Experimental Procedures

Synthesis

5-(4-chloro-3-methylphenyl)-N-(1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-1H-pyrazole-3-carboxamide (9). A mixture of **5** (237 mg, 1 mmol), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU) (417 mg, 1.1 mmol), and 1-Hydroxybenzotriazole (HOBT) (135 mg, 1 mmol) in 50/50 dichloromethane/DMF (10 mL) was stirred at RT for 5 min. DIEA (248 μL, 1.5 mmol) was added and the resulting mixture was stirred for another 10 min. A solution of **8** in 50/50 dichloromethane/DMF (10 mL) was then added to the mixture and the reaction mixture was stirred for another 2 h. The reaction solution was concentrated by vacuum rotary evaporation and purified with silica gel column chromatography eluted with 100% hexanes to 1:7 ethyl acetate/hexanes. Compound **9** was collected as a white solid (314 mg, 84%). ^{1}H NMR 400 MHz (CDCl3) δ 7.51 (s, 1H), 7.42 (s, 1H), 6.97 (s, 1H), 6.76 (bs, 1H), 3.84 (d, *J* = 9.6 Hz, 1H), 2.43 (s, 3H), 1.81 (d, *J* = 3.6 Hz, 1H), 1.73 (d, *J* = 10.8 Hz, 2H), 1.50-1.41 (m, 2H), 1.29-1.26 (m, 2H), 1.18 (s, 3H), 1.11 (s, 3H), 0.88 (s, 3H); MS (ESI)$^{+}$ $[M+Na]^{+}$ calcd 394.2, found 394.4.

1-(4-(bromomethyl)benzyl)-5-(4-chloro-3-methylphenyl)-N-(1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-1H-pyrazole-3-carboxamide (10) NaH (60% in mineral oil, 14.4 mg, 0.36 mmol) was added to a solution of **9** (111.6 mg, 0.3 mmol) in anhydrous toluene (30 mL) at room temperature. The mixture was heated at 70 °C for one hour and then cooled to room temperature. αα-Dibromo-p-xylene (791.9 mg, 3 mmol) was added and the resulting mixture was refluxed for 20 h. The reaction solution was then concentrated by vacuum rotary evaporation and the crude mixture was purified by column chromatography (silica gel) using gradient 100% hexanes to 1:9 ethyl acetate/hexanes as eluent. Compound **10** was collected

as an colorless oil (131.9 mg, 79%). ^{1}H NMR 400 MHz (CDCl3) δ 7.28 (s, 1H), 7.19 (s, 1H), 7.02 (s, 1H), 6.94 (d, *J* = 7.6 Hz, 4H), 6.77 (s, 1H), 5.24 (s, 2H), 4.40 (s, 2H), 3.75 (d, *J* = 9.6 Hz, 1H), 2.26 (s, 3H), 1.73 (s, 1H), 1.65 (d, *J* = 10.0 Hz, 2H), 1.43-1.34 (m, 2H), 1.20-1.45 (m, 2H), 1.10 (s, 3H), 1.04 (s, 3H), 0.80 (s, 3H); MS (ESI)$^+$ [M+Na]$^+$ calcd 578.1, found 578.2.

Mbc94 (11) A solution of **10** (22.2 mg, 40 μmol) in dichloromethane (5 mL) was added dropwise to a stirring solution of 1,6-hexane diamine (93 mg, 0.8 mmol) in dichloromethane (5 mL). The resulting mixture was stirred at room temperature for 5 h. The solution was then concentrated by rotary evaporation and chromatographed on a silica gel column using gradient 100% dichloromethane to 8:1:0.1 dichloromethane/methanol/ammonia as eluent. Mbc94 was collected as a pale yellow oil (19 mg, 70%). ^{1}H NMR 400 MHz (CDCl3) δ 7.33 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 1.6 Hz, 1H), 7.04-6.99 (m, 4H), 6.83 (s, 1H), 5.30 (s, 2H), 3.81 (dd, *J* = 9.6, 1.6 Hz, 1H), 3.76 (s, 2H), 2.68 (t, *J* = 2.4 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.32 (s, 3H), 1.79-1.78 (m, 1H), 1.72-1.70 (m, 5H), 1.53-1.41 (m, 6H), 1.34-1.30 (m, 4H), 1.26-1.21 (m, 2H), 1.16 (s, 3H), 1.11 (s, 3H), 0.86 (s, 3H); MS (ESI)$^+$ [M+H]$^+$ calcd 590.3, found 590.3.

NIRmbc94 A mixture of mbc94 (5 mg, 8.5 μmol) and IRDye^{TM} 800CW NHS ester (5 mg, 4.3 μmol) in DMSO (7 mL) was stirred under argon at room temperature overnight. HPLC analysis was performed to monitor the reaction on a Varian Polaris C-18 column (250 × 4.6 mm) at a flow rate of 0.8 mL/min. Flow A was 0.1% TEA in water and flow B was 0.1% TEA in acetonitrile. The elution method for analytical HPLC started with a linear gradient from 100% to 80% A over 30 min, then from 80% to 50% A for 5 min, arrived at 20% A in another 3 min, held at 20% A for 10 min, and finally returned to 100% A over 1 min. The elution profile was monitored by UV absorbance at 254 and 780 nm. Product was purified by preparative HPLC

using a Varian Polaris C-18 column (250 × 21.2 mm) at 17 mL/min. The collected solution was concentrated by vacuum rotary evaporation and frozen to -78ºC. The solvent (mostly water) was then removed via lyophilization. The amount of NIRmbc94 was determined by absorption in DMSO solution at 780 nm (3.0 mg, 44%). ^{1}H NMR 400 MHz (MeOD) δ 8.02 (d, *J* = 14.4 Hz, 1H), 7.89-7.78 (m, 5H), 7.74 (d, *J* = 1.2 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.34-7.32 (m, 4H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.20-7.17 (m, 3H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.84 (s, 1H), 6.33 (d, *J* = 14.0 Hz, 1H), 6.14 (d, *J* = 14.0 Hz, 1H), 5.49 (s, 2H), 4.20-4.15 (m, 4H), 4.09 (t, *J* = 2.4 Hz, 2H), 3.76 (dd, *J* = 9.2, 1.2 Hz, 1H), 3.01-2.93 (m, 4H), 2.89 (t, *J* = 2.8 Hz, 2H), 2.80 (t, *J* = 5.6 Hz, 2H), 2.74 (t, *J* = 5.6 Hz, 2H), 2.34 (s, 3H), 2.11 (t, *J* = 6.8 Hz, 2H), 2.05-2.02 (m, 2H), 1.95-1.92 (m, 5H), 1.79-1.71 (m, 6H), 1.64-1.49 (m, 8H), 1.40 (s, 6H), 1.34 (s, 6H), 1.25-1.21 (m, 7H), 1.15 (s, 3H), 1.08 (s, 3H), 0.92-0.86 (m, 2H), 0.85 (s, 3H). MS (ESI)$^-$ [M-H]$^-$ calcd 1572.6, found 1572.5.

Spectroscopic Characterization

The absorbance spectrum of NIRmbc94 was measured using a Shimadzu UV-VIS 1700 spectrophotometer (Columbia, MD) and the emission spectrum was measured using a PTI Technologies spectrofluorometer (Oxnard, CA; excitation wavelength: 780nm, 1 nm/second scan rate, 1.5 nm slit width and 75 watts Photomultiplier tube voltage).

Live Cell Imaging

The details of the generation and maintenance of wild-type and mouse CB_2-expressing DBT cells, a mouse astrocytoma cell line, will be described in a future manuscript. Briefly, mouse CB_2 receptor expression was obtained by transfection and generation of stable cell line, and confirmed by Q-PCR and radioligand binding. Both wild-type and CB_2-expressing DBT cells were expanded in DMEM containing Glutamax™, D-glucose (4.5g/l) and sodium pyruvate

(110 mg/ml) (Invitrogen/Gibco 10569), and supplemented with FBS (10%), HEPES (10 mM), $NaHCO_3$ (5 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were then cultured in MaTek dishes and incubated for 30 min with 5 μM NIRmbc94 or 5 μM free NIR dye in culture media. Cells were rinsed and re-incubated with culture media before imaging with a Nikon Eclipse TE2000-U microscope (Lewisville, TX) equipped with a Hamamatsu C4742-98 camera, Nikon Plan Apo 60x/1.40 oil objective, a mercury lamp and an ICG filter set (Figure 4&5).

***In Vitro* Competition Study**

CB_2-expressing DBT cells were plated in MaTek dishes. The culture media was removed and replaced with media containing molecular imaging agent. Cells were incubated for 30 minutes at 37 °C under 5% CO_2 with 5 μM NIRmbc94 or with 5 μM NIRmbc94 and 100 nM SR144528, after which cells were rinsed three times with culture media and imaged.