Supplementary Materials for

Betulinic acid analogs inhibit N- and T-type voltage-gated calcium channels to attenuate nerve-injury associated neuropathic and formalin models of pain

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Supplementary Methods

Synthesis and characterization of betulinic acid and betulin analogs 3–31.

General Experimental Procedures. Betulinic acid (**1**) and betulin (**2**) were purchased from Skin Acives Scientific, Gilbert, AZ, USA and Sigma Aldrich (St. Louis, MO, USA), respectively. All reagents used for chemical transformations were purchased from Sigma Aldrich (St. Louis, MO, USA) and Fisher Scientific (Pittsburgh, PA, USA). Solvents used for reactions were distilled before use. The progress of all reactions was monitored by thin-layer chromatography (TLC) on silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) and spots were visualized under UV light and spraying with a solution of anisaldehyde in H_2SO_4/HOAc followed by heating. Column chromatographic (CC) separations involved silica gel 40 μm flash chromatography packing (J. T. Baker, Jackson, TN, USA). Analytical and preparative TLCs were performed on pre-coated 0.20 mm thick plates of silica gel 60 F_{254} (E. Merck). Preparative HPLC was performed on a Waters Delta Prep 4000 system equipped with a Waters 996 photodiode array detector and a Waters Prep LC controller utilizing Empower Pro software and a reversed-phase (RP) column (Kromasil KR-100-7-C_{18}; 250 × 20 mm); chromatograms were acquired at 254 and 270 nm. 1D and 2D NMR spectra were recorded in $CDCl_3$ with a Bruker AVANCE III instrument at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR using residual $CHCl_3$ as the internal standard. The chemical shift (δ) values are given in parts per million (ppm) and the coupling constants (J values) are in Hz. High-resolution mass spectra (HRMS) were recorded on an Agilent G6224A TOF mass spectrometer.

Preparation of 3-*O*-acetylbetulinic acid (3). To a solution of betulinic acid (**1,** 20.0 mg) in pyridine (500 µL) was added Ac_2O (50 µL) and stirred at 25 ^{o}C. After 16 h (TLC control), EtOH was added to the reaction mixture and evaporated under reduced pressure. Residue thus obtained was chromatographed over a column of silica gel using hexanes/CH_2Cl_2 (40:60) followed by hexanes/CH_2Cl_2 (20:80). Fractions eluted with hexanes/CH_2Cl_2 (20:80) were

combined and evaporated to give **3** (20.5 mg, 98%) as a white solid. The spectroscopic data (NMR and LRMS) of **3** were identical with those reported.[1]

Preparation of 3-*O*-cyclopropanoyl betulinic acid (4). To a solution of **1** (25.0 mg) in anhy. EtOAc was added cyclopropanecarboxylic acid (14.1 mg), DCC (50.8 mg) and 4-pyrrolidinopyridine (4-pp, 2.0 mg) and stirred at 25 ^{o}C for 72 h (TLC control). The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue thus obtained was chromatographed over a column of silica gel (1.0 g) using hexanes/CH_2Cl_2 (1:1) as eluant to give **4** (27.5 mg, 96%) as awhite amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.71 (1H, brd, *J* = 1.8 Hz, Ha-29), 4.59 (1H, brt, *J* = 1.8 Hz, Hb-29), 4.44 (1H, dd, *J* = 10.5, 6.0 Hz, H-3), 2.98 (1H, dt, *J* = 5.0, 11.0 Hz, H-19), 2.25 (1H, dt, *J* = 12.6, 3.3 Hz, Ha-16), 2.15 (1H, dt, *J* = 3.8, 12.6 Hz, H-13), 1.96 (1H, dd, *J* = 9.9, 3.5 Hz, Ha-21), 1.94 (1H, m, Ha-22), 1.68 (1H, m, Ha-12), 1.67 (3H, s, H_3-30), 1.64 (1H, m, Ha-1), 1.59 (1H, m, H-18), 1.58 (2H, m, H_2-2), 1.57 (1H, m, H-2'), 1.50 (1H, m, Ha-15), 1.48 (1H, m, Ha-6), 1.47 (1H, m, Hb-22), 1.38 (1H, m, Hb-21), 1.36 (1H, m, Hb-6), 1.35 (2H, m, H_2-7), 1.27 (1H, m, H-9), 1.23 (1H, m, Hb-11), 1.16 (1H, m, Hb-15), 1.01 (1H, m, Hb-12), 0.96 (2H, m, Ha-3', Ha-4'), 0.94 (3H, s, H_3-27), 0.93 (1H, m, Hb-1), 0.91 (3H, s, H_3-26), 0.82 (6H, s, H_3-23, H_3-25), 0.81 (3H, s, H_3-24), 0.80 (2H, m, Hb-3', Hb-4'), 0.76 (1H, d, *J* = 9.4, Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.2 (C, C-28), 174.6 (C, C-1'), 150.3 (C, C-20), 109.7 (CH_2, C-29), 80.7 (CH, C-3), 56.4 (C, C-17), 55.4 (CH, C-5), 50.4 (CH, C-9), 49.2 (CH, C-18), 46.9 (CH, C-19), 42.4 (C, C-14), 40.7 (C, C-8), 38.4 (C, C-4), 38.3 (C, C-10), 37.9 (CH_2, C-1), 37.1 (CH_2, C-22), 34.2 (CH_2, C-7), 32.2 (CH_2, C-16), 30.6 (CH_2, C-21), 29.7 (CH_2, C-15), 27.9 (CH_3, C-23), 25.4 (CH_2, C-12), 23.7 (CH_2, C-2), 20.8 (CH_2, C-11), 19.3 (CH_3, C-30), 18.1 (CH_2, C-6), 16.5 (CH_3, C-24), 16.1 (CH_3, C-25), 16.0 (CH_3, C-26), 14.7 (CH_3, C-27), 13.3 (CH, C-1'), 8.1 (2*CH_2, C-3', C-4'); calcd for $C_{34}H_{52}NaO_4$ $[M+Na]^+$ 547.3763, found 547.3758.

Preparation of 3-*O*-(1*H*-imidazole-1-carbonyl)betulinic acid (5). To a solution of **1** (50.0 mg) in anhy. CH_2Cl_2 (10.0 mL) was added 1,1'-carbonyldiimidazole (53.0 mg) and stirred at 25

^{o}C for 16 h (TLC control). Reaction mixture was evaporated under reduced pressure and the residue was chromatographed over a column of silica gel (2.0 g) made up in EtOAc/hexanes (30:70) and eluted with the same solvent combination to give **5** (52.5, 87%) as a white amorphous solid. The spectroscopic data (NMR and LRMS) of **5** were identical with those reported.[2]

Preparation of 3-*O*-methoxycarbonylbetulinic acid (6). To a solution of **5** (20.0 mg) in anhy. MeOH (1.0 mL) was added NaOMe (1.0 mg) and stirred at 25 ^{o}C for 6 h (TLC control). The reaction mixture was evaporated under reduced pressure and the resulting residue was chromatographed over a column of silica gel (1.0 g) using hexanes/CH_2Cl_2 (60:40) as eluant to give **6** (15.2 mg, 81%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.71 (1H, brd, J = 1.8 Hz, Ha-29), 4.59 (1H, brt, J = 1.8 Hz, Hb-29), 4.29 (1H, dd, J = 10.7, 4.8 Hz, H-3), 3.74 (3H, s, OMe), 2.98 (1H, dt, J = 4.2, 10.4 Hz, H-19), 2.25 (1H, dt, J = 12.6, 3.3 Hz, Ha-16), 2.15 (1H, dt, J = 3.5, 12.2 Hz, H-13), 1.96 (1H, m, Ha-21), 1.94 (1H, m, Ha-22), 1.66 (1H, m, Ha-12), 1.67 (3H, s, H_3-30), 1.66 (1H, m, Ha-12), 1.59 (1H, m, H-18), 1.56 (2H, m, H_2-2), 1.48 (2H, m, Ha-15, Ha-6), 1.47 (1H, m, Hb-22), 1.39 (1H, m, Hb-21), 1.36 (1H, m, Hb-6), 1.35 (2H, m, H_2-7), 1.27 (1H, m, H-9), 1.23 (1H, m, Hb-11), 1.16 (1H, m, Hb-15), 1.01 (1H, m, Hb-12), 0.95 (3H, s, H_3-27), 0.93 (1H, m, Hb-1), 0.91 (3H, s, H_3-26), 0.89 (3H, s, H_3-25), 0.82 (3H, s, H_3-23), 0.81 (3H, s, H_3-24), 0.76 (1H, d, J = 9.8, Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9 (C, C-28), 155.8 (C, C-1'), 150.4 (C, C-20), 109.7 (CH_2, C-29), 85.5 (CH, C-3), 56.4 (C, C-17), 55.4 (CH, C-5), 54.5 (CH_3, OMe), 50.4 (CH, C-9), 49.2 (CH, C-18), 46.9 (CH, C-19), 42.4 (C, C-14), 40.7 (C, C-8), 38.4 (C, C-4), 38.3 (C, C-10), 38.0 (CH_2, C-1), 37.1 (CH_2, C-22), 34.2 (CH_2, C-7), 32.1 (CH_2, C-16), 30.6 (CH_2, C-21), 29.7 (CH_2, C-15), 27.8 (CH_3, C-23), 25.4 (CH_2, C-12), 23.6 (CH_2, C-2), 20.8 (CH_2, C-11), 19.3 (CH_3, C-30), 18.1 (CH_2, C-6), 16.3 (CH_3, C-24), 16.1 (CH_3, C-25), 16.0 (CH_3, C-26), 14.7 (CH_3, C-27); calcd for $C_{32}H_{50}NaO_5$ $[M+Na]^+$ 537.3556, found 537.3368.

Preparation of methyl betulinate (7). A solution of excess CH_2N_2 in Et_2O was added to a solution of **1** (40.0 mg) in Et_2O (10.0 mL) and stirred at 25 °C for 1 h (TLC control). The reaction mixture was evaporated under reduced pressure to give **6** (40.8 mg, 99%) as a white solid. The data (NMR and LRMS) of **6** were identical with those reported.[3]

Preparation of 28-*O*-5-methyl-2-furoylbetulinic anhydride (8). A solution of **1** (25.0 mg), 5-methyl-2-furoic acid (20.7 mg), N,N'-dicyclohexylcarbodiimide (DCC, 50.0 mg) and 4-pp (1.0 mg) in anhy. EtOAc (1.5 mL) was stirred at 25 °C for 72 h (TLC control). The reaction mixture was filtered, filtrate was evaporated under reduced pressure and the residue thus obtained was chromatographed over a column of silica gel (2.0 g) made up in hexanes/CH_2Cl_2 (80:20) and eluted with hexanes/CH_2Cl_2 (80:20) followed by CH_2Cl_2. Fractions eluted with CH_2Cl_2 were combined to give **8** (22.3 mg, 72%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (1H, d, J = 3.4 Hz, H-3'), 6.17 (1H, d, J = 3.4 Hz, H-4'), 4.72 (1H, brd, J = 1.8 Hz, Ha-29), 4.60 (1H, brt, J = 1.8 Hz, Hb-29), 3.16 (1H, dd, J = 11.9, 4.2 Hz, H-3), 2.99 (1H, dt, J = 5.0, 11.3 Hz, H-19), 2.39 (3H, s, H_3-6'), 2.31 (1H, dt, J = 12.6, 3.3 Hz, Ha-16), 2.27 (1H, dt, J = 3.9, 12.2 Hz, H-13), 2.11 (1H, m, Ha-22), 2.04 (1H, m, Ha-21), 1.71 (1H, m, Ha-12), 1.67 (3H, s, H_3-30), 1.65 (2H, m, Ha-1, H-18), 1.60 (1H, m, Ha-15), 1.57 (2H, m, H_2-2), 1.50 (2H, m, Hb-16, Hb-22), 1.49 (1H, m, Ha-6), 1.43 (1H, m, Hb-21), 1.40 (1H, m, Ha-11), 1.36 (3H, m, Hb-6, H_2-7), 1.28–1.22 (3H, m, H-9, Hb-11, Hb-15), 1.00 (1H, m, Hb-12), 0.97 (3H, s, H_3-27), 0.96 (3H, s, H_3-26), 0.95 (3H, s, H_3-23), 0.85 (1H, m, Hb-1), 0.80 (3H, s, H_3-25), 0.74 (3H, s, H_3-24), 0.65 (1H, d, J = 9.4 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (C, C-28), 159.6 (C, C-5'), 154.1 (C, C-1'), 150.0 (C, C-20), 142.8 (C, C-2'), 122.7 (CH, C-3'), 109.9 (CH_2, C-29), 109.3 (CH, C-4'), 78.9 (CH, C-3), 57.9 (C, C-17), 55.4 (CH, C-5), 50.6 (CH, C-9), 49.3 (CH, C-18), 46.6 (CH, C-19), 42.5 (C, C-14), 40.8 (C, C-8), 38.9 (CH_2, C-1), 38.7 (C, C-4), 38.1 (CH, C-13), 37.2 (C, C-10), 36.3 (CH_2, C-22), 34.3 (CH_2, C-7), 31.7 (CH_2, C-16), 30.3 (CH_2, C-21), 29.7 (CH_2, C-15), 28.0 (CH_3, C-23), 25.4 (CH_2, C-12), 23.6 (CH_2, C-2), 20.8 (CH_2, C-11), 19.3 (CH_3, C-30), 18.1

(CH_2, C-6), 16.2 (CH_3, C-26), 16.0 (CH_3, C-25), 15.4 (CH_3, C-24), 14.7 (CH_3, C-27), 14.2 (CH_3, C-6'); calcd for $C_{36}H_{52}NaO_5$ $[M+Na]^+$ 587.3712, found 587.3738.

Preparation of 28-*O*-(3′,4′,5′-trimethoxy)-benzoylbetulinic anhydride (9). A solution of **1** (25.0 mg), 3,4,5-trimethoxybenzoic acid (34.8 mg), DCC (50.0 mg) and 4-pp (1.0 mg) in anhy. EtOAc (1.5 mL) was stirred at 25 °C for 72 h (TLC control). The reaction mixture was filtered, filtrate was evaporated under reduced pressure and the rsulting residue was chromatographed over a column of silica gel (2.0 g) made up in toluene/acetone (95:5) and eluted with the same solvent mixture to give **9** (24.5 mg, 69%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (2H, s, H-3', H-7'), 4.73 (1H, brd, J = 2.1 Hz, Ha-29), 4.61 (1H, brt, J = 2.1 Hz, Hb-29), 3.91 (3H, s, OMe), 3.88 (6H, s, 2*OMe), 3.16 (1H, dd, J = 11.5, 5.0 Hz, H-3), 3.03 (1H, dt, J = 5.0, 11.2 Hz, H-19), 2.33 (1H, m, Ha-16), 2.32 (1H, m, H-13), 2.09 (1H, m, Ha-22), 2.05 (1H, m, Ha-21), 1.71 (1H, m, Ha-12), 1.67 (3H, s, H_3-30), 1.65–1.63 (3H, m, Ha-1, Ha-15, H-18), 1.57 (2H, m, H_2-2), 1.54 (2H, m, Hb-16, Hb-22), 1.49 (1H, m, Ha-6), 1.46 (1H, m, Hb-21), 1.42 (1H, m, Ha-11), 1.36 (3H, m, Hb-6, H_2-7), 1.28–1.22 (3H, m, H-9, Hb-11, Hb-15), 1.02 (1H, m, Hb-12), 0.98 (3H, s, H_3-27), 0.96 (3H, s, H_3-26), 0.94 (3H, s, H_3-23), 0.85 (1H, m, Hb-1), 0.80 (3H, s, H_3-25), 0.74 (3H, s, H_3-24), 0.66 (1H, d, J = 8.7 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1 (C, C-28), 159.6 (C, C-5'), 162.4 (C, C-2'), 153.1 (C, C-1', C-4', C-6'), 149.8 (C, C-20), 143.5(C, C-5'), 110.0 (CH_2, C-29), 107.6 (CH, C-3', C-7'), 78.9 (CH, C-3), 61.0 (CH_3, OMe), 58.2 (C, C-17), 56.2 (CH_3, OMe), 55.4 (CH, C-5), 50.6 (CH, C-9), 49.3 (CH, C-18), 46.6 (CH, C-19), 42.5 (C, C-14), 40.8 (C, C-8), 38.9 (CH_2, C-1), 38.7 (C, C-4), 38.1 (CH, C-13), 37.2 (C, C-10), 36.2 (CH_2, C-22), 34.3 (CH_2, C-7), 31.9 (CH_2, C-16), 30.3 (CH_2, C-21), 29.8 (CH_2, C-15), 27.9 (CH_3, C-23), 25.4 (CH_2, C-12), 23.6 (CH_2, C-2), 20.8 (CH_2, C-11), 19.4 (CH_3, C-30), 18.3 (CH_2, C-6), 16.1 (CH_3, C-26), 16.0 (CH_3, C-25), 15.3 (CH_3, C-24), 14.7 (CH_3, C-27); calcd for $C_{40}H_{58}NaO_7$ $[M+Na]^+$ 673.4080, found 673.4086.

Preparation of 3-*O*-acetylmethyl betulinate (10). To a solution of **7** (10.0 mg) in pyridine (500 µL) was added Ac_2O (50 µL) and stirred at 25 °C. After 16 h (TLC control), EtOH was

added to the reaction mixture and evaporated under reduced pressure. The residue thus obtained was chromatographed over a column of silica gel using hexanes/CH_2Cl_2 (60:40) followed by hexanes/CH_2Cl_2 (50:50). Fractions eluted with hexanes/CH_2Cl_2 (50:50) were combined to give **7** (10.5 mg, 96%) as a white solid. The spectroscopic data (NMR and LRMS) of **7** were identical with those reported.[1,3]

Preparation of betulonic acid (11). To a solution of **1 (**20.0 mg) in *N*,*N*-dimethylformamide (400.0 µL), Cr_2O_3 (20.0 mg) and conc. H_2SO_4 (3.0 µL) were added and stirred at 25 ^oC. After 12 h (TLC control), the reaction mixture was diluted with EtOAc (15.0 mL) and washed with brine (3 x 5 mL), dried over anhy. Na_2SO_4 and evaporated under reduced pressure. The resulting residue was chromatographed over a column of silica gel using CH_2Cl_2/MeOH (99:1) as eluant to afford **11** (19.2 mg, 96%) as a white solid. The spectroscopic data (NMR and LRMS) of **11** were identical with those reported.[4]

Preparation of methyl betulonate (12). A solution of excess CH_2N_2 in Et_2O was added to a solution of **3** (10.0 mg) in Et_2O (2.5 mL) and stirred at 25 ^oC for 1 h (TLC control). The reaction mixture was evaporated under reduced pressure to afford **12** (10.2 mg, 99%) as a white solid. The spectroscopic data (NMR and LRMS) of **12** were identical with those reported.[4]

Preparation of 20(29)-epoxybetulinic acid (14). To a solution of **1** (10.0 mg) in CH_2Cl_2 (2.5 mL) at 0 ^oC was added *m*-CPBA (8.0 mg) and stirred at 0 ^oC for 5 min and then at 25 ^oC for 4h (TLC control). The reaction mixture was diluted with $NaHSO_3$ and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhy. Na_2SO_4, evaporated under reduced pressure and the resulting residue was chromatographed over a column of silica gel using CH_2Cl_2 as eluant to give **14** (7.0 mg, 68%) as a white solid. The spectroscopic data (NMR and LRMS) of **14** were identical with those reported.[6]

Preparation of 20(29)-epoxymethyl betulinate (15). To a solution of **14** (30.0 mg) in CH_2Cl_2 (5.0 mL) at 0 ^oC was added *m*-CPBA (25.0 mg) and stirred at 0 ^oC for 5 min and then at 25 ^oC for 4h (TLC control). The reaction mixture was diluted with $NaHSO_3$ and extracted with

CH_2Cl_2. The combined organic extracts was washed with brine, dried over anhy. Na_2SO_4, evaporated under reduced pressure and the residue thus obtained was chromatographed over a column of silica gel using CH_2Cl_2/MeOH (98:2) as eluant to afford **15** (22.0 mg, 71%) as a white solid. The spectroscopic data (NMR and LRMS) of **15** were identical with those reported.[7]

Preparation of 3-*O*-acetyl-20(29)-epoxymethyl betulinate (16). To a solution of **15** (10.0 mg) in pyridine (500 µL) was added Ac_2O (50 µL) and stirred at 25 °C. After 16 h, EtOH was added to the reaction mixture and evaporated under reduced pressure. The residue thus obtained was chromatographed over a column of silica gel using hexanes/CH_2Cl_2 (1:1) to yield **16** (10.5 mg, 97%) as a white solid. The spectroscopic data (NMR and LRMS) of **16** were identical with those reported.[7]

Preparation of 3,28-di-*O*-tetrahydropyranylbetulin (17) and 28-*O*-tetrahydropyranylbetulin (32). To a solution of betulin (**2**, 100.0 mg) in THF (2.5 mL) was added 3,4-dihydro-2*H*-pyran (DHP, 200.0 *µ*L) and *para*-toluenesulfonic acid (*p*-TSA, 0.5 mg) and stirred at 25 °C for 1.5 h (TLC control). The reaction mixture was evaporated under reduced pressure and the resulting residue was chromatographed over a column of silica gel (5.0 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2. Fractions having similar TLC profiles were combined and evaporated to give **17** (60.5 mg, 45%) and **32** (42.7 mg, 37%) as amorphous solids.

3,28-Di-*O*-tetrahydropyranylbetulin (17). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 4.64 (1H, brd, *J* = 2.1 Hz, Ha-29), 4.53 (1H, brt, *J* = 2.1 Hz, Hb-29), 3.90 (1H, d, *J* = 11.0 Hz, Ha-28), 3.81 (2H, m), 3.50 (1H, d, *J* = 11.0 Hz, Hb-28), 3.44 (2H, m), 3.42 (2H, m), 3.18 (1H, dd, *J* = 11.9, 5.0 H-3), 2.43 (1H, m, H-H-19), 1.67 (3H, s, H_3-30), 1.00 (3H, s), 0.94 (3H, s), 0.85 (3H, s), 0.80 (3H, s), 0.76 (3H, s), 0.65 (1H, d, J = 9.4 Hz, H-5).

The spectroscopic data (NMR and LRMS) of **32** were identical with those reported.[4]

Acetylation of 28-*O*-tetrahydropyranylbetulin (32). To a solution of **32** (40.0 mg) in pyridine (1.0 mL) was added Ac_2O (200.0 *µ*L) and stirred at 25 °C for 5 h (TLC control). The

reaction mixture was diluted with EtOH and evaporated under reduced pressure and the resulting residue was passed through a short column of silica gel to afford 3-*O*-acetyl-28-*O*-tetrahydropyranylbetulin (**18,** 40.9 mg, 95%) as an amorphous solid. The spectroscopic data (NMR and LRMS) of **18** were identical with those reported.[4]

Preparation of 3-*O*-acetylbetulin (19). To a solution of **18** (20.0 mg) in EtOH (1.0 mL) was added *p*-TSA (1.0 mg) and stirred at 25 °C for 6 h (TLC control). The reaction was quenched with a saturated solution of aqueous $NaHCO_3$ (5.0 mL) and extracted with EtOAc (3 x 5.0 mL). The combined organic extracts was washed with brine, dried over anhy. Na_2SO_4 and evaporated under reduced pressure. The resulting residue was chromatographed over a column of silica gel using CH_2Cl_2 as eluant to provide **19** (16.5 mg, 90%) as an amorphous solid. The spectroscopic data (NMR and LRMS) of **19** were identical with those reported.[4]

Preparation of 3-*O*-cyclopropanoyl betulin (20). A solution of **32** (20.0 mg), cyclopropanecarboxylic acid (10.0 mg), DCC (27.0 mg) and 4-pp (1.0 mg) in anhy. EtOAc (1.0 mL) was stirred at 25 °C for 16 h (TLC control). The reaction mixture was filtered, the filtrate was evaporated under reduced pressure and the resulting residue was chromatographed over a column of silica gel. The product (21.0 mg) thus obtained was dissolved in EtOH (1.0 mL), stirred with *p*-TSA (2.0 mg) at 25 °C for 6 h (TLC control), quenched with a solution of saturated aqueous $NaHCO_3$ (5.0 mL), and extracted with EtOAc (3 x 5.0 mL). The combined organic extracts was washed with brine, dried over anhy. Na_2SO_4 and evaporated under reduced pressure. The resulting residue was then chromatographed over a column of silica gel using CH_2Cl_2 as eluant to give **20** (17.2 mg, 89%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (1H, brd, J = 2.1 Hz, Ha-29), 4.56 (1H, brt, J = 2.1 Hz, Hb-29), 4.43 (1H, dd, J = 10.4, 5.2 Hz, H-3), 3.77 (1H, d, J = 11.0 Hz, Ha-28), 3.30 (1H, d, J = 11.0 Hz, Hb-28), 2.35 (1H, dt, J = 5.6, 10.8 Hz, H-19), 1.98–1.80 (H, m, Ha-16, Ha-21, Ha-22), 1.66 (3H, s, H_3-30), 1.63–1.46 (9H, m, Ha-1, H_2-2, Ha-6, Ha-12, H-13, Ha-15, H-18, H-2'), 1.44–1.30 (5H, m, Hb-6, H_2-7, Ha-11, Hb-21), 1.28–1.22 (3H, m, H-9, Hb-11, Hb-16), 1.00 (3H, s, H_3-23), 0.96 (2H, m, Ha-3',

Ha-4'), 0.95 (3H, s, H_3-27), 0.82 (9H, s,H_3-24, H_3-25, H_3-26), 0.76 (1H, d, *J* = 8.7 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 174.6 (C, C-1'), 150.5 (C, C-20), 109.7 (CH_2, C-29), 80.6 (CH, C-3), 60.5 (CH_2, C-28), 55.4 (CH, C-5), 50.3 (CH, C-9), 48.7 (CH, C-18), 47.8 (CH, C-19), 47.7 (C, C-17), 42.7 (C, C-14), 40.9 (C, C-8), 38.3 (CH_2, C-1), 37.9 (C, C-4), 37.3 (CH, C-13), 37.0 (C, C-10), 34.1 (CH_2, C-7), 33.9 (CH_2, C-22), 29.7 (CH_2, C-16), 29.1 (CH_2, C-21), 27.9 (CH_3, C-23), 27.0 (CH_2, C-15), 25.1 (CH_2, C-12), 23.7 (CH_2, C-2), 20.8 (CH_2, C-11), 19.0 (CH_3, C-30), 18.1 (CH_2, C-6), 16.5 (CH_3, C-26), 16.1 (CH_3, C-25), 15.9 (CH_3, C-24), 14.7 (CH_3, C-27), 13.3, (CH, C-1'), 8.1 (2*CH_2, C-3', C-4'); calcd for $C_{33}H_{54}NaO_3$ $[M+Na]^+$ 533.3971, found 533.3967.

Preparation of 28-*O*-acetylbetulin (21) and 3,28-di-*O*-acetylbetulin (22). To a solution of **2** (100.0 mg) in pyridine (2.0 mL) was added Ac_2O (45 *μ*L) and stirred at 25 °C for 5 h (TLC control). The reaction mixture was diluted with EtOH and evaporated under reduced pressure. The resulting residue was chromatographed over a column of silica gel (4.0 g) made up in hexanes/CH_2Cl_2 (1:1) and eluted with hexanes/CH_2Cl_2 (1:1) followed by hexanes/CH_2Cl_2 (1:3). The fractions eluted with hexanes/CH_2Cl_2 (1:1) were combined and evaporated to give **22** (17.6, 15%) as an amorphous solid and those eluted with hexanes/CH_2Cl_2 (1:3) were combined and evaporated to give **21** (90.1, 82%) as an amorphous solid. The spectroscopic data (NMR and LRMS) of **21** and **22** were identical with those reported.[4]

Preparation of ((1*R*,3a*S*,5a*R*,5b*R*,9*S*,11a*R*)-9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl) icosahydro-3a*H*-cyclopenta[a]chrysen-3a-yl)methyl 5-methylfuran-2-carboxylate [28-*O*-(5-methyl-2-furoyl)betulin (23). A solution of **2** (50.0 mg), 5-methyl-2-furoic acid (15.6 mg), DCC (30.0 mg) and 4-pp (ca 1.0 mg) in anhy. EtOAc was stirred at 25 °C. After 16 h (TLC control), the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The resulting residue was chromatographed over a column of silica gel (2.0 g) made up in EtOAc/hexanes (5:95) and eluted with the same solvent to afford **23** (42.8 mg, 69%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.03 (1H, d, *J* = 3.7 Hz, H-3'), 6.08 (1H, d, *J* = 3.7 Hz, H-4'), 4.68 (1H, brd, *J* = 1.8 Hz, Ha-29), 4.57 (1H, brt, *J* = 1.8 Hz,

Hb-29), 4.45 (1H, d, *J* = 11.4 Hz, Ha-28), 4.02 (1H, d, J = 11.4 Hz, Hb-28), 3.16 (1H, dd, *J* = 11.9, 4.2 Hz, H-3), 2.48 (1H, dt, *J* = 5.8, 11.1 Hz, H-19), 2.36 (3H, s, H_3-6'), 1.99 (1H, m, Ha-21), 1.90 (1H, m, Ha-16), 1.84 (1H, m, Ha-22), 1.69 (1H, m, H-13), 1.67 (3H, s, H_3-30), 1.65 (2H, m, Ha-1, Ha-12), 1.60 (1H, m, H-18), 1.57–1.52 (2H, m, H_2-2), 1.49 (1H, m, Ha-6), 1.40 (2H, m, Ha-11, Ha-15), 1.36 (5H, m, Hb-6, H_2-7, Ha-11, Hb-21), 1.27 (1H, m, Hb-16), 1.24 (2H, m, H-9, Hb-11), 1.11 (1H, m, Hb-22), 1.03 (2H, m, Hb-12, Hb-15), 1.02 (3H, s, H_3-26), 0.96 (3H, s, H_3-27), 0.94(3H, s, H_3-23), 0.80 (3H, s, H_3-25), 0.85 (1H, m, Hb-1), 0.80 (3H, s, H_3-25), 0.74 (3H, s, H_3-24), 0.66 (1H, d, *J* = 9.1 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 159.3 (C, C-1'), 157.2 (C, C-5'), 150.2 (C, C-20), 143.1 (C, C-2'), 119.0 (CH, C-3'), 109.9 (CH_2, C-29), 108.3 (CH, C-4'), 78.9 (CH, C-3), 62.9 (CH_2, C-28), 55.3 (CH, C-5), 50.4 (CH, C-9), 48.8 (CH, C-18), 47.7 (CH, C-19), 46.8 (C, C-17), 42.7 (C, C-14), 40.9 (C, C-8), 38.8 (C, C-4), 38.7 (CH_2, C-1), 37.6 (CH, C-13), 37.1 (C, C-10), 34.7 (CH_2, C-22), 34.2 (CH_2, C-7), 29.8 (CH_2, C-16), 29.6 (CH_2, C-21), 27.9 (CH3, C-23), 27.4 (CH_2, C-2), 27.0 (CH_2, C-15), 25.2 (CH_2, C-12), 20.8 (CH_2, C-11), 19.1 (CH_3, C-30), 18.3 (CH_2, C-6), 16.1 (CH_3, C-26), 16.0 (CH_3, C-25), 15.3 (CH_3, C-24), 14.7 (CH_3, C-27), 14.0 (CH_3, C-6'); calcd for $C_{36}H_{54}NaO_4$ $[M+Na]^+$ 573.3920, found 573.3927.

Preparation of ((1*R*,3a*S*,5a*R*,5b*R*,9*S*,11a*R*)-9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-3a*H*-cyclopenta[a]chrysen-3a-yl)methyl thiophene-2-carboxylate [28-*O*-(2-thiophenecarboxyloyl)betulin (24). A solution of **2** (50.0 mg), 2-thiophenecarboxylic acid (15.8 mg), DCC (30.0 mg) and 4-pp (ca 1.0 mg) in anhy. EtOAc was stirred at 25 ^{o}C. After 16 h (TLC control), the reaction mixture was filtered, filtrate was evaporated under reduced pressure and the resulting residue was chromatographed over a column of silica gel (2.0 g) made up in EtOAc/hexanes (5:95) and eluted with the same solvent to give **24** (44.2 mg, 71%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.79 (1H, dd, *J* = 3.8, 1.3 Hz, H-5'), 7.53 (1H, dd, *J* = 4.9, 1.3 Hz, H-3'),7.08 (1H, dd, *J* = 4.9, 3.8 Hz, H-4'), 4.69 (1H, brd, *J* = 1.7 Hz, Ha-29), 4.58 (1H, brt, *J* = 1.7 Hz, Hb-29), 4.47 (1H, dd, *J* = 11.0, 1.8

Hz, Ha-28), 4.04 (1H, d, *J* = 11.0, 1.0 Hz, Hb-28), 3.16 (1H, dd, *J* = 11.1, 5.0 Hz, H-3), 2.49 (1H, dt, *J* = 5.5, 11.1 Hz, H-19), 2.02 (1H, m, Ha-21), 1.94 (1H, m, Ha-16), 1.87 (1H, m, Ha-22), 1.72 (1H, m, Ha-15), 1.69 (1H, m, H-13), 1.68 (3H, s, H_3-30), 1.67 (2H, m, Ha-1, Ha-12), 1.62 (1H, m, H-18), 1.57–1.52 (2H, m, H_2-2), 1.49 (1H, m, Ha-6), 1.40 (2H, m, Ha-11, Hb-21), 1.38 (3H, m, Hb-6, H_2-7), 1.29 (1H, m, Hb-16), 1.24 (1H, m, H-9), 1.21 (1H, m, Hb-11), 1.13 (1H, m, Hb-22), 1.04 (2H, m, Hb-12, Hb-15), 1.03 (3H, s, H_3-26), 0.97 (3H, s, H_3-27), 0.94 (3H, s, H_3-23), 0.85 (1H, m, Hb-1), 0.81 (3H, s, H_3-25), 0.80 (3H, s, H_3-25), 0.74 (3H, s, H_3-24), 0.66 (1H, d, *J* = 9.1 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 162.7 (C, C-1'), 150.1 (C, C-20), 134.0 (C, C-2'), 133.2 (CH, C-5'), 132.2 (CH, C-3'), 127.4 (CH, C-4'), 109.9 (CH_2, C-29), 78.9 (CH, C-3), 63.5 (CH_2, C-28), 55.3 (CH, C-5), 50.3 (CH, C-9), 48.8 (CH, C-18), 47.8 (CH, C-19), 46.7 (C, C-17), 42.8 (C, C-14), 40.9 (C, C-8), 38.9 (C, C-4), 38.7 (CH_2, C-1), 37.7 (CH, C-13), 37.2 (C, C-10), 34.7 (CH_2, C-22), 34.2 (CH_2, C-7), 29.9 (CH_2, C-16), 29.7 (CH_2, C-21), 28.0 (CH_3, C-23), 27.4 (CH_2, C-2), 27.2 (CH_2, C-15), 25.2 (CH_2, C-12), 20.8 (CH_2, C-11), 19.2 (CH_3, C-30), 18.3 (CH_2, C-6), 16.1 (CH_3, C-26), 16.0 (CH_3, C-25), 15.4 (CH_3, C-24), 14.8 (CH_3, C-27); calcd for $C_{35}H_{52}NaO_3S$ $[M+Na]^+$ 575.3535, found 575.3529.

Preparation of 3*β*-hydroxy-lup-20(29)-en-28-yl-1*H*-imidazole-1-carboxylate [28-*O*-(1*H*-imidazole-1-carbonyloxy)betulin (25). To a solution of **2** (50.0 mg) in CH_2Cl_2 (2.0 mL) was added CDI (17.0 mg) and stirred at 25 $_o$C for 16 h (TLC control). The reaction mixture was evaporated under reduced pressure and the resulting residue was chromatographed over a column of silica gel (1.0 g) using EtOAc as eluant to give **25** (40.2 mg, 66%) as an amorphous solid. The spectroscopic data (NMR and LRMS) of **25** were identical with those reported.[2]

Preparation of ((1*R*,3a*S*,5a*R*,5b*R*,9*S*,11a*R*)-9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-3a*H*-cyclopenta[a]chrysen-3a-yl)methyl methyl carbonate [28-*O*-methoxycarbonylbetulin] (26). To a solution of **25** (20.0 mg) in anhy. MeOH (1.0 mL) was added NaOMe (ca 0.5 mg) and stirred at 25 ^{o}C for 5 h (TLC control). The reaction mixture was evaporated under reduced pressure and the residue was chromatographed over a column

of silica gel (1.0 g) using hexanes/CH_2Cl_2 (1:1) as eluant to give 2**6** (15.8 mg, 85%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.67 (1H, brd, J = 1.6 Hz, Ha-29), 4.57 (1H, brt, J = 1.6 Hz, Hb-29), 4.32 (1H, dd, J = 10.8, 1.9 Hz, Ha-28), 3.89 (1H, d, J = 10.8, 1.0 Hz, Hb-28), 3.77 (3H, s, OMe), 3.16 (1H, dd, J = 11.5, 4.8 Hz, H-3), 2.41 (1H, dt, J = 5.9, 11.0 Hz, H-19), 1.96 (1H, m, Ha-21), 1.86 (1H, m, Ha-16), 1.80 (1H, m, Ha-22), 1.69 (1H, m, Ha-15), 1.68 (3H, s, H_3-30), 1.62 (3H, m, Ha-1, Ha-12, H-13), 1.57 (3H, m, H_2-2, H-18), 1.48 (1H, m, Ha-6), 1.36 (2H, m, H_a-11, Hb-21), 1.37 (3H, m, Hb-6, H_2-7), 1.24 (2H, m, H-9, Hb-16), 1.18 (1H, m, Hb-11), 1.05 (1H, m, Hb-22), 1.04 (1H, m, Hb-15), 1.03 (1H, m, Hb-12), 1.01 (3H, s, H_3-26), 0.95 (3H, s, H_3-27), 0.94 (3H, s, H_3-23), 0.85 (1H, m, Hb-1), 0.81 (3H, s, H_3-25), 0.80 (3H, s, H_3-25), 0.74 (3H, s, H_3-24), 0.66 (1H, d, J = 9.1 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3 (C, C-1'), 150.0 (C, C-20), 109.9 (CH_2, C-29), 78.9 (CH, C-3), 66.6 (CH_2, C-28), 55.2 (CH, C-5), 54.7 (CH_3, OMe), 50.3 (CH, C-9), 48.8 (CH, C-18), 47.7 (CH, C-19), 46.5 (C, C-17), 42.7 (C, C-14), 40.8 (C, C-8), 38.8 (C, C-4), 38.7 (CH_2, C-1), 37.6 (CH, C-13), 37.1 (C, C-10), 34.3 (CH_2, C-22), 34.1 (CH_2, C-7), 29.5 (CH_2, C-16), 27.9 (CH_3, C-23), 27.9 (CH_2, C-21), 27.4 (CH_2, C-2), 27.0 (CH_2, C-15), 25.1 (CH_2, C-12), 20.7 (CH_2, C-11), 19.1 (CH_3, C-30), 18.2 (CH_2, C-6), 16.1 (CH_3, C-26), 16.0 (CH_3, C-25), 15.3 (CH_3, C-24), 14.7 (CH_3, C-27); calcd for $C_{32}H_{52}NaO_4$ $[M+Na]^+$ 523.3763, found 523.3759.

Preparation of 3,28-di-*O-tert*-butyldimethylsilylbetulin (27). To a solution of **2** (50.0 mg) in anhy. CH_2Cl_2 (1.5 mL) was added *t*-BDMSCl (35.0 mg) and 4-pp (12.0 mg) and stirred at 25 ^{o}C for 16 h (TLC control). The reaction mixture was transferred to a column of silica gel (1.0 g) and eluted using CH_2Cl_2/hexanes (1:1) as eluant and evaporated to afford **27**[9] (52.5 mg, 70%) as a gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (1H, brd, J = 1.6 Hz, Ha-29), 4.55 (1H, brt, J = 1.6 Hz, Hb-29), 3.64 (1H, d, J = 9.9 Hz, Ha-28), 3.23 (1H, d, J = 9.9 Hz, Hb-28), 3.13 (1H, dd, J = 11.9, 4.9 Hz, H-3), 2.37 (1H, td, J = 5.8, 10.5 Hz, H-19), 1.92 (1H, m, Ha-21), 1.88 (1H, m, Ha-16), 1.87 (1H, m, Ha-22), 1.66 (3H, s, H_3-30), 1.63–1.50 (6h, m), 1.49–1.30 (5H, m), 1.28–0.99 (7H, m), 0.98 (3H, s), 0.95 (3H, s), 0.88 (9H, s, -C(CH_3)$_3$), 0.86 (9H, s, -C(CH_3)$_3$), 0.85 (3H, s),

0.80 (3H, s), 0.70 (3H, s), 0.64 (1H, d, *J* = 9.3 Hz, H-5), 0.02 (6H, s, $Si(CH_3)_2$), 0.01 (6H, s, $Si(CH_3)_2$).

Preparation of 28-*O*-acetyl-3-oxobetulin (28). To a solution of **21** (20.0 mg) in CH_2Cl_2 (1.5 mL) was added pyridinium chlorochromate (20.0 mg) and stirred at 25 ^oC for 4 h (TLC control). The reaction mixture was introduced to a column of silica gel using CH_2Cl_2 as eluant and evaporated to afford **28** (14.8 mg, 74%) as an amorphous solid. The spectroscopic data (NMR and LRMS) of **28** were identical with those reported.[4]

Preparation of betulinaldehyde (29) and betulonaldehyde (30). To a solution of **2** (100.0 mg) in CH_2Cl_2 (5.0 ML) at 0^oC was added pyridinium chlorochromate (80.0 mg) and stirred at 0 ^oC for 2 h (TLC control). The reaction mixture was introduced to a column of silica gel (2.0 g), eluted with CH_2Cl_2 and evaporated to give **29** (29.5 mg, 30%) and **30** (28.3 mg, 29%) as amorphous solids. The spectroscopic data (NMR and LRMS) of **29** and **30** were identical with those reported.[4]

Preparation of 20(29)-epoxybetulin (31). To a solution of **2** (100.0 mg) in CH_2Cl_2 (10.0 mL) was added *m*-CPBA (80.0 mg) and stirred at 25 ^oC for 3 h (TLC control). The reaction mixture was concentrated and chromatographed over a clumn of silica gel (2.0 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 followed by CH_2Cl_2/MeOH (99:1) to afford **31** (90.0 mg, 89%) as an amorphous solid. The spectroscopic data (NMR and LRMS) of **31** were identical with those reported.[7]

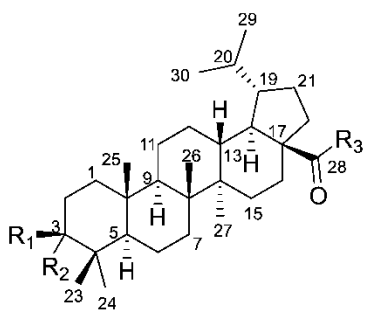
Table S1. Details of statistical analyses for all figures.

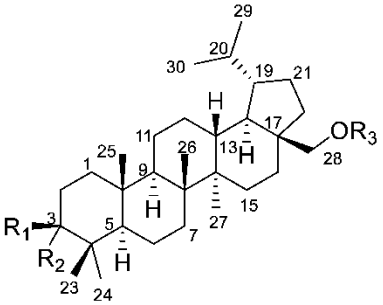
Figure panel	Assay	Statistical test; findings	Post-hoc analysis (adjusted p-value)	Number of subjects
Figure 1	% Change in average response to 40 mM KCl	One-way ANOVA; p<0.0001	DMSO 0.1% vs. **1** (20 μM): p<0.0001 DMSO 0.1% vs. **4**: p<0.0001 DMSO 0.1% vs. **5**: p<0.0001 DMSO 0.1% vs. **6**: p<0.0001 DMSO 0.1% vs. **7**: p<0.0001 DMSO 0.1% vs. **8**: p<0.0001 DMSO 0.1% vs. **9**: p<0.0001 DMSO 0.1% vs. **10**: p=0.013 DMSO 0.1% vs. **14**: p<0.0001 DMSO 0.1% vs. **16**: p=0.0089 DMSO 0.1% vs. **17**: p<0.0001 DMSO 0.1% vs. **18**: p<0.0001 DMSO 0.1% vs. **19**: p<0.0001 DMSO 0.1% vs. **20**: p<0.0001 DMSO 0.1% vs. **21**: p=0.0061 DMSO 0.1% vs. **22**: p<0.0001 DMSO 0.1% vs. **23**: p<0.0001 DMSO 0.1% vs. **24**: p<0.0001 DMSO 0.1% vs. **25**: p<0.0001 DMSO 0.1% vs. **26**: p<0.0001 DMSO 0.1% vs. **27**: p=0.0005 DMSO 0.1% vs. **31**: p=0.005	Normalized average peak response: mean ± SEM and (number of cells) 0.1% DMSO: 1 ± 0.032 (n=559) **1**: 0.505 ±0.0336 (n=230) **4**: 0.411 ± 0.0251 (n=180) **5**: 0.607 ± 0.0627 (n=116) **6**: 0.5039 ± 0.0950 (n=47) **7**: 0.547 ± 0.0192 (n=179) **8**: 0.355 ± 0.0270 (n=81) **9**: 0.455 ± 0.0542 (n=42) **10**: 0.8054 ± 0.0239 (n=124) **14**: 0.303 ± 0.0272 (n=62) **16**: 0.7846 ± 0.056 (n=104) **17**: 0.653 ± 0.0477 (n=139) **18**: 0.3827 ± 0.033 (n=55) **19**: 0.578 ± 0.0325 (n=171) **20**: 0.4044 ± 0.03655 (n=113) **21**: 0.8189 ± 0.0525 (n=171) **22**: 0.574 ± 0.0452 (n=115) **23**: 0.5356 ± 0.0832 (n=82) **24**: 0.4255 ± 0.0305 (n=97) **25**: 0.739 ± 0.0273 (n=365) **26**: 0.7062 ± 0.0608 (n=128)

				27: 0.6717 ± 0.079 (n=60) **31**: 0.8144 ± 0.04818 (n=166)
Figure 2B	Peak total calcium current density	One-way ANOVA, Turkey's test	DMSO 0.1% vs. **8** (20 μM): p=0.0026 DMSO 0.1% vs.**18** (20 μM) p=0.4535 **8** (20 μM) vs. **18** (20 μM) p=0.065	DMSO 0.1% (-70.4 ± 6.266, n=13) **8** (-31.65 ± 3.24, n=12) **18** (-58.28 ± 11.86, n=12)
Figure 3B	Peak N-type calcium current	Students' t-test	DMSO (0.1%) vs. **8**: p=0.0050	DMSO 0.1% (-43.93 ± 7.518, n=11) **8** (-18.77 ± 2.654, n=11)
Figure 4B	Peak T-type calcium current	Students' t-test	DMSO (0.1%) vs **8**: p=0.0042	DMSO 0.1% (-60.08 ± 5.02, n=13) **8** (-35.26 ± 5.714, n=9)
	Cell size (pF)	Students' t-test	DMSO (0.1%) vs **8**: p=0.0617	DMSO 0.1% (18.7 ± 1.527, n=13) **8** (24.57 ± 2.814, n=9)
Figure 5A	Peak L-type	Students' t-test	CaV1.2, DMSO (0.1%) vs **8**: 0.2824 CaV1.3, DMSO (0.1%) vs **8**: 0.0658	CaV1.2 DMSO 0.1% (-35.19 ± 5.98, n=10) **8** (-25.458 ± 6.43 n=10) CaV1.3 DMSO 0.1% (-111.2 ± 25.115, n=10) **8** (-54.626 ± 14.26, n=10)
Figure 5B	Peak N-type	Students' t-test	CaV2.2, DMSO (0.1%) vs **8**: <0.0001	CaV2.2 DMSO 0.1% (-76.55 ± 26.89, n=11) 8 (-8.67 ± 7.19 n=12)
Figure 5C	Peak T-type	Students' t-test	CaV3.1, DMSO (0.1%) vs **8**: 0.9187 CaV3.2, DMSO (0.1%) vs **8**: 0.0004 CaV3.3, DMSO (0.1%) vs **8**: 0.2541	CaV3.1 DMSO 0.1% (-52.47 ± 7.02, n=10) **8** (-53.43 ± 6.01, n=10) CaV3.2 DMSO 0.1% (-38.58 ± 4.23 n=19) **8** (-20.59 ± 1.73, n=19) CaV3.3 DMSO 0.1% (-34.55 ± 3.83 n=10) **8** (-42.43 ± 5.48, n=10)
Figure 6A	Paw withdrawal threshold (g), i.t. injection	2-way ANOVA	Vehicle - **8** i.t. 2 μg in 5 μl vs. Pre-SNI: Predrug: p>0.9999 0.5 h: p>0.9999 1 h: p=0.9970 2 h: p=0.0762 3 h: p=0.0061 4 h: p=0.1873	3 h vehicle control (18.42 ± 8.225, n=6) **8** 2 μg in 5 μl (1.917 ± 0.6804, n=5)

			5 h: p=0.8189	
Figure 6B	Area under the curve, i.t. injection	Mann-Whitney test	p=0.0303	DMSO 0.1% (18.4 ± 8.189, n=6) **8** (0.9895 ± 1.131, n=5)

[a] p values are indicated. Values are mean ± SEM; n, indicates number of subjects.

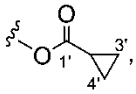


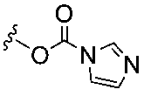


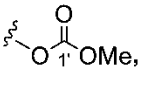
1 $R_1 = R_3 = OH$, $R_2 = H$, $\Delta^{20,29}$

2 $R_1 = OH$, $R_2 = R_3 = H$, $\Delta^{20,29}$

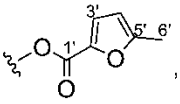
3 $R_1 = OAc$, $R_2 = H$, $R_3 = OH$, $\Delta^{20,29}$

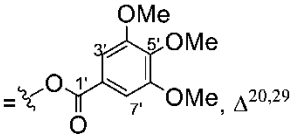
4 R_1 =  , $R_2 = H$, $R_3 = OH$, $\Delta^{20,29}$

5 R_1 =  , $R_2 = H$, $R_3 = OH$, $\Delta^{20,29}$

6 R_1 =  , $R_2 = H$, $R_3 = OH$, $\Delta^{20,29}$

7 $R_1 = OH$, $R_2 = H$, $R_3 = OMe$, $\Delta^{20,29}$

8 $R_1 = OH$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

9 $R_1 = OH$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

10 $R_1 = OAc$, $R_2 = H$, $R_3 = OMe$, $\Delta^{20,29}$

11 $R_1, R_2 = O$, $R_3 = OH$, $\Delta^{20,29}$

12 $R_1, R_2 = O$, $R_3 = OMe$, $\Delta^{20,29}$

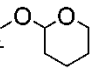
14 $R_1 = R_3 = OH$, $R_2 = H$, 20(29)epoxy

15 $R_1 = OH$, $R_2 = H$, $R_3 = OMe$, 20(29)epoxy

16 $R_1 = OAc$, $R_2 = H$, $R_3 = OMe$, 20(29)epoxy

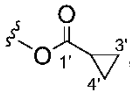
29 $R_1 = OH$, $R_2 = R_3 = H$, $\Delta^{20,29}$

30 $R_1, R_2 = O$, $R_3 = H$, $\Delta^{20,29}$

17 R_1 =  , $R_2 = H$, R_3 =  , $\Delta^{20,29}$

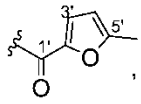
18 $R_1 = OAc$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

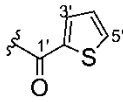
19 $R_1 = OAc$, $R_2 = R_3 = H$, $\Delta^{20,29}$

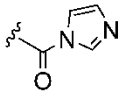
20 R_1 =  , $R_2 = R_3 = H$, $\Delta^{20,29}$

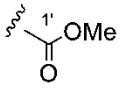
21 $R_1 = OH$, $R_2 = H$, $R_3 = Ac$, $\Delta^{20,29}$

22 $R_1 = OAc$, $R_2 = H$, $R_3 = Ac$, $\Delta^{20,29}$

23 $R_1 = OH$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

24 $R_1 = OH$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

25 $R_1 = OH$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

26 $R_1 = OH$, $R_2 = H$, R_3 =  , $\Delta^{20,29}$

27 $R_1 = OtBDMS$, $R_2 = H$, R_3= tBDMS, $\Delta^{20,29}$

28 $R_1, R_2 = O$, $R_3 = Ac$, $\Delta^{20,29}$

31 $R_1 = OH$, $R_2 = R_3 = H$, 20(29)epoxy

Figure S1. Structures of betulinic acid (**1**) and betulin (**2**) analogues.

Figure S2. ^{1}H NMR spectrum (400 MHz) of 3-*O*-cyclopropanoyl-betulinic acid (**4**) in $CDCl_3$

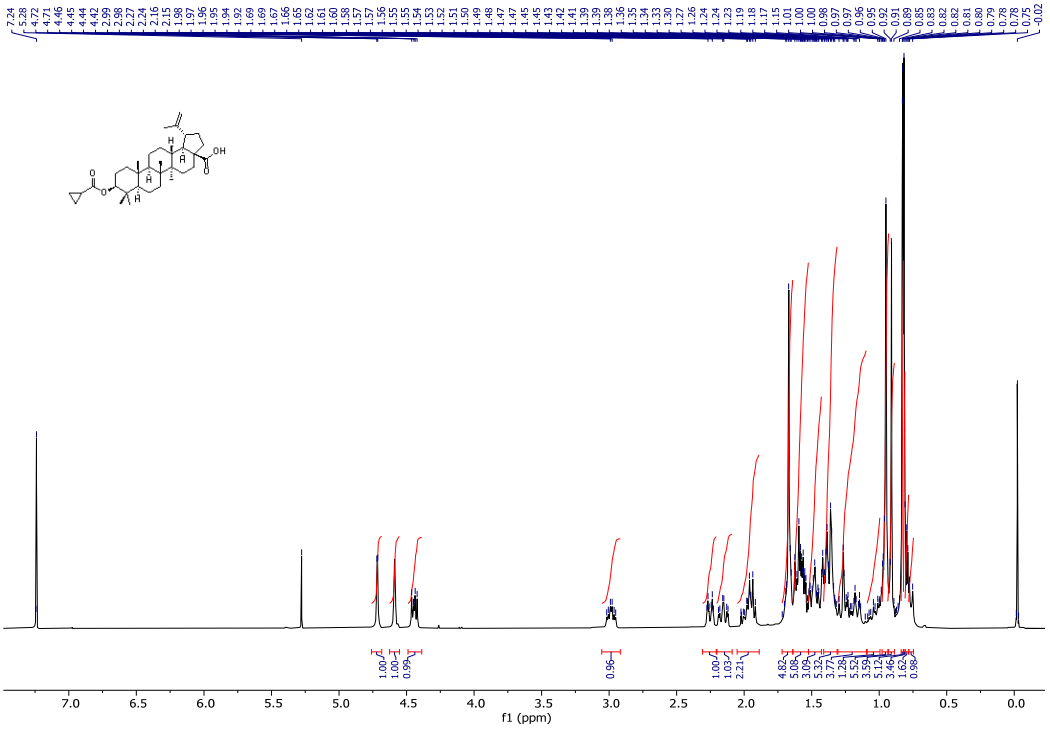


Figure S3. ^{13}C NMR spectrum (100 MHz) of 3-*O*-cyclopropanoyl-betulinic acid (**4**) in $CDCl_3$

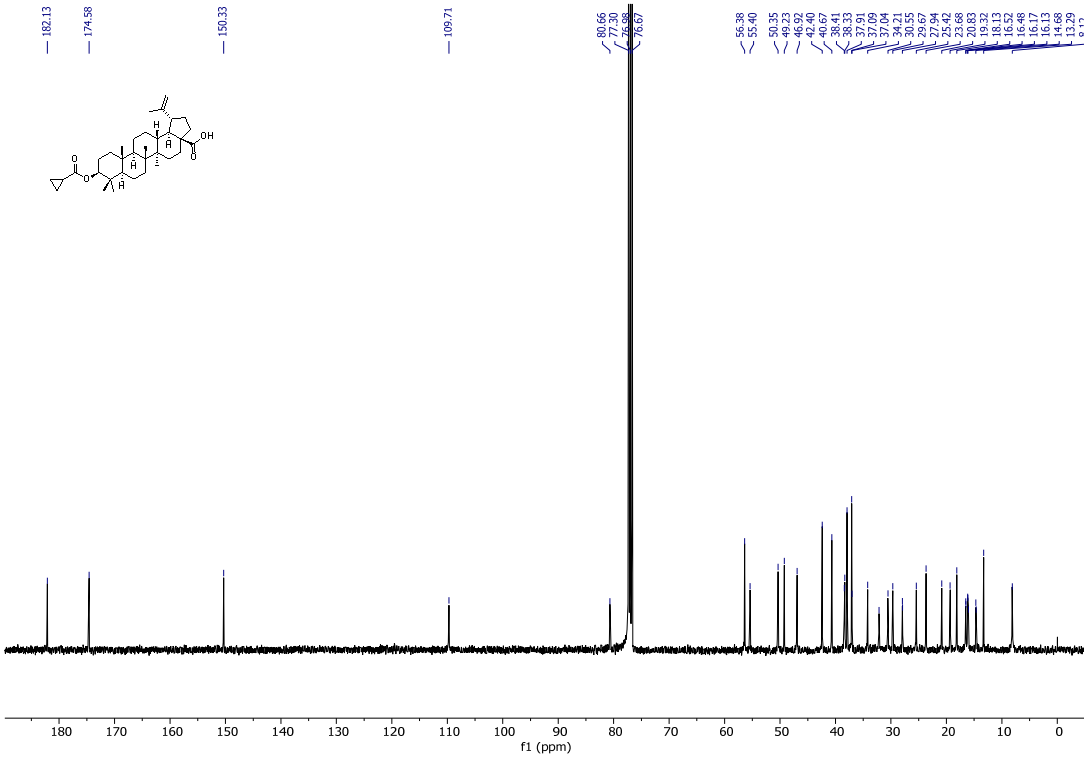


Figure S4. HSQC spectrum (400 MHz) of 3-*O*-cyclopropanoyl-betulinic acid (**4**) in $CDCl_3$

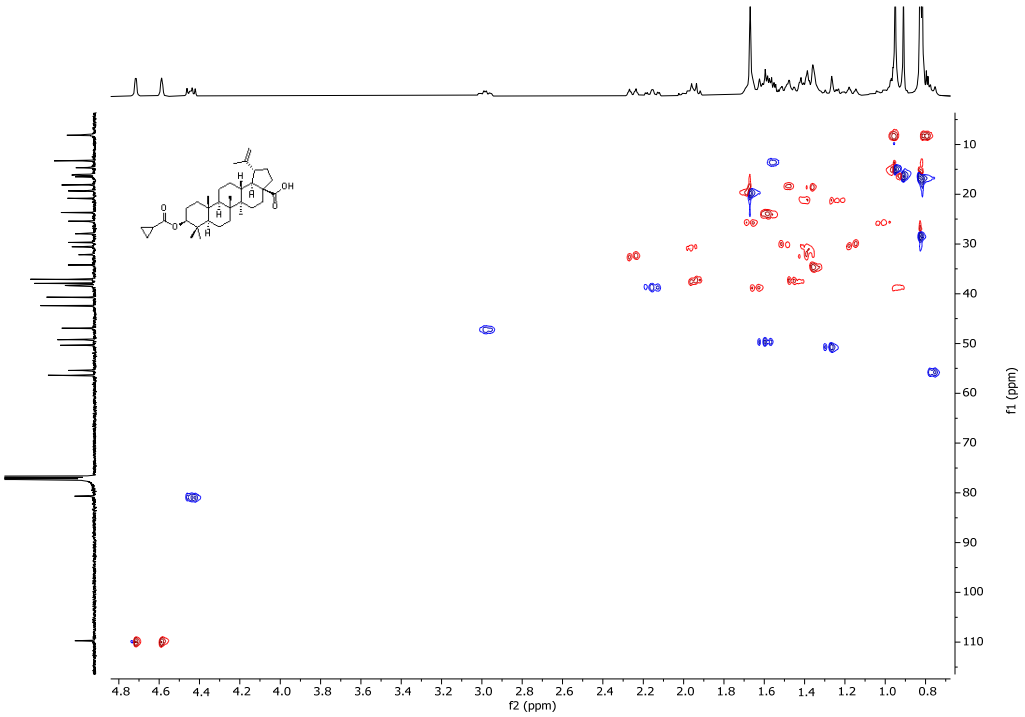


Figure S5. HMBC spectrum (400 MHz) of 3-*O*-cyclopropanoyl-betulinic acid (**4**) in $CDCl_3$

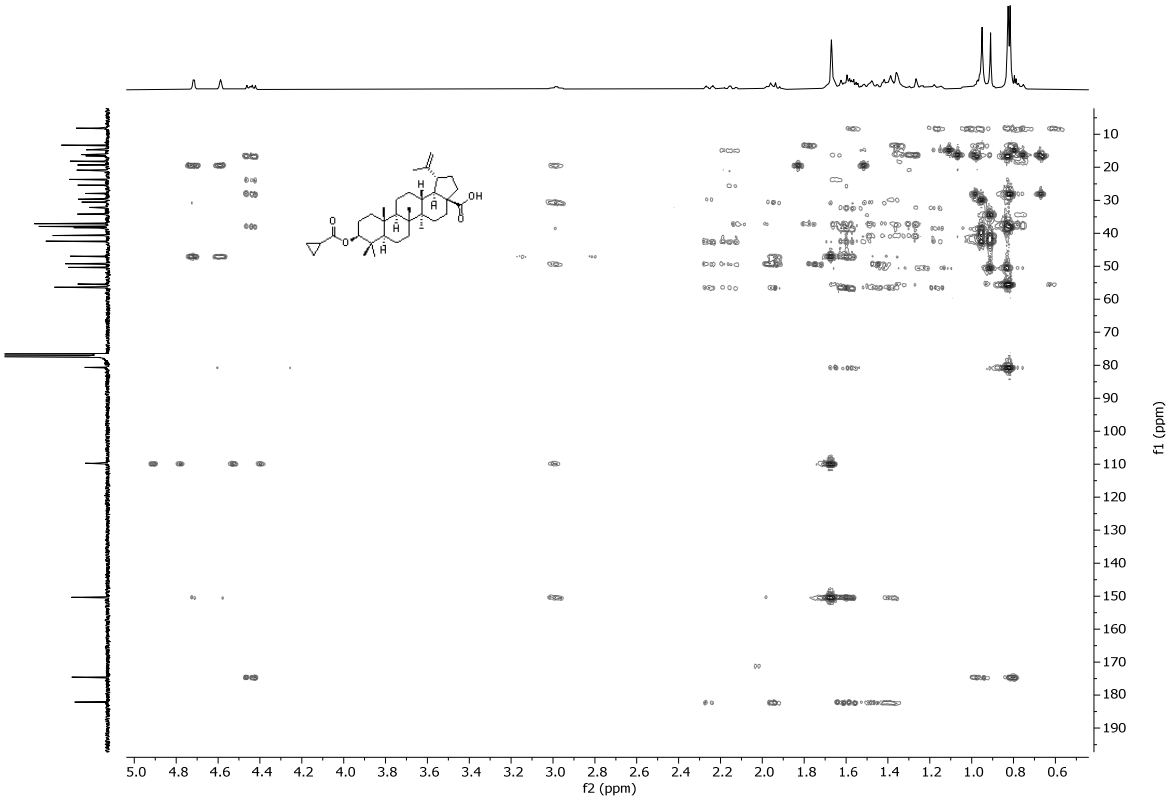


Figure S6. ^{1}H NMR spectrum (400 MHz) of 3-*O*-methoxycarbonyl-betulinic acid (**6**) in $CDCl_3$

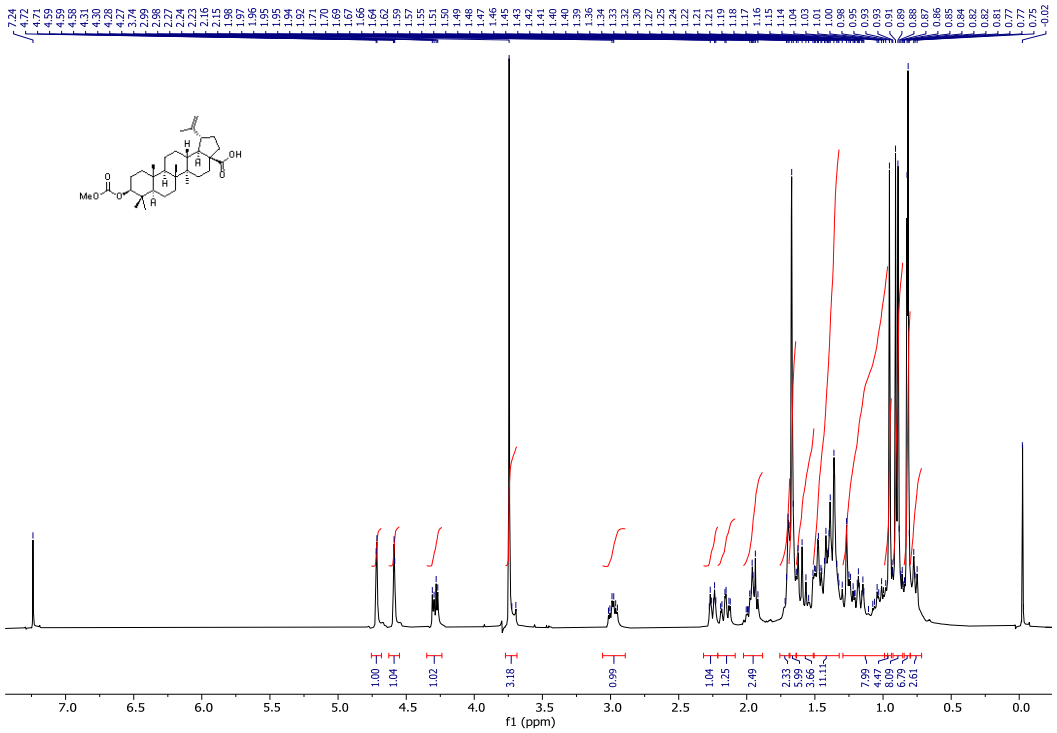


Figure S7. ^{13}C NMR spectrum (100 MHz) of 3-*O*-methoxycarbonyl-betulinic acid (**6**) in $CDCl_3$

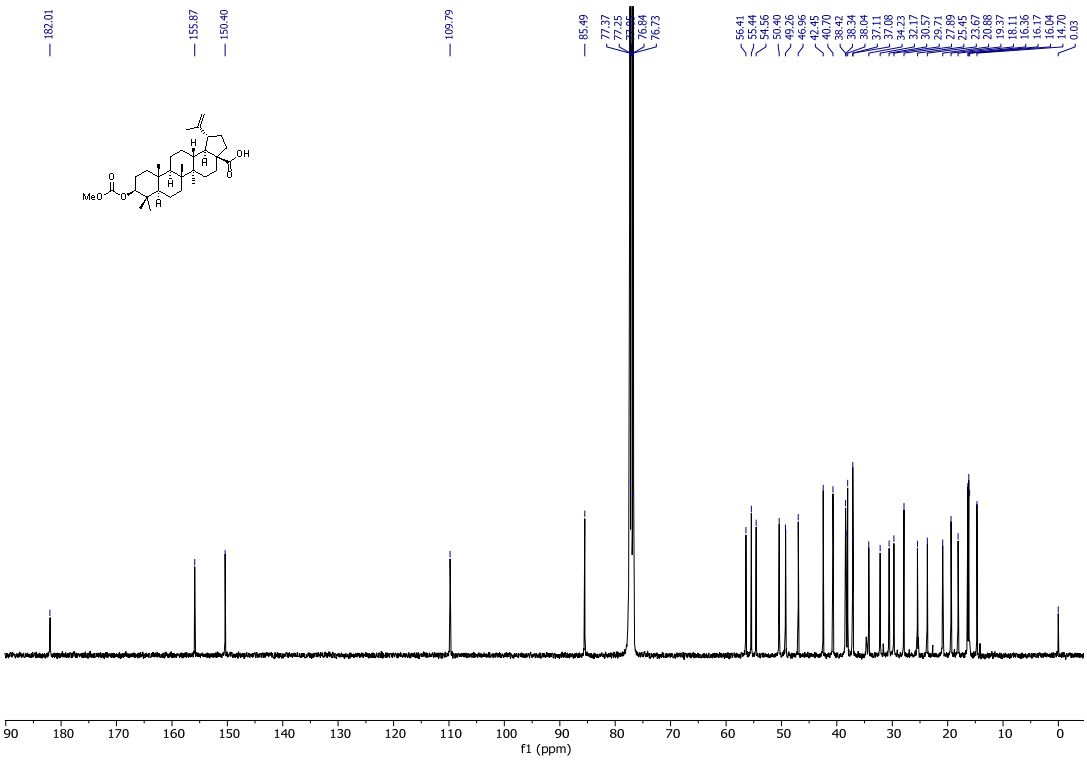


Figure S8. ^{1}H NMR spectrum (400 MHz) of 28-*O*-5-methyl-2-furoyl-betulinic anhydride (**8**) in $CDCl_3$

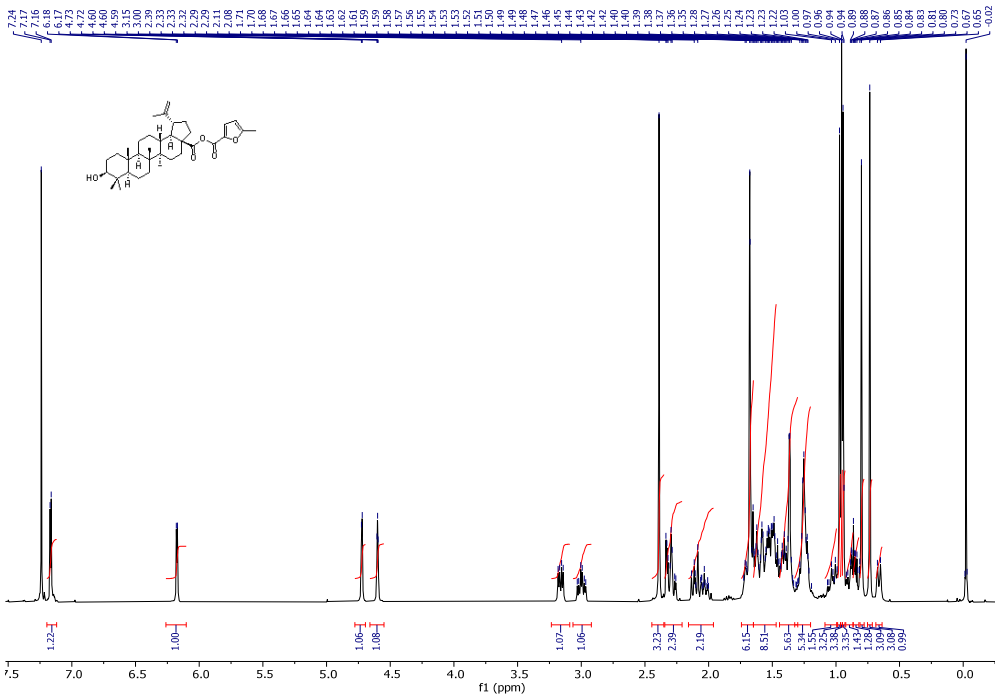


Figure S9. ^{13}C NMR spectrum (100 MHz) of 28-*O*-5-methyl-2-furoyl-betulinic anhydride (**8**) in $CDCl_3$

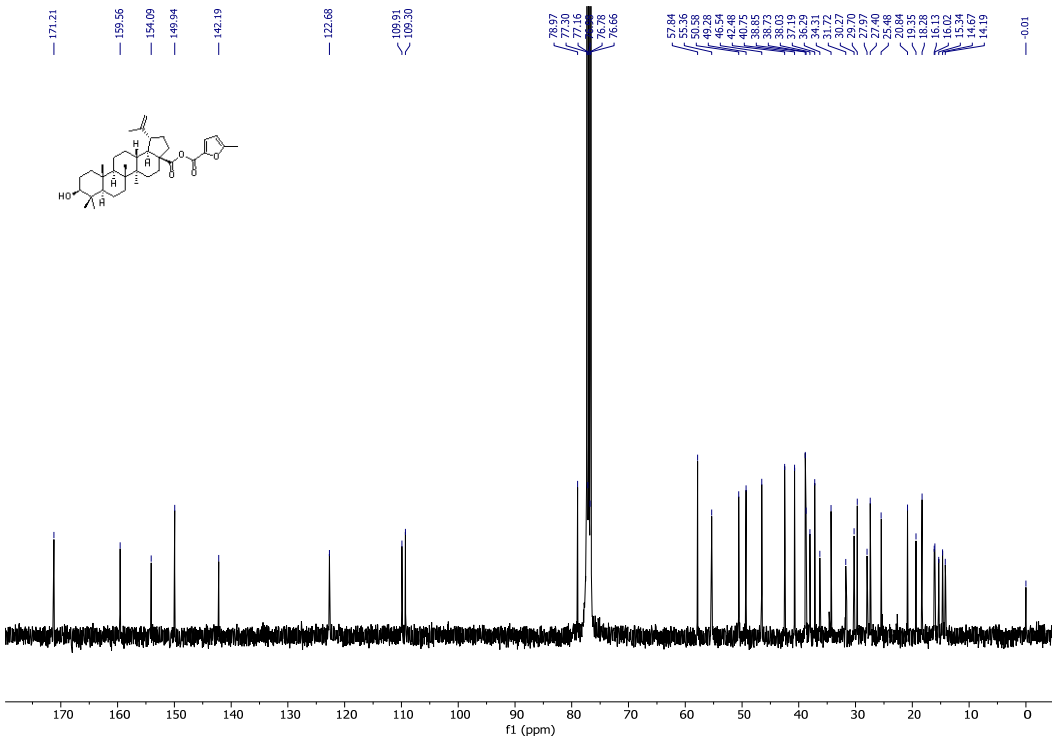


Figure S10. $^{1}H–^{1}H$ COSY spectrum (400 MHz) of 28-*O*-5-methyl-2-furoyl-betulinic anhydride (**8**) in $CDCl_3$

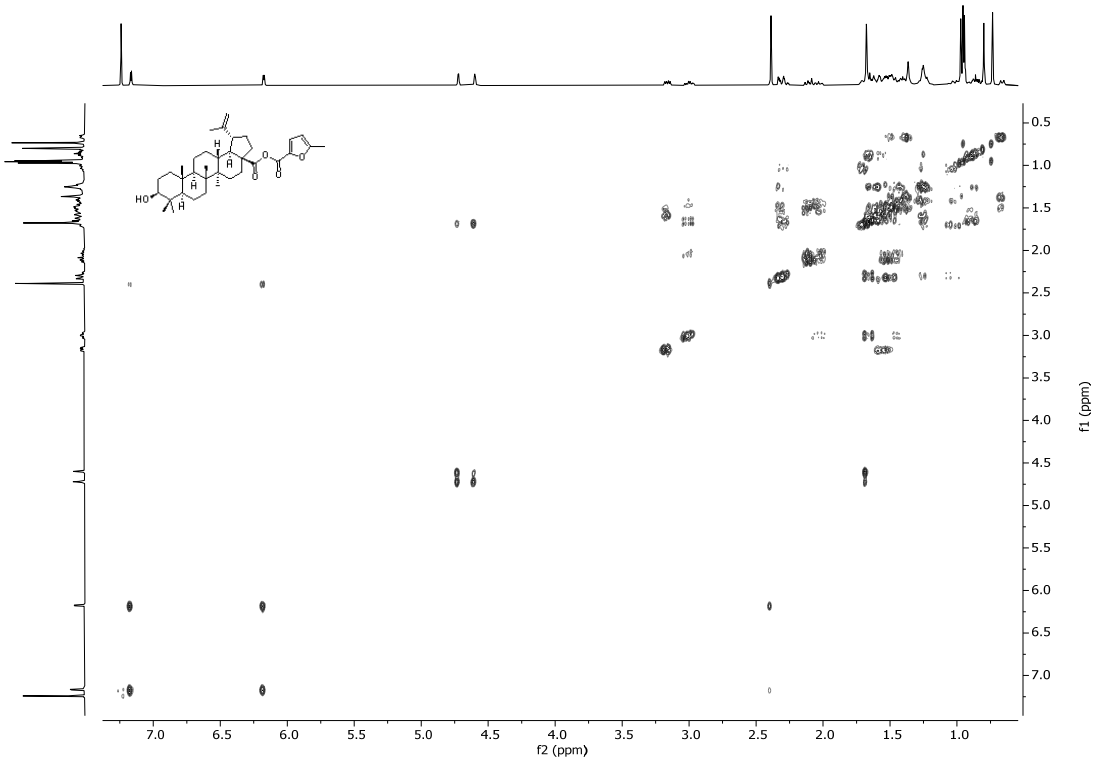


Figure S11. HSQC spectrum (400 MHz) of 28-*O*-5-methyl-2-furoyl-betulinic anhydride (**8**) in $CDCl_3$

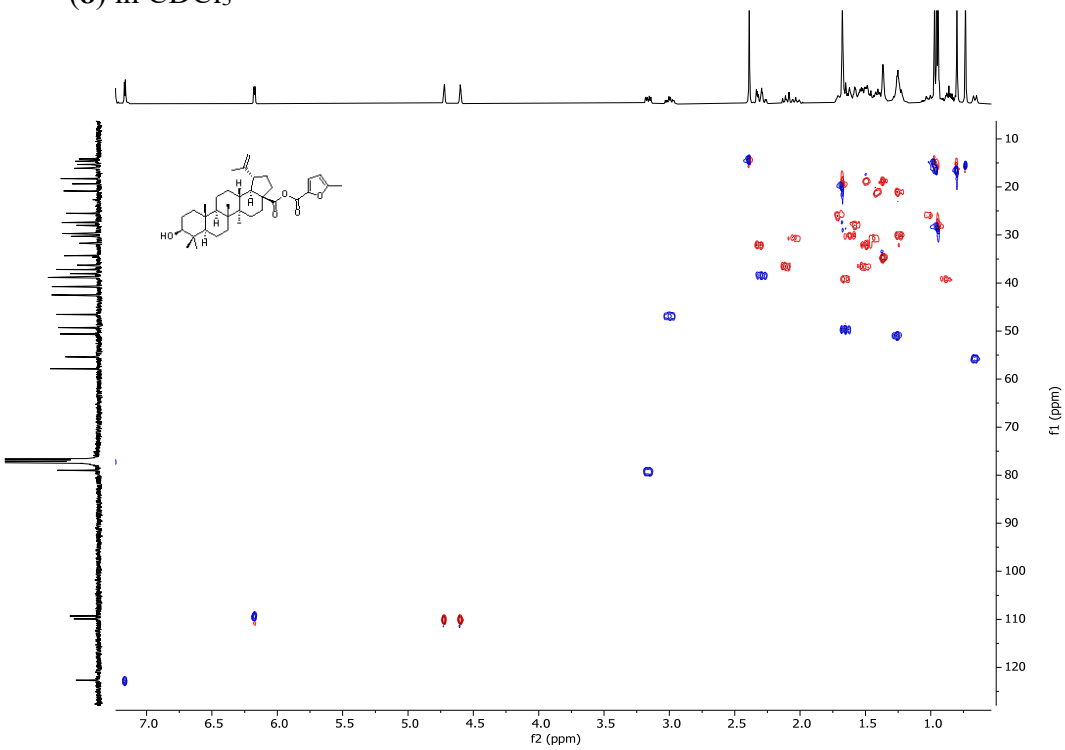


Figure S12. HMBC spectrum (400 MHz) of 28-*O*-5-methyl-2-furoyl-betulinic anhydride (**8**) in $CDCl_3$

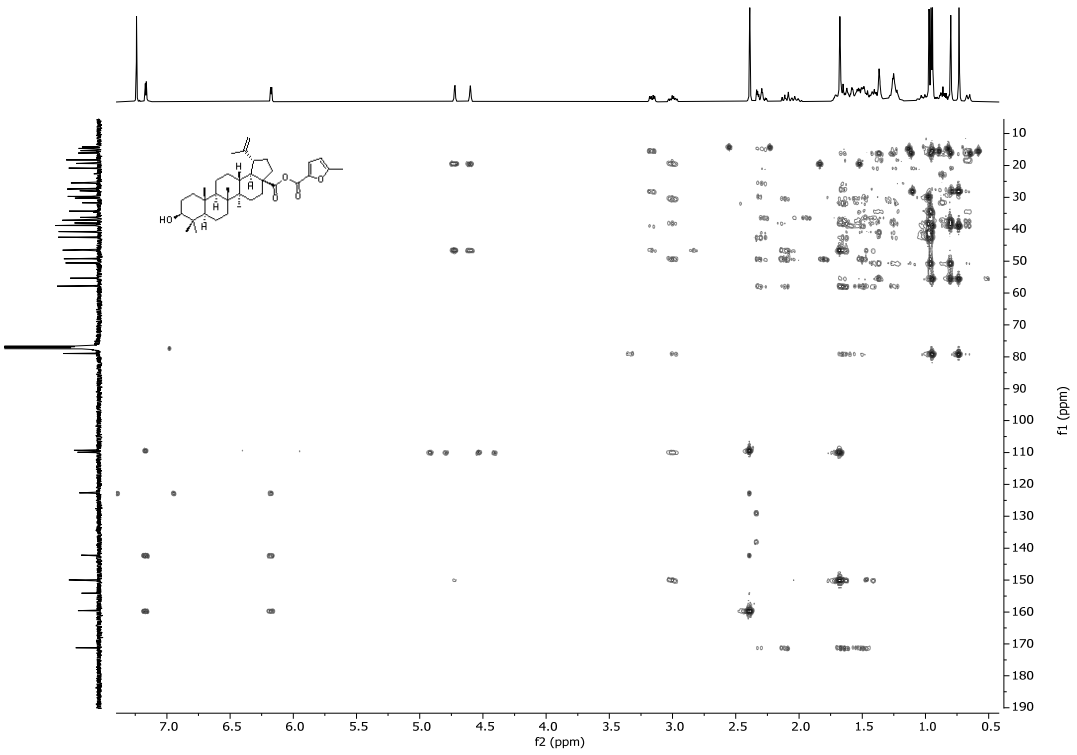


Figure S13. ^1H NMR spectrum (400 MHz) of 28-*O*-(3′,4′,5′-trimethoxy)-benzoyl-betulinic anhydride (**9**) in $CDCl_3$

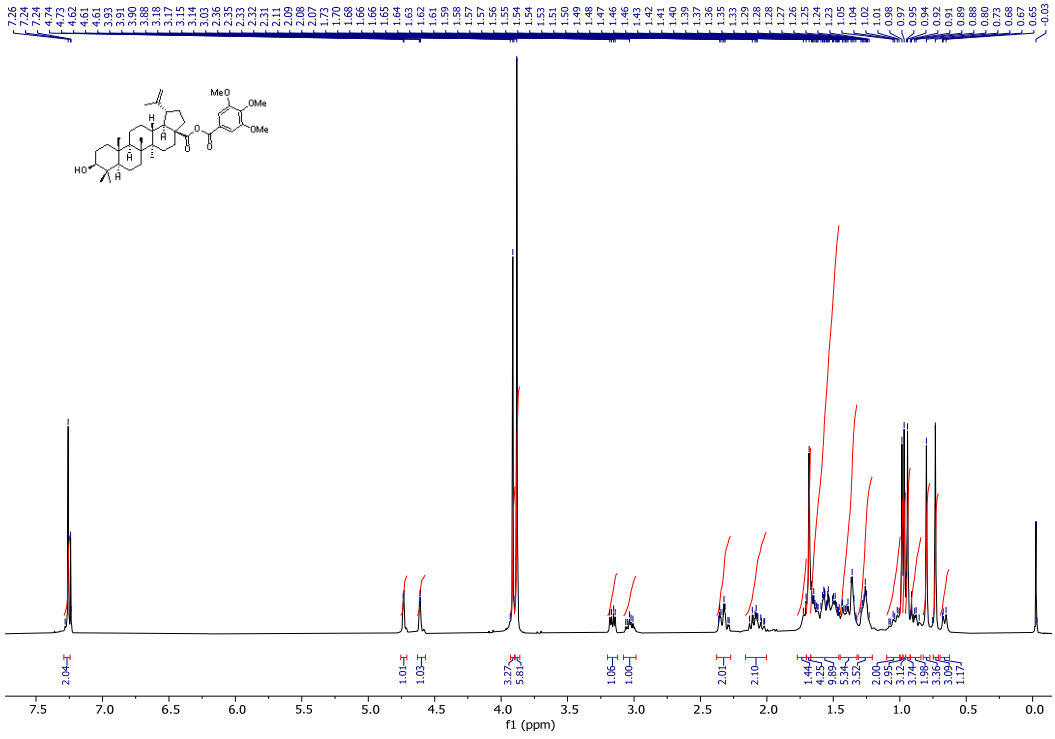


Figure S14. ^{13}C NMR spectrum (100 MHz) of 28-*O*-(3′,4′,5′-trimethoxy)-benzoyl-betulinic anhydride (**9**) in $CDCl_3$

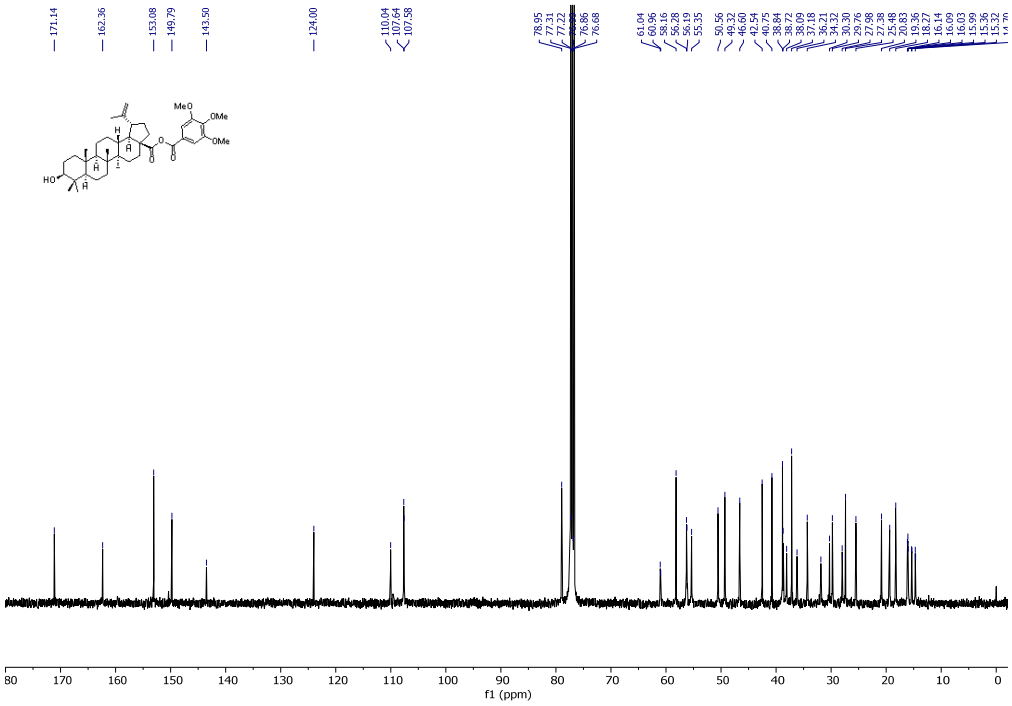


Figure S15. HSQC spectrum (400 MHz) of 28-*O*-(3′,4′,5′-trimethoxy)-benzoyl-betulinic anhydride (**9**) in $CDCl_3$

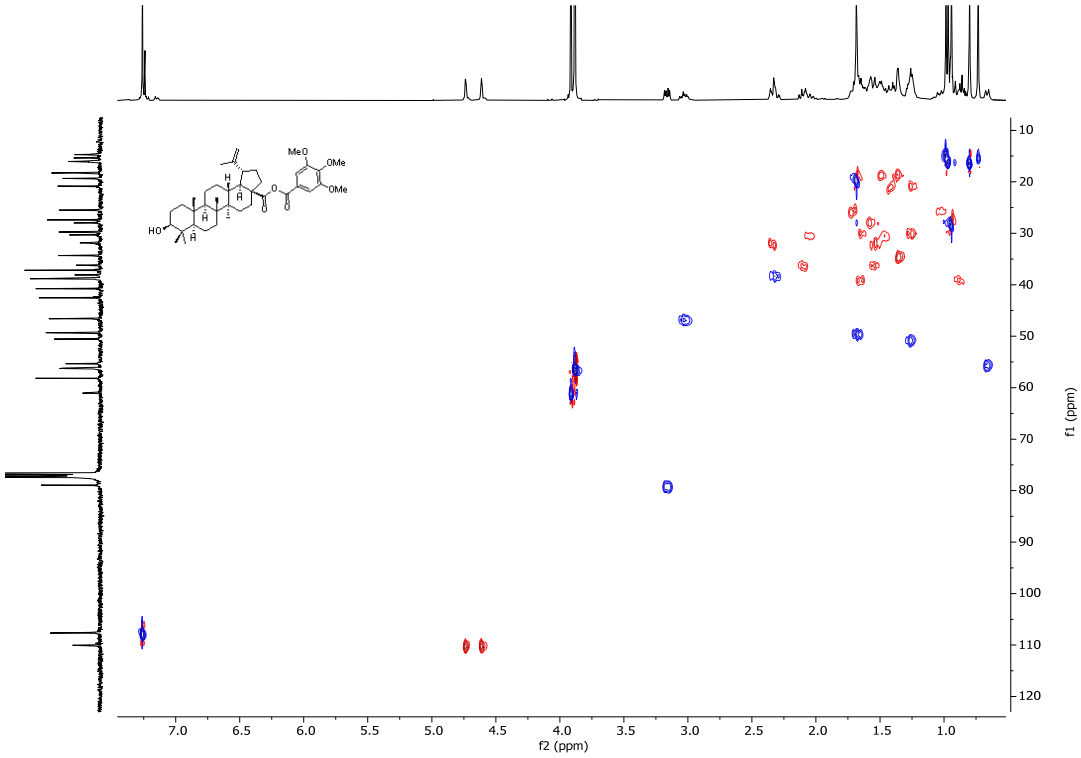


Figure S16. HMBC spectrum (400 MHz) of 28-*O*-(3′,4′,5′-trimethoxy)-benzoyl-betulinic anhydride (**9**) in $CDCl_3$

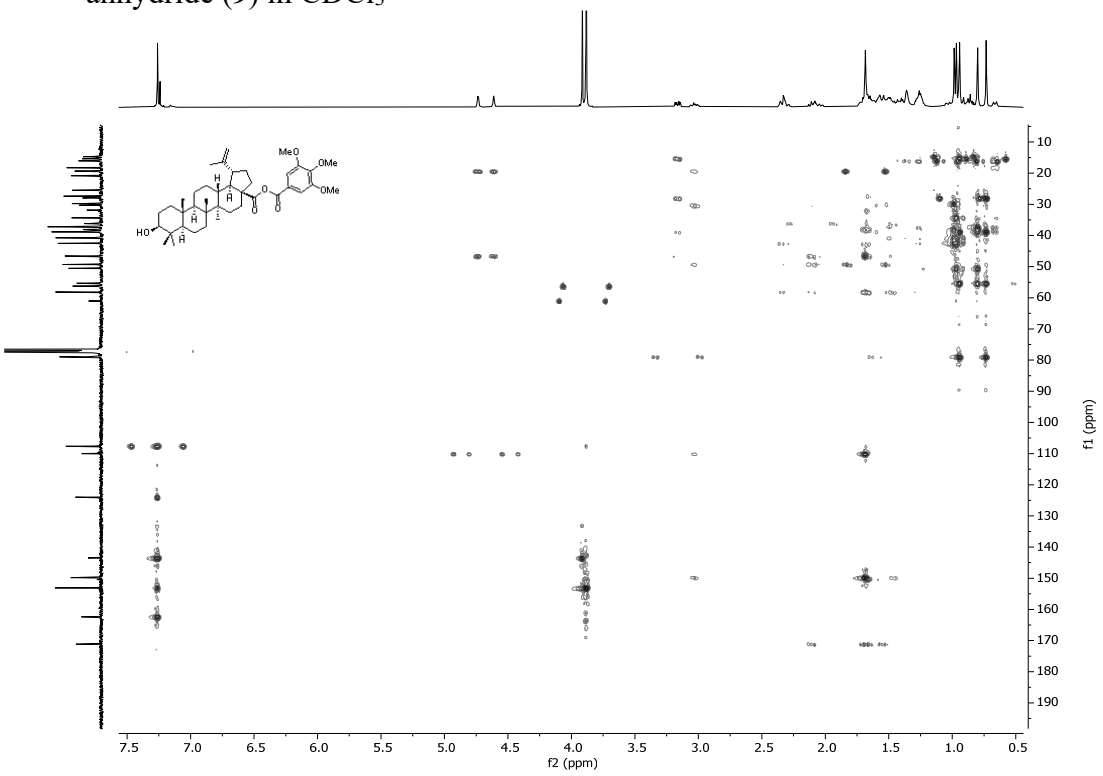


Figure S17. ^1H NMR spectrum (400 MHz) of 3,28-di-*O*-tetrahydropyranyl-betulin (**17**) in $CDCl_3$

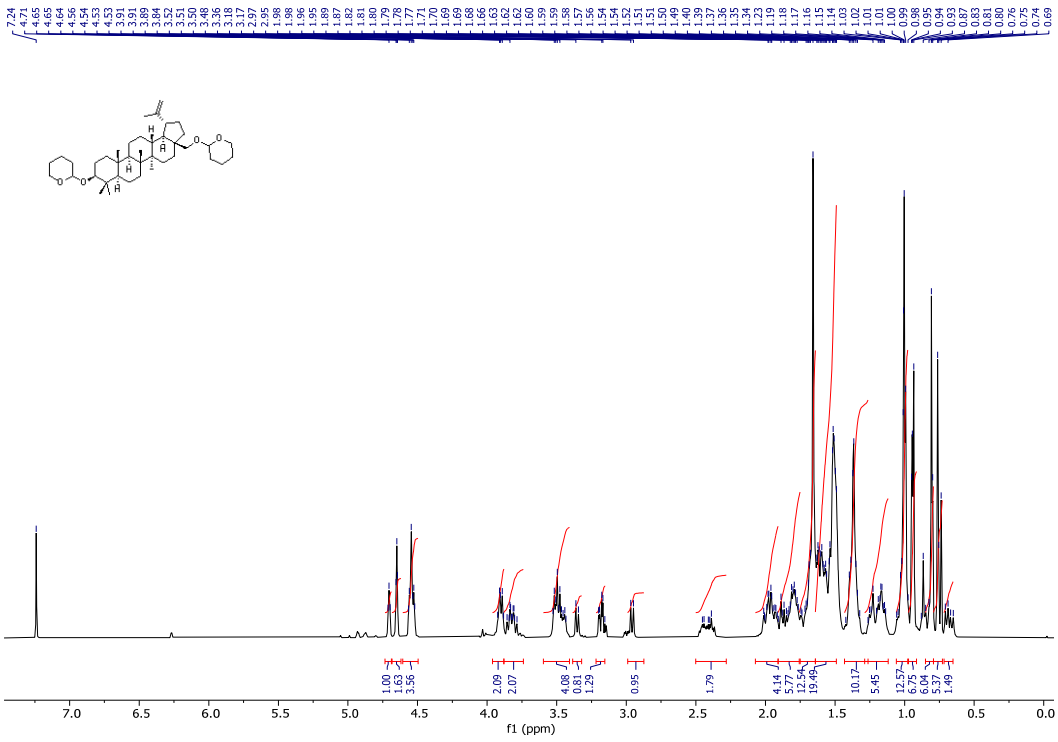


Figure S18. ^{1}H NMR spectrum (400 MHz) of 3-*O*-cyclopropanoyl-betulin (**20**) in $CDCl_3$

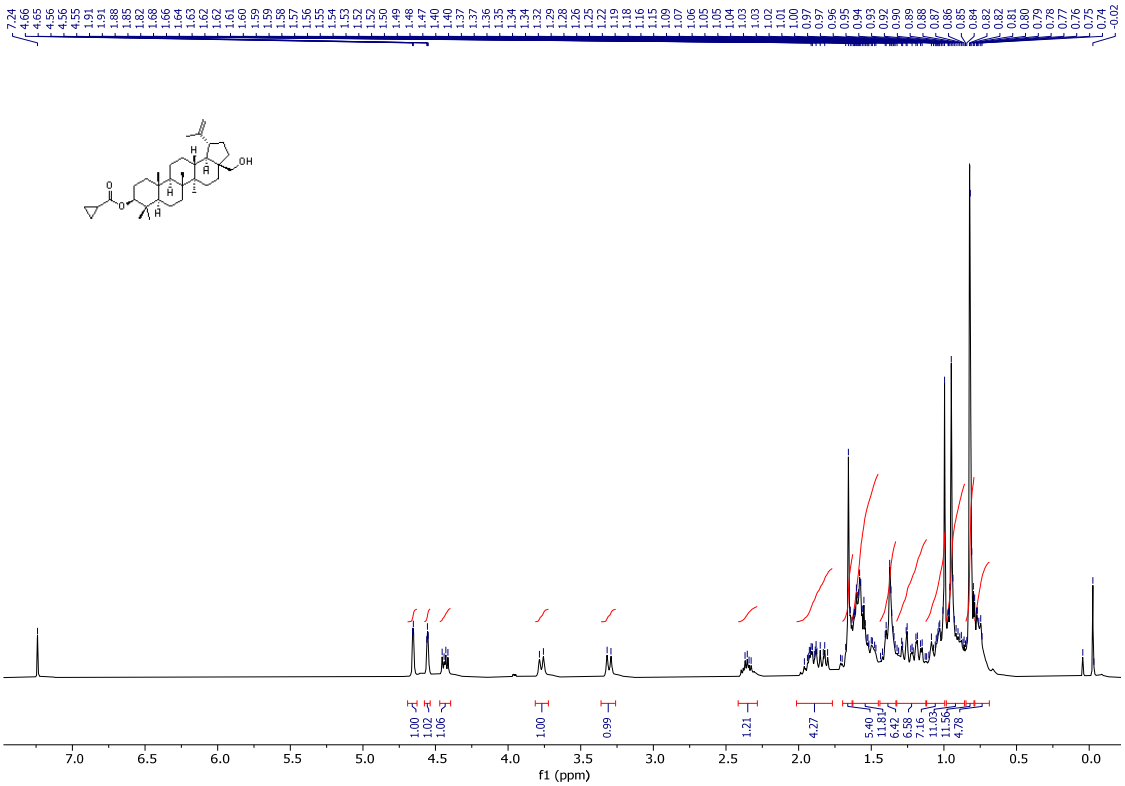


Figure S19. ^{1}H NMR spectrum (400 MHz) of 3-*O*-cyclopropanoyl-betulin (**20**) in $CDCl_3$

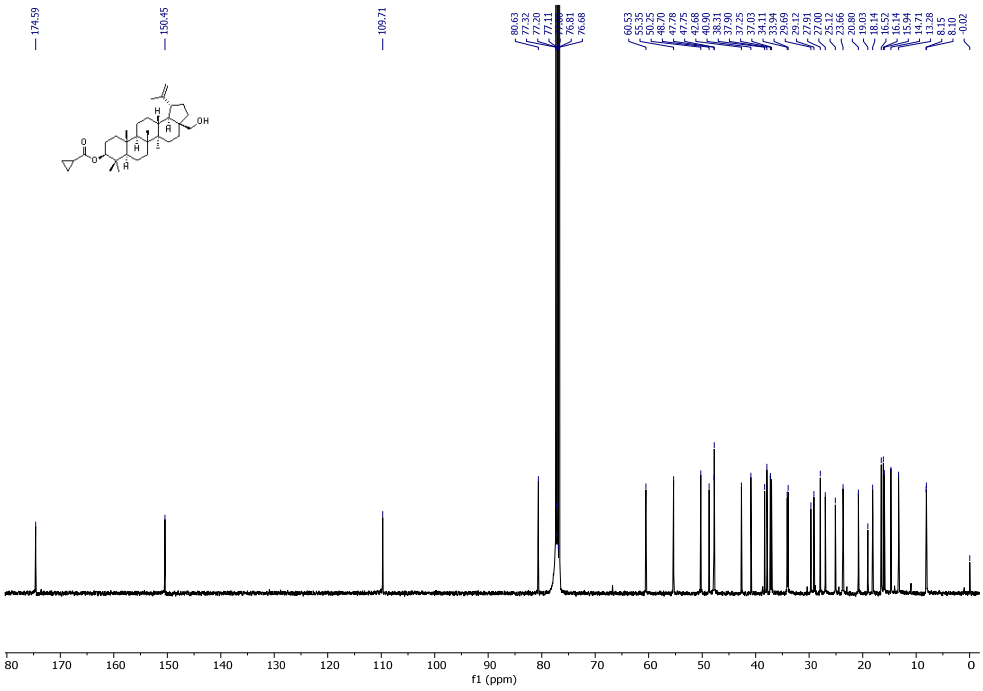


Figure S20. ^{1}H NMR spectrum (400 MHz) of 28-*O*-(5-methyl-2-furoyl)betulin (**23**) in $CDCl_3$

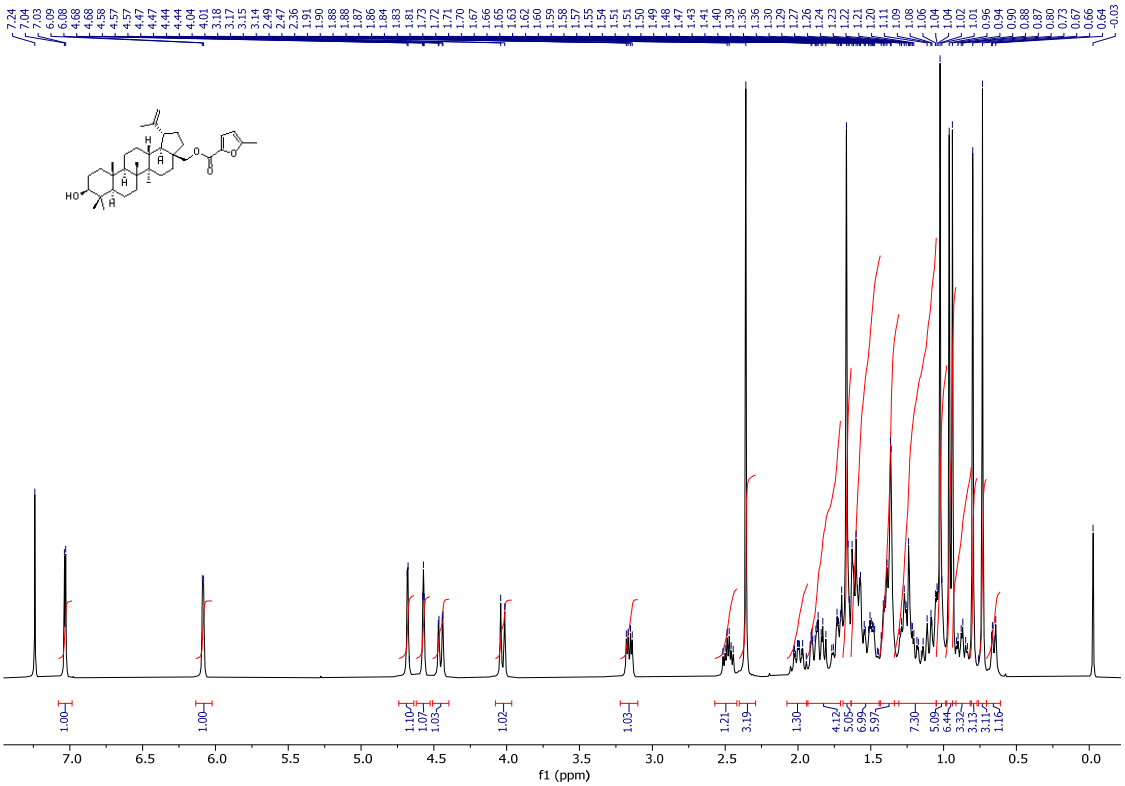


Figure S21. ^{13}C NMR spectrum (100 MHz) of 28-*O*-(5-methyl-2-furoyl)betulin (**23**) in $CDCl_3$

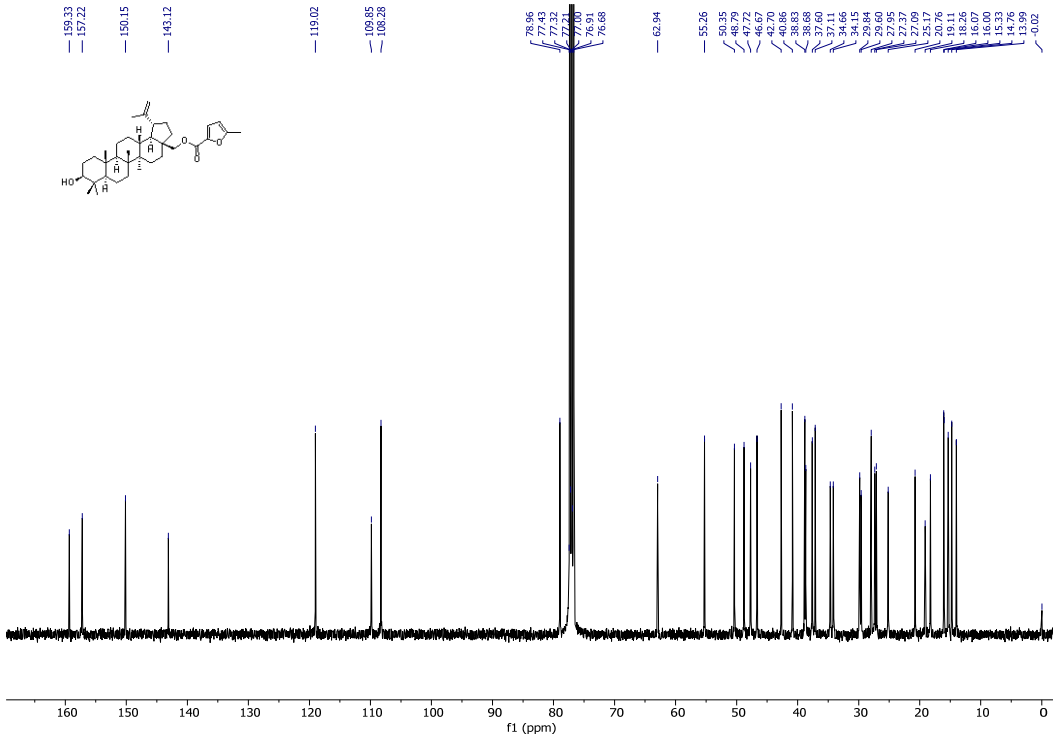


Figure S22. HSQC spectrum (400 MHz) of 28-*O*-(5-methyl-2-furoyl)betulin (**23**) in $CDCl_3$

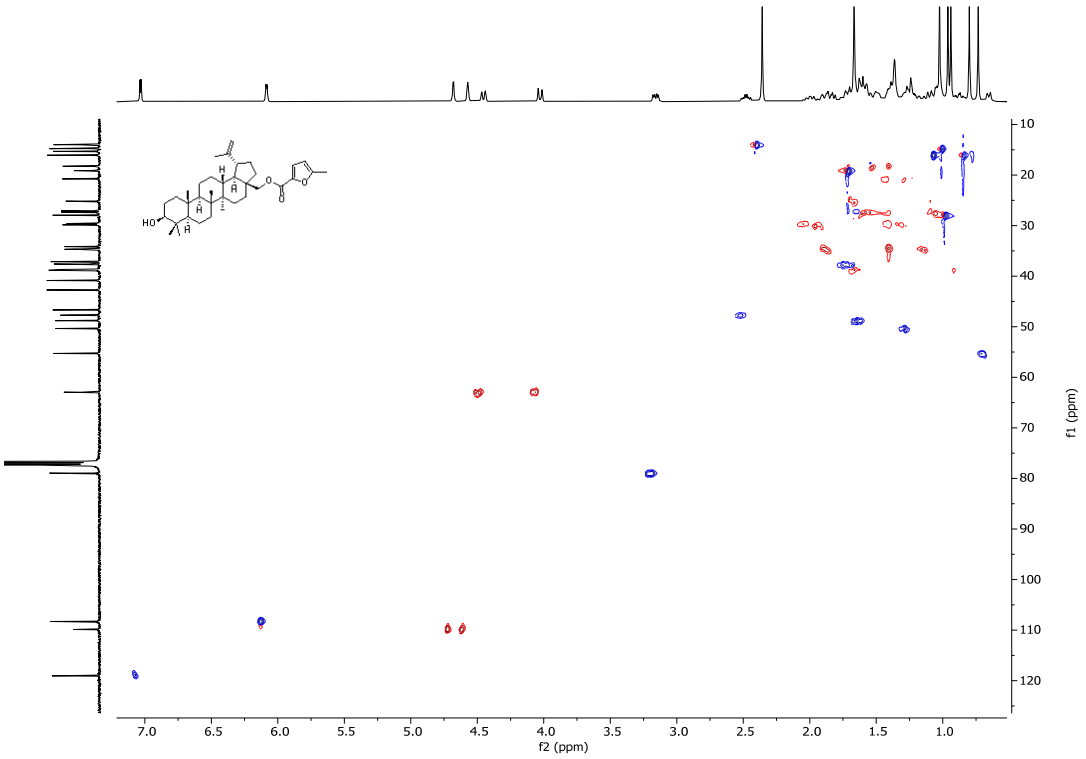


Figure S23. HMBC spectrum (400 MHz) of 28-*O*-(5-methyl-2-furoyl)betulin (**23**) in $CDCl_3$

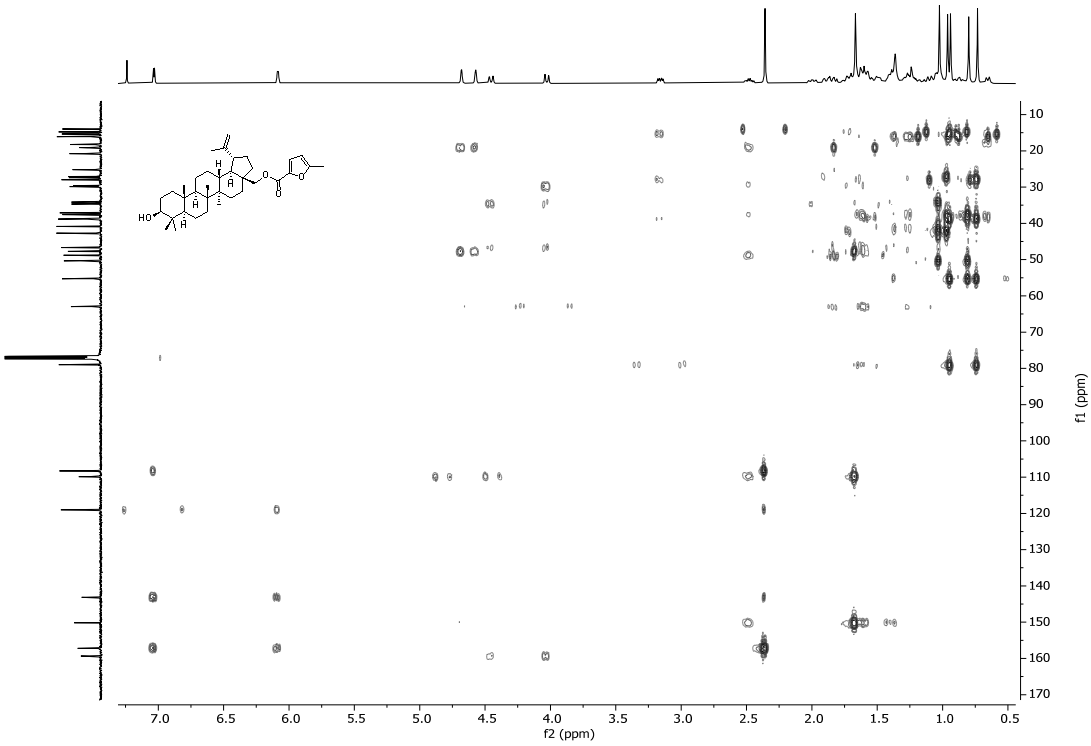


Figure S24. ^{1}H NMR spectrum (400 MHz) of 28-*O*-(2-thiophenecarboxyloyl)betulin (**24**) in $CDCl_3$

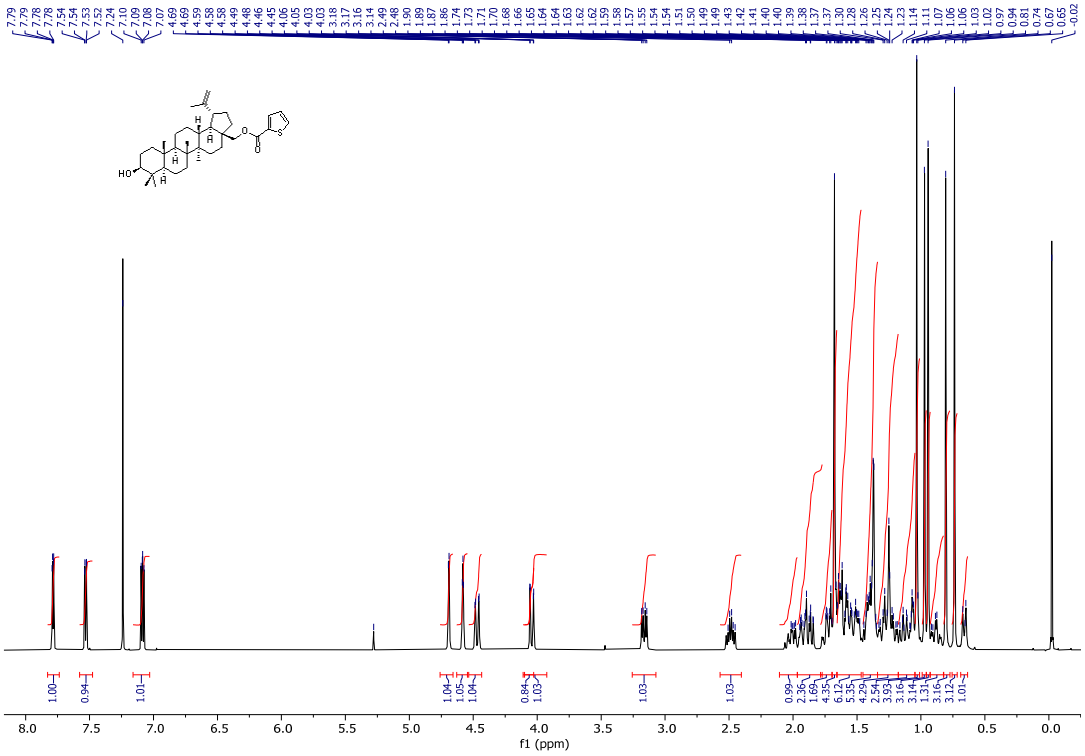


Figure S25. ^{13}C NMR spectrum (100 MHz) of 28-*O*-(2-thiophenecarboxyloyl)betulin (**24**) in $CDCl_3$

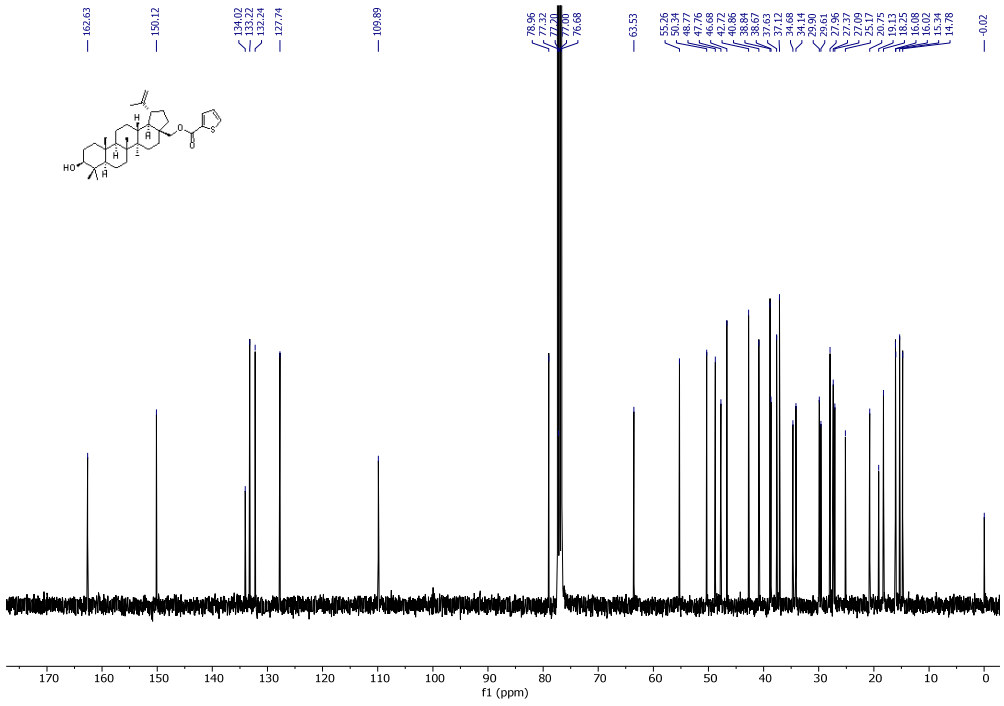


Figure S26. HSQC spectrum (400 MHz) of 28-*O*-(2-thiophenecarboxyloyl)betulin (**24**) in $CDCl_3$

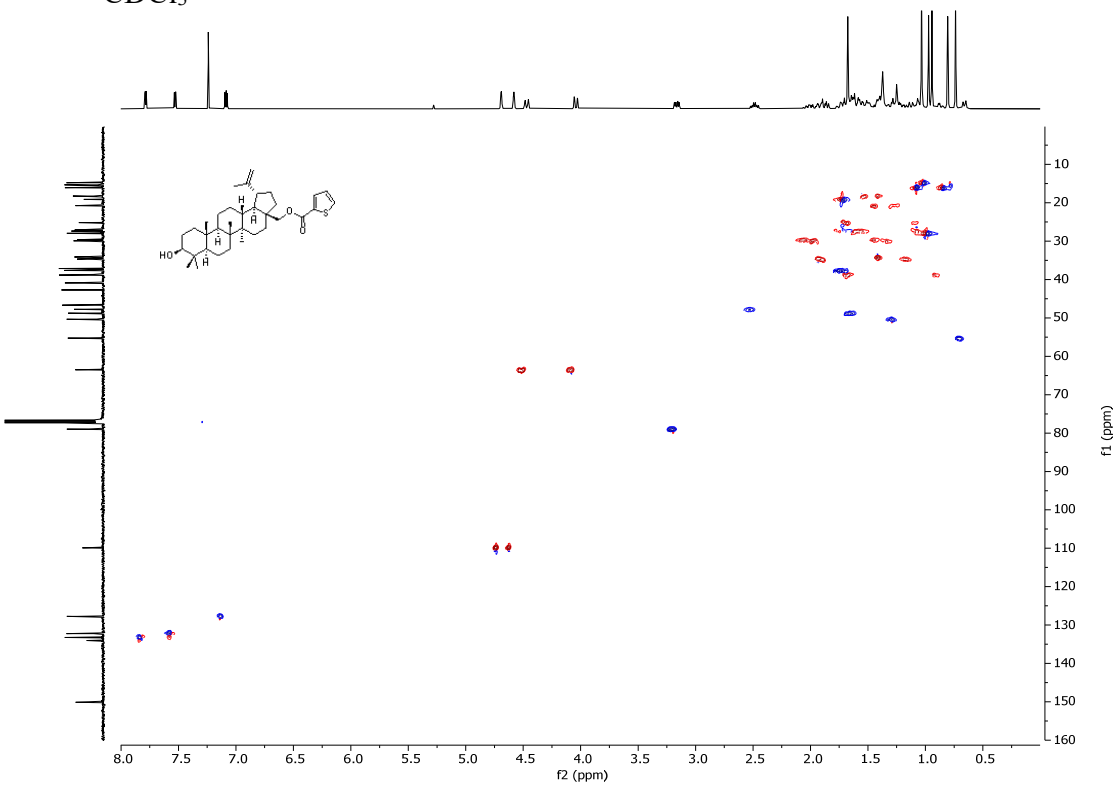


Figure S27. HMBC spectrum (400 MHz) of 28-*O*-(2-thiophenecarboxyloyl)betulin (**24**) in $CDCl_3$

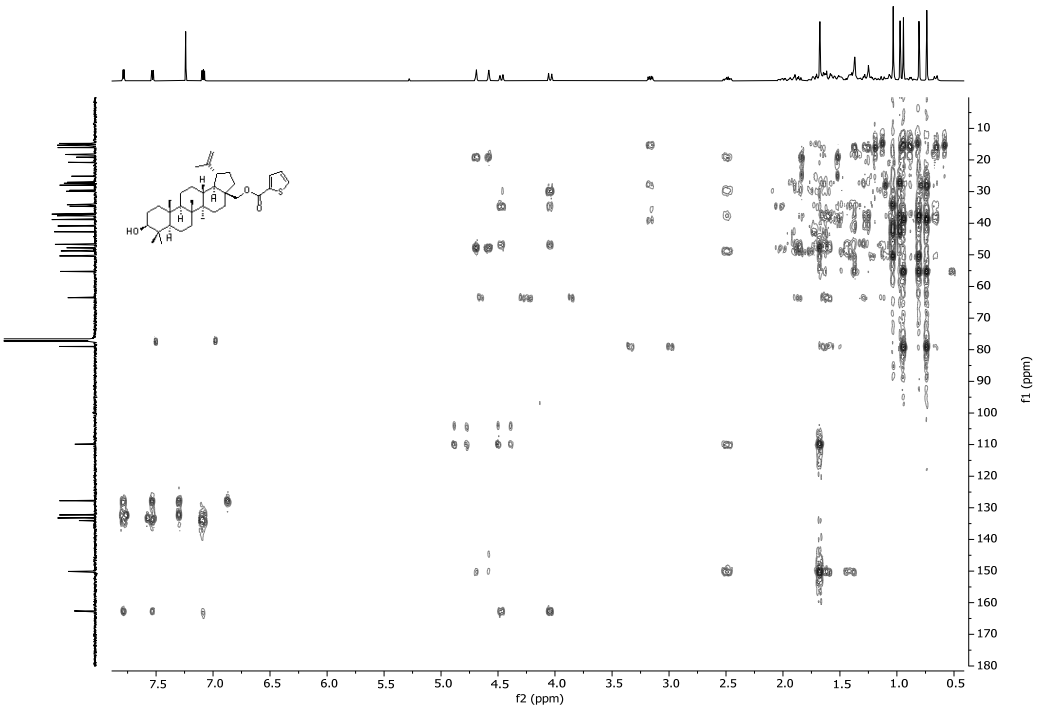


Figure S28. ^{1}H NMR spectrum (400 MHz) of 28-*O*-methoxycarbonyl-betulin (**26**) in $CDCl_3$

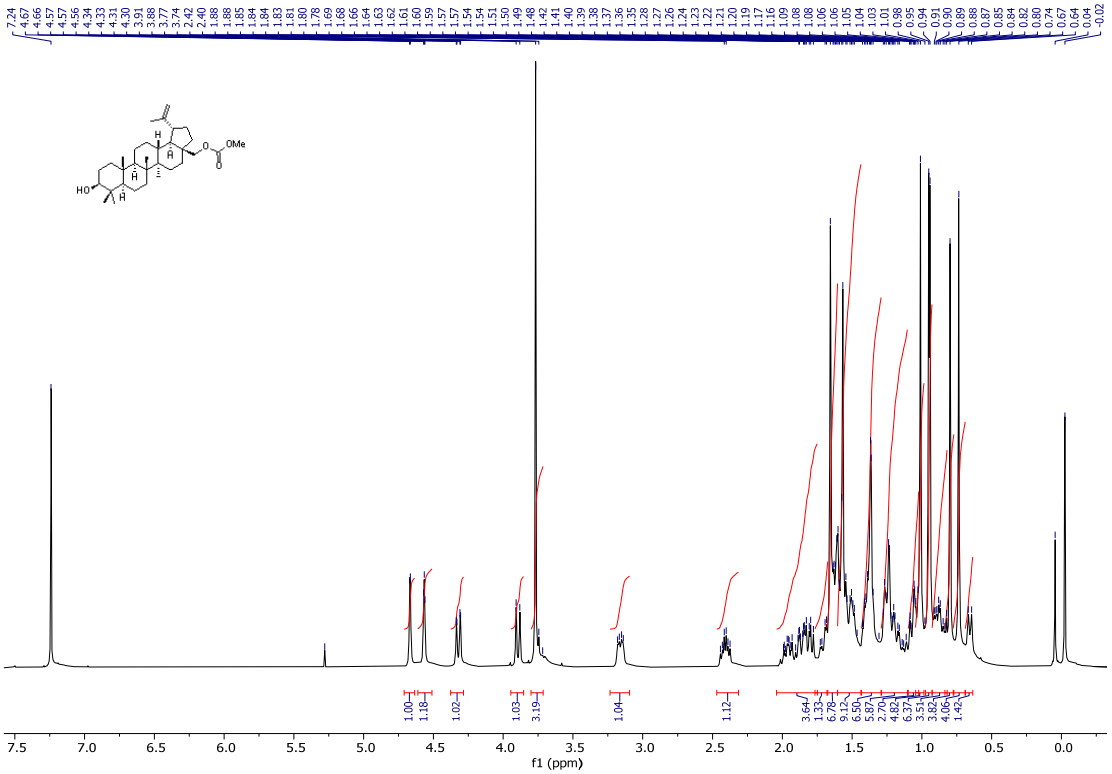


Figure S29. ^{13}C NMR spectrum (100 MHz) of 28-*O*-methoxycarbonyl-betulin (**26**) in $CDCl_3$

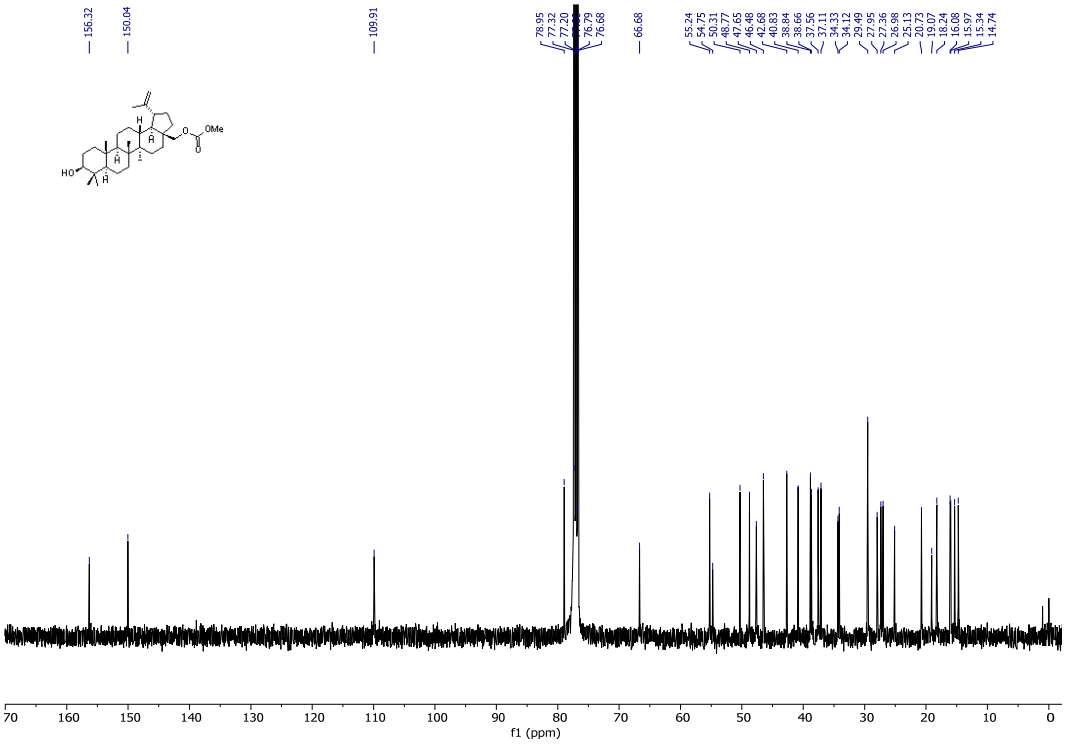


Figure S30. HSQC spectrum (400 MHz) of 28-*O*-methoxycarbonyl-betulin (**26**) in $CDCl_3$

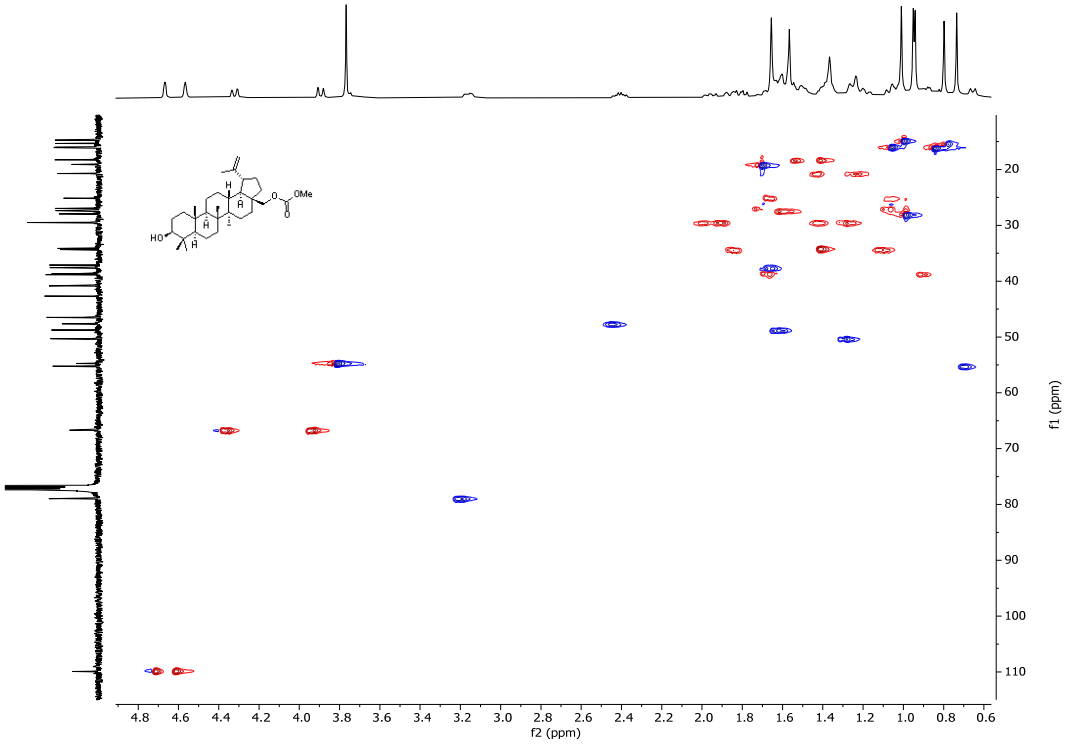


Figure S31. HMBC spectrum (400 MHz) of 28-*O*-methoxycarbonyl-betulin (**26**) in $CDCl_3$

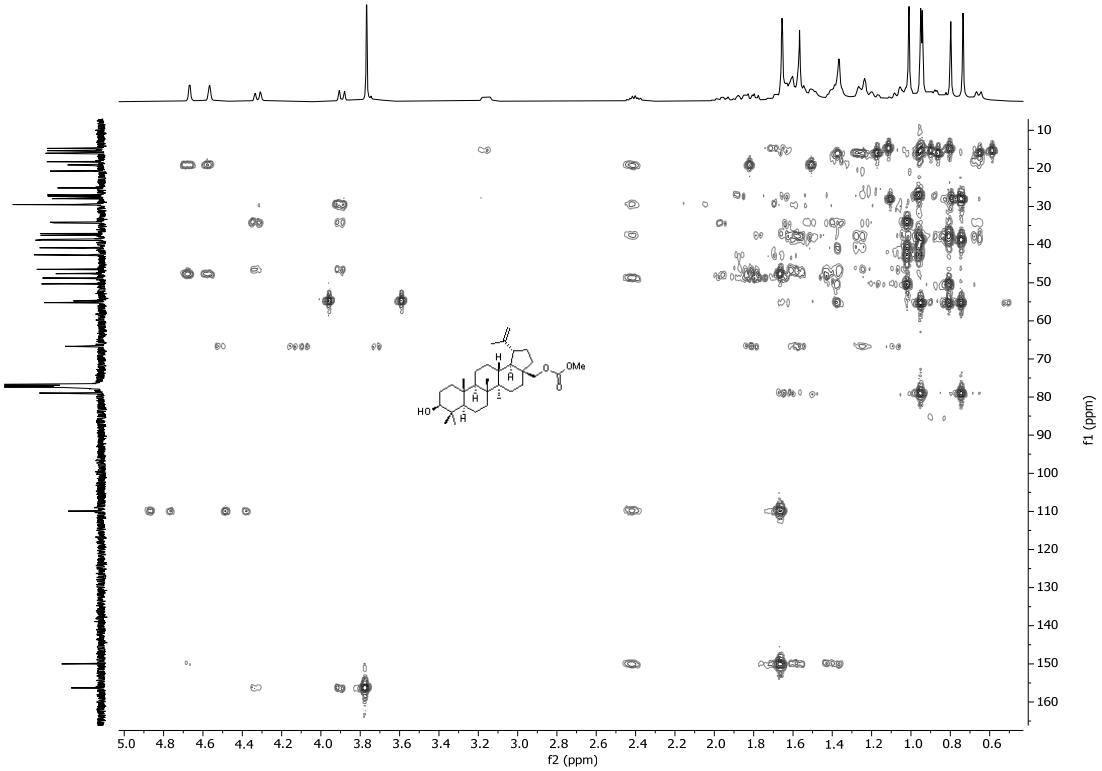
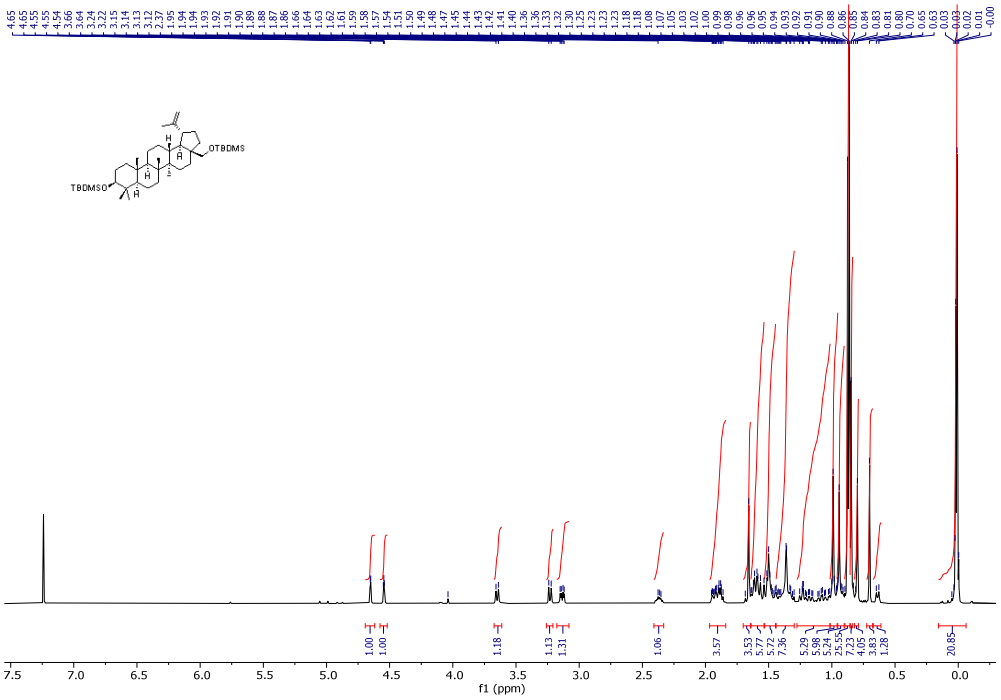


Figure S32. ^{1}H NMR spectrum (400 MHz) of 3,28-di-*O*-*tert*-butyldimethtysilylbetulin (**27**) in $CDCl_3$



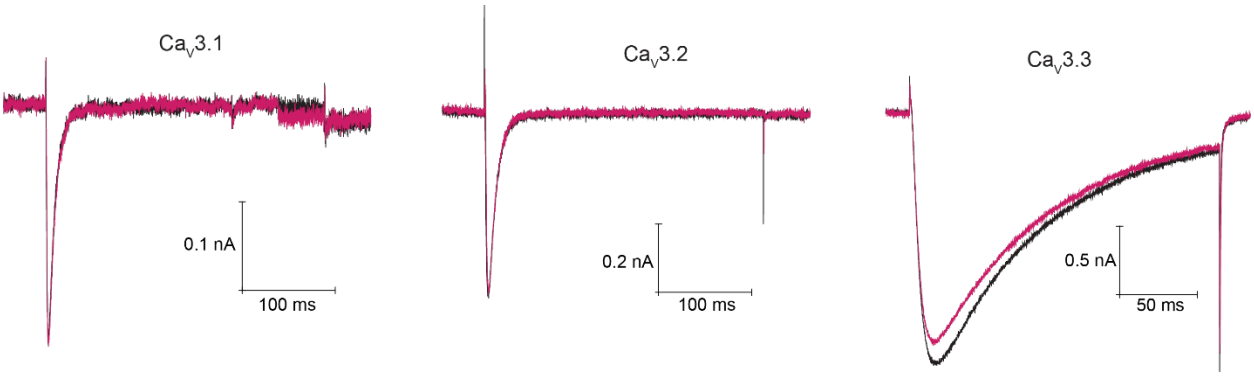


Figure S33. Acute treatment with betulinic acid analog **8** does not affect T-type currents expressed in HEK cells. Representative traces before (black) and after (pink) application of 20 μM of NPC-1-11 recorded from HEK cells transiently expressing CaV3.1, CaV3.2 or CaV3.3 channels as indicated. Currents were evoked from -100 to -30 mV for 100 ms.

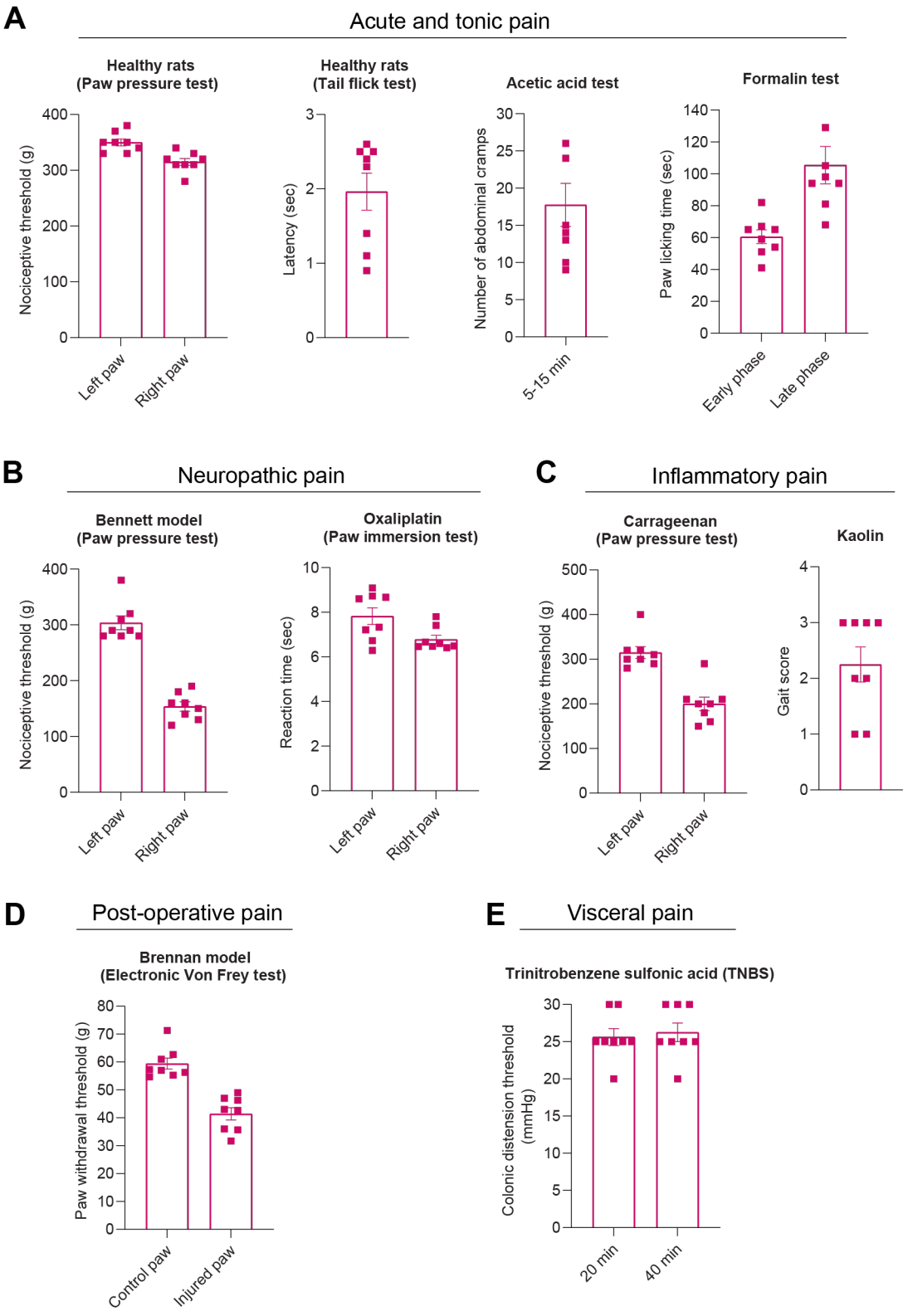


Figure S34. Raw behavior data of the effects of a single administration (0.4 µg/µl, i.t.) Analog **8** in ALGOGram™. (A) Acute and tonic pain area: in healthy rats, nociceptive threshold (g) and

latency (sec) were determined using the paw pressure test and the tail flick test. The cut off was 680 g and 10 sec respectively. For the acetic acid test and formalin test, the number of abdominal cramps and paw licking time (sec) were measured. (B) Neuropathic pain area: In the Bennet model of peripheral mononeuropathy, paw pressure test was employed to assess nociceptive threshold (g; cut off: 680 g). For oxaliplatin-induced neuropathy, paw immersion test was used to measure the reaction time (sec). (C) Inflammatory pain area: In carrageenan-induced mechanical hyperalgesia, paw pressure test was applied to measure nociceptive threshold (g; cut off: 680 g). For the kaolin-induced arthritis model, gait score was reported. (D) Post-operative pain area: For the Brennan model of incisional pain, paw withdrawal threshold was measured with electronic Von Frey test. (E) Visceral pain area: Trinitrobenzene sulfonic acid (TNBS) induced chronic colonic hypersensitivity and colonic distension threshold was assessed (mmHg; cut off: 75 mmHg)

Supplementary References

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