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Discovery of a novel, CNS penetrant M₄ PAM chemotype based on a 6-fluoro-4-(piperidin-1-yl)quinoline-3-carbonitrile core

Blake R. Bewley^a, Paul K. Spearing^a, Rebecca L. Weiner^a, Vincent B. Luscombe^a, Xiaoyan Zhan^a, Sichen Chang^a, Hyekyung P. Cho^a, Alice L. Rodriguez^a, Colleen M. Niswender^{a,b,d}, P. Jeffrey Conn^{a,b,d}, Thomas M. Bridges^{a,b}, Darren W. Engers^{a,b}, and Craig W. Lindsley^{a,b,c,*}

^aVanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, TN 37232, USA

^bDepartment of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

^cDepartment of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

^dVanderbilt Kennedy Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

Abstract

This Letter details the discovery and subsequent optimization of a novel M₄ PAM scaffold based on an 6-fluoro-4-(piperidin-1-yl)quinoline-3-carbonitrile core, which represents a distinct departure from the classical M₄ PAM chemotypes. Optimized compounds in this series demonstrated improved M₄ PAM potency on both human and rat M₄ (4 to 5-fold relative to HTS hit), and displayed attractive physicochemical and DMPK profiles, including good CNS penetration (rat brain:plasma K_p = 5.3, K_{p,uu} = 2.4; MDCK-MDR1 (P-gp) ER = 1.1).

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*To whom correspondence should be addressed: darren.engers@vanderbilt.edu and craig.lindsley@vanderbilt.edu.

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Keywords

M4; Muscarinic acetylcholine receptor; Positive allosteric modulator (PAM); Non-human primate (NHP); Structure-Activity Relationship (SAR)

While M₄ positive allosteric modulators (PAMs) hold great therapeutic promise for schizophrenia^{1–4} and a number of other CNS disorders,^{5,6} a lack of translatable chemotypes (and very limited structural diversity within known chemotypes) has hampered the field from realizing the true therapeutic potential of selective M₄ activation. The area has been dominated by differentially functionalized 3-amino-thieno[2,3-*b*]pyridine cores, typified by **1**, or 5-amino-3,4-dimethylthieno[2,3-*c*]pyridazine such as **2** (Figure 1).^{7–16} Multiple HTS campaigns revealed similar chemotypes, with the exception of **3** and **4**;^{17,18} however, these also failed to progress to development candidates. However, **4** (based on a 5,6-dimethyl-4-(piperidin-1-yl)thieno[2,3-*d*]pyrimidine core) was optimized to **5**, which demonstrated exceptional CNS penetration.¹⁸ With the goal of arriving at a development candidate, we recently reported on a scaffold hopping exercise that afforded **6**.¹⁹ In this Letter, we will discuss the synthesis, SAR and DMPK profiles of M₄ PAMs based on a novel 6-fluoro-4-(piperidin-1-yl)quinoline-3-carbonitrile core, which demonstrated robust SAR beyond that of related **4–6**, and holds promise for translation.

Recently, we performed a new functional M₄ high-throughput screen (HTS) in 1536-well assay format against the ~100,000 compound Vanderbilt collection. Of the four structurally novel M₄ PAMs that were identified, VU0619856 (**7**) represented an exciting new chemotype for the M₄ field, and while related to **4** and **5**, M₄ PAM potency peaked at ~600 nM in these earlier series.¹⁸ New M₄ PAM **7** was active on both rat and human (EC₅₀s of 2565 nM and 897 nM respectively) with promising points of diversity. The initial strategy was to utilize an iterative parallel synthesis to explore the amide portion of the molecule followed by evaluation of the 3-cyano quinoline core. Would the 3-cyano quinoline be a quinazoline mimetic as in **5**, or would SAR diverge?

The synthesis of analogs of M₄ PAM **7**, congeners **11** (secondary amides) and **12** (tertiary amides), was straightforward (Scheme 1), and employed a diverse collection of amines (benzyl, aryl/heteroaryl amines and secondary amines). Starting from commercial 4-chloro-6-fluoroquinoline-3-carbonitrile **8**, an S_NAr with ethyl piperidine-4-carboxylate provided **9** in 98% yield. After hydrolysis under basic conditions to provide the acid **10**, a library of amides **11** and **12** were generated under standard HATU conditions.

While subtle improvement in potency was observed with a variety of substituted benzyl amines (**11b–d**), compounds such as **11d** had high predicted hepatic clearance, CL_{hep} (h = 19, r = 61), and low brain distribution in rat (brain:plasma K_p = 0.29, K_{p,uu} = 0.26). The aniline **11e**, substituted with a primary sulfonamide, showed very promising hM₄ potency (248 nM, 6% ACh), but later was determined to be a P-gp substrate (MDCK-MDR1 ER = 83). Analog **11g** proved to be the most potent secondary amide in the series, with an hM₄ PAM potency of 147 nM; however, its DMPK profile was lacking. Importantly, this new chemotype already surpassed the PAM potency floor of **4** and **5** (hM₄ PAM EC₅₀s ~ 600 nM).

As disposition and CNS penetration were low with the secondary amides, we profiled tertiary amides to assess the impact of removing one hydrogen-bond donor. As shown in Table 2, representative tertiary amide analogs **12** suffered a diminution in hM₄ PAM potency relative to secondary amides **11**. However, we still advanced **12d**, a 648 nM M₄ PAM into *in vitro* and *in vivo* DMPK profiling. PAM **12d**, like analogs **11**, displayed high predicted hepatic clearance, CL_{hep} (h = 20, r = 64), but removal of the secondary amide H-bond donor improved CNS penetration (rat brain:plasma K_p = 1.3, K_{p,uu} = 0.68). These data led us to generate chimeras between the new 3-cyano quinoline core and the piperidinyl ethers of **5** and explore if this structural change would bring balance to hM₄ PAM potency while maintaining favorable CNS penetration.

Synthesis of the targeted chimera ligands **14** was facile (Scheme 2). Once again, commercial 4-chloro-6-fluoroquinoline-3-carbonitrile **8** underwent an S_NAr reaction with 4-hydroxypiperidine to provide **13** in 90% yield. Then, a Mitsunobu reaction with various functionalized phenols and heteroaryl alcohols delivered analogs **14** in yields ranging from 10–80%.

As shown in Table 3, these chimeric ligands proved to possess hM₄ PAM activity, but SAR was steep with few active PAMs. Of these, **14f** (VU6009003) stood out as a potent hM₄ PAM (EC₅₀ = 197 nM, pEC₅₀ = 6.78 ± 0.17, ACh Max = 79 ± 10). Prior to deeper characterization of **14f**, we elected to survey the necessity of the 3-cyano quinolone core, and assess if deletion of the 3-CN moiety, or additional functionalization of the quinoline core would afford hM₄ PAMs for further evaluation. To assess this possibility, analogs **17** were readily prepared in two steps from commercial **15** and 5-fluoro-2-methylpyridine via an S_NAr reaction to provide, after Boc deprotection **16**. A second S_NAr reaction with various 4-halo quinolines produced analogs **17**.

Deletion of the 3-CN moiety as in **17a** led to a greater than 10-fold loss in hM₄ PAM potency, highlighting the necessity of this novel moiety. Moreover, this result suggests that the 3-CN moiety in this core is not bioequivalent to the nitrogen of the quinazoline-based hM₄ PAMs represented by **5**. Replacement of the 6-fluoro moiety with either a 6-Cl atom (**17b**, hM₄ PAM EC₅₀ = 905 nM) results in a ~4-fold loss of potency, while replacement with a methyl group (**17c**, hM₄ PAM EC₅₀ = 3018 nM) results in a ~15-fold loss in hM₄ PAM potency relative to **14f**. Of these analogs **17**, only a 6,8-difluorocongener **17e**, proved of equivalent potency to **14f**.

Thus, **14f** (VU6009003) emerged from this effort as a novel M₄ PAM worthy of further profiling. PAM **14f** was potent at both human M₄ (197 nM, 80% ACh Max) and rat M₄ (437 nM, 74% ACh Max) with very modest species difference in PAM potency, and a marked improvement over the HTS hit **7**. In terms of physicochemical properties, **14f** was a low molecular weight compound (MW = 362) with an attractive xLogP (2.83) for a CNS agent (Table 5). In a microsomal intrinsic clearance assay, **14f** displayed moderate clearance in both species (predicted human CL_{hep} = 15 mL/min/kg, predicted rat CL_{hep} = 52 mL/min/kg) with moderate fraction unbound in human plasma (*f*_u = 0.024), rat plasma (*f*_u = 0.031) and rat brain (*f*_u = 0.014). In our standard rat plasma:brain level IV cassette paradigm, **14f** showed excellent CNS penetration with a rat brain:plasma partitioning coefficient (K_p) of

5.3, and when corrected for fraction unbound in plasma and brain, a $K_{p,uu}$ of 2.4. We also assessed human P-gp efflux potential in a MDCK-MDR1 transwell assay, and found no P-gp liability ($ER = 1.1$) coupled with high passive permeability ($P_{app} = 28 \times 10^{-6}$ cm/s). *In vivo*, **14f** displayed lower clearance ($CL_p = 36$ mL/min/kg) in rat (IV PK cassette, 0.2 mg/kg) than predicted ($CL_{hep} = 52$ mL/min/kg), with a short $t_{1/2}$ (1.7 hours) and moderate volume ($V_{ss} = 3.2$ L/kg). In a dog IV PK cassette study (0.2 mg/kg), **14f** displayed clearance ($CL_p = 41$ mL/min/kg) at hepatic blood flow with a $t_{1/2}$ of 1.6 hours (likely as a consequence of moderate-to-high volume, $V_{ss} = 4.0$ L/kg); however, **14f** did display a reasonable IVIVC with predicted hepatic clearance in dog ($CL_{hep} = 27$ mL/min/kg) and good fraction unbound ($f_u = 0.045$). While attractive overall in terms of novel chemotype, favorable CNS MPO score (>5),²¹ fraction unbound and CNS penetration, the disposition of **14f** remains to be addressed (after a deeper understanding of metabolic soft spots).

In summary, an HTS campaign identified a new M_4 PAM chemotype **7**, related to **4** and **5**, but displaying distinct SAR, improved M_4 PAM functional potency and enhanced CNS penetration. The discovery of new M_4 PAM chemical matter is of critical importance to enable translation to the clinic, as the field has been largely dominated by limited chemotypes **1** and **2**, fraught with development challenges. Metabolite identification studies and further optimization efforts are underway and will be reported in due course.

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Figure 1.

Structures of representative M₄ PAMs **1–4**, possessing the classical β -amino carboxamide pharmacophore (circled in **1**) in either a 3-amino-4,6-dimethylthieno[2,3-*b*]pyridine core, represented by **1**, or a 5-amino-3,4-dimethylthieno[2,3-*c*]pyridazine core, exemplified by **2**. The only structurally distinct M₄ PAM chemotypes reported are represented by **3–6**.

Figure 2.
Structures and profile of new HTS hit **7**, and the optimization plan.

Scheme 1. Synthesis of M₄ PAM **11** and **12**.^a^aReagents and conditions: (a) ethyl piperidine-4-carboxylate, DIEA, NMP, MW, 80 °C, 98%; (b) LiOH, THF/MeOH/H₂O (4/1/1), rt, 53%; (c) HNR₁R₂, HATU, DMF, rt, 22–76%.

Scheme 2. Synthesis of M₄ PAM analogs **14**.^a^aReagents and conditions: (a) 4-hydroxypiperidine, DIEA, NMP, rt, 90%; (b) ArOH or Het-OH, PPh₃, DIAD, THF, rt, 10–80%.

Scheme 3.

Synthesis of M₄ PAM analogs **17**.^a

^aReagents and conditions: (a) 5-fluoro-2-methylpyridine, DIEA, NMP, rt, then HCl, dioxane, rt, 90%; (b) Het-X (X = Cl or Br), DIEA, NMP, rt, 22–84%.

Table 1Structures and activities for human M₄ PAM analogs **11**.

<p style="text-align: center;">11</p>			
Cmpd	R	hM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	hM ₄ pEC ₅₀ (±SEM)
11a VU0619856		1307 [78 ± 8]	5.91 ± 0.08
11b VU0619858		651 [48 ± 4]	6.23 ± 0.12
11c VU0619859		664 [60 ± 5]	6.21 ± 0.11
11d VU6008714		1089 [65 ± 4]	5.98 ± 0.08
11e VU6008848		248 [63 ± 6]	6.64 ± 0.12
11f VU6009062		565 [52 ± 2]	6.25 ± 0.04
11g VU6009025		147 [73 ± 10]	6.83 ± 0.02
11h VU6009063		1756 [81 ± 10]	5.78 ± 0.10
11i VU6009064		940 [59 ± 3]	6.03 ± 0.01
11j VU6009067		912 [44 ± 3]	6.06 ± 0.10
11k VU6009066		619 [68 ± 5]	6.21 ± 0.04

^a Calcium mobilization assays with hM₄/Gq15-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine; values represent means from at least three (*n*=3) independent experiments performed in triplicate.

Table 2Structures and activities for analog **12**.

<p style="text-align: center;">12</p>			
Cmpd	R	hM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	hM ₄ PEC ₅₀ (±SEM)
12a VU6008847		1415 [88 ± 6]	5.85 ± 0.04
12b VU6009031		6436 [78 ± 17]	5.20 ± 0.05
12c VU6009288		652 [81 ± 8]	6.19 ± 0.05
12d VU6009289		648 [76 ± 8]	6.20 ± 0.08

^a Calcium mobilization assays with hM₄/Gq15-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine; values represent means from at least three (*n*=3) independent experiments performed in triplicate.

Table 3Structures and activities for human M₄ PAM analogs **14**.

<p style="text-align: center;">14</p>			
Cmpd	R	hM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	hM ₄ pEC ₅₀ (±SEM)
14a VU6009005		3256 [81 ± 12]	5.50 ± 0.06
14b VU6009117		1298 [80 ± 7]	5.89 ± 0.05
14c VU6009158		512 [83 ± 10]	6.31 ± 0.09
14d VU6009220		577 [80 ± 6]	6.25 ± 0.05
14e VU6009159		>10,000 [49 ± 7]	
14f VU6009003		197 [79 ± 10]	6.78 ± 0.17
14g VU6009160		5640 [53 ± 8]	5.27 ± 0.08
14h VU6009216		>10,000	<4.5
14i VU6009219		>10,000	<4.5

^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 4Structures and activities for analog **17**.

17			
Cmpd	R	hM₄ EC₅₀ (nM)^a [% ACh Max ±SEM]	hM₄ pEC₅₀ (±SEM)
17a VU6009451		2207 [62 ± 5]	5.67 ± 0.06
17b VU6009533		905 [72 ± 5]	6.04 ± 0.01
17c VU6009842		3018 [67 ± 4]	5.53 ± 0.05
17d VU6009436		571 [92 ± 8]	6.25 ± 0.05
17e VU6009532		223 [93 ± 4]	6.68 ± 0.09
17f VU6009845		2065 [82 ± 10]	5.69 ± 0.05

^aCalcium mobilization assays with hM₄/Gq15-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine; values represent means from at least three (*n*=3) independent experiments performed in triplicate.

Table 5In vitro and in vivo DMPK profiles of selected M₄ PAMs.

Property	11e	12d	14f	5
MW	453.5	444.5	362.4	354.5
cLogP	2.01	3.94	2.83	3.47
TPSA	129	69.5	62.0	51.1
<i>In vitro</i> PK parameters				
CL _{INT} (mL/min/kg), rat	66	709	198	1432
CL _{HEP} (mL/min/kg), rat	34	64	52	67
CL _{INT} (mL/min/kg), human	31	480	58	290
CL _{HEP} (mL/min/kg), human	13	20	15	20
Rat fu _{plasma}	0.016	0.013	0.031	0.011
Human fu _{plasma}	0.064	0.011	0.024	0.013
Rat fu _{brain}	0.010	0.007	0.014	0.007
Brain Distribution (0.25 hr) (SD Rat; 0.1–0.2 mg/kg IV)				
K _{pbrain:plasma}	0.16	1.26	5.25	1.16
K _{puu, brain:plasm}	0.10	0.68	2.37	0.74
MDCK-MDR1 (P-gp) ER	83	ND	1.1	ND

ND = not determined.