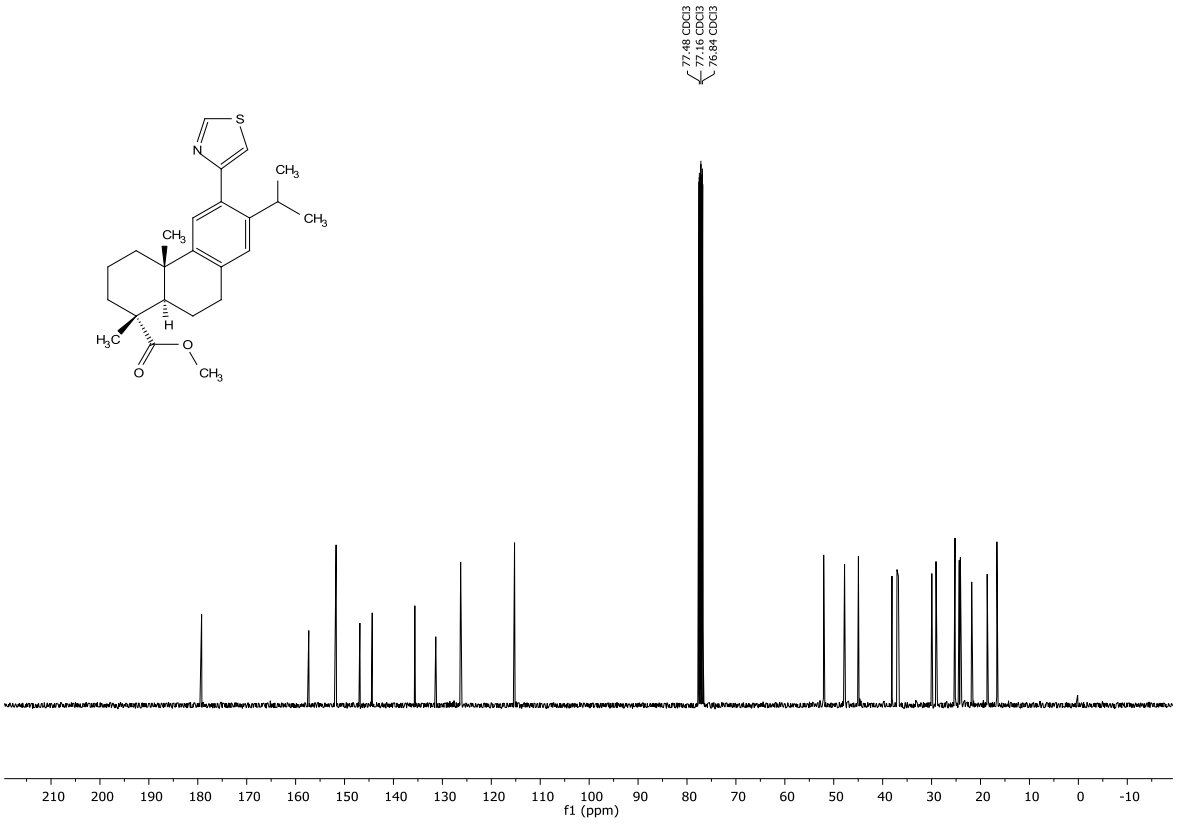
^{1}H NMR of compound **5** in $CDCl_3$



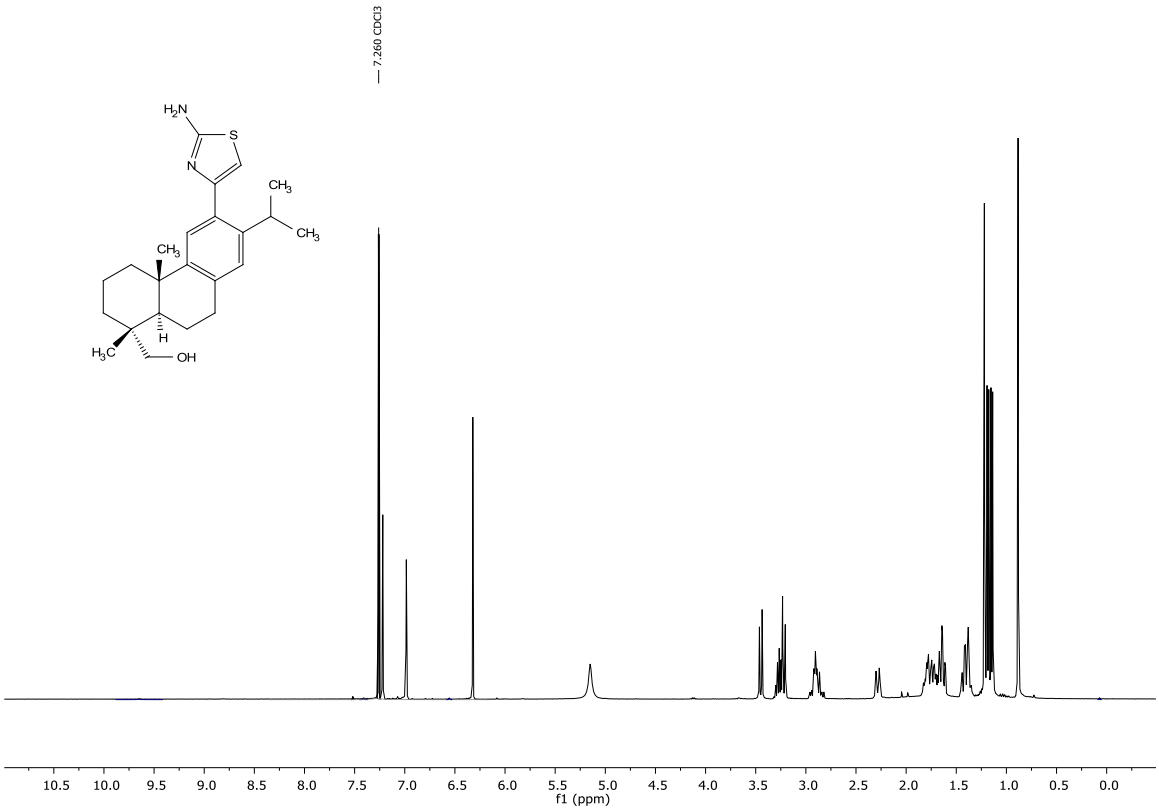
^{13}C NMR of compound **5** in $CDCl_3$



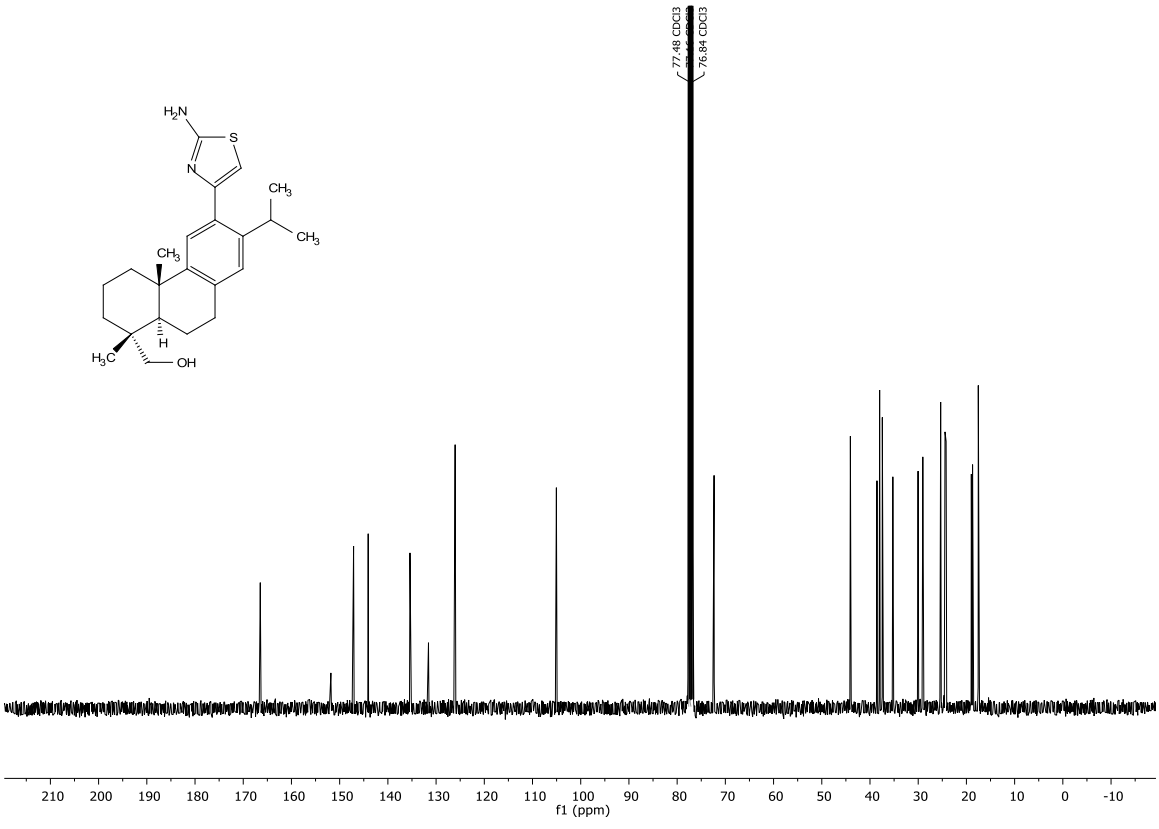
^{1}H NMR of compound **6** in $CDCl_3$



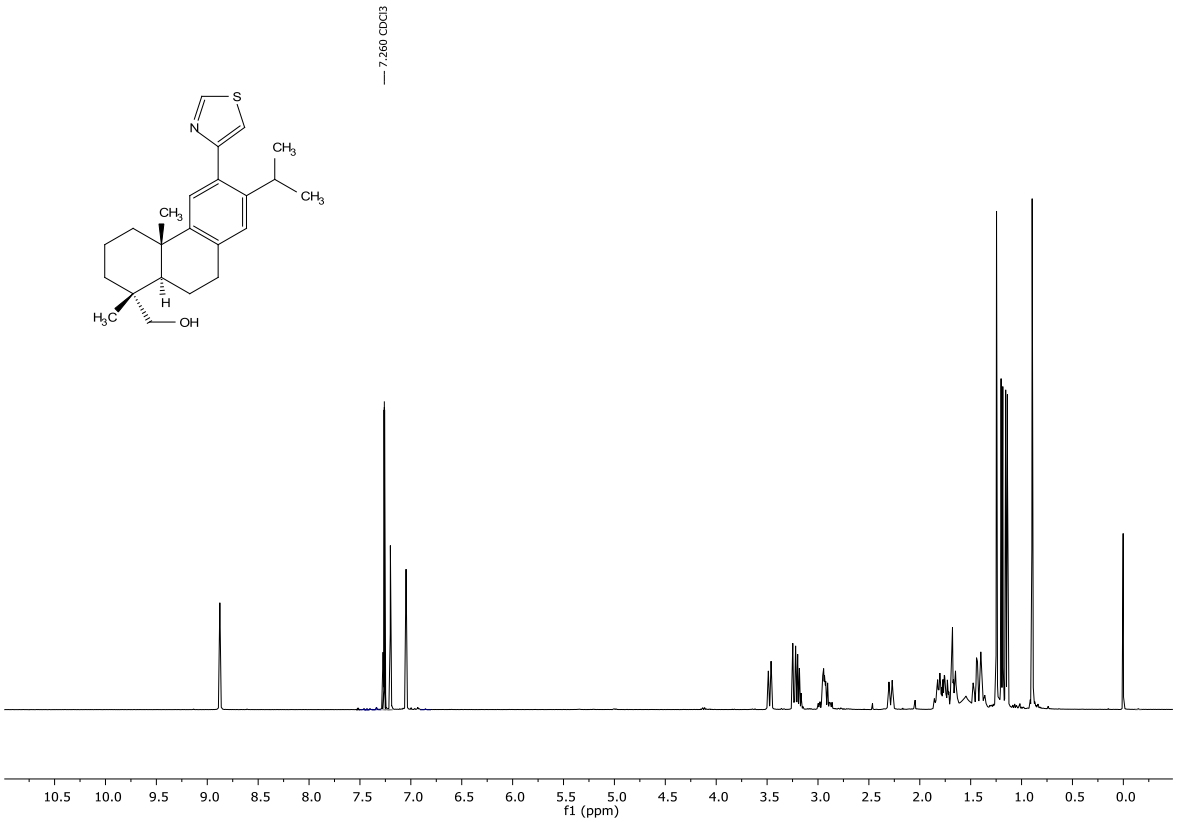
^{13}C NMR of compound **6** in $CDCl_3$



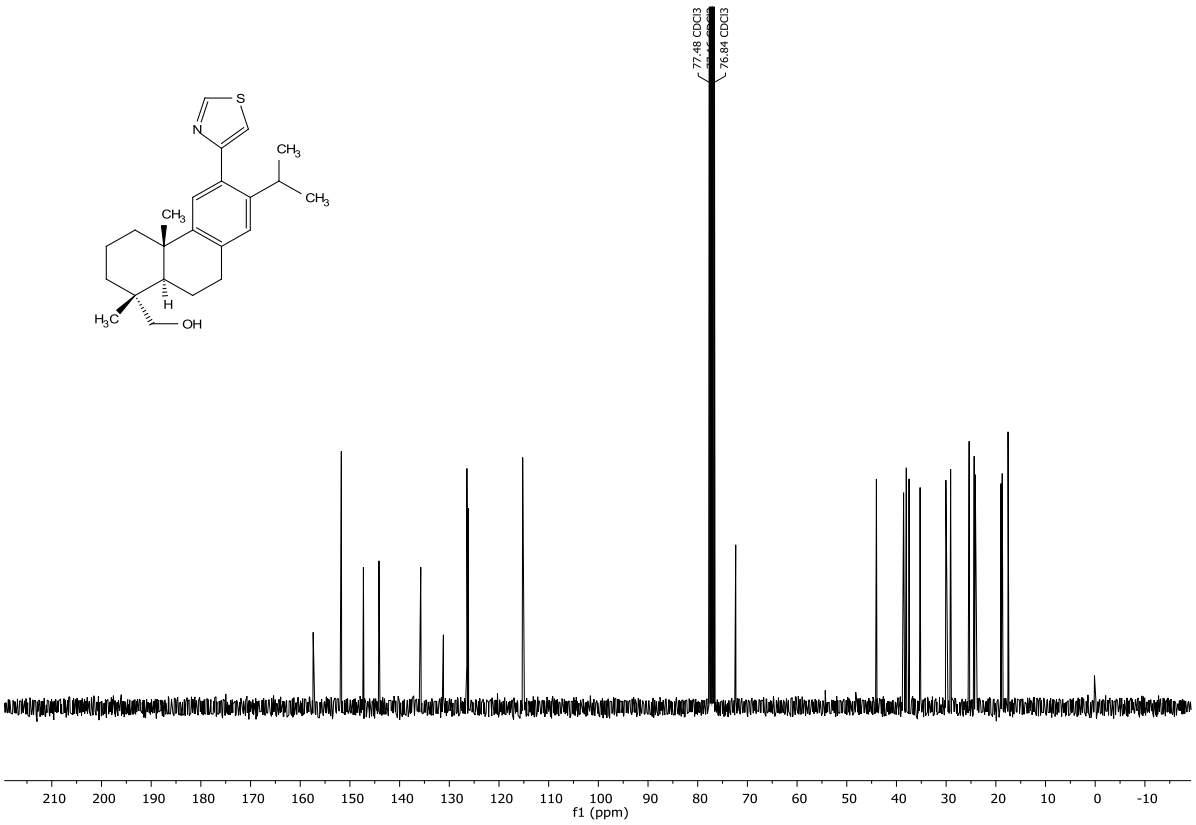
^{1}H NMR of compound **7** in $CDCl_3$



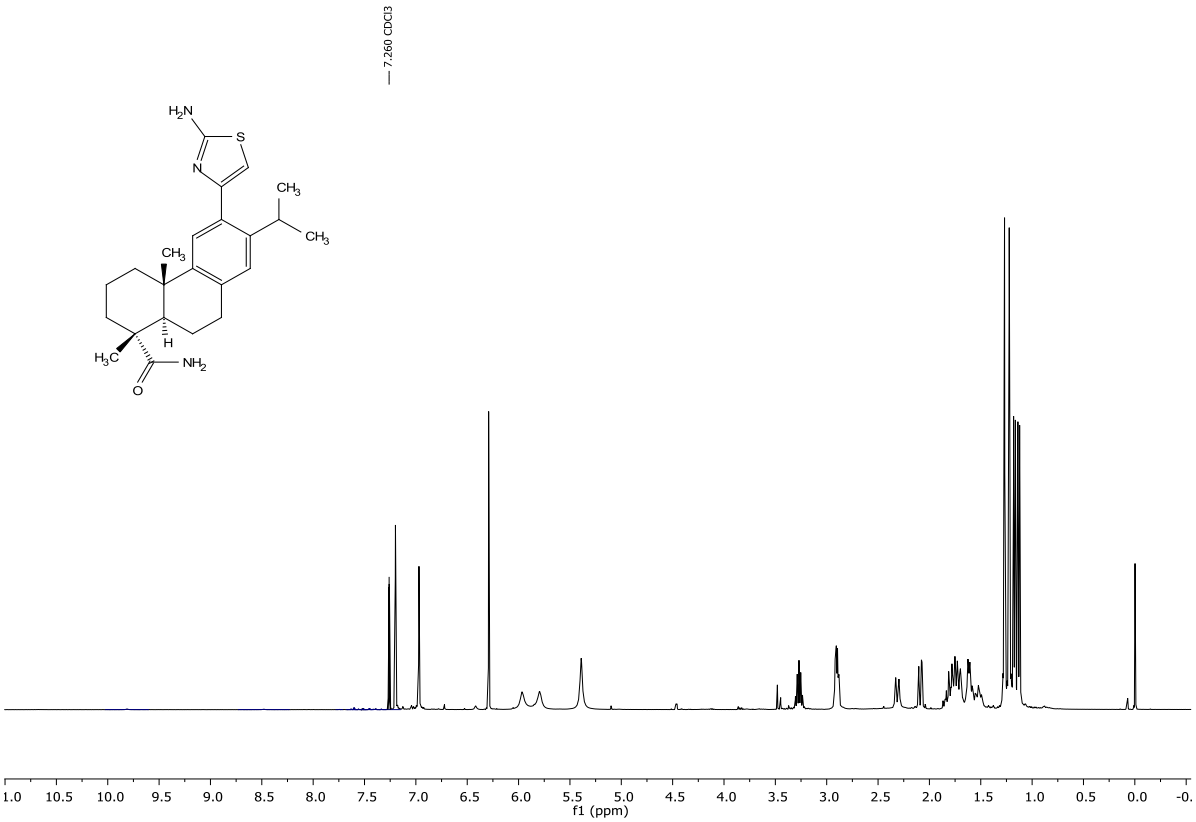
^{13}C NMR of compound **7** in $CDCl_3$



^{1}H NMR of compound **8** in $CDCl_3$



^{13}C NMR of compound **8** in $CDCl_3$



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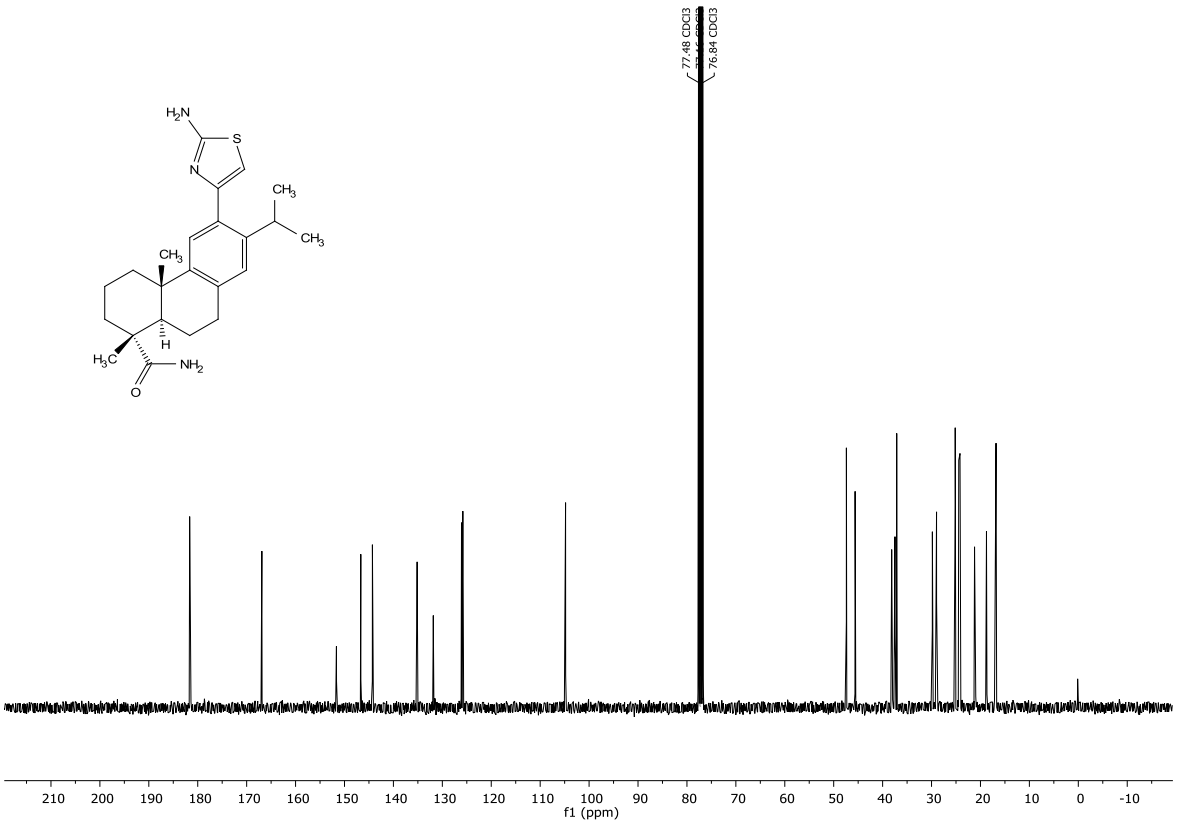

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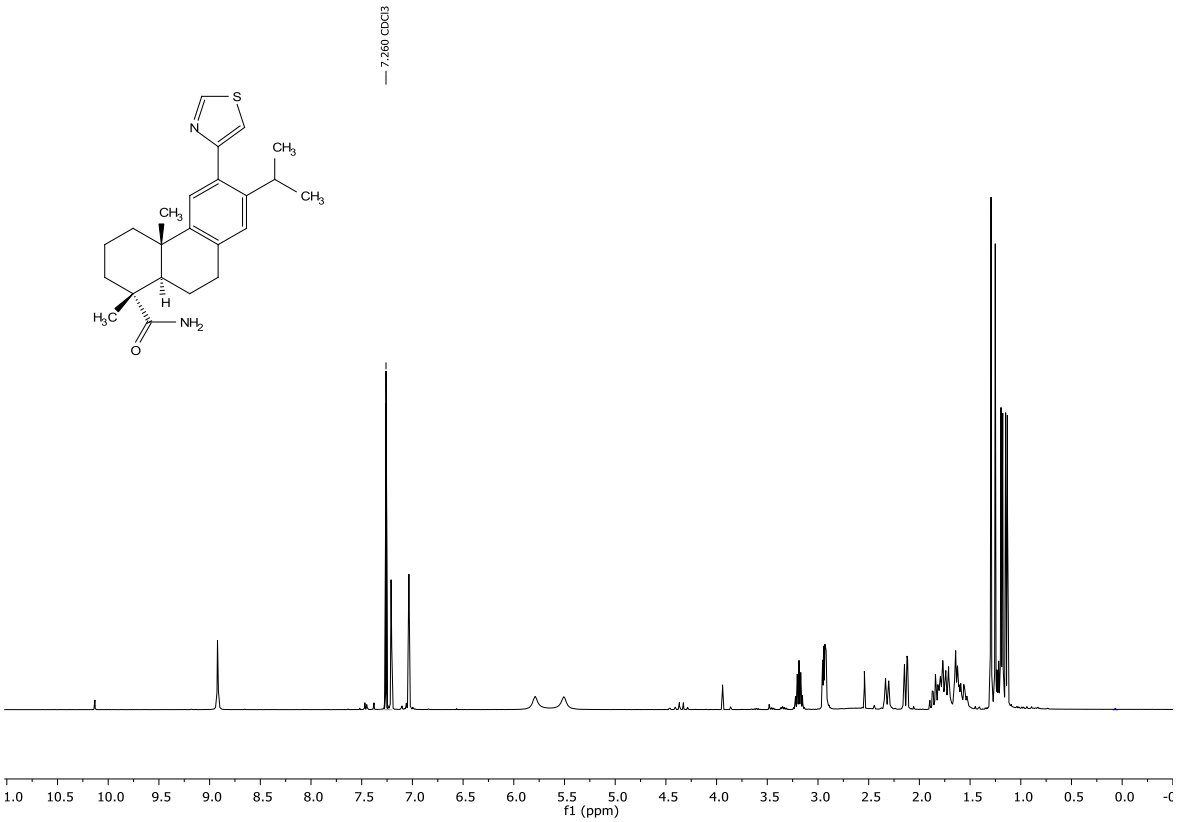

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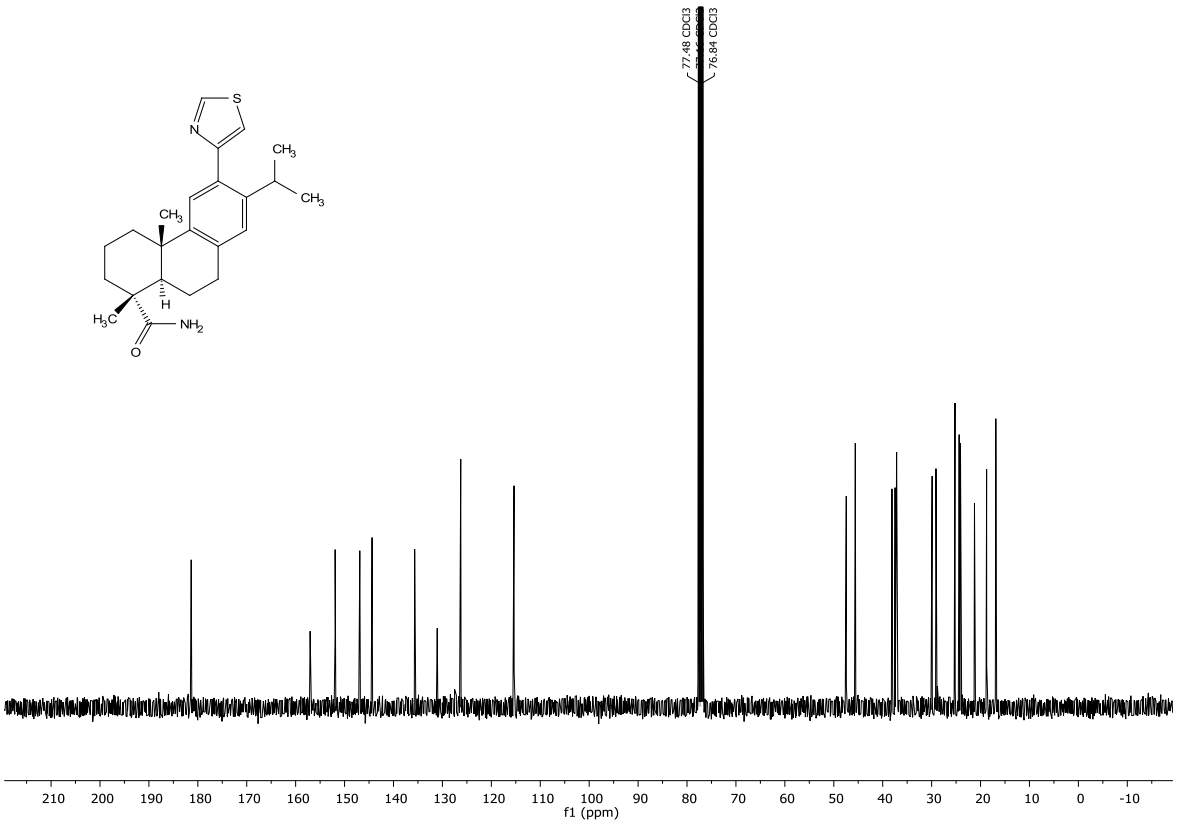
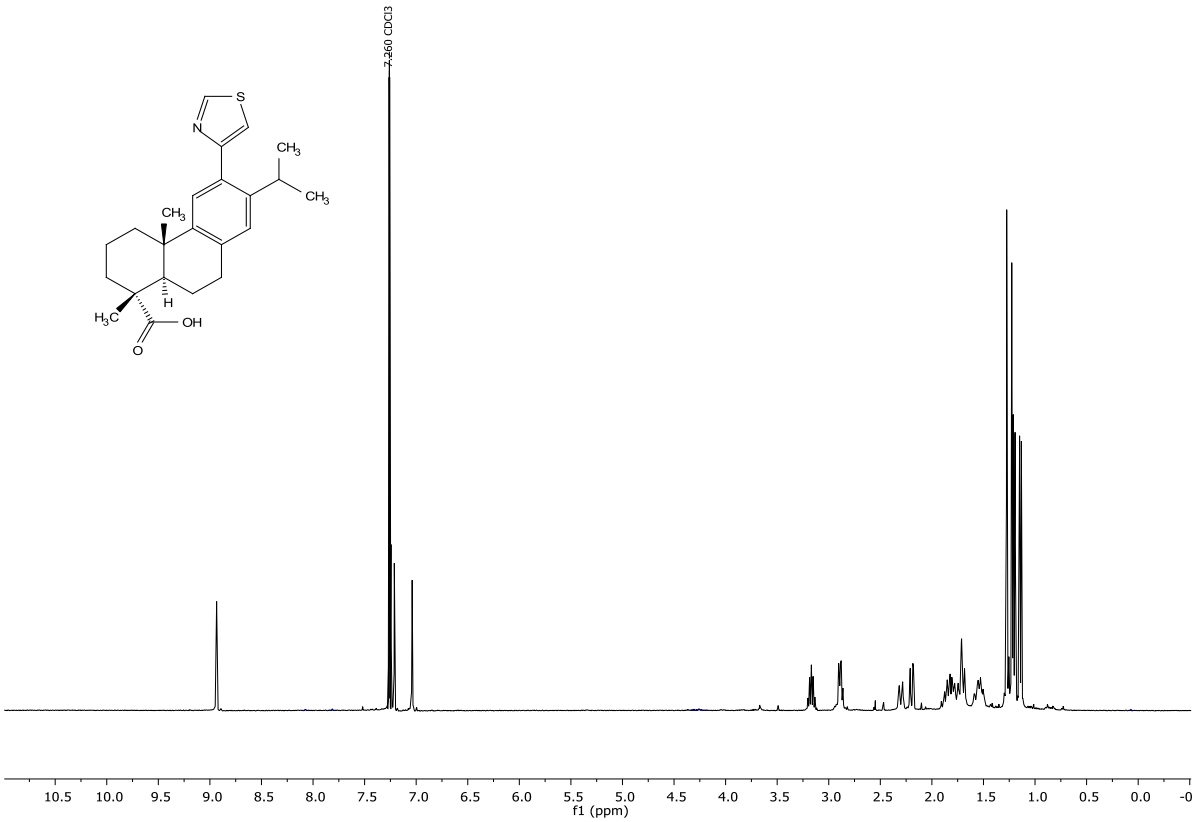
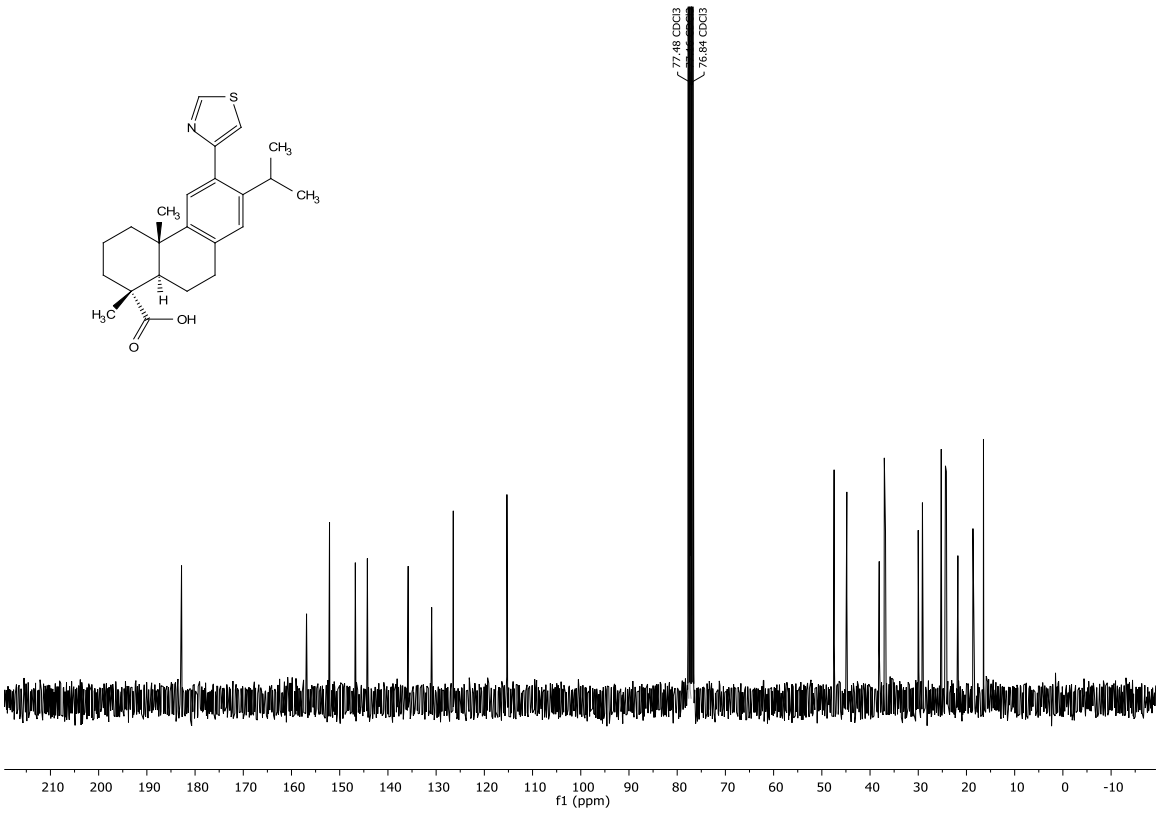


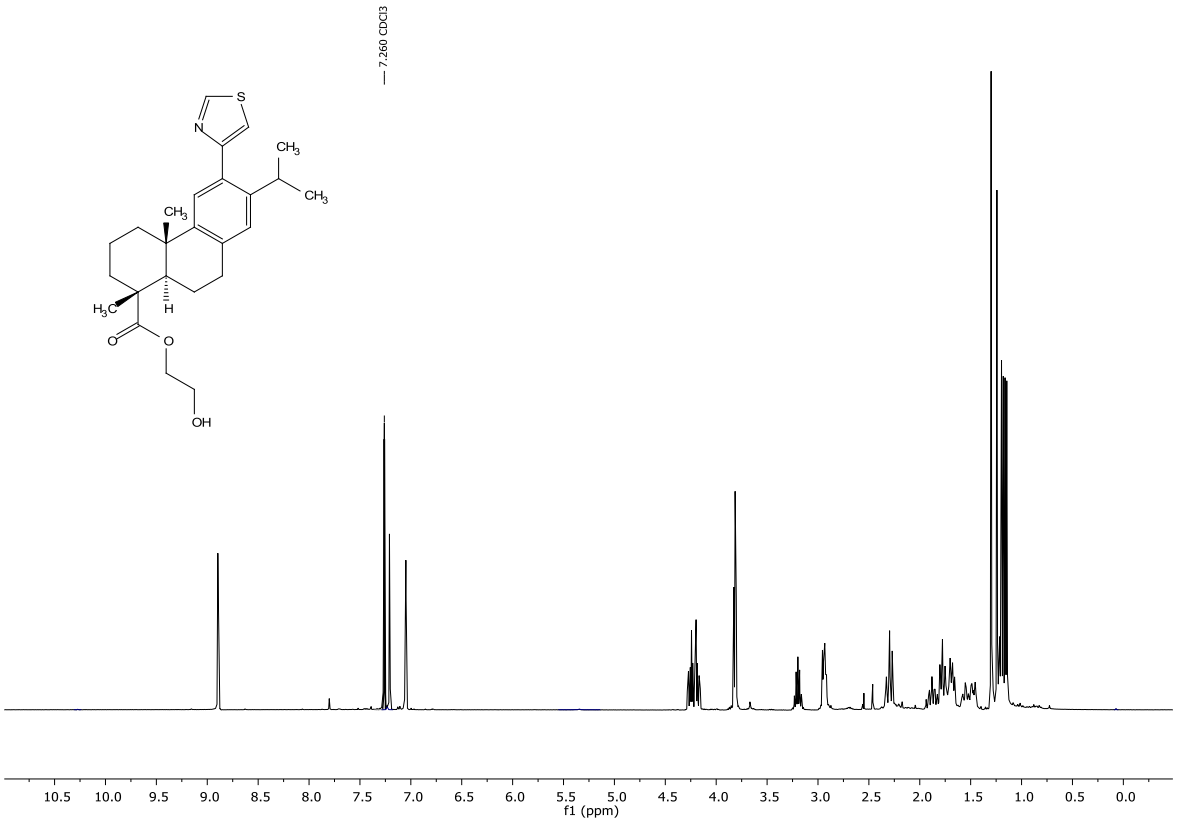
^{13}C NMR of compound **12** in $CDCl_3$



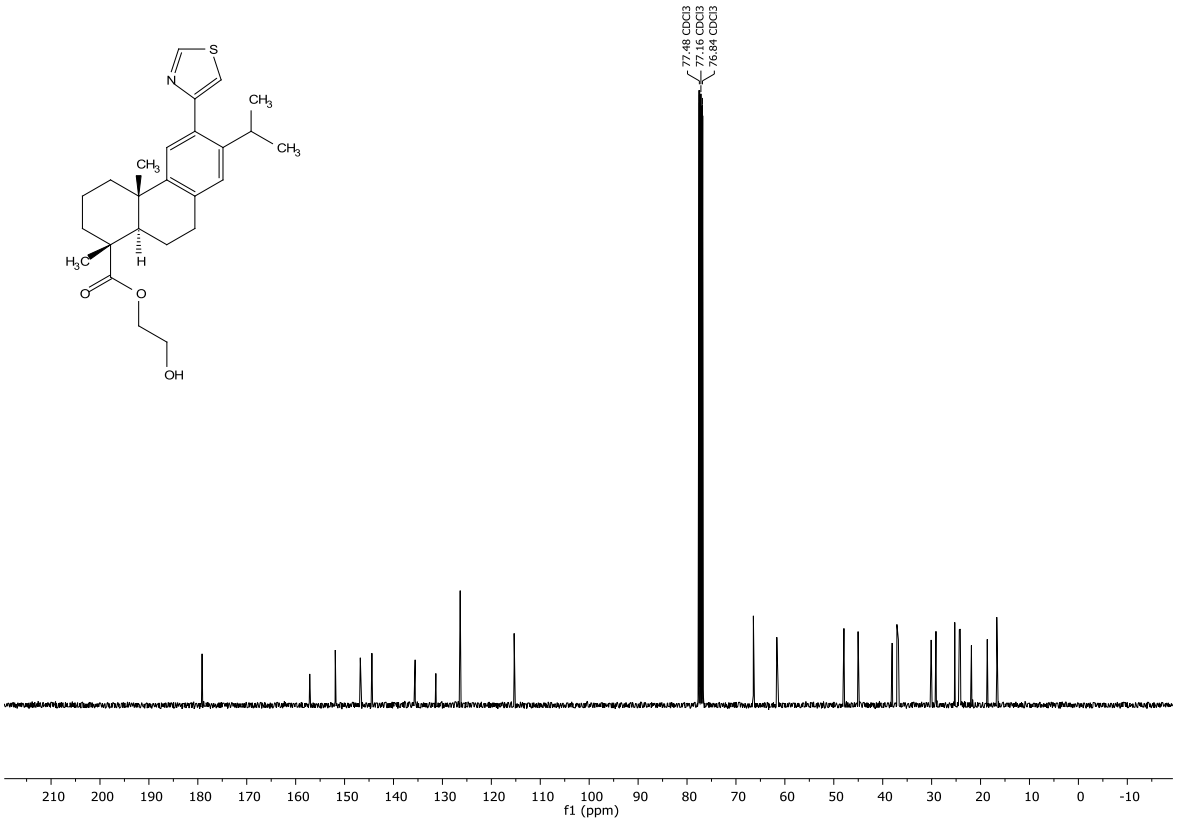
^{1}H NMR of compound **13** in $CDCl_3$



^{13}C NMR of compound **13** in $CDCl_3$



^{1}H NMR of compound **14** in $CDCl_3$



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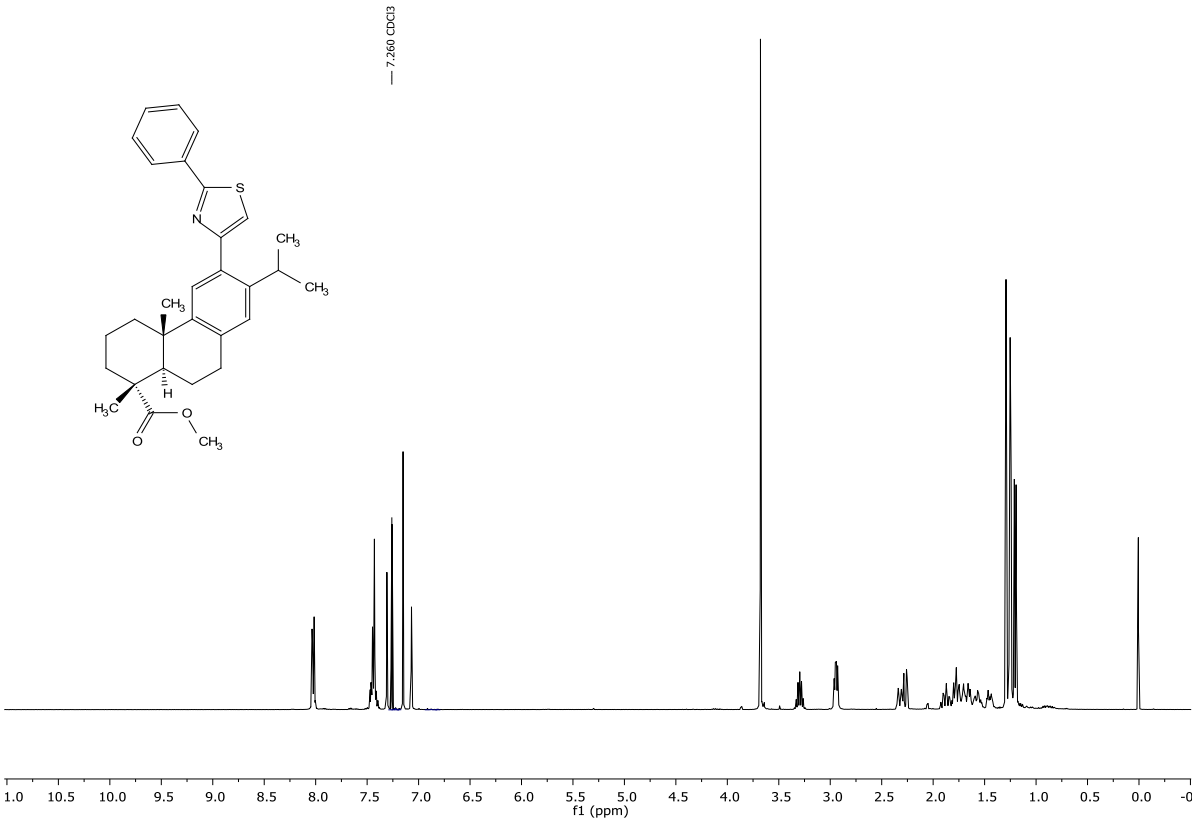

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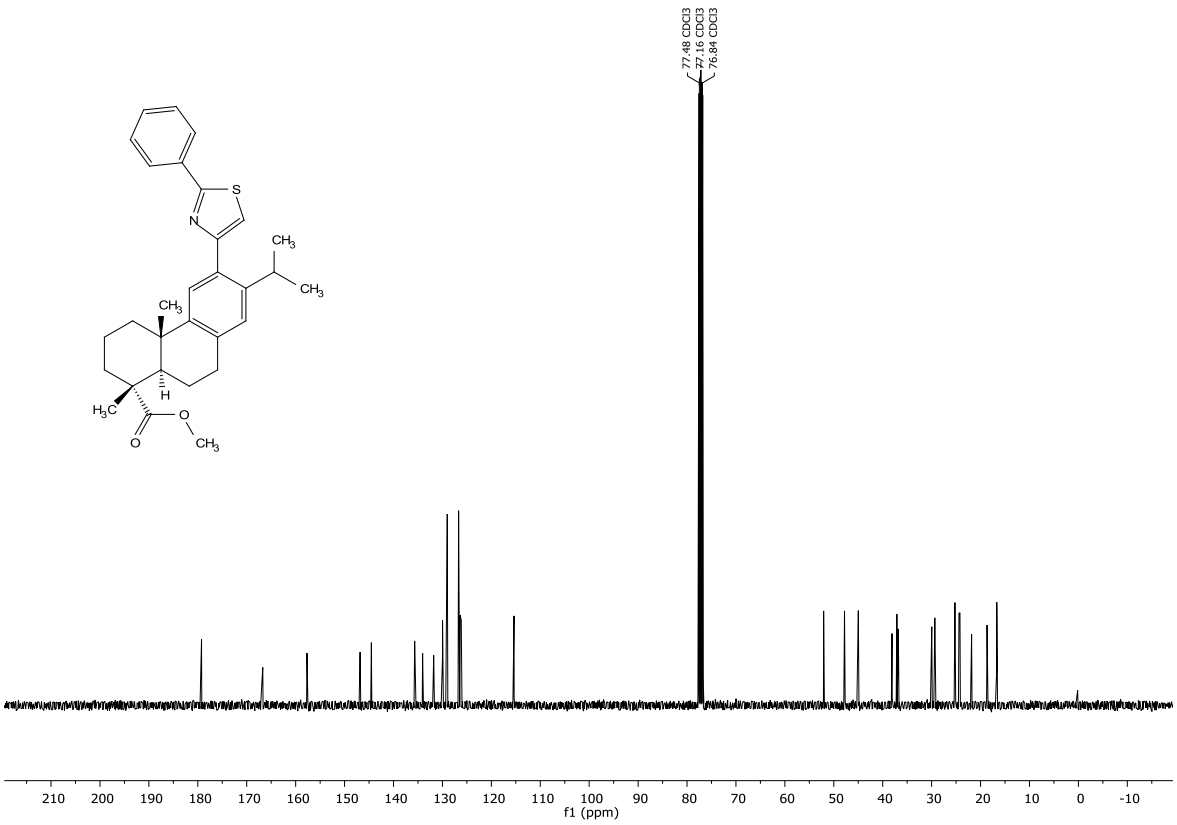

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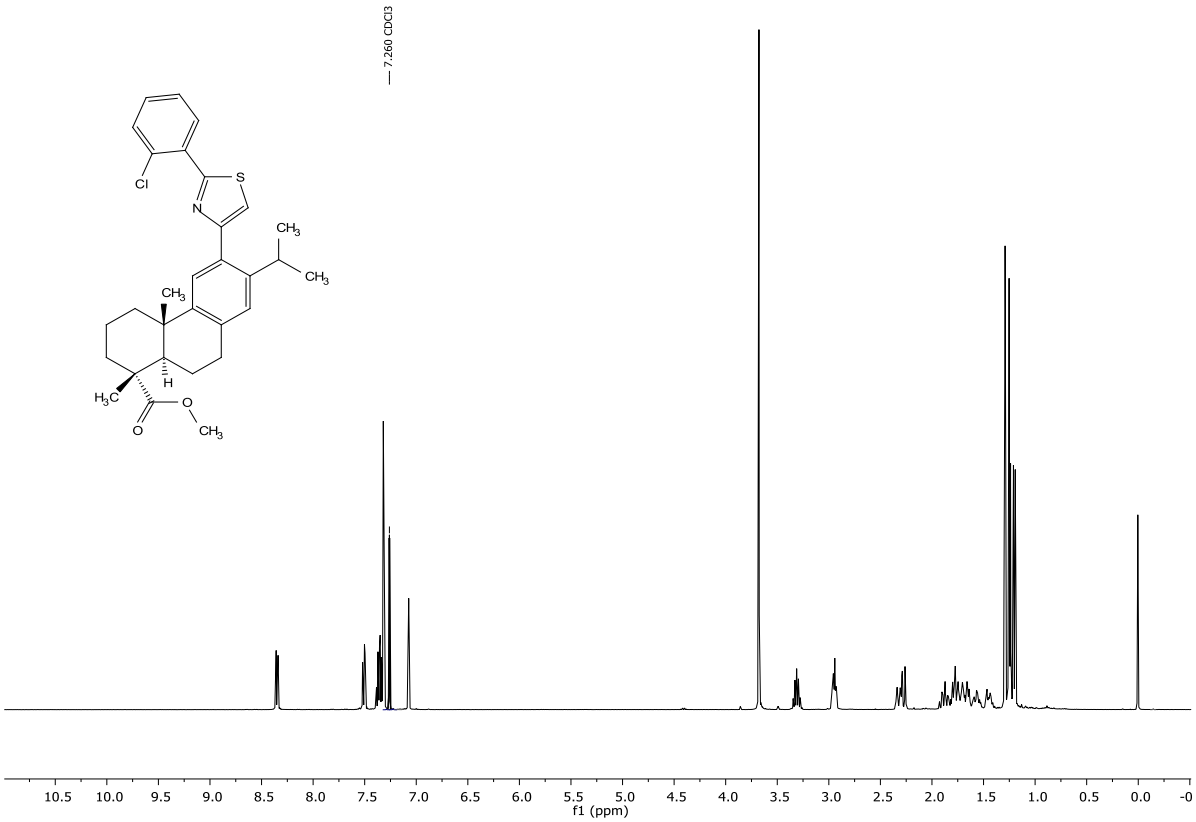
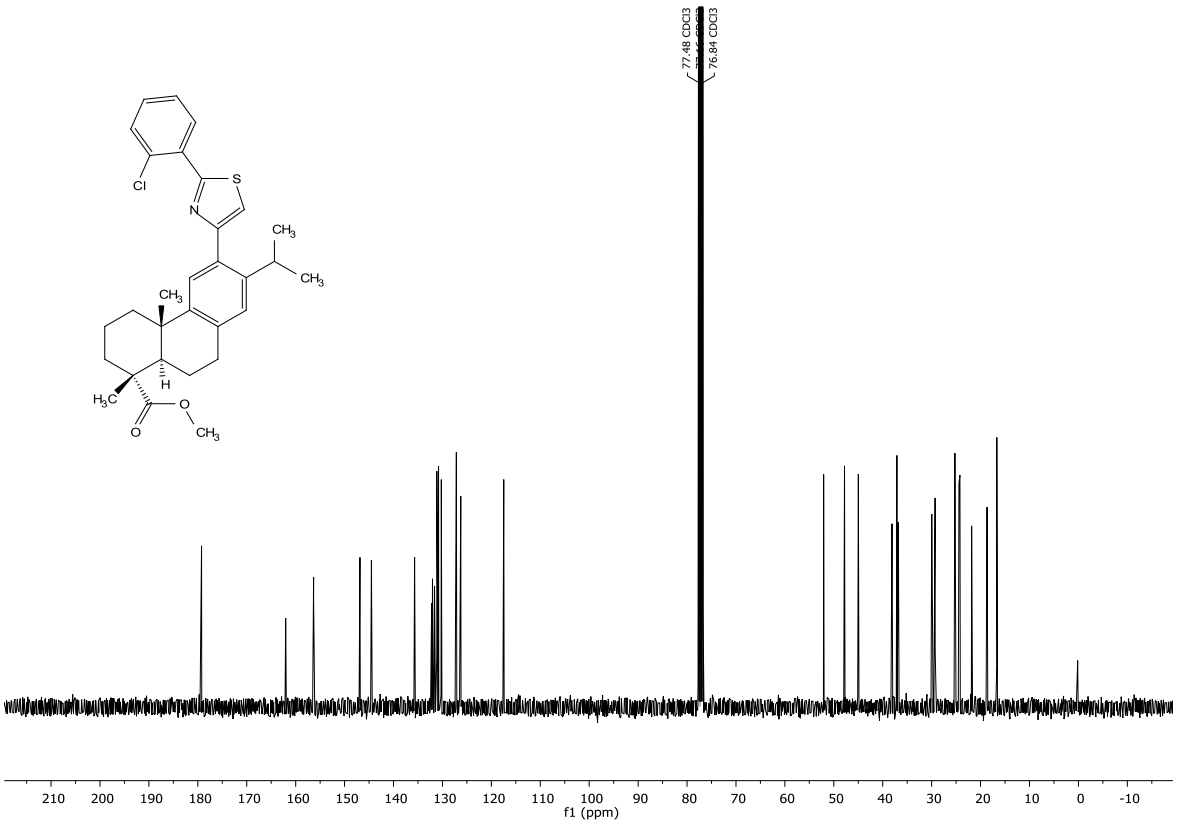


^{1}H NMR of compound **16** in $CDCl_3$



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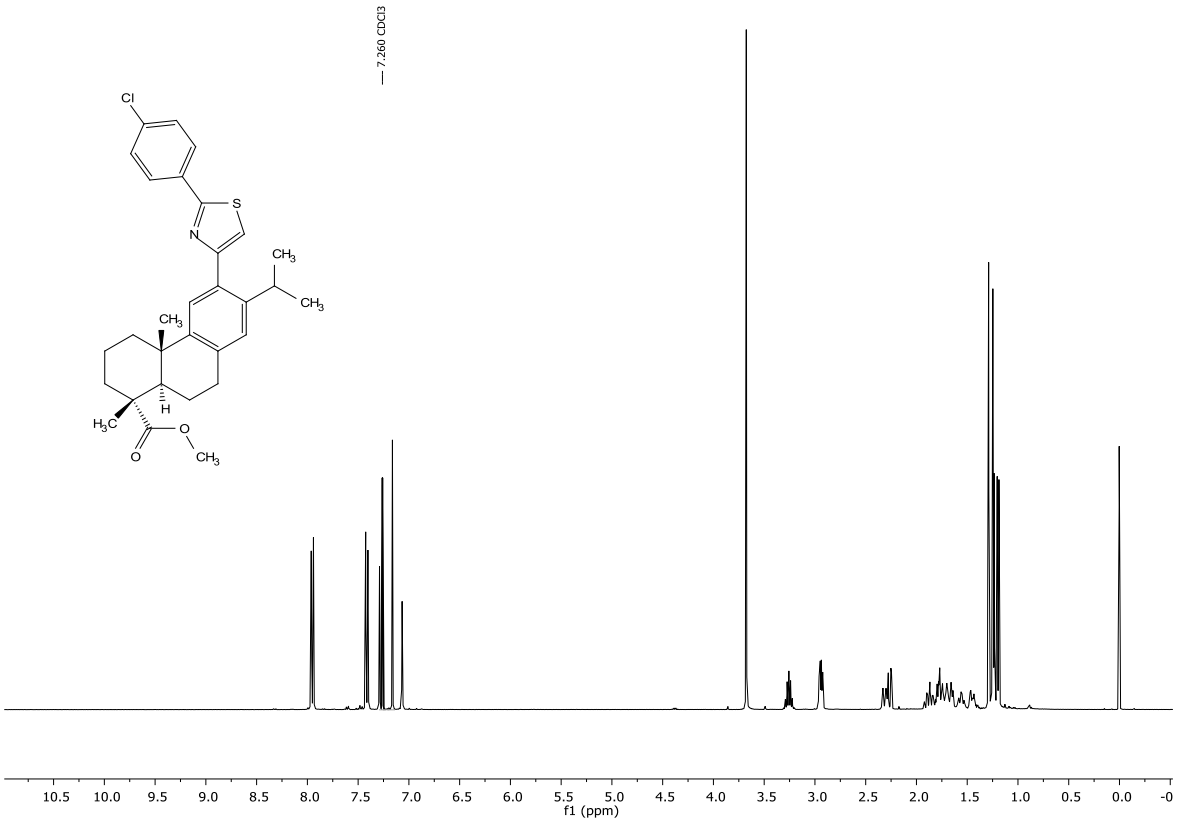

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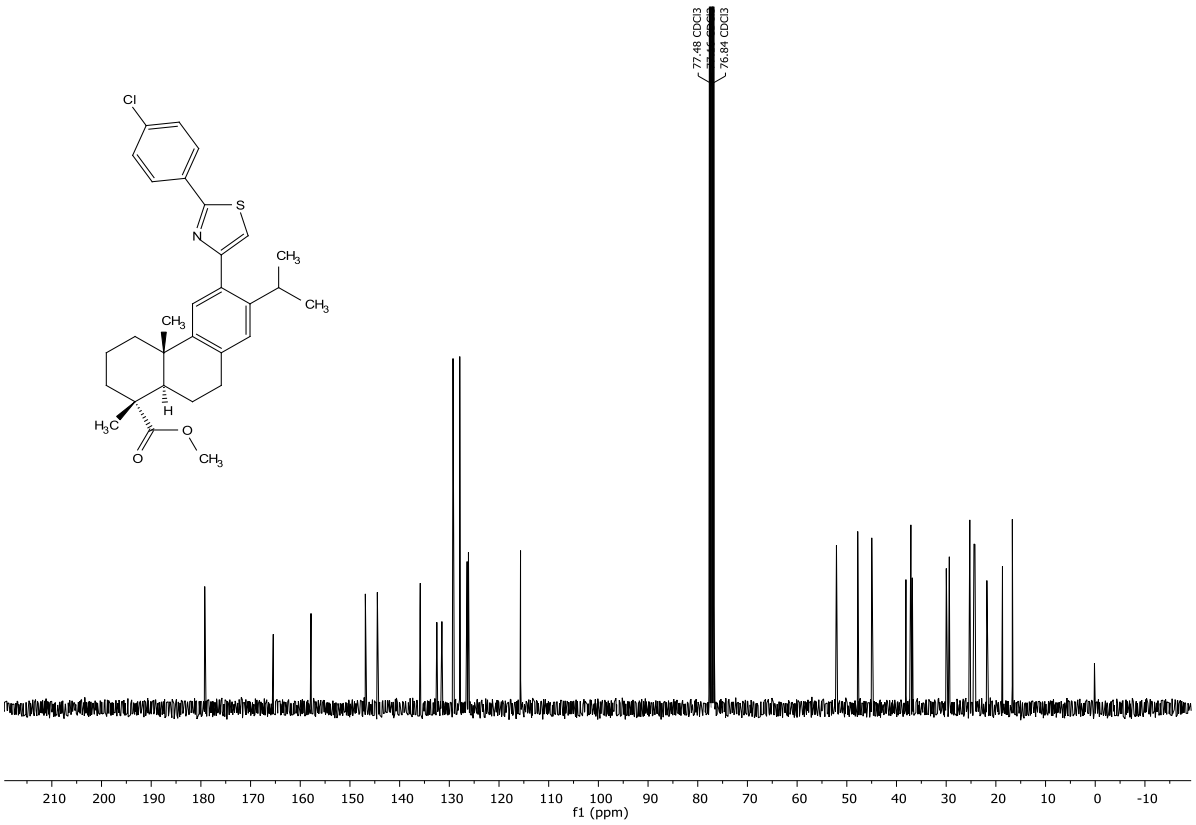
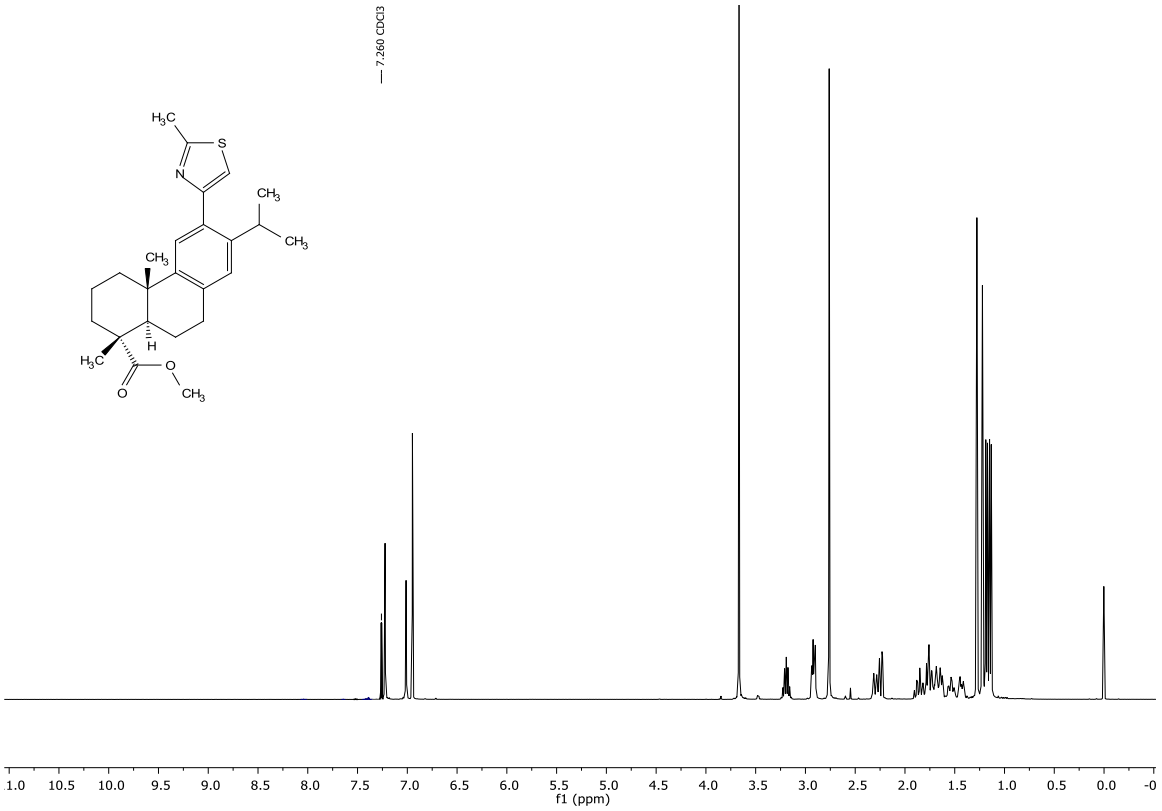


^{13}C NMR of compound **17** in $CDCl_3$



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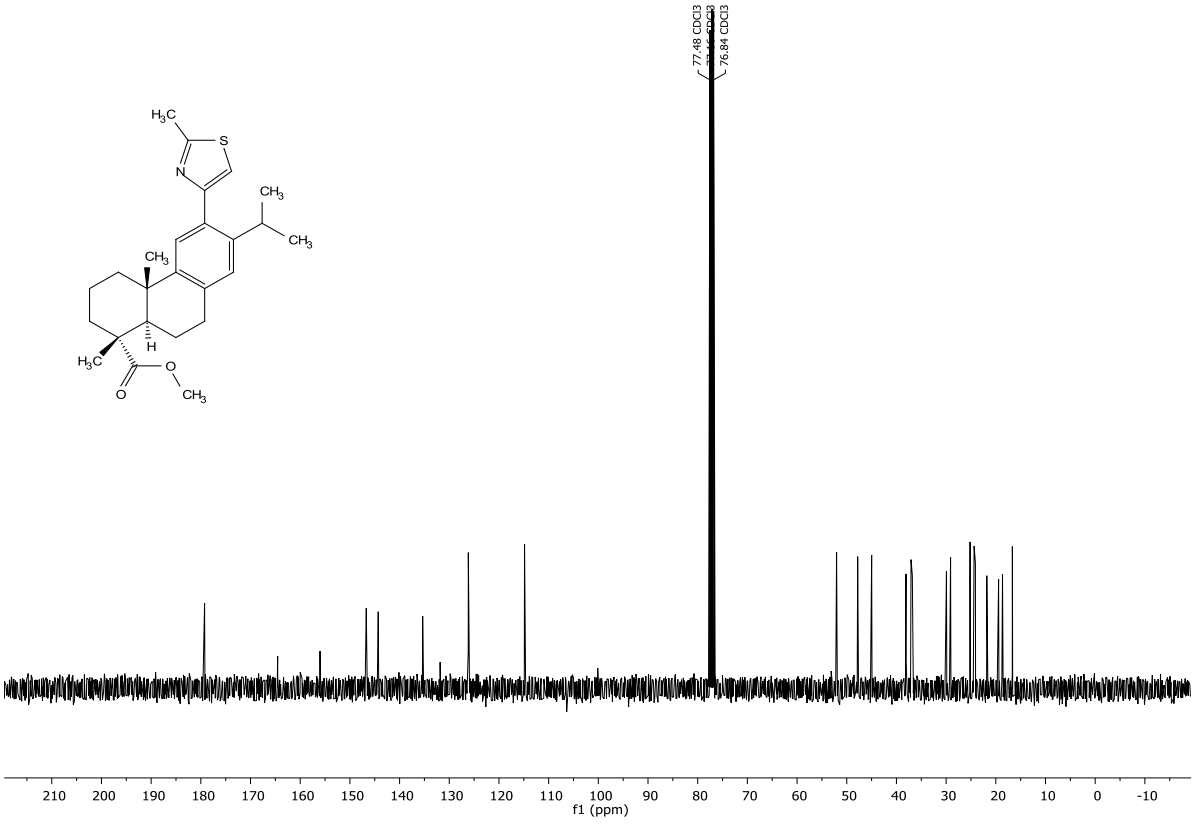

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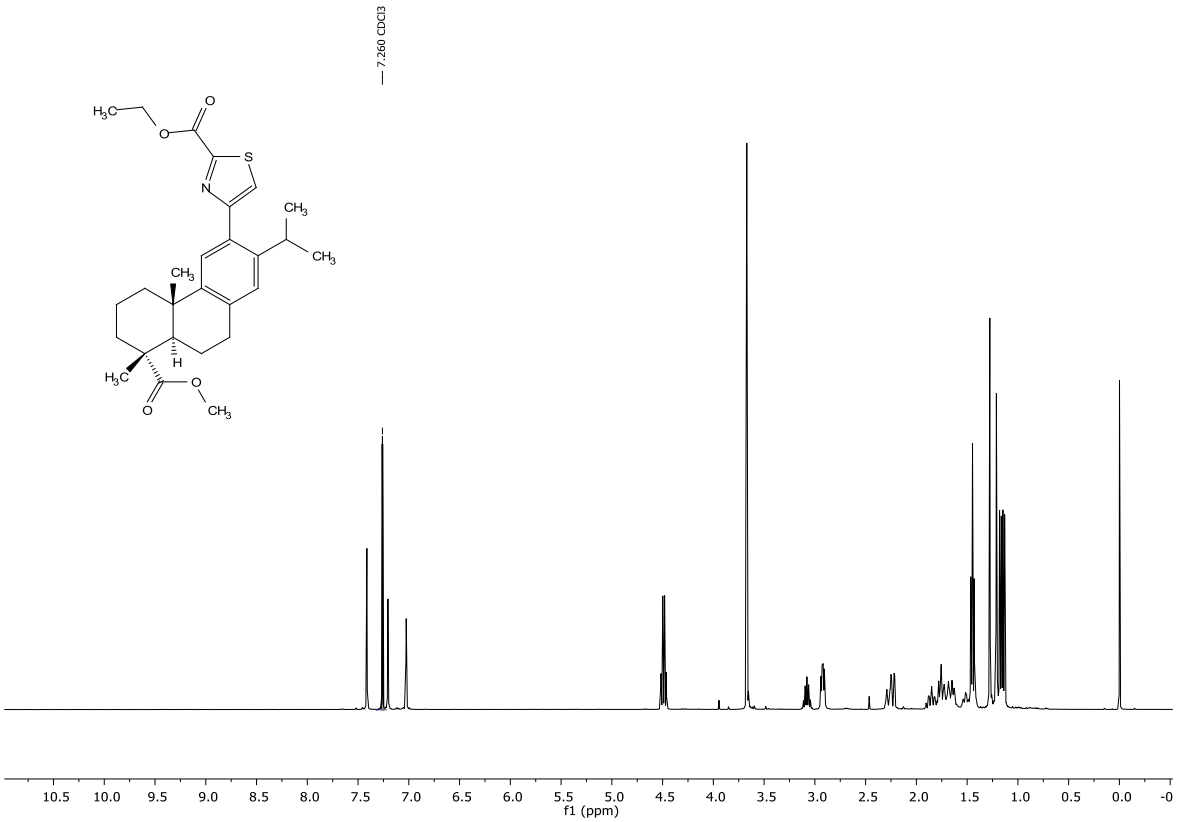
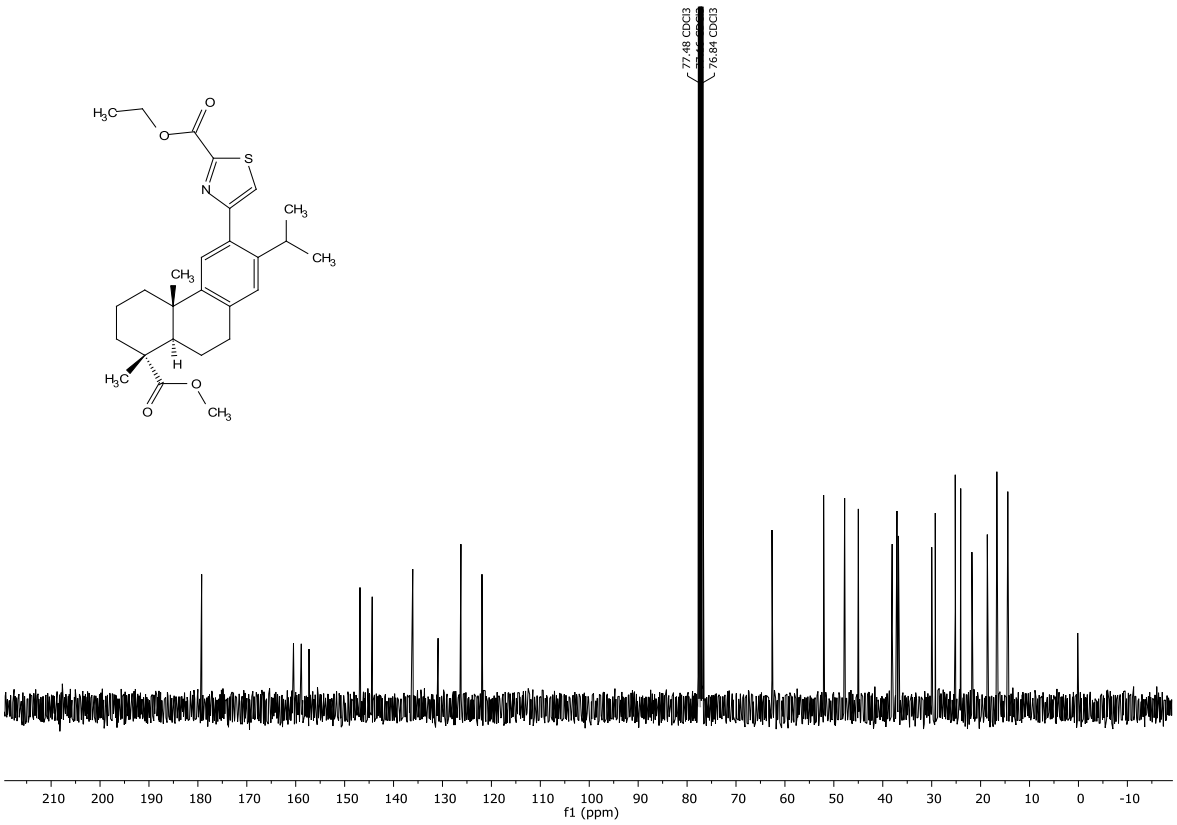


^{1}H NMR of compound **19** in $CDCl_3$



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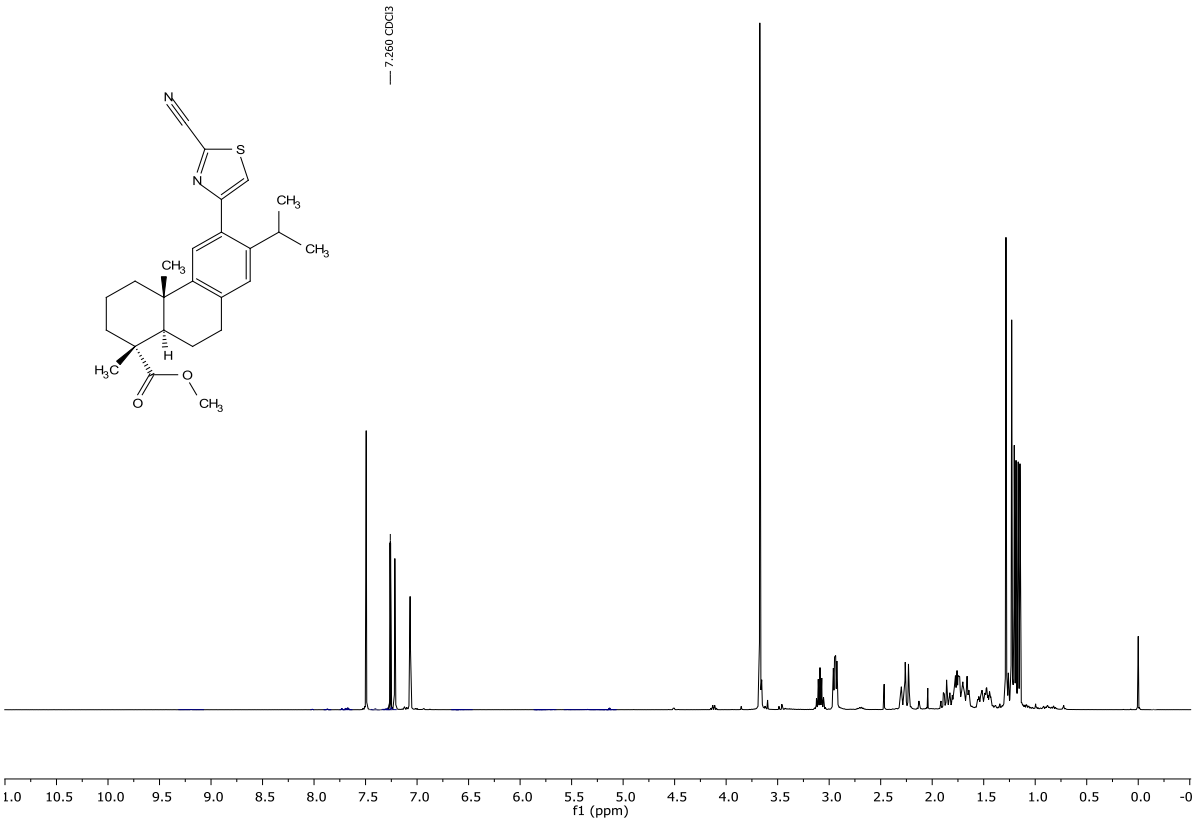
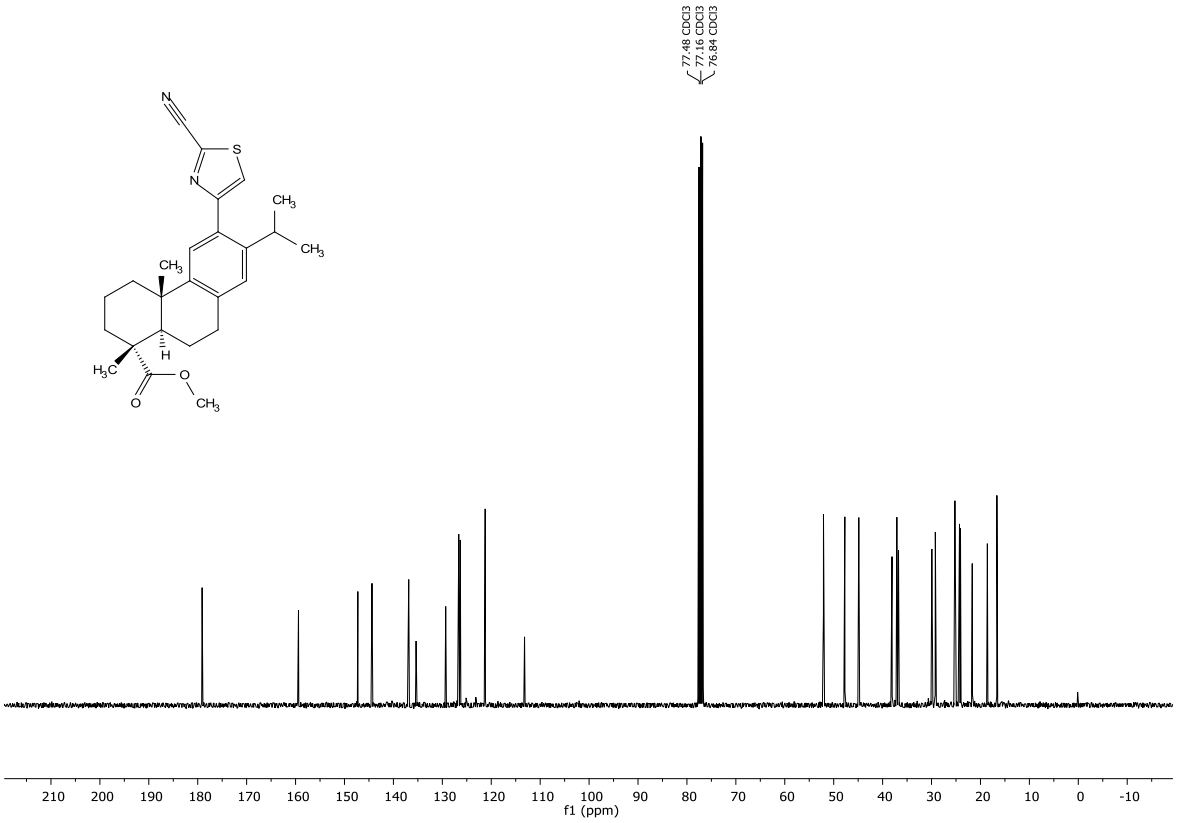
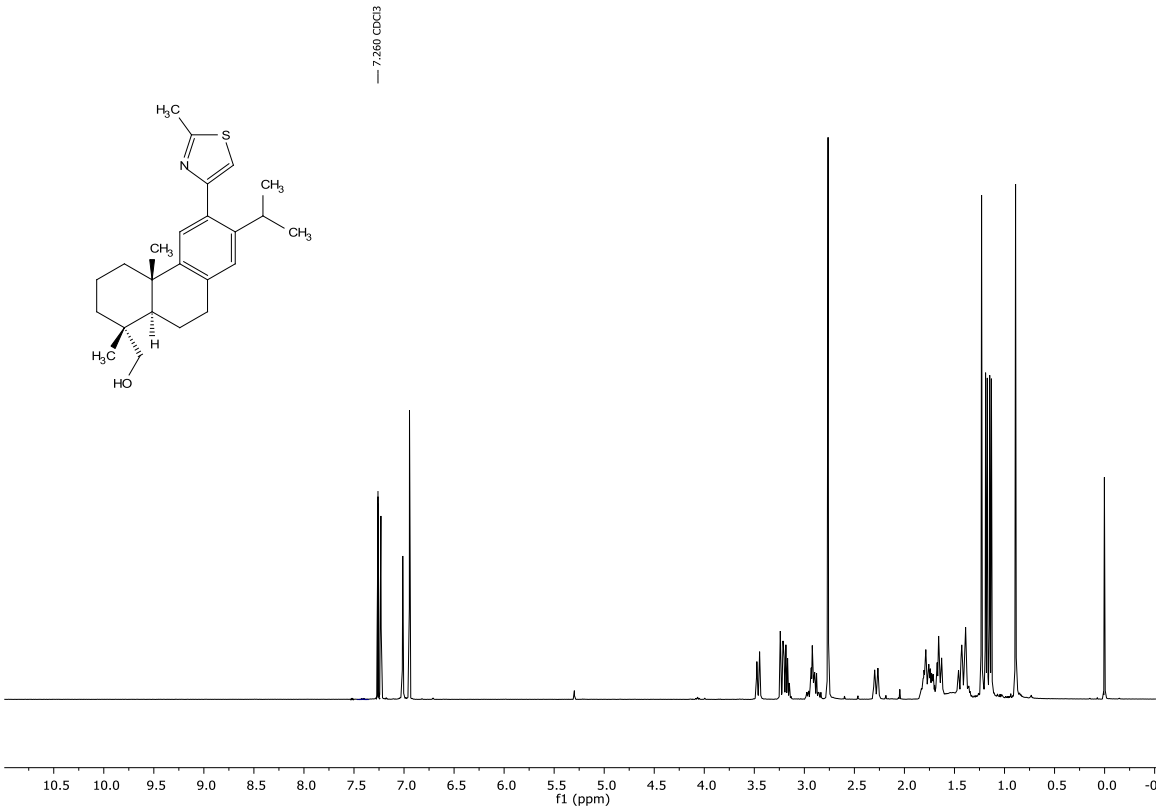


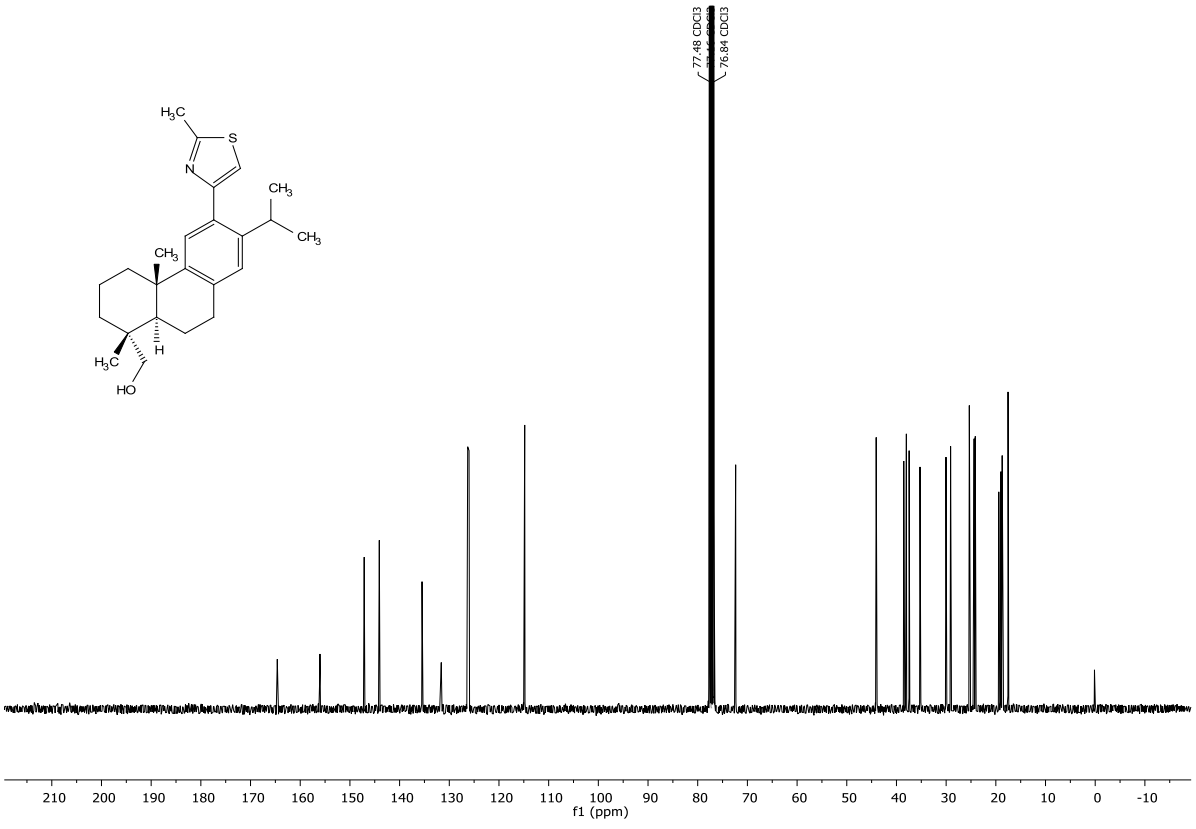
^{1}H NMR of compound **20** in $CDCl_3$



^{13}C NMR of compound **20** in $CDCl_3$



^{1}H NMR of compound **21** in $CDCl_3$



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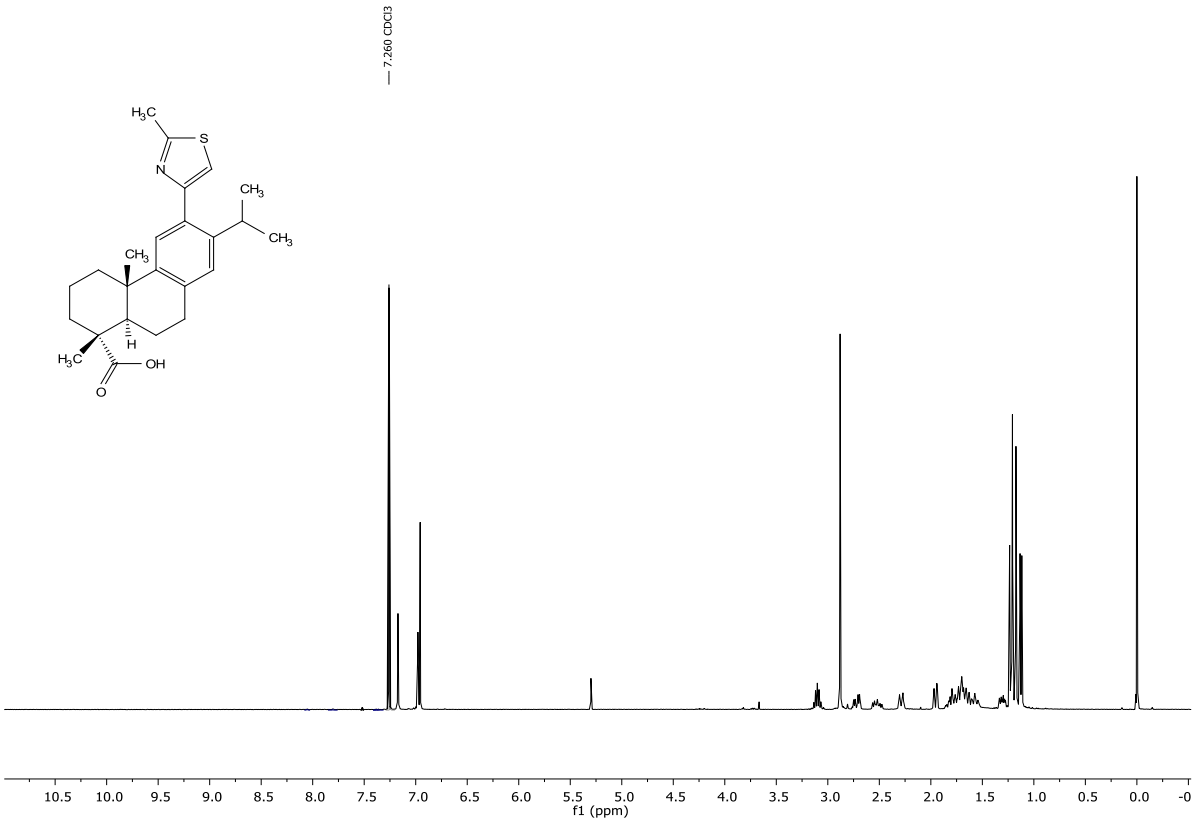

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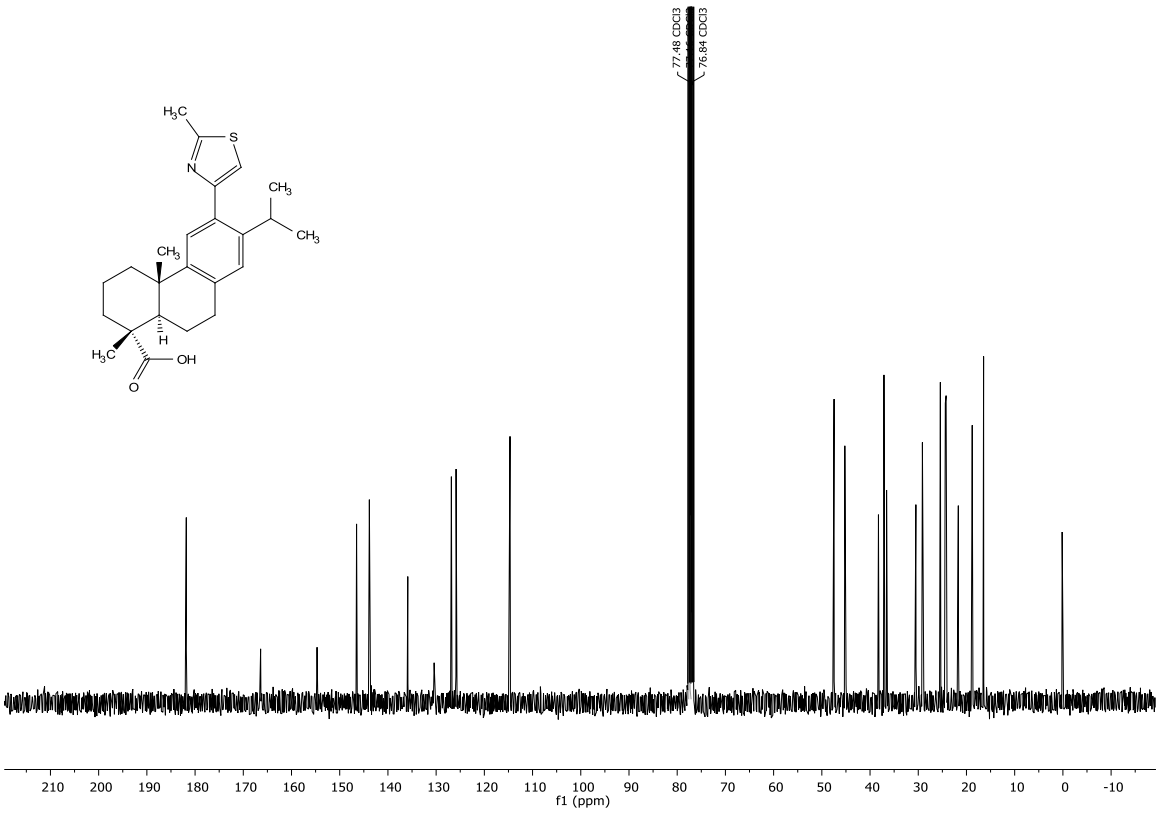
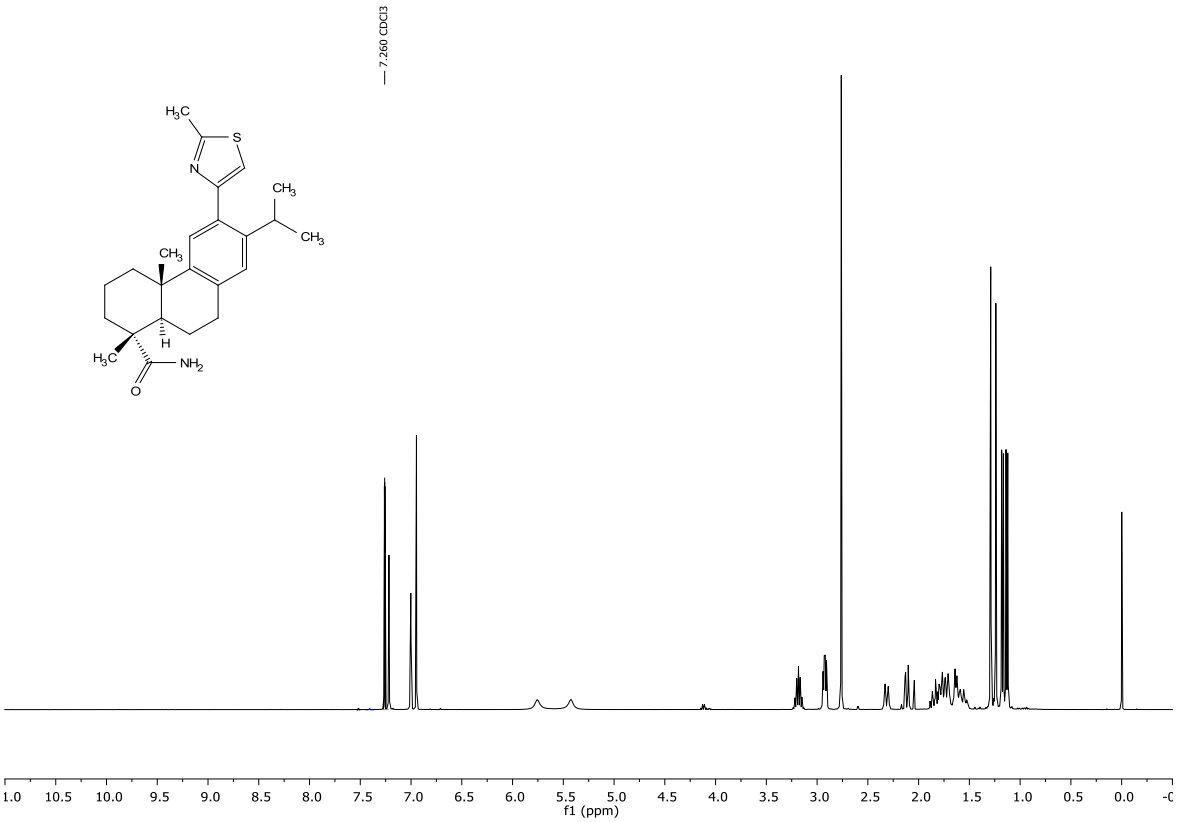


^{13}C NMR of compound **22** in $CDCl_3$



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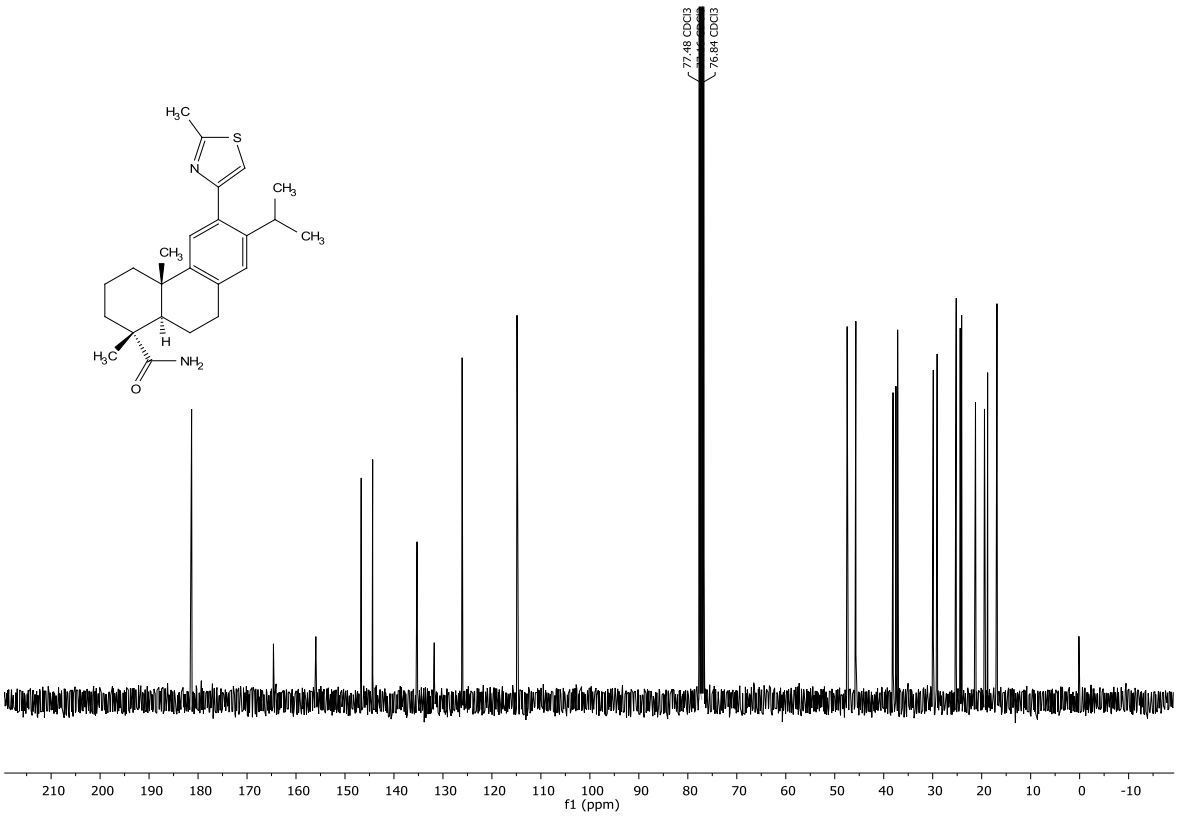

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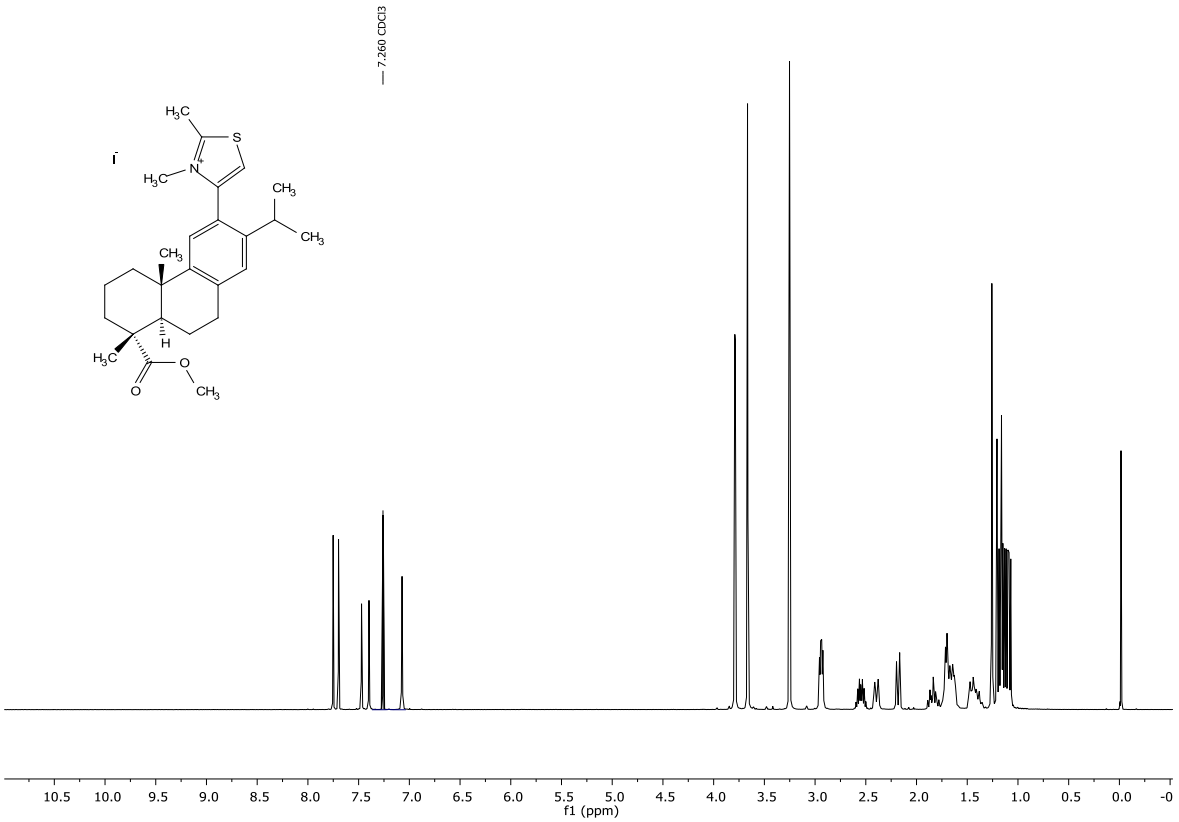
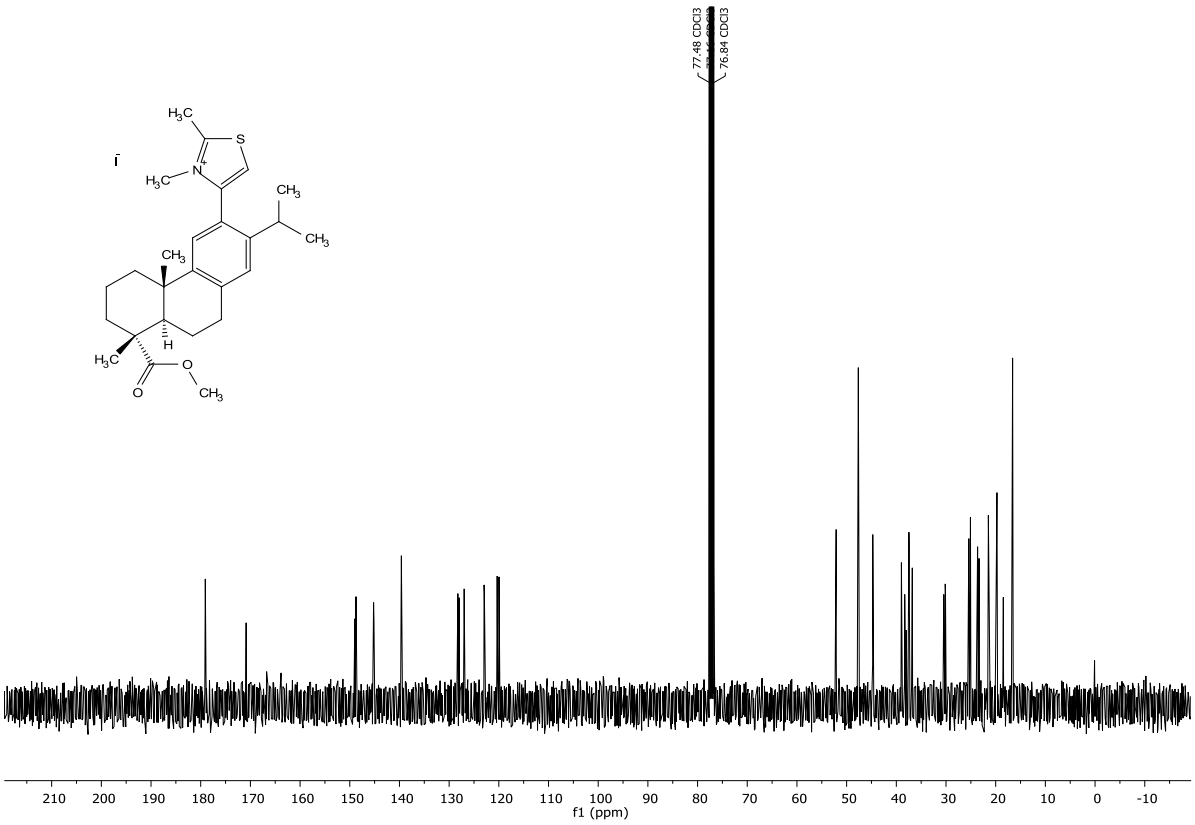
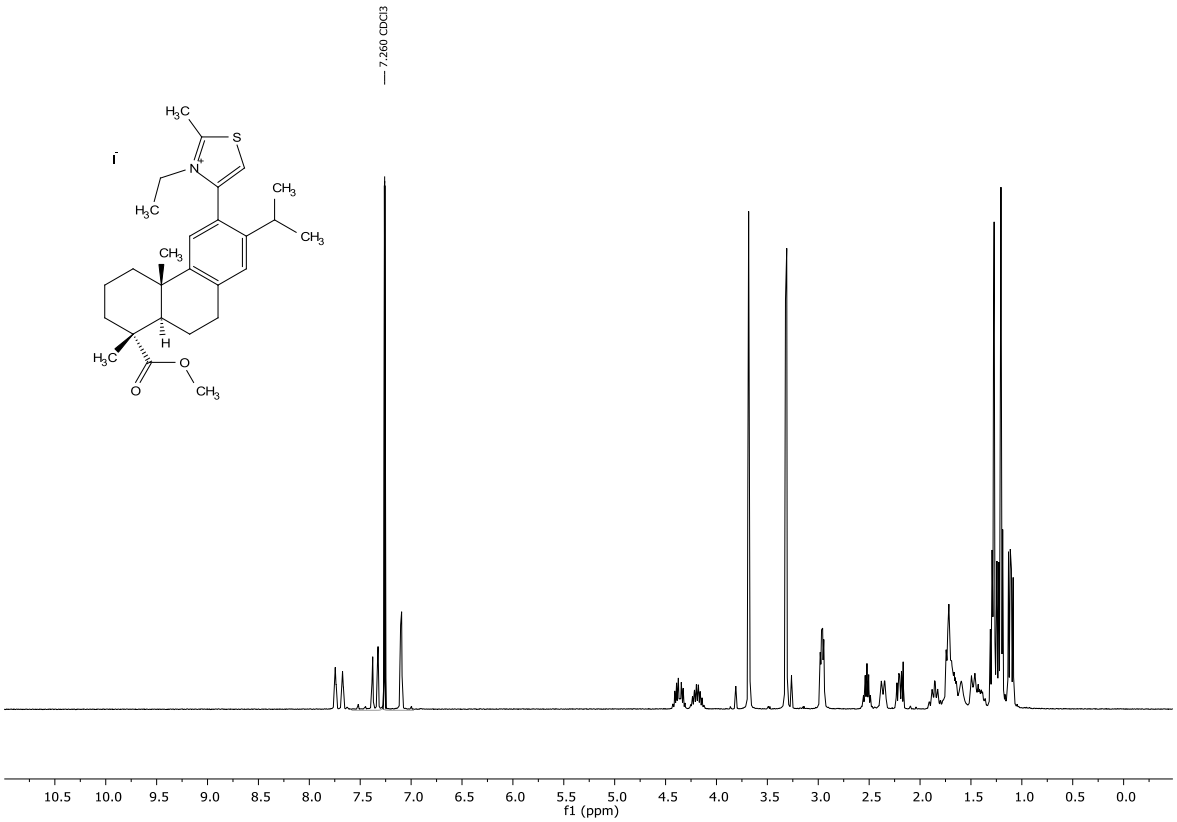


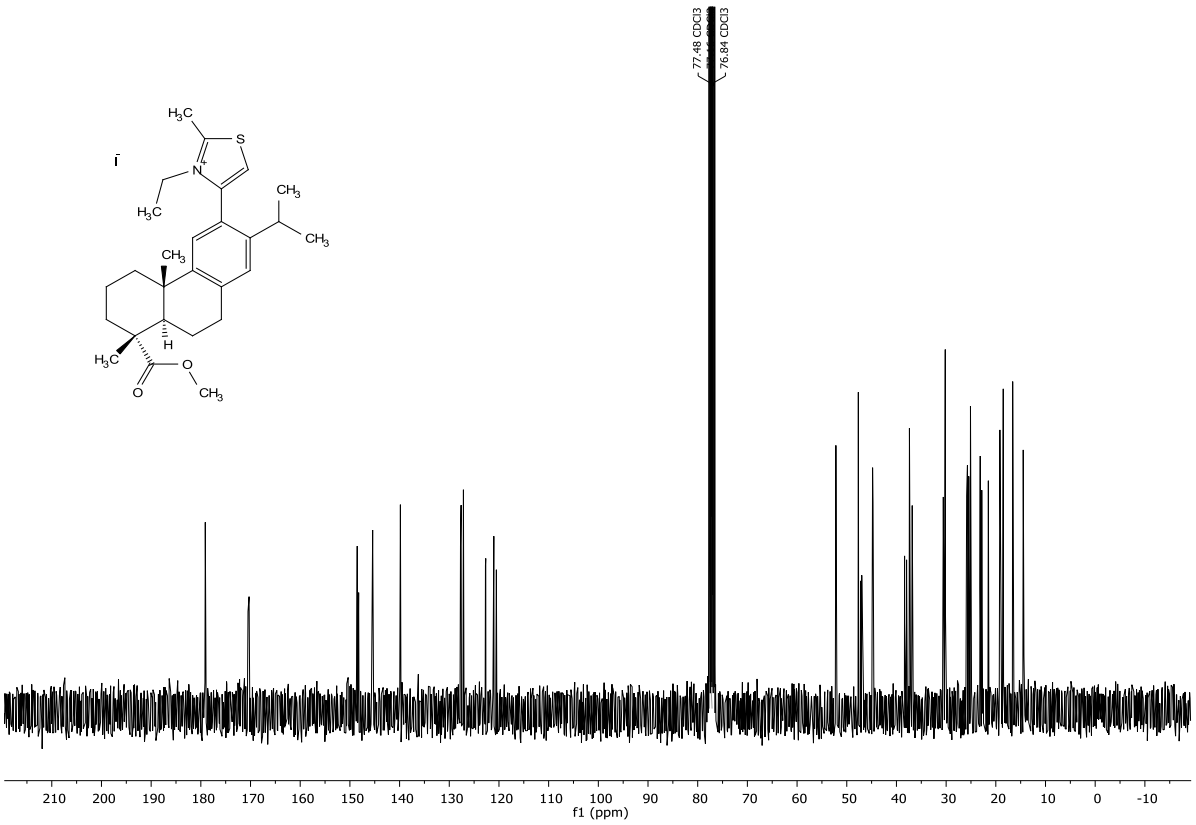
^{1}H NMR of compound **24** in $CDCl_3$



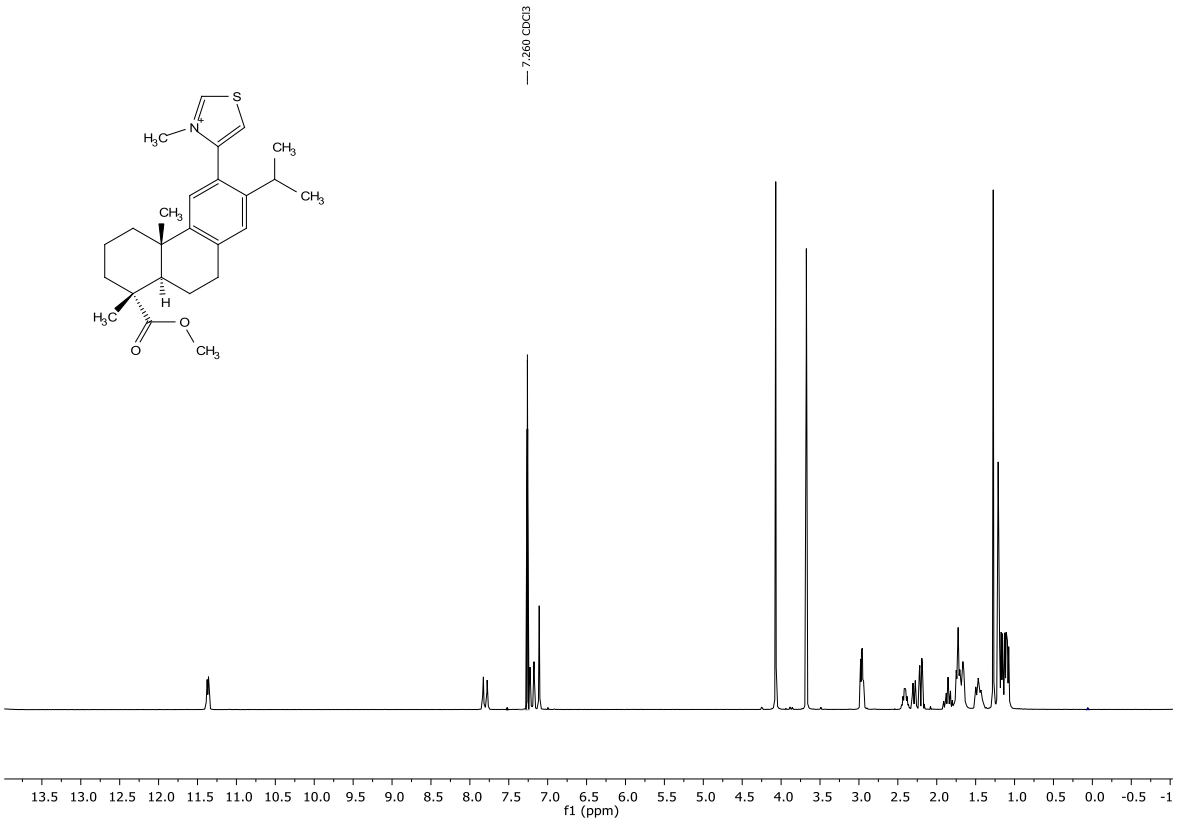
^{13}C NMR of compound **24** in $CDCl_3$



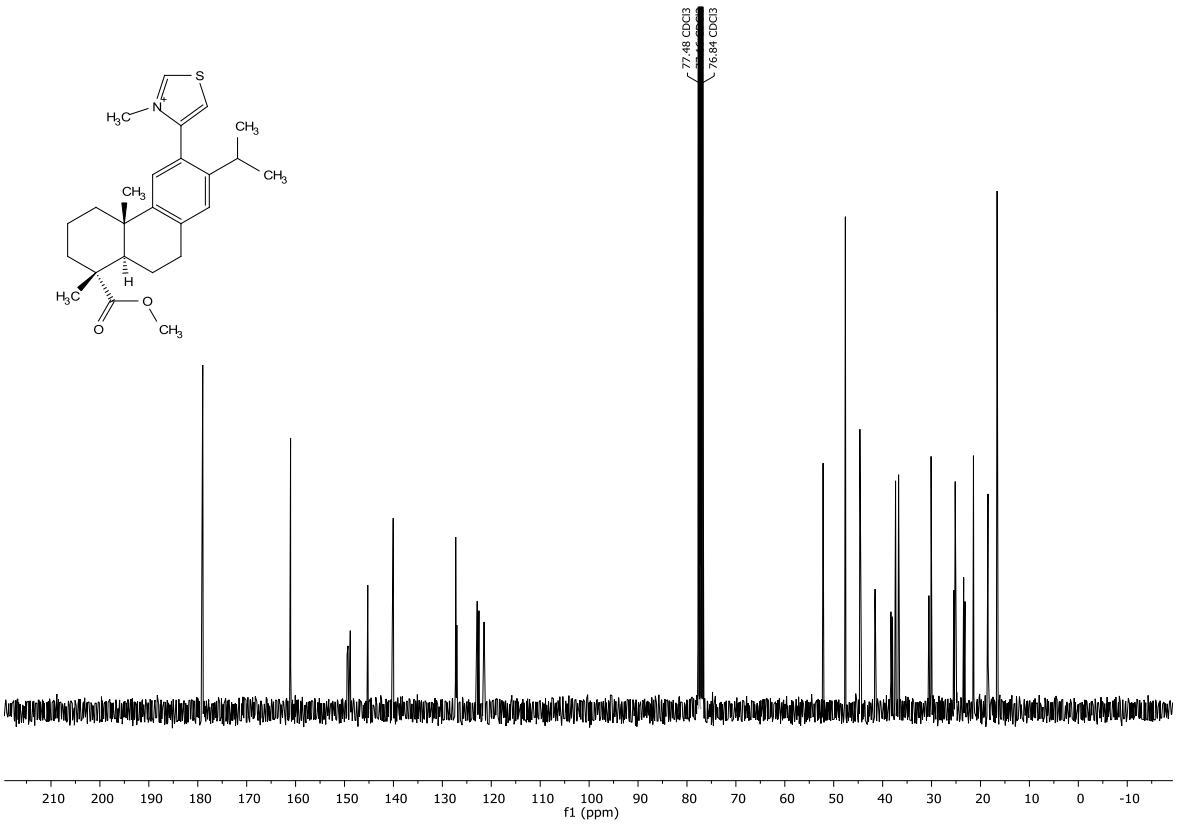
^{1}H NMR of compound **25** in $CDCl_3$



^{13}C NMR of compound **25** in $CDCl_3$



^{1}H NMR of compound **26** in $CDCl_3$



^{13}C NMR of compound **26** in $CDCl_3$

9. References

(1) Kolsi, L. E.; Krogerus, S.; Brito, V.; Rüffer, T.; Lang, H.; Yli-Kauhaluoma, J.; Silvestre, S. M.; Moreira, V. M. Regioselective Benzylic Oxidation of Aromatic Abietanes: Application to the Semisynthesis of the Naturally Occurring Picealactones A, B and C. *ChemistrySelect* **2017**, *2* (24), 7008–7012.

(2) Narayana, B.; Vijaya Raj, K. K.; Ashalatha, B. V; Kumari, N. S.; Sarojini, B. K. Synthesis of Some New 5-(2-Substituted-1,3-thiazol-5-yl)-2-hydroxy Benzamides and Their 2-Alkoxy Derivatives as Possible Antifungal Agents. *Eur. J. Med. Chem.* **2004**, *39* (10), 867–872.

(3) Navia-Paldanius, D.; Savinainen, J. R.; Laitinen, J. T. Biochemical and Pharmacological Characterization of Human α/β-Hydrolase Domain Containing 6 (ABHD6) and 12 (ABHD12). *J. Lipid Res.* **2012**, *53* (11), 2413–2424.

(4) Savinainen, J. R.; Patel, J. Z.; Parkkari, T.; Navia-Paldanius, D.; Marjamaa, J. J. T.; Laitinen, T.; Nevalainen, T.; Laitinen, J. T. Biochemical and Pharmacological Characterization of the Human Lymphocyte Antigen B-Associated Transcript 5 (BAT5/ABHD16A). *PLoS One* **2014**, *9* (10), 1–17.

(5) Parkkari, T.; Haavikko, R.; Laitinen, T.; Navia-Paldanius, D.; Rytilahti, R.; Vaara, M.; Lehtonen, M.; Alakurtti, S.; Yli-Kauhaluoma, J.; Nevalainen, T.; Savinainen, J. R.; Laitinen, J. T. Discovery of Triterpenoids as Reversible Inhibitors of α/β-Hydrolase Domain Containing 12 (ABHD12). *PLoS One* **2014**, *9* (5), e98286.