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The discovery of VU0486846: steep SAR from a series of M₁ PAMs based on a novel benzomorpholine core

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Abstract

This letter describes the chemical optimization of a new series of M₁ positive allosteric modulators (PAMs) based on a novel benzomorpholine core, developed via iterative parallel synthesis, and culminating in the highly utilized rodent *in vivo* tool compound, VU0486846 (**7**), devoid of adverse effect liability. This is the first report of the optimization campaign (SAR and DMPK profiling) that led to the discovery of VU0486846 and details all of the challenges faced in allosteric modulator programs (both steep and flat SAR, as well as subtle structural changes affecting CNS penetration and overall physiochemical and DMPK properties).

Keywords

M₁; Muscarinic acetylcholine receptor; Positive allosteric modulator (PAM); Schizophrenia; Structure-Activity Relationship (SAR)

Introduction

M₁ (muscarinic acetylcholine receptor subtype 1) positive allosteric modulators (PAMs) represent an exciting therapeutic strategy to treat multiple domains of cognitive dysfunction in CNS disorders such as schizophrenia and Alzheimer's disease.^{1–5} However, the great potential of this target has been hindered, originally by non-selective orthosteric ligands (**1**),⁶ and then by potent ago-PAMs **2–5** (in cell lines (M₁ PAM EC₅₀s < 100 nM) and M₁ agonist

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potency <1 μM) and native tissues) that proved to be cognitive disrupting and proconvulsive due to over stimulation of the M_1 receptor (Fig. 1).^{7–12} Recently, we disclosed the first M_1 PAM free from adverse events, VU6004256 (**6**),¹³ and with robust efficacy in NMDA receptor 1 (NR1) knock-down mice.¹⁴ With the goal of providing the community with an improved, and much needed, M_1 PAM *in vivo* tool compound, here we report on VU0486846 (**7**). PAM **7** (M_1 PAM EC_{50} ~250 nM for human and rat), based on a novel benzomorpholine core, is a significant structural departure within the M_1 PAM field that has proven to be a valuable rodent *in vivo* M_1 PAM tool compound, devoid of agonist activity in native systems, free from adverse effects and highly efficacious in multiple rodent cognition models.¹⁵ Here, we describe for the first time the optimization campaign (SAR and DMPK profiles).

As briefly detailed in the initial disclosure of **7**,¹⁵ we scaffold-hopped from the prototypical 6,6-fused ring system of **2** to a novel benzomorpholine core (as in **7**) wherein we simultaneously increased sp^3 character while bringing the Lewis basic oxygen of the quinolone into the 6,6-ring system. These modifications of the core also created a new stereogenic center, which, in the case of **7**, the (*R*)-enantiomer displayed enantiopreference.¹⁵ Here, we will detail the synthesis, SAR, and DMPK profiles of a multi-dimensional optimization campaign within the benzomorpholine series that ultimately led to the discovery of **7**, an M_1 PAM with a balance of overall properties as a new *in vivo* tool compound, free from adverse effect liability.

The synthesis of diverse analogs **13** was straightforward and starting materials were readily available from commercial sources (see Scheme 1).¹⁵ Ethyl 2,3-dibromopropanoate **8** was condensed with various substituted 2-aminophenols to provide the racemic heterocyclic cores **9** in 52–80% yields. Alkylation with substituted 4-bromo benzyl bromides or the analogous heterocyclic congeners proceeded smoothly delivering **10** in yields ranging from 66 to 74%. A quantitative hydrolysis of the ester gave **11**, which then underwent a HATU-mediated amide coupling with diverse primary and secondary amines to provide derivatives **12** as mixtures of diastereomers. Finally, either a copper-mediated coupling with heterocycles, or a Suzuki coupling protocol, produced the putative M_1 PAMs **13** in 35–86% yield. To facilitate more rapid SAR, we initially screened analogs **13** as racemates, and used the racemic version of **7**, **14** (VU0484043, M_1 EC_{50} = 0.92 μM , pEC_{50} = 6.10 $\pm$ 0.13, ACh Max 77 $\pm$ 7; M_2 - M_5 EC_{50} s > 30 μM) as a reference compound.¹⁵

Initial SAR around **14** with analogs **15–20** showed overall ‘flat’ SAR in terms of M_1 PAM potency, but significant impact on physiochemical and DMPK properties. Of the structural changes in Fig. 2, introduction of a quaternary carbon at the chiral center, as in **16**, led to a diminution in potency (M_1 EC_{50} = 7.3 μM , 61% ACh Max); however, all other modifications were within 2- to 3-fold of **14**. A major finding was that the *des*-oxy tetrahydroisoquinoline analog **15** was essentially equipotent to **14**, suggesting that the key intramolecular hydrogen bond (IMHB) of PAMs **4–7**^{7–14} may not be a key tenet in this new series (however, this will be disclosed in a subsequent publication).¹⁶ Other changes, represented by **17–20**, while active as M_1 PAMs, negatively impacted plasma protein

binding ($f_u < 0.001$) and/or decreased CNS penetration (brain/plasma $K_{ps} < 0.05$) relative to **14** ($f_u = 0.11$, rat $K_p = 0.17$, mouse $K_p = 0.7$).

Based on these data, we next explored the ‘fluorine walk’, a strategy that has been highly successful in allosteric modulator optimization, particularly for other M_1 PAM scaffolds.^{17,18} This exercise led to intriguing SAR (Fig. 3) relative to **14**. While incorporation of a fluorine atom at the 5-position (**21**) led to an approximate 3-fold decrease in activity (M_1 PAM $EC_{50} = 3.2 \mu M$, $pEC_{50} = 5.51 \pm 0.12$, ACh Max 76 ± 2), installation at the 6-position (**22**) led to a ~3-fold increase in M_1 PAM potency (M_1 PAM $EC_{50} = 0.32 \mu M$, $pEC_{50} = 6.51 \pm 0.08$, ACh Max 88 ± 1). Further movement to the 7-position (**23**) led to a diminution in potency (M_1 PAM $EC_{50} = 2.2 \mu M$, $pEC_{50} = 5.66 \pm 0.03$, ACh Max 80 ± 2). An almost 7-fold increase in M_1 PAM potency (M_1 PAM $EC_{50} = 0.14 \mu M$, $pEC_{50} = 6.85 \pm 0.06$, ACh Max 89 ± 1) was realized by incorporation of a single fluorine atom at the 8-position (**24**). Moreover, the rat CNS penetration was improved, relative to **14** ($K_p = 0.17$), with K_{ps} ranging from 0.3 to 0.4. Despite the improvement in M_1 PAM potency and modest gains in CNS penetration, incorporation of a single (see Fig 4). Fluorine atom negatively impacted the DMPK profiles of **21–24**. Unlike **14** (with good fraction unbound, $f_u > 0.11$ in rat and human plasma, and moderate predicted hepatic clearance, $CL_{hep} = 11.1$ and 43.2 mL/min/kg for human and rat, respectively), analogs **21–23** all showed unacceptable fraction unbound ($f_u < 0.01$) and high predicted hepatic clearance (CL_{hep} s of ~20 and >65 mL/min/kg, for human and rat, respectively). Thus, the ‘fluorine walk’ improved potency, but at the cost of disposition; therefore, a ‘balanced’ M_1 PAM *in vivo* tool compound would not be derived from this sub-series, and alternate regions of **14** needed to be evaluated.

We returned to the unsubstituted benzomorpholine core of **14**, and surveyed alternative amide moieties for the (1*S*,2*S*)-2-aminocyclohexan-1-ol amide with analogs **25** (Table 1).

Removal of the chiral hydroxyl moiety (**25a**) led to a complete loss of M_1 PAM activity ($EC_{50} > 10 \mu M$). However, the rat K_p for **25a** was >1, suggesting that this key hydroxyl pharmacophore engendered rat P-gp susceptibility and was responsible for the generally low brain levels ($K_p < 0.4$) observed with this moiety across multiple M_1 PAM chemotypes (note, the ER for human is generally <1.2, suggesting this is rat specific). Interestingly, all these analogs possess favorable CNS MPO scores (>4.5), despite rodent CNS exposure issues.¹⁹ The ring in **14** could be contracted to a cyclopentyl congener (**25b**) with comparable activity, but the well-known pyran homolog (**25c**) afforded a substantial increase in M_1 PAM potency (M_1 PAM $EC_{50} = 0.19 \mu M$, $pEC_{50} = 6.73 \pm 0.13$, ACh Max 91 ± 4). Deletion of the hydroxyl group on the pyran ring as in **25d** similarly led to a ~34-fold loss in activity. Replacement of the hydroxyl with either a primary amine (**25e**) or a ketone (**25f**) retained reasonable M_1 PAM activity. Acyclic congeners (**25 g–i**) of the (1*S*,2*S*)-2-aminocyclohexan-1-ol amide of **14** could begin to buy back M_1 PAM activity as steric bulk increased, but proved inferior to their cyclic counterparts. We then evaluated functionalized pyridinyl amides (**25j–l**), and were pleased to see that these too retained M_1 PAM activity despite the significant structural departure. Of these, the 3-hydroxy pyridinyl amide (**25j**) was equipotent to **14**. For all of these new analogs **25**, their DMPK profiles were inferior compared to the parent **14**, displaying an undesired combination of high predicted hepatic

clearance ($CL_{\text{hep}} \sim 20$ and >65 mL/min/kg in human and rat, respectively), unacceptable plasma protein binding (human and rat $f_u < 0.01$) and/or poor CNS penetration ($K_{ps} < 0.1$). Interestingly, metabolite identification studies with representative analogs, **14** and **25j**, showed that the route of metabolism differed between the saturated amides (driven by P_{450} -mediated debenzoylation to afford **26**) and pyridinyl amides (amide hydrolysis by esterases liberating **27**). Of all the analogs evaluated thus far in this benzomorpholine series, **7** was an outlier with a balance of M_1 PAM potency, pharmacological, and DMPK properties that made it an ideal *in vivo* tool compound.

As the pyridine biaryl derivative **18** (Fig. 2) was equipotent to **14**, we elected to evaluate analogs of this motif in an effort to identify an *in vivo* tool (see Table 2). Simple substituents, such as Cl, Br and $N(\text{CH}_3)_2$ (**28a-c**) demonstrated that such modifications were indeed tolerated as M_1 PAMs, but a methyl group (**28d**) proved equipotent (M_1 PAM $EC_{50} = 0.85$ μM , $pEC_{50} = 6.09 \pm 0.07$, ACh Max 85 ± 3) to **14**, but with reduced molecular weight and hydrogen bond acceptors. Interestingly, bromine (**28b**) was more potent than chlorine (**27a**), yet CF_3 (**28e**) and cyclopropyl (**28f**) were weaker than methyl (**28d**), suggesting size alone was not a driving factor for M_1 PAM potency. A simple vinyl group (**28g**) was also equipotent (M_1 PAM $EC_{50} = 0.70$ μM , $pEC_{50} = 6.17 \pm 0.10$, ACh Max 89 ± 3) to **14**. However, similar to analogs **25**, the DMPK profiles of these compounds precluded further advancement as *in vivo* tools. Thus, we decided to evaluate additional heterobiaryl derivatives of **18**. Moving from the *N*-linked pyrazole (**18**) to an *N*-linked imidazole (**28h**), and introduction of basic amine, led to an increase in M_1 PAM potency (M_1 PAM $EC_{50} = 0.29$ μM , $pEC_{50} = 6.55 \pm 0.12$, ACh Max 87 ± 2), as did replacement with a pyrrole (**28j**). Non-*N*-linked, *N*-methyl pyrazoles (**28k** and **28l**) also proved to be active, but regioisomeric preference was noted. Here, **28l** was the most potent analog in the series (M_1 PAM $EC_{50} = 0.22$ μM , $pEC_{50} = 6.75 \pm 0.07$, ACh Max 84 ± 5). Once again, however, none of these analogs possessed a balanced profile that positioned them to serve as M_1 PAM *in vivo* tools, and **7** (VU0486846) remained the most attractive compound from the overall series, offering robust efficacy without adverse effect liability or cholinergic side effects in multiple preclinical species.¹⁵ As recently detailed, **7** was devoid of M_1 agonism in cells and native systems, was devoid of cholinergic toxicity in mice, rats and non-human primates, afforded robust efficacy in a rat novel object recognition assay (MED = 3 mg/kg p.o.) and for the first time, demonstrated that M_1 PAMs can reverse the cognitive deficits induced by atypical antipsychotics, such as risperidone.¹⁵

In summary, we have detailed for the first time the progression and SAR from the prototypical quinolone-based M_1 PAM core to a novel benzomorpholine core. A multi-dimensional optimization campaign extensively surveyed diverse regions of the racemic counterpart **14** of **7** (VU0486846). While many new pharmacophores were identified that enhanced M_1 PAM potency, and challenged some notions regarding the critical need for an IMHB, physiochemical properties and DMPK profiles preclude their advancement as *in vivo* tool compounds. Importantly, the scaffold hopping exercise from the quinolone core to the benzomorpholine core gave rise to the highly valuable rodent M_1 PAM *in vivo* tool compound **7** (VU0486846), with a balanced profile; this compound demonstrated utility in target validation studies without adverse effect liability. Further optimization efforts in route

to M₁ PAM clinical candidates, and further evaluation of the tetrahydroisoquinoline core, will be reported in due course.

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Fig. 1.

Structures representative of a classical 'M₁ agonist' (1), that engenders cholinergic side effects, M₁ ago-PAMs 2–5, that are M₁ agonists in native systems and engender adverse effects, and M₁ PAMs 6 and 7, which are devoid of adverse effects.

Fig. 2.
Initial SAR around **7**, surveying multiple dimensions with analogs of **13**, M_1 PAMs **15–20**.

Fig. 3.
Impact of the ‘fluorine walk’ around the benzomorpholine core of **14**, to deliver M₁ PAM analogs **21–24**.

Fig. 4.
Divergent metabolic pathways leading to high predicted human and rat hepatic clearance for M1 PAMs **14** and **25j**.

Scheme 1.

Synthesis of M_1 PAM analogs **13**.^a ^aReagents and conditions: (a) substituted 2-aminophenols, CH_3CN , K_2CO_3 , 60 °C, 52–80%; (b) 4-bromoAr(Het)bromide, K_2CO_3 , CH_3CN , 80 °C, 66–74%; (c) KOH , $\text{THF}/\text{H}_2\text{O}$ (2:1), rt, 95–98%; (d) HN_2R_3 , HATU, DIEA, DMF , rt, 66–80%; (e) Het-NH, (1*S*,2*S*)- N^1,N^2 -dimethylcyclohexane-1,2-diamine, CuI , K_3PO_4 , dioxane, rt, 35–50%, or Ar(Het)- $\text{B}(\text{OH})_2$, 5 mol% $\text{Pd}(\text{PPh}_3)_4$, $\text{THF}:\text{H}_2\text{O}$, 45 °C, 54–86%.

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Table 1

Structures and human activities for M₁ PAM analogs **25**.

Cpd	R	hM ₁ EC ₅₀ (μM) ^a [% ACh Max ± SEM]	hM ₁ pEC ₅₀ (±SEM)	Rat K _p ^b
14		0.92 [77 ± 7]	6.10 ± 0.13	0.17
25a		>10 [35]	>5.0	1.1
25b		1.2 [77 ± 5]	5.93 ± 0.07	0.09
25c		0.19 [91 ± 4]	6.73 ± 0.03	0.08
25d		6.6 [69 ± 6]	5.20 ± 0.07	1.0
25e		2.3 [62 ± 3]	5.65 ± 0.07	ND
25f		2.7 [78 ± 5]	5.58 ± 0.06	ND
25g		6.4 [60 ± 3]	5.23 ± 0.19	ND
25h		2.2 [78 ± 3]	5.67 ± 0.02	ND
25i		1.9 [80 ± 4]	5.72 ± 0.07	ND
25j		0.71 [75 ± 7]	6.16 ± 0.04	0.11
25k		2.3 [81 ± 2]	5.64 ± 0.04	ND
25l		1.7 [80 ± 1]	5.79 ± 0.10	ND

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^aCalcium mobilization assays with hM1-CHO cells performed in the presence of an EC20 fixed concentration of acetylcholine; values represent means from three ($n = 3$) independent experiments performed in triplicate.

^bTotal 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 rats ($n = 1$), ND = not determined.

Table 2Structures and human activities for M₁ PAM analogs **28**.

Cpd	R	hM ₁ EC ₅₀ (μM) ^a [% ACh Max ± SEM]	hM ₁ pEC ₅₀ (±SEM)
28a	Cl	7.6 [73 ± 6]	5.15 ± 0.12
28b	Br	1.2 [91 ± 3]	5.97 ± 0.17
28c	N(CH ₃) ₂	3.8 [89 ± 2]	5.42 ± 0.02
28d	CH ₃	0.85 [85 ± 3]	6.09 ± 0.07
28e	CF ₃	3.7 [84 ± 5]	5.44 ± 0.06
28f		2.5 [90 ± 3]	5.65 ± 0.13
28 g		0.70 [89 ± 3]	6.17 ± 0.10
18		0.78 [92 ± 3]	6.15 ± 0.14
28 h		0.29 [87 ± 2]	6.55 ± 0.12
28i		0.67 [91 ± 5]	6.18 ± 0.06
28j		0.50 [94 ± 4]	6.32 ± 0.08
28 k		1.3 [94 ± 4]	5.90 ± 0.02
28 l		0.22 [84 ± 5]	6.75 ± 0.17

^aCalcium mobilization assays with hM₁-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine; values represent means from three (*n* = 3) independent experiments performed in triplicate.