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Abstract

Starting from a known non-specific agonist (**1**) of nicotinic acetylcholine receptors (nAChRs), rationally guided structural-based design resulted in the discovery of a small series of 5′-phenyl-1,2,5,6-tetrahydro-3,3′-bipyridines (**3a** – **3e**) incorporating a phenyl ring off the pyridine core of **1**. The compounds were synthesized *via* successive Suzuki couplings on a suitably functionalized pyridine starting monomer **4** to append phenyl and pyridyl substituents off the 3- and 5-positions, respectively, and then make subsequent modifications on the flanking pyridyl ring to provide target compounds. Compound **3a** is a novel antagonist which is highly selective for α3β4 nAChR (K_i = 123 nM) over the α4β2, and α7 receptors.

Graphical Abstract

Structure-based molecular design starting from a known non-specific agonist (**1**) of nicotinic acetylcholine receptors has generated a novel antagonist (**3a**) that is a reasonably potent and highly selective antagonist for the α3β4 receptor over α4β2 and α7.

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Supplemental data

Supplementary data (details of the chemical syntheses and assays performed) associated with this article can be found, in the online version, at http://dx.doi...

a: X = F; Y = H
b: X = F; Y = F
c: X = F; Y = Cl
d: X = F; Y = SO$_2$CH$_3$
e: X = OH; Y = H
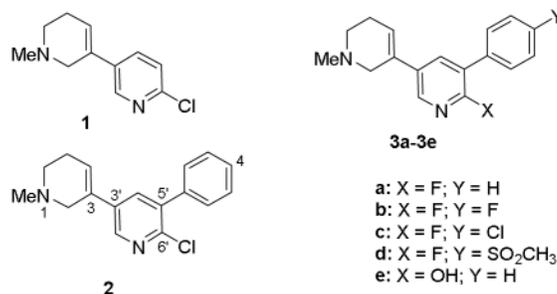
Introduction

Nicotinic acetylcholine receptors (nAChRs) are a family of well-studied ligand-gated ion channels that directly mediate fast signal transmission at chemical synapses through binding with neurotransmitter molecules.[1–5] This family of receptors consists of various subtypes, including α3β4, α4β2, and α7 *etc.* in the central nervous system (CNS). Abnormal opening-closing of these ion channels contributes to neurodegenerative disorders, resulting in several severe diseases, including Alzheimer's disease, Parkinson's disease, dyskinesias, Tourette's syndrome, schizophrenia, attention deficit disorder, anxiety, pain, and nicotine addiction.[2, 6–13] Hence, there is a need to develop subtype-selective agonists/antagonists of nAChRs as potential therapeutic agents.

For the rational design of potent agonists or antagonists of nAChRs, one first needs to understand the detailed three-dimensional (3D) structures and physiological functions of nAChRs. According to various reported X-ray diffraction and molecular modeling studies, [14–22] a ligand binding to the nAChR receptor in the acetylcholine (ACh)-binding site can be either an agonist or an antagonist, because the receptor can exist in the open- or closed-channel state.[19] An agonist can more favorably bind with the open-channel state of the receptor, whereas an antagonist can more favorably bind with the closed-channel state.[19] Various agonists and antagonists of nAChRs have been reported in the literature. However, compared to agonists, there are relatively few reported antagonists of nAChRs, particularly for α3β4, α4β2, and α7 subtypes.[6, 10, 13, 23] We are particularly interested in subtype-specific antagonists of α3β4 and α4β2 nAChRs, and toward that end set out to design and synthesize new antagonists of these nAChRs as potential treatments for drug addiction.

In this study, we tested the feasibility of converting a known agonist to an antagonist by modifying the agonist structure. Our strategy is based on a previous demonstration[19] that an agonist may bind to both the open- and closed-channel states of the nAChR, but with a relatively higher binding affinity to the open state. If we could appropriately modify the agonist structure in such a way that it no longer fits the binding pocket of the open-channel state, but still fits the binding pocket of the closed state, then it could become an antagonist.

Compound **1**, depicted in Figure 1, is a known non-specific agonist of nAChRs in rat brain membranes (with K_d = ~2.2 nM).[24, 25] According to our modeling inspection using the 3D model of the open-channel structures[19] of α3β4, α4β2, and α7 nAChRs, compound **2** (modified from compound **1** with a phenyl ring attached to the pyridine ring) would not fit

the binding pocket of the open-channel state of any of the above three receptors. But it might still fit the binding pocket of the closed-channel state. To test this hypothesis, we targeted the synthesis of five 5′-phenyl-1,2,5,6-tetrahydro-3,3′-bipyridine analogues (**3a** – **3e**, Figure 1) related to compound **2** to determine whether we could identify a new antagonist. Our decision to focus initially on 2′-fluoropyridines rather than 2′-chloropyridine **2** related to their ease of synthesis (*vide infra*).

Our strategy toward constructing the novel 5′-phenyl-1,2,5,6-tetrahydro-3,3′-bipyridine core was to carry out successive Suzuki couplings on a suitably functionalized pyridine starting monomer to append desired aromatic substituents at the 3- and 5-positions, and then make subsequent modifications on the flanking 5-pyridyl ring to provide target compounds (Scheme 1). Our decision to start with the core 5-bromo-2-fluoropyridine-3-boronic acid (**4**) rather than the 2-chloro congener (toward securing analogues of **2**) was predicated on the assumption that the second-stage Suzuki coupling would have been highly unselective. The initial coupling of commercially available boronic acid **4** with phenyl halides (iodo, bromo) to provide 3-(phenyl-substituted)pyridyl intermediates **5a**–**5d** was carried out by a modification of a literature procedure [26]. Yields were poor to modest (16 – 37%) with TLC showing the presence of multiple products, likely due to competitive palladium-catalyzed hetero- and homo bimolecular coupling side reactions. A second stage Suzuki coupling of **5a**–**5d** with pyridine-3-boronic acid under similar conditions proceeded much more cleanly to give 3,3′-bipyridine products **6a**–**6d** in 71 – 93 % yield. Two-step elaboration of these to target 1-methyl-1,2,5,6-tetrahydro-3,3′-bipyridine derivatives **3a** – **3d** was achieved by following a literature procedure with close precedence [27]. Thus, treatment of **6a** – **6d** with excess iodomethane in acetone resulted in selective methylation of the flanking pyridine ring to give **7a** – **7d** in 72 – 100% yields. Subsequent reduction of these with sodium borohydride in ethanol then gave target compounds **3a** – **3d** in 35 – 85% yields as free bases. These were then converted to hydrochloride salts for biological testing. Further modification of **3a** with concentrated HCl utilized a literature procedure [28] to provide 6′-pyridinol congener **3e** in 53 % yield. Complete experimental procedures can be found in Supplemental Materials.

Calculated lipophilicity (clog*P*), topological polar surface area (TPSA), and logBB-derived values provide information as to the potential of a compound for development as a pharmacotherapy for treating CNS disorders. These molecular descriptors were calculated for compounds **1**, **2 3a**–**3e**, as well as reference compounds nicotine and epibatidine (Table 1). In general, successful drugs used for treating CNS disorders have a clog*P* in the range 2–4[29], TPSA less than 76 Å[30] and logBB greater than −1[31]. All the compounds have clogP values (2.08 – 4.04) within the range of CNS drugs, and run higher than the reference compounds nicotine and epibatidine. TPSA values (16.1 – 58.6 Å) and logBB values (−0.38 – 0.51) also meet the criteria of successful CNS drugs and except for **3d** and **3e** are higher than for the reference compounds (0.04 – 0.06). All compounds have molecular weight (< 400 daltons) that predict for good BBB penetration.

Of the five analogues (**3a**–**3e**) synthesized, **3a** should be the most selective according to our previously reported molecular modeling and docking protocols.[19] This is because the modeling suggested that compound **3a** can reasonably fit the binding pocket of the closed-

channel state of α3β4 nAChR, but not α4β2 or α7 nAChR. None of these analogues appeared to fit the binding pocket of the open-channel state of any receptor examined (α3β4, α4β2, α7). Hence, we proposed that compound **3a** should be a selective antagonist of the α3β4 receptor. In the modeled binding structure of α3β4 nAChR with compound **3a** (depicted in Figure 2), **3a** stays on the interface between an α3 subunit and a β4 subunit in the usual agonist-binding site, with the protonated amine group of **3a** interacting with the aromatic residues αY93 (*i.e.*, Y93 residue of the α3 subunit), αY190, αW149, and βW54 (*i.e.,* W5 residue of the β4 subunit). The F atom of **3a** is close to the aromatic sides of αY197 and αW149. The phenyl ring of **3a** is packed with the hydrophobic side chains of residues βI108, βL116, and βL118 from β4 subunit and, hence, any hydrophilic substitution on the *p*- or *m*-position of the phenyl ring would be incompatible with the hydrophobic residues nearby.

The insights from molecular modeling were tested by carrying out electrophysiological experiments on compound **3a** with α3β4, α4β2, and α7 nAChRs using OpusXpress 6000A (Molecular Devices, Union City, CA), as we had done previously.[18, 32] OpusXpress is an integrated system that provides automated impalement and voltage clamp of up to eight oocytes in parallel (see Supplementary Information for complete experimental methods). First, we tried to detect possible agonist activity of compound **3a** at a concentration of 100 μM for α3β4, 30 μM for α4β2, and 60 μM for α7 as compared with 100 μM for acetylcholine (ACh) as a control response. The data were normalized to the corresponding control ACh responses, showing no significant agonist activity for any of the receptors tested.

Further, electrophysiological experiments were performed to test the antagonist activity against the nAChR receptors with ACh at the same concentrations of the three receptors specified above. The data revealed that at a concentration of 100 μM, compound **3a** significantly antagonized the α3β4 receptor, but showed no significant antagonist activity against the α4β2 or α7 receptors. With this finding, we ran a dose-response curve for compound **3a** against the α3β4 receptor at 100 μM ACh, calculating an $IC_{50} = 20 \pm 1$ μM (Figure 3). Hence, our data show that structural modification of the known agonist **1** has indeed resulted in the discovery of an α3β4 antagonist (**3a**) in vitro.

It should be noted that, in general, s a generated IC_{50} value is dependent on the substrate (ACh in this study) concentration [S] used in the assay. The higher the substrate concentration compared to the protein-substrate binding affinity ($K_d \approx K_m$), the larger the IC_{50} value. But the IC_{50} value can be converted to an inhibition constant (K_i), which is independent of the substrate concentration. It was reported[6] that $K_d = 619.63$ nM for ACh binding with α3β4 nAChR. Hence, based on the well-known relationship between the IC_{50} and K_i for a competitive antagonist/inhibitor,

$$IC_{50} = (1 + [S]/K_m)K_i \approx (1 + [S]/K_d)K_i, \quad (1)$$

Using this equation, this computes to $IC_{50} \approx 162 K_i$ for the α3β4 nAChR antagonist, assuming competitive antagonism in our assays using [S] = 100 μM. So, K_i = ~123 nM for compound **3a** against α3β4 nAChR.

In conclusion, we have used structure-based design on a known non-specific agonist (**1**) of the nicotinic acetylcholine receptor to generate a novel antagonist (**3a**) that is a reasonably potent and highly selective antagonist for the α3β4 receptor over α4β2 and α7. Future studies will detail ongoing studies of **3a** and other compounds of this series, including *in vivo* evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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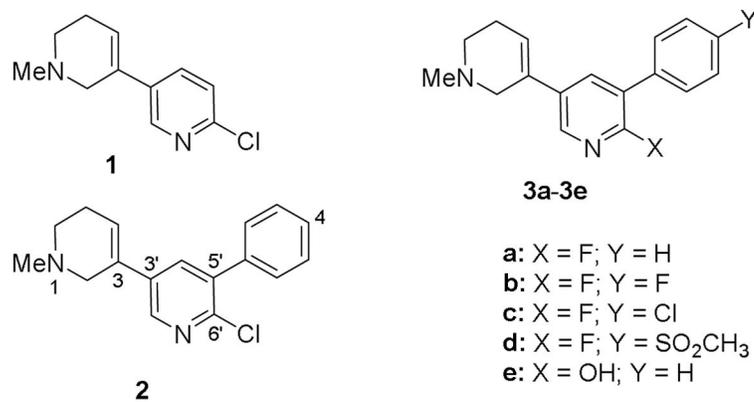
Figure 1.
Structures of the known nAChR agonist (**1**) and hypothesized analogues (**2**, **3a–3e**) acting as antagonists.

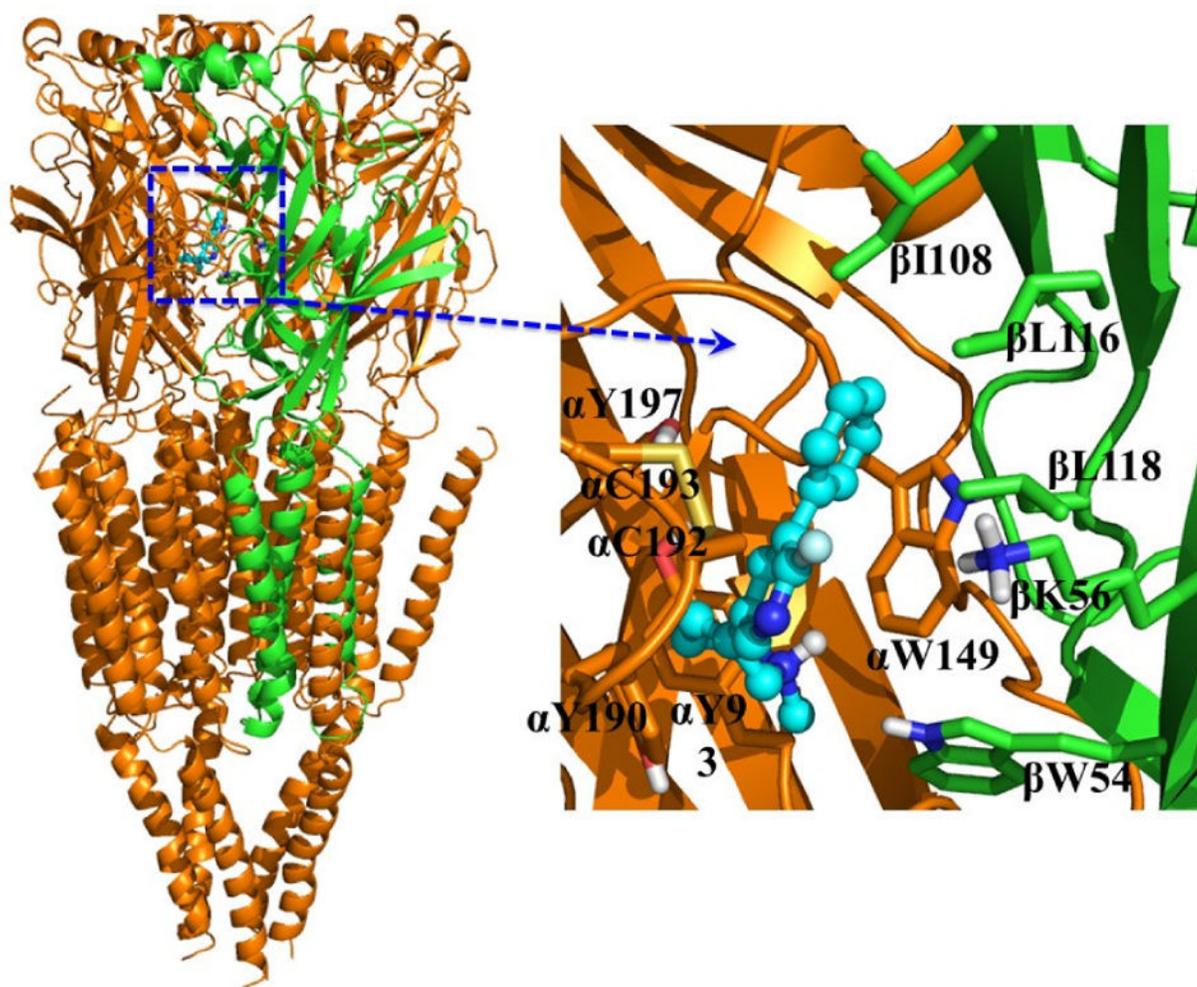**Figure 2.**
The modeled structure of compound **3a** binding with the closed-channel state of α3β4
nAChR. Left panel: The protein is represented in colored ribbon, with one β4 subunit in
green, and compound **3a** is in ball-stand-stick style with cyan color. Right panel: the details
of intermolecular interactions. Amino acid residues of the protein are represented in sticks
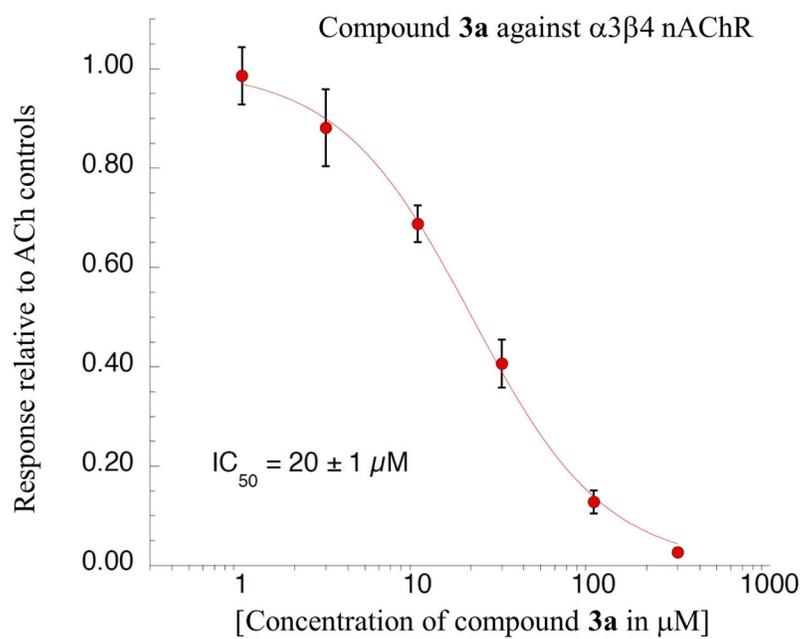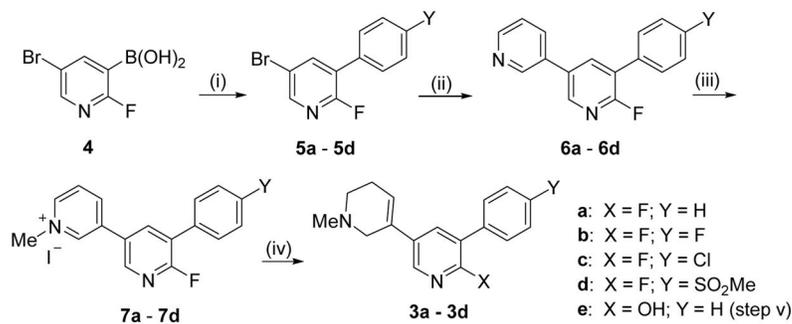and are colored by atom types.

Figure 3.
Dose-dependent antagonism of compound **3a** against α3β4 nAChR with 100 μM ACh.

Scheme 1.
Synthesis of 5′-phenyl-1,2,5,6-tetrahydro-3,3′-bipyridine analogues (**3a** – **3e**). (i) 4-Y-Ph-Z (Z = Br, I), Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, aq. p-dioxane, 90 °C (16 – 37 % yield); (ii) Pyridine-3-boronic acid, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, aq. p-dioxane, 90 °C (71 – 93 % yield); (iii) MeI, acetone, rt (72 – 100 % yield); (iv) NaBH$_4$, EtOH, 0 °C – rt (35–85 % yield); (v) for **3a** to **3e**, conc. HCl, aq. p-dioxane, 75 °C (53 % yield). See Supplemental Materials for experimental details.

Table 1

Calculated Physico Chemical Properties of 1, 2, and 3a–3e

Compd ID	Mol. Wt.	Log P^a	TPSA^a	logBB^b
nicotine	162.2	1.02	16.13	0.06
epibatidine	208.7	1.78	24.92	0.04
1	208.7	2.13	16.13	0.22
2	284.8	3.81	16.13	0.48
3a	268.3	3.52	16.13	0.44
3b	286.3	3.66	16.13	0.46
3c	302.8	4.04	16.13	0.51
3d	346.4	2.29	58.65 (50.27)^c	−0.38 (−0.26)^c
3e	266.3	2.08	32.34	−0.02

^{a}ChemAxon Calculator Plugins, Marvin 16.8.8, 2016.

^{b}logBB = −0.0148 × TPSA + 0.152 × log P + 0.139.[31]

^{c}Excludes sulfur from calculation.