



Chemical lead optimization of a pan G_q mAChR M₁, M₃, M₅ positive allosteric modulator (PAM) lead. Part II: Development of a potent and highly selective M₁ PAM

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ABSTRACT

This Letter describes a chemical lead optimization campaign directed at VU0119498, a pan G_q mAChR M₁, M₃, M₅ positive allosteric modulator (PAM) with the goal of developing a selective M₁ PAM. An iterative library synthesis approach delivered a potent (M₁ EC₅₀ = 830 nM) and highly selective M₁ PAM (>30 μM vs M₂–M₅).

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Recently, we described the identification of VU0119498, a pan G_q mAChR M₁, M₃, M₅ positive allosteric modulator (PAM), from a functional high throughput screen (Fig. 1).¹ In subsequent Letters, we described chemical lead optimization efforts based on VU0119498 (1) that delivered the first highly M₅-preferring PAM (VU0238429 (2)) and a highly M₅-selective PAM (VU0400265 (3)).^{2,3}

Incorporation of a 5-OCF₃ moiety on the isatin ring was essential for M₅ PAM activity and can be viewed as a 'molecular switch' to modulate mAChR subtype selectivity.^{1–3} As we described previously, other substituents on the isatin ring led to pan mAChR PAMs with varying degree of potency and efficacy across M₁–M₅.^{2,3}

Selective M₁ activation is an attractive therapeutic approach for the treatment of cognitive impairment, Alzheimer's disease, schizophrenia and a number of other CNS disorders.^{4–14} Until recently, no highly selective M₁ activators existed, and those that claimed to be highly M₁ selective were either not centrally penetrant or possessed significant ancillary pharmacology which prohibited their use as probes to study M₁ receptor function.^{15,16} We have disclosed three selective M₁ activators (Fig. 2): BQCA (4),^{17,18} a highly selective M₁ PAM, TBPB (5) a second generation M₁ allosteric agonist^{19–21} and VU0357017 (6), a best-in-class M₁ allosteric agonist.²² While BQCA

was a key compound (calcium mobilization assay M₁ EC₅₀ = 845 nM, 100% ACh Max, 100-fold left-shift of ACh CRC at 100 μM), brain penetration was acceptable, but not optimal, due presumably to the carboxylic acid moiety.^{17,18} Our initial report on the discovery of VU0119498 also described three other series of weak M₁ PAMs, and identified that different M₁ PAM chemotypes displayed different modes of activity on downstream receptor signaling.¹ Thus, all allo-

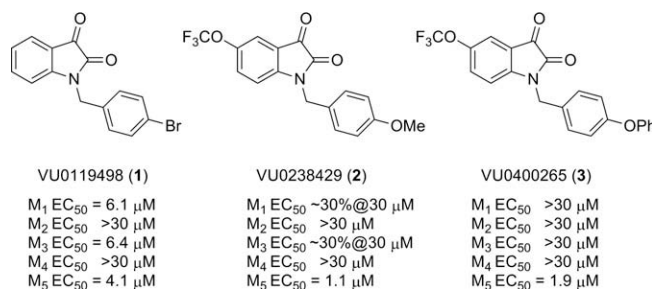
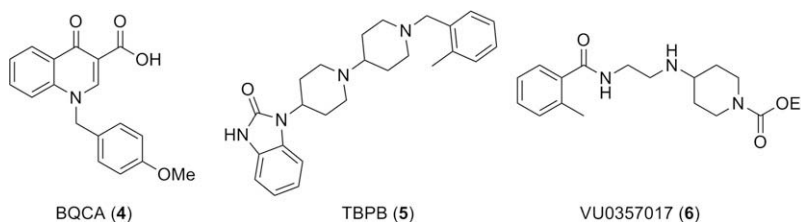


Figure 1. HTS lead VU0119498, a pan G_q mAChR M₁, M₃, M₅ PAM, VU0238429, a highly M₅-preferring PAM and VU0400265, a highly selective M₅ PAM. Data represent means from at least three independent determinations with similar results using mobilization of intracellular calcium in M₁–M₅ CHO cells (M₂ and M₄ cells co-transfected with G_qi5).

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steric M₁ activation is not equivalent, and additional tool compounds representing diverse chemotypes are required to truly dissect and study M₁ function in the CNS. Based on our ability to develop an M₅-selective PAM from a pan G_q M₁, M₃, M₅ PAM,^{2,3} we initiated an effort to optimize VU0119498 for M₁ PAM activity in an attempt to add a unique chemotype to our tool kit of selective M₁ activators.

Our initial optimization strategy is outlined in [Figure 3](#), and as SAR with allosteric ligands is often shallow, we employed an iterative parallel synthesis approach. From our M₅ work where we counter-screened on M₁, we quickly learned that most substitutions on the isatin ring led to pan mAChR activation profiles with various degrees of potency, efficacy, and subtype-selectivity.^{2,3} Thus, our first libraries employed a naked isatin core and surveyed diversity on the southern benzyl moiety.

Libraries were prepared according to [Scheme 1](#), wherein commercial indoline-2,3-dione **7** was alkylated with *p*-bromobenzylbromide to deliver key intermediate **8**. A 12-member Suzuki library was then prepared to explore the effect of introduction of biaryl and heterobiaryl motifs into VU0119498 providing analogs **9** ([Fig. 4](#)). In parallel, **7** was alkylated with functionalized phenethyl bromides **10** to probe the effect of chain homologation in analogs **11**. Compound libraries were triaged by a single point (10 μ M) screen for their ability to potentiate an EC₂₀ concentration of ACh on M₁ CHO cells. SAR was extremely shallow, with only one analog **9a** demonstrating robust M₁ potentiation ([Fig. 5](#)). VU0365137 (**9a**), possessing an *N*-methyl pyrazole in the 4-position of the southern benzyl ring displayed an M₁ EC₅₀ of 2.3 μ M, and good selectivity versus M₃ and M₅. Moreover, **9a** afforded a ~5-fold leftward shift of the M₁ ACh CRC at 10 μ M, and a larger ~14-fold shift at 30 μ M, with ~30% intrinsic allosteric agonism. Intriguingly, the 5-OCF₃ congener of **9a** is an equipotent M₅-preferring PAM,^{2,3} highlighting the aforementioned ‘molecular switch’ to engender M₅ preference. However, it was exciting to see that we could develop an M₁-preferring PAM from our initial pan G_q M₁, M₃, M₅ PAM lead.¹

Since SAR was incredibly shallow, we next incorporated subtle changes, in the form of fluorine atoms, to the VU0365137 (**9a**) scaffold, as we had previously shown was productive in optimizing BQCA, **4**.²³ Interestingly, there was some, but highly limited SAR overlap between these two series of M₁ PAMs. Following the synthetic route outlined in [Scheme 1](#), analogs with fluorine on both the isatin scaffold and the benzyl ring were readily prepared and evaluated for their ability to potentiate an EC₂₀ of ACh at M₁. This effort was more productive ([Table 1](#)) with five of the analogs **12** dis-

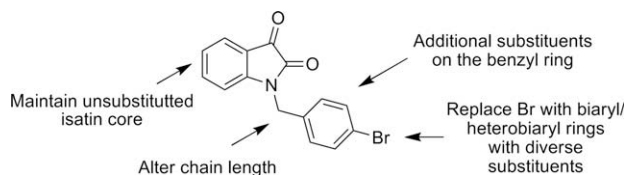
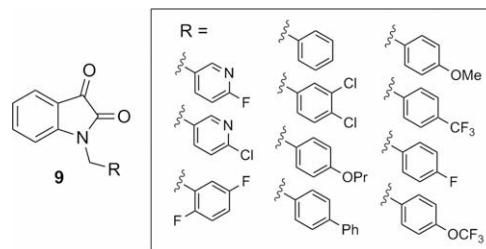
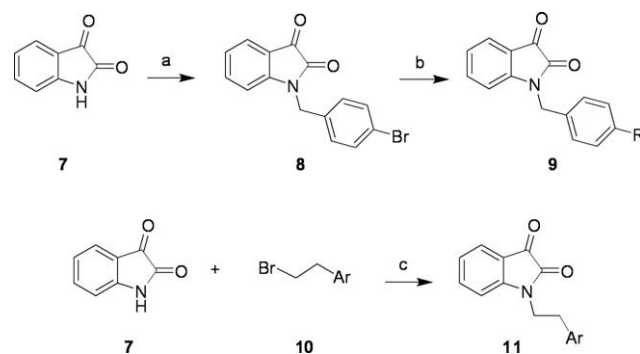


Figure 3. Initial optimization strategy for VU0119498, a pan $G_{\alpha} M_1, M_3, M_5$ PAM.

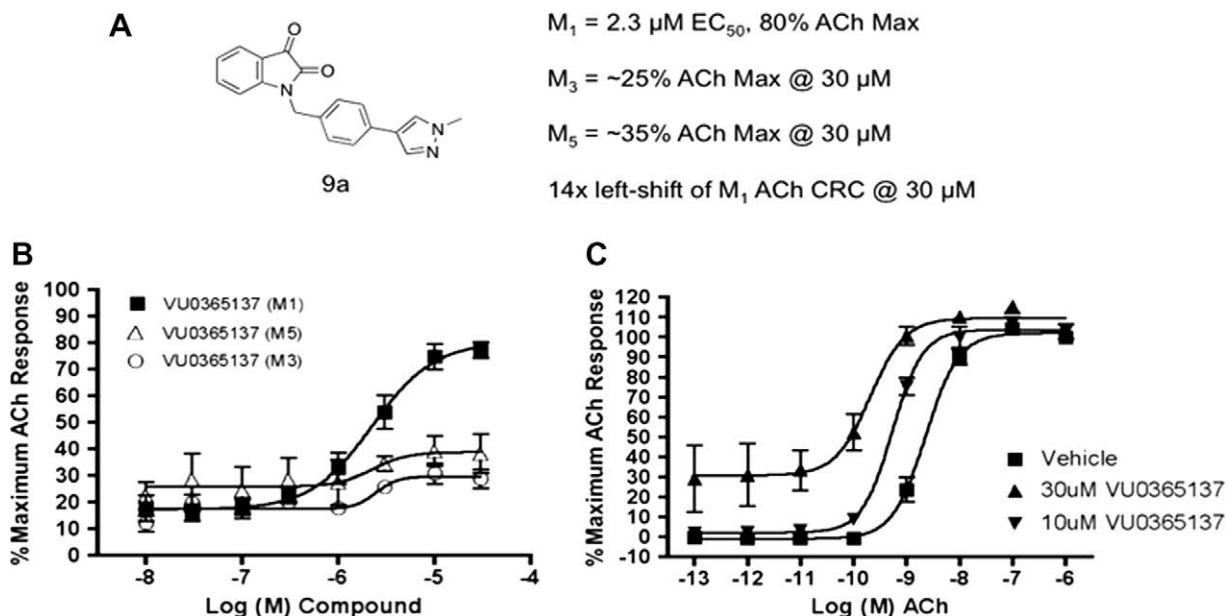
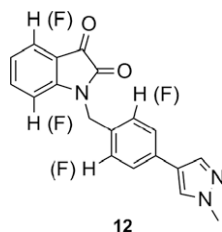


Figure 5. (A) Structure and activity of VU0365137 (**9a**); (B) CRCs for VU0365137 (**9a**) in the presence of a submaximal ($\sim\text{EC}_{20}$) concentration of ACh at M_1 , M_3 and M_5 ; (C) Fold-shift experiments of the ACh CRC at M_5 with both 10 μM and 30 μM concentrations of **9a**, providing an approximately fivefold and 14-fold shift, respectively. Data represent means of at least three independent determinations with similar results using mobilization of intracellular calcium in M_1 , M_3 , or M_5 CHO cells.

Table 1
Structures and activities of analogs **12**



Compd	VU number	Compound	$M_1\text{EC}_{50}^a$ (μM)	% ACh max ^a
12a	0366369		0.83	65
12b	0366368		0.86	60
12c	0366370		2.3	55
12d	0366367		1.1	40
12e	0366372		1.2	50

^a Average of at least three independent determinations. All compounds $M_1\text{EC}_{50} > 30 \mu\text{M}$.

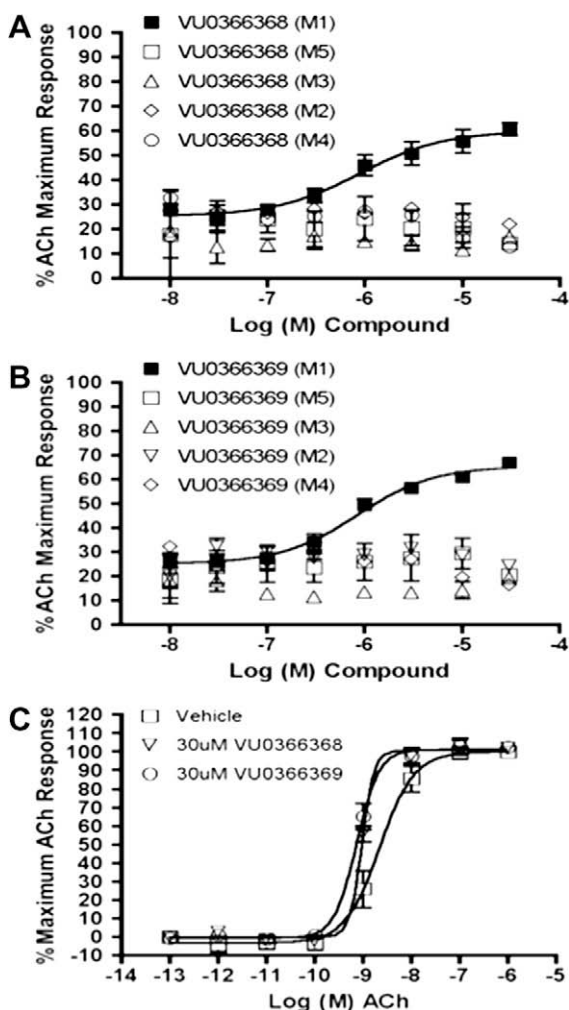


Figure 6. (A) and (B) CRCs for VU0366368 (**12b**) and VU0366369 (**12a**) in the presence of a submaximal (\sim EC₂₀) concentration of ACh at M₁, M₂/G_{q15}, M₃, M₄/G_{q15} and M₅; (C) Fold-shift experiments of the ACh CRC at M₅ with 30 μ M of **12a** and **12b**, providing an approximately 3- and 2-fold-shift, respectively. Data represent means of at least two independent determinations with similar results using mobilization of intracellular calcium in M₁, M₂/G_{q15}, M₃, M₄/G_{q15} and M₅ CHO cells.

the second known chemotype to provide potent and selective M₁ positive allosteric modulation.

Having been able to optimize a pan G_q M₁, M₃, M₅ PAM to deliver a potent and selective M₁ PAM (VU0366369, **12a**) and a potent and selective M₅ PAM (VU0400265, **3**),^{2,3} we hoped to identify 'molecular switches' within this chemotype that would engender M₃ PAM selectivity. We began by evaluating all analogs synthesized to date, that did not potentiate an EC₂₀ of ACh at M₁ or M₅, for their ability to potentiate an EC₂₀ of ACh at M₃ at a 10 μ M concentration. Surprisingly, identification of an M₃ PAM within this chemotype remains elusive.

Thus, optimization of a pan G_q mAChR M₁, M₃, M₅ PAM, which previously led to the discovery of the first selective M₅ PAM (VU0400265), provided VU0366369 (**12a**), a highly selective and

potent M₁ PAM. VU0366369 possesses comparable potency to BQCA and represents only the second known chemotype to provide highly selective M₁ potentiation. Efforts to develop an M₃ PAM from this chemotype have thus far proven unsuccessful; however, the ability to dial in or out M₁ and M₅ PAM activity within a single scaffold is unprecedented. Further in vitro and in vivo characterization of VU0400265 and VU0366369 is in progress with exciting results, which will be reported in due course.

Acknowledgments

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