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Diazaspirocyclic compounds as selective ligands for the α4β2 nicotinic acetylcholine receptor

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ABSTRACT

Diazaspirocyclic ligands have been synthesized in four steps as selective α4β2 nicotinic acetylcholine receptor antagonists. Structural assignment of 1-(pyridin-3-yl)-2-spiropyrrolidino-3,2′-1-azabiclo[2.2.1]heptane **2**, was confirmed using a combination of NMR experiments on a key intermediate, spirolactam **9**. All three target compounds synthesized in this diazaspirocyclic series exhibited high affinity (K_i <35 nM) at the human α4β2 nAChR subtype, and very low affinity for the human α7, α3β4 (ganglion) and α1β1γδ (muscle) subtypes (K_i >500 nM).

Nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated ion channels, and are widely distributed in the mammalian central nervous system (CNS) and peripheral nervous system (PNS). The two most prevalent nAChR subtypes in the CNS are α4β2 and α7.[1] Ligands for these receptors have been recognized as possessing potential for treatment of a variety of conditions and disorders characterized by substantial unmet medical need, including schizophrenia, various pain states, neurodegenerative diseases, and cognitive disorders.[2–19]

S-(-)-Nicotine, Figure 1, the principal alkaloid in tobacco and the prototypical nAChR ligand, possesses high affinity for the α4β2 nAChR (K_i ~2 nM).[1] It is also recognized as a non-selective ligand, with activity at multiple nAChR subtypes.[1,20] This lack of selectivity, particularly with respect to ganglionic α3β4 nAChR subtype, is assumed to be responsible for the undesirable side effects, such as nausea and elevation of heart rate and blood pressure, associated with nicotine use.[20] To create an effective nAChR-based R&D strategy, it is important to design ligands that selectively interact with specific receptor subtypes. Our goal in this particular project was therefore to create ligands with enhanced selectivity for α4β2 receptors over the ganglionic nAChRs in order to minimize the potential for adverse side effects. The lack of available crystal structures of the nicotinic receptors necessitated ligand-based design, in which compounds possessing pharmacophoric elements consistent with nicotinic activity serve as the basis for creation of new ligands.[21] Examples of such selective ligands include the metanicotines and the 2-(arylmethyl)-3-substituted quinuclidines identified by Targacept.[22–26]

Analogs in which the pyrrolidine ring of nicotine has been replaced by an azabicyclic (e.g., the frog toxin epibatidine, TC-2429[27] and TC-2531) scaffold are particularly useful in probing the effects of steric bulk, rigidity and lone pair orientation on

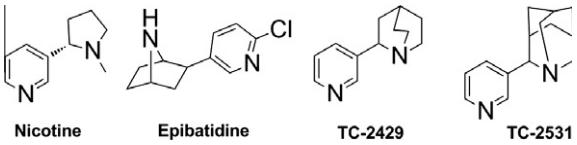

Figure 1. Nicotinic ligands.

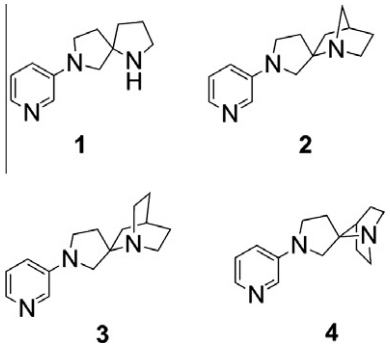

Figure 2. Diazaspirocyclic ligands.

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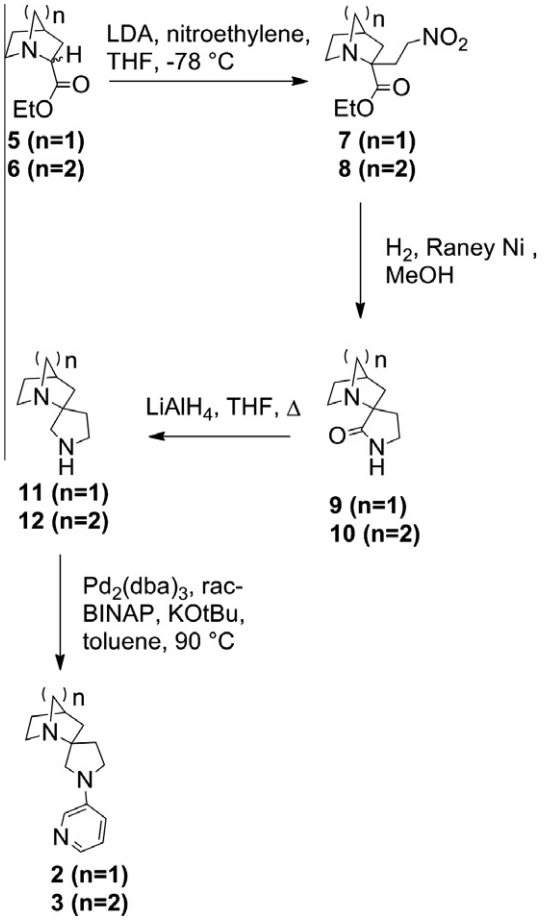
Table 1
In vitro data for 7-(pyridin-3-yl)-1,7-diazaspiro[4.4]nonane (**1**)

Structure	K_i (nM)					Ca Flux	
	H α4β2[a]	R α4β2[b]	Hα7[c]	H Ganglion[d]	H Muscle[e]	H Ganglion[d] EC_{50} (nM)	H Ganglion[d] E_{max} (%)
1	29	75	6900	1200	5700	55,000	9.6

H, human; R, rat. Affinity K_i values were obtained by competitive inhibition of [3H]-nicotine.
[a] SH-EP1 Hα4β2 cells and [3H]-epibatidine.
[b] Rat cortex.
[c] HEK Hα7/RIC3.
[d] SHSY-5Y.
[e] TE-671 cells respectively.
Functional assay (Ca Flux) was performed using a calcium-sensitive fluorescent dye in [a]SH-EP1 Hα4β2 and [d]SHSY-5Y cells respectively.



Scheme 1. Synthesis of 1′-pyridin-3-ylspiro[1-azabicyclo[2.2.1]heptane-2,3′-pyrrolidine] (**2**) and 1′-pyridin-3-ylspiro[1-azabicyclo[2.2.2]octane-2,3′-pyrrolidine] (**3**).

binding and functional activity (Fig. 1).[28–30] Bhatti and co-workers[31] have also shown that a slight modification of the pyrrolidine ring of nicotine has a marked effect on the binding affinity of these molecules toward the α4β2 nAChR subtype.

Thus, by increasing the steric bulk around the cationic nitrogen (i.e., progression from secondary to tertiary amines and then to increasingly congested tertiary amines), a clear progression towards antagonism is observed. These compounds were thus used as templates for the design of a new and novel class of nAChR antagonists which have the unique diazaspirocyclic structural

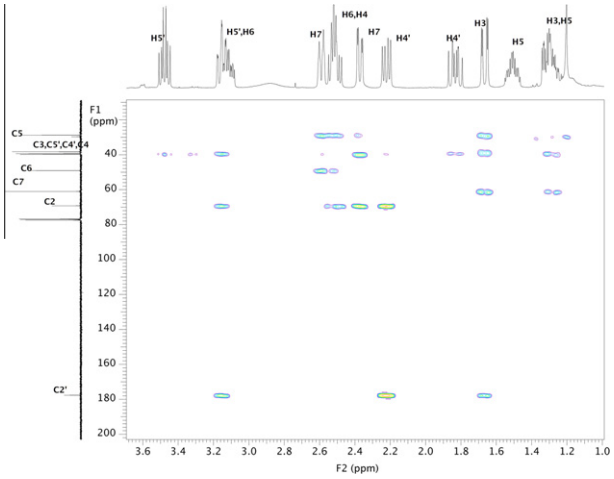


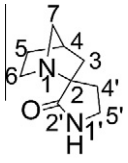
Figure 3. ^1H–^{13}C gHMBC of spirolactam **9**.

feature, exemplified by 7-(pyridin-3-yl)-1,7-diazaspiro[4.4]nonane (**1**) (Fig. 2).[32,33]

The in vitro data for compound **1** is summarized in Table 1.[34] In this Letter we describe the synthesis of structurally related diazaspirocyclic compounds that were designed as competitive antagonists for α4β2 nAChR subtype and which contain a bridged tertiary amine.

In a previous Letter, we described the non-stereoselective synthesis of tertiary bicyclic α-amino acid esters **5, 6** and **13** via the alkylation of either benzophenone imines or nitroacetates.[35] We have now used similar synthetic technology to access new nAChR ligands **2–4**. Alkylation of tertiary bicyclic α-amino acid esters **5** and **6** with LDA and nitroethylene at −78 °C followed by reduction and cyclization with Raney® Ni and H_2 (50 psi) afforded **9** and **10** in ~70% yield for the two steps combined (Scheme 1). Analysis (GCMS, LCMS and ^1H NMR) of **9** indicated that only one of the two diastereomers, which might be expected from this alkylation, had been formed. Indeed, intermediate **7** consists of a 19:1 mixture of diastereomers by ^1H NMR. These diastereomers (of **7**) were separated (only partially, in the case of the minor diastereomer) by flash chromatography, but it was not possible to assign exo or endo configuration by ^1H NMR (see NMR spectra in the Supplementary

Table 2
Peak assignments for 1-azaspiro[bicyclo[2.2.1]heptane-2,3′-pyrrolidin]-2′-one (**9**)



9

^1H Peak at (ppm)	Number scheme	^{13}C Peak at (ppm)
	1	
	2	69.5
1.65, 1.33	3	40.2
2.52	4	38.2
1.52, 1.29	5	28.7
3.12, 2.51	6	49
2.60, 2.38	7	61
	1′	
	2′	177.5
2.21, 1.83	4′	39.3
3.49, 3.16	5′	39.6

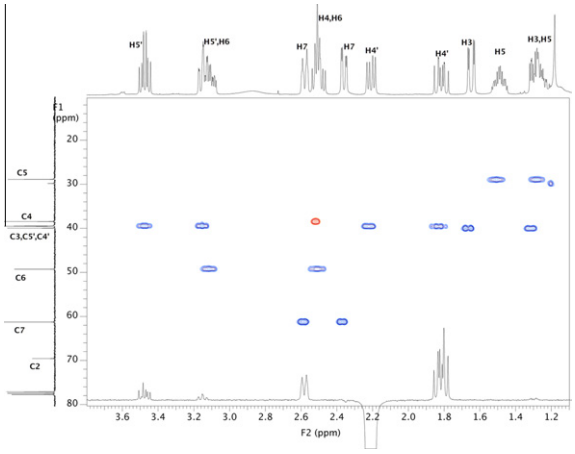


Figure 4. $^{1}H–^{13}C$ gHSQC of spirolactam **9** with a ^{1}H DPFGSE-NOESY 1D spectrum with the resonance at 2.21 ppm selected for excitation on the bottom axis.

data). Thus, the assignment of stereochemistry for the diastereomers of **7** was made based upon the NMR analysis of spirolactam **9**.

The stereochemistry of spirolactam **9** was determined using a combination of NMR experiments collected on a 400MR (Agilent, f/k/a Varian) with VnmrJ 2.2C software. The 2D $^{1}H–^{13}C$ gHMBC experiment optimized for 8 Hz coupling provided identification of the protons in the γ-lactam moiety (Fig. 3). The long-range correlation at 3.16 ppm (H-5′) to 177.5 ppm (C-2′) indentified the proton adjacent to the nitrogen in the γ-lactam moiety. The resonance at 3.16 ppm (H-5′) also displayed correlations to a non-protonated carbon at 69.5 ppm (C-2) and methylene carbon at 39.3 ppm (C-4′), both part of the γ-lactam moiety. The correlations at 2.21 ppm (H-4′) and 1.65 ppm (H-3) to 177.5 ppm (C-2′) indicated those protons are also long-range coupled to the carbonyl. The proton at 1.65 ppm (H-3) showed a correlation to the methine at 38.2 ppm (C-4), and methylenes at 28.7 ppm (C-5) and 61 ppm (C-7). Thus, the proton resonance at 1.65 ppm (H-3) belongs to the methylene on the bicyclo moiety adjacent to the quaternary carbon and the proton resonance at 2.21 ppm (H-4′) belongs to the methylene on the γ-lactam adjacent to the quaternary carbon. For complete proton and carbon assignments see Table 2.

The multiplicity edited 2D $^{1}H–^{13}C$ gHSQC experiment optimized for 140 Hz coupling provided identification of the carbon at 61 ppm (C-7) attached to 2 protons at 2.60 and 2.38 ppm. Selective excitation of the resonance at 2.21 ppm (H-4′) for the ^{1}H DPFGSE-NOESY 1D experiment with a mixing time of one second showed NOE enhancements for resonances on the γ-lactam at 3.49 (H-5′), 3.16 (H-5′), and 1.83 ppm (H-4′), and on the bicyclo moiety at 2.60 ppm (H-7), Figure 4. By observing this NOE to the bicycle moiety, the exo nature of the alkylation of the ethyl ester **5** to give intermediate **7** was determined. This is in accord with the approach of the electrophile from the less hindered exo face of the enolate, Figure 5.

Reduction of spirolactams **9** and **10** with $LiAlH_4$ gave the diazaspirocyclic scaffolds **11** and **12** in almost quantative yield. Using

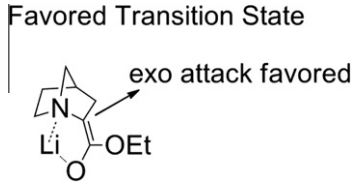
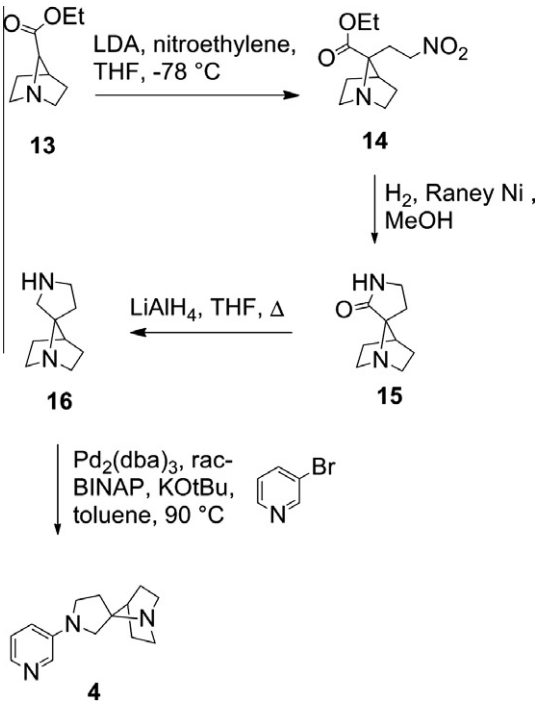


Figure 5. Proposed transition state for alkylation of **5**.



Scheme 2. Synthesis of 1′-pyridin-3-ylspiro[1-azabicyclo[2.2.1]heptane-7,3′-pyrrolidine] **4**.

standard Buchwald[36–40] coupling conditions, **11** and **12** were coupled with 3-bromopyridine to afford the desired target compounds **2** and **3** in ~80% yield. The 2nd targeted chemotype, 1′-pyridin-3-ylspiro[1-azabicyclo[2.2.1]heptane-7,3′-pyrrolidine] (**4**), was synthesized in good yield following chemistry established for **2** and **3** as illustrated in Scheme 2.

The diazaspirocyclic compounds synthesized (**2–4**) exhibited high affinity to the α4β2 nAChR subtype, as demonstrated by their inhibition of radiolabeled [^{3}H]-nicotine binding in SH-EP1 H α4β2 cells, with binding affinity (K_i) values below 35 nM.[41] High throughput screening indicates that none of the compounds bound to α7 receptors[41] with any significant affinity (K_i values >7.5 μM). Compounds **2–4** showed good antagonist activity at hα4β2 receptors (92–97% of nicotine response, data not shown in tables). In addition, compounds showed little activity at activation of

Table 3
In vitro data for 1′-pyridin-3-ylspiro[1-azabicyclo[2.2.1]heptane-2,3′-pyrrolidine] (**2**),1′-pyridin-3-ylspiro[1-azabicyclo[2.2.2]octane-2,3′-pyrrolidine] (**3**) and 1′-pyridin-3-ylspiro[1-azabicyclo[2.2.1]heptane-7,3′-pyrrolidine] (**4**)

Structure	K_i (nM)					Ca Flux	
	H α4β2[a]	R α4β2[b]	H α7[c]	H Ganglion[d]	H Muscle[e]	H Ganglion[d] EC_{50} (nM)	H Ganglion[d] E_{max} (% nic)
2	29	40	7900	2400	9900	31,000	3.1
3	32	5.7	8000	1100	11,000	6900	4.2
4	10	34	7600	560	580	2600	29

H, human; R, rat. Affinity K_i values were obtained by competitive inhibition of [3H]-nicotine.
[a] SH-EP1 Hα4β2 cells, [3H]-epibatidine.
[b] Rat cortex.
[c] HEK Hα7/RIC3.
[d] SHSY-5Y.
[e] TE-671 cells respectively. Functional assay (Ca Flux) was performed using a calcium-sensitive fluorescent dye in [a] SH-EP1 Hα4β2 and [d] SHSY-5Y cells respectively.

ganglion-type receptors (α3β4 subtype in human SHSY-5Y clonal cells, 1–30% of nicotine response, Table 3). The binding data for target compounds **2–4** indicate selectivity for α4β2 nAChRs. The diazaspirocylic compounds are selective antagonists at the α4β2 with little activity at ganglion-type nAChR subtype, are novel chemotypes, representing new and potentially useful pharmacologic tools.

Acknowledgments

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Supplementary data

Supplementary data (full experimental details and NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.108.

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41. Binding affinity (K_i) values were determined from two to six competitive inhibition of [3H]-nicotine or [3H]-epibatidine binding for each compound. Values for concentrations producing 50% of the maximal activation response (EC_{50}) and the maximal activation response (E_{max}) were determined from two to six functional assay (Ca Flux) performed using a calcium-sensitive fluorescent dye. Both K_i and Ca Flux data are expressed as mean.