Design, Synthesis, and Biological Evaluation of Novel Alkenylthiophenes as Potent and Selective CB1 Cannabinoid Receptor Antagonists

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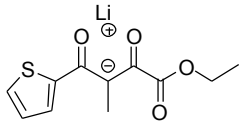
Supporting Information

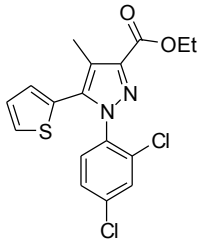
Synthetic Chemistry:

Lithium Salt of Ethyl 2,4-Dioxo-3-methyl-4-thiophenylbutanoate (3)



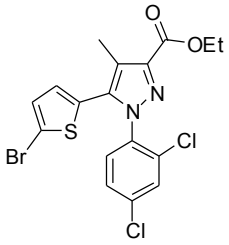
To a magnetically stirred solution of lithium *bis*-(trimethylsilyl)amide (48.0 mL, 1.0 M solution in THF, 48.00 mmol) in diethyl ether (20 mL) was added a solution of 1-(2-thiophene)-1-propanone (5.1 mL, 40.01 mmol) in dry diethyl ether (5 mL) at -78 °C. After the mixture was stirred at the same temperature for additional 45 min, diethyl oxalate (7.1 mL, 52.03 mmol) was added to the mixture. The reaction mixture was allowed to warm to the room temperature and stirred for 16 h. The precipitate was filtered, washed with diethyl ether, and dried under vacuum to afford the lithium salt **3** (8.37 g, 85% yield).

1-(2,4-dichloro-phenyl)-4-methyl-5-thiophen-2-yl-1*H*-pyrazole-3-carboxylic acid ethyl ester (4)



To a solution of lithium salt **3** (2.98 g, 12.10 mmol) in ethanol (35 mL) was added 2,4-dichlorophenylhydrazine hydrochloride (3.02 g, 14.30 mmol) in one portion at room temperature. The resulting mixture was kept stirring under a nitrogen atmosphere at the same temperature for 16 h. The precipitate was filtered, washed with ethanol and diethyl ether, and then dried under vacuum to give a light yellow solid (3.27 g, 68%). The crude solid, without further purification, was dissolved in acetic acid (30 mL) and refluxed for 24 h. The reaction mixture was poured into ice water and extracted with ethyl acetate (3 × 20 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated to give the crude residue. Flash chromatography of the residue on silica gel (*n*-hexane/ethyl acetate (9:1)) gave ester **4** (2.40 g, 74%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.1 Hz, 1H), 7.36 (s, 2H), 7.32 (d, *J* = 2.1 Hz, 1H), 7.00 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.89 (d, *J* = 3.6 Hz, 1H), 4.45 (q, *J* = 7.2 Hz, 2H), 2.44 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.7, 142.8, 137.8, 136.3, 136.0, 133.9, 131.0, 129.6, 128.8, 128.5, 127.7, 127.6, 127.2, 119.9, 60.9, 14.4, 9.9; ESMS *m/z*: 381.0 (MH^+).

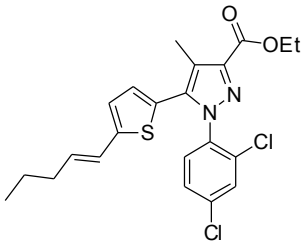
5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (5)



To a magnetically stirred solution of **4** (1.11 g, 2.91 mmol) was added NBS (0.62 g, 3.47 mmol) in acetonitrile (10 mL) in small portions at 0 ^{o}C. The resulting mixture was allowed to warm to room temperature and kept stirring for 16 h. Saturated aqueous sodium sulfite was added to quench the reaction. The solvent of the mixture was first evaporated under reduced pressure followed by extracting the residue with ethyl acetate (3 × 20 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was subjected to purification by flash chromatography on silica gel (*n*-hexane/ethyl acetate (9:1)) to give bromo ester **5** (1.27 g, 95%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 1.8 Hz, 1H), 7.36-7.35 (m, 1H), 7.34 (d, *J* = 1.8 Hz, 1H), 6.96 (d, *J* = 3.9 Hz, 1H), 6.64 (d, *J* = 3.9 Hz, 1H), 4.44 (q, *J* = 7.2 Hz, 2H), 2.42 (s,

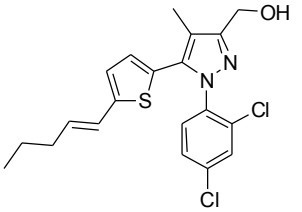
3H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.7, 142.2, 136.1, 135.8, 134.9, 133.0, 130.3, 129.5, 129.3, 128.6, 127.2, 119.5, 114.2, 114.1, 60.2, 13.7, 9.2; ESMS *m/z*: 460.9 (MH^+); Anal. Calcd for $C_{17}H_{13}BrCl_2N_2O_2S$: C 44.37; H 2.85; N 6.09. Found C 44.61; H 3.16; N 6.25.

1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazole-3-carboxylic acid ethyl ester (6)



A mixture of bromo ester **5** (2.28 g, 4.96 mmol), (*E*)-pent-1-enylboronic acid (0.68 g, 5.95 mmol), tetrakis-triphenylphosphinopallidum and cesium carbonate (3.23 g, 9.91 mmol) was stirred in DME (10 mL) under reflux for 3 h. The reaction mixture was cooled to room temperature, and then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography with *n*-hexane/ethyl acetate (8:1) to afford compound **6** as a white solid (1.78 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 1.8 Hz, 1H), 7.39-7.36 (m, 1H), 7.33 (d, J = 2.1 Hz, 1H), 6.72 (d, J = 3.6 Hz, 1H), 6.66 (d, J = 3.6 Hz, 1H), 6.38 (d, J = 14.7 Hz, 1H), 6.07-5.97 (m, 1H), 4.44 (q, J = 7.2 Hz, 2H), 2.45 (s, 3H), 2.12 (q, J = 6.9 Hz, 2H), 1.49-1.39 (m, 5H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 145.4, 142.7, 137.8, 136.0, 135.9, 133.6, 132.4, 130.8, 129.8, 128.9, 127.6, 125.5, 124.0, 122.4, 119.7, 60.8, 34.7, 22.1, 14.3, 13.5, 9.9; ESMS *m/z* : 449.0 (MH^+).

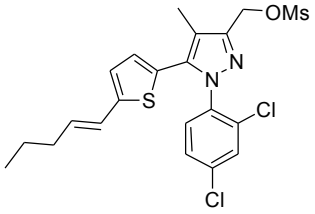
[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-yl]-methanol (7)



To a solution of compound **6** (0.64 g, 1.55 mmol) in dry THF (20 mL) at 0 °C was added lithium aluminum hydride (0.12 g, 3.15 mmol) in small portions. After stirring for 30 min at 0 °C, water was added to quench the reaction. The aqueous layer was separated and extracted with ethyl acetate (2 × 15 mL). The extracts were combined, washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash chromatography on silica gel with *n*-hexane/ethyl acetate

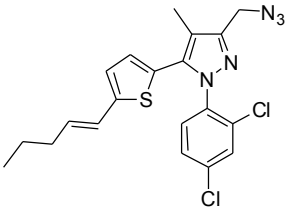
(4:1) to give alcohol **7** as a colorless oil (0.55 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (t, *J* = 1.5 Hz, 1H), 7.30 (d, *J* = 1.5 Hz, 2H), 6.70 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.06-5.96 (m, 1H), 4.74 (d, *J* = 5.4 Hz, 2H), 2.23 (brs, 1H), 2.14 (s, 3H), 2.12 (q, *J* = 6.3 Hz, 2H), 1.46 (sextet, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.0, 144.1, 136.2, 135.8, 135.1, 133.3, 131.5, 130.6, 129.4, 127.5, 127.3, 126.6, 123.8, 122.2, 114.2, 56.3, 34.4, 21.7, 13.2, 8.3; ESMS *m/z*: 407.1(MH^+).

Methanesulfonic acid 1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl ester (8)



To a magnetically stirred solution of alcohol **7** (0.42 g, 1.03 mmol) and triethylamine (0.29 mL, 2.11 mmol) in THF (10 mL) at 0 °C was added methanesulfonyl chloride (135 µL, 1.74 mmol) dropwise. The resulting mixture was warmed to room temperature and stirred for 8 h. The reaction was then quenched with water and the aqueous layer was separated and extracted with ethyl acetate (2 × 15 mL). The extracts were combined, washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product thus obtained was purified by flash chromatography on silica gel with *n*-hexane/ethyl acetate (8:1) to give compound **8** as a colorless liquid (0.48 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, *J* = 2.4 Hz, 1H), 7.33 (d, *J* = 1.8 Hz, 2H), 6.72 (d, *J* = 3.6 Hz, 1H), 6.63 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.07-5.97 (m, 1H), 5.33 (s, 2H), 3.02 (s, 3H), 2.27 (s, 3H), 2.12 (q, *J* = 7.2 Hz, 2H), 1.45 (sextet, *J* = 7.2 Hz, 2H), 0.96 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.5, 145.1, 137.4, 136.0, 136.0, 133.6, 132.4, 130.7, 130.1, 128.4, 127.8, 126.1, 124.1, 122.4, 116.0, 64.0, 38.0, 34.8, 22.1, 13.6, 8.7; ESMS *m/z*: 485.0 (MH^+).

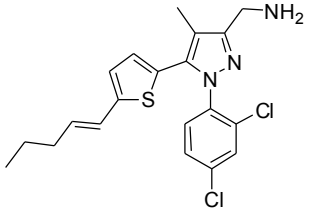
3-Azidomethyl-1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazole (9)



To a solution of compound **8** (0.41 g, 0.84 mmol) in dry DMF (10 mL) at room temperature, sodium azide (0.17 g, 2.54 mmol) was added in one portion. The resulting

mixture was heated up to 75 °C and kept stirring for 3 h. The reaction was cooled to room temperature and quenched with water. The aqueous layer was separated and extracted with ethyl acetate (2 × 15 mL). The organic layers were combined and washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product thus obtained was purified by flash chromatography on silica gel with *n*-hexane/ethyl acetate (10:1) to give azide **9** as a colorless liquid (0.31 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 1.8 Hz, 1H), 7.33 (d, *J* = 1.8 Hz, 2H), 6.72 (d, *J* = 3.6 Hz, 1H), 6.63 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.07-5.97 (m, 1H), 4.41 (s, 2H), 2.24 (s, 3H), 2.12 (q, *J* = 7.2 Hz, 2H), 1.45 (sextet, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.2, 144.8, 137.1, 136.2, 135.7, 133.8, 132.2, 130.7, 130.0, 128.2, 127.7, 126.6, 124.1, 122.5, 114.7, 46.5, 34.8, 22.1, 13.6, 8.8; ESMS *m/z*: 454.0 (M+23).

***(E)*-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-yl]-methylamine (10)**



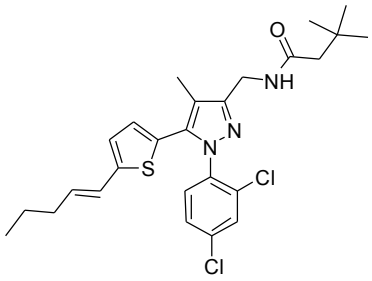
To a solution of azide **9** (0.31 g, 0.68 mmol) in THF (10 mL) at room temperature was added triphenylphosphine (0.19 g, 0.72 mmol) and water (2 mL) sequentially in one portion. After stirring at room temperature for 48 h, the mixture was extracted with ethyl acetate (2×15 mL). The extracts were combined, washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was subjected to purification by flash chromatography on silica gel with ethyl acetate/methanol (4:1) to afford amine **10** as a white solid (0.20 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 1.5 Hz, 1H), 7.34-7.28 (m, 2H), 6.71 (d, *J* = 4.5 Hz, 1H), 6.60 (d, *J* = 4.5 Hz, 1H), 6.39 (d, *J* = 11.7 Hz, 1H), 6.07-5.97 (m, 1H), 3.92 (s, 2H), 2.20 (s, 3H), 2.12 (q, *J* = 6.3 Hz, 2H), 1.78 (br s, 2H), 1.46 (sextet, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 144.5, 136.6, 136.6, 135.4, 133.8, 132.0, 130.9, 130.0, 127.9, 127.6, 127.3, 124.1, 122.6, 113.3, 38.5, 34.8, 22.2, 13.6, 8.8; ESMS *m/z*: 406.1 (MH^+).

The General Procedure for Acylation in the Synthesis of Compounds 11a-11m

To a solution of amine **10** (1.0 mmol) in dry dichloromethane (5 mL) at 0 °C were added triethylamine (1.3 mmol) and acid chloride (1.2 mmol) sequentially in one portion. The resulting mixture was allowed to warm to room temperature and kept stirring for 3~ 8 h. Ice-cold water was added to quench the reaction followed by extracting the aqueous layer with dichloromethane (2 × 20 mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to give the crude residue,

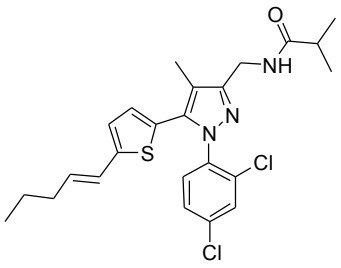
which was further purified by flash chromatography on silica gel with n-hexane/ethyl acetate (3:1) to afford the desired products **11a-11m** in 51-78% yields.

***N*-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-3,3-dimethyl-butyramide (11a)**



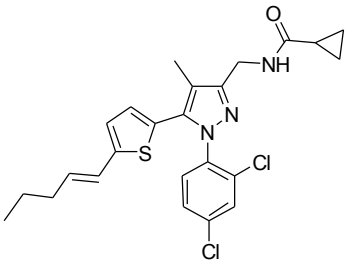
Treatment of amine **10** (0.10 g, 0.25 mmol) with triethylamine (45 μL, 0.32 mmol) and 3,3-Dimethyl-butyryl chloride (42 μL, 0.30 mmol) gave compound **11a** (98 mg, 78%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 1.8 Hz, 1H), 7.33-7.29 (m, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.19 (t, *J* = 4.8 Hz, 1H), 6.07-5.96 (m, 1H), 4.51 (d, *J* = 4.8 Hz, 2H), 2.18 (s, 3H), 2.12 (q, *J* = 7.8 Hz, 2H), 2.08 (s, 2H), 1.44 (sextet, *J* = 7.2 Hz, 2H), 1.03 (s, 9H), 0.92 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 149.5, 145.0, 136.7, 135.9, 134.0, 132.5, 131.1, 130.3, 128.3, 128.0, 127.1, 124.4, 122.8, 114.1, 50.6, 36.2, 35.1, 31.1, 30.0, 29.9, 22.4, 13.9, 9.0; ESMS *m/z*: 504.2 (MH^+).

***N*-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-isobutyramide (11b)**



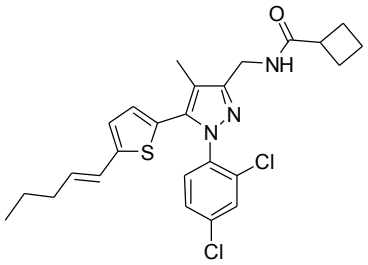
Treatment of amine **10** (0.10 g, 0.25 mmol) with triethylamine (45 μL, 0.32 mmol) and isobutyryl chloride (31 μL, 0.30 mmol) gave compound **11b** (82 mg, 70%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.32-7.31 (m, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.21 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.51 (d, *J* = 4.8 Hz, 2H), 2.41 (quintet, *J* = 6.8 Hz, 1H), 2.17 (s, 3H), 2.12 (q, *J* = 6.6 Hz, 2H), 1.47 (sextet, *J* = 7.2 Hz, 2H), 1.16 (d, *J* = 7.2 Hz, 6H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 149.2, 144.8, 137.0, 136.4, 135.6, 133.8, 132.2, 130.9, 130.1, 128.1, 127.8, 126.8, 124.1, 122.6, 114.0, 36.1, 35.5, 34.9, 22.2, 19.6, 13.7, 8.7; ESMS *m/z*: 476.2 (MH^+).

Cyclopropanecarboxylic acid [1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-amide (11c)



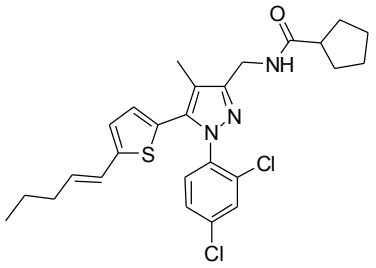
Treatment of amine **10** (0.03 g, 0.08 mmol) with triethylamine (15 µL, 0.11 mmol) and cyclopropanecarbonyl chloride (15 µL, 0.09 mmol) gave compound **11c** (22 mg, 57%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (t, *J* = 3.9 Hz, 1H), 7.36-7.31 (m, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.21 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.53 (d, *J* = 4.5 Hz, 2H), 2.17 (s, 3H), 2.11 (q, *J* = 7.2 Hz, 2H), 1.50-1.37 (m, 3H), 1.02-0.85 (m, 5H), 0.76-0.70 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 149.3, 144.8, 137.0, 136.4, 135.7, 133.8, 132.2, 130.9, 130.1, 128.1, 127.8, 126.8, 124.1, 122.6, 114.0, 36.3, 34.8, 22.2, 14.6, 13.6, 8.8, 8.7, 7.1; ESMS *m/z*: 474.1 (MH^+).

Cyclobutanecarboxylic acid [1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-amide (11d)



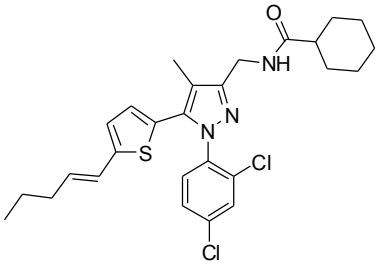
Treatment of amine **10** (0.05 g, 0.12 mmol) with triethylamine (22 µL, 0.16 mmol) and cyclobutanecarbonyl chloride (15 µL, 0.14 mmol) gave compound **11d** (33 mg, 57%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.31 (s, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.15 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.51 (d, *J* = 4.8 Hz, 2H), 3.04 (quintet, *J* = 8.7 Hz, 1H), 2.37-2.09 (m, 8H), 2.02-1.83 (m, 3H), 1.46 (sextet, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 149.2, 144.8, 136.9, 136.4, 135.6, 133.2, 132.2, 130.8, 130.1, 128.1, 127.7, 126.8, 124.1, 122.5, 114.0, 39.8, 36.0, 34.8, 25.3, 22.2, 18.1, 13.6 8.7; ESMS *m/z*: 488.1 (MH^+).

Cyclopentanecarboxylic acid [1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-amide (11e)



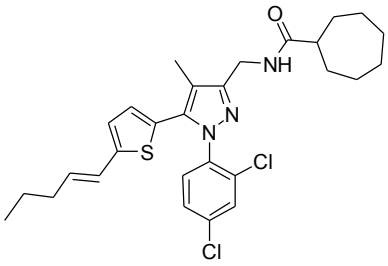
Treatment of amine **10** (0.05 g, 0.12 mmol) with triethylamine (22 μL, 0.16 mmol) and cyclopentanecarbonyl chloride (17 μL, 0.14 mmol) gave compound **11e** (41 mg, 69%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.31 (s, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 16.2 Hz, 1H), 6.26 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.51 (d, *J* = 5.8 Hz, 2H), 2.56 (quintet, *J* = 8.1 Hz, 1H), 2.17 (s, 3H), 2.23 (q, *J* = 7.2 Hz, 2H), 1.87-1.69 (m, 7H), 1.57-1.42 (m, 3H), 0.92 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 149.3, 144.8, 136.9, 136.4, 135.6, 133.8 132.2, 130.9, 130.1, 128.1, 127.7, 126.8, 124.1, 122.6, 114.0, 45.7, 36.1, 43.8, 30.4, 25.8, 22.2, 13.6, 8.7; ESMS *m/z*: 502.1 (MH^+).

Cyclohexanecarboxylic acid [1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-amide (11f)



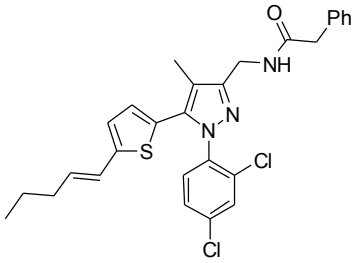
Treatment of amine **10** (0.10 g, 0.25 mmol) with triethylamine (45 μL, 0.32 mmol) and cyclohexanecarbonyl chloride (43 μL, 0.30 mmol) gave compound **11f** (85 mg, 68%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 3.6 Hz, 1H), 7.32-7.30 (m, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.27 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.51 (d, *J* = 4.8 Hz, 2H), 2.16-2.09 (m, 5H), 1.88-1.64 (m, 6H), 1.46 (sextet, *J* = 7.2 Hz, 3H), 1.26-1.20 (m, 4H), 0.92 (t, *J* = 8.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 149.3, 144.3, 136.9, 136.4, 135.6, 133.8, 132.2, 130.9, 130.1, 128.1, 127.7, 126.8, 124.1, 122.5, 114.0, 45.3, 35.9, 34.8, 29.5, 25.7, 22.2, 13.6, 8.7; ESMS *m/z*: 516.1 (MH^+).

Cycloheptanecarboxylic acid [1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1H-pyrazol-3-ylmethyl]-amide (11g)



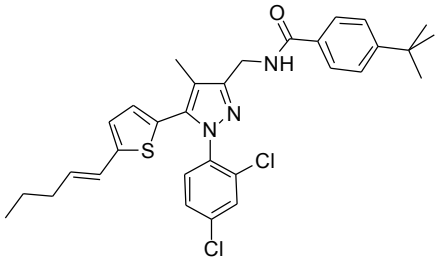
Treatment of amine **10** (0.05 g, 0.13 mmol) with triethylamine (24 μL, 0.17 mmol) and cycloheptanecarbonyl chloride (29 μL, 0.16 mmol) gave compound **11g** (43 mg, 62%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.32-7.27 (m, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.27 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.50 (d, *J* = 4.8 Hz, 2H), 2.31-2.22 (m, 1H), 2.19-2.09 (m, 5H), 1.95-1.84 (m, 2H), 1.79-1.65 (m, 4H), 1.63-1.52 (m, 4H), 1.49-1.40 (m, 4H), 0.92 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0, 149.3, 144.8, 136.9, 136.4, 135.6, 133.8, 132.2, 130.8, 130.1, 128.0, 127.7, 126.8, 124.1, 122.5, 114.0, 47.3, 36.0, 34.8, 31.6, 28.0, 26.5, 22.2, 13.6, 8.7; ESMS *m/z*: 530.3 (MH^+).

_N_-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiphen-2-yl)-1_H_-pyrazol-3-ylmethyl]-2-phenyl-acetamide (11h)



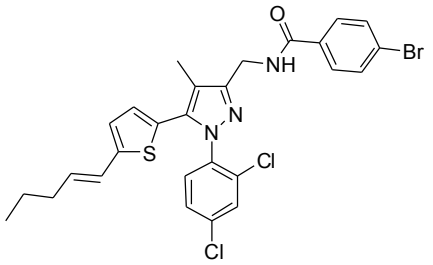
Treatment of amine **10** (0.06 g, 0.15 mmol) with triethylamine (27 μL, 0.20 mmol) and phenylacetyl chloride (30 μL, 0.18 mmol) gave compound **11h** (42 mg, 54%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, *J* = 2.1 Hz, 1H), 7.33-7.24 (m, 7H), 6.69 (d, *J* = 3.6 Hz, 1H), 6.57 (d, *J* = 3.6 Hz, 1H), 6.38 (d, *J* = 15.6 Hz, 1H), 6.19 (t, *J* = 4.8 Hz, 1H), 6.05-5.95 (m, 1H), 4.48 (d, *J* = 5.4 Hz, 2H), 3.59 (s, 2H), 2.16-2.08 (m, 5H), 1.44 (sextet, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 148.9, 144.8, 136.3, 135.6, 134.7, 133.3, 132.2, 130.8, 130.1, 129.3, 129.3, 128.8, 128.1, 127.7, 127.2, 126.8, 124.1, 122.5, 114.0, 43.6, 36.2, 34.9, 22.2, 13.7, 8.7; ESMS *m/z*: 524.2 (MH^+).

4-tert-Butyl-N-[1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1_H_-pyrazol-3-ylmethyl]-benzamide (11i)



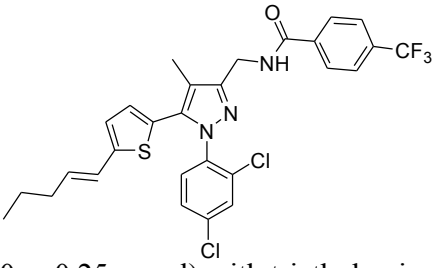
Treatment of amine **10** (0.06 g, 0.15 mmol) with triethylamine (27 μL, 0.20 mmol) and 4-tert-butylbenzoyl chloride (35 μL, 0.18 mmol) gave compound **11i** (43 mg, 51%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 3H), 7.32-7.24 (m, 3H), 6.68 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.38 (d, *J* = 15.3 Hz, 1H), 6.05-5.95 (m, 1H), 4.73 (d, *J* = 5.1 Hz, 2H), 2.22 (s, 3H), 2.11 (q, *J* = 7.2 Hz, 2H) 1.44 (sextet, *J* = 7.2 Hz, 2H), 1.33-1.26 (m, 9H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 154.8, 149.2, 144.8, 137.0, 136.4, 135.6, 133.8, 132.2, 131.4, 130.8, 130.1, 128.1, 127.7, 126.9, 126.8, 125.3, 124.1, 122.5, 114.1, 36.5, 34.9, 34.8, 31.1, 22.2, 13.6, 8.8; ESMS *m/z*: 566.2 (MH^+), Anal. Calcd for $C_{31}H_{33}Cl_2N_3OS$: C 65.72; H 5.87; N 7.42; Found C 66.00; H 5.74; N 7.05.

4-Bromo-*N*-[1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl]-benzamide (11j)



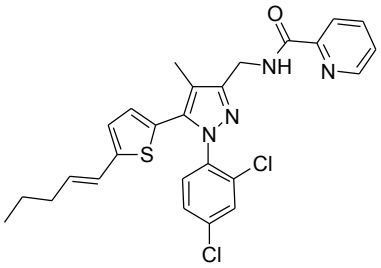
Treatment of amine **10** (0.06 g, 0.15 mmol) with triethylamine (27 μL, 0.20 mmol) and 4-bromobenzoyl chloride (39 mg, 0.18 mmol) gave compound **11j** (45 mg, 51%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.7 Hz, 2H), 7.57-7.54 (m, 2H), 7.47 (s, 1H), 7.31 (d, *J*=1.8 Hz, 2H), 6.92 (t, *J* = 4.8 Hz, 1H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.62 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.06-5.96 (m, 1H), 4.70 (d, *J* = 4.8 Hz, 2H), 2.18 (s, 3H), 2.13 (q, *J* = 7.2 Hz, 2H) 1.44 (sextet, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 149.1, 144.8, 137.1, 136.1, 135.6, 133.6, 132.9, 132.2, 131.8, 131.4, 130.7, 129.9, 128.6, 128.1, 127.6, 126.5, 125.9, 124.1, 122.5, 114.1, 36.4, 34.8, 22.1, 13.6, 8.7; ESMS *m/z*: 610.1 (M+23); Anal. Calcd for $C_{27}H_{24}BrCl_2N_3OS$: C 55.02; H 4.10; N 7.13; Found C 55.11; H 4.28; N 6.82.

***N*-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl]-4-trifluoromethyl-benzamide (11k)**



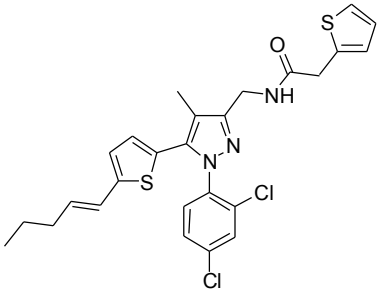
Treatment of amine **10** (0.10 g, 0.25 mmol) with triethylamine (45 μL, 0.33 mmol) and 4-trifluoromethyl-benzoyl chloride (45 μL, 0.30 mmol) gave compound **11k** (83 mg, 58%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12, (d, *J* = 8.4Hz, 1H), 7.94 (d, *J* = 8.4Hz, 2H), 7.72-7.65 (m, 3H), 7.46 (s, 1H), 7.39 (t, *J* = 4.8 Hz, 1H), 7.29 (s, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.62 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.06-5.97 (m, 1H), 4.75 (d, *J* = 4.8 Hz, 2H), 2.25 (s, 3H), 2.13 (q, *J* = 7.2 Hz, 2H) 1.44 (sextet, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 148.9, 145.1, 136.0, 135.9, 133.8, 132.4, 130.8, 130.3, 130.1, 128.3, 127.8, 127.6, 126.5, 125.5, 125.4, 124.2, 122.5, 114.1, 36.7, 34.8, 29.6, 22.2, 13.6, 8.7; ESMS *m/z*: 600.2 (M+23).

Pyridine-2-carboxylic acid [1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl]-amide (11l)



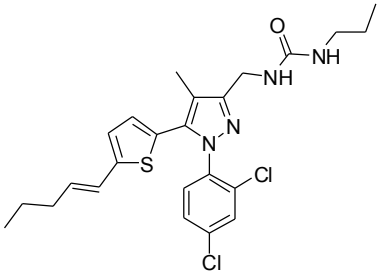
Treatment of amine **10** (0.06 g, 0.14 mmol) with triethylamine (25 μL, 0.18 mmol) and pyridine 2-carbonyl chloride (0.03 g, 0.17 mmol) gave compound **11l** (52 mg, 74%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56-8.54 (m, 1H), 8.46 (t, *J* = 4.8 Hz, 1H), 8.23 (d, *J* = 7.8 Hz, 1H), 7.88-7.82 (m, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 7.44-7.37 (m, 1H), 7.34-7.29 (m, 2H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.06-5.95 (m, 1H), 4.76 (d, *J* = 5.7 Hz, 2H), 2.22 (s, 3H), 2.13 (q, *J* = 7.2 Hz, 2H) 1.44 (sextet, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 149.7, 149.1, 148.1, 144.7, 137.2, 136,9, 136.5, 135.5, 133.7, 132.1, 130.9, 130.0, 128.0, 127.7, 127.0, 126.1, 124.1, 122.6, 122.2, 114.4, 36.0, 34.8, 22.2, 13.7, 8.8; ESMS *m/z*: 511.2 (MH^+); Anal. Calcd for $C_{26}H_{24}Cl_2N_4OS$: C 61.06; H 4.73; N 10.95; Found C 60.35; H 4.77; N 10.63.

***N*-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl]-2-thiophen-2-yl-acetamide (11m)**



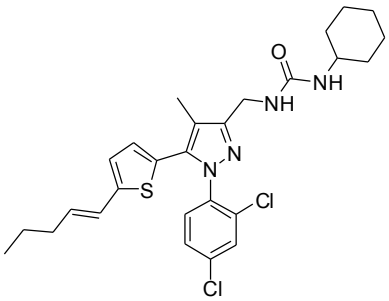
Treatment of amine **10** (0.06 g, 0.15 mmol) with triethylamine (27 µL, 0.19 mmol) and 2-thiopheneacetyl chloride (20 µL, 0.18 mmol) gave compound **11m** (45 mg, 57%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 1.8 Hz, 1H), 7.32-7.25 (m, 2H), 7.22-7.20 (m, 1H), 6.97-6.94 (m, 2H), 6.70 (d, *J* = 3.9 Hz, 1H), 6.60 (d, *J* = 3.9 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.25 (t, *J* = 4.8 Hz, 1H), 6.06-5.96 (m, 1H), 4.50 (d, *J* = 5.1 Hz, 2H), 3.82 (s, 2H), 2.17-2.09 (m, 5H), 1.44 (sextet, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 148.8, 144.8, 136.9, 136.3, 135.9, 135.6, 133.7, 132.2, 130.7, 130.1, 128.1, 127.7, 127.2, 127.1, 126.8, 125.3, 124.1, 122.5, 114.0, 37.4, 34.8, 29.6, 22.2, 13.6, 8.7; ESMS *m/z*: 552.2 (M+23).

1-[1-(2,4-Dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl]-3-propyl-urea (11n)



To a magnetically stirred solution of amine **10** (0.04 g, 0.11 mmol) and triethylamine (20 µL, 0.14 mmol) in THF (5 mL) at 0 ^{o}C was added *n*-propylisocyanate (20 µL, 0.13 mmol) dropwise. After stirring at room temperature for 8 h, the reaction was quenched with water and the aqueous layer was separated and extracted with ethyl acetate (2 x 10 mL). The extracts were combined, washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue thus obtained was subjected to purification by flash chromatography on silica gel with *n*-hexane/ethyl acetate (1:1) to give compound **11n** as a white solid (55 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, *J* = 1.8 Hz, 1H), 7.31-7.24 (m, 2H), 6.70 (d, *J* = 3.6 Hz, 1H), 6.58 (d, *J* = 3.6 Hz, 1H), 6.38 (d, *J* = 15.9 Hz, 1H), 6.05-5.95 (m, 1H), 5.66 (t, *J* = 4.8 Hz, 1H), 5.16 (t, *J* = 5.4 Hz, 1H), 4.37 (d, *J* = 5.4 Hz, 2H), 3.08 (q, *J* = 6.4 Hz, 2H), 2.16-2.08 (m, 5H), 1.49-1.38 (m, 4H), 0.92 (t, *J* = 7.8 Hz, 3H), 0.84 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.2, 150.2, 144.8, 137.0, 136.4, 135.6, 133.8, 132.2, 130.8, 130.1, 128.1, 127.7, 126.8, 124.1, 122.5, 114.0, 42.2, 37.1, 34.9, 23.2, 22.2, 13.7, 11.3, 8.7; ESMS *m/z*: 491.2 (MH^+).

1-Cyclohexyl-3-[1-(2,4-dichloro-phenyl)-4-methyl-5-(5-pent-1-enyl-thiophen-2-yl)-1*H*-pyrazol-3-ylmethyl]-urea (11o)



To a magnetically stirred solution of amine **10** (0.04 g, 0.11 mmol) and triethylamine (20 µL, 0.14 mmol) in THF (5 mL) at 0 °C was added isocyanatocyclohexane (20 µL, 0.14 mmol) dropwise. After the mixture was stirred at room temperature for 8 h, the reaction was quenched with water and the aqueous layer was separated and extracted with ethyl acetate (2 x 10 mL). The extracts were combined, washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude residue thus obtained was purified by flash chromatography on silica gel with *n*-hexane/ethyl acetate (1:1) to give compound **11o** as a white solid (33 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.31-7.25 (m, 2H), 6.70 (d, *J* = 3.6 Hz, 1H), 6.58 (d, *J* = 3.6 Hz, 1H), 6.38 (d, *J* = 15.9 Hz, 1H), 6.06-5.96 (m, 1H), 5.25 (t, *J* = 4.8 Hz, 1H), 4.74 (d, *J* = 7.5 Hz, 1H), 4.38 (d, *J* = 5.1 Hz, 2H), 3.58-3.48 (m, 1H), 2.17-2.09 (m, 5H), 1.93-1.84 (m, 3H), 1.66-1.62 (m, 2H), 1.56-1.42 (m, 2H), 1.37-1.25 (m, 3H), 1.16-1.03 (m, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.5, 150.3, 144.8, 137.0, 136.4, 135.6, 133.8, 132.2, 130.9, 130.0, 128.1, 127.7, 126.9, 124.1, 122.6, 114.0, 48.9, 37.0, 34.8, 33.7, 25.5, 24.7, 22.2, 13.6, 8.7; ESMS *m/z*: 553.1 (M+23).

Biological Evaluation:

The following test methods were followed to generate the data in Table 1.

Establishment of human CB1 (hCB1) and CB2 (hCB2) stable cell lines and membrane purification

The hCB1 cDNA tagged with Flag at the N terminus or hCB2 cDNA was subcloned into the pIRES2-EGFP vector (Clontech Laboratories, Inc., Mountain View, CA). After transfected to HEK 293 cells, clones stably expressed either hCB1 or hCB2 were selected by GFP and G418 sulfate, and maintained in DMEM supplemented with 10 % fetal bovine serum and 0.5 mg/ml G418 sulfate under 5% CO_2 at 37 °C. For membrane purification, cells were homogenized in ice-cold buffer A (50 mM Tris, 5 mM $MgCl_2$, 2.5 mM EDTA, pH 7.4, 10% sucrose) with 1 mM PMSF. The homogenate was centrifuged for 15 min at 2000xg at 4 °C. The resulting supernatant was centrifuged for another 30

minutes at 43000xg at 4 °C. The final pellet was resuspended in buffer A and stored at -80 °C.

Radioligand binding assay[1]

0.2~8 μg of the purified membrane was incubated with 0.75 nM [^{3}H] CP55,940 and compounds of interest in the incubation buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.3% BSA, pH 7.4). The non-specific binding was defined in the presence of 1 μM of CP55,940. The reactions were incubated for one and a half hours at 30 °C in Multiscreen microplates (Millipore Corp., Billerica, MA). The reactions were terminated by manifold filtration and washed with ice-cold wash buffer (50 mM Tris, pH 7.4, 0.25% BSA) for four times. The radioactivity bound to the filters was measured by Topcount (PerkinElmer Inc., Waltham, MA). IC_{50} was determined by the concentration of compounds required to inhibit 50% of the binding of [^{3}H] CP55,940 and calculated by non-linear regression (GraphPad software, San Diego, CA).

Eu-GTP binding assay[2]

The Eu-GTP binding assay was performed using the DELFIA Eu-GTP binding kit (Perkin Elmer Inc., Waltham, MA) based on methods developed by Frang *et al*. with minor modifications as described in the following: 1~4 μg of purified membrane was incubated with compounds of interest and 20 nM CP55,940 in assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 100 μg/mL saponin, 5 mM $MgCl_2$, 2 μM GDP, 0.5% BSA) at 30 °C for 60 minutes in acroplates (Pall Life Sciences, Ann Arbor, Mich.). Following the addition of Eu-GTP and incubation of 30 minutes at 30 °C, the assay was terminated by washing four times in washing buffer provided in the kit. The fluorescence signal of Eu-GTP was determined by Victor 2 multilabel reader (Perkin Elmer Inc., Waltham, MA). EC_{50} of tested compounds in inhibiting 50% of CP55,940-stimulated Eu-GTP binding was determined by dose-response curve using nonlinear regression (GraphPad software, San Diego, CA).

References

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