Supplemental Data

Molecular Pharmacology

Identification of novel functionally selective Kappa Opioid Receptor scaffolds

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Supplemental table 1 legend.

GR89696 was identified as a potent agonist for KOR for both G-protein activation and arrestin mobilization. However, GR89696 is more potent in activating arrestin than G-protein relative to salvinorin A. This compound was the only potent functionally selective ligand identified in the NCC library. Brucine, Doxapram, and Diphenoxylate show some activity at higher doses (1uM and higher) but do not generate reliable dose response curves.

Supplemental Table 1. Functional results from hits from NCC library

Compound	G-Protein EC_{50}	Emax	Arrestin EC_{50}	Emax
GR8969	0.515nM (-9.29 +/-0.11)	95.38	0.25nM (-9.60+/-0.06)	93.92
Bestatin	-	-	-	-
2-(2-aminoethyl) pyridine	1050nM (-5.98+/-0.68)	184	550nM (-6.26+/-0.09)	110
N-cyano-*N*'-(1,1-dimethylpropyl)-N''-3-pyridinylguanidine	159nM (-6.81+/-0.34)	85.0	233nM (-6.63+/-0.32)	73
Doxapram	-	-	-	-
Brucine	-	-	-	-
Diphenyoxylate	-	-	-	-

Supplemental Table 2. Comparison of Bias Factor and EC_{50} generated with Tango and BRET assays

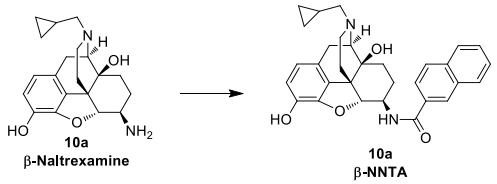
Compound	EC_{50} and Emax GloSensor	EC_{50} and Emax Tango	EC_{50} and Emax BRET	Bias Factor (Tango)	Bias Factor (BRET)
Salvinorin A	5.18 nM 99.7	5.75 nM 97.2	5.54 nM 98.8	1	1
GR89696	0.970 nM 96.4	0.259 nM 92.8	0.265 nM 104	5 Arrestin	5 Arrestin
ICI 199,441	1.63 nM 101	0.428 nM 84.8	0.461 nM 100	4 Arrestin	4 Arrestin
U62066	1.01 nM 103	6.21 nM 92.3	19.8 nM 101	6 G-Protein	18 G-Protein
RB 64	5.29 nM 102	391 nM 103	118 nM 105	35 G-Protein	13 G-Protein
RB 48	8.82 nM 101	143 nM 63.2	45.0 nM 101	25 G-Protein	4 G-Protein
RB 55	31.3 nM 103	229 nM 86.9	196 nM 79.0	8 G-Protein	10 G-Protein
RB 59	35.8 nM 95.7	4290 nM 76.6	3560 nM 177	95 G-Protein	35 G-Protein
Dyn 1-13	2.07 nM 96.6	97.8 nM 72.4	78.2 nM 86.3	34 G-Protein	32 G-Protein
Dyn 1-11	3.26 nM 101	450 nM 75.8	253 nM 92.0	44 G-Protein	27 G-Protein
Dyn 1-9	10.2 nM 101	600 nM 64.6	132 nM 86.9	16 G-Protein	15 G-Protein
Dyn 1-8	57.7 nM 106	720 nM 89.9	1068 nM 103	4 G-Protein	8 G-Protein
Dyn A	8.12 nM 101	268 nM 74.8	112 nM 99.2	34 G-Protein	20 G-Protein

Supplemental Table 3. LogTau/KA values for all ligands tested

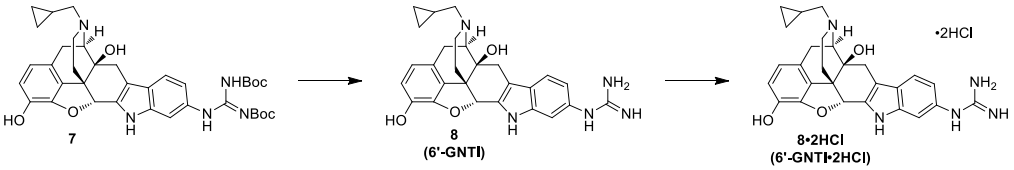
Drug	LogTau/KA GloSensor	LogTau/KA Tango	LogTau/KA BRET
Salvinorin A	8.197 +/-0.08	8.175 +/-0.07	8.182 +/-0.04
U69593	8.140 +/-0.08	8.126 +/-0.06	
(+) U50488	6.783 +/-0.09	5.873 +/-0.09	
U62066	8.979 +/-0.09	8.173 +/-0.08	7.563 +/-0.11
DIPPA	7.838 +/-0.09	7.765 +/-0.09	
N-MPPP	8.621 +/-0.09	8.423 +/-0.08	
BRL 52537	8.843 +/-0.09	8.702 +/-0.07	
ICI 204488	8.025 +/-0.08	8.255 +/-0.12	
ICI 199441	8.587 +/-0.07	9.189 +/-0.05	9.188 +/-0.05
GR8969	8.819 +/-0.08	9.492 +/-0.06	9.506 +/-0.05
(-)U50488	8.600 +/-0.09	8.910 +/-0.09	
Beta-NNTA	9.395 +/-0.13	9.354 +/-0.09	
6' GNTI	8.252 +/-0.08	7.489 +/-0.23	
Diprenorphine	8.615 +/-0.11	8.404 +/-0.10	
Butorphanol	8.611 +/-0.09	8.249 +/-0.19	
Nalbuphine	6.735 +/-0.14	7.240 +/-0.16	
Cyclazocine	8.771 +/-0.09	8.804 +/-0.14	
RB 48	7.87 +/-0.07	6.44 +/-0.09	7.221 +/-0.06
RB 64	7.94 +/-0.07	6.38+/-0.06	6.824 +/-0.06
RB 50	6.89 +/-0.12	5.03 +/-0.13	

RB 65	6.56 +/-0.13	5.08 +/-0.22	
RB 59	6.98 +/-0.10	4.97 +/-0.12	5.400 +/-0.70
RB 55-2	6.74 +/-0.08	5.19 +/-0.15	
RB 55-1	6.85 +/-0.09	5.49 +/-0.15	
RB 55	7.32 +/-0.09	6.42 +/-0.07	6.286 +/-0.14
Salvinorin B	6.89 +/-0.10	6.30 +/-0.05	
Dyn 1-13	8.497 +/-0.04	6.94 +/-0.09	6.979 +/-0.16
Dyn 1-9	7.636 +/-0.07	6.415 +/-0.13	6.439 +/-0.12
Dyn 1-11	8.263 +/-0.07	6.594 +/-0.12	6.816 +/-0.22
Dyn 1-8	7.249 +/-0.07	6.574 +/-0.09	6.344 +/-0.14
Dyn A	8.149 +/-0.06	6.590 +/-0.12	6.825 +/-0.09

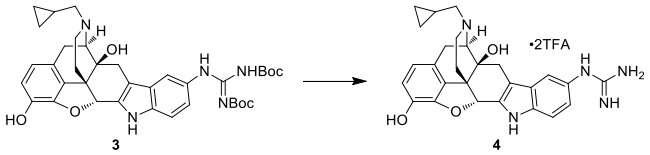
Compound Synthesis Procedures



β-NNTA (11a).[5b] An oven-dried 2-dram vial with a sepcap, cooled under N_2, was charged with **10a** (40.0 mg, 0.117 mmol), dry $CHCl_3$ (0.8 mL) and dry pyridine (25.0 µL, 0.309 mmol). The solution was cooled to 0 °C and 2-naphthoyl chloride (33.4 mg, 0.175 mmol) was added in $CHCl_3$ (0.5 mL) dropwise via syringe over 20 min down the wall of the vial. The solution was stirred at 0 °C for 0.5 h, then at room temperature for 5 h. The solution was concentrated under a stream of N_2 to a residue that was dissolved in MeOH (1 mL) and K_2CO_3 (81 mg, 0.59 mmol) was added in one portion. The mixture was stirred for 2 h then brine (5 mL) and H_2O (5 mL) were added, the pH of the solution was adjusted to 7-8 with saturated NH_4Cl solution and extracted with CH_2Cl_2 (3 x 10 mL). The organic extracts were pooled, washed with H_2O (2 x 20 mL) and brine (20 mL), dried ($NaSO_4$) and filtered. Concentration under vacuum provided 67.3 mg of a yellow residue. Purification by silica (10 g) flash column (1.5 x 16 cm) chromatography, eluting with 97:2.5:0.5 (150 mL) CH_2Cl_2/MeOH/concd $NH_4OH_{(aq)}$ yielded 46.9 mg (81%) of the title compound as a white solid: **^{1}H NMR** (400 MHz, acetone-d_6) δ 8.53 (s, 1H), 8.18-7.82 (m, 5H), 7.65-7.54 (m, 2H), 6.69 (d, *J* = 8.0 Hz; 1H), 6.58 (d, *J* = 8.0 Hz; 1H), 4.97 (br s, 1H), 4.69 (d, *J* = 7.3 Hz; 1H), 4.03 (dd, *J* = 5.5, 11.8 Hz; 1H), 3.16-3.04 (m, 2H), 2.83 (d, *J* = 13.1 Hz; 2H), 2.75-2.61 (m, 2H), 2.45 (dd, *J* = 6.8, 12.8 Hz; 1H), 2.39 (dd, *J* = 6.8, 12.8 Hz; 1H), 2.27 (ddd, *J* = 4.8, 12.3, 12.3 Hz; 1H), 2.15 (ddd, *J* = 2.3, 11.8, 11.8 Hz; 1H), 2.03-1.91 (m, 1H), 1.77-1.65 (m, 1H), 1.63-1.44 (m, 2H), 1.4 (d, *J* = 11.5 Hz; 1H), 0.97-0.84 (m, 1H), 0.60-0.44 (m, 2H), 0.24-0.08 (m, 2H); **LC-MS** (ESI+) m/z: $[M + H]^+$ Calcd for $C_{31}H_{33}N_2O_4$ 497.60; Found 497.34.



***6'-Guanidino*-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (8, 6'-GNTI).**[2,3b] A tared 50 mL flask was charged with di-Boc-guanidine **7** (367 mg, 0.546 mmol) and TFA (4.5 mL). The grey solution was stirred for 75 min, then concentrated to dryness from toluene (1 x 10 mL and 2 x 5 mL) to afford 442 mg of an off-white solid, to which was added MeOH (4.5 mL). The slight suspension was filtered under positive pressure through a plug (0.8 x 1 cm) of Celite in a pipet (4 mL) and the clear filtrate was added in equal portions to three auto sampler vials. Purification of each portion was accomplished by reverse phase preparative-LC (Agilent) using a phenyl-cyclohexyl capped column, eluting at 70 mL/min, detecting at 232 and 288 nm; solvent A = 99.95:0.05 H_2O/TFA, solvent B = MeOH; method: 10→70% B (0-9 min; linear gradient), 70→100% B (9-9.01 min; linear gradient) and 100% B (9.01→10 min; isocratic). Pooled all appropriate fractions, concentrated under vacuum and azeotropically dried the remaining residue with toluene (3 x 5 mL). Obtained 328 mg of the bis-TFA salt as a white solid. The solid was dissolved in MeOH (30 mL), MP-carbonate resin (*ca.* 200 mg, 2.5-3.5 mmol/g) was added and the mixture was stirred until a pH of 7-8 (pH paper) was achieved (10-15 min). The resin was removed by vacuum filtration (fine porosity sintered glass funnel; washed resin with 5 mL MeOH) and the filtrate was concentrated under vacuum to leave 220 mg (85%) of 6'-GNTI freebase as a white solid (^{1}H and ^{13}C NMR analyses performed). The majority of the solid (200 mg, 0.424 mmol) was dissolved in MeOH (10 mL) and HCl (220 µL, 4 M solution in 1,4-dioxane, 0.88 mmol) was added dropwise over 1 min. After stirring for 10 min the solution was concentrated to a volume of 3-4 mL on a rotary evaporator and then diluted (while stirring) with 35-40 mL of Et_2O. The resulting precipitate was collected by vacuum filtration (medium porosity sintered glass funnel). Further drying under high vacuum (12 h) yielded 201 mg (87%) of the title compound bis-hydrochloride salt as a white powder: **^{1}H NMR** (400 MHz, methanol-d_4) δ 7.45 (d, *J* = 8.4 Hz; 1H), 7.21 (d, *J* = 1.6 Hz; 1H), 6.83 (dd, *J* = 1.8, 8.3 Hz; 1H), 6.51 (d, *J* = 8.3 Hz; 1H), 6.49 (d, *J* = 8.3 Hz; 1H), 5.54 (s, 1H), 3.39 (d, *J* = 6.5 Hz; 1H), 3.17 (d, *J* = 18.6 Hz; 1H), 2.85-2.71 (m, 3H), 2.62 (d, *J* = 15.7 Hz; 1H), 2.53-2.41 (m, 2H), 2.41-2.29 (m, 2H), 1.82-1.68 (m, 1H), 1.00-0.89 (m, 1H), 0.63-0.52 (m, 2H), 0.25-0.15 (m, 2H); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.30 (d, *J* = 8.4 Hz; 1H), 6.70 (d, *J* = 1.1 Hz; 1H), 6.64 (dd, *J* = 1.7, 8.3 Hz; 1H), 6.50 (d, *J* = 8.1 Hz; 1H), 6.47 (d, *J* = 8.1 Hz; 1H), 5.49 (s, 1H), 4.72 (br s, 1H), 3.27 (d, *J* = 6.3 Hz; 1H), 3.06 (d, *J* = 18.6 Hz; 1H), 2.79-2.63 (m, 3H), 2.46-2.34 (m, 3H), 2.31 (ddd, *J* = 4.9, 12.5, 12.5 Hz; 1H), 2.15 (ddd, *J* = 2.9, 11.9, 11.9 Hz; 1H), 1.59 (d, *J* = 11.3 Hz; 1H), 0.96-0.82 (m, 1H), 0.58-0.43 (m, 2H), 0.20-0.10 (m, 2H); **^{13}C NMR** (100 MHz, methanol-d_4) δ 158.6, 145.4, 143.8, 139.0, 133.4, 131.8, 130.7, 127.9, 124.6, 120.9, 119.9, 119.3, 118.1, 111.6, 110.3, 85.4, 74.6, 63.7, 60.7, 45.1, 32.9, 29.9, 24.2, 10.4, 4.8, 4.3; **^{13}C NMR** (100 MHz, DMSO-d_6) δ 154.7, 143.0, 139.8, 137.2, 131.1, 129.8, 124.2, 123.7, 118.9, 118.2, 116.7, 116.5, 110.1, 107.1, 83.9, 72.1, 61.6, 58.6, 47.2, 43.3, 31.1, 28.7, 22.7, 9.2, 3.8, 3.4; **LC-MS** (ESI+) m/z: $[M + H]^+$ Calcd for $C_{27}H_{30}N_5O_3$ 472.24; Found 472.57.



***5'-Guanidino*-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (4, 5'-GNTI).**[1,2,3] To a tared 50 mL flask, containing TFA (3.5 mL, ~100 equiv.), was added **3** (319 mg, 0.475) in portions over 1-2 min. The resulting grey-green solution was stirred for 45 min and then concentrated to dryness from toluene (3 x 5 mL) and $CHCl_3$ (5 mL). Continued drying under high vacuum gave *ca.* 400 mg of an off-white solid. Addition of 95:5 MeOH/DMF (~5 mL) gave a slight suspension, which was filtered under positive pressure through a plug of Celite (0.5 x 2 cm) in a pipet (5¾ inch). The clear filtrate was added in equal portions to five auto sampler vials (1.6 mL capacity) and purified by reverse-phase preparative-LC with a phenyl-hexyl column, eluting at 70 mL/min, and detecting at 222 and 274 nm; solvent A = 99.95:0.05 H_2O/TFA, solvent B = MeOH; method: 10→100% B (0→9 min; linear gradient) and 100% B (9→10 min; isocratic). Obtained 298 mg (90%) of the title compound (**4•2TFA**) as a white solid: $[\alpha]_D^{25}$ –176.6 (*c* 0.53, MeOH); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 9.63 (s, 1H), 9.26 (br s, 1H), 8.96 (br s, 1H), 7.42 (d, *J* = 8.6 Hz; 1H), 7.26-7.11 (m, 5H), 6.95 (dd, *J* = 1.8, 8.6 Hz; 1H), 6.62 (d, *J* = 8.1 Hz; 1H), 6.58 (d, *J* = 8.1 Hz; 1H), 6.39 (br s, 1H), 5.71 (s, 1H), 4.08 (d, *J* = 6.3 Hz; 1H), 3.45 (d, *J* = 19.6 Hz; 1H), 3.38 (dd, *J* = 7.0, 13.9 Hz; 1H), 3.24 (dd, *J* = 6.9, 19.8 Hz; 1H), 3.12 (d, *J* = 11.7 Hz; 1H), 3.00-2.90 (m, 1H), 2.95 (d, *J* = 15.9 Hz; 1H), 2.79-2.56 (m, 2H), 1.83 (d, *J* = 11.4 Hz; 1H), 1.16-1.04 (m, 1H), 0.77-0.69 (m, 1H), 0.68-0.59 (m, 1H), 0.54-0.40 (m, 2H); **^{1}H NMR** (400 MHz, methanol-d_4) δ 7.46 (d, *J* = 8.5 Hz; 1H), 7.36 (d, *J* = 1.8 Hz; 1H), 7.04 (dd, *J* = 2.0, 8.6 Hz; 1H), 6.68 (d, *J* = 8.2 Hz; 1H), 6.65 (d, *J* = 8.2 Hz; 1H), 5.74 (s, 1H), 4.23 (d, *J* = 6.5 Hz; 1H), 3.44-3.35 (m, 2H), 3.20 (dd, *J* = 4.2, 12.6 Hz; 1H), 3.05-2.97 (m, 1H), 3.01 (d, *J* = 16.2 Hz; 1H), 2.94 (dd, *J* = 3.7, 12.9 Hz; 1H), 2.77 (ddd, *J* = 4.8, 13.4, 13.4 Hz; 1H), 2.73 (d, *J* = 16.1 Hz; 1H), 1.98 (dd, *J* = 2.6, 13.5 Hz; 1H), 1.22-1.11 (m, 1H), 0.93-0.85 (m, 1H), 0.83-0.75 (m, 1H), 0.60-0.50 (m, 2H); **^{13}C NMR** (100 MHz, methanol-d_4) δ 159.0, 145.0, 142.3, 138.4, 132.7, 130.4, 128.7, 127.1, 122.7, 122.4, 120.8, 119.6, 118.2, 114.0, 110.2, 85.0, 73.7, 63.8, 59.1, 48.3, 47.8, 30.4, 29.9, 25.2, 7.0, 6.4, 3.5; **HRMS** (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{27}H_{30}N_5O_3$ 472.2349; Found 472.2349

References (Supplemental)

(1) (a) Jones, R. M.; Hjorth, S. A.; Schwartz, T. W.; Portoghese, P. S. *J. Med. Chem.* **1998**, *41*, 4911–4914. (b) Stevens, W. C., Jr.; Jones, R. M.; Subramanian, G.; Metzger, T. G.; Ferguson, D. M.; Portoghese, P. S. *J. Med. Chem.* **2000**, *43*, 2759–2769.
(2) A κ-opioid receptor agonist: Sharma, S. K.; Jones, R. M.; Metzger, T. G.; Ferguson, D. M.; Portoghese, P. S. *J. Med. Chem.* **2001**, *44*, 2073–2079.
(3a) Portoghese, P. S.; Jones, R. M. Kappa (OP_2) Opioid Receptor Antagonist. U.S. Patent 6,500,824 B1, Dec 31, 2002.
(3b) Portoghese, P. S.; Jones, R. M.; Sharma, S. K. Therapeutic Compounds and Methods. U.S. Patent 7,232,829 B2, Jun 19, 2007.
(5b) Le Naour, M.; Lunzer, M. M.; Powers, M. D.; Portoghese, P. S. *J. Med. Chem.* **2012**, *55*, 670–677.