Synthesis, Opioid Receptor Binding, and Bioassay of Naltrindole Analogues Substituted in the Indolic Benzene Moiety

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A series of analogues of the δ opioid receptor antagonist naltrindole (**1**) possessing a phenyl, phenoxy, or benzyloxy group at the 4′-, 5′-, 6′-, or − 7′-positions (**4**−**15**) and a 2-(2-pyridinyl)-ethenyl group at the 5′-position (**16**) on the indolic benzene ring were synthesized through Fischer indolization of naltrexone. Compounds **4**−**16** were evaluated for their affinities in opioid receptor binding assays in rat or guinea pig brain membranes and for their opioid antagonist and agonist activities in vitro on the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations. All of the compounds displayed δ selectivity in binding to the δ, μ, and κ opioid receptors. The binding potencies of most of the compounds at the δ, μ, and κ sites, however, were lower than that of **1**. Among positional isomers, the 7′-substituted compounds in general had higher affinities than 6′-, 5′-, or 4′-substituted analogues, indicating that bulky groups are tolerated better at the 7′-position than at other positions. The affinity of the compounds were also determined at putative subtypes of the δ and κ receptors: δ_{cx-1} (μ-like), δ_{cx-2} (δ-like), and the κ_{2b} site in an attempt to identify subtype selective agents. Although none were identified, the data revealed a different rank-order of potency beteween μ vs δ_{cx-1}, δ_{cx-2} vs δ, and the κ_{2b} vs μ, δ, and κ_1. The antagonist potencies of the compounds in the MVD were in agreement with their binding affinities at the δ site in rat brain membrane. The most potent member of the series, the 7′-phenoxy compound **14**, binds to the δ site with a K_i of 0.71 nM, shows >40-fold δ over μ and δ over κ binding selectivity, and exhibits δ receptor antagonist potency in the MVD with a K_e of 0.25 nM, properties which are comparable to the δ receptor affinity and antagonist potency of naltrindole (K_i = 0.29 nM, K_e = 0.49 nM). Interestingly, many members of the series were found to possess significant partial to full agonist activities in the MVD (**6**, **9**, **10**, **13**, **16**) or GPI (**6**, **11**, **14**, **15**). Among the compounds studied, the highest agonist activity in the MVD was displayed by **16** (IC_{50} = 220 nM), and the highest agonist activity in the GPI was displayed by **14** (IC_{50} = 450 nM). The overall affinity and activity profile of compound **14** is, therefore, that of a nonpeptide ligand possessing mixed μ agonist/δ antagonist properties. Recently there has been considerable interest in such compounds possessing μ agonist/δ antagonist activities because of their potential therapeutic usefulness as analgesics with low propensity to produce tolerance and dependence side effects. The results of the present study suggest that morphinan derivatives related to **16** and **14** may provide useful leads for the development of potent nonpeptide ligands possessing δ agonist or mixed δ antagonist/μ agonist activities.

The existence of three major types of opioid receptors, referred to as mu (μ), delta (δ), and kappa (κ) receptors is widely accepted and well supported by a variety of experimental data including recent cloning of μ-, δ-, and κ-opioid receptors.[1−8] Among these three major classes of opioid receptors, recent research efforts have focused on the development of agonist and antagonist ligands selective for the δ opioid receptors.[9−14] Various studies suggest that δ selective agonists could be potentially useful as analgesics devoid of side effects such as respiratory depression, physical dependence, and gastrointestinal effects.[15] Selective antagonists of δ receptors have been shown to modulate the development of tolerance and dependence to μ agonists such as morphine,[16] modulate the behavioral effects of drugs of abuse such as cocaine,[17,18] and to elicit immunomodulatory effects.[19,20] Thus, the δ opioid receptor system has become an attractive candidate for the development of analgesics, immunomodulatory agents, and treatment agents for drug addiction.

Pharmacological studies in vitro and in vivo have suggested the existence of subtypes of the δ opioid receptors, termed δ_1 and δ_2.[21−25] On the basis of ligand binding studies the δ receptor sites have also been distinguished as δ sites associated with a putative $\mu-\delta$

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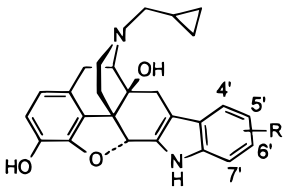
opioid receptor complex,[26] termed the δ_{cx} site, and δ sites not associated with the $\mu-\delta$ opioid receptor complex, termed the δ_{ncx} site.[26−29] Binding, pharmacological, and molecular biological studies indicate that the cloned δ receptor is related to the pharmacologically defined δ_2 receptor[30,31] and likely to the δ_{ncx-2} binding site.[32] The relationship between the δ subtypes defined in binding assays[26−29] and the δ_1 and δ_2 receptors remains to be fully elucidated. Among nonpeptide opioid ligands, the indolomorphinan naltrindole (**1**, NTI) developed by Portoghese and co-workers is widely used as a highly selective and potent δ opioid receptor antagonist and has served as a prototype molecule for structural manipulations in the search for novel agonist and antagonist ligands possessing opioid receptor subtype selectivity.[33−49] Although NTI itself does not display selectivity for the putative subtypes of the δ receptor, substituted analogues of NTI such as the 5′-isothiocyanato derivative **2** (NTII) and the 1′-benzyl derivative **3** (BNTI) have been found to possess pharmacological selectivity for the δ_2 receptor.[23,36,41]

The high δ-opioid antagonist potency and selectivity of NTI have been ascribed to the indolic benzene moiety, which mimics a portion of the putative "address" component (Phe[4]) of enkephalin.[45] Previous structure−activity studies on NTI analogues focusing on the indolic benzene ring have included ligands with benzannulations at the 5′,6′- and 6′,7′-positions, as well as compounds possessing substituents such as F, Br, Me, NO_2, NCS, OH, OMe, OEt at the 4′-, 5′-, 6′-, or 7′-positions and basic alkylamidinomethyl groups at the 5′-position.[33,34,36,37,40] Steric tolerance for bulky groups on the indolic benzene moiety of the indolomorphinans at the ligand binding sites of the opioid receptors, however, has remained largely unexplored. The present study was therefore undertaken to probe the effect of bulky aromatic group substitutions at the indolic benzene ring of NTI on the opioid receptor binding profile and intrinsic activity. The affinities of the compounds were also determined at putative subtypes of the δ and κ receptors, δ_{cx-1} (μ-like), δ_{cx-2} (δ-like), and the κ_{2b} site, in an attempt to identify subtype selective agents. Herein we report the synthesis and evaluation of a series of such analogues **4**−**16** (Chart 1) possessing primarily a phenyl group directly attached to the indolic benzene ring of NTI or tethered to it through a one- or two-atom spacer group. The choice of the O or OCH_2 linkage for tethering the phenyl group to the indolic benzene ring was primarily based on the synthetic accessibility of the needed intermediates.

Chart 1



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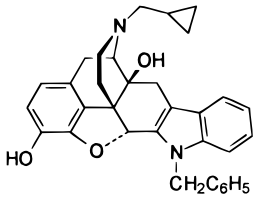


3, BNTI

1 H (NTI)

2 5′-NCS (NTII)

4 4′-C_6H_5

5 4′-OC_6H_5

6 4′-$OCH_2C_6H_5$

7 5′-C_6H_5

8 5′-OC_6H_5

9 5′-$OCH_2C_6H_5$

10 6′-C_6H_5

11 6′-OC_6H_5

12 6′-$OCH_2C_6H_5$

13 7′-C_6H_5

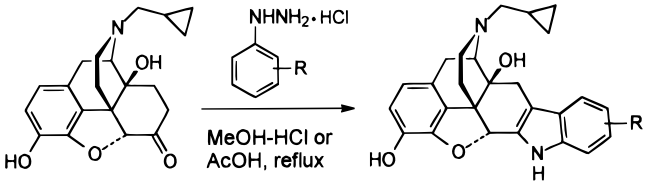
14 7′-OC_6H_5

15 7′-$OCH_2C_6H_5$

16 5′-(*E*)-CH=CH- 

17 5′-$CH_2NH-C(=NH)-R'$

Scheme 1

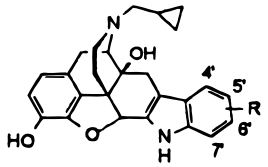


Naltrexone **4 - 17**

Chemistry

The target compounds **4**−**16** were synthesized by the Fischer indolization of naltrexone with the appropriate phenylhydrazine (Scheme 1). With the exception of 2-(benzyloxy)phenylhydrazine, all of the required phenylhydrazines carrying phenyl-, phenoxy-, and benzyloxy substituents at the *ortho*-, *meta*-, and *para*-positions were synthesized essentially according to literature methods starting from the corresponding anilines via diazotization and reduction.[46−50] The 2-(benzyloxy)-phenylhydrazine was prepared by a literature procedure involving sydnone hydrolysis.[51] The Fischer indolizations were carried out using hydrogen chloride-saturated methanol or glacial acetic acid as the reaction medium. The *ortho*- and *para*-substituted phenylhydrazines yielded the corresponding 7′-or 5′-substituted target compounds **13**−**15** and **7**−**9**, respectively. As expected, the condensation of naltrexone with *meta*-substituted phenylhydrazines yielded predominantly the 6′-substituted isomers **10**−**12** along with minor amounts of the 4′-isomers **4**−**6**. In the condensation of naltrexone with 3-(benzyloxy)phenylhydrazine, when methanolic hydrogen chloride was used as the acid catalyst the indolization proceeded with concomitant O-debenzylation to yield predominantly 6′-hydroxynaltrindole as the isolable product. This debenzylation could be avoided by using acetic acid as the reaction medium to obtain the desired 4′- and 6′-benzyloxy target compounds **6** and **12**. The 5′-(2-pyridinylethenyl) compound **16** was obtained by condensation of naltrexone

Table 1. Opioid Receptor Binding Affinities of NTI Analogues in Homogenates of Rat or Guinea Pig Brain Membranes



		K_i (nM) ± SEM						selectivity ratio	
compd	R	δ[a]	μ[b]	κ_1[c]	δ_{cx1}[d]	δ_{cx2}[e]	κ_{2b}[f]	μ/δ	k_1/δ
4	4′-C_6H_5	52.4 ± 9.2	1510 ± 106	2970 ± 368	284 ± 14.3	127 ± 13.7	435 ± 35.3	29	57
5	4′-OC_6H_5	67.9 ± 1.9	1430 ± 126	1310 ± 114	395 ± 85.7	114 ± 8.5	353 ± 21.7	21	19
6	4′-$OCH_2C_6H_5$	14.8 ± 1.5	360 ± 32	539 ± 85	74.1 ± 10.2	17.2 ± 4.8	109 ± 10.3	24	36
7	5′-C_6H_5	15.8 ± 1.3	165 ± 29	354 ± 23	121 ± 24.1	24.7 ± 2.2	186 ± 10.6	10	22
8	5′-OC_6H_5	17.9 ± 1.4	292 ± 29	75.2 ± 19	204 ± 50.5	4.9 ± 0.5	199 ± 40.6	16	4
9	5′-$OCH_2C_6H_5$	4.4 ± 0.51	81 ± 6.5	82.1 ± 14	14.9 ± 2.6	1.7 ± 0.17	85.8 ± 17.4	18	19
10	6′-C_6H_5	18.0 ± 2.0	1280 ± 219	632 ± 85	299 ± 46.0	14.2 ± 3.5	660 ± 43.6	71	35
11	6′-OC_6H_5	6.4 ± 1.2	235 ± 14	236 ± 66	89.4 ± 20.5	4.9 ± 0.49	175 ± 23.7	37	37
12	6′-$OCH_2C_6H_5$	6.7 ± 0.9	676 ± 69	470 ± 52	201 ± 58.4	6.3 ± 0.5	461 ± 43.4	101	70
13	7′-C_6H_5	2.1 ± 0.26	124 ± 10	312 ± 23	51.4 ± 7.1	0.46 ± 0.06	89.9 ± 7.5	59	149
14	7′-OC_6H_5	0.71 ± 0.08	34.2 ± 9.4	33.6 ± 2.6	18.7 ± 1.4	0.61 ± 0.04	56.8 ± 5.1	48	47
15	7′-$OCH_2C_6H_5$	3.1 ± 0.54	159 ± 17	258 ± 66	73.3 ± 10.7	2.5 ± 0.13	112 ± 15.1	51	83
16	5′-(*E*)-CH=CH-2-Py	3.7 ± 0.29	76 ± 9.4	20.1 ± 2.4	17.4 ± 2.5	4.0 ± 0.59	21.4 ± 1.2	21	5
1	H	0.29 ± 0.06	50 ± 2.3	34.1 ± 4.1	12.3 ± 1.4	0.24 ± 0.02	19.9 ± 1.4	172	118

[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 (1.4−2.0 nM) in rat brain membranes. [c] Displacement of [^{3}H]U69,593 (1.2−2.2 nM) in guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with irreversible ligands BIT and FIT. [d] Displacement of [^{3}H]DADLE (2.0 nM) in the presence of 300 nM DELT-II in rat brain membranes depleted of δ_{ncx} sites by treatment with δ_{ncx}-selective acylating agent, (+)-trans-SUPERFIT. [e]Diplacement of [^{3}H]DADLE (2.0 nM) in the presence of 50 nM morphine using rat brain membranes depleted of δ_{ncx} sites by pretreatment with the δ_{ncx}-selective acylating agent, (+)-trans-SUPERFIT. [f]Displacement of [^{125}I]IOXY (0.01 nM) in the presence of 5 μM (−)U50,488 using guinea-pig brain membranes depleted of μ and δ binding sites by pretreatment with BIT and FIT.

with the commercially available 4-(2-pyridinylethenyl)-phenylhydrazine.

Results and Discussion

Opioid Receptor Binding. The binding affinities of the target compounds for the μ and δ receptors were determined by inhibition of binding of [^{3}H]DAMGO[52] and [^{3}H]DADLE[53] to rat brain membranes. The affinities of the compounds for the κ receptors were determined by inhibition of binding of [^{3}H]U69,593[54] to guinea pig brain membranes. Since NTI binds with high affinity and selectivity for the morphine-insensitive δ_{cx-2} sites over the morphine-sensitive δ_{cx-1} sites and with moderate affinity to the κ_{2b} site, the target compounds were also evaluated for their affinities at the δ_{cx-1}, δ_{cx-2}, and κ_{2b} sites using previously reported methods.[28,55] The affinities of the target compounds for binding at the μ, δ, κ_1, δ_{cx-1}, δ_{cx-2}, and κ_{2b} sites are listed in Table 1.

All of the compounds bind with greater affinity at the δ receptor than at μ, κ_1, or κ_{2b} sites. The μ/δ binding selectivity of the target compounds are in the range of 10- to 100-fold. The overall affinity pattern for the phenyl-, phenoxy-, and benzyloxy naltrindoles at the δ receptor indicates that these bulky substituents, in general, are better tolerated at the 7′-position than at 4′-, 5′-, or 6′-positions, the δ receptor affinity generally decreasing in the following order: 7′ > 6′ ≃ 5′ > 4′. The most potent member of the series, the 7′-phenoxy compound **14**, is nearly as potent as naltrindole at the δ, μ, and κ_1 sites. Among the phenyl, phenoxy, and benzyloxy substituents at the 4′-, 5′-, or 6′-positions, the benzyloxy group appears to be better tolerated at the δ binding site. It is possible that the increased conformational flexibility of the benzyloxy side chain may allow for a better accommodation of this substituent at the δ receptor sites. The 6′-benzyloxy compound **12** shows the highest (101-fold) μ/δ selectivity ratio among this group of compounds. In general, the 6′- and 7′-substituted compounds display greater δ selectivities than the 4′- and 5′-substituted compounds. The affinities of the compounds at the δ_{cx-2} site parallel their affinities at the δ site. While most of the compounds show equal or slightly reduced affinities at the δ_{cx-2} site in comparison to their potency at δ site, a slight enhancement in affinities is observed for compounds **8**, **9**, and **13**. The δ_{cx-1} and δ_{cx-2} affinity profiles of the target compounds are in conformity with previous studies, indicating that the ligand selectivity profiles at the δ_{cx-1} and δ_{cx-2} sites in general reflect the selectivity profiles at the classical μ- and δ-sites, respectively.[28]

The affinities of the target compounds at the κ_1 site are in the same range as their affinities at the μ-site, their κ_1/δ selectivity ratios ranging from 4-fold for **8** to 149-fold for **13**. Highest affinity for binding at the κ_1 site is displayed by the 5′-pyridinylethenyl compound **16**. With the exception of **8** and **14**, the affinities of all of the compounds are greater at the κ_{2b} site than they are at the κ_1 site. Of interest are the affinity profiles of the two-atom bridged 5′-benzyloxy and 5′-pyridinylethenyl compounds **12** and **16** at the δ and κ_1 sites. Compared to NTI, compound **12** possesses lower affinities at both the δ and κ_1 sites with 23-fold reduction at the δ-site and 14-fold reduction at the κ_1 site. While the affinity of **16** is also reduced 13-fold at the δ site, relative to NTI, the affinity of **16** at the κ_1 site shows 1.7-fold enhancement. Earlier studies on naltrindole analogues of the type **17** have revealed that the introduction of basic groups such as alkylamidinomethyl groups at the 5′-position leads to a remarkable change

in opioid receptor selectivity from δ to κ.[34] The enhanced κ receptor affinity and selectivity of **17** as well as that of ligands such as norbinaltorphimine have been attributed to favorable interaction of the basic moieties present in these ligands with an acidic residue such as Glu297 at the TM6 of the κ receptor.[56,57] Therefore it appears likely that weak, favorable interactions provided by the basic nitrogen of the pyridinylethenyl substituent of **16** may be contributing to its enhanced binding affinity at the κ_1 site.

All of the benzene ring substituted naltrindole targets have retained the δ-selective binding property of the parent compound, albeit with moderate to significant reductions in their affinities in binding to the δ site. The inability of the benzene ring substituents to substantially modify the binding selectivity profile of NTI at the putative δ receptor subtypes suggests that the environment in the ligand binding pocket that accommodates the indolic benzenoid moiety of the indolomorphinans may be somewhat similar in these opioid receptor subtypes. As noted above, the ligand-selectivity pattern of the δ_{cx-2} site is similar to that of the δ site, and the ligand-selectivity pattern of the δ_{cx-1} site is similar to that of the μ site. Moreover, the ligand-selectivity pattern of the κ_{2b} site is somewhat similar to the μ and δ sites.[55] To probe for differences in ligand-selectivity patterns, the data was first normalized to that of the reference compound, naltrindole, by dividing the K_i values of each analogue by the K_i of naltrindole for that site to yield a "normalized K_i" value. To compare two sites, for example, μ vs δ, the following calculation was made: $1 - ([\text{normalized } K_i\ \mu]/[\text{normalized } K_i\ \delta])$. The term $[\text{normalized } K_i\ \mu]/[\text{normalized } K_i\ \delta]$ expresses the relative affinities. If two sites are identical, the expected ratio would be 1.0. Subtracting this value from 1 expresses the difference between expected and observed values. If two sites are identical, then the difference will be zero. Deviation from the expected value of 0 indicates a different ligand-selectivity profile. We made the following comparisons shown in Figure 1: μ vs δ_{cx-1} (panel A), δ vs δ_{cx-2} (panel B), μ vs κ_{2b} (panel C), δ vs κ_{2b} (panel D), and κ_1 vs κ_{2b} (panel E). These results show that compounds **7** and **8** best distinguish between μ and δ_{cx-1}, that compounds **8** and **13** best distinguish between δ and δ_{cx-2}, that compounds **7** and **14** best distinguish between μ and κ_{2b}, that compounds **5** and **16** best distinguish between δ vs κ_{2b}, and that compounds **4** and **6** best distinguish between κ_1 and κ_{2b}.

Bioassays in Smooth Muscle Preparations. The target compounds were evaluated for antagonist and agonist activities on electrically stimulated guinea pig ileal longitudinal muscle myenteric plexus (GPI) and the mouse vas deferens (MVD) smooth muscle preparations using previously described bioassays.[58] The standard agonist ligands DPDPE and PL 017 possessing pharmacological selectivity for μ and δ opioid receptor, respectively, were employed for the evaluation of antagonist potency. The compounds were incubated with the preparations for 30 min and washed with buffer prior to testing with the standard agonists. The antagonist potency is expressed as IC_{50} ratios and as K_e values. The IC_{50} ratio represents the agonist potency in the presence of the test compound divided by the control IC_{50} in the same preparation. The K_e values were calculated from the expression $K_e = [\text{antagonist}]/(IC_{50}\ \text{ratio} - 1)$. The agonist activities of the compounds were evaluated in the MVD and GPI and are expressed as percent of maximal response at 1 μM or, for more potent ligands, as IC_{50} values.

The opioid antagonist and agonist potencies of the target compounds and the reference ligand NTI are listed in Table 2. All of the compounds, with the exception of **6** and **16**, exhibited antagonist activity against the agonist DPDPE in the MVD. The δ-antagonist potencies of the compounds in the MVD correspond fairly closely with their δ-site affinities determined in the ligand binding assays. Indeed a comparison of the K_i and K_e values afforded a highly significant relationship ($r^2 = 0.87$, $P < 0.000006$). Among the positional isomers, high antagonist potencies in the MVD are displayed by the 7′-substituted compounds **13**−**15**. The most potent member of this series, the 7′-phenoxy compound **14** ($K_e = 0.25$ nM), is twice as potent as NTI in its antagonist activity at the δ receptor. The 5′- and 6′-substituted analogues **7**−**12** display diminished antagonist potencies with K_e values in the range of 4−20 nM. These results are in harmony with the observations of Portoghese and co-workers who found a similar reduction in δ antagonist potencies in compounds bearing substituents at the 5′- or 6′-position of naltrindole.[34] In comparison to their antagonist potencies in the MVD, all of the compounds are much weaker as antagonists in the GPI and, as a consequence, these analogues possess 10 to >170-fold δ over μ antagonist selectivities.

Most of the target compounds displayed partial agonist activity at 1 μM concentration in the MVD with inhibition of maximal response in the range of 11−36%. Two compounds that showed higher agonist responses were compounds **6** (70% maximal response) and **16** (79% maximal response, $IC_{50} = 220$ nM). Interestingly, several compounds displayed agonist activity in the GPI preparations. Compounds exhibiting >50% inhibition of maximal response in the GPI include **11**, **14** and **15** as well as **6** and **16**. Among the four compounds (**11**, **14**, **15**, **16**) for which agonist IC_{50} values were determined, the most potent was found to be the 7′-phenoxy compound **14** ($IC_{50} = 450$ nM). Regarding the MVD/GPI agonist selectivity, **11** is relatively nonselective, **16** is δ-selective, while **14** and **15** are μ-selective. The agonist effects of **14** and **16** in the GPI and the MVD were antagonized by naloxone. In the presence of 1 μM of naloxone, the agonist dose−response curve of **14** was shifted to higher concentration by a factor of 5.1 in the GPI and 2.8 in the MVD. Similarly, in the presence of naloxone (1μM), the agonist IC_{50} values of **16** were increased 2.5-fold in the GPI and 9.1-fold in the MVD. Further work is needed to determine whether the observed agonist activities of **14** in GPI and **16** in MVD smooth muscle preparations are indeed mediated through the μ- and δ- receptors, respectively, and whether these activities correlate with their modest binding potencies at the μ and δ sites in the rat brain membranes.

Studies in mice and rats have demonstrated that δ opioid antagonists such as NTI and TIPP can prevent the development of tolerance and dependence to μ agonists such as morphine.[15,59] On the basis of these observations it has been suggested that the development

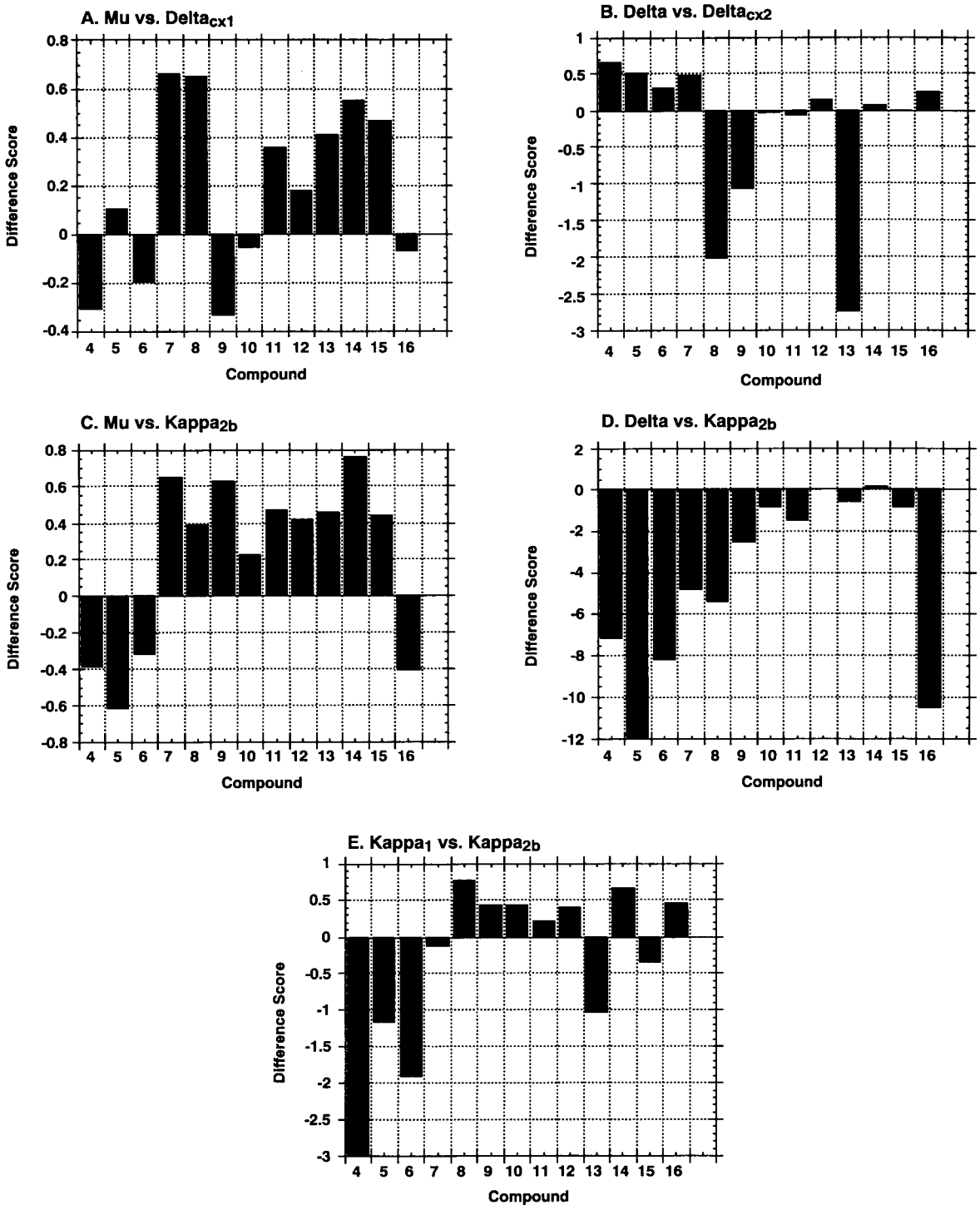


Figure 1. Difference scores for compounds **4**−**16** for binding at μ vs δ_{cx-1} (panel A), δ vs δ_{cx-2} (panel B), μ vs κ_{2b} (panel C), δ vs κ_{2b} (panel D) and κ_1 vs κ_{2b} sites (panel E). Difference scores were calculated using the formula $1 - [(K_i$ of compound at the first site/K_i of naltrindole at the first site)]/[(K_i of compound at the second site/K_i of naltrindole at the second site)]. As described in the text, the difference scores reflect the magnitude of differences in binding affinities at two binding sites relative to that of naltrindole (**1**).

of compounds possessing mixed μ agonist/δ antagonist properties may have considerable therapeutic potential as analgesic drugs for treating chronic pain.[16,59−61] Among the compounds examined in the present study, the 7′-phenoxy compound **14** is therefore of particular interest in that it displays the properties of a mixed δ antagonist/μ agonist with δ antagonist potency equal to that of NTI and μ agonist potency nearly 1/4 that of morphine in the GPI bioassay.[62]

Summary and Conclusions

We have investigated the opioid receptor affinity and activity profile of a series of naltrindole analogues possessing phenyl, phenoxy, and benzyloxy substituents in the indolic benzene moiety. The results of the present study indicate that the introduction of bulky aromatic substituents such as phenyl, phenoxy, and benzyloxy groups at the 4′-, 5′-, 6′-, or 7′-position on the indolomorphinan framework of naltrindole affects the affinity

Table 2. Opioid Antagonist and Agonist Potencies of NTI Analogues in the MVD and GPI Preparations

	antagonist activity					agonist activity		
	DPDPE (δ)[a]		PL017 (μ)[b]					
compd	IC_{50} ratio	K_e (nM)[c]	IC_{50} ratio	K_e (nM)[c]	K_e selectivity ratio μ/δ	MVD IC_{50} (nM) or % max resp[d]	GPI IC_{50} (nM) or % max resp[d]	IC_{50} selectivity ratio GPI(μ)/MVD(δ)
4	16 ± 7	66	2.4 ± 0.2	710	11	22%	0%	
5	61 ± 16	17	2.7 ± 0.1	590	35	27%	28%	
6	e		e			70%	86%	
7	110 ± 0.3	9.4	10 ± 3	110	12	25%	0%	
8	93 ± 7	11	2.3 ± 0.5	770	70	11%	8%	
9	210 ± 29	4.8	2.8 ± 0.1	560	117	35%	24%	
10	49 ± 7	21	f			36%	2%	
11	220 ± 3	4.6	e			3000 ± 1000	2300 ± 200	0.8
12	260 ± 50	3.9	f			25%	0%	
13	560 ± 90	1.8	4.2 ± 1	310	172	35%	37%	
14	4000 ± 200	0.25	e			4100 ± 2100	450 ± 40	0.11
15	610 ± 110	1.7	e			9500 ± 5200	1700 ± 500	0.18
16	e		e			220 ± 70	2600 ± 200	12
1	2000 ± 400	0.49	24 ± 2	43	88	16%	18%	

[a] DPDPE in the MVD preparation. [b] PL-017 in the GPI preparation. [c] K_e (nM) = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio is the IC_{50} of the agonist in the presence of antagonist divided by the control IC_{50} in the same preparation ($n \geq 3$). [d] Partial agonist activity is expressed as the percentage inhibition of contraction at a concentration of 1 μM. [e]The agonist effects precluded the determination of antagonist effects. [f]IC_{50} ratio was not statistically different from 1.

as well as the intrinsic activity to varying degrees depending upon the nature of the substituent and the substitution pattern. Among the various indolic benzene ring positions, bulky substitutions are better tolerated at the 7′-position than at others. The results also demonstrate moderate ligand-selectivity differences between the μ-like sites (μ vs δ_{cx-1}), the δ-like sites (δ vs δ_{cx-2}), and the κ_{2b} sites and μ, δ and κ_1 sites, supporting the hypothesis that these are distinct binding sites. While most of the substituted naltrindole analogues retain the δ antagonist property of the parent compound, some of the analogues display moderate to significant opioid agonist activity in the MVD (**6**, **9**, **10**, **13**, **16**) and GPI (**6**, **11**, **14**, **15**). Of special interest is the finding that compound **14** possesses affinity and activity profile of a nonpeptide opioid ligand with mixed μ agonist/δ antagonist properties, since such compounds may have considerable potential for development as analgesics with a low propensity to produce tolerance and dependence side effects. Further structural manipulations of the indolomorphinan framework may, therefore, lead to more potent nonpeptide ligands possessing δ antagonist or δ agonist as well as mixed μ agonist/δ antagonist properties.

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. Elemental analyses were performed by Atlantic Microlab, Inc. (Atlanta, GA) or the Molecular Spectroscopy Section 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. Preparative TLC was performed on Analtech silica gel GF 2.0 mm plates. Flash column chromatography was performed with E. Merck silica gel 60 (230−400 mesh). All organic extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness on a rotary evaporator under reduced pressure. All reagents were obtained from Aldrich Chemical Co. Naltrexone hydrochloride was obtained from Mallinckrodt. 4′-Hydrazino-2-stilbazole dihydrochloride was purchased from TCI America.

Chemistry. General Procedures. Fischer Indole Synthesis. Method A. 17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxy-4′-phenylindolo[2′,3′:6,7]morphinan (4) and 17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxy-6′-phenylindolo[2′,3′:6,7]morphinan (10). A suspension of naltrexone hydrochloride (1.51 g, 4.0 mmol) and 3-biphenylylhydrazine hydrochloride (1.32 g, 6.0 mmol) in glacial acetic acid (40 mL) was refluxed for 5 h with stirring in an oil bath at 120 °C. After cooling, the reaction mixture was concentrated under reduced pressure, the residue was suspended in water (20 mL), and the pH of the mixture was adjusted to 7.0 with saturated aqueous $NaHCO_3$. The solid obtained was collected by filtration and dried. The crude product was chromatographed on a column of silica gel using $CHCl_3$−MeOH (9:1) as the eluent to obtain a major less polar fraction containing the 6′-isomer and a minor fraction containing the 4′-isomer. Further purification of the polar fraction by preparative TLC ($CHCl_3$−MeOH 12:1), followed by crystallization from MeOH/H_2O yielded 0.057 g (3%) of **4**: mp >186 °C dec; TLC, R_f 0.47 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.08 and 0.43 (2m, 4H, cyclopropyl CH_2CH_2), 0.82 (m, 1H, cyclopropyl CH), 1.54 (d, 1H, C-15 H), 1.93−1.98 (m, 1H, C-8 H), 2.02−2.40 (m, 5H, C-8 H, C-15 H, C-16 H, NCH_2-cyclopropyl), 2.40−2.58 (m, 1H, C-10 H), 2.66 (m, 1H, C-16 H), 2.84 (d, 1H, C-9 H), 2.99 (d, 1H, C-10 H), 4.56 (bs, 1H, C-14 OH), 5.48 (s, 1H, C-5 H), 6.45−6.57 (m, 2H, C-1 H, C-2 H), 6.77 (dd, 1H, C-7′ H), 7.12 (t, 1H, C-6′ H), 7.24 and 7.32−7.44 (d and m, 6H, C-5′ H, C-2″ H, C-3″ H, C-4″ H, C-5″ H, C-6″ H), 8.97 (s, 1H, C-3 OH), 11.38 (s, 1H, NH); MS m/z 491 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_3$·0.5H_2O) C, H, N. Recrystallization of the less polar product from EtOAc/cyclohexane yielded 0.355 g (18%) of **10**: mp >198 °C dec; TLC, R_f 0.52 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.17 and 0.53 (2m, 4H, cyclopropyl CH_2CH_2), 0.92 (m, 1H, cyclopropyl CH), 1.61 (d, 1H, C-15 H), 2.18 (m, 1H, C-16 H), 2.27−2.53 (m, 4H, C-8 H, C-15 H, NCH_2-cyclopropyl), 2.66−2.82 (m, 3H, C-8 H, C-10 H, C-16 H), 3.09 (d, 1H, C-10 H), 3.30 (d, 1H, C-9 H), 4.77 (s, 1H, C-14 OH), 5.54 (s, 1H, C-5 H), 6.45−6.54 (m, 2H, C-1 H, C-2 H), 7.28 (m, 2H, C-4′ H, C-5′ H), 7.44 (t, 3H, C-3″ H, C-4″ H, C-5″ H), 7.56 (s, 1H, C-7′ H), 7.65 (d, 2H, C-2″ H, C-6″ H), 8.94 (s, 1H, C-3 OH), 11.24 (s, 1H, NH); MS m/z 491 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_3$·0.2$CH_3CO_2C_2H_5$) C, H, N.

Fischer Indole Synthesis. Method B. 17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxy-4′-phenoxyindolo[2′,3′:6,7]morphinan (5) and 17-(Cyclo-

propylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxy-6′-phenoxyindolo[2′,3′:6,7]morphinan (11). A suspension of naltrexone hydrochloride (1.89 g, 5.0 mmol) and 3-phenoxyphenylhydrazine hydrochloride (1.98 g, 7.5 mmol) in methanolic HCl (100 mL) was heated with stirring under reflux at 90° C under N_2 for 5 h. The reaction mixture was concentrated and diluted with ice water (200 mL), and the pH was adjusted to 7.0 with saturated aq. $NaHCO_3$. The solid obtained was collected by filtration and dried. Column chromatography of the crude product on silica gel using hexane/EtOAc/Et_3N, 1:1:0.04 as the eluent yielded a major fraction containing the less polar 6′-isomer and a minor fraction containing the 4′-isomer. Preparative TLC ($CHCl_3$−MeOH 9.5:0.5) of the more polar fraction gave 0.024 g (0.9%) of **5**: mp >154° C dec; TLC, R_f 0.45 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.13 and 0.48 (2m, 4H, cyclopropyl CH_2CH_2), 0.87 (m, 1H, cyclopropyl C*H*), 1.57 (d, 1H, C-15 H), 2.13 (m, 1H, C-16 H), 2.24−2.90 (m, 7H, C-8 H_2, C-10 H, NCH_2-cyclopropyl, C-15 H, C-16 H), 3.00 (d, 1H, C-10 H), 3.14 (s, 1H, C-9 H), 4.71 (bs, 1H, C-14 OH), 5.49 (s, 1H, C-5 H), 6.30 (d, 1H, C-5′ H), 6.42−6.55 (m, 2H, C-1 H, C-2 H), 6.97 (m, 3H, C-6′ H, C-2″ H, C-6″ H), 7.09 (m, 2H, C-7′ H, C-4″ H), 7.32 (m, 2H, C-3″ H, C-5″ H), 8.98 (s, 1H, C-3 OH), 11.35 (s, 1H, NH); MS *m*/*z* 507 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_4$·0.25 H_2O) C, H, N

Recrystallization of the less polar product from EtOAc/cyclohexane yielded 622 mg (25%) of **11**: mp >154 °C dec; TLC, R_f 0.59, $CHCl_3$/MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.15 and 0.50 (2m, 4H, cyclopropyl CH_2CH_2), 0.9 (m, 1H, cyclopropyl C*H*), 1.58 (d, 1H, C-15 H), 2.15 (m, 1H, C-16 H), 2.24−2.52 (m, 4H, C-8 H, C-15 H, NCH_2-cyclopropyl), 2.64−2.80 (m, 3H, C-8 H, C-10 H, C-16 H), 3.05 (d, 1H, C-10 H), 3.28 (d, 1H, C-9 H), 4.75 (s, 1H, C-14 OH), 5.55 (s, 1H, C-5 H), 6.45−6.55 (m, 2H, C-1 H, C-2 H), 6.70 (dd, 1H, C-5′ H), 6.93 (m, 3H, C-7′ H, C-2″ H, C-6″ H), 7.05 (m, 1H, C-4″ H), 7.34 (m, 3H, C-4′ H, C-3″ H, C-5″ H), 8.96 (s, 1H, C-3 OH), 11.12 (s, 1H, NH); MS *m*/*z* 507 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_4$·0.5H_2O) C, H, N.

4′-(Benzyloxy)-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxyindolo[2′,3′:6,7]morphinan (6) and 6′-(Benzyloxy)-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxyindolo[2′,3′:6,7]morphinan (12). From naltrexone hydrochloride and 3-(benzyloxy)-phenylhydrazine hydrochloride using method A. 4′-Isomer **6**: yield 0.7%, mp >146 °C dec; TLC, R_f 0.42, $CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.15 and 0.50 (2m, 4H, cyclopropyl CH_2CH_2), 0.90 (m, 1H, cyclopropyl C*H*), 1.57 (d, 1H, C-15 H), 2.15 (m, 1H, C-15 H), 2.30 (m, 1H, C-16 H), 2.39 (d, 2H, NCH_2-cyclopropyl), 2.58 (m, 1H, C-8H), 2.70 (m, 2H, C-10 H, C-16 H), 3.08 (m, 2H, C-8 H, C-10 H), 3.18 (m, 1H, C-9 H), 4.71 (bs, 1H, C-14 OH), 5.15 (dd, 2H, OCH_2-phenyl), 5.47 (s, 1H, C-5 H), 6.42−6.54 (m, 3H, C-1 H, C-2 H, C-5′ H), 6.92 (m, 2H, C-6′ H, C-7′ H), 7.30 (m, 1H, C-4″ H), 7.37 (m, 2H, C-3″ H, C-5″ H), 7.43 (m, 2H, C-2″ H, C-6″ H),8.94(s,1H, C-3 OH), 11.13 (s, 1H, NH); MS *m*/*z* 521$(MH)^+$. Anal. ($C_{33}H_{32}N_2O_4$·0.25$CH_3CO_2C_2H_5$) C, H, N.

6′-Isomer **12**: yield 33%, mp >136 °C dec; TLC, R_f 0.58, ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.15 and 0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.89 (m, 1H, cyclopropyl C*H*), 1.56 (d, 1H, C-15 H), 2.16 (m, 1H, C-16 H), 2.24−2.52 (m, 4H, C-8 H, C-15 H, NCH_2 cyclopropyl), 2.62−2.79 (m, 3H, C-8 H, C-10 H, C-16 H), 3.05 (d, 1H, C-10 H), 3.26 (d, 1H, C-9 H), 4.73 (s, 1H, C-14 OH), 5.10 (s, 2H, OCH_2 phenyl), 5.46 (s, 1H, C-5 H), 6.43−6.53 (m, 2H, C-1 H, C-2 H), 6.68 (dd, 1H, C-5′ H), 6.88 (d, 1H, C-7′ H), 7.24 (d, 1H, C-4′ H), 7.28−7.48 (m, 5H, C-2″ H, C-3″ H, C-4″ H, C-5″ H, C-6″ H), 8.92 (s, 1H, C-3 OH), 10.95 (s, 1H, NH); MS *m*/*z* 521 $(MH)^+$. Anal. ($C_{33}H_{32}N_2O_4$·0.6H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,-14-dihydroxy-5′-phenylindolo[2′,3′:6,7]morphinan (7). This compound was prepared by condensing naltrexone hydrochloride with 4-biphenylylhydrazine hydrochloride using method B. Yield 29%, mp >164 °C dec; TLC, R_f 0.40 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.16 and 0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.90 (m, 1H, cyclopropyl C*H*), 1.60 (d, 1H, C-15 H), 2.17 (m, 1H, C-16 H), 2.27−2.54 (m, 4H, C-8 H, C-15 H, NCH_2-cyclopropyl), 2.66−2.84 (m, 3H, C-8 H, C-10 H, C-16 H), 3.08 (d, 1H, C-10 H), 3.3 (d, 1H, C-9 H), 4.76 (s, 1H, C-14 OH), 5.53 (s, 1H, C-5 H), 6.45−6.55 (m, 2H, C-1 H, C-2 H), 7.26 (m, 1H, C-4″ H), 7.40 (m, 4H, C-4′ H, C-7′ H, C-3″ H, C-5″ H), 7.62 (m, 3H, C-6′ H, C-2″ H, C-6″ H), 8.96 (s, 1H, C-3 OH), 11.22 (s, 1H, NH); MS *m*/*z* 491 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_3$·1.2H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,-14-dihydroxy-5′-phenoxyindolo[2′,3′:6,7]morphinan (8). This compound was prepared by condensing naltrexone hydrochloride with 4-phenoxyphenylhydrazine hydrochloride using method A. Yield 18%, mp (HCl) >270 °C dec; TLC, R_f 0.50 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.38−0.55 and 0.57−0.78 (2m, 4H, cyclopropyl CH_2CH_2), 1.08 (m, 1H, cyclopropyl C*H*), 1.82 (d, 1H, C-15 H), 2.44−2.79 (m, 3H, C-8 H, C-15 H, C-16 H), 2.83−3.00 (m, 2H, C-8 H, NC*H*-cyclopropyl), 3.06−3.48 (m, 4H, C-10 H_2, C-16 H, NC*H*-cyclopropyl), 4.06 (d, 1H, C-9 H), 5.69 (s, 1H, C-5 H), 6.36 (s, 1H, C-14 OH), 6.55−6.69 (m, 2H, C-1 H, C-2 H), 6.88 (d, 3H, C-6′ H, C-2″ H, C-6″ H), 6.94−7.06 (m, 2H, C-4′ H, C-4″ H), 7.30 (t, 2H, C-3″ H, C-5″ H). 7.39 (d, 1H, C-7′ H), 8.94 (bs 1H, H^+), 9.25 (s, 1H, C-3 OH), 11.41 (s, 1H, NH); MS *m*/*z* 507 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_4$·HCl·0.5H_2O) C, H, N.

5′-(Benzyloxy)-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxyindolo[2′,3′:6,7]morphinan (9). This compound was prepared by condensing naltrexone hydrochloride with 4-benzyloxyphenylhydrazine hydrochloride using Method A. Yield 46%, mp (HCl) >360 °C dec; TLC, R_f 0.60 ($CHCl_3$−MeOH−NH_4OH 9:1:0.5); ^{1}H NMR (DMSO-d_6) δ 0.38−0.56 and 0.58−0.79 (2 m, 4H, cyclopropyl CH_2CH_2), 1.10 (m, 1H, cyclopropyl C*H*), 1.80 (d, 1H, C-15 H), 2.44−2.78 (m, 3H, C-8 H, C-15 H, C-16 H), 2.84−3.00 (m, 2H, C-8 H, NC*H*-cyclopropyl), 3.06−3.49 (m, 4H, C-10 H_2, C-16 H, NC*H*-cyclopropyl), 4.08 (d, 1H, C-9 H), 5.04 (s, 2H, OCH_2-phenyl), 5.65 (s, 1H, C-5 H), 6.36 (s, 1H, C-14 OH), 6.55−6.66 (m, 2H, C-1 H, C-2 H), 6.84 (dd, 1H, C-6′ H), 6.90 (d, 1H, C-4′ H), 7.22−7.45 (m, 6H, C-7′ H, C-2″ H, C-3″ H, C-4″ H, C-5″ H, C-6″ H), 8.94 (bs, 1H, H^+), 9.22 (s, 1H, C-3 OH), 11.17 (s, 1H, NH); MS *m*/*z* 521 $(MH)^+$. Anal. ($C_{33}H_{32}N_2O_4$·HCl·0.25H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,-14-dihydroxy-7′-phenylindolo[2′,3′:6,7]morphinan (13). This compound was prepared by condensing naltrexone hydrochloride with 2-biphenylylhydrazine hydrochloride using method B. Yield 38%, mp >160 °C dec; TLC, R_f 0.44 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.15 and 0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.90 (m, 1H, cyclopropyl C*H*), 1.59 (d, 1H, C-15 H), 2.18 (m, 1H, C-16 H), 2.25−2.54 (m, 4H, C-8 H, C-15 H, NCH_2–cyclopropyl), 2.64−2.8 (m, 3H, C-8 H, C-10 H, C-16 H), 3.08 (d, 1H, C-10 H), 3.30 (d, 1H, C-9 H), 4.75 (s, 1H, C-14 OH), 5.49 (s, 1H, C-5 H), 6.45−6.55 (m, 2H, C-1 H, C-2 H), 7.08 (m, 2H, C-4′ H, C-5′ H), 7.40 (m, 2H, C-6′ H, C-4″ H), 7.55 (t, 2H, C-3″ H, C-5″ H), 7.64 (d, 2H, C-2″ H, C-6″ H), 8.92 (s, 1H, C-3 OH), 10.99 (s, 1H, NH); MS *m*/*z* 491 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_3$·0.5$CH_3CO_2C_2H_5$) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,-14-dihydroxy-7′-phenoxyindolo[2′,3′:6,7]morphinan (14). This compound was prepared by condensing naltrexone hydrochloride with 2-phenoxyphenylhydrazine hydrochloride using method B. Yield 35%, mp (HCl) >240 °C dec; TLC, R_f 0.40 ($CHCl_3$−MeOH 9.5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.40−0.56, 0.64, and 0.74 (3m, 4H, cyclopropyl CH_2CH_2), 1.11 (m, 1H, cyclopropyl C*H*), 1.82 (d, 1H, C-15 H), 2.52−2.80 (m, 3H, C-8 H, C-15 H, C-16 H), 2.90−3.04 (m, 2H, C-8 H, NC*H*-cyclopropyl), 3.06−3.52 (m, 4H, C-10 H_2, C-16 H, NC*H*-cyclopropyl), 4.12 (d, 1H, C-9 H), 5.62 (s, 1H, C-5 H), 6.22 (s, 1H, C-14 OH), 6.57−6.71 (m, 3H, C-1 H, C-2 H, C-6′ H), 6.91−7.03 (m, 3H, C-2″ H, C-6″ H, C-5′ H), 7.08−7.20 (m, 2H, C-4′ H, C-4″ H), 7.37 (m, 2H, C-3″ H, C-5″ H), 8.96 (bs, 1H, H^+), 9.23 (s, 1H, C-3 OH), 11.61 (s, 1H, NH); MS *m*/*z* 507 $(MH)^+$. Anal. ($C_{32}H_{30}N_2O_4$·HCl·C_2H_5OH) C, H, N.

7′-(Benzyloxy)-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxyindolo[2′,3′:6,7]morphinan (15). This compound was prepared by condensing naltrexone hydrochloride with 2-(benzyloxy)phenylhydrazine hydrochloride

using method A. Yield 30%, mp >163 °C dec; TLC, R_f 0.37 ($CHCl_3$−MeOH 9.5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.15 and 0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.89 (m, 1H, cyclopropyl C*H*), 1.59 (d, 1H, C-15 H), 2.08−2.22 (m, 1H, C-16 H), 2.24−2.55 (m, 4H, C-8 H, C-15 H, NCH_2-cyclopropyl), 2.61−2.80 (m, 3H, C-8 H, C-10 H, C-16 H), 3.05 (d, 1H, C-10 H), 3.25 (d, 1H, C-9 H), 4.72 (s, 1H, C-14 OH), 5.24 (dd, 2H, OCH_2-phenyl), 5.46 (s, 1H, C-5 H), 6.44−6.54 (m, 2H, C-1 H, C-2 H), 6.72 (d, 1H, C-6′ H), 6.83 (t, 1H, C-5′ H), 6.94 (d, 1H, C-4′ H), 7.30−7.45 (m, 3H, C-3″ H, C-4″ H, C-5″ H), 7.59 (m, 2H, C-2″ H, C-6″ H), 8.93 (s, 1H, C-3 OH), 11.26 (s, 1H, NH); MS *m*/*z* 521 $(MH)^+$. Anal. ($C_{33}H_{32}N_2O_4 \cdot 0.5H_2O$) C, H, N.

***trans*-17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxy-5′-[2-(2-pyridinyl)ethenyl]indolo[2′,3′:6,7]morphinan (16).** This compound was prepared by condensing naltrexone hydrochloride with 4′-hydrazino-2-stilbazole dihydrochloride using method A. Yield 44%, mp (HCl) >198 °C dec; TLC, R_f 0.48 ($CHCl_3$−MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ 0.16 and 0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.90 (m, 1H, cyclopropyl C*H*), 1.59 (d, 1H, C-15 H), 2.16 (m, 1H, C-16 H), 2.25−2.53 (m, 4H, C-8 H, C-15 H, NCH_2-cyclopropyl), 2.65−2.82 (m, 3H, C-8 H, C-10 H, C-16 H), 3.08 (d, 1H, C-10 H), 3.30 (d, 1H, C-9 H), 4.76 (s, 1H, C-14 OH), 5.51 (s, 1H, C-5 H), 6.45−6.55 (m, 2H, C-1 H, C-2 H), 7.15 (d, J = 16 Hz, 1H, C-5″ H, C*H*═CH-py), 7.18 (t, 1H, C-5″ H), 7.34 (d, 1H, C-7′ H), 7.46 (d, 2H, C-6′ H, C-3″ H) 7.62 (s, 1H, C-4′ H), 7.70 (d, J = 16 Hz, 1H, CH═C*H* py), 7.73 (dt, 1H, C-4″ H), 8.53 (d, 1H, C-6″ H), 8.94 (s, 1H, C-3 OH), 11.28 (s, 1H, NH); MS *m*/*z* 518 $(MH)^+$. Anal. ($C_{33}H_{31}N_3O_3 \cdot HCl \cdot 0.4H_2O$) C, H, N.

Biological Assays. Radioligand Binding Assays for μ, δ and κ Receptors. Mu binding sites were labeled using [^{3}H]-DAMGO (1−3 nM) and rat brain membranes as previously described.[52] Briefly, incubations proceeded for 4 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. Delta binding sites were labeled using [^{3}H]DADLE (1.7−2.9 nM) and rat brain membranes as previously described.[53] Incubations proceeded for 3−4 h at 25 °C in 10 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, and 100 nM DAMGO to block binding to μ sites, and PIC. Nonspecific binding was determined using 20 μM levallorphan. Kappa$_1$ binding sites were labeled using [^{3}H]U69,593 (3.4−4.6 nM) and guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with irreversible ligands BIT and FIT as previously described,[54] except that the incubation temperature was 25 °C. Incubations proceeded for 4−6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing PIC and 1 μg/mL captopril. Nonspecific binding was determined using 1 μM U69,593. Delta-cx$_1$ binding sites were labeled using [^{3}H]DADLE (2.0 nM) in the presence of 300 nM DELT-II in rat brain membranes depleted of δ_{ncx} sites by pretreatment with the δ_{ncx}-selective acylating agent (+)-*trans*-SUPERFIT as previously described.[28] Delta-cx$_2$ binding sites were labeled using [^{3}H]DADLE (2.0 nM) in the presence of 50 nM morphine using rat brain membranes depleted of δ_{ncx} sites by pretreatment with the δ_{ncx}-selective acylating agent, (+)-*trans*-SUPERFIT as previously described.[28] For δ_{cx1} and δ_{cx2} binding, incubations proceeded for 4−6 h at 25 °C in 10 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 3 mM $MnCl_2$, 2 μM GTP, 5 mM 2-mercaptoethanol, and PIC. Kappa$_{2b}$ sites were labeled with [^{125}I] IOXY (0.01 nM) in the presence of 5 μM (−)U50,488 using guinea-pig brain membranes depleted of μ and δ binding sites by pretreatment with BIT and FIT as previously described.[55] Briefly, incubations proceeded for 4−6 h at 4 °C in 50 mM Tris-HCl, pH 7.4, containing 10 mM NaCl, PIC and 1 μg/mL of captopril.

Each ^{3}H ligand was displaced by 8−10 concentrations of test drug, two times. All drug dilutions were done in 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL bovine serum albumin. Compounds were prepared as 1 mM solution with 10 mM Tris buffer (pH 7.4) containing 10% DMSO before drug dilution. The IC_{50} and slope factor (N) were obtained by using the program MLAB−PC (Civilized Software, Bethesda, MD). K_i values were calculated according to the equation $K_i = IC_{50}/(1 + [L]/K_d)$.

GPI and MVD Bioassays.[62] 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 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 previously determined to be 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, 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 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. The tissues were then 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.[63]

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