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Syntheses and Biological Properties of Cysteine-Reactive Epibatidine Derivatives

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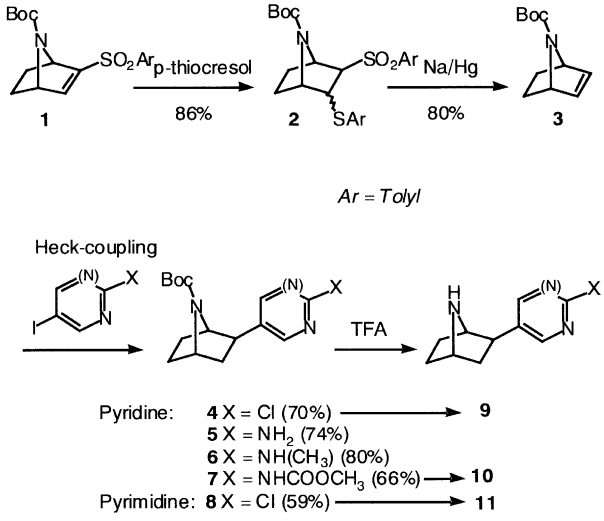
Abstract—The synthesis of epibatidine derivatives modified at the 2-position of the pyridine or pyrimidine rings by reactive functions are described for potential irreversible site-directed coupling reactions on cysteine mutants of neuronal nicotinic acetylcholine receptors. An improved synthesis of the 7-azabicyclo[2,2,1]hepta-2,5-diene key intermediate has been developed to allow reproducible syntheses of the epibatidine derivatives. Binding tests and electrophysiological experiments allowed to select the 2-substituted α-chloroacetamido **13** and the chloropyrimidine derivative **11** as potential site-directed probes for the epibatidine binding site.

Epibatidine, one of the most potent nicotinic agonist, has aroused considerable interest in therapeutic research because of its pronounced analgesic properties with a non-opioid mode of action.[1] The toxic side effects displayed by epibatidine prompted active structure–activity research, seeking for non-toxic derivatives and promoting different total syntheses of this alkaloid.[2] In the meantime, the recent publication of the 3-D structure of an acetylcholine binding protein (AChBP) from snail glial cells[3] has directed homology modeling of the extracellular portion of typical neuronal nicotinic receptors such as the α7 and the α4β2 subtypes.[4] These models enabled the docking of relevant nicotinic agonists including epibatidine. To validate these docking models we decided to apply the newly developed methodology of engineered site-directed coupling reactions using cysteine mutants.[5] This study required epibatidine-derived molecules to react with cysteines and therefore we undertook the syntheses of epibatidine derivatives modified at the pyridine ring of the molecule. This aim required an efficient synthetic method using mild final *N*-deprotection steps compatible with chemically reactive functions incorporated on our probes. We used a previously described method[6] which was improved in the production of the key intermediate **3**: *N*-Boc-7-azabicyclo[2,2,1]hept-2-ene (Scheme 1).



Scheme 1. Synthetic scheme for epibatidine-derived molecules.

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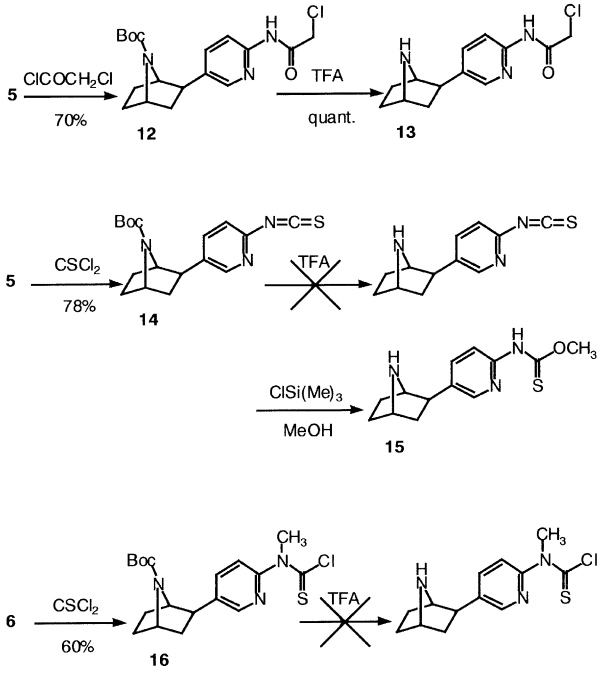
The synthesis of the epibatidine backbone used the described Diels–Alder approach[7] leading to a 7-azabicyclo[2,2,1]hepta-2,5-diene derivative which was selectively hydrogenated to compound **1**. The described direct conversion[6] of compound **1** into **3**, using sodium amalgam, occurred, in our hands with poor yields and reproducibility, as was also elsewhere reported.[8] The syntheses of the reactive epibatidine derivatives derived all from this key intermediate **3**, therefore an improved synthetic method was necessary. This was achieved in two steps by Michael addition of *p*-thiocresol to precursor **1**, forming the bicyclic 2-*p*-tolylsulfonyl-3-tolylsulfide derivative **2** which was in turn, in a reproducible manner, efficiently reduced by Na/Hg to the desired hept-2-ene derivative **3** in 69% overall yields. Gramme-scale synthesis of **3** is easily achievable using this modified procedure.[9] A similar two-steps transformation forming a 2-(*p*-tolylsulfonyl)-3-trimethylsilyl intermediate has been described to improve the synthesis of compound **3**.[8] A series of iodo-pyridine and-pyrimidine derivatives where coupled to this bicyclic intermediate using the described Heck methodology[6–8,10] which allowed us to synthesize the precursors of the reactive derivatives with satisfactory yields (Scheme 1: compounds **4**–**8**).

Removal of the *tert*-BOC protecting group was directly carried out on molecules **4**, **7** and **8** using standard acidic conditions (pure TFA or TFA in anhydrous CH_2Cl_2) leading to the quantitative formation of compounds **9**, **10** and **11**, respectively.

The syntheses of amino-derived reactive epibatidine compounds modified at the 2-position of the pyridine ring are outlined in Scheme 2. The *N*-protected derivatives: α-chloroacetamide **12**, isothiocyanate **14** or *N*-methyl-*N*-chlorothiocarbamoyl **16** were obtained from their amino pyridine precursors **5** or **6**. Deprotecting these reactive molecules to their bicyclic free amino counterpart was only successful for the α-chloroacetamide **12**, leading to compound **13**. Both isothiocyanate **14** and chlorothiocarbamoyl **16** were highly sensitive to the reaction conditions and did not permit the removal of the protecting group to occur satisfactorily. The use of milder reaction conditions ($ClSi(Me)_3$ in the presence of methanol),[10] fully converted the isothiocyanate to the thiocarbamate **15**. Obviously both the isothiocyanate and chlorothiocarbamoyl moieties were chemically too sensitive to be used in further biological experiments. Consequently, from this synthetic study, epibatidine **9**, its pyrimidine analogue **11**, carbamate **10** (or the corresponding thiocarbamate **15**) and the α-chloroacetamide derivative **13** will be tested further for biological evaluation.



Scheme 2. Syntheses of reactive epibatidine derivatives.

These molecules are intended to react with cysteine mutants incorporated by site-directed mutagenesis within the acetylcholine receptor-binding site. To evaluate such reactivity we used model reactions on *N*-acetyl-L-cysteine methyl ester in buffered medium, to be coupled to reactive pyridine-derived analogues. High concentrations are used for these bimolecular reactions[11] to mimic the situation occurring in receptor binding sites. Epibatidine **9** and the corresponding pyrimidine derivative **11** might undergo aromatic SN reactions even though, model reactions on chloro pyridine or chloro pyrimidine did not indicate the occurrence of such reactions. Similarly, no apparent reaction was noticeable on the carbamate **10** or thiocarbamate **15**. Despite this apparent lack of reactivity, these molecules will be tested on the mutant receptors knowing that the reactivity within a receptor-binding site could still be different from a bimolecular reaction occurring in buffer. Alternatively, α-chloroacetamido pyridine displayed the expected reactivity towards cysteine derivatives (half-life in the presence of excess Cys: 85 min)[12] indicative of an excellent potentiality for the corresponding probe **13**.

Biological Results

Binding studies on rat brain membranes and HEK 293 cells expressing the $\alpha7/5HT_3$ chimera, are given in Table 1, while Figure 1 shows the electrophysiological results on recombinant α7, α4β2 and α3β4 rat nicotinic receptors expressed in oocytes. Maximal responses are given for each compound, comparatively to epibatidine and acetylcholine, respectively.

Table 1. K_i and K_p values for compounds **9**, **10**, **11** and **13** in the [^{3}H]-cytisine[13] and [^{125}I]-αBgTx[14] binding assays using rat brain membranes (α4β2) and transfected $\alpha7\text{-}5HT_3$ HEK cells (α7), respectively

Compd[15]	α4β2, K_i (nM) [^{3}H]-cytisine	α7, Kp (nM) [^{125}I]-αBgTx
9	0.18	75
10	8100	$>10^6$
11	0.31	760
13	16.3	23,000

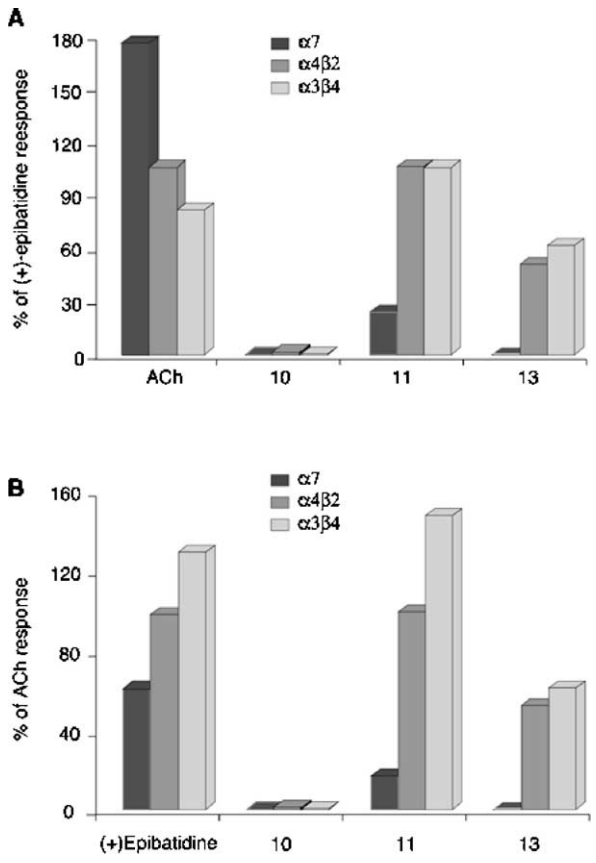


Figure 1. Fraction of current evoked by epibatidine derivatives. Ratio of peak currents obtained at α7, α4β2 and α3β4 subtypes[16] for three epibatidine derivatives. Currents evoked by brief pulses of epibatidine (2 μM, 3 s) or ACh (1 mM, 3 s) were compared with those evoked by the tested compounds (2 μM, 3 s). The α7 receptors were challenged with 1.6 mM ACh.

Conclusion

To challenge the docking model of epibatidine in neuronal nicotinic receptors, we synthesized cysteine-reactive epibatidine analogues for potential site-directed labeling reactions with mutant cysteine receptors. A reliable synthesis of the 7-azabicyclo[2,2,1]hepat-2;5-diene key intermediate has been developed to improve the syntheses. The chemical modification introduced at the 2-position of the pyridine ring of epibatidine had marked effects on the biological responses. The methylcarbamate derivative **10** became totally inactive on all receptors tested. This complete loss of affinity is somehow surprising when compared to the isosteric α-chloroacetamido **13** derivative, which displayed interesting properties. This molecule retained sufficient affinity on the α4β2 subtype while remaining a partial agonist on both α4β2 and α3β4 subtypes. According to its chemical reactivity, this molecule represents therefore a promising site directed probe for the cysteine mutants on the heteromeric receptor subtypes. The chloropyrimidine derivative (molecule **11**) displays interesting binding and functional properties. While remaining a high affinity ligand as well as a full agonist on both α4β2 and α3β4 subtypes, it displays an excellent α4β2/α7 and α3β4/α7 selectivity. Provided that this molecule reacts with the receptor cysteine mutants, this probe **11** would represent an interesting tool to validate the epibatidine docking model.[3]

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9. Synthesis of compounds **2** and **3**: a solution of compound **1** (5 g, 14.3 mmol), *p*-thiocresol (4.9 g, 42.9 mmol), and triethylamine (5.96 mL, 42.9 mmol) in dry THF (180 mL), was stirred at room temperature for 2 h. The reaction mixture was quenched with a solution of sodium hydrogenocarbonate (50 mL). Extraction with EtOAc (3×50 mL) followed by chromatography (EtOAc/heptane, 2/8) afforded **2** (5.86 g, 86%) as white powder; mp 140–142 °C. ^{1}H NMR (300 MHz, $CDCl_3$) 7.80 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 7.22 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 4.56 (m, 1H), 4.23 (m, 1H), 3.55 (brs, 1H), 3.37 (m, 1H), 2.46 (s, 3H), 2.34 (s, 3H), 1.94–1.62 (m, 4H), 1.45 (brs, 9H) ; MS (ES) *m/e* 474 [M + H$^+$]. To a solution of **2** (5.75 g, 12.14 mmol) in dry THF (360 mL), and MeOH (180 mL) was added successively 16 g (41.36 mmol) and after 1 h, 25 g (64.66 mmol) of 6% Na/Hg with vigorous stirring. After 4–5 h, the mixture was quenched with brine (200 mL), extracted with CH_2Cl_2 (5×150 mL) and pentane (3×150 mL). Removal of solvent and chromatography with neutral alumina (CH_2Cl_2/pentane, 1/1) afforded **3** (1.90 g, 80%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$), 6.22 (s, 2H), 4.66 (s, 2H), 1.84 (m, 2H), 1.42 (brs, 9H), 1.09 (m, 2H); MS (ES) *m/e* 196 [M + H$^+$].
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achieved on a membrane preparation of transfected α7-5HT$_3$/HEK cells (Corringer, P.-J.; Galzi, J.-L.; Eiselé, J.-L.; Bertrand, S.; Changeux, J.-P.; Bertrand, D. *J. Biol. Chem.* **1995**, *270*, 11749) according to *Torpedo* nicotinic receptors procedure (Grutter, T.; Goeldner, M.; Kotzyba-Hibert, F. *Biochemistry* **1999**, *38*, 7476).

15. **10**: ^{1}H NMR (300 MHz, $CDCl_3$) 9.32 (s, NH), 8.24 (s, 1H), 7.92 (d, $J=8.7$ Hz, 1H), 7.71 (m, 1H), 3.82 (brs, 4H), 3.58 (s, 1H), 2.79 (m, 1H), 2.05–1.41 (m, 6H); MS (ES) *m*/*e* 248 [M + H$^+$]. **11**: ^{1}H NMR (200 MHz, $CDCl_3$) 8.64 (s, 2H), 3.81 (brs, 1H), 3.55 (s, 1H), 2.69 (dd, $J=4.7$ and 9.0 Hz, 1H), 1.90 (dd, $J=9.0$ and 12.2 Hz, 1H), 1.76–1.48 (m, 5H); ^{13}C NMR (200 MHz, $CDCl_3$) 159.2, 139.1, 134.3, 62.9, 56.7, 43.0, 40.5, 31.7, 30.9; MS (ES) *m*/*e* 210/212 [M + H$^+$]. **13**: ^{1}H NMR (300 MHz, D_2O) 8.44 (s, 1H), 8.37 (dd, $J=2.0$ and 8.7 Hz, 1H), 7.77 (d, 8.7, 1H), 4.56 (s, 1H), 4.53 (s, 2H), 3.69–3.62 (m, 2H), 2.60 (dd, $J=9.4$ and 13.6 Hz, 1H), 2.31–1.96 (m, 5H); ^{13}C NMR (300 MHz, D_2O) 163.6, 157.1, 145.0, 138.6, 138.3, 117.3, 62.9, 59.7, 46.0, 42.2, 35.7, 27.7, 26.0; MS (ES) *m*/*e* 266/268 [M + H$^+$].

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