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Synthesis and SAR of *N*-(4-(4-alkylpiperazin-1-yl)phenyl)benzamides as muscarinic acetylcholine receptor subtype 1 (M_1) anatgonists

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ABSTRACT

This Letter describes the synthesis and SAR, developed through an iterative analog library approach, of a novel series of selective M_1 mAChR antagonists, based on an *N*-(4-(4-alkylpiperazin-1-yl)phenyl)benzamide scaffold for the potential treatment of Parkinson's disease, dystonia and other movement disorders. Compounds in this series possess M_1 antagonist IC_{50}s in the 350 nM to >10 μM range with varying degrees of functional selectivity versus M_2–M_5.

There are five subtypes of muscarinic acetylcholine receptors (mAChR1-5 or M_1–M_5), members of the G protein-coupled receptor (GPCR) family A, that mediate the metabotropic actions of the neurotransmitter acetylcholine.[1,2] M_1, M_3, and M_5 activate phospholipase C and calcium mobilization through G_q whereas M_2 and M_4 block the action of adenylyl cyclase through $G_{i/o}$.[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 to mention only a few.[1–4] As a result, agents that can selectively modulate the activity of mAChRs have the potential for therapeutic use in multiple peripheral and central pathological states. 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] Based on brain expression and cellular localization, data from mAChR knock-out mice and clinical trials with muscarinic agents, the M_1 subtype is an attractive molecular target for the treatment of CNS disorders. M_1 has been implicated in the pathologies of Alzheimer's disease (AD), Parkinson's disease (PD), and dystonia due to its role in cognition and motor control.[6]

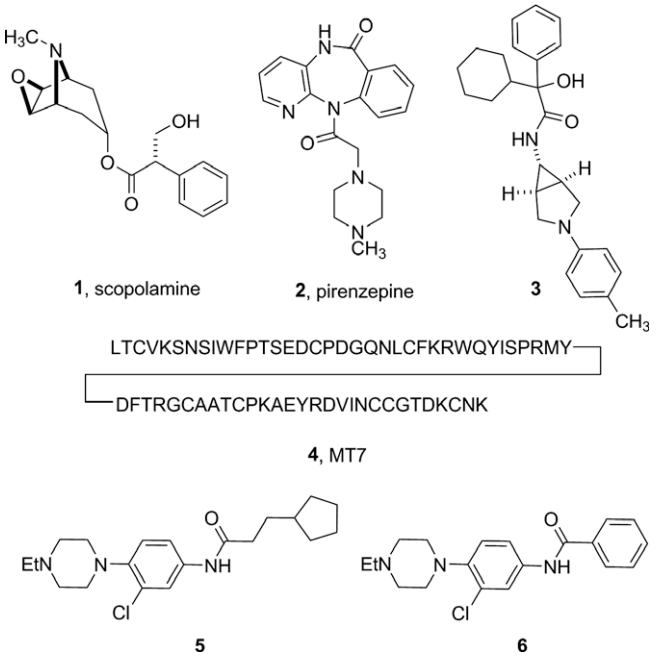

Figure 1. Structures of representative mAChR antagonists.

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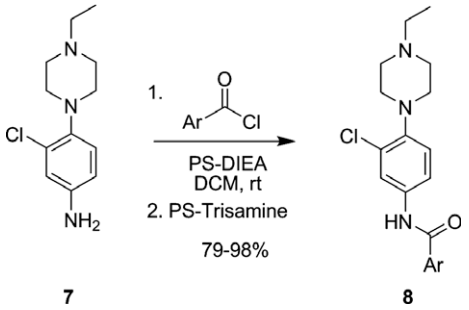
The majority of reported muscarinic antagonists are unselective, such as a scopolamine, **1**.[7] Recently, pirenzapine, **2** has emerged as a relatively selective M_1 receptor antagonist (20–50-fold vs M_2–M_5) and there are numerous reports of moderately selective M_3 antagonists (20–50-fold vs M_2) such as **3**.[8] Interestingly, the most selective M_1 antagonist, MT7, **4**, the 65 amino acid peptide, (>1000-fold vs M_2–M_5) was derived from venom extracts of the green mamba snake (Fig. 1).[9] From an M_1 functional screen within the MLSCN, we identified M_1 antagonists such as **5** (M_1 IC_{50} of 441 nM and with >340-fold selectivity versus M_4, but modest selectivity versus M_2, M_3, and M_5 (7.9-fold, 7-fold, and 2.4-fold, respectively)) and **6** (M_1 IC_{50} of 5.0 μM and with >30-fold selective vs M_2–M_5).[10–12] Based on the M_1 selectivity of **6**, attractive physiochemical properties (MW <350, *c*log *P* 3.6) and the fact that it was the only benzamide-containing analog in the series, we initiated a library synthesis effort[13] to develop SAR around **6**.

As shown in Scheme 1, the first round of library synthesis focused on benzamide analogs of **6**. Commercially available 3-chloro-(4-(4-ethylpierazin-1yl)aniline **7** was acylated under standard conditions employing polymer-supported reagents and scavengers[13] to afford a 24-member library of analogs **8**, along with re-synthesized **6**. All analogs were then purified by mass-guided HPLC to analytical purity.[14] To effectively screen small libraries of potential mAChR ligands, we have adopted a strategy to triage compounds in single-point screens (at 10 μM) at M_1, M_3 and M_5—the G_q-coupled mAChRs—to identify active and selective compounds prior to running full concentration–response curves (CRCs).[15] Figure 2 shows the 10 μM single-point screens for the first 25-member library of benzamide analogs **8**.



Scheme 1. Library synthesis of first generation analogs **8**. All library compounds were purified by mass-guided HPLC to >98% purity.[14]

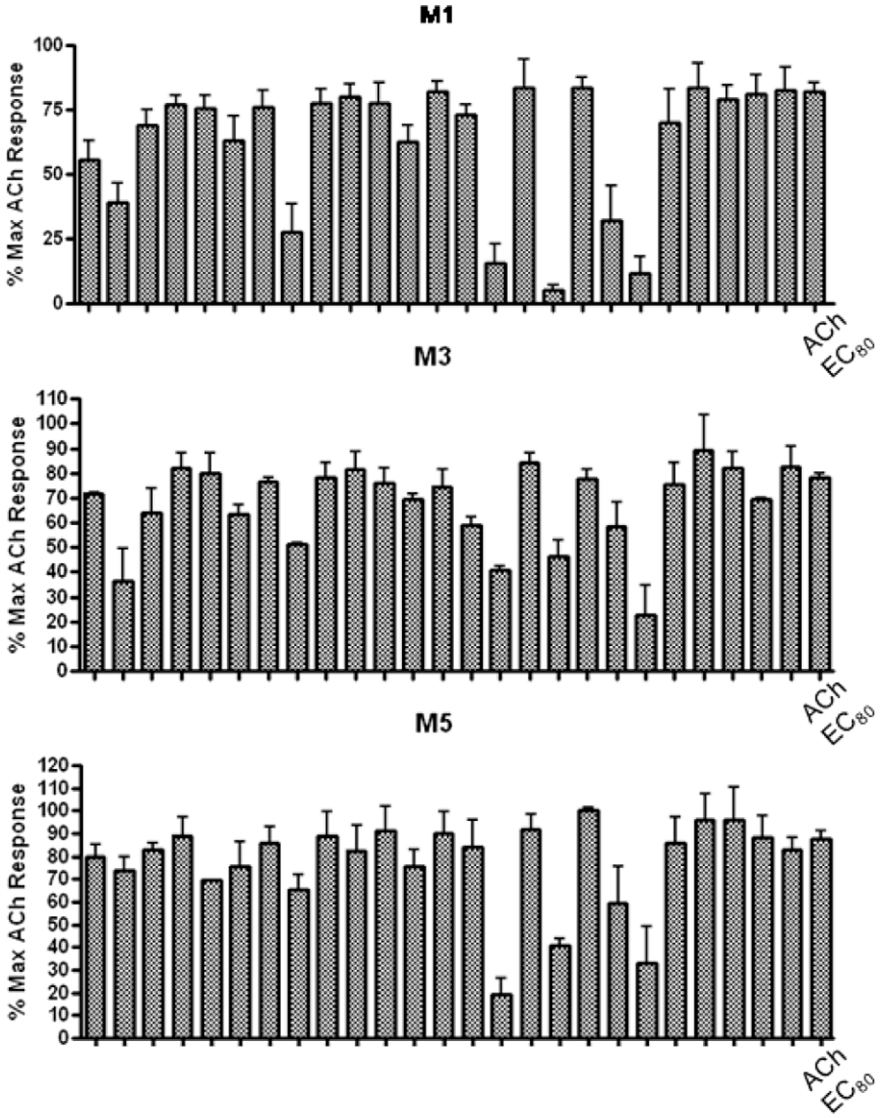
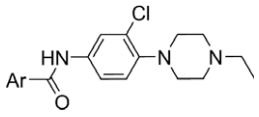
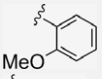
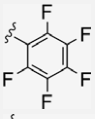
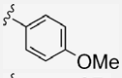
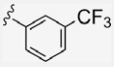
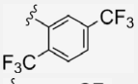
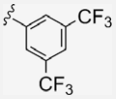


Figure 2. Single-point EC_{80} plus 10 μM compound triage screen at M_1, M_3, and M_5 to select compounds for full CRCs.

Table 1
Structures and mAChR activities of analogs **8**



8

Compd	Ar	M_1 IC_{50} (μM)[a]	M_2 IC_{50} (μM)[a]	M_3 IC_{50} (μM)[a]	M_4 IC_{50} (μM)[a]	M_5 IC_{50} (μM)[a]
6		3.2	>10	>10	>10	>10
8a		0.96	ND	0.82	ND	2.3
8b		0.82	ND	5.6	ND	1.3
8c		2.9	6.9	>10	3.7	>10
8d		2.1	ND	>10	ND	3.5
8e		0.35	ND	3.7	ND	0.83
8f		3.2	ND	>10	ND	>10
8g		2.9	>10	4.3	3.7	4.1
8h		0.49	2.7	4.2	1.5	4.1
8i		2.6	>10	>10	3.7	>10
8j		4.7	>10	>10	>10	>10

ND = not determined.
[a] IC_{50}s are an average of three independent experiments using mAChR (CHO) cell lines.

As Shown in Table 1, re-synthesized **6** displayed comparable potency and mAChR selectivity to the original sample (M_1 IC_{50} = 3.2 μM, IC_{50} >>10 μM for M_2–M_5). Functionalized benzamide analogs **8** possessed a wide range of M_1 potency and mAChR selectivity, and we initially evaluated analogs **8** against M_1, M_3, and M_5. Substitution in the 2-position, **8a** (2-Cl) and **8b** (2-OMe) possessed submicromolar M_1 IC_{50}s (960 nM and 820 nM, respectively), but also showed low micromolar activity at M_3 and M_5. A pentafluorophenyl congener **8e** (Fig. 3A) proved to be a submicromolar antagonist of both M_1 and M_5 (IC_{50}s of 350 nM and 830 nM, respectively). Substitution at the 4-position, as with the 4-OMe derivative **8f**, was comparable to the original **6**. Interestingly, a 2,5-bisCF_3 analog **8h** had an M_1 IC_{50} of 490 nM, with ~ninefold functional selectivity versus M_3 and M_5 (Fig. 3B). Intrigued by this potent and selective M_1 antagonist, we screened against M_2 and M_4 as well, but found that **8h** possessed only 3–4-fold selectivity versus the $G_{i/o}$-coupled mAChRs (Table 1). **8i**, a 3,5-bisCF_3 analog possessed a unique profile as a dual M_1/M_4 antagonist (IC_{50}s of 2.6 μM and 3.7 μM, respectively), with little effect on an ACh EC_{80} at 10 μM on M_2, M_3 or M_5. Finally, a 3,4-difluoro **8j** derivative was also comparable to the original **6**. While this library afforded interesting results, further optimization was required.

Having surveyed the amide moiety while maintaining the *N*-ethyl piperazine, we next generated two-dimensional libraries wherein the nature of the alkyl group was varied (**9–12**) while also surveying diverse benzamides to generate analogs **9a–f**, **10a–f**, **11a–f**, and **12a–f** (Scheme 2).

Application of the same strategy to triage compounds in single-point screens (at 10 μM) at M_1, M_3, and M_5 to identify active and selective compounds prior to running full (CRCs) was employed, but >75% of these new analogs possessed no M_1 antagonist activity. The SAR for this series was incredibly shallow, with only an *N*-propyl congener with the 3,5-dicholrobenzamide moiety **11i** displaying reasonable activity (M_1 IC_{50} = 3.7 μM, IC_{50} > 10 μM for M_3 and M_5), and all other analogs possessing M_1 IC_{50}s in the 6–9 μM range.

In summary, a two-dimensional parallel synthesis library campaign was performed around **6**, an M_1 antagonist identified in a functional HTS screen. SAR for this series was shallow, but we were able to improve the M_1 antagonist activity of **6** into the 350–500 nM range with analogs **8**, while maintaining good mAChR selectivity. Interestingly, **8i** is the first reported dual M_1/M_4-preferring antagonist, which compliments the prototypical M_1/M_4-preferring agonist xanomeline. Other chemical series from our M_1 functional screen are currently under chemical optimization, and further refinements will be reported in due course.

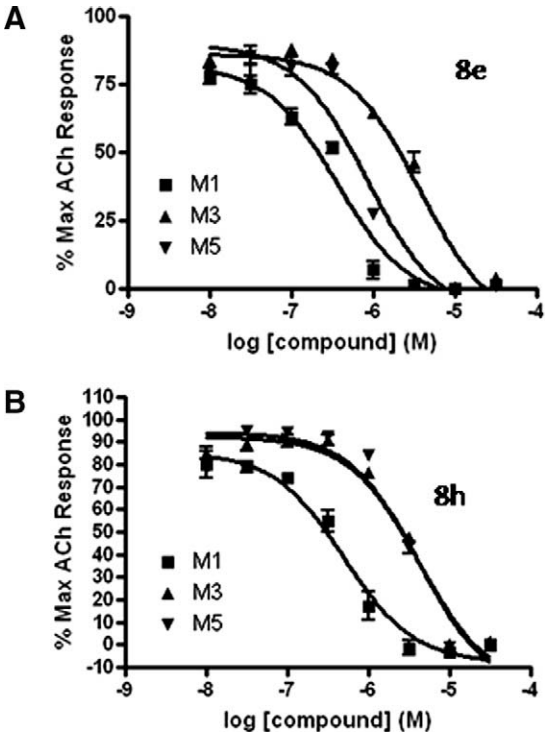
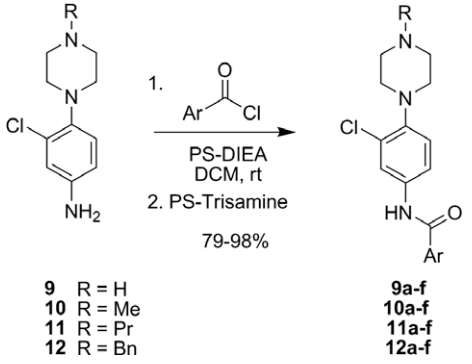


Figure 3. CRCs for M_1, M_3, and M_5 for (**A**) compound **8e** (M_1 IC_{50} = 350 nM) and (**B**) compound **8h** (M_1 IC_{50} = 490 nM), showing ~ninefold functional selectivity versus M_3 and M_5.



Scheme 2. Library synthesis of second generation analogs **9a–f**, **10a–f**, **11a–f** and **12a–f**. All library compounds were purified by mass-guided HPLC to >98% purity.[14]

Acknowledgments

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15. *Details of the calcium mobilization assays*: Chinese Hamster Ovary (CHO-K1) cells stably expressing human (h) M_1, hM_3, and hM_5 were used for calcium mobilization assays. hM_2 and hM_4 were adapted to this assay and signaling pathway after stably transfecting G_{qi5} chimeric G protein. To measure agonist-induced calcium mobilization and determine effect of novel compounds, stable muscarinic cell lines plated overnight in Costar 96-well cell culture plates (Corning) were incubated with 50 μL of 2 μM Fluo-4 AM diluted in assay buffer [HBSS (Invitrogen) supplemented with 20 mM HEPES and 2.5 mM probenecid, pH 7.4] for 45 min at 37 °C. Dye was then removed and replaced with assay buffer. Cells were pre-incubated with 10 μM or a concentration–response curve of novel compound, followed by a sub-maximal concentration of acetylcholine or carbachol. The signal amplitude was first normalized to baseline and then expressed as a percentage of the maximal response to acetylcholine.