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Synthesis and evaluation of 4,6-disubstituted pyrimidines as CNS penetrant *pan*-muscarinic antagonists with a novel chemotype

Aaron M. Bender [a,b], Rebecca L. Weiner [a,b], Vincent B. Luscombe [a,b], Hyekyung P. Cho [a,b], Colleen M. Niswender [a,b,d], Darren W. Engers [a,b], Thomas M. Bridges [a,b], P. Jeffrey Conn [a,b,d], Craig W. Lindsley [a,b,c,*]

[a] *Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*
[b] *Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*
[c] *Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA*
[d] *Vanderbilt Kennedy Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*

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ABSTRACT

This letter describes the synthesis and structure activity relationship (SAR) studies of structurally novel M_4 antagonists, based on a 4,6-disubstituted core, identified from a high-throughput screening campaign. A multi-dimensional optimization effort enhanced potency at both human and rat M_4 (IC_{50}s < 300 nM), with no substantial species differences noted. Moreover, CNS penetration proved attractive for this series (brain:plasma $K_{p,uu}$ = 0.87), while other DMPK attributes were addressed in the course of the optimization effort, providing low *in vivo* clearance in rat (CL_p = 5.37 mL/min/kg). Surprisingly, this series displayed *pan*-muscarinic antagonist activity across M_{1-5}, despite the absence of the prototypical basic or quaternary amine moiety, thus offering a new chemotype from which to develop a next generation of *pan*-muscarinic antagonist agents.

Introduction

The muscarinic acetylcholine receptors (mAChRs) are members of the class A family of G protein-coupled receptors (GPCRs), which modulate the activity of the neurotransmitter acetylcholine. Currently, five mAChR subtypes (M_1-M_5) have been characterized. M_1, M_3 and M_5 are G_q-coupled, and activate phospholipase C and calcium mobilization. M_2 and M_4 are coupled to $G_{i/o}$, and thus inhibit the actions of adenylyl cyclase. The mAChRs are distributed throughout the central and peripheral nervous systems, where they modulate a wide variety of neuronal and autocrine functions relating to memory, nociception, gastrointestinal function, and many others. The development of therapeutics targeting the mAChRs is therefore widely pursued, and such agents have the potential to be useful for a number of different pathologies.[1,2]

The majority of known muscarinic antagonists such as atropine (**1**) and the anti-emetic scopolamine (**2**) (Fig. 1) are non-selective across the five receptor subtypes (*pan*-muscarinic antagonists). Other muscarinic antagonists include tiotropium bromide (**3**), a quaternary ammonium salt currently used for the treatment of chronic obstructive pulmonary disease (COPD),[3] and AZD8683 (**4**), an M_3-preferring antagonist with a long duration of action and reduced side effect profile compared to tiotropium (Fig. 1).[4] Muscarinic antagonists are also used to treat overactive bladder and have shown promise for the treatment of irritable bowel syndrome (IBS).[5–7]

Since the M_4 receptor subtype is co-localized with dopaminergic D_1 receptors in the striatum, M_4 modulation has been identified as potentially playing a role in various movement disorders such as Parkinson's disease and dystonia; hence, our interest in development of selective M_4 antagonists.[8–11] Development of agents that are selective for M_4, or any of the individual mAChRs, has traditionally proven difficult due to the high sequence homology amongst the receptor subtypes.[2] In an effort to identify structurally novel and selective M_4 antagonist chemotypes, a high-throughput screen was performed, and we identified the 4,6-disubstituted pyrimidine **5** (Fig. 1) as a putative lead. This result was exciting, as **5** (VU0623863) represents a novel muscarinic antagonist scaffold, and we surmised that the potential for selectivity across the mAChR subtypes was high (especially given the lack of the basic nitrogen found in other non-selective muscarinic scaffolds **1–4**), as we have previously demonstrated for both M_1 and M_5.[12–18]

* Corresponding author at: Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University School of Medicine, Nashville, TN 37232, USA.
E-mail address: craig.lindsley@vanderbilt.edu (C.W. Lindsley).

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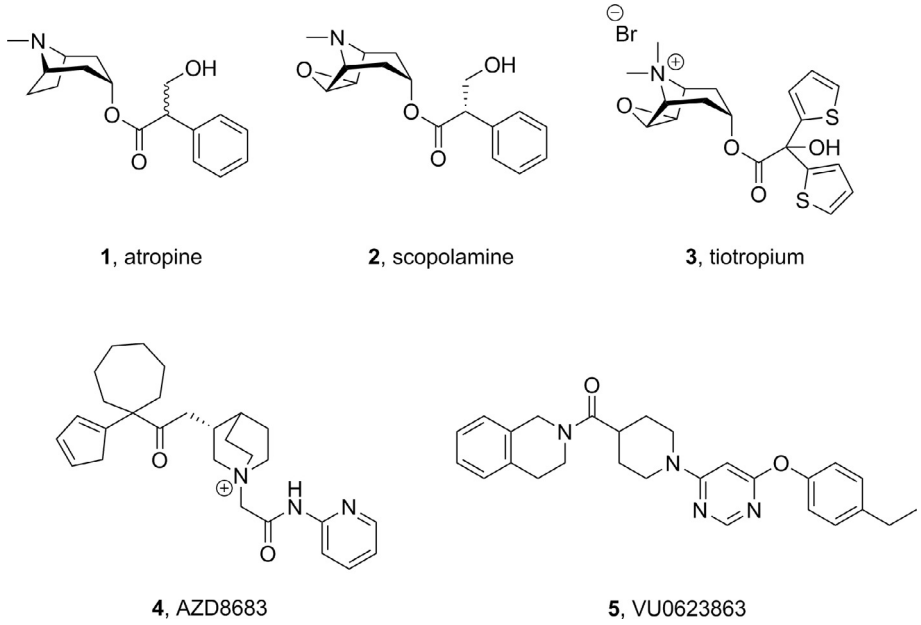
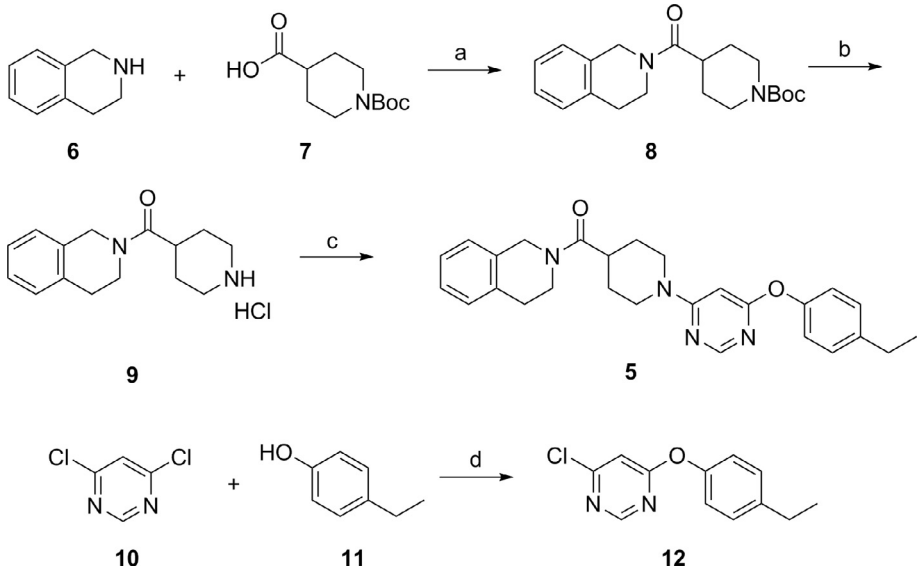


Fig. 1. Chemical structures of known muscarinic antagonists **1**–**4**, and the newly identified hit **5** from an M_4 antagonist high-throughput screen.



Scheme 1. Synthesis of compound **5** and route for analog synthesis.[a]. [a]Reagents and conditions: (a) HATU, DIPEA, DCM, 91%; (b) HCl, 1,4-dioxane, 99%; (c) **12**, DIPEA, NMP, microwave, 150 °C, 59%; (d) K_2CO_3, DMF, 100 °C, 38%.

Compound **5** was resynthesized as shown in Scheme 1. Briefly, a HATU-mediated amide coupling between 1,2,3,4-tetrahydroisoquinoline (THIQ) **6** and *N*-Boc-isonipecotic acid **7** affords **8** in 91% yield. Subsequent Boc-deprotection yielded HCl salt **9** in quantitative yield. Nucleophilic aromatic substitution (S_NAr) between 4,6-dichloropyrimidine **10** and 4-ethylphenol **11** provided **12**, which could be coupled to **9** via microwave-assisted S_NAr.[19]

After resynthesis, compound **5** was found to have comparable, sub-micromolar potency at both human and rat M_4 (IC_{50}s of 380 nM ($pIC_{50} = 6.46 \pm 0.09$, $5.3 \pm 0.7\%$ ACh Min) and 590 nM ($pIC_{50} = 6.25 \pm 0.10$, $13.1 \pm 2.5\%$ ACh Min), respectively). Due to the unique and non-basic chemotype, we anticipated that this would be selective for M_4, akin to our related efforts on M_1 and M_5.[12–18] Surprisingly, the compound was also found to have substantial antagonist activity at all of the mAChRs ($M_{1-3,5}$ IC_{50}s < 1.5 μM), but was weakly M_4-preferring (2- to 5-fold). We also assessed the DMPK profile of **5**, and found it to be highly bound in plasma ($f_{u,plasma}$ (r, h) = 0.004, 0.001), with a high rat brain:plasma K_p (1.45), but lower $K_{p,uu}$ (0.36)(rat $f_{u,brain}$ = 0.001). Furthermore, the predicted hepatic clearance of **5** was near hepatic blood flow in both human and rat (CL_{hep} = 20.3 and 68.6 mL/min/kg, respectively) based on CL_{int} data from microsomes. Despite these blemishes, this scaffold was attractive from an academic standpoint to assess if either M_4 selectivity could be improved or if a next generation *pan*-mAChR antagonist could be generated with favorable PK and CNS penetration to compliment **1**–**4**.

Toward these parallel goals, an SAR campaign focused around modifications to the eastern diaryl ether motif was pursued. In order to streamline the synthesis of these compounds for a more high-throughput approach, chloropyrimidine intermediate **13** was first synthesized from HCl salt **9** (Scheme 2). This intermediate