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Developments in Treatments Targeting Muscarinic Receptors for Alzheimer's Disease

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Abstract

Alzheimer's Disease (AD) is the most common neurodegenerative disease to date and affects over 50 million patients worldwide, thus making it a major area of focus in drug discovery. Muscarinic receptors, especially the M1 and M4 subtypes, are promising therapeutic targets due to their selectivity, high expression, and function in the central nervous system. Although the past research in AD has shifted away from muscarinic receptors due to difficulties involving receptor selectivity, adverse effects, and failure in clinical trials, new discoveries in the functions of these receptors have opened up the possibility for the development of treatments with better efficacy and less risk, resulting in a resurgence of interest. This review aims to first examine previous research and data that have helped recognize the functions of muscarinic receptors in AD, specifically where they are expressed in the brain, their intracellular mechanisms, and how these can help us find pathways to treat AD. This paper then analyzes the status of developments in different classes of treatments based on performance in trials, efficacy data, pathways targeted, and adverse effects. Finally, this review identifies potential areas for further discovery and development of pharmaceuticals by highlighting the flaws of previous treatments, unknown aspects of AD pathology, and how more recent and less studied areas of research could guide future development and discovery.

Keywords: muscarinic receptors, Alzheimer's disease (AD), cholinergic dysfunction, amyloid pathology, tau pathology, neurodegeneration, drug discovery, allosteric modulators

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive impairment, and behavioral changes. It is responsible for 60-70% of cases of dementia, which is a leading cause of death, disability, and dependency among older populations (World Health Organization, 2025). Despite AD's widespread burden, current treatments are limited and not effective enough to treat AD fully. Over the past few decades, researchers have developed

various hypotheses for AD. Today, the widely accepted understanding of AD pathogenesis stems from the cholinergic, amyloid cascade, and tau hypotheses, as well as less recognized theories like the calcium homeostasis hypothesis (Zhang et al., 2024).

First proposed in 1979, the cholinergic hypothesis is one of the earliest models of AD formation. This hypothesis links a deficiency in acetylcholine (ACh) with the cognitive effects experienced by individuals with Alzheimer's (Davies & Maloney, 1976). Acetylcholine is a neurotransmitter, or chemical messenger, that is synthesized in a neuron before being released into a synaptic cleft and then binds to a receptor. The hypothesis attributed the shortage of ACh to the loss of cholinergic neurons—neurons that use ACh to communicate—found in AD patients. These changes mostly occur in the hippocampus, cortex, and other cortical regions important for cognitive function. This discovery influenced the development of treatments that increased ACh levels in the brain, such as acetylcholinesterase inhibitors (AChEIs). AChEIs work by inhibiting the enzyme acetylcholinesterase (AChE), which breaks down acetylcholine, increasing acetylcholine levels in the brain and improving synaptic function, specifically in the cholinergic system. Currently, Donepezil, Rivastigmine, and Galantamine are FDA-approved AChEIs for clinical use (Alzheimer's Association, 2025). These drugs are often used in conjunction with an NMDA antagonist, Memantine. However, AChEIs plateau in their effectiveness as AD progresses in severity, calling for alternative means of increasing ACh levels (Hogan, 2014).

Muscarinic receptors (mAChRs) and Nicotinic receptors (nAChRs)—the two types of acetylcholine receptors—have built on the cholinergic hypothesis, showing substantial potential as effective targets of therapeutics for AD. This review focuses on developments in drugs targeting mAChRs. Muscarinic receptors are a family of class AG protein-coupled receptors (GPCRs) subdivided into 5 subtypes (M1-M5), which bind to and are activated by ACh in the central and peripheral nervous systems, a mechanism significant to the cholinergic system. Although no treatments targeting muscarinic receptors have been approved yet, they have regained increasing interest in Alzheimer's research. Previously, research in the field shifted away from targeting muscarinic receptors and instead focused on targeting biomarkers associated with AD, especially amyloid-beta (Aβ) plaques and neurofibrillary tangles(NFTs). Aβ plaques are abnormal accumulations of Aβ peptides, which then trigger a series of pathways, further progressing AD (Hardy & Higgins, 1992). One of these pathways is the hyperphosphorylation of tau proteins, misfolding the tau proteins that form NFTs and hindering synaptic function (Frost et al., 2009). Understanding of these pathways has come mainly from the amyloid cascade and tau hypotheses. Treatments that have come out of these discoveries include anti-amyloid therapies like Aducanumab, Lecanemab, and Donanemab (Alzheimer's Association, 2025). However, these treatments also had limited efficacy. For instance, Lecanemab showed a 27% slower decline in CDR-SB (Clinical Dementia Rating-Sum of Boxes) scores over 18 months compared to a placebo. This result is statistically meaningful but modest, and the treatment accompanied adverse effects such as brain swelling or bleeding (Hampel et al., 2023; Ren et al., 2025). Furthermore, by the time amyloid plaques accumulate, neurodegeneration has already advanced, limiting their ability to reverse damage. Nevertheless, the cholinergic, tau, and amyloid hypotheses have helped us gain a better idea of the mechanisms and pathways behind AD [Figure 1]. Most notably, the M1 and M4 receptors have frequently been researched for their role in cholinergic dysfunction and relationship with amyloid pathways (Davis et al., 2010; Foster et al., 2014).

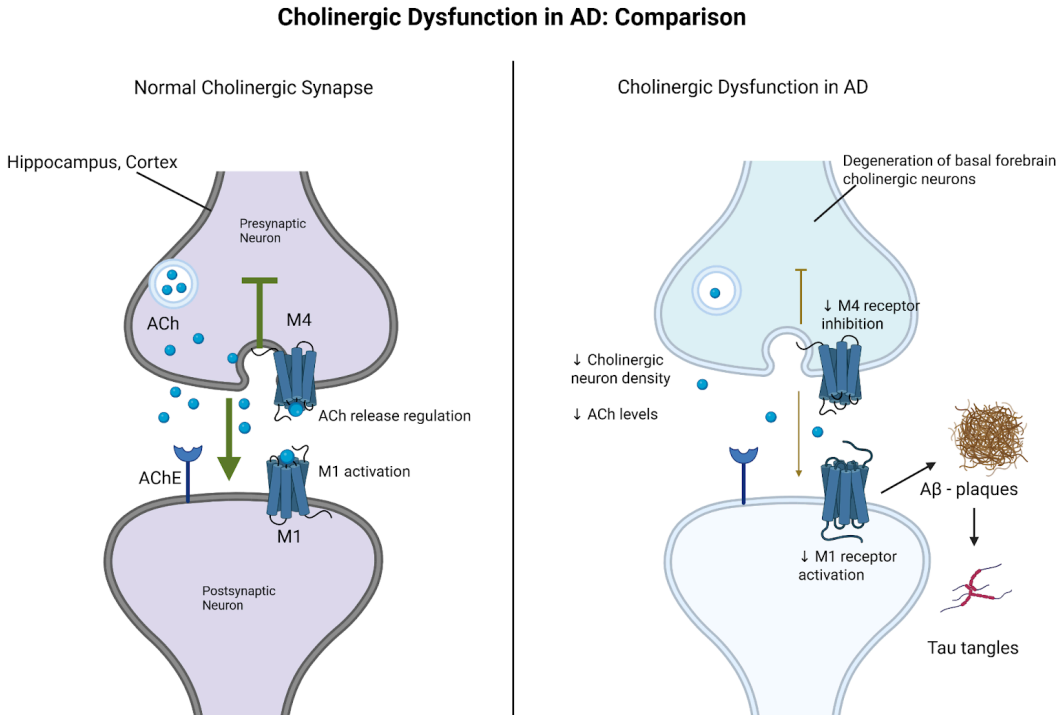

Figure 1: Cholinergic dysfunction in AD: comparison (created with BioRender)

Note: In healthy conditions, acetylcholine (ACh) is efficiently released from presynaptic neurons and inhibited by M4 muscarinic receptors, particularly in forebrain regions such as the hippocampus and cortex. This leads to substantial activation of M1 muscarinic receptors and regulation by acetylcholinesterase (AChE), allowing for normal cognitive function. In AD, degeneration of basal forebrain cholinergic neurons leads to reduced cholinergic neuron density, lower ACh levels, and ultimately less muscarinic activity. This dysfunction contributes to the development of Aβ plaques and NFTs, which collectively contribute to the progressive cognitive decline associated with Alzheimer's disease. (BioRender, n.d.; Davis et al., 2010; Foster et al., 2014)

Given the current landscape of Alzheimer's research and its treatments, this literature review aims to analyze past research to more clearly identify the potential roles muscarinic receptors can play in progressing the field. It will first examine the foundational studies that identified the functions, distributions, and intracellular mechanisms of mAChRs in the context of AD. This paper will then examine both the pathways that have been researched as a result and the status of drug developments and therapeutics that have attempted to target them. Finally, it will identify more recent and less studied discoveries that could hold potential solutions and discoveries in the field.

1. Foundational Discoveries of mAChR Roles in AD

Many aspects of Alzheimer's pathology are still unknown. However, understanding of the disease has come a long way since the proposal of the cholinergic hypothesis. As more pathways and locations of biomarkers involved in specific stages of AD have been identified, the roles mAChRs play have become clearer. While multiple factors impact mAChRs' significance to AD pathology, their structures and distributions provide the fundamental rationale for their targeting in treatments.

2.1. Distribution and Concentration of mAChRs in Areas Impacted by Alzheimer's

In its early stages, AD impacts areas significant to memory in the temporal lobe, specifically the hippocampus and entorhinal cortex, as well as the posterior cingulate gyrus, amygdala, and parahippocampal gyrus. Using MRIs, atrophy—brain shrinkage due to loss of neurons and synapses—has been found in these areas (Schuff et al., 2009). As the disease progresses, it expands to other parts of the cerebral cortex, like the frontal and parietal lobes (Ramos Bernardes da Silva Filho et al., 2017).

The M1 receptor, the most studied mAChR in AD research, is found throughout the central nervous system (CNS) in the cerebral cortex, hippocampus, and striatum, as well as the autonomic ganglia in the peripheral nervous system (PNS). The CNS consists of the brain and the spinal cord, acting as the control center of the nervous system. The PNS, consisting of the nerves outside of the CNS, acts as the communication network that connects the CNS to the rest of the body. M1 is most densely distributed in layers II/III and V in the frontal, parietal, and temporal cortex and the hippocampus (Levey et al., 1991) [Figure 2a]. Due to its prominence in these memory circuits, it is the most commonly targeted muscarinic receptor in the CNS for AD research and treatment. While the number of M1 receptors has been found to be unaffected by AD, their signaling efficacy is impaired, most likely attributed to impaired coupling with G proteins (Caccamo et al., 2006; Flynn et al., 1991).

Showing the highest overlap with the M1 receptor in the regions experiencing early neurodegeneration in AD, M4 receptors are mostly expressed in the caudate nucleus, putamen, and nucleus accumbens of the basal ganglia, as well as in the hippocampus and neocortex [Figure 2d]. In particular, their high expression in the basal ganglia is significant because they form part of the cortico–basal ganglia–thalamic loops, which integrate cognitive, motor, and reward-related information. In Alzheimer's, impairments in these circuits lead to cognitive impairment as well as neuropsychiatric symptoms such as apathy, agitation, and impaired decision-making. Similarly, M2 is an autoreceptor expressed in the hippocampus, cerebral cortex, and presynaptic terminals throughout the CNS, as well as in autonomic nuclei of the brainstem (Levey et al., 1991), [Figure 2b]. While not yet proven to be as effective a target due to its primary role being in the PNS, M2 provides major cholinergic pathways to the cortex and hippocampus (Smiley et al., 1999). Acting as inhibitory autoreceptors regulating ACh release, its loss in Alzheimer's patients contributes significantly to the cholinergic deficits associated with AD.

Finally, while the M3 and M5 receptors are mostly distributed in the PNS, they have displayed some therapeutic potential as targets [Figure 2b], [Figure 2e]. In neurons, M3 enables excitatory cholinergic signaling and plays a role in neurovascular coupling, mediating vasodilation—the widening of blood vessels—through Gq-protein pathways in endothelial cells of the cerebral vascular system (Kruse et al., 2012). The M5 receptor, while the least abundant muscarinic subtype in the CNS, also regulates cerebral blood flow [Figure 2e]. Hence, the M3 and M5 receptors' ability to manage cerebral blood flow could make them promising targets in early disease stages.

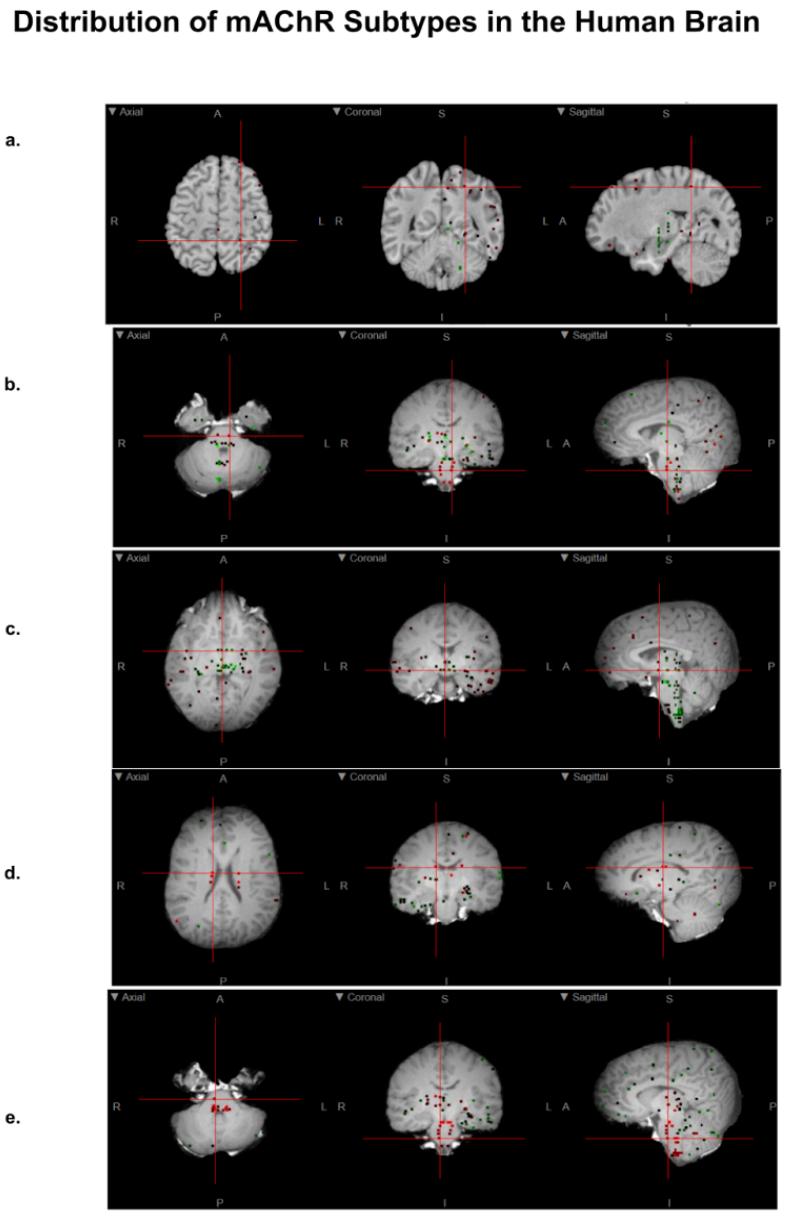

Figure 2: Distribution of muscarinic acetylcholine receptor (mAChR) subtypes in the human brain

Note: Coronal, axial, sagittal, and 3D projections from the Allen Human Brain Atlas showing gene expression patterns of mAChR subtypes in the areas they are most heavily distributed in (the brighter the red, the more concentrated). Allen Human Brain Atlas:

https://human.brain-map.org and https://atlas.brain-map.org.

a. M1 expression in the cerebral cortex
b. M2 expression in the pons
c. M3 expression in the hypothalamus
d. M4 expression in the striatum
e. M5 expression in the pons (Allen Institute for Science, n.d.)

2.2 Structure of mAChRs

Since mAChRs are GPCRs, all five subtypes have seven transmembrane (7TM) helices connected by extracellular and intracellular loops with an extracellular N-terminus and intracellular C-terminus. The orthosteric, or primary, binding site for ACh is located deep inside the 7TM bundle, which allows for precise ligand binding. This conformational change—change in receptor shape after binding—initiates intracellular signaling through heterotrimeric G proteins. The G protein that each mAChR couples with influences the pathways they signal. The M1, M3, and M5 receptors couple to Gq/11, which activates the phospholipase C (PLC)–IP_3–Ca^{2+} signaling cascade that promotes neuronal excitability and synaptic plasticity. On the other hand, the M2 and M4 receptors couple to Gi/o, which inhibit adenylyl cyclase and modulate ion channel activity to regulate neurotransmitter release [Figure 3]. GPCR structure makes mAChRs a more ideal target for therapeutics than the other family of acetylcholine receptors, nAChRs. Unlike nAChRs, which are ligand-gated ion channels with rapid but less modulatable responses, mAChRs transduce signals through G protein-mediated cascades. This allows for a broader range of pharmacological modulation, like biased agonism, allosteric regulation, and subtype selectivity (Wess, 1996). Furthermore, research using high-resolution crystallography and cryo-electron microscopy has discovered pocket structures and loop conformations that are unique to each mAChR subtype.

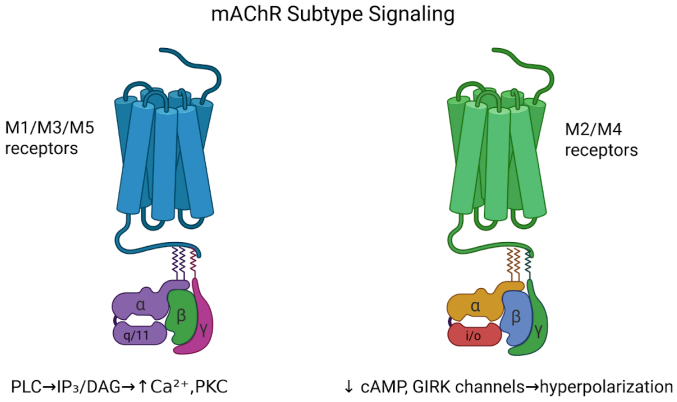

Figure 3: mAChR subtype signaling (created with BioRender)

Note: M1, M3, and M5 receptors couple to Gq/11 proteins, activating phospholipase C (PLC) and leading to production of IP_3 and second messenger Diacylglycerol (DAG), which increase intracellular Ca^{2+} and activate protein kinase C (PKC). M2 and M4 receptors couple to Gi/o proteins, which inhibit adenylate cyclase, decrease cAMP levels, and activate G protein-coupled receptor kinase (GIRK) channels, resulting in membrane hyperpolarization. (BioRender, n.d.)

2. Connections to Other Pathways and Diseases

In addition to being central to cholinergic neurotransmission, mAChRs also intersect with multiple pathological mechanisms relevant to AD and other neurological disorders. Understanding these cross-pathway interactions is critical, as it helps create the bigger picture, allowing for the development of therapies with greater efficacy and fewer adverse effects.

3.1 Amyloid and Tau Pathologies

As previously mentioned, muscarinic receptors influence the development of amyloid and tau pathologies [Figure 1]. Early research in vitro demonstrated that muscarinic stimulation increases sAPPα release, which inhibits and suppresses Aβ generation, an effect blocked by non-selective muscarinic antagonism and PKC inhibition (Lin et al., 1999). The M1 receptor, however, has a direct effect as it modulates enzymes involved in the processing activation of amyloid precursor protein (APP). More specifically, it promotes non-amyloidogenic processing of APP through the stimulation of alpha-secretase, reducing the production of pathogenic Aβ peptides (Cisse et al., 2011; Fisher, 2008). In vivo, the 3xTg-AD mouse model—chronic treatment with the M1 agonist AF267B—increased cortical and hippocampal sAPPα through ADAM17 activation, reduced Aβ42 levels and plaque burden, and improved memory (Caccamo et al., 2006). In addition to rodent models, AF267B lowered CSF Aβ in rabbits (Beach et al., 2001).

In parallel, M1 receptor stimulation also helps decrease the tau pathology associated with AD. Through PLC/Ca^2-dependent protein kinase C signaling and downstream inhibition of GSK3β, M1 activation reduces tau phosphorylation in vitro and in vivo. In vitro, muscarinic activation-modulated tau kinase cascades that include PKC and mitogen-activated protein kinase (MAPK) impact sites relevant to tangle formation (Forlenza et al., 2000). Additionally, in the same study, which found that M1 activation by AF267B in 3xTg-AD mice decreased amyloid pathology, AF267B treatment also decreased hyperphosphorylated tau. (Caccamo et al., 2006)

3.2. Links to Dopaminergic and Glutamatergic Systems

In addition to cholinergic dysfunction, imbalances in the dopaminergic and glutamatergic systems significantly contribute to AD pathogenesis and progression. The dopaminergic system is primarily involved in brain functions like motivation, reward, and motor control, all of which are impacted by Alzheimer's. Muscarinic receptors, especially the M4 subtype, play essential roles in modulating dopaminergic transmissions. More specifically, activation of M4 limits dopamine release, making it a useful target for countering the dopamine imbalances associated with AD. Nicotinic receptors, which, unlike mAChRs, promote dopamine release through a quicker and more direct pathway, are also significant to dopamine balance. However, targeting nAChRs has been more challenging due to obstacles like downregulation in AD, receptor selectivity, receptor desensitization, and subtype selectivity. More specifically, in AD, nicotinic receptor expression of subtypes α4β2 and α7 is significantly diminished, reducing their efficacy as therapeutic targets (Hoskin et al., 2018; Pimlott et al., 2004). Furthermore, issues like receptor desensitization with chronic activation and lack of subtype selectivity can lead to off-target effects (Choi, 1988).

On the other hand, dysfunction in the glutaminergic system—the brain's primary excitatory network with glutamate as a

neurotransmitter—is directly linked to the excitotoxicity and neuronal death found in AD. Among glutamate receptors, N-methyl-D-aspartate (NMDA) receptors are especially important because they are highly permeable to calcium and directly mediate synaptic plasticity, long-term potentiation (LTP), and memory encoding (Malenka & Bear, 2004). In Alzheimer's, NMDARs are overactivated and disrupted by Aβ plaques, which is why antagonists like memantine are used clinically to reduce excitotoxicity. However, memantine only dampens overactivation and does not restore the physiological pro-plasticity role of NMDA receptors. M1 receptors modulate NMDAR currents heavily. In particular, M1 activation has been shown to enhance NMDA receptor-mediated excitatory postsynaptic currents in hippocampal pyramidal neurons (Marino et al., 1998). Additionally, M1 increases neuronal excitability and calcium entry through NMDA receptors by inhibiting small-conductance calcium-activated potassium (SK) channels (Conn et al., 2009). Together, these mechanisms potentiate NMDAR currents and facilitate LTP, making M1 a useful target in countering this aspect of AD progression.

3.3. Connection to Other Diseases

In addition to AD, mAChRs have been proven to interact with many of the pathways in other neuropsychiatric and neurodegenerative disorders, such as schizophrenia (SZ), Parkinson's, and Huntington's diseases. This has led to the testing of the same drugs for both SZ and AD, most notably xanomeline. Parkinson's, which is mainly characterized by the loss of dopaminergic neurons, has been associated with the loss of M4 receptors in the striatum due to their role in modulation (Nielsen & Ford, 2024). Similarly, a decrease in the density and total of M2 has been found in post-mortem Huntington patients (Lange et al., 1992). Although SZ is mostly classified as a neuropsychiatric disorder, multiple studies have demonstrated that M1 and M4 interact with many of the same neural circuits as AD. This has led to the testing of the same drugs for both SZ and AD, most notably xanomeline.

3. The Progress and Current Status of mAChR-Targeted Therapeutics

Despite the advantageous aspects of muscarinic acetylcholine receptors (mAChRs) identified previously, early attempts to develop effective therapeutics faced major obstacles. These limitations revolved mainly around the lack of available compounds that were selective enough to target only individual mAChR subtypes, peripheral cholinergic side effects, and, while broader than nicotinic modulation, the still narrow window for modulation of mAChRs. Fortunately, recent research has shifted to improving subtype selectivity, blood–brain barrier (BBB) penetration, and utilizing novel mechanisms that modulate receptors to achieve cognitive benefits that have previously been unachievable. Due to their role in AD pathology, M1 and M4 are usually the targets of these compounds.

4.1. Subtype Selective Orthosteric Ligands

Orthosteric ligands target the acetylcholine binding site, deep inside the 7TM bundle. This site is common among all mAChRs, making subtype selectivity challenging [Figure 4.1, Figure 5]. For instance, Cevimeline (AF102B), developed as an orthosteric M1 agonist for AD, could cross the BBB and showed improved memory and reduced Aβ pathology in rodents (Mitoh et al., 2017). In human trials, the drug exhibited the same benefits but also showed peripheral adverse effects, preventing FDA approval (Oleksak et al., 2021). An older agent, which revealed that nonselective orthosteric ligands caused peripheral adverse effects, was the M1 and M4 agonist, xanomeline. Although now recognized as a dual orthosteric and allosteric M1 agonist, xanomeline exhibited similar adverse effects associated with its orthosteric off-target activations of M2 and M3 subtypes,

such as nausea, vomiting, excessive salivation, gastrointestinal disturbances, and bradycardia (Burger et al., 2023; Noetzel et al., 2009).

An emerging agent known as Kar-XT has combined xanomeline with trospium chloride, a peripherally restricted muscarinic antagonist that does not cross the BBB. This design preserves xanomeline's central M1/M4 activity while significantly reducing activity in the PNS. In a phase 1 healthy volunteer study (NCT02831231), the presence of trospium chloride reduced composite cholinergic adverse events by 46% compared to xanomeline alone. Adverse effects such as nausea, vomiting, diarrhea, sweating, and salivary hypersecretion were also reduced by at least 29% (Breier et al., 2023). Additionally, in prior phase 2 schizophrenia trials, KAR-XT demonstrated good efficacy and tolerability (Correll et al., 2022). While these clinical trials were conducted for schizophrenia, they target the same mechanism—muscarinic activation in the CNS protected from peripheral side effects—relevant to Alzheimer's disease, where similar challenges of peripheral toxicity have long limited the development of orthosteric mAChR agonists.

Beyond Kar-XT, recent research using high-resolution crystallography and cryo-electron microscopy has discovered pocket geometries and loop conformations that are unique to each mAChR subtype, allowing for the design of ligands with improved bias (Thal et al., 2016). For instance, VU0357017 and AC-260584 are M1-preferring agonists that have shown high efficacy while having little to no activation of the M2-M5 subtypes (S. R. Bradley et al., 2010; Digby et al., 2012). AC-260584 exhibited potent functional activity (pEC50 of 7.7 ± 0.1) and high efficacy with reduced activity at M2-M5. VU0357017 activated calcium signaling and ERK1/2 phosphorylation—pathways downstream of G-protein-coupled activation—while showing minimal β-arrestin recruitment, indicating a bias toward cognitive-relevant signaling cascades. While orthosteric agents offer a direct mechanism of action, even highly selective compounds can have adverse effects due to powerful signaling even at low levels of off-target activation. This risk has driven interest in allosteric modulators and bitopic ligands, which offer refined, tissue-specific modulation with potentially wider therapeutic scopes.

4.2. Allosteric Modulators

Allosteric modulators bind to sites on the muscarinic receptor other than the orthosteric site. Unlike orthosteric ligands, which compete directly with endogenous ACh, allosteric modulators change receptor activity by influencing the receptor's reaction to naturally released ACh [Figure 4.2]. Positive Allosteric Modulators (PAMs) do this by increasing the response to acetylcholine, while Negative Allosteric Modulators (NAMs) regulate the response. Subtype selectivity is more achievable since allosteric sites are less conserved and more structurally unique among mAChR subtypes [Figure 5]. Furthermore, endogenous ACh is released in specific locations and at precise times to regulate physiological functions. Allosteric modulation only occurs where endogenous ACh is present, so the risk of unwanted targeting in the PNS is reduced.

The most studied allosteric modulator has been M1-selective PAMs. The prototype M1 PAM, benzyl quinolone carboxylic acid (BQCA), has been developed as a preclinical research tool. It substantially promoted downstream signaling in medial prefrontal cortex (mPFC) pyramidal neurons, with effects absent in M1 knockout (KO) mice (Shirey et al., 2009). Most notably, BQCA restored discrimination reversal learning, often lost in AD patients, in a transgenic AD mouse model and regulated non-amyloidogenic APP processing in vitro. This suggested that they could possess symptomatic and disease-modifying potential. Additionally, in prion-diseased mice—an in vivo model with cholinergic dysfunction similar to AD—daily BQCA doses restored cognitive function and significantly increased survival, with minimal side effects compared to treatment with orthosteric agonists or acetylcholinesterase inhibitors (S. J. Bradley et al., 2017). Despite this, BQCA displayed poor

pharmacokinetic properties. More specifically, although it reached peak brain concentrations around 1.5 hours post-administration, systemic and tissue distribution favored organs like the kidney and lung over the brain, limiting its translational potential (Dwomoh et al., 2022). Building on BQCA's model, MK-7622 became the first human-tested M1 PAM for AD. The phase II trial evaluated it as an adjunct to standard AChEI therapy but was terminated early due to the lack of significant cognitive or functional benefit compared to placebo. Importantly, cholinergic-like adverse events such as diarrhea and nausea were more frequent than in control groups (Voss et al., 2018). Displaying similar reactions in rodents, PF-06767832 and PF-06764427 helped identify intrinsic agonism or activation of the receptor as a cause for these effects (Davoren et al., 2016; Moran et al., 2018). As a result, M1-PAMs like VU319, which minimize agonist activity, have shown a lack of adverse effects and are being further researched (Poslunsey et al., 2024).

Given that M4 receptors act as inhibitory autoreceptors on cholinergic interneurons and as modulators of dopaminergic signaling, recent research has also studied how they could potentially be targeted using PAMs to establish an excitatory–inhibitory balance in the networks affected by AD. Due to their recent rise to attention, most data on M4 PAMs for Alzheimer's treatment is preclinical and builds on schizophrenia research, which shares some common pathways. A compound that has shown promise in this class of treatments is VU0152100, which is being developed at Vanderbilt University. VU0152100 reversed amphetamine-induced hyperlocomotion in rodent models but not M4 knockout (KO) mice, demonstrating high selectivity, lacked intrinsic agonist activity, and did not produce the adverse effects associated with non-selective agonists (Byun et al., 2014). Although initially researched for schizophrenia, the mechanism of reducing excessive excitability through M4 signaling is relevant to Alzheimer's pathology, where similar dysregulation occurs. Among the next generation of M4 PAMs are VU0467154, CVL-231, and NMRA-861, which have shown better potency as well as many of the same effects seen in VU0152100, showing progress and potential in this type of treatment (Berezovskaia et al., 2025; Butler et al., 2024). Additionally, in 2018, it was revealed that Xanomeline could bind to the allosteric site on M4, allowing for further understanding of the mechanisms at work behind Kar-XT, and the potential for Xanomeline to be used as a PAM. (Burger et al., 2023)

Although less popular, negative allosteric modulators have also been explored for their roles in AD. Their purpose, unlike positive allosteric modulators, is to reduce the potency of endogenous ACh. Therefore, they target areas where mAChRs overactivation could be problematic, like the M2 receptor, which limits ACh in the cortex and hippocampus (Zuchner et al., 2005). It has been suggested that selective M2 antagonism can increase synaptic ACh and potentially improve cognition in AD by complementing AChEIs. The M2-selective NAM, Gallamine—a neuromuscular blocker—demonstrated negative cooperativity at muscarinic receptors, guiding research into M2 NAMs (Mash et al., 1985). Since then, there has been some research in developing more subtype-selective and CNS-suitable M2-selective NAM candidates for AD. Despite some early compounds having demonstrated potential, factors like the possibility of increased Aβ formation, lessened distribution in the CNS, and an incomplete understanding of the M2 receptor and its mechanisms, focus has remained on the more promising M1 and M4 receptors. While allosteric modulators solve many of the issues regarding subtype selectivity that orthosteric ligands couldn't, intrinsic agonism and their efficacy in general have driven focus to another type of agent, bitopic ligands.

4.3. Bitopic Ligands

Bitopic or dualsteric ligands are a more recent method of targeting GPCRs that often have greater affinity and selectivity than orthosteric ligands or allosteric modulators alone [Figure 4.3]. Engineered to engage the orthosteric site and a more structurally unique allosteric site at the same time, they can activate only designated downstream pathways through a

process known as biased signaling. This is useful for targeting mAChRs in the CNS, where peripheral side effects limit traditional agonists. Therefore, bitopic ligands can potentially deliver CNS-selective effects with fewer off-target actions.

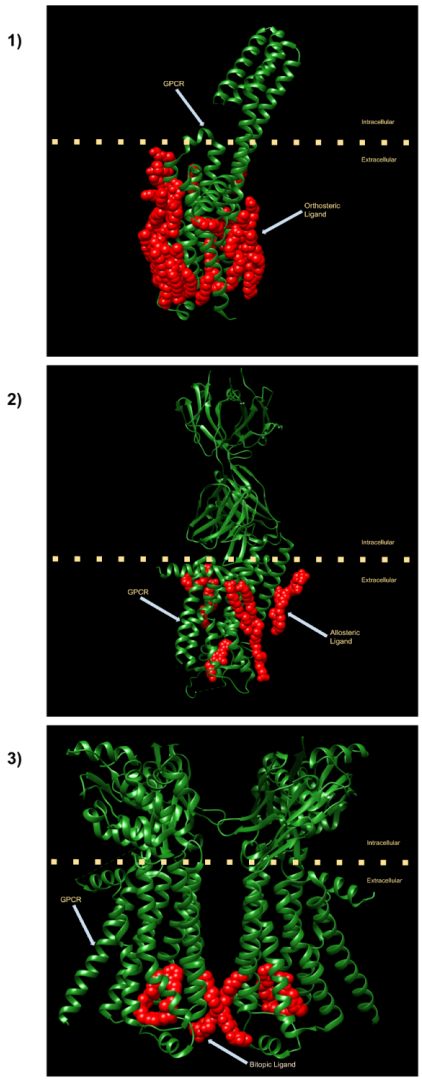

Figure 4: Binding structures of different agent types

Note: UCSF Chimera structure visualizations of class A GPCRs (green) bound to orthosteric, allosteric, and bitopic ligands (red) found on the

RCSB Protein Data Bank. A2A and beta2 adrenergic receptors were used due to limited access to PDBs of mAChR binding.

1. The A2A adrenergic receptor bound to an orthosteric ligand (PDB ID: 4EIY)
2. The A2A adrenergic receptor bound to an allosteric ligand (PDB ID: 3VG9)
3. The beta2 adrenergic receptor bound to a bitopic ligand (PDB ID: 8W1V)
   (Bitopic Ligands Support the Presence of a Metastable Binding Site at the B2 Adrenergic Receptor | Journal of Medicinal Chemistry, n.d.; G-Protein-Coupled Receptor Inactivation by an Allosteric Inverse-Agonist Antibody | Nature, n.d.; Structural Basis for Allosteric Regulation of GPCRs by Sodium Ions | Science, n.d.; Ef et al., n.d.-a, n.d.-b)

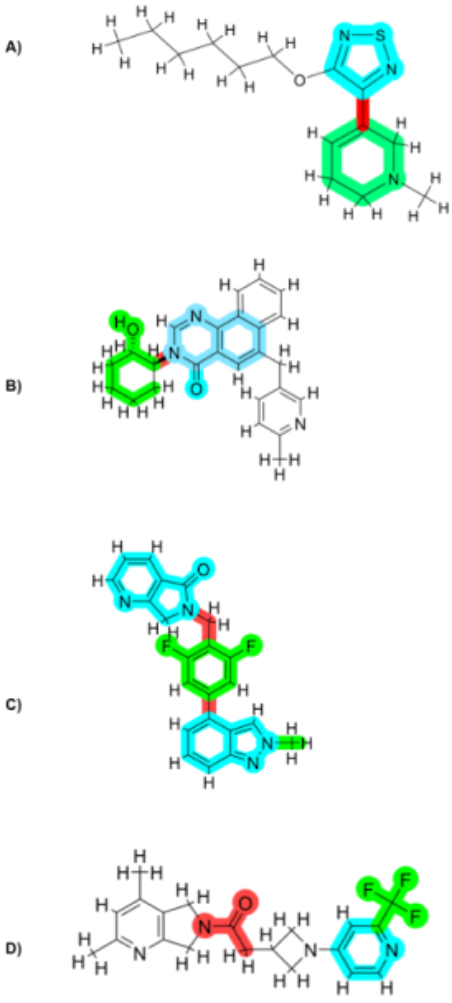

Figure 5: Chemical structures of prominent mAChR ligands investigated in AD

Note: Primary aromatic scaffolds (blue) provide the conserved features necessary for receptor interaction, linkers (red) influence binding orientation within the orthosteric pocket, and hydrophobic or bulky substituents (green) improve bitopic interactions and selectivity. The structures were obtained from PubChem and highlighted using ChemDraw.

A. Xanomeline, an orthosteric agonist with secondary allosteric engagement
B. MK-7622, a selective M1-PAM
C. VU0467319, a bitopic PAM
D. CVL-231, a recent allosteric PAM (PubChem, n.d.-d, n.d.-c, n.d.-a, n.d.-b)

Like PAMs, bitopic ligands are a promising drug class for targeting M1. One of the earliest M1-targeting bitopic ligands, tert-butyl peroxybenzoate (TBPB), was initially thought to achieve its selectivity only through allosteric binding. However, later studies showed that mutations in both the orthosteric and allosteric binding sites affected the binding and signaling of TBPB (Keov et al., 2014). In rodent models, TBPB enhanced cortical acetylcholine release, improved performance in object recognition tasks, and promoted non-amyloidogenic APP processing through increased amyloid-secretase activity while still not inducing gastrointestinal side effects (Jones et al., 2008). Due to pharmacokinetic limitations, including poor solubility and modest brain penetration, development of TBPB ceased; nevertheless, this serves as proof that M1-selective bitopic ligands could modulate pathways significant to Alzheimer's. 77-LH-28-1, another M1-directed bitopic ligand, improved spatial and working memory, in part by modulating calcium homeostasis and synaptic plasticity in hippocampal neurons without peripheral adverse effects (Langmead et al., 2008). Bitopic ligands targeting M4 for AD are less developed than those for M1, and none have yet emerged as especially promising.

4.4. Limitations of Agents In Current Trials and Future Directions

In addition to barriers relating to tolerability, pharmacokinetics, and target selectivity, recent trials of muscarinic-based therapies for AD are encountering a new set of challenges. A persistent translational gap remains, with many compounds showing efficacy in preclinical animal models [Table 2] but limited or inconsistent benefits in humans. This is frequently associated with species differences in receptor mechanisms and disease progression. For instance, although mouse models are able to emulate the basic aspects of cholinergic dysfunction, such as ACh release and amyloid pathology, they lack the degeneration found in the basal forebrain. This is often due to the inability of mouse models to exhibit the same characteristics found in aging humans, who suffer the most from AD (R & T, 2011). Additionally, data collected from mouse models may not be able to indicate the effects of treatments on the more complex cognitive functions of humans, such as language and executive function. Furthermore, the lack of known biomarkers for target engagement makes it difficult to determine whether disappointing outcomes reflect insufficient receptor modulation or true therapeutic inefficacy.

Safety concerns also continue to emerge. Although recently developed ligands in clinical trials [Table 1] have shown improved receptor selectivity, they have not fully eliminated the adverse effects associated with PNS activation. Both the selective M1 agonist HTL0018318 and M4 PAM CVL-231 were associated with transient increases in blood pressure, posing a risk for elderly patients with cardiovascular comorbidities (Jh et al., 2022; Nathan et al., 2022). Additionally, broader concerns about unethical practices and trial recruitment strategies that fail to account for all groups of individuals remain unresolved. Recently, a prominent paper on amyloid pathology in AD, published in 2006 by a team led by Sylvain Lesné, was found to have manipulated images and data, misleading the direction of Alzheimer's research for over 15 years.

Given these obstacles, muscarinic research for AD treatments is shifting toward precision medicine approaches that find therapeutic strategies depending on patient-specific biology. The development of Positron Emission Tomography (PET) tracers for muscarinic receptors, such as [11C]VC-002 for M1, provides a tool to measure receptor availability in vivo (Haense et al., 2012). This technology could allow for the grouping of patients based on receptor density and track target engagement during clinical trials. As a result, researchers can confirm with more confidence the efficacy of drug treatments. Genetic insights are also guiding muscarinic research. For instance, individuals carrying the APOE4 gene showed significant vulnerability to cholinergic dysfunction (Hill et al., 2007). Hence, they may particularly benefit from muscarinic-targeted therapies early on. Additionally, mutations and polymorphisms in the CHRM1 and CHRM4 genes have been linked to altered receptor expression or signaling, influencing the individual responses of drugs being tested (Dean & Scarr, 2021). The integration of genomic profiling into trials could reduce discrepancies like these. Combination therapy could also be helpful, given the complex nature of AD. More specifically, M4 PAMs could enhance cognition, while anti-amyloid drugs like Aducanumab, Lecanemab, and Donanemab slow progression. Similarly, given that Memantine is taken in conjunction with AChEIs, pairing them with mAChR targeting therapeutics could improve balance in the glutamatergic system.

In the future, large molecule treatments may complement or even surpass small molecules in muscarinic receptor drug discovery. Allosteric antibodies, while not yet applied clinically to muscarinic receptors, have already been developed for other GPCRs such as CCR5 and GLP-1R, for which they have stabilized receptor conformations (Cong et al., 2021; Olson & Jacobson, 2009). Applying this strategy to M1 or M4 could lead to the development of therapeutics with improved selectivity, pharmacokinetics, and tissue specificity, potentially overcoming the adverse effects seen with small-molecule drugs. Nanobodies (VHHs) are another promising platform, as they have already been used to stabilize muscarinic receptor conformations. For instance, nanobodies Nb9-8 and Nb10-6 were found to lock the M2 receptor in active states (Kruse et al., 2013). With further engineering, nanobodies could be developed as therapeutic allosteric modulators of M1 or M4. Finally, chemogenetic approaches offer proof-of-concept for circuit-specific cholinergic modulation. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), which were originally engineered from muscarinic receptors, have demonstrated this. The hM3Dq receptor, derived from M3, produces excitatory Gq signaling, while hM4Di, engineered from M4, produces inhibitory Gi signaling (Smith et al., 2016). Although DREADDs are primarily research tools, they have shown how muscarinic receptor scaffolds can be modified to enable precise spatiotemporal control of signaling, showing the potential for future gene-therapy-based interventions in Alzheimer's disease.

Table 1: Prominent Muscarinic Agents Currently Under Research for Relevance to AD in Clinical Trials

Agent	Class / Mechanism	Development Stage & Context	Relevance to AD
KarXT (xanomeline + trospium chloride)	Dual orthosteric and allosteric M1/M4 agonist + peripheral orthosteric antagonist	Phase 3 trials for psychosis and agitation in AD; FDA-approved for schizophrenia as of 2024. Invented at PureTech Health; advanced by	Central activation of M1/M4 while minimizing peripheral side effects; cognitive and behavioral relevance in AD.

		Karuna Therapeutics; acquired by Bristol Myers Squibb (BMS).	
HTL0018318	Orthosteric M1-selective agonist	Phase 1b/2a adjunct to donepezil in mild–moderate AD. Developed by Sosei Heptares in collaboration with Allergan.	Designed for enhanced cognitive benefit with improved receptor specificity.
HTL0016878	Orthosteric M4-selective agonist	Phase 1 in healthy volunteers Originated at Heptares Therapeutics; advanced by Neurocrine Biosciences.	Targeting neurobehavioral symptoms via M4 pathways.
HTL9936	Orthosteric M1-selective agonist	Phase 1 in healthy (elderly inclusive) subjects Developed by Heptares Therapeutics.	High selectivity addressing cognitive impairment with a focus on safety.
VU319	M1-positive allosteric modulator (PAM) with minimal intrinsic agonism	Phase 1 completed; proof-of-concept planned. Developed within Vanderbilt University's Warren Center for Neuroscience Drug Discovery (WCNDD).	Reduced side effects compared to earlier PAMs like MK-7622.
CVL-231 (Emraclidine)	M4 PAM	Phase 1 multiple-dose; includes AD dementia cohort Developed by Cerevel Therapeutics; now under AbbVie.	Balances excitatory/inhibitory signaling relevant to AD.

NS-136	M4 PAM	Phase 1 for schizophrenia; preclinical AD development Developed by NeuShen Therapeutics.	Forward-looking candidate, bridging schizophrenia and AD.

Table 2: Prominent Muscarinic Agents Currently Under Research for Relevance to AD in Preclinical Trials

Compound 24	M4 PAM (tool compound)	Preclinical in rodents and rhesus monkeys —	Demonstrated procognitive and antipsychotic-like effects in primate models.
VU0467154	M4 PAM	Preclinical (rodent) Originated at Vanderbilt University.	Enhanced learning and memory, strengthening the case for cognitive benefit.
VU0152100 / VU0152099 / LY2033298	M4-selective PAMs (tool compounds)	Preclinical (rodents) —	Validated M4 as an attractive target for cognition and behavior modulation.
AF267B (also AF102B / AF150(S))	Orthosteric M1-selective agonist	Preclinical (various animal models) —	Reduced Aβ and tau pathology; rescued memory in transgenic AD mice.
AF710B	M1-selective agonist with σ1 activity (bitopic-like)	Preclinical (rodents) —	Improved cognition and APP processing with high selectivity.
GSK1034702	Bitopic M1 agonist	Clinical testing in humans; problematic due to side effects (not discontinued yet). Developed by GlaxoSmithKline (GSK).	Demonstrated bitopic engagement and cognitive effects, but poor safety highlights the need for refined ligands.

4. Conclusion

Research in Alzheimer's pathogenesis, specifically regarding the mAChRs, has certainly undergone significant progress since the proposition of the cholinergic hypothesis in 1976. Moving beyond the initial setbacks of earlier orthosteric agonists, more refined approaches with subtype selectivity, allosteric modulation, and biased signaling have been discovered. The M1 and M4 subtypes remain the most prominent targets due to their dense expression in memory-related and dopaminergic circuits and their direct influence on amyloid and tau pathology. Understanding the overlap between these systems and other neurological diseases has contributed to the development of treatments like Kar-XT, which were successful in trials for related disorders like schizophrenia and have since shown promise in AD trials. Nevertheless, much remains unknown and preclinical. Translation from animal models to humans continues to be inconsistent, trial design is slowed by the lack of distinguishable biomarkers, and the heterogeneity of Alzheimer's demands more precision in clinical trials.

Muscarinic-targeted therapies with genetic profiling, PET tracers, and potentially combined strategies with anti-amyloid or anti-tau drugs could help overcome these challenges. Furthermore, beyond small molecules, new techniques such as allosteric antibodies, nanobodies, and receptor-based gene therapies are beginning to gain attention. Together, these developments have improved understanding of AD as well as served as a guide for the potential for innovation in treatments targeting mAChRs.

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Mentor Contribution Statement

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