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Discovery of a novel 2,4-dimethylquinoline-6-carboxamide M₄ positive allosteric modulator (PAM) chemotype via scaffold hopping

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Abstract

This Letter details our efforts to replace the 3-amino moiety, an essential pharmacophore for M₄ PAM activity in most M₄ PAMs to date, within the thieno[2,3-*b*]pyridine core, as the β -amino carboxamide motif has been shown to engender poor solubility, varying degrees of P-gp efflux and represents a structural alert. A scaffold hopping exercise identified a novel 2,4-dimethylquinoline carboxamide core that provided M₄ PAM activity and good CNS penetration without an amino moiety. In addition, MacMillan photoredox catalysis chemistry was essential for construction of the 2,4-dimethylquinoline core.

Graphical Abstract

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Keywords

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

Muscarinic acetylcholine receptor subtype 4 (M₄) positive allosteric modulators (PAMs) have garnered a great deal of attention as a new therapeutic approach for the treatment of schizophrenia (both the positive and cognitive symptom clusters),^{1–4} Huntington's disease⁵ and Parkinson's disease.⁶ However, the dominant M₄ PAM chemotypes, either a 3-amino-5-chloro-6-methoxy-4-methylthieno[2,3-*b*]pyridine core, such as **1**,^{7,8} a 3-amino-4,6-dimethylthieno[2,3-*b*]pyridine core such as **2**,^{9,10} or a 5-amino-3,4-dimethylthieno[2,3-*c*]pyridazine core, such as **3** and **4**,^{11–13} possess the β -amino carboxamide moiety as a key pharmacophore (Figure 1), essential for M₄ PAM activity. While necessary for M₄ PAM activity, this moiety engenders species differences in potency, varying degrees of P-gp efflux, poor solubility as well as eliciting a structural alert. To date, only two series of M₄ PAMs have been reported, exemplified by **5** and **6**,^{14,15} that are structurally distinct from **1–4**; however, these series failed to deliver development candidates. After several M₄ PAM high-throughput screening (HTS) campaigns provided only the usual suspects (congeners of **1–6**),¹⁶ we elected to pursue a scaffold hopping approach.^{17–19} In this Letter, we will discuss the scaffold hopping exercise and the identification and optimization of a fundamentally new M₄ PAM chemotype based on a 2,4-dimethylquinoline carboxamide core, devoid of the classical β -amino carboxamide moiety.

In short order, many proposals for new cores via scaffold hopping were envisioned and executed upon, but true to PAM SAR, the vast majority of novel cores provided compounds that proved inactive as M₄ PAMs. Fortunately, one design concept proved fruitful (Figure 2), based on 2,4-dimethylquinoline carboxamide cores **8** and **9**. Shown simplistically below, but confirmed in modeling, a quinolone core could serve as a replacement for the 3-amino thiophene moiety in **7**, but the pendent carboxamide could be either in the 6-position (**8**) or the 7-position (**9**). While modeling indicated that either regioisomer was potentially active, only analogs **8** proved to be M₄ PAMs (all analogs **9**, M₄ EC₅₀ > 10 μ M).

The synthesis of analogs **8** was expected to rely on recent photoredox-mediated Minisci C-H alkylation of *N*-heteroarenes developed by Macmillan, where MeOH serves as the alkylating agent,²⁰ as the requisite 2,4-dimethylquinoline starting materials were not readily available (Scheme 1). However, 6-bromo-2-methylquinoline **10** was available; an Iridium-catalyzed C-H insertion photoredox reaction under blue LEDs afforded a single product, the desired 6-bromo-2,4-dimethylquinoline **11**, in 87% isolated yield on gram scales. With **11** in hand, a Pd-catalyzed carbonylation reaction provided **12**, which was smoothly hydrolyzed to acid **13** and finally, HATU coupling with various amines delivered the desired analogs **8**.

Initial SAR of analogs **8**, employing amide moieties optimal in M₄ PAM series **1–4**,^{9–15} were weakly potent PAMs with modest efficacy (Table 1). Of these, the recently disclosed *N*-aryl azetidine amides were the most potent analogs (such as **8d**, rM₄ EC₅₀ = 856 nM) and warranted further inspection of additional analogs.²¹

Due to the physicochemical properties of **8d** (xLogP = 5.3 and poor solubility), future analogs would replace the fluorinated aryl ring with various heterocycles in an attempt to lower xLogP into acceptable CNS drug ranges and offer opportunities for salt formation to enhance solubility. As shown in Scheme 2, heteroaryl aminoazetidines were synthesized through common intermediate **14**, prepared via Scheme 1, by a Buchwald amination protocol to deliver analogs **15**.

SAR for select analogs **15** are highlighted in Table 2, and this exercise resulted in analogs with M₄ PAM potencies that rival **1–6** (EC₅₀s as low as 225 nM). In general, pyrimidines (**15a** and **b**) were less potent than pyrazines (**15c** and **d**), which were less potent than functionalized pyridines (**15e–t**). Moreover, several of the *N*-pyridyl azetidine amides possessed M₄ PAM functional potencies less than 500 nM: **15j** (EC₅₀ = 401 nM), **15l** (EC₅₀ = 449 nM), **15q** (EC₅₀ = 383 nM), **15r** (EC₅₀ = 307 nM) and **15t** (EC₅₀ = 332 nM). Of these, **15l**, **15q**, **15r** and **15t** were advanced into a battery of *in vitro* DMPK assays (Table 3) and our standard rat plasma:brain level (PBL) cassette paradigm. In terms of physicochemical properties, all four possessed molecular weights less than or equal to 400 Da, with attractive CNS xLogPs (2.38 to 2.76) except for the dichloro congener **15r** (xLogP = 3.42); however, analogs **15** were improved over lead **8d** (xLogP = 5.2). All four displayed moderate to high predicted hepatic clearance based on microsomal CL_{int} data (human CL_{hep}s of 13 to 19 mL/min/kg and rat CL_{hep}s of 40 to 56 mL/min/kg) and all were highly bound to plasma proteins (*f*_us (human and rat) ~0.005) except **15t** (rat *f*_u = 0.013 and rat *f*_u brain = 0.023). While **15l** proved to have limited CNS penetration (rat brain:plasma K_p = 0.05, K_{p,uu} = 0.20), M₄ PAM **15q** showed excellent CNS penetration (rat K_p = 3.4, K_{p,uu} = 0.84) and the other analogs **15r** and **15t** were modest (K_ps <0.2, K_{p,uu}s ~ 0.3).

In summary, a scaffold hopping exercise based on M₄ PAMs **1** and **2** identified a novel 2,4-dimethylquinoline carboxamide core that exhibited M₄ PAM activity in the absence of the classical β-amino carboxamide pharmacophore. Moreover, regiospecificity of the pendent carboxamide (6-versus 7-position) was noted, to afford potent and CNS penetrant M₄ PAMs, on par with **1–6**. While this initial foray did not produce PAMs with the DMPK profiles to advance to development candidates, it did provide leads for further refinements towards that goal, which 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 **2**, or a 5-amino-3,4-dimethylthieno[2,3-*c*]pyridazine core, exemplified by **3** and **4**. The only other structurally distinct M₄ PAM cores reported are represented by those in **5** and **6**, but these series failed to deliver clinical candidates.

Figure 2. Scaffold hopping concept from β -amino amide-containing M₄ PAMs **7** to 2,4-dimethylquinoline carboxamide cores **8** and **9**.

Scheme 1. Synthesis of M₄ PAM analogs **8**.^a

^aReagents and conditions: (a) 1 mol% Ir(ppy)₂(dtbbpy)PF₆, 5 mol% ethyl 2-mercaptopropanoate, MeOH, TsOH, DMSO, Blue LEDs, 23 °C, 87%; (b) Pd(OAc)₂, PdCl₂(dppf)₂, Et₃N, MeOH/DMF, CO (balloon), 80 °C, 83%; (c) LiOH, THF:H₂O (4:1), rt, 94%; (d) HNR¹R², HATU, DMF, rt, 37–99%.

Scheme 2. Synthesis of M₄ PAM analogs **15**.^a^aReagents and conditions: (a) Pd₂(dba)₃, Cs₂CO₃, Heteroaryl-X, 1,4-dioxane, 100 °C, 15–67%.

Table 1Structures and activities for M₄ PAM analogs **8**.

8			
Cpd	NHR	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)
8a VU6008407		>10,000 [63±7]	<5.00
8b VU6008408		Inactive [18.3]	Inactive
8c VU6008406		3910 [66±7]	5.41±0.08
8d VU6008536		856 [55±6]	6.07±0.14

^a Calcium mobilization assays with rM₄/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 **15**.

15			
Cpd	Het	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)
15a VU6008537		>10,000 [61±7]	<5.00
15b VU6008540		6140 [73±7]	5.21±0.06
15c VU6008539		1070 [82±4]	5.97±0.07
15d VU6009660		1160 [75±8]	5.94±0.02
15e VU6010002		1080 [77±2]	5.97±0.01
15f VU6009980		967 [84±4]	6.01±0.04
15g VU6009981		440 [77±4]	6.36±0.19
15h VU6009983		935 [82±4]	6.03±0.05
15i VU6009979		587 [70±3]	6.23±0.04

<p style="text-align: center;">15</p>			
Cpd	Het	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)
15j VU6009977		401 [72±3]	6.40±0.03
15k VU6009978		453 [82±3]	6.34±0.04
15l VU6009454		449 [85±5]	6.35±0.07
15m VU6009661		Inactive [43±12]	Inactive
15n VU6009663		820 [61±4]	6.09±0.04
15o VU6009543		1130 [86±4]	5.95±0.06
15p VU6009538		1060 [71±3]	5.97±0.02
15q VU6009453		383 [62±2]	6.42±0.03
15r VU6008541		307 [57±7]	6.51±0.07

<p style="text-align: center;">15</p>			
Cpd	Het	rM ₄ EC ₅₀ (nM) ^a [% ACh Max ±SEM]	rM ₄ pEC ₅₀ (±SEM)
15s VU6009541		225 [44±5]	6.65±0.24
15t VU6009548		332 [82±4]	6.48±0.06

^a Calcium mobilization assays with rM₄/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 3In vitro DMPK and rat PBL data for select analogs **15**.

Property	15l	15q	15r	15t
MW	400.4	396.9	401.3	368.4
xLogP	2.69	2.76	3.42	2.38
TPSA	58.1	67.3	58.1	58.1
<i>In vitro</i> PK parameters				
CL _{INT} (mL/min/kg), rat	94.0	178	295	232
CL _{HEP} (mL/min/kg), rat	40.1	50.2	56.6	53.8
CL _{INT} (mL/min/kg), human	36.0	106	160	129
CL _{HEP} (mL/min/kg), human	13.2	17.5	18.6	18.1
Rat fu _{plasma}	0.004	0.002	0.002	0.013
Human fu _{plasma}	0.005	0.002	0.002	0.009
Rat fu _{brain}	0.016	0.008	0.006	0.023
Brain Distribution (0.25 hr) (SD Rat; 0.2 mg/kg IV)				
K _{p, brain:plasma}	0.05	0.84	0.10	0.17
K _{puu, brain:plasma}	0.20	3.36	0.30	0.30