Med Chem Res 14:5 (2005) 241–259

DOI: 10.1007/s00044-005-0137-8

MEDICINAL CHEMISTRY RESEARCH

BIOISOSTERIC REPLACEMENT IN THE DESIGN AND SYNTHESIS OF LIGANDS FOR NICOTINIC ACETYLCHOLINE RECEPTORS

Weilin Sun,[†] Michael P. Blanton,[±] Jerome L. Gabriel,[‡] Daniel J. Canney[†,*]

[†]Temple University, School of Pharmacy, Department of Pharmaceutical Sciences,
[‡]School of Medicine, Department of Biochemistry,
Philadelphia, Pennsylvania 19140.
[±]Department of Pharmacology and Neuroscience,
Texas Tech University HSC, Lubbock, TX 79430

Abstract. A series of ethers containing pyrrolidine and/or pyridine bioisosteres was synthesized and evaluated as nicotinic ligands. The dimethylaminoethoxypyridines **6** and **7** inhibited the specific binding of (-)-[^{3}H]Nicotine with *Ki* values of 300 nM and 450 nM, respectively. Compounds **8** and **9** were found to have *Ki* values of 3390 nM and 360 nM. These results suggest that dialkylamino and appropriately substituted benzene rings (NO_2, **8**; OH, **9**) are bioisosteric replacements for pyrrolidine and pyridine, respectively.

The endogenous neurotransmitter acetylcholine (ACh) and the alkaloid nicotine bind nonselectively to nicotinic acetylcholine receptor (nAChR) subtypes and elicit a wide range of pharmacological actions. Evidence suggests that nAChR subtypes may serve as novel drug targets for treatment of CNS disorders including Alzheimer's Disease (AD) and Parkinson's Disease (PD).[2] Ligands capable of binding to nAChRs in the CNS could serve as important pharmacological tools and might lead to the development of therapeutic agents.[3]

Corresponding author: e-mail: canney@temple.edu

Pharmacophoric models are useful in the development of new ligands for nAChRs. Beers and Reich reported that nicotinic ligands should possess a hydrogen-bond acceptor (HBA) group (the lone pair of electrons on the pyridine) and a cationic center (the protonated nitrogen of the pyrrolidine ring).[4] The cationic center is positioned 5.9 Å from the center of the van der Waals surface of the HBA. Later Sheridan et al., used a distance geometry approach to propose a three dimensional model defining the key molecular dimensions of nicotinic ligands.[5] The distance between key functional groups for nicotine ligands in that model was postulated to be 4.8 Å.[5] Recent data suggest that nicotinic receptors are more heterogeneous than earlier appreciated and that interatomic distances for high affinity nicotinic ligands (e.g., epibatidine, 5.5 Å) exceed those proposed in these early models. A re-evaluation of the nicotine pharmacophore model has resulted in proposals by Glennon,[6] Tønder,[7,8] Katjusa Brejc,[9] Schmitt[10] and others.[11] A review of the recent structure-affinity literature suggests that ligands binding to the $\alpha4\beta2$ and $\alpha7$ receptor subtypes: should include a cationic center which is preferably nitrogenous; favor a HBA and/or π-electron rich moiety; should include a relative separation of cationic and HBA/π moieties of ca. 4-8Å; exhibit the tendency toward stereospecific interaction; may prefer some degree of HBA-cation coplanarity. Most recently Sixma et al., proposed that the pyridine of nicotine binds to nAChR by hydrogen bonding through a water bridge and that ligands with longer nitrogen to nitrogen distances (7-8 Å) may displace the water molecule and interact directly with specific residues of the receptor.[12] The pharmacophoric requirements for nicotinic receptor ligands continue to evolve as more SAR data become available.

The ether linkage has been shown to be an effective means of connecting the cationic center to the HBA moiety of nicotinic ligands.[13,14] Our ongoing interest in the design of cholinergic ligands led to the identification of a series of quinuclidine-containing ethers (CLZ-12, CLZ-13; Figure 1) and esters as nAChR ligands.[14b,15] These studies suggest that nitrobenzene and phenol groups may serve as useful bioisosteres for the pyridine ring of nicotinic ligands.[14b] As a continuation of that work, a series of ether-linked compounds containing potential pyridine and pyrrolidine bioisosteres has been synthesized. Interatomic distances and the results of receptor binding assays for the compounds are reported.

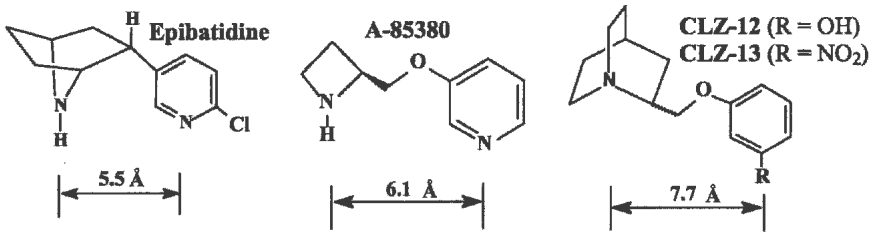
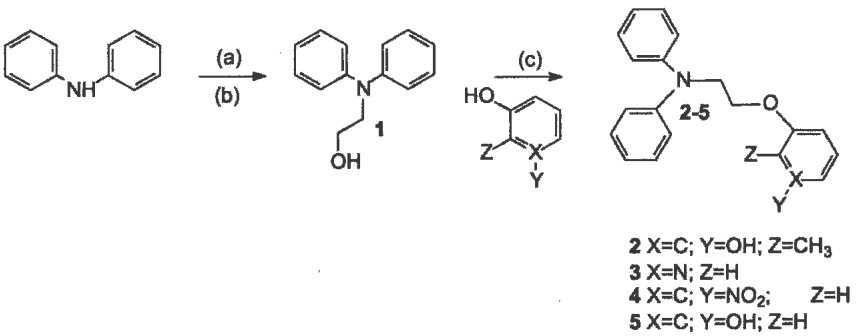


Figure 1. Interatomic distance of known compounds as determined by molecular modeling.[14b]

Chemistry

The synthetic routes to the target compounds are illustrated in Schemes 1–3. Scheme 1 shows the synthesis of **2-5**. These compounds contain the diphenylamine moiety as a potential bioisostere for the cationic portion of the ligands. Reaction of the amine with protected bromoethanol in the presence of NaH provides the corresponding protected alcohol.[16]

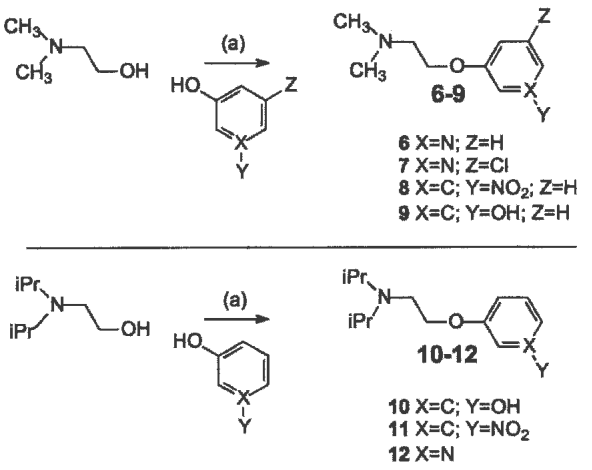


Scheme 1. Synthesis of the Diphenyl Series (**2-5**) of Compounds.

Conditions: (a) 2-Bromoethoxytrimethylsilane, NaH, Butyl ether. (b) 1% HCl, EtOH. (c) DEAD, Ph_3P, THF.

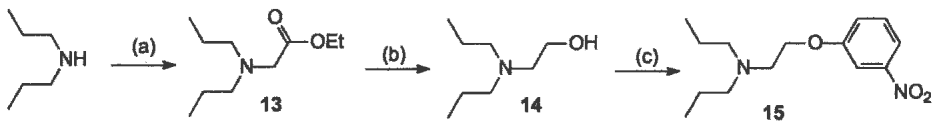
Deprotection in dilute HCl afforded the alcohol which was reacted with the appropriate pyridinol, phenol, or nitrobenzene to provide target compounds **2-5**.[17] Compound **3** possesses the same pyridine ring found in nicotine while 2, 4 and 5 contain pyridine bioisosteres in the form of phenol (**2** and **5**) or nitrobenzene (**4**) groups.

Scheme 2 outlines the synthesis of compounds **6-12** which contain dialkylamino groups as bioisosteres for the pyrrolidine ring and pyridine bioisosteres in the form of phenols or nitrobenzene. The synthesis of compounds **6-9** involved coupling of the commercially available dimethylaminoethanol with substituted phenols and pyridinols using Mitsunobu reaction conditions. The dimethylamino group has been shown to be useful in nicotinic ligands by other investigators.[18-20] Similarly, compounds **10-12** were prepared by reacting diisopropylaminoethanol with substituted phenols and pyridinol under the same Mitsunobu conditions.



Scheme 2. Synthesis of the Dimethyl (**6-9**) and the Diisopropyl (**10-12**) Series of Compounds. Conditions: (a) DEAD, Ph_3P, THF

The synthesis of ether **15** is shown in Scheme 3. The amine (di-*n*-propylamine) was treated with ethyl bromoacetate in the presence of NaH to provide amino ester **13**. The ester was reduced to the alcohol using lithium aluminum hydride and coupled to 3-nitrophenol using Mitsunobu reaction conditions to provide **15** in modest overall yield.



Scheme 3. Synthesis of Compound **15**.

Conditions: (a) Ethyl 2-Bromoacetate, NaH, Butyl Ether (b) LAH, THF (c) DEAD, Ph_3P, THF; 3-Nitrophenol.

Molecular Modeling

All molecular modeling was performed on a Silicon Graphics Personal IRIS 4D/25 workstation using the DREIDING II force field and Biograf^R (BIOSYM/Molecular Simulations, San Diego, CA). Individual models of compounds were constructed using the organic builder contained within the main Biograf^R program. In order to ascertain whether the internuclear atomic distances for test compounds were within the appropriate range for the nitrogen to nitrogen distances reported for nicotine and epibatidine, a model of each compound was constructed and analyzed as previously described.[21] In order to test the method, the interatomic distances of known high affinity nicotinic ligands were determined using the current technique and compared to the previously reported values.

Results

A series of ether-based nicotinic ligands was prepared in good to modest yields using well precedented synthetic routes.[14b] Interatomic distances were calculated for the test compounds. In order to evaluate the method, the interatomic distances for known nicotinic ligands were determined using the present technique and compared with values reported in the literature. As

shown in Table 1, the distances calculated herein were consistent with reported values. Distances ranging from 6.44Å for **11** to 8.29Å for **8** were obtained.

Table 1. Comparison of Internuclear Distances Calculated for Known Ligands and Novel Compounds Using Literature Methods to Those Obtained with the Current Method.

Compd	**Interatomic Distances Reported in Å.**			**Ki Values (nM)**
	Literature Values[a]	Values Based on Current Methods[b]		
	N to N (Å)	**N to O (Å)**	**N to N (Å)**	
Nicotine	4.87[a]		4.74	1.05[a]
Epibatidine	5.51[a]		5.83	0.07[a]
A-85380	6.1-6.3[a]		6.38	0.04[a]
CLZ-12	NA	3.71[b]	7.71[b]	4[b]
CLZ-13	NA	3.77[b]	7.76[b]	734[b]
2	NA	3.71[c]	7.86 (N to OH)[c]	(0%)[d]
3	NA	3.71	6.66	(0%)[d]
4	NA	3.74	6.65 (N to NO_2)	(0%)[d]
5	NA	3.70	7.71 (N to OH)	(0%)[d]
6	NA	3.75	**6.90**	300[e] (95%)[d]
7	NA	3.78	**6.95**	450[e] (84%)[d]
8	NA	3.77	8.29 (N to NO_2)	3390[e] (33%)[d]
9	NA	3.18	**7.22** (N to OH)	360[e]
10	NA	3.75	7.79 (N to OH)	(0%)[d]
11	NA	3.25	6.44 (N to NO_2)	(0%)[d]
12	NA	3.81	6.89	11200[e] (64%)
15	NA	3.75	6.58 (N to NO_2)	NB[e](21%)[d]

a) Distances calculated or Ki values as reported in Ref 5, 6 & 13 b) Distances and Ki values as reported in Ref 14b c) Distances calculated for compounds **2-15** as described in Ref 21 d) Test compounds **2-8**, **10-12** and **15** were evaluated in preliminary binding screens using [^{3}H]Cytisine in rat brain homogenates. Initial screening was performed in triplicate at 10 μM. Values in parenthesis represent % inhibition of specific binding of [^{3}H]Cytisine by the test compounds at 10 μM. Ki values were determined for compounds that were considered active in preliminary screens. Assay procedures are described in the experimental section. e) Ki values of selected compounds to human α4β2 AChRs; NB, no binding.

The test compounds were evaluated first in preliminary nicotinic binding screens using [^{3}H]Cytisine in rat brain homogenates. Initial screening of each compound was performed in triplicate at a concentration of 10 μM. In the preliminary assays, **2-5** (diphenylamine series) and

10 and **11** (diisopropylamine series) did not inhibit specific binding of the radioligand. Compounds **6** and **7** inhibited specific binding by 95% and 84% respectively, while, **8**, **12** and **15** inhibited binding by 33%, 64%, and 21%, respectively. Compounds exhibiting positive results in this screen (% inhibition > 20%) were evaluated further. Compounds **6**, **7**, **8**, **9**, **12**, and **15** were tested for dose-dependent inhibition of [^{3}H]Nicotine binding to human α4β2 nAChRs as described in the experiment section. Compound **9** was not tested in the preliminary assays but was included here based on previous SAR studies suggesting that phenols can serve as a bioisostere for pyridine in nicotinic ligands.[14b]

All compounds tested except **15** inhibited the specific binding of [^{3}H]Nicotine with *Ki* values ranging from 300 nM to 11,200 nM. The rank order of affinity for these compounds is **6** (300 nM) > **9** (360 nM) >**7** (450 nM) > **8** (3390 nM) > **12** (11 200 nM) >> **15** (no inhibition). Interatomic distances for the highest affinity compounds, **6** (300 nM), **7** (450 nM), and **9** (360 nM), were 6.90Å, 6.95Å, and 7.22Å respectively. The low affinity compounds **8** (3390 nM) and **12** (11200 nM) had interatomic distances of 8.29Å and 6.89Å, respectively.

Discussion

Previous reports by the authors and by other investigators suggest that bioisosteric replacement is a useful approach when designing nicotinic ligands.[13,14] For example, the quinuclidine nucleus serves as a template on which to design moderate affinity nicotinic ligands with interatomic distances that are consistent with literature values (e.g., CLZ-12, CLZ-13, Table 1). In the present work, the bioisosteric replacement approach has been used to design a series of ether-based nAChR ligands that contain pyrrolidine and/or pyridine bioisosteres. Replacing the pyrrolidine ring of nicotine with diphenyl or dialkyl substituents had a dramatic effect on receptor binding. In the diphenylaminoethoxy series, the compounds possess the requisite pharmacophoric elements (HBA and a cationic moiety) and interatomic distances within the range (6.7Å to 7.9 Å) reported for other moderate affinity nicotinic ligands (e.g., **6**). However, the diphenyl series of compounds did not inhibit the specific binding of radioligand in the preliminary binding assay. These data suggest that the diphenyl group is not an appropriate bioisostere for the pyrrolidine ring in nicotinic ligands. The lack of affinity observed for these

compounds may be due, in part, to the basicity of the nitrogen, to steric hindrance and/or the electrostatic potential of the aromatic rings. Pharmacophoric models of neuronal nACh receptors suggest that π-cation interactions between aromatic residues in the receptor binding site and the protonated nitrogen atom play an important role in ligand-receptor binding. The presence of two bulky aromatic rings around the sp3 nitrogen and the low pKa value (~3-4) of the nitrogen of the diphenylamine moiety may abolish this critical π-cation interaction. The sensitivity of nicotinic receptors to these factors is consistent with previous reports.[22]

The dimethylaminoethoxy-pyridines **6** and **7** inhibited the specific binding of [^{3}H]Nicotine to human α4β2 nicotinic acetylcholine receptors with *Ki* values of 300 nM and 450 nM, respectively. These data are generally consistent with dimethylaminoethoxy-pyridines reported simultaneously by Lin et al.[14,18] The *Ki* value of **6** is different from the value reported in the literature.[18-20] This discrepancy may be explained, in part, by species differences.[23] The present work was performed in human cell lines while the other investigators used rat brain homogenates for binding studies. Compounds **8** and **9** in the same series contain pyridine bioisosteres and were found to have *Ki* values of 3390 nM and 360 nM, respectively. These results suggest that a phenol (**9**) group may serve as a bioisosteric replacement in nicotinic ligands. The nitrobenzene group (**8** and **11**) may be useful but appears to reduce binding affinity. These data are consistent with other reports suggesting these moieties may serve as pyridine bioisosteres.[14b]

Preliminary screening of the diisopropylaminoethoxy compounds **10-12** showed that **12** inhibited the specific binding of [^{3}H]Cytisine by 64% at 10 μM. The *Ki* value of **12** is only 11.2 μM when tested for dose-dependent inhibition of [^{3}H]Nicotine binding to human α4β2 nAChRs. Steric bulk resulting from the isopropyl groups around the amine nitrogen may hinder efficient binding in this series.

The compounds designed, synthesized and tested in the present study possess the requisite pharmacophoric elements (HBA, a cationic moiety, and an appropriate interatomic distance) for nicotinic ligands. The interatomic distances calculated for the test compounds and those obtained for known nicotinic ligands are shown in Table 1. The distances calculated for the

present compounds are close to the a-b distance proposed by Tønder et al (7.3-8 Å).[7] With the exception of **8** (8.29Å), all compounds that bound to the receptor had distances approximating 7Å. However, it is important to note that the flexibility of the test compounds, coupled with the small number of compounds investigated, limits the usefulness of interatomic distances in predicting binding affinity. The flexibility of the ligands permits the molecules to adopt conformations in aqueous solution that may differ significantly from the low energy conformer used for the distance measurements. In addition, the actual conformation at the time of binding may be influenced by, for example, components of the receptor protein and/or the phospholipid membrane. However, the data provide a starting point for the design of novel nicotinic ligands. In order to better define optimal interatomic distances for nicotinic ligands, the rotatable bonds in these ligands could be constrained. The development of conformationally constrained ligands with fewer rotatable bonds between the putative pharmacophoric elements might be useful in the development of high affinity nicotinic ligands.

Conclusion

Bioisosteric replacement is a useful technique in lead modification that may produce favorable changes in pharmacokinetic and/or pharmacodynamic parameters. A series of ethers containing pyrrolidine and/or pyridine bioisosteres was synthesized and evaluated as nicotinic ligands. The dimethylaminoethoxypyridines **6** and **7** inhibited the specific binding of [^{3}H]Nicotine to human $\alpha4\beta2$ nicotinic acetylcholine receptors with *K*i values of 300 nM and 450 nM, respectively. Compounds **8** and **9** were found to have *K*i values of 3390 nM and 360 nM respectively. The data presented herein suggest that the dimethylamino group is a useful bioisostere for the pyrrolidine ring and that phenols are useful bioisosteres for the pyridine ring of nicotinic ligands. These finding are consistent with other reports on bioisosteric replacement in nicotinic ligands.[14b] The bioisosteres reported herein warrant further investigation and may be useful in the development of novel nicotinic ligands.

Experimental Section

Chemistry. General Procedure for preparation of 2-12 and 15 using the Mitsunobu reaction. A solution of the appropriate alcohol in dry THF was added to a phenol and triphenylphosphine (Ph_3P). The mixture was cooled in an ice bath and diethyl azodicarboxylate (DEAD) was added dropwise, stirred at 0 °C for 3 h, and allowed to warm to room temperature overnight. The following day the reaction mixture was condensed under reduced pressure and the residue was purified by flash chromatography to afford the target compounds.

2-Diphenylamino ethanol (1). A solution of diphenyl amine (7.1 g, 42 mmol) in anhydrous di-*n*-butyl ether (80 mL) was added sodium hydride (60% in oil dispersion, 2.0 g, 50 mmol). The resulting mixture was stirred at 135 °C for 3 h, cooled in an ice bath, and a solution of (2-bromoethoxy)*tert*-butyldimethysilane (10 g, 42 mmol) in anhydrous dibutyl ether was added dropwise with stirring. When addition was complete, the reaction was heated to 120 °C, stirred for 3 h, quenched with water (150 mL), and the mixture extracted with ethyl acetate (150 mL × 3). The combined organic phases were dried over Na_2SO_4 and condensed in *vacuo*. The residue was dissolved in 2-propanol (150 mL) and 1% HCl in ethanol was added to adjust the pH to 1. The mixture was stirred overnight, neutralized with 1% NaOH and extracted with ethyl acetate (200 mL × 3). The combined organic phases were dried over Na_2SO_4 and condensed in *vacuo*. The residue was purified by flash chromatography (silica gel; 4:1, Hexane/THF) to afford compound **1** (6.68 g, 75% yield for two steps) as a brown oil: ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.14 (t, J = 7.4 Hz, 4H), 6.93 (d, J = 7.6 Hz, 4H), 6.84 (t, J = 7.3Hz, 2H), 3.76 (t, J = 5.6 Hz, 2H), 3.66 (t, J = 5.6 Hz, 2H), 2.00 (bs, 1H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 148.5, 130.1, 122.2, 121.7, 59.6, 54.6.

2-Methyl-3-(2'-diphenylaminoethoxy)-phenol (2). Following the general procedure for Mitsunobu reactions described above, a solution of **1** (0.85 g, 4 mmol), 2-methylresorcinol (0.74 g, 6.0 mmol) and triphenylphosphine (1.26 g, 4.8 mmol) in dried THF (25 mL) was cooled in an ice bath and DEAD (0.75 mL, 4.8 mmol) added dropwise with stirring. The reaction mixture was condensed under reduced pressure and purified by flash column chromatography (silica gel; 4:1, hexane/EtOAc) to yield **2** (0.6 g, 47%) as a yellow solid: mp 108-109 °C. ^{1}HNMR (400

MHz, $CDCl_3$): δ 7.16-7.21 (t, J = 7.4Hz, 4H), 6.98-7.01 (d, J =7.6 Hz, 4H), 6.86-6.90 (t, J =7.4 Hz, 3H), 6.31-6.35 (dd, J = 8.3, 8.4Hz, 2H), 4.62 (s, 1H), 4.06-4.13 (m, 4H), 1.97 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 158.2, 154.9, 148.2, 129.8, 126.8, 121.9, 121.4, 112.8, 108.6, 104.4, 65.9, 51.7, 8.5. Anal. ($C_{21}H_{21}NO_2$) C, H, N.

3-(2'-Diphenylaminoethoxy)pyridine (3). Following the general procedure for Mitsunobu reactions described above, a solution of 1 (0.76 g, 3.5 mmol), 3-hydroxypyridine (0.51 g, 5.4 mmol) and triphenyphosphine (1.12 g, 4.3 mmol) in dried THF (25 mL) was cooled in an ice bath and DEAD (0.67 mL, 4.3 mmol) was added dropwise with stirring. Purification by flash column chromatography (silica gel; 2:1, hexane/EtOAc) yielded 3 (0.81 g, 78%) as a yellow solid: mp 54-56 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.12-8.19 (m, 2H), 7.18-7.23 (t, J = 7.3 Hz, 4H), 7.09 (m, 1H), 7.06 (m, 1H), 6.97-7.00 (d, J = 7.6 Hz, 4H), 6.89-6.92 (t, J = 7.3 Hz, 2H), 4.16 (m, 2H), 4.09 (m, 2H). ^{13}CNMR (100 MHz,$CDCl_3$): δ 153.1, 145.8, 136.3, 127.7, 122.0, 119.9, 119.4, 119.2, 63.6, 49.3. Anal. ($C_{19}H_{18}N_2O$) C, H, N.

1-Nitro-3-(2'-diphenylaminoethoxy)-benzene (4). Following the general procedure for Mitsunobu reactions described above, a solution of 1 (0.79 g, 3.7 mmol), 3-nitrophenol (0.77 g, 5.6 mmol) and triphenylphosphine (1.17 g, 4.5 mmol) in dried THF (25 mL) was cooled in an ice bath and DEAD (0.70 ml, 4.5 mmol) was added dropwise with stirring. Purification by flash column chromatography (silica gel; 6:1, hexane/EtOAc) to afford 4 (1.1 g, 88%) as yellow solid: mp 52-53 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.72-7.75 (dd, J = 2.1, 8.1 Hz, 1H), 7.59 (t, J = 2.1 Hz, 1h), 7.30-7.34 (t, J = 8.2 Hz, 1H), 7.19-7.24 (m, 4H), 7.08-7.11 (dd, J = 2.5, 8.3 Hz, 1H), 6.97-7.00 (d, J = 8.7 Hz, 4H), 6.89-6.94 (t, J = 7.3 Hz, 2H), 4.20 (dd, J = 1.7, 5.3Hz, 2H), 4.1 (dd, J = 1.6, 5.3 Hz, 2H). ^{13}CNMR(100 MHz, $CDCl_3$): δ: 159.6, 149.6, 147.9, 130.4, 129.9, 122.2, 122.0, 121.4, 116.4, 109.2, 66.1, 51.3. Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

3-(2'-Diphenylaminoethoxy)-phenol (5). Following the general procedure for Mitsunobu reactions described above, a solution of 1 (0.33 g, 1.6 mmol), resorcinol (0.26 g, 2.3 mmol) and triphenylphosphine (1.2 g, 1.9 mmol) in dried THF (25 mL) was cooled in an ice bath and DEAD (0.29 mL, 1.9 mmol) was added dropwise with stirring. Purification by flash column

chromatography (silica gel; 6:1, hexane/EtOAc) afforded **5** (0.24 g, 52%) as an off-white solid: mp 50-52 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.16–7.21 (t, J = 7.8 Hz, 4H), 6.96-7.02(m, 5H), 6.86-6.90 (t, J = 7.3 Hz, 2H), 6.29-6.37 (m, 2H), 6.24 (m, 1H), 4.72 (s, 1H), 4.05 (s, 4H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 160.4, 157.0, 148.1, 130.6, 130.1, 129.8, 122.0, 121.5, 108.4, 107.4, 102.6, 102.3, 65.3, 51.5. Anal. ($C_{20}H_{19}NO_2$) C, H, N.

3-(2'-Dimethylaminoethoxy) pyridine HCl Salt (6). Following the general procedure for Mitsunobu reactions described above, a solution of 2-dimethylaminoethanol (1 mL, 9.9 mmol), 3-hydroxypyridine (1.42 g, 14.9 mmol) and triphenylphosphine (3.13 g, 11.9 mmol) in dry THF (50 mL) was cooled in an ice bath and DEAD (1.9 mL, 11.9 mmol) was added dropwise with stirring. Purification by flash column chromatography (silica gel; 1:1, CH_2Cl_2/MeOH) afforded **6** as a yellow oil. The hydrochloride salt of the title compound was prepared by bubbling gaseous HCl through a chilled ether solution (30 mL) of **6**. Filtration of the resulting white precipitate, followed by recrystallization from CH_2Cl_2-MeOH provided **6** (0.16 g, 67%) as a yellow solid: ^{1}HNMR (400 MHz, MeOD-d4): δ 8.82 (s, 1H), 8.62 (d, J = 5.5 Hz, 1H), 8.42 (d, J = 6.5 Hz, 1H), 8.14 (m, 1H), 4.74 (t, J = 4.7 Hz, 2H), 3.78 (t, J = 4.7 Hz, 2H), 3.08 (s, 6H). ^{13}CNMR (100 MHz, MeOD-d4): δ 158.4, 136.8, 134.4, 132.6, 129.9, 66.0, 57.5, 49.0. Anal. ($C_9H_{16}Cl_2N_2O$) C, H, N.

3-(2'-Dimethylaminoethoxy)-5-chloro-pyridine HCl Salt (7). Compound **7** was obtained (70% yield) by reacting 2-dimethylaminoethanol (1 mL, 10 mmol) with 5-chloro-3-hydroxypyridine (1.93 g, 15 mmol), following the general reaction conditions (Mitsunobu reaction and HCl salt formation) described above for **6**. ^{1}HNMR (400 MHz, MeOD-d4): δ 8.35 (d, J = 2.2 Hz, 1H), 8.27 (s, 1H), 7.69 (t, J = 2.2 Hz, 1H), 4.53 (t, J = 4.9 Hz, 2H), 3.69 (t, J = 4.9 Hz, 2H), 3.04 (s, 6H). ^{13}CNMR (100 MHz, MeOD-d4): δ 156.5, 142.7, 137.8, 134.1, 64.5, 57.6, 44.4. Anal. ($C_9H_{15}Cl_3N_2O$) C, H, N.

1-Nitro-3-(2'-dimethylaminoethoxy)-benzene HCl salt (8). Compound **8** was obtained (49% yield) by reacting 2-dimethylaminoethanol (1 mL, 10 mmol) with 3-nitrophenol (2.08 g, 15 mmol), following the general reaction conditions (Mitsunobu reaction and HCl salt formation)

described for **6**. ^{1}HNMR (400 MHz, MeOD-d4): δ 7.79 (s, 1H), 7.77 (d, J = 1.5 Hz, 1H), 7.47 (t, J = 8.6 Hz, 1H), 7.35-7.38 (dt, J = 1.6, 8.1 Hz, 1H), 4.40 (t, J = 4.8 Hz, 2H), 3.58 (t, J = 4.8 Hz, 2H), 2.92 (s, 6H). ^{13}CNMR (100 MHz, MeOD-d4): δ 160.1, 151.0, 132.1, 122.9, 118.1, 111.0, 64.3, 57.9, 44.4. Anal. ($C_{10}H_{15}ClN_2O_3 \cdot 0.5\ H_2O$) C, H, N; N: calcd, 10.96; found, 11.47.

3-(2'-Dimethylaminoethoxy)-phenol (9). Following the general reaction conditions for the Mitsunobu reaction described above, 2-dimethylaminoethanol (1 mL, 10 mmol) was reacted with resorcinol (1.64 g, 15 mmol) to provide **9** in 65% yield. ^{1}HNMR (400 MHz, MeOD-d4): δ 7.18-7.23 (t, J = 8.4 Hz, 1H), 6.52-6.58 (m, 3H), 4.19-4.24 (t, J =5.5 Hz, 2H), 2.93-2.96 (t, J = 5.1 Hz, 2H), 2.52 (s, 6H). ^{13}CNMR (100 MHz, MeOD-d4): δ 161.7, 160.2, 131.4, 109.6, 106.9, 103.4, 66.6, 59.4, 46.1. GC-MS (EI): 181 (M+), 165, 135, 109, 93, 71, 65, 58, 51. Anal. ($C_{10}H_{15}NO_2$) C, H, N.

3-(2'-Di-iso-propylaminoethoxy)-phenol HCl Salt (10). Compound **10** was obtained (56% yield) by reacting 2-di-isopropylaminoethanol (3 mL, 17.1 mmol) with resorcinol (3.76 g, 34.2 mmol), using the general reaction conditions (Mitsunobu reaction and HCl salt formation) described for **6**. ^{1}HNMR (400 MHz, MeOD-d4): δ 6.98-7.02 (t, J = 8.1 Hz, 1H), 6.31-6.37 (m, 3H), 4.18 (t, J = 4.8 Hz, 2H), 3.63-3.74 (m, 2H), 3.48 (t, J = 4.9 Hz, 2H), 1.32 (d, J = 4.0Hz, 12H). ^{13}CNMR (100 MHz, MeOD-d4): δ 160.8, 160.4, 131.7, 110.4, 106.9, 103.5, 65.6, 58.1, 19.5, 17.8. Anal. ($C_{14}H_{24}ClNO_2$) C, H, N.

1-Nitro-3-(2'-di-iso-propylaminoethoxy)-benzene HCl Salt (11). Three-nitrophenol (1.78 g, 12.8 mmol) was reacted with 2-di-isopropylaminoethanol (1.5 mL, 7.5 mmol) to provide **11** in 43% yield using the general reaction conditions (Mitsunobu reaction and HCl salt formation) described for **6**. ^{1}HNMR (400 MHz, MeOD-d4): δ 7.78 (d, J = 7.8 Hz, 1H), 7.72 (s, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 4.37 (s, 2H), 3.76 (m, 2H), 3.60 (s, 2H), 1.38 (dd, J = 5.8, 5.9 Hz, 12H). ^{13}CNMR (100 MHz, MeOD-d4): δ 160.1, 151.0, 132.2, 122.9, 117.9, 110.7, 66.7, 58.0, 19.7, 17.8. Anal. ($C_{14}H_{23}ClN_2O_3$) C, H, N: Calcd, C, 55.53; Found: 54.97.

3-(2'-Di-iso-propylaminoethoxy)-pyridine HCl Salt (12). Compound **12** was obtained (61% yield) by reacting 2-di-isopropylaminoethanol (0.5 mL, 2.8 mmol) with 3-hydroxypyridine (0.41 g, 4.3 mmol), using the general reaction conditions (Mitsunobu reaction and HCl salt formation) described for **6**. ^{1}HNMR (400 MHz, MeOD-d4): δ 8.77 (s, 1H), 8.59 (s, 1H), 8.34 (d, J = 7.7 Hz, 1H), 8.11 (d, J = 7.4 Hz, 1H), 4.67 (t, J = 4.5 Hz, 2H), 3.86-3.92 (m, 2H), 3.78 (t, J = 4.5 Hz, 2H), 1.46-1.55 (dd, J = 6.5, 6.5 Hz, 12H). ^{13}CNMR (100 MHz, MeOD-d4): δ 158.6, 136.6, 134.1, 131.7, 129.9, 68.0, 58.3, 19.5, 17.7. Anal. ($C_{13}H_{24}Cl_2N_2O$•1.5H_2O) C, H, N.

Ethyl 2-(di-*n*-propylamino)-acetate (13). Ethyl bromoacetate (10.1 mL, 91.2 mmol) was added dropwise to a cooled solution of di-n-propylamine (10 mL, 72.9 mmol) and anhydrous K_2CO_3 (12.6 g, 91.2 mmol) in anhydrous *n*-butyl ether (50 mL) with stirring. The reaction mixture was heated to 120 °C for 5 hrs, cooled to room temperature, quenched with water, and extracted with benzene (100 mL x 3). The organic layer was dried over $MgSO_4$ and condensed under reduced pressure. Flash chromatography (silica gel; 4:1, hexane/EtOAc) of the residue afforded **13** (6.1 g, 45%) as a pale-yellow oil: ^{1}HNMR (400 MHz, $CDCl_3$): δ 4.14-4.19 (q, J = 7.1 Hz, 2H), 3.32 (s, 2H), 2.52-2.56 (t, J = 7.6 Hz, 4H), 1.43-1.52 (m, 4H), 1.25-1.29 (t, J = 7.1 Hz, 3H), 0.86-0.90 (t, J = 7.3 Hz, 6H). GC-MS (EI): 187 (MH^+), 158, 114, 86, 72, 42, 30.

2-di-n-Propylaminoethanol (14). Lithium aluminum hydride (LAH, 95% powder, 3.03 g, 80 mmol) was added slowly under nitrogen to a cooled (ice bath) solution of **13** (5 g, 26.7 mmol) in dried THF (30 mL). The resulting mixture was allowed to warm to room temperature, stirred for 2 h, quenched with H_2O (15 mL) and 1% NaOH (15 mL), and filtered. The filtrate was extracted with EtOAc (50 mL x 4), the organic layers combined, washed with brine, dried over $MgSO_4$ and condensed under reduced pressure. Flash chromatography (silical gel; 10:1, CH_2Cl_2/MeOH) of the recovered oil afforded **14** (1.97 g, 51%) as a pale-yellow oil: ^{1}HNMR (400 MHz, $CDCl_3$): δ 3.51 (t, J = 6.2 Hz, 2H), 2.56 (t, J = 6.1 Hz, 2H), 2.39 (t, J = 7.5 Hz, 4H), 1.45 (m, 4H), 0.89 (t, J = 7.3 Hz, 6H). GC-MS (EI): 145 (MH^+), 114, 72, 43, 30.

1-Nitro-3-(2'-di-*n*-propylaminoethoxy)-benzene (15). Compound **14** (0.32 g, 2.2 mmol) was reacted with resorcinol (0.36 g, 3.3 mmol) following the general procedure for Mitsunobu

reactions described above to provide **15** in 43% yield. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.70-7.73 (m, 1H), 7.64 (m, 1H), 7.33 (t, J = 8.2 Hz, 1H), 7.12-7.15 (m, 1H), 4.02 (t, J = 6.1 Hz, 2H), 2.83 (t, J = 6.1Hz, 2H), 2.43 (t, J = 7.5Hz, 4H), 1.37-1.47 (m, 4H), 0.82 (t, J = 7.3Hz, 6H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 159.6, 149.5, 130.3, 121.8, 116.1, 111.1, 64.9, 58.1, 53.6, 20.7, 12.2. Anal. ($C_{14}H_{22}N_2O_3$) C, H, N.

Biological Evaluation of Nicotinic Ligands

Preliminary Binding Assay. Neuronal α-BGTX-insensitive receptor originated from rat cerebral cortex was incubated with [^{3}H]Cytisine (1.5 nM) and non-specific compound (-)-nicotine bitartrate (10 μM) at 4 °C for 75 min. After incubation, the membranes were rapidly filtered under vacuum through glass fiber filters and washed several times with an ice-cold buffer using a cell harvester. Bound radioactivity was measured with a scintillation counter (Betaplate, Wallac) using a solid scintillant. (MeltiLex B/HS, Wallac). The specific radioligand binding to the receptors was defined as the difference between total binding and non-specific binding determined in the presence of an excess of unlabelled ligand. Results were expressed as a percent of control specific binding and as a percent inhibition of control specific binding obtained in the presence of the test compounds. IC_{50} value and Hill coefficient were determined for the reference compound by non-linear regression analysis of its competition curve. There parameters were obtained by Hill equation curve fitting. The IC_{50} value obtained for the reference compound is within accepted limits of the historic average obtained ± 0.5 log unit.

Binding Assay (ki values) of Selected Compounds

Materials. (L-(-)-[N-methyl-^{3}H]Nicotine ([^{3}H]Nicotine, 81 Ci/mmol) was obtained from Amersham Biosciences (Piscataway, NJ).

Cell culture and membrane preparation. Human epithelial (SH-EP1) cells stably transfected with human α4β2 AChRs were maintained on 140 mm cell culture plates at 37°C and 5% CO_2 in a humidified atmosphere. They were cultured in DMEM with high glucose

(Dulbecco's Modified Eagle Medium/ supplemented with 5% fetal calf serum, 10% heat-inactivated horse serum, 0.25 mg/mL Zeocin, 0.13 mg/mL Hygromycin B). Cells were grown to confluence, with feedings every 2-3 days and the cells harvested. For harvesting, all but 5 ml of media were removed and the cells scraped into 5 ml of ice-cold VDB buffer (10 mM MOPS, 100 mM NaCl, 0.1 mM EDTA, and 0.02% NaN_3, pH 7.5) and pelleted by centrifugation (600 rpm for 10 minutes). Cells were resuspended in 10 ml VDB buffer, pelleted by centrifugation, and resuspended in 10 ml VDB supplemented with 40 µL of protease cocktail inhibitor set III (Calbiochem). Cells were homogenized using a glass hand-held homogenizer and the membranes pelleted by centrifugation (18,000 rpm for 1 h at 4°C). The membrane pellet was resuspended in 5 ml VDB and stored at -80°C.

Inhibition of [^{3}H]Nicotine binding to human α4β2 AChRs. To determine the effect of nicotinic ligands on the specific binding of [^{3}H]Nicotine to human α4β2 AChRs, 0.6 nM α4β2 AChR membranes were suspended in 8 ml of VDB buffer with 1.6 nM [^{3}H]Nicotine. The total volume was then divided into aliquots, and increasing concentrations of a given nicotinic ligand were added from ethanolic stock solutions (ethanol concentration <1%) to each tube and the membrane suspension allowed to incubate for 2.5 h at RT. Following centrifugation (18,000 rpm for 1 h) of membrane samples, the [^{3}H]-containing pellets were resuspended in 100 µl 10% SDS and transferred to a scintillation vial with 5 ml Bio-Safe II. The bound fraction was determined by scintillation counting, with nonspecific binding determined in the presence of 100 µM nicotine. Nonspecific binding was between 5 and 10% of the total binding.

The concentration-response data were curve-fit by nonlinear least squares analysis (one-site competition) using the program Prism (Graphpad) and the corresponding IC_{50} values calculated. The observed IC_{50} values were transformed into K_i values using the Cheng-Prusoff relationship:

$$K_i = IC_{50} / \{1 + ([L] / K_d)\}$$

where [L] is the initial concentration of the [^{3}H]Nicotine and K_d is the dissociation constant for nicotine determined by saturation binding analysis (0.365 nM).

Acknowledgement.

The authors would like to thank the Office of the Vice President for Research and Graduate Studies and the Graduate School of Temple University (Research Incentive Funds) for financial support during the course of this research. D. J. C. and W. S. are grateful to Wyeth Research and the Dean's Office, School of Pharmacy, for financial support. M.P.B. would like to acknowledge Elizabeth McCardy for excellent technical assistance.

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Received: *06-20-05* *Accepted:* *09-21-05*