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Discovery and optimization of a novel series of highly CNS penetrant M₄ PAMs based on a 5,6-dimethyl-4-(piperidin-1-yl)thieno[2,3-*d*] pyrimidine core

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Abstract

This Letter describes the chemical optimization of a novel series of M₄ positive allosteric modulators (PAMs) based on a 5,6-dimethyl-4-(piperidin-1-yl)thieno[2,3-*d*]pyrimidine core, identified from an MLPCN functional high-throughput screen. The HTS hit was potent and selective, but not CNS penetrant. Potency was maintained, while CNS penetration was improved (rat brain:plasma K_p = 0.74), within the original core after several rounds of optimization; however, the thieno[2,3-*d*]pyrimidine core was subject to extensive oxidative metabolism. Ultimately, we identified a 6-fluoroquinazoline core replacement that afforded good M₄ PAM potency, muscarinic receptor subtype selectivity and CNS penetration (rat brain:plasma K_p > 10). Moreover, this campaign provided fundamentally distinct M₄ PAM chemotypes, greatly expanding the available structural diversity for this exciting CNS target.

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Keywords

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

Positive allosteric modulators (PAMs) of the muscarinic acetylcholine receptor subtype 4 (M₄) have garnered a great deal of attention as a novel therapeutic approach for the treatment of multiple symptom domains of schizophrenia,^{1–6} Huntington's disease⁷ and Alzheimer's disease.⁸ However, the diversity and breadth of small molecule M₄ PAMs is quite limited, and challenged by many of the most concerning issues with allosteric modulators (non-obvious, subtle structural modifications that engender steep SAR, species differences in affinity/cooperativity and subtype selectivity, solubility, and/or P-gp-mediated efflux).^{9–12} From the beginning, Lilly's LY2033298 (**1**) demonstrated that major species' disconnects in activity were possible with M₄ PAMs;^{1,2} however, second generation M₄ PAMs, such as VU0152100 (**2**, also known as ML108),^{3,4} were similarly potent on human and rat M₄, and displayed antipsychotic-like activity in rats by potentiation of receptor activation by endogenous acetylcholine (Fig. 1).^{3,4,13} Structurally related M₄ PAMs were also reported (VU0010010,¹⁴ ML253,¹⁵ ML173¹⁶), which reverted back to steep SAR and/or species differences in potency. VU0448088 (**3**, ML293) was optimized from an HTS hit, but again proved to be a human-preferring M₄ PAM with steep, intractable SAR.^{17,18} Recently, we disclosed the pyridazine congener VU0467154 (**4**), which displayed robust in vivo efficacy across a broad range of rodent antipsychotic and cognition models, further increasing interest in the target.¹⁸ However, all of the known chemotypes of M₄ PAMs (**1–4**) posed significant challenges en route to a clinical candidate. In this Letter, we detail the chemical optimization, SAR, DMPK profile and in vivo efficacy of a fundamentally new M₄ PAM chemotype.

In order to identify a new M₄ PAM chemotype, we performed a new functional M₄ HTS in 1536-well assay format against the ~360,000 compound MLSMR collection, and four structurally novel M₄ PAMs were identified.¹⁹ Of the four, VU0473619 (**5**) represented an exciting lead (Fig. 2), as it was similarly potent on both human and rat M₄ (EC₅₀s of 410 nM and 160 nM, respectively, i.e., little or no apparent species difference), and was inactive on both human M₁ and M₅ (built-in HTS counterscreen). For a lead, the DMPK profile was acceptable; however, **5** was not CNS penetrant, likely due to the primary sulfonamide moiety that engendered a *cLogP* of 1.2. Thus, the optimization effort would focus on replacing this detrimental functional group, and, in addition, assess the 5,6-dimethyl moieties as potential CYP₄₅₀ metabolic liabilities as well as the amide linker as another potentially CNS-limiting functional group. Upon resynthesis, the potency of **5** for human M₄ confirmed, but as a weaker hM₄ PAM (EC₅₀ = 1.20 μM, pEC₅₀ = 5.92 ± 0.10, 91 ± 4%).

The chemistry to produce amide analogs of **5** was straightforward (Scheme 1), and the initial optimization focused on replacing the sulfonamide. Commercially available 4-chloro-5,6-dimethylthieno[2,3-*d*]pyrimidine **6** was subjected to a standard S_NAr reaction with piperidine **7**, to afford **8** in 90% yield. Saponification delivered **9**, which then underwent a

HATU-mediated coupling with diverse amines to afford analogs **10** or **11** in yields ranging from 22% to 90%.

Table 1 highlights select SAR for the first generation of analogs **10** employing primary amines/anilines. Efficacy (% ACh Max) proved to be relatively low across all of the analogs **10**, but we were pleased to see that the primary sulfonamide of **5** could be replaced with other moieties such as a sulfone (**10e**) or the more lipophilic halogenated anilines **10g** and **10h** while retaining selectivity versus M₁ and M₅ (data not shown). However, SAR was steep, efficacy was uniformly low, and physiochemical and DMPK properties remained poor.

We then elected to evaluate tertiary amides, where we found greater success in terms of M₄ potency, efficacy and disposition. As shown in Table 2, many analogs **11** displayed submicromolar potency and improvements in efficacy. The thiomorpholine congener **11e** was considerably more potent and efficacious than the analogous morpholine derivative **11d**. Further exploration of pyrrolidine amides **11a** and **11b** provided encouraging results. In vitro DMPK assays, PAM **11a** displayed good free fraction in both rat ($f_u = 0.04$) and human ($f_u = 0.06$) plasma and importantly proved to be CNS penetrant (rat brain:plasma $K_p = 0.45$, $K_{p,uu} = 0.45$). However, in intrinsic clearance studies, **11a** was highly metabolized with predicted hepatic clearances near the organ blood flow rate (predicted CL_{hep} = 67 and 16 mL/min/kg for rat and human respectively). The fluorinated congener, **11b**, showed increased free fraction in both rat ($f_u = 0.12$) and human ($f_u = 0.05$) plasma, but CNS penetration decreased (rat brain:plasma $K_p = 0.17$, $K_{p,uu} = 0.12$) and intrinsic clearance remained high (predicted CL_{hep} = 68 and 14 mL/min/kg for rat and human, respectively).

These data suggested that efforts needed to focus on replacement of the amide linker as well as the 5,6-dimethylthieno[2,3-*d*]pyrimidine core. We first elected to replace the amide linker with ether linkages (Scheme 2), which could be readily accessed. Here, 4-chloro-5,6-dimethylthieno[2,3-*d*]pyrimidine **6** was subjected to a standard S_NAr reaction with piperidine **12** to afford **13** in 70–95% yield. A subsequent Mitsunobu reaction installed the aryl/heteroaryl ether moiety, producing analogs **14**. These ether analogs, devoid of the amide linker, displayed high CNS distribution ($K_p > 1$), but variable unbound CNS distribution $K_{p,uu}$. Of these, **14b** (VU6002703) stood out as a potent M₄ PAM (hM₄ EC₅₀ = 600 nM, 65% ACh Max) but exhibited high intrinsic clearance in vitro (hepatic microsomes) with predicted hepatic clearances near organ blood flow rates in both human and rat (CL_{hep} = 20 and 68 mL/min/kg, respectively), as did all analogs **14**. Clearly, a metabolic instability issue rested within the 5,6-dimethylthieno[2,3-*d*]pyrimidine core. Therefore, we performed metabolite identification (MetID) experiments in rat and human hepatic microsomes (Fig. 3) with **14b**, and found hydroxylation of the 5,6-dimethyl moiety was the major pathway of metabolism in rat, while heteroatom (likely the thiophene sulfur) oxidation was the major metabolic route in human. Tying back the 5,6-dimethyl moiety into five- and six-membered unsaturated rings was tolerated for potency and CNS penetration, but had no impact on lowering in vitro clearance (data not shown). Therefore, we needed to identify an alternate core (see Table 3).

Thus, we maintained the 2-methyl-5-(piperidin-4-yloxy)pyridine moiety in **14b** and surveyed a variety of alternative heteroaryl and heterobiaryl ring systems to replace the 5,6-dimethylthieno [2,3-*d*]pyrimidine core. New analogs **17** were readily obtained by a Mitsunobu reaction, Boc deprotection and S_NAr reaction (Scheme 3) in good overall yields. SAR for analogs **17** was steep, but substituted quinazoline derivatives **17a** and **17b**, a quinoline congener **17c**, and a pyrrolotriazine, **17d**, were active (M₄ PAM EC₅₀s 600 nM to 1.2 μM) and highly CNS penetrant (*K*_ps 0.94–21.1). Intrinsic clearance remained high (predicted CL_{hep} near hepatic blood flow in both human and rat) for all analogs **17** except **17b** (VU6003130). **17b** was the most potent human M₄ PAM in this new series (human M₄ EC₅₀ = 600 nM, 62%) with moderate activity at rat M₄ (rat EC₅₀ = 950 nM, 57%) and selective versus M_{1–3,5} (EC₅₀s > 10 μM). Moreover, **17b** had a moderate to high free fraction in rat (*f*_u = 0.02) and human (*f*_u = 0.16) plasma as well as in rat brain homogenate (*f*_u = 0.03). In addition, **17b** was highly CNS penetrant (rat brain:plasma *K*_p = 11.1, *K*_{p,uu} > 10) and was the only analog to show a notable improvement in metabolic stability in vitro (predicted CL_{hep} = 12 and 50 mL/min/kg in human and rat, respectively), moving it into the moderate clearance range. Thus, this optimization effort provided two fundamentally novel, potent and selective M₄ PAMs, **14b** (VU6002703) and **17b** (VU6003130), with excellent CNS distribution (see Table 4).

Before further advancement of **14b** and **17b**, we also evaluated replacement of the ether linkage in **14b** with an amine linker, as in **18** (Fig. 4). The majority of analogs were inactive, but once the alkyl chain reached 3-carbons in length, a ‘molecular switch’ was noted (rare amongst muscarinic allosteric modulators^{20–22} and the first report for M₄), wherein the ligands became M₄ antagonists. For example, **19** (human M₄ IC₅₀ = 2.4 μM, 9.3% ACh min) and **20** (human M₄ IC₅₀ = 1.5 μM, 8.7% ACh min) were robust M₄ antagonists. Analogs **19** and **20** had attractive DMPK profiles (*f*_us > 0.10 across species, *K*_ps ~ 0.2 and acceptable CYP₄₅₀ inhibition profiles); however, both were similarly potent at the other muscarinic receptors (M_{1–3,5}), suggesting binding had switched from an allosteric site to the orthosteric site. While not the desired outcome, this finding was interesting and will enable computational/docking strategies moving forward.

In summary, an HTS campaign identified a fundamentally new M₄ PAM chemotype, but with unfavorable disposition and no CNS penetration. Iterative parallel synthesis developed SAR and afforded two novel M₄ PAMs, **14b** (VU6002703) and **17b** (VU6003130), with excellent CNS distribution and enhanced M₄ PAM potency relative to the HTS hit. Importantly, this effort also identified a ‘molecular switch’ that modulated the mode of pharmacology from PAM to orthosteric antagonist. Further study and optimization within this series is ongoing, and will be reported in due course.

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Figure 1. Structures of representative M₄ PAMs **1–4**, highlighting the limited diversity of known chemotypes.

Figure 2.

Structure and key biological data for the M₄ PAM HTS hit VU0473619 (**5**), and three areas to address in the lead optimization campaign. Potency values represent means from at least three independent determinations in a functional Ca²⁺ mobilization assay with rat or human M₄/Gq α 5-CHO cells performed in the presence of an ~EC₂₀ fixed concentration of acetylcholine.

Figure 3.

Metabolism of **14b** (VU6002703) in rat and human hepatic microsomes. All biotransformation pathways shown are NADPH-dependent.

Figure 4. Generic structure of amine-linked analogs **18** and specific analogs **19** and **20** that underwent a ‘molecular switch’ into *pan*-orthosteric mAChR antagonists.

Scheme 1.

Reagents and conditions: (a) DIEA, NMP, MW, 200 °C, 15 min, 90%; (b) LiOH, aqueous MeOH, rt, 95%; (c) HNR¹R², HATU, DCE/DMF (9:1), rt, 22–90%.

Scheme 2.

Reagents and conditions: (a) DIEA, NMP, MW, 200 °C, 15 min, 70–95%; (b) ArOH/Het-OH, PPh₃, DABAD, THF, rt, 55–86%.

Scheme 3.

Reagents and conditions: (a) 6-methylpyridin-3-ol, PPh_3 , DABAD , THF, rt, 88%; (b) Het-Cl, DIEA, NMP, MW, 200 °C, 15 min, 62–94%.

Table 1Structures and activities for M₄ PAM analogs **10**

Compd	R	hM ₄ EC ₅₀ ^a (μM) [% ACh Max ± SEM]	hM ₄ pEC ₅₀ (±SEM)
10a	H	>10 [25]	>5
10b		2.3 [67 ± 1%]	5.63 ± 1.1
10c		0.39 [56 ± 6%]	6.41 ± 0.01
10d		0.57 [70 ± 3%]	6.24 ± 0.13
10e		0.85 [62 ± 6%]	6.07 ± 0.11
10f		6.2 [40 ± 8%]	5.21 ± 0.20
10g		1.6 [55 ± 5%]	5.80 ± 0.18

Compd	R	hM ₄ EC ₅₀ ^a (μM) [% ACh Max ± SEM]	hM ₄ pEC ₅₀ (±SEM)
10h		3.8 [69 ± 5%]	5.42 ± 0.09

^a Calcium mobilization assays with hM₄/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 2Structures and activities for M₄ PAM analogs **11**

Compd	NR ¹ R ²	hM ₄ EC ₅₀ ^a (μM) [% ACh Max ± SEM]	hM ₄ pEC ₅₀ (±SEM)
11a		1.1 [68 ± 3%]	5.94 ± 0.11
11b		0.97 [79 ± 10%]	6.01 ± 0.03
11c		1.9 [60 ± 5%]	5.73 ± 0.21
11d		1.7 [65 ± 4%]	5.77 ± 0.10
11e		0.82 [75 ± 7%]	6.09 ± 0.27
11f		0.96 [69 ± 5%]	6.02 ± 0.06

^a Calcium mobilization assays with hM4/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.

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Table 3

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

Compd	NR ¹ R ²	hM ₄ EC ₅₀ ^a (μM) [% ACh Max ± SEM]	hM ₄ pEC ₅₀ (±SEM)	Rat K _p (K _{p,uu}) ^b
14a		4.4 [60 ± 5%]	5.36 ± 0.09	1.77 (0.65)
14b		0.60 [65 ± 4%]	6.22 ± 0.04	1.16 (0.74)
14c		1.8 [68 ± 4%]	5.76 ± 0.03	2.80 (0.34)
14d		4.3 [64 ± 4%]	5.37 ± 0.11	2.56 (0.17)
14e		2.3 [74 ± 5%]	5.64 ± 0.09	1.25 (1.38)

^aCalcium mobilization assays with hM₄/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.

^bTotal 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.

Table 4Structures and activities for M₄ PAM analogs **17**

Compd	NR ¹ R ²	hM ₄ hEC ₅₀ ^a (μM) [% ACh Max ± SEM]	hM ₄ pEC ₅₀ (±SEM)	Rat K _p (K _{p,uu}) ^b
17a		1.2 [49 ± 7%]	5.93 ± 0.08	0.94 (0.62)
17b		0.6 [62 ± 7%]	6.24 ± 0.07	11.1 (>10)
17c		0.69 [64 ± 5%]	6.16 ± 0.03	21.4 (18.2)
17d		1.1 [58 ± 3%]	5.98 ± 0.17	1.42 (1.95)

^a Calcium mobilization assays with hM₄/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.

^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.