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Synthesis and in Vitro Biological Evaluation of Carbonyl Group-Containing Analogues for σ_1 Receptors

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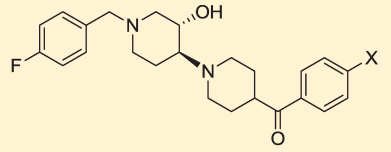
Supporting Information

ABSTRACT: To identify the ligands for σ_1 receptors that are potent and selective, analogues of prezamicol and trozamicol scaffolds of carbonyl-containing vesicular acetylcholine transporter (VAChT) inhibitors were explored. Of the 23 analogues synthesized and tested, 5 displayed very high affinity for σ_1 (K_i = 0.48−4.05 nM) and high selectivity for σ_1 relative to σ_2 receptors (σ_1/σ_2 selectivity of >749-fold). Four of the five compounds (**14a**, **14b**, **14c**, and **14e**) showed very low affinity for VAChT (K_i > 290 nM), and the fifth compound (**14g**) showed moderate affinity for VAChT (K_i = 44.2 nM). The compound [1′-(4-fluorobenzyl)-3′-hydroxy[1,4′]bipiperidinyl-4-yl]-(4-fluorophenyl)methanone (**14a**) displayed very high affinity and selectivity for σ_1 receptor (K_i = 0.48 nM, σ_1/σ_2 > 3600). All four of these most promising compounds (**14a**, **14b**, **14c**, and **14e**) can be radiosynthesized with fluorine-18 or carbon-11, which will allow further evaluation of their properties as PET probes for imaging σ_1 receptor in vivo.



X= F
14a

$K_{i\text{-}\sigma_1}$ = 0.48 ± 0.14 nM
$K_{i\text{-}\sigma_2}$ = 1740 ± 280 nM
σ_1/σ_2 = 3600
$K_{i\text{-}VAChT}$ = 1360 ± 295 nM
Ki-D_2 = 1350 ± 53 nM
Ki-D_3 > 20,000 nM
Log P = 2.83

X= OCH_3
14e

$K_{i\text{-}\sigma1}$ = 2.51± 0.34 nM
$K_{i\text{-}\sigma2}$ = 2788 ± 71 nM
σ_1/σ_2 = 1111
$K_{i\text{-}VAChT}$ = 294 ±16.1 nM
Ki-D_2 = 1489 ± 114 nM
Ki-D_3 > 15,000 nM
Log P = 2.82

1. INTRODUCTION

σ receptors were discovered in the 1970s,[1] and they were found to exhibit σ_1 and σ_2 subtypes.[2,3] Instead of opioid receptors as originally thought, they now are widely accepted as unique and independent.[4] The σ_1 receptor shares no homology with other mammalian proteins[5] and consists of 223 amino acids. It has been cloned and functionally expressed.[6] The σ_2 receptor has not been cloned, and its structure has not been identified yet.[7]

Studies discovered that σ_1 receptors are highly expressed in the brain and several peripheral organs that include heart, spleen, kidney, liver, lung, ovary, testes, and placenta.[8,9] The σ_1 receptors are located in cytoplasmic, endoplasmic reticulum, and mitochondrial membranes.[9] During development, elevated levels of σ_1 receptors are found in embryonic stem cells at all stages of embryogenesis.[9] In the central nervous system (CNS), the highest level of σ_1 transcripts is expressed in various cranial nerve nuclei, followed by mesencephalic structures that include red nucleus, periaqueductal gray matter, and substantia nigra, and in some diencephalic structures that include habenula and arcuate and paraventricular and ventromedial hypothalamic nuclei. Moderate levels of σ_1 transcripts were found in superficial and deeper cortical laminar layers, and mRNA of σ_1 receptor is found in the pyramidal cell layer and the dentate gyrus of the hippocampus formation.[10] Also in the brain, thalamus and amygdaloid body express the σ_1 receptor.[10]

Investigations have revealed that the σ_1 receptor is involved in different functions associated with the central nervous, endocrine, motor, and immune systems.[11,12] In the central nervous system, σ_1 receptor regulates neurotransmitter release, modulation of neurotransmitter receptor functions, learning and memory, and movement and posture control. On the basis of its broad modulatory effects,[13] the σ_1 receptor is believed to play an important role in neuropsychiatric and neurodegenerative diseases.[14−16] Recent studies have shown that several σ_1 receptor agonists have positive therapeutic effects on diseases such as schizophrenia,[17] major depression,[16] Alzheimer's disease (AD),[18] and substance abuse.[19] Thus, σ_1 receptors are now considered as a therapeutic target for the treatment of depression, anxiety, schizophrenia, and Alzheimer's disease.[20−22]

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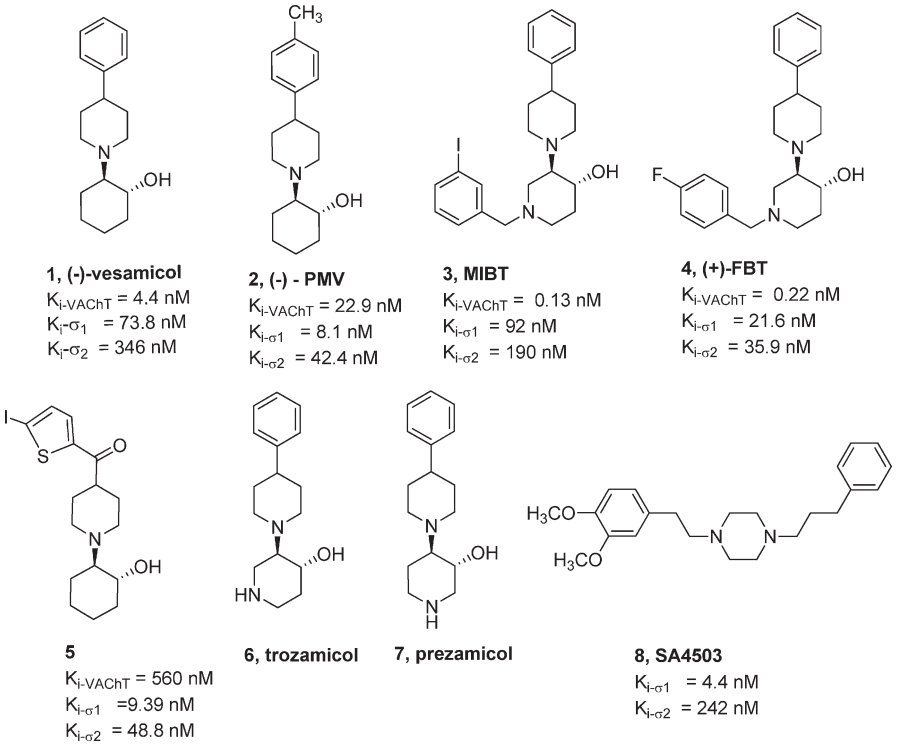

Figure 1. Representative structures that are potent for σ_1 receptors.

Studies of cancer have revealed that σ_1 receptors are overexpressed in breast cancer, small-cell and non-small-cell lung carcinoma, renal carcinoma, colon carcinoma, sarcoma, brain tumors, melanoma, glioblastoma, neuroblastoma, and prostate cancers.[23−26] On the basis of the expression pattern of σ_1 receptors in numerous tumors, ligands for these receptors have potential for both imaging and therapy.[27] Administration of σ ligands inhibits ex vivo growth of tumor cells derived from human mammary adenocarcinoma, colon carcinoma, and melanoma.[28,29] For example, the σ_1 receptor ligand rimcazole inhibits cellular proliferation and induces cell death, although the mechanism is not clear.[30]

The σ_1 receptor regulates the activity of diverse ion channels via protein−protein interactions, exhibits stereoselective drug binding, and binds different types of drugs that exhibit structural diversity.[31−33] The activation of σ_1 receptors promotes both neuronal differentiation and antiapoptotic action, potentially leading to cancer. The σ_1 receptor is a ligand-regulated mitochondrial membrane-associated endoplasmic reticulum (ER) protein receptor. σ_1 receptor ligands as potential therapeutic drugs may lead to decreased oxidative stress in mammalian cells and may have antitumorigenic activity.[30] Specific σ_1 ligands that have high specific binding and suitable pharmaceutical properties may be potential therapeutic agents for the treatment of cancer.[29,34] Although the importance of σ_1 receptor in CNS disorders and cancer is recognized, details of the pathophysiological functions of σ_1 receptor in the brain are still not clear.

Positron emission tomography (PET) is an elegant noninvasive imaging modality that can provide functional information about cellular processes. It has been used to quantify the density of σ_1 receptors in brain in vivo. A suitable clinical PET probe targeting the σ_1 receptors will advance our understanding of the functions of σ_1 receptors in the CNS and allow monitoring of the efficacy of disease treatment with σ_1 ligands.[35] Investigators have made tremendous efforts to identify PET probes for imaging σ_1 in vivo.[36−39] To date, the ^{11}C-labeled PET radiotracer [^{11}C]-SA4503 is the only PET probe for imaging σ_1 receptor in neurological applications.[40,41] Compared to ^{11}C ($t_{1/2}$ = 20.4 min), ^{18}F has a longer half-life ($t_{1/2}$ = 109.8 min) that places fewer time constraints on radiotracer synthesis and permits longer scan sessions usually resulting in higher target/nontarget ratios. However, there is no report of an ^{18}F-labeled probe for the σ_1 receptor that is clinically suitable.[17] Identifying such a probe is imperative for diagnostic and therapeutic applications in CNS disorders and cancer.

In the search for highly selective and clinically suitable σ_1 ligands, investigations have revealed that high affinity σ_1 ligands must contain a basic amine having two hydrophobic appendages.[7,42] Structure−activity relationship analysis of vesamicol ligands (Figure 1), which bind to the vesicular acetylcholine transporter (VAChT) in presynaptic cholinergic nerve terminals, showed that many vesamicol ligands contain a pharmacophore that also has high affinity for σ_1 receptors.[43] In fact, some of the VAChT ligands show moderate to high binding affinities for σ_1 receptor. As shown in Figure 1, (−)-vasamicol (**1**) has moderate σ_1 receptor binding affinity of 73.8 nM,[44] and its methyl substituted derivative (−)-*p*-methylvesamicol (**2**) has high σ_1 receptor binding affinity of 8.10 nM;[44] the trozamicol analogues *m*-iodobenzyltrozamicol (**3**) and (+)-4-fluorobenzyltrozamicol (**4**) have σ_1 receptor binding affinities of 92 and 21.6 nM, respectively.[43,45] Several carbonyl containing VAChT analogues also bind to σ_1 very well; for example, the binding affinity of (1*S*,2*S*)-2-(4-(5-iodothiophen-2-yl)piperidin-1-yl)cyclohexanol (**5**) is high with a K_i of 9.39 nM for σ_1 receptors.[46] To identify new, highly selective ligands for σ_1 receptors, our strategies are to (1) replace the 4-phenylpiperidinyl group in the prezamicol structure

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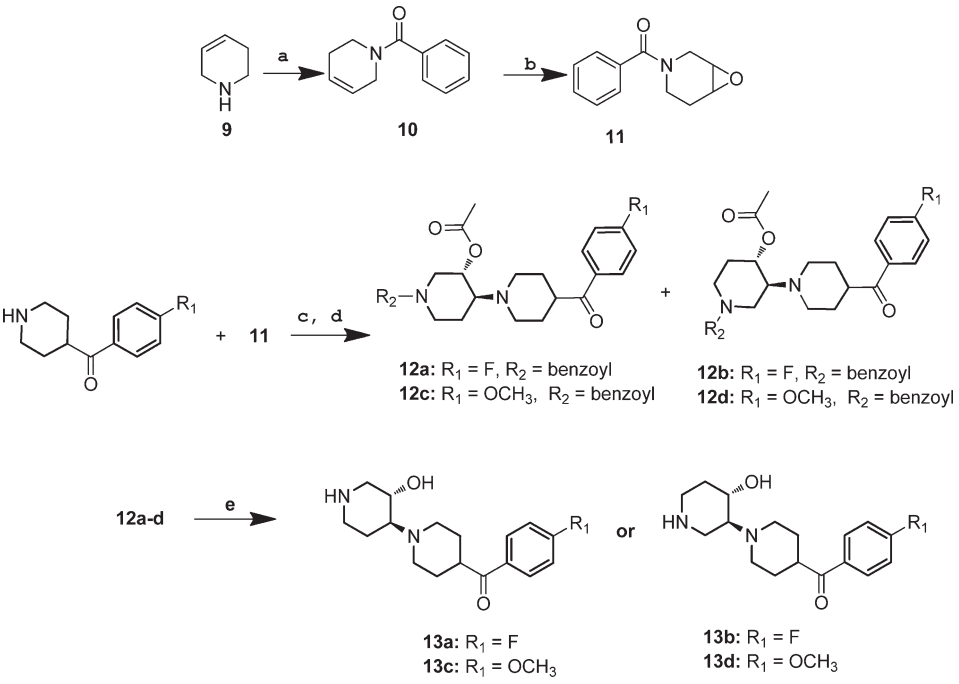
Scheme 1. Synthesis of Compounds 13a−d[a]



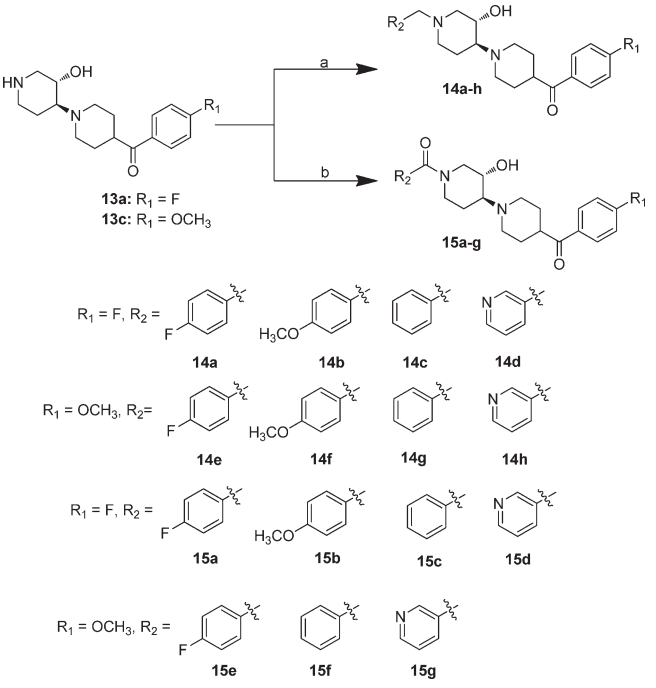
[a] Reagents: (a) benzoyl chloride, $(C_2H_5)_3N$; (b) *m*-CPBA, CH_2Cl_2; (c) 4-substituted benzoyl piperidinehydrochloride, $(C_2H_6)_3N$, ethanol, 70 °C; (d) acetic anhydride, CH_2Cl_2; (e) 6 N HCl, reflux.

with a 4-substituted benzoylpiperidinyl group, which is known to favor σ receptors,[46] (2) alkylate or acylate the secondary amine in (3′-hydroxy-1,4′-bipiperidin-4-yl)(4-substituted phenyl)methanone, which is structurally similar to the prezamicol scaffold, and (3) alkylate the secondary amine in (4′-hydroxy-1,3′-bipiperidin-4-yl)-(4-substituted phenyl)methanone, which is structurally similar to the trozamicol scaffold. In the current paper, we detail our exploration of these new compounds to identify the ligands that have high affinity for σ_1 receptors and selectivity for σ_1 versus σ_2 receptors. This investigation was inspired by (1) the observation that σ_1 receptor ligands may be potential therapeutic drugs for the treatment of neurological disorders[4] and cancer[34] and (2) the need for highly selective and potent σ_1 receptor ligands that can be labeled with fluorine-18 or Carbon-11. Moreover, a novel clinical PET probe for imaging the σ_1 receptor will provide a unique tool to assess the relationship between changes in σ_1 receptors in the brain during the progression of CNS disorders and provide a useful tool to monitor the treatment efficacy of the CNS disorders and cancer.

2. RESULTS AND DISCUSSION

Chemistry. The target compounds were synthesized as depicted in Schemes 1−3. The synthesis started with 1,2,3,6-tetrahydropyridine, in which the secondary amine was first protected by reacting with benzoyl chloride to form benzamide **10**. The alkenyl double bond of compound **10** was oxidized by *m*-chloroperoxybenzoic acid (*m*-CPBA) to form epoxide **11**. The epoxide **11** was refluxed with substituted 4-benzoylpiperidine hydrochloride salts, namely, either 4-(4′-fluorobenzoyl)piperidine hydrochloride or 4-(4′-methoxybenzoyl)piperidine hydrochloride, in ethanol with triethylamine as the base to afford tertiary amino alcohol intermediates. To improve the yield, commercially

Scheme 2. Synthesis of Compounds 14a−h and 15a−g[a]



[a] Reagents: (a) substituted benzyl halide, $(C_2H_5)_3N$, CH_2Cl_2; (b) substituted benzoic acid, BOP-Cl, $(C_2H_5)_3N$, CH_2Cl_2.

available 4′-substituted 4-benzoylpiperidine hydrochloride salts were used in excess and the reaction temperature was kept below 75 °C. This reaction condition afforded (3′-hydroxy-1,4′-bipiperidin-4-yl)(4-substituted phenyl)methanone intermediates

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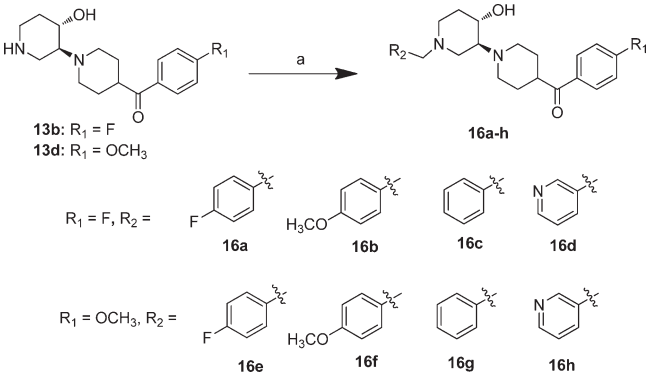
Scheme 3. Synthesis of Compounds 16a−h[a]



[a] Reagents: (a) substituted benzyl halide, $(C_2H_5)_3N$, CH_2Cl_2.

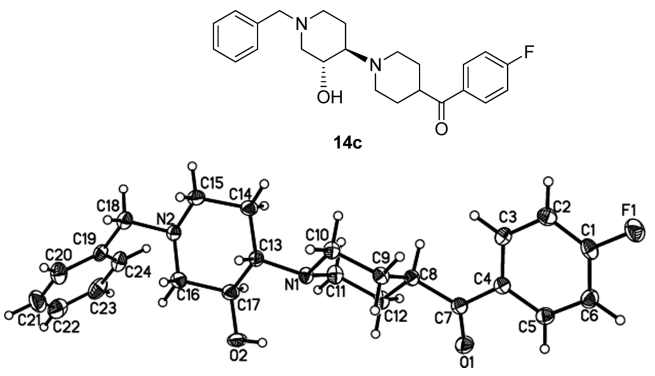


Figure 2. Chemical structure and X-ray crystal structure of **14c**.

and their regioisomers, (4′-hydroxy-1,3′-bipiperidin-4-yl)(4-substituted phenyl)methanone intermediates. The separation of these regioisomers by silica gel chromatography was difficult. To overcome the difficulty, the mixture of regioisomers was reacted with acetic anhydride to convert the free hydroxyl group to the corresponding acetates **12a**−**d**. Subsequently, **12a** and **12b** were separated easily by silica gel chromatography.

Under strongly acidic conditions, hydrolysis of **12a** and **12b** to remove both the acetyl and benzoyl groups afforded key intermediates **13a** and **13b**. A similar procedure was used to make corresponding regioisomers **13c** and **13d**. Intermediates **13a** and **13c** are similar in structure to prezamicol, and **13b** and **13d** are similar in structure to trozamicol (Scheme 1). Starting with key intermediates **13a** and **13c**, the target compounds **14a**−**h** were obtained via N-alkylation with various substituted benzyl halides in yields ranging from 34% to 84%. To assign the regiochemistry and relative stereochemistry of the compounds obtained in the oxirane ring-opening reaction, X-ray crystal structure of compound, **14c** was carried out. The regiochemistry (the nitrogen of piperidine ring is on C13 and hydroxyl group on C17, Figure 2) was assigned based on the X-ray structure of **14c**. The oxirane ring-opening reaction was performed under the basic conditions. As predicted, the relative stereochemistry of the ring-opening resulted in the trans stereochemistry (C13 and C17 in Figure 2). All the substituted benzyl halides were obtained commercially and were used in slight excess (Scheme 2). Target compounds **15a**−**g** were obtained by coupling various substituted benzoic acids with piperidine intermediates **13a** and **13c** using bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl) in yields ranging from 22% to 90%. Compounds **16a**−**h** were synthesized by following the similar procedure to synthesize compounds **14a**−**h** except that the trozamicol analogues **13b** and **13d** were utilized (Scheme 3). All the products were converted into oxalates for determining the bioactivity affinities.

Biological Binding Affinity Studies. The σ_1 and σ_2 binding affinities (K_i, nM) of the new compounds were determined by using the competitive inhibition method with tritiated σ ligands according to reported procedures.[7,42] The σ_1 binding sites were assayed by using guinea pig brain membranes with the selective radioligand (+)-[^{3}H]pentazocine. The σ_2 binding sites were assayed in rat liver membranes, a rich source of these sites, with [^{3}H]DTG in the presence of (+)-pentazocine (100 nM) to mask σ_1 sites. VAChT binding was assayed using highly expressed human VAChT assayed with homogenized and partially clarified PC12[123.7] cells by displacement of bound 5 nM (−)-[^{3}H]vesamicol. Apparent dissociation constants for binding of the novel compounds in vitro are shown in Table 1.

Binding affinity data identified a number of potentially useful structure−activity trends. First, these new analogues generally have very low affinity for σ_2 receptors (K_i > 1000 nM). For VAChT, only compounds **14g**, **16e**, and **16g** display moderate affinity of 44.2 ± 3.03, 48.6 ± 8.37, and 35.5 ± 11.1 nM. For the other ligands, the K_i values are greater than 100 nM. On the other hand, compounds **14a**, **14b**, **14c**, **14e**, and **14g** displayed very high affinity for σ_1 receptor (K_i < 5 nM) and very good selectivity for σ_1 versus σ_2 receptors (>1000-fold). Among these latter compounds, **14a** displayed the highest affinity for σ_1 receptors (K_i = 0.48 ± 0.14 nM) and very high selectivity for σ_1 versus σ_2 receptors (>3000 fold) and for σ_1 receptors vs VAChT (2800 fold). These results suggest that **14a** has high potency and high selectivity for the σ_1 receptor.

More importantly, **14a** has two fragments that contain a fluorine atom. One is in the para-position of the carbonyl group in 4-fluorobenzoylpiperidine fragment, which is easy to label by displacement of a nitro group with $^{18}F^-$. The other fluorine atom is in the para-position of the 1-(4-fluorobenzyl)piperidin-3-ol fragment. The position of this fluorine atom allows incorporation of fluorine-18 in two steps. The first step is to make 4-[^{18}F]-fluorobenzyl iodide,[47] and the second step is to make the final labeled radiotracer by N-alkylation of **13a** with 4-[^{18}F]-fluorobenzyl iodide. Labeling **14a** with fluorine-18 at different positions also provides a unique way to investigate the metabolic stability of **14a** in vivo by determining which fragment of the structure contains the radioactivity from fluorine-18. This information will be very useful for guiding further structural optimization of **14a** to identify a metabolically stable PET tracer favorable for clinical use. In addition, the moderate lipophilicity of **14a** (log P = 2.83) suggests that compound **14a** has good ability to enter the brain and has high potential to be a suitable PET imaging probe or therapeutic agent for CNS disorders.

In a comparison of compounds **14a**−**h** with **15a**−**g**, *N*-benzyl substituted ligands displayed significantly higher binding affinities for σ receptors, particularly for σ_1 receptor sites, whereas *N*-benzoyl substituted ligands displayed low binding affinities for σ receptor. For example, when the 4-fluorobenzoyl group in **15a** was replaced by the 4-fluorobenzyl group in **14a**, the σ_1 receptor affinity had increased dramatically, changing the K_i from 3144 nM for **15a** to 0.48 nM for **14a**. The same trend was observed in all of the other corresponding compounds. In general, compounds containing substituted benzyl groups preferred to bind to σ_1

Table 1. Affinities of New Analogues for σ_1 Receptor, σ_2 Receptor, and VAChT[a]

	K_i, nM					
compd	σ_1 [b]	σ_2 [c]	VAChT [d]	selective ratio of σ_1 vs σ_2	selective ratio of σ_1 vs VAChT	log P [e]
1[f]	73.8 ± 11.2	346 ± 37	4.4 ± 0.6	4.6	16.7	1.40
14a	0.48 ± 0.14	1741 ± 286	1360 ± 295	3627	2833	2.83
14b	4.03 ± 0.47	5521 ± 1352	3310 ± 907	1370	821	2.61
14c	1.36 ± 0.28	2301 ± 249	401 ± 42.0	1692	295	2.73
14d	22.8 ± 2.32	4208 ± 115	2030 ± 385	184	89	1.48
14e	2.51 ± 0.34	2788 ± 718	294 ± 16.1	1111	117	2.82
14f	25.9 ± 0.96	5157 ± 202	14800 ± 3460	199	569	2.61
14g	4.05 ± 0.88	3033 ± 248	44.2 ± 3.03	749	11	2.72
14h	59.64 ± 2.22	4540 ± 1606	137 ± 14.3	76	2.2	1.46
15a	3144 ± 140	8642 ± 812	3600 ± 499	2.7	1.1	1.92
15b	2238 ± 271	>10000	30900 ± 6400	>4.5	14	2.25
15c	2833 ± 374	20450 ± 4887	683 ± 90	7.2	0.24	1.67
15d	7739 ± 662	13565 ± 1435	3340 ± 706	1.7	0.43	0.57
15e	2088 ± 154	32850 ± 443	3460 ± 476	15.7	1.66	1.90
15f	2061 ± 113	15862 ± 3045	555 ± 65.4	7.7	0.27	1.65
15g	26646 ± 8738	17041 ± 8626	372 ± 65.7	0.64	0.01	0.56
16a	50.0 ± 7.9	3443 ± 928	136 ± 13.8	69	2.72	1.98
16b	91.1 ± 19.9	4979 ± 507	1970 ± 196	54	22	1.73
16c	106 ± 28	832 ± 147	149 ± 22.5	7.8	1.4	1.88
16d	1159 ± 128	13018 ± 2626	237 ± 41.4	11	0.20	0.79
16e	137 ± 21	5598 ± 1033	48.6 ± 8.37	41	0.35	2.00
16f	297 ± 27	4208 ± 439	1080 ± 296	14	3.6	1.75
16g	208 ± 53	8539 ± 1900	35.5 ± 11.1	41	0.17	1.89
16h	2225 ± 168	17135 ± 3863	107 ± 11	7.7	0.40	0.80

[a] K_i values (mean ± SEM) were determined in at least three experiments. [b] The σ_1 binding assay used membrane preparations of guinea pig brain. [c] The σ_2 binding assay used homogenates of rat liver. [d] The VAChT binding assay used expressed human VAChT. [e] Calculated value at pH 7.4 with ACD/I-Lab, version 7.0 (Advanced Chemistry Development, Inc., Canada). [f] Values from ref 44.

receptor sites; **14a**−**h** displayed very high σ_1 binding affinities in which the K_i ranged from 0.48 nM for **14a** to 59.6 nM for **14h**. However, compound **14g** showed moderate VAChT binding affinity (44.2 ± 3.03 nM), whereas the other compounds **14a**−**f** and **14h** displayed lower VAChT binding affinities. The observation that compounds containing the substituted benzoyl groups **15a**−**g** led to a dramatic decrease in σ_1 receptor binding affinities (K_i > 1000 nM) is consistent with results reported in the literature.[42] This confirms that a carbonyl group between the substituted aromatic ring and the piperidine moiety plays an important role by affecting σ_1 receptor binding affinities. Incorporating the carbonyl group leads to more rigidity in the structure and might prevent optimal interaction of ligands with the σ_1 receptor binding site.

When the electron-withdrawing group fluoride (**14a**−**d**) was replaced with the electron-donating group methoxy (**14e**−**h**) at the para-position of the 4-substitued benzoylpiperidine fragment, the σ_1 binding affinities (nM) were decreased. For compounds **14a**−**d**, σ_1 binding affinities (nM) were 0.48 ± 0.14, 4.03 ± 0.47, 1.36 ± 0.28, and 22.8 ± 2.32, respectively; for compounds **14e**−**h**, the σ_1 binding affinities (nM) were 2.51 ± 0.34, 25.9 ± 0.96, 4.05 ± 0.88, and 59.64 ± 2.22, respectively. The decrease in affinity ranged from 2.6-fold for **14h** vs **14d** to 6.4-fold for **14b** vs **14f**.

From a comparison of the structures of **14a**, **14b**, and **14c**, the only difference among them is the substitution at the para-position of the benzyl moiety on the terminal piperidine ring of (3′-hydroxy-1,4′-bipiperidin-4-yl)(4-fluorophenyl)methanone. The σ_1 K_i values (nM) are displayed in the order of −F > −H > −OCH_3 with the dissociation constant values being 0.48 ± 0.14 for **14a**, 1.36 ± 0.28 for **14c**, and 4.03 ± 0.47 for **14b**. Compared with **14b**, incorporating an electron-withdrawing fluorine atom at the 4-position of the benzyl group (**14a**) diminished the basicity of the nitrogen atom in the terminal piperidine ring and the ligand affinity for σ_1 receptor was increased by 2.8 fold; in contrast, incorporating an electron-donating methoxyl group (**14b**) enhanced the basicity of the nitrogen atom in the terminal piperidine ring and the ligand affinity for σ_1 receptor was decreased by approximately 3-fold. A similar trend was observed in 4-methoxybenzoyl containing compounds **14e**, **14f**, and **14g**. To further test the effect of the electron density on the benzyl ring, compounds **14d** and **14h** containing the relatively high electron-density pyridine ring, with the N-atom in the meta-position, were synthesized and screened. However, a decrease in σ_1 binding affinities was observed. For **14d**, the σ_1 binding affinity was decreased more than 17-fold as expressed by K_i changing from 1.36 ± 0.28 nM for **14c** to 22.8 ± 2.32 nM for **14d**. For **14h**, the σ_1 binding affinity was decreased 15-fold as expressed by K_i changing from 4.05 ± 0.88 nM for **14g** to 59.64 ± 2.22 nM for **14h**. To clearly understand how the electron density of the benzyl ring affects basicity of the nitrogen atom in the piperidine rings and results in the changes of σ_1 binding affinity, further structure−affinity investigation of these analogues is necessary.

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Table 2. D_2, D_3, and 5-HT_{1A} Affinities ($K_i \pm$ SD, nM) of Compounds 14a and 14e[a]

compd	σ_1, K_i (nM)	D_2, K_i (nM)[b]	D_3, K_i (nM)[b]	5-HT_{1A}, K_i (nM)[c]
14a	0.48 ± 0.14	1350 ± 53	>20,000	2540 ± 5.6
14e	2.51 ± 0.34	1489 ± 114	>15,000	ND

[a] K_i values (mean ± SEM) were determined in at least three experiments. [b] K_i values (mean ± SEM) for D_2 and D_3 binding assays were determined according to our published procedure.[49] [c] K_i values (mean ± SEM) for 5-HT_{1A} binding assay were determined according to the protocol with minor modification.[50]

The compounds with a prezamicol scaffold (**14a**−**h**) displayed substantially higher affinities for σ_1 receptors than compounds with a trozamicol scaffold (**16a**−**h**). The only structural difference between prezamicol and trozamicol is the position of the nitrogen atom in the terminal piperidine ring. Some trozamicol scaffold analogues displayed moderate σ_1 receptor binding affinities, for example, **16a** (50.0 ± 7.9 nM) and **16b** (91.1 ± 19.9 nM). The difference in binding affinities for prezamicol and trozamicol analogues is 11-fold for **14f** vs **16f** (25.9 ± 0.96 nM vs 297 ± 27 nM) and 104-fold for the most potent **14a** vs **16a** (0.48 ± 0.14 nM vs 50.0 ± 7.9 nM).

Since our initial in vitro screening showed that **14a** ($K_{i\text{-}\sigma 1}$ = 0.48 ± 0.14 nM, 3627-fold for σ_1 vs σ_2, 2833-fold for σ_1 vs VAChT) and **14e** ($K_{i\text{-}\sigma 1}$ = 2.51 ± 0.34 nM, 1111-fold for σ_1 vs σ_2, 117-fold for σ_1 vs VAChT) had high binding specificity for σ_1 receptors, both compounds **14a** and **14e** will be easy to label with carbon-11 or fluorine-18 to test the feasibility of imaging σ_1 receptors in animals. We also determined the D_2 and D_3 affinities for both **14a** and **14e** and 5-HT_{1A} affinity for **14a.** The in vitro binding data suggested that **14a** and **14e** had very low affinities for D_2, D_3, and $5HT_{1A}$ as shown in Table 2. This observation eliminates the concern that D_2, D_3, and 5-HT_{1A} receptor binding might interfere with the imaging signal when ^{11}C or ^{18}F labeled ligands of **14a** or **14e** are used for PET imaging studies of the σ_1 receptors in vivo.

Overall, (1) N-alkylation with benzyl groups of (3′-hydroxy-1,4′-bipiperidin-4-yl)(4-substituted phenyl)methanone to form tertiary amines generally gives very high σ_1 receptor binding affinities and high selectivity for σ_1 receptor vs σ_2 receptor and VAChT sites, (2) N-acylation with benzoyl groups of (3′-hydroxy-1,4′-bipiperidin-4-yl)(4-substituted phenyl)methanone to form tertiary amides diminishes the σ_1 receptor binding affinity, and (3) prezamicol analogues display high σ_1 receptor binding affinities, whereas trozamicol analogues display moderate to low σ_1 receptor binding affinities. Although it is well-known that σ_1 receptor distinguishes between enantiomers,[31,32] only racemic mixtures are used in this study. Further study will be focused on resolving the racemic mixtures to obtain enantiomeric isomers and conduct the binding affinity screening for the enantiomeric isomers. It is expected that enantiomers of compounds **14a**−**c** and **14e** will provide compounds with improved σ_1 binding affinity and selectivity. In addition, the in vitro affinities of **14a** and **14a**−**c** and **14e** binding to other neurotransmitter receptors, transporters, enzymes, and ion channels will be performed to determine if **14a**−**c** and **14e** bind to σ_1 receptor specifically.

3. CONCLUSION

In the present study, we synthesized a new class of compounds targeting σ_1 receptors. In an effort to identify compounds that have high affinity and selectivity for σ_1 receptors, we explored carbonyl containing VAChT inhibitors by introducing substituted benzyl or benzoyl groups on the nitrogen of the terminal piperidine ring. From this study, we have identified five racemic compounds, **14a** (0.48 nM), **14b** (4.03 nM), **14c** (1.36 nM), **14e** (2.51 nM), and **14g** (4.05 nM), that have very high binding affinities for σ_1 receptors. The first four compounds also displayed high selectivity for σ_1 vs σ_2 receptors and do not bind well to VAChT. They have the potential to be useful pharmacological agents targeting σ_1 receptors. More importantly, introducing fluorine-18 or carbon-11 isotopes into these compounds can be achieved easily. This accomplishment should permit further evaluation of these ligands to identify clinical PET probes for imaging σ_1 receptors in human beings. Particularly for **14a** and **14c**, the σ_1/σ_2 ratios are greater than 3600- and 1600-fold and the σ_1/VAChT ratios are greater than 2800- and 290-fold, respectively. After screening of the in vitro binding affinities of **14a**−**c** and **14e** with other neurotransmitter receptors, transporters, enzymes, and ion channels to confirm the σ_1 receptor binding specificity, the fluorine-18 or carbon-11 analogues of **14a**−**c** and **14e** will be synthesized to test their feasibility as useful PET probes to quantify the density of σ_1 receptors in humans. In addition, the SAR information from the current study provides new insight into σ_1 receptor ligands.

4. EXPERIMENTAL SECTION

General. All reagents and chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. All anhydrous reactions were carried out in an oven-dried and nitrogen purged glassware unless otherwise stated. Flash column chromatography was conducted using silica gel 60A, "40 Micron Flash" [32−63 μm] (Scientific Absorbents, Inc.); the mobile phase used is reported in the experimental procedure for each compound. Melting points were determined using the MEL-TEMP 3.0 apparatus and are uncorrected. ^1H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard unless otherwise stated. All chemical shift values are reported in parts per million (PPM) (δ). Peak multiplicities are singlet, s; doublet, d; triplet t; multiplet, m; broad, br. Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc. Elemental analysis and HPLC were used to determine the purity of the target compounds that were used for binding assay. All the final compounds with the biologic activity reported in the manuscript have a purity of ≥95%.

(5,6-Dihydropyridin-1(2*H*)-yl)(phenyl)methanone (10). Into a solution of 1,2,3,6-tetrahydropyridine (1.00 g, 12.0 mmol) and triethylamine (3 mL) in CH_2Cl_2 (30 mL), benzoyl chloride (1.69 g, 12.0 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 2 h and washed with saturated Na_2CO_3 (20 mL × 3) and brine solution (20 mL), dried over Na_2SO_4, and concentrated under vacuum to afford the crude product. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate (10/3, v/v) to afford target product **10** as a colorless oil (2.27 g, 99.5%). ^1H NMR ($CDCl_3$): δ 2.16−2.25 (m, 2H), 3.46−4.20 (m, 4H), 5.53−5.91 (m, 2H), 7.29−7.53 (m, 5H).

7-Oxa-3-azabicyclo[4.1.0]heptan-3-yl(phenyl)methanone (11). Into a solution of **10** (2.27 g, 12.1 mmol) in CH_2Cl_2 (20 mL), a solution of 3-chloroperoxybenzoic acid (5.43 g, 77% pure, 24.3 mmol) in CH_2Cl_2 (40 mL) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 5 h. After the reaction was complete as determined by TLC (hexane/ethyl acetate, 1/1, v/v), saturated Na_2CO_3 solution (50 mL) was added into the reaction vial slowly with stirring.

The reaction mixture was stirred for 30 min. The organic layer was washed with saturated Na_2CO_3 solution (50 mL × 3), brine solution (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate (3/1, v/v) as the mobile phase to afford the product as a sticky oil (1.54 g, 62%). ^{1}H NMR ($CDCl_3$): δ 2.04−2.18 (m, 2H), 3.09−4.40 (m, 6H), 7.28−7.47 (m, 5H).

Procedure A: General Method for Preparation of 1′-Substituted Benzoyl-4-acyl-[1,4′-bipiperidin]-3′-yl Acetates (12a and 12c) and 1′-Substituted Benzoyl-4-acyl-[1,3′-bipiperidin]-4′-yl Acetates (12b and 12d). A mixture of **11** (0.50 g, 1.97 mmol), 4-(4-fluorobenzoyl)piperidine hydrochloride (1.20 g, 4.92 mmol), and triethylamine (1 mL) in ethanol (50 mL) was heated to 70 °C with stirring for over 24 h. The reaction mixture was cooled, filtered, and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (50 mL) and washed with water (50 mL), saturated Na_2CO_3 (50 mL), and brine solution. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude product as an oil. Acetic anhydride (1.11 g, 10.92 mmol) was added to a solution of this crude product in CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature overnight. The mixture was washed with saturated Na_2CO_3 (30 mL × 3) and brine solution. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using ethyl acetate/hexane (2/1, v/v) as the mobile phase to afford **12a** (0.41 g, 37%), the first eluted compound as a colorless oil. ^{1}H NMR ($CDCl_3$): δ: 1.40−1.74 (m, 3H), 2.04 (s, 3H), 2.30−2.37 (m, 1H), 2.51−2.69 (m, 2H), 2.86−3.21 (m, 6H), 4.30−4.70 (m, 1H), 4.80−5.10 (br s, 1H), 7.13 (t, J = 5.7 Hz, 2H), 7.40−7.41 (m, 5H), 7.93−7.97 (m, 2H). At the same time, 1′-benzoyl-4-(4-fluorobenzoyl)-[1,3′-bipiperidin]-4′-yl acetate (**12b**) (0.50 g, 45%) was eluted as the second compound and **12b** was a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.50−1.90 (m, 3H), 2.07 (s, 3H), 2.81−3.29 (m, 6H), 2.20−2.80 (m, 4H), 3.50−4.00 (m, 2H), 4.64 (br s, 1H), 5.10−5.18 (m, 1H), 7.12 (t, J = 5.7 Hz, 2H), 7.41−7.42 (m, 5H), 7.93 (m, 2H).

1′-Benzoyl-4-(4-methoxybenzoyl)-[1,4′-bipiperidin]-3′-yl Acetate (12c) and 1′-Benzoyl-4-(4-methoxybenzoyl)-1,3′-bipiperidin-4′-yl Acetate (12d). Procedure A was followed with 4-(4-methoxybenzoyl)piperidine hydrochloride, **11**, triethylamine, and acetic anhydride to afford **12c** (0.42 g, 37%) as a colorless oil that was eluted first and **12d** (0.34 g, 37%) as a colorless oil that was eluted second. ^{1}H NMR of **12c** ($CDCl_3$): δ 1.40−1.90 (m, 8H), 2.04 (s, 3H), 2.22−2.40 (m, 1H), 2.24−2.70 (m, 2), 2.80−3.22 (m, 4H), 3.87 (s, 3H), 4.00−4.60 (m, 1H), 6.94 (d, J = 8.7 Hz, 2H), 7.41 (s, 5H), 7.91 (d, J = 8.7 Hz, 2H). ^{1}H NMR of **12d** ($CDCl_3$): δ 1.45−1.90 (m, 7H), 2.04 (s, 3H), 2.22−2.75 (m, 2H), 2.80−3.30 (m, 5H), 3.64 (br s, 1H), 3.86 (s, 3H), 4.64 (br s, 1H), 5.10−5.20 (m, 1H), 6.92 (d, J = 8.1 Hz, 2H), 7.64 (br s, 5H), 7.89 (m, 2H).

Procedure B. General Method for Preparation of (3′-Hydroxy-[1,4′-bipiperidin]-4-yl)(4-substituted-phenyl)methanones (13a and 13c) and (4′-Hydroxy-[1,3′-bipiperidin]-4-yl)-(4-substituted-phenyl)methanones (13b and 13d). To a solution of **12a** (0.66 mmol) in ethanol (10 mL), 6 N HCl (4 mL, 2.40 mmol) was added. The reaction mixture was heated to reflux for 14 h. The mixture was cooled and concentrated under vacuum. Then 1 N NaOH solution (20 mL) was added to the residue. The aqueous phase was extracted with CH_2Cl_2 (20 mL × 3). The combined organic phases were washed with saturated sodium carbonate (20 mL × 3) and brine solution, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel column chromatography using methanol/ethyl acetate/triethylamine (50/50/2, v/v/v) as the mobile phase to afford **13a** as a colorless oil (132 mg, 66%). ^{1}H NMR ($CDCl_3$): δ 1.37−1.89 (m, 8H), 2.24−2.77 (m, 6H), 2.98−3.01 (m, 1H), 3.12−3.24 (m, 2H), 3.35−3.51 (m, 2H), 7.11−7.17 (t, J = 5.7 Hz, 2H), 7.94−7.99 (m, 2H).

(3′-Hydroxy-[1,4′-bipiperidin]-4-yl)(4-methoxyphenyl)-methanone (13b). Procedure B was followed with **12b** and 6 N HCl to afford **13b** as a colorless oil (173 mg, 76%). ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 1.56−1.92 (m, 7H), 2.41−3.47 (m, 11H), 3.62−3.70 (m, 1H), 3.87 (s, 3H), 6.99−7.02 (m, 2H), 7.94−7.98 (m, 2H).

(1′-Benzoyl-4′-hydroxy-[1,3′-bipiperidin]-4-yl)(4-fluorophenyl)methanone (13c). Procedure B was followed with **12c** to afford **13c** as a colorless oil (0.22 g, 67%). ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 1.43−1.51 (m, 1H), 1.74−1.90 (m, 5H), 2.10−2.12 (m, 1H), 2.29−2.40 (m, 2H), 2.49−2.61 (m, 2H), 2.77−2.88 (m, 2H), 3.03−3.06 (m, 2H), 3.17−3.26 (m, 2H), 3.51−3.59 (m, 1H), 7.14 (t, J = 5.4 Hz, 2H), 7.94−7.98 (m, 2H).

(1′-Benzoyl-4′-hydroxy-[1,3′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (13d). Procedure B was followed with **12d** to afford **13d** as a colorless oil (0.18 g, 82%). ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 1.56−1.92 (m, 6H), 2.07−2.11 (m, 1H), 2.41−2.80 (m, 5H), 2.80−3.00 (m, 1H), 3.00−3.18 (m, 2H), 3.20−3.40 (m, 2H), 3.62−3.70 (m, 1H), 3.87 (s, 3H), 6.99−7.02 (m, 2H), 7.94−7.98 (m, 2H).

Procedure C. General Method for Preparation of Benzyl Compounds (14a−h). (1′-(4-Fluorobenzyl)-3′-hydroxy-[1,4′-bipiperidin]-4-yl)(4-fluorophenyl)methanone (14a). Into a solution of **13a** (132 mg, 0.42 mmol) and triethylamine (17.1 mg, 1.69 mmol) in CH_2Cl_2 (15 mL), a solution of 4-fluorobenzyl bromide (80 mg, 0.42 mmol) in CH_2Cl_2 (5 mL) was slowly added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with water (20 mL × 2) and brine solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatograph using triethylamine/ethyl acetate (1/50, v/v) as mobile phase to afford **14a** as a pale yellow oil (78.9 mg, 45%). ^{1}H NMR ($CDCl_3$): δ 1.53−2.04 (m, 9H), 2.20−2.32 (m, 2H), 2.71−2.76 (m, 2H), 2.91−3.01 (m, 2H), 3.17−3.22 (m, 2H), 3.51−3.63 (m, 3H), 6.96−7.02 (m, 2H), 7.10−7.17 (m, 2H), 7.23−7.27 (m, 2H), 7.93−7.98 (m, 2H). The free base was converted to the corresponding oxalate salt by dissolving it in acetone and mixing with 1 equiv of oxalic acid in acetone. Mp (oxalate salt): 234 °C (dec). Anal. ($C_{24}H_{28}F_2N_2O_2 \cdot H_2C_2O_4 \cdot 0.25H_2O$) C, H, N.

(4-Fluorophenyl)(3′-hydroxy-1′-(4-methoxybenzyl)-[1,4′-bipiperidin]-4-yl)methanone (14b). Procedure C was followed with **13a**, triethylamine, and 4-methoxybenzyl bromide to afford **14b** (54 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 1.55−1.87 (m, 7H), 2.23−2.27 (m, 2H), 2.70−2.75 (m, 2H), 2.92−3.00 (m, 2H), 3.19−3.24 (m, 2H), 3.48−3.60 (m, 4H), 3.80 (s, 3H), 6.83−6.86 (m, 2H), 7.10−7.21 (m, 4H), 7.93−7.98 (m, 2H). Mp (oxalate salt): 237 °C (dec). Anal. ($C_{25}H_{31}FN_2O_3 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

(1′-Benzyl-3′-hydroxy-[1,4′-bipiperidin]-4-yl)(4-fluorophenyl)-methanone (14c). Procedure C was followed with **13a**, triethylamine, and benzyl bromide to afford **14c** (54 mg, 84%). ^{1}H NMR ($CDCl_3$): δ 1.57−2.03 (m, 8H), 2.17−2.28 (m, 2H), 2.71−2.76 (m, 2H), 2.93−3.01 (m, 2H), 3.20−3.25 (m, 2H), 3.50−3.63 (m, 4H), 7.10−7.16 (m, 2H), 7.24−7.31 (m, 5H), 7.93 −7.98 (m, 2H). Mp (oxalate salt): 243 °C (dec). Anal. ($C_{24}H_{29}FN_2O_2 \cdot H_2C_2O_4 \cdot 0.25H_2O$) C, H, N.

(4-Fluorophenyl)(3′-hydroxy-1′-(pyridin-3-ylmethyl)-[1,4′-bipiperidin]-4-yl)methanone (14d). Procedure C was followed with **13a**, triethylamine, and pyridin-4-methyl bromide to yield **14d** (19 mg, 50%). The mobile phase used for column chromatographic separation was triethylamine/ethyl acetate/methanol (1/9/1, v/v/v). ^{1}H NMR ($CDCl_3$): δ 1.71−2.02 (m, 8H), 2.24−2.28 (m, 2H), 2.74−2.76 (m, 2H), 2.91−3.00 (m, 2H), 3.17−3.21 (m, 2H), 3.50−3.60 (m, 4H), 7.10−7.17 (m, 2H), 7.23−7.27 (m, 1H), 7.62−7.65 (m, 1H), 7.94−7.98 (m, 2H), 8.50−8.52 (m, 2H). Mp (oxalate salt): 178 °C (dec). Anal. ($C_{23}H_{28}FN_3O_2 \cdot 1.5H_2C_2O_4 \cdot H_2O$) C, H, N.

(1′-(4-Fluorobenzyl)-3′-hydroxy-[1,4′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (14e). Procedure C was followed

with **13c**, triethylamine, and 4-fluorobenzyl bromide to afford **14e** (24.5 mg, 63%). ^{1}H NMR ($CDCl_3$): δ 1.56−2.28 (m, 8H), 2.18−2.30 (m, 2H), 2.70−2.74 (m, 2H), 2.92−2.99 (m, 2H), 3.19−3.23 (m, 2H), 3.48−3.62 (m, 4H), 3.87 (s, 3H), 6.83−6.95 (m, 4H), 7.18−7.21 (m, 2H), 7.90−7.94 (m, 2H). Mp (oxalate salt): 231 °C (dec). Anal. ($C_{25}H_{31}FN_2O_3 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

(3′-Hydroxy-1′-(4-methoxybenzyl)-[1,4′-bipiperidin]-4-yl)-(4-methoxyphenyl)methanone (14f). Procedure C was followed with **13c**, triethylamine, and 4-methoxybenzyl bromide to afford **14f** (30 mg, 73%). ^{1}H NMR ($CDCl_3$): δ 1.51−2.00 (m, 8H), 2.20−2.32 (m, 2H), 2.71−2.76 (m, 2H), 2.91−3.01 (m, 2H), 3.17−3.22 (m, 2H), 3.51−3.63 (m, 4H), 3.79 (s, 3H), 3.86 (s, 3H), 6.96−7.02 (m, 2H), 7.10−7.17 (m, 2H), 7.23−7.27 (m, 2H), 7.93−7.98 (m, 2H). Mp (oxalate salt): 225 °C (dec). Anal. ($C_{25}H_{31}FN_2O_3 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

(1′-Benzyl-3′-hydroxy-[1,4′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (14g). Procedure C was followed with **13c**, triethylamine, and benzyl bromide to afford **14g** (32 mg, 34%). ^{1}H NMR ($CDCl_3$): δ 1.54−2.04 (m, 8H), 2.22−2.27 (m, 2H), 2.70−2.75 (m, 2H), 2.93−3.00 (m, 2H), 3.20−3.25 (m, 2H), 3.54−3.62 (m, 4H), 3.86 (s, 3H), 6.94 (d, J = 9 Hz, 2H), 7.26−7.31 (m, 5H), 7.93 (d, J = 9 Hz, 2H). Mp (oxalate salt): 234 °C (dec). Anal. ($C_{25}H_{32}N_2O_3 \cdot 2H_2C_2O_4$).

(3′-Hydroxy-1′-(pyridin-3-ylmethyl)-[1,4′-bipiperidin]-4-yl)-(4-methoxyphenyl)methanone (14h). Procedure C was followed with **13c**, triethylamine, and pyridin-4-methyl bromide to prepare **14h** (20 mg, 52%). The mobile phase used for column chromatographic separation was triethylamine/ethyl acetate/methanol (1/9/1, v/v/v). ^{1}H NMR ($CDCl_3$): δ 1.72−2.03 (m, 8H), 2.25−2.29 (m, 2H), 2.74−2.76 (m, 2H), 2.91−3.00 (m, 2H), 3.19−3.21 (m, 2H), 3.55−3.61 (m, 4H), 3.87 (s, 3H), 6.95 (d, J = 9 Hz, 2H), 7.23−7.27 (m, 1H), 7.62−7.65 (m, 1H), 7.93 (d, J = 9 Hz, 2H), 8.50−8.52 (m, 2H). The free base was converted to the oxalate salt. Mp: 188 °C (dec). Anal. ($C_{24}H_{31}N_3O_3 \cdot 2H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

Procedure D. General Method for Preparation of Benzamides (15a−g). (3′-Hydroxy-[1,4′-bipiperidine]-1′,4-diyl)bis-(4-fluorophenyl)methanone (15a). Into a solution of **13a** (50 mg, 0.163 mmol), BOP-Cl (100 mg, 0.40 mmol), and triethylamine (1 mL) in methylene chloride (30 mL) was added 4-fluorobenzoic acid (40 mg, 0.28 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was washed with saturated sodium carbonate (20 mL × 5) and brine solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using triethylamine/ethyl acetate (1/50, v/v) as mobile phase to afford **15a** (62 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 1.54−1.93 (m, 7H), 2.27−2.34 (m, 1H), 2.44−2.52 (m, 1H), 2.80−2.98 (m, 5H), 3.23−3.26 (m, 1H), 3.30−3.60 (m, 1H), 4.80 (br s, 1H), 7.07−7.18 (m, 4H), 7.40−7.44 (m, 2H), 7.94−7.99 (m, 2H). The free base was converted to the oxalate salt. Mp: 213 °C (dec). Anal. ($C_{24}H_{26}F_2N_2O_5 \cdot H_2C_2O_4$) C, H, N.

(4-(4-Fluorobenzoyl)-3′-hydroxy-[1,4′-bipiperidin]-1′-yl)-(4-methoxyphenyl)methanone (15b). Procedure D was followed with **13a**, BOP-Cl, triethylamine, and 4-methoxybenzoic acid to afford **15b** (48 mg, 22%). ^{1}H NMR ($CDCl_3$): δ 1.54−1.93 (m, 7H), 2.31−2.34 (m, 1H), 2.44 −2.51 (m, 1H), 2.79 −2.98 (m, 5H), 3.23−3.26 (m, 1H), 3.47 (m, 2H), 3.82 (s, 3H), 6.91 (d, J = 9 Hz, 2H), 7.14 (t, J = 6 Hz, 2H), 7.38 (d, J = 9 Hz, 2H), 7.94−7.99 (m, 2H). The free base was converted to the oxalate salt. Mp: 206 °C (dec). Anal. ($C_{24}H_{27}FN_2O_3 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

(1′-Benzoyl-3′-hydroxy-[1,4′-bipiperidin]-4-yl)(4-fluorophenyl)methanone (15c). Procedure D was followed with **13a**, BOP-Cl, triethylamine, and benzoic acid to afford **15c** (0.34 g, 78%). ^{1}H NMR ($CDCl_3$): δ 1.75−1.80 (m, 2H), 1.80−1.95 (m, 4H), 2.20−2.40 (m, 1H), 2.40−2.60 (m, 1H), 2.60−3.00 (m, 5H), 3.10−3.30 (m, 1H), 4.10 (s br s, 1H), 4.86 (br s, 1H), 7.12−7.18 (m, 2H), 7.28−7.42 (m, 5H), 7.95−7.99 (m, 2H). The free base was converted to the oxalate salt. Mp: 152 °C (dec). Anal. ($C_{24}H_{27}FN_2O_3 \cdot H_2C_2O_4 \cdot H_2O$) C, H, N.

(4-(4-Fluorobenzoyl)-3′-hydroxy-[1,4′-bipiperidin]-1′-yl)-(pyridin-3-yl)methanone (15d). Procedure D was followed with **13a**, BOP-Cl, triethylamine, and pyridine-3-carboxylic acid to afford **15d** (33 mg, 83%). The mobile phase used for column chromatographic separation was triethylamine/ethyl acetate/methanol (1/9/1, v/v/v). ^{1}H NMR ($CDCl_3$): δ 1.44−2.11 (m, 6H), 2.20−2.40 (m, 1H), 2.40−2.60 (m, 1H), 2.60−3.10 (m, 5H), 3.10−3.30 (m, 1H), 3.38−3.85 (m, 2H), 3.90−4.30 (m, 1H), 4.80−5.10 (m, 1H), 7.12−7.18 (m, 2H), 7.34−7.38 (m, 1H), 7.73−7.75 (m, 1H), 7.95−7.99 (m, 2H), 8.66−8.68 (m, 2H). The free base was converted to the oxalate salt. Mp: 215 °C (dec). Anal. ($C_{23}H_{26}FN_3O_3 \cdot H_2C_2O_4 \cdot 0.25H_2O$) C, H, N.

(1′-(4-Fluorobenzoyl)-3′-hydroxy-[1,4′-bipiperidin]-4-yl)-(4-methoxyphenyl)methanone (15e). Procedure D was followed with **13c**, BOP-Cl, triethylamine, and 4-fluorobenzoic acid to prepare **15e** (84 mg, 67%). ^{1}H NMR ($CDCl_3$): 1.30−1.98 (m, 5H), 2.16−2.33 (m, 1H), 2.34−2.41 (m, 1H), 2.45−3.65 (m, 8H), 3.77 (s, 3H), 4.03 (br s, 1H), 4.72 (br s, 1H), 6.83−6.87 (m, 2H), 6.97−7.03 (m, 2H), 7.29−7.35 (m, 2H), 7.81−7.85 (m, 2H). The free base was converted to the oxalate salt. Mp: 216 °C (dec). Anal. ($C_{25}H_{29}FN_2O_4 \cdot H_2C_2O_4$) C, H, N.

(1′-Benzoyl-3′-hydroxy-[1,4′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (15f). Procedure D was followed with **13c**, BOP-Cl, triethylamine, and benzoic acid to afford **15f** (0.14 g. 80%). ^{1}H NMR ($CDCl_3$): δ 1.60−1.90 (m, 5H), 2.10−2.50 (m, 2H), 2.60−3.00 (m, 5H), 3.20−3.50 (m, 2H), 3.65 (s, 1H), 3.87 (s, 3H), 4.10 (s br, 1H), 5.30 (br s 1H), 6.94 (d, J = 8.7 Hz, 2H), 7.38−7.40 (m, 5H), 7.92 (d, J = 9 Hz, 2H). The free base was converted to the oxalate salt. Mp: 188 °C (dec). Anal. ($C_{25}H_{30}N_2O_8 \cdot H_2C_2O_4 \cdot 1.5H_2O$) C, H, N.

(3′-Hydroxy-1′-isonicotinoyl-[1,4′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (15g). Procedure D was followed with **13c**, BOP-Cl, triethylamine, and pyridine-3-carboxylic acid to afford **15g** (36 mg, 90%). The mobile phase used for column chromatographic separation was triethylamine/ethyl acetate/methanol (1/9/1, v/v/v). ^{1}H NMR ($CDCl_3$): δ 1.61−1.96 (m, 6H), 2.04−2.08 (m, 1H), 2.27−2.56 (m, 2H), 2.80−2.09 (m, 4H), 2.09−3.27 (m, 2H), 3.44−3.69 (m, 2H), 3.87 (s, 3H), 4.85−5.15 (m, 1H), 6.93−6.97 (m, 2H), 7.28−7.38 (m, 1H), 7.73−7.80 (m, 1H), 7.85−7.94 (m, 2H), 8.67−8.68 (m, 2H). The free base was converted to the oxalate salt. Mp: 220 °C (dec). EIMS: calcd, 429.1843; found, 429.1843. Anal. ($C_{24}H_{29}N_3O_4 \cdot H_2C_2O_4$) C, H, N.

(1′-(4-Fluorobenzyl)-4′-hydroxy-[1,3′-bipiperidin]-4-yl)(4-fluorophenyl)methanone (16a). Procedure C was followed with **13b**, triethylamine and 4-fluorobenzyl bromide to yield **16a** as a pale yellow oil (28.7 mg, 43%). ^{1}H NMR ($CDCl_3$): δ 1.55−2.05 (m, 9H), 2.24−2.31 (m, 1H), 2.49−2.57 (m, 1H), 2.71−2.83 (m, 3H), 2.94−3.04 (m, 2H), 3.17−3.20 (m, 1H), 3.40−3.49 (m, 3H), 6.98−7.05 (m, 2H), 7.10−7.17 (m, 2H), 7.25−7.30 (m, 2H), 7.92−7.97 (m, 2H). The free base was converted to the oxalate salt. Mp: 209 °C (dec). Anal. ($C_{24}H_{28}F_2N_2O_2 \cdot H_2C_2O_4$) C, H, N.

(4-Fluorophenyl)(4′-hydroxy-1′-(4-methoxybenzyl)-[1,3′-bipiperidin]-4-yl)methanone (16b). Procedure C was followed with **13b**, triethylamine, and 4-methoxybenzyl bromide to afford **16b** (28 mg, 45%). ^{1}H NMR ($CDCl_3$): δ 1.56−2.06 (m, 8H), 2.26−2.30 (m, 1H), 2.53− 2.56 (m, 1H), 2.72−2.86 (m, 3H), 3.00−3.03 (m, 2H), 3.17−3.20 (m, 1H), 3.40−3.50 (m, 4H), 3.81 (s, 3H), 6.86−6.89 (m, 2H), 7.10−7.27 (m, 4H), 7.92−7.97 (m, 2H). The free base was converted to the oxalate salt. Mp: 204 °C (dec). Anal. ($C_{25}H_{31}FN_2O_3 \cdot H_2C_2O_4$) C, H, N.

(1′-Benzyl-4′-hydroxy-[1,3′-bipiperidin]-4-yl)(4-fluorophenyl)methanone (16c). Procedure C was followed with **13b**, triethylamine, and benzyl bromide to afford **16c** (40 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 1.56−2.06 (m, 8H), 2.24−2.3 (m, 1H), 2.51−2.59

(m, 1H), 2.72−2.86 (m, 3H), 3.00−3.04 (m, 2H), 3.17−3.20 (m, 1H), 3.40−3.54 (m, 4H), 7.10−7.15 (m, 2H), 7.26−7.33 (m, 5H), 7.92−7.97 (m, 2H). The free base was converted to the oxalate salt. Mp: 200 °C (dec). Anal. ($C_{24}H_{29}FN_2O_2 \cdot H_2C_2O_4$) C, H, N.

(4-Fluorophenyl)(4′-hydroxy-1′-(pyridin-3-ylmethyl)-[1,3′-bipiperidin]-4-yl)methanone (16d). Procedure C was followed with **13b**, triethylamine, and pyridine-4-methyl bromide to afford **16d** (22 mg, 56%). ^{1}H NMR ($CDCl_3$): δ 1.56−2.08 (m, 8H), 2.24−2.32 (m, 1H), 2.48−2.53 (m, 1H), 2.70−3.04 (m, 5H), 3.19−3.59 (m, 5H), 7.13 (t, J = 5.7 Hz, 2H), 7.26−7.31 (m, 1H), 7.66−7.69 (m, 1H), 7.92−7.97 (m, 2H), 8.52−8.54 (m, 2H). The free base was converted to the oxalate salt. Mp: 182 °C. Anal. ($C_{23}H_{28}FN_3O_2 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

(1′-(4-Fluorobenzyl)-4′-hydroxy-[1,3′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (16e). Procedure C was followed with **13d**, triethylamine, and 4-fluorobenzyl bromide to afford **16e** (38 mg, 55%). ^{1}H NMR ($CDCl_3$): δ 1.55−2.06 (m, 8H), 2.28 (td, J = 2.1, 13.8 Hz, 1H), 2.52 (td, J = 3.3, 10.5 Hz, 1H), 2.71−2.84 (m, 3H), 2.90−3.10 (m, 2H), 3.04−3.20 (m, 1H), 3.40−3.49 (m, 4H), 3.86 (s, 3H), 6.92−7.04 (m, 4H), 7.25−7.30 (m, 2H), 7.89−7.92 (m, 2H). The free base was converted to the oxalate salt. Mp: 221 °C (dec). Anal. ($C_{25}H_{31}FN_2O_3 \cdot H_2C_2O_4$) C, H, N.

(4′-Hydroxy-1′-(4-methoxybenzyl)-[1,3′-bipiperidin]-4-yl)-(4-methoxyphenyl)methanone (16f). Procedure C was followed with **13d**, triethylamine, and 4-methoxybenzyl bromide to afford **16f** (32 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 1.54−2.06 (m, 8H), 2.24−2.28 (m, 1H), 2.50−2.53 (m, 1H), 2.72−2.86 (m, 3H), 2.98−3.02 (m, 2H), 3.17−3.20 (m, 1H), 3.41−3.48 (m, 3H), 3.62 (br s, 1H), 3.81 (s, 3H), 3.86 (s, 3H), 6.85−6.95 (m, 4H), 7.22 (dd, J = 2.1, 6.9 Hz, 2H), 7.90 (dd, J = 2.1, 6.9 Hz, 2H). The free base was converted to the oxalate salt. Mp: 182 °C (dec). Anal. ($C_{26}H_{34}N_2O_4 \cdot H_2C_2O_4$) C, H, N.

(1′-Benzyl-4′-hydroxy-[1,3′-bipiperidin]-4-yl)(4-methoxyphenyl)methanone (16g). Procedure C was followed with **13d**, triethylamine, and benzyl bromide to afford **16g** (90 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 1.57−2.04 (m, 8H), 2.25−2.32 (t, J = 7.0 Hz, 1H), 2.51−2.60 (m, 1H), 2.70−2.86 (m, 3H), 3.00−3.04 (m, 2H), 3.17−3.20 (m, 1H), 3.40−3.59 (m, 4H), 3.86 (s, 3H), 6.91−6.94 (d, J = 9.0 Hz, 2H), 7.26−7.33 (m, 5H), 7.89−7.92 (d, J = 9.0 Hz, 2H). The free base was converted to the oxalate salt. Mp: 220.3 °C (dec). Anal. ($C_{25}H_{32}N_2O_3 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

(4′-Hydroxy-1′-(pyridin-3-ylmethyl)-[1,3′-bipiperidin]-4-yl)-(4-methoxyphenyl)methanone (16h). Procedure C was followed with **13d**, triethylamine, and pyridine-4-methyl bromide to afford **16h** (13 mg, 34%). ^{1}H NMR ($CDCl_3$): δ 1.58−2.08 (m, 8H), 2.22−2.29 (m, 1H), 2.52−2.53 (m, 1H), 2.70−3.04 (m, 5H), 3.18−3.20 (m, 1H), 3.42−3.54 (m, 4H), 3.87 (s, 3H), 6.91−6.96 (m, 2H), 7.26−7.30 (m, 1H), 7.63−7.70 (m, 1H), 7.89−7.94 (m, 2H), 8.52−8.54 (m, 2H). The free base was converted to the oxalate salt. Mp: 202 °C. Anal. ($C_{24}H_{31}N_3O_3 \cdot H_2C_2O_4$) C, H, N.

In Vitro Biological Evaluation. σ Receptor Binding Assays. The compounds were dissolved in DMF, DMSO, or ethanol and then diluted in 50 mM Tris-HCl buffer containing 150 mM NaCl and 100 mM EDTA at pH 7.4 prior to performing the σ_1 and σ_2 receptor binding assays. The procedures for isolating the membrane homogenates and performing the σ_1 and σ_2 receptor binding assays have been previously described in detail.[42]

Briefly, the σ_1 receptor binding assays were conducted in 96-well plates using guinea pig brain membrane homogenates (~300 μg of protein) and ~5 nM (+)-[^{3}H]pentazocine (34.9 Ci/mmol, Perkin-Elmer, Boston, MA). The total incubation time was 90 min at room temperature. Nonspecific binding was determined from samples that contained 10 μM cold haloperidol. After 90 min, the reaction was quenched by adding 150 μL of ice-cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) using a 96-channel transfer pipet (Fisher Scientific, Pittsburgh, PA). The samples were harvested and filtered rapidly through a 96-well fiberglass filter plate (Millipore, Billerica, MA) that had been presoaked with 100 μL of 50 mM Tris-HCl buffer at pH 8.0 for 1 h. Each filter was washed 3 times with 200 μL of ice-cold wash buffer and the filter counted in a Wallac 1450 MicroBeta liquid scintillation counter (Perkin-Elmer, Boston, MA).

The σ_2 receptor binding assays were conducted using rat liver membrane homogenates (~300 μg of protein) and ~5 nM [^{3}H]-DTG (58.1 Ci/mmol, Perkin-Elmer, Boston, MA) in the presence of 1 μM (+)-pentazocine to block σ_1 sites. The incubation time was 2 h at room temperature. Nonspecific binding was determined from samples that contained 10 μM cold haloperidol. All other procedures were identical to those described for the σ_1 receptor binding assay above.

Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration that inhibits 50% of the specific binding of the radioligand (IC_{50}). Competitive curves were best fit to a one-site fit and gave pseudo Hill coefficients of 0.6−1.0. K_i values were calculated using the method of Cheng and Prusoff[48] and are presented as the mean (±1 SEM). For these calculations, we used a K_d of 7.89 nM for (+)-[^{3}H]pentazocine and guinea pig brain and a K_d of 30.73 nM for [^{3}H]DTG and rat liver.

Vesicular Acetylcholine Transporter Binding Assays. In vitro VAChT binding assays of these novel compounds were performed on human vesicular acetylcholine transporter (VAChT) permanently expressed in PC12$^{A123.7}$ cells at about 50 pmol/mg crude extract. The radioligand used was 5 nM (−)-[^{3}H]vesamicol, and the assay was conducted as described at final concentrations of 10^{-11}−10^{-5} M novel compound.[43] Unlabeled (−)-vesamicol was used as an external standard, for which K_i = 15 nM, and the mixture was allowed to equilibrate for 20 h. Duplicate data were averaged and fitted by regression with a rectangular hyperbola to estimate the K_i of the novel compound. All compounds were independently assayed at least two times.

Dopamine Receptor Binding Assays. In vitro D_2 and D_3 binding assays were performed on human D_2 and D_3 receptor expressed HEK 293 cells according our recently published protocol.[49]

5-HT$_{1A}$ Receptor Binding Assays. The binding affinity at 5-HT$_{1A}$ receptors was characterized by a filtration assay following the reported procedure[50] with minor modification. Human 5-HT$_{1A}$ serotonin receptor membranes (~10 μg of protein) (ChanTest, Cleveland, OH, U.S.) were diluted in 50 mM Tris-HCl buffer (10 mM $MgCl_2$, 0.1 mM EDTA, pH 7.4) and incubated in a total volume of 150 μL with ~0.89 nM [^{3}H]8-OH-DPAT (Perkin-Elmer, Boston, MA, U.S.) at 25 °C in 96-well sample plates for 60 min. Then 10 μM WAY-100635 was used to define the nonspecific binding. The radioligand concentration was equal to approximately twice the K_d, and the concentration of the competitive inhibitor ranged over 6 orders of magnitude for competition experiments (0.1 nM to 10 μM or 0.01 nM to 1 μM). The reactions were terminated by the addition of 150 μL of cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4, at 4 °C) using a 96-channel transfer pipet (Fisher Scientific), and the samples were harvested and filtered rapidly to Millipore MultiScreen$_{HTS}$ 96-well filter plates that had been presoaked with 100 μL of 50 mM Tris-HCl buffer, pH 8.0, for 1 h. Each filter was washed with 200 μL of ice-cold wash buffer for a total of three washes. Filters were dissolved in 2.5 mL of scintillation fluid, and a Wallac 1450 MicroBeta liquid scintillation counter (Perkin-Elmer, Boston, MA, U.S.) was used to measure the radioactivity. The concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC_{50}) was determined by using nonlinear regression analysis to analyze the data of competitive inhibition experiments. Competition curves were modeled for a single site, and the IC_{50} values were converted to equilibrium dissociation constants (K_i) using the Cheng and Prusoff equation with equilibrium dissociation constant K_d = 0.35 nM.

■ ASSOCIATED CONTENT

Supporting Information. Elemental analysis data of oxalate salts, HPLC spectra for **14a**−**h**, **15a**−**g**, and **16a**−**h**, and X-ray crystallographic data for 14c. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

Anal., analysis; BOP-Cl, bis(2-oxo-3-oxazolidinyl)phosphonic chloride; calcd, calculated; CIMS, chemical ionization mass spectrometry; CNS, central nervous system; SAR, structure−activity relationship; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMF, N, N-dimethylformamide; DMSO, dimethylsulfoxide; DTG, 1,3-ditolylguanidine; ND, not determined; PET, positron emission tomography; prezamicol, 3-hydroxy-4-(4-phenylpiperidinyl)-piperidine; THF, tetrahydrofuran; TLC, thin layer chromatography; trozamicol, 4-hydroxy-3-(4-phenylpiperidinyl)piperidine; VAChT, vesicular acetylcholine transporter; vesamicol, (−)-*trans*-2-(4-phenylpiperidino)cyclohexanol

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