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Chemical lead optimization of a pan G_q mAChR M_1, M_3, M_5 positive allosteric modulator (PAM) lead. Part I: Development of the first highly selective M_5 PAM

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ABSTRACT

This Letter describes a chemical lead optimization campaign directed at VU0238429, the first M_5-preferring positive allosteric modulator (PAM), discovered through analog work around VU0119498, a pan G_q mAChR M_1, M_3, M_5 PAM. An iterative library synthesis approach delivered the first selective M_5 PAM (no activity at M_1–M_4 @ 30 μM), and an important tool compound to study the role of M_5 in the CNS.

The muscarinic acetylcholine receptors (mAChRs) are members of the family A G-protein-coupled receptors (GPCRs) and include five subtypes denoted M_1–M_5. M_1, M_3 and M_5 couple to G_q and activate phospholipase C, whereas M_2 and M_4 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 M_1 positive allosteric modulators (PAMs) we identified VU0119498, an M_1, M_3, M_5 PAM (Fig. 1). This was a unique hit from the screen, as it was not selective for M_1, but the first example of a pan-G_q mAChR PAM, devoid of activity at the $G_{i/o}$-coupled M_2 and M_4.[10]

Relative to M_1–M_4, little is known about the precise role(s) of M_5 in the CNS. However, localization data and studies using M_5-knockout (KO) mice suggest that M_5 activation is highly important to regulation of central dopaminergic pathways and to ACh-induced cerebrovasodilation. In light of these findings, drugs targeting M_5 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 M_5 has seriously hindered progress in this area.

Starting from a pan M_1, M_3, M_5 PAM, VU0119498, we felt it may be possible to maintain M_5 PAM activity and dial out M_1 and M_3 PAM activity through a chemical lead optimization campaign. In a recent Letter,[15] we reported on the discovery and characterization of VU0238429, the first M_5-preferring PAM. At 30 μM, VU0238429 displayed a 14-fold leftward shift of the ACh concentration-response-curve (CRC), increased ACh affinity for M_5 by ~11-fold and did not displace [^{3}H]-NMS from binding to M_5.[15] While this was a major advance in the field, we hoped to develop a truly M_5 selective PAM to dissect the role of M_5 in the CNS. In this Letter, we describe an iterative parallel synthesis approach[16] for the further optimization of VU0238429, and the discovery of M_5 PAMs with unprecedented mAChR selectivity (≫30 μM vs M_1–M_4).

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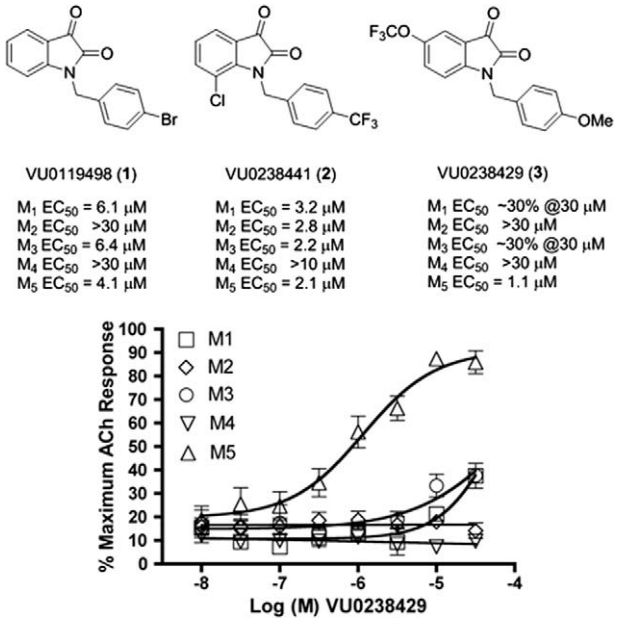


Figure 1. HTS hit VU0119498, a pan G_q mAChR M_1, M_3, M_5 PAM, VU0238441, a pan mAChR PAM and VU0238429, a highly M_5-preferring PAM. Data represent means of at least three independent determinations with similar results using mobilization of intracellular calcium as a functional readout for receptor activation M_1–M_5 CHO cells (M_2 and M_4 cells co-transfected with G_{qi5}).

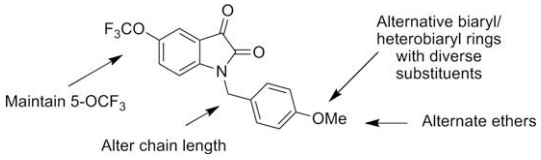
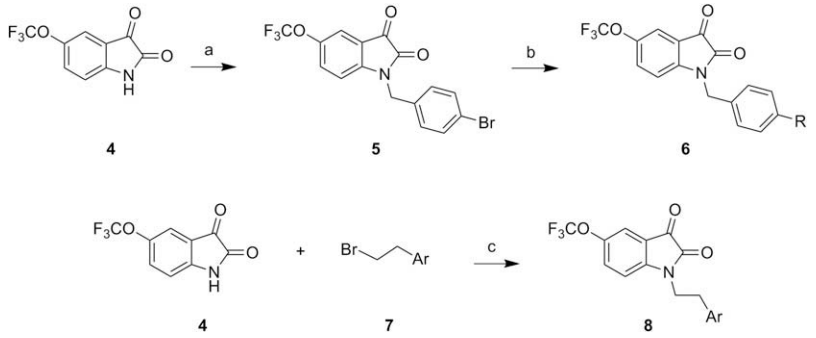


Figure 2. Optimization strategy for VU0238429 (**1**), a highly M_5-preferring PAM.

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-OCF_3 group was essential for M_5-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**. A 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 μM) screen for their ability to potentiate an EC_{20} of ACh in M_5-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



Scheme 1. Reagents and conditions: (a) *p*-bromobenzylbromide, K_2CO_3, KI, ACN, rt, 16 h (99%); (b) $RB(OH)_2$, $Pd(Pt\text{-}Bu_3)_2$, Cs_2CO_3, THF:H_2O, mw, 120 °C, 20 min (10–90%); (c) K_2CO_3, KI, ACN, rt, 16 h (50–90%).

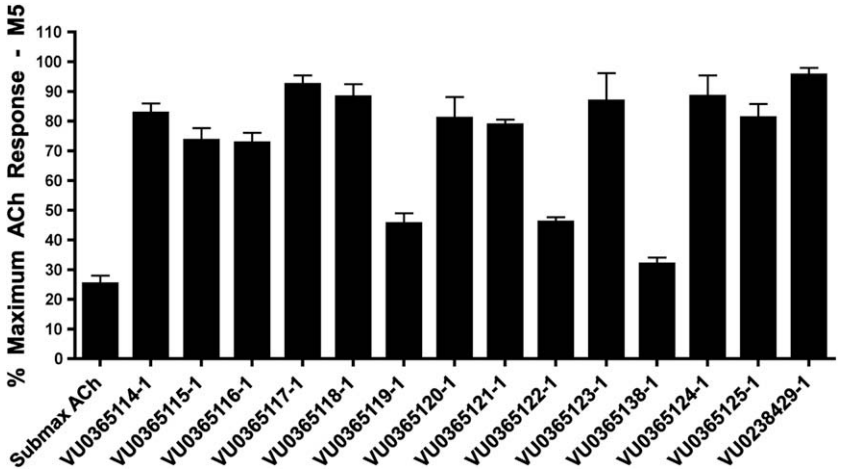
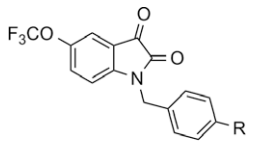
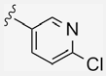
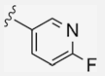
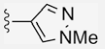
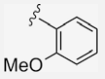


Figure 3. ACh EC_{20} triage screen of libraries of analogs **6** and **8** at 10 μM in M_5 CHO cells by intracellular calcium mobilization assay. Data represent means from at least three independent determinations with similar results.

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



6

Compd	VU number	R	hM5 EC_{50}[a] (μM)	%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

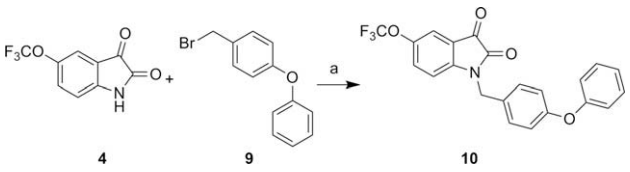
[a] Average of at least three independent determinations. All compounds M_1 EC_{50} >30 μM.

at higher concentrations. For example, 8a, the direct phenethyl analog of VU0238429 possessed an M_5 EC_{50} of 4.9 μM and 80% ACh maximum response (data not shown). Biaryl and heterobiaryl analogs **6** proved far more productive, affording a number of M_5 PAMs with high selectivity versus M_1 (>30 μM EC_{50}s) and low micromolar M_5 EC_{50}s (Table 1). All other analogs **6** possessed M_5 EC_{50}s > 10 μM. In general, both 5- (**6b** and **6e**) and six-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 (M_5 EC_{50}s 2.7– 4.8 μM) with similar ACh Max values (70–85%). Shallow SAR was again noted with compounds either being active in this potency range or inactive as M_5 PAMs.

Analogs **6a** (VU0365114) and **6b** (VU0365117) were selected for additional follow-up. Figure 4 depicts G_q mAChR (M_1, M_3 and M_5) CRCs for **6a** and **6b**. Note **6a** possesses improved M_5 selectivity versus VU0238429 (**3**), with only modest activation of M_3 at 30 μM. 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 M_1 PAM BQCA[18–20] and other ago-potentiators for class C GPCRs,[21–23] Figure 4C indicates moderate intrinsic allosteric agonism at 30 μM.

Encouraged by the potency and mAChR selectivity of VU03 65114 (**6a**) and VU0238429 (**3**), we synthesized (Scheme 2) a hybrid analog possessing a biphenyl ethermoiety, VU0400265 (**10**). VU0400265 possessed an M_5 EC_{50} of 1.9 μM with a 75% ACh Max. Importantly, VU0400265 was completely selective versus M_1–M_4, affording no elevation of an ACh EC_{20} at M_1–M_4 at 30 μM (Fig. 5). Notably, VU0400265 (**10**) represents the most selective M_5 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 M_5 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-OCF_3 moiety to the 6-position of the



Scheme 2. Reagents and conditions: (a) K_2CO_3, KI, ACN, mw, 160 °C, 10 min (68%).

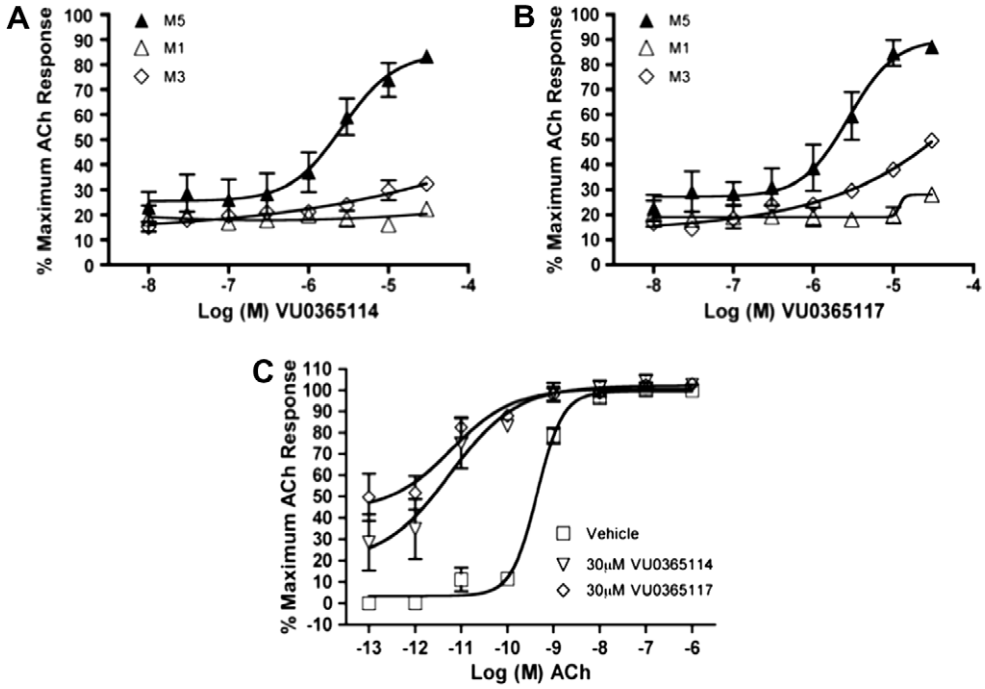


Figure 4. (A) CRCs for VU0365114 (**6a**) at M_1, M_3 and M_5 CHO cells; (B) CRCs for VU0365117 (**6b**) at M_1, M_3 and M_5 CHO cells; (C) M_5 Fold-shift experiments of the ACh CRC with 30 μM of either **6a** or **6b** (both >50×) in M_5 CHO cells. Data represent means from at least three independent determinations with similar results.

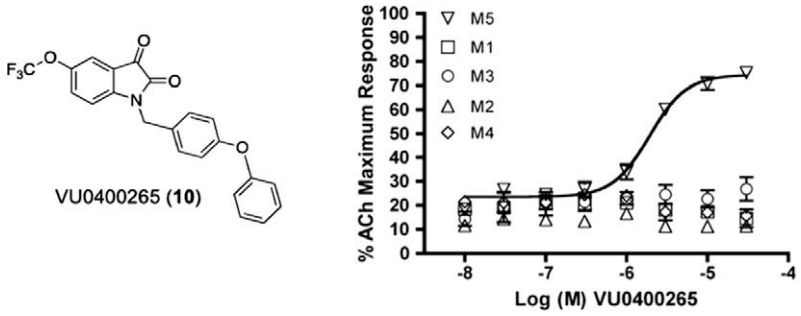


Figure 5. CRCs for VU0400265 (**10**) at M_1, M_2, M_3, M_4 and M_5 CHO cells (EC_{50} = 1.9 μM) with a submaximal (~EC_{20}) of ACh. VU0400265 (**10**) is the most selective M_5 PAM reported to date. Data represent means from at least three independent determinations with similar results (M2 and M4 cells co-transfected with G_{qi5}).

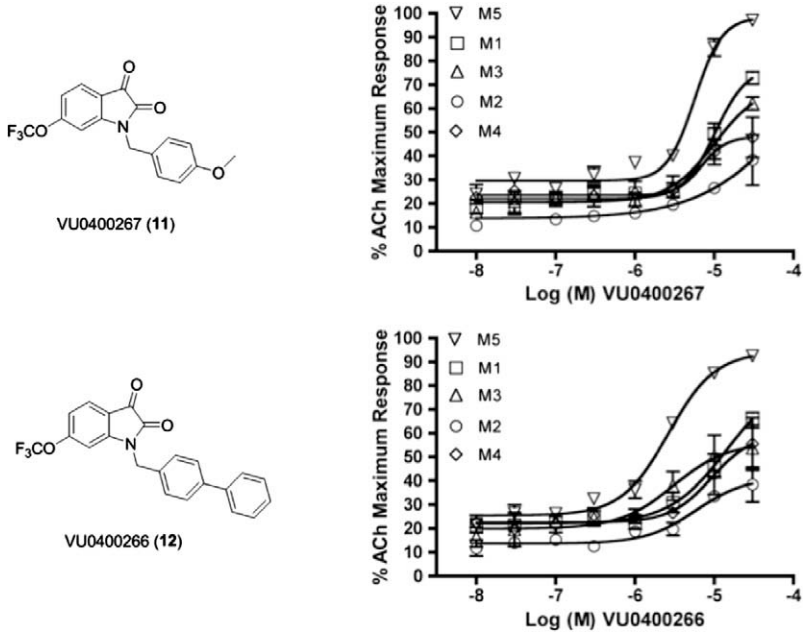


Figure 6. CRCs for VU0400267 (**11**) and VU0400266 (**12**) at M_1, M_2, M_3, M_4 and M_5 CHO Cells with a submaximal (EC_{20}) of ACh. Data represent means from at least three independent determinations with similar results (M_2 and M_4 cells co-transfected with G_{qi5}).

isatin core. Following the route in Scheme 1, we quickly prepared the 6-OCF_3 analogs **11** and **12** of our initial M_5-preferring PAM VU0119498 (**3**) and the biphenyl congener VU0365114 (**6a**), respectively. Interestingly, M_5 potency was relatively maintained (M_5 EC_{50}s 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-OCF_3 congener of VU0400265 (**10**) provided similar erosion in mAChR selectivity. Due to the loss in M_5 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 M_5 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 M_5-preferring PAM VU0238249 (**3**), derived from a pan G_q mAChR PAM, provided two highly selective M_5 PAMs—VU0365114 (**6a**) and VU0400265 (**10**). While VU0400265 (**10**) is the most selective M_5 PAM reported to date, **6a** is highly selective for M_5 and displays a >50-fold shift of the ACh CRC. These selective tool compounds will finally allow researchers to dissect the role of M_5 in the CNS, the one mAChR that has remained a mystery due to the lack of tool compounds. Since selective M_5 PAMs could be obtained from a pan G_q PAM lead, we are currently optimizing VU0119498 to provide highly selective M_1 PAMs as well as M_3 PAMs. Efforts in this arena are in progress with exciting results, which will be reported in due course.

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