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Structure–Activity Relationship Studies of 6 α - and 6 β -Indolylacetamidonaltrexamine Derivatives as Bitopic Mu Opioid Receptor Modulators and Elaboration of the “Message-Address Concept” To Comprehend Their Functional Conversion

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Abstract

Structure–activity relationship (SAR) studies of numerous opioid ligands have shown that introduction of a methyl or ethyl group on the tertiary amino group at position 17 of the epoxymorphinan skeleton generally results in a mu opioid receptor (MOR) agonist while introduction of a cyclopropylmethyl group typically leads to an antagonist. Furthermore, it has been shown that introduction of heterocyclic ring systems at position 6 can favor antagonism. However, it was reported that 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(2'-indolyl)acetamido]morphinan (INTA), which bears a cyclopropylmethyl group at position 17 and an indole ring at position 6, acted as a MOR agonist. We herein report a SAR study on INTA with a series of its complementary derivatives to understand how introduction of an indole moiety with α or β linkage at position 6 of the epoxymorphinan skeleton may influence ligand function. Interestingly, one of INTA derivatives, compound 15 (NAN) was identified as a MOR antagonist both in vitro and in vivo. Molecular modeling studies revealed that INTA and NAN may interact with different domains of the MOR allosteric binding site. In addition, INTA may interact with W293 and N150 residues found in the orthosteric site to stabilize MOR activation conformation while NAN does not. These results suggest that INTA and NAN may be bitopic ligands and the type of allosteric interactions with the MOR influence their functional activity. These insights

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Author Contributions

Y.Z. conceived and oversaw the project and finalized the manuscript. S.O. conducted the chemical synthesis and drafted the manuscript under the supervision of Y.Z. H.W. conducted the molecular modeling study. The binding assays were conducted by S.O. and A.J. under the supervision of D.E.S. The in vivo study was conducted by D.L.S. under the supervision of W.L.D. and H.I.A.

Supporting Information

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along with our enriched comprehension of the “message-address” concept will to benefit future ligand design.

Graphical Abstract

Keywords

Opioids; SAR; bitopic; allosteric; functional conversion; message-address concept

INTRODUCTION

Since opioid binding sites were first proposed in the early 1950s and 1960s, and discovered in mammalian brain tissue in 1973, extensive pharmacological studies have identified several types of opioid receptors.^{1–5} To date, four opioid receptors have been cloned, the mu opioid receptor (MOR), kappa opioid receptor (KOR), delta opioid receptor (DOR), and the opioid receptor-like orphan receptor (ORL) or nociceptin/orphanin FQ receptor (NOP).^{6–9} Opioid receptors share high sequence homology and belong to the superfamily of seven transmembrane-spanning G protein-coupled receptors (GPCRs).¹⁰ GPCRs play important roles in mediating the actions of many known neurotransmitters and hormones.^{11,12} Effects of opioids are mainly inhibitory resulting in significant inhibition of nerve firing and reduction in neurotransmitter release. The rewarding, dependence-producing, and analgesic effects of opioids result from activation of MORs in several brain regions.^{13–15} KOR agonists have been shown to produce dysphoric and psychotomimetic effects,¹⁶ while DOR activation results in inhibition of anxiety and stress.^{17,18} As a result, some opioid agonists and antagonists have been applied in the treatment of a number of diseases.¹⁹ In general, a compound is characterized as an opioid agonist only if its effect is competitively inhibited by an opioid antagonist.^{20–22}

Structure–activity relationship (SAR) studies of opioids have revealed that introduction of a methyl or ethyl group on the 17-amino group of the epoxymorphinan skeleton generally results in a MOR agonist while introduction of an allyl or cyclopropylmethyl group at the same position typically leads to an opioid antagonist. Also, a hydroxyl group at position 14 may strengthen the binding to the opioid receptors, like naltrexone.^{23–25} In addition, some compounds with hetero-cyclic rings at position 6 of the epoxymorphinan group such as 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-(isoquinoline-3-carboxamido)morphinan (NAQ) and 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-(4'-pyridylcarboxamido)morphinan (NAP) have been shown to be MOR selective antagonists (Figure 1).^{26–33} However, introduction of an allyl or cyclopropylmethyl

group on the 17-amino group of the epoxymorphinan skeleton may also lead to an opioid agonist.^{34–36} For example, Le Naour et al. reported that 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β [(2'-indolyl)acetamido]morphinan (INTA), which is similar to NAQ and NAP structure-wise, actually acted as an opioid agonist and activated KOR-DOR and KOR-MOR heterodimers in HEK293 cells (Figure 1).³⁴ It was shown that INTA produced potent antinociception and was devoid of tolerance and dependence while it showed no aversive effects in the conditioned place preference assay. Similar to NAQ and NAP, INTA carries a heterocyclic ring at position 6 and a cyclopropylmethyl group at position 17, thus it was intriguing how INTA acted as an agonist while NAQ and NAP acted as antagonists. Therefore, a series of compounds were prepared as INTA complementary analogues in order to understand how introduction of an indole moiety with an α or β linkage at position 6 of the epoxymorphinan skeleton would influence ligand function.

Interestingly, we identified 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-7-carboxamido)morphinan (NAN) as a potent MOR antagonist. Per the “message-address concept”, ligands that bind opioid receptors have distinct pharmacophores responsible for efficacy (message) or selectivity (address).^{37–39} Both NAN and INTA bear an identical epoxymorphinan message portion with subtle differences in the linker and address regions of the molecule. Thus, it would be expected that they carry similar efficacies at the opioid receptors; however, the opposite was observed.

Studies have shown that allosteric ligands of the MOR show varying abilities to modulate the orthosteric ligand's affinity, efficacy, or both.^{40,41} Allosteric ligands bind to a site on the receptor, termed as allosteric binding site, that is distinct from the site that endogenous orthosteric ligands bind (orthosteric binding site).^{42–44} An allosteric ligand can act as a positive allosteric modulator (PAM) which enhances the affinity or efficacy of an orthosteric ligand, a negative allosteric modulator (NAM) which inhibits the intrinsic affinity or efficacy, or a silent allosteric modulator (SAM) which occupies the allosteric binding site and has no effect on the affinity or efficacy. In addition, bitopic (dual steric) ligands which bind to both the orthosteric and allosteric binding sites of a single receptor monomer have been shown to have higher affinity, increase or decrease intrinsic efficacy, improve off-rates and induce functional selectivity.^{42,43,53,45–52} Thus, bitopic compounds may combine the advantages of orthosteric and allosteric ligands and carry unique pharmacological properties, e.g., higher selectivity.

We herein describe our effort to understand the SAR of INTA analogues as well as to investigate the molecular mechanisms of how structurally similar MOR ligands, NAN and INTA, result in the opposite functional effects on the MOR by application of classic “message-address” concept in addition to allosteric modulation of GPCRs.

RESULTS AND DISCUSSION

Chemistry.

The INTA analogues were synthesized in a similar way as described previously.^{27,31} Briefly, stereoselective reductive amination of naltrexone with benzyl amine followed by catalytic hydrogenation under acidic conditions produced 6 α -naltrexamine (6 α -NTA)

dihydrochloride salt in a total yield of 67%, while reductive amination of naltrexone with dibenzyl amine followed by catalytic hydrogenation under acidic conditions led to 6 β -naltrexamine (6 β -NTA) dihydrochloride salt in a total yield of 59%.⁵⁴ A variety of commercially available indole carboxylic acids were coupled with 6 α -NTA and 6 β -NTA using the EDCI/HOBt coupling method. The reaction mixture was then treated with K₂CO₃ to obtain the 6- monosubstituted NTA derivatives with yields ranging from 23 to 81% which were then converted to HCl salts (Scheme 1).

Biological Results.

The binding affinity and selectivity of these naltrexamine derivatives in three opioid receptors (mouse MOR, KOR, and DOR) were determined using monocloned opioid receptor-expressed Chinese hamster ovary (CHO) cells as described previously^{27,28,31} through radio-ligand binding assay. The [³⁵S]-GTP γ S functional assay using mouse MOR was applied to determine whether the synthesized compounds acted as agonist, partial agonist, or antagonist of the MOR by determining their efficacy for G-protein activation relative to a full agonist, DAMGO. An in vivo study was further conducted in mice using the tail immersion assay to determine whether the compounds had antinociceptive effects or antagonized the antinociceptive effects of morphine.

In Vitro Radioligand Binding and [³⁵S]-GTP γ S Functional Assays.

The binding affinities of the synthesized compounds for the MOR, KOR, and DOR together with their efficacy at the mouse MOR (mMOR) were compared with those of INTA. [³H]Naloxone was used to label the mMOR, while [³H]diprenorphine was used to label both the mKOR and mDOR. The [³⁵S]-GTP γ S functional assay was used to determine the potency and efficacy of these derivatives at the mMOR, and the results were interpreted as potency (EC₅₀) and efficacy (%E_{max} relative to DAMGO). The binding, selectivity, potency, and efficacy data for the 6 β -INTA analogues are summarized in Table 1, and the results for the 6 α -INTA analogues are summarized in Table 2.

As shown in Table 1, compounds **1–9** showed subnano-molar affinity for the MOR and KOR while having a lower affinity at the DOR except for compound **3** that bound to KOR with nanomolar affinity. Particularly, INTA showed similar affinity for the MOR and KOR and was about 5-fold selective for MOR over DOR and 9-fold selective for KOR over DOR. These results corroborated the findings from Le Naour et. al, where they reported that INTA produced similar stimulation in the MOR and KOR and the least stimulation in the DOR.³⁴ INTA showed the lowest selectivity for the MOR over the DOR compared to other compounds.

Among the 6 α -INTA analogues (compounds 10–18), it was observed that all the compounds bound to the MOR with subnanomolar affinity while having relatively lower affinity at the KOR, and even lower at the DOR. The reason for the greater binding affinity of these ligands for the MOR and KOR compared to other opioid antagonist with different heterocyclic rings attached to position 6 (examples include NAP and NAQ) could be due to additional hydrogen bonding interactions between the hydrogen atom at position 1 of the indole ring and an acceptor group in the binding pocket.³¹ It has been shown that

methylation of the indole 1-nitrogen atom led to reduction in activation of singly or coexpressed opioid receptors.³⁴ Even though MOR and DOR have a relatively higher percent sequence identity (62%) than that of MOR and KOR (57%), the percent sequence identities for TM3 and TM7 where the address region is located is relatively greater for the MOR and KOR (TM3 = 100%, TM7 = 86%) than those of the MOR and DOR (TM3 = 90%, TM7 = 78%).²⁶ This could account for the overall similar binding affinities at the MOR and KOR over the DOR.

It was also noticed that the position of substitution on the indole ring (compounds **1–6** and **10–15**) did not result in a significant change in the MOR binding affinity except for compound **13**. Also, increasing the alkyl chain length of the indole analogues (compounds **7–9** and **16–18**) at position 3 did not result in significant change in their MOR binding affinity, except a slightly lower affinity of compound **16**. This suggests that the binding pocket of the address site of the MOR may be spacious enough to allow the rotation and extension of the indole group.

Not surprisingly, INTA was identified as a MOR full agonist with subnanomolar potency (Table 1) in the [³⁵S]-GTP γ S functional assay, while compound **2** and **5** also showed high MOR efficacies relative to DAMGO. In addition to INTA, compounds **2** and **4** have previously been synthesized by Le Naour et al. and the results we obtained were similar to theirs.³⁴ For other compounds, even though they carried the same substitution (indole moiety) as the side chain, most of them showed moderate to low efficacy at the MOR. Particularly, compound **3** and compound **15** (NAN) acted as MOR antagonists since both had efficacies lower than 20%. Based on our previous studies, we have observed that compounds with less than 20% relative efficacies for G-protein activation in MOR-expressing CHO cells tend to be antagonists in vivo.^{26,28,31}

Furthermore, INTA and NAN together with their respective diastereomers (compound **10** and compound **6**) were compared to understand how the stereochemistry at position 6 of the epoxymorphinan skeleton would affect binding affinity and efficacy. It was observed that the configuration of the linker at position 6 did not lead to a significant difference in their MOR binding affinity but influenced their potency and efficacy on the MOR more evidently. For instance, changing the linker of INTA from β -configuration to α -configuration (compound **10**) did not result in a significant change in MOR binding affinity (INTA $K_i = 0.29 \pm 0.04$ nM, compound **10** $K_i = 0.36 \pm 0.03$ nM) but produced a 32-fold and 2.5-fold reduction in potency and efficacy, respectively (INTA $EC_{50} = 0.21 \pm 0.01$ nM, INTA $E_{max} = 92.42 \pm 2.81\%$, compound **10** $EC_{50} = 6.75 \pm 3.20$ nM, compound **10** $E_{max} = 36.91 \pm 2.40\%$). Meanwhile, changing the configuration of the linker in NAN from α to β (compound **6**) did not result in a significant change in MOR binding affinity either (NAN $K_i = 0.23 \pm 0.02$ nM, compound **6** $K_i = 0.19 \pm 0.01$ nM), but resulted in an increase in efficacy (NAN $E_{max} = 19.11 \pm 3.31\%$, compound **6** $E_{max} = 37.94 \pm 5.85\%$). In all, it seemed that changing the linker from the α -configuration to the β -configuration at position 6 resulted in an increase in efficacy on the MOR without affecting binding affinity on the receptor significantly.

Warm-Water Immersion Assay.

To corroborate the findings of the [35 S]-GTP γ S functional assay, an in vivo assay was conducted using the warm-water immersion assay to evaluate whether the compounds synthesized produced acute antinociceptive effects (opioid agonists) or antagonized morphine's antinociceptive effect (opioid antagonists). The warm-water immersion assay was performed as described previously.²⁷ The test was conducted 20 min after injection of the compounds because morphine's effect starts to peak 20 min after s.c. administration. The results were interpreted as the percentage of maximum possible effect (% MPE). A higher % MPE indicates a stronger antinociception effect by the ligand. Figure 2A and B shows the antinociceptive effects of these INTA analogues at a single dose of 10 mg/kg. Similar to the report from Le Naour et. al, among all the ligands tested, INTA, identified as the most potent and efficacious MOR agonists in the [35 S]-GTP γ S functional assay, also acted as an opioid agonist with the highest antinociception in the warm-water immersion assay. The % MPE values of the remaining INTA analogues, except for compounds **6** and **7**, were less than 20%, which corresponded to their lower efficacy observed in the [35 S]-GTP γ S functional assay. The low % MPE values for compounds **2** and **5**, which showed high potency and efficacy in the [35 S]-GTP γ S binding assay, could be due to their poor pharmacokinetic properties such as low CNS permeability, since the tail immersion response is mainly mediated at the level of the spinal cord in the CNS.⁵⁵

Figure 2C and D shows the inhibition of morphine's antinociceptive effect at 10 mg/kg in the presence of each INTA indole analogue at a single dose of 10 mg/kg. As expected, NAN, which showed a high potency and low efficacy in the [35 S]-GTP γ S functional assay, was identified as the most potent opioid antagonist and its AD₅₀ value was determined as 2.07 (0.40–10.74) mg/kg (95% CL) from a following dose–response study (Figure 3). We also observed that compound **3** which had a low efficacy in the [35 S]-GTP γ S functional assay did not significantly inhibit morphine's antinociceptive effect. This could be due to its poor pharmacokinetic properties preventing it from getting into the CNS.

Molecular Modeling Study.

SAR studies of numerous opioids have revealed that a cyclopropylmethyl group on the tertiary amino group at position 17 typically leads to opioid antagonism as observed with naltrexone⁵⁶ as well as NAN. On the contrary, INTA, which also carries a cyclopropylmethyl group at position 17, acted as an MOR agonist. Hence, docking studies were conducted to understand how these two structurally similar compounds, INTA and NAN (Figure 4), with the identical “message” structure but slightly different “address” portions produced almost opposite functional effects. The ligands INTA and NAN were both sketched using SybylX2.1, and then docked into the agonist-bound (PDB ID: 5C1M)⁵⁷ and antagonist-bound (PDB ID: 4DKL)⁵⁸ MORs using GOLD 5.4, respectively.⁵⁹ The highest scored (CHEMPLP) docking solutions were chosen as the optimal binding poses, named INTA_MOR^{active} and NAN_MOR^{inactive} complexes.

From the docking results, it was observed that the epoxymorphinan moiety (“message” portion) of both INTA and NAN bound to the MOR orthosteric binding site in a similar fashion as reported for other known MOR ligands.^{26,60} The “message” portions formed

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ionic interactions with D147, hydrogen bonding interactions with Y148, and hydrophobic interactions with residues M151, W293, and H297. Meanwhile, the indole ring (“address” portion) of INTA seemed to be able to form hydrophobic interactions with H54 (Figure 5A), while this interaction did not exist in the NAN__{MOR}^{inactive} complex. Moreover, the “address” portions of both INTA and NAN could form π – π stacking interactions with W318 located at the allosteric binding site, but the interaction in the INTA__{MOR}^{active} complex was stronger than that in the NAN__{MOR}^{inactive} complex based on the distances between the ligand and the residue (Table 3). Another observation from the docking study of INTA was that in order to accommodate the β -configuration of the indole side chain in the protein, the cyclohexyl ring that the side chain attached to had to adopt a twisted boat conformation. In the case of NAN, the cyclohexyl ring actually was in a twisted chair conformation to retain the α -configuration of the indole side chain.

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Many studies have proved that molecular dynamics (MD) simulation is an effective method to explore the binding modes between proteins and their ligands.^{61–64} Therefore, an MD simulation study was further conducted on the INTA__{MOR}^{active} and NAN__{MOR}^{inactive} complexes using NAMD 2.8 to investigate the potential allosteric modulation mechanisms of the “address” portions of INTA and NAN to the binding of their “message” portions with the orthosteric binding site of the MOR.⁶⁵ Prior to conducting the MD simulations, the two ligand–receptor complexes were first embedded in a lipid (POPC) bilayer membrane using VMD 1.9.3.⁶⁶ Next, each complex was solvated by TIP3 water layers. After that, any water molecules and POPC lipid molecules at a distance of 0.75 Å or less from the ligand–receptor complex were removed. Finally, sodium and chloride ions were added to the solvated system to make the ion concentration approximately 0.1 nM. A representative ligand–receptor complex embedded in a POPC bilayer membrane and solvated by a water box is displayed in the Supporting Information (Figure S1A).

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Here, 10 ns MD simulations were then conducted for the INTA__{MOR}^{active} and NAN__{MOR}^{inactive} complexes. After the simulation, the root-mean-square deviation (rmsd) values of all the protein backbone atoms relative to the respective starting structures were calculated to evaluate the stability of the two systems (Figure S1B). The average rmsd values of the INTA__{MOR}^{active} and NAN__{MOR}^{inactive} complexes were determined as 1.45 and 1.31 Å, respectively.

Previous studies have shown that when a system’s rmsd value is less than 3 Å, it indicates that the system has achieved dynamic equilibrium.^{67–69} In addition, it seemed that the rmsd values of both systems remained stable after 5 ns MD simulations (Figure S1B). Therefore, it can be concluded that both systems achieved stability after 10 ns MD simulations and snapshots from the last 4 ns of MD simulations were collected to analyze the interactions between the ligand and the receptor.

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As shown in Figure 6, owing to the hydrophobic interactions with W318 and H54, the “address” portion of INTA still interacted with the same domain (K303 and W318) of the allosteric binding site in the active MOR after 10 ns MD simulations (Table 3, Figure S2). On the other hand, the “address” portion of NAN formed new hydrophobic interactions with L232 and K233 and its binding domain seemed to shift from K303 and W318 to L232 and

K233 (a residue that formed a covalent bond with β -FNA in the MOR inactive crystal structure)⁵⁸ after 10 ns MD simulations (Table 3). As a consequence, the cyclopropylmethyl group of INTA was buried deeply into the orthosteric binding site, and much closer to N150 than that of NAN (Table 3) to induce a hydrophobic interaction. This hydrophobic interaction could stabilize the conformation of the side chain of N150, which may further help secure the hydrogen bonding interaction between the side chain of N150 and the backbone carbonyl of I146 in the INTA_MOR^{active} complex. Such a postulation was based on the fact that the distances between the nitrogen atom at the side chain of N150 and the backbone carbonyl of I146 in the INTA_MOR^{active} complex was 3.2 Å, (Figure 6A) very close to the same measurement in the BU72-bound active MOR crystal structure (2.8 Å) respectively.⁵⁷

Another observation from the binding of the “address” portion of both INTA and NAN with the allosteric binding site was that the distance between the aromatic moiety of epoxymorphinan skeleton in INTA and W293 (4.1 Å) was 1.6 Å shorter than that of NAN to the same residue (5.7 Å) (Table 3, Figure 6). As previous studies reported, the hydrophobic interaction between an agonist and W293 could stabilize the conformation of the side chain of W293 in its active state.⁵⁷ Therefore, this may also provide another possible explanation on INTA acting as an agonist.

Bitopic ligands typically carry two distinct parts that interact with the orthosteric binding site and allosteric binding site and are connected by a linker. Since the indole side chain (the “address” portion of the two molecules) attached to the “message” portion of the epoxymorphinan skeleton through opposite configurations, we further looked into how different linkers would influence their function on the MOR. As INTA has a β -configuration linker while NAN has an α one, this led to opposite conformations of the cyclohexyl ring in the skeletons in order to accommodate the “address” portion of the molecules when the “message” portions of NAN and INTA bound to the orthosteric binding site of the MOR. This may further make the “message” portion and cyclopropylmethyl group of INTA reach deeply into the orthosteric binding site and form interactions with W293 and N150, respectively, to stabilize their active conformations.

CONCLUSION

In summary, a series of 6 α - and 6 β -indolylacetamidonaltrexamine derivatives were prepared to understand the SAR of INTA. NAN, as one of INTA analogues, was identified as an MOR antagonist from in vitro radioligand competition binding and functional assays. Further in vivo studies showed that NAN antagonized morphine's antinociceptive effects while INTA produced potent antinociception. Molecular modeling studies were conducted to further understand how two structurally similar compounds, NAN and INTA, showed almost opposite functional activities. The hydrophobic interactions between the “address” portion of INTA and residues W318 and H54 at the allosteric binding site may cause the “message” portion and cyclopropylmethyl group of INTA to reach deeply into the orthosteric binding site and form favorable interactions with W293 and N150 to stabilize the MOR in its active state conformation. On the other hand, the “address” portion of NAN formed hydrophobic interactions with residues L232 and K233 at another allosteric binding site, which prevented

the message portion of NAN to reach deep enough into the orthosteric binding site. In other words, the indole moiety of INTA may be acting as a PAM while that of NAN may be acting as a NAM at the MOR. Since NAN and INTA have the same “message” portion that bind to the orthosteric binding site and distinct “address” portions that may interact with the allosteric binding site, NAN and INTA may be called bitopic ligands. These results further suggested that even though two ligands may carry identical “message” portion, they could “deliver” totally opposite function if the “address” portion (even with some minor structural differences) of the compounds do not interact with the same domain of the allosteric binding site of MOR. It is believed that such an elaboration of the “message-address” concept may enhance our comprehension of structure–activity relationship of opioid ligands and provide insight for future rational molecule design.

METHODS

Chemical Synthesis.

General Methods.—Chemical reagents were purchased from either Sigma-Aldrich or Alfa Aesar. Reactions were monitored using Analtech Uniplat F254 TLC plates. Compounds were purified using silica gel columns (230–400 mesh, Merck). The IR spectra were obtained using a Thermo Scientific smart iTR instrument. Proton (400 MHz) and carbon-13 (100 MHz) nuclear magnetic resonance (NMR) spectra were acquired at ambient temperature with tetramethylsilane as the internal standard on a Bruker Ultrashield 400 Plus spectrometer. MS analysis was performed using the PerkinElmer AxION TOF mass spectrometer. HPLC analysis was done with a Varian ProStar 210 system on Microsorb-MV 100–5 C8/C18 column (250 mm × 4.6 mm) at 254 nm, eluting with acetonitrile (0.1% TFA)/water (40/60) at 1 mL/min over 30 min. All above analytical methods were used to determine purity of the newly synthesized compounds, and their purity is confirmed as 95%.

General Procedure for Amide Coupling Reaction.

The carboxylic acid derivatives (3 equiv), hydrobenzotriazole (HOBt) (3 equiv), 4 Å molecular sieves, triethylamine (9 equiv), *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI) (3 equiv), and dimethylformamide (DMF) (2 mL) were added into a three-necked flask on an ice–water bath and stirred for 15 min. Naltrexamine (1 equiv) suspended in 2 mL of DMF was added dropwise and then stirred on the water bath for 24 h. When the reaction was completed, the reaction mixture was filtered with Celite and the filtrate was concentrated to remove DMF. MeOH (7 mL) and K₂CO₃ (3 equiv) were added to the concentrate and stirred at ambient temperature. When hydrolysis of the ester at position 3 was complete, the mixture was filtered through Celite again and concentrated to remove MeOH. The mixture was then purified using column chromatography with dichloromethane/MeOH (20:1) and 1% NH₄OH to give the free base.²⁷ Upon confirmation by ¹H NMR and ¹³C NMR, the free base was then transformed into hydrochloride salt by dissolving in MeOH (0.1 mL) and dichloro-methane (2 mL), adding HCl methanol solution (1.25 M, 4 equiv) on an ice–water bath, and stirring for 5 min. Diethyl ether (10 mL) was then added. Two hours later, the precipitate was collected by filtration and dried in vacuum to give the target compound as a hydrochloride salt, which

was characterized by ^1H NMR, ^{13}C NMR, IR, MS and the purity determined using HPLC (for spectra, see the Supporting Information).

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(indole-2-carboxamido)morphinan (1).—The title compound was prepared by following the

general procedure in 32% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 11.60 (s, 1H, exchangeable), 9.33 (s, 1H, exchangeable), 8.88 (s, 1H, exchangeable), 8.73 (d, J = 7.92 Hz, 1H, exchangeable), 7.62 (d, J = 7.96 Hz, 1H), 7.42 (d, J = 8.24 Hz, 1H), 7.18 (m, 2H), 7.04 (t, J = 7.52 Hz, 1H), 6.73 (d, J = 8.08 Hz, 1H), 6.66 (d, J = 7.96 Hz, 1H), 6.24 (s, 1H, exchangeable), 4.84 (d, J = 7.72 Hz, 1H), 3.89 (s, 1H), 3.71 (m, 1H), 3.33 (m, 2H), 3.11–3.06 (m, 2H), 2.88 (m, 1H), 2.50 (m, 2H), 1.91 (q, J = 11.84 Hz, 1H), 1.80 (d, J = 13.56 Hz, 1H), 1.63 (m, 1H), 1.49–1.40 (m, 2H), 1.09 (m, 1H), 0.68 (m, 1H), 0.60 (m, 1H), 0.52 (m, 1H), 0.42 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 160.73, 142.02, 141.09, 136.23, 131.30, 129.62, 126.97, 125.26, 123.46, 121.52, 120.64, 119.83, 119.39, 117.87, 112.23, 102.63, 89.85, 69.62, 61.67, 56.69, 50.66, 46.37, 45.61, 29.37, 23.78, 22.91, 5.62, 5.07, 2.55. Mp 235–237 °C. HRMS (m/z) [M] $^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found [MH] $^+$ 486.2411. IR (ATR, cm^{-1}) ν_{max} 3212, 2167, 1975, 1617, 1551, 1506, 1315, 1239, 1125, 1034, 815, 747, 668.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(indole-3-carboxamido)morphinan (2).—The title compound was prepared by following the

general procedure in 27% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 11.64 (s, 1H, exchangeable), 9.34 (s, 1H, exchangeable), 8.89 (s, 1H, exchangeable), 8.13–8.10 (m, 3H, 1 proton exchangeable), 7.42 (d, J = 7.88 Hz, 1H), 7.16–7.06 (m, 2H), 6.73 (d, J = 8.08 Hz, 1H), 6.65 (d, J = 8.12 Hz, 1H), 6.24 (s, 1H, exchangeable), 4.82 (d, J = 7.76 Hz, 1H), 3.90 (d, J = 4.28 Hz, 1H), 3.70 (m, 1H), 3.36 (m, 1H), 3.12–3.05 (m, 3H), 2.88 (m, 2H), 1.89 (q, J = 12.88 Hz, 1H), 1.78 (d, J = 13.2 Hz, 1H), 1.63 (m, 1H), 1.48–1.41 (m, 2H), 1.09 (m, 1H), 0.68 (m, 1H), 0.50 (m, 1H), 0.52 (m, 1H), 0.42 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.46, 142.18, 141.03, 135.90, 129.70, 127.60, 125.86, 121.98, 120.89, 120.63, 120.43, 119.29, 117.89, 111.79, 110.30, 90.17, 69.69, 61.70, 56.70, 50.21, 46.38, 45.60, 29.40, 27.30, 23.99, 22.93, 5.61, 5.12, 2.53. Mp 272–274 °C. HRMS (m/z) [M] $^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found [MH] $^+$ 486.2411. IR (ATR, cm^{-1}) ν_{max} 3267, 1671, 1621, 1539, 1505, 1455, 1313, 1176, 1119.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(indole-4-carboxamido)morphinan (3).—The title compound was prepared by following the

general procedure in 31% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 11.31 (s, 1H, exchangeable), 9.34 (s, 1H, exchangeable), 8.87 (s, 1H, exchangeable), 8.39 (d, J = 8.12 Hz, 1H, exchangeable), 7.55 (d, J = 8.04 Hz, 1H), 7.48 (d, J = 7.16 Hz, 1H), 7.43 (m, 1H), 7.15 (t, J = 7.72 Hz, 1H), 6.88 (s, 1H), 6.73 (d, J = 8.12 Hz, 1H), 6.66 (d, J = 8.16 Hz, 1H), 6.19 (s, 1H, exchangeable), 4.89 (d, J = 7.76 Hz, 1H), 3.88 (d, J = 4.92 Hz, 1H), 3.77–3.72 (m, 1H), 3.32 (m, 1H), 3.13–3.04 (m, 2H), 2.87 (m, 1H), 1.93 (q, J = 12.08 Hz, 1H), 1.78 (d, J = 13.68 Hz, 1H), 1.65 (m, 1H), 1.46 (m, 2H), 1.09 (m, 2H), 0.70–0.68 (m, 1H), 0.60 (m, 1H), 0.53–0.50 (m, 1H), 0.43–0.41 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 167.77, 142.15, 141.02, 136.35, 129.67, 126.30, 126.09, 125.75, 120.64, 120.19, 119.34, 118.50, 117.90,

114.31, 101.78, 89.92, 69.68, 61.68, 56.68, 50.89, 46.37, 45.61, 29.43, 27.29, 23.66, 22.90, 5.58, 5.11, 2.50. Mp 259–261 °C. HRMS (m/z) [M]⁺ calculated for C₂₉H₃₁N₃O₄ 485.2315, found [MH]⁺ 486.2434. IR (ATR, cm⁻¹) $\nu_{\max}$ 3207, 2166, 1639, 1503, 1455, 1319, 1237, 1195, 1123, 1037, 917.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(indole-5-carboxamido)morphinan (4).—The title compound was prepared by following the general procedure in 61% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.36 (s, 1H, exchangeable), 9.32 (s, 1H, exchangeable), 8.88 (s, 1H, exchangeable), 8.48 (d, J = 8.08 Hz, 1H, exchangeable), 8.18 (s, 1H), 7.66 (dd, J_1 = 1.56 Hz, J_2 = 8.56 Hz, 1H), 7.42 (m, 2H), 6.73 (d, J = 8.12 Hz, 1H), 6.65 (d, J = 8.16 Hz, 1H), 6.53 (t, J_1 = 2.04 Hz, J_2 = 2.00 Hz, 1H), 6.20 (s, 1H, exchangeable), 4.89 (d, J = 7.80 Hz, 1H), 3.88 (d, J = 4.96 Hz, 1H), 3.71 (m, 1H), 3.35 (m, 2H), 3.11–3.04 (m, 2H), 2.87 (m, 1H), 1.90 (q, J = 12.6 Hz, 1H), 1.78 (d, J = 13.76 Hz, 1H), 1.62 (m, 1H), 1.49–1.39 (m, 2H), 1.09 (m, 1H), 0.68 (m, 1H), 0.59 (m, 1H), 0.52 (m, 1H), 0.42 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.77, 142.30, 141.30, 137.41, 129.77, 126.99, 126.60, 125.29, 120.56, 120.49, 119.91, 119.16, 117.92, 110.80, 102.05, 90.06, 69.80, 61.81, 56.74, 51.11, 46.52, 45.62, 29.44, 27.41, 23.93, 23.06, 5.72, 5.09, 2.65. Mp 290–292 °C. HRMS (m/z) [M]⁺ calculated for C₂₉H₃₁N₃O₄ 485.2315, found [MH]⁺ 486.2434. IR (ATR, cm⁻¹) $\nu_{\max}$ 3125, 2162, 1980, 1622, 1595, 1547, 1360, 1311, 1249, 1124, 1037, 919, 760, 672.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(indole-6-carboxamido)morphinan (5).—The title compound was prepared by following the general procedure in 29% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.43 (s, 1H, exchangeable), 9.33 (s, 1H, exchangeable), 8.87 (s, 1H, exchangeable), 8.54 (d, J = 7.4 Hz, 1H, exchangeable), 7.99 (s, 1H), 7.58 (s, 2H), 7.50 (s, 1H), 6.73 (d, J = 6.36 Hz, 1H), 6.66 (d, J = 7.68 Hz, 1H), 6.49 (s, 1H), 6.20 (s, 1H, exchangeable), 4.90 (d, J = 5.92 Hz, 1H), 3.88 (s, 1H), 3.71 (m, 1H), 3.17–3.06 (m, 2H), 2.89 (m, 2H), 2.5 (s, 1H), 1.90 (m, 1H), 1.78 (m, 1H), 1.62 (m, 1H), 1.49–1.39 (m, 2H), 1.09 (m, 2H), 0.68 (m, 1H), 0.60 (m, 1H), 0.52 (m, 1H), 0.42 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.85, 142.16, 140.23, 135.05, 129.76, 127.74, 127.20, 119.34, 118.39, 117.95, 116.98, 111.16, 101.20, 90.74, 69.62, 61.76, 58.35, 51.50, 49.99, 39.96, 39.76, 30.60, 29.99, 24.65, 22.25, 3.72, 3.46. Mp 254–256 °C. HRMS (m/z) [M]⁺ calculated for C₂₉H₃₁N₃O₄ 485.2315, found [MH]⁺ 486.2396. IR (ATR, cm⁻¹) $\nu_{\max}$ 3223, 2152, 1970, 1608, 1551, 1312, 1035, 920, 820, 740.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -(indole-7-carboxamido)morphinan (6).—The title compound was prepared by following the general procedure in 56% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.07 (s, 1H, exchangeable), 9.37 (s, 1H, exchangeable), 8.90 (s, 1H, exchangeable), 8.75 (d, J = 8.04 Hz, 1H, exchangeable), 7.78–7.75 (m, 2H), 7.35 (m, 1H), 7.10 (t, J = 7.60 Hz, 1H), 6.74 (d, J = 8.12 Hz, 1H), 6.67 (d, J = 8.16 Hz, 1H), 6.49 (m, 1H), 6.26 (s, 1H, exchangeable), 4.93 (d, J = 7.8 Hz, 1H), 3.91 (d, J = 4.84 Hz, 1H), 3.82–3.78 (m, 2H), 3.18–3.06 (m, 3H), 2.91–2.86 (m, 1H), 2.02–1.93 (q, J = 11.56 Hz, 1H), 1.82 (d, J = 13.64 Hz, 1H), 1.65 (m, 1H), 1.56–1.41 (m, 2H), 1.10 (t, J = 7.00 Hz, 2H), 0.71–0.68 (m, 1H), 0.62–0.60 (m, 1H), 0.55–0.52 (m, 1H), 0.44–0.42 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 164.50, 139.51,

138.46, 131.48, 127.15, 126.67, 123.95, 121.72, 118.20, 117.28, 117.02, 115.79, 115.40, 113.77, 98.68, 87.35, 67.21, 62.45, 59.20, 54.25, 48.23, 43.86, 43.16, 26.96, 24.75, 21.14, 20.39, 12.43, 3.07, 2.65. Mp 246–248 °C. HRMS (*m/z*) [M]⁺ calculated for C₂₉H₃₁N₃O₄ 485.2315, found [MH]⁺ 486.2411. IR (ATR, cm⁻¹) $\nu_{\max}$ 3090, 2162, 1979, 1633, 1540, 1327, 1248, 1040.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[2-(indol-3-yl)acetamido)morphinan (7).—The title compound was prepared by following the general procedure in 49% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.89 (s, 1H, exchangeable), 9.32 (s, 1H, exchangeable), 8.82 (s, 1H, exchangeable), 8.20 (d, *J* = 7.92 Hz, 1H, exchangeable), 7.54 (d, *J* = 7.76 Hz, 1H), 7.34 (d, *J* = 8.04 Hz, 1H), 7.18 (d, *J* = 2.08 Hz, 1H), 7.06 (t, *J* = 7.08 Hz, 1H), 6.98 (t, *J* = 7.24 Hz, 1H), 6.70 (d, *J* = 8.12 Hz, 1H), 6.62 (d, *J* = 8.16 Hz, 1H), 6.14 (s, 1H, exchangeable), 4.60 (d, *J* = 7.84 Hz, 1H), 3.82 (d, *J* = 5.2 Hz, 1H), 3.50 (q, *J* = 14.96 Hz, 2H), 3.40 (m, 1H), 3.32 (m, 1H), 3.28 (m, 1H), 3.07–3.01 (dd, *J*₁ = 5.96 Hz, *J*₂ = 19.24 Hz, 2H), 2.84 (m, 1H), 2.43–2.39 (m, 2H), 1.68 (m, 2H), 1.49–1.28 (m, 3H), 1.07 (m, 1H), 0.67–0.66 (m, 1H), 0.60–0.56 (m, 1H), 0.51–0.48 (m, 1H), 0.41–0.39 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.94, 170.86, 141.99, 140.99, 135.88, 129.55, 127.03, 123.57, 121.02, 120.58, 119.36, 118.53, 118.42, 117.83, 111.28, 108.49, 99.49, 69.56, 61.54, 56.62, 46.32, 45.49, 32.78, 29.17, 27.21, 23.45, 22.82, 5.55, 5.08, 2.46. Mp 292–294 °C. HRMS (*m/z*) [M]⁺ calculated for C₃₀H₃₃N₃O₄ 499.2471, found [MH]⁺ 500.2539. IR (ATR, cm⁻¹) $\nu_{\max}$ 3270, 1666, 1549, 1463, 1303, 1274, 1232, 1174, 1124, 1030, 898.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[3-(indol-3-yl)propanamido)morphinan (8).—The title compound was prepared by following the general procedure in 70% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.77 (s, 1H, exchangeable), 9.34 (s, 1H, exchangeable), 8.83 (s, 1H, exchangeable), 8.14 (d, *J* = 7.8 Hz, 1H, exchangeable), 7.52 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.04 Hz, 1H), 7.10 (s, 1H), 7.06 (t, *J* = 7.96 Hz, 1H), 6.97 (t, *J* = 7.88 Hz, 1H), 6.72 (d, *J* = 8.12 Hz, 1H), 6.63 (d, *J* = 8.16 Hz, 1H), 6.18 (s, 1H, exchangeable), 4.55 (d, *J* = 7.8 Hz, 1H), 3.85 (s, 1H), 3.45 (m, 1H), 3.34–3.29 (m, 2H), 3.09–3.00 (m, 2H), 2.92–2.83 (m, 3H), 2.45–2.42 (m, 4H), 1.71–1.68 (m, 2H), 1.53–1.50 (m, 1H), 1.42 (d, *J* = 9.76 Hz, 1H), 1.33 (t, *J* = 12.32 Hz, 1H), 1.08 (m, 1H), 0.68–0.63 (m, 1H), 0.60–0.57 (m, 1H), 0.52–0.48 (m, 1H), 0.42–0.38 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 141.98, 140.93, 135.97, 129.53, 126.86, 121.96, 120.98, 120.64, 119.41, 118.26, 117.89, 113.57, 111.27, 89.91, 69.58, 61.58, 56.67, 50.47, 46.31, 45.49, 39.62, 29.21, 27.21, 23.43, 22.83, 20.85, 5.55, 5.11, 2.47, 0.012. Mp 279–281 °C. HRMS (*m/z*) [M]⁺ calculated for C₃₁H₃₅N₃O₄ 513.2628, found [MH]⁺ 514.2714. IR (ATR, cm⁻¹) $\nu_{\max}$ 3187, 2160, 1640, 1541, 1505, 1450, 1325, 1271, 1234, 1124, 1032, 854.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[4-(indol-3-yl)butanamido)morphinan (9).—The title compound was prepared by following the general procedure in 39% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.79 (s, 1H, exchangeable), 9.35 (s, 1H, exchangeable), 8.84 (s, 1H, exchangeable), 8.09 (d, *J* = 7.88 Hz, 1H, exchangeable), 7.51 (d, *J* = 7.84 Hz, 1H), 7.33 (d, *J* = 8.04 Hz, 1H), 7.12 (d, *J* = 2.08 Hz, 1H), 7.08 (t, *J* = 7.46 Hz, 1H), 6.97 (t, *J* = 7.42 Hz, 1H), 6.72 (d, *J* = 8.12 Hz, 1H), 6.64 (d, *J*

= 8.16 Hz, 1H), 6.18 (s, 1H, exchangeable), 4.56 (d, J = 7.84 Hz, 1H), 3.85 (d, J = 4.84 Hz, 1H), 3.09–3.03 (dd, J_1 = 5.88 Hz, J_2 = 19.48 Hz, 2H), 2.88–2.84 (m, 1H), 2.68 (t, J = 7.48 Hz, 2H), 2.44–2.41 (m, 2H), 2.16 (t, J = 7.32 Hz, 2H), 1.89–1.85 (m, 2H), 1.75–1.70 (m, 2H), 1.56–1.53 (m, 1H), 1.43 (d, J = 9.68 Hz, 1H), 1.38–1.31 (m, 1H), 1.12–1.05 (m, 2H), 0.69–0.67 (m, 1H), 0.61–0.58 (m, 1H), 0.53–0.50 (m, 1H), 0.43–0.40 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 142.02, 140.99, 136.07, 129.56, 127.05, 122.03, 120.88, 120.60, 119.35, 118.28, 118.18, 117.87, 114.11, 111.27, 89.88, 69.59, 67.88, 61.59, 56.65, 50.44, 46.33, 45.49, 29.24, 27.22, 26.15, 24.05, 23.53, 22.85, 15.00, 5.56, 5.10, 2.48. Mp 210–212 °C. HRMS (m/z) [M] $^+$ calculated for $\text{C}_{32}\text{H}_{37}\text{N}_3\text{O}_4$ 527.2784, found [MH] $^+$ 528.2874. IR (ATR, cm^{-1}) ν_{max} 3268, 1671, 1633, 1505, 1455, 1423, 1316, 1178, 1127, 1033.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-2-

carboxamido)morphinan (10).—The title compound was prepared by following the general procedure in 81% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 11.72 (s, 1H, exchangeable), 9.22 (s, 1H, exchangeable), 8.92 (s, 1H, exchangeable), 8.10 (d, J = 7.72 Hz, 1H, exchangeable), 7.62 (d, J = 8.00 Hz, 1H), 7.45 (d, J = 8.24 Hz, 1H), 7.25 (s, 1H), 7.20 (t, J = 7.24 Hz, 1H), 7.05 (t, J = 7.44 Hz), 6.74 (d, J = 8.04 Hz, 1H), 6.58 (d, J = 8.12 Hz, 1H), 6.43 (s, 1H, exchangeable), 4.78 (d, J = 3.68 Hz, 1H), 4.65 (m, 1H), 3.98 (d, J = 6.48 Hz, 1H), 3.39–3.37 (m, 2H), 3.09–2.99 (m, 3H), 2.73 (m, 1H), 1.99 (m, 1H), 1.65 (d, J = 11.52 Hz, 1H), 1.56–1.43 (m, 2H), 1.19–1.07 (m, 3H), 0.71–0.61 (m, 2H), 0.52–0.49 (m, 1H), 0.51–0.40 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 160.45, 146.16, 138.79, 136.50, 131.46, 128.74, 126.95, 123.40, 122.16, 121.44, 119.73, 119.15, 118.55, 112.26, 103.77, 87.41, 69.41, 61.07, 59.70, 57.08, 45.57, 45.28, 30.23, 29.27, 23.57, 19.56, 5.70, 5.16, 2.60. Mp 269–271 °C. HRMS (m/z) [M] $^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found [MH] $^+$ 486.2414. IR (ATR, cm^{-1}) ν_{max} 3146, 1633, 1544.97, 1505, 1457, 1341, 1312, 1235, 1173, 1116, 989, 810, 746.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-3-
carboxamido)morphinan (11).—The title compound was prepared by following the

general procedure in 29% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 11.65 (s, 1H, exchangeable), 9.21 (s, 1H, exchangeable), 8.85 (s, 1H, exchangeable), 8.14 (d, J = 2.88 Hz, 1H, exchangeable), 8.11 (d, J = 7.56 Hz, 1H), 7.45 (t, J = 6.68 Hz, 2H), 7.18–7.09 (m, 2H), 6.71 (d, J = 8.08 Hz, 1H), 6.58 (d, J = 8.12 Hz, 1H), 6.27 (s, 1H, exchangeable), 4.80 (d, J = 3.8 Hz, 1H), 4.66–4.60 (m, 1H), 3.91 (d, J = 6.68 Hz, 1H), 3.12–3.05 (m, 2H), 2.95 (m, 1H), 2.74 (m, 1H), 1.90 (m, 1H), 1.66 (d, J = 10.64 Hz, 1H), 1.56–1.44 (m, 2H), 1.11–1.06 (m, 2H), 0.71–0.61 (m, 2H), 0.51–0.40 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.32, 145.79, 138.41, 135.91, 128.82, 128.09, 125.73, 122.18, 122.07, 120.62, 119.31, 118.06, 111.90, 110.04, 87.85, 69.23, 60.97, 56.99, 45.13, 44.82, 39.02, 30.12, 29.16, 23.36, 19.59, 5.55, 5.19, 2.41. Mp 250–252 °C. HRMS (m/z) [M] $^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found [MH] $^+$ 486.2357. IR (ATR, cm^{-1}) ν_{max} 3269, 1667, 1540, 1504, 1459, 1318, 1174, 1118.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-4-
carboxamido)morphinan (12).—The title compound was prepared by following the

general procedure in 81% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.37 (s, 1H, exchangeable), 9.18 (s, 1H, exchangeable), 8.92 (s, 1H, exchangeable), 7.65 (d, J = 8.00 Hz, 1H, exchangeable), 7.57 (d, J = 8.00 Hz, 1H), 7.46–7.44 (m, 2H), 7.16 (t, J = 7.72 Hz, 1H), 6.85 (s, 1H), 6.71 (d, J = 8.04 Hz, 1H), 6.58 (d, J = 8 Hz, 1H), 6.33 (s, 1H, exchangeable), 4.83 (d, J = 3.24 Hz, 1H), 4.66 (m, 1H), 3.94 (d, J = 6.08 Hz, 1H), 3.32 (m, 1H), 3.10 (m, 2H), 3.05 (m, 2H), 2.74 (m, 2H), 1.94 (m, 1H), 1.67 (d, J = 12.92 Hz, 1H), 1.58 (m, 1H), 1.47 (m, 1H), 1.09 (m, 2H), 0.69 (m, 1H), 0.62 (m, 1H), 0.49 (m, 1H), 0.41 (m, 1H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 167, 145.62, 137.71, 136.30, 130.81, 126.53, 126.19, 125.36, 125.20, 120.34, 118.93, 118.77, 117.22, 114.36, 101.16, 88.85, 69.20, 61.27, 58.73, 54.59, 46.52, 45.99, 42.74, 29.27, 22.31, 20.32, 9.03, 3.80, 3.34. Mp 220–222 °C. HRMS (m/z) $[\text{M}]^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found $[\text{MH}]^+$ 486.2391. IR (ATR, cm^{-1}) ν_{max} 3212, 2152, 1980, 1606, 1505, 1462, 1318, 1118, 771.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-5-carboxamido)morphinan (13).—The title compound was prepared by following the

general procedure in 85% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.39 (s, 1H, exchangeable), 9.21 (s, 1H, exchangeable), 8.89 (s, 1H, exchangeable), 8.16 (s, 1H), 7.80 (d, J = 7.76 Hz, 1H), 7.65 (d, J = 8.44 Hz, 1H), 7.44 (d, J = 8.84 Hz, 1H, exchangeable), 7.43 (s, 1H), 6.72 (d, J = 8.04 Hz, 1H), 6.58 (d, J = 8.08 Hz, 1H), 6.55 (s, 1H), 6.37 (s, 1H, exchangeable), 4.79 (d, J = 3.52 Hz, 1H), 4.66–4.61 (m, 1H), 3.95 (d, J = 6.24 Hz, 1H), 3.35 (s, 2H), 3.09–2.98 (m, 4H), 2.73 (m, 2H), 1.97–1.93 (m, 1H), 1.65 (d, J = 12.04 Hz, 1H), 1.56–1.43 (m, 2H), 1.18–1.09 (m, 2H), 0.69–0.62 (m, 2H), 0.50 (m, 1H), 0.41 (m, 1H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 167.01, 146.10, 138.81, 137.43, 128.80, 126.92, 126.64, 125.33, 122.09, 120.66, 120.08, 119.06, 118.34, 110.85, 102.03, 87.55, 69.41, 61.02, 57.02, 45.79, 45.21, 39.54, 30.23, 29.29, 23.54, 19.61, 5.71, 5.17, 2.58. Mp 240–242 °C. HRMS (m/z) $[\text{M}]^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found $[\text{MH}]^+$ 486.2421. IR (ATR, cm^{-1}) ν_{max} 3147, 1627, 1603, 1522, 1502, 1464, 1351, 1323, 1118, 1036, 771, 755, 726.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-6-carboxamido)morphinan (14).—The title compound was prepared by following the

general procedure in 75% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.43 (s, 1H, exchangeable), 9.21 (s, 1H, exchangeable), 8.88 (s, 1H, exchangeable), 7.98 (s, 1H), 7.85 (d, J = 7.72 Hz, 1H, exchangeable), 7.61 (d, J = 8.28 Hz, 1H), 7.54 (dd, J_1 = 1.32 Hz, J_2 = 8.28 Hz, 1H), 7.50 (t, J = 2.72 Hz, 1H), 6.73 (d, J = 8.04 Hz, 1H), 6.58 (d, J = 8.12 Hz, 1H), 6.49 (d, J = 1.88 Hz, 1H), 6.35 (s, 1H, exchangeable), 4.81 (d, J = 3.76 Hz, 1H), 4.63 (m, 1H), 3.94 (d, J = 6.76 Hz, 1H), 3.3 (m, 2H), 3.11–3.05 (m, 2H), 2.98 (m, 1H), 2.72 (m, 1H), 1.94 (m, 1H), 1.65 (d, J = 10.96 Hz, 1H), 1.57–1.43 (m, 2H), 1.23–1.09 (m, 3H), 0.71–0.59 (m, 2H), 0.50 (m, 1H), 0.40 (m, 1H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 167.16, 145.92, 138.53, 134.98, 129.89, 128.76, 127.88, 127.01, 122.15, 119.46, 119.24, 118.21, 118.10, 111.31, 101.26, 87.53, 69.29, 61.04, 57.03, 45.73, 45.17, 39.28, 30.17, 29.16, 23.44, 19.44, 5.60, 5.18, 2.49. Mp 241–243 °C. HRMS (m/z) $[\text{M}]^+$ calculated for $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_4$ 485.2315, found $[\text{MH}]^+$ 486.2371. IR (ATR, cm^{-1}) ν_{max} 3225, 2162, 1975, 1605, 1540, 1503, 1318, 1119, 1066, 780, 735.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-(indole-7-

carboxamido)morphinan (15).—The title compound was prepared by following the general procedure in 22% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.13 (s, 1H, exchangeable), 9.25 (s, 1H, exchangeable), 8.91 (s, 1H, exchangeable), 8.14 (d, *J* = 7.52 Hz, 1H, exchangeable), 7.74 (m, 2H), 7.36 (t, *J* = 2.72 Hz, 1H), 7.09 (t, *J* = 7.60 Hz, 1H), 6.71 (d, *J* = 8.04 Hz, 1H), 6.58 (d, *J* = 8.12 Hz, 1H), 6.50 (m, 1H), 6.39 (s, 1H, exchangeable), 4.88 (d, *J* = 3.68 Hz, 1H), 4.70 (m, 1H), 3.95 (d, *J* = 6.64 Hz, 1H), 3.12–3.04 (m, 3H), 2.94 (m, 1H), 2.73 (m, 2H), 2.00–1.92 (m, 1H), 1.68 (d, *J* = 11.20 Hz, 1H), 1.56–1.45 (m, 3H), 1.24–1.18 (m, 1H), 1.08 (m, 1H), 0.70 (m, 1H), 0.62 (m, 1H), 0.50 (m, 1H), 0.41 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.63, 145.93, 138.61, 133.95, 129.03, 128.69, 126.40, 123.86, 122.10, 120.24, 119.20, 118.17, 118.06, 116.80, 101.06, 87.30, 69.31, 69.01, 57.00, 45.61, 45.18, 30.22, 29.15, 23.45, 19.27, 5.62, 5.16, 2.53. Mp 227–229 °C. HRMS (*m/z*) [M]⁺ calculated for C₂₉H₃₁N₃O₄ 485.2315, found [MH]⁺ 486.2408. IR (ATR, cm^{−1}) ν_{max} 3060, 2166, 1634, 1585, 1504, 1456, 1312, 1280, 1123, 1030, 984.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-[2-(indol-3-yl)acetamido)morphinan (16).

—The title compound was prepared by following the general procedure in 80% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.85 (s, 1H, exchangeable), 9.20 (s, 1H, exchangeable), 8.78 (s, 1H, exchangeable), 7.80 (d, *J* = 7.96 Hz, 1H, exchangeable), 7.58 (d, *J* = 7.92 Hz, 1H), 7.33 (d, *J* = 8.08 Hz, 1H), 7.20 (d, *J* = 2.28 Hz, 1H), 7.06 (t, *J* = 7.50 Hz, 1H), 6.96 (t, *J* = 7.88 Hz, 1H), 6.71 (d, *J* = 8.08 Hz, 1H), 6.56 (d, *J* = 8.16 Hz, 1H), 6.15 (s, 1H, exchangeable), 4.60 (d, *J* = 3.88 Hz, 1H), 4.40 (m, 1H), 3.84 (d, *J* = 6.84 Hz, 1H), 3.58 (s, 2H), 3.37 (s, 2H), 3.07–3.00 (m, 2H), 2.91 (m, 1H), 2.69 (m, 1H), 1.18 (m, 1H), 1.61 (d, *J* = 10.76 Hz, 1H), 1.40 (m, 2H), 1.10 (m, 2H), 0.68 (m, 1H), 0.59 (m, 1H), 0.46 (m, 1H), 0.39 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 174.63, 185.37, 146.81, 139.41, 138.03, 131.58, 128.34, 126.14, 125.26, 122.75, 120.82, 120.16, 119.49, 118.91, 112.80, 109.25, 90.01, 71.39, 63.20, 60.22, 45.06, 37.47, 34.11, 33.81, 32.11, 29.98, 23.93, 21.85, 9.43, 5.13, 4.01. Mp 242–244. HRMS (*m/z*) [M]⁺ calculated for C₃₀H₃₃N₃O₄ 499.2471, found [MH]⁺ 500.2539. IR (ATR, cm^{−1}) ν_{max} 3218, 1640, 1506, 1317, 1234, 117, 1032, 746.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-[3-(indol-3-yl)propanamido)morphinan (17).

—The title compound was prepared by following the general procedure in 55% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.78 (s, 1H, exchangeable), 9.20 (s, 1H, exchangeable), 8.85 (s, 1H, exchangeable), 7.71 (d, *J* = 8.08 Hz, 1H exchangeable), 7.53 (d, *J* = 7.88 Hz, 1H), 7.32 (d, *J* = 8.04 Hz, 1H), 7.12 (d, *J* = 2.16 Hz, 1H), 7.05 (t, *J* = 7.08 Hz, 1H), 6.97 (t, *J* = 7.88 Hz, 1H), 6.71 (d, *J* = 8.08 Hz, 1H), 6.55 (d, *J* = 8.16 Hz, 1H), 6.28 (s, 1H, exchangeable), 4.57 (d, *J* = 3.84 Hz, 1H), 4.42 (m, 1H), 3.90 (d, *J* = 6.80 Hz, 1H), 3.25 (m, 1H), 3.17 (s, 2H), 3.07–3.01 (m, 2H), 2.93 (t, *J* = 7.72 Hz, 3H), 2.74–2.66 (m, 1H), 2.53 (m, 1H), 2.44 (dd, *J*₁ = 4.76 Hz, *J*₂ = 13.36 Hz, 1H), 1.90–1.82 (m, 1H), 1.60 (d, *J* = 10.92 Hz, 1H), 1.42–1.36 (m, 2H), 1.11–1.06 (m, 1H), 0.96–0.90 (m, 1H), 0.70–0.59 (m, 2H), 0.50–0.46 (m, 1H), 0.42–0.37 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 169.37, 169.29, 143.31, 136.02, 133.54, 126.26, 124.50, 119.45, 118.46, 116.73, 115.85, 115.57, 111.26, 108.78, 85.09, 66.74, 58.46, 54.49, 45.97, 42.61, 42.29, 33.51, 27.59, 26.55, 20.90, 18.48, 17.08, 3.12, 2.70. Mp 202–204 °C. HRMS (*m/z*) [M]⁺ calculated

for $C_{31}H_{35}N_3O_4$ 513.2628, found $[MH]^+$ 514.2691. IR (ATR, cm^{-1}) ν_{max} 3267, 3146, 2157, 1670, 1616, 1543, 1504, 1463, 1427, 1343, 1316, 1172, 1118, 1031.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -[4-(indol-3-yl)

butanamido)morphinan (18).—The title compound was prepared by following the general procedure in 34% yield. 1H NMR (400 MHz, DMSO- d_6) δ 10.79 (s, 1H, exchangeable), 9.17 (s, 1H, exchangeable), 8.86 (s, 1H, exchangeable), 7.64 (d, J = 8.04 Hz, 1H, exchangeable), 7.51 (d, J = 7.84 Hz, 1H), 7.32 (d, J = 8.04 Hz, 1H), 7.11 (d, J = 2.16 Hz, 1H), 7.05 (t, J = 7.04 Hz, 1H), 7.96 (t, J = 7.88 Hz, 1H), 6.71 (d, J = 8.08 Hz, 1H), 6.54 (d, J = 8.12 Hz, 1H), 6.29 (s, 1H, exchangeable), 4.59 (d, J = 3.88 Hz, 1H), 4.45–4.38 (m, 1H), 3.91 (d, J = 6.80 Hz, 1H), 3.32 (d, J = 19.72 Hz, 1H), 3.25 (m, 1H), 3.02 (m, 2H), 2.96 (m, 1H), 2.69 (t, J = 7.4 Hz, 3H), 2.43 (dd, J_1 = 4.92 Hz, J_2 = 13.32 Hz, 1H), 2.22 (t, J = 7.32 Hz, 2H), 1.92–1.83 (m, 1H), 1.60 (d, J = 13.16 Hz, 1H), 1.38 (m, 2H), 1.07 (m, 1H), 0.99–0.87 (m, 1H), 0.69–0.59 (m, 2H), 0.50–0.45 (m, 1H), 0.41–0.36 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 173.08, 146.69, 139.36, 137.00, 129.64, 127.98, 123.04, 122.95, 121.78, 120.15, 119.20, 119.07, 118.97, 115.05, 112.19, 88.45, 70.11, 61.85, 57.90, 45.98, 45.66, 35.98, 30.97, 29.95, 27.11, 25.11, 24.28, 20.41, 15.92, 6.46, 6.08, 3.34. Mp 191–193 °C. HRMS (m/z) $[M]^+$ calculated for $C_{32}H_{37}N_3O_4$ 527.2784, found $[MH]^+$ 528.2876. IR (ATR, cm^{-1}) ν_{max} 3267, 3124, 2162, 1671, 1623, 1506, 1456, 1373, 1119.

Biological Evaluation. Drugs.

Morphine sulfate was purchased from Mallinckrodt (St. Louis, MO) or provided by NIDA. Naloxone and naltrexone were 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. All radioligands were purchased from PerkinElmer, Boston, MA.

Animals.

Male Swiss Webster mice were used for the warm-water immersion assay. The mice weighed 23–35 g and were housed five to a cage in animal care quarters maintained at 22 °C on a 12 h light–dark cycle with food and water available ad libitum. The mice were transferred to the test room and the tests were then conducted 18 h later. Protocols and procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University Medical Center and complied with the recommendations of the International Association for the Study of Pain.

In Vitro Competitive Radioligand Binding Assay.

The competition binding assay was conducted to determine the affinity of the synthesized compounds to the MOR, KOR and DOR. $[^3H]$ NLX was used to label MOR while $[^3H]$ DPN was used to label both DOR and KOR. The K_d and B_{max} values for $[^3H]$ NLX at MOR and $[^3H]$ DPN at the KOR and DOR had been determined previously in Dr. Selley's laboratory. Both the radioligand binding assay and the $[^{35}S]$ -GTP γ S binding assay were conducted using monoclonal mice opioid receptor expressed in Chinese hamster ovary (CHO) cell lines. In the radioligand binding assay, 30 μ g of membrane protein was incubated with the corresponding radioligand in the presence of different concentrations of test compounds in

TME buffer (50 mM Tris, 3 mM MgCl₂, and 0.2 mM EGTA, pH 7.7) for 1.5 h at 30 °C. The bound radioligand was separated by filtration using the Brandel harvester. Specific (i.e., opioid receptor-related) binding at the MOR, KOR, and DOR was determined as the difference in binding obtained in the absence and presence of 5 μ M naltrexone, U50,488 and SNC80, respectively. The IC₅₀ values were determined and converted to K_i values using the Cheng–Prusoff equation.⁷⁰

In Vitro [³⁵S]-GTP γ S Functional Assay.

The [³⁵S]-GTP γ S functional assay was conducted to determine the efficacy of the compounds at the MOR.⁷¹ In this assay, 10 μ g of MOR-CHO membrane protein was incubated with 10 μ M GDP, 0.1 nM [³⁵S]-GTP γ S and varying concentrations of the compound under investigation for 1.5 h in a 30 °C water bath. GTP γ S was used instead of GTP because GTP γ S cannot be hydrolyzed by GTPase. Therefore, when GTP γ S binds to the G α subunit it remains bound and the G α subunit remains in the active form. Since GTP γ S is radiolabeled, the amount of GTP γ S that is bound to the G α subunit can be quantified to determine the relative efficacy of the compounds. The Bradford protein assay was utilized to determine and adjust the concentration of protein required for the assay. Nonspecific binding was determined with 20 μ M unlabeled GTP γ S. TME buffer (50 mM Tris-HCl, 3 mM MgCl₂, 0.2 mM EGTA, pH 7.4) with 100 mM NaCl was used to increase agonist stimulated binding and the final volume in each assay tube was 500 μ L. Furthermore, 3 μ M of DAMGO was included in the assay as maximally effective concentration of a full agonist for MOR. After the incubation, the bound radioactive ligand was separated from the free radioligand by filtration through a GF/B glass fiber filter paper and rinsed three times with ice-cold wash buffer (50 mM Tris-HCl, pH 7.2) using the Brandel harvester. The results were determined by utilizing a scintillation counter. All assays were determined in triplicate and repeated at least three times. Percent DAMGO-stimulated [³⁵S]-GTP γ S binding was defined as (net-stimulated binding by ligand/net-stimulated binding by 3 μ M DAMGO) $\times$ 100%.

Warm Water Immersion Assay.

Antinociception for the 6 α - and 6 β -naltrexamine derivatives were determined using the warm-water tail immersion assay described by Coderre and Rollman.⁷² The assay was conducted using male Swiss Webster mice. The water bath temperature was maintained at 56 $\pm$ 0.1 °C. The baseline latency (control) was determined before injecting the compounds into the mice. The average baseline latency obtained for this experiment was 3.0 $\pm$ 0.1 s and only mice with a baseline latency of 2–4 s were used. To test for agonism, the tail immersion was done 20 min (time that morphine effect starts to peak) after injecting the indole analogues of 6 α and 6 β -naltrexamine. To prevent tissue damage, a 10 s maximum cut off time was imposed. Antinociceptive response was calculated as the percentage maximum possible effect (%MPE), where %MPE = [(test – control)/(10 – control)] $\times$ 100. In the antagonism study, the 6 α and 6 β -naltrexamine derivatives were given 5 min before morphine. The tail immersion test was then conducted 20 min after giving morphine. %MPE was calculated for each mouse using at least five mice per drug. AD₅₀ values were calculated using the least-squares linear regression analysis followed by calculation of 95% confidence interval by Bliss method.

Statistical Analysis.

One-way ANOVA followed by the posthoc Dunnett test were performed to assess significance using Prism 6.0 software (GraphPad Software, San Diego, CA).

Molecular Modeling Study.

Molecular modeling studies were conducted to observe the binding modes of NAN and INTA with the MOR which helped in understanding their molecular mechanism of action. Chemical structures of the compounds were sketched in SybylX2.1, and Gasteiger–Hückel charges were assigned before energy minimization (10,000 iterations) with the Tripos Force Fields. The X-ray crystal structure of the antagonist-bound (4DKL)⁵⁸ and agonist-bound (5C1M)⁵⁷ MOR were retrieved from the Protein Data Bank (PDB) and prepared for docking by adding hydrogen atoms and deleting water molecules and bound ligands inside the binding pocket. Automated docking was done utilizing the genetic algorithm docking program GOLD 5.4. The binding site was defined to include all atoms within 10 Å of the γ -carbon atom of D147 along with a distance constraint between the 17-N of the ligand's epoxymorphinan skeleton and the carboxylate group of D147. The best CHEM-PLP scored solutions were chosen for further analyses. Pictures of the binding modes were generated using PyMOL Molecular Graphics System, version 1.7.4.5 Schrödinger, LLC. The distances in the active and inactive MOR crystal structures were measured using PyMOL Molecular Graphics System, version 1.7.4.5 Schrödinger, LLC. The best scored solutions of NAN and INTA in MOR were further analyzed to obtain a 2D image using LigPlot+. ⁷³ In the molecular dynamics (MD) study, the best CHEM-PLP-scored solutions were selected and the force field parameter and topology files for NAN and INTA were generated utilizing CGenFF. ^{74–76} Coordinates for the spatial arrangement of the receptors within the lipid bilayer were retrieved from the Orientations of Proteins in Membranes (OPM) database. The simulation system, consisting of the receptor–ligand complex embedded in a lipid (POPC) bilayer surrounded with saline solution (0.1 nM NaCl) was created in VMD 1.9.3⁶⁶ using the CHARMM force field topology file. All simulations were performed under hybrid CHARMM force field parameters that included protein, lipids and ligand with a time-step of 2 fs (fs). Periodic boundary conditions were employed, and Particle Mesh Ewald (PME) summation was used to calculate long-range electrostatic interactions. Nonbonded interactions were calculated with a smooth cutoff at 14 Å with a frequency of 2 fs. The temperature was maintained at 310 K via Langevin dynamics. All molecular modeling simulations were performed using NAMD 2.8.⁶⁵ MD simulations were carried out in four stages. In the first stage, equilibration of the fluidlike lipid bilayer was performed via minimization (1000 iterations) followed by NPT equilibration (pressure equilibration, 0.5 ns) of the lipid tails only. In the second stage, an NPT equilibration of the system was run for a period of 1 ns with harmonic constraints placed on protein, INTA, and NAN atoms (5 kcal/(mol·Å)). The harmonic restraint was released in stage 3 and the entire system was equilibrated using the NVT canonical ensemble for a further 1 ns. The final production run was conducted for 10 ns using an NVT ensemble. The snapshots from the last 4 ns MD simulations were selected for further analysis and the distances in the receptor–ligand complexes were calculated using VMD 1.9.3.⁶⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

CHO	chinese hamster ovary
DAMGO	[D-Ala2-MePhe4-Gly(ol)5]enkephalin
DOR	delta opioid receptor
DPN	diprenorphine
β-FNA	β -funaltrexamine
INTA	17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[(2'-indolyl)-acetamido]morphinan
KOR	kappa opioid receptor
MOR	mu opioid receptor
NAN	17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(indole-7-carboxamido)morphinan
NAP	17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[(4'-pyridyl)carboxamido]morphinan
NAQ	17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(isoquinoline-3-carboxamido)morphinan
norBNI	nor-binaltorphimine
NLX	naloxone
NTI	naltrindole
NTX	naltrexone
TFF	TRIPOS force field

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Figure 1.
Structural similarities between INTA and some known MOR ligands.

Figure 2.

Warm-water tail immersion assay in mice ($n = 5$) at 56 ± 0.1 °C. All tested compounds were administered subcutaneously (s.c.). Antinociceptive effects of (A) INTA analogues based on 6β -naltrexamine and (B) INTA analogues based on 6α -naltrexamine. Compounds (10 mg/kg) were injected at time 0. Twenty minutes after injection, tail withdrawal response was assessed with warm water. Antagonism of the antinociceptive effect of morphine by (C) INTA analogues based on 6β -naltrexamine and (D) INTA analogues based on 6α -naltrexamine. Tested compounds (10 mg/kg) were injected at time 0. Five minutes later, morphine (10 mg/kg) was administered. Twenty minutes after morphine injection, tail flick was tested using warm water. * $P < 0.05$ compared to vehicle; ** $P < 0.01$ compared to vehicle; *** $P < 0.001$. ns indicates $P > 0.05$. In (A) and (B), all compounds except **1** had significantly less antinociception than morphine. While in (B) and (C), only compounds **6**, **7**, **15**, and naltrexone significantly antagonized morphine's antinociception compared to distilled water treated mice.

Figure 3.

Dose dependent studies on NAN for opioid antagonist effect. NAN had an AD₅₀ value of 2.07 (0.40–10.74) mg/kg (95% CL).

Figure 4. Chemical structures of INTA (A) and NAN (B) together with their atom notation derived from the complexes obtained from docking studies. The “message” and “address” portions of the molecules are marked out in different colors.

Figure 5.

Binding poses of (A) INTA in the agonist-bound (5C1M) and (B) NAN in the antagonist-bound (4DKL) MOR crystal structures from docking results. Protein shown as cartoon model in light-green (A) and light-pink (B); INTA, NAN, and key amino acid residues shown as stick model. Carbon atoms: INTA (cyan), NAN (magenta), amino acid residues in (A) (green) and in (B) (orange); oxygen atoms (red); nitrogen atoms (blue). Two-dimensional representations of binding modes of (A) INTA and (B) NAN in 5C1M and 4DKL, respectively, are also shown.

Figure 6.

Binding modes of INTA_MOR^{active} (A) and NAN_MOR^{inactive} (B) complexes after MD simulations. Protein shown as cartoon model in light-green (INTA_MOR^{active} complex) and light-pink (NAN_MOR^{inactive} complex); INTA, NAN, and key amino acid residues shown as stick model. Carbon atoms: INTA (cyan); NAN (magenta); key amino acid residues in INTA_MOR^{active} complex (green) and in NAN_MOR^{inactive} complex (orange). Oxygen atoms (red); nitrogen atoms (blue). The red dashed lines represent the critical distances

discussed. Two-dimensional representations of binding modes of (A) INTA and (B) NAN in INTA_MOR^{active} and in NAN_MOR^{inactive} complexes, respectively, are also shown.

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Scheme 1.
Synthetic Route for INTA Analogues

Table 1.

Opioid Receptor Binding Affinity and MOR [³⁵S]-GTPγS Functional Assay Results for 6β-INTA Analogues

Compd.	R	K_i (nM)			Selectivity		MOR [³⁵ S]-GTPγS assay	
		μ	κ	δ	κ/μ	δ/μ	EC ₅₀ (nM)	% E_{max} of DAMGO
1 (INTA)		0.29 ± 0.04	0.18 ± 0.00	1.54 ± 0.47	0.6	5.3	0.21 ± 0.01	92.42 ± 2.81
2		0.19 ± 0.02	0.16 ± 0.01	7.17 ± 1.23	0.8	37.7	0.22 ± 0.07	79.48 ± 7.41
3		0.24 ± 0.03	1.94 ± 0.30	49.84 ± 12.69	8.1	207.6	8.16 ± 4.72	16.22 ± 1.40
4		0.26 ± 0.04	0.51 ± 0.04	17.67 ± 4.78	2.0	68.0	2.98 ± 1.25	22.32 ± 3.50

Compd.	R	K_i (nM)			Selectivity		MOR [35 S]-GTP γ S assay	
		μ	κ	δ	κ/μ	δ/μ	EC ₅₀ (nM)	% $E_{\max}$ of DAMGO
5		0.25 ± 0.04	0.17 ± 0.01	6.23 ± 0.83	0.7	24.9	0.19 ± 0.02	71.79 ± 4.37
6		0.19 ± 0.01	0.52 ± 0.09	30.45 ± 9.49	2.7	160.3	1.96 ± 1.01	37.94 ± 5.85
7		0.24 ± 0.03	0.95 ± 0.11	37.02 ± 1.21	4.0	154.3	1.44 ± 0.75	48.27 ± 4.45
8		0.17 ± 0.01	0.39 ± 0.05	77.10 ± 28.29	2.3	453.5	1.73 ± 1.10	45.08 ± 5.18
9		0.20 ± 0.03	0.18 ± 0.04	25.30 ± 4.34	0.9	126.5	1.04 ± 0.17	36.29 ± 4.11

Table 2.

Opioid Receptor Binding Affinity and MOR [³⁵S]-GTPγS Functional Assay Results for 6α-INTA Analogues

Compd.	R	K_i (nM)			Selectivity		MOR [³⁵ S]-GTPγS assay	
		μ	κ	δ	κ/μ	δ/μ	EC ₅₀ (nM)	% E _{max} of DAMGO
10		0.36 ± 0.03	0.93 ± 0.13	14.24 ± 2.78	2.6	39.5	6.75 ± 3.20	36.91 ± 2.40
11		0.29 ± 0.04	0.98 ± 0.13	10.54 ± 2.89	3.4	36.3	1.00 ± 0.13	33.34 ± 3.51
12		0.26 ± 0.04	1.49 ± 0.35	9.26 ± 2.83	5.7	35.6	4.99 ± 2.19	28.90 ± 0.95
13		0.76 ± 0.11	3.45 ± 0.99	26.72 ± 7.72	4.5	35.2	1.55 ± 0.08	32.24 ± 1.26
14		0.43 ± 0.05	1.63 ± 0.28	12.78 ± 3.36	3.8	29.7	6.06 ± 1.25	26.62 ± 0.66

Compd.	R	K_i (nM)			Selectivity		MOR [^{35}S]-GTP γS assay	
		μ	κ	δ	κ/μ	δ/μ	EC_{50} (nM)	% E_{max} of DAMGO
15 (NAN)		0.23 ± 0.02	1.69 ± 0.35	13.69 ± 1.39	7.3	59.5	3.85 ± 2.32	19.11 ± 3.31
16		0.84 ± 0.12	3.14 ± 0.45	9.17 ± 2.67	3.7	10.9	4.75 ± 0.31	34.14 ± 0.97
17		0.44 ± 0.04	1.36 ± 0.18	25.07 ± 6.83	3.1	57.0	2.09 ± 0.19	41.55 ± 6.14
18		0.29 ± 0.04	0.15 ± 0.03	6.73 ± 0.40	0.5	23.2	1.89 ± 1.04	54.89 ± 6.35

Table 3.

Measured Shortest Distances between Atoms on Critical Amino Acid Residues and Atoms on the Ligands from INTA-MOR^{active} and NAN-MOR^{inactive} Complexes before and after MD Simulations

complex	atom of ligand	atom of residue	distance from docking study (Å)	distance from MD (Å)
INTA_MOR ^{active}	N37@INTA	ND1@H54	3.0	4.3
	N21@INTA	OD2@D147	4.0	3.1
	O15@INTA	OH@Y148	3.4	4.0
	C37@INTA	CB@N150	5.5	4.5
	C5@INTA	CE@M151	3.8	4.7
	C4@INTA	CZ3@W293	3.8	4.1
	C6@INTA	CE1@H297	3.3	4.0
	C63@INTA	NZ@K303	3.1	4.3
	C58@INTA	CH2@W318	3.2	3.9
NAN_MOR ^{inactive}	N21@NAN	OD2@D147	3.0	3.1
	O15@NAN	OH@Y148	3.0	3.5
	C37@NAN	CG@N150	6.5	6.8
	C26@NAN	CE@M151	3.6	4.2
	C34@NAN	CD2@L232	8.6	4.8
	O54@NAN	NZ@K233	5.2	4.5
	C52@NAN	CE@K233	5.3	3.9
	C4@NAN	CZ3@W293	5.4	5.7
	C6@NAN	CE1@H297	4.5	5.4
	C63@NAN	NZ@K303	3.6	6.9
	C63@NAN	CZ2@W318	3.7	5.4