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Development of novel M_1 antagonist scaffolds through the continued optimization of the MLPCN probe ML012

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ABSTRACT

This Paper describes the continued optimization of an MLPCN probe molecule M_1 antagonist (ML012) through an iterative parallel synthesis approach. After several rounds of modifications of the parent compound, we arrived at a new azetidine scaffold that displayed improved potency while maintaining a desirable level of selectivity over other muscarinic receptor subtypes. Data for representative molecules **7w** (VU0452865) and **12a** (VU0455691) are presented.

Acetylcholine (ACh) is a critical neurotransmitter with diverse functions both within the central nervous system (CNS) and in peripheral signaling pathways.[1–4] ACh operates by interacting with two very distinct groups of receptors; a set of ligand-gated ion channels—the nicotinic acetylcholine receptors (nAChRs)—and a set of family A, G protein-coupled receptors (GPCRs)—the muscarinic acetylcholine receptors (mAChRs). The muscarinic family of acetylcholine receptors is divided into five subtypes (M_{1-5}).[5] These subtypes can be further classified into two subsets based on their G protein-coupling partners, with the $M_{1,3,5}$ receptors preferentially coupling to G_q (stimulating PLC and intracellular calcium mobilization) and the $M_{2,4}$ receptors preferentially coupling to $G_{i/o}$ (inhibiting adenylate cyclase (AC), thereby decreasing cAMP production).[4] The specific subtypes of mAChRs are expressed throughout the body with varying degrees of expression levels based on the particular site or organ.[6] As a result, mAChRs play significant roles in a wide range of physiological functions such as memory and attention, motor control, nociception, regulation of sleep-wake cycles, cardiovascular function, secretory functions, and mediators of inflammation, renal and gastrointestinal (GI) function, among many others.[6,7]

It has been postulated that $M_{1,4,5}$ receptors are the relevant subtypes for CNS therapies; however, specific functions for each receptor subtype are still being investigated.[7] This is a direct result of the highly conserved orthosteric binding site for the endogenous ligand (ACh) that is shared across all five subtypes of mAChRs. This similarity has stymied the discovery and development of muscarinic ligands with high selectivity for a particular subtype.[8] Yet, this lack of selectivity has not precluded the development of pharmaceuticals with activity at mAChRs for a range of indications. Many of these non-selective compounds have undesirable side-effects that are attributed to activity at the other mAChRs (often M_2 and M_3), limiting their clinical impact. For example, xanomeline, a reported M_1- and M_4-selective agonist, showed robust clinical efficacy in Phase II trials for Alzheimer's disease and schizophrenia,[9,10] but also has nearly equivalent agonist activity at M_3.[8] Even in the absence of off-target mAChR activity, the debate still remains whether a single mAChR subtype (M_1 or M_4) is responsible for the positive outcomes in these trials; although, recent studies using mAChR genetic knockout (KO) mice have shed additional light on this topic.[11] More highly selective mAChR ligands would allow for more direct pharmacological insight and a better understanding of the individual roles for

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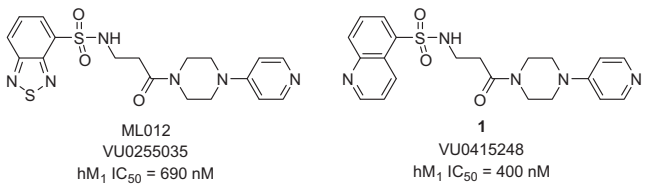
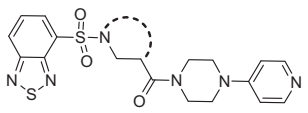


Figure 1. MLPCN Probe ML012 and VU0415248, a selective M_1 antagonist.

Table 1
Structures and potencies of M_1 antagonist analogs **2a–d** with cyclic constraints



Compd	Aza ring constraints[a]	pIC_{50} ±SEM[b]	hM_1 IC_{50} (μM)[b]	%EC min ± SEM[b]
2a			>10	33.4 ± 3.9
2b			Inactive	
2c			>10	47.1 ± 4.0
2d		5.68 ± 0.07	2.2	10.1 ± 3.3

[a] Examples **2a** and **2c** were prepared and screened as racemic mixtures.
[b] Values represent the mean ± standard error mean of at least three independent determinations performed in triplicate.

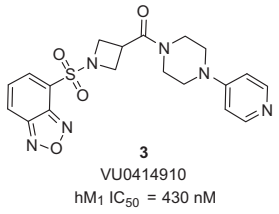


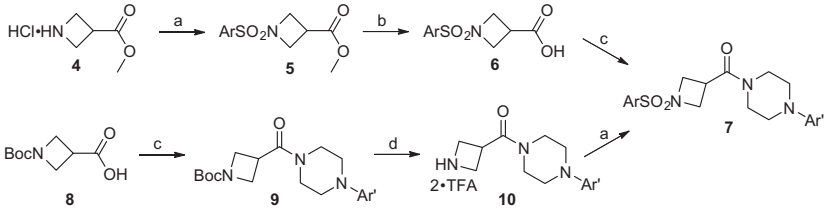
Figure 2. VU0414910, an M_1 antagonist.

each of the five mAChRs. We envision two ways to obtain mAChR subtype selectivity with synthetic ligands: 1) simultaneous binding to the orthosteric site and into adjacent areas which may be less structurally conserved among the other mAChRs[12] or 2) binding to a completely distinct region of the mAChR at an allosteric site, imparting a level of selectivity to the ligand not found relative to the other four mAChRs. This allosteric approach has been highly successful for a number of the individual mAChRs: M_1,[13–15] M_4,[16] and M_5. [17]

We have previously reported on the selective M_1 antagonist, ML012 (VU0255035, Fig. 1), and progress on optimization of the ML012 scaffold. ML012 showed 45- to 159-fold selectivity for M_1 over the other subtypes.[12] ML012 also reduced pilocarpine-induced seizures in rodents at doses that had no negative impact on contextual fear conditioning, a behavioral model of hippocampal-dependent cognitive function. These findings demonstrated that selective M_1 antagonists have therapeutic potential over non-selective muscarinic antagonists. Given the potential for M_1 antagonists in such indications as Parkinson's disease, movement disorders, and Fragile X syndrome,[18,19] we engaged in an optimization campaign of ML012. Our efforts yielded compound **1** (VU0415248, Fig. 1), a more potent antagonist with better selectivity for M_1.[20] These efforts also expanded the structure–activity relationship (SAR) of ML012 and other compounds in this series. Herein, we report further modifications which provided a panel of compounds with improved potency and good selectivity for the M_1 muscarinic receptor, and more importantly, divergent SAR from the ML012 series.

In our previous work on ML012 optimization, the central linker was modified through the introduction of methyl substitution and fluorination at the alpha position of the beta-alanine moiety. Of these modifications, none provided a desirable increase in potency or selectivity and many abolished activity altogether.[20] Concurrently, we prepared a limited series of cyclic constrained analogs and screened these compounds for antagonism at M_1 (Table 1). For compounds **2a–c**, potency was significantly decreased while some slight activity at M_1 remained (for **2a**, 33% activity and for **2c**, 47% activity). We were encouraged that an azetidine analog, **2d,** was tolerated, albeit threefold less active than ML012. This provided an opportunity to enter into new chemical space and investigate compounds for improved potency and selectivity over ML012. Previous modifications to the Western thiadiazole of ML012 led us to determine that an oxadiazole was a suitable replacement and generally maintained potency.[20] We procured **3** (Fig. 2), which contained the desired oxadiazole and a central azetidine linker, and found that it was equipotent to **1** (vida supra), our improved M_1 antagonist.

With **3** in hand, we explored the SAR at both termini of the molecule through an iterative parallel synthesis approach. These routes, illustrated in Scheme 1, made use of the commercially available azetidine central linkers **4** and **8**. For the Eastern SAR, azetidine **4** and aryl sulfonyl chlorides were reacted to provide sulfonamide **5**, followed by saponification which yielded acid **6**. Amide coupling with substituted piperazines provided the target compound (**7**). As with our previous findings from the optimization of ML012, Eastern SAR around the pyridine ring was unforgivingly steep.[20] Indeed, only compound **3** (hM_1 IC_{50} = 430 nM) or substitution with a (5-bromopyridin-2-yl)piperazine moiety (hM_1 IC_{50} = 280 nM, structure not shown) maintained activity.[21] Focusing on the Western SAR and starting with azetidine **8**, amide coupling to give **9** was followed by deprotection of the *N*-Boc with TFA in DCM to provide bis-TFA salt **10**. Sulfonation with aryl sulfonyl



Scheme 1. Reagents: (a) $ArSO_2Cl$, NEt_3, DCM; (b) NaOH (aq), MeOH; (c) amine, EDCI, HOBt, DIEA, DMF; (d) TFA/DCM (1:1).