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bis-Azaaromatic Quaternary Ammonium Analogues: Ligands for α4β2* and α7* Subtypes of Neuronal Nicotinic Receptors

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Abstract—A series of bis-nicotinium, bis-pyridinium, bis-picolinium, bis-quinolinium and bis-isoquinolinium compounds was evaluated for their binding affinity at nicotinic acetylcholine receptors (nAChRs) using rat brain membranes. *N*,*N*′-Decane-1,12-diyl-bis-nicotinium diiodide (bNDI) exhibited the highest affinity for [^{3}H]nicotine binding sites ($K_i = 330$ nM), but did not inhibit [^{3}H]methyllycaconitine binding ($K_i > 100$ μM), indicative of an interaction with α4β2*, but not α7* receptor subtypes, respectively. Also, bNDI inhibited ($IC_{50} = 3.76$ μM) nicotine-evoked $^{86}Rb^+$ efflux from rat thalamic synaptosomes, indicating antagonist activity at α4β2* nAChRs. *N*,*N*′-Dodecane-1,12-diyl-bis-quinolinium dibromide (bQDDB) exhibited highest affinity for [^{3}H]methyllycaconitine binding sites ($K_i = 1.61$ μM), but did not inhibit [^{3}H]nicotine binding ($K_i > 100$ μM), demonstrating an interaction with α7*, but not α4β2* nAChRs. Thus, variation of *N*-*n*-alkyl chain length together with structural modification of the azaaromatic quaternary ammonium moiety afforded selective antagonists for the α4β2* nAChR subtype, as well as ligands with selectivity at α7* nAChRs.

S(−)-Nicotine (NIC; Fig. 1) produces many effects following stimulation of neuronal nicotinic acetylcholine receptors (nAChRs), including stimulant effects, analgesia, anxiolytic activity, and enhancement of learning and memory. Nicotine and compounds acting at nAChRs have been suggested as potential therapeutic agents for several pathological conditions, including Parkinson's disease, Tourette's syndrome and Alzheimer's disease.[1–5] nAChRs are pentameric ligand-gated ion channels, composed of two varieties of subunits, α and β.[6,7] To date, ten α (α1–α10) and four β (β1–β4) subunits have been identified.[8] nAChRs have the general stoichiometry of 2α and 3β subunits that constitute the ion channel.[9] Expression of these nAChRs in *Xenopus* oocytes has shown that association of α2, α3, and α4 subunits with β2 and β4 subunits forms functional heterologous receptor channels, whereas α7, α8, and α9 subunits assemble to form homologous receptor channels.[10–12] Differences in subunit composition contribute to nAChR pharmacology and functional diversity.[13,14] Research is currently underway to identify the specific subunit composition of native nAChRs,[8] the putative nature of which is indicated by an asterisk beside the subunit designation herein.

Over the last decade, efforts have focused on the development of nAChR agonists as therapeutic agents.[1–4,15,16] However, relatively little attention has focused on the development of nAChR antagonists as potential drug candidates, and there are very few nAChR subtype selective antagonists available.[17,18]

bis-Quaternary ammonium salts, such as hexamethonium chloride (HEX) and decamethonium bromide (DEC), are regarded as simplified analogues of *d*-tubocurarine (TBC) [19,20] (see Fig. 1), and have been utilized to differentiate between subtypes of peripheral nicotinic receptors.[20–22] Thus, DEC inhibits neuromuscular nicotinic receptors, while HEX primarily inhibits ganglionic nicotinic receptors.[23,24] Recently, quaternary ammonium *N*-*n*-alkyl analogues of NIC [i.e., *N*-*n*-octylnicotinium iodide (NONI), *N*-*n*-decylnicotinium iodide (NDNI) and *cis*-6-decyl-1-methyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[2,3-*f*]quinolin-6-ium iodide (BCD; Fig. 1)] have been reported as nAChR subtype selective

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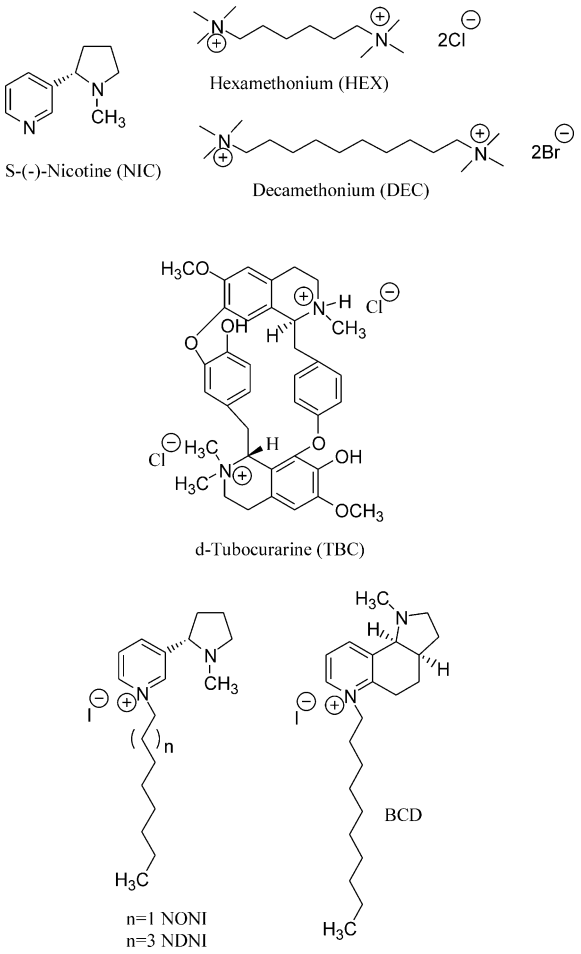

Figure 1. Structures of S-(−)-nicotine, hexamethonium, decamethonium, *d*-tubocurarine, NONI, NDNI and BCD.

antagonists.[25–30] These latter findings prompted us to evaluate a series of bis-quaternary ammonium compounds containing azaaromatic head groups for their interaction with α4β2* and α7* nAChR subtypes.

Chemistry

Compounds **1**–**6** (Fig. 2) were prepared by first dissolving NIC in glacial acetic acid, stirring the mixture for 5 min, and adding the appropriate diiodo- or dibromoalkane. The solution was stirred under reflux for 3 days, the solvent was evaporated under reduced pressure, and the resulting residue was treated with an aqueous saturated solution of $NaHCO_3$. The mixture was then extracted with diethyl ether (3×50 mL), and then with chloroform (3×50 mL). The aqueous layer was collected, lyophilized and the resulting solid triturated with chloroform. After filtration, the filtrate was dried over anhydrous $MgSO_4$. Filtration and removal of the solvent afforded the desired bis-nicotinium salt. Compounds **7**–**31** (Fig. 2) were prepared by reacting an excess of the appropriate azaaromatic compound with a variety of diiodo- or dibromoalkanes for 24 h at 65 °C in the absence of solvent. The resulting solid was collected by filtration, dissolved in water and the aqueous solution washed with diethyl ether (3×50 mL). The aqueous solution was then lyophilized to afford either a solid or a viscous hygroscopic oil. All compounds were characterized by ^1H and ^{13}C NMR spectroscopy, mass spectroscopy and elemental analysis.[31]

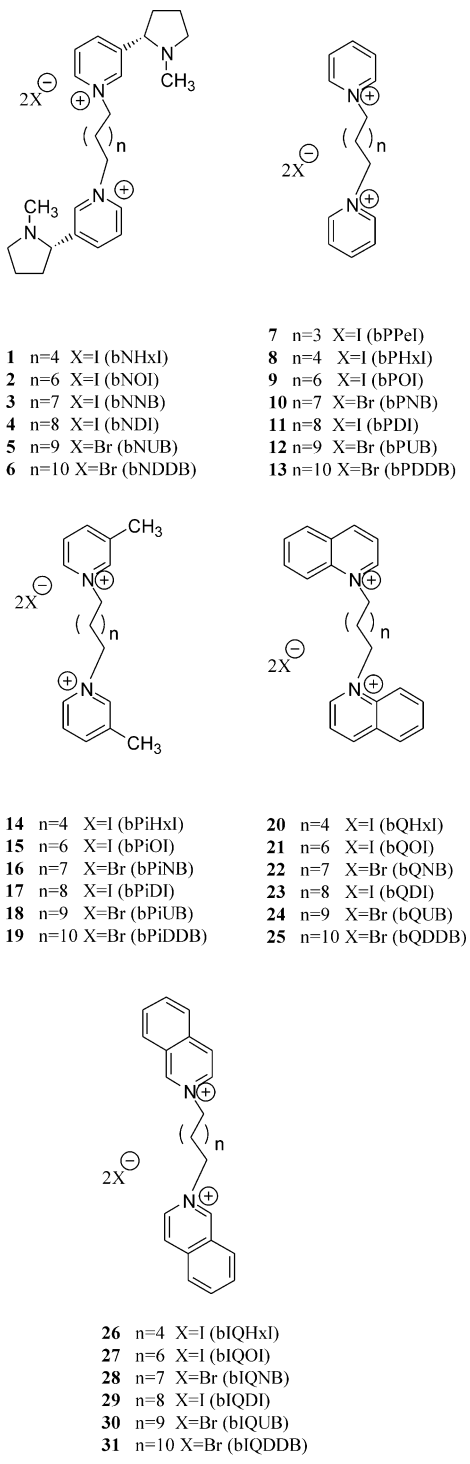

Figure 2. Structures of bis-azaaromatic quaternary ammonium analogues **1**–**31**.

Biological Assays

Compounds **1**–**31** were evaluated for their ability to inhibit [^3H]NIC and [^3H]methyllycaconitine ([^3H]MLA)

binding to rat brain membranes, indicative of affinity for α4β2* and α7* nAChRs, respectively.[30,32] Analogue-induced inhibition of binding was expressed as a percent of control and fitted by nonlinear, non-weighted least squares regression using a fixed slope sigmoidal function. The log IC_{50} value represented the logarithm of analogue concentration required to decrease binding by 50%. IC_{50} values were corrected for ligand concentration according to the Cheng–Prussoff equation[33] to yield inhibition constants (K_i), such that $K_i = IC_{50}/(1 + c/K_d)$, where c is the concentration of free radioligand and K_d is the equilibrium dissociation constant of the ligand. Protein concentration was determined using published methods.[34] Results are reported as K_i values (±SEM) in Table 1. Previous results have demonstrated a high correlation between inhibition of [^{3}H]NIC binding and inhibition of NIC-evoked $^{86}Rb^+$ efflux, such that the latter assay has been utilized to assess the functional interaction of ligands at the α4β2* nAChR subtype.[35] To ascertain if the most potent analogues in the [^{3}H]NIC binding assay act as antagonists at α4β2* nAChRs, inhibition of NIC (3 μM)-evoked $^{86}Rb^+$ efflux from rat thalamic synaptosomes was determined.

NIC exhibited a high affinity ($K_i = 1.4$ nM) for the α4β2* nAChR, and a relatively lower affinity ($K_i = 0.45$ μM) for the α7* nAChR subtype when compared to classical antagonists, α-bungarotoxin (αBTX; $K_i = 7$ nM) and MLA ($K_i = 2.3$ nM). Although HEX and DEC had 3-orders of magnitude lower affinity for the α4β2* subtype compared to NIC, DEC had 6-fold higher affinity ($K_i = 5.73$ μM) for the α4β2* subtype compared to HEX. Neither HEX nor DEC had affinity for the α7* nAChR subtype. TBC exhibited K_i values of 4.79 and 9.72 μM at α7* and α4β2* nAChRs, respectively.

In a homologous series of *N*-*n*-alkyl nicotinium analogues, with alkyl groups ranging from C_1–C_{12}, the C_{10} analogue NDNI exhibited highest affinity ($K_i = 64$ nM) for the α4β2* subtype.[26,28] Furthermore, NDNI inhibited NIC-evoked $^{86}Rb^+$ efflux, indicating that it acts as a pharmacological antagonist of α4β2* nAChRs.[26,29] Interestingly, BCD, a conformationally restricted analogue of NDNI, had no affinity for the α4β2* subtype.[30] The C_8 analogue, NONI, which has a shorter n-alkyl chain length, exhibited a significantly lower affinity ($K_i = 20$ μM) at α4β2*.[26,28] Neither NONI nor NDNI exhibited high affinity for the α7* nAChR subtype.

Table 1. K_i Values for compounds **1**–**31** in the [^{3}H]NIC and [^{3}H]MLA binding assays using rat brain membranes

Compd	K_i [^{3}H]NIC binding assay (μM)	K_i [^{3}H]MLA binding assay (μM)
NIC	0.0014±0.0001[a]	0.45±0.09
αBTX	>100	0.007±0.0003
MLA	1.56±0.01	0.0023±0.0008
HEX	21.7±3.80	>100
DEC	5.73±0.76	>100
TBC	9.72±2.23	4.79±0.20
NONI	19.7±1.50	23.6±4.85
NDNI	0.064±0.004	>100
1	20.0±2.84	>100
2	1.54±0.17	>100
3	5.03±0.80	>100
4	0.33±0.01	>100
5	0.43±0.06	>100
6	1.95±0.19	>100
7	>100	>100
8	97.8±17.5	>100
9	34.3±2.74	>100
10	24.2±1.19	>100
11	18.7±3.09	>100
12	14.0±3.21	57.0±4.89
13	9.15±0.17	33.0±4.60
14	>100	>100
15	>100	>100
16	79.9±16.6	>100
17	>100	>100
18	69.2±28.9	>100
19	48.6±17.2	>100
20	39.3±5.59	13.3±2.12
21	>100	2.39±0.69
22	22.5±1.30	3.58±0.45
23	>100	7.81±1.90
24	37.8±8.65	2.58±0.46
25	>100	1.61±0.21
26	17.9±3.59	16.0±1.92
27	65.5±14.5	11.7±5.63
28	7.89±0.92	5.95±1.01
29	>100	7.25±3.02
30	9.02±1.56	5.46±0.78
31	6.10±0.73	2.21±0.25

[a]Data represent K_i value expressed mean ± SEM.

Based on the interesting pharmacological profile of the *N*-*n*-alkylnicotinium analogues and the well-established pharmacology of the bis-trimethylammonium alkanes, including HEX and DEC, a series of bis-azaaromatic quaternary ammonium analogues were synthesized and evaluated for interaction with α4β2* and α7* nAChR subtypes in order to identify ligands which are both potent and selective at these nAChRs. The bis-nicotinium compounds (**1**–**6**) were thought to be a particularly interesting series of target compounds, since they are related structurally to NONI and NDNI.

The bis-nicotinium analogues **1**–**6** exhibited K_i values for the α4β2* subtype ranging from 0.33–20 μM, the most potent being the C_{10} analogue, bNDI (Table 1). None of the bis-nicotinium analogues had affinity for the α7* nAChR subtype. bNDI had a similar potency to NDNI at α4β2* nAChRs. Moreover, bNDI and bNUB inhibited NIC-evoked $^{86}Rb^+$ efflux from rat thalamic synaptosomes ($IC_{50} = 3.76 \pm 1.1$ μM and 5.22 ± 1.4 μM, respectively), without producing intrinsic activity, indicating that these bis-nicotinium analogues are functional antagonists at the α4β2* nAChR subtype.

The simple bis-pyridinium analogues (**7**–**13**) generally had a 10-fold lower affinity for the α4β2* subtype compared to the bis-nicotinium analogues (**1**–**6**). As the carbon chain in the bis-pyridinium analogues increased from C_5 to C_{12}, the affinity for the α4β2* subtype also increased by an order of magnitude. Thus, the C_{12} analogue **13** was the most potent ($K_i = 9.15$ μM) in the series at the α4β2* subtype. None of the compounds in the bis-pyridinium series exhibited high affinity for the α7* nAChR subtype. Interestingly, the structurally related bis-picolinium analogues (**14**–**19**) generally had low affinity for both α4β2* and α7* subtypes.

The bis-quinoliniums analogues (**20**–**25**) generally exhibited low affinity for the α4β2* subtype. The structure activity relationship for the bis-isoquinoliniums analogues (**26**–**31**) resulted in a range of affinity values for the α4β2* subtype ($K_i = 6.1$–65.5 μM; except bIQDI, $K_i > 100$ μM). As a group, the bis-quinolinium and bis-isoquinolinium analogues exhibited K_i values ranging from 1.61 - 16.0 μM for the α7* nAChR subtype. Thus, the two most potent analogues in these two groups were the C_{12} analogues **25**, bQDDB, and **31**, bIQDDB; and moreover, bQDDB demonstrates excellent selectivity for the α7* nAChR subtype as well.

From the structure activity trends in compounds **1**–**31**, it is apparent that replacing the *N*-trimethylammonium moieties such as HEX and DEC with azaaromatic moieties can afford molecules with affinity for α4β2* and α7* nAChR subtypes. Of particular interest is the finding that two compounds in this study exhibit high selectivity at these nAChR subtypes. In this respect, the bis-nicotinium analogue, bNDI (**4**) exhibited a K_i value of 330 nM at the α4β2* nAChR subtype, and functionally inhibited NIC-evoked $^{86}Rb^+$ efflux ($IC_{50} = 3.76$ μM), while demonstrating no affinity for the α7* nAChR subtype, indicating that this analogue is a selective α4β2* nAChRs antagonist. In contrast, the bis-quinolinium analogue, bQDDB (**25**) exhibited a K_i value of 1.61 μM for the α7* nAChR and no affinity for the α4β2* nAChR subtype. Importantly, replacing the bis-nicotinium headgroups with quinolinium moieties afforded a compound with no affinity for α4β2* nAChR, but had similar affinity to NIC at the α7* nAChR subtype. Thus, two interesting bis-azaaromatic quaternary ammonium compounds have emerged from this structure activity study, bNDI and bQDDB, which exhibit good affinity and selectivity for α4β2* and α7* nAChR subtypes, respectively. Subtype-selective nAChR ligands, such as bNDI and bQDDB, constitute useful agents for both basic and clinical research aimed at determining the role of nAChRs in physiological function.

Acknowledgements

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31. **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (1H, s, C2-H), 9.44 (1H, d, C6-H), 8.53 (1H, d, C4-H), 8.09 (1H, m, C5-H), 4.98 (2H, t, C″ 1-CH_2), 3.74 (1H, t, pyrrolidine CH_2), 3.30 (1H, t, pyrrolidine CH_2, 2.50 (2H, m, C″ 2-CH_2), 2.29 (3H, s, pyrrolidine N-CH_3), 1.65.20 (5H, m, pyrrolidine CH_2CH_2), 1.30.57 (6H, m, C″ 3–5-CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.6, 143.9×2, 143.4, 128.4, 66.9, 61.9, 57.0, 40.8, 36.1, 32.1, 28.5, 28.2, 25.8, 23.5, $C_{30}H_{48}I_2N_4$. Yellow oil, 27% yield. **25**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.58 (1H, d, C2-H), 9.29 (1H, d, C3-H), 8.61 (1H, d, C8-H), 8.50 (1H, d, C4-H), 8.29 (1H, t, C7-H), 8.18 (1H, t, C5-H), 8.04 (1H, d, C6-H), 5.04 (2H, t, C'1-CH_2), 1.95 (2H, m, C′2-CH_2), 1.2.4 (8H, m, C′3-6-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.5, 147.4, 137.4, 135.6, 130.7, 129.9, 129.7, 122.1, 118.9, 57.3, 29.5,28.9, 28.8, 28.5, 25.7, $C_{30}H_{38}Br_2N_2$. glassy solid, 92% yield.
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