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Identification of Opioid Ligands Possessing Mixed μ Agonist/δ Antagonist Activity among Pyridomorphinans Derived from Naloxone, Oxymorphone, and Hydropmorphone

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A series of pyridomorphinans derived from naloxone, oxymorphone, and hydromorphone (**7a**−**k**) were synthesized and evaluated for binding affinity at the opioid δ, μ, and κ receptors in brain membranes using radioligand binding assays and for functional activity in vitro using [^{35}S]GTP-γ-S binding assays in brain tissues and bioassays using guinea pig ileum (GPI) and mouse vas deferens (MVD) smooth muscle preparations. The pyridine ring unsubstituted pyridomorphinans possessing the oxymorphone and hydromorphone framework displayed nearly equal binding affinity at the μ and δ receptors. Their affinities at the κ site were nearly 10-fold less than their binding affinities at the μ and δ sites. Introduction of aryl substituents at the 5′-position on the pyridine ring improved the binding affinity at the δ site while decreasing the binding affinity at the μ site. Nearly all of the ligands possessing an *N*-methyl group at the17-position with or without a hydroxyl group at the 14-position of the morphinan moiety displayed agonist activity at the μ receptor with varying potencies and efficacies. In the [^{35}S]GTP-γ-S binding assays, most of these pyridomorphinans were devoid of any significant agonist activity at the δ and κ receptors but displayed moderate to potent antagonist activity at the δ receptors. In antinociceptive evaluations using the warm-water tail-withdrawal assay in mice, the pyridomorphinans produced analgesic effects with varying potencies and efficacies when administered by the intracerebroventricular route. Among the ligands studied, the hydromorphone-derived 4-chlorophenylpyridomorphinan **7h** was identified as a ligand possessing a promising profile of mixed μ agonist/δ antagonist activity in vitro and in vivo. In a repeated administration paradigm in which the standard μ agonist morphine produces significant tolerance, repeated administration of the μ agonist/δ antagonist ligand **7h** produced no tolerance. These results indicate that appropriate molecular manipulations of the morphinan templates could provide ligands with mixed μ agonist/δ antagonist profiles and such ligands may have the potential of emerging as novel analgesic drugs devoid of tolerance, dependence, and related side effects.

Introduction

Chronic pain represents a major health and economic problem throughout the world. Despite significant advances in understanding the physiological and pathological basis of pain, an ideal analgesic is yet to be discovered. Among analgesic drugs, the opioid class of compounds still remains the effective treatment agents for severe and chronic pain.[1,2] The opioid drugs produce their biological effects through their interaction with the opioid receptors, which belong to the family of seven transmembrane G-protein-coupled receptors. The existence of three opioid receptor types μ, δ, and κ has been clearly established and is confirmed by cloning of these three receptors from mouse, rat, and human cDNAs.[3,4] All three opioid receptor types are located in the human central nervous system, and each has a role in the mediation of pain. Morphine and related opioids currently prescribed as potent analgesics for the treatment of pain produce their analgesic activity primarily through their agonist action at the μ opioid receptors. The general administration of these medications is limited by significant side effects such as respiratory depression, muscle rigidity, emesis, constipation, tolerance, and physical dependence.[5,6]

A large body of evidence indicates the existence of physical or functional interactions between μ and δ receptors. Ligands with agonist or antagonist action at the δ receptor, for example, have been shown to modulate the analgesic and adverse effects of μ agonists.[7−11] Whereas agonist action at the δ receptors potentiate μ-mediated analgesic effects, antagonist action at the δ receptor suppresses the tolerance, physical dependence, and related side effects of μ agonists without affecting their analgesic activity. In a study using the non-peptide ligand naltrindole (**1**, Chart 1), Abdelhamid and co-

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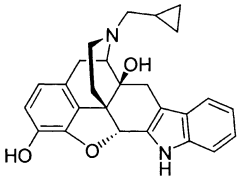
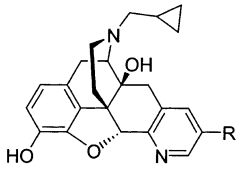
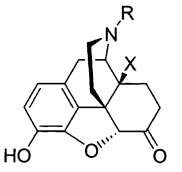
Chart 1



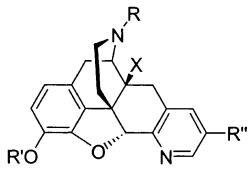
1



2a, R = H
2b, R = Ph
2c, R = 4-ClPh



3, R = CPM, X = OH
4, R = Allyl, X = OH
5, R = Me, X = OH
6, R = Me, X = H



7

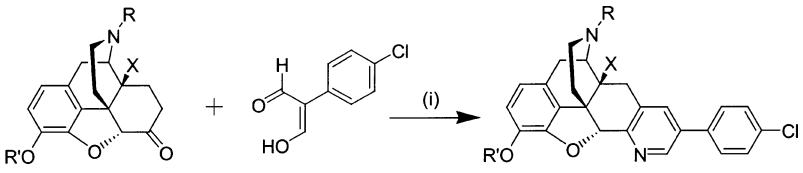
R = CPM, Allyl, Me
R' = H, Me
X = H, OH
R" = H, Ph, 4-ClPh

workers demonstrated that the δ receptor antagonist greatly reduced the development of morphine tolerance and dependence in mice in both the acute and chronic models without affecting the analgesic actions of morphine.[12] Fundytus and co-workers reported that continuous infusion of the δ selective antagonist TIPP[Ψ] by the intracerbroventricular (icv) route in parallel with continuous administration of morphine by the subcutaneous route to rats attenuated the development of morphine tolerance and dependence to a large extent.[13] Schiller and co-workers found that the peptide ligand DIPP-NH_2[Ψ] displayed mixed μ agonist/δ antagonist properties in vitro and that the compound given icv produced an analgesic effect with no physical dependence and less tolerance than morphine in rats.[14,15] Studies with antisense oligonucleotides of the δ receptor have demonstrated that reduction of δ receptor expression diminishes the development and/or expression of morphine dependence without compromising antinociception produced by μ agonists.[16,17] Furthermore, genetic deletion studies using δ receptor knockout mice have shown that these mutant mice retain supraspinal analgesia and do not develop analgesic tolerance to morphine.[18] These observations suggest that the development of opioid ligands, especially non-peptide ligands possessing mixed μ agonist/δ antagonist activity, may provide a novel approach for the development of analgesic agents with low propensity to produce tolerance, physical dependence, and other side effects.

In our studies on naltrexone-derived heterocycle annulated morphinan ligands, we found that the pyridomorphinan **2a** displayed high-affinity binding at the opioid receptors and that the binding affinity and antagonist potency of the pyridomorphinans at the δ receptors are modulated by the substituents placed at the 5′-position on the pyridine moiety. For example, the introduction of aromatic groups such as a phenyl group (**2b**) or a 1-pyrrolyl group at this position gave ligands with high binding affinity and improved δ antagonist potency as determined in bioassays using mouse vas deferens smooth muscle preparations.[19,20] Interestingly, among phenyl ring substituted analogues of **2b**, the *p*-chlorophenyl compound (**2c**) displayed a mixed μ agonist/δ antagonist profile of activity in the smooth muscle assays in vitro.[19] In analgesic activity evaluations, this compound displayed partial agonist activity in the warm-water tail-withdrawal assay and a full agonist activity in the acetic acid writhing assay after icv or ip administration in mice, and it did not produce tolerance to antinociceptive effects on repeated ip injections. Studies in mice with selective antagonists characterized this compound as a partial μ agonist/δ antagonist.[21] Paradoxically, however, in the in vitro biochemical assays using [^{35}S]GTP-γ-S binding, compound **2c** failed to display μ agonist activity in guinea pig caudate membranes as well as in cloned cells expressing human μ receptors.[22] In view of the weak μ agonist activity and the confounding in vitro/in vivo profile of this compound, we decided to explore newer analogues to identify compounds possessing a μ agonist/δ antagonist profile of activity in vitro and in vivo.

Toward this goal, we initially explored a series of analogues of **2c** in which the *p*-chlorophenyl substituent on the pyridine ring was replaced with a number of substituted aryl and heteroaryl groups and found that none of these displayed any significant μ agonist activity in the [^{35}S]GTP-γ-S binding assay in guinea pig caudate membranes even at the high concentration of 10 μM.[23] These results indicated that naltrexone-derived pyridomorphinans possessing the cyclopropylmethyl (CPM) group at the N-17 position of the morphinan unit, in general, may bind to and stabilize the receptors in the antagonist state. It is known that the nature of the nitrogen substituent on the basic nitrogen plays an important role in the agonist or antagonist functional activity among morphinan and 6,7-benzomorphan ligands possessing selectivity toward μ receptors. For example, the 4,5-epoxymorphinan ketones naltrexone (**3**) and naloxone (**4**) possessing the *N*-cyclopropylmethyl and *N*-allyl groups interact as antagonists whereas oxymorphone (**5**) and hydromrophone (**6**) possessing the *N*-methyl group display agonist activity at the μ receptors.[24−26] In contrast, among epoxymorphinan ligands possessing selectivity toward δ receptors, the influence of the N-substituent on the agonist or antagonist functional activity remains unpredictable. For example, the replacement of the *N*-CPM group in naltrindole (**1**) with a methyl group yielded a ligand (oxymorphindole) possessing only partial agonist activity at δ receptors. Replacement with other alkyl groups, such as ethyl and higher alkyl groups, produced ligands that displayed only an antagonist profile of intrinsic activity.[26−28] In addition to the N-substituent, the presence or absence of a hydroxyl group at the 14-position also influences the agonist or antagonist potency of 4,5-epoxymorphinan ligands.[29] In view of these results, it was of interest to synthesize and evaluate N-17 and C-14 modified analogues of **2a**−**c** to study the effect of such modifications on the binding affinity and functional activity of the resulting compounds at the opioid μ, δ, and κ receptors. Herein, we describe the results of this investigation that led to the identification of ligands with varying intrinsic activity profiles including those that displayed the desired mixed μ agonist/δ antagonist profile of activity among the group of ligands generically represented by **7**.

Scheme 1



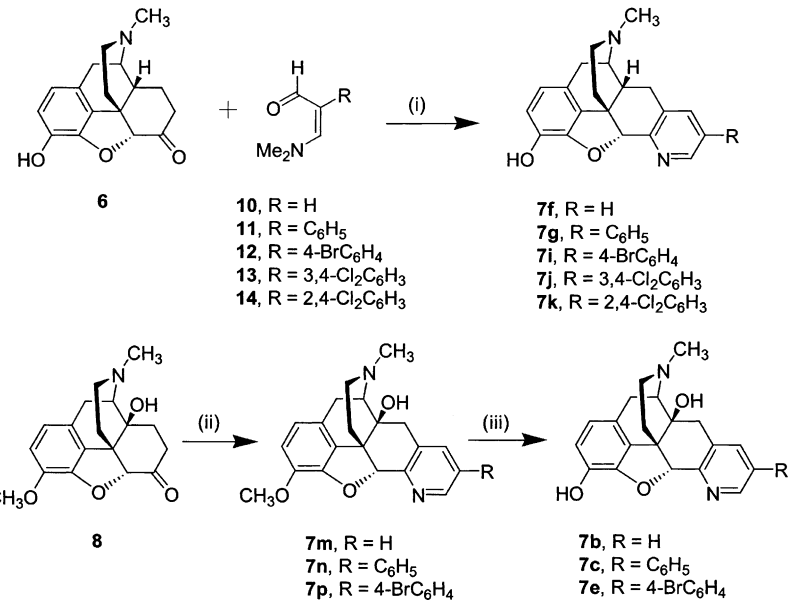
4, R = $CH_2CH{=}CH_2$, R' = H, X = OH
6, R = CH_3, R' = H, X = H
8, R = CH_3, R' = CH_3, X = OH

9

7a, R = $CH_2CH{=}CH_2$, R' = H, X = OH
7h, R = CH_3, R' = H, X = H
7o, R = CH_3, R' = CH_3, X = OH
7d, R = CH_3, R' = H, X = OH (ii)

[a] Reagents and reaction conditions: (i) $AcONH_4$, AcOH, reflux, 18 h; (ii) BBr_3, CH_2Cl_2, −20 °C, 4 h.

Scheme 2



6

10, R = H
11, R = C_6H_5
12, R = 4-BrC_6H_4
13, R = 3,4-$Cl_2C_6H_3$
14, R = 2,4-$Cl_2C_6H_3$

7f, R = H
7g, R = C_6H_5
7i, R = 4-BrC_6H_4
7j, R = 3,4-$Cl_2C_6H_3$
7k, R = 2,4-$Cl_2C_6H_3$

8

7m, R = H
7n, R = C_6H_5
7p, R = 4-BrC_6H_4

7b, R = H
7c, R = C_6H_5
7e, R = 4-BrC_6H_4

[a] Reagents and reaction conditions: (i) $AcONH_4$, AcOH, reflux, 18 h; (ii) **10**, **11**, or **12**, $AcONH_4$, AcOH, reflux, 18 h; (iii) BBr_3, CH_2Cl_2, −20 °C, 4 h.

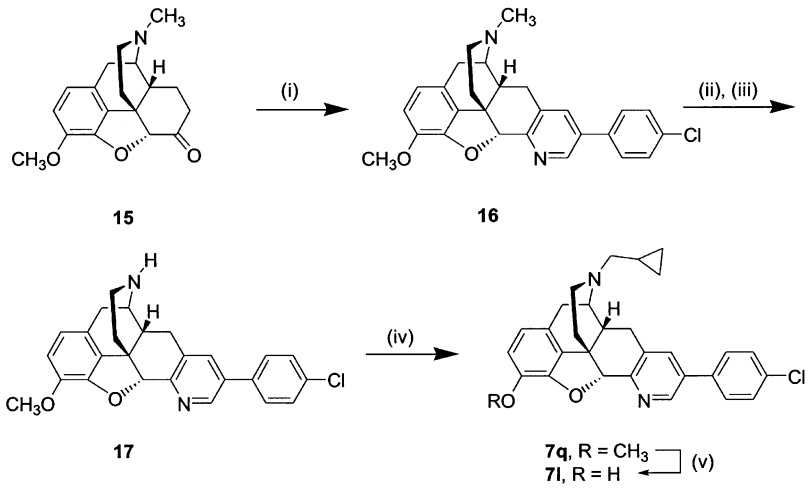
Chemistry

The synthesis of the pyridomorphinan target compounds was accomplished from commercially available morphinan ketones using the pyridine annulation methodolgy that we had earlier developed for the prototype compounds.[19,20,23] As depicted in Scheme 1, the condensation of naloxone (**4**) or hydromorphone (**6**) with 4-chlorophenylmalondialdehyde (**9**) in the presence of ammonium acetate in acetic acid gave the corresponding pyridine compounds **7a** and **7h**, respectively. Since oxymorphone (**5**) was not commercially available, we utilized oxycodone (**8**) as the starting material for the preparation of the target compounds possessing oxymorphone framework. Thus, the condensation of **8** with the aldehyde **9** under the standard reaction conditions gave the methyl ether **7o**, which was then converted to **7d** by phenolic O-demethylation using BBr_3. The target compounds **7f**, **7g**, and **7i**−**k** were obtained by reacting hydromorphone (**6**) with the enaminoaldehydes **10**−**14** and ammonium acetate (Scheme 2). Oxycodone (**8**) was reacted with the aldehydes **10**−**12** to obtain the corresponding methyl ethers **7m**, **7n**, and **7p**, which were then demethylated with BBr_3 to yield the target compounds **7b**, **7c**, and **7e**, respectively. The 14-deoxy analogue of **2c** was synthesized by the sequence of reactions shown in Scheme 3. The pyridine ring annulation reaction of hydrocodone (**15**) with the malondialdehyde **9** gave the pyridomorphinan **16**, which was then converted to the *N*-nor compound **17** by reaction with vinyl chloroformate followed by hydrolysis of the resulting carbamate intermediate. Alkylation of **17** with cyclopropylmethyl bromide followed by removal of the methyl group from the ether function gave the desired target compound **7l**.

Biology

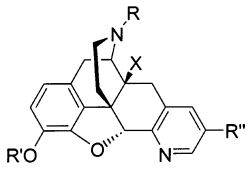
Opioid Receptor Binding. The binding affinities of the target compounds for the opioid δ and μ receptors were determined by inhibition of binding of [^{3}H]DADLE[30] and [^{3}H]DAMGO[31] to rat brain membranes. [^{3}H]DADLE binding to μ receptors was blocked using 100 nM DAMGO. The affinities of the compounds for the κ receptors were determined by inhibition of binding of [^{3}H]U69,593[32] to guinea pig brain membranes using previously reported procedures.[19,33] The δ, μ, and κ opioid receptor binding affinities along with binding selectivity ratios for the target compounds **7a**−**l** are given in Table 1. The phenolic methyl ether compounds **7m**−**q** were prepared as intermediates leading to the corresponding phenolic targets. These methyl ethers were also evaluated for their binding affinities. The affinity data for these ethers as well as the previously reported data for the prototype compounds **2a**−**c** are also listed in Table 1.

Scheme 3



[a] Reagents and reaction conditions: (i) **9**, $AcONH_4$, AcOH, reflux, 18 h; (ii) vinyl chloroformate, K_2CO_3, 1,2-dichloroethane, reflux, 36 h; (iii) 2 N HCl, EtOH, reflux, 2 h; (iv) cyclopropylmethyl bromide, $NaHCO_3$, EtOH, reflux, 14 h; (v) BBr_3, CH_2Cl_2, −20 °C, 4 h.

Table 1. Binding Affinities of the Pyridomorphinans at the Opioid δ, μ, and κ Receptors in Rodent Brain Membranes



					$K_i \pm$ SEM (nM)			selectivity ratio	
compd	R	X	R′	R″	δ[a]	μ[b]	κ[c]	μ/δ	κ/δ
7a	allyl	OH	H	4-chlorophenyl	8.2 ± 0.1	467 ± 19	75 ± 5	57	9
7b	Me	OH	H	H	18 ± 1.4	7.9 ± 0.2	264 ± 18	0.4	15
7c	Me	OH	H	phenyl	2.9 ± 0.1	26 ± 1.0	360 ± 17	9	124
7d	Me	OH	H	4-chlorophenyl	3.9 ± 0.2	230 ± 10	468 ± 17	59	120
7e	Me	OH	H	4-bromophenyl	4.0 ± 0.3	196 ± 4	432 ± 18	49	108
7f	Me	H	H	H	8.0 ± 0.8	13 ± 0.5	66 ± 2	1.6	8
7g	Me	H	H	phenyl	1.9 ± 0.1	24 ± 2	81 ± 5	13	43
7h	Me	H	H	4-chlorophenyl	4.4 ± 0.2	148 ± 9.5	78 ± 13	34	18
7i	Me	H	H	4-bromophenyl	5.0 ± 0.6	200 ± 11	91 ± 6	40	18
7j	Me	H	H	3,4-dichlorophenyl	3.7 ± 0.1	93 ± 4	278 ± 7	25	75
7k	Me	H	H	2,4-dichlorophenyl	1.1 ± 0.1	97 ± 4	403 ± 9	88	366
7l	CPM	H	H	4-chlorophenyl	2.6 ± 0.1	62 ± 3	6.0 ± 0.3	24	2.3
7m	Me	OH	Me	H	143 ± 9	325 ± 16	6400 ± 350	2.3	45
7n	Me	OH	Me	phenyl	34 ± 0.6	894 ± 19	>10000	26	>294
7o	Me	OH	Me	4-chlorophenyl	21 ± 2	2050 ± 95	>7100	98	>338
7p	Me	OH	Me	4-bromophenyl	23 ± 1.3	1890 ± 70	7370 ± 520	82	320
7q	CPM	H	Me	4-chlorophenyl	41 ± 3	1970 ± 50	539 ± 20	48	13
2a[d]	CPM	OH	H	H	0.78 ± 0.06	1.5 ± 0.1	8.8 ± 0.7	1.9	11
2b[d]	CPM	OH	H	phenyl	0.87 ± 0.07	13.5 ± 1.0	17.6 ± 1.6	16	20
2c[d]	CPM	OH	H	4-chlorophenyl	2.2 ± 0.2	51.0 ± 8.0	20.0 ± 1.0	23	9
1, NTI[e]					1.6 ± 0.1	151 ± 14	75 ± 7	94	47
SNC-80[e]					5.6 ± 0.5	8070 ± 930	8760 ± 710	1440	1560
DAMGO[e]					469 ± 39	6.1 ± 0.7	5820 ± 540	0.01	12
morphine[e]					157 ± 11	11.0 ± 1.0	188 ± 20	0.07	1.2
U69,593[e]					>5000	7250 ± 660	4.8 ± 0.5	<1.5	<0.01
naltrexone[e]					7.5 ± 1.8	2.4 ± 0.3	2.2 ± 0.2	0.3	0.3

[a] Displacement of [^{3}H]DADLE (1.3−2.0 nM) in rat brain membranes using 100 nM DAMGO to block binding to μ sites. [b] Displacement of [^{3}H]DAMGO (1.4−3.0 nM) in rat brain membranes. [c] Displacement of [^{3}H]U69,593 (1.2−2.2 nM) in guinea pig brain membranes. [d] Data from ref 19. [e] Data included for comparison.

[^{35}S]GTP-γ-S Binding Assays. All compounds were screened at a 10 μM concentration for agonist and antagonist activity at μ, δ, and κ receptors in vitro using [^{35}S]GTP-γ-S binding assays in guinea pig caudate membranes as described previously.[34−36] Agonist activity was tested by measuring the stimulation of [^{35}S]GTP-γ-S binding by the compounds in the absence and presence of fixed concentrations of selective antagonists to block receptors other than the one being studied. The selective antagonist ligands used were CTAP (2 μM) to block μ receptors, TIPP (1 μM) to block δ receptors, and nor-BNI (6 nM) to block κ receptors.[34] The antagonist properties of the compounds were determined by measuring the test compound's ability to inhibit stimulation of [^{35}S]GTP-γ-S binding produced by the selective agonists (10 μM): SNC-80 for δ receptor, DAMGO for μ receptor, and U69,593 for κ receptor.[35,36] Compounds were selected for more detailed study, using concentra-

Table 2. Antagonist and Agonist Functional Activity of Selected Compounds in [^{35}S]GTP-γ-S Binding Assays in Guinea Pig Caudate Membranes

	antagonist activity apparent $K_i \pm$ SD (nM)			agonist activity $EC_{50} \pm$ SD (nM), E_{max} (%)		
compd	δ[a]	μ[b]	κ[c]	δ[d]	μ[e]	κ[f]
7b	>1000	>1000	>1000	*g*	3000 ± 670 40 ± 2%	*g*
7c	920 ± 240[h]	>1000	>1000	*g*	660 ± 190 27 ± 2%	*g*
7d	17 ± 2.6	>1000	767 ± 54	*g*	*g*	*g*
7f	595 ± 48	>1000	>1000	*g*	500 ± 80 44 ± 1%	*g*
7g	>1000	>1000	>1000	g	1030 ± 100 60 ± 1%	*g*
7h	10.9 ± 1.0	*i*	333 ± 25	g	900 ± 170 48 ± 2%	*g*
7i	55 ± 16[h]	>1000	265 ± 29	g	3220 ± 570 59 ± 3%	g
7j	74 ± 14[h]	>1000	4770 ± 1500[h]	*g*	1310 ± 240 63 ± 3%	*g*
7k	1.1 ± 0.1	>1000	308 ± 21	*g*	225 ± 30 51 ± 5%	*g*
7l	1.56 ± 0.14	9.2 ± 0.86	11.2 ± 0.6	*g*	*g*	*g*
2c	0.18 ± 0.01	7.8 ± 0.42	11.2 ± 0.4	*g*	*g*	*g*
1, NTI	0.062 ± 0.006	3.2 ± 0.2	8.8 ± 0.8	*g*	*g*	*g*
SNC-80	na[j]	na[j]	na[j]	760 ± 130 100%	na[j]	na[j]
DAMGO	na[j]	na[j]	na[j]	na[j]	414 ± 50 100%	na[j]
morphine	na[j]	na[j]	na[j]	*g*	290 ± 80 32 ± 2%	*g*
U69,593	na[j]	na[j]	na[j]	na[j]	na[j]	380 ± 40 100%

[a] SNC-80 (10 μM) was used as the agonist selective for the δ receptor. [b] DAMGO (10 μM) was used as the agonist selective for the μ receptor. [c] U69,593 (10 μM) was used as the agonist selective for the κ receptor. [d] The μ and κ sites were blocked with the antagonists CTAP (2 μM) and nor-BNI (6 nM). [e] The δ and κ sites were blocked with the antagonists TIPP (1 μM) and nor-BNI (6 nM). [f] The δ and μ sites were blocked with TIPP (1 μM) and CTAP (2 μM). [g] Not active as an agonist. [h] IC_{50} values. K_i values could not be calculated because of partial inhibition of agonist stimulated [^{35}S]GTP-γ-S binding. [i] K_i value could not be calculated because of agonist activity. [j] na = not applicable.

tion−response curves, based on their binding K_i values (Table 1) and their profile of agonist and antagonist activity in the initial [^{35}S]GTP-γ-S binding assay. The agonist efficacy of the compounds is expressed as a percent of stimulation compared to that produced by the standard agonist. The results are presented in Table 2.

Bioassays in Smooth Muscle Preparations. The functional activity profiles of selected ligands were also determined in the mouse vas deferens (MVD) and guinea pig ileum (GPI) smooth muscle preparations as described previously.[37,38] The agonist activity was determined by the ability of the compound to inhibit electrically stimulated contractions of the GPI and MVD. The GPI is primarily a μ receptor preparation, even though the ileum does also contain κ receptors. In the MVD, the opioid effects are predominantly mediated through δ receptors, but μ and κ receptors also exist in this tissue. Testing for antagonist activity was carried out by preincubating the muscle preparations with the test compound 30 min prior to washing with buffer and testing with the standard δ agonist DPDPE in the MVD and the μ agonist PL-017 in the GPI. The antagonist and agonist potencies of the tested compounds are listed in Table 3.

Analgesic Testing and Assessment of Tolerance Development. The analgesic activity of selected ligands was tested in mice using the 55 °C warm-water tail-withdrawal test as previously described.[21] The test compounds were administered by the intracerbroventricular (icv) route. The analgesic effects of the compounds that were evaluated are given in Table 4.

Table 3. Antagonist and Agonist Functional Activity of Selected Compounds in Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI) Smooth Muscle Assays

	antagonist activity		agonist activity	
compd	MVD (δ) K_e (nM)[a]	GPI (μ) K_e (nM)[b]	MVD (δ) IC_{50} (nM) or % max response[c]	GPI (μ) IC_{50} (nM) or % max response[c]
7b	*d*	*d*	49%	523 ± 95
7c	*d*	*d*	213 ± 56	211 ± 36
7d	38.2 ± 14.7	*d*	58%	8.7%
7f	*d*	*d*	152 ± 26	67.7 ± 10
7g	*d*	*d*	48.4 ± 8.7	98.7 ± 20.1
7h	21.9 ± 2.14	*d*	565 ± 13	177 ± 41
7i	20.0 ± 6.6	*d*	44%	666 ± 127
7j	*d*	*d*	489 ± 164	447 ± 163
7k	5.02 ±1.56	*d*	28.3%	724 ± 131
7l	6.00 ± 1.33	*d*	7.9%	109 ± 27
2a[e]	37.0 ± 1.0	190 ± 65	0%	0%
2b[e]	3.7 ± 1.0	43 ± 6.6	4.7%	0%
2c[e]	0.91 ± 0.48	*d*	21%	163 ± 22
1, NTI[f]	0.53 ± 0.18	43 ± 3.0	16%	18%
DPDPE[f]	*d*	*d*	5.81 ± 1.63	11600 ± 2990
PL-017[f]	*d*	*d*	243 ± 38	59.4 ± 3.6

[a] Determined using DPDPE as the agonist ligand for the δ receptor. [b] Determined using PL-017 as the agonist ligand for the μ receptor. [c] Partial agonist activity is expressed as the percentage inhibition of contraction at 1 μM. [d] The agonist effects precluded the determination of antagonist effects. [e] Data from ref 19. [f] Data included for comparison.

The A_{50} values were calculated for compounds that produced full antinociceptive effects with minimal or no toxicity. For those compounds for which the A_{50} values could not be calculated, the percentage antinociception

Table 4. Analgesic Activity of Selected Ligands in the Mouse Warm-Water Tail-Withdrawal Assay[a]

compd	A_{50} or % nociception	95% confidence limits (nmol)	naloxone sensitivity[b]
7b	67.1 nmol	49.0−91.8	yes
7c	64% @ 100 nmol	*c*	no
7d	43% @ 300 nmol	*c*	partial
7f	75% @100 nmol	*c*	no
7g	48% @ 100 nmol	*c*	yes
7h	42.8 nmol	30.6−59.8	yes
7i	47.2 nmol	31.3−71.2	yes
7j	44% @ 300 nmol	*c*	ND[d]
7k	40% @ 60 nmol[e]	*c*	ND[d]
7l	18% @ 300 nmol	*c*	ND[d]
2c	21% @ 100 nmol[f]	*c*	yes
morphine	4.2 nmol	3.0−6.9	yes

[a] Compounds were administered icv with A_{50} values calculated at time of peak drug effect. [b] Compounds exhibiting greater than 80% reduction in the antinociceptive effect to a fixed dose of naloxone are designated "yes" for naloxone sensitivity. [c] 95% confidence levels could not be calculated. [d] ND = not determined. [e] Doses of 100−600 nmol produced less than 40% MPE. [f] Doses of 300 and 600 nmol produced less than 10% MPE.

at the given dose is listed in the table. To determine whether the analgesic activity of the tested compounds is mediated through opioid receptors, the blockade of antinociceptive activity by pretreatment with naloxone was carried out. The analgesic activity was considered as naloxone-sensitive if greater than 80% reduction in the antinociceptive response was observed. Selected compounds were also tested for antinociception in mice pretreated with the μ selective antagonist β-FNA (19 nmol, icv, −24 h). The development of tolerance to analgesic effect was evaluated using a repeated administration procedure as previously described.[21]

Results and Discussion

An examination of the affinities of the phenolic target compounds **7a**−**l** reveals that, with the exception of **7b**, all of the ligands display high-affinity binding at the δ site with K_i values less than 10 nM and are δ selective, their binding affinities at δ site being higher than their affinities at the μ and κ sites. Compounds **7b** and **7f** possess the basic morphinan unit present in oxymorphone and hydromorphone, respectively, and do not carry any substituent on the fused pyridine ring system. These two compounds displayed a relatively nonselective binding profile between μ and δ receptors [$K_i(\mu)/K_i(\delta)$ = 0.4 for **7b**; $K_i(\mu)/K_i(\delta)$ = 1.6 for **7f**]. Their affinities at the κ site were significantly lower than their affinities at δ and μ sites. The introduction of a phenyl group at the 5′-position on these two templates gave compounds **7c** and **7g**, which displayed 4- to 6-fold enhanced affinity at the δ site in comparison to the parent compounds. This improvement in the binding affinity of the phenyl-substituted analogues at the δ site is accompanied by a decrease in affinity at μ and κ sites, thus leading to an enhancement in δ selectivity profile of these compounds. These improvements in the binding affinity and binding selectivity brought about by the introduction of an aryl group at the 5′-position of the pyridomorphinan is in conformity with the structure−affinity relationships observed among closely related 5′-aryl analogues of **2a**.[19,20,23] Thus, it appears that an aryl group placed at the 5′-position of the pyridomorphinan templates encounters favorable interactions at the binding site of the δ receptor as opposed to unfavorable interactions at the μ and κ receptors. Introduction of a chlorine or bromine substituent at the para position of the free-rotating phenyl ring in **7c** or **7g** brings about a modest decrease in binding affinity at the δ site. Of the two isomeric dichlorophenyl compounds **7j** and **7k**, the 2,4-dichlorophenyl compound **7k** binds with higher affinity at the δ site than the 3,4-dichlorophenyl compound. This structure−affinity trend parallels that observed among dichlorophenyl analogues of **2b**.[23] Among phenolic compounds **7a**−**l**, the 2,4-dichlorphenyl compound **7k** displayed the highest δ receptor binding affinity (K_i = 1.1 nM) and the highest δ receptor binding selectivity with μ/δ and κ/δ selectivity ratios of 88 and 366, respectively.

A comparison of the affinities of compounds possessing an *N*-CPM group (**2a**−**c**) with those possessing an *N*-methyl group (**7b**−**d**) indicates that replacing the CPM group with a methyl group in general led to reduction in affinities at all three receptors. The reductions in affinities at the κ sites are relatively larger than the reductions in binding affinities at δ or μ sites. Compared to the *N*-CPM compound **2c**, the *N*-allyl analogue **7a** also displayed reduced affinities at the δ, μ, and κ receptors with greater reduction in affinity at the μ site (9-fold) than at δ (4-fold) or κ sites (4-fold). Comparison of the affinities of **7b**−**e** with **7f**−**i** and those of **2c** with **7l** indicates that the replacement of the 14-hydroxyl group with a hydrogen atom brings about only a modest change in the affinity at the δ and μ sites (less than 3-fold change in affinity). At the κ site, however, the deoxy compounds displayed 3- to 6-fold higher affinity than their 14-hydroxy counterparts. The presence of a free phenolic hydroxyl group is usually considered essential for high-affinity binding at opioid receptors. It was not surprising, therefore, to find that the affinities of the phenolic methyl ethers **7m**−**q** were lower than the affinities of their corresponding phenolic compounds **7b**−**e** and **7l** at all three binding sites. The magnitude of reduction in affinity at the δ site, in general, was less (5- to 16-fold) than the reduction in affinity at the μ (9- to 41-fold) or κ sites (15- to 90-fold).

One of the primary goals of the present investigation was to identify ligands with a mixed profile of agonist activity at the μ receptor and antagonist activity at the δ receptor. As shown by the functional activity data in the [^{35}S]GTP-γ-S assays (Table 2), most of the compounds examined in the present study in general displayed the desired profile of μ agonist/δ antagonist activity. Compounds **7c**, **7i**, and **7j** were devoid of agonist activity at the δ receptors but failed to inhibit SNC-80 stimulated binding of [^{35}S]GTP-γ-S to 100% in the antagonist assays at the δ receptors. The maximum percentage inhibition displayed by **7c**, **7i**, and **7j** were 61 ± 3%, 69 ± 3%, and 58 ± 3%, respectively. A similar partial inhibition profile (maximum inhibition of 66 ± 6%) was also observed for **7j** at the κ receptors. The partial inhibition profile displayed by these ligands is exemplified by the concentration−response curve for **7j** shown in Figure 1. For these compounds, the calculated IC_{50} values instead of K_i values are listed in Table 2. The intriguing observation of partial antagonist inhibition is currently under investigation and will be the subject of a separate report.

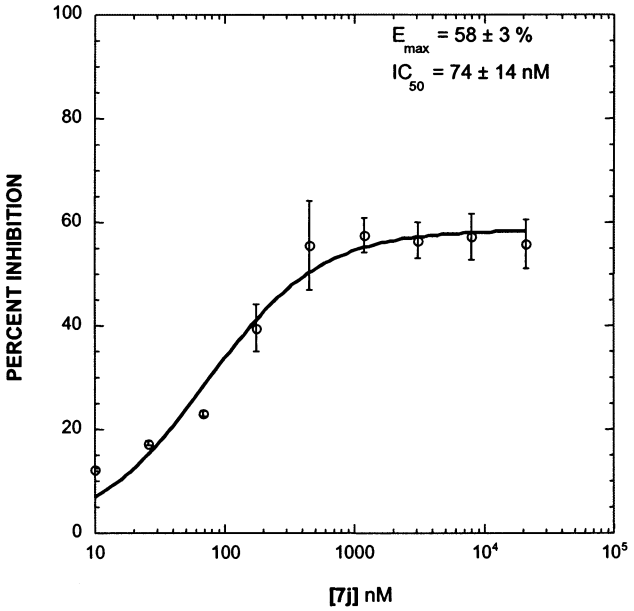


Figure 1. Concentration−response curve for inhibition of SNC-80 stimulated [^{35}S]GTP-γ-S binding by **7j**.

Among the compounds studied, only two compounds, **7d** and **7l**, failed to display agonist activity at the μ receptor. Compound **7l** is the 14-deoxy analogue of **2c** and carries the *N*-CPM group at the 17-position. The lack of μ agonist activity for **7l** is therefore not surprising because removal of the hydroxyl function from the 14-position by itself may not be sufficient for inducing μ agonist interactions for the morphinan ligands carrying the *N*-CPM group. With the exception of **7d**, all of the ligands carrying an *N*-methyl group displayed μ agonist activity with varying potencies. Among the ligands that displayed μ agonist activity, compound **7k** was the most potent with an EC_{50} value of 225 nM, which is comparable to the EC_{50} values of morphine (290 nM) and DAMGO (414 nM). The rank order of potencies for the agonist ligands was **7k** > **7f** > **7c** > **7h** > **7g** > **7j** > **7b** > **7i**, and there appears to be no strict correlation between the agonist potency and the binding affinity of these ligands at the μ receptor. This is not surprising not only in view of the substantial differences in assay conditions between the binding and functional [^{35}S]GTP-γ-S binding assays but also because the EC_{50} of stimulation of [^{35}S]GTP-γ-S binding is a function of binding affinity and intrinsic efficacy.[39] The agonist efficacies of these ligands, as indicated by their percentage maximum stimulation (E_{max}) values, were in the range of 27% (**7c**) to 63% (**7j**). With the exception of **7c**, all of these ligands were more efficacious than morphine (E_{max} = 32%) but less efficacious than DAMGO (E_{max} = 100%). With regard to antagonist activity, the *N*-CPM compound **7l** displayed significant antagonist potency at all three receptors. All of the *N*-methyl compounds examined displayed no or only weak antagonist activity at the μ and κ receptors. Interestingly, however, most of the compounds displayed moderate antagonist potencies at the δ receptor. The pyridomorphinans **7d** and **7h** possessing the 4-chlorophenyl substituent are *N*-methyl analogues of the *N*-CPM compound **2c**. These two compounds **7d** and **7h** displayed δ antagonist K_i values of 17 and 10.9 nM, respectively, compared to the K_i value of 0.18 nM for **2c**. Thus, the exchange of the *N*-CPM group with a methyl group brings about a significant reduction in the δ antagonist potency but without altering the innate antagonist profile of these ligands at the δ receptor. Interestingly, while the introduction of a second chlorine atom at the meta position of the chlorophenyl ring of **7h** (compound **7j**) did not significantly change the binding affinity or the antagonist potency at the δ receptor, the introduction of the chlorine atom at the ortho position (compound **7k**) provided a 4-fold enhancement in binding affinity and a 10-fold improvement in antagonist potency at the δ receptor. Among the ligands studied, the 2,4-dichlorophenyl compound **7k** is not only the most potent δ antagonist but also the most potent μ agonist, thus making it the best mixed μ agonist/δ antagonist ligand in vitro. The profile of **7h** is similar to that of **7k** but with somewhat weaker antagonist and agonist potencies at the δ and μ receptors, respectively, in vitro. As observed in an earlier work with κ receptor antagonists,[35] some of the δ antagonist ligands (**7b**, **7c**, **7f**, **7g**) displayed a marked discrepancy between the binding K_i values (Table 1) and the corresponding functional K_i values (Table 2). The reason for this is unclear.

The functional activity results obtained for the selected compounds in the smooth muscle assays (Table 3) were somewhat similar to that obtained in the [^{35}S]GTP-γ-S assays. All of the ligands that displayed agonist activity in the [^{35}S]GTP-γ-S assays at the μ site also displayed agonist activity in the GPI. One significant exception is the activity of **7l**, which was a potent agonist in the GPI (IC_{50} = 109 nM) but was found to be an antagonist at the μ site in the [^{35}S]GTP-γ-S assays. This intriguing activity profile of **7l** is similar to that displayed by its 14-hydroxy analogue **2c** studied earlier.[19,22] While none of the compounds displayed any significant agonist activity at the δ site in the [^{35}S]GTP-γ-S assays, a few compounds, **7c**, **7f**, **7g**, **7h**, and **7j**, displayed agonist activity in the MVD smooth muscle preparations. It appears that the agonist activity displayed by these compounds in the MVD may be due to their agonist effects at the μ receptors. Although not investigated in depth, we found that the agonist activity of **7f** and **7h** in the MVD was blocked by the nonselective antagonist naloxone but not by the δ selective antagonist ICI-174,864. Among the compounds studied, δ antagonist K_e values could be determined for **7d**, **7h**, **7i**, **7k**, and **7l** in the MVD. The antagonist K_e values for these compounds were in the range of 5 nM (**7k**) to 38 nM (**7d**). Compounds **7h** and **7k** were the two compounds that displayed an in vitro μ agonist/δ antagonist profile of activity in both the [^{35}S]GTP-γ-S and the smooth muscle assay systems.

The structure−activity relationships observed in the present study, together with those observed in our earlier investigations, suggest that fusion of an unsubstituted pyridine ring at the 6,7-position of 4,5-epoxymorphinans has the effect of increasing the binding affinity at the δ receptor and decreasing the binding affinity at the μ receptor, thus leading to templates that bind with nearly equal affinity to the δ and μ receptors. These pyridomorphinans appear to have a basic tendency to interact with δ receptors as antagonists irrespective of the nature of the alkyl substituent (CPM or methyl) on the morphinan nitrogen. Their functional

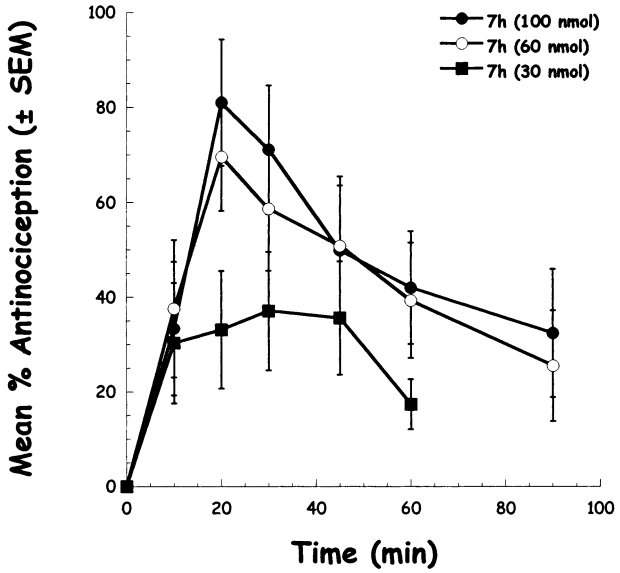


Figure 2. Antinociceptive dose− and time−response curves for **7h** (icv) in the 55 °C warm-water tail-withdrawal assay.

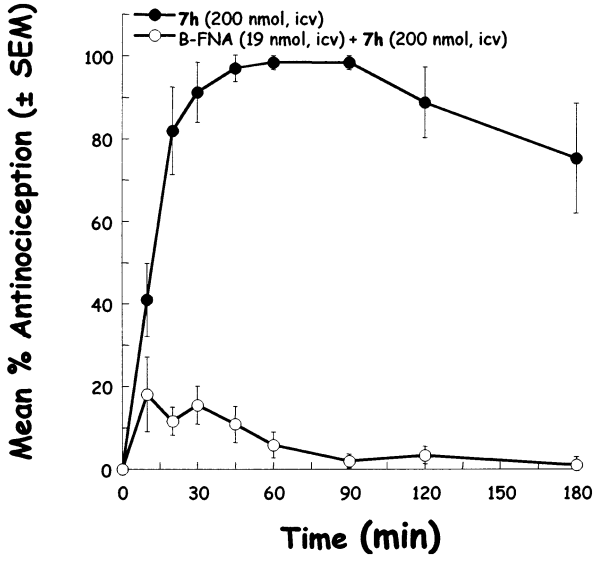


Figure 3. Antinociceptive dose− and time−response curve for **7h** (icv) with and without pretreatment with β-FNA (19 nmol, icv, −24 h).

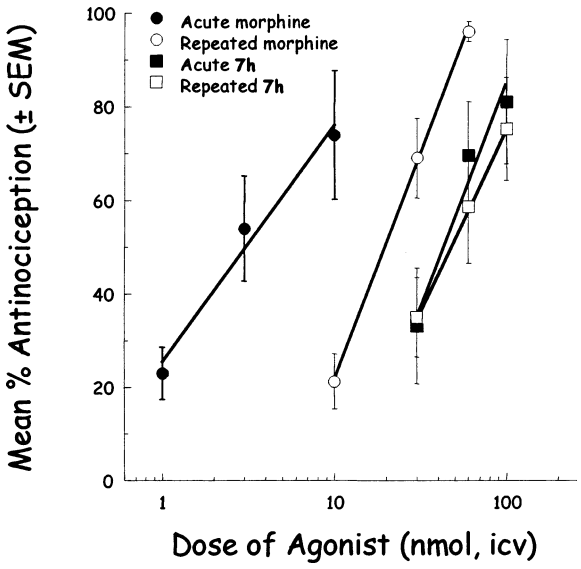


Figure 4. Antinociceptive dose−response curves for naive control mice and mice injected repeatedly with A_{90} doses of icv morphine or **7h** given twice daily for 3 days.

activity at the μ receptor, however, appears to be governed by the nature of the *N*-alkyl substituent, those with the CPM group interacting as antagonists and those with the *N*-methyl group interacting as agonists. Further modulations in binding and functional activity of the pyridomorphinans could be achieved through introduction of appropriate substituents, particularly at the 5′-position of the pyridine ring.

All of the compounds that were evaluated in the functional assays in vitro were evaluated for antinociceptive activity in mice using the warm-water tail-withdrawal assay (Table 4). Among the compounds tested, the antinociceptive A_{50} values could be determined for only three compounds: **7b**, **7h**, and **7i**. Factors that prevented us from determining the A_{50} values for other compounds include lack of efficacy (**7c**, **7j**, **7k**, and **7l**), lack of potency (**7d**), insensitivity to naloxone (**7f**), and toxicity (**7g**). All of the compounds examined in the present study were found to be more efficacious in the warm-water tail-withdrawal assay than the previously studied compound **2c**. From the results obtained in the in vitro functional assays, **7k** and **7h** were identified as compounds of interest as mixed μ agonist/δ antagonist ligands. Of these two compounds, the δ antagonist/μ agonist profile of **7k** was superior to that of **7h** in vitro. In the antinociceptive evaluations, however, compound **7k** was found to be not as efficacious as **7h**. Compound **7h** displayed full agonist efficacy with an A_{50} potency value of 42.8 nmol in the warm-water tail-withdrawal assay in mice (Figure 2). The antinociceptive activity of this compound was completely blocked by the μ selective antagonist β-FNA (Figure 3), confirming that the analgesic activity of this compound is indeed mediated through opioid μ receptors. From these studies, the pyridomorphinan **7h** emerged as a ligand possessing mixed μ agonist/δ antagonist activity in vitro and in vivo. It was gratifying to find that this compound when tested in the tolerance development assays involving repeated injections of the compound for 3 days induced an insignificant shift in the antinociceptive potency (less than 1.1-fold increase in A_{50} value), indicating very little development of tolerance. This is in contrast to morphine, which in the same paradigm, produced a significant 6.4-fold shift in the A_{50} values, indicating the development of tolerance to its analgesic effects (Figure 4). The lack of tolerance displayed by this non-peptide μ agonist/δ antagonist ligand **7h** is an exciting finding that supports the hypothesis that ligands with a mixed μ agonist/δ antagonist profile of activity have the potential of becoming therapeutically useful analgesic agents devoid of tolerance and dependence development commonly associated with pure μ agonist analgesics such as morphine.

Conclusions

The fusion of an unsubstituted pyridine ring on the oxymorphone and hydromorphone framework gave pyridomorphinans that bind with nearly equal affinity at the μ and δ receptors and with much less affinity at the κ receptors. Introduction of aryl substituents at the 5′-position on these pyridomorphinan scaffolds in gen-

eral improved the affinity and antagonist potency at the δ receptor with retention of agonist activity at the μ receptor, thus leading to mixed μ agonist/δ antagonist ligands. The in vitro functional activity evaluations led to the identification of hydromorphone-derived chlorophenylpyridomorphinan **7h** and dichlorophenylpyridomorphinan **7k** as ligands with the desired μ agonist/δ antagonist properties in vitro. Antinociceptive evaluations with **7h** by the warm-water tail-withdrawal test in mice demonstrated that the compound produces antinociceptive effects without inducing analgesic tolerance on repeated administration. This finding is very significant because it demonstrates the validity of the hypothesis that opioid ligands with a mixed μ agonist/δ antagonist profile of activity may have diminished propensity to induce tolerance and therefore may have therapeutic advantages over μ agonist analgesics for long-term treatment of pain. Further studies are clearly warranted to fully explore the potential of such ligands as novel analgesic drugs superior to those currently available.

Experimental Section

General Methods. Melting points were determined in open capillary tubes with a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Nicolet 300NB spectrometer operating at 300.635 MHz. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Spectral assignments were supported by proton decoupling. Mass spectra were recorded on a Varian MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode or on a Bruker BIOTOF II in electrospray ionization (ESI) mode. Elemental analyses were performed by Atlantic Microlab, Inc. (Atlanta, GA) or by the Spectroscopic and Analytical Laboratory of Southern Research Institute. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on Analtech silica gel GF 0.25 mm plates. Flash column chromatography was performed with E. Merck silica gel 60 (230−400 mesh). Yields are of purified compounds and were not optimized. Naloxone hydrochloride, oxycodone hydrochloride, hydromorphone hydrochloride, and hydrocodone bitartrate were obtained from Mallinckrodt. 2-(4-Chlorophenyl)malondialdehyde was purchased from Acros Organics. All other reagents were obtained from Aldrich.

17-(Allyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-5′-(4-chlorophenyl)pyrido[2′,3′:6,7]morphinan (7a). A solution of naloxone hydrochloride (1.0 g, 2.74 mmol), 2-(4-chlorophenyl)malondialdehdye (0.552 g, 3.02 mmol), and ammonium acetate (0.421 g, 5.48 mmol) in acetic acid (20 mL) was heated to reflux in an oil bath at 130−135 °C under an argon atmosphere for 18 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was treated with water, and the pH of the mixture was adjusted to 8 with saturated aqueous $NaHCO_3$ solution. The solid that separated was collected by filtration, dissolved in CH_2Cl_2, and washed with brine. The organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed under reduced pressure. The crude product was chromatographed over a column of silica using $CHCl_3$−MeOH−NH_4OH (98.5:1:0.5) as the eluent to obtain (0.385 g, 30%) the desired product **7a**: mp 168−172 °C; TLC R_f = 0.2 (CH_2Cl_2−MeOH−NH_4OH, 97:2.5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.82−1.85 (m, 1H, C-16 H), 2.31−2.43 (m, 2H, C-15 H, C-16 H), 2.62 (m, 4H, C-8 H_2, C-10 H, C-15 H), 3.11−3.25 (m, 4H, C-9 H, C-10 H, CH_2 CH=CH_2), 4.80−5.50 (broad hump, 2H, C-3 OH, C-14 OH), 5.18−5.28 (m, 2H, CH=CH_2), 5.59 (s, 1H, C-5 H), 5.78−5.91 (m, 1H, CH=CH_2), 6.59 (d, 1H, J = 8.1 Hz, C-1 H), 6.68 (d, 1H, J = 8.1 Hz, C-2 H), 7.37−7.45 (m, 4H, C-2″ H, C-3″ H, C-5″ H, C-6″ H), 7.47 (d, 1H, J = 2.1 Hz, C-4′ H), 8.69 (d, 1H, J = 1.8 Hz, C-6′ H); MS m/z 473 (MH)$^+$. Anal. ($C_{28}H_{25}ClN_2O_3$·0.1H_2O) C, H, N.

6,7-Didehydro-3,14-dihydroxy-4,5α-epoxy-17-methylpyrido[2′,3′:6,7]morphinan (7b). Oxycodone hydrochloride (2.0 g, 5.69 mmol), 3-(dimethylamino)acrolein (0.845 g, 8.52 mmol), ammonium acetate (1.31 g, 17.04 mmol), and acetic acid (30 mL) was refluxed in an oil bath at 130−135 °C under an atmosphere of argon for 18 h. Workup of the reaction mixture and purification of the crude product as described above for the preparation of **7a** gave 6,7-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylpyrido[2′,3′:6,7]morphinan (**7m**) (0.792 g, 40%): mp 210−212 °C; TLC R_f = 0.4 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.80−1.83 (m, 1H, C-15 H), 2.35−2.40 (m, 2H, C-15 H, C-16 H), 2.43 (s, 3H, NCH_3), 2.50−2.78 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.95 (d, 1H, J = 6.5 Hz, C-9 H), 3.26 (d, 1H, J = 18.7 Hz, C-10 H), 3.79 (s, 3H, OCH_3), 4.5−5.8 (broad hump, 1H, C-14 OH), 5.53 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.66 (d, 1H, J = 8.1 Hz, C-1 H), 7.10 (dd, 1H, J = 7.7 and 4.6 Hz, C-5′ H), 7.34 (d, 1H, J = 7.7 Hz, C-4′ H), 8.56−8.58 (m, 1H, C-6′ H); MS m/z 351 (MH)$^+$. Anal. ($C_{21}H_{22}N_2O_3$·0.2H_2O) C, H, N.

A solution of **7m** (0.67 g, 1.91 mmol) in CH_2Cl_2 (25 mL) was cooled to −78 °C and treated dropwise with BBr_3 (19.0 mL of 1 M solution in CH_2Cl_2, 19.0 mmol). After 30 min, the reaction mixture was allowed to warm to −15 to − 20 °C and stir for 4 h. The mixture was then treated with Et_2O (2 mL) and allowed to warm to room temperature. After being stirred for an additional 30 min, the mixture was diluted with water and extracted twice with CH_2Cl_2. The organic layer was washed with brine and dried (Na_2SO_4). The solvents were removed under reduced pressure and the crude product obtained was chromatographed over a column of silica using CH_2Cl_2−MeOH−NH_4OH (97.5:2:0.5) as the eluent to obtain **7b** (0.179 g, 28%): mp >230 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR (DMSO-d_6) δ 1.52−1.56 (m, 1H, C-15 H), 2.14−2.32 (m, 2H, C-15 H, C-16 H), 2.35 (s, 3H, NCH_3), 2.44−2.62 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.91 (d, 1H, J = 6.1 Hz, C-9 H), 3.16 (d, 1H, J = 18.6 Hz, C-10 H), 4.75 (s, 1H, C-14 OH), 5.28 (s, 1H, C-5 H), 6.49−6.54 (m, 2H, C-1 H, C-2 H), 7.23 (dd, 1H, J = 7.7 and 4.7 Hz, C-5′ H), 7.46 (dd, 1H, J = 7.7 and 1.4 Hz, C-4′ H), 8.48 (dd, 1H, J = 4.7 and 1.5 Hz, C-6′ H), 9.01 (s, 1H, C-3 OH); MS m/z 337 (MH)$^+$. Anal. ($C_{20}H_{20}N_2O_3$·0.3H_2O) C, H, N.

6,7-Didehydro-3,14-dihydroxy-4,5α-epoxy-17-methyl-5′-phenylpyrido[2′,3′:6,7]morphinan (7c). Oxycodone hydrochloride (2.0 g, 5.68 mmol), was reacted with 3-(dimethylamino)-2-phenylacrolein[40] (1.40 g, 8.52 mmol) and ammonium acetate (1.31 g, 17.04 mmol) in acetic acid (20 mL) by the same procedure as described for the preparation of **7a** to obtain 6,7-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methyl-5′-phenylpyrido[2′,3′:6,7]morphinan (**7n**) (1.325 g, 55%): mp >250 °C; TLC R_f = 0.5 (CH_2Cl_2−MeOH−NH_4OH, 96.5:3:0.5); ^{1}H NMR ($CDCl_3$) δ 1.82−1.86 (m, 1H, C-15 H), 2.37−2.42 (m, 2H, C-15 H, C-16 H), 2.44 (s, 3H, NCH_3), 2.52−2.84 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.99 (d, 1H, J = 6.4 Hz, C-9 H), 3.28 (d, 1H, J = 18.7 Hz, C-10 H), 3.82 (s, 3H, OCH_3), 5.58 (s, 1H, C-5 H), 6.63 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.37−7.53 (m, 6H, C-4′ H, C-5′ phenyl-H), 8.78−8.79 (m, 1H, C-6′ H); MS m/z 427 (MH)$^+$. Anal. ($C_{27}H_{26}N_2O_3$·0.2H_2O) C, H, N.

The methyl ether **7n** (0.189 g, 0.44 mmol) in CH_2Cl_2 (10 mL) was reacted with BBr_3 (4.4 mL of 1 M solution in CH_2Cl_2, 4.4 mmol) as described for the preparation of **7b** from **7m** to yield 0.112 g (61%) of **7c**: mp 190−192 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 96.5:3:0.5); ^{1}H NMR ($CDCl_3$) δ 1.82−1.85 (m, 1H, C-15 H), 2.38−2.42 (m, 2H, C-15 H, C-16 H), 2.44 (s, 3H, NCH_3), 2.53−2.82 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.98 (d, 1H, J = 6.4 Hz, C-9 H), 3.27 (d, 1H, J = 18.7 Hz, C-10 H), 4.20−5.68 (broad hump, 2H, C-3 OH, C-14 OH), 5.59 (s, 1H, C-5 H), 6.60 (d, 1H, J = 8.1 Hz, C-2 H), 6.69 (d, 1H, J = 8.1 Hz, C-1 H), 7.37−7.51 (m, 6H, C-4′ H, C-5′ phenyl-H), 8.72−8.73 (m, 1H, C-6′ H); MS m/z 413 (MH)$^+$. Anal. ($C_{26}H_{24}N_2O_3$·0.5H_2O) C, H, N.

5′-(4-Chlorophenyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-17-methylpyrido[2′,3′:6,7]morphinan (7d). Oxycodone hydrochloride (1.0 g, 2.84 mmol) was reacted with 2-(4-chlorophenyl)malondialdehdye (0.584 g, 3.13 mmol) and ammonium acetate (0.438 g, 5.68 mmol) in acetic acid (20 mL) by the same procedure as described for the preparation of **7a** to obtain 5′-(4-chlorophenyl)-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylpyrido[2′,3′:6,7]morphinan (**7o**) (0.527 g, 40%): mp 112−114 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 96.5:3:0.5); ^{1}H NMR ($CDCl_3$) δ 1.82−1.86 (m, 1H, C-15 H), 2.37−2.41 (m, 2H, C-15 H, C-16 H), 2.44 (s, 3H, NCH_3), 2.52−2.83 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.98 (d, 1H, J = 6.4 Hz, C-9 H), 3.29 (d, 1H, J = 18.7 Hz, C-10 H), 3.81 (s, 3H, OCH_3), 4.7−4.8 (broad s, 1H, C-14 OH), 5.57 (s, 1H, C-5 H), 6.63 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.34−7.47 (m, 5H, C-4′ H, C-2″ H, C-3″ H, C-5″ H, C-6″ H), 8.74 (d, 1H, J = 2.1 Hz, C-6′ H); MS m/z 461 $(MH)^+$. Anal. ($C_{27}H_{25}ClN_2O_3\cdot0.1H_2O$) C, H, N.

The methyl ether **7o** (0.352 g, 0.76 mmol) in CH_2Cl_2 (15 mL) was reacted with BBr_3 (7.6 mL of 1 M solution in CH_2Cl_2, 7.6 mmol) as described for the preparation of **7b** from **7m** to yield 0.132 g (39%) of **7d**: mp 196−198 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.80−1.88 (m, 1H, C-15 H), 2.34−2.40 (m, 2H, C-15 H, C-16 H), 2.44 (s, 3H, NCH_3), 2.49−2.81 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.98 (d, 1H, J = 6.4 Hz, C-9 H), 3.27 (d, 1H, J = 18.7 Hz, C-10 H), 4.8−5.7 (broad hump, 2H, C-3 OH, C-14 OH), 5.58 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.69 (d, 1H, J = 8.1 Hz, C-1 H), 7.34−7.44 (m, 4H, C-2″ H, C-3″ H, C-5″ H, C-6″ H), 7.47 (d, 1H, J = 2.0 Hz, C-4′ H), 8.69 (d, 1H, J = 2.0 Hz, C-6′ H); MS m/z 447 $(MH)^+$. Anal. ($C_{26}H_{23}ClN_2O_3\cdot0.5H_2O$) C, H, N.

5′-(4-Bromophenyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-17-methylpyrido[2′,3′:6,7]morphinan (7e). Oxycodone hydrochloride (2.0 g, 5.68 mmol) was reacted with 2-(4-bromophenyl)-3-(dimethylamino)acrolein[41] (2.16 g, 8.52 mmol) and ammonium acetate (1.31 g, 17.04 mmol) in acetic acid (30 mL) by the same procedure as described for the preparation of **7a** to obtain 5′-(4-bromophenyl)-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylpyrido[2′,3′:6,7]morphinan (**7p**) (0.79 g, 28%): mp >230 °C; TLC R_f = 0.4 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.82−1.85 (m, 1H, C-15 H), 2.37−2.41 (m, 2H, C-15 H, C-16 H), 2.44 (s, 3H, NCH_3), 2.51−2.83 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.98 (d, 1H, J = 6.4 Hz, C-9 H), 3.28 (d, 1H, J = 18.7 Hz, C-10 H), 3.81 (s, 3H, OCH_3), 4.5−5.2 (broad hump, 1H, C-14 OH), 5.56 (s, 1H, C-5 H), 6.63 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.36−7.40 (m, 2H, C-2″ H, C-6″ H), 7.46 (d, 1H, J = 2.1 Hz, C-4′ H), 7.54−7.59 (m, 2H, C-3″ H, C-5″ H), 8.74 (d, 1H, J = 2.1 Hz, C-6′ H); MS m/z 505 $(MH)^+$. Anal. ($C_{27}H_{25}BrN_2O_3$) C, H, N.

The methyl ether **7p** (0.508 g, 1.0 mmol) in CH_2Cl_2 (20 mL) was reacted with BBr_3 (10.0 mL of 1 M solution in CH_2Cl_2, 10.0 mmol) as described for the preparation of **7b** from **7m** to yield 0.198 g (40%) of **7e**: mp 196−198 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.82−1.85 (m, 1H, C-15 H), 2.38−2.41 (m, 2H, C-15 H, C-16 H), 2.44 (s, 3H, NCH_3), 2.53−2.81 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.97 (d, 1H, J = 6.4 Hz, C-9 H), 3.27 (d, 1H, J = 18.7 Hz, C-10 H), 4.4−5.8 (broad hump, 2H, C-3 OH, C-14 OH), 5.57 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.69 (d, 1H, J = 8.1 Hz, C-1 H), 7.34−7.38 (m, 2H, C-2″ H, C-6″ H), 7.48 (d, 1H, J = 2.0 Hz, C-4′ H), 7.54−7.58 (m, 2H, C-3″ H, C-5″ H), 8.68 (d, 1H, J = 2.0 Hz, C-6′ H); MS m/z 491 $(MH)^+$. Anal. ($C_{26}H_{23}BrN_2O_3\cdot0.25H_2O$) C, H, N.

6,7-Didehydro-4,5α-epoxy-3-hydroxy-17-methylpyrido-[2′,3′:6,7]morphinan (7f). A mixture of hydromorphone hydrochloride (1.0 g, 3.10 mmol), 3-(dimethylamino)acrolein (0.369 g, 3.72 mmol), ammonium acetate (0.477 g, 6.20 mmol), and acetic acid (20 mL) was refluxed in an oil bath at 130−135 °C for 18 h. Workup of the reaction mixture and purification of the crude product as described for the preparation of **7a** gave the desired product **7f** (0.215 g, 22%): mp 164−166 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 95:4.5:0.5); ^{1}H NMR ($CDCl_3$) δ 2.01−2.34 (m, 2H, C-15 H_2), 2.54−2.77 (m, 6H, C-8 H_2, C-10 H, C-14 H, C-16 H_2), 2.69 (s, 3H, NCH_3), 3.16 (d, 1H, J = 18.7 Hz, C-10 H), 3.39−3.41 (m, 1H, C-9 H), 5.51 (s, 1H, C-5 H), 6.58 (d, 1H, J = 8.1 Hz, C-2 H), 6.66 (d, 1H, J = 8.1 Hz, C-1 H), 7.15 (dd, 1H, J = 7.8 and 4.7 Hz, C-5′ H), 7.34 (m, 1H, C-4′ H), 8.52 (dd, 1H, J = 4.7 and 1.1 Hz, C-6′ H), 8.52−8.58 (br s, 1H, C-3 OH); MS m/z 321 $(MH)^+$. Anal. ($C_{20}H_{20}N_2O_2\cdot0.6H_2O$) C, H, N.

6,7-Didehydro-4,5α-epoxy-3-hydroxy-17-methyl-5′-phenylpyrido[2′,3′:6,7]morphinan (7g). Hydromorphone hydrochloride (1.0 g, 3.10 mmol) was reacted with 3-(dimethylamino)-2-phenylacrolein[40] (0.651 g, 3.72 mmol) and ammonium acetate (0.477 g, 6.20 mmol) in acetic acid (20 mL) by the same procedure as described for the preparation of **7a** to obtain **7g** (0.54 g, 44%): mp 182−184 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 95:4.5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.95−2.14 (m, 2H, C-15 H_2), 2.31−2.67 (m, 6H, C-8 H_2, C-10 H, C-14 H, C-16 H_2), 2.46 (s, 3H, NCH_3), 3.11 (d, 1H, J = 18.7 Hz, C-10 H), 3.29−3.31 (m, 1H, C-9 H), 4.8−5.6 (broad hump, 1H, C-3 OH), 5.58 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.35−7.52 (m, 6H, C-4′ H, C-5′ phenyl-H), 8.74 (d, 1H, J = 1.6 Hz, C-6′ H); MS m/z 397 $(MH)^+$. Anal. ($C_{26}H_{24}N_2O_2\cdot0.6H_2O$) C, H, N.

5′-(4-Chlorophenyl)-6,7-didehydro-4,5α-epoxy-3-hydroxy-17-methylpyrido[2′,3′:6,7]morphinan (7h). Hydromorphone hydrochloride (10.0 g, 31.1 mmol) was reacted with 2-(4-chlorophenyl)malondialdehdye (6.81 g, 37.3 mmol) and ammonium acetate (4.79 g, 62.2 mmol) in acetic acid (140 mL) by the same procedure as described for the preparation of **7a** to obtain **7h** (3.033 g, 23%): mp 188−190 °C; TLC R_f = 0.35 (CH_2Cl_2−MeOH−NH_4OH, 96.5:3:0.5); ^{1}H NMR ($CDCl_3$) δ 1.97−2.14 (m, 2H, C-15 H_2), 2.30−2.48 (m, 3H, C-8 H, C-10 H, C-16 H), 2.46 (s, 3H, NCH_3), 2.55−2.64 (m, 3H, C-8 H, C-14 H, C-16 H), 3.11 (d, 1H, J = 18.6 Hz, C-10 H), 3.28−3.30 (m, 1H, C-9 H), 5.2−5.9 (broad hump, 1H, C-3 OH), 5.57 (s, 1H, C-5 H), 6.60 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.40−7.46 (m, 5H, C-4′ H, C-2″ H, C-3″ H, C-5″ H, C-6″ H), 8.72 (d, 1H, J = 2.1 Hz, C-6′ H); MS m/z 431 $(MH)^+$. Anal. ($C_{26}H_{23}ClN_2O_2$) C, H, N.

A solution of the compound in EtOH was treated with a 2 M solution of hydrogen chloride in Et_2O. Removal of the solvent under reduced pressure and trituration with Et_2O gave the **7h**·2HCl salt: mp 276−278 °C dec; MS m/z 431 $(MH)^+$. Anal. ($C_{26}H_{23}ClN_2O_2\cdot2HCl\cdot2H_2O$) C, H, N.

5′-(4-Bromophenyl)-6,7-didehydro-4,5α-epoxy-3-hydroxy-17-methylpyrido[2′,3′:6,7]morphinan (7i). Hydromorphone hydrochloride (3.0 g, 9.32 mmol) was reacted with 2-(4-bromophenyl)-3-(dimethylamino)acrolein[41] (2.60 g, 10.25 mmol) and ammonium acetate (1.45 g, 18.64 mmol) in acetic acid (60 mL) by the same procedure as described for the preparation of **7a** to obtain **7i** (0.93 g, 21%): mp 186−188 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.99−2.13 (m, 2H, C-15 H_2), 2.27−2.44 (m, 1H, C-16H), 2.46 (s, 3H, NCH_3), 2.33−3.08 (broad hump, 2H, C-3 OH, C-14 H), 2.49−2.65 (m, 4H, C-8 H_2, C-10 H, C-16 H), 3.11 (d, 1H, J = 18.7 Hz, C-10 H), 3.31 (dd, 1H, J = 5.8 and 2.4 Hz, C-9 H), 5.55 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.31−7.35 (m, 2H, C-3″ H, C-5″ H), 7.41 (d, 1H, J = 2.0 Hz, C-4′ H), 7.53−7.57 (m, 2H, C-2″ H, C-6″ H), 8.65 (d, 1H, J = 2.0 Hz, C-6′ H); MS m/z 475 $(MH)^+$. Anal. ($C_{26}H_{23}BrN_2O_2\cdot0.5H_2O$) C, H, N.

5′-(3,4-Dichlorophenyl)-6,7-didehydro-4,5α-epoxy-3-hydroxy-17-methylpyrido[2′,3′:6,7]morphinan (7j). Hydromorphone hydrochloride (1.00 g, 3.1 mmol) was reacted with 2-(3,4-dichlorophenyl)-3-(dimethylamino)acrolein[40] (1.135 g, 4.65 mmol) and ammonium acetate (0.478 g, 6.2 mmol) in acetic acid (20 mL) by the same procedure as described for the preparation of **7a** to obtain **7j** (0.25 g, 17%): mp 184−186 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.97−2.14 (m, 2H, C-15 H_2), 2.30−2.48 (m, 3H, C-8 H, C-10 H, C-16 H), 2.46 (s, 3H, NCH_3), 2.55−2.64 (m, 3H, C-8 H, C-14 H, C-16 H), 3.12 (d, 1H, J = 18.7 Hz, C-10 H), 3.27−3.30 (m, 1H, C-9 H), 4.8−5.6 (broad hump, 1H, C-3 OH), 5.57 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.68 (d, 1H, J = 8.1 Hz, C-1 H), 7.34 (dd, 1H, J = 8.3 and 2.1

Hz, C-5 H), 7.44 (d, 1H, J = 1.9 Hz, C-4 H′), 7.52 (d, 1H, J = 8.3 Hz, C-6″ H), 7.59 (d, 1H, J = 2.2 Hz, C-2″ H), 8.70 (d, 1H, J = 1.8 Hz, C-6′ H); MS m/z 465 $(MH)^+$. Anal. ($C_{26}H_{22}Cl_2N_2O_2 \cdot 0.5H_2O$) C, H, N.

5′-(2,4-Dichlorophenyl)-6,7-didehydro-4,5α-epoxy-3-hydroxy-17-methylpyrido[2′,3′:6,7]morphinan (7k). Hydromorphone hydrochloride (1.00 g, 3.1 mmol) was reacted with 2-(2,4-dichlorophenyl)-3-(dimethylamino)acrolein[42] (1.135 g, 4.65 mmol) and ammonium acetate (0.716 g, 9.3 mmol) in acetic acid (20 mL) by the same procedure as described for the preparation of **7a** to obtain **7k** (0.464 g, 32%): mp 198−200 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 94.5:5:0.5); ^{1}H NMR ($CDCl_3$) δ 1.96−2.15 (m, 2H, C-15 H_2), 2.30−2.71 (m, 6H, C-8 H_2, C-10 H, C-14 H, C-16 H_2), 2.46 (s, 3H, NCH_3), 3.11 (d, 1H, J = 18.7 Hz, C-10 H), 3.30−3.33 (m, 1H, C-9 H), 5.56 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.69 (d, 1H, J = 8.1 Hz, C-1 H), 7.20 (d, 1H, J = 8.4 Hz, C-6″ H), 7.31 (dd, 1H, J = 8.2 and 2.1 Hz, C− 5″ H), 7.38 (d, 1H, J = 2.0 Hz, C-4′ H), 7.49 (d, 1H, J = 2.1 Hz, C-3″ H), 8.58 (d, 1H, J = 1.8 Hz, C-6′ H); MS m/z 465 $(MH)^+$. Anal. ($C_{26}H_{22}Cl_2N_2O_2 \cdot 0.5H_2O$) C, H, N.

5′-(4-Chlorophenyl)-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3-hydroxypyrido[2′,3′:6,7]morphinan (7l). Hydrocodone (5.838 g, 19.52 mmol), obtained from the bitartrate salt by conventional methods, was reacted with 2-(4-chlorophenyl)malondialdehdye (5.46 g, 29.28 mmol) and ammonium acetate (4.51 g, 58.56 mmol) in acetic acid (100 mL) by the same procedure as described for the preparation of **7a** to obtain 5′-(4-chlorophenyl)-6,7-didehydro-4,5α-epoxy-3-methoxy-17-methylpyrido[2′,3′:6,7]morphinan (**16**) (4.70 g, 55%): mp 234−237 °C; TLC R_f = 0.57 ($CHCl_3$−MeOH, 9:1); ^{1}H NMR ($CDCl_3$) 1.99−2.13 (m, 2H, C-15 H, C-16 H), 2.32−2.54 (m 3H, C-8 H, C-10 H, C-16 H), 2.46 (s, 3H, NCH_3), 2.55−2.64 (m, 3H, C-8 H, C-10 H, C-16 H), 3.18 (d, 1H, J = 18.5 Hz, C-10 H), 3.25−3.28 (m, 1H, C-9 H), 3.8 (s, 3H, OCH_3), 5.5 (s, 1H, C-5 H), 6.64 (d, 1H, J = 8.2 Hz, C-1 H), 6.69 (d, 1H, J = 8.2 Hz, C-2 H), 7.5−7.6 (m, 2H, C-3″ H, C-5″ H), 7.70−7.73 (m, 3H, C-3′ H, C-2″ H, C-6″ H), 8.74 (d, 1H, J = 2.3 Hz, C-6′ H); MS m/z 445 $(MH)^+$. Anal. ($C_{27}H_{25}ClN_2O_2 \cdot 0.2H_2O$) C, H, N.

To a solution of compound **16** (2.85 g, 6.61 mmol) in 1,2-dichloroethane (50 mL) potassium carbonate (3.11 g, 22.5 mmol) was added. The mixture was stirred in an inert atmosphere, and vinyl chloroformate (4.1 g, 38.52 mmol) was added dropwise. The reaction mixture was refluxed for 36 h and filtered. The filtrate was evaporated to dryness and the residue was dissolved in ethanol (15 mL). To this solution was added 2 N HCl (5.0 mL) and the mixture was refluxed for 2 h. The solvent was removed under reduced pressure. The residue was treated with water and the pH of the mixture was adjusted to 7−8 by addition of saturated aqueous $NaHCO_3$ solution. The mixture was then extracted with $CHCl_3$ (4 × 100 mL). The extracts were combined, washed with brine, and dried (Na_2SO_4). Filtration and removal of the solvent yielded the crude product, which was purified by chromatography over a column of silica using CH_2Cl_2−MeOH−NH_4OH 98.5:1:0.5 as the eluent to obtain 5′-(4-chlorophenyl)-6,7-didehydro-4,5α-epoxy-3-methoxypyrido[2′,3′:6,7]morphinan (**17**) (1.47 g, 53%): mp 246−248 °C; TLC R_f = 0.44 ($CHCl_3$−MeOH, 9:1); ^{1}H NMR (DMSO-d_6) δ 1.69−1.73 (m, 1H, C-15 H), 1.82−1.92 (m, 1H, C-15 H), 1.99−2.08 (m, 1H, C-16 H), 2.36−2.44 (m, 1H, C-14 H), 2.59−2.78 (m, 4H, C-8 H_2, C-10 H, C-16 H), 2.87−2.96 (m, 1H, C-10 H), 3.37−3.39 (m, 1H, C-9 H), 3.67 (s, 3H, OCH_3), 5.54 (s, 1H, C-5 H), 6.64 (d, 1H, J = 8.2 Hz, C-1 H), 6.62 (d, 1H, J = 8.1 Hz, C-2 H), 7.5−7.6 (m, 2H, C-3″ H, C-5″ H), 7.70−7.73 (m, 3H, C-3′ H, C-2″ H, C-6″ H), 8.8 (d, 1H, J = 2.1 Hz, C-6′ H); MS m/z 431 $(MH)^+$. Anal. ($C_{26}H_{23}ClN_2O_2 \cdot 0.5H_2O$) C, H, N.

Compound **17** (1.37 g, 3.19 mmol) was dissolved in ethanol (70 mL), and $NaHCO_3$ (5.33 g, 6.37 mmol) was added. To this mixture was added cyclopropylmethyl bromide (2.16 g, 16.0 mmol), and the reaction mixture was refluxed under nitrogen for 16 h. The mixture was then concentrated, and water (180 mL) was added to the residue. The mixture was extracted with $CHCl_3$ (4 × 100 mL) and dried over Na_2CO_3. Removal of the solvent under reduced pressure gave the crude product, which was purified by chromatography over a column of silica using CH_2Cl_2−MeOH−NH_4OH (98.5:1:0.5) as the eluent to give 5′-(4-chlorophenyl)-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3-methoxypyrido[2′,3′:6,7]morphinan (**7q**) (0.93 g, 76%): mp 196−198 °C; TLC R_f = 0.5 (CH_2Cl_2−MeOH−NH_4OH, 96.5:3:0.5); ^{1}H NMR ($CDCl_3$) δ 0.14−0.19 and 0.53−0.59 (2m, 4H, cyclopropyl CH_2CH_2), 0.85−0.89 (m, 1H, cyclopropyl CH), 1.97−2.15 (m, 2H, C-15 H_2), 2.28−2.66 (m, 7H, C-8 H_2, C-10 H, C-14 H, C-16 H, and NCH_2-cyclopropyl), 2.81−2.86 (m, 1H, C-16 H), 3.02 (d, 1H, J = 18.7 Hz, C-10 H), 3.57−3.60 (m, 1H, C-9 H), 3.81 (s, 3H, OCH_3), 5.56 (s, 1H, C-5 H), 6.61 (d, 1H, J = 8.1 Hz, C-2 H), 6.67 (d, 1H, J = 8.1 Hz, C-1 H), 7.39−7.46 (m, 5H, C-4′ H, C-2″ H, C-3″ H, C-5″ H, C-6″ H), 8.75 (d, 1H, J = 1.6 Hz, C-6′ H); MS m/z 485 $(MH)^+$. Anal. ($C_{30}H_{29}ClN_2O_2$) C, H, N.

A solution of the methyl ether **7q** (0.83 g, 1.71 mmol) in CH_2Cl_2 (20 mL) was reacted with BBr_3 (17.0 mL of 1 M solution in CH_2Cl_2, 17.0 mmol) as described for the preparation of **7b** from **7m** to yield 0.227 g (28%) of **7l**: mp 178−180 °C; TLC R_f = 0.3 (CH_2Cl_2−MeOH−NH_4OH, 96.5:3:0.5); ^{1}H NMR ($CDCl_3$) δ 0.13−0.19 and 0.51−0.57 (2m, 4H, cyclopropyl CH_2CH_2), 0.85−0.93 (m, 1H, cyclopropyl CH), 1.96−2.16 (m, 2H, C-15 H_2), 2.28−2.66 (m, 7H, C-8 H_2, C-10 H, C-14 H, C-16 H, and NCH_2-cyclopropyl), 2.82−2.87 (m, 1H, C-16 H), 3.00 (d, 1H, J = 18.7 Hz, C-10 H), 3.60−3.62 (m, 1H, C-9 H), 5.2−5.8 (broad hump, 1H, C-3 OH), 5.57 (s, 1H, C-5 H), 6.58 (d, 1H, J = 8.1 Hz, C-2 H), 6.67 (d, 1H, J = 8.1 Hz, C-1 H), 7.39−7.45 (m, 5H, C-4′ H, C-2″ H, C-3″ H, C-5″ H, C-6″ H), 8.70 (d, 1H, J = 2.0 Hz, C-6′ H); MS m/z 471 $(MH)^+$. Anal. ($C_{29}H_{27}ClN_2O_2$) C, H, N.

Biological Assays. Radioligand Binding Assays for μ, δ, and κ Receptors. The μ binding sites were labeled using [^{3}H]DAMGO (1−3 nM) and rat brain membranes as previously described[31] with several modifications. Rat membranes were prepared each day using a partially thawed frozen rat brain that was homogenized with a polytron in 10 mL/brain of ice-cold 10 mM Tris-HCl, pH 7.0. Membranes were then centrifuged twice at 30000g for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (50 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, along with a protease inhibitor cocktail (PIC). The nonspecific binding was determined using 20 μM of levallorphan. The δ binding sites were labeled using [^{3}H]DADLE (2 nM) and rat brain membranes as previously described,[30] with several modifications. Rat membranes were prepared each day using a partially thawed frozen rat brain that was homogenized with a polytron in 10 mL/brain of ice-cold 10 mM Tris-HCl, pH 7.0. Membranes were then centrifuged twice at 30000g for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (50 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, 100 nM DAMGO to block binding to μ sites, and PIC. Nonspecific binding was determined using 20 μM levallorphan. The κ binding sites were labeled using [^{3}H]U69,593 (2 nM) as previously described,[32] with several modifications. Guinea pig brain membranes were prepared each day using partially thawed guinea pig brain that was homogenized with a polytron in 10 mL/brain of ice-cold 10 mM Tris-HCl, pH 7.0. The membranes were then centrifuged twice at 30000g for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (75 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing 1 μg/mL of captopril and PIC. Nonspecific binding was determined using 1 μM U69,593. Each ^{3}H ligand was displaced by 8−10 concentrations of test drug, two times. Compounds were prepared as 1 mM solution with 10 mM Tris buffer (pH 7.4) containing 10% DMSO before drug dilution. All drug dilutions were done in 10 mM Tris-HCl, pH

7.4, containing 1 mg/mL bovine serum albumin. All washes were done with ice-cold 10 mM Tris-HCl, pH 7.4.

[^{35}S]GTP-γ-S Functional Assays. The [^{35}S]GTP-γ-S binding assays were performed according to the methods described previously.[35] Guinea pig caudate membranes (10−20 μg of protein in 300 μL of 50 mM Tris-HCl, pH 7.4, with 1.67 mM DTT and 0.15% BSA) were added to polystyrene 96-well plates filled with 200 μL of a reaction buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 100 μM GDP, 0.1% BSA, 0.05−0.1 nM [^{35}S]GTP-γ-S, and varying concentrations of drugs. The reaction mixture was incubated for 3 h at 22 °C (equilibrium). The reaction was terminated by the addition of 0.5 mL of ice-cold Tris-HCl, pH 7.4 (4 °C), followed by rapid vacuum filtration through Whatman GF/B filters previously soaked in ice-cold Tris-HCl, pH 7.4 (4 °C). The filters were washed twice with 0.5 mL of ice-cold H_2O (4 °C). Bound radioactivity was counted at an efficiency of 98% by liquid scintillation spectroscopy. Nonspecific binding was determined in the presence of 10 μM GTP-γ-S.

In initial screening experiments, each test agent was tested to determine agonist and antagonist activity using a 10 μM concentration in the absence and presence of selective antagonists (6000 nM CTAP, 6 nM nor-BNI or 20 nM NTI or 500 nM TIPP) and selective agonists (10 μM SNC-80, 10 μM DAMGO, or 10 μM U69,593). Compounds showing significant agonist activity were further characterized. In this case, agonist dose−response curves (10 data points each) were generated in the presence of selective antagonists using previously defined "blocking" concentrations:[34] μ receptors (1000 nM TIPP, 6 nM nor-BNI), δ receptors (2000 nM CTAP, 6 nM nor-BNI), and κ receptors (1000 nM TIPP, 2000 nM CTAP). Each curve was run with a 10 μM concentration of the standard agonist (DAMGO, U69,593, or SNC-80). The data were expressed as a percent of the stimulation produced by the standard agonist. As described elsewhere,[35,36] compounds showing significant antagonist activity were further assessed with full dose−response curves to determine the functional K_i values for inhibition of agonist-stimulated [^{35}S]GTP-γ-S binding using 10 μM DAMGO, 10 μM SNC-80, or 10 μM U69,593.

Data Analysis. The data of the two separate experiments (opioid binding assays) or three experiments ([^{35}S]GTP-γ-S assays) were pooled and fit by applying the nonlinear least-squares curve-fitting program MLAB-PC (Civilized Software, Bethesda, MD) to the two-parameter logistic equation[43] for the best-fit estimates of the IC_{50} and slope factor. The K_i values were then calculated using the equation $K_i = IC_{50}/(1 + [L]/K_d)$.

GPI and MVD Bioassays.[37,38] Electrically induced smooth muscle contractions of mouse vas deferens and strips of guinea pig ileum longitudinal muscle myenteric plexus were used. Tissues came from male ICR mice weighing 25−40 g and male Hartley guinea pigs weighing 250−500 g. The tissues were tied to a gold chain with suture silk, suspended in 20 mL baths containing 37 °C oxygenated (95% O_2, 5% CO_2) Krebs bicarbonate solution (magnesium free for the MVD), and allowed to equilibrate for 15 min. The tissues were then stretched to optimal length with previously determined 1 g tension (0.5 g for MVD) and allowed to equilibrate for 15 min. The tissues were stimulated transmurally between platinum wire electrodes at 0.1 Hz with 0.4 ms pulses (2 ms pulses for MVD) and supramaximal voltage. An initial dose−response curve of DPDPE or PL-017 was constructed at the start of each assay to establish tissue effects, allowing each tissue to be used as its own control. Tissues not producing typical results were not used. Experimental compounds were added to the baths in 14−60 μL volumes. Succeeding doses of agonist were added cumulatively to the bath at 3 min intervals to produce a concentration−response curve. The tissues were then washed extensively with fresh buffer until the original contraction height was reestablished. Agonist effects of the compounds at 1 μM were measured as the percent inhibition of contraction height 10 min after addition to the bath. Antagonist effects to DPDPE and PL-017 were assayed after incubation of the tissues with 1 μM concentration of the compound in the bath for 30 min. In the testing of compounds **7d**, **7h**, **7i**, and **7k**, a concentration causing less than 20% inhibition of contraction height was used in the 30 min incubation to allow for a more accurate determination of the antagonist potency against DPDPE in the MVD. After the 30 min incubation, the tissues were washed with fresh buffer for 30 min, and the agonist dose−response curve was repeated. Rightward shifts in the dose−response curves were calculated by dividing the antagonized dose−response curve IC_{50} value by the unantagonized IC_{50} value. IC_{50} values represent the mean of two to four tissues. IC_{50} estimates and their associated standard errors were determined by using a computerized nonlinear least-squares method.[44]

Antinociceptive Studies. Male ICR mice (Harlan) were used for all evaluations. Mice were housed in a temperature and humidity controlled vivarium on a (12 h):(12 h) light:dark cycle with unlimited access to food and water prior to the formal procedures. Graded doses of morphine or the test compounds were injected intracerebroventricularly (icv) under light ether anesthesia.[21] Morphine sulfate was dissolved in distilled water and injected in a volume of 5 μL. The dihydrochloride salt of **7h** was dissolved in water and injected in a volume of 5 μL. All other compounds were dissolved in 100% DMSO and injected in a volume of 5 μL. Antinociceptive assays were performed at various times after injection.

Warm-Water Tail-Withdrawal Assay. Naive mice were baselined in the 55 °C warm-water tail-withdrawal test as previously described.[21,45] Doses of morphine or the test compound were injected icv, and antinociception was assessed at 10, 20, 30, 45, 60, 80, 120, and 180 min postinjection. Percent antinociception was calculated using the formula %MPE (maximal possible effect) = 100 × (test − control)/(cutoff − control) where control is the predrug observation, test is the postdrug observation, and cutoff is the maximal length of stimulus allowed (10 s for 55 °C tail-withdrawal). Antinociceptive A_{50} values and 95% confidence intervals were determined using linear regression software (FlashCalc). Opioid activity of the test compounds was assessed by pretreating animals with naloxone (10 mg/kg ip, −10 min) followed by an icv injection of an approximate A_{90} dose of test compound. If a compound did not produce a full agonist effect, then the dose that produced the greatest antinociceptive effect was used. Antinociception was assessed in the 55 °C warm-water tail-withdrawal test at 10, 20, and 30 min. A positive response to a fixed dose of naloxone was indicated when greater than 80% reduction in the antinociceptive effect of the agonist was observed.

Tolerance Regimen. Mice were injected twice daily (8 a.m. and 8 p.m.) with an approximate A_{90} dose of morphine or A_{90} doses of **7h** for 3 days. Antinociceptive dose−response curves in the warm-water tail-withdrawal assay were generated on the morning of the fourth day using the procedures outlined above.

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