Published in final edited form as:

J Med Chem. 2012 November 26; 55(22): 10118–10129. doi:10.1021/jm301247n.

Design, Synthesis, and Biological Evaluation of 17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4'-pyridyl)carboxamido]morphinan Derivatives as Peripheral Selective Mu Opioid Receptor Agents

Yunyun Yuan[†], Orgil Elbegdorj[†], Jianyang Chen[†], Shashidhar K. Akubathini[†], Feng Zhang[†], David L. Stevens[§], Irina O. Beletskaya[§], Krista L. Scoggins[§], Zhenxian Zhang[¶], Phillip M. Gerk[¶], Dana E. Selley[§], Hamid I. Akbarali[§], William L. Dewey[§], and Yan Zhang^{†,*}

[†]Department of Medicinal Chemistry, Virginia Commonwealth University, 800 East Leigh Street, Richmond, VA 23298, USA

[§]Department of Pharmacology and Toxicology, Virginia Commonwealth University, 410, North 12th Street, Richmond, VA 23298, USA

[¶]Department of Pharmaceutics, Virginia Commonwealth University, 410 North 12th Street, Richmond, VA 23298, USA

Abstract

Peripheral selective mu opioid receptor (MOR) antagonists could alleviate the symptoms of opioid-induced constipation (OIC) without compromising the analgesic effect of opioids. However, a variety of adverse effects were associated with them, partially due to their relatively low MOR selectivity. NAP, a 6β-*N*-4'-pyridyl substituted naltrexamine derivative, was identified previously as a potent and highly selective MOR antagonist mainly acting within the peripheral nervous system. The noticeable diarrhea associated with it prompted the design and synthesis of its analogues in order to study its structure activity relationship. Among them, compound **8** showed improved pharmacological profiles compared to the original lead, acting mainly at peripheral while increasing the intestinal motility in morphine-pelleted mice (ED₅₀=0.03 mg/kg). The slight decrease of the ED₅₀ compared to the original lead was well compensated by the unobserved adverse effect. Hence, this compound seems to be a more promising lead to develop novel therapeutic agents toward OIC.

Keywords

peripheral selective MOR antagonist; GI transit; NAP; NMP

Introduction

Opioids are the mainstay for cancer and non-cancer pain management.^{1–3} However, their use is often associated with multiple side effects, such as dependence, respiratory depression, sedation, dizziness, pruritus, urinary retention and bowel dysfunction.⁴ Among them, the most common and distressing one is probably constipation. The prevalence of

*To whom correspondence should be addressed. Phone: 804-828-0021; Fax: 804-828- 7625; yzhang2@vcu.edu.

Supporting Information Available: Acid side chain synthesis and characterization, HPLC spectra and elemental analysis results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

opioid-induced constipation (OIC) varies from 9.3 to 95% among different population investigated.^{5–13} Moreover, unlike other adverse effects of opioids, tolerance to constipation rarely develops.¹⁴

Three subtypes of opioid receptors are implicated in their pharmacology, designated as the mu opioid receptor (MOR), the kappa opioid receptor (KOR), and the delta opioid receptor (DOR), respectively. The pathomechanism of OIC is mainly attributed to the activation of the peripheral MOR in the gastrointestinal (GI) tract,^{15–17} although central effects cannot be fully ruled out.^{18–20} It has been demonstrated that “excitation” of MOR delayed gastric emptying and intestinal transit, reduced water and electrolytes secretion, and increased intestinal liquid reabsorption, which subsequently led to OIC.^{21–25}

The traditional treatment of OIC employing laxatives provides less than satisfying and predictable results. A survey showed that only 46% of opioid-treated patients who required laxative therapy achieved the desired results half of the time, comparing to an 84% satisfaction rate in the control group.²⁶ Several other pharmacological interventions have also been applied to address OIC with some encouraging outcomes, for example, opioid switch (such as switching from morphine to transdermal fentanyl,^{27–30} transdermal buprenorphine,³¹ methadone,³² or a novel MOR agonist/norepinephrine reuptake inhibitor, tapentadol^{33–35}), 5-HT₄ agonists (such as Prucalopride³⁶), and type-2 chloride channel (ClC-2) activators (such as Lubiprostone³⁷). However, controversial results have also been reported for each of these agents.^{38–40}

An essential reason that the aforementioned therapies are less effective and satisfactory for OIC is that they do not directly address the underlying mechanism of OIC. As pointed out earlier, a molecule that could selectively block the peripheral MOR function would be of therapeutic interest for OIC. Naloxone (Figure 1), a relative mu and kappa-selective opioid antagonist (K_i value ratios, $\delta/\mu \approx 96$, $\delta/\kappa \approx 69$),⁴¹ has low systemic bioavailability (~2%) due to its significant hepatic first-pass,⁴² and its role in OIC has been extensively studied for over a decade. Yet reversal of desired analgesia and/or precipitation of withdrawal symptoms are frequently seen with modestly improved laxation for immediate-release naloxone.^{43–46} A fixed combination of prolonged-release (PR) naloxone and PR oxycodone (1:2) overcame these drawbacks and significantly improved bowel function.^{47–49} Given its predetermined “recipe”, this medication is not applicable to patients who have either liver disease or need other opioid analgesics and it is only approved in thirteen European countries.^{49, 50} Therefore, a systemic MOR antagonist still doesn't seem to be an ideal and universal resolution for OIC.

As for peripheral selective MOR antagonists, due to their restricted ability to cross the blood-brain barrier (BBB), they were able to relieve OIC without compromising the central analgesic effect and inducing withdrawal symptoms by themselves.⁵¹ The first drug of this class, methylnaltrexone (MNTX, Figure 1),⁵² in its subcutaneous formulation, was approved in 2008 for palliative-care patients who are suffering from OIC when laxative therapy is insufficient.⁵³ The FDA approval was based on two major clinical trials, in which 48%, and 62% patients had laxation within 4 hours after the first dose of MNTX, as compared with 15%, and 14% patients on placebo, respectively.^{54, 55} Several clinical trials investigating the efficacy of oral MNTX for OIC/opioid-induced bowel dysfunction (OIBD) were completed, but no results have been published. Although three of four reported clinical trials have shown that oral alvimopan (Figure 1), another peripheral selective MOR antagonist, is efficacious in improving spontaneous bowel movement (SBM) compared to placebo, the myocardial infarction associated with long term use restrained its application for OIC.^{56–60}

In light of the debilitating and troublesome impact of OIC on patients' quality of life and the relative low efficiency of MNTX to induce SBM (62%),^{54, 55} it has been the center of great interest to develop novel peripheral MOR antagonists. At least four new agents are under clinical development right now.⁶¹ Among them, one is a PEGylated modified naloxone that was well reviewed.⁶² The structures of the rest of the compounds have not been disclosed. But all molecules showed enhanced SBMs versus placebo without impeding central antinociception in early clinical trials.^{62, 63} Preliminary research published not long ago by Yancey-Wrona and colleagues revealed that 6 β -naltrexol (Figure 1) inhibits morphine-induced slowing of GI transit in healthy opioid-naïve volunteers by acting as a peripheral selective opioid antagonist.⁶⁴ Although it is too early to tell whether any of these new molecules will eventually replace MNTX, they do serve as "proof-of-concept" that specifically blocking MOR in the GI tract can improve symptoms of OIC.

In an effort to develop highly selective opioid antagonists, a 6 β -*N*-4'-pyridyl substituted naltrexamine derivative, NAP (Figure 1), was identified as a peripheral selective MOR antagonist based on its *in vitro/in vivo* pharmacological assays and pharmacokinetic studies.^{65–68} The ED₅₀ of NAP is 0.0088 mg/kg in the GI transit assay, which makes it an interesting compound to address the peripheral side effects of opioids. However, the incidence of diarrhea associated with high doses of NAP requires further structure-activity relationship (SAR) studies with concentration on its C(6)-pyridyl ring system, which was proposed to interact as an alternate "address" domain with MOR based on the "message-address" concept.⁶⁵ Thus a series of new ligands were rationally designed, synthesized and biologically evaluated as the second generation of molecular modeling aided drug design. One new compound with improved pharmacological profiles compared to the initial lead was identified for future optimization.

Results and Discussion

Molecular Design

Docking studies of NAP into the homology models of the three opioid receptors revealed a preferred binding mode for NAP to MOR over DOR and KOR through aromatic stacking and a putative hydrogen-bonding via the nitrogen atom on the pyridyl ring.⁶⁵ On the basis of our modeling study and the Craig Plot, the following features were taken into account when designing the new NAP analogues to facilitate its structure-selectivity relationship (SSR) study: electronic/steric/hydrophobic effects of the C(6)-pyridyl ring, the length of the spacer between the C(6)-pyridyl ring and the morphinan skeleton, and the aromatic property of the C(6) side chain (Figure 2).

Chemistry

Fifteen NAP derivatives were synthesized in a similar way as reported previously (Scheme 1).^{65, 69} Briefly, stereo-selective reductive amination of naltrexone with dibenzylamine followed by catalytic hydrogenation-deprotection under acidic condition furnished 6 β -naltrexamine (6 β -NTA)⁷⁰ dihydrochloride in a total yield of 50%. Then a variety of substituted *N*-containing heterocyclic acids, obtained either through commercial resources or prepared in house (see Supporting Information), were coupled with 6 β -NTA employing EDCI/HOBt method. After treated with K₂CO₃, the 6-monosubstituted NAP analogues were thus obtained, with yield ranging from 44 to 88%.

Biology

The synthesized NAP analogues were first evaluated in the radioligand binding assay and ³⁵S-GTP[γ S] functional assay (*in vitro*). Then selected ligands were further advanced to

in vivo behavioral (tail flick) and functional activity (charcoal gavage and intestinal motility) tests.

In Vitro Radioligand Binding and ^{35}S -GTP[γS] Functional Assays

To determine the binding affinity and selectivity of these novel NAP analogues to three subtype opioid receptors, the competitive radioligand binding assay was performed on monocloned opioid receptor-expressed Chinese Hamster Ovarian (CHO) cells as described previously.^{65, 66} [^3H]naloxone, [^3H]naltrindole (NTI), and [^3H]nor-binaltorphimine (norBNI) or [^3H]diprenorphine (DPN) were used to label MOR, DOR and KOR, respectively. The results are summarized in Table 1.

As seen in Table 1, all second generation derivatives retained sub-nanomolar to nanomolar affinity for MOR, but the selectivity of MOR over DOR and KOR varied among different substituents on the pyridyl ring, the spacer length between the pyridyl ring and the morphinan skeleton, and the side chain saturation state. Overall most of the ligands bound to the DOR with low affinity of K_i values at three-digit nanomolar concentration. This was in line with their parent compound NAP's high selectivity over the DOR. More particularly, the 3'-analogues appeared to be more potent than their 2'- counterparts for DOR binding (**1** vs. **6**, **13** vs. **14**), except for the methyl substitutions. In contrast, chloro- substitution (**1**, **6**) and introduction of a second nitrogen into the pyridyl ring (**13**, **14**) tended to have lower affinity for the DOR whereas bromo- and methoxygroups (**2**, **5**, **7**, **9**) were relatively favorable for the DOR binding. Similarly, it seemed the increased spacer length between the pyridyl ring and the morphinan skeleton (**10–12**) did not influence their low affinity to the DOR very significantly. It thus seemed that a balance between electronic property (affecting hydrogen bonding) and steric hindrance (affecting aromatic stacking) was desired to reach high MOR selectivity over DOR. Nevertheless, the majority of the new analogues displayed over 150-fold MOR selectivity over DOR. Ligands with 2'-chloro substitution on the pyridyl ring (**1**), one methylene spacer (**10**), and saturated piperidyl ring (**15**) even achieved over 1000-fold selectivity. Replacement of the aromatic system in the side chain of NAP with a non-aromatic one (**15**) caused 10-fold decrease in its binding affinity for DOR, probably due to the loss of aromatic staking interaction⁶⁵.

Compared to NAP, two derivatives, **8** and **12**, obtained comparable or slightly improved MOR selectivity over KOR, whereas the rest derivatives exhibited decreased selectivity. Nonetheless, 3'-substitution on the pyridyl ring seemed to favor the MOR selectivity over the KOR compared with 2'-substitution regardless of the electronic characteristics while a longer spacer seemed to be beneficial to the MOR selectivity over the KOR (**10–12**).

Collectively, the structure selectivity relationship study of NAP derivatives thus supported the original hypothesis that interactions of aromatic stacking and hydrogen bonding between an alternative "address" domain in the receptor binding pocket and the 6-position side chain of NAP would facilitate attaining high MOR selectivity over DOR and KOR.^{65, 69} To be noticed, compounds **6**, **8–10**, and **12** displayed improved MOR selectivity profile comparing to the marketing drugs MNTX (K_i value ratios, $\delta/\mu \approx 24$, $\kappa/\mu \approx 10$)⁷¹, and alvimopan (K_i value ratios, $\delta/\mu \approx 6$, $\kappa/\mu \approx 52$)⁷².

The ^{35}S -GTP[γS] binding assay was first conducted on the MOR-expressed CHO cells to determine the efficacy of each new ligand and define whether it acts as a full agonist, a partial agonist, or an antagonist of MOR as illustrated before.^{65, 66} The results were interpreted as EC_{50} and the relative efficacy of each molecule to the full MOR agonist [D-Ala²-MePhe⁴-Gly(ol)⁵]enkephalin (DAMGO) to stimulate G-protein (Table 1). MOR antagonists naltrexone (NTX; 0.1–100 nM) and D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP; 1–300 nM) were tested along as controls. Both control compounds produced

minimal stimulation (< 8% relative to DAMGO at 100 nM, Table 1). From Table 1, it seemed most of the NAP analogs act as MOR partial agonists under the tested condition with 1 or 2-digit nanomolar potency. Compound **14** had the lowest efficacy, followed by compounds **12** and **11**. As pointed out in the previous report, the 2'-substitution on the pyridyl ring, except for the methoxyl group, appeared to favor MOR agonism (for example, **1** vs. **6**, **4** vs. **8**),⁶⁹ whereas the electronic characteristics, the spacer length, and the side chain saturation state had rather inconsistent/irregular impact.

Given that compounds **8** and **12** showed comparable binding affinity, selectivity and efficacy to the initial lead NAP, they were further characterized by the [³⁵S]-GTP[γS] functional assay on DOR and KOR-expressed CHO cell membranes, respectively (Table 2). Both compounds exhibited partial agonism at KOR with relative low efficacy (% $E_{\max}$ of U50,488H = 35%) and low potency (EC₅₀s are double digit nanomolar versus single digit nanomolar at MOR). Meanwhile, both ligands **8** and **12** behaved as low potency DOR agonists. The fact that compounds **8** and **12** possessed higher efficacy at DOR but lower efficacy at KOR compared to those of NAP is very crucial since it was believed that KOR activation may cause sedation and dysphoric effects, whereas DOR agonism was regarded to associate with fewer side effects.^{73, 74} Encouraged by the results from these *in vitro* assay results, compounds **8** and **12** were subjected to *in vivo* study to further characterize their pharmacological properties.

Tail Flick Test

Compounds **8** and **12** were first evaluated for their acute antinociception effects in the tail flick test as previously described.⁶⁵ They also were tested for their ability to antagonize the antinociceptive effects of morphine. The percentage maximum possible effect (% MPE) for compounds **8** (10 mg/kg) and **12** (3 mg/kg) are $4.4 \pm 2.8\%$, and $11.2 \pm 3.1\%$, respectively, comparing to a 100% MPE of morphine (10 mg/kg, Figure 3). Thus, compound **8** seemed to have no apparent CNS antinociception whereas compound **12** looked like a partial agonist with relatively low efficacy. There was no statistically significant blockage of antinociceptive effect of morphine (10 mg/kg) for compound **8** at the dose as high as 10 mg/kg (Figure 3A), and no apparent antagonism effect was noticed for compound **12** even up to 30 mg/kg (Figure 3B). Collectively, both ligands appeared to have marginal effects in CNS at doses = 10 mg/kg by themselves or challenged with 10 mg/kg of morphine, which makes them more preferable as peripheral selective MOR ligands.

Charcoal Gavage and Intestinal Motility Assays

The GI transit assay was employed to examine the effects of compounds **8** and **12** on the GI function of morphine-pelleted and morphine naive mice (2 mg/kg, chronic, or 10 mg/kg, acute, respectively). Two mg/kg of morphine was found to decrease GI motility.^{75, 76} Studies were conducted as described in the literature.^{68, 77} Results are shown in Figures 4–5, correspondingly.

As seen in Figure 4A, compound **8** showed a dose-response increase of GI motility in the chronic assay in morphine-pelleted mice. Treatments with 0.1, 0.3, 1, and 10 mg/kg of **8** significantly restored the GI transit compared to the control. Similarly, 0.3, 1, and 10 mg/kg of compound **12** also statistically significantly reversed the morphine inhibition of GI motility versus saline (Figure 4B). No incidence of diarrhea happened at any tested doses of either compound. The calculated ED₅₀ for compounds **8** and **12** to reverse the inhibitory effect of morphine are 0.03 and 0.08 mg/kg, respectively. Their relatively reduced potency compared to parent lead NAP might be correlated to their higher efficacy on the DOR as indicated in the *in vitro* [³⁵S]-GTP[γS] functional assay. As reported by Smith *et al.*, the mouse ileum express both DOR and KOR.⁷⁸ Thus, compounds **8** and **12** may inhibit

acetylcholine release through their DOR agonism, which might be in facilitation to the GI transit delayed by morphine. It is thus very tempting to speculate that the intestinal motility activity of NAP and its derivatives is associated with both MOR antagonism and DOR agonism for mice. Although the DOR activation effect has a negative impact on GI transit, its presence might also somehow be advantageous as no diarrhea was observed. However, further characterization will be needed to test this hypothesis.

Due to the improved side effect profile of compounds **8** and **12** comparing to that of NAP in the chronic intestinal motility assay, i.e. no incidence of diarrhea, the acute effects of these two novel NAP derivatives on GI transit were further evaluated in morphine-naïve mice that were later challenged by 10 mg/kg morphine (Figure 5). Morphine (10 mg/kg) significantly reduced the intestinal motility compared to saline (12.4 ± 2.0 % vs. 58.4 ± 8.5 %, Figure 5A) while compounds **8** and **12** alone (10 mg/kg) had negligible effect on GI transit versus saline. This was more promising compared to the results of NAP in a similar assay where the intestinal motility decreased at a similar dose of NAP.⁶⁸ The acute effect of treatment of compound **8** at 10 mg/kg dose appeared to be a significant recovery of GI motility challenged by 10 mg/kg morphine compared to control while a positive trend was observed as the dose increased, which demonstrated that compound **8** could antagonize the negative impact of morphine on GI tract in morphine-naïve mice (ED_{50} value was 7.85 mg/kg). In contrast, compound **12** was not able to restore the GI motility as effective as compound **8** (Figure 5B), which is consistent with the observed partial agonism in the tail flick assay for compound **12**.

Preliminary pharmacokinetics studies on bidirectional transport of compounds **8** and **12** in Caco-2 Cells

To further characterize the permeability of compounds **8** and **12**, they were evaluated in Caco-2 cells for their bidirectional transport capacity (Figure 6). As reported previously,^{66, 67} the apparent permeability of NAP was significantly low in corresponding to its apparent decreased CNS activity in the *in vivo* assays. Similarly compound **8** (NMP) also showed low permeability, which is in line with its peripheral nervous system activity. On the other hand, compound **12** (NGP) showed no significant difference in its bidirectional transport capacity, which matched well with its apparent partial agonism observed in the tail flick assay.

Conclusions

The second generation MOR antagonists were designed and synthesized based on the original modeling study and the structure of the lead compound, NAP, from the first generation designed molecules. Structure selectivity study of the new series supported the hypothesis that an alternative “address” domain in the receptors distinguished ligand selectivity among three opioid receptors. Among them, compounds **8** and **12**, which showed comparable MOR selectivity comparing to that of NAP and marginal CNS effects, also restored the intestinal motility in morphine-pelleted mice, with ED_{50} of 0.03 and 0.08 mg/kg, respectively. The slight decrease of the ED_{50} might be due to the activation of the DOR in the mouse ileum. Nevertheless, the overall pharmacological profiles were enhanced as no diarrhea occurred at tested doses up to 10 mg/kg for both compounds. Due to the high MOR selectivity of compound **8** (NMP) over DOR and KOR, compared to that of MNTX and alvimopan, together with its apparent low CNS permeability, it seems to be a very promising agent of therapeutic interest in the peripheral system, such as for OIC 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, Merck). 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 (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 on 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 C8/C18 column (250 mm × 4.6 mm) at 254 nm eluting with acetonitrile (0.1% TFA)/water (50/50 or 35/65) at 1 mL/min over 30 min. Elemental analysis was conducted in Atlantic Microlab, Inc. Specific rotation was gained on the JASCO DIP-1000 Digital Polarimeter and given as the mean value of three measurements. All above analytical methods were used to determine purity of the newly synthesized compounds and their purity is confirmed so forth as 95%.

General procedure for amide coupling/hydrolysis reaction

On an ice-water bath, to a solution of acid (3 eq) 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) with N₂ protection. Fifteen minutes later, a solution of 6β-naltroxamine hydrochloride (1.0 eq) in DMF (1 mL) was added dropwise. The resulted 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. Methanol (5 mL), and K₂CO₃ (2 eq) were then added to the residue and stirred at ambient temperature overnight. The mixture was then filtered through celite again. The filtrate was concentrated to remove methanol. The residue was 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. Upon confirmation by ¹H NMR and ¹³C NMR, the free base was then transformed into hydrochloride salt by soluting in MeOH (0.1 mL) and DCM (2 mL), adding HCl methanol solution (1.25 M, 4 eq) 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 used in the HPLC, MS, specific rotation and elemental analysis.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[[4'-(2'-chloropyridyl)]carboxamido}morphinan (1)

The title compound was obtained following the general procedure as a yellow solid, in 88% yield. $[\alpha]_D^{25} -105.64^\circ$ ($c = 1.0$, MeOH). ¹H NMR (300 MHz, CDCl₃) δ 8.43 (d, $J = 5.1$ Hz, 1H), 8.08 (d, $J = 8.7$ Hz, 1H), 7.73 (m, 1H), 7.62 (dd, $J = 5.4$ Hz, 1.5 Hz, 1H), 6.61 (d, $J = 8.1$ Hz, 1H), 6.54 (d, $J = 8.1$ Hz, 1H), 4.71 (d, $J = 6.0$ Hz, 1H), 3.98 (m, 1H), 3.16 (d, $J = 5.7$ Hz, 1H), 3.03 (d, $J = 18.6$ Hz, 1H), 2.65 (m, 2H), 2.38 (d, $J = 6.6$ Hz, 2H), 2.19 (m, 2H), 2.02 (m, 1H), 1.67 (m, 2H), 1.49 (m, 2H), 0.85 (m, 1H), 0.55 (m, 2H), 0.13 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 200.8, 164.1, 154.6, 152.5, 150.6, 144.6, 139.5, 130.7, 124.9, 122.5, 120.3, 117.9, 92.3, 70.6, 62.4, 59.6, 51.5, 47.6, 44.1, 35.3, 31.9, 29.5, 23.5, 9.6, 4.3, 4.1. MS m/z found 482.6 (M + H)⁺ IR (KBr, cm⁻¹) $\nu_{\max}$ 3250.3, 1660.3, 1550.4, 1498.7, 1317.8, 1136.8. mp >250 °C. Anal. (C₂₆H₂₈ClN₃O₄·2HCl·1.5H₂O) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[[4'-(2'-bromopyridyl)]carboxamido]morphinan (2)

The title compound was obtained following the general procedure as a light yellow solid, in 62% yield. $[\alpha]_D^{25} -141.75^\circ$ ($c = 1.0$, MeOH). ^1H NMR (300 MHz, CDCl_3) δ 8.41 (d, $J = 5.1$ Hz, 1H), 8.19 (d, $J = 8.7$ Hz, 1H), 7.91 (m, 1H), 7.68 (dd, $J = 5.4$ Hz, 1.5 Hz, 1H), 6.60 (d, $J = 8.4$ Hz, 1H), 6.55 (d, $J = 8.1$ Hz, 1H), 4.80 (d, $J = 6.6$ Hz, 1H), 3.91 (m, 1H), 3.19 (d, $J = 5.4$ Hz, 1H), 3.05 (d, $J = 18.3$ Hz, 1H), 2.68 (m, 2H), 2.39 (m, 2H), 2.19 (m, 3H), 1.70 (m, 2H), 1.46 (m, 2H), 0.85 (m, 1H), 0.55 (m, 2H), 0.15 (m, 2H); ^1H NMR (300 MHz, CD_3OD) δ 8.54 (d, $J = 4.8$ Hz, 1H), 8.04 (d, $J = 1.2$ Hz, 1H), 7.81 (dd, $J = 5.4$ Hz, 1.5 Hz, 1H), 6.71 (m, 2H), 4.71 (d, $J = 7.8$ Hz, 1H), 3.90 (m, 1H), 3.43 (m, 1H), 3.24 (m, 1H), 2.85 (m, 3H), 2.62 (m, 1H), 2.40 (m, 2H), 2.02 (m, 1H), 1.77–1.53 (m, 4H), 1.02 (m, 1H), 0.67 (m, 2H), 0.32 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 204.7, 166.2, 152.1, 146.4, 143.7, 143.6, 142.4, 132.1, 127.4, 122.2, 120.6, 119.0, 92.5, 71.8, 64.0, 59.9, 53.8, 48.7, 46.4, 31.4, 31.1, 25.3, 23.9, 9.4, 5.2, 4.1. MS m/z found 526.1 ($\text{M} + \text{H}$) $^+$. IR (KBr, cm^{-1}) ν_{max} 3398.9, 1673.2, 1544.0, 1498.7, 1472.9, 1324.4, 1130.3. mp $>250^\circ\text{C}$. Anal. ($\text{C}_{26}\text{H}_{28}\text{BrN}_3\text{O}_4 \cdot 2\text{HCl} \cdot 1.5\text{H}_2\text{O}$) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[[4'-(2'-cyanopyridyl)]carboxamido]morphinan (3)

The title compound was obtained following the general procedure as a light yellow solid, in 48% yield. $[\alpha]_D^{25} -146.12^\circ$ ($c = 0.5$, MeOH). Hydrochloride salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.15 (s, 1H), 8.76 (m, 1H), 8.68 (brs, 1H), 8.25 (dd, $J = 0.64$, 1.44 Hz, 1H), 7.95 (dd, $J = 1.62$, 5.06 Hz, 1H), 6.55 (d, $J = 8.16$ Hz, 1H), 6.49 (d, $J = 8.16$ Hz, 1H), 6.02 (s, 1H), 4.62 (d, $J = 7.76$ Hz, 1H), 3.69 (d, $J = 5.16$ Hz, 1H), 3.54–3.48 (m, 1H), 3.19 (m, 2H), 2.94–2.85 (m, 2H), 2.68 (m, 1H), 2.27 (m, 1H), 2.26 (m, 1H), 1.78–1.69 (m, 1H), 1.60 (m, 1H), 1.43 (m, 1H), 1.34–1.21 (m, 2H), 0.89 (m, 1H), 0.50 (m, 1H), 0.42 (m, 1H), 0.35 (m, 1H), 0.24 (m, 1H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 162.16, 152.15, 142.42, 142.00, 141.34, 133.25, 129.51, 126.43, 125.28, 120.60, 119.41, 117.96, 117.19, 89.54, 69.63, 61.65, 56.69, 51.64, 46.46, 45.60, 29.27, 27.30, 23.45, 23.01, 5.70, 5.10, 2.62. MS m/z found 473.6 ($\text{M} + \text{H}$) $^+$. IR (Diamond, cm^{-1}) ν_{max} 3084.0, 2234.1, 1655.9, 1536.6, 1503.1, 1323.1, 1128.0, 1031.0, 919.8, 857.9, 747.8. mp 251°C dec.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[[4'-(2'-methylpyridyl)]carboxamido]morphinan (4)

The title compound was obtained following the general procedure as a light yellow solid, in 66% yield. $[\alpha]_D^{25} -202.18^\circ$ ($c = 1.0$, MeOH). ^1H NMR (400 MHz, CDCl_3) δ 8.58 (d, $J = 5.2$ Hz, 1H), 7.49 (s, 1H), 7.39 (d, $J = 8.8$ Hz, 1H), 7.31 (d, $J = 5.2$ Hz, 1H), 6.73 (d, $J = 8.0$ Hz, 1H), 6.57 (d, $J = 8.4$ Hz, 1H), 4.49 (d, $J = 6.0$ Hz, 1H), 4.12 (m, 1H), 3.13 (d, $J = 6.0$ Hz, 1H), 3.04 (d, $J = 18.4$ Hz, 1H), 2.65 (m, 2H), 2.60 (s, 3H), 2.38 (d, $J = 4.8$ Hz, 2H), 2.21 (d, $J = 7.6$ Hz, 2H), 1.81 (m, 1H), 1.67 (m, 1H), 1.58 (m, 1H), 1.53 (m, 2H), 0.86 (m, 1H), 0.54 (d, $J = 8.0$ Hz, 2H), 0.14 (d, $J = 4.8$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 164.88, 158.92, 148.87, 143.11, 141.90, 140.04, 130.53, 123.97, 121.26, 119.11, 118.27, 118.01, 91.47, 70.23, 62.13, 59.15, 51.06, 47.23, 43.87, 31.39, 29.21, 23.67, 23.41, 22.53, 9.30, 3.94, 3.66; Hydrochloride salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.34 (brs, 1H, exchangeable), 9.01 (d, $J = 7.2$ Hz, 1H, exchangeable), 8.88 (brs, 1H, exchangeable), 8.64 (d, $J = 4.8$ Hz, 1H), 7.76 (s, 1H), 7.66 (d, $J = 4.8$ Hz, 1H), 6.73 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.66 (d, $J = 8.0$ Hz, 1H, Ar-H), 6.23 (brs, 1H), 4.82 (d, $J = 8.0$ Hz, 1H), 3.89 (m, 1H), 3.69 (m, 1H), 3.36 (m, 2H), 3.06 (m, 2H), 2.85 (m, 1H), 2.57 (s, 3H), 2.45 (m, 2H), 1.90 (m, 1H), 1.78 (m, 1H), 1.59 (m, 1H), 1.44 (m, 2H), 1.07 (m, 1H), 0.67 (m, 1H), 0.59 (m, 1H), 0.50 (m, 1H), 0.42 (m, 1H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 163.92, 158.14, 148.51, 142.23, 142.04, 141.34, 129.60, 121.23, 120.60, 119.30, 118.81, 117.90, 89.62, 69.66, 61.53, 56.64, 51.37, 46.46, 45.58, 29.29, 27.28, 23.59, 23.43, 23.02, 5.73, 5.13, 2.62. MS m/z found 462.4 ($\text{M} + \text{H}$) $^+$. IR

(Diamond, cm^{-1}) ν_{max} 3181.9, 3057.7, 2936.5, 1661.1, 1609.4, 1543.5, 1505.1, 1452.0, 1346.1, 1273.8, 1240.9, 1125.0, 1032.3. mp 248 °C dec. Anal. ($\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_4 \cdot \text{HCl} \cdot 2\text{H}_2\text{O}$) C, H, N.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -{[4'-(2'-methoxypyridyl)]carboxamido}morphinan (5)

The title compound was prepared by following the general procedure in 62% yield. $[\alpha]_{\text{D}}^{25} -179.44^\circ$ ($c = 0.8$, MeOH). Hydrochloride salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.94 (d, $J = 8.04$ Hz, 1H), 8.88 (brs, 1H), 8.31 (d, $J = 5.24$ Hz, 1H), 7.40 (dd, $J = 5.2$ Hz, 1.2 Hz, 1H), 7.25 (m, 1H), 6.73 (d, $J = 8.0$ Hz, 1H), 6.65 (d, $J = 8.4$ Hz, 1H), 4.83 (d, $J = 8.0$ Hz, 1H), 3.90 (m, 4H), 3.67 (m, 1H), 3.34 (m, 2H), 3.05 (m, 2H), 2.86 (m, 1H), 2.45 (m, 2H), 1.90 (m, 1H), 1.78 (m, 1H), 1.58 (m, 1H), 1.43 (m, 2H), 1.08 (m, 1H), 0.67 (m, 1H), 0.59 (m, 1H), 0.52 (m, 1H), 0.41 (m, 1H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 164.10, 163.63, 147.53, 144.34, 141.98, 141.24, 129.49, 120.52, 119.26, 117.86, 114.55, 108.25, 89.51, 69.60, 61.59, 56.61, 53.51, 51.27, 46.39, 45.51, 29.23, 27.23, 23.47, 22.94, 5.63, 5.02, 2.54. MS m/z found 478.2 ($\text{M} + \text{H}$) $^+$. IR (Diamond, cm^{-1}) ν_{max} 3390.5, 3172.6, 3116.7, 1659.7, 1617.9, 1547.9, 1422.0, 1372.2, 1329.2, 1276.0, 1131.8, 1033.6, 919.4, 859.7, 811.6. mp 244–248 °C dec. Anal. ($\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_5 \cdot 2\text{HCl} \cdot 2.5\text{H}_2\text{O}$) C, H, N.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -{[4'-(3'-chloropyridyl)]carboxamido}morphinan (6)

The title compound was obtained following the general procedure as a light yellow solid, in 68% yield. $[\alpha]_{\text{D}}^{25} -155.77^\circ$ ($c = 1.0$, MeOH). ^1H NMR (300 MHz, CDCl_3) δ 8.61 (m, 1H), 8.51 (d, $J = 4.8$ Hz, 1H), 7.56 (d, $J = 9.3$ Hz, 1H), 7.39 (m, 1H), 6.72 (d, $J = 8.4$ Hz, 1H), 6.57 (d, $J = 8.1$ Hz, 1H), 4.51 (d, $J = 6.6$ Hz, 1H), 4.04 (m, 1H), 3.10 (d, $J = 5.7$ Hz, 1H), 3.04 (d, $J = 18.3$ Hz, 1H), 2.63 (m, 2H), 2.38 (d, $J = 6.6$ Hz, 2H), 2.18 (d, $J = 8.1$ Hz, 2H), 1.90 (m, 1H), 1.74–1.62 (m, 2H), 1.54–1.47 (m, 2H), 0.82 (m, 1H), 0.54 (m, 2H), 0.14 (m, 2H); ^1H NMR (300 MHz, CD_3OD) δ 8.62 (m, 1H), 8.54 (d, $J = 4.5$ Hz, 1H), 7.50 (m, 1H), 6.62 (m, 2H), 4.51 (d, $J = 7.8$ Hz, 1H), 3.83 (m, 1H), 3.13 (m, 2H), 2.68 (m, 2H), 2.41 (m, 2H), 2.17 (m, 2H), 1.94 (m, 1H), 1.76 (m, 1H), 1.58 (m, 2H), 1.39 (m, 1H), 0.87 (m, 1H), 0.54 (m, 2H), 0.16 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.0, 150.8, 149.1, 145.1, 143.7, 142.1, 132.5, 129.8, 125.4, 124.2, 120.3, 120.7, 92.8, 71.8, 63.7, 60.3, 53.8, 49.0, 45.5, 31.8, 31.4, 25.5, 23.7, 10.3, 4.7, 4.4. MS m/z found 482.4 ($\text{M} + \text{H}$) $^+$ IR (KBr, cm^{-1}) ν_{max} 3198.6, 1653.9, 1498.7, 1317.8, 1123.9, 1033.4. mp 230 °C dec. Anal. ($\text{C}_{26}\text{H}_{28}\text{ClN}_3\text{O}_4 \cdot 2\text{HCl} \cdot \text{H}_2\text{O}$) C, H, N.

17-Cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -{[4'-(3'-bromopyridyl)]carboxamido}morphinan (7)

The title compound was obtained following the general procedure as a light yellow solid, in 51% yield. $[\alpha]_{\text{D}}^{25} -141.16^\circ$ ($c = 1.0$, MeOH). ^1H NMR (300 MHz, CDCl_3) δ 8.71 (m, 1H), 8.53 (d, $J = 5.1$ Hz, 1H), 7.48 (d, $J = 9.0$ Hz, 1H), 7.29 (d, $J = 4.5$ Hz, 1H), 6.71 (d, $J = 8.1$ Hz, 1H), 6.56 (d, $J = 8.4$ Hz, 1H), 4.52 (d, $J = 6.6$ Hz, 1H), 4.03 (m, 1H), 3.09 (d, $J = 5.7$ Hz, 1H), 3.03 (d, $J = 18.6$ Hz, 1H), 2.65 (m, 2H), 2.36 (d, $J = 6.0$ Hz, 2H), 2.17 (d, $J = 8.1$ Hz, 2H), 1.89 (m, 1H), 1.74–1.61 (m, 2H), 1.53–1.46 (m, 2H), 0.82 (m, 1H), 0.53 (d, $J = 7.5$ Hz, 2H), 0.13 (d, $J = 4.5$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) 168.0, 153.2, 149.5, 147.5, 143.7, 142.1, 132.5, 125.4, 124.3, 120.3, 119.0, 118.7, 92.8, 71.8, 63.7, 60.3, 53.8, 49.0, 45.5, 31.8, 31.4, 25.5, 23.7, 10.3, 4.7, 4.4. MS m/z found 526.6 ($\text{M} + \text{H}$) $^+$ IR (KBr, cm^{-1}) ν_{max} 3398.9, 1653.9, 1550.4, 1505.2, 1330.7, 1123.9. mp 235 °C dec. Anal. ($\text{C}_{26}\text{H}_{28}\text{BrN}_3\text{O}_4 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[[4'-(3'-methylpyridyl)]carboxamido]morphinan (8)

The title compound was obtained following the general procedure as a white solid, in 88% yield. $[\alpha]^{25}_{\text{D}} -141.45^{\circ}$ ($c = 1.0$, MeOH). ^1H NMR (300 MHz, CDCl_3) δ 8.53 (m, 2H), 7.23 (d, $J = 5.4$ Hz, 1H), 6.87 (d, $J = 9.0$ Hz, 1H), 6.78 (d, $J = 7.8$ Hz, 1H), 6.62 (d, $J = 7.8$ Hz, 1H), 4.48 (d, $J = 5.7$ Hz, 1H), 4.12 (m, 1H), 3.14 (d, $J = 5.7$ Hz, 1H), 3.07 (d, $J = 18.9$ Hz, 1H), 2.67 (m, 2H), 2.43 (s, 3H), 2.39 (m, 2H), 2.22 (m, 2H), 1.89 (m, 1H), 1.69 (m, 2H), 1.55 (m, 2H), 0.85 (m, 1H), 0.56 (m, 2H), 0.16 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ 167.5, 151.3, 147.0, 143.6, 143.0, 140.3, 131.1, 130.8, 124.1, 121.1, 119.3, 118.3, 92.0, 70.5, 62.3, 59.4, 53.6, 51.4, 47.6, 44.1, 29.7, 24.0, 22.7, 16.7, 9.5, 4.2, 3.9. MS m/z found 462.2 ($\text{M} + \text{H}$) $^+$ IR (KBr, cm^{-1}) ν_{max} 3424.8, 1653.9, 1544.0, 1505.2, 1317.8, 1130.3. mp 211 °C dec. Anal. $\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_4 \cdot 2\text{HCl} \cdot 0.2\text{H}_2\text{O}$ C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[[4'-(3'-methoxypyridyl)]carboxamido]morphinan (9)

The title compound was obtained following the general procedure as a light yellow solid, in 60% yield. $[\alpha]^{25}_{\text{D}} -145.47^{\circ}$ ($c = 0.5$, MeOH). Hydrochloride salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.88 (brs, 1H), 8.76 (brs, 1H), 8.71 (d, $J = 8.16$ Hz, 1H), 8.54 (brs, 1H), 7.80 (m, 1H), 6.74 (d, $J = 8.00$ Hz, 1H), 6.66 (d, $J = 8.12$ Hz, 1H), 4.87 (d, $J = 7.64$ Hz, 1H), 4.08 (s, 3H), 3.88–3.70 (m, 2H), 3.36–3.32 (m, 2H), 3.12–3.05 (m, 2H), 2.87 (m, 1H), 2.47 (m, 2H), 2.02–1.89 (m, 1H), 1.74 (m, 1H), 1.62 (m, 1H), 1.46–1.35 (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, $\text{DMSO}-d_6$) δ 162.95, 155.65, 153.65, 152.38, 142.15, 141.28, 129.68, 121.76, 120.60, 119.26, 117.97, 92.59, 89.91, 69.71, 61.45, 56.92, 56.69, 51.27, 46.49, 45.68, 29.52, 27.29, 23.58, 23.00, 21.18, 5.72, 5.10, 2.63. MS m/z found 478.6 ($\text{M} + \text{H}$) $^+$ IR (Diamond, cm^{-1}) ν_{max} 3068.3, 1655.7, 1525.6, 1503.5, 1319.4, 1255.5, 1125.2, 1032.1, 1006.7, 800.7. mp 225 °C dec.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[2'-(pyridine-4"-yl)acetamido]morphinan (10)

The title compound was obtained following the general procedure as a yellow solid, in 44% yield. $[\alpha]^{25}_{\text{D}} -115.30^{\circ}$ ($c = 1.0$, MeOH). ^1H NMR (300 MHz, CDCl_3) δ 8.32 (d, $J = 5.1$ Hz, 2H), 7.45 (d, $J = 7.5$ Hz, 1H), 7.18 (d, $J = 5.1$ Hz, 2H), 6.69 (d, $J = 8.1$ Hz, 1H), 6.54 (d, $J = 7.8$ Hz, 1H), 4.59 (d, $J = 7.2$ Hz, 1H), 3.87 (m, 1H), 3.68 (d, $J = 15.0$ Hz, 1H), 3.48 (d, $J = 15.6$ Hz, 1H), 3.05 (m, 2H), 2.62 (m, 2H), 2.38 (d, $J = 6.0$ Hz, 2H), 2.17 (d, $J = 6.9$ Hz, 2H), 1.81 (m, 2H), 1.58–1.28 (m, 3H), 0.84 (m, 1H), 0.55 (d, $J = 8.1$ Hz, 2H), 0.14 (d, $J = 4.5$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.8, 150.1 ($\times 2$), 147.7, 143.7, 142.0, 132.5, 126.3 ($\times 2$), 125.3, 120.2, 118.7, 93.2, 71.8, 63.8, 60.3, 53.3, 49.0, 45.5, 43.2, 31.8, 31.4, 25.7, 23.7, 10.3, 4.7, 4.3. MS m/z found 462.3 ($\text{M} + \text{H}$) $^+$ IR (KBr, cm^{-1}) ν_{max} 3398.9, 3243.8, 3069.3, 1660.3, 1640.0, 1556.9, 1501.8, 1317.8, 1130.3. mp 210 °C dec. Anal. $(\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_4 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O})$ C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[3'-(pyridine-4"-yl)propanamido]morphinan (11)

The title compound was obtained following the general procedure as a yellow solid, in 49% yield. $[\alpha]^{25}_{\text{D}} -102.63^{\circ}$ ($c = 1.5$, MeOH). ^1H NMR (400 MHz, CDCl_3) δ 8.47 (m, 2H), 7.14 (m, 3H), 6.71 (d, $J = 8.0$ Hz, 1H), 6.53 (d, $J = 8.0$ Hz, 1H), 4.30 (d, $J = 6.4$ Hz, 1H), 3.84 (m, 1H), 3.07 (d, $J = 5.6$ Hz, 1H), 3.00 (d, $J = 18.4$ Hz, 1H), 2.94 (t, $J = 7.4$ Hz, 2H), 2.58 (m, 2H), 2.48 (t, $J = 7.6$ Hz, 2H), 2.35 (d, $J = 6.4$ Hz, 2H), 2.11 (m, 2H), 1.69 (m, 1H), 1.61–1.54 (m, 2H), 1.43 (m, 2H), 0.86 (m, 1H), 0.52 (d, $J = 8.0$ Hz, 2H), 0.12 (d, $J = 4.8$ Hz, 2H); Hydrochloride salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.36 (brs, 1H, exchangeable), 8.82 (brs, 1H, exchangeable), 8.55 (d, $J = 4.4$ Hz, 2H), 8.22 (d, $J = 7.6$ Hz, 1H, exchangeable),

7.44 (d, $J = 5.2$ Hz, 2H), 6.72 (d, $J = 8.0$ Hz, 1H), 6.63 (d, $J = 8.0$ Hz, 1H), 6.17 (brs, 1H, exchangeable), 4.51 (d, $J = 8.0$ Hz, 1H, C₅-H), 3.83 (m, 1H, C₆-H), 3.45–3.20 (m, 3H, buried in water peak), 3.10–2.97 (m, 2H), 2.91 (t, $J = 7.4$ Hz, 2H), 2.84 (m, 1H), 2.48–2.32 (m, 4H), 1.64 (m, 2H), 1.44 (m, 2H), 1.32 (m, 1H), 1.06 (m, 1H), 0.67 (m, 1H), 0.58 (m, 1H), 0.50 (m, 1H), 0.40 (m, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.44, 146.83 (× 2), 141.92, 141.03, 139.0, 129.49, 124.73 (× 2), 120.54, 119.26, 117.72, 89.72, 69.49, 61.46, 56.52, 50.44, 46.28, 45.41, 35.33, 30.30, 29.15, 27.14, 23.45, 22.81, 5.55, 5.03, 2.46. MS *m/z* found 476.4 (M + H)⁺ IR (Diamond, cm⁻¹) ν_{max} 3065.4, 1652.1, 1556.5, 1501.4, 1463.3, 1319.1, 1159.8, 1128.6. mp 205 °C dec. Anal. (C₂₈H₃₃N₃O₄·2HCl·0.5H₂O) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-{2'-[(pyridine-4"-yl)carboxamido]acetamido}morphinan (12)

The title compound was obtained following the general procedure as a white solid, in 74% yield. [α]_D²⁵ -126.34° (*c* = 1.0, MeOH). ¹H NMR (300 MHz, CDCl₃) δ 8.55 (d, $J = 4.5$ Hz, 2H), 8.41 (m, 1H, exchangeable), 7.74 (d, $J = 6.9$ Hz, 1H, exchangeable), 7.64 (d, $J = 4.8$ Hz, 2H), 6.67 (d, $J = 7.8$ Hz, 1H), 6.53 (d, $J = 8.1$ Hz, 1H), 4.57 (d, $J = 6.6$ Hz, 1H), 4.28 (m, 1H), 4.08 (d, $J = 13.2$ Hz, 1H), 3.82 (m, 1H), 3.03 (m, 2H), 2.58 (m, 2H), 2.35 (d, $J = 5.1$ Hz, 2H), 2.11 (m, 2H), 1.89 (m, 1H), 1.60 (m, 2H), 1.37 (m, 2H), 0.81 (m, 1H), 0.52 (d, $J = 7.5$ Hz, 2H), 0.11 (d, $J = 3.9$ Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 169.5, 166.0, 150.2 (× 2), 142.5, 140.7, 140.3, 131.3, 124.4, 121.5 (× 2), 119.5, 117.8, 92.5, 70.5, 62.4, 59.3, 52.1, 47.8, 44.2, 43.6, 30.9, 30.2, 24.7, 22.8, 9.6, 4.2, 4.0. MS *m/z* found 505.7 (M + H)⁺ IR (KBr, cm⁻¹) ν_{max} 3398.9, 1653.9, 1544.0, 1498.7, 1317.8, 1246.7, 1123.9. mp 215 °C dec. Anal. (C₂₈H₃₂N₄O₅·2HCl·3H₂O) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(2'-pyridazine)carboxamido]morphinan (13)

The title compound was prepared by following the general procedure in 68% yield. [α]_D²⁵ -150.27° (*c* = 0.8, MeOH). Hydrochloride salt: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.60 (dd, $J = 1.8$ Hz, 1.2 Hz, 1H), 9.47 (dd, $J = 5.2$ Hz, 1.2 Hz, 1H), 9.33 (d, $J = 8.0$ Hz, 1H, exchangeable), 8.89 (brs, 1H, exchangeable), 8.08 (dd, $J = 5.6$ Hz, 2.4 Hz, 1H), 6.73 (d, $J = 8.0$ Hz, 1H), 6.66 (d, $J = 8.4$ Hz, 1H), 6.25 (brs, 1H, exchangeable), 4.82 (d, $J = 7.6$ Hz, 1H), 3.88 (d, $J = 4.8$ Hz, 1H), 3.73 (m, 1H), 3.37 (m, 2H), 3.07 (m, 2H), 2.86 (m, 1H), 2.44 (m, 2H), 1.91 (m, 1H), 1.80 (m, 1H), 1.61 (m, 1H), 1.42 (m, 2H), 1.09 (m, 1H), 0.67 (m, 1H), 0.60 (m, 1H), 0.51 (m, 1H), 0.41 (m, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.48, 152.09, 148.58, 141.92, 141.27, 130.96, 129.43, 124.03, 120.54, 119.34, 117.88, 89.43, 69.57, 64.79, 56.61, 51.45, 46.38, 45.53, 29.21, 27.21, 23.40, 22.93, 5.63, 5.02, 2.53. MS (ESI) *m/z*: 449.54 (M + H)⁺ IR (Diamond, cm⁻¹) ν_{max}: 3172.3, 3054.0, 1659.2, 1541.3, 1503.4, 1455.8, 1124.8, 1032.3, 1012.5, 919.0, 896.2. mp 213–216 °C dec. Anal. (C₂₅H₂₈N₄O₄·2HCl) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(2'-pyrimidine)carboxamido]morphinan (14)

The title compound was prepared by following the general procedure in 62% yield. [α]_D²⁵ -190.45° (*c* = 0.5, MeOH). Hydrochloride salt: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.37 (s, 1H), 9.30 (d, $J = 8.8$ Hz, 1H), 9.09 (d, $J = 4.8$ Hz, 1H), 8.87 (brs, 1H), 8.0 (d, $J = 4.4$ Hz, 1H), 6.68 (d, $J = 8.4$ Hz, 1H), 6.66 (d, $J = 8.4$ Hz, 1H), 6.25 (brs, 1H), 5.0 (d, $J = 7.6$ Hz, 1H), 3.87 (d, $J = 4.8$ Hz, 1H), 3.70 (m, 1H), 3.32 (m, 2H), 3.06 (m, 2H), 2.86 (m, 1H), 2.44 (m, 2H), 2.02 (m, 1H), 1.76 (d, $J = 14.0$ Hz, 1H), 1.53 (m, 1H), 1.42 (m, 2H), 1.09 (m, 1H), 0.67 (m, 1H), 0.6 (m, 1H), 0.51 (m, 1H), 0.41 (m, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.32, 159.67, 157.86, 156.36, 142.09, 141.32, 129.68, 120.59, 119.26, 118.60, 117.88, 89.59, 69.67, 64.88, 56.63, 51.15, 46.45, 45.59, 29.49, 27.27, 23.50, 23.01, 5.74, 5.13, 2.63. MS (ESI) *m/z*: 449.50 (M + H)⁺ IR (Diamond, cm⁻¹) ν_{max} 3071.0, 1667.7, 1514.3, 1455.3,

1322.3, 1236.1, 1127.1, 1033.9, 986.3, 857.9, 664.0. mp 214–217 °C. Anal. (C₂₅H₂₈N₄O₄•2HCl•H₂O) C, H, N

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-(1'-methylpiperidine-4'-carboxamido)morphinan (15)

The title compound was obtained following the general procedure as a light yellow solid, in 57% yield. $[\alpha]_D^{25} -88.06^\circ$ ($c = 1.0$, MeOH). ¹H NMR (300 MHz, CDCl₃) δ 7.02 (d, $J = 8.4$ Hz, 1H), 6.65 (d, $J = 8.1$ Hz, 1H), 6.51 (d, $J = 8.1$ Hz, 1H), 4.47 (d, $J = 6.3$ Hz, 1H), 3.86 (m, 1H), 3.11 (d, $J = 5.7$ Hz, 1H), 3.06 (d, $J = 18.3$ Hz, 1H), 2.96–2.89 (m, 2H), 2.64–2.56 (m, 2H), 2.43–2.35 (m, 4H), 2.27–2.26 (m, 4H), 2.19–2.08 (m, 4H), 2.00 (m, 2H), 1.80 (m, 1H), 1.58 (d, $J = 10.5$ Hz, 2H), 1.47 (d, $J = 8.7$ Hz, 2H), 0.83 (m, 1H), 0.53 (d, $J = 7.2$ Hz, 2H), 0.12 (d, $J = 4.5$ Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 175.0, 143.7, 140.5, 130.8, 123.8, 119.0, 118.6, 91.8, 77.4, 70.3, 62.3, 59.4, 55.1, 50.6, 47.4, 46.1, 44.1, 42.6, 31.8, 29.3, 28.5, 28.4, 23.6, 22.7, 9.5, 4.2, 3.9. MS m/z found 468.6 (M + H)⁺ IR (KBr, cm⁻¹) $\nu_{\max}$ 3437.7, 1647.4, 1544.0, 1460.0, 1311.3, 1123.9. mp >250 °C. Anal. (C₂₇H₃₇N₃O₄•2HCl•2.5H₂O) C, H, N.

Biological Evaluation. Drugs

Morphine sulfate was purchased from Mallinckrodt, St. Louis, MO. Naloxone was purchased from Sigma-Aldrich (St. Louis, MO). All drugs and test compounds were dissolved in pyrogen-free isotonic saline (Baxter Healthcare, Deerfield, IL).

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 and Functional Assay

The radioligand binding assay and ³⁵S-GTP[γS]-binding assay were conducted using monocloned opioid receptor expressed in Chinese hamster ovarian (CHO) cell lines as described previously.^{65, 66, 79–81} [³H]naloxone, [³H]NTI, and [³H]norBNI (or [³H]DPN) were used to label the μ, δ, and κ opioid receptors, respectively. Aliquots of a membrane protein (30 μg) were incubated with the corresponding radioligand in the presence of different concentrations of the drug under investigation at 30 °C for 1 h. Specific (i.e., opioid receptor related) binding was determined as the difference in binding obtained in the absence and presence of 10 μM naltrexone. The potency of the drugs in displacing the specific binding of the radioligand was determined from data 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. Functional assays were conducted in the same cell membranes used for the receptor binding assays. Membrane proteins (10 μg) were incubated with varying concentrations of drugs, GDP (μ: 10 μM; κ and δ: 20 μM) and 0.1 nM ³⁵S-GTP[γS] in assay buffer for 2 h (μ) or 1.5 h (κ and δ) at 30 °C. Nonspecific binding was determined with 10 μM unlabeled GTP[γS]. DAMGO (3 μM), U50,488H (5 μM), and SNC80 (5 μM) were included in the assay for a maximally effective concentration of a full agonist for the μ, κ, and δ opioid receptor, respectively.

In Vivo Assays. Tail Flick Test

The warm-water tail flick test was performed according to Coderre and Rollman⁸² using a water bath with the temperature maintained at 56 ± 0.1 °C. 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 Pierson⁸³ as the percentage of maximum possible effect (% MPE), which was calculated as: $\% \text{ MPE} = [(\text{test latency} - \text{control latency}) / (10 - \text{control latency})] \times 100$. Percent MPE was calculated for each mouse using at least six mice per drug.

Intestinal Motility Assay

The GI transit assay was conducted as reported in the literature.^{68, 77} Briefly, each group of four or six mice received a subcutaneous (s.c.) injection of testing compound at different concentrations or saline at time zero. Five minutes later, morphine (2, or 10 mg/kg) was given subcutaneously. After twenty minutes, a forced meal of charcoal suspension was given via gavage. Thirty minutes following the meal, mice were euthanized and the small intestine was dissected. The distance traveled by the charcoal in the intestine was then measured and expressed as a percentage of the total length of the intestine, from pylorus to cecum.

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).

Pharmacokinetics. Bidirectional Transport of NAP, NMP, and NGP in Caco-2 Cells

Caco-2 (passages 45–47; ATCC, Manassas, VA) cell culture and bidirectional permeability studies with polyester Transwell filters were performed as described previously.⁶⁷ Briefly, cells were cultured in Dulbecco's modified Eagle's medium (9.6 g/L glucose) with 10% fetal bovine serum and supplemented with 100 unit/mL penicillin, 100 µg/mL streptomycin, and 5% nonessential amino acids for 22 days after seeding on 12 mm 0.4 µm #3460 Transwell-Clear inserts (Fisher Scientific) at a density of 80,000 cells/cm². Drug solutions in Hank's balanced salt solution (buffered with 10 mM HEPES) were added to either the apical or basolateral chambers, with sampling from the receiver chambers up to 2 h. Acetonitrile (50 µL) was then added to the samples and centrifuged. A portion of the supernatants was analyzed by HPLC-UV using an Alltima HP C18 column (3 µm, 4.6×100 mm; Alltech, Deerfield, IL) at 270 nm for NAP, and a Microsorb-MV 100-3 C18 (4.6×100 mm; Varian) column for NMP or NGP at 266 nm. Analytes concentration was quantified from standard curves prepared in transport buffer:acetonitrile (4:1). Calibration curves for NAP, NMP, and NGP were all linear in the range of 0.01 to 100 µM ($R^2 = 0.999$). Apparent permeability was then calculated using the following equation: $P_{app} = J / (A \times C_i)$, where J is the transport rate, A is the surface area of the cell monolayer, and C_i is the initial concentration of the dosing solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful for Drs. Lee-Yuan Liu-Chen (Temple University) and Ping-Yee Law (University of Minnesota) for the generous gift of opioid receptor expressed CHO cell lines. The work was funded by PHS grants from NIH/

NIDA, DA024022. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health.

a Abbreviations

BBB	blood-brain barrier
CHO	Chinese hamster ovarian
CTAP	DPhe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH ₂
DAMGO	[D-Ala ² -MePhe ⁴ -Gly(ol) ⁵]enkephalin
DOR	delta opioid receptor
GI	gastrointestinal
KOR	kappa opioid receptor
MNTX	methylnaltrexone
MOR	mu opioid receptor
MPE	maximum possible effect
6β-NTA	6β-naltrexamine
NTX	naltrexone
norBNI	nor-binaltorphimine
NTI	naltrindole
NAP	17-cyclopropylmethyl-3, 14β-dihydroxy-4,5α-epoxy-6β-[(4' - pyridyl)carboxamido]morphinan
OIBD	opioid-induced bowel dysfunction
OIC	opioid-induced constipation
PR	prolonged-release
SAR	structure-activity relationship
SBM	spontaneous bowel movement
SSR	structure-selectivity relationship.

References

1. Manchikanti L, Singh A. Therapeutic opioids: A ten-year perspective on the complexities and complications of the escalating use, abuse, and nonmedical use of opioids. *Pain Physician*. 2008; 11:S63–S88. [PubMed: 18443641]
2. Svendsen KB, Andersen S, Arnason S, Arnér S, Breivik H, Heiskanen T, Kalso E, Kongsgaard UE, Sjogren P, Strang P, Bach FW, Jensen TS. Breakthrough pain in malignant and non-malignant diseases: a review of prevalence, characteristics and mechanisms. *Eur. J. Pain*. 2005; 9:195–206. [PubMed: 15737812]
3. Cohen MZ, Easley MK, Ellis C, Hughes B, Ownby K, Rashad BG, Rude M, Taft E, Westbrooks JB. JCAHO. Cancer pain management and the JCAHO's pain standards: an institutional challenge. *J. Pain Symptom Manage*. 2003; 25:519–527. [PubMed: 12782432]
4. McNicol E, Horowicz-Mehler N, Fisk RA, Bennett K, Gialeli-Goudas M, Chew PW, Lau J, Carr D. Management of opioid side effects in cancer-related and chronic noncancer pain: a systematic review. *J. Pain*. 2003; 4:231–256. [PubMed: 14622694]

5. Candrilli SD, Davis KL, Iyer S. Impact of constipation on opioid use patterns, health care resource utilization, and costs in cancer patients on opioid therapy. *J. Pain Palliat. Care Pharmacother.* 2009; 23:231–241. [PubMed: 19670020]
6. Twycross, RG.; Lack, SA. Symptom control in far advanced cancer: pain relief. London: Pitman; 1983.
7. Moore RA, McQuay HJ. Prevalence of opioid adverse events in chronic nonmalignant pain: systematic review of randomised trials of oral opioids. *Arthritis Res. Ther.* 2005; 7:R1046–R1051. [PubMed: 16207320]
8. Allan L, Hays H, Jensen N-H, de Waroux BLP, Bolt M, Donald R, Kalso E. Randomised crossover trial of transdermal fentanyl and sustained release oral morphine for treating chronic non-cancer pain. *BMJ.* 2001; 322:1154–1158. [PubMed: 11348910]
9. Kalso E, Edwards JE, Moore RA, McQuay HJ. Opioids in chronic non-cancer pain: systematic review of efficacy and safety. *Pain.* 2004; 112:372–380. [PubMed: 15561393]
10. Vainio A, Auvinen A. Prevalence of symptoms among patients with advanced cancer: an international collaborative study. *J. Pain Symptom Manage.* 1996; 12:3–10. [PubMed: 8718910]
11. Cook SF, Lanza L, Zhou X, Sweeney CT, Goss D, Hollis K, Mangel AW, Fehnel SE. Gastrointestinal side effects in chronic opioid users: results from a population-based survey. *Aliment Pharmacol. Ther.* 2008; 27:1224–1232. [PubMed: 18363893]
12. Bell TJ, Panchal SJ, Miaskowski C, Bolge SC, Milanova T, Williamson R. The prevalence, severity, and impact of opioid-induced bowel dysfunction: results of a US and European Patient Survey (PROBE 1). *Pain Med.* 2009; 10:35–42. [PubMed: 18721170]
13. Sykes NP. The relationship between opioid use and laxative use in terminally ill cancer patients. *Palliat. Med.* 1998; 12:375–382. [PubMed: 9924600]
14. Ling GSF, Paul D, Simantov R, Pasternak GW. Differential development of acute tolerance to analgesia, respiratory depression, gastrointestinal transit and hormone release in a morphine infusion model. *Life Sci.* 1989; 45:1627–1636. [PubMed: 2555641]
15. Manara L, Bianchi G, Ferretti P, Tavani A. Inhibition of gastrointestinal transit by morphine in rats results primarily from direct drug action on gut opioid sites. *J. Pharmacol. Exp. Ther.* 1986; 237:945–949. [PubMed: 3012075]
16. Tavani A, Bianchi G, Ferretti P, Manara L. Morphine is most effective on gastrointestinal propulsion in rats by intraperitoneal route: evidence for local action. *Life Sci.* 1980; 27:2211–2217. [PubMed: 7207014]
17. Shook JE, Pelton JT, Hrubby VJ, Burks TF. Peptide opioid antagonist separates peripheral and central opioid antitransit effects. *J. Pharmacol. Exp. Ther.* 1987; 243:492–500. [PubMed: 2824748]
18. Kolso RJ, Vaught JL, Cowan A, Gmerek DE, Porreca F. Intrathecal morphine slows gastrointestinal transit in rat. *Eur. J. Pharmacol.* 1985; 119:243–246. [PubMed: 3841514]
19. Galligan JJ, Burks TF. Centrally mediated inhibition of small intestinal transit and motility by morphine in the rat. *J. Pharmacol. Exp. Ther.* 1983; 226:356–361. [PubMed: 6875849]
20. Stewart JJ, Weisbrodt NW, Burks TF. Centrally mediated intestinal stimulation by morphine. *J. Pharmacol. Exp. Ther.* 1977; 202:174–181. [PubMed: 874814]
21. De Luca A, Coupar IM. Insights into opioid action in the intestinal tract. *Pharmacol. Ther.* 1996; 69:103–115. [PubMed: 8984506]
22. Holzer P. Treatment of opioid-induced gut dysfunction. *Expert Opin. Investig. Drugs.* 2007; 16:181–194.
23. Fiocchi R, Bianchi G, Petrillo P, Tavani A, Manara L. Morphine inhibits gastrointestinal transit in the rat primarily by impairing propulsive activity of the small intestine. *Life Sci.* 1982; 31:2221–2223. [PubMed: 7162339]
24. McKay JS, Linaker BD, Higgs NB, Turnberg LA. Studies of the antisecretory activity of morphine in rabbit ileum in vitro. *Gastroenterol.* 1982; 82:243–247.
25. Warhust G, Smith G, Tonge A, Turnberg L. Effects of morphine on net water absorption, mucosal adenylate cyclase activity and PGE2 metabolism in rat intestine. *Eur. J. Pharmacol.* 1983; 86:77–82.

26. Pappagallo M. Incidence, prevalence, and management of opioid bowel dysfunction. *Am. J. Surg.* 2001; 182:11S–18S. [PubMed: 11755892]
27. Hunt R, Fazekas B, Thorne D, Brooksbank M. A comparison of subcutaneous morphine and fentanyl in hospice cancer patients. *J. Pain Symptom Manage.* 1999; 18:111–119. [PubMed: 10484858]
28. Haazen L, Noorduyn H, Megens A, Meert T. The constipation-inducing potential of morphine and transdermal fentanyl. *Eur. J. Pain.* 1999; 3:9–15.
29. Ahmedzai S, Brooks D. Transdermal fentanyl versus sustained-release oral morphine in cancer pain: preference, efficacy and quality of life. *J. Pain Symptom Manage.* 1997; 13:254–261. [PubMed: 9185430]
30. Donner B, Zenz M, Tryba M, Strumpf M. Direct conversion from oral morphine to transdermal fentanyl: a multicenter study in patients with cancer pain. *Pain.* 1996; 64:527–534. [PubMed: 8783318]
31. Bach V, Kamp-Jensen M, Jensen N-H, Eriksen J. Buprenorphine and sustained release oral morphine—effect and side-effects in chronic use. *Pain Clinic.* 1991; 4:87–93.
32. Mercadante S, Casuccio A, Fulfaro F, Groff L, Boffi R, Villari P, Gebbia V, Ripamonti C. Switching from morphine to methadone to improve analgesia and tolerability in cancer patients: a prospective study. *J. Clin. Oncol.* 2001; 19:2898–2904. [PubMed: 11387363]
33. Tzschentke TM, Christoph T, Kögel B, Schiene K, Hennies HH, Englberger W, Haurand M, Jahnel U, Cremers TI, Friderichs E, De Vry J. (-)-(1R,2R)-3-(3-Dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride (tapentadol HCl): a novel mu-opioid receptor agonist/norepinephrine reuptake inhibitor with broad-spectrum analgesic properties. *J. Pharmacol. Exp. Ther.* 2007; 323:265–276. [PubMed: 17656655]
34. Vorsanger G, Xiang J, Biondi D, Upmalis D, Delfgaauw J, Allard R, Moskovitz B. Post hoc analyses of data from a 90-day clinical trial evaluating the tolerability and efficacy of tapentadol immediate release and oxycodone immediate release for the relief of moderate to severe pain in elderly and nonelderly patients. *Pain Res. Manag.* 2011; 16:245–251. [PubMed: 22059194]
35. Etropolski M, Kelly K, Okamoto A, Rauschkolb C. Comparable efficacy and superior gastrointestinal tolerability (nausea, vomiting, constipation) of tapentadol compared with oxycodone hydrochloride. *Adv. Ther.* 2011; 28:401–417. [PubMed: 21494892]
36. Sloots CE, Rykx A, Cools M, Kerstens R, De Pauw M. Efficacy and safety of prucalopride in patients with chronic noncancer pain suffering from opioid-induced constipation. *Dig. Dis. Sci.* 2010; 55:2912–2921. [PubMed: 20428949]
37. Sucampo Pharmaceuticals, Inc.. website. Available at: <http://investor.sucampo.com/phoenix.zhtml?c=201197&p=irolnewsArticle&ID=1655607&highlight=lubiprostone>.
38. Wirz S, Wittmann M, Schenk M, Schroeck A, Schaefer N, Mueller M, Standop J, Kloecker N, Nadstawek J. Gastrointestinal symptoms under opioid therapy: a prospective comparison of oral sustained-release hydromorphone, transdermal fentanyl, and transdermal buprenorphine. *Eur. J. Pain.* 2009; 13:737–743. [PubMed: 18977159]
39. Jeong ID, Camilleri M, Shin A, Iturrino J, Boldingh A, Busciglio I, Burton D, Ryks M, Rhoten D, Zinsmeister AR. A randomised, placebo-controlled trial comparing the effects of tapentadol and oxycodone on gastrointestinal and colonic transit in healthy humans. *Aliment. Pharmacol. Ther.* [Online early access]. DOI: 10.1111/j.1365-2036.2012.05040.x. Published Online: February 21, 2012.
40. Wong BS, Camilleri M. Lubiprostone for the treatment of opioid-induced bowel dysfunction. *Expert Opin. Pharmacother.* 2011; 12:983–990. [PubMed: 21385112]
41. Peng X, Knapp BI, Bidlack JM, Neumeyer JL. Pharmacological properties of bivalent ligands containing butorphan linked to nalbuphine, naltrexone, and naloxone at μ and κ opioid receptors. *J. Med. Chem.* 2007; 50:2254–2258. [PubMed: 17407276]
42. Handal KA, Schauben JL, Salamone FR. Naloxone. *Ann. Emerg. Med.* 1983; 12:438–445. [PubMed: 6309038]
43. Liu M, Wittbrodt E. Low-dose oral naloxone reverses opioid-induced constipation and analgesia. *J. Pain Symptom Manage.* 2002; 23:48–53. [PubMed: 11779668]

44. Meissner W, Schmidt U, Hartmann M, Kath R, Reinhart K. Oral naloxone reverses opioid-induced constipation. *Pain*. 2000; 84:105–109. [PubMed: 10601678]
45. Sykes NP. An investigation of the ability of oral naloxone to correct opioid-related constipation in patients with advanced cancer. *Palliat. Med.* 1996; 10:135–144. [PubMed: 8800821]
46. Culpepper-Morgan JA, Inturrisi CE, Portenoy RK, Foley K, Houde RW, Marsh F, Kreek MJ. Treatment of opioid-induced constipation with oral naloxone: a pilot study. *Clin. Pharmacol. Ther.* 1992; 52:90–95. [PubMed: 1623695]
47. Ahmedzai SH, Nauck F, Bar-Sela G, Bosse B, Leyendecker P, Hopp M. A randomized, double-blind, active-controlled, double-dummy, parallel-group study to determine the safety and efficacy of oxycodone/naloxone prolonged-release tablets in patients with moderate/severe, chronic cancer pain. *Palliat. Med.* 2012; 26:50–60. [PubMed: 21937568]
48. Löwenstein O, Leyendecker P, Lux EA, Blagden M, Simpson KH, Hopp M, Bosse B, Reimer K. Efficacy and safety of combined prolonged-release oxycodone and naloxone in the management of moderate/severe chronic nonmalignant pain: results of a prospectively designed pooled analysis of two randomised, double-blind clinical trials. *BMC Clin. Pharmacol.* 2010; 10:12. [PubMed: 20920236]
49. Holzer P. Opioid antagonists for prevention and treatment of opioid-induced gastrointestinal effects. *Curr. Opin. Anaesthesiol.* 2010; 23:616–622. [PubMed: 20543677]
50. Mueller-Lissner S. Fixed combination of oxycodone with naloxone: a new way to prevent and treat opioid-induced constipation. *Adv. Ther.* 2010; 27:581–590. [PubMed: 20714946]
51. Yuan CS. Clinical status of methylnaltrexone, a new agent to prevent and manage opioid-induced side effects. *J. Support. Oncol.* 2004; 2:111–122. [PubMed: 15328815]
52. Relistor [package insert]. Philadelphia, PA, and Tarrytown, NY: Wyeth Pharmaceuticals Inc and Progenics Pharmaceuticals; 2009.
53. FDA. Drug approval package: Relistor (methylnaltrexone bromide) subcutaneous injection. 2008 Apr 24. Available at: <http://tinyurl.com/2uby2uf>.
54. Thomas J, Karver S, Cooney GA, Chamberlain BH, Watt CK, Slatkin NE, Stambler N, Kremer AB, Israel RJ. Methylnaltrexone for opioid-induced constipation in advanced illness. *N. Engl. J. Med.* 2008; 358:2332–2343. [PubMed: 18509120]
55. Slatkin N, Thomas J, Lipman AG, Wilson G, Boatwright ML, Wellman C, Zhukovsky DS, Stephenson R, Portenoy R, Stambler N, Israel R. Methylnaltrexone for opioid-induced constipation in advanced illness patients. *J. Support. Oncol.* 2009; 7:39–46. [PubMed: 19278178]
56. Jansen JP, Lorch D, Langan J, Lasko B, Hermanns K, Kleoudis CS, Snidow JW, Pierce A, Wurzelmann J, Mortensen ER. A randomized, placebo-controlled phase 3 trial (Study SB-767905/012) of alvimopan for opioid-induced bowel dysfunction in patients with non-cancer pain. *J. Pain.* 2011; 12:185–193. [PubMed: 21292169]
57. Irving G, Pénez J, Ramjattan B, Cousins M, Rauck R, Spierings EL, Kleoudis CS, Snidow JW, Pierce A, Wurzelmann J, Mortensen ER. A randomized, placebo-controlled phase 3 trial (Study SB-767905/013) of alvimopan for opioid-induced bowel dysfunction in patients with non-cancer pain. *J. Pain.* 2011; 12:175–184. [PubMed: 21292168]
58. Webster L, Jansen JP, Peppin J, Lasko B, Irving G, Morlion B, Snidow J, Pierce A, Mortensen E, Kleoudis C, Carter E. Alvimopan, a peripherally acting mu-opioid receptor (PAM-OR) antagonist for the treatment of opioid-induced bowel dysfunction: results from a randomized, double-blind, placebo-controlled, dose-finding study in subjects taking opioids for chronic non-cancer pain. *Pain.* 2008; 137:428–440. [PubMed: 18164818]
59. Paulson DM, Kennedy DT, Donovan RA, Carpenter RL, Cherubini M, Techner L, Du W, Ma Y, Schmidt WK, Wallin B, Jackson D. Alvimopan: an oral, peripherally acting, mu-opioid receptor antagonist for the treatment of opioid-induced bowel dysfunction—a 21-day treatment-randomized clinical trial. *J. Pain.* 2005; 6:184–192. [PubMed: 15772912]
60. Bream-Rouwenhorst HR, Cantrell MA. Alvimopan for postoperative ileus. *Am. J. Health-Syst. Pharm.* 2009; 66:1267–1277. [PubMed: 19574601]
61. [Accessed on April 14, 2012] <http://clinicaltrials.gov>. Key words: OIC
62. Diego L, Atayee R, Helmons P, Hsiao G, von Gunten CF. Novel opioid antagonists for opioid-induced bowel dysfunction. *Expert Opin. Investig. Drugs.* 2011; 20:1047–1056.

63. AISK 37, Alkermes website. Available at: <http://investor.alkermes.com/phoenix.zhtml?c=92211&p=irolnewsArticle&ID=1561623&highlight=>
64. Yancey-Wrona J, Dallaire B, Bilsky E, Bath B, Burkart J, Webster L, Magiera D, Yang X, Phelps M, Sadee W. 6 β -Naltrexol, a peripherally selective opioid antagonist that inhibits morphine-induced slowing of gastrointestinal transit: an exploratory study. *Pain Med.* 2011; 12:1727–1737. [PubMed: 22123184]
65. Li G, Aschenbach LC, Chen J-Y, Cassidy MP, Stevens DL, Gabra BH, Selley DE, Dewey WL, Westkaemper RB, Zhang Y. Design, synthesis, and biological evaluation of 6 α - and 6 β -N-heterocyclic substituted naltrexamine derivatives as μ -opioid receptor selective antagonists. *J. Med. Chem.* 2009; 52:1416–1427. [PubMed: 19199782]
66. Yuan Y, Li G, He H-J, Stevens DL, Kozak P, Scoggins KL, Mitra P, Gerk PM, Selley DE, Dewey WL, Zhang Y. Characterization of 6 α - and 6 β -N-heterocyclic substituted naltrexamine derivatives as novel leads to development of mu opioid receptor selective antagonists. *ACS Chem. Neurosci.* 2011; 2:346–351. [PubMed: 22816021]
67. Mitra P, Venitz J, Yuan Y, Zhang Y, Gerk PM. Preclinical disposition (in vitro) of novel μ -opioid receptor selective antagonists. *Drug Metab. Dispos.* 2011; 39:1589–1596. [PubMed: 21685245]
68. Yuan Y, Stevens DL, Braithwaite A, Scoggins KL, Bilsky E, Akbarali HI, Dewey WL, Zhang Y. 6 β -N-Heterocyclic substituted naltrexamine derivative as potential lead to develop peripheral mu opioid receptor selective antagonists. *Bioorg. Med. Chem. Lett.* 2012; 22:4731–4734. [PubMed: 22683223]
69. Yuan Y, Elbegdorj O, Chen J, Akubathini SK, Beletskaya IO, Selley DE, Zhang Y. Structure selectivity relationship studies of 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[(4'-pyridyl)carboxamido]morphinan derivatives toward the development of the mu opioid receptor antagonists. *Bioorg. Med. Chem. Lett.* 2011; 21:5625–5629. [PubMed: 21788135]
70. Sayre LM, Portoghese PS. Stereospecific synthesis of the 6 α - and 6 β -amino derivatives of naltrexone and oxymorphone. *J. Org. Chem.* 1980; 45:3366–3368.
71. Magnan J, Paterson SJ, Tavani A, Kosterlitz HW. The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1982; 319:197–205. [PubMed: 6125900]
72. Zimmerman DM, Gidda JS, Cantrell BE, Schoepp DD, Johnson BG, Leander JD. Discovery of a potent, peripherally selective trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine opioid antagonist for the treatment of gastrointestinal motility disorders. *J. Med. Chem.* 1994; 37:2262–2265. [PubMed: 8057274]
73. Spealman RD, Bergman J. Modulation of the discriminative stimulus effects of cocaine by mu and kappa opioids. *J. Pharmacol. Exp. Ther.* 1992; 261:607–615. [PubMed: 1315861]
74. Rapaka RS, Porreca F. Development of delta opioid peptides as nonaddicting analgesics. *Pharm. Res.* 1991; 8:1–8. [PubMed: 1849640]
75. Esmaeili-Mahani S, Javan M, Motamedi F, Ahmadiani A. Post-adrenalectomy changes in the gene expression of specific G-protein subunits involved in morphine sensitization. *Neuropeptides.* 2008; 42:169–175. [PubMed: 18237777]
76. Fukuda H, Suenaga K, Tsuchida D, Mantyh CR, Pappas TN, Hicks GA, Dehaven-Hudkins DL, Takahashi T. The selective mu opioid receptor antagonist, alvimopan, improves delayed GI transit of postoperative ileus in rats. *Brain Res.* 2006; 1102:63–70. [PubMed: 16797494]
77. Ross GR, Gabra BH, Dewey WL, Akbarali HI. Morphine Tolerance in the Mouse Ileum and Colon. *J. Pharmacol. Exp. Ther.* 2008; 327:561–572. [PubMed: 18682567]
78. Smith CF, Waldron C, Brook NA. Opioid receptors in the mouse ileum. *Arch. Int. Pharmacodyn. Ther.* 1988; 291:122–131. [PubMed: 2835021]
79. Bonner G, Meng F, Akil H. Selectivity of mu-opioid receptor determined by interfacial residues near the third extracellular loop. *Eur. J. Pharmacol.* 2000; 403:37–44. [PubMed: 10969141]
80. Zhu J, Xue J-C, Law P-Y, Claude PA, Luo L-Y, Yin J, Chen C, Liu-Chen L-Y. The region in the mu opioid receptor conferring selectivity for sufentanil over the delta receptor is different from that over the kappa receptor. *FEBS Lett.* 1996; 384:198–202. [PubMed: 8612823]
81. Thompson CM, Wojno H, Greiner E, May EL, Rice KC, Selley DE. Activation of G-proteins by morphine and codeine congeners: insights to the relevance of O- and N-demethylated metabolites

- at mu- and delta-opioid receptors. *J. Pharmacol. Exp. Ther.* 2004; 308:547–554. [PubMed: 14600248]
82. Coderre TJ, Rollman GB. Naloxone hyperalgesia and stress-induced analgesia in rats. *Life Sci.* 1983; 32:2139–2146. [PubMed: 6843288]
83. Harris LS, Pierson AK. Some narcotic antagonists in the benzomorphan series. *J. Pharmacol. Exp. Ther.* 1964; 143:141–148. [PubMed: 14163985]

Figure 1.
Mechanism-based pharmacological interventions of OIC.

R = Me, OMe, CN, Cl etc;
X = CH₂, C₂H₄, CH₂NHCO;
Y/Y' = N;
Z = N or NMe;

Figure 2.
 Schematic representative of NAP derivatives—the second generation.

(A)

(B)

Figure 3.
NMP (8, A), NGP (12, B) tail flick assay in morphine naive mice challenged with 10 mg/kg morphine (n = 4, *** P < 0.0005).

(A)

(B)

Figure 4.
NMP (8, A), NGP (12, B) chronic charcoal gavage and intestinal motility assay in morphine-pelleted mice challenged with 2 mg/kg morphine ($n = 6$, $** P < 0.005$, $*** P < 0.0005$).

(A)

(B)

Figure 5. NMP (8, A), NGP (12, B) acute charcoal gavage and intestinal motility assay in morphine naive mice challenged with 10 mg/kg morphine ($n = 4$, * $P < 0.05$, ** $P < 0.005$).

Figure 6.
Bidirectional transport of NAP, NMP (8), and NGP (12) in Caco-2 cells.

Scheme 1.
NAP Analogues Synthetic Route.

Table 1
Binding Affinity and MOR ^{35}S -GTP[γS] Binding Assay Results for NAP Derivatives^a

Compd	R	K_i (nM)			Selectivity		MOR ^{35}S -GTP[γS] Binding	
		μ	δ	κ	δ/μ	κ/μ	EC_{50} (nM)	$\%E_{\text{max}}$ of DAMGO
NTX ^b	N/A	0.26 $\pm$ 0.02	117.1 $\pm$ 8.9	5.15 $\pm$ 0.26	450	20	ND	7.75 $\pm$ 0.20 ^c
CTAP ^b	N/A	2.02 $\pm$ 0.71	1441 $\pm$ 106	1013 $\pm$ 175	713	501	ND	1.99 $\pm$ 0.92 ^c
NAP ^b		0.37 $\pm$ 0.07	277.5 $\pm$ 8.0	60.7 $\pm$ 5.6	747	163	1.14 $\pm$ 0.38	22.72 $\pm$ 0.84
1		0.10 $\pm$ 0.04	602.2 $\pm$ 22.3	0.15 $\pm$ 0.04	6111	1.5	0.83 $\pm$ 0.03	58.20 $\pm$ 1.47
2		0.63 $\pm$ 0.18	173.7 $\pm$ 134.9	0.18 $\pm$ 0.03	276	0.3	1.19 $\pm$ 0.57	50.80 $\pm$ 2.85
3		1.25 $\pm$ 0.55	75.3 $\pm$ 7.0	0.13 $\pm$ 0.01 ^d	60	0.1	10.00 $\pm$ 0.80	43.13 $\pm$ 1.82
4		0.39 $\pm$ 0.20	90.1 $\pm$ 17.1	0.58 $\pm$ 0.12	232	1.5	1.13 $\pm$ 0.14	43.32 $\pm$ 2.80
5		0.60 $\pm$ 0.23	160.4 $\pm$ 6.6	0.46 $\pm$ 0.04 ^d	267	0.8	7.89 $\pm$ 0.61	25.32 $\pm$ 4.36
6		0.67 $\pm$ 0.28	502.4 $\pm$ 70.1	19.9 $\pm$ 7.7	746	30	2.32 $\pm$ 1.76	24.86 $\pm$ 1.83
7		0.45 $\pm$ 0.13	128.0 $\pm$ 61.2	2.83 $\pm$ 0.60	285	6	1.31 $\pm$ 0.59	23.23 $\pm$ 0.35

Compd	R	K_i (nM)			Selectivity		MOR ^{35}S -GTP[γS] Binding		
		μ	δ	κ	δ/μ	κ/μ	EC_{50} (nM)	$\%E_{\text{max}}$ of DAMGO	
8 (NMP)		0.58 ± 0.25	273.6 ± 1.8	96.7 ± 12.2	470	166	1.52 ± 0.26	30.63 ± 0.55	
9		0.43 ± 0.02	103.9 ± 3.9	4.28 ± 0.46^d	242	10.0	— ^e	— ^e	
10		0.85 ± 0.16	865.6 ± 135.2	9.01 ± 0.61	1017	10.6	2.32 ± 0.64	32.78 ± 1.80	
11		0.57 ± 0.41	294.0 ± 75.4	1.65 ± 0.19	516	2.9	1.46 ± 0.11	22.62 ± 1.24	
12 (NGP)		0.73 ± 0.59	526.1 ± 78.3	203.2 ± 67.0	719	278	2.84 ± 0.53	22.62 ± 0.66	
13		2.07 ± 0.41	419.9 ± 50.0	1.57 ± 0.18^d	203	0.7	28.3 ± 4.3	27.64 ± 4.54	
14		2.13 ± 0.31	339.6 ± 27.4	1.21 ± 0.10^d	159	0.6	7.49 ± 0.79	17.78 ± 2.71	
15		0.87 ± 0.38	2586 ± 1704	6.49 ± 2.36	2968	7.5	5.08 ± 0.43	37.24 ± 0.97	

^aThe values are the means $\pm$ S.E.M. of three independent experiments. [^3H]naloxone, [^3H]NTI, and [^3H]norBNI were used to label MOR, DOR and KOR, respectively, unless as otherwise stated. The percentage stimulation to DAMGO is the E_{max} of the compound compared to that of DAMGO (normalized to 100%). Naltrexone (NTX), and CTAP were tested along as controls under same conditions. N/A not applicable. N.D. could not be determined.

^bSee reference 65.

^cAt 100 nM, see reference 66.

\$watermark-text

\$watermark-text

\$watermark-text

d^3 [H]DPN was used as the radioligand.

c Not tested.

Table 2KOR/DOR ^{35}S -GTP[γS] Binding Assay Results for Compounds **8** and **12**^a

Compd	KOR ^{35}S -GTP[γS] Binding		DOR ^{35}S -GTP[γS] Binding	
	EC ₅₀ (nM)	% E_{max} of U50,488H	EC ₅₀ (nM)	% E_{max} of SNC80
NAP ^b	28.8 ± 14.4	45.5 ± 4.4	15.2 ± 15.2	10.2 ± 3.1
8(NMP)	26.4 ± 8.4	31.4 ± 3.9	38.6 ± 10.0	90.2 ± 21.0
12(NGP)	25.0 ± 22.4	23.5 ± 1.6	19.0 ± 2.9	84.3 ± 23.2

^aThe values are the means ± S.E.M. of three independent experiments. The percentage stimulation to U50, 488H or SNC80 is the E_{max} of the compound compared to that of U50,488H or SNC80 (normalized to 100%).

^bSee reference 66.