Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Experimental Procedures

Chemistry

General Remarks

Melting points were obtained on a Yanagimoto micro melting point apparatus without correction. ^{1}H-NMR spectra were recorded at 400 MHz on Bruker AVANCE 400. ^{13}C-NMR spectra were recorded at 175 MHz on a Bruker AVANCE 700 spectrometer. Chemical shifts are reported in ppm as δ values from tetramethylsilane. Data are reported as follows; chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q quartet; br, broad; m, multiplet), coupling constants (Hz), integration. Mass spectra were recorded on a Bruker FT-ICR (9.4T) for ESI (electrospray ionization)-Mass.

1-(3-Cyanophenyl)-1,12-dicarba-*closo*-dodecaborane (13)

Under argon atmosphere, *n*-butyllithium (1.59 mol/L in *n*-hexane, 14.4 ml, 22.9 mmol) was added dropwise to a solution of *p*-carborane (3.00 g, 20.8 mmol) in dimethoxyethane (20 ml) at 0°C. The reaction mixture was warmed to room temperature for 30 min, then copper(I) chloride (2.68 g, 27.0 mmol) was added to the reaction. After the reaction mixture was stirred for 1 h, pyridine (7 ml) and 3-iodobenzonitrile (5.24 g, 22.9 mmol) was further added and stirred for overnight at 80°C. The reaction mixture was cooled for room temperature and diluted with diethyl ether. Insoluble materials were filtered off through Celite. The filtrate was washed with 5% sodium thiosulfate, 2 mol/L hydrochloric acid, water and brine, dried over sodium sulfate and then concentrated. The crude product was purified by silica gel column chromatography (eluent: n-hexane/dichlorimethane 10:1) to give compound **13** (3.82 g, 15.6 mmol, 75%) as colorless solid: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.50 (dt, *J* = 7.6 Hz, 1.1 Hz, 1 H), 7.47 (m, 1 H), 7.42 (ddd, *J* = 8.3 Hz, 2.1 Hz, 1.1 Hz, 1 H), 7.29 (t, *J* = 7.9 Hz, 1 H), 3.5-1.0 (br m, 10 H), 2.83 (br s, 1 H).

12-(3-Cyanophenyl)-1,12-dicarba-*closo*-dodecaborane-1-carboxylic acid (13)

Under argon atmosphere, lithium diisopropylamine (1.14 mol/L in nhexane-THF, 9.30 ml, 10.6 mmol) was added dropwise to a solution of **13** (2.00 g, 8.15 mmol) in tetrahydrofuran (40 ml) at 0°C. The reaction mixture was stirred for 10 min, large excess of dry ice was added and stirred for 4.5 h. The reaction was quenched with 2 mol/L hydrochloric acid and diluted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and then concentrated. The crude product was purified by silica gel column chromatography (eluent: n-hexane /ethyl acetate 10:1 to ethyl acetate/methanol 10:1) to give compound **14** (1.90 g, 6.80 mmol, 83%) as colorless solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.53 (dt, *J* = 7.7 Hz, 1.1 Hz, 1 H), 7.47 (m, 1 H), 7.42 (m, 1 H), 7.32 (t, *J* = 7.9 Hz, 1 H), 3.5-1.0 (br m, 10 H).

General procedure for preparation of amide compounds 8a-i and 9a-b:

To a solution of **14** (200 mg, 0.69 mmol) in dichloromethane (4.0 mL) were added oxalyl chloride (0.090 mL, 1.04 mmol) and catalytic amount of DMF at 0°C, and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and the resulting material was dissolved in THF (4.0 mL). Appropriate amine (2.07 mmol) and pyridine (0.28 mL, 3.45 mmol) were added to the mixture at 0°C, and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent: hexane/ethyl acetate) gave the products as colorless solids. The products were further purified by recrystallization from *n*-hexane.

1-(3-Cyanophenyl)-12-phenylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (8a)

Colorless solid, mp: 193.0-197.5 °C, ^{1}H-NMR (400 MHz, acetone-d_6) δ 8.50 (br s, 1 H), 7.78 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.68 (m, 2 H), 7.56 (m, 3 H), 7.33 (t, *J* = 7.6 Hz, 1 H), 7.16 (t, *J* = 7.4 Hz, 2 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, acetone-d_6) δ 158.4, 137.5, 137.1, 132.7, 131.6, 130.3, 129.9, 128.6, 125.1, 121.2, 121.1 117.6, 112.8, 82.8, 81.2; HRMS Calcd for $C_{16}H_{20}B_{10}N_2O$ Na [M + Na]: 387.2471. Found 387.2477.

1-(3-Cyanophenyl)-12-(4-methoxyphenylcarbamoyl)-1,12-dicarba-*closo*-dodecaborane (8b)

Colorless solid, mp: 223.0-229.5 °C, ^{1}H-NMR (400 MHz, acetone-d_6) δ 8.49 (br s, 1 H), 7.77 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.65 (m, 2 H), 7.56 (t, *J* = 7.6 Hz, 1 H), 7.43 (d, *J* = 9.1 Hz, 2 H), 6.88 (d, *J* = 9.1 Hz, 2 H), 3.6-1.5 (br m, 10 H), 3.78 (s, 3 H). ^{13}C-NMR (175 MHz, acetone-d_6) δ 158.2, 157.1, 137.1, 132.7, 131.6, 130.4, 129.9, 122.9, 125.1, 121.2, 117.6 113.6, 112.7, 82.6, 81.2, 54.8; HRMS Calcd for $C_{17}H_{22}B_{10}N_2O_2$ Na [M + Na]: 417.2577. Found 417.2590.

1-(3-Cyanophenyl)-12-(2-pyridylcarbamoyl)-1,12-dicarba-*closo*-dodecaborane (8c)

Colorless solid, mp: 177.5-181.0 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 4.0 Hz, 1 H), 7.99 (d, *J* = 8.4 Hz, 1 H), 7.64 (br s, 1 H), 7.69 (dt, *J* = 1.8 Hz, 8.0 Hz, 1 H), 7.56 (dt, *J* = 7.8 Hz, 1.3 Hz, 1 H), 7.50 (t, *J* = 1.6 Hz, 1 H), 7.45 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.1 Hz, 1 H), 7.35 (t, *J* = 7.8 Hz, 1 H), 7.09 (ddd, *J* = 7.4 Hz, 5.0 Hz, 0.8 Hz, 1 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 158.7, 150.0, 148.0, 138.6, 137.2, 132.3, 131.4, 130.7, 129.3, 120.9, 118.0, 113.8, 112.8, 82.8, 80.2; HRMS Calcd for $C_{15}H_{19}B_{10}N_3O$ Na [M + Na]: 388.2424. Found 388.2424.

1-(3-Cyanophenyl)-12-(3-pyridylcarbamoyl)-1,12-dicarba-*closo*-dodecaborane (8d)

Colorless solid, mp: 192.0-194.5 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.87 (br s, 1 H), 8.38 (d, *J* = 5.0 Hz,

1 H), 8.27 (d, *J* = 8.4 Hz, 1 H), 7.94 (br s, 1 H), 7.57 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.50 (t, *J* = 1.6 Hz, 1 H), 7.45 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.1 Hz, 1 H), 7.41 (dd, *J* = 8.4 Hz, 5.0 Hz, 1 H), 7.35 (t, *J* = 7.8 Hz, 1 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 159.5, 142.8, 139.4, 137.1, 135.1, 132.4, 131.4, 130.6, 130.3, 129.3, 124.6, 117.9, 112.8, 83.1, 79.6; HRMS Calcd for $C_{15}H_{19}B_{10}N_3O$ Na [M + Na]: 388.2424. Found 388.2423.

1-(3-Cyanophenyl)-12-(4-pyridylcarbamoyl)-1,12-dicarba-*closo*-dodecaborane (8e)

Colorless solid, mp: 238.5-244.5 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 5.7 Hz, 2 H), 7.57 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.49 (t, *J* = 1.6 Hz, 1 H), 7.45 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.1 Hz, 1 H), 7.40-7.34 (m, 4 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 158.8, 150.9, 143.5, 137.0, 132.4, 131.3, 130.6, 129.4, 124.6, 117.9, 113.7, 112.8, 83.1, 79.7; HRMS Calcd for $C_{15}H_{20}N_3O$ [M + H]: 366.2604. Found 366.2603.

1-(3-Cyanophenyl)-12-(2-pyrimidylcarbamoyl)-1,12-dicarba-*closo*-dodecaborane (8f)

Colorless solid, mp: 224.0-225.5 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.62 (d, *J* = 4.8 Hz, 2 H), 8.06 (br s, 1 H), 7.56 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.50 (t, *J* = 1.6 Hz, 1 H), 7.45 (ddd, *J* = 8.2 Hz, 2.0 Hz, 1.1 Hz, 1 H), 7.35 (t, *J* = 7.8 Hz, 1 H), 7.09 (t, *J* = 4.8 Hz, 1 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 158.5, 157.5, 156.4, 137.2, 132.3, 131.4, 130.6, 129.3, 118.0, 117.8, 112.8, 82.9, 80.2; HRMS Calcd for $C_{14}H_{18}B_{10}N_4O$ Na [M + Na]: 389.2376. Found 389.2394.

1-(3-Cyanophenyl)-12-(2-pyrazylcarbamoyl)-1,12-dicarba-*closo*-dodecaborane (8g)

Colorless solid, mp: 186.0-187.3 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1 H), 8.40 (d, *J* = 2.4 Hz, 1 H), 8.26 (dd, *J* = 2.4 Hz, 1.6 Hz, 1 H), 7.87 (br s, 1 H), 7.57 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.50 (t, *J* = 1.6 Hz, 1 H), 7.45 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.1 Hz, 1 H), 7.36 (t, *J* = 7.8 Hz, 1 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 158.7, 146.8, 142.2, 141.4, 137.0, 136.7, 132.4, 131.3, 130.6, 129.4, 117.9, 112.8, 83.2, 79.4; HRMS Calcd for $C_{14}H_{18}B_{10}N_4O$ Na [M + Na]: 389.2376. Found 389.2380.

1-(3-Cyanophenyl)-12-(2-thiazolyl)-1,12-dicarba-*closo*-dodecaborane (8h)

Colorless solid, mp: 226.8-228.5 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.89 (br s, 1 H), 7.57 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.49 (t, *J* = 1.6 Hz, 1 H), 7.47 (br d, 1 H), 7.44 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.1 Hz, 1 H), 7.36 (t, *J* = 7.8 Hz, 1 H), 7.04 (br d, 1 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) □ 158.4, 156.9, 137.8, 137.0, 132.4, 131.3, 130.6, 129.4, 117.9, 115.0, 112.8, 83.5, 78.1; HRMS Calcd for $C_{13}H_{17}B_{10}N_3OS$ Na [M + Na]: 394.1988. Found 394.1991.

1-(3-Cyanophenyl)-12-cyclohexylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (8i)

Colorless solid, mp: 183.8-185.0 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.54 (dt, *J* = 7.7 Hz, 1.3 Hz, 1 H),

7.48 (t, *J* = 1.6 Hz, 1 H), 7.43 (ddd, *J* = 8.2 Hz, 2.0 Hz, 1.1 Hz, 1 H), 7.33 (t, *J* = 7.8 Hz, 1 H), 5.46 (br d, *J* = 7.4 Hz, 1 H), 3.57 (m, 1 H), 3.5-1.5 (br m, 10 H), 1.78 (m, 2 H), 1.60 (m, 4 H), 1.34 (m, 2 H), 1.20 (m, 2 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 159.5, 137.3, 132.2, 131.4, 130.7, 129.2, 118.0, 112.7, 82.0, 80.6, 49.5, 32.3, 25.3, 24.4; HRMS Calcd for $C_{16}H_{26}B_{10}N_2O$ Na [M + Na]: 393.2941. Found 393.2952.

1-(3-Cyanophenyl)-12-methylphenylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (**9a**)

Colorless solid, mp: 180.5-182.5 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.49 (dt, *J* = 7.4 Hz, 1.4 Hz, 1 H), 7.45 (m, 3 H), 7.38 (t, *J* = 1.6 Hz, 1 H), 7.34 (ddd, *J* = 8.2 Hz, 2.0 Hz, 1.3 Hz, 1 H), 7.28 (t, *J* = 8.2 Hz, 1 H), 7.10 (m, 2 H), 3.5-1.5 (br m, 10 H), 3.16 (s, 3 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 160.7, 143.0, 137.5, 132.0, 130.6, 129.8, 129.2, 129.1, 128.7, 118.1, 112.5, 84.4, 81.8, 42.8; HRMS Calcd for $C_{17}H_{22}B_{10}N_2O$ Na [M + Na]: 401.2628. Found 401.2636.

1-(3-Cyanophenyl)-12-[(methyl-*o*-tolyl)carbamoyl]-1,12-dicarba-*closo*-dodecaborane (**9b**)

Colorless solid, mp: 191.5-193.0 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.49 (dt, *J* = 7.4 Hz, 1.4 Hz, 1 H), 7.39 (t, *J* = 1.6 Hz, 1 H), 7.34 (ddd, *J* = 8.2 Hz, 2.0 Hz, 1.3 Hz, 1 H), 7.27 (t, *J* = 8.2 Hz, 1 H), 7.22 (d, *J* = 8.2 Hz, 2 H), 6.97 (d, *J* = 8.2 Hz, 2 H), 3.5-1.5 (br m, 10 H), 3.13 (s, 3 H), 2.44 (s, 3 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 160.7, 140.4, 139.2, 137.6, 132.0, 131.3, 130.6, 130.4, 129.1, 128.3, 118.1, 112.5, 84.4, 81.9, 42.9, 21.3; HRMS Calcd for $C_{18}H_{24}B_{10}N_2O$ Na [M + Na]: 415.2784. Found 415.2797.

12-(3-Cyanophenyl)-1,12-dicarba-*closo*-dodecaborane-1-carboxyamide (**15**)

To a solution of **14** (400 mg, 1.38 mmol) in dichloromethane (10 mL) were added oxalyl chloride (263 mg, 2.07 mmol) and catalytic amount of DMF at 0°C, and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and the resulting material was dissolved in THF (10 mL). A 17% solution of aqueous ammonia (0.934 mL, 13.8 mmol) was added to the mixture at 0°C, and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent: hexane/ethyl acetate, 1:1) gave **15** (quant) as colorless solid: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.55 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.48 (t, *J* = 1.6 Hz, 1 H), 7.43 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.2 Hz, 1 H), 7.34 (t, *J* = 7.8 Hz, 1 H), 5.63 (br s, 1 H), 5.57 (br s, 1 H), 3.6-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 162.5, 137.2, 132.2, 131.4, 130.6, 129.3, 118.0, 112.7, 82.5, 79.2; HRMS Calcd for $C_{10}H_{16}B_{10}N_2O$ Na [M + Na]: 311.2158. Found 311.5157.

1-(3-Cyanophenyl)-12-*tert*-butoxycarbonylamino-1,12-dicarba-*closo*-dodecaborane (**16**)

To a solution of **15** (1.0 g, 3.47 mmol) in *tert*-butylalcohol (50 mL) were added [bis(trifluoroacetoxy)iodo]benzene (4.47 g, 10.4 mmol) and the mixture was heated at 80 °C for 3 h. After

cooling, the reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with 5% aqueous sodium thiosulfate, brine, water and brine, dried over sodium sulfate, then concentrated. Purification by silica gel flash column chromatography (eluent: hexane/ethyl acetate, 5:1) gave **16** (60%) as colorless solid and **17** (13%) as a byproduct. **16**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.50 (m, 2 H), 7.43 (m, 1 H), 7.34 (t, *J* = 7.8 Hz, 1 H), 4.85 (br s, 1 H), 3.6-1.5 (br m, 10 H), 1.39 (s, 9 H).

1-Amino-12-(3-cyanophenyl) -1,12-dicarba-*closo*-dodecaborane (**17**)

To a solution of **16** (1.75 g, 4.85 mmol) in dichloromethane (20 mL) was added dropwise trifluoroacetic acid (3.7 mL) and the mixture was stirred at room temperature for 18 h. The reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent: hexane/ethyl acetate, 4:1) gave **17** (87%) as colorless solid: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.50 (m, 2 H), 7.43 (m, 1 H), 7.34 (t, *J* = 7.8 Hz, 1 H), 3.8-1.5 (br m, 12 H); HRMS Calcd for $C_{14}H_{24}B_{10}N_2O_2$ Na [M + Na]: 383.2733. Found 383.2737.

General procedure for preparation of compounds 10a-c and 11a-b:

To a solution of **17** (50 mg, 0.19 mmol) in pyridine (2.0 mL) was added acid chloride or sulfonyl chloride (0.58 mmol) and the mixture was stirred at room temperature until the reaction had completed. The reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent: hexane/ethyl acetate) gave the products as a colorless solids. The products were further purified by recrystallization from *n*-hexane.

1-(3-Cyanophenyl)-12-(picolinamido)-1,12-dicarba-*closo*-dodecaborane (**10a**)

Colorless solid, mp: 168.5-169.5 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.54-8.47 (m, 2 H), 8.10 (d, *J* = 7.8 Hz, 1 H), 7.84 (dt, *J* = 1.6 Hz, 7.8 Hz, 1 H), 7.55-7.42 (m, 4 H), 3.5-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 161.2, 148.4, 147.9, 137.6, 137.2, 132.0, 131.6, 130.9, 129.1, 126.8, 122.4, 118.0, 112.7, 86.2, 77.4; HRMS Calcd for $C_{15}H_{19}B_{10}N_3O$ Na [M + Na]: 388.2424. Found 388.2423.

1-(3-Cyanophenyl)-12-(nicotinamido)-1,12-dicarba-*closo*-dodecaborane (**10b**)

Colorless solid, mp: 231.0-233.5 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.77 (s, 1 H), 8.73 (d, *J* = 3.8 Hz, 1 H), 7.95 (d, *J* = 7.9 Hz, 1 H), 7.53 (t, *J* = 8.0 Hz, 1 H), 7.52 (s, 1 H), 7.47 (d, *J* = 8.1 Hz, 1 H), 7.38 (dd, *J* = 7.7 Hz, 4.9 Hz, 1 H), 7.33 (t, *J* = 7.9 Hz, 1 H), 3.5-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 162.6, 153.0, 147.6, 136.9, 135.1, 132.1, 131.5, 130.8, 129.1, 123.6, 117.9, 112.7, 86.2, 77.4; HRMS Calcd for $C_{15}H_{19}B_{10}N_3O$ Na [M + Na]: 388.2424. Found 388.2432.

1-(3-Cyanophenyl)-12-(isonicotinamido)-1,12-dicarba-*closo*-dodecaborane (10c)

Colorless solid, mp: 282.5-284.5 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.71 (d, *J* = 6.1 Hz, 1 H), 7.55-7.50 (m, 2 H), 7.46 (ddd, *J* = 8.0 Hz, 2.0 Hz, 1.0 Hz, 1 H), 7.40 (d, *J* = 6.1 Hz, 2 H), 7.32 (t, *J* = 7.9 Hz, 1 H), 5.56 (br s, 1 H), 3.5-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 162.6, 150.7, 140.5, 136.9, 132.1, 131.4, 130.8, 129.1, 120.5, 117.8, 112.9, 86.0, 77.8; HRMS Calcd for $C_{15}H_{19}B_{10}N_3O$ Na [M + Na]: 388.2424. Found 388.2428.

1-(3-Cyanophenyl)-12-(benzenesulfonamido)-1,12-dicarba-*closo*-dodecaborane (11a)

Colorless solid, mp: 207.5-210.0 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, *J* = 8.2 Hz, 2 H), 7.66 (t, *J* = 8.0 Hz, 1 H), 7.56 (t, *J* = 8.2 Hz, 2 H), 7.50 (dt, *J* = 1.3 Hz, 7.6 Hz, 1 H), 7.41 (t, *J* = 1.6 Hz, 1 H), 7.36 (ddd, *J* = 8.2 Hz, 2.1 Hz, 1.2 Hz, 3 H), 7.28 (t, *J* = 8.0 Hz, 1 H), 5.56 (s, 1 H), 3.5-1.5 (br m, 10 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 138.9, 136.7, 133.6, 132.1, 131.5, 130.7, 129.14, 129.11, 128.0, 117.9, 112.7, 86.8, 77.4; HRMS Calcd for $C_{15}H_{20}B_{10}N_2O_2S$ Na [M + Na]: 423.2142. Found 423.2156.

1-(3-Cyanophenyl)-12-(*p*-toluenesulfonamido)-1,12-dicarba-*closo*-dodecaborane (11b)

Colorless solid, mp: 214.0-217.0 °C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, *J* = 8.4 Hz, 2 H), 7.51 (dt, *J* = 7.6 Hz, 1.3 Hz, 1 H), 7.42 (t, *J* = 1.5 Hz, 1 H), 7.39-7.34 (m, 3 H), 7.29 (t, *J* = 7.8 Hz, 1 H), 5.41 (s, 1 H), 3.5-1.5 (br m, 10 H), 2.49 (s, 3 H). ^{13}C-NMR (175 MHz, $CDCl_3$) δ 144.7, 136.7, 135.8, 132.1, 131.5, 130.8, 129.8, 129.2, 128.1, 118.0, 112.6, 86.8, 77.4, 21.7; HRMS Calcd for $C_{16}H_{22}B_{10}N_2O_2S$ Na [M + Na]: 437.2298. Found 437.2299.

Biology

LNCaP cell proliferation assay

The human prostate adenocarcinoma cell line, LNCaP was routinely cultivated in RPMI-1640 supplemented with 10% FBS and 100 IU/mL penicillin and 100 mg/mL streptomycin at 37 °C in a 5% CO_2 humidified incubator. Cells were trypsinized from the maintenance dish with trypsin-EDTA and seeded in a 96-well plate at a density of 3000 cells per final volume of 100 µL RPMI-1640 supplemented with 10% s.FBS. After 24 h, 10 µL of the medium was removed and 10 µL of the drug solution, supplemented with serial dilutions of the test compounds or DMSO as dilute control in the presence or absence of 1 nM testosterone, was added to triplicate microcultures. Cells were incubated for 6 days, and half of the media was removed and medium with the test compounds or DMSO as dilute control in the presence or absence of 1 nM testosterone was replaced once after 3 days. At the end of the incubation time, proliferation was evaluated by using the WST-8. 10 µM of WST-8 was added to microcultures and cells were incubated for 2-4 h. The absorbance at 450 nm was measured. This parameter relates to the number of living cells in the culture.

SC-3 Growth Promotion/Inhibition Assay

SC-3 cells were cultured in the presence of MEMα (Wako Co.) supplemented with 2% FBS and 10 nM testosterone at 37°C under 5% CO_2. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 20000 cells/mL with MEMα supplemented with 2% stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL and incubated at 24 h. After removal of 10 μL of medium from each well, 10 μL of the drug solution, which was supplemented with serial dilutions of the test compounds or DMSO as a dilution control in the presence or absence of 1 nM testosterone, was added. Then the plates were incubated at 37°C under 5% CO_2 for 3 days, and the cell number was determined using a Cell Counting Kit-8 (DOJINDO). A 10 μL aliquot of WST-8 was added to each well of microcultures, and the cells were incubated for 4 h. The absorbance at 450 nm was measured with a model 680 microplate reader (BIO-RAD). This parameter is related to the number of living cells in the culture.

Competitive Binding Assay for hAR-LBD

A hAR-LBD expression plasmid vector which encodes GST-hARLBD (627-919 aa, EF domain) fusion protein under the lac promoter (provided by Prof. S. Kato, University of Tokyo) was transfected into E. coli strain HB-101. An overnight culture (10 mL) of the bacteria was added to 1 L of LB medium and incubated at 27 °C until its optical density reached 0.6-0.7 at 600 nm. Following the addition of IPTG to a concentration of 1 mM, incubation was continued for an additional 4.5 h. Cells were harvested by centrifugation at 4000g at 4 °C for 15 min and stored at -80 °C until use. All subsequent operations were performed at 4 °C. The bacterial pellet obtained from 40 mL of culture was resuspended in 1 mL of ice-cold TEGDM buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 10% glycerol, 10 mM DTT, 10 mM sodium molybdate). This suspension was subjected to sonication using 10 × 10 s bursts on ice, and crude GST-hARLBD fraction was prepared by centrifugation of the suspension at 12000g for 30 min at 4 °C. This crude receptor fraction was diluted with buffer (20 mM Tris-HCl pH 8.0, 0.3 M KCl, 1 mM EDTA) to a protein concentration of 0.3-0.5 mg/mL and used in binding assays as hAR-LBD fraction. Aliquots of the hARLBD fraction were incubated in the dark at 4 °C with [^{3}H]-DHT (PerkinElmer, 4 nM final concentration), triamcinolone acetonide (1 μM final concentration), and reference or test compounds (dissolved in DMSO). Nonspecific binding was assessed by addition of a 200-fold excess of nonradioactive DHT. After 15 h, a Dextran T-70/γ-globulin-coated-charcoal suspension was added to the ligand/protein mixture (1% Norit A, 0.05% γ-globulin, 0.05% Dextran T-70 final concentration each) and the whole was incubated at 4 °C for 10 min. The charcoal was removed by centrifugation for 5 min at 1300g, and the radioactivity of the supernatant was measured in scintillation cocktail (ACS-2) by using a liquid scintillation counter.