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Improved synthesis and *in vitro* evaluation of quinuclidin-2-ene based ligands for the nicotinic acetylcholine receptor

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In the search for compounds with agonistic or antagonistic effects at the muscarinic receptors a series of achiral 3-heteroaryl substituted quinuclidin-2-ene derivatives have been synthesized and evaluated [1, 2]. The most potent ligands comprising a monocyclic heteroaromatic ring were the 2-thienyl- and 2-furanyl-substituted ligands **1** and **2** with moderate affinities for the cortical muscarinic receptor ($M_1 : K_i = 290$ and 300 nM, respectively) (Scheme). Structure-activity relationship (SAR) studies demonstrated that the affinity of type **2** ligands for muscarinic receptors could be enhanced more than 1000-fold by an appropriate substitution e.g. to the corresponding *m*-hydroxyphenyl variant **3** [1]. Bioisosteric replacement of the furanyl moiety by a pyridine ring proved to be detrimental lowering the affinity of the resulting ligand **4** more than sevenfold as compared to the furan **2** [2].

However the obvious structural relationship of **4** to the highly potent semirigid nAChR agonists (−)-epibatidine (**5**) [3] and UB 165 (**6**) [4, 5] gave rise to investigate compounds of type **4** as novel nAChR ligands. It was anticipated that the design, synthesis and biological evaluation of quinuclidin-2-ene derivatives such as **4** might achieve selectivity for central versus ganglionic nAChRs and possibly contribute to a further understanding of SAR also at the nicotinic in addition to the muscarinic receptor family [6, 7].

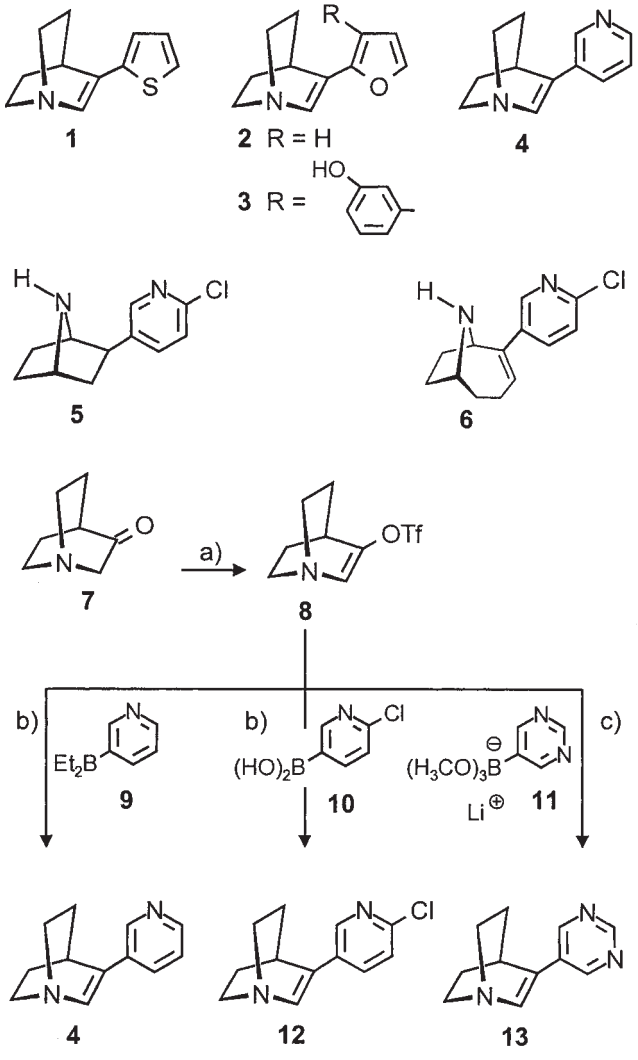
A particularly attractive feature for the synthesis of quinuclidin-2-ene based compounds such as **4** seemed to be an approach using commercial 3-quinuclidone (**7**) as starting material. Unfortunately the known multistep synthesis using this useful precursor only afforded moderate yields of ligand **4** [8]. Thus we undertook an alternative more efficient synthetic pathway distinctly different from the previous approaches starting with the novel vinyl triflate **8**. This was easily accessible using lithium diisopropylamide (LDA) in tetrahydrofuran at −80 °C to prepare the corresponding lithium enolate of **7**, which was converted with Comins reagent [*N*-(5-chloro-2-pyridyl-triflimide)] to the ketone-derived vinyl triflate **8** in more than 90% yield [5]. Most promising for the introduction of the 3-pyridyl unit into the bulky quinuclidine moiety seemed to be an approach utilizing a Suzuki-type cross coupling [5, 9], the well-known palladium-catalyzed reaction of organoboron compounds with an organic electrophile as the pivotal step. Thus, the vinyl triflate **8** and 3-diethylboranylpyridine (**9**) [9] were examined as appropriate starting materials for the synthesis of the target ligand **4**. The 3-pyridyl

Table: Radioligand binding affinities of three quinuclidin-2-ene based ligands 4, 12 and 13 to $(\alpha4)_2(\beta2)_3$ and α7* nAChRs in comparison with (−)-nicotine, (±)-epibatidine and UB 165[a]

Compd.	$(\alpha4)2(\beta2)_3$[b] (±)-[^{3}H]-epibatidine rat brain K_i(nM)	α7*[b] [^{3}H]MLA rat brain K_i(nM)
(−)-Nicotine	0.84 ± 0.132	130 ± 10 [^{125}I] α-BTX
(±)-Epibatidine	0.008 ± 0.001	4 ± 0.5 [^{125}I] α-BTX
UB 165	0.04 ± 0.004	12 ± 2.5
4	7.6 ± 0.49	85.2 ± 2.7
12	2.2 ± 0.52	26.8 ± 4.1
13	12.2 ± 0.18	751 ± 52.3

[a] Values represent mean ± SEM obtained from n independent experiments where n = 3–5
[b] Naturally expressed nAChRs

Scheme



Reagents and conditions: (a) 1. LDA, THF, −80 °C; 2. 2-N(Tf_2)-5-chloropyridine, 12 h, −80 °C ⇒ RT. 94%. (b) $Pd(PPh_3)_2Cl_2$, THF, 2 M aqueous Na_2CO_3, 18 h, 80 °C, 53% and 67%. (c) analogous (b) in THF : ethanol = 3 : 1, 58%

group was successfully introduced into the 3-position of the azabicycle by reacting binyl triflate **8** with the borane **9** in THF using bis(triphenylphosphane)palladium(II) chloride as catalyst (0.01 equiv.) and 2 M aqueous sodium carbonate as a nucleophilic activator. The coupling proceeded with satisfying success to give the target compound **4** in 53% isolated yield. A similar approach for introduction of the 2-chloropyridine nucleus into the azabicycle utilized the stable 2-chloro-5-pyridyl boronic acid **10**. Under the same reaction conditions as before cross coupling of the vinyl triflate **8** proceeded in 67% yield to the hitherto unknown nAChR ligand **12**. In addition, lithium trimethoxy(5-pyrimidyl)boronate (**11**) offered an elegant access to the pyrimidine-substituted target ligand **13** [10]. Cross-coupling with the vinyl triflate **8** could be achieved under similar conditions as described before affording the coupling product **13** with 68% yield. The nAChR ligands **4**, **12** and **13** exhibited the expected ^{1}H and ^{13}C NMR, IR, and MS characteristics and gave satisfactory high-resolution MS data.

Studies of the in vitro affinity for $(\alpha4)_2(\beta2)_3$ and $\alpha7$* nAChR subtypes, predominant in the central nervous system, by previously described [5, 11–13] radioligand binding assays demonstrated that the quinuclidin-2-ene based compounds **4**, **12** and **13** can be considered as nAChR ligands featuring affinities at neuronal nAChRs in the low nanomolar range (Table). Compared to (−)-nicotine, (±)-epibatidine or UB 165 ligands **4**, **12** and **13** bind with distinctly lower affinity and selectivity to the nAChR subtypes under consideration. The 2-chloropyridine-containing ligand **12** turned out to be the most active quinuclidin-2-ene based species, 3-fold less active at the $(\alpha4)_2(\beta2)_3$ subtype and 13-fold less selective than (−)-nicotine.

Experimental

For "general procedures", "*in vitro* binding studies", "membrane preparation", "binding assays" and "data analysis" see literature [5, 11–13].

1. Trifluoromethansulfonic acid-1-azabicyclo[2.2.2]oct-2-en-3-yl ester (**8**)

A solution of ketone **7** (0.60 g, 4.8 mmol) in dry THF (5 mL) was added dropwise to a freshly prepared solution of LDA [from diisopropylamine (0.56 g, 5.6 mmol) in THF (15 mL) and BuLi (3.4 mL of a 1.6 M solution, 5.4 mmol in hexane)]. After stirring for 2 h under argon a solution of *N*-(5-chloro-2-pyridyl)triflimide (2.20 g, 5.1 mmol, freshly Kugelrohr destilled) in dry THF (5 mL) was added in one portion. The mixture was stirred at −80 °C for 12 h, then allowed to warm to RT and stirred again for 12 h. The solvent was evaporated in vacuo and the residue purified by column chromatography on silica gel (column 4 × 30 cm with ethyl acetate) to provide **8** as a yellowish oil (123 mg, 94%): R_f 0.24 (ethyl acetate); IR (film): 3062 cm^{-1}, 2957, 1644, 1298. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.71–1.74 (m, 4 H, 5-H and 8-H), 2.50–2.60 (m, 2 H, 7-H), 2.74–2.75 (m, 1 H, 4-H), 2.83–2.90 (m, 2 H, 6-H), 6.36 (d, 1 H, 2-H, J = 2.2 Hz). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ = 25.6, 29.3, 31.5, 46.8, 48.8, 119.2 (q, CF_3, J_{CF} = 320 Hz), 130.3, 156.1. MS (70 eV) m/z (%) = 257 (M^+, 47), 96 (100). Exact mass calcd for $C_8H_{10}F_3NO_3S$: 257.0333, found: 257.0327.

2. 3-(Pyridin-3-yl)-1-azabicyclo[2.2.2]oct-2-ene (4)

To a solution of bis(triphenylphosphane)palladium(II) chloride (8 mg, 0.01 mmol) and the organoborane **9** (220 mg, 1.4 mmol) in THF (5 mL) an aqueous solution of sodium carbonate (2 M, 2 mL) was added and the mixture heated to 80 °C. Then a solution of triflate **8** (260 mg, 1.0 mmol) in THF (5 mL) was added dropwise and the mixture heated at 80 °C for 18 h. Water (20 mL) was added and the mixture extracted with dichloromethane (4 × 30 mL). The combined organic phases were dried with Na_2SO_4, filtered and the solvent evaporated in vacuo. The residue was purified by column chromatography on silica gel (column 2 × 10 cm, CH_2Cl_2 : CH_3OH = 3 : 1) to yield an amorphous powder (99 mg, 53%), m.p. 54–56 °C, R_f = 0.35 (CH_2Cl_2/CH_3OH 3 : 1). IR (KBr): 2955 cm^{-1}, 2866, 1610, 1484. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.51–1.52 (m, 2 H, 5-H or 8-H), 1.72–1.74 (m, 2 H, 5-H or 8-H), 2.58–2.60 (m, 2 H, 6-H or 7-H), 2.95–2.98 (m, 2 H, 6-H or 7-H), 3.07–3.08 (m, 1 H, 4-H), 6.81 (d, ^{4}J = 1.6 Hz, 1 H, 2-H), 7.20–7.21 (m, 1 H, 4′-H), 7.61–7.62 (m, 1 H, 5′-H), 8.43–8.44 (m, 1 H, 6′-H), 8.60–8.61 (d, J = 1.5 Hz, 1 H, 2′H,). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ = 28.2 (2C), 29.2, 48.9 (2C), 123.4, 132.0, 132.5, 139.0, 144.2, 146.3, 148.5. MS (70 eV) m/z (%) = 186 (M^+, 26), 43 (100). Exact mass calcd. for $C_{12}H_{14}N_2$: 186.1156; found: 186.1152.

3. 3-(6-Chloro-pyridin-3-yl)-1-azabicyclo[2.2.2]oct-2-ene (12)

According to the same protocol as described for the synthesis of ligand **4** 147 mg (67%) of compound **12** were obtained as a yellow wax, m. p. 57–59 °C, from 260 mg (1 mmol) of triflate **8** and 220 mg (1.4 mmol) of the organoborane **10**. IR (KBr): 3024 cm^{-1}, 2947, 1611, 1580. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.45–1.60 (m, 2 H, 5-H or 8-H), 1.75–1.85 (m, 2 H, 5-H or 8-H), 2.53–2.65 (m, 2 H, 6H- or 7-H), 2.92–3.02 (m, 2 H, 6-H or 7-H), 3.03–3.06 (d, ^{4}J = 1.6 Hz, 1 H, 4-H), 6.81–6.82 (d, ^{4}J = 1.6 Hz, 1 H, 2-H), 7.19–7.22 (m, 1 H, 3′-H), 7.58–7.59 (m, 1 H, 4′-H), 8.35 (d, ^{4}J = 2.3 Hz, 6′-H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ = 28.0 (2C), 29.2, 48.9 (2C), 124.1, 131.3, 134.9, 139.0, 143.1, 145.9, 150.2. MS (70 eV) m/z (%) = 220 (M^+, 100). Exact mass calcd for $C_{12}H_{13}ClN$: 220.0767, found 220.0762.

4. 3-(Pyrimidin-5-yl)-1-azabicyclo[2.2.2]oct-2-ene (13)

According to the same protocol as described for the synthesis of ligand **4** 127 mg (68%) of compound **13** were obtained as a yellowish powder, m. p. 67–69 °C from 260 mg (1 mmol) of triflate **8** and 530 mg (3 mmol) of the organoborane **11**. IR (KBr): 2948 cm^{-1}, 2876, 1550, 1413. ^{1}H NMR (500 MHz, [D_6]DMSO) δ = 1.46–1.47 (m, 2 H, 5-H or 8-H), 1.71–1.72 (m, 2 H, 5-H or 8-H), 2.48–2.49 (m, 2 H, 6-H or 7-H), 2.88–2.90 (m, 2 H, 6-H or 7-H), 3.19 (d, ^{4}J = 1.7 Hz, 1 H, 4-H), 7.05 (d, ^{4}J = 1.7 Hz, 1 H, 2-H), 8.91 (s, 2 H, 4′-H and 6′-H), 9.05 (s, 1 H, 2′-H). ^{13}C NMR (125.8 MHz, [D_6]DMSO) δ = 27.5 (2C), 39.9, 48.2 (2C), 129.6, 141.0, 141.1, 152.7 (2C), 156.9. MS (70 eV) m/z (%) = 187 (M^+, 100). Exact mass calcd for $C_{11}H_{13}N_3$: 187.1109, found 187.1115.

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