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Neuronal Nicotinic Acetylcholine Receptor Binding Affinities of Boron-Containing Nicotine Analogues

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Abstract—A series of boron-containing nicotine (NIC) analogues **7**–**9** was synthesized and evaluated for binding to α4β2 and α7 nicotinic receptors. Compound ACME-B inhibited [^{3}H]methyllycaconitine binding to rat brain membranes with a similar potency compared to NIC ($K_i = 2.4$ and 0.77 μM, respectively), but was markedly less potent in inhibiting [^{3}H]NIC binding when compared to NIC ($K_i = 0.60$ μM and 1.0 nM, respectively). Thus, tethering a two-carbon bridge between the 2-pyridyl and 3′-pyrrolidino carbons of NIC or **7** affords analogues that bind to the α7 receptor in a manner similar to NIC, but with a dramatic loss of affinity for the α4β2 receptor.

Over the last 12 years, there has been a substantial increase in the number of studies on neuronal nicotinic receptors (nAChRs).[1–5] nAChRs are composed of two types of subunits (α and β), and are believed to assemble as pentameric receptor cation channel complexes with a general stoichiometry of 2α and 3β, or 5α subunit in the case of α7, α8, α9, and α10.[6–10] Presently, nine α (α2–α10) and three β (β2–β4) subunits have been isolated and cloned.[11–13] Numerous combinations of α and β subunits can be expressed in *Xenopus* oocytes or other cell expression systems, resulting in functional receptors with diverse pharmacological properties.[11,14,15]

It is generally accepted that the predominant nAChR in the CNS that binds [^{3}H]NIC with high affinity ($K_d = 0.5$–5 nM) is composed of α4 and β2 subunits (α4β2).[14–20] This receptor subtype contains two ligand binding domains located at the interface of each αβ subunit.[21] The α4β2 subtype has been implicated as important in cognition, neurodegeneration, pain, anxiety, and depression.[2] A second predominant nicotinic receptor subtype contains protein encoded by the α7 subunit gene and represents the major α-bungarotoxin binding site in the CNS.[22–25] The α7 receptor subtype has been implicated as important in nicotine-induced improvement of learning and memory, and nicotine-induced slowing of neuronal degeneration, as may occur in aging, dementia, and neurodegenerative diseases.[26] Activation of α7 receptors may also be a beneficial therapeutic approach for the treatment of schizophrenia.[27]

Syntheses have recently been reported for an extensive series of boron-containing compounds,[28] which offer exciting new possibilities in drug development due to their unique structure and interesting biological activities. The amine-carboxyboranes and their derivatives are regarded as being isoelectronic and isosteric boron analogues of amino acids. Interest is increasing regarding the synthesis of such compounds because of their antitumor, antiarthritic, and hypocholesteremic activity.[29–31] For example, pyridine-carboxyborane has been shown to inhibit tumor growth in mice.[32] As a result of the interest in the biological activity of new borane adducts, and as part of our continuing efforts to develop subtype-selective nAChR ligands, we undertook a study to synthesize boron-containing nicotine analogues. This paper reports for the first time the synthesis, characterization, and evaluation of a number of nicotine-borane adducts and their interaction with nAChRs.

Compounds **3**–**6** (Fig. 1) were prepared by modifications of reported literature methods.[33–35] Boron-containing analogues **7**, **8**, and **9** were synthesized by refluxing a

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suspension of $NaBH_3CN$ and hydrochloride salts of the corresponding precursors (**1**, **5**, and **6**) in THF under N_2 overnight. The complexes were purified by column chromatography over silica gel and were then converted to hydrobromide or hydrochloride salts. All three compounds were characterized by elemental analyses, and IR, ^1H, ^{11}B, and ^{13}C NMR spectroscopy.[36] The IR spectra exhibited characteristic absorptions for B–H and C≡N groups. The ^1H, ^{11}B, and ^{13}C NMR spectral data were consistent with the proposed structures of these compounds. X-ray crystallographic analysis unequivocally confirmed the structure of **8** (Fig. 2).[37]

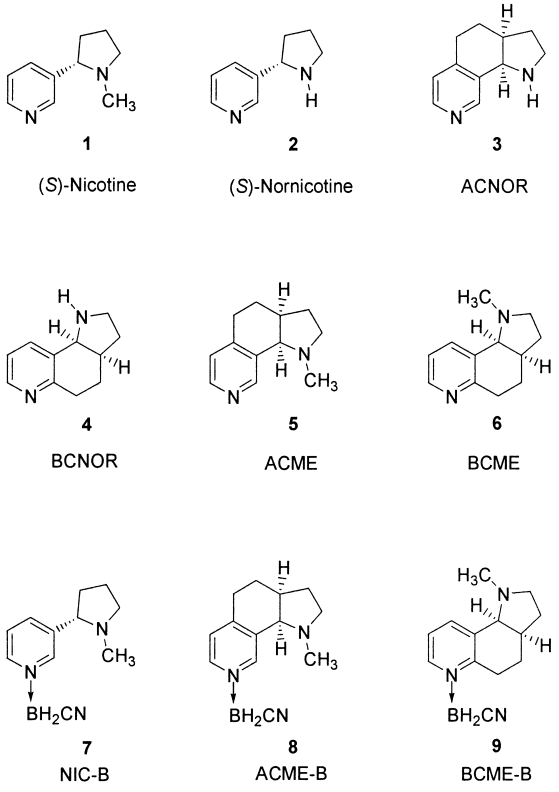

Figure 1. Structures of (*S*)-nicotine, (*S*)-nornicotine, bridged nicotine analogues and boron-containing nicotine analogues.

All three boron-containing analogues were found to be very stable in water. Hydrolytic stability at pH 7 is critical for biological testing. No decomposition was observed after a 0.1 M solution of **7** in D_2O was kept at room temperature for 8 days, as indicated by ^1H NMR. Hydrolysis of **7** also occurred very slowly in alkali. However, significant decomposition (8%) was observed when **7** was exposed to 1 N HCl for 1 week.

The borane-containing analogues **7**–**9** were evaluated as their hydrobromide or hydrochloride salts for their binding affinities for α4β2 and α7 nAChRs. Methyllycaconitine (MLA), alpha bungarotoxin (BTX), NIC (**1**), nornicotine (**2**), and compounds **3**–**6** were also tested for comparison. The affinity of the analogues for the α4β2 receptor subtype was assayed by analogue-induced inhibition of [^{3}H]NIC binding to rat striatal membranes.[38] The affinity of the analogues for the α7 receptor subtype was assayed by inhibition of [^{3}H]MLA binding to rat brain membranes.[39] Results are reported as K_i values (Table 1).

Both MLA and BTX bind with very high affinity to the α7 receptor ($K_i = 0.0023$ and 0.0072 μM, respectively), and MLA also exhibited low affinity for the α4β2 receptor ($K_i = 1.56$ μM). In contrast, NIC showed about 100-fold higher affinity for the α4β2 receptor than for the α7 receptor.

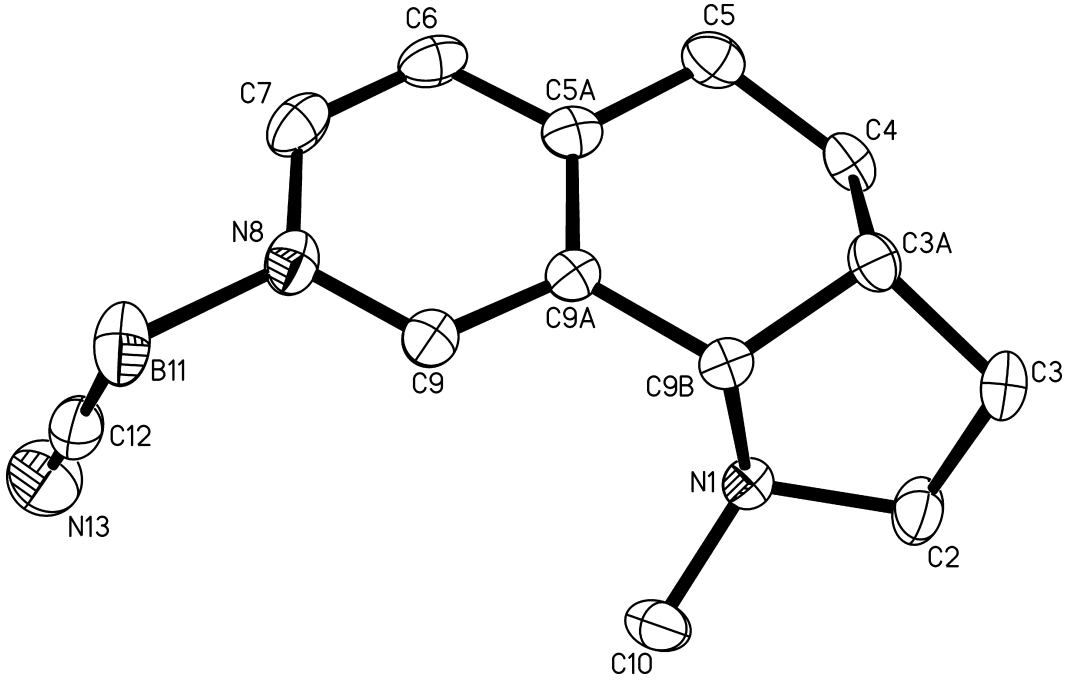

Figure 2. X-ray structure of compound **8** with atomic numbering scheme.

Table 1. K_i values for MLA, αBTX and compounds **1**–**9** in the [^{3}H]MLA and [^{3}H]NIC binding assays[a]

Compounds	K_i [^{3}H]MLA binding assay (μM)	K_i [^{3}H]NIC bnding assay (μM)
MLA	0.0023	1.56
αBTX	0.0072	>10
1	0.77	0.001
2	1.34	0.047
3	20.2	0.22
4	>100	10.5
5	0.59	0.40
6	>100	12.2
7	15.2	0.041
8	2.4	0.60
9	>100	>100

[a]N = at least three independent determinations using triplicate nine-point inhibition curves.

NIC is a conformationally flexible molecule. The conformational freedom mainly arises from the rotation of the pyrrolidine ring relative to the pyridine ring about the C_3–$C_{2'}$ bond. Compounds **5** and **6** represent the *syn*- and *anti*-rotamers of NIC, respectively. Similarly, compounds **3** and **4** can be viewed as conformationally-restricted analogues of nornicotine. Thus, compounds **8** and **9** can be regarded as rigid analogues of **7**. Interestingly, all the *anti*-rotameric analogues **4**, **6**, and **9** failed to inhibit [^{3}H]MLA binding at concentrations >100 μM. These three compounds also bind with low affinity (>10 μM) to the α4β2 receptor (Table 1). The *syn*-rotameric analogue ACME (**5**) inhibited [^{3}H]MLA binding with a similar potency to NIC (K_i = 0.59 and 0.77 μM, respectively) and was markedly less potent in inhibiting [^{3}H]NIC binding than was NIC (K_i = 0.4 μM and 1.0 nM, respectively). Thus, tethering a two-carbon bridge between the 2-pyridyl carbon and 3′-pyrrolidino carbon of NIC results in comparable binding affinity for the α7 receptor, but a dramatic loss in affinity for the α4β2 receptor. *N*-Demethylation of ACME to ACNOR reduced affinity for the α_7 receptor by 33-fold, but the affinity for the α4β2 receptor was retained. The NIC–borane complex **7** binds with 20-fold lower affinity than NIC at the α7 receptor (K_i = 15.2 μM) and with 40-fold lower affinity at the α4β2 receptor (K_i = 41 nM). However, the *syn*-rotamer ACME-B (**8**) had an affinity (K_i = 2.4 μM) 7-fold higher than **7** at the α7 receptor subtype, and was 15-fold less potent (K_i = 0.60 μM) than **7** at the α4β2 receptor. The results suggest that restricting the rotation of the C_3–$C_{2'}$ bond of NIC-like molecules to the *syn* conformation affords analogues that interact with the α7 receptor in a manner similar to NIC, but in contrast, disrupts the interaction with the α4β2 receptor. In the *syn*-rotameric analogues, NIC was locked into a conformation that retains its activity and interaction with the α7 receptor, indicating that the *syn* conformation is likely to be that responsible for interaction of nicotine with this receptor subtype. In addition, in the *syn*-rotamer series, loss of the pyridino-nitrogen lone pair, as a consequence of boron complexation, does not appear to greatly affect α7 receptor affinity (compare compounds **5** and **8**, K_i = 0.59 μM and 2.4 μM, respectively), and both **5** and **8** have comparable affinities to *S*-(−)-nicotine (K_i = 0.77 μM). Thus, the crucial interaction of the pyridino-nitrogen lone pair of nicotine and nicotine analogues with a hydrogen donor moiety at the α4β2 binding site[40] does not appear to be a structural requirement in the binding of nicotine and the nicotine analogues **5** and **8** at the α7 receptor. These conformationally restrained analogues can be used to study the structural requirements for binding to different nAChRs and to determine the pharmacophore characteristics of specific nicotinic receptor ligands.

Acknowledgements

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36. **7** (hydrobromide salt): mp 150–151 °C; IR (KBr) 2422 (BH), 2200 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.25 (1H, br s), 8.91 (1H, s), 8.80 (1H, d, J = 5.7 Hz), 8.65 (1H, m), 8.04 (1H, dd, J = 8.1, 5.7 Hz), 4.69 (1H, m), 3.81 (1H, m), 3.25 (1H, m), 2.75 (3H, d, J = 4.2 Hz), 2.10–2.70 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.99, 147.61, 142.68, 132.72, 127.35, 67.83, 55.75, 38.48, 30.66, 21.47; ^{11}B NMR (64 MHz, DMSO-d_6) δ −15.93. Anal. calcd for $C_{11}H_{17}BBrN_3$: C, 46.85; H, 6.08; N, 14.90. Found: C, 46.77; H, 6.11; N, 14.79. **8**: (hydrobromide salt): mp 202–204 °C; IR (KBr) 2426, 2411 (BH), 2206 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.11 (1H, br s), 8.34 (1H, s), 8.64 (1H, d, J = 6.0 Hz), 7.83 (1H, d, J = 6.0 Hz), 4.75 (1H, t, J = 7.5 Hz), 3.64 (1H, m), 3.25 (1H, m), 3.13 (1H, m), 2.92 (3H, s), 2.70–3.00 (2H, m), 2.38 (1H, m), 1.89 (2H, m), 1.75 (1H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.62, 148.34, 146.92, 128.40, 127.15, 63.71, 54.09, 39.02, 34.41, 27.58, 26.33, 24.22; ^{11}B NMR (64 MHz, DMSO-d_6) δ −16.02. Anal. calcd for $C_{13}H_{19}BBrN_3$: C, 50.69; H, 6.22; N, 13.64. Found: C, 50.55; H, 6.22; N, 13.48. **9**: (hydrochloride salt): mp 178–179 °C; IR (KBr) 2431 (BH), 2221, 2194 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (1H, br s), 8.86 (1H, d, J = 5.7 Hz), 8.47 (1H, d, J = 7.8 Hz), 7.80 (1H, dd, J = 7.8, 6.0 Hz), 4.68 (1H, t, J = 7.5 Hz), 3.69 (1H, m), 3.05–3.35 (3H, m), 2.93 (3H, d, J = 4.2 Hz), 2.87 (1H, m), 2.36 (1H, m), 1.90 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.56, 149.31, 144.92, 128.32, 123.96, 65.81, 53.71, 39.04, 34.31, 27.46, 26.72, 23.76; ^{11}B NMR (64 MHz, DMSO-d_6) δ −17.94. Anal. calcd for $C_{13}H_{19}BClN_3$: C, 59.24; H, 7.27; N, 15.94. Found: C, 59.41; H, 7.39; N, 15.98.
37. Crystals were obtained by slow evaporation from an isopropanol solution. A crystal with approximate dimensions of 0.35×0.12×0.07 mm was used for data collection on a Nonius Kappa CCD diffractometer equipped with Mo$K\alpha$ radiation and a graphite monochromator. A total of 5174 unique reflections were measured within the range $-9 \le h \le 9$, $-15 \le k \le 15$, $-17 \le l \le 17$. The structure was solved by direct methods using SHELX program (Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1990**, *A46*, 467) and refined using full-matrix least-squares fit on F^2. Crystallographic data: unit cell parameters a = 7.878(1) Å, α = 76.98(1)°, b = 12.702(2) Å, β = 87.99(1)°, c = 15.069(2) Å, γ = 88.67 (1)°; formula $C_{13}H_{19}BBrN_3$; formula weight 308.03 g/mol; radiation wavelength 0.71073 Å; volume 1468.0(4) Å^3; Z = 4; calculated density 1.394 g/cm^3; space group triclinic $P\bar{1}$; temperature 173(1) K. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary material.
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