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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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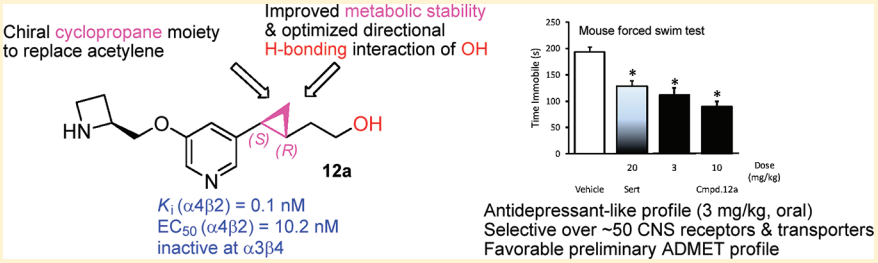
Supporting Information



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.[1−4] 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). Homomeric 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

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effects such as emesis and nausea.[5,6] 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,[7,8] 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.[9−11] 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.[12] In addition, nicotinic ligands targeting α4β2*−nAChRs may likewise be used to treat neuropathic pain or attention deficit hyperactivity disorder (ADHD).[4] 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 efficacy.[13] 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).[14] Pfizer's varenicline (**3**), an α4β2−nAChR partial agonist and a full agonist at α7− and α3β4*−nAChR, is at present the most successful synthetic small molecule on the market for smoking cessation pharmacotherapy (Figure 1).[15]

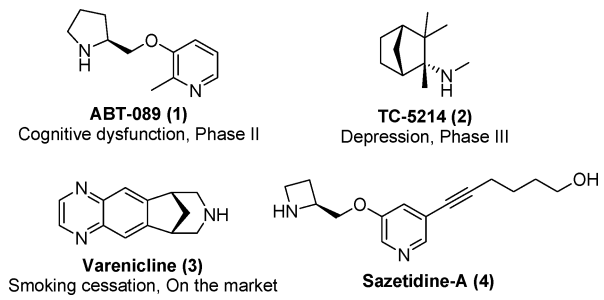

Figure 1. Selected examples of synthetic nAChR ligands.

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,[4] 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.[16,17] Compound **4** has been shown to possess extremely promising antidepressant and anxiolytic effects in rodent studies, including nicotine-like effects in drug discrimination studies.[18−20] In addition, analgesic effects of compound **4**, without any neurological side effects, have been reported using the rat formalin model.[21] 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.[22] 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.[23,24] 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.[25] 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.[26] 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[27] to afford the α,β-unsaturated ester **6**. Conversion of the ester group to the Weinreb amide[28] 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.[29,30] 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 carbamate using various amines or phenyl isocyanate. Removal of the Boc group from **11a** or the carbamate 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

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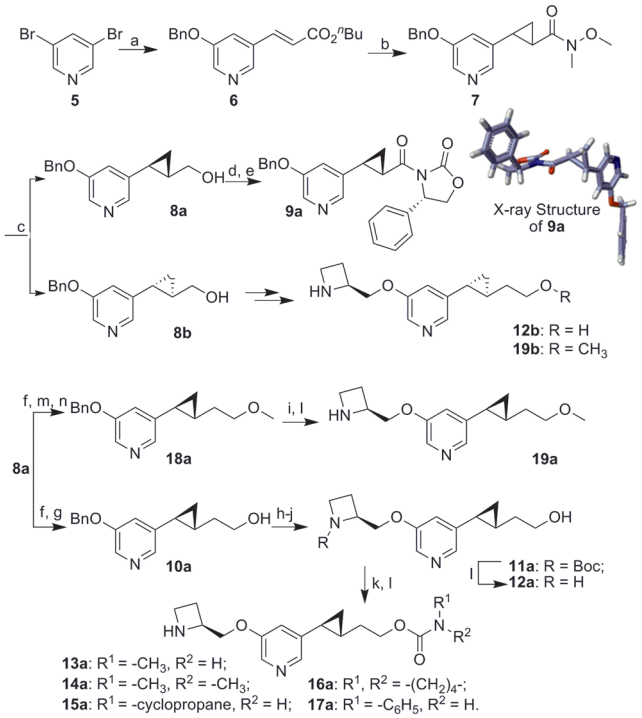
Scheme 1.[a]



[a]Reagents and conditions: (a) (i) BnOH, NaH, DMF, rt, (ii) *n*-butyl acrylate, 1% $Pd(OAc)_2$, 2% $PhNHCONH_2$, K_2CO_3, 130 °C; (b) (i) 2N NaOH, MeOH/THF (1:1), rt, (ii) $MeNH_2^+OMeCl^-$, EDCI, DMAP, CH_2Cl_2, rt, (iii) $Me_3S(O)^+I^-$, NaH, DMSO, rt; (c) (i) DIBAL-H, THF, −78 °C to −20 °C, (ii) $NaBH_4$, MeOH, rt, (iii) Chiralpak AD, EtOH; (d) (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, (ii) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, *tert*-butanol/H_2O, rt; (e) (i) trimethylacetyl chloride, Et_3N, THF, −78 to 0 °C, (ii) (*S*)-(+)-4-phenyl-2-oxazolidinone lithium salt, THF, −78 to 0 °C; (f) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (g) (i) $Ph_3P{=}CH_2$, THF, 0 °C, (ii) dicyclohexylborane, THF, 0 °C to rt, (iii) 30% H_2O_2, 3N NaOH, 55 °C; (h) isobutyric anhydride, cat. DMAP, Et_3N, CH_2Cl_2, rt; (i) (i) 10% Pd/C, H_2, EtOAc/MeOH, rt, (ii) 1-(*tert*-butoxycarbonyl)-(2*S*)-azetidinylmethanol, azodicarbonyldipiperidide (ADDP), P(*n*-Bu)$_3$, PhMe, 0 °C to rt; (j) NaOMe, MeOH, 40 °C; (k) 1,1′-carbonyldiimidazole, PhMe, THF, amine, rt, or phenyl isocyanate, PhMe, reflux; (l) CF_3COOH, CH_2Cl_2, rt; (m) $Ph_3P{=}CHOCH_3$, THF, 0 °C; (n) PtO_2, H_2, CH_2Cl_2, rt.

12b and **19b**, diastereoisomers of **12a** and **19a**, were synthesized from **8b** by the same sequence of steps.

IN VITRO RADIOLIGAND BINDING AND FUNCTIONAL STUDIES

The K_i values of all the synthesized cyclopropane compounds were evaluated by [^{3}H]epibatidine binding competition assays at seven heterologously expressed rat nAChR subtypes. As shown in Table 1, compound **12a** with a (1*S*,2*R*)-configured cyclopropane ring exhibited subnanomolar binding affinity for both the $\alpha4\beta2-$ (K_i = 0.1 nM) and $\alpha4\beta2^*-$ (K_i = 0.5 nM) nAChRs, thus having K_i values similar to those found for compound **4**. Compound **12a** was approximately 7-fold more potent than its diastereoisomer **12b** at both the $\alpha4\beta2-$ and $\alpha4\beta2^*-$nAChRs (**12b**: K_i = 0.7 and 3.7 nM, respectively). Similarly, the (1*R*,2*S*)-configured ether analogue **19b** was less active than **19a** at both the $\alpha4\beta2-$ (K_i = 0.6 vs 0.1 nM) and $\alpha4\beta2^*-$nAChR subtype (K_i = 6.2 vs 0.3 nM). All four of these compounds demonstrated good selectivity for nAChRs containing $\beta2$ subunits ($\alpha2\beta2-$, $\alpha3\beta2-$, $\alpha4\beta2-$, and $\alpha4\beta2^*-$nAChRs), which are associated with the regulation of dopamine release in the nucleus accumbens,[31] over nAChRs containing $\beta4$ subunits ($\alpha3\beta4-$, $\alpha2\beta4-$, and $\alpha4\beta4-$nAChRs). Of considerable importance in the identification of therapeutically useful nicotinic ligands is the selectivity away from the $\alpha3\beta4-$nAChR subtype. The $\alpha3\beta4-/\alpha4\beta2-$nAChR K_i ratios of the two cyclopropane analogues featuring the (1*S*,2*R*)-configuration, **12a** and **19a**, were 65200 and 100000, respectively, which are much higher than that of nicotine ($\alpha3\beta4-/\alpha4\beta2-$nAChR = 53) or even compound **4** ($\alpha3\beta4-/\alpha4\beta2-$nAChR = 24000). Collectively, these outcomes clearly suggest that the (1*S*,2*R*)-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 $\alpha4\beta2-$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 $\alpha4\beta2-$nAChR ligands, in our efforts to further optimize the side chain.[32] The carbamate analogues **13a**−**17a**, which contain the preferred (1*S*,2*R*)-configured cyclopropane ring, also exhibited subnanomolar to low nanomolar binding affinities at both the $\alpha4\beta2-$ and $\alpha4\beta2^*-$nAChRs (Table 1). Their binding preference for the $\beta2-$ over the $\beta4-$containing

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

	K_i (nM)[a]							
compd	$\alpha2\beta2$	$\alpha2\beta4$	$\alpha3\beta2$	$\alpha3\beta4$	$\alpha4\beta2$	$\alpha4\beta2^*$[b]	$\alpha4\beta4$	selectivity ($\alpha3\beta4/\alpha4\beta2$)
12a	0.1	249[c]	3.0 ± 0.4	6520	0.1	0.5 ± 0.1	82.6 ± 9	65200
12b	0.5 ± 0.1	65.0 ± 7	17.0 ± 7	1040	0.7 ± 0.1	3.7 ± 0.6	29.0 ± 4.3	1500
13a	0.3 ± 0.1	1890	9.8 ± 2.9	>10000	0.6 ± 0.1	1.7 ± 0.2	441	>100000
14a	0.3 ± 0.1	261	15.0 ± 2.1	>10000	0.4	2.1 ± 0.3	281	>100000
15a	0.9 ± 0.1	720	16.0 ± 3	>10000	0.7 ± 0.1	2.4 ± 0.3	234	>100000
16a	1.5 ± 0.1	2330	21.5 ± 5.2	>10000	0.8 ± 0.1	4.0 ± 0.3	162	>100000
17a	1.2 ± 0.1	241	24.4 ± 5.7	8850	0.6 ± 0.1	5.9 ± 1.1	551	14800
19a	0.1	236	2.4 ± 0.4	>10000	0.1	0.3	50.2 ± 11	>100000
19b	0.5 ± 0.1	405	20 ± 7.3	>10000	0.6 ± 0.1	6.2 ± 1.6	96.5 ± 21	>16700
nicotine[d]	5.5	70	29	260	4.9	9.8	23	53
4[e]				10000	0.4	0.9		24000

[a]See Experimental Section. [b]$\alpha4\beta2^*$, 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.

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nAChRs was consistent with that of the corresponding alcohols and ethers. For both the $\alpha3\beta2-$ and $\alpha4\beta2^*-$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 $\alpha7-$nAChRs. Ten μM concentrations of test ligand inhibited binding of 10 nM ^{3}H-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 $^{86}Rb^+$ ion flux assays using SH-EP1-h$\alpha4\beta2$ cells, which heterologueously and stably express human $\alpha4\beta2-$nAChRs assembled from individual subunits.[33,34] SH-SY5Y and TE671/RD cells were used to assess activities of tested compounds at human $\alpha3\beta4^*-$ or $\alpha1\beta1\gamma\delta-$nAChRs, respectively.[35,36] All of the cyclopropane ligands had agonist activity at $\alpha4\beta2-$nAChRs with EC_{50} values <50 nM (Table 2). Consistent with the radioligand binding studies, compounds **12a** and **19a** bearing the (1*S*,2*R*)-configured cyclopropane ring exhibited about 3-fold higher potencies than their diastereoisomers **12b** and **19b** (EC_{50}: 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 $\alpha4\beta2-$nAChRs to a full agonist at IC_{50} values similar to the agonism EC_{50} values (Table 2). All tested ligands had neither agonist nor antagonist activity at ganglionic $\alpha3\beta4^*-$ or muscle-type $\alpha1\beta1\gamma\delta-$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) $\alpha4\beta2-$nAChRs. The efficacy values at the HS $\alpha4\beta2-$nAChRs were extrapolated using compound **4** defined as a full agonist at the HS $\alpha4\beta2-$nAChR with 100% efficacy (see Supporting Information for more details).[20] All of the tested ligands were found to be partial agonists at HS $\alpha4\beta2-$nAChRs with efficacy values ranging from 60 to 92%.

Table 2. Sensitivities and Efficacies of Ligand Agonism and Inactivation at $\alpha4\beta2$ nAChRs[a]

	agonism			desensitization	
compd	EC_{50} (nM)	efficacy (%)[b]	efficacy (%) HS[c]	IC_{50} (nM)	efficacy (%)[b]
12a	10.2	21	92	9.4	63
12b	34.6	10	65	50.9	69
13a	15.7	17	77	18.2	85
14a	18.2	15	71	19.1	85
15a	23.3	27	80	19.6	82
16a	44.4	23	69	48.5	84
17a	48.9	24	78	50.2	87
19a	17.5	6	60	5.6	71
19b	43.1	8	62	50.4	75
nicotine[d]	290	88		430	93
4[d]	5.8	55	100	4.8	63

[a]See Experimental Section. [b]The efficacies were measured in a mixture of HS and LS $\alpha4\beta2$-nAChRs. [c]The efficacy values were extrapolated using compound **4** defined as a full agonist at the HS $\alpha4\beta2$-nAChR (see Supporting Information for details). [d]Results for nicotine and compound **4** were obtained from Reference 17.

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 $\beta2^*$ receptors, whereas the carbamate analogue **13a** showed only approximately 65% occupancy (Figure 3). These RO findings are

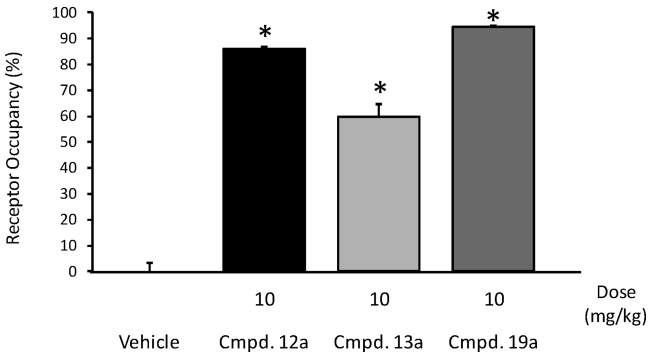

Figure 3. Receptor occupancy studies of compounds **12a**, **13a**, and **19a** in mice showed a significant occupancy level. (*Mann–Whitney U: $p < 0.05$). All drugs were injected intraperitoneally; n = 4—6/group.

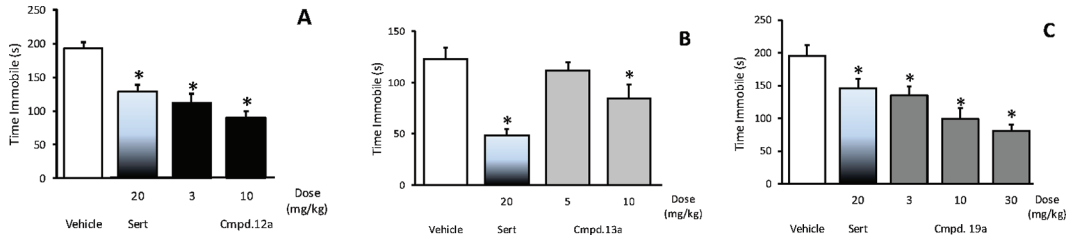

Figure 2. Mouse forced swim data for compounds **12a** (A), **13a** (B), and **19a** (C). The selective serotonin reuptake inhibitor, sertraline, produced the expected decrease in immobility. (ANOVAs: F (3,35) = 13.43, $p < 0.001$ (A); F (3,36) = 11.46, $p < 0.001$ (B); F (4,44) = 9.29, $p < 0.001$ (C). *Fisher's PLSD posthoc test: $ps < 0.05$ vs vehicle). All drugs were administered orally; n = 9—10/group.

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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.[39] 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 a concentrations up to 10 μM, none of the CYP isoforms tested (CYP1A, 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 isoform, 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 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 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 ($t_{1/2}$ = 150 min) as well as in plasma ($t_{1/2}$ = 144 min). The concentration of compound **19a** reached a value of 133 ng/mL ($C_{max\text{-}brain}$) in 30 min ($T_{max\text{-}brain}$) in brain, and of 359 ng/mL ($C_{max\text{-}plasma}$) in 10 min ($T_{max\text{-}plasma}$) in plasma. The brain to plasma ratio of compound **19a** was found to be 0.37 ($C_{max\text{-}brain}/C_{max\text{-}plasma}$), 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.[38]

CONCLUSION

In summary, a series of chiral cyclopropane analogues of the lead structure, compound **4**, were identified as highly potent, $\alpha4\beta2$-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 $\alpha4\beta2$−nAChRs. The best compounds, **12a**, **13a**, and **19a**, exhibited subnanomolar to low-nanomolar binding affinity for both $\alpha4\beta2$− and $\alpha4\beta2$*−nAChRs with negligible interaction with $\alpha3\beta4$−nAChRs. In functional studies, these ligands acted as highly potent, partial agonists at HS $\alpha4\beta2$−nAChRs and were totally inactive at both ganglionic $\alpha3\beta4$*− or muscle-type $\alpha1\beta1\gamma\delta$−nAChRs. Compounds **12a**, **13a**, and **19a** were found to display antidepressant-like properties in the mouse forced swim test, associated with high levels of $\beta2$* 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 ChemImpex, and solvents were used as obtained from Fisher Scientific or Sigma-Aldrich without further purification. Anhydrous THF and CH_2Cl_2 were obtained by distillation over sodium wire or CaH_2, 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 SiO_2. Spots were visualized by their quenching of the fluorescence of an indicator admixed to the SiO_2 layer or by dipping into I_2/SiO_2 mixture. Products were purified by column chromatography on 230−400 mesh SiO_2. 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 $CHCl_3$ (^{1}H NMR), the δ 4.80 signal of HDO (^{1}H NMR), and the δ 77.23 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 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 CF_3COOH) 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 Synergi 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 CF_3COOH) in 18 min. See Supporting Information for detailed experimental procedures and NMR spectral data (^{1}H and ^{13}C) of all intermediates.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-(2-hydroxyethyl)-cyclopropyl]pyridine Trifluoroacetate (12a). ^{1}H NMR (D_2O): 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), 1.98 (m, 1H), 1.68 (q, J = 6.8 Hz, 2H), 1.33 (m, 1H), 1.20−1.14 (m, 2H). ^{13}C NMR (D_2O): 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. $[\alpha]_D^{20}$ = +36.5 (c 0.40, MeOH). Anal. Calcd for $C_{14}H_{20}N_2O_2{\cdot}2.15CF_3COOH{\cdot}0.5H_2O$: 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-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*R*,2*S*)-2-(2-hydroxyethyl)-cyclopropyl]pyridine Trifluoroacetate (12b). ^{1}H NMR (400 MHz, D_2O): 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). ^{13}C NMR (100 MHz, D_2O): 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. $[\alpha]_D^{20}$ = −49.4 (c 0.17, MeOH). Anal. Calcd for $C_{14}H_{20}N_2O_2{\cdot}2CF_3COOH{\cdot}0.15H_2O$: 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.

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3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-[2-(*N*-methylcarbamoyloxy)ethyl]cyclopropyl]pyridine Trifluoroacetate (13a). ^{1}H NMR (D_2O): 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), 4.27−4.05 (m, 4H), 2.70 (q, J = 8.4 Hz, 2H), 2.62 (s, 3H), 1.99 (m, 1H), 1.88 (m, 1H), 1.65 (m, 1H), 1.34 (m, 1H), 1.16 (m, 2H). ^{13}C NMR (D_2O): 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. $[\alpha]_D^{20}$ = +43.3 (c 0.18, MeOH). Anal. Calcd for $C_{16}H_{23}N_3O_3{\cdot}1.95CF_3COOH{\cdot}1.8H_2O$: C, 42.67; H, 5.14; F, 19.84; N, 7.50. Found: C, 42.77; H, 4.99; F, 19.86; N, 7.35.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-[2-(*N*,*N*-dimethylcarbamoyloxy)ethyl]cyclopropyl]-pyridine Trifluoroacetate (14a). ^{1}H NMR (D_2O): δ 8.31 (s, 1H), 8.19 (s, 1H), 7.80 (s, 1H), 4.94 (m, 1H), 4.48 (d, J = 4.0 Hz, 2H), 4.20−4.03 (m, 4H), 2.79 (s, 6H), 2.66 (q, J = 8.4 Hz, 2H), 1.95 (m, 1H), 1.83 (m, 1H), 1.65 (m, 1H), 1.33 (m, 1H), 1.11 (m, 2H). ^{13}C NMR (D_2O): δ 162.3 (TFA), 157.8, 155.9, 146.2, 131.9, 127.9, 125.3, 115.9 (TFA), 67.1, 65.0, 58.2, 43.2, 35.1, 32.2, 21.9, 19.8, 19.4, 16.3. $[\alpha]_D^{20}$ = +31.3 (c 0.07, MeOH). Anal. Calcd for $C_{17}H_{25}N_3O_3{\cdot}2.15CF_3COOH{\cdot}1.55H_2O$: C, 43.18; H, 5.15; F, 20.68; N, 7.09. Found: C, 43.35; H, 5.12; F, 20.51; N, 6.89.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-[2-(*N*-cyclopropylcarbamoyloxy)ethyl]cyclopropyl]-pyridine Trifluoroacetate (15a). ^{1}H NMR (D_2O): δ 8.34 (s, 1H), 8.22 (s, 1H), 7.84 (s, 1H), 4.98 (m, 1H), 4.52 (d, J = 4.0 Hz, 2H), 4.19−4.05 (m, 4H), 2.69 (q, J = 8.4 Hz, 2H), 2.37 (m, 1H), 1.97 (m, 1H), 1.85 (m, 1H), 1.65 (m, 1H), 1.31 (m, 1H), 1.14 (m, 2H), 0.63 (m, 2H), 0.39 (m, 2H). ^{13}C NMR (D_2O): δ 162.3 (TFA), 159.3, 155.9, 146.2, 131.9, 128.0, 125.4, 115.9 (TFA), 67.2, 64.4, 58.2, 43.3, 32.1, 21.9, 21.8, 19.8, 19.4, 16.1, 5.3, 5.2. $[\alpha]_D^{20}$ = +40.6 (c 0.68, MeOH). Anal. Calcd for $C_{18}H_{25}N_3O_3{\cdot}1.95\ CF_3COOH{\cdot}0.7H_2O$: C, 46.44; H, 5.05; F, 19.62; N, 7.42. Found: C, 46.19; H, 4.74; F, 19.42; N, 7.16.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-[2-(1-pyrrolidinylcarbonyloxy)ethyl]cyclopropyl]-pyridine Trifluoroacetate (16a). ^{1}H NMR (D_2O): δ 8.32 (s, 1H), 8.20 (s, 1H), 7.81 (s, 1H), 4.95 (m, 1H), 4.49 (d, J = 4.0 Hz, 2H), 4.20−4.04 (m, 4H), 3.22 (m, 4H), 2.67 (q, J = 8.8 Hz, 2H), 1.94 (m, 1H), 1.87−1.76 (m, 5H), 1.66 (m, 1H), 1.33 (m, 1H), 1.11 (m, 2H). ^{13}C NMR (D_2O): δ 162.3 (TFA), 156.1, 155.9, 146.2, 131.9, 128.0, 125.3, 115.9 (TFA), 67.2, 64.7, 58.2, 45.5, 43.2, 32.2, 24.3, 21.9, 19.8, 19.4, 16.3. $[\alpha]_D^{20}$ = +39.7 (c 0.66, MeOH). Anal. Calcd for $C_{19}H_{27}N_3O_3{\cdot}2.25CF_3COOH{\cdot}0.45H_2O$: C, 46.26; H, 4.98; F, 21.02; N, 6.89. Found: C, 46.11; H, 4.81; F, 20.95; N, 6.91.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-[2-(*N*-phenylcarbamoyloxy)ethyl]cyclopropyl]pyridine Trifluoroacetate (17a). ^{1}H NMR (D_2O): δ 8.02 (s, 1H), 7.96 (s, 1H), 7.58 (s, 1H), 7.20 (t, J = 8.0 Hz, 2H), 7.07 (d, J = 7.6 Hz, 2H), 7.01 (t, J = 7.2 Hz, 1H), 4.41 (m, 1H), 4.21 (m, 1H), 4.10 (m, 3H), 3.97 (m, 1H), 2.59 (m, 2H), 2.02 (m, 1H), 1.83 (m, 1H), 1.45 (m, 1H), 1.28 (m, 1H), 1.04 (m, 2H). ^{13}C NMR (D_2O): δ 162.3 (TFA), 155.5, 154.9, 145.9, 137.2, 131.4, 128.6, 127.4, 125.0, 123.1, 118.1, 115.9 (TFA), 66.8, 64.1, 58.0, 43.2, 32.4, 21.7, 19.9, 19.8, 16.3. $[\alpha]_D^{20}$ = +36.4 (c 0.91, MeOH). Anal. Calcd for $C_{21}H_{25}N_3O_3{\cdot}2.15CF_3COOH{\cdot}1.0H_2O$: C, 48.19; H, 4.66; F, 19.43; N, 6.66. Found: C, 48.16; H, 4.51; F, 19.48; N, 6.51.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*S*,2*R*)-2-(2-methoxyethyl)-cyclopropyl]pyridine Trifluoroacetate (19a). ^{1}H NMR (D_2O): δ 8.35 (s, 1H), 8.24 (s, 1H), 7.86 (s, 1H), 5.00 (m, 1H), 4.54 (d, J = 4.0 Hz, 2H), 4.18−4.09 (m, 2H), 3.61 (t, J = 6.4 Hz, 2H), 3.36 (s, 3H), 2.71 (q, J = 8.4 Hz, 2H), 2.00 (m, 1H), 1.74 (m, 2H), 1.34 (m, 1H), 1.21−1.15 (m, 2H). ^{13}C NMR (D_2O): δ 162.3 (TFA), 155.9, 146.2, 132.0, 128.0, 125.4, 115.9 (TFA), 71.5, 67.1, 58.2, 57.3, 43.3, 32.2, 22.0, 19.8, 19.4, 16.2. $[\alpha]_D^{20}$ = +40.0 (c 0.20, MeOH). Anal. Calcd for $C_{15}H_{22}N_2O_2{\cdot}2.05CF_3COOH{\cdot}0.5H_2O$: C, 45.42; H, 5.00; F, 23.13; N, 5.55. Found: C, 45.43; H, 4.90; F, 23.05, N, 5.57.

3-[(2(*S*)-Azetidinyl)methoxy]-5-[(1*R*,2*S*)-2-(2-methoxyethyl)-cyclopropyl]pyridine Trifluoroacetate (19b). ^{1}H NMR (D_2O): δ 8.31 (s, 1H), 8.20 (s, 1H), 7.82 (s, 1H), 4.96 (m, 1H), 4.50 (d, J = 4.0 Hz, 2H), 4.14−4.05 (m, 2H), 3.55 (t, J = 6.4 Hz, 2H), 3.31 (s, 3H), 2.67 (q, J = 8.4 Hz, 2H), 1.96 (m, 1H), 1.69 (m, 2H), 1.28 (m, 1H), 1.16−1.10 (m, 2H). ^{13}C NMR (D_2O): δ 162.3 (TFA), 155.8, 146.2, 131.9, 127.9, 125.3, 115.9 (TFA), 71.4, 67.1, 58.3, 57.3, 43.3, 32.2, 21.9, 19.8, 19.4, 16.2. $[\alpha]_D^{20}$ = −42.9 (c 0.84, MeOH). Anal. Calcd for $C_{15}H_{22}N_2O_2{\cdot}2.05CF_3COOH{\cdot}0.6H_2O$: C, 45.26; H, 5.02; F, 23.05; N, 5.53. Found: C, 45.22; H, 4.88; F, 22.91; N, 5.47.

In Vitro Studies. [^{3}H]Epibatidine competition studies: For experimental details, please refer to the PDSP Web site http://pdsp.med.unc.edu/ for all nAChR subtypes except α7. For assay at α7, membrane preparations from SH-EP1 cells heterologously expressing human α7 nAChRs were used to test 10 μM concentrations of test ligands in competition with 10 nM [^{3}H]epibatidine with an experimental protocol similar to that utilized by the PDSP.[33,34]

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 α1*−nAChRs, containing α1, β1, γ, and δ subunits, with function detectable using $^{86}Rb^+$ efflux assays.[36] 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 $^{86}Rb^+$ efflux assays.[33] SH-SY5Y cells also express homopentameric α7−nAChRs; however, their function is not detected in the $^{86}Rb^+$ efflux assay under the conditions used. SH-EP1 human epithelial cells stably transfected with cDNAs separately encoding human α4 or β2 subunits (SH-EP1-hα4β2 cells) have been established and characterized with both ion flux and radioligand binding assays.[34] These cells thus express a mixture of so-called "high sensitivity" (HS) α4β2−nAChRs, having the presumed subunit ratios of 2:3 α4:β2 and comparatively high sensitivity to nicotinic agonists, and "low sensitivity" (LS) α4β2−nAChRs presumably having a 3:2 ratio of α4:β2 subunits at which nicotinic agonists have lower observed potency.[17]

TE671/RD, SH-SY5Y, and transfected SH-EP1 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-EP1) 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.

Drugs. Compounds **12a**, **13a**, and **19a** were 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 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). 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 PLSD test.

β2-nAChR ex Vivo 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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