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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4727–4730
Bioorganic & Medicinal Chemistry Letters

High affinity ligands for the α7 nicotinic receptor that show no cross-reactivity with the 5-HT_3 receptor

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Received 28 August 2003; revised 14 July 2005; accepted 26 July 2005
Available online 13 September 2005

Abstract—Potent and selective ligands of the α7 nicotinic acetylcholine receptor are required to understand the pharmacological effect of α7 activation. A common cross-reactivity occurs with serotonergic 5-HT_3 receptors with which α7 receptors have a high sequence homology. We demonstrate that certain quinuclidine 3-biaryl carboxamides are high affinity α7 ligands with an excellent binding selectivity over 5-HT_3 receptors.

1. Introduction

There is a high sequence homology between 5-HT_3 serotonergic receptors (5-HT_3Rs) and α7 nicotinic acetylcholine receptors (nAChRs).[1] Both of these receptors are ligand-gated ion channels and are thought to play important roles in cognition and schizophrenia.[2] Recent reports have highlighted the crossover in functional activity of ligands with affinity for both these receptors.[3,4,6] Typically, agonist activity at one receptor translates into antagonist activity at the other. For example, tropisetron (**1**), a 5-HT_3 antagonist, was shown to act as a weak α7 partial agonist, whilst the well-known α7 partial agonist DMXB (GTS-21) acted as a 5-HT_3 antagonist.[4,7] We examined the cross-reactivity profile of a potent α7 agonist reported by AstraArcus (**2**).[5] It was demonstrated that **2** was able to evoke currents in native neuronal 5-HT_3Rs with potency and efficacy similar to those evoked in native α7 nAChRs.[6] An understanding of the dual nature of ligands for these two receptors is relevant in the study of the pharmacological effects of receptor activation. There is clearly a need to develop selective compounds to undertake this. This is of particular interest for the α7 nAChR, as the dearth of selective and potent tools for pharmacological mapping has left us with only tantalizing hints on the role of α7 nAChRs in human physiology.

From a small selection of ligands profiled on both 5-HT_3Rs and α7 nAChRs, it is not clear what structural features will lead to selectivity for one over the other. Sequence homology modeling of the ligand binding domain of α7 and 5-HT_3 based on the snail ACh binding protein could be a useful tool for understanding the structural requirements for selectivity,[8] but so far there have been no reports of it being used in this context. Ligands with good binding affinity for the α7 receptor are typically constrained analogues of ACh. The azabicycle quinuclidine is a common structural motif in many reported α7 ligands, as is an aryl group extended at some point into space from the tertiary nitrogen.[9] In a recent study, Macor et al.[3] highlighted that modification in either domain could influence the affinity for both α7 nAChRs and 5-HT_3Rs. When quinuclidine is replaced by the azabicyclo[3.2.1]octane skeleton of tropisetron (**1**), activity is retained at both receptors, but N-methylation of the indazole of structurally related LY-278584 (**3**) (also a 5-HT_3 receptor antagonist) removed the α7 activity. We decided that the effect of such simple structural modifications on selectivity merited investigation.

A series of structurally related compounds has been disclosed in the patent literature as α7 agonists, but their pharmacology has not been reported.[10] A recent disclosure of quinuclidine benzamides as α7 agonists, profiled on functional α7 (α7-5HT_3) chimeric receptors, highlights some SAR differences between α7 nAChR and 5-HT_3R activity.[11] We had synthesized several α7 ligands in-house (Fig. 1) (**2**,[5] **4**,[10a] and

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doi:10.1016/j.bmcl.2005.07.070

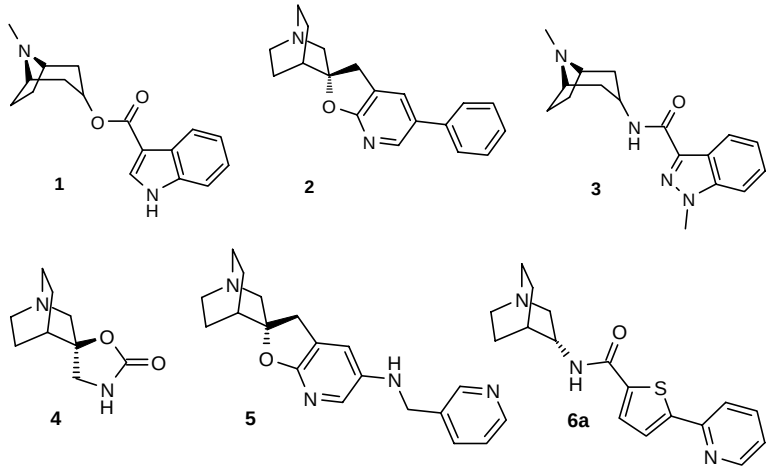


Figure 1. Literature $5HT_3R$ antagonists and α7 nAChR agonists.

5[10b]) and profiled them across human recombinant 5-HT_3 and α7 receptors in radioligand binding assays (Table 1).[12] Independently, we had also been investigating quinuclidine biaryl carboxamides as α7 agonists, for example, **6a**, subsequently disclosed in the literature,[10c] and had discovered that **6a** was selective for α7 receptors over 5-HT_3 receptors. To investigate this finding further, a small set of associated biaryl carboxamide ligands were prepared and assayed for cross-reactivity.

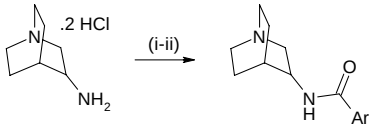
2. Chemistry

A general method was devised to prepare racemic compounds via parallel synthesis, as described in Scheme 1.[13] (*rac*)-*3*-Amino quinuclidine, liberated from the dihydrochloride salt, was treated with an excess of the appropriate aryl carboxylic acid in the presence of 1-hydroxybenzotriazole monohydrate and *N*-cyclohexylcarbodiimide-*N*′-methylpolystyrene in amine-free DMF. Following agitation at ambient temperature for 3 days, the product was isolated by cation-exchange chromatography (SCX) and purified further by preparative LC–MS. Compounds were characterized by ^1H NMR and LC–MS, and tested for their affinity on 5-HT_3 and α7 receptors in radioligand binding assays.

Table 1. Binding affinities for compounds **2** and **4–6**

Compounds	α7 nAChR[a] K_i (nM)	5-HT_3R[b] K_i (nM)
MLA[c]	7.3 (±0.2)	16.5% at 10 μM (1)
MDL-72222	Not tested	10 (2)
(R)-**2**	3.1 (±5.8)	73 (2)
(R)-**4**	420 (±36)	2.5% at 10 μM (1)
(R)-**5**	8.3 (±1.2)	2225 (2)
(R)-**6a**	1.3 (±0.2)	15.5% at 10 μM (1)

[a] SEM for $n \geqslant 3$.
[b] Number of determinations in parentheses.
[c] MLA is methyllycaconitine, a selective nicotinic α7 receptor antagonist.

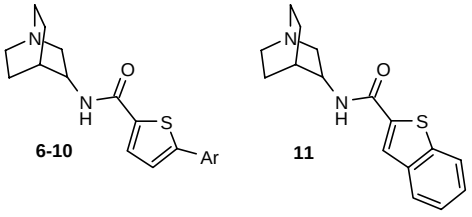


Scheme 1. Reagents and conditions: Ar = aryl acids for **6–14**. (i) Free base liberation; (ii) $ArCO_2H$, HOBT, resin-CDI, DMF, room temperature, 3 days.

3. Results and discussion

It is clear from the data in Table 2 that the biaryl compounds **6–10** were highly selective for α7 nAChRs over 5-HT_3Rs, in contrast to the fused heteroaryl benzothiophene (**11**), which was essentially non-selective. The influence of electron density in the distal aryl ring on both α7 and 5-HT_3 affinities in this series is not clear (**6–10**). Although the pyridyl-containing ligand **6** had the best α7 affinity/5-HT_3 selectivity profile, the more electron-rich bis-thiophene **8** was the most potent α7

Table 2. Binding affinities for compounds **6–11**



(*rac*)-Ar	α7 nAChR[a] K_i (nM)	5-HT_3R[b] K_i (nM)
2-Pyridine (**6**)	6.9 (±0.6)	28% at 10 μM (1)
Ph (**7**)	3.4 (±0.9)	2024 (2)
2-Thiophene (**8**)	1.1 (±0.1)	1043 (2)
p-$MeOC_6H_4$ (**9**)	268.4 (±29.8)	27% at 10 μM (1)
p-ClC_6H_4 (**10**)	524.5 (±51.0)	45% at 10 μM (1)
11	14.6 (±0.8)	49.8 (2)

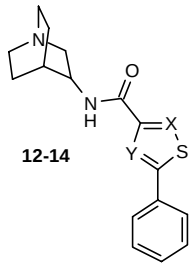
[a] SEM for $n \geqslant 3$.
[b] Number of determinations in parentheses.

ligand prepared and was still 1000-fold selective for α7 receptors over 5-HT_3 receptors.

The degree of selectivity attained was not universal within the biaryl-containing compounds. Although we did not fully investigate the effect of positional isomers on selectivity, we discovered that moving the connectivity of the thienyl amide substituent from C2 to C3 decreased selectivity from >500-fold to 20-fold in the phenyl series (Table 3, **12**). Introduction of a nitrogen atom into the C3-linked thiophene series also had an interesting effect. The thiazole analogue (**13**) proved essentially non-selective, whilst the isothiazole analogue (**14**) only displayed affinity for the α7 receptor (Table 3).

In contrast to the previous observation of minimal influence of stereogenicity on the quinuclidine-tropisetron hybrid ligands,[3] we established that the α7 affinity of the quinuclidine biaryl carboxamide series, for example, **7** resided in the (*R*)-enantiomer (Table 4).[14] Selectivity for α7 nAChRs over 5-HT_3Rs was also improved by resolution in this instance.

Table 3. Binding affinities for compounds **12–14**

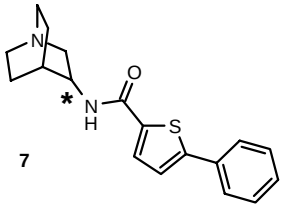


12-14

(*rac*)-X, Y	α7 nAChR[a] K_i (nM)	5-HT_3R[b] K_i (nM)
X=Y=CH (**12**)	16.7 (±2.6)	409 (2)
X=CH, Y=N (**13**)	121.2 (±7.1)	665 (2)
X=N, Y=CH (**14**)	4.1 (±0.2)	28% at 10 μM (1)

[a] SEM for $n \geqslant 3$.
[b] Number of determinations in parentheses.

Table 4. Binding affinities for compound **7**



7

Compounds	α7 nAChR[a] K_i (nM)	5-HT_3R[b] K_i (nM)
Racemate (**7**)	3.4 (±0.9)	2024 (2)
(*R*)-(**7a**) (AcOH salt)	1.8 (±0.9)	24% at 10 μM (1)
(*S*)-(**7b**) (AcOH salt)	26.9 (±0.2)	1547 (2)

[a] SEM for $n \geqslant 3$.
[b] Number of determinations in parentheses.

4. Summary

We have prepared and pharmacologically characterized a set of quinuclidine biaryl carboxamides that displayed high affinity for α7 nAChRs and excellent selectivity over 5-HT_3Rs. Whilst the structural requirements for selectivity remain to be fully defined, the connectivity of the biaryl motif and relative electron density of the biaryl system have been shown to be influential. As interest in the α7 nAChR as a therapeutic target grows, the ability to design selective ligands to help understand any associated pharmacology becomes ever more important.

Acknowledgment

The authors thank Dr. John Schaus for help in preparing this manuscript.

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12. Binding assay conditions: (a) α7 nAChR: 10 nM [^{3}H]methyllycaconitine (MLA), human nAChR α7 membranes (expressed in GH4C1 cells), 500 μg/mL; buffer (50 Trizma, 150 NaCl, 5 KCl, pH 7.4, 4 °C; nM), 4 h, ambient temperature; NSB = unlabeled MLA, 10 μM; Wheatgerm Agglutinin SPA Bead (Amersham Biosciences PRNQ0001), 10 mg/mL; (b) 5-HT$_3$R: [^{3}H]GR65630 0.34 nM, human 5-HT$_3$ serotonin receptor membrane (Receptor Biology Inc. RB-HS3) 0.143 mg/mL; buffer (50 Trisma, 5 $MgCl_2$, 1 EDTA, pH 7.5, 4 °C, nM); wash buffer PBS; 1 h, ambient temperature; NSB = MDL-72222, 10 μM; 3 washes with wash buffer through Whatman GF/B filters.
13. Synthesis of **8**. 3-Aminoquinuclidine hydrochloride (24.4 mg, 0.15 mmol) was suspended in amine-free *N*,*N*-dimethylformamide (DMF, 1.5 mL) and treated with Amberlite IRA-440C strong anion-exchange resin. The mixture was agitated until all of the quinuclidine was basified and dissolved, forming a 0.05 M solution. A fritted vessel was charged sequentially with *N*-cyclohexylcarbodiimide-*N*′-methylpolystyrene (Nova, 56 mg, 1.8 mmol/g, 0.1 mmol), an aliquot (0.5 mL, 0.025 mmol) of the 0.05 M quinuclidine solution, an aliquot (0.5 mL, 0.05 mmol) of a 0.1 M solution of 2,2′-bithiophene-5-carboxylic acid in amine-free DMF, and an aliquot (0.5 mL, 0.05 mmol) of a 0.1 M solution of 1-hydroxybenzotriazole monohydrate in amine-free DMF. The vessel was sealed and agitated by orbital rotation for 3 days at room temperature and then the solution-phase materials were collected by filtration into a vial. The spent resin was washed with DMF (1 mL), methanol (1 mL), and DMF (1 mL). The combined filtrate and washings were applied to a methanol-conditioned 500 mg SCX-2 cation-exchange cartridge (Argonaut, 0.5 mmol/g SO_3H). Solution-phase materials were allowed to pass to waste. The cartridge was washed with one volume of methanol, which was also passed to waste. Then the cartridge was then treated with one volume of 2 N ammonia in methanol, which eluted the product into a tared vial. Evaporation of solvents in vacuo yielded **8** (7.9 mg, 0.0248 mmol, 98%) in 100% purity. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.81 (1H, d, J = 6.03 Hz), 7.93 (1H, d, J = 3.77 Hz), 7.58 (1H, d, J = 5.27 Hz), 7.42 (1H, d, J = 3.39 Hz), 7.34 (1H, d, J = 3.77 Hz), 7.08–7.17 (1H, m), 4.22–4.33 (1H, m), 3.11–3.69 (6H, m), 2.17 (2H, m), 1.82–1.96 (2H, m), 1.63–1.81 (1H, m); LC–MS 100% pure (single peak, $[M+H]^+$ 319).
14. (*R*)-(+)- and (*S*)-(−)-3-Aminoquinuclidine dihydrochloride 98%, available from the Aldrich Chemical Company Inc.