# A Bivalent Ligand (KDAN-18) Containing δ-Antagonist and κ-Agonist Pharmacophores Bridges $\delta_2$ and $\kappa_1$ Opioid Receptor Phenotypes†

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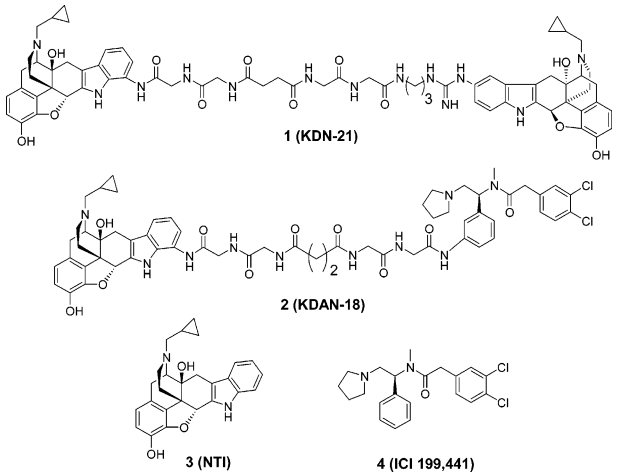
**Abstract:** To characterize δ- and κ-opioid receptor phenotypes, bivalent ligands (KDAN series) containing δ-antagonist (naltrindole) and $\kappa_1$-agonist (ICI-199,441) pharmacophores were synthesized and evaluated by the intrathecal route using the mouse tail-flick assay and binding studies. The data have suggested that KDAN-18 (**2**) bridges phenotypic $\delta_2$- and $\kappa_1$-receptors. A conceptual model is presented to explain the organizational differences between the opioid receptors that give rise to the phenotypes ($\delta_1$, $\delta_2$, $\kappa_1$, $\kappa_2$).

A major question currently addressed in opioid research relates to the fact that receptor cloning has identified fewer receptors than pharmacology predicts. One explanation for this phenomenon is that the pharmacological effects thought to be mediated by putative opioid receptor subtypes may in fact arise through activation of opioid receptor dimers.[1−3] The idea that two distinct δ-receptor subtypes exist was proposed in 1991 on the basis of in vivo pharmacological studies.[4,5] Given the recent evidence that the $\delta_1$-opioid receptor agonist [D-Pen$^{2,5}$]enkephalin[6] (DPDPE) activates an apparently allosterically coupled δ−κ heterodimer[7] and in view of the very recent pharmacological studies with the δ−κ antagonist bivalent ligand KDN-21 (**1**),[8] we have concluded that the $\delta_1$ and $\kappa_2$ phenotypes in the spinal cord are characteristic of this heterodimer. These studies have raised questions concerning the nature of opioid receptors that give rise to the $\delta_2$ and $\kappa_1$ phenotypes. We have addressed this by the synthesis and biological evaluation of a series of δ−κ bivalent ligands with $\kappa_1$-agonist and δ-antagonist pharmacophores. Here, we report on a member (**2**, KDAN-18)[9] of this series whose pharmacology and binding data suggest that the $\delta_2$ and $\kappa_1$ opioid receptor phenotypes are associated but not allosterically coupled δ- and κ-opioid receptors in the mouse spinal cord.

In the spinal cord, the $\delta_2$-agonist [D-Ala$_2$,Glu$_4$]deltorphin[10] (deltorphin II) interacts with a subpopulation of opioid receptors that is different from that activated by the $\delta_1$-agonist DPDPE.[5,11] The pharmacological features distinguishing the spinal $\delta_1$ from the $\delta_2$ phenotype are that the former is antagonized by both the $\delta_1$-antagonist benzylidenenaltrexone[12] (BNTX) and the κ-opioid antagonist norbinaltorphimine[13] (norBNI), whereas the latter is antagonized only by the $\delta_2$-antagonist naltriben[11] (NTB).[7] In this regard, the antagonism of DPDPE by a κ-opioid antagonist has been




attributed to cooperativity between a subpopulation of δ- and κ-opioid receptors organized as heterodimers that give rise to the $\delta_1$ and $\kappa_2$ phenotypes.[7,8] The lack of antagonism of deltorphin II by norBNI is apparently due to δ-receptors that are not allosterically coupled with κ-opioid receptors. Given these differences, we have investigated the organization of δ- and κ-opioid receptors that are manifested as $\delta_2$ and $\kappa_1$ phenotypes.

Since it appeared unlikely that all colocalized spinal δ- and κ-opioid receptors are associated as heterodimers, we considered the possibility that the $\delta_2$ and $\kappa_1$ phenotypes might represent an arrangement of δ- and κ-opioid receptors that are not heteromeric. One example would be δ homodimers that are associated with κ homodimers because there are reports of such homodimeric associations of both δ and κ receptors in cultured cells.[14−18] If this were the case, a mixture of δ- and κ-opioid receptors could contain δ−κ, δ−δ, and κ−κ dimers in an oligomeric array similar to that reported for rhodopsin.[19] Under such conditions, we might expect intradimer allosteric interaction but little, if any, cooperativity between homodimeric δ- and κ-opioid receptors. The inability of norBNI to antagonize deltorphin II in the mouse spinal cord[7] therefore might reflect the interaction of this agonist with a δ−δ homodimer rather than with a δ−κ heterodimer.

To investigate the organizational features of receptors that give rise to the $\delta_2$ and $\kappa_1$ phenotypes, we have employed bivalent ligands containing $\kappa_1$-agonist and δ-antagonist pharmacophores. The rationale for this approach was based on preliminary modeling studies that suggested it might be feasible for a single bivalent ligand to bridge two neighboring δ−δ and κ−κ homodimers in an oligomeric array.[20] The oligomerization of dimeric opioid receptors is consistent with recent direct evidence for the oligomerization of rhodopsin dimers in native membranes.[19]

The use of a combination of agonist and antagonist pharmacophores was employed in the design of bivalent ligands for exploring the interaction between δ- and κ-receptors that are not coupled because we anticipated that bridging of interdimeric recognition sites should be more favorable when compared to the intradimer bridging of an allosteric δ−κ opioid receptor. This is

† Dedicated to the memory of Dr. Paul Janssen, a great scientist.

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**Table 1.** Opioid Agonist Selectivity of Bivalent and Monovalent Ligands in the Mouse Tail-Flick Assay upon Intrathecal Administration[a]

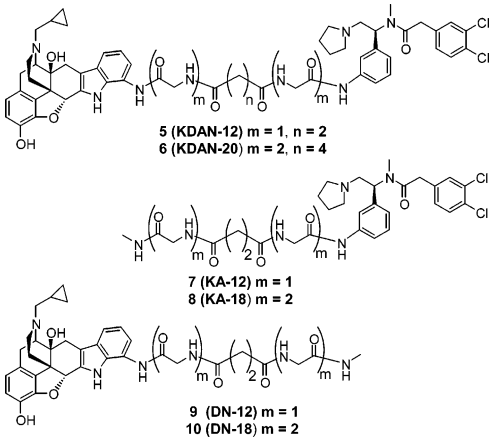
| | | | $ED_{50}$ ratio[b,c] (95% CL) | | |
|---|---|---|---|---|---|
| compd | name | $ED_{50}$ (nmol/mouse)[d] | BNTX $\delta_1$ | NTB $\delta_2$ | NorBNI $\kappa$ |
| **2** | KDAN-18[e] | 0.19 (0.15−0.26) | 3.0 (1.8−4.8) | 25.9 (16.2−41.4) | 39.3 (23.6−58.9) |
| **5** | KDAN-12 | 0.44 (0.36−0.56) | 2.1 (1.3−3.3) | 1.1 (0.8−1.5) | 33.0 (25.8−42.2) |
| **6** | KDAN-20 | 0.50 (0.40−0.63) | 3.4 (1.4−10.4) | 2.0 (0.6−5.5) | 3.4 (1.4−8.5) |
| **7** | KA-12 | 1.4 (0.84−2.75) | ∼1[f] | ∼1[f] | 11.0 (4.4−29.5) |
| **8** | KA-18 | 0.11 (0.03−0.21) | 1.3 (0.6−4.4) | 1.5 (0.6−4.0) | 10.8 (4.9−27.2) |
| **8** + **10** | KA-18 + DN-18[g] | 0.14 (0.10−0.19) | ∼1[f] | ∼1[f] | 44.8 (28.9−68.7) |
| | U50488 | 21.0 (18.2−24.7)[h] | 0.94 (0.7−1.3)[h] | 0.81 (0.1−1.5)[h] | 4.23 (1.76−8.28) |

[a] At least three groups of 10 male CD1 mice weighing between 20 and 25 g were employed in a modified tail-flick assay. Antinociception was considered positive if the tail-flick latency was more than the control latency plus 3 SD of the mean of the reaction time. [b] $ED_{50}$ ratio = $ED_{50}$ of agonist in the presence of antagonist divided by the $ED_{50}$ of the agonist alone. $ED_{50}$ ratios were considered significant when the 95% confidence intervals of the ratio was >1.0. [c] Peak times and doses of it. administered antagonists were as follows: norBNI (2.5 nmol, 16 min), BNTX (25 pmol, 10 min), NTB (50 pmol, 10 min). [d] All the agonists behaved as full agonists. [e] The icv $ED_{50}$ of KDAN-18 was 0.03 (0.025−0.26) nmol/mouse. Its $ED_{50}$ ratios were as follows: BNTX, 1.43 (1.19−1.68); NTB, 18.25 (13.02−24.76); norBNI, 14.65 (7.63−27.95). [f] The $ED_{50}$ ratio was determined at a control ED dose of agonist that produced 70% antinociception. No change in the % antinociception was observed in the presence of antagonist. [g] Both monovalent analogues KA-18 and DN-18 were coadministered (i.t.) in an equimolar ratio. [h] Values from Portoghese and Lunzer.[7]

proposed because the allosterically coupled $\delta-\kappa$ heterodimer interacts selectively with the antagonist−antagonist bivalent ligand KDN-21,[8] thereby promoting the antagonist state in both the $\delta$ and $\kappa$ receptors. One interpretation is that both receptors in the dimer are in the agonist or antagonist state because of cooperativity. According to this model, a KDAN ligand with an appropriate length spacer linking agonist and antagonist pharmacophores should therefore favor bridging with neighboring $\delta$ and $\kappa$ receptors that are associated as homodimers versus those that are organized as allosterically coupled $\delta-\kappa$ heterodimers.

The pharmacophores chosen for the KDAN[9] series of bivalent ligands incorporate the $\delta$-opioid antagonist naltrindole[21] **3** (NTI) and the $\kappa_1$-agonist ICI-199441[22] **4**, tethered through a variable length spacer. The pharmacophores were selected because of the high affinity and selectivity of the parent ligands. The spacer is comprised of oligoglycyl units attached to a methylenediacyl core containing a variable number of methylenes. Spacer length was varied by the number of glycyl residues and methylenes. The constitution of the spacers was motivated by our desire to maintain a favorable hydrophilic−hydrophobic balance of the bivalent ligands. Course adjustment of spacer length was accomplished by varying the number of glycyl residues, whereas incremental changes of methylene groups afforded fine adjustment. The spacers varied from 12 atoms (KDAN-12, **5**) to 20 atoms (KDAN-20, **6**). Matched monovalent control compounds were synthesized in an effort to factor out possible effects of the spacer on activity in the bivalent ligands. The synthesis of these ligands is described in the Supporting Information.

The target compounds were administered intrathecally to CD1 mice and evaluated using the tail-flick assay[23] (Table 1). The bivalent ligands (**2**, **5**, **6**) and the $\kappa$ monovalent control compounds (**7** and **8**) exhibited antinociceptive activity and were full agonists. The pharmacological selectivity was determined using the selective antagonists BNTX ($\delta_1$), NTB ($\delta_2$), norBNI ($\kappa$), and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-$NH_2$[24] (CTOP) ($\mu$). The bivalent ligands KDAN-18 and KDAN-12 (**2** and **5**) were potently antagonized by norBNI. Significantly, NTB potently antagonized only the agonist effect of KDAN-18 (**2**), which contains an 18-atom spacer. The lack of antagonism by CTOP [(5.9 pmol; $ED_{50}$ ratio, 0.80 (0.50−1.23)] suggested that $\mu$ receptors are not involved in the agonist effect of KDAN-18.




We attribute the pharmacologic antagonism by NTB to competition with the $\delta$-antagonist pharmacophore of KDAN-18 at the $\delta$-receptor associated with the $\kappa$-receptor. Such competition should promote a change from bivalent to univalent binding, thereby reducing the potency of KDAN-18. The finding that NTB did not antagonize the monovalent analogue KA-18 (**8**), or a mixture of DN-18 (**10**) and KA-18 (**8**), supports this interpretation. This explanation was consistent with the results of binding studies with coexpressed $\delta$ and $\kappa$ opioid receptors. Specifically, NTB substantially reduced the binding affinity of KDAN-18 to the coexpressed $\delta-\kappa$ cell line when the $\kappa_1$ radioligand U69593 was employed but did not affect the binding affinity of KDAN-12 (Table 2). Moreover, the observation that the $\kappa_1$-agonist U50,-488[25] and the other bivalent ligands (KDAN-12, **5** and KDAN-20, **6**) were not antagonized in intrathecal (i.t.) studies by NTB indicates that the pharmacological profile of KDAN-18 is dependent on a spacer of specific length.[7] This is in harmony with the interaction of KDAN-18 with neighboring $\delta_2$ and $\kappa_1$ phenotypes through a bridging mechanism. Moreover, the reported[7] inability of norBNI and NTB to antagonize deltorphin II and U50,488, respectively, suggests that no allosteric cooperativity exists between the associated $\delta_2$ and $\kappa_1$ phenotypic receptors.

In an additional effort to evaluate bridging, binding studies were carried out on human embryonic kidney

**Table 2.** Effects of Selective δ Antagonists on the Binding Affinity of KDAN-18 and KDAN-12 to Coexpressed δ−κ Receptors in HEK293 Cells

| | $K_i$ (nM)[a] | | | |
|---|---|---|---|---|
| | KDAN-18 (**2**) | | KDAN-12 (**5**) | |
| antagonist[b] | coexpressed δ−κ[c] | mixed δ + κ[c] | coexpressed δ−κ[c] | mixed δ + κ[c] |
| none | 0.06 ± 0.04 | 2.51 ± 1.66 | 1.66 ± 1.06 | 1.90 ± 1.66 |
| BNTX ($\delta_1$) | 0.19 ± 0.11 | 1.14 ± 0.47 | 3.79 ± 2.46 | 2.75 ± 0.96 |
| NTB ($\delta_2$) | 2.34 ± 1.59 | 1.94 ± 0.88 | 4.06 ± 2.23 | 2.02 ± 0.63 |

[a] Binding was conducted on intact HEK cells using [$^3$H]U69593 as the radioligand. [b] Binding was done alone, in the presence of 10 nM NTB, or in the presence of 100 nM BNTX. [c] Coexpressed δ and κ opioid receptors in HEK cells or mixed HEK cells containing singly expressed δ or κ receptors; see Supporting Information for more details.

**Table 3.** Binding of Bivalent and Monovalent Ligands to δ and κ Opioid Receptors in HEK Cells

| | | $K_i$ (nM) [$^3$H]deltorphin II[a] | | $K_i$ (nM) [$^3$H]U69593[a] | |
|---|---|---|---|---|---|
| compd | name | coexpressed δ−κ[b] | mixed δ + κ[b] | coexpressed δ−κ[b] | mixed δ + κ[b] |
| **2** | KDAN-18 | 0.008 ± 0.004 | 0.52 ± 0.24 | 0.06 ± 0.04 | 2.51 ± 1.66 |
| **5** | KDAN-12 | 1.23 ± 1.02 | 0.43 ± 0.18 | 1.66 ± 1.06 | 1.90 ± 1.05 |
| **7** | KA-12 | 281.2 ± 122.3 | >1000 | 41.6 ± 32.7 | 6.75 ± 4.89 |
| **8** | KA-18 | >1000 | >1000 | 36.2 ± 21.7 | 23.9 ± 16.6 |
| **9** | DN-12 | 0.46 ± 0.27 | 0.15 ± 0.13 | >1000 | >1000 |
| **10** | DN-18 | 0.91 ± 0.62 | 1.07 ± 0.93 | >1000 | >1000 |

[a] Binding was conducted on intact HEK cells using [$^3$H]deltorphin II or [$^3$H]U69593 as the radioligand. [b] Coexpressed δ and κ opioid receptors in HEK cells or mixed HEK cells containing singly expressed δ or κ receptors; see Supporting Information for more details.

(HEK) cells that contained singly expressed or coexpressed δ and κ opioid receptors. This included studies using a mixture of HEK cells with singly expressed δ or κ receptors to distinguish between bivalent and univalent binding of the bivalent ligands. The binding data (Table 3) revealed that the bivalent ligand with an 18 atom spacer, KDAN-18, had ∼65-fold higher affinity for the coexpressed cell line compared to the mixed cell lines when the $\delta_2$-selective [$^3$H]deltorphin II was used. In contrast, the shorter bivalent ligand KDAN-12 possessed nearly equal affinity (∼3-fold difference) for the coexpressed δ−κ cell line when compared to the mixed cell lines. A similar binding pattern was found when the $\kappa_1$-selective ligand [$^3$H]U69593 was employed (Table 3). Additionally, the δ and κ monovalent ligands possessed very low affinity for coexpressed δ−κ cell line and mixed cell lines.

These data suggest that KDAN-18 bridges associated δ and κ receptors in the coexpressed cell line, whereas the shorter bivalent ligand KDAN-12 does not. The finding that KDAN-18 had a higher affinity for coexpressed cell line over the mixed cell lines in competition studies with [$^3$H]deltorphin II and [$^3$H]U69593 identifies these bridged opioid receptors as $\delta_2$ and $\kappa_1$ phenotypes. This phenotype characterization was further supported by binding studies that employed $\delta_1$- and $\delta_2$-selective antagonists BNTX and NTB (Table 2). Specifically, NTB significantly reduced the binding affinity of KDAN-18 to the coexpressed δ−κ cell line by ∼42-fold, while BNTX only reduced the binding affinity ∼3-fold. In contrast, KDAN-12 was not affected by BNTX or NTB. These results support the i.t. studies (Table 1) and are consistent with the idea that KDAN-18 interacts with associated $\delta_2$- and $\kappa_1$-opioid receptors.

It therefore appears that KDAN-18 (**2**) is capable of interacting with associated δ- and κ-opioid receptors ($\delta_2$ and $\kappa_1$ phenotypes) that are organized differently from the allosterically coupled δ−κ heterodimer that gives rise to the $\delta_1$ and $\kappa_2$ phenotypes.[7] The observation that KDN-21 (**1**) (0.03 nmol/mouse), which selectively bridges $\delta_1$ and $\kappa_2$ phenotypes arising from δ−κ heterodimers, did not antagonize KDAN-18 [$ED_{50}$ ratio, 0.65 (0.37−1.04)] supports this view.

In contrast to KDN-21, whose spinal antagonist selectivity profile differs from that in the brain,[8] the KDAN-18 agonist selectivity profiles are essentially the same (Table 1, footnote e). This points to a similar structural organization of δ- and κ-opioid receptors in the spinal cord and brain that give rise to $\delta_2$- and $\kappa_1$-phenotypes and to the localization of $\delta_1$-$\kappa_2$ phenotypes in the cord but not the brain.

What is the difference in organization between the opioid receptor phenotypes that are allosterically coupled ($\delta_1-\kappa_2$) and those that are associated but not coupled ($\delta_2$ and $\kappa_1$)? On the basis of the organizational arrangement of rhodopsin receptors in native tissue,[19] we propose that two distinct populations of associated δ−κ spinal receptors are responsible for the $\delta_1-\kappa_2$, $\delta_2$, and $\kappa_1$ opioid receptor phenotypes. One possibility is that δ- and κ-opioid receptors exist in the cord as dimers (δ−κ heterodimers, δ−δ homodimers, and κ−κ homodimers) that are oligomerized. We have termed the interface between the monomeric subunits in the dimers as a "privileged" interface when it participates in allosteric coupling between the individual receptors in the dimer, whereas "passive" interfaces are involved in interdimer contacts that do not produce significant cooperativity. For example, if a mixture of dimeric δ−δ, δ−κ, and κ−κ receptors associate to form an oligomeric array of dimers (Figure 1) in an arrangement similar to that observed for rhodopsin in native membranes,[19] they may give rise to two separate populations of associated δ and κ receptors: the δ−κ heterodimer, whose subunits are allosterically coupled, and associated δ and κ receptors formed when two homodimeric receptors contact one another in the oligomerized state. In the latter case, no allosteric cooperativity would be expected between the associated δ−κ receptors because the contact is "passive". In view of the pharmacological actions of KDAN-18, we propose a model in which a population of δ- and κ-opioid receptors are associated through a passive interface that represents the $\delta_2$ and $\kappa_1$ receptor phenotypes that are recognized by deltorphin II and U50488, respectively.

In conclusion, we have designed and synthesized a novel bivalent ligand, KDAN-18 (**2**), whose selectivity suggests bridging between δ- and κ-opioid receptors that correspond to the $\delta_2$ and $\kappa_1$ phenotypes. In contrast to bivalent ligands with a 12- or 20-atom spacer, the length of the 18-atom spacer appears to facilitate bridging. There appears to be a clear distinction between δ−κ heterodimeric receptors that recognize KDN-21 (**1**) and associated δ and κ receptors that are selective for KDAN-18 (**2**). The former displays cooperativity via a putative privileged interface between receptors within the heterodimer that gives rise to the $\delta_1$ and $\kappa_2$ phenotypes. The absence of an allosteric effect between associated δ and κ receptors that bind KDAN-18 is

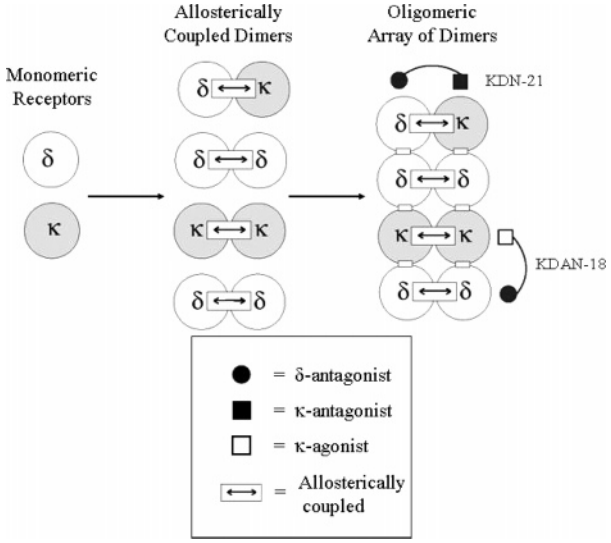



**Figure 1.** Cartoon illustrating the proposed model for association of $\delta$- and $\kappa$-opioid receptors and bridging by bivalent ligands. Monomeric $\delta$- and $\kappa$-opioid receptors form dimers that are allosterically coupled. According to this model, the dimers form an oligomeric array similar to that reported for rhodopsin.[19] Two populations of associated $\delta$ and $\kappa$ receptors are thus formed, one whose subunits are coupled through a privileged interface and a second whose subunits are associated through a passive interface. KDN-21 interacts with allosteric $\delta-\kappa$ heterodimers whose properties are characteristic of $\delta_1$ and $\kappa_2$ phenotypes, while KDAN-18 bridges neighboring $\delta$ and $\kappa$ homodimers that give rise to $\delta_2$ and $\kappa_1$ phenotypes.

proposed to be due to their interaction through a passive interface that is manifested as $\delta_2$ and $\kappa_1$ phenotypes. The fact that KDN-21 does not antagonize KDAN-18 highlights the selectivity of these bivalent ligands for their respective $\delta$ and $\kappa$ phenotypes.

Finally, this study raises the possibility that differences in the organization of oligomerized opioid receptors may have implications in regard to changes in signal transduction pathways and screening paradigms.[8,16,26] Since the $\delta_1-\kappa_2$ phenotype appears to be localized primarily in the spinal cord, it may be possible to develop spinally selective analgesics and thereby reduce the supraspinal side effects of opioid agonists.[8]

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**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Jordan, B. A.; Cvejic, S.; Devi, L. A. Opioids and their complicated receptor complexes. *Neuropsychopharmacology* **2000**, *23*, S5−S18.
(2) Simonin, F.; Slowe, S.; Becker, J. A.; Matthes, H. W.; Filliol, D.; Chluba, J.; Kitchen, I.; Kieffer, B. L. Analysis of [$^3$H]bremazocine binding in single and combinatorial opioid receptor knockout mice. *Eur. J. Pharmacol.* **2001**, *414*, 189−195.
(3) Levac, B. A.; O'Dowd, B. F.; George, S. R. Oligomerization of opioid receptors: generation of novel signaling units. *Curr. Opin. Pharmacol.* **2002**, *2*, 76−81.
(4) Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Differential antagonism of delta opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for delta opioid receptor subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 676−680.
(5) Jiang, Q.; Takemori, A. E.; Sultana, M.; Portoghese, P. S.; Bowen, W. D.; Mosberg, I.; Porreca, F. Differential antagonism of opioid delta antinociception by [D-Ala2,Leu5,Cys6]enkephalin and naltrindole 5′-isothiocyanate: evidence for delta receptor subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 1069−1075
(6) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Bis-penicillamine enkephalins possess highly improved specificity toward delta opioid receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871−5874.
(7) Portoghese, P. S.; Lunzer, M. M. Identity of the putative $\delta$1-opioid receptor as a $\delta-\kappa$ heteromer in the mouse spinal cord. *Eur. J. Pharmacol.* **2003**, *467*, 233−234.
(8) Bhushan, R. G.; Sharma, S. K.; Xie, Z.; Daniels, D. J.; Portoghese, P. S. A Bivalent Ligand (KDN-21) Reveals Spinal $\delta$ and $\kappa$ Opioid Receptors Are Organized as Heterodimers That Give Rise to $\delta_1$ and $\kappa_2$ Phenotypes. Selective Targeting of $\delta-\kappa$ Heterodimers. *J. Med. Chem.* **2004**, *47*, 2969−2972.
(9) Nomenclature employed in this series is as follows: K = $\kappa$ pharmacophore, D = $\delta$ pharmacophore, A = agonist, N = antagonist; the digits refer to the number of atoms in the spacer.
(10) Erspamer, V.; Melchiorri, P.; Falconieri-Erspamer, G.; Negri, L.; Corsi, R.; Severini, C.; Barra, D.; Simmaco, M.; Kreil, G. Deltorphins: a family of naturally occurring peptides with high affinity and selectivity for delta opioid binding sites. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5188−92.
(11) Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Differential antagonism of delta opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for delta opioid receptor subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 676−680.
(12) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. A highly selective $\delta$1-opioid receptor antagonist: 7-benzylidenenaltrexone. *Eur. J. Pharmacol.* **1992**, *218*, 195−196.
(13) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Binaltorphimine and nor-binaltorphimine, potent and selective kappa-opioid receptor antagonists. *Life Sci.* **1987**, *40*, 1287−1292.
(14) Cvejic, S.; Devi, L. A. Dimerization of the $\delta$ Opioid Receptor: Implications for a Role in Receptor Internalization. *J. Biol. Chem.* **1997**, *272*, 26959−26964.
(15) Jordan, B.; Devi, L. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* **1999**, *399*, 697−700.
(16) George, S. R.; Fan, T.; Xie, Z.; Tse, R.; Tam, V.; Varghese, G.; O'Dowd, B. F. Oligomerization of mu and delta-Opioid Receptors. *J. Biol. Chem.* **2000**, *275*, 26128−26135.
(17) McVey, M.; Ramsay, D.; Kellett, E.; Rees, S.; Wilson, S.; Pope, A. J.; Milligan, G. Monitoring receptor oligomerization using time-resolved fluorescence resonance energy transfer and bioluminescence resonance energy transfer. *J. Biol. Chem.* **2001**, *276*, 14092−14099.
(18) Ramsay, D.; Kellett, E.; McVey, M.; Rees, S.; Milligan, G. Homo- and hetero-oligomeric interaction between G-protein-coupled receptors in living cells monitored by two variants of bioluminescence resonance energy transfer (BRET): hetero-oligomers between receptor subtypes form more efficiently than between less closely related sequences. *Biochem. J.* **2002**, *365*, 429−440.
(19) Liang, Y.; Fotiadis, D.; Filipek, S.; Saperstein, D. A.; Palczewski, K.; Engel, A. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. *J. Biol. Chem.* **2003**, *278*, 21655−21662.
(20) Daniels, D. J.; Portoghese, P. S. Unpublished studies.
(21) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of peptidomimetic delta opioid receptor antagonists using the message−address concept. *J. Med. Chem.* **1990**, *33*, 1714−1720.
(22) Barlow, J. J.; Blackburn, T. P.; Costello, G. F.; James, J.; Le Count, D. J.; Main, B. G.; Pearce, R. J.; Russel, K.; Shaw, J. S. Structure activity studies related to 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-1-substituted-ethyl]-acetamide: a novel series of potent and selective $\kappa$-opioid agonists. *J. Med. Chem.* **1991**, *34*, 3149−3158.
(23) Tulunay, F. C.; Takemori, A. E. The increased efficacy of narcotic antagonists induced by various narcotic analgesics. *J. Pharmacol. Exp. Ther.* **1974**, *190*, 395−400.
(24) Gulya, K.; Krivan, M.; Nyolczas, N.; Sarnyai, Z.; Kovacs, G. L. Central effects of the potent and highly selective mu opioid antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) in mice. *Eur. J. Pharmacol.* **1988**, *150*, 355−360.
(25) Szmuszkovicz, J.; Von Voigtlander, P. F. Benzeneacetamide amines: structurally novel non-$\mu$ opioids. *J. Med. Chem.* **1982**, *25*, 1125−1126.
(26) Mattia, A.; Vanderah, T.; Mosberg, H. I.; Porreca, F. Lack of antinociceptive cross-tolerance between [D-Pen2, D-Pen5]enkephalin and [D-Ala2]deltorphin II in mice: evidence for delta receptor subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 583−587.

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