Probes for Narcotic Receptor-Mediated Phenomena. 21. Novel Derivatives of 3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-6*H*-azocino[4,5-*b*]indol-6-yl)-phenols with Improved δ Opioid Receptor Selectivity

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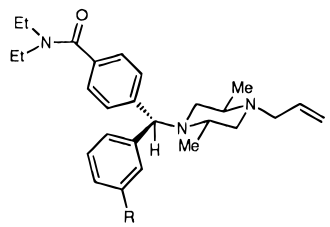
Derivatives of racemic and optically pure levorotatory 3-(1,2,3,4,5,11-hexahydro-3-methyl-2,6-methano-6*H*-azocino[4,5-*b*]indol-6-yl)phenols containing methoxy substituents in the C10′, C9′, and C8′ positions (compounds **9**−**11**, respectively) were synthesized and characterized by spectroscopic and X-ray methods. The binding affinities for the μ, δ, and κ_1 opioid receptors and activity in the guinea pig ileum (GPI) and mouse vas deferens (MVD) functional bioassays were determined for these compounds. A methoxy substituent in the C8′ position decreases the binding affinity for both the μ and δ receptors, while a C10′ methoxy substituent has little effect on either binding affinity. Interestingly, a methoxy group at the C9′ position in the levorotatory series provides compound (−)-**10** which exhibits both enhanced *in vitro* affinity and selectivity for the δ opioid receptor relative to the unsubstituted derivative (−)-**8** and is the most selective (μ/δ IC_{50} ratio 17.9, κ_1/δ IC_{50} ratio 314) and highest affinity (IC_{50} 3.7 nM) δ receptor ligand for this novel class of compounds. The results of the GPI and MVD bioassays are more dramatic and indicate that (−)-**10** is an agonist for the δ receptor (IC_{50} 49.0 nM) with substantial selectivity for the δ versus the μ receptor borne out by a GPI/MVD IC_{50} ratio of >612.

Introduction

The development of highly selective δ opioid agonists is a key component of our research. One aim of our program focuses on opioid analgesics which do not act through μ receptors, thereby circumventing the side effects associated with the μ receptor active compounds, namely, respiratory depression, physical dependence, and gastrointestinal effects. Various studies suggest that agonists that selectively act through the δ receptor might be ideal replacements for traditional μ agonists.[1] Whereas much of the initial research defining the potential clinical application of selective δ receptor ligands involved peptidic ligands,[2] the inherent inability for systemically administered peptides to cross the blood−brain barrier has hindered further research. Currently there are very few selective nonpeptidic δ agonists available. SNC80 (**1**),[3] a compound related to BW373U86 (**2**), and heterocyle-condensed derivatives of octahydroisoquninolines[4] were recently reported δ agonists. The limited number of nonpeptidic δ agonists available, our interest in their potential medical utility, and their potential for use as tools for characterization of the δ receptor have been the impetus for the work presented below.

Design Rationale

We recently reported our results on the development of nonpeptidic δ opioid ligands prepared via the addition of various aromatic moieties to the μ opioid 5-(3-hydroxyphenyl)morphans.[5] The most significant finding was that an indole moiety fused at the C6−C7 position of the parent (−)-phenylmorphan nucleus produced a compound with >180-fold enhancement of affinity for δ receptors; however, the affinity for μ receptors of these indole derivatives was unchanged relative to the parent (−)-phenylmorphan compound. Functional bioassays (mouse vas deferens and guinea pig ileum) revealed that the novel (−)-indole derivatives were agonists at the δ receptor. Furthermore, the δ binding affinity exhibited by the racemic indole derivatives was found to be due exclusively to the levorotary indole derivatives. Our initial assumption was that these new δ agonists, based on the (−)-phenylmorphans, and the highly potent and selective nonpeptidic δ agonist SNC80 (**1**) were all binding to the same domain on the δ receptor. However, we were aware that various binding studies with



R = OMe, SNC80 (**1**)
R = OH, (+)-BW373U86 [(+)-**2**]

BW373U86 (**2**) and the SNC80 (**1**)-related derivative [^{3}H]SNC-121, showing the effects of added GppNHp and NaCl, collectively indicated that these ligands might be binding to δ receptors in an atypical manner when compared to other peptidic and nonpeptidic δ receptor ligands.[6] Thus, we began to examine the similarities

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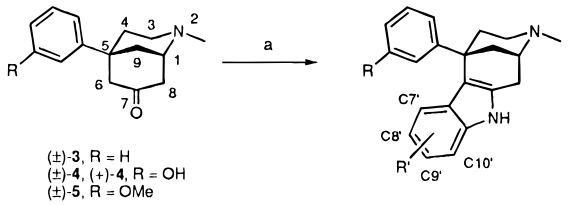
Table 1. Inhibition of Radioligand Binding to Rat Brain μ and δ Receptors and Guinea Pig κ_1 Receptors

compd	IC_{50} (±SD) (nM) [^{3}H]DAMGO[13] (μ)	[^{3}H]DADLE[14] (δ)	[^{3}H]U69,593[15] (κ_1)	μ/δ	κ_1/δ
(±)-**4**[a]	21.2 ± 1.8	720 ± 58	2026 ± 173	0.03	2.81
(+)-**4**[a]	136.8 ± 20.0	1136 ± 255	5071 ± 739	0.12	4.46
(±)-**7**	7267 ± 2467	1967 ± 352	>10 000	3.69	5.08
(±)-**8**[a,b]	29.8 ± 3.9	7.1 ± 0.9	>1000	4.20	141
(−)-**8**[a]	14.2 ± 1.0	5.6 ± 1.5	ND	2.54	ND
(±)-**9**	28.8 ± 5.5	8.7 ± 0.8	3959 ± 868	3.31	455
(−)-**9**	29.4 ± 7.2	4.4 ± 0.5	1770 ± 369	6.68	402
(±)-**10**	48.4 ± 9.0	6.3 ± 1.3	>1000	7.68	>159
(−)-**10**	66.4 ± 4.3	3.7 ± 0.3	1162 ± 249	17.9	314
(±)-**11**	24.2 ± 2.7	90.1 ± 17.9	>1000	0.27	11.1
(−)-**11**	474.2 ± 40.6	143.7 ± 17.2	>2500	3.30	17.4
SNC80[c]	2467 ± 200	2.9 ± 0.4	>1000	851	ND
morphine[d]	1.94 ± 0.31	684 ± 83	271 ± 34	2.84×10^{-3}	0.396
DADLE[d]	41.9 ± 7.4	1.71 ± 0.23	4404 ± 786	24.5	2575
(−)-etorphine[d]	0.14 ± 0.03	7.37 ± 0.52	1.82 ± 0.33	0.019	0.247

[a] See ref 5. [b] *O*-Methyl derivative (±)-**8** had IC_{50} values (μ, δ, and κ_1) >1000 nM. [c] Data from Calderon, S. N.; Rothman, R. B.; Porreca, F.; Flippen-Anderson, J. L.; Kayakiri, H.; Xu, H.; Becketts, K.; Smith, L. E.; Bilsky, E. J.; Davis, P.; Horvath, R.; Rice, K. C. Probes for narcotic receptor mediated phenomena. 22. Synthesis of novel nonpeptide delta opioid receptor ligand derivatives of the highly selective delta agonist SNC80. Submitted for publication. [d] See Xu, H.; Kim, C.-H.; Zhu, Y. C.; Weber, R. J.; Jacobson, A. E.; Rice, K. C.; Rothman, R. B. *Neuropharmacology* **1991**, *30*, 455−462.

Scheme 1[a]



[a] Reagents and conditions: (a) with R = R′ = H and R = OH and OMe, R′ = H, phenylhydrazine·HCl, HCl(g)-saturated EtOH, reflux; with R = OH, R′ = OMe, (*o*-, *m*-, or *p*-methoxyphenyl)-hydrazine·HCl, propanoic acid, reflux.

and differences between these two ligand classes. Both classes of compounds exhibit two phenyl moieties and a basic amino group. Whereas methylation or removal of the phenolic hydroxyl function of compound (±)-**8**, resulting in compounds (±)-**6** and (±)-**7**, respectively, caused the loss of all affinity for the δ receptor, the methylation of the phenolic group of (+)-BW373U86 [(+)-**2**], producing SNC80 (**1**), does not significantly affect the affinity for the δ receptor.[3] Consideration of these facts and a CAMM comparison[7] resulted in our hypothesis that the indole phenyl group, not the phenolic moiety of the indole (−)-phenylmorphan derivatives, might be structurally analogous to the methoxyphenyl moiety of SNC80 (**1**). Therefore, we believed that preparing the various methoxyindole isomer adducts of (−)-5-(3-hydroxyphenyl)morphan might provide δ agonists with improved selectivity for the δ receptor.

Chemical Synthesis

All of the products, chiral and racemic **6**−**11**, were prepared under Fischer indole synthesis conditions starting with the appropriate 7-ketone 5-phenylmorphan, **3**−**5** (see Scheme 1). The assignment of the regiochemistry of the indole in each case was accomplished by comparison of the proton NMR data with that of compound (±)-**6**, whose single-crystal X-ray analysis and characteristic proton NMR have been reported previously.[5] The assignment of the regiochemistry for compounds (±)-**9** and (±)-**10** was confirmed by single-crystal X-ray analyses, *vide infra*.

X-ray Crystallography of (±)-9 and (±)-10. Compound (±)-**9** crystallized as a hydrobromide salt with one molecule of butanol in the asymmetric unit. Compound (±)-**10** crystallized as the free base with no included solvent. The conformation of the fused ring system is the same in both molecules with the saturated 6-membered rings having a normal chair conformation. With relation to the indole moiety of each molecule, the phenolic aromatic rings are rotated by ca. 180° relative to each other, and this has an effect on the N···O1 intramolecular distances. For compound (±)-**9** the O1 to N3′ and O1 to N11′ distances are 7.52 and 6.58 Å, respectively. For compound (±)-**10** these distances are 7.03 and 8.33 Å. For compound (±)-**9** the methoxy group is attached to C10′, whereas in (±)-**10** it is bonded to C9′ which also has an effect on the N···O intramolecular distances (N3′···O10 = 7.08 Å and N11′···O10 = 2.86 Å for (±)-**9**; N3′···O9 = 8.76 Å and N11′···O9 = 4.92 Å for (±)-**10**).

Results and Discussion

Biological Data. In order to determine if the methoxy group substitutions on the C6−C7 indolo derivatives of the 5-(3-hydroxyphenyl)morphans had increased the affinity or the selectivity of the compounds for the δ receptor relative to the unsubstituted compounds, *in vitro* binding assays were determined for μ and δ receptors in rat brain and for κ_1 receptors in guinea pig brain (see Table 1). None of the C6−C7 methoxyindole derivatives [(±)- and (−)-**9**−**11**] had any substantial affinity for the κ_1 receptor (IC_{50} >1000 nM). As was previously seen,[5] the indole moiety in the C6−C7 position of compound (±)-**8** increases its affinity for the δ receptor by a remarkable 101-fold relative to the 7-ketone precursor (±)-**4**. Likewise, the binding affinity of (−)-**8** for the δ receptor is 5.6 nM, an increase of 203-fold relative to the chiral 7-ketone precursor (+)-**4**. However, the affinity of (±)- and (−)-**8** for the μ receptor remains high (29.8 and 14.2 nM, respectively), thus resulting in μ/δ IC_{50} ratios of small magnitude (4.20- and 2.54-fold, respectively). The binding affinities of the racemic methoxy derivatives (±)-**9**−**11** for the μ receptor

are substantially unchanged from that of the unsubstituted derivative (±)-**8** (29.8 nM). Furthermore, the affinities of the racemic C10′ and C9′ methoxy derivatives (±)-**9** and (±)-**10** for the δ receptor have also remained unchanged relative to the unsubstituted (±)-**8** (7.1 nM). Interestingly, the racemic C8′ methoxy derivative (±)-**11** (90.1 nM) had a 13-fold decrease in affinity for the δ receptor over the unsubstituted (±)-**8**.

Earlier studies[5] have shown that the levorotatory isomers of the C6−C7 indolo derivatives of the 5-(3-hydroxyphenyl)morphans have the appropriate stereochemistry for binding to the δ receptor (*vide supra*). Therefore it was of interest to study the optically pure levorotatory isomers of (±)-**9**−**11**. Compound (−)-**9**, with a methoxy group in the C10′ position, had a μ/δ IC_{50} ratio of 6.68, which is a 2.6-fold improvement resulting mainly from a decrease in μ receptor affinity and a virtually unchanged δ receptor affinity (4.4 nM) relative to the unsubstituted (−)-**8**. Compound (−)-**11**, which has a methoxy group in the C8′ position on the indole phenyl moiety, had a μ/δ IC_{50} ratio of 3.30; however, the affinity for both the μ and δ receptors was decreased by 33- and 26-fold relative to (−)-**8**. The compound with the highest affinity and selectivity for the δ receptor was compound (−)-**10**. This was displayed by a μ/δ IC_{50} ratio of 17.9 and an IC_{50} of 3.7 nM for binding to the δ receptor. The increase in the μ/δ IC_{50} ratio for (−)-**10** relative to the unsubstituted (−)-**8** results mainly from a decrease in μ receptor affinity. The δ receptor affinity represents a 307-fold increase for this C9′ methoxy indolo 5-(3-hydroxyphenyl)morphan derivative relative to the precursor 7-ketone derivative (+)-**4**. The μ/δ IC_{50} ratio for (−)-**10** therefore shows a favorable increase of 7.0- and 149-fold relative to the unsubstituted indole derivative (−)-**8** and the precursor (+)-**4**, respectively. For comparison, the binding affinities of SNC80 have been included in Table 1. SNC80 has an IC_{50} of 2.9 nM for the δ receptor, which is comparable to that of (−)-**8**. However, the μ/δ IC_{50} ratio of SNC80 is 851 or 48 times that for (−)-**8**, clearly indicating the superior *in vitro* selectivity of SNC80 for the δ receptor.

From Table 1 it is apparent that methoxy substitution sequentially in the C10′, C9′, and C8′ positions steadily decreases μ receptor binding affinities before the δ binding affinity is affected with the substitution at C8′. These results are in line with our previous findings for racemic derivatives of (±)-**8** containing phenyl rings fused to the indole moiety.[5] Whereas a phenyl ring fused at the C7′−C8′ position removed all μ and δ binding affinity, a phenyl at the C9′−C10′ position substantially decreased the μ binding affinity while only decreasing the δ receptor binding affinity by a small amount. Considering the continued difficulty in preparing compounds based on the C6−C7 indolo derivatives of the 5-(3-hydroxyphenyl)morphans that are substantially selective for the δ receptor versus the μ receptor (compared to SNC80-like compounds), it seems plausible that the binding interaction with the μ and δ receptors are similar. This is in line with our previous SAR (structure−activity relationship) studies involving the *N*-nor *N*-alkyl derivatives related to (±)-**8** which indicated that only minor changes in the μ/δ IC_{50} ratio could be obtained by major changes in the amine substituent even though the binding affinities could be substantially affected.[5] Further pharmacological studies would be necessary to determine if the C6−C7 indolo 5-(3-hydroxyphenyl)morphan derivatives are binding to the δ receptor in the typical manner as do compounds such as naltrindole, deltorphin II, and DPDPE or in what is suspected to be an atypical fashion indicative of the SNC80 type compounds.[6]

Table 2. Agonist Activity of Selected Compounds in the Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI) Bioassays (Data presented as mean ± SEM)

	IC_{50} ± SEM (nM)		
compd	GPI (μ receptors)	MVD (δ receptors)	GPI (μ)/ MVD (δ)
(±)-**8**[a]	2073 ± 556	347 ± 30	6
(−)-**8**[a]	2345 ± 64	393 ± 67	6
(±)-**9**	1949 ± 571	270 ± 58	7.2
(−)-**9**	2893 ± 1185	165 ± 27	17.5
(±)-**10**	862 ± 155	118 ± 21	7.3
(−)-**10**	38% at 30 μM	49.0 ± 12.3	>612
(±)-**11**	6767 ± 147	4366 ± 1589	1.5
(−)-**11**	25 901 ± 9200	0% at 30 μM	ND
SNC80[b]	5457 ± 2052	2.7 ± 0.5	2021
DPDPE[c]	7300 ± 1700	4.1 ± 0.5	1800
DADLE[c]	28.8 ± 8.8	447 ± 133	9.3

[a] See ref 5. [b] Data from Calderon, S. N.; Rothman, R. B.; Porreca, F.; Flippen-Anderson, J. L.; Kayakiri, H.; Xu, H.; Becketts, K.; Smith, L. E.; Bilsky, E. J.; Davis, P.; Horvath, R.; Rice, K. C. Probes for narcotic receptor mediated phenomena. 22. Synthesis of novel nonpeptide delta opioid receptor ligand derivatives of the highly selective delta agonist SNC80. Submitted for publication. [c] See ref 8.

Whereas the results from the *in vitro* binding studies indicated only minor improvement in δ receptor selectivity upon the addition of a methoxy moiety to the C9′ indole position in the levorotatory series, the results from bioassays revealed substantial enhancement in agonist activity from δ receptors relative to μ receptors. The agonist activity (Table 2) of the C6−C7 indolo 5-(3-hydroxyphenyl)morphan derivatives was next evaluated in the isolated mouse vas deferens (MVD) and guinea pig ileum (GPI) bioassays.[8] The data indicate that none of the levorotatory methoxy isomers, (−)-**9**−**11**, had appreciable agonist activity in the GPI bioassay (μ), and for (−)-**10** and (−)-**11**, the IC_{50} values were substantially lower than for the unsubstituted [(−)-**8**] and C10′ methoxy substituted [(−)-**9**] compounds. The MVD bioassay (δ) for (−)-**9** indicated that a C10′ methoxy-substitution resulted in a 2-fold increase of δ agonist activity relative to the unsubstituted compound (−)-**8**. The substitution of a methoxy moiety at the C9′ position in the levorotatory series yields compound (−)-**10** and has the effect of increasing the δ agonist activity 8-fold relative to the unsubstituted compound (−)-**8**. Interestingly, substitution of methoxy at the C8′ position in the levorotatory series [(−)-**11**] totally eliminates the agonist activity at the δ receptor. This result is in line with the *in vitro* binding data for (−)-**11**. The result of these trends indicates that a methoxy group substitution at the C9′ position in the levorotatory series results in a compound with good agonist activity at the δ receptor (IC_{50} 49.0 nM) and a GPI/MVD IC_{50} ratio of >612. For comparison, the results of the MVD and GPI bioassays for SNC80 are given in Table 2 as well. The increase in the GPI/MVD IC_{50} ratio for (−)-**10** (GPI/MVD IC_{50} ratio >612) relative to the unsubstituted compound (−)-**8** (GPI/MVD IC_{50} ratio 6) is remarkably >100-fold and results in a compound with MVD selectivity approaching that displayed by SNC80 (GPI/MVD IC_{50} ratio 2021).

Experimental Section

General Instrumentation and Methods. Proton NMR spectra were recorded for the free bases of all compounds in $CDCl_3$ (unless otherwise specified) on a Varian Gemini-300 spectrometer, and the data are reported in the following format: chemical shift (all relative to Me_4Si), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, ap = apparent), integration, coupling constants, and exchangeability after D_2O addition. Electron impact (EI) mass spectra were recorded on a VG Micromass 7070F spectrometer, and chemical ionization (CI) mass spectra were recorded on a Finnigan 4600 spectrometer. UV spectra were recorded on a Hewlett-Packard 8450A UV/vis spectrophotometer, and IR spectra were recorded on a Bio-Rad FTS-45 spectrophotometer. Polarimetric measurements were taken using a Perkin-Elmer 241MC polarimeter. Thin layer chromatography (TLC) was performed on Analtech silica gel GHLF 0.25-mm plates. Column chromatography was performed with Fluka silica gel 60 (mesh 220−440). Elemental microanalyses were performed by Atlantic Microlab, Inc. Melting points were recorded on a Thomas-Hoover capillary apparatus or on a Mel-Temp II apparatus (>260 °C) and are uncorrected. The yields reported are not optimized.

1,2,3,4,5,11-Hexahydro-6-phenyl-3-methyl-2,6-methano-6*H*-azocino[4,5-*b*]indole Hydrobromide [(±)-7]. A solution of (±)-**3**·HBr[9] (310 mg, 1.00 mmol) and phenylhydrazine·HCl (289 mg, 2.00 mmol) in a solution of ethanol (30 mL) saturated with HCl(g) was heated to reflux under an atmosphere of argon for 24 h. The solvent was evaporated, and the residue was taken up in a mixture of 1 M NH_4OH/brine (15 mL:15 mL), and this was extracted with chloroform (25 mL). The chloroform extract was dried (Na_2SO_4) and evaporated onto silica gel (3 g). This residue was loaded onto a silica gel column (40 g), and the column was eluted with chloroform/methanol/28% NH_4OH (95:5:0.5). The fractions containing the major product were combined, and evaporation gave a red oil. This was dissolved in a minimum amount of hot 2-propanol and acidified with 48% HBr(aq). The crystalline salt obtained, 278 mg (72%), was recrystallized from ethanol/water (1:1) yielding 211 mg of an off-white salt: mp 324−325 °C; ^{1}H NMR δ 7.84 (br s, 1H, ex w/D_2O), 7.45 (m, 2H), 7.32 (m, 4H), 7.00 (t, 1H, J = 7.8 Hz), 6.73 (t, 1H, J = 7.5 Hz), 6.29 (d, 1H, J = 7.9 Hz), 3.41 (m, 1H), 3.12 (d, 1H, J = 17.4 Hz), 2.81 (dd, 1H, J = 5.4, 17.5 Hz), 2.67 (ddd, 1H, J = 2.0 4.9, 11.9 Hz), 2.51 (m, 1H), 2.45 (s, 3H), 2.17 (m, 3H), 1.94 (dd, 1H, J = 2.6, 12.0 Hz); MS (CI-NH_3) m/z 303 (MH^+). Anal. ($C_{21}H_{22}N_2$·HBr·0.25H_2O) C, H, N.

3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-10-methoxy-6*H*-azocino[4,5-*b*]indol-6-yl)phenol Hydrobromide [(±)-9]. A solution of (±)-**4**·HBr[5] (500 mg, 1.53 mmol) and (*o*-methoxyphenyl)hydrazine·HCl (536 mg, 3.06 mmol) in propanoic acid (10 mL) was heated to reflux for 1 h under an atmosphere of argon. The reaction solution was evaporated, and the residue was taken up in H_2O (30 mL) and adjusted to pH 9 with 28% NH_4OH. This was extracted with chloroform (4 × 30 mL), and the extracts were dried (Na_2SO_4). This solution was evaporated onto silica gel 60 (2.5 g). This was loaded onto a silica gel column (60 g) packed in chloroform. The column was eluted with chloroform/methanol/28% NH_4OH (90:10:0.5), and the fractions containing the major product (R_f = 0.18 with the eluting solvent) were combined and evaporated. The solid residue was dissolved in 2-propanol, and the solution was made acidic with 48% hydrobromic acid. The filtered off-white salt was filtered, rinsed with cold 2-propanol, and dried; yield 208 mg (28%). An analytical sample was prepared by recrystallization from methanol/propanol: yield 202 mg; mp >200 °C; ^{1}H NMR δ 8.06 (s, 1H, ex w/D_2O), 7.19 (t, 1H, J = 7.9 Hz), 6.98 (d, 1H, J = 7.3 Hz), 6.92 (br s, 1H), 6.74 (dd, 1H, J = 2.4, 7.4 Hz), 6.69 (t, 1H, J = 8.1 Hz), 6.50 (d, 1H, J = 7.8 Hz), 6.02 (d, 1H, J = 8.0 Hz), 3.92 (s, 3H), 3.39 (m, 1H), 3.11 (d, 1H, J = 17.5 Hz), 2.79 (dd, 1H, J = 5.4, 17.5 Hz), 2.66 (m, 1H), 2.49 (dt, 1H, J = 4.5, 12.0 Hz), 2.43 (s, 3H), 2.11 (m, 3H), 1.90 (m, 1H); MS (CI-NH_3) 349 (MH^+). Anal. ($C_{22}H_{24}N_2O_2$·HBr·C_3H_8O) C, H, N.

(−)-(2*S*,6*R*)-3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-10-methoxy-6*H*-azocino[4,5-*b*]indol-6-yl)phenol Methanesulfonate [(−)-9]. A procedure analogous to the preparation of (±)-**9** was carried out with (+)-**4**,[5] except that the salt prepared was a methanesulfonate from 2-propanol; yield 173 mg (25%). An analytical sample was prepared by recrystallization from methanol, yielding 162 mg: mp >300 °C dec; $[\alpha]_D^{23}$ (methanesulfonate salt in DMSO, c = 0.22) = −74.5°; ^{1}H NMR and MS (CI-NH_3) of the free base matched that of (±)-**9**. Anal. ($C_{22}H_{24}N_2O_2$·CH_4SO_3) C, H, N.

3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-9-methoxy-6*H*-azocino[4,5-*b*]indol-6-yl)phenol Methanesulfonate [(±)-10]. A solution of (±)-**4**[5] (415 mg, 1.69 mmol) and (*m*-methoxyphenyl)hydrazine·HCl (592 mg, 3.38 mmol) in propanoic acid (10 mL) was heated to reflux for 2 h under an atmosphere of argon. The reaction solution was evaporated, and the residue was dissolved in *N*,*N*-dimethylformamide (2 mL) and chloroform (20 mL). H_2O (10 mL) was added, and the pH was adjusted to 9 with 28% NH_4OH. The chloroform layer was separated, and the aqueous layer was extracted with chloroform (8 × 10 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated. The residual oil was dissolved in chloroform, and this was evaporated onto silica gel 60 (3 g). This was loaded onto a silica gel column (75 g) packed in chloroform. The column was eluted with chloroform/methanol/28% NH_4OH (90:10:0.5), and the fractions containing the major product (R_f = 0.45 with the eluting solvent) were combined and evaporated. The solid residue was dissolved in 2-propanol, and the solution was made acidic with methanesulfonic acid. The filtered off-white salt was filtered, rinsed with cold 2-propanol, and dried; yield 330 mg (44%). An analytical sample was prepared by recrystallization from methanol/2-propanol; yield 283 mg. The free base compound was crystallized from methanol. The salt had mp 290−292 °C; ^{1}H NMR δ 7.71 (s, 1H, ex w/D_2O), 7.17 (t, 1H, J = 7.9 Hz), 6.92 (br d, 1H, J = 6.8 Hz), 6.85 (br s, 1H), 6.78 (d, 1H, J = 2.5 Hz), 6.73 (dd, 1H, J = 2.5, 7.9 Hz), 6.46 (dd, 1H, J = 2.5, 8.8 Hz), 6.27 (d, 1H, J = 8.6 Hz), 3.78 (s, 3H), 3.35 (m, 1H), 3.04 (d, 1H, J = 17.5 Hz), 2.75 (dd, 1H, J = 5.2, 17.5 Hz), 2.59 (m, 1H), 2.41 (s, 3H), 2.41 (m, 1H), 2.10 (m, 3H), 1.81 (d, 1H, J = 11.9 Hz); MS (CI-NH_3) 349 (MH^+). Anal. ($C_{22}H_{24}N_2O_2$·CH_4SO_3) C, H, N.

(−)-(2*S*,6*R*)-3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-9-methoxy-6*H*-azocino[4,5-*b*]indol-6-yl)phenol Methanesulfonate [(−)-10]. A procedure analogous to the preparation of (±)-**10** was carried out with (+)-**4**;[5] yield 362 mg (48%). An analytical sample was prepared by recrystallization from methanol, yielding 298 mg; mp >300 °C dec; $[\alpha]_D^{23}$ (methanesulfonate in DMSO, c = 0.42) = −74.3°; ^{1}H NMR and MS (CI-NH_3) of the free-base matched that of (±)-**10**. Anal. ($C_{22}H_{24}N_2O_2$·CH_4SO_3) C, H, N.

3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-8-methoxy-6*H*-azocino[4,5-*b*]indol-6-yl)phenol Methanesulfonate [(±)-11]. A solution of (±)-**4**[5] (700 mg, 2.86 mmol) and (*p*-methoxyphenyl)hydrazine·HCl (1.00 g, 5.71 mmol) in propanoic acid (15 mL) was heated to reflux for 2 h under an atmosphere of argon. The reaction mixture was cooled and left to stand overnight. The crystalline solid was filtered and rinsed with acetic acid. This was dissolved in a minimum amount of warm *N*,*N*-dimethylformamide, and 1 M NH_4OH (50 mL) was added. This was extracted with chloroform (2 × 50 mL), and the organic layer was separated, dried (Na_2SO_4), and evaporated. The resulting oil was dissolved in hot methanol (10 mL) and acidified with methanesulfonic acid. The resulting off-white crystalline solid was filtered and rinsed with 2-propanol followed by petroleum ether; yield 807 mg (63%). An analytical sample was prepared by free basing as above and again preparing the salt: yield 750 mg; mp >300 °C dec; ^{1}H NMR δ 7.70 (s, 1H, ex w/D_2O), 7.19 (t, 1H, J = 7.8 Hz), 7.12 (d, 1H, J = 8.7 Hz), 6.96 (br, 1H), 6.84 (br s, 1H), 6.73 (dd, 1H, J = 2.4, 7.9 Hz), 6.67 (dd, 1H, J = 2.5, 8.8 Hz), 5.84 (d, 1H, J = 2.3 Hz), 3.49 (s, 3H), 3.38 (m, 1H), 3.07 (d, 1H, J = 17.7 Hz), 2.77 (dd, 1H, J = 5.4, 17.6 Hz), 2.59 (m, 1H), 2.42 (s, 3H), 2.36 (dd, 1H, J = 4.0, 12.1 Hz), 2.14 (m, 3H), 1.80 (d, 1H, J = 11.2 Hz); MS (CI-NH_3) 349 (MH^+). Anal. ($C_{22}H_{24}N_2O_2$·CH_4SO_3) C, H, N.

(−)-(2*S*,6*R*)-3-(1,2,3,4,5,11-Hexahydro-3-methyl-2,6-methano-8-methoxy-6*H*-azocino[4,5-*b*]indol-6-yl)phenol Methanesulfonate [(−)-11]. A procedure analogous to the preparation of (±)-**11** was carried out with (+)-**4**;[5] yield 423 mg (66%). An analytical sample was prepared as for (±)-**11**: mp >300 °C dec; $[\alpha]_D^{23}$ (methanesulfonate in DMSO, $c = 0.46$) = −60.0°; ^{1}H NMR and MS of the free base matched that of (±)-**11**. Anal. ($C_{22}H_{24}N_2O_2 \cdot CH_4SO_3$) C, H, N.

Single-Crystal X-ray Analyses of (±)-9 and (±)-10. Crystals of (±)-**9** (as HBr salt cocrystallized with 1-butanol) were grown by slow evaporation from a mixture of 1-butanol/hexane. Crystals of (±)-**10** were obtained as the free base from methanol. Data for both compounds were collected on a computer-controlled automatic diffractometer and corrected for Lorentz and polarization effects. Data for (±)-**9** was also corrected for absorption effects. The structures were solved by direct methods with the aid of the program SHELXTL[10] and refined by full-matrix least-squares on F^2 values using the program SHELXLS93.[11] The parameters refined included the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. For the most part hydrogen atoms were included using a riding model in which the coordinate shifts of their covalently bonded atoms were applied to the attached hyrdogens with C−H = 0.96 Å and N−H = 0.86 Å. H angles were idealized and U_{iso}(H) set at fixed ratios of U_{iso} values of bonded atoms. Coordinates were refined for H atoms bonded to oxygen. Additional experimental and structural analysis details are given in the tables of refinement parameters, crystal coordinates, bond distances, bond angles, and hydrogen bonds which are available as Supporting Information.[12]

Biological Assays: [^{3}H]DAMGO, [^{3}H]DADLE, and [^{3}H]-U69,593 Radioligand Binding Assays. μ binding sites were labeled using [^{3}H]DAMGO (1−3 nM) and rat brain membranes as previously described.[13] 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 levallorphan. δ binding sites were labeled using [^{3}H]DADLE (1.7−2.5 nM) and rat brain membranes as previously described.[14] 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. κ_1 binding sites were labeled using [^{3}H]-U69,593 (3.5−5.0 nM) and guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with irreversible ligands BIT and FIT as previously described,[15] except that the incubation temperature was at 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.

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 solutions with 10 mM Tris buffer (pH 7.4) containing 10% DMSO before drug dilution. Compound (±)-**11** was prepared as a 1 mM solution with 10 mM Tris buffer (pH 7.4) containing 10% DMSO and 5% Emulphor EL-620 before drug dilution. The IC_{50} and slope factor (N) were obtained by using the program MLAB.

GPI and MVD Bioassays. Electrically induced smooth muscle contraction of mouse vas deferens and strips of guinea pig ileum longitudinal muscle-myenteric plexus were used as a bioassay.[16] 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. Drugs were added to the baths in 14−60 μM volumes. The agonists remained in contact with the tissue until maximum inhibition was reached before the addition of the next cumulative dose. Percent inhibition was calculated by using the average contraction height for 1 min preceding the addition of the agonist divided by the contraction height at maximal inhibition after exposure to the dose of agonist. 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.[17]

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Supporting Information Available: Tables of refinement parameters and crystallographic data for (±)-**9** and (±)-**10** including bond lengths, bond angles, and atomic coordinates (10 pages). Ordering information is given on any current masthead page.

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