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Discovery and SAR of a novel series of potent, CNS penetrant M₄ PAMs based on a non-enolizable ketone core: Challenges in disposition

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Abstract

This letter describes the chemical optimization of a novel series of M₄ PAMs based on a non-enolizable ketone core, identified from an MLPCN functional high-throughput screen. The HTS hit was potent, selective and CNS penetrant; however, the compound was highly cleared *in vitro* and *in vivo*. SAR provided analogs for which M₄ PAM potency and CNS exposure were maintained; yet, clearance remained high. Metabolite identification studies demonstrated that this series was subject to rapid, and near quantitative, reductive metabolism to the corresponding secondary alcohol metabolite that was devoid of M₄ PAM activity.

Graphical abstract

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Keywords

M₄; Muscarinic acetylcholine receptor; Positive allosteric modulator (PAM); Schizophrenia; Structure-Activity Relationship (SAR)

Selective activation of the M₄ muscarinic acetylcholine receptor has emerged as a very promising, and mechanistically distinct, therapeutic approach for the treatment of numerous neuropsychiatric and rare genetic CNS disorders.¹⁻⁸ Until recently,⁹ the M₄ PAMs reported to date have been primarily within a single amide-bearing chemotype (Figure 1),¹⁰⁻¹⁹ wherein non-obvious, subtle structural modifications engender steep SAR, species differences in PAM potency as well as affinity/cooperativity and subtype selectivity, solubility, and/or P-gp efflux.¹⁰⁻²¹ As such, all of the known chemotypes of M₄ PAMs (**1-6**) pose one or more major challenges en route to a clinical development candidate. In this Letter, we detail the identification, SAR and DMPK profile of a new, non-enolizable ketone chemotype, related to **1**, **2** and **4**, identified in a high-throughput screening (HTS) campaign.²²

Under the auspice of the MLPCN, we performed a functional M₄ HTS in 1536-well assay format against the ~360,000 compound MLSMR collection, and four structurally novel M₄ PAMs were identified.²² Of the hits, VU0009153 (**7**, CID:682264) represented an interesting lead (Figure 2), as it was similarly potent on both human and rat M₄ (EC₅₀s of 120 nM (76% ACh Max) and 76 nM (71% ACh Max), respectively (using DMSO stock from the source plates). In addition to no apparent species differences, **7** was also inactive on both human M₁ and M₅ (built-in HTS counterscreen). Upon re-synthesis of **7**, M₄ PAM potency remained intact (hM₄ EC₅₀ = 138 nM, pEC₅₀ = 6.86±0.07, ACh Max = 76.8±4.1 and rM₄, EC₅₀ = 145 nM, pEC₅₀ = 6.84±0.10, ACh Max = 80.5±6.3). Moreover, it was a *des*-NH variant of the prototypical M₄ PAM chemotypes **1**, **2**, and **4**, replacing the amide linker with a non-enolizable ketone. PAM **7** displayed very high *in vitro* intrinsic clearance (CL_{int}) in both rat and human hepatic microsomes with predicted hepatic clearance (CL_{hep}) near hepatic blood flow rates (Q_{hep}), but was this due to the trimethyl moiety, or an inherent feature of the ketone-linker? Figure 2 also highlights the three regions of **7** targeted for SAR exploration with focus on alternate aryl/heteroaryl moieties, evaluating our preferred aza systems of **2** and **4**, while also exploring ketone bioisosteres.

The chemistry to produce analogs of **7** was straightforward (Scheme 1), and employed advanced mercaptonitrile intermediates **8-10** previously described^{3,15-19} in a one-step protocol. Here, intermediates **8-10** are treated with a diverse array of commercial bromomethylketones **11** under basic conditions to afford, via a one-pot alkylation/cyclization sequence analogs **12-14**, respectively in yields ranging from 55-90%.

Table 1 highlights select SAR for the first generation of analogs **12** surveying diverse aryl/heteroaryl moieties. The direct analog of **7**, **12a**, lost ~7- to 10-fold potency at both human and rat M₄, whereas the direct ketone congener of **2** (**12c**) was essentially equipotent at both human and rat M₄, but with diminished efficacy (~50% ACh Max). The 2,4-difluorophenyl analog **12e** and the 4-pyridyl congener **12f** displayed good activity at human M₄, but exhibited a significant (4- to 7-fold) rightward shift in potency at rat M₄. In general, ketone-linked subseries **12** possessed weaker efficacy than the corresponding amide-linked derivatives. Relative to **7**, these findings suggest substitution in the 5-position may be required for M₄ PAM potency.

While in a very limited number of cases (Table 2), substitution at the 5-position with a chlorine atom afforded active M₄ PAMs with good efficacy as in **13a-b**, solubility with other analogs proved poor (driven by cLogPs >5) and hindered evaluation. Thus, our attention focused on the 5,6-dimethyl pyridazine core of **4** and ketone analogs **14**.

As shown in Table 3, analogs **14** restored M₄ PAM potency (hM₄, EC₅₀s 102 nM to 891 nM) and efficacy (ACh Max 55-76%), akin to **7**; however, unpredictable species differences emerged (cf, **14a** versus **14d-e**) with a trend towards diminished potency at rat M₄. Encouragingly, analogs within these three subseries retained selectivity for M₄ versus M_{1-3,5} (data not shown).

From our ongoing M₄ PAM program, we had additional mercaptonitrile intermediates beyond **8-10**, so we elected to convert them into ketone analogs **15-16** and assess their profiles (Figure 3). Gratifyingly, significant diversity was tolerated, providing, in the case of **15**, our most potent M₄ PAM in the ketone-linked series (hM₄ EC₅₀ = 37 nM, pEC₅₀ = 7.12±0.09, ACh Max = 63.8±5.9 and rM₄ EC₅₀ = 182 nM, pEC₅₀ = 6.74±0.05, ACh Max = 52.7±5.4).

Initial *in vitro* and *in vivo* DMPK evaluation of analogs **12-17** showed a range of low to moderate fraction unbound in rat and human plasma (*f*_us from 0.005 to 0.180) coupled with high CNS distribution (*K*_ps of 0.83 to 6.95 and *K*_{p,uu}s of 0.48 to 2.12, but low absolute concentrations in plasma and brain).² In addition, analogs **12-17** showed generally clean CYP₄₅₀ inhibition profiles with the exception of often potent CYP1A2 inhibition (data not shown). However, **12-17** all shared one common property, predicted *in vitro* clearance in hepatic microsomes near hepatic blood flow rates (hCL_{hep} 20 mL/min/kg and rCL_{hep} 68 mL/min/kg) despite their structural diversity. The only commonality amongst them was the non-enolizable ketone linker. *In vitro* metabolite identification (Met ID) studies performed with **12f** (as a representative of the series) in rat and human cryopreserved hepatocytes demonstrated a rapid, and near quantitative, reductive metabolism route affording the generic secondary alcohol **18** as the single major metabolite (Figure 4). We synthesized these known and putative metabolites by simple reduction of the ketones, and found that unfortunately, analogs **18** were devoid of M₄ PAM activity (human and rat EC₅₀s > 10 μM), i.e., a primary pharmacology inactivation metabolism pathway. Subsequent rat IV PK studies using a subset of representative compounds demonstrated that these PAMs were also highly cleared *in vivo* (CL_ps >70 mL/min/kg) indicating a good *in vitro:in vivo* correlation

(IVIVC). Interestingly, even the secondary alcohols **18** had high predicted hepatic clearance ($CL_{\text{hep}} > 15$ and > 55 mL/min/kg for human and rat, respectively).

Based on these data, we decided to pursue two divergent courses of action: 1) survey enolizable ketone congeners, and 2) survey alternatives for the ketone functional group. Figure 5 highlights two representative examples, **19** and **20**, of the first approach employing a phenethylketone derivative. Despite the significant structural change, **19** (hM_4 $EC_{50} = 479$ nM, $pEC_{50} = 6.32 \pm 0.12$, ACh Max = 59.1 ± 4.8 and rM_4 $EC_{50} = 540$ nM, $pEC_{50} = 6.27 \pm 0.02$, ACh Max = 67.9 ± 5.9) and **20** (hM_4 , $EC_{50} = 380$ nM, $pEC_{50} = 6.42 \pm 0.11$, ACh Max = 69.1 ± 4.3 and rM_4 $EC_{50} = 254$ nM, $pEC_{50} = 6.60 \pm 0.10$, ACh Max = 77.2 ± 4.4) were potent M_4 PAMs. Once again high brain distribution was observed ($K_{ps} \sim 11$, with very low absolute concentrations)²³, but both compounds were highly cleared *in vitro* (predicted $CL_{\text{hep}} = 18$ and 62 mL/min/kg for human and rat, respectively). Thus, this approach was terminated in favor of exploring alternatives for the ketone moiety.

A number of substitutions for the ketone moiety were prepared and assessed as M_4 PAMs. In short, we synthesized and assessed mono-fluoro, gem-difluoro, ether and amino (1° and 2°) derivatives; however, all were devoid of M_4 PAM activity ($EC_{50}s > 10$ μ M). All attempts to make the 2- and 3-spirooxetane ketone bioisosteres proved unsuccessful, as the 3-amino moiety of the azathiophene bicycle precluded successful chemistry. By this time, it was clear that the ketone series was not tractable for the development of a clinical candidate, despite similar non-enolizable ketone moieties found in commercial drugs wherein ketone reduction is not a major route of metabolism.^{24,25} In the present system, the presence of the 3-amino moiety, essential for M_4 PAM activity, likely serves as internal H-bond donor to activate the carbonyl moiety for metabolic reduction.

In summary, an HTS campaign identified a new M_4 PAM chemotype, based on a non-enolizable ketone moiety, as a substitute for the classical amide linker in **1-4**. Despite attractive M_4 PAM potency and CNS penetration, the compounds uniformly were unstable in hepatic microsomes as well as *in vivo*, and underwent a rapid and near quantitative reductive metabolism to the corresponding secondary alcohol – an inactive metabolite, confirmed via synthesis and biological evaluation. All attempts to replace the ketone were unsuccessful, rendering this new core not viable to deliver a clinical candidate. Further study and optimization with other HTS series are ongoing, and will be reported in due course.

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Figure 1.
Structures of reported M_4 PAMs **1-6**.

Figure 2.

Structure and key pharmacology and in vitro DMPK data for the M₄ PAM HTS hit VU0009153 (7), and three areas to address in the lead optimization campaign. Potency, efficacy, and selectivity was determined via calcium mobilization assays with hM₄/Gqi5-CHO or rM₄/Gqi5-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. Plasma protein binding and hepatic microsomal intrinsic clearance values represent data from a single (*n*=1) experiment performed in triplicate.

Figure 3.

More highly functionalized M₄ PAMs **15-17** in the ketone series. Potencies were determined via calcium mobilization assays with hM₄/Gqi5-CHO or rM₄/Gqi5-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.

Figure 4. Metabolite identification studies in rat and human cryopreserved hepatocytes with **12f** suggest **12-17** undergo rapid and near quantitative reductive metabolism to the corresponding inactive secondary alcohols, **18**. Predicted hepatic clearance values (from microsomal CL_{int} assays) represent data from a single (*n*=1) experiment performed in triplicate.

Figure 5.

Phenethylketone-based M₄ PAMs **19** and **20**. Potencies were determined via calcium mobilization assays with hM₄/Gq α 5-CHO or rM₄/Gq α 5-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.

Scheme 1.

Reagents and conditions: a) KOH, EtOH, rt, 6 hr, 55-90%.

Table 1

Structures and activities for M₄ PAM analogs **12**.

Cpd	Ar (Het)	hM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	hM ₄ pEC ₅₀ (±SEM)	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)	
12a	Ph	891 [47±6%]	6.05±0.12	1,500 [46±4%]	5.82±0.03	
12b	4-OHPh	355 [70±6%]	6.45±0.04	380 [73±4%]	6.42±0.02	
12c	4-OMePh	479 [48±6%]	6.32±0.11	490 [54±4%]	6.31±0.05	
12d	3-Cl,4-FPh	240 [45±9%]	6.62±0.11	195 [41±2%]	6.71±0.03	
12e	2,4-diFPh	562 [39±3%]	6.25±0.28	3,980 [35±7%]	5.40±0.31	
12f	4-pyridyl	589 [71±5%]	6.23±0.12	2,239 [64±6%]	5.65±0.22	

^a Calcium mobilization assays with hM₄/Gq15-CHO or rM₄/Gq15-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.

Table 2

Structures and activities for M₄ PAM analogs **13**.

Cpd	Ar (Het)	hM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	hM ₄ pEC ₅₀ (±SEM)	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)
13a	Ph	372 [45±5%]	6.43±0.13	692 [43±4%]	6.16±0.12
13b	3-pyridyl	105 [74±5%]	6.98±0.16	417 [95±7%]	6.38±0.06

^a Calcium mobilization assays with hM₄/Gq15-CHO or rM₄/Gq15-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.

Table 3

Structures and activities for M₄ PAM analogs **14**.

14					
Cpd	Ar (Het)	hM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	hM ₄ pEC ₅₀ (±SEM)	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)
14a	Ph	102 [76±3%]	6.99±0.04	166 [67±8%]	6.78±0.04
14b	4-OMePh	646 [63±6%]	6.19±0.21	1,450 [70±7%]	5.84±0.20
14c	2,4-diFPh	331 [55±5%]	6.48±0.28	380 [66±4%]	6.42±0.05
14d	3-pyridyl	105 [74±5%]	6.98±0.16	417 [95±7%]	6.38±0.06
14e	4-pyridyl	891 [76±4%]	6.05±0.05	3,550 [68±3%]	5.45±0.07

^a Calcium mobilization assays with hM₄/Gq15-CHO or rM₄/Gq15-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.