Investigation of the N-Substituent Conformation Governing Potency and μ Receptor Subtype-Selectivity in (+)-(3*R*,4*R*)-Dimethyl-4-(3-hydroxyphenyl)-piperidine Opioid Antagonists

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A study of the binding site requirements associated with the N-substituent of (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**4**) derivatives was undertaken using a set of rigid vs flexible N-substituents. The study showed that compounds **7**−**9** bearing the *trans*-cinnamyl N-substituent most closely reproduced the potency at the opioid receptor of the flexible *N*-propylphenyl or *N*-propylcyclohexyl analogues previously reported. Neither the N-substituted *cis*-cinnamyl nor the *cis*-phenylcyclopropylmethyl compounds **10** and **11**, respectively, showed high affinity for the opioid receptor. However, the *N*-*trans*-phenylcyclopropylmethyl compound **12** closely approximated the affinity of compounds **7**−**9**. Additionally, we found that free rotation of the phenyl ring is necessary for high affinity binding and μ receptor subtype selectivity as the planar N-substituted thianaphthylmethyl and benzofuranylmethyl compounds **13** and **14** had significantly lower binding affinities. Altogether, these findings suggest that the high binding affinity, selectivity, and antagonist potency of *N*-propylphenyl or *N*-propylcyclohexyl analogues of (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**4**) are achieved via a conformation wherein the connecting chain of the N-substituents is extended away from piperidine nitrogen with the appended ring system rotated out-of-plane relative to the connecting chain atoms. This conformation is quite similar to that observed in the solid state for **5**, as determined by single crystal X-ray analysis. Additionally, it was found that, unlike naltrexone, N-substituents bearing secondary carbons attached directly to the piperidine nitrogen of **4** suffer dramatic losses of potency vs analogues not substituted in this manner. Using a functional assay which measured stimulation or inhibition of [^{35}S]GTP-γ-S binding, we show that the *trans*-cinnamyl analogues of (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)-piperidine (**4**) retain opioid pure antagonist activity and possess picomolar antagonist potency at the μ receptor.

Introduction

The development of potent, highly receptor-selective opioid binding ligands that display pure antagonist activity has been a goal of medicinal chemists since the identification of multiple opioid receptor types.[1,2] These molecular probes have served as useful tools in the study of both the structure and the physiological function of the highly complex opioid receptor system. The elegant work of Portoghese and co-workers over the past decade, which ultimately led to the conversion of the nonselective opioid antagonist naltrexone (**1**) into the receptor subtype selective antagonists norbinaltorphimine (**2**, nor-BNI) and naltrindole (**3**, NTI) for the κ and δ opioid receptors, respectively, represented milestones in opioid research.[3−5] One of our specific research aims has been the discovery of reversibly binding ligands that display pure antagonist activity in the opioid receptor system for use not only as molecular probes but also as potential drug candidates for the treatment of substance abuse. In 1978, Zimmerman and co-workers reported the discovery of a structurally unique series of opioid pure antagonists based on N-substituted analogues of (±)-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (LY272922, **4**).[6] These compounds were novel opioid antagonists because their intrinsic antagonist activity was not mediated by the structure of the N-substituent (i.e., the *N*-methyl and *N*-cyclopropylmethyl analogues in the phenylpiperidine series are both pure antagonists).[7] Instead, the antagonist activity in the phenylpiperidine series appears to arise from the 3,4-dimethyl substituents. Furthermore, potent antagonist activity was only obtained with *trans*-3,4-dimethyl substituted piperidines with the (3*R*,4*R*) enantiomer being the most potent.[8−14] Some of these compounds, especially (+)-*N*-[(3*S*)-3-hydroxy-3-cyclohexylpropyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**5**, LY255582), possessed potent anorectant activity in vivo which Rothman et al. correlated with high affinity of these compounds for the κ_{2b} receptor subtype.[15] It is somewhat surprising that 4-phenylpiperidine derived antagonists have received little atten-

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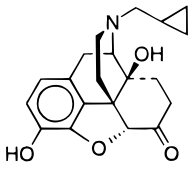

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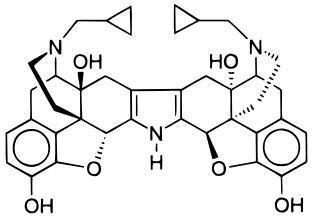

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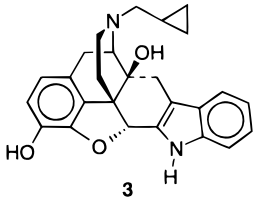

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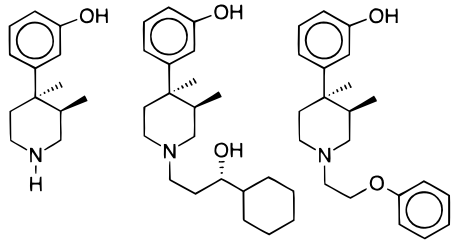
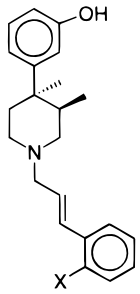


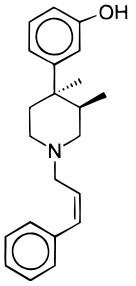
4 5 6

tion in comparison with the oxymorphone series from which naltrexone is derived.

Early investigations in the 4-phenylpiperidine series suggested that their antagonist activity was mediated through a phenylequatorial mode of binding at opioid receptors.[16] Naltrexone on the other hand is structurally constrained to interact via a phenyl axial mode of binding. The different binding behavior between the two classes of antagonists, as related to their N-substituents, suggests that two different regions of receptor space are occupied by the respective N-substituents when bound to the opioid receptors. In the initial work, a number of (+)-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogues possessing a wide variety of N-substituent structures were synthesized and evaluated for opioid receptor binding affinity and antagonist potency.[8] It was discovered that both the binding potency and efficacy of these antagonists were directly related to the structure of the N-substituent, with the most potent analogues having a lipophilic entity (an aromatic or a cyclohexyl ring) separated from the piperidine nitrogen by three atoms, as exemplified by **5** and **6**. However, the flexibility inherent in the N-substituents of these analogues provided no information concerning the effect of conformation on potency or receptor subtype selectivity. Given our desire to build novel opioid antagonists based on the (+)-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine scaffold, we felt that a more thorough understanding of the conformational requirements of the N-substituent and the receptor space to which it binds was needed. This prompted us to synthesize and test a series of analogues of **4** bearing rotationally restricted or otherwise rigidified N-substituents. In this study, we report the synthesis of the N-substituted (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidines **7**−**18**, wherein the rotational degrees



7, X= H
8, X= CH_3
9, X= Cl



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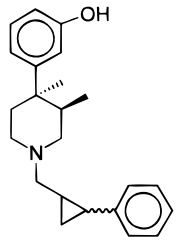
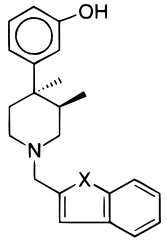
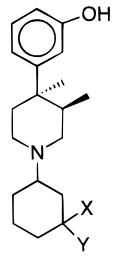


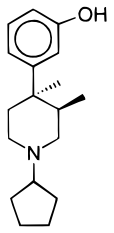
11, cis isomer
12, trans isomer



13, X= S
14, X= O



15, X = OH, Y = C_6H_5
16, X = Y = H
18, X = OH, Y = H



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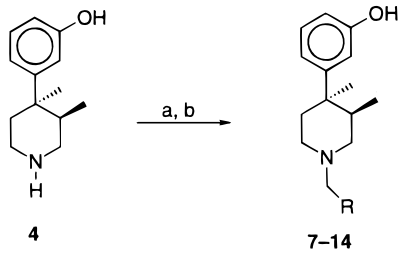
of freedom of the N-substituent are limited by incorporation of carbon−carbon double bonds and cyclic or heterocyclic ring systems, and report their evaluation for inhibition of radioligand binding at the μ, δ, and κ opioid receptors. The most potent analogues discovered were also evaluated in a functional assay to assess their ability to stimulate and/or inhibit agonist-stimulated GTP binding.

Chemistry

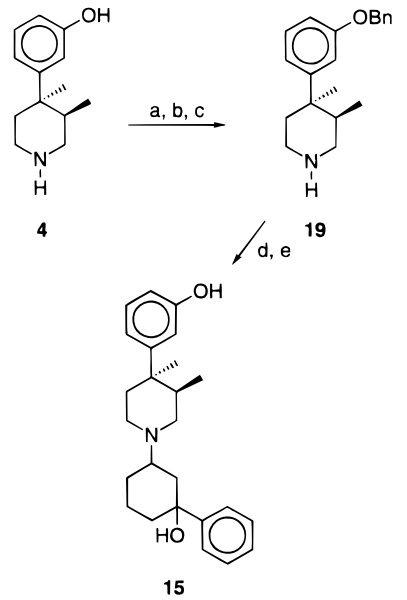
The preparation of compounds **7**−**14** followed the general sequence shown in Scheme 1, wherein the appropriate acid derivative was coupled to (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**4**) using benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (Bop, Castro's reagent) in tetrahydrofuran followed by bis-(2-methoxyethoxy)aluminum hydride (Red-Al) reduction to give the target amine compound.

Scheme 1[a]



[a] Reagents: (a) RCO_2H, BOP, TEA, THF; (b) Red-Al, THF.

Scheme 2[a]



[a] Reagents: (a) $(Boc)_2O$, THF; (b) BnBr, THF, NaH; (c) TFA, CH_2Cl_2; (d) cyclohexenone, H_2O; PhMgBr; (e) Pd/C, H_2, EtOH, HCl.

Compound **15** was prepared as outlined in Scheme 2. Direct addition of **4** to cyclohexenone failed under a variety of conditions. Eventually, we found that the Michael reaction with cyclohexenone would take place when **4** was protected as its benzyl ether and the reaction was conducted in the presence of a small amount of water.[17] Compound **15** was then obtained by treating the Michael adduct of **19** with excess phenylmagnesium bromide followed by deprotection of the phenol. The same reaction sequence using cyclopentenone did not provide useful amounts of material. The synthesis of the cyclic derivatives **16**−**18** followed the steps shown in Scheme 3. Condensation of **4** with tosylated cyclohexene-1,3-dione or tosylated 1,3-cyclopentanedione gave **20a** and **20b**, respectively. Catalytic reduction of **20a** and **20b** using platinum oxide gave the N-cycloalkanes **16** and **17**, respectively. Reduction of **20a** with lithium aluminum hydride gave the N-cycloalkanol **18**.

Results and Discussion

The results from the inhibition of radioligand binding of compounds **7**−**18** along with compound **5** and naltrexone (**1**) at all three opioid receptors are presented in Table 1. Inspection of these data reveals that compounds **7**−**9** containing the *trans*-cinnamyl moiety are the most potent of the series at all of the opioid receptors. The difference in affinities between the trans

Scheme 3

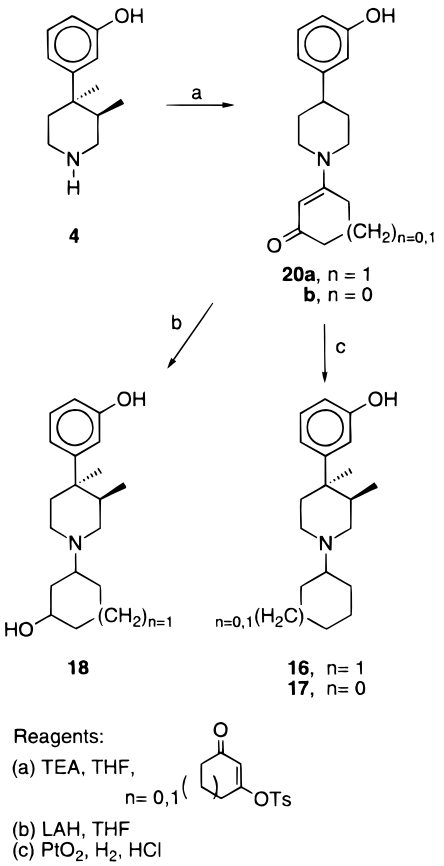


Table 1. Radioligand Binding Results of Rotationally Restricted Derivatives of LY272922 in the μ, δ and κ Opioid Receptor Binding Assays

	K_i (nM ± SD)		
compd	[^{3}H]DAMGO[a]	[^{3}H]DADLE[b]	[^{3}H]U69,593[c]
5	0.32 ± 0.006	198 ± 5.6	28.0 ± 1.2
7	0.74 ± 0.05	322 ± 38.1	122 ± 11.9
8	0.86 ± 0.09	142 ± 22.8	38.9 ± 3.74
9	1.12 ± 0.15	168 ± 36.7	35.8 ± 3.06
10	11.4 ± 1.33	931 ± 235	298 ± 22.5
11	56.1 ± 12.8	827 ± 160	388 ± 30.6
12	1.75 ± 0.14	709 ± 130	81.8 ± 8.6
13	21.2 ± 3.24	1580 ± 304	>690
14	22.2 ± 3.28	1450 ± 251	>690
15	203 ± 25.2	>7300	674 ± 61
16	1280 ± 213	>7300	315 ± 18
17	444 ± 61.9	>7300	199 ± 30
18	1570 ± 296	>7300	1150 ± 189
naltrexone (**1**)	1.39 ± 0.40	94.9 ± 6.6	4.71 ± 0.12

[a] [^{3}H]DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Tritiated ligand selective for μ opioid receptor. [b] [^{3}H]DADLE [(D-Ala^2,D-Leu^5)enkephalin]. Tritiated ligand selective for δ opioid receptor. [c] [^{3}H]U69,593 {[^{3}H](5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide}. Tritiated ligand selective for κ opioid receptor.

compound **7** and the cis compound **10** strongly suggests that it is the spatial orientation or placement of the phenyl ring within the binding site relative to the piperidine ring and not the double bond itself that is responsible for the differences or similarities in potency observed between these compounds and compound **5**. This is further supported by the difference in affinities observed for the *cis*- and *trans*-cyclopropane analogues **11** and **12** which have a similar spatial arrangement of their phenyl rings. Since **7** and **10** as well as **11** and

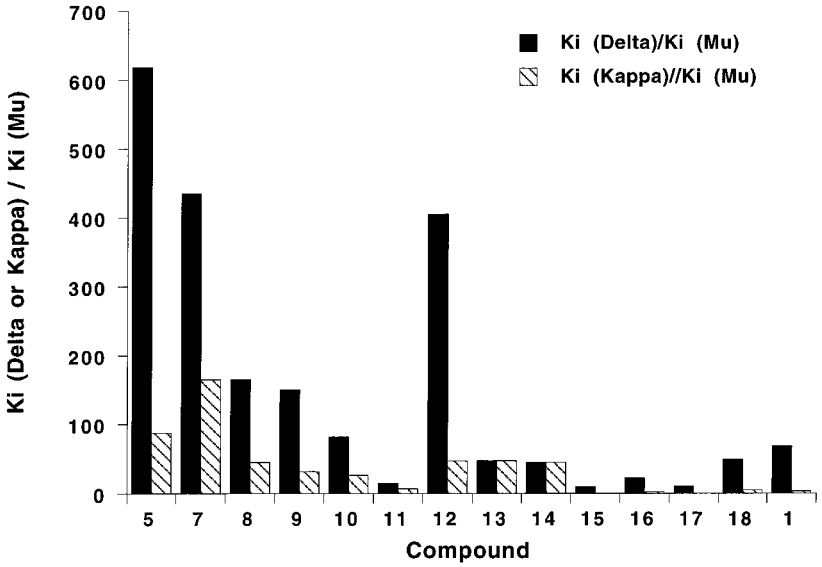


Figure 1. Comparison of the μ opioid receptor selectivity relative to that of the δ and κ receptors using radioligand binding data listed in Table 1.

12 will possess almost identical lipophilicity, these results also suggest that conformational properties are more important than lipophilicity. The nearly 32-fold loss of affinity of the planar N-substituted thianaphthylmethyl and benzofuranylmethyl compounds **13** and **14**, respectively, relative to compounds **7**−**9**, suggests that maximum overlap with the binding site requires the phenyl ring to rotate out of plane relative to the plane of the three connecting chain atoms. The high affinity binding demonstrated by compounds **8** and **9**, which possess ortho methyl and chloro groups, respectively, further strengthens the proposed out-of-plane conformation for **5** or **7**. While solution conformations are not directly comparable with those found in the solid state, it is of interest that the N-substituent conformation suggested above is matched by that found in the single-crystal X-ray structure of **5**.[12]

Compound **15** represents a different type of conformationally constrained analogue of **5** wherein flexibility is limited via a three carbon bridge spanning the α and γ carbons of the N-substituent. The dramatically lower binding affinity displayed by **15** relative to that of the other analogues suggested that some factor other than conformation was at work. The high affinity binding evident in compound **12** with its β,γ bridge illustrates that steric bulk is acceptable in the distal region of the N-substituent, as long as the conformational requirements are also met. This pointed to the proximal end of the connecting chain as the probable cause for the low affinity of **15**. The realization of low affinity binding in all three cycloalkyl compounds **16**−**18** continued the trend found in compound **15** and suggests that substituents bearing a secondary carbon attached directly to the nitrogen atom are not allowed in this class of opioid antagonist. This is in contrast to the high affinity binding displayed by derivatives bearing *n*-propyl, *n*-butyl, and other alkyl substituents larger than methyl.[8] Interestingly, this information also supports the assertion that the N-substituents of these two classes of antagonist interact with different regions of the opioid receptors since similar changes to naltrexone did not convert high affinity ligands into low affinity ligands.[2] The magnitude of the potency loss displayed by this substitution pattern in the phenylpiperidine series coupled with its proximity to the piperidine nitrogen suggests that a disruption may occur in an ionic (salt bridge or cation-π) ligand/receptor interaction which in turn suggests a constriction or crowding in the receptor space near the piperidine nitrogen binding domain.

The conformation of the N-substituent was also found to directly impact the degree of receptor subtype selectivity displayed by a given ligand. As illustrated in Figure 1, compounds **5**, **7**, **8**, and **9** displayed radioligand binding behavior substantially different from that of naltrexone in terms of their selectivity for the μ receptor relative to that for the δ receptor. Whereas **7** was over 400-fold μ-selective, **8** and **9** were substantially less so. Nevertheless, both **8** and **9** were much more μ-selective than **1** (naltrexone). Although **12** was considerably less potent at the μ receptor than **7**, **8**, and **9**, it was 400-fold selective for the μ site relative to that for the δ site. In this series, compound **5** was the most selective agent for the μ receptor relative to the δ receptor and is most closely mimicked by compound **7**, the unsubstituted *trans*-cinnamyl derivative.

These compounds also showed selectivity for the μ receptor relative to that for the κ receptor with compound **7** being 147-fold more selective for μ than for the κ receptor (Figure 1). In comparison, **5** was 87.5-fold selective, and naltrexone was 3.4-fold selective. In all cases, the compounds were more selective for μ relative to δ receptors than for μ relative to κ receptors and were also more selective than naltrexone.

To assess their potency as antagonists and to verify that the *trans*-cinnamyl structure retains pure antagonist activity, compounds **7**−**9** were further analyzed for either stimulation or inhibition of agonist-stimulated GTP binding in comparison with compound **5** and naltrexone (Table 2). In the functional assay, none of the analogues **5**, **7**, **8**, or **9** stimulated GTP binding as measured at concentrations of up to 10 μM. As mentioned previously, retention of pure antagonist activity regardless of the N-substituent structure is a key feature that separates this class of antagonist from oxymorphone-based antagonists which display pure antagonism only for certain N-substitutents such as the

Table 2. Inhibition by Antagonists of [^{35}S]GTP-γ-S Binding in Guinea Pig Caudate Stimulated by DAMGO (μ), SNC-80 (δ), and U69,593 (κ) Selective Opioid Agonists

	K_i (nM ± SD) (N)		
compd	μ (DAMGO[a])	δ (SNC-80[b])	κ (U69,593[c])
5	0.021 ± 0.002 (1.13 ± 0.08)	0.312 ± 0.032 (0.99 ± 0.09)	0.330 ± 0.027 (1.10 ± 0.09)
7	0.039 ± 0.003 (1.06 ± 0.07)	1.48 ± 0.094 (1.19 ± 0.08)	1.04 ± 0.061 (1.07 ± 0.06)
8	0.013 ± 0.001 (1.13 ± 0.08)	0.355 ± 0.020 (1.19 ± 0.07)	0.170 ± 0.013 (0.97 ± 0.07)
9	0.026 ± 0.002 (1.2 ± 0.09)	1.07 ± 0.080 (0.91 ± 0.06)	0.567 ± 0.034 (0.95 ± 0.05)
naltrexone (**1**)	0.930 ± 0.208 (1.03 ± 0.22)	19.3 ± 2.25 (1.13 ± 0.14)	2.06 ± 0.210 (0.76 ± 0.05)

[a] DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Agonist selective for μ opioid receptor. [b] SNC-80 ([(+)-4-[(αR)-α-(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide). Agonist selective for δ opioid receptor. [c] U69,593 [(5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide]. Agonist selective for κ opioid receptor.

N-allyl or *N*-cyclopropylmethyl derivatives. In their ability to reverse agonist-stimulated GTP binding, compounds **7**−**9** displayed a potency significantly higher than that of naltrexone. With a potency 70 times greater than that of naltrexone (13 vs 930 pM), compound **8** is, to our knowledge, the most potent μ antagonist yet reported. Interestingly, this derivative achieved potencies which surpassed that of the more flexible derivative **5** at the μ and κ opioid receptors, indicating that flexibility in the N-substituent is not a necessary attribute for high antagonist potency.

It should be noted that, in general, the test agents were more potent as inhibitors of agonist-stimulated [^{35}S]GTP-γ-S binding than as inhibitors in the binding assays. Figure 2 shows the ratio of the K_i values in the binding assay to the K_i values in the GTP assay. The most pronounced differences were seen with the δ and κ receptors. For example, compound **5** was 634-fold more potent in the [^{35}S]GTP-γ-S assay than in the binding assay. For μ receptors, the ratios varied from 1.5-fold for **1** to 55-fold for **8**. While these differences between the assays at μ and δ receptors are at least partially linked to changes in tissue and standard ligands (alkaloid vs peptide), no such changes are available to explain the shift in K_i for the κ assay. In view of the observations that agonist and antagonist drugs bind to different domains of G-protein-linked receptors,[18,19] these differences may reflect the fact that binding sites were labeled with agonist ligands rather than with antagonist ligands. Importantly, the differences seen between the two assay methods highlight the need to evaluate compounds in more than one manner.

The more potent actions in the [^{35}S]GTP-γ-S assay translated into the compounds having less selectivity for μ relative to both δ and κ in the GTP assay than in the binding assays (Figure 3). The order of potency for **5**, **7**, **8**, **9**, and **1** for μ vs δ selectivity in binding was **5** > **7** > **8** > **9** > **1**, Figure 1. In contrast, the order of potency for the same set of compounds for μ vs δ selectivity in the GTP assay was **9** > **7** > **8** > **1** > **5**. It is also worthy of note that in this assay, the phenylpiperidine antagonists begin to display selectivity behavior similar to that of naltrexone even though the former compounds maintain vastly different potencies (Table 2).

It is clear from the data that N-substituent structures possessing three connecting chain atoms and a pendant cyclohexyl or phenyl ring impart a distinct μ receptor subtype selectivity to **4**. Furthermore, these selectivities are mediated through a *trans*-cinnamyl-like conformation. Note that all derivatives lacking this spatial relationship also lack the high selectivity observed for compounds **7** and **12**. Through this conformation the appended ring reaches a binding site which expresses high potency and selectivity for the μ receptor. This site is apparently a subsite that exists within a larger lipophilic binding domain with which all N-substituents of this class of opioid antagonist interact. This is supported by the generally good affinities of many of the compounds presented here as well as the high affinities displayed by simple *n*-alkyl or phenylethyl derivatives of **4**. Due to the fact that the whole cinnamyl side chain can rotate relative to the piperidine ring, it is not possible to pinpoint the exact location of this binding site relative to the rest of the molecule. However, the extreme potencies of both the rigid and flexible analogues, **7** and **5**, support the hypothesis that very little reorganization is required for these ligands when they are moving between receptor-bound and -unbound states, which in turn implies that a low energy solution conformation is very nearly equal to the conformation required for docking with the receptor.

On the basis of the fact that the cyclohexyl ring in **5** and the phenyl ring in **7** give nearly identical potencies in the μ receptor, it is reasonable to conclude that the μ selective binding subsite is made up of the side chains of lipophilic but not aromatic amino acid residues. This is not an unreasonable assumption based upon the large lipophilic pockets that are proposed to be created by such residues within the G-protein-coupled receptors.[20] Since these ligands do not bind either the δ or the κ receptors well and since the change of even a single residue within a binding site can introduce dramatic changes in receptor subtype selectivity, it is not possible to draw conclusions about this area of receptor space within the κ or δ receptors[21] except to say that they are different from the μ receptor.

In summary, we have shown that incorporation of rigid N-substituent linkers on the (+)-(3R,4R)-dimethyl-4-(3-hydroxy)phenylpiperidine (**4**) can dramatically influence the antagonist potency and selectivity at all of the opioid receptors. In radioligand binding studies, compound **7** displayed a combination of potency and μ receptor subtype selectivity which is superior to any alkaloid antagonist yet reported. Also, compound **8**, though less selective than **7**, displayed the highest functional assay potency at the μ receptor. The superior potencies and μ selectivity demonstrated in both binding and functional assays by analogues possessing the *trans*-cinnamyl moiety support the hypothesis that the active conformation for compounds such as **5** have the connecting chain and appended ring extended away from the piperidine nitrogen in a manner consistent with the *trans*-cinnamyl skeleton. Furthermore, it was deduced that, when interacting with the μ binding site described herein, the appended ring in these analogues is rotated out of plane with respect to an imaginary plane which contains all three connecting chain carbon atoms. We have also shown that ligands possessing a

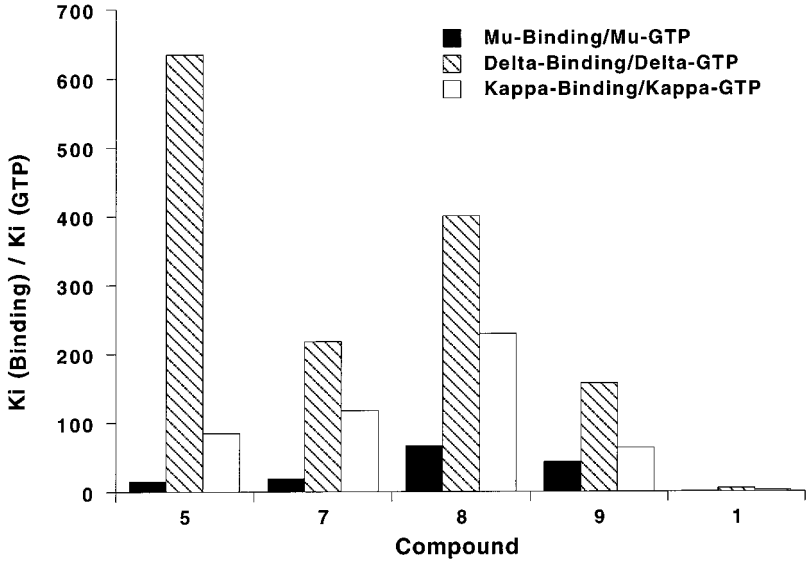


Figure 2. Comparison of the radioligand binding data (Table 1) to the [^{35}S]GTP-γ-S binding data (Table 2).

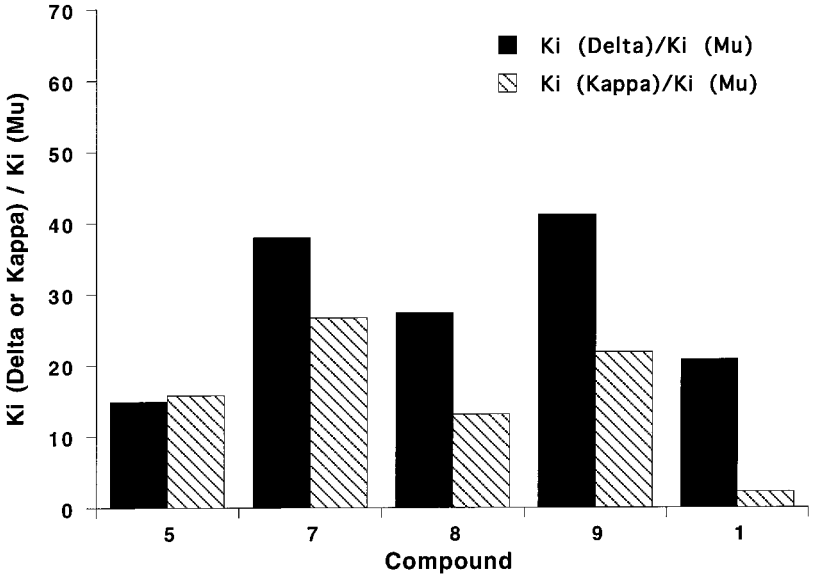


Figure 3. Comparison of the μ opioid receptor selectivity relative to that of the δ and κ receptors using the [^{35}S]GTP-γ-S binding data listed in Table 2.

secondary carbon atom bound directly to the piperidine nitrogen of **4** lose high affinity binding for all of the opioid receptors. This loss of high affinity binding was attributed to either a direct or an indirect disruption of an ionic interaction between the ammonium proton of the ligand and an anionic site within the receptor which indicates a constriction in that region of receptor space. This discovery further strengthens the notion that the N-substituents of the phenylpiperidine- and oxymorphone-based antagonists interact with different regions of the opioid receptors.

The information provided herein should be valuable for future work with this class of antagonists and should aid in developing novel potent, receptor-selective antagonists based upon the 3,4-dimethyl-4-phenylpiperidinyl structural architecture as well as in providing a framework for understanding the spatial relationship of these antagonists as they occupy the opioid antagonist binding site. Furthermore, the identification of a binding site selective for μ antagonists within the larger lipophilic domain described herein suggests that other subsites may be available which will provide selectivity for the δ or κ opioid receptors.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus and are not corrected. Elemental analyses were obtained by Atlantic Microlabs, Inc., and are within $\pm$0.4% of the calculated values. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1-dm cell). ^{1}H NMR were determined on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. Silica gel 60 (230−400 mesh) was used for all column chromatography. All reactions were followed by thin-layer chromatography using Whatman silica gel 60 TLC plates and were visualized by UV or by charring using 5% phosphomolybdic acid in ethanol. All solvents were reagent grade. Tetrahydrofuran and diethyl ether were dried over sodium benzophenone ketyl and distilled prior to use.

The [^{3}H]DAMGO, DAMGO, and [^{3}H][D-Ala^2,D-Leu^5]enkephalin were obtained via the Research Technology Branch, NIDA, and were prepared by Multiple Peptide Systems (San Diego, CA). The [^{3}H]U69,593 and [^{35}S]GTP-γ-S (specific activity = 1250 Ci/mmol) were obtained from DuPont New

England Nuclear (Boston, MA). U69,593 was obtained from Research Biochemicals International (Natick, MA). Levallorphan was a generous gift from Kenner Rice, Ph.D., NIDDK, NIH (Bethesda, MD). GTP-γ-S and GDP were obtained from Sigma Chemical Co. (St. Louis, MO). The sources of other reagents are published.[22]

General Coupling Procedure. (+)-(3*R*,4*R*)-Dimethyl-4-(3-hydroxyphenyl)piperidine (**4**, LY272922) (0.04 mmol) and TEA (0.088 mmol) were combined in THF (1.5 mL), and to this were added the appropriate carboxylic acid (0.04 mmol) and BOP reagent (0.04 mmol) at room temperature. The vial was closed with a Teflon-lined cap. After the mixture was agitated for at least 1 h at room temperature, ethyl ether (3 mL) and water (1.5 mL) were added. The mixture was shaken and the aqueous layer removed via pipet. This procedure was repeated using 1.5 mL of saturated $NaHCO_3$ and 1.5 mL of brine. The dry (Na_2SO_4) organic layer was removed via pipet and filtered through a prepacked cotton filter into a 6 in. pipet draining into a scintillation vial. Chloroform (2 mL) was added to the first vial as a rinse. The collection vial was evaporated in a nitrogen stream and moved to a high vacuum desiccator.

General Reduction Procedure. To the coupling reaction vial was added 3 mL of THF followed by Red-Al 65 wt % in toluene (0.08 mmol) with stirring. After 1 h at room temperature, pH 10 buffer ($NaHCO_3/Na_2CO_3$, 5%) was added to quench excess Red-Al. The pH was adjusted to 1 using 1 N HCl, followed by adjustment to a pH of 10 using saturated sodium bicarbonate. The solution was diluted with 6 mL of ethyl ether, and the organic layer was withdrawn with a pipet and transferred to a second scintillation vial containing sodium sulfate. The aqueous layer was extracted once more with ethyl ether and combined with the first layer. After drying, the mixture was filtered as before and the solvent removed in a nitrogen stream.

(+)-*N*-(*trans*-4′-Phenyl-2′-butenyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Fumarate (7). The free base was prepared from commercially available *trans*-cinnamic acid according to the general procedure in 40% yield. The crude mixture was purified by flash chromatography on silica gel using three fractions of neat hexanes, followed by elution with 20% (5% EtOH in EtOAc) in hexanes. Concentration of the product fraction gave the free amine as a pale yellow resin: ^{1}H NMR ($CDCl_3$) δ 0.8 (d, 3H), 1.3 (s, 3H), 1.65 (m, 1H), 2.0 (m, 1H), 2.45 (m, 2H), 2.65 (m, 2H), 3.0 (bs, 1H), 3.25 (d, 2H), 6.3 (m, 1H), 6.5−6.9 (m, 4H), 7.25 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 16.2, 27.4, 30.9, 38.3, 38.9, 49.9, 56.1, 61.5, 112.7, 113.2, 117.6, 125.7, 126.3, 127.5, 128.5, 129.2, 133.5, 136.7, 151.1, 156.3. The fumarate salt was prepared and recrystallized from ethyl acetate to provide a white crystalline solid: mp 187−189 °C; $[\alpha]^{25}_D$ +39.7 (*c* 0.355, CH_3OH). Anal. ($C_{26}H_{31}NO_5 \cdot 0.25H_2O$) C, H, N.

(+)-*N*-[*trans*-4′-(2-Methylphenyl)-2′-butenyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Fumarate (8). The free base was prepared from commercially available 2-methylcinnamic acid according to the general procedure. The crude mixture was purified by flash chromatography on silica gel using three fractions of neat hexanes, followed by gradient elution with 5−25% (5% EtOH in EtOAc) in hexanes. Concentration of the product fractions gave the free amine as a pale yellow resin in 82% yield: ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3H), 1.3 (s, 3H), 1.63 (d, 1H), 2.0 (m, 1H), 2.48 (m, 9H), 2.92 (bt, 1H), 3.22 (d, 2H), 3.63 (dt, 1H), 6.19 (dt, 1H), 6.62 (d, 1H), 6.78 (m, 3H); 7.22 (m, 5H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 16.4, 19.9, 27.7, 31.3, 38.4, 39.1, 50.0, 56.4, 61.9, 113.1, 113.7, 117.7, 125.9, 126.2, 127.0, 127.5, 128.8, 129.3, 130.3, 131.6, 135.3, 135.9, 151.0, 156.5. The fumarate salt was prepared in CH_3OH/2-propanol (1:3) and triturated with diethyl ether. This provided the salt as a white solid: mp 97−101 °C; $[\alpha]^{25}_D$ +38.03 (c 0.22, CH_3OH). Anal. ($C_{27}H_{33}NO_5 \cdot 0.25H_2O$) C, H, N.

(+)-*N*-[*trans*-4′-(2-Chlorophenyl)-2′-butenyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Fumarate (9). The free base was prepared from commercially available *trans*-2-chlorocinnamic acid according to the general procedure in 71% yield. The crude mixture was purified by flash chromatography on silica gel using three fractions of neat hexanes, followed by gradient elution with 5−25% (5% EtOH in EtOAc) in hexanes. The free amine was obtained as a pale yellow resin: ^{1}H NMR ($CDCl_3$) δ 0.70 (d, 3H), 1.3 (s, 3H), 1.65 (m, 1H), 2.0 (m, 1H), 2.42 (m, 2H), 2.67 (m, 2H), 3.92 (bs, 1H), 3.22 (d, 2H), 6.3 (dt, 1H), 6.62 (d, 1H), 6.78 (m, 2H); 6.94 (d, 1H); 7.25 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 16.4, 27.7, 31.2, 38.4, 39.1, 50.1, 56.5, 61.6, 113.07, 113.6, 117.78, 126.9, 127.1, 128.6, 128.8, 129.3, 129.7, 130.5, 132.9, 134.9, 151.2, 156.4. The fumarate salt was prepared in CH_3OH/2-propanol (1:3) and triturated with diethyl ether. This provided the salt as a white solid: mp 105−108 °C; $[\alpha]^{25}_D$ +34.4 (*c* 0.19, CH_3OH). Anal. ($C_{26}H_{30}ClNO_5 \cdot 0.25H_2O$) C, H, N.

(+)-*N*-(*cis*-4′-Phenyl-2′-butenyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (10). The free base was prepared from *cis*-cinnamic acid, available by the method of Trumbull et al.[23] from commercially available phenylpropiolic acid, according to the general acylation/reduction procedure in 27% yield. The crude mixture was purified by flash chromatography on silica gel using three fractions of neat hexanes, followed by elution with 20% (5% EtOH in EtOAc) in hexanes. The free amine was obtained as a clear resin: ^{1}H NMR ($CDCl_3$) δ 0.74 (d, 3H), 1.28 (s, 3H), 1.62 (m, 1H), 1.91 (m, 1H), 2.40 (t, 2H), 2.63 (AB, 2H), 2.95 (m, 1H), 3.35 (d, 2H), 5.85 (m, 1H), 6.61 (m, 2H), 6.84 (m, 2H), 7.1−7.4 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 16.4, 27.3, 30.8, 38.4, 39.0, 50.1, 56.3, 56.7, 112.6, 113.1, 117.8, 126.9, 128.2, 129.0, 129.26, 129.3, 131.4, 137.2, 151.6, 156.1. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white crystalline solid: mp 97 °C (fus); $[\alpha]^{25}_D$ +47.57 (*c* 0.37, CH_3OH). Anal. ($C_{22}H_{28}ClNO \cdot 0.5H_2O$) C, H, N.

(+)-*N*-(*cis*-3′-Phenylcyclopropylmethyl)−(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (11). The free base was prepared from *cis*-2-phenylcyclopropane carboxylic acid, available by the method of Kaiser et al.[24] from commercially available styrene and ethyl diazoacetate, according to the general acylation/reduction procedure in 44% yield. The crude mixture was purified by flash chromatography on silica gel using three fractions of neat hexanes, followed by gradient elution with 5−25% (5% EtOH in EtOAc) in hexanes. The free amine was obtained as a clear, plastic resin in a diastereomeric ratio of 1:1; ^{1}H NMR ($CDCl_3$) δ 0.65 (t, 3H), 0.75 (q, 1H), 0.98 (m, 1H), 1.14 (d, 3H), 1.25 (m, 1H), 1.45 (m,1H), 1.65 (m,1H), 1.85 bs, 1H), 2.08 (q, 1H), 2.24 (q, 2H), 2.41 (m, 2H), 2.68 (m, 2H), 2.96 (m, 1/2H), 6.5 (d, 1H), 6.64 (m, 2H), 7.08 (m, 6H), 7.95 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 10.3, 15.5, 15.80, 16.9, 20.0, 27.5, 31.15, 38.4, 39.0, 49.7, 50.1, 56.1, 56.7, 58.7, 58.9, 112.9, 113.5, 117.5, 125.9, 128.0, 129.0, 129.1, 129.2, 138.6, 151.0, 156.5. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white powder: mp 122 °C (fus); $[\alpha]^{25}_D$ +60.6 (*c* 0.485, CH_3OH). Anal. ($C_{23}H_{30}ClNO \cdot 0.25H_2O$) C, H, N.

(+)-*N*-(*trans*-3′-Phenylcyclopropylmethyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (12). The free base was prepared from commercially available *trans*-2-phenylcyclopropane carboxylic acid according to the general acylation/reduction procedure in 49% yield. The crude mixture was purified by flash chromatography on silica gel using three fractions of neat hexanes, followed by gradient elution with 2.5−10% (5% EtOH in EtOAc) in hexanes. The free amine was obtained as a clear, plastic resin in a diastereomeric ratio of 1:1; ^{1}H NMR ($CDCl_3$) δ 0.71 (q, 3H), 0.85 (m, 1H), 0.95 (m, 1H), 1.29 (d, 4H), 1.56 (m, 1H), 1.70 (m,1H), 1.95 (m,1H), 2.45 (band, 3.5H), 2.66 (band, 2.5H), 3.05 (t, 1H), 6.58 (d, 1H), 6.68 (s, 1H), 6.76 (d, 1H), 7.15 (band, 6H); ^{13}C NMR ($CDCl_3$) δ 15.1, 16.3, 19.9, 22.7, 23.0, 27.3, 30.88, 38.3, 38.9, 49.6, 49.8, 56.2, 56.4, 63.4, 112.7, 113.2, 117.6, 125.5, 125.8, 128.3, 129.2, 142.7, 151.2, 156.3. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white powder: mp 146

°C (dec); $[\alpha]^{25}_D$ +49.7 (*c* 0.33, CH_3OH). Anal. ($C_{23}H_{30}ClNO\cdot 1.0H_2O$) C, H, N.

(+)-*N*-[(2′-Thianaphthenyl)methyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (13). The free base was prepared from commercially available 2-thianaphthene carboxylic acid according to the general acylation/reduction procedure in 65% yield. The crude mixture was purified by flash chromatography on silica gel using gradient elution with 0−10% (5% EtOH in EtOAc) in hexanes. The free amine was obtained as a plastic resin. ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3H), 1.39 (s, 3H), 1.59 (d, 1H), 1.95 (m,1H), 2.45 (td, 1H), 2.50 (td, 1H), 2.65 (d 2H), 2.95 (dt, 1H), 3.84 (AB, 2H), 6.62 (dd, 1H), 6.78 (s, 1H), 6.85 (d, 1H), 7.25 (m, 4H), 7.70 (dd, 1H), 7.80 (dd, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 15.9, 27.4, 30.6, 38.5, 38.9, 50.1, 55.4, 58.1, 112.3, 112.9, 118.1, 121.7, 122.3, 123.0, 123.7, 124.0, 127.2, 139.73, 140.1, 144.4, 152.3, 155.5. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white powder: mp 151−154 °C; $[\alpha]^{25}_D$ +41.4 (*c* 0.355, CH_3OH). Anal. ($C_{22}H_{26}ClNOS\cdot 0.5H_2O$) C, H, N, S.

(+)-*N*-[(2′-Benzofuranyl)methyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (14). The free base was prepared from commercially available 2-benzofuran carboxylic acid according to the general acylation/reduction procedure in 78% yield. The crude mixture was purified by flash chromatography on silica gel using gradient elution with 0−10% (5% EtOH in EtOAc) in hexanes in three fraction increments of 2.5%. The free amine was obtained as a clear, plastic resin: ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3H), 1.20 (s, 3H), 1.58 (d, 1H), 1.94 (d, 1H), 2.38 (td, 1H), 2.66 (m, 3H), 2.92 (m, 1H), 3.72 (AB, 2H), 6.55 (s, 1H), 6.60 (dd, 1H), 6.71 (s, 1H), 6.78 (d, 1H), 7.15 (m, 3H), 7.38 (d, 1H), 7.50 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 11.2, 16.2, 27.6, 31.0, 38.3, 38.9, 49.9, 55.5, 55.7, 105.8, 112.8, 113.4, 117.8, 120.8, 122.7, 123.9, 128.4, 129.2, 151.3, 154.5, 155.0, 156.07. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white powder: mp 130−133 °C; $[\alpha]_D$ +43.3 (*c* 0.27, CH_3OH). Anal. ($C_{22}H_{26}ClNO_2\cdot 0.5H_2O$) C, H, N.

(+)-*N*-(3′-Hydroxy-3′-phenylcyclohexyl)-(3*R*,4*R*)-dimethyl-(3-hydroxyphenyl)piperidine Bitartrate (15). To 3.44 g (16.75 mmol) of (3*R*,4*R*)-dimethyl-(3-hydroxyphenyl)-piperidine in 50 mL of dry THF was added portionwise 4.0 g (18.32 mmol) of ditertbutoxypyrocarbonate. Portionwise addition was necessary to keep foaming to a minimum. After the addition of the carbonate was complete, 2.61 mL of triethylamine (18.73 mmol) was added, and the whole mixture was aged for 2 h. The reaction mixture was poured into 150 mL of ethyl ether and washed consecutively with 50 mL portions of 5% HCl, water, and brine. Drying over magnesium sulfate followed by removal of the solvent under vacuum gave 5.58 g of unpurified product which was pure enough to carry forward without further purification: ^{1}H NMR ($CDCl_3$) δ 0.62 (d, 3H), 1.31 (s, 3H), 1.41 (s, 9H), 1.95 (bs, 1H), 2.17 (td, 1H), 3.05 (bt, 1H), 3.30 (bt, 1H), 3.85 (bt, 1H), 4.20 (band, 1H), 6.75 (m, 3H), 7.15 (t, 1H). The crude material 1 g (3.26 mmol) obtained above was dissolved in 5 mL of dry DMF to which was added 1 g of finely divided potassium carbonate and 0.387 mL (3.26 mmol) of benzyl bromide. This mixture was allowed to stir overnight at room temperature. The following day, the reaction mixture was diluted with 95 mL of ethyl ether and washed four times with water, and the organic layer was dried over magnesium sulfate. The solvent was removed in vacuo and the residue subjected to chromatography on silica gel 0−5% (5% EtOH in EtOAc) in hexanes using a fraction ratio of 3:3:6. This provided 1.07 g (83%) of the desired product. ^{1}H NMR ($CDCl_3$) δ 0.63 (d, 3H), 1.32 (s, 3H), 1.45 (s, 9H), 1.52 (m, 1H), 1.95 (bm, 1H), 2.15 (td, 1H), 3.01 (bm, 1H), 3.28 (bm, 1H), 3.68 (bm, 1H), 4.15 (band, 1H), 5.05 (s, 2H), 6.85 (m, 3H), 7.31 (band, 6H); ^{13}C NMR ($CDCl_3$) δ 14.4, 26.0, 28.5, 29.4, 38.6, 38.9, 39.4, 40, 45.0, 46.1, 69.9, 79.1, 111.2, 113.0, 118.1, 127.6, 127.9, 128.6, 129.2, 137.1, 151.4, 155.7, 158.9.

To 8 mL of TFA in 26 mL of dry CH_2Cl_2 was added 2.61 g (6.58 mmol) of 1-*tert*-butoxycarbonyl-(3*R*,4*R*)-dimethyl-4-(3-benzyloxyphenyl)piperidine and the mixture aged at −5 °C for 30 min. After this time, the mixture was carefully quenched with saturated Na_2CO_3 and the pH checked to ensure that it was basic. The aqueous layer was extracted once with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4. Removal of the solvent under vacuum provided a crude viscous residue which was purified by chromatography over silica gel using 2% CH_3OH in $CHCl_3$ to remove the unreacted starting material and $CH_3OH/NH_4OH/CHCl_3$ (9:1:40) to remove the product from the column. This provided 1.75 g (90%) of intermediate **19** of sufficient purity to be used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3H), 1.38 (s, 3H), 1.65 (d, 1H), 2.05 (m, 1H), 2.28 (td, 1H), 2.89 (dd, 1H), 3.22 (band, 3H), 5.05 (s, 2H), 6.85 (m, 3H), 7.35 (band, 6H).

A one-neck, round-bottom flask was charged with 1.93 g (6.53 mmol) of (3*R*,4*R*)-dimethyl-(3-benzyloxyphenyl)piperidine (**19**), 0.63 mL (6.53 mmol) of cyclohexenone, and five drops of water. The mixture was allowed to stir at room temperature for 1 h at which point 10 mL of EtOH was added in such a manner so as to wash any material on the sides of the flask into the bottom of the flask. A nitrogen line was inserted into the open neck of the flask, and a continuous stream of nitrogen was introduced. The nitrogen flow was continued until all of the solvent was removed and a viscous oil remained. This oil may solidify on standing.

In a separate three-neck, round-bottom flask, 6.2 g (25.4 mmol) of $CeCl_3$ (previously dried) is slurried in 75 mL of dry THF. Sonication is needed in order to completely slurry the solid. Once slurried, the mixture is heated to reflux for ∼5 min and cooled to −78 °C, whereupon 7.8 mL (23.5 mmol) of 3 M phenylmagnesium bromide was added via syringe. After the addition, the mixture was allowed to stir at −78 °C for 45 min. Next, the previously prepared Michael adduct was added neat as quickly as possible. Any material that remained in the flask was washed forward with THF, but this process is inefficient since the THF slurry becomes gelatinous. The combined reaction mixture was allowed to stir for 2 h at −78 °C and warmed to 5 °C. Once the temperature reached 5 °C, the reaction was recooled to −78 °C, and saturated NH_4Cl was added to quench the reaction. After this, the cooling bath was removed, and the mixture was allowed to warm to room temperature and stir there for 15 min. After this time, the crude mixture was filtered through Celite and the filter cake washed thoroughly with THF. When separated, the aqueous layer was extracted two times with methylene chloride, and the combined organic layers were dried over sodium sulfate. Removal of the solvent under vacuum provided a crude residue which was purified on silica gel using CH_3OH in chloroform as an eluent. Neat chloroform was first used, followed by 1% CH_3OH to remove brown, fast-moving impurities. The product was eluted from the column by using 10% CH_3OH in chloroform. It was noticed that the product moved with the solvent front when using this eluent. This gave 1 g (33%) of the desired material as a 1:1 mixture of diastereomers as indicated by ^{1}H NMR: ^{1}H NMR ($CDCl_3$) δ 0.75 (m, 3H), 1.29 (m, 3H), 1.71 (band, 4H), 2.02 (m, 2H), 2.30 (m, 1H), 2.61 (d, 1H), 2.82 (m, 2H), 3.10 (t, 1H), 5.05 (s, 2H), 6.71 (d, 1H), 6.90 (m, 2H), 7.25 (band, 11H). The material isolated above was dissolved in EtOH which had been treated with acetyl chloride to which was added a catalytic amount of 10% Pd/C. The bottle was attached to a Parr apparatus and hydrogenated at 20 psi for 7 h. After this time, the mixture was filtered though Celite and the cake washed well with CH_3OH. The solvent was removed in vacuo, and the residue treated with saturated sodium bicarbonate solution and chloroform, and the organic layer dried over sodium sulfate. Removal of the solvent followed by silica gel chromatography to remove starting material provided 610 mg (76%) of the desired material as a 1:1 mixture of diastereomers. The *d*-tartrate salt was prepared and crystallized from ethyl ether/isopropyl alcohol. ^{1}H NMR analysis indicated a ∼5:1 mixture of diastereomers which was shown to be predominately the slower moving isomer in TLC analysis on silica gel: mp 160−165 °C; $[\alpha]^{25}_D$ +28.0 (*c* 0.15, CH_3OH); ^{1}H NMR (250 MHz, CD_3OD) δ 0.90

(bs, 3H), 1.45 (m, 4H), 1.6−2.5 (band, 12H), 3.4 (m, 4H), 3.6 (bs, 1H), 4.4 (s, 1.3H), 6.65 (d, 1H), 6.79 (d, 2H), 7.1−7.4 (band, 4H), 7.55 (d, 2H); ^{13}C NMR (lower R_f isomer, 125.7 MHz, CD_3OD) δ 16.4, 22.4, 28.4, 29.1, 32.7, 39.3, 39.6, 40.1, 40.6, 47.4, 52.2, 61.5, 75.3, 79.5, 113.1, 114.1, 118.3, 125.6, 127.6, 129.1, 130.1, 151.0, 152.0, 158.3. Anal. ($C_{27}H_{35}NO_5 \cdot H_2O$) C, H, N.

(+)-*N*-Cyclohexyl-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (16). To a dry 50-mL round-bottom flask containing 20 mL of dry THF under a nitrogen atmosphere were charged 1 equiv (8.9 mmol) of cyclohexane 1,3-dione, 1 equiv (8.9 mmol) of tosyl chloride, and 1.2 equiv (10.68 mmol) of triethylamine at room temperature. The mixture was aged for 6 h or until TLC indicated the consumption of the starting material. The crude reaction mixture was diluted with ethyl ether (3 × volume), washed with 20 mL of water and 20 mL of NaCl (saturated), and dried over sodium sulfate/sodium chloride. Removal of the solvent at reduced pressure provided the unstable, crude tosylate in 90% yield: ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 2H), 2.40 (m, 2H), 2.5 (m, 5H), 5.81 (s, 1H), 7.38 (d, 2H), 7.85 (d, 2H). To a mixture of 1.43 g (7 mmol) of (3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**4**) and 2 mL (14.2 mmol) of triethylamine in 50 mL of dry THF at room temperature was added 1.78 g (7 mmol) of the cyclohexenyl tosylate prepared in the previous step. The mixture was heated under reflux for 8 h. After the starting material was consumed as indicated by TLC on silica gel using 1:1 ethyl acetate in hexane (1% EtOH, 2% triethylamine), the reaction mixture was poured into 600 mL of ethyl ether and 150 mL of 10% HCl. After vigorous agitation, the layers were separated, and the acid layer was made basic (pH 10) in a 1 L beaker. The aqueous layer was extracted three times with 150 mL of chloroform and dried over sodium sulfate. Removal of the solvent under reduced pressure gave 3.94 g (90% yield) of **20a** as a foamy plastic resin: ^{1}H NMR ($CDCl_3$) δ 0.61 (d, 3H), 1.35 (s, 3H), 1.62 (d, 1H), 1.91−2.55 (band, 8H), 3.18 (t, 1H), 3.40 (d, 1H), 3.61 (d, 1H), 3.86 (d, 1H), 5.42 (s, 1H), 6.67 (d, 1H), 6.81 (m, 2H), 7.12 (m, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.6, 22.0, 26.0, 27.0, 30.1, 34.9, 38.7, 39.2, 42.9, 48.7, 98.1, 113.0, 113.8, 116.1, 129.2, 149.7, 157.5, 167.0, 197.7.

A solution of dry HCl in CH_3OH was prepared by adding 0.15 mL of AcCl to 20 mL of CH_3OH carefully and allowing this mixture to stir for 5 min. After this, 0.5 g (1.66 mmol) of 1-(3′-oxo-1′-cyclohexenyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxylphenyl)piperidine (**20a**) and a catalytic amount of platinum oxide were added, and the flask was flushed with hydrogen at atmospheric pressure. The mixture was allowed to stir under a hydrogen atmosphere for 48 h. After this time, the mixture was filtered through Celite and the cake washed well with CH_3OH. The solvent was removed under vacuum and the residue shaken with chloroform and saturated sodium bicarbonate solution. The organic layer was washed once with water and dried over sodium sulfate. Removal of the solvent under vacuum provided 300 mg (63%) of **16** as a clear plastic resin: ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3H), 1.20 (m, 8H), 1.65 (m, 2H), 1.76 (m, 2H), 1.91 (m, 3H), 6.63 (dd, 1H), 6.75 (s, 1H), 6.81 (d, 1H), 7.12 (t, 1H); ^{13}C NMR ($CDCl_3$) δ 16.2, 26.0, 26.3, 27.9, 28.1, 28.7, 32.4, 38.8, 39.2, 45.9, 51.4, 64.0, 113.0, 114.1, 117.7, 128.9, 150.8, 155.4. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white powder: mp 230−232 °C (dec); $[\alpha]^{25}_D$ +64.0 (*c* 0.1, CH_3OH). Anal. ($C_{19}H_{30}ClNO$) C, H, N. Low resolution MS (EI): M^+ *m*/*e* 287, calcd 287.

(+)-*N*-Cyclopentyl-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (17). To a dry 50-mL round-bottom flask containing 20 mL of dry THF under a nitrogen atmosphere were charged 1 equiv (8.9 mmol) of 1,3-cyclopentanedione, 1 equiv (8.9 mmol) of tosyl chloride, and 1.2 equiv (10.68 mmol) of triethylamine at room temperature. The mixture was aged for 6 h or until TLC indicated consumption of starting material. The crude reaction mixture was diluted with ethyl ether (3 × volume), washed with 20 mL of water and 20 mL of NaCl (saturated), and dried over sodium sulfate/sodium chloride. Removal of the solvent at reduced pressure provided the unstable, crude tosylate which was used without further purification. The product, obtained as a white granular solid, is more unstable than the cyclohexane derivative but works well in the reaction if used within a day of preparation. The crude material obtained in 93% yield is used without further purification: ^{1}H NMR ($CDCl_3$) δ 2.41 (m, 5H), 2.68 (m, 2H), 5.92 (s, 1H), 7.43 (d, 2H), 7.96 (d, 2H).

The procedure used to prepare 1-(3′-oxo-1′-cyclopentenyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine is identical to that described for the six-membered ring compound. The crude material **20b** was used without further purification: ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 3H), 1.38 (s, 3H), 1.68 (m, 1H), 2.15 (m, 2H), 2.48 (s, 2H), 2.68 (m, 2H), 3.56 (band, 4H), 5.13 (s, 1H), 5.21 (s, 1H), 6.68 (d, 2H), 6.80 (m, 2H), 7.13 (t, 1H); ^{13}C NMR ($CDCl_3$) δ 14.5, 26.6, 27.6, 29.0, 30.3, 33.7, 38.5, 38.8, 43.7, 45.0, 49.8, 50.8, 99.4, 112.7, 113.2, 116.0, 129.8, 149.7, 157.6, 178.1, 178.4, 204.4.

The procedure used to prepare (+)-1-cyclopentyl-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**17**) hydrochloride is identical to that used to prepare the six-membered ring compound. The free base was obtained as a plastic resin which was purified by silica gel chromatography using 20% CH_3OH in chloroform. The yield for the reduction step was 83%: ^{1}H NMR ($CDCl_3$) δ 0.72 (d, 3H), 1.38 (s, 3H), 1.52 (s, 3H), 1.68 (m, 3H), 1.92 (m, 3H), 2.52 (band, 5H), 3.02 (bs, 1H), 6.62 (d, 1H), 6.80 (m, 2H), 7.12 (t, 1H); ^{13}C NMR ($CDCl_3$) δ 16.4, 24.2, 27.5, 29.8, 31.4, 38.2, 39.1, 49.1, 55.5, 68.5, 112.9, 113.4, 117.6, 129.1, 151.0, 156.4. The hydrochloride salt was prepared and crystallized from ethyl ether/CH_3OH using 1 N HCl in ethyl ether. This provided a white crystalline powder: mp 255 °C (dec); $[\alpha]^{25}_D$ +59.6 (*c* 0.28, CH_3OH). Anal. ($C_{18}H_{28}ClNO \cdot 0.5H_2O$) C, H, N.

(+)-*N*-(3′-Hydroxycyclohexyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine Hydrochloride (18). A 500-mg portion of crude 1-(3′-oxo-1′-cyclohexenyl)-(3*R*,4*R*)-dimethyl-(3-hydroxyphenyl)piperidine (**20a**, 1.65 mmol) was heated under reflux with 1 g of lithium aluminum hydride (LAH, excess) for 1 week in 50 mL of dry THF. After this time, 15% NaOH was added to the mixture to quench any unreacted reducing agent. The whole mixture was filtered through Celite and the cake washed thoroughly with THF. This was diluted with ether, washed with water and brine, and dried over magnesium sulfate. Removal of the solvent under vacuum provided a residue that required purification over silica gel using 0−20% CH_3OH in chloroform. From this residue, the HCl salt was prepared using isopropyl alcohol/ethyl ether and dry HCl in ethyl ether. The salt was recrystallized from the same mixture and gave 54 mg (11%) of **18** as a 4:1 mixture of diastereomers: mp 214−217 °C; $[\alpha]^{25}_D$ +48.0 (*c* 0.1, CH_3OH); ^{1}H NMR (CD_3OD) δ 0.82 (d, 3H), 1.33 (m, 7H), 1.92 (m, 4H), 2.25 (m, 3H), 2.75 (bt, 1H), 3.05 (m, 4H), 3.60 (m, 1H), 6.60 (d, 1H), 6.75 (m, 2H), 7.12 (t, 1H); ^{13}C NMR ($CDCl_3$) δ 16.0, 22.4, 22.6, 27.1, 27.7, 28.2, 32.1, 35.6, 35.7, 36.9, 37.8, 39.4, 39.7, 47.7, 48.0, 52.4, 52.6, 61.8, 64.0, 64.2, 67.2, 70.3, 113.7, 113.9, 114.1, 118.2, 130.3, 130.4, 151.1, 158.5. Anal. ($C_{19}H_{30}ClNO_2 \cdot H_2O$) C, H, N.

Opioid Binding Assays. μ Binding sites were labeled using [^{3}H][D-Ala^2-MePhe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO) (2.0 nM, specific activity = 45.5 Ci/mmol), and δ binding sites were labeled using [^{3}H][D-Ala^2,D-Leu^5]enkephalin (2.0 nM, specific activity = 47.5 Ci/mmol) using rat brain membranes prepared as described.[25] κ-1 binding sites were labeled using [^{3}H]U69,593 (2.0 nM, specific activity = 45.5 Ci/mmol) and guinea pig membranes pretreated with BIT and FIT to deplete the μ and δ binding sites.[22]

[^{3}H]DAMGO binding proceeded as follows: 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in binding buffer (BB: 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL BSA), followed by 50 μL of BB, and 100 μL of [^{3}H]DAMGO in a protease inhibitor cocktail (10 mM Tris-HCl, pH 7.4, which contained bacitracin (1 mg/mL), bestatin (100 μg/mL), leupeptin (40 μg/mL), and chymostatin (20 μg/mL). Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.2 mg/mL protein and proceeded for 4 to 6 h at 25 °C. The

ligand was displaced by 10 concentrations of test drug, in triplicate, two times. Nonspecific binding was determined using 20 μM levallorphan. Under these conditions, the K_d of [^{3}H]DAMGO binding was 4.35 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash-buffer (ice-cold 10 mM Tris-HCl, pH 7.4).

[^{3}H][D-Ala^2,D-Leu^5]Enkephalin binding proceeded as follows: 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in BB, followed by 100 μL of a salt solution containing choline chloride (1 M, final concentration of 100 mM), $MnCl_2$ (30 mM, final concentration of 3.0 mM), and, to block μ sites, DAMGO (1000 nM, final concentration of 100 nM), followed by 50 μL of [^{3}H][D-Ala^2,D-Leu^5]enkephalin in the protease inhibitor cocktail. Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.41 mg/mL of protein and proceeded for 4 to 6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, two times. Nonspecific binding was determined using 20 μM levallorphan. Under these conditions, the K_d of [^{3}H][D-Ala^2,D-Leu^5]enkephalin binding was 2.95 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4).

[^{3}H]U69,593 binding proceeded as follows: 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in BB, followed by 50 μL of BB, followed by 100 μL of [^{3}H]U69,593 in the standard protease inhibitor cocktail with the addition of captopril (1 mg/mL in 0.1 N acetic acid containing 10 mM 2-mercapto-ethanol to give a final concentration of 1 μg/mL). Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.4 mg/mL of protein and proceeded for 4 to 6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, two times. Nonspecific binding was determined using 1 μM U69,593. Under these conditions the K_d of [^{3}H]U69,593 binding was 3.75 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4) containing 1% PEI.

For all three assays, the filtration step proceeded as follows: 4 mL of the wash buffer was added to the tubes, was rapidly filtered, and was followed by two additional wash cycles. The tritium retained on the filters was counted, after an overnight extraction into ICN Cytoscint cocktail, in a Taurus beta counter at 44% efficiency.

[^{35}S]GTP-γ-S Binding Assay. Ten frozen guinea pig brains (Harlan Bioproducts for Science, Inc., Indianapolis, IN) were thawed, and the caudate putamen were dissected and homogenized in buffer A (3 mL/caudate) (buffer A = 10 mM Tris-HCl, pH 7.4 at 4 °C containing 4 μg/mL leupeptin, 2 μg/mL chymostatin, 10 μg/mL bestatin, and 100 μg/mL bacitracin) using a polytron (Brinkman) at setting 6 until a uniform suspension was achieved. The homogenate was centrifuged at 30000*g* for 10 min at 4 °C and the supernatant discarded. The membrane pellets were washed by resuspension and centrifuged twice more with fresh buffer A, aliquotted into microfuge tubes, and centrifuged in a Tomy refrigerated microfuge (model MTX 150) at maximum speed for 10 min. The supernatants were discarded, and the pellets were stored at −80 °C until assayed.

For the [^{35}S]GTP-γ-S binding assay, all drug dilutions were made up in buffer B [50 mM TRIS−HCl, pH 7.7/0.1% BSA]. Briefly, 12 × 75 mm polystyrene test tubes received the following additions: (a) 50 μL of buffer B with or without an agonist, (b) 50 μL of buffer B with or without 60 μM GTP-γ-S for nonspecific binding, (c) 50 μL of buffer B with or without an antagonist, (d) 50 μL of salt solution which contained in buffer B 0.3 nM [^{35}S]GTP-γ-S, 600 mM NaCl, 600 μM GDP, 6 mM dithiothreitol, 30 mM $MgCl_2$, and 6 mM EDTA, and (e) 100 μL of membranes in buffer B to give a final concentration of 10 μg per tube. The final concentration of the reagents were 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 100 μM GDP, 0.1% BSA, 0.05−0.1 nM [^{35}S]GTP-γ-S, 500 nM or 10 μM agonists, and varying concentrations (at least 10 different concentrations) of antagonists. The reaction was initiated by the addition of membranes and terminated after 4 h by addition of 3 mL of ice-cold (4 °C) purified water (Milli-Q UV-Plus, Millipore) followed by rapid vacuum filtration through Whatman GF/B filters presoaked in purified water. The filters were then washed once with 5 mL of ice-cold water. Bound radioactivity was counted by liquid scintillation spectroscopy using a Taurus (Micromedic) liquid scintillation counter at 98% efficiency after an overnight extraction in 5 mL of Cytoscint scintillation fluid. Nonspecific binding was determined in the presence of 10 μM GTP-γ-S. Assays were performed in triplicate, and each experiment was performed at least three times.

Data Analysis. The data of the two separate experiments (opioid binding assays) or three experiments ([^{35}S]GTP-γ-S assay) were pooled and fit, using the nonlinear least-squares curve-fitting language MLAB-PC (Civilized Software, Bethesda, MD), to the two-parameter logistic equation[26] for the best-fit estimates of the IC_{50} and slope factor. The K_i values were then determined using the equation: $IC_{50}/[1 + ([L]/K_d)]$.

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