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Quinuclidines as selective agonists for alpha-7 nicotinic acetylcholine receptors

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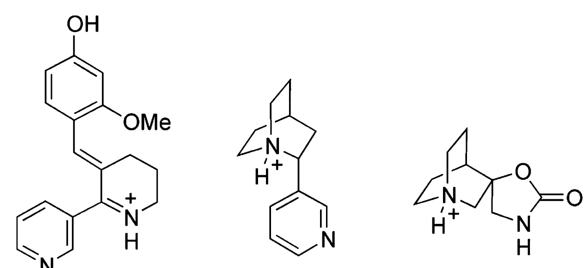
Abstract—The α7 subtype of the neuronal nicotinic acetylcholine receptors (nAChRs) was targeted for the design of selective agonists deriving from the quinuclidine scaffold. Arylidene groups at the 3-position and *N*-methyl quinuclidine were found to be selective agonists with EC_{50}s of 1.5 and 40 μM, respectively.

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels, permeable to alkali cations once they have been activated by extracellular binding of an agonist.[1] Numerous subtypes of the receptor are known and may be distinguished both by their subunit composition and pharmacological behavior. In the brain one of two major nAChRs is the α4β2 subtype, distinguished by its high affinity binding of acetylcholine and nicotine. The α7-subtype receptors are homopentameric and may be distinguished by their tight binding of α-bungarotoxin. Prior to the molecular cloning of the neuronal nAChR genes differential binding of α-bungarotoxin and the agonists ACh and nicotine was used to demonstrate the localization of these two subtypes in unique regions within the brain.[2] The α7 receptors have been proposed as therapeutic targets for pathologies such as Alzheimer's, inflammation, and for neuroprotection.[3–5]

While the traditional pharmacophore model for nAChR agonists consists of a charged nitrogen and a hydrogen bond acceptor, such as found in acetylcholine, anabaseine, and nicotine, the model has been the subject of ongoing scrutiny and refinement.[6] Benzylidene anabaseine (BA) compounds such as 4-hydroxy-GTS-21,[7a] **1**, (Fig. 1) are α7-selective agonists. Interestingly, 4-hydroxy-GTS-21 has been shown to be cytoprotective in both human and rat cells.[5] An interesting property of α7 receptors is that they are far more permissive than



1 **2** **3**

Figure 1. Compounds which are α7 selective AChR agonists. GTS-21, **1**; TC1698, **2**; and AR-R17779, **3**.

other nicotinic receptor types in responding to structurally diverse agonists; large hydrophobic agonists such as benzylidene anabaseines yet, smaller agonists such as choline are selective for α7 receptors.[8] It has been suggested that a larger, more hydrophobic environment in the α7 ligand-binding domain compared to other receptor subtypes is the basis for the selectivity of these receptors for BA-type agonists.[9] However, since even simple *N*,*N*-dialkyl piperidines are α7-selective[10] we suspected that a secondary hydrophobic pocket might exist in the region of the receptor closer to the charged nitrogen pharmacophore. In the past, the quinuclidine framework has been utilized for development of nAChR agonists that may be classified within the traditional pharmacophore model consisting of a charged nitrogen and nearby H-bond acceptor as seen in **2** and **3**[7b,c] (Fig. 1). We sought to use the quinuclidine framework in a new way, as a platform for probing these two hypothetical hydrophobic pockets, by utilizing aryl or

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alkyl groups in positions that would resemble either the aryl group in selective α7 agonists like GTS-21, or small alkyl groups as in *N*,*N*-dialkyl piperidines (Fig. 2, compounds **4** and **5**). Note that these compounds lack the hydrogen bond acceptor moiety of the traditional pharmacophore, and unsubstituted quinuclidine is a non selective nAChR agonist.[11] In this way, we tested the hypothesis that states that a selective α7 agonist can utilize interactions in a hydrophobic pocket to provide selectivity.

We therefore synthesized compounds **4** and **5** (Fig. 3) and tested their selectivity as agonists of neuronal α7 and α4β2 nAChRs. To the best of our knowledge, surprisingly little has been reported regarding one-step 3-benzylidene quinuclidine syntheses,[12] though olefination via organometallic additions to 3-quinuclidinone and dehydrative eliminations are known.[13] We envisioned a straightforward synthesis of the 3-benzylidine analogs of quinuclidine via Wittig-type olefinations and report the results of these studies.

Alkylation of quinuclidine hydrochloride with methyl or ethyl iodide in methanolic solution in the presence of K_2CO_3 afforded *N*-methyl and *N*-ethyl quinuclidines **5a** and **b** in 99% and 90% yields, respectively. Our initial attempt to synthesize 3-benzylidene quinuclidine **4a** utilized the Wittig reaction[14] between 3-quinuclidinone and the ylid derived from treatment of triphenyl benzyl phosphonium iodide with *n*-BuLi. Unfortunately, only an 8% yield of an E/Z mixture of **4a** was obtained, with ~30% conversion of the starting 3-quinuclidinone based on ^{1}H NMR analysis of the crude reaction mixture. Based on this result, we considered that the acidity of the 2-position in 3-quinuclidinone required a less basic olefination reagent. Benzyl phosphonates were utilized in the Wadsworth-Emmons reaction[15] to provide more satisfactory yields. In this case, diethyl benzyl phosphonate for Z/E-**4a** and diethyl-4-methoxy benzyl phosphonate for Z/E-**4b** were used as olefination reagents.(Scheme 1).

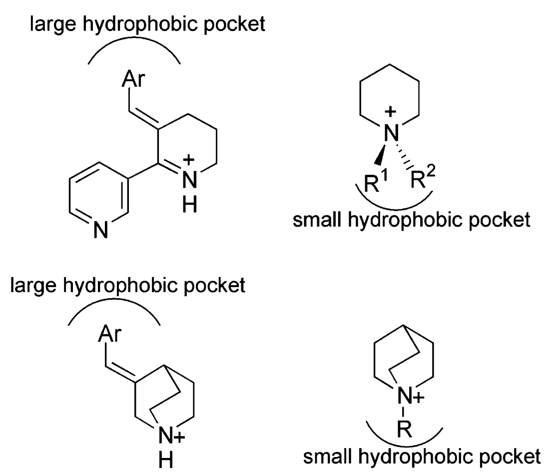
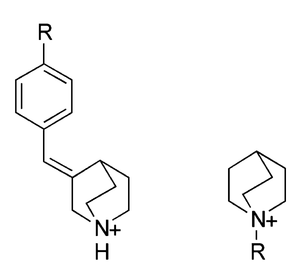


Figure 2. Proposed α7-binding pockets that confer selectivity. The top row represents the model developed to account for α7 selectivity of benzylidene anabaseines and *N*,*N*-dimethylpiperidine. The bottom row represents how 3-arylidine quinuclidines **4** or *N*-alkyl quinuclidines **5** would bind to the α7 receptor.



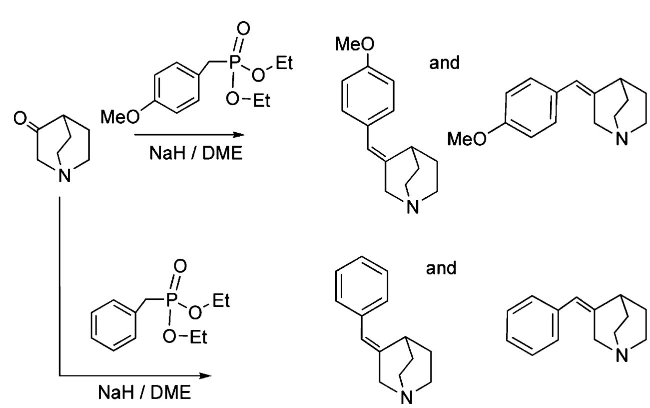
4a, R = H 5a, R = Me
4b, R = MeO 5b, R = Et

Figure 3. Target quinuclidine α7 agonists.

The best solvent for this reaction was found to be 1,2-dimethoxy ethane (DME). Chromatographic separation of the geometric isomers proved to be difficult; a variety of binary and ternary systems failed to provide a clean separation in a single step. After two successive chromatographic steps on silica in $CHCl_3$/MeOH mixtures, the two isomers were obtained in pure form, providing **4a** in 46% yield (Z/E ratio, 2:1). Compound **4b** was obtained in 23% yield with a Z/E ratio of 7:1 after chromatographic purification. The olefin geometry for each isomer was unambiguously established based on analysis of NOESY spectra and analysis of chemical shifts (Fig. 4).

The NOESY spectrum for Z-**4b** revealed crosspeaks for interactions between H_2–H_6, and H_4–H_5. The E-isomer of **4b** presented a complementary set of data, with crosspeaks corresponding to interactions between H_2–H_5 and H_4–H_6. The NOESY spectrum of Z-**4a** displayed crosspeaks for interactions between H_2–H_6, and H_4–H_5 as was observed for Z-**4b**. Finally, characteristic chemical shifts were identified for H_2 and H_4 depending on the isomer in question. Thus, for the E-isomers of **4a**,**b**, the chemical shift of H_4 was found downfield relative to the shift for H_4 of the Z-isomers, while in the case of the Z-isomers, H_2 was shifted downfield relative to the chemical shift of H_2 in the E-isomers. These effects may be attributed to deshielding from the phenyl ring.

Xenopus oocytes expressing mRNAs corresponding to α7, α3β4, or α4β2 subunits of nAChRs[10] were used to determine agonism of compounds **4a**,**b** and **5a**,**b**. We



Scheme 1. Synthesis of benzylidene quinuclidines.

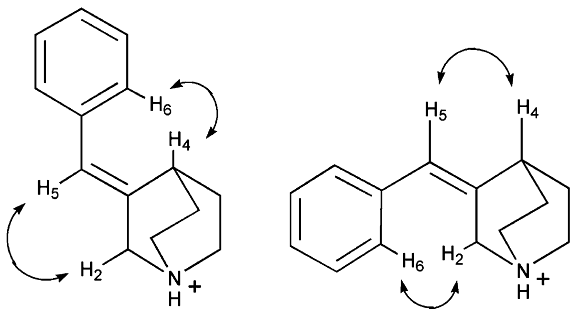


Figure 4. NOE enhancements used for assignment of olefin geometry of **4a**.

found that E-**4a** and E-**4b** were α7 selective partial agonists with respective EC_{50}'s of 1.5 and 1.3 μM.[16] Compound **5a** was an α7-selective full agonist with an EC_{50} of 40 μM, and the data for **5b** suggested it was a weak α7 selective agonist,[16] but receptor-independent currents were observed upon application of the compound to oocytes making detailed interpretation of this compound's activity difficult.

In summary, the results of these experiments are consistent with the proposed model for selectivity of α7 agonists, showing that selectivity and activity may be obtained with molecules possessing a charged nitrogen and suitable hydrophobic residue. We believe the EC_{50} values indicate that the binding site for the aryl group is tolerant of substitution and therefore amenable to further development of agonists. Further details[11] of the biological activity of these and other quinuclidine compounds will be reported elsewhere.[16]

Acknowledgments

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