Articles

(*E*)-8-Benzylidene Derivatives of 2-Methyl-5-(3-hydroxyphenyl)morphans: Highly Selective Ligands for the σ_2 Receptor Subtype†

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The determination of the structure and function of the σ receptor subtypes and their physiological role(s) has been impeded by the unavailability of selective ligands. We have developed a new class of σ subtype selective receptor ligands that are (*E*)-8-benzylidene derivatives of the synthetic opioid (±)-, (+)-, and (−)-2-methyl-5-(3-hydroxyphenyl)morphan-7-one (**1**). The derivatives can be prepared by reaction of **1**, (+)-**1**, and (−)-**1** with the appropriate benzaldehyde under Claisen–Schmidt conditions. Incorporation of substituted (*E*)-8-benzylidene moieties onto the 7-keto precursor of (+)-2-methyl-5-(3-hydroxyphenyl)morphan, (+)-**1**, produces compounds (−)-**2** through (−)-**7** (5.8–32.0 nM, σ_1), which have between a 25- and 131-fold increase in affinity for the σ_1 receptor subtype relative to the keto precursor (+)-**1** (K_i = 762 nM, σ_1). Compound (−)-**2** is the most selective of this group (16-fold) for the σ_1 subtype versus σ_2. Substitution of an (*E*)-8-benzylidene moiety onto the 7-keto precursor of (−)-2-methyl-5-(3-hydroxyphenyl)morphan, (−)-**1**, produces compounds (+)-**2**–(+)-**9** (6.4–52.6 nM, σ_2), which have at least a 475–3906-fold increase in affinity for the σ_2 receptor subtype relative to the keto precursor (−)-**1** (K_i = 25 × 10^3 nM). This enhancement of σ_2 receptor affinity is accompanied by substantial selectivity of all of these dextrorotatory products for the σ_2 relative to the σ_1 subtype (32–238-fold), and thus, they are among the most σ_2 subtype selective compounds currently known. Furthermore, the σ_1 subtype is highly enantioselective for the levorotatory isomers, (−)-**2**–(−)-**7** (41–1034-fold), whereas the σ_2 subtype is only somewhat enantioselective for the dextrorotatory isomers, (+)-**2**–(+)-**7** (2.6–9.3-fold). All of these derivatives retain substantial affinity for the μ opioid receptor. Despite the high affinity of the dextrorotatory derivatives for the μ opioid receptor, the high affinity and selectivity for σ_2 over σ_1 sites will surely prove beneficial as tools for the delineation of the function and physiological role of σ_2 receptors.

Introduction

The development of highly selective ligands for σ receptor subtypes is a key preliminary to studies investigating the properties, function, and physiological role of these entities. The σ receptor was originally proposed by Martin et al. as an opioid receptor that mediated the effects of racemic SKF-10,047 (*N*-allylnormetazocine) and other benzomorphans in dogs.[1] Other studies supported the contention that the σ receptor and the phencyclidine (PCP)-binding site were equivalent.[2] However, later studies utilizing more selective ligands classified the σ receptor as a non-opioid entity independent of the PCP-binding site.[3] The perplexity in determining the nature of the σ receptor was due to the lack of selectivity of racemic SKF-10,047 for the receptor. Subsequently, it was found that (−)-SKF-10,047 binds mainly to μ and κ opioid receptors,[4] and (+)-SKF-10,047 binds to PCP receptors as well as to the σ receptor.[2c,3g,5] Therefore, σ receptors are not the 'sigma opiate receptor' that were first proposed by Martin in 1976.[1] These initial difficulties involving the characterization of the σ receptor have warranted the current trend toward the production of σ receptor selective as well as subtype selective ligands and clearly demonstrate the problems that can be encountered from the use of racemates in pharmacological studies.

A wide variety of structurally diverse compounds have been classified as σ receptor ligands,[6] and they have been implicated in a wide array of physiological processes,[7] including neuroprotection,[8] cytotoxicity,[9] and ulceroprotective effects,[10] as well as motor activity[11] and disorders, especially those seen during the clinical use of antipsychotics.[12] However, some believe that these receptors may contribute to the therapeutic action of neuroleptics and antipsychotics and represent targets for the development of novel antipsychotic agents.[13] Whether or not σ antagonists have potential to be used as antipsychotic agents is still under investigation.[14]

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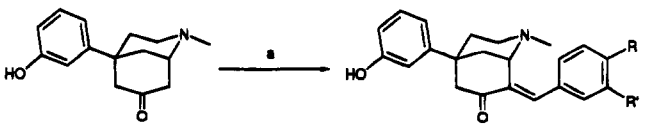
Support for the connection with motor effects comes from studies that show motoric alteration upon microinjection of σ ligands into σ receptor rich brain regions.[15,12a,c,7a] Furthermore, other studies have identified altered σ receptors in genetically dystonic rats.[16] σ Receptors appear to modulate the *N*-methyl-D-aspartate (NMDA) receptor complex and therefore might play a role in neuroprotection.[17] As well as having therapeutic potential for use as antipsychotics, and for neuroprotection, σ receptor antagonists are seen by some as possible clinical agents for the treatment of drug abuse.[18] However, a universally accepted functional assay defining the properties of σ agonists and antagonists is needed.[19]

Recent reports propose radiolabeled σ receptor ligands as potential tools for the diagnosis of certain cancers.[20] Also, the ability of σ ligands to kill tumor-derived cells expressing σ receptors has suggested possible applicability to cancer chemotherapy.[9] σ Receptors have been implicated in other physiological processes such as intestinal ion transport,[21] synthesis of pineal hormone,[22] colonic motility,[23] and inhibition of carbachol-stimulated phosphoinositide turnover.[24] Furthermore, the affinity of certain steroids for σ receptors has led to the suggestion that these receptors might somehow link the endocrine, nervous, and immune systems.[25] A recent finding that a key component of rat liver σ receptors may be a cyclophilin also suggests its link to the immune system,[26] and indeed, certain σ ligands have shown effects on immune response.[27] Other research has centered on the proposal that σ receptors may be xenobiotic metabolizing enzymes and not neurotransmitter receptors.[28] The effects of guanine nucleotides and G-protein function modifiers on the binding properties of σ ligands might suggest interaction of σ receptors with G-proteins, whereas the physical characteristics of σ receptors might suggest otherwise, thus impeding their unquestionable establishment as neurotransmitter-like receptors.[29]

Additional evidence describing the heterogeneity of the σ receptor has further complicated the comprehension of its function.[30,31] Currently, two distinct σ receptor subtypes have been defined. Whereas σ_1 sites strongly bind (+)-benzomorphans, DTG, and haloperidol, σ_2 receptors preferentially bind (−)-benzomorphans and have high affinity for both DTG and haloperidol.[30c,h] The effects of GTP on ligand binding to the σ receptor subtypes have led to the postulation that the σ_1 but not the σ_2 subtype is coupled to G-protein(s). Haloperidol treatment has also been shown to preferentially downregulate the σ_1 relative to the σ_2 subtype *in vivo*. It also appears that the σ_2 subtype but not the σ_1 subtype is associated with the effects on motor behavior seen upon the microinjection of σ ligands into the substantia nigra or red nucleus of rats.[12,32] It is imperative that subtype selective σ ligands be produced to further elucidate the functional roles of the subtypes. Additionally, these ligands would have potential therapeutic value for the clinic.

Recently we reported that the incorporation of an (*E*)-8-benzylidene moiety into the 2-methyl-5-(3-hydroxyphenyl)morphan-7-one produces compounds with decreased opioid-binding affinity and greatly increased σ receptor-binding affinity.[33] Now we wish to report further structure−activity relationship (SAR) studies

Scheme 1[a]



1
(+)-1
(−)-1

2, (−)-2, (+)-2, R,R' = H
3, (−)-3, (+)-3, R,R' = Cl
4, (−)-4, (+)-4, R = OMe, R' = H
5, (−)-5, (+)-5, R = Cl, R' = H
6, (−)-6, (+)-6, R = H, R' = OMe
7, (−)-7, (+)-7, R = H, R' = Cl
(+)-8, R = I, R' = H
(+)-9, R = H, R' = I

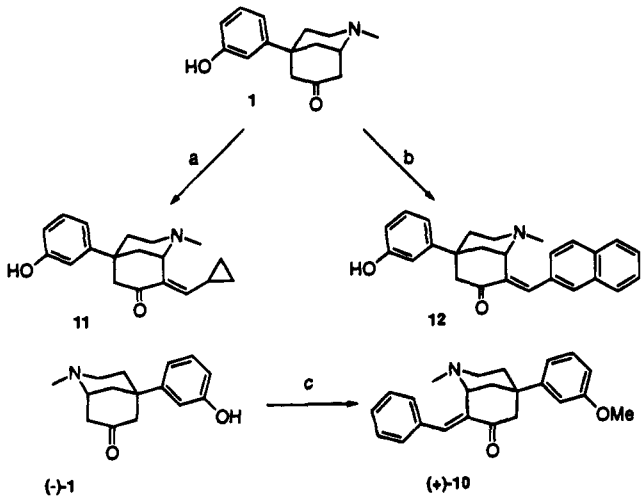
[a] Reagents and conditions: (a) aldehyde, KOH, methanol, reflux.

and that the enantiomers of several of these derivatives have selectivity for the σ_1 and σ_2 subtypes and are among the most selective derivatives for the σ_2 subtype currently known.[34,35]

Chemical Synthesis.[36] All of the compounds were prepared by reaction of **1**, (+)-**1**, or (−)-**1** with the appropriately substituted benzaldehyde under the conditions of the Claisen−Schmidt reaction with the exception of compound (+)-**10** which was prepared from (−)-(1*R*,5*R*)-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo-[3.3.1]nonan-7-one.[37] The *E*-8 configuration assigned to all of the compounds in Scheme 1 was based on the similarities of their ^{1}H NMR spectra (chemical shifts and multiplicity) with that of compound **2**, the configuration of which was previously determined by an X-ray crystallographic study.[33] The 1-H proton of **2** resonates at 4.48 ppm, whereas the 1-H proton of the assigned isomers ranges from 4.30 to 4.54 ppm. Furthermore, the chemical shifts of the N-2 methyl and benzylidene protons of **2** are 2.02 and 7.79 ppm, respectively, while those of the assigned isomers resonate in the range 2.03−2.10 and 7.63−7.75 ppm, respectively. The chemical shifts of the 6-H protons of **2** are 2.95 and 2.61 ppm, and the 6-H protons of the assigned isomers range from 2.93 to 2.96 and 2.59 to 2.61 ppm. Compound **12** was also assigned as having an *E*-8 configuration for the naphthylidene moiety on the basis of the similarity of the ^{1}H NMR spectrum with that of **2**: 1-H proton (4.59 ppm), N-Me (2.05), benzylidene proton (7.94), and 6-H protons (2.98, 2.65). The larger aromatic system of **12** relative to the benzylidene derivatives **2**−**11** results in slightly more deshielded 1-H, benzylidene, and 6-H protons for **12**.[38] The *E*-8 configuration of the cyclopropylmethylidene moiety of compound **11** was confirmed by an X-ray crystallographic analysis, *vide infra*.

X-ray Crystallography of 11. The six-membered heterocyclic ring has a normal chair conformation with the methyl group equatorial to the ring (ORTEP is shown in Figure 1). The six-membered ring fused to the heterocyclic ring has an envelope conformation with C-9 being 0.69 Å out of the plane (±0.07 Å) formed by the remaining five ring atoms. The aromatic rings and the cyclopropyl moiety are on opposite sides of the fused ring system with the cyclopropyl group attached at C-8. The configuration of the C-8−C-16 double bond is E. There is one intermolecular hydrogen bond which links the molecules into pairs in the unit cell (O-H···N where OH = 0.98 Å, H···N = 1.85 Å, O···N = 2.80 Å, and the angle of O−H···N = 159.4°).

Scheme 2[a]



[a] Reagents and conditions: (a) cyclopropanecarboxaldehyde, KOH, methanol, reflux; (b) 2-naphthaldehyde, KOH, methanol, reflux; (c) (1) diazomethane/ether, (2) benzaldehyde, KOH, methanol, reflux.

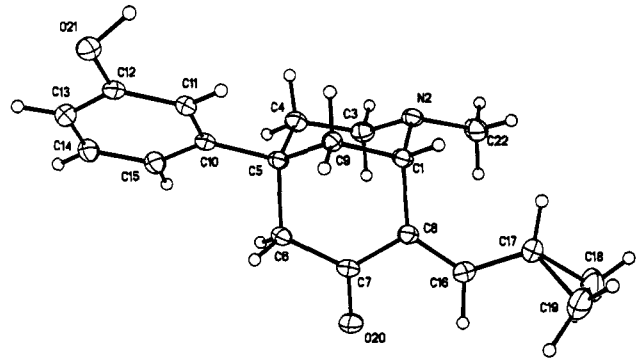


Figure 1. Results of the X-ray study on 11. The figure is drawn using the experimentally determined coordinates with the thermal ellipsoids at the 20% probability level.

Results and Discussion

Biological Data: Binding to σ Receptors. Binding assays for the σ receptor subtypes, σ_1 and σ_2, were performed. The σ receptor binding data are shown in Table 1. As was shown previously, the (*E*)-8-benzylidene functionality is necessary for imparting σ receptor affinity to the 2-methyl-5-(3-hydroxyphenyl)-morphans.[33] However, the smaller (*E*)-8-cyclopropylmethylidene moiety of **11** and the larger (*E*)-8-naphthylidene group of **12** do not significantly alter their σ_1 subtype affinities (62.3 and 16.6 nM, respectively) relative to the (*E*)-8-benzylidene derivatives. We had reported[33] that (*E*)-8-benzylidene derivatives of the 2-methyl-5-(3-hydroxyphenyl)morphans appeared to have the structural features necessary for σ_1 receptor binding consistent with the proposed model of Glennon et al.,[39] which postulates a primary hydrophobic site (B) and a secondary hydrophobic site (A), with distances from the basic nitrogen ranging from 6 to 10 and 2.5 to 3.9 Å, respectively. X-ray crystallographic data for compound **2** (compound **5** in ref 33) revealed distances between the amine nitrogen and the centroids of the phenolic and benzylidene phenyl ring of 5.71 and 4.81 Å, respectively. Although it should be kept in mind that the above model was derived for binding data for nonrigid molecules, thereby limiting the certainty with which the spatial relationships between the binding sites can be determined, it appeared that the benzylidene moiety of (−)-**2** (compound (−)-**5** in ref 33) was consistent with site A of the model of Glennon et al. Furthermore, the model proposed that steric bulk was tolerated by site A. The high σ_1 receptor-binding affinity of **12** (16.6 nM), which contains a more bulky (*E*)-8-naphthylidene moiety, is consistent with this observation. The σ_2 subtype appears to be more sensitive to the size of the substituent at the C-8 position of the 2-methyl-5-(3-hydroxyphenyl)-morphan nucleus, which is evident from the affinities for compounds **11** (356.0 nM, σ_2) and **12** (80.2 nM, σ_2), which are somewhat lower than the affinities for the racemic (*E*)-8-benzylidene derivatives **2**−**7** (9.5−71.8 nM, σ_2). In general, the racemic (*E*)-8-benzylidene derivatives are potent σ receptor ligands but display only slight subtype selectivity.

Table 1. Inhibition of Radioligand Binding to Guinea-Pig Brain σ_1 Receptors and Rat Liver σ_2 Receptors

	K_i (±SEM) (nM)		
compd	[^{3}H]-(+)-pentazocine (σ_1)	[^{3}H]DTG + dextrallorphan (σ_2)	$K_i(\sigma_1)/K_i(\sigma_2)$
(+)-**1**	762 ± 81.6[a]	>80 × 10^3	0.01
(−)-**1**	31.8 (±1.8) × 10^3 [a]	25.0 (±2.5) × 10^3	1.27
2	23.3 ± 4.0[a]	37.0 ± 1.3	0.63
(−)-**2**	9.4 ± 1.3[a]	154.0 ± 3.0	0.06
(+)-**2**	1.3 (±0.1) × 10^3 [a]	16.5 ± 2.6	79
3	65.3 ± 5.0[a]	71.8 ± 3.4	0.91
(−)-**3**	32.0 ± 2.9[a]	35.5 ± 8.8	0.90
(+)-**3**	3.2 (± 0.2) × 10^3 [a]	13.4 ± 2.0	238
4	18.4 ± 1.7	35.0 ± 8.9	0.53
(−)-**4**	7.8 ± 0.7	81.3 ± 1.4	0.10
(+)-**4**	1.91 (±0.16) × 10^3	21.6 ± 4.4	88
5	38.1 ± 4.4	9.5 ± 1.3	4.0
(−)-**5**	18.4 ± 1.7	26.5 ± 0.7	0.69
(+)-**5**	759.3 ± 59.8	6.4 ± 0.4	119
6	7.9 ± 1.8	44.3 ± 5.5	0.18
(−)-**6**	5.8 ± 1.5	46.3 ± 0.1	0.12
(+)-**6**	6.0 (±0.75) × 10^3	38.2 ± 3.5	157
7	51.3 ± 14.2	34.5 ± 5.2	1.5
(−)-**7**	30.3 ± 10.5	123 ± 7.6	0.25
(+)-**7**	3.1 (±0.35) × 10^3	22.4 ± 2.1	138
(+)-**8**	1.71 (±0.14) × 10^3	52.6 ± 0.3	32
(+)-**9**	1.62 (±0.17) × 10^3	24.0 ± 1.5	68
10	138 ± 9.6[a]	81.7 ± 4.6	1.7
(+)-**10**	145 ± 22.2	52.0 ± 4.8	2.8
11	62.3 ± 3.9	356 ± 30.1	0.18
12	16.6 ± 1.9	80.2 ± 4.7	0.21

[a] Data from ref 33.

In vitro binding assay data for the racemic compounds do not allude to the more profound σ subtype selectivity that is seen for some of the individual enantiomers of these racemates. Incorporation of substituted (*E*)-8-benzylidene moieties onto the 7-keto precursor (+)-2-methyl-5-(3-hydroxyphenyl)morphan, (+)-**1**, produces compounds (−)-**2**−(−)-**7** (σ_1-binding affinities 5.8−32.0 nM), which have between a 25- and 131-fold increase in affinity for the σ_1 receptor subtype relative to the keto precursor (+)-**1** (K_i = 762 nM, σ_1), thereby substantiating the necessity of the (*E*)-8-benzylidene substituents for σ_1 receptor binding. Whereas levorotatory derivatives (−)-**2** (*m*,*p*-H), (−)-**4** (*p*-OMe), (−)-**6** (*m*-OMe), and (−)-**7** (*m*-Cl) have subtype selectivity for the σ_1 receptor (4−16-fold), (−)-**3** (*m*,*p*-Cl) and (−)-**5** (*p*-Cl) are not selective and bind equally well to either σ subtype. Relative to the unsubstituted derivative and the *p*-OMe derivative, the chloro-substituted derivatives (−)-**3** and **5** have a decreased affinity for the σ_1 subtype with a concomitant increase in σ_2 affinity. Furthermore, substitution of an (*E*)-8-benzylidene moiety onto the 7-keto

precursor (−)-2-methyl-5-(3-hydroxyphenyl)morphan, (−)-**1**, produces compounds (+)-**2**−(+)-**9**, (6.4−52.6 nM, σ_2), which have at least a 475−3906-fold increase in affinity for the σ_2 receptor subtype relative to the keto precursor (−)-**1** ($K_i = 25 \times 10^3$ nM). This remarkable enhancement of σ_2 receptor affinity is accompanied by substantial selectivity of all of these dextrorotatory products for the σ_2 relative to the σ_1 subtype (32−238-fold), and thus, they are among the most σ_2 subtype selective compounds currently reported. It should be noted that the degree of σ_2 selectivity reported here for (+)-**2** and (+)-**3** (79- and 238-fold, respectively) is lower than that described previously in our preliminary report[34] (185- and 554-fold, respectively). This is due to the observation of slightly lower σ_1 affinities (higher K_i values) for (+)-**2** and (+)-**3** in the previous study. The reason for this is not clear but may be related to differences in the preparation of the guinea pig brain membranes.[30j,33]

The enantioselectivities of the σ receptor subtypes for this class of compounds are quite distinct. Whereas the σ_1 subtype is highly enantioselective for the levorotatory isomers, (−)-**2**−(−)-**7** (41−1034-fold), the σ_2 subtype is only somewhat enantioselective for the dextrorotatory isomers, (+)-**2**−(+)-**7** (2.6−9.3-fold). The sizable difference in enantioselectivity for the dextrorotary and levorotatory isomers of the σ receptor subtypes provides further support for their classification as distinct entities. The potent affinities of all of the levorotatory isomers for the σ_1 receptor, regardless of the substituents on the (*E*)-8-benzylidene moiety, further support the contention that site B of the Glennon et al. model tolerates bulk and also indicate that electronic effects associated with substituents on the aromatic benzylidene moiety have limited affect on the binding affinities. The Glennon et al. model is two-dimensional and, therefore, does not provide any explanation for the high degree of enantioselectivity of the σ_1 receptor for the levorotatory isomers (−)-**2**−(−)-**7**. Perhaps these isomers, along with other classes of compounds whose enantiomers discriminate the σ_1 receptor, might allow further refinement of the model.[40]

Binding to Non-σ Receptors. Binding assays for the PCP site and opioid receptors (μ, δ, and κ_1) were determined (see Table 2). Many classical opioid σ ligands typically bind to PCP sites,[41] and this concern led to the determination of binding affinities at this site on the NMDA complex. All of the racemic and pure enantiomers of the (*E*)-8-benzylidene derivatives of **1**, compounds **2**−**10**, as well as compounds **11** and **12**, had only negligible affinity for PCP sites with all having K_i's of ≥100 μM, with the exception of (+)-**6**, (+)-**9**, and **10**, whose binding affinities for the PCP site were 29.9, >50, and >50 μM, respectively. The 2-methyl-5-(3-hydroxyphenyl)morphans are known to have high affinity for the μ opioid receptor,[42] and thus a study of the opioid receptor-binding affinities of these derivatives was thought to be important when considering the goal to be the production of σ receptor subtype selective ligands. In general, all of the racemic and enantiomerically pure (*E*)-8-benzylidene derivatives **2**−**10** and racemates **11** and **12** had no significant affinity for the κ_1 opioid receptor (>500 nM) with the exception of (±)-**7** (246 nM). These low binding affinities for the κ_1 receptor were in line with the binding affinities for the parent 7-keto precursors (+)-**1** and (−)-**1**. Furthermore, where the affinity of (−)-(1*R*,5*R*)-**1** for both the μ and δ opioid receptor is higher than that of the (+)-(1*S*,5*S*)-**1** isomer, this same trend is seen in the respective (*E*)-8-benzylidene products (+)-(1*R*,5*R*)-**2**−**9** and (−)-(1*S*,5*S*)-**2**−**7**. With the exception of (−)-**2** and (+)-**2**, the binding affinities of the (*E*)-8-benzylidene products **3**−**9** for the μ opioid receptor were roughly unchanged from that of the 7-keto precursors (+)-**1** and (−)-**1**. This result indicates that the (*E*)-8-benzylidene side chains for these 2-methyl-5-(3-hydroxyphenyl)morphans are easily accommodated in the μ opioid receptor binding site. Furthermore, the 1*S*,5*S* enantiomers of both the precursor **1** and the products consistently had higher affinities for the μ receptor (2−11-fold) versus the 1*R*,5*R* isomers, possibly indicating that both the 7-keto precursors and the (*E*)-8-benzylidene products are binding in a similar manner to the μ opioid receptor. The binding affinities for the precursors and the (*E*)-8-benzylidene products for the δ opioid receptor were consistently about 1 order of magnitude less than the respective μ receptor-binding affinities. However, as was found with the binding affinities for the μ receptor, the 1*S*,5*S* enantiomers of both the precursor **1** and the products consistently had higher affinities for the δ receptor versus the 1*R*,5*R* isomers. This might be an indication of a similar binding interaction of these 2-methyl-5-(3-hydroxyphenyl)morphan derivatives with both the μ and δ opioid receptors.

It is well established that the phenolic hydroxyl is necessary for imparting opioid activity to the 5-phenylmorphan compounds, and subsequently, the 3-deoxy and 3-methoxyphenyl derivatives have limited opioid activity.[43] Because of our desire to obtain σ_2 subtype selective ligands devoid of opioid receptor affinity, we prepared compound (+)-**10**, the 3-methoxyphenyl derivative of (+)-**2**. Although it was apparent that the opioid-binding affinities for (+)-**10** were decreased relative to (+)-**2**, the selectivity for the σ_2 subtype was

Table 2. Inhibition of Radioligand Binding to Rat Brain μ and δ Receptors and Guniea Pig κ_1 Receptors

	K_i (±SD) (nM)		
compd	[^{3}H]DAMGO (μ)	[^{3}H]DADLE (δ)	[^{3}H]U69,593 (κ_1)
(+)-**1**[a]	35.5 ± 5.2	505 ± 113	2536 ± 370
(−)-**1**[a]	8.7 ± 0.9	122 ± 29	491 ± 47
2[a]	33.9 ± 3.5	>500	576 ± 31
(−)-**2**[a]	392 ± 48	1989 ± 283	889 ± 97
(+)-**2**[a]	37.6 ± 2.2	735 ± 109	1091 ± 102
3[a]	6.1 ± 1.6	240 ± 45	>500
(−)-**3**[a]	45.1 ± 4.3	>500	>500
(+)-**3**[a]	4.5 ± 1.3	271 ± 35	>500
4	4.1 ± 0.9	7.1 ± 0.3	>500
(−)-**4**	27.0 ± 5.8	>500	>500
(+)-**4**	3.9 ± 1.4	127 ± 29	>500
5	21.6 ± 2.8	>1000	>500
(−)-**5**	48.2 ± 4.6	>500	>500
(+)-**5**	6.4 ± 0.8	336 ± 58	>500
6	11.8 ± 1.6	>400	>500
(−)-**6**	31.4 ± 2.8	>400	>500
(+)-**6**	12.6 ± 1.2	175 ± 117	>500
7	23.2 ± 1.6	222 ± 49	246 ± 90
(−)-**7**	47.6 ± 5.0	386 ± 96	>500
(+)-**7**	11.8 ± 0.8	217 ± 68	>500
(+)-**8**	26.7 ± 4.9	321 ± 59	>1000
(+)-**9**	9.1 ± 1.3	141 ± 30	>500
10[a,b]	>250	>500	>1000
(+)-**10**	>250	>500	>500
11	11.2 ± 1.4	>1000	>500
12	2.2 ± 0.2	142 ± 39	>500

[a] Data from ref 33. [b] Equivalent to compound **3** in ref 33.

diminished by 22-fold. This selectivity decrease results from a concomitant increase of σ_1 affinity of 7-fold and a decrease of σ_2 affinity of 3-fold for (+)-**10** relative to (+)-**2**. The opposing effects of the *O*-methylation of (+)-**2**, which produces (+)-**10**, on the binding affinities for the σ_1 and σ_2 receptors provide additional evidence supporting the distinct nature of these subtypes.

σ Receptor Ligands as Tumor-Imaging Agents. Although the cellular role of σ receptors has remained elusive, their high expression in many human tumor cell lines[20] and solid tumors[44] has prompted the use of radiolabeled σ receptor ligands as tumor-imaging agents. While σ_1 ligands, such as ^{131}I-labeled [2-(piperidinylamino)ethyl]-4-iodobenzamide, have been used to visibly delineate tumors in nude mice,[45] it appears that σ_2 ligands are found in much higher density in tumor cells,[20b] suggesting that radiolabeled σ_2 ligands would constitute superior tumor-imaging agents. Therefore, it was of interest to prepare iodinated derivatives in the dextrorotatory series and determine their affinity and selectivity for σ_2 receptors. We prepared the *p*- and *m*-I derivatives (+)-**8** and (+)-**9**, and the binding affinities revealed that both compounds were σ_2 selective ligands. The *m*-I derivative (+)-**9** had superior affinity (24.0 nM) and selectivity (68-fold) for the σ_2 versus σ_1 subtype as compared to (+)-**8**. Therefore, utilizing standard methodology,[46] it should be possible to prepare an ^{123}I- or ^{131}I-labeled derivative of (+)-**9** as a potential SPECT (single-photon emission computed tomography) imaging agent for tumors.

Conclusion

The high degree of selectivity of the dextrorotatory isomers of this novel class of σ receptor ligands for the σ_2 subtype strongly supports the hypothesis that the σ_1 and σ_2 subtypes are pharmacologically discrete binding sites.[30] The probable association of the σ_2 receptor subtype with the motor side effects of antipsychotic and neuroleptic drugs,[32] the high density of σ_2 sites in tumor cells,[20b] and elucidation of the physiological role of the σ receptor subtypes have prompted the pursuit of σ_2 selective ligands. Recent progress has been made by several laboratories in the development of σ_2 subtype selective compounds with excellent affinity. Among these are represented a benzimidazolone,[35a] 3-(ω-aminoalkyl)-1*H*-indoles,[35b] and spiropiperidine derivatives.[35c] Despite their high affinity for the μ opioid receptor, the (*E*)-8-benzylidene-5-phenylmorphans reported here, with their high selectivity and affinity for the σ_2 receptors versus the σ_1 sites, should be useful tools for further delineation of the function and physiological role of these receptors. Further modification of this new class of σ subtype selective ligands to decrease their affinity for opioid receptors is currently in progress.

Experimental Section

General Instrumentation and Methods. Proton NMR spectra were recorded for the free bases of all compounds in $CDCl_3$ (unless otherwise specified) on a Varian Gemini 300 spectrometer, and the data are reported in the following format: chemical shift (all relative to Me_4Si), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, ap = apparent), integration, coupling constants, and exchangeability after D_2O addition. Electron impact (EI) mass spectra were recorded on a VG 7070F spectrometer, and chemical ionization (CI) mass spectra were recorded on a Finnigan 4600 spectrometer. Polarimetric measurements were taken using a Perkin-Elmer 241MC polarimeter. Thin layer chromatography (TLC) was performed on Analtech silica gel GHLF 0.25-mm plates. Preparative TLC was performed on Analtech silica gel GF 2.00-mm plates. Radial disk chromatography was performed on 2.00- or 4.00-mm Merck silica gel 60 GF_{254} (mean particle size 15 μm) disks. Column chromatography was performed with Fluka silica gel 60 (mesh 220–440). Chloroform/methanol/28% NH_4OH solvent systems for chromatography are as follows: A, 90:10:0.5; B, 95:5:0.5; C, 98:2:0.2; D, 98:2:0.1; E, 99:1:0.1, by volume. Evaporations were done using a Buchi rotary evaporator unless specified otherwise. Ethyl acetate/hexanes solvent systems for chromatography are as follows: F, 3:1; G, 4:1, by volume. Products were visualized on TLC with short-wave UV or iodoplatinate reagent (Sigma Chemical Co.). Elemental microanalyses were performed by Atlantic Microlab, Inc. Melting points were recorded on a Thomas-Hoover capillary apparatus or on a Mel-Temp II apparatus (>260 °C) and are uncorrected. The yields reported are not optimized.

General Procedure for the Preparation of 4, (−)-4, (+)-4, 5, (−)-5, (+)-5, 6, (−)-6, (+)-6, 7, (−)-7, (+)-7, (+)-8, and (+)-9. A solution of the appropriate ketone free base precursor 1, (+)-1, or (−)-1, 5–7 mequiv of 87% KOH (s), and 2.5–6 mequiv of the appropriately substituted benzaldehyde in methanol (24–33 mL/mmol ketone) was heated to reflux under an atmosphere of argon for the amount of hours indicated below. The solvent was evaporated, and the residue was taken up in saturated brine and extracted with chloroform. The chloroform extracts were combined, dried (Na_2SO_4/K_2CO_3), and evaporated. The resulting crude compounds were further purified as indicated below.

5-(3-Hydroxyphenyl)-(*E*)-8-(4-methoxybenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate (4) from 1.[33] Reaction time was 153 h; the resulting oil was purified by spinning disk chromatography (4.00 mm, eluting solvent system B). The major short-wave UV-absorbing band (R_f = 0.26 in eluting solvent) was removed and extracted with the eluting solvent. Evaporation gave a yellow residue which was further purified with two preparative TLC plates (eluting 2× with solvent system G). The compound was extracted with solvent system A, and evaporation of the extracts gave a light yellow foam that weighed 147 mg (43%) after thorough drying with high vacuum at room temperature. This foam was dissolved in 2-propanol (4 mL) and made acidic with 61% perchloric acid. The light yellow crystalline salt was filtered and rinsed with 2-propanol and petroleum ether: yield 155 mg; mp 224–225 °C dec; ^{1}H NMR δ 7.74 (s, 1H), 7.40 (d, 2H, J = 8.7 Hz), 7.23 (m, 1H), 6.94 (ap d, 3H, J = 8.7 Hz), 6.84 (m, 1H), 6.71 (dd, 1H, J = 2.4, 8.0 Hz), 5.3 (br, 1H, ex w/D_2O), 4.54 (m, 1H), 3.85 (s, 3H), 2.93 (dd, 1H, J = 3.0, 18.0 Hz), 2.67 (dd, 1H, J = 3.9, 13.7 Hz), 2.59 (d, 1H, J = 19.0 Hz), 2.49 (m, 2H), 2.25 (td, 1H, J = 2.7, 12.9 Hz), 2.10 (s, 3H), 2.04 (m, 1H), 1.89 (m, 1H); MS (CI-NH_3) m/z 364 (MH^+). Anal. ($C_{23}H_{25}NO_3 \cdot HClO_4$) C, H, N.

(−)-(1*S*,5*S*)-5-(3-Hydroxyphenyl)-(*E*)-8-(4-methoxybenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(−)-4] from (+)-(1*S*,5*S*)-1.[33] A procedure analogous to the preparation of **4** was carried out with (+)-1 except that the crude product was purified by passing through a silica gel 60 (15 g) pad eluting with solvent system A. The product was then further purified by spinning disk chromatography (2.00 mm, eluting with solvent system F). The yield of yellow foam was 128 mg (34%), and this was converted to a perchlorate salt by dissolving this in hot 2-propanol (3 mL) and acidifying with 61% perchloric acid yielding a light yellow salt: 134 mg; mp 218–219 °C; $[\alpha]^{21}_D$ (perchlorate salt in DMSO, c = 0.59) = −176.5°; $[\alpha]^{21}_D$ (free base in MeOH, c = 0.50) = −149.9°; ^{1}H NMR and MS of the free base matched that of **4**. Anal. ($C_{23}H_{25}NO_3 \cdot HClO_4$) C, H, N.

(+)-(1*R*,5*R*)-5-(3-Hydroxyphenyl)-(*E*)-8-(4-methoxybenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-4] from (−)-(1*R*,5*R*)-1.[33] A procedure analogous to the preparation of (−)-**4** was carried out yielding a yellow foam that weighed 134 mg (36%), and this was converted to a perchlorate salt by dissolving this in hot 2-propanol (3 mL) and acidifying with 61% perchloric acid

yielding a light yellow salt: 137 mg; mp 218-219 °C; $[\alpha]^{21}_D$ (perchlorate salt in DMSO, $c = 0.70$) = +175.4°; $[\alpha]^{21}_D$ (free base in MeOH, $c = 0.44$) = +155.4°; ^{1}H NMR of the free base matched that of **4**; MS (EI) m/z 363 ($M^{+\cdot}$). Anal. ($C_{23}H_{25}NO_3 \cdot HClO_4$) C, H, N.

(*E*)-8-(4-Chlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate (5) from 1. Reaction time was 14 h; the residue was purified by preparative TLC (eluting solvent system B). The major short-wave UV-absorbing band ($R_f = 0.46$ in eluting solvent) was removed and extracted with the eluting solvent. Evaporation gave a yellow residue which was dissolved in 2-propanol (2 mL) and made acidic with 61% perchloric acid. The yellow crystalline salt was filtered and rinsed with 2-propanol and petroleum ether: yield 156 mg (59%); mp 237-239 °C dec; ^{1}H NMR δ 7.71 (s, 1H), 7.39 (d, 2H, J = 8.5 Hz), 7.32 (d, 2H, J = 8.5 Hz), 7.23 (d, 1H, J = 7.9 Hz), 6.94 (d, 1H, J = 8.8 Hz), 6.84 (m, 1H), 6.71 (dd, 1H, J = 2.4, 7.9 Hz), 5.2 (br, 1H, ex w/D_2O), 4.38 (m, 1H), 2.95 (dd, 1H, J = 3.0, 17.8 Hz), 2.67 (m, 1H), 2.60 (d, 1H, J = 19.3 Hz), 2.47 (m, 2H), 2.25 (td, 1H, J = 3.0, 12.8 Hz), 2.05 (m, 1H), 2.04 (s, 3H), 1.90 (dd, 1H, J = 2.1, 12.9 Hz); MS (CI-NH_3) m/z 368 (MH^+). Anal. ($C_{22}H_{22}ClNO_2 \cdot HClO_4 \cdot 0.5C_3H_8O$) C, H, N.

(−)-(1*S*,5*S*)-(*E*)-8-(4-Chlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(−)-5] from (+)-(1*S*,5*S*)-1. A procedure analogous to the preparation of **5** was carried out with (+)-1 except that the crude product was purified by spinning disk chromatography (2.00 mm, eluting with solvent system B) yielding a light yellow foam that weighed 202 mg (67%) after thorough drying on a high-vacuum line. An analytical sample was prepared by preparative TLC (eluting solvent system G), yield 175 mg. This was dissolved in hot 2-propanol (4 mL) and made acidic with 61% perchloric acid. The light yellow crystalline solid was filtered, rinsed with 2-propanol, and dried: yield 215 mg; mp 257-258 °C dec; $[\alpha]^{21}_D$ (perchlorate salt in DMSO, $c = 0.55$ excluding solvent of crystallization) = −214.0°; $[\alpha]^{22}_D$ (free base in MeOH, $c = 0.78$) = −133.5°; ^{1}H NMR and MS of the free base matched that of **5**. Anal. ($C_{22}H_{22}ClNO_2 \cdot HClO_4 \cdot C_3H_8O$) C, H, N.

(+)-(1*R*,5*R*)-(*E*)-8-(4-Chlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-5] from (−)-(1*R*,5*R*)-1. A procedure analogous to the preparation of (−)-**5** was carried out with (−)-1 yielding a light yellow foam that weighed 206 mg (67%) after thorough drying on a high-vacuum line. An analytical sample was prepared by preparative TLC (eluting solvent system G), yield 180 mg. This was dissolved in hot 2-propanol (4 mL) and made acidic with 61% perchloric acid. The light yellow crystalline solid was filtered, rinsed with 2-propanol, and dried: yield 214 mg; mp 256-257 °C dec; $[\alpha]^{21}_D$ (perchlorate salt in DMSO, c = 0.61) = +206.6.°; $[\alpha]^{22}_D$ (free base in MeOH, $c = 0.74$) = +128.5°; ^{1}H NMR and MS of the free base matched that of **5**. Anal. ($C_{22}H_{22}ClNO_2 \cdot HClO_4$) C, H, N.

5-(3-Hydroxyphenyl)-(*E*)-8-(3-methoxybenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate (6) from 1. Reaction time was 47 h; the residue was dissolved in chloroform and evaporated onto silica gel 60 (2.5 g, mesh 220-440). This was loaded onto a 50-g silica gel column that was eluted with solvent system C. The fractions containing the product ($R_f = 0.33$ with solvent system B) were combined and evaporated. The resulting oil was dissolved in hot 2-propanol (4 mL), and this solution was made acidic with 61% perchloric acid. The light yellow salt was filtered and rinsed with 2-propanol, yield 278 mg (49%). An analytical sample was prepared by recrystallization from methanol/2-propanol: yield 248 mg; mp 212-213 °C; ^{1}H NMR δ 7.74 (s, 1H), 7.32 (t, 1H, J = 7.8 Hz), 7.24 (t, 1H, J = 7.9 Hz), 6.90 (m, 5H), 6.71 (dd, 1H, J = 2.6, 8.1 Hz), 4.48 (t, 1H, J = 3.4 Hz), 3.83 (s, 3H), 2.95 (dd, 1H, J = 3.0, 17.7 Hz), 2.67 (m, 1H), 2.61 (d, 1H, J = 17.4 Hz), 2.46 (m, 2H), 2.28 (td, 1H, J = 2.8, 12.8 Hz), 2.05 (s, 3H), 2.04 (m, 1H), 1.91 (m, 1H); MS (CI-NH_3) m/z 364 (MH^+). Anal. ($C_{23}H_{25}NO_3 \cdot HClO_4 \cdot 0.5C_3H_8O$) C, H, N.

(−)-(1*S*,5*S*)-5-(3-Hydroxyphenyl)-(*E*)-8-(3-methoxybenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(−)-6] from (+)-(1*S*,5*S*)-1. A procedure analogous to the preparation of **6** was carried out with (+)-1: yield 40%; mp 243-245 °C; $[\alpha]^{23}_D$ (perchlorate salt in DMF, $c = 0.63$) = −226.3°; $[\alpha]^{23}_D$ (free base in methanol, $c = 0.70$) = −127.0°; ^{1}H NMR and MS of the free base matched that of **6**. Anal. ($C_{23}H_{25}NO_3 \cdot HClO_4$) C, H, N.

(+)-(1*R*,5*R*)-5-(3-Hydroxyphenyl)-(*E*)-8-(3-methoxybenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-6] from (−)-(1*R*,5*R*)-1. A procedure analogous to the preparation of **6** was carried out with (−)-1: yield 39%; mp 242-243 °C; $[\alpha]^{23}_D$ (perchlorate salt in DMF, $c = 0.65$) = +229.3°; $[\alpha]^{23}_D$ (free base in methanol, $c = 0.60$) = +124.5°; ^{1}H NMR and MS of the free base matched that of **6**. Anal. ($C_{23}H_{25}NO_3 \cdot HClO_4$) C, H, N.

(*E*)-8-(3-Chlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate (7) from 1. Reaction time was 23 h; the residue was dissolved in chloroform, and this was evaporated onto silica gel 60 (2.5 g, mesh 220-440). This was loaded onto a 50-g silica gel column that was eluted with solvent system B. The fractions containing the product ($R_f = 0.57$ with solvent system B) were combined and evaporated. The resulting oil was dissolved in hot 2-propanol (4 mL), and this solution was made acidic with 61% perchloric acid. The light yellow salt was filtered and rinsed with 2-propanol: yield 313 mg (55%); mp 241-242 °C; ^{1}H NMR δ 7.70 (s, 1H), 7.29 (m, 5H), 6.92 (d, 1H, J = 7.8 Hz), 6.84 (m, 1H), 6.70 (dd, 1H, J = 2.5, 7.9 Hz), 5.64 (br, 1H, ex w/D_2O), 4.37 (t, 1H, J = 3.6 Hz), 2.96 (dd, 1H, J = 3.0, 17.9 Hz), 2.69 (ddd, 1H, J = 1.9, 4.2, 13.7 Hz), 2.61 (d, 1H, J = 17.2 Hz), 2.54-2.41 (m, 2H), 2.26 (td, 1H, J = 2.8, 13.0 Hz), 2.04 (m, 1H), 2.05 (s, 3H), 1.90 (dd, 1H, J = 1.9, 13.1 Hz); MS (CI-NH_3) m/z 368 (MH^+). Anal. ($C_{22}H_{22}NClO_2 \cdot HClO_4$) C, H, N.

(−)-(1*S*,5*S*)-(*E*)-8-(3-Chlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(−)-7] from (+)-(1*S*,5*S*)-1. A procedure analogous to the preparation of **7** was carried out with (+)-1: yield 51%; mp 251-252 °C; $[\alpha]^{23}_D$ (perchlorate salt in DMF, $c = 0.70$) = −206.2°; $[\alpha]^{23}_D$ (free base in methanol, $c = 0.50$) = −104.8°; ^{1}H NMR and MS of the free base matched that of **7**. Anal. ($C_{22}H_{22}NClO_2 \cdot HClO_4$) C, H, N.

(+)-(1*R*,5*R*)-(*E*)-8-(3-Chlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-7] from (−)-(1*R*,5*R*)-1. A procedure analogous to the preparation of **7** was carried out with (−)-1: yield 39%; mp 252-253 °C; $[\alpha]^{23}_D$ (perchlorate salt in DMF, $c = 0.56$) = +217.9°; $[\alpha]^{23}_D$ (free base in methanol, $c = 0.41$) = +100.1°; ^{1}H NMR and MS of the free base matched that of **7**. Anal. ($C_{22}H_{22}NClO_2 \cdot HClO_4$) C, H, N.

(+)-(1*R*,5*R*)-5-(3-Hydroxyphenyl)-(*E*)-8-(4-iodobenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-8] from (−)-(1*R*,5*R*)-1. Reaction time was 23 h; the residue was taken up in chloroform and evaporated onto silica gel 60 (2 g, mesh 220-440). This was loaded onto a 50-g silica gel column that was eluted with solvent system C. The fractions containing the product (R_f = 0.45 with solvent system B) were combined and evaporated. The resulting light yellow crystalline solid was dissolved in hot 2-propanol (7 mL), and this solution was made acidic with 61% perchloric acid. The off-white crystals were filtered and rinsed with 2-propanol: yield 263 mg (53%); mp 259-260 °C dec; $[\alpha]^{22}_D$ (perchlorate salt in DMSO, $c = 0.75$) = +191.8°; $[\alpha]^{22}_D$ (free base in DMSO, $c = 0.61$) = +41.8°; ^{1}H NMR δ 7.75 (d, 2H, J = 8.4 Hz), 7.67 (s, 1H), 7.24 (t, 1H, J = 8.0 Hz), 7.12 (d, 2H, J = 8.4 Hz), 6.94 (d, 1H, J = 7.8 Hz), 6.84 (m, 1H), 6.71 (dd, 1H, J = 2.6, 8.2 Hz), 4.8 (br, 1H, ex w/D_2O), 4.37 (m, 1H), 2.95 (dd, 1H, J = 3.0, 17.8 Hz), 2.67 (dd, 1H, J = 3.2, 13.4 Hz), 2.60 (d, 1H, J = 17.7 Hz), 2.46 (m, 2H), 2.25 (td, 1H, J = 2.9, 12.9 Hz), 2.04 (m, 1H), 2.03 (s, 3H), 1.90 (d, 1H, J = 13.1 Hz); MS (CI-NH_3) m/z 460 (MH^+). Anal. ($C_{22}H_{22}INO_2 \cdot HClO_4 \cdot 0.75C_3H_8O$) C, H, N.

(+)-(1*R*,5*R*)-5-(3-Hydroxyphenyl)-(*E*)-8-(3-iodobenzylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-9] from (−)-(1*R*,5*R*)-1. Reaction time was 26 h; the residue was dissolved in chloroform and evaporated onto silica gel 60 (2 g, mesh 220-440). This was loaded onto a 25-g silica gel column that was eluted with solvent system C. The

fractions containing the product (R_f = 0.40 with solvent system B) were combined and evaporated. The resulting light yellow foam was dissolved in hot 2-propanol (10 mL), and this solution was made acidic with 61% perchloric acid. The light yellow crystals were filtered and rinsed with 2-propanol: yield 242 mg (48%); mp 246–247 °C dec (MelTemp); $[\alpha]^{23}_D$ (perchlorate salt in DMSO, c = 0.74) = +146.2°; $[\alpha]^{21}_D$ (free base in DMSO, c = 0.68) = −0.2°; ^{1}H NMR δ 7.75 (s, 1H), 7.68 (d, 1H, J = 7.9 Hz), 7.66 (s, 1H), 7.33 (d, 1H, J = 7.8 Hz), 7.24 (m, 1H), 7.14 (t, 1H, J = 7.8 Hz), 6.93 (d, 1H, J = 7.8 Hz), 6.84 (t, 1H, J = 2.0 Hz), 6.71 (dd, 1H, J = 2.6, 7.9 Hz), 5.2 (br, 1H, ex w/D_2O), 4.34 (t, 1H, J = 3.4 Hz), 2.95 (dd, 1H, J = 3.0, 18.0 Hz), 2.69 (ddd, 1H, J = 2.0, 5.5, 13.4 Hz), 2.60 (d, 1H, J = 17.0 Hz), 2.48 (m, 2H), 2.25 (td, 1H, J = 2.8, 13.0 Hz), 2.07 (s, 3H), 2.03 (m, 1H), 1.89 (m, 1H); MS (CI-NH_3) m/z 460 (MH^+). Anal. ($C_{22}H_{22}INO_2 \cdot HClO_4 \cdot C_3H_8O$) C, H, N.

(+)-(1*R*,5*R*)-(*E*)-8-Benzylidene-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-10]. A solution of (−)-(1*R*,5*R*)-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one[37] (121.9 mg, 0.47 mmol), benzaldehyde (0.40 mL, 3.9 mmol), and 87% KOH (0.11 g, 1.7 mmol) in methanol (10 mL) was held at reflux under an atmosphere of argon for 10 h. The reaction solution was evaporated, and the residue was taken up in half-saturated brine (15 mL). This was extracted with chloroform (2 × 15 mL), and the extracts were dried and evaporated onto silica gel 60 (1.5 g, mesh 220–440). This was purified on a 25-g silica gel column with solvent system E. The appropriate fractions were combined and evaporated. The yellow oil was dissolved in hot 2-propanol (3 mL), and the insoluble material was filtered. The filtrate was acidified with 61% perchloric acid (3 drops). A light yellow crystalline perchlorate was filtered and rinsed with 2-propanol and petroleum ether: yield 150.1 mg (71%); mp 238–239 °C dec; ^{1}H NMR was identical with that for racemic (*E*)-8-benzylidene-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one;[33] $[\alpha]^{23}_D$ (perchlorate salt in DMF, c = 0.80) = +228.7°; $[\alpha]^{23}_D$ (free base in MeOH, c = 0.61) = +164.7°. Anal. ($C_{23}H_{25}NO_2 \cdot HClO_4$) C, H, N.

(*E*)-8-(Cyclopropylmethylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one (11) from 1. A solution of 1 (301 mg, 1.23 mmol), 87% potassium hydroxide (0.63 g, 9.8 mmol), and cyclopropanecarboxaldehyde (0.65 mL, 8.7 mmol) in methanol (40 mL) was heated to relflux for 70 h in the presence of type 3A molecular sieves (3.0 g). The solids were removed by filtration through a pad of Celite. The filtrate was evaporated, and 0.5 M citric acid (11 mL) was added to the residue. Chloroform was added, and the pH of the aqueous layer was adjusted to 9 with saturated K_2CO_3. This was extracted with chloroform (3 × 25 mL). The extracts were dried (Na_2SO_4) and evaporated, and the residue was purified on four preparative TLC plates (eluting solvent system A). The major short-wave UV-absorbing bands (R_f = 0.32 with solvent system A) were removed, combined, and extracted with the eluting solvent. Evaporation gave an off-white foam that was purified using spinning disk chromatography (2.00 mm, eluting solvent system A) which gave a white foam that weighed 269 mg (74%) after drying on a high-vacuum line. The foam crystallized upon treatment with ether and yielded white crystals: mp 192–194 °C; ^{1}H NMR δ 7.20 (t, 1H, J = 7.9 Hz), 6.88 (d, 1H, J = 7.8 Hz), 6.81 (m, 1H), 6.69 (dd, 1H, J = 2.6, 8.2 Hz), 6.33 (d, 1H, J = 11.1 Hz), 4.22 (m, 1H), 2.82 (dd, 1H, J = 2.8, 18.3 Hz), 2.69 (m, 1H), 2.50 (m, 2H), 2.30 (s, 3H), 2.26 (m, 2H), 2.03 (dt, 1H, J = 4.6, 12.5 Hz), 1.90 (m, 1H), 1.70 (m, 1H), 1.05 (ap d, 2H, J = 7.8 Hz), 0.75 (m, 2H); MS (CI-NH_3) m/z 298 (MH^+). Anal. ($C_{19}H_{23}NO_2$) C, H, N.

5-(3-Hydroxyphenyl)-(*E*)-8-(naphth-2-ylidene)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one (12). A solution of 1 (201 mg, 0.819 mmol), 87% potassium hydroxide (0.19 g, 2.9 mmol), and 2-naphthaldehyde (514 mg, 3.29 mmol) in methanol (20 mL) was heated to reflux for 14 h. The reaction solution was evaporated, and the residue was taken up in half-saturated brine (20 mL). This was extracted with chloroform (2 × 20 mL). The extracts were dried (Na_2SO_4) and evaporated onto silica gel 60 (3 g, mesh 220–440). This was loaded as a slurry onto a silica gel 60 column packed in chloroform. The column was eluted with solvent system D, and the fractions containing the major compound (R_f = 0.41 in solvent system B) were combined and evaporated yielding a yellow foam that weighed 182 mg (58%) after drying on a high-vacuum line. An analytical sample was prepared by purifying with a preparative TLC plate (eluting solvent system G), and the resulting foam gave light yellow crystals from ether: mp 177–178 °C; ^{1}H NMR δ 7.94 (s, 1H), 7.86 (m, 4H), 7.52 (m, 3H), 7.24 (t, 1H, J = 7.9 Hz), 6.96 (d, 1H, J = 7.8 Hz), 6.87 (m, 1H), 6.71 (dd, 1H), 5.2 (br, 1H, ex w/D_2O), 4.59 (t, 1H, J = 3.2 Hz), 2.98 (dd, 1H, J = 2.9, 17.8 Hz), 2.68 (m, 1H), 2.65 (d, 1H, J = 17.2 Hz), 2.50 (m, 2H), 2.34 (td, 1H, J = 2.9, 12.9 Hz), 2.06 (m, 1H), 2.05 (s, 3H), 1.92 (m, 1H); MS (CI-NH_3) m/z 384 (MH^+). Anal. ($C_{26}H_{25}NO_2$) C, H, N.

Table 3. Crystal and Refinement Data for 11

formula	$C_{19}H_{23}NO_2$
formula weight	297.38
crystal color, habit	colorless, prism
crystal dimensions, mm	0.40 × 0.24 × 0.14
crystal system	orthorhombic
space group	$P2_12_12_1$
a, Å	6.208(2)
b, Å	12.211(3)
c, Å	20.970(5)
V, Å^3	1589.6(7)
Z	4
ρ(calcd), g cm^{-3}	1.24
μ, absorption coef, cm^{-1}	0.63
temperature, °C	22
diffractometer	Siemens R3m/V
cell determination (no. of reflections, 2θ range)	25, 47–72
λ, wavelength, Å	Cu Kα, 1.54178
2θ max, deg, scan mode	115, $\theta/2\theta$
total reflections measured	1111
unique data	1067
observed data ($I > 2\sigma I$)	944
R_{int}	0.012
refinement on F^2 using all data parameters refined	221
R,[a] R_w[b]	0.042, 0.106
R,[a] R_w,[b] S[c] (for all data)	0.050, 0.112, 1.05
data:parameter ratio	5:1
final Δ_{max}/σ	0.018
Fourier excursions, eÅ^{-3}	0.12, −0.15

[a] $\Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $[\Sigma(w(F_o^2 - F_c^2)^2)/\Sigma(wF_o^2)^2]^{1/2}$. [c] $[\Sigma(w(F_o^2 - F_c^2)^2)/(N_o - N_p)]^{1/2}$.

Single-Crystal X-ray Analysis of 11. Crystals of 11 were grown by evaporation from 2-butanone/octane. Data were collected on a computer-controlled automatic diffractometer and corrected for Lorentz and polarization effects. The structure was solved by direct methods with the aid of program SHELXTL[47] and refined by full-matrix least-squares on F^2 values using program SHELXL-93.[48] The parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms and the coordinates for the hydrogen atoms on the cyclopropyl moiety and the hydroxyl oxygen. All other hydrogen atoms were included using a riding model in which the coordinate shifts of their covalently bonded atoms were applied to the attached hyrdogens with C−H = 0.96 Å. H angles were idealized and U_{iso}(H) values set at fixed ratios of U_{iso} values of bonded atoms. Additional experimental and structural analysis details are given in Table 3, and tables of crystal coordinates, bond distances, and bond angles are available as supporting information as well as from the Cambridge Crystallographic Data Base.[49]

Radioligand-Binding Assays. [^{3}H]-*N*-[1-(2-Thienyl)-cyclohexyl][3,4-^{3}H]piperidine ([^{3}H]TCP) Binding Assay (PCP Site). The methods used were as previously described.[33]

Membrane Preparation for σ_1 Receptor Assay. Membranes were prepared from the brains of male Hartley guinea pigs, as described previously.[33]

Membrane Preparation for σ_2 Receptor Assay. Rat liver membranes were used for σ_2 assays. Male Sprague–Dawley rats (Charles River, Wilmington, MA; 150–200 g) were killed by decapitation, and the livers were removed to ice-cold 10 mM Tris-HCl, pH 7.4, containing 0.9% saline or 0.32 M

sucrose. Livers were homogenized (after mincing with scissors) using a Potter-Elvehjem Teflon–glass homogenizer in 10 mL/g tissue wet weight of ice-cold 10 mM Tris-HCl/0.32 M sucrose, pH 7.4. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in 2 mL/g ice-cold Tris/sucrose and centrifuged again at 1000*g* for 10 min. The combined 1000*g* supernatants were centrifuged at 31000*g* for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/g 10 mM Tris-HCl, pH 7.4, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellets were resuspended by gentle Potter-Elvehjem homogenization to a final volume of 1.53 mL/g original tissue wet weight in 10 mM Tris-HCl, pH 7.4. Aliquots were stored at −80 °C until use. Protein concentration was determined by the method of Lowry et al.[50]

[^{3}H]-(+)-Pentazocine Binding Assay (σ_1 Receptor). σ_1-Binding assays were carried out as previously described,[33] using the σ_1 selective probe [^{3}H]-(+)-pentazocine.[30j]

[^{3}H]DTG and Dextrallorphan (σ_2 Receptor). σ_2-Binding assays were carried out using [^{3}H]DTG[51] and the method described previously for selective labeling of σ_2 sites.[52] Assays were performed using rat liver membranes in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 500 μL with 160 μg of membrane protein and 5 nM radioligand. Assays with [^{3}H]DTG included 1 μM dextrallorphan to mask σ_1 binding. Nonspecific binding was determined in the presence of 10 μM haloperidol. Assays were terminated by addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass fiber filters (Schleicher and Schuell, Keene, NH) using a Brandel cell harvester (Gaithersburg, MD). Filters were soaked in 0.5% poly(ethylenimine) for at least 30 min at 25 °C prior to use.

Competition Assays and Data Analysis for σ_2 Receptor. Test compound was incubated in σ_2 assays at 12 concentrations ranging from 0.05 to 10 000 nM or 0.5 to 100 000 nM. IC_{50} values were obtained using the iterative curve-fitting program GraphPad InPlot (San Diego, CA). K_i values were then calculated from IC_{50} values using the Cheng–Prusoff equation[53] and K_d values determined in independent experiments by Scatchard analysis. K_i values are the averages of two to three experiments, ±SEM. Each experiment was carried out in duplicate.

[^{3}H]DAMGO, [^{3}H]DADLE, and [^{3}H]U69,593 Binding Assays. μ Binding sites were labeled using [^{3}H]DAMGO (1–3 nM) and rat brain membranes as previously described.[54] Briefly, incubations proceeded for 4 h at 25 °C in 50 mM Tris-HCl, pH 7.4, along with a protease inhibitor cocktail (PIC). The nonspecific binding was determined using 20 μM levallorphan. δ Binding sites were labeled using [^{3}H]DADLE (1.7–2.5 nM) and rat brain membranes as previously described.[55] Briefly, incubations proceeded for 3–4 h at 25 °C in 10 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, and 100 nM DAMGO to block binding to μ sites and PIC. Nonspecific binding was determined using 20 μM levallorphan. k_1 binding sites were labeled using [^{3}H]U69,593 (1.2–2.3 nM) and guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with irreversible ligands BIT and FIT as previously described,[56] except that the incubation temperature was at 25 °C. Briefly, incubations proceeded for 4–6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing PIC and 1 μg/mL captopril. Nonspecific binding was determined using 1 μM U69,593.

Each [^{3}H]ligand was displaced by 8–10 concentrations of test drug, two times. All drug dilution was done in 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL bovine serum albumin. Compounds **5**, **6**, (+)-**6**, (−)-**6**, **7**, (+)-**7**, (−)-**7**, (+)-**8**, (+)-**9**, (+)-**10**, and **12** were prepared as 1 mM solutions with 10 mM Tris buffer (pH 7.4) containing 10% DMSO and 5% Emulphor EL-620 before drug dilution. The IC_{50} and slope factor (N) were obtained by using the program MLAB.

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Supporting Information Available: Tables of crystallographic data for 11 including bond lengths, bond angles, and atomic coordinates (3 pages). Ordering information is given on any current masthead page.

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