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Design, Syntheses, and Biological Evaluation of 14-Heteroaromatic Substituted Naltrexone Derivatives: Pharmacological Profile Switch from Mu Opioid Receptor Selectivity to Mu/Kappa Opioid Receptor Dual Selectivity

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Abstract

Based on a mu opioid receptor (MOR) homology model and the “isosterism” concept, three generations of 14-heteroaromatically substituted naltrexone derivatives were designed, synthesized, and evaluated as potential MOR selective ligands. The first generation ligands appeared to be MOR selective, whereas the second and the third generation ones showed MOR/kappa opioid receptor (KOR) dual selectivity. Docking of ligands **2** (MOR selective) and **10** (MOR/KOR dual selective) to the three opioid receptor crystal structures revealed a non-conserved residue facilitated “hydrogen bonding network” that could be responsible for their distinctive selectivity profiles. The MOR/KOR dual selective ligand **10** showed no agonism and acted as a potent antagonist in the tail flick assay. It also produced less severe opioid withdrawal symptoms than naloxone in morphine dependent mice. In conclusion, ligand **10** may serve as a novel lead compound to develop MOR/KOR dual selective ligands, which might possess unique therapeutic value for opioid addiction treatment.

Keywords

MOR; MOR/KOR dual selective; antagonist; isosterism

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Supporting Information

Synthesis and characterization of 14-aminonaltrexone **21**, acid side chain for ligands **19–20**, and HPLC spectra of ligands **2–20**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Notes

The authors declare no competing financial interest.

INTRODUCTION

Opium, the dried latex obtained from the immature seed pod of a poppy flower (*Papaver Somniferum*), has been used for medical and euphoric purposes since ancient time. The major active ingredient was later identified to be morphine (Figure 1).¹ Opioids is a generic term referring to alkaloids isolated from opium poppy, their synthetic analogues, and endogenous substances synthesized in the human body.² Opioids exert their function through interacting with one or more of the three major opioid receptors, designated as the mu opioid receptor (MOR), the kappa opioid receptor (KOR), and the delta opioid receptor (DOR).^{3,4} Among them, the MOR plays a major role in mediating the antinociception and other unwanted adverse effects of opioids, such as abuse/addiction liability, respiratory depression, sedation, and constipation.⁵ Amongst the side effects, the abuse/addiction liability is the major concern for prescription of opioid analgesics.

According to the 2012 World Drug Report, the global annual prevalence of illicit opioids use ranged from 0.6 to 0.8% of the adult population in 2010. North America (3.8–4.2%), Oceania (2.3–3.4%), and Eastern and South-Eastern Europe (1.2–1.3%) have higher prevalence of opioid illicit use than the global average. The non-medical use of prescription opioids plays a dominant and problematic role in North America. For the United States alone, the overdose deaths involving prescription opioids in 2010 was four times of that in 1999.² Therefore, efficacious medications are still highly desired to treat acute (overdose) and chronic (abuse/addiction) side effects of opioids.

The current FDA approved pharmacologic treatment for long term opioid dependence/addiction based on the MOR mechanisms includes methadone (a full agonist), buprenorphine (a partial agonist), and naltrexone (an antagonist) (Figure 1).^{6–8} While methadone has shown great efficacy for opioid addiction maintenance, it could cause lethal respiratory depression when taken in overdose due to its MOR full agonist property.⁶ Furthermore, sudden cessation of methadone would precipitate a longer period of withdrawal symptoms than that following morphine termination, which makes methadone become an abused drug itself.⁹ Similarly, buprenorphine also suffered these drawbacks of methadone, albeit to a lesser extent due to its partial agonism on the MOR¹⁰, while it was associated with fewer drug–drug interactions compared to methadone.⁶ In contrast, as a MOR antagonist, naltrexone did not induce respiratory suppression and showed no abuse liability. However, it has precipitated considerable withdrawal syndrome which has compromised its therapeutic efficiency.^{11–14} The extended-release formulation of naltrexone significantly improved the adherence rate and treatment outcome.^{15, 16} Nevertheless, its application is restricted from people who have end-stage liver disease or need a long period of chronic pain management.⁶ Collectively, these three drugs have associated shortcomings, while they do serve as “proof-of-concept” that targeting on the MOR could provide effective treatment for opioid dependence and addiction.

In this respect, a number of chemical entities have been developed as MOR ligands. Some of the representatives are depicted in Figure 2. Among them, β -funaltrexamine (β -FNA), clocinnamox, and methocinnamox are potential MOR irreversible antagonists. β -FNA binds equally potent to the MOR and the KOR, whereas clocinnamox and methocinnamox bind to

all three opioid receptors in mouse brain homogenates with similar affinity.¹⁷ β -FNA also possessed reversible KOR agonist activity.^{18–20} Cyprodime is another intensively studied MOR antagonist, which has moderate selectivity and potency at the MOR (K_i value ratios are $\delta/\mu \approx 39$, $\kappa/\mu \approx 10$; K_i at the MOR is 10.6 ± 0.7 nM).^{21, 22} Sally *et al.* recently disclosed (-)-3-cyclopropylmethyl-2,3,4,4a,5,6,7,7a-octahydro-1H-benzofuro[3,2-e]isoquinolin-9-ol (LTC-274)²³ (Figure 2) as a novel MOR antagonist, which showed the least inverse agonist activity at the MOR among twenty-one ligands tested. Meanwhile, this compound bound to the KOR as strongly as to the MOR, and it acted as a KOR partial agonist ($EC_{50} = 2.7$ nM, $E_{max} = 23\%$).²³ 2-Methyl-N-([2'-(pyrrolidin-1-ylsulfonyl)biphenyl-4-yl]methyl)propan-1-amine (PF-04455242),²⁴ and c[Ala¹-pro²-Phe³-trp⁴]²⁵ (a cyclic peptidyl derivative), have also been reported to function as MOR/KOR dual antagonists (Figure 2). Yet both of them were more potent and selective at the KOR.^{24, 25} Therefore, MOR selective ligands with high potency are still highly desirable.

From another standpoint, accumulating evidence showed that the release of dynorphins and activation of the KOR mediated dysphoria and anhedonia associated with drug withdrawal, stress-induced aversion states, and stress-induced relapse-like behavior.²⁶ For example, the KOR selective agonist 2-(3,4-dichlorophenyl)-N-methyl-N-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide **1** (U50,488) mimicked stress exposure and significantly potentiated cocaine conditioned place preference.²⁷ Furthermore, **1** effectively reinstated cocaine-seeking behavior in mice previously conditioned to cocaine.²⁸ Such effects of **1** were abolished by the KOR selective antagonist nor-binaltorphimine (norBNI) in both studies.^{27, 28} Meanwhile, it has also been reported that blockade of the dynorphin/KOR system decreased dependence-induced ethanol self-administration in male Wistar rats.²⁹ In contrast, the effects of the dynorphin/KOR system on opioid dependence and addiction seemed to be dose, ligand and species specific. For instance, low doses of **1** first increased (0.1 mg/kg) and then decreased (0.5 mg/kg) heroin self-administration in mice.³⁰ KOR selective agonist (2*E*)-N-[(5 α ,6 β)-17-(cyclopropylmethyl)-3,14-dihydroxy-4,5-epoxymorphinan-6-yl]-3-(3-furyl)-N-methylacrylamide (TRK-820) but not N-methyl-2-phenyl-N-[(5*R*,7*S*,8*S*)-7-(pyrrolidin-1-yl)-1-oxaspiro[4.5]dec-8-yl]acetamide (U69,593) decreased morphine-induced conditional place preference.^{31, 32} NorBNI potentiated naloxone-precipitated morphine withdrawal symptoms in rats,³³ while KOR gene disruption in mice showed reduced naloxone-precipitated morphine withdrawal syndrome³⁴. Collectively, these data implicated that the dynorphin/KOR system may act as an important modulator in the neurobiology of drug dependence and addiction, and it could serve as a co-target along with the MOR for drug dependence and addiction treatment.^{35–37}

The continuing interest in developing novel, non-peptidic, and reversible opioid receptor ligands in our lab led to the identification of two potent and highly selective MOR ligands, NAP and NAQ, the C6-heteroaromatic substituted naltrexone derivatives based on a MOR homology model and the “message-address” concept (Figure 2).³⁸ Both compounds acted as MOR partial agonists with low efficacy in the [³⁵S]-GTP γ S binding assay, but antagonized the effects of the MOR full agonists both in vitro and in vivo.^{38, 39} Docking experiments of naltrexone to the same MOR homology model led to the identification of an alternative “address” domain which located around “the extracellular loop III and the upper-level

region of transmembrane VI/VII⁴⁰, which might have the potential to interact with some putative substitutions at the 14-position of naltrexone so as to increase the MOR binding affinity and selectivity. To test this hypothesis, three generations of 14-heteroaromatic substituted naltrexone derivatives were designed and synthesized consecutively. All the newly synthesized compounds were tested in the opioid receptor radioligand competition binding assay and the [³⁵S]-GTP γ S binding assay for their affinity, selectivity, and function. Selected compounds were further evaluated for the acute antinociceptive agonistic/antagonistic effects in the tail flick assay, and the opioid withdrawal symptoms in morphine dependent mice. Two representative ligands were also docked into the crystal structures of three opioid receptors to further validate our original hypothesis.

RESULTS AND DISCUSSION

Molecular Design

A previous molecular modeling study revealed that the 14-hydroxyl group of naltrexone might putatively point to some non-conserved yet potential hydrogen bond donor/acceptor residues (i.e. Tyr210 and Trp318) in one of the MOR-naltrexone binding modes,⁴⁰ which might be able to act as a unique MOR “address” domain. We thus hypothesized that a “functionalized moiety” that could interact with these residues (through hydrogen-bonding and/or aromatic stacking) might provide enhanced MOR affinity and thus increase the MOR selectivity over the KOR and the DOR. Six hetero-aromatic ring side chains containing one nitrogen atom (a hydrogen bond donor under physiological conditions) and two aromatic rings (as the control compounds) were thus chosen to test this hypothesis. These “functionalized moieties” were introduced to the 14-hydroxyl group of naltrexone via an ester bond (compounds **2–9**) based on a straightforward chemical synthetic method. Compound **2** was then identified as the most selective MOR molecule among the first generation ligands. However, there have been reports that sterically hindered tertiary esters (such as compounds **2–9**) could possibly be hydrolyzed by certain esterases and lipases.^{41, 42} To avoid such potential shortcomings and also facilitate future in vivo studies, the isosteres of compounds **2–9**, the amide analogues **10–17** were then synthesized as the second generation compounds to test whether the high MOR selectivity induced by these heteroaromatic ring would be retained. Interestingly, the amide derivatives became selective to both the MOR and the KOR. Among them, ligand **10** appeared to have the highest selectivity for these two receptors over the DOR. To investigate whether the selectivity profile change for ligand **10** was due to different projection of the 2'-pyridyl ring as a result of the restricted rotation of the amide bond compared to the ester linkage, different lengths of spacers were introduced between the epoxymorphinan skeleton and the 2'-pyridyl ring in compound **10** to yield the third generation compounds **18–20**. Surprisingly, these ligands with an extended spacer were still MOR/KOR dual selective. It thus suggested that the amide bond itself might be involved in the receptor-ligand interactions, which was later supported by our molecular modeling study based on the crystal structures of three opioid receptors.

Chemistry. Synthesis of 14-substituted naltrexone derivatives

The 14-*O*-substituted naltrexone derivatives (**2–9**, Table 1) were readily obtained by reacting naltrexone with the corresponding acyl chloride in TEA/DMF at 100 °C with subsequent saponification under either acidic (compounds **2–4**, **6–8**) or basic (compounds **5**, **9**) conditions (Scheme 1).⁴⁰

An established synthetic route shown in Scheme 2 was adopted to prepare 14-aminonaltrexone **21**.⁴³ Compounds **10–20** were then obtained by coupling intermediate **21** with an acid/acyl chloride, commercially available or prepared in house (see Supporting Information), followed by saponification with K₂CO₃ as described previously (Scheme 1).^{38, 43, 44}

Biology. In vitro and in vivo pharmacological studies

The synthesized three generations of naltrexone derivatives were first evaluated in the radioligand competition binding assay and the [³⁵S]-GTPγS functional assay on opioid receptor-transfected CHO cell membranes for their binding affinity, selectivity and MOR agonism/antagonism (in vitro). Then selected compounds were further advanced to behavioral tail flick and opioid withdrawal assays for their functional activity (in vivo). Naltrexone (NTX) was tested along for comparison in all the assays.

In vitro radioligand binding assay and [³⁵S]-GTPγS functional assay

The competitive radioligand binding assay was performed on monocloned opioid receptor-expressed CHO cell membranes as described previously.^{38, 39, 44} [³H]naloxone (NLX), [³H]naltrindole (NTI), and [³H]norBNI (for compounds **2–9**) or [³H]diprenorphine (DPN, for compounds **10–20**) were used to label the MOR, the DOR and the KOR, respectively. The MOR [³⁵S]-GTPγS binding assay was conducted to determine whether each of the new ligands would act as a full agonist, a partial agonist, or an antagonist at the MOR as illustrated before.^{38, 39, 44} The results were interpreted as the relative efficacy of each compound to a MOR full agonist [D-Ala²-MePhe⁴-Gly(ol)⁵]enkephalin (DAMGO) for MOR activation.

As seen in Table 1, all the first generation of naltrexone derivatives retained subnanomolar to one-digit nanomolar binding affinity at the MOR, except for compounds **5**, **6**, and **9**. The relative low binding affinity of control compounds **5** and **9** compared to the rest of the ester congeners indicated that the nitrogen atom in the “functionalized moiety” of these derivatives did play an important role in the MOR-ligand interactions. In detail, nitrogen atom substitutions with 2', 3'-pyridyl (compounds **2** and **3**, respectively), and 2', 3'-quinolyl (compounds **7** and **8**, respectively) were favorable for MOR binding, 11 to 40-fold higher compared to other isomers (compounds **3**, **4**, and **6**). Meanwhile, the first generation of naltrexone derivatives **2–9** generally all displayed considerably decreased binding affinities at the KOR and the DOR compared to naltrexone (except compounds **2** and **7** at the DOR), and compounds **2**, **3**, **6**, and **8** showed above 30-fold more selective to the MOR over the KOR and the DOR. In this sense, the nitrogen atom position seemed to be critical as well. Compound **2** (ONP) appeared to be the most selective and potent MOR ligand in this first generation of naltrexone derivatives. As for the MOR [³⁵S]-GTPγS binding assay, all the

first generation compounds, except for ligand **8**, did not show any detectable MOR agonism under the tested conditions.

In contrast, the second generation of naltrexone derivatives (**10–17**) also bound to the MOR at subnanomolar to one-digit nanomolar affinity (Table 2). But the presence/absence and the position of the nitrogen atom in the “functionalized moiety” did not seem to have a significant impact on their MOR binding affinity. In the meantime, increased binding affinities at the KOR and the DOR were observed for these second generation compounds **10–17**, in comparison to both naltrexone and the first generation of ligands, which significantly changed their opioid receptor selectivity profile. Some of the ligands were both MOR and KOR selective over the DOR (compounds **10–12**), whereas others were even more KOR selective over the MOR and the DOR (compounds **13–15**, and **17**). Nevertheless, the majority of the second generation of naltrexone derivatives produced less than 20% MOR stimulation relative to DAMGO. Compounds **10** (NNP), **11**, and **14** showed relatively lower MOR efficacy among this series of compounds. Comparing to the undetectable MOR agonism of the first generation ligands, it thus appeared that the isostere replacement of an ester linkage with an amide bond not only decreased MOR selectivity over the KOR, but also slightly enhanced ligand MOR efficacy.

Compounds **18–20** were then prepared to test whether the changed opioid receptor selectivity was due to the restricted rotation of the amide bond, compared to the ester linkage. As shown in Table 2, ligands **18** and **19** were still MOR/KOR dual selective, whereas ligand **20** bound to all three opioid receptors with nearly equal potency. It thus seemed that the conventionally restricted rotation of the amide bond might not be the reason for the ligand opioid receptor selectivity change from MOR (ligand **2**) to MOR/KOR (ligand **10**). Meanwhile, the relative lower K_i values of ligands **18–20** with an extended spacer compared to that of ligand **10** suggests that a less restricted “functionalized moiety” could facilitate the ligand to bind to the MOR. Ligand **18** behaved as a MOR partial agonist with relatively high efficacy, while ligands **19** and **20** acted as low efficacy MOR partial agonists.

Collectively, the radioligand competition binding assay identified three potent MOR selective ligands (**2**, **3**, and **8**) and three potent MOR/KOR dual selective ligands (**10**, **11**, and **19**). These six novel ligands all displayed marginal MOR agonism in the [35 S]-GTP γ S binding assay. To our notice, a similar opioid receptor selectivity profile change upon isosterism has also been reported for methocinamox (amide linkage) and its ester analogue. Methocinamox has no selectivity among the three opioid receptors, whereas its ester analogue is moderately selective to the MOR over the KOR and the DOR (the K_i value ratios for methocinamox are: delta/ μ $\approx$ 1, kappa/ μ $\approx$ 3; for its ester isostere are: delta/ μ $\approx$ 31, kappa/ μ $\approx$ 15).⁴⁵ Such findings somewhat coincided with our observation for the selectivity profile change reported herein.

Since compounds **10** and **11** showed relatively higher MOR/KOR dual selectivity over the DOR among all the new ligands, the [35 S]-GTP γ S binding assays of these two compounds on the monocloned KOR and DOR-expressed CHO cell membranes were further conducted. Compound **14** was also included in these assays due to its minimal efficacy at the MOR (Table 3). Naltrexone, and compounds **10** and **14** appeared to carry similar efficacy at the

KOR while compound **11** appeared to show the highest efficacy. On the other hand, naltrexone, and compounds **10** and **11** exhibited marginal efficacy at the DOR, while compound **14** showed the highest efficacy though its potency was relatively low.

Tail flick assay

Due to their minimum MOR activation as shown in the [^{35}S]-GTP γ S functional assay, compounds **10**, **11**, and **14** were chosen for further evaluation of their acute agonistic and antagonistic effects in the tail flick assay in mice. None of the compounds produced any significant agonist effect up to 30 mg/kg dose while they were found to potentially antagonize the analgesic effect of morphine (Table 4). Compound **10** seemed to be the most potent one among these three new ligands. The in vivo assay results thus in general were consistent with the in vitro MOR [^{35}S]-GTP γ S functional data for these compounds.

Opioid withdrawal assays

As the ultimate goal of the study is to identify selective opioid receptor ligands for opioid dependence and addiction treatment, it is necessary to evaluate whether these new ligands would precipitate severe withdrawal symptoms in morphine dependent mice (Figure 3). Similar to previous reports,^{39, 46} the well-known opioid antagonist naltrexone precipitated significant withdrawal syndrome manifested as over twenty times of escape jumps (Figure 3A) and above seven times of wet dog shakes (Figure 3B) in morphine-pelleted mice while naloxone showed even more significant syndrome (Figure 3). In the first assay, interestingly compound **10** displayed clear decrease of escape jumps as its dose increased (not significantly though), with similar jumping numbers at 30 mg/kg to that of 1 mg/kg of naltrexone (Figure 3A). In contrast, there seemed to be a dose-response increasing effect for compound **11** and no significant dose-response effect for compound **14**, respectively. Both compounds **11** and **14** at 30 mg/kg yielded similar counts of jumps as 1 mg/kg of naloxone did.

There was a minimal number of wet dog shakes for compound **10** at 3 mg/kg, but a dramatic increase was observed as the dose went up to 10 mg/kg and remained the same level at 30 mg/kg. Nevertheless, the effects at high doses of compound **10** were still lower than that of 1 mg/kg naloxone (Figure 3B). Both compounds **11** and **14** appeared to show a dose-response increasing effect with the wet dog shakes while at their highest doses tested (30 mg/kg) resembled the effect of 1 mg/kg naloxone and naltrexone. Since all three compounds only showed marginal efficacy at the MOR, their partial agonism at the KOR and the DOR could be one of the factors for their distinct profiles in the withdrawal assays. Nevertheless, the withdrawal symptoms of these MOR/KOR dual selective ligands seemed to be less significant than naloxone, which sheds some light for their potential application in drug abuse/addiction treatment.

Molecular modeling study

As discussed earlier, the selectivity profile of the first and second generations of naltrexone derivatives switched from MOR selective (ester analogues) to MOR/KOR dual selective (amide analogues). In order to understand such a phenomena in the context of ligand-receptor interaction, automated docking of ligands **2** and **10** (two compounds which showed

the highest selectivity in each generation) to the crystal structures of three opioid receptors^{47–49} was performed employing GOLD5.1. In majority of the obtained docking modes, the known “morphinan type” pose was scored the highest for both ligands, and the docking poses of ester bond-linked ligand **2** were similar to those of amide bond-linked ligand **10**.

In order to identify the possible explanations for the high MOR selectivity of ligand **2** over the KOR and the DOR, amino acid residues around the binding pocket for each opioid receptor along with docked ligand **2** were allowed to attain a lower energy conformation by a 10 ps NVT dynamic simulation (moles N, volume V and temperature T are conserved) at 300 K under TRIPOS force field (TFF). After averaging the last 1 ps of simulation, the obtained energy minimized structures were then studied. Interestingly, conformational changes observed for Gln^{2.60} of the MOR allowed for a possible hydrogen bonding interaction with the pyridyl nitrogen of ligand **2** (Figure 4A, green dots). However, this conserved Gln residue was directed differently in both the KOR and the DOR, diminishing the possibility of hydrogen bonding interactions between the Gln residue and the pyridyl nitrogen of ligand **2** in these two receptors (Figure 4A). Looking further within the same docking pocket, it was noticed that the residues at position 2.63 (directly above Gln^{2.60}) for the three opioid receptors projected and functioned differently. Asn^{2.63} in the MOR presented a helical turn above Gln^{2.60} and helped to direct Gln^{2.60} towards ligand **2** through a hydrogen bond (Figure 4A, pink dashes). However, for the KOR and the DOR, the 2.63 position was occupied by Val118 and Lys108, respectively, which were not able to interact with the Gln^{2.60} residue through hydrogen bonds (Figure 4A). Based on the receptor competition binding assay results and the current modeling studies, we thus hypothesized that a “hydrogen bonding network” among the pyridyl nitrogen of ligand **2**, the conserved Gln residue at 2.60 position, and the non-conserved residue at 2.63 position of the three opioid receptors is responsible for the receptor selectivity of this ligand. The presence of such a “hydrogen bonding network” enables ligand **2** to be more MOR selective over the KOR and the DOR.

In contrast, the MOR selectivity over the KOR was lost for ligand **10** although it has the same “functionalized moiety” as ligand **2**. To understand the possible conformational changes in residues around the binding pocket that might cause the decreased MOR selectivity for ligand **10**, the ester ‘O’ atom of ligand **2** was replaced with amide ‘NH’ atoms to put ligand **10** in the same docking pose as ligand **2**. This was then followed by the same dynamic simulation experiment as described earlier. Figure 4B presented the highest scored binding mode of ligand **10** in the MOR. The model suggested that for ligand **10**, due to a possible internal hydrogen bond between the amide NH and the pyridyl nitrogen (Figure 4B, orange dots), the pyridyl nitrogen preferred to stay close to the amide NH (potential energy under TRIPOS force field calculated for ligand **10** pose with the internal hydrogen bond was 12.5 kcal/mol lower than that without the internal hydrogen bond). Thus the pyridyl nitrogen was no longer available to form a hydrogen bond with the Gln^{2.60}, which seemed to be a key component of the “hydrogen bonding network” that decided the opioid receptor selectivity of ligand **2** according to our observation. Furthermore, this internal hydrogen bond in ligand **10** also facilitated the amide NH to form a hydrogen bonding interaction

(Figure 4B, brown dots) with the conserved residue Asp^{3.32} in all three opioid receptors, which would enhance the binding affinities of ligand **10** to all three opioid receptors. Therefore, disruption of the “hydrogen bonding network” and formation of a hydrogen bond with a conserved residue in all three opioid receptors together might account for the reduced MOR selectivity for ligand **10**, compared to its ester isostere ligand **2**.

Taken together, the current molecular modeling study based on the crystal structures of three opioid receptors identified an alternative MOR “address” domain composed of Gln^{2.60} and Asn^{2.63} in transmembrane helix II. Interestingly, such finding is significantly different from our previous studies which applied opioid receptor homology models based on the crystal structure of bovine rhodopsin. Similar discrepant results have also been observed by other research groups.⁵⁰ We also noticed that the introduction of a substitution at the 14-position of morphinan skeleton could be crucial in altering the pharmacological profile⁵¹ while the orientation (in the manner of rotation freedom) of such substitution at this position may lead to high potency MOR agonism activity during the development of some pseudo-irreversible opioid receptor antagonists.⁵²⁻⁵⁴ In combination of our observation, a more vivid picture of 14-substitution impact on the pharmacology of morphinan skeleton derivatives is now available.

CONCLUSIONS

In conclusion, based on a previous MOR homology model, three generations of 14-heteroaromatic substituted naltrexone derivatives (esters **2–9**, and amides **10–20**) were designed, synthesized, and biologically evaluated. The majority of these new ligands bound to the MOR with subnanomolar to nanomolar affinity. The selectivity profile of the compounds switched from MOR selective to MOR/KOR dual selective upon application of the concept of isosterism with marginal increase of the functional activity in the MOR [³⁵S]-GTP γ S binding assay. Further molecular modeling studies based on the crystal structures of three opioid receptors revealed that a “hydrogen bonding network” among the “functionalized moiety” (a nitrogen atom on an aromatic ring system), the Gln residue at the 2.60 position, and the non-conserved residue at the 2.63 position might decide the opioid receptor selectivity of these ligands. Presence of such a network led to high MOR selectivity over the KOR and the DOR for ligand **1** (ONP). Among the amide series of naltrexone derivatives, compounds **10**, **11**, and **14** showed minimal MOR efficacy in the [³⁵S]-GTP γ S binding assay. These three compounds also had no agonist-like property and acted as potent MOR antagonists in the tail flick assay. Furthermore, compound **10** produced less severe withdrawal symptoms than naloxone at high doses and compounds **11** and **14** behaved similarly to naltrexone in the opioid withdrawal assay. Collectively, due to the highest MOR/KOR selectivity over the DOR, minimal MOR/DOR efficacy and low KOR efficacy in the [³⁵S]-GTP γ S binding assay, lack of agonist property and potent MOR antagonism in the tail flick assay, and less severe withdrawal symptoms compared to naloxone, ligand **10** (NNP) thus represents a new lead compound to develop MOR/KOR dual selective ligands, which might possess unique therapeutic value for opioid abuse/dependence treatment.

EXPERIMENTAL SECTION

Chemical Synthesis. General Methods

Chemical reagents were purchased from either Sigma-Aldrich or Alfa Aesar. TLC analyses were carried out on Analtech Uniplate F254 plates. Chromatographic purification was accomplished on silica gel columns (230–400 mesh, Bodman). Melting points were obtained with a Fisher scientific micro melting point apparatus without correction. IR spectra were recorded on either a Nicolet iS10 or a Nicolet Avatar 360 FT-IR Instrument. Proton (300, 400 MHz) and Carbon-13 (75, 100 MHz) nuclear magnetic resonance (NMR) spectra were acquired at ambient temperature with tetramethylsilane as the internal standard on a Varian Gemini spectrometer or Bruker Ultrashield 400 Plus spectrometer, respectively. MS analysis was performed on a Quattro II triple quadrupole mass spectrometer, or a Waters Micromass QTOF-II instrument (ESI source), or an Applied Bio Systems 3200 Q trap with a turbo V source for TurbolonSpray. HPLC analysis was done with a Varian ProStar 210 system on Microsorb-MV 100–5 C18 column (250 mm × 4.6 mm) at 210/254 nm eluting with acetonitrile (0.1% TFA)/water at 1 mL/min over 15 to 50 min. All of the above analytical methods were used to determine the purity of the newly synthesized compounds and their purity is confirmed so forth as 95%.

General procedure 1. 14-*O*-substituted naltrexone derivatives synthesis

The mixture of naltrexone (1 equiv), acyl chloride (3 equiv), and triethylamine (6 equiv) in dry DMF was heated at 100 °C for 6 h under N₂ protection. After cooled down, the reaction mixture was concentrated under vacuum to remove DMF. The resulting crude intermediate was then dissolved in MeOH and 4% H₂SO₄ aqueous solution (potassium carbonate aqueous, pH ≈ 10, for compounds **5**, and **9**) and stirred overnight at ambient temperature. After concentration, the residue was partitioned between water and CH₂Cl₂. The water layer was extracted with CH₂Cl₂ three times. The combined organic phase was then washed with brine, and dried over Na₂SO₄. After removal of the solvent under reduced pressure, the resulting residue was purified using a silica gel column with a CH₂Cl₂/ MeOH (150:1→100:1) (1 % NH₃·H₂O) solvent system as eluent to give the target products **2–9** as free base.

General procedure 2. 14-*N*-substituted naltrexone derivatives synthesis with acyl chloride

To a solution of 14β-amino-7,8-dihydro-17-cyclopropylmethyl-normorphinone (**21**, 1 equiv) in dry CH₂Cl₂ was added acyl chloride (2 equiv) and Et₃N (4 equiv) on ice-water bath under N₂ protection. The mixture was then allowed to stir overnight at ambient temperature and then concentrated under reduced pressure.

General procedure 3. 14-*N*-substituted naltrexone derivatives synthesis with acid

On an ice-water bath, to a solution of acid (3 equiv) in anhydrous DMF (3 mL), was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI, 3 equiv), hydrobenzotriazole (HOBt, 3 equiv), 4 Å molecular sieves, and TEA (5.0 eq) under N₂ protection. Fifteen minutes later, **21** (1.0 eq) in DMF (1 mL) was added dropwise. The resultant mixture was allowed to warm up to ambient temperature gradually. Upon

completion of the reaction, the mixture was then filtered through celite. The filtrate was concentrated to remove DMF.

General procedure 4. Saponification of 14-*N*-substituted amide intermediates

Methanol (5 mL), and K₂CO₃ (2 eq) were added to the residue obtained from procedure 2 or 3, and stirred at ambient temperature overnight. The mixture was then filtered through celite. The filtrate was concentrated to remove methanol. The residue was then partitioned between CH₂Cl₂ (50 mL) and brine (50 mL). The organic layer was separated and dried over anhydrous MgSO₄, concentrated under reduced pressure. The residue was then purified by column chromatography, eluting with CH₂Cl₂/MeOH (1% NH₃·H₂O) to afford the corresponding compound as free base.

General procedure 5. Hydrochloride salt formation

Upon confirmation by ¹H NMR, the free base was then transformed into hydrochloride salt by dissolving the base in MeOH (0.1 mL) and CH₂Cl₂ (2 mL), HCl methanol solution (1.25 M, 4 eq) was then added with an ice-water bath, and stirred for 5 min. Diethyl ether (10 mL) was then added. Two hours later, the precipitate was collected by filtration, dried in vacuum to give the target compound as hydrochloride salt, which was then used in the further analysis and biological assays.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(pyridyl-2'-carboxy)morphinan-6-one (2)

Hydrochloride salt: ¹H NMR (300 MHz, CD₃OD) δ 8.97 (m, 1 H, Ar-H), 8.71 (m, 1 H, Ar-H), 8.27 (m, 1 H, Ar-H), 8.18 (m, 1 H, Ar-H), 6.75 (d, *J* = 8.1 Hz, 1 H, C1-H), 6.73 (d, *J* = 8.1 Hz, 1 H, C2-H), 4.92 (s, 1 H, C5-H), 4.10 (d, *J* = 6.6 Hz, 1 H), 3.50–3.27 (m, 4 H), 3.07–2.92 (m, 2 H), 2.92–2.67 (m, 2 H), 2.30 (m, 1 H), 2.10 (m, 1 H), 1.72 (m, 2 H), 1.15 (m, 1 H), 0.86 (m, 1 H), 0.78 (m, 1 H), 0.58 (m, 2 H); ¹³C NMR (75 MHz, CD₃OD) δ 207.59, 161.31, 146.38, 142.36, 139.87, 138.89, 129.90, 127.39, 125.40, 119.98, 117.95, 117.50, 88.54, 61.69, 56.92, 52.99, 37.51, 34.17, 30.23, 29.30, 27.89, 26.90, 22.49, 4.97, 4.34, 1.56. MS (ESI) *m/z*: 447 (M + H)⁺, 342. IR (KBr, cm⁻¹) $\nu_{\max}$: 3411, 1660, 1259, 794. mp 250 °C (dec).

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(pyridyl-3'-carboxy)morphinan-6-one (3)

Free base: ¹H NMR (300 MHz, CDCl₃) δ 9.57 (d, *J* = 1.5 Hz, 1 H), 8.86 (dd, *J* = 1.8 Hz, 5.1 Hz, 1 H), 8.45 (m, 1 H), 7.52 (dd, *J* = 4.95 Hz, 7.95 Hz, 1 H), 6.80 (d, *J* = 7.8 Hz, 1 H), 6.66 (d, *J* = 8.1 Hz, 1 H), 4.82 (s, 1 H), 4.68 (d, *J* = 5.4 Hz, 1H), 3.18 (d, *J* = 18.3 Hz, 1 H), 3.00 (m, 1 H), 2.82–2.68 (m, 3 H), 2.61 (dd, *J* = 5.85 Hz, 18.45 Hz, 1 H), 2.40–2.20 (m, 4 H), 1.85 (dt, *J* = 3.8 Hz, 14.32 Hz, 1 H), 1.71 (m, 1 H), 0.67 (m, 1 H), 0.40 (m, 2 H), 0.10 (m, 2 H); ¹³C NMR (75 MHz, CDCl₃) δ 207.43, 163.37, 152.17, 149.96, 143.14, 139.31, 137.56, 137.42, 127.42, 123.75, 123.37, 119.76, 118.21, 93.97, 89.35, 58.86, 55.33, 50.85, 43.47, 35.29, 30.30, 26.56, 22.87, 9.00, 3.46, 3.30. MS (ESI) *m/z*: 447 (M + H)⁺, 342. IR (KBr, cm⁻¹) $\nu_{\max}$: 2946, 1716, 1282, 1108, 737. mp 202 °C (dec).

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(pyridyl-4'-carboxy)morphinan-6-one (4)

Free base: ^1H NMR (300 MHz, CDCl_3) δ 8.84 (m, 2 H), 8.02 (m, 2 H), 6.96 (d, J = 8.1 Hz, 1 H), 6.75 (d, J = 8.4 Hz, 1 H), 4.73 (s, 1 H), 3.24 (d, J = 6.3 Hz, 1 H), 3.14 (d, J = 18.9 Hz, 1 H), 3.09–2.98 (m, 2 H), 2.79–2.60 (m, 3 H), 2.49–2.30 (m, 4 H), 2.18 (m, 1 H), 1.93 (m, 1 H), 0.89 (m, 1 H), 0.58 (m, 2 H), 0.17 (m, 2 H); Hydrochloride salt: ^{13}C NMR (75 MHz, CD_3OD) δ 207.27, 160.13, 147.69, 145.32, 143.50, 132.56, 129.71, 129.13, 127.53, 127.02, 123.80, 121.02, 120.88, 90.43, 70.16, 69.69, 62.11, 57.76, 49.17, 34.68, 30.97, 27.21, 23.81, 5.80, 5.23, 2.46. MS (ESI) m/z : 447 ($\text{M} + \text{H}$) $^+$, 342, 224. IR (KBr, cm^{-1}) ν_{max} : 3385, 1755, 1724, 1270, 1241, 749. mp 190–195 $^\circ\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(benzoyloxy)morphinan-6-one (5)

Free base: ^1H NMR (300 MHz, CDCl_3) δ 8.21 (m, 2 H), 7.62 (m, 1 H), 7.49 (m, 2 H), 6.97 (d, J = 8.1 Hz, 1 H), 6.73 (d, J = 8.4 Hz, 1H), 4.72 (s, 1H), 3.24 (m, 1 H), 3.13 (d, J = 19.2 Hz, 1 H), 3.10–2.95 (m, 1 H), 2.80–2.60 (m, 3 H), 2.50–2.40 (m, 4 H), 2.34 (m, 1 H), 2.18 (m, 1 H), 1.90 (m, 1 H), 0.89 (m, 1 H), 0.58 (m, 2 H), 0.17 (m, 2 H); Hydrochloride salt: ^{13}C NMR (75 MHz, CD_3OD) δ 205.92, 163.75, 147.54, 133.52, 132.66, 132.18, 129.39, 129.31, 123.44, 122.50, 119.74, 96.46, 93.10, 89.12, 69.35, 69.12, 62.11, 61.64, 58.04, 56.98, 33.76, 30.03, 26.89, 22.87, 4.91, 4.34, 1.56. MS (ESI) m/z : 446 ($\text{M} + \text{H}$) $^+$, 342. IR (KBr, cm^{-1}) ν_{max} : 3398, 1730, 1239, 1055, 710. mp 161–165 $^\circ\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(isoquinolyl-3'-carboxy)morphinan-6-one (6)

Free base: ^1H NMR (300 MHz, CDCl_3) δ 8.73 (s, 1 H); 8.46 (m, 1 H), 8.06 (m, 1 H), 7.89 (m, 3 H), 7.07 (d, J = 8.1 Hz, 1 H), 6.78 (d, J = 8.1 Hz, 1 H), 4.74 (s, 1 H), 3.66 (d, J = 4.5 Hz, 1 H), 3.27 (d, J = 5.4 Hz, 1 H), 3.16 (d, J = 18.6 Hz, 1 H), 2.80–2.64 (m, 2 H), 2.54–2.42 (m, 2 H), 2.37 (m, 1 H), 2.22 (dt, J = 3.3 Hz, 12.08 Hz, 1 H), 1.94 (m, 1 H), 1.78–1.62 (m, 2 H), 1.18 (m, 1 H), 0.90 (m, 1 H), 0.60 (m, 2 H), 0.20 (m, 2 H); ^{13}C NMR (75 MHz, CDCl_3) δ 207.23, 161.70, 147.36, 138.94, 136.64, 132.23, 131.66, 130.75, 130.19, 129.85, 128.34, 128.16, 126.34, 125.01, 122.62, 119.05, 90.27, 69.63, 61.48, 58.79, 53.68, 50.25, 43.05, 33.68, 30.79, 30.38, 22.61, 8.97, 3.62, 3.45. MS (ESI) m/z : 497 ($\text{M} + \text{H}$) $^+$. IR (KBr, cm^{-1}) ν_{max} : 3392, 2921, 1725, 1182, 781. mp 201–204 $^\circ\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(quinolyl-2'-carboxy)morphinan-6-one (7)

Hydrochloride salt: ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.17 (brs, 1 H, exchangeable), 8.70 (d, J = 8.1 Hz, 1 H), 8.27 (d, J = 8.7 Hz, 2 H), 8.18 (d, J = 7.5 Hz, 1 H), 7.95 (m, 1 H), 7.83 (m, 1 H), 7.24 (d, J = 8.1 Hz, 1 H), 6.94 (d, J = 8.7 Hz, 1 H), 5.20 (s, 1 H), 4.10 (m, 1 H), 3.57–3.35 (m, 2 H), 3.30–3.14 (m, 2 H), 3.10–2.90 (m, 2 H), 2.77 (m, 1 H), 2.60 (m, 1 H), 2.25–2.08 (m, 2 H), 1.70–1.50 (m, 2 H), 1.14 (m, 1 H), 0.71 (m, 1 H), 0.65 (m, 1 H), 0.56 (m, 1 H), 0.46 (m, 1 H); Free base: ^{13}C NMR (75 MHz, CDCl_3) δ 209.92, 163.00, 143.76, 143.11, 140.65, 138.64, 133.72, 131.41, 130.28, 128.51, 127.38, 123.52, 122.34, 121.09, 119.50, 117.66, 115.70, 90.06, 69.90, 61.56, 58.76, 50.60, 43.20, 35.79, 30.94, 30.18, 22.20,

8.96, 3.62, 3.39. MS (ESI) m/z : 497 ($M + H$)⁺, 342. IR (KBr, cm⁻¹) $\nu_{\max}$: 3179, 1731, 1660, 1453, 1240, 730. mp 85–88 °C.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(quinolyl-3'-carboxy)morphinan-6-one (8)

Hydrochloride salt: ¹H NMR (300 MHz, CD₃OD) δ 9.64 (m, 1 H, Ar-H), 9.58 (m, 1 H, Ar-H), 8.40–8.17 (m, 3 H, Ar-H), 7.98 (m, 1 H, Ar-H), 7.27 (d, $J = 8.1$ Hz, 1 H, C1-H), 7.08 (d, $J = 8.1$ Hz, 1 H, C2-H), 5.10 (s, 1 H), 4.25 (m, 1 H), 3.49 (m, 2 H), 3.37 (m, 2 H), 3.20 (m, 1 H), 3.10 (m, 1 H), 2.90 (m, 1 H), 2.85 (m, 1 H), 2.35 (m, 1 H), 2.20 (m, 1 H), 2.00 (m, 1 H), 1.80 (m, 1 H), 1.20 (m, 1 H), 0.90 (m, 1 H), 0.80 (m, 1 H), 0.60 (m, 2 H); ¹³C NMR (75 MHz, CD₃OD) δ 206.38, 160.69, 147.10, 146.82, 144.14, 143.32, 134.82, 132.07, 129.70, 129.02, 128.44, 128.19, 127.06, 123.67, 123.29, 122.14, 120.07, 89.52, 69.37, 64.98, 61.44, 56.97, 33.87, 30.16, 26.93, 22.96, 13.57, 4.97, 4.42, 1.63. MS (ESI) m/z : 497 ($M + H$)⁺, 342. IR (KBr, cm⁻¹) $\nu_{\max}$: 3386, 1725, 1189, 762. mp 187 °C (dec).

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -O-(2-naphthoyloxy)morphinan-6-one (9)

Hydrochloride salt: ¹H NMR (300 MHz, CD₃OD) δ 8.97 (m, 1 H, Ar-H), 8.10 (m, 4 H, Ar-H), 7.70 (m, 2 H, Ar-H), 7.21 (d, $J = 8.1$ Hz, 1 H, C1-H), 7.02 (d, $J = 8.1$ Hz, 1 H, C2-H), 4.94 (s, 1 H, C5-H), 4.19 (m, 1 H), 3.68 (m, 1 H), 3.56 (m, 3 H), 3.06 (m, 2 H), 2.87 (m, 1 H), 2.76 (m, 1 H), 2.53 (m, 1 H), 2.38 (m, 1 H), 2.16 (m, 1 H), 1.86 (m, 1 H), 1.34 (m, 1 H), 1.10–0.75 (m, 2 H), 0.61 (m, 2 H); ¹³C NMR (75 MHz, CD₃OD) δ 206.04, 164.81, 135.49, 132.78, 132.02, 131.25, 128.70, 128.15, 127.72, 127.36, 127.04, 126.62, 124.37, 123.53, 122.32, 121.53, 119.80, 119.17, 96.52, 93.17, 89.18, 76.69, 69.37, 61.65, 56.96, 33.79, 26.90, 22.86, 4.92, 4.39, 1.50. MS (ESI) m/z : 496 ($M + H$)⁺, 342. IR (KBr, cm⁻¹) $\nu_{\max}$: 3386, 1732, 1189, 1056, 776. mp 137–140 °C.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(2'-pyridyl)carboxamido]morphinan-6-one (10)

Compound **10** was prepared by following the general procedures 3 and 4 in 45% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.21 (s, 1 H, exchangeable), 9.19 (s, 1 H, exchangeable), 8.69 (m, 1 H), 8.13–8.01 (m, 2 H), 7.65 (m, 1 H), 6.63 (d, $J = 8.08$ Hz, 1 H), 6.60 (d, $J = 8.12$ Hz, 1 H), 4.91 (s, 1 H), 3.67 (d, $J = 5.4$ Hz, 1 H), 3.04 (d, $J = 18.4$ Hz, 1 H), 2.76 (m, 1 H), 2.55 (m, 2 H), 2.42 (dd, $J = 6.56$ Hz, 12.72 Hz, 1 H), 2.30 (dd, $J = 6.70$ Hz, 12.66 Hz, 1 H), 2.15 (m, 1 H), 2.10–1.88 (m, 3 H), 1.71 (m, 1 H), 1.35 (m, 1 H), 0.83 (m, 1 H), 0.44 (m, 2 H), 0.16 (m, 2 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 207.26, 163.73, 149.58, 148.36, 143.36, 138.96, 138.01, 128.31, 126.70, 124.06, 121.56, 119.01, 117.29, 88.59, 58.97, 58.27, 55.85, 47.96, 43.31, 36.34, 30.01, 29.28, 21.03, 9.25, 3.75, 3.46. MS m/z found 446.3 ($M + H$)⁺. IR (diamond, cm⁻¹) $\nu_{\max}$ 2949, 1722, 1682, 1505, 1302, 1112. mp 195 °C (dec).

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(3'-pyridyl)carboxamido]morphinan-6-one (11)

Compound **11** was prepared by following the general procedures 2 and 4 in 62% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.19 (s, 1 H, exchangeable), 9.03 (d, $J = 1.64$ Hz, 1 H), 8.74 (dd, $J = 1.62$ Hz, 4.78 Hz, 1 H), 8.26 (s, 1 H, exchangeable), 8.17 (m, 1 H), 7.55 (m, 1 H),

6.60 (d, $J = 8.08$ Hz, 1 H), 6.56 (d, $J = 8.12$ Hz, 1 H), 5.00 (s, 1 H), 4.06 (d, $J = 5.4$ Hz, 1 H), 2.97 (d, $J = 18.4$ Hz, 1 H), 2.71 (m, 2 H), 2.47 (m, 1 H), 2.44–2.23 (m, 4 H), 2.11 (m, 1 H), 2.04 (m, 1 H), 1.56 (m, 1 H), 1.32 (m, 1 H), 0.78 (m, 1 H), 0.39 (m, 2 H), 0.08 (m, 1 H), 0.04 (m, 1 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 208.30, 165.59, 151.61, 148.36, 143.38, 139.02, 135.26, 131.55, 128.37, 124.28, 123.44, 118.94, 117.11, 88.52, 58.86, 57.66, 57.23, 48.57, 43.14, 36.38, 29.36, 28.12, 21.42, 9.60, 3.85, 3.20. MS m/z found 446.5 ($\text{M} + \text{H}$) $^+$. IR (diamond, cm^{-1}) ν_{max} 2924, 1712, 1677, 1523, 1241. mp 192–195 $^{\circ}\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(4'-pyridyl)carboxamido]morphinan-6-one (12)

Compound **12** was prepared by following the general procedures 2 and 4 in 77% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 9.19 (s, 1 H, exchangeable), 8.76 (d, $J = 6.0$ Hz, 2 H), 8.28 (s, 1 H, exchangeable), 7.75 (d, $J = 6.0$ Hz, 2 H), 6.60 (d, $J = 8.04$ Hz, 1 H), 6.56 (d, $J = 8.12$ Hz, 1 H), 5.00 (s, 1 H), 4.07 (d, $J = 5.2$ Hz, 1 H), 2.97 (d, $J = 18.4$ Hz, 1 H), 2.69 (m, 2 H), 2.47 (m, 1 H), 2.44–2.23 (m, 4 H), 2.11 (m, 1 H), 2.04 (m, 1 H), 1.55 (m, 1 H), 1.31 (m, 1 H), 0.78 (m, 1 H), 0.40 (m, 2 H), 0.09 (m, 1 H), 0.05 (m, 1 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 208.37, 165.68, 150.09 ($\times 2$), 143.40, 142.98, 139.06, 128.35, 124.32, 121.59 ($\times 2$), 119.04, 117.18, 88.54, 58.88, 57.79, 57.15, 48.61, 43.21, 36.39, 29.38, 28.03, 21.40, 9.62, 3.85, 3.37. MS m/z found 446.5 ($\text{M} + \text{H}$) $^+$. IR (diamond, cm^{-1}) ν_{max} 3270, 1710, 1686, 1604, 1510, 1221. mp 195 $^{\circ}\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-(benzamido)morphinan-6-one (13)

Compound **13** was prepared by following the general procedures 3 and 4 in 60% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 9.18 (s, 1 H, exchangeable), 8.06 (s, 1 H, exchangeable), 7.86 (m, 2 H), 7.53 (m, 3 H), 6.62 (d, $J = 8.08$ Hz, 1 H), 6.57 (d, $J = 8.12$ Hz, 1 H), 5.00 (s, 1 H), 3.95 (d, $J = 5.2$ Hz, 1 H), 2.98 (d, $J = 18.5$ Hz, 1 H), 2.70 (m, 2 H), 2.32 (m, 3 H), 2.23 (m, 1 H), 2.07 (m, 2 H), 1.59 (m, 1 H), 1.32 (m, 1 H), 1.23 (m, 1 H), 0.81 (m, 1 H), 0.42 (m, 2 H), 0.12 (m, 1 H), 0.07 (m, 1 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 208.10, 167.23, 143.40, 138.97, 135.79, 131.09, 128.47, 128.28 ($\times 2$), 127.29 ($\times 2$), 124.31, 118.92, 117.14, 88.59, 58.82, 57.84, 57.04, 48.40, 43.15, 36.38, 29.53, 28.44, 21.32, 9.58, 3.83, 3.30. MS m/z found 445.3 ($\text{M} + \text{H}$) $^+$. IR (diamond, cm^{-1}) ν_{max} 2958, 1721, 1659, 1508, 1302, 1112. mp 180–185 $^{\circ}\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(3'-isoquinolyl)carboxamido]-morphinan-6-one (14)

Compound **14** was prepared by following the general procedures 3 and 4 in 44% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 9.43 (s, 1 H), 9.42 (s, 1 H, exchangeable), 9.20 (s, 1 H, exchangeable), 8.63 (s, 1 H), 8.27 (d, $J = 8.1$ Hz, 1 H), 8.19 (d, $J = 8.1$ Hz, 1 H), 7.89 (m, 1 H), 7.82 (m, 1 H), 6.63 (d, $J = 8.0$ Hz, 1 H), 6.59 (d, $J = 8.16$ Hz, 1 H), 4.93 (s, 1 H), 3.68 (d, $J = 5.4$ Hz, 1 H), 3.06 (d, $J = 18.4$ Hz, 1 H), 2.84 (m, 1 H), 2.47 (m, 1 H), 2.32 (dd, $J = 6.78$ Hz, 12.62 Hz, 1 H), 2.39 (m, 2 H), 2.19 (m, 1 H), 2.05 (m, 3 H), 1.78 (m, 1 H), 1.36 (d, $J = 11.7$ Hz, 1 H), 0.89 (m, 1 H), 0.50 (m, 2 H), 0.23 (m, 2 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 207.39, 164.26, 151.54, 143.41, 138.94, 135.39, 131.53, 129.28, 129.23, 128.42, 127.94, 127.86, 124.23, 119.62, 119.12, 117.36, 88.70, 59.19, 58.33, 55.89, 54.69,

48.01, 43.38, 36.39, 30.13, 29.46, 21.06, 9.32, 3.85, 3.53. MS m/z found 497.7 (M + H)⁺. IR (diamond, cm⁻¹) $\nu_{\max}$ 2953, 1721, 1676, 1508, 1305, 1111. mp 251–253 °C.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(2'-quinolyl)carboxamido]morphinan-6-one (15)

The title compound was prepared by following the general procedures 3 and 4 in 40% yield. Hydrochloride salt: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.23 (s, 1 H, exchangeable), 9.12 (b, 1 H, exchangeable), 8.65 (d, *J* = 8.6 Hz, 1 H), 8.34 (d, *J* = 8.4 Hz, 1 H), 8.21 (d, *J* = 8.5 Hz, 1 H), 8.14 (d, *J* = 8.1 Hz, 1 H), 7.94 (m, 1 H), 7.78 (m, 1 H), 6.79 (d, *J* = 8.1 Hz, 1 H), 6.71 (d, *J* = 8.1 Hz, 1 H), 5.57 (s, 1 H), 5.49 (d, *J* = 5.8 Hz, 1 H), 3.46 (m, 2 H), 3.17 (m, 2 H), 2.93 (m, 1 H), 2.68 (m, 4 H), 2.23 (m, 1 H), 1.73 (m, 1 H), 1.61 (m, 1 H), 1.07 (m, 1 H), 0.73 (m, 1 H), 0.66 (m, 1 H), 0.55 (m, 1 H), 0.44 (m, 1 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 204.73, 163.58, 147.50, 143.19, 141.16, 137.77, 135.94, 128.41, 126.94, 126.66, 126.18, 125.74, 124.35, 118.50, 117.89, 116.33, 115.92, 85.60, 55.57 ($\times$ 2), 54.92, 45.49 ($\times$ 2), 43.65, 33.12, 24.56, 19.73, 3.22, 3.09. MS m/z found 496.7 (M + H)⁺. IR (diamond, cm⁻¹) $\nu_{\max}$ 2946, 1722, 1680, 1499, 1320, 1112. mp 200–202 °C.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(3'-quinolyl)carboxamido]morphinan-6-one (16)

Compound **16** was prepared by following the general procedures 3 and 4 in 48% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.29 (d, *J* = 2.12 Hz, 1 H), 9.20 (s, 1 H, exchangeable), 8.82 (d, *J* = 1.84 Hz, 1 H), 8.42 (brs, 1 H, exchangeable), 8.13 (m, 2 H), 7.88 (m, 1 H), 7.72 (m, 1 H), 6.62 (d, *J* = 8.08 Hz, 1 H), 6.58 (d, *J* = 8.12 Hz, 1 H), 5.04 (s, 1 H), 4.10 (d, *J* = 5.3 Hz, 1 H), 2.99 (d, *J* = 18.4 Hz, 1 H), 2.77 (m, 2 H), 2.55 (m, 1 H), 2.37 (m, 4 H), 2.15 (m, 1 H), 2.08 (m, 1 H), 1.61 (m, 1 H), 1.35 (m, 1 H), 0.84 (m, 1 H), 0.41 (m, 2 H), 0.13 (m, 1 H), 0.06 (m, 1 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 208.28, 165.62, 148.96, 148.29, 143.41, 139.04, 135.57, 131.09, 128.93, 128.66, 128.61, 128.41, 127.41, 126.46, 124.31, 118.97, 117.15, 88.57, 58.86, 57.76, 57.42, 48.61, 43.19, 36.42, 29.46, 28.22, 21.48, 9.62, 3.88, 3.25. MS m/z found 496.3 (M + H)⁺. IR (diamond, cm⁻¹) $\nu_{\max}$ 2929, 1715, 1659, 1499, 1305, 1102. mp 185 °C.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[(2'-naphthamido]morphinan-6-one (17)

The title compound was prepared by following the general procedures 3 and 4 in 45% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.19 (s, 1 H, exchangeable), 8.46 (s, 1 H), 8.24 (s, 1 H, exchangeable), 8.03 (m, 3 H), 7.94 (m, 1 H), 7.62 (m, 2 H), 6.61 (d, *J* = 8.08 Hz, 1 H), 6.57 (d, *J* = 8.12 Hz, 1 H), 5.03 (s, 1 H, C₅-H), 4.00 (d, *J* = 5.3 Hz, 1 H), 3.01 (d, *J* = 18.4 Hz, 1 H), 2.73 (m, 2 H), 2.56 (m, 1 H), 2.34 (m, 4 H), 2.09 (m, 2 H), 1.63 (m, 1 H), 1.35 (m, 1 H), 0.83 (m, 1 H), 0.43 (m, 2 H), 0.16 (m, 1 H), 0.09 (m, 1 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 208.05, 167.19, 143.42, 138.98, 138.0, 134.02, 133.05, 132.02, 128.64, 128.48, 127.88, 127.59, 127.52, 127.40, 126.76, 124.30, 118.92, 117.14, 88.62, 58.81, 58.00, 57.15, 48.42, 43.17, 36.41, 34.0, 28.53, 21.36, 9.61, 3.90, 3.31. MS m/z found 495.7 (M + H)⁺. IR (diamond, cm⁻¹) $\nu_{\max}$ 3029, 1717, 1668, 1506, 1302, 1021. mp 192–195 °C.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[2'-(pyridin-2''-yl)acetamido]morphinan-6-one (18)

The title compound was prepared by following the general procedures 3 and 4 in 43% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 9.17 (s, 1 H, exchangeable), 8.51 (d, J = 4.16 Hz, 1 H), 8.10 (b, 1 H, exchangeable), 7.74 (m, 1 H), 7.52 (d, J = 7.80 Hz, 1 H), 7.25 (m, 1 H), 6.58 (d, J = 8.0 Hz, 1 H), 6.53 (d, J = 8.0 Hz, 1 H), 4.82 (s, 1 H), 3.92 (s, 1 H), 3.78 (m, 2 H), 2.91 (d, J = 18.12 Hz, 1 H), 2.71 (m, 1 H), 2.59 (m, 1 H), 2.41 (m, 1 H), 2.25 (m, 4 H), 2.02 (m, 2 H), 1.47 (m, 1 H), 1.24 (m, 1 H), 0.70 (m, 1 H), 0.41 (m, 2 H), 0.05 (m, 2 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 208.02, 169.21, 156.41, 148.85, 143.35, 139.06, 136.60, 128.53, 124.28, 123.67, 121.80, 119.04, 117.14, 88.45, 58.90, 56.83, 56.76, 48.36, 45.79, 43.45, 36.21, 29.04, 27.86, 21.24, 9.12, 3.62, 3.47. MS m/z found 460.2 ($M + \text{H}$) $^+$. IR (diamond, cm^{-1}) ν_{max} 3011, 1720, 1689, 1537, 1304, 1015. mp 195–198 $^{\circ}\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-[3'-(pyridin-2''-yl)propanamido]morphinan-6-one (19)

The title compound was prepared by following the general procedures 3 and 4 in 38% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 9.15 (s, 1 H, exchangeable), 8.46 (m, 1 H), 7.68 (m, 1 H), 7.67 (s, 1 H, exchangeable), 7.30 (d, J = 7.8 Hz, 1 H), 7.19 (m, 1 H), 6.56 (d, J = 8.1 Hz, 1 H), 6.51 (d, J = 8.1 Hz, 1 H), 4.80 (s, 1 H), 3.96 (d, J = 5.1 Hz, 1 H), 3.03 (m, 2 H), 2.89 (d, J = 18.4 Hz, 1 H), 2.68 (m, 2 H), 2.55 (m, 2 H), 2.38 (m, 1 H), 2.20 (m, 4 H), 1.96 (m, 2 H), 1.41 (m, 1 H), 1.18 (m, 1 H), 0.76 (m, 1 H), 0.42 (m, 2 H), 0.09 (m, 2 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 208.44, 171.68, 160.43, 148.76, 143.34, 138.97, 136.44, 128.63, 124.46, 122.82, 121.35, 118.99, 117.03, 88.49, 58.81, 56.64, 56.58, 48.40, 43.39, 36.17, 35.32, 33.18, 28.96, 27.83, 21.25, 9.35, 3.61, 3.52. MS m/z found 474.3 ($M + \text{H}$) $^+$. IR (diamond, cm^{-1}) ν_{max} 3020, 1721, 1674, 1505, 1304, 1031. mp 210–212 $^{\circ}\text{C}$.

17-cyclopropylmethyl-4,5 α -epoxy-3-hydroxy-14 β -N-{2'-[(pyridin-2''-yl)carboxamido]acetamido}morphinan-6-one (20)

The title compound was prepared by following the procedures 3 and 4 in 37% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 9.15 (s, 1 H, exchangeable), 9.08 (t, J = 5.64 Hz, 1 H, exchangeable), 8.66 (m, 1 H), 8.07 (m, 1 H), 8.00 (m, 1 H), 7.84 (s, 1 H, exchangeable), 7.62 (m, 1 H), 6.58 (d, J = 8.04 Hz, 1 H), 6.51 (d, J = 8.08 Hz, 1 H), 4.74 (s, 1 H), 4.06 (d, J = 5.76 Hz, 2 H), 3.69 (d, J = 5.32 Hz, 1 H), 2.89 (d, J = 18.4 Hz, 1 H), 2.71 (m, 1 H), 2.52 (m, 1 H), 2.46 (m, 1 H), 2.16 (m, 4 H), 1.97 (m, 2 H), 1.55 (m, 1 H), 1.23 (m, 1 H), 0.53 (m, 1 H), 0.27 (m, 2 H), 0.02 (m, 2 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 207.64, 168.78, 164.19, 149.30, 148.52, 143.30, 138.97, 137.81, 128.54, 126.74, 124.20, 121.86, 119.04, 117.24, 88.43, 58.59, 57.58, 56.13, 48.20, 43.47, 42.95, 36.25, 29.31, 28.37, 21.06, 9.06, 3.48, 3.25. MS m/z found 503.2 ($M + \text{H}$) $^+$. IR (diamond, cm^{-1}) ν_{max} 3014, 1718, 1691, 1652, 1532, 1319, 1029. m.p. 205–208 $^{\circ}\text{C}$.

Biological Evaluation. Drugs

Morphine sulfate was purchased from Mallinckrodt, St. Louis, MO or provided by NIDA. Morphine pellets (75mg) and placebo pellets were provided by NIDA. Naloxone and Naltrexone was purchased from Sigma-Aldrich (St. Louis, MO). All drugs and test

compounds were dissolved in pyrogen-free isotonic saline (Baxter Healthcare, Deerfield, IL) or sterile-filtered distilled/deionized water.

Animals

Male Swiss-Webster mice (Harlan, Indianapolis, IN) weighing 25 to 30 g were housed six per cage in animal care quarters at 22 ± 2 °C on a 12 h light/dark cycle. Food and water were available *ad libitum*. The mice were brought to a test room (22 ± 2 °C, 12 h light/dark cycle), marked for identification, and allowed 18 h to recover from transport and handling. Protocols and procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University Medical Center and comply with the recommendations of the International Association for the Study of Pain.

In Vitro Competitive Radioligand Binding Assay

The radioligand binding assay and the [35 S]-GTP γ S binding assay were conducted using monocloned mice opioid receptor expressed in Chinese hamster ovarian (CHO) cell lines as described previously.^{38, 39, 44} [3 H]NLX, [3 H]NTI, and [3 H]norBNI (or [3 H]DPN) were used to label the MOR, the DOR, and the KOR, respectively. Aliquots of a membrane protein (30 μ g) were incubated with the corresponding radioligand in the presence of different concentrations of the drug under investigation in the TME buffer (50 mM Tris, 3 mM MgCl₂, 0.2 mM EGTA, pH 7.7) at 30 °C for 1.5 h. The bound radioactive ligand was separated from the free radioligand by filtration using the Brandel harvester (Biomedical Research & Development Laboratories, MD). Specific (i.e., opioid receptor related) binding was determined as the difference in binding obtained in the absence and presence of 5 μ M naltrexone, 5 μ M **1**, and 5 μ M 4-[(R)-[(2*S*,5*R*)-4-allyl-2,5-dimethylpiperazin-1-yl](3-methoxyphenyl)methyl]-*N,N*-diethylbenzamide **30** (SNC80)⁵⁸ for the MOR, the KOR, and the DOR, respectively. The potency of the drugs in displacing the specific binding of the radioligand was determined from the specific binding using linear regression analysis of Hill plots. The IC₅₀ values will then be determined and corrected to *K*_i values using the Cheng-Prusoff equation.

In Vitro Functional Assay: Stimulation of [35 S]-GTP γ S Binding

[35 S]-GTP γ S functional assays were conducted in the same cell membranes used for the receptor binding assays. Membrane proteins (10 μ g) were incubated with varying concentrations of drugs, GDP (10 μ M) and 0.1 nM [35 S]-GTP γ S in assay buffer (50 mM Tris, 3 mM MgCl₂, 100 mM NaCl, 0.2 mM EGTA, pH 7.7) for 1.5 h at 30 °C. Nonspecific binding was determined with 20 μ M unlabeled GTP[γ S]. DAMGO (3 μ M), **1** (5 μ M), and **30** (5 μ M) were included in the assay for a maximally effective concentration of a full agonist for the MOR, the KOR and the DOR, respectively.

In Vivo Pharmacology. Tail Flick Test

The warm-water tail flick test was performed according to Coderre and Rollman⁵⁵ using a water bath with the temperature maintained at 56 ± 0.1 °C. All drugs and test compounds were administered to mice subcutaneously (sc). Before injecting, the baseline latency (control) of the mice was determined. Only mice with a reaction time from 2 to 4 s were

used. The average baseline latency for the experiment was 3.0 ± 0.1 s. The test latency after drug treatment was assessed at the appropriate time, and a 10 s maximum cutoff time was imposed to prevent tissue damage. Antinociception was quantified according to the method of Harris and Pierson⁵⁶ as the percentage of maximum possible effect (% MPE), which was calculated as: % MPE = [(test latency – control latency)/(10 – control latency)] $\times$ 100. Percent MPE was calculated for each mouse using at least six mice per drug.

In Vivo Pharmacology. Opioid Withdrawal Assay

A 75 mg morphine pellet was implanted into the base of the neck of male Swiss Webster mice following the reported procedure.³⁹ The animals were allowed to recover in their home cages before testing. Mice were allowed for 30 minutes to habituate to an open-topped, square, clear Plexiglas observation chamber ($26 \times 26 \times 26$ cm³) with lines partitioning the bottom into quadrants before given antagonist. All drugs and test compounds were administered to mice subcutaneously (sc). Withdrawal was precipitated at 72 hours from pellet implantation with naloxone (1.0 mg/kg, s.c.), naltrexone (1.0 mg/kg, s.c.), and the test compounds at indicated doses. Withdrawal commenced within 1 minute after antagonist administration. Escape jumps and wet dog shakes were quantified by counting their occurrences over 20 minutes for each mouse using at least four mice per drug.

Statistical Analysis

One-way ANOVA followed by the post hoc “Dunnett” test were performed to assess significance using the Prism 3.0 software (GraphPad Software, San Diego, CA).

Molecular modeling study

Chemical structures of the ligands were sketched in SybylX-2.0, and their Gasteiger-Hückel charges were assigned before energy minimization (10,000 iterations) with the TFF. The X-ray crystal structures for MOR (4DKL), KOR (4DJH) and DOR (4EJ4) were retrieved from PDB Data Bank at <http://www.rcsb.org>. Automated docking on these retrieved receptor structures was done utilizing a genetic algorithm docking program GOLD 5.1. The binding site was defined to include all atoms within 10 Å of the γ -carbon atom of Asp^{3.32} for the three opioid crystal structures along with a hydrogen bond constraint between the 14-N of the ligand's morphinan skeleton and the carboxylate group of Asp^{3.32}. Best CHEM-PLP scored solutions were chosen for further analyses. All molecular dynamic (MD) simulations were performed in SybylX-2.0 for 10 ps under NVT ensemble. All the residues outside 15 Å sphere radius of 14-C of the ligand were defined as aggregates and MD simulations were run after assigning Gasteiger-Hückel charges and initial temperature at 300 K. Average structure of last 1 ps of the simulation was again energy minimized after assigning Gasteiger-Hückel charges for 1000 iterations. Pictures were generated using PyMOL Molecular Graphics System, Version 1.5.0.4.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

CHO	Chinese hamster ovarian
DAMGO	[D-Ala ² -MePhe ⁴ -Gly(ol) ⁵]enkephalin
DOR	delta opioid receptor
DPN	diprenorphine
β-FNA	β-funaltrexamine
KOR	kappa opioid receptor
MOR	mu opioid receptor
NLX	naloxone
NTX	naltrexone
norBNI	nor-binaltorphimine
NTI	naltrindole
NAP	17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4'-pyridyl)carboxamido]morphinan
NAQ	17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-(isoquinoline-3-carboxamido)morphinan
TFF	TRIPOS force field

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Figure 1.
Morphine and the current available drugs for opioid dependence/addiction treatment based on the MOR mechanisms.

Figure 2.
Representatives of the small molecules possessing the MOR antagonist characteristics.

(A)

(B)

Figure 3.

14-*N*-heteroaromatic substituted naltrexone derivatives **10**, **11**, and **14** in withdrawal assays in chronic morphine exposed mice: (A) Escape jumps; (B) Wet dog shakes.

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Figure 4.

(A) Superimposed binding mode of ligand **2** (Orange balls and stick) in three opioid receptors: conserved residues (Cyan), MOR residues (green), KOR residues (black), and DOR residues (purple). Pink dashes and green dots represent possible hydrogen bonding interactions. Asn^{2.63} (MOR), but not Val^{2.63} (KOR) or Lys^{2.63} (DOR), facilitated the hydrogen bonding interaction (green dots) between the Gln^{2.60} and the pyridyl nitrogen atom. The thus formed “hydrogen bonding network” yields the high MOR selectivity of ligand **2** over the KOR and the DOR. (B) The highest scored binding mode of ligand **10** (green balls and stick) in the MOR (cyan). Conserved hydrogen bonding interactions as seen for ligand **2** are shown in pink dashes. A potential internal hydrogen bond (orange dots) between the amide NH and the pyridyl nitrogen in ligand **10** disrupts the “hydrogen bonding network” as observed for ligand **2**. Furthermore, a hydrogen bonding interaction (brown dots) also formed between the amide NH and the conserved residue Asp^{3.32} in all three opioid receptors and thus enhanced the binding affinities of ligand **10** in all three opioid receptors. Together, a reduced MOR selectivity for ligand **10** was observed as compared to that of ligand **2**.

Scheme 1.

Synthetic route of the three generations of 14-substituted naltrexone derivatives **2–20**. (a) RCOCl or RCOOH; (b) K₂CO₃ or H₂SO₄.

Scheme 2.

Synthetic route of 14-aminonaltrexone **21**. (a) DEAD, benzene, reflux; (b) Pyridine·HCl, EtOH/H₂O, reflux to rt, 83%, two steps; (c) TEA, DCM, 0 °C to rt, quantitative; (d) LiAlH₄, THF, reflux, 77%; (e) NaIO₄, NaOAc, EtOAc, 73%; (f) Pd/C, H₂/60 psi, AcOH, NaOAc, MeOH, 40%; (g) BBr₃, DCM, 44%.

Table 1

Opioid receptor binding affinity, selectivity, and the MOR [35 S]GTP γ S functional assay results of the first generation of 14-substituted naltrexone derivatives^a

Compd	R	K_i (nM)			Selectivity			% $E_{\max}$ of DAMGO
		μ	κ	δ	κ/μ	δ/μ	δ/κ	
NTX ^b	NA	0.26 $\pm$ 0.02	5.15 $\pm$ 0.26	117.0 $\pm$ 8.9	20	450	23	ND
2 (ONP)		0.14 $\pm$ 0.03	25.5 $\pm$ 6.5	117.4 $\pm$ 18.0	182	838	4.6	ND
3		1.59 $\pm$ 0.61	47.8 $\pm$ 8.5	170.3 $\pm$ 12.6	30	107	3.6	ND
4		5.58 $\pm$ 1.34	49.2 $\pm$ 20.4	405.3 $\pm$ 234.7	8.8	73	8.2	ND
5 (control)		123.2 $\pm$ 38.2	586.4 $\pm$ 32.4	>10,000.00	4.7	>81	>17	ND
6		68.4 $\pm$ 6.0	>10,000	>10,000	>146	>146	NA	ND
7		1.44 $\pm$ 0.32	67.2 $\pm$ 36.7	22.8 $\pm$ 19.5	47	16	0.34	ND
8		2.69 $\pm$ 0.72	148.2 $\pm$ 55.5	818.4 $\pm$ 507.2	55	304	5.5	22.0 $\pm$ 10.3
9 (control)		225.3 $\pm$ 46.6	46.6 $\pm$ 13.5	907.2 $\pm$ 193.0	0.21	4.0	19	ND

^aThe values are the means $\pm$ S.E.M. of three independent experiments. [³H]NLX, [³H]NTI, and [³H]norBNI were used to label MOR, DOR and KOR, respectively. The percentage stimulation to DAMGO is the $E_{\max}$ of the compound compared to that of 3 μ M DAMGO (normalized to 100%). NA, not applicable. ND, not detectable.

^bPercentage stimulation 55 produced at the maximum concentration of 10 μ M. ^c These controls were also reported previously in Reference 57.

Table 2

Opioid receptor binding affinity, selectivity, and the MOR [^{35}S]GTP γ S functional assay results of the second and third generations of 14-substituted naltrexone derivatives^a

Compd	R	K_i (nM)			Selectivity			% E_{max} of DAMGO
		μ	K	δ	μ/k	δ/k	δ/μ	
NTX ^b	NA	0.34 ± 0.03	0.90 ± 0.11	95.5 ± 6.1	0.4	106	281	7.2 ± 0.6
10 (NNP)		1.51 ± 0.34	0.36 ± 0.01	94.5 ± 6.5	4.2	263	63	0.9 ± 0.4^b
11		0.75 ± 0.28	0.16 ± 0.01	39.9 ± 0.5	4.7	249	53	5.1 ± 0.6^b
12		0.82 ± 0.33	0.33 ± 0.01	10.9 ± 1.3	2.5	33	13	7.7 ± 0.7
13 (control)		4.34 ± 0.70	0.12 ± 0.001	57.3 ± 4.3	36	477	13	5.8 ± 1.4
14		3.50 ± 1.87	0.27 ± 0.02	25.1 ± 1.8	13	93	7.2	2.8 ± 1.6^b
15		9.09 ± 4.94	0.26 ± 0.01	15.1 ± 0.6	35	58	1.7	34.4 ± 5.4
16		1.13 ± 0.25	0.13 ± 0.02	1.48 ± 0.05	8.7	11	1.3	15.8 ± 5.6
17 (control) ^c		6.22 ± 4.01	0.33 ± 0.02	10.5 ± 1.4	19	32	1.7	16.5 ± 2.0

Compd	R	K_i (nM)			Selectivity			% $E_{\max}$ of DAMGO
		μ	K	δ	μ/k	δ/k	$\delta\mu$	
18		0.29 ± 0.04	0.19 ± 0.03	3.92 ± 0.12	1.5	21	14	78.0 ± 2.7
19		0.32 ± 0.04	0.17 ± 0.02	9.1 ± 0.5	1.9	54	29	12.7 ± 0.2
20		0.30 ± 0.01	0.14 ± 0.01	0.37 ± 0.04	2.1	2.6	1.2	11.8 ± 0.4

^aThe values are the means $\pm$ S.E.M. of three independent experiments. [^3H]NLX, [^3H]NTI, and [^3H]DPN were used to label MOR, DOR and KOR, respectively. The percentage stimulation to DAMGO is the $E_{\max}$ of the compound compared to that of 3 μM DAMGO (normalized to 100%). NA, not applicable.

^bPercentage stimulation produced at the maximum concentration of 10 μM .

^cThese controls were also reported previously in Reference 57.

Table 3The KOR/DOR [³⁵S]-GTP γ S Binding Results for Compounds **10**, **11**, and **14**^a

Compd	KOR [³⁵ S]-GTP γ S Binding		DOR [³⁵ S]-GTP γ S Binding	
	EC ₅₀ (nM)	% E _{max} of 1	EC ₅₀ (nM)	% E _{max} of 30
NTX	0.81 ± 0.08	20.8 ± 0.9	4.4 ± 1.6	5.6 ± 0.6
10 (NNP)	1.74 ± 0.50	24.9 ± 2.0	19.7 ± 7.4	8.6 ± 0.8
11	0.56 ± 0.06	35.3 ± 6.8	3.2 ± 2.0	10.3 ± 0.6
14	2.75 ± 0.71	16.8 ± 0.6	14.2 ± 1.9	23.8 ± 0.7

^aThe values are the means ± S.E.M. of three independent experiments. The percentage stimulation to **1** or **30** is the E_{max} of the compound compared to that of **1** or **30** (normalized to 100%).

Table 4

AD₅₀ Values of Compounds **10**, **11**, and **14** for Antagonizing Morphine (10 mg/kg) Antinociception Effect in Warm-Water Tail Flick Assay^a

Compound	AD ₅₀ values (mg/kg (95% CL))
Naloxone	0.05 (0.03-0.09)
Naltrexone	0.006 (0.003-0.014)
10 (NNP)	0.25 (0.18-0.36)
11	0.55 (0.35-0.87)
14	0.87 (0.52-1.47)

^a All drugs and test compounds were administered to a group of six mice subcutaneously (s.c).