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Synthesis and pharmacological characterization of bivalent ligands of epibatidine at neuronal nicotinic acetylcholine receptors

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Abstract—A series of bivalent ligands **6a**–**d** of epibatidine were synthesized. All four ligands showed nanomolar binding affinities at six neuronal nicotinic acetylcholine receptor (nAChR) subtypes in competition binding assays. In contrast to epibatidine, these bivalent ligands are weak partial agonists at the α3β4 nAChR as shown by functional assays.

The nicotinic acetylcholine receptors (nAChRs) belong to a superfamily of ligand-gated ion channels, which includes muscle-type and neuronal-type nAChRs, 5-hydroxytryptamine receptors (5-HT_3), γ-aminobutyric acid receptors ($GABA_A$ and $GABA_C$), glycine receptors, and invertebrate glutamate and histidine receptors.[1] In mammalian and avian tissues, 5 muscle-type nAChR subunits (α1, β1, γ, ε, and δ) and 12 neuronal nAChR subunits (α2–α10 and β2–β4) have been found. Several combinations of α and β subunits, and in the case of α7–α9, the α subunits alone, can be expressed in oocytes or other heterologous expression systems resulting in functional ion channels with distinct biophysical, physiological, and pharmacological properties.[1,2] Neuronal nAChRs hold considerable promise as therapeutic targets for the treatment of disorders of the central nervous system. Drugs aimed at nAChRs have potential for the treatment of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, schizophrenia, Tourette's syndrome, and certain epilepsies, as well as nicotine addiction.[3] nAChRs are allosteric, and contain multiple agonist-binding sites, noncompetitive-antagonist sites, and gates that interact at a distance through changes in the quaternary structure of the receptor.[1,3] Site-directed mutagenesis has shown that the binding sites on the nAChR are located at the interface between the α and β subunits in heteropentameric receptors and between the α subunits in homopentameric receptors. Recently, crystal structure analysis of the molluscan ACh-binding protein was solved by Brejc et al,[4] and the extracellular domains of some nAChRs were homologously modeled by Changeux et al.[5]

The novel alkaloid epibatidine (**2**), originally isolated from the skin of the Ecuadorian poison frog, *Epipedobates tricolor*,[6] has been shown to possess powerful analgesic activity and high binding affinity to nicotinic

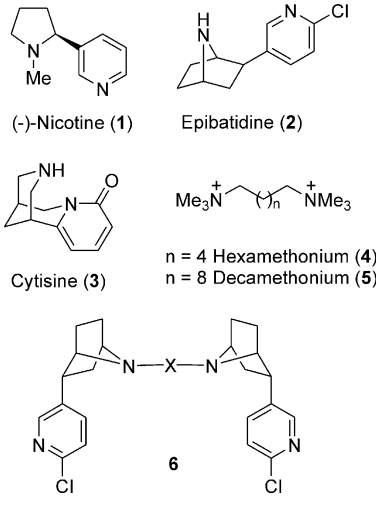

Figure 1.

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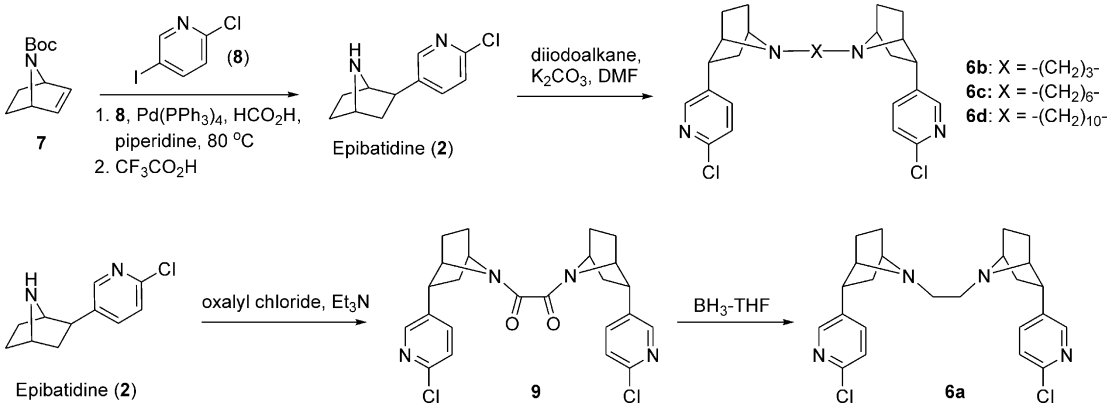
acetylcholine receptors.[7] Epibatidine is the most powerful natural nicotinic agonist known. Interestingly, both the (+)- and (−)-forms of this molecule possess similar biological activity.[6b] Epibatidine has become an important research tool for the study of nicotinic receptors, and it has served as an attractive lead in the design of new ligands selective for distinct nAChR subtypes. Studies aimed at probing the SAR of this molecule have largely focused on modification of its heteroaryl group,[8] alterations in the position of the aliphatic ring nitrogen,[9] and expansion of the two-carbon bridge.[10] Recently, we described the synthesis and binding activity of epibatidine analogues in which we attached certain heteroatom groups onto the azanorbornane core in an effort to achieve nAChR subtype selectivity.[11] Moreover, we and other groups have also reported on the structure/affinity relationships of some conformationally constrained analogues of epibatidine.[12] However, few modifications of the nitrogen atom of the bicycle have been made.[13] It is well known that the nAChR is quite sensitive to changes in the nature of the protonatable nitrogen of the agonist.[14] For example, *N*-methylation of (−)-cytisine (**3**) results in decreased affinity, while in some cases, introduction of an *N*-methyl group increases affinity (as with nornicotine versus nicotine). However, *N*-methylation of (−)-epibatidine has little effect on affinity.[13] Accordingly, we felt that it would be of interest to examine the activity of certain bivalent ligands of epibatidine. We hypothesized that such bivalent ligands might show unusual subtype selectivity and/or interesting functional activity, as the presence of the second epibatidine unit could allow for additional ligand–receptor interactions with accessory binding sites or with the ion channel itself. It is well known that the bis-quaternary ammonium ligand, hexamethonium (**4**), is a potent noncompetitive ganglionic nAChRs antagonist. In the case of ligand **4**, elongation of the carbon linker between the ammonium head groups as in the case of decamethonium (**5**) leads to the formation of a competitive antagonist for muscle-type nAChRs.[2] Similarly, a series of alkane-1,ω-diguanidinium compounds have been found to act as subtype selective blockers in their interaction with neuronal nAChRs.[15] Quite recently, Sparatore et al.[16] studied some bivalent cytisine analogues and based upon their results suggested that the second cytisine unit is likely to interact with some additional sites at or near the acetylcholine binding pocket. Additionally, Trudell et al.[17] recently synthesized some ethylene glycol bis(tropane-3-carboxylates) as potential nAChR ligands. Herein, we report our preliminary results pertaining to the synthesis and pharmacological characterization of some bivalent ligands **6** of epibatidine (Fig. 1).

The synthesis of our carbon-linked bivalent ligands **6a–d** of epibatidine is shown in Scheme 1. Compounds **6b–d** with 3, 6, and 10 methylene groups, respectively, in their linker were readily prepared by treatment of racemic epibatidine (**2**) with the corresponding 1,ω-diiodoalkane. Epibatidine (**2**) was readily prepared from olefin **7**[8g] by reductive Heck reaction with 2-chloro-5-iodopyridine (**8**),[18] followed by removal of the Boc protecting group. The two-carbon-linked epibatidine dimer **6a** was synthesized by treatment of **2** with one half equivalent of oxalyl chloride to provide the oxamide **9**, followed by reduction with an excess of borane.[19]

Competition binding assays were carried out to measure the binding affinities (K_i values) of **6a–d** at six defined rat nicotinic receptor subtypes using conditions previously reported.[12a] The results are summarized in Table 1. As is readily apparent, linkage of two epibatidine molecules through the amine nitrogen brings about a 50- to 200-fold decrease in affinity at the six nAChR subtypes in comparison to epibatidine (**2**). The observed reduction in the binding affinities of the epibatidine dimers **6a–d** is similar to that found for *N*-ethylepibatidine,[13] which shows an about 500-fold decreased affinity at the α4β2 subtype. In contrast, the *N*-methylation of epibatidine has little effect on affinity. While our results further confirm the observation that attachment of a group larger than methyl to the amine nitrogen reduces binding affinity, all ligands still show low nM affinity. These results are to be contrasted with the data reported for the cytisine dimers.[16] The cytisine dimers possessing a linker of 3–4 methylene groups displayed good affinity (K_i = 25–30 nM), while those with two or six methylene groups displayed reduced affinity, with K_i = 96 and 113 nM, respectively; further chain lengthening to 10–16 methylene groups resulted in a strong reduction in binding affinity (K_i = 1040–1230 nM).[16a]

Lastly, we have begun to examine the functional properties of these bivalent ligands of epibatidine. In



Scheme 1.

Table 1. Binding affinities of (−)-nicotine (**1**), (±)-epibatidine (**2**) and four bivalent ligands **6a–d** of epibatidine to six nAChR subtypes[a]

Compds	K_i (nM)					
	α2β2	α2β4	α3β2	α3β4	α4β2	α4β4
Nicotine (**1**)	12±2	112±21	47±11	443±60	10±2	40±6
Epibatidine (**2**)	0.025±0.001	0.095±0.017	0.035±0.011	0.565±0.121	0.061±0.009	0.157±0.006
6a	2.08±0.4	26.8±12.1	5.44±0.31	47.8±18.1	9.33±0.69	27.7±6.3
6b	5.53±0.93	57.3±20.8	8.99±1.93	112±26	15.7±3.6	54.2±17.1
6c	3.13±0.65	47.8±5.2	6.19±0.35	82.2±9.9	9.05±0.99	40.4±5.7
6d	7.51±1.12	71.7±24.1	12.6±2.9	138±35	21.6±6.1	61.3±21.4

[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 (Xiao and Kellar, 2003, submitted for publication). The K_i values of (−)-nicotine (**1**) and epibatidine (**2**) shown are the mean±SEM of 3 to 6 independent measurements. The K_i values of **6a–d** shown are the mean±SEM of 3 independent measurements.

contrast to epibatidine,[20] the dimers are weak partial agonists at the α3β4 nAChR subtype. Their EC_{50} values range from 6.8 μM to 24 μM. Their E_{max} range from 25% to 60% of that of epibatidine. In the functional assay, ligand **6b** has the lowest potency and efficacy among the four bivalent ligands.

In summary, we have described the synthesis of a series of epibatidine bivalent ligands **6a–d** linked by an oligomethylene chain of varying length attached to the ring nitrogen. Competition binding assays reveal that these four new ligands possess similar low nanomolar affinities at each of the six nAChR subtypes tested. As these ligands show weak, partial agonist properties at the α3β4 nAChR subtype, we believe that further elaboration of this compound class is warranted, as they may prove to be less toxic than the parent structure from which they are derived. Such ligands may therefore be of possible therapeutic value. Based upon these results, we are encouraged to examine the activity of such bivalent ligands that have been prepared in optically pure form.

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