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Discovery and optimization of 3-(4-aryl/heteroarylsulfonyl)piperazin-1-yl)-6-(piperidin-1-yl)pyridazines as novel, CNS penetrant *pan*-muscarinic antagonists

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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 3-(4-aryl/heteroarylsulfonyl)piperazin-1-yl)-6-(piperidin-1-yl)pyridazine core, identified from a high-throughput screening campaign. A multi-dimensional optimization effort enhanced potency at human M_4 (hM_4 IC_{50}s < 200 nM), with only moderate species differences noted, and with enantioselective inhibition. Moreover, CNS penetration proved attractive for this series (rat brain:plasma K_p = 2.1, $K_{p,uu}$ = 1.1). Despite the absence of the prototypical mAChR antagonist basic or quaternary amine moiety, this series displayed pan-muscarinic antagonist activity across M_{1-5} (with 9- to 16-fold functional selectivity at best). This series further expands the chemical diversity of mAChR antagonists.

Selective inhibition of the M_4 receptor, one of five muscarinic acetylcholine receptors (mAChRs or M_{1-5}), a class A family of G protein-coupled receptors (GPCRs), has emerged as an exciting new approach for the symptomatic treatment of Parkinson's disease.[1–4] However, the development of small molecule ligands that are selective for M_4, or any of the individual mAChRs, has proven to be challenging due to the high sequence homology amongst the receptor subtypes.[5–8] Historically, mAChR antagonists possessed somewhat conserved chemotypes, exemplified by a basic tertiary or quaternary amine, and compounds with these functional groups represent the bulk of high-throughput screening (HTS) hits (the 'usual suspects') and marketed drugs **1**–**4** (Fig. 1).[9–11] Thus, we were delighted to identify fundamentally new chemotypes in an M_4 functional HTS campaign, which was subsequently optimized to deliver potent and CNS penetrant antagonists such as **5**; however, despite the absence of the classical pharmacophore, **5** proved to be a *pan*-muscarinic antagonist that bound to the orthosteric (ACh) site.[12] Another departure from the classical mAChR chemotype was found in HTS hit **6**, an ~1 μM hM_4 antagonist. This Letter details the synthesis, SAR, pharmacology and DMPK profiles of analogs of **6**, and the finding, once again, of *pan*-mAChR inhibition.

Compound **6** was resynthesized as shown in Scheme 1 as both the racemate, as well as the single (*R*)- and (*S*)-enantiomers. Briefly, 3,6-dichloropyridazine was subjected to an S_NAr reaction with 2-methylpiperidine in NMP at 200 °C, followed by a second S_NAr with piperazine to afford racemic **8** or chiral **8a**/**8b** in 32–39% isolated yields in a one-pot procedure. Standard sulfonamide formation with 2-chlorobenzenesulfonyl chloride delivered racemic **6** and chiral **6a**/**6b** in moderate yields.[13]

After resynthesis, racemic **6** was found to have sub-micromolar potency at human M_4 (hM_4 IC_{50} = 530 nM, pIC_{50} = 6.28 ± 0.07, 9.2 ± 5.4% ACh Min). Excitingly, **6a**, the (*S*)-enantiomer showed enhanced potency (hM_4 IC_{50} = 440 nM, pIC_{50} = 6.37 ± 0.08, 4 ± 0% ACh Min), while **6b**, the (*R*)-enantiomer was significantly less active (hM_4 IC_{50} = 6.08 μM, pIC_{50} = 5.24 ± 0.09, 12 ± 2% ACh Min). Based on the unique and non-basic chemotype, coupled with the

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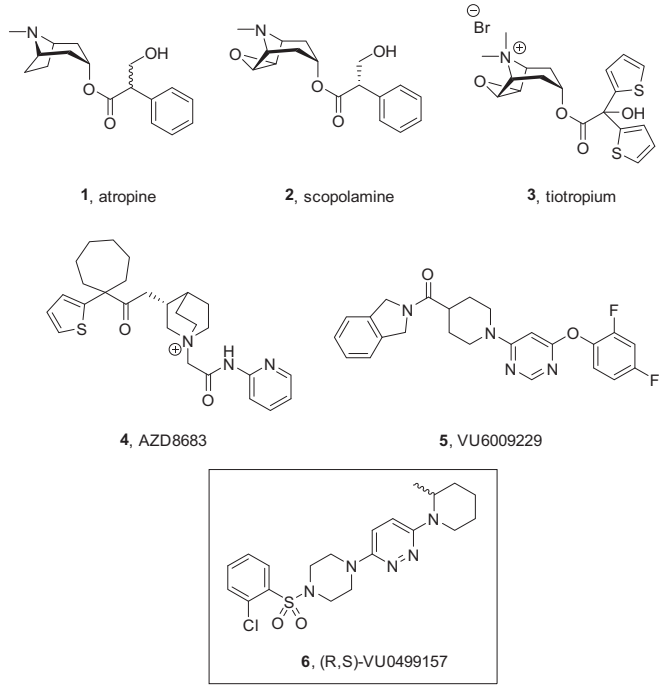


Fig. 1. Chemical structures of known muscarinic antagonists **1**–**4**, the newly optimized *pan*-mAChR antagonist **5**, and a novel hit **6** from an M_4 antagonist high-throughput screen.

noted enantioselectivity (~14-fold) of hM_4 inhibition, we anticipated that **6a** would be selective for M_4, akin to our related efforts on M_1 and M_5.[14–20] Interestingly, **6a** inhibited the other four mAChRs (hM_1 IC_{50} = 1.3 μM, 2.8% ACh Min; hM_2 IC_{50} = 1.2 μM, 2.9% ACh Min; hM_3 IC_{50} = 7.0 μM, 4.3% ACh Min; hM_5 IC_{50} = 6.5 μM, 3.1% ACh Min), but was moderately M_4-preferring (2.7- to 16-fold). We then determined activity at rat mAChRs, and here, **6a** was a weaker antagonist (rM_1 IC_{50} = 1.4 μM, 2.6% ACh Min; rM_2 IC_{50} = 5.9 μM, 7.1% ACh Min; rM_3 IC_{50} > 10 μM, 40% ACh Min; rM_4 IC_{50} = 1.0 μM, 4.8% ACh Min; rM_5 IC_{50} > 10 μM, 37% ACh Min), only partially diminishing an EC_{80} of ACh at M_3 and M_5. While not the result we were hoping for in terms of mAChR selectivity, the series was still deemed worthy of further optimization. Fig. 2 highlights the chemical optimization plan for **6a**.

Our initial survey held the eastern portion of **6a** constant while evaluating alternative sulfonamides, according to the route depicted in Scheme 1, which afforded analogs **9** (Table 1). Clear SAR was noted with analogs **9**. Substitutions in the 2-position of the phenylsulfonamide were preferred, as potency decreased from **6a** (hM_4 IC_{50} = 440 nM), to the 3-Cl congener **9a** (hM_4 IC_{50} = 760 -

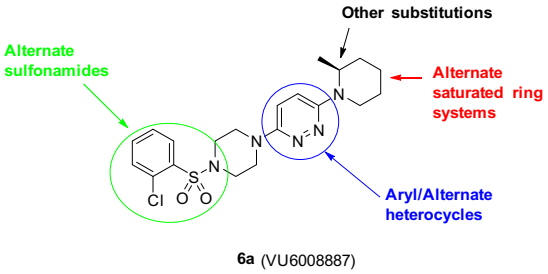
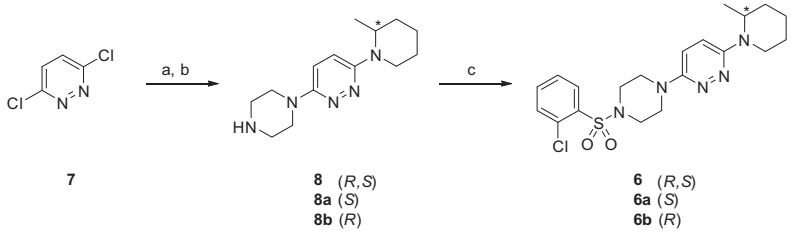


Fig. 2. Chemical optimization plan for **6a**, surveying multiple dimensions of the novel mAChR antagonist chemotype.

nM), and to the 4-Cl analog **9b** (hM_4 IC_{50} = 2.34 μM). Unsubstituted phenyl, **9c**, was weak, as were electron-donating moieties in the 2-positon (**9d**). A 2-CF_3 derivative (**9e**) was essentially equipotent to **6a**. A 2,5-dimethylisoxazole (**9g**) afforded the best activity in this series (hM_4 IC_{50} = 90 nM), and a piperonyl congener **9i** (hM_4 IC_{50} = 200 nM) was 10-fold more potent than a 3,4-dimethoxy analog **9j** (hM_4 IC_{50} = 2.80 μM). However, all analogs **9** (and including **6a**) displayed high predicted hepatic clearance (CL_{hep}) based on microsomal intrinsic clearance (CL_{int}) data in both rat and human near hepatic blood flow rates (>60 mL/min/kg and > 20 mL/min/kg, respectively), as well as high plasma protein binding. Based on these results, we performed metabolite identification studies in rat and human hepatic microsomes to assess soft spots and attempt to understand the high clearance, which appeared to be independent of the nature of the sulfonamide moiety. For this work, we evaluated **6a** (VU6008887) in the presence and absence of NADPH, and found no NADPH-independent metabolism. **6a** was more stable in human, forming 8 NADPH-dependent metabolites, but the parent remained the major species. In rat microsomes, the major peak (by UV and extracted ion chromatograms) was metabolite F, resulting from extensive oxidative metabolism of the piperidine moiety (Fig. 3), and very little parent remained.

Based on these data, we first explored alternative heteroaryl replacements for the pyridazine ring, to assess if electronics could modulate metabolism of the piperidine ring (or the pendant methyl group) while maintaining M_4 inhibitory activity. Employing variations of **7** in Scheme 1, the two regioisomeric pyridine cores **10** and **11** were prepared, as well as a pyrazine core **12** and a phenyl congener **13** (Fig. 4). Clearly, the pyridazine of **6a** and **9a**–**k** is essential for M_4 activity, and these alternate heterocyclic analogs did not provide superior compounds.

Therefore, all efforts now focused on a multidimensional array surveying the most active sulfonamide moieties (**6a**, **9g** and **9i**) in combination with replacements for the 2-methylpiperidine moiety (Table 2). SAR was steep, with chiral 2-methyl morpholine surrogates (**14a**–**c**) devoid of M_4 activity (hM_4 IC_{50}s > 10 μM), as was a



Scheme 1. Synthesis of racemic compound **6** as well as discrete enantiomers.[a] [a]Reagents and conditions: (a) (*R*,*S*), (*R*) or (*S*)-2-methylpiperidine, DIPEA, NMP, microwave, 200 °C; (b) piperazine, 200 °C, 36% (*R*,*S*), 32% (*R*), 39% (*S*); (c) 2-chlorobenzenesulfonyl chloride, DIPEA, DCM, r.t., 38% (*R*,*S*), 35% (*R*), 44% (*S*).