Supporting Information

A Bivalent Ligand (KDAN-18) Containing δ Antagonist and κ Agonist Pharmacophores Bridges δ_2 and κ_1 Opioid Receptor Phenotypes.

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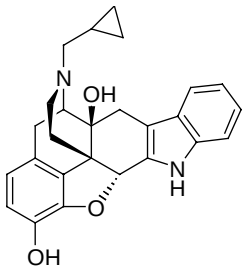
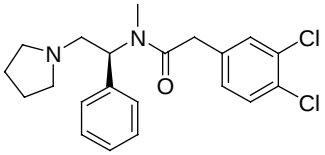
Experimental

All reactions involving moisture sensitive reagents were conducted in oven-dried glassware under nitrogen atmosphere. Solvents were dried when necessary. All other chemicals and solvents were reagent grade unless specified otherwise and were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Naltrexone was obtained from Mallinckrodt & Co. ^{1}H NMR Spectra were recorded on a Varian 300 MHz spectrometer and referenced to the solvent. Chemical shifts are expressed in ppm and coupling constants (J) are in hertz (Hz). Peak multiplicities are abbreviated: broad, br; singlet, s; doublet, d; triplet, t; quartet, q; pentet, p, and multiplet, m. Fast-atom bombardment (FAB) mass spectra (MS) were obtained on a VG 7070E-HF instrument. Flash chromatography was performed on Merck Science silica gel 60 (230-400) mesh. Thin layer chromatography (TLC) was performed on analytical Uniplate silica gel GF glass plates (250 mm by 2.5 x 20 cm^2). Preparative TLC was performed on 1.0 or 0.5 mm Analtech silica gel plates. Plates were visualized by UV light, iodine vapor or ninhydrin solution.

Chemistry

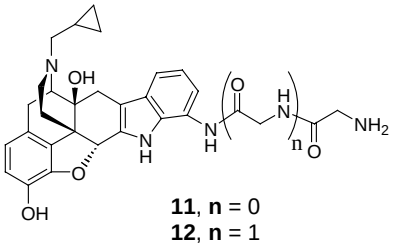


3 (NTI)

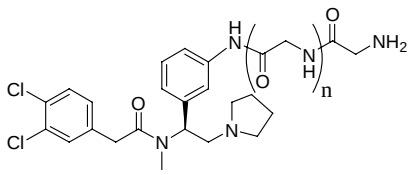


4 (ICI 199,441)

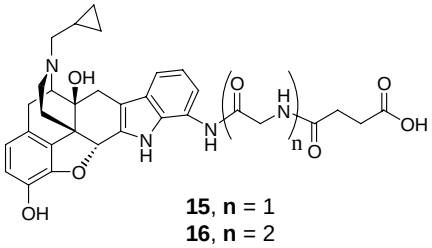
The key intermediates were the 7'-amino derivative[1] of naltrindole (**3**) and the *m*-amino derivative[2] of ICI-199441 (**4**). The amino group does not radically change the selectivity or potency in either of these derivatives and it serves as a point of attachment for the spacer. The first step in this convergent pathway was to couple the amino derivatives of **3** and **4** to either N-Cbz-glycine or N-Cbz-glycylglycine, followed by deprotection to afford **11–14** . The naltrindole intermediates were then reacted with succinic or adipic anhydride to give **15-17**. Coupling of the carboxylic acid derivatives (**15-17**) with the arylacetamide intermediates (**13** or **14**) afforded the target bivalent ligands (**2**, **5**, **6**). Matched monovalent analogs **7-10** were prepared by a similar route using the succinyl or adipyl derivatives of N-methylglycinamide or N-methylglycylglycinamide.



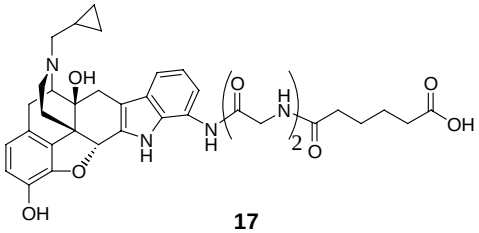
11, **n** = 0
12, **n** = 1



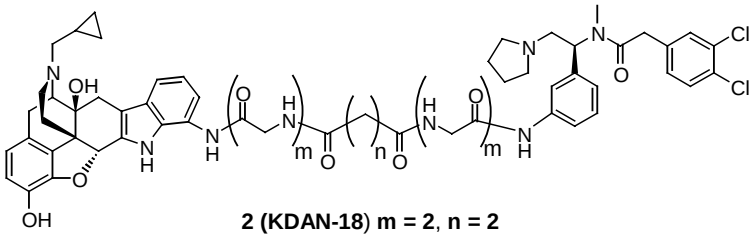
13, **n** = 0
14, **n** = 1



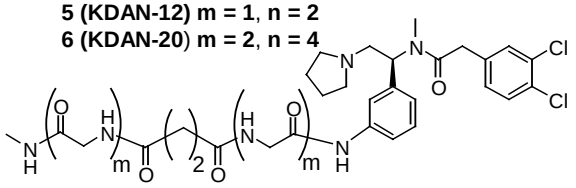
15, **n** = 1
16, **n** = 2



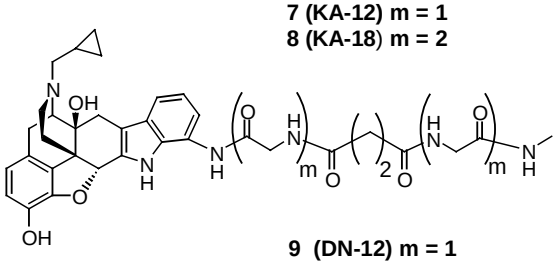
17



2 (KDAN-18) **m = 2**, **n = 2**
5 (KDAN-12) m = 1, **n = 2**
6 (KDAN-20) **m = 2**, **n = 4**



7 (KA-12) m = 1
8 (KA-18) **m = 2**



9 (DN-12) m = 1
10 (DN-18) **m = 2**

N-{([(3-{(S)-α-[N-{2-(3,4-Dichlorophenyl)-acetyl}-N'-methyl-amino]-α-[pyrrolidin-1-yl-methyl]-methyl}-phenylaminocarbonyl)-methyl]-aminocarbonyl)-methyl}-N'-({[(naltrindol-7'-ylaminocarbonyl)-methyl]-aminocarbonyl}-methyl)-succinamide
(**2**, KDAN-18). A solution of carboxylic acid **16** (0.175 g, 0.272 mmol, 1.0 eq), DCC (0.056 g, 0.272 mmol, 1.0 eq), and HOBt (0.110 g, 0.816 mmol, 3.0 eq) in DMF (0.75 mL) was reacted with stirring at 0 ˚C for 30 min. Amine **14** (0.312 g, 0.641 mmol, 1.0 eq) was added in one portion, and the reaction mixture was stirred under N_2 at 0 ˚C for 2 h than rt for another 48 h. The DCU precipitate was collected via vacuum filtration and the solvent was removed in vacuo from the filtrate to give the crude product. Further purification via flash chromatography (silica gel, D/M/A, 89.0/10.0/1.0, v/v/v (2L), gave **2** as an off-white solid (69.0 %); R_f 0.26 (silica gel,

D/M/A. 87/12/1. v/v/v); mp 176 °C (softens), 211 °C (decomposes); ^{1}H NMR (DMSO-d6) δ 10.63 (s, 1H), 9.63 (s, 1H), 9.50 (s, 1H), 8.83 (br s, 1H), 8.21 (m, 2H), 8.13-8.09 (m, 2H), 7.45-7.38 (m, 3H) 7.33 (s, 1H) 7.29 (d, *J* = 7.5 Hz, 1H), 7.14-7.08 (m, 2H), 7.00 (d, *J* = 7.8 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 6.75 (t, *J* = 8.1 Hz, 1H), 6.38-6.31 (m, 2H), 5.70-5.65 (m, 1H), 5.41 (s, 1H), 4.63 (br s, 1H), 3.87 (m, 2H), 3.81 (unresolved, 1H), 3.74-3.70 (m, 2H), 3.62-3.57 (m, 4H), 3.52 (unresolved, 1H), 3.15 (unresolved, 1H), 2.92 (d, *J* = 18.9 Hz, 1H), 2.77 (m, 1H), 2.63 (unresolved, 1H), 2.56 (s, 3H), 2.53-2.50 (m, 4H), 2.35-2.27 (unresolved m, 10H), 2.18-2.14 (m, 1H), 2.01 (m, 1H), 1.50 (s, 4H), 1.42 (unresolved, 1H), 0.74 (m, 1H), 0.36 (m, 2H), 0.02 (m, 2H); UHR-ESI MS *m/z* 1167.6 $(M + Na)^+$, $C_{59}H_{66}Cl_2N_{10}O_{10}$ requires 1144.43. Anal. ($C_{59}H_{66}Cl_2N_{10}O_{10}$) C, H, N.

N-[(3-{(S)-α-[N-{2-(3,4-Dichlorophenyl)-acetyl}-N'-methyl-amino]-α-[pyrrolidin-1-yl-methyl]-methyl}-phenylaminocarbonyl)-methyl]-N'-[(naltrindol-7'-ylaminocarbonyl)-methyl]-succinamide (**5**, KDAN-12). A solution of carboxylic acid **15** (0.165 g, 0.281 mmol, 1.0 eq), DCC (0.058 g, 0.281 mmol, 1.0 eq), and HOBt (0.114 g, 0.844 mmol, 3.0 eq) in DMF (0.75 mL) was reacted with stirring at 0˚ C for 30 min. Amine **13** (0.312 g, 0.641 mmol, 1.0 eq) was then added in one portion, and the reaction mixture was stirred under N_2 at 0˚ C for 2 h then rt for another 72 h. Analysis by TLC (D/M/A [dichloromethane/methanol/ammonium hydroxide], 89/10/1, v/v/v) showed the reaction was complete. The DCU precipitate was collected via vacuum filtration and the solvent was removed in vacuo from the filtrate to give the crude product. Further purification via flash chromatography (silica gel, starting with D/M/A, 96.5/3.0/0.5, v/v/v (1L), then 96.0/3.5/0.5, v/v/v (1L), finally 95.5/4/0.5 v/v/v (2L)) gave **5** as an off-white solid (55.2 %); R_f 0.55 (silica gel, D/M/A. 89/10/1. v/v/v); mp 167 °C (softens), 186 °C (decomposes); ^{1}H NMR (DMSO-d6) δ 10.69 (s, 1H), 9.86 (s, 1H), 9.67 (s, 1H), 8.93 (br s, 1H), 8.31 (t, 1H), 8.28 (t, 1H), 7.55-7.47 (m, 2H) 7.46 (s, 1H) 7.39 (d, *J* = 7.6 Hz, 1H), 7.30-7.20 (m, 3H) 7.13 (d, *J* = 7.6 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.87 (t, *J* = 8.0 Hz, 1H), 6.48 (m, 2H), 5.80 (m, 1H), 5.54 (s, 1H), 4.73 (br s, 1H), 4.01 (d, *J* = 5.4 Hz, 2H), 3.89 (1H), 3.84 (d, *J* = 6.0 Hz, 2H), 3.68 (d, *J* = 15.8 Hz, 1H), 3.15 (1H) 3.05 (d, *J* = 17.6 Hz, 1H) 2.91 (m, 1H), 2.78 (1H), 2.69 (s, 3H), 2.64 (s, 1H), 2.57-2.39 (m, 13H), 2.25-2.13 (m, 2H), 1.62 (s, 4H), 1.54 (1H), 0.88 (m, 1H), 0.49 (m, 2H), 0.13 (m, 2H); HR-ESI MS *m/z* 1031.396 $(M + H)^+$, $C_{55}H_{60}Cl_2N_8O_8$ requires 1030.391. Anal. ($C_{55}H_{60}Cl_2N_8O_8 \bullet 1H_2O$) C, H, N.

N-{([(3-{(S)-α-[N-{2-(3,4-Dichlorophenyl)-acetyl}-N'-methyl-amino]-α-[pyrrolidin-1-yl-methyl]-methyl}-phenylaminocarbonyl)-methyl]-aminocarbonyl)-methyl}-N'-({[(naltrindol-7'-ylaminocarbonyl)-methyl]-aminocarbonyl}-methyl)-adipamide (**6**, KDAN-20). A solution of carboxylic acid **17** (0.131 g, 0.195 mmol, 1.0 eq), DCC (0.040 g, 0.195 mmol, 1.0 eq), and HOBt (0.053 g, 0.390 mmol, 2.0 eq) in DMF (1 mL) was reacted with stirring at 0 ˚C for 30 min. Amine **14** (0.102 g, 0.195 mmol, 1.0 eq) was added in one portion, and the reaction mixture was stirred under N_2 at 0 ˚C for 2 h then rt for another 48 h. The DCU precipitate was collected via vacuum filtration and the solvent was removed in vacuo to give the crude product. Further purification via flash chromatography (silica gel, with D/M/A, 91.5/7.0/0.5, v/v/v (1L), then D/M/A, 91.0/7.5/0.5, v/v/v (1L), then D/M/A, 90.0/9.0/1.0, v/v/v (1L)) gave **6** as an off-white solid (46.7 %); R_f 0.28 (silica gel, D/M/A. 87/12/1. v/v/v); mp 184 °C (softens), 217 °C (decomposes); ^{1}H NMR (DMSO-d6) δ 10.65 (s, 1H), 9.65 (s, 1H), 9.49 (s, 1H), 8.81 (br s, 1H), 8.08-8.00 (m, 4H), 7.41-7.36 (m, 3H) 7.31 (s, 1H) 7.28 (d, *J* = 7.5 Hz, 1H), 7.15-7.03 (m, 2H) 6.98 (d, *J* = 8.1 Hz, 1H), 6.81 (d, J = 7.8 Hz, 1H), 6.73 (t, *J* = 7.8 Hz, 1H),

6.37-6.30 (m, 2H), 5.68-5.63 (m, 1H), 5.39 (s, 1H), 4.62 (br s, 1H), 3.86-3.84 (m, 2H), 3.72-3.65 (m, 3H), 3.60 (d, *J* = 5.7 Hz, 2H), 3.55 (d, *J* = 5.1 Hz, 2H), 3.50 (unresolved, 1H), 3.16 (unresolved, 1H), 2.95-2.88 (m, 2H), 2.57 (unresolved, 1H), 2.54 (s, 3H), 2.48 (s, 1H), 2.40 (m, 2H), 2.33-2.25 (m, 7H), 2.18-2.13 (m, 1H), 2.00-1.99 (m, 5H), 1.48 (s, 4H), 1.35 (m, 5H), 0.79-0.66 (m, 1H), 0.37-0.32 (m, 2H), 0.00 (m, 2H); HR-FAB MS *m/z* 1173.472 $(M + H)^+$, $C_{60}H_{68}Cl_2N_{10}O_{10}$ requires 1172.4653. Anal ($C_{60}H_{68}Cl_2N_{10}O_{10} \bullet 2H_2O$) C, H, N.

N-[(3-{(S)-α-[N-{2-(3,4-Dichlorophenyl)-acetyl}-N'-methyl-amino]-α-[pyrrolidin-1-yl-methyl]-methyl}-phenylaminocarbonyl)-methyl]-N'-[(methylaminocarbonyl)-methyl]-succinamide (**7**, KA-12). A solution of carboxylic acid N-methylaminocarbonylmethyl-succinamic acid (0.252 g, 1.338 mmol, 1.3 eq), DCC (0.276 g, 1.338 mmol, 1.3 eq), and HOBt (0.181 g, 1.338 mmol, 1.3 eq) in DMF (1.0 mL) were incubated with stirring at rt for 30 min. Amine **13** (0.477 g, 1.029 mmol, 1.0 eq) was dissolved in DMF (1.0 mL) and then added in one portion to the above reaction mixture. This was stirred under N_2 at 50˚ C for 18 h. Analysis by TLC (D/M/A, 89/10/1, v/v/v) showed the starting amine was completely consumed. The DCU precipitate was collected via vacuum filtration and the solvent was removed in vacuo from the filtrate to give the crude product. Further purification via flash chromatography (silica gel, starting with D/M/A, 96.5/3.0/0.5, v/v/v (1L), then 96.0/3.5/0.5, v/v/v (1L), then 95.0/4.5/0.5 v/v/v (1L), finally 94.5/0.5/0.5, v/v/v (1.5L)) gave **7** as an off-white solid (34.0 %); R_f 0.33 (silica gel, D/M/A. 89/10/1. v/v/v); mp 91 °C (softens), 102 °C (melts); ^{1}H NMR (DMSO-d6) δ 9.88 (s, 1H), 8.32 (t, *J* = 6.0 Hz, 1H), 8.23 (t, *J* = 6.0 Hz, 1H), 7.73 (d, *J* = 4.5 Hz, 1H), 7.60-7.53 (m, 3H) 7.49 (s, 1H), 7.29-7.34 (m, 2H), 6.99 (d, *J* = 7.8 Hz, 1H), 5.83 (m, 1H), 3.89 (unresolved, 1H), 3.85 (d, *J* = 5.1 Hz, 2H), 3.71 (d, *J* = 15.9 Hz, 1H), 3.62 (d, *J* = 5.7 Hz, 2H), 3.09 (m, 1H), 2.71 (s, 3H), 2.65 (1H), 2.54 (d, *J* = 4.5 Hz, 3H), 2.44-2.41 (m, 8H), 1.66 (s, 4H); HR-FAB MS *m/z* 633.234 $(M + H)^+$, $C_{30}H_{38}Cl_2N_6O_5$ requires 632.2281. Anal. ($C_{30}H_{38}Cl_2N_6O_5$) C, H, N.

N-{([(3-{(S)-α-[N-{2-(3,4-Dichlorophenyl)-acetyl}-N'-methyl-amino]-α-[pyrrolidin-1-yl-methyl]-methyl}-phenylaminocarbonyl)-methyl]-aminocarbonyl)-methyl}-N'-({[(methylaminocarbonyl)-methyl]-aminocarbonyl}-methyl)-succinamide (**8**, KA-18). A solution of carboxylic acid N-[(methylaminocarbonylmethyl-aminocarbonyl)-methyl]-succinamic acid (0.132 g, 0.538 mmol, 1.4 eq), DCC (0.111 g, 0.538 mmol, 1.4 eq), and HOBt (0.208 g, 1.536 mmol, 4.0 eq) in DMSO (0.7 mL) was reacted with stirring at rt for 5 minutes. A solution of amine **14** (0.200 g, 0.384 mmol, 1.0 eq) in DMF (0.7 mL) was added in one portion to the reaction mixture. This was stirred under N_2 at 0 ˚C for 2h then rt for an additional 18 h. The DCU precipitate was collected via vacuum filtration and the DMF was removed in vacuo from the filtrate to give the crude product along with residual DMSO. The filtrate was added to ethyl ether (100 mL) to facilitate precipitation of the crude product and then collected by vacuum filtration; further purification via flash chromatography (silica gel, with D/M/A, 87/12/1, v/v/v) gave **8** as an off-white solid (38.3 %); R_f 0.17 (silica gel, D/M/A. 87/12/1. v/v/v); mp 219 °C (decomposes);^{1}H NMR (DMSO-d6) δ 9.78 (s, 1H), 8.35 (t, *J* = 5.1 Hz, 1H), 8.29-8.22 (m, 2H), 8.11 (d, *J* = 6.0 Hz, 1H), 7.63-7.61 (m, 1H), 7.58-7.53 (m, 3H), 7.47 (s, 1H), 7.31-7.18 (m, 2H), 6.96 (d, *J* = 7.5 Hz, 1H), 5.82 (m, 1H), 3.89 (unresolved, 1H), 3.84 (d, *J* = 4.5 Hz, 2H), 3.70 (unresolved, 1H), 3.70 (d, *J* = 4.2 Hz, 2H), 3.68 (d, *J* = 4.2 Hz, 2H), 3.61 (d, *J* = 6.0 Hz, 2H), 3.08 (m, 1H), 2.70 (s, 3H), 2.64 (s, 1H), 2.55 (d, *J* = 4.5 Hz, 3H), 2.48 (m, 4H), 2.41 (s, 4H), 1.65

(s, 4H); HR-FAB MS *m/z* 769.261 $(M + Na)^+$, $C_{34}H_{44}Cl_2N_8O_7$ requires 746.271. Anal. ($C_{34}H_{44}Cl_2N_8O_7$) C, H, N.

N-[(Methylaminocarbonyl)-methyl]-N'-[(naltrindol-7'-ylaminocarbonyl)-methyl]-succinamide (**9**, DN-12). A solution of carboxylic acid N-methylaminocarbonylmethyl-succinamic acid (0.060 g, 0.321 mmol, 1.3 eq), DCC (0.066 g, 0.321 mmol, 1.3 eq), and HOBt (0.133 g, 0.986 mmol, 4.0 eq) in DMF (0.5 mL) were incubated with stirring at 0˚ C for 10 min. Amine **11** (0.120 g, 0.247 mmol, 1.0 eq) was added in one portion, and the reaction mixture was stirred under N_2 at 0˚ C for 2 h then rt for another 36 h. The DCU precipitate was collected via vacuum filtration and the solvent was removed in vacuo from the filtrate to give the crude product. Further purification via flash chromatography (silica gel, starting with D/M/A, 93.0/6.5/0.5, v/v/v (1L), then 91.65/7.6/0.75, v/v/v (1L), finally 91.45/7.8/0.75 v/v/v (1L)) gave **9** as an off-white solid (84.6 %); R_f 0.30 (silica gel, D/M/A. 89/10/1. v/v/v); mp 194 °C (softens), 211 °C (decomposes); ^{1}H NMR (DMSO-d6) δ 10.73 (s, 1H), 9.69 (s, 1H), 8.97 (s, 1H), 8.31 (t, *J* = 5.6 Hz, 1H), 8.19 (t, *J* = 5.8 Hz, 1H), 7.71 (d, *J* = 4.6 Hz, 1H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.89 (, *J* = 7.4 Hz, 1H), 6.54-6.45, (m, 2H), 5.56 (s, 1H), 4.00 (d, *J* = 5.2 Hz, 2H), 3.61 (d, *J* = 6.0 Hz, 2H), 3.14 (m, 1H), 3.04 (unresolved, 1H), 2.83-2.81 (m, 1H), 2.75-2.67 (m, 2H), 2.55 (d, *J* = 4.4 Hz, 3H), 2.49-2.24 (m, 9H), 1.59 (d, *J* = 11.4 Hz, 1H), 0.90 (m, 1H), 0.52 (m, 2H), 0.16 (m, 2H); HR-FAB MS *m/z* 657.306 $(M + H)^+$, $C_{35}H_{40}N_6O_7$ requires 656.296. Anal. ($C_{35}H_{40}N_6O_7 \bullet 2H_2O$) C, H, N.

N-{([(Methylaminocarbonyl)-methyl]-aminocarbonyl)-methyl}-N'-({[(naltrindol-7'-ylaminocarbonyl)-methyl]-aminocarbonyl}-methyl)-succinamide (**10**, DN-18). A solution of the carboxylic acid N-[(methylaminocarbonylmethyl-aminocarbonyl)-methyl]-succinamic acid (0.129 g, 0.525 mmol, 1.2 eq), DCC (0.108 g, 0.525 mmol, 1.2 eq), and HOBt (0.118 g, 0.875 mmol, 2.0 eq) in DMSO (1.0 mL) was reacted with stirring at rt for 15 min. Amine **12** (0.238 g, 0.438 mmol, 1.0 eq) was added in one portion to the reaction mixture. This was stirred under N_2 at rt for 18 h. The DCU precipitate was collected via vacuum filtration and the filtrate was added to ethyl ether (100 mL) to facilitate precipitation of the crude product. The product was collected by vacuum filtration and washed with ethyl ether (100 mL); further purification via flash chromatography (silica gel, with D/M/A, 86/13/1, v/v/v) gave **10** as an off-white solid (57.0 %); R_f 0.12 (silica gel, D/M/A. 87/12/1. v/v/v); mp 220 °C (decomposes);^{1}H NMR (DMSO-d6) δ 10.62 (s, 1H), 9.48 (s, 1H), 8.82 (s, 1H), 8.19 (t, *J* = 6.0 Hz, 1H), 8.12-8.06 (m, 2H), 7.96 (t, *J* = 6.0 Hz, 1H), 7.46-7.44 (m, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 6.74 (t, *J* = 7.5 Hz, 1H), 6.34-6.30 (m, 2H), 5.40 (s, 1H), 3.86-3.83 (m, 2H), 3.60 (d, *J* = 5.7 Hz, 2H), 3.52 (d, *J* = 5.7 Hz, 2H), 3.46 (d, *J* = 6.0 Hz, 2H), 3.16 (d, 1H), 2.92 (d, *J* = 18.9 Hz, 1H), 2.64-2.52 (m, 3H), 2.41 (d, *J* = 4.2 Hz, 3H), 2.32-2.26 (m, 6H), 2.18-2.14 (m, 1H), 2.10-2.02 (m, 2H), 1.44 (d, J = 11.1 Hz, 1H), 0.74 (m, 1H), 0.38-0.33 (m, 2H), 0.00 (m, 2H); HR-FAB MS *m/z* 771.348 $(M + H)^+$, $C_{39}H_{46}N_8O_9 \bullet H^+$ requires 771.347. Anal. ($C_{39}H_{46}N_8O_9$) C, H, N.

Expression and co-expression of the DOR and KOR in HEK293 cells: cDNAs encoding the rat KOR and mouse DOR were inserted separately into the mammalian expression vector pcDNA3 and tagged with FLAG or c-Myc epitope respectively. HEK293 cells were cultured at 37°C in DMEM medium supplemented with 10% fetal bovine serum and P/S antibiotics. The cells, grown to about 50% confluence, were transfected with the expression vectors containing DOR or KOR cDNA using Calcium Phosphate Transfection Kit. For co-epression, the cells

were first transfected with the pcDNA3 vecotr of KOR and then with the vector for DOR. An equal amount of pcDNA3 vector was co-transfected with each receptor construct to keep the total DNA used equivalent. Geneticin and hygromycin were used to obtain stable KOR and DOR cells.

Radioligand Binding Assays: HEK293 cells of each 100 mm plate, expressing single or a combination of DOR and KOR were suspended in 2.5 mL of 25 mM HEPES Buffer (pH 7.4). Saturation binding was performed on whole cells using either [^{3}H]deltorphin II or [^{3}H]U69593 to determine Bmax and Kd of the receptors. Each concentration was examined in duplicate. The IC_{50} values for the tested compounds were determined by competition binding is which whole cells were incubated at 25°C for 2 hours with either [^{3}H]deltorphin II or [^{3}H]U69593 and 10 different concentrations (10^{-15} – 10^{-6} M) of compound, in a final reaction volume of 500 μL. The concentration of the radioligand in the competition assay was ~ equivalent to its Kd value. Nonspecific binding was determined in the presence of 10 μM of naloxone. The samples were filtered and washed 3 times through GF/C filters (Whatman) presoaked in 0.25% PEI using a Brandel 48-well harvester. After filtration, the filters were incubated in 4 mL of Econo-Safe cocktail and counted in a LS3801 Beckman counter. The experiments were determined from displacement curves using Kaleidograph 3.1, and the Ki values were calculated using the Cheng-Prussoff equation.[3]

Appendix

Elemental Analyses:

Cmpd No.	Compound	Formula		C	H	N
2	**KDAN-18**	$C_{59}H_{66}Cl_2N_{10}O_{10}$	Calc	61.83	5.80	12.22
			Found	61.67	5.73	12.05
5	**KDAN-12**	$C_{55}H_{60}Cl_2N_8O_8 \cdot 1H_2O$	Calc	62.91	5.95	10.67
			Found	63.20	6.11	10.35
6	**KDAN-20**	$C_{60}H_{68}Cl_2N_{10}O_{10} \cdot 2H_2O$	Calc	60.54	6.16	11.57
			Found	60.80	6.30	11.69
7	**KA-12**	$C_{30}H_{38}Cl_2N_6O_5$	Calc	56.87	6.05	13.26
			Found	56.74	5.87	13.12
8	**KA-18**	$C_{34}H_{44}Cl_2N_8O_7$	Calc	54.62	5.93	14.99
			Found	54.52	6.12	14.86
9	**DN-12**	$C_{35}H_{40}N_6O_7 \cdot 2H_2O$	Calc	60.68	6.40	12.13
			Found	60.89	6.46	11.83
10	**DN-18**	$C_{39}H_{46}N_8O_9$	Calc	60.77	6.01	14.54
			Found	60.59	5.98	14.41

References

1. Portoghese, P.S.; Sultana, M.; Nelson, W.L.; Klein, P.; Takemori, A.E. δ- Opioid receptor antagonistic activity and binding studies of regioisomeric isothiocyanate derivatives of naltrindole: Evidence for opioid δ- receptor subtypes. *J. Med. Chem.* **1992**, 35, 4089-4091

2. Chang, A.; Takemori, A.E.; Ojala, W.H.; Gleason, W.B.; Portoghese, P.S. κ-Opioid receptor selective affinity labels: electrophilic benzeneacetamides as κ-selective opioid antagonists. *J. Med. Chem.* **1994**, 37, 4490-4498

3. Cheng, Y.C.; Prusoff, W.H. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzyme reaction. *Biochem. Pharmacol*. **1973**, 22, 3099-3102