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

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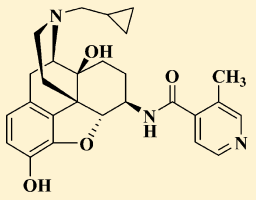
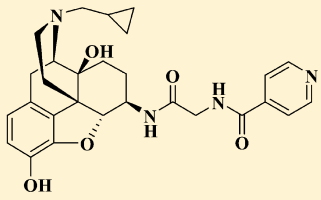
Supporting Information

ABSTRACT: Peripheral selective μ 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 (ED_{50} = 0.03 mg/kg). The slight decrease of the ED_{50} 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.



8, NMP



12, NGP

INTRODUCTION

Opioids are the mainstay for cancer and noncancer 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 opioid-induced constipation (OIC) varies from 9.3% to 95% among different populations 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 μ opioid receptor (MOR), the κ opioid receptor (KOR), and the δ opioid receptor (DOR). 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, compared 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-HT_4 agonists (such as prucalopride[36]), and type-2 chloride channel (ClC-2) activators (such as lubiprostone[37]). 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

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peripheral MOR function would be of therapeutic interest for OIC. Naloxone (Figure 1), a relative μ- and κ-selective opioid

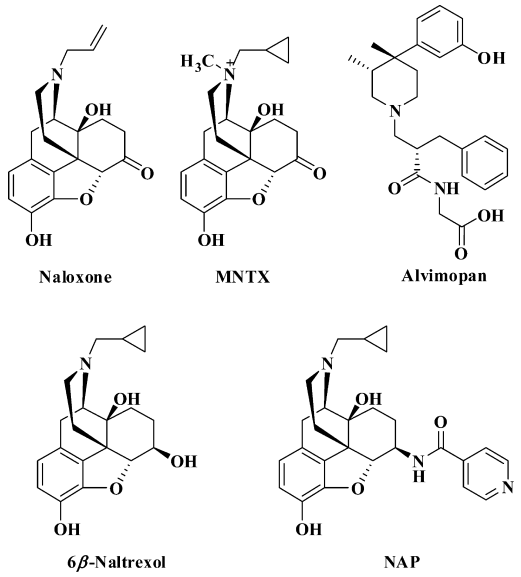

Figure 1. Mechanism-based pharmacological interventions of OIC.

antagonist (K_i 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 13 European countries.[49,50] Therefore, a systemic MOR antagonist still does not seem to be an ideal and universal resolution for OIC.

As for peripheral selective MOR antagonists, because of 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 formation, 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 h after the first dose of MNTX 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 6β-naltrexol (Figure 1) inhibits morphine-induced slowing of GI transit in healthy opioid-naive 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 6β-*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 ED_{50} 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 alternative "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, stereoselective reductive amination of naltrexone with dibenzyl-

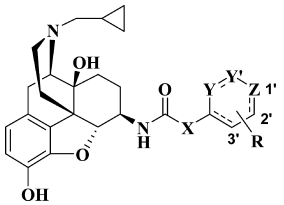

Figure 2. Schematic representative of NAP derivatives, the second generation.

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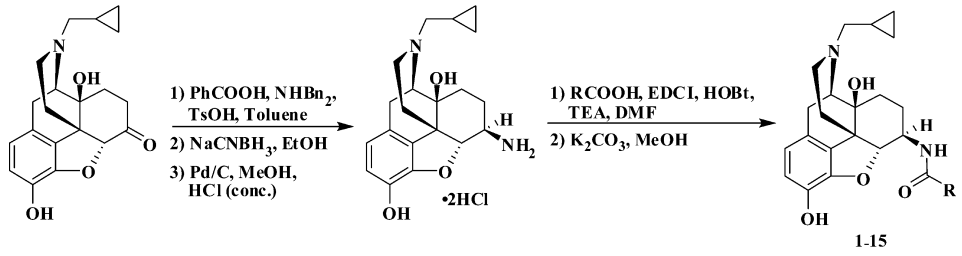
Scheme 1. Synthesis Route of NAP Analogues



amine followed by catalytic hydrogenation−deprotection under acidic conditions furnished 6β-naltrexamine (6β-NTA)[70] dihydrocholoride in a total yield of 50%. Then a variety of substituted N-containing heterocyclic acids, obtained through commercial resources or prepared in house (see Supporting Information), were coupled with 6β-NTA employing the EDCI/HOBt method. After treatment with K_2CO_3, the 6-monosubstituted NAP analogues were thus obtained, with yields ranging from 44% to 88%.

Biology. The synthesized NAP analogues were first evaluated in the radioligand binding assay and ^{35}S-GTP[γS] 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 ovary (CHO) cells as described previously.[65,66] [^{3}H]Naloxone, [^{3}H]naltrindole (NTI), and [^{3}H]nor-binaltorphimine (norBNI) or [^{3}H]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 subnanomolar 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 methoxy groups (**2**, **5**, **7**, **9**) were relatively favorable for the DOR binding. Similarly, it seemed that 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 nonaromatic one (**15**) caused 10-fold decrease in its binding affinity for DOR, probably because of the loss of aromatic staking interaction.[65]

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 compared to the marketing drugs MNTX (K_i ratios $\delta/\mu \approx 24$, $\kappa/\mu \approx 10$),[71] and alvimopan (K_i 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-Ala^2-MePhe^4-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-NH_2 (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 analogues act as MOR partial agonists under the tested conditions with one- or two-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 ^{35}S-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 of $\leq$35%) and low potency (EC_{50} values 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

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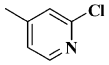
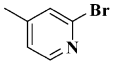
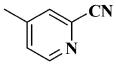
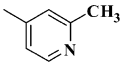
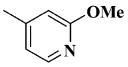
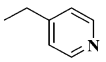
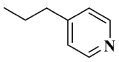
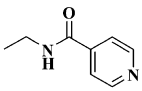
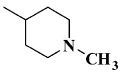
Table 1. Binding Affinity and MOR ^{35}S-GTP[γS] Binding Assay Results for NAP Derivatives[a]

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

[a]The values are the mean ± SEM of three independent experiments. [^{3}H]Naloxone, [^{3}H]NTI, and [^{3}H]norBNI were used to label MOR, DOR, and KOR, respectively, unless 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 the same conditions. N/A: not applicable. ND: could not be determined. [b]See ref 65. [c]At 100 nM; see ref 66. [d][^{3}H]DPN was used as the radioligand. [e]Not tested.

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

	KOR ^{35}S-GTP[γS] binding		DOR ^{35}S-GTP[γS] binding	
compd	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

[a]The values are the mean ± SEM 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%). [b]See ref 66.

activation may cause sedation and dysphoric effects, whereas DOR agonism was regarded to be associated 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 ± 2.8% and 11.2 ± 3.1%, respectively, compared 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 the antinociceptive effect of morphine (10 mg/kg) for compound **8** at a 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 of ≤10 mg/kg by themselves or challenged with 10 mg/kg 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). An amount of 2 mg/kg 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 and 5.

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 **8** significantly restored the GI transit compared to the control. Similarly, 0.3, 1, and 10 mg/kg 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 ED_{50} values 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 ^{35}S-GTP[γS] functional assay. As reported by Smith et al., the mouse ileum expresses 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

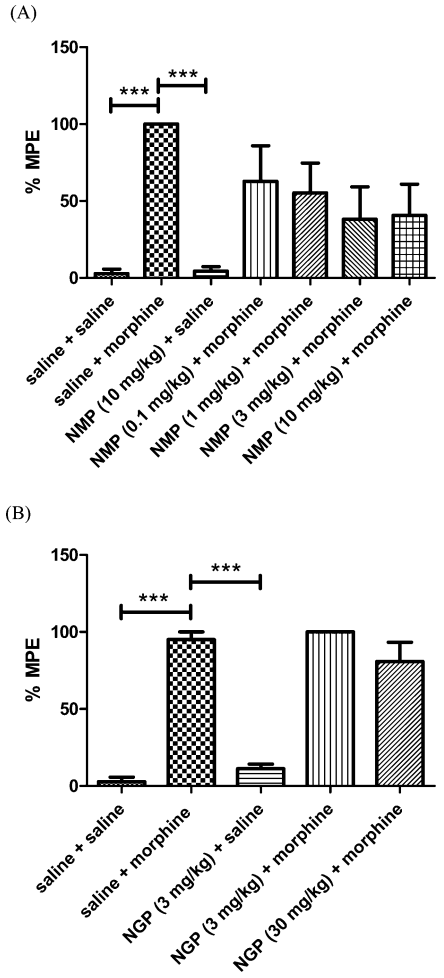

Figure 3. NMP (**8**, A) and NGP (**12**, B) tail flick assay in morphine naive mice challenged with 10 mg/kg morphine (n = 4, (***) P < 0.0005).

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.

Because of the improved side effect profile of compounds **8** and **12** compared 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-naive 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-naive mice (ED_{50} value was 7.85 mg/kg). In contrast, compound **12** was not able to restore the GI motility as effectively as compound **8** (Figure 5B), which is

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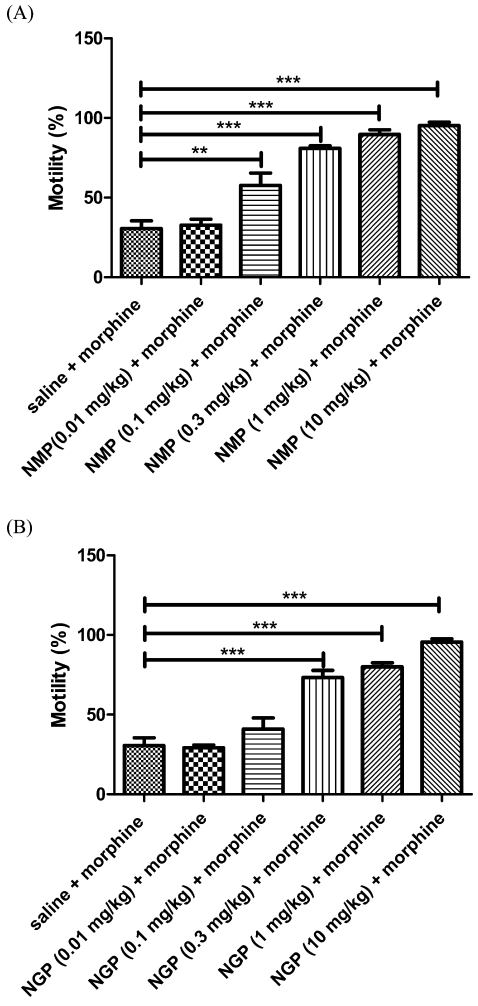

Figure 4. NMP (**8**, A) and NGP (**12**, B) chronic charcoal gavage and intestinal motility assay in morphine-pelleted mice challenged with 2 mg/kg morphine (n = 6, (**) P < 0.005, (***) P < 0.0005).

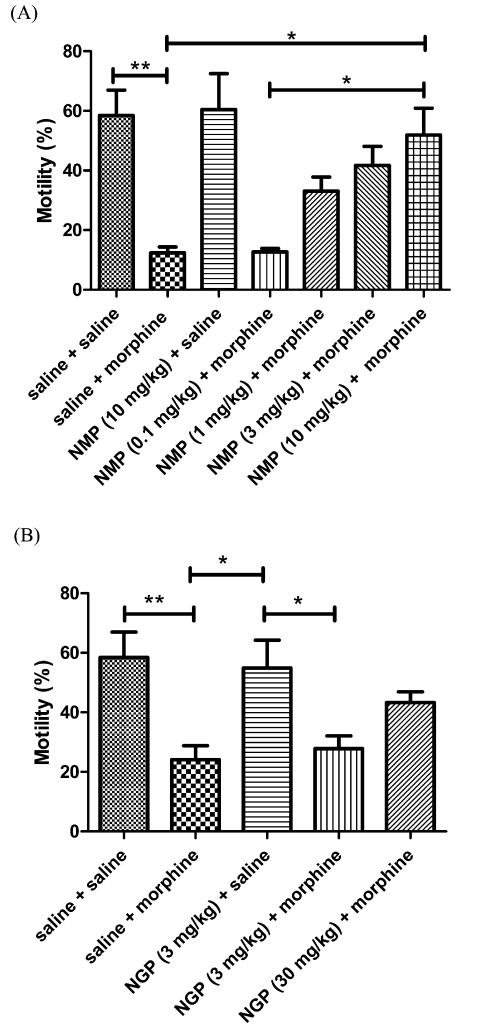

Figure 5. NMP (**8**, A) and NGP (**12**, B) acute charcoal gavage and intestinal motility assay in morphine naive mice challenged with 10 mg/kg morphine (n = 4, (*) P < 0.05, (**) P < 0.005).

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.

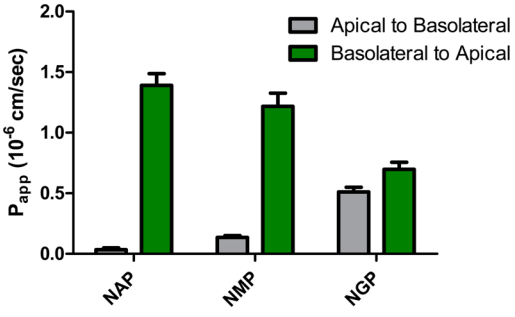

Figure 6. Bidirectional transport of NAP, NMP (**8**), and NGP (**12**) in Caco-2 cells.

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 compared to 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. Because of the high MOR selectivity of compound **8** (NMP) over DOR and KOR, compared to that of MNTX

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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 as ≥95%.

General Procedure for Amide Coupling/Hydrolysis Reaction. On an ice−water bath, to a solution of acid (3 equiv) in anhydrous DMF (3 mL) was added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI, 3 equiv), hydrobenzotriazole (HOBt, 3 equiv), 4 Å molecular sieves, and TEA (5.0 equiv) with N_2 protection. Fifteen minutes later, a solution of 6β-naltroxamine hydrochloride (1.0 equiv) in DMF (1 mL) was added dropwise. The resulting mixture was allowed to warm 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 K_2CO_3 (2 equiv) 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 CH_2Cl_2 (50 mL) and brine (50 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was then purified by column chromatography, eluting with CH_2Cl_2/MeOH (1% $NH_3 \cdot H_2O$) to afford the corresponding compound as free base. Upon confirmation by ^{1}H NMR and ^{13}C NMR, the free base was then transformed into hydrochloride salt by dissolving in MeOH (0.1 mL) and DCM (2 mL), adding HCl methanol solution (1.25 M, 4 equiv) with 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 used in 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. $[\alpha]^{25}_D$ −105.64° (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) 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, 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); ^{13}C NMR (75 MHz, $CDCl_3$) 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}) ν_{max} 3250.3, 1660.3, 1550.4, 1498.7, 1317.8, 1136.8. Mp >250 °C. Anal. ($C_{26}H_{28}ClN_3O_4 \cdot 2HCl \cdot 1.5H_2O$) 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. $[\alpha]^{25}_D$ −141.75° (*c* 1.0, MeOH). ^{1}H 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, 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); ^{1}H 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, 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 $(M + H)^+$. IR (KBr, cm^{-1}) ν_{max} 3398.9, 1673.2, 1544.0, 1498.7, 1472.9, 1324.4, 1130.3. Mp >250 °C. Anal. ($C_{26}H_{28}BrN_3O_4 \cdot 2HCl \cdot 1.5H_2O$) 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. $[\alpha]^{25}_D$ −146.12° (*c* 0.5, MeOH). Hydrochloride salt: ^{1}H NMR (400 MHz, 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, 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 $(M + H)^+$. 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. $[\alpha]^{25}_D$ −202.18° (*c* 1.0, MeOH). ^{1}H 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: ^{1}H NMR (400 MHz, 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, 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 $(M + H)^+$. 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. ($C_{27}H_{31}N_3O_4 \cdot HCl \cdot 2H_2O$) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-{[4′-(2′-methoxypyridyl)]carboxamido}morphinan (5). The title compound was prepared by following the general procedure, in 62% yield. $[\alpha]^{25}_D$ −179.44° (*c* 0.8, MeOH). Hydrochloride salt: ^{1}H NMR (400 MHz, 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, 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, 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 (M + 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. ($C_{27}H_{31}N_3O_5$·2HCl·2.5H_2O) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-{[4′-(3′-chloropyridyl)]carboxamido}morphinan (6). The title compound was obtained following the general procedure as a light yellow solid, in 68% yield. $[\alpha]^{25}_D$ −155.77° (c 1.0, MeOH). ^{1}H 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); ^{1}H 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 (M + H)$^+$. IR (KBr, cm^{-1}) ν_{max} 3198.6, 1653.9, 1498.7, 1317.8, 1123.9, 1033.4. Mp 230 °C, dec. Anal. ($C_{26}H_{28}ClN_3O_4$·2HCl·H_2O) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-{[4′-(3′-bromopyridyl)]carboxamido}morphinan (7). The title compound was obtained following the general procedure as a light yellow solid, in 51% yield. $[\alpha]^{25}_D$ −141.16° (c 1.0, MeOH). ^{1}H 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 (M + H)$^+$. IR (KBr, cm^{-1}) ν_{max} 3398.9, 1653.9, 1550.4, 1505.2, 1330.7, 1123.9. Mp 235 °C, dec. Anal. ($C_{26}H_{28}BrN_3O_4$·2HCl·2H_2O) 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. $[\alpha]^{25}_D$ −141.45° (c 1.0, MeOH). ^{1}H 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 (M + H)$^+$. IR (KBr, cm^{-1}) ν_{max} 3424.8, 1653.9, 1544.0, 1505.2, 1317.8, 1130.3. Mp 211 °C, dec. Anal. ($C_{27}H_{31}N_3O_4$·2HCl·0.2H_2O) C, H, N.

17-Cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-{[4′-(3′-methoxylpyridyl)]carboxamido}morphinan (9). The title compound was obtained following the general procedure as a light yellow solid, in 60% yield. $[\alpha]^{25}_D$ −145.47° (c 0.5, MeOH). Hydrochloride salt: ^{1}H NMR (400 MHz, 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, 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 (M + 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. $[\alpha]^{25}_D$ −115.30° (c 1.0, MeOH). ^{1}H 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 (×2), 147.7, 143.7, 142.0, 132.5, 126.3 (×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 (M + 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. ($C_{27}H_{31}N_3O_4$·2HCl·2H_2O) 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. $[\alpha]^{25}_D$ −102.63° (c 1.5, MeOH). ^{1}H 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: ^{1}H NMR (400 MHz, 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, C_5−H), 3.83 (m, 1H, C_6−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); ^{13}C 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. ($C_{28}H_{33}N_3O_4$·2HCl·0.5H_2O) 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. $[\alpha]^{25}_D$ −126.34° (c 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) 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. ($C_{28}H_{32}N_4O_5$·2HCl·3H_2O) 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. $[\alpha]^{25}_{D}$ −150.27° (*c* 0.8, MeOH). Hydrochloride salt: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (dd, *J* = 1.8, 1.2 Hz, 1H), 9.47 (dd, *J* = 5.2, 1.2 Hz, 1H), 9.33 (d, *J* = 8.0 Hz, 1H, exchangeable), 8.89 (brs, 1H, exchangeable), 8.08 (dd, *J* = 5.6, 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); ^{13}C 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. ($C_{25}H_{28}N_4O_4$·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. $[\alpha]^{25}_{D}$ −190.45° (*c* 0.5, MeOH). Hydrochloride salt: ^{1}H 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); ^{13}C 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. ($C_{25}H_{28}N_4O_4$·2HCl·H_2O) 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. $[\alpha]^{25}_{D}$ −88.06° (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) 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}) ν_{max} 3437.7, 1647.4, 1544.0, 1460.0, 1311.3, 1123.9. Mp >250 °C. Anal. ($C_{27}H_{37}N_3O_4$·2HCl·2.5H_2O) 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 25−30 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 ^{35}S-GTP[γS]-binding assay were conducted using monocloned opioid receptor expressed in Chinese hamster ovary (CHO) cell lines as described previously.[65,66,79−81] [^{3}H]Naloxone, [^{3}H]NTI, and [^{3}H]norBNI (or [^{3}H]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 IC_{50} 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 ^{35}S-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 receptors, respectively.

In Vivo Assays. Tail Flick Test. The warm-water tail flick test was performed according to Coderre and Rollman[82] using a water bath with the temperature maintained at 56 ± 0.1 °C. Before injection, 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 Pierson[83] as the percentage of maximum possible effect (% MPE), which was calculated as % MPE = [(test latency − control latency)/(10 − control latency)] × 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 (sc) 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 20 min, 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 was 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 no. 3460 Transwell-Clear inserts (Fisher Scientific) at a density of 80 000 cells/cm^2. 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 mm × 100 mm; Alltech, Deerfield, IL) at 270 nm for NAP and a Microsorb-MV 100-3 C18 (4.6 mm × 100 mm, Varian) column for NMP or NGP at 266 nm. Analyte 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 0.01−100 μM (R^2 = 0.999). Apparent permeability was then calculated using the following equation: $P_{app} = J/(AC_i)$, 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.

■ ASSOCIATED CONTENT

Supporting Information

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.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

BBB, blood−brain barrier; CHO, Chinese hamster ovary; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2; DAMGO, [D-Ala^2-MePhe^4-Gly(ol)^5]enkephalin; DOR, δ opioid receptor; GI, gastrointestinal; KOR, κ opioid receptor; MNTX, methylnaltrexone; MOR, μ 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

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