Journal of Medicinal Chemistry

Article

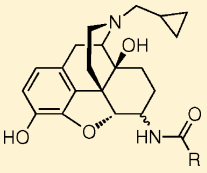
pubs.acs.org/jmc

Opioid Activity of Spinally Selective Analogues of *N*-Naphthoyl-β-naltrexamine in HEK-293 Cells and Mice

Morgan Le Naour, Mary M. Lunzer, Mike D. Powers, and Philip S. Portoghese*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, United States

ABSTRACT: Using the selective mu−kappa agonist, *N*-naphthoyl-β-naltrexamine **1**, as the prototype ligand, a series of closely related naphthalene analogues were synthesized to study the chemical space around the naphthalene moiety in an effort to evaluate how receptor selectivity is affected by chemical modification. Nine analogues (**2**−**10**) of compound **1** were synthesized and tested on HEK-293 cells expressing homomeric and heteromeric opioid receptors, and in the mouse tail-flick assay. It was found that a small change in structure produces profound changes in selectivity in this series. This is exemplified by the discovery that introduction of a 6-fluoro group transforms **1** from a selective mu−kappa heteromeric receptor agonist to a delta-preferring agonist **7**. The in vivo studies reveal that many of the ligands are more potent spinally than supraspinally and devoid of tolerance.



INTRODUCTION

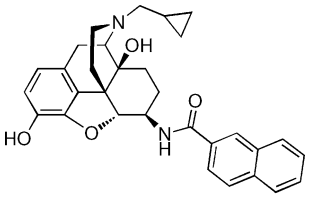
Morphine and other opiates derived from opium have been employed as analgesics for more than a century. A feature common to these analgesics is that they produce side effects that include tolerance, physical dependence, constipation, and respiratory depression. These ligands produce their effects through interaction with opioid receptors that are class A members of the G protein-coupled receptor (GPCR) superfamily.[1,2]

The three major classes of opioid receptors known to mediate antinociception upon activation are designated mu (MOP), delta (DOP), and kappa (KOP).[2] Since the establishment of these receptor types through binding, pharmacology, and cloning, selective opioid ligands have played an important role in deconvoluting the effects of opioid ligands acting at multiple opioid receptors.[3] This classification was based primarily on the assumption that opioid receptors exist as monomers.[4]

The concept of mu, delta, and kappa receptors as monomers was first challenged nearly three decades ago through studies with bivalent ligands that could bridge putative homomeric opioid receptors.[5,6] Later studies in cultured cells revealed that class A−C GPCRs can exist as homodimers or heteromers.[7−9] This was followed by the discovery of mu−delta, kappa−delta, and mu−kappa heteromers.[10−13]

The existence of opioid receptor heteromers suggests a level of action and regulation more complex than the traditional view of monomeric receptor pharmacology. For example, morphine, fentanyl, and methadone, traditionally viewed as mu-selective analgesics, have been reported to be more potent in activating mu−delta heteromers in cultured cells.[14] Moreover, mu−delta heteromers also appear to be the principal target for producing antinociception in monkeys.[15]

Molecular tools that target opioid heteromers have been developed in an effort to sort out the effects mediated by such receptors. These have included ligands that selectively target mu−delta, mu−kappa, and delta−kappa heteromers. The recently reported, spinally selective mu−kappa agonist, *N*-naphthoyl-β-naltrexamine **1** (NNTA),[16] selectively activates mu−kappa heteromers in HEK-293 cells and produces exceptionally potent antinociception upon intrathecal (i.t.) administration in mice. Given the ∼100-fold greater spinal potency of **1** compared to that by the intracerebroventricular (i.c.v.) route, it is believed that the mu−kappa heteromers that mediate antinociception are localized in the spinal cord rather than supraspinally. Significantly, no tolerance was produced i.t., and only marginal tolerance was observed i.c.v. This profile prompted us to synthesize and evaluate derivatives related to ligand **1** to explore the structural requirements for selectivity.



1 (NNTA)

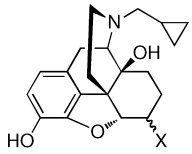
CHEMISTRY

The series was designed on the basis of conservative structural changes in the amide moiety. In this regard, the key question that we wished to address was the structural requirements for the mu−kappa selectivity of **1**. Consequently, all members (**2**−**10**) of the series contain a naltrexamine opioid pharmacophore linked through an amide moiety to a substituted naphthalene or a heterocycle isosteric with naphthalene.

β-Naltrexamine **11** was employed as the opioid pharmacophore for all members of the series except **2**, which was derived from α-naltrexamine **12**, for evaluation of the stereochemical role of the amide moiety in conferring selectivity. Both α- and β-naltrexamine were derived from naltrexone.[17]

Received: July 9, 2011
Published: December 2, 2011

The general procedure for synthesis of analogues **2**–**10** involved coupling of naltrexamine **11** or **12** in the presence of benzotriazol-1-yl-oxy-tris(dimethyamino)phosphonium hexafluorophosphate (BOP) and diisopropylethylamine (DIPEA) in dichloromethane (DCM).[18] The target compounds **2**–**10** were thus obtained in moderate to high yields (58–84%) after chromatographic purification.



11, X= β–NH_2
12, X= α–NH_2

BIOLOGICAL RESULTS

Intracellular Ca Release Studies. The target compounds were tested for agonist activity using an intracellular calcium release assay. Briefly, HEK-293 cells were transfected with a chimeric Δ6-$G_{\alpha qi4\text{-}myr}$ protein[15] employed to measure intracellular Ca^{2+} ion release upon receptor activation.[1] Cells stably expressing the chimeric protein were selected from transiently transfected cells in zeocin-containing media (DMEM, 10% fetal bovine serum, 1% penicillin/streptomycin, and 0.1 μg/mL zeocin). Opioid receptors were transiently transfected using different combinations of DNA for heteromers (mu–delta, mu–kappa, and kappa–delta) or for singly expressing homomers (mu, delta, and kappa). Intracellular calcium release was measured using a FLIPR calcium kit (Molecular Devices) in a FlexStation3 apparatus. For each compound, concentration–response profiles were established by measuring the fluorescence for 90 s after addition of the compound and determining the peak effect (maximum − minimum). A dose–response curve was plotted with the change in relative fluorescence units (ΔRFU) versus concentration (Table 1). Because the efficacy of **1** was substantially higher than those of other members of the series, the RFU scale for the activity of **2**–**10** is expanded to 2.5 times (0–1000) that of the ligand of reference **1** (0–2500).

The selectivity profile of **1** is displayed as the structurally related reference compound for the closely related series of compounds. Its 6α epimer **2** was synthesized to evaluate the role of the C-6 stereocenter in the activation of mu–kappa heteromers. It is noteworthy that its selectivity profile differs substantially from that of **1**, in that the extent of mu–kappa receptor activation has been greatly reduced. Other opioid receptors also showed reduced levels of activation. These data led us to explore only close structural modifications in the 6β series.

The activity of the *N*-methyl analogue **3**[20] differed significantly from that of compound **1** in that activation of mu–kappa receptors was lost. There was an apparent small increase in the level of activation at delta receptors in the higher concentration range. Conformational differences in the naphthyl moiety induced by the *N*-methyl group make up one of several possibilities that could contribute to loss of mu–kappa selectivity.

Regioisomer **4** activated both mu–kappa and delta–kappa heteromers to the same degree, but the mu–kappa component was ∼3-fold less effective than **1**. The curves for mu–kappa, mu–delta, and mu receptors were grouped together and have lower RFU values than the kappa-containing heteromers. No activation of delta receptors is apparent.

Various substituents were introduced at the 3′ and 6′ positions of the naphthoyl group. Introduction of a 6′-methoxy group (**5**) resulted in a loss of efficacy, suggesting that an electron-donating group in that position is not well tolerated. However, the same substituent in position 3′ (**6**) afforded moderate delta selectivity. As the 3′-methoxy group could engage in intramolecular hydrogen bonding with the carboxamide NH group, the delta selectivity might arise by restricting the naphthyl moiety to a conformation that differs from that of **5**.

Interestingly, the congener with a 6′-fluoro group (**7**) was delta-selective and exhibited considerably greater agonist potency and efficacy for delta receptors than **6**. The fact that the activity of **7** is substantially more potent than that of **5** is consistent with the idea that an electron-withdrawing group favors delta receptor activation in view of the inactivity of the 6′-methoxy analogue.

We also explored heterocyclic analogues (**8**–**10**) of **1** whose aromatic rings are isosteric with naphthalene. They included quinazoline **8** and isomeric quinolines **9** and **10**. All of these analogues were inactive at delta receptors but possessed nonselective opioid activity at heteromeric and homomeric receptors in the nanomolar range. In this regard, **8** and **9** had similar profiles with higher efficacy at mu–delta and mu–kappa receptors.

Pharmacological Evaluation in Mice. The in vivo profiles of compounds **2**–**10** were evaluated using the mouse tail-flick assay after the ligands had been administered by the i.c.v. and/or i.t. routes[21–23] (Table 2). Tolerance was measured only for selected compounds displaying a full agonist profile i.c.v. and/or i.t. by comparing the ED_{80-90} dose measured on day 1 to the same dose measured 24 h later on the same mice.

The 6α stereoisomer **2** and *N*-methyl analogue **3** possessed partial agonist antinociceptive activity when administered by both routes. These data were supported by their low efficacy in the calcium mobilization assay as shown previously (Table 1).

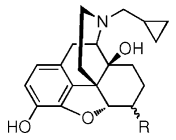
Compound **4** displayed an i.t. potency similar to that of the parent compound (20.98 pmol/mouse vs 18.7 pmol for **1**), but it did not exhibit a full agonist profile when given supraspinally. The exceptional spinal activity was accompanied by tolerance 24 h after i.t. administration.

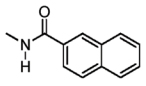
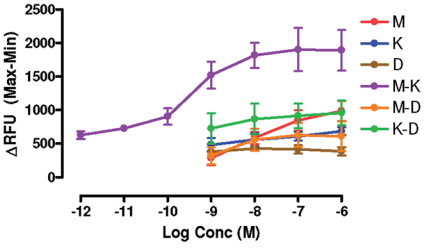
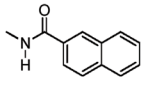
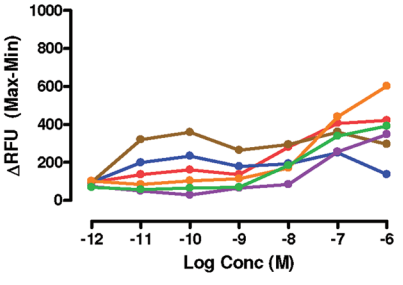
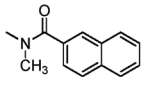
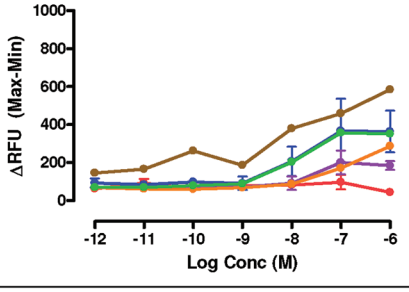
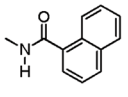
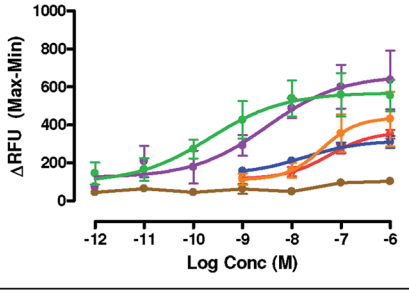
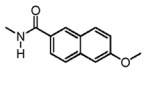
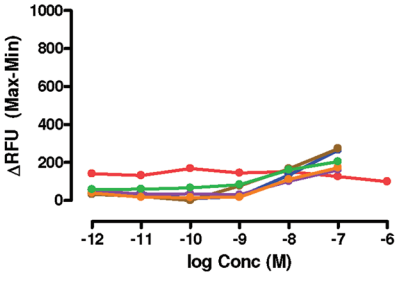
The negative effect of a 6′-methoxy group (**5**) on efficacy, as revealed in the calcium mobilization assay, was supported by its i.t. and i.c.v. partial agonist profile. The activity of the 3′ isomer **6** was greater than that of **5** when they were injected spinally (ED_{50} = 115 pmol/mouse) or supraspinally (ED_{50} = 312 pmol) and consistent with the cell-based results. No i.t. tolerance was apparent, while some tolerance was observed after i.c.v. injection.

The 6′-fluoro analogue **7** was found to be a fairly potent agonist by the i.t. route, with an ED_{50} of 81.1 pmol. No tolerance was observed. Because **7** was found to be delta-selective in the calcium release assay, it was further evaluated using the selective antagonists, nor-BNI[24] (kappa) and NTI[25] (delta), and in mu receptor knockout mice (Figure 1). After i.t. administration of **7**, NTI produced a 26-fold shift consistent with the delta selectivity observed in the cell-based assay. The finding that its activity was also antagonized by nor-BNI may be due to the antagonism of the combined agonist effect mediated by kappa, kappa–delta, and kappa–mu receptors, given that **7** activates these receptor systems in the nanomolar range as

dx.doi.org/10.1021/jm200902v | J. Med. Chem. 2012, 55, 670–677

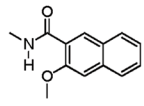
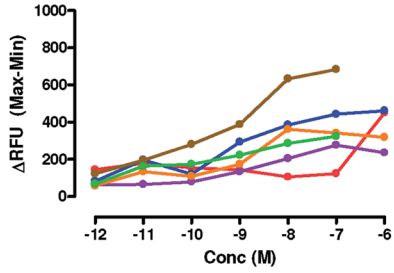
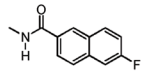
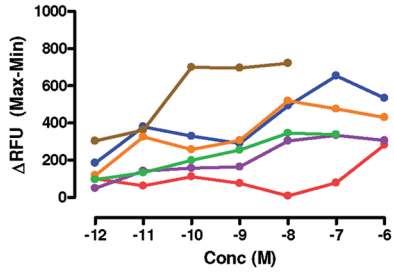
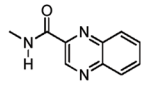
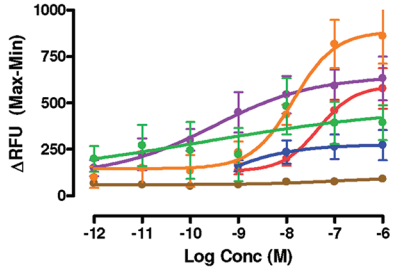
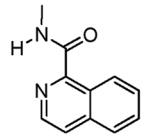
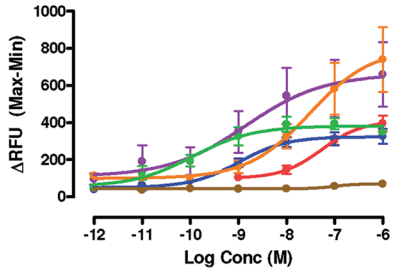
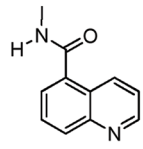
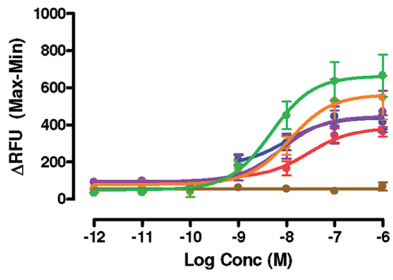
Table 1. Intracellular Ca^{2+} Release Profiles at Multiple Opioid Receptors



Compound	C-6 configuration	R	Calcium mobilization[a]
1[b] (NNTA)	β		
2	α		
3	β		
4	β		
5	β		

dx.doi.org/10.1021/jm200902v | J. Med. Chem. 2012, 55, 670−677

Table 1. continued

Compound	C-6 configuration	R	Calcium mobilization[a]
6	β		
7	β		
8	β		
9	β		
10	β		

[a]Data are means ± the standard error of the mean (n = 3–8). [b]Data from ref 16.

shown in the calcium release assay. Such antagonism by nor-BNI may be mediated through a negative allosteric effect mediated via the kappa receptor, as suggested previously.[26] Thus, nor-BNI may antagonize a mu or delta receptor in a heteromer via an associated kappa protomer in vivo. This is consistent with the decreased level of antinociception of **7** in the mu opioid receptor knockout mice due to the absence of mu–delta heteromers.

The quinoxaline analogue **8** was found to be a potent agonist by both i.c.v. and i.t. routes (ED_{50} values of 50.76 and 757.2 pmol, respectively), with 15-fold greater spinal potency. It is noteworthy that **8** exhibited no apparent tolerance by either route of administration. Quinoline analogue **9** also exhibited

dx.doi.org/10.1021/jm200902v | J. Med. Chem. 2012, 55, 670–677

Table 2. Antinociceptive Activity and Tolerance of 2−10 after i.c.v. or i.t. Administration in Mice

compd[a]	injection site	ED_{50} (pmol/mouse) (95% confidence interval)	24 h tolerance[b]
1 (NNTA)	i.t.	18.7 (10.3−32.8)	no
	i.c.v.	2060 (1090−3270)	yes
2	i.t.	partial agonist[c]	−
	i.c.v.	partial agonist[c]	−
3	i.t.	partial agonist[c]	−
	i.c.v.	partial agonist[c]	−
4	i.t.	20.9 (13.5−32.7)	yes
	i.c.v.	partial agonist[c]	−
5	i.t.	partial agonist[c]	−
	i.c.v.	partial agonist[c]	−
6	i.t.	115.1 (95.5−138.7)	no
	i.c.v.	312.4 (168.6−578.7)	yes
7	i.t.	81.1 (40.7−161.7)	no
	i.c.v.	partial agonist[c]	−
8	i.t.	50.8 (30.0−95.6)	no
	i.c.v.	757.2 (561.7−1021)	no
9	i.t.	1408 (931−2128)	no
	i.c.v.	2070 (1490−2876)	no
10	i.t.	partial agonist[c]	−
	i.c.v.	partial agonist[c]	−

[a]Peak times for the dose−response curves were as follows: 5 min for **1**, **5**, **6**, and **8** for i.t., 20 min for **7** and **9** for i.t., 10 min for **5** and **9** for i.c.v., and 20 min for NNTA. [b]The 24 h tolerance was calculated using the highest dose of the dose−response curve on day 1 and repeated on day 2. If there was no significant difference between the two days, the animals were said to be not tolerant. [c]Maximal percent maximal possible effect of ≤60%.

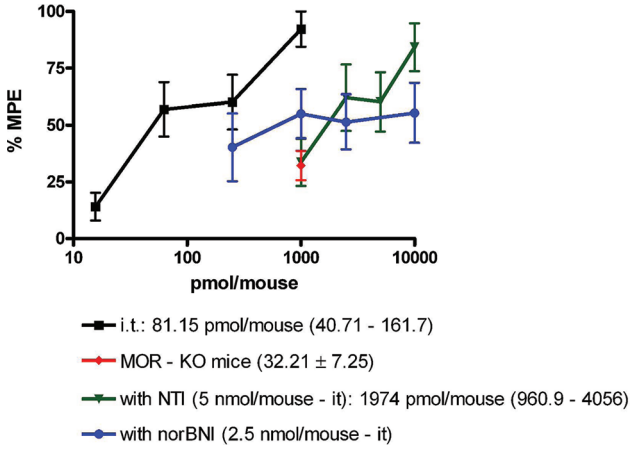


Figure 1. Antinociception of compound **7** after treatment with opioid antagonists naltrindole (NTI) and norbinatorphimine (norBNI) after i.t. administration in wild-type mice and in mu opioid receptor knockout (MOR-KO) mice.

full i.t. and i.c.v. agonist activity without tolerance, but it was substantially less potent than **8**. Interestingly, compound **4**, the isosteric naphthalene analogue of **9**, was exceptionally potent i.t. but displayed tolerance. The isomeric quinoline analogue **10** behaved as a partial agonist when administered spinally or supraspinally.

DISCUSSION

Using the selective mu−kappa agonist **1**, as the prototype ligand, a series of closely related naphthalene analogues were synthesized to study the chemical space around the naphthalene moiety in an effort to evaluate how receptor selectivity is affected by chemical modification. Nine analogues (**2**−**10**) were synthesized and tested on HEK-293 cells expressing homomeric and heteromeric opioid receptors, and in the mouse tail-flick assay.

Because **1** is derived from 6β-naltrexamine, we first explored the effect of configuration at the C-6 chiral center by the synthesis of **2**. As it appeared from the cell-based data that **2** gave a profound reduction in the level of activation of opioid receptors, particularly mu−kappa receptors, relative to that of **1**, we decided to focus on ligands in the 6β series.

In this regard, we first evaluated the effect of substitution on the 6β nitrogen atom with compound **3**, as we suspected an *N*-methyl group would alter the rotational conformational preference of the naphthalene moiety. Once again, the level of activation of heteromeric and homomeric receptors was greatly reduced, suggesting that an NH group hydrogen bonding with the receptor(s) and/or conformational factors might be involved.

Interestingly, the 1-naphthyl analogue **4** exhibited improved ΔRFU values for activation of heteromeric mu−kappa and delta−kappa receptors relative to compound **3**. As both of these heteromers have been reported to be potently activated in the spinal cord of rodents, it is not surprising that **4** produces a high level of i.t. antinociception. Other receptors were activated to a lesser degree, and delta showed no activation.

The effect of substitution of the naphthyl group had a profound effect on selectivity and potency. Thus, a 6′-OMe substituent (**5**) greatly reduced the level of activation of all homomers and heteromers, whereas its 3′ regioisomer (**6**) selectively activated delta receptors. These results are consistent with several possible explanations that include an unfavorable electronic or steric effect by the 6′-OMe group in **5** and a favorable effect on the conformation of the naphthyl group of **6** due to possible intramolecular H-bonding between the 6β-NH group and the 3′-OMe group.

The 6′-fluoro-substituted analogue **7** appeared to be more delta-selective than **6**. One possible explanation could be the electron withdrawing effect of the 6′-fluoro group, in view of the electron-donating 6′-OMe group of **5** having the opposite effect on activity. It is noteworthy that **7** did not produce tolerance and was highly potent in mice by the i.t. route.

The quinoxaline analogue **8** and quinoline analogues (**9** and **10**) were essentially nonselective agonists in HEK-293 cells. The trend seems to support a greater level of activation for mu−delta and mu−kappa receptors. Interestingly, these ligands failed to activate delta receptors.

Of the nine analogues tested in mice, five (**4** and **6**−**9**) were highly potent full agonists after i.t. administration. Among these, only compound **4** exhibited tolerance. Analogues **2**, **3**, **5**, and **10** produced partial agonism by this route. Partial agonism among members of the series was observed more frequently upon i.c.v. administration, and in this regard, **2**−**5**, **7**, and **10** produced this effect. The only members of the series that were full agonists both i.c.v. and i.t. were compounds **6**, **8**, and **9**. It is noteworthy that i.t. administration resulted in potency greater than that produced by the i.c.v. route when the ED_{50} values were determined after both i.t. and i.c.v. administration.

dx.doi.org/10.1021/jm200902v | J. Med. Chem. 2012, 55, 670−677

CONCLUSIONS

Judging from the calcium release studies of compounds **2**−**10** in HEK-293 cells, the high selectivity of **1** for activation of heteromeric mu−kappa receptors appears to be unique in this series. Intriguingly, the 6-fluoro analogue **7** appeared to selectively activate homomeric delta receptors in vitro and induced potent i.t. antinociception without tolerance in mice. The finding that a small change in structure produces a profound change in selectivity exemplifies the complexity of the structure−activity relationships in this series. The complex profiles of these compounds in activating the six combinations of opioid receptors make it difficult to come to any firm conclusions regarding the relationship of receptor activation to potency and tolerance in vivo. Nevertheless, on the basis of the i.t. and i.c.v. data, most of the analogues possess potent antinociceptive activity that arises from the selective activation of spinal opioid receptors.

EXPERIMENTAL SECTION

All commercial reagents and anhydrous solvents were purchased from vendors and were used without further purification or distillation. Analytical thin-layer chromatography (TLC) was performed on EM Science silica gel 60 F_{254} (0.25 mm). Compounds were visualized with UV light. Flash column chromatography was performed on Fisher Scientific silica gel (230−400 mesh).

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Bruker Avance 400 MHz instruments and calibrated using an internal reference. Chemical shifts are expressed in parts per million, and coupling constants (J) are in hertz. Peak multiplicities are abbreviated: br, broad; s, singlet; d, doublet; t, triplet; and m, multiplet. ESI mode mass spectra were recorded on a BrukerBioTOF II mass spectrometer. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ). Analytical data confirmed the purity of the products was ≥95%.

General Procedure for the Amidation of β-Naltrexamine with a Carboxylic Acid. β-Naltrexamine (100 mg, 0.29 mmol), heterocyclic carboxylic acid (0.58 mmol), and BOP (258 mg, 0.58 mmol) were dissolved in DCM (5 mL). To this solution was added DIPEA (150 mL, 0.81 mmol), and the mixture was stirred at room temperature for 3−16 h. The solution was concentrated under reduced pressure and the residue taken up in MeOH (5 mL), and K_2CO_3 was added (300 mg). After 1 h at room temperature, the mixture was concentrated to dryness. The final crude was purified by SiO_2 chromatography to afford the target compound.

The title compound was then subsequently converted into the HCl salt for biological testing.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-[(2′-naphthyl)acetamido]morphinan (2). Compound **2** was prepared according to the general procedure described above; combining α-naltrexamine (100 mg, 0.29 mmol), 2-naphthoic acid (117 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (70:30 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **2** as a white solid (116 mg, 81%): ^{1}H NMR (DMSO-d_6) δ 0.13 (m, 2H), 0.49 (m, 2H), 0.88 (m, 1H), 1.37−1.69 (m, 4H), 2.14−2.37 (m, 4H), 2.96−3.08 (m, 2H), 4.54 (m, 1H), 4.69 (d, 1H, J_{H5-H6} = 3.8), 4.92 (bs, 1H, OH-14), 6.48 (d, 1H, J_{H1-H2} = 8.1), 6.59 (d, 1H, J_{H2-H1} = 8.1), 7.59−7.63 (m, 2H), 7.93−8.04 (m, 4H), 8.10 (d, 1H amide, J = 7.7), 8.47 (s, 1H), 8.89 (bs, 1H, OH-3); ^{13}C NMR (DMSO-d_6) δ 3.40, 3.81, 9.13, 19.98, 22.31, 29.44, 33.53, 42.78, 46.52, 46.65, 58.75, 61.36, 69.23, 88.27, 117.22, 118.49, 124.40, 124.85, 126.67, 127.51, 127.58 (×2), 127.74, 128.76, 130.68, 131.88, 132.05, 134.06, 137.97, 145.82, 165.91; mp 232−234 °C; ESI-TOF MS calcd for $C_{31}H_{32}N_2O_4$ m/z 496.236, found 519.303 (M + Na)$^+$. Anal. Calcd for $C_{31}H_{33}ClN_2O_4$: C, 69.85; H, 6.24; N, 5.26. Found: C, 69.98; H, 6.13; N, 5.32.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(1′-naphthyl)acetamido]morphinan (4). Compound **4** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), 1-naphthoic acid (117 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (75:25 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **4** as a white solid (120 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 0.11 (m, 2H), 0.52 (m, 2H), 0.81 (m, 1H), 1.47 (m, 2H), 1.62−1.75 (m, 2H), 1.91 (m, 1H), 2.17 (m, 2H), 2.35 (m, 2H), 2.61 (m, 2H), 3.01 (d, 1H), 3.08 (m, 1H), 4.18 (m, 1H), 4.50 (d, 1H, J_{H5-H6} = 7.8), 6.55 (d, 1H, J_{H1-H2} = 8.1), 6.72 (d, 1H, J_{H2-H1} = 8.1), 6.87 (d, 1H, J = 8.9), 7.38 (m, 1H), 7.48−7.53 (m, 2H), 7.58 (Dd, 1H, J = 7, 1.2), 7.79−7.92 (m, 2H), 8.32 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 3.77, 3.97, 9.38, 22.64, 23.91, 31.23, 31.45, 43.96, 47.54, 51.00, 59.26, 62.26, 70.14, 93.40, 117.83, 119.19, 124.54, 124.72, 125.04, 125.42, 126.36, 127.12, 128.27, 130.10, 130.58, 130.86, 133.65, 134.44, 139.82, 142.97, 169.46; mp 224−226 °C; ESI-TOF MS calcd for $C_{31}H_{32}N_2O_4$ m/z 496.236, found 497.139 (M + H)$^+$, 993.260 (2M + H)$^+$. Anal. Calcd for $C_{31}H_{33}ClN_2O_4$: C, 74.98; H, 6.50; N, 5.64. Found: C, 75.27; H, 6.57; N, 5.74.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(6′-methoxy-2′-naphthyl)acetamido]morphinan (5). Compound **5** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), 6-methoxy-2-naphthoic acid (117 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (75:25 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **5** as a white solid (114 mg, 75%): ^{1}H NMR (DMSO-d_6) δ 0.15 (m, 2H), 0.50 (m, 2H), 0.88 (m, 1H), 1.23−1.62 (m, 4H), 1.82−1.91 (m, 2H), 2.08 (m, 1H), 2.27 (m, 2H), 2.36 (m, 2H), 3.03 (m, 2H), 3.75 (m, 1H), 3.90 (s, 3H), 4.77 (d, 1H, J_{H5-H6} = 7.1), 4.91 (bs, 1H, OH-14), 6.57−6.60 (m, 2H), 7.23 (Dd, 1H, J = 9, 2.4), 7.38 (d, 1H, J = 1.9), 7.86−7.94 (m, 3H), 8.42 (s, 1H), 8.72 (D, 1H amide, J_{NH-H6} = 8.1), 9.04 (bs, 1H, OH-3); ^{13}C NMR (DMSO-d_6) δ 3.27, 3.45, 9.03, 22.32, 24.46, 29.89, 31.14, 38.81, 41.66, 45.71, 51.47, 55.27, 61.17, 69.63, 90.48, 105.82, 117.20, 118.76, 119.33, 123.45, 124.61, 126.57, 127.30, 127.42, 127.55, 129.28, 130.37, 135.70, 140.60, 142.11, 158.50, 165.67; mp 199−201 °C; ESI-TOF MS calcd for $C_{32}H_{34}N_2O_5$ m/z 526.247, found 527.298 (M + H)$^+$. Anal. Calcd for $C_{32}H_{35}ClN_2O_5$: C, 68.25; H, 6.27; N, 4.97. Found: C, 68.38; H, 6.13; N, 5.04.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-methoxy-2′-naphthyl)acetamido]morphinan (6). Compound **6** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), 6-methoxy-2-naphthoic acid (117 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (75:25 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **6** as a white solid (119 mg, 78%): ^{1}H NMR (DMSO-d_6) δ 0.12 (m, 2H), 0.47 (m, 2H), 0.85 (m, 1H), 1.26−1.66 (m, 4H), 1.81−1.90 (m, 2H), 2.08 (m, 1H), 2.27 (m, 2H), 2.36 (m, 2H), 3.03 (m, 2H), 3.75 (m, 1H), 3.90 (s, 3H), 4.77 (d, 1H, J_{H5-H6} = 7.1), 4.91 (bs, 1H, OH-14), 6.57−6.60 (m, 2H), 7.23 (Dd, 1H, J = 9, 2.4), 7.38 (d, 1H, J = 1.9), 7.86−7.94 (m, 3H), 8.23 (s, 1H), 8.50 (D, 1H amide, J_{NH-H6} = 8.1), 9.04 (bs, 1H, OH-3); ^{13}C NMR (DMSO-d_6) δ 3.45, 3.71, 9.17, 22.17, 24.30, 29.97, 30.36, 41.23, 45.67, 47.13, 51.56, 55.83, 61.72, 69.63, 90.63, 106.46, 115.91, 117.96, 124.20, 125.30, 126.33, 127.41, 127.66, 127.77, 128.17, 128.34, 130.66, 134.94, 140.43, 142.17, 154.41, 164.73; mp 215−26 °C; ESI-TOF MS calcd for $C_{32}H_{34}N_2O_5$ m/z 526.247, found 527.301 (M + H)$^+$. Anal. Calcd for $C_{32}H_{35}ClN_2O_5$: C, 68.25; H, 6.27; N, 4.97. Found: C, 68.47; H, 6.21; N, 5.02.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(6′-fluoro-2′-naphthyl)acetamido]morphinan (7). Compound **7** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), 6-fluoro-2-naphthoic acid (110 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA

dx.doi.org/10.1021/jm200902v | J. Med. Chem. 2012, 55, 670−677

(150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (80:20 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **7** as a white solid (118 mg, 79%): ^{1}H NMR (CD_3OD) δ 0.23 (m, 2H), 0.59 (m, 2H), 0.94 (m, 1H), 1.47−1.79 (m, 4H), 2.03 (m, 1H), 2.17−2.35 (m, 2H), 2.44−2.52 (m, 2H), 2.70−2.76 (m, 2H), 3.13−3.23 (m, 2H), 4.01 (m, 1H), 4.72 (d, 1H, J = 7.7), 6.63 (d, 1H, J = 8.1), 6.68 (d, 1H, J = 8.1), 7.42 (td, 1H, J = 2.5, 9.8), 7.62 (dd, 1H, J = 2.4, 9.9), 7.93−7.99 (m, 2H), 8.08 (m, 1H), 8.45 (s, 1H); ^{13}C NMR (CD_3OD) δ 4.14, 4.58, 10.23, 23.61, 25.59, 31.34, 31.42, 43.86, 49.01, 53.49, 60.19, 63.80, 71.82, 93.04, 111.69, 111.90, 117.93, 118.18, 118.67, 120.13, 126.10 (×2), 128.70, 128.91, 131.12, 132.55, 132.88, 134.05, 140.47, 143.74, 169.76; ^{19}F NMR (CD_3OD) δ −114.03; mp 189−190 °C; ESI-TOF MS calcd for $C_{31}H_{31}FN_2O_5$ m/z 514.227, found 515.300 $(M + H)^+$. Anal. Calcd for $C_{31}H_{32}ClFN_2O_5$: C, 67.57; H, 5.85; N, 5.08. Found: C, 67.91; H, 5.85; N, 5.17.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(2′-quinoxalyl)acetamido]morphinan (8). Compound **8** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), 2-quinoxalinecarboxylic acid (101 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (75:25 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **8** as a white solid (110 mg, 77%): ^{1}H NMR (DMSO-d_6) δ 0.01 (m, 2H), 0.37 (m, 2H), 0.72 (m, 1H), 1.28 (m, 1H), 1.49 (m, 2H), 1.85 (m, 2H), 2.05 (m, 2H), 2.25 (m, 2H), 2.55 (m, 2H), 2.93−3.02 (m, 2H), 4.00 (m, 1H), 4.37 (d, 1H, J_{H5-H6} = 7.8), 6.46 (d, 1H, J_{H1-H2} = 8.1), 6.55 (d, 1H, J_{H2-H1} = 8.1), 7.31−7.36 (m, 3H), 7.44 (m, 1H), 7.81 (d, 1H, J = 8.3), 7.89 (d, 2H, J = 8.2), 8.15 (d, 1H, J = 8.7), 8.39 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 3.52, 3.64, 9.36, 24.40, 25.26, 30.06, 30.27, 43.6, 47.43, 52.67, 58.37, 61.75, 69.56, 90.22, 110.87, 115.64, 118.31, 119.08, 126.54 129.07, 129.38, 131.26, 139.76, 142.08, 142.84, 143.66, 144.36, 156.54, 162.88; mp 177−180 °C; ESI-TOF MS calcd for $C_{29}H_{30}N_4O_4$ m/z 498.227, found 499.121 $(M + H)^+$, 1019.221 $(2M + Na)^+$. Anal. Calcd for $C_{29}H_{31}ClN_4O_4$: C, 65.10; H, 5.84; N, 10.47. Found: C, 64.81; H, 5.81; N, 10.55.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(1′-isoquinolyl)acetamido]morphinan (9). Compound **9** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), isoquinoline-1-carboxylic acid (100.4 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (90:10 EA/hexanes) and then recrystallized from an acetone/hexanes mixture to provide **9** as a white solid (90 mg, 63%): ^{1}H NMR ($CDCl_3$) δ 0.13 (m, 2H), 0.53 (m, 2H), 0.85 (m, 1H), 1.17−1.68 (m, 4H), 1.83−2.04 (m, 3H), 2.18 (m, 2H), 2.25 (m, 2H), 2.38 (m, 2H), 2.61−2.67 (m, 2H), 4.06 (m, 1H), 4.60 (d, 1H, J_{H5-H6} = 7.8), 6.58 (d, 1H, J_{H1-H2} = 8.1), 6.73 (d, 1H, J_{H2-H1} = 8.1), 7.58−7.78 (m, 4H), 8.39 (d, 1H, J = 5.5), 8.58 (d, 1H, J = 9.1), 9.49 (d, 1H amide, J_{NH} = 8.2); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.97, 9.44, 22.70, 24.54, 30.27, 30.57, 44.11, 47.84, 51.34, 59.23, 62.37, 70.21, 93.82, 117.73, 119.17, 124.36, 124.44, 126.69, 126.92, 127.76, 128.56, 130.38, 131.15, 137.32, 140.06, 140.12, 142.56, 147.93, 165.89; mp 152−154 °C; ESI-TOF MS calcd for $C_{30}H_{31}N_3O_4$ m/z 497.231, found 498.226 $(M + H)^+$, 1017.425 $(2M + Na)^+$. Anal. Calcd for $C_{30}H_{32}ClN_3O_4$: C, 67.47; H, 6.04; N, 7.87. Found: C, 67.11; H, 6.08; N, 7.93.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(5′-quinolyl)acetamido]morphinan (10). Compound **10** was prepared according to the general procedure described above; combining β-naltrexamine (100 mg, 0.29 mmol), 5-quinoline-1-carboxylic acid (100.4 mg, 0.58 mmol), BOP (258 mg, 0.58 mmol), and DIPEA (150 μL, 0.81 mmol) followed by basic solvolysis with K_2CO_3 gave the target compound that was purified by flash chromatography (4% MeOH/DCM) and then recrystallized from an acetone/hexanes mixture to provide **10** as a white solid (83 mg, 58%): ^{1}H NMR (CD_3OD) δ 0.18 (m, 2H), 0.56 (m, 2H), 0.91 (m, 1H), 1.45 (m, 1H), 1.58−1.65 (m, 2H), 1.84 (m, 1H), 2.00 (m, 1H), 2.13−2.36 (m, 2H), 2.43−2.51 (m, 2H), 2.69−2.73 (m, 2H), 3.10−3.20 (m, 2H), 3.99 (m, 1H), 4.59 (d, 1H, J_{H5-H6} = 7.8), 6.62 (d, 1H, J_{H1-H2} = 8.1), 6.69 (d, 1H, J_{H2-H1} = 8.1), 7.62 (m, 1H), 7.82−7.85 (m, 2H), 8.14 (t, 1H, J = 5.3), 8.78 (d, 1H, J = 9.5), 8.90 (dd, 1H, J = 1.6, 4.2); ^{13}C NMR (CD_3OD) δ 4.15, 4.54, 10.13, 23.59, 25.66, 31.44, 31.71, 45.44, 48.78, 53.54, 60.20, 63.75, 71.80, 92.95, 118.61, 120.18, 123.25, 125.36, 127.02, 127.24, 130.18, 131.60, 132.54, 136.16, 136.27, 142.08, 143.73, 148.69, 151.74, 170.64; mp 165−167 °C; ESI-TOF MS calcd for $C_{30}H_{31}N_3O_4$ m/z 497.231, found 498.133 $(M + H)^+$. Anal. Calcd for $C_{30}H_{32}ClN_3O_4$: C, 67.47; H, 6.04; N, 7.87. Found: C, 67.82; H, 6.09; N, 7.79.

MATERIALS AND METHODS

Intracellular Calcium Release. To determine the functional selectivity of the ligands described above, a modified intracellular calcium release assay was employed. As activation of opioid receptors does not regulate signaling mechanisms that cause calcium release because they are coupled to Gi/o, a chimeric G protein ($\Delta 6\text{-}G_{\alpha qi4\text{-}myr}$) was employed. The construction of this chimera has been reported.[19] Human embryonic kidney (HEK-293) cells stably expressing human Giq protein were grown at 37 °C and 10% CO_2 in Dulbelcco's modified medium (GIBCO) using zeocin as the antibiotic for selecting cells expressing the Gi G protein.

HEK-293 cells containing the various opioid receptors were created by transiently transfecting opioid recptor DNA in OptiMEM medium (Invitrogen) at a concentration of 200 ng/20000 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Twenty-four hours later, 20000 cells/well were seeded into 96-well black plates (Corning Inc.). The FLIPR Calcium Explorer kit (Molecular Devices) was used for the assay. On the third day, cells were incubated with a Ca^{2+} chelating dye from the kit and incubated for 60 min at 37 °C. The plates were then assayed in a FlexStation 3 apparatus (Molecular Devices) using a range of concentrations of the ligand tested. The response was measured as the change in relative fluorescence units ($\Delta RFU = RFU_{max} - RFU_{min}$), and the time of the response was measured in seconds. Following a 30 s equilibration period, the ligand was added to each well of the plate. RFUs were measured continuously for 60 s following the addition of the ligand (the response before calcium ion reuptake mechanisms brings the Ca^{2+} ion concentration back to basal levels). The ΔRFU was computed for each concentration, which was plotted as a concentration response curve and analyzed, yielding the IC_{50} value using nonlinear regression. The peak effect was also recorded. At least three independent replications with four internal replicates were used to evaluate each ligand in each of the cell types.

Animals. Male ICR-CD1 mice (17−25 g; Harlan, Madison, WI) employed in the testing were housed in groups of eight in a temperature- and humidity-controlled environment with unlimited access to food and water. They were maintained on a 12 h light/dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Antinociceptive Testing. The tail-flick assay for antinociception described by D'Amour and Smith[21] and modified by Dewey at al.[22] was employed. For the measurement of tail-flick latency, mice were held gently with the tail positioned in the apparatus (Tail Flick Analgesia Meter, Columbus Instruments, Columbus, OH) for radiant heat stimulus. The tail-flick response was elicited by applying radiant heat to the dorsal side of the tail. The intensity of the heat was set so that the mouse flicks its tail within 2−3 s. The test latency was measured before drug treatment (control) and again after the drug treatment (test) at the peak time of the compound; a 10 s maximal cutoff time was used to prevent damage to the tail. Antinociception was quantified according to the method of Harris and Pierson[23] as the percent maximal possible effect (%MPE): %MPE = (test − control/10 − control) × 100.

At least three groups of 8−10 mice were used for each dose−response curve, and each mouse was used only once. ED_{50} values with 95% confidence intervals were computed with GraphPad Prism 4 by using nonlinear regression methods.

dx.doi.org/10.1021/jm200902v | J. Med. Chem. 2012, 55, 670−677

Agonism. All the compounds were dissolved in 10% DMSO and then diluted to <1% DMSO in the test solutions. Controls when given either i.c.v. or i.t. with ≤1% DMSO did not show any antinociception. All compounds were administered in a 5 μL volume in conscious mice according to the method of Haley and McCormick[27] for i.c.v. and Hylden and Wilcox[28] for i.t. injections. A time course study (10, 20, 30, and 60 min) was used to determine the peak antinociception. Administrations of ligands and the antagonist were timed so that they peaked at the same time [nor-BNI (2.5 nmol/mouse, 20 min peak time), NTI (5 nmol/mouse, 20 min peak time)]. Potency ratios (test ED_{50}/control ED_{50}) were deemed significant if their 95% confidence intervals did not overlap.

Acute tolerance was measured on select compounds by comparing the initial ED_{80-90} dose to the same dose measured 24 h later on the same mouse.

Knockout Studies. MOR-KO mice (male and female, 13−15 weeks old) were grouped with equal numbers of male and females so that there were eight mice per group. Each mouse was used only once. The MOR-KO mice were injected i.t. with the ED_{80-90} of the given agonist to see if there was a change in the %MPE. The point was considered significant if the 95% confidence intervals did not overlap.

AUTHOR INFORMATION

Corresponding Author

*E-mail: porto001@umn.edu. Phone: (612) 624-9174. Fax: (202) 513-8609.

ACKNOWLEDGMENTS

We thank Dr. Evi Kostenis for providing us with the cDNA for the Δ6-$G_{\alpha qi4\text{-}myr}$ chimeric Gα subunit and Dr. Sabita Roy for the MOR-KO mice. This research was supported by National Institutes of Health Grant DA01533.

REFERENCES

(1) IUPHAR receptor database, opioid receptors. http://uphar-db.org/ PRODGPCR.

(2) Gutstein, H. B.; Akil, H. Opioid analgesics. In *Goodman and Gillman's the Pharmacological Basis of Therapeutics*, 11th ed.; McGraw-Hill: New York, 2006; Chapter 21.

(3) (a) Takemori, A. E.; Portoghese, P. S. Selective naltrexone-derived opioid receptor antagonists. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 239−269. (b) Eguchi, M. Recent advances in selective opioid receptor agonists and antagonists. *Med. Res. Rev.* **2004**, *24* (2), 182−213.

(4) Gershengorn, M. C.; Osman, R. Minireview: Insights into G protein-coupled receptor function using molecular models. *Endocrinology* **2001**, *142*, 2−10.

(5) Erez, M.; Takemori, A. E.; Portoghese, P. S. Narcotic antagonistic potency of bivalent ligands which contain β-naltrexamine. Evidence for bridging between proximal recognition sites. *J. Med. Chem.* **1982**, *25*, 847−849.

(6) Portoghese, P. S.; Ronsisvalle, G.; Larson, D.; Takemori, A. E. Synthesis and opioid antagonist bivalent ligands with conformationally restricted spacers. *J. Med. Chem.* **1986**, *29*, 1650−1653.

(7) Angers, S.; Salahpour, A.; Bouvier, M. Dimerization: An emerging concept for G protein-coupled receptor ontogeny and function. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 409−435.

(8) Pin, J.-P.; Neubig, R.; Bouvier, M.; Devi, L.; Filizola, M.; Javitch, J. A.; Lohse, M. J.; Milligan, G.; Palczewski, K.; Parmentier, M.; spedding, M. International union of basic and clinical pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. *Pharmacol. Rev.* **2007**, *59*, 5−13.

(9) Devi, L. A. Heterodimerization of G-protein-coupled receptors: Pharmacology, signaling and trafficking. *Trends Pharmacol. Sci.* **2001**, *22*, 532−537.

(10) Jordan, B. A.; Devi, L. A. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* **1999**, *5*, 697−700.

(11) Gupta, A.; Decaillot, F. M.; Devi, L. A. Targeting opioid receptor heterodimers: Strategies for screening and drug development. *AAPS J.* **2006**, *8* (1), E153−E159.

(12) Aldrich, J. V.; Vigil-Cruz, S. C. Narcotic analgesics. In *Burger's Medicinal Chemistry and Drug Discovery*; Abraham, D. J., Ed.; John Wiley & Sons: New York, 2003; Vol. *6*, pp 329−481.

(13) Friderichs, E. Opioids. In *Analgesics, from Chemistry and Pharmacology to Clinical Application*; Buschmann, H., Christoph, T., Friderichs, E., Maul, C., Sundermann, B., Eds.; Wiley-VCH: Weinheim, Germany, 2002; pp 127−150.

(14) Yekkirala, A. S.; Kalyuzhny, A. E.; Portoghese, P. S. Standard opioid agonists activate heteromeric opioid receptors: Evidence for morphine and [D-Ala^2-MePhe4-Glyol5]enkephalin as selective m-d agonists. *ACS Chem. Neurosci.* **2010**, *1*, 146−154.

(15) Yekkirala, A. S.; Banks, M. L.; Lunzer, M. M.; Negus, S. S.; Rice, K. C.; Portoghese, P. S. Unpublished results.

(16) Yekkirala, A. S.; Lunzer, M. M.; McCurdy, C. R.; Powers, M. D.; Kalyuzhny, A. E.; Roerig, S. C.; Portoghese, P. S. N-Naphtoyl-β-Naltrexamine (NNTA), a highly selective and potent activator of heteromeric μ/κ opioid receptors. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (12), 5098−5103.

(17) Sayre, L. M.; Portoghese, P. S. Stereospecific synthesis of the 6α- and 6β-amino derivatives of naltrexone and oxymorphone. *J. Org. Chem.* **1980**, *45*, 3366−3368.

(18) (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. BOP: A new peptide coupling reagent exemplified in the synthesis of somatostatin. *Pept., Proc. Eur. Pept. Symp.* **1976**, *14*, 79−84. (b) Ghirmai, S.; Azar, M. R.; Polgar, W. E.; Berzetzei-Gurske, I.; Cashman, J. R. Synthesis and biological evaluation of α- and β-6-amido derivatives of 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxymorphinan: Potential alcohol-cessation agents. *J. Med. Chem.* **2008**, *51*, 1913−1924.

(19) Kostenis, E. Is G-α16 the optimal tool for fishing ligands of orphan G-protein-coupled receptors? *Trends Pharmacol. Sci.* **2001**, *22*, 560−564.

(20) (a) Kawai, K.; Hayakawa, J.; Miyamoto, T.; Imamura, Y.; Yamane, S.; Wakita, H.; Fujii, H.; Kawamura, K.; Matsuura, H.; Izumimoto, N.; Kobayashi, R.; Endoh, Y.; Nagase, H. Design, synthesis and structure-activity relationship of novel opioid κ-agonists. *Bioorg. Med. Chem.* **2008**, *16* (20), 9188−9201. (b) Nagase, H.; Hayakawa, J.; Kawamura, H.; Kawai, K.; Endoh, T. Preparation of morphinan derivatives as brain cell protecting agents. PCT Int Appl. CODEN: PIXXD2 WO 9503308 A1 19950202.

(21) D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74−79.

(22) Dewey, W. L.; Harris, L. S.; Howes, J. F.; Nuite, J. A. The effect of various neurochemical modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J. Pharmacol. Exp. Ther.* **1970**, *175*, 435−442.

(23) Harris, L. S.; Pierson, A. K. Some narcotic antagonists in the benzomorphan series. *J. Pharmacol. Exp. Ther.* **1964**, *143*, 141−148.

(24) 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.

(25) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of peptidomimetic δ opioid receptor antagonists using the message−address concept. *J. Med. Chem.* **1990**, *33*, 1714−1720.

(26) Lunzer, M. M.; Portoghese, P. S. Selectivity of δ- and κ-opioid ligands depends on the route of central administration in mice. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 166−171.

(27) Haley, T. J.; McCormick, W. G. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br. J. Pharmacol. Chemother.* **1957**, *12*, 12−15.

(28) Hylden, J. L.; Wilcox, G. L. Intrathecal morphine in mice: A new technique. *Eur. J. Pharmacol.* **1980**, *67*, 313−316.