

# Enantiopure Cyclopropane-Bearing Pyridyldiazabicyclo[3.3.0]octanes as Selective $\alpha 4\beta 2$ -nAChR Ligands

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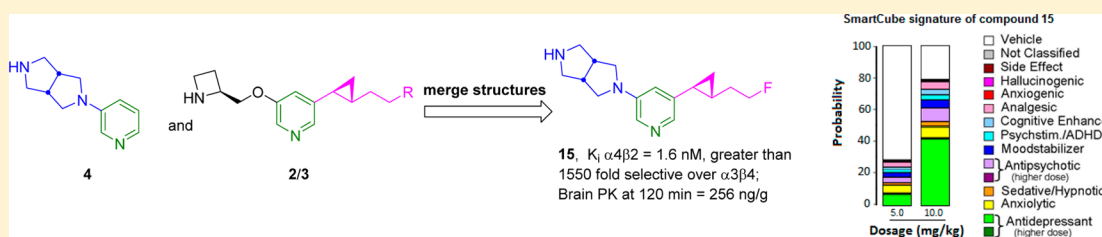
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## S Supporting Information



**ABSTRACT:** We report the synthesis and characterization of a series of enantiopure 5-cyclopropane-bearing pyridyldiazabicyclo[3.3.0]octanes that display low nanomolar binding affinities and act as functional agonists at  $\alpha 4\beta 2$ -nicotinic acetylcholine receptor (nAChR) subtype. Structure–activity relationship studies revealed that incorporation of a cyclopropane-containing side chain at the 5-position of the pyridine ring provides ligands with improved subtype selectivity for nAChR  $\beta 2$  subunit-containing nAChR subtypes ( $\beta 2^*$ -nAChRs) over  $\beta 4^*$ -nAChRs compared to the parent compound 4. Compound 15 exhibited subnanomolar binding affinity for  $\alpha 4\beta 2$ - and  $\alpha 4\beta 2^*$ -nAChRs with negligible interaction. Functional assays confirm selectivity for  $\alpha 4\beta 2$ -nAChRs. Furthermore, using the SmartCube assay system, this ligand showed antidepressant, anxiolytic, and antipsychotic features, while mouse forced-swim assay further confirm the antidepressant-like property of 15.

**KEYWORDS:** Nicotinic acetylcholine receptor, selective  $\alpha 4\beta 2$  partial agonist, N-pyridyldiazabicyclo[3.3.0]octane

Nicotinic acetylcholine receptors (nAChRs) are expressed as pentameric complexes of single (homomeric) or multiple (heteromeric) subunits, which are encoded by 17 different genes (in vertebrates), thus creating a wide variety of nAChR subtypes. The most common nAChR subtypes present in the central nervous system (CNS) are heteropentamers containing  $\alpha 4$  and  $\beta 2$  subunits or the homopentamer comprising  $\alpha 7$  subunits, while the peripheral nervous system (PNS) consist mainly of  $\alpha 3$  subunit combinations (predominately  $\alpha 3\beta 4$  heteromer).<sup>1</sup> nAChR subtypes possess unique pharmacological and physiological properties depending on their subunit makeup and identifying ligands that offer selectivity among these subtypes affords opportunities to develop novel therapeutic agents for use in various central nervous system disorders including schizophrenia, depression, Alzheimer's disease, tobacco addiction, and attention deficit hyperactivity disorder (ADHD).<sup>2–4</sup> Moreover, identifying selective ligands would help to attenuate adverse side effects associated with actions at ganglionic  $\alpha 3\beta 4^*$ -nAChRs (the asterisk indicates that the receptor complex is known to or may contain other subunits than those specified).<sup>5,6</sup> A growing body

of evidence indicates that  $\alpha 4\beta 2^*$ -nAChR subtypes appear to play an essential role in depression as well as in cognition, attention, anxiety, and nicotine dependence.<sup>2,4,7–10</sup> For instance, varenicline, marketed as a smoking cessation pharmacotherapy, is an  $\alpha 4\beta 2$ -nAChR partial agonist.<sup>11,12</sup> Studies have shown that varenicline possesses antidepressant-like effects and also improves cognition in animal models.<sup>13,14</sup> However, several side effects such as nausea, mood changes, sleep disturbance, and constipation have been associated with the use of varenicline for smoking cessation, effects that may be related in part to its  $\alpha 3\beta 4$  subtype activity coupled with its  $5HT_3$  activity.<sup>10,15–18</sup> Additionally, efforts have been made to advance the noncompetitive nicotinic antagonist mecamylamine for use in depression; however, this compound failed to show efficacy in human clinical trials.<sup>19</sup> The development of other nAChR ligands for use in depression thus still represents a therapeutic opportunity.

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Sazetidine-A (**1**), a 3-pyridyl ether possessing an alkynyl substituent at its 5-position, is a highly potent  $\alpha 4\beta 2$ -nAChR partial agonist ( $K_i = 0.4$  nM) that possesses a 24,000-fold selectivity for  $\alpha 4\beta 2$ - over  $\alpha 3\beta 4$ -nAChRs (Figure 1).<sup>20</sup> Sazetidine-A also displays potent anxiolytic, analgesic, and antidepressant features as revealed in studies using animal models.<sup>21–23</sup>

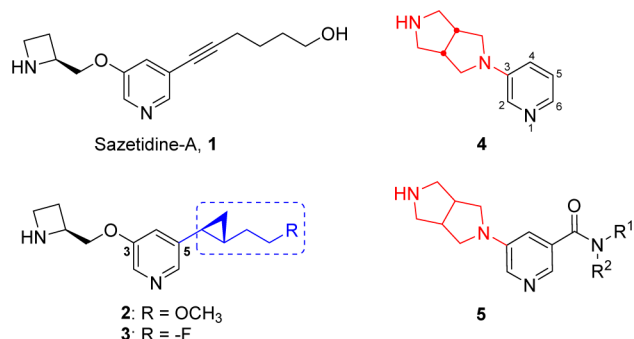


Figure 1. Selected nicotinic receptor ligands (**1**–**5**).

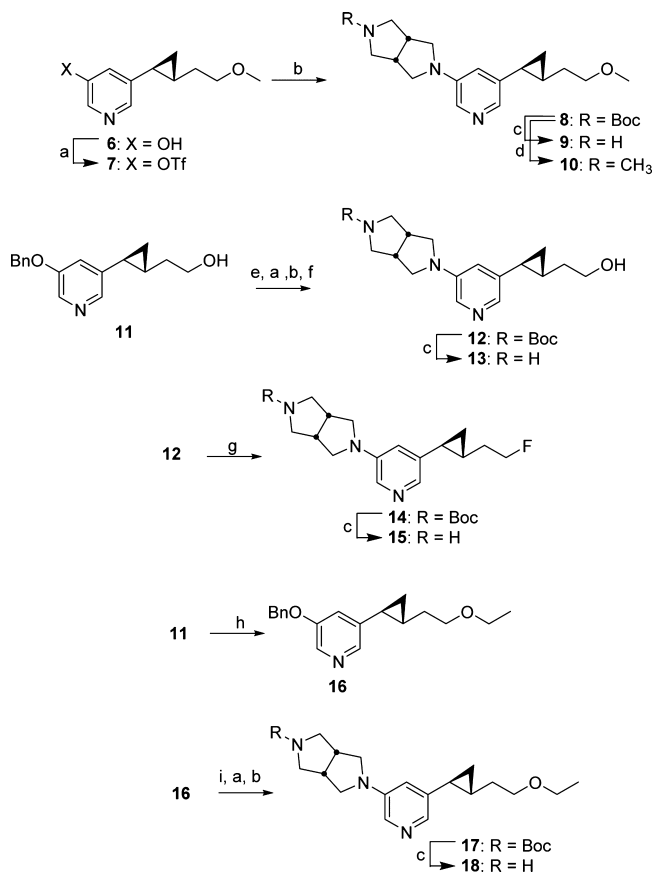
We recently reported the synthesis and biological characterization of a novel series of  $\alpha 4\beta 2$ -nAChR partial agonists bearing a cyclopropane ring in place of the acetylenic bond present in Sazetidine-A (**1**). The cyclopropane ring enforces an orientation of the side chain such that compounds possessing this motif maintained subtype selectivity for  $\alpha 4\beta 2$ -nAChRs.<sup>24</sup> Compounds **2** and **3** are highly selective  $\alpha 4\beta 2$ -nAChR partial agonists with subnanomolar binding affinities ( $K_i = 0.1$  and  $0.2$  nM, respectively) and excellent subtype selectivity over  $\alpha 3\beta 4$ - and  $\alpha 7$ -nAChRs.<sup>24,25</sup> These compounds also show antidepressant activity in mouse forced swim studies. Compound **4**, a 3-pyridyl diazabicyclo[3.3.0]octane, is an  $\alpha 4\beta 2$ -nAChR agonist having subnanomolar binding affinity ( $K_i = 0.12$  nM for a rat brain  $\alpha 4\beta 2$ \* subtype) and approximately 400-fold selectivity over  $\alpha 7$ -nAChRs.

The selectivity of these compounds for  $\alpha 4\beta 2$ - versus  $\alpha 7$ -nAChRs can be improved depending on the nature of the R group at position 5 of the pyridine ring, with larger R groups generally showing improved selectivity for the  $\alpha 4\beta 2$ -nAChR subtype.<sup>26</sup> Compound **5**, a 3-pyridyl diazabicyclo[3.3.0]octane with a carboxamide group at position 5, resulted in compounds that generally possessed high binding affinity and selectivity for  $\alpha 4\beta 2$ - compared to  $\alpha 7$ -nAChRs.<sup>27</sup> In this study, we selected our best ligands<sup>24,25</sup> and incorporated the cyclopropane-containing side chain scaffold onto the 5-position of the *N*-pyridyldiazabicyclo[3.3.0]octane motif **4**, in an attempt to improve subtype selectivity toward  $\alpha 4\beta 2$ \*- over ganglionic  $\alpha 3\beta 4$ \*-nAChRs.

The synthesis of the chiral cyclopropylpyridine ligands **9**, **10**, **13**, **15**, and **18** is summarized in Scheme 1. The hydroxyl group of the optically pure pyridine intermediate **6**<sup>24</sup> was activated as its triflate **7** and subsequently reacted with *tert*-butyl *cis*-hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate,<sup>26</sup> under slightly modified Buchwald–Hartwig conditions to obtain the precursor **8**. Deprotection with trifluoroacetic acid (TFA) yielded the secondary amine **9** as its trifluoroacetate salt. The Boc-protected amine **8** was reduced with LiAlH<sub>4</sub> to yield the *N*-methyl derivative **10**.

Next, acylation of **11** using isobutyric anhydride followed by removal of the benzyl group and Buchwald–Hartwig reaction

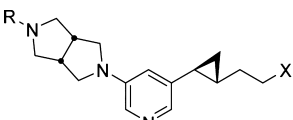
Scheme 1. Synthesis of Derivatives **9**, **10**, **13**, **15**, and **18**<sup>a</sup>



<sup>a</sup>Reagents and conditions: (a) (CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>O, C<sub>5</sub>H<sub>5</sub>N, 0 °C; (b) *tert*-butyl *cis*-hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate,<sup>26</sup> tris-(dibenzylideneacetone)dipalladium, 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl, K<sub>3</sub>PO<sub>4</sub>, 1,4-dioxane, microwave, 160 °C, 10 min; (c) CF<sub>3</sub>COOH, CH<sub>2</sub>Cl<sub>2</sub>, rt; (d) LiAlH<sub>4</sub>, THF, reflux; (e) (i) isobutyric anhydride, cat. 4-(dimethylamino)pyridine, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt; (ii) 10% Pd/C, MeOH/EtOAc (4:1), rt, 2 h; (f) NaOMe, CH<sub>3</sub>OH, 40 °C; (g) (i) TsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to rt, (ii) (*n*-Bu)<sub>4</sub>NF, THF, rt; (h) NaH, CH<sub>3</sub>CH<sub>2</sub>I, DMF, rt; (i) 10% Pd/C, MeOH/EtOAc (4:1), rt, 2 h.

generated **12** after hydrolysis of the isobutyrate protecting group. Cleavage of the Boc group with TFA gave the alcohol **13** as its TFA salt. To generate the fluoride **14**, the hydroxyl group of compound **12** was activated as its tosylate and this intermediate treated with *n*-tetrabutylammonium fluoride to yield the corresponding protected fluoride. Boc deprotection of **14** with TFA afforded the final product **15** as its TFA salt. To prepare the ethyl ether analogue **18**, the intermediate **11** was first subjected to the Williamson ether synthesis using ethyl iodide as the alkylating agent. Next, the benzyl group was removed via hydrogenolysis, the amine coupling reaction carried out, and then the Boc group cleaved from **17** to afford **18** as its TFA salt.

*In vitro* binding affinities ( $K_i$ ) of all the synthesized 5-cyclopropane-bearing pyridyldiazabicyclo[3.3.0]octanes (**9**, **10**, **13**, **15**, and **18**) were determined using [<sup>3</sup>H] epibatidine binding competition assays at seven rat nAChR subtypes.<sup>28</sup> As illustrated in Table 1, most of these compounds demonstrated relatively high binding affinities for both  $\alpha 4\beta 2$ - ( $K_i$  ranging from 0.4 to 60 nM) and  $\alpha 4\beta 2$ \*-nAChRs ( $K_i$  ranging from 1.2 to 120 nM) with poor binding affinities for  $\alpha 3\beta 4$ -nAChRs ( $K_i > 2000$  nM). This profile suggests a reduced risk of undesirable

Table 1. Binding Affinities of 11 Ligands at Seven nAChR Subtypes Defined by Competition for [<sup>3</sup>H]Epibatidine Binding


9: R = H, X = OCH<sub>3</sub>  
 10: R = CH<sub>3</sub>, X = OCH<sub>3</sub>  
 13: R = H, X = OH  
 15: R = H, X = F  
 18: R = H, X = OCH<sub>2</sub>CH<sub>3</sub>

compd	<i>K<sub>i</sub></i> (nM) <sup>a</sup>							selectivity (α3β4/α4β2)
	α2β2	α2β4	α3β2	α3β4	α4β2	α4β2* <sup>b</sup>	α4β4	
1 <sup>f</sup>				10 <sup>4</sup>	0.40	0.90		24000
2 <sup>g</sup>				>10 <sup>4</sup>	0.10	0.30		>100000
3 <sup>h</sup>				3200	0.20	0.90		16000
4	0.3	46	2.7	200	0.50	1.5	27	400
9	0.30 ± 0.10	380	26 ± 7.0	>10 <sup>4</sup>	4.1 ± 2.0	1.2 ± 0.20	170	>2400
10	25 ± 3.0	>10 <sup>4</sup>	>10 <sup>4</sup>	2500	58 ± 18	120	1400	40
13	0.60 ± 0.10	260	19 ± 5.0	7900	3.1 ± 1.0	2.4 ± 0.40	110	2500
15	0.40	65	10	2500	1.6	3.6	37	1600
18	0.20	390	6.8	>10 <sup>4</sup>	0.40	1.9	160	>25000
nicotine <sup>c</sup>	5.5	70	29	260	4.9	9.8	23	53
varenicline <sup>e</sup>				86	0.40		110	210

<sup>a</sup>See Supporting Information. <sup>b</sup>α4β2\*, endogenous receptors prepared from rat forebrain. Besides α4 and β2, other unidentified subunits may also be present. <sup>c</sup>*K<sub>i</sub>* values for nicotine were taken from the PDSP Assay Protocol Book (<http://pdsp.med.unc.edu/>). <sup>d</sup>NA: not active, defined as <50% inhibition of binding in the primary assay at 10 μM. <sup>e</sup>*K<sub>i</sub>* values for varenicline are from the literature. <sup>f</sup>*K<sub>i</sub>* values for 1 are from the literature. <sup>g</sup>*K<sub>i</sub>* values for 2 are from the literature. <sup>h</sup>*K<sub>i</sub>* values for 3 are from the literature. <sup>25</sup>

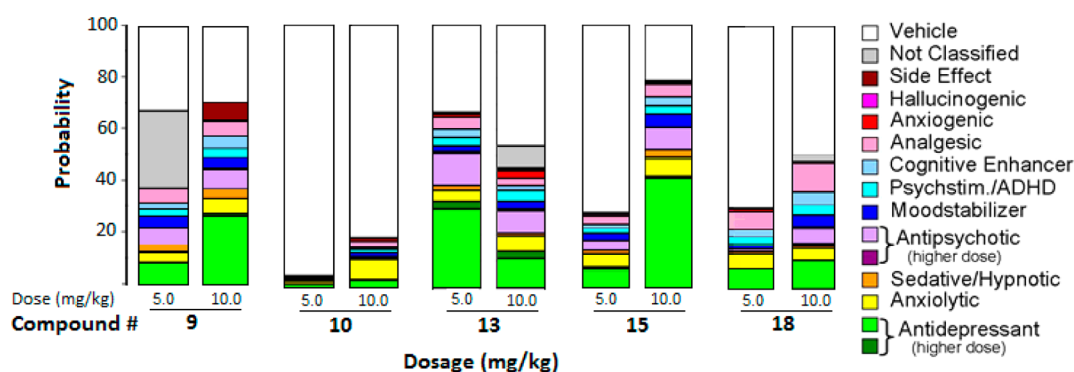
Table 2. Functional Potencies and Efficacies of Ligands: Agonism and inactivation of Human α4β2-nAChRs.<sup>a</sup>

compd	agonism				inactivation		
	EC <sub>50</sub> (nM)	pEC <sub>50</sub>	HS-α4β2 efficacy (%)	LS-α4β2 efficacy (%)	IC <sub>50</sub> (nM)	pIC <sub>50</sub>	efficacy (%)
1 <sup>b</sup>	5.8		100		4.8		63
2 <sup>c</sup>	18		60		5.6		71
9	14	7.9 ± 0.10	76 ± 14	0.40 ± 5.1	11	8.0 ± 0.04	77 ± 1.0
10	>1000	<6.0	ND <sup>d</sup>	ND	>10 <sup>3</sup>	<6.0	ND
13	18	7.8 ± 0.10	100 ± 7.0	1.7 ± 4.0	15	7.8 ± 0.04	73 ± 2.0
15	25	7.6 ± 0.10	110 ± 7.0	−9.4 ± 4.0	20	7.7 ± 0.04	74 ± 2.0
18	20	7.7 ± 0.10	88 ± 4.0	−7.0 ± 4.0	12	7.9 ± 0.10	76 ± 4.0
nicotine	300	6.5 ± 0.10	120 ± 9.0	70 ± 6.0	430	6.4 ± 0.10	92 ± 2.0

<sup>a</sup>See Supporting Information for details. The term “inactivation” is used because compounds may be acting to desensitize receptors or as competitive or noncompetitive antagonists, and further work is needed to make such a distinction. Potencies (EC<sub>50</sub> or IC<sub>50</sub> values) and efficacies were measured for actions at a mixture of high-sensitivity (HS) and low-sensitivity (LS) α4β2-nAChRs. Reported errors are the standard error of the mean (SEM) for all values. <sup>b</sup>Results for compound 1 were obtained from ref 18. <sup>c</sup>Results for compound 2 were obtained from ref 20. <sup>d</sup>ND: Not determined. The efficacy was not determined if the EC<sub>50</sub> or the IC<sub>50</sub> value was greater than 1000 nM.

side effects associated with binding to ganglionic α3β4-nAChRs. These compounds also showed good selectivity for β2\*-nAChRs (α2β2-, α3β2-, α4β2-, and α4β2\*-nAChRs) over β4\*-nAChRs (α2β4-, α3β4-, and α4β4-nAChRs) compared to nicotine. The *N*-methyl-bearing analogue 10 showed decreased binding affinity for α4β2-nAChRs (*K<sub>i</sub>* = 58 nM) compared to the corresponding unsubstituted compound 9 (*K<sub>i</sub>* = 4.1 nM), resulting in a 60-fold reduction in selectivity for α4β2- over α3β4-nAChRs. The binding affinity of ligand 13, bearing a terminal hydroxyl group, at α4β2-nAChRs was similar to its fluoro-containing counterpart 15. However, alcohol 13 has an improved selectivity ratio (α3β4/α4β2) compared to 15. Of the new analogues made and tested herein, the ethoxy derivative 18 displayed the best binding affinity for α4β2-nAChRs (*K<sub>i</sub>* = 0.40 nM), and it was found to be inactive at α3β4-nAChRs (*K<sub>i</sub>* = >10000 nM). Selected ligands were also tested at α7- and α7\*-nAChRs, (α7\*, endogenous receptors prepared from rat forebrain) and they were found to be devoid of activity at the highest concentration used (10 μM), with the exception of 18 (*K<sub>i</sub>* = 680 nM at α7-nAChRs) (data not shown).

Functional activity of all compounds was characterized at human α4β2-, α3β4\*, and α1β1γδ-nAChRs using SH-EP1-hα4β2, SH-SY5Y, and TE671/RD cells, respectively, and <sup>86</sup>Rb<sup>+</sup> ion efflux assays. Note that α4β2-nAChRs actually exist as two isoforms differing in sensitivity to nicotine or acetylcholine: high sensitivity (HS) (α4)<sub>2</sub>(β2)<sub>3</sub>-nAChRs and low sensitivity (LS) (α4)<sub>3</sub>(β2)<sub>2</sub>-nAChRs. Sazetidine-A is unusual in that it is a fully efficacious agonist at HS (α4)<sub>2</sub>(β2)<sub>3</sub>-nAChRs, but it has much weaker efficacy at the LS isoform relative to conventional agonists like ACh and nicotine. This is because sazetidine-A only activates the HS phase of LS receptor function but does not activate the LS phase due to its lack of activity at the α4/α4 subunit interface present in the LS isoform.<sup>30</sup> Efficacy of ligands at HS vs LS α4β2-nAChR can be assayed by reference to proportions of those isoforms expressed in a given preparation of cells being studied as defined by function elicited by sazetidine-A. As seen in Table 2, the analogues tested share sazetidine-A's characteristic discrimination between HS- and LS-α4β2 nAChR isoforms. Tested compounds had agonist activity at α4β2-nAChRs with EC<sub>50</sub> < 30 nM, with the exception of 10, which showed no activity (Table 2). For the



**Figure 2.** Behavioral SmartCube signatures of all diazabicyclo[3.3.0]octane. Compounds 9, 13, 15, and 18 produced a signature of activity suggesting a potential antidepressant-like effect. The drug was injected ip, 15 min before testing.

ligands evaluated, there was neither agonist nor antagonist activity at ganglionic  $\alpha 3\beta 4^{*}$ - or muscle-type  $\alpha 1\beta 1\gamma\delta$ -nAChRs or the potency was too low to characterize without testing at concentrations above 10  $\mu$ M. The methoxy analogue **9** showed similar  $EC_{50}$  and  $IC_{50}$  values (14 and 11 nM) to those for the azetidine-containing ligand **2** ( $EC_{50}$  = 18 nM and  $IC_{50}$  = 5.6 nM, Table 2). Interestingly, the hydroxyl (**13**) and fluoro (**15**) analogues showed the highest efficacies at 100% and 110%, respectively, for stimulation of HS ( $\alpha 4$ )<sub>2</sub>( $\beta 2$ )<sub>3</sub>-nAChRs.

Compounds **13** and **15**, however, had functional inactivation efficacies similar to those of other compounds tested in this study (Table 2). The ethoxy analogue **18**, which had the best binding affinity to  $\alpha 4\beta 2$ -nAChRs ( $K_i$  = 0.40 nM) among the compounds tested here, also showed excellent activity at  $\alpha 4\beta 2$ -nAChRs in functional agonism and inactivation assays ( $EC_{50}$  = 20 nM and  $IC_{50}$  = 12 nM). Of note, none of the tested ligands appear to have any significant intrinsic activity at LS ( $\alpha 4$ )<sub>3</sub>( $\beta 2$ )<sub>2</sub>-nAChRs but have apparent efficacies ranging from 76% to 110% at HS ( $\alpha 4$ )<sub>2</sub>( $\beta 2$ )<sub>3</sub>-nAChRs.

Preliminary *in vivo* evaluation of the nicotinic ligands for behavioral effects was carried out using SmartCube, an automated system that analyzes the behaviors of compound-treated mice captured on digital video with the aid of computer algorithms.<sup>31</sup> The behavioral signature of a test compound is compared with a database of behavioral signatures obtained from a large set of diverse reference compounds. Thus, we are able to make predictions as to the possible neuropharmacological activity of a test compound relative to major classes of compounds such as anxiolytics, antipsychotics, and antidepressants. All compounds were administered at doses of 5 or 10 mg/kg. Compounds **9**, **13**, **15**, and **18** were found to produce behavioral signatures that have features of antidepressants, anxiolytics, and antipsychotics with little or no side effect profiles (Figure 2). Consistent with its lower potency in the radioligand binding and functional studies, compound **10** is relatively inactive in SmartCube and does not show the behavioral signature of compounds **9**, **13**, **15**, and **18**.

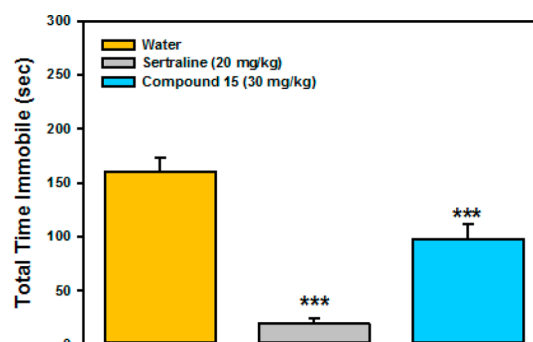
Next, to further establish the ability of these compounds to penetrate the blood–brain barrier, compound **15** was selected for mouse *in vivo* pharmacokinetic (PK) studies. The plasma and brain concentrations of compound **15** in male CD-1 mice after a single intraperitoneal (IP) injection at a dose of 10 mg/kg were measured. The concentration of **15** reached a value of 197 and 256 ng/g at 30 and 120 min in the brain and 828 and 146 ng/mL at 30 and 120 min in plasma (Table 3). The brain to plasma ratio of compound **15** was found to be 0.24 at 30 min and 1.75 at 120 min, indicating acceptable CNS penetration.

**Table 3.** Pharmacokinetic Parameters of **15** in Mouse Plasma and Brain Following IP (10 mg/kg) Administration

dose of <b>15</b> (mg/kg)	plasma		brain	
	time (min)	concentration (ng/mL)	time (min)	concentration (ng/g)
10	30	828	30	197
10	120	146	120	256

Furthermore, the binding of **15** to protein in male CD-1 mouse plasma and brain tissue was determined using equilibrium dialysis. Binding of **15** was evaluated at a final concentration of 1  $\mu$ M. The percentage of binding of compound **15** in mouse plasma and brain tissue was 27% and 73%, respectively, after a 6 h incubation period. These results thus indicate that sufficient amounts of the unbound drug are available in the brain to exert a pharmacological action.

On the basis of the SmartCube data and brain concentration levels of compound **15**, we decided to further probe the possible antidepressant action of compound **15**. We thus examined the effects of compound **15** in the classical mouse forced-swim test,<sup>32</sup> an assay in which mice are placed into a beaker of water, and the time spent passively floating in the water (immobility) is recorded (Figure 3). Most traditional



**Figure 3.** Mouse forced-swim data for compound **15**.

antidepressants decrease the amount of time the mouse spends immobile. Mice were administered compound **15** (30 mg/kg of the free base) 15 min prior to testing or the selective serotonin reuptake inhibitor sertraline, as a positive control (20 mg/kg). Compound **15** exhibited an antidepressant-like effect when administered IP with a significant reduction in immobility at a single dose as displayed in the bar graph in Figure 3.



In summary, we describe the synthesis, pharmacological evaluation, and behavioral characterization of some 5-cyclopropane-bearing pyridyldiazabicyclo[3.3.0]octanes as nAChR ligands. All tested ligands with the exception of compound **10** showed excellent binding affinities for both  $\alpha 4\beta 2$ - and  $\alpha 4\beta 2^*$ -nAChRs from the rat ( $K_i$  values ranging from 0.4 to 4.1 nM) and poor affinity for rat  $\alpha 3\beta 4$ -nAChRs ( $K_i > 2400$  nM). In functional studies, these ligands acted as potent agonists at human  $\alpha 4\beta 2$ -nAChRs and were inactive at both ganglionic  $\alpha 3\beta 4^*$ - or muscle-type  $\alpha 1\beta 1\gamma\delta$ -nAChRs. In this series, the fluoro-analogue **15** was found to possess subnanomolar binding affinity, a 1550-fold selectivity for  $\alpha 4\beta 2$ - versus  $\alpha 3\beta 4$ -nAChRs, as well as good agonist efficacy in the functional studies. Compound **15** achieves a brain concentration of  $\sim 0.70 \mu\text{M}$  at 30 min, and this is over 400-times more than its binding affinity at the  $\alpha 4\beta 2$ -nAChR. Compound **15** was found to display antidepressant-like properties in the mouse forced-swim test. The above data support our hypothesis that the incorporation of the cyclopropane side chain at the 5-position of the pyridyldiazabicyclo[3.3.0]octanes would improve subtype selectivity for  $\alpha 4\beta 2$ - over  $\alpha 3\beta 4$ -nAChRs when compared to the parent compound **4**, thus implying that the nature of the substitution at the position 5 plays a vital role in attenuating possible side effects associated with ganglionic  $\alpha 3\beta 4^*$ -nAChRs. These potent and selective nAChR ligands produced antidepressant/anxiolytic-like properties in the SmartCube test, and thus, they may serve as chemical probes in further exploring various aspects of nicotinic receptor function related to mood disorders.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Experimental procedures and analytical data. 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

nAChR, nicotinic acetylcholine receptor; ADHD, attention deficit hyperactivity disorder; TFA, trifluoroacetic acid; NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; HS, high sensitivity; LS, low sensitivity; IP, intraperitoneal

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