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Articles

Structure–Activity Relationships of *trans*-3,4-Dimethyl-4-(3-hydroxyphenyl)piperidine Antagonists for μ- and κ-Opioid Receptors

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A series of racemic N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines were evaluated for opioid agonist and antagonist activity at μ and κ receptors. Several highly potent μ and κ antagonists were discovered; however, no compounds with high selectivity for either the μ or κ receptor were identified. Importantly, no derivative was found to have significant opioid agonist activity. Two derivatives were resolved, and the activities of the enantiomers were investigated. Only a limited stereochemical effect on opioid receptor selectivities was observed. The structure–activity relationships described establish the existence of an important lipophilic binding site distal to the nitrogen for both μ and κ receptors and confirm the pure opioid antagonist pharmacophore nature of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine structure.

Previously we described the discovery of opioid antagonist activity in a series of *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines[1,2] (1, Figure 1). These 4-phenylpiperidine antagonists were structurally unique, since prior to their discovery, opioid antagonists were generally *N*-allyl or *N*-methylcyclopropyl analogs of morphine and morphine-like agonists derived from the morphine structure (e.g., in 4,5-epoxymorphinan-6-one,[3] morphinan,[4] benzomorphan,[5] and isoquinoline series[6]). Unlike these polycyclic structures, the antagonist activity in the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines was shown to be a consequence of substitution at the 3 position of the piperidine ring rather than substitution at the nitrogen. Compound **2** (the *N*-methyl derivative, Figure 1), has antagonist potency comparable to that of nalorphine (the *N*-allyl analog of morphine), but is without opioid agonist effects. In contrast, the des-3-methyl analog **10** is a morphine-like agonist.[1,7] Antagonist potency in the *trans*-3,4-dimethyl-4-phenylpiperidines is not altered by *N*-allyl or *N*-methylcyclopropyl substitution (e.g., **3** and **4**) but is increased in a stepwise manner from *N*-methyl to β-phenethyl (**5**), 2-(phenylcarbonyl)ethyl (**6**), and 3-hydroxy-3-phenylpropyl (**7**, LY117413). Resolution of the 2-(phenylcarbonyl)ethyl (**6**) provided only partial separation of activity as both are opiate antagonists with the (+)-3*R*,4*R*-isomer **8** (Figure 2) being 2–6 times more potent than the (−)-3*S*,4*S*-isomer **9**. As with **2**, compounds **3**–**7**, including both enantiomers of **6**, have no measurable opioid agonist properties.

Prior to the discovery of compounds **2**–**7**, only two other pharmacologically pure opioid antagonists (antagonists devoid of any opioid agonists effects) had been well characterized, naloxone and naltrexone.[3] Even today, there are still relatively few pure opioid antagonists known, and the structural requirements for such activity appear to be quite precise.[8,9] In accordance with this, minor alterations of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)-piperidine structure often impart opioid agonist activity to the molecule. The *cis*-3,4-dimethyl analog (**11**, Figure 1) has mixed agonist–antagonist properties. Substitution of *n*-propyl for the 4-methyl group of **1** and **11** increases opioid agonist activity and led to the discovery of the opioid analgesic, picenadol, which has mixed opioid agonist and antagonist properties.[2,10]

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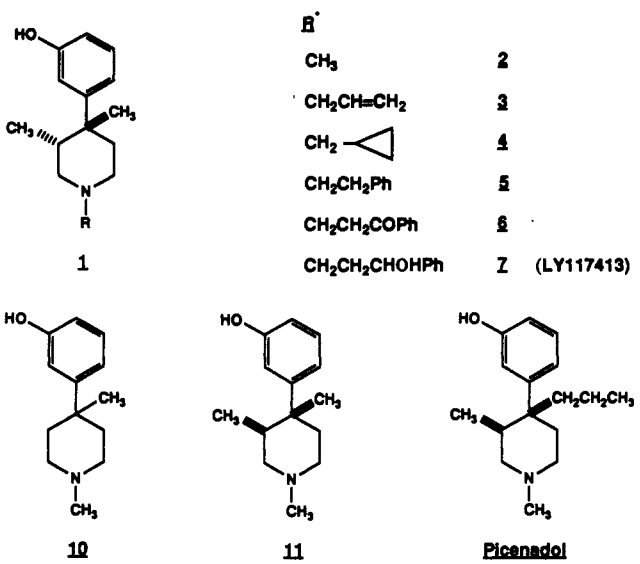


Figure 1. Compounds 2–7, 11, and picenadol are racemic mixtures. Compound 7 is also a diasteriomeric mixture.

Since the original disclosure of opioid antagonist activity in the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines, the understanding of opioid receptor mediated pharmacology has advanced considerably. Three opioid receptors have been characterized (μ, κ, and δ), endogenous ligands for these receptors have been identified and the existence of opioid receptor subtypes has been postulated.[11-17] These findings have led to the realization that opioid receptors are involved in a multitude of physiological processes and to suggestions of several new therapeutic uses for opioid receptor antagonists.[18] These findings have also demonstrated the need for selective opioid antagonists to further study this complex receptor system.

The 4-phenylpiperidine antagonists were originally identified as μ-receptor antagonists; however, κ-antagonist effects were later identified within this series.[19] Compounds 2 and 5–7 were also shown to have significant affinity for the δ receptor.[20] It was further determined that the (+) and (−)-isomers of 6 had similar relative activity for μ, κ, and δ receptors ($\mu > \kappa \cong \delta$ compounds 8 and 9, Table I). In this manuscript, we report the further characterization of the structure–activity relationships (SAR) within this opioid antagonist series. Substituents bound to nitrogen were varied systematically, and the effects at μ and κ receptors, both *in vitro* and *in vivo*, were evaluated. Because of ease of synthesis and the limited stereochemical effect observed with the enantiomers of 6, SAR comparisons were made on racemic mixtures and in a few cases on diasterameric mixtures. The intent was to further document the pure opioid antagonist nature of *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines and rapidly characterize effects which maximize activities for μ and κ receptors. Selected compounds with high potency for either μ and/or κ receptors were to be studied further, including separation and evaluation of individual stereoisomers and evaluation for δ-receptor activities.[21]

Chemistry

The synthesis of the 4-(3-methoxyphenyl)piperidine 16 has been recently described and is outlined in Scheme I.[22] The tetrahydropyridine 12,[23] was converted to the metalloenamine 13 and then alkylated regioselectively with methyl iodide giving 14. Metalloenamines such as 13 have proven to be highly versatile intermediates in the synthesis

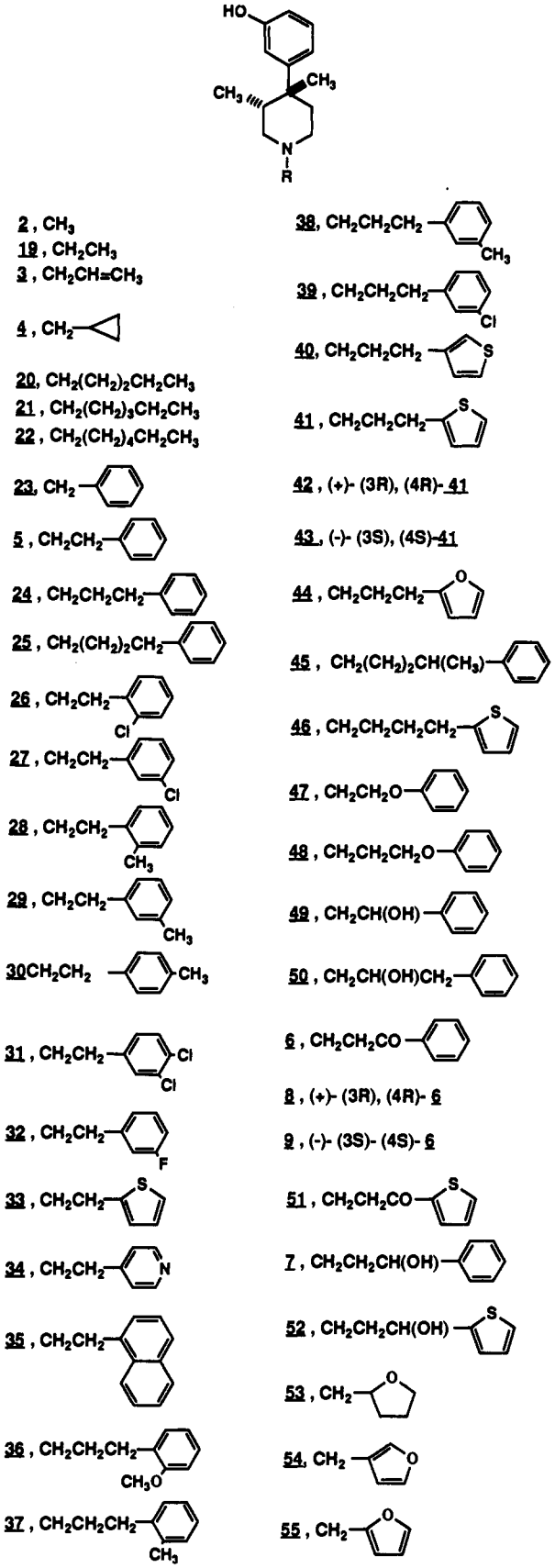
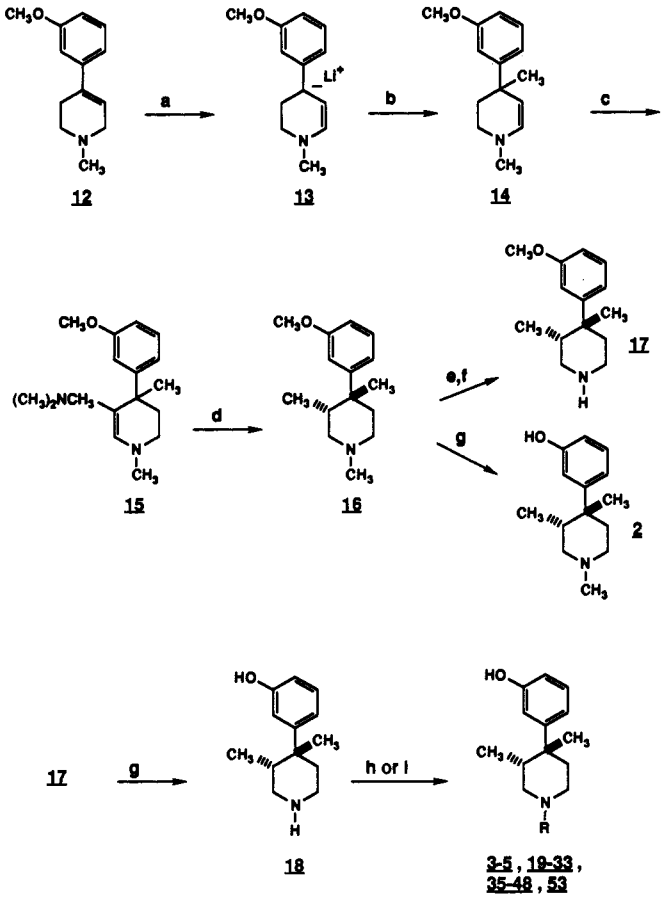


Figure 2. All compounds, unless otherwise indicated, are racemic mixtures. Compounds 7, 48, and 49 are also diasteriomeric mixtures.

of opioid related structures.[6,24-27] Treatment of 14 with dimethylamine and formaldehyde gave 15 in high yield[28] which was reduced with high stereoselectivity to the *trans*-3,4-dimethylarylpiperidine 16. N-Demethylation with

Scheme I[a]



[a] Reagents: (a) *n*-BuLi, THF; (b) CH_3I; (c) $(CH_3)_2NH$, CH_2O, H_2SO_4; (d) H_2, 5% Pt/C, EtOH; (e) $ClCOCH{=}CH_2$, proton sponge; (f) HCl, EtOH; (g) 48% HBr, HOAc; (h) RCOCl, Red-Al or $LiAlH_4$; (i) R-X, $NaCO_3$.

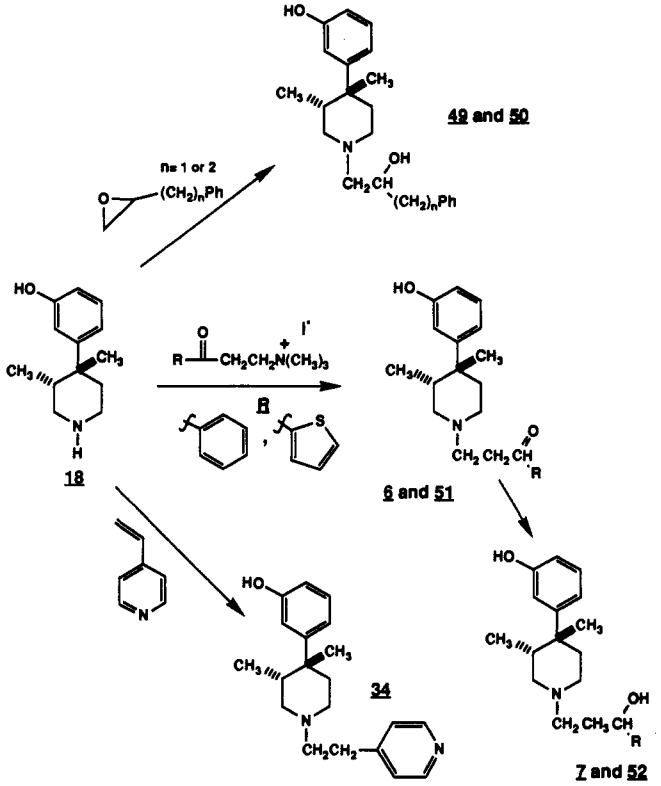
vinyl chloroformate[29] followed by O-demethylation with HBr in acetic acid afforded 18, while O-demethylation of 16 gave 2. The trans configuration of the 3,4-dimethyl groups was proven by analysis of ^1H and ^{13}C NMR spectra and this assignment was confirmed by X-ray crystallography.[30] Alkylation or acylation of 18 followed by reduction of the intermediate amide provided the N-substituted derivatives 3–5, 19–33, 35–48, and 53 (Scheme I and Figure 2). Alkylation of 18 with a 1-aryl-3-(dimethylamino)-1-propanone methiodide afforded 6 and 51 (Scheme II) which were subsequently reduced to give the 1-aryl-3-propanols 7 and 52. Alkylation of 18 with styrene oxide and 2,3-epoxy-1-phenylpropane gave 49 and 50 while reaction with 4-vinylpiperidine gave 34 (Scheme II and Figure 2). The *N*-furanylmethyl derivatives 54 and 55 were synthesized through reduction of the intermediate Schiff bases derived from 17 and 2 or 3-furaldehyde and subsequent O-demethylation (Scheme III and Figure 2).

The (+)-(3*R*,4*R*)- and (−)-(3*S*,4*S*)-isomers of 3,4-dimethyl-4-phenylpiperidine 16 were resolved by fractional crystallization of the dibenzoyl tartrate salts as previously described.[21] Their absolute configurations have been established by synthesis using Sharpless asymmetric expoxidation.[21,30] Compounds 8, 9, 42, and 43 were synthesized from 18 as described for 6 and 41.

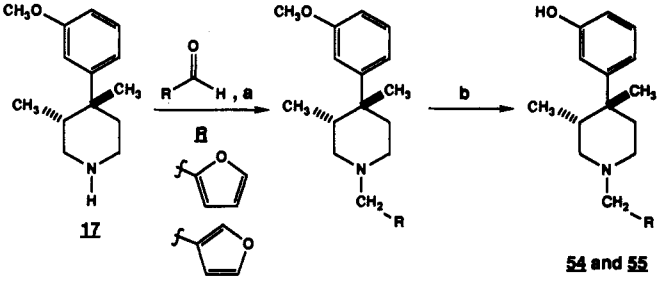
Biological Assays

Affinity for the μ-opioid receptor was determined by assaying a compound's ability to displace [^{3}H]naloxone

Scheme II



Scheme III[a]



[a] Reagents: (a) 5% Pd/C; (b) *n*-PrSH, Kt-BuO.

([^{3}H]NAL) binding from rat brain homogenates. Affinity for the κ receptor was determined by the ability of a compound to displace [^{3}H]ethylketocyclazocine ([^{3}H]-EKC) from guinea pig cortical tissue. In the κ-receptor binding assay, fentanyl and D-Ala^2-D-Leu^5-enkephalin (DADL) were added to inhibit binding of [^{3}H]EKC to μ and δ receptors. Affinity for the δ receptor was determined using [^{3}H]DADL with rat brain homogenates.[31]

Opioid antagonist activity was determined using the mouse writhing analgesic assay, measuring the test compound's ability to block morphine-induced (μ-receptor-mediated) and U50,488-induced (κ-receptor-mediated) analgesia.

Results

The results of this structure–activity relationship study are shown in Tables I and II. In accord with previous findings, compounds 2–7 antagonize morphine-induced analgesia, and the antagonist AD_{50} values obtained using the mouse writhing test correlate well to those obtained by other measures.[1] Comparison of the values in Tables I and II show that there appears to be a correlation between the ability of compounds 2–7 to block morphine-induced analgesia and affinity for the μ receptor. Compounds 2–7

Table I. Affinities of the 4-Phenylpiperidine Antagonists for the μ- and κ-Opioid Receptors

	% displacement					
	[³H]NAL[a] binding assay (μ receptor):			[³H]EKC[b] binding assay (κ receptor)		
compd	K_i (nM)[d]	10 nM	100 nM	K_i (nM)[d]	10 nM	100 nM
2	80			833		
3	16			313		
4	21					36
5	1.5			52		
6	5.4			208		
7	0.96			12.5		
8[e]	3.6			163		
9[e]	12			318		
19	52			18		
20	4.3				44	78
21	0.29			9.6		
22	0.62				57	87
23		65	89		57	82
24	0.69			15		
25	2.0				22	70
26	3.5				55	83
27	0.30				54	82
28		90	100	7.5		
29		83	100		35	82
30		52	95		13	61
31		50	90		26	58
32	3.2				60	93
33	5.3				34	72
34		54	99		20	51
35	1.1				22	77
36	0.95				53	80
37	0.62			8.9		
38	2.8				49	73
39		89	100		19	63
40	0.26			10		
41	0.56			6.1		
42	0.20			3.3		
43	1.8			13		
44	1.4			14		
45		63	100		41	78
46		23	69		20	72
47	1.3			57		
48	1.0			61		
49		48	97		34	70
50		88	100		42	77
51	1.5			15		
52	0.50			11.7		
53		26	60		9.3	29
54		82	100		63	90
55	5.8			71		
naloxone	3.7			66		
naltrexone	0.56			3.9		

[a] Naloxone. [b] Ethylketocyclazocine. [c] Percent stereospecific displacement of either [³H]NAL or [³H]EKC run in triplicate at the concentration indicated. [d] K_is were derived from six different concentrations each run in triplicate. [e] K_i values (nM) for [³H]-D-Ala²-D-Leu⁵-enkephalin (δ receptor) displacement for *8* and *9* are 93 and 246, respectively.

Table II. Opioid Antagonist Effects of the 4-Phenylpiperidine Antagonists

	antagonism of opioid analgesia mouse writhing [AD_{50} (mg/kg, sc)][a]		antagonism of κ diuresis: AD_{50} (mg/kg, sc)[b]
compd	μ receptor (morphine)	κ receptor (U50,488)	
2	0.74	>5.0	>10
3	1.3	>10	
4	1.3	6.1[d]	
5	0.16	1.4	1.8
6	0.14	4.5	3.2
7	0.050	0.92	2.5
8			
9			
19	>1.25	>1.25	
20	0.37	0.60	0.69
21	0.052	0.11	0.70
22	0.21[c]	>0.64[c]	3.3
23	0.29	0.45	2.5
24	2.4[c]	5.3[c]	1.1
25	0.28	>1.25[c]	4.1
26	0.31	0.51	3.4
27	0.44	0.38	3.6
28	0.19	0.33	0.50
29	0.27	0.95	5.7
30	>1.25	>1.25	11
31			NA
32	0.14	0.84	1.4
33	0.25	0.25	3.8
34	>1.25	>1.25	14
35	0.08	0.51	>5.0
36	0.18	>0.64	2.2
37	0.05	0.09	1.4
38	>1.25	>0.32	10
39	0.76	>1.25	11
40	0.12	0.24	1.9
41	0.22	0.30	1.0
42	0.03	0.25	
43	0.24	0.65	
44	0.08	0.26	
45	0.25	0.91	8.4
46	0.16	0.43	3.3
47	0.14[c]	>40[c]	3.0
48	>1.25[c]	>1.25[c]	8.4
49	2.4[c]	11.8[c]	6.9
50	0.27	0.85	3.7
51			
52	0.07	0.14	1.4
53	0.60	0.75	3.2
54	0.40	0.71	3.8
55	0.71	1.35	
naloxone	0.08	1.1	3.5
naltrexone	0.05	0.06	2.5

[a] Dose required for 50% reduction in the analgesic response to either morphine (1.25 mg/kg, sc) or U50,488 (2.5 mg/kg, sc). [b] Dose required to decrease the 5-h bremazocine-induced (0.08 mg/kg, sc) urination by 50%. [c] At higher doses this compound inhibited writhing itself and the AD_{50} had to be approximated.

also have affinity for the κ receptor and are antagonists of κ-induced analgesia and diuresis. These compounds exhibited varying degrees of selectivity for μ vs κ receptors, but in general, the effects of substitution at the nitrogen on potency are similar for both μ and κ receptors. Of these compounds, **7** (LY117413), is the most potent μ- and κ-receptor antagonist. Its μ- and κ-receptor *in vivo* antagonist activities are equivalent to those of naloxone.

These early SAR findings supported the existence of an important lipophilic binding region for both μ and κ receptors in the proximity of the nitrogen binding site. Interaction with this region could be achieved with appropriate N-substitution and this accounts for the increase in antagonist potencies with compounds **2–7**. In this SAR study, different strategies were used to take advantage of this effect and increase opioid receptor affinity. It was hoped that this strategy would also identify the means to achieve selectivity for a particular opioid receptor.

The alkylphenyl analogs (**5** and **23–25**) with one to four carbon atom spacers were synthesized to define the optimal distance between the nitrogen and a phenyl substituent. Affinity for the the μ and κ receptors is found to be maximized with the 3-phenylpropyl derivative (**24**); however, all four alkylphenyl analogs show significant affinity for both receptors and, *in vivo*, are μ- and κ-receptor antagonists (Table II). Unexpectedly, on the basis of its affinity for μ and κ receptors, **24** is only a weak antagonist of opioid mediated analgesia. Instead, **24** produces an

antinociceptive response in the writhing test (ED_{50} = 15 mg/kg, sc), and it is this effect which appears to mask its abilities to antagonize opioid-induced analgesia. The antinociceptive activity of **24** is not blocked by a high dose of the opioid antagonist naloxone (10 mg/kg, sc) and thus appears to be a nonopioid effect. As noted in Table II, a limited number of other compounds significantly inhibit mouse writhing at relatively high doses, but at doses which appear to affect their AD_{50} values. In all cases these antinociceptive activities were judged to be nonopioid effects.

Other compounds with various nitrogen substituents were synthesized to further increase opioid antagonist activities and to characterize structural requirements for maximum binding at μ and κ receptors. The phenoxyethyl (**47**) and phenoxypropyl (**48**) derivatives both have high affinity for the μ receptor, but relatively weak affinity for the κ receptor. Compound **47** is a potent antagonist of morphine-induced analgesia but has relatively weak nonopioid agonist effects in the writhing test (ED_{50} = 6.8 mg/kg, sc). Substitution of a hydroxyl α to the phenyl of **24**, giving **7**, has little effect on affinity for either μ or κ receptors; however, **7** is devoid of the nonopioid agonist effects observed with **24**. Several different lipophilic groups in addition to phenyl apparently bind tightly to the hydrophobic site distal to nitrogen including thiophene (**33**, **40**, **41**, **51**, and **52**), furan (**44**, **54**, and **55**), and *n*-alkyl (**20–22**). The 3-(2-furanyl)propyl (**44**), 3-(2- and 3-thienyl)-propyl (**40** and **41**), and 1-hydroxy-1-(2-thienyl)propanol-3-yl (**52**) derivatives are highly potent μ- and κ-receptor antagonists.

With *N*-alkyl substitution, replacement of methyl (**2**) with ethyl (**19**) leads to a loss in affinity for the μ receptor while increasing affinity for the κ receptor. Increasing the alkyl chain length to 5, 6, and 7 carbons markedly increases opioid antagonist activities. The *n*-hexyl (**21**) and heptyl (**22**) derivatives are found to have high affinity for the μ receptor. Compound **21** is a highly potent antagonist of μ- and κ-mediated analgesia and of κ-induced diuresis. The *n*-pentyl derivative **20** is a potent antagonist of κ-agonist-induced diuresis.

The *N*-phenylethyl and *N*-phenylpropyl derivatives (**5** and **24**) were chosen to explore the effects on opioid antagonist activity of substitutions on the distal phenyl ring. In general, methyl or chloro substitution produced relatively minor effects on μ- and κ-receptor activities. For **5**, substitution of methyl in the ortho position (**28**) enhances affinity at the κ receptor. In accord with this, **28** is a potent blocker of κ diuresis. *p*-Methyl substitution of **5** (**30**) reduces activity at both μ and κ receptors. Addition of *o*-methyl to **24** gives compound **37** which has highly potent μ- and κ-receptor antagonist activities but is devoid of the nonopioid agonist effects found with **24** in the mouse writhing test.

The 3-(2-thienyl)propyl derivative **41** was resolved and the activities of the enantiomers (**42** and **43**, Tables I and II) were investigated. As with the 2-propiophenone derivative **6** only a limited stereochemical effect on μ and κ activities was observed. The (+)-3*R*,4*R*-isomer **44** has the highest affinity and antagonist activity for both μ and κ receptors. Its potencies are approximately 8–9 (μ receptor) and 2–4 (κ receptor) times that of the (−)-3*S*,4*S*-isomer.

Discussion

Further exploration of the effects of N-substitution with *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine has led to the discovery of several new, highly potent opioid antagonists. No antagonists with high selectivity for the μ or even moderate selectivity for the κ receptor were discovered and in general, the consequence of structural changes at the nitrogen is similar for μ and κ receptors, with all antagonists possessing somewhat higher affinity for the μ receptor. The SAR presented here further establishes the existence of an important lipophilic binding site distal to the nitrogen for both μ and κ receptors to which a variety of different substituents can tightly bind. Our data suggest that this lipophilic pocket is either very large or is a domain which has considerable malleability. These conclusions have been derived principally from evaluation of racemic mixtures. While it is possible that further separations of μ and κ activities could be achieved through separation of individual stereoisomers, the very limited stereochemical effects observed with the isomers of **6** and **41** suggest that this is unlikely.[34]

Significant opioid agonist activity (analgesia that is naloxone reversible) in the mouse writhing assay was not detected with any of the newly synthesized antagonists. This is particularly noteworthy because the mouse writhing test is known to be highly sensitive for detecting analgesic activities of opioid partial agonists. The opioid agonist and antagonist effects of a representative series of 4-phenylpiperidines have been further assessed in the isolated MVD. Potent μ-, κ-, and δ-receptor antagonist activities were observed; however, no opioid agonist activities were detected with any N-substituted derivatives tested (M. L. Cohen, personal communication).[34] Consequently, the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine structure appears to be a pure opioid antagonist pharmacophore in that structural changes at nitrogen affect only the molecule's affinity for a particular opioid receptor. This feature differentiates these compounds from other series of opioid antagonists.

We have used molecular modeling techniques in an attempt to identify the 3-dimensional structure of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines that binds to opioid receptors. Conformational energy analyses (Macromodel, MM2 force field) indicate that these 4-phenylpiperidines can exist in either an axial-phenyl or equatorial-phenyl conformation. The energy difference between these conformations is relatively small with the equatorial-phenyl conformer being of lower energy (ΔG = 2.6 kcal/mol). Others using high-field NMR spectroscopy (^1H, ^{13}C) have noted a preference for the 1-methyl-*trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine to exist in an equatorial phenyl conformation.[35,36] These data and SAR studies exploring the effect of 6-methyl substitution led us to conclude that the opioid antagonist activity is mediated through the equatorial-phenyl conformation.[37] We have compared equatorial-phenyl low-energy conformations of the phenylpiperidine antagonists with other opioid antagonists and agonists. These studies have led us to postulate that the 3-hydroxyphenyl substituent of the piperidine antagonists binds to the site occupied by the 3-hydroxyphenyl substituent of rigid, multicyclic opioids such as naloxone[38] and WIN44,441.[39] We have also concluded that the lipophilic substituent distal to the nitrogen in the N-substituted 4-phenylpiperidine antagonists binds in the same region as does the cyclopentyl

group, extended from the 5 position of the benzomorphan nucleus, of WIN44,441. A more thorough discussion of these 3-dimensional structural comparisons will be the subject of a future publication.

The failure to discover highly selective antagonists for either the μ or κ receptor within this series of racemic *trans*-3,4-dimethyl-4-arylpiperidines was disappointing; however, compounds with significant selectivity for the μ receptor were identified and the degree of selectivity for μ versus κ receptors could be altered with N-substitution. This implies that additional SAR work could lead to the discovery of receptor selective opioid antagonists. Most importantly, we were able to more firmly establish the antagonist pharmacophore nature of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine nucleus.[40] Thus, there are now available for the first time a large number of highly potent pure opioid antagonists for pharmacological study.

Experimental Section

Melting points were determined for all solids on a Thomas-Hoover apparatus and are unconnected. NMR spectra were recorded on either a Varian T-60, brucker WM-270, or GE QE-300 spectrometer and were consistent with assigned structures. Mass spectra and microanalyses were determined by the Structural and Organic Chemistry Research Department of the Lilly Research Laboratories. Mass spectra were consistent with assigned structures for all compounds. All compounds were elementally analyzed within 0.4% of theoretical value unless othersise indicated. Column chromatography was performed by gravitational flow with use of Allied Fisher Silica (70–150 mesh).

General Acylation/Reduction Procedures for Preparing N-Substituted 3(*R),4(*R**)-Dimethyl-4-(3-hydroxyphenyl)-piperidines (Method A).** To a solution of 1.00 g (0.0049 mol) of 18 and 1.25 g (0.012 mol) of triethylamine in 70 mL of DMF was added dropwise 0.012 mol of the appropriate acid chloride at room temperature under nitrogen. After the reaction mixture was heated for 2 h at 90 °C, the solution was cooled and poured into 100 mL of water. The desired amide was extracted into ether. The ether layer was washed two times with water, dried over K_2CO_3, and concentrated under vacuum.

Red-Al Reduction. To 6 mL of Red-Al in 20 mL toluene was added dropwise a solution of the crude amide dissolved in approximately 50 mL of toluene. The reaction was heated to 60–70 °C for 2 h and quenched by the addition of 400 mL of a pH 10 buffer. The pH of the mixture was adjusted to approximately 9.8 with 1 N hydrochloric acid, and the mixture was extracted with toluene. The organic extracts were combined and dried over anhydrous sodium sulfate. The filtrate was concentrated under vacuum, and the resulting residue was chromatographed over silica gel.

$LiAlH_4$ Reduction. The crude amide was dissolved into 75 mL of anhydrous THF and added dropwise to 0.75 g of $LiAlH_4$ dispersed in 50 mL of anhydrous THF. After a 4-h reflux, the reaction mixture was cooled, and the excess $LiAlH_4$ was neutralized by the careful addition of 10 mL of ethyl acetate with ice cooling. Saturated NH_4Cl solution was then added to precipitate the lithium salts. The solution containing the desired product was separated, evaporated to dryness, and worked up as above. Overall yields with either reducing agent were generally 20–35%.

(±)-1-(Cyclopropylmethyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (4)** was prepared from 18 and cyclopropane carboxylic acid chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with a solvent gradiant of ethyl acetate to ethyl acetate/methanol (1:1). The HCl salt was prepared and recrystallized from isopropyl ether/acetone/ethanol: mp 217.5–218.5 °C. Anal. ($C_{17}H_{26}ClNO$) C, H, N.

(±)-1-*n*-Heptyl-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)-piperidine (22)** was prepared from 18 and heptanoyl chloride. The crude amide was reduced with $LiAlH_4$ and chromatographed over silica gel eluting with hexane/ethyl acetate (3:1) containing 0.5% triethylamine. Treatment with HCl and trituration in ether gave the HCl salt: mp 155–157 °C. Anal. ($C_{20}H_{34}ClNO$) C, H, N.

(±)-1-Benzyl-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)-piperidine (23)** was prepared from 18 and benzoyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with hexane/ethyl acetate (2.5:1.5). The HCl salt was prepared and triturated in ether: mp 127–130 °C. Anal. ($C_{20}H_{27}ClNO$) C, H, N.

(±)-1-(4-Phenylbutyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (25)** was prepared from 18 and 4-phenylbutyryl chloride. The crude amide was reduced with $LiAlH_4$ and chromatographed over silica gel eluting with hexane/ethyl acetate (2.5:1.5). Treatment with HCl and trituration in ether gave the HCl salt: mp 159–160 °C. Anal. ($C_{23}H_{32}ClNO$) C, H, N.

(±)-1-[2-(2-Chlorophenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (26)** was prepared from 18 and (2-chlorophenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with hexane/ethyl acetate (4:1). Treatment with HCl and trituration in ether gave the HCl salt: mp 98–104.5 °C, with foaming. Anal. ($C_{21}H_{27}Cl_2NO$) C, H, N.

(±)-1-[2-(3-Chlorophenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (27)** was prepared from 18 and (3-chlorophenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed with silica gel eluting with hexane/ethyl acetate (4:1). Treatment with HCl and trituration in ether gave the HCl salt: mp 179–185 °C. Anal. ($C_{21}H_{27}Cl_2NO$) C, H, N.

(±)-1-[2-(2-Methylphenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (28)** was prepared from 18 and (2-methylphenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with ethyl acetate. The HCl salt was prepared and triturated in ethyl acetate: mp 127–132 °C with foaming. Anal. ($C_{22}H_{30}ClNO$) C, H, N.

(±)-1-[2-(3-Methylphenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (29)** was prepared from 18 and (3-methylphenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with hexane/ethyl acetate (1:1). The HCl salt was prepared and triturated in ether: mp 198–200 °C. Anal. ($C_{22}H_{30}ClNO$) C, H, N.

(±)-1-[2-(4-Methylphenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (30)** was prepared from 18 and (4-methylphenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with hexane/ethyl acetate (1:1). The HCl salt was prepared and triturated in ether: mp 185–187 °C. Anal. ($C_{22}H_{30}ClNO$) C, H, N.

(±)-1-[2-(3,4-Dichlorophenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (31)** was prepared from 18 and (3,4-dichlorophenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 107–114.5 °C. Anal. ($C_{21}H_{26}Cl_3NO$) C, H, N.

(±)-1-[2-(3-Fluorophenyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (32)** was prepared from 18 and (3-fluorophenyl)acetyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with ethyl acetate. The HCl salt was recrystallized from ethyl acetate/ethanol/isopropyl ether: mp 185–186 °C. Anal. ($C_{21}H_{27}ClFNO$) C, H, N.

(±)-1-[2-(2-Thienyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (33)** was prepared from 18 and 2-thienylacetyl chloride. The crude amide was reduced with $LiAlH_4$ and chromatographed over silica gel eluting with hexane/ethyl acetate (3:1) containing 0.5% triethylamine. Treatment with HCl and trituration in ether gave the HCl salt: mp 98–100 °C. Anal. ($C_{19}H_{26}ClNOS$) C, H, N.

(±)-1-[3-(2-Methoxyphenyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (36)** was prepared from 18 and 3-(2-methoxylphenyl)propionyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel

eluting with hexane/ethyl acetate (1:1). The HCl salt was prepared and triturated in ether: mp 94–97 °C. Anal. ($C_{23}H_{32}ClNO_2$) C, H, N.

(±)-1-[3-(2-Methylphenyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (37)** was prepared from 18 and 3-(2-methylphenyl)propionyl chloride. The crude amide was reduced with Red-Al and then chromatographed over silica gel eluting with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 91–95 °C. Anal. ($C_{23}H_{32}ClNO$) C, H, N.

(±)-1-[3-(3-Methylphenyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (38)** was prepared from 18 and 3-(3-methylphenyl)propionyl chloride. The crude amide was reduced with Red-Al and then chromatographed over silica gel eluting with hexane/ethyl acetate (1:4). The HCl salt was prepared and triturated in ether: mp 83–89 °C. Anal. ($C_{23}H_{32}ClNO$) C, H, N.

(±)-1-[3-(3-Chlorophenyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (39)** was prepared from 18 and 3-(3-chlorophenyl)propionyl chloride. The crude amide was reduced with Red-Al and then chromatographed over silica gel eluting with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 73–77 °C. Anal. ($C_{22}H_{29}Cl_2NO$) C, H, N.

(±)-1-[3-(3-Thienyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (40)** was prepared from 18 and 3-(3-thienyl)propionyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with hexane/ethyl acetate (2:1). Treatment with HCl and trituration in ether gave the HCl salt: mp decomposed. Anal. ($C_{20}H_{28}ClNOS$) C, H, N.

(±)-1-[3-(2-Thienyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (41)** was prepared from 18 and 3-(2-thienyl)propionyl chloride. The crude amide was reduced with $LiAlH_4$ and was chromatographed over silica gel eluding with hexane/ethyl acetate (3:1) containing 0.5% triethylamine. Treatment with HCl and trituration in ether gave the HCl salt: mp 101–103 °C. Anal. ($C_{20}H_{28}ClNOS$) C, H, N.

(+)-1-[3-(2-Thienyl)propyl]-3(*R),4(*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (42)** was prepared from (+)-3(*R*),4(*R*)-18 and as described for 41. Treatment with HCl and trituration in ether gave the HCl salt: mp 110–112 °C; $[\alpha]^{25}_D$ = +54.9 (*c* = 1.0, MeOH). Anal. ($C_{20}H_{28}ClNOS$), C, H, N.

(−)-1-[3-(2-Thienyl)propyl]-3(*S*),4(*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine (43) was prepared from (−)-3(*S*),4(*S*)-18 and as described 42. Treatment with HCl and trituration in ether gave the HCl salt: mp 110–112 °C; $[\alpha]^{25}_D$ = −55.2 (*c* = 1.0, MeOH). Anal. ($C_{20}H_{28}ClNOS$), C, H, N.

(±)-1-[3-(2-Furanyl)propyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (44)** was prepared from 18 and 3-(2-furanyl)propionyl chloride. The crude amide was reduced with Red-Al and chromatographed over silica gel eluting with hexane/ethyl acetate (1:1). The HCl salt was prepared and triturated in ether: mp 78–81 °C. Anal. ($C_{20}H_{28}ClNO_2$) C, H, N.

(±)-1-[4-(2-Thienyl)butyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (46)** was prepared from 18 and 4-(2-thienyl)butyl chloride. The crude amide was reduced with $LiAlH_4$ and chromatographed over silica gel eluting with hexane/ethyl acetate (3:1) containing 0.5% triethylamine. Treatment with HCl and trituration in ether gave the HCl salt: mp 148–150 °C. Anal. ($C_{21}H_{30}ClNOS$) C, H, N.

General Alkylation Procedure for Preparing N-Substituted 3(*R),4(*R**)-Dimethyl-4-(3-hydroxyphenyl)piperidines (Method B).** Compound 18 (500 mg) was refluxed for 1 h in 35 mL DMF, with 1.1 equiv of the appropriate alkyl halide and 1.1 equiv of $NaHCO_3$. The mixture was cooled and poured into 100 mL of water. The pH was adjusted to 9.8, and the product was extracted into ether. The ether layer was washed, dried over K_2CO_3, and concentrated under vacuum. The products were purified using silica gel column chromatography. The HCl salts were prepared and further purified by recrystallization or trituration. Yields of purified products were generally 35–80%.

(±)-1-Alyl-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (3)** was prepared from 18 and allyl bromide. The HCl salt was recrystallized from ethanol/isopropyl ether: mp 200.5–203 °C. Anal. ($C_{16}H_{24}ClNO$), C, H, N.

(±)-1-(2-Phenylethyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (5)** was prepared from 18 and 2-phenylethyl bromide and eluted from a silica gel column with a solvent gradient of ethyl acetate to ethyl acetate/methanol (1:1). The HCl salt was prepared and dissolved in ethanol and precipitated with isopropyl ether: mp 125–128 °C. Anal. ($C_{21}H_{28}ClNO$) C, H, N.

(±)-1-Ethyl-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (19)** was prepared from 18 and ethyl iodide and eluted from a silica gel column with a solvent gradient of ethyl acetate to ethyl acetate/methanol (1:1). The HCl salt was prepared and triturated in ether: mp 150–152 °C. Anal. ($C_{15}H_{24}ClNO$) C, H, N.

(±)-1-*n*-Pentyl-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (20)** was prepared from 18 and *n*-pentyl bromide and eluted from a silica gel column with ethyl acetate. The HCl salt was triturated in ether: mp 83–86 °C. Anal. ($C_{18}H_{30}ClNO$) C, H. N.

(±)-1-*n*-Hexyl-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (21)** was prepared from 18 and *n*-hexyl iodide and eluted from a silica gel column with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 140–143 °C with decomposition. Anal. ($C_{19}H_{32}ClNO$) C, H, N.

(±)-1-(3-Phenylpropyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (24)** was prepared from 18 and 3-phenylpropyl bromide and eluted from a silica gel column with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 103–107 °C. Anal. ($C_{22}H_{30}ClNO$) C, H, N.

(±)-1-(2-Naphthalen-1-yl)ethyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (35)** was prepared from 18 and 2-naphthalen-1-ylethyl bromide. The crude free base was recrystallized from ethyl acetate: mp 201–202 °C. Anal. ($C_{25}H_{29}NO$) C, H, N.

(±)-1-(4-Phenylpentyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (45)** was prepared from 18 and 4-phenylpentyl bromide and eluted from silica with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 187–189 °C. Anal. ($C_{24}H_{34}ClNO$) C, H, N.

(±)-1-(2-Phenoxyethyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (47)** was prepared from 18 and 2-phenoxyethyl bromide and eluted from a silica gel column with a solvent gradient of ethyl acetate to ethyl acetate/methanol (1:1). The HCl salt was prepared, dissolved in ethanol, and precipitated with isopropyl ether: mp 105–106 °C. Anal. ($C_{21}H_{28}ClNO_2$) C, H, N.

(±)-1-(3-Phenoxypropyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (48)** was prepared from 18 and 3-phenoxypropyl bromide and eluted from a silica gel column with ethyl acetate. The HCl salt was prepared and triturated in ether: mp 78–81 °C. Anal. ($C_{22}H_{30}ClNO_2$) C, H, N.

(±)-1-(Tetrahydro-2(*R*,*S*)-furanylmethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (53)** was prepared from 18 and tetrahydrofuranyl bromide and eluted from a silica gel column with hexane/ethyl acetate (1:7). The HCl salt was prepared and triturated in ethanol/isopropyl ether: mp 177–179 °C. Anal. ($C_{18}H_{29}ClNO_2$) C, H, N.

(±)-1-(1-Phenyl-1-oxoprop-3-yl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (6).** A mixture of 3.70 g (0.018 mol) of 18, 3.82 g (0.036 mol) of Na_2CO_3, and 6.41 g (0.020 mol) of 1-phenyl-3-(dimethylamino)-1-propanone methiodide in 37 mL DMF was stirred at room temperature for 4 h with nitrogen continuously bubbling through. The mixture was poured into 200 mL of water and extracted with ether. The ether layer was washed, dried over K_2CO_3, and concentrated under vacuum. The maleic acid salt was prepared and recrystallized from ethyl acetate to give 6.8 g (86%): mp 70–71 °C. Anal. ($C_{26}H_{31}NO_6$) C, H, N.

(+)-1-(1-Phenyl-1-oxoprop-3-yl)-3(*R*),4(*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (8). A mixture of 2.54 g (0.012 mol) of (+)-3(*R*),4(*R*)-18, 2.63 g (0.025) of Na_2CO_3, and 4.35 g (0.014 mol) of 1-phenyl-3-(dimethylamino)-1-propanone methiodide in 45 mL of DMF was stirred at room temperature for 4 h with nitrogen continuously bubbling through. The ether layer was washed, dried over K_2CO_3, and concentrated under vacuum. The HCl salt was prepared and recrystallized from 2-isopropyl alcohol/isopropyl ether: mp 184-186 °C; $[\alpha]^{25}_D$ = +48.7 (c = 1.0, MeOH). Anal. ($C_{22}H_{28}ClNO_2$) C, H, N.

(−)-1-(1-Phenyl-1-oxoprop-3-yl)-3(*S*),4(*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine (9) was prepared as described for 8 from (−)-3(*S*),4(*S*)-18: mp 184–185.5 °C; $[\alpha]^{25}_D$ = −50.1 (c = 1.0, MeOH). Anal. ($C_{22}H_{28}ClNO_2$) C, H, N.

(±)-1-[*R,S*-(1-Hydroxy-1-phenylprop-3-yl)]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (7).** Compound 6 (3.50 g, 0.010 mol) in 30 mL of benzene was added dropwise to 8.0 mL of Red-Al, and the mixture was reflexued for 5 h. The reaction was cooled and carefully added to 100 mL water, and the pH was adjusted to 9.8. The product was extracted into ether, dried over K_2CO_3, and concentrated under vacuum. The HCl salt was prepared and triturated in ether to give 2.8 g (74%): mp 86–89 °C with decomposition. Anal. ($C_{22}H_{30}ClNO_2$) C, H, N.

(±)-1-[2-(4-Pyridinyl)ethyl]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (34).** A mixture of 18 as the hydrochloride salt (1.17 g, 0.0049 mol), 4-vinylpyridine (1.05 mL, 0.0097 mol), and 0.37 mL of water was heated at 100 °C for 4.5 h. After cooling the mixture was diluted with 1 N HCl poured into water and the pH adjusted to 9.8. The product was extracted into ether, dried over K_2CO_3, and concentrated under vacuum. The crude product was chromatographed over silica gel eluting with ethyl acetate containing 1% methanol. The product crystallized as the free base on standing and was triturated with hexane to give 0.16 g (9%): mp 170–172 °C. Anal. ($C_{20}H_{26}N_2O$) C, H, N.

(±)-1-[*R,S*-(1-Hydroxy-1-phenyleth-3-yl)]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (49).** To a solution of 0.50 g (0.0025 mol) of 18 in 50 mL of ethanol was added 0.46 g (0.0038 mol) of styrene oxide in 1.5 mL of methylene chloride. The reaction mixture was heated at 50 °C for 7 h, cooled to room temperature, and concentrated under vacuum. The resulting residue was chromatographed over silica gel eluting with ethyl acetate/methanol (1:1). The HCl salt was prepared and triturated in acetone/ethyl ether to give 0.25 g (28%): mp 120 °C with decomposition. Anal. ($C_{21}H_{28}ClNO_2$) C, H, N.

(±)-1-[*R,S*-(1-Hydroxy-1-(phenylmethyl)eth-3-yl)]-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (50)** was prepared by the procedure described for 49. The HCl salt was prepared and triturated in acetone/ethyl ether: mp 90 °C with decomposition. Anal. ($C_{22}H_{30}ClNO_2$) C, H, N.

(±)-1-[1-Oxo-1-(2-thienyl)prop-3-yl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (51)** was prepared by the procedure described for 6 except the crude product was extracted into ethyl acetate and purified by flash chromatography over silica gel eluting with hexane/ethyl acetate (1:1) containing 0.5% triethylamine. The HCl salt was prepared and triturated in ether: mp 118–120 °C. Anal. ($C_{20}H_{26}ClNO_2S$) C, H, N.

(±)-1-[1-Hydroxy-1-(2-thienyl)prop-3-yl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (52).** $NaBH_4$ (0.25 g, 0.0066 mol) was added to a solution of 51 (2.00 g, 0.0058 mL) in 50 mL isopropyl alcohol at 0 °C. After 30 min of stirring, a second 0.25 g (0.0066 mol) portion of $NaBH_4$ was added. The mixture was stirred for an additional 30 min following which 50 mL of 1 N HCl was carefully added. The mixture was concentrated under vacuum, and the residue was dissolved into 50 mL of water. The pH was adjusted to 9.8 and extracted with ethyl acetate. The extracts were washed with NaCl solution, dried over NaCl/Na_2SO_4 and concentrated under vacuum. The crude product was chromatographed over silica gel eluting with hexane/ethyl acetate (1:1) containing 0.5% triethylamine. The HCl salt was prepared and triturated in ether: mp 90–92 °C. Anal. ($C_{20}H_{28}ClNO_2S$) C, H. N.

(±)-1-(3-Furanylmethyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (54).** A solution of 2.40 g (0.011 mol) of 17, 1.6 g (0.017 mol) of 3-furaldehyde, and 0.030 g of 5% Pd/C in 100 mL of ethanol was adjusted to pH 6 with ethanolic HCl and hydrogenated at 45 psi for 1 h at room temperature. The catalyst was filtered, and the solvent was concentrated under vacuum. The residue was partitioned between 1 N NaOH and ether. The ether extracts were washed with aqueous NaCl, dried over NaCl/Na_2SO_4, and concentrated under vacuum. The residue was chromatographed over silica gel using hexane/ethyl acetate (3:1) containing 0.1% triethylamine, giving 1.10 g (0.0011 mol) of (±)-1-(3-furanylmethyl)-3(*R**),4(*R**)-dimethyl-4-(3-methoxyphenyl)piperidine. This was dissolved in 25 mL DMF and added to a solution of *n*-propanethiol (2.0 mL, 0.0022 mol), 1.25 g (0.0011 mol) of potassium *tert*-butoxide and 25 mL of DMF at room temperature. The solution was then heated at 145 °C for 5 h. The volatiles were removed under vacuum at 75 °C. To the residue was added 50 mL of water and the pH was adjusted to 9.8 by the addition of 1 N HCl. The product was extracted into toluene/1-butanol (3:1), dried over NaCl/Na_2SO_4, and concentrated under vacuum. The crude product was chromatographed over silica gel eluting with hexane/ethyl acetate (3:1) containing 0.1% triethylamine. The HCl salt was prepared and recrystallized from isopropyl alcohol/isopropyl ether: mp 217–219 °C. Anal. ($C_{18}H_{24}ClNO_2$) C, H, N.

(±)-(2-Furanylmethyl)-3(*R),4(*R**)-dimethyl-4-(3-hydroxyphenyl)piperidine (55).** This compound was prepared from 17 and 2-furaldehyde as described for the synthesis of 54. The HCl salt was prepared and triturated in methylene chloride/isopropyl ether: mp 197–199 °C. Anal. ($C_{18}H_{24}ClNO_2$) C, H, N.

Mouse Writhing Analgesic and Opioid Antagonist Assay. Five CF-1 male mice (Charles River Portage, MI), weighing approximately 20 g after being fasted overnight, were observed simultaneously for the writhing response. The writhing response was induced by the intraperitoneal administration of 0.6% acetic acid in a volume of 1 mL/100 g of body weight. The observation period was 10 min in duration, beginning 5 min after injection of acetic acid. The percent inhibition of writhing was calculated from the average number of writhes in the control (nondrug) group. Each data point is the mean (+ standard error) for five mice. Dose–response effects were measured by varying the antagonist 2-fold, until minimum and maximum doses were determined. A minimum of five different doses were used. The response for each dose had a standard error < 25%. The AD_{50} was defined as the dose of antagonist that reduced the inhibition of writhing produced by a standard dose of the agonist (1.25 mg/kg for morphine or 2.5 mg/kg for U-50, 488H) to 50%. Each mouse was used only once. All drugs were administered subcutaneously (1 mL/100 g of body weight) 20 min before the injection of acetic acid. The drugs used and the forms in which the doses were calculated are as follows: morphine sulfate, and U-50,488H methane sulfonate (The Upjohn Co., Kalamazoo, MI).

Antagonism of κ (Bremazocine-Induced) Diuresis in Rats. The animals used were a pool of 50 male Long-Evans hooded rats (Charles River, Portage, MI) weighing 350–500 g. They were individually housed in a colony room (23 °C) illuminated between 0600 and 1800 h. Rodent chow and tap water were freely available except during the measurement of urinary output. The animals were used repeatedly, but no more frequently than twice (separated by two days) during a week. To measure urinary output, the animals were removed from their home cages at about 1000 h, weighed, injected, and placed individually in metabolism cages for the next 5 h. Excreted urine was funneled into graduated cylinders, and the volume was recorded at 2 h and 5 h after injection. Bremazocine hydrochloride (Sandoz Ltd., Basle, Switzerland) was used as the κ agonist to induce urination. Bremazocine HCl was injected subcutaneously in a dose of 0.08 mg/kg, and without delay doses of the potential antagonists were injected subcutaneously on the opposite side of the rat. For each test compound three rats per dose were used. The response for each dose had a standard error < 25%. Test doses were varied 2-fold until minimum and maximum effect doses were identified.

Opioid Receptor Binding Assays. The affinities of test compounds of μ- and κ-opioid receptors were determined by a modification of previously published methods.[41] To perform μ-receptor binding, washed crude synaptosomal membranes from rat whole brain tissue (minus cerebellum) were prepared and stored at −125 °C until use. Samples were incubated for 20 minutes at 37 °C in the presence of tissue, [^{3}H]naloxone (0.5 nM), and the test compound. The incubation buffer consisted of a modified Krebs-HEPES buffer (NaCl 118.2 mM; KCl 4.6 mM; $CaCl_2$ 1.6 mm: $MgSO_4$ 1.2 mM; KH_2PO_4 1.2 mM; glucose 10 mM; and HEPES 25 mM pH 7.4). Nonspecific binding was determined in the presence of 1000 nM of unlabeled naloxone. Bound radioactivity was separated from free ligand by filtration through Whatman GF-C glass fiber filters. Filters were further washed with 2 × 5 mL of ice-cold buffer. Bound radioactivity on the filter was quantitated using liquid scintillation spectrometry.

For κ-receptor binding, washed crude synaptosomal membranes were prepared from guinea pig cortical tissue and stored at −125 °C until use. Tissue was suspended in modified Krebs-HEPES buffer and incubated at 37 °C for 45 min with 1.0 nM [^{3}H]ethylketocyclazocine and the test compound. Fentanyl and D-Ala^2-D-Leu^5-enkephalin were also added to the samples at 100 nM concentration to inhibit binding to μ and δ receptors, respectively. Nonspecific binding was determined in the presence of 1000 nM unlabeled ethylketocyclazocine. Bound radioactivity was separated and quantitated as described above for μ binding. Methods for δ receptor binding are described in the accompanying paper.

For all opioid receptor binding assays K_i values were derived from at least six different concentrations each run in triplicate. The standard errors from the extrapolated K_i values from the regression lines were less than 50% of the K_i. The correlation coefficient for calculating the K_i values were >0.9 and the triplicate values generally different by less than 10%.

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