Identification of (3*R*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide as a Novel Potent and Selective Opioid κ Receptor Antagonist

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(3*R*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic) was identified as a potent and selective κ opioid receptor antagonist. Structure−activity relationship (SAR) studies on JDTic analogues revealed that the 3*R*,4*R* stereochemistry of the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine core structure, the 3*R* attachment of the 7-hydroxy-1,2,3,4-tetrahydroisoquinoline group, and the 1*S* configuration of the 2-methylpropyl (isopropyl) group were all important to its κ potency and selectivity. The results suggest that, like other κ opioid antagonists such as nor-BNI and GNTI, JDTic requires a second basic amino group to express potent and selective κ antagonist activity in the [^{35}S]GTPγS functional assay. However, unlike previously reported κ antagonists, JDTic also requires a second phenol group in rigid proximity to this second basic amino group. The potent and selective κ antagonist properties of JDTic can be rationalized using the "message−address" concept wherein the (3*R*,4*R*)-3,4-dimethyl-4-(hydroxyphenyl)piperidinyl group represents the message, and the basic amino and phenol group in the N substituent constitutes the address. It is interesting to note the structural commonality (an amino and phenol groups) in both the message and address components of JDTic. The unique structural features of JDTic will make this compound highly useful in further characterization of the κ receptor.

Introduction

Opium, the exudate of the opium poppy (*Papaver somniferum*), has been used for beneficial medicinal purposes for thousands of years. For an equally long time however, mankind has known the hardship associated with abuse of this powerfully addictive substance. Early in the past century, attempts to harness the better nature of this natural product led to the development of heroin (**1**, Chart 1), the diacetyl derivative of the principle constituent of opium, morphine (**2**). Ironically, heroin is much more addictive than opium and has since become the most abused opiate worldwide. To counter this problem, a scientific campaign was mounted early in the 20th century to probe the fundamentals of addiction and the addiction process.[1,2] The wealth of knowledge accumulated since its inception is enormous and includes examples of milestone discoveries commensurate with its breadth from the original demonstration of an endogenous opioid receptor[3] to the more recent cloning of the opioid receptor. With distinct cDNAs encoding μ, δ, and κ receptors for animals and humans identified, sequenced, and cloned, it is now well established that opioid receptors belong to the superfamily of G-protein-coupled receptors (GPCRs).[4−11]

An integral part of the effort to characterize the opioid receptor has been the discovery of antagonists for the opioid receptors. In addition, it has long been thought that antagonists for the opioid receptors might provide treatment medications for those suffering from opiate abuse, especially those antagonists that are selective for particular receptor subtypes. One of the more useful compounds for studying the opioid receptor has been the κ selective opioid antagonist nor-BNI (**3**), which is derived from the nonselective opioid antagonist naltrexone (**4**).[12] We recently reported the discovery of (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (**5**, JDTic) as a potent and selective antagonist for the κ opioid receptor.[13] Compound **5** arose via modification of the R group in N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines (**6**) first to give the analogue **7**, which was optimized by additional structural modification to give the κ selective **5**.[13,14] Preliminary results from some of these studies have been reported.[13] In this article, we report the results of an SAR investigation of JDTic and present additional results on the in vitro pharmacological characterization of JDTic.

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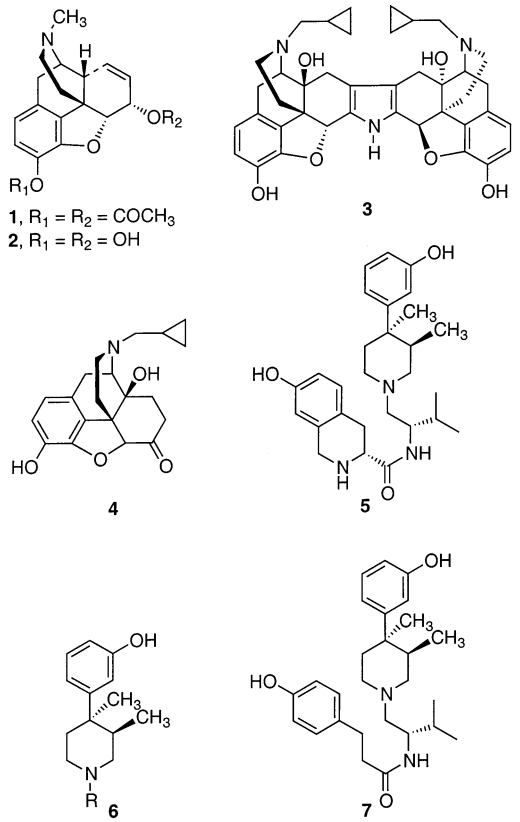
Chart 1



Chemistry

In this study to develop a potent and κ-selective opioid receptor antagonist, we synthesized and evaluated JDTic (**5**) and the JDTic analogues **9**−**17**. JDTic (**5**), JLTic (**9**), the desphenolic analogue **10**, and the tyrosine analogues **11** and **12** were all prepared from the common intermediate **8**[14] (Scheme 1). Coupling of Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Boc-L-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, and Boc-D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid with **8** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) in THF, followed by removal of the Boc protecting group with trifluoroacetic acid in methylene chloride, yielded **5**, **9**, and **10**, respectively. The tyrosine analogues **11** and **12** were synthesized by coupling Boc-D-tyrosine and Boc-L-tyrosine to **8** using BOP followed by removal of the Boc protecting group with trifluoroacetic acid in methylene chloride. The reduced and N-substituted analogues **13**−**16** were all synthesized from **5**. The desired products were obtained via carbonyl reduction with borane dimethyl sulfide (**13**), methylation using sodium triacetoxy borohydride, and formaldehyle (**14**), and acylation using BOP and acetic acid (**15**) or *N*,*N*-dimethylglycine (**16**) (Scheme 2).

The 3*S*,4*S* diastereomer (**17**) was synthesized from (3*S*,4*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**18**), which was in turn prepared according to the method of Werner and co-workers starting from 1,3-dimethyl-4-piperidone (**19**) as depicted in Scheme 3.[15] Thus, **19** was treated with 3-isopropoxyphenyllithium followed by formation of the ethyl carbonate and recrystallization of the (+)-diethyl ditoluyltartrate salt to give optically pure carbonate **20**. Heating **20** in refluxing Decalin provided 3-methyltetrahydropyridine **21**, which was alkylated with dimethyl sulfate following deprotonation with *n*-butyllithium. N-demethylation using phenyl chloroformate followed by deprotection of the phenol group with HBr in acetic acid gave (3*S*,4*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**18**). Acylation of **18** with Boc-L-valine followed by reduction with the borane dimethyl sulfide complex and deprotection with TFA gave diamine **22**. The completion of the synthesis of **17** from this point followed the same route employed in the preparation of **5** from **8**.

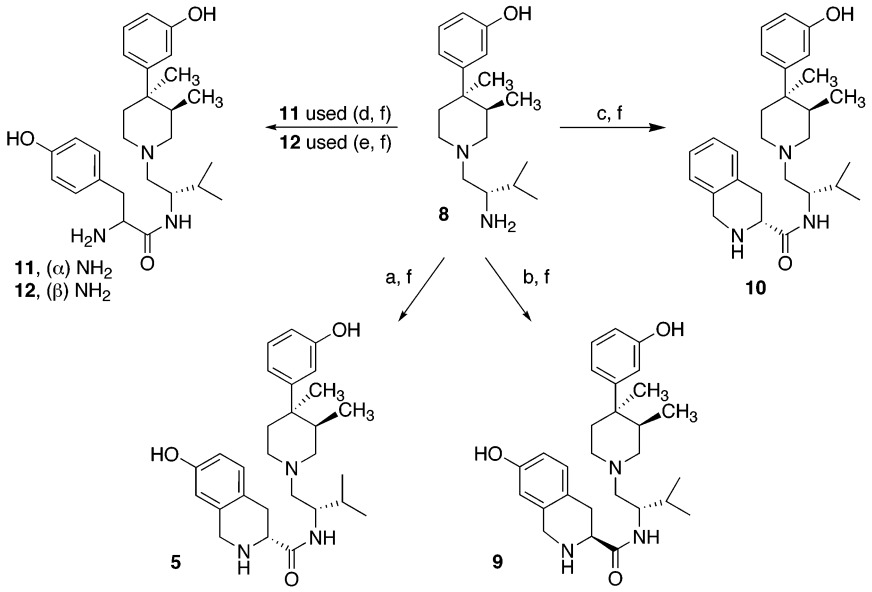
Biological Results

The binding affinities of the test compounds for the μ, δ, and κ opioid receptors listed in Table 1 were determined using previously reported competitive binding assays.[13,14,16] [^{3}H]DAMGO, [^{3}H]DADLE, and [^{3}H]-U69,593 radioligands were used to label the μ, δ, and κ receptors, respectively. The tissues used in the binding assays included rat brain (μ and δ receptors) and guinea pig brain (κ receptors). Measures of functional antagonism were obtained by monitoring the ability of test compounds to inhibit stimulation of [^{35}S]GTPγS binding produced by the selective agonists (D-Ala^2,MePhe^4,Gly-ol^5)enkephalin (DAMGO, μ receptor), (+)-4-[(αR)-α-(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80, δ receptor), and 5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro-[4,5]dec-8-yl]benzeneacetamide (U69,593, κ receptor) in guinea pig caudate membranes (Table 2).[13,14,16] The binding affinities of selected compounds for the μ, δ, and κ receptors using [^{3}H]DAMGO, [^{3}H]Cl-DPDPE, and [^{3}H]-U69,593, respectively (Table 3), and the ability to inhibit [^{35}S]GTPγS binding stimulated by DAMGO (μ), DPDPE (δ), and U69,593 (κ), using cloned human opioid receptors transfected into CHO cells, were also determined (Table 4).[17,18]

Results and Discussion

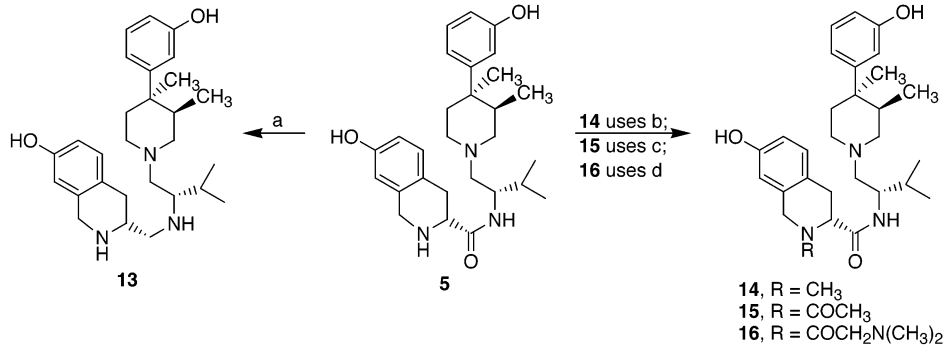
In our studies of novel opioid agonists and antagonists,[16,19] we commonly utilized radioligand binding assays as a primary screen. Once compounds possessing desirable biological properties, i.e., enhanced affinity or selectivity for a particular receptor subtype, were identified, the selected compounds were evaluated in the in vitro [^{35}S]GTPγS functional assay to reveal their efficacy, potency, and selectivity. This progression scheme proved to be reliable when dealing with μ and δ receptor ligands, where a good correlation was observed between a compound's receptor affinity in binding studies and its potency in functional assays. However, this scheme proved to be less useful in our studies directed toward the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine-based κ opioid receptor antagonists. The data from the radioligand binding assays did not accurately predict those compounds that would be most selective for the κ over the μ and δ receptors in the in vitro efficacy assays. In fact, some compounds exhibited an inverse behavior between κ selectivity in binding relative to selectivity in functional assays. For example, we reported that compound **7** with radioligand binding K_i values of 171, >3400, and 3.84 nM at the μ, δ, and κ receptors, respectively, and μ/κ and μ/δ selectivity ratios of 45 and

Scheme 1[a]



[a] Reagents: all coupling reactions used BOP and Et_3N in THF; (a) Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; (b) Boc-L-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; (c) Boc-D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; (d) Boc-D-tyrosine; (e) Boc-L-tyrosine; (f) TFA, CH_2Cl_2.

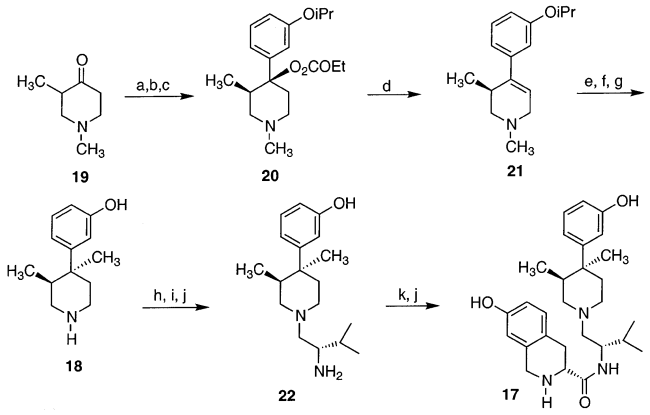
Scheme 2[a]



[a] Reagents: (a) $BH_3{\cdot}SMe_2$, THF; (b) $NaBH(OAc)_3$, 37% aqueous CH_2O; (c) BOP, TEA, CH_3CO_2H; (d) BOP, TEA, $(CH_3)_2NCH_2CO_2H$.

>885 were superior to **5** (JDTic), which possessed K_i values of 3.73, 301, and 0.32 nM at the μ, δ, and κ receptors and μ/κ and μ/δ ratios of 12 and 940, respectively (Table 1). In fact, compound **7** possesses a μ/κ K_i selectivity ratio comparable to that of nor-BNI and a far superior δ/κ K_i ratio and, thus, is selective for the κ opioid receptor over the μ or δ receptor. In contrast, **5** shows very little κ versus μ selectivity relative to **7** or nor-BNI in the radioligand binding assays. The principle reason for this lack of κ selectivity is the greater affinity of **5** for the μ receptor relative to **7** and nor-BNI. The overall result is that **5** possesses low κ versus μ selectively in the radioligand binding test. In contrast, **5** shows a 16-fold improvement in its κ receptor K_e value in the [^{35}S]GTPγS assay relative to the radioligand binding assay (0.02 versus 0.32 nM). Since the K_e values for **5** in the μ and δ assays do not increase substantially, the shift to higher potency for **5** in the κ receptor functional assay results in greater than 100-fold μ versus κ selectivity and a remarkable >15000-fold selectivity for the δ versus κ receptor. Similar to JDTic (**5**), nor-BNI (**3**) shows a 28-fold increase in its K_e value for the κ receptor relative to its K_i value in the radioligand binding assay and only a 4- and 8-fold increase at the μ and δ receptors. Overall, this translates into a significant increase in μ versus κ and δ versus κ selectivity in [^{35}S]-

Scheme 3[a]



[a] Reagents: all coupling reactions used BOP and Et_3N in THF, and all deprotections used TFA in CH_2Cl_2; (a) 3-isopropoxyphenyllithium, THF; (b) EtOCOCl, EtOAc; (c) (+)-DTTA; (d) Decalin reflux; (e) n-BuLi, $(CH_3)_2SO_4$; (f) C_6H_5OCOCl; (g) 48% HBr, HOAc; (h) Boc-L-valine; (i) $BH_3{\cdot}SMe_2$, THF; (j) TFA; (k) Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

Table 1. Radioligand Binding Results at the μ, δ, and κ Opioid Receptors Using Opioid Receptors Obtained from Brain Tissue (μ and δ of Rat, κ of Guinea Pig)

	$K_i \pm$ SD, nM				
compd	μ [^{3}H]DAMGO[a]	δ [^{3}H]DADLE[b]	κ [^{3}H]U69,593[c]	μ/κ	δ/κ
Nor-BNI, **3**	65 ± 5.6[d]	86 ± 7.2[d]	1.09 ± 0.14[d]	60	79
JDTic, **5**	3.73 ± 0.17[d]	301 ± 50[d]	0.32 ± 0.05[d]	12	940
7	171 ± 15[d]	>3400	3.84 ± 0.26[d]	45	>885
9	596 ± 29	>4900	9.8 ± 1.6	61	>500
10	775 ± 75	>4900	2.1 ± 0.17	369	>2333
11	208 ± 14	>5051	20.4 ± 2	10	>247
12	633 ± 34	>5051	16 ± 1.1	40	>315
13	107 ± 11	>4900	0.63 ± 0.05	170	>7777
14	37 ± 1.4	616 ± 59	0.053 ± 0.003	700	11,622
15	764 ± 66	>4900	146 ± 15	5	>34
16	164 ± 10	>3400	15.5 ± 1.2	11	>219
17	138 ± 8	144 ± 15	17.5 ± 2.5	7.8	8.2

[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. [d] Data taken from ref 13.

Table 2. Inhibition by Antagonists of [^{35}S]GTPγS Binding in Guinea Pig Caudate Stimulated by the Opioid Receptor Subtype-Selective Agonists, DAMGO (μ), SNC80 (δ), and U69,593 (κ)

	$K_e \pm$ SD, nM				
compd	μ DAMGO[a]	δ SNC-80[b]	κ U69,593[c]	μ/κ	δ/κ
3	16.7 ± 1.5[d]	10.2 ± 1.0[d]	0.038 ± 0.005[d]	440	268
5	2.16 ± 0.75[d]	>300[d]	0.02 ± 0.002[d]	108	>15000
7	7.25 ± 0.52	450 ± 74.1	4.7 ± 0.56	1.5	96
9	11 ± 0.8	327 ± 35	4.2 ± 0.20	2.6	78
10	68.6 ± 6.5	147 ± 11	11.5 ± 1	5.9	12.8
11	12 ± 0.8	334 ± 41	44.6 ± 6	0.3	7.5
12	16.5 ± 2	452 ± 35	30 ± 2.9	0.55	15
13	12.8 ± 1.4	>300	0.20 ± 0.03	64	>1500
14	12.7 ± 1.4	>300	0.37 ± 0.04	34	810
15	17.4 ± 1.2	>300	19.6 ± 1.6	0.9	15
16	29 ± 2	628 ± 50	0.16 ± 0.03	181	3925
17	178 ± 17	>300	16.7 ± 1.9	10.7	>18

[a] DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Agonist selective for μ opioid receptor. [b] SNC-80 ([(+)-4-[(α*R*)-α-(2*S*,5*R*)-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. [d] Taken from ref 13.

GTPγS assay relative to that observed in the binding assay.

As already mentioned, compound **7** also shows significant changes in behavior between these two assays, but the changes do not parallel those found for JDTic (**5**) and nor-BNI (**3**). Instead, compound **7** shows no μ versus κ selectivity in the [^{35}S]GTPγS assay compared with its 45-fold selectivity seen in the radioligand binding assay, an effect driven primarily by its 25-fold increase in μ receptor K_e. Overall, compound **7** is not selective in [^{35}S]GTPγS assay and does not compare favorably with JDTic (**5**) and nor-BNI (**3**). Similar differences in the radioligand and [^{35}S]GTPγS assays seen for compounds **3**, **5**, and **7** were also noted for other compounds assayed (Tables 1 and 2). Figure 1 shows a comparison of the μ versus κ selectivity ratios from the radioligand binding and [^{35}S]GTPγS functional assays, as listed in Tables 1 and 2, respectively, for a relevant set of N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)-piperidine-based antagonists. In this representation, the nature of this phenomenon of a lack of correlation between the radioligand and [^{35}S]GTPγS assays is readily appreciated with compounds **5**, **16**, **10**, and **14**, which illustrate the two observable extremes. As depicted, the selectivity ratios obtained from binding data for **5** and **16** suggest that these ligands would possess low selectivity for the κ receptor versus the μ receptor in the functional assay, but as is clear from the [^{35}S]-GTPγS functional assay selectivity ratios, this is not the case. Indeed, in the more relevant functional assay, these compounds were found to be among the most potent and selective κ antagonists ever identified. At the opposite end of the spectrum, the binding assay data for compounds **10** and **14** indicate that these compounds should possess extraordinary selectivity in the functional assay, yet as was the case for **7**, this stands in contrast to the actual selectivity found in the functional assay. Nevertheless, it is clear that the development of JDTic required input from both series of data. Radioligand binding data provided the novel lead structure **7**, and the [^{35}S]GTPγS assay, which is more correlated with in vivo functional assays, led to the identification of JDTic as a potent and κ-selective opioid antagonist. On the basis of the higher correlation of [^{35}S]GTPγS data with in vivo functional data, the SAR analysis of the JDTic analogues is limited to the data obtained in the functional assays.

The data obtained for the test compounds in the [^{35}S]-GTPγS functional assay using guinea pig caudate membranes are presented in Table 2. By means of comparison, the SAR for compound **5** and that of the original lead compound **7** guided the design of additional compounds to reveal those structural features underpinning the dramatic change in biological activity observed between these two compounds. The addition of a methyleneamino bridge to compound **7**, giving compound **5**, has a tremendous impact on conformational flexibility, overall basicity, and solubility; yet it allows **5** to still meet all five of Lipinski's requirements for a compound to possess druglike properties.[20] For example, from a comparison of the data for **5** and **7** in Table 2, the overall result to antagonist activity of this structural change is expressed as a 3-fold increase of potency in the μ receptor assay (K_e = 7.25 versus 2.16 nM) and a 235-fold increase in potency at the κ receptor (K_e = 4.75 versus 0.02 nM).

The presence of pendant amino groups in the naltrexone-based κ antagonists such as nor-BNI (**3**) has been shown to be essential to its selectivity for the κ receptor versus the μ or δ opioid receptors.[21] Thus,

Table 3. Radioligand Binding Results for Test Compound Using Human Cloned μ, δ, and κ Receptors

	$K_i \pm$ SD, nM				
compd	μ [^{3}H]DAMGO	δ [^{3}H]Cl-DPDPE	κ [^{3}H]U69,593	μ/κ	δ/κ
nor-BNI, **3**[a]	21 ± 5.0	5.7 ± 0.9	0.2 ± 0.05	105	29
GNTI, **23**[b]	36.9 ± 2.3	70.0 ± 0.3	0.18 ± 0.10	206	389
JDTic, **5**	0.96 ± 0.01	29.6 ± 11.9	0.41 ± 0.10	2.3	72
10	33.1 ± 0.89	1090 ± 174	2.18 ± 0.12	15	500
13	3.80 ± 0.30	83.6 ± 8.09	1.82 ± 0.20	2.1	46
14	8.86 ± 0.69	118 ± 6.66	1.01 ± 0.17	8.8	117
16	13.2 ± 1.16	175 ± 25.5	16.2 ± 2.58	0.81	11

[a] Taken from ref 24. [b] Taken from ref 23.

Table 4. Inhibition of Agonist Stimulated [^{35}S]GTPγS Binding by JDTic and JDTic Analogues in Cloned Human μ, δ, and κ Opioid Receptors

	μ, DAMGO[a]		δ, DPDPE[b]		κ, U69,593[c]			
compd	K_e (nM)	pA_2	K_e (nM)	pA_2	K_e (nM)	pA_2	μ/κ	δ/κ
nor-BNI,**3**[d]	19 ± 1.8		4.4 ± 0.38		0.04		475	115
GNTI, **23**[e]		8.49 ± 0.09		7.81 ± 0.06		10.40 ± 0.10		
JDTic, **5**	3.41 ± 0.36		79.3 ± 9.3	*f*	0.01 ± 0.00		341	7930
10	8.38 ± 0.30		ND		2.95 ± 0.42		2.8	
13	14.3 ± 2.27		427 ± 109		0.14 ± 0.02		102	3100
14	8.92 ± 1.23		120 ± 11		0.10 ± 0.02		89	1200
16	21.2 ± 1.48		299 ± 54		1.10 ± 0.09		19	272

[a] DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin] (selective for μ opioid receptor). [b] DPDPE cyclo[D-Pen^2,D-Pen^5]enkephalin (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). [d] pA_2 values taken from ref 24; no K_e values were given in the reference. [e] Data taken from ref 23. [f] The pA_2 value could not be determined because this compound is a noncompetitive antagonist at the δ opioid site.

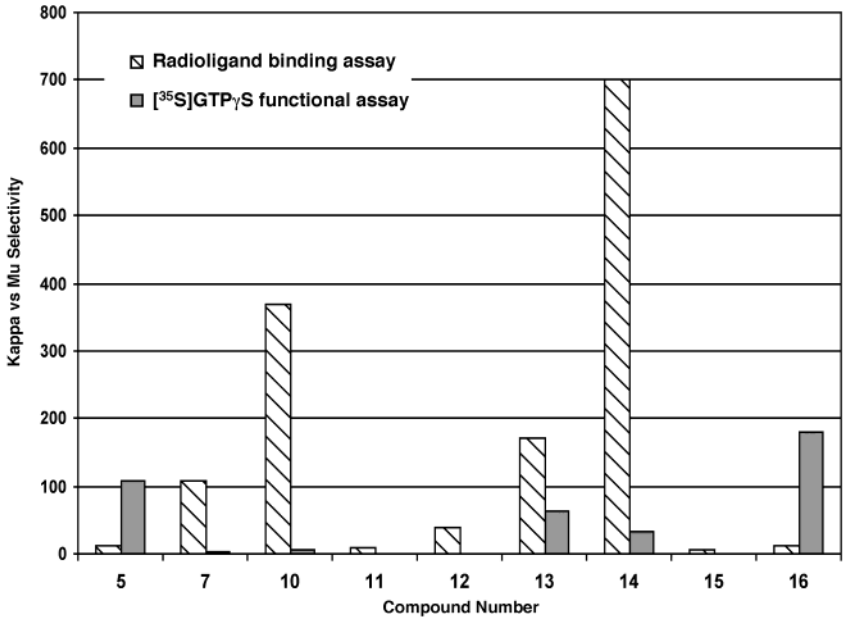


Figure 1. Comparison of the κ to μ ratio for radioligand binding and [^{35}S]GTPγS assays.

addition of pendant amino groups to **7** might be expected to have the same κ directing effect. However, the data for compounds **11** and **12** in Table 2, both of which possess pendant amino groups, clearly show that this modification does not produce κ selective compounds. In fact, at the μ and δ receptors there is little change in behavior for these amine-bearing ligands relative to **7**, and with respect to κ receptor potency, compounds **11** and **12** were found to be less potent than **7**. On the whole, the net affect of this change provided a decrease in κ antagonist activity and leads to the conclusion that the amino group alone is insufficient to promote κ receptor selectivity in the absence of a methylene bridge. Viewed from a different perspective, one must consider compounds **11** and **12** as analogues of compound **5**, which lack this methylene bridge. The 2230- and 1500-fold losses in κ antagonist potency displayed by **11** and **12** relative to **5** clearly highlight the importance of this conformationally constraining methylene bridge.

In a previous report, it was established that the pendant phenol group in the structure of **7** is a requirement for its κ binding affinity.[14] This suggested that the corresponding phenol group in **5** might also be required for the expression of its κ antagonist activity. The data for compound **10** (Table 2), which differs from **5** by the absence of this phenol group, support such an assertion. In fact, this structural change impacts activity at all of the opioid receptors. At the μ receptor, the loss of this hydroxyl group provides a 32-fold decrease in potency (K_e = 68.6 nM for **10** versus 2.16 nM for **5**), while at

the δ receptor the same change results in an improvement in potency. The most dramatic change, however, is seen in the κ receptor assay where **10** shows a 575-fold loss of antagonist potency relative to **5**. Clearly, the phenol group plays an integral part in the action of this compound at the κ receptor. More importantly, this is the first example of a pendant phenol group showing such a profound effect upon the potency of a κ opioid receptor antagonist.

Compound **9** was prepared to examine the importance of the configuration of the carbon adjacent to the amino group in **5**. Opioid receptors have been shown to distinguish between enantiomers as well as changes in a single stereocenter. The data for compound **9** indicate that the stereocenter of interest is also critical to κ receptor potency and selectivity but has little affect on μ or δ activity. Overall, **9** is not a selective κ compound, and this change in behavior is driven primarily by the 210-fold loss of potency of this compound for the κ receptor relative to **5**. Taken together, it is clear that this stereocenter is a key structural determinant of the selectivity and potency displayed by compound **5**.

Reduction of the amide carbonyl in compound **5**, giving **13**, provided a means of probing the contribution of this functional group to the overall activity of **5**. The data obtained from **13** indicated that the loss of this group does not impact the potency or selectivity of the resulting compound to the same degree as that seen in other modifications. For example, compared to compound **5**, **13** shows only a 10-fold loss of potency for the κ receptor, maintains about the same affinity for the δ receptor, and loses about 4-fold activity in the μ receptor assay. On the whole, the change in this functional group had little overall impact on antagonist activity.

Compounds **14**−**16** were produced to examine the requirements of basicity of the pendant amino group of **5** as well as to uncover a potential hydrogen-bonding interaction or size constraints. Substitution of the amine hydrogen for methyl to give compound **14** provided an 18-fold loss of potency at the κ receptor and a 6-fold decrease in potency for the μ opioid receptor. The activity in the δ assay remained unchanged. Together, these shifts in potency combine to produce a less selective κ antagonist, 34-fold for **14** versus 108-fold for **5**, and suggest that a hydrogen-bonding interaction could contribute to the activity of compound **5**.

Acylation of **5** to give compound **15** effectively removes the pendant basic center that has historically been a requirement for κ antagonist selectivity. As the data from Table 2 show, compound **15** fits the pattern established for earlier κ compounds because removal of its basic center affords a 980-fold loss of κ potency. To demonstrate that the loss of activity was not the result of a steric interaction between receptor and binding site, compound **16** was prepared that incorporates the acyl group from **15** but that also contains a basic amino group. The addition of this basic center to **15** affords a dramatic 122-fold increase in potency relative to **15** and effectively restores most of the κ antagonist potency lost by the acylation. Interestingly, this modification of **5** produces a significant decrease in μ antagonist potency that, coupled with the subnanomolar activity of **16** at the κ receptor, results in a compound with improved μ versus κ selectivity compared to that found in compound **5** (181-fold versus 108-fold, respectively).

In the initial investigation of the N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine antagonists, it was found that the relative position of the 3,4-dimethyl substituents on the piperidine ring affected the overall selectivity and potency but not the agonist versus antagonist activity of the resulting compounds.[22] To establish a link with this historical information, the diastereomer of **5**, compound **17**, which has a 3*S*,4*S* versus the 3*R*,4*R* configuration for the 3,4-dimethyl groups found in **5**, was prepared and tested. A comparison of the data for compounds **5** and **17** in Table 2 clearly shows that the stereochemistry of these methyl groups contributes substantially to the observed potency and selectivity of compound **5**. In the κ receptor assay, the shift of the 3-methyl group from one side of the piperidine ring to the other resulted in an 835-fold loss of antagonist potency. On the whole, the behavior observed for these two compounds is congruent with historical precedent because the placement of the methyl groups does impact overall activity but the magnitude of the observed difference for these diastereomers is far greater than any other pair yet studied in this series. Importantly, however, while compound **17** was found to lack the selectivity and potency of **5**, it still retains full antagonist character because it showed no agonist properties up to 10 μM (data not shown). Taken together, this information suggests that the methyl group in the piperidine 3-position of compound **5** could be interacting directly with the κ receptor in a highly specific manner or that the placement of the methyl group in **17** is such that it makes the "κ critical" conformation energetically unavailable.

Compounds JDTic (**5**), **10**, **13**, **14**, and **16** were also evaluated in radioligand binding and [^{35}S]GTPγS assays using cloned human μ, δ, and κ opioid receptors transfected into Chinese hamster ovary (CHO) cells and compared to reported values for nor-BNI (**3**) and GNTI (**23**).[17,23,24] The results are listed in Tables 3 and 4. In general, we observed a similar pattern of results in both assay systems. Interestingly, some compounds were more potent at cloned receptors than native receptors. As noted above, there were at times striking differences in potency between the binding K_i value and the functional K_e value. For example, JDTic (**5**) and nor-BNI (**3**) possessed K_e values of 0.01 and 0.04 nM, respectively, at the κ receptor and showed a 40- and 20-fold increase, respectively, in the [^{35}S]GTPγS assay relative to the radioligand binding assay. The K_i (radioligand binding) to K_e ([^{35}S]GTPγS binding) ratio was very small for both compounds at the μ and δ receptors. This is represented graphically in Figure 2.

Unlike JDTic, GNTI with K_i values of 36.9, 70, and 0.18 nM at the μ, δ, and κ receptor was selective for the κ receptor in the radioligand binding assays. However, the pA_2 values of 8.49 and 10.40 for the μ and κ receptor, respectively, in the [^{35}S]GTPγS test are almost identical to the pA_2 values of 8.50 and 10.46 at the μ and κ receptors found for JDTic. Since JDTic is a noncompetitive antagonist at the δ receptor, a comparison of pA_2 values was not possible. The 79.3 nM K_e values for JDTic and 7.81 pA_2 for GNTI show that neither compound has significant potency at the δ receptor.

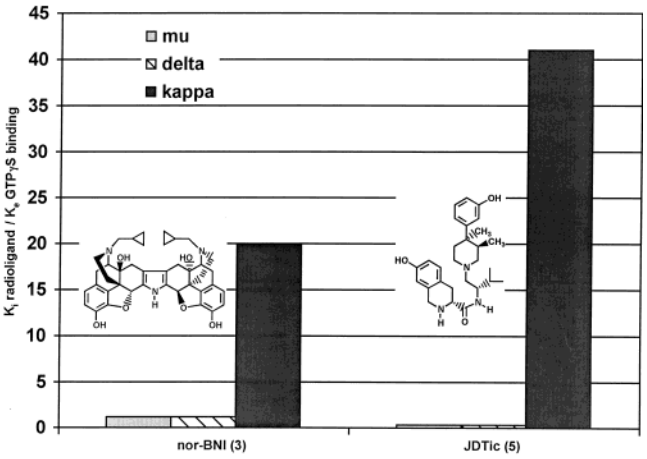
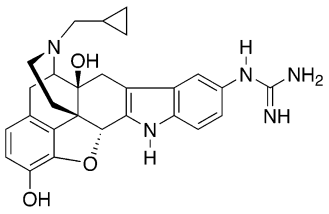
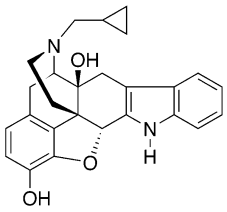


Figure 2. Comparison of ratios of radioligand binding and GTPγS assays for JDTic (**5**) and nor-BNI (**3**) using cloned human opioid receptors.

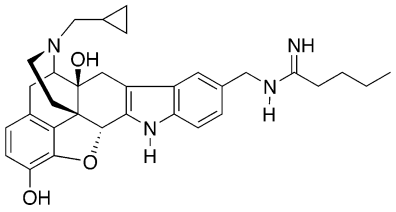
Chart 2



23, GNTI, κ-selective antagonist



24, NTI, δ-selective antaognist



25, κ-selective antagonist

Portoghese has explained the selectivity of opioid ligands in terms of the "message−address" concept of Schwyzer,[25] wherein the "message" component of the ligand specifies primary receptor recognition and the "address" portion confers selectivity by specific recognition of a particular receptor subsite.[26] Portoghese provided an elegant demonstration of the "message−address" concept by showing that the selectivity of naltrindole (NTI, **24**) could be changed from a δ- to a κ-selective ligand by attaching a methyleneamidino group to its 5′ position to give 5′-[(*N*2-butylamidino)-methyl]naltrindole (**25**) (Chart 2).[21] Further refinements of this concept, targeting the distance between the amino address group and the naltrexone message fragment in **25**, led to the more potent and selective κ antagonist, C5′-guanidinylnaltrindole (GNTI, **23**).[27] Taken together, the studies in the naltrexone-derived series clearly illustrated that the second basic amino group in these compounds is critical to their κ selectivity and that the second phenolic hydroxyl group in nor-BNI did not have to be present. The present SAR study of JDTic (**5**) revealed that this structurally unique κ antagonist also requires a second basic amino group to express κ potency and in this sense behaves similarly to previously identified naltrexone-based κ antagonists such as nor-BNI (**3**). In the case of JDTic, the *trans*-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine could be viewed as the "message" and some portion of the N substituent as the "address". If one assumes that this "message−address" is as suggested, the JDTic studies reveal that the κ potency is due to at least two structural features in the N substituent "address" moiety. The first involves the interaction of the N−H moiety of the D-hydroxy Tic group, possibly via a charged interaction with Glu297 in the κ receptor similar to the interaction found for N′-17 group for nor-BNI and the guanidino amino group in GNTI. Equally important is the interaction of the phenol moiety in the D-hydroxy Tic group with the κ receptor. This interaction is unique to JDTic and opens the possibility that site-directed mutagenesis studies might identify the complementary residues in the κ receptor, leading to further characterization of the κ receptor. The κ selectivity of JDTic is due to the combination of high κ affinity resulting from these two D-hydroxy Tic interactions plus possible steric hindrance of these groups at the address-recognition locus present in the μ and δ opioid receptors.

Conclusions

The discovery of the potent and selective κ opioid receptor antagonist (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic, **5**) represents a significant advancement in the development of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonist. To our knowledge, JDTic is the only potent and selective κ opioid receptor antagonist not derived from the opiate class of compounds. On the basis of historical precedent, we believe the potent and pure opioid antagonist activity results from the (3*R*,4*R*)-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine core structure. An SAR study of JDTic analogues suggests that the κ opioid receptor potency and selectivity result from a combination of (a) the isoquinoline amino group and 7-hydroxy group held in a rigid orientation by the 1,2,3,4 tetrahydroisoquinoline structure in its 3*R* attachment to the amide carboxyl, (b) an *S* configuration of the 2-methylpropyl group in the spacer between the piperidine ring and the D-hydroxy Tic acyl group, and (c) the lack of a substituent on the amide nitrogen. The unique structure of JDTic provides an additional pharmacological tool to further characterize the κ opioid receptor. Using site-directed mutagenesis studies, Portoghese et al. have shown that Glu297 in the κ receptor is critical to the κ selectivity shown by nor-BNI and GNTI.[28] It will be particularly interesting to determine if Glu297 is also critical for the κ selectivity of JDTic. In addition, since the 7-hydroxy (phenolic) group is important to the κ selectivity of JDTic, it will be of interest to see if site-directed mutagenesis studies can identify the amino acid residue in the κ receptor responsible for this interaction. It will

be of great interest to determine if the potent and κ opioid receptor properties of JDTic seen in the in vitro binding and functional assay translate to in vivo tests.

Experimental Section

^{1}H NMR were determined on a Bruker WM-250 or a Bruker 300 spectrometer using tetramethylsilane as an internal standard. Mass spectral data were obtained using a Finnegan LCQ electrospray mass spectrometer in positive ion mode at atmospheric pressure. 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. Methylene chloride and chloroform were distilled from calcium hydride if used as reaction solvents. HCl in dry ethyl ether was purchased from Aldrich Chemical Co. and used while fresh before discoloration.

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]GTPγS (SA = 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 C. Rice, Ph.D., NIDDK, NIH (Bethesda, MD). GTPγS and GDP were obtained from Sigma Chemical Company (St. Louis, MO). Boc-D- and Boc-L-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid were purchased from CSPS Pharmaceuticals, Inc. (San Diego, CA). The sources of other reagents are published.

General Amide Coupling Procedure. The appropriate Boc-protected carboxylic acid derivative (1.2 equiv) was added to a solution of the appropriate amine intermediate (1 equiv) in dry THF (0.5 mL/mg) followed by BOP reagent (1.2 equiv) and triethylamine (5 equiv). The reaction mixture was stirred for 2 h at room temperature, and then ether was added (2 mL/mL THF) and the mixture was washed with saturated $NaHCO_3$ and then water. The organic layer was collected and dried over magnesium sulfate, and the solvent was removed under reduced pressure.

General Boc-Deprotection Procedure. The material obtained from the coupling reaction was dissolved in dry CH_2Cl_2 (50 mL/g of Boc intermediate) and cooled to −20 °C whereupon TFA (27 mL/g of Boc intermediate) was added dropwise. The reaction flask was left in a MeOH/ice bath for 10 min and then was allowed to warm to room temperature. The solvent was removed under reduced pressure, and the residue diluted with CH_2Cl_2. To this was added saturated $NaHCO_3$. The organic layer was separated, and the solvent was removed under reduced pressure. The material obtained from the coupling reactions was purified using flash column chromatography, if necessary, and was then converted to its HCl salt by dissolving in methanol at 0 °C followed by addition of 1 equiv of 1 N HCl in ethyl ether.

(3*R*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (5). 5 was prepared according to the general procedures starting from Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and 3-[1-(2*S*-amino-3-methylbutyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol. The impure product was purified by silica gel preparative plate chromatography (50% ($CHCl_3$/MeOH/NH_4-OH, 80:18:2) in $CHCl_3$ to give **5** as a white solid that was converted to its HCl salt via the general procedures. ^{1}H NMR (CD_3OD): δ 7.11 (t, 1H, J = 7.9 Hz), 6.92 (d, 1H, J = 8.3 Hz), 6.74 (m, 2H), 6.59 (m, 2H), 6.50 (m, 1H), 4.03 (m, 1H), 3.94 (d, 2H, J = 5.9 Hz), 3.54 (dd, 1H, J = 4.8, 10.2 Hz), 2.94 (dd, 1H, J = 4.7, 15.7 Hz), 2.80 (m, 2H), 2.67−2.37 (m, 5H), 2.27 (dt, 1H, J = 4.2, 12.6 Hz), 1.99−1.85 (m, 2H), 1.57 (d, 1H, J = 12.7 Hz), 1.30 (s, 3H), 0.95 (m, 6H), 0.74 (d, 3H, J = 6.7 Hz). Anal. ($C_{28}H_{39}N_3O_3$·2HCl·H_2O) C, H, N.

(2*R*)-Amino-3-(4-hydroxyphenyl)-*N*-{1-[4-(3-hydroxyphenyl)-3*R*,4*R*-dimethyl-piperidin-1-ylmethyl]-2-methyl-1-propyl}propanamide (11). 11 was prepared according to the general procedures from Boc-D-tyrosine and 3-[1-(2*S*-amino-3-methylbutyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol (**8**, 1 equiv).[14] The impure product was purified by flash chromatography using 67% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ to afford **11** (99%) as a white foam. ^{1}H NMR (CD_3OD): δ 7.02 (m, 3H), 6.71 (m, 4H), 6.55 (d, 1H, J = 7.8 Hz), 3.91 (m, 1H), 3.50 (t, 1H, J = 7.1 Hz), 2.90−2.21 (m, 9H), 1.97 (br, 1H), 1.74 (m, 1H), 1.56 (d, 1H, J = 12.6 Hz), 1.28 (s, 3H), 0.74 (m, 9H). ^{13}C NMR (CD_3OD): δ 176.8, 158.6, 157.7, 153.5, 131.8, 130.4, 129.9, 118.3, 116.7, 114.1, 113.6, 61.4, 58.3, 57.2, 52.7, 52.3, 41.9, 40.5, 39.8, 32.2, 32.1, 28.4, 20.0, 18.2, 17.0. Anal. ($C_{27}H_{39}N_3O_3$) C, H, N.

(2*S*)-Amino-3-(4-hydroxyphenyl)-*N*-{1-[4-(3-hydroxyphenyl)-3*R*,4*R*-dimethylpiperidin-1-ylmethyl]-2-methyl-1-propyl}propanamide (12). 12 was prepared according to the general procedures from Boc-L-tyrosine and 3-[1-(2*S*-amino-3-methylbutyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol (**8**, 1 equiv).[14] The crude was purified by flash chromatography using 67% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ to afford **12** (94%) as a white foam. ^{1}H NMR (CD_3OD): δ 6.94 (m, 3H), 6.60 (m, 4H), 6.45 (d, 1H, J = 8.0 Hz), 3.80 (m, 1H), 3.36 (m, 1H), 2.81 (m, 1H), 2.66 (m, 1H), 2.57−2.07 (m, 7H), 1.84 (br, 1H), 1.73 (m, 1H), 1.43 (d, 1H, J = 12.7 Hz), 1.16 (s, 3H), 0.76 (m, 6H), 0.6 (d, 3H, J = 6.9 Hz). ^{13}C NMR (CD_3OD): δ 176.3, 158.1, 157.3, 153.2, 131.4, 130.0, 129.4, 117.9, 116.3, 113.7, 113.1, 61.0, 57.8, 56.8, 52.4, 51.7, 41.6, 40.1, 39.3, 31.7, 28.0, 19.7, 17.8, 16.6. Anal. ($C_{27}H_{39}N_3O_3$) C, H, N.

(3*R*)-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-Hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (10). 10 was prepared according to the general procedures from Boc-D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and 3-[1-(2*S*-amino-3-methylbutyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol (**8**, 1 equiv).[14] The crude product was purified by silica preparative plate chromatography 50% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$, yielding **10** as a white foam. ^{1}H NMR ($CDCl_3$) 7.22 (d, J = 8.8 Hz, 1H), 7.05−7.13 (m, 3H), 6.98−7.01 (m, 1H), 6.79 (s, 1H), 6.73 (d, J = 7.8 Hz, 1H), 6.65 (dd, J = 7.9, 1.8 Hz, 1H), 4.05−4.14 (m, 1H), 3.99 (s, 2H), 3.56 (dd, J = 10.7, 4.9 Hz, 1H), 3.18 (dd, J = 16.5, 4.8 Hz, 1H), 2.67−2.82 (m, 3H), 2.31−2.54 (m, 4H), 2.18−2.25 (m, 1H), 1.86−1.96 (m, 2H), 1.52 (d, J = 12.9 Hz, 1H), 1.25 (s, 3H), 0.92 (t, J = 7.6 Hz, 6H), 0.67 (d, J = 6.9 Hz, 3H). Anal. ($C_{28}H_{39}N_3O_2$) C, H, N.

(3*S*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (9). 9 was prepared according to the general procedures starting from Boc-L-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and 3-[1-(2*S*-amino-3-methylbutyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol (**8**, 1 equiv).[14] The crude product was purified using silica gel column chromatography gradient elution starting with neat $CHCl_3$ and moving to 50% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ to give **9** as an off-white solid. ^{1}H NMR (CD_3-OD): δ 7.09 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 4.1 Hz, 1H), 6.77 (s, 1H), 6.74 (d, J = 0.8 Hz, 1H), 6.58 (dd, J = 8.3, 2.3 Hz, 2H), 6.48 (d, J = 2.3 Hz, 1h), 4.01−3.95 (m, 1H), 3.90 (s, 2H), 3.51−3.48 (m, 1H), 3.35 (s, 3H), 2.94−2.21 (m, 9H), 1.96 (d, J = 13 Hz, 1H), 1.28 (s, 3H), 0.89 (t, 7.2 Hz, 6H), 0.74 (d, J = 6.9 Hz, 3H). Anal. ($C_{28}H_{39}N_3O_3$) C, H, N.

(3*R*)-3-{[((1*S*)-1-{[(3*R*,4*R*)-4-(3-Hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)amino]-methyl}-1,2,3,4-tetrahydro-7-isoquinolinol (13). A solution of 2 M BH_3·SMe_2 in THF (0.495 mL, 0.99 mmol) was added dropwise to a −20 °C solution of (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (**3**, 46 mg, 0.099 mmol) in 5 mL of dry THF.[13] The reaction mixture was heated to reflux overnight and was cooled to −20 °C, and 0.647 mL of methanol was added. The contents were stirred at room temperature for 1 h, 1 M HCl in ether (0.142 mL, 0.142 mmol) was added, and

the mixture was stirred for 30 min. The solvent was then removed under reduced pressure, the oil obtained was dissolved in 3:1 CH_2Cl_2/THF, and the pH was adjusted to 10 with saturated $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted 5× with 3:1 CH_2Cl_2/THF. The combined organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure to give a yellow foam. The crude product (0.03 g) was purified by silica gel preparative plate chromatography using a solvent gradient, starting with 25% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ and then 40% and finally 65%. This afforded 6 mg of **13**. ^{1}H NMR (CD_3OD): δ 7.12 (t, J = 5.1 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.8 (d, J = 3.9 Hz, 1H), 6.76 (d, J = 1.1 Hz, 1h), 6.61 (dd, J = 5.8, 2.2 Hz, 2H), 6.48 (d, J = 1.2 Hz, 1H), 2.88−2.43 (m, 16H), 2.4−1.8 (m, 2H), 1.67 (d, J = 13 Hz, 1H), 1.34 (s, 3H), 1.01 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H), 0.81 (d, J = 3.4 Hz, 3H). Anal. ($C_{28}H_{41}N_3O_2$) C, H, N.

(3*S*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-2-methyl-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (14). Formalin (0.02 mL, 0.215 mmol) was added to a stirring solution of (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (**3**, 100 mg, 0.215 mmol) dissolved in 5 mL of dry DCE.[13] To this mixture was added $Na(OAc)_3BH$ (205 mg, 0.97 mmol). The reaction mixture was stirred at room temperature for 1.5 h, and then the reaction was quenched by the addition of saturated $NaHCO_3$ until the bubbling subsided. The reaction mixture was extracted three times with a solution of 3:1 CH_2Cl_2/THF and the residue was purified using silica gel preparative plate chromatography in 60% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ to give pure **14** as a white solid. ^{1}H NMR (CD_3OD): δ 7.09 (t, J = 3.8 Hz, 1H), 6.9 (d, J = 4.5 Hz, 1H), 6.76 (s, 1H), 6.73 (d, J = 1.5 Hz, 1H), 6.57 (dd, J = 9, 5.3 Hz, 2H), 6.51 (s, 1H), 3.98−3.83 (m, 3H), 3.80 (s, 2H), 3.47 (d, J = 16 Hz, 1H), 3.31 (s, 1H), 3.13−2.44 (m, 11H), 2.37 (t, J = 18 Hz, 1H), 1.27 (s, 3H), 0.90 (t, J = 3 Hz, 6H), 0.71 (d, J = 3 Hz, 3H). Anal. ($C_{29}H_{41}N_3O_3$) C, H, N.

(3*R*)-2-Acetyl-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (15). 15 was prepared according to the general procedures starting from (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (**3**)[13] and acetic acid. The crude product was purified using silica gel preparative plate chromatography in 45% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$. ^{1}H NMR (CD_3OD): δ 7.68 (t, J = 1.5 Hz, 1H), 7.10 (d, J = 3 Hz, 1H), 6.99 (d, J = 3 Hz, 2H), 6.69 (dd, J = 13.5, 6 Hz, 2H), 6.61 (d, J = 1.5 Hz, 1H), 4.73 (m, 1H), 4.63 (s, 2H), 3.36−2.56 (m, 8H), 2.46 (t, J = 9 Hz, 1H), 2.31−1.49 (m, 9H), 1.29 (s, 3H), 0.73 (t, J = 2.3 Hz, 6H), 0.68 (d, J = 6 Hz, 3H). Anal. ($C_{30}H_{41}N_3O_4$) C, H, N.

(3*R*)-2-(*N*,*N*-Dimethylglycyl)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]-methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (16). 16 was prepared according to the general procedures starting from (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]-methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (**3**) and *N*,*N*-dimethylglycine. The crude product was purified by flash chromatography using 50% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ to afford **16** as a white foam. 7.09 (t, J = 7.91 Hz, 1H), 6.96 (d, J = 8.29 Hz, 1H), 6.72 (m, 2H), 6.63 (m, 1H), 6.57 (m, 2H), 4.83−4.33 (m, 4H), 3.82 (m, 1H), 3.37 (m, 1H), 3.28 (m, 1H), 3.14 (m, 1H), 3.00 (m, 1H), 2.74−2.17 (m, 12H), 1.91 (m, 1H), 1.69 (m, 1H), 1.51 (m, 1H), 1.24 (m, 3H), 0.78 (d, J = 6.8 Hz, 3H), 0.69 (m, 6H). Anal. ($C_{32}H_{46}N_4O_4$) C, H, N.

(3*R*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*S*,4*S*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (3*S*,4*S*)-Dimethyl-4-(3-hydroxyphenyl)piperidine (17). The (3*S*,4*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**18**) used in the synthesis of **17** was prepared according to the method of Werner et al. with a single modification.[15] The intermediate tartrate salt (**20**) was recrystallized twice rather than once from ethanol. The (3*S*,4*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**18**) thus obtained was found to be identical in all respects to an authentic sample of **18**. The 3-[1-(2*S*-amino-3-methylbutyl)-3*S*,4*S*-dimethyl-4-piperidinyl]phenol (**22**) illustrated in Scheme 3 was prepared from **18** and Boc-L-valine according to the method described for the synthesis of **8** in Scheme 1.[14] The impure product was purified via silica gel flash chromatography using a gradient of 0−10% MeOH in $CHCl_3$ to afford **22** as a yellow-white foam. ^{1}H NMR ($CDCl_3$): δ 7.13 (t, J = 7.9 Hz, 1H), 6.76 (d, J = 7.9 Hz, 1H), 6.69 (s, 1H), 6.64 (dd, J = 7.9 Hz, 1H), 4.25 (br s, 3H), 2.89 (d, J = 5.1 Hz, 1H), 2.79 (dd, J = 11.2, 2.5 Hz, 1H), 2.72−2.75 (m, 1H), 2.42 (d, J = 11.2 Hz, 1H), 2.36 (dd, J = 12.4, 2.9 Hz, 1H), 2.22 (d, J = 8.4 Hz, 2H), 1.98 (d, J = 6.5 Hz, 1H), 1.61−1.72 (m, 1H), 1.56 (d, J = 9.7 Hz, 1H), 1.28 (s, 3H), 0.96 (d, 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 0.79 (d, J = 6.9 Hz, 3H). The final product (**17**) was obtained according to the general procedures starting from **22** and Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. The product was purified by silica gel preparative plate chromatography using 60% ($CHCl_3$/MeOH/NH_4OH, 80:18:2) in $CHCl_3$ to give **17** as a white solid. ^{1}H NMR (CD_3OD): δ 7.09 (t, J = 7.9 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.77 (s, 1H), 6.73 (d, J = 2.3 Hz, 1H), 6.56−6.61 (m, 2H), 6.49 (d, J = 2.3 Hz, 1H), 3.96−4.03 (m, 1H), 3.92 (d, J = 6.5 Hz, 2H), 3.56 (dd, J = 10.0, 4.9 Hz, 1H), 2.93 (dd, J = 15.7, 4.9 Hz, 1H), 2.83 (d, J = 9.9 Hz, 2H), 2.52−2.66 (m, 2H), 2.18−2.48 (m, 5H), 1.86−1.97 (m, 2H), 1.53 (d, J = 12.5 Hz, 1H), 1.27 (s, 3H), 0.92 (t, J = 6.6 Hz, 6H), 0.76 (d, J = 7.0 Hz, 3H). Anal. ($C_{28}H_{39}N_3O_3$) C, H, N.

Pharmacological Methods. The biological methods followed published procedures.[14,29] The sources of reagents are also descrbied in these papers. For [^{35}S]GTPγS binding assays, guinea pig caudate nuclei (from 10 brains) were homogenized in 30 mL of ice-cold buffer containing 50 mM Tris, pH 7.4, 4 μg/mL leupeptin, 10 μg/mL bestatin, 100 μg/mL bacitracin, and 2 μg/mL chymostatin. The homogenate was centrifuged at 30000*g* and 4 °C for 10 min and then was washed by repeated centrifugation and resuspension in buffer two times. After the final wash, the pellets were resuspended in 50 mL of homogenization buffer. The suspension was divided into 1 mL aliquots and centrifuged. Pellets were stored at −80 °C until use.

[^{35}S]GTPγS Binding Assay. The [^{35}S]GTPγS binding assay proceeded as described elsewhere.[14,29] Guinea pig caudate membranes (10−20 μg protein in 300 μL of 50 mM Tris-HCl, pH 7.4, with 1.67 mM DTT and 0.15% BSA) were added to either polystyrene 96-well plates or 12 mm × 75 mm polystyrene test tubes, filled with 200 μL of a reaction buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 100 μM GDP, 0.1% BSA, 0.05−0.01 nM [^{35}S]GTPγS, and varying concentrations of drugs. The reaction mixture was incubated for 3 h at 25 °C (equilibrium). The reaction was terminated by the addition of 0.5 mL of ice-cold Tris-HCl, pH 7.4 (4 °C), followed by rapid vacuum filtration through Whatman GF/B filters previously soaked in ice-cold Tris-HCl, pH 7.4 (4 °C). The filters were washed twice with 0.5 mL of ice-cold distilled H_2O (4 °C). Bound radioactivity was counted at an efficiency of 98% by liquid scintillation spectroscopy. Nonspecific binding was determined in the presence of 10 μM GTPγS.

Opioid Binding Assays. Opioid binding assays proceeded according to published procedures.[14,30] The μ receptors were labeled with [^{3}H]DAMGO. Rat membranes for μ and δ receptor binding assays were prepared each day using a partially thawed frozen rat brain that was homogenized with a Polytron in 10 mL/brain of ice-cold 10 mM Tris-HCl, pH 7.0. Membranes were then centrifuged twice at 30000*g* for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (60 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl,

pH 7.4, along with a protease inhibitor cocktail (PIC). The nonspecific binding was determined using 20 μM of levallorphan. The δ binding sites were labeled using [^{3}H]DADLE (2 nM) and rat brain membranes. Rat membranes were prepared each day as described above. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (50 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, 100 nM DAMGO to block binding to μ sites, and PIC. Nonspecific binding was determined using 20 μM levallorphan. The κ binding sites were labeled using [^{3}H]-U69,593 (2 nM). Guinea pig brain membranes were prepared each day using partially thawed guinea pig brain, which was homogenized with a Polytron in 15 mL/brain of ice-cold 10 mM Tris-HCl, pH 7.0. The membranes were then centrifuged twice at 30000*g* for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (85 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing 1 μg/mL of captopril and PIC. Nonspecific binding was determined using 1 μM U69,593. Each radioligand was displaced by 8−10 concentrations of test drug. Compounds were prepared as 1 mM solution with 10 mM Tris buffer (pH 7.4) containing 10% DMSO before drug dilution. All drug dilutions were done in 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL bovine serum albumin. All washes were done with ice-cold 10 mM Tris-HCl, pH 7.4. The IC_{50} and slope factor (N) were obtained by using the program MLAB−PC (Civilized Software, Bethesda, MD). K_i values were calculated according to the equation $K_i = IC_{50}/(1 + [L]/K_d)$.

Data Analysis. The data of the two separate experiments (opioid binding assays) or three experiments ([^{35}S]GTPγS assay) were pooled and fit, using the nonlinear least-squares curve-fitting language MLAB-PC (Civilized Software).

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