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Synthesis and Biological Evaluation of Novel Hybrids of Highly Potent and Selective $\alpha 4\beta 2$ -Nicotinic Acetylcholine Receptor (nAChR) Partial Agonists

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Abstract

We previously reported the cyclopropylpyridine and isoxazolylpyridine ether scaffolds to be versatile building blocks for creating potent $\alpha 4\beta 2$ nicotinic acetylcholine receptor (nAChR) partial agonists with excellent selectivity over the $\alpha 3\beta 4$ subtype. In our continued efforts to develop therapeutic nicotinic ligands, seven novel hybrid compounds were rationally designed, synthesized, and evaluated in [³H]epibatidine binding competition studies. Incorporation of a cyclopropane- or isoxazole-containing side chain onto the 5-position of 1-(pyridin-3-yl)-1,4-diazepane or 2-(pyridin-3-yl)-2,5-diazabicyclo[2.2.1]heptane led to highly potent and selective $\alpha 4\beta 2$ * nAChR partial agonists with K_i values of 0.5–51.4 nM for $\alpha 4\beta 2$ and negligible affinities for $\alpha 3\beta 4$ and $\alpha 7$. Moreover, compounds **21**, **25**, and **30** maintained the functional profiles (EC_{50} and IC_{50} values of 15–50 nM) of the parent azetidine-containing compounds **3** and **4** in the ⁸⁶Rb⁺ ion flux assays. *In vivo* efficacy of the most promising compound **21** was confirmed in the mouse SmartCube[®] platform and classical forced swim tests, supporting the potential use of $\alpha 4\beta 2$ partial agonists for treatment of depression.

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Graphical Abstract

Keywords

$\alpha_4\beta_2$ -nAChRs; partial agonists; hybrids; antidepressant-like effects

Introduction

Nicotinic acetylcholine receptors (nAChRs) belong to the cys-loop superfamily of ligand-gated ion channels, which are widely distributed in the central and peripheral nervous systems. Common nAChRs are homomeric or heteromeric pentamers assembled from a varying combination of subunits (α_2 – α_{10} , β_2 – β_4 , γ , and δ), with α_4 , β_2 , and α_7 as the most widespread in mammalian brain. The heteromeric $\alpha_4\beta_2^*$ -nAChR (the asterisk denotes the possible integration of other subunits into the pentamer) complexes are the predominant form of nAChRs in the central nervous system (CNS) and have been pursued as drug targets for various CNS disorders including, but not limited to, nicotine addiction, Parkinson's disease, depression, and pain[1].

The best-known drug targeting the $\alpha_4\beta_2^*$ receptors is the partial agonist varenicline (**1**)[2], which is considered to be the treatment of choice for long-term smoking cessation. This is consistent with the well-established role of $\alpha_4\beta_2^*$ -nAChR mediation of the reinforcing properties of nicotine[3, 4]. Of particular interest for the clinical use of varenicline is the observation of improved mood and cognition both in non-depressed and depressed smokers attempting to quit[5]. Another $\alpha_4\beta_2^*$ -nAChR partial agonist, CP-601,297 (**2**), was also recently shown to possess clinical antidepressant efficacy in non-obese subjects[6]. The use of varenicline, however, is also associated with unwanted side effects, such as nausea, constipation, and dizziness, which may be due to its activity at the $\alpha_3\beta_4^*$ -nAChRs[7]. As such, potent partial agonists of $\alpha_4\beta_2^*$ -nAChRs that are highly selective over the $\alpha_3\beta_4^*$ subtypes are expected to exert higher efficacy and likely fewer side effects in rodent behavioral models of mood disorders.

Previously our group reported the discovery of highly potent and selective $\alpha_4\beta_2^*$ -nAChR partial agonists containing a cyclopropyl- or isoxazolylpyridine ether scaffold as versatile building blocks for achieving selectivity for $\alpha_4\beta_2$ - over $\alpha_3\beta_4$ -nAChRs[8–11]. Compounds **3** and **4** bind potently to $\alpha_4\beta_2$ -nAChRs ($K_i < 1 \text{ nM}$) but not to $\alpha_3\beta_4^*$ - ($K_i > 10^4 \text{ nM}$) and α_7 -

nAChRs ($K_i > 10^4$ nM) or other neurotransmitter receptors and transporters widely distributed throughout the CNS, while possessing favorable ADMET profiles. During our lead optimization campaign, however, the hydrochloride or trifluoroacetate salts of compound **3** or **4** were found to be hygroscopic and gradually decomposed to form a dimer of the parent structure. This was consistent with the literature findings in the case of the related compound ABT-594 (**5**), which was reported to dimerize to compound **6** in the presence of HCl or TFA following exposure to water[12]. Synthetic efforts were thus made to overcome this issue by utilizing an *N*-methylpyrrolidine or diazabicyclo[3.3.0]octane moiety to replace the azetidine group present in compound **3**[13, 14].

N-Pyridyldiamine compounds exemplified by 1-(pyridin-3-yl)-1,4-diazepane (NS3531, **7**) and 2-(pyridin-3-yl)-2,5-diazabicyclo[2.2.1]heptane (**8**), were reported in the literature as potent $\alpha 4\beta 2$ -nAChR agonists with K_i values of 0.7 nM and 0.15 nM, respectively, in [3 H]cytisine binding competition assays[15, 16]. In oocytes expressing human nAChRs, the functional EC₅₀ value of compound **7** is 21 nM at the high sensitivity (HS) $\alpha 4\beta 2$ -nAChR with an efficacy of 41 % relative to that of acetylcholine. While the potency at $\alpha 4\beta 2$ subtypes is excellent, compound **7** also behaved as an agonist at the $\alpha 7$ subtype with a K_i value of 136 nM. Substituents at the 5-position of the pyridine core were found to be beneficial for the selectivity for $\alpha 4\beta 2$ over $\alpha 7$ and ganglionic nAChRs, consistent with our own findings employing sazetidine-A analogs[8-11, 13]. In our continued efforts to develop highly selective $\alpha 4\beta 2$ -nAChR partial agonists with improved stability, we herein report preliminary SAR findings focused on the incorporation of the diazepane and diazabicycloheptane moieties into our cyclopropyl- or isoxazolyipyridine ether scaffolds.

Chemistry

The syntheses of the hybrid compounds **21–23**, **25**, and **26** are depicted in Scheme 1. 1,3-Dibromopyridine (**9**) was advanced to the optically pure chiral intermediates **16** and **17** according to published methods[9]. Thus, one of the bromine atoms was replaced by a benzyloxy group to obtain compound **10** which underwent a Heck reaction with *n*-butyl acrylate to afford the α,β -unsaturated ester **11**. Following ester hydrolysis, the Weinreb amide **13** of carboxylic acid **12** was formed by reaction with *N,O*-dimethylhydroxylamine hydrochloride. This functional group interchange was found to be necessary since the α,β -unsaturated Weinreb amide **13** gave a much superior yield in the subsequent Corey-Chaykovsky cyclopropanation compared to the ester **11**. The cyclopropanecarboxylate **14** was sequentially reduced with DIBAL-H and NaBH₄ to furnish the primary alcohol **15**. Both enantiomers, **16** and **17**, were acquired in essentially 100% enantiomeric excess and high recovery through resolution of the racemate **15** by HPLC on a chiral stationary phase, ChiralPak AD[®] (Chiral Technologies, Inc.). The optically pure alcohol **17** was subjected to Swern oxidation followed by Wittig reaction to obtain the chain-extended olefin **18**. Sequential hydrogenolysis in the presence of PtO₂ and 10% Pd/C yielded the saturated ether **19**, which was transformed to triflate **20** and subsequently reacted with *tert*-butyl 1,4-diazepane-1-carboxylate, 1-methyl-1,4-diazepane, or *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate under modified Buchwald–Hartwig conditions followed by treatment with trifluoroacetic acid to afford the corresponding trifluoroacetates

21, **22**, and **23**. The diastereoisomers **25** and **26** were synthesized from intermediates **16** and **24** by employing the same sequence of steps.

3-(Benzyloxy)-5-ethynylpyridine (**27**) was prepared via the Sonogashira coupling of **10** and ethynyl(trimethyl)silane followed by removal of the TMS group with tetrabutylammonium fluoride (TBAF). Compound **27** then underwent 1,3-dipolar cycloaddition with propionitrile oxide generated from 1-nitropropane to form isoxazole **28**, which was subsequently subjected to hydrogenolysis to give hydroxyisoxazole **29**. Successive triflation and amination afforded the trifluoroacetates **30** and **31** after removal of the Boc group under acidic conditions.

Results and Discussion

In vitro Characterization—Radioligand Binding Studies

The K_i values of all the synthesized hybrid compounds were evaluated by [^3H]epibatidine binding competition assays at eight heterologously expressed rat nAChR subtypes (Table 1) [17]. Compound **21** bearing a (1*S*,2*R*)-configured cyclopropane side chain exhibited high binding affinities at both $\alpha 4\beta 2$ - and $\alpha 4\beta 2^*$ -nAChRs, which are comparable to those found for parent compound **7**. Its diastereoisomer **25** with a (1*R*,2*S*)-configured cyclopropane side chain displayed a similar binding profile. The presence of an *N*-methyl group (compound **22**) caused more than 10-fold drop in binding affinities at $\alpha 4\beta 2$ and $\alpha 4\beta 2^*$ -nAChRs. Importantly, all three of these compounds (**21**, **22**, and **25**) were confirmed to show improved selectivity for nAChRs containing $\beta 2$ subunits ($\alpha 2\beta 2$ -, $\alpha 3\beta 2$ -, $\alpha 4\beta 2$ -, and $\alpha 4\beta 2^*$ -nAChRs) over nAChRs containing $\beta 4$ subunits ($\alpha 2\beta 4$ -, $\alpha 3\beta 4$ -, and $\alpha 4\beta 4$ -nAChRs) when compared to parent compound **5**, consistent with our previous findings. In particular, the selectivity for $\alpha 4\beta 2$ - over $\alpha 3\beta 4$ -nAChRs of these three compounds was > 10,000-fold, essentially abolishing the potential peripheral side effects thought to be associated with $\alpha 3\beta 4$ -nAChRs. Similarly, incorporation of an isoxazole-containing side chain to compound **7**, as exemplified in compounds **30** and **31**, resulted in retention of the activity at both $\alpha 4\beta 2$ - and $\alpha 4\beta 2^*$ -nAChRs and greatly improved the subtype selectivity for $\beta 2$ - over $\beta 4$ -nAChRs. *N*-methylation (compound **31**) caused a > 60-fold decrease in binding affinity for $\alpha 4\beta 2$ - or $\alpha 4\beta 2^*$ -nAChRs, which is consistent with that found for compound **22**. In reference to diazabicyclo[2.2.1]heptane derivatives (**23** and **26**), at least a 6-fold decline in binding affinities at $\beta 2$ -nAChRs was observed upon attachment of a cyclopropane-containing side chain to the parent compound **8**. Compound **23** with a (1*S*,2*R*)-configured cyclopropane was much more potent than its enantioisomer **26**, while both exhibited improved subtype selectivity for $\beta 2$ - over $\beta 4$ -nAChRs compared to parent compound **8**. Additionally, all of the tested ligands were found to have very low affinities for $\alpha 7$ - or $\alpha 7^*$ -nAChRs (less than 50% inhibition of binding in the primary assay at 10 μM).

In vitro Functional Characterization

In functional studies, all the synthesized compounds were tested using $^{86}\text{Rb}^+$ ion efflux assays and SH-EP1-h $\alpha 4\beta 2$ (expressing human $\alpha 4\beta 2$ -nAChRs), SH-SY5Y ($\alpha 3\beta 4^*$ -nAChRs), or TE671/RD ($\alpha 1\beta 1\gamma\delta$ -nAChRs) cells. Ligand potencies in the stimulation of ion flux were quantified as EC_{50} values, and inhibition by ligands of ion flux responses to a

challenge concentration of a nicotinic full agonist following 10 min preincubation were quantified as inactivation IC₅₀ values. In general, the observed trend in the binding affinities of seven tested compounds (**21–23**, **25**, **26**, **30**, and **31**) correlated with functional potencies (Table 2), and none of the tested ligands was active at $\alpha 3\beta 4^{*}$ - or $\alpha 1\beta 1\gamma\delta$ -nAChRs (EC₅₀ or IC₅₀ values were > 10000 nM; data not shown). The most promising ligands **21**, **25**, and **30** were found to be highly potent, partial agonists at the mixture of high sensitivity (HS) and low sensitivity (LS) $\alpha 4\beta 2$ -nAChRs. Their EC₅₀ values were comparable to those previously reported for the azetidine-containing analogues **3** and **4**. Efficacy of agonism was 58–81% 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. All three of these compounds were found to functionally inactivate the response of the $\alpha 4\beta 2$ -nAChRs to carbamylcholine at IC₅₀ values similar to the agonism EC₅₀ values.

***In vivo* Behavioral Studies—SmartCube® and Mouse Forced Swim Test**

Preliminary *in vivo* evaluation of our nicotinic ligands for behavioral effects relevant to psychiatric diseases was carried out using SmartCube®, a proprietary, automated assay platform in which behaviors of compound-treated mice are captured by digital video and analyzed with advanced computer algorithms[11, 18–20]. The behavioral signature of a test compound is matched to a database of behavioral signatures previously obtained using a large set of diverse reference compounds, including clinically used antipsychotics, anxiolytics, and antidepressants. The neuropharmacological effects of a test compound can therefore be predicted by comparing its signature similarity to the existing database. The behavioral signatures of selected synthesized compounds (**21**, **23**, **25**, **26**, and **30**) in the SmartCube® assay at 5 and 10 mg/kg after 15 min of preinjection (i.p.) are shown in **Figure 2**. Consistent with the binding profiles shown in Table 2, compounds **21**, **25**, and **30** displayed the most prominent effects in this assay. Both compounds **25** and **30** showed a marked increase in their behavioral signatures at 10 mg/kg compared to the lower dose of 5 mg/kg. In contrast, administration of compound **21** at 5 mg/kg elicited a fairly similar behavioral signature compared to that of a 10 mg/kg dose. To assess the antidepressant effect, compound **21** was further investigated in the classical Porsolt's forced swim test[21] with the selective serotonin reuptake inhibitor, sertraline, as a positive control at 20 mg/kg dose (Figure 3). Briefly, mice were placed into a beaker of water, and the time spent passively floating in the water (immobility) was recorded. Most traditional antidepressants decrease the amount of time mice spend immobile. Acute oral (PO) administration of **21** at 1 and 10 mg/kg significantly decreased (*P < 0.05) total immobility time compared to the control group (water), indicating an antidepressant-like effect in a dose-dependent manner.

Conclusions

In this paper we describe the synthesis and biological evaluation of novel hybrid compounds which combine the substituted cyclopropyl or isoxazolyl side chains of our recently identified $\alpha 4\beta 2$ -nAChR partial agonists (**3** and **4**) with *N*-pyridyldiamines (**7** and **8**). In general, the hybrid compounds **21–23**, **25**, **26**, **30**, and **31** carried over the binding profiles of parent compounds **3** and **4**. All hybrids exhibited high binding affinities for $\beta 2$ -containing nAChRs and superior selectivity for $\alpha 4\beta 2$ (K_i = 0.5–51.4 nM) over $\alpha 3\beta 4$ (K_i > 8000 nM or

< 50% inhibition of binding in the primary assay at 10 μ M) and α 7 (50% inhibition of binding in the primary assay at 10 μ M), which is a significant improvement of selectivity for α 4 β 2 when compared to parent compounds **7** and **8**. Functional assays identified three compounds (**21**, **25**, and **30**) as α 4 β 2-nAChR partial agonists with EC₅₀ values that were comparable to the azetidine ethers **3** and **4**. In vivo efficacy of the most promising compound **21** in the mouse SmartCube® and forced swim tests suggest the potential use of these potent and selective α 4 β 2-nAChR partial agonists in depression.

Experimental Section

Chemical Synthesis

General Methods—Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification, unless otherwise stated. Anhydrous CH₂Cl₂ and THF were obtained by distillation over CaH₂ or sodium wire, respectively. All non-aqueous reactions were run under an argon atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. The progress of reactions was monitored by TLC on SiO₂. Spots were visualized by their quenching of the fluorescence of an indicator admixed to the SiO₂ layer. SiO₂ for column chromatography (CC) was of 230–400 mesh particle size, and an EtOAc/hexane mixture or gradient was used for elution unless stated otherwise. ¹H NMR spectra were recorded at a spectrometer frequency of 400 MHz, ¹³C NMR spectra at 100 MHz. ¹H chemical shifts are reported in δ (ppm) using the δ 7.26 signal of CDCl₃ or the δ 4.80 signal of D₂O as internal standards. ¹³C chemical shifts are reported in δ (ppm) using the δ 77.23 signal of CDCl₃ as internal standard. ¹³C NMR spectra in D₂O were not adjusted.

Purities of all final compounds (> 98 %) were established by analytical HPLC, which was carried out on an Agilent 1100 HPLC system with a Synergi 4 Hydro-RP 80A column, with detection at 280 or 254 nm on a variable wavelength detector G1314A; flow rate = 1.4 mL/min; gradient of 0 to 100% methanol in water (both containing 0.05 vol% of TFA) in 18 min. Final products were purified by preparative HPLC under the following conditions: column, ACE AQ, 250 $\times$ 20 mm; flow, 17 mL/min; all solvents containing 0.05 vol% TFA; UV detection at 254 nm and 280 nm; gradient: 0–50% MeOH in water in 20 min, to 100% in 5 min, 100% for another 5 min, return to 0% in 5 min, and equilibration at 0% for 1 min.

General Procedure for Conversion of a Hydroxyl Group to a Triflate (Method A)

—The substrate (1 mmol) was dissolved in anhydrous pyridine (3 mL) and stirred at 0 °C before trifluoromethanesulfonic anhydride (1.1 mmol) was added. The mixture was allowed to warm to room temperature. The reaction was monitored via TLC until no starting material was observed, water (30 mL) was added, and the mixture was extracted with EtOAc (3 $\times$ 15 mL). The combined organic solutions were washed with 1N HCl (3 $\times$ 20 mL) and brine (20 mL), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified on silica gel (CH₂Cl₂/CH₃OH, 10:1) to obtain the desired product.

General Procedure for the Buchwald-Hartwig Coupling of Diamines with

Pyridyl Triflates (Method B)—In an oven dried 2–5 mL microwave reactor vessel was placed Pd₂(dba)₃ (0.03 mmol), X-Phos (0.05 mmol), K₃PO₄ (3 mmol), and 1,4-dioxane (2

mL). The mixture was stirred and purged with a light stream of argon, then a solution of the triflate (1 mmol) and the diamine (1.1 mmol) in anhydrous 1,4-dioxane (2 mL) was added. The mixture was again purged with argon, cap-sealed, and irradiated with microwaves at 165 °C for 10–20 min. The resulting mixture was filtered through celite and concentrated, and the residue was purified by column chromatography on silica gel with hexane/EtOAc (3:2) to obtain the desired product.

General Procedure for the Deprotection of *N*-Boc-Amines to Afford TFA Salts (Method C)—To a solution of the *N*-Boc protected precursor (1 mmol) in CH₂Cl₂ (10 mL) was added TFA (1 mL) under argon with ice cooling. The mixture was stirred overnight at rt. After the solvent was evaporated, the residue was dissolved in distilled water (5 mL). The solution was filtered over a syringe filter (polytetrafluoroethylene, 17 mm diameter, 0.45 μm pore size), then concentrated to 2–3 mL under reduced pressure at 30 °C bath temperature. The crude product was purified by preparative HPLC. After the solvent was evaporated, the residue was dissolved in distilled water (about 2–3 mL). The solution was lyophilized to obtain the TFA salt.

General Procedure for the [3+2] Cycloaddition to Form Isoxazoles (Method D)—To a solution of nitro compound (2.0–3.0 equiv.) and alkyne (1.0 equiv.) in anhydrous toluene were added phenyl isocyanate (1.1 equiv.) and triethylamine (1.1 equiv.). The reaction mixture was stirred at 60 °C for 12–48 h, then cooled to room temperature. After removal of the solvent, the residue was purified by CC on SiO₂ with hexane/acetone (2:1) to give the isoxazoles.

General Procedure for O-Debenzylation to Afford Hydroxypyridines (Method E)—To a solution of the Bn-protected hydroxypyridine (1 mmol) in MeOH (10 mL) was added 10% Pd/C. A H₂ balloon was connected after the atmosphere was exchanged three times with Ar. The reaction was allowed to proceed at rt for 2 h, then the catalyst was filtered off over celite. The filtrate was evaporated, and the residue was purified by CC on SiO₂ (CHCl₃/MeOH 30:1) to furnish the hydroxypyridine as a colorless oil.

5-[(1*S*,2*R*)-2-(2-Methoxyethyl)cyclopropyl]pyridin-3-yl

trifluoromethanesulfonate (20)—This compound was obtained from compound **19** employing Method A; colorless oil; 92% yield. ¹H NMR (CDCl₃) δ 8.42 (s, 1H), 8.35 (s, 1H), 7.19 (s, 1H), 3.51–3.48 (m, 2H), 3.36 (s, 3H), 1.78–1.63 (m, 3H), 1.22–1.16 (m, 1H), 1.01 (m, 2H). ¹³C NMR (CDCl₃) δ 148.2, 147.1, 142.1, 139.5, 125.7, 72.3, 59.0, 34.3, 21.9, 20.3, 16.5.

1-[5-[(1*S*,2*R*)-2-(2-Methoxyethyl)cyclopropyl]pyridin-3-yl]-1,4-diazepane

trifluoroacetate (21)—This compound was obtained from compound **20** and *tert*-butyl 1,4-diazepane-1-carboxylate employing Methods B and C; colorless oil; 41% yield over two steps; purity: 99.9%. ¹H NMR (D₂O) δ 7.93 (s, 1H), 7.74 (s, 1H), 7.45 (s, 1H), 3.84 (s, 2H), 3.62 (t, 2H, *J* = 6.0 Hz), 3.57 (t, 2H, *J* = 6.0 Hz), 3.45 (s, 2H), 3.33–3.31 (m, 5H), 2.23–2.19 (m, 2H), 1.88–1.84 (m, 1H), 1.70–1.66 (m, 2H), 1.26–1.22 (m, 1H), 1.10–1.03 (m, 2H). ¹³C NMR (D₂O) δ 162.6 (TFA), 146.4, 144.9, 126.2, 123.7, 121.4, 116.2 (TFA), 72.0, 57.7, 46.8, 45.6, 45.4, 44.4, 32.7, 24.2, 21.5, 20.0, 15.9. Anal Calcd for C₁₆H₂₅N₃O · 2.5

$C_2HF_3O_2 \cdot 0.65 H_2O$: C, 44.08; H, 5.07; F, 24.90; N, 7.34. Found: C, 44.04; H, 5.00; F, 24.85; N, 7.28. M.W.: 572.1. Free amine MW: 275.4. $[\alpha]_D^{20}$: 37.0 ($c = 0.32$, CH_3OH).

1-[5-[(1*S*,2*R*)-2-(2-methoxyethyl)cyclopropyl]pyridin-3-yl]-4-methyl-1,4-diazepane trifluoroacetate(22)—This compound was obtained from **20** and 1-methyl-1,4-diazepane employing Method B; colorless oil; 22% yield; purity: 99.3%. 1H NMR (D_2O) δ 7.91 (s, 1H), 7.75 (s, 1H), 7.43 (s, 1H), 3.92–3.80 (m, 2H), 3.72–3.52 (m, 6H), 3.32–3.25 (m, 5H), 2.93 (s, 3H), 2.36–2.32 (m, 2H), 1.86 (s, 1H), 1.69–1.65 (m, 2H), 1.25–1.21 (m, 1H), 1.09–1.03 (m, 2H). δ ^{13}C NMR (D_2O) δ 162.6 (TFA), 146.6, 144.9, 126.3, 123.7, 121.4, 116.2 (TFA), 72.0, 57.7, 56.1, 56.0, 46.7, 44.0, 43.3, 32.7, 23.7, 21.5, 21.4, 20.0, 16.0, 15.9. Anal Calcd for $C_{17}H_{27}N_3O \cdot 2.6 C_2HF_3O_2 \cdot 0.75 H_2O$: C, 44.49; H, 5.23; F, 24.72; N, 7.01. Found: C, 44.33; H, 4.94; F, 24.47; N, 6.84. M.W.: 599.4. Free amine MW: 289.4. $[\alpha]_D^{20}$: +58.4 ($c = 0.25$, CH_3OH).

(1*S*,4*S*)-2-[5-[(1*S*,2*R*)-2-(2-methoxyethyl)cyclopropyl]pyridin-3-yl]-2,5-diazabicyclo[2.2.1]heptane(23)—This compound was obtained from **20** and *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate employing Methods B and C; colorless oil; 51% yield over two steps; purity: 99.9%. 1H NMR (D_2O) δ 7.84 (s, 1H), 7.80 (s, 1H), 7.34 (s, 1H), 4.84 (s, 1H), 4.66 (s, 1H), 3.78 (dd, 1H, $J = 1.6, 10.8$ Hz), 3.59 (t, 2H, $J = 6.4$ Hz), 3.50–3.43 (m, 3H), 3.35 (s, 3H), 2.33 (d, 1H, $J = 11.6$ Hz), 2.16 (d, 1H, $J = 11.6$ Hz), 1.91–1.87 (m, 1H), 1.73–1.65 (m, 2H), 1.29–1.24 (m, 1H), 1.14–1.05 (m, 2H). ^{13}C NMR (D_2O) δ 162.7 (TFA), 145.2, 143.9, 126.8, 124.0, 121.6, 116.2 (TFA), 72.0, 58.1, 57.7, 55.7, 51.7, 50.9, 35.8, 32.7, 21.6, 19.9, 16.1. Anal Calcd for $C_{16}H_{23}N_3O \cdot 2.5 C_2HF_3O_2$: C, 45.17; H, 4.60; F, 25.52; N, 7.52. Found: C, 45.00; H, 4.39; F, 25.41; N, 7.47. M.W.: 558.4. Free amine MW: 273.4. $[\alpha]_D^{20}$: –13.5 ($c = 0.56$, CH_3OH).

1-[5-[(1*R*,2*S*)-2-(2-methoxyethyl)cyclopropyl]pyridin-3-yl]-1,4-diazepane (25)—This compound was obtained from **16** following the same procedure as for the preparation of compound **21**; colorless oil; yield: 20% over three steps; purity: 99.2%. 1H NMR (D_2O) δ 7.90 (s, 1H), 7.72 (s, 1H), 7.43 (s, 1H), 3.84 (t, 2H, $J = 4.0$ Hz), 3.60 (t, 2H, $J = 6.0$ Hz), 3.55 (t, 2H, $J = 8.0$ Hz), 3.43 (t, 2H, $J = 4.0$ Hz), 3.33–3.31 (m, 5H), 2.22–2.18 (m, 2H), 1.67–1.64 (m, 1H), 1.67–1.64 (m, 2H), 1.26–1.18 (m, 1H), 1.09–1.00 (m, 1H). ^{13}C NMR (D_2O) δ 162.6 (TFA), 146.3, 144.9, 126.2, 123.6, 121.4, 116.1 (TFA), 71.9, 57.7, 46.8, 45.6, 45.3, 44.4, 41.6, 32.7, 24.1, 21.4, 20.0, 15.9. Anal Calcd for $C_{16}H_{25}N_3O \cdot 2.9 C_2HF_3O_2 \cdot 0.55H_2O$: C, 42.51; H, 4.75; F, 26.83; N, 6.82. Found: C, 42.44; H, 4.53; F, 26.62; N, 6.69. M.W.: 615.9. Free amine MW: 275.4. $[\alpha]_D^{20}$: –29.7 ($c = 0.13$, CH_3OH).

(1*S*,4*S*)-2-[5-[(*R*,2*S*)-2-(2-methoxyethyl)cyclopropyl]pyridin-3-yl]-2,5-diazabicyclo[2.2.1]heptane (26)—This compound was obtained from **16** following the same procedure as for the preparation of compound **23**; colorless oil; yield: 37% over three steps; purity: 99.7%. 1H NMR (D_2O) δ 7.83 (s, 1H), 7.79 (s, 1H), 7.33 (s, 1H), 4.83 (s, 1H), 4.65 (s, 1H), 3.77 (dd, 1H, $J = 2.0, 11.2$ Hz), 3.58 (t, 2H, $J = 6.4$ Hz), 3.49–3.45 (m, 3H), 3.34 (s, 3H), 2.32 (d, 1H, $J = 11.6$ Hz), 2.15 (d, 1H, $J = 11.6$ Hz), 1.88 (m, 1H), 1.69 (m, 2H), 1.25 (m, 1H), 1.09 (m, 2H). ^{13}C NMR (D_2O) δ 162.4 (TFA), 144.8, 143.5, 126.4, 123.6, 121.2, 115.8 (TFA), 71.5, 57.6, 57.3, 55.3, 51.3, 50.5, 35.4, 32.3, 21.3, 19.5, 15.6.

Anal Calcd for $C_{16}H_{23}N_3O \cdot 2.55 C_2HF_3O_2 \cdot 0.25 H_2O$: C, 44.57; H, 4.62; F, 25.56; N, 7.39. Found: C, 44.70; H, 4.47; F, 25.47; N, 7.24. M.W.: 568.6. Free amine MW: 273.4. $[\alpha]_D^{20}$: -102.7 ($c = 0.39$, CH_3OH).

2-([5-[5-(1,4-Diazepan-1-yl)pyridin-3-yl]isoxazol-3-yl]ethanol (30)—This compound was obtained from compound **29** and *tert*-butyl 1,4-diazepane-1-carboxylate employing Methods A, B, and C; colorless oil; yield: 45% over three steps; purity: 99.9%. 1H NMR (D_2O) δ 8.46 (s, 1H), 8.25 (s, 1H), 8.11 (s, 1H), 7.04 (s, 1H), 3.97 (t, 2H, $J = 4.0$ Hz), 3.93 (t, 2H, $J = 6.0$ Hz), 3.73 (t, 2H, $J = 6.0$ Hz), 3.52 (t, 2H, $J = 4.0$ Hz), 3.39 (t, 2H, $J = 4.0$ Hz), 2.98 (t, 2H, $J = 6.0$ Hz), 2.30–2.25 (m, 2H). ^{13}C NMR (D_2O) δ 163.9, 163.6, 162.6 (TFA), 146.9, 127.0, 125.6, 125.2, 122.7, 116.2 (TFA), 103.9, 59.4, 47.2, 45.6, 45.3, 44.5, 28.4, 24.1. Anal Calcd for $C_{15}H_{20}N_4O_2 \cdot 2.8 C_2HF_3O_2$: C, 40.72; H, 3.78; F, 26.26; N, 9.22. Found: C, 41.03; H, 3.84; F, 26.54; N, 9.29. M.W.: 607.6. Free amine MW: 288.3.

2-[5-[5-(4-Methyl-1,4-diazepan-1-yl)pyridin-3-yl]isoxazol-3-yl]ethanol (31)—This compound was obtained from **29** and *tert*-butyl 4-methyl-1,4-diazepane-1-carboxylate employing Methods A, B, and C; colorless oil; yield: 42% over three steps; purity: 99.7%. 1H NMR (D_2O) δ 8.48 (s, 1H), 8.25 (s, 1H), 8.12 (s, 1H), 7.05 (s, 1H), 4.07–3.92 (m, 4H), 3.80–3.71 (m, 2H), 3.67–3.60 (m, 2H), 3.43–3.31 (m, 2H), 3.00–2.97 (m, 5H), 2.42–2.31 (m, 2H). ^{13}C NMR (D_2O) δ 163.9, 163.7, 162.6 (TFA), 147.1, 127.0, 125.7, 125.2, 122.8, 116.2 (TFA), 103.9, 59.4, 56.1, 56.0, 47.1, 44.0, 43.3, 28.5, 23.7. Anal Calcd for $C_{16}H_{22}N_4O_2 \cdot 2.4 C_2HF_3O_2 \cdot 1.5 H_2O$: C, 41.43; H, 4.58; F, 22.68; N, 9.29. Found: C, 41.36; H, 4.26; F, 22.38; N, 9.06. M.W.: 603.0. Free amine MW: 302.3.

General Procedures for Binding and Functional Studies

In vitro Binding Studies— $[^3H]$ Epibatidine competition studies were carried out by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # 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 $^{86}Rb^+$ efflux assays.[22] 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 $^{86}Rb^+$ efflux assays[23]. SH-EP1 human epithelial cells stably transfected with human $\alpha 4$ and $\beta 2$ subunits 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[24]. They are known to express a mixture of high sensitivity (HS- $\alpha 4\beta 2$ ($\alpha 4\beta 2$) $_2\beta 2$) and low sensitivity (LS- $\alpha 4\beta 2$ ($\alpha 4\beta 2$) $_2\alpha 4$) stoichiometries. TE671/RD, SH-SY5Y, and transfected SH-EP1 cell lines were maintained as low passage number (1–26 from our frozen stocks) cultures to ensure stable expression of native or heterologously expressed nAChRs as previously described.[22] Cells were passaged once a week by splitting just-

confluent cultures 1/300 (TE671/RD), 1/10 (SH-SY5Y), or 1/40 (transfected SH-EP1) in serum-supplemented medium to maintain log-phase growth.

$^{86}\text{Rb}^+$ Efflux Assays—Function of nAChR subtypes was investigated using a previously established $^{86}\text{Rb}^+$ efflux assay protocol[22]. Cells harvested at confluence from 100 mm plates under a stream of fresh medium only (SH-SY5Y cells) or after mild trypsinization (for TE671/RD or transfected SH-EP1 cells) were then suspended in complete medium and evenly seeded into 24-well plates. After cells had adhered and grown to confluence, the medium was removed and replaced with 250 μL per well of complete medium supplemented with ~ 350000 cpm of $^{86}\text{Rb}^+$. After at least 4 h and typically overnight, $^{86}\text{Rb}^+$ efflux was measured. Each well was rinsed with 2 mL of fresh $^{86}\text{Rb}^+$ efflux buffer (pH 7.4; 2 mM CaCl_2 , 5.4 mM KCl, 130 mM NaCl, 50 mM HEPES, and 5 mM glucose) after aspiration of the bulk of $^{86}\text{Rb}^+$ loading medium. The flip-plate technique was used again to simultaneously introduce 1.5 mL of fresh efflux buffer containing drugs at indicated final concentrations from a 24-well “efflux/drug plate” into the wells of the cell plate. After a 9.5 min incubation, the solution was “flipped” back into the efflux/drug plate, and any remaining buffer in the cell plate was removed by aspiration. 10 min after the initiation of the first drug treatment, a second efflux/drug plate was used to reintroduce the same concentrations of drugs with the addition of an $\sim \text{EC}_{90}$ concentration of carbamylcholine for 5 min. The second drug treatment was then flipped back into its drug plate, and the remaining cells were lysed and suspended by addition of 1.5 mL of 0.1 M NaOH with 0.1% sodium dodecyl sulfate to each well. Suspensions in each well were then subjected to Cerenkov counting after placement of inserts (Wallac 1450–109) into each well to minimize cross-talk between wells.

Total $^{86}\text{Rb}^+$ efflux was assessed in the presence of a fully efficacious concentration of carbamylcholine alone (3 mM for SH-SY5Y cells, or 1 mM for SH-EP1-ha4 β 2 and TE671/RD cells). Nonspecific $^{86}\text{Rb}^+$ efflux was measured in the presence of the fully efficacious concentration of carbamylcholine plus 100 μM mecamylamine. Both determinations of nonspecific efflux were equivalent. Specific efflux was then taken as the difference in control samples between total and nonspecific $^{86}\text{Rb}^+$ efflux. The same approaches were used to define total, nonspecific, and specific ion flux responses in samples subjected to the second, 5 min exposure to test drug with or without carbamylcholine at its EC_{90} concentration.

Intrinsic agonist activity of tested drugs was ascertained during the first 9.5 min of the initial 10 min exposure period using samples containing only test drug at different concentrations and was normalized, after subtraction of nonspecific efflux, to specific efflux in carbamylcholine control samples. Ion flux assays ($n = 3$ separate studies for each drug and cell line combination) were fit to the Hill equation, $F_{\text{max}} = F / (1 + (X/\text{EC}_{50})^n)$, where F is the percentage of control, F_{max} , for EC_{50} ($n > 0$ for agonists) or IC_{50} ($n < 0$ for antagonists) values using Prism 5 (GraphPad, San Diego, USA). To determine the efficacies of tested ligands at both HS and LS $\alpha 4\beta 2$ -nAChR, an unusual property of sazetidine-A was exploited. The SHEP1-ha4 $\beta 2$ cell line used expresses a mixture of HS and LS $\alpha 4\beta 2$ -nAChR that varies, and the proportion of HS $\alpha 4\beta 2$ -nAChR can usually be increased by incubation of cells at 29 °C. Plotting the efficacy of test ligands relative to carbamylcholine (ordinate axis)

versus the efficacy of sazetidine-A relative to carbamylcholine (abscissa) reveals a linear relationship in which the intercepts where the abscissa value (sazetidine-A efficacy) is 0 % or 100 % correspond to the test ligand efficacies at LS or HS $\alpha 4\beta 2$ -nAChR, respectively.

General Procedures for Behavioral Studies

Animals—Male C57 BL6 mice used in the SmartCube[®] assay were purchased from Taconic Farms. BALB/cJ male mice used in the forced swim test were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Mice were group-housed in OptiMICE ventilated cages with four mice per cage 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 **21**, **23**, **25**, **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 PO or intraperitoneal injection in a volume of 10 mL/kg.

SmartCube[®] Test—General procedures were based on those previously described.[18] Male C57 BL6 mice were administered intraperitoneally compound **21**, **23**, **25**, or **30** (5 and 10 mg/kg) 15 min before the test. Mice were then taken from their home cage to the SmartCube[®] suite of experimental rooms. A standard SmartCube[®] protocol runs for a single testing session for 45 min. The mouse behavior is captured by digital video and analyzed with computer algorithms. The neuropharmacological effects of the test compound were predicted by similarity to major classes of compounds.

Mouse Forced Swim Test—Procedures have been previously described[21]. BALB/cJ male mice (8–10 weeks) 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, ip) as a positive control, or compound **21** (1 and 10 mg/kg, po). Thirty minutes following administration mice were placed in the water, and the time the animal spent immobile was recorded over a 6-min trial.

Statistical Analysis—Data were analyzed with analysis of variance (ANOVA). Total time immobile was used as the dependent variable and treatment (vehicle, sertraline, or compound **21**) as the independent variable. Main effects were compared between groups and analyzed for significance with the post hoc Fisher's PLSD test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CNS	central nervous system
AD	Alzheimer's disease
ADHD	attention deficit hyperactivity disorder
NIMH-PDSE	National Institute of Mental Health Psychoactive Drug Screening Program
nAChR(s)	nicotinic acetylcholine receptor(s)
TCA	tricyclic antidepressant
FST	forced swim test
SAR	structure-activity relationship
SSRI	selective serotonin reuptake inhibitor
CC	column chromatography
rt	room temperature
TFA	trifluoroacetic acid
HS	high-sensitivity
LS	low-sensitivity

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Highlights

Seven novel hybrid compounds were rational designed and synthesized.

All compounds were found to be potent and selective $\alpha 4\beta 2$ -nAChR binders.

Compounds **21**, **25**, and **30** maintained the functional profiles of the parent compounds.

In vivo efficacy of compound **21** was confirmed in SmartCube[®] and forced swim tests.

Figure 1.
Structures of compounds **1–8**.

Scheme 1.

a Reagents and conditions: (a) BnOH, NaH, DMF; (b) *n*-butyl acrylate, 1 % Pd(OAc)₂, 2 % PhNHCONH₂, K₂CO₃, 130 °C; (c) 2N NaOH, MeOH/THF (1:1); (d) MeNH₂⁺OMeCl⁻, EDCI, DMAP, CH₂Cl₂; (e) Me₃S(O)⁺I⁻, NaH, DMSO; (f) (I) DIBAL-H, THF, -78 °C to -20 °C; (II) NaBH₄, MeOH; (g) ChiralPak AD, EtOH; (h) (I) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -78 °C; (II) Ph₃P=CHOCH₃, THF, 0 °C; (i) PtO₂, H₂, CH₂Cl₂; (j) 10 % Pd/C, H₂; (k) (CF₃SO₂)₂O, pyridine; (l) *tert*-butyl 1,4-diazepane-1-carboxylate or 1-methyl-1,4-diazepane or *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate, tris-(dibenzylideneacetone)dipalladium, 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl, K₃PO₄, 1,4-dioxane, microwave, 160 °C, 10 min; (m) CF₃CO₂H, CH₂Cl₂; (n) (I) ethynyl(trimethyl)silane, CuI, PdCl₂(PPh₃)₂, PPh₃, Et₃N, 60 °C, (II) TBAF, THF; (o) O₂N(CH₂)₂OTBS, PhNCO, Et₃N, PhMe.

Figure 2.
SmartCube® signatures of 5 selected hybrids.

Figure 3.

A: Time course for the effects of sertraline and **21** on time immobile in the FS test in mice. Data represent mean $\pm$ SEM. **B:** Effects of sertraline and **21** in mice on total time immobile in the forced swim test. Data were summed over the 6-min test and represent mean $\pm$ SEM. * $p < 0.05$ indicates statistical significance compared to water. **C:** Baseline body-weights of all treatment groups tested in FS. Data are presented as mean $\pm$ SEM.

Table 1

Affinities of ligands for rat nAChR subtypes defined by competition for [³H]epibatidine binding.

Compound	K_i (nM) ^a								
	$\alpha 2\beta 2$	$\alpha 2\beta 4$	$\alpha 3\beta 2$	$\alpha 3\beta 4$	$\alpha 4\beta 2$	$\alpha 4\beta 2^*^b$	$\alpha 4\beta 4$	$\alpha 7$	$\alpha 7^*^b$
21	0.3	460	17.0	>10 ⁴	0.7	2.7	151	NA	NA
22	18.1	NA	681	NA	25.4	33.8	4806	NA	NA
23	3.1	4811	141	NA	6.2	16.1	1340	NA	NA
25	0.3	256	15.8	>10 ⁴	0.5	2.7	168	NA	NA
26	43.1	NA	617	NA	35.6	269	8451	NA	NA
30	0.8	331	42	8098	0.8	3.5	67.6	NA	NA
31	46.3	>10 ⁴	1037	NA	51.4	274	4936	NA	NA
7	1.1	62	5.1	366	3.4	3.3	71.6	ND ^d	ND
8	0.3	15.2	3.1	64.6	1.0	0.8	7.7	ND	ND
3	0.1	236	2.4	NA	0.1	0.3	50.2	NA	NA
4	1.0	935	15.4	>10 ⁴	0.6	3.0	1790	NA	NA
Nicotine ^c	5.5	70	29	260	4.9	9.8	23	ND	ND
1 ^f	-	-	-	86	0.4	-	110	125	-

^e NA: not active, defined as < 50% inhibition of binding in the primary assay at 10 μ M.

^a See Experimental Section.

^b $\alpha 4\beta 2^*$ or $\alpha 7^*$, endogenous receptors prepared from rat forebrain. Besides $\alpha 4$, $\beta 2$, or $\alpha 7$, other unidentified subunits may also be present. Details are provided in the Experimental Section.

^c K_i values for nicotine are taken from the PDSP Assay Protocol Book.

^d ND: not detected.

^f K_i values for **3**, **4**, and **1** are from the literature[8, 9].

Table 2Potencies and Efficacies of Ligand Agonism at and Inactivation of Human $\alpha 4\beta 2$ - nAChRs^a

Compound	Agonism				Inactivation		
	EC ₅₀ (nM)	pEC ₅₀	HS- $\alpha 4\beta 2$ Efficacy (%)	LS- $\alpha 4\beta 2$ Efficacy (%)	IC ₅₀ (nM)	pIC ₅₀	Inactivation Efficacy (%)
21	15.9	7.80 ± 0.18	81 ± 14	-9.3 ± 7.3	16.0	7.80 ± 0.11	74 ± 2.8
22	> 1000	< 6	ND	ND	> 1000	< 6	ND
23	125	± 1.4	ND	ND	169	± 1.3	ND
25	17.1	7.77 ± 0.15	63 ± 7.2	-5.6 ± 4.1	15.7	7.80 ± 0.18	79 ± 3.2
26	> 1000	< 6	ND	ND	> 1000	< 6	ND
30	30.9	7.51 ± 1.3	58 ± 6.2	-5 ± 3.9	50.8	7.29 ± 1.40	68 ± 1.7
31	> 1000	< 6	ND	ND	> 1000	< 6	ND
3^c	17.5	-	60	-	5.6	-	71
4^c	42.4	7.37 ± 0.19	69 ± 5.6	-7.8 ± 3.8	32	7.50 ± 0.26	68 ± 4.9
Nicotine ^c	295	6.53 ± 0.05	124 ± 8.5	70 ± 5.9	427	6.37 ± 0.06	92 ± 2.1
1^c	1096	5.96 ± 0.18	106 ± 5.9	34 ± 3.3	39	7.41 ± 0.08	83 ± 1.0

^bND: Not Determined. The efficacy was not determined if the EC₅₀ or the IC₅₀ value was greater than 1000 nM.^aSee Experimental Section for details. The term “inactivation” is used because compounds may be acting to desensitize receptors or as competitive or non-competitive antagonists, and further work is needed to make such a distinction. Potencies (EC₅₀ or IC₅₀ values) and efficacies were measured for actions at a mixture of high-sensitivity (HS) and low-sensitivity (LS) $\alpha 4\beta 2$ -nAChRs. Reported errors are the standard error of the mean (SEM) for all values.^cData for **3**, **4**, and **1** are from the literature[8, 9].