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Chemistry and Pharmacological Studies of 3-Alkoxy-2,5-Disubstituted-Pyridinyl Compounds as Novel Selective α4β2 Nicotinic Acetylcholine Receptor Ligands That Reduce Alcohol Intake in Rats

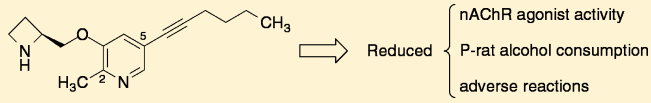
Yong Liu,[†] Janell Richardson,[‡] Thao Tran,[‡] Nour Al-Muhtasib,[‡] Teresa Xie,[‡] Venkata Mahidhar Yenugonda,[†] Hannah G. Sexton,[§] Amir H. Rezvani,[§] Edward D. Levin,[§] Niaz Sahibzada,[‡] Kenneth J. Kellar,[‡] Milton L. Brown,[†] Yingxian Xiao,*[,‡] and Mikell Paige*[,†]

[†]Center for Drug Discovery, Georgetown University Medical Center, Research Building EP07, 3970 Reservoir Road, NW, Washington, D.C. 20057, United States

[‡]Department of Pharmacology and Physiology, Georgetown University School of Medicine, 3970 Reservoir Road, NW, Washington, D.C. 20057, United States

[§]Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina 27710, United States

ABSTRACT: Neuronal acetylcholine receptors mediate the addictive effects of nicotine and may also be involved in alcohol addiction. Varenicline, an approved smoking cessation medication, showed clear efficacy in reducing alcohol consumption in heavy-drinking smokers. More recently, sazetidine-A, which selectively desensitizes α4β2 nicotinic receptors, was shown to significantly reduce alcohol intake in a rat model. To develop novel therapeutics for treating alcohol use disorder, we designed and synthesized novel sazetidine-A analogues containing a methyl group at the 2-position of the pyridine ring. In vitro pharmacological studies revealed that some of the novel compounds showed overall pharmacological property profiles similar to that of sazetidine-A but exhibited reduced agonist activity across all nicotinic receptor subtypes tested. In rat studies, compound (*S*)-**9** significantly reduced alcohol uptake. More importantly, preliminary results from studies in a ferret model indicate that these novel nAChR ligands have an improved adverse side-effect profile in comparison with that of varenicline.



INTRODUCTION

Tobacco use, mainly via cigarette smoking, and alcohol consumption are among the leading preventable risk factors for premature mortality in the world. In 2000, tobacco use was the number one risk factor for mortality in the United States, which resulted in 435,000 premature deaths. In that same year, alcohol consumption was the number three risk factor, which was responsible for 85,000 premature deaths.[1] In addition to being an immense medical and public health problem, tobacco use and alcohol consumption also impose a huge social and economic burden to society. Given the grave consequences of nicotine and alcohol addiction, there is obviously a great need for significant improvement in existing therapies for treating these disorders.

Although alcohol and nicotine addictions are usually categorized as separate disorders, heavy alcohol consumption and smoking occur very often in the same individual.[2,3] It has been well established that there are potent interactions between alcohol and nicotinic systems and that alcohol potentiates nicotine reward and self-administration.[4−7]

Neuronal nicotinic acetylcholine receptors (nAChRs) are found throughout the central nervous system (CNS) and peripheral nervous system (PNS). The receptors mediate the pharmacological effects of nicotine and play a key role in regulating nicotine self-administration behavior.[8−10] The predominant nAChR subtype in mammalian CNS, which contains both α4 and β2 subunits, is essential for nicotine addiction.[11−18] Several lines of evidence show that nAChRs are involved in mediating alcohol consumption as well. The classical noncompetitive antagonist of nAChRs, mecamylamine, significantly reduced alcohol consumption in rats and mice.[6,19−21] Several other nicotinic ligands were reported to attenuate alcohol seeking and consumption in rats.[22,23] Furthermore, genetic studies showed that nicotinic receptor subunits are directly involved in alcohol consumption in mouse models.[24−26]

Varenicline, an nAChR ligand developed by Pfizer, was approved by the US Food and Drug Administration (FDA) in 2006 as a smoking cessation medication.[27−30] Interestingly, smokers treated with varenicline reported that the medication also reduced their alcohol consumption.[31] In a double-blind, placebo-controlled clinical trial, varenicline significantly reduced alcohol self-administration in heavy-drinking smokers.[32] Furthermore, several studies using animal models showed that varenicline effectively reduced alcohol consumption in rats and mice.[24,25,31,33] Although varenicline appears to be safe for most

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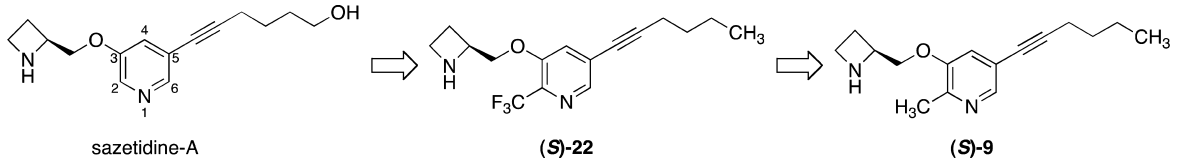


Figure 1. Development of nicotinic desensitizers with reduced agonist activity.

people, significant side effects such as nausea have been reported.[28,29,34]

In 2006, we proposed a new approach in the design of novel nicotinic therapeutics, which is based on the ability of nicotinic ligands to desensitize nAChRs.[35] The actions of nicotine and other nicotinic agonists to activate and then desensitize nAChRs have been known for more than 100 years[36] and conceptualized for more than 50 years.[37] A fundamental question about nicotinic cholinergic signaling in the CNS is how each of these two opposite actions contributes to the overall pharmacological effects of nicotine. It has been well-known that nicotine, as well as all other known nicotinic agonists, is more potent in desensitizing nAChRs than in activating them.[38−45]

Sazetidine-A is a highly selective ligand for nAChRs that contain the β2 subunit, especially the $\alpha4\beta2$ nAChR subtype.[35] Sazetidine-A was shown to potently and selectively desensitize $\alpha4\beta2$ nAChRs (see Table 2). In studies using animal behavioral models, sazetidine-A reduced nicotine self-administration,[46,47] improved performance in tests of attention,[48,49] and showed antidepressant and/or antianxiety effects.[50−52] Interestingly, sazetidine-A also significantly decreased alcohol intake in rats.[47]

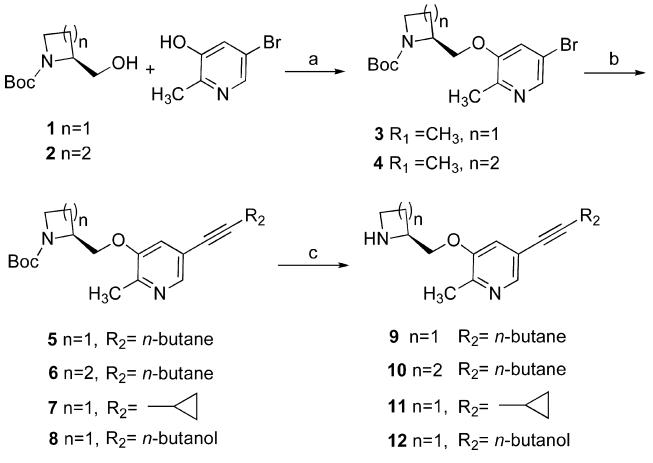
Herein we report the design, synthesis, and biological characterization of a new class of sazetidine-A analogues that have a reduced agonist effect on several nAChR subtypes and a reduced adverse side-effect profile in ferrets as compared with varenicline. These novel compounds could lead to more effective treatments for alcohol addiction.

RESULTS

Chemistry. Previous studies have shown that O-substitution at the 3-position of pyridine with a 2(*S*)-azetinylmethanol substituent and the presence of an aliphatic chain at the 5-position, such as that found in sazetidine-A, are key structural components for the selective desensitization of $\alpha4\beta2$ nAChRs.[35] During our studies of sazetidine-A and related analogues, it became clear that the in vivo stability of these compounds should be an important consideration for clinical development. Recently, the Baran and the MacMillan groups independently reported that the innate susceptibility for heteroaromatic hydroxylation could be investigated under oxidative conditions in the presence of a trifluoromethylating reagent.[53,54] We subjected sazetidine-A (Figure 1) to the conditions reported by Baran and co-workers and observed that trifluoromethylation occurred at the 2-position of the pyridine ring, albeit in 10% yield. This result suggested that the 2-position of the pyridine moiety could be susceptible to P450-mediated oxidation. We hypothesized that blocking the 2-position of the pyridine ring of sazetidine-A with a CF_3 group could result in a compound with increased stability. Therefore, we synthesized compound (*S*)-**22**, a sazetidine-A analogue that incorporates a CF_3 group at the 2-position of the pyridine moiety (Figure 1). However, this analogue exhibited a tremendous decrease in binding affinity to the target receptors (Table 1). It is conceivable that the electronegativity of the CF_3 group, or the large volume of the group, is responsible for the significant alteration in the pharmacological properties for this structural class. To test this possibility, we then synthesized compound (*S*)-**9** (Figure 1), which contains a 2-methyl substituent on the pyridine moiety in place of the CF_3 group in compound (*S*)-**22**. The 2-methyl group of compound (*S*)-**9** provides an electron-donating substituent at the 2-position in contrast to the CF_3 group of compound (*S*)-**22**, as well as a smaller volume than that of the CF_3 group. We were delighted that in the binding assays compound (*S*)-**9** showed a high binding affinity (K_i = 12 nM) to $\alpha4\beta2$ nAChRs and high selectivity for the $\alpha4\beta2$ nAChR subtype over two other major subtypes, $\alpha3\beta4$ and $\alpha7$ (Table 1). Furthermore, in comparison with varenicline and sazetidine-A, compound (*S*)-**9** exhibited significantly lower agonist activities at both the $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs (Table 2). This discovery indicated a possibility for developing a series novel nicotinic ligands that selectively bind to $\alpha4\beta2$ nAChRs with high affinities and potently desensitize the receptor subtype with very low agonist activities across all major nAChR subtypes. To further study this new class of nAChR ligands, we explored the importance of the ring size and the stereocenter of the cyclic amine. The effect of changing the terminal group of the alkynyl appendage was also investigated.

The new sazetidine-A analogues designed in our study that contain a 2-methyl group on the pyridine moiety were synthesized by an optimized three-step procedure as shown in Scheme 1. Each enantiomer of Boc-protected 2-azetidinylmethanol or 2-pyrrolidinylmethanol was reacted with 5-bromo-2-methylpyridin-3-ol via standard Mitsunobu coupling conditions to afford bromides **3** and **4**. The alkynyl appendage was

Scheme 1. Synthesis of Analogues 9−12[a]



[a]Reagents and conditions: (a) DEAD, PPh_3, THF, 0 °C-rt, 48 h, 68−77%; (b) 1-hexyne, $Pd(PPh_3)_2Cl_2$ (cat.), CuI (cat.), PPh_3 (cat.), Et_3N, DMSO, sealed tube, 95 °C, 60 h, 89−98%; (c) method A, TFA, CH_2Cl_2, 0 °C-rt, 3 h then 10% aqueous NaOH solution−methanol, 75−83%; method B, HCl in methanol, 0 °C-rt, 3 h, 87−92%.

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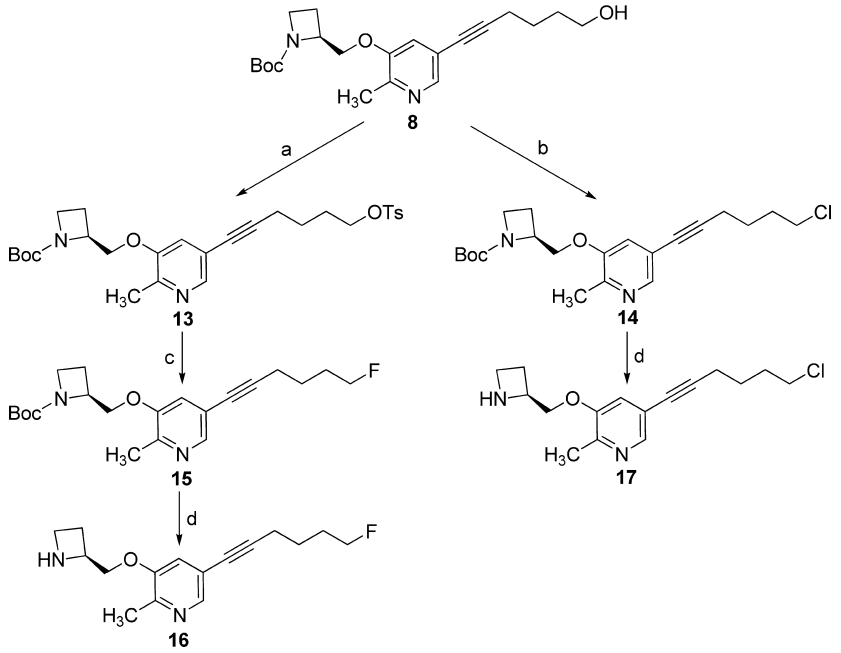
Scheme 2. Synthesis of Analogues 16−17[a]



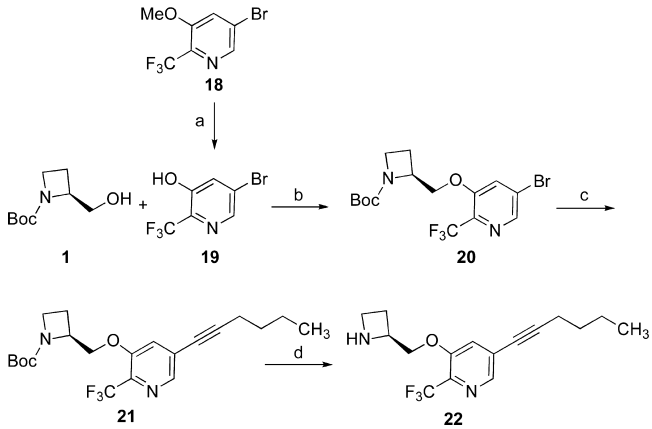
[a]Reagents and conditions: (a) p-TsCl, DMAP, Et_3N, DCM, 0 °C-rt, overnight, 80%; (b) Ph_3P, CCl_4, cat. $NaHCO_3$, sealed tube, 80 °C, 48 h, 69%; (c) Kryptofix 2.2.2, KF, THF, reflux, overnight, 85%; (d) HCl in methanol, 0 °C-rt, 3 h, 90%.

incorporated by Sonogashira homologation in the presence of triphenylphosphine in triethylamine with DMSO as a cosolvent. The *N*-Boc protecting group was then removed by treatment with trifluoroacetic acid or hydrogen chloride to afford the analogues **9** through **12** as the corresponding salt.

Analogues **16** and **17**, which contain a terminal halogen substituent, were synthesized as outlined in Scheme 2. The hydroxide group of compound **12** was tosylated by treatment with *p*-toluenesulfonyl chloride in methylene chloride at 0 °C. The resulting product was then treated with potassium fluoride in the presence of Kryptofix 2.2.2 in anhydrous THF to afford fluoroalkane **15**.[55] Compound **16** was obtained as the hydrochloride salt after deprotection of the *N*-Boc group by treatment with hydrogen chloride in methanol. The chloroalkane analogue **17** was originally afforded by a side reaction during the tosylation step when compound **12** was treated with *p*-toluenesulfonyl chloride in pyridine at room temperature. We have optimized the synthesis of the chloroalkane analogue by treating compound **12** with triphenylphosphine and carbon tetrachloride in a sealed tube at 80 °C for 48 h.[56] The deprotection step of the chlorinated product **14** was accomplished by treatment with hydrogen chloride in methanol to afford the hydrochloride salt of compound **17**.

The 2-trifluoromethylpyridinyl analogue **22** was synthesized as shown in Scheme 3. The methyl aryl ether **18** was treated with hydrogen bromide in acetic acid at elevated temperatures to afford phenol **19**.[57] Mitsunobu homologation of phenol **19** with alcohol **1** then gave aryl bromide **20**. Homologation of 1-hexyne to compound **20** was accomplished following the Sonogashiro protocol as outlined in Scheme 1. However, for this substrate the reaction was accomplished at lower temperature and shorter reaction time than for the 2-methylpyridinyl substituted substrates, presumably because the electron-withdrawing properties of the trifluoromethyl substituent assists in the initial oxidative insertion of the palladium between the carbon and bromine atoms. It is

Scheme 3. Synthesis of Analogue 22[a]



[a]Reagents and conditions: (a) HBr, AcOH, 110 °C, sealed tube, overnight, 79%; (b) DEAD, PPh_3, THF, 0 °C-rt, 24 h, 88%; (c) 1-hexyne, $Pd(PPh_3)_2Cl_2$ (cat.), CuI (cat.), PPh_3 (cat.), Et_3N, DMSO, 50 °C, overnight, 91%; (d) HCl in methanol, 0 °C-rt, 3 h, 73%.

important to note that higher temperatures and longer reaction times resulted in diminished yields. Deprotection of the *N*-Boc group was then effected by treatment with hydrogen chloride in methanol to afford compound **22** as the hydrogen chloride salt.

In Vitro Binding Affinities for nAChR Subtypes. The binding affinities of the novel 2,5-disubstituted sazetidine-A analogues for the defined rat nAChR subtypes as well as for native nAChRs of rat forebrain were examined in binding competition studies against [^{3}H]-epibatidine. For comparison, binding affinities of (−)-nicotine, varenicline, and sazetidine-A were obtained from parallel binding experiments. To determine the selectivities of these compounds in binding assays among the three predominant nAChR subtypes, $\alpha3\beta4$, $\alpha4\beta2$, and $\alpha7$, the ratios of the corresponding K_i values ($\alpha3\beta4/\alpha4\beta2$ and $\alpha7/\alpha4\beta2$) were determined.

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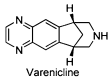
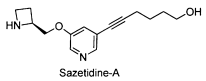
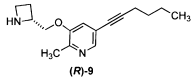
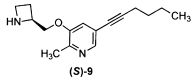
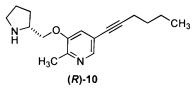
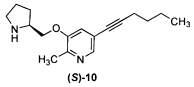
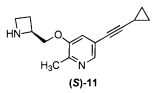
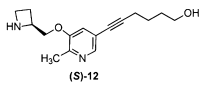
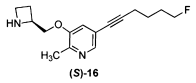
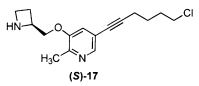
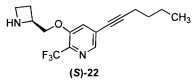
Table 1. Comparison of the Binding Affinities of Nicotine, Varenicline, and Sazetidine-A for Rat nAChR Subtypes to Those of the 3-Alkoxy-2,5-Substituted-Pyridyl Compounds

Compound	K_i (nM) [a]								K_i Ratio	
	α2β2	α2β4	α3β2	α3β4	α4β2	α4β4	α7	Fore-brain	α3β4/α4β2	α7/α4β2
 Nicotine	12	110	47	440	10	40	517	12	44	520
 Varenicline	0.48	94	2.5	390	0.12	28	37	1.1	3,300	310
 Sazetidine-A	0.087	210	0.38	1,900	0.062	52	670	0.17	31,000	11,000
 (*R*)-9	970	250,000	3,600	200,000	740	160,000	160,000	4,300	270	220
 (*S*)-9	16	31,000	390	230,000	12	3,500	93,000	52	19,000	7,800
 (*R*)-10	22,000	370,000	19,000	220,000	9,900	270,000	110,000	110,000	22	11
 (*S*)-10	130	59,000	2,500	94,000	73	23,000	130,000	540	1,300	1,800
 (*S*)-11	13	9,600	340	150,000	9.6	750	150,000	31	16,000	16,000
 (*S*)-12	5.3	26,000	220	300,000	4.3	3,300	170,000	11	70,000	40,000
 (*S*)-16	8.7	24,000	230	150,000	5.1	3,000	150,000	15	29,000	29,000
 (*S*)-17	8.6	29,000	300	92,000	5.3	3,700	67,000	32	17,000	13,000
 (*S*)-22	NT	NT	NT	>100,000	>100,000	NT	NT	>100,000	--	--

[a] K_i values of the compounds shown are the mean of 3 to 5 independent measurements (for clarity, SEM values were omitted, which were within 20% of the mean in most cases).

As reported previously[35] and shown in Table 1, sazetidine-A bound to $\alpha4\beta2$ nAChRs with a very high affinity (K_i = 0.062 nM), which was 31,000 and 11,000 times higher than its affinities for the $\alpha3\beta4$ and $\alpha7$ subtypes, respectively. The data suggest that sazetidine-A bound to each $\beta2$-containing nAChR subtype with a much higher affinity than to the corresponding $\beta4$-containing counterparts having the same α subunit.

As shown in Scheme 1, the incorporation of a methyl group at the 2-position of the pyridine moiety in sazetidine-A afforded analogue (*S*)-**12**. The K_i value of this compound for $\alpha4\beta2$ nAChRs is 4.3 nM, representing a lower binding affinity than that of sazetidine-A (Table 1). However, the binding affinity of (*S*)-**12** for $\alpha4\beta2$ nAChRs is 2-fold higher than that of (−)-nicotine. More importantly, analogue (*S*)-**12** retained the excellent selectivity pattern seen with sazetidine-A with a slightly more favorable selectivity for the $\alpha4\beta2$ nAChRs over the $\alpha3\beta4$ and $\alpha7$ subtypes.

We then synthesized four analogues of compound (*S*)-**12**. Replacing the hydroxyl group at the terminal end of the alkyl chain with a fluorine atom (analogue (*S*)-**16**) or a chlorine atom (analogue (*S*)-**17**) did not lead to significant changes in the binding property profiles (Table 1). Similarly, removing the hydroxyl group (analogue (*S*)-**9**) or replacing the end of the side chain with a cyclopropyl ring (analogue (*S*)-**11**) resulted in

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little change in the binding profiles for this class of compounds (Table 1).

All closely related *S* enantiomers, including (*S*)-**9**, (*S*)-**11**, (*S*)-**16**, and (*S*)-**17**, showed binding property profiles similar to that of (*S*)-**12**. In contrast, compound (*R*)-**9** showed significantly lower binding affinities and reduced nAChR subtype selectivities than those of compound (*S*)-**9** (Table 1). Consistent with this finding, all analogues containing an *R* stereocenter in this study showed lower binding affinities and less nAChR subtype selectivities than analogues containing the *S* stereochemistry.

It is important to note that compound (*S*)-**10**, which has a five-membered ring pyrrolidine group, showed much lower affinities and nAChR subtype selectivites than those of compound (*S*)-**9**, which has a four-member ring azetidinyl group (Table 1). However, compound (*S*)-**10** still had binding affinities at $\alpha4\beta2$ nAChRs in the nanomolar range and maintained a greater than 1,000-fold selectivity for the $\alpha4\beta2$ nAChR subtype over the $\alpha3\beta4$ or $\alpha7$ subtypes. In line with our emerging SAR, the *R* enantiomer of compound **10** showed reduced binding affinity and nAChR subtype selectivities in comparison to the corresponding *S* enantiomer.

Compound **22**, which contains a trifluoromethyl group at the 2-position of the pyridine ring, showed minimal binding affinity to every nAChR subtype tested. This may indicate that reducing the electron density in the pyridine ring and/or increasing steric bulk at the 2-position of the pyridine moiety can lead to lower binding affinities.

In Vitro Effects on nAChR Function. Six new compounds showing high binding affinities for $\alpha4\beta2$ nAChRs and high selectivities for this subtype over $\alpha3\beta4$ and $\alpha7$ receptors in binding assays were chosen for functional studies (Table 2). The agonist activities for these analogues were assessed by measuring stimulated $^{86}Rb^+$ efflux from stably transfected cells, either expressing human $\alpha4\beta2$ nAChRs or rat $\alpha3\beta4$ receptors. The ability of the analogues to desensitize the two nAChR subtypes were determined by measuring nicotine-stimulated $^{86}Rb^+$ efflux after cells were preincubated with the test compounds for 10 min. For comparison, nicotine, varenicline, and sazetidine-A were included in these experiments.

Table 2. Comparison of Activation and Desensitization of nAChR Function by Ligands

	$\alpha4\beta2$ nAChRs[a]			$\alpha3\beta4$ nAChRs[a]		
compd	EC_{50}[b] (nM)	E_{max}[c] (%)	$IC_{50(10')}$[d] (nM)	EC_{50} (nM)	E_{max} (%)	$IC_{50(10')}$ (nM)
(−)-nicotine	2,400	100	370	23,000	100	>10,000
varenicline	950	45	94	21,000	82	>10,000
sazetidine-A	24	40	11	30,000	12	>10,000
(*S*)-**9**	310	15	260	NA	NA	>10,000
(*S*)-**10**	NA[e]	NA	1,600	NA	NA	6,400
(*S*)-**11**	160	17	110	NA	NA	>10,000
(*S*)-**12**	450	20	51	NA	NA	>10,000
(*S*)-**16**	NA	NA	130	NA	NA	>10,000
(*S*)-**17**	NA	NA	260	NA	NA	>10,000

[a]Functional properties of each compound were determined using stable cell lines expressing human $\alpha4\beta2$ or rat $\alpha3\beta4$ nAChRs. [b]EC_{50} values show potencies of agonist activities. [c]E_{max} (%) values show relative efficacies of agonist activities, which were normalized to the E_{max} value of nicotine. [d]$IC_{50(10')}$ values show potencies of desensitizer activities. [e]NA indicates that no significant stimulated efflux was detected. All values shown are the means of 3 to 9 independent experiments (for clarity, SEM values were omitted, which were within 20% of the mean in most cases).

As expected, nicotine showed full agonist activities at both human $\alpha4\beta2$ and rat $\alpha3\beta4$ nAChRs (Table 2). Consistent with previous reports,[27,30,58] in comparison with (−)-nicotine our studies show that varenicline had 45% of the efficacy in stimulating efflux from cells expressing the $\alpha4\beta2$ nAChRs and 90% of the efficacy from cells expressing the $\alpha3\beta4$ nAChRs. Sazetidine-A showed lower efficacy at the $\alpha4\beta2$ nAChRs than that of varenicline. In contrast, we found that sazetidine-A's agonist activity at the $\alpha3\beta4$ nAChRs was lower than that of (−)-nicotine or varenicline. Consistent with the purposes of developing this line of novel ligands, all 6 novel compounds tested showed much lower agonist activities than those of sazetidine-A at both receptor subtypes. Compounds (*S*)-**9**, (*S*)-**11**, and (*S*)-**12** had less than 20% of the agonist efficacy at the human $\alpha4\beta2$ nAChRs in comparison to sazetidine-A. Therefore, though these three new compounds showed partial agonist activities at the $\alpha4\beta2$ nAChR subtype, their efficacies of activations were much lower than that of varenicline. Compounds (*S*)-**10**, (*S*)-**16**, and (*S*)-**17** showed no detectable agonist activity at the human $\alpha4\beta2$ receptor subtype. Additionally, all 6 compounds showed no detectable agonist activity at the rat $\alpha3\beta4$ nAChRs.

Consistent with our previous report,[35] (−)-nicotine, varenicline, and sazetidine-A potently and selectively desensitize $\alpha4\beta2$ nAChRs with $IC_{50(10')}$ values in the nanomolar range (Table 2). To our delight, compounds (*S*)-**9**, (*S*)-**11**, (*S*)-**12**, (*S*)-**16**, and (*S*)-**17** selectively desensitized the $\alpha4\beta2$ nAChRs. The $IC_{50(10')}$ values for these compounds to desensitize the $\alpha4\beta2$ nAChRs ranged from 51 nM for analogue (*S*)-**12** to 260 nM for analogue (*S*)-**17**. In contrast, the $IC_{50(10')}$ values for these compounds in desensitizing $\alpha3\beta4$ nAChRs were higher than 10,000 nM.

Analogue (*S*)-**10** has the lowest potency in desensitizing the $\alpha4\beta2$ nAChRs among all of the new compounds studied. Furthermore, the potency of analogue (*S*)-**10** to desensitize $\alpha4\beta2$ nAChRs is only slightly higher than that to desensitize $\alpha3\beta4$ nAChRs. This observation confirmed the importance of the four-member ring azetidinyl group in sazetidine-A, (*S*)-**9**, (*S*)-**11**, (*S*)-**12**, (*S*)-**16**, and (*S*)-**17** for selective desensitization of the $\alpha4\beta2$ nAChRs.

In Vivo Effects on Alcohol Intake in Rats. In our initial behavioral studies in an animal model, effects of compound (*S*)-**9** on alcohol self-administration in selectively bred alcohol-preferring rats (P rats) were assessed (Figure 2). Three doses of compound (*S*)-**9** were tested: 0.33, 1, and 3 mg/kg (s.c.). Compared with the control vehicle, compound (*S*)-**9** significantly reduced alcohol intake in a dose-dependent manner. At 0.33 mg/kg, compound (*S*)-**9** did not show a significant effect at any time point. The medium dose of 1 mg/kg reduced alcohol intake significantly ($p < 0.05$) over the 24-h time point after administration. At 3 mg/kg, the compound had significant effects on alcohol intake at both the 6-h ($p < 0.05$) and 24-h time points ($p < 0.0005$) (Figure 2).

Emetic Effects of Compound (*S*)-9 in Ferrets. The effects of compound (*S*)-9 on causing nausea and emesis were assessed in a ferret model. Varenicline was used as a control. Administration of compound (*S*)-**9** (1 to 30 mg/kg; s.c.) did not elicit any emetic episodes or stereotypical behaviors that are indicative of nausea ($n = 6$). In general, all animals remained alert and active with periods of inactivity that mirrored their baseline behavior within the 1-h observation period.

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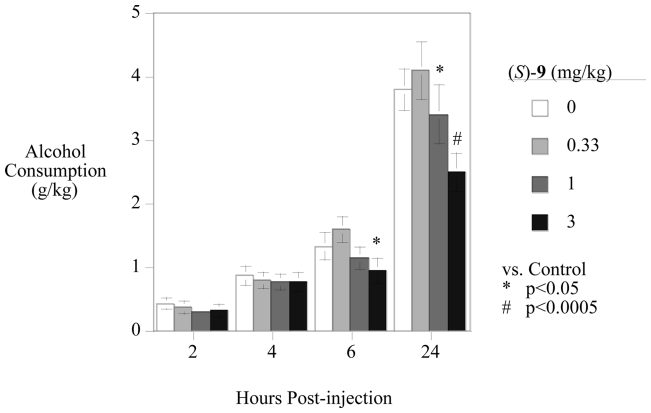

Figure 2. Effect of acute administration of compound (*S*)-**9** on alcohol intake in P-rats. Alcohol intakes (g/kg) were measured at 2, 4, 6, and 24 h after an injection of (*S*)-**9**. All values are expressed as the mean ± SEM (n = 18).

Varenicline (0.5 mg/kg; s.c.; n = 5) was administered to ferrets to assess its effects on emesis or stereotypical behaviors associated with nausea. Whereas no emetic episodes were observed, drug-induced nausea as evidenced by gagging (retching; mean # of episodes 2.80 + 1.63) were evident following varenicline administration in the majority of the animals (n = 4/5) tested. In one animal, this behavior was accompanied by oral stereotypy (licking), whereas in two animals it was interspersed by episodes of backward walking. On the average, these behaviors manifested around ∼2.20 min. In general, the locomotor behavior of these animals was noticeably reduced at ∼1.05 min postinjection and remained so for the duration of the observation period (∼1 h). Occasionally, when animals tried to ambulate they would immediately cease their movement after each attempt. This would be followed by a momentary increase in breathing giving the impression that the animal was in distress.

DISCUSSION AND CONCLUSIONS

We have developed a series of sazetidine-A analogues that contain a methyl group at the 2-position of the pyridine ring. Similar to sazetidine-A, compounds (*S*)-**9**, (*S*)-**10**, (*S*)-**11**, (*S*)-**12**, (*S*)-**16**, and (*S*)-**17** are highly selective to the $\alpha4\beta2$ nAChR subtype over the $\alpha3\beta4$ and $\alpha7$ subtypes in binding assays. In vitro pharmacological studies to assess their functional effects at nicotinic receptors showed that these compounds selectively desensitize $\alpha4\beta2$ nAChRs. Particularly noteworthy, we found that these compounds exhibit much lower agonist activities at all nAChR subtypes tested in comparison with that of sazetidine-A. In rat modeling, compound (*S*)-**9** significantly reduced alcohol self-administration. It is important to note that preliminary studies in a ferret model show that compound (*S*)-**9** has a better adverse effects profile than that of varenicline.

In our previous studies, sazetidine-A exhibited much higher binding affinity for $\alpha4\beta2$ nAChRs than for $\alpha3\beta4$ or $\alpha7$ receptors.[35,59] After cells expressing $\alpha4\beta2$ nAChRs were exposed to sazetidine-A for 10 min, sazetidine-A potently inhibited nicotine-stimulated function mediated by the $\alpha4\beta2$ nAChRs. Consistent with its high selectivity for the $\alpha4\beta2$ receptor, sazetidine-A at concentrations up to 10 μM had no effect on the function of $\alpha3\beta4$ or $\alpha7$ nAChRs. These observations are consistent with our hypothesis that sazetidine-A selectively desensitizes $\alpha4\beta2$ receptors. In addition to its ability to cause long lasting desensitization of $\alpha4\beta2$ receptors, Zwart and co-workers reported that sazetidine-A also elicited agonist activity at the $\alpha4\beta2$ receptors.[60] It is very interesting that this agonist effect is specific to the stoichiometry of the subunits. Of the two stoichiometric forms of the $\alpha4\beta2$ receptors, sazetidine-A showed full agonist activity at the $(\alpha4)_2(\beta2)_3$ receptors but nearly no agonist activity at the $(\alpha4)_3(\beta2)_2$ receptors.[61] Thus, sazetidine-A, like nicotine itself, has both agonist and desensitizer effects on $\beta2$-containing nAChRs, but in contrast to nicotine, its desensitizing effects appear to predominate and be long lasting.[35,59,62] For example, initial activation of the $\alpha4\beta2$ nAChRs in vitro by sazetidine-A is brief, lasting only seconds, whereas the receptor desensitization that immediately follows activation lasts more than an hour even with continual washing of the cells.

Many studies to further understand the in vitro pharmacological properties of sazetidine-A and its behavioral effects in animal models have been reported.[47−50,52,63−69] The results from these studies are highly supportive of our proposal to develop novel nicotinic therapeutics based on their ability to selectively desensitize $\alpha4\beta2$ nAChRs. In the present study, we investigated the agonist and desensitization properties of these new analogues at human $\alpha4\beta2$ nAChRs and rat $\alpha3\beta4$ nAChRs (Table 2). For the efficacies of agonist activities at $\alpha4\beta2$ nAChRs in comparison to nicotine, compounds (*S*)-**9**, (*S*)-**11**, and (*S*)-**12** showed very low relative E_{max} values at the $\alpha4\beta2$ nAChRs as follows: 15%, 17%, and 20%, respectively. Compounds (*S*)-**10**, (*S*)-**16**, and (*S*)-**17** did not show any measurable agonist activities at this subtype, and none of the compounds tested exhibited agonist activities at rat $\alpha3\beta4$ nAChRs. It is important to note that all of the new analogues tested showed significantly lower agonist activities at both $\alpha4\beta2$ and $\alpha3\beta4$ receptor subtypes than those of varenicline or sazetidine-A. With regards to desensitization activities, compounds (*S*)-**9**, (*S*)-**11**, (*S*)-**12**, (*S*)-**16**, and (*S*)-**17** potently and selectively desensitized the $\alpha4\beta2$ nAChRs after a 10-min incubation period (Table 2). These in vitro pharmacological functional properties indicate the possibility for these compounds to desensitize $\alpha4\beta2$ nAChRs in vivo.

As shown in Figure 2, compound (*S*)-**9** significantly reduced alcohol intake in a dose-dependent manner in the animal efficacy model using selectively bred alcohol-preferring rats. On the basis of the in vitro pharmacological property profile of compound (*S*)-**9**, we speculate that the ability of compound (*S*)-**9** in reducing alcohol intake resulted mainly from its $\alpha4\beta2$ nAChR desensitization properties. In our previous postdeprivation studies,[47] sazetidine-A at 3 mg/kg significantly reduced alcohol intake at the 2-, 4-, and 6-h time points but not at the 24-h time point. Interestingly, (*S*)-**9** showed a different pattern of the inhibition, significantly reducing the alcohol intake at the 6- and 24-h time points but not at the 2- and 4-h points. It is important to note that though the binding affinity of (*S*)-**9** to $\alpha4\beta2$ nAChRs is lower than that of sazetidine-A (Table 1), both compounds reduced alcohol intake at 3 mg/kg. It is conceivable that (*S*)-**9** might have reached a higher brain concentration than sazetidine-A at the same dose given systematically.

Varenicline is marketed in the U.S. and many other countries as a smoking cessation aid. Clinical trials before and after its approval as well as postmarketing reports demonstrated its superior efficacy compared with placebo or other marketed smoking cessation therapeutics.[28,29,70−72] In addition to its use as a smoking cessation drug, the significant efficacy of

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varenicline in reducing alcohol consumption in heavy-drinking smokers indicated the possibility for it to be used to treat alcohol use disorders.[32] However, notable adverse events related to varenicline have been reported,[28,29,34] which resulted in the recent ruling by the FDA that varenicline carry a black box label for suicidal ideation and suicidal behavior (FDA Drug Safety Newsletter 2009). The most common adverse events reported for varenicline are nausea and vomiting. For example, 30−40% of people taking varenicline reported nausea in randomized clinical trials, which is greater than 3 times that reported for the placebo group.[73] The adverse side effects of varenicline are thought to be mediated through its strong agonist activities at the $\alpha3\beta4$ and $\alpha7$ nAChR subtypes.[58] As shown in Table 2, compound (*S*)-**9** did not show any agonist activity at the rat $\alpha3\beta4$ nAChRs. In addition, preliminary studies using whole cell current measurements showed that compound (*S*)-**9** did not elicit any detectable current in cells expressing rat $\alpha7$ nAChRs (Sahibzada et al., data not shown). On the basis of a comparison of the in vitro pharmacological property profile of compound (*S*)-**9** with that of varenicline, it is conceivable that compound (*S*)-**9** might be much less likely to induce nausea and vomiting than varenicline. The animal studies in a ferret nausea and emetic model showed a clear difference between varenicline and compound (*S*)-**9** in their potential to induce nausea and vomiting. Varenicline at 0.5 mg/kg (s.c., $n = 5$) caused evident signs related to nausea and vomiting in 4 ferrets. In contrast, compound (*S*)-**9** at doses up to 30 mg/kg (s.c.) did not elicit any emetic episodes or stereotypical behaviors that are indicative of nausea ($n = 6$).

In summary, a series of novel sazetidine-A analogues were designed and synthesized. The in vitro pharmacological evaluations revealed that these compounds were selective $\alpha4\beta2$ nAChR desensitizers. Analogue (*S*)-**9**, our lead compound, showed significant efficacy in reducing alcohol intake in a rat model. More importantly, analogue (*S*)-**9** did not induce nausea and vomiting up to 30 mg/kg in ferrets, exhibiting a potentially better adverse side effect profile than that of varenicline. Therefore, we concluded that this series of analogues should be further studied in the development of new treatments for alcohol use disorders.

EXPERIMENTAL SECTION

General Chemistry Methods. All solvents and reagents were used as obtained from commercial sources unless otherwise indicated. All reactions were performed under nitrogen atmosphere unless otherwise noted. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400MR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Deuterated chloroform or deuterated methanol was used as the solvent for NMR experiments. ^{1}H Chemical shifts values (δ) are referenced to the residual nondeuterated components of the NMR solvents (δ = 7.26 ppm for $CHCl_3$ and δ = 3.31 ppm for CHD_2OD). The ^{13}C chemical shifts (δ) are referenced to $CDCl_3$ (central peak, δ = 77.0 ppm) or CD_3OD (δ = 49.0 ppm) as the internal standard. Optical rotation was detected on an ADP220 Automatic polarimeter from Bellingham & Stanley Limited. Mass spectra were measured in positive mode electrospray ionization (ESI). The HRMS data were obtained on a Waters Q-TOF Premier mass spectrometer. TLC was performed on silica gel 60 F_{254} plastic sheets. Column chromatography was performed using silica gel (35−75 mesh). Combustion analyses were performed at Atlantic Microlabs, Inc. Norcross, GA.

General Procedure for the Mitsunobu Reaction. To a mixture of the N-Boc protected alcohol (1.0 equiv), the 5-halogen-3-pyridinol (1.0 equiv), and Ph_3P (1.3 equiv) in anhydrous THF (0.10 M) was added DEAD (1.3 equiv) dropwise at 0 °C under nitrogen atmosphere. After stirring for 2 days at room temperature, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of hexane−ethyl acetate (10:1 to 5:1) as the eluent to give the product in 68−90% yield.

General Procedure for the Sonogashira Coupling Reaction. A mixture of the Mitsunobu adduct (1.0 equiv), alkyne (4.0 equiv), $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (10 mol %), and PPh_3 (10 mol %) in Et_3N/DMSO (10:1, 0.12 M) was heated to 95 °C under a nitrogen atmosphere in a sealed tube for 60 h. The cooled reaction mixture was taken up in ethyl acetate, and the organic phase was washed with water, brine, and then dried over Na_2SO_4. The extract was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel using a gradient of CH_2Cl_2−ethyl acetate (16:1 to 10:1) as the eluent to give the product in 88−98% yield.

General Procedure (Method A) for Deprotection of the N-Boc Group. To a stirred solution of the Sonogashira adduct (1.0 equiv) in dichloromethane (0.10 M) was added trifluoroacetic acid (32 equiv) dropwise at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 3 h at room temperature. The solvent and excess TFA was then removed under reduced pressure. To the residue was added 2−3 mL of methanol followed by dropwise addition of 10% aqueous NaOH solution at 0 °C until the pH value of the mixture was 9−10. After the mixture was stirred at room temperature for 30 min, the solution was taken up in dichloromethane, and the organic phase was washed with brine and dried over Na_2SO_4. The extract was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of CH_2Cl_2−methanol (20:1 to 10:1) as the eluent to give the product in 75−83% yield.

General Procedure (Method B) for Deprotection of the N-Boc Group. To the Boc-protected compound (1.0 mmol), a solution of 2 M HCl in methanol (10 mL) was added at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel using a gradient of CH_2Cl_2−methanol (10:1 to 5:1) as the eluent to give the product as a white solid in 73−92% yield.

*(S)-tert-Butyl 2-((5-bromo-2-methylpyridin-3-yloxy)methyl)-azetidine-1-carboxylate ((S)-**3**).* Yield: 69% (light red solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (s, 1H), 7.26 (s, 1H), 4.52 (m, 1H), 4.33 (m, 1H), 4.05 (m, 1H), 3.90 (m, 2H), 2.45 (s, 3H), 2.34 (m, 2H), 1.41 (s, 9H).

*(R)-tert-Butyl 2-((5-bromo-2-methylpyridin-3-yloxy)methyl)-azetidine-1-carboxylate ((R)-**3**).* Yield: 77% (light red solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16 (s, 1H), 7.26 (s, 1H), 4.53 (m, 1H), 4.34 (m, 1H), 4.06 (m, 1H), 3.91 (m, 2H), 2.45 (s, 3H), 2.35 (m, 2H), 1.42 (s, 9H).

*(S)-tert-Butyl 2-((5-bromo-2-methylpyridin-3-yloxy)methyl) pyrrolidine-1-carboxylate ((S)-**4**).* Yield: 68% (light red solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.13 (s, 1H), 7.26 (br s, 1H), 4.15 (m, 2H), 4.00 (m, 0.5H), 3.85 (m, 0.5H), 3.41 (m, 2H), 2.41 (s, 3H), 1.96 (m, 4H), 1.48 (s, 9H).

*(R)-tert-Butyl 2-((5-bromo-2-methylpyridin-3-yloxy)methyl) pyrrolidine-1-carboxylate ((R)-**4**).* Yield: 70% (light red solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (s, 1H), 7.26 (br s, 1H), 4.14 (m, 2H), 3.99 (m, 0.5H), 3.84 (m, 0.5H), 3.41 (m, 2H), 2.40 (s, 3H), 1.95 (m, 4H), 1.47 (s, 9H).

*(S)-tert-Butyl 2-((5-(hex-1-ynyl)-2-methylpyridin-3-yloxy)methyl) azetidine-1-carboxylate ((S)-**5**).* Yield: 95% (light yellow oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (d, 1H, J = 1.2 Hz), 7.10 (d, 1H, J = 1.2 Hz), 4.52 (m, 1H), 4.32 (m, 1H), 4.05 (dd, 1H, J = 10, 2.8 Hz), 3.90 (m, 2H), 2.48 (s, 3H), 2.41 (t, 2H, J = 7.2 Hz), 2.33 (m, 2H), 1.59 (m, 2H), 1.48 (m, 2H), 1.41 (s, 9H), 0.95 (t, 3H, J = 7.2 Hz).

*(R)-tert-Butyl 2-((5-(hex-1-ynyl)-2-methylpyridin-3-yloxy)methyl) azetidine-1-carboxylate ((R)-**5**).* Method B was used. Yield: 93% (light yellow oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (d, 1H, J = 1.2 Hz), 7.09 (d, 1H, J = 1.2 Hz), 4.50 (m, 1H), 4.30 (m, 1H), 4.03 (dd, 1H, J = 10, 2.8 Hz), 3.89 (m, 2H), 2.46 (s, 3H), 2.39 (t, 2H, J = 7.2

Hz), 2.31 (m, 2H), 1.57 (m, 2H), 1.46 (m, 2H), 1.39 (s, 9H), 0.94 (t, 3H, J = 7.2 Hz).

*(S)-tert-Butyl 2-((5-(hex-1-ynyl)-2-methylpyridin-3-yloxy)methyl) pyrrolidine-1-carboxylate ((S)-**6**).* Yield: 98% (light yellow oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (s, 1H), 7.11 (br s, 1H), 4.14 (m, 2H), 4.00 (m, 0.5H), 3.83 (m, 0.5H), 3.42 (m, 2H), 2.44 (s, 3H), 2.41 (t, 2H, J = 7.2 Hz), 1.96 (m, 4H), 1.60 (m, 2H), 1.48 (m, 11H), 0.95 (t, 3H, J = 7.2 Hz).

*(R)-tert-Butyl 2-((5-(hex-1-ynyl)-2-methylpyridin-3-yloxy)methyl) pyrrolidine-1-carboxylate ((R)-**6**).* Method B was used. Yield: 92% (light yellow oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (s, 1H), 7.10 (br s, 1H), 4.13 (m, 2H), 3.99 (m, 0.5H), 3.82 (m, 0.5H), 3.41 (m, 2H), 2.43 (s, 3H), 2.40 (t, 2H, J = 7.2 Hz), 1.95 (m, 4H), 1.59 (m, 2H), 1.48 (m, 11H), 0.95 (t, 3H, J = 7.2 Hz).

*(S)-tert-Butyl 2-((5-(cyclopropylethynyl)-2-methylpyridin-3-yloxy)methyl)azetidine-1-carboxylate ((S)-**7**).* Yield: 97% (light yellow oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, 1H, J = 1.2 Hz), 7.08 (d, 1H, J = 1.2 Hz), 4.51 (m, 1H), 4.29 (m, 1H), 4.03 (dd, 1H, J = 10, 2.8 Hz), 3.89 (m, 2H), 2.46 (s, 3H), 2.32 (m, 2H), 1.42 (m, 1H), 1.40 (s, 9H), 0.87 (m, 2H), 0.80 (m, 2H).

*(S)-tert-Butyl 2-((5-(6-hydroxyhex-1-ynyl)-2-methylpyridin-3-yloxy) methyl) azetidine-1-carboxylate ((S)-**8**).* Yield: 89% (yellow oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.04 (br s, 1H), 7.06 (s, 1H), 4.46 (m, 1H), 4.25 (m, 1H), 3.98 (dd, 1H, J = 10, 2.0 Hz), 3.84 (m, 2H), 3.62 (t, 2H, J = 6.0 Hz), 2.91 (br s, 1H), 2.41 (s, 3H), 2.39 (t, 2H, J = 6.0 Hz), 2.26 (m, 2H), 1.65 (m, 4H), 1.34 (s, 9H).

*(S)-3-(Azetidin-2-ylmethoxy)-5-(hex-1-ynyl)-2-methylpyridine ((S)-**9**).* Method A was used. Yield: 83% (light red oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08 (d, 1H, J = 1.6 Hz), 7.04 (d, 1H, J = 1.6 Hz), 4.25 (m, 1H), 3.96 (m, 2H), 3.67 (m, 1H), 3.47 (m, 1H), 2.42 (s, 3H), 2.38 (m, 4H), 2.24 (m, 1H), 1.57 (m, 2H), 1.46 (m, 2H), 0.92 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.3, 148.0, 143.1, 119.7, 118.8, 92.7, 77.4, 72.4, 57.1, 44.3, 30.6, 23.9, 22.0, 19.2, 19.1, 13.5. HRMS (ESI) m/z calcd for $C_{16}H_{22}N_2O$ (M + H)$^+$ 259.1810; found, 259.1813; $[\alpha]_D^{24}$ = −4.3 (c 0.77, $CHCl_3$). Anal. Calcd for $C_{16}H_{22}N_2O{\cdot}0.625H_2O$: C, 71.28; H, 8.69; N, 10.39; O, 9.64. Found: C, 71.25; H, 8.46; N, 10.13; O, 9.89.

*(R)-3-(Azetidin-2-ylmethoxy)-5-(hex-1-ynyl)-2-methylpyridine ((R)-**9**).* Method A was used. Yield: 75% (light red oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07 (d, 1H, J = 1.6 Hz), 7.04 (d, 1H, J = 1.6 Hz), 4.24 (m, 1H), 3.94 (m, 2H), 3.66 (m, 1H), 3.45 (m, 1H), 2.41 (s, 3H), 2.36 (m, 3H), 2.24 (m, 2H), 1.56 (m, 2H), 1.45 (m, 2H), 0.92 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.3, 148.0, 143.1, 119.7, 118.8, 92.7, 77.4, 72.5, 57.1, 44.3, 30.6, 24.0, 22.0, 19.2, 19.0, 13.5. HRMS (ESI) m/z calcd for $C_{16}H_{22}N_2O$ (M + H)$^+$ 259.1810; found, 259.1816; $[\alpha]_D^{25}$ = +10.8 (c 0.62, $CHCl_3$). Anal. Calcd for $C_{16}H_{22}N_2O{\cdot}0.375H_2O$: C, 72.49; H, 8.65; N, 10.57. Found: C, 72.72; H, 8.67; N, 10.43.

*(S)-**5**-(Hex-1-ynyl)-2-methyl-3-(pyrrolidin-2-ylmethoxy)pyridine ((S)-**10**).* Method A was used. Yield: 80% (light red oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07 (d, 1H, J = 1.6 Hz), 7.02 (d, 1H, J = 1.6 Hz), 3.84 (m, 2H), 3.52 (m, 1H), 2.98 (m, 2H), 2.42 (s, 3H), 2.38 (t, 2H, J = 7.2 Hz), 2.11 (br s, 1H), 1.93 (m, 1H), 1.78 (m, 2H), 1.56 (m, 3H), 1.45 (m, 2H), 0.92 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.3, 147.8, 143.0, 119.6, 118.8, 92.6, 77.5, 71.6, 57.0, 46.7, 30.6, 28.0, 25.4, 22.0, 19.3, 19.0, 13.5. HRMS (ESI) m/z calcd for $C_{17}H_{24}N_2O$ (M + H)$^+$ 273.1967; found, 273.1964; $[\alpha]_D^{25}$ = +6.4 (c 1.05, $CHCl_3$). Anal. Calcd for $C_{17}H_{24}N_2O{\bullet}0.375H_2O$: C, 73.15; H, 8.94; N, 10.04. Found: C, 73.29; H, 8.83; N, 9.99.

*(R)-5-(Hex-1-ynyl)-2-methyl-3-(pyrrolidin-2-ylmethoxy) Pyridine ((R)-**10**).* Method A was used. Yield: 83% (light red oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06 (d, 1H, J = 1.6 Hz), 7.02 (d, 1H, J = 1.6 Hz), 3.84 (m, 2H), 3.52 (m, 1H), 2.97 (m, 2H), 2.66 (br s, 1H), 2.40 (s, 3H), 2.37 (t, 2H, J = 7.2 Hz), 1.93 (m, 1H), 1.78 (m, 2H), 1.55 (m, 3H), 1.44 (m, 2H), 0.91 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.2, 147.8, 143.0, 119.6, 118.8, 92.6, 77.4, 71.4, 57.0, 46.6, 30.6, 27.9, 25.3, 21.9, 19.2, 19.0, 13.5. HRMS (ESI) m/z calcd for $C_{17}H_{24}N_2O$ (M + H)$^+$ 273.1967; found, 273.1960; $[\alpha]_D^{24}$ = −4.4 (c 0.85, $CHCl_3$). Anal. Calcd for $C_{17}H_{24}N_2O{\cdot}0.75H_2O$: C, 71.42; H, 8.99; N, 9.80. Found: C, 71.58; H, 8.80; N, 9.51.

*(S)-3-(Azetidin-2-ylmethoxy)-5-(cyclopropylethynyl)-2-methylpyridine Dihydrochloride ((S)-**11**).* Method B was used. Yield: 91% (off-white solid). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.18 (s, 1H), 7.72 (s, 1H), 4.93 (m, 1H), 4.47 (m, 2H), 4.11 (m, 2H), 2.71 (m, 2H), 2.60 (s, 3H), 1.54 (m, 1H), 0.95 (m, 2H), 0.81 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ 154.4, 147.3, 140.2, 125.7, 122.8, 100.2, 71.5, 68.9, 60.3, 45.0, 21.9, 17.5, 9.3 (2C), 0.7. HRMS (ESI) m/z calcd for $C_{15}H_{18}N_2O$ (M + H)$^+$ 243.1497; found, 243.1509. $[\alpha]_D^{26}$ = −8.1 (c 0.82, MeOH). Anal. Calcd for $C_{15}H_{18}N_2O{\cdot}2HCl{\cdot}2.5H_2O$: C, 50.01; H, 6.99; N, 7.78. Found: C, 49.64; H, 6.82; N, 8.12.

*(S)-6-(5-(Azetidin-2-ylmethoxy)-6-methylpyridin-3-yl)hex-5-yn-1-ol Dihydrochloride ((S)-**12**).* Method B was used. Yield: 87% (white solid). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.37 (s, 1H), 8.12 (s, 1H), 4.97 (m, 1H), 4.59 (m, 2H), 4.13 (m, 2H), 3.61 (t, 2H, J = 5.2 Hz), 2.71 (m, 5H), 2.56 (m, 2H), 1.71 (m, 4H). ^{13}C NMR (CD_3OD, 100 MHz): δ 155.6, 146.2, 136.2, 129.4, 124.4, 99.3, 75.5, 69.6, 62.3, 60.0, 44.9, 32.7, 25.8, 21.8, 19.9, 15.7. HRMS (ESI) m/z calcd for $C_{16}H_{22}N_2O_2$ (M + H)$^+$ 275.1760; found, 275.1761. $[\alpha]_D^{26}$ = −7.1 (c 0.94, MeOH). Anal. Calcd for $C_{16}H_{22}N_2O_2{\cdot}2HCl{\cdot}H_2O$: C, 52.61; H, 7.17; N, 7.67. Found: C, 52.54; H, 6.92; N, 7.92.

*(S)-tert-Butyl 2-(2-methyl-5-(6-(tosyloxy) hex-1-ynyl) pyridine-3-yloxy) methyl)azetidine-1-carboxylate ((S)-**13**).* To a stirred solution of **8** (374 mg, 1 mmol) in dry dichloromethane (25 mL) was added DMAP (12 mg, 0.1 mmol), Et_3N (0.34 mL, 2.4 mmol), and tosyl chloride (228 mg, 1.2 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The mixture was taken up in dichloromethane, and the organic phase was washed with saturated aqueous $NaHCO_3$ solution, brine, and then dried over Na_2SO_4. The extract was concentrated under reduced pressure, and then the residue was purified by column chromatography on silica gel using a gradient of hexane−ethyl acetate (6:1 to 3:1) as the eluent to give the product (**13**) 422 mg as light yellow oil, Yield: 80%. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.05 (s, 1H), 7.75 (d, 2H, J = 8.4 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.07 (s, 1H), 4.49 (m, 1H), 4.29 (m, 1H), 4.06 (t, 2H, J = 6.4 Hz), 4.01 (dd, 1H, J = 10.4, 2.4 Hz), 3.87 (m, 2H), 2.44 (s, 3H), 2.40 (s, 3H), 2.36 (t, 2H, J = 6.8 Hz), 2.28 (m, 2H), 1.80 (m, 2H), 1.61 (m, 2H), 1.37 (s, 9H).

*(S)-tert-Butyl 2-((5-(6-chlorohex-1-ynyl)-2-methylpyridin-3-yloxy)methyl)azetidine-1-carboxylate ((S)-**14**).* A mixture of the compound **8** (374 mg, 1 mmol), PPh_3 (288 mg, 1.1 mmol), and $NaHCO_3$ (20 mg, 0.24 mmol) in dry CCl_4 (15 mL) was heated to 80 °C under a nitrogen atmosphere in a sealed tube for 48 h. The cooled reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel using a gradient of hexane−ethyl acetate (6:1 to 3:1) as the eluent to give the product **14** (271 mg) as a light yellow oil, Yield: 69%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, 1H, J = 1.2 Hz), 7.08 (d, 1H, J = 1.2 Hz), 4.49 (m, 1H), 4.30 (m, 1H), 4.02 (dd, 1H, J = 10, 2.8 Hz), 3.88 (m, 2H), 3.57 (t, 2H, J = 6.4 Hz), 2.45 (s, 3H), 2.44 (t, 2H, J = 6.8 Hz), 2.31 (m, 2H), 1.92 (m, 2H), 1.74 (m, 2H), 1.38 (s, 9H).

*(S)-tert-Butyl 2-((5-(6-fluorohex-1-ynyl)-2-methylpyridin-3-yloxy)-methyl)azetidine-1-carboxylate ((S)-**15**).* A mixture of **13** (67 mg, 0.13 mmol), Kryptofix 2.2.2 (62 mg, 0.16 mmol), and KF (10 mg, 0.17 mmol) in dry THF (3 mL) was heated to reflux overnight under a nitrogen atmosphere. The cooled reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of hexane-ethyl acetate (4:1) as the eluent to give the product 40 mg as light yellow oil, Yield: 85%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.11 (s, 1H), 7.10 (s, 1H), 4.56 (t, 1H, J = 6.0 Hz), 4.51 (m, 1H), 4.44 (t, 1H, J = 6.0 Hz), 4.31 (m, 1H), 4.05 (dd, 1H, J = 10, 2.8 Hz), 3.90 (m, 2H), 2.48 (m, 5H), 2.33 (m, 2H), 1.90 (m, 1H), 1.83 (m, 1H), 1.74 (m, 2H), 1.41 (s, 9H).

*(S)-3-(Azetidin-2-ylmethoxy)-5-(6-fluorohex-1-ynyl)-2-methylpyridine dihydrochloride ((S)-**16**).* Method B was used. Yield: 92% (white solid). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.09 (s, 1H), 7.49 (s, 1H), 4.91 (m, 1H), 4.54 (t, 1H, J = 6.0 Hz), 4.42 (t, 1H, J = 6.0 Hz), 4.41 (m, 2H), 4.11 (m, 2H), 2.69 (m, 2H), 2.53 (m, 5H), 1.89 (m, 1H), 1.82 (m, 1H), 1.73 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ 153.6, 148.6, 143.1, 123.0, 121.5, 94.6, 84.5 (d, J = 163.1 Hz), 77.9, 68.4, 60.5, 45.0, 30.8 (d, J = 20.3 Hz), 25.6 (d, J = 4.9 Hz), 22.0, 19.8, 18.7. ^{19}F

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NMR (CD_3OD, 376 MHz): −220.6. HRMS (ESI) m/z calcd for $C_{16}H_{21}FN_2O$ $(M + H)^+$ 277.1716; found, 277.1714. $[\alpha]_D^{25} = -7.2$ (c 1.16, MeOH). Anal. Calcd for $C_{16}H_{21}FN_2O{\cdot}2HCl{\cdot}2.5H_2O$: C, 48.74; H, 7.16; N, 7.10. Found: C, 48.69; H, 6.84; N, 7.06.

*(S)-3-(Azetidin-2-ylmethoxy)-5-(6-chlorohex-1-ynyl)-2-methylpyridine Dihydrochloride ((S)-**17**).* Method B was used. Yield: 90% (white solid). ^1H NMR (CD_3OD, 400 MHz): δ 8.39 (s, 1H), 8.17 (s, 1H), 4.98 (m, 1H), 4.62 (m, 2H), 4.13 (m, 2H), 3.64 (t, 2H, J = 6.4 Hz), 2.73 (m, 5H), 2.58 (t, 2H, J = 6.8 Hz), 1.96 (m, 2H), 1.80 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ 154.3, 144.8, 134.4, 128.4, 122.9, 97.5, 74.1, 68.2, 58.6, 43.8, 43.4, 31.4, 25.1, 20.3, 18.0, 14.1. HRMS (ESI) m/z calcd for $C_{16}H_{21}ClN_2O$ $(M + H)^+$ 293.1421; found, 293.1430. $[\alpha]_D^{25} = -4.6$ (c 0.72, MeOH). Anal. Calcd. for $C_{16}H_{21}ClN_2O{\bullet}2HCl{\bullet}0.75H_2O$: C, 50.67; H, 6.51; N, 7.39. Found: C, 50.31; H, 6.12; N, 7.59.

*5-Bromo-2-(trifluoromethyl)pyridin-3-ol (**19**).* A solution of 5-bromo-3-methoxy-2-(trifluoromethyl)pyridine (**18**) (0.87 g, 3.4 mmol) in a mixture of acetic acid (6 mL) and 33% HBr in acetic acid (12 mL) was stirred at 110 °C in a sealed tube overnight. Then, potassium sodium tartrate tetrahydrate (7.8 g) was added slowly at 0 °C. The mixture was taken up in ethyl acetate after stirring for 10 min, and the organic phase was washed with brine and dried over sodium sulfate. The extract was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of hexane−ethyl acetate (1:2) as the eluent to give 0.65 g of the product (**19**) as a gray solid with a yield of 79%. ^1H NMR (CD_3OD, 400 MHz): δ 8.18 (s, 1H), 7.58 (s, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 154.6, 141.4, 134.7 (q, J = 34 Hz), 128.8, 125.2, 123.3 (q, J = 272 Hz). ^{19}F NMR ($CDCl_3$, 376 MHz): −68.0. HRMS (ESI) m/z calcd for $C_6H_3BrF_3NO$ $(M + H)^+$ 241.9428; found, 241.9434.

*(S)-tert-Butyl 2-((5-bromo-2-(trifluoromethyl)pyridin-3-yloxy)-methyl)azetidine-1-carboxylate ((S)-**20**).* The general procedure for the Mitsunobu Reaction was used. Yield: 88% (brown oil). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.31 (s, 1H), 7.62 (s, 1H), 4.55 (m, 2H), 4.11 (m, 1H), 3.88 (m, 2H), 2.36 (m, 2H), 1.41 (s, 9H).

*(S)-tert-Butyl 2-((5-(hex-1-ynyl)-2-(trifluoromethyl)pyridin-3-yloxy)methyl)azetidine-1-carboxylate ((S)-**21**).* A mixture of compound (**20**) (290 mg, 0.7 mmol), 1-hexyne (0.3 mL, 2.8 mmol), $Pd(PPh_3)_2Cl_2$ (25 mg, 0.036 mmol), CuI (13.5 mg, 0.071 mmol), and PPh_3 (18.6 mg, 0.071 mmol) in Et_3N/DMSO (5 mL/0.5 mL) was heated to 50 °C under a nitrogen atmosphere overnight. The cooled reaction mixture was taken up in ethyl acetate, and the organic phase was washed with water, brine, and then dried over Na_2SO_4. The extract was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel using a gradient of CH_2Cl_2−ethyl acetate (50:1 to 20:1) as the eluent to give the product (265 mg) as a yellow oil. Yield: 91%. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.22 (s, 1H), 7.40 (s, 1H), 4.52 (m, 2H), 4.10 (m, 1H), 3.89 (m, 2H), 2.44 (t, 2H, J = 7.2 Hz), 2.36 (m, 2H), 1.59 (m, 2H), 1.48 (m, 2H), 1.41 (s, 9H), 0.96 (t, 3H, J = 7.2 Hz).

*(S)-3-(Azetidin-2-ylmethoxy)-5-(hex-1-ynyl)-2-(trifluoromethyl) Pyridine Hydrochloride ((S)-**22**).* Method B was used. Yield: 73% (white solid). ^1H NMR (CD_3OD, 400 MHz): δ 8.25 (s, 1H), 7.74 (s, 1H), 4.81 (m, 1H), 4.46 (m, 2H), 4.02 (m, 2H), 2.72 (m, 1H), 2.60 (m, 1H), 2.51 (t, 2H, J = 7.2 Hz), 1.63 (m, 2H), 1.52 (m, 2H), 0.98 (t, 3H, J = 7.2 Hz). ^{13}C NMR (CD_3OD, 100 MHz): δ 153.8, 144.3, 135.5 (q, J = 34 Hz), 127.4, 125.2, 123.1 (q, J = 272 Hz), 98.3, 77.1, 69.9, 59.7, 44.7, 31.6, 23.0, 22.1, 19.8, 13.9. ^{19}F NMR (CD_3OD, 376 MHz): −67.3. HRMS (ESI) m/z calcd for $C_{16}H_{19}F_3N_2O$ $(M + H)^+$ 313.1528; found, 313.1525. $[\alpha]_D^{25} = -3.9$ (c 0.86, MeOH). Anal. Calcd for $C_{16}H_{19}F_3N_2O{\cdot}HCl$: C, 55.10; H, 5.78; N, 8.03. Found: C, 55.49; H, 5.65; N, 7.87.

Cell Lines and Cell Culture. The cell lines expressing defined rat nAChR subtypes were established previously by stably transfecting HEK 293 cells with rat nAChR subunit genes.[74,75] The cell line expressing human $\alpha4\beta2$ nAChRs, YX$\alpha4\beta2$H1, was established recently. These cell lines were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 100 units/mL penicillin G, 100 mg/mL streptomycin, and selective antibiotics at 37 °C with 5% CO_2 in a humidified incubator. Fetal bovine serum was provided by Gemini Bio-Products (Woodland, CA). Tissue culture medium and antibiotics were obtained from Invitrogen Corporation (Carlsbad, CA), unless otherwise stated.

[^{3}H]-Epibatidine Radioligand Binding Assay. Membrane preparation procedures and binding assays were described previously.[74,75] Briefly, cultured cells at >80% confluence were removed from their flasks (80 cm^2) with a disposable cell scraper and placed in 10 mL of 50 mM Tris·HCl buffer (pH 7.4, 4 °C). The cell suspension was centrifuged at 10,000g for 5 min, and the pellet was collected. The cell pellet was then homogenized in 10 mL of buffer with a polytron homogenizer and centrifuged at 36,000g for 10 min at 4 °C. The membrane pellet was resuspended in fresh buffer, and aliquots of the membrane preparation were used for binding assays. The concentration of [^{3}H]-epibatidine used was ∼500 pM for competition binding assays. Nonspecific binding was assessed in parallel incubations in the presence of 300 μM nicotine. Bound and free ligands were separated by vacuum filtration through Whatman GF/C filters treated with 0.5% polyethylenimine. The filter-retained radioactivity was measured by liquid scintillation counting. Specific binding was defined as the difference between total binding and nonspecific binding. Data from competition binding assays were analyzed using Prism 5 (GraphPad Software, San Diego, CA). The K_d values for the [^{3}H]-epibatidine used for calculating K_i values of nAChR subtypes were 0.02 nM for $\alpha2\beta2$, 0.08 nM for $\alpha2\beta4$, 0.03 nM for $\alpha3\beta2$, 0.3 nM for $\alpha3\beta4$, 0.04 nM for $\alpha4\beta2$, 0.09 nM for $\alpha4\beta4$, 1.8 nM for $\alpha7$, and 0.05 for rat forebrain.

$^{86}Rb^+$ Efflux Assay. Functional properties of compounds at nAChRs expressed in the transfected cells were measured using $^{86}Rb^+$ efflux assays as described previously.[35,75] In brief, cells expressing human $\alpha4\beta2$ or rat $\alpha3\beta4$ nAChRs were plated into 24-well plates coated with poly-D-lysine. The plated cells were grown at 37 °C for 18 to 24 h to reach 85−95% confluence. The cells were then incubated in growth medium (0.5 mL/well) containing $^{86}Rb^+$ (2 μCi/mL) for 4 h at 37 °C. The loading mixture was then aspirated, and the cells were washed four times with 1 mL of buffer (15 mM HEPES, 140 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 1.8 mM $CaCl_2$, and 11 mM glucose, pH 7.4). One milliliter of buffer with or without compounds to be tested was then added to each well. After incubation for 2 min, the assay buffer was collected for measurements of $^{86}Rb^+$ released from the cells. Cells were then lysed by adding 1 mL of 100 mM NaOH to each well, and the lysate was collected for the determination of the amount of $^{86}Rb^+$ that was in the cells at the end of the efflux assay. Radioactivity of assay samples and lysates was measured by liquid scintillation counting. Total loading (cpm) was calculated as the sum of the assay sample and the lysate of each well. The amount of $^{86}Rb^+$ efflux was expressed as a percentage of $^{86}Rb^+$ loaded. Stimulated $^{86}Rb^+$ efflux was defined as the difference between efflux in the presence and absence of nicotine. For obtaining EC_{50} and E_{max} values, stimulation curves were constructed in which 8 different concentrations of a ligand were included in the assay. For obtaining an $IC_{50(10')}$ value, inhibition curves were constructed in which eight different concentrations of a compound were applied to cells for 10 min before 100 μM nicotine was applied to measure stimulated efflux. EC_{50}, E_{max}, and $IC_{50(10')}$ values were determined by nonlinear least-squares regression analyses (GraphPad, San Diego, CA).

General Procedures for Effect on Alcohol Intake in Rats. Adult male rats were obtained from a colony of selectively bred alcohol-preferring rats (P rats) maintained at Indiana University School of Medicine. Rats were housed in cages that were fitted with two 100 mL Richter tubes for the recording of water and alcohol intakes. Animals were kept under a constant room temperature of 22 ± 1 °C and a 12:12 light−dark cycle (7:00 a.m.−7:00 p.m. dark). Animals were fed 5001 Rodent Chow (Lab Diet, Brentwood, MO, USA). All procedures were approved by the IACUC at Duke University Medical Center.

After a week of handling and habituation, rats were given free access to water in a graduated Richter tube for 1 day. Next, they were given free access only to a solution of 10% (v/v) alcohol for 3 consecutive days. Thereafter, rats were given free access to water and a solution of

10% alcohol throughout the study. Water and alcohol intake were indexed by graduated Richter drinking tubes.

An acute study was conducted to determine a dose−response for compound (*S*)-**9**. After the establishment of a stable baseline for alcohol and water intake, rats were injected subcutaneously with 0.33, 1, and 3 mg/kg of compound (*S*)-**9** or the same volume of the vehicle (1 mg/kg). Alcohol and water intakes were measured at, 2, 4, 6, and 24 h after drug administration. The preference for alcohol solution, [(alcohol volume)/(alcohol + water volume) × 100], was calculated for the same time points as alcohol and water intake.[47] All animals (n = 18) received all treatments following a crossover design with random assignment. The interval between injections was at least 3 days.

The solution of 10% (v/v) ethyl alcohol was prepared twice weekly from a solution of 200% ethanol mixed with tap water. Solutions of compound (*S*)-**9** was prepared weekly in 100 mM HCl solution and isotonic saline and was injected subcutaneously in a volume of 1 mL/kg.

The data were assessed by an analysis of variance with a between subjects factor of strain and a repeated measures factor of compound (*S*)-**9** dose. $P < 0.05$ (two-tailed) was used as the threshold for significance.

General Procedures for Emesis Studies in Ferrets. Studies were undertaken in male ferrets (*Mustela putorius furo*) weighing 0.9−1.5 kg (Marshall Farms, NY). They were housed in controlled conditions of room temperature (22 °C) and light (12:12 h light−dark cycle) with free access to food and water. The technique that was employed to study emesis is one that was adapted from Osinski, et al.[76] Prior to each experiment, food was withheld overnight, whereas water was provided ad libitum. All tested compounds were constituted in 0.9% saline. Each ferret was tested with either varenicline (0.5 mg/kg) or compound (*S*)-**9** (1−30 mg/kg).

Emetic testing consisted of placing animals individually in a polycarbonate enclosure. Following a 30 min acclimation period, each animal randomly received a subcutaneus injection of varencline or compound (*S*)-**9** in the subscapular region. A 60 min observation and video recording followed drug administration. Following this period, animals that showed drug effects of either nausea and/or emesis were administered intraperitoneally a 1 mg/kg combination of granisetron and dexamethasone and returned to their home enclosure.[77] All drugs were dissolved in sterile saline.

Video analysis of the incidence of emesis, stereotypical behaviors (such as licking, mouth-clawing, backward walking, gagging, and burying of the head in the cage), and GI side effects (diarrhea) was done off-line. (Note: stereotypical behaviors are thought to be reflective of a subjective sensation of nausea.)

AUTHOR INFORMATION

Corresponding Authors

*(M.P.) Phone: +1-202-687-5341. E-mail: map65@georgetown.edu.

*(Y.X.) Phone: +1-202-687-5390. E-mail: yxiao02@georgetown.edu.

Notes

The authors declare the following competing financial interest(s): Y.L., K.J.K., A.H.R., E.D.L., M.L.B., Y.X., and M.P. are co-inventors on the novel compounds reported in this article.

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ABBREVIATIONS USED

nAChR, nicotinic acetylcholine receptors; CNS, central nervous system; PNS, peripheral nervous system

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