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Chemical Lead Optimization of a pan G_q mAChR M₁, M₃, M₅ Positive Allosteric Modulator (PAM) Lead. Part I. Development of the first highly selective M₅ PAM

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Abstract

This Letter describes a chemical lead optimization campaign directed at VU0238429, the first M₅-preferring positive allosteric modulator (PAM), discovered through analog work around VU0119498, a pan G_q mAChR M₁, M₃, M₅ PAM. An iterative library synthesis approach delivered the first selective M₅ PAM (no activity at M₁–M₄ @ 30 μM), and an important tool compound to study the role of M₅ in the CNS.

The muscarinic acetylcholine receptors (mAChRs) are members of the family A G-protein-coupled receptors (GPCRs) and include five subtypes denoted M₁–M₅. M₁, M₃ and M₅ couple to G_q and activate phospholipase C, whereas M₂ and M₄ couple to G_{i/o} and associated effector systems. All five of the mAChRs are known to play critical roles in multiple basic physiological processes.^{1–3} As such cholinergic agents that activate or inhibit one or more subtypes have found success both preclinically and clinically for a number of peripheral and CNS pathologies.^{3,4} Within the mAChRs, a major challenge has been a lack of subtype selective ligands to study the specific contribution of discrete mAChRs in various disease states. To address this limitation, we have focused on targeting allosteric sites on mAChRs as a means to develop subtype selective small molecules, both allosteric agonists and positive allosteric modulators (PAMs).^{5–11}

From a functional cell-based high-throughput screen (HTS) to identify M₁ positive allosteric modulators (PAMs) we identified VU0119498, an M₁, M₃, M₅ PAM (Fig. 1). This was a unique hit from the screen, as it was not selective for M₁, but the first example of a pan-G_q mAChR PAM, devoid of activity at the G_{i/o}-coupled M₂ and M₄.

Relative to M₁–M₄, little is known about the precise role(s) of M₅ in the CNS. However, localization data and studies using M₅-knockout (KO) mice suggest that M₅ activation is

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highly important to regulation of central dopaminergic pathways and to ACh-induced cerebrovasodilation. In light of these findings, drugs targeting M₅ may have therapeutic potential for numerous CNS disorders, including cerebrovascular dementia, stroke, Parkinson's disease, Alzheimer's disease, and Schizophrenia^{2,4,12–14}. Historically, lack of selective pharmacological tools available to confirm the putative role(s) of M₅ has seriously hindered progress in this area.

Starting from a pan M₁, M₃, M₅ PAM, VU0119498, we felt it may be possible to maintain M₅ PAM activity and dial out M₁ and M₃ PAM activity through a chemical lead optimization campaign. In a recent Letter,¹⁵ we reported on the discovery and characterization of VU0238429, the first M₅-preferring PAM. At 30 μ M, VU0238429 displayed a 14-fold leftward shift of the ACh concentration-response-curve (CRC), increased ACh affinity for M₅ by ~11-fold and did not displace [³H]-NMS from binding to M₅¹⁵. While this was a major advance in the field, we hoped to develop a truly M₅ selective PAM to dissect the role of M₅ in the CNS. In this Letter, we describe an iterative parallel synthesis approach¹⁶ for the further optimization of VU0238429, and the discovery of M₅ PAMs with unprecedented mAChR selectivity ($\gg$ 30 μ M vs. M₁–M₄).

Our optimization strategy is outlined in Figure 2, and as SAR with allosteric ligands is often shallow,^{5,6,17} we employed an iterative parallel synthesis approach.¹⁶ From our earlier work, the 5-OCF₃ group was essential for M₅-preferring activity, so this moiety was maintained.¹⁵ Libraries were prepared according to Scheme 1, wherein commercial 5-(trifluoromethoxy)indoline-2,3-dione **4** was alkylated with *p*-bromobenzylbromide to deliver key intermediate **5**. An 11-member Suzuki library was then prepared to explore the effect of introduction of biaryl and heterobiaryl motifs into VU0238429 providing analogs **6**. In parallel, **4** was alkylated with functionalized phenethyl bromides **7** to probe the effect of chain homologation in analogs **8**. Compound libraries were then triaged by a single point (10 μ M) screen for their ability to potentiate an EC₂₀ of ACh in M₅-CHO cells (Fig. 3).¹⁵ Based on this screen, select compounds were assayed in 8-point CRCs based on their potentiation efficacy.

In general, chain homologation in analogs **8** proved unsuccessful as potency was compromised despite retention of PAM efficacy at higher concentrations. For example **8a**, the direct phenethyl analog of VU0238429 possessed an M₅ EC₅₀ of 4.9 μ M and 80% ACh maximum response (data not shown). Biaryl and heterobiaryl analogs **6** proved far more productive, affording a number of M₅ PAMs with high selectivity versus M₁ (>30 μ M EC₅₀s) and low micromolar M₅ EC₅₀s (Table 1). All other analogs **6** possessed M₅ EC₅₀s >10 μ M. In general, both 5- (**6b** and **6e**) and 6-membered heterocycles (**6c** and **6d**) were tolerated as were simple phenyl (**6a**) and substituted phenyl (**6f**). Potency was virtually identical for all of these analogs (M₅ EC₅₀s 2.7 μ M to 4.8 μ M) with similar ACh Max values (70–85%). Shallow SAR was again noted with compounds either being active in this potency range or inactive as M₅ PAMs.

Analog **6a** (VU0365114) and **6b** (VU0365117) were selected for additional follow-up. Figure 4 depicts G_q mAChR (M₁, M₃ and M₅) CRCs for **6a** and **6b**. Note **6a** possesses improved M₅ selectivity versus VU0238429 (**3**), with only modest activation of M₃ at 30 μ M. Both analogs **6a** and **6b** elicit significant leftward shifts (>50 -fold) of the ACh CRC, as compared to the 14-fold shift of VU0238429 (**3**). As seen with the M₁ PAM BQCA^{18–20} and other ago-potentiators for class C GPCRs,^{21–23} figure 4C indicates moderate intrinsic allosteric agonism at 30 μ M.

Encouraged by the potency and mAChR selectivity of VU0365114 (**6a**) and VU0238429 (**3**), we synthesized (Scheme 2) a hybrid analog possessing a biphenyl ether moiety,

VU0400265 (**10**). VU0400265 possessed an M₅ EC₅₀ of 1.9 μM with a 75% ACh Max. Importantly, VU0400265 was completely selective versus M₁–M₄, affording no elevation of an ACh EC₂₀ at M₁–M₄ at 30 μM (Fig. 5). Notably, VU0400265 (**10**) represents the most selective M₅ PAM described to date; however, unlike **6a** and **6b**, analog **10** only afforded a ~5-fold shift of the ACh CRC at 30 μM.

Efforts next centered on maintaining mAChR selectivity and M₅ potency, while attempting to improve fold-shift. Subtle structural changes have been shown in this series to have dramatic effects on potency, selectivity and fold-shift. Therefore, we next explored the effect of moving the 5-OCF₃ moiety to the 6-position of the isatin core. Following the route in Scheme 1, we quickly prepared the 6-OCF₃ analogs **11** and **12** of our initial M₅-preferring PAM VU0119498 (**3**) and the biphenyl congener VU0365114 (**6a**), respectively. Interestingly, M₅ potency was relatively maintained (M₅ EC₅₀s of 5.7 μM and 2.7 μM, for **11** (VU0400267) and **12** (VU0400266), respectively); however, both afforded ~95% of ACh Max, suggesting the fold-shift might be improved with this modification. Quite unexpectedly, mAChR selectivity was lost (Fig. 6), once again highlighting the difficulty in developing SAR for allosteric ligands^{5,6,17}. A 6-OCF₃ congener of VU0400265 (**10**) provided similar erosion in mAChR selectivity. Due to the loss in M₅ selectivity, ACh fold-shift experiments were not performed.

All of these analogs displayed moderate to poor PK in rats with limited brain exposure (AUC_{Brain}/AUC_{Plasma} ~0.25), presumably due to the bis-carbonyl of the isatin moiety. However, these are important tools to study M₅ function in cells, in electrophysiology and by icv injection. We did not examine the brain exposure when a DMSO-containing vehicle was employed, and this may improve brain levels.^{21–22}

Thus, further optimization of an M₅-preferring PAM VU0238249 (**3**), derived from a pan G_q mAChR PAM, provided two highly selective M₅ PAMs - VU0365114 (**6a**) and VU0400265 (**10**). While VU0400265 (**10**) is the most selective M₅ PAM reported to date, **6a** is highly selective for M₅ and displays a >50-fold shift of the ACh CRC. These selective tool compounds will finally allow researchers to dissect the role of M₅ in the CNS, the one mAChR that has remained a mystery due to the lack of tool compounds. Since selective M₅ PAMs could be obtained from a pan G_q PAM lead, we are currently optimizing VU0119498 to provide highly selective M₁ PAMs as well as M₃ PAMs. Efforts in this arena are in progress with exciting results, which will be reported in due course.

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Figure 1.

HTS hit VU0119498, a pan G_q mAChR M₁, M₃, M₅ PAM, VU0238441, a pan mAChR PAM and VU0238429, a highly M₅-preferring PAM. Data represent means of at least three independent determinations with similar results using mobilization of intracellular calcium as a functional readout for receptor activation M₁–M₅ CHO cells (M₂ and M₄ cells co-transfected with G_qi5).

Figure 2.
Optimization strategy for VU0238429 (**1**), a highly M_5 -preferring PAM.

Figure 3. ACh EC₂₀ triage screen of libraries of analogs **6** and **8** at 10 μ M in M₅ CHO cells by intracellular calcium mobilization assay. Data represent means from at least three independent determinations with similar results.

Figure 4.

A) CRCs for VU0365114 (**6a**) at M₁, M₃ and M₅ CHO cells; B) CRCs for VU0365117 (**6b**) at M₁, M₃ and M₅ CHO cells; C) M₅ Fold-shift experiments of the ACh CRC with 30 μ M of either **6a** or **6b** (both >50x) in M₅ CHO cells. Data represent means from at least three independent determinations with similar results.

Figure 5. CRCs for VU0400265 (**10**) at M₁, M₂, M₃, M₄ and M₅ CHO cells (EC₅₀ = 1.9 μM) with a submaximal (~EC₂₀) of ACh. VU0400265 (**10**) is the most selective M₅ PAM reported to date. Data represent means from at least three independent determinations with similar results (M₂ and M₄ cells co-transfected with G_{qi5}).

Figure 6. CRCs for VU0400267 (**11**) and VU0400266 (**12**) at M₁, M₂, M₃, M₄ and M₅ CHO Cells with a submaximal (EC₂₀) of ACh. Data represent means from at least three independent determinations with similar results (M₂ and M₄ cells co-transfected with G_{qi5}).

Scheme 1.

a) *p*-bromobenzylbromide, K₂CO₃, KI, ACN, rt, 16 h (99%); b) RB(OH)₂, Pd(*P**t*-Bu₃)₂, Cs₂CO₃, THF:H₂O, mw, 120 °C, 20 min (10–90%); c) K₂CO₃, KI, ACN, rt, 16 h (50–90%).

Scheme 2.

a) K_2CO_3 , KI, ACN, mw, $160^\circ C$, 10 min (68%).

Table 1Structures and activities of analogs **6**.

Cmpd	VU Number	R	hM5 EC ₅₀ (μM) ^a	% ACh Max ^a
6a	0365114		2.7	85
6b	0365117		2.8	85
6c	0365118		4.8	85
6d	0365121		3.6	80
6e	0365123		3.3	85
6f	0365116		3.9	70

^a Average of at least three independent determinations. All compounds M₁ EC₅₀ > 30 μM.