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Further optimization of the M_1 PAM VU0453595: Discovery of novel heterobicyclic core motifs with improved CNS penetration

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ABSTRACT

This Letter describes the continued chemical optimization of the VU0453595 series of M_1 positive allosteric modulators (PAMs). By surveying alternative 5,6- and 6,6-heterobicyclic cores for the 6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine-5-one core of VU453595, we found new cores that engendered not only comparable or improved M_1 PAM potency, but significantly improved CNS distribution (K_ps 0.3–3.1). Moreover, this campaign provided fundamentally distinct M_1 PAM chemotypes, greatly expanding the available structural diversity for this valuable CNS target, devoid of hydrogen-bond donors.

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Positive allosteric modulators (PAMs) of the muscarinic acetylcholine receptor subtype 1 (M_1) have garnered a great deal of attention as a novel therapeutic approach for the treatment of the cognitive and negative symptom domains of schizophrenia, especially targeting NMDA receptor hypofunction.[1–8] Moreover, M_1 PAMs are also of interest in general cognition enhancement and Alzheimer's disease.[1–4,9–12] Since we reported on the discovery of the first M_1 PAM, BQCA,[13,14] numerous M_1 PAMs have been reported in the primary and patent literature (most by scaffold hopping VU and Merck series) with many conserved moieties that consistently engender low CNS penetration (K_ps < 0.3).[15–25] Our latest entry into M_1 PAMs was the result of three distinct high-throughput screening campaigns,[26] which resulted in novel indole-, azaindole- and isatin-based M_1 PAM scaffolds.[25,27–29] Of these, the isatin VU0119498 (**1**) was a unique PAM in that it potentiated all of the G_q-coupled mAChRs (M_1, M_3 and M_5) with equivalent potency and efficacy.[27] Subsequent optimization efforts identified 'molecular switches' that gave rise to a series of highly selective M_5 PAMs,[30–32] as well as ML137 (**2**), a highly selective M_1 PAM by virtue of the fluorophenyl pyrazole moiety.[28] Carbonyl deletion provided lactam **3**, and surveying regioisomeric lactams afforded VU0451725 (**4**), with improved DMPK properties over **2** and **3**.[33] Finally, an aza-scan identified the the 6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine-5-one core of VU0453595 (**5**), which proved a useful in vitro and in vivo tool, demonstrating efficacy in rodent models of pharmacologically-induced NMDA hypofunction (Fig. 1).[33] In this

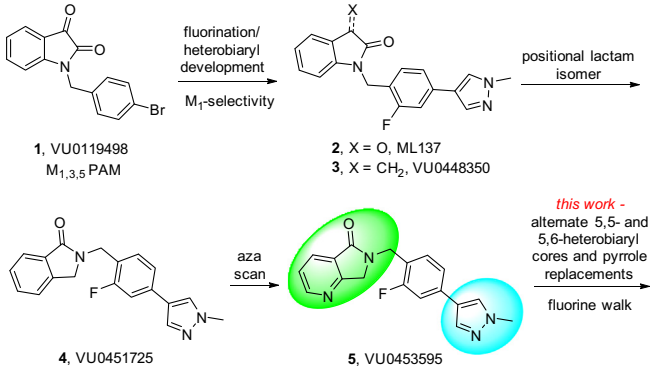

Figure 1. Evolution of the development of the VU0119498 series of M_1 PAMs, culminating in VU0453595 (**5**), a moderately potent PAM (rM_1 EC_{50} = 3.2 μM, 75% ACh Max and 3× less potent on hM_1) with modest CNS penetration (K_p = 0.3). In this work, we survey alternative 5,6- and 6,6-heterobicyclic cores and pyrrole replacements in an attempt to increase CNS penetration.

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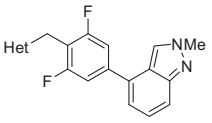
Letter, we detail an optimization campaign surveying alternative 5,6- and 6,6-heterobicyclic cores, alternate moieties for the pyrazole, and walking additional fluorines around the central phenyl ring to ultimately provide multiple novel M_1 PAM scaffolds with comparable or improved rat M_1 PAM potencies and improved CNS distribution (K_ps 0.4–3.1).

The chemistry to access new analogs, if not commercially available, was straightforward (Scheme 1).[34] The fluorinated heterobiaryl tail moities were readily prepared in two steps as either a benzyl chloride **7** or a benzyl mesylate **8** from commercial benzyl alcohols **6**. Various 5,6- and 6,6-heterobiaryl systems were then alkylated with either **7** or **8** to provide analogs **10**. A subsequent Suzuki coupling installed the heterobiaryl motif, delivering analogs **11**. Quinolinone and naphthyridinone analogs **11** of **9**, were made in a single step from **12**, and based on our previous work, cores such as **15** were also accessed in a simple three step procedure.[34]

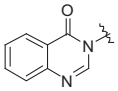
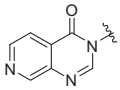
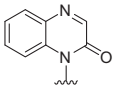
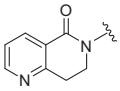
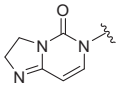
SAR was steep for the diverse analogs **11**, with many compounds devoid of M_1 PAM activity on both human or rat M_1, or displaying species bias towards rat M_1 PAM activity. In general, the 2,6-difluoro analogs were active whereas mono-fluoro and *des*-fluoro phenyl congeners were inactive as M_1 PAMs. Representative SAR is shown in Table 1 for a subset of analogs **16**, possessing an *N*-Me-indazole attached at the 4-position to the 2,6-difluorophenyl ring. While only rat M_1 data is shown, analogs **16** were uniformly 2- to 3-fold less potent on human M_1 (with many >10 μM). Here, various 6,6-hetrobicyclic ring systems were comparably active (rM_1 EC_{50}s 4.3–4.9 μM) across quinazolin-4(3*H*)-ones (**16a**), pyrido[3,4-*d*]pyrimidin-4(3*H*)-ones (**16b**), quinoxalin-2(1*H*)-ones (**16c**) and naphthyridin-5(6*H*)-ones (**16d**). These analogs possessed favorable in vitro DMPK profiles (rat and human f_us of 0.01–0.04) and moderate predicted hepatic clearance (CL_{hep}s of 40–44 mL/min/kg). However, they were superior to the lead **5** in terms of brain distribution (K_p), wherein **16a–d** displayed K_ps (rat brain:plasma ratios) of 0.35–2.16, and when corrected for fraction unbound in plasma and brain homogenate binding, the K_{puu}s ranged from 0.3 to 0.77—a major advance in the context of M_1 PAMs. Notably, **16c** (VU0478436) afforded a >6-fold increase in CNS penetration over **5**. The 5,6-congener **16e** (VU0486691), based on a dihydroimidazol[1,2-*c*]pyrimidin-5(3*H*)-one core, showed enhanced M_1 PAM potency (rM_1 EC_{50} = 1.7 μM, 50% ACh Max), improved in vitro DMPK profile (rat and human f_us of 0.08 and 0.04, respectively and moderate rat predicted hepatic clearance (CL_{hep} = 40 mL/min/kg)). Moreover, **16e** demonstrated a rat K_p of 0.35 and a K_{puu} of 0.3. This finding led us to explore additional 5,6-heterobicyclic cores.

SAR proved steep as additional 5,6-hetrobicyclic cores were prepared and evaluated, with the vast majority devoid of M_1 PAM activity. During this effort, it was also shown that regioisomeric *N*-Me indazoles had a profound effect on M_1 PAM activity (Fig. 2). Interestingly, the 4-positional isomer **17** was devoid of M_1 PAM activity, while in contrast, the 5-positional isomer **18** was a potent M_1 PAM (EC_{50} = 1.7 μM, 50% ACh Max, pEC_{50} = 5.76 + 0.02) with very attractive in vitro DMPK properties (rat and human f_us of 0.06 and 0.05, respectively, low rat hepatic clearance (CL_{hep} = 29 mL/min/kg) and a large free fraction in rat brain homogenate binding, f_u = 0.098). Moreover, **18** (VU0484061) possessed a rat brain:plasma ratio (K_p) of 0.40 and a K_{puu} of 0.70. Once again, and in comparison to the known M_1 PAMs with low K_ps and K_{puu}s, both **16c** and **18** truly stand out. It

Table 1
Structures and activities of analogs **16**

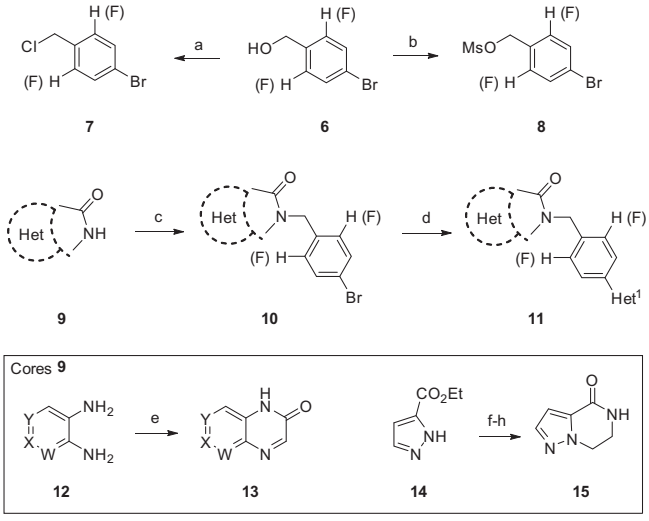


16

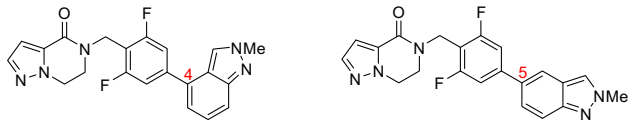
Compd	Het	rM_1 EC_{50} (μM)[a] [% ACh Max ± SEM]	rM_1 pEC_{50} (±SEM)	Rat K_p ($K_{p,uu}$)[b]
16a		4.7 [45 ± 4%]	5.32 ± 0.10	0.79 (0.32)
16b		4.7 [73 ± 5%]	5.32 ± 0.08	0.77 (0.60)
16c		4.9 [67 ± 3%]	5.31 ± 0.02	2.16 (0.77)
16d		4.3 [52 ± 4%]	5.37 ± 0.07	0.52 (0.49)
16e		1.7 [50 ± 5%]	5.77 ± 0.04	0.35 (0.30)

[a] Calcium mobilization assays with rM_1-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine; values represent means from three (n = 3) independent experiments performed in triplicate.

[b] Total and calculated unbound brain:plasma partition coefficients determined at 0.25 h post-administration of an IV cassette dose (0.20–0.25 mg/kg) to male, SD rat (n = 1), in conjunction with in vitro rat plasma protein and brain homogenate binding assay data.



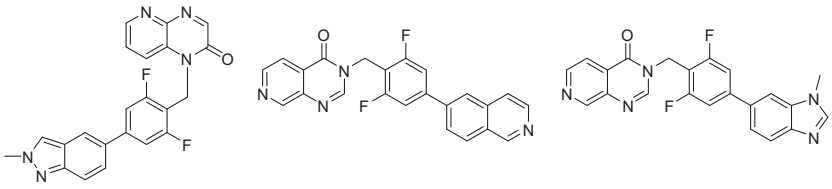
Scheme 1. *Reagents and conditions*: (a) Ghosez's reagent or $SOCl_2$, DCM, rt, 65–78%; (b) MsCl, Et_3N, DCM, 0 °C, 75–88%; (c) **7** or **8**, Cs_2CO_3, MeCN, 70 °C, 52–80%; (d) Het-$B(OH)_2$, $Pd(dppf)Cl_2$, Cs_2CO_3, THF:H_20 (10:1), μw 140 °C, 22–69%; (e) ethyl glyoxylate, 51–68%; (f) $Br(CH_2)_2NHBoc$, Cs_2CO_3, DMF, rt, 16 h, 98%; (g) HCl, 1,4-dioxane, rt, 1.5 h, 90%; (h) Na_2CO_3, 1,4-dioxane/H_2O, rt 3 h, 90%.



17, VU0478499
rM_1 EC_{50} >10 μM

18, VU0484061
rM_1 EC_{50} = 1.7 μM
K_p = 0.4, K_{puu} = 0.7

Figure 2. The impact of positional isomers of the *N*-Me indazole in the context of dihydropyrazolo[1,5-*a*]pyrazin-4-(5*H*)-ones **17** and **18**.



19, VU0486384
rM_1 EC_{50} = 2.8 μM
K_p = 1.64, K_{puu} = 1.1

20, VU0483225
rM_1 EC_{50} = 6.1 μM
K_p = 3.1, K_{puu} = 2.7

21, VU0483232
rM_1 EC_{50} = 5.3 μM
K_p and K_{puu} = BLQ

Figure 3. Additional M_1 PAMs **19**–**21** based on 6,6-heterobicyclic cores with a diverse range of pharmacological and DMPK properties.

is important to point out that the majority of M_1 PAMs possess two or more hydrogen bond donors (typically a *trans*-2-hydroxy cyclohexyl amide moiety) that likely engenders the poor CNS penetration due to P-gp efflux or low permeability.[13–25]

Prior to leaving this unique sub-series of M_1 PAMs, one last library of analogs was prepared with more diverse tail pieces within the **16b** and **16c** 6,6-heterobicyclic cores. Again, SAR was steep, and few active M_1 PAM resulted. However, this last campaign afforded three M_1 PAMs **19**–**21** with diverse profiles (Fig. 3). Here, the pyrido[2,3-*b*]pyrazin-2(1*H*)-one **19** (VU0486384) was a potent and efficacious rat M_1 PAM (EC_{50} = 2.8 μM, 80 ± 1% ACh Max, pEC_{50} = 5.56 ± 0.12), with a favorable fraction unbound in plasma (rat and human f_us of 0.04), high predicted hepatic clearance (CL_{hep} = 60 mL/min/kg), yet excellent CNS penetration (K_p = 1.64 and K_{puu} = 1.1). The nature of the heterobiaryl moiety played a key role in analogous pyrido[3,4-*d*]pyrimidin-4-(3*H*)-one congeners **20** and **21**. The isoquinoline analog **20** was a weak rat M_1 PAM (EC_{50} = 6.1 μM, 42 ± 3% ACh Max, pEC_{50} = 5.21 ± 0.11) with equivalent plasma fraction unbound (f_us of 0.03) for rat and human, and the best K_p to date for an M_1 PAM of 3.1 (and a K_{puu} of 2.7). In sharp contrast, the more basic *N*-Me benzimidazole congener **21** was of comparable potency (EC_{50} = 5.3 μM, 65 ± 4% ACh Max, pEC_{50} = 5.28 ± 0.14), good plasma fraction unbound (rat and human f_us of 0.04 and 0.06, respectively), but no detectable CNS penetration (brain levels below the level of quantitation, BLQ). These data show that subtle pK_a modulation can dramatically impact K_p.

Finally, the concept of divergent signal bias, mediated by stabilization of unique conformers of the GPCR by the allosteric ligand, has emerged, and in many instances is critical for avoiding adverse effect liabilites.[1–4,35–38] Thus, our lab surveys the propensity of new M_1 PAM ligands to display signal bias.[38-41] For VU0453595 (**5**), DiscoverRX assessed activities of the M_1 PAM against human M_1 in a calcium flux assay, as well on β-arrestin recruitment and internalization.[39] PAM **5** was shown to be a modest human M_1 PAM (EC_{50} = 1.9 μM, 79% ACh Max) with no effect on receptor internalization (EC_{50} >10 μM) and modest effect on β-arrestin recruitment (EC_{50} = 2.6 μM, 57% Max). New M_1 PAM **16b** was evaluated similarly, and was found to be a modest human M_1 PAM (EC_{50} = 5.3 μM, 65% ACh Max) with no effect on receptor internalization (EC_{50} >10 μM), yet a submicromolar effect on β-arrestin recruitment (EC_{50} = 980 nM, 33% Max). At this point, the in vivo ramification of these profiles across signal transduction pathways are unclear, but we are tracking and noting differences between M_1 PAMs and plan to investigate more thoroughly once a collection of M_1 PAM ligands with diverse profiles (and comparable PK) are accumulated.

In summary, we report on the further optimization of the in vivo tool M_1 PAM, VU0453595 (**5**). A diverse array of 5,6- and 6,6-heterobicyclic cores were developed as novel M_1 PAMs with unprecedented levels of CNS penetration (K_ps 0.3–3.1 and K_{puu}s of 0.3–2.7) and lacking the prototypical hydrogen-bond donor motifs. While these M_1 PAMs are too weak to advance as clinical candidates, the improved disposition of these new chemotypes represent fundamentally new starting points for further chemical optimization. Additional refinements are in progress and will be reported in due course.

Acknowledgments

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39. For full information on the assays, see: https://www.discoverx.com/targets/gpcr-target-biology.