N-Substituent Modulation of Opiate Agonist/Antagonist Activity in Resolved 3-Methyl-3-(m-hydroxyphenyl)piperidines

Alice Cheng,[†,‖] Edward Uyeno,[†] Willma Polgar,[†] Lawrence Toll,[†] John A. Lawson,[†] Joseph I. DeGraw,[†] Gilda Loew,*[†] Arthur Camerman,[‡] and Norman Camerman[§]

SRI International, Menlo Park, California 94025, Departments of Medicine and Pharmacology, University of Washington, Seattle, Washington 98195, and Biochemistry Department, University of Toronto, Toronto, Canada M5S 1A8.
Received March 22, 1985

A series of 3-methyl-3-(*m*-hydroxyphenyl)piperidines with N-substituent variations have been synthesized and resolved, and an X-ray crystal structure of one analogue was determined. The compounds have been characterized, pharmacologically, by detailed opiate receptor binding studies and determination of in vivo analgesia and opiate antagonism. The results indicate that all compounds bind with high selectivity and moderate affinity to μ-receptors with no qualitative difference between enantiomeric pairs. By contrast a striking difference in activities is found, with the (−) enantiomers being pure agonists and the (+) enantiomers having both agonist and antagonist activity. The effect of N-substituents on relative agonist and antagonist potency does not mimic that of fused ring opiates with the *N*-phenethyl compound, the most potent antagonist. These results together with the X-ray structure obtained suggest that agonist and antagonist activity is initiated by a bimodel binding of the compounds in two different orientations at the μ-receptor site.

In many families of opiate narcotic analgesics, small changes in chemical structure have been shown to modify the extent to which an analogue exhibits agonist and antagonist activity. For example, in the morphine family, and in fused-ring opiates in general, N-substituent variation modulates relative agonist/antagonist potency.[1–3] Among such fused-ring opiates, *N*-methyl and *N*-phenethyl compounds are almost always pure agonists while *N*-allyl and *N*-cyclopropylmethyl compounds usually have antagonist activity. In other families of opioids, however, modulation of agonist/antagonist activity need not follow the same pattern.

The discovery of the analgesic properties of meperidine[4] represented a major advance in the analgesic field because it demonstrated that partial similarity to the morphine structure can lead to opiate analgesic activity. Many N-substituted meperidine analogues have since been prepared with varying degrees of analgesic potency and side effects.[5] Moreover, the discovery of meperidine aroused interest in other classes of phenylpiperidines. First, 2-phenyl and 4-phenyl analogues[6] and, later, the subject of this paper, the 3-arylpiperidine congeners, were synthesized and tested in several laboratories for opiate agonist and antagonist activity.[7–13] However, no variation in any of the arylpiperidines produced significant antagonism until *m*-hydroxyaryl derivatives were made, establishing this feature as a minimum requirement for antagonist activity in all known arylpiperidine series.[7–13]

While these investigations established the requirement of a *m*-hydroxyphenyl group for antagonism, they could not, by themselves, elucidate the modes of receptor binding of these 3-arylpiperidines leading to agonism and antagonism. Nor was the role in modulating relative agonist and antagonist potency of other features, such as the second 3-substituent, the N-R variations, and the presence or absence of a 2-methyl group, clearly understood.

In previous studies, we have attempted to address these questions,[14–17] by extensive energy conformation calculations for a series of (2*R*,3*R*′)-3-(*m*-hydroxyphenyl)-piperidines for which agonist and antagonist potency data were available. Combining calculated energy-conformation profiles with known experimental structure–activity profiles, we were able to postulate specific types of drug–receptor interactions that could modulate relative analgesic or antagonist potencies. We were, however, unable to fully compare our predictions with pharmacological data on the 3-aryl-3-alkylpiperidine family reported by Kugita et al.[7] because these compounds were evaluated as racemic mixtures. And, while the related benzomorphan opioids manifest their analgesic angonist/antagonist potency almost entirely from the (−) or "natural" *l*-enantiomer, we had no assurance that only one enantiomer would be active in the 3-aryl family.

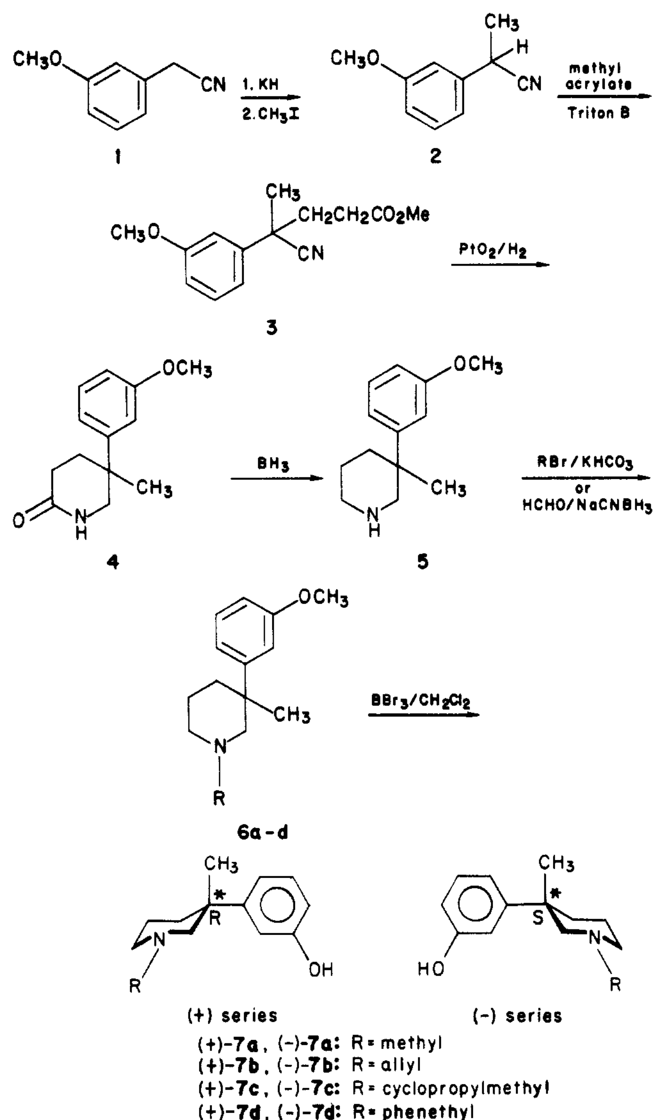
Therefore, to more completely address the requirements

[†] SRI International.
[‡] University of Washington.
[§] University of Toronto.
[‖] Present address: Allergan Pharmaceuticals, 2525 DuPont Drive, Irvine CA 92713.

(1) Michne, W. F.; Lewis, T. R.; Michalec, S. J. "Characteristics and Function of Opiates"; van Ree, J. M., Terenius, L., Eds.; Elsevier/North Holland: Amsterdam, 1978; pp 197–206.
(2) Fries, D. S. "Annual Report in Medicinal Chemistry"; Clark, F. H., Ed.; Academic Press: New York, 1978; Vol. 13, Chapter 5.
(3) DeGraw, J. I.; Lawson, J. A.; Crase, J. L.; Johnson, H. L.; Ellis, M.; Uyeno, E. T.; Loew, G. H.; Berkowitz, D. S. *J. Med. Chem.* **1978**, *21*, 415.
(4) Eisleb, O.; Schaumann, O. *Dtsch. Med. Wochenscher.* **1939**, *65*, 967.
(5) Weiglard, J.; Orahovats, P. D.; Sullivan, A. P.; Purdue, G.; Heath, F. K.; Pfister, K., III *J. Am. Chem. Soc.* **1956**, *78*, 2342.
(6) Lee, J.; Ziering, A.; Heineman, S. D.; Berger, L. *J. Org. Chem.* **1947**, *12*, 885.
(7) Kugita, H.; Inoue, H.; Oine, T.; Hayashi, G.; Nurimoto, S. *J. Med. Chem.* **1964**, *7*, 298.
(8) Kugita, H.; Oine, T.; Inoue, H.; Hayashi, G. *J. Med. Chem.* **1965**, *8*, 313.
(9) Nurimoto, S.; Hayashi, G. *Jpn. J. Pharmacol.* **1973**, *23*, 742.
(10) Jacoby, R. L.; Nieforth, K. A.; Willette, R. E. *J. Med. Chem.* **1974**, *17*, 453.
(11) Iorio, M.; Casy, A. F. *J. Pharm. Pharmacol.* **1975**, *27*, 140.
(12) Iorio, M.; Casy, A. F. *J. Med. Chem.* **1978**, *21*, 812.
(13) Jacoby, R. L.; Boon, D.; Darling, L. E.; Willette, R. E. *J. Med. Chem.* **1981**, *24*, 218.
(14) Burt, S. K.; Loew, G. H. In "Endogenous and Exogenous Opiate Agonist and Antagonist"; Way, E. L., Ed.; Pergamon Press: Elmsford, NY, 1980; p 25.
(15) Burt, S. K.; Loew, G. H.; Loew, Hashimoto, G. M. In "Quantum Chemistry in Biomedical Sciences"; Weinstein, H., Green, J. P., Eds.; The New York Academy of Sciences, New York, 1981; p 219.
(16) Loew, G. H.; Burt, S. K. In "Endogenous and Exogenous Opiate Agonists and Antagonists"; Way, E. L., Ed.; Pergamon Press: Elmsford, NY, 1980; p 39.
(17) Loew, G. H.; Burt, S. K.; Hashimoto, G. M. Proceedings of the 42nd Annual Scientific Meeting, The Committee on Problems of Drug Dependence; Harris, L. S., Ed.; NIDA Research Monograph 34, February 1981; p 399.

0022-2623/86/1829-0531$01.50/0 © 1986 American Chemical Society

Scheme I



for agonist/antagonist activity, we have resynthesized and resolved the known series of N-substituted 3-methyl-3-(*m*-hydroxyphenyl)piperidines (+)- and (−)-**7a**-**d** (Scheme I) in order to investigate their opiate receptor binding behavior and opioid agonist and antagonist pharmacological activities. To the best of our knowledge, no other studies have appeared in which the actions of resolved 3-(*m*-hydroxyphenyl)piperidines have been examined for their in vitro receptor binding and in vivo pharmacological activities. Moreover an X-ray structure determination of one enantiomer, (+)-**5**, has permitted the determination of absolute configurations for this series.

Comparisons of the structure–activity profiles within this family and with fused-ring opiates have aided in the further elucidation of the conformations and orientations (pharmacophores) in which these flexible opiates can bind to opioid receptors and initiate agonist and antagonist activity.

Methods and Materials

1. Chemistry. Our synthesis of the 3-methyl-3-(*m*-hydroxyphenyl)piperidine analogues (Scheme I) follows the procedure of Kugita et al.,[7] but with some modification, which afforded (±)-**5** in 40% overall yield from **1**, compared to the literature result of an 8% yield. Thus, methylation of (*m*-methoxyphenyl)acetonitrile (**1**) in the presence of potassium hydride and methyl iodide provided the known[20] 2-(*m*-methoxyphenyl)propionitrile (**2**). Addition of methyl acrylate to **2** in the presence of a catalytic amount of Triton B afforded the cyano ester **3**. Reductive cyclization of this cyano ester with platinum oxide in glacial acetic acid at room temperature generated the 2-piperidone **4**. Reduction of lactam **4** with diborane in THF gave the piperidine **5**. Direct N-alkylation of **5** with the appropriate alkyl halide and $KHCO_3$ in 2-butanone yielded the desired racemic N-substituted compounds **6**-**d**. A reductive alkylation, using formaldehyde and sodium cyanoborohydride, was employed in the preparation of the *N*-methyl analogue **6a** to avoid quaternization. The aryl methyl esters were cleaved with boron tribromide at 0 °C to give the final racemic products (±)-**7a**-**d**.

Resolved enantiomers of **7a**-**d** were prepared beginning by resolution of (±)-**5**. For the resolution of (±)-**5**, we found that (−)-D-tartaric acid was a convenient resolving agent. After three recrystallizations from 95% EtOH, a pure diastereomeric salt of (+)-**5** was obtained. The combined mother liquors containing enriched (−)-**5**, when treated with (+)-L-tartaric acid, led to the isolation of (−)-**5**, which exhibits an opposite rotation of similar magnitude to (+)-**5**.

Determination of the optical purity of the enantiomers of **5** was achieved by Mosher's method.[21] Compound (±)-**5** was converted to the diastereomeric amides with optically pure (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The ^{19}F NMR spectrum of these diastereomeric amides in $CDCl_3$ displayed two equally intense singlets at δ 9.38 and 8.58 for the two trifluoromethyl resonances. The amide generated from (+)-**5** displayed a major signal at δ 8.58 and a minor one at δ 9.38, while integration of the two peaks showed a ratio of 96:4. This, coupled with the specific rotations of the two enantiomers, establishes that they are equally resolved and suggests that they are 92% optically pure.

Direct N-alkylation of the enantiomers of **5** with the three appropriate alkyl halides, as indicated above and in Scheme I, yielded the desired resolved N-substituted compounds and (−)-**6b**-**d**. Again, the same reductive alkylation, using formaldehyde and sodium cyanoborohydride, was employed in the preparation of the resolved *N*-methyl analogues (−)-**6a** and (+)-**6a**. The aryl methyl ethers were cleaved by boron tribromide to give the resolved target compounds, enantiomers of (+)- and (−)-**7a**-**d**.

2. Opiate Receptor Binding Assay and Data Analysis. Opiate receptor binding assays were performed essentially as described by Pasternak et al.[22] Briefly, rat whole brain homogenates were prepared, preincubated at 37 °C for 1 h and resuspended in Tris, pH 7.7, containing 100 mM NaCl when appropriate, at 6.7 mg of tissues/mL. Receptor-binding incubations contained 1.8 mL of tissue suspension and 0.1 mL of labeled ligand and unlabeled drugs in a total volume of 2.0 mL. The tubes were incubated in triplicate at 25 °C for 45 min prior to filtration.

In the present studies, self- and cross-competition experiments were conducted for four labeled ligands, naloxone, dihydromorphine (DHM), D-Ala[2]-D-Leu[5]-enkephalin

(18) Abdel-Monem, M. M.; Larson, D. L.; Kupfenberg, H. J.; Portoghese, P. S. *J. Med. Chem.* **1972**, *15*, 494.
(19) Sullivan, H. R.; Due, S. L.; McMahon, R. E. *J. Pharmacol.* **1975**, *27*, 728.
(20) Kugita, H.; Oine, T. *Chem. Pharm. Bull.* **1963**, *11*, 253.
(21) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(22) Pasternak, G. W.; Snowman, A. M.; Snyder, S. H. *Mol. Pharmacol.* **1975**, *11*, 735.

Table I. Analgesic Agonist and Antagonist Activity of Racemic 3-methyl-3-(*m*-hydroxyphenyl)piperidine with N-Substituent Variation

compound	writhing test[a] ED$_{50}$, μmol/kg sc	writhing test[a] 95% confidence limits	Straub-tail test[b] AD$_{50}$, μmol/kg sc	Straub-tail test[b] 95% confidence limits
(±)-**7a** *N*-methyl HBr	26.90	16.71–43.32	18.17	11.08–29.80
(±)-**7b** *N*-allyl HCl	418.22	258.16–677.52	38.83	25.06–60.19
(±)-**7c** *N*-cyclopropylmethyl HCl	83.45	52.82–131.85	7.50	3.95–14.24
(±)-**7d** *N*-phenethyl HCl	14.12	8.50–23.44	33.94	22.04–52.57
morphine sulfate	0.81 (0.72)[c]	0.51–1.29 (0.27–2.08)		
nalorphine hydrochloride	1.40	0.84–2.33	0.71 (0.74)[d]	0.43–1.17 (0.42–1.28)
meperidine hydrochloride	20.16	13.44–30.23	*e*	*e*

[a] Inhibition of acetic acid writhing by procedure in ref 24 and 25. [b] Counteraction of the Straub-tail reaction produced by morphine sulfate (56.07 μmol/kg) by method in ref 27. [c] Literature value from ref 13. [d] Literature value from ref 27. [e] No antagonist activity up to 846 μmol/kg.

(DADL), and ethylketocyclazocine (EKC), at two different concentrations of labeled ligand. The resulting data constitute what we call a four-by-four "matrix" of competitive inhibition behavior. In addition to this matrix, inhibition of binding of all four labeled ligands with each pair of enantiomers was performed. Data obtained from all three binding studies were analyzed by a modified version of the program LIGAND,[23] which predicts receptor-binding affinities and capacities using weighted nonlinear, least-squares regression analysis.

In the procedure used, all self- and cross-competition studies involving the four labeled ligands were analyzed together assuming one-, two-, three-, four-, five-, and six-site models of receptor binding, and results were compared for statistical significance and other indications of reliability. Inhibition data for each of the four enantiomeric pairs of N-substituted-3-methyl-3-(*m*-hydroxyphenyl)-piperidines were then added to the matrix obtained for the labeled and unlabeled ligands and the data reanalyzed simultaneously for self-consistent receptor-binding affinities and capacities. Again, one to six site models for receptor binding were systematically explored.

3. Animal Testing. Analgesic potencies of the chemicals and the reference drug (morphine sulfate) were determined by the mouse writhing test, developed by Koster et al.[24] and Blumberg at al.[25] The writhing test was chosen as a measure of analgesic activity since it is sensitive enough to detect activity in these compounds suspected of having antagonist activity. The median effective doses (ED$_{50}$) were derived from the dose–response curves, and the 95% confidence limits were calculated according to the graphic method of Litchfield and Wilcoxon.[26]

In a preliminary assessment of antagonist activities of racemic mixtures, a test of antagonism of the morphine-induced Straub-tail[27] was used. Mice were injected (sc) with a test substance, *N*-allylnormorphine hydrochloride (a reference drug), or the diluent, and immediately afterward with 56.07 μmol/kg (sc) of morphine sulfate. The total number of mice showing Straub-tail reaction during 15–25 min after treatment was recorded. The Straub-tail response was defined as elevation of the tail at angles greater than 45°. In a more complete test for antagonist potency, the resolved enantiomers were evaluated in the test of antagonism of morphine analgesia to the mouse tail-flick response. In this tail-flick assay, the radiant heat

Table II. Effect of Na$^+$ on IC$_{50}$ Value in Inhibition of [^{3}H]Naloxone Receptor Binding

compd	N-R	IC$_{50}$: [^{3}H]naloxone[a] −Na$^+$	+Na$^+$	ratio
(±)-**7a**	methyl	800	1400	1.8
(±)-**7b**	allyl	350	440	1.3
(±)-**7c**	cyclopropylmethyl	165	250	1.6
(±)-**7d**	phenethyl	140	600	4.3
(−)-**7a**	methyl	2000	3200	1.6
(+)-**7a**	methyl	1050	1600	1.5
(−)-**7c**	cyclopropylmethyl	100	230	2.3
(+)-**7c**	cyclopropylmethyl	335	350	1.0
(−)-**7d**	phenethyl	90	440	4.9
(+)-**7d**	phenethyl	55	350	6.4
DHM		2.2	29	13.2
DADL		20	210	10.5
EKC		1.7	18	10.5
naloxone		3.0	3.2	1.1
meperidine		6000	80000	13.3
etorphine		0.35	0.50	1.4

[a] Binding studies conducted as described in the Methods and Materials section. Values shown are averages from at least two experiments with less then 20% variation.

method described by D'Amour and Smith,[28] Harris and Pierson,[29] and Harris et al.[30] has been adapated for use in our laboratory.

4. X-ray Structure Determination: The (−)-D-tartrate salt of the (+) enantiomer of 3-methyl-3-(*m*-methoxyphenyl)piperidine [(+)-**5**] crystalized in an orthorhombic space group $P_{2_12_12_1}$ with cell dimensions: a = 7.534 (3) Å, b = 7.677 (3) Å, c = 30437 (7) Å and $d_{X\text{-ray}}$ = 1.34, Z = 4. Cu Kα radiation was used for measuring 1660 independent reflections. Final values of R = 0.055 and R_w = 0.060 were obtained for 1045 observed reflections.

The crystal data (diffractometer) were reduced by using standard procedures[31] with no absorption correction applied. Routine runs of the program MULTAN 80 did not succeed in solving the structure. Manual input to MULTAN 80 also failed. The structure was finally solved by using the program RANTAN (written by Yao, York University). Refinement was done by full least-squares matrix techniques, anisotropic thermal parameters for non-hydrogen atoms, and fixed isotropic B values for H atoms. All positional parameters were refined. All hydrogen atom positions were located by electron density difference Fourier maps, except for one H atom, which is probably disordered between positions attached to O_{20} and O_{25}

(23) Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.
(24) Koster, R.; Anderson, M.; de Beer, E. J. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1959**, *18*, 412.
(25) Blumberg, H.; Wolf, P. S.; Dayton, H. B. *Proc. Soc. Exp. Biol. Med.* **1965**, *118*, 763.
(26) Litchfield, J. T., Jr.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
(27) Aceto, M. D.; McKean, D. B.; Pearl, J. *Br. J. Pharmacol.* **1969**, *36*, 225.
(28) D'Amour, F. E.; Smith, D. L. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74.
(29) Harris, L. S.; Pierson, A. K. *J. Pharmacol. Exp. Ther.* **1964**, *143*, 141.
(30) Harris, L. S.; Dewey, W. L.; Howes, J. F.; Kennedy, J. S.; Pars, H. *J. Pharmacol. Exp. Ther.* **1969**, *169*, 17.
(31) Karle, I. L.; Karle, J. K.; Mastropaolo, D.; Cammerman, A.; Cammerman, N. *Acta Crystallogr., Sect. B* **1983**, *B39*, 625.

Table III. Receptor Affinities and Maximum Binding Capacities for a Five-Receptor-Site Model

	K_D, nM				
	site 1[a] "μ_1"	site 2 "μ_2"	site 3 "δ"	site 4 "κ"	site 5
naloxone[b,c]	0.4	5.0	18.0	1.0	154
DADL	0.9	22.0	2.1	625.0	10000
EKC	0.5	6.7	14.0	0.3	4160
DHM	0.2	10.0	100.0	830.0	200
3-phenylpiperidines					
7a (–)-*N*-CH_3	114	1170	30000	625	8330
7a (+)-*N*-CH_3	132	1000	15900	714	19200
7b (–)-*N*-allyl	24	110	4000	370	1720
7b (+)-*N*-allyl	100	370	4000	137	3700
7c (–)-*N*-CPM	32	43	4350	122	1700
7c (+)-*N*-CPM	120	256	2700	83	833
7d (–)-*N*-phenethyl	13	130	2170	625	1390
7d (+)-*N*-phenethyl	5.0	6.2	588	179	26300
B_{max}, pmol/g	3.0	30.0	7.8	4.2	233

[a] Values determined by using the curve-fitting program LIGAND, as described in the Methods and Materials section. [b] For computer analysis each of the 3-phenylpiperidines was used to inhibit the four labeled ligands naloxone, DADL, EKC, and DHM. [c] All experiments were conducted at two difference labeled ligand concentrations with 13 concentrations of inhibitor.

(tartrate). The nitrogen N_9 is protonated in a tetrahedral configuration.

Results

Table I presents the results of in vivo pharmacological studies to determine the extent of agonist and antagonist activity displayed by a series of racemic 3-methyl-3-(*m*-hydroxyphenyl)piperidines with varying N-substituents. As indicated in the table, all of the compounds appear to possess both analgesic agonist and antagonist activity as judged by their abilities to inhibit acetic acid induced writhing and morphine-induced Straub-tail, respectively.

As shown in Table II, the moderate affinities of these compounds for inhibition of [^{3}H]naloxone binding indicate that their agonist and antagonist activities are mediated through opioid receptors.

Compounds (±)-**7a**, (±)-**7b**, and (±)-**7d** were previously tested for analgesia by Kugita et al.[7] While our methods are different (hot plate vs. writhing), our order of decreasing agonist activity (±)-**7d** > (±)-**7a** > (±)-**7c** † (±)-**7b** is consistent with that found by Kugita. Though Kugita did not report antagonism data for his compounds, he did note that one compound, (±)-**7b**, appeared to reverse the analgesic effect of morphine at approximately the dose that nalorphine is effective as a morphine antagonist. As shown in Table I, (±)-**7b** shows antagonist activity but at a dose 50 times higher than nalorphine.

Shown in Table III are the affinities of the resolved 3-phenylpiperidine compounds at the multiple opioid receptors obtained by analysis of receptor-binding data by the computer program LIGAND. As described previously,[32] a four-by-four matrix of competitive inhibition data using the labeled and unlabeled analogues of naloxone, DADL, DHM, and EKC yielded a five-receptor-site model that was statistically much more significant than one-, two-, three-, or four-site models and equivalent to a six-site model. Shown in Table III are the affinities of the four compounds used as radiolabeled ligands at sites we labeled "μ_1," "μ_2," "δ," and "κ" to be consistent with values reported by other investigators using a variety of techniques to determine receptor affinities at the various sites.[33-36] The fifth site may be a composite of sites of unknown relation to opioid analgesic activity.

Examination of the affinities at each site for each of the four resolved enantiomeric pairs of 3-phenylpiperidine compounds indicates that, in general, the compounds are μ-selective, with affinities highest at the site we labeled μ_1, the site of highest affinity also for the four standard labeled opioids. A similar site has been proposed by Pasternak and colleagues to be the one that mediates CNS analgesia.[33] Affinities are also moderate at the site labeled κ but substantially lower at δ sites. There are no striking qualitative differences between affinities of (+) and (–)-enantiomers at any site, for any compound.

In contrast to the similarity in binding affinities of the enantiomeric pairs, striking differences were found in their relative agonist and antagonist activities (Table IV). The most obvious differences between in vitro binding and in vivo pharmacology is apparent in the antagonist assay. For all four pairs of enantiomers, the (+) isomers have antagonist activity, while the (–) isomers are inactive as antagonists up to 80 mg/kg (240–300 μmol/kg). Thus, the (–) isomers appear to be pure agonists, while the (+) isomers are mixed agonist/antagonist. Apparently, all of the antagonism found in the racemic mixtures (Table I) resides in the (+) enantiomers. Some stereospecificity is also revealed in the writhing assay for agonism as well. In particular, the (+) isomers of the *N*-allyl and *N*-cyclopropylmethyl analogues are much weaker agonists than the (–) isomers; and in fact, (+)-**7c** is inactive as an agonist up to 700 μmol/kg. Furthermore it is conceivable that some of the agonist component of (+)-**7b** is due to the small amount of contamination from (–)-**7b**. In contrast, the (+) and (–) isomers of the *N*-methyl and *N*-phenethyl analogues have comparable agonist potencies in their inhibition of the writhing response.

For most classes of opiates, Na^+ affects receptor binding by agonists to a much greater extent than binding by antagonists.[37] In general, agonists, but not antagonists, are much less potent in inhibiting [^{3}H]naloxone binding in the presence than in the absence of 100 mM NaCl. Because agonism and antagonism appear to be regulated by different requirements in 3-(*m*-hydroxyphenyl)piperidines, it was of interest to determine whether the differential value of IC_{50} vs. [^{3}H]naloxone in the presence and absence of Na^+ would correlate with the extent of agonist and

(32) Toll, L; Keys, C.; Polgar, W.; Loew, G. *Neropeptides* **1984**, *5*, 205.
(33) Wolozin, B.; Paternak, G. W. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6181.
(34) Chang, K. J.; Hzaum, E.; Cautrecases, P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4141.
(35) Pfeiffer, A.; Sadee, W.; Herz, A. *J. Neurosci.* **1982**, *2*, 912.
(36) James, I. F.; Goldstein, A. *J. Mol. Pharmacol.* **1984**, *25*, 337.
(37) Pert, C. B.; Snyder, S. H. *J. Mol. Pharmacol.* **1974**, *10*, 868.

Table IV. Analgesic and Narcotic Antagonist Potencies of Resolved 3-Methyl-3-(*m*-hydroxyphenyl)piperidines with N-Substituent Variation

compd	agonism mouse writhing test[a] ED50, μmol/kg sc	(95% confidence limits)	antagonism mouse tail-flick test[b] ED50, μmol/kg sc	(95% confidence limits)
N-methyl				
(−)-**7a**	25.69	(14.68–44.96)	c	
(+)-**7b**	41.36	(21.55–79.42)	101.34	(71.45–143.90)
N-allyl				
(−)-**7b**	14.34	(8.96–22.94)	c	
(+)-**7b**	160.94	(106.58–243.02)	112.02	(69.58–180.36)
N-cyclopropylmethyl				
(−)-**7c**	12.23	(2.83–21.41)	c	
(+)-**7c**	d		139.32	(83.93–231.27)
N-phenethyl				
(−)-**7d**	18.38	(11.08–30.52)	c	
(+)-**7d**	11.53	(7.21–18.44)	13.07	(8.71–19.58)
morphine sulfate	0.81	(0.51–1.29)		
nalorphine	1.40	(0.84–2.33)	2.04	(1.44–2.87)

[a] Inhibition of acetic acid induced writhing.[24,25] [b] Antagonism of tail-flick inhibition induced by morphine sulfate (21.08 μmol/kg sc).[28–30] [c] Compounds showed no significant antagonist activity up to dose of 240–300 μmol/kg. [d] Showed no significant agonist activity at a dose of 707.21 μmol/kg.

Table V. Methods and Yields for the Preparation of Racemic *N*-R-3-methyl-3-(3-X-phenyl)piperidines

compound[a]	X	R	method	yield, %	mp, °C (lit.)	empirical formula
(±)-**6a**	OCH_3	methyl	A	90	199–200	$C_{14}H_{22}ClNO$
(±)-**6b**	OCH_3	allyl	B	69	153–154 (149–150)[7]	$C_{16}H_{24}ClNO$
(±)-**6c**	OCH_3	cyclopropylmethyl	B	83	170–171	$C_{17}H_{26}ClNO$
(±)-**6d**	OCH_3	phenethyl	B	72	164–166 (166–188)[7]	$C_{21}H_{23}ClNO$
(±)-**7a**	OH	methyl	C	58	208–209 (209–211)[20]	$C_{13}H_{20}BrNO$
(±)-**7b**	OH	allyl	C	70	170–171 (170–173)[6]	$C_{15}H_{22}ClNO$
(±)-**7c**	OH	cyclopropylmethyl	C	90	115–120	$C_{16}H_{24}ClNO$
(±)-**7d**	OH	phenethyl	C	72	123–125	$C_{20}H_{26}ClNO$

[a] NMR spectra are in accordance with the expected structures.

antagonist potency in this series.

Table II shows IC_{50} values vs. [^{3}H]naloxone in the presence and absence of 100 mM NaCl for the racemic 3-phenylpiperidines, for three of the enantiomeric pairs and for various other opioid ligands. It is evident from this table that Na^+ does not differentially affect binding of the 3-(*m*-hydroxyphenyl)piperidine agonists and antagonists as it does for some other classes of opiates.

The IC_{50} ratios obtained have no relationship to the observed agonist/antagonist potency ratios (Table IV). All (−) enantiomers are pure agonists but have IC_{50} ratios varying from 2.3 to 4.9, while all (+) enantiomers with mixed agonist/antagonist activity have ratios varying from 1.5 to 6.5. Moreover, the *N*-phenethyl analogue (+)-**7d**, which is the most potent antagonist, has a higher IC_{50} ratio than the other (+) isomers. In each instance, the Na^+ shift in IC_{50} obtained for both partners of each enantiomeric pair was similar. Indeed, it appears for this series as if the nature of the N-substituent is more of a factor in determining the effect of Na^+ on receptor binding than is relative agonist or antagonist activity.

X-ray crystal structure of (+)-**5** as a (−)-D-tartrate salt was determined as described in and Methods Materials section. Figure 1 is a representation of the molecule derived from the crystal coordinates[38] and a simulated enantiomer (−)-**5** produced by reflecting the coordinates through a mirror plane. As seen in Figure 1 the phenyl group appears equatorial, and, as expected,[14–17] the piperidine ring is in a chair conformation.

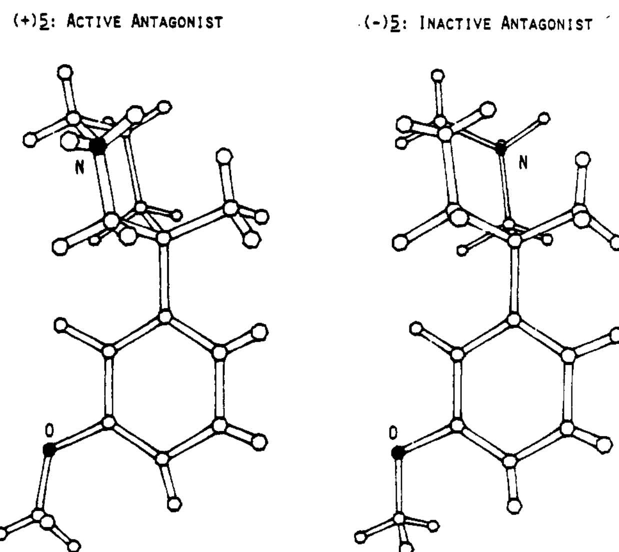


Figure 1. Molecular structure from X-ray determination of (−)-D-tartrate salt of the resolved (+)-**5** analogue 3-methyl-3-(*m*-methoxyphenylpiperidine, and its simulated enantiomer in proposed antagonist orientations. The drawing shows the correct absolute configuration of the molecule based on the known configuration (−)-D-tartrate salt acid. The interring torsion angle $J(C_2C_1C_7C_8)$ = 62.8°.

Discussion

The apparent mixed agonist/antagonist activity determined from preliminary studies on the racemic 3-(*m*-hydroxyphenyl)piperidine compounds was confirmed by more detailed analysis of the resolved enantiomers. Although receptor-binding affinities do not vary significantly among racemic and resolved enantiomers, all of the antagonist activity resides in the (+) isomer of the enantiomeric pair. Some stereospecificity is also apparent in agonist activity of the *N*-allyl and *N*-cyclopropylmethyl but not *N*-methyl or *N*-phenethyl analogues. These results

(38) Crystal coordinates available upon request from author.

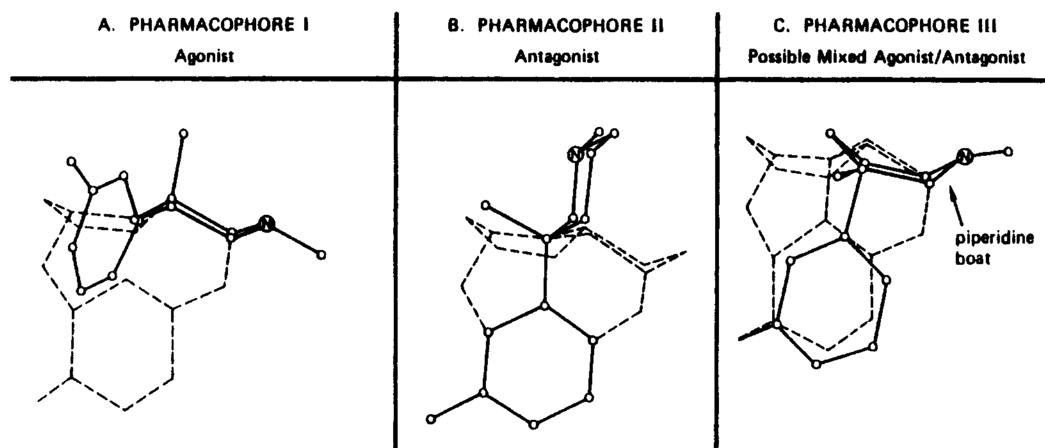


Figure 2. Postulated 3-phenylpiperidine modes of initiating agonist/antagonist activity.

indicate that determination of receptor affinities is not predictive of agonist or antagonist activity.

In this series of compounds, *N*–R variations do not modulate agonist and antagonist activity as they do in fused-ring opiates. For the (−) enantiomers, the N-substituent has very little effect on the activity of the compounds. All the (−) isomers are pure agonists with approximately equal analgesic activity. *N*-Allyl and *N*-cyclopropylmethyl substitutions decrease agonist activity for the (+) enantiomers, as they do for the fused ring opiates. An *N*-phenethyl substitution, however, greatly increases antagonist activity. This is in contrast to fused-ring opiates in which an antagonist with an *N*-phenethyl substituent has never been demonstrated.

For opioid,[37] as well as histamine,[39] and α-adrenergic[40] receptors, it is well known that Na^+ decreases apparent affinity of agonists but not antagonists. This "Na^+ effect" has been used as in vitro predictor of in vivo analgesic activity. The unusual behavioral profile of the resolved 3-phenylpiperidines provided an interesting system to further explore this phenomenon.

In the 3-phenylpiperidines, the extent of agonism or antagonism is unrelated to binding affinity in the presence or absence of Na^+. Even though all of the (−) enantiomers are pure agonists, their abilities to inhibit [^{3}H]naloxone binding do not decrease in the presence of Na^+ to the extent of the fused-ring, flexible, or peptide opioids tested. The lack of similarity between 3-phenylpiperidines and other opioid classes with respect to Na^+ sensitivity may be due to qualitative differences between binding modes possible for these classes of compounds.

The rigidity of the fused-ring opiates probably precludes binding of these compounds in more than a single orientation at receptor sites. On the basis of energy calculations of 3-phenylpiperidine compounds and on the strict requirement for a *m*-hydroxyphenyl moiety for antagonist activity, we have postulated three qualitatively different receptor binding orientations that might lead to agonism and antagonism (Figure 2). In these binding modes, an overlap of the cationic amine nitrogen with that of morphine will lead to agonism (pharmacophore I), while an overlap of *m*-hydroxyphenyl moieties without an amine overlap (pharmacophore II) will produce antagonism regardless of N–R variations. Accordingly, a single compound could produce both agonist and antagonist activity by binding in this bimodal fashion. Conversely, if the 3-phenylpiperidines bind in a piperidine boat conformation with simultaneous overlap with morphine's amine nitrogen and *m*-hydroxyphenyl groups (pharmacophore III), we would expect N–R variations to modulate agonism and antagonism in a manner similar to that in fused-ring opiates.

If agonism and antagonism are mediated by different opiate receptor subtypes, as has been suggested for the mixed agonist/antagonist nalorphine,[41,42] this same scheme may well apply. The presence or absence of the *m*-hydroxyphenyl may direct binding in the two different orientations to two distinct receptor subtypes.

For these 3-methyl-3-(*m*-hydroxyphenyl)piperidines pharmacophores I and II involve a piperidine chair conformation. Pharmacophore III necessitates a piperidine boat conformer, which, although slightly higher in energy, cannot be excluded from receptor-site interactions. The fact that N–R variations do not modulate activity as they do in fused-ring opiates suggests, however, that mixed agonist/antagonist activity is probably due to binding of the piperidine chair conformer at the receptor site(s) in different orientations. X-ray structure determination of the (+) enantiomer of the analogue **5**, 3-methyl-3-(*m*-methoxyphenyl)piperidine reveals that configuration at C_3 is as shown in Scheme I. Furthermore, it was found that the piperidine ring was in a chair conformation, strengthening the argument for bimodal binding orientations leading to agonism and antagonism.

The surprising finding of strict stereospecificity for antagonism but not agonism, along with the X-ray structure determination, provides some clues to the spatial requirements necessary for mediation of a certain response by this class of flexible opioids. As shown in Figure 1, the two enantiomers differ in the proposed antagonist pharmacophore in the position of the cationic piperidine nitrogen relative to the *m*-hydroxyphenyl moiety. In the active enantiomer (+)-**5** the N atom is 1.62 Å above the plane of the phenyl ring and is on the same side of the C_1–C_7 (interring bond) as the oxygen atom. In the inactive antagonist, the N atom is 1.62 Å below the plane of the phenyl ring and on the opposite side of the C_1–C_7 (interring bond) as the oxygen atom. Apparently, with the *m*-OH in a position to initiate antagonist activity, only the cationic amine positioned as in the (+) enantiomer finds a complementary (anionic) receptor site compatible with this type of receptor interaction. By contrast, when the 3-(*m*-hydroxyphenyl)piperidine is being directed by the amine

(39) Chang, R. S. L.; Snyder, S. H. *J. Neurochem.* **1980**, *34*, 916.
(40) Greenberg, D. A.; O'Prichard, D. C.; Sheehan, P.; Snyder, S. H. *Brain Res.* **1978**, *140*, 378.
(41) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Hoppler, R. E.; Gilbert, P. E. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517.
(42) Takemori, A. E.; Rufferberg, H. J.; Miller, J. W. *J. Pharmacol. Exp. Ther.* **1969**, *169*, 39.

nitrogen to an agonist pharmacophore (I), the relative position of the *m*-OH appears to be less crucial, allowing some agonist activity in both enantiomeric pairs of all but the cyclopropylmethyl analogue (−)-**7c**.

Experimental Section

All reactions were performed under a nitrogen or argon atmosphere, and solvents were removed on a rotary evaporator under vacuum. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on Varian EM-360 or 390 instruments. Chemical shift values are reported in parts per million (δ) relative to Me_4Si. MS were determined on a LKB 9000 spectrometer equipped with a gas chromatograph and a PDP12 computer. Analytical HPLC was carried out on a Waters Radialpak Column, and preparative liquid chromatography was performed on a Waters Prep LC/500 system. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and are within ±0.4% of theoretical values.

2-(*m*-Methoxyphenyl)propionitrile (2). Into a 1-L, three-necked, round-bottomed flask were placed 350 mL of THF (freshly distilled) and KH (35% in mineral oil, 19.5 g, 0.17 mol). A solution of (*m*-methoxyphenyl)acetonitrile (25 mg, 0.17 mol) in 100 mL of dry THF was added dropwise over a period of 1.5 h with ice cooling. After 1 h, the reaction mixture was treated with iodomethane (10.6 mL, 0.17 mol), added dropwise, and stirred overnight at room temperature. After workup, 29 g of crude product was revealed by ^1H NMR and HPLC to be a 25:55:20 ratio of dimethylated material to monomethylated product (**2**) to starting material. The oil was chromatographed on a silica gel prep-LC column, eluting with a mixture of hexane/CH_2Cl_2 (4:6), and pure **2** was recovered: 15.5 g (55%); NMR ($CDCl_3$) δ 6.90–7.47 (m, 4 H, Ar H), 3.83 (q, 1 H, CH), 3.76 (s, 3 H, OCH_3), 1.56 (d, 3 H, CH_3).

Methyl 4-Cyano-4-(*m*-methoxyphenyl)pentanoate (3). By the Kugita method,[20] **2** (15.5 g, 90 mmol) and Triton B (2.2 mL, 13.2 mmol) in 80 mL of dioxane was added to methyl acrylate (16 g, 180 mmol) to produce the crude adduct **3**. This was then subjected to silica gel column chromatography (Prep-LC) and eluted with a mixture of CH_2Cl_2/hexane (1:1) and finally with CH_2Cl_2, to give 22 g (92%) of **3**: NMR ($CDCl_3$) δ 6.77–7.53 (m, 4 H, Ar H), 3.83 (s, 3 H, OCH_3), 3.63 (s, 3 H, $COOCH_3$), 1.80–2.50 (m, 4 H, CH_2), 1.73 (s, 3 H, CH_3).

5-Methyl-5-(*m*-methoxyphenyl)-2-piperidone (4). A mixture of **3** (23 g, 93.1 mmol), platinum oxide (82%, 3 g), and 170 mL of glacial acetic acid was shaken in a Parr hydrogenator under H_2 (63 psi) for 2.5 days at room temperature. The mixture was worked up to give 19.4 g (95%) of **4**, a colorless oil that crystallized upon standing at room temperature: mp <55 °C (lit.[7] mp 58–60 °C); NMR ($CDCl_3$) δ 7.73 (br s, 1 H, NH), 6.70–7.50 (m, 4 H, Ar H), 3.80 (s, 3 H, OCH_3), 3.13–3.67 (m, 2 H, CH_2N), 2.0–2.53 (m, 4 H, CH_2), 1.33 (s, 3 H, CH_3).

3-Methyl-3-(*m*-methoxyphenyl)piperidine (5). Piperidone **4** (10.3 g, 47 mmol) in 50 mL of dry THF was reduced with diborane in THF (1 M, 80 mL, 30 mmol) as described by Brown and Heim[43] to give 8.2 g (85%) of **5** as a colorless oil: NMR ($CDCl_3$) δ 6.60–7.43 (m, 4 H, Ar H), 3.80 (s, 3 H, OCH_3), 1.33–3.50 (m, 8 H, CH_2), 1.17 (s, 3 H, CH_3). An analytical sample was obtained by converting the free base to the tartrate salt, mp 188–190 °C. Anal. ($C_{17}H_{25}NO_7$) C, H, N.

1,3-Dimethyl-3-(*m*-methoxyphenyl)piperidine (6a; Method A, Table V). To a stirred solution of piperidine **5** (0.49 g, 2.4 mmol) and 37% aqueous formaldehyde (1 mL, 3.8 mmol) in 10 mL of acetonitrile (sieve-dried) was added sodium cyanoborohydride (0.23 g, 3.6 mmol) and then glacial acetic acid was added dropwise until the solution tested neutral. Reaction was immediate, and after routine workup the reaction afforded 0.47 g (90%) of **6a** as a colorless oil: NMR ($CDCl_3$) δ 6.60–7.50 (m, 4 H, Ar H), 3.77 (s, 3 H, OCH_3), 1.33–2.80 (m, 8 H, CH_2), 2.27 (s, 3 H, NCH_3), 1.27 (s, 3 H, CH_3). The analytical sample was prepared by dissolving the free base in dry Et_2O and adding, dropwise at 0 °C, a small amount of ether saturated with dry HCl gas. The white hydrochloride salt was recrystallized from a mixture of 2-propanol/ether to yield **6a**·HCl as a white solid: mp 199–200 °C. Anal. ($C_{14}H_{22}ClNO$) C, H, N, Cl.

General Procedure for the Preparation of *N*-Alkyl-3-phenylpiperidines (6b–d; Method B). 1-Allyl-3-methyl-3-(*m*-methoxyphenyl)piperidine (6b). A mixture of piperidine **5** (0.83 g, 4.0 mmol), $KHCO_3$ (3.35 g, 36.4 mmol), and allyl bromide (0.32 mL, 4.2 mmol) in 15 mL of 2-butanone was stirred at reflux for 24 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum. The residue was taken up in 25 mL of dilute HCl solution and washed with Et_2O (2 × 20 mL). The acid layer was made basic with solid K_2CO_3 and then extracted with Et_2O (3 × 25 mL). The organic layers were dried (Na_2SO_4) and concentrated in vacuo to yield 0.68 g (69%) of **6b** as a colorless liquid: NMR ($CDCl_3$) δ 6.57–7.50 (m, 4 H, Ar H), 5.50–6.30 (m, 1 H, C*H*=CH_2), 4.83–5.43 (m, 2 H, CH=C*H*$_2$), 3.76 (s, 3 H, OCH_3), 2.99 (d, 2 H, NCH_2), 1.27–3.00 (m, 8 H, CH_2), 1.27 (s, 3 H, CH_3). The analytical sample was prepared as for **6a** to provide **6b**·HCl: mp 153–154 °C (lit.[7] mp 149–150 °C). Anal. ($C_{17}H_{26}ClNO$) C, H, N, Cl.

General Procedure for the O-Demethylation of N-Substituted 3-Phenylpiperidines (7a–d; Method C). 1,3-Dimethyl-3-(*m*-hydroxyphenyl)piperidine (7a). To amine **6a** (0.31 g, 1.4 mmol) in 30 mL of CH_2Cl_2 was added, dropwise with stirring, boron tribromide (0.27 mL, 2.8 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and then quenched by dropwise addition of 10 mL of anhydrous methanol. The solvent was removed in vacuo, and the residue was treated with an additional 20 mL of methanol. The solvent was again removed, leaving a foamy residue which was dissolved in 10 mL of 3 N HCl and stirred for 0.5 h at room temperature. This was then made basic by solid $NaHCO_3$ and extracted with $CHCl_3$ (3 × 30 mL). The organic extracts were dried (Na_2SO_4) and concentrated to give 0.20 g (58%) of **7a**·HBr: mp 208–209 °C (lit.[7] mp 209–211 °C); NMR ($CDCl_3$) of the free base δ 8.00 (br s, 1 H, OH), 6.34–7.35 (m, 4 H, Ar H), 1.25–3.00 (m, 8 H, CH_2), 2.30 (s, 3 H, NCH_3), 1.20 (s, 3 H, CH_3). An analytical sample was obtained by converting the free base to the picrate salt, mp 168–170 °C. Anal. ($C_{19}H_{22}N_4O_8$) C, H, N.

Acknowledgment. Support for this work by National Institute of Drug Abuse Grant No. DA 02622 is gratefully acknowledged.

Registry No. 1, 19924-43-7; (±)-**2**, 100189-39-7; (±)-**3**, 100189-40-0; (±)-**4**, 100189-41-1; (±)-**5**, 100189-42-2; (+)-**5**·(-)-D-tartrate, 100295-35-0; (-)-**5**·(+)-L-tartrate, 100295-37-2; (+)-**5** ((-)-α-methoxy-α-trifluoromethyl)phenylacetamide), 100189-51-3; (-)-**5** ((-)-α-methoxy-α-trifluoromethyl)phenylacetamide), 100189-50-2; (±)-**6a**, 55803-46-8; (±)-**6a**·HCl, 100189-43-3; (±)-**6b**, 100189-44-4; (±)-**6b**·HCl, 100189-45-5; (±)-**6c**, 100205-32-1; (±)-**6c**·HCl, 100189-46-6; (±)-**6d**, 100189-48-8; (±)-**6d**·HCl, 100189-47-7; (-)-**7a**, 100187-20-0; (+)-**7a**, 100187-16-4; (±)-**7a**·HBr, 100295-28-1; (±)-**7a**·picrate, 100295-30-5; (-)-**7b**, 100187-21-1; (+)-**7b**, 100187-17-5; (±)-**7b**·HCl, 100295-31-6; (-)-**7c**, 100187-22-2; (+)-**7c**, 100187-18-6; (±)-**7c**·HCl, 100295-32-7; (-)-**7d**, 100189-49-9; (+)-**7d**, 100187-19-7; (±)-**7d**·HCl, 100295-33-8; methyl acrylate, 96-33-3; allyl bromide, 106-95-6; cyclopropylmethyl bromide, 7051-34-5; phenethyl bromide, 103-63-9.

(43) Brown, H. C.; Heim, P. *J. Am. Chem. Soc.* 1964, *86*, 3566.