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Discovery of Tricyclic Triazolo- and Imidazopyridine Lactams as M₁ Positive Allosteric Modulators

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Abstract

This Letter describes the chemical optimization of a new series of muscarinic acetylcholine receptor subtype 1 (M₁) positive allosteric modulators (PAMs) based on novel tricyclic triazolo- and imidazopyridine lactam cores, devoid of M₁ agonism, e.g., no M₁ ago-PAM activity, in high expressing recombinant cell lines. While all the new tricyclic congeners afforded excellent rat pharmacokinetic (PK) properties (CL_p < 8 mL/min/kg and t_{1/2} > 5 h), regioisomeric triazolopyridine analogues were uniformly not CNS penetrant (K_p < 0.05), despite a lack of hydrogen bond donors. However, removal of a single nitrogen atom to afford imidazopyridine derivatives proved to retain the excellent rat PK and provide high CNS penetration (K_p > 2), despite inclusion of a basic nitrogen. Moreover, **24c** was devoid of M₁ agonism in high expressing recombinant cell lines and did not induce cholinergic seizures in vivo in mice. Interestingly, all of the new M₁ PAMs across the diverse tricyclic heterocyclic cores possessed equivalent CNS MPO

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Author Contributions

C.W.L. and K.A.E. oversaw and designed the chemistry. P.J.C., H.P.C., and C.M.N. oversaw and designed the molecular pharmacology, and A.L.B. oversaw the DMPK. J.L.E., A.M.B., J.J.K., C.H., and B.J.M. performed synthetic/medicinal chemistry and scaled-up key compounds. H.P.C. designed and executed advanced molecular pharmacology assays. K.S.L. and V.B.L. performed molecular pharmacology assays. A.L.B. performed DMPK and bioanalysis. J.M.R. and J.W.D. performed and analyzed data from the in vivo mouse study, and C.W.L. wrote the manuscript.

Notes

The authors declare the following competing financial interest(s): The authors hold patents on M1 PAMs, M1 ago-PAMs and M1 agonists.

scores (>4.5), highlighting the value of both “medicinal chemist’s eye” and experimental data, e.g., not sole reliance (or decision bias) on in silico calculated properties, for parameters as complex as CNS penetration.

Graphical Abstract

Keywords

Positive allosteric modulator; PAM; M₁; muscarinic acetylcholine receptor 1; SAR; CNS

Recently, progress in understanding the ideal molecular pharmacology profile of muscarinic acetylcholine receptor subtype 1 (M₁) positive allosteric modulators (PAMs) to avoid cholinergic adverse events in vivo, while maximizing procognitive efficacy (for the treatment of Alzheimer’s disease profile of an M₁ PAM to advance as a clinical candidate is a “pure” M₁ PAM, devoid of agonist activity. within M₁ PAM chemotypes, e.g., M₁ ago-PAMs, as an undesired pharmacological profile (in both high expressing cell lines and in native tissues).^{1–7} Excessive activation of M₁ by robust ago-PAMs leads to cholinergic toxicity, seizures, induction of long-term depression (LTD) and, as a result, greatly diminished pro-cognitive efficacy.^{2–7} Thus, the ideal profile of an M₁ PAM to advance as a clinical candidate is a “pure” M₁ PAM, devoid of agonist activity. However, the first M₁ PAM, BQCA (**1**),^{8,9} and the progenitor of the vast majority of “M₁ PAMs” (**2–4**),^{10–14} was a highly potent and robust M₁ ago-PAM (Figure 1). Thus, **2–4** retained the undesirable M (AD) and schizophrenia), has identified M₁ agonist activity ago-PAM activity of **1**, from which they were designed (or scaffold-hopped), and subsequently have been shown to possess limited efficacy in combination with cholinergic toxicity.^{2–14} However, the ago-PAM profile is not absolute, and structurally related M₁ PAMs can be derived with significantly limited M₁ ago-PAM activity, such as **5**,³ or M₁ PAMs, such as **6**⁴ and **7**,¹⁵ devoid of agonist activity. Indeed, synthetic derivatives of these scaffolds can have dramatically different modes of pharmacology from the parent compounds. Importantly, all three afforded robust efficacy without cholinergic toxicity. However, the latter is very rare, and it is challenging to surmount the inherent bias of M₁ ago-PAM pharmacology within an ago-PAM scaffold.

Interestingly, both **5** and **6** resulted from the chemical optimization of hits from an M₁ triple-add functional highthroughput screen (Figure 2).^{3,4} The most productive “hit” was both novel, and an unlikely candidate for hit-to-lead chemistry. The compound in question was VU0119498 (**9**), a hit with *pan*-G_q-muscarinic acetylcholine receptor (mAChR) PAM activity (M₁, M₃, and M₅ PAM), yet inactive at M₂ and M₄.^{16,17} Despite the electrophilic and potentially reactive isatin moiety of **9**, which showed virtually no detectable M₁ agonist

activity, the unprecedented “pure” M₁ PAM pharmacology profile led to its prioritization. Optimization of **9** quickly led to the first M₅ PAMs¹⁶ and the identification of divergent SAR to dial-out activity at M₃ and M₅,¹⁷ while simultaneously eliminating the isatin moiety for a more preferred lactam functionality provided M₁ PAM VU0451725 (**10**).⁶ An aza scan then led to the discovery of the key in vivo tool VU0453595 (**11**)⁶ and the recently published “pure” M₁ PAM electrophysiology tool, VU0550164 (**12**).² These data highlight the value of not only the quality of the initial hit (an isatin would usually be discarded during HTS triage), but also caveats in the pharmacological profile from which to select hits to launch an optimization campaign.

In parallel with the above-described optimization efforts, we also envisioned further modifications of **10**, and specifically: incorporation of various fused tricyclic motifs (**13**), possibly with an incorporation of a basic amine to enhance aqueous solubility (as the pyridine nitrogen in **11** and **12** proved to be unable to form salts), while hopefully maintaining the favorable (and necessary) “pure PAM” pharmacology (Figure 3).^{3,6} Ideally, we also sought to develop common intermediates from which all desired tricyclic cores could be rapidly prepared. In this Letter, we detail the discovery and optimization of multiple, novel tricyclic cores, unexpected challenges with CNS penetration (despite favorable CNS MPO scores),¹⁸ maintenance of no detectable M₁ agonism across all the tricyclic subseries (in high expressing recombinant cell lines), and finally, a new M₁ PAM chemotype with high CNS penetration and exceptional rat PK.

RESULTS AND DISCUSSION

We recently identified robust chemistry for the synthesis of triazolo- and imidazopyridine based allosteric modulators of another GPCR target.¹⁹ Thus, we quickly developed a streamlined chemistry approach to rapidly access diverse tricyclic congeners **13** from two common intermediates.²⁰ Commercial methyl-6-chloro-3-methylpicolinate **14** proved a viable starting material to provide benzylic bromide **15**. Treatment of **15** with an appropriately substituted benzylic amine (see the Supporting Information for a benzylic amine synthetic example) generates the chloro-lactam derivatives **16** (Scheme 1). With **16** in hand, a series of novel tricyclic analogues were rapidly synthesized. Reaction of **16** with hydrazine followed by condensation with a substituted orthoester provided substituted 6,7-dihydro-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[4,3-*a*]pyridin-8-ones **17** in 28–50% yields.²⁰ Unsubstituted congeners, 6,7-dihydro-8*H*-pyrrolo[3,4-*e*]-[1,2,4]triazolo[4,3-*a*]pyridin-8-ones, **18** were readily prepared in 22–70% yield by treatment of **16** with hydrazine, followed by condensation with triethyl orthoformate (or by direct condensation with formyl hydrazide).

Regioisomeric triazole analogues **23**, substituted 6,7-dihydro-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*a*]pyridin-8-ones, and imidazo analogues **24**, 2,3-dihydro-1*H*-imidazo[1,2-*a*]pyrrolo[3,4-*e*]pyridine-1-ones, were accessed as described in Scheme 2.²⁰ Methyl-6-chloro-3-methylpicolinate **14** again proved a viable starting material and provided the *N*-Boc congener **19** in 84% yield via a Buchwald reaction. Benzylic bromination to deliver **20** proceeded in moderate yield (36%). Treatment with an appropriately substituted benzylic amine again generated the lactam derivatives **21** (in 41–65% yield), followed by acidic

deprotection of the Boc group to provide linchpins **22** in >95% yield. Triazoles **23** were derived by treatment of **22** with DMF-DMA in 2-propanol at reflux, followed by cooling and the addition of hydroxylamine hydrochloride at 50 °C. After workup and concentration, treatment with TFAA at 0 °C delivered **23** in 13–32% yields. From **22**, imidazo analogues **24** are prepared via condensation with aqueous chloroacetaldehyde in yields ranging from 50 to 65%.²⁰ Thus, from two late-stage intermediates, we could readily access a range of fused heterocyclic systems **17**, **18**, **23**, and **24**, enabling an assessment of varying hydrogen bond accepting networks and basicity on M₁ PAM potency, rat PK and CNS penetration.

Evaluation of tricyclic lactams **17**, **18**, **23**, and **24** showed great promise early on, with no detectable M₁ agonism at either rat or human M₁ (agonist EC₅₀ >>30 μM in high expressing cell lines), in sharp contrast to **1–4**,^{2–7} and displaying no significant M₁ PAM species differences. Here, we will systematically walk through the SAR across the various tricyclic lactams, beginning with **17**, wherein the C1 substituent is a methyl group (Table 1). While initial SAR indicated a loss in M₁ PAM potency (EC₅₀'s 2 to >10 μM) relative to **10**, **17a**, **c** and **d** proved to be pure M₁ PAMs, with no detectable agonism. Despite weak PAM potency, these novel tricycles did impart improved drug metabolism and pharmacokinetics (DMPK) properties. For example, **17a** showed an enhanced fraction unbound in plasma (rat f_u = and human f_u = 0.18), low predicted hepatic clearance (rat CL_{hep} = 19.2 mL/min/kg and human CL_{hep} = 8.5 mL/min/kg) and attractive in vivo rat PK parameters (CL_p = 7.7 mL/min/kg, $t_{1/2}$ = 5.75 h, V_{ss} = 3.8 L/kg). The indazole congener **17c** showed similar, favorable properties: fraction unbound in plasma (rat f_u = 0.03 and human f_u = 0.04), low predicted hepatic clearance (rat CL_{hep} = 15.0 mL/min/kg and human CL_{hep} = 6.8 mL/min/kg) and good in vivo rat PK parameters (CL_p = 9.5 mL/min/kg, $t_{1/2}$ = 4.4 h, V_{ss} = 3.6 L/kg). Analogues **17** were found to be poorly CNS penetrant in rat, perhaps as a result of the increase in total number of polar atoms, with rat brain:plasma partitioning coefficients (K_p) between 0.08 and 0.09, despite favorable CNS MPO scores (>4.5)¹⁸ and no hydrogen bond donors. Thus, our initial foray into the tricyclic lactam series was both positive (maintenance of pure M₁ PAM activity, enhanced in vitro and in vivo DMPK profiles) and negative (loss of M₁ PAM potency coupled with loss of CNS penetration). From a medicinal chemistry viewpoint, we felt variations in the tricycle would ultimately offer opportunities to address the negative issues with series **17**, and hopefully maintain all the positive attributes.

Evaluation of the related *des*-methyl congeners, 7-benzyl-6,7-dihydro-8*H*-pyrrolo[3,4-*e*] [1,2,4]triazolo[4,3-*a*]pyridine-8-ones, **18** (Table 2) led to a breakthrough in terms of M₁ PAM potency. In this subseries, many analogues displayed submicromolar M₁ PAM potency (and good efficacy), and still no detectable M₁ agonism (at rat or human) in cell lines. Without the C1 methyl group on the triazole ring, a wide range of mono- and difluorobenzyl motifs were tolerated, as were a diverse collection of heterocycles. The direct analogue of **10**, **18a**, was effectively equipotent, while the difluoro variant, **18b**, proved to be a submicromolar PAM. We paused to assess the in vitro and in vivo DMPK profiles of these first two analogues in this subseries **18** to see if the favorable attributes were retained, and indeed they were. PAM **18a**, possessed a good fraction unbound in plasma (rat f_u = 0.16 and human f_u = 0.19) and very low (stable) predicted hepatic clearance (rat CL_{hep} = 0 mL/min/kg and human CL_{hep} = 11.1 mL/min/kg). The slightly more potent **18b** was similar:

fraction unbound in plasma (rat $f_u = 0.17$ and human $f_u = 0.19$) and very low (stable) predicted hepatic clearance (rat $CL_{\text{hep}} = 0$ mL/min/kg and human $CL_{\text{hep}} = 5.6$ mL/min/kg), coupled with a good in vitro/in vivo correlation (IVIVC) [in vivo rat PK parameters: ($CL_p = 5.2$ mL/min/kg, $t_{1/2} = 2.9$ h, $V_{ss} = 1.2$ L/kg)]. Analogues bearing indazoles, **18c–18f** were uniformly more potent, delivering M_1 PAMs in the 300–400 nM potency range at rM_1 and 600–900 nM for hM_1 . Like **18a/b**, these analogues showed comparable in vitro and in vivo DMPK profiles. Of note, **18e** displayed a highly desirable profile: fraction unbound in plasma (rat $f_u = 0.05$ and human $f_u = 0.07$) and stable predicted hepatic clearance (rat $CL_{\text{hep}} = 0$ mL/min/kg and human $CL_{\text{hep}} = 0$ mL/min/kg), coupled with a robust IVIVC in rat ($CL_p = 4.0$ mL/min/kg, $t_{1/2} = 3.2$ h, $V_{ss} = 1.1$ L/kg). Similar to analogues **17**, all analogues **18** once again displayed little (rat $K_p < 0.08$) or no ($K_p =$ below level of quantitation, BLQ) brain penetration, in contrast to the favorable CNS MPO scores.¹⁸ Moreover, analogue **18b** was not only peripherally restricted in rat, but also predicted to have low CNS penetration in humans based on high P-gp efflux in an MDR1 transfected MDCK bidirectional flux assay (ER = 35).

We next evaluated novel heterocycles in the M_1 PAM space, such as quinolines **18g** and **18h**, and while active these modifications did not offer any advantages over **18a–f**. Replacing the benzyl moiety in series **18** with a piperidinelinked heterocycle (**18i**) afforded an unexpected result (Figure 4), a “molecular switch” to a potent and selective M_1 antagonist (rat $IC_{50} = 150$ nM, 4% acetylcholine (ACh) min and human $IC_{50} = 350$ nM, 3% ACh min).^{21,22} This is the first instance, across multiple M_1 PAM chemical series, where we have observed a “molecular switch” modulating the mode of M_1 pharmacology, and possibly suggests the allosteric site might be in close proximity to the orthosteric ACh binding site, although the possibility of a binding profile more distal to the orthosteric site and stabilization of a receptor conformation that precludes ACh binding cannot be ruled out. Clearly, this finding diminished enthusiasm for the subseries and raised concerns for metabolite pharmacology.

Regioisomeric congeners of **18**, 6,7-dihydro-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*a*]pyridin-8-ones **23**, were generally less potent than **18**, and SAR was very flat. Of note, **23a** and **23b** (Figure 5) were moderately active M_1 PAMs (devoid of agonist activity at rat or human M_1). Like series **17** and **18**, **23b** showed a favorable in vitro DMPK profile with fraction unbound in plasma (rat $f_u = 0.12$ and human $f_u = 0.08$) and low predicted hepatic clearance in rat (rat $CL_{\text{hep}} = 11$ mL/min/kg) and stable in human (human $CL_{\text{hep}} = 0$ mL/min/kg). Rat in vivo PK was excellent ($CL_p = 0.60$ mL/min/kg, $t_{1/2} = 7.2$ h, and $V_{ss} = 0.37$ L/kg). Unfortunately, **23b** was peripherally restricted (rat $K_p = 0.03$, $K_{p,uu} = 0.05$). These experimental CNS penetration studies with **17**, **18**, and **23** and favorable CNS MPO scores,¹⁸ led us to consider that the presence of too many hydrogen bond acceptors (HBAs) on one face on the tricycle was leading to potential P-gp recognition and CNS penetration issues. Thus, we proceeded to evaluate 2-benzyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]pyrrolo-[3,4-*e*]pyridine-1-ones **24**, wherein one of the potentially P-gp offending nitrogen atoms was removed. However, this modification also afforded a potentially basic tricyclic core, and the impact of pK_a modulation on M_1 PAM potency, agonism and in vitro/in vivo DMPK profiles was uncertain.

Incorporation of the imidazo ring system in analogues **24** diminished M₁ PAM potency (Table 3), but a submicromolar PAM, **24c**, did result (Figure 6). We first profiled **24b**, and were pleased to find a favorable DMPK profile with fraction unbound (rat $f_u = 0.08$ and human $f_u = 0.10$), low predicted hepatic clearance (rat $CL_{hep} = 1.8$ mL/min/kg and human $CL_{hep} = 9.1$ mL/min/kg and desirable in vivo rat PK ($CL_p = 7.3$ mL/min/kg, $t_{1/2} = 2.5$ h, and $V_{ss} = 1.4$ L/kg). Importantly, **24b** showed moderate CNS penetration ($K_p = 0.32$, $K_{p,uu} = 0.14$), a >10-fold improvement over analogues **17** and **18**. However, **24c** (VU6005877) was significantly improved. PAM **24c** displayed only a moderate in vitro DMPK profile: fraction unbound in plasma (rat $f_u = 0.016$ and human $f_u = 0.01$) and moderate predicted hepatic clearance (rat $CL_{hep} = 31.7$ mL/min/kg and human $CL_{hep} = 13.6$ mL/min/kg). Here, we noted a lack of IVIVC with regards to clearance, as **24c** showed a good in vivo rat PK profile ($CL_p = 7.3$ mL/min/kg, $t_{1/2} = 6.7$ h, and $V_{ss} = 3.4$ L/kg), however, for the first time among the tricyclic series, exceptional CNS penetration (rat $K_p = 2.2$, $K_{p,uu} = 0.82$) was observed. PAM **24c** was also highly selective ($EC_{50} > 30$ μ M vs human M₂₋₅; see the Supporting Information), and displayed improved solubility versus **10** (SGF, pH 1.6: **10**, <5 μ g/mL; **24c**, 170 μ g/mL). **24c** was not characterized for selectivity in rat cell lines. Moreover, **24c** (VU6005877) was a PAM (unlike ago-PAM MK-7622 (**2**)), with no detectable agonism in our high-expressing cell line (up to 30 μ M), and did not induce seizures (e.g., cholinergic toxicity) in our standard phenotypic mouse model (100 mg/kg, IP, Racine scoring, data not shown). While potency in the imidazo series **24** could not be improved to provide a clinical candidate, **24c** represents a novel M₁ PAM chemotype with high CNS penetration and exceptional rat PK, a distinct profile versus literature M₁ ago-PAMs **1–4**.^{2–7}

CONCLUSION

Further chemical optimization of bicyclic lactam **10** generated several series of M₁ positive allosteric modulators (PAMs) based on novel tricyclic triazolo- and imidazopyridine lactam cores. Notably, these novel tricyclic M₁ PAMs were devoid of M₁ agonism, e.g., no M₁ ago-PAM activity in high expressing cell lines, and did not induce cholinergic-mediated seizures in mice. Moreover, we also discovered an unexpected, and the first example of, a “molecular switch” that modulated the mode of pharmacology from highly selective M₁ potentiation to highly selective M₁ antagonism. While all the new tricyclic congeners afforded excellent rat PK ($CL_p < 8$ mL/min/kg and $t_{1/2} > 5$ h), regioisomeric triazolopyridine analogues were uniformly not CNS penetrant ($K_p < 0.05$), despite a lack of hydrogen bond donors. However, removal of a single nitrogen atom to afford imidazopyridine derivatives, such as VU6005877 (**24c**), proved to retain the excellent rat PK and provide high CNS penetration ($K_p > 2$), despite inclusion of a basic nitrogen. Interestingly, all of the new M₁ PAMs across the diverse tricyclic heterocyclic cores possessed equivalent CNS MPO scores (>4.5), highlighting the value of both “medicinal chemist’s eye” and experimental data, e.g., not sole reliance (or decision bias) on in silico calculated properties, for parameters as complex as CNS penetration.

Supplementary Material

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ABBREVIATIONS

CNS	central nervous system
SAR	structure–activity relationship
mAChR	muscarinic acetylcholine receptor
M₁	muscarinic acetylcholine receptor subtype 1
K_p	brain/plasma partitioning coefficient
MPO	multiparameter optimization
DMPK	drug metabolism and pharmacokinetics
PK	pharmacokinetics
CL_{hep}	hepatic clearance
f_u	fraction unbound
IVIVC	in vitro/in vivo correlation
P-gp	P-glycoprotein
HBAs	hydrogen bond acceptors
ACh	acetylcholine

REFERENCES

- (1). Bradley SJ, Molloy C, Bundgaard C, Mogg AJ, Thompson KJ, Dwomoh L, Sanger HE, Crabtree MD, Brooke SM, Sexton PM, Felder CC, Christopoulos A, Broad LM, Tobin AB, and Langmead CJ (2018) Bitopic binding mode of an M₁ muscarinic acetylcholine receptor agonist associated with adverse clinical trial outcomes. *Mol. Pharmacol* 93, 645–656. [PubMed: 29695609]
- (2). Moran SP, Dickerson JW, Plumley HC, Xiang Z, Maksymetz J, Remke DH, Doyle CA, Niswender CM, Engers DW, Lindsley CW, Rook JM, and Conn PJ (2018) M₁ positive allosteric modulators lacking agonist activity provide the optimal profile for enhancing cognition. *Neuropsychopharmacology* 43, 1763–1771. [PubMed: 29581537]
- (3). Rook JM, Abe M, Cho HP, Nance KD, Luscombe VB, Adams JJ, Dickerson JW, Remke DH, Garcia-Barrantes PM, Engers DW, Engers JL, Chang S, Foster JJ, Blobaum AL, Niswender CM, Jones CK, Conn PJ, and Lindsley CW (2017) Diverse effects on M₁ signaling and adverse effect liability within a series of M₁ ago-PAMs. *ACS Chem. Neurosci* 8, 866–883. [PubMed: 28001356]
- (4). Rook JM, Berton JL, Cho HP, Garcia-Barrantes PM, Moran SP, Maksymetz JT, Nance KD, Dickerson JW, Remke DH, Chang S, Harp JM, Blobaum AL, Niswender CM, Jones CK, Stauffer

SR, Conn PJ, and Lindsley CW (2018) A novel M1 PAM VU0486846 exerts efficacy in cognition models without displaying agonist activity or cholinergic toxicity. *ACS Chem. Neurosci*, DOI: 10.1021/acscchemneuro.8b00131.

- (5). Moran SP, Cho HP, Maksymetz J, Remke D, Hanson R, Niswender CM, Lindsley CW, Rook JM, and Conn PJ (2018) PF-06827443 displays robust allosteric agonist and positive allosteric modulator activity in high receptor reserve and native systems. *ACS Chem. Neurosci*, DOI: 10.1021/acscchemneuro.8b00106.
- (6). Ghoshal A, Rook J, Dickerson J, Roop G, Morrison R, Jalan-Sakrikar N, Lamsal A, Noetzel M, Poslunsey M, Stauffer SR, Xiang Z, Daniels JS, Niswender CM, Jones CK, Lindsley CW, and Conn PJ (2016) Selective potentiation of M₁ muscarinic receptors reverses deficits in plasticity and negative and cognitive symptoms in repeated phencyclidine treated mouse model of schizophrenia. *Neuropsychopharmacology* 41, 598–610. [PubMed: 26108886]
- (7). Grannan MD, Mielnik CA, Moran SP, Gould RW, Ball J, Bubser M, Ramsey AJ, Abe M, Cho HP, Nance KD, Blobaum AL, Niswender CM, Conn PJ, Lindsley CW, and Jones CK (2016) Prefrontal cortex-mediated impairments in a genetic model of NMDA receptor hypofunction are reversed by the novel M₁ PAM VU6004256. *ACS Chem. Neurosci* 7, 1706–1716. [PubMed: 27617634]
- (8). Ma L, Seager M, Wittman M, Bickel N, Burno M, Jones K, Graufelds VK, Xu G, Pearson M, McCampbell A, Gaspar R, Shughrue P, Danzinger A, Regan C, Garson S, Doran S, Kreatsoulas C, Veng L, Lindsley CW, Shipe W, Kuduk S, Jacobson M, Sur C, Kinney G, Seabrook GR, and Ray WJ (2009) Selective activation of the M1 muscarinic acetylcholine receptor achieved by allosteric potentiation. *Proc. Natl. Acad. Sci. U. S. A* 106, 15950–15955. [PubMed: 19717450]
- (9). Shirey JK, Brady AE, Jones PJ, Davis AA, Bridges TM, Jadhav SB, Menon U, Christain EP, Doherty JJ, Quirk MC, Snyder DH, Levey AI, Watson ML, Nicolle MM, Lindsley CW, and Conn PJ (2009) A selective allosteric potentiator of the M₁ muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and can restore impairments of reversal learning. *J. Neurosci* 29, 14271–14286. [PubMed: 19906975]
- (10). Lange HS, Cannon CE, Drott JT, Kuduk SD, and Uslaner JM (2015) The M1 muscarinic positive allosteric modulator PQCA improves performance on translatable tests of memory and attention in rhesus monkeys. *J. Pharmacol. Exp. Ther* 355, 442–450. [PubMed: 26446308]
- (11). Beshore DC, Di Marco CN, Chang RK, Greshock TJ, Ma L, Wittman M, Seager MA, Koeplinger MT, Thompson CD, Furest J, Hartman GD, Bilodeau MT, Ray WJ, and Kuduk SD (2018) MK-7622: A first-in-class M₁ positive allosteric modulator development candidate. *ACS Med. Chem. Lett* 9, 652–656. [PubMed: 30034595]
- (12). Melancon BJ, Tarr JC, Panarese JD, Wood MR, and Lindsley CW (2013) Allosteric modulation of the M₁ muscarinic acetylcholine receptor: improving cognition and a potential treatment for schizophrenia and Alzheimer's disease. *Drug Discovery Today* 18, 1185–1199. [PubMed: 24051397]
- (13). Davoren JE, O'Neil SV, Anderson DP, Brodney MA, Chenard L, Dlugolenski K, Edgerton JR, Green M, Garnsey M, Grimwood S, Harris AR, Kauffman GW, LaChapelle E, Lazzaro JT, Lee C-W, Lotarski SM, Nason DM, Obach RS, Reinhart V, Salomon-Ferrer R, Steyn SJ, Webb D, Yan J, and Zhang L (2016) Design and optimization of selective azaindole amides M1 positive allosteric modulators. *Bioorg. Med. Chem. Lett* 26, 650–655. [PubMed: 26631313]
- (14). Davoren JE, Lee C-W, Garnsey M, Brodney MA, Cordes J, Dlugolenski K, Edgerton JR, Harris AR, Helal CJ, Jenkinson S, Kauffman GW, Kenakin TP, Lazzaro JT, Lotarski SM, Mao Y, Nason DM, Northcott C, Nottebaum L, O'Neil SV, Pettersen B, Popiolek M, Reinhart V, Salomon-Ferrer R, Steyn SJ, Webb D, Zhang L, and Grimwood S (2016) Discovery of the potent and selective M1 PAM-agonist N-[(3R,4S)-3-Hydroxytetrahydro-2H-pyran-4-yl]-5-methyl-4-[4-(1,3-thiazol-4-yl)-benzyl]pyridine-2-carboxamide (PF-06767832): evaluation of efficacy and cholinergic side effects. *J. Med. Chem* 59, 6313–6328. [PubMed: 27275946]
- (15). Engers JL, Childress ES, Long MF, Capstick RA, Luscombe VB, Cho HP, Dickerson JW, Rook JM, Blobaum AL, Niswender CM, Engers DW, Conn PJ, and Lindsley CW (2018) VU6007477, a novel M1 PAM based on a pyrrolo[2,3-b]pyridine carboxamide core devoid of cholinergic adverse events Submitted manuscript.

- (16). Bridges TM, Kennedy JP, Cho HP, Conn PJ, Lindsley CW, et al. (2010) Chemical optimization of an M₁, M₃, M₅ positive allosteric modulator (PAM) lead. Part I. Development of a highly selective M₅ PAM. *Bioorg. Med. Chem. Lett* 20, 558–562. [PubMed: 20004578]
- (17). Bridges TM, Kennedy JP, Cho HP, Conn PJ, and Lindsley CW (2010) Chemical optimization of an M₁, M₃, M₅ positive allosteric modulator (PAM) lead. Part II. Development of a highly selective M₁ PAM. *Bioorg. Med. Chem. Lett* 20, 1972–1975. [PubMed: 20156687]
- (18). Wager TT, Hou X, Verhoest PR, and Villalobos A (2016) Central nervous system multiparameter optimization desirability: application in drug discovery. *ACS Chem. Neurosci* 7, 767–775. [PubMed: 26991242]
- (19). Felts AS, Rodriguez AL, Morrison RD, Bollinger KA, Venable DF, Blobaum AL, Byers FW, Thompson Gray A, Daniels JS, Niswender CM, Jones CK, Conn PJ, Lindsley CW, and Emmitte KA (2017) Discovery of imidazo[1,2-a]-, [1,2,4]triazolo[4,3-a]-, and [1,3,4]triazolo[1,5-a]pyridine-8-carboxamide negative allosteric modulators of metabotropic glutamate receptor subtype 5. *Bioorg. Med. Chem. Lett* 27, 4858–4866. [PubMed: 28958625]
- (20). See the Supporting Information for full details.
- (21). Sharma S, Kedrowski J, Rook JM, Smith JM, Jones CK, Rodriguez AL, Conn PJ, and Lindsley CW (2009) Discovery of molecular switches that modulate modes of mGluR5 pharmacology in vitro and in vivo within a series of functionalized 5-(phenylethynyl)pyrimidines. *J. Med. Chem* 52, 4103–4106. [PubMed: 19537763]
- (22). Wood MR, Hopkins CR, Brogan JT, Conn PJ, and Lindsley CW (2011) ‘Molecular switches’ on allosteric ligands that modulate modes of pharmacology. *Biochemistry* 50, 2403–2410. [PubMed: 21341760]

Figure 1.
Structures, mode of M₁ pharmacology, and indication of presence or absence of cholinergic side effects, e.g., seizures, of reported M₁ ago-PAMs and M₁ PAMs.

Figure 2.

Structurally distinct M₁ PAM HTS hit **9** (a *pan*-M_{1,3,5} hit), optimized to deliver pure M₁ PAM tool compounds **10–12**.

Figure 3. Multidimensional chemical optimization plan of **10** to potentially afford “pure” M₁ PAMs with improved potency, rat PK, CNS penetration, and physiochemical properties.

Figure 4.

“Molecular switch” in the mode of pharmacology from **18a–h** to **18i**, affording a potent M₁ antagonist (rat M₁ pIC₅₀ = 6.81 ± 0.04, 4 ± 0, human M₁ pIC₅₀ = 6.45 ± 0.12, 3 ± 0) with high selectivity (>30 μM versus M_{2–5}).

23a

rat M_1 EC_{50} = 1.3 μ M, 98%
hum M_1 EC_{50} = 0.76 μ M, 83%

23b

rat M_1 EC_{50} = 1.1 μ M, 89%
hum M_1 EC_{50} = 2.8 μ M, 85%

Figure 5.
Representative M_1 PAMs within the 6,7-dihydro-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*a*]pyridin-8-one core **23**.

Figure 6.

Molecular pharmacology profile of an M_1 PAM (VU6005877, **24c**). Rat M_1 functional assay in high expressing M_1 cell line with the PAM VU6005877 (**24c**) showing the agonist activity (closed circle) and the PAM activity (open circle). In sharp contrast to M_1 ago-PAMs **1–4**, no agonist activity is noted up to 30 μ M.

Scheme 1.
Synthesis of Triazolopyridines 17 and 18^a

Scheme 2. Synthesis of Triazolo Analogues 23 and Imidazo Analogues 24^a

Structures and Activities of 7-Benzyl-1-methyl-6,7-dihydro-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[4,3-*a*]pyridine-8-ones 17

Table 1.

Cmpd	R ₁	R ₂	Het	rM ₁ EC ₅₀ (μM) ^a [pEC ₅₀ ±SEM]	rM ₁ ACh Max ±SEM	hM ₁ EC ₅₀ (μM) ^a [pEC ₅₀ ±SEM]	hM ₁ ACh Max ±SEM
17a	F	F		5.8 [5.24±0.08]	71±4	3.5 [5.45±0.15]	58±11
17b	F	F		>10 [>5]	66±4	>10 [>5]	49±1
17c	F	H		3.1 [5.51±0.13]	82±3	2.8 [5.56±0.18]	77±3
17d	F	F		2.3 [5.64±0.12]	81±2	3.8 [5.42±0.22]	77±2

^aCalcium mobilization assays with either rM₁-CHO cells or hM₁-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine for PAM, no exogenous acetylcholine (ACh) for the agonist EC₅₀; values represent means from three (*n* = 3) independent experiments performed in triplicate.

Structures and Activities of 7-Benzyl-6,7-dihydro-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[4,3-*d*]pyridine-8-ones 18

Cmpd	R ₁	R ₂	Het	rM ₁ EC ₅₀ (μM) ^a [pEC ₅₀ ±SEM]	rM ₁ ACh Max ±SEM	hM ₁ EC ₅₀ (μM) ^a [pEC ₅₀ ±SEM]	hM ₁ ACh Max ±SEM
18a	F	H		1.1 [5.94±0.12]	87±1	2.6 [5.59±0.11]	78±2
18b	F	F		0.95 [6.02±0.13]	88±2	2.6 [5.58±0.15]	80±2
18c	F	H		0.49 [6.31±0.14]	88±3	0.95 [6.02±0.07]	81±2
18d	F	F		0.32 [6.50±0.17]	88±4	0.85 [6.07±0.04]	80±5
18e	F	H		0.39 [6.41±0.15]	90±2	0.63 [6.20±0.05]	87±2
18f	F	F		0.30 [6.52±0.15]	88±3	0.60 [6.22±0.05]	86±1
18g	F	H		0.76 [6.12±0.08]	78±1	1.4 [5.87±0.03]	75±5
18h	F	F		0.62 [6.21±0.10]	78±2	1.1 [5.95±0.09]	77±2

^aCalcium mobilization assays with either rM₁-CHO cells or hM₁-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine for PAM, no exogenous ACh for the agonist EC₅₀; values represent means from three (*n* = 3) independent experiments performed in triplicate.

Structures and Activities of Fluorinated Benzyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]pyrrolo[3,4-*e*]pyridine-1-ones 24

^aCalcium mobilization assays with either rM₁-CHO cells or hM₁-CHO cells performed in the presence of an EC₂₀ fixed concentration of acetylcholine for PAM, no exogenous ACh for the agonist EC₅₀; values represent means from three (*n* = 3) independent experiments performed in triplicate.