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

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Abstract

This Letter describes the synthesis and structure activity relationship (SAR) studies of structurally novel M₄ 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 M₄ (IC₅₀s < 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.

Graphical Abstract

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Keywords

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

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**) (Figure 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 (Figure 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** (Figure 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}

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 ± 0.7 % ACh Min) and 590

nM ($pIC_{50} = 6.25 \pm 0.10$, 13.1 ± 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 ($M_{1–3,5}$ $IC_{50}s < 1.5$ μ M), 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 ($f_{u,p\ plasma} (r, h) = 0.004, 0.001$), 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 could then be used as a platform for library synthesis, and a number of substituted phenols were coupled to **13** through microwave-assisted S_NAr in moderate yields (**14a–o**).

All analogs **14** were of comparable potency at rat M_4 ($IC_{50}s$ within 2- to 3-fold, $n = 1$), and all were uniformly predicted to be highly cleared (predicted CL_{hep} of ~ 18 – 20 mL/min/kg and ~ 60 – 68 mL/min/kg, for human and rat respectively). Similarly, SAR was rather ‘flat’ with little texture with respect to M_4 inhibition, suggesting other areas of the molecule may be better targets for future optimization. However, analog **14g** showed high brain distribution (rat $K_p \sim 0.68$, $K_{p,uu} \sim 0.75$), but its high clearance hindered any further advancement. Moreover, when evaluated in a [3H]-NMS binding assay with human M_4 cell membranes, **14k** (VU6008913) displaced the radioligand binding with a K_i of 10.3 nM (cf. to atropine, $K_i = 1.6$ nM),²⁰ which translated into no mAChR selectivity (M_1 $IC_{50} = 250$ nM, M_2 $IC_{50} = 330$ nM, M_3 $IC_{50} = 380$ nM, M_5 $IC_{50} = 510$ nM, all $n = 1$ and $<6\%$ ACh Min). Similarly, other potent antagonists such as **14g** (M_1 $IC_{50} = 322$ nM, M_2 $IC_{50} = 384$ nM, M_3 $IC_{50} = 404$ nM, M_5 $IC_{50} = 732$ nM, all $n = 1$ and $<6\%$ ACh Min) and **14h** (M_1 $IC_{50} = 313$ nM, M_2 $IC_{50} = 352$ nM, M_3 $IC_{50} = 365$ nM, M_5 $IC_{50} = 695$ nM, all $n = 1$ and $<6\%$ ACh Min) were *pan*-mAChR antagonists. Thus, it became readily apparent that this series was not likely to afford selective M_4 antagonists, but that the goal should instead focus on development of a next generation *pan*-mAChR antagonist with exceptional PK and CNS penetration to compliment **1–4**.

Due to a putative blockade of oxidative metabolism of the phenyl moiety via fluoro substitution, we next decided to hold the 2,4-difluoro phenylether eastern piece constant and explore an amide library to evaluate alternatives for the western THIQ moiety. Starting from commercially available methyl 1-(6-chloropyrimidin-4-yl)piperidine-4-carboxylate **15**, a one-pot S_NAr /methyl ester hydrolysis was performed under microwave irradiation to give carboxylic acid **16** in moderate yield, which could then be used for HATU-mediated amide couplings to give final analogs **17a–h** (Scheme 3).

As shown in Table 2, SAR was steep with respect to replacements for the THIQ moiety. Benzyl amines such as **17g** and **17h** were inactive, as was a CF₃-substituted THIQ, **17a**. Electron-donating moieties, such as the regioisomeric OMe-substituted THIQ analogs **17b** and **17c**, retained M₄ inhibitory activity, as did a fully saturated congener **17d**. However, the isoindoline derivative **17e** emerged as the most exciting analog. **17e** was a potent antagonist at both human and rat M₄ (hM₄ IC₅₀ = 340 nM, pIC₅₀ = 6.49±0.06, 18.4±2.3% ACh Min and rM₄ IC₅₀ = 560 nM, pIC₅₀ = 6.32±0.18, 42.7±3.2% ACh Min), but displayed partial antagonist activity.^{21,22} Moreover, **17e** proved to also be a *pan*-mAChR antagonist (Figure 2). However, the disposition of **17e** was superior to all other analogs **14** and **17** evaluated. **17e** (VU6009229) displayed good CNS penetration (rat brain:plasma K_p = 0.67, K_{p,uu} = 0.87) and low to moderate predicted hepatic clearance (rat CL_{hep} = 36.5 mL/min/kg and human CL_{hep} = 5.56 mL/min/kg). A robust *in vitro:in vivo* correlation (IVIVC) was noted, with **17e** displaying a low rat *in vivo* clearance (CL_p = 5.37 mL/min/kg; predicted CL_{hep} with inclusion of binding terms in the well-stirred model = 5.93 mL/min/kg) with a 3.7 hour elimination half-life and modest volume (V_{ss} = 1.35 L/kg) in an IV cassette (0.2 mg/kg; *n* = 1) study. Thus, **17e** emerged as a next generation *pan*-mAChR partial antagonist tool compound, with attractive *in vivo* rat PK and excellent CNS penetration, suitable for *in vitro* and *in vivo* studies.

In summary, an M₄ HTS campaign identified **5** as a novel M₄ antagonist chemotype, that proved to lack mAChR subtype selectivity. Further optimization afforded **17e**, a potent and highly CNS penetrant *pan*-mAChR antagonist with an attractive rat *in vivo* PK profile. Additionally, **17e** and related analogs do not feature the prototypical tropane structure of classical muscarinic antagonists, nor a strong basic amine. Thus, these analogs represent a next generation of *pan*-mAChR antagonists that could serve as leads for the development of potential safer or differentiating anti-cholinergic agents. The expedient and straightforward synthesis of these analogs will allow us to further explore the requirements for muscarinic selectivity, as well as fine-tune the DMPK properties of this series.

Acknowledgments

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19. 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. Automated flash column chromatography was performed on an Isolera One by Biotage. 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 experimental for **5**. **(3,4-dihydroisoquinolinyl)methanone**. *N*-Boc-isonipecotic acid **7** (2.0 g, 8.72 mmol) and 1,2,3,4-tetrahydroisoquinoline **6** (1.39 g, 10.5 mmol) were dissolved in DCM (50 mL), and DIPEA (3.04 mL, 17.4 mmol) was added, followed by HATU (3.98 g, 10.5 mmol). The resulting solution was stirred at r.t. overnight, after which time the reaction mixture was quenched with sat. NaHCO₃, and extracted with DCM. Combined organic extracts were filtered through a phase separator and concentrated. Crude residue was purified by column chromatography (hex/EtOAc) to give product **8** as a yellow solid (2.73 g, 91%). ¹H NMR (400 MHz, CDCl₃) δ 7.17 – 7.07 (m, 4H), 4.65 (s, 1H), 4.60 (s, 1H), 4.08 (br, 2H), 3.76 (br, 1H), 3.66 (t, J = 5.7, 1H), 2.86 – 2.64 (m, 5H), 1.75 – 1.58 (m, 4H), 1.39 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 173.43, 173.24, 154.68, 135.11, 133.85, 133.48, 132.53, 129.07, 128.29, 127.04, 126.70, 126.63, 126.55, 126.38, 125.96, 79.53, 47.35, 44.46, 43.07, 40.02, 39.03, 38.89, 29.82, 28.44, 28.39, 28.28. LCMS (215 nm) RT = 0.924 min (>98%); m/z 289.2 [M+H - *t*-butyl]. Compound **8** (2.73 g, 7.93 mmol) was dissolved in 1,4-dioxane (40 mL), and 4M HCl in dioxanes solution (40 mL) was then added dropwise. The resulting solution was stirred at r.t. for 1 h, after which time solvents were concentrated under reduced pressure, and the resulting white solid **9** was dried under vacuum and used without additional purification (2.21 g, 99%). ¹H NMR (400 MHz, CD₃OD) δ 7.25 – 7.15 (m, 4H), 4.82 (s, 1H), 4.70 (s, 1H), 3.86 (t, J = 5.8, 1H), 3.80 (t, J = 6.0, 1H), 3.49 – 3.46 (m, 2H), 3.27 – 3.14 (m, 3H), 2.97 (t, J = 5.7, 1H), 2.86 (t, J = 5.9, 1H), 2.04 – 1.91 (m, 4H); ¹³C NMR (101 MHz, CD₃OD) δ 173.11, 172.93, 134.63, 134.18, 132.82, 132.76, 128.33, 128.06, 126.73, 126.43, 126.23, 126.13, 126.09, 125.89, 48.48, 46.92, 44.28, 43.14, 42.95, 40.41, 35.77, 35.60, 29.20, 27.82, 25.20, 25.08. LCMS (215 nm) RT = 0.527 min (>98%); m/z 245.2 [M+H]. 4-ethylphenol **11** (205 mg, 1.68 mmol) was dissolved in DMF (10 mL) and K₂CO₃ (471 mg, 3.36 mmol) was added. 4,6-dichloropyrimidine **10** (250 mg, 1.68 mmol) was then added. The resulting solution was stirred at 100 °C until complete. Reaction mixture was cooled to r.t., and diluted with H₂O and DCM. Aqueous layer was extracted with DCM, and combined organic extracts were filtered through a phase separator and concentrated. Crude residue was purified by RP-HPLC. Fractions containing product were basified with sat. NaHCO₃ and extracted with DCM. Solvents were concentrated to give product **12** as a brown oil (149 mg, 38%). ¹H NMR (400 MHz, CDCl₃) δ 8.50 (s, 1H), 7.18 (d, J = 8.1, 2H), 6.96 (d, J = 8.1, 2H), 6.80 (s, 1H), 2.60 (q, J = 7.6, 2H), 1.18 (t, J = 7.6, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 170.61, 161.94, 158.65, 149.88, 142.33, 129.36, 121.16, 107.76, 28.33, 15.47. LCMS (215 nm) RT = 0.958 min (>95%); m/z 235.2 [M+H]. Compound **9** (50.2 mg, 0.18 mmol), compound **12** (28 mg, 0.12 mmol) and DIPEA (0.042 mL, 0.24 mmol) were combined in a microwave vial, and NMP (1 mL) was added. The resulting solution was heated to 150 °C with microwave irradiation for 20 min, after which time crude residue was purified directly by RP-HPLC. Fractions containing product were basified with sat. NaHCO₃, and extracted with 3:1 chloroform/IPA. Solvents were concentrated to give product **5** (as a white solid (31 mg, 59%). ¹H NMR (400 MHz, CDCl₃) δ 8.23 (s, 1H), 7.16 – 7.09 (m, 6H), 6.96 (d, J = 8.0, 2H), 5.88 (br, 1H), 4.66 (s, 1H), 4.63 (s, 1H), 4.28 (br, 2H), 3.77 (t, J = 5.7, 1H), 3.69 (t, J = 5.7, 1H), 2.98 – 2.77 (m, 5H), 2.59 (q, J = 7.6, 2H), 1.82 – 1.76 (m, 4H), 1.18 (t, J = 7.5, 3H). LCMS (215 nm) RT = 0.955 min (>98%); m/z 443.2 [M+H]. Representative experimental for **17e** (**VU6009229**). **(1-(6-(2,4-difluorophenoxy)pyrimidin-4-yl)piperidin-4-yl)(isoindolin-2-yl)methanone**. methyl 1-(6-chloropyrimidin-4-yl)piperidine-4-carboxylate **15** (500 mg, 1.96 mmol), 2,4-difluorophenol (1.02 g, 7.82 mmol) and Cs₂CO₃ (2.56 g, 7.82 mmol) were combined in a microwave vial, and NMP (11 mL) was added. The resulting solution was heated to 180 °C with microwave irradiation for 20 min, after which time solids were removed by filtration, and crude residue was purified by RP-HPLC. Fractions containing product were extracted with 3:1 chloroform/IPA. Solvents were dried with MgSO₄, filtered and concentrated to give product **16** as a brown, spongy solid (320 mg, 49%). ¹H NMR (400 MHz, CDCl₃) δ 8.27 (s, 1H), 7.20 – 7.14 (m, 1H), 6.97 – 6.88 (m, 2H), 6.05 (s, 1H), 4.27 – 4.24 (m, 2H), 3.17 – 3.10 (m, 2H), 2.70 – 2.63 (m, 1H), 2.06 – 2.02 (m, 2H), 1.82 – 1.73 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 179.03, 169.60, 163.87, 161.28, 161.18, 158.82, 158.72, 157.72, 156.01, 155.89, 153.51, 153.38, 136.62, 124.63, 124.54, 111.68, 111.64, 111.45, 111.41, 105.68, 105.46, 105.41, 105.19, 85.88, 43.83, 40.68, 27.46 (multiple Cs coupled to F). LCMS (215 nm) RT = 0.764 min (>98%); m/z 336.2 [M+H]. Compound **16** (50 mg, 0.15 mmol) and isoindoline (0.028 mL, 0.22 mmol) were dissolved in DMF (2 mL), and DIPEA (0.13 mL, 0.75 mmol) and was stirred at r.t. for 1 h, after which time crude residue was purified directly by RP-HPLC. Fractions containing product were basified with

sat. NaHCO_3 , and extracted with 3:1 chloroform/IPA. Organic extracts were filtered through a phase separator and concentrated to give product **17e** as a light brown solid (42 mg, 65%). ^1H NMR (400 MHz, CDCl_3) δ 8.26 (s, 1H), 7.32 – 7.27 (m, 4H), 7.20 – 7.14 (m, 1H), 6.96 – 6.87 (m, 2H), 6.09 (s, 1H), 4.93 (s, 2H), 4.82 (s, 2H), 4.48 – 4.41 (m, 2H), 3.10 – 3.03 (m, 2H), 2.83 – 2.75 (m, 1H), 1.93 – 1.88 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.19, 169.70, 163.99, 161.12, 161.02, 158.67, 158.57, 157.87, 156.02, 155.90, 153.52, 153.39, 136.74, 136.70, 136.62, 136.58, 136.54, 136.01, 128.10, 127.72, 124.64, 124.62, 124.54, 124.52, 123.22, 122.70, 111.55, 111.51, 111.32, 111.29, 105.56, 105.34, 105.29, 105.07, 85.94, 52.51, 52.47, 43.96, 40.47, 27.59 (multiple Cs coupled to F). LCMS (215 nm) RT = 0.950 min (>98%); m/z 437.2 $[\text{M}+\text{H}]$. 20.

20.

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Figure 1. Chemical structures of known muscarinic antagonists **1–4**, and the newly identified hit **5** from an M₄ antagonist high-throughput screen.

Figure 2.

Concentration response curves (CRCs) for **17e** (VU6009229) in calcium mobilization assays with recombinant hM₁₋₅ Chinese hamster ovary cells (co-transfected with G_{q15}) in the presence of an approximate EC₈₀ of ACh. (M₁ IC₅₀ = 540 nM (18.2% ACh min), M₂ IC₅₀ = 520 nM (8.7% ACh min), M₃ IC₅₀ = 660 nM (2.3% ACh min), M₅ IC₅₀ = 1,130 nM (42.6% ACh min), all n = 1.

Scheme 1.

Synthesis of compound **5** and route for analog synthesis.^a

^aReagents and conditions: (a) HATU, DIPEA, DCM, 91%; (b) HCl, 1,4-dioxane, 99%; (c)

12, DIPEA, NMP, microwave, 150 °C, 59%; (d) K₂CO₃, DMF, 100 °C, 38%.

Scheme 2. Synthesis of analogs **14**.^a^aReagents and conditions: (a) 4,6-dichloropyrimidine, DIPEA, DMF, 100 °C, 88%; (b) substituted phenol, Cs₂CO₃, NMP, microwave, 180 °C, 45–67%.

Scheme 3. Synthesis of compounds **17a-h**.^a

^aReagents and conditions: (a) 2,4-difluorophenol, Cs_2CO_3 , NMP, microwave, 180 °C, 49%; (b) amine, HATU, DIPEA, DMF, 32–67%.

Table 1Structures and mAChR activities of analogs **5**, **14a–o**.

<p style="text-align: center;">14a-o</p>			
Compound	R	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
5	4-Et	0.38 [5.3±0.7]	6.46±0.09
14a	H	0.65 [2.7±0.3]	6.21±0.09
14b	4-Me	0.31 [3.3±0.2]	6.52±0.08
14c	2-naphthyl	0.71 [6.8±2.2]	6.16±0.06
14d	3,4-methylenedioxy	0.51 [4.1±0.3]	6.30±0.09
14e	4-F	0.24 [3.5±0.2]	6.64±0.02
14f	3-F	0.31 [3.2±0.3]	6.52±0.06
14g	2-F	0.13 [3.2±0.5]	6.91±0.08
14h	4-Cl	0.14 [3.0±0.2]	6.88±0.07
14i	4-OMe	0.46 [5.3±1.0]	6.30±0.06
14j	2,3-diF	0.19 [3.1±0.1]	6.77±0.15
14k	2,4-diF	0.10	7.00±0.08
14l	2,5-diF	0.08 [3.1±0.2]	7.11±0.11
14m	2,6-diF	2.72 [7.0±0.9]	5.57±0.05
14n	2-F, 4-Cl	0.15 [3.1±0.3]	6.93±0.06
14o	2,4-diCl	0.27 [3.0±0.1]	6.58±0.09

^aMean 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.

Table 2Structures and mAChR activities of analogs **17a–h**.

<p style="text-align: center;">17a-h</p>			
Compound	R	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
17a		>10	>5
17b		0.58 [5.5±2.9]	6.25±0.09
17c		2.47 [11.8±1.5]	5.62±0.08
17d		0.41 [5.1±0.6]	6.39±0.05
17e		0.34 [18.4±2.3]	6.49±0.06
17f		1.04 [6.0±0.5]	6.00±0.05

17a-h

Compound	R	hM ₄ IC ₅₀ (μM) ^a [% ACh Min ±SEM]	hM ₄ pIC ₅₀ (±SEM) ^a
17g		>10	>5
17h		>10	>5

^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.