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VU6007477, a Novel M_1 PAM Based on a Pyrrolo[2,3-*b*]pyridine Carboxamide Core Devoid of Cholinergic Adverse Events

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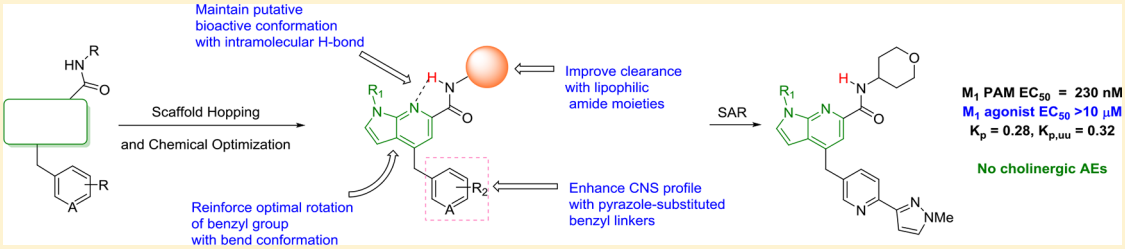
Supporting Information



ABSTRACT: Herein, we report the chemical optimization of a new series of M_1 positive allosteric modulators (PAMs) based on a novel pyrrolo[2,3-*b*]pyridine core, developed via scaffold hopping and iterative parallel synthesis. The vast majority of analogs in this series proved to display robust cholinergic seizure activity. However, by removal of the secondary hydroxyl group, VU6007477 resulted with good rat M_1 PAM potency (EC_{50} = 230 nM, 93% ACh max), minimal M_1 agonist activity (agonist EC_{50} > 10 μM), good CNS penetration (rat brain/plasma K_p = 0.28, $K_{p,uu}$ = 0.32; mouse K_p = 0.16, $K_{p,uu}$ = 0.18), and no cholinergic adverse events (AEs, e.g., seizures). This work demonstrates that within a chemical series prone to robust M_1 ago-PAM activity, SAR can result, which affords pure M_1 PAMs, devoid of cholinergic toxicity/seizure liability.

KEYWORDS: *Positive allosteric modulator (PAM), muscarinic acetylcholine receptor 1 (M_1), cholinergic toxicity, VU6007477*

Muscarinic acetylcholine receptor subtype 1 (M_1) positive allosteric modulators (PAMs) hold great promise for the treatment of cognitive impairment, schizophrenia, and Alzheimer's disease.[1−7] Originally, the clinical promise of M_1 as a target was derailed by M_1 agonists lacking true M_1 selectivity,[1,2,8] and later, by robust M_1 ago-PAMs that overstimulated the M_1 receptor, resulting in cholinergic toxicity and adverse events (AEs).[9−18] While these ago-PAMs, represented by **1**−**4** (Figure 1), dampened enthusiasm for the translational utility of selective M_1 activation, next generation M_1 PAMs **5** and **6**, devoid of agonism in cells and native systems (and without cholinergic toxicity/seizures), provided a new path to the clinic.[14,19] However, due to the voluminous literature with ago-PAMs **1**−**4**, multiple new M_1 PAMs are required for the community to evaluate the safety and efficacy of the "pure" M_1 PAM mechanism and independently embrace the therapeutic potential of M_1 PAMs. Herein, a novel series of M_1 PAMs featuring a pyrrolo[2,3-*b*]pyridine carboxamide core is described, including the design, SAR, DMPK properties, pharmacology, and cholinergic adverse effect profiles to provide the community with another new, pure M_1 PAM tool for *in vivo* efficacy and tolerability studies.

Scaffold-hopping has been a major strategy in the M_1 PAM arena, and this approach led to the discovery of the benzomorpholine-based pure M_1 PAM **6**.[19] We once again held the key carboxamide and heterobiaryl tail moieties of **3**, **5**, and **6** constant while surveying new heterobiaryl cores, as in **7**, that might engender pure M_1 PAM pharmacology (Figure 2). From this exercise, a novel pyrrolo[2,3-*b*]pyridine carboxamide core, represented generically by **8**, resulted, with a spectrum of M_1 pharmacology from potent ago-PAMs to pure PAMs.

The synthesis of diverse analogs **13** (Scheme 1) proved straightforward with readily available starting materials from commercial sources.[20] 4-Chloro-1*H*-pyrrolo[2,3-*b*]pyridine-6-carbonitrile **9** could be alkylated with various R_1 moieties, but we first explored methyl. Conversion to the boronate ester under standard conditions provided **10** in >90% yield for the two steps. Suzuki coupling with either benzyl halides or heteroaryl methyl halides **11** generated derivatives **12** in 50−84% yield. Finally,

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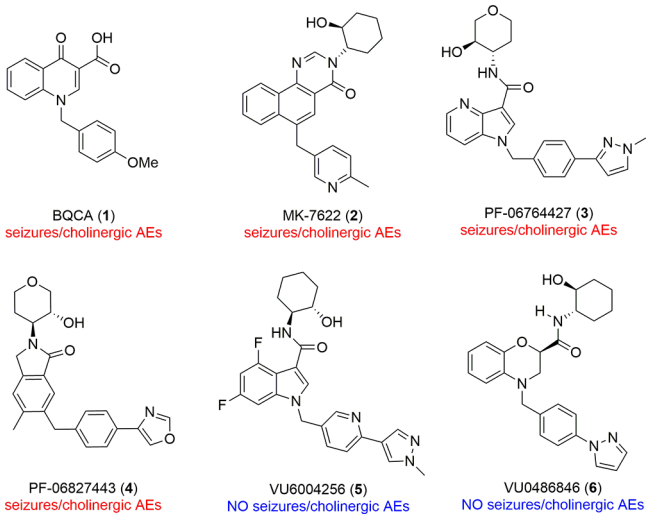


Figure 1. Structures of representative M_1 PAMs **1**–**6**. Of these, **1**–**4** are robust ago-PAMs in high expressing cell lines and native systems, resulting in severe seizures/cholinergic AEs due to overstimulation of M_1. M_1 PAMs **5** and **6** are devoid of seizures/cholinergic AEs and show little to no M_1 agonism in high expressing cell lines and native systems.

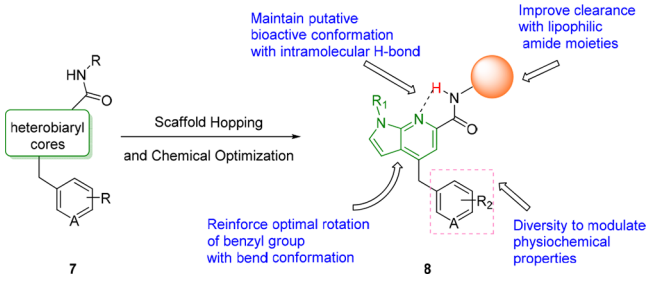
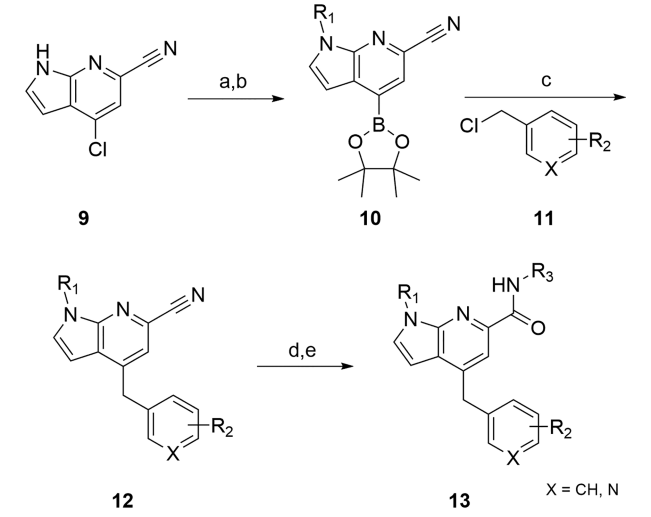


Figure 2. Scaffold hopping approach based on M_1 PAMs **3**, **5**, and **6**, which led to the discovery of a novel pyrrolo[2,3-*b*]pyridine carboxamide-based series of M_1 ago-PAMs and pure PAMs, **8**.

Scheme 1. Synthesis of M_1 PAM Analogs 13[a]

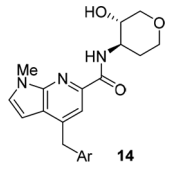


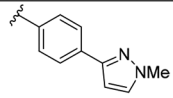
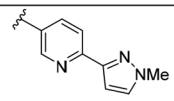
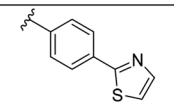
[a]Reagents and conditions: (a) R_1I, NaH, DMF, 0 °C–rt, 85–90%; (b) bis(pinacolato)diboron, KOAc, Pd(dppf)Cl_2, 1,4-dioxane, 100 °C, 16 h, >98%; (c) benzyl chloride, Cs_2CO_3, Pd(dppf)Cl_2, THF/H_2O, 90 °C, 16 h; 50–84%; (d) conc. HCl, reflux, 2 h, >98%; (e) amine, HATU, DIEA, DMF, rt, 20 min, 35–66%.

hydrolysis of the nitrile and standard HATU amide coupling reactions delivered the putative M_1 PAMs **13** in 35–66% yield.[20]

We then surveyed this new core with a variety of heterobiaryl tails and amide congeners, grouped based on internal knowledge regarding SAR to engender robust M_1 ago-PAM versus PAM activity. The first amide moiety surveyed was the (3*S*,4*R*)-3-hydroxy-4 amino tetrahydropyranyl (THP) amide, well documented to engender potent M_1 PAMs, but with undesired potent M_1 agonist activity.[11,13−17,19] As we previously reported, M_1 PAM potencies below 100 nM, coupled with M_1 agonist activity, generally lead to robust cholinergic AEs.[19] As anticipated, analogs **14** (Table 1) with diverse southern

Table 1. Structures and M_1 Pharmacology for Analogs 14[a]



Cpd	R	rM_1 PAM EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)	rM_1 ago-nist EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)
14a		0.04 [87±0]	7.41±0.06	2.24 [65±2]	5.65±0.04
14b		0.014 [86±1]	7.86±0.06	0.575 [67±3]	6.24±0.06
14c		0.10 [90±1]	7.00±0.03	5.62 [41±3]	5.25±0.06

[a]Calcium mobilization assays with rat M_1-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine for PAM, and no exogenous ACh for the agonist EC_{50}; values represent means from three (n = 3) independent experiments performed in triplicate.

heterobiaryl tails provided potent M_1 ago-PAMs. Analog **14b** stands out as a 14 nM M_1 PAM (86% ACh max), with an agonist EC_{50} of 575 nM (67% ACh max) and a direction not worthy of further pursuit, as overstimulation of M_1 would definitely result.

As the analogous cyclohexyl congener **15** of THP **14** generally afforded a lower degree of M_1 agonism, we next evaluated such analogs. As shown in Table 2, analogs **15** were also potent M_1 ago-PAMs (**15a**–**c**), but with reduced M_1 agonism relative to **14**,[11,13−17,19] as well as a moderately potent M_1 PAM (**15d**). Interestingly, both **15b** and **15c** were not CNS penetrant in rat, with K_p values below level of quantitation (BLQ), despite favorable CNS MPO scores (4–5).[21] Previously, we have shown that the secondary hydroxyl moiety engenders poor CNS penetration in rodents, and future analogs would avoid this moiety.[19]

Thus, we evaluated the profile of simple, unsubstituted pyranyl amides, as we hoped this would engender a balance between M_1 PAM potency (and diminished M_1 agonism) and

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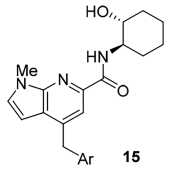
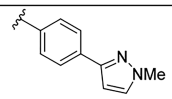
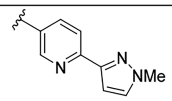
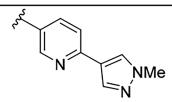
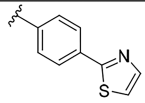
Table 2. Structures and M_1 Pharmacology for Analogs 15[a]

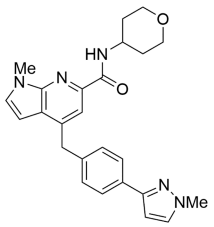


15

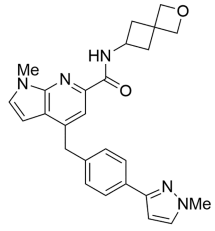
Cpd	R	rM_1 PAM EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)	rM_1 agonist EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)
15a		0.18 [87±1]	6.74±0.03	3.24 [41±2]	5.49±0.02
15b		0.09 [87±1]	7.02±0.04	2.29 [53±2]	5.64±0.04
15c		0.04 [89±1]	7.38±0.04	1.21 [58±3]	5.92±0.05
15d		0.36 [91±1]	6.44±0.01	>30	<4.5

[a]Calcium mobilization assays with rM_1-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine for PAM, and no exogenous ACh for the agonist EC_{50}; values represent means from three (n = 3) independent experiments performed in triplicate.

CNS penetration. Our initial evaluation produced pyranyl amide **16** and the spiro-cyclic congener **17** (Figure 3); importantly,



16
rM_1 PAM EC_{50} = 560 nM, 89% ACh Max
rM_1 agonist EC_{50} >10 μM



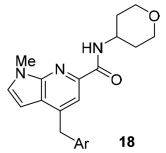
17
rM_1 PAM EC_{50} = 604 nM, 91% ACh Max
rM_1 agonist EC_{50} >10 μM

Figure 3. SAR evaluation of nonhydroxylated amides **16** and **17**.

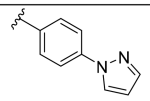
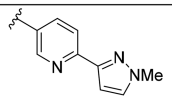
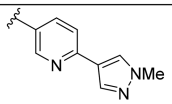
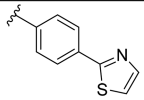
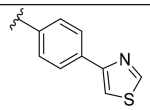
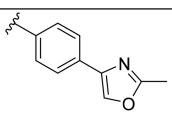
both proved to be pure M_1 PAMs (agonist EC_{50}s > 10 μM). However, M_1 PAM potency was modest (**16**: rM_1 PAM EC_{50} = 560 nM, pEC_{50} = 6.25 ± 0.05, ACh max = 89 ± 1; **17**: rM_1 PAM EC_{50} = 604 nM, pEC_{50} = 6.22 ± 0.04, ACh max = 91 ± 1). Still, similar pyranyl amides (lacking the secondary hydroxyl moiety) in other series (such as **5** and **6**) were inactive or weak, suggesting this is a unique core. To further optimize M_1 PAM potency with the pyranyl amide, we next explored alternative heterobiaryl tails.

Surprisingly, while holding the pyranyl amide moiety constant in analogs **18** and varying the heterobiaryl tail (Table 3), the lack

Table 3. Structures and M_1 Pharmacology for Analogs 18[a]



18

Cpd	R	rM_1 PAM EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)	rM_1 agonist EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)
18a		0.49 [87±1]	6.31±0.03	>10 [26±5]	<5
18b		0.76 [90±4]	6.12±0.05	>10 [34±16]	<5
18c		0.23 [93±0]	6.64±0.07	>10	<5
18d		1.74 [78±1]	5.76±0.02	>30	<4.5
18e		0.76 [86±2]	6.12±0.01	>30	<4.5
18f		0.76 [88±3]	6.12±0.03	>10	<5

[a]Calcium mobilization assays with rM_1-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine for PAM, and no exogenous ACh for the agonist EC_{50}; values represent means from three (n = 3) independent experiments performed in triplicate.

of M_1 agonism in these des-hydroxy congeners proved to be a generally conserved pharmacological property. Of these, **18c** emerged as an attractive M_1 PAM (EC_{50} = 230 nM, 93% ACh max; log K_b = −4.82; log $\alpha\beta$ = 2.6) with minimal to no agonism in our high expressing cell line (M_1 agonist EC_{50} > 10 μM).[20] Replacement of the *N*-Me pyrazole in **16** for an oxazole analog (e.g., **18f**) provided an M_1 PAM of comparable potency (EC_{50} = 760 nM, 88% ACh max) to **16** and still devoid of M_1 agonism. Regioisomeric pyridyl pyrazole heterobiaryls **18b** and **18c** showed similar M_1 PAM activity, but **18b** displayed weak M_1 agonism (~34% @ 10 μM). An *N*-linked pyrazole congener, **18a**, displayed similar PAM potency and weak M_1 agonism (~26% at 10 μM) as well, highlighting the sensitivity of this series for M_1 agonist activity.

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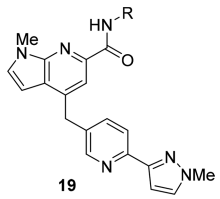
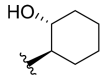
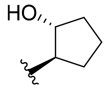
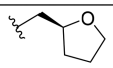
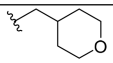
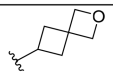
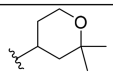
A larger amide scan of the pyrazole regioisomers **19** based on the **18b** heterobiaryl tail motif (Table 4) similarly identified a

Table 4. Structures and M_1 Pharmacology for Analogs 19[a]



19

Cpd	R	rM_1 PAM EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)	rM_1 agonist EC_{50} (μM)[a] [% ACh Max ±SEM]	rM_1 pEC_{50} (±SEM)
15b		0.10 [87±1]	7.02±0.04	2.29 [53±2]	5.64±0.02
19a		0.26 [86±1]	6.59±0.04	>10	<5
19b		0.49 [84±2]	6.31±0.07	>10	<5
19c		1.58 [87±1]	5.80±0.04	> 30	< 4.5
19d		0.57 [89±1]	6.24±0.06	>30	< 4.5
19e		0.55 [94±1]	6.26±0.05	>10	<5

[a]Calcium mobilization assays with rM_1-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine for PAM, and no exogenous ACh for the agonist EC_{50}; values represent means from three (n = 3) independent experiments performed in triplicate.

number of pure M_1 PAMs and ago-PAMs (with novel amides), but none showed advantages over analogs highlighted in Tables 1−3. However, unlike predecessor series **1**−**4**, this scaffold afforded pure M_1 PAMs, devoid of agonist activity in high expressing M_1 cell lines.

Prior to assessing physiochemical and DMPK properties, we wanted to ensure good species alignment for key M_1 PAMs at both human and rat M_1. Gratifyingly, there was high species conservation (Table 5), indicating translational potential for this novel series of M_1 PAMs.

The M_1 PAMs highlighted in Table 5 displayed favorable physiochemical properties (MWs < 450, cLogPs between 1.2 and 4.0, TPSAs 89−102 Å^2, CNS MPO scores between 3.2 and

Table 5. Human and Rat M_1 PAM Activities for Select Analogs 14−18

Cpd	rat M_1 PAM EC_{50} (μM)[a] [% ach Max ± SEM]	rat M_1 pEC_{50} (±SEM)	hum M_1 PAM EC_{50} (μM)[a] [% ACh max ± SEM]	hum M_1 pEC_{50} (±SEM)
14a	0.04, [87 ± 0]	7.41 ± 0.06	0.06, [83 ± 3]	7.22 ± 0.10
14c	0.10, [90 ± 1]	7.00 ± 0.03	0.09, [80 ± 8]	7.05 ± 0.20
15a	0.18, [87 ± 1]	6.74 ± 0.03	0.18, [84 ± 2]	6.74 ± 0.10
15b	0.10, [87 ± 1]	7.02 ± 0.04	0.12, [79 ± 4]	6.91 ± 0.10
16	0.56, [89 ± 1]	6.25 ± 0.05	0.66, [87 ± 3]	6.18 ± 0.10
18c	0.23, [93 ± 0]	6.64 ± 0.07	0.32, [84 ± 7]	6.49 ± 0.05

[a]Calcium mobilization assays with rM_1-CHO or hM_1-CHO cells performed in the presence of an EC_{20} fixed concentration of acetylcholine for PAM; values represent means from three (n = 3) independent experiments performed in triplicate.

4.3) and acceptable *in vitro* DMPK profiles (Table 6).[20] The majority show modest predicted hepatic clearance in human and

Table 6. *In Vitro* DMPK Data and PBL Data for Select M_1 PAMs 14−18[a]

property	14a	14c	15b	15c	16	18c
MW	445	446	443	444	427	430
cLogP	2.40	1.22	4.02	2.84	5.21	1.77
TPSA	89.7	102.1	80.5	92.9	60.3	81.9
In Vitro PK Parameters						
rat CL_{HEP} (mL/min/kg)	39.1	42.0	49.6	27.3	47.9	44.2
human CL_{HEP} (mL/min/kg),	6.97	10.6	17.8	9.3	10.7	6.78
rat f_u (plasma)	0.03	0.02	0.03	0.06	0.04	0.06
human f_u (plasma)	0.02	0.02	0.12	0.04	0.02	0.04
rat f_u (brain)	0.01	0.01	0.04	0.07	0.01	0.08
Rat PBL (IV, 0.2 mg/kg)						
K_p	0.11	0.21	BLQ	BLQ	BLQ	0.28
$K_{p,uu}$	0.05	0.13	BLQ	BLQ	BLQ	0.32
Mouse PBL (IP, 100 mg/kg)						
K_p	0.09	ND	0.28	0.03	ND	0.16
$K_{p,uu}$	0.04	ND	0.17	0.04	ND	0.18

[a]BLQ: brain concentration is below limit of quantitation (BLQ) in low dose rat cassette (0.2 mg/kg). ND = not determined.

rat, good free fraction (human, rat, and rat brain homogenate binding), but low to modest CNS penetration in rat (rat brain/plasma K_ps and $K_{p,uu}$s ≤ 0.3). Moreover, as we have seen significant variations between brain penetration in mice and rats, we also assessed mouse K_p at 100 mg/kg ip. Despite low but measurable K_p in mice, brain levels were high (both total and unbound). The low K_ps were driven by very high plasma concentrations (Supplemental Figure 1).[20]

As we have documented previously,[11,13−17,19] robust ago-PAM activity can lead to over stimulation of the M_1 receptor and/or induce M_1 agonist-dependent long-term depression in the prefrontal cortex leading to cognitive dysfunction and, in mice (the most susceptible species to excessive M_1 activation), Racine scale 4 to 5 seizures.[11,14,17,19] Thus, a quick phenotypic screen in mice (at 100 mg/kg IP) to assess seizure liability has become a proven method of choice to eliminate compounds from the lead progression flow-chart and deem them non-developable. Interestingly, this pyrrolo[2,3-*b*]pyridine carboxamide-based series showed a pronounced tendency for cholinergic adverse effect liability in the mouse seizure model

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(Figure 4), with all analogs showing robust Racine scale seizures within 30 min of IP administration (100 mg/kg), save **18c**

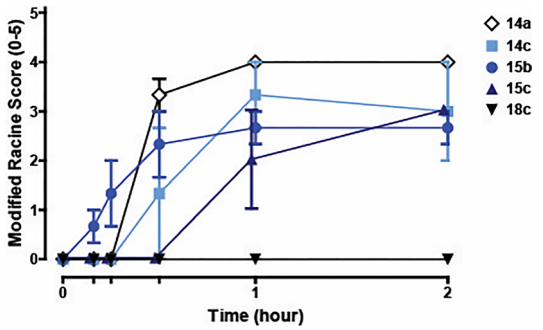

Figure 4. Phenotypic mouse seizure assay with compounds dosed at 100 mg/kg i.p. VU6007477 (**18c**) was devoid of seizure liability (mouse brain levels: 7 μM total, 546 nM unbound), whereas **14a**, **14c**, **15b**, and **15c** elicited robust Racine scale 4/5 seizures.[20]

(VU6007477). These data further highlight the range of clean versus adverse event (AE) *in vivo* pharmacology that can occur within a highly conserved chemical series of M_1 PAMs (and the value of an informative phenotypic triage screen).[14,19]

Due to the significant AE liability within this series, we were hesitant to further advance this series down the lead optimization flow-chart; however, additional characterization quickly led to a no-go decision for both **18c** and this series. While **18c** was highly selective for M_1 (M_2–M_5 EC_{50}s > 30 μM, Supplemental Figure 2),[20] it proved to be a human P-gp substrate (ER = 4.5), with only moderate permeability (Papp = 1.2×10^{-5} cm/s). Thus, **18c** is a new *in vitro*/*in vivo* rodent M_1 "pure" PAM tool compound but is not suitable for translation to the clinic.

In summary, a scaffold-hopping exercise identified a novel pyrrolo[2,3-*b*]pyridine carboxamide-based series of M_1 PAMs that displayed a range of potent ago-PAM and pure PAM pharmacology. While congeners possessing the prototypical (3*S*,4*R*)-3-hydroxy-4 amino tetrahydropyranyl (THP) amide moiety (or the cylcohexyl analog) engendered a range of M_1 ago-PAM activity, they also resulted in severe Racine scale 4/5 seizures that correlated with the presence of M_1 agonist activity (which is predicted to result in overstimulation of M_1). In contrast, a simple pyranyl amide derivative, **18c** (VU6007477), was a pure M_1 PAM in high expressing cell lines (M_1 agonist EC_{50} > 10 μM), displayed improved CNS penetration over the hydroxylated congeners, and was devoid of seizure liability in mice. While **18c** was not advanceable as a clinical candidate, it remains an important new rodent tool compound to study selective M_1 activation without concern for cholinergic toxicity and related AEs. The optimization of other pure M_1 PAMs, devoid of cholinergic toxicity and adverse effect liability, with translational potential, will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00261.

General methods for the synthesis and characterization of all compounds, and methods for the *in vitro* and *in vivo* DMPK protocols and supplemental figures (PDF)

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C.W.L., C.M.N., P.J.C., H.P.C., and J.M.R. drafted/corrected the manuscript. J.L.E., E.S.C., M.F.L., R.A.C., and D.W.E. performed the chemical synthesis. C.W.L., P.J.C., C.M.N., J.K.R., and H.P.C. oversaw the medicinal chemistry and target selection and interpreted the biological data. V.B.L. and H.P.C. performed the *in vitro* molecular pharmacology studies. A.L.B. performed the *in vitro* and *in vivo* DMPK studies. J.M.R. oversaw the *in vivo* experiments. J.W.D. performed the *in vivo* studies. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): Hold IP on M1 PAMs.

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■ ABBREVIATIONS

PAM, positive allosteric modulator; PBL, plasma/brain level; AE, adverse event; dppf, 1′-ferrocenediyl-bis(diphenylphosphine); HATU, 1-[Bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; DIEA, diisopropylethyl amine; DMPK, drug metabolism and pharmacokinetics; MPO, multiparameter optimization

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