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Designing bifunctional NOP receptor-mu opioid receptor ligands from NOP-receptor selective scaffolds. Part II

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Abstract

The nociceptin opioid receptor (NOP) and its endogenous peptide ligand nociceptin/orphanin FQ have been shown to modulate the pharmacological effects of the classical opioid receptor system. Suppression of opioid-induced reward associated with mu-opioid receptor (MOP)-mediated analgesia, without decreasing anti-nociceptive efficacy, can potentially be achieved with NOP agonists having bifunctional agonist activity at MOP, to afford ‘non-addicting’ analgesics. In Part II of this series, we describe a continuing structure-activity relationship (SAR) study of the NOP-selective piperidin-4-yl-1,3-dihydroindol-2-one scaffold, to obtain bifunctional activity at MOP, and a suitable ratio of NOP/MOP agonist activity that produces a non-addicting analgesic profile. The SAR reported here is focused on the influence of various piperidine nitrogen aromatic substituents on the ratio of binding affinity and intrinsic activity at both the NOP and MOP receptors.

Keywords

N/OFQ; Nociceptin/orphanin FQ; Nociceptin receptor; Bifunctional ligands; NOP/opioid

1. Introduction

Mu opioid receptor (MOP) agonists such as morphine, used in the treatment of acute and chronic pain, are well known to induce a host of undesirable effects, most notably dependence and tolerance. Nevertheless, MOP agonists remain the analgesic therapies of choice, due to their potent antinociceptive effects. Significant effort has been expended in exploring a multi-target approach to reduce MOP-related liabilities, by opposing activities at other pharmacological targets. For example, compounds with bifunctional mu agonist/ delta opioid receptor antagonist activity have been explored as an approach to decrease tolerance development of MOP agonists.^{1–3} Even non-opioid systems known to modulate opioid-

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mediated pharmacology have been investigated as potential targets in a ‘multi-target’ drug approach for analgesics lacking opioid-related side effects. An example of such an approach is the investigation of bifunctional opioid agonists/cholecystokinin antagonists, for neuropathic pain treatment without side effects such as opioid tolerance and sedation.⁴

Within the opioid family of receptors and endogenous ligands, the nociceptin opioid receptor NOP and its endogenous ligand, nociceptin/orphanin FQ (N/OFQ) have been shown to modulate the pharmacology of the mu opioid receptor, particularly reward, tolerance and antinociception.^{5–7} We have hypothesized that compounds that possess bifunctional NOP agonist and MOP agonist activity in the same molecule, may yield ‘non-addicting’ analgesics. Indeed, our detailed pharmacological characterization of several bifunctional NOP/MOP agonists in assays of acute and chronic nociception, and reward paradigms in rodents, suggests that a NOP/MOP bifunctional profile may yield a desirable profile of potent antinociceptive activity, but without opioid-mediated reward.^{8–11} However, these pharmacological studies suggest that the ratio of NOP agonist activity to the MOP activity needs to be carefully controlled, to provide a suitable nonaddicting profile, while maintaining antinociceptive efficacy. Our studies suggests that NOP /MOP receptor selectivity, binding affinity as well as intrinsic activity (efficacy) of the agonist at both receptors plays an important role in the overall pharmacological profile of the bifunctional agonist.

As part of our continuing effort in exploring the structure-activity relationships (SAR) that lead to useful bifunctional NOP/MOP agonist profiles, we recently reported, in part I of this series,¹² several potent bifunctional NOP/ MOP agonist ligands, developed from our 2-indolinone class of NOP-selective ligands.¹³ We carried out SAR exploration of the 2-indolinone heterocyclic moiety (the A-moiety of our 2-D pharmacophore¹⁴) to increase the MOP affinity and efficacy of the NOP-selective 2-indolinone ligands. Small alkyl substitutions (Me-, Et-) on the 3-position of the indolinone A-moiety increased MOP binding affinity as well as agonist efficacy, compared to the unsubstituted analogs.¹² From our previous SAR exploration of the piperidine nitrogen substituent (C-moiety of our 2-D pharmacophore¹⁴) we found that that arylalkyl substitution on the piperidine nitrogen modulated MOP binding affinity and efficacy.¹² Here we report a detailed exploration of various aromatic groups on the piperidine nitrogen of the 2-indolinone class of NOP ligands, to explore various bifunctional profiles of NOP/MOP affinity and efficacy. We used the 3-ethyl indolinone as the common scaffold to investigate the SAR of different aromatic groups at the piperidine nitrogen, as in compounds **5a–d**, **7e–j** (Table 1).

2. Results and Discussion

2.1. Chemistry

The synthesis of bifunctional ligands **5a–d** with aromatic piperidine nitrogen substituents (C-moieties in our 2-D pharmacophore¹⁴) directly attached to the piperidine ring was carried out as described previously,^{12,13} by introducing the C-moiety on to the piperidine nitrogen prior to construction of the 3-ethyl indolinone ring (Scheme 1). For compounds **5a** and **5b** (Table 1), piperidones **1a–b** were synthesized and separated by flash chromatography into their anti and syn diastereomers according to the procedures reported in Wichmann et al.¹⁵

Each diastereomer was then subjected to reductive amination with di-tert-butyl 2-(2-aminophenyl)malonate¹³ to furnish intermediates **2a–b**, which were then cyclized to 2-indolinones **3a–b** using TsOH. Indolinones **3c–d**, containing α - and β -tetralone C-moieties, were prepared as reported previously.¹³ Aldol condensation of the 3-unsubstituted 2-indolinones **3a–d** with acetaldehyde afforded methyl olefins **4a–d**,¹⁶ (Scheme 1) which were then reduced using catalytic hydrogenation with PtO₂/H₂(g) to give the 3-ethylindolinones **5a–d**.

Alternately, for compounds **7e** and **7g–j**, the alkyl-linked aromatic groups were installed directly onto the piperidine nitrogen of the 3-Et indolinone scaffold, by standard reductive amination conditions using 3-ethyl-1-(piperidin-4-yl)indolin-2-one **6**¹² and the appropriate commercially available ketone or aldehyde (Scheme 2). Compound **7f** was prepared by displacement of diphenylpropyl bromide with amine **6** (Scheme 2).¹⁷

2.2. In Vitro Binding Affinity and Functional Efficacy at the Nociceptin and other Opioid Receptors

The binding affinity of **3a–b**, **5a–d** and **7e–j** at NOP, MOP, DOP (delta opioid receptor) and KOP (kappa opioid receptor) was determined in radioligand displacement assays, as we have reported previously,¹³ using [³H]N/OFQ¹⁸, [³H]DAMGO, [³H]CI-DPDPE and [³H]U69,593, respectively.¹⁹ Functional activity was determined by stimulation of [³⁵S]GTP γ S binding to cell membranes, and compared to the standard agonists N/OFQ (NOP), DAMGO (MOP) and U69,593, (Table 1), as reported previously.^{9,13,19,20} The in vitro binding affinity and functional efficacy data for the NOP, MOP and KOP receptors is shown in Table 1. Most compounds reported here did not show appreciable binding affinity for the DOP receptor.

2.3 SAR affecting the NOP/MOP ratio of binding affinity and functional efficacy

We have previously reported a limited SAR around aromatic moieties at the piperidine nitrogen on our 2-indolinone-based NOP ligands.^{12–14} The NOP agonist SR16835 (now AT-202), containing the hexahydro-phenalen-1-yl piperidine substituent, has modest selectivity (8-fold) for NOP and very low agonist potency at MOP. Its separated diastereomers **3a** (anti) and **3b** (syn) (Table 1) have equipotent affinity at NOP, and similarly modest selectivity versus MOP. Interestingly, a 3-Et substitution of the indolinone ring (**5a** (AT-089) and **5b**) significantly increases MOP binding affinity as well as functional efficacy, without adversely affecting its NOP affinity or efficacy. This trend is similar to what we observed previously, where small alkyl substituents at the 3-indolinone position resulted in increased MOP binding affinity and a bifunctional NOP/MOP profile (Table 1).¹² We therefore used the 3-Et-2-indolinone scaffold, to investigate the influence of various other aromatic substituents on the piperidine nitrogen on the binding affinity and efficacy at both receptors (Table 1). The α -tetralone moiety (**5c**) results in a 3-fold drop in NOP affinity, compared to bifunctional agonists **5a–b**, but not in the MOP affinity. However, the β -tetralone isomer **5d** had low affinity for both NOP and MOP, suggesting that the piperidine nitrogen substituent may play a role in the fit of the ligand in the NOP and MOP receptor binding pockets. A similar decrease in NOP and MOP activity was also observed for α - and β -tetralone moieties in the parent 2-indolinone series,¹³ indicating that

the β -tetralone C-moiety is suboptimal for binding at both receptor subtypes. Substitution with a heteroaromatic indole moiety (**7e**) results in a modest decrease in NOP and MOP affinity, however; this analog has equipotent binding affinity at both receptors. It is interesting to note that **7e** is a potent full agonist at the MOP receptor, but is a partial agonist at the NOP receptor.

Introducing various alkyl and alkenyl linkers between the piperidine nitrogen and the aromatic groups (**7f-7j**) largely afforded a loss in NOP and MOP binding affinity. Freely rotating aromatic rings **7f** and **7h** yield low affinity at NOP, however; conformational restriction (**7g** and **7i**) and linear arrangement of the aromatic rings (**7j**) abolish NOP affinity. MOP affinity, albeit reduced, is still retained in these compounds, which share somewhat similar N-1 aromatic substitution as the 4-anilidopiperidine class of MOP agonists such as fentanyl.²¹ In contrast, the 3,3-diphenyl propyl analog (**7f**) retains potent MOP agonism, which is not surprising given that this moiety is also present in the MOP agonist DiPOA.²²

The SAR observed in our series shows that aromatic substituents similar to those in potent MOP agonists, when introduced on the NOP-selective 2-indolinone scaffold, result in a loss of affinity at the NOP receptor. Nonetheless, our SAR studies reported here and earlier¹² show that designing ‘bifunctional activity’ at two closely related targets, is affected by the same subtle differences in the NOP and MOP binding pockets that are responsible for high selectivity at either of the two receptors. Our SAR studies have resulted in several NOP/MOP agonists, with varying profiles of NOP and MOP efficacies, which we continue to investigate pharmacologically in vivo, to identify a ‘non-addicting’ analgesic profile.

2.4. In vivo characterization of antinociceptive and rewarding effects

The anti-nociceptive effect of the bifunctional NOP/MOP agonist **5a** (AT-089) was characterized in the mouse tail-flick model of antinociception, whereas its rewarding effects were characterized in the conditioned place preference (CPP) paradigm. **5a** has nanomolar binding affinity at both the NOP and MOP receptors, and is a full agonist at NOP, with partial agonist activity at MOP. We have previously characterized other bifunctional NOP/MOP agonists, of varying selectivities and functional profiles in similar assays.^{8, 10}

2.4.1. Assessment of Acute Thermal Nociception—Acute antinociception was assessed in the tail-flick assay in mice by application of radiant heat to the tail, as described in the Experimental Methods. The compound **5a** was administered via subcutaneous (s.c.) injection at different doses, and the tail-flick latencies measured at 30-min, 1-h, 2-h, 4-h and 6-h post-injection. The effect of morphine on tail-flick latency was also determined, as a positive control. Morphine was dosed s.c. at two doses, 3 mg/kg and 10 mg/kg, and its effect measured up to the 4-h timepoint.

As seen in Figure 1, **5a** showed modest anti-nociceptive efficacy (30–40% MPE) in the tail-flick assay irrespective of the dose, compared to 3 and 10 mg/kg morphine. Although there was no effect of increasing doses on anti-nociceptive potency, the 10 mg/kg dose of **5a** showed sustained anti-nociceptive efficacy up to 4-h post-injection. No anti-nociceptive effect of **5a** was observed at the 6-h timepoint, possibly indicating the clearance of the drug

by that time. To determine the relative involvement of the MOP and NOP agonist activity in the overall anti-nociceptive efficacy of **5a**, the animals were pre-treated with opioid antagonist naloxone and NOP antagonist SB-612111. We have previously shown that the NOP antagonist SB-612111 (10 mg/kg), administered alone 10-min prior to a vehicle injection, has no effect on tail-flick latency in mice.²³ As shown in Figure 2, opioid antagonist naloxone completely inhibited the anti-nociceptive efficacy of the 3 mg/kg dose of **5a**, suggesting that the anti-nociceptive efficacy observed was due to the MOP agonist activity of **5a**. When NOP antagonist SB-612111 (10 mg/kg) was given as pretreatment to **5a**, the anti-nociceptive activity was potentiated at all the four time-points tested. We have shown previously that NOP full agonist activity in a NOP/MOP bifunctional agonist can reduce its effective antinociceptive efficacy, particularly in thermal anti-nociceptive assays, and that this effect may be reversed by a NOP antagonist, restoring and even potentiating the full MOP-mediated antinociceptive effect, as seen for **5a** (Figure 2).²³

2.4.2. Assessment of the Rewarding and Behavioral Effects of Bifunctional NOP/MOP Ligands using the Conditioned Place Preference (CPP) Paradigm—

The rewarding effects of **5a** were assessed in the well-accepted CPP assay in mice (Figure 3). The behavioral effect of **5a** on locomotion and overall activity (global activity) were also assessed in the same assay throughout the conditioning experiment (Figure 4). Morphine, at 10 mg/kg, was used as a positive control. As seen in Figure 3, animals treated with morphine showed the expected preference for the drug-paired compartment, as determined by the percent time spent in the drug-paired compartment (details of the CPP testing in the Experimental Methods). On the other hand, animals treated with **5a** did not show a preference for the drug treatment compartment, compared to vehicle, at all three increasing doses tested (Figure 3). These results show that the NOP/MOP bifunctional agonist **5a** has no rewarding effects of its own. Therefore, although **5a** has a modest anti-nociceptive effect, mediated by its MOP agonist activity, it does not show the typical rewarding effects of a classical MOP agonist like morphine, likely due to the NOP full agonist activity present in **5a**, which attenuates its MOP-mediated rewarding effects.

Further confirmation that NOP full agonist activity can suppress the behavioral effects of MOP agonist activity was obtained by assessing the effect of morphine and **5a** on locomotion and global activity of the treated animals during the CPP conditioning. As seen in Figure 4, morphine (10 mg/kg) increases global activity in treated animals acutely (Figure 4, injection 1) as well as following repeated injections (Figure 4, injection 4) compared to vehicle controls. **5a**, however, does not have an effect on global activity relative to vehicle controls, and following repeated injections, tolerance to global activity was evident as a decrease in activity after the third injection relative to the first, indicating that animals habituated to their environment following repeated exposure. Habituation to the environment is also evident in vehicle controls (Figure 4). The finding that there was no increase in activity with **5a** indicates that the NOP agonist activity attenuates the MOP-mediated hyperactivity in the bifunctional NOP-MOP agonist **5a** (Figure 4)

Although **5a** has no rewarding or hyperactivity effects of a MOP agonist, its anti-nociceptive potency was modest compared to morphine. Therefore the ratio of NOP to MOP agonist

activity to produce a useful non-addicting antinociceptive profile depends on the NOP-MOP selectivity and the efficacy at each of the two target receptors. From our previously reported NOP/MOP bifunctional compounds,¹⁰ it appears that MOP partial agonist activity of at least 40–50% efficacy is required to obtain antinociceptive activity, whereas full agonist activity at NOP is required to attenuate the rewarding effects of the MOP component. Modulation of the NOP-MOP selectivity will then lead to a non-addicting antinociceptive profile. Such bifunctional compounds are currently being characterized and will be reported in due course.

3. Conclusions

We have hypothesized that bifunctional NOP/MOP agonists may be developed as ‘non-addicting’ analgesics, such that attenuation of MOP-mediated effects by the anti-opioid NOP component, while maintaining MOP analgesia is achieved. SAR studies described here provide further insights into modification of our NOP pharmacophore¹⁴ for designing bifunctional NOP/MOP compounds. Our previous paper in this series¹² details the importance of a 3-Et indolinone group at the A-moiety pharmacophore of our NOP scaffold for increasing MOP activity (AT-212, formerly SR16507). Here, we note that on the 3-Et indolinone scaffold, substitution at the C-moiety with aromatic groups affords compounds of a bifunctional NOP/MOP profile. While the SAR approaches described here are useful to modulate the ratio of NOP/MOP activity, the desirable ratio of NOP agonist activity to MOP agonist activity to obtain a useful ‘non-addicting’ analgesic profile is yet to be determined.

4. Experimental section

Thin layer chromatography was performed on Analtech silica gel GF 250 um TLC plates. The plates were visualized with a 254 nm UV light and staining with iodine. Flash chromatography was carried out on F60 silica gel, 230–400 mesh, (Silicycle SiliaFlash) 60 Å. NMR (300 MHz) was recorded on a Varian Mercury Plus NMR using CDCl₃ or DMSO-*d*₆. Mass spectra were obtained on a LCQ Fleet Ion Trap LC/MSⁿ using either electrospray ionization (ESI) or the atmospheric pressure chemical ionization (APCI) mode or a micromass ZMD1000 using ESI mode. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

4.1. General procedure for the preparation of t-butyl malonates 2a–b

To a mixture of di-tert-butyl 2-(2-aminophenyl)malonate (1 equiv)¹³ in 1,2-dichloroethane (0.15 M) was added the appropriate ketone (1 equiv), followed by NaBH(OAc)₃ (2.5 equiv) and HOAc (2.5 equiv), and stirred at RT for 19 h. The mixture was adjusted to pH 9 by addition of satd. NaHCO₃ (aq), and extracted in EtOAc. Organic layers were collected, washed with satd. NaCl(aq), dried over Na₂SO₄, filtered and concentrated. The residue was purified via flash chromatography using DCM:EtOAc (100:0→ 93:7) as the eluent to afford the title material.

4.1.1. di-tert-butyl 2-(2-((1-((1*RS*,3*aRS*)-2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)amino)phenyl)malonate (2*a*, anti diastereomer)—Prepared according to the general procedure using (1*RS*,3*aRS*)-1-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-piperidin-4-one **1a**¹⁵ (950 mg, 3.53 mmol) to afford 1.04 g of the title

material (53%). ^1H NMR (300 MHz, CDCl_3) δ : 1.28-1.44 (m, 2H), 1.49 (s, 18H), 1.54-1.65 (m, 2H), 1.75-1.84 (m, 2H), 1.91-1.96 (m, 2H), 2.01-2.03 (m, 2H), 2.09-2.12 (m, 2H), 2.28 (t, 1H, $J = 7.8$ Hz), 2.56-2.70 (m, 3H), 2.82-2.84 (m, 3H), 3.31 (s, 1H), 3.90-3.94 (m, 1H), 4.46 (s, 1H), 4.55 (s, 1H), 6.66-6.72 (m, 2H), 6.95-6.97 (m, 1H), 7.11 (t, 1H, $J = 5.7$ Hz), 7.15-7.19 (m, 2H), 7.55 (d, 1H, $J = 5.7$ Hz). MS(ESI) m/z : 561.5 $[\text{M}+\text{H}]^+$.

4.1.2. di-tert-butyl 2-((1-((1*RS*,3*aSR*)-2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)amino)phenyl)malonate (2*b*, syn diastereomer)—Prepared according to the general procedure using (1*RS*,3*aSR*)-1-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-piperidin-4-one **1b**¹⁵ (1.27 g, 4.71 mmol) afford 1.51 g of the title material (57%). ^1H NMR (300 MHz, CDCl_3) δ : 1.24-1.39 (m, 2H), 1.49 (s, 18H), 1.60-1.65 (m, 2H), 1.73-1.80 (m, 2H), 1.86-1.93 (m, 2H), 1.96-2.00 (m, 2H), 2.06-2.09 (m, 2H), 2.29 (t, 1H, $J = 7.5$ Hz), 2.43-2.45 (m, 1H), 2.65-2.73 (m, 2H), 2.83-2.85 (m, 3H), 3.33 (s, 1H), 3.77-3.78 (m, 1H), 4.46 (s, 1H), 4.60 (s, 1H), 6.67-6.72 (m, 2H), 6.97-6.99 (m, 1H), 7.12-7.19 (m, 3H), 7.51 (d, 1H, $J = 5.7$ Hz). MS(APCI) m/z : 561.16 $[\text{M}+\text{H}]^+$.

4.2. General procedure for the preparation of indolinones 3*ab*

To a mixture of *t*-butyl malonate (1 equiv) in toluene (0.06-0.1 M) was added $\text{pTsOH}\cdot\text{H}_2\text{O}$ (2 equiv), and refluxed for 2 h. The mixture was concentrated, then extracted in 2*N* $\text{NaOH}(\text{aq})$ and EtOAc . The residue was purified via flash chromatography using Hexanes: EtOAc 9:1 $\rightarrow$ 85:15 (for **3a**) or Hexanes: $\text{DCM}:\text{EtOAc}$ 49.5:49.5:1 $\rightarrow$ 48:48:4 (for **3b**).

4.2.1. 1-(1-((1*RS*,3*aRS*)-2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)indolin-2-one (3*a*, anti diastereomer)—Prepared according to the general procedure from **2a** (390 mg, 0.700 mmol) to afford 240 mg of the title material (89%). ^1H NMR (300 MHz, CDCl_3) δ : 1.29-1.42 (m, 2H), 1.61-1.64 (m, 2H), 1.77-1.81 (m, 2H), 1.91-1.94 (m, 2H), 2.03-2.05 (m, 2H), 2.29-2.36 (m, 2H), 2.55-2.62 (m, 2H), 2.72-2.79 (m, 2H), 2.82-2.85 (m, 2H), 2.99-3.02 (m, 1H), 3.53 (s, 2H), 3.96-3.99 (m, 1H), 4.31-4.32 (m, 1H), 6.97-7.06 (m, 2H), 7.16 (t, 1H, $J = 5.7$ Hz), 7.21-7.34 (m, 3H), 7.63 (d, 1H, $J = 5.7$ Hz). MS(APCI) m/z : 387.14 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}$: C, 80.79; H, 7.82; N, 7.25; found: C, 80.67; H, 7.81; N, 7.17.

4.2.2. 1-(1-((1*RS*,3*aSR*)-2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)indolin-2-one (3*b*, syn diastereomer)—Prepared according to the general procedure from **2b** (1.12 g, 1.98 mmol) to afford 650 mg of the title material (84%). ^1H NMR (300 MHz, CDCl_3) δ : 1.27-1.39 (m, 2H), 1.61-1.67 (m, 2H), 1.76-1.79 (m, 2H), 1.89-1.97 (m, 2H), 1.98-2.08 (m, 2H), 2.23-2.31 (m, 2H), 2.43-2.47 (m, 1H), 2.54-2.62 (m, 1H), 2.74-2.79 (m, 1H), 2.82-2.84 (m, 3H), 2.98-3.01 (m, 1H), 3.53 (s, 2H), 3.82 (s, 1H), 4.33 (s, 1H), 6.99-7.05 (m, 2H), 7.16-7.21 (m, 2H), 7.25-7.31 (m, 2H), 7.58 (d, 1H, $J = 5.7$ Hz). MS(APCI) m/z : 387.14 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}$: C, 80.79; H, 7.82; N, 7.25; found: C, 80.61; H, 7.70; N, 7.13.

4.3. General procedure for the preparation of 3-ethyl indolinones 5a–d

The appropriate indolinone (1 equiv) was charged to a sealed tube and dissolved in 1:1 v/v MeOH/THF (0.04–0.12 M). To this mixture was added acetaldehyde (9 equiv), sodium acetate (1 equiv) and molecular sieves, and heated to 80°C for 1.5–4 h. The mixture was filtered, concentrated and chromatographed with hexanes:EtOAc (100:0→95:5 for **4a–b**, 95:5→7:3 for **4c–d**) to yield the corresponding E/Z isomers, which were used directly in the next reaction.

To a solution of E/Z isomers (1 equiv) in EtOH (0.02–0.12 M) was added 10 wt % PtO₂, and the mixture was hydrogenated at 1 atm for 0.5–6 h. The mixture was filtered through a pad of Celite, concentrated, and chromatographed with hexanes: ether (98:2→80:20) for **5a–b,d** or DCM: ether (95:5→85:15) for **5c** as the eluent. For **5a–b,d**, the title material was converted to an HCl salt by addition of 2M HCl/ether.

4.3.1. 3-ethyl-1-(1-((1R,3aR)-2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)piperidin-4-yl)indolin-2-one (5a, anti diastereomer)—Prepared according to the general procedure from **3a** (200 mg, 0.517 mmol) to yield 120 mg (56%) of E/Z isomers, which was then converted to the title material according to the general procedure (89 mg, 73%). ¹H NMR (free base, 300 MHz, CDCl₃) δ : 0.851 (t, 3H, *J* = 5.7 Hz), 1.27–1.44 (m, 2H), 1.55–1.64 (m, 2H), 1.71–1.81 (m, 3H), 1.91–1.94 (m, 2H), 2.03–2.05 (m, 3H), 2.25–2.39 (m, 2H), 2.55–2.62 (m, 2H), 2.71–2.78 (m, 2H), 2.81–2.83 (m, 2H), 2.98–3.01 (m, 1H), 3.40 (t, 1H, *J* = 3.6 Hz), 3.95–4.00 (m, 1H), 4.29–4.31 (m, 1H), 6.97–6.99 (m, 1H), 7.03–7.07 (m, 1H), 7.14–7.18 (m, 1H), 7.19–7.22 (m, 1H), 7.25–7.31 (m, 2H), 7.64 (d, 1H, *J* = 5.7 MHz). MS(APCI) *m/z*: 415.2 [M+H]⁺. Anal. Calcd. for C₂₈H₃₄N₂O·1.00 HCl·1.30 H₂O: C, 70.88; H, 7.99; N, 5.90; found: C, 70.80; H, 7.76; N, 5.81.

4.3.2. 3-ethyl-1-(1-((1R,3aR)-2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)piperidin-4-yl)indolin-2-one (5b, syn diastereomer)—Prepared according to the general procedure from **3b** (510 mg, 1.32 mmol) to yield 387 mg (71%) of E/Z isomers, which was then converted to the title material according to the general procedure (143 mg, 75%). ¹H NMR (free base, 300 MHz, CDCl₃) δ : 0.821–0.868 (m, 3H), 1.27–1.37 (m, 2H), 1.55–1.65 (m, 2H), 1.71–1.81 (m, 2H), 1.86–1.93 (m, 2H), 1.96–2.08 (m, 4H), 2.26–2.34 (m, 2H), 2.43–2.49 (m, 1H), 2.56–2.62 (m, 1H), 2.73–2.75 (m, 1H), 2.79–2.85 (m, 3H), 2.97–3.00 (m, 1H), 3.40 (t, 1H, *J* = 4.2 Hz), 3.82 (s, 1H), 4.31 (s, 1H), 6.99–7.07 (m, 2H), 7.16–7.21 (m, 2H), 7.25–7.31 (m, 2H), 7.58–7.59 (m, 1H). MS(APCI) *m/z*: 415.2 [M+H]⁺. Anal. Calcd. for C₂₈H₃₄N₂O·0.9HCl·0.9H₂O: C, 72.54; H, 7.98; N, 6.04; found: C, 72.63; H, 7.92; N, 6.01.

4.3.3. 3-ethyl-1-(1-(1,2,3,4-tetrahydronaphthalen-1-yl)piperidin-4-yl)indolin-2-one (5c)—Prepared using the general procedure starting from 1-(1-(1,2,3,4-tetrahydronaphthalen-1-yl)piperidin-4-yl)indolin-2-one ¹³ (400 mg, 1.16 mmol) to yield 330 mg (77%) of E/Z isomers, which was then converted to the title material according to the general procedure (240 mg, 77%). ¹H NMR (300 MHz, CDCl₃) δ : 0.816–0.869 (m, 3H), 1.55–1.80 (m, 4H), 2.01–2.05 (m, 4H), 2.23–2.38 (m, 2H), 2.57–2.87 (m, 5H), 2.99–3.02 (m, 1H), 3.41 (t, 1H, *J* = 3.9 Hz), 3.91 (s, 1H), 4.28–4.32 (m, 1H), 7.03–7.09 (m, 2H), 7.15 (t,

1H, $J = 5.4$ Hz), 7.19-7.32 (m, 4H), 7.81 (d, 1H, $J = 5.7$ Hz). MS(ESI) m/z : 375.3 [M+H]⁺. Anal. Calcd. for C₂₅H₃₀N₂O: C, 80.17; H, 8.07; N, 7.48; found: C, 79.93, H, 8.01, N, 7.42.

4.3.4. 3-ethyl-1-(1-(1,2,3,4,4a,8a-hexahydronaphthalen-2-yl)piperidin-4-yl)indolin-2-one (5d)—Prepared using the general procedure starting from 1-(1-(1,2,3,4,4a,8a-hexahydronaphthalen-2-yl)piperidin-4-yl)indolin-2-one **13** (250 mg, 0.722 mmol) to yield 110 mg (41%) of E/Z isomers, which was then converted to the title material according to the general procedure (59 mg, 58%). ¹H NMR (free base) (300 MHz, CDCl₃) δ : 0.791-0.890 (m, 3H), 1.70-1.77 (m, 4H), 2.02 (qn, 2H, $J = 5.1$ Hz), 2.11-2.14 (m, 1H), 2.45-2.52 (m, 3H), 2.82-2.98 (m, 5H), 3.13 (s, 2H), 3.40 (t, 1H, $J = 3.9$ Hz), 4.37-4.38 (m, 1H), 7.01-7.05 (m, 1H), 7.09-7.12 (m, 4H), 7.22-7.24 (m, 3H). MS(ESI) m/z : 375 [M+H]⁺. Anal. Calcd. for C₂₅H₃₀N₂O·1.00 HCl·0.7 H₂O: C, 70.89; H, 7.71; N, 6.61; found: C, 70.69; H, 7.44; N, 6.45.

4.4. General procedure for the preparation of 3-ethyl indolinones **7e,7g-j**

3-ethyl-1-(piperidin-4-yl)indolin-2-one **6** ¹² (1 equiv), requisite aldehyde or ketone (1.3 equiv), acetic acid (3 equiv) and NaBH(OAc)₃ (2.6 equiv) were dissolved in 1,2-dichloroethane (0.1-0.2 M) and stirred at RT for 3-29 h. The mixture was adjusted to pH = 9 by addition of satd. NaHCO₃ (aq), and extracted in DCM. The organic layers were collected, washed with satd. NaCl(aq), dried over Na₂SO₄, filtered and concentrated. The residue was purified via flash chromatography using hexanes: diethyl ether 100:0→5:5 or hexanes: EtOAc 100:0→5:5 as the eluent to afford the title material. For **7f-7j**, the title material was converted to the HCl salt by addition of 2M HCl/ether.

4.4.1. 1-(1-((1H-indol-4-yl)methyl)piperidin-4-yl)-3-ethylindolin-2-one (7e)—

Prepared using the general procedure with indole-4-carboxaldehyde to afford 140 mg of the title material in 46% yield. ¹H NMR (free base, 300 MHz, CDCl₃) δ : 0.835 (t, 3H, $J = 5.7$ Hz), 1.61-1.69 (m, 2H), 1.98-2.05 (m, 2H), 2.20-2.26 (m, 2H), 2.48-2.53 (m, 2H), 3.12 (app d, 2H, $J = 8.4$ Hz), 3.39 (t, 1H, $J = 4.2$ Hz), 3.88 (s, 2H), 4.33-4.35 (m, 1H), 6.79 (s, 1H), 7.01-7.05 (t, 1H, $J = 5.7$ Hz), 7.14-7.25 (m, 6H), 7.34 (d, 1H, $J = 5.7$ Hz). MS(ESI) m/z : 374 [M+H]⁺. Anal. Calcd. for C₂₄H₂₇N₃O·0.2EtOAc: C, 76.16; H, 7.37; N, 10.74; found: C, 76.47; H, 7.39; N, 10.67.

4.4.2. 1-(1-(3,3-diphenylpropyl)piperidin-4-yl)-3-ethylindolin-2-one (7f)—3-

ethyl-1-(piperidin-4-yl)indolin-2-one **6** ¹² (160 mg, 0.655 mmol, 1 equiv), 3,3-diphenylpropyl bromide (360 mg, 1.32 mmol, 2.02 equiv) and K₂CO₃ (912 mg) were combined in acetonitrile and stirred at RT for 20 h. The mixture was extracted in H₂O and EtOAc. The organic layers were collected, washed with satd. NaCl(aq), dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified via flash chromatography using hexanes: EtOAc 100:0→75:25 to afford 192 mg of the title material in 67% yield. ¹H NMR (HCl salt, 300 MHz, DMSO-*d*₆) δ : 0.699 (t, 3H, $J = 5.4$ Hz), 1.74-1.80 (m, 2H), 1.87-1.92 (m, 2H), 2.55-2.58 (m, 2H), 2.82-2.91 (m, 4H), 3.12-3.20 (m, 2H), 3.47-3.59 (m, 3H), 4.05 (t, 1H), 4.50 (s, 1H), 7.04 (t, 1H, $J = 5.7$ Hz), 7.18-7.26 (m, 3H), 7.32 (t, 5H, $J = 5.4$ Hz), 7.36-7.38 (m, 4H), 7.52 (s, 1H), 11.0 (s, 1H). MS(ESI) m/z :

439.3 [M+H]⁺. Anal. Calcd. for C₃₀H₃₄N₂O·1.00 HCl·1.30H₂O: C, 72.28; H, 7.60; N, 5.62; found: C, 72.53; H, 7.41; N, 5.34.

4.4.3. 1-(1-(4,4-diphenylbutan-2-yl)piperidin-4-yl)-3-ethylindolin-2-one (7g)—

Prepared using the general procedure with 4,4-diphenyl-2-butanone to afford 113 mg of the title material in 34% yield. ¹H NMR (HCl salt, 300 MHz, DMSO-*d*₆) δ : 0.703 (t, 3H, *J* = 5.1 Hz), 1.66-1.93 (m, 5H), 2.66-2.69 (m, 2H), 2.81 (s, 2H), 3.10-3.20 (m, 4H), 3.49-3.58 (m, 3H), 4.54 (s, 1H), 7.05 (t, 1H, *J* = 5.7 Hz), 7.25 (t, 1H, *J* = 5.7 Hz), 7.31-7.36 (m, 5H), 7.45 (t, 2H, *J* = 5.7 Hz), 7.51-7.53 (m, 1H), 7.60 (d, 2H, *J* = 6 Hz), 7.65 (d, 2H, *J* = 5.7 Hz), 10.6 (s, 1H). MS(ESI) *m/z*: 453.3 [M+H]⁺. Anal. Calcd. for C₃₁H₃₆N₂O·1.00 HCl·0.7 H₂O: C, 74.21; H, 7.72; N, 5.58; found: C, 74.04; H, 7.48, N, 5.38.

4.4.4. 3-ethyl-1-(1-(3-phenylpropyl)piperidin-4-yl)indolin-2-one (7h)—

Prepared using the general procedure with 3-phenylpropionaldehyde to afford 150 mg of the title material in 56% yield. ¹H NMR (HCl salt, 300 MHz, DMSO-*d*₆) δ : 0.699 (t, 3H, *J* = 5.7 Hz), 1.75 (t, 2H, *J* = 12.6 Hz), 1.86-1.92 (m, 2H), 2.08-2.1 (m, 2H), 2.66 (t, 2H, *J* = 5.4 Hz), 2.84-2.90 (m, 2H), 3.05-3.15 (m, 4H), 3.48-3.51 (m, 1H), 3.56-3.59 (m, 2H), 4.55 (s, 1H), 7.04 (t, 1H, *J* = 5.7 Hz), 7.20-7.34 (m, 8H), 10.9 (s, 1H). MS(ESI) *m/z*: 363.3[M+H]⁺. Anal. Calcd. for C₂₄H₃₀N₂O·1.00 HCl·0.3H₂O: C, 71.29; H, 7.88; N, 6.93; found: C, 71.23; H, 7.85; N, 6.75.

4.4.5. (E,Z)-1-(1-(cinnamyl)piperidin-4-yl)-3-ethylindolin-2-one (7i)—

Prepared using the general procedure with trans cinnamaldehyde to afford 127 mg of the title material in 57% yield. ¹H NMR (HCl salt, 300 MHz, DMSO-*d*₆) δ : 0.705 (t, 3H, *J* = 5.4 Hz), 1.79-1.91 (m, 4H), 2.82-2.90 (m, 2H), 3.18-3.30 (m, 2H), 3.50-3.60 (m, 3H), 3.85-3.95 (m, 2H), 4.56 (s, 1H), 6.45-6.48 (m, 1H), 6.86 (d, 1H, *J* = 11.7 Hz), 7.04 (t, 1H, *J* = 5.1 Hz), 7.24-7.53 (m, 8H), 11.2 (s, 1H). MS(ESI) *m/z*: 361.2[M+H]⁺. Anal. Calcd. for C₂₄H₂₈N₂O·1.00 HCl·1.00H₂O: C, 69.47; H, 7.53; N, 6.75; found: C, 69.45; H, 7.40; N, 6.55.

4.4.6. 1-(1-(4-([1,1'-biphenyl]-4-yl)butyl)piperidin-4-yl)-3-ethylindolin-2-one (7j)—

Prepared using the general procedure with 4-(4-biphenyl)butanal to afford 100 mg of the title material in 38% yield. ¹H NMR (HCl salt, 300 MHz, DMSO-*d*₆) δ : 0.703 (t, 3H, *J* = 5.4 Hz), 1.66-1.77 (m, 6H), 1.88-1.91 (m, 2H), 2.674 (t, 2H, *J* = 5.7 Hz), 2.75-2.85 (m, 2H), 3.10-3.15 (m, 4H), 3.50-3.58 (3H), 4.54 (s, 1H), 7.05 (t, 1H, *J* = 5.7 Hz), 7.31-7.36 (m, 4H), 7.45 (t, 3H, *J* = 5.7 Hz), 7.51-7.52 (m, 1H), 7.60 (d, 2H, *J* = 6 Hz), 7.65 (d, 2H, *J* = 5.7 Hz), 10.6 (s, 1H). MS(ESI) *m/z*: 453.4 [M+H]⁺. Anal. Calcd. for C₃₁H₃₆N₂O·1.00 HCl·0.4H₂O: C, 77.02; H, 7.68; N, 5.64; found: C, 75.32; H, 7.80; N, 5.35.

In vivo Characterization

Animals—Male ICR mice weighing 25–30 g at the start of the experiment were used.

Animals were group-housed under standard laboratory conditions on a 12:12-h day/night cycle. For the experiments examining tail flick latency, animals were housed 10/cage and for the conditioned place preference experiments the animals were housed 4/cage. Animals were handled for 3–4 days before the experiments were conducted. On behavioral test days,

animals were transported to the testing room and acclimated to the environment for 1 h. Mice were maintained in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003).

Drugs—5a was dissolved in 2% DMSO and 0.5% aqueous hydroxypropylcellulose. Morphine hydrochloride (Eli Lilly & Co., Indianapolis, IN) was dissolved in water. Drugs were injected in a volume of 0.1 mL/30 g s.c. Controls received 0.1 mL/30 g of the appropriate vehicle.

Assessment of Thermal Nociception

Tail-Flick Assay—Acute nociception was assessed in the tailflick assay with an analgesia instrument (Stoelting) that uses radiant heat. This instrument is equipped with an automatic quantification of tail-flick latency, and a 15 sec cutoff to prevent damage to the animal's tail. During testing, the focused beam of light was applied to the lower half of the animal's tail, and tail-flick latency was recorded. Baseline values for tail-flick latency were determined before drug administration in each animal. The mean basal tail-flick latency was 5.5 ± 0.16 SEM.

Drug Regimen—Following baseline measures, animals (n=8–10 per group) received a s.c. injection of their assigned dose of drug and were tested for tail-flick latencies at 0.5-, 1-, 2-, 4-, and 6-h post-injection. Controls received an injection of vehicle prior to testing. The experimenters were blinded to the treatment and condition of each animal.

To determine the relative contributions of NOP and MOP activity to the anti-nociceptive effects observed, separate groups of animals were pre-treated with MOP antagonist naloxone and with NOP antagonist SB-612111 (synthesized in our laboratory). **5a** (3 mg/kg) was given 10-min following pretreatment with naloxone (1 mg/kg, s.c.) and SB-612111 (10 mg/kg, s.c.), and tail-flick latencies measured at 0.5-, 1-, 2- and 4-h timepoints. A group of animals served as controls and received vehicle injections prior to testing. This resulted in four separate groups with N=8–10/group.

Statistical Analyses—Antinociception (% maximum potential effect; % MPE) was quantified by the following formula: $\% \text{ MPE} = 100 * [(\text{test latency} - \text{baseline latency}) / (15 - \text{baseline latency})]$. If the animal did not respond before the 15-s cutoff, the animal was assigned a score of 100%. Behavioral results were analyzed by use of repeated measures ANOVAs with drug treatment (**5a**, naloxone, SB612111) as between group variables and post druginjection time (0.5-, 1-, 2-, 4-, and 6-h) as the repeated measure followed by Bonferroni post hoc tests where appropriate. The level of significance was set at $P=0.05$.

Assessment of Rewarding/Aversive Effects of 5a Using the Conditioned Place Preference (CPP) Paradigm

CPP Apparatus—The apparatus consisted of rectangular Plexiglas chambers divided into two distinct equal-sized compartments (19 cm×22.8 cm×18 cm high, Lafayette Instruments). One compartment had cedar-scented bedding underneath a bar grid floor and all but the front walls were black. The other compartment had pine-scented bedding beneath a mesh

floor and all but the front wall were white. The front walls were transparent so that the animal's behavior could be monitored. A removable partition divided the two compartments. During conditioning, the compartments were divided by a solid partition. On the test day, the solid partition was replaced with a partition that had an opening, allowing the animal free access to both compartments. Previous experiments using this set-up have indicated that the apparatus is unbiased, as untreated animals do not show a preference for one compartment over the other.

Conditioning Training—Each conditioning trial was composed of two sessions conducted over two consecutive days. During the drug session, animals received a s.c. injection of their respective dose of **5a** (1, 3, 10 mg/kg; N=8/group) and were confined to one of the compartments for 30 min. On the following consecutive day, the vehicle session, animals received an injection of vehicle and were confined to the alternate compartment for 30 min. The control group of mice received vehicle in both compartments. The positive control group of mice received morphine (10 mg/kg; N=8) during their drug session. Each two-day conditioning trial was repeated over six consecutive days (3 trials) such that animals received three drug sessions and three vehicle sessions. The particular compartment paired with the drug and the order of placement into the drug-paired versus vehicle-paired compartment was counterbalanced across groups.

CPP Test Day—24 h following the last conditioning session, animals were given access to both compartments simultaneously for 15 min and the amount of time animals spent in each compartment was recorded.

Assessment of Global Activity—Previous data from our laboratory and others have indicated that compounds with NOP activity can produce a decrease in activity.^{8,10} Thus, during conditioning, overall activity of the animals following acute and repeated drug injections was recorded. These data were captured by the Spontaneous Motor Recording and Tracking software system (SMART; Panlab, Barcelona, Spain), a color image-capturing system that works in real time and tracks all the movements of the animal, for a given amount of time, via a video camera connected to the computer. This system tracks and records all behavior that results in any movement/positional changes. We term this as “global activity,” because it encompasses all movement detected including fine movement, movement due to rearing, grooming, sniffing, and locomotor activity.

Statistical Analyses—Global activity was analyzed using an ANOVA with drug treatment (**5a**, morphine) as between-subjects measures and injection day (first versus third) as a repeated measure. Significant interactions were further analyzed with one-way ANOVAs and Bonferroni post-hoc tests. For the data obtained on the CPP test day, the percentage of time animals spent in the drug-paired compartment was analyzed using one-way ANOVAs and significant effects were further analyzed with Bonferroni post-hoc tests. A CPP was evident if animals spent significantly more time in their drug-paired compartment, resulting in a positive difference score relative to control animals. A conditioned place aversion (CPA) was evident if animals spent significantly less time in

their drug-paired compartment resulting in a negative difference score compared to vehicle controls. The level of significance was set at $P < 0.05$.

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Figure 1.

The effect of **5a** on antinociception as measured by the tail-flick assay at 0.5-, 1-, 2-, 4-, and 6-h post-injection time points. Morphine was used as positive control and tail-flick latencies measured at the 0.5-, 1-, 2-, and 4-h timepoints. The data are mean %MPE $\pm$ SEM. *, $P < 0.05$, significant difference from vehicle controls.

Figure 2.

Attenuation of **5a** antinociception by pretreatment with opioid antagonist naloxone (1 mg/kg, s.c.), and potentiation of **5a** antinociception by pretreatment with NOP antagonist SB-612111 (10 mg/kg, s.c.). Data are mean %MPE $\pm$ SEM. *, $P < 0.05$, significant difference from vehicle controls, Δ , $P < 0.05$, significant difference from **5a** alone.

Figure 3.

The effect of **5a** (three doses) and morphine (10 mg/kg) on conditioned place preference. Data are mean ($\pm$ SEM) percentage of time spent in the drug-paired compartment after conditioning conducted over 6 consecutive days (See Experimental details) *, significant difference from vehicle controls,

Figure 4.

The effect of **5a** (three doses) and morphine on global activity on the first (open bars) and 3rd (filled bars) injection, during the conditioning trials. Data are mean ($\pm$ SEM) activity (cm) following first and last drug injection. *, significant difference from vehicle controls, $P < 0.05$; †, significant difference from the first injection, $P < 0.05$.

Scheme 1.

Synthesis of compounds **3a-b**, **5a-d**. Reagents and conditions: (a) $\text{NaBH}(\text{OAc})_3$, HOAc , DCE, 19 h, RT, 53–57%; (b) $\text{pTsOH} \cdot \text{H}_2\text{O}$, toluene, reflux 2 h, 84–89%; (c) acetaldehyde, sodium acetate, molecular sieves, MeOH/THF , sealed tube, 80°C , 1.5–2 h, 41–77%; (d) PtO_2 , EtOH , 1 atm $\text{H}_2(\text{g})$, 0.5–6 h, 58–77%.

Scheme 2.

Synthesis of compounds **7e-j**. Reagents and conditions: (a) aldehyde or ketone, NaBH(OAc)₃, HOAc, DCE, RT, 3–22 h, 34–57% or 3,3-diphenylpropyl bromide, K₂CO₃, ACN, 29 h RT, 67% (for **7f**).

Table 1

Binding Affinities and Functional Activities of Bifunctional NOP/ μ ligands.*

Structure		Receptor Binding K_i (nM)			$[^{35}\text{S}]\text{GTP-}\gamma\text{S}$ NOP			$[^{35}\text{S}]\text{GTP-}\gamma\text{S}$ μ			$[^{35}\text{S}]\text{GTP-}\gamma\text{S}$ κ	
R ₁	R ₂	NOP	μ	κ	EC ₅₀ nM	% Stim	EC ₅₀ nM	EC ₅₀ nM	% Stim	EC ₅₀ nM	% Stim	
3a	H	2.10 ± 0.14	41.5 ± 3.71	516 ± 57.9	84.9 ± 1.12	97.6 ± 19.1	409 ± 219	86.8 ± 7.3		NT		
3b	H	3.41 ± 0.23	30.3 ± 6.55	814 ± 35.6	52.7 ± 4.6	109 ± 14.1	745 ± 257	85.9 ± 0.9		NT		
5a	Et	3.16 ± 0	8.19 ± 0.16	152 ± 25.7	21.7 ± 5.6	96.5 ± 2.45	56.9 ± 19.3	52.3 ± 6.7		NT		
5b	Et	2.99 ± 1.16	1.46 ± 0.28	184 ± 31.8	40.9 ± 4.9	100.7 ± 0.7	81.4 ± 19.3	45.2 ± 7.34		NT		
5c	Et	8.63 ± 0.96	1.05 ± 0.14	56.3 ± 10.2	50.2 ± 8.8	70.9 ± 5.0	28.7 ± 5.5	93.8 ± 10.8				
5d	Et	12.0 ± 2.58	10.2 ± 1.76	15.13 ± 1.6	27.9 ± 17.1	29.3 ± 2.5	22.3 ± 0.1	54.4 ± 7.8		152.6 ± 23	12.4 ± 7	
7e	Et	11.1 ± 0.28	10.6 ± 2.55	12.82 ± 1.6	82.0 ± 16.4	40.4 ± 12.2	23.4 ± 5.6	99.5 ± 5.5		>10K	4.1 ± 3	
7f	Et	76.6 ± 3.6	1.97 ± 0.2	88.4 ± 14.2	123.5 ± 6.1	71.4 ± 1.4	39.5 ± 7.4	96.1 ± 6.6		>10K	0	
7g	Et	>10K	100 ± 4.17	4171	-	-	>10K	0

Structure	R ₁	R ₂	Receptor Binding K _i (nM)		[³⁵ S]GTPγS NOP		[³⁵ S]GTPγS μ		[³⁵ S]GTPγS κ	
			NOP	μ	κ	EC ₅₀ nM	% Stim	EC ₅₀ nM	% Stim	EC ₅₀ nM
7h	Et		188.7 ± 43	10.2 ± 0.26	467.7 ± 14.7	72.2 ± 19.4	10.1 ± 1.0	673 ± 334	54.9 ± 0.9	
7i	Et		>10K	33.4 ± 6.4	1367 ± 185	-	-	2110 ± 135	38.6 ± 3.2	
7j	Et		>10K	167.8 ± 33	>10K	-	-	>10K	0	
N/OFQ			0.12 ± 0.01			3.6 ± 0.7	100			
DAMGO				2.96 ± 0.5				32.6 ± 4.06	100	
U69,593					1.05 ± 0.02			60.14 ± 7.5	100	

* All compounds tested showed no appreciable DOP affinity. NT: not tested, usually in cases where binding affinity K_i greater than 100 nM.