Synthesis and Quantitative Structure−Activity Relationships of *N*-(1-Benzylpiperidin-4-yl)phenylacetamides and Related Analogues as Potent and Selective σ_1 Receptor Ligands

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A series of *N*-(1-benzylpiperidin-4-yl)phenylacetamide derivatives was synthesized and evaluated for affinity at σ_1 and σ_2 receptors. Most of these compounds showed a high affinity for σ_1 receptors and a low to moderate affinity for σ_2 receptors. The unsubstituted compound *N*-(1-benzylpiperidin-4-yl)phenylacetamide, **1**, displayed a high affinity and selectivity for σ_1 receptors (K_i values of 3.90 nM for σ_1 receptors and 240 nM for σ_2 receptors). The influence of substitutions on the phenylacetamide aromatic ring on binding at both the σ_1 and σ_2 receptor has been examined through Hansch-type quantitative structure−activity relationship (QSAR) studies. In general, all 3-substituted compounds, except for the OH group, had a higher affinity for both σ_1 and σ_2 receptors when compared with the corresponding 2- and 4-substituted analogues. The selectivity for σ_1 receptors displayed a trend of 3 > 2 ≈ 4 for Cl, Br, F, NO_2, and OMe substituted analogues. Halogen substitution on the aromatic ring generally increased the affinity for σ_2 receptors while maintaining a similar affinity for σ_1 receptors. Substitution with electron-donating groups, such as OH, OMe, or NH_2, resulted in weak or negligible affinity for σ_2 receptors and a moderate affinity for σ_1 receptors. The 2-fluoro-substituted analogue, **11**, exhibited the highest selectivity for σ_1 receptors among all compounds tested, with a K_i value of 3.56 nM for σ_1 receptors and 667 nM for σ_2 receptors. Compounds **1**, **5**, **9**, **11**, and **20** had no affinity for dopamine D_2 (IC_{50} > 10 000 nM) and D_3 (IC_{50} > 10 000 nM) receptors. The nanomolar binding affinity and high selectivity for σ_1 receptors suggest that these compounds may be developed as potential radiotracers for positron emission tomography or single photon emission computerized tomography imaging studies.

Introduction

σ receptors were once viewed as subtypes of the opioid class of receptors.[1] They were later believed to be part of the phencyclidine (PCP) binding site[2] and part of the NMDA (*N*-methyl-D-aspartic acid) receptor complex.[3] The development of σ-selective ligands, such as (+)-pentazocine, DTG, and 3-PPP, allowed σ binding sites to be distinguished as a separate receptor system.[4] Specific σ ligands have facilitated tissue distribution studies and the subclassification of σ receptors. It is now generally agreed that there are at least two subtypes of σ receptors, termed σ_1 and σ_2.[4−6] σ_1 sites are enantioselective for the (+)-benzomorphans, whereas the (−)-benzomorphans are mixed σ_1/σ_2 ligands with moderate affinity. σ_1 receptors are sensitive to G-protein modifying reagents, whereas σ_2 receptors are not.[7−9] Finally, σ_1 receptors from guinea pig brain have a molecular size of 25 kDa, whereas the σ_2 receptors in PC12 cells have an apparent molecular weight of 18−21 kDa.[4,10]

Binding studies have revealed that both σ_1 and σ_2 receptors not only are found in the central nervous system but also are widely distributed in many endocrine, immune, and peripheral organs and tissues, such as heart, spleen, adrenal, kidney, ovary, testes, gastrointestinal tract, and liver (for a review, see ref 4). The precise biochemical and physiological roles of σ receptors are not yet completely understood. However, evidence indicates that σ receptors have a number of biological functions, including motor effects,[11−14] regulation of dopamine and acetylcholine release,[15−18] modulation of the NMDA-evoked norepinephrine release,[19] antagonism of opioid analgesia,[20−22] and mediation of the functional activity of the immune cells.[23] A potential application of σ ligands is in the treatment of psychosis, since a number of antipsychotic drugs have a much higher affinity for σ receptors than for dopamine D_2 receptors.[24] It was postulated that σ ligands may be used as antipsychotics without the extrapyramidal side effects.[25] σ receptors have also been suggested to play a role in dystonia and in the regulation of motor functions.[26]

A number of studies have revealed that many tumor cell lines in vitro contain high concentrations of σ receptors.[27−31] σ receptor ligands have been shown to inhibit cell proliferation in tumor cell lines under cell culture conditions.[32,33] These results suggest that σ receptors may play a role in cell growth, or proliferation, and development. The potential use of σ ligands as external biomarkers and characterization of tumor progression is encouraging, and σ ligands that possess high affinity for either σ_1 or σ_2 receptors could potentially be used to image primary tumor and metastatic sites in conjunction with single photon emission com-

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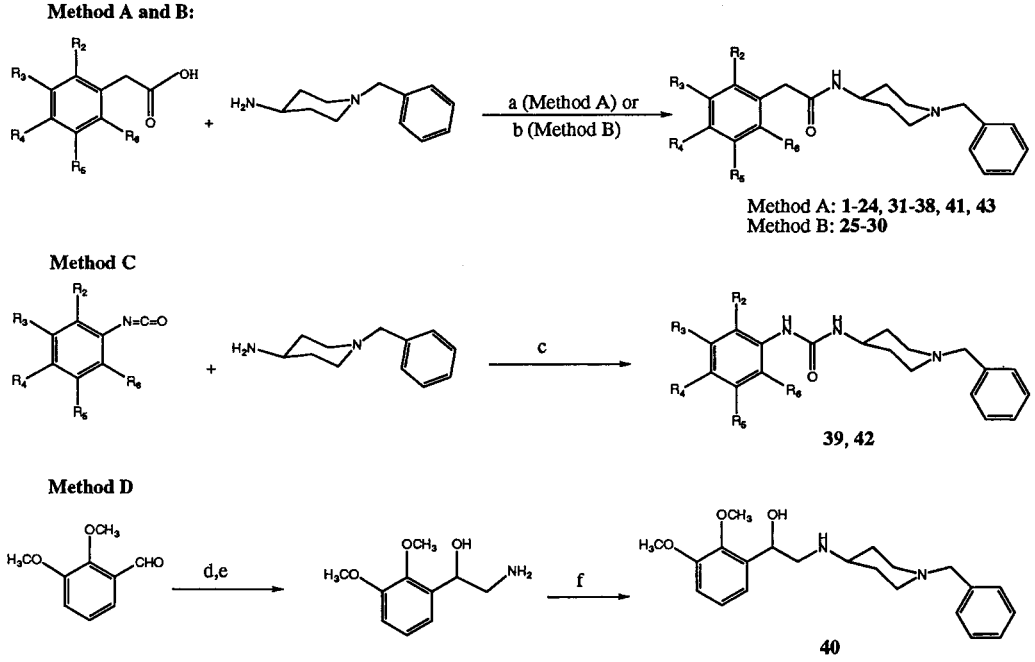
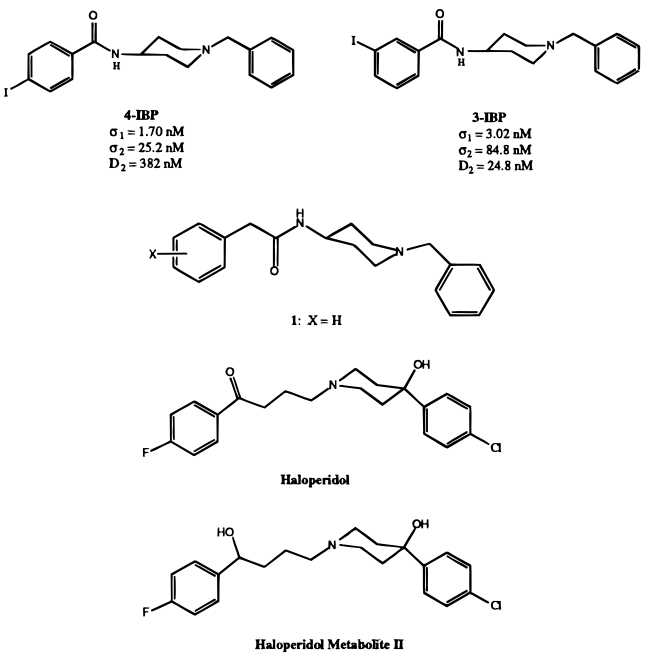
Scheme 1[a]



[a] Reagents: (a) DCC/THF; (b) BOP/CH_2Cl_2; (c) dibutyltin diacetate/CH_2Cl_2; (d) trimethylsilyl cyanide/ZnI_2/benzene; (e) $LiAlH_4$/THF; (f) 1-benzyl-4-piperidone/$NaBH_3CN$/methanol.

Chart 1



puted tomography (SPECT) and positron emission tomography (PET).

Previous studies have shown that the benzamide derivative 4-[^{125}I](*N*-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP), a potential radiotracer for SPECT imaging studies of breast tumors, and its 3-isomer (Chart 1) have a high affinity for σ_1 receptors and a moderate affinity for σ_2 and dopamine D_2 receptors.[34] In the current study, a series of phenylacetamide analogues of 4-IBP (Chart 1) were prepared and their affinity for σ_1 and σ_2 receptors measured in vitro. Since a benzamide moiety is usually required for D_2 receptor affinity for this class of compounds,[45] the addition of a methylene unit to give the corresponding phenylacetamide is predicted to generate compounds having a reduced affinity for dopamine D_2 receptors while retaining a high affinity for σ receptors. A structure−activity relationship study was conducted to explore the substituent effects on σ_1 and σ_2 receptor affinity in the phenylacetamide aromatic ring of **1**. The results of this study are reported below.

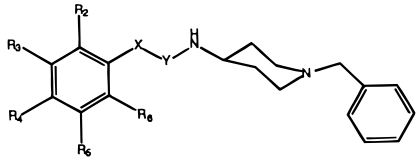
Chemistry

The preparation of the desired compounds is shown in Scheme 1. Most of the compounds were synthesized directly by using the coupling reagents DCC (1,3-dicyclohexylcarbodiimide) (method A) or BOP (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) (method B).[35] The amino group of the phenylacetic acid precursor of **43** was protected as the *N*-*tert*-butyloxycarbonyl group and deprotected after the coupling process with trifluoroacetic acid. By using the BOP reagent, hydroxy groups could be unprotected during the coupling process. The ureas, **39** and **42**, were obtained by reacting the corresponding isocyanate with the amine (method C). Compound **40** was synthesized from 2,3-dimethoxybenzaldehyde which was treated with trimethylsilyl cyanide in the presence of zinc iodide to give 2-[(trimethylsilyl)oxy](2,3-dimethoxy)phenylacetonitrile. The subsequent product was reduced with $LiAlH_4$ to form 2-hydroxy-2-(2,3-dimethoxyphenyl)ethylamine, which was then condensed with the *N*-benzyl-4-piperidone in the presence of $NaBH_3CN$ to give the *N*-benzyl and *N*-alkylated product (method D).

Results and Discussion

The goal of this study was to prepare a series of *N*-(1-benzylpiperidin-4-yl)phenylacetamide analogues of the σ ligand, *N*-(1-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP),[34] and to measure their affinity at σ_1 and σ_2 receptors. The strategy of incorporating a methylene unit into the structure of 4-IBP to form a phenylacetamide analogue was expected to afford compounds with

Table 1. σ Receptor Binding Profiles of *N*-(1-Benzylpiperidin-4-yl)phenylacetamide Analogues in Cell Membranes



								K_i (nM)[a]		
no.	R_2	R_3	R_4	R_5	R_6	X	Y	σ_1[b]	σ_2[c]	σ_2/σ_1 ratio[d]
1	H	H	H	H	H	CH_2	CO	3.90 ± 0.82	240 ± 30	62
2	Cl	H	H	H	H	CH_2	CO	3.08 ± 0.25	174 ± 6	56
3	H	Cl	H	H	H	CH_2	CO	1.40 ± 0.66	36.9 ± 1.8	26
4	H	H	Cl	H	H	CH_2	CO	6.46 ± 0.59	80.7 ± 7.5	12
5	H	Cl	Cl	H	H	CH_2	CO	5.22 ± 0.84	14.6 ± 2.4	3
6	Cl	H	Cl	H	H	CH_2	CO	9.66 ± 2.21	24.5 ± 3.4	3
7	Cl	H	H	H	Cl	CH_2	CO	7.28 ± 2.99	70.8 ± 3.6	10
8	Br	H	H	H	H	CH_2	CO	2.89 ± 0.83	86.6 ± 8.3	28
9	H	Br	H	H	H	CH_2	CO	0.87 ± 0.02	15.2 ± 2.0	18
10	H	H	Br	H	H	CH_2	CO	3.97 ± 0.73	23.9 ± 1.3	6
11	F	H	H	H	H	CH_2	CO	3.56 ± 1.07	667 ± 98	187
12	H	F	H	H	H	CH_2	CO	2.45 ± 0.09	153 ± 6	62
13	H	H	F	H	H	CH_2	CO	3.43 ± 1.94	69.2 ± 0.5	20
14	F	H	F	H	H	CH_2	CO	3.87 ± 0.22	130 ± 31	33
15	F	H	H	F	H	CH_2	CO	4.21 ± 0.73	129.3 ± 4.9	31
16	F	H	H	H	F	CH_2	CO	5.80 ± 1.86	207 ± 9	36
17	H	F	F	H	H	CH_2	CO	3.17 ± 0.63	53.5 ± 3.8	17
18	H	F	H	F	H	CH_2	CO	7.63 ± 1.78	37.3 ± 6.8	5
19	CF_3	H	H	H	H	CH_2	CO	8.70 ± 2.41	61.9 ± 6.5	7
20	H	CF_3	H	H	H	CH_2	CO	3.92 ± 0.79	37.8 ± 3.8	10
21	H	H	CF_3	H	H	CH_2	CO	9.84 ± 1.42	53.4 ± 0.6	5
22	NO_2	H	H	H	H	CH_2	CO	26.9 ± 3.3	493 ± 115	18
23	H	NO_2	H	H	H	CH_2	CO	4.56 ± 1.52	79.2 ± 9.9	17
24	H	H	NO_2	H	H	CH_2	CO	18.0 ± 2.3	105.4 ± 3.6	6
25	NO_2	H	NO_2	H	H	CH_2	CO	14.2 ± 0.9	243 ± 69	17
26	OH	H	H	H	H	CH_2	CO	18.8 ± 1.3	343 ± 19	18
27	H	OH	H	H	H	CH_2	CO	110 ± 27	>1000	>9
28	H	H	OH	H	H	CH_2	CO	286.5 ± 7.9	>1000	>3
29	H	OH	OH	H	H	CH_2	CO	>1000	>1000	0
30	H	Cl	OH	H	H	CH_2	CO	23.8 ± 4.6	757 ± 13	32
31	OMe	H	H	H	H	CH_2	CO	30.8 ± 2.6	>1000	33
32	H	OMe	H	H	H	CH_2	CO	10.5 ± 3.1	326 ± 40	31
33	H	H	OMe	H	H	CH_2	CO	66.4 ± 3.3	483 ± 12	7
34	OMe	H	H	OMe	H	CH_2	CO	43.4 ± 9.3	>1000	>23
35	H	OMe	OMe	H	H	CH_2	CO	352 ± 91	>1000	>3
36	H	OMe	H	OMe	H	CH_2	CO	121 ± 22	>1000	>8
37	H	OMe	OMe	OMe	H	CH_2	CO	80.0 ± 28.1	>1000	>12
38	H	OCH_2O		H	H	CH_2	CO	7.32 ± 0.65	290 ± 57	40
39	OMe	H	OMe	H	H	NH	CO	30.1 ± 7.4	227 ± 6	8
40	OMe	OMe	H	H	H	CHOH	CH_2	32.1 ± 3.9	535 ± 27	17
41	H	H	SMe	H	H	CH_2	CO	27.1 ± 6.3	83.3 ± 10.0	3
42	H	H	SMe	H	H	NH	CO	63.9 ± 1.6	80.5 ± 5.8	1
43	H	H	NH_2	H	H	CH_2	CO	470 ± 36	>1000	>2
haloperidol								2.53 ± 0.25	28.5 ± 1.6	11
haloperidol metabolite II								14.34 ± 0.08	28.6 ± 4.2	2

[a] Mean ± SEM, K_i values were determined by at least three experiments. Each inhibition curve consisted of eight points from each binding assay. [b] K_i values for σ_1 receptors were measured on quinea pig brain membranes using [^{3}H]-(+)-pentazocine as the radioligand. [c] K_i values for σ_2 receptors were measured on rat liver membranes using [^{3}H]DTG as the radioligand in the presence of (+)-pentazocine. [d] K_i for σ_2 receptor/K_i for σ_2 receptor.

low affinity for dopamine D_2 receptors while retaining a high affinity for σ receptors. Substitutions were made on the phenyl ring of the phenylacetamide moiety to investigate the effect of different lipophilic and hydrophilic substituents on σ receptor potency and selectivity. Binding assays were performed on guinea pig brain membranes for σ_1 receptors with [^{3}H]-(+)-pentazocine as the radioligand and on rat liver membranes for σ_2 receptors with [^{3}H]DTG as the radioligand in the presence of (+)-pentazocine to mask the σ_1 sites. The binding affinities (K_i values) for the new ligands, as well as the reference compounds haloperidol and its corresponding benzylic alcohol analogue (i.e., haloperidol metabolite II, Chart 1), at the σ_1 and σ_2 receptors are shown in Table 1. Substitution on the phenylacetamide aromatic ring influences both σ_1 and σ_2 receptor affinity as well as selectivity. The unsubstituted compound **1** had K_i values of 3.90 nM for σ_1 receptors and 240 nM for σ_2 receptors and a σ_1:σ_2 selectivity ratio of 62. Results show a spectrum of σ_1 receptor affinities ranging from 0.87 nM for compound **9** to >1000 nM for compound **29**, with σ_2 receptor affinities ranging from 14.6 nM for compound **5** to >1000 nM for several compounds.

Hansch-type analyses were carried out on all *N*-(1-benzylpiperidin-4-yl)phenylacetamides in the current study (**1**−**38**, **41**, and **43**, n = 40) to examine the influence of substitutions on the phenylacetamide aromatic ring on binding at both the σ_1 and σ_2 receptors. Models relating log(1/K_i) values for both the σ_1 and σ_2 receptor binding data were evaluated using both forward stepwise and backward stepwise multiple regression calculations.[36] Both stepwise in and stepwise out gave very similar equations for set of data. Parameters used in these analyses were taken from Hansch and Leo[37,38,45] and represent electronic, hydrophobic, and steric bulk effects for the various substituents. Param-

eters selected included: π values; Hammett σ values, with the value used based on the position of the substituent on the aromatic ring, that is, σ_o values used for ortho substituents, σ_m values used for meta substituents, and σ_p values used for para substituents; and MR (molar refractivity, scaled by 0.1). Along with individual descriptor variables for each compound (σ_2, σ_3, π_4, etc.), additional parameters examined were composites of the effects at both the ortho and para positions (σ_{2+4}, π_{2+4}, MR_{2+4}), effects at the two possible meta positions (σ_{3+5}, π_{3+5}, MR_{3+5}), summation of all electronic ($\sum\sigma$), hydrophobic ($\sum\pi$) and steric ($\sum$MR) constants, and MR_4^2. The correlation matrix for all parameters was examined to assure that any components used in final equations showed low cross-correlation. Analyses were carried out separately, using both the individual and composite descriptors as well as the combined set of parameters.

While a variety of regression equations were identified as significant, the regression equations shown below represent those models which seem to best account for the binding data variance. Along with each model is provided the squared correlation coefficient (r^2), the F statistic, and the standard error of estimate (SE). Confidence intervals for the coefficients are given in parentheses. For all equations, individual variables were significant at less than the 0.05 level, and $P = 0.0001$ for the overall equation. Equations 1−3 were derived for σ_1 binding data.

$$1\log(1/K_i) = -0.77(\pm0.12) + 0.89(\pm0.23)\pi_3 + 0.96(\pm 0.19)\pi_4 - 0.86(\pm0.23)MR_4 - 1.01(\pm0.40)MR_5$$

$$n = 40, \quad r^2 = 0.64, \quad SE = 0.46, \quad F_{4,35} = 15.7 \quad (1)$$

$$\log(1/K_i) = -0.82(\pm0.18) + 0.36(\pm0.17)\sum\sigma + 0.68 (\pm0.12)\sum\pi - 0.48(\pm0.14)\sum MR$$

$$n = 40, \quad r^2 = 0.61, \quad SE = 0.47, \quad F_{3,36} = 19.1 \quad (2)$$

$$\log(1/K_i) = -0.70(\pm0.16) + 0.85(\pm0.29)\sigma_4 + 0.74(\pm 0.29)MR_3 + 0.67(\pm0.11)\sum\pi - 0.70(\pm0.16)\sum MR$$

$$n = 40, \quad r^2 = 0.68, \quad SE = 0.43, \quad F_{4,35} = 18.3 \quad (3)$$

Equation 1, based on parameters at single positions on the aromatic ring, indicates that hydrophobic substituents at both the 3- and 4-positions increase binding, while steric bulk at the 4-position, and 5-position in disubstituted compounds, decreases potency. Equation 2, derived from composite parameters, is similar to eq 1, showing a general increase in binding affinity for hydrophobic groups and a decrease in potency for bulky groups. Additionally, the inclusion of the $\sum\sigma$ term indicates that electron-withdrawing substituents increase activity, even though the aromatic ring is isolated from the active nitrogen in these compounds. Equation 3, which shows the highest correlation of the three equations ($r^2 = 0.68$), is similar to eq 2 but includes parameters that reflect both general trends as well as specific interactions with the receptors. Electron-withdrawing substituents in the 4-position and hydrophobic groups at all positions increase binding affinity, while steric bulk has in general a negative effect on potency. Unlike eq 2, the additional MR_3 term may indicate some tolerance for steric bulk at the 3-position, with substituents in that position interacting with a polar region of the receptor.

In accord with eq 3, substitution at the 3-position with electron-withdrawing, hydrophobic groups increases activity relative to the unsubstituted compound **1**. It is of note that compound **9**, whose 3-bromo substituent is both hydrophobic and has a relatively large molar refractivity term, shows the highest activity at the σ_1 receptor of all compounds studied. Conversely, eq 1 indicates that electron-donating groups at the 4-position should not be well tolerated, particularly if these substituents have low hydrophobicity. Compounds **28** (4-OH) and **43** (4-NH_2) both have these characteristics and show binding affinities at the σ_1 receptor of 287 and 470 nM, respectively. In fact, for monohydroxylated derivatives, only the 2-substituted analogue (**26**) retained significant σ_1 receptor affinity, with a K_i value of 18.8 nM.

$\log(1/K_i)$ values for σ_2 binding data were predicted well by somewhat different equations:

$$\log(1/K_i) = -2.29(\pm0.07) + 0.54(\pm0.16)\pi_2 + 0.57(\pm 0.17)\pi_3 + 0.59(\pm0.14)\pi_4 + 0.82(\pm0.30)\sigma_3 + 0.54(\pm 0.21)\sigma_4 - 0.90(\pm0.26)MR_5$$

$$n = 40, \quad r^2 = 0.79, \quad SE = 0.29, \quad F_{6,33} = 20.1 \quad (4)$$

$$\log(1/K_i) = -2.17(\pm0.12) + 0.27(\pm0.12)\sigma_{2+4} + 0.49 (\pm0.16)MR_4^2 + 0.97(\pm0.26)\sigma_{3+5} + 0.61(\pm0.08)\sum\pi - 0.41(\pm0.11)\sum MR$$

$$n = 40, \quad r^2 = 0.77, \quad SE = 0.30, \quad F_{5,34} = 22.4 \quad (5)$$

$$\log(1/K_i) = -2.32(\pm0.06) + 0.59(\pm0.18)\sigma_4 - 0.98(\pm 0.24)MR_5 + 0.85(\pm0.22)\sigma_{3+5} + 0.54(\pm0.07)\sum\pi$$

$$n = 40, \quad r^2 = 0.80, \quad SE = 0.27, \quad F_{4,35} = 34.8 \quad (6)$$

Equation 4 shows a general increase in binding affinity for hydrophobic substituents at the 2-, 3-, and 4-positions, as well as an increase in potency for electron withdrawing substituents at the 3- and 4-positions. For disubstituted compounds, steric bulk at the 5-position decreases affinity, as was the case for σ_1 receptor affinity (eq 1). Equation 5, derived using composite parameters, indicates an increase in affinity for electron-withdrawing and hydrophobic substituents but a small decrease in potency correlated with bulky substituents. This latter effect is counterbalanced by a weak positive effect on affinity by bulky substituents at the 4-position.

A slightly better equation than 5 was derived using both individual and composite parameters and is shown in eq 6. As was the case for eq 3 for σ_1 receptor affinity, eq 6 predicts an increase in binding for electron-withdrawing substituents in the 4-position, as well as for electron-withdrawing groups at the 3-position and the 3- and 5-positions for disubstituted compounds. This latter effect is counterbalanced by a sensitivity to steric

size at 5-position, as reflected by the relatively large (and negative) MR_5 term in eq 6. Equation 6 predicts a small increase in binding affinity with substitution of hydrophobic groups at all positions.

These influences on σ_2 binding predicted by eq 6 may be illustrated by examination of the monosubstituted compounds **3**, **4**, **9**, **10**, **12**, **13**, **20**, and **21**. These compounds all show higher activity at the σ_2 receptor than did the unsubstituted compound **1** and all have groups that are both hydrophobic and electron-withdrawing at either the 3- or 4-position. As was noted earlier for σ_1 binding affinity, σ_2 binding appears to be enhanced to a larger extent by electron-withdrawing substituents at the 3-position than it is for substitutions made at the 2- or 4-positions. This is the case for **3** (3-Cl), **9** (3-Br), **20** (3-CF_3), and for **23** (3-NO_2). Equation 6 may reflect this sensitivity through the larger coefficient for the σ_{3+5} term compared to that of the σ_4 term (see also eq 5). One exception to this trend appears to be for **12** (3-F) and **13** (4-F), where **13** shows approximately a 2-fold greater affinity to the σ_2 receptor than does compound **12**.

It is of note that all three bromo-substituted analogues, compounds **8**−**10**, show a 2−3-fold higher σ_2 receptor affinity than the corresponding chloro-substituted analogues, perhaps due to the greater hydrophobicity of the bromo substituent (eq 6). One outcome of this effect is that the bromo-substituted analogues have a reduced σ_1:σ_2 selectivity ratio relative to the corresponding chloro-substituted analogues. In general, 2- and 3-substitution with Cl, Br, and F led to the following relative order of potency at both the σ_1 and σ_2 receptor: Br > Cl > F.

An interesting contrast between the effects of monosubstitution on binding at σ_1 and σ_2 receptors is found for 2-substitution with fluorine. While 2-substitution with either Cl, Br, or F had a minimal effect on σ_1 receptor affinity, and showed a slight increase in affinity at the σ_2 receptor for bromo and chloro derivatives, 2-substitution of a fluoro group dramatically reduced the affinity at σ_2 receptors (K_i = 667 nm), thereby resulting in the compound (**11**) with the highest for σ_1:σ_2 selectivity ratio (~187) among all of the new ligands tested in this study. None of the QSAR equations above appear to account for this selectivity. For σ_2 binding, substitution by fluorine at the 3- and 5-positions appears to be favored over the 3- and 4-positions for the two disubstituted compounds **17** and **18**, in agreement with the larger coefficient for the σ_{3+5} term compared to the σ_4 term in eq 6. In this case, the MR_5 term should play a small part since the molar refractivity for fluorine is slightly smaller than that of hydrogen.

A difference between eqs 3 and 6 is the lack of an overall negative effect on activity for steric bulk as shown by the absence of the ∑MR term in eq 6. However, the inclusion of the MR_5 term in eq 6 does indicate some sensitivity to steric bulk specifically at the 5-position in polysubstituted compounds. For example, compounds **34**, **36**, and **37** all show essentially no affinity (K_i > 1000 nM) at the σ_2 receptor and all contain the 5-OCH_3 moiety.

As was the case for σ_1 binding, substituents that are electron donating and those with low hydrophobicity cause a substantial decrease in binding affinity. While the 2-hydroxy-substituted analogue **26** had measurable affinity for σ_2 receptors (K_i value of 343 nM), both the 3-hydroxy-substituted (**27**) and 4-hydroxy-substituted (**28**) analogues had no affinity. A similar result was observed with the 4-NH_2-substituted analogue, **43**, which had no measurable affinity for σ_2 receptors.

Dihydroxy substitution had a further detrimental effect on both σ_1 and σ_2 receptor affinity, with the 3,4-dihydroxy-substituted analogue (**29**) having no measurable affinity for either receptor. When the dimethoxy substitution pattern of **35** was replaced with a methylenedioxy moiety, the resultant compound, **38**, had a 48-fold increase in σ_1 receptor affinity from 352 nM for **35** to 7.32 nM for **38**. This change in substitution also resulted in a significant increase in σ_2 receptor affinity, from >1000 nM for **35** to 290 nM for **38**. These results may indicate a sensitivity of both receptors to steric bulk outside the plane of the aromatic ring.

An unexpected observation was the relatively high σ_1 affinity of the urea compound, **39**, and the hydroxyethylamino analogue, **40**. Compound **39** had higher affinity for σ_1 and σ_2 receptors than the dimethoxy-substituted phenylacetamide analogues **34**, **35**, and **36**. Although no 2,4-dimethoxy-substituted phenylacetamide analogue was made to compare in this study, we anticipate that the 2,4-dimethoxy-substituted phenylacetamide analogue would have σ_1 and σ_2 receptor affinities lower than those of the urea analogue, **39**. The 4-methythio-substituted analogue **41** had an affinity for both receptors higher than that of the corresponding 4-methoxy-substituted analogue, **33**. This may be attributed to the higher lipophilicity of the SMe than that of OMe.

In order to confirm that the phenylacetamide analogues do not bind to dopamine D_2 class of receptors, in vitro binding assays were conducted with genetically engineered Sf9 cells containing either the rat dopamine D_{2-long} or rat D_3 receptor.[39] The dopamine receptor binding assays were conducted on analogues **1**, **5**, **9**, **11**, and **20** and revealed that these compounds do not bind (IC_{50} > 10 000 nM) to D_2 or D_3 receptors (data not shown).

In conclusion, the present study investigated a series of *N*-(1-benzylpiperidin-4-yl)phenylacetamide derivatives for their binding affinities for σ_1 and σ_2 receptors. Substituents were introduced into various positions on the phenyl ring of the phenylacetamide moiety. Lipophilic substituents, such as Cl, Br, and CF_3 groups, gave either higher or similar affinities for σ_1 receptors and a higher σ_2 receptor affinity in comparison to compound **1**. All 3-substitutions afforded the highest affinities for both σ_1 and σ_2 receptors. The bromo-substituted analogues gave the highest increase in affinity for both receptors. All *N*-(1-benzylpiperidin-4-yl)phenylacetamide analogues evaluated in this study have higher affinities for σ_1 receptors than for σ_2 receptors. The 2-F analogue, **11**, was the most selective σ_1 ligand (σ_1:σ_2 ratio of 187); compounds **1**, **2**, and **12** also showed high σ_1:σ_2 selectivity ratios of 62, 56, and 62, respectively. Compounds with hydrophilic substituents (NO_2, OH, OMe, and NH_2) had decreased affinities for σ_1 receptors and a low affinity for σ_2 receptors when compared to that of compound **1**. These results are in agreement with the generally accepted model of the σ receptor binding site, suggesting that the binding site is com-

posed of a primary lipophilic site, a basic nitrogen binding site, and a secondary lipophilic site.[40] Compounds **1**, **11**, and **12** may be developed as potential radiotracers for PET or SPECT imaging of σ_1 receptors in vivo.

Experimental Section

Chemistry. Melting points were measured on a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA; where molecular formulae were indicated, analyses were found to be within ±0.4% of the theoretical values for these elements. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker ASPECT3000 spectrometer. All ^{1}H NMR spectra were obtained in either $CDCl_3$ or DMSO-d_6, and results are recorded as parts per million (ppm) downfield to tetramethylsilane (TMS). The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, dq = double quartet, br = broad. All starting materials and solvents were purchased from either Aldrich or Fisher and were used without further purification.

General Method A. Preparation of *N*-(1-Benzylpiperidin-4-yl)phenylacetamide (1). To an ice-cold solution of phenylacetic acid (1.36 g, 10 mmol) in dry THF (35 mL) was added DCC (2.06 g, 10 mmol). After 30 min of stirring, 1-benzyl-4-aminopiperidine (1.90 g, 10 mmol) was added. The reaction was continued at room temperature overnight. The solid was removed by filtration, the solvent was removed, and the residue was partitioned between CH_2Cl_2 and water. The combined organic layer was washed with 1 N NaOH and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel column using $CHCl_3$ and then $CHCl_3$−EtOH (9.5:0.5) as the eluents. The product was then recrystallized from ethyl acetate/hexane to give 0.87 g of free amine, mp 136−137 °C. The mother liquid was concentrated and converted into the HCl salt by treatment with HCl gas in ethyl acetate. After removal of the solvent, the HCl salt was recrystallized from ethyl acetate−ethanol to give 0.96 g (56% overall yield): mp 204−206 °C; ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.37 (m, 10H, aromatic), 5.17−5.20 (d, 1H, NH), 3.73−3.85 (m, 1H, NHCH), 3.55 (s, 2H, CH_2CO), 3.44 (s, 2H, CH_2-phenyl), 2.68−2.72 (d, 2H 2α-H_{eq} of piperidine), 2.03−2.12 (dt, 2H, 2α-H_{ax} of piperidine), 1.80−1.86 (m, 2H, 2β-H_{eq} of piperidine), 1.24−1.37 (dq, 2H, 2β-H_{ax} of piperidine). Anal. ($C_{20}H_{24}N_2OHCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2-chlorophenylacetamide (2):** yield 74%; mp 138−139 °C (free amine), 215−217 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.23−7.41 (m, 9H), 5.25−5.28 (d, 1H), 3.79−3.82 (m, 1H), 3.66 (s, 2H), 3.45 (s, 2H), 2.69−2.73 (d, 2H), 2.05−2.13 (dt, 2H), 1.83−1.88 (m, 2H), 1.28−1.41 (dq, 2H). Anal. ($C_{20}H_{23}N_2OCl\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-chlorophenylacetamide (3):** yield 64%; mp 115−116 °C (free amine), 170−172 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.13−7.30 (m, 9H), 5.22−5.24 (d, 1H), 3.74−3.84 (m, 1H), 3.50 (s, 2H), 3.46 (s, 2H), 2.72−2.76 (d, 2H), 2.05−2.13 (dt, 2H), 1.83−1.88 (m, 2H), 1.29−1.42 (dq, 2H). Anal. ($C_{20}H_{23}N_2OCl\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-chlorophenylacetamide (4):** yield 44%; mp 139−140 °C (free amine), 200−202 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.16−7.33 (m, 9H), 5.21−5.23 (d, 1H), 3.72−3.85 (m, 1H), 3.50 (s, 2H), 3.45 (s, 2H), 2.71−2.75 (d, 2H), 2.04−2.12 (dt, 2H), 1.82−1.87 (m, 2H), 1.26−1.40 (dq, 2H). Anal. ($C_{20}H_{23}N_2OCl\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,4-dichlorophenylacetamide (5):** yield 46%; mp 197−199 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 8.43−8.46 (d, 1H), 7.44−7.64 (m, 7H), 7.21−7.25 (m, 1H), 4.21−4.23 (d, 2H), 3.71−3.73 (m, 1H), 3.43 (s, 2H), 3.25−3.35 (m, 2H), 2.92−3.02 (m, 2H), 1.77−1.97 (m, 4H). Anal. ($C_{20}H_{22}N_2OCl_2\cdot HCl\cdot H_2O$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2,4-dichlorophenylacetamide (6):** yield 59%; mp 148−150 °C (free amine), 228−230 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.41−7.42 (d, 1H), 7.21−7.33 (m, 7H), 5.27−5.30 (d, 1H), 3.68−3.86 (m, 1H), 3.60 (s, 2H), 3.46 (s, 2H), 2.72−2.76 (d, 2H), 2.05−2.17 (dt, 2H), 1.84−1.89 (m, 2H), 1.31−1.44 (dq, 2H). Anal. ($C_{20}H_{22}N_2OCl_2\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2,6-dichlorophenylacetamide (7):** yield 39%; mp 167−169 °C (free amine), 203−205 °C (HCl salt). ^{1}H NMR (free amine in $CDCl_3$) δ 7.16−7.36 (m, 8H), 5.16−5.19 (d, 1H), 3.90 (s, 2H), 3.76−3.87 (m, 1H), 3 45 (s, 2H), 2.70−2.74 (d, 2H), 2.05−2.13 (dt, 2H), 1.85−1.95 (m, 2H), 1.30−1.42 (dq, 2H). Anal. ($C_{20}H_{22}N_2OCl_2\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2-bromophenylacetamide (8):** yield 42%; mp 170−172 °C (free amine), 228−231 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.54−7.60 (m, 1H), 7.13−7.36 (m, 8H), 5.27−5.30 (d, 1H), 3.75−3.86 (m, 1H), 3.67 (s, 2H), 3.45 (s, 2H), 2.69−2.73 (d, 2H), 2.05−2.13 (dt, 2H), 1.83−1.96 (m, 2H), 1.29−1.42 (dq, 2H). Anal. ($C_{20}H_{23}N_2OBr\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-bromophenylacetamide (9):** yield 40%; mp 115−116 °C (free amine), 180−182 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.16−7.47 (m, 9H), 5.26 (br, 1H), 3.70−3.85 (m, 1H), 3.49 (s, 2H), 3.46 (s, 2H), 2.72−2.76 (d, 2H), 2.04−2.13 (dt, 2H), 1.83−1.88 (m, 2H), 1.26−1.42 (dq, 2H). Anal. ($C_{20}H_{23}N_2OBr\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-bromophenylacetamide (10):** yield 26%; mp 142−144 °C (free amine), 197−200 °C (HCl salt). tH-NMR (free amine in $CDCl_3$) δ 7.44−7.49 (m, 2H), 7.21−7.33 (m, 5H), 7.10−7.14 (m, 2H), 5.19−5.22 (d, 1H), 3.72−3.84 (m, 1H), 3.48 (s, 2H), 3.46 (s, 2H), 2.71−2.75 (d, 2H), 2.04−2.12 (dt, 2H), 1.82−1.95 (m, 2H), 1.28−1.40 (dq, 2H). Anal. ($C_{20}H_{23}N_2OBr\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2-fluorophenylacetamide (11):** yield 78%; mp 131−132 °C (free amine); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.34 (m, 6H), 6.95−7.04 (m, 3H), 5.25−5.28 (d, 1H), 3.76−3.82 (m, 1H), 3.52 (s, 2H), 3.48 (s, 2H), 2.71−2.75 (d, 2H), 2.04−2.12 (dt, 2H), 1.82−1.88 (m, 2H), 1.28−1.41 (dq, 2H). Anal. ($C_{20}H_{23}N_2OF$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-fluorophenylacetamide (12):** yield 95%; mp 115−116 °C (free amine); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.33 (m, 7H), 7.03−7.15 (m, 2H), 5.34−5.37 (d, 1H), 3.74−3.82 (m, 1H), 3.55 (s, 2H), 3.45 (s, 2H), 2.71−2.75 (d, 2H), 2.04−2.13 (dt, 2H), 1.83−1.88 (m, 2H), 1.29−1.42 (dq, 2H). Anal. ($C_{20}H_{23}N_2OF$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-fluorophenylacetamide (13):** yield 43%; mp 124−126 °C (free amine), 192−194 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 6.97−7.33 (m, 9H), 5.19−5.22 (d, 1H), 3.72−3.85 (m, 1H), 3.50 (s, 2H), 3.45 (s, 2H), 2.70−2.74 (d, 2H), 2.04−2.13 (dt, 2H), 1.82−1.87 (m, 2H), 1.27−1.40 (dq, 2H). Anal. ($C_{20}H_{23}N_2OF\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2,4-difluorophenylacetamide (14):** yield 59%; mp 137−138 °C (free amine), 189−191 °C (HCl salt). ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.33 (m, 6H), 6.79−6.90 (m, 2H), 5.33−5.35 (d, 1H), 3.72−3.85 (m, 1H), 3.49 (s, 2H), 3.46 (s, 2H), 2.73−2.77 (d, 2H), 2.05−2.13 (dt, 2H), 1.84−1.89 (m, 2H), 1.32−1.45 (dq, 2H). Anal. ($C_{20}H_{22}N_2OF_2\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2,5-difluorophenylacetamide (15):** yield 52%; mp 122 123 °C (free amine), 185−187 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.36 (m, 5H), 6.91−7.06 (m, 3H), 5.36−5.38 (d, 1H), 3.73−3.86 (m, 1H), 3.51 (s, 2H), 3.47 (s, 2H), 2.74−2.78 (d, 2H), 2.05−2.14 (dt, 2H), 1.85−1.95 (m, 2H), 1.292−1.42 (dq, 2H). Anal. ($C_{20}H_{22}N_2OF_2\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2,6-difluorophenylacetamide (16):** yield 40%; mp 148−149 °C (free amine). ^{1}H NMR (free amine in $CDCl_3$) δ 7.20−7.33 (m, 6H), 6.87−6.95 (m, 2H), 5.33−5.35 (d, 1H), 3.74−3.85 (m, 1H), 3.58 (s, 2H), 3.46 (s, 2H), 2.73−2.77 (d, 2H), 2.05−2.14 (dt, 2H), 1.85−1.95 (m, 2H), 1.33−1.46 (dq, 2H). Anal. ($C_{20}H_{22}N_2OF_2\cdot HCl$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,4-difluorophenylacetamide (17):** yield 55%; mp 125−126 °C (free amine), 173−175 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.33 (m, 5H), 7.06−7.14 (m, 2H), 6.94−6.99 (m, 1H), 5.25−5.28 (d, 1H), 3.72−3.85 (m, 1H), 3.46 (s, 4H), 2.73−2.77 (d, 2H), 2.05−2.13

(dt, 2H), 1.85−1.96 (m, 2H), 1.31−1.44 (dq, 2H). Anal. ($C_{20}H_{22}N_2OF_2$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,5-difluorophenylacetamide (18):** yield 32%; mp 117−118 °C (free amine), 180−182 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.22−7.36 (m, 5H), 6.70−6.83 (m, 3H), 5.26−5.29 (d, 1H), 3.73−3.86 (m, 1H), 3.49 (s, 2H), 3.47 (s, 2H), 2.74−2.78 (d, 2H), 2.05−2.14 (dt, 2H),1.86−1.90 (m, 2H), 1.32−1.45 (dq, 2H). Anal. ($C_{20}H_{22}N_2OF_2$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl-2-(trifluoromethyl)phenylacetamide (19):** yield 64%; mp 128−129 °C (free amine), 239−241 °C (HCl salt). ^{1}H NMR (free amine in $CDCl_3$) δ 7.66−7.69 (m, 1H), 7.52−7.57 (m, 1H),7.37−7.47 (m, 2H), 7.21−7.32 (m, 5H), 5.13−5.16 (d, 1H), 3.73−3.84 (m, 1H), 3.73 (s, 2H), 3.46 (s, 2H), 2.71−2.75 (d, 2H), 2.04−2.12 (dt, 2H), 1.83−1.87 (m, 2H), 1.27−1.40 (dq, 2H). Anal. ($C_{21}H_{23}N_2OF_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-(trifluoromethyl)phenylacetamide (20):** yield 48%; mp 110−111 °C (free amine), 184−186 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.43−7.61 (m, 4H), 7.22−7.33 (m, 5H), 5.22−5.24 (d, 1H), 3.74−3.87 (m, 1H), 3.58 (s, 2H), 3.46 (s, 2H), 2.72−2.76 (d, 2H), 2.05−2.14 (dt, 2H), 1.84−1.88 (m, 2H), 1.31−1.44 (dq, 2H). Anal. ($C_{21}H_{23}N_2OF_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-(trifluoromethyl)phenylacetamide (21):** yield 28%; mp 124−125 °C (free amine), 209−211 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.58−7.61 (d, 2H), 7.36−7.39 (d, 2H), 7.21−7.32 (m, 5H), 5.20−5.22 (d, 1H), 3.73−3.86 (m, 1H), 3.58 (s, 2H), 3.46 (s, 2H), 2.72−2.76 (d, 2H), 2.04−2.13 (dt, 2H), 1.84−1.87 (m, 2H), 1.30−1.42 (dq, 2H). Anal. ($C_{21}H_{23}N_2OF_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2-nitrophenylacetamide (22):** yield 59%; mp 124−125 °C (free amine), 209−211 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 8.01−8.04 (m, 1H), 7.57−7.62 (m, 1H), 7.42−7.49 (m, 2H), 7.24−7.31 (m, 5H), 5.66−5.68 (d, 1H), 3.73−3.84 (m, 1H), 3.80 (s, 2H), 3.47 (s, 2H), 2.74−2.78 (d, 2H), 2.06−2.14 (dt, 2H), 1.85−1.91 (m, 2H), 1.38−1.50 (dq, 2H). Anal. ($C_{20}H_{23}N_3O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-nitrophenylacetamide (23):** yield 58%; mp 170−173 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 8.47−8.50 (m, 1H, CONH), 8.09−8.18 (m, 2H), 7.69−7.76 (m, 1H), 7.57−7.62 (m, 3H), 7.28−7.46 (m, 3H), 4.22−4.24 (d, 2H), 3.71−3.76 (m, 1H), 3.59 (s, 2H), 3.21−3.35 (m, 2H), 2.96−3.03 (m, 2H), 1.76−2.01 (m, 4H). Anal. ($C_{20}H_{23}N_3O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-nitrophenylacetamide (24):** yield 43%; mp 162−164 °C (free amine), 213−215 °C (HCl salt); ^{1}H NMR(free amine in $CDCl_3$) δ 8.18−8.26 (d, 2H), 7.43−7.46 (d, 2H), 7.22−7.33 (m, 5H), 5.30−5.33 (d, 1H), 3.73−3.86 (m, 1H), 3.61 (s, 2H), 3.47 (s, 2H), 2.75−2.79 (d, 2H), 2.05−2.14 (dt, 2H), 1.86−1.90 (m, 2H), 1.33−1.46 (dq, 2H). Anal. ($C_{20}H_{23}N_3O_3$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2-methoxyphenylacetamide (31):** yield 48%; mp 211−213 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 8.13−8.16 (d, 1H, CONH), 7.58−7.61 (m, 2H), 7.44−7.47 (m, 3H), 7.11−7.23 (m, 2H), 6.83−6.96 (m, 2H), 4.22−4.24 (d, 2H), 3.73−3.80 (m, 1H), 3.73 (s, 3H), 3.20−3.36 (m, 2H), 2.97−3.01 (m, 2H), 1.73−1.99 (m, 4H). Anal. ($C_{21}H_{26}N_2O_2$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-methoxyphenylacetamide (32):** yield 60%; mp 117−118 °C (free amine), 165−167 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.32 (m, 6H), 6.77−6.85 (m, 3H), 5.22−5.24 (d, 1H), 3.73−3.84 (m, 1H), 3.81 (s, 3H), 3.52 (s, 2H), 3.44 (s, 2H), 2.69−2.74 (d, 2H), 2.03−2.11 (dt, 2H), 1.81−1.91 (m, 2H), 1.23−1.37 (dq, 2H). Anal. ($C_{21}H_{26}N_2O_2$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-methoxyphenylacetamide (33):** yield 87%; mp 151−152 °C (free amine), 177−180 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.32 (m, 5H), 7.12−7.16 (d, 2H), 6.85−6.90 (d, 2H), 5.17−5.20 (d, 1H), 3.73−3.85 (m, 1H), 3.81 (s, 3H), 3.48 (s, 2H), 3.36 (s, 2H), 2.68−2.73 (d, 2H), 2.03−2.11 (dt, 2H), 1.81−1.84 (m, 2H), 1.23−1.36 (dq, 2H). Anal. ($C_{21}H_{26}N_2O_2$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2,5-dimethoxyphenylacetamide (34):** yield 71%; mp 113−114 °C (free amine), 187−190 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.32 (m, 5H), 6.75−6.80 (m, 3H), 5.68−5.71 (d, 1H), 3.72−3.83 (m, 1H), 3.79 (s, 3H), 3.76 (s, 3H), 3.49 (s, 2H), 3.45 (s, 2H), 2.67−2.72 (d, 2H), 2.05−2.12 (dt, 2H), 1.79−1.85 (m, 2H), 1.25−1.38 (dq, 2H). Anal. ($C_{22}H_{28}N_2O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,4-dimethoxyphenylacetamide (35):** yield 50%; mp 190−192 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 8.26−8.29 (d, 1H, CONH), 7.59−7.62 (m, 2H), 7.44−7.46 (m, 3H), 6.83−6.86 (m, 2H), 6.72−6.78 (m, 1H), 4.21−4.23 (d, 2H), 3.73−3.80 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.36−3.46 (m, 2H), 3.30 (s, 2H), 2.92−3.03 (m, 2H), 1.74−7.92 (m, 4H). Anal. ($C_{22}H_{28}N_2O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,5-dimethoxyphenylacetamide (36):** yield 56%; mp 129−130 °C (free amine), 185−187 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.20−7.32 (m, 5H), 6.38 (m, 3H), 5.29−5.32 (d, 1H), 3.72−3.86 (m, 1H), 3.78 (s ,6H), 3.48 (s, 2H), 3.44 (s, 2H), 2.71−2.74 (d, 2H), 2.03−2.12 (dt, 2H), 1.82−1.85 (m, 2H), 1.26−1.39 (dq, 2H). Anal. ($C_{22}H_{28}N_2O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,4,5-trimethoxyphenylacetamide (37):** yield 79%; mp 111−112 °C (free amine), 198−200 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.20−7.32 (m, 5H), 6.45 (s, 2H), 5.31−5.34 (d, 1H), 3.73−3.86 (m, 1H), 3.85 (s, 9H), 3.48 (s, 2H), 3.45 (s, 2H), 2.72−2.76 (d, 2H), 2.04−2.13 (dt, 2H), 1.74−1.84 (m, 2H), 1.29−1.42 (dq, 2H). Anal. ($C_{23}H_{30}N_2O_4$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,4-(methylenedioxy)phenylacetamide (38):** yield 30%; mp 144−145 °C (free amine), 210−212 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.20−7.32 (m, 5H), 6.76−6.79 (d, 12H), 6.71−6.72 (d, 1H), 6.65−6.68 (dd, 1H), 5.97 (s, 2H, OCH_2O), 5.22−5.25 (d, 1H), 3.73−3.83 (m, 1H), 3.45 (s, 4H), 2.71−2.75 (d, 2H), 2.04−2.11 (dt, 2H), 1.82−1.86 (m, 2H), 1.26−1.39 (dq, 2H). Anal. ($C_{21}H_{24}N_2O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-(methylthio)phenylacetamide (41):** yield 73%; mp 188−190 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 8.33−8.36 (d, 1H, CONH), 7.60−7.62 (m, 2H), 7.44−7.46 (m, 3H), 7.18−7.24 (m, 4H), 4.21−4.23 (d, 2H), 3.69−3.74 (m, 1H), 3.35 (s, 2H), 3.19−3.35 (m, 2H), 2.95−2.99 (m, 2H), 2.45 (s, 3H, SCH_3), 1.87−2.00 (m, 4H). Anal. ($C_{21}H_{26}N_2OS$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-aminophenylacetamide (43).** To a solution of 4-aminophenylacetic acid (4.54 g, 0.03 mol) in aqueous NaOH (0.2 N, 35 mL) was added di-*tert*-butyl dicarbonate (6.55 g, 0.03 mol). The mixture was stirred at room temperature overnight, adjusted to pH = 2 using 1 N HCl, extracted with ethyl acetate (3 × 30 mL), and dried over Na_2SO_4. After removal of the solvent, the product was recrystallized from ethanol/pentane to give 7.43 g (98.5%) of 4-[(*tert*-butoxycarbonyl)amino]phenylacetic acid: ^{1}H NMR ($CDCl_3$) δ 7.30−7.33 (d, 2H), 7.18−7.21 (d, 2H), 3.59 (s, 2H), 1.51 (s, 9H).

The 4-[(*tert*-butoxycarbonyl)amino]phenylacetic acid was condensed with 1-benzyl-4-aminopiperidine according to method A to give *N*-(1-benzylpiperidin-4-yl)-4-[(*tert*-butoxycarbonyl)-amino]phenylacetamide: ^{1}H NMR ($CDCl_3$) δ 7.33−7.36 (d, 2H), 7.21−7.29 (m, 5H), 7.13−7.16 (d, 2H), 6.53 (s, 1H, CONH-phenyl), 5.19−5.22 (d, 1H), 3.76−3.82 (m, 1H), 3.45 (s, 2H), 3.49 (s, 2H), 2.69−2.73 (d, 2H), 2.04−2.11 (dt, 2H), 1.80−1.83 (m, 2H), 1.53 (s, 9H), 1.28−1.42 (dq, 2H).

N-(1-Benzylpiperidin-4-yl)-4-[(*tert*-butoxycarbonyl)amino]-phenylacetamide (1.0 g) was treated with trifluoroacetic acid to give *N*-(1-benzylpiperidin-4-yl)-4-aminophenylacetamide (0.74 g, 97%): mp 171−172 °C (free amine), 230−232 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.21−7.32 (m, 5H), 6.98−7.01 (d, 2H), 6.64−6.67 (d, 2H)), 5.21−5.24 (d, 1H), 3.72−3.82 (m, 1H), 3.68 (s, 2H, NH_2), 3.44 (s, 2H), 3.43 (s, 2H), 2.68−2.73 (d, 2H), 2.03−2.11 (dt, 2H), 1.80−1.83 (m, 2H), 1.23−1.36 (dq, 2H). Anal. ($C_{20}H_{25}N_3O$·2HCl) C, H, N.

General Method B. Preparation of *N*-(1-Benzylpiperidin-4-yl)-2,4-dinitrophenylacetamide (25). To a solu-

tion of 2,4-dinitrophenylacetic acid (2.26 g, 10 mmol) in dry CH_2Cl_2 (50 mL) was added BOP (4.42 g, 10 mmol). 1-Benzyl-4-aminopiperidine (1.90 g, 10 mmol) was added with stirring. After 2 h of stirring at room temperature, the mixture was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$, washed with saturated aqueous NaCl, and dried over Na_2SO_4. The product was purified by silica gel column chromatography, eluting with $CHCl_3$−EtOH (9.5:0.5). Recrystallization from ethanol/pentane gave 0.98 g of free amine, mp 212−214 °C. The rest was converted into HCl salt by treatment with ethanol−HCl and recrystallized from ethanol/ethyl acetate to give 2.96 g (93% overall yield): mp 207−210 °C; ^{1}H NMR (free amine in DMSO-d_6) δ 8.50 (m, 1H), 8.36 (m, 1H), 7.81 (m, 1H), 7.49−7.52 (m, 5H), 4.26−4.28 (br, 2H), 4.07 (s, 2H), 3.71−3.85 (m, 1H), 2.95−3.16 (m, 2H), 1.88−1.99 (m, 4H), 1.55−1.67 (m, 2H). Anal. ($C_{20}H_{22}N_4O_5$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-2-hydroxyphenylacetamide (26):** yield 40%; mp 196−198 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 9.62 (s, 1H, OH), 8.28−8.31 (d, 1H, CONH), 7.58−7.62 (m, 2H), 7.41−7.47 (m, 3H), 7.00−7.08 (m, 2H), 6.68−6.82 (m, 2H), 4.22−4.24 (d, 2H), 3.72−3.77 (m, 1H), 2.94−3.04 (m, 2H), 1.78−1.99 (m, 4H), CH_2CO peak and the 2α-H_{eq} peaks overlapped with DMSO-d_6 peaks at around 3.36 ppm. Anal. ($C_{20}H_{24}N_2O_2$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-hydroxyphenylacetamide (27):** yield 57%; mp 218−220 °C (free amine), 212−214 °C (HCl salt); ^{1}H NMR (free amine in DMSO-d_6) δ 9.30 (s, 1H, OH), 8.16−8.18 (d, 1H, CONH), 7.48−7.51 (m, 5H), 7.03−7.11 (m, 1H), 6.59−6.67 (m, 3H), 4.26−4.28 (d, 2H), 3.68−3.75 (m, 1H), 3.28 (s, 2H), 2.99−3.10 (m, 2H), 1.87−1.99 (m, 2H), 1.51−1.63 (m, 2H). Anal. ($C_{20}H_{24}N_2O_2$·HCl·H_2O) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-4-hydroxyphenylacetamide (28):** yield 98%; mp 215−217 °C (free amine), 214−216 °C (HCl salt); ^{1}H NMR (free amine in DMSO-d_6) δ 9.23 (s, 1H, OH), 8.10−8.12 (d, 1H, CONH), 7.48−7.51 (m, 5H), 7.00−7.03 (d, 2H), 6.66−6.69 (d, 2H), 4.26−4.28 (d, 2H), 3.70−3.73 (m, 1H), 3.25 (s, 2H), 2.98−3.09 (m, 2H), 1.86−1.99 (m, 2H), 1.51−1.62 (m, 2H). Anal. ($C_{20}H_{24}N_2O_2$·HCl + $^1/_2H_2O$) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3,4-dihydroxyphenylacetamide (29):** yield 21%; mp 203−205 °C (HCl salt); ^{1}H NMR (HCl salt in DMSO-d_6) δ 10.84 (br, 1H), 8.79 (br, 1H), 8.17−8.20 (d, 1H), 7.58−7.60 (m, 2H), 7.44−7.46 (m; 3H), 6.60−6.63 (m, 2H), 6.44−6.50 (m, 1H), 4.22−4.24 (d, 2H), 3.68−3.72 (m, 1H), 3.17 (s, 2H), 2.92−3.64 (m, 2H), 1.71−1.92 (m, 4H). Anal. ($C_{20}H_{24}N_2O_3$·HCl) C, H, N.

***N*-(1-Benzylpiperidin-4-yl)-3-chloro-4-hydroxyphenylacetamide (30):** yield 99%; mp 85−87 °C (free amine); ^{1}H NMR (free amine in $CDCl_3$) δ 7.23−7.30 (m, 5H), 7.21−7.22 (m, 1H), 7.01−7.05 (m, 1H), 6.92−6.95 (m, 1H), 5.21−5.24 (d, 1H), 3.71−3.81 (m, 1H), 3.47 (s, 2H), 3.44 (s, 2H), 2.74−2.78 (d, 2H), 2.05−2.13 (dt, 2H), 1.84−1.87 (m, 2H), 1.28−1.42 (dq, 2H). Anal. ($C_{20}H_{23}N_2O_2Cl$) C, H, N.

General Method C. Preparation of 1-(2,4-Dimethoxyphenyl)-3-(1-benzylpiperidin-4-yl)urea (39). A mixture of 2,4-dimethoxyphenyl isocyanate (1.79 g, 10 mmol), 1-benzyl-4-aminopiperidine (1.90 g, 10 mmol), and dibutyltin diacetate (0.35 g, 1 mmol) in 40 mL of CH_2Cl_2 was stirred at room temperature for 60 min. The mixture was partitioned between CH_2Cl_2 and water, and the organic layer was washed with saturated aqueous NaCl and dried over Na_2SO_4. The solvent was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography using $CHCl_3$−EtOH (9.5:0.5) as the eluent. The product was recrystallized from ethanol/ethyl acetate to give 1.04 g of free amine, mp 155−156 °C. The rest was converted into HCl salt using ethanolic hydrogen chloride and recrystallized from ethanol−water to give 1.99 g (77% overall yield): mp 245−247 °C; ^{1}H NMR (free amine in $CDCl_3$) δ 7.63−7.66 (d, 1H), 7.21−7.34 (m, 5H), 6.43−6.47 (m, 2H), 6.26 (s, 1H, phenyl-NHCO), 4.45−4.48 (d, 1H, CONH-piperidinyl), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.63−3.73 (m, 1H), 3.48 (s, 2H, CH_2-phenyl), 2.77−2.81 (d, 2H, 2α-H_{eq} of piperidine), 2.08−2.17 (dt, 2H, 2α-H_{ax} of piperidine), 1.93−1.96 (dd, 2H, 2β-H_{eq} of piperidine), 1.36−1.49 (dq, 2H, 2β-H_{ax} of piperidine). Anal. ($C_{21}H_{27}N_3O_3$·HCl) C, H, N.

1-(4-Methylthiophenyl)-3-(1-benzylpiperidin-4-yl)-urea (42): yield 74%; mp 165−166 °C (free amine), 238−240 °C (HCl salt); ^{1}H NMR (free amine in $CDCl_3$) δ 7.18−7.34 (m, 9H), 6.07 (s, 1H, phenyl-NHCO), 4.58−4.61 (d, 1H, CONH-piperidinyl), 3.65−3.75 (m, 1H), 3.48 (s, 2H, CH_2-phenyl), 2.77−2.81 (d, 2H), 2.46 (s, 3H, SCH_3), 2.08−2.15 (dt, 2H), 1.92−1.95 (m, 2H), 1.36−1.49 (dq, 2H). Anal. ($C_{20}H_{25}N_3OS$·HCl) C, H, N.

Method D. Preparation of 1-Benzyl-4-[[2-hydroxy-2-(2,3-dimethoxyphenyl)ethyl]amino]piperidine (40). A suspension of 2,3-dimethoxybenzaldehyde (16.62 g, 0.1 mol), TMSCN (11.0 g, 0.11 mol), and ZnI_2 (0.01 g) in benzene (100 mL) was stirred at room temperature for 4 h. The mixture was concentrated in vacuo to give a residue which was dissolved in 100 mL of dry THF, to which was added $LiAlH_4$ (4.0 g, 0.12 mol) in THF (50 mL) dropwise. After 1 h of refluxing, H_2O (6 mL) and NaOH (15%, 6 mL) were added to consume the excess $LiAlH_4$. The solvent was removed in vacuo to yield a residue which was extracted with ether (3 × 30 mL), washed with aqueous NaCl, and dried over Na_2SO_4. After removal of the Na_2SO_4, HCl gas was added to the ether solution to give the HCl salt as a white solid, which was recrystallized from ethanol to give 7.66 g (33%): mp 156−158 °C; ^{1}H NMR (HCl salt in DMSO-d_6) δ 6.99−7.13 (m, 3H), 5.96−5.98 (d, 1H, OH), 5.08−5.12 (m, 1H, CH−OH), 3.08 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.90−2.94 (m, 2H, CH_2), 2.71−2.78 (t, 2H, NH_2).

The above HCl salt (2.34 g, 10 mmol) was added to *N*-benzylpiperid-4-one (1.89 g, 10 mol) in 30 mL of methanol. The mixture was stirred at room temperature for 2 h, $NaBH_3$-CN (0.63 g, 10 mol) was added into the solution, and the mixture was stirred at room temperature overnight. Solvent was removed in vacuo, and the residue was dissolved in 1 N HCl (30 mL) and extracted with ether (3 × 30 mL). The aqueous layer was basified with aqueous 2 N NaOH to pH >10 and then extracted with CH_2Cl_2 (3 × 30 mL), washed with saturated aqueous NaCl, and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was dissolved in ethanol (10 mL) and converted into HCl salt by treatment with HCl gas. Recrystallization from ethanol gave 3.32 g (82%): mp 224−226 °C; ^{1}H NMR (HCl salt in DMSO-d_6) δ 7.45−7.62 (m, 5H), 7.00−7.14 (m, 3H), 6.05 (br, 1H, OH), 5.21−5.24 (m, 1H, CHOH), 4.24−4.26 (m, 2H, CH_2NH), 3.80 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 2.93−2.96 (m, 4H), 2.24−2.30 (m, 2H), 2.03−2.15 (m, 2H), 2.36−2.56 (br). Anal. ($C_{21}H_{30}N_2O_3$·2HCl) C, H, N.

Pharmacology. Tissue Source and Radioligands. σ_1 binding sites were labeled with the σ_1-selective radioligand, [^{3}H]-(+)-pentazocine (DuPont-NEN, Billerica, MA) in guinea pig brain membranes (Rockland Biological, Gilbertsville, PA) according to published procedures.[4,41] σ_2 sites were assayed in rat liver membranes with [^{3}H]DTG (DuPont-NEN, Boston, MA) in the presence of (+)-pentazocine (100 nM) to mask σ_1 sites.[4,41]

Membrane Preparation. Crude synaptosomal (P_2) membrane homogenates were prepared from frozen guinea pig brains without cerebellum.[41,42] Brains were allowed to thaw slowly on ice before homogenization. Crude P_2 membranes were also prepared from the livers of male Sprague−Dawley rats (300−350 g). Animals were sacrificed by decapitation, and the livers were removed and minced before homogenization. Tissue homogenization was carried out at 4 °C in 10 mL/g tissue weight of 10 mM Tris-HCl/0.32 M sucrose, pH 7.4, using a Potter-Elvehjem tissue grinder. The crude homogenate was centrifuged for 10 min at 1000*g* and the supernatant saved on ice. The pellet was resuspended in 2 mL/g tissue weight of ice-cold 10 nM Tris-HCl/0.32 M sucrose, pH 7.4 by vortexing. After being centrifuged at 1000*g* for 10 min, the pellet was discarded and the supernatants were combined and centrifuged at 31000*g* for 15 min. The pellet was resuspended in 3 mL/g 10 mM Tris-HCl, pH 7.4, by vortexing, and the

suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the aliquots were stored at −80 °C until used. The protein concentration of the suspension was determined by the method of Bradford[43] and generally ranged from 6 to 11 mg of protein/mL.

σ_1 Binding Assay. Guinea pig brain membrane homogenates (100 μg of protein) were incubated with 3 nM [^{3}H]-(+)-pentazocine (31.6 Ci/mmol) in 50 mM Tris-HCl (pH 8.0) at 25 °C for either 120 or 240 min. Test compounds were dissolved in ethanol and then diluted in buffer for a total incubation volume of 0.5 mL. Test compounds were added in concentrations ranging from 0.005 to 1000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl (pH 8.0) followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL of ice cold buffer. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals, Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%.

σ_2 Binding Assay. Rat liver membrane homogenates (35 μg of protein) were incubated with 3 nM [^{3}H]DTG (38.3 Ci/mmol) in the presence of 100 nM (+)-pentazocine to block σ_1 sites. Incubations were carried out in 50 mM Tris-HCl (pH 8.0) for 120 min at 25 °C in a total incubation volume of 0.5 mL. Test compounds were added in concentrations ranging from 0.005 to 1000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl (pH 8.0) followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 5 μM DTG. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals, Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%.

Data Analysis. The IC_{50} values at σ sites were generally determined in triplicate from nonlinear regression of binding data as analyzed by JMP (SAS Institute; Cary, NC), using eight concentrations of each compound. K_i values were calculated using the method of Cheng−Prusoff[44] and represent mean values ±SEM. The K_d value used for [^{3}H]DTG in rat liver was 17.9 nM and was 4.8 nM for [^{3}H]-(+)-pentazocine in guinea pig brain.[4,41]

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References

(1) Martin, W. R.; Eades, C. E.; Thompson, J. A.; Happler, R. E.; Gilbert, P. E. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517−532.

(2) Zukin, S. R; Fitz-Syage, M. L.; Nichtenhauser, R.; Zukin, R. S. Specific binding of [^{3}H]phencyclidine in rat central nervous tissue: further characterization and technical considerations. *Brain Res.* **1983**, *258*, 277−284.

(3) Quirion, R.; Chicheportiche, R.; Contreras, P. C.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and nomenclature of phencyclidine and sigma receptor sites. *Trends Neurosci.* **1987**, *10*, 444−446.

(4) Walker, J. M.; Brown, W. D.; Walker, F. O.; Matsumoto, R. E.; De Costa, B.; Rice, K. R. Sigma receptors: biology and function. *Pharmacol. Rev.* **1990**, *42*, 355−402.

(5) Bowen, W. D.; Hellewell, S. B.; McGarry, K. A. Evidence for a multi-site mode of the rat brain σ receptor. *Eur. J. Pharmacol.* **1989**, *163*, 309−318.

(6) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, J. M. A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85−86.

(7) Itzhak, Y.; Khouri, M. Regulation of the binding of σ- and phencyclidine (PCP)-receptor ligands in rat brain membranes by guanidine nucleotide and ions. *Neurosci. Lett.* **1988**, *85*, 147−152.

(8) Beart, P. M.; O'Shea, R. D.; Mallack, D. T. Regulation of σ-receptors: high and low affinity agonist states, GTP shifts- and up-regulation by rimcazole and 1,3-di-(2-tolyl)guanidine. *J. Neurochem.* **1989**, *53*, 779−788.

(9) Connick, J. H.; Hanlon, G.; Roberts, J.; France, L.; Fox, P. K.; Nicholson, C. D. Multiple σ binding sites in guinea-pig and rat brain membranes: G-protein interactions. *Br. J. Pharmacol.* **1992**, *107*, 726−731.

(10) Hellewell, S. B.; Bowen, W. D. A sigma-like binding site in the rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor from that of quinea pig brain. *Brain Res.* **1990**, *527*, 244−253.

(11) Menkel, M.; Terry, P.; Pontecorvo, M.; Katz, J. L.; Witkins, J. M. Selective σ ligands block stimulant effects of cocaine. *Eur. J. Pharmacol.* **1991**, *201*, 251−252.

(12) Walker, J. M.; Bowen, W. D.; Patrick, S. L.; Williams, W. E.; Mascarella, S. W.; Bai, X.; Carroll, F. I. A comparison of (−)-deoxybenzomorphans devoid of opiate activity with their dextrarotatory phenolic counterparts suggests role of σ_2 receptors in motor function. *Eur. J. Pharmacol.* **1993**, *231*, 61−68.

(13) Mash, D. C.; Zabetian, C. Sigma receptors are associated with cortical limbic areas in the primate brain. *Synapse* **1992**, *12*, 195−205.

(14) Rukert, N. G. H.; Schmidt, W. J. The σ receptor ligand 1,3-di-(2-tolyl)guanidine in animal models of schizophrenia. *Eur. J. Pharmacol.* **1993**, *233*, 261−267.

(15) Kanzaki, A.; Okumura, K.; Ujike, H.; Tsuchida, K.; Akiyama, K.; Otsuki, S. BMY-14802 reverses the reduction of striatal dopamine release induced by (+)-3-[3-hydroxyphenyl]-N-(1-propyl)piperidine. *J. Neural. Transm.* **1992**, *90*, 137−144.

(16) Weiser, S. D.; Patrick S. L.; Mascarella, S. W.; Downing-Park, J.; Bai, X.; Carroll, F. I.; Walker, J. M.; Patrick, R. L. Stimulation of rat striatal tyrosine hydroxylase activity following intranigral administration of σ receptor ligands. *Eur. J. Pharmacol.* **1995**, *275*, 1−7.

(17) Gonzalez-Alvear, G. M.; Werling, L. L. Regulation of [^{3}H]-dopamine release from rat striatal slices by sigma receptor ligands. *J. Pharmacol Imp. Ther.* **1994**, *271*, 212−219.

(18) Matsuno, K.; Senda, T.; Kobayashi, T.; Tita, S. Involvament of σ_1 receptor in (+)-N-allylnormetazocine stimulated hippocampal cholinergic functions in rats. *Brain Res.* **1995**, *690*, 200−206.

(19) Gonzalez-Alvear, G. M.; Werling, L. L. Sigma receptor regulation of norepinephrine release from rat hippocampal slices. *Brain Res.* **1995**, *673*, 61−69.

(20) Chien, C. C.; Pasternak, G. W. Selective antagonism of opioid analgesia by a sigma system. *J. Pharmacol. Imp. Ther.* **1994**, *271*, 1583−1590.

(21) Chien, C. C.; Pasternak, G. W. Functional antagonism of morphine analgesia by (+)-pentazocine: evidence for an anti-opioid σ_1 system. *Eur. J. Pharmacol.* **1993**, *250*, R7−R8.

(22) Chien, C. C.; Pasternak, G. W. Sigma antagonists potentiate opioid analgesia in rats. *Neurosci. Lett.* **1995**, *190*, 137−139.

(23) Liu, Y.; Whitlock B. B.; Pultz, J. A.; Wolfe, S. A., Jr. Sigma-1 receptors modulate functional activity of rat spleocytes. *J. Neuroimmunol.* **1995**, *59*, 143−154.

(24) Largent, B. L.; Wikstrom, H.; Snowman, A. M.; Synder, S. H. Novel antipsychotic drugs share high affinity for σ receptors. *Eur. J. Pharmacol.* **1988**, *155*, 345−347.

(25) Snyder, S. H.; Largent, B. L. Receptor mechanism in antipsychotic drug action: focus on sigma receptors. *J. Neuropsych Clin. Neurosci.* **1989**, *1*, 7−15.

(26) Goldstein, S. R.; Matsumoto, R. R.; Thompson, T. L.; Patrick, R. L.; Bowen, W. D.; Walker, J. M. Motor effects of two sigma ligands mediated by nigrostriatal dopamine neurons. *Synapse* **1989**, *4*, 254−258.

(27) Thomas, G. E.; Szucs, M.; Mammone, J. Y.; Bem, W. T.; Rush, M. D.; Johnson, F. E.; Coscia, C. T. Sigma and opioid receptors in human brain tumors. *Life Sci.* **1990**, *46*, 1279−1286.

(28) Bem, W. T.; Thomas, G. E.; Mamone, J. Y.; Homan, S. M.; Levy, B. K.; Johnson, F. E.; Coscia, C. J. Overexpression of σ receptors in nonneural human tumors. *Cancer Res.* **1991**, *51*, 6558−6562.

(29) Vilner, B. J.; John, C. S.; Bowen, W. D. Sigma-1 and sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines. *Cancer Res.* **1995**, *55*, 408−413.

(30) Mach, R. H.; Smith, C. R.; Al-Nabulsi, I.; Whirrett, B. R.; Childers, S. R.; Wheeler, K. T. σ_2 receptors as potential biomarkers of proliferation in breast cancer. *Cancer Res.* **1997**, *57*, 156−161.

(31) John, C. S.; Bowen, W. D.; Varma, V. M.; McAfee, J. G.; Moody, T. W. Sigma receptors are expressed in human non-small cell lung carcima. *Life Sci.* **1995**, *56*, 2385−2392.

(32) Vilner, B. J.; DeCosta, B. R.; Bowen, W. D. Cytotoxic effects of sigma ligands: sigma receptor-mediated alterations in cellular morphology and viability. *J. Neurosci.* **1995**, *15*, 117−134.
(33) Brent, P. J.; Pang, G. T. σ binding site ligands inhibit cell proliferation in mammary and colon carcinoma cell lines and melanoma cells in culture. *Eur. J. Pharmacol.* **1995**, *278*, 151−160.
(34) John, C. S.; Vilner, G. J.; Gulden, M. E.; Efange, S. M. N.; Langason, R. B.; Moody, T. W.; Bowen, W. D. Synthesis and pharmacological characterization of 4-[^{125}I]-N-(N-benzylpiperidin-4-yl)-4-iodobenzamide: a high affinity σ receptor ligand for potential imaging of breast cancer. *Cancer Res.* **1995**, *55*, 3022−3027.
(35) Ye, Y.; Huang, Y.; Wang, Z.; Chen, S.; Tia, Y. Synthesis of new amino acid and peptide derivatives of estradiol and their binding affinities for the estrogen receptor. *Steroids* **1993**, *58*, 35−39.
(36) The SAS System, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513-2414.
(37) Hansch, C.; Leo, A.; Hoekman, D. In *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*; Heller, S. R., Ed.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1995.
(38) Hansch, C.; Leo, A. *In Exploring QSAR: Fundamentals and Applications in Chemistry and Biology*; Heller, S. R., Ed.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1995.
(39) Mach, R. H.; Nader, M. A.; Ehrenkaufer, R. L. E.; Line, S. W.; Smith, C. R.; Luedtke, R. R.; Kung, M.-P.; Kung, H. F.; Lyons, D.; Morton, T. E. Comparison of two fluorine-18 labeled benzamide derivatives that bind reversibly to dopamine D_2 receptors: in vitro binding studies and positron emission tomography. *Synapse* **1996**, *24*, 322−333.
(40) Carroll, F. I.; Abraham, P.; Parham, K.; Bai, X.; Zhang, X.; Brine, G. A.; Mascarella, S. W.; Martin, B. R.; May, F. L.; Sauss, C.; Paolo, D.; Wallace, P.; Walker, J. M.; Bowen, W. D. Enantiomeric N-substituted N-Normetazocines: A comparative study of affinities at σ, PCP, and μ opioid receptors. *J. Med Chem.* **1992**, *35*, 2812−2818.
(41) Hellewell, S. B.; Bruce, A.; Feinstein, G.; Orringer, J.; Williams, W.; Bowen, W. D. Rat liver and kidney contain high densities of σ_1 and σ_2 receptors: characterization by ligand binding and photoaffinity labeling. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1994**, *268*, 9−18.
(42) Mach, R. H.; Smith, C. R.; Childers, S. R. Ibogaine possesses a selective affinity for σ_2 receptors. *Life Sci.* **1995**, *57*, PL 57−62.
(43) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(44) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which cause 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−4022.
(45) Hogberg, T.; Norinder, U. Quantitative structure-activity relationships and experimental design. In *A Textbook of Drug Design and Development*; Krogsgaard-Larsen, P., Liljefors, T., Madsen, U., Eds.; Harwood Academic Publishers: Amsterdam, 1996; pp 94−130.

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