

Novel $\alpha 3\beta 4$ Nicotinic Acetylcholine Receptor-Selective Ligands. Discovery, Structure–Activity Studies, and Pharmacological Evaluation

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Antagonist activity at the $\alpha 3\beta 4$ nicotinic acetylcholine receptor (nAChR) is thought to contribute to the antiaddictive properties of several compounds. However, truly selective ligands for the $\alpha 3\beta 4$ nAChR have not been available. We report the discovery and SAR of a novel class of compounds that bind to the $\alpha 3\beta 4$ nAChR and have no measurable affinity for the $\alpha 4\beta 2$ or $\alpha 7$ subtype. In functional assays the lead compound antagonized epibatidine-induced Ca^{2+} flux in $\alpha 3\beta 4$ -transfected cells in a noncompetitive manner.

Introduction

Nicotine, the addictive active ingredient in tobacco smoke, acts by binding to nicotinic acetylcholine receptors (nAChRs^a) in the central and peripheral nervous system. nAChRs are ligand-gated ion channels in the same family as the GABA and glutamate receptors and mediate cation flux.^{1,2} The neuronal nAChR is a pentameric protein made up of a combination of α and β subunits,³ although fully functional homomeric proteins, particularly the prominent $\alpha 7$ nAChR, are also present in the brain. The $\alpha 4\beta 2$ and the $\alpha 7$ nAChRs are by far the most prevalent in the central nervous system (CNS),^{4,5} whereas the $\alpha 3\beta 4$ subtype is predominant in the sensory and autonomic ganglia and in a subpopulation of neurons in the medial habenula (MHN) and interpeduncular nucleus (IPN) in the CNS.⁶

Although nAChRs are implicated in nicotine reward, dependence, and expression of withdrawal, little is known definitively about which subtypes are involved in each of these aspects of tobacco addiction. Since nicotine increases dopamine levels in the mesocorticolimbic reward circuitry, most attention has been focused on nAChR subtypes present in these pathways. Behavioral and functional studies in knockout mice have shown that the predominant $\alpha 4\beta 2$ nAChR present in this circuitry is required for nicotine addiction and dependence.^{7–9} The $\alpha 4\beta 2$ nAChR is therefore an important target for the development of smoking cessation medications, and significant efforts have been directed toward the development of ligands selective for this nAChR subtype. Indeed, the $\alpha 4\beta 2$ nAChR partial agonist varenicline, which was recently approved by the FDA as a smoking cessation medication, is effective in reducing craving and preventing relapse to smoking during abstinence.^{10–13}

Although $\alpha 4$ and $\beta 2$ subunits appear to be crucial for nicotine dependence, other nAChR subtypes, particularly the $\alpha 3\beta 4$ nAChR, have also been implicated in addiction to nicotine and other drugs of abuse.¹⁴ The $\alpha 3\beta 4$ receptor, which appears to predominate in sensory and autonomic ganglia and in the adrenal gland, is sometimes referred to as the “ganglionic

nAChR”.^{6,15,16} In the CNS, $\alpha 3\beta 4$ nAChR is present, although not in large amounts, in the mesolimbic dopamine pathway, which is known to be crucial for the rewarding effects of drugs of abuse.⁴ Differences in desensitization rates may, however, increase its relative influence in this brain region.⁴ It is abundant, however, in the medial habenula and interpeduncular nucleus, regions that receive inputs from the nucleus accumbens and send efferents to the ventral tegmental area (VTA).^{4,6,16,17} As discussed below, these receptors may influence drug dependence. A recent study has further indicated the importance of the $\alpha 3\beta 4$ nAChR to nicotine addiction by demonstrating that nicotine-induced hypolocomotion is reduced in $\beta 4$ null mice.¹⁸ Furthermore, after chronic nicotine treatment, mecamylamine-induced withdrawal is greatly diminished in $\beta 4$ null mice but not in $\beta 2$ null mice.¹⁸

Although recent studies seem to implicate a role for $\alpha 3\beta 4$ nAChR in psychostimulant and drug-seeking behavior, researchers have no good tools to explore this hypothesis. Some compounds, such as epibatidine, have high affinity at this site, but virtually no available compound has sufficient selectivity for this site to allow the study of the pharmacological role of $\alpha 3\beta 4$ nAChR. 18-Methoxycoronaridine (18-MC), a semisynthetic iboga alkaloid congener, has been shown to block the $\alpha 3\beta 4$ nAChR and has shown intriguing effectiveness in a variety of animal models of drug dependence against morphine, methamphetamine, and nicotine.^{19–24} Although 18-MC is an antagonist at $\alpha 3\beta 4$ nAChR,²⁵ it is not particularly selective and binds to opioid and other receptors. Studies by Glick and colleagues, which include the synthesis and testing of more selective congeners, have further implicated $\alpha 3\beta 4$ nAChR in dependence induced by nicotine and other drugs of abuse.^{22,26}

Another nonselective $\alpha 3\beta 4$ nAChR antagonist mecamylamine is an antagonist at several nAChRs.²⁷ Mecamylamine has been evaluated previously in clinical trials as a smoking cessation pharmacotherapy²⁸ and as an antidepressant.²⁹ Dextromethorphan has also been shown to have antagonist activity at $\alpha 3\beta 4$ nAChR;³⁰ however, it is also an antagonist at the NMDA receptor. Potent, selective ligands for the $\alpha 3\beta 4$ nAChR have been unavailable thus far.

We recently discovered a novel compound, **5** (SR16584), which binds to the $\alpha 3\beta 4$ nAChR and has no apparent affinity for $\alpha 4\beta 2$ or $\alpha 7$ nAChR. We report here the structure–activity relationships of **5** and its congeners to understand the molecular features that afford the selectivity toward the $\alpha 3\beta 4$ nAChR. We

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^a Abbreviations: nAChR, nicotinic acetylcholine receptor; CNS, central nervous system; FDA, Food and Drug Administration; MHN, medial habenula; IPN, interpeduncular nucleus; VTA, ventral tegmental area; 18-MC, 18-methoxycoronaridine; FLIPR, fluorometric imaging plate reader.

also characterized the binding of these compounds to the $\alpha 3\beta 4$ nAChR and compared them to epibatidine.

Results and Discussion

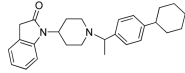
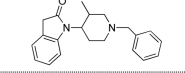
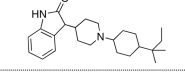
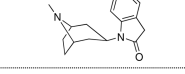
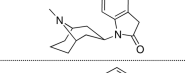
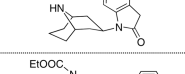
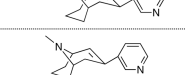
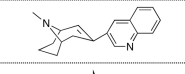
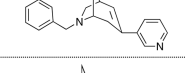
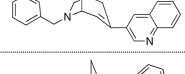
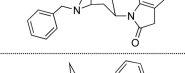
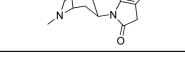
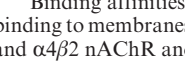
Lead compound **5** was discovered from screening a limited, selective library of small molecule compounds that possessed a protonatable nitrogen in an alicyclic ring, keeping in mind the three-point pharmacophoric features of the Sheridan nicotinic pharmacophore.³¹ Most of the compounds screened contained a piperidine core ring, monocyclic or bicyclic, with a lipophilic substituent on the alicyclic nitrogen and a heterocyclic aromatic substituent on the 4-position of the piperidine ring. These compounds were screened against the $\alpha 3\beta 4$ and $\alpha 4\beta 2$ nAChR transfected into HEK293 cells (obtained from the laboratory of Dr. Kenneth Kellar, Georgetown University; Washington, DC). Compounds were evaluated for binding affinity, determined by inhibition of [³H]epibatidine binding to cell membranes, and for functional activity, measuring stimulation or inhibition of calcium flux using the fluorometric imaging plate reader (FLIPR). Selected compounds were also tested for binding affinity to the $\alpha 7$ nAChR on rat brain membranes. Table 1 provides the structures of some of the compounds screened and their binding affinities.

From the binding affinities in Table 1, it was apparent that compounds that contain the essential protonatable nitrogen in a monocyclic piperidine ring (**1–3**) have no affinity for the $\alpha 3\beta 4$ or the $\alpha 4\beta 2$ nAChR, whereas those in which the nitrogen is present as part of a bicyclic core ring (**4–13**) possessed measurable binding affinity at one or both nAChRs. This is not surprising and is also a trend seen in the natural product nAChR ligands such as epibatidine (Figure 1), which contains a bicyclic ring and has a 1000-fold higher affinity for nAChR than does nicotine, which contains a monocyclic pyrrolidine ring. In fact, several potent nAChR ligands contain bicyclic core rings.^{32–35} From the 65 compounds we tested, we identified [3.2.1]azabicyclooctane-containing **4** and [3.3.1]azabicyclononane-containing **5** as potential lead compounds; both have binding affinity at the $\alpha 3\beta 4$ nAChR and no affinity for the $\alpha 4\beta 2$ or $\alpha 7$ nAChR up to 100 μ M. The binding affinity of **5** at $\alpha 3\beta 4$ is similar to that of nicotine and cytosine at $\alpha 3\beta 4$ (see Table 1); however, **5** has over 200-fold selectivity for the $\alpha 3\beta 4$ nAChR and offers a good starting point for developing potent and selective $\alpha 3\beta 4$ nAChR ligands. More importantly, our SAR studies suggest insights into the structural basis of subtype selectivity between the $\alpha 3\beta 4$ and $\alpha 4\beta 2$ nAChRs.

Both **4** and **5** contain an azabicyclic core ring, with a dihydroindolin-2-one ring distal to the protonatable nitrogen. Although the azabicyclic rings differ by only one carbon (azabicyclooctane vs azabicyclononane), **5** is more potent than **4** by almost a whole order of magnitude at the $\alpha 3\beta 4$ nAChR. Moreover, both these bicyclic rings are larger than the azabicycloheptane ring of epibatidine, indicating that, among other factors, the $\alpha 3\beta 4$ receptor may have a larger binding pocket than does $\alpha 4\beta 2$, which may be distinguished by compounds containing larger rings, like **5**, in contrast to epibatidine, which contains an optimally sized bicyclic ring that fits into all the nAChRs and thus lacks selectivity.

We synthesized a small series of analogues to investigate the importance of the three major pharmacophoric elements, (i) the heteroaromatic ring, (ii) the bicyclic core ring, and (iii) the nitrogen substituent, for selectivity to the $\alpha 3\beta 4$ nAChR. The synthesis of these analogues is shown in Schemes 1–3 and followed standard methodology as shown. These analogues, shown in Table 1, were tested for their binding affinity and functional activity at the $\alpha 3\beta 4$ and $\alpha 4\beta 2$ nAChR and showed

Table 1. Structures and Binding Affinities (K_i , nM)^a of Selected Compounds Screened for nAChR Affinity

Compound/Structure	Entry	$\alpha 3\beta 4$	$\alpha 4\beta 2$	$\alpha 7$
Epibatidine		0.15 ± 0.05	0.06 ± 0.0	4.2 ± 0.5
Nicotine		481 ± 59	11.1 ± 1.1	
Cytisine		203 ± 19	1.53 ± 0.2	
Acetylcholine		620 ± 130	37.7 ± 3.7	
	1	>10,000	>10,000	
	2	>10,000	>10,000	
	3	>10,000	>10,000	
	4	2790 ± 169	>100,000	
	5	508 ± 162	>100,000	>100,000
	6	422 ± 53	>100,000	
	7	>10,000	>10,000	
	8	241 ± 30	10.4 ± 0.2	
	9	2615 ± 277	423 ± 5.9	
	10	170 ± 48	1.95 ± 0.2	424
	11	2478 ± 123	29.3 ± 9.3	
	12	1836 ± 210	>10,000	
	13	>10,000	>10,000	

^a Binding affinities were determined by inhibition of [³H]epibatidine binding to membranes derived from HEK cells transfected with rat $\alpha 3\beta 4$ and $\alpha 4\beta 2$ nAChR and rat brain membranes for $\alpha 7$ nAChR.

some interesting trends that suggested the basis for the selectivity of lead **5** for the $\alpha 3\beta 4$ nAChR.

The dihydroindolin-2-one ring plays a significant role in the selectivity of **5** for $\alpha 3\beta 4$ and, as discussed later, also on conferring antagonist activity at the $\alpha 3\beta 4$ nAChR. When the indolinone ring of **5** was replaced with a 3-pyridyl ring, as in **8**, the compound retained affinity for $\alpha 3\beta 4$ but now gained significant affinity for the $\alpha 4\beta 2$ receptor (10 nM) and was a potent ligand for $\alpha 4\beta 2$ nAChR. This surprising result indicates that it is the dihydroindolinone ring of **5** that provides selectivity for the $\alpha 3\beta 4$ receptor. In the published SAR on epibatidine, the 3-pyridyl ring is a favored heteroaromatic ring for several $\alpha 4\beta 2$ ligands.^{32,35,36} The gain in $\alpha 4\beta 2$ affinity by introducing the 3-pyridyl ring onto the [3.3.1]azabicyclo core ring of **8** agrees with published SAR trends and confirms our observation that the dihydroindolinone ring somehow precludes binding to the $\alpha 4\beta 2$ receptor, thereby affording selectivity for

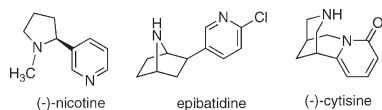


Figure 1. Structures of known nonselective nAChR ligands with affinity for the $\alpha 3\beta 4$ nAChR.

$\alpha 3\beta 4$. Replacing the heteroaromatic ring with a quinoline, as in **9**, decreases binding affinity for $\alpha 4\beta 2$ and $\alpha 3\beta 4$, indicating that the larger 3-quinolinyl ring is not optimal for binding at either of these receptors.

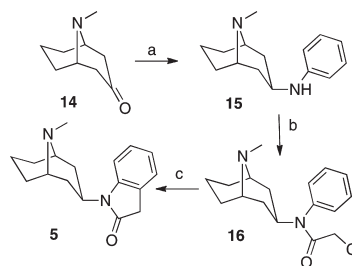
We explored the effect of substitution on the basic nitrogen and found that removal of the *N*-methyl group of **5** (as in **6**) retained the affinity and selectivity for $\alpha 3\beta 4$. This finding agrees with most reported observations for nAChR ligands.^{32,35} Interestingly, carbamate-type *N*-substituents, such as those present in **7** (*N*-ethoxycarbamate analogue of **8**), result in complete loss of binding affinity for $\alpha 3\beta 4$ and $\alpha 4\beta 2$, confirming previous SAR that the protonatable basic nitrogen is an important requisite for binding affinity at all nAChRs.

We determined the mode of binding and functional activity of **5** at $\alpha 3\beta 4$ nAChR. Saturation binding experiments at $\alpha 3\beta 4$ using [³H]epibatidine indicated that **5** and cytisine bind noncompetitively with epibatidine, as indicated by a change in K_d and B_{max} when using increasing concentrations of **5** (Figure 2). However, the moderate K_i of both compounds in displacing [³H]epibatidine in binding experiments appears to indicate that both compounds may bind at sites partially overlapping the epibatidine binding.

We used the high-throughput FLIPR and calcium flux to evaluate the functional activity of these compounds. As seen in Figure 3, addition of the nAChR agonist epibatidine leads to a 1000-fold increase in intracellular Ca^{2+} -induced fluorescence. The potencies of epibatidine ($0.030 \pm 0.004 \mu M$) and nicotine ($8.7 \pm 0.9 \mu M$, data not shown) are similar to those reported by Fitch et al.³⁷ Both **5** and its desmethyl analogue **6** were antagonists in the FLIPR assay. They showed no agonist activity alone and fully reversed the epibatidine-induced Ca^{2+} fluorescence, with potencies in the micromolar range (as shown for **5** in Figure 3). The 3-pyridyl containing analogue **8**, on the other hand, showed partial agonist activity in the FLIPR assay. This result supports our hypothesis that the larger bicyclic benzofused dihydroindolinone ring not only plays a role in the selectivity but also confers antagonistic activity to this class of azabicyclononane-type nAChR ligands. To the best of our knowledge, the dihydroindolinone ring is a new heterocyclic ring not present in any nAChR ligands reported to date. Notably, **5** has no affinity at the $\alpha 7$ nAChR (Table 1). Compound **5** therefore represents a novel class of $\alpha 3\beta 4$ -selective nAChR ligands that may be valuable for studying the pharmacological role of this receptor in drug abuse and reward pathways.

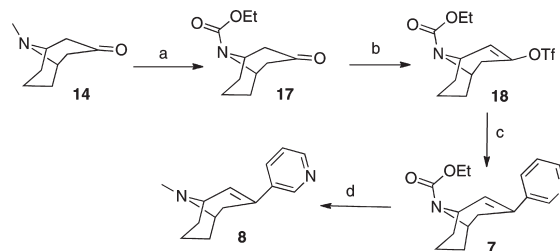
From our screening efforts, we also identified a new bicyclic core ring, the 2-azabicyclo[3.2.1]octane (an isomer of tropane) that provided potent binding affinity for $\alpha 4\beta 2$ nAChR. The compound containing this isotropane core scaffold and the $\alpha 4\beta 2$ -favored 3-pyridyl ring, **10** (Table 1), has a 2 nM binding affinity for $\alpha 4\beta 2$ and about 100-fold selectivity over $\alpha 3\beta 4$ receptors and greater than 200-fold selectivity over $\alpha 7$ nAChR. Interestingly, **10** contains an *N*-benzyl substituent on the basic nitrogen. Furthermore, in functional assays, **10** is a partial agonist at $\alpha 4\beta 2$ nAChR. This is unusual because most SARs on $\alpha 4\beta 2$ show that for agonist activity, only small alkyl substituents are tolerated at the basic nitrogen.^{32,35} Our results show that in this isotropane class, the larger *N*-benzyl substituent can still impart agonist activity at the $\alpha 4\beta 2$ receptor. At $\alpha 3\beta 4$

Scheme 1^a



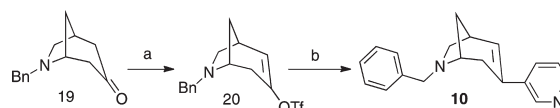
^a Reagents and conditions: (a) aniline, molecular sieves, toluene, reflux, sodium cyanoborohydride, methanol; (b) chloroacetyl chloride, triethylamine, methylene chloride, reflux; (c) aluminum chloride, 130–160 °C.

Scheme 2^a



^a Reagents and conditions: a. ethyl chloroformate, K_2CO_3 , toluene, 90–100 °C; b. $NaN(TMS)_2$, *N*-(5-chloro-2-pyridyl)triflimide, THF, –78 °C; c. 3-pyridineboronic acid, $Pd(PPh_3)_4$, K_3PO_4 , dioxane, 85 °C; d. LAH, THF.

Scheme 3^a



^a Reagents and conditions: (a) $NaN(TMS)_2$, *N*-(5-chloro-2-pyridyl)triflimide, THF, –78 °C; (b) 3-pyridineboronic acid, $Pd(PPh_3)_4$, K_3PO_4 , dioxane, 85 °C.

nAChR, the 3-pyridyl containing **10** is also a partial agonist, similar to the 3-pyridyl containing azabicyclononane **8** (Figure 3B). The 3-quinolinyl analogue **11** in the isotropane series has decreased activity at both receptors, similar to the trend also seen with the in the azabicyclononane series (**9**). However, when the 3-pyridyl ring in the isotropane series is replaced with the dihydroindolinone ring (**12**), it completely abolished affinity at the $\alpha 4\beta 2$ nAChR, although it did bind to the $\alpha 3\beta 4$ nAChR, albeit with lower affinity than the isotropane **10** or the azabicyclononane **5**. This further confirms our SAR that the dihydroindolinone ring somehow prevents binding to the $\alpha 4\beta 2$ nAChR, a trend also seen with the azabicyclononane series of ligands.

The SAR on the two pharmacophoric features, the heteroaromatic ring and the core bicyclic ring, provides approaches to designing $\alpha 3\beta 4$ -selective nAChR ligands. The heteroaromatic dihydroindolinone ring precludes binding to the $\alpha 4\beta 2$ nAChR when present on any of the core bicyclic rings (see **4**, **5**, and **12**), thus conferring selectivity for the $\alpha 3\beta 4$ nAChR. Among the azabicyclic core rings, the larger azabicyclononane affords good binding affinity for the $\alpha 3\beta 4$ nAChR and the possibility of preferential binding to the $\alpha 3\beta 4$ nAChR, with an appropriate heteroaromatic pharmacophore, as in **5**. We are currently optimizing the pharmacophoric elements of the azabicyclononane class of $\alpha 3\beta 4$ ligands to improve binding affinity, and these results will be reported in due course.

In summary, we have discovered a new class of nAChR ligands that do not appear to bind other major nAChR

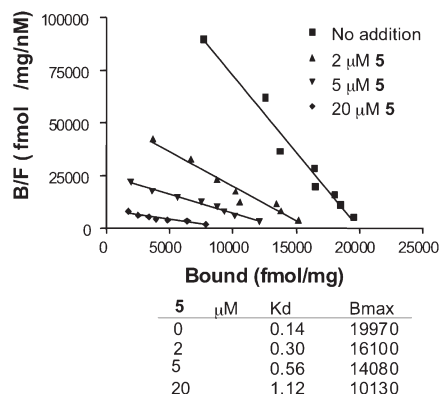


Figure 2. Scatchard analysis of [³H]epibatidine binding at $\alpha 3\beta 4$ nAChR alone and in the presence of various concentrations of the inhibitor **5**.

subtypes at concentrations greater than 100 μM and therefore show excellent selectivity (> 200-fold) for the $\alpha 3\beta 4$ nAChR. Our SAR studies show that the azabicyclo[3.3.1]nonane core ring and the dihydroindolinone heteroaromatic ring play a role in conferring the selectivity for the $\alpha 3\beta 4$ nAChR, whereas only the dihydroindolinone-containing ligands possess antagonist activity at $\alpha 3\beta 4$ nAChR. These SARs may lead to a better understanding of structural features for selectivity and functional activity at the nAChR. Such selective and potent $\alpha 3\beta 4$ nAChR antagonists will be useful for testing the hypothesis that $\alpha 3\beta 4$ receptor antagonists can attenuate rewarding properties of nicotine and other drugs of abuse.^{14,38}

Experimental Details

General. ¹H and ¹³C NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer (300 and 75 MHz, respectively) and are internally referenced to chloroform at δ 7.27. Data for ¹³C are reported in terms of chemical shift. Mass spectra were obtained using a ThermoFinnigan LCQ Duo LC/MS/MS instrument and an electrospray ionization probe. Thin-layer chromatography was run on Analtech Uniplate silica gel TLC plates. Flash chromatography was carried out using silica gel, Merck grade 9385, 230–400 mesh. The purity of the final compounds reported was confirmed by HPLC and mass spectra, using the following conditions: column, Phenomenex Synergi 4 m Fusion RP, 250 mm \times 4.60 mm; mobile phase, (A) MeCN (0.1% TFA)/(B) H₂O (0.1% TFA) (gradient 20% A to 100% A, 15 min); flow rate, 1 mL/min; detection, PDA 254 nm. The purity of all final compounds was greater than 95%. Experimental details for intermediates in Schemes 1–3 are reported in the Supporting Information.

1-(9-Methyl-9-azabicyclo[3.3.1]non-3-yl)-1,3-dihydro-2H-indol-2-one (5). A mixture of **16** (576 mg, 1.88 mmol) and aluminum chloride (1.00 g, 7.51 mmol) under argon was placed in a 160 °C oil bath and stirred for 15 min and then allowed to cool to 130 °C and kept at this temperature 1.75 h. A solution of 1 N sodium hydroxide (10 mL) was added, and the resulting mass was sonicated to homogeneity and extracted three times with methylene chloride, dried (sodium sulfate), and evaporated to an oil. The oil was purified by flash chromatography, eluting with 0–6% methanol containing 5% of 28% ammonium hydroxide/methylene chloride to give **5** as a colorless oil (free base, 219 mg, 43%). This was treated with HCl in ether and evaporated to dryness to give an off-white solid, mp 268–269 °C. ¹H NMR (HCl salt; 300 MHz, CD₃OD) δ 1.63 (br d, J = 14.4 Hz, 2H, H-6_{ax}, H-8_{ax}), 1.75 (br d, J = 14.4 Hz, 1H, H-7_{ax}), 2.22 (m, 2H, 2-H, H-4), 2.35–2.48 (m, 3H, H-6_{eq}, H-7_{eq}, H-8_{eq}), 2.79 (ddd, J = 10.4, 10.1, 2.3 Hz, 2H, H-2, H-4), 2.98 (s, 3H, N-CH₃), 3.55 (s, 2H, indolinyl CH₂), 3.74 and 3.77 (2 br s, J = 11.2 Hz, 2H, H-1, H-5), 4.90–5.05 (m, 1H, H-3), 7.05

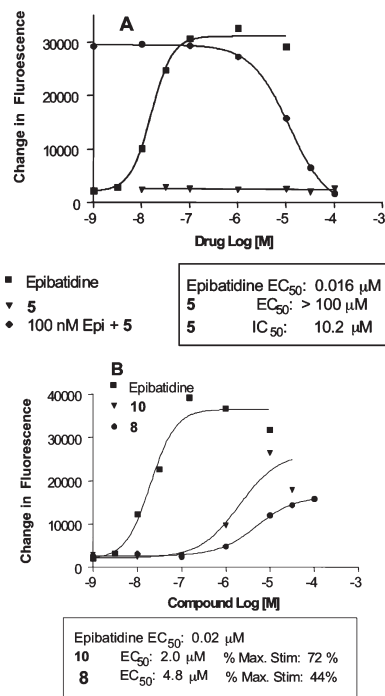


Figure 3. Ligand-induced Ca²⁺ fluorescence in $\alpha 3\beta 4$ nAChR-containing HEK cells using FLIPR: (A) agonist activity of epibatidine and antagonist activity of **5**; (B) agonist activity of epibatidine and the partial agonist activity of **10** and **8**.

(t, J = 7.6 Hz, 1H, Ar-H8), 7.25–7.32 (m, 3H, Ar-H5, Ar-H6, Ar-H7). MS (ESI) m/z : 271.2 (M + H)⁺.

9-Methyl-3-(pyridin-3-yl)-9-azabicyclo[3.3.1]non-2-ene (8). To a solution of **19** (67 mg, 0.25 mmol) in THF (10 mL) was added lithium aluminum hydride (108 mg, 4.74 mmol). The resultant mixture was stirred at room temperature for 30 min, and the excess of lithium aluminum hydride was destroyed with ethyl acetate until no gas was released. Then water (0.5 mL) was added. The mixture was filtered and the solid washed with ethyl acetate (10 mL). The filtrate and washings were combined, dried over sodium sulfate, and evaporated to dryness. The residue was subjected to chromatography on silica gel, eluting with a solvent mixture of dichloromethane and methanol (20%) to afford 14 mg of desired product **8** (35%) as a light yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 1.36–1.62 (m, 4H, H-6, H-7, H-8), 1.78–1.96 (m, 2H, H-6, H-8), 2.03 (dd, J = 18.3, 1.5 Hz, 1H, H-4), 2.37 (s, 3H, N-CH₃), 2.74 (ddd, J = 18.4, 6.9 Hz, 1.0 Hz, 1H, H-4), 3.14 (br, 1H, H-5), 3.37 (br, 1H, H-1), 6.06 (dt, J = 4.8, 2.1 Hz, 1H, H-2), 7.19 (ddd, J = 8.1, 4.8, 0.9 Hz, 1H, Ar-H5), 7.63 (ddd, J = 8.8, 2.4, 1.5 Hz, 1H, Ar-H4), 8.43 (dd, J = 4.8, 1.5 Hz, 1H, Ar-H6), 8.63 (d, J = 2.1 Hz, 1H, Ar-H2). ¹³C NMR (300 MHz, CDCl₃) δ 15.4 (C-7), 26.5 (C-6), 28.1 (C-8), 32.9 (C-4), 41.8 (C-9, N-CH₃), 52.7 (C-1), 55.4 (C-5), 123.2 (Ar-C5), 125.5 (C-2), 132.0 (Ar-C4), 133.9 (Ar-C3), 135.6 (C-3), 146.5 (Ar-C2), 148.3 (Ar-C6). MS (APCI) m/z : 215.1 (M + H)⁺.

6-Benzyl-3-(pyridin-3-yl)-6-azabicyclo[3.2.1]oct-2-ene (10). A mixture of **21** (140 mg, 0.40 mmol), 3-pyridineboronic acid (55 mg, 0.45 mmol), Pd(PPh₃)₄ (23 mg, 0.02 mmol), K₃PO₄ (128 mg, 0.60 mmol), and dioxane (4 mL) was stirred at 85 °C overnight (17 h). The mixture was treated with NaOH (2 M) until strongly basic (pH > 12) and extracted with ethyl acetate (3 \times 10 mL). The extract was washed with brine, dried over sodium sulfate, and evaporated to dryness. The residue was subjected to chromatography on silica gel, eluting with a mixture solvent of ethyl acetate/hexanes/methanol (5:5:1) to afford 36 mg of **10** (32%) as a light yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 1.71 (d, J = 10.5 Hz, 1H, H-8), 1.96–1.86 (m, 1H, H-8), 2.43 (d, J = 17.1 Hz, 1H, H-4), 2.55 (d, J = 17.1 Hz, 1H, H-4), 2.66–2.71 (m, 1H, H-5), 2.83 (dd, J = 8.7, 4.8 Hz, 1H, H-7_{eq}), 2.97 (d, J = 9.0 Hz, 1H, H-7_{ax}), 3.46 (m, 1H, H-1), 3.74 (d, J = 10.5 Hz, 1H,

CH₂-Ph), 3.87 (d, *J* = 10.5 Hz, 1H, CH₂-Ph), 6.48 (d, *J* = 6.1, 1H, H-2), 7.10–7.36 (m, 6H, Ar-H, pyridyl H-5), 7.58 (ddd, *J* = 8.8, 2.3, 1.6 Hz, 1H, pyridyl H-4), 8.38 (dd, *J* = 5.0, 1.6 Hz, 1H, pyridyl H-6), 8.58 (dd, *J* = 2.4, 0.6 Hz, 1H, pyridyl H-2). ¹³C NMR (300 MHz, CDCl₃) δ 32.8 (C-1), 35.7 (C-4), 35.8 (C-8), 57.6 (C-5), 59.6 (C-7), 62.4 (Ar-CH₂-N), 123.0 (pyridyl C-5), 126.8 (Ar C-4), 128.2 (Ar C-3, Ar C-5), 128.5 (Ar C-2), 131.6 (C-2), 131.7 (C-3), 131.9 (pyridyl C-4), 135.9 (pyridyl C-3), 146.5 (pyridyl C-2), 148.0 (pyridyl C-6). MS (APCI) *m/z*: 277.0 (M + H)⁺.

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Supporting Information Available: Experimental details and analytical data for all intermediates in Schemes 1–3, biological evaluation, and in vitro assay protocols. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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