Published in final edited form as:

Bioorg Med Chem Lett. 2017 August 01; 27(15): 3576–3581. doi:10.1016/j.bmcl.2017.05.042.

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 M₄ 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 multidimensional optimization effort enhanced potency at human M₄ (hM₄ IC₅₀s < 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 M₁₋₅ (with 9- to 16-fold functional selectivity at best). This series further expands the chemical diversity of mAChR antagonists.

Graphical abstract

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Keywords

pyridazine; muscarinic acetylcholine receptor; *pan*-antagonist; DMPK; Structure-Activity Relationship (SAR)

Selective inhibition of the M₄ receptor, one of five muscarinic acetylcholine receptors (mAChRs or M₁₋₅), 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 M₄, 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** (Figure 1).⁹⁻¹¹ Thus, we were delighted to identify fundamentally new chemotypes in an M₄ 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 μ M hM₄ 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 submicromolar potency at human M₄ (hM₄ IC₅₀ = 530 nM, pIC₅₀ = 6.28 ± 0.07, 9.2 ± 5.4 % ACh Min). Excitingly, **6a**, the (*S*)-enantiomer showed enhanced potency (hM₄ IC₅₀ = 440 nM, pIC₅₀ = 6.37 ± 0.08, 4 ± 0 % ACh Min), while **6b**, the (*R*)-enantiomer was significantly less active (hM₄ IC₅₀ = 6.08 μ M, pIC₅₀ = 5.24 ± 0.09, 12 ± 2 % ACh Min). Based on the unique and non-basic chemotype, coupled with the noted enantioselectivity (~14-fold) of hM₄ inhibition, we anticipated that **6a** would be selective for M₄, akin to our related efforts on M₁ and M₅.¹⁴⁻²⁰ Interestingly, **6a** inhibited the other four mAChRs (hM₁ IC₅₀ = 1.3 μ M, 2.8% ACh Min; hM₂ IC₅₀ = 1.2 μ M, 2.9% ACh Min; hM₃ IC₅₀ = 7.0 μ M, 4.3% ACh Min; hM₅ IC₅₀ = 6.5 μ M, 3.1% ACh Min), but was moderately M₄-preferring (2.7- to 16-fold). We then determined activity at rat mAChRs, and here, **6a** was a weaker antagonist (rM₁ IC₅₀ = 1.4 μ M, 2.6% ACh Min; rM₂ IC₅₀ = 5.9 μ M, 7.1% ACh Min; rM₃ IC₅₀ > 10 μ M, 40 % ACh Min; rM₄ IC₅₀ = 1.0 μ M, 4.8% ACh Min; rM₅ IC₅₀ > 10 μ M, 37% ACh Min), only partially diminishing an EC₈₀ of ACh at M₃ and M₅. While not the result we were hoping for in terms of mAChR selectivity, the series was still deemed worthy of further optimization. Figure 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** (hM₄ IC₅₀ = 440 nM), to the 3-Cl congener **9a** (hM₄ IC₅₀ = 760 nM), and to the 4-Cl analog **9b** (hM₄ IC₅₀ = 2.34 μM). Unsubstituted phenyl, **9c**, was weak, as were electron-donating moieties in the 2-position (**9d**). A 2-CF₃ derivative (**9e**) was essentially equipotent to **6a**. A 2,5-dimethylisoxazole (**9g**) afforded the best activity in this series (hM₄ IC₅₀ = 90 nM), and a piperonyl congener **9i** (hM₄ IC₅₀ = 200 nM) was 10-fold more potent than a 3,4-dimethoxy analog **9j** (hM₄ IC₅₀ = 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 (Figure 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 M₄ 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** (Figure 4). Clearly, the pyridazine of **6a** and **9a-k** is essential for M₄ 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 M₄ activity (hM₄ IC₅₀s > 10 μM), as was a piperidinone (**14o**). Ring contraction to a chiral 2-methyl pyrrolidine as in **14d-f** lost 5- to 6-fold in hM₄ inhibitory activity relative to **6a**, as did deletion of the chiral methyl group (**14g, h**). Interestingly, ring expansion to an unsubstituted homopiperidine, as in **14i-k**, afforded potent hM₄ antagonists (hM₄ IC₅₀s as low as 290 nM). This result led us to re-explore the piperidine core, but with a *gem*-dimethyl moiety in the 3-position (**14l-n**) to mimic the steric bulk of the homopiperidine. From this effort, **14n** (hM₄ IC₅₀ = 120 nM, 12% ACh min) emerged as an attractive antagonist for further profiling, as it was also active on rat M₄ (rM₄ IC₅₀ = 365 nM, pIC₅₀ = 6.43±0.01, 32±1% ACh Min).

Based on the unexpected results with **5** and **6a**, we next evaluated the molecular pharmacology profile of **14n** (VU6009833, Figure 5). Like **5**, **14n** proved to bind at the

orthosteric ACh site (or possibly overlapping with the orthosteric site), affording a K_i of 7.78 ± 0.86 nM in a radioligand binding assay displacing [3 H]-NMS in human M_4 membranes. Compound **14n** inhibited all five human mAChRs, but displayed weak, partial antagonism^{21,22} of M_3 and M_5 , and was a potent full antagonist of M_1 and M_2 (rat data similar but not shown). Thus, like atypical pyrimidine **5**, **14n** represents another novel chemotype with *pan*-mAChR inhibitory activity.

14n possessed attractive physiochemical properties (clogP of 2.9, TPSA of 96 and a molecular weight of 434), which translated into acceptable plasma protein binding (f_u (r,h) = 0.07 and 0.08) and rat brain homogenate binding (f_u = 0.04). In our standard rat plasma:brain level (PBL) cassette study,²³ **14n** displayed a K_p of 2.1 and a $K_{p,uu}$ of 1.1, indicating excellent total and free CNS distribution. However, predicted hepatic clearance remained near hepatic blood flow rates in both rat and human (CL_{hep} = 66.6 mL/min/kg and 20 mL/min/kg, respectively), suggesting that co-administration with an inhibitor of metabolism (putatively CYP₄₅₀) and/or non-oral route(s) of administration may be required for *in vivo* studies with **14n**. Currently, efforts are focused on improving metabolic stability of **14n** and related analogs, as well as homology modeling and mutagenesis to better understand the mode of interaction with the ACh binding site.

In summary, an M_4 HTS campaign identified **6** as a novel M_4 antagonist chemotype, devoid of the ‘usual suspect’ pharmacophores. Synthesis of the discrete enantiomers indicated enantioselectivity with regards to M_4 inhibition, yet this series proved to lack mAChR subtype selectivity. Further optimization afforded **14n**, a potent and highly CNS penetrant *pan*-mAChR antagonist with attractive physiochemical properties. Additionally, **14n** and related analogs do not feature either a strong basic amine or the prototypical tropane structure of classical muscarinic antagonists. Thus, these analogs represent a next generation of *pan*-mAChR antagonists that could serve as leads for the development of potentially safer or differentiating anti-cholinergic agents. Studies are underway to better understand the nature of the binding mode, as well as efforts to reduce metabolic clearance and ultimately improve *in vivo* pharmacokinetics.

Acknowledgments

We thank the NIH for funding via the NIH Roadmap Initiative 1X01 MH077607 (C.M.N.), the Molecular Libraries Probe Center Network (U54MH084659 to C.W.L.) and U01MH087965 (Vanderbilt NCDDG). We also thank William K. Warren, Jr. and the William K. Warren Foundation who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.). We thank Q² Solutions’ discovery metabolism group for the contracted metabolite identification experiments and analysis.

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13. **Representative Experimental.** All reactions were carried out employing standard chemical techniques under inert atmosphere. Solvents used for extraction, washing, and chromatography were HPLC grade. All reagents were purchased from commercial sources and were used without further purification. Analytical HPLC was performed on an Agilent 1200 LCMS with UV detection at 215 nm and 254 nm along with ELSD detection and electrospray ionization, with all final compounds showing > 95% purity and a parent mass ion consistent with the desired structure. All NMR spectra were recorded on a 400 MHz Bruker AV-400 instrument. ¹H chemical shifts are reported as δ values in ppm relative to the residual solvent peak (MeOD = 3.31, CDCl₃ = 7.26). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz), and integration. ¹³C chemical shifts are reported as δ values in ppm relative to the residual solvent peak (MeOD = 49.0, CDCl₃ = 77.16). Low resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization, with a gradient of 5–95% MeCN in 0.1% TFA water over 1.5 min. Microwave synthesis was performed on an Initiator+ by Biotage. Preparative purification (RP-HPLC) of library compounds was performed on a Gilson 215 preparative LC system. Representative synthesis of **14n**: 3-(3,3-dimethylpiperidin-1-yl)-6-(piperazin-1-yl)pyridazine. 3,6-dichloropyridazine (150 mg, 1.01 mmol) and 3,3-dimethylpiperidine (137 mg, 1.21 mmol, 1.2 eq) were dissolved in NMP (3 mL) in a 5 mL microwave vial. DIPEA (0.88 mL, 5.03 mmol, 5 eq) was then added. The resulting solution was heated to 200 °C in the microwave for 1 h, after which time piperazine (651 mg, 5.03 mmol, 5 eq) was added. The resulting solution was heated to 200 °C in the microwave for 1 h, after which time solids were removed by filtration. Crude residue was purified by RP-HPLC, and fractions containing product were concentrated to give product as the TFA salt (213 mg, 54%). ¹H NMR (400 MHz, CD₃OD) δ 7.82 – 7.72 (m, 2H), 3.67 (t, J = 5.2, 4H), 3.54 (t, J = 5.6, 2H), 3.26 (t, J = 5.3, 4H), 3.22 (s, 2H), 1.73 – 1.67 (m, 2H), 1.46 (t, J = 6.2, 2H), 0.90 (s, 6H); ¹³C NMR (101 MHz, CD₃OD) δ 151.77, 149.22, 126.18, 123.55, 57.20, 46.72, 42.44, 36.43, 31.79, 24.53, 21.06. LCMS (215 nm) RT = 0.317 min (>98%); m/z 276.4 [M+H]⁺. (**14n**): 4-[4-[6-(3,3-dimethyl-1-piperidyl)pyridazin-3-yl]piperazin-1-yl]sulfonyl-3,5-dimethylisoxazole. 3-(3,3-dimethylpiperidin-1-yl)-6-(piperazin-1-yl)pyridazine. (10 mg, 0.026 mmol) was dissolved in DCM (1 mL) and DIPEA (9 μ M, 0.051 mmol, 2 eq) was added, followed by 3,5-dimethylisoxazole-4-sulfonyl chloride (8 mg, 0.039 mmol, 1.5 eq). The resulting solution was stirred at r.t. for 1 h, after which time solvents were concentrated, and crude residue was purified by RP-HPLC. Fractions containing product were basified with sat. NaHCO₃, and extracted with 3:1 chloroform/IPA solution. Solvents were filtered through a phase separator and concentrated to give the title compound (3.5 mg, 31%). ¹H NMR (400 MHz, CDCl₃) δ 6.93 – 6.84 (m, 2H), 3.55 (t, J = 5.0, 4H), 3.43 (t, J = 5.6, 2H), 3.20 (t, J = 5.0, 4H), 3.14 (s, 2H), 2.62 (s, 3H), 2.38 (s, 3H), 1.69 – 1.63 (m, 2H), 1.39 (t, J = 6.1, 2H), 0.92 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 173.95, 158.00, 156.88, 154.27, 118.04, 117.18, 113.11, 57.97, 46.37, 46.15, 44.90, 37.73, 31.21, 26.59, 21.80, 13.10, 11.47. LCMS (215 nm) RT = 0.839 min (>98%); m/z 435.4 [M+H]⁺
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Figure 1. Chemical structures of known muscarinic antagonists **1-4**, the newly optimized *pan*-mAChR antagonist **5**, and a novel hit **6** from an M₄ antagonist high-throughput screen.

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

Figure 3.

Metabolite identification studies in rat and human liver microsomes in the presence or absence of NADPH. No NADPH-independent metabolites were noted in either species, but rat specifically produced metabolite F.

Figure 4. Structures and activities of pyridazine replacements **10-13**. Note, the parent pyridazine analog, **9i**, has an hM₄ IC₅₀ of 200 nM.

Figure 5.

In vitro molecular pharmacology profile of **14n** (VU6009833). A) Radioligand binding assay displacing [³H]-NMS in human M₄ membranes. **14n** afforded a K_i of 7.78±0.86 nM, while atropine, in agreement with historical data afforded a K_i of 0.64±0.09 nM. B) Concentration response curves (CRCs) for **14n** in calcium mobilization assays in stably expressing hM₁₋₅ Chinese hamster ovary cells (for Gi-coupled hM₂ and hM₄, co-expressed with Gq_{i5}) in the presence of an approximate EC₈₀ of ACh. Data are presented as the percentage of the EC₈₀ ACh response of each cell line. All data points represent the mean of triplicate in single assay. (M₁ IC₅₀ = 288 nM (11% ACh min), M₂ IC₅₀ = 352 nM (17% ACh min), M₃ IC₅₀ = 782 nM (55% ACh min), M₅ IC₅₀ = 1,030 nM (43% ACh min).

Scheme 1.

Synthesis of racemic compound **6** as well as discrete enantiomers.^a

^aReagents 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*).

Table 1Structures and mAChR activities of analogs **6a**, **9a-k**.

<p style="text-align: center;">9</p>			
Compound	R	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
6a		0.44 [4.0±0]	6.37±0.08
9a		0.76 [22±2]	6.15±0.11
9b		2.34 [30±2]	5.64±0.05
9c		2.57 [8.0±1]	5.59±0.02
9d		2.38 [11±0]	5.62±0.02
9e		0.52 [4.0±0]	6.28±0.02

Compound	R	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
9f		1.60 [8.0±0]	5.80±0.06
9g		0.09 [6.0±0]	7.04±0.03
9h		1.17 [7.0±1]	5.95±0.09
9i		0.20 [9.0±1]	6.71±0.06
9j		2.80 [46±3]	5.58±0.10
9k		4.02 [51±0]	5.40±0.08

^a Mean of three independent determinations in a calcium mobilization assay using recombinant hM₄-expressing Chinese hamster ovary cells co-transfected with chimeric Gq15 in the presence of an ACh EC₈₀.

Table 2Structures and mAChR activities of analogs **14**.

14				
Cmpd	R	R ₁	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
14a			>10 [56]	>5
14b			>10 [63]	>5
14c			>10 [66]	>5
14d			2.90 [9.0±0]	5.54±0.02
14e			2.73 [14±5]	5.59±0.12
14f			3.31 [10±1]	5.48±0.03

Cmpd	R	R ₁	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
14g			5.58 [12±1]	5.27±0.08
14h			0.73 [55±8]	6.18±0.14
14i			1.27 [6.0±0]	5.90±0.06
14j			0.29 [20±3]	6.54±0.02
14k			0.43 [8.0±1]	6.39±0.09
14l			0.94 [5.0±0]	6.03±0.03

<p style="text-align: center;">14</p>				
Cmpd	R	R ₁	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
14m			0.47 [14±2]	6.34±0.06
14n			0.12 [12±1]	6.96±0.14
14o			>10 [51]	>5

^a Mean of three independent determinations in a calcium mobilization assay using recombinant hM₄-expressing Chinese hamster ovary cells co-transfected with chimeric G_qi5 in the presence of an ACh EC₈₀.