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Chemistry and Pharmacological Characterization of Novel Nitrogen Analogues of AMOP-H-OH (Sazetidine-A, 6-[5-(Azetidin-2-ylmethoxy)pyridin-3-yl]hex-5-yn-1-ol) as α4β2-Nicotinic Acetylcholine Receptor-Selective Partial Agonists

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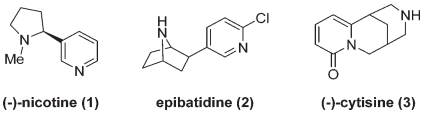
In order to advance therapeutic applications of nicotinic ligands, continuing research efforts are being directed toward the identification and characterization of novel nicotinic acetylcholine receptor (nAChR) ligands that are both potent and subtype selective. Herein we report the synthesis and pharmacological evaluation of members of a new series of 3-alkoxy-5-aminopyridine derivatives that display good selectivity for the α4β2-nAChR subtype based on ligand binding and functional evaluations. The most potent ligand in this series, compound **64**, showed high radioligand binding affinity and selectivity for rat α4β2-nAChR with a K_i value of 1.2 nM and 4700-fold selectivity for α4β2- over α3β4-nAChR, and ~100-fold selectivity for functional, high-sensitivity, human α4β2-nAChR over α3β4*-nAChR. In the mouse forced swim test, compound **64** exhibited antidepressant-like effects. Structure−activity relationship (SAR) analyses suggest that the introduction of additional substituents to the amino group present on the pyridine ring of the *N*-demethylated analogue of compound **17** can provide potent α4β2-nAChR-selective ligands for possible use in treatment of neurological and psychiatric disorders including depression.

Introduction

Nicotinic acetylcholine receptors (nAChR[a])[1,2] are members of the cys-loop superfamily of ligand-gated ion channel receptors and widely distributed in the central and peripheral nervous systems. nAChR in the brain can regulate neurotransmitter release and neuronal excitability. They are thus considered to be promising therapeutic targets for the treatment of central nervous system (CNS) diseases because of their important roles in a variety of critical physiological functions.[1,2] Drugs aimed at nAChR are now in clinical development for the treatment of depression,[3−6] smoking cessation,[7,8] Alzheimer's disease (AD),[9] Parkinson's disease (PD),[10] attention deficit hyperactivity disorder (ADHD),[11] anxiety,[12,13] pain,[14] and schizophrenia.[15,16] Recently, the expression of nAChR in non-neuronal cells was reported to be associated with the nicotine-mediated proliferation of cancer cells, suggesting that nAChR may also serve as a potential anticancer targets.[17]

nAChR are pentameric structures that assemble to create a cation-permeable hydrophilic pore, the opening of which is gated by nicotinic agonist binding.[1,2] To date, 17 nAChR subunit genes have been identified in vertebrates. nAChR subtypes are defined by their subunit composition and can exist as homomeric or heteromeric pentamers.[1,2] Each nAChR subtype and subunit has a unique pattern of expression. For example, an abundant nAChR subtype in the mammalian brain that displays a high affinity for nicotine contains both α4 and β2 subunits,[1,2] while the homopentameric α7-nAChR appears to play a role in sensory gating/schizophrenia.[15,16] On the other hand, α3β4*-nAChR (where the * indicates that subunits in addition to those specified are known or possible components of the assembly) mediate autonomic nicotinic signaling.[1,2] Because each nAChR subtype has distinctive biophysical, pharmacological, and physiological properties,[18,19] it is expected that the subtype selectivity of ligands targeting nAChR will be crucial in achieving a desirable biological outcome while avoiding undesired side effects. Pertinent to our current efforts, preclinical and clinical data suggest that α4β2-nAChR play a role in the control of mood and may therefore be excellent targets for the development of unique antidepressants.[3−6]



(-)-nicotine (1) epibatidine (2) (-)-cytisine (3)

Figure 1. Naturally occurring nAChR ligands.

Considerable progress has been made in the identification of novel nAChR ligands (Figures 1 and 2).[20,21] Compounds **1** ((−)-nicotine), **2** (epibatidine), and **3** ((−)-cytisine) are potent natural nAChR agonists that have been used as templates for

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[a]Abbreviations: AChBP, acetylcholine binding protein; AD, Alzheimer's disease; ADHD, attention deficit hyperactivity disorder; AMOP-H-OH (sazetidine A), 6-[5-(azetidin-2-ylmethoxy)pyridin-3-yl]hex-5-yn-1-ol; CNS, central nervous system; BBB, blood−brain barrier; HS, high sensitivity; LS, low sensitivity; nAChR, nicotinic acetylcholine receptor(s); PD, Parkinson's disease; PNS, peripheral nervous system; SAR, structure−activity relationship.

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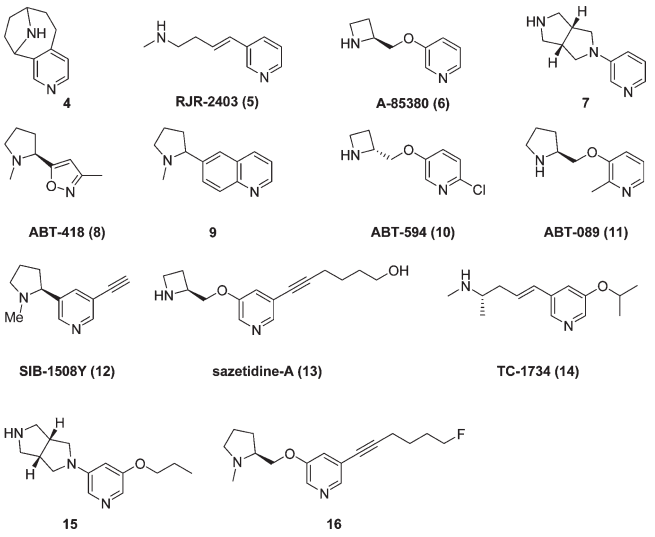


Figure 2. Reported synthetic nAChR ligands.

the design of derivatives possibly having enhanced therapeutic value. As compound **1** represents the prototypical nAChR agonist, studies of its analogues aimed at the identification of more potent and selective compounds continue to represent a vigorous area of research. Three different strategies for analogue design have emerged, and these include: (1) Modification of the pyrrolidine ring generated a number of promising nAChR ligands. For example, the rigid nicotine analogue **4**[22] displayed a K_i value of 1.3 nM in the displacement of [^{3}H]-nicotine at α4β2* receptors from mouse fibroblast M10 cells. Compound **5** (RJR-2403)[23,24] is an α4β2-nAChR-selective partial agonist with a K_i value of 26 nM, and it has been investigated for use in the treatment of cognitive dysfunction. Likewise the pyridyl ether **6** (A-85380)[25] showed high potency and selectivity for human α4β2-nAChR with a K_i value of 0.04 nM. Compound **7**[26] has recently been reported as one of a new series of α4β2-nAChR-selective agonists with a K_i value of 0.12 nM. (2) Replacement of the pyridine with other aromatic rings is a strategy exemplified by the discovery of compound **8** (ABT-418) and quinoline **9**. Compound **8**[25] is a partial agonist at α4β2-nAChR (K_i = 3 nM) that showed some initial promise in the treatment of cognitive problems in AD and ADHD, but its clinical development has been suspended because of its side effects including dizziness and nausea reported in a clinical trial for ADHD, together with little differentiation from placebo discovered in a clinical trial with AD patients.[27,28] Although compound **9**[29,30] does not possess a high affinity for nAChR (K_i = 132 nM), it showed a promising analgesic activity in mice using the hot-plate test. (3) Introduction of various substituents to the pyridine ring can be combined with other structural changes. Chlorination at the 6-position of the pyridine ring in the stereoisomer of compound **6** led to compound **10** (ABT-594), which passed the test of potency in both in vitro and in vivo studies but was abandoned after phase II clinical trials because of the side effects due to insufficient selectivity away from the α3β4*-nAChR.[31] Compound **11** (ABT-089), as another analogue of **6**, showed promise in clinical phase II trials for the treatment of adult ADHD.[32] Substitution at the 5-position of the pyridine ring in nicotine led to compound **12** (SIB-1508Y), which is a partial agonist of α4β2-nAChR that shows high selectivity for β2*- over β4*-nAChR.[33]

Previously, in our efforts to design selective neuronal nAChR ligands, both hydrophobic and hydrogen-bonding groups were attached to an alkynyl substituent appended to the 5-position of the pyridyl ring of compound **6**. Such analogues were found to exhibit an improved selectivity for β2*- over β4*-nAChR. In particular, compound **13** (AMOP-H-OH; sazetidine-A; 6-[5-(azetidin-2-ylmethoxy)pyridin-3-yl]hex-5-yn-1-ol) was identified as a novel partial agonist with high selectivity for α4β2- over α3β4*-nAChR. More rigorous pharmacological studies demonstrated that this compound acts as a potent agonist acting at the high sensitivity (HS) α4β2-nAChR isoform as opposed to the low sensitivity (LS) α4β2-nAChR isoform.[34−36] Additional compounds with substitution at the 5-position of the pyridine such as compound **14** (TC-1734),[37] compound **15**,[26] and AMOP-H-OH analogue **16**[38] were reported to possess high selectivity for α4β2-nAChR, revealing relationships between substitution at the 5-position of the pyridine and α4β2-nAChR selectivity.

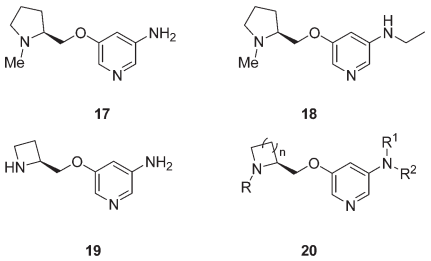


Figure 3. A new series of 3-alkoxy-5-aminopyridine derivatives.

As compound **13** demonstrated promising pharmacology, we sought to explore the activity of related structures in which the metabolically deleterious acetylene group[39] was replaced by other functional groups. In terms of synthetic tractability, we thus chose to explore the introduction of an amino group at the 5-position of the pyridine ring of compound **13** in place of the acetylene group. A search of the literature revealed that certain 3-alkoxy-5-aminopyridine compounds have been reported previously to be potent nAChR ligands (Figure 3, compounds **17**−**19**).[40,41] These compounds showed high affinity for α4β2-nAChR, with K_i values from [^{3}H]-(−)-cytisine binding competition studies of 0.63, 3.70, and 0.082 nM, respectively. However, no information was provided as to nAChR subtype selectivity. Herein, we report on the synthesis and pharmacological characterization of a new series of 3-alkoxy-5-aminopyridine derivatives (Figure 3, series **20**) targeting α4β2-nAChR and behavioral studies utilizing the mouse forced swim test, an assay that is predictive of antidepressant responsiveness in humans.[42]

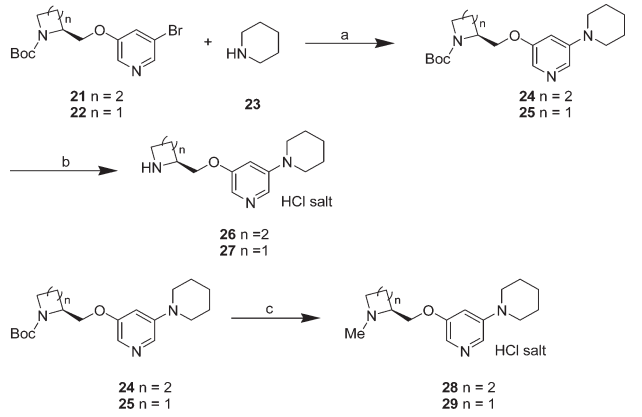
Results and Discussion

Initial Chemistry. When we first embarked on the synthesis of this 3-alkoxy-5-aminopyridine series, a piperidine was selected to replace the simple amine group of compounds **17** and **19**. Compounds **26**−**29** were synthesized by following the synthetic routes shown in Scheme 1. Amination of pyridyl bromides **21** and **22** was accomplished by using a palladium catalyzed amination reaction.[43,44] By employing Pd_2dba_3 as catalyst and xantphos as ligand, bromides **21** and **22** were reacted with piperidine to afford amination products **24** and **25**, respectively. Subsequent deprotection of compounds **24** and **25** with HCl−ether/methanol followed by purification with HPLC afforded the corresponding 3-alkoxy-5-aminopyridine compounds **26** and **27**. Reduction of compounds **24** and **25** with lithium aluminum hydride in refluxing

THF afford the *N*-methylated compounds, which were treated with HCl and sequentially purified by HPLC to provide the final compounds **28** and **29**. All compounds were transformed to their HCl salts.

In Vitro Characterization and Radioligand Binding Studies. These four compounds **26**−**29** were subjected to [^{3}H]-epibatidine binding competition assays, and their K_i values obtained at seven rat nAChR subtypes are presented in Table 1. The LogBB values were also calculated for each compound as presented in Table 1. Based upon these values the compounds are predicted to enter the brain.[45,46] All compounds exhibited high affinity for rat, heterologously expressed α4β2- or native α4β2*-nAChR with K_i values in the nanomolar range. There was as much as a 5- to 10-fold difference in K_i values for ligand inhibition of radioligand binding to α4β2- as opposed to α4β2*-nAChR, and this could reflect contributions of additional subunits in the native receptor population of rat forebrain not found in cells heterologously expressing just α4 and β2 subunits in isolation. These compounds also displayed much higher selectivity for β2*- over the corresponding β4*-nAChR and for α2β2- or α4β2- over α3β2-nAChR. These findings suggest that compounds **26**−**29** are potent and highly selective for α4β2- over α3β4-nAChR. In order to evaluate the influence of the ring size of the azacycle in the alkoxyl part of the 3-alkoxy-5-aminopyridine-based analogues on binding affinity, we compared the K_i values between the five-membered and the four-membered analogues (**26** vs **27** and **28** vs **29**).

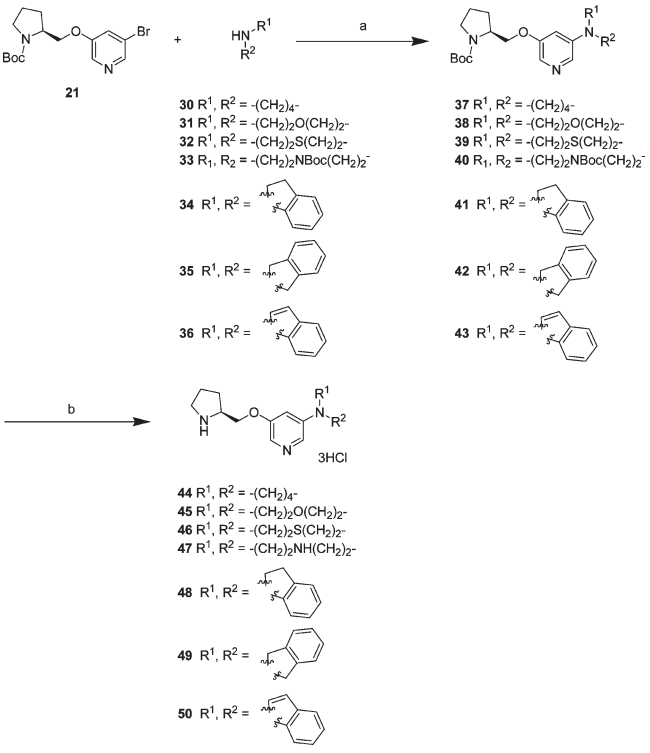
Scheme 1[a]



[a]Reagents and conditions: (a) Pd_2dba_3, xantphos, *t*-BuONa, toluene, 100 °C 4 h; (b) HCl−ether/methanol; (c) (1) $LiAlH_4$, THF, reflux; (2) HCl−ether/methanol.

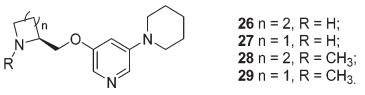
Compounds **26** and **27** exhibited comparable binding affinities, whereas compound **29** had a 5−10-fold lower affinity than compound **28** at α4β2-nAChR. This result suggests that ring size of the ether linked azacycle in these 3-alkoxy-5-aminopyridine-based analogues has some impact on affinity. However, this structural modification is not significant. The presence of the *N*-methyl group as in **29** does reduce binding affinity relative to **27**, suggesting the *N*-methyl azetidinyl group is not preferred in this series. Furthermore, taking into consideration the economy and stability of these two building blocks (pyrrolidinyl and azetidinyl), only 5-(pyrrolidin-2-ylmethoxy)pyridin-3-ylamine analogues were synthesized in the further rounds of our structure−activity relationship (SAR) studies, thus eliminating the somewhat more labile azetidinyl analogues.

Scheme 2[a]



[a]Reagents and conditions: (a) Pd_2dba_3, xantphos, *t*-BuONa, or *t*-BuOK, toluene, 100 °C, 4 h or MW 130 °C, 0.5 h (for compound **43**, CuI, K_3PO_4, *N*,*N*′-dimethylethylenediamine, toluene, MW 130 °C, 40 min); (b) HCl−ether/methanol.

Table 1. Binding Affinities of Compounds **26**−**29** at Seven Rat nAChR Subtypes

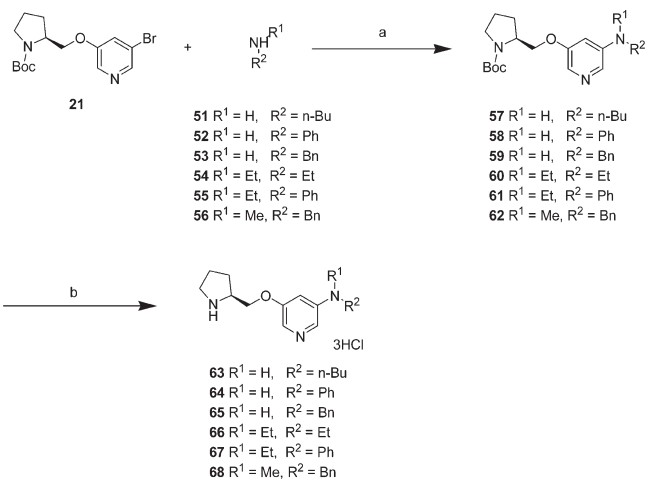


	K_i (nM)[a]							
compd	α2β2	α2β4	α3β2	α3β4	α4β2	α4β2*[b]	α4β4	LogBB[d]
26	38.3 ± 3.0	> 10000	957 ± 252	> 10000	23.3 ± 6.0	194 ± 50	5670[e]	0.24
27	16.2 ± 1.0	2500[e]	171 ± 74	> 10000	13.1 ± 1.6	60.2 ± 12.0	1230[e]	0.04
28	81.9 ± 10.0	> 10000	2840[e]	> 10000	28.2 ± 5.0	293 ± 63	5790[e]	0.08
29	250 ± 42	> 10000	5700[e]	> 10000	141 ± 31	838 ± 195	> 10,000	0.12
1[c]	5.5	70	29	260	4.9	9.8	23	0.03

[a] See Experimental Section. [b] α4β2*, prepared from rat forebrain, see Experimental Section for details. [c] The binding data for nicotine are from the PDSP Assay Protocol Book. [d] LogBB was calculated using the following equation: −0.0148PSA + 0.152CLogP + 0.139. [e] SEM values are not provided for K_i values > 1000 nM.

Second Round of SAR Studies. Further modifications to compound **26** were carried out. To investigate the role of different aliphatic rings on binding affinity, a group of cyclic amine compounds **44**−**50** was prepared by employing the same methodology as that used in Scheme 1 (Scheme 2), except for compound **43**, whose precursor was obtained from Cu (I) catalyzed amination by employing CuI as catalyst and *N*,*N*′-dimethylethylenediamine as ligand. Subsequently, a group of acyclic amine compounds **63**−**68** was also prepared (Scheme 3). The use of microwave irradiation[47] was found valuable in facilitating these amination reactions.

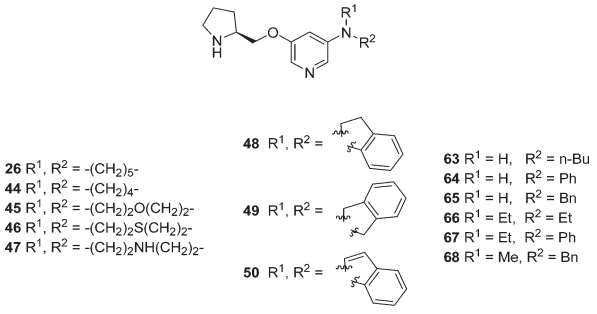
Scheme 3[a]



[a]Reagents and conditions: (a) Pd_2dba_3, xantphos, *t*-BuONa, or *t*-BuOK, toluene, 100 °C, 4 h or MW 130 °C, 0.5 h; (b) HCl−ether/methanol.

Follow-up In Vitro Characterization, Radioligand Binding Studies, and SAR Analysis. The cyclic and acyclic amine compounds **44**−**50** and **63**−**68** also were subjected to in vitro binding studies, and their K_i values at the seven rat nAChR subtypes are listed in Table 2. All tested compounds also exhibit high selectivity for $\beta2$*- over the corresponding $\beta4$*-nAChR subtypes and high selectivity for $\alpha2\beta2$- or $\alpha4\beta2$- over $\alpha3\beta2$-nAChR. Compounds **44**−**50** and **63**−**68** have K_i values for $\alpha4\beta2$-nAChR in the nanomolar range, although there again was as much as a 7-fold difference in K_i values at $\alpha4\beta2$- as opposed to $\alpha4\beta2$*-nAChR for the most of ligands. Compared to the piperidine analogue **26**, introduction of a pyrrolidine ring as in compound **44** resulted in lowered ligand-binding affinity. Other six-membered ring compounds **45**−**47** were found to have similar K_i value as the piperidine analogue **26**, suggesting the substituent at the 4-position of the six-membered ring is not a crucial factor. Attaching a phenyl ring (**48** and **49**) resulted in about a 5- to 10-fold increase in binding affinity when compared with the pyrrolidine compound **44**. These two bicyclic compounds **48** and **49** showed similar binding capability for nAChR as monocyclic compounds **45**−**47**. Indole compound **50** exhibited the best K_i for $\alpha4\beta2$-nAChR (K_i = 2.4 nM) in this series of cyclic ligands, and it also displayed higher affinity for other nAChR subtypes, including $\alpha3\beta4$-nAChR, than other cyclic ligands, suggesting that the introduction of an aryl group to the 5-position of the pyridine would increase affinity for all nAChR subtypes in the cyclic amine series. Secondary amines **63**−**65** were found to have lower K_i values

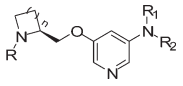
Table 2. Binding Affinities of Variously Substituted Amine Analogues at Seven Rat nAChR Subtypes



	K_i (nM)[a]							
compd	α2β2	α2β4	α3β2	α3β4	α4β2	α4β2*[b]	α4β4	LogBB[c]
26	38.3 ± 3.0	> 10000	957 ± 252	> 10000	23.3 ± 6.0	194 ± 50	5670[e]	0.24
44	445 ± 98	> 10000	4170[e]	> 10000	297 ± 110	1570[e]	1680[e]	−0.09
45	126 ± 22	> 10000	8070[e]	> 10000	54.3 ± 9.0	426 ± 45	8630[e]	−0.35
46	61.8 ± 14.0	> 10000	2130[e]	> 10000	27.7 ± 2.0	213 ± 32	4110[e]	−0.09
47	69.7 ± 7.0	8870[e]	166 ± 33	> 10000	39.2 ± 5.0	104 ± 24	7080[e]	−0.40
48	57.9 ± 14.8	349 ± 47	3220[e]	6270[e]	13.3 ± 2.0	366 ± 69	225 ± 36	0.20
49	158 ± 47	> 10000	812 ± 142	> 10000	50.9 ± 5.0	509 ± 124	3960[e]	0.01
50	6.5 ± 1.0	104 ± 30	208 ± 32	793 ± 195	2.4 ± 0.3	55.3 ± 7.0	70.6 ± 17.0	0.18
63	20.5 ± 2.5	4160[e]	383 ± 55	> 10000	18.8 ± 3.0	100 ± 12	1790[e]	−0.03
64	1.7 ± 0.3	559 ± 165	40.6 ± 4.0	5640[e]	1.2 ± 0.3	1.4 ± 0.1	16.9 ± 3.0	0.03
65	12.5 ± 2.0	937 ± 138	150 ± 25	> 10000	13.2 ± 1.0	63.4 ± 9.0	503 ± 114	−0.05
66	357 ± 64	1970[e]	1290[e]	> 10000	190 ± 49	1150[e]	108 ± 25	0.05
67	124 ± 38	631 ± 77	953 ± 192	9520[e]	59.3 ± 10.0	374 ± 51	378 ± 161	0.24
68	133 ± 15	3400[e]	628 ± 240	> 10000	48.8 ± 10.0	380 ± 50	192 ± 36	0.16
1[d]	5.5	70	29	260	4.9	9.8	23	0.03

[a] See Experimental Section; abbreviations: *n*-Bu, *n*-butyl; Ph, Phenyl; Bn,Benzyl; Et, Ethyl. [b] $\alpha4\beta2$*, prepared from rat forebrain; see Experimental Section for details. [c] LogBB was calculated using the following equation: $-0.0148PSA + 0.152CLogP + 0.139$. [d] The binding data for nicotine are from the PDSP Assay Protocol Book. [e] SEM values were not provided for K_i values > 1000 nM.

Table 3. Sensitivities and Efficacies of Ligand Agonism and Inactivation of Human α4*β*2-nAChR[a]



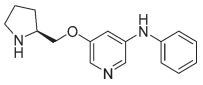
compd	n	R	R^1	R^2	Agonism		Inactivation		K_i (nM)
					EC_{50} (μM)	efficacy (%)[b]	IC_{50} (μM)	efficacy (%)[b]	α4β2
26	2	H			NA	0	19.9	100	23.3
27	1	H			1.14	9.7	0.47	80.6	13.1
28	2	CH_3			NA	0	3.82	97.7	28.2
29	1	CH_3			NA	0	14.1	100	141.0
44	2	H			1.56	5.6	6.46	100	297.0
45	2	H			NA	0	21	100	54.3
46	2	H			0.40	4	0.64	74	27.7
47	2	H			NA	0	>100	100	39.2
48	2	H			NA	0	3.9	100	13.3
49	2	H			NA	0	>100	100	50.9
50	2	H			NA	0	0.78	85	2.4
63	2	H	H	*n*-Bu	0.46	5.5	0.25	75.5	18.8
64	2	H	H	Ph	0.010, .1.98[c]	23	0.058	85.2	1.2
65	2	H	H	Bn	0.22, 12.6[c]	37.0	0.091, 3.13[c]	83.3	13.2
66	2	H	Et	Et	NA	0	16.3	100	190
67	2	H	Et	Ph	1.22	3.7	2.34	98.8	59.3
68	2	H	CH_3	Bn	0.76	3.6	1.24	85.5	48.8
1					0.3	88	0.43	93	4.9

[a] See Experiment Section for details. The term "inactivation" is used because compounds may be acting to desensitize receptors or as competitive or noncompetitive antagonists, and further work is needed to make such a distinction. SEM values were determined for each parameter and, although not presented here, typically are less than 3% and frequently less than 1% of the maximal carbamylcholine response for efficacy measures for ligands potent enough to reach maximal efficacy at 10 μM. SEM values for EC_{50} and IC_{50} values were no more than a factor of 2. See the legend to Table 2 for abbreviations. [b] For compounds that were not potent enough to cause maximal inhibition at the highest concentration tested, 10 μM, inactivation efficacy was fixed at 100% to allow IC_{50} values to be fit during graphical analysis. For compound **64**, the efficacy was fixed at 23% to optimize the regression analysis of the low sensitivity α4*β*2-nAChR phase of the agonism concentration−response curve. [c] The smaller number indicated is the agonist EC_{50} or inactivation IC_{50} value for compound interaction with the high sensitivity α4*β*2-nAChR isoform, and the larger number is the value for compound interaction with the low sensitivity isoform.

at α4*β*2-nAChR than tertiary amines **66**−**68**, suggesting that steric effects might play a role in this series. This conclusion also could be reached by comparing different substituted analogues in the same amine category such as secondary amines **63**−**65**. Thus, it is not surprising that compound **64**, featuring the smallest phenylamino moiety, displayed the most potent binding affinity at each nAChR subtype, especially at the α4*β*2- and α4*β*2*-nAChR.

In Vitro Characterization, Functional Assays. Compounds were assayed for their intrinsic activity as agonists across several, human nAChR subtypes. However, the pharmacological end point for a nAChR exposed to a chronically administered nAChR agonist or partial agonist is likely to be functional inactivation or desensitization. Thus, compounds also were assayed for their ability to inactivate functional responses of nAChR to a full agonist after cells expressing receptors had been exposed to test compounds for 10 min. Compounds were first tested using SH-EP1-hα4*β*2 cells stably expressing human α4*β*2-nAChR assembled from loose subunits (Table 3). For compounds having IC_{50} values < 600 nM for functional inactivation of α4*β*2-nAChR, they also were tested using SH-SY5Y and TE671/RD cells for activity at human α3*β*4*- and α1*β*1*δγ*-nAChR, respectively. All compounds were highly selective for α4*β*2-nAChR, having little or no activity at α3*β*4*- or α1*β*1*δγ*-nAChR at the highest concentrations tested. Note that only compounds **64** and **65** had >10% aggregate efficacy when acting as agonists at human α4*β*2-nAChR. Only **64** had any appreciable agonist

Table 4. Sensitivities and Efficacies of Ligand Agonism and Inactivation at α4β2[a], α1β1δγ,[b] and α3β4[b] nAChR for Compound **64**



agonism (α4β2)		agonism (α1β1δγ)		agonism (α3β4)	
EC_{50} (μM)	efficacy (%)	EC_{50} (μM)	efficacy (%)	EC_{50} (μM)	efficacy (%)
0.010, 1.98[a]	23	NA	0	1.35	29.7
inactivation (α4β2)		inactivation (α1β1δγ)		inactivation (α3β4)	
IC_{50} (μM)[c]	efficacy (%)	IC_{50} (μM)	efficacy (%)	IC_{50} (μM)	efficacy (%)
0.058	85.2	9.48	100	2.04	100

[a] See the legend to Table 3 for details. [b] See Experimental Section for details about α1βγδ- and α3β4-nAChR. [c] The SEM for the α4β2-nAChR ic50 value is 0.13 (log molar) or × /÷ 0.74 and the SEM of the inactivation efficacy is 4.5%. At $n = 5$ for α4β2, both of these SEMs are 2−3 fold larger than is typical for the assay due to the shallowness of the Hill slope and because the highest dose tested may be just short of maximal inactivation efficacy (see Figure 4).

activity at α3β4*-nAChR (Table 4), and it was unique among this series of compounds in being a self-inhibiting agonist[48,49] at this receptor subtype (Figure 4). The biphasic agonist response seen for **64** at α4β2-nAChR is due to the expression of two isoforms operationally defined based on their sensitivities to nicotine or acetylcholine as agonists and presumed to have the subunit stoichiometries indicated as HS $(\alpha4)_2(\beta2)_3$-nAChR and LS $(\alpha4)_3(\beta2)_2$-nAChR.[34−36] The two phases are well-defined for **64** when acting acutely as an agonist, having EC_{50} values of 10 nM and 1.98 μM, respectively, for actions at HS and LS α4β2-nAChR, respectively. However, as a functional inactivator, **64** is insufficiently selective to calculate individual IC_{50} values for HS and LS α4β2-nAChR. For this reason, although the shallow Hill slope suggests actions at more than one α4β2-nAChR isoform, the 58 nM inactivation IC_{50} reported in Table 3 is slightly misleading and lies between the actual IC_{50} values for compound-mediated inactivation of HS and LS α4β2-nAChR isoforms. Compounds (**27**, **44**, **46**, **63**, **67**, and **68**) showed little efficacy as agonists (< 10%). Although compounds **26**, **28**, **29**, **45**, **47**−**50**, and **66** did not exhibit agonist activity, they might act as desensitizers or competitive/noncompetitive antagonists.

Comparisons between ligand binding and functional interactions with nAChR are somewhat more challenging, even accounting for possible, but unlikely to be major, differences across rat vs human nAChR of the same subunit composition. Binding affinity for ligand interactions with rat α4β2-nAChR does have a generally positive correlation with functional potency of ligands acting at human α4β2-nAChR. However, functional inactivation IC_{50} values for actions at human α4β2-nAChR can vary as much as 50-fold for ligands that have nearly identical K_i values at rat α4β2-nAChR (Figure 5), and compounds such as **46** and **50** can have greater than a 10-fold difference in binding affinity at rat α4β2-nAChR, yet functionally, they have nearly identical EC_{50} or IC_{50} values at human α4β2-nAChR. Nevertheless, functional inactivation could be due to compound action as a desensitizing agent, as a noncompetitive antagonist (even if there is competition for agonist binding), and/or as a competitive antagonist. Inactivation IC_{50} values might be expected to be lower than EC_{50} values if the compound acted as a desensitizing agent, but the data show that this is not

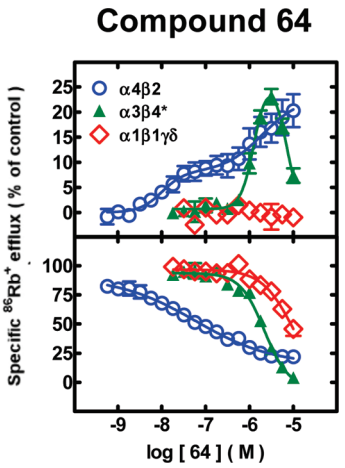


Figure 4. Functional assays show compound **64** to be potent and selective for α4β2-nAChR. Specific $^{86}Rb^+$ efflux (ordinate; percentage of control + SEM) was determined for human α4β2-nAChR (○), α3β4*-nAChR (▲), or α1β1δγ-nAChR (◇) naturally or heterologously expressed by SH-EP1-h α4β2, SH-SY5Y, or TE671/RD cells, respectively. The upper panel shows responses to an initial, 10 min exposure to the compound at the indicated concentrations (abscissa, log molar concentration) revealing any intrinsic activity of the compound as an agonist. The lower panel shows responses to an EC_{90} concentration of carbamylcholine in cells after the initial 10 min preincubation period and in the continuing presence of compound **64**. Results are normalized to responses to a fully efficacious concentration of carbamylcholine (see the Experimental Section for details). Micromolar agonist EC_{50} values and inactivation IC_{50} values are provided in Table 4, as are agonist and inactivation efficacies (normalized to those for a full agonist or antagonist, respectively). SEM values were determined for each parameter and, although not presented here, typically are less than 3% and frequently less than 1% of the maximal carbamylcholine response for efficacy measures and no more than a factor of 2 for molar EC_{50} or IC_{50} values. The biphasic profile for compound **64** acting as an agonist at α4β2-nAChR (upper panel, ○) and the shallow Hill slope for its inactivation of receptor function (lower panel, ○) are indicative of activity at both HS and LS α4β2-nAChR.

necessarily the case. Perhaps these findings indicate that some ligands act principally as antagonists, consistent with their low or absent intrinsic activities in tests for agonism, whereas others that have some agonist activity also engage in desensitization. Further complicating these comparisons are sometimes large differences in K_i values for heterologously expressed, rat α4β2-nAChR and for native α4β2*-nAChR from rat forebrain that may contain other subunits. In addition, the fact that α4β2-nAChR can exist as two different structural isoforms with different agonist sensitivities also complicates data interpretation, especially when ligand functional interactions with the two isoforms cannot be clearly delineated.

Interestingly, compounds **64** and **65** have the highest agonist potencies and efficacies and, like compound **13**, show significant agonist activity. Nevertheless, compounds **64** and **65** differ from **13**, which seems to have agonist activity exclusively at human HS α4β2-nAChR only, in that they have concentration−isotopic ion flux response curves that suggest activation of both HS and LS α4β2-nAChR. As can best be assessed, inactivation IC_{50} values are lower than agonist EC_{50} values for these ligands, consistent with their longer-term actions as desensitizing agents. Compound **64** has about 10-fold lower EC_{50}, IC_{50}, or K_i values than compound **65**, suggesting that steric effects play an important role in this

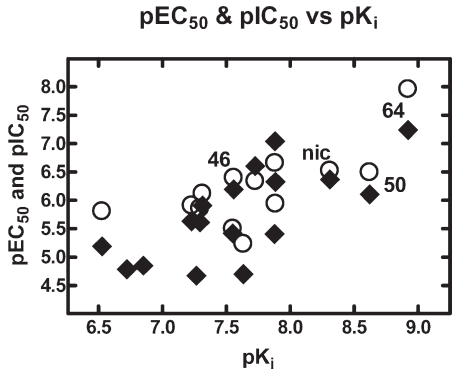


Figure 5. Relationships between ligand competition for radioligand binding and functional activities for tested compounds. Mean agonist pEC_{50} values (○) and inactivation pIC_{50} values (◆) for actions at functional, human α4β2-nAChR heterologously expressed by SH-EP1-hα4β2 cells are plotted (ordinate; potency increases down-to-up) vs corresponding radioligand binding inhibition pK_i values for drug interactions with heterologously expressed, rat α4β2-nAChR (abscissa, binding affinity increases left-to-right) for compounds tested including, for special reference, compounds **46**, **50**, and **64**. The data show reasonably good, but not perfect, agreement in rank order potency across ligand binding and functional dimensions but also indicate that radioligand binding assays have a limited ability to predict functional potency.

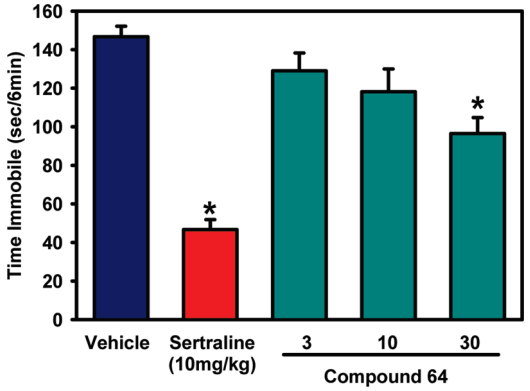


Figure 6. Compound **64** reduced immobility in the forced swim test in mice at the high (30 mg/kg) but not lower (3 and 10 mg/kg) doses. The selective serotonin reuptake inhibitor, sertraline, produced the expected decrease in immobility. (* $p < 0.05$ vs vehicle, Newman Keuls post hoc test; intraperitoneal. $n = 10$/group).

series of compounds. These ligands thus seem to belong to the same class of compounds and could be useful as research tools based on their different apparent potencies at functional α4β2-nAChR isoforms.

Overall, the current observations underscore the necessity of performing functional assays before advancing a potential new chemical entity down the drug discovery pipeline. Binding assay results can illuminate mechanisms involved in interactions with receptors, but functional effects must be defined and must exist if a ligand is to have hope of altering physiological processes mediated by the target. With an understanding of the effects compounds have on their targets, behavioral studies can be properly interpreted and used both to inform subsequent drug design and the decision to advance or halt drug development.

Behavioral Characterization, Mouse Forced Swim Test. Antidepressant efficacy was assessed with the mouse forced swim test, an assay in which mice are placed into a beaker of water and the time the mouse spends passively floating in the water (immobility) is recorded. Most traditional antidepressants decrease the amount of time the mouse spends immobile.[42] Mice were administered most potent compound **64** (3, 10, or 30 mg/kg) or the selective serotonin reuptake inhibitor antidepressant, sertraline, as a positive control (10 mg/kg). Drug administration produced a reduction in immobility ($F(4,44) = 21.3$, $p < 0.0001$). Post hoc tests showed that **64** reduced immobility at the high dose (30 mg/kg), suggestive of an antidepressant like effect. The two lower doses of that compound (3 and 10 mg/kg), however, were inactive. Sertraline produced the expected decrease in immobility (Figure 6).

Conclusion

In this study, a new series of 3-alkoxy-5-aminopyridine compounds have been designed and synthesized, and some of these were shown to bind with high affinity to α4β2-nAChR. All of the compounds possess K_i values based on radioligand binding assays in the nanomolar range at the rat α4β2-nAChR and high selectivity for rat α4β2- over α3β4-nAChR. Further structure−activity analyses suggested that: (1) The steric effect of the substituent group on the 5-position of the pyridine ring is a crucial factor for both ligand affinity and agonism. The secondary amines, such as the phenyl analogue **64** and the benzyl compound **65**, are more potent and selective than the tertiary amines including the cyclic amines. These results thus imply that less bulky groups are preferred. (2) Regarding the azacycle linked to the pyridine ring through the methyleneoxy group, the *N*-methyl compounds (**28** and **29**) show lower activity in both binding and functional studies than their N−H analogues (**26** and **27**), and the unsubstituted azetidine analogue shows better affinity and higher agonism compared to the unsubstituted pyrrolidine ring analogue. (3) An aryl group on the 5-position of the pyridine ring results in high binding affinity but low efficacy as an α4β2-nAChR agonist (e.g., see indole compound **50**). The most potent compound **64**, with a phenylamino moiety, exhibits low K_i values of 1.2 and 1.4 nM for binding to rat, heterologously expressed α4β2- and native, rat forebrain α4β2*-nAChR, respectively. The $^{86}Rb^+$ efflux studies reveal that most compounds had little or no agonist activity (efficacy < 10%). Only compounds **64** and **65** exhibited >10% efficacy. Further studies show that compound **64** is a partial agonist selective for human α4β2- over α3β4- and α1β1δγ-nAChR. Antidepressant-like activity of compound **64** also was revealed by behavioral assessment in the mouse forced swim test, and it is consistent with the antidepressant like activity of other α4β2* selective ligands including compound **13**[34−36] and compound **6**.[50] Compound **64** thus provides a useful starting point in the development of further novel classes of α4β2-nAChR-selective partial agonists. Besides compound **64** and **65**, most of our compounds exhibited partial agonism activity with low efficacy, and those that did not show agonism might be classified as desensitizers or competitive/noncompetitive antagonists.

Experimental Section

General. Proton and carbon NMR spectra were recorded on a 400 MHz spectrometer. NMR chemical shifts were reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ (^{1}H NMR), δ 4.80 signal of D_2O (^{1}H NMR), and δ 77.2 signal of $CDCl_3$ (^{13}C NMR) as internal standards. ^{13}C NMR spectra in D_2O were not adjusted. Optical rotation was detected on an Autopol IV automatic

polarimeter. Mass spectra were measured in the ESI mode at an ionization potential of 70 eV with LCMS MSD (Hewlett-Packard). Column chromatography was performed using Merck silica gel (40−60 mesh). Purity of compounds (≥95%) was established by HPLC, which was carried out with two methods: (1) On an ACE 5 AQ column (100 mm × 4.6 mm), with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector; flow rate = 3.6 mL/min; gradient of 8−100% acetonitrile (or methanol) in water (both containing 0.05 vol% of CF_3COOH) in 30 min, to 100% in another 5 min, return to 0% in next 4 min, finally balanced at 0% for the final 1 min. (2) On an Agilent 1100 HPLC system with a Synergi 4 μ Hydro-RP 80A column, with detection at 254 (or 280) nm on a variable wavelength detector G1314A; flow rate = 1.4 mL/min; gradient elution over 20−29 min, from 30% methanol−water to 100% methanol (both containing 0.05 vol % of CF_3COOH). Microwave-assisted reactions were performed with a Biotage initiator.

General Procedure for Amination (Method A). To a mixture of pyridylbromide (1.0 equiv) and amine (1.0−1.5 equiv) in anhydrous toluene (0.1 M) were added *t*-BuONa or *t*-BuOK (1.5 equiv), $Pd_2(dba)_3$ (0.02 equiv), and xantphos (0.06 equiv) successively. The mixture was degassed and purged with argon (3 cycles) and then heated to 98−100 °C for 4 h. The mixture was cooled to room temperature and diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using CH_2Cl_2−ethyl acetate (4:1 to 1:1) as the eluent to give the pure product (53% −100%).

General Procedure for Amination with Microwave Assistance (Method B). To a mixture of pyridylbromide (1.0 equiv) and amine (1.0−1.5 equiv) in anhydrous toluene (0.1 M) was added *t*-BuONa or *t*-BuOK (1.5 equiv), $Pd_2(dba)_3$ (0.02 equiv), and xantphos (0.06 equiv) successively. The mixture was degassed and purged with argon (three cycles) and then heated under microwave irradiation for 30−40 min at 130 °C. The mixture was cooled to room temperature and diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using CH_2Cl_2−ethyl acetate (4:1 to 1:1) as the eluent to give the pure product.

General Procedure for Deprotecting *N*-Boc to Afford Hydrochloride Salt (Method C). To a solution of *N*-Boc protected compound (1.0 equiv) in methanol was added 2 N HCl/ether (1 mL) under argon protection at room temperature. The mixture was stirred overnight. After the solvent was evaporated, the resulting residue was dissolved in distilled water (about 20−30 mL). After the resultant solution was filtered over a cotton plug, the water was removed under reduced pressure at 35 °C. The crude product was purified with HPLC (see HPLC conditions), and the resulting CF_3COOH salt was treated with AAA resin to afford free amine compound. The free amine then was dissolved in methanol and treated with 2 N HCl/ether (1 mL), again under argon protection at room temperature. The mixture was stirred overnight. After the solvent was evaporated, the resultant residue was dissolved in distilled water (about 20−30 mL). After the resultant solution was filtered over a cotton plug, the water was removed under reduced pressure at 35 °C. Pure final HCl salt product could be obtained after lyophilization.

Preparative HPLC Conditions (Water/Acetonitrile System−Gradient A). ACE AQ 150 mm × 21.2 mm column; UV detection at both 254 and 280 nm; flow 10.0 mL/min; gradient of 0−50% acetonitrile in water (both containing 0.05 vol% of CF_3COOH) for 25 min, to 100% for another 5 min, return to 0% for the next 5 min, finally balanced at 0% for the final 5 min.

Preparative HPLC Conditions (Water/Methanol System−Gradient B). ACE AQ 150 mm × 21.2 mm column; UV detection at both 254 and 280 nm; flow 10.0 mL/min; gradient of 0−50% methanol in water (both containing 0.05 vol% of CF_3COOH) for 20 min, to 100% for another 5 min, maintain 100% for another 5 min, return to 0% for the next 5 min, finally balanced at 0% for the final 5 min.

(*S*)-1-(*tert*-Butoxycarbonyl)-2-(5-bromo-3-pyridinyloxymethyl)-pyrrolidine (21). To a mixture of (*S*)-1-(*tert*-butoxycarbonyl)-2-hydroxymethyl pyrrolidine (1.39 g, 6.9 mmol), 5-bromo-3-pyridinol (0.796 g, 4.6 mmol), and Ph_3P (1.80 g, 6.9 mmol) in anhydrous THF (35 mL) was added DEAD (1.09 mL, 6.9 mmol) dropwise at 0 °C under argon protection. After stirring at room temperature for 5 days, the solvent was removed in vacuo. The mixture was diluted with hexane−ethyl acetate (4:1), and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using hexane−ethyl acetate (4:1 to 1:1) as the eluent to give **21** as a colorless solid (1.412 g, 86%). ^{1}H NMR ($CDCl_3$): δ 8.24 (m, 2H), 7.40 (m, 1H), 4.13 (m, 2H), 3.99−3.84 (m, 1H), 3.34 (m, 2H), 2.00−1.87 (m, 4H), 1.46 (s, 9H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-(piperidinyl)pyridine (24). Method A was used. Yield: 99% (colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.87 (m, 1H), 7.69 (s, 1H), 6.79−6.61 (m, 1H), 4.04 (m, 2H), 3.85−3.77 (m, 1H), 3.32 (m, 2H), 3.11 (s, 4H), 1.95−1.78 (m, 4H), 1.61 (m, 4H), 1.52 (m, 2H), 1.39 (s, 9H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-azetidinyl]methoxy]-5-(piperidinyl)pyridine (25). Method A was used. Yield: 85% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.94(s, 1H), 7.77 (s, 1H), 6.75 (s, 1H), 4.49 (m, 1H), 4.28 (m, 1H), 4.11 (dd, J = 2.8, 10.0 Hz, 1H), 3.88 (m, 2H), 3.18 (m, 4H), 2,29 (m, 2H), 1.70 (m, 4H), 1.61 (m, 2H), 1.41 (s, 9H).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-(piperidinyl)pyridine Hydrochloride (26). Method C was used. Yield: 99% (pale-yellow foam). ^{1}H NMR (D_2O): δ 7.91 (s, 1H), 7.73 (s, 1H), 7.41 (s, 1H), 4.42 (m, 1H), 4.23 (m, 1H), 4.03 (m, 1H), 3.29 (m, 6H), 2.20−2.15 (m, 1H), 2.06−1.96 (m, 2H), 1.86−1.81 (m, 1H), 1.55 (m, 6H).; ^{13}C NMR (D_2O) δ 156.5, 148.9, 122.2, 117.8, 114.5, 67.2, 58.1, 48.6, 45.5, 25.4, 23.8, 23.0, 22.7. HPLC Purity: 99.8%.HRMS (ESI) m/z calcd for $C_{15}H_{23}N_3O$ $(M + H)^+$ 262.1919, found 262.1913; $[\alpha]_D^{20}$ = +4.3 (c = 0.14, MeOH). Anal. Calcd for $C_{15}H_{23}N_3O \cdot 2.60HCl \cdot 2.25H_2O$: C, 45.42; H, 7.65; N, 10.59; Cl, 23.24. Found: C, 45.76; H, 7.37; N, 10.26; Cl, 23.11.

3-[(2(*S*)-Azetidinyl)methoxy]-5-(piperidinyl)pyridine Hydrochloride (27). Method C was used. Yield: 61% (yellow foam). ^{1}H NMR (D_2O): δ 8.02 (s, 1H), 7.78 (s, 1H), 7.57 (s, 1H), 4.92 (m, 1H), 4.46 (m, 2H), 4.04 (m, 2H), 3.37 (m, 4H), 2.61 (dt, J = 8.4, 8.4 Hz, 2H), 1.60 (m, 6H). ^{13}C NMR (D_2O): δ 159.1, 150.9, 125.1, 121.2, 117.8, 69.7, 60.8, 51.6, 45.8, 26.3, 25.0, 22.4. HPLC purity: 99.40%. HRMS (ESI) m/z calcd for $C_{14}H_{21}N_3O$ $(M + H)^+$ 248.1763, found 248.1755; $[\alpha]_D^{21}$ = −9.6 (c = 0.22, MeOH). Anal. Calcd for $C_{14}H_{21}N_3O \cdot 3.25HCl \cdot 1.2H_2O$: C, 43.40; H, 6.93; N, 10.85; Cl, 29.74. Found: C, 43.47; H, 6.89; N, 10.75; Cl, 29.67.

3-[(1-Methyl-2(*S*)-pyrrolidinyl)methoxy]-5-(piperidinyl)pyridine Hydrochloride (28). To a suspension of lithium aluminum hydride (27.4 mg, 0.72 mmol) in THF (1 mL) was added a solution of compound **24** (52.3 mg, 0.14 mmol) in THF (1 mL). The resulting mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction was poured into a flask containing 4−5 g of Na_2SO_4 and 40−50 mL of Et_2O. Water then was added to the mixture to quench the reaction until no more gas was generated, the mixture was filtrated, and the solid part was washed with CH_2Cl_2/methanol = 4:1 (100−150 mL). After the solvent was removed, 41.0 mg of product was obtained, which could used directly without further purification. Following method C, product **28** could be obtained (yield 83% for two steps) as a yellow foam solid. ^{1}H NMR (D_2O): δ 8.01 (s, 1H), 7.84 (s, 1H), 7.51 (s, 1H), 4.53 (dd, J = 2.8, 10.8 Hz, 1H), 4.38 (dd, J = 9.6, 10.8 Hz, 1H), 3.89 (m, 1H), 3.69 (m, 1H), 3.35 (m,4H), 3.18 (m, 1H), 2.83 (s, 3H), 2.33 (m, 1H), 2.14 (m, 1H), 2.02 (m, 2H), 1.60 (m, 6H). ^{13}C NMR (D_2O): δ 156.4, 148.4, 122.6, 118.2, 114.9, 66.9, 65.9, 56.8, 48.9, 40.2, 25.5, 23.8, 22.5, 21.7. HPLC purity: 99.63%. HRMS (ESI) m/z calcd for $C_{16}H_{25}N_3O$ $(M + H)^+$ 276.2076, found

276.2077; $[\alpha]_D^{23} = -5.7$ ($c = 0.35$, MeOH). Anal. Calcd for $C_{16}H_{25}N_3O \cdot 3.0HCl \cdot 2.2H_2O$: C, 45.28; H, 7.69; N, 9.90. Found: C, 45.28; H, 7.74; N, 9.81.

3-[(1-Methyl-2(*S*)-azetidinyl)methoxy]-5-(piperidinyl)pyridine Hydrochloride (29). The synthesis involved starting with **25** and following the same methodology as employed for the preparation of **28** from **24**. Yield: 58% (for two steps, yellow foam). ^{1}H NMR (D_2O): δ 8.00 (s, 1H), 7.86 (s, 1H), 7.54 (s, 1H), 4.66 (m, 1H), 4.39 (m, 2H), 4.17 (m, 1H), 3.89 (m, 1H), 3.33 (s, 4H), 2.80 (s, 3H), 2.54 (m, 1H), 1.57 (s, 6H). ^{13}C NMR (D_2O): δ 156.4, 148.0, 122.8, 119.0, 115.5, 67.7, 66.4, 52.9, 49.4, 40.6, 23.7, 22.4, 17.5. HPLC purity: 98.57%. HRMS (ESI) m/z calcd for $C_{15}H_{23}N_3O$ $(M + H)^+$ 262.1919, found 262.1915; $[\alpha]_D^{23} = -21$ (c = 0.13, MeOH).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-(pyrrolidinyl)pyridine (37). Method B was used. Yield: 85% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 8.31−8.20 (m, 1H), 7.74 (m, 1H), 6.46−6.30 (m, 1H), 4.14 (m, 2H), 3.93−3.81 (m, 1H), 3.40 (m, 2H), 3.27 (t, J = 8.4 Hz, 4H), 2.00−1.84 (m, 8H), 1.45 (s, 9H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-(morpholinyl)pyridine (38). Method A was used. Yield: 85% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 8.00−7.55 (m, 2H), 7.14−6.70 (m, 1H), 4.22−4.11 (m, 2H), 3.85 (m, 5H), 3.38−3.08 (m, 6), 2.00−1.78 (m, 4H), 1.46 (s, 9H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-(thiomorpholinyl)pyridine (39). Method B was used. Yield: 100% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 8.30 (m, 1H), 7.77 (s, 1H), 6.97−6.63 (m, 1H), 4.13 (m, 2H), 3.86 (m, 1H), 3.61 (m, 4H), 3.39 (m, 2H), 2.71 (m, 4H), 1.88 (m, 4H), 1.45 (s, 9H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-[4-(*tert*-butoxycarbonyl)piperazinyl]pyridine (40). Method B was used. Yield: 46% (colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.92−7.81 (m, 2H), 6.96−6.66 (m, 1H), 4.10 (m, 2H), 3.90 (m, 1H), 3.55 (m, 4H), 3.36 (m, 2H), 3.16 (m, 4H), 2.01−1.82 (m, 4H), 1.45 (s, 18H).

3-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-(1-indolinyl)pyridine (41). Method A was used. Yield: 90% (bright-yellow oil). ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H), 7.91 (s, 1H), 7.20−7.00 (m, 4H), 6.79 (t, J = 6.8 Hz, 1H), 4.11 (m, 2H), 3.90−3.86 (m, 3H), 3.41 (m, 2H), 3.13 (t, J = 8.4 Hz, 2H), 2.02 (m, 3H), 1.86 (m, 1H), 1.46 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 155.6, 146.0, 141.4, 132.1, 131.5, 130.1, 129.1, 127.3, 125.3, 120.0, 109.4, 108.7, 68.5, 56.1, 51.9, 47.1, 46.8, 28.9, 28.6, 28.2, 24.0.

3-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-(1,3-dihydro-2*H*-isoindol-2-yl)pyridine (42). Method A was used. Yield: 68% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.73 (m, 2H), 7.27 (m, 4H), 6.63−6.41 (m, 1H), 4.85 (s, 4H), 4.10 (m, 2H), 3.96 (m, 1H), 3.41 (m, 2H), 2.02 (m, 3H), 1.85 (m, 1H), 1.47 (s, 9H).

3-(1-Indolyl)-5-[[*N*-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinyl]-methoxy]pyridine (43). The mixture of (*S*)-1-(*tert*-butoxycarbonyl)-2-hydroxymethyl-pyrrolidine **21** (150.0 mg, 0.42 mmol), indole (59.0 mg, 0.50 mmol), CuI (8 mg, 0.042 mmol), and K_3PO_4 (187.2 mg, 0.88 mmol) in toluene (2 mL) was degassed and purged with Ar (3 cycles), then *N*,*N*′-dimethylethane-1,2-diamine (9 μL, 0.084 mmol) was added. After reacting in a microwave oven at 130 °C for 40 min, the mixture was poured into brine and extracted with ethyl acetate (3 times). The combined organic layer was dried over Na_2SO_4. After concentration, the residue was purified by gel column chromatography with CH_2Cl_2 to CH_2Cl_2/EA 4:1, and 3-(1-indolyl)-5-[[*N*-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-pyridine **52** was obtained 152.0 mg (yield 92%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.70 (m, 1H), 7.59 (m, 1H), 7.40 (m, 3H), 7.24 (m, 3H), 6.72 (m, 1H), 4.15 (m, 2H), 3.90 (m, 1H), 3.38 (m, 2H), 1.89 (m, 4H), 1.47 (s, 9H).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-(pyrrolidinyl)pyridine Hydrochloride (44). Method C was used. Yield: 92% (pale-yellow solid). ^{1}H NMR (D_2O): δ 7.64 (m, 2H), 7.06 (s, 1H), 4.54 (dd, J = 3.2, 10.4 Hz, 1H), 4.33 (dd, J = 8.0, 10.4 Hz, 1H), 4.15 (m, 1H), 3.43 (t, J = 7.2 Hz, 2H), 3.37 (t, J = 6.4 Hz, 4H), 2.29 (m, 1H), 2.10 (m, 6H), 1.96 (m, 1H). ^{13}C NMR (D_2O): δ 156.2, 139.4, 118.3, 114.6, 109.8, 67.0, 58.1, 47.3, 45.5, 25.4, 24.5, 23.0. HPLC purity: 99.7%. MS (ESI) m/z 248.2 $(M + H)^+$; $[\alpha]_D^{20} = +1.3$ (c = 0.16, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-(morpholinyl)pyridine Hydrochloride (45). Compound was synthesized via method C. Yield: 84% (orange solid). ^{1}H NMR (D_2O): δ 8.04 (d, J = 2.0 Hz, 1H), 7.91 (d, J = 2.0 Hz, 1H), 7.54 (t, J = 2.0 Hz, 1H), 4.56 (dd, J = 3.2, 10.4 Hz, 1H), 4.36 (dd, J = 7.6, 10.8 Hz, 1H), 4.16 (m, 1H), 3.92 (t, J = 4.8 Hz, 4H), 3.43 (m, 6H), 2.30 (m, 1H), 2.14 (m, 2H), 1.96 (m, 1H). ^{13}C NMR (D_2O): δ 156.5, 149.5, 121.5, 118.0, 114.2, 67.2, 65.4, 58.0, 46.2, 45.5, 25.3, 23.0. Purity by HPLC: 99.7%. MS (ESI, m/e) 264.2 $(M + 1)^+$; $[\alpha]_D^{23} = +3.0$ (c = 0.034, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-(thiomorpholinyl)pyridine Hydrochloride (46). Compound was synthesized via method C. Yield: 84% (pale-yellow solid). ^{1}H NMR (D_2O): δ 8.01 (d, J = 2.0 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.48 (t, J = 2.4 Hz, 1H), 4.56 (dd, J = 3.2, 10.4 Hz, 1H), 4.35 (dd, J = 7.2, 10.4 Hz, 1H), 4.17 (m, 1H), 3.86 (t, J = 4.8 Hz, 4H), 3.43 (t, J = 7.2 Hz, 2H), 2.77 (m, 4H), 2.31 (m, 1H), 2.11 (m, 2H), 1.96 (m, 1H). ^{13}CNMR (D_2O): δ 157.0, 139.0, 121.8, 116.9, 114.1, 67.2, 58.0, 49.3, 45.5, 40.8, 25.4, 24.2, 23.0. Purity by HPLC: 98.6%. MS (ESI, m/e) 280.2 $(M + 1)^+$; $[\alpha]_D^{22} = +8.8$ (c = 0.057, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-(piperazinyl)pyridine Hydrochloride (47). Compound was synthesized via method C. Yield: 100% (pale-yellow foam). ^{1}H NMR (D_2O): δ 8.08 (s, 1H), 7.95 (s, 1H), 7.59 (s, 1H), 4.54 (dd, J = 3.2 Hz, 1H), 4.35 (m, 1H), 4.13 (m, 1H), 3.70 (m, 4H), 3.40 (m, 6H), 2.23 (m, 1H), 2.10 (m, 2H), 1.94 (m, 1H). ^{13}CNMR (D_2O): δ 156.5, 148.4, 122.1, 119.0, 115.4, 67.4, 58.0, 45.5, 43,6, 42.2, 25.4, 23.0. Purity by HPLC: 99.4%. MS (ESI, m/e) 263.2 $(M + 1)^+$; $[\alpha]_D^{23} = +2.8$ (c = 0.50, MeOH).

3-(1-Indolinyl)-5-[(2(*S*)-pyrrolidinyl)methoxy]pyridine Hydrochloride (48). Compound was synthesized via method C. Yield: 97% (yellow solid). ^{1}H NMR (D_2O): δ 7.97 (s, 1H), 7.83 (s, 1H), 7.41 (s, 1H), 7.12 (d, J = 7.2 Hz, 1H), 7.05 (m, 2H), 6.82 (t, J = 7.2 Hz, 1H), 4.45 (dd, J = 3.2, 10.4 Hz, 1H), 4.31 (dd, J = 7.6, 10.4 Hz, 1H), 4.15 (m, 1H), 3.78 (t, J = 8.4 Hz, 2H), 3.45 (t, J = 7.2 Hz, 2H), 3.01 (t, J = 8.4 Hz, 2H), 2.32 (m, 1H), 2.14 (m, 2H), 1.97 (m, 1H). ^{13}C NMR (D_2O): δ 156.2, 142.5, 141.9, 132.2, 126.7, 125.3, 121.9, 121.5, 118.2, 114.2, 109.6, 67.3, 58.1, 51.0, 45.6, 26.6, 25.5, 23.0. Purity by HPLC: 99.9%. HRMS (ESI) calcd for $C_{18}H_{22}N_3O$ $(M + H^+)$ m/z 296.1763, found 296.1758; $[\alpha]_D^{20} = +7.0$ (c = 1.0, MeOH). Anal. Calcd for $C_{18}H_{21}N_3O \cdot 3.0HCl \cdot 0.1H_2O$: C, 53.18; H, 6.00; N, 10.34; Cl, 26.16. Found: C, 53.27; H, 6.18; N, 10.26; Cl, 26.03.

3-(1,3-Dihydro-2*H*-isoindol-2-yl)-5-[(2(*S*)-pyrrolidinyl)methoxy]-pyridine Hydrochloride (49). Compound was synthesized via method C. Yield: 18% (gray solid). ^{1}H NMR (D_2O): δ 7.54 (s, 1H), 7.40 (s, 1H), 7.17 (m, 4H), 6.83 (s, 1H), 4.38 (m, 1H), 4.22 (m, 5H), 4.10 (m, 1H), 3.46 (t, J = 7.2 Hz, 2H), 2.33 (m, 1H), 2.18 (m, 2H), 1.98 (m, 1H). ^{13}C NMR (D_2O): δ 157.3, 156.1, 144.9, 134.9, 127.2, 122.0, 115.9, 109.8, 67.0, 58.1, 52.9, 45.6, 25.4, 23.0. Purity by HPLC: 99.5%. HRMS (ESI) calcd for $C_{18}H_{22}N_3O$ $(M + H^+)$ m/z 296.1763, found 296.1761; $[\alpha]_D^{20} = +1.1$ (c = 0.90, MeOH). Anal. Calcd for $C_{18}H_{21}N_3O \cdot 2.9HCl \cdot 0.2H_2O$: C, 53.42; H, 6.05; N, 10.38; Cl, 25.40. Found: C, 53.56; H, 6.22; N, 10.31, Cl, 25.48.

3-(1-Indolyl)-5-(2(*S*)-pyrrolidinylmethoxy)pyridine Hydrochloride (50). Compound was synthesized via method C. Yield: 99% (yellow solid). ^{1}H NMR (D_2O): δ 8.22 (s 1H), 8.11 (s, 1H), 7.80 (s, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.34 (s, 1H), 7.20 (m, 2H), 6.67 (s, 1H), 4.28 (m, 2H), 4.13 (m, 1H), 3.44 (t, J = 6.6 Hz, 2H), 2.35 (m, 1H), 2.03 (m, 2H), 1.97 (m, 1H). ^{13}C NMR (D_2O): δ 156.9, 139.3, 134.1, 130.2, 127.9, 127.7, 126.5, 124.3, 123.1, 122.7, 122.1, 110.7, 107.5, 68.4, 58.7, 46.5, 26.3, 23.7. Purity by HPLC: 99.6%. MS (ESI, m/e) 294.2 $(M + 1)^+$; $[\alpha]_D^{22} = +14$ (c = 0.022, MeOH). Anal. Calcd for $C_{18}H_{19}N_3O \cdot 1.65HCl \cdot 1.95H_2O$: C, 55.63; H, 6.37; N, 10.81; Cl, 15.05. Found: C, 55.76; H, 6.21; N, 10.64; Cl, 15.16.

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-butylaminopyridine (57). Method A was used. Yield: 67% (colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.64 (m, 2H), 6.51−6.36 (m, 1H), 4.12 (m, 2H), 3.91−3.79 (m, 1H), 3.39 (m, 2H), 3.09 (t, J = 7.2 Hz, 2H), 2.00−1.84 (m, 4H), 1.59 (m, 2H), 1.45 (m, 12H), 0.94 (t, J = 7.2 Hz, 3H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-phenylaminopyridine (58). Method A was used. Yield: 79% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 8.01 (s, 1H), 7.81 (s, 1H), 7.27 (t, J = 7.6 Hz, 2H), 7.10 (d, J = 8.0 Hz, 2H), 6.99 (t, J = 7.6 Hz, 1H), 6.41−6.34 (m, 1H), 4.10 (m, 2H), 3.90−3.80 (m, 1H), 3.37 (m, 2H), 1.99 (m, 3H), 1.83 (m, 1H), 1.44 (s, 10H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-benzylaminopyridine (59). Method A was used. Yield: 91% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.99 (s, 1H), 7.52 (s, 1H), 7.37 (m, 5H), 6.80−6.25 (m, 1H), 4.45 (s, 2H), 4.22 (m, 1H), 4.06 (m, 1H), 3.86 (m, 1H), 3.32 (m, 3H), 1.92 (m, 4H), 1.46 (s, 1H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-diethylaminopyridine (60). Method A was used. Yield: 80% (colorless oil). ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 7.61 (s, 1H), 6.41−6.66 (m, 1H), 4.12 (m, 2H), 3.89 (m, 1H), 3.31 (m, 6H), 2.01 (m, 4H), 1.45 (s, 9H), 1.56 (t, J = 6.8 Hz, 6H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-[(*N*-ethyl-*N*-phenyl)amino]pyridine (61). Method A was used. Yield: 59% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.80 (m, 2H), 7.31 (t, J = 7.6 Hz, 2H), 7.08 (d, J = 7.6 Hz, 3H), 6.78−6.62 (m, 1H), 4.09 (m, 2H), 3.86−3.72 (m, 3H), 3.36 (m, 2H), 2.01−1.81 (m, 4H), 1.43 (s, 9H), 1.21 (t, J = 7.0 Hz, 3H).

3-[[*N*-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]-5-[(*N*-methyl-*N*-benzyl)amino]pyridine (62). Method A was used. Yield: 55% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.76 (m, 1H), 7.60 (s, 1H), 7.27−7.13 (m, 5H), 6.64−6.44 (m, 1H), 4.48 (s, 2H), 4.06 (m, 2H), 3.85−3.76 (m, 1H), 3.33 (m, 2H), 2.98 (s, 3H), 1.98−1.78 (m, 4H), 1.40 (s, 9H).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-butylaminopyridine Hydrochloride (63). Method C was used. Yield: 100% (yellow solid). ^{1}H NMR (D_2O): δ 7.70 (m, 2H), 7.20 (s, 1H), 4.52 (dd, J = 2.4, 10.4 Hz, 1H), 4.32 (m, 1H), 4.14 (m, 1H), 3.43 (t, J = 7.2 Hz, 2H), 3.21 (t, J = 6.8 Hz, 2H), 2.32 (m, 1H), 2.12 (m, 2H), 1.97 (m, 1H), 1.62 (m, 2H), 1.43 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H), ^{13}C NMR (D_2O): δ 156.9, 148.7, 119.4, 116.3, 111.1, 67.4, 58.4, 45.9, 42.4, 29.6, 25.7, 23.4, 19.4, 12.9, HPLC purity: 99.9%. MS (ESI) m/z 250.2 $(M + H)^+$; $[\alpha]_D^{22}$ = +6.0 (c = 0.050, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-phenylaminopyridine Hydrochloride (64). Method C was used. Yield: 100% (yellow solid). ^{1}H NMR (D_2O): δ 7.94 (d, J = 1.6 Hz, 1H), 7.83 (d, J = 1.6 Hz, 1H), 7.50 (s, 1H), 7.42 (t, J = 7.6 Hz, 2H), 7.23 (m, 3H), 4.46 (dd, J = 3.2, 10.4 Hz, 1H), 4.28 (dd, J = 7.6, 10.0 Hz, 1H), 4.12 (m, 1H), 3.40 (t, J = 6.8 Hz, 2H), 2.26 (m, 1H), 2.11 (m, 2H), 1.93 (m, 1H). ^{13}C NMR (D_2O): δ 156.5, 145.2, 138.4, 129.5, 124.4, 121.1, 120.8, 118.6, 113.5, 67.1, 58.0, 45.5, 25.3, 23.0. HPLC purity: 99.6%. MS (ESI) m/z 270.2 $(M + H)^+$; $[\alpha]_D^{23}$ = +9.9 (c = 0.28, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-benzylaminopyridine Hydrochloride (65). Method C was used. Yield: 99% (yellow solid). ^{1}H NMR (D_2O): δ 7.62 (m, 2H), 7.37 (m, 4H), 7.30 (m, 1H), 7.01 (s, 1H), 4.37 (m, 3H), 4.16 (m, 1H), 4.01 (m, 1H), 3.35 (t, J = 6.8 Hz, 2H), 2.21 (m, 1H), 2.05 (m, 2H), 1.86 (m, 1H). ^{13}C NMR (D_2O): δ 156.3, 147.5, 137.5, 128.8, 127.5, 127.2, 121.9, 118.8, 110.4, 67.1, 58.4, 46.1, 45.8, 25.7, 23.3. HPLC purity: 99.8%. MS (ESI) m/z 284.2 $(M + H)^+$; $[\alpha]_D^{23}$ = +8.5 (c = 0.56, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-diethylaminopyridine Hydrochloride (66). Method C was used. Yield: 74% (pale-yellow solid). ^{1}H NMR (D_2O): δ 7.76 (d, J = 2.0 Hz, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.16 (t, J = 2.0 Hz, 1H), 4.52 (dd, J = 3.2, 10.4 Hz, 1H), 4.33 (dd, J = 7.6, 10.4 Hz, 1H), 4.13 (m, 1H), 3.44 (m, 6H), 2.28 (m, 1H), 2.13 (m, 2H), 1.98 (m, 1H), 1.17 (t, J = 7.2 Hz, 6H). ^{13}C NMR (D_2O): δ 156.5, 146.4, 118.9, 114.3, 110.2, 67.0, 58.1, 45.5, 44.2, 25.4, 23.0, 10.4. HPLC purity: 99.5%. HRMS (ESI) m/z calcd for $C_{14}H_{23}N_3O$ $(M + H)^+$ 250.1919, found 250.1914; $[\alpha]_D^{23}$ = +7.2 (c = 0.14, MeOH).

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-[(*N*-ethyl-*N*-phenyl)amino]-pyridine Hydrochloride (67). Method C was used. Yield: 72% (yellow solid). ^{1}H NMR (D_2O): δ 7.69 (s, 1H), 7.54 (s, 1H), 7.41 (t, J = 7.6 Hz, 2H), 7.28 (t, J = 7.6 Hz, 1H), 7.18 (d, J = 7.6 Hz, 2H), 4.35 (dd, J = 2.8, 10.4 Hz, 1H), 4.19 (m, 1H), 4.02 (m, 1H), 3.66 (q, J = 7.2 Hz, 2H), 3.32 (t, J = 7.2 Hz, 2H), 2.15 (m, 1H), 2.03 (m, 2H), 1.85 (m, 1H), 1.06 (t, J = 7.2 Hz, 3H). ^{13}C NMR (D_2O): δ 156.3, 147.4, 142.7, 130.2, 127.3, 126.9, 120.3, 116.1, 112.3, 67.1, 58.0, 46.5, 45.5, 23.4, 23.0, 10.5. HPLC purity: 99.4%. HRMS (ESI) m/z calcd for $C_{18}H_{23}N_3O$ $(M + H)^+$ 298.1919, found 298.1920; $[\alpha]_D^{23}$ = +13 (c = 0.24, MeOH). Anal. Calcd for $C_{18}H_{23}N_3O \cdot 2.95HCl \cdot 0.05H_2O$: C, 53.27; H, 6.47; N, 10.35; Cl, 25.77. Found: C, 53.38; H, 6.62; N, 10.44; Cl, 25.67.

3-[(2(*S*)-Pyrrolidinyl)methoxy]-5-[(*N*-methyl-*N*-benzyl)amino]-pyridine Hydrochloride (68). Method C was used. Yield: 73% (pale-yellow solid). ^{1}H NMR (D_2O): δ 7.64 (m, 2H), 7.27 (m, 2H), 7.17 (m, 3H), 7.07 (s, 1H), 4.58 (s, 2H), 4.34 (dd, J = 3.2, 10.8 Hz, 1H), 4.17 (dd, J = 8.0, 10.4 Hz, 1H), 3.98 (m, 1H), 3.31 (t, J = 7.2 Hz, 2H), 3.09 (s, 3H), 2.15 (m, 1H), 2.02 (m, 2H), 1.82 (m, 1H). ^{13}C NMR (D_2O): δ 156.2, 147.8, 135.7, 128.4, 127.2, 126.1, 118.7, 115.6, 110.8, 67.0, 58.0, 54.9, 45.5, 38.5, 25.4, 23.0. HPLC purity: 99.6%. HRMS (ESI) m/z calcd for $C_{18}H_{23}N_3O$ $(M + H)^+$ 298.1919, found 298.1920; $[\alpha]_D^{23}$ = +22 (c = 0.036, MeOH). Anal. Calcd for $C_{18}H_{23}N_3O \cdot 2.25HCl \cdot 0.7H_2O$: C, 55.15; H, 6.85; N, 10.72; Cl, 20.35. Found: C, 54.91; H, 6.61; N, 10.74; Cl, 20.12.

In Vitro Studies. [^{3}H]-Epibatidine competition studies: K_i values were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, contract no. HHSN-271-2008-00025-C (NIMH PDSP). The indicated compounds were used in [^{3}H]-epibatidine binding competition assays to define their K_i values (nM) for blockade of specific binding of [^{3}H]-epibatidine to membrane fractions prepared from stably transfected cell lines (e.g., HEK293, COS, CHO, NIH3T3) ($\alpha2\beta2$-, $\alpha2\beta4$-, $\alpha3\beta2$-, $\alpha3\beta4$-, $\alpha4\beta2$-, or $\alpha4\beta4$-nAChR) or rat forebrain ($\alpha4\beta2$*-nAChR). Results are from three independent determinations. Asterisk in $\alpha4\beta2$*, means that other unidentified subunits also may be present because membrane fractions prepared from rat forebrain contain nAChR subtypes whose subunit composition has not been precisely determined, although they have features of nAChR containing $\alpha4$ and $\beta2$ subunits. For experimental details, please refer to the PDSP web site http://pdsp.med.unc.edu/

Cell Lines and Culture. Cell lines naturally or heterologously expressing specific, functional, human nAChR subtypes were used. The human clonal cell line TE671/RD naturally expresses human muscle-type $\alpha1$*-nAChR, containing $\alpha1$, $\beta1$, γ, and δ subunits, with function detectable using $^{86}Rb^+$ efflux assays.[51] The human neuroblastoma cell line SHSY5Y naturally expresses autonomic $\alpha3\beta4$*-nAChR, containing $\alpha3$, $\beta4$, probably $\alpha5$, and sometimes $\beta2$ subunits, and also displays function detectable using $^{86}Rb^+$ efflux assays.[52] SH-SY5Y cells also express homopentameric $\alpha7$-nAChR, however, their function is not detected in the $^{86}Rb^+$ efflux assay under the conditions used. SH-EP1 human epithelial cells stably transfected with human $\alpha4$ and $\beta2$ subunits (SHEP1-h$\alpha4\beta2$ cells) have been established and characterized with both ion flux and radioligand binding assays.[53]

TE671/RD, SH-SY5Y, and transfected SH-EP1-h$\alpha4\beta2$ cell lines were maintained as low passage number (1−26 from our frozen stocks) cultures to ensure stable expression of native or heterologously expressed nAChR as previously described.[51] Cells were passaged once a week by splitting just-confluent cultures 1/300 (TE671/RD), 1/10 (SH-SY5Y), or 1/40 (transfected SH-EP1-h$\alpha4\beta2$) in serum-supplemented medium to maintain log-phase growth.

$^{86}Rb^+$ Efflux Assays. Function of nAChR subtypes was investigated using an established $^{86}Rb^+$ efflux assay protocol.[51] The assay is specific for nAChR function under the conditions used, for example, giving identical results in the presence

of 100 nM atropine to exclude possible contributions of muscarinic acetylcholine receptors. Cells harvested at confluence from 100 mm plates under a stream of fresh medium only (SH-SY5Y cells) or after mild trypsinization (Irvine Scientific, USA; for TE671/RD or transfected SH-EP1 cells) were then suspended in complete medium and evenly seeded at a density of 1.25−2 confluent 100 mm plates per 24-well plate (Falcon; ∼100−125 mg of total cell protein per well in a 500 μL volume; poly L-lysine-coated for SH-SY5Y cells). After cells had adhered generally overnight, but no sooner than 4 h later, the medium was removed and replaced with 250 μL per well of complete medium supplemented with ∼350000 cpm of $^{86}Rb^+$ (NEN; counted at 40% efficiency using Cerenkov counting and the Packard TriCarb 1900 liquid scintillation analyzer). After at least 4 h and typically overnight, $^{86}Rb^+$ efflux was measured using the "flip-plate" technique.[53] Briefly, after aspiration of the bulk of $^{86}Rb^+$ loading medium from each well of the "cell plate," each well containing cells was rinsed with 2 mL of fresh $^{86}Rb^+$ efflux buffer (130 mM NaCl, 5.4 mM KCl, 2 mM $CaCl_2$, 5 mm glucose, 50 mM HEPES, pH 7.4) to remove extracellular $^{86}Rb^+$. Following removal of residual rinse buffer by aspiration, the flip-plate technique was used again to simultaneously introduce 1.5 mL of fresh efflux buffer containing drugs of choice at indicated final concentrations from a 24-well "efflux/drug plate" into the wells of the cell late. After a 10 min incubation, the solution was "flipped" back into the efflux/drug plate, and any remaining buffer in the cell plate was removed by aspiration. A second efflux/drug plate was then used to reintroduce the same concentrations of drugs of choice with the addition of an $\sim EC_{90}$ concentration of the full agonist carbamylcholine for 5 min ($\sim EC_{90}$ concentrations were 200 μM for SH-EP1-hα4β2 cells, 2 mM for SHSY5Y cells, and 464 mM for TE671/RD cells). The second drug treatment was then flipped back into its drug plate, and the remaining cells in the cell plate were lysed and suspended by addition of 1.5 mL of 0.1 M NaOH, 0.1% sodium dodecyl sulfate to each well. Suspensions in each well were then subjected to Cerenkov counting (Wallac Micobeta Trilux 1450; 25% efficiency) after placement of inserts (Wallac 1450−109) into each well to minimize cross-talk between wells.

For quality control and normalization purposes, the sum of $^{86}Rb^+$ in cell plates and efflux/drug plates was defined to confirm material balance (i.e., that the sum of $^{86}Rb^+$ released into the efflux/drug plates and $^{86}Rb^+$ remaining in the cell plate were the same for each well). Similarly, the sum of $^{86}Rb^+$ in cell plates and efflux/drug plates also determined the efficiency of $^{86}Rb^+$ loading (the percentage of applied $^{86}Rb^+$ actually loaded into cells). Furthermore, the sum of $^{86}Rb^+$ in cell plates and the second efflux/drug plates defined the amount of intracellular $^{86}Rb^+$ available at the start of the second, 5 min assay and were used to normalize nAChR function assessed.

For each experiment, in one set of control samples, total $^{86}Rb^+$ efflux was assessed in the presence of only a fully efficacious concentration of carbamylcholine (1 mM for SH-EP1-hα4β2 and TE671/RD cells, or 3 mM for SH-SY5Y cells). Nonspecific $^{86}Rb^+$ efflux in another set of control samples was measured either in the presence of the fully efficacious concentration of carbamylcholine plus 100 μM mecamylamine, which gave full block of agonist-induced and spontaneous nAChR-mediated ion flux, or in the presence of efflux buffer alone. Both determinations of nonspecific efflux were equivalent. Specific efflux was then taken as the difference in control samples between total and nonspecific $^{86}Rb^+$-efflux. The same approaches were used to define total, nonspecific, and specific ion flux responses in samples subjected to the second, 5-min, exposure to test drug with or without carbamylcholine at its $\sim EC_{90}$ concentration.

Intrinsic agonist activity of test drugs was ascertained during the initial 10 min exposure period using samples containing test drug only at different concentrations and was normalized, after subtraction of nonspecific efflux, to specific efflux in test drug-free control samples. Specific $^{86}Rb^+$ efflux elicited by test drug as a percentage of specific efflux in the absence of test drug was the same in these samples whether measured in absolute terms or as a percentage of loaded $^{86}Rb^+$. Even in samples previously giving an efflux response during the initial 10 min exposure to a partial or full agonist, residual intracellular $^{86}Rb^+$ was adequate to allow assessment of nAChR function in the secondary, 5 min assay. However, care was needed to ensure that data were normalized to the amount of intracellular $^{86}Rb^+$ available at the time of the assay, as absolute levels of total, nonspecific, or specific efflux varied in cells depleted of intracellular $^{86}Rb^+$ due to action of any agonist present during the 10 min drug exposure period. That is, calculations of specific efflux as a percentage of loaded $^{86}Rb^+$ typically corrected for any variation in the electrochemical gradient of $^{86}Rb^+$ created by intracellular ion depletion after the first (agonism/pretreatment) drug treatment.

Ion flux assays ($n \geq 3$ separate studies for each drug and cell line combination) were fit to the Hill equation, $F = F_{max}/(1 + (X/EC_{50})^n)$, where F is the percentage of control, F_{max}, for EC_{50} ($n > 0$ for agonists) or IC_{50} ($n < 0$ for antagonists) values using Prism 4 (GraphPad, San Diego, CA). In some cases, biphasic concentration−ion flux response curves were evident and were fit to a two-phase Hill equation from which EC_{50} values and Hill coefficients for the rising agonist phase, and IC_{50} values and Hill coefficients for the falling self-inhibitory phase could be determined. Most ion flux data were fit allowing maximum and minimum ion flux values to be determined by curve fitting, but in some cases, where antagonists or agonists had weak functional potency, minimum ion flux was set at 0% of control or maximum ion flux was set at 100% of control, respectively.

General Procedures for Behavioral Studies. Animals. BALB/cJ male mice (9 weeks old at testing) were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were housed four to a cage in a colony room maintained at 22 °C ± 2 on a 12 h light−dark cycle. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the PsychoGenics Animal Care and Use Committee.

Drugs. Compound **64** was synthesized according to procedures described in the text, and sertraline was purchased from Toronto Research Chemicals (Ontario, Canada). All compounds were dissolved in injectable water and administered by intraperitoneal (IP) injection in a volume of 10 mL/kg.

Mouse Forced Swim Test. Procedures were based on those previously described.[42] Mice were individually placed into clear glass cylinders (15 cm tall × 10 cm wide, 1 L beakers) containing 23 ± 1 °C water 12 cm deep (approximately 800 mL). Mice were administered vehicle, the selective serotonin reuptake inhibitor, sertraline (10 mg/kg), as a positive control or compound **64** (3, 10, or 30 mg/kg). Thirty min following IP injection, mice were placed in the water and the time the animal spent immobile was recorded over a 6 min trial. Immobility was defined as the postural position of floating in the water.

Statistical Analysis. Data were analyzed with Analysis of Variance (ANOVA) with treatment group (Vehicle, Sertraline, compound **64** (3, 10, and 30 mg/kg)) as the between-group variable and total time immobile (sec over the 6 min trial) as the dependent variable. Significant main effects were followed up with the post hoc Newman Keuls test.

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References

(1) Romanelli, M. N.; Gratteri, P.; Guandalini, L.; Martini, E.; Bonaccini, C.; Gualtieri, F. Central nicotinic receptors: structure, function, ligands, and therapeutic potential. *ChemMedChem* **2007**, *2*, 746–767.

(2) Gotti, C.; Clementi, F.; Fornari, A.; Gaimarri, A.; Guiducci, S.; Manfredi, I.; Moretti, M.; Pedrazzi, P.; Pucci, L.; Zoli, M. Structural and functional diversity of native brain neuronal nicotinic receptors. *Biochem. Pharmacol.* **2009**, *78*, 703–711.

(3) Shytle, R. D.; Silver, A. A.; Lukas, R. J.; Newman, M. B.; Sheehan, D. V.; Sanberg, P. R. Nicotinic acetylcholine receptors as targets for antidepressants. *Mol. Psychiatry* **2002**, *7*, 525–535.

(4) Fryer, J. D.; Lukas, R. J. Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 88–92.

(5) Fryer, J. D.; Lukas, R. J. Antidepressants noncompetitively inhibit nicotinic acetylcholine receptor function. *J. Neurochem.* **1999**, *72*, 1117–1124.

(6) Damaj, M. I.; Carroll, F. I.; Eaton, J. B.; Navarro, H. A.; Blough, B. E.; Mirza, S.; Lukas, R. J.; Martin, B. R. Enantioselective effects of hydroxy metabolites of bupropion on behavior and on function of monoamine transporters and nicotinic receptors. *Mol. Pharmacol.* **2004**, *66*, 675–682.

(7) Dwoskin, L. P.; Smith, A. M.; Wooters, T. E.; Zhang, Z.; Crooks, P. A.; Bardo, M. T. Nicotinic receptor-based therapeutics and candidates for smoking cessation. *Biochem. Pharmacol.* **2009**, *78*, 732–743.

(8) Carroll, F. I.; Blough, B. E.; Mascarella, S. W.; Navarro, H. A.; Eaton, J. B.; Lukas, R. J.; Damaj, M. I. Synthesis and biological evaluation of bupropion analogues as potential pharmacotherapies for smoking cessation. *J. Med. Chem.* **2010**, *53*, 2204–2214.

(9) Buckingham, S. D.; Jones, A. K.; Brown, L. A.; Sattelle, D. B. Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. *Pharmacol. Rev.* **2009**, *61*, 39–61.

(10) Quik, M.; Bordia, T.; O'Leary, K. Nicotinic receptors as CNS targets for Parkinson's disease. *Biochem. Pharmacol.* **2007**, *74*, 1224–1234.

(11) Wilens, T. E.; Decker, M. W. Neuronal nicotinic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: focus on cognition. *Biochem. Pharmacol.* **2007**, *74*, 1212–1223.

(12) Brody, A. L. Functional brain imaging of tobacco use and dependence. *J. Psychiatr. Res.* **2006**, *40*, 404–418.

(13) Bencan, Z.; Levin, E. D. The role of alpha7 and alpha4beta2 nicotinic receptors in the nicotine-induced anxiolytic effect in zebrafish. *Physiol. Behav.* **2008**, *95*, 408–412.

(14) Decker, M. W.; Meyer, M. D. Therapeutic potential of neuronal nicotinic acetylcholine receptor agonists as novel analgesics. *Biochem. Pharmacol.* **1999**, *58*, 917–923.

(15) Martin, L. F.; Freedman, R. Schizophrenia and the alpha7 nicotinic acetylcholine receptor. *Int. Rev. Neurobiol.* **2007**, *78*, 225–246.

(16) Ochoa, E. L.; Lasalde-Dominicci, J. Cognitive deficits in schizophrenia: focus on neuronal nicotinic acetylcholine receptors and smoking. *Cell Mol. Neurobiol.* **2007**, *27*, 609–639.

(17) Egleton, R. D.; Brown, K. C.; Dasgupta, P. Nicotinic acetylcholine receptors in cancer: multiple roles in proliferation and inhibition of apoptosis. *Trends Pharmacol. Sci.* **2008**, *29*, 151–158.

(18) Jensen, A. A.; Frolund, B.; Liljefors, T.; Krogsgaard-Larsen, P. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *J. Med. Chem.* **2005**, *48*, 4705–4745.

(19) Chavez-Noriega, L. E.; Crona, J. H.; Washburn, M. S.; Urrutia, A.; Elliott, K. J.; Johnson, E. C. Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors h alpha 2 beta 2, h alpha 2 beta 4, h alpha 3 beta 2, h alpha 3 beta 4, h alpha 4 beta 2, h alpha 4 beta 4 and h alpha 7 expressed in *Xenopus* oocytes. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 346–356.

(20) Steinlein, O. K.; Bertrand, D. Neuronal nicotinic acetylcholine receptors: from the genetic analysis to neurological diseases. *Biochem. Pharmacol.* **2008**, *76*, 1175–1183.

(21) Cassels, B. K.; Bermudez, I.; Dajas, F.; Abin-Carriquiry, J. A.; Wonnacott, S. From ligand design to therapeutic efficacy: the challenge for nicotinic receptor research. *Drug Discovery Today* **2005**, *10*, 1657–1665.

(22) Kanne, D. B.; Tomizawa, M.; Durkin, K. A.; Casida, J. E. 6′-Methylpyrido[3,4-*b*]norhomotropane: synthesis and outstanding potency in relation to the alpha4beta2 nicotinic receptor pharmacophore model. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 877–881.

(23) Bencherif, M.; Lovette, M. E.; Fowler, K. W.; Arrington, S.; Reeves, L.; Caldwell, W. S.; Lippiello, P. M. RJR-2403: a nicotinic agonist with CNS selectivity I. In vitro characterization. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1413–1421.

(24) Lippiello, P. M.; Bencherif, M.; Gray, J. A.; Peters, S.; Grigoryan, G.; Hodges, H.; Collins, A. C. RJR-2403: a nicotinic agonist with CNS selectivity II. In vivo characterization. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1422–1429.

(25) Sullivan, J. P.; Donnelly-Roberts, D.; Briggs, C. A.; Anderson, D. J.; Gopalakrishnan, M.; Piattoni-Kaplan, M.; Campbell, J. E.; McKenna, D. G.; Molinari, E.; Hettinger, A. M.; Garvey, D. S.; Wasicak, J. T.; Holladay, M. W.; Williams, M.; Arneric, S. P. A-85380 [3-(2(*S*)-azetidinylmethoxy) pyridine]: in vitro pharmacological properties of a novel, high affinity alpha 4 beta 2 nicotinic acetylcholine receptor ligand. *Neuropharmacology* **1996**, *35*, 725–734.

(26) Bunnelle, W. H.; Tietje, K. R.; Frost, J. M.; Peters, D.; Ji, J.; Li, T.; Scanio, M. J.; Shi, L.; Anderson, D. J.; Dyhring, T.; Gronlien, J. H.; Ween, H.; Thorin-Hagene, K.; Meyer, M. D. Octahydropyrrolo[3,4-*c*]pyrrole: a diamine scaffold for construction of either alpha4beta2 or alpha7-selective nicotinic acetylcholine receptor (nAChR) ligands. Substitutions that switch subtype selectivity. *J. Med. Chem.* **2009**, *52*, 4126–4141.

(27) Lloyd, G. K.; Williams, M. Neuronal nicotinic acetylcholine receptors as novel drug targets. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 461–467.

(28) Davis, J. B.; Bountra, C.; Richardson, J. Perspectives of Alzheimer's disease treatments. *Handb. Clin. Neurol.* **2008**, *89*, 273–290.

(29) Guandalini, L.; Martini, E.; Dei, S.; Manetti, D.; Scapecchi, S.; Teodori, E.; Romanelli, M. N.; Varani, K.; Greco, G.; Spadola, L.; Novellino, E. Design of novel nicotinic ligands through 3D database searching. *Bioorg. Med. Chem.* **2005**, *13*, 799–807.

(30) Guandalini, L.; Martini, E.; Gratteri, P.; Ghelardini, C.; Varani, K.; Romanelli, M. N. Design, synthesis and binding affinity of new nicotinic ligands. *ARKIVOC* **2006**, *VIII*, 50–65.

(31) Meyer, M. D. Neuronal nicotinic acetylcholine receptors as a target for the treatment of neuropathic pain. *Drug Dev. Res.* **2006**, *67*, 355–359.

(32) Rueter, L. E.; Anderson, D. J.; Briggs, C. A.; Donnelly-Roberts, D. L.; Gintant, G. A.; Gopalakrishnan, M.; Lin, N. H.; Osinski, M. A.; Reinhart, G. A.; Buckley, M. J.; Martin, R. L.; McDermott, J. S.; Preusser, L. C.; Seifert, T. R.; Su, Z.; Cox, B. F.; Decker, M. W.; Sullivan, J. P. ABT-089: pharmacological properties of a neuronal nicotinic acetylcholine receptor agonist for the potential treatment of cognitive disorders. *CNS Drug Rev.* **2004**, *10*, 167–182.

(33) Cosford, N. D.; Bleicher, L.; Herbaut, A.; McCallum, J. S.; Vernier, J. M.; Dawson, H.; Whitten, J. P.; Adams, P.; Chavez-Noriega, L.; Correa, L. D.; Crona, J. H.; Mahaffy, L. S.; Menzaghi, F.; Rao, T. S.; Reid, R.; Sacaan, A. I.; Santori, E.; Stauderman, K. A.; Whelan, K.; Lloyd, G. K.; McDonald, I. A. (*S*)-(−)-5-Ethynyl-3-(1-methyl-2-pyrrolidinyl)pyridine maleate (SIB-1508Y): a novel anti-Parkinsonian agent with selectivity for neuronal nicotinic acetylcholine receptors. *J. Med. Chem.* **1996**, *39*, 3235–3237.

(34) Xiao, Y.; Fan, H.; Musachio, J. L.; Wei, Z. L.; Chellappan, S. K.; Kozikowski, A. P.; Kellar, K. J. Sazetidine-A, a novel ligand that desensitizes alpha4beta2 nicotinic acetylcholine receptors without activating them. *Mol. Pharmacol.* **2006**, *70*, 1454–1460.

(35) Zwart, R.; Carbone, A. L.; Moroni, M.; Bermudez, I.; Mogg, A. J.; Folly, E. A.; Broad, L. M.; Williams, A. C.; Zhang, D.; Ding, C.; Heinz, B. A.; Sher, E. Sazetidine-A is a potent and selective agonist at native and recombinant alpha 4 beta 2 nicotinic acetylcholine receptors. *Mol. Pharmacol.* **2008**, *73*, 1838–1843.

(36) Kozikowski, A. P.; Eaton, J. B.; Bajjuri, K. M.; Chellappan, S. K.; Chen, Y.; Karadi, S.; He, R.; Caldarone, B.; Manzano, M.; Yuen, P. W.; Lukas, R. J. Chemistry and pharmacology of nicotinic ligands based on 6-[5-(azetidin-2-ylmethoxy)pyridin-3-yl]hex-5-yn-1-ol (AMOP-H-OH) for possible use in depression. *ChemMedChem* **2009**, *4*, 1279–1291.

(37) Gatto, G. J.; Bohme, G. A.; Caldwell, W. S.; Letchworth, S. R.; Traina, V. M.; Obinu, M. C.; Laville, M.; Reibaud, M.; Pradier, L.; Dunbar, G.; Bencherif, M. TC-1734: an orally active neuronal nicotinic acetylcholine receptor modulator with antidepressant, neuroprotective and long-lasting cognitive effects. *CNS Drug Rev.* **2004**, *10*, 147–166.

(38) Wei, Z. L.; Xiao, Y.; Yuan, H.; Baydyuk, M.; Petukhov, P. A.; Musachio, J. L.; Kellar, K. J.; Kozikowski, A. P. Novel pyridyl ring

C5 substituted analogues of epibatidine and 3-(1-methyl-2(*S*)-pyrrolidinylmethoxy)pyridine (A-84543) as highly selective agents for neuronal nicotinic acetylcholine receptors containing beta2 subunits. *J. Med. Chem.* **2005**, *48*, 1721–1724.

(39) Ortiz de Montellano, P. R.; Kunze, K. L. Self-catalyzed inactivation of hepatic cytochrome P-450 by ethynyl substrates. *J. Biol. Chem.* **1980**, *255*, 5578–5585.

(40) Lin, N. H.; Gunn, D. E.; Li, Y.; He, Y.; Bai, H.; Ryther, K. B.; Kuntzweiler, T.; Donnelly-Roberts, D. L.; Anderson, D. J.; Campbell, J. E.; Sullivan, J. P.; Arneric, S. P.; Holladay, M. W. Synthesis and structure−activity relationships of pyridine-modified analogs of 3-[2-((*S*)-pyrrolidinyl)methoxy]pyridine, A-84543, a potent nicotinic acetylcholine receptor agonist. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 249–254.

(41) Holladay, M. W.; Bai, H.; Li, Y.; Lin, N. H.; Daanen, J. F.; Ryther, K. B.; Wasicak, J. T.; Kincaid, J. F.; He, Y.; Hettinger, A. M.; Huang, P.; Anderson, D. J.; Bannon, A. W.; Buckley, M. J.; Campbell, J. E.; Donnelly-Roberts, D. L.; Gunther, K. L.; Kim, D. J.; Kuntzweiler, T. A.; Sullivan, J. P.; Decker, M. W.; Arneric, S. P. Structure−activity studies related to ABT-594, a potent nonopioid analgesic agent: effect of pyridine and azetidine ring substitutions on nicotinic acetylcholine receptor binding affinity and analgesic activity in mice. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2797–2802.

(42) Porsolt, R. D.; Bertin, A.; Jalfre, M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* **1977**, *229*, 327–336.

(43) Wolfe, J. P.; Wagaw, S.; Marcoux, J. F.; Buchwald, S. L. Rational development of practical catalysts for aromatic carbon−nitrogen bond formation. *Acc. Chem. Res.* **1998**, *31*, 805–818.

(44) Surry, D. S.; Buchwald, S. L. Biaryl phosphane ligands in palladium-catalyzed amination. *Angew. Chem., Int. Ed. Engl.* **2008**, *47*, 6338–6361.

(45) Nicolazzo, J. A.; Charman, S. A.; Charman, W. N. Methods to assess drug permeability across the blood−brain barrier. *J. Pharm. Pharmacol.* **2006**, *58*, 281–293.

(46) Fan, Y.; Unwalla, R.; Denny, R. A.; Di, L.; Kerns, E. H.; Diller, D. J.; Humblet, C. Insights for predicting blood−brain barrier penetration of CNS targeted molecules using QSPR approaches. *J. Chem. Inf. Model.* **2010**, *50*, 1123–1133.

(47) Mavandadi, F.; Pilotti, A. The impact of microwave-assisted organic synthesis in drug discovery. *Drug Discovery Today* **2006**, *11*, 165–174.

(48) Forman, S. A.; Firestone, L. L.; Miller, K. W. Is agonist self-inhibition at the nicotinic acetylcholine receptor a nonspecific action? *Biochemistry* **1987**, *26*, 2807–2814.

(49) Arias, H. R. Agonist self-inhibitory binding site of the nicotinic acetylcholine receptor. *J. Neurosci. Res.* **1996**, *44*, 97–105.

(50) Buckley, M. J.; Surowy, C.; Meyer, M.; Curzon, P. Mechanism of action of A-85380 in an animal model of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2004**, *28*, 723–730.

(51) Lukas, R. J. Fryer, J. D.; Eaton, J. B.; Gentry, C. L. Some methods for studies of nicotinic acetylcholine receptor pharmacology. In *Nicotinic Receptors and the Nervous System*; Levin, E. D., Ed.; CRC Press: Boca Raton, FL, **2002**; pp 3−27.

(52) Lukas, R. J.; Norman, S. A.; Lucero, L. Characterization of Nicotinic Acetylcholine Receptors Expressed by Cells of the SH-SY5Y Human Neuroblastoma Clonal Line. *Mol. Cell Neurosci.* **1993**, *4*, 1–12.

(53) Eaton, J. B.; Peng, J. H.; Schroeder, K. M.; George, A. A.; Fryer, J. D.; Krishnan, C.; Buhlman, L.; Kuo, Y. P.; Steinlein, O.; Lukas, R. J. Characterization of human alpha 4 beta 2-nicotinic acetylcholine receptors stably and heterologously expressed in native nicotinic receptor-null SH-EP1 human epithelial cells. *Mol. Pharmacol.* **2003**, *64*, 1283–1294.