

Institut für Pharmazeutische Chemie der Philipps-Universität Marburg and Institut für Pharmazeutische Chemie der Rheinischen Friedrich-Wilhelms-Universität Bonn, Germany

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

S. SEIFERT, D. GÜNDISCH, M. C. TILOTTA, G. SEITZ

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Univ.-Prof. Dr. Gunther Seitz, Institut für Pharmazeutische Chemie, Marbacher Weg 6, D-35032 Marburg/Lahn, Germany

seitzg@mail.uni-marburg.de

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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

Table: Radioligand binding affinities of three quinuclidin-2-ene based ligands **4, **12** and **13** to $(\alpha 4)_2(\beta 2)_3$ and $\alpha 7^*$ nAChRs in comparison with (–)-nicotine, (±)-epibatidine and UB 165^a**

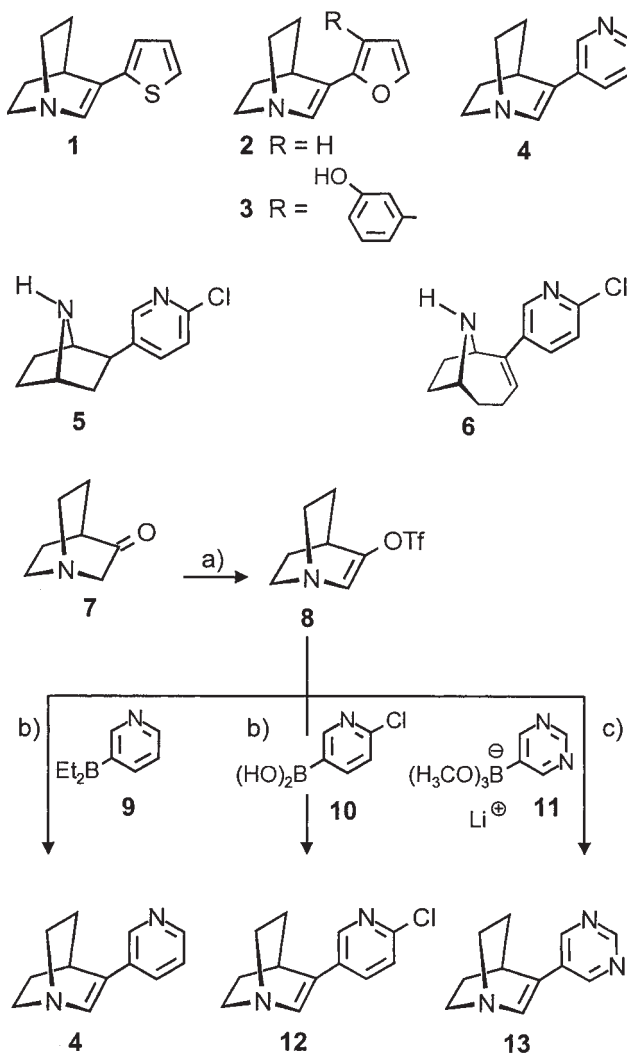
Compd.	$(\alpha 4)_2(\beta 2)_3^b$ (±)-[³ H]-epibatidine rat brain K_i (nM)	$\alpha 7^*b$ [³ H]MLA rat brain K_i (nM)
(–)-Nicotine	0.84 ± 0.132	130 ± 10 [¹²⁵ I] α -BTX
(±)-Epibatidine	0.008 ± 0.001	4 ± 0.5 [¹²⁵ I] α -BTX
UB 165	0.04 ± 0.004	12 ± 2.5
4	7.6 ± 0.49	85.2 ± 2.7
12	2.2 ± 0.52	26.8 ± 4.1
13	12.2 ± 0.18	751 ± 52.3

^a Values represent mean $\pm$ SEM obtained from *n* independent experiments where *n* = 3–5

^b Naturally expressed nAChRs

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

Scheme



Reagents and conditions: (a) 1. LDA, THF, -80°C ; 2. *N*-(5-chloro-2-pyridyl)-triflimide, 12 h, $-80^\circ\text{C} \Rightarrow \text{RT}$, 94%. (b) $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, 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 azabicyclo by reacting vinyl 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 azabicyclo 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 ^1H and ^{13}C NMR, IR, and MS characteristics and gave satisfactory high-resolution MS data.

Studies of the *in vitro* affinity for $(\alpha 4)_2(\beta 2)_3$ and $\alpha 7^*$ 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 $(\alpha 4)_2(\beta 2)_3$ 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 distilled) 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. ^1H NMR (400 MHz, CDCl_3) δ = 1.71–1.74 (m, 4H, 5-H and 8-H), 2.50–2.60 (m, 2H, 7-H), 2.74–2.75 (m, 1H, 4-H), 2.83–2.90 (m, 2H, 6-H), 6.36 (d, 1H, 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 $\text{C}_8\text{H}_{10}\text{F}_3\text{NO}_3\text{S}$: 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. ^1H NMR (400 MHz, CDCl_3) δ = 1.51–1.52 (m, 2H, 5-H or 8-H), 1.72–1.74 (m, 2H, 5-H or 8-H), 2.58–2.60 (m, 2H, 6-H or 7-H), 2.95–2.98 (m, 2H, 6-H or 7-H), 3.07–3.08 (m, 1H, 4-H), 6.81 (d,

4J = 1.6 Hz, 1H, 2-H), 7.20–7.21 (m, 1H, 4'-H), 7.61–7.62 (m, 1H, 5'-H), 8.43–8.44 (m, 1H, 6'-H), 8.60–8.61 (d, J = 1.5 Hz, 1H, 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 $\text{C}_{12}\text{H}_{14}\text{N}_2$: 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. ^1H NMR (400 MHz, CDCl_3) δ = 1.45–1.60 (m, 2H, 5-H or 8-H), 1.75–1.85 (m, 2H, 5-H or 8-H), 2.53–2.65 (m, 2H, 6-H or 7-H), 2.92–3.02 (m, 2H, 6-H or 7-H), 3.03–3.06 (d, 4J = 1.6 Hz, 1H, 4-H), 6.81–6.82 (d, 4J = 1.6 Hz, 1H, 2-H), 7.19–7.22 (m, 1H, 3'-H), 7.58–7.59 (m, 1H, 4'-H), 8.35 (d, 4J = 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 $\text{C}_{12}\text{H}_{13}\text{ClN}$: 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. ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$) δ = 1.46–1.47 (m, 2H, 5-H or 8-H), 1.71–1.72 (m, 2H, 5-H or 8-H), 2.48–2.49 (m, 2H, 6-H or 7-H), 2.88–2.90 (m, 2H, 6-H or 7-H), 3.19 (d, 4J = 1.7 Hz, 1H, 4-H), 7.05 (d, 4J = 1.7 Hz, 1H, 2-H), 8.91 (s, 2H, 4'-H and 6'-H), 9.05 (s, 1H, 2'-H). ^{13}C NMR (125.8 MHz, $[\text{D}_6]\text{DMSO}$) 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 $\text{C}_{11}\text{H}_{13}\text{N}_3$: 187.1109, found 187.1115.

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