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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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Supporting Information

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.1−10 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). Homomeric α7−nAChRs, which are typified by low ACh affinity and fast activation, are the other major component in the brain. Ganglionic α3/β4 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 α4β2−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 α4β2−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 α4β2−nAChRs over α3β4−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 α4β2−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 α4β2−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 hydroxyl, with the goal to optimize its hydrogen bonding interactions. Moreover, the chiral cyclopropane 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 (5) underwent Br displacement with benzyl alcohol, followed by a Heck reaction with n-butyl acrylate using a recently described, phosphine-free protocol to afford the αβ-unsaturated ester 6. Conversion of the ester group to the Weinreb amide using a standard procedure and subsequent Corey−Chaykowsky 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 hydroboration 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 isocyanoate. 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 substituent in the Wittig reagent, and unsaturation removed by catalytic hydrogenation. Compounds

Figure 1. Selected examples of synthetic nAChR ligands.

The emergence of compound 3 lends support to the use of α4β2−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 α4β2−nAChR partial agonist with excellent selectivity over α3β4−nAChRs. It 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 cyclopropane would endow the ligand with a unique restricted conformation in which the functional groups display a particular arrangement and might more effectively interact with the amino acid residues of the binding site of the target receptor. Moreover, the chiral cyclopropane 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.

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

<table>
<thead>
<tr>
<th>compd</th>
<th>$\alpha_2\beta_2$</th>
<th>$\alpha_2\beta_4$</th>
<th>$\alpha_3\beta_2$</th>
<th>$\alpha_4\beta_2$</th>
<th>$\alpha_4\beta_2^{n}$</th>
<th>$\alpha_4\beta_4$</th>
<th>selectivity ($\alpha_3\beta_4/\alpha_4\beta_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>0.1</td>
<td>249$^f$</td>
<td>3.0 ± 0.4</td>
<td>6520</td>
<td>0.1</td>
<td>0.5 ± 0.1</td>
<td>82.6 ± 9</td>
</tr>
<tr>
<td>12b</td>
<td>0.5 ± 0.1</td>
<td>65.0 ± 7</td>
<td>17.0 ± 7</td>
<td>&gt;10000</td>
<td>0.7 ± 0.1</td>
<td>3.7 ± 0.6</td>
<td>29.0 ± 4.3</td>
</tr>
<tr>
<td>13a</td>
<td>0.3 ± 0.1</td>
<td>1890</td>
<td>2.3 ± 2.9</td>
<td>&gt;10000</td>
<td>0.6 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>441</td>
</tr>
<tr>
<td>14a</td>
<td>0.3 ± 0.1</td>
<td>261</td>
<td>15.0 ± 2.1</td>
<td>&gt;10000</td>
<td>0.4</td>
<td>2.1 ± 0.3</td>
<td>281</td>
</tr>
<tr>
<td>15a</td>
<td>0.9 ± 0.1</td>
<td>720</td>
<td>16.0 ± 3</td>
<td>&gt;10000</td>
<td>0.7 ± 0.1</td>
<td>2.4 ± 0.3</td>
<td>234</td>
</tr>
<tr>
<td>16a</td>
<td>1.5 ± 0.1</td>
<td>2330</td>
<td>21.5 ± 5.2</td>
<td>&gt;10000</td>
<td>0.8 ± 0.1</td>
<td>4.0 ± 0.3</td>
<td>162</td>
</tr>
<tr>
<td>17a</td>
<td>1.2 ± 0.1</td>
<td>241</td>
<td>24.4 ± 5.7</td>
<td>8850</td>
<td>0.6 ± 0.1</td>
<td>9.5 ± 1.1</td>
<td>551</td>
</tr>
<tr>
<td>19a</td>
<td>0.1</td>
<td>236</td>
<td>2.4 ± 0.4</td>
<td>&gt;10000</td>
<td>0.1</td>
<td>0.3</td>
<td>50.2 ± 11</td>
</tr>
<tr>
<td>19b</td>
<td>0.5 ± 0.1</td>
<td>405</td>
<td>20 ± 7.3</td>
<td>&gt;10000</td>
<td>0.6 ± 0.1</td>
<td>6.2 ± 1.6</td>
<td>96.5 ± 21</td>
</tr>
<tr>
<td>nicotine$^d$</td>
<td>5.5</td>
<td>70</td>
<td>29</td>
<td>260</td>
<td>4.9</td>
<td>9.8</td>
<td>23</td>
</tr>
<tr>
<td>4$^d$</td>
<td>10000</td>
<td>0.4</td>
<td>0.9</td>
<td>24000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“See Experimental Section. $^b$/$\alpha_4\beta_2^{n}$, prepared from rat forebrain. $^c$SEM values are not provided for $K_i$ values >100 nM. $^d$The binding data for nicotine are from the PDSP Assay Protocol Book (http://pdsp.med.unc.edu/). $^e$The binding data for compound 4 were obtained from Reference 16.
nAChRs was consistent with that of the corresponding alcohols and ethers. For both the α3/β− and α4/β− 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-htα4/β2 cells, which heterogeneously and stably express human α4/β2−nAChRs assembled from individual subunits.33,34 SH-SY5Y and TE671/RD cells were used to assess activities of tested compounds at human α3/β4− or α1/β1γδ−nAChRs, respectively.35,36 All of the cyclopropane ligands had agonist activity at α4/β2−nAChRs with EC50 values <50 nM (Table 2). Consistent with the radioligand agonism EC50 values (Table 2). All tested ligands had neither agonist nor antagonist activity at ganglionic α3/β4− or muscle-type α1/β1γδ−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) α4/β2−nAChRs. The efficacy values at the HS α4/β2−nAChRs were extrapolated using compound 4 defined as a full agonist at the HS α4/β2−nAChR with 100% efficacy (see Supporting Information for more details).20 All of the tested ligands were found to be partial agonists at HS α4/β2−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,37 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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#### Table 2. Sensitivities and Efficacies of Ligand Agonism and Inactivation at α4/β2 nAChRs

<table>
<thead>
<tr>
<th>Compd</th>
<th>EC50 (nM)</th>
<th>Efficacy (%)</th>
<th>Efficacy (%)</th>
<th>IC50 (nM)</th>
<th>Efficacy (%)</th>
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<tbody>
<tr>
<td>12a</td>
<td>10.2</td>
<td>21</td>
<td>92</td>
<td>9.4</td>
<td>63</td>
</tr>
<tr>
<td>12b</td>
<td>34.6</td>
<td>10</td>
<td>65</td>
<td>50.9</td>
<td>69</td>
</tr>
<tr>
<td>13a</td>
<td>15.7</td>
<td>17</td>
<td>77</td>
<td>18.2</td>
<td>85</td>
</tr>
<tr>
<td>14a</td>
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<td>15</td>
<td>71</td>
<td>19.1</td>
<td>85</td>
</tr>
<tr>
<td>15a</td>
<td>23.3</td>
<td>27</td>
<td>80</td>
<td>19.6</td>
<td>82</td>
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<tr>
<td>16a</td>
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<td>23</td>
<td>69</td>
<td>48.5</td>
<td>84</td>
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<tr>
<td>17a</td>
<td>48.9</td>
<td>24</td>
<td>78</td>
<td>50.2</td>
<td>87</td>
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<tr>
<td>19a</td>
<td>17.5</td>
<td>6</td>
<td>60</td>
<td>5.6</td>
<td>71</td>
</tr>
<tr>
<td>19b</td>
<td>43.1</td>
<td>8</td>
<td>62</td>
<td>50.4</td>
<td>75</td>
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<tr>
<td>Nicotine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>290</td>
<td>88</td>
<td>430</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8</td>
<td>55</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>See Experimental Section. The efficacies were measured in a mixture of HS and LS α4/β2−nAChRs. The efficacy values were extrapolated using compound 4 defined as a full agonist at the HS α4/β2−nAChR (see Supporting Information for details). <sup>a</sup>Results for nicotine and compound 4 were obtained from Reference 17.

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![Figure 3. Receptor occupancy studies of compounds 12a, 13a, and 19a](image-url)

**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 injected intraperitoneally; n = 4−6/group.

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![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 administered orally; n = 9−10/group.](image-url)
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 (CYP1A, CYP2C9, CYP2C19, CYP2D6, and CYP3A) showed more than 25% inhibition, indicating minimal potential for hERG-related cardiovascular 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 exposure levels of compound 19a, which displayed about 80% and 70% inhibition of the tail current, respectively, were measured 15 min after administration and were at or below 3 ng/g at 1 mg/kg or ∼3 ng/g at 1 mg/kg in the presence of compounds 12a, 13a, and 19a. In contrast, brain exposure levels of compound 19a were measured 15 min after administration and were at or below 3 ng/g at 1 mg/kg or ∼3 ng/g at 3 mg/kg when measured 15 min after administration and were at or below detection level at later time points.

**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 ganglionic 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 (1H NMR), the δ 4.80 signal of HDD (2H NMR), and the δ 77.23 signal of CDCl3 (13C NMR) as internal standards. 13C NMR spectra in D2O and CD3OD were investigated using frequencies of 400 and 100 MHz, respectively. NMR chemical shifts were measured 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 ÅQ 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 Syngent 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 (1H and 13C) of all intermediates.

3-[(2S)-Azetidinyl]methoxy]-5-(15,2R)-2-(2-hydroxyethyl)cyclopropanecarboxylic acid Trifluoroacetate (12a). 1H NMR (D2O, D): 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, D): 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]20D = +36.5 (c 0.40, MeOH). Anal. Calc'd for C14H20N2O2 30.1% C, 45.1% H, 4.69% F, 16.3% N; Found: C, 43.55; H, 4.42; F, 24.38; N, 5.57. 3-[(2S)-Azetidinyl]methoxy]-5-(15,2S)-2-(2-hydroxyethyl)cyclopropanecarboxylic acid Trifluoroacetate (12b). 1H NMR (400 MHz, D2O, D): 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), 2.70 (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, D): δ 162.3 (TFA), 155.8, 146.3, 132.0, 128.0, 125.3, 115.9 (TFA), 67.1, 60.8, 58.3, 43.3, 35.1, 21.9, 19.8, 19.4, 16.3. [α]20D = +94.9 (c 0.17, MeOH). Anal. Calc'd for C14H20N2O2·2CF3COOH·0.5H2O: 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.
Significant main effects were followed up with the post hoc Fisher LSD test (p < 0.05). Compounds 12a, 13a, and 19a were synthesized according to procedures described in the text, and sterinaline 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.

**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.

Batches. Compounds 12a, 13a, and 19a were synthesized according to procedures described in the text, and sterinaline 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 SSR1 sertraline (10 or 20 mg/kg, IP or PO) as a positive control, or compounds 12a (PO), 13a (PO), and 19a (PO). Thirty 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 vs Vero 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 forced swim testing) for analysis of β2-nAChR occupancy in the...
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

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(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.