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Chemistry, Pharmacology, and Behavioral Studies Identify Chiral Cyclopropanes as Selective $\alpha4\beta2$ -Nicotinic Acetylcholine Receptor Partial Agonists Exhibiting an Antidepressant Profile. Part II

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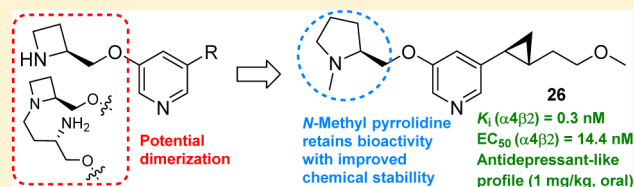
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S Supporting Information

ABSTRACT: A 3-pyridyl ether scaffold bearing a cyclopropane-containing side chain was recently identified in our efforts to create novel antidepressants that act as partial agonists at $\alpha4\beta2$ -nicotinic acetylcholine receptors. In this study, a systematic structure–activity relationship investigation was carried out on both the azetidine moiety present in compound 3 and its right-hand side chain, thereby discovering a variety of novel nicotinic ligands that retain bioactivity and feature improved chemical stability. The most promising compounds, 24, 26, and 30, demonstrated comparable or enhanced pharmacological profiles compared to the parent compound 4, and the *N*-methylpyrrolidine analogue 26 also exhibited robust antidepressant-like efficacy in the mouse forced swim test. The favorable ADMET profile and chemical stability of 26 further indicate this compound to be a promising lead as a drug candidate warranting further advancement down the drug discovery pipeline.



■ INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) belong to the ligand-gated ion channel superfamily of neurotransmitter receptors, which are widely distributed in the central and peripheral nervous system.¹ They are thought to be vital players modulating major brain functions and participating in pathophysiological processes, and the dysfunction of this family of receptors has been implicated in a variety of nervous system disorders such as Alzheimer's disease, Parkinson's disease, schizophrenia, nicotine addiction, and depression.¹ Brain nAChRs are homomeric or heteromeric pentamers assembled from diverse combinations of subunits ($\alpha2$ – $\alpha10$ and $\beta2$ – $\beta4$), and different subtype stoichiometries allow the assembly of varied pentamers with a wide range of physiological and pharmacological profiles.² Increasing evidence has implied that the $\alpha4\beta2^*$ -nAChRs (the asterisk denotes the possible integration of other subunits aside from those specified into the pentamer), the most abundant and widespread nAChRs in the brain, may play a role in mood and reward. Numerous preclinical studies in murine models have illustrated that administration of $\alpha4\beta2$ -nAChR partial agonists or antagonists potentiates antidepressant-like effects of conventional antidepressants, suggesting possible human use as an adjunctive therapy, but also indicate that nicotinic ligands acting alone can elicit antidepressant-like effects.³ The antidepressant effects of

mecamylamine, and even those of the tricyclic antidepressant amitriptyline, are abolished in nAChR $\beta2$ subunit knockout mice that lack $\alpha4\beta2^*$ -nAChRs.^{4,5} Moreover, results of clinical trials in depressed patients also demonstrate that the marketed drug varenicline, an $\alpha4\beta2$ -nAChR partial agonist for smoking cessation pharmacotherapy, has positive effects on relieving the symptoms of depression.⁶ Given that more than one-third of depressed individuals are resistant to currently available drug treatments,⁷ selective $\alpha4\beta2$ -nAChR ligands could offer hope as mechanistically novel antidepressant medications. In addition, considerable progress has recently been made in understanding nAChRs containing the $\alpha6$ subunit regarding their anatomical distribution, subunit composition, and physiological function. Although their expression in the brain is relatively restricted, these receptors are prevalent in midbrain dopaminergic regions and are thought to influence physiological mechanisms associated with mood and drug dependence.^{8,9}

Azetidine is a four-membered nitrogen-containing saturated heterocycle possessing reasonable chemical stability. With the increasing number of methods that have been developed for the synthesis of four-membered ring systems,¹⁰ azetidine-containing building blocks have received considerable interest by the

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medicinal chemistry community as components in various drugs or bioactive compounds. For instance, azetidine-2-one (β -lactam ring) is the key structural element of β -lactam antibiotics that were discovered more than 80 years ago and are still widely used clinically, as exemplified by penicillins, cephalosporins, and pemems.¹¹ Azelnidipine, a long-acting calcium channel blocker containing a azetidiny ether unit, has been approved in Japan as an antihypertensive drug.¹² Additionally, the azetidine moiety has also been introduced by Abbott laboratories for the development of neuronal nAChR ligands for treating specific CNS disorders with reduced side effect liabilities.¹³ Among them, tebanicline (**1**) is a highly potent $\alpha 4\beta 2$ -nAChR agonist with improved selectivity over other subtypes in comparison with the parent compound epibatidine (**2**). A chiral azetidiny methoxy group was used to replace the 7-azabicyclo[2.2.1]heptane structure in compound **2**, providing a protonated nitrogen atom as an essential pharmacophoric element of nicotinic ligands (Figure 1).

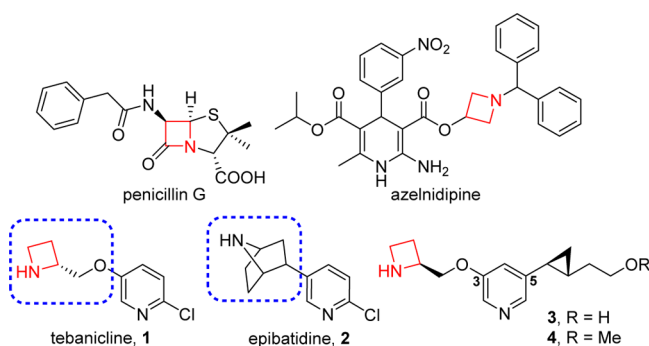


Figure 1. Selected azetidine-containing compounds and nicotinic ligands.

Although compound **1** was abandoned after completing phase II clinical trials for the treatment of neuropathic pain due to an unacceptably narrow therapeutic index,¹⁴ the 3-pyridyl ether scaffold offers new perspectives on the design of $\alpha 4\beta 2$ -nAChR ligands and holds promise for $\alpha 4\beta 2$ -nAChR modulators to be advanced to the clinic as novel therapeutic agents.

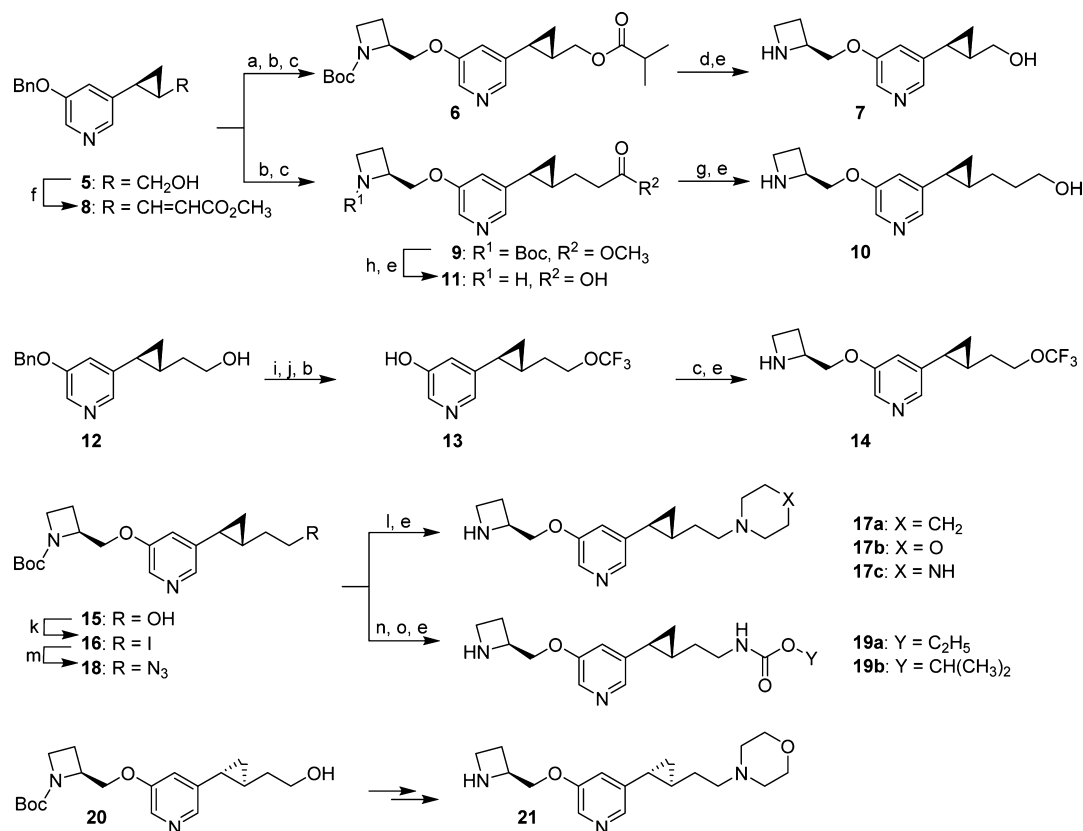
Previously, in our efforts to design nAChR ligands as potential antidepressants, a series of selective $\alpha 4\beta 2$ -nAChR partial agonists were identified based on the 3-pyridyl ether scaffold.¹⁵ The chiral cyclopropane-containing side chain appended to the 5-position of the pyridine ring confers substantial nAChR subtype selectivity to these ligands compared to the 5-unsubstituted compound A-85380, particularly over ganglionic $\alpha 3\beta 4^*$ -nAChRs that are thought to be associated with unwanted side effects *in vivo*¹⁶ while not affecting their high affinity for $\alpha 4\beta 2$ -nAChRs. Some of the most promising compounds (**3** and **4**) demonstrate favorable antidepressant-like effects in the mouse forced swim test as well as acceptable ADMET profiles and may thus serve as suitable lead compounds for the development of novel antidepressant agents. However, it is noteworthy that the hydrochloride salt of compound **3** was hygroscopic and partially decomposed after the absorption of water to give considerable quantities of a major byproduct. On the basis of analysis of LC-MS results and NMR spectra, the structure of the decomposition product was identified as a dimer of the parent compound, which resulted from attack of one azetidine nitrogen atom on the less-substituted α -methylene group of

another azetidine ring. Similar intermolecular dimerization was also observed by Abbott chemists during the process of salt screening and selection for compound **1**.¹⁷ Although this potential stability issue can be prevented by changing the salt form of these azetidine-containing ligands,¹⁷ replacement of the azetidine unit by other ring systems having improved chemical stability is a logical first choice. Thus, in continuation of our efforts to develop $\alpha 4\beta 2$ -nAChR ligands for treating depression, a novel series of nAChR ligands with different ring systems replacing the azetidine ring found in the lead compound **4** were designed and synthesized for pharmacological evaluation. Selected compounds were further assessed in behavioral tests used to gauge antidepressant drug action, and preliminary ADMET studies were carried out. Moreover, we detailed additional structural modifications of the side chain attached to the 5-position of the pyridine ring.

CHEMISTRY

In our previous study of the isoxazoly pyridine ether analogues, we found that the length of the side chain was crucial to their biological activity.¹⁸ We thus chose to synthesize cyclopropane ligands bearing a shorter or longer side chain in comparison with compound **3** to explore the optimal length of the side chain for the cyclopropane series. In addition, in order to better understand structure–activity relationship (SAR) of the right-hand side chain, analogues featuring a terminal carboxylic acid, trifluoromethoxy group, carbamate function, or six-membered ring were designed. The syntheses of the cyclopropane analogues **7**, **10**, **11**, **14**, **17a–c**, **19a–b**, and **21** are outlined in Scheme 1. The optically pure alcohol **5** was acylated with isobutyric anhydride, and the benzyl group was removed by hydrogenolysis, followed by installation of the azetidine moiety by a modified Mitsunobu reaction to obtain the intermediate **6**. Successive removal of the isobutyryl group and the Boc group from **6** gave the desired product **7**. For compound **10** with a longer side chain, alcohol **5** was subjected to standard Swern oxidation and Wittig reaction to furnish the α, β -unsaturated ester **8**. After removal of the benzyl group and saturation of the double bond by catalytic hydrogenation in the same step, the azetidine moiety was installed to give the ester **9**. Reduction of the ester group in the intermediate **9** and subsequent removal of the Boc group gave compound **10**. Hydrolysis of the ester **9** and then removal of the Boc group furnished the carboxylic acid compound **11**. For the terminal trifluoromethoxy compound **14**, the starting alcohol **12** was converted to dithiocarbonate, followed by reaction with 70% HF/pyridine to give hydroxypyridine **13** after removal of the benzyl group.¹⁹ Installation of the azetidine moiety and subsequent removal of Boc group gave the desired product **14**. The carbamate analogues **19a** and **19b** were prepared from the previously reported alcohol **15**,¹⁵ which was transformed to the corresponding iodide, followed by nucleophilic substitution with sodium azide to give the intermediate **18**. Reduction of the azide group in compound **18** and subsequent reaction with various chloroformates afforded the desired products after removal of the Boc protecting group. Similarly, the iodide **16** was substituted by morpholine, piperidine, or piperazine and the Boc group then removed to give **17a**, **17b**, and **17c**, respectively. Compound **21**, the diastereoisomer of **17b**, was synthesized from alcohol **20** by the same sequence of steps. All target compounds were isolated as their trifluoroacetate salts.

On the other hand, pyrrolidine, a higher homologue of azetidine and a common heterocyclic building block in

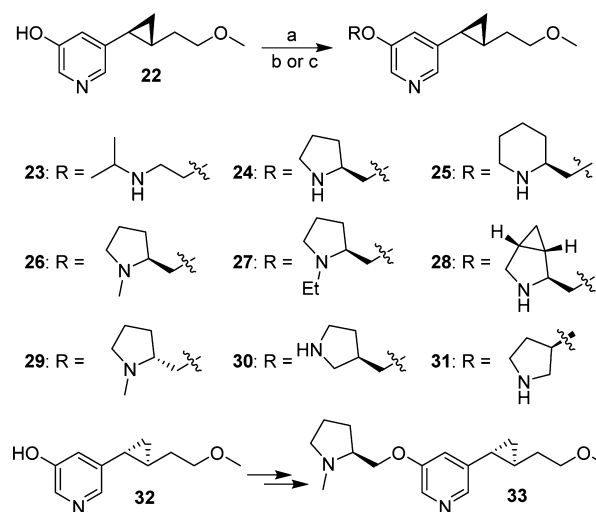
Scheme 1^a

^aReagents and conditions: (a) isobutyric anhydride, cat. 4-(dimethylamino)pyridine (DMAP), Et₃N, CH₂Cl₂, rt; (b) 10% Pd/C, H₂, EtOAc/MeOH, rt; (c) 1-(*tert*-butoxycarbonyl)-(2*S*)-azetidinylnmethanol, azodicarbonyldipiperidine (ADDP), P(*n*-Bu)₃, PhMe, 0 °C to rt; (d) NaOMe, MeOH, 40 °C; (e) CF₃COOH, CH₂Cl₂, rt; (f) (i) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -78 °C, (ii) Ph₃P=CHCO₂CH₃, THF, 0 °C to rt; (g) LiAlH₄, THF, rt; (h) 2N NaOH, MeOH/THF (1:1), rt; (i) (i) NaH, CS₂, DMF, 0 °C to rt, (ii) MeI, rt; (j) 70% HF/pyridine, 1,3-dibromo-5,5-dimethylhydantoin (DBH), CH₂Cl₂, -78 °C; (k) I₂, PPh₃, imidazole, 0 °C to rt; (l) morpholine, piperidine, or piperazine, CH₃CN; (m) NaN₃, DMF, 60 °C; (n) 10% Pd/C, H₂, ethanol, rt; (o) ethyl or isopropyl chloroformate, Et₃N, 0 °C to rt.

medicinal chemistry, was chosen to replace the azetidine ring and thus furnish a variety of pyrrolidine-containing derivatives. The target molecules 23–31 were prepared through a previously reported route employing the hydroxypyridine intermediate 22 as the starting material (Scheme 2).¹⁵ Coupling of 22 with various alcohols to afford the corresponding ethers was brought about using the Mitsunobu reaction. Removal of the Boc group from the coupling intermediates or reduction of the Boc or acetyl group to the corresponding methyl or ethyl groups, respectively, gave the desired products as their trifluoroacetate salts. Compound 33, the diastereoisomer of 26, was synthesized from alcohol 32 by the same sequence of steps.

RESULTS AND DISCUSSION

In Vitro Characterization: Radioligand Binding Studies. All of the synthesized compounds were first assayed for [³H]epibatidine binding competition at seven, heterologously expressed, rat nAChR subtypes. As shown in Table 1, these compounds generally demonstrated favorable subtype selectivity for β₂*-nAChRs (α₂β₂-, α₃β₂-, α₄β₂-, and α₄β₂*-nAChRs) over β₄*-nAChRs (α₂β₄-, α₃β₄-, and α₄β₄-nAChRs) compared to nicotine. Compound 7 exhibited subnanomolar binding affinity at both α₄β₂- and α₄β₂*-nAChRs, while the K_i values were slightly higher for compound 3. In contrast, the activity for α₄β₂- or α₄β₂*-nAChRs was

Scheme 2^a

^aReagents and conditions: (a) azodicarbonyldipiperidine (ADDP), P(*n*-Bu)₃, alcohol, PhMe, 0 °C to rt; (b) CF₃COOH, CH₂Cl₂, rt; (c) LiAlH₄, THF, reflux.

retained when extending the side chain in compound 3 by one more carbon atom (compound 10). In view of the existing pharmacological data for the cyclopropane ligands¹⁵ combined

Table 1. Affinities of Compounds 7, 10, 11, 14, 17a–c, 19a–b, and 21 for Rat nAChR Subtypes Defined by Competition for [³H]Epibatidine Binding

compd	K_i (nM) ^a						
	$\alpha 2\beta 2$	$\alpha 2\beta 4$	$\alpha 3\beta 2$	$\alpha 3\beta 4$	$\alpha 4\beta 2$	$\alpha 4\beta 2^{*b}$	$\alpha 4\beta 4$
7	0.2	233.4	2.8 ± 0.5	6119	0.2	0.7 ± 0.1	78.1 ± 11.9
10	0.1	211.8	3.8 ± 0.6	7514	0.1	0.4 ± 0.1	88.1 ± 10.4
11	0.5 ± 0.1	318	10.2 ± 1.9	4290	0.2	1.6 ± 0.3	75.7 ± 16
14	1.1 ± 0.1	20.2 ± 4.1	17 ± 3.3	612.7	0.8 ± 0.1	3.5 ± 0.6	4.2 ± 0.3
17a	15.3 ± 1.1	NA	224	NA	11.3 ± 0.9	9.8 ± 2.1	NA
17b	14.9 ± 3.5	>10 ⁴	403	NA	4.6 ± 0.4	10.4 ± 1.4	2930
17c	65.3 ± 14.4	NA	6.5 ± 1.9	NA	2.1 ± 0.3	1.7 ± 0.5	6237
19a	0.5 ± 0.1	203	8.9 ± 2.3	8830	0.4 ± 0.1	1.0 ± 0.1	125
19b	0.6 ± 0.1	453	11.1 ± 3.7	NA	0.5 ± 0.1	1.4 ± 0.2	158
21	89.7 ± 16	7850	1190	NA	56.1 ± 7.8	91.5 ± 14.3	2700
3 ^c	0.1	249	3.0	6520	0.1	0.5	82.6
4 ^c	0.1	236	2.4	NA	0.1	0.3	50.2
nicotine ^d	5.5	70	29	260	4.9	9.8	23
saz-A ^e	0.087	210	0.38	1900	0.062	0.17	52

^aSee Experimental Section. SEM values are not provided for K_i values >100 nM. ^b $\alpha 4\beta 2^{*}$, prepared from rat forebrain. ^c K_i values for compounds 3 and 4 are obtained from ref 15. ^d K_i values for nicotine are taken from the PDSP Assay Protocol Book (<http://pdsp.med.unc.edu/>). ^e K_i values for sazetidine-A were obtained from the literature.²² NA: not active, defined as <50% binding in the primary assay at 10 μ M.

Table 2. Affinities of Compounds 23–31 and 33 for Rat nAChR Subtypes Defined by Competition for [³H]Epibatidine Binding

compd	K_i (nM) ^a						
	$\alpha 2\beta 2$	$\alpha 2\beta 4$	$\alpha 3\beta 2$	$\alpha 3\beta 4$	$\alpha 4\beta 2$	$\alpha 4\beta 2^{*b}$	$\alpha 4\beta 4$
23	NA	NA	NA	NA	2176	NA	NA
24	0.5 ± 0.1	612	17.3 ± 5.3	NA	0.4 ± 0.1	1.1 ± 0.2	161
25	18.1 ± 2.3	>10 ⁴	2534	NA	38.8 ± 7	221	>10 ⁴
26	0.2	129.5	21 ± 5.7	NA	0.3	0.5 ± 0.1	584.8
27	167.9	NA	NA	NA	49.5 ± 8.9	173.7	NA
28	43.8 ± 10.7	NA	2094	NA	28.5 ± 5.6	193.8	NA
29	12.9 ± 1.7	9959	472.5	NA	21.7 ± 6.6	86.6 ± 15.3	9475
30	0.04	50.8 ± 10.5	0.8 ± 0.2	2074	0.05	0.2	30 ± 4.3
31	6.1 ± 0.6	NA	191.5	NA	90.9 ± 27	40.7 ± 5.3	NA
33	3.5 ± 0.5	546.6	137.3	NA	3.3 ± 1	15.7 ± 2.8	234.1
4 ^c	0.1	236	2.4	NA	0.1	0.3	50.2
nicotine ^d	5.5	70	29	260	4.9	9.8	23
saz-A ^e	0.087	210	0.38	1900	0.062	0.17	52

^aSee Experimental Section. SEM values are not provided for K_i values >100 nM. ^b $\alpha 4\beta 2^{*}$, prepared from rat forebrain. ^c K_i values for compound 4 are obtained from ref 15. ^d K_i values for nicotine are taken from the PDSP Assay Protocol Book (<http://pdsp.med.unc.edu/>). ^e K_i values for sazetidine-A were obtained from the literature.²² NA: not active, defined as <50% binding in the primary assay at 10 μ M.

with the knowledge obtained from the isoxazolopyridine ether analogues,¹⁸ a two-carbon alkyl linker between the chiral cyclopropane and the terminal functional groups was optimal for biological activity. In reference to the terminal hydroxyl group, an at least 2-fold decline in binding affinities at $\beta 2^{*}$ -nAChRs was observed when converting the hydroxyl group in compound 3 to a carboxylic acid (compound 11). The *O*-methylation of compound 3 did not alter binding affinities and selectivity for $\beta 2^{*}$ -nAChRs. Moreover, this group would preclude the possible metabolic liability of the hydroxyl group, which can undergo oxidation and/or bioconjugation reactions.²⁰ The replacement of the methoxy group in compound 4 with a more lipophilic and electron-withdrawing trifluoromethoxy moiety (compound 14) caused a 7-fold decrease in binding affinity for $\alpha 4\beta 2$ - or $\alpha 4\beta 2^{*}$ -nAChRs and significantly decreased the subtype selectivity for $\beta 2^{*}$ - over $\beta 4^{*}$ -nAChRs. As previously reported, derivation of the hydroxyl group in compound 3 to a variety of carbamate groups in general maintained the high binding affinities at $\beta 2^{*}$ -

nAChRs. The reversed carbamate derivatives 19a and 19b also exhibited subnanomolar to low nanomolar binding affinities at $\beta 2^{*}$ -nAChRs, and binding affinities decreased as the size of substituents at the carbamate oxygen increased. Introduction of a saturated six-membered heterocycle at the end of the side chain to replace the hydroxyl group (compounds 17a, 17b, and 17c) resulted in at least 2-fold less potency at $\beta 2^{*}$ -nAChRs, while a more polar heterocyclic ring was preferred in this position (potency at $\alpha 4\beta 2$ -nAChRs: piperazine > morpholine > piperidine). In addition, compound 21, bearing a (1*R*,2*S*)-configured cyclopropane ring, was found to be about 10-fold less active than its diastereoisomer 17b at both $\alpha 4\beta 2$ - and $\alpha 4\beta 2^{*}$ -nAChRs. Collectively, as shown in Table 1, these compounds bearing a varied cyclopropane-containing side chain generally were very potent at both $\alpha 4\beta 2$ - and $\alpha 4\beta 2^{*}$ -nAChRs, suggesting that variations in the terminal substituents on the right-hand side chain do not significantly affect their binding affinities, which is consistent with the previous finding²¹ that the cyclopropane-containing side chain points

Table 3. Potencies and Efficacies of Ligand Agonism and Inactivation of Human $\alpha 4\beta 2$ - and $\alpha 6^*$ - nAChRs^a

compd	agonism				inactivation			
	$\alpha 4\beta 2$		$\alpha 6^*$		$\alpha 4\beta 2$		$\alpha 6^*$	
	EC ₅₀ (nM)	efficacy at HS (%) ^{b,c}	EC ₅₀ (nM)	efficacy (%)	IC ₅₀ (nM)	efficacy (%) ^b	IC ₅₀ (nM)	efficacy (%)
24	23	29 ± 2.5	51.5	4.9 ± 0.6	108	84 ± 4.6	223	79 ± 5.6
26	14.4	49 ± 5.2	9.0	6.5 ± 0.4	20	88 ± 2.7	23.9	87 ± 1.5
30	6.2	92 ± 1.5	23.5	12 ± 0.6	5.5	70 ± 3.3	8.3	76 ± 0.7
4	17.5	60 ± 5.9	7.4	6.2 ± 0.3	5.6	71 ± 5.5	9.7	80 ± 3.5
nicotine	290	125 ± 10	127	56 ± 0.8	430	92 ± 2.1	84.8	26 ± 1.0

^aSee Experimental Section. ^bEfficacies were measured in a mixture of HS and LS $\alpha 4\beta 2$ -nAChRs. ^cEfficacy values for actions at HS $\alpha 4\beta 2$ -nAChR were extrapolated from results obtained using sazetidine-A as a full agonist at HS $\alpha 4\beta 2$ -nAChR (see Supporting Information for details).

outward from the binding pocket without engaging in any significant polar interactions based on the analysis of the cocrystal structure of the acetylcholine binding protein from *Capitella teleta* in complex with compound 3.

Next, we fixed the structure of the right-hand side chain and explored a series of functional groups to replace the somewhat labile azetidone unit. As shown in Table 2, replacement of the azetidone ring as in compound 4 with an aliphatic amine (compound 23) resulted in the complete loss of activity at all of the nAChR subtypes tested with the exception of weak binding affinity at $\alpha 4\beta 2$ -nAChRs (2.2 μ M), suggesting that the conformationally restricted azetidone ring plays an important role in maintaining high binding affinity for the nAChRs. As the smallest saturated azaheterocycle aziridine is highly reactive and thus an unsuitable replacement for the azetidone, we thus chose to examine the higher homologue of azetidone and thus prepared the pyrrolidine analogue 24. This compound exhibited subnanomolar to low nanomolar binding affinity at the $\alpha 4\beta 2$ - and $\alpha 4\beta 2^*$ -nAChRs and good selectivity for $\beta 2^*$ -nAChRs, similar to the parent compound 4. Further ring expansion of the pyrrolidine ring to a piperidine ring (compound 25) caused more than a 150-fold drop in binding affinities at $\beta 2^*$ -nAChRs. The presence of an *N*-methyl group, as in compound 26, increased binding at the $\alpha 4\beta 2$ - or $\alpha 4\beta 2^*$ -nAChRs relative to compound 24, however, an *N*-ethyl group (compound 27) remarkably reduced binding affinity for all seven nAChRs. The fusion of a cyclopropane ring to the pyrrolidine ring (compound 28) also caused a sharp decrease in binding potency toward all nAChR subtypes tested, indicating that the space in the cation-binding pocket accommodating the pyrrolidine ring is limited. With respect to stereoselectivity, compound 26 was at least 70-fold or 10-fold more potent at the $\alpha 4\beta 2$ - and $\alpha 4\beta 2^*$ -nAChRs than its diastereoisomers 29 and 33, respectively, indicating that an (*S*)-configuration of the *N*-methyl-pyrrolidine and a cyclopropane ring with the (1*S*,2*R*)-configuration were preferred. Shifting the nitrogen atom in the pyrrolidine ring of compound 24 to the less-substituted α -methylene position gave compound 30, having the highest binding affinity at $\beta 2^*$ -nAChRs in this series while exhibiting weak activity at $\alpha 3\beta 4$ -nAChRs (2 μ M). Modification of compound 30 by deletion of the methylene group connecting the pyrrolidine ring with the 3-pyridyl ether framework led to compound 31, which showed a significant decrease in binding potency at all nAChR subtypes tested. In spite of the fact that some of the compounds listed in Table 2 had only modest activity at $\alpha 4\beta 2$ -nAChRs, most of them generally presented good subtype-selectivity for $\beta 2^*$ -nAChRs over $\beta 4^*$ -nAChRs compared to nicotine. On the basis of previous findings that $\alpha 4\beta 2$ -nAChR ligands featuring distinct chemical scaffolds can

engage in similar receptor interactions,²¹ we anticipate that the cyclopropane-containing side chain could be appended to other $\alpha 4\beta 2$ -nAChR ligands with diverse scaffolds to improve their subtype selectivity.

In Vitro Functional Characterization. In functional studies, the most promising compounds, 24, 26, and 30, were tested using ⁸⁶Rb⁺ ion flux assays in SH-EP1- $\alpha 4\beta 2$ (expressing human $\alpha 4\beta 2$ -nAChRs), SH-EP1-h($\alpha 6/3$) $\beta 2\beta 3$ (expressing human ($\alpha 6/3$) $\beta 2\beta 3$ -nAChRs; referred to as $\alpha 6^*$ -nAChRs), SH-SY5Y ($\alpha 3\beta 4^*$ -nAChRs), or TE671/RD ($\alpha 1\beta 1\gamma\delta$ -nAChRs) cells. Abilities of ligands to stimulate ion flux were characterized as EC₅₀ values with efficacies normalized to that of carbamylcholine. Abilities of chronically administered ligands to inhibit ion flux were characterized as inactivation IC₅₀ values following the 10 min preincubation used to characterize receptor stimulation.

These pyrrolidine analogues were found to be highly potent, partial agonists at the mixture of high sensitivity (HS) and low sensitivity (LS) $\alpha 4\beta 2$ -nAChRs and to have weak if any agonist activity at $\alpha 3\beta 4^*$ - or $\alpha 1\beta 1\gamma\delta$ -nAChRs. Their EC₅₀ values were comparable to that found for parent compound 4, with the exception of compound 30, possessing an EC₅₀ value in the single-digit nanomolar range, and the trend was consistent with results observed in the binding studies (Table 3 and Supporting Information Figure S2). Response magnitude (efficacy) was 29–92% relative to a maximally efficacious concentration of the full agonist, carbamylcholine, for action at HS $\alpha 4\beta 2$ -nAChRs. At LS $\alpha 4\beta 2$ -nAChRs, these ligands had no measurable efficacy as agonists. Their inactivation of nAChR function was most potent for $\alpha 4\beta 2$ -nAChRs and weak or absent for actions at $\alpha 3\beta 4^*$ - or $\alpha 1\beta 1\gamma\delta$ -nAChRs. IC₅₀ values for compounds 26 and 30 were 4-fold higher or similar to that of compound 4, respectively, while compound 24 was 20-fold less potent. The absence or weakness of agonist or antagonist activity at ganglionic $\alpha 3\beta 4^*$ - or muscle-type $\alpha 1\beta 1\gamma\delta$ -nAChRs indicates that the occurrence of peripheral side effects is unlikely. All of the pyrrolidine analogues had agonist activity at $\alpha 6^*$ -nAChRs with EC₅₀ values in the range of 9.0–51.5 nM. Their agonism efficacies were relatively low, ranging from 4.9 to 12% of the maximum response to carbamylcholine. This low-efficacy agonism correlates with inactivation efficacies that are much higher than that seen for nicotine (Table 3). Inactivation IC₅₀ values of these ligands were lower than 24 nM, with the exception of compound 24, having an IC₅₀ value greater than 220 nM.

In Vivo Behavioral Pharmacology. To determine whether the excellent potency of the pyrrolidine analogues at $\alpha 4\beta 2$ -nAChRs would translate into antidepressant-like efficacy in a behavioral model, compounds 24, 26, and 30 were

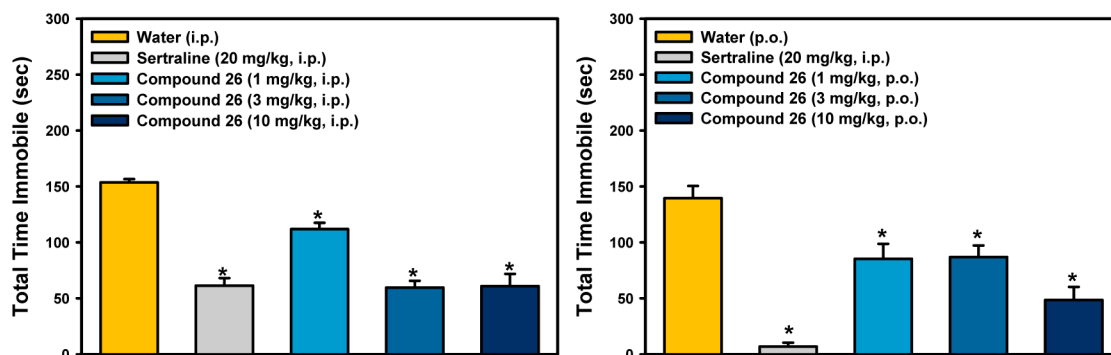


Figure 2. Mouse forced swim data for compound 26. The selective serotonin reuptake inhibitor, sertraline, produced the expected decrease in immobility. ANOVAs: $F(4,43) = 34.20$, $p < 0.001$ (left); $F(4,44) = 20.75$, $p < 0.001$ (right). *Fisher's PLSD posthoc test: $p_s < 0.05$ vs vehicle. $n = 9-10$ /group.

investigated in the mouse forced swim test,²³ 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 mice spend immobile. Mice were administered nicotinic ligands or the selective serotonin reuptake inhibitor, sertraline, as a positive control (20 mg/kg). Compound 24 showed no significant effects in the forced swim test when administered intraperitoneally at a dose of 1, 3, or 10 mg/kg (Supporting Information Figure S3), likely due to its lower inhibitory potency at both $\alpha 4\beta 2$ - and $\alpha 6^*$ -nAChRs relative to the parent compound 4. On the other hand, the *N*-methylpyrrolidine analogue 26 demonstrated an antidepressant-like effect when administered intraperitoneally or orally, with a significant reduction in immobility at the minimal dose of 1 mg/kg (Figure 2). It appears that the *N*-methyl group in compound 26 is crucial to maintaining in vivo activity. Intraperitoneal administration of the most potent compound 30 in vitro, however, produced abnormal behavioral effects, such as sedation and tremor at 5 mg/kg, and resulted in death of the mice during the pretreatment or trial at 10 mg/kg.

Preliminary ADMET Study. Encouraged by the favorable biological data, we submitted compound 26 for preliminary ADMET tests (Table 4).²⁴ When incubated with human or mouse (CD-1) liver microsomes, at least 90% of compound 26 remained unchanged after 1 h incubation at 0.1 μ M. Incubation

with human hepatocytes resulted in more than 85% of compound 26, remaining unchanged after 2 h incubation at 1 μ M. In the presence of compound 26 at a concentration of 10 μ M, none of the CYP isoforms tested (CYP1A, CYP2C9, CYP2C19, and CYP3A) showed more than 30% inhibition, suggesting minimal adverse drug–drug interactions, with the exception of 44% inhibition of the CYP2D6 isoform. Plasma protein binding (PPB) assays were conducted with both human and mouse (CD-1) plasma at a concentration of 10 μ M of compound 26. In human plasma, 31% binding was observed, while in mouse plasma the bound fraction was 11%. In the bidirectional Caco-2 cell permeability assay, compound 26 was found to exhibit high permeability and had an acceptable efflux ratio of 0.2. In addition, the efflux ratio increased to 0.7 when compound 26 was incubated in the presence of verapamil, a P-glycoprotein (P-gp) inhibitor, indicating that the test compound is not a P-gp substrate. The bacteria reversion mutation assay (Ames Test) was used to evaluate the mutagenic potential of compound 26. At all concentrations tested (5, 10, 50, and 100 μ M), no positive significance was observed for compound 26 in all tested strains (TA98, TA100, TA1535, and TA1537) in the presence and absence of a liver metabolic activation system (S9 fraction), with the exception of higher concentrations (50 and 100 μ M), at which compound 26 exhibited positive significance when tested using strain TA1537 in the presence of the S9 fraction. However, when the Ames genotoxicity testing was repeated using the tartrate salt of compound 26 and the TA1537 tester strain with or without metabolic activation, the compound was found not to be mutagenic at all concentrations tested (up to 3×10^5 μ M). Cardiotoxicity associated with the inhibition of human hERG was also evaluated at three test concentrations (0.1, 1, and 10 μ M). Compound 26 led to 4%, 7%, and 23% inhibition of tail current, respectively. Moreover, compound 26 was found to be quite chemically stable, as it could be stored at room temperature for several months showing little or no sign of decomposition.

Table 4. ADMET Properties of Compound 26

assay	compound 26
liver microsomes (human or mouse, 0.1 μ M) (%)	103.3 or 90.1
cryopreserved hepatocytes (human, 1 μ M) (%)	85.7
CYP inhibition (10 μ M) (%)	
CYP1A	17
CYP2C9	30
CYP2C19	21
CYP2D6	44
CYP3A	10
hERG inhibition (0.1, 1, 10 μ M) (%)	4, 7, 23
plasma protein binding (human or mouse, 10 μ M) (%)	31 or 11
A–B permeability (Caco-2, pH 7.4/7.4) (cm/s)	90.9×10^{-6}
A–B permeability (Caco-2, pH 7.4/7.4 + verapamil) (cm/s)	79.2×10^{-6}
B–A permeability (Caco-2, pH 7.4/7.4) (cm/s)	18.4×10^{-6}
B–A permeability (Caco-2, pH 7.4/7.4 + verapamil) (cm/s)	55.2×10^{-6}
Ames test (strain TA98, TA100, TA1535, TA1537, with/without S9)	negative

CONCLUSION

In this report, we disclose the synthesis, pharmacological characterization, and structure–activity relationships of a series of cyclopropane-containing nAChR ligands. Modifications to both the azetidine ring present in the lead compound 3 and its right-hand side chain have been explored to improve compound druggability. We successfully identified potent and highly selective $\alpha 4\beta 2$ -nAChR partial agonists 24, 26, and 30

that exhibited comparable or improved pharmacological parameters in comparison to compound **3** in [³H]epibatidine binding studies as well as functional assays based on ⁸⁶Rb⁺ ion flux measurements. In addition, minor changes such as incorporation of a methyl group (**24** vs **26**) or alteration of the position of the nitrogen atom as in the pyrrolidines **24** vs **30** may significantly affect the in vivo activity of these nicotinic ligands. Compound **26** demonstrated favorable antidepressant-like effects in the mouse forced swim test while possessing a favorable ADMET profile and chemical stability. Together, these findings support compound **26** as a promising lead candidate that deserves further evaluation in the design of antidepressants possessing a unique mechanism of action.

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 the 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 H₂O (¹H NMR), and the δ 77.23 signal of CDCl₃ (¹³C 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 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 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 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 CF₃COOH) in 18 min. See Supporting Information for detailed experimental procedures and NMR spectral data (¹H and ¹³C) of all intermediates.

3-[(2(S)-Azetidiny)methoxy]-5-[(1S,2S)-2-(hydroxymethyl)cyclopropyl]pyridine Trifluoroacetate (7). ¹H NMR (D₂O): δ 8.36 (s, 1H), 8.28 (s, 1H), 7.91 (s, 1H), 4.98 (m, 1H), 4.53 (d, J = 3.6 Hz, 2H), 4.12 (m, 2H), 3.69 (m, 1H), 3.54 (m, 1H), 2.70 (q, J = 8.4 Hz, 2H), 2.12 (m, 1H), 1.64 (m, 1H), 1.22 (t, J = 7.2 Hz, 2H). ¹³C NMR (D₂O): δ 162.3 (TFA), 155.9, 145.2, 132.2, 128.3, 125.7, 115.8 (TFA), 67.2, 64.1, 58.2, 43.3, 25.4, 19.8, 18.1, 14.1. [α]_D²⁰ = +27.7 (c 0.48, MeOH). Anal. Calcd for C₁₃H₁₈N₂O₂·2.15CF₃COOH·0.85H₂O: C, 42.0; H, 4.45; F, 24.77; N, 5.66. Found: C, 41.89; H, 4.18; F, 24.51; N, 5.55. HPLC purity = 99.1%; t_R = 6.1 min.

3-[(2(S)-Azetidiny)methoxy]-5-[(1S,2S)-2-(3-hydroxypropyl)cyclopropyl]pyridine Trifluoroacetate (10). ¹H NMR (D₂O): δ 8.32 (s, 1H), 8.22 (s, 1H), 7.84 (s, 1H), 4.98 (m, 1H), 4.53 (d, J = 4.0 Hz, 2H), 4.11 (m, 2H), 3.63 (t, J = 6.6 Hz, 2H), 2.70 (q, J = 8.4 Hz, 2H), 1.93 (m, 1H), 1.69 (m, 2H), 1.56–1.44 (m, 2H), 1.31 (m, 1H), 1.14 (m, 2H). ¹³C NMR (D₂O): δ 162.4 (TFA), 155.8, 146.6, 131.9, 127.8, 125.2, 115.8 (TFA), 67.1, 60.9, 58.2, 43.3, 30.4, 29.0, 25.0, 19.8, 19.7, 16.9. [α]_D²⁰ = +10.0 (c 1.1, MeOH). Anal. Calcd for C₁₅H₂₂N₂O₂·2.55CF₃COOH·0.75H₂O: C, 42.61; H, 4.63; F, 25.65; N, 4.94. Found: C, 42.70; H, 4.51; F, 25.55; N, 4.97. HPLC purity = 99.2%; t_R = 6.1 min.

3-[(1S,2S)-2-[5-[(S)-Azetidin-2-yl]methoxy]pyridin-3-yl]cyclopropyl]propanoic Acid Trifluoroacetate (11). ¹H NMR (D₂O): δ 8.34 (s, 1H), 8.22 (s, 1H), 7.85 (s, 1H), 4.98 (m, 1H), 4.52 (d, J = 4.0 Hz, 2H), 4.12 (m, 2H), 2.70 (q, J = 8.4 Hz, 2H), 2.53 (m, 2H), 1.99 (m, 1H), 1.84 (m, 1H), 1.68 (1H), 1.33 (m, 1H), 1.17 (m, 2H). ¹³C NMR (D₂O): δ 178.1, 162.4 (TFA), 155.8, 146.1, 132.0, 127.9, 125.4, 115.9 (TFA), 67.1, 58.2, 43.3, 33.1, 28.2, 24.2, 19.8, 19.7, 16.3. [α]_D²⁰ = +32.5 (c, 0.36 MeOH). Anal. Calcd for C₁₅H₂₀N₂O₃·2.15CF₃COOH·0.9H₂O: C, 43.11; H, 4.49; F, 22.79; N, 5.21. Found: C, 42.96; H, 4.24; F, 22.53; N, 5.16. HPLC purity = 99.8%; t_R = 7.0 min.

3-[(2(S)-Azetidiny)methoxy]-5-[(1S,2R)-2-(2-trifluoromethoxyethyl)cyclopropyl]pyridine Trifluoroacetate (14). ¹H NMR (D₂O): δ 8.33 (s, 1H), 8.22 (s, 1H), 7.85 (s, 1H), 4.96 (m, 1H), 4.51 (d, J = 3.6 Hz, 2H), 4.18–4.03 (m, 4H), 2.68 (q, J = 8.4 Hz, 2H), 2.02 (m, 1H), 1.92 (m, 1H), 1.73 (m, 1H), 1.37 (m, 1H), 1.19 (m, 2H). ¹³C NMR (D₂O): δ 162.5 (TFA), 156.2, 146.3, 132.4, 128.4, 125.9, 121.5 (q, J_{C-F} = 251.1 Hz), 116.2 (TFA), 67.7, 67.6, 58.6, 43.6, 32.1, 21.8, 20.2, 19.7, 16.1. ¹⁹F NMR (D₂O): δ –60.2, –75.6. [α]_D²⁰ = +28.6 (c 0.14, MeOH). Anal. Calcd for C₁₅H₁₉F₃N₂O₃·2.4CF₃COOH·0.5H₂O: C, 39.7; H, 3.77; F, 32.35; N, 4.68. Found: C, 39.47; H, 3.52; F, 32.14; N, 4.49. HPLC purity = 99.8%; t_R = 9.7 min.

3-[(2(S)-Azetidiny)methoxy]-5-[(1S,2R)-2-(2-(piperidin-1-yl)ethyl)cyclopropyl]pyridine Trifluoroacetate (17a). ¹H NMR (D₂O): δ 8.37 (s, 1H), 8.25 (s, 1H), 7.87 (s, 1H), 4.99 (m, 1H), 4.54 (s, 2H), 4.18–4.08 (m, 2H), 3.52 (d, J = 6.0 Hz, 2H), 3.23 (t, J = 8.0 Hz, 2H), 2.93 (t, J = 8.0 Hz, 2H), 2.67 (dd, J = 12.0, 4.0 Hz, 2H), 2.01 (m, 1H), 1.97–1.70 (m, 7H), 1.49–1.46 (m, 1H), 1.35–1.33 (m, 1H), 1.24–1.19 (m, 2H). ¹³C NMR (D₂O): δ 162.7 (TFA), 156.2, 145.6, 132.4, 128.5, 126.0, 116.3 (TFA), 67.5, 58.6, 53.1, 27.5, 22.7, 21.5, 21.0, 20.2, 19.8, 16.3. [α]_D²⁰ = +43.6 (c = 3.4, MeOH). Anal. Calcd for C₁₉H₂₉N₃O·3.1CF₃COOH·0.45H₂O: C, 44.71; H, 4.91; F, 26.1; N, 6.21. Found: C, 44.83; H, 4.77; F, 25.97; N, 6.14. HPLC purity = 99.8%; t_R = 4.1 min.

3-[(2(S)-Azetidiny)methoxy]-5-[(1S,2R)-2-(2-(morpholin-4-yl)ethyl)cyclopropyl]pyridine Trifluoroacetate (17b). ¹H NMR (D₂O) δ 8.33 (s, 1H), 8.21 (s, 1H), 7.82 (s, 1H), 4.96–4.94 (m, 1H), 4.50 (d, J = 4.0 Hz, 2H), 4.14–4.05 (m, 4H), 3.78 (t, J = 12.4 Hz, 2H), 3.50 (d, J = 12.8 Hz, 2H), 3.29 (t, J = 8.0 Hz, 2H), 3.16 (dt, J = 12.4, 3.2 Hz, 2H), 2.67 (q, J = 8.8 Hz, 2H), 2.05–2.03 (m, 1H), 1.91–1.85 (m, 2H), 1.30 (m, 1H), 1.21–1.15 (m, 2H). ¹³C NMR (D₂O): δ 162.2 (TFA), 155.8, 145.1, 132.0, 128.1, 125.7, 115.9 (TFA), 67.1, 63.3, 58.2, 55.9, 51.2, 43.2, 26.7, 20.8, 19.8, 19.4, 15.9. [α]_D²⁰ = +43.5 (c = 0.8, MeOH). Anal. Calcd for C₁₈H₂₇N₃O₂·3.4CF₃COOH·0.4H₂O: C, 41.82; H, 4.41; F, 27.2; N, 5.9. Found: C, 41.91; H, 4.31; F, 27.09; N, 5.96. HPLC purity = 99.6%; t_R = 3.9 min.

3-[(2(S)-Azetidiny)methoxy]-5-[(1S,2R)-2-(2-(piperazin-1-yl)ethyl)cyclopropyl]pyridine Trifluoroacetate (17c). ¹H NMR (D₂O): δ 8.37 (s, 1H), 8.25 (s, 1H), 7.85 (s, 1H), 4.98 (m, 1H), 4.53 (d, J = 4.0 Hz, 2H), 4.17–4.05 (m, 2H), 3.65 (br s, 8H), 3.44 (t, J = 8.4 Hz, 2H), 2.71 (q, J = 8.4 Hz, 2H), 2.09 (m, 1H), 2.02–1.87 (m, 2H), 1.36 (m, 1H), 1.26–1.18 (m, 2H). ¹³C NMR (D₂O): 162.3 (TFA), 155.9, 145.0, 132.1, 128.2, 125.7, 115.9 (TFA), 67.2, 58.2, 55.9, 48.0, 43.3, 40.2, 26.9, 20.6, 19.8, 19.4, 15.9. [α]_D²⁰ = +36.4 (c 1.52, MeOH). Anal. Calcd for C₁₈H₂₈N₄O·4.05CF₃COOH·0.45H₂O: C, 39.86; H, 4.22; F, 29.36; N, 7.12. Found: C, 39.97; H, 4.23; F, 29.26; N, 7.03. HPLC purity = 99.7%; t_R = 4.8 min.

Ethyl [2-[(1R,2S)-2-[5-[(S)-Azetidin-2-yl]methoxy]pyridin-3-yl]cyclopropyl]ethyl]carbamate Trifluoroacetate (19a). ¹H NMR (D₂O): δ 8.32 (s, 1H), 8.21 (s, 1H), 7.82 (s, 1H), 4.97 (m, 1H), 4.51 (d, J = 4.4 Hz, 2H), 4.12 (m, 2H), 3.94 (m, 2H), 3.23 (m, 2H), 2.68 (q, J = 8.4 Hz, 2H), 1.94 (m, 1H), 1.70 (m, 1H), 1.50 (m, 1H), 1.27 (m, 1H), 1.15–1.10 (m, 5H). ¹³C NMR (D₂O): δ 162.6 (TFA), 158.7, 156.2, 146.7, 132.2, 128.2, 125.6, 116.3 (TFA), 67.5, 61.5, 58.6, 43.6, 40.0, 33.1, 23.2, 20.2, 20.0, 16.5, 13.7. [α]_D²⁰ = +35.0 (c 0.20, MeOH). Anal. Calcd for C₁₇H₂₅N₃O₃·2.6CF₃COOH·0.55H₂O: C, 42.61; H, 4.62; F, 23.68; N, 6.71. Found: C, 42.75; H, 4.58; F, 23.55; N, 6.63. HPLC purity = 99.8%; t_R = 6.8 min.

Isopropyl [2-[(1R,2S)-2-[5-[(S)-Azetidin-2-yl]methoxy]pyridin-3-yl]cyclopropyl]ethyl]carbamate Trifluoroacetate

(19b). ¹H NMR (D₂O): δ 8.32 (s, 1H), 8.20 (s, 1H), 7.81 (s, 1H), 4.96 (m, 1H), 4.65 (m, 1H), 4.50 (d, *J* = 3.2 Hz, 2H), 4.10 (m, 2H), 3.22 (m, 2H), 2.67 (q, *J* = 8.4 Hz, 2H), 1.93 (m, 1H), 1.69 (m, 1H), 1.50 (m, 1H), 1.26 (m, 1H), 1.12 (m, 8H). ¹³C NMR (D₂O): δ 162.5 (TFA), 158.3, 156.2, 146.7, 132.2, 128.2, 125.6, 116.2 (TFA), 69.3, 67.5, 58.6, 43.6, 39.9, 33.1, 23.1, 21.1, 20.2, 20.0, 16.6. $[\alpha]_{\text{D}}^{20} = +40.0$ (c 0.24, MeOH). Anal. Calcd for C₁₈H₂₇N₃O₃·2.35CF₃COOH·0.2H₂O: C, 45.07; H, 4.96; F, 22.14, N, 6.95. Found: C, 44.99; H, 4.87; F, 22.07; N, 6.95. HPLC purity = 99.7%; *t*_R = 7.3 min.

3-[(2(S)-Azetidiny)methoxy]-5-[(1R,2S)-2-(2-(morpholin-4-yl)ethyl)cyclopropyl]pyridine Trifluoroacetate (21). ¹H NMR (D₂O) δ 8.37 (s, 1H), 8.26 (s, 1H), 7.87 (s, 1H), 5.00–4.98 (m, 1H), 4.54 (d, 2H, *J* = 4.0 Hz), 4.17–4.08 (m, 4H), 3.82 (t, 2H, *J* = 12.4 Hz), 3.54 (d, 2H, *J* = 12.8 Hz), 3.34 (t, 2H, *J* = 8.0 Hz), 3.20 (dt, 2H, *J* = 12.4, 3.2 Hz), 2.71 (q, 2H, *J* = 8.8 Hz), 2.10–2.08 (m, 1H), 1.97–1.88 (m, 2H), 1.35 (m, 1H), 1.25–1.98 (m, 2H). ¹³C NMR (D₂O): δ 162.2 (TFA), 155.9, 145.1, 132.1, 128.1, 125.7, 115.9 (TFA), 67.1, 63.3, 58.2, 55.9, 51.2, 43.2, 26.7, 20.8, 19.8, 19.4, 15.9. $[\alpha]_{\text{D}}^{20} = -46.4$ (c 0.78, MeOH). Anal. Calcd for C₁₈H₂₇N₃O₂·3.4CF₃COOH·0.05H₂O: C, 42.19; H, 4.35; F, 27.45; N, 5.95. Found: C, 42.22; H, 4.38; F, 27.38; N, 5.97. HPLC purity = 99.9%; *t*_R = 3.9 min.

3-[2-(Isopropylamino)ethoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (23). ¹H NMR (D₂O): δ 8.14 (s, 1H), 8.07 (s, 1H), 7.66 (s, 1H), 4.35 (t, *J* = 4.0 Hz, 2H), 4.46–4.36 (m, 5H), 3.20 (s, 3H), 1.83 (s, 1H), 1.57 (s, 2H), 1.23 (d, *J* = 6.4 Hz, 6H), 1.16 (m, 1H), 1.02 (m, 2H). ¹³C NMR (D₂O): δ 162.4 (TFA), 156.1, 146.5, 132.2, 128.2, 125.6, 116.2 (TFA), 71.8, 64.9, 57.7, 51.1, 43.3, 32.6, 22.3, 19.8, 18.0, 16.6. $[\alpha]_{\text{D}}^{20} = +33.4$ (c = 0.9, MeOH). Anal. Calcd for C₁₆H₂₆N₂O₂·2.15CF₃COOH·0.8H₂O: C, 45.32; H, 5.57; F, 22.78; N, 5.21. Found: C, 45.06; H, 5.24; F, 22.63; N, 5.09. HPLC purity = 99.8%; *t*_R = 9.3 min.

3-[(2(S)-Pyrrolidinyl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (24). ¹H NMR (D₂O): δ 8.26 (s, 1H), 8.19 (s, 1H), 7.77 (s, 1H), 4.53 (dd, *J* = 10.4, 2.4 Hz, 1H), 4.33 (m, 1H), 4.11 (m, 1H), 3.56 (t, *J* = 6.4 Hz, 2H), 3.39 (t, *J* = 7.2 Hz, 2H), 3.32 (s, 3H), 2.27 (m, 1H), 2.12–2.06 (m, 2H), 1.94 (m, 2H), 1.68 (m, 2H), 1.28 (m, 1H), 1.12 (m, 2H). ¹³C NMR (D₂O): δ 162.6 (TFA), 156.1, 146.5, 132.3, 128.2, 125.6, 116.2 (TFA), 71.8, 67.6, 58.3, 57.7, 45.9, 32.6, 25.7, 23.4, 22.3, 19.8, 16.6. $[\alpha]_{\text{D}}^{20} = +47.3$ (c 0.28, MeOH). Anal. Calcd for C₁₆H₂₄N₂O₂·2.05CF₃COOH·0.05H₂O: C, 47.24; H, 5.16; F, 22.86; N, 5.48. Found: C, 47.30; H, 5.24; F, 22.75, N, 5.46. HPLC purity = 99.5%; *t*_R = 7.6 min.

3-[(2(S)-Piperidinyl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (25). ¹H NMR (D₂O): δ 8.22 (s, 1H), 8.16 (s, 1H), 7.74 (s, 1H), 4.39 (m, 1H), 4.24 (m, 1H), 3.61 (m, 1H), 3.54 (t, *J* = 6.4 Hz, 2H), 3.43 (m, 1H), 3.30 (s, 3H), 3.03 (m, 1H), 1.97–1.88 (m, 4H), 1.68–1.58 (m, 5H), 1.25 (m, 1H), 1.10 (m, 2H). ¹³C NMR (D₂O): δ 162.2 (TFA), 155.7, 146.1, 131.9, 127.8, 125.2, 115.7 (TFA), 71.4, 68.7, 57.3, 54.9, 44.2, 32.2, 23.9, 21.9, 21.2, 20.6, 19.4, 16.2. $[\alpha]_{\text{D}}^{20} = +49.3$ (c 0.53, MeOH). Anal. Calcd for C₁₇H₂₆N₂O₂·2.6CF₃COOH·1.05H₂O: C, 44.02; H, 5.11; F, 24.46, N, 4.62. Found: C, 44.04; H, 4.89; F, 24.27; N, 4.78. HPLC purity = 98.7%; *t*_R = 7.9 min.

3-[(1-Methyl-2(S)-pyrrolidinyl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (26). ¹H NMR (D₂O): δ 8.30 (s, 1H), 8.23 (s, 1H), 7.81 (s, 1H), 4.61 (dd, *J* = 10.8, 2.8 Hz, 1H), 4.45 (m, 1H), 3.95 (m, 1H), 3.76 (m, 1H), 3.59 (t, *J* = 6.4 Hz, 2H), 3.34 (s, 3H), 3.25 (m, 1H), 3.04 (s, 3H), 2.40 (m, 1H), 2.18 (m, 1H), 2.09 (m, 2H), 1.98 (m, 1H), 1.71 (m, 2H), 1.31 (m, 1H), 1.15 (m, 2H). ¹³C NMR (D₂O): δ 162.0 (TFA), 155.6, 146.2, 132.0, 127.8, 125.2, 115.7 (TFA), 71.4, 66.9, 66.0, 57.3, 56.8, 40.2, 32.2, 25.4, 21.9, 21.7, 19.4, 16.2. $[\alpha]_{\text{D}}^{20} = +37.0$ (c 0.27, MeOH). Anal. Calcd for C₁₇H₂₆N₂O₂·2.4CF₃COOH·0.4H₂O: C, 45.83; H, 5.15; F, 23.94; N, 4.9. Found: C, 45.64; H, 4.91; F, 23.8, N, 4.9. HPLC purity = 98.9%; *t*_R = 7.8 min.

3-[(1-Ethyl-2(S)-pyrrolidinyl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (27). ¹H NMR (D₂O): δ 8.30 (s, 1H), 8.23 (s, 1H), 7.80 (s, 1H), 4.57 (m, 1H), 4.44 (m, 1H), 4.05 (m, 1H), 3.73 (m, 1H), 3.61–3.54 (m, 3H), 3.35

(s, 3H), 3.24 (2H), 2.34 (m, 1H), 2.09–1.97 (m, 4H), 1.71 (m, 2H), 1.38–1.32 (m, 4H), 1.15 (m, 2H). ¹³C NMR (D₂O): δ 161.9 (TFA), 155.6, 146.2, 132.1, 127.8, 125.2, 115.9 (TFA), 71.5, 66.5, 65.5, 57.3, 53.8, 50.1, 32.2, 25.5, 22.0, 21.9, 19.4, 16.2, 9.6. $[\alpha]_{\text{D}}^{20} = +39.8$ (c 0.61, MeOH). Anal. Calcd for C₁₈H₂₈N₂O₂·2.1CF₃COOH·0.85H₂O: C, 47.68; H, 5.73; F, 21.4, N, 5.01. Found: C, 47.4; H, 5.42; F, 21.32; N, 4.91. HPLC purity = 99.3%; *t*_R = 8.0 min.

3-[[[(1S,2S,5R)-3-Azabicyclo[3.1.0]hexan-2-yl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (28). ¹H NMR (D₂O): δ 8.25 (s, 1H), 8.17 (s, 1H), 7.76 (s, 1H), 4.67 (d, *J* = 14.0 Hz, 1H), 4.35–4.27 (m, 2H), 3.55–3.45 (m, 4H), 3.30 (s, 3H), 1.94–1.88 (m, 3H), 1.66 (m, 2H), 1.26 (m, 1H), 1.10 (m, 2H), 0.84 (m, 1H), 0.59 (m, 1H). ¹³C NMR (D₂O): δ 162.4 (TFA), 156.2, 146.5, 132.2, 128.2, 125.6, 117.6 (TFA), 71.8, 67.7, 59.3, 57.7, 47.6, 32.6, 22.3, 19.8, 16.6, 15.9, 14.1, 3.9. $[\alpha]_{\text{D}}^{20} = +34.3$ (c = 2.4, MeOH). Anal. Calcd for C₁₇H₂₄N₂O₂·2.15CF₃COOH·0.85H₂O: C, 46.61; H, 5.11; F, 22.33; N, 5.1. Found: C, 46.62; H, 4.92; F, 22.14; N, 5.13. HPLC purity = 99.5%; *t*_R = 12.7 min.

3-[(1-Methyl-2(R)-pyrrolidinyl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (29). ¹H NMR (D₂O): δ 8.29 (s, 1H), 8.21 (s, 1H), 7.79 (s, 1H), 4.60 (dd, *J* = 10.8, 2.8 Hz, 1H), 4.43 (m, 1H), 3.94 (m, 1H), 3.74 (m, 1H), 3.57 (t, *J* = 6.4 Hz, 2H), 3.32 (s, 3H), 3.24 (m, 1H), 3.02 (s, 3H), 2.39 (m, 1H), 2.19 (m, 1H), 2.07 (m, 2H), 1.96 (m, 1H), 1.69 (m, 2H), 1.29 (m, 1H), 1.13 (m, 2H). ¹³C NMR (D₂O): δ 162.2 (TFA), 155.7, 146.2, 132.1, 127.9, 125.2, 115.8 (TFA), 71.4, 66.9, 66.0, 57.3, 56.8, 40.1, 32.2, 25.4, 22.0, 21.7, 19.4, 16.2. $[\alpha]_{\text{D}}^{20} = +39.5$ (c 0.2, MeOH). Anal. Calcd for C₁₇H₂₆N₂O₂·2.1CF₃COOH·0.75H₂O: C, 46.86; H, 5.49; F, 22.03; N, 5.16. Found: C, 46.96; H, 5.14; F, 21.7, N, 5.12. HPLC purity = 99.2%; *t*_R = 7.4 min.

3-[(3(S)-Pyrrolidinyl)methoxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (30). ¹H NMR (D₂O): δ 8.23 (s, 1H), 8.16 (s, 1H), 7.75 (s, 1H), 4.28 (m, 1H), 4.20 (m, 1H), 3.58 (m, 3H), 3.46 (m, 1H), 3.38–3.23 (m, 5H), 2.96 (m, 1H), 2.30 (m, 1H), 1.96 (m, 2H), 1.69 (m, 2H), 1.27 (m, 1H), 1.13 (m, 2H). ¹³C NMR (D₂O): δ 162.2 (TFA), 156.5, 145.9, 131.4, 127.8, 125.1, 115.8 (TFA), 71.5, 69.6, 57.3, 46.9, 45.1, 36.2, 32.2, 26.0, 21.8, 19.4, 16.1. $[\alpha]_{\text{D}}^{20} = +42.0$ (c 0.70, MeOH). Anal. Calcd for C₁₆H₂₄N₂O₂·2.0CF₃COOH·1.05H₂O: C, 45.9; H, 5.41; F, 21.78, N, 5.35. Found: C, 45.52; H, 5.0; F, 21.5; N, 5.31. HPLC purity = 99.4%; *t*_R = 7.3 min.

3-[(3(R)-Pyrrolidinyl)oxy]-5-[(1S,2R)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (31). ¹H NMR (D₂O): δ 8.23 (s, 1H), 8.15 (s, 1H), 7.75 (s, 1H), 5.37 (s, 1H), 3.68–3.46 (m, 6H), 3.29 (s, 3H), 2.34 (m, 2H), 1.91 (m, 1H), 1.66 (m, 2H), 1.24 (m, 1H), 1.09 (m, 2H). ¹³C NMR (D₂O): δ 162.1 (TFA), 154.5, 146.2, 131.8, 128.7, 126.0, 115.8 (TFA), 77.2, 71.4, 57.3, 50.0, 43.4, 32.2, 29.3, 21.9, 19.4, 16.2. $[\alpha]_{\text{D}}^{20} = +34.0$ (c 0.1, MeOH). Anal. Calcd for C₁₅H₂₂N₂O₂·2.15CF₃COOH·0.1H₂O: C, 45.52; H, 4.82; F, 24.06; N, 5.5. Found: C, 45.38; H, 4.72; F, 24.08, N, 5.69. HPLC purity = 99.8%; *t*_R = 6.4 min.

3-[(1-Methyl-2(S)-pyrrolidinyl)methoxy]-5-[(1R,2S)-2-(2-methoxyethyl)cyclopropyl]pyridine Trifluoroacetate (33). ¹H NMR (D₂O): δ 8.30 (s, 1H), 8.22 (s, 1H), 7.80 (s, 1H), 4.60 (dd, *J* = 10.8, 2.8 Hz, 1H), 4.44 (m, 1H), 3.95 (m, 1H), 3.74 (m, 1H), 3.57 (t, *J* = 6.4 Hz, 2H), 3.33 (s, 3H), 3.25 (m, 1H), 3.03 (s, 3H), 2.40 (m, 1H), 2.20 (m, 1H), 2.08 (m, 2H), 1.97 (m, 1H), 1.70 (m, 2H), 1.30 (m, 1H), 1.14 (m, 2H). ¹³C NMR (D₂O): δ 162.2 (TFA), 155.7, 146.2, 132.1, 127.9, 125.2, 115.9 (TFA), 71.5, 66.9, 66.0, 57.3, 56.8, 40.2, 32.2, 25.4, 22.0, 21.7, 19.4, 16.2. $[\alpha]_{\text{D}}^{20} = -33.8$ (c 0.13, MeOH). Anal. Calcd for C₁₇H₂₆N₂O₂·2.05CF₃COOH·0.45H₂O: C, 47.61; H, 5.48; F, 21.95; N, 5.26. Found: C, 47.66; H, 5.24; F, 21.73, N, 5.19. HPLC purity = 99.8%; *t*_R = 5.8 min.

General Procedures for Binding and Functional Studies. In Vitro Binding Studies. [³H]Epibatidine competition studies and broad-range screening were carried out by the National Institute of Mental Health's Psychoactive Drug Screening Program, contract no. HHSN-271-2008-00025-C (NIMH PDSP). For experimental details, please refer to the PDSP Web site <http://pdsp.med.unc.edu/>.

Cell Lines and Culture. Cell lines naturally or heterologously expressing specific, functional, human nAChR subtypes were used. The human clonal cell line TE671/RD naturally expresses human muscle-type $\alpha 1^*$ -nAChRs, containing $\alpha 1$, $\beta 1$, γ , and δ subunits, with function detectable using $^{86}\text{Rb}^+$ efflux assays.²⁵ The human neuroblastoma cell line SH-SY5Y naturally expresses autonomic $\alpha 3\beta 4^*$ -nAChRs, containing $\alpha 3$, $\beta 4$, probably $\alpha 5$, and sometimes $\beta 2$ subunits, and also displays function detectable using $^{86}\text{Rb}^+$ efflux assays.²⁶ SH-SY5Y cells also express homopentameric $\alpha 7$ -nAChRs. However, their function is not detected in the $^{86}\text{Rb}^+$ efflux assay under the conditions used. SH-EP1 human epithelial cells stably transfected with human $\alpha 4$ and $\beta 2$ subunits (SH-EP1- $\alpha 4\beta 2$ cells) have been established and the human $\alpha 4\beta 2$ -nAChRs that they stably and heterologously express have been characterized with both ion flux and radioligand binding assays.²⁷ Details of experimental procedures used for $^{86}\text{Rb}^+$ efflux assays are as previously described.¹⁵

Also used was the recently established SH-EP1-h($\alpha 6/3$) $\beta 2\beta 3$ cell line.²⁸ The designation ($\alpha 6/3$) refers to a chimeric subunit that has amino acids from the extracellular, N-terminal, ligand-binding domain of the human nAChR $\alpha 6$ subunit fused to what otherwise is the nAChR $\alpha 3$ subunit. When expressed with the appropriate nAChR subunit assembly partners, in this case, human $\beta 2$ and $\beta 3$ subunits, functional nAChR are formed that have the pharmacological features of receptors containing wild-type $\alpha 6$ subunits such as sensitivity to the $\alpha 6\beta 2\beta 3$ -nAChR-selective functional antagonist, α -conotoxin MII.^{29,30} However, levels of functional expression are much higher, enabling assessment using assays such as $^{86}\text{Rb}^+$ efflux, probably because transmembrane and cytoplasmic domains in the nAChR $\alpha 3$ subunit are more permissive for assembly and/or function of receptors than the corresponding domains in nAChR $\alpha 6$ subunits. Briefly,²⁸ wild-type SH-EP1 cells were transfected using a cationic polymer (Qiagen, Valencia, CA) and nAChR ($\alpha 6/3$), $\beta 2$, and $\beta 3$ subunit gene cDNAs optimized for vertebrate expression (GeneArt, Inc., Burlingame, CA) and delivered in different variants on the pcDNA3.1 vector (Invitrogen, Carlsbad, CA). Following selection of triple transfectants based on triple antibiotic resistance, clonal lines were isolated and screened for nAChR radioligand binding capacity. Promising clones were verified as consistently expressing a high level of functional ($\alpha 6/3$) $\beta 2\beta 3$ -nAChR based on $^{86}\text{Rb}^+$ assays.

TE671/RD, SH-SY5Y, and transfected SH-EP1 cell lines were maintained as low passage number (1–26 from our frozen stocks) cultures, under continuing positive selection as appropriate, to ensure stable expression of native or heterologously expressed nAChRs as previously described.²⁵ Cells were passaged once a week by splitting just-confluent cultures 1/300 (TE671/RD), 1/10 (SH-SY5Y), or 1/20–1/40 (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 **24**, **26**, and **30** were synthesized as described above, and sertraline was purchased from Toronto Research Chemicals (Ontario, Canada). All compounds were dissolved in water and administered by intraperitoneal (IP) injection or administrated orally (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 \times 10 cm wide, 1 L beakers) containing 23 ± 1 °C water 12 cm deep (approximately 800 mL). Mice were administered vehicle, the SSRI sertraline (20 mg/kg) as a positive control, or test compound **24**, **26**, or **30**. Thirty minutes following IP or PO administration (as indicated in Figure 2), 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, or tested compounds) as the between-group variable and total time immobile (seconds over the 6 min trial) as the dependent variable. Significant main effects were followed up with the post hoc Fisher's LSD test.

■ ASSOCIATED CONTENT

📄 Supporting Information

Experimental details for synthesis of all compounds shown and detailed information for in vitro functional studies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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📄 Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

nAChR(s), nicotinic acetylcholine receptor(s); CNS, central nervous system; ADMET, absorption, distribution, metabolism, excretion, and toxicity; LC-MS, liquid chromatography–mass spectrometry; NMR, nuclear magnetic resonance; SAR, structure–activity relationship; HS, high sensitivity; LS, low sensitivity; P-gp, P-glycoprotein; hERG, human ether-a-go-related gene; TFA, trifluoroacetic acid

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