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Syntheses and structure–activity relationship (SAR) studies of 2,5-diazabicyclo[2.2.1]heptanes as novel α7 neuronal nicotinic receptor (NNR) ligands

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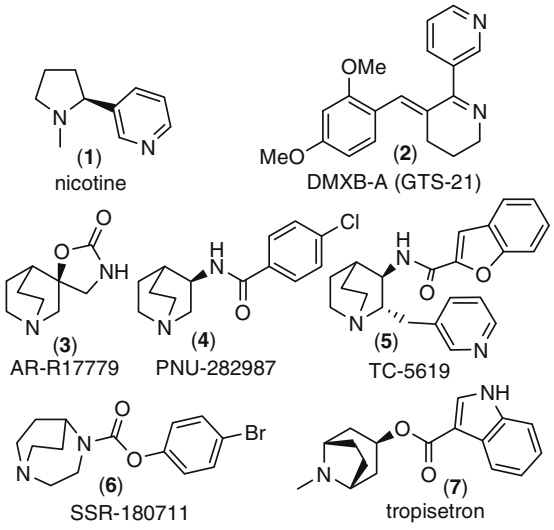
ABSTRACT

Biaryl substituted 2,5-diazabicyclo[2.2.1]heptanes have been synthesized and tested for their affinity toward α7 neuronal nicotinic receptors (NNRs). SAR studies established that 5-*N*-methyl substituent, heteroaryl linker and the nature of terminal aryl group are critical for the ligand to achieve potent α7 NNR agonist activity.

α7 Neuronal nicotinic receptors (α7 NNRs) belong to the family of ACh-gated cation channels, which includes multiple subtypes resulting from pentameric combinations of 12 known subunits (α2–α10 and β2–β4).[1] These homo-oligomeric channels are considered to play an important role in modulating biochemical and neurophysiological events underlying cognitive and attentive processes, sensory gating, and neuroprotection.[2] The convergence of evidence from pharmacological, genetic and early clinical studies suggests that selective α7 NNR agonists have potential for the treatment of a variety of cognitive deficits associated with Alzheimer's disease (AD) and schizophrenia.[3] Accordingly, a range of structurally diverse α7 NNR ligands, largely emerging from the anabaseine and quinuclidine scaffolds, have been identified towards further preclinical validation, and ultimately, clinical studies in humans.[4] As shown in Scheme 1, examples of α7 NNR agonists that have been extensively used for preclinical in vivo studies include nicotine (**1**),[5] the anabaseine derivative DMXB-A (GTS-21) (**2**),[6] spiro-oxazolidinone AR-R17779 (**3**),[7] quinuclidine amides PNU-282987 (**4**)[8] and TC-5619 (**6**),[9] SSR-180711 (**5**),[10] and tropisetron (**7**).[11] Although several selective α7 NNR agents have advanced to human clinical trials, to date none has been brought



Scheme 1. Structures of α7 NNR agents have been extensively profiled in literature.

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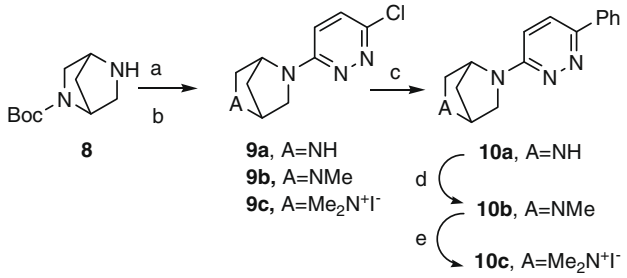
to market.[5–11] The search for novel and selective α7 NNR agents continues to receive considerable attention. In this communication, we describe the synthesis and structure–activity relationship (SAR) studies of biaryl substituted 2,5-diazabicyclo[2.2.1]heptanes as novel α7 NNR ligands.

Initially discovered by Barlocco and co-workers[12,13] 2-(6-chloropyridazin-3-yl)-2,5-diazabicyclo[2.2.1]heptane (**9a**) was found to be a potent α4β2 NNR ligand (K_i = 6 nM in the [^{3}H]cytisine binding assay). Barlocco also reported that the 5-*N*-methyl analogue (**9b**) exhibits diminished binding affinity (K_i = 65 nM), while a second methylation to the 5-*N*,*N*-dimethylated quaternary ammonium salt (**9c**) restores α4β2 NNR binding affinity (K_i = 4.3 nM).[11] Consistent with our studies on substituent control of NNR subtype selectivity,[14] we found that replacing the chloro substituent in **9a** with a phenyl group, as in (*1S,4S*)-**10a** (Scheme 2) caused a dramatic attenuation of α4β2 NNR binding affinity, but enhanced affinity at the α7 NNR subtype (Table 1). Likewise, the 5-*N*-methyl analog (*1S,4S*)-**10b** had weak α4β2 NNR affinity. In this case the *N*-methyl group does not have a detrimental effect on α4β2 NNR binding, but appears to enhance the α7 NNR affinity (Table 1, entry 7). Even more dramatically, the quaternary ammonium salt (*1S,4S*)-**10c** (A-585539). exhibited α7 NNR binding affinity in the picomolar range (Table 1, entry 8). Although (*1S,4S*)-**10c** has limited therapeutic potential due to poor CNS access, its favorable binding properties have made it our preferred radioligand for in vitro binding at α7 NNRs.[15] Interestingly, while the α4β2 binding is enhanced in the (*1R,4R*)-diamine series (Ref. 12 and Table 1), the opposite trend pertains to α7 NNR affinity, where (*1S,4S*)-**10b** and (*1S,4S*)-**10c** are significantly more potent than their (*1R,4R*) counterparts (Table 1, entries 7–10). Consistent with its low affinity for the native rat α4β2 receptor, (*1S,4S*)-**10b** did not produce an agonist response at concentrations ⩽100 μM in cells expressing the recombinant human α4β2 NNR, nor did it activate other heteromeric NNR subtypes (α3β4 and α4β4). This suggests that good selectivity for α7 over other NNR subtypes can be achieved with this scaffold.



Scheme 2. Synthesis of 2-(6-phenylpyridazin-3-yl)-2,5-diazabicyclo-[2.2.1]heptanes. Reagents and conditions: (a) 2,6-dichloropyridazine, *i*-Pr_2NEt, DMF, 100 °C, 16 h (78%); (b) HCl; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3 (aq), dioxane, 70 °C, 10 h, >70%; (d) HCHO, $NaBH(OAc)_3$, MeCN, rt, 10 h, >90%; (e) MeI, CH_2Cl_2, >80%.

Table 1
Rat α7 NNR binding affinity for 2-(6-phenylpyridazin-3-yl)-2,5-diazabicyclo [2.2.1]heptanes

Entry	Compound	α4β2 K_i[a] (nM)	α7 K_i[b] (nM)
1	(*1S,4S*)-**9a**	6	303
2	(*1R,4R*)-**9a**	0.2	486
3	(*1S,4S*)-**9b**	139	1480
4	(*1R,4R*)-**9b**	26	748
5	(*1S,4S*)-**9c**	4.3	21.5
6	(*1S,4S*)-**10a**	31,200	10.1
7	(*1S,4S*)-**10b**	12,700	9.3
8	(*1S,4S*)-**10c**	700	0.066
9	(*1R,4R*)-**10b**	26,700	212
10	(*1R,4R*)-**10c**	5500	2.2

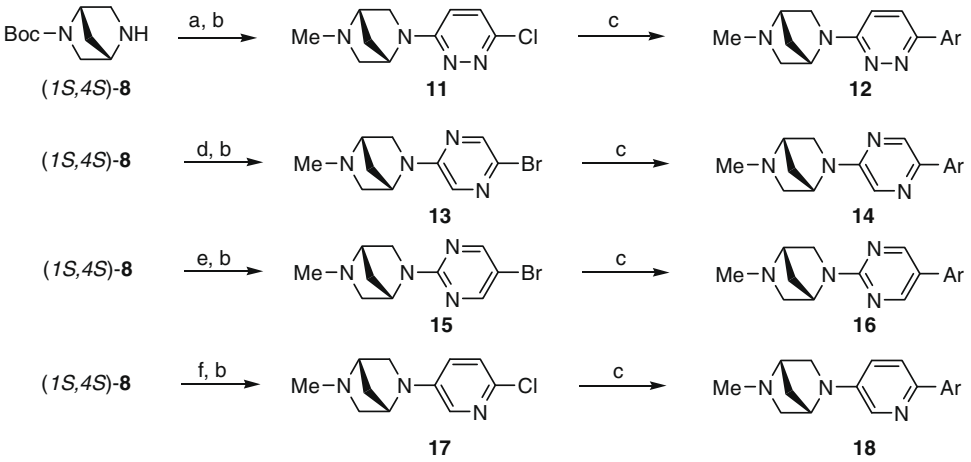
[a] Displacement studies with [^{3}H]cytisine using rat brain homogenates. The K_i represents mean values obtained from independent experiments ($n \geqslant 3$, SEM ⩽ 10%.
[b] Displacement of [^{3}H]A-585539.

Based on the preliminary SAR results shown in Table 1, aryl substitution on pyridazine and 5-N-methylation on 2,5-diazabicyclo[2.2.1]heptane led to an increase in α7 NNR affinity, but decrease in α4β2 NNR activity. Between enantiomers, (*1S,4S*) compounds are preferable because they are not only more selective with respect to α7 activity than α4β2, but also 20–30-fold more potent in the α7 binding assay. Based on these observations, 5-N-methylated (*1S,4S*) compounds were chosen for further exploration.

To further explore the potential of 2,5-diazabicyclo[2.2.1]heptane α7 NNR ligands, our efforts focused on the SAR of biaryl substituted (*1S,4S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptanes. As shown in Scheme 3, the condensation of 5-*N*-Boc-2,5-diazabicyclo[2.2.1]heptane (*1S,4S*)-**8** with a heteroaryl halide followed by reductive animation with formalin in formic acid provided an array of halo-heteroaryl intermediates **11**, **13**, **15**, and **17**. Aryl halides that are activated toward nucleophilic aromatic substitution, such as 3,6-dichloropyridazine, 2-chloropyrazine and 2,5-dibromopyrazine, reacted efficiently with (*1S,4S*)-**8** in the presence of Hünig's base in DMSO at 100 °C. Regioselective amination of 5-bromo-2-chloropyridine was accomplished by using the palladium mediated procedures initially developed by Buchwald and Hartwig, then by Ji.[16] Under standard Suzuki coupling reaction conditions,[17] halide intermediates (**11**, **13**, **15**, and **17**) reacted smoothly with a variety of boronic acids in the presence of a palladium catalyst, giving biaryl substituted (*1S,4S*)-5-methyl-2,5-diaza-bicyclo[2.2.1]heptanes **12**, **14**, **16**, and **18** in moderate to excellent chemical yield.

Biaryl substituted 2,5-diazabicyclo[2.2.1]heptane ligands were evaluated for their ability to inhibit radioligands [^{3}H]cytisine and [^{3}H]A-585539 binding to rat brain membranes. The functional activity of these ligands was investigated using recombinant human α7 NNR expressed in *Xenopus* oocytes. All of these compounds show low affinity for α4β2 NNR based on displacement of [^{3}H]cytisine binding (K_i >10 μM), which is consistent with our early finding for **10b**. On the other hand, many of them also exhibit potent α7 agonist activities. These results are summarized in Table 2.

As shown in Table 2, several SAR trends immediately emerged. First, the nature of the terminal Ar group plays a very important role on α7 NNR agonist activities. For instance, benzofuran-5-yl and benzothiophen-5-yl analogues (**12b** and **12c**) show comparable binding affinity relative to phenyl analog **12a**, but have approximately a 4–6-fold increase in α7 NNR functional potency (entries 1–3). N-Containing bicyclic aryl groups, such as indazol-5-yl and indol-5-yl, led to a significant boost on both α7 NNR binding affinity and functional potency (entries 4–5). Meanwhile, analog **12e** showed very weak [^{3}H]-dofetilide binding affinity (K_i >20 μM) and excellent human liver microsome stability (>97% remaining after 30 min incubation) thanks to the indol-5-yl substituent. An electron-donating 2-methyl substituent on the indole (**12i**) shows a slight increase in binding affinity and functional potency, but an electron-withdrawing 2-CF_3 group (**12f**) decreases α7 binding affinity by 5-fold relative to **12e** (entries 5–7). Second, the substitution site at the terminal aryl ring is important for activity. Indol-5-yl is more favorable than indol-4-yl and indol-6-yl. For example, **12e** is 35-fold more potent than **12g**. Third, the linking heteroaryl ring has an effect on α7 NNR activity. In terms of the α7 NNR binding affinities, there is a clear preference for pyridine and pyridazine over pyrimidine and pyrazine. Compounds **18** and **12** are at least several fold more potent than their **14** and **16** counterparts. The pyridin-3-yl linker appears to be optimal; ligands **18a**–**18h** show very potent α7 NNR activity. For instance, indol-5-yl compound



Scheme 3. Synthesis of biaryl substituted 2,5-diazabicyclo[2.2.1]heptanes. Reagents and conditions: (a) 3,6-dichloropyridazine (1.2 equiv), *i*-Pr_2NEt/DMF (v. 1/1, 0.5 M), 100 °C, 16 h, >70%; (b) HCHO (aq), HCOOH (aq), 100 °C, 1 h; (c) Ar-$B(OH)_2$ (1.2 equiv), $Pd(PPh_3)_4$ (0.05 equiv), K_2CO_3 (aq), dioxane, 70 °C, 10 h, >70%; (d) (1) 2-chloropyrazine (1.2 equiv) *i*-Pr_2NEt/DMSO (v. 1/1, 0.5 M), 100 °C, 16 h; (2) NBS (1.0 equiv), MeCN, rt, 3 h; (e) 5-bromo-2-chloropyrimidine, *i*-Pr_2NEt/DMSO (v. 1/1, 0.5 M), 100 °C, 16 h; (f) 5-bromo-2-chloropyridine, $Pd_2(dba)_3$ (0.02 equiv), Xantphos (0.06 equiv), *t*-BuONa (1.5 equiv), toluene (0.1 M), 100 °C, 3 h.

Table 2
Biological properties of biaryl substituted 2,5-diazabicyclo[2.2.1]heptanes

Entry	Compounds	Ar	α7 Binding assay	α7 Functional assay		[^{3}H]-Dofetilide binding assay
			K_i[a] (nM)	EC_{50}[b] (μM)	Max[c] (%)	K_i[d] (μM)
1	**12a**	Phenyl	10	9.5	55	
2	**12b**	Benzofuran-5-yl	14	2.7	75	8.8
3	**12c**	Benzo[*b*]thiophen-5-yl	3.1	1.5	78	1.8
4	**12d**	Indazol-5-yl	0.9	1.1	70	> 55
5	**12e**	Indol-5-yl	0.4	0.9	79	21
6	**12f**	2-CF_3-indol-5-yl	2.1	1.2	69	2.2
7	**12g**	Indol-4-yl	13	2.7	85	> 57
8	**12h**	Indol-6-yl	7.0	1.7	65	12
9	**12i**	2-Methyl-indol-5-yl	0.3	0.4	70	12
10	**14a**	Phenyl	18	5.6	75	26
11	**14b**	Benzofuran-5-yl	61	11	41	5.3
12	**14c**	Benzo[*b*]thiophen-5-yl	35	12	71	1.2
13	**14d**	Indazol-5-yl	6.3	1.5	77	> 65
14	**14e**	Indol-5-yl	1.0	0.54	90	14
15	**14f**	2-CF_3-indol-5-yl	17	12	71	1.9
16	**14g**	Indol-4-yl	26	2.1	84	> 51
17	**14h**	Indol-6-yl	50	3.2	59	9.0
18	**16a**	Phenyl	61	7.4	45	22
19	**16b**	Benzofuran-5-yl	95	27	20	1.3
20	**16c**	Benzo[*b*]thiophen-5-yl	33	33	31	0.56
21	**16d**	Indazol-5-yl	7.6	0.8	83	>35
22	**16e**	Indol-5-yl	1.1	0.7	77	4.3
23	**16f**	2-CF_3-indol-5-yl	15	18	37	1.85
24	**16g**	Indol-4-yl	73	4.6	69	13
25	**18a**	Phenyl	3.1	1.8	51	10
26	**18b**	Benzofuran-5-yl	4.4	4.7	6	2.7
27	**18c**	Benzo[*b*]thiophen-5-yl	2.9	5.3	56	0.55
28	**18d**	Indazol-5-yl	0.3	0.3	72	46
29	**18e**	Indol-5-yl	0.2	1.0	56	11
30	**18f**	2-CF_3-indol-5-yl	3.0	2.8	28	2.0
31	**18g**	Indol-4-yl	10	3.9	59	32
32	**18h**	Indol-6-yl	5.4	1.6	58	2.9

[a] Displacement experiments of [^{3}H]A-585539 in rat brain ($n \geqslant 3$, SEM $\leqslant$ 10%).
[b] Current activation measured by two-electrode voltage clamp using the automated parallel oocyte electrophysiology test station (POETs)[18] and EC_{50} values determined by analysis of concentration–response parameters using the nonlinear curve-fitting in Graphpad Prism.
[c] Responses evoked by test compounds were measured as the evoked peak (maximal) inward current relative to the baseline and normalized to responses evoked by 10 mM ACh in the same oocyte.
[d] [^{3}H]-Dofetilide-isolated membrane binding assay.[19]

18e shows a K_i value of 0.2 nM and indazol-5-yl ligand **18d** had an EC_{50} value of 0.3 μM.

Virtually all of the compounds listed in Table 2 are partial agonists at α7, although a few (entries 7, 14, and 17) do approach full efficacy. Nevertheless, our previous work[20] has indicated that procognitive effects of α7 agonists require very little channel opening, and occur at exposures well below those predicted by EC_{50}'s measured in vitro. Therefore, the compounds in Table 2 exhibit pharmacological profiles consistent with precognitive efficacy in animal models.

In this study, we have discovered that biaryl substituted 2,5-diazabicyclo[2.2.1]heptanes are potent and selective α7 NNR agents. SAR studies established that 5-*N*-methyl, heteroaryl linker and the nature of terminal aryl group are critical for the ligands to achieve potent α7 NNR agonist activity.

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