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Chemistry and Behavioral Studies Identify Chiral Cyclopropanes as Selective α4β2-Nicotinic Acetylcholine Receptor Partial Agonists Exhibiting an Antidepressant Profile

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ABSTRACT: Despite their discovery in the early 20th century and intensive study over the last 20 years, nicotinic acetylcholine receptors (nAChRs) are still far from being well understood. Only a few chemical entities targeting nAChRs are currently undergoing clinical trials, and even fewer have reached the marketplace. In our efforts to discover novel and truly selective nAChR ligands, we designed and synthesized a series of chiral cyclopropane-containing α4β2-specific ligands that display low nanomolar binding affinities and excellent subtype selectivity while acting as partial agonists at α4β2—nAChRs. Their favorable antidepressant-like properties were demonstrated in the classical mouse forced swim test. Preliminary ADMET studies and broad screening toward other common neurotransmitter receptors were also carried out to further evaluate their safety profile and eliminate their potential off-target activity. These highly potent cyclopropane ligands possess superior subtype selectivity compared to other α4β2—nAChR agonists reported to date, including the marketed drug varenicline, and therefore may fully satisfy the crucial prerequisite for avoiding adverse side effects. These novel chemical entities could potentially be advanced to the clinic as new drug candidates for treating depression.

INTRODUCTION

Over the past two decades, nicotinic acetylcholine receptors (nAChRs) have been investigated with the goal of developing drugs that can potentially treat a variety of nervous system disorders such as Alzheimer’s disease, Parkinson’s disease, schizophrenia, pathological pain, nicotine addiction, and depression.1−4 In vertebrates, nAChRs are pentameric ligand-gated ion channel proteins that are composed of 17 known homologous subunits (α1−α10, β1−β4, γ, δ, and ε) that are expressed widely throughout the central and peripheral nervous systems (CNS and PNS) and neuromuscular junctions. They broadly participate in physiological and pathophysiological processes by modulating the synaptic release of neurotransmitters such as dopamine (DA), serotonin (5-HT), glutamate (Glu), acetylcholine (ACh), and γ-aminobutyric acid (GABA) that are all involved in the aforementioned diseases.

There are 12 nAChR subunits expressed in the nervous system (α2−α10 and β2−β4), and different combinations of subunits allow the assembly of many functional pentamers although the actual number of functional pentamers expressed is far less than the theoretical number of possible combinations. The predominant form of nAChRs in the CNS are heteromeric α4β2*—nAChR complexes characterized by high-affinity ACh binding and slow desensitization (the asterisk denotes the possible integration of other subunits into the pentamer). Homeric α7—nAChRs, which are typified by low ACh affinity and fast activation, are the other major component in the brain. Ganglionic α3β4* nAChRs play a dominant role in the sensory and autonomic ganglia as well as in subpopulations of neurons in the brain and are frequently associated with adverse side
effects such as emesis and nausea. Less abundant in the brain overall, but nevertheless concentrated in dopaminergic, pleasure—reward centers putatively involved in mood and drug dependence, are α6−nAChRs.

It is now well established that the α4/β2−nAChRs have an essential role in mediating nicotine’s rewarding properties, and it is hypothesized that they are also responsible for the antidepressant effects of nicotinic agents. This notion is supported by the findings that knockout mice lacking the nAChR β2 subunit do not show any behavioral antidepressant response to mecamylamine or amitriptyline and that nAChR α4 subunit knock-in mice exhibit increased anxiety. Furthermore, social defeat, a behavioral model of depression in rodents, produced a robust increase in the expression of the nAChR β2 subunits in the brain. In addition, nicotinic ligands targeting α4/β2−nAChRs may likewise be used to treat neuropathic pain or attention deficit hyperactivity disorder (ADHD). Because there is a great deal of conservation between the primary structures of the nAChR subtypes, the design of ligands selective for α4/β2−nAChRs over α3/β4−nAChRs provides a challenge but not one that is insurmountable.

Among the natural nicotinic ligands and a number of synthetic small molecules that have been pharmacologically tested as agents targeting brain α4/β2−nAChRs, only a small fraction have been advanced to preclinical studies and even fewer have made it to clinical trials. Abbott Laboratories developed ABT-089 (1), an α4/β2−nAChR partial agonist that recently underwent clinical trials for the treatment of pediatric ADHD. Whereas this drug was found to be safe and well tolerated, it showed no significant difference from placebo in terms of efficacy. Another compound, TC-5214 (2) from Targacept, the S-enantiomer of mecamylamine, is now in phase III development as an adjunctive therapy for major depressive disorder (MDD). Pfizer’s varenicline (3), an α4/β2−nAChR partial agonist and a full agonist at α7− and α3/β4−nAChRs, is at present the most successful synthetic small molecule on the market for smoking cessation pharmacotherapy (Figure 1).

The emergence of compound 3 lends support to the use of α4/β2−nAChR partial agonists as clinical drugs to treat nervous system diseases. However, peripheral and central side effects of compound 3, such as nausea, gastrointestinal symptoms, changes in mood, and, perhaps, suicidal ideation are most likely due to its insufficient subtype selectivity, indicating that the nicotinic arena is still rife with both opportunities and challenges.

### RATIONAL DESIGN AND SYNTHESIS OF CHIRAL CYCLOPROPANE NACHR LIGANDS

There is still a need for antidepressants that exhibit fewer side effects, act pharmacologically in new ways, and that have a faster onset of action compared to currently available therapeutics. In pursuit of this goal, our group has identified sazetidine-A (4) as a highly potent α4/β2−nAChR partial agonist with excellent selectivity over α3/β4−nAChRs. Compound 4 has been shown to possess extremely promising antidepressant and anxiolytic effects in rodent studies, including nicotine-like effects in drug discrimination studies. In addition, analgesic effects of compound 4, without any neurological side effects, have been reported using the rat formalin model. However, the potential metabolic liability of the acetylenic bond in compound 4, which may be oxidized to generate a labile, highly reactive oxirene, thereby possibly giving rise to toxicity, discouraged further advancement of this compound down the drug discovery pipeline. Novel ligands were, therefore, designed to avoid the acetylene function while maintaining the important pharmacophoric elements of compound 4. For various reasons, we considered replacement of the acetylene by a small and rigid cyclopropane ring. Cyclopropanes widely occur in both natural products and synthetic, biologically active compounds. A cyclopropane ring in place of the acetylene group would not only function as a spacer but also might be directly involved in the ligand—receptor binding interaction. The rigid structure of the cyclopropanes could also be used to modify the areas of space accessible to the side chain hydroxyl, with the goal to optimize its hydrogen bonding interactions. In our previous studies on analogues of compound 4, we found that a side chain length of between 4 and 6 carbon atoms was optimal for biological activity. As our first goal, we chose to synthesize a cyclopropane ligand bearing a four-carbon side chain counting along the shortest path from the pyridine ring to the hydroxyl group.

The syntheses of the chiral cyclopropane ligands 12a, 13a−17a, and 19a are described in Scheme 1. 3,5-Dibromopyridine underwent Br displacement with benzyl alcohol, followed by a Heck reaction with n-butylicrylate using a recently described, phosphine-free protocol to afford the α,β-unsaturated ester 6. Conversion of the ester group to the Weinreb amide using a standard procedure and subsequent Corey–Chaykovsky cyclopropanation gave the racemic mixture of cyclopropanes 7, which were then reduced to the corresponding alcohols in two steps, followed by chiral resolution on a ChiralPak AD column to give alcohols 8a and 8b in gram quantities with essentially 100% ee values. The absolute configuration of the alcohol 8a was determined by the X-ray crystallography of its derivative 9a, which was obtained by subsequent oxidation and coupling with a chiral Evans oxazolidinone.

The optically pure alcohol 8a was subjected to standard Swern oxidation, Wittig reaction, and hydrogenation to obtain the chain-extended terminal alcohol 10a. Successive acylation of the alcohol, removal of the benzyl group, and Mitsunobu reaction to install the azetidine moiety furnished the intermediate 11a after removal of the isobutyrate group. The intermediate 11a was then converted to a carbonate using various amines or phenyl isocyanate. Removal of the Boc group from 11a or the carbonate intermediates gave the desired products 12a and 13a−17a. The methyl ether analogue 19a was prepared by a similar procedure in which the methoxy group was introduced as a substitute in the Wittig reagent, and unsaturation removed by catalytic hydrogenation. Compounds
IN VITRO RADIOLIGAND BINDING AND FUNCTIONAL STUDIES

The Ki values of all the synthesized cyclopropane compounds were evaluated by [3H]epibatidine binding competition assays at seven heterologously expressed rat nAChR subtypes. As shown in Table 1, compound 12a with a (1S,2R)-configured cyclopropane ring exhibited subnanomolar binding affinity for both the α4β2− (Ki = 0.1 nM) and α4β2*− (Ki = 0.5 nM) nAChRs, thus having Ki values similar to those found for compound 4. Compound 12a was approximately 7-fold more potent than its diastereoisomer 12b at both the α4β2− (Ki = 0.6 vs 0.1 nM) and α4β2*−nAChR subtype (Ki = 6.2 vs 0.3 nM). All four of these compounds demonstrated good selectivity for nAChRs containing β2 subunits ([αβ2−, α3β2−, α4β2−, and α4β2*−nAChRs]), which are associated with the regulation of dopamine release in the nucleus accumbens. Over nAChRs containing β4 subunits ([α3β4−, α2β4−, and αβ4−nAChRs]). Of considerable importance in the identification of therapeutically useful nictinic ligands is the selectivity away from the α3β4−nAChR subtype. The α3β4−/ α4β2−nAChR Ki ratios of the two cyclopropane analogues featuring the (1S,2R)-configuration, 12a and 19a, were 65200 and 100000, respectively, which are much higher than that of nicotine (α3β4−/ α4β2−nAChR Ki = 53) or even compound 4 (α3β4−/ α4β2−nAChR Ki = 24000). Collectively, these outcomes clearly suggest that the (1S,2R)-configuration of the cyclopropane ring improves subtype selectivity by conferring the proper orientation to the side chain, thereby improving upon the compounds’ affinity for the α4β2−nAChR.

Next, we derivatized the hydroxyl group with a variety of carbamate groups, a common functional group in medicinal chemistry that has been successfully employed in the design of other selective α4β2−nAChR ligands, in our efforts to further optimize the side chain. The carbamate analogues 13a−17a, which contain the preferred (1S,2R)-configured cyclopropane ring, also exhibited subnanomolar to low nanomolar binding affinities at both the α4β2− and α4β2*−nAChRs (Table 1). Their binding preference for the β2- over the β4-containing group was enhanced by up to 1500-fold compared to nicotine and the diastereoisomers of 12a and 19a, were synthesized from 8b by the same sequence of steps.

Table 1. Binding Affinities of Cyclopropane Ligands, Nicotine, and Sazetidine-A at Seven nAChR Subtypes

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<tr>
<th>compd</th>
<th>α2β2</th>
<th>α2β4</th>
<th>α3β2</th>
<th>α3β4</th>
<th>α4β2</th>
<th>α4β2*</th>
<th>α4β4</th>
<th>selectivity (α3β4/α4β2)</th>
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<td>249</td>
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<td>441</td>
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“See Experimental Section. aα4β2*, prepared from rat forebrain. SEM values are not provided for Ki values >100 nM. bThe binding data for nicotine are from the PDSP Assay Protocol Book (http://pdsp.med.unc.edu/). cThe binding data for compound 4 were obtained from Reference 16.
nAChRs was consistent with that of the corresponding alcohols and ethers. For both the α3β2− and α4β2− nAChRs, binding affinities gradually decreased as the size of substituents at the carbamate nitrogen increased.

Additionally, radioligand competition binding assays revealed the cyclopropane ligands tested (12a−17a, 19a, 12b, and 19b) to have very low affinity for α7−nAChRs. Ten μM concentrations of test ligand inhibited binding of 10 nM 3H-epibatidine by a maximum of 30% (16a) with other compounds showing less inhibition or no inhibition of radioligand binding at all (data not shown).

For functional studies, all compounds were tested in 86Rb+ ion flux assays using SH-EP1-human β2 cells, which heterologously and stably express human α4β2−nAChRs assembled from individual subunits.33,34 SH-SYSY and TE671/RD cells were used to assess activities of tested compounds at human α3β4− or α1β1γδ−nAChRs, respectively.35,36 All of the cyclopropane ligands had agonist activity at α4β2−nAChRs with EC50 values <50 nM (Table 2). Consistent with the radioligand binding studies, compounds 12a−19a bearing the (1S,2R)-configured cyclopropane ring exhibited about 3-fold higher potencies than their diastereoisomers 12b and 19b (EC50: 10.2 vs 34.6 nM; 17.5 vs 43.1 nM). The functional agonism in the carbamate series tended to decline stepwise with the increasing size of the terminal substituents of the side chain. All compounds were found to functionally inactivate the response of the α4β2−nAChRs to a full agonist at IC50 values similar to the agonism EC50 values (Table 2). All tested ligands had neither agonist nor antagonist activity at ganglionic α3β4− or muscle-type α1β1γδ−nAChRs even at the highest concentration (10 μM) tested.

In the functional agonism studies, the efficacies of the tested compounds were determined in a mixed population of high sensitivity (HS) and low sensitivity (LS) αβ2−nAChRs. The efficacy values at the HS α4β2−nAChRs were extrapolated using compound 4 defined as a full agonist at the HS α4β2−nAChR with 100% efficacy (see Supporting Information for more details).20 All of the tested ligands were found to be partial agonists at HS α4β2−nAChRs with efficacy values ranging from 60 to 92%.

### IN VIVO BEHAVIORAL PHARMACOLOGY

To assess the antidepressant effects of selected compounds in vivo, we used 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. Mice were administered the most potent compounds 12a, 13a, and 19a, or the selective serotonin reuptake inhibitor, sertraline, as a positive control (20 mg/kg) (Figure 2).

All of the three tested compounds exhibited antidepressant-like effects at the minimal dose of 10 mg/kg (compound 13a) or 3 mg/kg (compounds 12a and 19a). Receptor occupancy (RO) studies were also performed to quantify the relationship between drug concentration at the receptor and the observed antidepressant effects.38 When tested at a dose of 10 mg/kg, both the compounds 12a and 19a showed very high levels of ex vivo receptor occupancy (85−95%) at the β2* receptors, whereas the carbamate analogue 13a showed only approximately 65% occupancy (Figure 3). These RO findings are

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“See Experimental Section. *The efficacies were measured in a mixture of HS and LS αβ2−nAChRs. The efficacy values were extrapolated using compound 4 defined as a full agonist at the HS αβ2−nAChR (see Supporting Information for details). Results for nicotine and compound 4 were obtained from Reference 17.

Figure 2. Mouse forced swim data for compounds 12a (A), 13a (B), and 19a (C). The selective serotonin reuptake inhibitor, sertraline, produced the expected decrease in immobility. (ANOVA: F (3,35) = 13.43, p < 0.001 (A); F (3,36) = 11.46, p < 0.001 (B); F (4,44) = 9.29, p < 0.001 (C). *Fisher’s PLSD posthoc test: ps < 0.05 vs vehicle). All drugs were injected intraperitoneally; n = 4−6/group.

Figure 3. Receptor occupancy studies of compounds 12a, 13a, and 19a in mice showed a significant occupancy level. (*Mann−Whitney U: p < 0.05). All drugs were administered orally; n = 9−10/group.
consistent with their observed antidepressant potencies, with compounds 12a and 19a being more potent and compound 13a being less potent (Figure 2).

**BROAD SCREENING AND PRELIMINARY ADMET STUDIES**

Apart from assessment of ligand interactions with nAChRs, a broad-ranging screen was carried out for compounds 12a, 13a, and 19a to determine their off-target binding at 53 other neurotransmitter receptors and transporters that are widely distributed throughout the CNS. The PDSP broad screening studies indicated that none of the three tested compounds showed significant interactions with other neurotransmitter receptors and transporters (see Supporting Information for more details).

Compounds 12a, 13a, and 19a were further tested in preliminary ADMET assays. When incubated with human or mouse liver microsomes, at least 80% of compound 12a, 98% of compound 13a, or 84% of compound 19a remained unchanged after 1 h incubation at 1 μM. In the presence of compounds 12a, 13a, or 19a at concentrations up to 10 μM, none of the CYP isoforms tested (CYP1A1, CYP2C9, CYP2C19, CYP2D6, and CYP3A) showed more than 25% inhibition, indicating minimal adverse drug–drug interactions, with the exception of compound 13a and 19a, which displayed about 80% and 70% inhibition of the CYP1A2 isofrom, respectively. The plasma protein binding of compound 13a was investigated using human and mouse plasma (CD-1) at 10 μM. A mean percent binding of 8.4 and 23.8, respectively, was observed. Lastly, automated patch-clamp electrophysiology was employed to measure the inhibitory interactions between the test compounds (12a, 13a, and 19a) and hERG K⁺ channels using CHO cell lines in three test concentrations (0.1, 1, and 10 μM). At the highest concentration, compounds 12a, 13a, and 19a exhibited 19.1%, 16.6%, and 13.7% inhibition of tail current, respectively, indicating minimal potential for hERG-related cardiovasucular toxicity.

To further explore the metabolic stability of these cyclopropane ligands, compound 19a was selected for full mouse in vivo pharmacokinetic (PK) studies. The plasma and brain concentrations of compound 19a in male CD-1 mice after a single oral gavage administration at a dose of 5 mg/kg were measured. Compound 19a possessed a reasonable half-life in brain (t½ = 150 min) as well as in plasma (t½ = 144 min). The concentration of compound 19a reached a value of 133 ng/mL (C_{max, brain}) in 30 min (T_{max, brain}) in brain, and of 359 ng/mL (C_{max, plasma}) in 10 min (T_{max, plasma}) in plasma. The brain to plasma ratio of compound 19a was found to be 0.37 (C_{max, brain}/C_{max, plasma}), indicating acceptable CNS penetration. In contrast, brain exposure levels of compound 4 were relatively low (~3 ng/g at 1 mg/kg or ~10 ng/g at 3 mg/kg) when measured 15 min after administration and were at or below detection level at later time points.39

**CONCLUSION**

In summary, a series of chiral cyclopropane analogues of the lead structure, compound 4, were identified as highly potent, α4β2-selective nAChR partial agonists. To avoid possible issues relating to the metabolic instability of the acetylene bond, a rigid cyclopropane ring was introduced in its place. The cyclopropane ring is also virtuous because of its ability to direct the orientation of the side chain in a manner that improves subtype selectivity for α4β2-nAChRs. The best compounds, 12a, 13a, and 19a, exhibited subnanomolar to low-nanomolar binding affinity for both α4β2- and α4β2*–nAChRs with negligible interaction with α3β4–nAChRs. In functional studies, these ligands acted as highly potent, partial agonists at HS α4β2–nAChRs and were totally inactive at both gangli- onic α3β4*– or muscle-type α1β1γ6–nAChRs. Compounds 12a, 13a, and 19a were found to display antidepressant-like properties in the mouse forced swim test, associated with high levels of β2* receptor occupancy. Furthermore, our findings that these three compounds lack any significant off-target activities and show favorable ADMET profiles commend these chiral cyclopropane ligands as potential drug candidates for the treatment of depression.

**EXPERIMENTAL SECTION**

**General.** All chemicals were purchased from Sigma-Aldrich or Chem-Impex, and solvents were used as obtained from Fisher Scientific or Sigma-Aldrich without further purification. Anhydrous THF and CH₂Cl₂ were obtained by distillation over sodium wire or CaH₂, respectively. All nonaqueous reactions were run under an argon atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. The progress of reactions was monitored by TLC on SiO₂. Spots were visualized by their quenching of the fluorescence of an indicator admixed to the SiO₂ layer or by dipping into I₂/SiO₂ mixture. Products were purified by column chromatography on 230–400 mesh SiO₂. Proton and carbon NMR spectra were recorded at spectrometer frequencies of 400 and 100 MHz, respectively. NMR chemical shifts were reported in δ (ppm) using the δ 7.26 signal of CHCl₃ (δ H NMR), the δ 4.80 signal of HDO (δ D NMR), and the δ 77.23 signal of CDCl₃ (δ C NMR) as internal standards. ¹³C NMR spectra in D₂O were not adjusted. Optical rotation was detected on an Autopol IV automatic polarimeter. Mass spectra were measured at the ESI mode at an ionization potential of 70 eV with an LC-MS MSD (Hewlett-Packard). The final compounds were purified by preparative HPLC, which was carried out on an ACE 5 AQ column (150 mm × 20 mm), with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector; flow rate = 17.0 ml/min; gradient of 0–50% methanol in water (both containing 0.05 vol% of CF₃COOH) in 30 min. Purities of final compounds (≥98%) were established by both elemental analysis and by analytical HPLC, which was carried out on an Agilent 1100 HPLC system with a Symmetry 4 μm Hydro-RP 80A column, with detection at 254 or 280 nm on a variable wavelength detector G1314A; flow rate = 1.4 ml/min; gradient of 0–100% methanol in water (both containing 0.05 vol% of CF₃COOH) in 18 min. See Supporting Information for detailed experimental procedures and NMR spectral data (H and ¹³C) of all intermediates.

**3-[(2S)-Azetidinyl]methoxy]-5-[(15R,2R)-2-(2-hydroxyethyl)-cyclopropyl]pyridinetrifluoroacetaote (12a).** ¹H NMR (D₂O): δ 8.33 (s, 1H), 8.23 (s, 1H), 7.85 (s, 1H), 4.98 (m, 1H), 4.53 (d, J = 4.0 Hz, 2H), 4.17–4.07 (m, 2H), 3.71 (t, J = 6.4 Hz, 2H), 2.70 (q, J = 8.4 Hz, 2H), 1.96 (m, 1H), 1.68 (q, J = 6.8 Hz, 2H), 1.33 (m, 1H), 1.20–1.14 (m, 2H). ¹³C NMR (D₂O): δ 162.3 (TFA), 155.8, 146.3, 132.0, 128.0, 125.3, 115.9 (TFA), 67.1, 60.8, 58.2, 43.3, 35.1, 21.9, 19.8, 19.4, 16.3. [α]D²⁰ = +36.5 (c 0.40, MeOH). Anal. Calcld for C₂₉H₂₅N₂O₂·2HClO₂·COOH·0.5H₂O: C, 43.74; H, 4.64; F, 24.39; N, 5.57. Found: C, 43.55; H, 4.42; F, 24.38; N, 5.52.

**3-[(2S)-Azetidinyl]methoxy]-5-[(1R,2S)-2-(2-hydroxyethyl)-cyclopropyl]pyridinetrifluoroacetate (12b).** ¹H NMR (400 MHz, D₂O): δ 8.32 (s, 1H), 8.22 (s, 1H), 7.84 (s, 1H), 4.97 (m, 1H), 4.52 (d, J = 4.0 Hz, 2H), 4.16–4.06 (m, 2H), 3.70 (t, J = 6.4 Hz, 2H), 2.69 (q, J = 8.4 Hz, 2H), 1.97 (m, 1H), 1.67 (q, J = 6.8 Hz, 2H), 1.32 (m, 1H), 1.17–1.12 (m, 2H). ¹³C NMR (100 MHz, D₂O): δ 162.3 (TFA), 155.8, 146.3, 132.0, 127.9, 125.3, 115.9 (TFA), 67.1, 60.8, 58.3, 43.3, 35.1, 21.9, 19.8, 19.4, 16.3. [α]D²⁰ = −49.4 (c 0.17, MeOH). Anal. Calcld for C₂₉H₂₅N₂O₂·2CF₃COOH·0.15H₂O: C, 45.13; H, 4.69; F, 23.79; N, 5.85. Found: C, 45.10; H, 4.67; F, 23.90; N, 5.84.
3-((2S)-Azetidinyl)methoxy)-5-[[1S,2R]-2-[2-N-methylcarbamoyl]ethylylcyclopropyl]pyridine Trifluoroacetate (13a). H NMR (D2O): δ 8.35 (s, 1H), 8.23 (s, 1H), 7.84 (s, 1H), 4.98 (m, 1H), 4.52 (d, J = 3.6 Hz, 2H), 2.47–2.05 (m, 4H), 2.35 (m, 1H), 1.99 (m, 1H), 1.87 (m, 1H), 1.65 (m, 1H), 1.34 (m, 1H), 1.16 (m, 2H). 13C NMR (D2O): δ 162.3 (TFA), 159.2, 156.2, 146.5, 132.3, 128.2, 125.7, 115.9 (TFA), 67.5, 64.8, 58.6, 43.6, 32.6, 26.5, 22.3, 20.2, 19.8, 16.4. [α]D 20 = +43.3 (c 0.18, MeOH). Anal. Calc for C18H19N3O3·2H2O: C, 48.19; H, 4.66; F, 19.43; N, 6.66. Found: C, 48.16; H, 4.51; F, 19.48; N, 6.57.

13a.

13b.

13c.

13d.

13e.

13f.

13g.

13h.

13i.

13j.

13k.

13l.

13m.

13n.

13o.

13p.

13q.

13r.

13s.

13t.

13u.

13v.

13w.

13x.

13y.

13z.

In Vivo Studies. [3H]Epibatidine competition experiments: For experimental details, please refer to the PDPD Web site http://pdpd.med.unc.edu/ for all nAChR subtypes except α7. For assay at α7, membrane preparations from SH-EPI cells heterologously expressing human α7 nAChRs were used to test 10 μM concentrations of test ligands in competition with 10 nM [3H]epibatidine with an experimental protocol similar to that utilized by the PDPD. 3,34

Cell Lines and Culture. Cell lines naturally or heterologously expressing specific, functional, human nAChR subtypes were used. The human clonal cell line TE671/HD naturally expresses human muscle-type α14–nAChRs, containing α1, β2, γ, and δ subunits, with function detectable using 86Rb+ efflux assays.26 The human neuroblastoma cell line SH-SY5Y naturally expresses autonomic α3β4–nAChRs, containing α3, β4, probably α5, and sometimes β2 subunits, and also displays function detectable using 86Rb+ efflux assays.22 SH-SY5Y cells also express homopentameric α7–nAChRs; however, their function is not detected in the 86Rb+ efflux assay under the conditions used. SH-EPI human epithelial cells stably transfected with cDNAs separately encoding human α4 or β2 subunits (SH-EPI-hα4β2 cells) have been established and characterized with both ion flux and radioligand binding assays.34 These cell lines thus express a mixture of so-called “high sensitivity” (HS) α4β2–nAChRs, having the presumed subunit ratios of 2.3 α4β2 and comparatively high sensitivity to nicotinic agonists, and “low sensitivity” (LS) α4β2–nAChRs presumably having a 3.2 ratio of α4β2 subunits at which nicotinic agonists have lower observed potency.27

TE671/HD, SH-SY5Y, and transfected SH-EPI cell lines were maintained as low passage number (1–26 from our frozen stocks) cultures to ensure stable expression of native or heterologously expressed nAChRs as previously described. Cells were passaged once a week by splitting just-confluent cultures 1/300 (TE671/RD), 1/10 (SH-SY5Y), or 1/40 (transfected SH-EPI) in serum-supplemented medium to maintain log-phase growth.

General Procedures for Behavioral Studies. Animals. BALB/cj male mice (8–10 weeks old at testing) were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed four to a cage in a colony room maintained at 22 ± 2 °C 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.

Compounds. Compounds 12a, 13a, and 19a were synthesized in accordance with 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 oral gavage (PO) in a volume of 10 mL/kg.

Mouse Forced Swim Test. Procedures were based on those previously described. Mice were individually placed into clear glass cylinders (15 cm tall × 10 cm diameter, 1 L beakers) containing 23 ± 1 °C water 12 cm deep (approximately 800 mL). Mice were administered vehicle, the SSRI sertraline (10 or 20 mg/kg; IP or PO) as a positive control, or compounds 12a (PO), 13a (PO), and 19a (PO) 30 min after compound administration, 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, compounds 12a, 13a, and 19a) as the between group variable and total time immobile in sec (over the 6 min trial) as the dependent variable. Significant main effects were followed up with the post hoc Fisher’s LSD test. 

β2–nAChR evX Vise Receptor Occupancy. Compounds 12a, 13a, and 19a (10 mg/kg) or water were administered via intraperitoneal injection 30 min before brain collection (the same time point as in force swim testing) for analysis of β2–nAChR occupancy in the brain by competitive binding outside tissues.
thalamus (for compound 12a and 19a, n = 6; for compound 13a, n = 4) as described before.38

■ ASSOCIATED CONTENT

Supporting Information
Experimental details for synthesis of all compounds shown, procedures for in vitro functional studies, and detailed broad screening data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

nAChR(s), nicotinic acetylcholine receptor(s); CNS, central nervous system; PNS, peripheral nervous system; DA, dopamine; 5-HT, serotonin; Glu, glutamate; ACh, acetylcholine; GABA, γ-aminobutyric acid; ADHD, attention deficit hyperactivity disorder; MDD, major depressive disorder; ee, enantiomeric excess; HS, high-sensitivity; LS, low-sensitivity; ADMET, absorption, distribution, metabolism, excretion, and toxicity; hERG, human ether-a-go-go-related gene; PK, pharmacokinetic; TFA, trifluoroacetic acid

■ REFERENCES


(29) The Charette protocol was also applied to the allylic alcohol corresponding to ester 6 to obtain alcohol 8a with a modest ee of 77%. The major product was found to be identical with the (S,S)-isomer 8a, an outcome which is consistent with the prediction using Charette’s model.


(39) The preliminary ADMET studies were carried out by Cerep, Inc.