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Synthesis and Evaluation of Aryl-Naloxamide Opiate Analgesics Targeting Truncated Exon 11-Associated μ Opioid Receptor (MOR-1) Splice Variants

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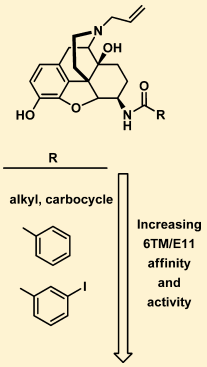
Supporting Information

ABSTRACT: 3-Iodobenzoylnaltrexamide **1** (IBNtxA) is a potent analgesic acting through a novel receptor target that lack many side-effects of traditional opiates composed, in part, of exon 11-associated truncated six transmembrane domain MOR-1 (6TM/E11) splice variants. To better understand the SAR of this drug target, a number of 4,5-epoxymorphinan analogues were synthesized. Results show the importance of a free 3-phenolic group, a phenyl ring at the 6 position, an iodine at the 3′or 4′ position of the phenyl ring, and an *N*-allyl or *c*-propylmethyl group to maintain high 6TM/E11 affinity and activity. 3-Iodobenzoylnaloxamide **15** (IBNalA) with a *N*-allyl group displayed lower δ opioid receptor affinity than its naltrexamine analogue, was 10-fold more potent an analgesic than morphine, elicited no respiratory depression or physical dependence, and only limited inhibition of gastrointestinal transit. Thus, the aryl-naloxamide scaffold can generate a potent analgesic acting through the 6TM/E11 sites with advantageous side-effect profile and greater selectivity.



INTRODUCTION

Opiates have been the subject of intense research since the isolation of morphine in the early 1800s.[1] Despite their efficacy and utility, side-effects greatly limit the usefulness of virtually all the clinically available opiates, including respiratory depression, constipation, and physical dependence, reward, and addiction. Attempts to separate these unwanted actions from analgesia have largely been unsuccessful. A number of studies exploring the pharmacology of naloxonazine dissociated morphine analgesia from respiratory depression,[2,3] inhibition of gastrointestinal transit,[4,5] and physical dependence.[6] Yet, dissociation of these actions functionally failed to yield selective analgesics lacking these side-effects. The identification of δ and κ_1 receptors led to extensive efforts to generate alternative analgesics acting through different receptors to achieve a better pharmacological profile. Although highly selective agents were developed, they have yet to fill the clinical need. The κ_1 receptor drugs that were tested clinically revealed unacceptable psychotomimetic effects, as well a profound diuresis, while the utility of δ-selective agents have not yet been fully validated. Thus, there still remains a need for potent analgesics with safer side-effect profiles.

Clinicians have long appreciated differences among μ opioids, with some patients responding better to one agent than another.[7−9] These clinical observations, along with preclinical pharmacological and binding studies, led to the initial suggestion of multiple μ receptors,[10] a concept that has been confirmed with the cloning of a vast array of splice variants of the μ opioid receptor (MOR-1) gene *Oprm1* in mice, rats, and humans.[11−20] Included in these variants is a set of truncated six transmembrane domain splice variants generated by an alternative promoter associated with exon 11 of the *OPRM1* gene of mice,[16] rats[21] and humans.[22] Although it was suggested that these variants might be active alone,[23,24] the poor affinity of the expressed variants for opiates and the very high doses of drug necessary to activate the receptors seem to make this unlikely.

Recently, we reported the synthesis[25] and pharmacological characterization[26] of a β-naltrexamine analogue, 3′-iodobenzoylnaltrexamide **1** (IBNtxA, Figure 1). Pharmacological studies revealed an analgesic with a potency 10-fold greater than morphine with no observable respiratory depression, physical dependence, cross tolerance to morphine, or rewarding behavior in the conditioned place preference assay and limited inhibition of gastrointestinal transit. This compound displayed high affinity for a binding site in the brain that was distinct from traditional μ, δ, or κ_1 receptors, most clearly shown by its persistent analgesia and binding in a triple knockout mouse lacking the traditional μ, δ, and κ_1 receptors. The loss of both its analgesia and its binding site in an exon 11 knockout mouse, further established a unique mechanism of action and implicated exon 11-associated 6 transmembrane domain splice variants (6TM/E11). Additional studies suggest that these

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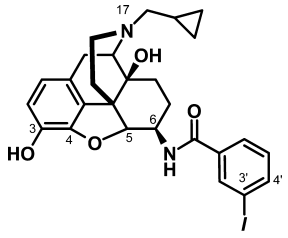
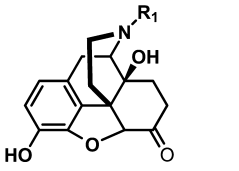


1 IBNtxA



R1

2 Oxymorphone -CH3
3 Naltrexone -CH2-
4 Naloxone -allyl

Figure 1. Structures of selected opiates.

truncated variants do not act alone. Rather, **1** appears to label with high affinity a heterodimer of a 6TM/E11 variant with an additional G-protein coupled receptor, although the partner is still unknown. We now describe the structure−activity relationships for this new drug target by synthesizing several analogues of **1** with substitutions on the 6, 17, and 3 positions of the 4,5-epoxymorphinan scaffold. All synthesized molecules were evaluated for their relative binding affinity for cloned μ (MOR-1), κ_1 (KOR-1), and δ (DOR-1) sites in cell lines and 6TM/E11 sites in mouse brain and for their analgesic activity in vivo with the radiant heat tail flick assay.

RESULTS

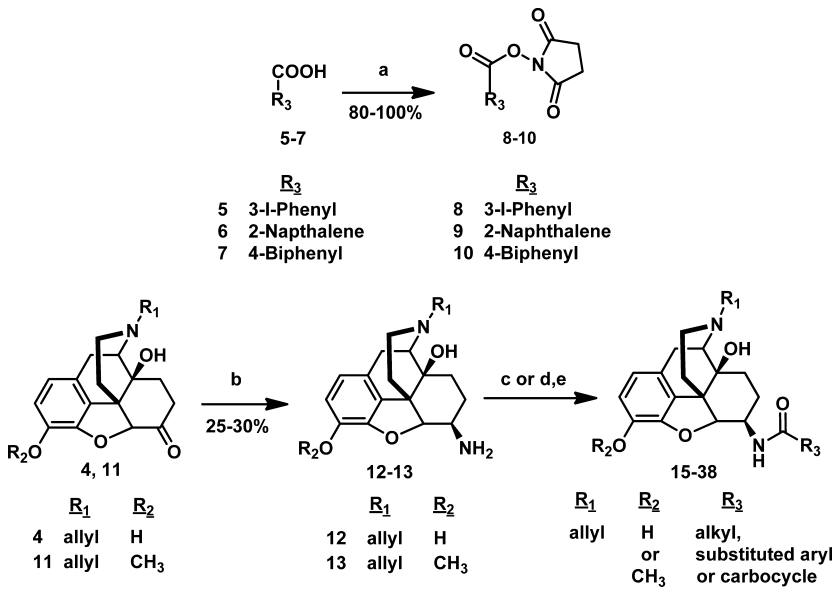
Chemistry. The ketone at the 6-position of naloxone **4** and 3-methoxynaloxone **11** were transformed to an amine (opiate-NH_2) by reductive amination using sodium cyanoborohydride ($NaBH_3CN$) and ammonium acetate (NH_4OAc) to yield a mixture of β and α isomers.[27] The β and α isomers were then purified by column chromatography. In a parallel synthesis, substituted carboxylic acids **5**−**7** were converted to their respective *N*-succinimidyl esters **8**−**10** by reacting them with *N*-hydroxysuccinimide (NHS) in the presence of DCC and THF. The corresponding activated esters were then reacted with the β or α isomer of the opiate-NH_2 in the presence of diisopropylethylamine (DIEA) and DCM. The corresponding aryl, alkyl, and carbocyclic amido derivatives of opiates were then purified by column chromatography. Alternatively, the substituted carboxylic acids were directly coupled to the opiate-NH_2 using benzotriazol-1yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) and DIEA in DCM to give 3, 6-disubstituted derivatives. The 3,6-disubstituted opiate derivatives were then subjected to basic hydrolysis with potassium carbonate to yield 6- alkyl, aryl, and carbocyclic derivatives of naloxamide and 3-methoxynaloxamide (Scheme 1; Table 1). **1**, **14**, and **15** were synthesized as previously reported.[25] The pharmacological characterization of **14** and **15** and all newly synthesized compounds (**16**−**38**) is summarized in Table 1.

Pharmacology. **1** is a potent analgesic lacking many of the problematic side-effects associated with traditional opiates. Our objective is to establish the criteria important for both potency and selectivity for the 6TM/E11 sites by exploring the structure−activity relationship (SAR).

The N-substituent is important for defining both affinity and analgesic activity. Receptor binding studies reveal that an *N*-methyl substituent dramatically lowers the affinity of the compound for the 6TM/E11 site, as shown with oxymorphone **2** (K_i >1 μM, compared to the *c*-propylmethyl group in naltrexone **3** (K_i 32 nM) or the allyl group in naloxone **4** (K_i 53 nM) (Figure 1; Table 1).

The increased affinity of **1** (K_i = 0.16 nM) illustrates the importance of substituents at the 6-position of β-naloxamine.

Scheme 1. Synthesis of Aryl and Alkyl Amido Derivatives of β-Naloxamine and 3-Methoxy-β-naloxamine[a]



[a]Reagents: (a) NHS, DCC, dry THF, 24 h, rt, N_2; (b) NH_4OAc, $NaBH_3CN$, dry MeOH, 24 h, rt, N_2; (c) **8**−**10**, DIEA, dry DCM, rt, N_2, 2 h; (d) R_3COOH, BOP, DIEA, DCM, 2 h; (e) K_2CO_3, MeOH, 3 h.

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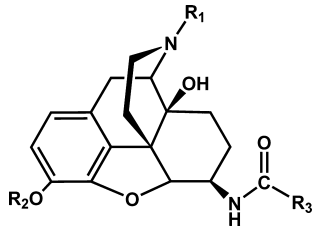
Table 1. Evaluation of Receptor Binding Affinity and Analgesic Activity[a]



				K_i (nM)				tail flick analgesia
compd	R_1	R_2	R_3	MOR-1	KOR-1	DOR-1	6TM/E11	ED_{50} mg/kg[d] (95%CL)
naltrexone **3**							32.7 ± 4.1[b]	
naloxone **4**							53.2 ± 7.13[b]	
oxymorphone **2**							>1 μM	
1	−CPM	H	Ph-3′-I	0.11 ± 0.02	0.03 ± 0.001	0.24 ± 0.05	0.16 ± 0.04[b]	0.39 (0.15, 0.58)[b]
14	$-CH_3$	H	Ph-3′-I	0.97 ± 0.2	47.22 ± 14.2	2.45 ± 1	41.22 ± 12.3	>10
15	−allyl	H	Ph-3′-I	0.22 ± 0.12	0.08 ± 0.06	2.55 ± 0.18	0.25 ± 0.12	0.60 (0.42, 0.9)
16	−allyl	H	Ph-3′-I[c]	5.07 ± 1.9	12.16 ± 3.8	7.64 ± 2.56	8.46 ± 2.9	4.9 (4.47, 5.37)
17	−allyl	H	Ph-2′-I	1.56 ± 0.4	1.00 ± 0.11	22.8 ± 10.3	29 ± 5.9	>10
18	−allyl	H	Ph-4′-I	0.11 ± 0.04	0.28 ± 0.08	3.36 ± 2.57	0.64 ± 0.22	0.14 (0.08, 0.24)
19	−allyl	H	Ph-3′-F	0.47 ± 0.03	2.05 ± 0.49	18.19 ± 3.81	8.09 ± 3.45	2.9 (2.4, 3.6)
20	−allyl	H	Ph-3′-Cl	1.15 ± 0.11	0.52 ± 0.04	4.87 ± 3.32	5.49 ± 0.98	1.6 (0.8, 3.2)
21	−allyl	H	Ph-3′-Br	3.85 ± 3.53	1.58 ± 0.89	23.37 ± 20.24	2.05 ± 1.3	0.71 (0.47, 1.1)
22	−allyl	H	Ph	4.03 ± 1.04	14.27 ± 3.99	60.78 ± 11.44	5.82 ± 1.79	5.3 (4.3, 6.5)
23	−allyl	H	Ph-3′-CH_3	0.29 ± 0.05	1.62 ± 0.28	8.24 ± 4.84	8.98 ± 0.1	2.25 (1.8, 2.9)
24	−allyl	H	Ph-3′-CF_3	0.85 ± 0.24	0.22 ± 0.13	2.96 ± 2.09	9.32 ± 1.3	0.21 (0.14, 0.3)
25	−allyl	H	Ph-3′-OCH_3	0.18 ± 0.1	4.97 ± 0.63	17.22 ± 3.53	1.64 ± 0.45	0.23 (0.13, 0.41)
26	−allyl	H	Ph-3′-NH_2	0.43 ± 0.12	0.4 ± 0.1	36 ± 11	7.62 ± 3.94	>10
27	−allyl	H	Ph-3′-$N(CH_3)_2$	6.4 ± 2.3	34.91 ± 10.5	51.35 ± 15.6	10.79 ± 1.31	>10
28	−allyl	H	Ph-3′-OH	0.23 ± 0.01	2.75 ± 0.87	11.25 ± 4.13	5.21 ± 1.51	>10
29	−allyl	H	Ph-3′-NO_2	1.41 ± 0.12	1.51 ± 0.14	18.13 ± 6.86	4.53 ± 1.2	5.3 (4.22, 6.7)
30	−allyl	H	Ph-4′-OCF_3	0.66 ± 0.04	3.16 ± 0.19	17.88 ± 3.25	7.43 ± 2.67	0.67 (0.52,0.88)
31	−allyl	H	naphthalene	0.74 ± 0.63	1.29 ± 0.45	5.51 ± 1.15	6.64 ± 1.82	1 (0.7,1.42)
32	−allyl	H	biphenyl	0.95 ± 0.2	25.79 ± 10.1	19.15 ± 5.6	7.17 ± 0.93	>10
33	−allyl	H	CH_3	20.46 ± 1.4	>100	>100	>100	>10
34	−allyl	H	C_6H_{13}	9.5 ± 26	9.15 ± 0.94	32.2 ± 9.45	29.65 ± 9.29	>10
35	−allyl	H	$C_{12}H_{25}$	0.61 ± 0.1	9.35 ± 1.55	15.25 ± 0.38	39.43 ± 1.54	>10
36	−allyl	H	*c*-hexane	11.54 ± 2.64	17.9 ± 3.59	>100	30.17 ± 7.79	>10
37	−allyl	H	adamantane	6.5 ± 1.94	7.1 ± 3.15	>100	30.27 ± 6.41	>10
38	−allyl	CH_3	Ph-3I	>100	>100	>100	>100	>10

[a]All analogues were the β-isomer at the 6 position, with the exception of **16**, which was the α-isomer of 6-naloxamine. Competition studies were performed with the indicated compounds against ^{125}I-BNtxA (0.1 nM) in membranes from CHO cells stably expressing the indicated cloned mouse opioid receptors or in mice brain membranes for 6TM/E11 sites with blockers to prevent binding to traditional μ, κ_1, and δ receptors as described in the Experimental Section. K_i values were calculated from the IC_{50} values[43] and represent the means ± SEM of at least three independent replications. ^{125}IBNtxA K_D values for MOR-1, KOR-1, DOR-1, and 6TM/E11 sites were 0.11, 0.03, 0.24, and 0.16 nM, respectively. [b]Values from the literature.[26] [c]Alpha isomer. [d]95% confidence limits.

To further explore the role of the 3′-iodophenyl moiety at the 6-position of the 4,5-epoxymorphinan, we synthesized **14** and **15**, which incorporated a 3′-iodophenyl amido moiety into the oxymorphone and naloxone scaffold, respectively. **14** has a K_i of 41 nM for 6TM/E11 sites. Although the addition of the 3′-iodophenyl amido moiety increased the affinity by at least 50-fold, its affinity remained over 100-fold poorer for the 6TM/E11 site than either the naloxone analogue (3-iodobenzoylnaloxamide **15** (IBNalA)), K_i = 0.25 nM, Figure 1) or the naltrexone analogue (**1**, K_i 0.16 nM). The 3′-iodophenyl amido moiety also influenced the pharmacology of the compounds. Oxymorphone **2** is a potent analgesic. Yet, its analogue, **14**, showed no analgesia at doses as high as 10 mg/kg sc despite a reasonable affinity for μ receptors. Conversely, the 3′-iodophenylamido substituent at the 6 position converted the naltrexone and naloxone analogues into potent analgesics, contrasting markedly with the antagonist character of naloxone and naltrexone themselves. It is important to note that **15** retained a similar affinity for the 6TM/E11 binding site and analgesic potency as **1** (Figure 2A). Compared to **1**, **15** had a 10-fold lower affinity for δ receptors and about 2-fold lower affinity for μ and κ receptors. In view of this increased selectivity, we decided to pursue the naloxone series of analogues.

The stereochemistry of the 6-position is important. The α-isomer, **16**, showed a 33-fold decreased binding affinity for the 6TM/E11 site, a 23-, 150-, and 3-fold lower affinity for μ, κ, and δ receptors and a 10-fold higher ED_{50} in analgesic assays (Table 1). Thereafter, we focused upon β-naloxamine compounds.

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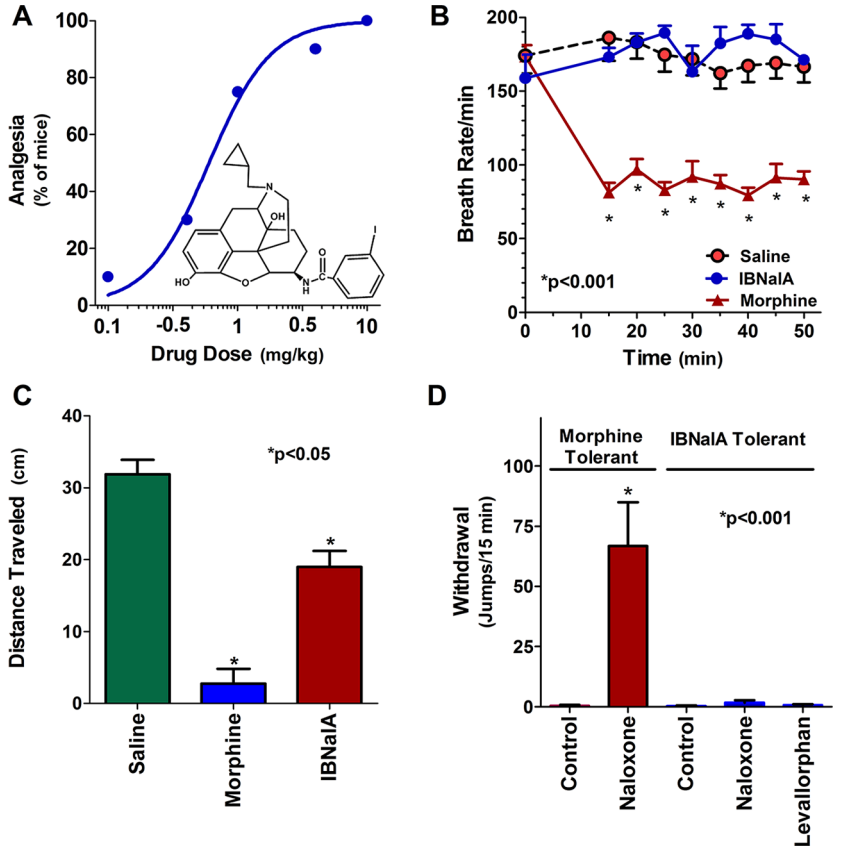

Figure 2. Pharmacology of 17-allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-iodo)benzamido]morphinan (**15**). (A) Analgesia: Cumulative dose–response curves were carried out on groups of mice (n = 10) with **15** at the indicated doses of **15** (sc) and analgesia tested 30 min later at peak effect. ED_{50} values (and 95% confidence limits) were 0.6 mg/kg (0.42, 0.87) in CD1 mice by using the radiant heat tail-flick assay. (B) Respiratory rate: Animals are randomly assigned to receive saline (n = 5, sc) or the opioid **15** (2.5 mg/kg, sc; n = 5) or morphine (20 mg/kg, sc; ED_{50}, n = 5) at 5-fold and 4-fold their respective ED_{50} and tested as described in the Experimental Section. Each animal's baseline average breath rate was measured every 5 min for 25 min prior to drug injection, and breath rates following drug injection were determined. **15** did not depress respiratory rate and was not significantly different from saline at any time point, while morphine decreased respiratory depression in comparison to both saline and **15** (p < 0.001) as determined by repeated measures ANOVA followed by Bonferroni multiple comparison test. (C) Gastrointestinal transit: Groups of mice (n = 10) received saline (sc), morphine at its analgesic ED_{50} (5 mg/kg, sc), or **15** at twice its analgesic ED_{50} (1 mg/kg, sc) before receiving a charcoal meal (0.2 mL; 2.5% gum tracaganth in 10% activated charcoal in water) by gavage and sacrificed 30 min later. The distance traveled by the charcoal was measured 30 min later. **15** lowered transit significantly compared to saline (p < 0.05) but less than morphine (p < 0.05) as determined by ANOVA followed by Tukey's multiple comparison test. (D) Physical dependence: Groups of mice ($n \geq 10$) received either morphine (10 mg/kg, sc) or **15** (1 mg/kg, sc) at twice their analgesic ED_{50} for 5 days. They then were challenged with the indicated antagonist. Naloxone precipitated a profound withdrawal syndrome in the morphine-treated animals, as shown by the number of jumps/15 min, which was significantly greater than that in the morphine or **15** controls (i.e., given no antagonist) or in **15** mice given the indicated antagonist. Mice chronically administered **15** showed no significant difference from controls when challenged by naloxone or levallorphan (1 mg/kg, sc).

Moving the position of the iodine on the aryl ring influences activity. Although an iodine at position 4′, **18**, retains a very similar profile compared to the 3′ position, **15**, placing it at position 2′, **17**, lowered its binding affinity for the 6TM/E11 sites by 100-fold with approximately 10-fold lower affinities for μ, κ, and δ receptors and eliminated its analgesic activity at doses as high as 10 mg/kg.

We next examined a series of halogens at the 3′ position of the aryl ring (**19**–**21**, Table 1) and compared it to hydrogen in **22**. We chose the 3′ position based on the higher affinity of the 3′-iodo-analogue, **15**, when compared to the 4′-iodo-analogue, **18**. **22** illustrates the importance of the iodine when compared to a hydrogen. Removal of the iodine at either the 3′- or the 4′-position lowered the affinity at the 6TM/E11 site by over 20-fold, while the affinity across μ, κ, and δ receptors also decreased by about 20-, 180-, and 25-fold, respectively. The 6TM/E11 affinity increased as the size of the halogen increased, and electron withdrawing nature of the halogen atom decreased while no observable trends in affinity were seen with the traditional μ, κ, and δ receptors. The analgesic activity correlated with the 6TM/E11 site binding, increasing from F to I. The existence of halogen bonding in protein–ligand complexes has been shown for adenosine kinase inhibitors,[28] HIV-RT inhibitors,[29,30] and PD5 inhibitors.[31] This raises the possibility that the ability of iodine to form weak halogen bonds[32] in the protein pocket, rather than the electron-withdrawing nature of the halogen atom, maybe responsible for its higher affinity for the 6TM/E11 sites.

To further evaluate the role of electron releasing or electron withdrawing groups on the aryl ring on 6TM/E11 affinity, we synthesized compounds with both electron releasing groups (CH_3, OCH_3, OH, and NMe_2) and electron withdrawing groups (NO_2, OCF_3, and CF_3; **23**–**30**, Table 1). No clear trend was seen with either series, with these substituents lowering the affinities for the 6TM/E11 site and the traditional opioid receptors when compared to **15**. However, the analgesic activity

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of **24** with a CF_3 at the 3′-position and **25** with OMe at the 3′-position of the aryl ring stand out from the others with regard to their potent analgesic actions. **24** is 42-, 4-, and 10-fold more selective for κ_1 sites than 6TM/E11, μ, or δ receptors. Similarly, **25** is 9-, 27-, and 100-fold more μ selective than 6TM/E11, κ, and δ receptors respectively, raising the question whether the strong analgesic actions of **24** and **25** might be due to traditional opioid receptors. If true, this would be unusual because the placement of an allyl group on the nitrogen in the 17-position typically yield antagonists for the traditional receptors.

An aryl ring may be sufficient to instill high affinity for the 6TM/E11 site in view of the similar affinity of analogues with either electron withdrawing or releasing groups. More complex aryl rings (**31**−**32**, Table 1) maintained a modest binding affinity for the 6TM/E11 site. **31**, with its naphthalene ring, also shows modest analgesic activity. Despite its similar binding affinity for the 6TM/E11 site, **32** was not analgesic. Both **31** and **32** have higher affinity for the μ receptor over 6TM/E11 sites and offer no additional advantage in terms of 6TM/E11 selectivity over the traditional opioid receptors.

Lipophilicity alone appears to have little role in defining binding affinity for the 6TM/E11 sites or activity. Methyl, hexyl, dodecyl, cyclohexyl, and adamantyl groups at the 6 position (**33**−**37**, Table 1) all showed poor binding affinity for the 6TM/E11 sites and moderate to poor affinities across the traditional opioid receptors and no analgesic activity at doses as high as 10 mg/kg sc. This contrasts markedly with the aryl substituent's. **22**, with a simple phenyl ring (K_i 5nM; ED_{50} 5 mg/kg, sc), is far superior to any of the above analogues, raising the question of a pi−pi stacking interaction between the aromatic ring at the 6-position and the 6TM/E11 protein binding site.

Finally, we examined the need for a free 3-OH group. It has long been known that traditional μ opiates of the 4,5-epoxymorphinan series require a free hydroxyl group at this position. Although codeine and oxycodone, which have a 3-methoxy group, are effective analgesics, many believe that the activity results from demethylation to morphine and oxymorphone, respectively. Like other opiates, the presence of a methyl group in **38** eliminates binding across the 6TM/E11 sites and the traditional opioid receptors and analgesia.

To see if these compounds are acting in a manner similar to **1**, we extended the pharmacological assessment of **15**, the naloxone analogue. The *N*-allyl analogue had an advantage in that its affinity for δ receptors is approximately 10-fold lower than the CPM compound, **1**. Despite its potent analgesic activity, **15** displayed no respiratory depression at doses approximately 4-fold greater than its analgesic activity, showed no evidence of physical dependence after repeated administration based upon the absence of withdrawal signs following the administration of naloxone or levallorphan, and far less inhibition of GI transit than morphine (Figure 2). Thus, the overall pharmacology of **15** mimics that of **1**, consistent with activity through the 6TM/E11 target.

DISCUSSION

Our earlier work with **1** illustrated the ability to develop opioids capable of acting through a novel target, which is composed, in part, by exon 11-associated splice variants of MOR-1.[26] The overall profile of this class of analgesic was quite unusual in that the agents had no respiratory depression, physical dependence, or cross tolerance to morphine and only limited effects on gastrointestinal transit. Furthermore, **1** retained full analgesic activity in a MOR-1/DOR-1/KOR-1 triple knockout mouse lacking traditional μ, δ, and κ_1 receptors while it was without activity in an E11 knockout mouse with a disruption of the exon 11-associated variants.[26] Our current study explores a series of analogues. The pharmacological profile of the analogous naloxone derivative, **15**, was quite similar. Again, it was a potent analgesic with no evidence of respiratory depression or physical dependence and limited inhibition of gastrointestinal transit.

The structure−activity relationships of these analogues are interesting. The N-substituent is important, as the analgesic activity is limited to the *N*-allyl, **15**, or *N*-methylcyclopropyl 6β-naltrexamine derivatives, **1**. No analgesia could be detected for the *N*-methyl derivative, **14**. This was opposite to what might be expected because analgesia is seen only with oxymorphone and not naloxone and naltrexone. This might reflect a reversal of the effect of the N-substituent on the traditional agonist/antagonist character of the molecules, but the *N*-methyl group also was associated with a marked decrease in affinity for the 6TM/E11 sites. It is interesting to note that an aryl ring at the 6-position enhances affinity for the 6TM/E11 sites. All analogues lacking the aryl ring had poorer affinity with K_i >10 nM. Placement of an iodine on the aryl ring at the 3′ or 4′ position further enhances the affinity for the 6TM/E11 sites as well as analgesic activity.

Structurally analogous aryl naltrexamides have been described by Ghirmai et al. as alcohol cessation agents[33,34] and by Zhang and co- workers as μ antagonists.[35,36] However, none of these molecules were noted to have analgesic activity. Similarly, 6-arylamido morphines have been reported as M6G analogues by McDougal et al.[37] More recently, Portoghese described a naphthoyl analogue of 6β-naltrexamine (NNTA) analogous to the naloxone version synthesized in our study **31** (Table 1) with analgesic activity and lacking physical dependence and rewarding behavior in the place preference assay that he ascribed to activation of a μ/κ_1 opioid receptor dimer.[38] As with the 6β-naltrexamine, the 6β-naloxamine analogue was active in the mouse radiant heat tail flick assay. However, it is unclear whether this compound is acting through the same target as **1** and **15**. Compared to **15**, the napthoyl derivative, **31**, showed over a 26-fold lower binding affinity for the 6TM/E11 site while its analgesic activity was only 1.5-fold lower. The loss of NNTA analgesia as reported in their MOR-1 knockout mouse[38] does not help because the disruption of exons 2 and 3 in their mouse leads to the loss of both the exon 11-associated 6TM variants and the full-length MOR-1 ones and therefore would be expected to abolish the actions of compounds acting through any of the 6TM or 7 TM MOR-1 variants.

There is, in general, a reasonably good correlation between binding affinity for the 6TM/E11 sites and analgesic potency for most of the compounds, consistent with activity through the 6TM/E11 sites. However, there are some exceptions. For example, the 3-OCH_3 analogue, **25**, shows an analgesic potency over 3-fold greater than **15** despite an affinity for the 6TM/E11 site that is 10-fold poorer. A similar dissociation of analgesic activity and binding affinity for the 6TM/E11 site is seen with **24** (4-CF_3) and **30** (4-OCF_3), raising questions about their targets as well. Thus, interpretation of the analgesic activity and correlating it with binding affinities must be done cautiously because a wide range of targets can all elicit the same pharmacological response. Finally, both **1** and **15** show a

remarkable side-effect profile, confirming that it is possible to develop superior analgesics. However, it is premature to extrapolate these observations to the other compounds that may, or may not, have a similar side-effect profiles.

CONCLUSION

A series of C-6-substituted 4,5-epoxymorphinans (i.e., aryl, alkyl, and carbocycle naloxamides) were synthesized as analogues of **1** to probe the structure−activity relationships of both affinity and selectivity of ligands for the 6TM/E11 sites. These compounds were prepared by the reaction of 6β-naloxamine with alkyl, aryl, and carbocyclic carboxylic acids or their corresponding NHS activated esters. This is the first report of the pharmacological properties of aryl naloxamides and their ability to demonstrate potent agonist activity, although structurally analogous aryl naltrexamides have been reported before. To our knowledge, this is the first study demonstrating aryl-naloxamide analgesics. Although many of the derivatives displayed high affinity for other opiate receptor targets, particularly μ and κ_1, there were clear predictors for the 6TM/E11 target. The most important was the aryl ring at the C-6 position of the 4,5-epoxymorphinan scaffold. All derivatives lacking a phenyl ring were completely inactive in analgesia assays and had lower affinity in 6TM/E11 binding assays. The presence of an iodine at either the 3′- or 4′-position, but not the 2′-position, of the phenyl ring also increased the 6TM/E11 affinity and activity. Replacing the N-CPMof **1** with an *N*-allyl substituent, **15**, produced an analogue with similar affinity for the 6TM/E11 site and a similar pharmacological profile, as well as increased selectivity over the δ opioid receptor. The superior pharmacological profile of **15** compared to traditional μ analgesics suggested that the 6TM/E11 site may provide a useful target in developing useful analgesics.

EXPERIMENTAL PROCEDURES

Materials. Naltrexone, oxymorphone, levallorphan, morphine, U50,488H, [D-Pen^2,D-Pen^5]enkephalin, [D-Ala^2,MePhe^4,Gly(ol)^5]-enkephalin, and naloxone were a generous gift from the Research Technology Branch of the National Institute of Drug Abuse (Rockville, MD) and other chemicals were purchased from Fischer Scientific (Pittsburgh, PA, USA) and Sigma (St Louis, MO, USA). Na^{125}I was bought from Perkin-Elmer (Waltham, MA, USA).

Synthesis of Compounds (15−38). *General Procedures.* All reactions were carried out under positive nitrogen atmosphere with a magnetic stirrer at ambient temperatures using oven-dried glassware. ^{1}H NMR were taken on a 500 MHz Bruker Advance III instrument using $CDCl_3$ as solvent and calibrated using an internal reference. Chemical shifts are expressed in parts per million (ppm) and coupling constants (J) in hertz . High resolution mass spectra were obtained with a Waters LCT premiere spectrometer, while a Waters SQD mass spectrometer instrument operating in the ESI mode was also used. A reversed-phase HPLC using a Perkin-Elmer LC pump series 200 and a 785A UV/vis detector (214 nM) was used. A Varian microsorb MV 100−5 reversed-phase column (5 μm × 4.6 mm × 250 mm) with the mobile phases being 0.1% TFA in water and 0.1% TFA in ACN with a gradient elution at a flow rate of 1 mL/min was used. Silica gel (230−400 mesh) was used in column chromatography. C18 sep paks were purchased from Waters (Milford, MA). Radioiodinated samples were counted on a Wizard 1470 automatic gamma counter from Perkin-Elmer (Waltham, MA, USA). All chemicals and were either purchased from Fischer Scientific (Pittsburgh, PA, USA) or Sigma (St Louis, MO). All synthesized compounds were characterized and purity determined by ^{1}H NMR, ^{13}C NMR, HPLC, MS, and HRMS. Analytical data confirmed the purity of the products ≥95%.

*Synthesis of β-/naloxamine **12** and β-3-Methoxynaloxamine **13**.* Reductive amination of naloxone **4** and 3-methoxynaloxone **11** was carried out using a literature protocol published by Jiang et al.[27] Typically, 10 g of opiate (30 mmol) was stirred with NH_4OAc (22 g, 0.3 mol, 10 equiv) in 40 mL of dry methanol for 10 min at room temperature. $NaBH_3CN$ (1.31 g, 21 mmol, 0.7 equiv) in 5 mL of dry methanol was then added to the reaction mixture and contents stirred overnight. The reaction was quenched by addition of 10 mL of 1N NaOH, and the solvents were evaporated on a rotavapor at 40 °C. The residue was then extracted with 30 mL of DCM three times; the organic extracts were combined and washed with 25 mL of water. The organic extracts were dried over Na_2SO_4 and concentrated to a white solid, which was purified by silica gel column chromatography. The reaction gave a mixture of α and β isomers. The respective isomers were isolated by column chromatography using 87:10:3 of EtOAc:MeOH:NH_4OH as the eluent. The β isomer had a higher R_f than the α isomer on a TLC plate and eluted first when the mixture was subjected to column chromatography. Yields for β isomer were about 2.5−3 g (25−30%). The stereochemistry of the diastereomers at the C-6 position were determined on the basis of the size of the NMR coupling constant, $J_{5,6}$. For the α-isomer, the chemical shift of the C-5 H is δ 4.58 with 3.5 Hz as the $J_{5,6}$ coupling constant, compared to the literature value[27] of δ 4.55 and 4.0 Hz; for the β-isomer, the chemical shift of the C-5 H is δ 4.24 with 7.1 Hz as the $J_{5,6}$ coupling constant, comparing to the literature value[25,27] of δ 4.18 and 7.4 Hz.

*Synthesis of N-Hydroxysuccinimide (NHS) Esters of Carboxylic Acids **8−10**.* Substituted carboxylic acid (**5−7**, 7.8 mmol), NHS (1 g, 8.6 mmol, 1.1 equiv), and DCC (1.79 g, 8.6 mmol, 1.1 equiv) in 20 mL of dry THF were stirred overnight. The white suspension was filtered, and the clear filterate was evaporated on a rotavapor at 40 °C. The white solid seen was purified by column chromatography using EtOAc/hexanes as eluents. A singlet at δ 2.9 integrating to four protons in ^{1}H NMR and corresponding to four protons of succinimide was seen in all NHS esters of substituted carboxylic acids. Crude yields were about 80−100%. The crude activated ester was used in the next step without any purification.

*Synthesis of Aryl-α-naloxamide, Aryl-β-naloxamides, and β-3-Methoxynaloxamides **15−38**.* Coupling of α-naloxamine, β-naloxamine **12**, or β-3-methoxynaloxamine **13** (opiate-NH_2) to the corresponding carboxylic acid was carried out by either reacting the amine to the NHS-activated carboxylic acid ester or by direct coupling of the amine to the carboxylic acid in presence of BOP.

Procedure I. Opiate-NH_2 (**12−13**, 200 mg, 0.6 mmol) was reacted with DIEA (116 μL, 0.66 mmol, 1.1 equiv) and NHS esters of substituted carboxylic acids (**8−10**, 0.66 mmol, 1.1 equiv) in dry DCM (5 mL) for 2 h. The reaction was diluted to 20 mL with DCM and washed with 5 mL of water. The organic extracts were dried over Na_2SO_4 and then concentrated to a white solid, which was purified by silica gel flash column chromatography using a gradient run of 1−5% MeOH:DCM as eluents.

Alternate Procedure II. Opiate-NH_2 (**12−13**, 200 mg, 0.6 mmol) was reacted with BOP (271 mg, 1.2 mmol, 2 equiv), DIEA (313 μL, 1.8 mmol, 3 equiv), and substituted carboxylic acid (1.2 mmol, 2 equiv) in dry DCM (5 mL) for 2 h. The reaction mixture poured into a small silica gel column and eluted with 100 mL of EtOAc. The ethyl acetate fraction was evaporated, and a white solid was obtained. The solid obtained was hydrolyzed in K_2CO_3 and MeOH. Briefly, the contents, usually a white suspension were stirred with K_2CO_3 (622 mg, 4.22 mmol, 7 equiv) and MeOH for 3 h.The white suspension seen was filtered and the filterate concentrated to a yellowish oil or a white solid. The oily residue or white solid obtained was then purified by flash column chromatography using a gradient run of 1−5% MeOH:DCM as the eluent.

Synthesis of Individual Embodiments. *17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-iodo)benzamido]morphinan (**15**).* Compound **15** was synthesized according to the general procedure (I) described above using β-naloxamine, NHS ester of 3-iodobenzoic acid **8**, and DIEA in DCM. A white solid was obtained. Yield: 255 mg (75%); mp 203−207 °C dec; $[\alpha]^{20}_D$ −161.2 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.16 (s, 1H), 7.8 (d, J = 8.9 Hz, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.15−7.11 (m, 1H), 6.69 (d, J = 10.6 Hz, 1H), 6.57 (d, J = 10.6 Hz, 1H), 5.8 (m, 1H), 5.23−5.16 (m, 2H), 4.57 (d, J = 8.85

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Hz, 1H), 4.13 (m, 1H), 3.14−1.2 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.4, 142.9, 140.3, 139.2, 136.4, 136.2, 135.2, 130.6, 130.1, 126.1, 124.7, 119.3, 118.1, 117.6, 94.3, 92.9, 70.2, 62.4, 57.8, 50.5, 47.3, 43.6, 31.5, 29.0, 23.2, 22.7 ppm. ESI-MS m/z: 559.1 (MH^+). HRMS calcd for $C_{26}H_{28}N_2O_4I$ (MH+), 559.1094; found, 559.1099. HPLC purity: 96.5%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6α-[(3′-iodo)benzamido]-morphinan (**16**).* Compound **16** was synthesized according to the general procedure (I) described above using α-naloxamine, NHS ester of 3-iodobenzoic acid **8** and DIEA in DCM. A white solid was obtained. Yield: 248 mg (73%); mp 134−136 °C; $[\alpha]^{20}_D$ −225.7 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.56 (d, J = 8.1 Hz, 1H), 6.37 (d, J = 8.2 Hz, 1H), 5.80 (m, 1H), 5.18 (d, J = 18.5 Hz, 1H,), 5.15 (d, J = 10.9 Hz, 1H), 4.74 (m, 2H), 3.50−1.00 (m, 15 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.5, 145.1, 140.3, 137.2, 136.6, 136.0, 135.2, 130.8, 130.1, 126.3, 125.9, 119.4, 118.0, 117.3, 94.2, 90.1, 69.7, 62.3, 58.1, 47.2, 46.7, 42.9, 33.3, 28.9, 23.0, 21.0 ppm. MS (ESI) m/z (%) 559 (MH+). HRMS calcd for $C_{26}H_{28}N_2O_4$ I (MH+), 559.1094; found, 559.1107. HPLC purity: 97.5%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(2′-iodo)benzamido]-morphinan (**17**).* Compound **17** was synthesized according to the general procedure (II) described above using β-naloxamine, 2-iodobenzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 204 mg (60%); mp 208−211 °C dec; $[\alpha]^{20}_D$ −180.0 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.87 (d, J = 8.35 Hz, 1H), 7.42 (d, J = 8.35, 1H), 7.38−7.36 (m, 1H), 7.11−7.08 (m, 1H), 6.75 (d, J = 8.35, 1H), 6.6 (d, J = 8.35, 1H), 6.41 (m, 1H), 5.78 (m, 1H), 5.14 (m, 2H), 4.51 (d, J = 8.35, 1H), 4.17 (m, 1H), 3.49−1.26 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 169.2, 142.9, 142.2, 139.9, 139.6, 135.2, 131.1, 130.8, 128.3, 128.2, 124.8, 119.3, 118.0, 117.6, 93.2, 92.4, 70.2, 62.4, 57.7, 50.8, 47.5, 43.6, 31.0, 29.5, 23.5, 22.7 ppm. MS(ESI) m/z (%) 559 (MH+). HRMS calcd for $C_{26}H_{28}N_2O_4$ I (MH+), 559.1094; found, 559.1115. HPLC purity: 96.5%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4′-iodo)benzamido]-morphinan (**18**).* Compound **18** was synthesized according to the general procedure (II) described above using β-naloxamine, 4-iodobenzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 146 mg (43%); mp 210−213 °C dec; $[\alpha]^{20}_D$ −163.2 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.78 (d, J = 9.8 Hz, 2H), 7.53 (d, J = 9.8 Hz, 2H), 6.7 (d, J = 9.8 Hz, 1H), 6.57 (d, J = 9.8 Hz, 1H), 5.82 (m, 1H), 5.23−5.2 (m, 2H), 4.51 (d, J = 8.2 Hz, 1H), 4.23 (m, 1H), 3.19−1.5 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.1, 143.2, 139.0, 137.7, 135.2, 133.9, 130.5, 128.7, 124.9, 119.3, 118.1, 117.5, 98.4, 92.7, 70.2, 62.4, 57.8, 49.9, 47.1, 43.5, 31.9, 28.7, 22.9, 22.7 ppm. MS(ESI) m/z (%) 559 (MH+). HRMS calcd for $C_{26}H_{28}N_2O_4$ I (MH+), 559.1094; found, 559.1099. HPLC purity: 90.1%; α-isomer, 8.6%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-fluoro)benzamido]-morphinan (**19**).* Compound **19** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-fluorobenzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 192 mg (70%); mp 193−196 °C dec; $[\alpha]^{20}_D$ −135.5 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.59 (d, J = 9.2 Hz, 1H), 7.55 (d, J = 9.2 Hz, 1H), 7.41−7.36 (m, 2H), 7.21−7.17 (m, 1H), 6.73 (d, J = 9.2 Hz, 1H), 6.59 (d, J = 10 Hz, 1H), 5.81 (m, 1H), 5.23−5.16 (m, 2H), 4.51 (d, J = 9.2 Hz, 1H), 4.25 (m, 1H), 3.14−1.28 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.6, 143.0, 140.1, 136.4, 130.3, 130.2, 122.8, 119.2, 118.8, 118.7, 118.6, 114.6, 114.4, 93.1, 70.6, 62.4, 57.7, 50.8, 47.4, 43.7, 31.2, 29.3, 23.9, 22.7 ppm. MS(ESI) m/z (%) 451 (MH+). HRMS calcd for $C_{26}H_{28}N_2O_4F$ (MH+), 451.2033; found, 451.2031. HPLC purity: 94.3%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-chloro)benzamido]-morphinan (**20**).* Compound **20** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-chlorobenzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 205 mg (72%); mp 207−211 °C dec; $[\alpha]^{20}_D$ −181.7 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.82 (s, 1H), 7.69 (d, J = 7.85 Hz, 1H), 7.47 (d, J = 7.85 Hz, 1H), 7.39−7.35 (m, 1H), 6.73 (d, J = 8.05 Hz, 1H), 6.59 (d, J = 8.05 Hz, 1H), 5.82−5.81 (m, 1H), 5.2−5.17 (m, 2H), 4.51−4.5 (d, J = 5 Hz, 1H), 4.25 (m, 1H), 3.14−1.28 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.7, 142.9, 139.2, 136.1, 135.2, 134.6, 131.5, 130.5, 129.8, 127.5, 125.1, 124.7, 119.3, 118.1, 117.6, 92.7, 70.3, 62.4, 57.8, 50.5, 47.2, 43.6, 31.6, 29.0, 23.2, 22.7 ppm. MS(ESI) m/z (%) 467 (MH+). HRMS calcd for $C_{26}H_{28}N_2O_4Cl$ (MH+), 467.1738; found, 467.1737. HPLC purity: 97.4%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-bromo)benzamido]-morphinan (**21**).* Compound **21** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-bromobenzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 218 mg (70%); mp 152−154 °C; $[\alpha]^{20}_D$ −193.1 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.72 (d, J = 8.75 Hz, 1H), 7.61 (d, J = 8.75 Hz, 1H), 7.31−7.28 (m, 1H), 7.24−7.22 (m, 1H), 6.72 (d, J = 8.75 Hz, 1H), 6.58 (d, J = 8.75 Hz, 1H), 5.8 (m, 1H), 5.23−5.16 (m, 2H), 4.52 (d, J = 8.75 Hz, 1H), 4.18 (m, 1H), 3.14−1.5 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.6, 143.2, 139.4, 136.4, 135.4, 135.2, 134.5, 130.4, 130.1, 125.6, 124.5, 122.7, 119.3, 118.1, 117.8, 92.5, 70.2, 62.4, 57.8, 50.2, 47.1, 43.5, 31.9, 28.7, 23.0, 22.7 ppm. MS (ESI) m/z (%) 511 (MH+). HRMS calcd for $C_{26}H_{28}N_2O_4Br$ (MH+), 511.1232; found, 511.1250. HPLC purity: 94.4%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-(benzamido)morphinan (**22**).* Compound **22** was synthesized according to the general procedure (II) described above using β-naloxamine, benzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 84 mg (32%); mp 177−181 °C; $[\alpha]^{20}_D$ −58.4 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.82 (d, J = 9.2 Hz, 2H), 7.51−7.42 (m, 3H), 7.20 (m, 1H), 6.74 (d, J = 9.2 Hz, 1H), 6.59 (d, J = 9.2 Hz, 1H), 5.82 (m, 1H), 5.23−5.17 (m, 2H), 4.5 (d, J = 7.65 Hz, 1H), 4.26 (m, 1H), 3.13−1.25 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.9, 143.3, 139.2, 135.2, 134.5, 131.5, 130.7, 128.6, 127.0, 125.0, 119.2, 118.1, 117.5, 93.3, 70.2, 62.5, 57.8, 49.8, 47.2, 43.6, 31.7, 28.9, 23.2, 22.7 ppm. MS (ESI) m/z (%) 433 (MH+). HRMS calcd for $C_{26}H_{29}N_2O_4$ (MH+), 433.2127; found, 433.2125. HPLC purity: 90.4%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-methyl)benzamido]-morphinan (**23**).* Compound **23** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-toluic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 133 mg (49%); mp 218−221 °C dec; $[\alpha]^{20}_D$ −163.4 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.67 (m, 2H), 7.51 (s, 1H), 7.35 (d, J = 8.1 Hz, 1H), 6.71 (d, J = 8.1 Hz, 1H), 6.61 (d, J = 8.1 Hz, 1H), 5.82 (m, 1H), 5.23−5.17 (m, 2H), 4.55 (d, J = 7.05 Hz, 1H), 4.06 (m, 1H), 3.36−1.5 (m, 16H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.3, 143.1, 139.3, 138.4, 135.2, 134.4, 132.3, 130.7, 128.4, 127.8, 124.8, 123.9, 119.2, 118.1, 117.6, 93.3, 70.2, 62.5, 57.8, 50.2, 47.3, 43.6, 31.5, 29.1, 23.5, 22.7, 21.4 ppm. MS(ESI) m/z (%) 447 (MH+). HRMS calcd for $C_{27}H_{31}N_2O_4$ (MH+), 447.2284; found, 447.2290. HPLC purity: 98.6%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-trifluoromethyl)-benzamido]morphinan (**24**).* Compound **24** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-trifluorotoluic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 210 mg (69%); mp 88−91 °C; $[\alpha]^{20}_D$ −126.7 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (s, 3H), 8.0 (m, 1H), 7.89−7.88 (m, 1H), 7.65 (m, 1H), 7.45 (m, 1H), 6.62 (d, J = 8.15 Hz, 1H), 6.5 (d, J = 8.15 Hz, 1H), 5.78−5.74 (m, 1H), 5.2−5.13 (m, 2H), 4.67 (d, J = 6.15 Hz, 1H), 4.11−4.02 (m, 1H), 3.54−1.24 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.5, 143.1, 139.2, 135.2, 130.5, 130.3, 129.1, 128.1, 124.8, 124.2, 119.3, 118.1, 117.5, 92.8, 70.2, 62.4, 57.8, 50.2, 47.2, 43.5, 31.8, 28.8, 23.0, 22.7 ppm. MS (ESI) m/z (%) 501 (MH+). HRMS calcd for $C_{27}H_{28}N_2O_4F_3$ (MH+), 501.2001; found, 501.2004. HPLC purity: 96.4%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-methoxy)-benzamido]morphinan (**25**).* Compound **25** was synthesized

according to the general procedure (II) described above using β-naloxamine, 3-anisic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 169 mg (60%); mp 113−115 °C; $[\alpha]^{20}_{D}$ −124.4 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 9 Hz, 1H), 7.39−7.26 (m, 3H), 7.0 (m, 1H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 5.78−5.74 (m, 1H), 5.24−5.17 (m, 2H), 4.52 (d, *J* = 6.2 Hz, 1H), 4.12−4.11 (m, 1H), 3.78 (s, 3H), 3.72−1.25 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.9, 159.8, 143.1, 139.3, 135.9, 134.9, 130.6, 129.5, 124.6, 119.2, 118.8, 117.8, 117.7, 112.4, 93.0, 70.2, 62.5, 57.8, 55.4, 50.2, 47.2, 43.7, 31.5, 29.0, 23.3, 22.7 ppm. MS (ESI) *m*/*z* (%) 463 (MH+). HRMS calcd for $C_{27}H_{31}N_2O_5$ (MH+), 463.2233; found, 463.2232. HPLC purity: 97.0%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-amino)benzamido]-morphinan (**26**).* Compound **26** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-amino benzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 82 mg (30%); mp 191−193 °C dec; $[\alpha]^{20}_{D}$ −148.7 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.2−7.16 (m, 1H), 7.1 (d, *J* = 7.95 Hz, 1H), 6.90 (d, *J* = 7.95 Hz, 1H), 6.8 (d, *J* = 7.95 Hz, 1H), 6.75 (d, *J* = 8.1 Hz, 1H), 6.69 (d, *J* = 8.1 Hz, 1H), 5.81−5.8 (m, 1H), 5.19−5.16 (m, 2H), 4.46 (d, *J* = 5.85 Hz, 1H), 4.21−4.19 (m, 1H), 3.48−1.22 (m, 16H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.6, 146.6, 143.0, 139.6, 135.5, 135.3, 130.7, 129.3, 124.4, 119.1, 118.3, 118.0, 117.9,116.8, 114.5, 92.8, 70.4, 62.5, 57.7, 50.7, 47.3, 43.6, 31.2, 29.2, 23.7, 22.7 ppm. MS (ESI) *m*/*z* (%) 448 (MH+). HRMS calcd for $C_{26}H_{30}N_3O_4$ (MH+), 448.2236; found, 448.2230. HPLC purity: 95.4%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-dimethylamino)-benzamido]morphinan (**27**).* Compound **27** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-dimethylamino benzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 174 mg (60%); mp 170−173 °C; $[\alpha]^{20}_{D}$ −127.3 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.21 (s, 1H), 7.02 (d, *J* = 10 Hz, 1H), 6.89−6.85 (m, 1H), 6.75 (d, *J* = 10 Hz, 1H), 6.59 (m, 1H), 5.79 (m, 1H), 5.22−5.16 (m, 2H), 4.48 (d, *J* = 10 Hz, 1H), 4.22 (m, 1H), 3.14−1.5 (m, 20H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.8, 150.7, 143.2, 139.4, 135.3, 130.7, 129.1, 119.1, 118.0, 117.6, 115.3, 114.2, 111.6, 93.1, 70.2, 62.5, 57.8, 53.4, 50.2,47.2, 43.6, 40.5, 31.5, 29.1, 23.4, 22.7 ppm. MS (ESI) *m*/*z* (%) 476 (MH+). HRMS calcd for $C_{28}H_{34}N_3O_4$ (MH+), 476.2549; found, 476.2544. HPLC purity: 96.5%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-hydroxy)-benzamido]morphinan (**28**).* Compound **28** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-hydroxy benzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 107 mg (39%); mp 201−204 °C dec; $[\alpha]^{20}_{D}$ −170.7 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 (m, 3H), 7.3−7.28 (m, 2H), 6.99 (d, *J* = 7.75 Hz, 1H), 6.71 (d, *J* = 7.75 Hz, 1H), 6.6 (d, *J* = 7.75 Hz, 1H), 5.82−5.8 (m, 1H), 5.22−5.17 (m, 2H), 4.51 (d, *J* = 7.75 Hz, 1H), 4.062 (m, 1H), 3.51−1.51 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 168.0, 156.7, 142.7, 139.8, 135.1, 134.8, 130.6, 129.7, 124.1, 119.5, 119.2, 118.2, 118.1, 115.1, 93.3, 70.6, 62.4, 57.7, 51.3, 47.4, 43.7, 30.9, 29.3, 24.2, 22.7 ppm. MS (ESI) *m*/*z* (%) 449 (MH+). HRMS calcd for $C_{26}H_{29}N_2O_5$ (MH+), 449.2076; found, 449.2080. HPLC purity: 95.5%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(3′-nitro)benzamido]-morphinan (**29**).* Compound **29** was synthesized according to the general procedure (II) described above using β-naloxamine, 3-nitro benzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 172 mg (59%); mp 228−231 °C dec; $[\alpha]^{20}_{D}$ −128.8 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.36−8.34 (m, 1H), 8.22 (d, *J* = 11.8 Hz, 1H), 7.67−7.63 (m, 2H), 6.69 (d, *J* = 11.8 Hz, 1H), 6.58 (d, *J* = 11.8 Hz, 1H), 5.81 (m, 1H), 5.2−5.17 (m, 2H), 4.59 (d, *J* = 9.8 Hz, 1H), 4.27 (m, 1H), 3.14−1.25 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.6, 148.1, 141.6, 139.4, 135.5, 135.2, 133.7, 130.6, 129.4, 125.8, 124.6, 121.9, 119.4, 118.1, 117.3, 92.1, 70.6, 62.4, 57.9, 51.9, 47.4, 43.4, 38.6, 31.0, 29.5, 23.7,22.7 ppm. MS(ESI) *m*/*z* (%) 478 (MH+). HRMS calcd for $C_{26}H_{28}N_3O_6$ (MH+), 479.1978; found, 478.1967. HPLC purity: 97.0%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4′-trifluoromethoxy)-benzamido]morphinan (**30**).* Compound **30** was synthesized according to the general procedure (II) described above using β-naloxamine, 4-(trifluoromethoxy)benzoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 248 mg (79%); mp 138−140 °C; $[\alpha]^{20}_{D}$ −136.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.87 (d, *J* = 11.75 Hz, 1H), 7.44 (d, *J* = 11.75 Hz, 1H), 7.24 (m, 2H), 6.72 (d, *J* = 11.75 Hz, 1H), 6.58 (d, *J* = 11.75 Hz, 1H), 5.81 (m, 1H), 5.23−5.16 (m, 2H), 4.53 (d, *J* = 9.8 Hz, 1H), 4.24 (m, 1H), 3.33−1.28 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.7, 151.4, 143.1, 139.1, 135.1, 132.8, 130.5, 129.0, 124.7, 120.5, 119.3, 118.2, 117.6, 92.6, 70.3, 62.4, 57.8, 50.2, 47.1, 43.5, 31.8, 28.8, 23.0, 22.6 ppm. MS (ESI) *m*/*z* (%) 517 (MH+). HRMS calcd for $C_{27}H_{28}N_2O_5F_3$ (MH+), 517.1950; found, 517.1956. HPLC purity: 99.9%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(2′-naphthyl)-acetamido]morphinan (**31**).* Compound **31** was synthesized according to the general procedure (I) described above using β-naloxamine, NHS ester of naphthalene-2-carboxylic acid **9**, and DIEA in DCM. A white solid was obtained. Yield: 261 mg (89%); mp 210−214 °C dec; $[\alpha]^{20}_{D}$ −260.2 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.17 (s, 1H), 7.78−7.70 (m, 4H), 7.47 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.51 (d, *J* = 8.1 Hz, 1H), 5.79 (m, 1H), 5.16 (m, 2H), 4.80 (m, 1H), 4.73 (d, *J* = 4.3 Hz, 1H), 3.10−1.05 (m, 15 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.3, 145.3, 137.4, 134.6, 132.5, 131.7, 128.9, 128.1, 127.6, 127.5, 127.4, 126.4, 123.9, 119.2, 117.4, 93.5, 90.2, 70.2, 69.7, 62.4, 58.1, 47.1, 46.6, 42.9, 33.3, 29.0, 22.9, 21.0 ppm. MS (ESI) *m*/*z* (%) 483 (MH+). HRMS calcd for $C_{30}H_{31}N_2O_4$ (MH+), 483.2284; found, 483.2293. HPLC purity: 97.3%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4′-phenyl)benzamido]-morphinan (**32**).* Compound **32** was synthesized according to the general procedure (I) described above using β-naloxamine, NHS ester of biphenyl-4-carboxylic acid **10**, and DIEA in DCM. A white solid was obtained. Yield: 263 mg (85%); mp 210−214 °C dec; $[\alpha]^{20}_{D}$ −145.8 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.15 Hz, 2H), 7.66−7.61 (m, 4H), 7.46 (m, 3H), 7.38 (m, 1H), 6.74 (d, *J* = 8.15 Hz, 1H), 6.61 (d, *J* = 8.15 Hz, 1H), 5.82−5.79 (m, 1H), 5.23−5.17 (m, 2H), 4.53−4.52 (d, *J* = 5.15 Hz, 1H), 4.31−4.29 (m, 1H), 3.15−1.25 (m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.7, 144.2, 143.2, 140.0, 139.2, 135.2, 133.1, 130.6, 128.9, 128.0, 127.6, 127.2, 124.8, 119.2,118.1,117.6, 92.9, 70.2, 62.5, 57.8, 50.1, 47.2, 43.6, 31.7, 28.9, 23.2, 22.7 ppm. ESI-MS m/*z*: 509.09 (MH^+). HPLC purity: 95.9%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-(acetylamido)-morphinan (**33**).* Compound **33** was synthesized according to the general procedure (II) described above using β-naloxamine, acetic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 120 mg (53%); mp 204−207 °C dec; $[\alpha]^{20}_{D}$ −157.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.70 (d, *J* = 8.2 Hz, 1H,), 6.56 (d, *J* = 8.2 Hz, 1H), 5.96 (d, *J* = 9.2 Hz, 1H), 5.76 (m, 1H,), 5.18 (d, *J* = 17.8 Hz, 1H,), 5.14 (d, *J* = 10.5 Hz, 1H,), 4.33 (d, *J* = 6.5 Hz, 1H), 3.89 (m, 1H), 3.15−0.80 (m, 18 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 170.6, 142.8, 139.8, 135.2, 130.8, 124.3, 119.1, 118.0, 117.8, 93.0, 70.4, 62.4, 57.7, 50.7, 47.4, 43.7, 30.9, 29.5, 23.9, 23.6, 22.7 ppm. MS(ESI) *m*/*z* (%) 371 (MH+). HRMS calcd for $C_{21}H_{27}N_2O_4$ (MH+), 371.1971; found, 371.1965. HPLC purity: 97.0%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-(hexylamido)morphinan (**34**).* Compound **34** was synthesized according to the general procedure (II) described above using β-naloxamine, hexanoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 130 mg (50%); mp 113−116 °C; $[\alpha]^{20}_{D}$ −101.6 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.71 (d, *J* = 8.2 Hz, 1H), 6.55 (d, *J* = 8.2 Hz, 1H), 6.07 (d, *J* = 9.2 Hz, 1H), 5.77 (m, 1H), 5.18 (d, *J* = 17.4 Hz, 1H), 5.14 (d, *J* = 10.1 Hz, 1H), 4.34 (d, *J* = 6.4 Hz, 1H), 3.91 (m, 1H), 3.15−0.80 (m, 26 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 173.0, 142.9, 139.6, 135.3, 130.8, 124.7, 119.2, 118.0,

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117.6, 93.7, 70.1, 62.5, 57.7, 50.1, 47.4, 43.6, 37.1, 31.4, 31.1, 29.5, 25.4, 23.9, 22.7, 22.4, 14.0 ppm. MS(ESI) m/z (%) 427 (MH+). HRMS calcd for $C_{26}H_{35}N_2O_4$ (MH+), 427.2597; found, 427.2591. HPLC purity: 96.0%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-(dodecylamido)-morphinan (**35**).* Compound **35** was synthesized according to the general procedure (II) described above using β-naloxamine, dodecanoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 202 mg (65%); mp 95−98 °C; $[\alpha]^{20}_D$ −99.9 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.71 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 8.2 Hz, 1H,), 6.07 (d, J = 9.2 Hz, 1H,), 5.76 (m, 1H), 5.18 (d, J = 17.4 Hz, 1H,), 5.14 (d, J = 10.1 Hz, 1H), 4.34 (d, J = 6.4 Hz, 1H), 3.91 (m, 1H), 3.10−0.86 (m, 38 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 173.1, 142.9, 139.7, 130.7, 119.2, 117.7, 93.7, 70.1, 62.5, 57.7, 53.4, 50.1, 47.4, 43.7, 37.1, 31.9, 29.6, 29.5, 29.3, 29.2, 25.7, 23.9, 22.7, 14.1 ppm. MS(ESI) m/z (%) 511 (MH+). HRMS calcd for $C_{31}H_{47}N_2O_4$ (MH+), 511.3536; found, 511.3550. HPLC purity: 95.3%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-(cyclohexylamido)-morphinan (**36**).* Compound **36** was synthesized according to the general procedure (II) described above using β-naloxamine, cyclohexanoic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 174 mg (65%); mp 236−239 °C dec; $[\alpha]^{20}_D$ −135.2 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.71 (d, J = 8.1 Hz, 1H), 6.55 (d, J = 8.0 Hz, 1H), 6.14 (d, J = 9.1 Hz, 1H), 5.77 (m, 1H), 5.18 (d, J = 17.4 Hz, 1H), 5.14 (d, J = 10.0 Hz, 1H), 4.33 (d, J = 6.1 Hz, 1H), 3.93 (m, 1H), 3.15−0.80 (m, 26 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 176.0, 143.1, 139.5, 135.3, 130.8, 124.7, 119.1, 118.0, 117.6, 93.7, 70.1, 62.5, 57.7, 49.7, 47.3, 45.7, 43.6, 31.3, 29.7, 29.6, 29.3, 25.8, 25.7, 23.6, 22.7 ppm. MS (ESI) m/z (%) 439 (MH+). HRMS calcd for $C_{26}H_{35}N_2O_4$ (MH+), 439.2597; found, 439.2602. HPLC purity: 93.2%.

*17-Allyl-3,14β-dihydroxy-4,5α-epoxy-6β-[2-adamantylamido]-morphinan (**37**).* Compound **37** was synthesized according to the general procedure (II) described above using β-naloxamine, 1-adamantyl carboxylic acid, BOP, and DIEA in DCM followed by base hydrolysis. A white solid was obtained. Yield: 227 mg (76%); mp 153−156 °C; $[\alpha]^{20}_D$ −129.0 (c 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.71 (d, J = 8.2 Hz, 1H,), 6.55 (d, J = 8.2 Hz, 1H), 6.22 (d, J = 9.5 Hz, 1H), 5.77 (m, 1H), 5.28 (s, 1H), 5.18 (d, J = 17.2 Hz, 1H), 5.14 (d, J = 10.2 Hz, 1H), 4.31 (d, J = 5.9 Hz, 1H), 3.97 (m, 1H), 3.15−0.76 (m, 29 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 178.1, 162.6, 143.2, 139.6, 135.3, 130.7, 124.4,119.0,118.0, 117.8, 93.0, 70.2, 62.4, 57.8, 49.6, 47.1, 43.6, 40.7, 39.1, 38.6, 36.5, 31.7, 31.5, 28.9, 28.1, 23.2, 22.6 ppm. MS (ESI) m/z (%) 491 (MH+). HRMS calcd for $C_{30}H_{39}N_2O_4$ (MH+), 491.2910; found, 491.2912. HPLC purity: 91.9%.

*17-Allyl-4,5α-epoxy-14β-hydroxy-3β-methoxy-6β-[(3′-iodo)-benzamido]morphinan (**38**).* Compound **38** was synthesized according to the general procedure (I) described above using 3-OMe-β-naloxamine, NHS ester of 3-iodobenzoic acid **10**, and DIEA in DCM. A white solid was obtained. Yield: 296 mg (85%); mp 125−127 °C; $[\alpha]^{20}_D$ −133.7 (c 0.1, $CHCl_3$). ^{1}H NMR δ: 8.19 (s, 1H), 7.8 (m, 1H), 7.42 (m, 1H), 7.16 (m, 1H), 6.75 (d, J = 10 Hz, 1H), 6.66 (d, J = 10 Hz, 1H), 5.85 (m, 1H), 5.18 (m, 2H), 4.61 (d, 1H), 4.08 (m, 1H), 3.85 (s, 2H), 3.15−0.1(m, 14H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.2, 144.9, 144.3, 136.4, 130.8, 130.1, 127.3, 126.3, 124.4, 120.9, 119.4, 115.8, 94.2, 91.9, 72.7, 71.8, 71.7, 60.8, 57.0, 56.9, 50.0, 47.1, 30.9, 28.1, 26.7, 22.6 ppm. MS (ESI) m/z (%) 573 (MH+). HRMS calcd for $C_{27}H_{30}N_2O_4I$ (MH+), 573.1250; found, 573.1252. HPLC purity: 96.6%.

Receptor-Binding Assays. Competition-binding assays in CHO cells stably expressing MOR-1 (μ), DOR-1 (δ), or KOR-1 (κ) were performed at 25 °C in potassium phosphate buffer (50 mM; pH 7.4), with the inclusion of $MgSO_4$ (5 mM) in the MOR-1 assays. All competition assays were carried out using ^{125}I-BNtxA (**1**) as described.[39] Specific binding was defined as the difference between total binding and nonspecific binding, determined in the presence of levallorphan (8 μM). Protein concentrations were between 30 and 40 μg/mL, and incubation times were 90 min. 6TM/E11 competition binding assays using ^{125}I-BNtxA (**1**; 0.15 nM) were carried out in whole brain membrane homogenates (0.5 mL; 0.5 mg protein) at 25 °C in potassium phosphate buffer (50 mM; pH 7.4) with magnesium sulfate (5 mM) for 90 min in the presence of CTAP, U50488H, and DPDPE, all at 200 nM, to block traditional opioid binding sites. Specific binding was defined as the difference between total binding and nonspecific binding, determined in the presence of levallorphan (8 μM). Protein concentration was determined as described by Lowry et al.,[40] using bovine serum albumin as the standard.

Tail Flick Analgesia Assays. Male CD-1 mice (25−35 g; Charles River Breeding Laboratories, Wilmington, MA) were maintained on a 12 h light/dark cycle with Purina rodent chow and water available ad libitum. Mice were housed in groups of five until testing. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center. Analgesia was determined using the radiant heat tail flick technique[41] using a machine from (Ugo Basile; model 37360). The intensity was set to achieve a baseline between 2 and 3 s. The latency to withdraw the tail from a focused light stimulus was measured electronically using a photocell. Baseline latencies (2.0−3.0 s) were determined before experimental treatments for all animals as the mean of two trials. Post-treatment tail flick latencies were determined as indicated for each experiment, and a maximal latency of 10 s for tail flick was used to minimize tissue damage. Drugs were given subcutaneously and cumulative dose−response experiments carried out with two independent assays with each group (n = 10). The combined results presented as the ED_{50} with 95% confidence limits (n = 20) presented. Analgesia was defined quantally as a doubling, or greater, of the baseline latency. Similar results were obtained analyzing the data in a graded response manner. Analgesic ED_{50}s and confidence limits were determined using nonlinear regression analysis Graph Pad Prism (Graphpad Software, La Jolla, CA).

Respiratory Depression Assay. Respiratory rate was assessed in awake, freely moving, adult male mice using the MouseOx pulse oximeter system (Starr Life Sciences, Pittsburgh, PA). Each animal was habituated to the device for 30 min and then tested. A 5 s average breath rate was assessed at 5 min intervals. A baseline for each animal was obtained over a 25 min period prior to drug injection, and testing began 15 min postinjection and continued for a period of 35 min. Groups of mice (n = 5) were treated subcutaneously with either morphine (20 mg/kg sc) or **15** (2.5 mg/kg sc) at doses approximately four times their analgesic ED_{50}. Groups were compared with repeated measures ANOVA followed by Bonferroni as a post test.

Gastrointestinal Motility Assay. Gastrointestinal transit was determined as described.[5] Animals received the indicated drug subcutaneously, followed by a charcoal meal by gavage, were sacrificed 30 min later and the distance traveled measured.

Physical Dependence Studies. Tolerance was induced by twice daily injections for at least five days with either morphine (10 mg/kg sc) or **15** (1 mg/kg sc). Dependence was determined on day 5 with naloxone or levallorphan (1 mg/kg, sc) to precipitate withdrawal and animals evaluated for signs of diarrhea and jumping.[6,42]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLCs of compounds **15**−**38**. 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

6TM/E11, six transmembrane/exon 11; MOR-1, μ opioid receptor; KOR-1, κ_1 opioid receptor; DOR-1, δ opioid receptor; IBNtxA, 3′-iodobenzoylnaltrexamide; IBNalA, 3′-iodobenzoylnaloxamide; CPM, cyclopropylmethyl; sc, subcutaneous; DIEA, diisopropyl ethyl amine; K_2CO_3, potassium carbonate; NHS, *N*-hydroxysuccinimide; NH_4OAc, ammonium acetate; $NaBH_3CN$, sodium cyanoborohydride; ACN, acetonitrile; NaOH, sodium hydroxide; N_2, nitrogen gas; MeOH, methanol; BOP, benzotriazol-1yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate; CHO, Chinese hamster ovary cells; CPM, cyclopropylmethyl; DPDPE, [D-Pen^2,D-Pen^5]enkephalin

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