Antipodal α-*N*-(Methyl through Decyl)-*N*-normetazocines (5,9α-Dimethyl-2′-hydroxy-6,7-benzomorphans): In Vitro and in Vivo Properties

Everette L. May,*,† Mario D. Aceto,† Edward R. Bowman,† Christine Bentley,† Billy R. Martin,† Louis S. Harris,† Fedor Medzihradsky,‡ Mariena V. Mattson,§ and Arthur E. Jacobson§

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298-0613; Departments of Biological Chemistry and Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109; and Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received April 29, 1994®

The enantiomeric (−)- and (+)-*N*-(methyl through decyl) normetazocines (5,9α-dimethyl-2′-hydroxy-6,7-benzomorphans) were synthesized and their in vitro and in vivo activities determined. Increasingly bulky enantiomeric *N*-alkyl homologs were prepared until their interaction with the σ_1 receptor decreased and their insolubility became a hindrance to their evaluation in vivo and/or in vitro. The (−)-methyl, -pentyl, -hexyl, and -heptyl homologs were essentially as potent as, or more potent than, morphine in the tail-flick, phenylquinone, and hot-plate assays for antinociceptive activity; the (−)-propyl homolog had narcotic antagonist activity between that of nalorphine and naloxone in the tail-flick vs morphine assay, and it also displayed antagonist properties in the single-dose suppression assay in the rhesus monkey. The antinociceptively potent (−)-heptyl homolog did not substitute for morphine in monkeys but did show morphine-like properties in a primary physical-dependence study in continuously infused rats. All five potent compounds showed high affinity for the μ opioid receptor from both rat and monkey preparations and the κ opioid receptor (<0.05 μM), and all except the (−)-methyl homolog interacted reasonably well at the δ receptor (K_i <0.1 μM). The (−)-propyl compound was equipotent (K_i 1.5–2.0 nM) at μ and κ receptors. The pattern of interaction of the (−)-enantiomeric homologs with μ receptors from rat and monkey preparations was similar, but not identical. The enantioselectivity of the homologs for μ receptors was greater in the rat than in the monkey preparation for all but the N-H and butyl compounds, and the enantioselectivity of the lower homologs (methyl through butyl) for the μ (monkey) receptor was greater than for the κ or δ receptors. However, bulkier homologs (hexyl through decyl) displayed higher enantioselectivity at κ or δ receptors than at the μ (monkey) receptor. The (+)-butyl through (+)-octyl homologs were essentially equipotent with, or more potent than, (+)-pentazocine at the σ receptor. Only the (+)-H and (+)-methyl homologs had high affinity (<0.05 μM) at PCP binding sites.

During the 1950s to 1970s, racemic 2-(methyl to hexyl) 5,9α-dimethyl-2′-hydroxy-6,7-benzomorphans were synthesized and their antinociceptive activity (in mice)[1–4] and physical-dependence capacity (in monkeys)[5–11] determined. A curious mixture of activities was observed. Essentially equipotent (morphine-like) antinociceptive activity was found for the racemic *N*-methyl, -pentyl, and -hexyl homologs, and only the racemic *N*-propyl homolog was noted to have narcotic antagonist activity. Racemic compounds with intermediate length alkyl groups (*N*-ethyl, -propyl, and -butyl) were much less potent or were inactive as agonists or antagonists. Both 2′-hydroxy-2,5,9α-trimethyl-6,7-benzomorphan (metazocine)[12–14] and *N*-normetazocine (5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan)[15,16] were previously resolved, and a separation of opioid-like effects was noted on optical resolution of several 2-(*N*)-methyl-6,7-benzomorphans.[14,17–19] Although the (+)-enantiomers were not generally further examined when they failed to show antinociceptive or narcotic antagonist activity, it was noted[15] that the (+)-isomers deserved further exploration.

Our present work to determine the in vitro and in vivo activities of the antipodal *N*-methyl to -hexyl compounds and the extension of this series by synthesis of the enantiomers of the longer-chain *N*-alkyl homologs (*N*-heptyl to -decyl), is aimed at the possibility of SAR rationalization of these data and possible future exploration of the relationship between their theoretical interaction with (cloned and sequenced) receptors and these experimental data using computer-assisted molecular modeling.

The receptors which have been found to interact with opioids such as the 6,7-benzomorphans have a long and complicated history. That history, and the postulated functions of the opioid receptor subtypes, has been described.[20–22] It is now well-established by a great deal of experimental work that there are three main classes of opioid receptors, μ, δ, and κ. Much work has also been expended in investigating subtypes of the μ,[23,24] δ,[25,26] and κ[27] receptors and, recently, the cDNA's of all three opioid receptors have been cloned and their amino acid sequences determined.[28–35]

N-Substituted normetazocine homologs (e.g., *N*-allyl) are also known to interact with the phencyclidine (PCP) binding site in the *N*-methyl-D-aspartate (NMDA) receptor system as well as with the σ receptor. The σ receptor recently received its current definition. This

† Virginia Commonwealth University.
‡ University of Michigan Medical School.
§ National Institutes of Health.
® Abstract published in *Advance ACS Abstracts*, September 1, 1994.

0022-2623/94/1837-3408$04.50/0

receptor is classified[36,37] as a haloperidol-sensitive, non-dopaminergic, non-PCP, non-opioid binding site, and it is thought to play a significant role in biological functions[38,39] including the regulation of motor behavior,[40-43] the modulation of smooth muscle contraction,[44-46] the enhancement of norepinephrine and dopamine release,[44] the negative modulation of phosphoinositide metabolism,[47,48] and the inhibition of neuronal firing rates.[49,50] Several benzomorphans, usually the (+)-enantiomers, are known to interact with the σ receptor. One such compound, (+)-[^{3}H]SKF 10,047 (*N*-allylnormetazocine), was formerly used as the radioligand for the σ-binding assay,[51] although a more potent and σ-selective benzomorphan, (+)-[^{3}H]pentazocine (5,9-dimethyl-2-(dimethylallyl)-2′-hydroxy-6,7-benzomorphan) is now more frequently used for these binding studies.[52-54] There are at least two subtypes of σ receptors.[37,55] The (+)-benzomorphan ligands which displace (+)-[^{3}H]pentazocine from membranes of guinea pig brains have been shown to have high σ_1 selectivity; the (−)-benzomorphans fail to discriminate between the two subtypes.[54,56] A considerable variety of compounds, of very different structural types, have been noted to interact with σ_1 and/or σ_2 sites.[36,57]

PCP (phencyclidine, 1-(1-phenylcyclohexyl)piperidine) sites are generally considered to exist in excitatory amino acid cation channels regulated by the NMDA/glutamate receptor complex and PCP-like ligands act as noncompetitive antagonists at that receptor.[58-60] The ion channels regulated by the NMDA/glutamate receptor complex under normal conditions are thought to be involved with cellular and molecular processes underlying learning and memory.[61,62] Drugs which act at PCP sites have been found to display anticonvulsant actions,[63] and some may eventually find a medical role as antitrauma agents since they are known to inhibit excessive Ca^+ flow caused by hypoxic−ischemic injury[64] which may lead to neuronal destruction. Various ligands from the benzomorphan class of opioids have been found to interact with PCP receptors.[51,65]

Thus, benzomorphans have been found which interact with opioid and σ receptors and with PCP sites in the NMDA/glutamate receptor complex. In that context, we thought that it would be of interest to study a complete series of *N*-alkyl substituted *N*-normetazocines in both the (+)- and the (−)-enantiomeric series (Table 1), both in vitro (through binding studies at opioid and σ receptors, and PCP sites (Table 2)) and in vivo (antinociceptive assays in mice, and single-dose suppression studies in monkeys (Table 5) to determine their ability to substitute for morphine and, thus, demonstrate morphine-like side effects in primates). A similar study of *N*-aralkyl and -alkenyl normetazocine homologs was recently published.[57]

Chemistry

The α-(−)-(1*R*,5*R*,9*R*) and α-(+)-(1*S*,5*S*,9*S*)-*N*-normetazocines (**1a** and **1l**) were used as starting materials.[13,16,66] Compounds **1c−k** and **1n−v** (*N*-ethyl- to *N*-decyl-α-*N*-normetazocines, Table 1) were prepared from α-(−)- and -(+)-*N*-normetazocines (**1a** and **1l**), respectively, and appropriate alkyl bromides (iodides for *N*-ethyl, *N*-nonyl and *N*-decyl) with $KHCO_3$ as the hydrogen halide acceptor. The solvent system was THF−DMSO (ca. 5:1).[15] For the *N*-heptyl to *N*-decyl homologs refluxing EtOH (dried over K_2CO_3) was also satisfactory. Some O,N-dialkylation was noted for higher homologs. The course of reaction was monitored by thin-layer chromatography. Yields were 70−95%. The melting points and optical rotations of the compounds are listed in Table 1. The enantiomers of each of the *N*-alkyl compounds showed equal and opposite optical rotations, and their melting points were essentially identical.

Binding Studies

The binding studies were carried out with opioid receptors obtained from rat whole brain minus cerebellum and rhesus monkey cortex. The former were useful for comparison with data from others,[57] and the latter for correlation between in vitro binding and in vivo single-dose suppression studies in monkeys. Membranes from guinea pig brain plus cerebellum were used for the σ assay; PCP assays used the rat whole brain minus cerebellum.

Pharmacology

Antinociceptive studies were determined through hotplate (HP), tail-flick (TF), and phenylquinone (PPQ) assays. The tail-flick assay vs morphine (TFA) was used to determine the narcotic antagonist activity of the various compounds. All of these assays were carried out in the mouse. Single dose suppression (SDS) studies were carried out in rhesus monkeys (*M. mulatta*). Substitution for morphine (SM) and a primary physical dependence study (PPD) were carried out in continuously infused rats for a few compounds.

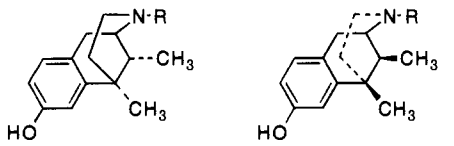
Results

In Vitro Binding: (−)-Enantiomers. In the μ opioid preparation from rhesus monkey cortex the rank order of potency was found to be: propyl > butyl ≈ methyl > pentyl ≈ ethyl > heptyl > hexyl > H ≈ octyl > nonyl > decyl, and at the κ opioid receptor: propyl > butyl > ethyl ≈ methyl > pentyl > heptyl > hexyl > octyl > H > nonyl > decyl (Table 2). In general, although the potencies were higher overall in the μ than the κ assay, the rank order for potency at the μ and κ receptors were very similar (Figure 1). The compounds were much less potent in the δ receptor assay, and the rank order was different: propyl > heptyl ≈ hexyl ≈ butyl > pentyl ≈ octyl > methyl ≈ ethyl > H ≈ nonyl > decyl, although the overall pattern of activity was similar to the displacements observed with the μ and κ receptors (Figure 1).

In the μ opioid rat whole brain minus cerebellum preparation, a different rank order was displayed: hexyl > propyl ≈ heptyl > methyl ≈ butyl > pentyl > H > octyl ≈ ethyl > nonyl > decyl (Figure 2). However, as shown in Figure 2, the overall pattern of interaction of these compounds in the rat and monkey preparations was similar.

At the σ receptor, the *N*-H, -methyl, -ethyl, and -propyl homologs had little activity. Potency increased with the (−)-butyl homolog and maximized with the bulkier (more lipophilic) pentyl, hexyl, heptyl, octyl, nonyl, and decyl homologs (Table 2 and Figure 3), which were found to be essentially equipotent. The σ activity of the (−)-enantiomers bore little or no relationship to their potency in the μ, κ, or δ opioid assays (Table 2).

Table 1. Physical Constants of (−)- and (+)-α-*N*-(Methyl through Decyl)-*N*-normetazocines[a]



1

1a - 1k = (-) series (**1a** = (-)-H)

1l - 1v = (+)-series (**1l** = (+)-H)

compound	R	$[\alpha]^{20-25}_D$	concn	mp, °C
1b[5]	(−)-CH_3	−84.8	0.90	183−4.5
·HCl		−52.0	2.00 (H_2O)	249−50 (d)
1m[5]	(+)-CH_3	+84.3	0.83	183−4.5
·HCl		+52.1	1.46 (H_2O)	249−50 (d)
1c	(−)-C_2H_5	−94.0	0.298	144−5 (149−150)
·HCl		−69.6	0.460	282−4
1n	(+)-C_2H_5	+94.5	0.505	144−5 (149−150)
·HCl		+70.1	0.455	281−3 (d)
1d	(−)-C_3H_7	−99.9	0.711	175−6
·HCl·0.5(2-PrOH)		−71.8	0.464	223−4.5
1o	(+)-C_3H_7	+100.3	0.395	174−5
·HCl·0.5(2-PrOH)		+71.6	0.398	223−4 (d)
1e	(−)-C_4H_9	−102.3	0.346	128.5−30
·HCl·0.5H_2O		−75.2	0.536	105−10 (froth)
1p	(+)-C_4H_9	+107.2	0.493	128−9.5
·HCl·H_2O		+75.6	0.748	105−10 (froth)
1f	(−)-C_5H_{11}	−98.5	0.460	114−5
·HCl		−80.1	0.574	234−6
1q	(+)-C_5H_{11}	+98.2	0.540	114−5
·HCl		+80.1	0.778	234−6
1g·HCl	(−)-C_6H_{13}	−77.7	0.701	236−7
1r·HCl	(+)-C_6H_{13}	78.8	0.495	236−7
1h·HCl	(−)-C_7H_{15}	−77.2	0.421	190.1
1s·HCl	(+)-C_7H_{15}	+77.7	0.475	190.1
1i·HCl	(−)-C_8H_{17}	−72.2	0.331	200−1
1t·HCl	(+)-C_8H_{17}	+72.2	0.297	201−2
1j·HCl	(−)-C_9H_{19}	−70.6	0.361	206−7
1u·HCl	(+)-C_9H_{19}	+70.0	0.361	206−7
1k·HBr	(−)-$C_{10}H_{21}$	−62.2	0.226	198−9
1v·HBr	(+)-$C_{10}H_{21}$	+62.8	0.519	198−9

[a] Elemental analyses for CHN were found to be within 0.4% of theory.

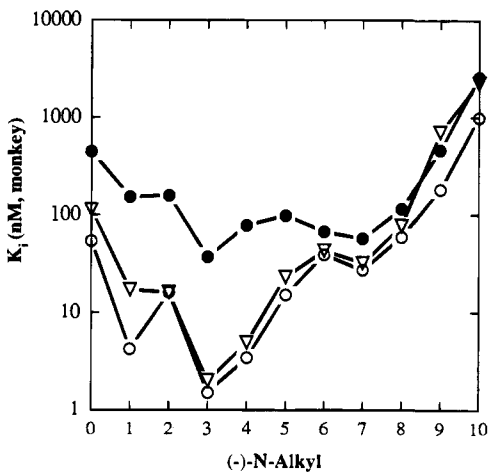


Figure 1. Comparison of potencies of (−)-α-*N*-(H through decyl)-*N*-normetazocines in opioid receptor assays (K_i, nM) using rhesus monkey cortex membranes. Radioligands were [^{3}H]Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO) for μ opioid receptors (○), [^{3}H]-D-Pen^2,D-Pen^5)enkephalin (DPDPE) for δ opioid receptors (●), and [^{3}H]-(5a,7a,8b)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]-dec-8-yl]benzeneacetamide (U69,-593) for κ opioid receptors (▽), as described in the Methods section.

With the exception of the (−)-*N*-H and -propyl homologs, none of the compounds showed affinity <0.5 μM in the PCP binding site assay (Table 2 and Figure 3).

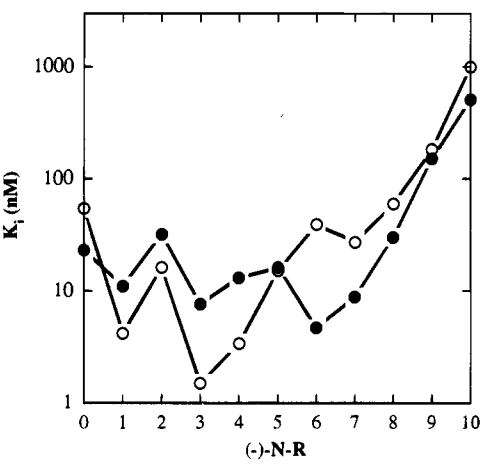


Figure 2. Comparison of potencies (K_i, nM) of (−)-α-*N*-(H through decyl)-*N*-normetazocines for the μ opioid receptors in rhesus monkey cortex membranes (○) and rat whole brain minus cerebellum (●), by displacement of [^{3}H]Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO) as described in the Methods section.

There was no relationship between the affinity of the homologs for the PCP and σ binding sites. The potency ratio (PCP/σ, Table 3) varied from 0.03 for the (−)-H homolog to 228 for the N-octyl compound. The (−)-*N*-H, -methyl, -ethyl, and -propyl homologs were more potent at PCP sites than at σ receptors and the remain-

Table 2. Displacement Studies (K_i, nM) of (−)- and (+)-α-*N*-(Methyl through Decyl)-*N*-normetazocines

N-R	μ (monkey)[a]	μ (rat)[b]	δ (monkey)[a]	κ (monkey)[a]	σ[c]	PCP[d]
(−)-H	54	23	449	113	6750	197
(−)-methyl	4.2	11	152	17	18100	1190
(−)-ethyl	16	32	158	16	16600	1865
(−)-propyl	1.5	7.6	37	2	739	264
(−)-butyl	3.4	13	78	4.9	104	613
(−)-pentyl	15	16	98	23	45	2468
(−)-hexyl	39	4.7	67	43	40	3347
(−)-heptyl	27	8.8	57	32	49	5634
(−)-octyl	59	30	114	77	35	7975
(−)-nonyl	183	151	464	709	32	6166
(−)-decyl	997	507	2584	2265[e]	38	10000
(+)-H	1721	470	7000	3776	2733	30
(+)-methyl	1378	4970	7000	3776	1330	41
(+)-ethyl	4186	10000	7000	3776	1090	130
(+)-propyl	943	5218	7000	452	43	606
(+)-butyl	1241	2876	7000	783	6	893
(+)-pentyl	776	1742	7000	738	3.8	1703
(+)-hexyl	318	795	788	2265	2.3	7305
(+)-heptyl	259	243	7000	723	1.9	2567
(+)-octyl	160	522	1586	2265	2.8	786
(+)-nonyl	126	1379	2934	2265	12	4146
(+)-decyl	756	4622	4200	2265	69	4193

[a] Opioid K_i values from rhesus monkey cortex membranes were obtained from displacement studies in the presence of NaCl using 0.5 nM [^{3}H]DAMGO (Tyr-D-Ala-Gly-NMe-Phe-Gly-ol) (μ), 1.5 nM [^{3}H]DPDPE (D-Pen^2,D-Pen^5)enkephalin) (δ), or 1.5 nM [^{3}H]U69,593 ((5a,7a,8b-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]-dec-8-yl]benzeneacetamide) (κ), as described in the Methods section. In duplicate experiments the radiolabeled ligand was displaced by five different concentrations of the test compound. IC_{50} values were determined by linear regression analysis and converted to K_i values using the Cheng−Prusoff equation[73] and the following K_d values (nM): [^{3}H]DAMGO (0.36); [^{3}H]DPDPE (3.50); [^{3}H]U69,593 (0.91). Shown are averages of two experiments with less than 10% difference between the respective K_i values. For reference, the K_i of U69,593 was found to be 1.18 nM by displacement of [^{3}H]U69,593 (κ), the K_i of DPDPE was 2.95 nM by displacement of [^{3}H]DPDPE (δ), and the K_i of DAMGO was 0.46 nM by displacement of [^{3}H]DAMGO. [b] [^{3}H]DAMGO was displaced from homogenates of rat brain minus cerebellum using five concentrations of the test drug as described in the Methods section. Conversion of IC_{50} to K_i values was performed using the Cheng−Prusoff equation[73] and the [^{3}H]DAMGO K_d value of 1.9 nM. The results represent the mean ± SEM for three experiments. The average standard error of the mean for all of the compounds was 12.8%. [c] For the σ receptor assay, displacement of 3 nM [^{3}H]pentazocine from homogenate of guinea pig brain plus cerebellum was conducted as described in the Methods section. The K_i values shown were calculated from the Cheng−Prusoff equation[73] using the K_d of (+)-[^{3}H]pentazocine of 3.4 nM obtained by Scatchard analysis and are the mean (± SEM) of three or more separate experiments. The average standard error of the mean for all of the compounds was 11.4%. For reference, the K_i values for the standard drugs (+)-SKF 10,047 (*N*-allylnormetazocine), (−)-SKF 10,047, and (+)-pentazocine are 124 ± 18, 4300 ± 300 and 4.4 ± 0.3 nM, respectively. [d] [^{3}H]TCP (1-[1-(2-thienylcyclohexyl)]piperidine) was displaced from homogenates of rat brain minus cerebellum using five concentrations of the test drug as described in the Methods section. Conversion of IC_{50} to K_i values was performed using the Cheng−Prusoff equation[73] and the [^{3}H]TCP K_d value of 7.7 nM. The results represent the mean ± SEM for three experiments. The average standard error of the mean for all of the compounds was 12.0%. [e] Less than 50% displacement at 2265 nM.

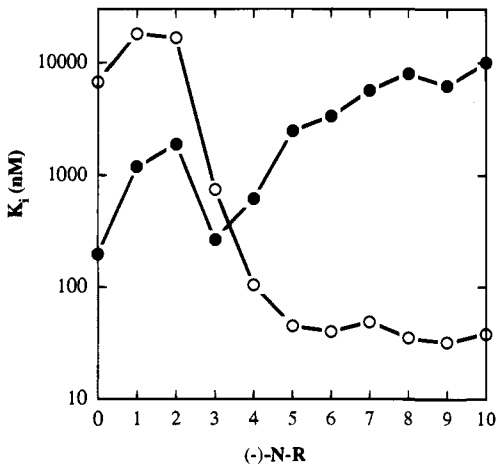


Figure 3. Comparison of potencies (K_i, nM) of (−)-α-*N*-(H through decyl)-*N*-normetazocines for σ (○) and PCP (●) binding sites. The σ assay was carried out in guinea pig brain plus cerebellum homogenates by displacement of [^{3}H]pentazocine and PCP assays were carried out using rat whole brain minus cerebellum as described in the Methods section.

ing (−)-homologs were much more potent at σ receptors than at PCP sites.

(+)-Enantiomers. The (+)-nonyl (K_i 126 nM), octyl (K_i 160 nM), heptyl (K_i 259 nM), and hexyl (K_i 318 nM) homologs were reasonably potent in the μ opioid monkey preparation; only the heptyl (K_i 243 nM), H (K_i 470 nM), and octyl (522 nM) homologs showed K_i <0.6 μM in the μ opioid rat preparation (Table 2). None of the other (+)-N-substituted compounds showed much affinity for the μ receptor. The hexyl homolog had a little affinity for the δ receptor (K_i 788 nM); the K_i's of the remaining compounds were >1.5 μM. In the κ receptor assay, only the propyl homolog was found to have K_i < 0.5 μM. The potency of the (+)-enantiomers at the σ receptor (Figure 4) was similar to the profile shown by the (−)-enantiomers, except that the more potent (+)-compounds showed exceptional activity. Although the (+)-*N*-butyl to -octyl homologs were all very potent (the heptyl homolog (K_i 1.9 nM) was about twice as potent as (+)-pentazocine (K_i 4.4 nM)), a drop-off in potency was noted with the nonyl and decyl homologs. The σ activity bore little or no relationship to the potency of these (+)-enantiomers in the μ, κ, or δ opioid assays (Table 2)).

Only the (+)-H, (+)-methyl, and (+)-ethyl enantiomers were reasonably potent (K_i < 0.5 μM) in the PCP binding assay (Table 2), and they, unlike all the other (+)-homologs, were more potent at PCP sites than at the σ receptor. With the (+)-enantiomers, the PCP/σ potency ratio varied from 0.01 for the *N*-H homolog to 3176 for the hexyl.

Receptor Specificity. The (−)-methyl, (+)-heptyl, (−)-propyl, (+)-nonyl, and (−)-butyl homologs were best discriminated between the μ (monkey) and δ receptors

Table 3. Comparison (Ratios) of Potencies of (−)- and (+)-α-*N*-(H through Decyl)-*N*-normetazocines in Receptor Assays

N-R	μ (monkey)/μ (rat)	κ/μ (monkey)	PCP/σ	σ/μ (rat)	PCP/μ (rat)
(−)-H	2.4	2.1	0.03	293	8.6
(−)-methyl	0.4	4.0	0.07	1645	108
(−)-ethyl	0.5	1.0	0.1	519	58
(−)-*n*-propyl	0.2	1.3	0.4	97	35
(−)-*n*-butyl	0.3	1.4	5.9	8.0	47
(−)-*n*-pentyl	0.9	1.5	55.	2.8	154
(−)-*n*-hexyl	8.3	1.1	84.	8.5	712
(−)-*n*-heptyl	3.1	1.2	115.	5.6	640
(−)-*n*-octyl	2.0	1.3	228.	1.2	266
(−)-*n*-nonyl	1.2	3.9	193.	0.2	41
(−)-*n*-decyl	2.0	2.3	263.	0.08	20
(+)-H	3.7	0.5	0.01	5.8	0.06
(+)-methyl	0.3	2.7	0.03	0.3	0.008
(+)-ethyl	0.4	0.9	0.1	0.1	0.01
(+)-*n*-propyl	0.2	0.5	14.1	0.008	0.1
(+)-*n*-butyl	0.4	0.6	149.	0.002	0.3
(+)-*n*-pentyl	0.5	1.0	448.	0.002	1.0
(+)-*n*-hexyl	0.4	7.1	3176.	0.003	9.2
(+)-*n*-heptyl	1.1	2.8	1351.	0.008	11
(+)-*n*-octyl	0.3	14.2	281.	0.005	1.5
(+)-*n*-nonyl	0.1	18.0	346.	0.009	3.0
(+)-*n*-decyl	0.2	3.0	61.	0.02	0.9

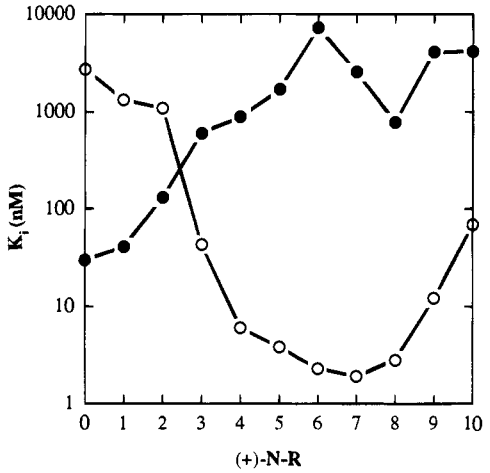


Figure 4. Comparison of potencies (K_i, nM) of (+)-α-*N*-(H through decyl)-*N*-normetazocines for σ (○) and PCP (●) binding sites. The σ assay was carried out in guinea pig brain plus cerebellum homogenates by displacement of [^{3}H]pentazocine and PCP assays were carried out using rat whole brain minus cerebellum as described in the Methods section.

(δ/μ ratio of 36, 27, 25, 23, and 23, respectively), and the (−)-propyl, (−)-butyl, and (+)-propyl homologs were best discriminated between the δ and κ receptors (δ/κ ratios of 18 and 16, 15, respectively). The μ (monkey) and κ receptors did not discriminate the (−)-enantiomers very well; the best discriminated were the (−)-methyl and (−)-nonyl homologs which were found to have similar κ/μ ratios of about 4 (Table 3). Higher κ/μ ratios were observed for three (+)-enantiomers (ratios of 7, 14, and 18 for the hexyl, octyl, and nonyl homologs, respectively). The (+)-hexyl enantiomer was surprisingly potent and opioid receptor-selective at the μ (monkey) receptor, and the (+)-heptyl was somewhat more potent than the (+)-hexyl at the μ receptor but was less selective for that receptor.

Except for the (−)-octyl, nonyl, and decyl homologs, the (−)-enantiomers were considerably more potent and selective for the μ (rat) receptor than the σ receptor (σ/μ (rat) ratio varied from 1600 for the methyl homolog to 3 for the pentyl). The (−)-nonyl and (−)-decyl homologs were much more potent and selective in their interaction with the σ receptor than with any of the opioid receptors (σ/μ ratio 0.2 and 0.08, respectively). Only the (+)-H and (+)-methyl enantiomers were potent (<0.05 μM) and selective for the PCP binding site.

Enantioselectivity. The *N*-alkyl enantiomers interact somewhat differently with the μ receptors in the rat and monkey. Although enantioselectivity among the *N*-alkyl compounds ((+)-enantiomer potency/(−)-enantiomer potency, Table 4) at the μ receptor in the monkey preparation varied considerably among the homologs, from a ratio of 0.7 for the *N*-nonyl enantiomers to 629 for the propyl enantiomers, in the rat preparation the enantioselectivity at the μ receptor was even greater. It was highest for the propyl enantiomers (687) and least for the nonyl or decyl enantiomers (9.1). Enantioselectivity was also observed at the κ and δ receptors in the monkey preparation. The ethyl enantiomers were most selective at κ (+/− ratio of 236) and propyl at δ (+/− ratio of 190). The decyl enantiomers were least selective for both κ and δ receptors. In all, the propyl homolog displayed the consistently highest enantioselectivity and potency at the various opioid receptors.

At the σ-site, all of the (+)-enantiomers were more potent than their (−)-counterparts (Table 4) with the exception of the decyl enantiomers. The heptyl enantiomers displayed the greatest difference in their affinity for the σ receptor (+/− ratio of 0.04, a 25-fold enantioselectivity). In the PCP assay, the methyl and ethyl homologs were the most enantioselective, displaying 33- and 14-fold enantioselectivity, respectively (Table 4). Three (−)-enantiomers were more potent than their (+)-counterparts in that assay (propyl, butyl, and hexyl, with +/− ratios of 2.3, 1.5, and 2.2, respectively).

Activity in Vivo. The in vivo results are summarized in Table 5. Both *N*-H enantiomers were essentially inactive in the mouse antinociception tests and did not substitute for morphine in the monkey SDS model. The (−)-methyl homolog displayed potent antinociceptive activity and in the rat-infusion study (SM) and monkey (SDS) assay, nearly substituted or partially substituted for morphine, respectively. In addition, it produced physical dependence in the rat PPD model at a lower dose regimen than morphine. In sharp contrast, the (+)-methyl enantiomer was antinociceptively inactive. The (−)-ethyl through -butyl compounds showed

Table 4. Comparison (Ratios) of Potencies of Enantiomers of α-*N*-(H through Decyl)-*N*-normetazocines in Receptor Assays

N-R	(+)-μ/(−)-μ (monkey)	(+)-μ/(−)-μ (rat)	(+)-κ/(−)-κ (monkey)	(+)-δ/(−)-δ (monkey)	(+)-σ/(−)-σ (guinea pig)	(+)-PCP/(−)-PCP (rat)
H	32	20	33	16	0.4	0.2
methyl	328	452	222	46	0.07	0.03
ethyl	262	313	236	44	0.07	0.07
propyl	629	687	226	190	0.06	2.3
butyl	365	221	160	90	0.06	1.5
pentyl	52	109	32	71	0.08	0.7
hexyl	8.2	169	53	12	0.06	2.2
heptyl	9.6	28	23	123	0.04	0.5
octyl	2.7	17	29	14	0.08	0.1
nonyl	0.7	9.1	3.2	6.3	0.4	0.7
decyl	0.8	9.1	1.0	1.6	1.8	0.4

Table 5. In Vivo Activities[a] of (−)- and (+)-α-*N*-(H through Decyl)-*N*-normetazocines

N-R	TF	TF vs M	PPQ	HP	SDS, or rat SM or PPD
(−)-H	13% at 30.0	inactive (I)	66% at 30.0	38% at 30.0	NT
(−)-methyl	0.8 (0.3−2.0)	I	0.3 (0.1−0.9)	0.6 (0.5−0.9)	*SDS*: partial substitution at 4.0; salivation, drowsiness, and slowing observed *SM*: nearly substituted at 50.0/day *PPD*: produced physical dependence at lower dose regimen than morphine
(−)-ethyl	I	3.7 (1.8−7.8)	1.4 (0.5−4.1)	13% at 1.0, 25% at 10.0 and 30.0	*SDS*: no substitution at 0.5 and 2.0; may have exacerbated withdrawal; Retching, head tremors, seizure observed
(−)-propyl	I	0.4 (0.2−1.0)	22.0 (16.0−30.1)	I	*SDS*: exacerbated withdrawal at 0.025 and 0.1
(−)-butyl	I	1.5 (0.5−4.2)	40% at 30.0	I	*SDS*: slight reduction of withdrawal signs but severe ataxia, slowing, ptosis, increased retching and vomiting, wet dog shakes and tremors observed; drug introduction in normal nondependent monkeys produced signs which naloxone could not reverse
(−)-pentyl	1.3 (0.7−2.4)	I	0.2 (0.08−0.6)	2.4 (1.2−4.7)	*SDS*: substitutes completely at 4.0; convulsions and vomiting observed
(−)-hexyl	1.1 (0.6−2.3)	I	0.3 (0.1−0.7)	3.4 (1.7−6.8)	*SDS*: partial substitution at 5.0
(−)-heptyl	1.7 (1.1−2.7)	I	0.13 (0.02−0.99)	2.4 (1.1−5.1)	*SDS*: I at 1.25 and 5.0; convulsions observed *SM*: partial suppression at 10.0 *PPD*: produced physical dependence like morphine
(−)-octyl	10.3 (4.3−23.2)	I	0.5 (0.2−1.5)	5.4 (3.4−8.6)	*SDS*: I at 4.0 and 16.0; may have exacerbated withdrawal
(−)-nonyl	I	I	I	I	*SDS*: neither substituted nor exacerbated withdrawal at 5.0 and 15.0
(−)-decyl	I	I	I	I	*SDS*: I at 2.0 and 10.0; may have exacerbated withdrawal
(+)-H	27% at 30.0	I	23% at 10.0	13% at 10.0	*SDS*: NT
(+)-methyl	I	NT	43% at 20	I	*SDS*: NT *SM*: I at 50 and 100/day *PPD*: dextromethorphan-like—only overt withdrawal signs, no loss of body weight
(+)-ethyl	I	I	33.5 (15.5−72.1)	I	*SDS*: no substitution at 0.5 and 2.0; ataxia, sagging, seizure, frequent retching, biting hands and feet, muscle spasms observed
(+)-propyl	I	20% at 30.0	I	I	*SDS*: partial substitution at 8.0; severe ataxia, jaw sag, stupor observed
(+)-butyl	I	I	43% at 30.0	I	*SDS*: partial substitution at 12.0; ataxia, disorientation, head tremor observed
(+)-pentyl	21.3 (15.0−30.3)	I	5.6 (1.8−1.6)	I	*SDS*: partial substitution at 3.0; ataxia, disorientation, head tremors observed
(+)-hexyl	20.4 (12.9−32.2)	I	1.0 (0.4−2.8)	38% at 10 and 30	*SDS*: neither substituted nor exacerbated withdrawal at 3.0 or 12.0; ataxia, slowing, ptosis, head tremors noted
(+)-heptyl	12.9 (6.2−26.7)	I	3.5 (1.1−11.4)	11.5 (4.4−30.2)	*SDS*: I at 1.25 or 10.0
(+)-octyl	14% at 30.0	I	7.8 (1.2−49.5)	11.1 (3.1−40.3)	*SDS*: I at 4 and 16
(+)-nonyl	I	I	I	I	*SDS*: neither substituted nor exacerbated withdrawal at 5.0 or 15.0
(+)-decyl	I	I	I	I	*SDS*: I at 4.0 and 16.0; may have exacerbated withdrawal

[a] All doses are given in mg/kg; I = inactive; NT = not tested. Antinociceptive assays (in mice, parenthesized numbers represent 95% confidence limits): TF = tail flick (morphine sulfate = 0.73 (0.35−1.53)); TF vs M = tail flick vs morphine (naloxone hydrochloride = 0.04 (0.01−0.09); nalorphine hydrochloride = 2.6 (0.7−10.0)); PPQ = phenylquinone (morphine sulfate = 0.4 (0.20−0.8)); HP = hot plate (morphine sulfate = 3.1 (1.5−6.4)); SDS = single-dose suppression studies in rhesus monkeys; SM = substitution for morphine studies in the continuously-infused rat; PPD = primary physical dependence study in the continuously-infused rat. In the mouse antinociception tests, the following vehicles were used: for *N*-methyl through -hexyl enantiomers, sterile H_2O; for the *N*-heptyl enantiomers, propylene glycol and H_2O; for the *N*-octyl enantiomers, DMSO and H_2O; for the *N*-nonyl enantiomers; DMSO, propylene glycol, and H_2O; and for the *N*-decyl enantiomers, propylene glycol, Tween 80, and H_2O. In the SDS study in monkeys, the same vehicles were used for the *N*-methyl through -heptyl enantiomers. The following vehicles were used for the *N*-octyl and -nonyl enantiomers, 25% gum tragacanth and H_2O; and for the (−)- and (+)-decyl enantiomers, 25% (hydroxypropyl)-β-cyclodextrin and H_2O, or propylene glycol, Tween 80, and H_2O, respectively. In the SM and PPD studies in continuously-infused rats, the vehicle was H_2O.

activity in the PPQ test, were inactive in the TF and HP assays, and showed varying degrees of antagonist activity vs morphine in the TF test; the (−)-propyl derivative was the most potent antagonist. It was about 1/10 as potent as naloxone in this respect. In morphine-dependent monkeys, the (−)-propyl clearly

exacerbated withdrawal at 0.025 and 0.01 mg/kg. The (−)-butyl enantiomer produced behavioral signs suggesting exacerbation of withdrawal in the monkey; some of these signs were not antagonized by naloxone which suggests non-opioid behavior. In the (+)-ethyl through -butyl series, all the (+)-enantiomers were essentially inactive in all the antinociceptive tests. In the monkey SDS assay, these compounds were either inactive (*N*-ethyl) or substituted partially. However, all of these compounds produced severe ataxia and other overt signs including disorientation. Interestingly, the (+)-methyl homolog produced dextrorphan-like behavior in the rat PPD model.

The (−)-pentyl through -octyl homologs showed antinociceptive activity in all tests; (−)-octyl was clearly the least potent. In the monkey SDS test, the (−)-pentyl and (−)-hexyl enantiomers substituted completely ((−)-pentyl) or partially ((−)-hexyl) for morphine. The (−)-heptyl enantiomer was inactive in the monkey SDS test regarding relief from withdrawal, and (−)-*N*-octyl may have exacerbated withdrawal. The (−)-heptyl enantiomer was also evaluated in the rat infusion assay; it partially substituted for morphine (SM) and produced physical dependence (PPD) indistinguishable from that produced by morphine. Although the (+)-pentyl through -octyl compounds had varying degrees of antinociceptive activity, they were inactive in the monkey SDS model. In addition, the (+)-pentyl and -hexyl compounds produced ataxia and head tremors. The (+)-pentyl enantiomer-treated monkey was also disoriented.

The (−)- and (+)-nonyl and -decyl compounds were inactive antinociceptively as agonists or antagonists. Interestingly, both the (−)- and (+)-decyl enantiomers may have exacerbated withdrawal in the monkey SDS model.

Discussion

Previous studies of the *N*-H and *N*-alkyl substituted benzomorphans were generally limited to observations on racemic mixtures, with the easily envisioned difficulty in interpreting in vitro and in vivo data from a mixture. One exception, a recent compilation by Carroll et al.,[57] included the enantiomeric *N*-H and *N*-methyl homologs in a study of the affinities of the *N*-alkenyl and *N*-aralkyl series of *N*-norbenzomorphans. Their receptor-binding work (μ opioid, σ_1 and σ_2, and the PCP site) differs from this study, in which we examined opioid receptor subtypes (using monkey cortex, which could provide a more realistic link to in vivo results obtained with that animal model, and rat brain preparations for correlation with rodent antinociceptive studies), as well as the σ_1 and PCP sites. The four compounds examined by both groups ((+)- and (−)-*N*-H and (+)- and (−)-*N*-methyl) provide a link for comparison of the data between laboratories. The affinity of these ligands, determined by displacement of [^{3}H]pentazocine to the σ receptor in membranes of frozen[57] vs fresh (see Methods) guinea pig brain with or without[57] cerebellum, are reasonably similar in both studies (e.g., (+)-*N*-methyl and (+)-*N*-H K_i 2100 ± 269[57] vs 1330 ± 130 (Table 2), and 3380 ± 503[57] vs 2733 ± 570 (Table 2), respectively).

Our finding that the rank order of potency of the (−)-enantiomers for the μ and the κ receptors are quite similar, but not identical, may point to their interaction with similar, but not identical, areas in these receptors. The seven transmembrane α helical regions of μ and κ receptors are known to be highly homologous, much more so than those parts of these receptors which exist in extracellular space.[28-35] The three-dimensional pattern of the helical regions of at least one G protein-coupled receptor has been determined,[67,68] and considerably more is known about the pattern in that area than is known about their 3-D pattern in extracellular space. If in fact these ligands do interact with the transmembrane helical regions, the different rank order for the interaction of these compounds with the μ and κ receptors may prove useful for computer-assisted molecular modeling experiments.

The antinociceptively potent (−)-homologs (*N*-methyl, -pentyl, -hexyl, and -heptyl), each of which was essentially equipotent with morphine in the TF, PPQ, and hot-plate assays (Table 5), interacted mostly with the μ opioid receptor. Although all of these homologs had good affinity for the κ receptor, the pentyl and hexyl compounds were almost equipotent at μ and κ. The (−)-propyl, -butyl, and -ethyl enantiomers were as potent as, or more potent than, nalorphine as antagonists in the TF vs M assay (Table 5). Of these three enantiomers, only the (−)-propyl compound clearly and potently exacerbated withdrawal in the SDS study in monkeys. The overt behavioral signs induced by the (−)-butyl enantiomer in nondependent monkeys were not reversible by naloxone. This finding is generally considered indicative of non-opioid properties and may have been caused by its interaction with σ receptors or PCP sites. This enantiomer also interacts potently with both μ and κ opioid receptors in the monkey and rat (Table 2). With the exception of the (+)-pentyl through -octyl homologs, the homologs in the (+)-series were found to be devoid of antinociceptive or narcotic antagonist properties. The (+)-pentyl and -hexyl showed some activity both in the TF and PPQ assays, and the (+)-heptyl showed antinociceptive activity in TF, PPQ, and hot-plate assays. The (+)-octyl compound showed antinociceptive activity in both PPQ and hot-plate assays, Although the aforementioned (+)-enantiomers had antinociceptive activity about $^1/_4$ to $^1/_{10}$ the potency of morphine, all of these (+)-enantiomers were considerably less potent than their counterparts in the (−)-enantiomeric series in the various antinociceptive assays. In the SDS assay, the (+)-ethyl, (+)-propyl, (+)-butyl, and (+)-hexyl enantiomers produced what could be interpreted as PCP-like overt signs and/or signs induced by interaction with σ receptors (Table 5). In the rat PPD assay, dextrorphan-like withdrawal was noted with the (+)-methyl homolog. Also, (+)-pentyl-treated monkeys were disoriented. No significant activity was observed in the SDS assay with the (+)-heptyl, (+)-octyl, and (+)-nonyl compounds, although these compounds did bind to μ opioid receptors (Table 2). Thus, in these cases binding may not reflect efficacy or, perhaps, their solubility presented a problem in vivo. The (+)-heptyl and (+)-octyl compounds were essentially inactive in the SDS assay (Table 5) and showed weak antinociceptive activity in one or more antinociceptive assays. These enantiomers could theoretically be useful as mild analgesics since they might not have morphine-like side effects in humans. However, they exhibit high affinity for σ sites, and this interaction could be delete-

rious to their use as analgesics (σ-site directed 6,7-benzomorphans such as cyclazocine and pentazocine have been noted to interact synergistically with neuroleptics, or under some conditions act directly to induce marked changes in C6 glioma cell morphology and eventually cause cell death).[69,70] The (−)-heptyl homolog was found to be considerably less potent for σ sites. It is very potent in antinociceptive assays and inactive in the SDS assay in monkeys. However, in a primary physical dependence study in rats (Table 5), the compound had morphine-like physical dependence properties.

We found that a few of the longer chain alkyl compounds in the (+)-series (pentyl through octyl) were among the most potent ligands known for the σ binding site. The affinities of the (−)-enantiomeric ligands for the σ_1 receptor appear to follow the general rule noted for ligand interaction with that receptor.[71] That is, as the N-substituent increases in bulk (or lipophilicity increases), the interaction with the σ site also increases (or reaches a plateau). This relationship does not appear to be completely true for the (+)-enantiomers, where the affinity of the ligands for the σ binding site appears to lessen with the most bulky (*N*-nonyl and *N*-decyl) compounds. Thus, this general rule does not appear to be completely valid for the (+)-enantiomeric series. It was noteworthy that the enantiomeric compounds with the smaller *N*-alkyl substituents interacted better at PCP sites than at σ receptors.

All of the compounds from (−)-methyl through -octyl show antinociceptive activity in at least one of the antinociceptive assays (Table 5), and three of them display narcotic antagonist activity in the TF vs M assay. Of all of the tested compounds, only the (−)-propyl homolog is a potent narcotic antagonist. It displayed weak antinociceptive activity in the PPQ assay. The transition, which is easily measured, from agonist ((−)-methyl) to agonist–antagonist ((−)-ethyl, -propyl, and -butyl) and back to agonist ((−)-pentyl, -hexyl, -heptyl, and -octyl) in this (−)-*N*-alkyl series (Table 5), is not readily explainable. Conceivably, future exploration of the series at the molecular level (perhaps through examination of their interaction with specific amino acids in the transmembrane helices of the various opioid receptors) will resolve this enigma.

Experimental Section

Melting points (uncorrected) were taken in a Thomas-Hoover capillary apparatus. IR spectra (Beckman Acculab 8 instrument) were consistent with the structures shown. Optical rotations (EtOH as solvent unless otherwise noted) were measured in a Perkin-Elmer 141 digital polarimeter. Free bases were recrystallized from Me_2CO (lower homologs) and "hexanes", HCl and HBr salts from MeOH–Me_2CO. Thin-layer chromatography (TLC) was performed on 250 μm silica gel GHLF, Analtech Uniplates using a CH_2Cl_2–MeOH–concentrated aqueous NH_3 (90:9:1) solvent system with visualization by iodine vapor. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA, and were found to be within ±0.4% of theory.

General Procedure for *N*-Alkyl-*N*-normetazocines (1h·HCl as example). (−)-5,9α-Dimethyl-2-heptyl-2′-hydroxy-6,7-benzomorphan Hydrochloride. A mixture of **1a** (5 g, 2.35 mmol), 1-bromoheptane (Aldrich, 4.5 g, 2.56 mmol), and $KHCO_3$ (6 g) in (dried over K_2CO_3) THF (25 mL) and DMF (5 mL) was stirred and refluxed for 8 h. The solvents were removed in vacuo, and the residue was treated with water and ether. The water-washed, dried (Na_2SO_4) ethereal solution (two extracts) was evaporated to dryness in vacuo to give 8.0 g of residue. The residue was dissolved in Me_2CO (10 mL)–AcOEt (10 mL) and yielded 7.6 g (95%) of 1h·HCl (mp 190–192 °C) upon acidification with gaseous HCl (30–33% HBr in glacial HOAc was used to form the HBr salts of **1k** and **1v**). This solid was dissolved in boiling MeOH (20 mL), Me_2CO (35–40 mL) was added, and the solution was concentrated to 20–25 mL to give a precipitate, after cooling gradually to −5 °C, which was filtered and washed with Me_2CO to give pure 1h·HCl (6.6 g, mp 192–193 °C). An additional amount of solid was recovered from the filtrate (0.5 g, mp 184–187 °C). Anal. ($C_{21}H_{34}ClNO$) C, H, N.

Opioid Receptor Subtype Assays Using Rhesus Monkey Cortex Membranes. The isolation of membranes from rhesus monkey cortex, the determination of protein, and the binding assays using tritiated Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO), (D-Pen^2,D-Pen^5)enkephalin (DPDPE), and (5a,7a,-8b)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide (U69,593) were carried out as described previously.[72] The assays were implemented at 25 °C in a buffer medium consisting of 50 mM Tris-HCl (pH 7.4) and 150 mM NaCl. Specific binding of the radioligand was defined as the difference between binding in the absence and presence of an appropriate excess of the corresponding unlabeled opioid. After incubation at 25 °C to reach binding equilibrium, the samples were quickly filtered and the glass-fiber disks subjected to liquid scintillation counting. The IC_{50} was determined by linear regression from plots relating inhibition of specific binding in probit units to the log of five different ligand concentrations. The correlation coefficient of the log-probit plot (r^2) was higher than 0.98 in all cases. The IC_{50}'s were converted to K_i values using the Cheng–Prusoff equation[73] and the following K_d values (nM): [^{3}H]DAMGO (0.36); [^{3}H]DPDPE (3.50); [^{3}H]U69,593 (0.91).

μ Opioid Receptor Assay Using Rat Whole Brain Minus Cerebellum. Adult male Sprague–Dawley rats (Dominion Laboratories, Dublin, VA) were decapitated, and the whole brain minus cerebellum was used to prepare the homogenate. The brain was homogenized in 10 volumes of 50 mM Tris-HCL buffer (pH 7.4) and centrifuged at 45000*g* for 15 min at 4 °C. The pellet was homogenized again in 10 volumes of buffer and centrifuged as before. The final pellet was resuspended in 10 volumes of buffer. Aliquots (200 μL) of freshly prepared homogenate were incubated in triplicate (30 °C, 2.5 h) with the appropriate concentration of [^{3}H]-DAMGO in a total volume of 2 mL. Nonspecific binding was determined in the presence of 1 μM levorphanol. The reaction was terminated by rapid filtration on a Brandel cell harvester (Gaithersburg, MD). Schleicher and Schuell (Keene, NH) no. 32 glass fiber filters were soaked for 5 min in a 0.05% polyethylenimine solution prior to filtration. Filters were washed two times with 5 mL of cold buffer, suspended in 10 mL of Budgetsolve (Research Products International, Mount Prospect, IL), and shaken for 1 h. Radioactivity was determined by liquid scintillation spectrometry at a counting efficiency of approximately 50% which was corrected by external standardization. Specific [^{3}H]DAMGO binding was defined as total binding minus nonspecific binding. Protein concentrations were determined by the method of Bradford.[74] Displacement studies were conducted by incubating the drugs with 1 nM [^{3}H]DAMGO as described above. Conversion of IC_{50} to K_i values was performed using the Cheng–Prusoff equation[73] and the [^{3}H]DAMGO K_d value of 1.9 nM. The results represent the mean ± SEM for three experiments. The average standard error of the mean for all of the compounds was 12.8%.

σ Receptor Assay. Male Hartley guinea pigs (Charles River, Kingston, NY) were decapitated, and the whole brains plus cerebellum were rapidly removed and disrupted with 40 volumes of 50 mM Tris-HCl buffer (pH 8.0) using a Brinkman polytron (setting 6, 20 s). The homogenates were centrifuged at 27000*g* for 20 min at 5 °C. The pellet was resuspended in the original volume with fresh assay buffer and recentrifuged for a total of three times. The final resuspension was kept on ice until needed.

Binding to homogenates was determined in a 1 mL incubation volume, consisting of 900 μL of tissue, 50 μL of [^{3}H]-(+)-pentazocine[52] (51.7 Ci/mmol) for a final concentration of 3 nM, and 50 μL of either buffer, test compound, or 10 μM (+)-pentazocine (for determination of nonspecific binding). After a 120 min incubation at 25 °C, the reaction was terminated by rapid filtration using a Brandel cell harvester (Brandel, Inc., Gaithersburg, MD) through no. 32 Schleicher and Schuell (Keene, NH) glass fiber filters which had been presoaked in 0.5% polyethylenimine at 25 °C during the incubation period. The filters were washed with three 5 mL aliquots of ice-cold 10 mM Tris buffer (pH 8.0), placed in counting vials with 4 mL of CytoScint ES scintillation cocktail (ICN Biomedicals, Inc., Irvine, CA), and allowed to stand overnight before counting in a Packard Tri-Carb 2200CA liquid scintillation counter (Packard Instrument Co., Downers Grove, IL).

Data were analyzed using GraphPAD software (ISI Software, Philadelphia, PA) using a K_d for (+)-pentazocine of 3.4 nM as determined by Scatchard analysis. Each concentration was performed in triplicate, and the resulting values are displayed as the arithmetic mean and standard error of three or more experiments. The average standard error of the mean for all of the compounds was 11.4%.

PCP Receptor Assay. Adult male Sprague–Dawley rats (Dominion Laboratories, Dublin, VA) were decapitated, and the whole brain minus cerebellum was used to prepare the homogenate. The brain homogenate was prepared in a 5 mM Tris-HCl buffer (pH 7.7) as described for the μ opioid assay (in rats). The homogenate was incubated at 25 °C for 20 min with 1 nM [^{3}H]TCP (1-[1-(2-thienylcyclohexyl)]piperidine) for displacement studies. Nonspecific binding was determined in the presence of 1 μM unlabeled TCP. The reactions were filtered and counted for radioactivity as described for the μ opioid assay. Conversion of IC_{50} to K_i values was performed using the Cheng–Prusoff equation[73] and the [^{3}H]TCP K_d value of 7.7 nM. The results represent the mean ± SEM for three experiments. The average standard error of the mean for all of the compounds was 12.0%.

In Vivo Assays.[75] Mouse Antinociception Tests. Male mice, weighing 20–30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (sc). At least three doses were tested, and 6–10 animals per dose were used. When applicable, ED50's were calculated by using computerized probit analysis.

Tail-Flick (TF) and Tail-Flick vs Morphine (TF vs M) Assays.[76,77] Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2–4 s under control conditions. Mice were injected with drug or vehicle and tested 20 min later. In the assay for antagonism of the antinociceptive effect (TF vs M), the potential antagonists were administered 10 min before the agonist, and evaluation occurred 20 min later.

Phenylquinone Abdominal-Stretching (PPQ) Assay.[78] The mice were injected with test drugs and 10 min later received 2.0 mg/kg ip of a freshly prepared paraphenylquinone (PPQ) solution. They were then placed in cages in groups of two each. Ten minutes after the PPQ injection, the total number of stretches per group were counted over a 1 min period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

Hot-Plate (HP) Assay.[79,80] The hot plate was held at 55 °C. Mice were placed on the hot plate and activity was scored if the animal jumped or licked its paws after a delay of 5 s or more, but no more than 30 s beyond the control time.

Dependence-Liability Studies in Rhesus Monkeys. Single-Dose Substitution (SDS) Test. Male and female rhesus monkeys (*M. mulatta*) weighing 2.5–7.5 kg were used; they received 3 mg/kg sc of morphine sulfate every 6 h. All the animals had received morphine for at least 3 months and were maximally dependent on morphine.[81] A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay[82,83] was initiated by a sc injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14–15 h and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: (a) a dose of the compound under investigation; (b) morphine control, 3.0 mg/kg; and (c) vehicle control, 1 mL/kg. The animals were scored for suppression of withdrawal signs during a 2.5 h observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score ± SEM was calculated.

Rat Infusion Studies. The continuous-infusion method[84] was modified as follows. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7–10 mL of solution every 24 h.

Substitution for Morphine (SM) Test Using Rat Infusion. The rats received morphine sulfate (50 mg/kg/24 h on the first day, 100 mg/kg/24 h on the second day, and 200 mg/kg/24 h from days 3 to 6). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of water. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 30 min at 6, 24, 48, 72, and/or 96 h after stopping the infusion of morphine.

Primary Physical Dependence (PPD) in Rat. The rats received test compound as specified above for 6 days and then were placed in abrupt withdrawal and observed for overt behavioral signs.

Acknowledgment. The authors would like to thank the Research Technology Branch, National Institute on Drug Abuse, NIH (Dr. R. Hawks, Chief), Dr. F. Ivy Carroll (Research Triangle Institute), and Drs. William F. Michne and A. E. Soria (Sterling-Winthrop Research Institute, now Eastman Laboratories) for providing the resolved *N*-normetazocines used as starting materials in the syntheses, Susan M. Tucker, Zhen Ji, Larry Hughes, and Frank Winckler (all at the Medical College of Virginia), and Sandra Lee (University of Michigan) for their expert assistance. Portions of this work were supported by PHS Grants DA02396 (B.R.M.) and DA00490 (E.L.M. and L.S.H.), Contracts DA 271-90-7200 and N01DA-3-8200 (M.D.A., L.S.H., E.R.B.) from the National Institute on Drug Abuse, NIH, and support from the College on Problems of Drug Dependence is also gratefully acknowledged.

References

(1) May, E. L.; Fry, E. M. Structures Related to Morphine. VIII. Further Syntheses in the Benzomorphan Series. *J. Org. Chem.* **1957**, *22*, 1366–1369.
(2) Eddy, N. B.; Murphy, J. G.; May, E. L. Structures Related to Morphine. IX. Extension of the Grewe Morphinan Synthesis in the Benzomorphan Series and Pharmacology of Some Benzomorphans. *J. Org. Chem.* **1957**, *22*, 1370–1372.
(3) Ager, J. H.; May, E. L. Structures Related to Morphine. XIII. 2-Alkyl-2′-hydroxy-5,9-dimethyl-6,7-benzomorphans and a More Direct Synthesis of the 2-Phenethyl Compound (NIH 7519). *J. Org. Chem.* **1960**, *25*, 984–986.
(4) Joshi, B. C.; Chignell, C. F.; May, E. L. Structures Related to Morphine. XXX. N-Hexyl and 5-Butyl-, Amyl- and Hexyl-6,7-Benzomorphans. *J. Med. Chem.* **1965**, *8*, 694–696.
(5) Villarreal, J. E.; Seevers, M. H. Evaluation of New Compounds for Morphine-Like Physical Dependence in the Rhesus Monkey. In *Problems of Drug Dependence 1972*; National Academy of Sciences: Washington, D.C., 1972; p 1044.

(6) Swain, H. H.; Seevers, M. H. Evaluation of New Compounds for Morphine-like Physical Dependence in the Rhesus Monkey. In *Problems of Drug Dependence 1975*; National Academy of Sciences: Washington, D.C., 1975; p 778.
(7) Aceto, M. D.; Harris, L. S.; Dewey, W. L.; Balster, R. L. Dependence Studies of New Compounds in the Rhesus Monkey. In *Problems of Drug Dependence 1975*; National Academy of Sciences: Washington, D.C., 1975; p 748.
(8) Swain, H. H.; Fly, C. L.; Seevers, M. H. Evaluation of New Compounds for Morphine-like Physical Dependence in the Rhesus Monkey. In *Problems of Drug Dependence 1977*; Committee on Problems of Drug Dependence, Inc.: Washington, D.C., 1977; pp 618–619.
(9) Aceto, M. D.; Bowman, E. R.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey, Rat, and Mouse. In *Problems of Drug Dependence 1988*; Harris, L. S., Ed.; NIDA Research Monograph 90, U.S. Government Printing Office: Washington, D.C., 1989; pp 473.
(10) Aceto, M. D.; Bowman, E. R.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey, Rat, and Mouse. In *Problems of Drug Dependence 1989*; Harris, L. S., Ed.; NIDA Research Monograph 95; U.S. Government Printing Office: Washington, D.C., 1990; p 610.
(11) Aceto, M. D.; Bowman, E. R.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey and Mouse. In *Problems of Drug Dependence 1989*; Harris, L. S., Ed.; NIDA Research Monograph 105; U.S. Government Printing Office: Washington, D.C., 1991; pp 644–645.
(12) May, E. L.; Eddy, N. B. Structures Related to Morphine. XII. (±)-2′-Hydroxy-5,9-dimethyl-2-phenethyl-6,7-benzomorphans (NIH 7519) and its Optical Forms. *J. Med. Chem.* **1959**, *24*, 1435–1437.
(13) Tullar, B. F.; Harris, L. S.; Perry, R. L.; Pierson, A., K.; Soria, A. E.; Wetterau, W. F.; Albertson, N. F. Benzomorphans. Optically Active and Trans Isomers. *J. Med. Chem.* **1967**, *10*, 383–386.
(14) Ager, J. H.; Jacobson, A. E.; May, E. L. Separation of Morphine-like Effects by Optical Resolution. Levo Isomers as Strong Analgetics and Narcotic Antagonist. *J. Med. Chem.* **1969**, *12*, 288–289.
(15) Aceto, M. D.; May, E. L.; Harris, L. S.; Jacobson, A. E.; Awaya, H. Agonist-Antagonist Actions and Stereoselectivity of Optical Pairs of Some N-Substituted α-N-Normetazocines. *J. Pharm. Sci.* **1984**, *73*, 1864–1866.
(16) Brine, G. A.; Berrang, B.; Hayes, J. P.; Carroll, F. I. An Improved Resolution of (±)-cis-N-Normetazocine. *J. Heterocycl. Chem.* **1990**, *27*, 2139–2143.
(17) May, E. L.; Takeda, M. Optical Isomers of Miscellaneous Strong Analgetics. *J. Med. Chem.* **1970**, *13*, 805–807.
(18) Villarreal, J. E. Recent Advances in the Pharmacology of Morphine-like Drugs. In *Drug Dependence, Advances in Mental Science II*; Harris, R. T., McIsaac, W. M., Schuster, C. R., Jr., Eds.; University of Texas Press: Houston, 1970; pp 83–116.
(19) Rice, K. C.; Jacobson, A. E. Optical Resolution of (±)-2,5-Dimethyl-2′-hydroxy-9α- and -9β-propyl-6,7-benzomorphans and Their Pharmacological Properties. *J. Med. Chem.* **1976**, *19*, 430–432.
(20) Portoghese, P. S. The Role of Concepts in Structure-Activity Relationship Studies of Opioid Ligands. *J. Med. Chem.* **1992**, *35*, 1928–1937.
(21) Jaffe, J. H.; Martin, W. R. Opioid Analgesics and Antagonists. In *The Pharmacological Basis of Therapeutics*; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 485–521.
(22) Robson, L. E.; Patterson, S. J.; Kosterlitz, H. W. Opiate Receptors. *Handbook Psychopharmacol.* **1988**, *17*, 13–80.
(23) Rothman, R. B.; Jacobson, A. E.; Rice, K. C.; Herkenham, M. Autoradiographic Evidence for Two Classes of μ-Opioid Binding Sites in Rat Brain Using [^{125}I]FK33824. *Peptides* **1987**, *8*, 1015–1021.
(24) Itzhak, Y. Multiple Opioid Binding Sites. In *The Opiate Receptors*; Pasternak, G. W., Ed.; Humana Press: Clifton, 1988; pp 95–142.
(25) Xu, H.; Partilla, J. S.; de Costa, B. R.; Rice, K. C.; Rothman, R. B. Interaction of Opioid Peptides and Other Drugs with Multiple δ_{ncx} Binding Sites in Rat Brain - Further Evidence for Heterogeneity. *Peptides* **1992**, *13*, 1207–1213.
(26) Porreca, F.; Takemori, A. E.; Sultana, M.; Portoghese, P. S.; Bowen, W. D.; Mosberg, H. I. Modulation of μ-Mediated Antinociception in the Mouse Involves Opioid δ_2 Receptors. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 147–152.
(27) Rothman, R. B.; Bykov, V.; de Costa, B. R.; Jacobson, A. E.; Rice, K. C.; Brady, L. S. Interaction of Endogenous Opioid Peptides and Other Drugs with Four Kappa Opioid Binding Sites in Guinea Pig Brain. *Peptides* **1990**, *11*, 311–331.
(28) Fukuda, K.; Kato, S.; Mori, K.; Nishi, M.; Takeshima, H. Primary Structures and Expression from cDNAs of Rat Opioid Receptor δ- and μ-Subtypes. *FEBS Lett.* **1993**, *327*, 311–314.
(29) Evans, C. J.; Keith, D. E.; Morrison, H.; Magendzo, K.; Edwards, R. H. Cloning of a Delta Opioid Receptor by Functional Expression. *Science* **1992**, *258*, 1952–1954.
(30) Kieffer, B. L.; Befort, K.; Gaveriaux-Ruff, C.; Hirth, C. G. The δ-Opioid Receptor: Isolation of a cDNA by Expression Cloning and Pharmacological Characterization. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 12048–12052.
(31) Meng, F.; Xie, G. X.; Thompson, R. C.; Mansour, A.; Goldstein, A.; Watson, S. J.; Akil, H. Cloning and Pharmacological Characterization of a Rat κ-Opioid Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9954–9958.
(32) Wang, J. B.; Imai, Y. S.; Eppler, C. M.; Gregor, P.; Spivak, C. E.; Uhl, G. R. μ-Opiate Receptor: cDNA Cloning and Expression. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10230–10234.
(33) Chen, Y.; Mestek, A.; Liu, J.; Hurley, J. A.; Yu, L. Molecular Cloning and Functional Expression of a μ-Opioid Receptor from Rat Brain. *Mol. Pharmacol.* **1993**, *44*, 8–12.
(34) Minami, M.; Toya, T.; Katao, Y.; Maekawa, K.; Nakamura, S.; Onogi, T.; Kaneko, S.; Satoh, M. Cloning and Expression of a cDNA for the Rat Kappa-Opioid Receptor. *FEBS Lett.* **1993**, *329*, 291–295.
(35) Nishi, M.; Takeshima, H.; Fukuda, K.; Kato, S.; Mori, K. cDNA Cloning and Pharmacological Characterization of an Opioid Receptor with High Affinities for Kappa-Subtype-Selective Ligands. *FEBS Lett.* **1993**, *330*, 77–80.
(36) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. A Proposal for the Classification of Sigma Binding Sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85–86.
(37) Quirion, R.; Chicheportiche, R.; Contreras, P. C.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and Nomenclature of Phencyclidine and Sigma Receptor Sites. *Trends Neurosci.* **1987**, *10*, 444–446.
(38) Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; de Costa, B.; Rice, K. C. Sigma-Receptors - Biology and Function. *Pharmacol. Rev.* **1990**, *42*, 355–402.
(39) Rothman, R. B.; Reid, A.; Mahboubi, A.; Kim, C. H.; de Costa, B. R.; Jacobson, A. E.; Rice, K. C. Labeling by [^{3}H]1,3-Di(2-tolyl)-Guanidine of Two High Affinity Binding Sites in Guinea Pig Brain: Evidence for Allosteric Regulation by Calcium Channel Antagonists and Pseudoallosteric Modulation by σ Ligands. *Mol. Pharmacol.* **1991**, *39*, 222–232.
(40) Bowen, W. D.; Walker, J. M.; Yashar, A. G.; Matsumoto, R. R.; Walker, F. O.; Lorden, J. F. Altered Haloperidol-Sensitive Sigma Receptors in the Genetically Dystonic (dt) Rat. *Eur. J. Pharmacol.* **1988**, *147*, 153–154.
(41) Goldstein, S. R.; Matsumoto, R. R.; Thompson, T. L.; Patrick, R. L.; Bowen, W. D.; Walker, J. M. Motor Effects of Two Sigma Ligands Mediated by Nigrostriatal Dopamine Neurons. *Synapse* **1989**, *4*, 254–258.
(42) Matsumoto, R. R.; Hemstreet, M. K.; Lai, N. L.; Thurkauf, A.; de Costa, B. R.; Rice, K. C.; Hellewell, S. B.; Bowen, W. D.; Walker, J. M. Drug Specificity of Pharmacological Dystonia. *Pharmacol. Biochem. Behav.* **1990**, *36*, 151–155.
(43) Walker, J. M.; Matsumoto, R. R.; Bowen, W. D.; Gans, D. L.; Jones, K. D.; Walker, F. O. Evidence for a Role of Haloperidol-Sensitive Sigma-'Opiate' Receptors in the Motor Effects of Antipsychotic Drugs. *Neurology* **1988**, *38*, 961–965.
(44) Campbell, B. G.; Bobker, D. H.; Leslie, F. M.; Mefford, I. N.; Weber, E. Both the Sigma Receptor-Specific Ligand (+)-PPP and the PCP Receptor Specific Ligand TCP Act in the Mouse Vas Deferens via Augmentation of Electrically Evoked Norepinephrine Release. *Eur. J. Pharmacol.* **1987**, *138*, 447–449.
(45) Campbell, B. G.; Scherz, M. W.; Keana, J. F.; Weber, E. Sigma Receptors Regulate Contractions of the Guinea Pig Ileum Longitudinal Muscle/Myenteric Plexus Preparation Elicited by Both Electrical Stimulation and Exogenous Serotonin. *J. Neurosci.* **1989**, *9*, 3380–3391.
(46) Vaupel, D. B.; Su, T. P. Guinea-pig Vas Deferens Preparation May Contain Both Sigma Receptors and Phencyclidine Receptors. *Eur. J. Pharmacol.* **1987**, *139*, 125–128.
(47) Bowen, W. D.; Tolentino, P. J.; Kirschner, B. N.; Varghese, P.; de Costa, B. R.; Rice, K. C. Sigma Receptors and Signal Transduction: Negative Modulation of Signaling Through Phosphoinositide-Linked Receptor System. In *Sigma, PCP, and NMDA Receptors*; DeSouza, E. B., Clouet, D. H., London, E. D., Eds.; NIDA Monograph 133; U.S. Government Printing Office: Washington, D.C., 1993; pp 69–93.
(48) Bowen, W. D.; Kirschner, B. N.; Newman, A. H.; Rice, K. C. Sigma Receptors Negatively Modulate Agonist-Stimulated Phosphoinositide Metabolism in Rat Brain. *Eur. J. Pharmacol.* **1988**, *149*, 399–400.
(49) Ceci, A.; Smith, M.; French, E. D. Activation of the A10 Mesolimbic System by the Sigma Receptor Agonist (+)-SKF10,-047 Can be Blocked by Rimcazole, a Novel Putative Antipsychotic Agent. *Eur. J. Pharmacol.* **1988**, *154*, 53–57.
(50) Matsumoto, R. R.; Walker, J. M. Inhibition of Rubral Neurons by a Specific Ligand for Sigma Receptors. *Eur. J. Pharmacol.* **1988**, *158*, 161–165.

(51) Goldman, M. E.; Jacobson, A. E.; Rice, K. C.; Paul, S. M. Differentiation of [^{3}H]Phencyclidine and (+)-[^{3}H]SKF-10,047 Binding Sites in Rat Cerebral Cortex. *FEBS Lett.* **1985**, *190*, 333–336.
(52) de Costa, B. R.; Bowen, W. D.; Hellewell, S. B.; Walker, J. M.; Thurkauf, A.; Jacobson, A. E.; Rice, K. C. Synthesis and Evaluation of Optically Pure [^{3}H]-(+)-Pentazocine, a Highly Potent and Selective Radioligand for Sigma Receptors. *FEBS Lett.* **1989**, *251*, 53–58.
(53) Bowen, W. D.; de Costa, B.; Hellewell, S. B.; Thurkauf, A.; Walker, J. M.; Rice, K. C. Characterization of [^{3}H] (+)-Pentazocine, a Highly Selective Sigma-Ligand. In *Progress in Clinical and Biological Research, vol. 328: The International Narcotics Research Conference (INRC) '89*; Quirion, R., Jhamandas, K., Gianoulakis, C., Eds.; Alan R. Liss, Inc.: New York, 1990; pp 117–120.
(54) Bowen, W. D.; de Costa, B. R.; Hellewell, S. B.; Walker, M.; Rice, K. C. [^{3}H](+)-Pentazocine: a Potent and Highly Selective Benzomorphan-Based Probe for Sigma-1 Receptors. *Mol. Neuropharmacol.* **1993**, *3*, 117–126.
(55) Bowen, W. D.; Hellewell, S. B.; McGarry, K. A. Evidence for a Multi-Site Model of the Rat Brain Sigma Receptor. *Eur. J. Pharmacol.* **1989**, *163*, 309–318.
(56) Hellewell, S. B.; Bowen, W. D. A Sigma-Like Binding Site in Rat Pheochromocytoma (PC12) Cells - Decreased Affinity for (+)-Benzomorphans and Lower Molecular Weight Suggest a Different Sigma Receptor Form from That of Guinea Pig Brain. *Brain Res.* **1990**, *527*, 244–253.
(57) Carroll, F. I.; Abraham, P.; Parham, K.; Bai, X.; Zhang, X.; Brine, G. A.; Mascarella, S. W.; Martin, B. R.; May, E. L.; Sauss, C.; Dipaolo, L.; Wallace, P.; Walker, J. M.; Bowen, W. D. Enantiomeric N-Substituted N-Normetazocines - A Comparative Study of Affinities at σ, PCP, and μ Opioid Receptors. *J. Med. Chem.* **1992**, *35*, 2812–2818.
(58) Lodge, D.; Aram, J. A.; Church, J.; Davies, S. N.; Martin, D.; O'Shaughnessy, C. T.; Zeman, S. Excitatory Amino Acids and Phencyclidine Like Drugs. In *Excitatory Amino Acid Transmission, Neurology and Neurobiology*; Hicks, T. P., Lodge, D., McLennan, H., Eds.; Alan R. Liss, Inc.: New York, 1987; Vol. 24; pp 83–90.
(59) Fagg, G. E.; Baud, J. Characterization of NMDA-Receptor-Ionophore Complexes in the Brain. In *Excitatory Amino Acids in Health and Disease*; Lodge, D., Ed.; John Wiley and Sons: New York, 1988; pp 63–90.
(60) Corddry, A. H.; Domino, E. F. Quantitative Structure Activity Relationships (QSAR) and Prediction of Molecular Requirements for Phencyclidine Binding to the NMDA Receptor. In *Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection*; Kamenka, J.-M., Domino, E. F., Eds.; NPP Books: Ann Arbor, 1992; pp 45–52.
(61) Collingridge, G. L.; Singer, W. Excitatory Amino Acid Receptors and Synaptic Plasticity. *Trends Pharmacol. Sci.* **1990**, *11*, 290–296.
(62) Trujillo, K. A.; Akil, H. Inhibition of Morphine Tolerance and Dependence by the NMDA Receptor Antagonist MK-801. *Science* **1991**, *251*, 85–87.
(63) Leander, J. D.; Rathbun, R. C.; Zimmerman, D. M. Anticonvulsant effects of Phencyclidine-like Drugs: Relation to N-Methyl-D-Aspartic Acid Antagonism. *Brain Res.* **1988**, *454*, 368–372.
(64) Church, J.; Zeman, S.; Lodge, D. Ketamine and MK-801 as Neuroprotective Agents in Cerebral Ischemia/Hypoxia. In *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology*; Domino, E. F., Kamenka, J.-M., Eds.; NPP Books: Ann Arbor, 1988; pp 747–756.
(65) Wang, Y.; Palmer, M. R.; Freedman, R.; Rice, K. C.; Lessor, R. A.; Jacobson, A. E.; Hoffer, B. J. Electrophysiological Interactions of Isomers of Cyclazocine with the Phencyclidine Antagonist Metaphit in Rat Cerebellar Purkinje Neurons. *J. Neurosci.* **1986**, *6*, 3189–3196.
(66) Dr. F. Ivy Carroll (Research Triangle Institute) and Drs. William F. Michne and A. E. Soria (Sterling-Winthrop Research Institute, now Eastman Laboratories) provided the (+)- and (−)-*N*-normetazocine used as starting material.
(67) Hibert, M. F.; Trumpp-Kallmeyer, S.; Bruinvels, A.; Hoflack, J. Three-Dimensional Models of Neurotransmitter G-Binding Protein-Coupled Receptors. *J. Pharmacol. Exp. Ther.* **1991**, *40*, 8–15.
(68) Baldwin, J. M. The Probable Arrangement of the Helices in G Protein-Coupled Receptors. *EMBO J.* **1993**, *12*, 1693–1703.
(69) Vilner, B. J.; Bowen, W. D. Sigma Receptor-Active Neuroleptics are Cytotoxic to C6 Glioma Cells in Culture. *Eur. J. Pharmacol.* **1993**, *244*, 199–201.
(70) Bowen, W. D.; Vilner, B. J.; de Costa, B. R. Sigma Receptor-Mediated Changes in Morphology, Division, and Viability of C6 Glioma Cells. In *Society for Neuroscience Abstracts*; 1993; pp 421, no. 177.9.
(71) Kaiser, C.; Pontecorvo, M. J.; Mewshaw, R. E. Sigma Receptor Ligands: Function and Activity. In *Neurotransmissions*; Research Biochemicals Inc.: 1991; Vol. VII (1).
(72) Clark, M. J.; Carter, B. D.; Medzihradsky, F. Selectivity of Ligand Binding to Opioid Receptors in Brain Membranes From the Rat, Monkey and Guinea Pig. *Eur. J. Pharmacol.* **1988**, *148*, 343–351.
(73) Cheng, Y.-C.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes Fifty Percent Inhibition (IC50) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3103.
(74) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(75) Aceto, M. D.; Bowman, E. R.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse (1993). In *Problems of Drug Dependence 1993*; Harris, L. S., Ed.; NIDA Research Monograph 140; U.S. Government Printing Office: Washington, D.C., 1994; Vol. I; pp 103–178.
(76) D'Amour, F. E.; Smith, D. L. A Method for Determining Loss of Pain Sensation. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74–79.
(77) Dewey, W. L.; Harris, L. S. Antinociceptive Activity of the Narcotic Antagonists Analogues and Antagonistic Activity of Narcotic Analgesics in Rodents. *J. Pharmacol. Exp. Ther.* **1971**, *179*, 652–659.
(78) Pearl, J.; Harris, L. S. Inhibition of Writhing by Narcotic Antagonists. *J. Pharmacol. Exp. Ther.* **1966**, *154*, 319–323.
(79) Eddy, N. B.; Leimbach, D. Synthetic Analgesics. II. Dithienylbutenyl- and Dithienylbutylamines. *J. Pharmacol. Exp. Ther.* **1953**, *107*, 385–393.
(80) Jacobson, A. E.; May, E. L. Structures Related to Morphine. XXXI. 2′-Substituted Benzomorphans. *J. Med. Chem.* **1965**, *8*, 563–566.
(81) Seevers, M. H.; Deneau, G. A. Physiological Aspects of Tolerance and Physical Dependence. In *Physiological Pharmacology*; Root, W. S., Hofman, F. G., Eds.; Academic Press: New York, 1963; Vol. I, pp 565–570.
(82) Aceto, M. D.; Flora, R. E.; Harris, L. S. The Effects of Naloxone and Nalorphine During the Development of Morphine Dependence in Rhesus Monkeys. *Pharmacology* **1977**, *15*, 1–9.
(83) Aceto, M. D.; Flora, R. E.; Harris, L. S. Caffeine Elicited Withdrawal Signs in Morphine-Dependent Rhesus Monkeys. *Eur. J. Pharmacol.* **1978**, *50*, 203–207.
(84) Teiger, D. G. Induction of Physical Dependence on Morphine, Codeine, and Meperidine in the Rat by Continuous Infusion. *J. Pharmacol. Exp. Ther.* **1974**, *190*, 408–415.