Halogenated 4-(Phenoxymethyl)piperidines as Potential Radiolabeled Probes for σ-1 Receptors: *In Vivo* Evaluation of [^{123}I]-1-(Iodopropen-2-yl)-4-[(4-cyanophenoxy)methyl]piperidine

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Several halogenated 4-(4-phenoxymethyl)piperidines were synthesized as potential σ receptor ligands. The affinity and selectivity of these compounds were determined using *in vitro* receptor binding assays, and their log *P* values were estimated using HPLC analysis. The effect of various N-substituents on the σ-1 and σ-2 dissociation constants was examined. These substituents included fluoroalkyl, hydroxyalkyl, iodopropenyl, and selected *ortho*-, *meta*-, and *para*-substituted benzyl groups. Also determined were the effects of various moieties on the phenoxy ring; specifically 4-iodo, 4-bromo, 4-nitro, 4-cyano, 3-bromo, and pentafluoro substituents were examined. The ranges in the dissociation constants of these compounds for σ-1 and σ-2 receptors were 0.38−24.3 and 3.9−361 nM, respectively. The ratio of K_i (σ-2/σ-1) ranged from 1.19 to 121. One of the most promising of the iodinated ligands, 1-(*trans*-iodopropen-2-yl)-4-[(4-cyanophenoxy)methyl]piperidine (**4**), was labeled with ^{123}I and studied *in vivo* in adult male rats. High uptake and good retention of radioactivity was observed in the brain and many other organs known to possess σ receptors. Blocking studies revealed high specific binding of [^{123}I]-**4** to σ receptors in the brain, lung, kidney, heart, muscle, and other organs known to possess these sites. These results indicate that [^{123}I]-**4** and other halogenated 4-(phenoxymethyl)piperidines of this series may provide useful probes for *in vivo* tomographic studies of σ receptors.

Introduction

Recently, the importance of the demonstrated interactions between the brain, immune, and endocrine systems have been given recognition, adding to a new field called "neuroimmunology" or "psychoneuroimmunology".[1,2] Sigma (σ) receptors represent a potentially important and interesting area of research as these sites are distributed heterogeneously throughout the central nervous system and are also found in the organs of the endocrine and immune systems.[3−5] On the basis of the differential binding and pharmacological properties of structurally diverse σ receptor ligands, at least two subtypes are known to exist, termed σ-1 and σ-2.[6,7] The molecular size of σ-1 and σ-2 receptors has been determined by photoaffinity labeling and SDS gel electrophoresis to be 21−29 and 18−21 kDa, respectively.[8,9] Despite their relatively small size, there is evidence to suggest that these receptors belong to the G-protein-coupled family of receptors, and current work is aimed at determining the nature of the interactions of σ receptors and G-proteins.[10,11] There is evidence that σ receptors modulate certain neuroreceptors, most notably dopaminergic and other catecholaminergic systems.[12,13]

While endogenous ligands for σ receptors are yet to be identified, it has been found that several hormonal steroids, most notably progesterone, bind with moderate affinity to σ-1 receptors, and Zn^{2+} has been suggested as an endogenous ligand for the σ-2 site.[14−16] The distribution of σ sites in the primate brain has been investigated using [^{3}H]-(+)-PPP.[17] High densities of σ sites were found over the paralimbic belt cortices, including the parahippocampal, insular, cingulate, orbitofrontal, and temporopolar gyri. These results suggested a role of σ receptors in limbic system functions. A separate study, using [^{3}H]DTG, also revealed high concentrations of σ receptor in many areas of the limbic system, suggesting that these sites may be involved in emotional, endocrine, and motor behavior.[18] Using the 2-deoxy-D-1-[^{14}C]glucose autoradiographic method,[19] della Puppa and London have demonstrated that σ receptor binding compounds influence the regulation of cerebral function and energy metabolism in the brain.[20]

Several compounds with high binding affinity for σ receptors are presented in Figure 1. Most known σ receptor ligands are either selective for the σ-1 site or are relatively nonselective, such as haloperidol and DTG. The benzomorphans (+)-SKF 10047 and (+)-pentazocine are among the compounds which show the highest σ-1/σ-2 selectivity; however, these compounds also bind to other neuroreceptors.[21] Highly selective σ-2 ligands are yet to be reported. It has been shown that various antipsychotic agents, including haloperidol, rimcazol, remoxipride, and BMY 14802 bind σ receptors with moderate to high affinity.[22−24] There is increasing evidence to implicate σ-2 sites in the adverse motor effects that arise from the chronic administration of neuroleptics,[25] whereas σ-1 receptors are down-regulated by the exposure of rats to haloperidol.[26] σ-1 receptor densities, however, are upregulated in the frontal cortex and substantia nigra of methamphetamine-treated rats, leading to the suggestion of σ receptor sensitization as a result of exposure to psychostimulants.[27] It has been demonstrated in postmortem examinations that there are alterations in σ receptor densities in the cerebral cortex of schizophrenic pa-

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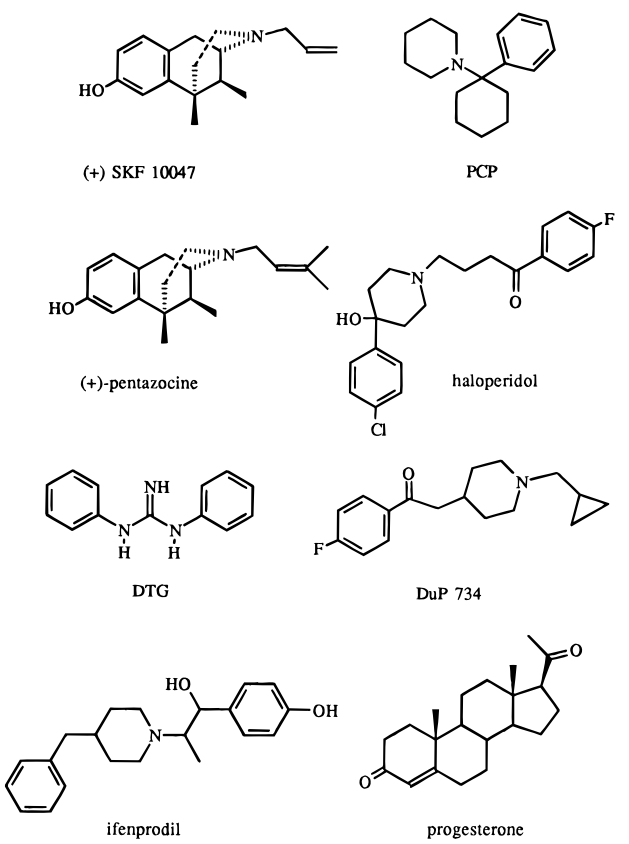


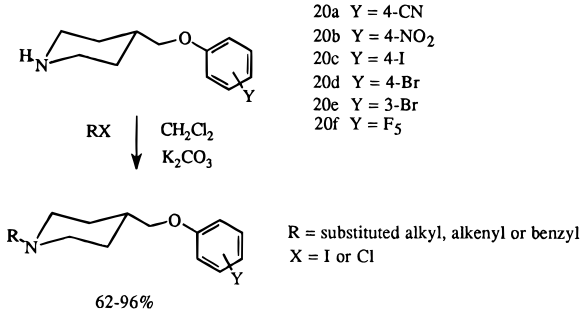
Figure 1. Structures of some σ receptor binding compounds.

tients.[28,29] Understanding the biological functions of σ receptors may, therefore, be important in understanding the etiology of schizophrenia and in developing better drug therapies for psychosis.[6,24] It is possible that the development of σ receptor radioligands would allow for the study of the intact human brain to assess the occupancy of neuroleptics and their metabolites at these sites via tomographic imaging. The lack of selective σ radioligands possessing the required *in vivo* properties has prevented the successful tomographic imaging of these sites in humans.[30]

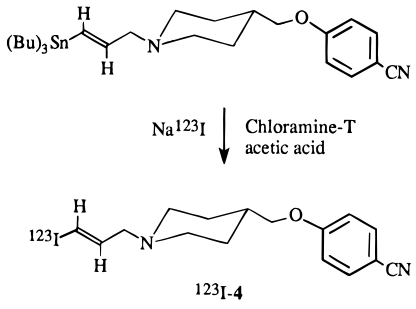
Several radiolabeled σ receptor binding compounds possessing antipsychotic or other therapeutic properties have been synthesized as potential probes for the imaging of these sites by positron emission tomography (PET) or single photon emission computed tomography (SPECT). Examples include [^{18}F]-α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol,[31,32] [^{125}I]-iodobenzovesamicol,[33] ^{11}C- and ^{18}F-labeled DTG derivatives,[34] [^{125}I]iodophenyl-3-(adamantyl)guanidine,[35] [^{18}F]haloperidol,[36] and [^{11}C]-*N*-benzyl-*N*-normetazocine.[37] However, in general, these tracers exhibit low brain uptake and significant *in vivo* metabolism or are not sufficiently selective for σ receptors, and therefore, the synthesis of more suitable σ receptor probes is the focus of current research.[30]

In vivo pharmacokinetic studies of the high-affinity σ ligand [^{3}H]-1-(cyclopropylmethyl)-4-(2-fluoro-2′-oxoethyl)piperidine ([^{3}H]DuP 734) in mouse brain has been recently reported.[38] This ligand was found to exhibit maximum brain uptake at 45 min and specific binding ranging from 56% to 72% of the total binding in several mouse brain regions. However, DuP 734 is nonselective and strongly binds serotonin 5HT$_2$ receptors (K_i = 15 nM) as well as σ-1 and σ-2 receptors *in vitro*. The

Scheme 1. Alkylation of Para-Substituted 4-(Phenoxymethyl)piperidines



Scheme 2. Synthesis of [^{123}I]-**4**



synthesis, *in vitro* characterization, and *in vivo* examination in mouse antipsychotic models of a series of more selective DuP 734 analogs has also been reported.[39] Examples include 1-(cyclopropylmethyl)-4-[(4-cyanophenoxy)methyl]piperidine ($K_i(\sigma)$ = 10 nM) and 1-(cyclopropylmethyl)-4-[(4-nitrophenoxy)methyl]piperidine ($K_i(\sigma)$ = 13 nM), both of which exhibit only moderate affinity (K_i > 200 nM) for serotonin 5HT$_2$ receptors. As an extension of the series to include compounds which can be radiolabeled with PET or SPECT isotopes, such as ^{123}I, ^{18}F, and ^{76}Br, using known radiochemical methods, we report here the synthesis and *in vitro* characterization of several halogenated 4-(phenoxymethyl)piperidines, their *in vitro* affinities for σ-1 and σ-2 receptors, and the preliminary *in vivo* studies to characterize one of the most promising iodinated ligands of the series.

Chemistry

The 4-(4-phenoxymethyl)piperidines **20a**−**f** were prepared in four steps from 4-(hydroxymethyl)piperidine as previously described.[39−42] Alkylation of the piperidine nitrogen of **20a**−**e** with the appropriately substituted alkyl or benzyl halides provided compounds **1**−**3** and **5**−**19** in yields of 71−96% (Scheme 1). We have previously reported the synthesis of compounds **2** and **9** as well as the *N*-iodopropenyl ligand **4**.[40−42] All of the compounds, in their free base form, were purified by column chromatography. The structure and chemical purity of each compound was determined by ^{1}H-NMR, CI-MS, and elemental analysis. Melting point analysis was also obtained for each solid.

The radioligand [^{123}I]-**4** was prepared as described previously by reacting the corresponding *trans*-vinylstannane precursor with Na^{123}I in the presence of *N*-chloramine-T at a pH = 6.5 (Scheme 2).[40] The radiotracer was purified by HPLC using a reverse-phase base-deactivated column and a mobile phase consisting

of methanol and water (85:15 v/v). The radiochemical yield, after purification, was 60−80% EOS ($n = 5$), and the radiochemical purity of the product was >99%. To prepare suitable solutions of [^{123}I]-**4** for use *in vivo*, the eluted radioactive peak corresponding to [^{123}I]-**4** was collected, the mobile phase was removed *in vacuo*, and the product was redissolved in saline. The resulting solution was passed through a sterile filter into an evacuated sterile vial and diluted with saline to provide approximately 10 μCi of the tracer per 100 μL of solution.

Results and Discussion

Research into the biological roles of σ receptors has been hindered by the lack of σ subtype specific ligands with which to probe these sites. The aim of this work was to develop high affinity σ receptor selective radioligands suitable for *in vivo* PET or SPECT imaging. We have synthesized a total of 36 halogenated 4-(phenoxymethyl)piperidine derivatives that can be radiolabeled with PET or SPECT isotopes using established procedures. A representative few of these are presented in this paper. Iodoaryl or iodovinyl radioligands can be rapidly prepared from the corresponding tributyltin precursors using oxidative radioiododestannylation reactions.[40, 41, 46, 47] The [^{18}F]fluoroalkyl derivatives can be readily synthesized from alkyl mesylate or alkyl tosylate precursors using nucleophilic ^{18}F.[42] Once synthesized, compounds **1**−**19** were screened in neuroreceptor binding assays to determine their affinity and selectivity for σ-1 and σ-2 receptor subtypes (Table 1). In general, all compounds were found to be selective for σ receptors *in vitro* and exhibited negligible affinity (>10000 nM) for other neuroreceptors, including PCP, NMDA, histamine, dopamine D_1, D_2, and serotonin $5HT_1$, $5HT_2$ receptors. Excluding compound **19**, which did not exhibit significant affinity for either subtype (K_i > 1000 nM), the ranges in the dissociation constants of these compounds for σ-1 and σ-2 receptors were 0.38−24.3 and 3.9−361 nM, respectively. The ratio of K_i (σ-2/σ-1) ranged from 1.19 to 121.

In general, the *N*-haloalkyl, *N*-hydroxyalkyl, and *N*-haloalkenyl derivatives exhibited the best σ-1 selectivity, whereas the *N*-4-fluorobenzyl ligands **3** and **7** had comparably high affinities for both subtypes. By a comparison of **3** and **7**, it was evident that 4-cyano substitution provided ligands with a slightly higher affinity and better selectivity for the σ-1 site as compared to the corresponding 4-nitro derivatives. Furthermore, a comparison of **1** and **8** demonstrated that replacement of the 4-cyano group with an iodine atom increased the σ-1 binding affinity by a factor of 30 and increased the σ-1/σ-2 selectivity by 8-fold. The *N*-cyclopropylmethyl derivatives **10**, **14**, and **15** had comparable affinities for both σ subtypes, and the range in K_i (σ-2/σ-1) for these compounds was 11.3−23.4. This indicated that, for this class of compounds, only small effects on the subtype selectivity would be realized by movement of iodine or bromine atoms at the 3- or 4-position of the phenoxy ring.

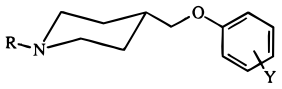
As indicated above, a comparison of ligands **2** and **3** showed that the incorporation of the *N*-(3-fluoropropyl) group provided compounds having better σ-1 receptor affinity than corresponding *N*-(4-fluorobenzyl) derivatives. This is due in large part to the 27.7-fold lower σ-2 affinity for **2** as compared with **3**. It is of interest that this trend was not observed for the pentafluorophenoxy derivatives as the **16** exhibited only a 1.2-fold lower affinity for σ-2 sites as compared with **17.** It has been recently reported by Moltzen *et al.* that the placement of fluorine atoms at certain positions of the aromatic ring of spiro-joined isobenzofuran piperidines can significantly influence the σ-1/σ-2 selectivity of that class of compounds.[43] The isobenzofuran piperidines are similar in structure, *albeit* more rigid than the compounds discussed in this paper. The affects on σ-1/σ-2 selectivity of fluorine substitution at various positions of the 4-(phenoxymethyl)piperidines warrants further investigation. It is interesting the *N*-pentafluorobenzyl derivative **19** was not active in either the σ-1 or σ-2 assay at a concentration of 10^{-6} M. Clearly there is scope for a further investigation into the effects of fluorine subtituents at different positions of N-alkylated 4-phenoxypiperidine derivatives and related compounds.

It was recently reported that placement of an iodine at the *ortho* position of the σ-1 selective ligand *N*-benzyl-(+)-normetazocine reduced the affinity of the ligand for that receptor by approximately 1000-fold as compared to the corresponding 4-fluorobenzyl derivative.[44] To examine the effect of an *o*-iodo- or -bromo-substituted benzyl group on the σ-1 and σ-2 affinity of this class of compounds, **5** and **6** were synthesized. The effect of these *ortho* substituents on the σ-1 dissociation constants was not nearly as pronounced as was observed for the normetazocine analogs. This may be due to the fact that these 4-(phenoxymethyl)piperidines are less rigid in structure as compared with N-alkylated normetazocines, and as such, they could be able to more easily take on the three-dimensional structure required for interaction with the σ-1 site in spite of the addition of the *o*-iodo and -bromo groups. A similar reduction of binding affinity can be obtained by the addition of an *o*-cyanobenzyl group as is revealed by the analysis of compound **12**. Finally, a comparison of **17** and **18** revealed that *N*-(3-fluorobenzyl) substituents provide more selective σ-1 ligands as compared with the corresponding *N*-(4-fluorobenzyl) derivatives. This was mainly due to a 5-fold reduction in the σ-2 binding affinity of **18** as compared with that of **17**.

The compounds described in this paper possess the basic structural features reported by Gilligan *et al.* for optimal σ binding and good *in vivo* potency.[39] The distances from the basic nitrogen to the points of attachment of the proximal hydrophobic group and the distal hydrophobic group (aromatic ring) were 2.48 ± 0.05 and 6.49 ± 0.10 Å, respectively. These values were determined using standard MM2 energy minimization calculations contained in the molecular modeling program CAChe.[53]

Lipophilicity is an important property of receptor-targeted imaging agents as compounds having high log *P* values (log P > 4.0) will generally exhibit a significant degree of nonspecific binding, reducing the target to nontarget ratios.[45] To address this issue, the log $P_{7.5}$ value of each potential ligand was estimated using HPLC methods as previously described.[46] The log $P_{7.5}$ values for this series of compounds ranged from 2.42 to 6.67 (Table 1). Taking into consideration the *in vitro* binding data and the lipophilicity estimations of these compounds, ligands **2** and **4** appear to be the most

Table 1. Receptor Binding of Halogenated 4-(4-Phenoxymethyl)piperidine Derivatives **1**−**19**



			K_i (nM)					
	R	Y	σ-1	σ-2	$5HT_2$	D_2	K_i (σ-2/σ-1)	log *P*
1	$(CH_2)_2F$	*p*-CN	24.3	361	>10000	>10000	14.9	2.42
2[b]	$(CH_2)_3F$	*p*-CN	4.3	144	>10000	>10000	33.5	2.93
3	*p*-FBn	*p*-CN	0.76	5.2	>10000	>10000	6.84	3.22
4[b]	CH_2CHCHI	*p*-CN	0.67	38.8	>10000	>10000	57.9	3.36
5	OBrBn	*p*-CN	2.02	31.1	>1000	ND	15.40	4.09
6	OIBn	*p*-CN	9.24	93.6	>1000	ND	10.13	4.30
7	*p*-FBn	*p*-NO2	1.2	3.9	>10000	>10000	3.25	3.62
8	$(CH_2)_2F$	4-I	0.84	102	>10000	>10000	121.1	4.02
9[b]	$(CH_2)_2OH$	4-I	2.3	139	>10000	>10000	60.4	3.80
10[c]	CH_2-c-C_3H_5	4-I	0.5	11.7	>10000	>10000	23.4	6.67
11	*m*-CNBn	4-I	2.9	69.5	>1000	ND	24.0	4.19
12	*o*-CNBn	4-I	10.5	142	>1000	ND	13.5	4.32
13	$(CH_2)_3F$	4-Br	0.38	13.1	>10000	>10000	34.5	5.88
14[c]	CH_2-c-C_3H_5	4-Br	0.6	13.6	>10000	>10000	22.7	6.22
15[c]	CH_2-c-C_3H_5	3-Br	0.88	9.9	>10000	>10000	11.3	6.36
16	$(CH_2)_3F$	F_5	1.7	5.3	>10000	ND	2.05	4.11
17	4-F-Bn	F_5	3.6	4.3	>10000	ND	1.19	ND
18	3-F-Bn	F_5	3.3	25	>10000	ND	7.48	4.72
19	F5-Bn	F_5	>1000	>1000	>10000	ND	ND	ND

[a] Assays were carried out through the NIMH/NovaScreen Drug Discovery and Development Program (Contract No. NIMH 2003) using the conditions provided in the refs 4, 50, and 51. [b] The synthesis and characterization of these compounds have been reported elsewhere in references 40−42. [c] Described in DuPont patent PCT WO91/03243; however, the synthesis and characterization has not previously been reported as far as the authors are aware. ND = not determined.

Table 2. Biodistribution of [^{123}I]-**4** in Rats[a]

organ	0.33 h	1 h	2 h	4 h	24 h
liver	0.33 ± 0.04	0.45 ± 0.03	0.43 ± 0.09	0.50 ± 0.12	0.55 ± 0.20
spleen	0.91 ± 0.13	0.92 ± 0.09	0.89 ± 0.19	0.93 ± 0.14	0.87 ± 0.12
lung	6.59 ± 1.00	6.35 ± 1.05	3.75 ± 0.84	3.14 ± 0.55	0.79 ± 0.08
heart	1.25 ± 0.12	1.21 ± 0.07	0.81 ± 0.29	0.67 ± 0.18	0.18 ± 0.02
kidneys	1.64 ± 0.08	1.64 ± 0.07	1.30 ± 0.29	1.31 ± 0.27	0.59 ± 0.06
stomach	0.46 ± 0.09	0.65 ± 0.10	0.30 ± 0.12	0.27 ± 0.06	0.18 ± 0.04
intestine	0.54 ± 0.11	0.82 ± 0.21	0.84 ± 0.21	0.81 ± 0.19	0.74 ± 0.25
muscle	0.23 ± 0.08	0.29 ± 0.05	0.22 ± 0.13	0.20 ± 0.07	0.09 ± 0.01
blood	0.05 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.001
brain	1.06 ± 0.08	1.09 ± 0.08	0.91 ± 0.23	1.07 ± 0.20	0.84 ± 0.10
brain[b]	1.97 ± 0.09	1.96 ± 0.17	2.01 ± 0.30	2.03 ± 0.31	1.61 ± 0.12
thyroid[b]	0.09 ± 0.02	0.30 ± 0.06	0.27 ± 0.10	0.34 ± 0.09	1.19 ± 0.28

[a] Data are means of %ID/g of tissue ± SD; $n = 3$. [b] %ID/organ.

suitable candidates for *in vivo* imaging of σ-1 receptors. With this in mind, compound **4** was deemed the most promising iodinated compound of this series and the analogous SPECT tracer, [^{123}I]-**4**, was synthesized for *in vivo* use. Preliminary reports of this work have been published.[47,48]

***In Vivo* Distribution, Pharmacological Blocking, and Saturation Studies.** The radiotracer [^{123}I]-**4** was administered *via* tail vein injection at a concentration of 10 μCi/100 μL of sterile saline. Table 2 summarizes the uptake of radioactivity in selected organs at several time points postinjection (PI). Of importance is the high uptake and prolonged retention of radioactivity in the brain, which was 1.97 ± 0.09%ID at 20 min and 1.61 ± 0.12%ID at 24 h PI. Also noted was the retention of radioactivity in other organs known to possess σ binding sites, such as the lung, kidney, heart, muscle, and spleen. The amount of radioactivity in the thyroid was initially low and increased only moderately from 20 min (0.09 ± 0.02%ID) to 24 h (1.19 ± 0.28%ID) postinjection. This is an indication that [^{123}I]-**4** is relatively stable to *in vivo* deiodination. The initial liver uptake of radioactivity was relatively low (0.33 ± 0.04%ID/g at 15 min) and slowly increased over time (0.55 ± 0.20%ID/g at 24 h), suggesting an accumulation of labeled metabolites.

Table 3. Distribution of [^{123}I]-**4** in Selected Rat Brain Regions[a]

brain region	20 min	60 min
medulla pons	0.95 ± 0.07	1.00 ± 0.09
cerebellum	1.01 ± 0.08	1.09 ± 0.10
midbrain	1.06 ± 0.08	1.17 ± 0.08
diencephalon	1.03 ± 0.10	1.18 ± 0.24
hippocampus	1.02 ± 0.13	1.06 ± 0.11
striatum	1.17 ± 0.14	1.13 ± 0.13
frontal cortex	1.28 ± 0.14	1.29 ± 0.09
posterior cortex	1.43 ± 0.20	1.51 ± 0.12

[a] Data are means of %ID/g of tissue ± SD; $n = 3$.

The relative distributions of σ-1 and σ-2 binding sites in the rat brain has been reported;[4] however, it should be noted that strain variations may occur.[49] In order to determine that the radioligand was distributed throughout the rat brain as expected, regional brain dissections were performed (Table 3). These results demonstrate the uptake of radioactivity in all eight gross brain regions assayed in a similar pattern to that expected for a σ receptor probe. The highest average density of radioactivity was found in the posterior and frontal cortices, and slightly lower uptake was observed

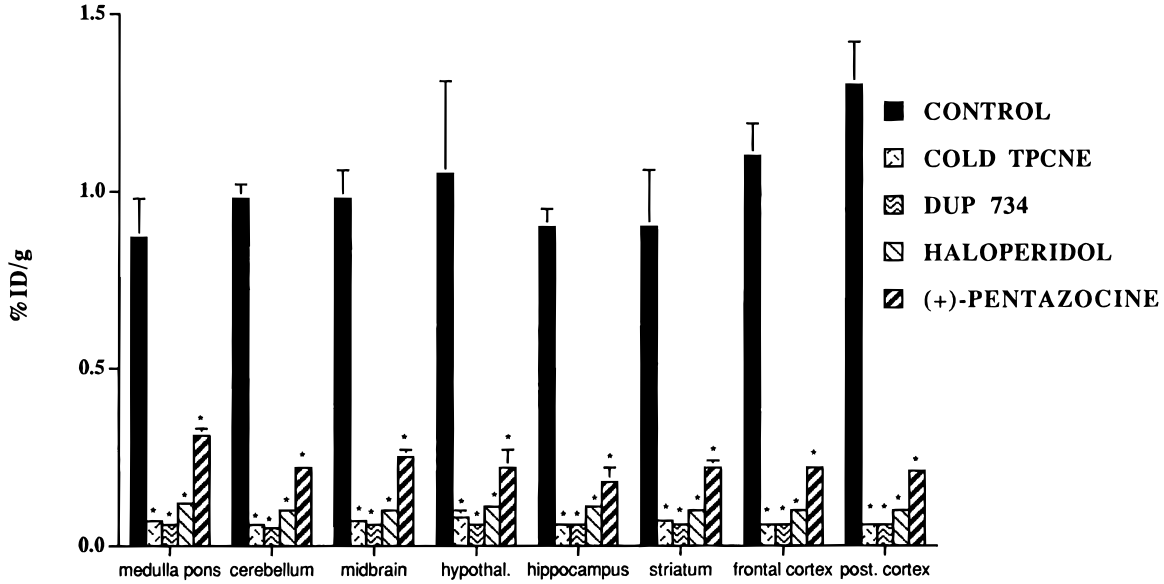


Figure 2. Effects of selected σ ligands on the uptake of [^{123}I]-**4** in rat brain regions. Data are means of %ID/g of tissue ± SD; $n = 3$; *$p < 0.01$.

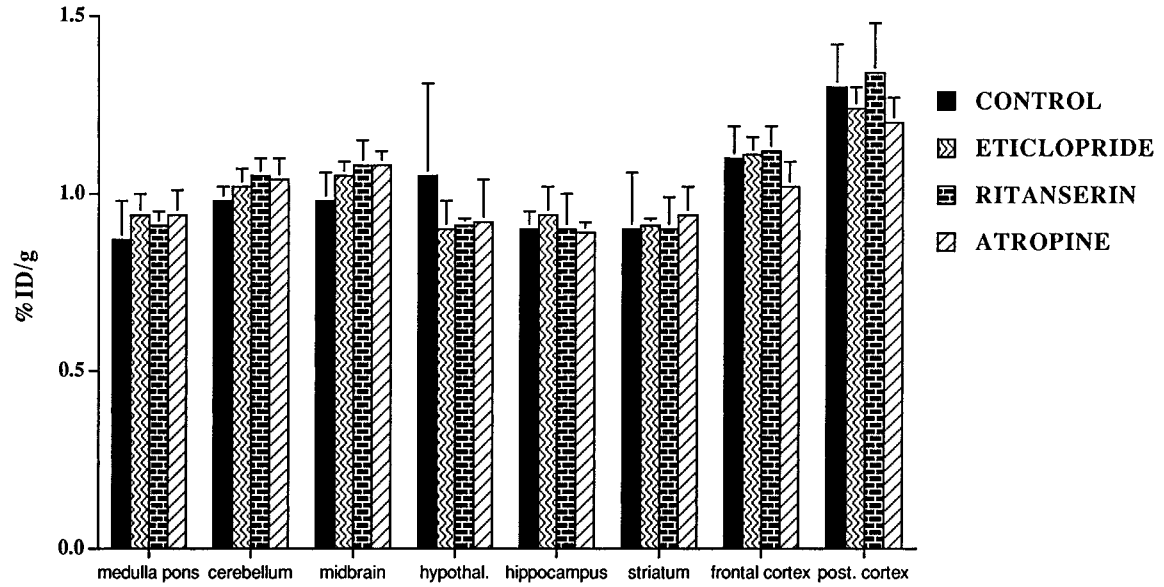


Figure 3. Effects of drugs on the uptake of [^{123}I]-**4** in rat brain regions. Data are means of %ID/g of tissue ± SD; $n = 3$; $p > 0.05$ for all determinations.

in all other regions examined, including the striatum, cerebellum, medulla pons, midbrain, hippocampus, and hypothalamus.

To assess the specific binding of [^{123}I]-**4** to σ receptors *in vivo*, the effect of preadministration of σ receptor binding drugs on the distribution of the radioligand in various organs was examined. For these studies, the specific binding of the radioligand was determined in eight different brain regions in lieu of studying the intact whole brain. By the separate examination of each brain region, a more accurate estimation of the binding of a tracer to neuroreceptors localized in that region can be obtained. The compounds used for pharmacological challenge were unlabeled **4**, DuP 734 (σ-1, σ-2, serotonin $5HT_2$), haloperidol (dopamine D_2, σ-1, σ-2), (+)-pentazocine (σ-1 ≫ σ-2), (−)-eticlopride (dopamine D_2), ritanserin (serotonin $5HT_2$, $5HT_{1C}$), and atropine (muscarinic M_1, M_2, M_3). The drugs were administered intravenously at a dose of 1 μmol/kg 5 minutes prior to the injection of [^{123}I]-**4**.

A significant reduction in the uptake of radioactivity ($p < 0.01$) occurred in all brain regions and in organs known to contain σ receptors including the lung, heart, muscle, and kidney (Figures 2 and 4). For example, in the posterior cortex, the uptake of the radiotracer was blocked 91% by haloperidol and 84% by (+)-pentazocine. In contrast, the preadministration of eticlopride, ritanserin, or atropine did not alter the brain uptake of [^{123}I]-**4** (Figure 3). Also, the uptake of the tracer was prevented by a greater extent by DuP 734 as compared to haloperidol in all brain regions. This is in accordance with the observations reported in the characterization of [^{3}H]DuP 734 in mice[38] and [^{123}I]-1-(4-cyanobenzyl)-4-[[(*trans*-iodopropen-2-yl)oxy]methyl]piperidine in AAW rats.[46] This result indicates that [^{123}I]-**4** binds the haloperidol insensitive σ binding site in the rat brain, *albeit* only to a small extent. It should be noted that, although **4** exhibits good selectivity for the σ-1 subtype *in vitro*, it is difficult to determine the *in vivo* selectivity

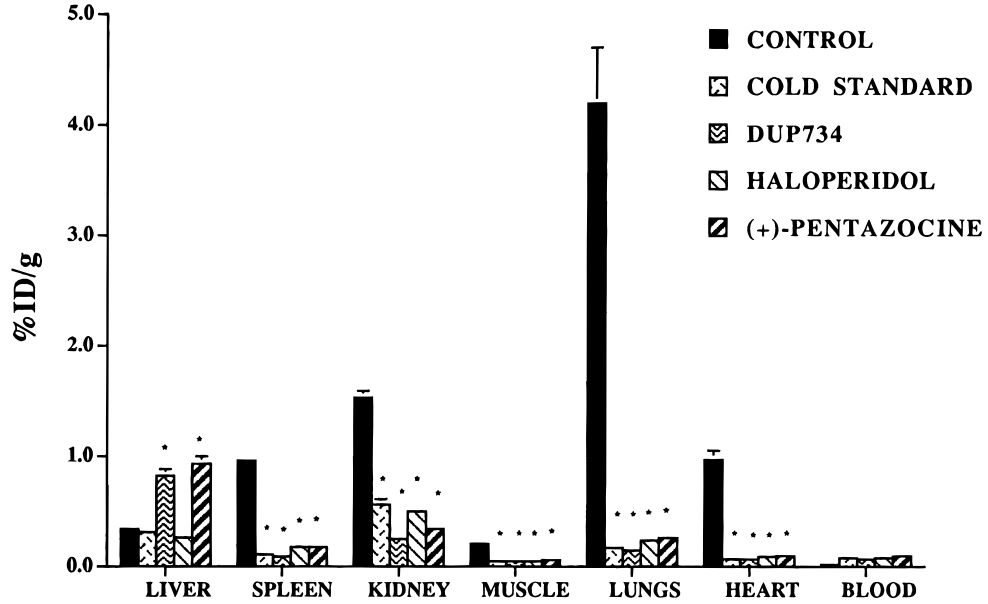


Figure 4. Effects of selected σ ligands on the uptake of [^{123}I]-**4** in various rats organs. Data are means of %ID/g of tissue ± SD; $n = 3$; *$p < 0.01$.

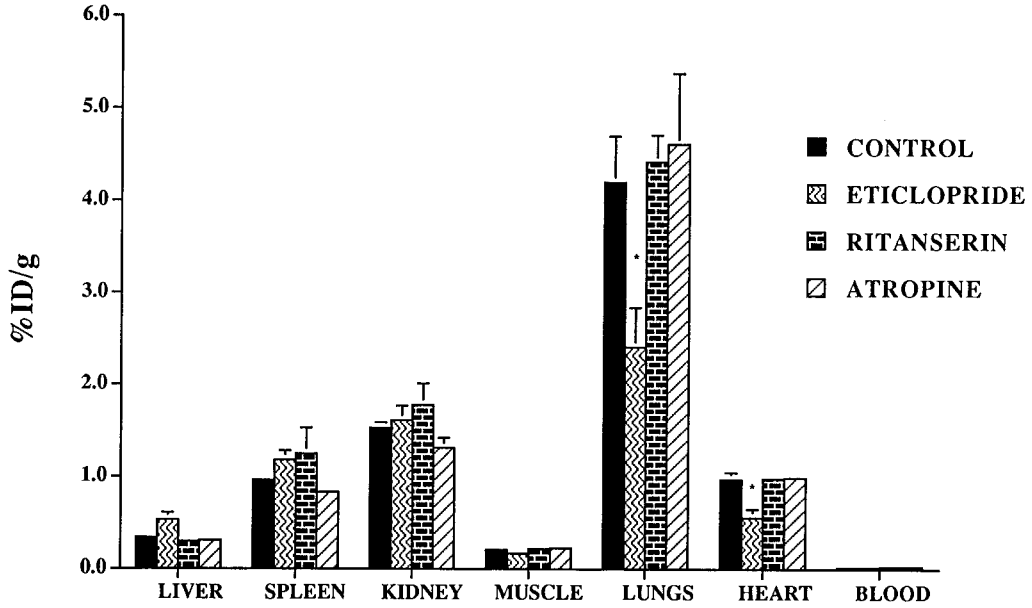


Figure 5. Effects of selected drugs on the uptake of [^{123}I]-**4** in rat organs. Data are means of %ID/g of tissue ± SD; $n = 3$; *$p < 0.01$.

of radiotracers for this site with the current lack of σ-2 receptor specific compounds.[37]

The specific binding of [^{123}I]-**4** to σ receptors in the lung was particularly high and was reduced from 4.2 ± 0.50%ID/g to 0.24 ± 0.02 and 0.26 ± 0.02%ID/g by the preadministration of haloperidol and (+)-pentazocine, respectively (Figure 4). Similar blocking effects were seen in other organs known to possess σ binding sites, such as the kidney, heart, muscle, and spleen. In general, the uptake of [^{123}I]-**4** in these organs was not reduced by pretreatment with atropine, ritanserin, or eticlopride (Figure 5). However, some effects were observed on the uptake of radioactivity in the heart and lung as a result of the preadministration of (−)-eticlopride. It is not evident why this effect was seen in the lung and heart but not in the brain or other organs. No specific binding was detected in the liver; however, as mentioned previously, the presence of σ receptors in the liver may be species and strain dependent.[49] The presence or absence of σ receptors in the liver of the AAW rat has not been demonstrated to date, and as metabolite accumulation is likely to occur in the liver, the degree of *in vivo* specific binding in that organ may be difficult to observe using blocking studies.

A study was performed to show that the binding of [^{123}I]-**4** to brain σ receptor was saturable at 20 min postinjection. The method used has been previously described for the characterization of [^{3}H]DuP 734.[38] The specific binding in the control group was determined by the preadministration of haloperidol at 1 mg/kg. In separate groups of animals, DuP 734 was injected in several concentrations between 8×10^{-4} and 1.0 mg/kg 5 min prior to injection of the radioligand. Saturable σ receptor binding of [^{123}I]-**4** versus DuP 734 was demonstrated with an ID_{50} of approximately 0.008−0.010 mg/kg in all brain regions examined (Figure 6).

Conclusions. Several halogenated 4-(phenoxymethyl)piperidines have been synthesized and character-

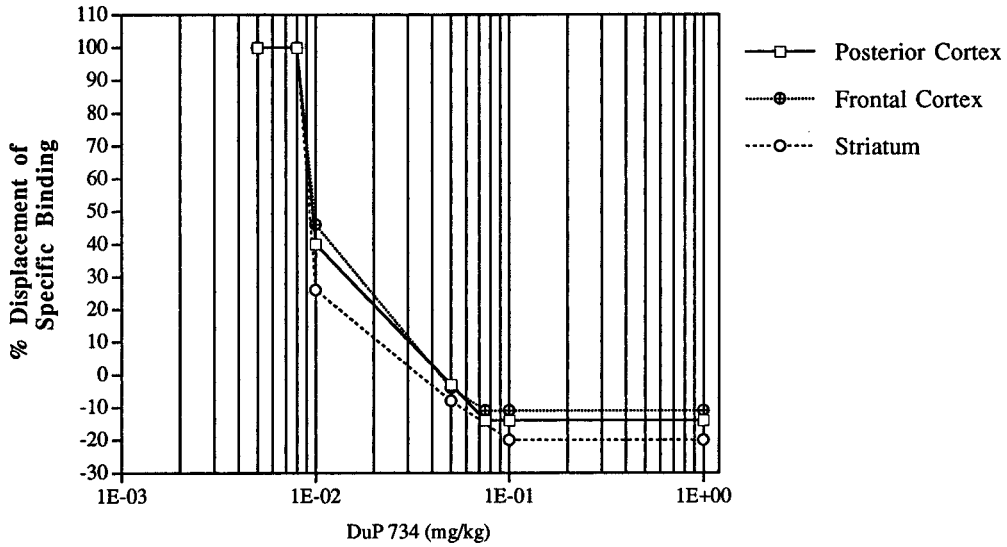


Figure 6. Effect of DuP 734 on the uptake of [^{123}I]-**4** in rat cortex and striatum. Data are means of %ID/g of tissue ± SD; $n = 3$. The SD is within ±20% of the mean for all determinations.

ized *in vitro* in receptor binding assays, and their log $P_{7.5}$ values were estimated using HPLC analysis. All of these compounds were highly selective for σ receptors and possessed lower dissociation constants for σ-1 as compared to σ-2 sites. The lipophilicity of these compounds was moderate to high; however, the log *P* values for a few of the ligands were within acceptable limits (log $P < 4.0$), suggesting their appropriateness for use in receptor imaging experiments.[45] On the basis of these results, one of the most promising of the iodinated ligands, **4**, was labeled with ^{123}I and studied *in vivo* in adult male AAW rats. High uptake, specific binding, and good retention of radioactivity was observed in brain and many other organs known to possess σ receptors, including the lung, kidney, heart, muscle, and other organs. Also, the binding of [^{123}I]-**4** to brain σ receptors was demonstrated to be saturable, with an ID_{50} of 0.008−0.01 mg/kg.

It is also worthwhile to note that [^{123}I]-**4** has demonstrated high cortical and cerebellar uptake and apparent low *in vivo* metabolism in rhesus macaques and that a preliminary report of this work has been presented.[48] These results indicate that [^{123}I]-**4** is a suitable candidate for the *in vivo* SPECT assessment of σ receptor densities. Furthermore, other compounds of this series, in their radiolabeled form, may also provide useful probes for σ receptors. The excellent retention of radioactivity in the brain observed using [^{123}I]-**4** leads to the hypothesis that compounds of this series possessing relatively high log *P* values might also be useful for *in vivo* studies if the nonspecific binding is reduced adequately over time to allow acceptable target to nontarget ratios to be realized. Therefore, it is suggested that compound **8**, which has a log *P* of 4.0 but better selectivity than **4** for σ-1 sites and can be labeled with either ^{123}I for SPECT or ^{18}F for PET, is also a reasonable candidate for further characterization.

Experimental Section

Chemical Materials and Methods. Proton NMR spectra were recorded on a JEOL 400 MHz FT-NMR spectrometer. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in deuteriochloroform, and coupling constants (*J*) are reported in hertz. Low-resolution mass spectral analysis was performed using a VG Quattro 81 triple quadrupole mass spectrometer [VG Biotech (now Micromass), Altrincham, UK]. High-resolution fast atom bombardment mass spectroscopy (HRMS) was performed using a ZAB-EQ mass spectrometer at the Department of Chemistry, The University of Tennessee (Knoxville, TN). Melting points were recorded using a Gallenkamp melting point apparatus and are uncorrected. Elemental analysis was performed by Atlantic Microlabs Inc. (Norcross, GA). Gravity chromatography was performed using silica gel (Fluka, 70−230 mesh, ASTM) using the solvent systems as indicated in the text. For mixed solvent systems, the ratios are given with respect to volumes.

All reagents were purchased from commercial sources and were used without further purification. Sodium [^{123}I]iodide was obtained from the National Medical Cyclotron (Sydney, Australia) as a solution in sodium hydroxide (0.1 M). HPLC analysis of the radioligand was performed using a Spectraphysics P1000 HPLC pump, a Spectraphysics UV 1000 detector, and a EG&G NaI scintillation detector connected to a Model NS276 photomultiplier preamplifier and an EG&G 925-SCINT-S Ace Mate amplifier and bias supply. The columns used were a reverse-phase base-deactivated column [Activon, Goldpak Exsil, ODS B, 10 μm, 4.6 × 250 mm (analytical) or 10 × 250 mm (semiprep)], and the mobile phases used are indicated in the text below. The secondary amine precursors **20a**−**f** were prepared using methods previously described,[39−42] and their structures were confirmed by ^{1}H-NMR, mass spectral analysis, and melting point determination. We have previously reported the synthesis and characterization of compounds **2**, **4**, and **9**.[40−42]

1-(2-Fluoroethyl)-4-[(4-cyanophenoxy)methyl]piperidine (1). General Alkylation Procedure. To ethanol-free dichloromethane (6 mL) was added 4-[(4-cyanophenoxy)methyl]piperidine (280 mg, 1.3 mmol), potassium carbonate (716 mg, 5.18 mmol), and 3-bromo-1-fluoroethane (104 μL, 1.09 mmol). The resulting solution was stirred at room temperature for 24 h. The reaction mixture was then diluted with water (100 mL) and the product extracted into dichloromethane (2 × 30 mL). The organic extracts were combined and dried over magnesium sulfate, and the solvent was removed *in vacuo* to provide the crude product as a yellow oil. Purification was accomplished by column chromatography (silica gel; ethyl acetate/ethanol (9:2 v/v)) to provide a white solid (311 mg, 91%): mp = 46−47 °C; ^{1}H-NMR δ 1.35−1.49 (m, 2H, piperidine CH_2), 1.77−1.99 (m, 5H, piperidine CH_2, CH, NCH_2), 2.75 (dt, 2H, $J_{HF} = 28.40$, $J_{HH} = 4.85$, CH_2CH_2F), 3.02 (d, 2H, $J = 11.40$, piperidine NCH_2), 3.85 (d, 2H, $J = 6.00$, CH_2O), 4.60 (dt, 2H, $J_{HF} = 47.50$, $J_{HH} = 4.85$, CH_2F), 6.93 (d, 2H, $J = 9.0$, $ArH^{2,6}$), 7.58 (d, 2H, $J = 9.0$, $ArH^{3,5}$); MS *m/z* 263.0. Anal. Calcd for $C_{15}H_{19}N_2OF$: C, H, N.

1-(4-Fluorobenzyl)-4-(4-cyanophenoxymethyl)piperidine (3). Compound **3** was prepared using the general method provided above for compound **1** by alkylation of 4-[(4-cyanophenoxy)methyl]piperidine (230 mg, 1.1 mmol) with 4-fluorobenzyl bromide (164 μL, 1.3 mmol) in the presence of potassium carbonate (588 mg, 4.3 mmol). After purification, the product was obtained as a white solid (331 mg, 96%): mp = 122−123 °C; ^{1}H-NMR δ 1.38−1.47 (m, 2H, piperidine CH_2), 1.76−1.87 (m, 3H, piperidine CH_2, CH), 1.96−2.05 (m, 2H, piperidine CH_2), 2.82 (d, 2H, J = 11.50, piperidine NCH_2), 3.49 (s, 2H, NCH_2Ar), 3.86 (d, 2H, J = 6.0, CH_2O), 6.93 (d, 2H, J = 9.0 $ArH^{2,6}$), 6.96−7.03 (m, 2H, ArH), 7.22−7.30 (m, 2H, ArH), 7.58 (d, 2H, J = 9.0, $ArH^{3,5}$); MS m/z 325. Anal. Calcd for $C_{20}H_{21}N_2OF$: C, H, N.

1-(2-Bromobenzyl)-4-[(4-cyanophenoxy)methyl]piperidine (5). Compound **5** was prepared using the general method provided above for compound **1** by alkylation of 4-[(4-cyanophenoxy)methyl]piperidine (100 mg, 0.46 mmol) with 2-bromobenzyl bromide (127 mg, 0.51 mmol) in the presence of potassium carbonate (190 mg, 1.40 mmol). After purification, the desired product was obtained as a white solid (149 mg, 83%): mp = 103−104 °C; ^{1}H-NMR δ 1.38−1.52 (m, 2H, piperidine CH_2), 1.78−1.93 (m, 3H, piperidine CH_2, CH), 2.11−2.20 (t, 2H, J = 5.80, piperidine NCH_2), 2.95 (d, 2H, J = 9.50, piperidine NCH_2), 3.62 (s, 2H, NCH_2Ar), 3.78 (d, 2H, J = 4.80, CH_2O), 6.93 (d, 2H, J = 8.90, $ArH^{2,6}$), 7.11 (dt, 1H, J = 9.0, 1.5, ArH), 7.25−7.55 (m, 3H, ArH), 7.57 (d, 2H, J = 8.90, $ArH^{3,5}$); MS m/z 384.8. Anal. Calcd for $C_{20}H_{21}N_2OBr$: C, H, N, I.

1-(2-Iodobenzyl)-4-[(4-cyanophenoxy)methyl]piperidine (6). Compound **3** was prepared using the general method provided above for **1** by alkylation of 4-[(4-cyanophenoxy)-methyl]piperidine (100 mg, 0.46 mmol) with 2-iodobenzyl chloride (128 mg, 0.51 mmol) in the presence of potassium carbonate (190 mg, 1.40 mmol) and potassium iodide (23 mg, 0.14 mmol). After purification, the product was obtained as a white solid (189 mg, 94%): mp = 90−91 °C; ^{1}H-NMR δ 1.38−1.52 (m, 2H, piperidine CH_2), 1.78−1.93 (m, 3H, piperidine CH_2, CH), 2.11−2.20 (t, 2H, J = 5.80, piperidine NCH_2), 2.95 (d, 2H, J = 6.50, piperidine NCH_2), 3.52 (s, 2H, NCH_2Ar), 3.78 (d, 2H, J = 4.80, CH_2O) 6.90−6.97 (m, 3H, ArH), 7.32 (dt, 1H, J = 7.63, 1.1, ArH), 7.42 (dd, 1H, J = 6.11, 1.10, ArH), 7.57 (d, 2H, J = 8.90, $ArH^{3,5}$), 7.83 (dd, 1H, J = 7.63, 1.10, ArH); MS m/z 433.1. Anal. Calcd for $C_{20}H_{21}N_2OI$: C, H, N, I.

1-(4-Fluorobenzyl)-4-[(4-nitrophenoxy)methyl]piperidine (7). Compound **7** was prepared using the general method provided above for **1** by alkylation of 4-[(4-nitrophenoxy)-methyl]piperidine (250 mg, 1.1 mmol) with 4-fluorobenzyl bromide (150 μL, 1.2 mmol) in the presence of potassium carbonate (585 mg, 4.2 mmol). After purification, the product was obtained as a white solid (302 mg, 82%): mp = 118−119 °C; ^{1}H-NMR δ 1.45−1.59 (m, 2H, piperidine CH_2), 1.80−1.95 (m, 3H, piperidine CH_2, CH), 2.08−2.18 (m, 2H, piperdine NCH_2), 3.05 (d, 2H, J = 11.60, piperidine NCH_2), 3.59 (s, 2H, NCH_2Ar), 3.92 (d, 2H, J = 5.76, CH_2O), 6.94 (d, 2H, J = 9.50, ArH), 6.98−7.03 (m, 2H, ArH), 7.28−7.36 (m, 2H, ArH), 8.18 (d, 2H, J = 9.50, $ArH^{3,5}$); MS m/z 345.6. Anal. Calcd for $C_{19}H_{21}N_2O_3F$: C, H, N.

1-(2-Fluoroethyl)-4-[(4-iodophenoxy)methyl]piperidine (8). Compound **8** was prepared using the general method provided above for **1** by alkylation of 4-[(4-iodophenoxy)methyl]-piperidine (250 mg, 0.79 mmol) with 2-bromo-1-fluoroethane (60 μL, 0.83 mmol) in the presence of potassium carbonate (436 mg, 3.2 mmol). After purification, the desired product was obtained as a white solid (205 mg, 71%): mp = 78−79 °C; ^{1}H-NMR δ 1.51−1.73 (m, 2H, piperidine CH_2), 1.80−1.92 (m, 3H, piperidine CH_2, CH), 2.18−2.30 (m, 2H, piperidine NCH_2), 2.82 (dt, 2H, J_{HF} = 28.20, J_{HH} = 4.70, CH_2CH_2F), 3.14, (d, 2H, J = 11.50, piperidine NCH_2), 3.77 (d, 2H, J = 5.80, CH_2O), 4.68 (dt, 2H, J_{HF} = 48.0, J_{HH} = 4.70, CH_2F), 6.65 (d, 2H, J = 8.85, $ArH^{2,6}$), 7.54 (d, 2H, J = 8.85, $ArH^{3,5}$); MS m/z 362.5. Anal. Calcd for $C_{14}H_{19}NOFI$: C, H, N.

1-(Cyclopropylmethyl)-4-[(4-iodophenoxy)methyl]-piperidine (10). Compound **10** was prepared using the general method provided above for **1** by alkylation of 4-[(4-iodophenoxy)methyl]piperidine (350 mg, 1.10 mmol) with (bromomethyl)cyclopropane (120 μL, 1.21 mmol) in the presence of potassium carbonate (616 mg, 4.40 mmol). After purification, the desired product was obtained as a white solid (320 mg, 78%): mp = 139−140 °C; ^{1}H-NMR δ 0.90−1.20 (m, 2H, cyclopropyl CH_2), 0.50−0.55 (m, 2H, cyclopropyl CH_2), 1.85−1.93 (m, 1H, cyclopropyl CH), 1.38−1.48 (m, 2H, piperidine CH_2), 1.75−1.87 (m, 3H, piperidine CH_2, CH), 1.92−2.02 (m, 2H, piperidine NCH_2), 2.25 (d, 2H, J = 6.60, $NCH_2C_3H_5$), 3.10 (d, 2H, J = 9.60, piperidine NCH_2), 3.76 (d, 2H, J = 6.25, CH_2O), 6.95 (d, 2H, J = 8.90, $ArH^{2,6}$), 7.57 (d, 2H, J = 8.90, $ArH^{3,5}$); MS m/z 370.5. Anal. Calcd for $C_{16}H_{22}NOI$: C, H, N, I.

1-(2-Cyanobenzyl)-4-[(4-iodophenoxy)methyl]piperidine (11). Compound **11** was prepared using the general method provided above for **1** by alkylation of 4-[(4-iodophenoxy)methyl]piperidine (200 mg, 0.63 mmol) with 2-cyanobenzyl bromide (136 mg, 0.69 mmol) in the presence of potassium carbonate (261 mg, 1.89 mmol). After purification, the desired product was obtained as a white solid (222 mg, 81%); mp = 102−103 °C; ^{1}H-NMR δ 1.33−1.45 (m, 2H, piperidine CH_2), 1.75−1.85 (m, 3H, piperidine CH_2, CH), 2.10−2.20 (m, 2H, piperidine NCH_2), 2.93 (d, 2H, J = 11.0, piperidine NCH_2), 3.70 (s, 2H, NCH_2Ar), 3.80 (d, 2H, J = 5.80, CH_2O), 6.65 (d, 2H, J = 10.2, ArH), 7.40 (t, 1H, J = 7.76, ArH), 7.50−7.58 (m, 4H, ArH), 7.67 (s, 2H, ArH); MS m/z 432.7. Anal. Calcd for $C_{20}H_{21}N_2OI$: C, H, N, I.

1-(3-Cyanobenzyl)-4-[(4-iodophenoxy)methyl]piperidine (12). Compound **5** was prepared using the general method provided above for **1** by alkylation of 4-[(4-iodophenoxy)methyl]piperidine (205 mg, 0.65 mmol) with 3-cyanobenzyl bromide (159 mg, 0.81 mmol) in the presence of potassium carbonate (270 mg, 1.90 mmol). After purification, the desired product was obtained as a white solid (236 mg, 84%); mp = 133−134 °C; ^{1}H-NMR δ 1.33−1.45 (m, 2H, piperidine CH_2), 1.75−1.85 (m, 3H, piperidine CH_2, CH), 2.10−2.20 (m, 2H, piperidine NCH_2), 2.93 (d, 2H, J = 11.0, piperidine NCH_2), 3.70 (s, 2H, NCH_2Ar), 3.80 (d, 2H, J = 5.80, CH_2O), 6.65 (d, 2H, J = 10.2, $ArH^{2,6}$), 7.30−7.68 (m, 6H, ArH); MS m/z 333. Anal. Calcd for $C_{20}H_{21}N_2OI$: C, H, N, I.

1-(3-Fluoropropyl)-4-[(4-bromophenoxy)methyl]-piperidine (13). Compound **14** was prepared using the general method provided above for **1** by alkylation of 4-[(4-bromophenoxy)methyl]piperidine (200 mg, 0.74 mmol) with 3-bromo-1-fluoropropane (110 μL, 0.78 mmol) in the presence of potassium carbonate (360 mg, 2.2 mmol). The desired product was obtained as a white solid (190 mg, 78%): mp = 59−60 °C; ^{1}H-NMR δ 1.30−1.45 (m, 2H, piperidine CH_2), 1.70−2.15 (m, 7H, piperidine CH_2, CH, NCH_2, CH_2CH_2F), 2.47 (t, 2H, J = 7.33, $NCH_2(CH_2)_2F$), 2.95 (d, 2H, J = 11.59, piperidine NCH_2), 3.90 (d, 2H, J = 6.0, CH_2O), 4.50 (dt, 2H, J_{HF} = 47.30, J_{HH} = 5.95, CH_2F), 6.75 (d, 2H, J = 9.00, $ArH^{2,6}$), 7.34 (d, 2H, J = 9.00, $ArH^{3,5}$); MS m/z 330.2. Anal. Calcd for $C_{15}H_{21}$-NOBrF: C, H, N.

1-(Cyclopropylmethyl)-4-[(4-bromophenoxy)methyl]-piperidine (14). Compound **14** was prepared using the general method provided above for **1** by alkylation of 4-[(4-bromophenoxy)methyl]piperidine (500 mg, 1.85 mmol) with (bromomethyl)cyclopropane (205 μL, 2.0 mmol) in the presence of potassium carbonate (1.0 g, 7.4 mmol). After purification, the desired product was obtained as a white solid (495 mg, 82%); mp = 53−54 °C; ^{1}H-NMR δ 0.90−1.20 (m, 2H, cyclopropyl CH_2), 0.50−0.55 (m, 2H, cyclopropyl CH_2), 1.85−1.93 (m, 1H, cyclopropyl CH), 1.38−1.48 (m, 2H, piperidine CH_2), 1.75−1.87 (m, 3H, piperidine CH_2, CH), 1.92−2.02 (m, 2H, piperidine NCH_2), 2.25 (d, 2H, J = 6.60, $NCH_2C_3H_5$), 3.10 (d, 2H, J = 9.60, piperidine NCH_2), 3.76 (d, 2H, J = 6.25, CH_2O), 6.76 (d, 2H, J = 9.00, $ArH^{2,6}$), 7.35 (d, 2H, J = 9.00, $ArH^{3,5}$); MS m/z 324.3. Anal. Calcd for $C_{16}H_{22}NOBr$: C, H, N, Br.

1-(Cyclopropylmethyl)-4-[(3-bromophenoxy)methyl]-piperidine (15). Compound **15** was prepared using the general method provided above for **1** by alkylation of 4-[(3-bromophenoxy)methyl]piperidine (200 mg, 0.74 mmol) with (bromomethyl)cyclopropane (80 μL, 0.80 mmol) in the presence of potassium carbonate (307 mg, 2.2 mmol). After purification, the desired product was obtained as a white solid (200 mg, 83%); mp = 41−42 °C; ^{1}H-NMR δ 0.90−1.20 (m, 2H, cyclo-

propyl CH_2), 0.50−0.55 (m, 2H, cyclopropyl CH_2), 1.85−1.93 (m, 1H, cyclopropyl CH), 1.38−1.48 (m, 2H, piperidine CH_2), 1.75−1.87 (m, 3H, piperidine CH_2, CH), 1.92−2.02 (m, 2H, piperidine NCH_2), 2.25 (d, 2H, J = 6.60, $NCH_2C_3H_5$), 3.10 (d, 2H, J = 9.60, piperidine NCH_2), 3.76 (d, 2H, J = 3.75, CH_2O), 6.79−6.83 (m, 1H, ArH), 7.03−7.08 (m, 2H, ArH), 7.12 (t, 1H, J = 7.94, ArH); MS m/z 324.3. Anal. Calcd for $C_{16}H_{22}NOBr$: C, H, N, Br.

1-(3-Fluoropropyl)-4-[(pentafluorophenoxy)methyl]piperidine (16). Compound **16** was prepared using the general method provided above for **1** by alkylation of 4-[(pentafluorophenoxy)methyl]piperidine (160 mg, 0.57 mmol) with 3-bromo-1-fluoropropane (90 μL, 0.57 mmol) in the presence of potassium carbonate (236 mg, 1.7 mmol). After purification, the desired product was obtained as a clear, colorless oil (120 mg, 62%): ^{1}H-NMR δ 1.30−1.45 (m, 2H, piperidine CH_2), 1.70−2.15 (m, 7H, piperidine CH_2, CH, NCH_2, CH_2CH_2F), 2.47 (t, 2H, J = 7.33, $NCH_2(CH_2)_2F$), 2.95 (d, 2H, J = 11.59, piperidine NCH_2), 3.99 (d, 2H, J = 6.26, CH_2O), 4.51 (dt, 2H, J_{HF} = 47.30, J_{HH} = 5.96, CH_2F); MS m/z 342.5. Anal. Calcd for $C_{15}H_{17}N_2OF_6$: C, H, N.

1-(4-Fluorobenzyl)-4-[(pentafluorophenoxy)methyl]piperidine (17). Compound **17** was prepared using the general method provided above for **1** by alkylation of 4-[(pentafluorophenoxy)methyl]piperidine (100 mg, 0.36 mmol) with 4-fluorobenzyl bromide (45 μL, 0.36 mmol) in the presence of potassium carbonate (200 mg, 1.4 mmol). After purification, the desired product was obtained as a white solid (120 mg, 86%); mp = 56−57 °C; ^{1}H-NMR δ 1.32−1.45 (m, 2H, piperidine CH_2), 1.70−1.90 (m, 3H, piperidine CH_2, CH), 1.99 (t, 2H, J = 9.80, piperdine NCH_2), 2.90 (d, 2H, J = 11.45, piperidine NCH_2), 3.47 (s, 2H, NCH_2Ar), 3.98 (d, 2H, J = 5.95, CH_2O), 6.99 (t, 2H, J = 8.69, ArH), 7.25−7.30 (m, 2H, ArH); MS m/z 390.2. Anal. Calcd for $C_{19}H_{17}NOF_6$: C, H, N.

1-(3-Fluorobenzyl)-4-[(pentafluorophenoxy)methyl]piperidine (18). Compound **18** was prepared using the general method provided above for **1** by alkylation of 4-{(pentafluorophenoxy)methyl]piperidine (100 mg, 0.36 mmol) with 3-fluorobenzyl bromide (45 μL, 0.36 mmol) in the presence of potassium carbonate (200 mg, 1.4 mmol). After purification, the desired product was obtained as a white solid (125 mg, 90%); mp = 46−47 °C; ^{1}H-NMR δ 1.28−1.50 (m, 2H, piperidine CH_2), 1.70−1.90 (m, 3H, piperidine CH_2, CH), 2.08 (t, 2H, J = 11.60, piperidine NCH_2), 2.93 (d, 2H, J = 10.98, piperidine NCH_2), 3.54 (s, 2H, NCH_2Ar), 3.96 (d, 2H, J = 6.40, CH_2O), 6.92−7.00 (m, 1H, ArH), 7.08−7.15 (m, 2H, ArH), 7.24−7.30 (m, 1H, ArH); MS m/z 390.2. Anal. Calcd for $C_{19}H_{17}NOF_6$: C, H, N.

1-(Pentafluorobenzyl)-4-[(pentafluorophenoxy)methyl]piperidine (19). Compound **19** was prepared using the general method provided above for **1** by alkylation of 4-[(pentafluorophenoxy)methyl]piperidine (100 mg, 0.37 mmol) with pentafluorobenzyl bromide (105 μl, 0.40 mmol) in the presence of potassium carbonate (153 mg, 1.1 mmol). After purification, the desired product was obtained as a white solid (148 mg, 87%): mp = 179−181 °C; ^{1}H-NMR δ 1.28−1.50 (m, 2H, piperidine CH_2), 1.70−1.90 (m, 3H, piperidine CH_2, CH), 2.08 (t, 2H, J = 11.60, piperidine NCH_2), 2.93 (d, 2H, J = 10.98, piperidine NCH_2), 3.73 (s, 2H, NCH_2Ar), 3.96 (d, 2H, J = 6.40, CH_2O); MS m/z 462.5. Anal. Calcd for $C_{19}H_{13}NOF_{10}$: C, H, N.

Radiolabeling. The synthesis and purification of [^{123}I]-**4** was accomplished as previously described.[40] Briefly, to sodium [^{123}I]iodide in aqueous sodium hydroxide solution were added glacial acetic acid (30 μL), and chloramine-T (0.5 mg) dissolved in a solution of methanol and water (100 μL, 80:20 v/v), followed immediately by a solution of the vinylstannane precursor in ethanol (1.0 mg, 200 μL). The reaction mixture was quenched with aqueous sodium metabisulfite (20 μL, 1 M) and then made basic by the addition of aqueous sodium carbonate. The product was purified by HPLC (mobile phase: methanol/water, 80:20 v/v), providing the radiotracer in 60−80% yield (EOB). The radiochemical purity was >99%, and the specific activity was >74 000 MBq/μmol (>2000 mCi/μmol).

To obtain suitable preparations of the tracer for use *in vivo*, the eluted radioactive peak corresponding to [^{123}I]-**4** was collected, the mobile phase removed *in vacuo*, and the product redissolved in saline (0.9% NaCl, sterile). The saline solution was passed through a sterile filter into an evacuated sterile vial and diluted with saline to provide approximately 10 μCi of activity per 100 μL of solution.

Lipophilicity Estimations. The lipophilicity of each new compound was examined by determination of the log $P_{7.5}$ value using a HPLC method previously described.[40−42,46] Samples were analyzed using a C18 column (Goldpak Exsil, 10 μm, 4.6 × 250 mm) and a mobile phase of MeOH and phosphate buffer (85:15 v/v, pH = 7.5) with a flow rate of 1.0 mL/min. The lipophilicity of each ligand was estimated by a comparison of its retention time to that of standards having known log P values. The standards used were catechol, aniline, benzene, bromobenzene, ethyl benzene, trimethylbenzene, and hexachlorobenzene dissolved in an appropriate solvent. All sample injections were done in triplicate and the results averaged to provide the final values. Relative retention times, RRT (to catechol), were calculated, and a calibration curve of log P vs log RRT was generated. The calibration equations were polynomial with r^2 of 0.994 or greater.

Ligand Binding Assays. Potential σ ligands were tested through the NIMH/NovaScreen Drug Discovery & Development Program (Contract No. NIMH-2003). Briefly, competitive binding assays were performed in either 250 or 500 μL volumes containing, by volume, 80% receptor preparations, 10% radiolabeled competing ligand, and 10% of each new compound (nonspecific binding determinant/4% DMSO (total binding determinant)). All compounds were solubilized in neat DMSO and diluted with water to a final concentration of 0.4% DMSO for use in the assay. Three independent determinations were made at each concentration, and the results were averaged. Assays were terminated by rapid vacuum filtration over glass fiber filters (Whatman) followed by rapid washing with cold buffer. Radioactivity was determined by either liquid scintillation or γ spectrometry. Data was reduced by a software program proprietary to NOVASCREEN. Details of methods used in the *in vitro* receptor binding assays are provided in the references.[4,50,51]

σ-1 Binding Assay. The assay was performed following the method described by Chaki *et al.*[52] Guinea pig membranes (5 mg/tube) were incubated with 2.0 nM [^{3}H]-(+)-pentazocine (30−60 Ci/mmol) in 50 mM Tris-HCl (pH 8.0) at 25 °C for 120 minutes. Nonspecific binding was determined in the presence of 10.0 μM (+)-3-PPP. Test compounds were added over the concentration range of 10^{-11}−10^{-6} M, and either triplicate analysis at five concentrations or duplicate analysis at eight concentrations was used in each assay. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values obtained with known amounts of (+)-3-PPP in order to ascertain any interactions of the test compound with the σ-1 binding site.

σ-2 Binding Assay. The assay was performed following the method described by Chaki *et al.*[52] Guinea pig membranes (10 mg/tube) were incubated with 2.0 nM [^{3}H]DTG (30−60 Ci/mmol) in the presence of 100 nM (+)-pentazocine in 50 mM Tris-HCl (pH 8.0) at 25 °C for 120 min. Nonspecific binding was determined in the presence of 1.0 μM haloperidol. Test compounds were added over the concentration range 10^{-11}−10^{-6} M, and either triplicate analysis at five concentrations or duplicate analysis at eight concentrations was used in each assay. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values obtained with know amounts of haloperidol in order to ascertain any interactions of the test compound with the σ-2 binding site. It should be noted that this method is an indirect assay for σ-2 receptor binding but is an accepted method with the current lack of a suitably selective σ-2 receptor ligand.

Biological Materials and Methods. All *in vivo* procedures were carried out in compliance with Australian laws governing animal experimentation.

***In Vivo* Tissue Distribution Studies.** The radiotracer was administered to 12−16-week-old male Australian Albino Wistar (AAW) rats via tail vein injection ($n = 3$). At selected times postinjection of the radioligand, the rats were sacrificed by CO_2 administration followed by cervical fracture. Various organs were removed, weighed, and assayed for radioactivity using an automated γ counter. The percent injected dose (%ID) for each organ was calculated by comparison of a diluted standard solution of the initial injected dose. The density of radioactivity in each organ (%ID/g) was found by dividing the %ID for each tissue by the weight of the tissue.

Receptor Blocking and Saturation Studies. Studies were performed to examine the *in vivo* specificity and saturability of [^{123}I]-**4** binding to σ receptors. Selected drugs having known pharmacological activity, including haloperidol, DuP 734, (+)-pentazocine, unlabeled **4**, ritanserin, (−)-eticlopride, and atropine, were dissolved in sterile saline. If required, solubilization was aided by the addition of a small amount of glacial acetic acid (10−20 μL). Each drug was administered to male AAW rats at a dose of 1 mg/kg by tail vein injection, and after 5 min, the radioligand [10 μCi in sterile saline (100 μL)] was administered *via* the tail vein. After 60 min, the rats were sacrificed by cervical fracture under the influence of CO_2. Various organs were removed, weighed, and assayed for radioactivity, and the %ID and %ID/g determined exactly as described above for the tissue distribution studies.

Statistical Analysis. Statistical significance was evaluated using ANOVA and an unpaired Students *t*-test. The criterion for significance was $p < 0.05$.

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