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

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### Abstract

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, six display very high affinity for VACHT ( $K_i$ , 0.25 – 0.66 nM) and greater than 500-fold selectivity for VACHT over  $\sigma_1$  and  $\sigma_2$  receptors. Twelve compounds have high affinity ( $K_i$ , 1.0–10 nM) and good selectivity for VACHT. Furthermore, three 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-iodothiencarbonyl)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*.

### Keywords

PET imaging; vesicular acetylcholine transporter; cholinergic function; vesamicol

### 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

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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>.

cholinergic neurons and synapses in the brain.<sup>1–5</sup> 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.<sup>6–12</sup> Until recently, cholinergic neurons were identified by the expression of only three marker proteins: choline acetyltransferase (ChAT), 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 neuropathologies of neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease are associated with alterations in the levels of AChE.<sup>13</sup> With the discovery of the anticholinergic properties of the lipophilic aminoalcohol (–)-*trans*-2-(4-phenylpiperidino)cyclohexanol (vesamicol, Figure 1), and the subsequent demonstration of a unique binding site for this molecule (the erstwhile vesamicol receptor) on cholinergic synaptic vesicles,<sup>14–16</sup> 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 [<sup>3</sup>H]vesamicol was found to exhibit a regional heterogeneity which is correlated with ChAT activity and [<sup>3</sup>H]hemicholinium-3 binding (an indicator of SDHAcHT).<sup>17,18</sup> These results suggested that the vesamicol receptor may be a reliable cholinergic marker. Although problems were subsequently encountered with [<sup>3</sup>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.<sup>19–24</sup> 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.<sup>25–29</sup> Consequently, VAcHT is now firmly established as a reliable cholinergic marker.

Recently, Prado and colleagues<sup>30</sup> 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.<sup>30</sup> 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.<sup>31,32</sup> 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.<sup>32</sup>

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.<sup>33</sup> We also reported (a) the radiosynthesis of an <sup>18</sup>F-labeled Positron Emission Tomography (PET) lead radiotracer, namely (–)-*trans*-2-hydroxy-3-(4-(4-[<sup>18</sup>F]fluorobenzoyl)piperidino)tetralin, in this new class, and (b) *in vivo* evaluation of the compound in rodents and monkeys. The initial evaluation suggest this radiotracer has specific binding to VAcHT enriched striatum region of the midbrain. The process of determining the potential clinical utility of this tracer for PET imaging of VAcHT is still underway.

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.<sup>33</sup> 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;<sup>34–37</sup> 2) the marginal selectivity of [<sup>3</sup>H]vesamicol<sup>14,38,39</sup> and the inability of this ligand to adequately reflect the loss of cholinergic neurons in experimental animals<sup>17,18,40–42</sup> or in Alzheimer's disease;<sup>35</sup> and 3) the need for a suitable radioligand that could serve as PET probe for imaging VACHT *in vivo*.

## Results and Discussion

Previously, we and others have described several high-affinity VACHT ligands.<sup>5,39,43–50</sup> 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.<sup>43</sup> 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  $\sigma$  receptors,<sup>51</sup> many vesamicol analogues bind to both VACHT and  $\sigma$  receptors.<sup>5,38,39</sup> 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**,<sup>43</sup> that is comparable in affinity to the parent.<sup>35</sup> On the basis of these observations, we proposed that within 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<sup>52,53</sup> 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.<sup>33</sup> 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-Crafts 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 literature.<sup>33</sup> 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**.

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<sub>3</sub>N 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  $\sigma_1$  and  $\sigma_2$  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  $K_i(\sigma_1 \text{ or } \sigma_2)/K_i(\text{VAcHT})$ . In the ensuing discussion, binding affinities are characterized as very high ( $K_i < 1.0$  nM), high ( $K_i = 1\text{--}10$  nM), moderate ( $K_i = 11\text{--}50$  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,<sup>43</sup> 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). 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** versus **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-arylpiperidines **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.

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-arylpiperidines **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-arylpiperidines **27a–b** and **27f** may be due in part to unfavorable steric interaction of the C4-aryl 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** versus **27b**, **27c** and **27f** versus **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** versus **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** versus **28b**); however, a 10–20 fold increase in affinity was observed when substitution was extended to include a bromo or nitro group (compare **28a** versus **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 carboxy group (**34e**) and a small selection of carbamoyl fragments (**34a–d**). While the 1-piperidylcarbonyl group brought about 7- and 545-fold reductions in affinity relative to **28a** and **2**, respectively, analogues containing substituted piperidyl/piperazinyl groups (**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** ( $K_i = 0.0065 \pm 0.0005$  nM) the most potent VACHT inhibitor reported to date.<sup>54</sup> 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** versus **30b** and **28c** versus **30c**) or the 4-(thien-2-yl)carbonylpiperidines (**28f** versus **30f** and **28g** versus **30g**). There was, nevertheless, a separation in affinity between the regioisomeric pairs (**30b** versus **31b**, **30f** versus **31f**, **30g** versus **31g** and **36** versus **37**) similar to that observed with **2** and its 8-amino isomer.<sup>5</sup> Thus, it would appear that the structure-activity relationships of the 4-arylpiperidines 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  $\sigma_1$  and  $\sigma_2$  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  $\sigma_1$  or  $\sigma_2$  receptors; only one compound (**27h**) displayed high affinity for  $\sigma_1$  receptors while five compounds displayed moderate affinity for either  $\sigma_1$  or  $\sigma_2$  receptors. All other compounds exhibited poor affinity for  $\sigma$  receptors. As in previous studies of vesamicol analogues,<sup>5,39,45</sup> 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  $\sigma_1$  and  $\sigma_2$  receptors determined from rat or guinea pig brain preparations. In the present study, all cyclohexyl-containing analogues examined (**27a–h**) displayed poor VACHT/ $\sigma$  receptors (Table 1). Among the benzovesamicol analogues, two compounds (**28a**, **b**) showed moderated selectivity while three others (**28c**, **g**, **h**) displayed high VACHT/ $\sigma$  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/ $\sigma$  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 the high affinity and high selectivity of VACHT, we modified the vesamicol structure by interposing a carbonyl group between the B region and the C region of the vesamicol structure, and exploiting 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*  $\sigma_1$  and  $\sigma_2$  receptors, and could serve as useful pharmacological probes. For **28c** and **30g** the VACHT/ $\sigma$  selectivity ratios are greater than 1000, while **30c**, the selectivity ratio is greater than 500 fold. We also have identified 12 compounds that have high affinities for VACHT ( $K_i$ , 1.0 – 10 nM) and some of them display very high selectivity for VACHT *versus* sigma receptors. Furthermore, some analogues contain fluorine, iodine and bromide and thus provide the possibility to make corresponding radioactive ligands to serve as PET/SPECT probes. Further evaluation of these radioligands could provide useful tools for studying the relationship between alternation of VACHT expression in brain and the cholinergic deficiency associated with the severity of dementia 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.  $^1\text{H}$  NMR spectra were recorded on a 200 MHz IBM-Brucker 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 ( $\text{CHCl}_3$ ) or 4.78 ( $\text{CD}_3\text{OH}$ ). 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 id 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 by HPLC method were used to determine the purity of the target compounds that were assessed the biological data. All the compounds reported in the manuscript have a purity  $\geq 95\%$ .

### 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 **16** (5.5 g, 88%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to give **18a** (1.4 g, 99%) of the desired compound. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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**, <sup>1</sup>H NMR (CDCl<sub>3</sub>) is 2.0–3.45 (m, 8H), 7.15–7.50 (m, 7H), 7.75–8.15 (2H); for **18c**, <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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<sub>24</sub>H<sub>29</sub>NO<sub>2</sub>·HCl) C, H, N.

#### Procedure D: General method of preparing tetralins (22a–c & 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<sub>3</sub> (25 mL) and 2N 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<sub>2</sub>SO<sub>4</sub> 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 hours, 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 re-dissolved in methylene chloride (60 mL). The solution was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to a residue. The crude product was purified on radial flow



chromatography to give **22a** (1.0 g, 46%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{28}\text{H}_{29}\text{NO}_2 \cdot \text{HCl} \cdot 0.25\text{H}_2\text{O}$ ) 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.  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{28}\text{H}_{28}\text{FNO}_2 \cdot \text{HCl} \cdot 0.25\text{H}_2\text{O}$ ) 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.  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{29}\text{H}_{32}\text{NO}_2 \cdot \text{HCl} \cdot 0.25\text{H}_2\text{O}$ ) C, H, N.

Compounds **26a–e** were obtained as reported in literature.<sup>33</sup> Compounds **26f–h** were made by using similar procedure of making compounds **26a–e**.<sup>33</sup>

**1-Benzoyl-4-(thien-2-yl)carbonylpiperidine (25f)**: Yield, 80%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.60–5.00 (m, 9H), 7.10–7.90 (m, 8H).

**1-Benzoyl-4-(5-bromothien-2-yl)carbonylpiperidine (25g)**: Yield, 80%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  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%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.60–5.00 (m, 9H), 7.10–7.80 (m, 8H).

**4-(Thien-2-yl)carbonylpiperidine hydrochloride (26f)**: Yield, 72%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  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%.  $^1\text{H}$  NMR Non provided.

**4-(5-Iodothien-2-yl)carbonylpiperidine hydrochloride (26h)**: Yield, 67%.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  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)cyclohexanol hydrochloride (27a)**: Compound **27a** was prepared by using procedure C to give (0.45 g, 51%), Mp 232–233 °C.  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{18}\text{H}_{25}\text{NO}_2 \cdot \text{HCl}$ ) C, H, N.

**2-[4-(p-Fluorobenzoyl)piperidinyl]cyclohexanol hydrochloride (27b)**: Compound **27b** was prepared by using procedure C to give (0.35 g, 63%). mp 263–264 °C.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  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. ( $\text{C}_{18}\text{H}_{24}\text{FNO}_2 \cdot \text{HCl}$ ) C, H, N.

**2-[4-(p-Bromobenzoyl)piperidinyl]cyclohexanol hydrochloride (27c)**: Compound **27c** was prepared by using procedure C to give (1.4 g, 80%); mp 294–296 °C.  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{18}\text{H}_{24}\text{BrNO}_2 \cdot \text{HCl}$ ) 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. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>) 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<sup>+</sup>); Found, 293.1483 (M<sup>+</sup>). Anal. (C<sub>16</sub>H<sub>23</sub>NO<sub>2</sub>S.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. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>) 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<sub>16</sub>H<sub>22</sub>BrNO<sub>2</sub>S.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. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.80–3.80 (m, 18H), 3.80–4.80 (m, 2H), 7.00–7.90 (m, 2H). CIMS: Calcd, 420.0449 (M<sup>+</sup>); Found, 420.0449 (M<sup>+</sup>).

Compounds **28a–e** were made as reported.<sup>33</sup>

**trans-2-Hydroxy-3-[4-(thien-2-yl)carbonylpiperidinyl]-1,2,3,4-tetrahydronaphthalene hydrochloride (28f):** Compound **28f** was prepared by using procedure D to give (0.6 g, 20%); mp 253–255 °C. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>) 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<sup>+</sup>); Found, 342.1485 (M<sup>+</sup>). 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. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>) 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<sub>20</sub>H<sub>22</sub>BrNO<sub>2</sub>S.HCl.0.5H<sub>2</sub>O) 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 (0.65g, 42%); Mp 275–277 °C. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>) δ 1.60–2.80 (m, 14H), 4.10–4.40 (m, 2H), 7.00–7.90 (m, 6H). CIMS: Calcd, 468.0449 (M<sup>+</sup>); Found, 468.0449 (M<sup>+</sup>). 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<sub>2</sub>SO<sub>4</sub>, 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)  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{22}\text{H}_{25}\text{FN}_2\text{O}_2 \cdot 2\text{HCl} \cdot 1.25\text{H}_2\text{O}$ ) C, H, N. For compound **31b**, mp 217 °C (decomposed),  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{22}\text{H}_{25}\text{FN}_2\text{O}_2 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$ ) 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 **31b** (0.69 g, 25%). For compound **30c**,  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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. ( $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_2\text{Br} \cdot 2\text{HCl}$ ) C, H, N. For compound **31c**,  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  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)carbonylpiperidinyl]-2-hydroxy-1,2,3,4-tetrahydronaphthalene hydrochloride (30f) and 8-Amino-3-[4-(thien-2-yl)carbonylpiperidinyl]-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.  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  1.60–3.40 (m, 14H), 3.50–4.20 (m, 4H), 6.40–8.10 (m, 6H). Anal. ( $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2 \cdot \text{HCl}$ ) C, H, N. For compound **31f**, mp 243–247 °C.  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  1.80–3.5 (m, 14H), 3.50–4.20 (m, 4H), 6.40–8.10 (m, 6H). Anal. ( $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2 \cdot \text{HCl}$ ) 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),  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  1.70–3.40 (m, 14H), 3.40–4.75 (m, 4H), 6.40–8.10 (m, 5H). Anal. ( $\text{C}_{20}\text{H}_{23}\text{BrN}_2\text{O}_2 \cdot 2\text{HCl}$ ) H, N, C. For compound **31g**, mp 215 °C (decomposed),  $^1\text{H}$  NMR [free base] ( $\text{CDCl}_3$ )  $\delta$  1.70–3.40 (m, 14H), 3.40–4.75 (m, 4H), 6.40–8.10 (m, 5H). Anal. ( $\text{C}_{20}\text{H}_{23}\text{BrN}_2\text{O}_2 \cdot 2\text{HCl}$ ) C, H, N.

**1-(*t*-Butoxycarbonyl)piperidine 4-carboxylic acid succinimide ester (32):** A mixture of ethyl isonipecotatate (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  $\text{CHCl}_3$  ( $2 \times 35$  mL). The organic extracts were combined, dried over anhydrous  $\text{Na}_2\text{SO}_4$  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  $\text{Na}_2\text{SO}_4$ . After concentrated 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 and set

aside. The filtrate was washed with saturated aqueous sodium bicarbonate (2 × 35 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and then concentrated to a white solid. The latter was triturated with hot hexane, the mixture cooled and filtered to give **32** (16 g, 98%) of the activated ester. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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 re-dissolved into methylene chloride (80 mL), the solution washed with saturated aqueous sodium bicarbonate (2 × 20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to an off-white solid. The solid was re-dissolved 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 re-dissolved 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. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) 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<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub>.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. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) 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)<sup>+</sup>. Anal. (C<sub>27</sub>H<sub>34</sub>N<sub>2</sub>O<sub>2</sub>.HCl.0.5H<sub>2</sub>O) C, H, N.

##### **trans-3-[4-(4-Benzylpiperidyl)carbonylpiperidyl]-2-hydroxy-1,2,3,4-**

**tetrahydronaphthalene hydrochloride (34c):** Compound **34c** was prepared by using procedure F to give (0.6 g, 32%), mp 246–248 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) 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<sup>+</sup>); Found, 433.2812 (M<sup>+</sup>). Anal. (C<sub>28</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub>.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. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) 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<sup>+</sup>); Found, 434.2780 (M<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>35</sub>N<sub>3</sub>O<sub>2</sub>.2HCl.0.75H<sub>2</sub>O) 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 over night, and concentrated to a residue which was partitioned between water (60 mL) and CH<sub>2</sub>Cl<sub>2</sub> (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 <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  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<sup>+</sup>); Found, 304.1832 (M<sup>+</sup>).

**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. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>)  $\delta$  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<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub>).

For compound **37**, mp 264–266 °C. <sup>1</sup>H NMR [free base] (CDCl<sub>3</sub>)  $\delta$  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<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub>) 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 [<sup>3</sup>H]vesamicol, and the assay conditions were conducted as described.<sup>43</sup> (–)-vesamicol was used as an external standard in this assay. Averaged data were fit by regression with a rectangular hyperbola to estimate K<sub>i</sub> 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  $\sigma_1$  and  $\sigma_2$  receptor binding assays. The procedures for isolating the membrane homogenates and performing the  $\sigma_1$  and  $\sigma_2$  receptor binding assays have been described in detail previously.<sup>55,56</sup>

Briefly, the  $\sigma_1$  receptor binding assays were conducted in 96-well plates using guinea pig brain membrane homogenates (~300  $\mu$ g protein) and ~5 nM [<sup>3</sup>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  $\mu$ M of cold haloperidol. After 90 min, the reaction was terminated by the addition of 150  $\mu$ L of ice-cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) using a 96 channel transfer pipette (Fisher Scientific, Pittsburgh, PA). The samples were harvested and filtered rapidly through a 96-well fiber glass filter plate (Millipore, Billerica, MA) that had been presoaked with 100  $\mu$ L of 50 mM Tris-HCl buffer at pH 8.0 for 1 h. Each filter was washed 3 times with 200  $\mu$ L of ice-cold wash buffer, and the filter counted in a Wallac 1450 MicroBeta liquid scintillation counter (Perkin Elmer, Boston, MA).

The  $\sigma_2$  receptor binding assays were conducted using rat liver membrane homogenates (~300  $\mu$ g protein) and ~5 nM [<sup>3</sup>H]DTG (58.1 Ci/mmol, Perkin Elmer, Boston, MA) in the presence of 1  $\mu$ M (+)-pentazocine to block  $\sigma_1$  sites. The incubation time was 2 h at room temperature. Nonspecific binding was determined from samples that contained 10  $\mu$ M of cold haloperidol. All other procedures were identical to those described for the  $\sigma_1$  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<sup>57</sup> and are presented as the mean  $\pm$  1 SEM. For these calculations, we used a  $K_d$  value of 7.89 nM for [<sup>3</sup>H](+)-pentazocine and guinea pig brain and a  $K_d$  value of 30.73 nM for [<sup>3</sup>H] DTG and rat liver.<sup>58</sup>

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Abbreviations List

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	(-)- <i>trans</i> -2-(4-phenylpiperidino)cyclohexanol.

## References and notes

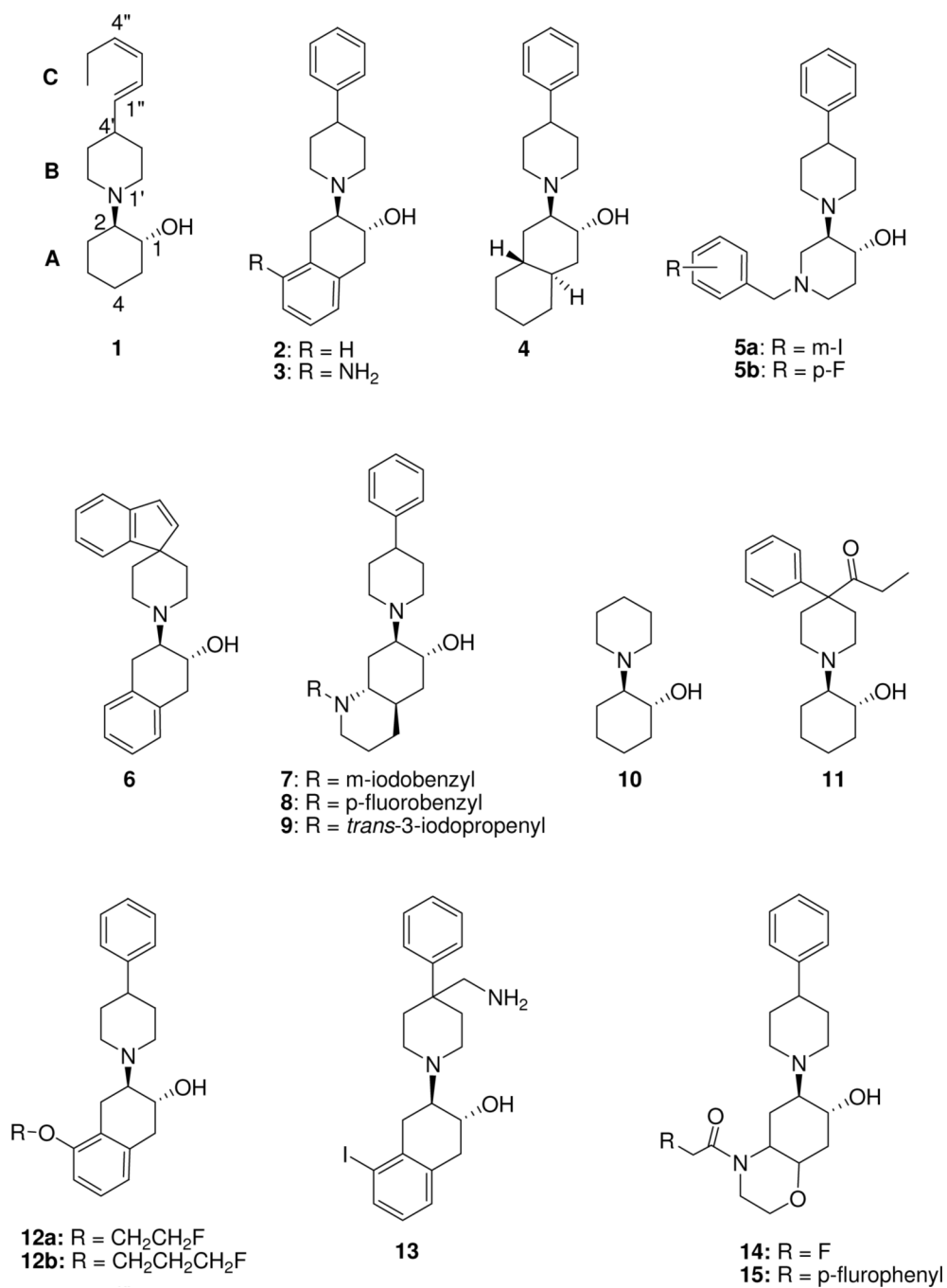
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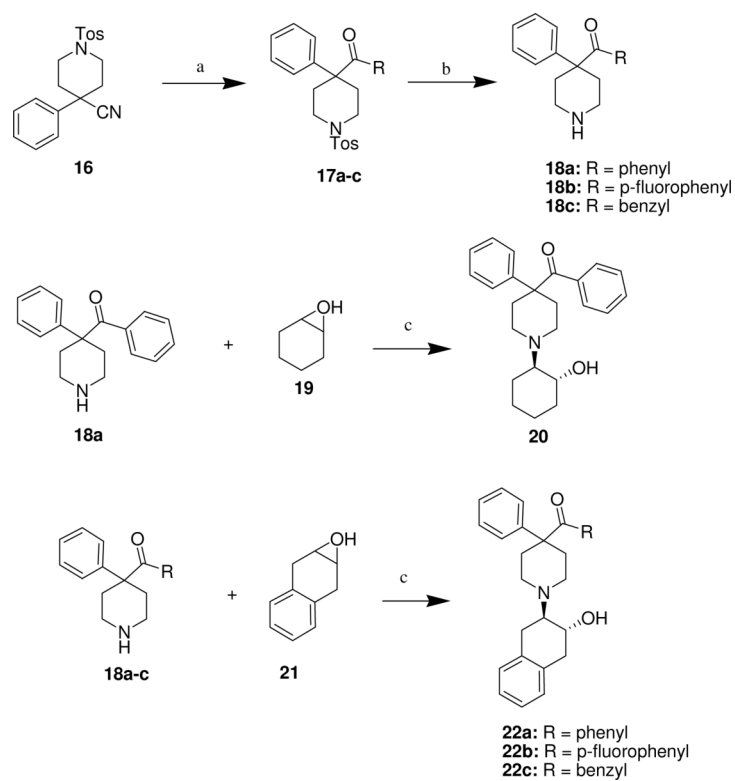
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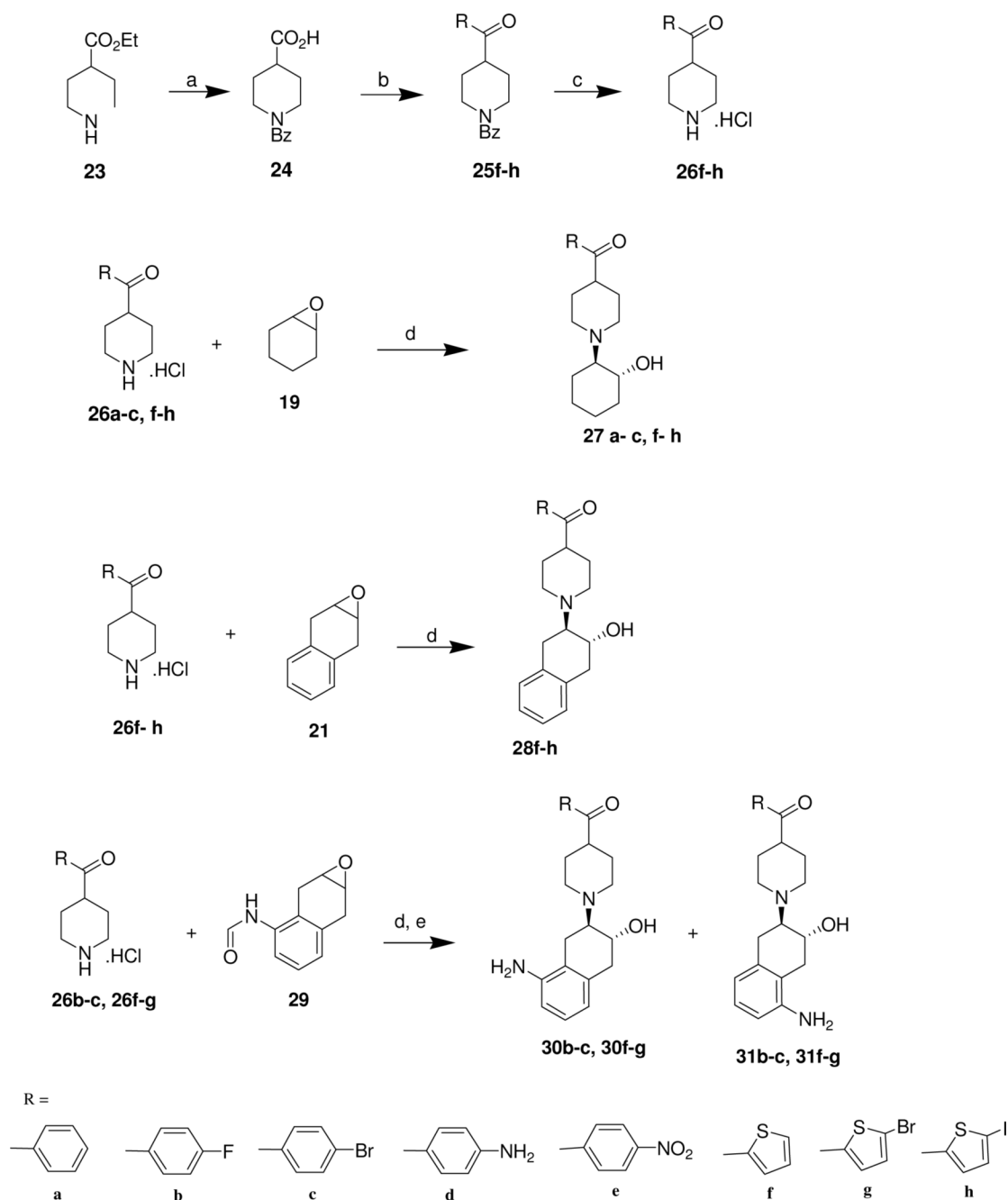
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**Figure 1.**  
Representative compound structures for VACHT.

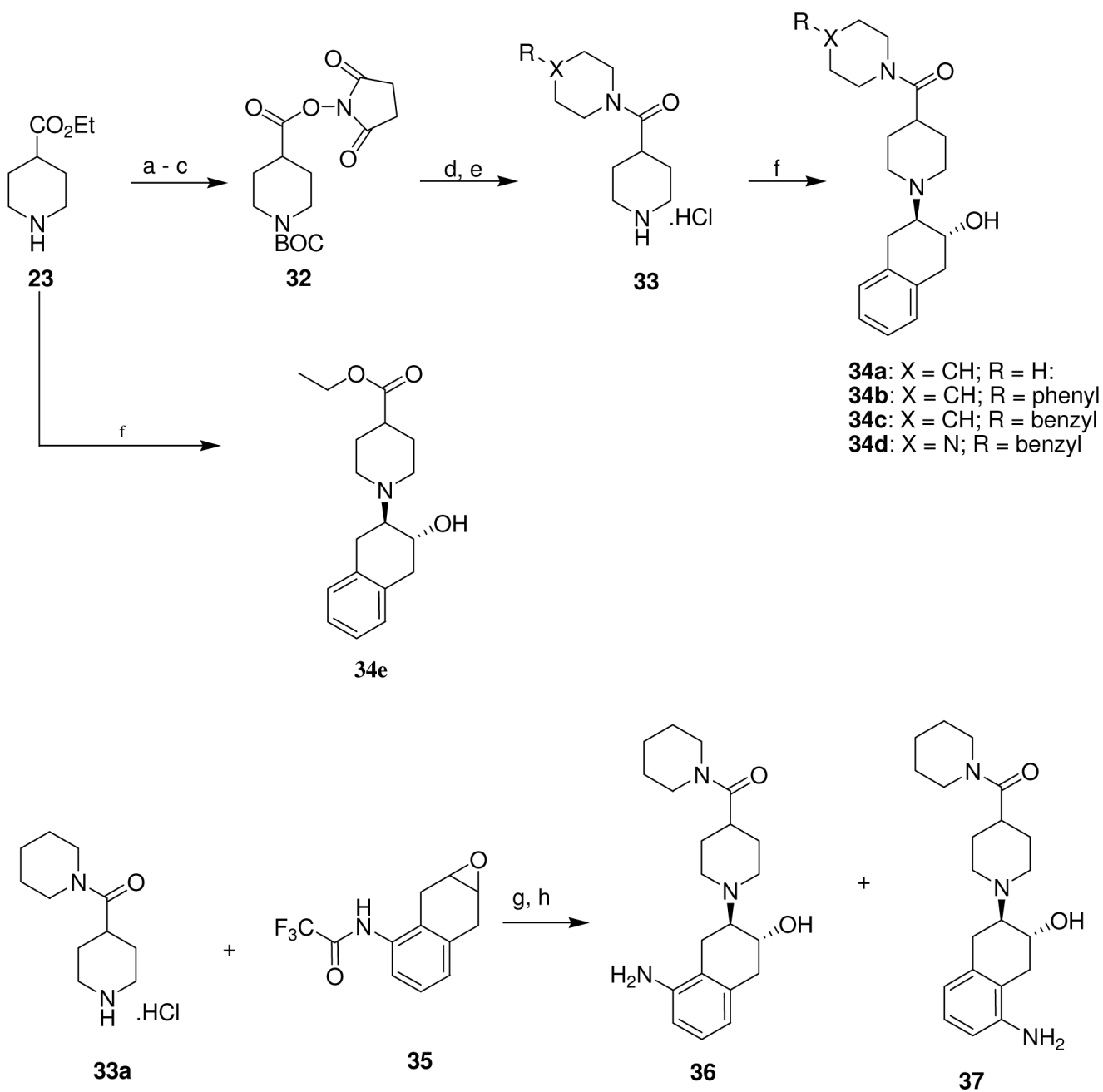
**Scheme 1.**

<sup>a</sup> Reagents and conditions: (a) aryl Grignard reagents/toluene, reflux; (b) 70% of sulfuric acid, 160 – 170°C; (c) Ethanol/Na<sub>2</sub>CO<sub>3</sub>, refluxed.

**Scheme 2.**

<sup>a</sup>Reagents and conditions: (a) (i) BzCl, Et<sub>3</sub>N; (ii) NaOH, aq. EtOH; (b) (i) Oxalyl chloride, CH<sub>2</sub>Cl<sub>2</sub>; (ii) arene, AlCl<sub>3</sub>, CS<sub>2</sub>, reflux; (c) 6N HCl, heat; (d) EtOH, Et<sub>3</sub>N, reflux; e: NaOH, aq. EtOH.



**Scheme 3.**

<sup>a</sup>Reagents and conditions: (a) di-tert-butylidicarbonate,  $\text{CHCl}_3$ , NaCl,  $\text{H}_2\text{O}$ , reflux; (b) NaOH (aq.), EtOH; (c) N-hydroxysuccinimide, DCC,  $\text{CH}_2\text{Cl}_2$ ; (d) 4-substituted piperidine,  $\text{CH}_3\text{CN}$ , reflux; (e) HCl(g), EtOAc,  $0^\circ\text{C}$ ; (f) trans-2-hydroxy-3-bromo-1,2,3,4-tetrahydronaphthalene, EtOH,  $\text{Et}_3\text{N}$ , reflux; (g) EtOH,  $\text{Et}_3\text{N}$ , reflux; h: NaOH, aq. EtOH.

**TABLE 1**  
<sup>a</sup> Affinities ( $K_i \pm$  SD, nM) of Vesamicol and Selected Analogues for *Torpedo* VACht and  $\sigma_1$  and  $\sigma_2$  Receptors.

Compounds	VACht <sup>b</sup> ( $K_i$ [nM])	$\sigma_1^c$ ( $K_i$ [nM])	$\sigma_2^d$ ( $K_i$ [nM])	Selectivity Index	
				$K_1\text{-}\sigma_1/K_1\text{-VACht}$	$K_1\text{-}\sigma_2/K_1\text{-VACht}$
Vesamicol (1) <sup>e</sup>	2.0 $\pm$ 1.0	25.8 $\pm$ 8.0	34.5 $\pm$ 2.0	12.9	17.25
Benzovesamicol <sup>f</sup>	0.055 $\pm$ 0.010	ND	ND	ND	ND
11g	50	ND	ND	ND	ND
20	68.5 $\pm$ 2.54	ND	ND	ND	ND
22a	1.64 $\pm$ 0.26	ND	ND	ND	ND
22b	1.83 $\pm$ 0.39	ND	ND	ND	ND
22c	1.38 $\pm$ 0.36	ND	ND	ND	ND
27a	650 $\pm$ 330	280.8 $\pm$ 18.8	341.4 $\pm$ 28.7	0.4	0.5
27b	423 $\pm$ 2	302.79 $\pm$ 25.23	112.74 $\pm$ 8.14	0.7	0.3
27c	51.4 $\pm$ 6.0	15.91 $\pm$ 2.82	30.13 $\pm$ 3.80	0.3	0.6
27f	763 $\pm$ 94	ND	ND	ND	ND
27g	46.8 $\pm$ 5.0	29.46 $\pm$ 0.94	35.73 $\pm$ 1.13	0.6	0.8
27h	560 $\pm$ 50	9.39 $\pm$ 1.75	48.86 $\pm$ 3.26	0.02	0.1
28a	4.30 $\pm$ 1.00	219.6 $\pm$ 17.3	320.0 $\pm$ 6.6	51.1	74.4
28b	2.70 $\pm$ 0.40	191.1 $\pm$ 57.7	251.3 $\pm$ 39.2	70.8	93.1
28c	0.25 $\pm$ 0.02	297.69 $\pm$ 18.73	592.61 $\pm$ 95.19	1190.8	2370.4
28d	1.68 $\pm$ 0.14	ND	ND	ND	ND
28e	0.48 $\pm$ 0.03	ND	ND	ND	ND
28f	5.00 $\pm$ 1.20	ND	ND	ND	ND
28g	0.54 $\pm$ 0.08	128.92 $\pm$ 1.65	72.02 $\pm$ 9.40	238.7	133.4
28h	0.66 $\pm$ 0.06	194.03 $\pm$ 28.70	344.50 $\pm$ 13.34	294	522
30b	2.40 $\pm$ 0.26	> 1,000	> 1,000	> 416.7	> 416.7
30c	0.53 $\pm$ 0.09	395.11 $\pm$ 33.8	> 1,000	745.5	1886.8
30f	4.90 $\pm$ 1.90	> 1,000	> 1,000	> 204.1	> 204.1
30g	0.41 $\pm$ 0.02	> 1,000	> 1,000	> 2439.0	> 2439.0

Compounds	VACHT <sup>b</sup> (K <sub>i</sub> [nM])	$\sigma_1^c$ (K <sub>i</sub> [nM])	$\sigma_2^d$ (K <sub>i</sub> [nM])	Selectivity Index	
				K <sub>i</sub> $\sigma_1$ /K <sub>i</sub> -VAChT	K <sub>i</sub> $\sigma_2$ /K <sub>i</sub> -VAChT
<b>31b</b>	7.60 ± 0.96	497.1 ± 42.1	352.7 ± 13.2	65.4	46.4
<b>31f</b>	134 ± 17	> 1,000	> 1,000	>7.5	>7.5
<b>31g</b>	29.4 ± 3.3	483.24 ± 33.71	120.66 ± 31.54	16.4	4.1
<b>34a</b>	31.0 ± 4.7	>1,000	> 1,000	>32.3	>32.3
<b>34b</b>	2.07 ± 0.34	50.24 ± 13.07	> 1,000	24.3	>483.1
<b>34c</b>	2.40 ± 0.25	19.88 ± 5.71	> 1,000	8.3	>416.7
<b>34d</b>	2.08 ± 0.14	118.12 ± 47.24	> 1,000	56.8	>480.8
<b>34e</b>	68.5 ± 9.0	> 1,000	> 1,000	>14.6	>14.6
<b>36</b>	89.5 ± 1.1	ND	ND	ND	ND
<b>37</b>	447 ± 55	ND	ND	ND	ND

<sup>a</sup> K<sub>i</sub> values (mean ± SEM) were determined in at least three experiments.

<sup>b</sup> K<sub>i</sub> for VACHT is measured using *Torpedo* synaptic vesicles.

<sup>c</sup>  $\sigma_1$  binding is measured using guinea pig brain membranes.

<sup>d</sup>  $\sigma_2$  binding is measured using rat liver preparations.

<sup>e</sup>Reference<sup>59</sup>.

<sup>f</sup>Reference<sup>54</sup>.

<sup>g</sup>Reference<sup>43</sup>.