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5-Azidoepibatidine: An Exceptionally Potent Photoaffinity Ligand for Neuronal α4β2 and α7 Nicotinic Acetylcholine Receptors

Nanjing Zhang, Motohiro Tomizawa and John E. Casida*

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720-3112, USA

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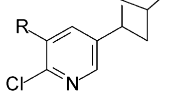
Abstract—Racemic 5-azidoepibatidine [(±)-**1**] was synthesized via 5-aminoepibatidine as a candidate photoaffinity ligand with exceptionally high affinity at the mammalian neuronal nicotinic receptors (K_i values of 0.027 nM for α4β2 and 9.7 nM for α7) and excellent photoreactivity.

The nicotinic acetylcholine receptor (nAChR) is the prototype of ligand-gated ion channels. It consists of five homologous subunits surrounding a transmembrane ionotropic pore. Different subunit combinations serve as the basis for the diversity of nAChR subtypes.[1,2] Various subtypes are responsible for different biological functions and pharmacological profiles which are modulated by agonists and antagonists, leading to the current search for subtype-selective ligands as potential therapeutic agents for analgesia and neural disorders including neurodegenerative Alzheimer's and Parkinson's diseases.[2,3] nAChR ligands bind at specific sites which are presumably located at the interface between subunits.[4] Despite the recent determination of the crystal structure of an acetylcholine binding protein,[5,6] current knowledge on the ligand-binding environment is mostly derived from studies involving photoaffinity labeling or site-directed mutagenesis.[4,7] A photoaffinity probe specifically-bound at the receptor will be activated into highly reactive species upon UV irradiation and covalently link to nearby amino acid residues, allowing identification of the binding site environment. A suitable photoaffinity probe with very high affinity to the site will greatly facilitate characterizing the functional architecture of various nAChR subtypes.

We report here an exceptionally potent candidate photoaffinity ligand for mammalian neuronal nAChRs. The rationale to design and synthesize 5-azidoepibatidine (**1**) (Fig. 1) as a photoaffinity probe is based on the following facts: (1) epibatidine (**2**), a neurotoxin isolated from the skin of the Ecuadoran poison frog, *Epipedobates tricolor*,[8] is of very high potency towards all mammalian neuronal nAChR subtypes;[2,9] (2) our previous structure–activity studies on azidopyridinyl neonicotinoids establish that introduction of an azido group at the 5-position of the pyridine ring[10] does not decrease the potency at the mammalian neuronal α4β2 nAChR.[11]

Our initial attempt to prepare **1** by direct lithiation of *N*-Boc-protected **2** followed by reaction with tosyl azide provided no success. We therefore focused on the synthesis of 5-aminoepibatidine (**3**) which could be potentially converted to **1**. It was envisioned that **3** might be accessible through stereoselective Heck-type reductive coupling[12] of a 3-iodopyridine with suitably-protected 7-aza-bicyclo[2.2.1]hept-2-ene. Thus, 5-amino-6-chloro-3-iodopyridine (**7**) was synthesized from 2-hydroxy-3-nitropyridine (**4**) (Scheme 1). Iodination[13] of **4** provided the corresponding 3-iodopyridine (**5**), which was converted to **6** by $POCl_3$. Reduction of **6**



1 R=N_3, 5-azidoepibatidine
2 R=H, epibatidine
3 R=NH_2, 5-aminoepibatidine

Figure 1. nAChR ligands epibatidine (**2**), its photoaffinity analogue 5-azidoepibatidine (**1**) and the synthesis intermediate 5-aminoepibatidine (**3**).

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*Corresponding author. Tel.: +1-510-642-5424; fax: +1-510-642-6497; e-mail: ectl@nature.berkeley.edu

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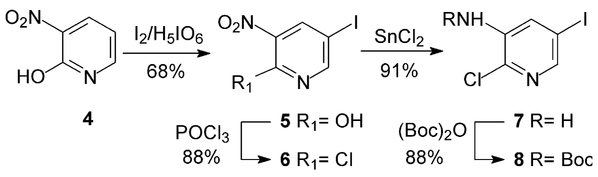
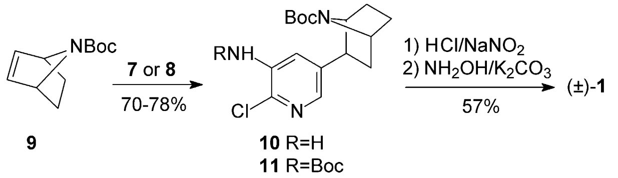


Scheme 1.



Scheme 2.

with $SnCl_2$ converted the nitro group to an amino substituent to provide **7**.

Reductive Heck-type coupling[12] of **7** with *N*-Boc-protected 7-aza-bicyclo[2.2.1]hept-2-ene (**9**) provided the desired *exo*-*N*-Boc-protected 5-aminoepibatidine (**10**) as a racemate in 78% yield (Scheme 2). The coupling reaction of Boc-protected iodopyridine **8** provided racemic **11** in similar yield (70%). Finally **10** and **11** were converted to (±)-**1** by a two-step and one-pot reaction in 57% yield.[10,14]

5-Azidoepibatidine [(±)-**1**] has the desired photoreactivity (Fig. 2). The azido group is activated upon irradiation with 254 nm UV and decomposed completely within 120 s.

The binding affinity of (±)-**1** was evaluated with mammalian neuronal α4β2 and α7 receptors as well as the *Drosophila* receptor (Fig. 3; Table 1). As a control, (±)-**2** was also tested under the same conditions. It was interesting to find that (±)-**1** has the same or even slightly higher affinity than that of (±)-**2** to both the α4β2 and α7 nAChRs but is 10 times less active on *Drosophila* nAChR. The affinity of (±)-**1** to the α4β2 receptor is exceptionally high as indicated by a K_i value of 0.027 nM.[22] The affinity to the α7 subtype is also very high with a K_i value of 9.7 nM. These binding affinities make **1** the most potent photoaffinity probe for the α4β2 and α7 nAChRs reported to date, with high selectivity towards mammalian versus insect nAChRs.

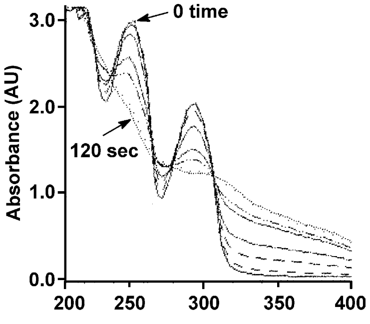


Figure 2. Photolysis of 5-azidoepibatidine [(±)-**1**] (0.46 mM in ethanol) upon UV irradiation at 254 nm. The times shown are 0, 5, 15, 30, 45, 60, 90 and 120 s.

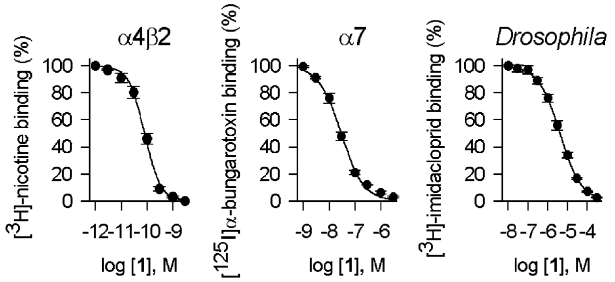


Figure 3. Displacement curves for 5-azidoepibatidine [(±)-**1**] of [^{3}H]nicotine, [^{125}I]α-bungarotoxin, and [^{3}H]imidacloprid binding to the α4β2, α7 and *Drosophila* nAChRs, respectively, as percent of controls.

Table 1. Binding affinities of 5-azidoepibatidine [(±)-**1**] and epibatidine [(±)-**2**] to the α4β2, α7 and *Drosophila* nicotinic receptors

Compd	K_i (nM±SD, n = 3–4)[a]		
	α4β2	α7	*Drosophila*
(±)-**1** [b]	0.027±0.001	9.7±0.23	2100±260
(±)-**2** [b]	0.035±0.004	10.2±1.33	200±22

[a] α4β2 subtype was immunoisolated by monoclonal antibody (mAb) 299 from mouse fibroblast M10 cells and assayed with 9 nM [^{3}H]nicotine binding.[15] In the same manner, α7 receptor subtype was immunoisolated by mAb 306 from human neuroblastoma SH-SY5Y cells and assayed with 1 nM [^{125}I]α-bungarotoxin binding.[16] *Drosophila* receptor was assayed with 2.5 nM [^{3}H]imidacloprid binding.[17] K_i values are calculated with the equation of Cheng and Prusoff,[18] that is $K_i = IC_{50}/(1 + [L]/K_D)$, with K_D 3.8 nM for the α4β2 subtype in M10 cells,[19] 1.06 nM for the α7 subtype in SH-SY5Y cells [20] and 2.4 nM for the *Drosophila* nAChR.[17]

[b] Compounds **1** and **2** are racemates but it is known that (+)-**2** and (−)-**2** do not differ significantly in level of activity[9,21] on nAChRs.

In conclusion, we have synthesized a very potent photoaffinity probe 5-azidoepibatidine [(±)-**1**] for neuronal α4β2 and α7 nAChRs. The exceptionally high potency as well as the excellent photoreactivity make it an ideal candidate for radiolabeling and photoaffinity studies to precisely characterize the agonist binding site environment of the mammalian neuronal nAChRs.

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14. 5-Azidoepibatidine [(±)-**1**]. ^{1}H NMR: δ, 8.06 (1H, d, $J=2.0$ Hz), 7.77 (1H, d, $J=2.0$ Hz), 3.81 (1H, br s), 3.57 (1H, br s), 2.76 (1H, q, $J=5.1$, 9.2 Hz), 1.91 (1H, dd, $J=8.7$, 11.8 Hz), 1.64–1.52 (6H, m). ^{13}C NMR: δ, 144.4, 143.2, 139.8, 134.2, 126.8, 62.8, 56.2, 44.3, 40.5, 31.5, 30.4. FAB-HRMS calcd for $C_{11}H_{13}N_5Cl$ (MH^+), 250.0859, found, 250.0862. Anal. calcd for $C_{11}H_{12}N_5Cl$: C, 52.91, H, 4.84, N, 28.05. Found: C, 52.76, H, 4.98, N, 28.03.

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22. 5-Aminoepibatidine [(±)-**3**], the immediate precursor to 5-azidoepibatidine [(±)-**1**], also shows very high affinity with the α4β2 receptor ($K_i = 0.025 \pm 0.004$ nM, n = 3). The very high affinity of (±)-**3** assayed with [^{3}H](±)-**2** binding in rat brain membrane preparation was reported by others after submission of the present paper. See: Carroll, F. I.; Lee, J. R.; Navarro, H. A.; Ma, W.; Brieaddy, L. E.; Abraham, P.; Damaj, M. I.; Martin, B. R. *J. Med. Chem.* **2002**, *45*, 4755.