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Synthesis and *in Vitro* Biological Evaluation of Carbonyl Group-Containing Inhibitors of Vesicular Acetylcholine Transporter

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To identify selective high-affinity inhibitors of the vesicular acetylcholine transporter (VAChT), we have interposed a carbonyl group between the phenyl and piperidyl groups of the prototypical VAChT ligand vesamicol and its more potent analogues benzovesamicol and 5-aminobenzovesamicol. Of 33 compounds synthesized and tested, 6 display very high affinity for VAChT (K_i, 0.25−0.66 nM) and greater than 500-fold selectivity for VAChT over σ_1 and σ_2 receptors. Twelve compounds have high affinity (K_i, 1.0−10 nM) and good selectivity for VAChT. Furthermore, 3 halogenated compounds, namely, *trans*-3-[4-(4-fluorobenzoyl)piperidinyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene (**28b**) (K_i = 2.7 nM, VAChT/sigma selectivity index = 70), *trans*-3-[4-(5-iodothienylcarbonyl)piperidinyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene (**28h**) (K_i = 0.66 nM, VAChT/sigma selectivity index = 294), and 5-amino-3-[4-(*p*-fluorobenzoyl)piperidinyl]-2-hydroxy-1,2,3,4,-tetrahydronaphthalene (**30b**) (K_i = 2.40 nM, VAChT/sigma selectivity index = 410) display moderate to high selectivity for VAChT. These three compounds can be synthesized with the corresponding radioisotopes so as to serve as PET/SPECT probes for imaging the VAChT in vivo.

Introduction

Alzheimer's disease, Down's syndrome, Parkinson's disease, and schizophrenia are generally characterized by progressive diminution in cognitive function associated with the loss of cholinergic neurons and synapses in the brain.[1−5] For example, in Alzheimer's disease, the severity of cognitive dysfunction is correlated with loss of cholinergic synaptic elements in the cortex and subcortical brain areas.[6−12] Until recently, cholinergic neurons were identified by the expression of only three marker proteins: choline acetyltransferase (ChAT[a]), the sodium-dependent high-affinity choline transporter (SDHAChT), and acetylcholinesterase (AChE). To date, ChAT and SDHAChT are widely accepted as reliable cholinergic markers. However, AChE is viewed as less reliable because of its occurrence in cholinergic and noncholinergic neurons and oligodendrocytes. This view has persisted even though studies have revealed that the neurophathologies of neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease are associated with alterations in the levels of AChE.[13] With the discovery of the anticholinergic properties of the lipophilic aminoalcohol (−)-*trans*-2-(4-phenylpiperidino)cyclohexanol (vesamicol (**1**), Figure 1), and the subsequent demonstration of a unique binding site for this molecule (the erstwhile vesamicol receptor) on cholinergic synaptic vesicles,[14−16] efforts were initiated to further characterize this site and to evaluate its potential utility as a cholinergic marker. In rat brain sections, the binding of [^{3}H]vesamicol was found to exhibit a regional heterogeneity which is correlated with ChAT activity and [^{3}H]hemicholinium-3 binding (an indicator of SDHAChT).[17,18] These results suggested that the vesamicol receptor may be a reliable cholinergic marker. Although problems were subsequently encountered with [^{3}H]vesamicol, these earlier predictions have been confirmed with the help of molecular biology. The vesamicol binding protein hereinafter referred to as the vesicular acetylcholine transporter (VAChT), was cloned from the nematode *Caenorhabditis elegans,* three species of *Torpedo*, rat, and human beings.[19−24] ACh accumulation and vesamicol binding are expressed by a single polypeptide, suggesting that all the essential components of ACh storage reside in that polypeptide. Thus, the terms vesamicol receptor and VAChT are synonymous.

In several studies of the rat brain, staining for VAChT protein and mRNA and ChAT protein and mRNA has unequivocally revealed that VAChT is localized to synaptic vesicles within cholinergic terminals.[25−29] Consequently,

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[a]Abbreviations: ABV, aminobenzovesamicol; ACh, acetylcholine; AChE, acetylcholinesterase; Anal., Analysis; Calcd., Calculated; ChAT, choline acetyltransferase; CIMS.,Chemical ionization mass spectrometry (CIMS) choline acetyltransferase; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DTG, 1,3-ditolylguanidine; NBS, *N*-bromosuccinimide; ND, not determined; PET, positron emission tomography; SPECT, single photon emission computed tomography; SDHAChT, sodium-dependent high-affinity choline transporter; THF, tetrahydrofuran; TLC, thin layer chromatography; VAChT, vesicular acetylcholine transporter; Vesamicol, (-)-*trans*-2-(4-phenylpiperidino)cyclohexanol.

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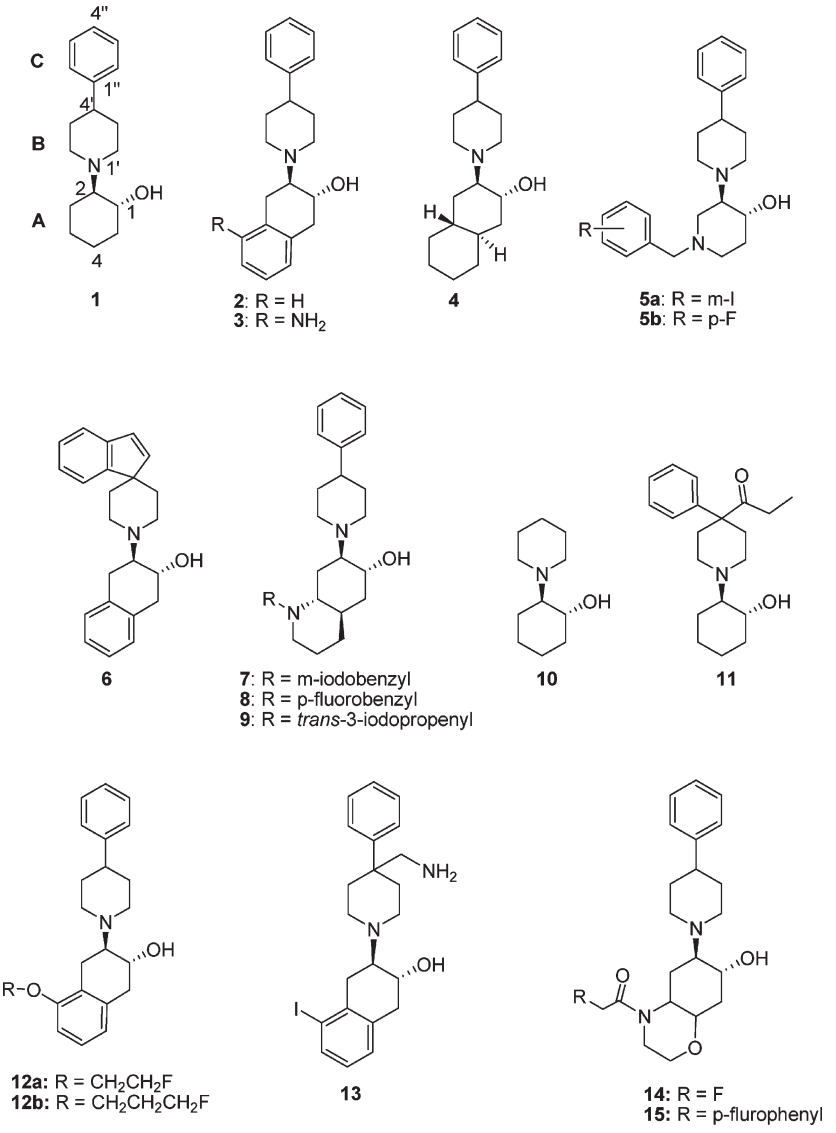


Figure 1. Representative compound structures for VAChT.

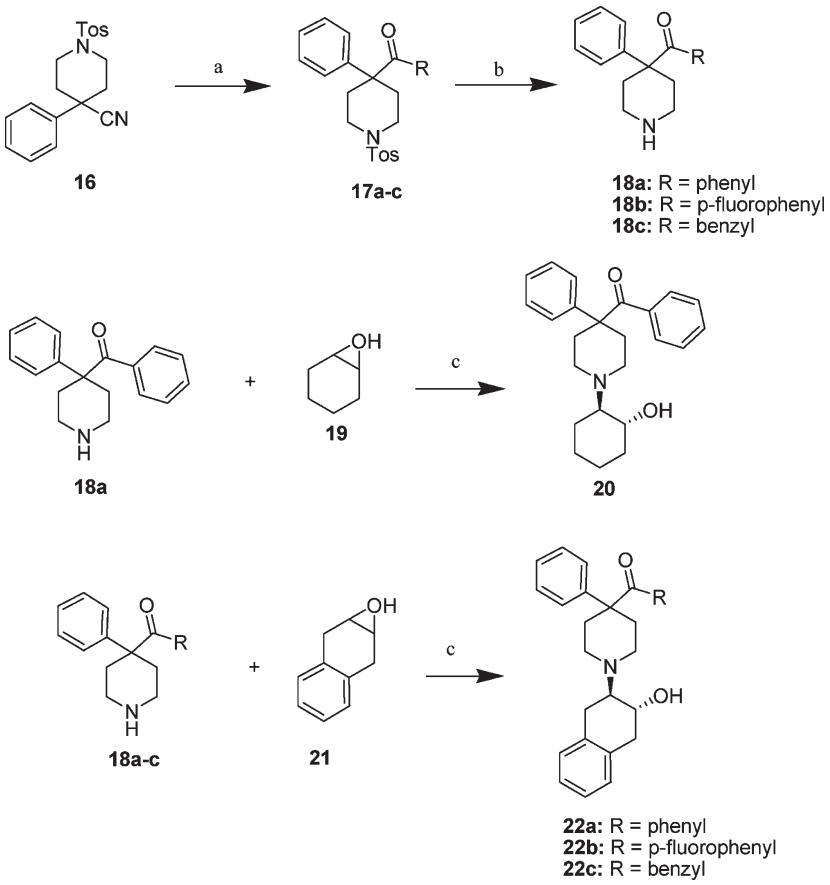
VAChT is now firmly established as a reliable cholinergic marker.

Recently, Prado and colleagues[30] reported a strong relationship between the levels of VAChT expression and ACh release in both the peripheral and central nervous systems. A marked reduction of VAChT expression can affect neurotransmission at the neuromuscular junction. Even a modest deficiency of VAChT is sufficient to interfere with release of ACh in the brain and to alter cognitive behavior in object and social recognition and memory function.[30] Alterations in VAChT expression levels have also been implicated in drug addiction, including addiction to nicotine, ethanol, and neurostimulants such as cocaine and amphetamine and opiates.[31,32] The levels of VAChT are subject to both up- and down-regulation as part of compensatory processes that attempt to maintain homeostasis of neuronal cholinergic activity.[32]

Recently, we reported the synthesis and in vitro characterization of five analogues of a new class of VAChT ligands in which a carbonyl group was interposed between the two rings of the 4-phenylpiperidinyl fragment in the vesamicol structure.[33] We also reported (a) the radiosynthesis of an ^{18}F-labeled positron emission tomography (PET) lead radiotracer, namely, (−)-*trans*-2-hydroxy-3-(4-(4-[^{18}F]fluorobenzoyl)piperidino)-tetralin, in this new class, and (b) in vivo evaluation of the compound in rodents and monkeys. The initial evaluation suggests that this radiotracer has specific binding to the VAChT enriched striatum. The process of determining the potential clinical utility of this tracer for PET imaging of VAChT is still in progress.

In this paper, we will detail our exploration of this new class of compounds to identify selective high-affinity analogues for future investigation of VAChT in vivo. In order to discuss the comprehensive structure−activity relationships, we include in this article the molecular structures and in vitro data for compounds that we reported earlier.[33] The investigations were inspired by (1) the observation that VAChT expression in brain is correlated with the severity of dementia associated with cholinergic deficiency in patients with neurodegenerative diseases;[34−37] (2) the marginal selectivity of [^{3}H]vesamicol[14,38,39] and the inability of this ligand to adequately reflect the loss of cholinergic neurons in experimental animals[17,18,40−42] or in Alzheimer's disease;[35] and (3) the need for a suitable radioligand that could serve as PET probe for imaging VAChT in vivo.

Scheme 1[a]



[a] Reagents and conditions: (a) aryl Grignard reagents/toluene, reflux; (b) 70% of sulfuric acid, 160−170 °C; (c) ethanol/Na_2CO_3, refluxed.

Results and Discussion

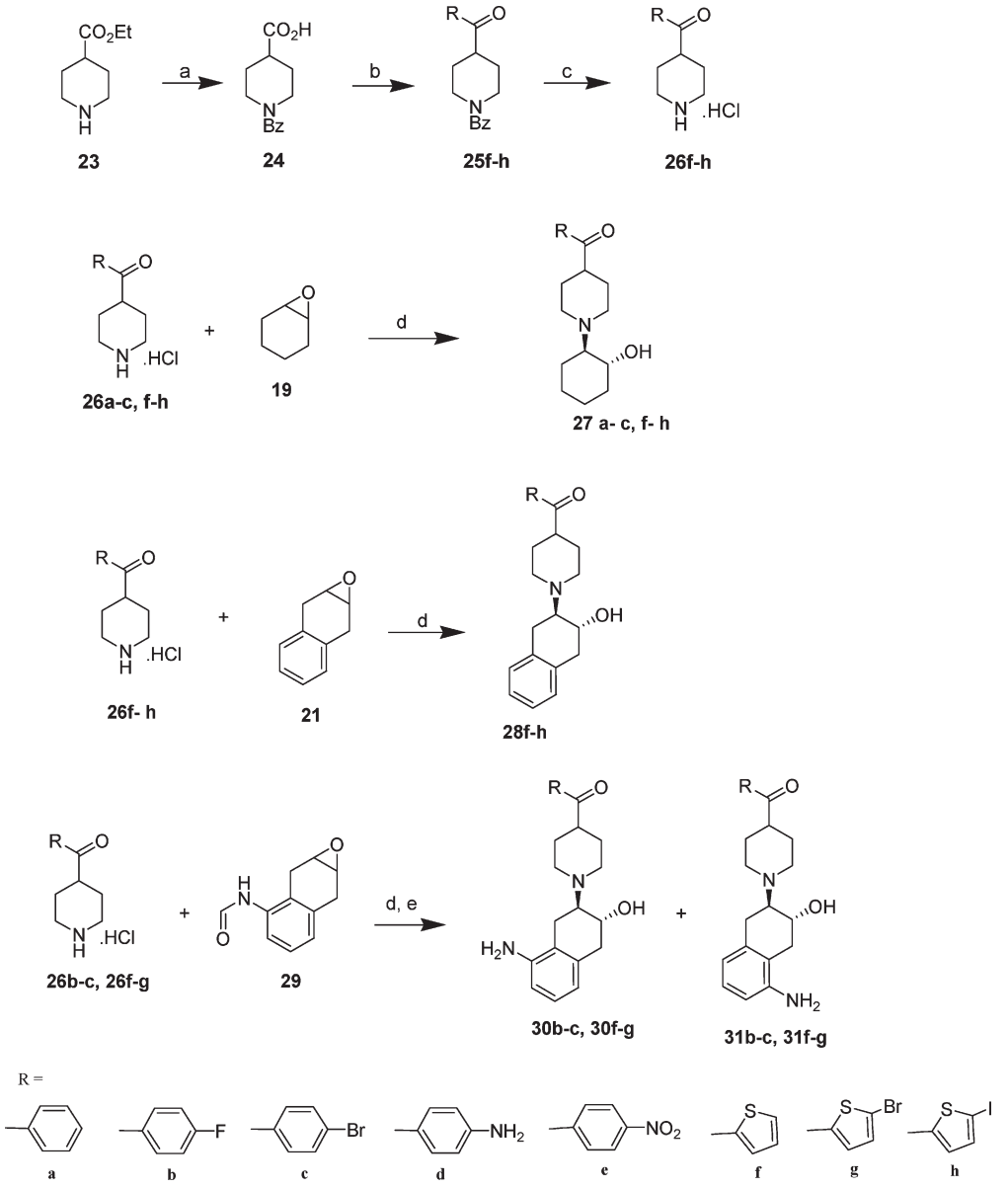
Previously, we and others have described several high-affinity VAChT ligands.[5,39,43−50] However, the majority of these can be represented by only a few structural classes (Figure 1, **1**−**15**). A common attribute of these ligands is the presence of a 4-phenylpiperidyl substructure. The recurrence of this structural motif is based on the structure of the parent compound and further justified by the observation that removal of the phenyl group (to yield **10**) or replacement of the piperidyl fragment with the aminoethyl moiety results in complete loss of potency.[43] However, the continued use of the 4-phenylpiperidyl fragment in the design of new VAChT ligands limits the scope of structure modification in this class of ligands. Since the 4-phenylpiperidyl group is also recognized by σ receptors,[51] many vesamicol analogues bind to both VAChT and σ receptors.[5,38,39] The discovery of new VAChT ligands that are devoid of this structural attribute therefore presents an attractive prospect for the development of novel selective VAChT ligands.

The present study was initiated by analyzing previously reported structure−activity data. The structure of vesamicol can be divided into distinct fragments: A, B, and C (Figure 1). In reviewing the data, we observed that, while removal of the phenyl group of vesamicol (to yield **10**) causes a drastic reduction in potency, addition of a propionyl group to the C4′ position of vesamicol yields a ligand, **11**,[43] that is comparable in affinity to the parent.[35] On the basis of these observations, we proposed that, within the vesamicol binding site, the binding pocket around fragment C is large enough to accommodate simultaneously both the phenyl group and a second fragment. Because the existence of a large binding pocket would allow for greater flexibility in structure modification, we synthesized and tested a number of analogues designed to (a) probe the size of the pocket and (b) extend structure modification beyond the 4-phenylpiperidine backbone.

Chemistry. The target compounds were synthesized as depicted in Schemes 1−3. The previously reported[52,53] 4-cyanopiperidine **16** reacted with Grignard reagents to produce **17a**−**c** in moderate yields (Scheme 1). Hydrolytic removal of the tosyl group in **17a**−**c** was followed by reaction of the resulting secondary amines **18a**−**c** with epoxides **19** or **21** to yield the target compounds **20** or **22a**−**c** in moderate yield.

The synthesis of aryl 4-piperidyl ketones **26a**−**e** has been reported.[33] Construction of the aryl 4-piperidyl ketones **26f**−**h** was used similar procedure of making **26a**−**e** by starting with **23** (Scheme 2). Conversion of the latter into the N-benzoyl protected isonipecotic acid **24** provided a versatile intermediate for Friedel−Crafts acylation. Hydrolytic removal of the tosyl group from the Friedel−Cratfts acylation products **25f**−**h** provided **26f**−**h**. Refluxing these aminoketones **26a**−**c**, **f**−**h** with the epoxides **19** or **21** provided the compounds of type **27a**−**c**, **f**−**h**, or **28f**−**h** in moderate yields (Scheme 2). The procedure for making **28a**−**e** was reported in the literature.[33] Similarly, **26b**−**c** and **26f**−**g** were reacted with **29** and then hydrolyzed by sodium hydroxide aqueous solution to produce the regioisomeric pairs **30b**,**c**, **30f**,**g**, and **31b**,**c**, **31f**,**g**.

Scheme 2[a]



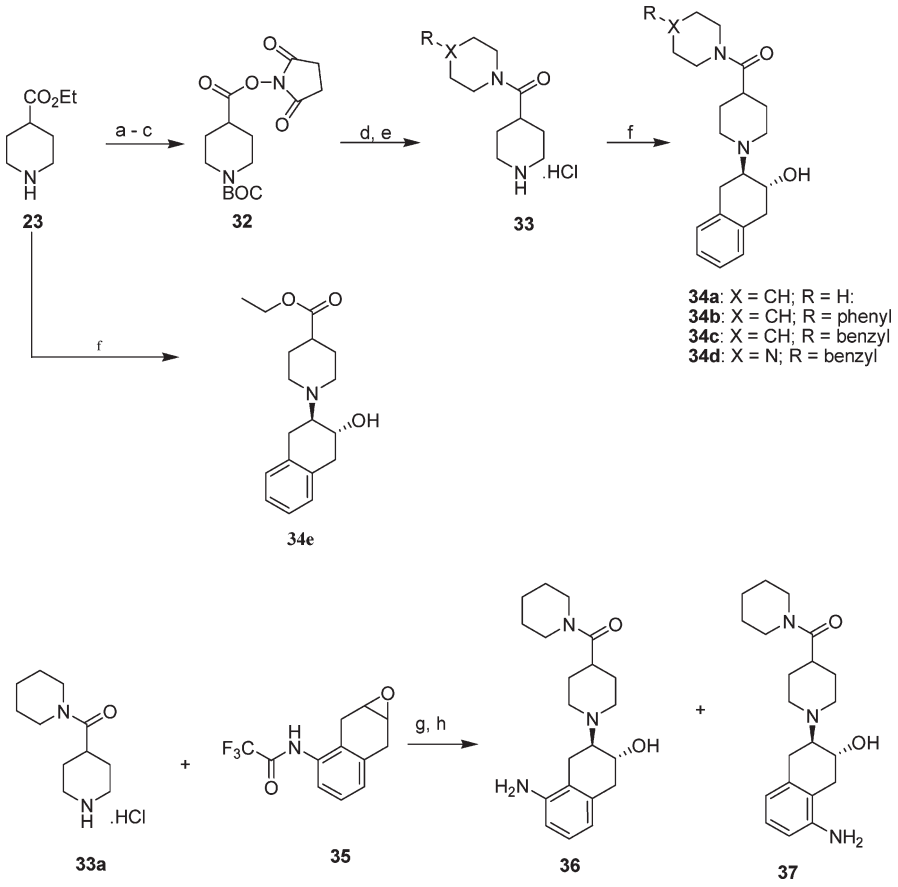
[a] Reagents and conditions:(a) (i) BzCl, Et_3N; (ii) NaOH, aq. EtOH; (b) (i) Oxalyl chloride, CH_2Cl_2; (ii) arene, $AlCl_3$, CS_2, reflux; (c) 6 N HCl, heat; (d) EtOH, Et_3N, reflux; (e) NaOH, aq. EtOH.

To obtain the target amides **34a**−**d**, ethyl isonipecotate was converted in three steps to the activated ester **32** (Scheme 3). The latter was reacted with a number of substituted piperidines and the products were treated with HCl (g) to yield the hydrochlorides **33a**−**d**. Intermediates **33a**−**d** and **23** were refluxed with *trans*-2-hydroxy-3-bromo-1,2,3,4-tetrahydronaphthalene in EtOH in the presence of Et_3N to provide **34a**−**e**. Similarly, **33a** was reacted with the epoxide **35** to produce **36** and **37**. All compounds were tested as the hydrochlorides.

In Vitro Binding Studies. Compounds were tested for VAChT binding using highly purified *Torpedo* synaptic vesicles. The σ_1 and σ_2 binding affinities were tested in rat brain and in guinea pig membranes, respectively. Equilibrium binding constants (K_i) are reported in Table 1. As in previous studies, we define ligand selectivity in terms of a selectivity index which is calculated as K_i (σ_1 or σ_2)/K_i (VAChT). In the ensuing discussion, binding affinities are characterized as very high (K_i < 1.0 nM), high (K_i = 1−10 nM), moderate (K_i = 11−50 nM), or poor (K_i > 50 nM).

In general, the study identified several potent new analogues. Moreover, a number of potentially useful trends are discernible. In contrast to 4′-propionylvesamicol,[43] 4′-benzoylvesamicol **20** displayed 35-fold lower affinity for VAChT than vesamicol, while compound **22a** was 30-fold less potent than the corresponding tetralin benzovesamicol (**2**). No change in affinity was observed following the introduction of a fluorine atom or replacement of the benzoyl group with the phenylacetyl fragment (compare **22a** with **22b** and **22c**). Given the nanomolar affinity of **22a** (and its homologues), it does appear that there is indeed a large binding pocket within the receptor around fragment-C. However, the lower potency of the 4-phenyl-4-aroylpiperidines **20** and **22a** relative to the corresponding 4-phenylpiperidines suggests that, in contrast to the propionyl group, the more rigid benzoyl substituent is not as well tolerated in this binding pocket.

Scheme 3[a]



[a] Reagents and conditions: (a) di-*tert*-butyldicarbonate, $CHCl_3$, NaCl, H_2O, reflux; (b) NaOH (aq.), EtOH; (c) *N*-hydroxysuccinimide, DCC, CH_2Cl_2; (d) 4-substituted piperidine, CH_3CN, reflux; (e) HCl(g), EtOAc, 0 °C; (f) *trans*-2-hydroxy-3-bromo-1,2,3,4-tetrahydronaphthalene, EtOH, Et_3N, reflux; (g) EtOH, Et_3N, reflux; (h) NaOH, aq. EtOH.

To further probe the binding pocket around fragment-C, we sought to examine vesamicol analogues that are devoid of the 4-phenyl group. Since potent VAChT ligands can be obtained from both the 4-phenylpiperidines and 4-phenyl-4-acylpiperidines, we reasoned that the corresponding 4-acylpiperidines would also yield VAChT ligands of high affinity. Surprisingly, the 4-aroylpiperidines **27a**,**b** and **27f** were 200−380 times less potent than **1** and 6−11-fold less potent than the corresponding 4-phenyl-4-acylpiperidine **20**. To explain the difference in affinity, molecular models of 4-phenylpiperidine and 4-benzoylpiperidine were constructed and inspected. When these models are rotated around the long axis (which passes through N1 and C4 of the piperidyl group), it is observed that the C4-phenyl and 4-benzoyl groups sweep through different regions of conformational space. Moreover, the C4-benzoyl group appears to sweep through a larger spatial volume than the C4-phenyl group. Therefore, the diminished affinity of the 4-aroylpiperidines **27a**,**b** and **27f** may be due in part to unfavorable steric interaction of the C4-aroyl groups with the binding pocket around fragment-C. Since compound **1** displays higher affinity than compound **27a**, it would appear that when fragment-A is cyclohexyl, the 4-phenyl group interacts more efficiently with the binding pocket than the angular benzoyl group. We note, however, that the affinity of the aroyl compounds can be enhanced by appropriate substitution on the aroyl group (compare **27a** vs **27b** or **27c**, and **27f** vs **27g**).

In the tetralin series, replacement of the 4-phenyl group in **2** with a benzoyl group also resulted in reduction of affinity for VAChT (**2** vs **28a**). However, the 4-benzoylpiperidine **28a** was more closely matched with the 4-benzoyl-4-phenylpiperidine **22a**, suggesting that the tetrahydronaphthyl fragment partially compensates for the unfavorable interaction between the benzoyl group and the binding pocket around fragment-C. As observed in the cyclohexane series, the *para*-fluoro group was ineffective at altering affinity for VAChT (**28a** vs **28b**); however, a 10−20-fold increase in affinity was observed when substitution was extended to include a bromo or nitro group (compare **28a** with **28c** or **28e**), thus bringing the affinity of the benzoylpiperidines closer to that of the potent 4-phenylpiperidines. The use of a 2-thienylcarbonyl fragment in place of the 4-benzoylpiperidyl group provided trends similar to those observed above. Compound **28f** displayed comparable affinity with **28a**, while the 2-bromo and iodothienyl compounds, **28g** and **28h**, respectively, showed 8−10-fold higher affinity. We therefore conclude that, with suitable substitution, the benzoyl and related aroyl groups can replace the phenyl group in fragment C.

To extend our structure modification studies, the benzoyl group was replaced with the carbethoxy group (**34e**) and a small selection of carbamoyl fragments (**34a**−**d**). While the 1-piperidylcarbonyl group in **34a** brought about 7- and 545-fold reductions in affinity relative to **28a** and **2**, respectively; analogues containing substituted piperidyl/piperazinyl groups

Table 1. [a]: Affinities ($K_i \pm$ SD, nM) of Vesamicol and Selected Analogues for *Torpedo* VAChT and σ_1 and σ_2 Receptors

				selectivity index	
compounds	VAChT[b] (K_i [nM])	σ_1[c] (K_i [nM])	σ_2[d] (K_i[nM])	K_i-σ_1/K_i-VAChT	K_i-σ_2/K_i-VAChT
Vesamicol (**1**)[e]	2.0 ± 1.0	25.8 ± 8.0	34.5 ± 2.0	12.9	17.25
Benzovesamicol (**2**)[f]	0.055 ± 0.010	ND	ND	ND	ND
11[g]	50	ND	ND	ND	ND
20	68.5 ± 2.54	ND	ND	ND	ND
22a	1.64 ± 0.26	ND	ND	ND	ND
22b	1.83 ± 0.39	ND	ND	ND	ND
22c	1.38 ± 0.36	ND	ND	ND	ND
27a	650 ± 330	280.8 ± 18.8	341.4 ± 28.7	0.4	0.5
27b	423 ± 2	302.79 ± 25.23	112.74 ± 8.14	0.7	0.3
27c	51.4 ± 6.0	15.91 ± 2.82	30.13 ± 3.80	0.3	0.6
27f	763 ± 94	ND	ND	ND	ND
27g	46.8 ± 5.0	29.46 ± 0.94	35.73 ± 1.13	0.6	0.8
27h	560 ± 50	9.39 ± 1.75	48.86 ± 3.26	0.02	0.1
28a	4.30 ± 1.00	219.6 ± 17.3	320.0 ± 6.6	51.1	74.4
28b	2.70 ± 0.40	191.1 ± 57.7	251.3 ± 39.2	70.8	93.1
28c	0.25 ± 0.02	297.69 ± 18.73	592.61 ± 95.19	1190.8	2370.4
28d	1.68 ± 0.14	ND	ND	ND	ND
28e	0.48 ± 0.03	ND	ND	ND	ND
28f	5.00 ± 1.20	ND	ND	ND	ND
28g	0.54 ± 0.08	128.92 ± 1.65	72.02 ± 9.40	238.7	133.4
28h	0.66 ± 0.06	194.03 ± 28.70	344.50 ± 13.34	294	522
30b	2.40 ± 0.26	> 1000	> 1000	> 416.7	> 416.7
30c	0.53 ± 0.09	395.11 ± 33.8	> 1000	745.5	1886.8
30f	4.90 ± 1.90	> 1000	> 1000	> 204.1	> 204.1
30g	0.41 ± 0.02	> 1000	> 1000	> 2439.0	> 2439.0
31b	7.60 ± 0.96	497.1 ± 42.1	352.7 ± 13.2	65.4	46.4
31f	134 ± 17	> 1000	> 1000	> 7.5	> 7.5
31g	29.4 ± 3.3	483.24 ± 33.71	120.66 ± 31.54	16.4	4.1
34a	31.0 ± 4.7	> 1000	> 1000	> 32.3	> 32.3
34b	2.07 ± 0.34	50.24 ± 13.07	> 1000	24.3	> 483.1
34c	2.40 ± 0.25	19.88 ± 5.71	> 1000	8.3	> 416.7
34d	2.08 ± 0.14	118.12 ± 47.24	> 1000	56.8	> 480.8
34e	68.5 ± 9.0	> 1000	> 1000	> 14.6	> 14.6
36	89.5 ± 1.1	ND	ND	ND	ND
37	447 ± 55	ND	ND	ND	ND

[a] K_i values (mean ± SEM) were determined in at least three experiments. [b] K_i for VAChT is measured using *Torpedo* synaptic vesicles. [c] σ_1 binding is measured using guinea pig brain membranes. [d] σ_2 binding is measured using rat liver preparations. [e] Ref 59. [f] Ref 54. [g] Ref 43.

(**34b**−**d**) displayed affinities comparable to **28a**. The angular carbamoyl group thus permits deep penetration of substituents into the binding pocket around fragment-C without a substantial loss of binding affinity.

The introduction of an amino group into **2** (benzovesamicol) increases affinity for VAChT by an order of magnitude, making **3** (K_i = 0.0065 ± 0.0005nM) the most potent VAChT inhibitor reported to date.[54] In the present study, we therefore sought to investigate the effect of the 5-amino group on the affinity of 4-benzoylpiperidines. Surprisingly, no improvement was observed for the 4-benzoylpiperidines (**28b** vs **30b** and **28c** vs **30c**) or the 4-(thien-2-yl)carbonylpiperidines (**28f** vs **30f** and **28g** vs **30g**). There was, nevertheless, a separation in affinity between the regioisomeric pairs (**30b** vs **31b**, **30f** vs **31f**, **30g** vs **31g**, and **36** vs **37**) similar to that observed with **2** and its 8-amino isomer.[5] Thus, it would appear that the structure−activity relationships of the 4-aroylpiperidines largely parallel, but are not identical to, those of the 4-phenylpiperidines.

The moderate to high affinity of vesamicol and many of its analogues for σ_1 and σ_2 receptors reduces the selectivity of these compounds for the VAChT and potentially compromises their utility as VAChT ligands. Consequently, we screened the target compounds for sigma binding to identify selective VAChT ligands from this new structural class of agents. Among the compounds tested, there was wide variability in affinity for σ_1 or σ_2 receptors; only one compound (**27h**) displayed high affinity for σ_1 receptors, while five compounds displayed moderate affinity for either σ_1 or σ_2 receptors. All other compounds exhibited poor affinity for σ receptors. As in previous studies of vesamicol analogues,[5,39,45] we have derived estimates of ligand selectivity, expressed as a selectivity index, by comparing K_i values for VAChT obtained from highly purified *Torpedo* synaptic vesicles, with corresponding values for σ_1 and σ_2 receptors determined from rat or guinea pig brain preparations. In the present study, all cyclohexyl-containing analogues examined (**27a**−**h**) displayed poor VAChT/σ receptors (Table 1). Among the benzovesamicol analogues, two compounds (**28a**,**b**) showed moderate selectivity while three others (**28c**,**g**,**h**) displayed high VAChT/σ selectivity. In the latter group, the bromo analogue **28c** emerged as the most selective compound. Among the aminobenzovesamicol analogues **30b**−**g**, all compounds except **31b** displayed high VAChT/σ selectivity. In contrast, among the amide-containing analogues **34a**−**d**, only **34d** exhibited moderate selectivity while the rest showed poor selectivity for VAChT. Collectively, these observations are consistent with previous studies, which suggest that benzovesamicol (**2**) and aminobenzovesamicol (**3**) are more selective VAChT ligands than vesamicol (**1**).

We have therefore shown that the phenyl group of vesamicol can be successfully replaced with aroyl groups. The current demonstration serves as a useful point of departure for expanding the search for new structural classes of VAChT inhibitors.

Conclusion

In the present study, we have synthesized a new class of compounds targeting VAChT. In an effort to identify compounds that have high affinity and high selectivity for VAChT, we modified the vesamicol structure by interposing a carbonyl group between the B region and the C region of the vesamicol structure, and exploited the observation that benzovesamicol analogs have increased VAChT binding affinity compared with vesamicol. From this study, we have identified six compounds, **28c** (0.25 nM), **28e** (0.48 nM), **28g** (0.54 nM), **28h** (0.66 nM), **30c** (0.53 nM), and **30g** (0.41 nM), that have very high affinity for VAChT. These compounds also display very high selectivity for VAChT versus σ_1 and σ_2 receptors, and could serve as useful pharmacological probes. For **28c** and **30g**, the VAChT/σ selectivity ratios are greater than 1000, while for **30c**, the selectivity ratio is greater than 500-fold. We also have identified 12 compounds that have high affinity for VAChT (K_i, 1.0−10 nM) some of these also display very high selectivity for VAChT vs σ receptors. Furthermore, promising analogues which contain fluorine, iodine, or bromine provide the possibility to make corresponding radio halogenated ligands to serve as PET/SPECT probes. Further evaluation of these radioligands could provide useful tools for studying the relationship between alteration of VAChT expression in the brain and the cholinergic deficiency associated with the severity of dementia associated with cholinergic deficiency in patients with neurodegenerative diseases.

Experimental Section

General. Synthetic intermediates were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Lancaster Synthesis (Windham, MA) and used as received. Tetrahydrofuran (THF) was distilled from sodium hydride immediately prior to use. All other reagents and solvents were purchased as reagent grade and used without further purification.

All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Yields were not optimized. Melting points were determined on a Haake-Buchler or Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 200 MHz IBM-Bruker spectrometer or a 300 MHz GE spectrometer. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. With this condition, the chemical shifts (in ppm) of residual solvents are observed at 7.26 ($CHCl_3$) or 4.78 (CD_3OH). The following abbreviations were used to describe peak patterns wherever appropriate: b = broad, d = doublet, t = triplet, q = quartet, m = multiplet. Preparative chromatography was performed on Harrison Research Chromatotron using Merck 60 PF254 silica gel or a preparative HPLC system (Rainin Instrument Co.) using a 41.1 mm i.d. Dynamax silica gel column (delivering solvent at 80 mL/min). Analytical TLC was carried out on Analtech GHLF silica gel glass plates, and visualization was aided by UV. Elemental analysis and HPLC method were used to determine the purity of the target compounds that were assessed biological data. All the compounds reported in the manuscript have a purity ≥95%.

Procedure A: General Method of Preparing 4-Acyl-4-phenyl-*N*-tosylpiperidines (17a−c). 4-Benzoyl-4-phenyl-1-(*p*-toluenesulfonyl)piperidine (17a). Phenylmagnesium bromide was freshly prepared from bromobenzene (4.74 g, 30.2 mmol) and Mg (0.73 g, 30.0 mmol) in 10 mL of dry THF. A solution of **16** (5.1 g, mmol) in dry toluene (20 mL) was added under nitrogen to the stirring solution of phenylmagnesium bromide in THF. The latter solvent was removed by distillation (at 70 °C oil bath temp), and the resulting mixture was refluxed overnight, cooled, and acidified with 10% aqueous HCl. The precipitate was collected by filtration, washed with diethyl ether, and recrystallized from ethanol to give **17**a (5.5 g, 88%). ^{1}H NMR ($CDCl_3$) δ 2.12−2.63 (m, 9H), 3.60−3.71 (m, 2H), 7.10−7.70 (m, 14H).

4-Fluorobenzoyl-4-phenyl-1-(*p*-toluenesulfonyl)piperidine (17b). Procedure A was used to prepare **17b** (1.2 g, 90%). ^{1}H NMR ($CDCl_3$) δ 2.11−2.62 (m, 9H), 3.62−3.74 (m, 2H), 6.91−7.71 (m, 13H).

4-Phenylacetyl-4-phenyl-1-(*p*-toluenesulfonyl)piperidine (17c). Procedure A was used to prepare **17c** (0.9 g, 61%). ^{1}H NMR ($CDCl_3$) δ 2.10−2.62 (m, 9H), 3.39 (s, 2H), 3.55−3.59 (m, 2H), 6.74−7.59 (m, 14H).

Procedure B: General Method of Preparing 4-Acyl-4-phenylpiperidines (18a−c). 4-Benzoyl-4-phenylpiperidine (18a). Compound **17a** (2.0 g, 4.8 mmol) was dissolved in 7 mL of 70% sulfuric acid and the solution was heated at 160−170 °C for 2 h. The reaction mixture was cooled to room temperature, and then carefully neutralized with dilute aqueous NaOH and extracted with methylene chloride. The organic extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **18a** (1.4 g, 99%) of the desired compound. ^{1}H NMR ($CDCl_3$) 2.05−3.41 (m, 8H), 7.16−7.44 (m, 10H). **18a** was used without further purification.

Procedure B was used to prepare **18b** and **18c** in 95% and 45% yield, respectively, starting with the corresponding sulfonamides. For **18b**, ^{1}H NMR ($CDCl_3$) is 2.0−3.45 (m, 8H), 7.15−7.50 (m, 7H), 7.75−8.15 (2H); for **18c**, ^{1}H NMR ($CDCl_3$) is 2.09−2.70 (m, 8H), 3.56−3.61 (m, 2H), 7.08−7.50 (m, 10H).

Procedure C: General Method of Preparing Cyclohexanols (20,27a−c and 27f−h). 2-(4-Benzoyl-4-phenylpiperidinyl)cyclohexanol Hydrochloride (20). A mixture of **18a**, anhydrous sodium carbonate, and cyclohexene oxide in absolute ethanol (25 mL) was refluxed for 48 h, and then it was cooled and filtered to remove insoluble material. The precipitate was washed with a small amount of ethanol (5 mL) and set aside. Concentration of the filtrate provided a residue that was purified by radial flow chromatography using acetone/hexane (20/80) as mobile phase to give **20** (1.0 g, 45%). ^{1}H NMR ($CDCl_3$). δ 1.10−2.79 (m, 17H), 3.30−3.40 (m, 1H), 3.80−4.20 (s, 1H), 7.20−7.50 (m, 10H). The hydrochloride was obtained from methanolic HCl and recrystallized from i-PrOH; mp 240−241 °C. Anal. ($C_{24}H_{29}NO_2\cdot$HCl) C, H, N.

Procedure D: General Method of Preparing Tetralins (22a−c and 28f−h). *trans*-3-(4-Benzoyl-4-phenylpiperidinyl)-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (22a). A mixture of dihydronaphthalene bromohydrin (2.0 g, 8.8 mmol) in $CHCl_3$ (25 mL) and 2 N NaOH (25 mL) was refluxed for 2 h and allowed to cool. The organic layer was separated and the aqueous phase was extracted with methylene chloride (2 × 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and then concentrated to yield crude dihydronaphthalene oxide (**21**). The latter was dissolved in ethanol, and to this solution was added **18a** (2.2 g, 8.3 mmol) and sodium carbonate (4.0 g, 37.7 mmol). The resulting mixture was refluxed for 42 h, and then cooled and filtered. The precipitate was washed with a small amount of ethanol and set aside. The filtrate was concentrated to a residue that was redissolved in methylene chloride (60 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated to a residue. The crude product was purified on radial flow chromatography to give **22a** (1.0 g, 46%). ^{1}H NMR ($CDCl_3$) δ 2.72−3.70 (m, 13H), 4.19−4.22 (m, 1H), 5.00−5.50 (s, 1H), 7.07−7.56 (m, 14H). The free base was converted to the hydrochloride by dissolving in methanolic HCl and recrystallizing from isopropyl alcohol−diethyl

ether; mp 248−249 °C. Anal. ($C_{28}H_{29}NO_2 \cdot HCl \cdot 0.25H_2O$) C, H, N.

***trans*-3-(4-Fluorobenzoyl-4-phenylpiperidinyl)-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (22b).** Procedure D was used to prepare **22b** (0.72 g, 40%). mp 242−243 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 2.10−3.34 (m, 13H), 3.79−3.88 (m, 1H), 4.10−4.50 (s, 1H), 6.91−7.54 (m, 13H). Anal. ($C_{28}H_{28}FNO_2 \cdot HCl \cdot 0.25H_2O$) C, H, N.

***trans*-2-Hydroxy-3-(4-phenylacetyl-4-phenylpiperidinyl)-1,2,3,4-tetrahydronaphthalene Hydrochloride (22c).** Compound **22c** was prepared using procedure D to give (0.14 g, 13%). mp 243−244 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 2.09−3.58 (m, 13H), 3.53 (s, 2H), 3.83−3.87 (m, 1H), 4.27 (s, 1H), 6.87−7.47 (m, 14H). Anal. ($C_{29}H_{32}NO_2 \cdot HCl \cdot 0.25H_2O$) C, H, N.

Compounds **26a−e** were obtained as reported in literature.[33] Compounds **26f−h** were made by using similar procedure of making compounds **26a−e**.[33]

1-Benzoyl-4-(thien-2-yl)carbonylpiperidine (25f). Yield, 80%. ^{1}H NMR ($CDCl_3$) δ 1.60−5.00 (m, 9H), 7.10−7.90 (m, 8H).

1-Benzoyl-4-(5-bromothien-2-yl)carbonylpiperidine (25g). Yield, 80%. ^{1}H NMR ($CDCl_3$) δ 1.70−4.85 (m, 9H), 7.26−7.52 (m, 5H), 7.11−7.50 (d, 2H).

1-Benzoyl-4-(5-iodothien-2-yl)carbonylpiperidine (25h). Yield, 76%. ^{1}H NMR ($CDCl_3$) δ 1.60−5.00 (m, 9H), 7.10−7.80 (m, 8H).

4-(Thien-2-yl)carbonyliperidine Hydrochloride (26f). Yield, 72%. ^{1}H NMR ($CDCl_3$) δ 1.60−3.60 (m, 9H), 7.00−7.90 (m, 3H), 6.00−6.80 (s, 1H).

4-(5-Bromothien-2-yl)carbonylpiperidine Hydrochloride (26g). Yield, 68%. ^{1}H NMR not provided.

4-(5-Iodothien-2-yl)carbonylpiperidine Hydrochloride (26h). Yield, 67%. ^{1}H NMR (DMSO-d_6) δ 1.50−3.80 (m, 9H), 7.10−8.30 (m, 2H), 8.70−8.60 (s, 2H).

Compounds **27a−c**, **27f−h** were made by using procedure C described above.

2-(4-Benzoylpiperidinyl)cycohexanol Hydrochloride (27a). Compound **27a** was prepared by using procedure C to give (0.45 g, 51%). mp 232−233 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.00−3.20 (m, 18H), 3.30−3.60 (s, 1H), 3.80−4.20 (s, 1H), 7.40−8.20 (m, 5H). Anal. ($C_{18}H_{25}NO_2 \cdot HCl$) C, H, N.

2-[4-(*p*-Fluorobenzoyl)piperidinyl]cycohexanol Hydrochloride (27b). Compound **27b** was prepared by using procedure C to give (0.35 g, 63%). mp 263−264 °C. ^{1}H NMR (DMSO-d_6) δ 1.20−1.36 (m, 4H), 1.60−1.70 (m, 2H), 1.98 (m, 4H), 2.86−3.66 (m, 8H), 4.62 (t, 1H), 7.36 (m, 2H), 8.08 (m, 2H). Anal. ($C_{18}H_{24}FNO_2 \cdot HCl$) C, H, N.

2-[4-(*p*-Bromobenzoyl)piperidinyl]cycohexanol Hydrochloride (27c). Compound **27c** was prepared by using procedure C to give (1.4 g, 80%). mp 294−296 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.00−3.30 (m, 18H), 3.30−3.60 (s, 1H), 3.80−4.25 (s, 1H), 7.50−8.20 (d, 4H). Anal. ($C_{18}H_{24}BrNO_2 \cdot HCl$) C, H, N.

2-[4-(Thien-2-yl)carbonylpiperidinyl]cyclohexanol Hydrochloride (27f). Compound **27f** was prepared by using procedure C to give (0.80 g, 42%). mp 247−248 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 0.80−3.25 (m, 18H), 3.25−3.50 (m, 1H), 3.80−4.25 (s, 1H), 7.00−7.85 (m, 3H). CIMS: Calcd, 294.1483 (M+); Found, 293.1483 (M+). Anal. ($C_{16}H_{23}NO_2S \cdot HCl$) C, H, N.

2-[4-(5-Bromothien-2-yl)carbonylpiperidinyl]cyclohexanol Hydrochloride (27g). Compound **27g** was prepared by using procedure C to give (0.43 g, 30%). mp 283−285 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.00−3.20 (m, 18H), 3.20−3.60 (s, 1H), 3.70−4.20 (s, 1H), 6.90−7.80 (m, 2H). Anal. ($C_{16}H_{22}BrNO_2S \cdot HCl$) C, H, N.

2-[4-(5-Iodothien-2-yl)carbonylpiperidinyl]cyclohexanol Hydrochloride (27h). Compound **27h** was prepared by using procedure C to give (1.0 g, 71%); mp 236−238 °C. ^{1}H NMR ($CDCl_3$) δ 0.80−3.80 (m, 18H), 3.80−4.80 (m, 2H), 7.00−7.90 (m, 2H). CIMS: Calcd 420.0449 (M+); Found 420.0449 (M+).

Compounds **28a−e** were made as reported.[33]

***trans*-2-Hydroxy-3-[4-(thien-2-yl)carbonylpiperidinyl]-1,2,3,4-tetrahydronaphthalene Hydrochloride (28f).** Compound **28f** was prepared by suing procedure D to give (0.6 g, 20%). mp 253−255 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.70−3.40 (m, 14H), 3.80−4.00 (m, 1H), 4.00−4.60 (s, 1H), 7.00−7.85 (m, 7H). CIMS: Calcd 342.1485 (M+); Found 342.1485 (M+). The purity of **28f** was greater than 95% (determined by HPLC).

***trans*-3-[4-(5-Bromothienylcarbonyl)piperidinyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (28g).** Compound **28g** was prepared by using procedure D to give (1.3 g, 30%). mp 273−274 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.80−3.14 (m, 13H), 3.28−3.36 (m, 1H), 3.83−3.92 (m, 1H), 4.17 (s, 1H), 7.00−7.60 (m, 6H). Anal. ($C_{20}H_{22}BrNO_2S \cdot HCl \cdot 0.5H_2O$) C, H, N.

***trans*-3-[4-(5-Iodothienylcarbonyl)piperidinyl]-2-hydroxy-1,2,-3,4-tetrahydronaphthalene Hydrochloride (28h).** Compound **28h** was prepared by using procedure D to give (0.65 g, 42%). mp 275−277 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.60−2.80 (m, 14H), 4.10−4.40 (m, 2H), 7.00−7.90 (m, 6H). CIMS: Calcd, 468.0449 (M+); Found, 468.0449 (M+). The purity of **28h** was greater than 95% (determined by HPLC).

Procedure E: General Method of Preparing Amino Tetralins 30b−c and 30f−g, 31b−c and 30f−g, as well as 36 and 37. 5-Amino-3-[4-(*p*-fluorobenzoyl)piperidinyl]-2-hydroxy-1,2,3,4,-tetrahydronaphthalene Hydrochloride (30b) and 8-Amino-3-[4-(*p*-fluorobenzoyl)-piperidinyl]-2-hydroxy-1,2,3,4,-tetrahydronaphthalene Hydrochloride (31b). A mixture of **26b** (2.2 g, 9.1 mmol), **29** (0.94 g, 3.7 mmol), and sodium carbonate (3.86 g, 36 mmol) in absolute ethanol (50 mL) was refluxed for 48 h. The reaction mixture was cooled to room temperature and filtered to remove insoluble material. The filtrate was concentrated to a residue that was redissolved into acetone (150 mL). More insoluble material was removed by filtration. The solvent was removed by rotary evaporator. The residue was dissolved into 70% aqueous ethanol (50 mL). NaOH pellets (0.3 g, 7.5 mmol) were added into the solution. The reaction mixture was stirred overnight and then concentrated to a residue in vacuum. The residue was treated with water (20 mL) and extracted with methylene chloride (3 × 20 mL). After drying over anhydrous Na_2SO_4, the organic extract was concentrated to a residue. The residue was purified on silica gel column using isopropyl alcohol/hexane (20/80) as mobile phase to give **30b** (1.24 g, 37%) and **31b** (1.24 g, 37%) as 1:1 ratio. In this system, compound **30b** displayed greater chromatographic mobility. The free bases were converted to the corresponding hydrochlorides by using methanolic HCl. The hydrochloride salt was recrystallized from *i*-PrOH. For compound **30b**, mp 212 °C (decomposed) ^{1}H NMR [free base] ($CDCl_3$) δ 1.82−1.94 (m, 4H), 2.44−3.22 (m, 8H), 3.26−3.33 (m, 2H), 3.62 (s, 2H), 3.87 (m, 1H), 4,20 (br s, 1H), 6.57 (t, 2H, J = 9 Hz), 6.99 (t, 1H, J = 9 Hz), 7.16 (t, 2H, J = 9 Hz), 7.97 (m, 2H). Anal. ($C_{22}H_{25}FN_2O_2 \cdot 2HCl \cdot 1.25H_20$) C, H, N. For compound **31b**, mp 217 °C (decomposed), ^{1}H NMR [free base] ($CDCl_3$) δ 1.71−1.96 (m, 4H), 2.36−2.44 (m, 2H), 2.75−3.30 (m, 8H), 3.63 (s, 2H), 3.93 (m, 1H), 4.30 (br s, 1H), 6.55 (d, 2H, J = 7.5 Hz), 6.99 (t, 1H, J = 7.5 Hz), 7.16 (t, 2H, J = 9 Hz), 7.97 (m, 2H). Anal. ($C_{22}H_{25}FN_2O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N.

5-Amino-3-[4-(*p*-bromobenzoyl)piperidinyl]-2-hydroxy-1,2,3,4,-tetrahydronaphthalene Hydrochloride (30c) and 8-Amino-3-[4-(*p*-bromobenzoyl)-piperidinyl]-2-hydroxy-1,2,3,4,-tetrahydronaphthalene (31c). Procedure E was used to prepare **30c** (1.0 g, 36%) and **31c** (0.69 g, 25%). For compound **30c**, ^{1}H NMR [free base] ($CDCl_3$) δ 1.70−3.40 (m, 14H), 3.40−4.75 (m, 4H), 6.45−7.20 (m, 3H), 7.50−8.20 (d, 4H). Anal. ($C_{22}H_{25}N_2O_2Br \cdot 2HCl$) C, H, N. For compound **31c**, ^{1}H NMR [free base] ($CDCl_3$) δ 1.50−3.40 (m, 14H), 3.40−4.70 (m, 4H), 6.40−7.20 (m, 3H), 7.50−8.20 (d, 4H).

5-Amino-3-[4-(thien-2-yl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (30f) and 8-Amino-3-[4-(thien-2-yl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (31f). Procedure E was used to prepare **30f** (0.8 g, 41%) and **31f** (0.78 g, 40%). For compound **30f**, mp 225−227 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.60−3.40 (m, 14H), 3.50−4.20 (m, 4H), 6.40−8.10 (m, 6H). Anal. ($C_{20}H_{24}N_2O_2 \cdot HCl$) C, H, N. For compound **31f**, mp 243−247 °C. ^{1}H NMR

[free base] ($CDCl_3$) δ 1.80−3.5 (m, 14H), 3.50−4.20 (m, 4H), 6.40−8.10 (m, 6H). Anal. ($C_{20}H_{24}N_2O_2 \cdot HCl$) C, H, N.

5-Amino-3-[4-(5-bromothien-2-yl)carbonylpiperidinyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (30g) and 8-Amino-3-[4-(5-bromothien-2-yl)carbonylpiperidinyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (31g). Procedure E was used to prepare **30g** (1.0 g, 50%) and **31g** (1.0 g, 50%) as 1:1 ratio. For compound **30g**, mp 195 °C (decomposed), ^{1}H NMR [free base] ($CDCl_3$) δ 1.70−3.40 (m, 14H), 3.40−4.75 (m, 4H), 6.40−8.10 (m, 5H). Anal. ($C_{20}H_{23}BrN_2O_2 \cdot 2HCl$) H, N, C. For compound **31g**, mp 215 °C (decomposed), ^{1}H NMR [free base] ($CDCl_3$) δ 1.70−3.40 (m, 14H), 3.40−4.75 (m, 4H), 6.40−8.10 (m, 5H). Anal. ($C_{20}H_{23}BrN_2O_2 \cdot 2HCl$) C, H, N.

1-(*t*-Butoxycarbonyl)piperidine 4-Carboxylic Acid Succinimide Ester (32). A mixture of ethyl isonipecotate (24.8 g, 158 mmol), di-*tert*-butyldicarbonate (36.3 g, 166 mmol), sodium chloride (42 g, 1.05 mmol) in chloroform (100 mL), and water (125 mL) was refluxed for 2.5 h and cooled to room temperature. The layers were separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 35 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, and then concentrated to pale yellow syrup. The latter was dissolved into EtOH (60 mL), and the resulting solution treated with 6.25 M NaOH solution (40 mL). After stirring at room temperature for 14 h, the reaction mixture was concentrated to half its original volume, and treated with a saturated aqueous solution of NaCl (80 mL). The resulting mixture was cooled in ice bath, stirred vigorously, and treated carefully with glacial acetic acid (21.5 mL). Extraction of the resulting mixture followed by drying over anhydrous Na_2SO_4. After concentrating the organic extracts, it gave the N-protected isonipecotic acid as a white solid (33.1 g, 91%). The latter was used without further purification. Accordingly, a cold (ice bath) solution of this acid (11.46 g, 50.0 mmol) in methylene chloride (90 mL) was treated consecutively with *N*-hydroxysuccinimide (6.33 g, 55.0 mmol) and DCC (11.35 g, 55.0 mmol) dissolved in 30 mL of methylene chloride). Cooling was stopped after 20 min and stirring was continued at room temperature. After 16 h, the reaction mixture was cooled in an ice bath and filtered. The suspension was washed with a minimum volume of methylene chloride. The filtrate was washed with saturated aqueous sodium bicarbonate (2 × 35 mL), dried over anhydrous Na_2SO_4, and then concentrated to a white solid. The latter was triturated with hot hexane, and the mixture cooled and filtered to give **32** (16 g, 98%) of the activated ester. ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 9H), 1.7−1.85 (m 1H), 1.95−2.05 (m, 1H), 2.75−3.05 (m, 2H), 4.00 (m, 1H).

Procedure F: General Method of Preparing Isonipecotamide Derivatives (34a−d). *Trans*-3-[4-(piperidyl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (34a). A mixture of piperidine (0.90 g, 10.6 mmol) and **32** (3.26 g, 10.1 mmol) in dry acetonitrile (50 mL) was refluxed for 26 h and concentrated to a residue. The latter was redissolved into methylene chloride (80 mL), and the solution was washed with saturated aqueous sodium bicarbonate (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated to an off-white solid. The solid was redissolved in ethyl acetate (50 mL) and the solution cooled in an ice bath. HCl(g) was bubbled vigorously through the solution for 20 min, and stirring was continued at room temperature. After 3 h, the reaction mixture was concentrated to give crude **33a**. The latter was redissolved in EtOH (60 mL), and the solution treated with sodium bicarbonate (2.15 g, 20 mmol) and dihydronaphthalene bromohydrin (1.82 g, 8.0 mmol). The resulting mixture was refluxed for 17 h, and concentrated to a residue that was partitioned between water (60 mL) and methylene chloride (2 × 60 mL). The organic extracts were combined, dried, and concentrated to a dark brown residue. The product was purified by silica gel radial flow chromatography using acetone/hexane (20/80) as mobile phase. The free base was converted to the hydrochloride in methanolic HCl, and then, the hydrochloride salt was recrystallized from isopropyl alcohol to give **34a** (0.50 g, 16%) of white powder. mp 252−254 °C. ^{1}H NMR (DMSO-d_6) δ 1.39−1.57 (m, 6H), 1.74−2.11 (m, 4H), 2.73−2.82 (m, 1H), 2.92−3.45 (m, 13H), 4.11 (m, 1H), 7.13 (m, 4H). Anal. ($C_{21}H_{30}N_2O_2 \cdot HCl$) C, H, N.

***trans*-3-[4-(4-Phenylpiperidyl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (34b).** Compound **34b** was prepared by using procedure F to give (0.3 g, 8%). mp 238−240 °C. ^{1}H NMR (DMSO-d_6) δ 1.34−1.56 (m, 2H), 1.74−2.18 (m, 5H), 2.54−2.62 (t, 1H), 2.74−2.79 (m, 2H), 2.99−3.45 (m, 8H), 4.11 (m, 2H), 4.52 (d, 1H), 7.09−7.30 (m, 9H). EIMS: Calcd, 454.2387; Found, 418.2625 $(M-HCl)^+$. Anal. ($C_{27}H_{34}N_2O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

***trans*-3-[4-(4-Benzylpiperidyl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (34c).** Compound **34c** was prepepared by using procedure F to give (0.6 g, 32%), mp 246−248 °C. ^{1}H NMR (DMSO-d_6) δ 0.89−1.11 (m, 2H), 1.57 (m, 2H), 2.47 (m, 3H), 2.73−3.41 (m, 12H), 4.00 (d, 1H), 4.11 (m, 1H), 4.33 (d, 1H), 6.08 (s, 1H), 7.08−7.29 (m, 9H). EIMS: Calcd, 433.2810 (M+); Found, 433.2812 (M+). Anal. ($C_{28}H_{36}N_2O_2 \cdot HCl$) C, H, N.

***trans*-3-[4-(4-Benzylpiperazinyl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (34d).** Procedure F was used to prepare **34d** (0.82 g, 77%). mp 250−252 °C. ^{1}H NMR (DMSO-d_6) δ 1.80−2.15 (m, 5H), 2.73−3.72 (m, 14H), 4.02−4.48 (m, 6H), 6.08 (s, 1H), 7.12 (m, 5H), 7.38 (m, 2H), 7.66 (m, 2H). EIMS: Calcd, 434.2780 (M+); Found, 434.2780 (M+). Anal. ($C_{27}H_{35}N_3O_2 \cdot 2HCl \cdot 0.75H_2O$) C, H, N.

***trans*-3-[4-Ethoxycarbonylpiperidyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene Hydrochloride (34e).** The ethyl isonipecotate (3.63 g, 23 mmol) was dissolved in EtOH (60 mL) and the solution treated with sodium bicarbonate (8.0 g, 75 mmol) and dihydronaphthalene bromohydrin (5.22 g, 23 mmol). The resulting mixture was refluxed overnight and concentrated to a residue which was partitioned between water (60 mL) and CH_2Cl_2 (2 × 60 mL). The organic extracts were combined, dried, and concentrated to a dark brown residue. The product was purified by silica gel column chromatography using ethyl acetate/hexane (30/70) as mobile phase to give **34e** free base, which was converted to the hydrochloride in methanolic HCl and recrystallized from ethanol to give **34e**·HCl (5.0 g, 71%) of white powder. mp 191−192 °C ^{1}H NMR ($CDCl_3$): δ 1.25−1.31 (t, 3H), 1.70−2.94 (m, 9H), 3.27−3.35 (m, 1H), 3.80−3.90 (m, 1H), 4.12−4.19 (q, 2H), 4.24 (s, 1H), 7.00−7.27 (m, 4H). EIMS: Calcd, 303.1832 (M+); Found, 304.1832 (M+).

***trans*-5-Amino-3-[4-(piperidyl)carbonylpiperidyl]-2-hydroxy-1,2,3,4,-tetrahydronaphthalene Hydrochloride (36) and *trans*-8-Amino-3-(4-(piperidyl)carbonylpiperidyl)-2-hydroxy-1,2,3,4,-tetrahydronaphthalene Hydrochloride (37).** Using procedure E gave compound **36** (1.2 g, 43%) and compound **37** (1.2 g, 43%). For compound **36**, mp 256−258 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.25−3.80 (m, 26H), 3.80−4.00 (m, 1H), 6.50−7.10 (m, 3H). EIMS: Calcd, 357.2416; Found, 357.2415. Anal. ($C_{21}H_{31}N_3O_2$).

For compound **37**, mp 264−266 °C. ^{1}H NMR [free base] ($CDCl_3$) δ 1.25−3.80 (m, 26H), 3.80−4.00 (m, 1H), 6.50−7.10 (m, 3H). EIMS: Calcd, 357.2416; Found, 357.2416. Anal. ($C_{21}H_{31}N_3O_2$) H, N, C.

***In Vitro* Biological Evaluation. Vesicular Acetylcholine Transporter Binding Assays.** In vitro assays of the binding of compounds to the VAChT were conducted with cholinergic synaptic vesicles isolated from the electric organ of *Torpedo californica*. The radioligand used was [^{3}H]vesamicol, and the assay conditions were conducted as described.[43] (−)-Vesamicol was used as an external standard in this assay. Averaged data were fit by regression with a rectangular hyperbola to estimate K_i value. All compounds were independently assayed at least two times.

Sigma 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 described in detail previously.[55,56]

Briefly, the σ_1 receptor binding assays were conducted in 96-well plates using guinea pig brain membrane homogenates (~300 μg 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 of cold haloperidol. After 90 min, the reaction was terminated by the addition of 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 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 of 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} value). 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[57] and are presented as the mean ± 1 SEM. For these calculations, we used a K_d value of 7.89 nM for [^{3}H](+)-pentazocine and guinea pig brain and a K_d value of 30.73 nM for [^{3}H]DTG and rat liver.[58]

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Supporting Information Available: Analytical data of new analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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