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Synthesis and SAR of selective muscarinic acetylcholine receptor subtype 1 (M1 mAChR) antagonists

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Abstract—This Letter describes the synthesis and SAR, developed through an iterative analogue library approach, of a novel series of selective M1 mAChR antagonists for the potential treatment of Parkinson's disease, dystonia and other movement disorders. Compounds in this series possess M1 antagonist IC_{50}s in the 441 nM–19 μM range with 8- to >340-fold functional selectivity versus rM2–rM5.

The muscarinic acetylcholine receptors (mACHRs) are members of the G protein-coupled receptor (GPCR) family A that mediate the metabotropic actions of the neurotransmitter acetylcholine.[1,2] To date, five distinct subtypes of mAChRs (M1–M5) have been cloned and sequenced. M1, M3, and M5 activate phospholipase C and calcium through Gq whereas M2 and M4 block the action of adenylyl cyclase through Gi/o.[1,2] The cholinergic system, mediated by mAChRs, plays a critical role in a wide variety of CNS and peripheral functions including memory and attention mechanisms, motor control, nociception, regulation of sleep wake cycles, cardiovascular function, renal and gastrointestinal function, and many others.[1–4] As a result, agents that can selectively modulate the activity of mAChRs have the potential for therapeutic use in multiple pathological states. However, due to high sequence conservation within the orthosteric binding site of the five mAChR subtypes, it has been historically difficult to develop mAChR subtype selective ligands.[1–5]

To date, the majority of reported muscarinic antagonists are unselective, such as a scopolamine, **1**.[6] Recently, pirenzapine, **2**, has emerged as a relatively selective M1 receptor antagonist (20- to 50-fold versus M2–M5) and there are numerous reports of moderately selective M3 antagonists (20- to 50-fold versus M2) such as **3**.[7] Interestingly, the most selective M1 antagonist, MT7, **4**, the 65 amino acid peptide (>1000-fold versus M2–M5) was derived from venom extracts of the green mamba snake (Fig. 1).[8] Based on brain expression and cellular localization, data from mAChR knock-out mice, and clinical trials with muscarinic agents, the M1 mAChR subtype is an attractive molecular target for the treatment of Alzheimer's disease (AD), Parkinson's disease (PD), and dystonia due to its role in cognition and motor control.[9] Indeed, pan-muscarinic agonists, such as the M1/M4 preferring xanomeline, showed efficacy in Phase III clinical trials in AD patients; however, activation of peripheral M2 and M3 receptors led to intolerable adverse side effects.[10] Moreover, anti-cholinergic agents have also demonstrated efficacy in both PD and dystonia patients, and this benefit is believed to be derived from antagonism of the M1 mAChR subtype;

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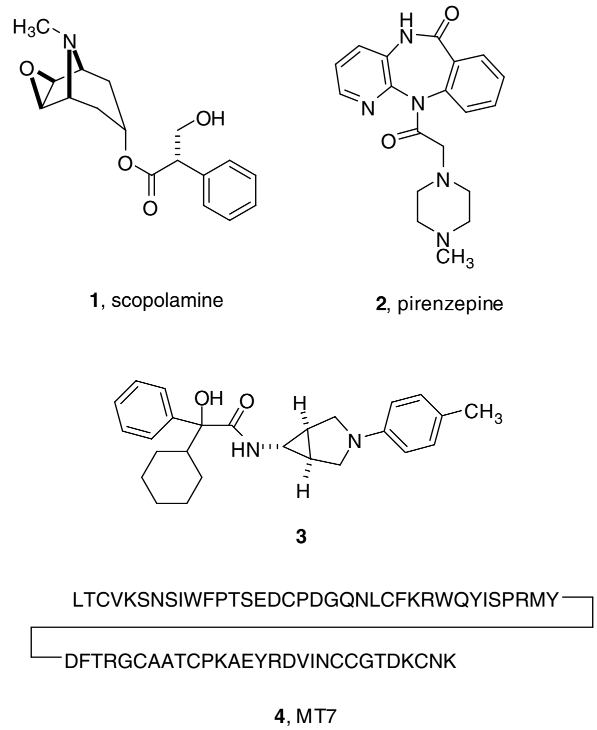


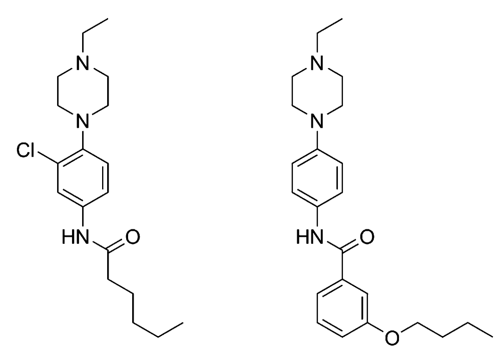
Figure 1. Structures of representative mAChR antagonists.

however, the relative contributions from M4 are unclear.[1–10] In order to probe the role of M1 antagonism as a potential therapeutic approach for Parkinson's disease, dystonia, and other movement disorders, potent small molecule mAChR antagonists are required with a high degree of M1 versus M4 selectivity for study in preclinical models.

The Vanderbilt Screening Center for GPCRs, Ion Channels and Transporters, and the companion Chemistry Center, were established as members of the Molecular Libraries Screening Center Network (MLSCN) initiated and supported by the NIH Molecular Libraries Roadmap.[11,12] The MLSCN is a nationwide consortium of facilities that provide high-throughput small molecule screening and medicinal chemistry expertise for the development of chemical probes for use as tools to explore biological targets/pathways for which small molecule tools are unavailable.[12] One such target which lacks the appropriate small molecule tools are the muscarinic acetylcholine receptors (mAChRs).[1–10]

Based on this unmet need in the scientific community, our MLSCN Center initiated an effort to identify potent small molecule mAChR antagonists with high specificity for M1 for use as a chemical probe and lead for further optimization toward a novel therapeutic. Toward this goal, we optimized a real-time cell-based calcium-mobilization assay employing a rat M1/CHO cell line (Z' averaged 0.7), screened a 63,656 member MLSCN compound library, and identified 2179 primary M1 antagonist hits.[13] Of these primary hits, 1665 were available from Biofocus-DPI for re-test, and duplicate testing afforded 723 confirmed hits (43%). These compounds were then counter-screened against an mGluR4/CHO cell line which eliminated 9 hits. The remaining compounds were tested in triplicate in 10-point concentration–response curves against both rat M1/CHO and rat M4/CHO cells to identify compounds with ~10-fold selectivity for M1 versus M4, our initial cutoff for a lead. While the vast majority of compounds displayed no selectivity for M1 versus M4, we identified two related structures based on a *N*-(4-(4-ethylpiperazin-1-yl)phenyl amide scaffold, **5** (rM1 $IC_{50} = 0.49\ \mu M$, rM4 $IC_{50} = 7.9\ \mu M$) and **6** (rM1 $IC_{50} = 0.58\ \mu M$, rM4 $IC_{50} = 5.1\ \mu M$), which displayed ~16- to ~9-fold selectivity, respectively, for rM1 versus rM4 and displayed comparable inhibition of human M1 (Fig. 2).

Analogues of **5** and **6** were synthesized in a library format according to Scheme 1. Both requisite anilines **7** and **8**, 3-chloro-(4-(4-ethylpiperazin-1yl)aniline and (4-(4-ethylpiperazin-1yl)aniline, were commercially available and acylated under standard conditions employing polymer-supported reagents and scavengers to afford 24-member libraries of analogues **9** and **10**, respectively.[14] In the initial lead optimization phase, we prepare a 24-member library employing a diversity set of acid chlorides containing aromatic, alphatic, polar, basic, and acidic moieties in order to rapidly probe the breadth and scope of the SAR; subsequent libraries will be more focused. As the chemistry was straightforward, we elected to re-synthesize the parent compounds **5** and **6** within the library. All analogues were purified by mass-guided HPLC to analytical purity.[15] Surprisingly, all analogues **10**, as well as the re-synthesized parent **6**, were found to be inactive on rM1. Moreover, upon re-synthesis in the library, **5** lost considerable efficacy as an M1 antagonist (rM1 $IC_{50} = 13\ \mu M$), but still displayed ~10-fold selectivity versus rM4 ($IC_{50} > 150\ \mu M$).[16] Not surprisingly, analysis of the original screening samples **5** and **6** indicated that there were several impurities in the wells, and we elected not to pursue a complex deconvolution exercise. Despite these findings, the strategy of employing library synthesis and exploding SAR around a primary HTS hit proved advantageous for **5**, as analogues **9** proved to possess intriguing mAChR selectivity profiles.



rM1 vs. rM4 ~10-fold
5 (Pubchem SID: 4264322)

rM1 vs. rM4 ~10-fold
6 (Pubchem SID: 4258852)

Figure 2. HTS leads **5** and **6**, rM1 antagonists with selectivity versus rM4 of ~10-fold in the primary assays.