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

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A series of *N*-(1-benzylpiperidin-4-yl)arylacetamides were synthesized and evaluated for their binding properties for σ_1 and σ_2 receptors. In agreement with previously reported σ_1/σ_2 receptor binding data for *N*-(1-benzylpiperidin-4-yl)phenylacetamide, all of the *N*-(1-benzylpiperidin-4-yl)arylacetamide compounds reported below displayed higher affinity for σ_1 vs σ_2 receptors. Replacement of the phenyl ring of the phenylacetamide moiety with a thiophene, naphthyl, or indole aromatic ring had no significant effect on the σ_1 receptor affinity. Replacement of the phenyl ring with an imidazole or pyridyl aromatic ring resulted in a >60-fold loss in affinity for σ_1 receptors and no significant binding affinity for σ_2 receptors. Substitution on the aromatic ring of the benzyl group showed a similar or slightly decreased affinity for σ_1 receptors. Substitution on the aromatic rings of both the phenylacetamide moiety and the benzyl group with a halogen resulted in a similar affinity for σ_1 receptors and a significantly increased affinity for σ_2 receptors. Comparative molecular field analysis revealed that electrostatic properties of the substituents in the phenylacetamide aromatic ring strongly influenced binding to σ_1 receptors. Compounds **1**, **10**, **18**, **22**, **37**, and **40** showed the highest selectivity for σ_1 receptors with K_i (σ_2) to K_i (σ_1) ratios of 100, >92, >122, 77, 74, and 80, respectively. In agreement with previously reported results, the phenylacetamide analogues had no binding affinity for dopamine receptors (D_2/D_3).

Introduction

σ receptors are present in high density in both the central nervous system and many peripheral organs.[1] The classification of sigma receptors into two distinct subtypes, designated σ_1 and σ_2,[1−3] has prompted interest in developing selective ligands for investigating the functional differences between σ_1 and σ_2 receptors. Over the past several years, tremendous effort has been focused on studying the physiological functions of σ_1 and σ_2 receptors, but the endogenous ligand for these receptors is still unidentified. The σ_1 receptor has been cloned and displays a 30% sequence homology with the enzyme, yeast sterol isomerase. These data, in concert with the observation that progesterone possesses a modest affinity for σ_1 receptors,[1,2] suggest that this subtype of the sigma receptor may play a role in steroid biochemistry. The σ_2 receptor has not been cloned, but evidence suggests that it is linked to potassium channels in NCB-20 cells.[1,2]

Recent evidence has indicated that σ_1 receptors may be involved in regulating a variety of neurotransmitters in the central nervous system, including cholinergic,[4,5] dopaminergic,[6,7] and glutamatergic systems.[8,9] σ_1 receptor agonists have been shown to elevate extracellular acetylcholine levels in rat frontal cortex without affecting striatal acetylcholine levels.[10,12] The ability of these compounds to increase the extracellular levels of acetylcholine in rat frontal cortex was positively correlated with their binding affinities for σ_1 receptors, and this facilitated release was fully reversed by σ_1 receptor antagonists.[11] Stimulated dopamine release by NMDA (*N*-methyl-D-aspartate) was inhibited by σ receptor agonists in a concentration-dependent manner, while this inhibition was reversed by σ_1 receptor antagonists.[7,13] Various σ_1 agonists were also shown to potentiate NMDA in a dose-dependent manner, and this effect was also reversed by selective σ_1 receptor antagonists.[8,14] σ receptor antagonists have also been shown to block the development of sensitization to cocaine and other psychostimulants.[15,16] These data suggest that σ_1 receptor antagonists may prevent schizophrenics from deteriorating or becoming susceptible to relapses.[16] Clinical studies using the σ ligand panamesine in patients with schizophrenia indicated improvement in psychometric variables and no extrapyramidal and other side effects were observed.[17]

We have previously reported the *N*-(1-benzylpiperidin-4-yl)phenylacetamide (**BPP**; Chart 1) as a σ receptor ligand with high affinity for σ_1 binding sites and relatively low affinity for σ_2 binding sites.[18] A structure−activity relationship (SAR) study of more than 40 derivatives of **BPP** was conducted with variable substitutions on the aromatic ring of the phenylacetamide moiety. Substitution with fluoro at the ortho-position to afford the *N*-(1-benzylpiperidin-4-yl)-2-fluorophenylacetamide gave the highest selectivity for σ_1 sites over σ_2 sites, while substitution with bromo at the meta-position to afford the *N*-(1-benzylpiperidin-4-yl)-3-bro-

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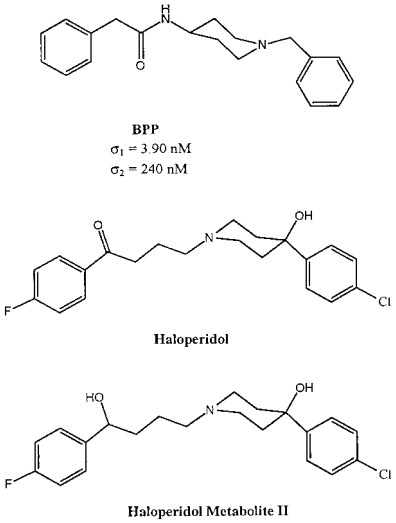
Chart 1



mophenylacetamide (**BBP**) had the highest affinity for both σ_1 and σ_2 binding sites.[18] These compounds exhibited no detectable binding affinity for dopamine D_2 and D_3 receptors, which distinguish these analogues from benzamides and some other σ ligands that showed high affinity for both σ and dopamine receptors.[19]

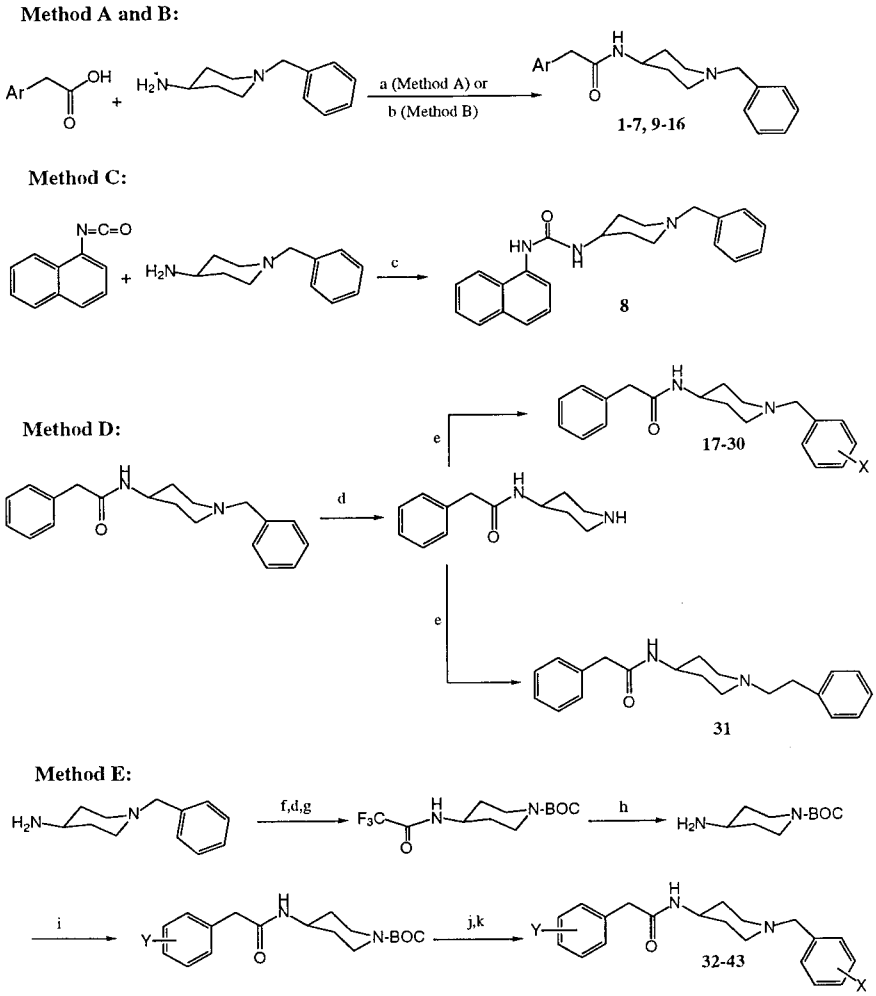
As a continued effort to further characterize the SARs within this class of compounds,[18] we report herein the preparation and in vitro evaluation of a series of *N*-(1-benzylpiperidin-4-yl)arylacetamide analogues as selective σ_1 ligands. In this study, SAR studies were conducted on three different regions of the template compound, **BPP**, by (a) replacing the phenyl ring of the phenylacetamide moiety with other aromatic rings, such as thiophene, pyridyl, naphthyl, etc. (**1**−**15**); (b) substituting the aromatic ring of the benzyl group with different substituents (**17**−**30**); and (c) substituting either a fluorine or an iodine on the aromatic ring of the benzyl group (**32**−**43**) to find suitable compounds for the development of ^{125}I- and ^{18}F-labeled imaging agents to be used with the functional imaging techniques, positron emission tomography (PET), and single photon emission computed tomography (SPECT). The in vitro binding affinities for σ_1 and σ_2 receptors were measured for all compounds, and selected compounds were also tested for their binding to dopamine D_2 and D_3 receptors. Also reported is a comparative molecular field analysis (CoMFA) study on 79 compounds from a combined trial set taken from this and our previous SAR study. The CoMFA model developed provides new insight into both steric and electrostatic influences that affect binding in both aromatic regions of **BPP** and its structural congeners.

Results

Chemistry. The desired compounds were prepared according to the reactions outlined in Schemes 1 and 2. Various *N*-(1-benzylpiperidin-4-yl)arylacetamides were prepared directly by a simple coupling reaction using either DCC (1,3-dicyclohexylcarbodiimide) (method A) or BOP (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) (method B).[20,21] Compound **8** was obtained by reacting the corresponding isocyanate with 1-benzyl-4-aminopiperidine in the presence of a catalytic amount of dibutyltin diacetate (method C). Compounds with substitution on the benzyl group were prepared from **BPP** by hydrogenation over a palladium hydroxide catalyst in methanol to give the corresponding secondary amine (method D). The amine was then alkylated with different substituted benzyl halides to afford the corresponding analogues, **17**−**31**. The disubstituted compounds with halogens on both aromatic rings (**32**−**43**) were prepared by the selective protection and deprotection of the amino groups of 4-aminopiperidine (method E). Thus, 1-benzyl-4-aminopiperidine was treated with trifluoroacetic anhydride to give *N*-(1-benzylpiperidin-4-yl)trifluoroacetamide, followed by hydrogenation with palladium hydroxide as the catalyst to give the debenzylated amine. The debenzylated amine was protected with a *tert*-butoxycarbonyl (BOC) group by reacting with di-*tert*-butyl-di-carbonate. The bis-protected aminopiperidine was reacted with ammonium hydroxide to remove the trifluoroacetyl group to generate 1-BOC-4-aminopiperidine, which was then condensed with different substituted phenylacetic acids to afford the *N*-(1-BOC-piperidin-4-yl)phenylacetamides. Deprotection of the BOC group gave the corresponding amine, which was then alkylated with different substituted benzyl halides to afford the desired analogues. 4-Iodobenzyl chloride, which was used to synthesize compounds **22**, **37**, **40** and **43**, was prepared from 4-bromobenzyl alcohol in three steps (Scheme 2).

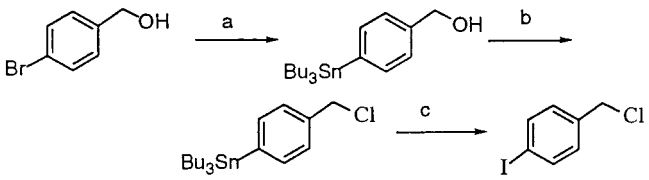
In Vitro Binding. Examination of the σ receptor affinities of those compounds with the phenylacetyl moiety being replaced by various types of arylacetyl moieties (**1**−**15**) reveals that lipophilicity of the aromatic moiety has a great impact on σ receptor binding affinity (Table 1). Compounds **1** and **2** had a thiophene ring replacement of the phenyl ring as compared to **BPP**. Both maintained high σ_1 receptor affinities and relatively low σ_2 receptor affinities. The 2-substituted thiophene, **1**, exhibited higher σ_1 to σ_2 selectivity (100-fold) than did both the **BPP** (62-fold) and the 3-substituted thiophene, **2** (47-fold). Compounds **3**−**6** have one basic nitrogen in the aryl aromatic ring and are more hydrophilic than compounds **1** and **2** and **BPP**. Their K_i values indicated that they had much lower affinities for σ_1 receptors as compared to **BPP** and no significant affinity for σ_2 receptors (K_i > 1000). It is possible that compounds **3**−**6** may be protonated in the binding environment of the σ receptors, which may generate an unfavorable interaction with the protein and result in a decreased binding affinity. Compounds **7**−**9** have a naphthyl group replacement of the phenyl ring. The 1-naphthylacetyl compound, **7**, had the highest σ_1 receptor affinity among the three naphthyl analogues, with a σ_1 receptor affinity that is comparable to that of **BPP**. The 2-naphthylacetyl compound, **9**, had a 13-fold decrease in σ_1 receptor affinity as compared to compound **7**. The urea analogue, **8**, displayed a moderate affinity for σ_1 and σ_2 receptors, which was in agreement with our previous results of the *N*-(1-benzylpiperidin-4-yl)-4-methylthiophenylacetamide and 1-(4-methylthiophenyl)-3-(1-benzylpiperidin-4-yl)urea.[18] All three compounds, **7**−**9**, had higher σ_2 receptor affinities as compared to **BPP**. Compounds **10**−**13** have an indole (**10**−**12**) or an indanone moiety (**13**) in place of the

Scheme 1[a]



[a]Reagents: (a) DCC/THF; (b) BOP/CH_2Cl_2; (c) dibutyltin diacetate/CH_2Cl_2; (d) H_2/Pd−C/MeOH; (e) $Br(CH_2)_nAr/CH_2Cl_2$; (f) $(CF_3CO)_2O/CH_2Cl_2$; (g) $(BOC)_2O/CH_2Cl_2$; (h) NH_4OH/MeOH; (i) $YC_6H_4CH_2COOH$/DCC/CH_2Cl_2; (j) CF_3COOH/CH_2Cl_2; (k) $ZCH_2C_6H_4X$ (Z = Br, Cl).

Scheme 2[a]

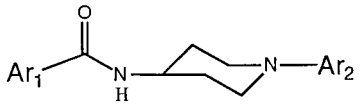


[a]Reagents: (a) BuLi/THF, Bu_3SnCl; (b) Ph_3P/CCl_4; (c) I_2/CH_2Cl_2.

phenyl ring. These compounds displayed a minor to significant decrease in σ_1 receptor affinity, with K_i values in the rank order of **10** < **11** < **12** < **13**. Compound **10** had a high σ_1 receptor affinity and possessed a σ_1:σ_2 selectivity ratio of >92. Substitution with a bromo (**11**) or methoxy (**12**) group decreased σ_1 receptor affinity, and replacement with an indanone ring (**13**) further reduced σ_1 receptor affinity. Compound **14**, which contained a 7-hydroxycoumarin ring, had no measurable affinity for σ_1 and σ_2 receptors. Compound **15**, which had a 2-pyrimidylthio moiety replacement of the phenyl ring, showed a significant decrease in σ_1 receptor affinity and no σ_2 receptor affinity.

Substitution on the phenyl ring of the benzyl group also showed some impact on σ_1 and σ_2 receptor affinity. Compounds **17**−**19** contained a 2-, 3-, and 4-fluoro-substituted benzyl group, respectively. Their σ_1 receptor affinities were similar to that of **BPP**, but their σ_2 receptor affinities were either decreased with ortho (**17**) and meta (**18**) substitution or increased with para substitution (**19**) when compared to that of **BPP**. The 3-fluoro-substituted analogue, **18**, showed no significant σ_2 receptor affinity and had the highest σ_1:σ_2 selectivity ratio (>122). The iodo-substituted compounds, **20**−**22**, displayed a slightly different pattern of binding to σ receptors. The 2-iodo-substituted analogue, **20**, had a low affinity for σ_1 receptors, whereas both the 3-iodo- and the 4-iodo-substituted analogues maintained a σ_1 receptor affinity that is comparable to that of **BPP**. The σ_2 receptor affinities of 2-iodo- and 4-iodo-substituted analogues were decreased as compared to that of **BPP**, while the σ_2 receptor affinity of the 3-iodo-substituted analogue was similar to that of **BPP**. As a result, the 4-iodo-substituted analogue **22** had the highest σ_1 to σ_2 selectivity (σ_1:σ_2 ratio = 77) among the three compounds. Substitution with a trifluoromethyl group (**23**−**25**) resulted in a significant reduction in σ_1 receptor affinity and a slight to significant reduction of σ_2 receptor affinity. The 4-nitro-substituted analogue (**26**) had a 5-fold lower σ_1 receptor affinity than **BBP**, whereas the σ_2 receptor affinity was only slightly reduced. All three 3,4-disubstituted analogues (**27**−**29**) maintained similar σ_1 and σ_2 receptor affinities to that of **BPP**. Replacement of the benzyl group with a

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



			K_i(nM)[a]		
no.	Ar_1	Ar_2	σ_1[b]	σ_2[c]	σ_2/σ_1[d]
1	2-thiopheneacetyl	benzyl	3.93 ± 0.80	392.82 ± 10.76	100
2	3-thiopheneacetyl	benzyl	7.20 ± 0.19	336.74 ± 50.65	47
3	4-imidazoleacetyl	benzyl	248.36 ± 2.42	>1000	>4
4	2-pyridylacetyl	benzyl	235.50 ± 3.06	>1000	>4
5	3-pyridylacetyl	benzyl	269.15 ± 4.33	>1000	>4
6	4-pyridylacetyl	benzyl	298.47 ± 1.45	>1000	>3
7	1-naphthylacetyl	benzyl	4.64 ± 1.01	39.44 ± 3.81	9
8	1-naphthylcarbamoyl	benzyl	22.66 ± 1.13	25.02 ± 1.85	1
9	2-naphthylacetyl	benzyl	61.77 ± 2.14	111.57 ± 7.52	2
10	3-indoleacetyl	benzyl	10.90 ± 0.02	>1000	>92
11	5-bromo-3-indoleacetyl	benzyl	51.94 ± 3.87	678.28 ± 3.42	13
12	5-methoxy-3-indoleacetyl	benzyl	125.41 ± 4.04	>1000	>8
13	5-methoxy-1-indanone-3-acetyl	benzyl	298.56 ± 2.13	416.41 ± 5.31	1
14	7-hydroxycoumarin-4-acetyl	benzyl	>1000	>1000	0
15	(2-pyrimidylthio)acetyl	benzyl	149.84 ± 10.17	>1000	>7
16	4-iodophenylacetyl	benzyl	13.82 ± 3.43	61.31 ± 1.70	4
17	phenylacetyl	2-fluorobenzyl	10.16 ± 1.92	682.70 ± 11.96	64
18	phenylacetyl	3-fluorobenzyl	8.22 ± 0.05	>1000	>122
19	phenylacetyl	4-fluorobenzyl	6.59 ± 0.89	82.89 ± 1.35	13
20	phenylacetyl	2-iodobenzyl	346.90 ± 4.66	631.00 ± 8.34	2
21	phenylacetyl	3-iodobenzyl	6.75 ± 0.32	191.05 ± 3.11	28
22	phenylacetyl	4-iodobenzyl	9.03 ± 0.34	693.54 ± 58.16	77
23	phenylacetyl	2-trifluoromethylbenzyl	>1000	>1000	0
24	phenylacetyl	3-trifluoromethylbenzyl	141.36 ± 5.78	513.16 ± 5.68	4
25	phenylacetyl	4-trifluoromethylbenzyl	87.05 ± 3.50	300.41 ± 3.64	3
26	phenylacetyl	4-nitrobenzyl	19.07 ± 0.22	319.07 ± 13.78	17
27	phenylacetyl	3,4-dichlorobenzyl	7.24 ± 1.04	423.17 ± 4.11	58
28	phenylacetyl	3,4-difluorobenzyl	6.30 ± 0.01	231.61 ± 29.36	37
29	phenylacetyl	3,4-methylenedioxybenzyl	3.97 ± 0.07	171.40 ± 15.68	43
30	phenylacetyl	2-naphthylmethyl	23.88 ± 1.70	189.47 ± 8.82	8
31	phenylacetyl	phenylethyl	31.25 ± 2.04	106.87 ± 4.82	3
32	2-fluorophenylacetyl	4-fluorobenzyl	3.15 ± 0.05	139.51 ± 21.89	44
33	2-fluorophenylacetyl	3-iodobenzyl	15.41 ± 0.37	181.30 ± 15.40	12
34	2-fluorophenylacetyl	4-iodobenzyl	4.20 ± 0.84	232.53 ± 23.54	55
35	3-fluorophenylacetyl	4-fluorobenzyl	5.51 ± 0.61	31.02 ± 0.18	6
36	3-fluorophenylacetyl	3-iodobenzyl	4.29 ± 1.16	50.26 ± 3.24	12
37	3-fluorophenylacetyl	4-iodobenzyl	2.25 ± 0.60	167.63 ± 15.09	74
38	3-chlorophenylacetyl	4-fluorobenzyl	1.15 ± 0.22	9.38 ± 0.27	8
39	3-chlorophenylacetyl	3-iodobenzyl	2.31 ± 0.46	30.34 ± 5.34	13
40	3-chlorophenylacetyl	4-iodobenzyl	3.21 ± 0.14	256.62 ± 19.04	80
41	3-bromophenylacetyl	4-fluorobenzyl	1.21 ± 0.16	6.06 ± 1.50	5
42	3-bromophenylacetyl	3-iodobenzyl	3.49 ± 0.35	59.32 ± 2.32	17
43	3-bromophenylacetyl	4-iodobenzyl	1.78 ± 0.20	90.31 ± 6.79	51
BBP	phenylacetyl	benzyl	3.90 ± 0.82	240 ± 30	62
	haloperidol		2.53 ± 0.25	28.52 ± 1.64	11
	haloperidol metabolite II		14.34 ± 0.08	28.55 ± 4.17	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 guinea 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_1 receptor.

2-naphthylmethyl (**30**) or phenethyl (**31**) group resulted in 6−8-fold loss in σ_1 receptor affinity and a similar or slightly reduced σ_2 receptor affinity.

CoMFA Model. The initial analysis for the full set of 79 compounds gave a moderate crossvalidated r_{cv}^2 value (q^2) of 0.47 using six components. Examination of residuals showed that three compounds, i.e., *N*-(1-benzylpiperidin-4-yl)-2-methoxyphenylacetamide, *N*-(1-benzylpiperidin-4-yl)-3-hydroxyphenylacetamide, and *N*-(1-benzylpiperidin-4-yl)-3-pyridylacetamide, **5**, were not well fit by the model. Dropping these three compounds markedly improved the analysis. Analysis of the remaining set of 76 compounds gave a satisfactory crossvalidated r_{cv}^2 value (q^2) of 0.64 with seven components recommended and a standard error of prediction (SEOP) of 0.49. Examination of the crossvalidated r_{cv}^2 values (q^2) and the SEOPs indicated that the optimum number of components was six since addition of a seventh component increased the final non-crossvalidated r^2 value by less than 5%. The final model (six components) showed a crossvalidated $q^2 = 0.61$ (SEOP = 0.50), with a final non-crossvalidated $r^2 = 0.91$. CoMFA analyses of the σ_1 binding data (log $1/K_i$) are

Table 2. Summary of Results for CoMFA on 76 Compound Set for σ_1 Binding Data

q^2	N_{pc}	SEOP	r^2	SEE	F	steric	electrostatic
0.61	6	0.50	0.91	0.24	116	0.54	0.46

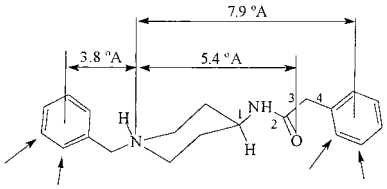


Figure 1. Rotatable bonds used in conformational searches, substituent orientations, and critical distances in **BPP** and related compounds.

summarized in Table 2. Analyses were also carried out using single-point AM1 charges rather than those calculated by the Gasteiger−Hückel method but showed no improvement in the model.

Similar CoMFA analyses of affinity data for the σ_2 binding data gave only low crossvalidated r_{cv}^2 (q^2) values and indicated that further work will be needed to derive a predictive model for those binding data.

Figure 2a,b shows the steric and electrostatic contour plots, respectively, for the final σ_1 CoMFA model. Figure 3 shows a plot of the predicted vs actual pK_i values, spanning three orders of magnitude, for the σ_1 binding affinities of the 76 phenylacetamides used in this model. In general, binding affinities were well fit, and the CoMFA model should be of use in the prediction of binding affinities for new compounds.

Discussion

Our interest in σ ligands was to discover compounds having a high affinity and selectivity for both subtypes of the sigma receptors, σ_1 and σ_2 receptors. A second goal was to develop suitable ligands as SPECT or PET radiotracers for functional imaging studies of these receptors. Our previous results had shown that the **BBP** displayed a high affinity for both σ_1 and σ_2 receptors, while the *N*-(1-benzylpiperidin-4-yl)-2-fluorophenylacetamide had the highest selectivity for σ_1 receptors in that series.[18] The 4-fluorobenzyl, 3-iodobenzyl, and 4-iodobenzyl are ideal substitutions for the development of ^{18}F- and $^{123/125}$I-labeled radiotracers. Therefore, a number of analogues were prepared containing either the 4-fluoro-substituted benzyl group (**32**, **35**, **38**, and **41**), the 3-iodo-substituted benzyl group (**33**, **36**, **39**, and **42**), or the 4-iodo-substituted benzyl group (**34**, **37**, **40**, and **43**). In addition to the 2-fluorophenylacetyl and 3-bromophenylacetyl analogues, the 3-fluorophenylacetyl (**35**−**37**) and 3-chlorophenylacetyl (**38**−**40**) analogues were prepared for comparison. The results of in vitro binding studies showed that compounds **32**−**43** maintained an affinity for the σ_1 receptor that is similar to that of the corresponding *N*-benzyl phenylacetamide.[18] However, the σ_2 receptor affinities were all increased as compared to that of the corresponding *N*-benzyl phenylacetamide, resulting in a lower selectivity for σ_1 vs σ_2 receptors. Compounds **38** and **41** had very high affinities for both σ_1 and σ_2 receptors, whereas **32** had a high affinity and high selectivity for σ_1 vs σ_2 receptors. Compounds **22**, **34**, **37**, **40**, and **43**, which contain a 4-iodobenzyl group, all showed high affinity and selectivity for σ_1 receptors. These compounds can be labeled with iodine-125 and used in in vitro and in vivo studies of the σ_1 receptor. Similarly, **32** can be labeled with fluorine-18 and be used in PET imaging studies of the σ_1 receptor. These compounds are currently under evaluation, and the results will be reported elsewhere.[30,31]

Only one CoMFA study of σ_1 ligands has been published to date.[33] This study utilized compounds with limited substitutions on the aromatic ring or rings. Examining a model based on a more diverse set of compounds with greater substitution in both the primary and the secondary hydrophobic sites should be valuable for identifying the binding requirements of σ_1 and σ_2 receptors. Initially, conformational search studies were carried out to identify low energy conformations as starting points for CoMFA studies. The conformation chosen for evaluation for parent compound **BPP** possessed similar features to the receptor features proposed originally by Glennon.[34] As shown in Figure 1, the distance from the piperidinyl−nitrogen to the centroid of the phenylacetamide aromatic ring was 7.9 Å (as compared to the optimal 8.3 Å proposed by Glennon) and represents the distance to the proposed primary hydrophobic site. The distance from the nitrogen to the centroid of the benzylic aromatic was 3.8 Å and is in good agreement with the distance to the secondary hydrophobic binding site proposed by Glennon (2.5−3.9 Å range). One further feature that may be derived from

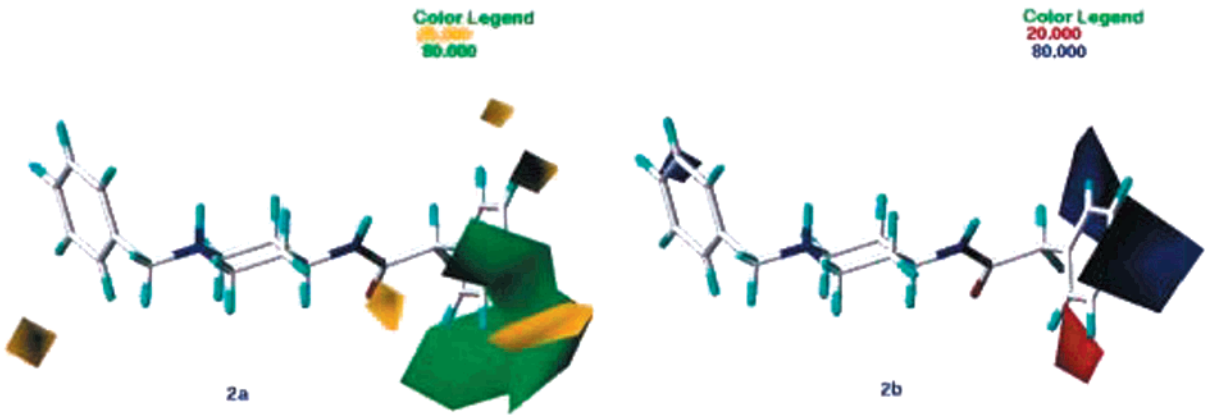


Figure 2. Steric and electrostatic contour plots for the final non-crossvalidated CoMFA model overlaid on the parent compound **BPP**. (a) Regions color-coded green correspond to areas where increased steric bulk is predicted to lead to greater affinity at the σ_1 receptor, while yellow regions represent areas where increased steric bulk should lead to decreased affinity. (b) Blue regions correspond to areas where an increase in positive charge should lead to greater affinity at the σ_1 receptor, while red regions correspond to areas where an increase in negative charge should lead to greater affinity.

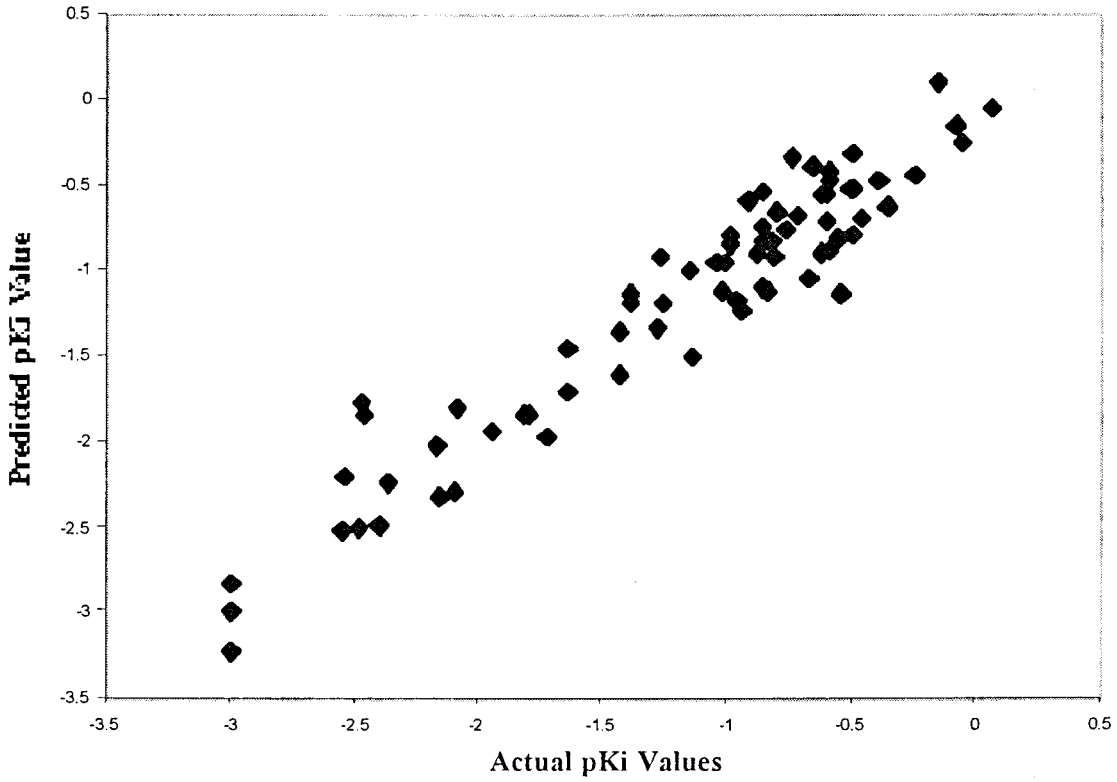


Figure 3. Plot of actual vs predicted pK_i values for σ_1 receptor binding data based on final CoMFA model.

the phenylacetamides is the distance to the proposed hydrogen bonding center, as represented by the distance from the cationic nitrogen to the carbonyl oxygen of 5.4 Å.

Figure 2 shows regions, overlaid on the parent compound **BPP**, where either steric or electrostatic features are expected to have a marked influence on binding affinity to the σ_1 receptor. In general, Figure 2 shows that substitutions on the benzylic ring appear to contribute much less than comparable substitutions on the phenylacetamide aromatic ring. Examining Figure 2a, the model predicts that steric bulk at either the 3-position or the 4-position on the phenylacetamide aromatic ring will be well-tolerated and will lead to increased affinity at the σ_1 receptor. However, note that a region where steric bulk will decrease binding affinity is predicted further from the 4-position of that same aromatic moiety. This may indicate a pocket with limited ability to accommodate larger groups in that region of the receptor. For example, while the 1-naphthylacetamide derivative (**7**) fits well in this region and shows affinity comparable to the phenyl derivative **BPP**, the 2-naphthyl-acetamide (**9**) extends past the sterically allowed region, with a subsequent 14-fold decrease in binding affinity.

There is a large area of steric bulk tolerance found near the 1- and 2-position of the phenylacetamide and behind the viewer's orientation. This may imply that larger, nonplanar rings might be tolerated by this receptor. Two regions near the 5- and 6-positions of the phenylacetamide ring indicate that steric bulk is not well-tolerated in these areas. Figure 2a also indicates that 2-position and in some cases 3-position substitutions on the benzylic aromatic ring will lead to lowered affinity when the steric bulk of a substituent becomes too large.

Electrostatic features that strongly influence binding are shown in Figure 2b. Two areas where increased positive charge should increase binding affinity were found near the 4- and 6-position of the phenylacetamide aromatic ring. A slightly smaller region, with opposite effects, is found near the 3-position, indicating that in this area, negative charge should increase binding affinity. Further, a small area where positive charge should enhance binding affinity was found near the benzylic aromatic.

These results appear to be in general agreement with our earlier conclusions drawn from a Hansch analysis of more limited data but provide more detail into interactions in specific areas. For example, the 3-bromophenylacetamide ($K_i = 0.87$ nM), the most potent σ_1 receptor ligand from both studies, places steric bulk well within the regions shown in Figure 2a and places negative charge in the area shown in Figure 2b where negative charge should lead to enhanced affinity. Conversely, 4-methoxyphenylacetamides, which show a decrease in binding affinity as compared to unsubstituted **BPP**, have negative charge near the 4-position region, a region predicted to show increased affinity with positive charge. Furthermore, these results provide a more detailed analysis, particularly for electrostatic interactions, than that provided by the compounds used in the CoMFA published by Ablordeppey.[33]

To confirm that these arylacetamide analogues do not bind to the 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 the rat D_3 receptors.[35] The dopamine receptor binding assays were conducted on analogues **1**, **10**, and **18** and revealed that the arylacetamide analogues had no affinity (IC_{50} > 10 000 nM) for both D_2 and D_3 receptors.

In summary, this study investigated a series of *N*-(1-benzylpiperidin-4-yl)arylacetamides for their binding affinities for σ_1 and σ_2 receptors. A SAR study was conducted based on structural modifications that were made in four regions of the template compound **BPP**. The σ receptor binding results are qualitatively comparable and in agreement with those of our previously reported series.[18] Specifically, (a) replacement of the phenyl ring of the phenylacetamide moiety with various aromatic rings maintains a high σ_1 receptor affinity when the aromatic ring is lipophilic and decreases the σ_1 receptor affinity when the aromatic ring is less lipophilic/more hydrophilic; (b) substitution on the phenyl ring of the benzyl group maintains a high or slightly lowered affinity for σ_1 receptors when the substitution is made at the 3- or 4-position and has a slightly to significantly decreased or no affinity for σ_2 receptors as the steric bulk of the substituent increases at the 2-position; and (c) substitution on both the phenylacetyl aromatic ring and the benzyl aromatic ring maintains a high affinity for σ_1 receptors with a generally increased σ_2 receptor affinity. Compounds **22**, **32**, **34**, **37**, **40**, and **43** can be used as PET and SPECT imaging agents to study the physiological functions of σ_1 receptors.

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 formulas were indicated, analyses were found to be within ±0.4% of the theoretical values for these elements. Proton nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz on a Bruker ASPECT3000 spectrometer. All proton 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, and 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)thiophene-2-acetamide (1). To an ice-cold solution of thiophene-2-acetic acid (1.42 g, 10 mmol) in dry tetrahydrofuran (THF) (50 mL) was added DCC (2.06 g, 10 mmol). After 30 min, 1-benzyl-4-aminopiperidine (1.90 g, 10 mmol) in THF (20 mL) was added. The reaction was stirred at room temperature overnight. The solid was removed by filtration, the solvent was evaporated in vacuo, and the residue was dissolved in CH_2Cl_2 (100 mL) and then washed with saturated aqueous NaCl and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column using $CHCl_3$ and $CHCl_3$−EtOH (9.5:0.5) as the eluents. The product was then recrystallized from ethyl acetate/hexane to give 0.86 g free amine, mp 122−123 °C. The mother liquid was concentrated and converted into the HCl salt by treatment with HCl gas in ethyl acetate. After the solvent was removed, the HCl salt was recrystallized from ethyl acetate−ethanol to give 1.46 g (69% overall), mp 208−210 °C. NMR (free amine in $CDCl_3$): δ 7.21−7.32 (m, 6H, aromatic), 6.97−7.00 (m, 1H, aromatic), 6.92−6.93 (m, 1H, aromatic), 5.42−5.44 (d, 1H, NH), 3.75 (s, 2H, CH_2CO), 3.73−3.85 (m, 1H, NH−CH), 3.45 (s, 2H, CH_2−phenyl), 2.69−2.74 (d, 2H, 2α-H_{eq} of piperidine), 2.05−2.14 (dt, 2H, 2α-H_{ax} of piperidine), 1.84−1.88 (m, 2H, 2β-H_{eq} of piperidine), 1.29−1.42 (dq, 2H, 2β-H_{ax} of piperidine). Anal. Calcd for $C_{18}H_{23}N_2OSCl$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-3-thiopheneacetamide (2).** Recrystallized from ethyl acetate/ethanol; yield, 64%; mp (HCl salt) 218−220 °C. NMR (free base; $CDCl_3$/TMS): δ 7.21−7.35 (m, 6H), 7.12 (d, J = 2.6 Hz, 1H), 6.97 (dd, J = 1.2, 5 Hz, 1H), 5.29 (d, J = 8 Hz, 1H), 3.71−3.81 (m, 1H), 3.58 (s, 2H), 3.45 (s, 2H), 2.71 (d, J = 12 Hz, 2H), 2.09 (dt, J = 2, 8 Hz, 2H), 1.83 (d, 12 Hz, 2H), 1.36 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{18}H_{22}N_2OS$·HCl: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-1-naphthylacetamide (7).** Recrystallized from ethyl acetate/ethanol; yield, 31%; mp (HCl salt) 215−217 °C. NMR (free base; $CDCl_3$/TMS): δ 7.82−7.91 (m, 3H), 7.39−7.53 (m, 4H), 7.18−7.26 (m, 5), 5.08 (d, J = 8 Hz, 1H), 3.81 (s, 2H), 3.72−3.82 (m, 1H), 3.35 (s, 2H), 2.55 (d, J = 12 Hz, 2H), 2.00 (dt, J = 2, 11 Hz, 2H), 1.62 (d, J = 12 Hz, 2H), 1.10 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{24}H_{26}N_2O$·HCl: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-2-naphthylacetamide (9).** Recrystallized from ethyl acetate/hexane; yield, 43%; mp (HCl salt) 206−208 °C. NMR (free base; $CDCl_3$/TMS): δ 7.81−7.85 (m, 4H), 7.48−7.51 (m, 2H), 7.22−7.37 (m, 6), 5.25 (d, J = 8 Hz, 1H), 3.73−3.83 (m, 1H), 3.71 (s, 2H), 3.42 (s, 2H), 2.69 (d, J = 12 Hz, 2H), 2.05 (dt, J = 2, 11 Hz, 2H), 1.80 (d, J = 12 Hz, 2H), 1.30 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{24}H_{26}N_2O$·HCl: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-5-bromoindole-3-acetamide (11).** Recrystallized from ethyl acetate/ethanol; yield, 58%; mp (free base) 149−150 °C. NMR ($CDCl_3$/TMS): δ 8.33 (s, 1H), 7.67 (s, 1H), 7.19−7.30 (m, 7H), 7.13 (s, 1H), 5.46 (d, J = 8 Hz, 1H), 3.77−3.83 (m, 1H), 3.65 (s, 2H), 3.42 (s, 2H), 2.60 (d, J = 9 Hz, 2H), 2.03−2.12 (m, 2H), 1.80 (dd, J = 2, 11 Hz, 2H), 1.26 (dq, J = 2, 10 Hz, 2H). Anal. Calcd for $C_{23}H_{24}N_3OBr$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-5-methoxy-1-indone-3-acetamide (13).** Recrystallized from ethyl acetate/hexane; yield, 20%; mp (HCl salt) 223−226 °C. NMR (free base; DMSO-d_6/TMS): δ 7.66 (d, J = 8 Hz, 1H), 7.26−7.32 (m, 5H), 6.90−6.93 (m, 2H), 5.36 (d, J = 7 Hz, 1H), 3.80−3.85 (m, 6H), 3.50 (s, 2H), 2.78 (d, J = 7 Hz, 2H), 2.31−2.39 (m, 2H), 2.10−2.16 (m, 1H), 2.08 (t, J = 15 Hz, 2H), 1.40−1.50 (m, 2H). Anal. Calcd for $C_{24}H_{28}N_2O_3$·HCl: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-(2-pyrimidylthio)acetamide (15).** Recrystallized from ethyl acetate/hexane; yield, 19%; mp (free base) 138−139 °C. NMR ($CDCl_3$/TMS): δ 8.54 (d, J = 5 Hz, 2H), 7.20−7.32 (m, 5H), 7.05 (t, J = 5 Hz, 2H), 6.86 (d, J = 8 Hz, 1H), 3.74−3.90 (m, 3H), 3.44 (s, 2H), 2.69 (d, J = 12 Hz, 2H), 2.07 (dt, J = 2, 11 Hz, 2H), 1.82 (d, J = 12 Hz, 2H), 1.36 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{18}H_{22}N_4OS$: C, H, N.

***N*-(1-Benzyl)piperidin-4-yl)-4-iodophenylacetamide (16).** Recrystallized from ethyl acetate/hexane; yield, 87%; mp (free base) 145−147 °C. NMR ($CDCl_3$/TMS): δ 7.66 (dd, J = 2, 5 Hz, 2H), 7.22−7.32 (m, 4H), 7.00 (d, J = 8 Hz, 2H), 5.22 (d, J = 8 Hz, 1H), 3.60−3.70 (m, 1H), 3.45 (s, 2H), 3.43 (s, 2H), 2.73 (d, J = 11 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.75−1.95 (m, 2H), 1.25−1.45 (m, 2H). Anal. Calcd for $C_{20}H_{23}N_2OI$: C, H, N.

General Method B. Preparation of *N*-(1-Benzylpiperidin-4-yl)imidazole-4-acetamide (3). To a solution of imidazole-4-acetic acid hydrochloride (1.25 g, 7.7 mmol) in DMSO (50 mL) and triethylamine (1 mL) were added BOP (3.40 g, 7.7 mmol) and 1-benzyl-4-aminopiperidine (1.46 g, 7.7 mmol) under stirring. After the solution was stirred at room temperature for 2 h, the mixture was poured into 100 mL of water and extracted with CH_2Cl_2 (3 × 20 mL), washed with aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried over Na_2SO_4. The product was purified on a silica gel column using $CHCl_3$−EtOH (7:3) as the eluent. Recrystallization from ethyl acetate/pentane gave 0.83 g of free amine (37%). mp 134−135 °C. NMR (free amine in $CDCl_3$): δ 7.60 (s, 1H), 7.17−7.33 (m, 5H), 6.88 (m, 2H), 3.73−3.84 (m, 1H), 3.52 (s, 2H), 3.47 (s, 2H), 2.73−2.77 (d, 2H), 2.04−2.15 (dt, 2H), 1.84−1.88 (m, 2H), 1.38−1.51 (dq, 2H). Anal. Calcd for $C_{17}H_{22}N_4O$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-2-pyridoacetamide (4).** Recrystallized from ethyl acetate/hexane; yield, 92%; mp (free base) 107−108 °C. NMR ($CDCl_3$/TMS): δ 8.69−8.79 (m, 1H), 7.63−7.67 (m, 1H), 7.18−7.33 (m, 7H), 3.75−3.82 (m, 1H), 3.69 (s, 2H), 3.47 (s, 2H), 2.72 (d, J = 12 Hz, 2H), 2.13 (dt, J = 2,

11 Hz, 2H), 1.85 (d, J = 12 Hz, 2H), 1.45 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{19}H_{23}N_3O$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-3-pyridoacetamide (5).** Recrystallized from ethyl acetate/hexane; yield, 44%; mp (free base) 111−112 °C. NMR ($CDCl_3$/TMS): δ 8.54 (dd, J = 1.5, 5 Hz, 1H), 8.50 (d, J = 2 Hz, 1H), 7.63−7.67 (m, 1H), 7.21−7.34 (m, 5H), 5.29 (d, J = 7 Hz, 1H), 3.70−3.86 (m, 1H), 3.52 (s, 2H), 3.46 (s, 2H), 2.76 (d, J = 12 Hz, 2H), 2.08 (dt, J = 2, 11 Hz, 2H), 1.86 (d, J = 12 Hz, 2H), 1.40 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{19}H_{23}N_3O$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-4-pyridoacetamide (6).** Recrystallized from ethyl acetate/ethanol; yield, 49%; mp (free base) 224−226 °C. NMR (DMSO-d_6/TMS): δ 8.48 (d, J = 4 Hz, 2H), 8.30 (d, J = 6 Hz, 1H), 7.49 (s, 5H), 7.27 (d, J = 5 Hz, 2H), 4.26 (s, 1H), 3.03−3.61 (m, 8H), 1.94 (d, J = 12 Hz, 2H), 1.59−1.62 (m, 2H). Anal. Calcd for $C_{19}H_{23}N_3O$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)indole-3-acetamide (10).** Recrystallized from ethyl acetate/ethanol; yield, 58%; mp (free base) 139−140 °C. NMR ($CDCl_3$/TMS): δ 8.40 (s, 1H), 7.53 (d, J = 8 Hz, 1H), 7.40 (d, J = 8 Hz, 1H), 7.22−7.27 (m, 6H), 7.11−7.16 (m, 2H), 5.56 (d, J = 8 Hz, 1H), 3.75−3.85 (m, 1H), 3.72 (s, 2H), 3.40 (s, 2H), 2.65 (d, J = 9 Hz, 2H), 1.76−2.09 (m, 4H), 1.21 (dq, J = 2, 10 Hz, 2H). Anal. Calcd for $C_{23}H_{25}N_3O$: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-5-methoxyindole-3-acetamide (12).** Recrystallized from ethyl acetate/ethanol; yield, 36%; mp (HCl salt) 177−178 °C. NMR (free base; $CDCl_3$/TMS): δ 8.15 (s, 1H), 7.19−7.30 (m, 6H), 7.09 (d, J = 2.4 Hz, 1H), 6.87−6.94 (m, 2H), 5.57 (d, J = 8 Hz, 1H), 3.79−3.86 (m, 4H), 3.68 (s, 2H), 3.40 (s, 2H), 2.64 (d, J = 11.4 Hz, 2H), 2.05 (dt, J = 2.3, 12 Hz, 2H), 1.76 (m, 2H), 1.20−1.30 (m, 2H). Anal. Calcd for $C_{23}H_{27}N_2O_2$·HCl: C, H, N.

***N*-(1-(Benzyl)piperidin-4-yl)-7-hydroxycoumarin-4-acetamide (14).** Recrystallized from ethyl acetate/ethanol; yield, 100%; mp (HCl salt) 189−192 °C. NMR (free base; DMSO-d_6/TMS): δ 10.58 (s, 1H), 8.38 (d, J = 7 Hz, 1H), 6.80−7.59 (m, 5H), 6.72−6.76 (m, 2H), 5.72 (s, 1H), 4.00−4.04 (m, 3H), 3.63−3.75 (m, 1H), 3.47−3.59 (m, 2H), 3.35−3.46 (m, 2H), 3.04−3.11 (m, 2H), 1.90−2.01 (m, 2H), 1.20−1.57 (m, 2H). Anal. Calcd for $C_{24}H_{28}N_2O_4$·HCl: C, H, N.

Method C. Preparation of 1-(1-Naphthyl)-3-(1-benzylpiperidin-4-yl)urea (8). A mixture of 1-naphthylisocyanate (1.69 g, 10 mmol), 1-benzyl-4-aminopiperidine (1.90 g, 10 mmol), and dibutyltin diacetate (3 drops) in 40 mL of CH_2Cl_2 was stirred at room temperature for 2 h. The mixture was poured into ice-cold water, extracted with CH_2Cl_2 (3 × 20 mL), washed with saturated aqueous NaCl, and dried over Na_2SO_4. It was concentrated in vacuo and purified by a silica gel column using $CHCl_3$−EtOH (9.5:0.5) as the eluent. The product was recrystallized from ethanol/ethyl acetate to give 3.42 g (100%) of free amine, mp 183−185 °C. A small amount was converted into HCl salt using ethanol−HCl and recrystallized from ethanol−water to yield the salt for biological assay, mp 247−250 °C. NMR (free amine in $CDCl_3$ + DMSO-d_6): δ 8.33 (s, 1H), 8.02−8.09 (m, 2H), 7.79−7.85 (m, 1H), 7.23−7.50 (m, 9H), 6.40−6.43 (d, 1H), 3.64−3.67 (m, 1H), 3.50 (s, 2H), 2.78−2.82 (d, 2H), 2.12−2.19 (dt, 2H), 1.93−1.97 (dd, 2H), 1.43−1.56 (dq, 2H). Anal. Calcd for $C_{23}H_{26}N_3OCl$: C, H, N.

General Method D. Preparation of *N*-(1-(2-Fluorobenzyl)piperidin-4-yl)phenylacetamide (17). BPP (4.0 g, 13 mmol) was dissolved in 100 mL of methanol containing 20 mg of palladium hydroxide on carbon and hydrogenated under 50 psi for 12 h. The catalyst was filtered, and the solution was evaporated under reduced pressure to give a residue that was recrystallized from ethyl acetate−hexane to give 2.57 g (90%) of 4-phenylacetamidopiperidine; mp 127−129. ^{1}H NMR (free amine in $CDCl_3$): δ 7.23−7.39 (m, 4H), 5.22−5.28 (d, 1H), 3.79−3.92 (m, 1H), 3.56 (s, 2H), 2.94−3.01 (dt, 2H), 2.60−2.69 (dt, 2H), 1.82−1.87 (m, 2H), 1.01−1.24 (dq, 2H).

A mixture of 4-phenylacetamidopiperidine (0.22 g, 1 mmol), 2-fluorobenzyl bromide (0.19 g, 1 mmol), and 0.5 mL triethylamine in 50 mL dichloromethane was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was dissolved in 50 mL of ethyl acetate, washed with 0.5 N NaOH and water, and dried over Na_2SO_4. Solvent was removed, and the residue was purified by a silica gel column with $CHCl_3$−EtOH (9.5:0.5) as the eluent and recrystallized from ethyl acetate−hexane to give 0.27 g (83%) of free amine; mp 108−109 °C. NMR (free amine in $CDCl_3$): δ 7.21−7.37 (m, 7H), 7.97−7.10 (m, 2H), 5.16−5.19 (d, 1H), 3.76−3.79 (m, 1H), 3.55 (s, 2H), 3.52 (s, 2H), 2.70−2.74 (d, 2H), 2.10−2.18 (dt, 2H), 1.82−1.85 (m, 2H), 1.26−1.37 (dq, 2H). Anal. Calcd for $C_{20}H_{23}N_2OF$: C, H, N.

***N*-(1-(3-Fluorobenzyl)piperidin-4-yl)phenylacetamide (18).** Recrystallized from ethyl acetate/hexane; yield, 58%; mp (free base) 117−118 °C. NMR ($CDCl_3$/TMS): δ 7.20−7.38 (m, 6H), 6.99−7.03 (m, 2H), 6.88−6.95 (dt, J = 2, 9 Hz, 1H), 5.20 (d, J = 8 Hz, 1H), 3.73−3.84 (m, 1H), 3.55 (s, 2H), 3.43 (s, 2H), 2.68 (d, J = 11 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.82 (d, J = 9 Hz, 2H), 1.32 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{20}H_{23}N_2OF$: C, H, N.

***N*-(1-(4-Fluorobenzyl)piperidin-4-yl)phenylacetamide (19).** Recrystallized from ethyl acetate/hexane; yield, 90%; mp (free base) 120−121 °C. NMR ($CDCl_3$/TMS): δ 7.20−7.35 (m, 7H), 6.94−7.00 (m, 2H), 5.20 (d, J = 8 Hz, 1H), 3.75−3.86 (m, 1H), 3.55 (s, 2H), 3.40 (s, 2H), 2.67 (d, J = 12 Hz, 2H), 2.06 (t, J = 8 Hz, 2H), 1.82 (d, J = 8 Hz, 2H), 1.25−1.31 (m, 2H). Anal. Calcd for $C_{20}H_{23}N_2OF$: C, H, N.

***N*-(1-(2-Iodobenzyl)piperidin-4-yl)phenylacetamide (20).** Recrystallized from ethyl acetate/hexane; yield, 74%; mp (free base) 135−136 °C. NMR ($CDCl_3$/TMS): δ 7.80 (d, J = 8 Hz, 1H), 7.23−7.38 (m, 7H), 6.93 (dt, J = 2, 8 Hz, 1H), 5.20 (d, J = 8 Hz, 1H), 3.77−3.94 (m, 1H), 3.56 (s, 2H), 3.45 (s, 2H), 2.72 (d, J = 11 Hz, 2H), 2.20 (dt, J = 2, 11 Hz, 2H), 1.85 (d, J = 9 Hz, 2H), 1.35 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{20}H_{23}N_2OI$: C, H, N.

***N*-(1-(3-Iodobenzyl)piperidin-4-yl)phenylacetamide (21).** Recrystallized from ethyl acetate/hexane; yield, 85%; mp (free base) 133−134 °C. NMR ($CDCl_3$/TMS): δ 7.64 (s, 1H), 7.56 (d, J = 8 Hz, 1H), 7.17−7.38 (m, 6H), 7.02 (t, J = 8 Hz, 1H), 5.19 (d, J = 8 Hz, 1H), 3.74−3.84 (m, 1H), 3.40 (s, 2H), 3.37 (s, 2H), 2.67 (d, J = 11 Hz, 2H), 2.07 (dt, J = 2, 11 Hz, 2H), 1.83 (d, J = 9 Hz, 2H), 1.30 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{20}H_{23}N_2OI$: C, H, N.

***N*-(1-(4-Iodobenzyl)piperidin-4-yl)phenylacetamide (22).** Recrystallized from ethyl acetate/hexane; yield, 41%; mp (free base) 136−138 °C. NMR ($CDCl_3$/TMS): δ 7.62 (d, J = 8 Hz, 2H), 7.17−7.38 (m, 5H), 7.01 (d, J = 8 Hz, 2H), 5.20 (d, J = 7 Hz, 1H), 3.74−3.84 (m, 1H), 3.55 (s, 2H), 3.37 (s, 2H), 2.66 (d, J = 12 Hz, 2H), 2.06 (dt, J = 2, 11 Hz, 2H), 1.82−1.90 (d, J = 8 Hz, 2H), 1.30 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{23}N_2OI$: C, H, N.

***N*-(1-(2-Trifluoromethylbenzyl)piperidin-4-yl)phenylacetamide (23).** Recrystallized from ethyl acetate; yield, 85%; mp (free base) 133−134 °C. NMR ($CDCl_3$/TMS): δ 7.70 (d, J = 8 Hz, 1H), 7.60 (d, J = 8 Hz, 1H), 7.47 (t, J = 8 Hz, 1H), 7.23−7.39 (m, 6H), 5.23 (d, J = 8 Hz, 1H), 3.72−3.92 (m, 1H), 3.59 (s, 2H), 3.56 (s, 2H), 2.68 (d, J = 11 Hz, 2H), 2.18 (dt, J = 2, 11 Hz, 2H), 1.85 (d, J = 9 Hz, 2H), 1.32 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{21}H_{23}N_2OF_3$: C, H, N.

***N*-(1-(3-Trifluoromethylbenzyl)piperidin-4-yl)phenylacetamide (24).** Recrystallized from ethyl acetate; yield, 56%; mp (free base) 141−142 °C. NMR ($CDCl_3$/TMS): δ 7.23−7.55 (m, 9H), 5.22 (d, J = 8 Hz, 1H), 3.72−3.92 (m, 1H), 3.55 (s, 2H), 3.48 (s, 2H), 2.68 (d, J = 11 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.82 (d, J = 9 Hz, 2H), 1.30 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{21}H_{23}N_2OF_3$: C, H, N.

***N*-(1-(4-Trifluoromethylbenzyl)piperidin-4-yl)phenylacetamide (25).** Recrystallized from ethyl acetate; yield, 66%; mp (free base) 128−129 °C. NMR ($CDCl_3$/TMS): δ 7.54 (d, J = 8 Hz, 2H), 7.23−7.41 (m, 7H), 5.22 (d, J = 8 Hz, 1H), 3.72−3.92 (m, 1H), 3.56 (s, 2H), 3.49 (s, 2H), 2.68 (d, J = 11 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.85 (d, J = 9 Hz, 2H), 1.30 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{21}H_{23}N_2OF_3$: C, H, N.

***N*-(1-(4-Nitrobenzyl)piperidin-4-yl)phenylacetamide (26).** Recrystallized from ethyl acetate/ethanol; yield, 74%; mp (free base) 140−142 °C. NMR ($CDCl_3$/TMS): δ 8.15 (d, J = 9 Hz, 2H), 7.46 (d, J = 9 Hz, 2H), 7.23−7.39 (m, 5H), 5.20 (d, J

= 8 Hz, 1H), 3.72−3.92 (m, 1H), 3.56 (s, 2H), 3.53 (s, 2H), 2.70 (d, J = 11 Hz, 2H), 2.15 (dt, J = 2, 11 Hz, 2H), 1.88 (d, J = 9 Hz, 2H), 1.38 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, H, N.

***N*-(1-(3,4-Dichlorobenzyl)piperidin-4-yl)phenylacetamide (27).** Recrystallized from ethyl acetate/ethanol; yield, 80%; mp (free base) 157−158 °C. NMR ($CDCl_3$/TMS): δ 7.22−7.38 (m, 7H), 7.09 (dd, J = 1.5, 8 Hz, 1H), 5.20 (d, J = 8 Hz, 1H), 3.72−3.92 (m, 1H), 3.55 (s, 2H), 3.37 (s, 2H), 2.65 (d, J = 11 Hz, 2H), 2.08 (dt, J = 2, 11 Hz, 2H), 1.82 (d, J = 9 Hz, 2H), 1.30 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{21}H_{22}N_2OCl_2$: C, H, N.

***N*-(1-(3,4-Difluorobenzyl)piperidin-4-yl)phenylacetamide (28).** Recrystallized from ethyl acetate/hexane; yield, 55%; mp (free base) 110−111 °C. NMR ($CDCl_3$/TMS): δ 7.23−7.38 (m, 5H), 6.96−7.15 (m, 3H), 5.20 (d, J = 8 Hz, 1H), 3.74−3.94 (m, 1H), 3.55 (s, 2H), 3.37 (s, 2H), 2.68 (d, J = 11 Hz, 2H), 2.08 (dt, J = 2, 11 Hz, 2H), 1.85 (d, J = 9 Hz, 2H), 1.30 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{21}H_{22}N_2OF_2$: C, H, N.

***N*-(1-(3,4-Methylenedioxybenzyl)piperidin-4-yl)phenylacetamide (29).** Recrystallized from ethyl acetate/ethanol; yield, 68%; mp (free base) 110−111 °C. NMR ($CDCl_3$/TMS): δ 7.22−7.38 (m, 5H), 6.67−6.79 (m, 3H), 5.90 (s, 2H), 5.18 (d, J = 8 Hz, 1H), 3.73−3.88 (m, 1H), 3.55 (s, 2H), 3.34 (s, 2H), 2.68 (d, J = 11 Hz, 2H), 2.05 (dt, J = 2, 11 Hz, 2H), 1.82 (d, J = 9 Hz, 2H), 1.38 (dt, J = 2, 9 Hz, 2H). Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, H, N.

***N*-(2-Naphthylmethyl)piperidin-4-yl)phenylacetamide (30).** Recrystallized from ethyl acetate/ethanol; yield, 56%; mp (free base) 145−146 °C. NMR ($CDCl_3$/TMS): δ 7.76−7.80 (m, 3H), 7.68 (s, 1H), 7.43−7.45 (m, 3H), 7.22−7.34 (m, 5H), 5.20 (d, J = 8 Hz, 1H), 3.76−3.86 (m, 1H), 3.60 (s, 2H), 3.54 (s, 2H), 2.75 (d, J = 11 Hz, 2H), 2.10 (t, J = 9 Hz, 2H), 1.83 (d, J = 9 Hz, 2H), 1.35 (dq, J = 4, 10 Hz, 2H). Anal. Calcd for $C_{24}H_{26}N_2O$: C, H, N.

***N*-(2-Phenylethyl)piperidin-4-yl)phenylacetamide (31).** Recrystallized from ethyl acetate/hexane; yield, 16%; mp (free base) 131−133 °C. NMR ($CDCl_3$/TMS): δ 7.16−7.38 (m, 10H), 5.20 (d, J = 8 Hz, 1H), 3.79−3.89 (m, 1H), 3.56 (s, 2H), 2.73−2.85 (m, 4H), 2.53−2.58 (m, 2H), 2.15 (t, J = 12 Hz, 2H), 1.88 (br d, J = 12 Hz, 2H), 1.34 (dq, J = 4, 10 Hz, 2H). Anal. Calcd for $C_{21}H_{26}N_2O \cdot 1/4H_2O$: C, H, N.

General Method E. Preparation of *N*-(1-(4-Fluorobenzyl)piperidin-4-yl)-2-fluorophenylacetamide (32). To a solution of 4-amino-1-benzylpiperidine (9.5 g, 50 mmol) in 200 mL of dry dicholoromethane were added trifluoroacetic anhydride (12.6 g, 60 mmol) and 5 mL triethylamine under ice and stirring. After the solution was stirred at room temperature for 12 h, the solvent was removed under vacuo, the residue was dissolved in ethyl acetate and washed with aqueous $NaHCO_3$ and water and dried over Na_2SO_4. The solvent was removed, and the product was recrystallized from ethyl acetate to give 13.5 g (94%) of 1-benzyl-4-trifluoroacetamidopiperidine. NMR (free amine in $CDCl_3$): δ 7.29−7.38 (m, 5H), 6.55−6.62 (d, 1H), 3.87−3.96 (m, 1H), 3.69 (s, 2H), 3.02−3.06 (d, 2H), 2.27−2.36 (dt, 2H), 1.98−2.01 (m, 2H), 1.67−1.81 (dq, 2H).

The above product was dissolved in 150 mL of methanol containing 50 mg of palladium hydroxide on carbon and hydrogenated at 50 psi for 12 h. The catalyst was removed by filtration, and the solution was concentrated in vacuo to give an oil that was reacted with di-*tert*-butyl-di-carbonate (11.0 g, 50 mmol) in 200 mL of dicholoromethane for 6 h. The reaction mixture was transferred into a separatory funnel and washed with aqueous $NaHCO_3$ and water and dried over Na_2SO_4. After the solvent was removed, the residue was dissolved in 100 mL of methanol and 50 mL of 30% ammonium hydroxide and refluxed for 6 h. The solvent was removed, and the residue was dissolved in 100 mL of dichloromethane, washed with 1 N NaOH and water, and dried over Na_2SO_4. The solvent was removed, and the product was recrystallized from ethyl acetate−hexane to give 5.75 g (58%) of 4-amino-1-(*tert*-butoxycarbonyl)piperidine. NMR (free amine in $CDCl_3$): δ 3.90−4.20 (m, 2H), 2.65−2.95 (m, 2H), 1.15−2.00 (m, 14H).

A mixture of 2-fluorophenylacetic acid (1.54 g, 10 mmol) and DCC (2.06 g, 10 mmol) in 50 mL of dichloromethane was stirred for 5 min, 4-amino-1-(*tert*-butoxycarbonyl)piperidine (2.0 g, 10 mmol) was added, and the solution was stirred at room temperature overnight. The solid was removed by filtration, and the solution was concentrated in vacuo to afford a residue that was then reacted with CF_3COOH to remove the *tert*-butoxycarbonyl group to afford 0.80 g (34%) of 4-(2-fluorophenyl)acetamidopiperidine; mp 126−128 °C. NMR (free amine in $CDCl_3$): δ 7.25−7.35 (m, 2H), 7.05−7.15 (m, 2H), 5.51−5.54 (d, 1H), 3.88−3.96 (m, 1H), 3.56 (s, 2H), 3.42−3.52 (m, 1H), 2.69−2.78 (dt, 2H), 1.91−1.96 (d, 2H), 1.36−1.46 (m, 2H), 1.03−1.20 (m, 2H).

4-(2-Fluorophenyl)acetamidopiperidine (0.118 g, 0.5 mmol) in 40 mL of dichloromethane was added with 4-fluorobenzyl bromide (0.095 g, 0.5 mmol) and triethylamine (0.5 mL). After the solution was stirred at room temperature overnight, the solvent was removed in vacuo. The residue was dissolved in 50 mL of ethyl acetate and washed with 0.5 N NaOH and water and dried over Na_2SO_4. The solvent was removed in vacuo, and the product was purified by silica gel column chromatography using $CHCl_3$−EtOH (9.5:0.5) as the eluent. The product was recrystallized from ethyl acetate−hexane to give 0.10 g (59%) of *N*-(1-(4-fluorobenzyl)piperidin-4-yl)-2-fluorophenylacetamide; mp 129−131 °C. NMR (free amine in $CDCl_3$): δ 7.20−7.32 (m, 4H), 6.93−7.15 (m, 4H), 5.32−5.34 (d, 1H), 3.73−3.84 (m, 1H), 3.55 (s, 2H), 3.41 (s, 2H), 2.68−2.72 (d, 2H), 2.03−2.11 (dt, 2H), 1.83−1.88 (m, 2H), 1.28−1.41 (dq, 2H). Anal. Calcd for $C_{20}H_{22}N_2OF_2$: C, H, N.

***N*-(1-(3-Iodobenzyl)piperidin-4-yl)-2-fluorophenylacetamide (33).** Recrystallized from ethyl acetate/hexane; yield, 83%; mp (free base) 135−136 °C. NMR ($CDCl_3$/TMS): δ 7.65 (s, 1H), 7.57 (d, J = 8 Hz, 1H), 7.22−7.32 (m, 3H), 6.99−7.15 (m, 3H), 5.35 (d, J = 8 Hz, 1H), 3.73−3.84 (m, 1H), 3.55 (s, 2H), 3.39 (s, 2H), 2.70 (d, J = 12 Hz, 2H), 2.08 (dt, J = 2, 12 Hz, 2H), 1.86 (d, J = 10 Hz, 2H), 1.32 (dq, J = 4, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OFI$: C, H, N.

***N*-(1-(4-Iodobenzyl)piperidin-4-yl)-3-fluorophenylacetamide (34).** Recrystallized from ethyl acetate/hexane; yield, 57%; mp (free base) 144−145 °C. NMR ($CDCl_3$/TMS): δ 7.26−7.30 (m, 6H), 7.00−7.20 (m, 2H), 5.35 (d, J = 7 Hz, 1H), 3.72−3.90 (m, 1H), 3.55 (s, 2H), 3.46 (s, 2H), 2.60 (d, J = 12 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.75−1.95 (m, 2H), 1.30−1.45 (m, 2H). Anal. Calcd for $C_{20}H_{22}N_2OFI$: C, H, N.

***N*-(1-(4-Fluorobenzyl)piperidin-4-yl)-3-fluorophenylacetamide (35).** Recrystallized from ethyl acetate/hexane; yield, 58%; mp (free base) 115−117 °C. NMR ($CDCl_3$/TMS): δ 7.15−7.40 (m, 4H), 6.90−7.10 (m, 4H), 5.70 (d, J = 8 Hz, 1H), 3.75−3.82 (m, 1H), 3.53 (s, 2H), 3.41 (s, 2H), 2.70 (d, J = 12 Hz, 2H), 2.00−2.10 (m, 2H), 1.60−1.70 (m, 2H), 1.05−1.15 (m, 2H). Anal. Calcd for $C_{20}H_{22}N_2OF_2$: C, H, N.

***N*-(1-(3-Iodobenzyl)piperidin-4-yl)-3-fluorophenylacetamide (36).** Recrystallized from ethyl acetate/hexane; yield, 74%; mp (free base) 111−112 °C. NMR ($CDCl_3$/TMS): δ 7.66 (s, 1H), 7.57 (d, J = 8 Hz, 1H), 7.23−7.38 (m, 2H), 6.96−7.05 (m, 4H), 5.26 (d, J = 8 Hz, 1H), 3.75−3.82 (m, 1H), 3.53 (s, 2H), 3.40 (s, 2H), 2.72 (d, J = 12 Hz, 2H), 2.09 (dt, J = 2, 12 Hz, 2H), 1.86 (d, J = 12 Hz, 2H), 1.35 (dq, J = 4, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OFI$: C, H, N.

***N*-(1-(4-Iodobenzyl)piperidin-4-yl)-3-fluorophenylacetamide (37).** Recrystallized from ethyl acetate/hexane; yield, 57%; mp (free base) 135−136 °C. NMR ($CDCl_3$/TMS): δ 7.26−7.30 (m, 6H), 6.90−7.05 (m, 2H), 5.25 (d, J = 7 Hz, 1H), 3.72−3.82 (m, 1H), 3.55 (s, 2H), 3.40 (s, 2H), 2.75 (d, J = 12 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.80−1.92 (m, 2H), 1.35 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OFI$: C, H, N.

***N*-(1-(4-Fluorobenzyl)piperidin-4-yl)-3-chlorophenylacetamide (38).** Recrystallized from ethyl acetate/hexane; yield, 67%; mp (free base) 101−102 °C. NMR ($CDCl_3$/TMS): δ 7.25−7.28 (m, 6H), 7.00 (t, J = 9 Hz, 2H), 5.20 (d, J = 8 Hz, 1H), 3.75−3.85 (m, 1H), 3.51 (s, 2H), 3.43 (s, 2H), 2.70 (d, J = 12 Hz, 2H), 2.08 (dt, J = 2, 11 Hz, 2H), 1.85 (d, J = 12 Hz, 2H), 1.32 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OFCl$: C, H, N.

***N*-(1-(4-Iodobenzyl)piperidin-4-yl)-3-chlorophenylacetamide (39).** Recrystallized from ethyl acetate/hexane; yield, 87%; mp (free base) 119−120 °C. NMR ($CDCl_3$/TMS): δ 7.20−7.35 (m, 7H), 7.10−7.15 (m, 1H), 5.20 (d, J = 7 Hz, 1H), 3.70−3.90 (m, 1H), 3.50 (s, 2H), 3.45 (s, 2H), 2.75 (d, J = 12 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.80−1.90 (m, 2H), 1.55 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OClI$: C, H, N.

***N*-(1-(4-Iodobenzyl)piperidin-4-yl)-3-chlorophenylacetamide (40).** Recrystallized from ethyl acetate/hexane; yield, 60%; mp (free base) 134−135 °C. NMR ($CDCl_3$/TMS): δ 7.62 (d, J = 8 Hz, 2H), 7.25−7.28 (m, 3H), 7.12−7.16 (m, 1), 7.03 (d, J = 8 Hz, 2H), 5.32 (d, J = 8 Hz, 1H), 3.72−3.85 (m, 1H), 3.57 (s, 2H), 3.39 (s, 2H), 2.70 (d, J = 12 Hz, 2H), 2.08 (dt, J = 2, 11 Hz, 2H), 1.85 (d, J = 12 Hz, 2H), 1.37 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OClI$: C, H, N.

***N*-(1-(4-Fluorobenzyl)piperidin-4-yl)-3-bromophenylacetamide (41).** Recrystallized from ethyl acetate/hexane; yield, 69%; mp (free base) 109−110 °C. NMR ($CDCl_3$/TMS): δ 7.42−7.44 (m, 2H), 7.20−7.55 (m, 4H), 6.98 (t, J = 9 Hz, 2H) 5.70 (d, J = 8 Hz, 1H), 3.72−3.82 (m, 1H), 3.50 (s, 2H), 3.42 (s, 2H), 2.72 (d, J = 12 Hz, 2H), 2.10 (dt, J = 2, 11 Hz, 2H), 1.80−2.00 (m, 2H), 1.45 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OFBr$: C, H, N.

***N*-(1-(3-Iodobenzyl)piperidin-4-yl)-3-bromophenylacetamide (42).** Recrystallized from ethyl acetate/hexane; yield, 85%; mp (free base) 136−137 °C. NMR ($CDCl_3$/TMS): δ 7.66 (s, 1H), 7.58 (d, J = 8 Hz, 1H), 7.41−7.44 (m, 2H), 7.22−7.26 (m, 3H), 7.02 (t, J = 8 Hz, 1H), 5.76 (d, J = 8 Hz, 1H), 3.74−3.84 (m, 1H), 3.50 (s, 2H), 3.39 (s, 2H), 2.72 (d, J = 12 Hz, 2H), 2.09 (dt, J = 2, 12 Hz, 2H), 1.86 (d, J = 10 Hz, 2H), 1.45 (dq, J = 4, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OBrI$: C, H, N.

***N*-(1-(4-Iodobenzyl)piperidin-4-yl)-3-bromophenylacetamide (43).** Recrystallized from ethyl acetate/hexane; yield, 66%; mp (free base) 140−141 °C. NMR ($CDCl_3$/TMS): δ 7.62 (d, J = 8 Hz, 2H), 7.41−7.44 (m, 2H), 7.17−7.26 (m, 2H), 7.03 (d, J = 8 Hz, 2H), 5.23 (d, J = 7 Hz, 1H), 3.72−3.82 (m, 1H), 3.49 (s, 2H), 3.39 (s, 2H), 2.70 (d, J = 12 Hz, 2H), 2.08 (t, J = 11 Hz, 2H), 1.84 (d, J = 9 Hz, 2H), 1.35 (dq, J = 3, 12 Hz, 2H). Anal. Calcd for $C_{20}H_{22}N_2OBrI$: C, H, N.

Preparation of 4-Iodobenzyl Chloride. A solution of 2.0 M *n*-BuLi (44 mL, 0.088 mol) was added dropwise under argon to a cooled (−78 °C) stirring solution of 4-bromobenzyl alcohol (7.48 g, 0.04 mol) in dry THF (150 mL). The resulting mixture was stirred at −78 °C for 90 min, and then, a solution of *n*-Bu_3SnCl (28.86 g, 0.088 mol) in dry THF (50 mL) was added dropwise. The reaction was continued at room temperature overnight. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (150 mL), washed with water, and dried over Na_2SO_4. The solvent was removed, and the product was purified by a silica gel column chromatography using hexane/acetone (9:1) as the eluent to give 4-tributyltinbenzyl alcohol, an oil (9.05 g, 57%). NMR (free amine in $CDCl_3$): δ 7.45−7.48 (d, 2H), 7.31−7.34 (d, 2 H), 4.65−4.68 (d, 2 H), 1.48−1.58 (m, 6H), 1.26−1.38 (m, 6H), 1.02−1.08 (m, 6H), 0.85−0.91 (t, 9H).

The above 4-tributyltinbenzyl alcohol (3.97 g, 0.01 mol) was added into a triphenylphosphine solution in carbon tetrachloride (30 mL). The mixture was stirred under reflux overnight. The solid was filtered off, and the filtrate was added into CH_2Cl_2 (60 mL), washed with water, and dried over Na_2SO_4. The solvent was removed, and the product was purified by a silica gel column chromatography using hexane/ethyl acetate (9.5:0.5) as the eluent to give 4-tributyltinbenzyl chloride (4.11 g, 99%). NMR (free amine in $CDCl_3$): δ 7.45−7.48 (d, 2H), 7.32−7.35 (d, 2 H), 4.54 (s, 2 H), 1.47−1.57 (m, 6H), 1.23−1.36 (m, 6H), 1.02−1.09 (m, 6H), 0.85−0.92 (t, 9H).

To a solution of 4-tributyltinbenzyl chloride (4.15 g, 0.01 mol) in CH_2Cl_2 (30 mL) was added iodine (4.0 g, 0.016 mol). The mixture was stirred at room temperature overnight. A $Na_2S_2O_5$ aqueous solution (10%, 30 mL) was added to consume excess I_2. The solution was extracted with CH_2Cl_2 (60 mL), washed with water, and dried over Na_2SO_4. The solvent was removed, and the product was purified by a silica gel column chromatography using hexane as the eluent and recrystallized from pentane to give 4-iodobenzyl chloride (2.46 g, 97%). NMR (free amine in $CDCl_3$): δ 7.67−7.72 (d, 2H), 7.11−7.15 (d, 2 H), 4.52 (s, 2 H). Anal. Calcd for $C_7H_6ClI \cdot 1/4H_2O$: C, H.

Pharmacology. Tissue Source and Radioligands. σ_1 binding sites were labeled with the σ_1-selective radioligand, [^{3}H](+)-pentazocine (DuPont-NEN, Bilerica, MA) in guinea pig brain membranes (Rockland Biological, Gilbertsville, PA) according to published procedures.[1,36] σ_2 sites were assayed in rat liver membranes with [^{3}H]DTG (DuPont-NEN, Bilerica, MA) in the presence of (+)-pentazocine (100 nM) to mask σ_1 sites.[1,36]

Membrane Preparation. Crude synaptosomal (P_2) membrane homogenates were prepared from frozen guinea pig brains without cerebellum.[36,37] 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 was saved on ice. The pellet was resuspended in 2 mL/g tissue weight of ice-cold 10 mM Tris-HCl/0.32 M sucrose, pH 7.4, by vortexing. After the pellet was centrifuged at 1000*g* for 10 min, the pellet was discarded and the supernatants were combined and centrifuged at 31 000*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 31 000*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[38] and generally ranged from 6 to 11 mg protein/mL.

***σ*$_1$ Binding Assay.** Guinea pig brain membrane homogenates (100 μg 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[39] and represent mean values ± standard error of the mean (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.[1,36]

Molecular Modeling

Molecular modeling studies were performed using Sybyl.[22] Calculations were carried out on a Silicon Graphics Indigo workstation. For all compounds, initial molecular models were based on **BPP**, the parent compound for the acetamide series. Structures were modified as needed with fragments or atoms added from the Sybyl structural library, using standard bond lengths and angles. Charges, except as noted, were calculated using the Gasteiger−Hückel[23] or AM1[24] method. Structures were minimized using the Tripos force field to gradient convergence using both steric and electrostatic components (criteria: gradient energy change, 0.01 kcal/mol; rms displacement, 0.001 Å; nonbonded cutoff, 8.000 Å; dielectric function as distance dependent; dielectric constant, 1.000). To identify low energy conformations as starting points for CoMFA,[25] systematic search procedures (as implemented in Sybyl) and random[26] conformational analyses were used to explore the conformational flexibility of **BPP**.

Rotatable bonds were defined as shown in Figure 1. For the systematic conformational search, rotatable bonds 1, 2, and 4 were examined using 5° increments with no charges on the molecule. An energy window of 5 kcal/mol greater than that of the starting conformation was used to limit the number of final conformations. For random searches, all four rotatable bonds were examined and calculations were carried out with Gasteiger−Hückel charges and a 10 kcal/mol energy window (only conformations with energies no more than 10 kcal/mol greater than the original conformation were retained). Conformations identified by the random search procedure were retained only when certain difference criteria were met (rms threshold, 0.20; data convergence, 0.005).

On the basis of these conformational analyses, a conformation for **BPP** was selected that showed a reasonable overlap with the crystal structure of the rigid σ_1 selective compound pentazocine.[27] $\tau_1 = -78°$ (C−C−NH−C(O)); $\tau_2 = -180°$ (C−NH−C(O)−C); $\tau_3 = 49°$ (NH−C(O)−C−C(ar)); and $\tau_4 = 55°$ (C(O)−C−C(ar)−C(ar)). This conformation was approximately 0.5 kcal/mol higher in energy than the global minimum conformation identified by the random search method. The benzylic group pendant to the piperidine nitrogen was also examined for low-energy conformations. Three low-energy conformational regions (with similar minima) were identified. The conformation most closely aligning to pentazocine was chosen for use in the modeling studies for all other compounds. Conformations of other compounds were prepared following modification of **BPP**. In some cases, where large divergence was observed from the beginning conformation on full minimization, a limited number of iterations were applied (200) to allow reduction of the most serious steric and electrostatic interactions. For a few compounds where large changes in conformation occurred with any degree of minimization, torsional constraints were introduced to maintain a similar structure to that of **BPP**. In cases where ortho and meta substituents on the aromatic rings could take on several orientations (for example, the OCH_3 group) and where the rings themselves could be oriented to place the substituents either above or below the plane of the piperidine ring, conformations were chosen in which substituents were oriented below the plane of the piperidine ring (as indicated by arrows in Figure 1) and placed in an orientation similar to other nonsymmetrical substituents. Energy differences are typically small (less than 0.2 kcal) for these various conformational changes.

CoMFA. Compounds chosen for inclusion in the analysis set were acetamides from this study and from our previous work.[18] Out of 81 possible acetamides, 79 were included in the initial compound set for analysis. Prior to CoMFA, conformations were aligned using a least squares fit (where bond lengths, bond angles, etc. were held fixed and all alignment points were given equal weight) to all heavy atoms of the piperidine ring and the amide nitrogen of **BPP**. Following alignment, conformations for each compound were placed in a grid of C-sp^3 probe atoms (+1 charges) spaced at 2 Å intervals and extending 4 Å from the overall molecular area. Regions were automatically defined, and steric and electrostatic interactions for each grid point were then evaluated for all atoms in each molecule and added to the table as a CoMFA field. Default values of ±30 kcal/mol were used to truncate the fields. Electrostatic energies within common steric regions[28] were not dropped in these calculations. Initially, crossvalidated (leave-one-out) partial least squares analyses[29] were performed with the number of groups equal to the number of molecules. Up to 10 components were allowed in these initial analyses, with the results used to identify the optimum number of components to use in the final non-crossvalidated analysis. Final analyses were carried out with CoMFA standard scaling. Crossvalidated q^2 (r_{cv}^2), conventional (non-crossvalidated) r^2, F statistic, standard error of the estimate, probability of $r^2 = 0$, steric and electrostatic field coefficients (normalized), and fraction of contribution values were calculated and are presented in the text or tables.

For CoMFA analyses, steric and electrostatic interactions that result in marked changes in activity are typically represented as three-dimensional coefficient contour diagrams. Points are mapped as polyhedra with the polyhedra outer surfaces drawn when the product of a specific term coefficient (steric or electrostatic) and the standard deviation of the associated column is greater than or less than a standard cutoff value, indicating a strong influence of change in that term in that region of space for one or more analogues. By color-coding these regions of space, information may be conveyed on the effects of increases or decreases in steric bulk or electrostatic charge in a given area. Steric CoMFA contributions were set at the 80 and 25% levels, with green areas as those where steric bulk increases binding and areas where steric bulk decreases binding color-coded yellow. Electrostatic CoMFA contributions were set at the 85 and 15% level, with areas where increasing negative charge correlates with an increase in binding color-coded red and areas where an increase in positive charge leads to an increase in binding color-coded blue.

CoMFA Analysis. All crossvalidated analyses were carried out with CoMFA standard scaling and a 2.0 kcal filter. Final non-crossvalidated analyses were carried

out with CoMFA standard scaling and no filter. Electrostatic and steric components of the CoMFA analyses were calculated using default values.

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