Quinuclidines as selective agonists for alpha-7 nicotinic acetylcholine receptors

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Received 2 November 2006; revised 16 December 2006; accepted 3 January 2007

Available online 12 January 2007

Abstract—The \( \alpha_7 \) 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 \( \mu \text{M} \), 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. 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 \( \alpha_4\beta_2 \) subtype, distinguished by its high affinity binding of acetylcholine and nicotine. The \( \alpha_7 \)-subtype receptors are homopentameric and may be distinguished by their tight binding of \( \alpha \)-bungarotoxin. Prior to the molecular cloning of the neuronal nAChR genes differential binding of \( \alpha \)-bungarotoxin and the agonists ACh and nicotine was used to demonstrate the localization of these two subtypes in unique regions within the brain. The \( \alpha_7 \) receptors have been proposed as therapeutic targets for pathologies such as Alzheimer’s, inflammation, and for neuroprotection.

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. Benzylidene anabaseine (BA) compounds such as 4-hydroxy-GTS-21, \( 1 \), (Fig. 1) are \( \alpha_7 \)-selective agonists. Interestingly, 4-hydroxy-GTS-21 has been shown to be cytoprotective in both human and rat cells. An interesting property of \( \alpha_7 \) receptors is that they are far more permissive than 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 \( \alpha_7 \) receptors. It has been suggested that a larger, more hydrophobic environment in the \( \alpha_7 \) ligand-binding domain compared to other receptor subtypes is the basis for the selectivity of these receptors for BA-type agonists. However, since even simple \( N,N \)-dialkyl piperidines are \( \alpha_7 \)-selective 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 (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

Keywords: Quinuclidine; Olefination; Agonist; nAChR; Nicotinic; Acetylcholine; Receptor.

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0960-894X/S - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2007.01.003
alkyl groups in positions that would resemble either the aryl group in selective $\alpha_7$ 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. In this way, we tested the hypothesis that states that a selective $\alpha_7$ 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 $\alpha_7$ and $\alpha_4\beta_2$ nAChRs. To the best of our knowledge, surprisingly little has been reported regarding one-step 3-benzylidene quinuclidine syntheses, though olefination via organometallic additions to 3-quinuclidinone and dehydrative eliminations are known. We envisioned a straightforward synthesis of the 3-benzylidene 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 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 Wads-worth-Emmons reaction 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).

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 cross-peaks corresponding to interactions between H$_2$–H$_5$ and H$_4$–H$_6$. The NOESY spectrum of Z-4a displayed cross-peaks 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 $\alpha_7$, $\alpha_3\beta_4$, or $\alpha_4\beta_2$ subunits of nAChRs were used to determine agonism of compounds 4a,b and 5a,b. We
found that E-4a and E-4b were a7 selective partial agonists with respective EC50's of 1.5 and 1.3 μM.16 Compound 5a was an a7-selective full agonist with an EC50 of 40 μM, and the data for 5b suggested it was a weak a7 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 a7 agonists, showing that selectivity and activity may be obtained with molecules possessing a charged nitrogen and suitable hydrophobic residue. We believe the EC50 values indicate that the binding site for the aryl group is tolerant of substitution and therefore amenable to further development of agonists. Further details11 of the biological activity of these and other quinuclidine compounds will be reported elsewhere.16

Acknowledgments

This work was supported by Grant R01 GM57481. We thank Dr. I. Ghiviriga for help with the NOESY experiments, and Lisa Jacobs and Dolan Abu-Aouf for technical assistance.

References and notes