Supporting Information

Towards the Development of Bivalent Ligand Probes of Cannabinoid CB1 and Orexin OX1 Receptor Heterodimers

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Experimental Procedures

General. All solvents and chemicals were reagent grade. Unless otherwise mentioned, all were purchased from commercial vendors and used as received. Flash column chromatography was done on a Teledyne ISCO CombiFlash Rf system using prepacked columns. Solvents used were hexane, ethyl acetate (EtOAc), dichloromethane, methanol and chloroform:methanol:ammonium hydroxide (80:18:2) (CMA-80). Purity and characterization of compounds was established by a combination of high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) analysis. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz) spectrometer and were determined in chloroform-d or methanol-d4 with tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in ppm relative to the reference signal, and coupling constant (J) values are reported in Hz. TLC was done on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light or iodine staining. Low resolution mass spectra were obtained using a Waters Alliance HT/Micromass ZQ system (ESI). All test compounds were greater than 90% pure as determined by HPLC on an Agilent 1100 system using an Agilent Zorbax SB-Phenyl, 2.1 mm x 150 mm, 5 μm column with gradient elution using the mobile phases (A) H_2O containing 0.1% CF_3COOH and (B) MeCN, with a flow rate of 1.0 mL/min.

Compounds **7a**, **7c**, **8a**, **8c**, **9a**, **9c**, **10**, **11**, **16d** and **25** are reported elsewhere.[1,2]

N-[2-(3,4-Dimethoxyphenyl)ethyl]-2-(3-hydroxy-4-methoxyphenyl)acetamide (7b). 3-Hydroxy-4-methoxyphenylacetic acid (1.0 g, 5.49 mmol), 3,4-dimethoxyphenethylamine (1.0 g, 0.93 mL, 5.49 mmol) and HBTU (2.08 g, 5.49 mmol)

were combined in dry dimethylformamide (55 mL) at RT under N_2. Diisopropylethylamine (1.77 g, 2.4 mL, 13.72 mmol) was added and the reaction stirred at RT overnight. The reaction was diluted with ethyl acetate, washed with 2N hydrochloric acid, sodium bicarbonate solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure to give the desired amide as a yellow oil which solidified upon standing (1.50 g, 79%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 6.82 - 6.88 (m, 1H), 6.71 (d, *J* = 8.10 Hz, 1H), 6.58 - 6.67 (m, 3H), 6.51 (dd, *J* = 1.88, 8.10 Hz, 1H), 5.74 (s, 1H), 5.44 (br. s., 1H), 3.86 (s, 3H), 3.83 (s, 6H), 3.38 - 3.49 (m, 4H), 2.67 (t, *J* = 6.88 Hz, 2H).

5-[(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl]-2-methoxyphenol (8b). Amide **7b** (1.51 g, 4.37 mmol) was suspended in anhydrous toluene (22 mL) and phosphorous oxychloride (4.02 g, 2.45 mL, 26.23 mmol) added slowly. The reaction was heated to 90 °C for 2 hours, during which the solid went into solution, then a red oil separated. The reaction was cooled, then quenched by slow addition of the reaction mixture to water and heated until a solution formed. Sodium hydroxide solution (2N) was added until pH was 8-9, then the solution was extracted 3 times with dichloromethane. The combined extracts were dried over $MgSO_4$ and the solvent removed under reduced pressure.

The crude dihydroisoquinoline was dissolved in methanol (25 mL) and cooled in an ice bath under N_2. Sodium borohydride (0.83 g, 21.99 mmol) was added portionwise and the reaction allowed to warm slowly to RT overnight. The reaction was quenched with water then the methanol removed under reduced pressure. The aqueous solution was extracted 3 times with dichloromethane and the combined extracts were dried over

$MgSO_4$ and the solvent removed under reduced pressure to give the desired tetrahydroisoquinoline **8b** as a frothy solid (0.73 g, 91%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 6.81 - 6.90 (m, 1H), 6.69 - 6.79 (m, 3H), 6.66 (s, 1H), 6.59 (s, 1H), 4.50 (br. s., 1H), 4.07 - 4.14 (m, 1H), 3.86 (s, 6H), 3.84 (s, 3H), 3.11 - 3.25 (m, 2H), 2.82 - 2.98 (m, 4H).

N-Benzyl-2-{1-[(3-hydroxy-4-methoxyphenyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}acetamide (9b). Amine **8b** (0.20 g, 0.61 mmol), N-benzyl-2-bromoacetamide (0.152 g, 0.67 mmol) and tetrabutylammonium iodide (0.045 g, 0.12 mmol) were combined in dry dimethylformamide (6 mL) and diisopropylethylamine (0.196 g, 0.26 mL, 1.52 mmol) was added. The reaction was stirred at RT overnight under N_2. The reaction was diluted with EtOAc, washed with $NaHCO_3$ solution, water and brine (x2), then dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by chromatography on silica (0-60% EtOAc in hexane) to obtain the desired product as a frothy white solid (0.097 g, 33%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.21 - 7.36 (m, 3H), 7.14 (d, *J* = 6.69 Hz, 2H), 6.93 - 7.04 (m, 1H), 6.76 - 6.84 (m, 1H), 6.66 - 6.73 (m, 1H), 6.64 (d, *J* = 1.51 Hz, 1H), 6.59 (s, 1H), 6.45 (s, 1H), 5.50 (s, 1H), 4.48 (dd, *J* = 8.01, 14.69 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 3.76 (s, 3H), 3.56 - 3.72 (m, 2H), 3.35 - 3.49 (m, 1H), 3.09 - 3.34 (m, 2H), 2.79 - 3.01 (m, 4H), 2.42 - 2.56 (m, 1H). *m/z* 477 (M+H).

4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenyl butanoate (12). Phenol **9b** (50 mg, 0.105 mmol) and BOP (46 mg, 0.105 mmol) were combined in dichloromethane (1 mL). Butyric acid (9 mg, 10 μL, 0.105 mmol) and diisopropylethylamine (34 mg, 46 μL, 0.262 mmol) were added

and the reaction stirred at RT under N_2 overnight. The reaction was diluted with EtOAc, washed with 2N HCl, saturated $NaHCO_3$ solution and saturated brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by chromatography on silica (0-75% EtOAc in hexane) to give the desired product (46 mg, 81%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.23 - 7.35 (m, 3H), 7.14 - 7.21 (m, 2H), 7.07 (t, *J* = 6.22 Hz, 1H), 6.78 - 6.85 (m, 1H), 6.66 - 6.74 (m, 2H), 6.58 (s, 1H), 6.36 (s, 1H), 4.40 - 4.50 (m, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.70 (s, 3H), 3.63 - 3.95 (m, 2H), 3.11 - 3.47 (m, 3H), 2.78 - 3.04 (m, 4H), 2.42 - 2.63 (m, 3H), 1.73 - 1.89 (m, 2H), 1.07 (t, *J* = 7.39 Hz, 3H). *m/z* 547 (M+H). Purity (HPLC) 95.7%.

N-Benzyl-2-(1-{[4-(hexyloxy)-3-methoxyphenyl]methyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)acetamide (13). Phenol **9b** (50 mg, 0.105 mmol), potassium carbonate (29 mg, 0.210 mmol) and tetrabutylammonium iodide (8 mg, 0.021 mmol) were combined in dimethylformamide (1 mL) and 1-bromohexane (19 mg, 16 μL, 0.115 mmol) was added. The reaction was heated at 50 °C overnight. It was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by chromatography on silica (0-75% EtOAc in hexane) to give the desired product (43 mg, 73%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.19 - 7.34 (m, 3H), 7.08 - 7.16 (m, 2H), 7.01 (dd, *J* = 5.23, 7.49 Hz, 1H), 6.63 - 6.73 (m, 3H), 6.59 (s, 1H), 6.44 (s, 1H), 4.49 (dd, *J* = 8.01, 14.88 Hz, 1H), 3.84 - 3.90 (m, 5H), 3.81 (s, 3H), 3.79 (s, 3H), 3.58 - 3.71 (m, 2H), 3.34 - 3.49 (m, 1H), 3.10 - 3.34 (m, 2H), 2.79 - 2.99 (m, 4H), 2.41 - 2.56 (m, 1H), 1.74 - 1.88 (m, 2H), 1.29 - 1.51 (m, 6H), 0.91 (t, *J* = 6.78 Hz, 3H). *m/z* 561 (M+H). Purity (HPLC) 96.6%.

2-{1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}-2-phenylacetic acid (14). The ester intermediate was prepared as per **9b**, using **8c** and ethyl α-bromophenylacetate and purified by chromatography on silica (0-80% EtOAc in hexane) to give the ester as a mixture of diastereomers (64% yield). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.58 (dd, *J* = 1.79, 7.63 Hz, 1H), 7.34 - 7.43 (m, 2H), 7.18 - 7.33 (m, 2H), 6.72 (t, *J* = 8.01 Hz, 1H), 6.58 (d, *J* = 6.03 Hz, 1H), 6.35 - 6.53 (m, 2H), 5.64 - 5.91 (m, 1H), 4.47 - 4.55 (m, 1H), 4.02 - 4.22 (m, 2H), 3.76 - 3.93 (m, 7H), 3.71 - 3.75 (m, 3H), 3.41 - 3.57 (m, 3H), 3.08 - 3.24 (m, 2H), 2.63 - 3.02 (m, 3H), 2.40 - 2.53 (m, 1H), 1.10 - 1.23 (m, 3H).

The ester (0.18 g, 0.36 mmol) was dissolved in ethanol (4 mL) and 2N NaOH solution (1.8 mL, 3.6 mmol) was added and the reaction stirred at RT overnight. The ethanol was removed under reduced pressure, diluted with water and the pH adjusted to 6 with 2N HCl. The solution was extracted twice with EtOAc, then the combined extracts were dried over MgSO4 and the solvent removed under reduced pressure to give the desired acid as a mixture of diastereomers (0.11 g, 64%). ^{1}H NMR (300 MHz, METHANOL-d_4) δ 7.46 - 7.72 (m, 2H), 7.32 - 7.46 (m, 2H), 7.13 - 7.22 (m, 1H), 6.73 - 6.96 (m, 2H), 6.47 - 6.70 (m, 2H), 5.57 - 6.00 (m, 1H), 4.47 - 4.65 (m, 1H), 3.74 - 3.86 (m, 7H), 3.68 - 3.73 (m, 3H), 3.47 - 3.54 (m, 2H), 3.21 - 3.37 (m, 4H), 3.01 - 3.20 (m, 2H), 2.61 - 3.00 (m, 2H).

2-{1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}-N-heptyl-2-phenylacetamide (15). Acid **14** (50 mg, 0.105 mmol), 1-heptylamine (12 mg, 16 μL, 0.105 mmol) and BOP (46 mg, 0.105 mmol) were combined in dichloromethane (1 mL). Diisopropylethylamine (27 mg, 37 μL, 0.210 mmol) was added

and the reaction stirred at RT overnight. It was diluted with EtOAc, washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude was purified by chromatography on silica (0-50% EtOAc in hexane) to give the desired product (37 mg, 62%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.30 - 7.40 (m, 2H), 7.13 - 7.24 (m, 2H), 6.90 - 6.99 (m, 1H), 6.76 - 6.88 (m, 2H), 6.67 (d, *J* = 8.19 Hz, 1H), 6.60 (d, *J* = 16.11 Hz, 2H), 5.99 - 6.27 (m, 1H), 4.18 - 4.34 (m, 1H), 3.83 - 3.94 (m, 9H), 3.65 - 3.75 (m, 3H), 3.48 (dd, *J* = 5.70, 9.94 Hz, 1H), 2.86 - 3.33 (m, 6H), 2.77 (dd, *J* = 5.46, 13.94 Hz, 1H), 2.51 (dd, *J* = 5.09, 16.58 Hz, 1H), 1.05 - 1.64 (m, 10H), 0.82 - 0.94 (m, 3H). *m/z* 575 (M+H). Purity (HPLC) 96.4%.

N-(7-Aminoheptyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (16a). 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid[1] (0.37 g, 0.97 mmol), 1,7-diaminoheptane (0.63 g, 4.85 mmol) and BOP (0.43 g, 0.97 mmol) were combined in anhydrous tetrahydrofuran (10 mL) and the reaction stirred under N_2 at RT overnight. The reaction was diluted with EtOAc, washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude was purified by chromatography on silica (0-80% CMA-80 in EtOAc) to give the desired product (0.29 g, 60%). ^{1}H NMR (300 MHz, METHANOL-d_4) δ 7.49 - 7.59 (m, 2H), 7.42 - 7.47 (m, 1H), 7.36 (d, *J* = 8.48 Hz, 2H), 7.19 (d, *J* = 8.67 Hz, 2H), 3.33 - 3.39 (m, 2H), 2.62 (t, *J* = 7.06 Hz, 2H), 2.30 (s, 3H), 1.61 (t, *J* = 6.69 Hz, 2H), 1.43 - 1.54 (m, 2H), 1.31 - 1.43 (m, 6H).

N-(10-Aminodecyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (16b). Prepared as per **16a** using 1,10-diaminodecane. Yield 33%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.32 Hz, 1H), 7.23 - 7.33

(m, 6H), 7.03 - 7.09 (m, 2H), 6.95 (t, *J* = 5.65 Hz, 1H), 3.35 - 3.45 (m, 2H), 2.61 - 2.71 (m, 2H), 2.35 - 2.40 (m, 3H), 1.51 - 1.65 (m, 2H), 1.23 - 1.48 (m, 14H).

N-(10-Aminododecyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (16c). Prepared as per **16a** using 1,12-diaminododecane. Yield 59%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.70 Hz, 1H), 7.24 - 7.34 (m, 5H), 7.03 - 7.09 (m, 2H), 6.95 (t, *J* = 5.84 Hz, 1H), 3.41 (q, *J* = 6.84 Hz, 2H), 2.68 (t, *J* = 6.97 Hz, 2H), 2.38 (s, 3H), 1.54 - 1.66 (m, 2H), 1.22 - 1.49 (m, 18H).

7-[(7-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}heptyl)(methyl)amino]heptanoic acid (17a). Ethyl 7-bromoheptanoate (0.33 g, 0.27 mL, 1.39 mmol) and amine **16a** (0.69 g, 1.39 mmol) were combined in toluene (15 mL) and triethylamine (0.28 g, 0.39 mL, 2.78 mmol) was added. The reaction was heated to reflux overnight. The reaction was cooled, solvent was removed under reduced pressure and the crude partitioned between EtOAc and $NaHCO_3$ solution. The layers were separated, the aqueous portion was extracted twice more with EtOAc, then the combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by chromatography on silica (0-30% CMA-80 in EtOAc) to give the desired product (0.33 g, 37%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.88 Hz, 1H), 7.25 - 7.33 (m, 4H), 7.03 - 7.09 (m, 2H), 6.97 (t, *J* = 5.75 Hz, 1H), 4.12 (q, *J* = 7.16 Hz, 2H), 3.36 - 3.45 (m, 2H), 2.53 - 2.61 (m, 4H), 2.37 (s, 3H), 2.29 (t, *J* = 7.44 Hz, 2H), 1.53 - 1.68 (m, 4H), 1.42 - 1.52 (m, 4H), 1.29 - 1.40 (m, 10H), 1.25 (t, *J* = 7.16 Hz, 3H).

Formaldehyde (37% aqueous solution, 0.21 g, 0.19 mL, 2.54 mmol) was added to a solution of the ester (0.33 g, 0.51 mmol) in 1,2-dichloroethane (5 mL), then sodium

triacetoxyborohydride (0.54 g, 2.54 mmol) was added portionwise. The reaction was stirred at RT for 2 hr, then the solvent was removed under reduced pressure and the crude purified by chromatography on silica (0-30% CMA-80 in EtOAc) to give the desired methylamine as a pale yellow oil (0.22 g, 65%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.88 Hz, 1H), 7.23 - 7.33 (m, 4H), 7.02 - 7.10 (m, 2H), 6.94 (t, *J* = 5.84 Hz, 1H), 4.12 (q, *J* = 7.10 Hz, 2H), 3.41 (q, *J* = 6.72 Hz, 2H), 2.38 (s, 3H), 2.24 - 2.32 (m, 6H), 2.18 (s, 3H), 1.55 - 1.68 (m, 4H), 1.43 (d, *J* = 5.65 Hz, 4H), 1.28 - 1.38 (m, 10H), 1.21 - 1.28 (m, 3H).

To a solution of the ester (0.22 g, 0.33 mmol) in ethanol (6 mL) was added 2N sodium hydroxide solution (0.66 mL, 1.32 mmol) and the reaction stirred at RT overnight. The ethanol was removed under reduced pressure, the aqueous solution washed with ether then acidified to pH 6 with 2N HCl. The solution was extracted 3 times with EtOAc, the combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent removed under reduced pressure to give the acid (0.21 g, 95%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 11.62 (br. s., 1H), 7.41 - 7.44 (m, 1H), 7.27 - 7.34 (m, 4H), 7.02 - 7.12 (m, 3H), 3.42 (q, *J* = 6.78 Hz, 2H), 2.72 - 2.79 (m, 4H), 2.32 - 2.40 (m, 5H), 2.10 (s, 3H), 1.74 - 1.91 (m, 4H), 1.54 - 1.70 (m, 4H), 1.34 - 1.47 (m, 10H).

7-[(10-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}decyl)(methyl)amino]heptanoic acid (17b). Prepared as per **17a** using **16b**. Yield 9% over 3 steps. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.88 Hz, 1H), 7.23 - 7.35 (m, 4H), 6.98 - 7.11 (m, 3H), 3.35 - 3.47 (m, 2H), 2.90 - 3.06 (m, 4H), 2.75 (s, 3H), 2.31 - 2.41 (m, 5H), 1.75 - 1.91 (m, 4H), 1.53 - 1.71 (m, 4H), 1.23 - 1.48 (m, 16H).

7-[(12-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}dodecyl)(methyl)amino]heptanoic acid (17c). Prepared as per **17a** using **16c**. Yield 15% over 3 steps. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.88 Hz, 1H), 7.24 - 7.35 (m, 4H), 7.06 (d, *J* = 8.48 Hz, 2H), 6.96 - 7.02 (m, 1H), 3.41 (q, *J* = 6.84 Hz, 2H), 2.93 - 3.04 (m, 4H), 2.76 (s, 3H), 2.30 - 2.41 (m, 5H), 1.75 - 1.92 (m, 4H), 1.54 - 1.70 (m, 4H), 1.21 - 1.47 (m, 20H).

1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}-12-methyl-3,6,9-trioxa-12-azanonadecan-19-oic acid (17d). Prepared as per **17a** using **16d**. Yield 22% over 3 steps. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 10.71 (br. s., 1H), 7.35 - 7.45 (m, 2H), 7.23 - 7.34 (m, 4H), 7.06 (d, *J* = 8.29 Hz, 2H), 3.83 - 3.96 (m, 2H), 3.55 - 3.77 (m, 12H), 2.97 - 3.41 (m, 4H), 2.88 (s, 3H), 2.27 - 2.39 (m, 5H), 1.74 - 1.88 (m, 2H), 1.56 - 1.70 (m, 2H), 1.32 - 1.44 (m, 4H).

2-[(Benzylcarbamoyl)methyl]-1-[(3,4-dimethoxyphenyl)methyl]-6-methoxy-1,2,3,4-tetrahydroisoquinolin-7-yl 7-[(7-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}heptyl)(methyl)amino]heptanoate (18). Acid **17a** (73 mg, 0.115 mmol), phenol **9a** (55 mg, 0.115 mmol) and BOP (51 mg, 0.115 mmol) were combined in dry dichloromethane (1 mL) and diisopropylethylamine (37 mg, 50 μL, 0.289 mmol) was added slowly. The reaction was stirred at RT under N_2 overnight. It was diluted with EtOAc, then washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by chromatography on silica (0-30% CMA-80 in EtOAc) to give the desired ester (21 mg, 17%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.18 - 7.34 (m, 8H), 7.01 - 7.13 (m, 4H), 6.85 - 6.98 (m, 2H), 6.75 (s, 1H), 6.56 - 6.71 (m, 4H), 4.47 (dd, *J* = 7.96,

15.02 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.73 (s, 3H), 3.57 - 3.70 (m, 2H), 3.34 - 3.46 (m, 3H), 3.10 - 3.33 (m, 2H), 2.77 - 3.03 (m, 4H), 2.51 - 2.60 (m, 3H), 2.37 (s, 3H), 2.25 - 2.35 (m, 4H), 2.20 (s, 3H), 1.77 (quin, *J* = 7.35 Hz, 2H), 1.54 - 1.68 (m, 2H), 1.26 - 1.53 (m, 14H). *m/z* 1096, 1094 (M+H). Purity (HPLC) 95.2%.

4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenyl 7-[(7-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}heptyl)(methyl)amino]heptanoate (19). This was prepared as per **18** using **17a** and **9b**. Yield 29%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.32 Hz, 1H), 7.21 - 7.34 (m, 7H), 7.17 (d, *J* = 6.69 Hz, 2H), 7.06 (d, *J* = 8.48 Hz, 3H), 6.94 (t, *J* = 5.60 Hz, 1H), 6.76 - 6.84 (m, 1H), 6.65 - 6.73 (m, 2H), 6.58 (s, 1H), 6.36 (s, 1H), 4.45 (dd, *J* = 7.54, 14.79 Hz, 1H), 3.88 - 3.96 (m, 1H), 3.86 (s, 3H), 3.78 (s, 3H), 3.64 - 3.71 (m, 5H), 3.11 - 3.46 (m, 5H), 2.78 - 3.03 (m, 4H), 2.58 (t, *J* = 7.44 Hz, 2H), 2.44 - 2.54 (m, 1H), 2.38 (s, 3H), 2.25 - 2.35 (m, 4H), 2.20 (s, 2H), 1.71 - 1.85 (m, 2H), 1.54 - 1.68 (m, 2H), 1.24 - 1.53 (m, 14H). *m/z* 1096, 1094 (M+H). Purity (HPLC) 96.7%.

4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenyl 7-[(10-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}decyl)(methyl)amino]heptanoate (20). This was prepared as per **18** using **17b** and **9b**. Yield 73%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.88 Hz, 1H), 7.21 - 7.35 (m, 7H), 7.13 - 7.20 (m, 2H), 7.06 (d, *J* = 8.29 Hz, 3H), 6.94 (t, *J* = 5.79 Hz, 1H), 6.77 - 6.84 (m, 1H), 6.66 - 6.73 (m, 2H), 6.58 (s, 1H), 6.36 (s, 1H), 4.45 (dd, *J* = 7.49, 14.83 Hz, 1H), 3.91 (d, *J* = 5.37 Hz, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.62 - 3.72 (m, 4H), 3.36 - 3.47 (m, 3H), 3.11 - 3.35 (m, 2H), 2.79 - 3.03 (m,

4H), 2.43 - 2.62 (m, 3H), 2.26 - 2.40 (m, 7H), 2.21 (s, 3H), 1.68 - 1.85 (m, 4H), 1.21 - 1.66 (m, 20H). *m/z* 1138, 1136 (M+H). Purity (HPLC) 97.5%.

4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenyl 7-[(12-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}dodecyl)(methyl)amino]heptanoate (21). This was prepared as per **18** using **17c** and **9b**. Yield 39%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.98 Hz, 1H), 7.22 - 7.35 (m, 7H), 7.17 (d, *J* = 6.78 Hz, 2H), 7.03 - 7.13 (m, 3H), 6.97 (t, *J* = 5.89 Hz, 1H), 6.77 - 6.84 (m, 1H), 6.66 - 6.74 (m, 2H), 6.59 (s, 1H), 6.37 (s, 1H), 4.44 (dd, *J* = 7.63, 15.07 Hz, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.61 - 3.73 (m, 5H), 3.32 - 3.45 (m, 3H), 3.11 - 3.31 (m, 2H), 2.79 - 3.03 (m, 4H), 2.46 - 2.62 (m, 3H), 2.37 (s, 3H), 2.29 - 2.40 (m, 4H), 2.23 (s, 3H), 1.78 (td, *J* = 7.51, 15.12 Hz, 2H), 1.15 - 1.67 (m, 26H). *m/z* 1166, 1164 (M+H). Purity (HPLC) 100%.

4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenyl 1-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}-12-methyl-3,6,9-trioxa-12-azanonadecan-19-oate (22). This was prepared as per **18** using **17d** and **9b**. Yield 34%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.42 (d, *J* = 1.04 Hz, 1H), 7.22 - 7.35 (m, 7H), 7.13 - 7.21 (m, 2H), 7.06 (d, *J* = 8.29 Hz, 4H), 6.76 - 6.85 (m, 1H), 6.66 - 6.74 (m, 2H), 6.58 (s, 1H), 6.36 (s, 1H), 4.45 (dd, *J* = 7.49, 14.84 Hz, 1H), 3.86 (s, 3H), 3.78 (s, 3H), 3.69 (s, 3H), 3.59 - 3.67 (m, 12H), 3.50 - 3.58 (m, 3H), 3.11 - 3.46 (m, 4H), 2.79 - 3.03 (m, 4H), 2.51 - 2.61 (m, 5H), 2.30 - 2.43 (m, 5H), 2.19 - 2.28 (m, 3H), 1.20 - 1.56 (m, 8H). *m/z* 1157, 1155 (M+H). Purity (HPLC) 94.1%.

7-[4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenoxy]heptanoic acid (23). Phenol **9b** (150 mg, 0.315 mmol), ethyl 7-bromoheptanoate (82 mg, 68 μL, 0.346 mmol), potassium carbonate (87 mg, 0.629 mmol) and tetrabutylammonium iodide (23 mg, 0.063 mmol) were combined in dry dimethylformamide (3 mL) and heated to 50 °C overnight. The reaction was cooled, diluted with EtOAc and then washed with water and brine. The aqueous portion was back-extracted with EtOAc and then the combined organic fractions were dried over $MgSO_4$ and the solvent removed under reduced pressure. TLC (3:1 EtOAc/hexane) showed one spot and the material was used in the next step without further purification. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.18 - 7.35 (m, 3H), 7.08 - 7.14 (m, 2H), 7.01 (dd, *J* = 5.18, 7.82 Hz, 1H), 6.62 - 6.72 (m, 3H), 6.58 (s, 1H), 6.44 (s, 1H), 4.49 (dd, *J* = 7.91, 14.88 Hz, 1H), 4.13 (q, *J* = 7.16 Hz, 2H), 3.86 (s, 3H), 3.83 - 3.89 (m, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.65 (dd, *J* = 4.90, 15.07 Hz, 2H), 3.35 - 3.46 (m, 2H), 3.11 - 3.33 (m, 2H), 2.79 - 2.94 (m, 4H), 2.43 - 2.54 (m, 1H), 2.30 (t, *J* = 7.54 Hz, 2H), 1.75 - 1.91 (m, 2H), 1.61 - 1.72 (m, 2H), 1.31 - 1.53 (m, 4H), 1.26 (t, *J* = 7.16 Hz, 3H).

To a solution of the ester (199 mg, 0.315 mmol) in ethanol (5 mL) was added 2N sodium hydroxide solution (0.63 mL, 1.26 mmol) and the reaction stirred at RT overnight. The ethanol was removed under reduced pressure, the aqueous solution washed with ether then acidified to pH 6 with 2N HCl. The solution was extracted 3 times with EtOAc, the combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent removed under reduced pressure to give the acid (164 mg, 86%). ^{1}H NMR (300 MHz, METHANOL-d_4) δ 7.19 - 7.32 (m, 3H), 7.06 (d, *J* = 6.97 Hz, 2H), 6.91 (s, 1H), 6.72 - 6.84 (m, 2H), 6.69 (d, *J* = 4.71 Hz, 2H), 4.37 (d, *J* = 15.26 Hz, 1H),

3.80 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.74 - 3.82 (m, 2H), 3.61 - 3.73 (m, 3H), 3.36 - 3.60 (m, 3H), 2.84 - 3.02 (m, 4H), 2.46 - 2.57 (m, 1H), 2.29 (t, *J* = 7.44 Hz, 2H), 1.68 - 1.91 (m, 2H), 1.55 - 1.68 (m, 2H), 1.32 - 1.52 (m, 4H).

7-[4-({2-[(Benzylcarbamoyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl}methyl)-2-methoxyphenoxy]-N-(2-{2-[2-(2-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}ethoxy)ethoxy]ethoxy}ethyl)-N-methylheptanamide (24). Acid **23** (29 mg, 0.048 mmol), amine **16d** (27 mg, 0.048 mmol) and BOP (21 mg, 0.048 mmol) were combined in dry dimethylformamide (1 mL). Diisopropylethylamine (15 mg, 21 μL, 0.120 mmol) was added and the reaction stirred at RT overnight. The reaction was diluted with EtOAc, washed with $NaHCO_3$ solution and brine, then the aqueous was back-extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude was purified by chromatography on silica (0-20% CMA-80 in EtOAc) to give the desired product (26 mg, 47%). ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.39 - 7.45 (m, 1H), 7.17 - 7.36 (m, 8H), 6.96 - 7.14 (m, 5H), 6.62 - 6.72 (m, 3H), 6.58 (s, 1H), 6.44 (s, 1H), 6.15 (br. s., 1H), 4.48 (dd, *J* = 8.01, 14.98 Hz, 1H), 3.86 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.76 - 3.89 (m, 2H), 3.59 - 3.69 (m, 12H), 3.53 - 3.59 (m, 2H), 3.47 - 3.53 (m, 2H), 3.35 - 3.46 (m, 3H), 3.10 - 3.33 (m, 2H), 2.79 - 2.98 (m, 4H), 2.42 - 2.54 (m, 1H), 2.37 (s, 3H), 2.17 (t, *J* = 7.49 Hz, 2H), 1.73 - 1.87 (m, 2H), 1.65 (td, *J* = 7.52, 14.62 Hz, 2H), 1.30 - 1.52 (m, 4H). *m/z* 1144, 1142 (M+H). Purity (HPLC) 96.0%.

N-{7-[(7-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]formamido}heptyl)(methyl)amino]heptyl}-2-{1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl}-2-phenylacetamide (26). This was

prepared as per **24** using acid **14** and amine **25**. Yield 40%. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.43 (d, *J* = 1.41 Hz, 1H), 7.26 - 7.39 (m, 6H), 7.20 - 7.26 (m, 1H), 7.12 - 7.20 (m, 1H), 7.02 - 7.10 (m, 2H), 6.95 (t, *J* = 5.98 Hz, 1H), 6.77 - 6.91 (m, 3H), 6.59 - 6.69 (m, 1H), 6.57 (d, *J* = 1.88 Hz, 1H), 6.52 (d, *J* = 1.70 Hz, 1H), 6.17 (s, 1H), 6.01 (s, 1H), 4.29 (s, 1H), 4.21 (s, 1H), 3.72 - 3.94 (m, 9H), 3.60 - 3.71 (m, 3H), 3.34 - 3.52 (m, 3H), 2.86 - 3.30 (m, 4H), 2.69 - 2.82 (m, 1H), 2.42 - 2.53 (m, 1H), 2.22 - 2.40 (m, 7H), 2.15 - 2.21 (m, 3H), 1.53 - 1.66 (m, 2H), 1.07 - 1.52 (m, 18H). *m/z* 1082, 1080 (M+H). Purity (HPLC) 98.9%.

Generation of Stable CB1-OX1 Coexpressing Cell Lines

The CB1/OX1 dual-expressing cell line was created by stably expressing OX1 receptors in our cells singly expressing CB1 receptors. Briefly, cDNA coding for OX1 (Open Biosystems) was subcloned into the pcDNA3.1/Zeocin mammalian expression vector (Life Technologies) and transfected into our CB1/RD-HGA16 cells using Fugene transfection reagent. Cells were then selected by Zeocin resistance and for functional response to Orexin A for OX1 expression and to CP55940 for CB1 expression using a FlexStation II 384 fluorometric imaging plate reader. A positive cell line with good efficacy and potency for both ligands was chosen for further studies and maintained in both zeomycin, for OX1 expression, and geneticin, for CB1 expression.

Radioligand Binding Studies

Dual-expressing cells were grown in tissue culture flasks overnight to near confluence. Cells were lifted from the culture flasks using versene and pelleted by centrifugation at

115 X g for 5 minutes at RT. The cell pellet was suspended in assay buffer (HBSS with 20 mM Hepes and 0.5% fatty acid-free BSA, pH=7.4). Binding assays were performed with a range of concentrations of [^{125}I]-Orexin A which were made by serial dilution in assay buffer. Each concentration of radioligand was incubated with a cell suspension (approximately 200,000 cells/tube) with or without the appropriate competitor - 100 nM unlabeled Orexin A. The reactions were incubated at RT for 1 hour and then centrifuged at 1000 X g for 5 minutes. The supernatants were discarded and the pellets were washed in assay buffer and centrifuged again. This wash was repeated for a total of two washes. After discarding the final supernatants, 10 μL of 1 N NaOH was added to each reaction to solubilize the pellets. The reactions were also incubated at 65^{o}C for 1 hour or overnight to further solubilize the pellets followed by transfer to polypropylene test tubes and determination of CPM in a Packard Cobra Quantum gamma counter. Receptor-specific binding was calculated as total CPM minus CPM in the presence of unlabeled Orexin A. Binding assays demonstrate 293.6 fmol OX1 receptor/10^6 cells.

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