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Synthesis and nicotinic receptor activity of a hydroxylated tropane

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Abstract—(±)-3α-Hydroxy homoepibatidine **4** has been synthesized from the alkaloid scopolamine **5** and its properties as a nicotinic agonist assessed. While still binding strongly, the compound showed reduced agonist potency for the $\alpha_4\beta_2$ nAChR compared with the parent compound epibatidine **1**. Compound **4** also displayed generally similar binding and selectivity profiles at $\alpha_4\beta_2$, $\alpha_2\beta_4$, $\alpha_3\beta_4$, and $\alpha_4\beta_4$ nAChR subtypes to those for nicotine.

Nicotinic acetylcholine receptors (nAChRs) are widely distributed throughout the PNS and CNS. Efforts are now being made to synthesize ligands that exhibit selectivity for different nAChR subtypes and that also display selectivity for central over peripheral nAChRs. As the $\alpha_4\beta_2$ nAChR is one of the most abundant in the CNS, this has been a major target.[1] The powerful analgesic epibatidine **1** [(+)-enantiomer shown] has a high affinity for the $\alpha_4\beta_2$ nAChR and has therefore acted as a structural template for many nicotinic ligands produced in this field. Epibatidine is also a full agonist at other nAChR subtypes, resulting in high toxicity as a result of deleterious actions on CNS responses and respiratory, gastrointestinal and cardiovascular function.[2]

Epibatidine analogues have so far failed to yield a highly selective agonist,[3] and the search is complicated by the complex pharmacology of the nAChR subtypes and lack of a reliable pharmacophore.[4] One of the most promising analogues to date is a 3,8-diazabicyclo[3.2.1]octane **2**. It showed no life-threatening side effects in vivo (mouse) at antinociceptive doses, evidently due to selective binding to the $\alpha_4\beta_2$ receptor subtype, while lacking strong binding to neuromuscular junction nAChRs.[5–7] Another seven-membered ring analogue of epibatidine, homoepibatidine **3**, retains analgesic potency but also shows little selectivity (Fig. 1).[8,9] Most bicyclic analogues experiment with positioning of the pyridyl substituent, retaining an aliphatic region, thus

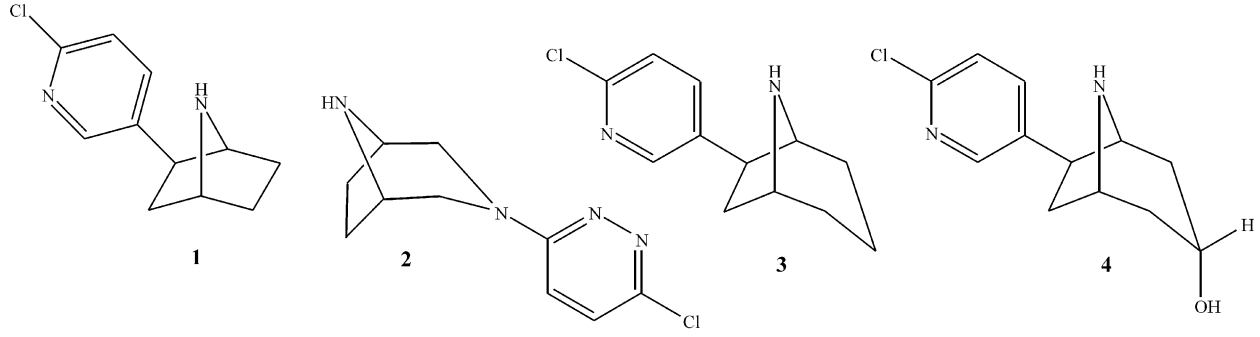

Figure 1. Chemical structure of epibatidine and analogues.

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incorporation of polar substituents in **3**, capable of both H-bond donation and acceptance, was of interest to explore the potential effects on receptor binding and selectivity.[10] To this end, the hydroxylated tropane derivative (±)-3α-hydroxy homoepibatidine **4** was synthesized from the tropane alkaloid scopolamine **5**, and receptor binding studies conducted. The results are discussed in this paper.

The synthesis of (±)-3α-hydroxy homoepibatidine **4** is summarized in Scheme 1. Scopolamine **5** was used as the starting material, as supplied by Phytex Australia. Zinc–copper reduction of **5** in ethanol gave 6,7-dehydrohyoscyamine **6** (96%),[11] followed by base hydrolysis to provide tropenol **7** (90%).[12] Protection of the hydroxy group as a TBDMS ether to give **8** (90%), followed by demethylation and protection of the nitrogen with ethyl chloroformate gave the carbamate **9** (58%). The reductive Heck coupling between the commercially available 2-chloro-5-iodopyridine and **9** was carried out in a solution of DMF containing tetrakis(triphenylphosphine)palladium(0), piperidine and formic acid.[9,10] The coupled product **10** was obtained in fair yield (49%). Finally, cleavage of both the carbamate and TBDMS ether with trimethylsilyl iodide afforded the target molecule **4** (45%).[13] The *exo* stereochemistry for the 2-chloropyridyl substituent was confirmed from the ^{1}H NMR spectrum with the coupling constants for the doublet of doublets signal being 9.3 and 5.4 Hz (H6 *endo*–H7 *endo* and H6 *endo*–H7 *exo*, respectively, with no coupling to H5); similar coupling constants were observed[14] in homoepibatidine **3**.

The pharmacological activity of **4** was evaluated in a [^{3}H]-epibatidine binding assay at native nAChRs and at recombinant nAChRs expressed in HEK293 or tsA cells.[15] Furthermore, the compound was characterized functionally at the $\alpha_3\beta_4$ nAChR in a FLIPR® Membrane Potential Assay using a NOVOstar™. The binding assays and functional assay were performed essentially as previously described.[15] Affinity and comparison data are shown in Table 1. At native nAChRs compound **4** had a lower binding affinity[8] than either enantiomer of homoepibatidine **3**. However, at these native receptors, analogue **4** showed a binding affinity similar to that of (*S*)-nicotine but 100-fold lower binding affinity than epibatidine. This was also true in the

Table 1. Binding affinities (K_i, nM) for **4** and standard nicotinic agonists in a [^{3}H]-epibatidine binding assay to native and recombinant nAChRs[a]

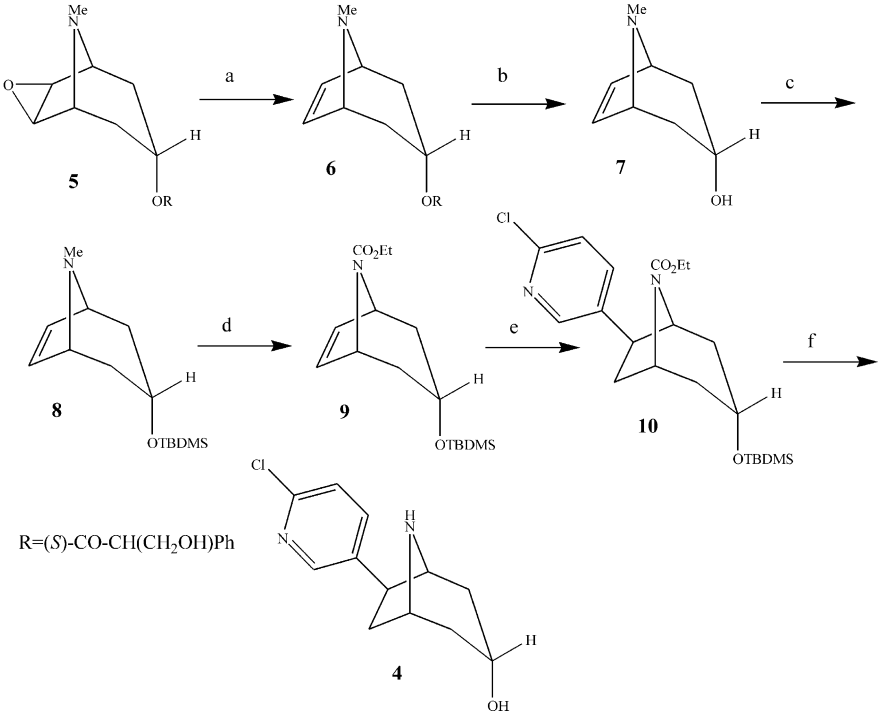
Compd	Rat brain tissue	$\alpha_4\beta_2$	$\alpha_2\beta_4$	$\alpha_3\beta_4$	$\alpha_4\beta_4$
Epibatidine	0.034	0.046	0.091	0.457	0.072
(*S*)-Nicotine	27	9.1	103	320	129
4	18	7.4	31	88	38

[a] Values are determined using the equation $K_i = IC_{50}/(1 + [L]/K_D)$, where [L] is the radioligand concentration, *n* the Hill coefficient, and K_D the dissociation constant and are the means of 3–4 independent assays.

Table 2. Agonistic potencies of **4** and standard nicotinic agonists at the $\alpha_3\beta_4$ nAChR in a FLIPR® membrane potential assay

Compd	EC_{50} (μM)[a]
Epibatidine	0.019
(*S*)-Nicotine	4.3
4	2.1

[a] Means of three independent assays.



Scheme 1. Synthesis of 3α-hydroxy homoepibatidine. Reagents and conditions: (a) Zn–Cu, EtOH; (b) NaOH, MeOH; (c) TBDMSTf, 2,6-lutidine, DCM; (d) $ClCO_2Et$, K_2CO_3, $CHCl_3$; (e) 2-chloro-5-iodopyridine, $Pd(PPh_3)_4$, HCO_2H, piperidine, DMF; (f) Me_3SiI, $CHCl_3$.

recombinant $\alpha_4\beta_2$ binding assay and the functional assay at the $\alpha_3\beta_4$ nAChR. Compound **4** displayed low nanomolar binding affinities at all recombinant nAChR subtypes studied and is an agonist at the $\alpha_3\beta_4$ nAChR (Table 2).

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