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# Functionalization of the alicyclic skeleton of epibatidine: synthesis and nicotinic acetylcholine receptor binding affinities of epibatidine analogues †‡

**Zhi-Liang Wei,[a] Yingxian Xiao,[b] Clifford George,[c] Kenneth J. Kellar[b] and Alan P. Kozikowski*[a]**

[a] *Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois, 60612-7231, USA. E-mail: kozikowa@uic.edu; Fax: +1-312-996-7107; Tel: +1-312-996-5972*

[b] *Department of Pharmacology, Georgetown University, 3900 Reservoir Road, NW Washington DC, 20057, USA*

[c] *Naval Research Laboratory, 4555 Overlook Avenue, SW Washington DC, 20375, USA*

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**A novel method for the epimerization of *endo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptan-3-one (12) on silica gel was developed and used as the key step to synthesize functionalized analogues of epibatidine which were evaluated for their nicotine receptor subtype selectivity in binding studies.**

Neuronal nicotinic acetylcholine receptors (nAChRs) are a class of pentameric ligand-gated ion channels comprised of combinations of one or more α and β subunits, and different subunit combinations define different receptor subtypes with distinct biophysical, physiological and pharmacological properties. Neuronal nAChRs hold considerable promise as therapeutic targets for the treatment of disorders of the central and peripheral nervous systems. Drugs aimed at nAChRs have potential for the treatment of neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, schizophrenia, Tourette's syndrome, and certain epilepsies, as well as nicotine addiction.[1] The novel alkaloid epibatidine (**1**), isolated from the skin of the Ecuadorian poison frog, *Epipedobates tricolor*,[2] was found to have powerful analgesic activity and high binding affinity to nicotinic acetylcholine receptors.[3] Though its high toxicity limits the therapeutic potential,[4] it provides an attractive lead for the design of new ligands selective for distinct nAChR subtypes and possibly for the treatment of nervous system disorders. However, in spite of numerous synthetic studies of this molecule,[5] analogue synthesis has been generally limited to modification of the heteroaryl group,[6] alterations in the position of the aliphatic ring nitrogen,[7] and expansion of the two-carbon bridge,[8] in some cases with introduction of a double bond[8c,8h–k] or an additional ring nitrogen atom.[8e,k] However, few modifications involving the introduction of heteroatom groups into the azanorbornane core have been made or pharmacologically investigated.[9] We anticipated that introduction of an additional polar group, such as hydroxyl, into the azanorbornane core might alter nAChR affinity and subtype selectivity through the possibility of engaging in additional H-bond donor–acceptor interactions with appropriately positioned amino acid residues present in the respective pentameric receptor subtypes. Moreover, the hydroxyl group can be manipulated chemically so as to provide access to other analogues, including $^{18}$F derivatives for positron emission tomography (PET) imaging purposes. In a previous communication, we described the synthesis of the 5- and 6-hydroxyl-substituted epibatidine analogues **2a**,**b** and **3a**,**b** (Fig. 1).[10] Herein, we present additional studies on the functionalization of the alicyclic skeleton of epibatidine along with the nAChR binding affinities of these new analogues.

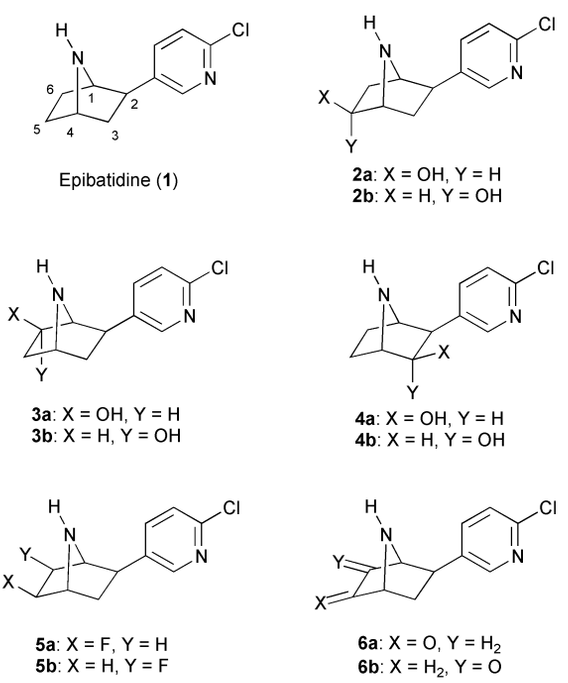


**Fig. 1**

As illustrated in Scheme 1, Michael addition of the aryllithium reagent prepared from 2-chloro-5-iodopyridine[11] to **7**, which itself was synthesized in accordance with a reported procedure,[9e] proceeded smoothly to give the adduct **8** in 86% yield. Elimination of the sulfonyl group of **8** with *t*-BuOK gave the olefin **9** in high yield. Hydroboration–oxidation of **9** provided exclusively the *trans* alcohol **10**, as was confirmed by single crystal X-ray analysis of its deprotection product **11** (Fig. 2).[12] § Although base-mediated epimerization of the *endo*-chloropyridine moiety to the corresponding *exo*-isomer has been reported in the synthesis of epibatidine (**1**),[13] treatment of **10** with *t*-BuOK in *t*-BuOH failed to afford the desired epimerized product. Thus, the alcohol **10** was oxidized with Dess–Martin periodinane to give the corresponding ketone **12** in 96% yield. Unfortunately, attempts to bring about epimerization of this

† Electronic supplementary information (ESI) available: detailed experimental procedures with spectroscopic data, and crystal data for compounds **11** and **4a**. See http://www.rsc.org/suppdata/ob/b3/b308906a/

‡ Dedicated to Dr John W. Daly of the NIH, who first discovered epibatidine.

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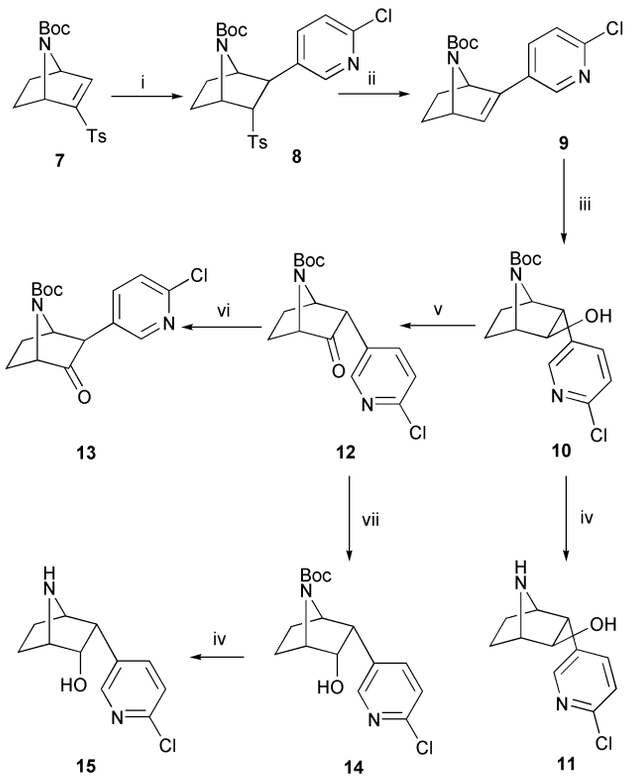


**Scheme 1** *Reagents and conditions:* (i) 2-chloro-5-iodopyridine, *n*-BuLi, THF, −78 °C, 86%; (ii) *t*-BuOK, THF, −78 °C to rt, 98%; (iii) $BH_3$·THF, THF, rt, overnight, then aq. NaOH, 35% $H_2O_2$, 63%; (iv) $CF_3COOH$, $CH_2Cl_2$; (v) Dess–Martin periodinane, $CH_2Cl_2$, 96%; (vi) silica gel, rt; (vii) L-Selectride, THF, −30 to 0 °C, 91%.

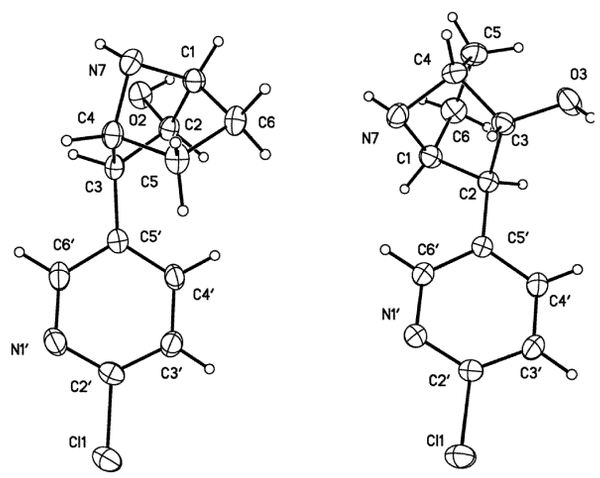
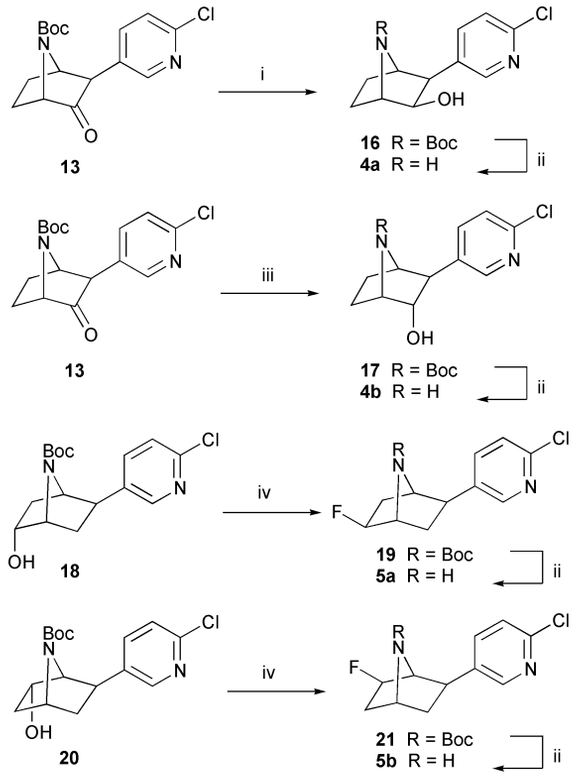


**Fig. 2** X-Ray structures of **11** (left) and **4b** (right).

2-*endo* ketone using standard protocols ($K_2CO_3$–MeOH, NaH–THF, and *p*-TsOH–toluene) afforded only the starting material or decomposition products. Gratifyingly, in a moment of serendipity, we found that treatment of **12** with silica gel at room temperature provided an effective means for its transformation into the 2-*exo*-epimer **13** in quantitative yield based on consumed **12** (Scheme 1). Further study showed that **13** and **12** could epimerize to each other on silica gel and reach a balance in a ratio of 1.7 : 1 (**13** : **12**) (Fig. 3). Reduction of **13** with the bulky reducing agent, L-Selectride, provided alcohol **16** in 92% yield. This reducing agent afforded alcohol **16** exclusively through hydride attack on the face opposite the pyridyl substituent. On the other hand, when **13** was reduced with $NaBH_4$, both the 3-*endo* alcohol **17** and the 3-*exo* alcohol **16** were isolated in a ratio of 3 : 1 as shown by the $^1$H NMR analysis of the crude products. Removal of the Boc protecting group in **16** and **17** provided the 3-*exo*-OH and 3-*endo*-OH epibatidine



**Scheme 2** *Reagents and conditions:* (i) L-Selectride, THF, −78 °C to rt, 92%; (ii) $CF_3COOH$, $CH_2Cl_2$; (iii) $NaBH_4$, THF, 95% (diastereoselectivity 3 : 1); (iv) DAST, $CH_2Cl_2$, −78 °C to rt, 83–87%.

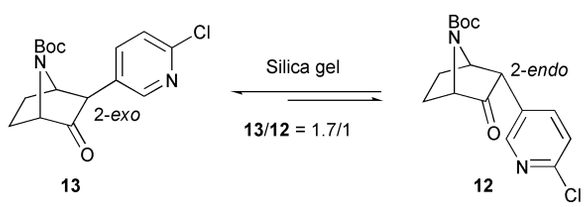


**Fig. 3**

analogues **4a** and **4b**, respectively (Scheme 2). Their stereochemistry was ascertained from their respective $^1$H NMR spectra and confirmed independently by X-ray analysis of **4b** (Fig. 2).[14]§ Two additional 3-hydroxyl-bearing epibatidine analogues containing the chloropyridyl group in the *endo* orientation, namely compounds **11** and **15**, were synthesized for comparison purposes (Scheme 1).

The 5-*exo*- and 6-*exo*-fluorinated epibatidine analogues **5a** and **5b** were readily prepared from compounds **18**[10] and **20**[10] by treatment with diethylaminosulfur trifluoride (DAST) followed by removal of the protecting group (Scheme 2). These two ligands were investigated, in particular, with the idea that if they possessed excellent receptor affinity, they could serve as PET ligands if appropriately labelled with $^{18}$F.

Competition binding assays were carried out to measure the binding affinities ($K_i$ values) of **11**, **15** and **2a**,**b**–**6a**,**b** at six defined rat nicotinic receptor subtypes using conditions previously reported.[15] The results are summarized in Table 1. As expected, **11** and **15** failed to show significant binding affinities to any of the receptor subtypes (data not shown), probably due to the orientation (*endo*) of the pyridine ring. Generally, the addition of extra functionality to the alicyclic skeleton of epibatidine reduced the nAChR binding affinities of the corresponding analogues in comparison with epibatidine (**1**). However, the electronic character and the ring position of the introduced groups, as well as their orientation on the alicyclic ring, resulted in considerable differences in their nAChR binding affinities. Introduction of a hydroxyl group at either the 3-*exo* or 5-*endo* position of epibatidine as in the

**Table 1** Binding affinities of (±)-epibatidine (**1**) and epibatidine analogues **2a**,**b**–**6a**,**b** to six nAChR subtypes[a]

| Ligand | Introduced group | $K_i$/nM[b] | | | | | |
|---|---|---|---|---|---|---|---|
| | | α2β2 | α2β4 | α3β2 | α3β4 | α4β2 | α4β4 |
| **1** | — | 0.025 | 0.095 | 0.035 | 0.565 | 0.061 | 0.157 |
| **4a** | 3-*exo*-OH | 552 ± 146 | 426 ± 102 | 1290 ± 160 | 915 ± 127 | 2320 ± 40 | 683 ± 91 |
| **4b** | 3-*endo*-OH | 1.92 ± 0.29 | 9.97 ± 2.67 | 3.91 ± 1.72 | 29.2 ± 6.7 | 3.56 ± 0.33 | 13.9 ± 1.7 |
| **2a** | 5-*exo*-OH | 19.7 ± 1.7 | 60.7 ± 5.4 | 24.3 ± 3.1 | 169 ± 34 | 28.6 ± 2.1 | 125 ± 27 |
| **2b** | 5-*endo*-OH | 71.9 ± 12.7 | 215 ± 17 | 169 ± 58 | 742 ± 88 | 78.7 ± 5.5 | 391 ± 77 |
| **3a** | 6-*exo*-OH | 5.78 ± 0.33 | 34.9 ± 4.1 | 13.5 ± 2.8 | 121 ± 15 | 13.7 ± 0.6 | 73.3 ± 20.4 |
| **3b** | 6-*endo*-OH | 1.27 ± 0.15 | 5.11 ± 0.79 | 1.92 ± 0.33 | 20.6 ± 5.5 | 1.44 ± 0.28 | 8.56 ± 2.35 |
| **5a** | 5-*exo*-F | **0.70 ± 0.19** | **5.29 ± 1.79** | **7.23 ± 6.53** | **11.5 ± 2.7** | **1.49 ± 0.21** | **6.38 ± 3.48** |
| **5b** | 6-*exo*-F | **0.18 ± 0.04** | **0.54 ± 0.18** | **0.25 ± 0.05** | **2.58 ± 0.77** | **0.45 ± 0.12** | **1.22 ± 0.66** |
| **6a** | 5-oxo | 971 ± 179 | 1230 ± 190 | 2260 ± 230 | 5190 ± 620 | 5710 ± 1160 | 2830 ± 390 |
| **6b** | 6-oxo | 1030 ± 90 | 3540 ± 260 | 1400 ± 100 | 8630 ± 1040 | 2050 ± 310 | 3510 ± 460 |

[a] $K_d$ values (nM) for [$^3$H]-epibatidine used for calculating $K_i$ values were 0.02 for α2β2, 0.08 for α2β4, 0.03 for α3β2, 0.30 for α3β4, 0.04 for α4β2 and 0.09 for α4β4 (ref. 15). [b] The $K_i$ values of epibatidine (**1**) were averages of several experiments. The $K_i$ values of **2a**,**b**–**6a**,**b** were means ± SEM of three independent measurements.

ligands **4a** or **2b** led to a drastic reduction of binding affinities at all six receptor subtypes. On the other hand, ligands **4b**, **2a**, **3a**, and **3b** which possess a 3-*endo*, 5-*exo*, 6-*exo*, and 6-*endo* hydroxyl group, respectively, exhibited higher nAChR binding affinities ($K_i$ values in the low nM range), although these $K_i$s are still lower than those of epibatidine (**1**). *Both the 5-exo and 6-exo fluorine-substituted epibatidine analogues **5a** and **5b** showed very high binding affinities.* For example, **5b** is only five to seven-fold less potent than epibatidine at all of the six subtypes. Interestingly, both 5- and 6-oxo epibatidine (**6a** and **6b**)[10] showed very low binding affinities to all of the receptor subtypes.

In summary, we have described a practical route to the 3-*exo*- and 3-*endo*-hydroxylated epibatidine analogues **4a** and **4b** using the silica gel catalyzed epimerization of the 2-*endo* ketone **12** as a key step. Competition binding assays of **2a**,**b**–**6a**,**b** at six defined rat nicotinic receptor subtypes revealed that the 3-*endo*, 5-*exo*, 6-*exo*, and 6-*endo* positions of the alicyclic skeleton of epibatidine can more readily tolerate an additional hydroxyl group than the 3-*exo* and 5-*endo* positions. Incorporation of a fluorine atom at the 5-*exo* or 6-*exo* position of epibatidine causes a smaller perturbation of the ligand–receptor complex than that occurring with the corresponding hydroxyl derivatives. Introduction of an oxo group at the 5- or 6-position of epibatidine leads to compounds of only micromolar affinity, a result that suggests a large, unfavorable electronic interaction with the receptor. In light of the high affinity found for the fluorinated analogues, efforts to explore these compounds in brain imaging studies (PET) are planned. The present structure–activity relationship study will also aid in constructing appropriate pharmacophore models of various nicotinic receptor subtypes through molecular modeling methods.

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§ CCDC reference numbers 216438 (**4b**) and 216439 (**11**). See http://www.rsc.org/suppdata/ob/b3/b308906a/ for crystallographic data in .cif or other electronic format.

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12 *Crystal data of* **11**: $C_{11}H_{13}ClN_2O$, $M$, = 224.68, orthorhombic, space group $P2_12_12_1$, $a$ = 9.595(1), $b$ = 9.689(1), $c$ = 11.318(1) Å, $V$ = 1052.17(14) Å$^3$, $T$ = 93(2) K, $Z$ = 4, $\mu$ = 0.336 mm$^{-1}$, $D_{calcd}$ = 1.418 g cm$^{-3}$, $\lambda$(MoKα) = 0.71073 Å, $F$(000) = 472, 8373 reflections collected, 2573 ($R_{int}$ = 0.0395) independent reflections, parameters = 142, goodness-of-fit = 1.036, final $R$ indices [$I$>2σ($I$)] $R$ = 0.0384, $R$w = 0.0928.

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