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N,*N*-Disubstituted Piperazines: Synthesis and Affinities at α4β2* and α7* Neuronal Nicotinic Acetylcholine Receptors

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Abstract—A series of *N*,*N*-disubstituted piperazines were prepared and evaluated for binding to α4β2* and α7* neuronal nicotinic acetylcholine receptors using rat striatum and whole brain membrane preparations, respectively. This series of compounds exhibited selectivity for α4β2* nAChRs and did not interact with the α7* nAChRs subtype. The most potent analogues were compounds **8b** and **8f** ($K_i = 32$ μM). Thus, linking together a pyridine π-system and a cyclic amine moiety via a piperazine ring affords compounds with low affinity, but good selectivity for α4β2* nicotinic receptors.

Structural analogues of neuronal nicotinic acetylcholine receptors (nAChR) agonists such as nicotine (NIC) and epibatidine have been suggested as potential therapeutic agents for several neurological disorders, including attention deficit hyperactivity disorder, Tourette's syndrome, schizophrenia and Alzheimer's and Parkinson's diseases.[1–3]

nAChRs are pentameric ligand-gated ion channels, composed of two types of subunits, α and β.[4,5] To date, ten α (α1-α10) and four β (β1-β4) subunits have been identified.[6] nAChRs have the general stoichiometry of 2α and 3β subunits that constitute the receptor/ion channel complex.[7] Expression of nAChRs in Xenopus oocytes has shown that associations of α2, α3 and α4 subunits with β2 and β4 subunits forming functional heterologous receptor channels, whereas α7, α8, and α9 subunits assemble to form homologous subunit receptor channels.[8–10] Differences in subunit composition contribute to nAChR pharmacology and functional diversity.[11,12] Research is currently underway to identify the specific subunit composition of native nAChRs,[6] the putative nature of which is indicated herein by an asterisk beside the subunit designation. The most prevalent nAChR subtypes in brain are the α4β2* and α7* subtypes. The α4β2* subtype binds [^{3}H]NIC with high affinity, and the α7* subtype binds α-bungarotoxin (α-BTX) and methyllycaconitine (MLA) with high affinity. These receptor subtypes have been implicated as important in mediating the effect of nicotine improvement of cognition, learning and memory, as well as nicotine-induced analgesia, anxiolytic effects, and antidepressant effects, in addition to protection from neurodegeneration.[13]

Numerous studies have focused on the development of ligands for nAChRs,[1,3,14,15] and several high affinity ligands have been reported.[16–26] However, limited knowledge of the localization, structure and function of nAChRs, and the lack of selectivity of available ligands for the various nAChRs subtypes have prevented the extensive use of nAChR agonist and antagonists as therapeutic agents. Current drug discovery efforts in this area are being directed towards the development of subtype-selective neuronal nAChR ligands as therapeutic agents.[27]

A number of established nAChR ligands, such as epibatidine (**1**),[28] (*S*)-NIC (**2**),[27] (*S*)-nornicotine (**3**)[13] and

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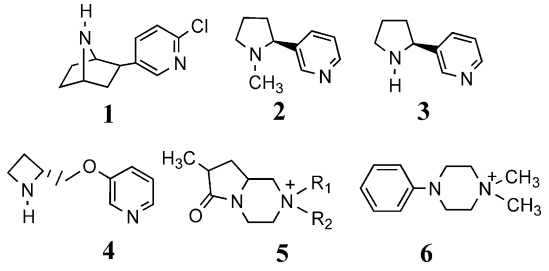
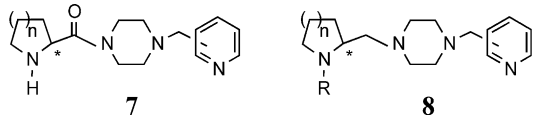
ABT-594 (**4**)[29] possess potent binding affinity for central nAChRs. Epibatidine (**1**) is a natural alkaloid with high affinity for the α4β2* nAChR subtype in rat brain.[28] The nAChR ligands **1**–**4** all contain pyridine and cyclic amine pharmacophoric moieties, and a comprehensive review, which summarized the structure–activity relationships of ligands for nAChRs,[30] has emphasized the importance in the ligand structure of a π-system, such as a heteroaromatic ring or carbonyl group as a hydrogen bond acceptor moiety, and a basic cyclic amine group.



1 **2** **3** **4** **5** **6**

In addition, a series of compound **5** with micromolar affinity for neuronal nAChRs was reported,[30] and *N*-dimethyl-*N*-phenylpiperazinium iodide (**6**, DMPP), a widely studied ligand, binds with high affinity to α4β2 and α7 receptors.[2] In our continuing efforts to develop subtype-selective nAChRs ligands, a novel series of piperazine derivatives **7** and **8** (Tables 1 and 2), in which the pyridine π-system and cylic amine moieties are linked together via a piperazine ring, was thus designed by strutural hybridization of **5** and **6** with the well-known ligands **1**–**4**. Like compound **4**, compounds **7** and **8** are conformationally more flexible compared with compounds **1**–**3**. The substituted pyridine units at ortho, meta or para position in these designed compounds are covered, and the chiral centers of these compounds are of S-configuration or racemate. We have now prepared and evaluated a series of *N*,*N*-disubstituted piperazines of general structure **7** and **8** for binding to α4β2* and α7* nAChRs,



7 **8**

Compounds **7a**–**7e** were prepared by the method summarized in Scheme 1. *N*-Pyridylpiperazines **10** were obtained by modification of reported methods.[31] Coupling of the N-Boc-protected cyclic amino acids **9** and compounds of general structure **10** with dicyclohexyl carbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT)[31] gave the corresponding Boc-protected intermediates **11,** which were further treated with TFA in CH_2Cl_2 to afford compounds **7a**–**7e**.

Table 1. Structures of compounds **7a**–**7e**

Compd	*n*	Stereochem	Substituted position of pyridine
7a	1	*S*	*ortho*
7b	1	*S*	*meta*
7c	2	*R*, *S*	*ortho*
7d	2	*R*, *S*	*meta*
7e	2	*R*, *S*	*para*

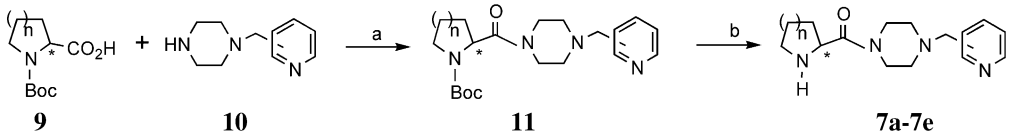
Table 2. Structures of compounds **8a**–**8h**

Compd	*n*	Stereochem	R	Substituted position of pyridine
8a	1	*S*	H	*meta*
8b	1	*S*	CH_3	*meta*
8c	1	*S*	H	*para*
8d	1	*S*	CH_3	*para*
8e	2	*R*, *S*	H	*meta*
8f	2	*R*, *S*	CH_3	*meta*
8g	2	*R*, *S*	H	*para*
8h	2	*R*, *S*	CH_3	*para*

The general method for the preparation of compounds **8a**, **8b**, and **8e**–**8h** is summarized in Scheme 2. Reduction of **7b**, **7d** and **7e** with BH_3·THF in refluxing THF[32] gave **8a**, **8e** and **8g**, respectively, each of which was *N*-methylated with a 40% aqueous solution of formaldehyde and HCO_2H to give **8b**, **8f** and **8h**, respectively. The reduction of **7a** and **7c** by BH_3·THF was unsuccessful. In a one-pot preparation of **8c** and **8d**, it was found that reduction of **12** with BH_3·THF in refluxing THF followed by quenching with 6N HCl gave the expected **8c**, and the methylated compound **8d** simultaneously (Scheme 3).

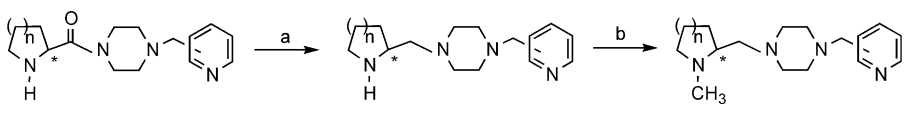
All novel compounds were characterized by IR, HRMS, ^{1}H NMR and ^{13}C NMR spectroscopy.[33]

Compounds listed in Table 3 were evaluated for their binding affinities for α4β2* and α7* nAChRs. MLA, α-BTX, NIC (**2**), and nornicotine (**3**) were also examined



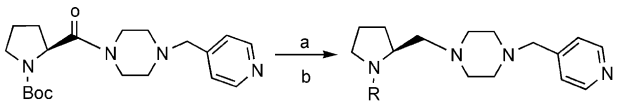
9 **10** **11** **7a-7e**

Scheme 1. Reagents and conditions: (a) DCC, HOBT, CH_2Cl_2, rt. (b) TFA, CH_2Cl_2, rt.



7b, 7d, 7e **8a, 8e 8g** **8b, 8f, 8h**

Scheme 2. Reagents and conditions: (a) BH_3·THF, THF, reflux. (b) 40% HCHO aqueous solution, HCO_2H, reflux.



12 **8c** R=H, **8d** R=CH3

Scheme 3. Reagents and conditions: (a) BH_3·THF, THF, reflux. (b) 6 N HCl.

Table 3. K_i values for **7a–7e** and **8a–8h** in [^{3}H]NIC and [^{3}H]MLA binding assays[a]

Compd	K_i [^{3}H]NIC binding assay (μM)	K_i [^{3}H]MLA binding assay (μM)
MLA	1.46±0.72	0.0048±0.0004
α-BTX	>10	0.0057±0.0002
2	0.001±0.00005	0.34±0.01
3	0.033±0.004	1.19±0.11
7a	>100	>100
7b	Nd[b]	Nd[b]
7c	>100	>100
7d	54.8±20.8	>100
7e	60.5±25.7	>100
8a	>100	>100
8b	31.7±14.3	>100
8c	46+1.3	>100
8d	38.9+3.6	>100
8e	>100	>100
8f	32.1±1.5	>100
8g	>100	>100
8h	42±10.7	>100

[a]N=at least three independent determinations using triplicate nine-point inhibition curves.
[b]Not determined.

for comparison. Compounds were evaluated for their ability to inhibit [^{3}H]NIC binding to rat striatal membranes and [^{3}H]MLA binding to whole brain membranes, demonstrating affinity for α4β2* and α7* nAChRs, respectively.[34,35] A brief description of the membrane preparation and ligand binding assays are provided below.[36] 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-Prusoff equation[37] to yield true 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.[38] Results are reported as K_i values (±SEM). Both MLA and BTX had very high affinity for the α7* nAChR (K_i=4.8 and 5.7 nM, respectively). MLA exhibited low affinity for the α4β2* nAChR (K_i=1.46 μM). In contrast, NIC showed ~100-fold higher affinity for the α4β2* than for the α7* nAChR.

Compared to NIC (**2**), compounds **7a–e** and **8a–h** generally exhibited lower affinity for the α4β2* nAChR; however generally these novel compounds were selective for the α4β2* nAChR, since none of the compounds exhibited affinity for α7* nAChR. It appears that the *N*-methyl compounds (**8b**, **8d**, **8f** and **8h**) have a slightly higher affinity than those of their *N*-demethylated counterparts, exhibiting a similar trend to the relative binding affinities of NIC (**2**) and nornicotine (**3**). Also, the position of substitution of the piperazine moiety on the pyridine ring has no substantial influence on binding affinity. The most potent analogues were compounds **8b** and **8f** (K_i=32 μM). Thus, linking together a pyridine π-system and a cyclic amine moiety via a piperazine ring affords compounds with low affinity but good selectivity for α4β2* nicotinic receptors.

Acknowledgements

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33. Selected analytical data: **7d**: IR (film, cm^{-1}) 3396, 2929, 1633, 1423, 1300, 1227, 1144, 1032, 1001, 716; HRMS (*m*/*z*): calcd for $C_{16}H_{24}N_4O$: 288.1950, found 288.1958; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.54 (d, $J=1.9$ Hz, 1H), 8.52 (dd, $J=1.7$, 4.9 Hz, 1H), 7.67 (d, $J=8.0$ Hz, 1H), 7.27 (m, 1H), 3.65–3.56 (m, 5H), 3.52 (s, 2H), 3.14 (m, 1H), 2.66 (m, 1H), 2.45–2.41 (m, 4H), 1.89 (br s, 1H), 1.71–1.31 (m, 6H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 170.6, 149.5, 147.9, 135.9, 132.4, 122.6, 59.1, 55.3, 52.3, 51.8, 44.5, 44.4, 40.9, 28.9, 25.3, 23.3. **8 h:** IR (film, cm^{-1}) 2931, 2808, 1653, 1605, 1562, 1460, 1416, 1373, 1290, 1159, 1138, 1011, 841, 609; HRMS (*m*/*z*): calcd for $C_{17}H_{28}N_4O$: 288.2314, found 288.2303; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.51 (d, $J=6.0$ Hz, 2H), 7.24 (d, $J=6.8$ Hz, 2H), 3.45 (s, 2H), 2.92 (d, $J=11.8$ Hz, 1H), 2.68 (dd, $J=15.4$, 7.7 Hz, 1H), 2.39 (m, 8H), 2.41(s, 3H), 2.26–2.18 (m, 3H), 1.83–1.61 (m, 3H), 1.41–1.25 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.6 (2 C), 147.6, 123.8 (2 C), 61.6, 61.3, 57.0, 53.7 (2 C), 53.1(2 C), 42.6, 30.0, 29.6, 24.6, 23.5.
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36. Membranes from rat striata and whole brain (excluding cerebellum, cortex and striatum) were prepared for the [^{3}H]NIC and [^{3}H]MLA binding assays, respectively. Striata or whole brain was homogenized with a Tekmar polytron in 10–20 vol of ice-cold modified Krebs-HEPES buffer (20 mM HEPES, 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, pH 7.5). Homogenates were incubated (5 min at 37 °C) and centrifuged (20 min at 29,000g at 4 °C). Tissue pellets were resuspended in 10 vol of ice-cold Milli-Q water, incubated (5–10 min at 37 °C) and centrifuged for 20 min (29,000g at 4 °C). Tissue pellets were again resuspended in 10 vol of ice-cold 10% Krebs-HEPES buffer, incubated and centrifuged as described and stored at −70 °C in 10% Krebs-HEPES buffer until use. Membrane suspension (150–200 μg protein/100 μL) was added to assay tubes containing analogue (7–9 concentrations, 1 nM–1 mM) and 3 nM [^{3}H]NIC or 3 nM [^{3}H]MLA for a final assay vol of 200–250 μL. Subsequently, [^{3}H]NIC and [^{3}H]MLA assays were incubated for 90 and 120 min, respectively. Reactions were terminated by addition of ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filters. Bound radioactivity was determined via liquid scintillation spectroscopy. Nonspecific binding was determined in the presence of 10 μM NIC ([^{3}H]NIC binding assay) and 1 mM NIC ([^{3}H]MLA binding assay).
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