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Targeting Muscarinic Acetylcholine Receptors for the Treatment of Psychiatric and Neurological Disorders

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Abstract

Muscarinic acetylcholine receptors (mAChR) play important roles in regulating complex behaviors such as cognition, movement, and reward, making them ideally situated as potential drug targets for the treatment of several brain disorders. Recent advances in the discovery of subtype-selective allosteric modulators for mAChRs has provided an unprecedented opportunity for highly specific modulation of signaling by individual mAChR subtypes in the brain. Recently, mAChR allosteric modulators have entered clinical development for Alzheimer's disease (AD) and schizophrenia, and have potential utility for other brain disorders. However, mAChR allosteric modulators can display a diverse array of pharmacological properties, and a more nuanced understanding of the mAChR will be necessary to best translate preclinical findings into successful clinical treatments.

Targeting Muscarinic Acetylcholine Receptors

Acetylcholine (ACh) plays a major role as a neurotransmitter and neuromodulator throughout the central nervous system (CNS) as well as in multiple peripheral systems [1,2]. In the CNS, cholinergic sources include local interneurons that are present in multiple brain regions, and also projections originating from the brainstem pedunculopontine and lateral dorsal tegmental nuclei as well as from the basal forebrain nuclei [1]. The latter provides long-range cholinergic projections and is the major source of ACh in the neocortex, hippocampus, and amygdala (Figure 1A, Key Figure), brain regions important in learning and memory.

ACh can signal through two distinct classes of receptors that include ligand-gated cation channels, termed nicotinic ACh receptors, and G protein-coupled muscarinic ACh receptors (mAChRs). Although both receptor classes play important roles in the central and peripheral nervous systems, in the CNS ACh acts primarily through mAChRs as a neuromodulator to shape ensembles of neurons and alter neuronal firing in response to changing environmental

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Resources

conditions [1,3], The five-member mAChR G protein-coupled receptor (GPCR) family consists of M_1, M_3, and M_5, which primarily couple to G_q to activate phospholipase C, and M_2 and M_4, which primarily couple to $G_{i/o}$ to inhibit adenylyl cyclase and modulate ion channels. Considerable evidence suggests that mAChRs are centrally involved in modulating complex behaviors such as cognition, motivation, and substance use disorder (SUD) [4,5], and their localization both pre- and postsynaptically throughout the CNS means that mAChRs are uniquely situated as potential targets for the treatment of multiple CNS disorders (Figure 1B–D).

Targeting mAChRs for the Treatment of AD and Schizophrenia

Cholinergic signaling is disrupted in AD, and several post-mortem studies have demonstrated a significant reduction in cholinergic projection neurons originating in the basal forebrain of patients with AD [6]. Current clinical strategies to combat the loss of cholinergic neurons and restore memory and cognition in AD include raising total cholinergic tone through systemic administration of acetylcholinesterase (AChE) inhibitors that block the breakdown of ACh [7]. Although AChE inhibitors such as tacrine and donepezil have demonstrated dose-dependent efficacy in improving cognition in patients with early-stage AD, they suffer from dose-limiting adverse effects attributed to generalized non-selective activation of cholinergic receptors in the CNS and periphery, thereby limiting their clinical utility [7]. Therefore, there is intense interest in developing more selective agents that activate specific receptor subtypes within the cholinergic system.

Robust preclinical and clinical evidence suggests that mAChRs are crucially involved in learning and memory [4], and significant investments have been made in developing ligands that engage mAChRs for the treatment of cognitive disruptions associated with AD, including the nonselective M_1/M_4 receptor-preferring agonist xanomeline. In a Phase III clinical study in patients with AD, xanomeline significantly reduced behavioral disturbances including vocal outbursts, suspiciousness, delusions, agitation, hallucinations, and had trending but not statistically significant improvements in cognition [8]. Although the clinical effects were promising, xanomeline has activity at all mAChR subtypes and induced severe dose-limiting gastrointestinal (GI) and other adverse effects that are mediated by activation of peripheral mAChRs [8,9]. Despite these peripheral effects, the promising reduction in behavioral disturbances and the trending effect on cognition prompted a small follow-up Phase II clinical trial in patients with schizophrenia [10]. Xanomeline produced significant improvements in the **brief psychiatric rating scale** (BPRS, see Glossary), **positive and negative syndrome scale** (PANSS), and the **clinical global impression** scale, compared to the placebo-controlled group [10]. Similar to the AD study, xanomeline produced GI disturbances, which halted further clinical development of xanomeline [9,10]. Follow-up preclinical studies suggest that activation of M_2 and M_3 in the periphery are responsible for the peripheral adverse effects of xanomeline [9]. Recently, Karuna pharmaceuticals renewed interest in xanomeline by advancing KarXT, a combination therapy of xanomeline with the peripherally restricted mAChR antagonist, trospium chloride [11], into a Phase II clinical trial for schizophrenia (Clinical Trial Number[i]:). Although this combination therapy may

[i] https://clinicaltrials.gov/

reduce the adverse effects of xanomeline in the periphery, and thereby increase the therapeutic window of xanomeline, a more targeted approach selectively activating specific mAChRs may provide the greatest clinical benefit.

Significant investment has been made to develop selective agonists devoid of M_2 and M_3 activation, including development of the M_1 agonist HTL0018318 by Sosei Heptares, who recently partnered with Allergan to sponsor a Phase I clinical trial for AD (Clinical Trial Number[i]:) and Phase II trial in patients with Lewy body dementia in Japan (JapicCTI[ii]: 183989) (Table 1). However, an unexpected HTL0018318 chronic dosing toxicology finding in nonhuman primates placed the clinical trial in Lewy body dementia patients on hold[iii] (Table 1). Unfortunately, despite major investments in medicinal chemistry to develop highly selective M_1 or M_4 orthosteric agonists, these efforts have largely failed owing to the highly conserved **orthosteric site** for ACh binding among the mAChRs.

Allosteric Modulators of mAChRs

To develop highly selective small-molecule ligands for specific mAChR subtypes, several groups have pursued the development of allosteric modulators that target less well conserved **allosteric sites** that are distinct from the orthosteric ACh binding site [12,13]. Significant progress has been made in understanding the structural basis of allosterism given the determination of the crystal structure for multiple mAChR subtypes [14–18], and the crystallization of several state-dependent receptor conformations [19]. Collectively, the insights into the exact nature of orthosteric and allosteric ligand interactions provided by these crystal structures, paired with state-of-the-art in silico docking of digital compound libraries, provide the exciting potential to screen large numbers of compounds in very little time and at low cost, thereby identifying new chemical scaffolds and novel selective ligands through rational drug design [20,21].

Positive allosteric modulators (PAMs) increase responses to orthosteric agonists, whereas negative allosteric modulators (NAMs) inhibit responses to orthosteric agonists [13]. PAMs and NAMs exert their effects by modulating the affinity of an orthosteric ligand to the receptor or by modulating coupling to intracellular signaling [13]. Follow-up functional studies, including work on receptor-knockout animals, have demonstrated that the procognitive and antipsychotic-like effects of xanomeline are likely mediated by M_1 and M_4 receptors, respectively [9,22,23]. Thus, multiple drug discovery efforts have focused on developing allosteric modulators for these two mAChR subtypes.

Potential Cognition-Enhancing Effects of M_1 PAMs

The M_1 mAChR is the most abundant of the five mAChR subtypes expressed in brain regions crucially involved in cognition, such as the prefrontal cortex (PFC) and hippocampus [24,25]. Pharmacological blockade [26–28] or genetic deletion [29] of M_1 produces disturbances in learning and memory. Based on these studies, and extensive clinical studies implicating central mAChRs in cognitive processing [30–32], selective potentiation of M_1

[ii] https://rctportal.niph.go.jp/
[iii] www.fiercebiotech.com/biotech/allergan-sosei-halt-alzheimer-s-trials-amid-safety-scare

signaling in the CNS using highly selective M_1 PAMs may hold promise to enhance cognition and reverse learning and memory disturbances.

Over the past decade, multiple studies have shown that M_1 PAMs have robust efficacy in reversing cognitive disruptions in preclinical animal models relevant for AD [33–35] and schizophrenia [36–41]. M_1 PAMs can potentiate a form of **synaptic plasticity** termed long-term depression (LTD) [37,42], enhance neuronal excitability [43], and reverse synaptic plasticity deficits in the PFC. In addition, several studies have demonstrated a role of the M_1 mAChRs in hippocampal function since M_1 PAMs can specifically potentiate LTD at the hippocampus–prefrontal cortex (PFC) synapse [44], and activation of M_1 in the hippocampus can induce long-term potentiation (LTP) [45,46] as well as facilitate spatial reversal learning, an important hippocampus-dependent task [47].

Overactivation of the M_1 mAChR May Be Detrimental to M_1 PAM Efficacy

Although these findings are very promising, recent studies have revealed that some but not all M_1 PAMs have adverse effects, including GI distress and behavioral convulsions in rodents and dogs [24,42,48]. It was previously demonstrated that the nonselective mAChR orthosteric agonist pilocarpine induced robust seizures in healthy adult mice and mice in which M_2, M_3, M_4, or M_5 receptors were genetically knocked out (KO), but produced no effect in M_1-KO mice, suggesting that overactivation of the M_1 receptor mediates these adverse effects [49,50]. Therefore, one possibility to account for the stark contrast between M_1 PAMs that produce adverse effects and those that do not is the hypothesis that some M_1 PAMs overactivate the M_1 receptor and therefore lead to similar adverse effects as traditional orthosteric agonists [42,48,51,52]. This is reminiscent of studies from allosteric modulators for other GPCRs, such as the metabotropic glutamate receptor subtype 5 ($mGlu_5$), which demonstrated that the allosteric agonist activity of $mGlu_5$ PAMs can cause severe behavioral convulsions in rodents [53].

In agreement with this hypothesis, M_1 PAMs such PF-06764427 and MK-7622 (Table 1) demonstrate robust agonist activity in addition to PAM activity (**ago-PAM**) and induce M_1-dependent behavioral convulsions in rodents [42,48] that were absent in M_1-KO mice. This contrasts with two structurally distinct M_1 PAMs, VU0453595 and VU0550164, that were optimized to eliminate agonist activity [42]. Similarly to previously described M_1 PAMs, VU0453595 and VU0550164 potentiate M_1 responses to ACh [42]. However, in contrast to PF-06764427 and MK-7622, VU0453595 and VU0550164 lack agonist activity in all assays tested [42]. Furthermore, the severe adverse effects observed with the M_1 ago-PAMs were not observed at any dose of VU0453595, an M_1 PAM optimized to avoid allosteric agonist activity [42]. Finally, VU0453595 (but not MK-7622) has robust efficacy in improving object recognition memory in rats [42]. These studies suggest that the ability of MK-7622 to activate M_1 mAChRs regardless of presynaptic ACh release may lead to aberrant receptor activity and may even disrupt cognition. These properties could therefore explain why MK-7622 did not meet clinical endpoints in a proof-of-concept clinical trial in AD patients [54]. Together, these studies have provided fundamental new insights into the impact of subtle differences in the modes of activity of different M_1 PAMs and the need to strictly avoid allosteric agonist activity in these compounds.

Interestingly, a newer M_1 PAM, PF-06827443, was reported to have minimal agonist activity in cell lines but still produced robust adverse effects in preclinical animal model studies [24]. However, allosteric agonist activity can vary dramatically depending on total receptor expression, and is much more evident in systems that contain high **receptor reserve**. A follow-up study demonstrated that PF-06827443 has robust agonist activity in moderate- and high-expressing cell lines, as well as in native brain tissue electrophysiological assays [52]. Thus, PF-06827443 is also an ago-PAM, and allosteric agonist activity likely contributes to the adverse effect liability of this compound.

M_1 PAMs That Display Bias Can Have Differential Effects in the CNS

In addition to differences in allosteric agonist activity, M_1 PAMs can also differ in their ability to confer bias to M_1 signaling. Signal bias is the phenomenon by which different GPCR ligands induce distinct active receptor-complex states that are biased toward or away from specific signaling pathways (Figure 2A) [55]. To date, GPCR signal bias has been well characterized for μ opioid receptor agonists that can signal through G proteins, β-arrestin, or both [56]. Recent work suggests that μ opioid receptor agonists that avoid β-arrestin activity and preferentially signal through G proteins can induce analgesia while minimizing respiratory suppression. Therefore, these biased ligands could provide a larger therapeutic window than fentanyl, which preferentially signals through β-arrestin and produces robust respiratory depression [56,57]. Thus, characterization of potential signal bias in muscarinic ligands may provide opportunities to understand specific signaling pathways involved in efficacy, and potentially increase *in vivo* efficacy while minimizing adverse effect liability.

Characterization of a broad range of structurally diverse M_1 PAMs revealed that some M_1 PAMs confer signal bias and potentiate receptor signaling through the canonical phospholipase C (PLC) pathway, but do not potentiate M_1 receptor-mediated activation of phospholipase D (PLD) [58]. Little was known about the role of PLD in M_1 signaling in the CNS or whether PLD is necessary for any M_1-dependent signaling. Using brain slice electrophysiology, follow-up studies demonstrated that not all M_1-dependent responses in the CNS are PLD-dependent, and biased M_1 PAMs function similarly to nonbiased M_1 PAMs in M_1 signaling that was PLD-independent [59]. However, M_1 PAMs that do not couple to PLD function dramatically differently from nonbiased M_1 PAMs in their ability to potentiate PLD-dependent M_1-mediated plasticity in the PFC [59]. These findings demonstrate that PLD plays a crucial role in the ability of M_1 PAMs to modulate particular CNS functions, and that biased M_1 PAMs function differently in synaptic plasticity in the cortex that is implicated in cognition. However, PLC and PLD are only two of many different signaling pathways downstream of the M_1 mAChR, and future studies are necessary to identify M_1 PAMs with favorable *in vivo* properties that may display signal bias for other signaling pathways, including ERK and β-arrestin, to fully dissect the downstream signaling pathways important for efficacy and adverse effect liability. Furthermore, additional studies are necessary to determine whether biased M_1 mAChR ligands have a lower propensity for inducing seizures and could therefore provide a larger therapeutic window, as is the case with biased $mGlu_5$ PAMs that avoid activation of NMDA receptors [60].

In conclusion, the high-profile failure of several experimental therapeutic approaches targeting the reduction of Aβ in patients with AD warrants the identification and development of novel therapeutic targets for the treatment of the cognitive disruptions in AD. Furthermore, current antipsychotics do not improve and may even worsen the cognitive deficits associated with schizophrenia [61]. The ability of M_1 PAMs to improve cognition in multiple animal models [27,36,37,40–42] suggests strong potential for success in the clinic and may help to mitigate the crucial issue common to animal models – that they often fail to recapitulate the full range of disease symptoms and etiology. However, with the recent Phase II failure of MK-7622 to significantly improve cognitive endpoints in AD patients [54], there is an urgent need to fully characterize M_1 PAMs with respect to agonist activity, signal bias, and other pharmacological properties (Box 1) so as to de-risk clinical candidates and move the M_1 PAM with the highest chance of success forward into the clinic.

Selective M_4 PAMs for the Treatment of Schizophrenia

The M_4 mAChR is abundantly expressed in the dorsal striatum, nucleus accumbens, and the nigros-triatal and mesolimbic dopaminergic pathways [25,62], circuitry that has been implicated in the positive symptoms of schizophrenia that include hallucinations, delusions, and disorganized thought [63,64], as well as motivational deficits that contribute to the negative symptoms observed in schizophrenia patients. Given this localization and previous studies suggesting that mAChRs can reduce striatal dopamine signaling [65–68], it was hypothesized that activation of M_4 mAChRs could reduce the hyperactivity of striatal dopaminergic pathways and exert antipsychotic-like efficacy. Consistent with this hypothesis, the antipsychotic-like effects of the $M_{1/4}$-preferring agonist xanomeline were absent in M_4-KO mice [23] as well as in mice in which M_4 was specifically deleted from D_1 dopamine receptor-expressing neurons [22]. Therefore, several research groups have aggressively pursued the development of highly selective, CNS-penetrant, M_4 PAMs for the treatment of the psychotic symptoms of schizophrenia as well as of other brain disorders. Excitingly, several M_4 PAMs including LY2033298 [69], VU0152099, and VU0152100 [70] have demonstrated robust antipsychotic-like efficacy in amphetamine- and apomorphine-induced models of psychosis including conditioned avoidance, hyperlocomotion, and disrupted prepulse inhibition (PPI). Moreover, M_4 PAMs have displayed efficacy in other preclinical models relevant to neuropsychiatric and neurological disorders (Box 2).

Of note, allosteric compounds targeting the M_4 mAChRs exhibit species-specific pharmacology that must be taken into account when designing *in vitro* and *in vivo* assays [69,71,72]. LY2033298 was initially identified in a screen utilizing cell lines expressing human M_4 receptors, and was later demonstrated to be significantly less potent at potentiating ACh responses in cell lines expressing rat M_4 compared to human M_4 [69]. Preclinical assessment of M_4 PAM efficacy was further confounded because LY2033298 displays **probe-dependence** [71]. In these studies, LY2033298 failed to demonstrate a significant behavioral effect when dosed alone, but displayed antipsychotic-like effects when co-dosed with an ineffective dose of the synthetic mAChR agonist oxotremorine [69,71]. Overall, these pharmacodynamic challenges are not limited to LY2033298 and have been reported by other research groups [73,74], thereby producing challenges in determining the mechanism of action of these ligands as well as in developing clinical compounds. However,

the findings from these early M_4 mAChR studies demonstrate that detailed characterization of M_4 mAChR pharmacology using cultured cells can be used to both predict and rationalize the subsequent design of *in vivo* studies to identify the mechanism by which M_4 PAMs exert their antipsychotic efficacy.

To understand the biological mechanisms of M_4 PAM antipsychotic efficacy in preclinical models, multiple rodent M_4 PAMs have been developed, including VU0152100 [70,75] and VU0467154 [72,73]. Consistent with the proposed mechanism that the M_4 mAChR is ideally localized to reduce hyperactivity of striatal dopamine signaling, VU0152100 attenuated amphetamine-induced activation of the dorsal striatum and nucleus accumbens as assessed by functional magnetic resonance imaging (fMRI) [75]. VU0152100 also attenuated amphetamine-induced striatal dopamine release [75], suggesting that M_4 activation may have direct effects on dopaminergic signaling despite the lack of M_4 expression on dopaminergic terminals in the striatum [76]. Further investigation into the biological mechanism of M_4 PAM antipsychotic efficacy revealed a previously undescribed signaling pathway by which M_4 activation of dopamine D_1 receptor-expressing spiny projection neurons (D_1-SPNs) in the dorsal striatum leads to the mobilization of endocannabinoids, which in turn activate cannabinoid CB_2 receptors on dopaminergic terminals to locally reduce dopamine release [67]. In support of the importance of this mechanism for the *in vivo* antipsychotic efficacy of M_4 PAMs, the ability of VU0467154 to reverse amphetamine-induced disruptions in PPI was lost in D_1-SPN M_4-KO mice and was blocked by the CB_2 receptor antagonist AM630 [67]. These novel findings were intriguing because it is classically thought that G_q activation, not $G_{i/o}$, leads to the production of endocannabinoids. Subsequent studies revealed that coactivation of the metabotropic glutamate receptor subtype 1 ($mGlu_1$) is required for both the M_4 PAM-mediated reductions in dopamine release and the *in vivo* antipsychotic efficacy of M_4 PAMs [68]. This finding has identified $mGlu_1$ PAMs as novel potential antipsychotic treatments [68] and highlights the importance of fully characterizing the detailed mechanism of action of M_4 PAMs *in vivo* because we could discover other druggable targets that act through similar pathways.

Finally, in addition to actions on dopamine release, it was recently demonstrated that M_4 receptors at D_1-SPN terminals in the substantia nigra pars reticulata (SNr) functionally antagonize D_1 receptor-mediated increases in direct pathway transmission [66]. This is another mechanism by which M_4 can counteract excessive dopamine signaling through D_1 receptors that may be relevant to M_4 antipsychotic-like efficacy. Together, the effects of M_4 PAMs on dopamine release and dopamine D_1 receptor signaling, and the fact that M_4 PAMs lack any observable peripheral cholinergic effects seen with xanomeline [72], highlight the potential clinical advantages of M_4 PAMs at reversing a hyperdo-paminergic state via a local, striatum-specific mechanism over the broad antagonism of dopamine receptors by current antipsychotic medications.

M_4 PAMs Improve Cognition in Preclinical Studies

Although current antipsychotics can interfere with dopaminergic regulation of cognitive function in the hippocampus and PFC [77], M_4 PAMs such as VU0467154 can improve cognitive function in multiple preclinical rodent models relevant to schizophrenia in addition

to their well-established antipsychotic-like efficacy [72,78]. The cognition-enhancing and antipsychotic effects of VU0467154 persisted during chronic dosing, suggesting that it may be possible to clinically treat the pervasive cognitive deficits of schizophrenia patients while also preventing the relapse or induction of a psychotic episode with a single, chronically dosed M_4 PAM. Therefore, the potential of M_4 PAMs to treat multiple symptom clusters in schizophrenia patients may provide a substantial advantage over current antipsychotic medications that fail to effectively treat the cognitive disruptions of the disease.

In parallel to investigations of the mechanisms underlying the preclinical efficacy of M_4 PAMs, efforts have been made to identify translatable biomarkers that could predict clinical efficacy. The use of quantitative electroencephalography (qEEG) provides a powerful approach that can discriminate between different behavioral states including arousal, sedation, and alertness [79]. Furthermore, dysfunctional qEEG measures have been correlated with psychotic symptoms and cognitive disruptions in schizophrenia [79]. In rodents, the M_4 PAM VU0467154 increased arousal, as measured by qEEG during periods of wake, but importantly did not promote sedation, whereas the atypical antipsychotic clozapine increased arousal but exhibited sedative-like effects [80]. In addition, VU0467154 attenuated the elevation of gamma power induced by MK-801 [80], an electrophysiological correlate associated with positive symptoms and acute psychosis [79]. Overall, this study demonstrated that an M_4 PAM may improve sleep, a considerable advantage over current antipsychotics, and that M_4 target engagement can produce changes in qEEG signals that could be predictive of clinical efficacy. Therefore, qEEG may provide a quantifiable readout of target engagement in the clinic in the absence of a M_4 mAChR specific radioligand.

Ultimately, the development of an M_4 PAM clinical candidate relies on the optimization of a compound with activity at the human M_4 receptor while also ideally retaining activity at multiple preclinical species (i.e., rat, dog, primate) to facilitate preclinical development and **investigational new drug (IND)-enabling studies**. Although the aforementioned species-specific pharmacology has complicated development, recent efforts have produced M_4 PAMs with activity at M_4 in multiple species including human and nonhuman primates [74,81]. The M_4 PAM VU0476406 was recently developed that has similar potency at rat, human, dog, and cynomolgus M_4 mAChR and favorable pharmacokinetic properties across species [81]. Unfortunately, suboptimal predicted human bioavailability and aqueous solubility prevented VU0476406 from being advanced into the clinic but allowed VU0476406 to become a useful tool compound for cross-species preclinical studies. Subsequently, it was shown that, similar to the effects of VU0467154 in mice, VU0476406 significantly reduced L-DOPA-induced dyskinesia in non-human primates [82], an effect related to excessive dopaminergic signaling in striatal circuitry [83]. Merck also recently disclosed the development of an M_4 PAM with comparable potencies at rat and human M_4 that was efficacious in reversing amphetamine-induced hyperlocomotion [74]. Finally, Sosei in collaboration with Allergan initiated a Phase I clinical trial of the purported human M agonist HTL0016878 (Clinical Trial Number[i]: , Table 1); however, no preclinical pharmacology or efficacy data have so far been disclosed. Interestingly, the Merck M_4 PAM exhibits moderate agonism at human M_4 [74], and HTL0016878 has been publicly described as an M_4 agonist. Altogether, these recent breakthroughs in developing compounds with

favorable human pharmacodynamic properties are promising for the potential of M_4 PAMs to treat multiple symptom domains of patients suffering from schizophrenia.

Concluding Remarks and Future Perspectives

A wealth of preclinical literature over the past decade suggest that allosteric modulators of several mAChRs hold great promise for the treatment of multiple devastating CNS disorders, including AD, schizophrenia, and SUD (Box 3), which have limited to no effective treatments. Recent advances in medicinal chemistry efforts to develop highly selective mAChR ligands have provided fundamental new insights into muscarinic receptor biology as well as key information for drug discovery efforts. As a consequence of these efforts, several allosteric modulators for M_1, M_4, and M_5 mAChRs have already entered clinical trials or are quickly advancing toward the clinic (Table 1).

Although much progress has been made in developing allosteric modulators of the various mAChR subtypes for the potential treatment of several CNS disorders, there are still many outstanding questions that the muscarinic field is primed to address (see Outstanding Questions). This is best illustrated by the crucial need to understand which pharmacological properties are important for allosteric modulator efficacy and which, if any, are responsible for adverse effects. Overall, a better understanding of how these distinct pharmacological properties (e.g., bias, total brain exposure, partial agonism [84], differences in binding sites [85] etc.) drive efficacy and/or adverse effect liability could potentially explain how distinct mAChR ligands display differences *in vivo*.

Recent characterization of biased allosteric ligands for the M_1 mAChR have provided useful insight into the mechanism of action of these allosteric modulators. To date, however, we have only identified a limited number of biased ligands, and more focused drug discovery efforts will be necessary to identify biased ligands for other distinct signaling pathways as well as for the other mAChR subtypes. Therefore, dedicated medicinal chemistry paired with pathway-specific but still high-throughput pharmacological assays will be necessary to identify a wider range of biased ligands for all the mAChR subtypes. Information gleaned from these studies could greatly advance our collective knowledge of mAChR biology as well as help to inform drug discovery programs. Even modest investments into pharmacological characterization of the signaling pathways and pharmacological properties involved in allosteric modulator action *in vivo* could pay huge dividends for drug discovery efforts. Through better understanding of the pharmacological properties that are important for efficacy and adverse effects, we as a field can ultimately advance mAChR allosteric modulators forward into the clinic with the highest chance of success.

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Disclaimer Statement

P.J.C. is an inventor on multiple patents protecting allosteric modulators for several classes of mAChRs. P.J.C. receives research support from Lundbeck, Boehringer Ingelheim, and Ancora Innovation.

Glossary

Ago-PAM or PAM-agonists
these positive allosteric modulators (PAMs) can activate the receptor in the absence of the orthosteric agonist in addition to increasing the potency and/or efficacy of orthosteric agonists when present.

Allosteric site
a binding site on a receptor that is topographically and structurally distinct from the orthosteric ligand binding site.

Brief psychiatric rating scale (BPRS)
a rating scale which a clinician or researcher can use to measure psychiatric symptoms such as depression, anxiety, hallucinations, and unusual behavior.

Clinical global impression
a brief clinician-administered scale that measures illness severity, global improvement or change, and therapeutic response in the patient.

Investigational new drug (IND)-enabling studies
IND status is a key FDA milestone before clinical testing on humans. Studies include repeat-dose toxicology in rodents, nonclinical safety studies, and others to facilitate FDA submission before clinical testing.

Orthosteric site
the binding site for the endogenous ligand on a receptor.

Positive and negative syndrome scale (PANSS)
a medical scale for measuring symptom severity of patients with schizophrenia.

Probe-dependence
the phenomenon whereby the ability of an allosteric ligand to enhance or reduce a response differs depending on the orthosteric ligand.

Receptor reserve
the concept that a full pharmacological response can be induced at ligand concentrations that do not saturate the total receptor population.

Synaptic plasticity
the biological process by which specific patterns of synaptic activity result in changes in synaptic strength; is thought to contribute to learning and memory.

Therapeutic index
a quantitative measurement of the relative safety of a drug. It is a comparison of the amount of a therapeutic agent that causes the desired therapeutic effect to the amount that causes toxicity or undesired effects.

References

1. Picciotto MR et al. (2012) Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron 76, 116–129 [PubMed: 23040810]
2. Soreq H (2015) Checks and balances on cholinergic signaling in brain and body function. Trends Neurosci. 38, 448–458 [PubMed: 26100140]
3. Sarter M et al. (2014) Deterministic functions of cortical acetylcholine. Eur. J. Neurosci 39, 1912–1920 [PubMed: 24593677]
4. Hasselmo ME and Sarter M (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. Neuropsychopharmacology 36, 52–73 [PubMed: 20668433]
5. Wess J et al. (2007) Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat. Rev. Drug Discov. 6, 721–733 [PubMed: 17762886]
6. Whitehouse PJ et al. (1981) Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol 10, 122–126 [PubMed: 7283399]
7. Birks J (2006) Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst. Rev, CD005593. [PubMed: 16437532]
8. Bodick NC et al. (1997) Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. Arch. Neurol 54, 465–473 [PubMed: 9109749]
9. Bender AM et al. (2017) Classics in chemical neuroscience: xanomeline. ACS Chem. Neurosci 8, 435–443 [PubMed: 28141924]
10. Shekhar A et al. (2008) Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. Am. J. Psychiatry 165, 1033–1039 [PubMed: 18593778]
11. Callegari E et al. (2011) A comprehensive non-clinical evaluation of the CNS penetration potential of antimuscarinic agents for the treatment of overactive bladder. Br. J. Clin. Pharmacol 72, 235–246 [PubMed: 21392072]
12. Conn PJ et al. (2009) Subtype-selective allosteric modulators of muscarinic receptors for the treatment of CNS disorders. Trends Pharmacol. Sci 30, 148–155 [PubMed: 19201489]
13. Conn PJ et al. (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat. Rev. Drug Discov. 8, 41–54 [PubMed: 19116626]
14. Thal DM et al. (2016) Crystal structures of the M1 and M4 muscarinic acetylcholine receptors. Nature 531, 335–340 [PubMed: 26958838]
15. Haga K et al. (2012) Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. Nature 482, 547–551 [PubMed: 22278061]
16. Kruse AC et al. (2012) Structure and dynamics of the M3 muscarinic acetylcholine receptor. Nature 482, 552–556 [PubMed: 22358844]
17. Liu H et al. (2018) Structure-guided development of selective M3 muscarinic acetylcholine receptor antagonists. Proc. Natl. Acad. Sci. U. S. A 115, 12046–12050 [PubMed: 30404914]
18. Vuckovic Z et al. (2019) Crystal structure of the M5 muscarinic acetylcholine receptor. bioRxiv Published online August 14, 2019 10.1101/730622
19. Miao Y et al. (2013) Activation and dynamic network of the M2 muscarinic receptor. Proc. Natl. Acad. Sci. U. S. A 110, 10982–10987 [PubMed: 23781107]
20. Burger WAC et al. (2018) Toward an understanding of the structural basis of allostery in muscarinic acetylcholine receptors. J. Gen. Physiol 150, 1360–1372 [PubMed: 30190312]
21. Macalino SJY et al. (2015) Role of computer-aided drug design in modern drug discovery. Arch. Pharm. Res 38, 1686–1701 [PubMed: 26208641]
22. Dencker D et al. (2011) Involvement of a subpopulation of neuronal M4 muscarinic acetylcholine receptors in the antipsychotic-like effects of the M1/M4 preferring muscarinic receptor agonist xanomeline. J. Neurosci 31, 5905–5908 [PubMed: 21508215]
23. Woolley ML et al. (2009) Attenuation of amphetamine-induced activity by the non-selective muscarinic receptor agonist, xanomeline, is absent in muscarinic M4 receptor knockout mice and attenuated in muscarinic M1 receptor knockout mice. Eur. J. Pharmacol 603, 147–149 [PubMed: 19111716]

24. Davoren JE et al. (2017) Design and synthesis of γ- and δ-lactam M1 positive allosteric modulators (PAMs): convulsion and cholinergic toxicity of an M1-selective PAM with weak agonist activity. J. Med. Chem 60, 6649–6663 [PubMed: 28598634]
25. Lein ES et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445, 168–176 [PubMed: 17151600]
26. Miyauchi M et al. (2017) Muscarinic receptor signaling contributes to atypical antipsychotic drug reversal of the phencyclidine-induced deficit in novel object recognition in rats. J. Psychopharmacol. (Oxford) 31, 1588–1604 [PubMed: 28946779]
27. Gould RW et al. (2015) Role for the M1 muscarinic acetylcholine receptor in top-down cognitive processing using a touchscreen visual discrimination task in mice. ACS Chem. Neurosci 6, 1683–1695 [PubMed: 26176846]
28. Sheffler DJ et al. (2009) A novel selective muscarinic acetylcholine receptor subtype 1 antagonist reduces seizures without impairing hippocampus-dependent learning. Mol. Pharmacol 76, 356–368 [PubMed: 19407080]
29. Anagnostaras SG et al. (2003) Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat. Neurosci 6, 51–58 [PubMed: 12483218]
30. Lebois EP et al. (2018) Muscarinic receptor subtype distribution in the central nervous system and relevance to aging and Alzheimer's disease. Neuropharmacology 136, 362–373 [PubMed: 29138080]
31. Foster DJ and Conn JP (2017) Allosteric modulation of GPCRs: new insights and potential utility for treatment of schizophrenia and other CNS disorders. Neuron 94, 431–446 [PubMed: 28472649]
32. Bubser M et al. (2012) Muscarinic receptor pharmacology and circuitry for the modulation of cognition. Handb. Exp. Pharmacol 208, 121–166
33. Ma L et al. (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]
34. Lebois EP et al. (2017) Disease-modifying effects of M1 muscarinic acetylcholine receptor activation in an Alzheimer's disease mouse model. ACS Chem. Neurosci 8, 1177–1187 [PubMed: 28230352]
35. Bradley SJ et al. (2017) M1 muscarinic allosteric modulators slow prion neurodegeneration and restore memory loss. J. Clin. Invest 127, 487–499 [PubMed: 27991860]
36. Grannan MD et al. (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]
37. Ghoshal A et al. (2016) Potentiation of M1 muscarinic receptor reverses plasticity deficits and negative and cognitive symptoms in a schizophrenia mouse model. Neuropsychopharmacology 41, 598–610 [PubMed: 26108886]
38. Kurimoto E et al. (2019) TAK-071, a muscarinic M1 receptor positive allosteric modulator, attenuates scopolamine-induced quantitative electroencephalogram power spectral changes in cynomolgus monkeys. PLoS One 14, e0207969 [PubMed: 30856192]
39. Vardigan JD et al. (2015) Improved cognition without adverse effects: novel M1 muscarinic potentiator compares favorably to donepezil and xanomeline in rhesus monkey. Psychopharmacology 232, 1859–1866 [PubMed: 25491927]
40. Uslaner JM et al. (2013) The muscarinic M1 receptor positive allosteric modulator PQCA improves cognitive measures in rat, cynomolgus macaque, and rhesus macaque. Psychopharmacology 225, 21–30 [PubMed: 22825578]
41. Lange HS et al. (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]
42. Moran SP et al. (2018) M1-positive allosteric modulators lacking agonist activity provide the optimal profile for enhancing cognition. Neuropsychopharmacology 43, 1763–1771 [PubMed: 29581537]

43. Shirey JK et al. (2009) A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. J. Neurosci 29, 14271–14286 [PubMed: 19906975]
44. Maksymetz J et al. (2019) M1 muscarinic receptors modulate fear-related inputs to the prefrontal cortex: implications for novel treatments of posttraumatic stress disorder. Biol. Psychiatry 85, 989–1000 [PubMed: 31003787]
45. Dennis SH et al. (2016) Activation of muscarinic M1 acetylcholine receptors induces long-term potentiation in the hippocampus. Cereb. Cortex 26, 414–426 [PubMed: 26472558]
46. Cui Y et al. (2008) Enhancement of memory function in aged mice by a novel derivative of xanomeline. Cell Res. 18, 1151–1153 [PubMed: 18936783]
47. Xiong C-H et al. (2019) M1 muscarinic receptors facilitate hippocampus-dependent cognitive flexibility via modulating GluA2 subunit of AMPA receptors. Neuropharmacology 146, 242–251 [PubMed: 30529302]
48. Rook JM et al. (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]
49. Hamilton SE et al. (1997) Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. Proc. Natl. Acad. Sci. U. S. A 94, 13311–13316 [PubMed: 9371842]
50. Bymaster FP et al. (2003) Role of specific muscarinic receptor subtypes in cholinergic parasympathomimetic responses, in vivo phosphoinositide hydrolysis, and pilocarpine-induced seizure activity. Eur. J. Neurosci 17, 1403–1410 [PubMed: 12713643]
51. Rook JM et al. (2018) A novel M1 PAM VU0486846 exerts efficacy in cognition models without displaying agonist activity or cholinergic toxicity. ACS Chem. Neurosci 9, 2274–2285 [PubMed: 29701957]
52. Moran SP et al. (2018) PF-06827443 displays robust allosteric agonist and positive allosteric modulator activity in high receptor reserve and native systems. ACS Chem. Neurosci 9, 2218–2224 [PubMed: 29683646]
53. Rook JM et al. (2013) Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor subtype 5 determine differences in in vivo activity. Biol. Psychiatry 73, 501–509 [PubMed: 23140665]
54. Voss T et al. (2018) Randomized, controlled, proof-of-concept trial of MK-7622 in Alzheimer's disease. Alzheimers Dement. 4, 173–181
55. Wootten D et al. (2018) Mechanisms of signalling and biased agonism in G protein-coupled receptors. Nat. Rev. Mol. Cell Biol. 19, 638–653 [PubMed: 30104700]
56. Conibear AE and Kelly E (2019) A biased view of μ-opioid receptors? Mol. Pharmacol 96, 542–549 [PubMed: 31175184]
57. Schmid CL et al. (2017) Bias factor and therapeutic window correlate to predict safer opioid analgesics. Cell 171, 1165–1175 [PubMed: 29149605]
58. Marlo JE et al. (2009) Discovery and characterization of novel allosteric potentiators of M1 muscarinic receptors reveals multiple modes of activity. Mol. Pharmacol 75, 577–588 [PubMed: 19047481]
59. Moran SP et al. (2019) Biased M1 positive allosteric modulators reveal novel role of phospholipase D in M1-dependent rodent cortical plasticity. bioRxiv Published online October 16, 2019 10.1101/806943
60. Rook JM et al. (2015) Biased mGlu5-positive allosteric modulators provide in vivo efficacy without potentiating mGlu5 modulation of NMDAR currents. Neuron 86, 1029–1040 [PubMed: 25937172]
61. Elie D et al. (2010) Cognitive effects of antipsychotic dosage and polypharmacy: a study with the BACS in patients with schizophrenia and schizoaffective disorder. J. Psychopharmacol. (Oxford) 24, 1037–1044 [PubMed: 19164494]
62. Hersch SM et al. (1994) Distribution of m1–m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. J. Neurosci 14, 3351–3363 [PubMed: 8182478]

63. Howes OD and Kapur S (2009) The dopamine hypothesis of schizophrenia: version III–the final common pathway. Schizophr. Bull 35, 549–562 [PubMed: 19325164]
64. Kesby JP et al. (2018) Dopamine, psychosis and schizophrenia: the widening gap between basic and clinical neuroscience. Transl. Psychiatry 8, 30 [PubMed: 29382821]
65. Pancani T et al. (2014) M4 mAChR-mediated modulation of glutamatergic transmission at corticostriatal synapses. ACS Chem. Neurosci 5, 318–324 [PubMed: 24528004]
66. Moehle MS et al. (2017) Cholinergic projections to the substantia nigra pars reticulata inhibit dopamine modulation of basal ganglia through the M4 muscarinic receptor. Neuron 96, 1358–1372.e4 [PubMed: 29268098]
67. Foster DJ et al. (2016) Antipsychotic-like effects of M4 positive allosteric modulators are mediated by CB2 receptor-dependent inhibition of dopamine release. Neuron 91, 1244–1252 [PubMed: 27618677]
68. Yohn SE et al. (2018) Activation of the mGlu1 metabotropic glutamate receptor has antipsychotic-like effects and is required for efficacy of M4 muscarinic receptor allosteric modulators. Mol. Psychiatry Published online August 16, 2018 10.1038/s41380-018-0206-2
69. Chan WY et al. (2008) Allosteric modulation of the muscarinic M4 receptor as an approach to treating schizophrenia. Proc. Natl. Acad. Sci. U. S. A 105, 10978–10983 [PubMed: 18678919]
70. Brady AE et al. (2008) Centrally active allosteric potentiators of the M4 muscarinic acetylcholine receptor reverse amphetamine-induced hyperlocomotor activity in rats. J. Pharmacol. Exp. Ther 327, 941–953 [PubMed: 18772318]
71. Suratman S et al. (2011) Impact of species variability and ‘probe-dependence’ on the detection and in vivo validation of allosteric modulation at the M4 muscarinic acetylcholine receptor. Br. J. Pharmacol 162, 1659–1670 [PubMed: 21198541]
72. Bubser M et al. (2014) Selective activation of M4 muscarinic acetylcholine receptors reverses MK-801-induced behavioral impairments and enhances associative learning in rodents. ACS Chem. Neurosci 5, 920–942 [PubMed: 25137629]
73. Wood MR et al. (2017) Challenges in the development of an M4 PAM in vivo tool compound: the discovery of VU0467154 and unexpected DMPK profiles of close analogs. Bioorg. Med. Chem. Lett 27, 171–175 [PubMed: 27939174]
74. Schubert JW et al. (2019) Discovery, optimization, and biological characterization of 2,3,6-trisubstituted pyridine-containing M4 positive allosteric modulators. ChemMedChem 14, 943–951 [PubMed: 30920765]
75. Byun NE et al. (2014) Antipsychotic drug-like effects of the selective M4 muscarinic acetylcholine receptor positive allosteric modulator VU0152100. Neuropsychopharmacology 39, 1578–1593 [PubMed: 24442096]
76. Weiner DM et al. (1990) Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proc. Natl. Acad. Sci. U. S. A 87, 7050–7054 [PubMed: 2402490]
77. Ott T and Nieder A (2019) Dopamine and cognitive control in prefrontal cortex. Trends Cogn. Sci 23, 213–234 [PubMed: 30711326]
78. Gould RW et al. (2018) Cognitive enhancement and antipsychotic-like activity following repeated dosing with the selective M4 PAM VU0467154. Neuropharmacology 128, 492–502 [PubMed: 28729220]
79. Uhlhaas PJ and Singer W (2015) Oscillations and neuronal dynamics in schizophrenia: the search for basic symptoms and translational opportunities. Biol. Psychiatry 77, 1001–1009 [PubMed: 25676489]
80. Gould RW et al. (2016) State-dependent alterations in sleep/wake architecture elicited by the M4 PAM VU0467154 – relation to antipsychotic-like drug effects. Neuropharmacology 102, 244–253 [PubMed: 26617071]
81. Melancon BJ et al. (2017) Optimization of M4 positive allosteric modulators (PAMs): the discovery of VU0476406, a non-human primate in vivo tool compound for translational pharmacology. Bioorg. Med. Chem. Lett 27, 2296–2301 [PubMed: 28442253]
82. Shen W et al. (2015) M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. Neuron 88, 762–773 [PubMed: 26590347]

83. Jenner P (2008) Molecular mechanisms of L-DOPA-induced dyskinesia. Nat. Rev. Neurosci 9, 665–677 [PubMed: 18714325]
84. Shannon HE et al. (1994) Xanomeline: a novel muscarinic receptor agonist with functional selectivity for M1 receptors. J. Pharmacol. Exp. Ther 269, 271–281 [PubMed: 7909557]
85. Bradley SJ et al. (2018) Bitopic binding mode of an M1 muscarinic acetylcholine receptor agonist associated with adverse clinical trial outcomes. Mol. Pharmacol 93, 645–656 [PubMed: 29695609]
86. Kenakin T (2017) Theoretical aspects of GPCR–ligand complex pharmacology. Chem. Rev 117, 4–20 [PubMed: 26856272]
87. Sako Y et al. (2019) TAK-071, a novel M1 positive allosteric modulator with low cooperativity, improves cognitive function in rodents with few cholinergic side effects. Neuropsychopharmacology 44, 950–960 [PubMed: 30089885]
88. Mandai T et al. (2019) In vivo pharmacological comparison of TAK-071, a positive allosteric modulator of muscarinic M1 receptor, and xanomeline, an agonist of muscarinic M1/M4 receptor, in rodents. Neuroscience 414, 60–76 [PubMed: 31299348]
89. Kurimoto E et al. (2018) An approach to discovering novel muscarinic M1 receptor positive allosteric modulators with potent cognitive improvement and minimized gastrointestinal dysfunction. J. Pharmacol. Exp. Ther 364, 28–37 [PubMed: 29025977]
90. Coyle JT et al. (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219, 1184–1190 [PubMed: 6338589]
91. Holschneider DP et al. (2019) Positive allosteric modulation of cholinergic receptors improves spatial learning after cortical contusion injury in mice. J. Neurotrauma 36, 2233–2245 [PubMed: 30688147]
92. Stoll K et al. (2018) Effects of muscarinic M1 and M4 acetylcholine receptor stimulation on extinction and reinstatement of cocaine seeking in male mice, independent of extinction learning. Psychopharmacology 235, 815–827 [PubMed: 29250738]
93. Pancani T et al. (2015) Allosteric activation of M4 muscarinic receptors improve behavioral and physiological alterations in early symptomatic YAC128 mice. Proc. Natl. Acad. Sci. U. S. A 112, 14078–14083 [PubMed: 26508634]
94. Thomsen M et al. (2012) Contribution of both M1 and M4 receptors to muscarinic agonist-mediated attenuation of the cocaine discriminative stimulus in mice. Psychopharmacology 220, 673–685 [PubMed: 21964721]
95. Dencker D et al. (2012) An allosteric enhancer of M4 muscarinic acetylcholine receptor function inhibits behavioral and neurochemical effects of cocaine. Psychopharmacology 224, 277–287 [PubMed: 22648127]
96. Dall C et al. (2017) Muscarinic receptor M4 positive allosteric modulators attenuate central effects of cocaine. Drug Alcohol Depend. 176, 154–161 [PubMed: 28544993]
97. Thomson SR et al. (2017) Cell-type-specific translation profiling reveals a novel strategy for treating fragile X syndrome. Neuron 95, 550–563 [PubMed: 28772121]
98. Gogliotti RG et al. (2018) Total RNA sequencing of rett syndrome autopsy samples identifies the M4 muscarinic receptor as a novel therapeutic target. J. Pharmacol. Exp. Ther 365, 291–300 [PubMed: 29523700]
99. Moehle MS and Conn PJ (2019) Roles of the M4 acetylcholine receptor in the basal ganglia and the treatment of movement disorders. Mov. Disord 34, 1089–1099 [PubMed: 31211471]
100. Bowman S et al. (2013) Reducing the health consequences of opioid addiction in primary care. Am. J. Med 126, 565–571 [PubMed: 23664112]
101. Joffe ME et al. (2014) Biological substrates of addiction. Wiley Interdiscip Rev Cogn Sci 5, 151–171 [PubMed: 24999377]
102. Yasuda RP et al. (1993) Development of antisera selective for m4 and m5 muscarinic cholinergic receptors: distribution of m4 and m5 receptors in rat brain. Mol. Pharmacol 43, 149–157 [PubMed: 8429821]
103. Wei J et al. (1994) m1–m5 muscarinic receptor distribution in rat CNS by RT-PCR and HPLC. J. Neurochem 63, 815–821 [PubMed: 7519660]

104. Basile AS et al. (2002) Deletion of the M5 muscarinic acetylcholine receptor attenuates morphine reinforcement and withdrawal but not morphine analgesia. Proc. Natl. Acad. Sci. U. S. A 99, 11452–11457 [PubMed: 12154229]

105. Thomsen M et al. (2005) Reduced cocaine self-administration in muscarinic M5 acetylcholine receptor-deficient mice. J. Neurosci 25, 8141–8149 [PubMed: 16148222]

106. Gentry PR et al. (2013) Discovery of the first M5-selective and CNS penetrant negative allosteric modulator (NAM) of a muscarinic acetylcholine receptor: (S)-9b-(4-chlorophenyl)-1-(3,4-difluorobenzoyl)-2,3-dihydro-1H-imidazo[2,1-a] isoindol-5(9bH)-one (ML375). J. Med. Chem 56, 9351–9355 [PubMed: 24164599]

107. Berizzi AE et al. (2018) Muscarinic M5 receptors modulate ethanol seeking in rats. Neuropsychopharmacology 43, 1510–1517 [PubMed: 29483658]

108. Gunter BW et al. (2018) Selective inhibition of M5 muscarinic acetylcholine receptors attenuates cocaine self-administration in rats. Addict Biol. 23, 1106–1116 [PubMed: 29044937]

109. Gould RW et al. (2019) Acute negative allosteric modulation of M5 muscarinic acetylcholine receptors inhibits oxycodone self-administration and cue-induced reactivity with no effect on antinociception. ACS Chem. Neurosci 10, 3740–3750 [PubMed: 31268669]

110. Shin JH et al. (2015) Muscarinic regulation of dopamine and glutamate transmission in the nucleus accumbens. Proc. Natl. Acad. Sci. U. S. A 112, 8124–8129 [PubMed: 26080439]

111. Foster DJ et al. (2014) M5 receptor activation produces opposing physiological outcomes in dopamine neurons depending on the receptor's location. J. Neurosci 34, 3253–3262 [PubMed: 24573284]

Box 1.

M_1 PAMs with Low a-Values May Minimize Adverse Effects

According to the operational allosteric ternary complex model [13,86], allosteric modulators can exert their effects by modulating the binding affinity of the orthosteric agonist (e.g., binding of ACh to the M_1 mAChR) or by modulating receptor efficacy (e.g., the ACh response), termed α and b respectively (Figure IA). When α >1, the allosteric modulator increases the affinity of the orthosteric agonist, whereas α <1 demonstrates a decrease in receptor–agonist affinity. Conversely, when β >1, the allosteric modulator potentiates cellular activation, whereas when β <1 the modulator inhibits cellular activation (Figure IB). Importantly, α and β values are independent of each other and in theory can occur in every combination [86]. Several recent papers from Takeda Pharmaceutical Company suggest that M_1 PAMs that possess low α values, such as TAK-071 (Table 1), have a wider therapeutic index in rodent models relevant for schizophrenia compared to M_1 PAMs with high α values [38,87,88]. However, TAK-071 was not completely devoid of adverse effects, and demonstrated a concentration-dependent increase in spontaneous ileum motility [87]. Nonetheless, TAK-071 provides a much greater margin between doses leading to cognition-enhancing effects and those with adverse effects (e.g., diarrhea) compared to T-662, an M_1 PAM with a high a value [38,87,89]. Furthermore, it is not known whether M_1 PAMs with higher α and β values may be more beneficial in later stages of AD where there is greater loss of endogenous ACh [6,90]. Overall, although these results are promising, more extensive studies will be necessary to understand the exact relationship between α value, agonist activity, and signal bias to fully characterize the pharmacological profiles of M_1 PAMs. Lastly, it is prudent to carefully consider the appropriate α and β values for a M_1 PAM clinical candidate depending on the stage of AD (e.g., early or late) chosen for clinical intervention.

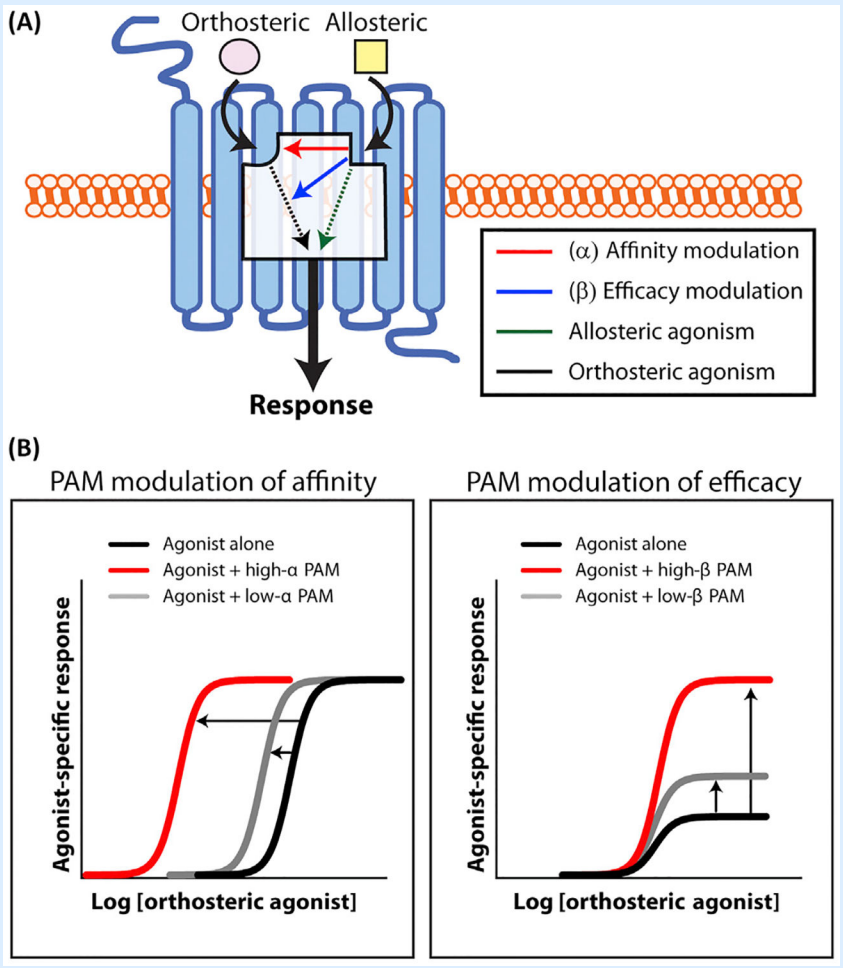

Figure I. Allosteric Modulator Modes of Action.

(A) Allosteric modulators (yellow squares) bind to a topographically and structurally distinct site on the muscarinic receptor to modulate orthosteric agonist (ACh, pink) affinity (red) and/or efficacy (blue). Binding of positive allosteric agonists (PAMs) or ago-PAMs can also directly induce receptor signaling in the absence of the orthosteric agonist (green). (B) (Left) Allosteric modulators that robustly modulate agonist affinity (high α value, red) will result in a large leftward shift in the orthosteric agonist concentration–response curve. By contrast, allosteric modulators that weakly enhance agonist affinity (low α value, grey) result in a modest leftward shift in the orthosteric agonist concentration–response curve. (Right) Allosteric modulators that strongly modulate agonist efficacy (high β value, red) may result in a large increase in the orthosteric agonist maximal response. By contrast, allosteric modulators that weakly enhance agonist efficacy (low β value, grey) result in a modest increase in the orthosteric agonist response. Sigmoidal curves were generated using Graphpad Prism8 (www.graphpad.com).

Box 2.

Potential Utility of M_1 and M_4 Allosteric Modulators for the Treatment of Other Schizophrenia Symptom Domains and Neurological Disorders

In addition to potential efficacy in reversing cognitive deficits in AD and schizophrenia patients, recent studies suggest that M_1 PAMs also improve social interactions in rodent models [37]. Previously, xanomeline demonstrated efficacy in reducing negative symptoms in schizophrenia patients [9,10], and it will therefore be important to fully evaluate the potential efficacy of M_1 PAMs in animal models that are relevant for negative symptoms. To this same end, the wide variety of M_1-and M_4-selective tool compounds developed over the past decade have identified other psychiatric and neurological disorders in which subtype-selective muscarinic modulation may be effective. Consistent with procognitive efficacy, the M_1 PAM BQCA improved learning and memory deficits in a rodent model of traumatic brain injury [91]. An M_1 PAM was also able to enhance the consolidation and recall of fear extinction in a rodent model of post-traumatic stress disorder (PTSD) [44], suggesting that M_1 PAMs could improve the efficacy of exposure therapy in the clinic for the treatment of PTSD and other anxiety disorders. In addition, M_1 activation in combination with an M_4 PAM accelerated the extinction of cocaine-seeking behavior [92], implying that potentiating M_1 activation may broadly facilitate and/or enhance extinction learning across multiple behavioral paradigms.

Given their direct actions on dopamine release, M_4 PAMs may provide benefit in other disorders that display exaggerated dopaminergic signaling. The M_4 PAMs VU0467154 and VU0476406 reduced L-DOPA-induced dyskinesia in mice and nonhuman primate models, respectively [82]. VU0467154 also alleviated synaptic and motor deficits in the YAC128 model of Huntington's disease [93], in part through effects on dopaminergic signaling but also via regulation of corticostriatal transmission [65]. Furthermore, M_1 and M_4 mAChRs may be viable targets for SUD because M_1 activation reduced cocaine discrimination [94], and M_4 PAMs effectively reduced cocaine self-administration and striatal dopamine release [95], facilitated extinction, and prevented reinstatement of cocaine-induced conditioned place preference [96].

Recent work suggests that M_4 PAMs could be useful in the treatment of neurodevelopmental disorders such as fragile X and Rett syndrome. The M_4 PAM VU0152100 normalized excessive protein synthesis and audiogenic seizures in *Fmr1*$^{-/y}$ mice, suggesting therapeutic potential in fragile X patients [97]. Further support for M_4 PAMs as potential therapeutics for neurodevelopmental disorders is demonstrated by a recent RNA sequencing study that identified a reduction in total *CHRM4* transcript levels (the mRNA encoding the M_4 mAChR), in human Rett syndrome autopsy samples compared to controls, and found that an M_4 PAM could rescue social and cognitive deficits in a mouse model of Rett syndrome [98]. The exact mechanisms underlying the procognitive effects of M_4 PAMs and their efficacy in these neurodevelopmental models remain unknown, and future studies are necessary to fully understand these mechanisms.

Conversely, reducing M_4 receptor signaling may be effective in disorders where dopamine is reduced, such as Parkinson's disease (PD) [99]. Anticholinergic and pan-mAChR antagonists were some of the first clinically used treatments for PD [99] but, similarly to non-selective muscarinic agonists, they suffer from a lack of tolerability [99]. Important to PD treatment, we found that the M_4 receptor may tonically inhibit D_1-SPN transmission to the substantia nigra pars reticulata (SNr), an effect counteracted by D_1 receptor signaling [66]. Therefore in states of reduced dopaminergic tone, such as in PD where the positive regulation of D_1-SPN to SNr transmission via D_1 is reduced or lost, M_4 antagonism might alleviate cholinergic-mediated inhibition at this synapse, restore direct pathway function, and thereby restore normal motor function [66,99].

Box 3.

Potential Utility of M_5 NAMs for the Treatment of Substance Use Disorder

Beyond dementia and schizophrenia, mAChRs are exciting targets for other CNS disorders such as SUD, a mental illness that afflicts >20 million people in the USA[iv]. SUD is a chronic, relapsing disorder characterized by compulsive drug-seeking behavior, continued use despite harmful consequences, and long-lasting changes in the brain[v]. Many current therapies for addiction (e.g., methadone) are inadequate because of their abuse liability or their inability to treat multiple distinct classes of addictive substances (e.g., naloxone or naloxone combination therapies) [100]. Many drugs of abuse alter mesolimbic dopamine reward circuitry, and targets that are exclusively found or highly enriched in this reward circuitry could therefore potentially provide treatments for multiple SUDs and thus have broad clinical efficacy [101].

The M_5 mAChR is uniquely situated as a promising target for SUD because it is the only muscarinic receptor expressed on dopaminergic neurons of the substantia nigra pars compacta and ventral tegmental area [102,103]. Furthermore, the rewarding effects of drugs of abuse are diminished in M_5-KO mice, including reduced cocaine self-administration, decreased cocaine- and morphine-induced place preference, and less severe cocaine and morphine withdrawal symptoms [104,105]. These M_5-KO studies provide compelling evidence that reducing M_5 receptor function could be a potential treatment for SUD. Importantly for human translation studies, the analgesic effects of morphine were completely unaltered in M_5-KO mice [104]. Therefore, reduction of M_5 receptor function through highly selective M_5 NAMs provides great promise as a potential treatment for SUD in human patients.

Unfortunately, despite major investments in medicinal chemistry, little progress had been made towards the generation of a highly selective and brain-penetrant M_5 NAM until the recent discovery of ML375, the first highly selective, potent, and brain-penetrant M_5 NAM [106]. In preclinical rodent models relevant to SUD, ML375 was found to dramatically reduce self-administration of alcohol [107], cocaine [108], and opiates [109]. Furthermore, microinjections of ML375 into the dorsal lateral striatum decreased ethanol self-administration [107], suggesting that M_5 mAChR expression on dopamine terminals may be important for M_5 NAM efficacy *in vivo* [110,111].

Importantly, ML375 did not alter motor function nor did it reduce the natural rewarding effects of food [107,109]. Collectively, these studies suggest that M_5 NAMs are poised to be a very promising treatment for SUD while avoiding unwanted effects on natural reward circuitry. Although the ability of M_5 NAMs to reduce addiction-like behavior across multiple substances of abuse is promising, ML375 displays an unfavorably long half-life (80 h), and considerable medicinal chemistry work will therefore be necessary to generate a clinical candidate with more favorable ‘drug-like’ properties.

[iv] www.drugabuse.gov/related-topics/trends-statistics
[v] www.psychiatry.org/psychiatrists/practice/dsm

Highlights

mAChR allosteric modulators demonstrate unparalleled subtype selectivity, can possess a wide array of distinct pharmacological properties, and are rapidly advancing into the clinic for the treatment of multiple central nervous system disorders.

M_1 mAChR positive allosteric modulators (PAMs) may enhance cognition and reverse memory deficits in AD and schizophrenia, and may display a larger therapeutic window than acetylcholinesterase inhibitors.

M_4 PAMs can reduce dopamine release and demonstrate antipsychotic-like effects in preclinical animal models.

Recent preclinical literature suggests that M_5 negative allosteric modulators may effectively treat an array of substance use disorders without reducing the effects of natural rewards.

Outstanding Questions

Can biased ligands for other M_1 signaling pathways (e.g., β-arrestin) and other mAChRs be developed?

Will M_1 or M_4 allosteric modulators have therapeutic potential for the negative symptoms (e.g., the disruptions in motivation) in patients with schizophrenia?

How do mAChRs function within key brain circuits that are important for complex behaviors such as learning, memory, motivation, and addiction?

How do allosteric modulators exert their effects at the brain-circuit level?

Key Figure

Muscarinic Receptor Distribution and Molecular Mechanisms Relevant to the Treatment of Neurological Disorders

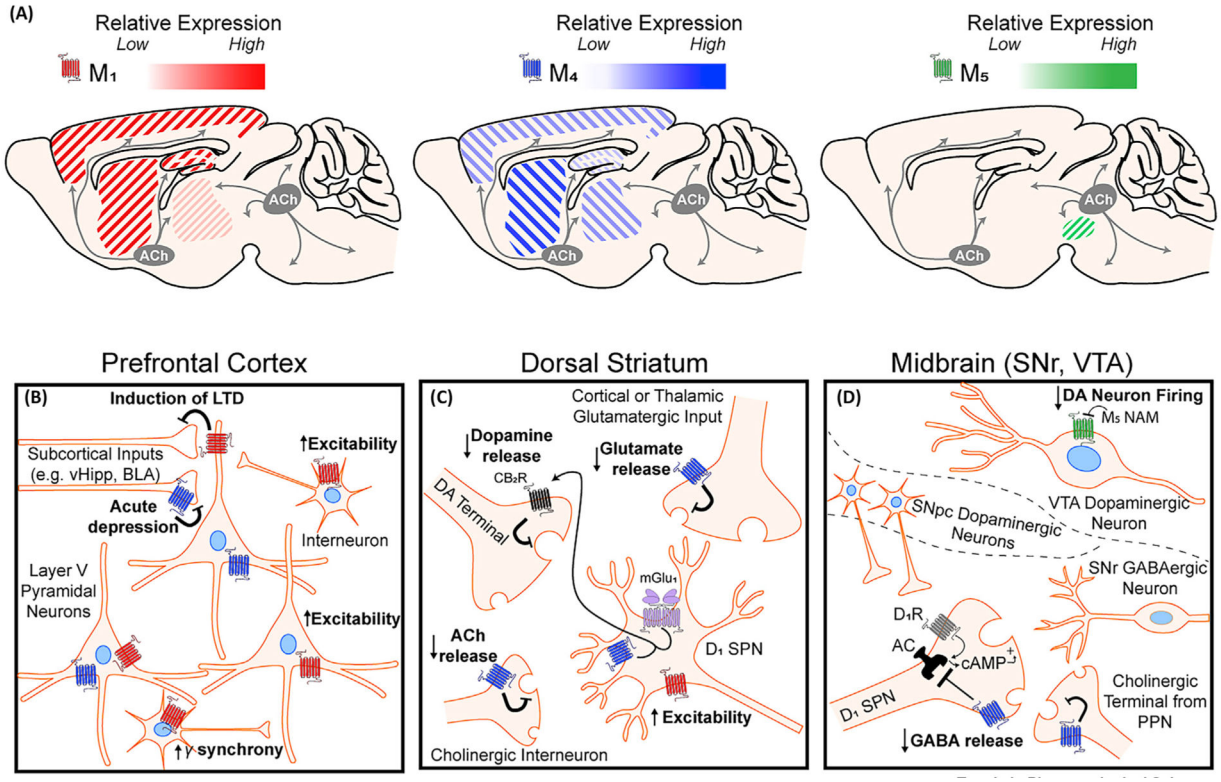

Figure 1.

(A) Distribution of M_1, M_4, and M_5 muscarinic acetylcholine (ACh) receptors in brain regions implicated in neurological dysfunction. The relative expression of each receptor subtype is indicated by its respective color gradient. M_2 and M_3 (not shown) muscarinic ACh receptors (mAChRs) are also expressed widely throughout the brain. M_1 mAChRs are highly expressed in the cortex, hippocampus, and dorsal and ventral striatum, and are expressed at low levels in thalamic areas. M_4 mAChRs are highly expressed in striatal regions, moderately expressed across the cortex and thalamus, and are poorly expressed in the hippocampus. M_5 mAChR expression is restricted to the midbrain. Cholinergic projection neurons produce and release ACh from two distinct clusters – the basal forebrain nuclei (grey circle, left) which innervates cortical, hippocampal, and thalamic areas, and the brain stem nuclei (grey circle, right) which innervates midbrain, hindbrain, thalamic, and cerebellar areas. Cholinergic tone in the dorsal and ventral striatum is primarily provided by large cholinergic interneurons (not depicted). (B) In the prefrontal cortex, M_1 mAChR activation induces a form of long-term depression (LTD) of glutamatergic inputs from subcortical areas including the ventral hippocampus (vHipp) and basolateral amygdala (BLA). M_1 mAChR activation also increases the excitability of pyramidal neurons and GABAergic interneurons. Activation of M_1 via interneurons can also increase gamma oscillation synchrony in the cortex. M_4 mAChRs can acutely inhibit neurotransmitter

release. (C) In the dorsal striatum, M_4 mAChRs expressed on direct pathway D_1 receptor-positive spiny projection neurons (SPNs) interact with metabotropic glutamate receptor 1 ($mGlu_1$) to produce endocannabinoids which then bind to cannabinoid type 2 (CB_2) receptors to inhibit local dopamine release. In addition, M_4 activation can reduce both ACh release from local cholinergic interneurons and act as a heteroreceptor on glutamatergic terminals from the cortex and thalamus to reduce glutamate release. M_1 mAChRs expressed on D_1-SPNs increase the excitability of these neurons. (D) In the midbrain, cholinergic modulation of dopaminergic (DA) neurons in the ventral tegmental area (VTA) and direct pathway input into the substantia nigra reticulata (SNr) are relevant to neurological disorders. In the VTA (top), M_5 mAChRs are expressed on VTA DA neurons and M_5 negative allosteric modulators (NAMs) are hypothesized to reduce DA neuron firing. In the SNr (bottom), M_4 mAChR activation on direct pathway D_1-SPN terminals directly opposes increased GABA release mediated through D_1-receptor activation by DA released from the substantia nigra pars compacta (SNpc, middle). M_4 can also act as an autoreceptor and reduce ACh release from cholinergic projection terminals.

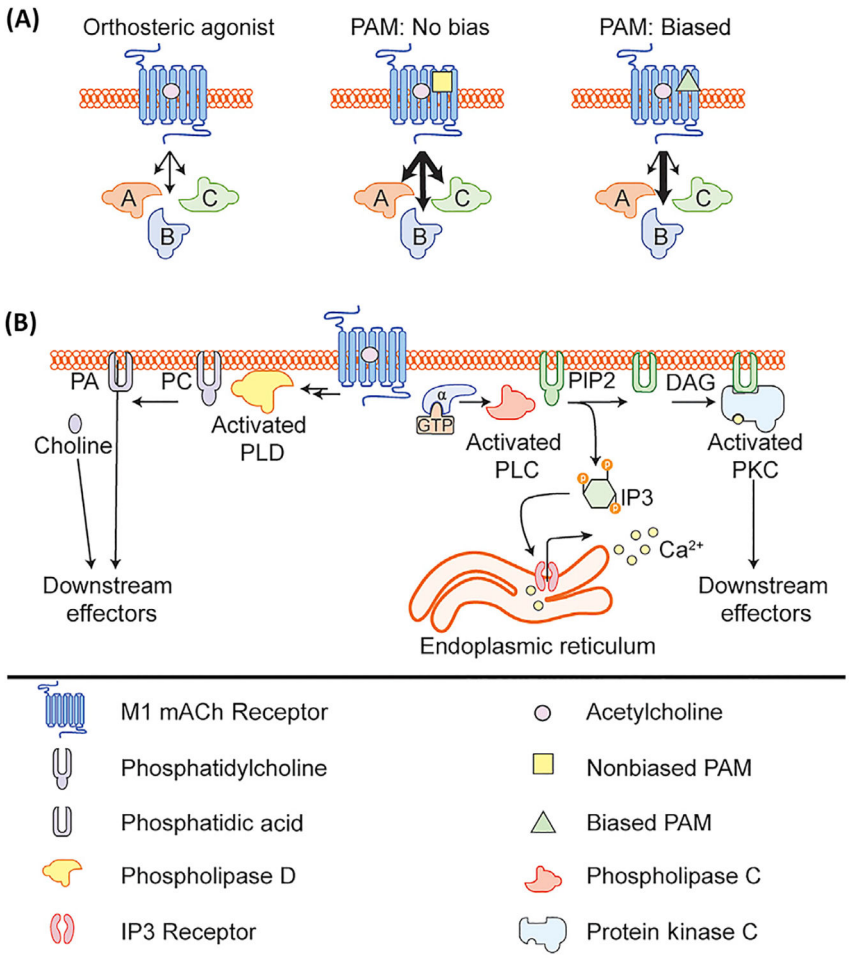

Figure 2. M_1 Muscarinic Acetylcholine Receptor (mAChR) Allosteric Modulator-Induced Signal Bias.

(A) Schematic depicting the effects of acetylcholine (ACh) alone, ACh plus a non-biased M_1 positive allosteric modulator (PAM), or ACh plus a biased M_1 PAM on various downstream signaling cascades. (B) Activation of the M_1 mAChR can lead to the activation of several downstream signaling pathways including canonical activation of G_{aq} signaling leading to activation of phospholipase C, release of calcium, and activation of PKC. M_1 mAChR activation can also lead to activation of phospholipase D, through an unknown mechanism. Abbreviations: DAG, diacylglycerol; IP3, inositol trisphosphate; PA, phosphatidic acid; PC, phosphatidylcholine; PIP2, phosphatidylinositol bisphosphate.

Table 1.

Recent Compounds Targeting the mAChR in Clinical Development[a]

mAChR	Compound	Ligand type	Therapeutic area	Refs	Preclinical or clinical stage	Clinical trial identifier
M_1	VU319	PAM	AD	-	Phase I; ongoing	(USA)
	MK-7622	PAM	AD	[56]	Phase II; discontinued	(USA)
	TAK-071	PAM	Mild cognitive impairment/AD	[39,88]	Phase I; concluded	(USA)
	HTL0018318	Agonist	AD	-	Phase Ib; completed	(USA)
			Lewy body dementia	-	Phase II (Japan); withdrawn	JapicCTI-183989 (Japan), secondary ID: (USA)
	NS	PAM	Schizophrenia	[37,38]	Preclinical	-
M_1/M_4	KarXT (xanomeline plus trospium chloride)	Nonselective M_1/M_4 preferring agonist plus peripherally restricted muscarinic antagonist	Schizophrenia	-	Phase II; ongoing	(USA)
M_4	HTL0016878	Agonist	AD	-	Phase I; ongoing	(USA)
	NS	PAM	Schizophrenia	[73,76,79]	Preclinical	-
	NS	NAM/antagonist	Parkinson's disease [99]	[98]	Preclinical	-
M_5	NS	NAM	Substance use disorder	[109,110]	Preclinical	-

[a]Abbreviations: AD, Alzheimer's disease, mAChR, muscarinic acetylcholine receptor; NAM, negative allosteric modulator; NS, not specified; PAM, positive allosteric modulator.