

# Activation of M<sub>1</sub> and M<sub>4</sub> muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia

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**Abstract:** Alzheimer's disease (AD) and schizophrenia (SZ) are neurological disorders with overlapping symptomatology, including both cognitive deficits and behavioral disturbances. Current clinical treatments for both disorders have limited efficacy accompanied by dose-limiting side effects, and ultimately fail to adequately address the broad range of symptoms observed. Novel therapeutic options for AD and SZ are needed to better manage the spectrum of symptoms with reduced adverse-effect liability. Substantial evidence suggests that activation of muscarinic acetylcholine receptors (mAChRs) has the potential to treat both cognitive and psychosis-related symptoms associated with numerous central nervous system (CNS) disorders. However, use of nonselective modulators of mAChRs is hampered by dose-limiting peripheral side effects that limit their clinical utility. In order to maintain the clinical efficacy without the adverse-effect liability, efforts have been focused on the discovery of compounds that selectively modulate the centrally located M<sub>1</sub> and M<sub>4</sub> mAChR subtypes. Previous drug discovery attempts have been thwarted by the highly conserved nature of the acetylcholine site across mAChR subtypes. However, current efforts by our laboratory and others have now focused on modulators that bind to allosteric sites on mAChRs, allowing these compounds to display unprecedented subtype selectivity. Over the past couple of decades, the discovery of small molecules capable of selectively targeting the M<sub>1</sub> or M<sub>4</sub> mAChR subtypes has allowed researchers to elucidate the roles of these receptors in regulating cognitive and behavioral disturbances in preclinical animal models. Here, we provide an overview of these promising preclinical and clinical studies, which suggest that M<sub>1</sub>- and M<sub>4</sub>-selective modulators represent viable novel targets with the potential to successfully address a broad range of symptoms observed in patients with AD and SZ.

**Keywords:** muscarinic receptors, schizophrenia, Alzheimer's disease

## Introduction

Schizophrenia (SZ) and Alzheimer's disease (AD) are two devastating disorders of the central nervous system (CNS) that present clinically with cognitive impairments and psychotic symptoms. Psychosis is the hallmark symptom of SZ and manifests as hallucinations, disordered thought/speech, and delusions. While these psychotic symptoms are commonly associated with SZ, it has become well documented that these patients also experience cognitive and behavioral disturbances that are not adequately addressed by currently prescribed typical and atypical psychotics.<sup>1</sup> Conversely, the most commonly associated symptoms of AD are cognitive in nature and include deficits in learning and memory. However, 50%–80% of AD patients display psychotic and behavioral disturbances that are correlated with poor social and functional outcomes.<sup>2</sup> While these two diseases arise from separate etiologies, there is a large amount of overlap

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in the cognitive deficits and psychotic symptoms that are observed. Currently available therapies for these conditions fail to alleviate the broad range of symptoms experienced by patients and are often hampered by dose-limiting side effects, emphasizing the need for novel therapeutics with which to treat these patients.

Another commonality between AD and SZ is the apparent involvement of dysregulated cholinergic signaling in the brain.<sup>3,4</sup> Acetylcholine (ACh) is a neurotransmitter that modulates neuronal function in several areas of the CNS associated with AD and/or SZ pathology, including the striatum, cortex, hippocampus, and prefrontal cortex.<sup>5</sup> ACh mediates its actions via two families of receptors, termed the muscarinic ACh receptors (mAChRs) and the nicotinic ACh receptors (nAChRs). Here, we review the potential of mAChR modulation for the treatment of AD and SZ; however, modulation of nAChRs could also provide novel therapeutic avenues for treating these diseases (see Taly et al<sup>6</sup> for a comprehensive review).

The mAChR family consists of five subtypes ( $M_1$ – $M_5$ ) that can be found throughout the CNS and periphery. These receptors are guanosine nucleotide-binding protein (G-protein)-coupled receptors and can be subdivided based on their canonical signaling pathways.  $M_1$ ,  $M_3$ , and  $M_5$  all signal primarily via the  $G_{\alpha q}$  G-protein and induce  $Ca^{2+}$  mobilization and inositol trisphosphate ( $IP_3$ ) production, while  $M_2$  and  $M_4$  signal via the  $G_{\alpha i}$  G-protein to inhibit cyclic adenosine monophosphate (cAMP) production. As discussed in further detail below, treatments that broadly augment cholinergic signaling have demonstrated clinical efficacy in treating the cognitive and behavioral deficits observed in AD and SZ patients. However, the clinical utility of these treatments is curtailed by peripherally mediated side effects. The recent discovery of compounds that selectively act at the  $M_1$  or  $M_4$  receptor have suggested that these receptors may provide viable drug targets with which to safely and effectively treat AD and SZ patients.

## Alzheimer's disease

AD is the most commonly diagnosed form of dementia and currently affects approximately 35 million individuals worldwide.<sup>7</sup> AD is a progressive neurodegenerative disease that is characterized by a host of cognitive deficits, including impairments in learning and memory. In addition to the well-documented cognitive impairments, AD patients also display behavioral disturbances, including anxiety, depression, and psychosis.<sup>8</sup> Age is the primary risk factor for AD, and the disease usually manifests in individuals after the age

of 60 years. Due to an aging population, the prevalence of AD is predicted to rise to 66 million people by the year 2030. This devastating disease burdens not only the afflicted and their families, but also generates a global financial burden, with dementias costing society approximately US\$604 billion in 2010 alone.<sup>7</sup> Given the necessity for increased attention and care, AD places a great burden and strain on the daily lives of patients, families, and caregivers.

The hallmarks of AD pathology are the accumulation of amyloid-beta ( $A\beta$ ) peptide aggregates (neuritic plaques) and hyperphosphorylated tau protein (neurofibrillary tangles).<sup>9,10</sup> The popular amyloid cascade hypothesis posits that the gradual build-up of  $A\beta$  plaques leads to neuronal inflammation, dysfunction, and, eventually, cell death. The two brain regions most critically affected by this degeneration are the cortex and hippocampus, both of which are involved in cognition, learning, and memory.<sup>11</sup> Several lines of evidence suggest that impaired cholinergic signaling plays a key role in mediating both the cognitive and the behavioral impairments observed in AD patients.<sup>12</sup> The basal forebrain cholinergic system is disproportionately affected in AD patients, with a robust loss of cholinergic neurons, including those innervating the hippocampus and cortex.<sup>4,13,14</sup> In addition, administration of nonselective muscarinic antagonists can produce or exacerbate cognitive deficits in animals,<sup>15</sup> as well as in AD patients and both young and old control subjects,<sup>16,17</sup> suggesting that mAChRs can directly modulate cognition. The current primary treatments for AD symptoms are acetylcholinesterase inhibitors (AChEIs) such as donepezil, tacrine, galantamine, and rivastigmine, which potentiate cholinergic signaling.<sup>18,19</sup> These treatments not only provide improvements in cognitive symptoms associated with AD,<sup>20,21</sup> but also show efficacy in treating the psychiatric symptoms.<sup>22,23</sup> Unfortunately, cardiovascular and gastrointestinal side effects are often observed with these treatments, effects thought to be mediated by peripherally located ACh receptors. Despite this, AChEIs remain modestly beneficial for treating AD and other forms of dementia.<sup>24</sup> Collectively, these findings highlight the importance of the cholinergic system in mediating the cognitive and behavioral deficits observed in AD patients and highlight the need to develop cholinergic therapeutics that can provide clinical efficacy in the absence of peripherally mediated side effects.

## Schizophrenia

SZ is a severe and debilitating psychiatric disease that affects approximately 1% of the population.<sup>25</sup> It is characterized by multiple symptom clusters, including positive

symptoms, negative symptoms, and cognitive impairments. The onset of SZ symptoms usually occurs early in life (before 30 years of age), between adolescence and young adulthood. There is marked variability in the symptomatology between individual SZ patients, and the neurobiology of the disease is complex. Accordingly, it has been hypothesized that SZ symptoms can arise as a result of numerous underlying etiologies that are poorly understood.<sup>26</sup> The hallmark psychotic symptoms of SZ are the positive cluster and include auditory hallucinations, delusional beliefs, and disorganized thoughts and speech. SZ patients also exhibit negative symptoms, including anhedonia, dysfunctional social interactions, and poverty of thoughts and speech, as well as cognitive disturbances affecting several behavioral domains, including working memory, attention, and executive function. Furthermore, the above-mentioned symptom groups are commonly accompanied by disruptions in mood and substance abuse, which affect 40%–80% of SZ patients.<sup>27,28</sup> Given the vast spectrum of symptoms observed in SZ patients, it is important to develop therapeutic treatments that can provide efficacy in treating positive, negative, and cognitive deficits.

Although the causes of SZ remain largely unknown, research over the past couple of decades has focused on the dysregulation of signaling by monoamines such as dopamine and serotonin. The prevailing dopaminergic hypothesis attributes positive symptoms to hyperdopaminergic activity in striatal and mesolimbic pathways, while negative symptoms are ascribed to hypodopaminergic activity in the medial prefrontal cortical and mesocortical pathways.<sup>29</sup> Current treatments include both typical (eg, haloperidol and chlorpromazine) and atypical (eg, risperidone and clozapine) antipsychotics, which act on the dopaminergic system and D<sub>2</sub> dopamine receptors in particular. These treatments show partial efficacy in reducing psychotic or positive symptoms;<sup>30</sup> however, they demonstrate little to no efficacy in addressing negative symptoms and cognitive impairments, which can prevent patients from participating fully and productively in society.<sup>31,32</sup> Despite the beneficial effects of treating positive symptoms in SZ patients, up to 74% of patients on atypical antipsychotics discontinue use after 18 months due to adverse parkinsonian-like and metabolic syndrome side effects.<sup>1</sup> Accumulating evidence suggests that the three clusters of SZ symptoms cannot be ascribed solely to alterations in monoaminergic signaling as dysregulation of glutamatergic,  $\gamma$ -aminobutyric acid (GABA)-ergic, and cholinergic systems have also been reported.<sup>3,33–35</sup> The therapeutic efficacy of AChEIs in ameliorating cognitive deficits in AD has led to the hypothesis that these same drugs could be effective as an

adjunct medication in SZ patients. Unfortunately, the results from clinical trials with AChEIs in SZ patients have been disappointing,<sup>36</sup> likely owing to dose-limiting effects caused by activation of peripheral receptors. Given the shortcomings of these current therapies, it is imperative that novel approaches are developed to provide more comprehensive clinical efficacy with reduced adverse side effects.

## Targeting muscarinic receptors for treatment of AD and SZ

The efficacy of AChEIs observed in patients with AD highlights the potential of cholinergic modulation in treating both cognitive- and psychosis-related behavioral disturbances. Furthermore, administration of nonselective muscarinic antagonists can induce cognitive deficits and psychosis in humans,<sup>16,37</sup> indicating that mAChR activation may provide pro-cognitive and antipsychotic efficacy. Accordingly, several mAChR agonists have been developed and have entered clinical testing with the goal of ameliorating the behavioral and cognitive deficits observed in numerous psychiatric diseases. Of these, the M<sub>1</sub>/M<sub>4</sub>-preferring agonist xanomeline was the only one to progress to a phase III clinical trial, where it was assessed for efficacy in ameliorating cognitive deficits observed in AD patients. While xanomeline showed a trend toward improving cognitive function in these patients, this effect did not reach statistical significance. However, this agonist did produce surprisingly robust and dose-dependent reductions in hallucinations, delusions, vocal outbursts, and other behavioral disturbances in these patients.<sup>38,39</sup> The efficacy of xanomeline in treating psychotic and behavioral disturbances in AD patients led to a more recent double-blinded, placebo-controlled outcome trial to determine if similar efficacy could be observed in patients with SZ. This study reported that xanomeline treatment produced robust improvements in both the positive and the negative symptoms of patients with SZ.<sup>40</sup> Both the magnitude and the time-course of xanomeline efficacy were superior to those previously reported with atypical antipsychotics, with statistically significant effects observed after only 1 week of treatment. In addition, xanomeline produced statistically significant improvements in verbal learning and short-term memory, indicating efficacy in treating cognitive symptoms.<sup>40</sup> Unfortunately, gastrointestinal side effects were observed, and dose limitations have removed it from consideration for long-term clinical use. However, these two seminal studies provide strong clinical validation of mAChRs as targets for the treatment of both psychotic and cognitive disturbances in AD and SZ.

## Allosteric modulation of muscarinic receptors

The efficacy of AChEIs in treating AD patients, in conjunction with the efficacy of xanomeline in improving both the cognitive and the behavioral disturbances observed in patients with either AD or SZ, highlight the potential of cholinergic modulation in treating these diseases. However, the poorly tolerated gastrointestinal side effects of AChEIs and xanomeline limit the clinical utility of these compounds.<sup>40,41</sup> The adverse side effects observed with nonselective modulation of the cholinergic system are thought to be primarily mediated by peripherally located  $M_2$  and  $M_3$  receptors.<sup>42</sup> Accordingly, it has been hypothesized that selective modulation of the  $M_1$ ,  $M_4$ , or  $M_5$  subtype could maintain the clinical efficacy observed with nonselective cholinergic treatments without the adverse-effect liability. A critical obstacle in these efforts has been the high conservation of the orthosteric ACh-binding site across the five mAChR subtypes, making it difficult to develop subtype-selective ACh-site ligands. However, an alternative strategy of targeting allosteric sites that are distinct from the ACh-binding site has been used with success at numerous G-protein-coupled receptors (for review, see Conn et al).<sup>43</sup> By targeting less conserved sites, it has been possible to develop compounds with unprecedented subtype selectivity. Allosteric ligands can modulate receptor signaling via multiple mechanisms. Allosteric agonists bind to an allosteric site and directly cause receptor activation. Alternatively, positive allosteric modulators (PAMs) bind to allosteric sites where they have no effect alone, but increase the affinity and/or efficacy of endogenous agonists. Because allosteric modulators do not directly activate the receptor, but instead potentiate activation by orthosteric ligands, they maintain the temporal and spatial signaling of cholinergic circuits. In some instances, molecules can act as both an allosteric agonist and an allosteric potentiator, indicating that these mechanisms of receptor regulation are not exclusive in nature.<sup>44</sup> As discussed below, the discovery of subtype-selective  $M_1$  and  $M_4$  agonists and modulators have greatly advanced our understanding of the importance of these receptors and emphasize the potential utility of targeting  $M_1$  and  $M_4$  in the treatment of AD and SZ.

## Targeting $M_1$ muscarinic receptors for cognitive symptoms observed with AD and SZ

The  $M_1$  mAChR subtype is the most predominantly expressed mAChR subtype in the CNS and is expressed in

several brain regions implicated in the regulation of cognitive processes, including the striatum, prefrontal cortex, and hippocampus.<sup>45,46</sup> Many of the studies examining the role of the  $M_1$  receptor in the CNS have utilized  $M_1$  knockout (KO) mice that do not express the  $M_1$  receptor. Interestingly, these  $M_1$ -deficient mice display increased amphetamine-induced hyperlocomotion and dopamine neurotransmission,<sup>47</sup> indicating that  $M_1$  modulation may have antipsychotic potential. However, the majority of studies have focused on the role of  $M_1$  in regulating cognitive processes. N-methyl-D-aspartate (NMDA) receptors play a critical role in regulating synaptic plasticity, and disrupted NMDA-receptor neurotransmission is thought to underlie the cognitive deficits observed in numerous psychiatric diseases.  $M_1$  mAChRs have been demonstrated to potentiate NMDA-receptor signaling in the hippocampus and cortex,<sup>48,49</sup> brain areas intimately associated with learning and memory. In addition,  $M_1$  KO mice displayed reduced hippocampal long-term potentiation, a mechanism heavily implicated in learning and memory. Behaviorally,  $M_1$  KO animals display deficits in several medial prefrontal cortex-dependent cognitive tasks, including non-matching-to-sample, win-shift radial arm maze, and social discrimination tasks.<sup>50</sup> Finally, studies in mice exhibiting AD-like A $\beta$  plaque pathologies found that deletion of  $M_1$  increased amyloidogenic processes, suggesting that  $M_1$  may play a role in regulating AD disease progression.<sup>51</sup> Collectively, these studies provided key rationale to pursue compounds targeting the  $M_1$  mAChR for the treatment of cognitive symptoms observed in neuropsychiatric diseases.

Numerous  $M_1$ -selective compounds have been discovered and subsequently tested in preclinical animal models of cognition (see Table 1 for complete list). A breakthrough came with the discovery of the first-generation  $M_1$  mAChR allosteric agonist AC-42. This compound was found to bind to an allosteric site and displayed  $M_1$ -selective functional activity when assessed at muscarinic subtypes *in vitro*.<sup>52</sup> However, this compound does not possess the physiochemical properties necessary for *in vivo* use.<sup>53</sup> Subsequent optimization produced two analogs of AC-42 (AC-260584 and 77-LH-28-1), which maintained  $M_1$  selectivity and possessed properties suitable for use in animal models. Both AC-260584 and 77-LH-28-1 displayed antipsychotic and cognition-enhancing efficacy in pre-clinical models.<sup>54–56</sup> Unfortunately, the efficacy of AC-260584 was confounded by nonselective effects on dopaminergic, adrenergic, and serotonergic receptors.<sup>57</sup> Another early allosteric agonist, TBPB (1-(1'-(2-methylbenzyl)-1,4'-bipiperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-1), also exhibited impressive selectivity for

**Table 1** Antipsychotic and pro-cognitive effects of M<sub>1</sub> and M<sub>4</sub> selective modulators

Receptor	Mode of action	Ligand	In vivo efficacy	Species
M <sub>1</sub>	Agonist	AC-260584	Increased retention of platform location in Morris water maze <sup>56</sup>	Mouse
			Increased novel object recognition <sup>54</sup>	Mouse
		77-LH-28-1	Increased hippocampal cell firing <sup>55</sup>	Rat
		TBPB	Reversed amphetamine-induced hyperlocomotion <sup>58</sup>	Rat
			Reversed apomorphine-induced disruption of prepulse inhibition <sup>59</sup>	Rat
			Reversed amphetamine-induced disruption of cued fear conditioning <sup>59</sup>	Rat
		VU0357017	Reversed scopolamine-induced disruption of contextual fear conditioning <sup>61</sup>	Rat
			Enhanced acquisition of contextual fear <sup>60</sup>	Rat
		VU0364572	Enhanced spatial learning in Morris water maze <sup>60</sup>	Rat
			Enhanced acquisition of contextual fear <sup>60</sup>	Rat
	PAM	GSK1034702	Reversed nicotine abstinence-induced impairment of episodic memory <sup>67</sup>	Human
		BQCA	Reversed scopolamine-induced disruption of contextual fear conditioning <sup>68</sup>	Mouse
			Increased wakefulness and inhibited delta sleep <sup>68</sup>	Rat
			Enhanced cerebral blood flow <sup>68</sup>	Rat
M <sub>1</sub> /M <sub>4</sub> M <sub>4</sub>	Agonist	Xanomeline	Decreased behavioral disturbances <sup>38,39</sup>	Human
	PAM	VU0152099	Reversed amphetamine-induced hyperlocomotion <sup>86</sup>	Rat
		VU0152100	Reversed amphetamine-induced hyperlocomotion <sup>86</sup>	Rat
		LY2033298	Reversed apomorphine-induced disruption of prepulse inhibition of the acoustic startle reflex <sup>84</sup>	Rat
			Decreased conditioned avoidance responding <sup>84</sup>	Rat
			Decreased conditioned avoidance responding <sup>87</sup>	Mouse
			Reversed amphetamine-induced hyperlocomotion <sup>88</sup>	Mouse

**Abbreviations:** AD, Alzheimer's disease; BQCA, benzyl quinolone carboxylic acid; mPFC, medial prefrontal cortex; M<sub>1</sub>, muscarinic acetylcholine receptor subtype 1; M<sub>4</sub>, muscarinic acetylcholine receptor subtype 4; PAM, positive allosteric modulator; TBPB, 1-(1'-(2-methylbenzyl))-1,4'-bipiperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-1.

M<sub>1</sub> mAChRs and potentiated NMDA receptor currents in CA1 hippocampal cells.<sup>58</sup> Moreover, additional pre-clinical studies with TBPB demonstrated efficacy in reducing antipsychotic-like behaviors and in reversing scopolamine-impaired acquisition of contextual fear.<sup>59</sup> Studies in cell lines also demonstrated that TBPB promoted a non-amyloidogenic pathway and decreased Aβ production, indicating that M<sub>1</sub> modulation may have efficacy in the treatment of both symptomatic and pathologic features of AD.<sup>58</sup> More recently, the M<sub>1</sub>-selective allosteric agonist VU0357017 was discovered, which displayed improved potency via binding to a novel allosteric site on the M<sub>1</sub> mAChR. VU0357017 significantly blocked scopolamine-impaired contextual fear conditioning and enhanced spatial and contextual fear learning.<sup>60,61</sup> Interestingly, recent studies suggest that many M<sub>1</sub> allosteric agonists, including 77-LH-28-1 and VU0357017, may act in a 'bitopic' manner, simultaneously binding at both allosteric and orthosteric sites.<sup>62-66</sup> In addition to the confounding issue of bitopic binding, some M<sub>1</sub> allosteric agonists display 'signal bias' or context-dependent pharmacology and differentially activate various downstream signaling pathways such as Ca<sup>2+</sup> mobilization and β-arrestin activation.<sup>60</sup> A recent

clinical study utilizing the M<sub>1</sub>-selective allosteric agonist GSK1034702 demonstrated pro-cognitive efficacy in a nicotine abstinence model of episodic memory impairment in smokers,<sup>67</sup> providing exciting evidence that M<sub>1</sub>-selective activation can provide pro-cognitive benefits in humans. Collectively, these preclinical and clinical findings with allosteric agonists highlight the potential utility of M<sub>1</sub>-selective activation in treating cognitive deficits observed with numerous CNS disorders.

Another approach extensively studied in recent years involves the development of PAM compounds that bind to allosteric sites on the M<sub>1</sub> mAChR and indirectly promote activity by enhancing the affinity and/or efficacy of the endogenous ligand ACh. The first subtype-selective M<sub>1</sub> PAM to be characterized was benzyl quinolone carboxylic acid (BQCA);<sup>68</sup> BQCA exhibited high selectivity with no activity at mAChR subtypes M<sub>2</sub>-M<sub>5</sub> and induced up to a 129-fold leftward shift in ACh potency at the M<sub>1</sub> mAChR.<sup>68,69</sup> In brain slice electrophysiology studies, BQCA enhanced excitatory postsynaptic currents in medial prefrontal cortical neurons,<sup>69</sup> an area critical for higher cognitive, learning, and memory functions.<sup>70</sup> In pre-clinical animal studies, BQCA reversed

scopolamine-impaired contextual fear conditioning and rescued medial prefrontal cortex-dependent discrimination reversal learning deficits in a transgenic mouse model of AD.<sup>68,69</sup> Additionally, recent studies demonstrated that BQCA was effective in reversing memory deficits in Y-maze object recognition and spontaneous alternation tasks in rats.<sup>71,72</sup> The discovery and characterization of BQCA exemplified the promising potential for allosteric modulators to selectively target M<sub>1</sub> mAChRs, and has since provided a basis for further development of similar M<sub>1</sub> PAMs.<sup>73</sup> Recent drug discovery efforts in our group have yielded novel M<sub>1</sub>-selective PAMs VU0405652 (ML169) and VU0456940, both of which potentiate M<sub>1</sub>-mediated non-amyloidogenic amyloid precursor protein (APP $\alpha$ ) processing, suggesting disease-modifying potential in AD.<sup>74,75</sup> Collectively, these studies provide evidence supporting the therapeutic potential of selectively targeting M<sub>1</sub> mAChRs in the treatment of AD and SZ. However, continued development and characterization of M<sub>1</sub>-selective compounds is needed to fully elucidate the potential of M<sub>1</sub>-modulation in mediating symptomatic and disease-modifying efficacy in AD and SZ.

## Targeting M<sub>4</sub> muscarinic receptors for psychotic symptoms observed with AD and SZ

Recent evidence suggests that modulation of the M<sub>4</sub> receptor may provide a novel avenue for the development of antipsychotic drugs. The psychotic symptoms associated with SZ are thought to be intimately associated with hyperactive dopaminergic signaling in striatal and mesocortical pathways. Clinically prescribed typical and atypical psychotics show efficacy in reducing psychosis and exert their effects primarily via antagonizing the D<sub>2</sub> dopamine receptor. Nonselective mAChR agonists can reduce striatal dopamine release,<sup>76</sup> while administration of nonselective mAChR antagonists can induce psychosis in humans<sup>37</sup> and disrupt sensorimotor gating in the preclinical prepulse inhibition rodent model.<sup>77</sup> Conversely, the nonselective mAChR agonist BuTAC ([5R-(exo)]-6-[4-butylthio-1,2,5-thiadiazol-3-yl]-1-azabicyclo-[3.2.1]-octane) shows an antipsychotic profile when tested in numerous preclinical animal models. Administration of BuTAC reduces apomorphine-induced climbing and apomorphine-induced disruptions of prepulse inhibition<sup>78,79</sup> and reduces conditioned avoidance responding in wild-type, but not M<sub>4</sub> KO mice.<sup>80</sup> Collectively, these results suggest that activation of mAChRs, and M<sub>4</sub> in particular, may provide a novel strategy for treating psychotic symptoms in AD and SZ.

As mentioned above, clinical trials for xanomeline yielded the striking finding that administration of the M<sub>1</sub>/M<sub>4</sub>-preferring agonist significantly reduced psychosis-related symptoms in both AD and SZ patients.<sup>38–40</sup> The M<sub>4</sub> receptor is highly expressed in the striatum, hippocampus, and neocortex,<sup>45,46</sup> suggesting that this mAChR subtype is ideally located to modulate dopaminergic signaling. In support of this hypothesis, M<sub>4</sub> KO mice exhibit a hyperdopaminergic phenotype that is resistant to mAChR agonist-induced attenuation of dopamine levels.<sup>76,81</sup> Selective deletion of M<sub>4</sub> mAChRs on D<sub>1</sub> dopamine receptor-expressing neurons resulted in increased locomotor activity and behavioral sensitization to psychostimulants.<sup>82</sup> Additionally, the antipsychotic efficacy of xanomeline in preclinical animal models is attenuated in animals in which this subpopulation of M<sub>4</sub> receptors is deleted.<sup>83</sup> These findings support the hypothesis that M<sub>4</sub> mAChRs represent a viable novel drug target for the treatment of psychosis in SZ, AD, and other neurological disorders.

Two novel M<sub>4</sub>-selective compounds, VU10010 and LY2033298, represented a breakthrough when they were described in 2008.<sup>84,85</sup> VU10010 is a potent M<sub>4</sub>-selective PAM that increases affinity/efficacy of ACh to promote M<sub>4</sub> mAChR activation. In brain slices, VU10010 selectively potentiated mAChR-mediated reductions in glutamatergic, but not GABAergic, signaling in hippocampal neurons, indicating a key role for M<sub>4</sub> in regulating hippocampal function, and possibly in modulating cognition. Though these findings were major advances in validating the concept of selective M<sub>4</sub> PAMs at a cellular and molecular level, VU10010 does not possess physiochemical properties suitable for in vivo dosing (displaying a high log *P*-value of 4.5), limiting the utility of this tool compound.<sup>85</sup> Subsequent optimization of VU10010 led to the discovery of VU0152100 and VU0152099, both of which possessed improved chemical and pharmacokinetic characteristics making them suitable for use in rodent models. Both compounds possess log *P*-values a full order of magnitude less lipophilic than VU10010, resulting in improved solubility and affording homogeneous dosing solutions in multiple vehicles acceptable for in vivo studies.<sup>86</sup> Additionally, VU0152100 and VU0152099 exhibited substantial systemic absorption and brain penetration following intraperitoneal administration.<sup>86</sup> Both VU0152100 and VU0152099 effectively reversed amphetamine-induced hyperlocomotion, demonstrating antipsychotic-like activity in preclinical models. LY2033298, a structurally distinct M<sub>4</sub>-selective PAM, was similarly efficacious in several preclinical models of psychosis, including conditioned avoidance responding and

apomorphine-impaired prepulse inhibition.<sup>84,87,88</sup> Together, these preliminary efforts suggest that the antipsychotic effects of xanomeline may be primarily due to M<sub>4</sub> mAChR activation. The development of M<sub>4</sub>-selective PAMs has proven to be very fruitful in substantiating the efficacy in targeting M<sub>4</sub> mAChRs for the potential treatment of the positive symptoms of SZ. In addition, preliminary studies suggest that selective activation may enhance cognitive domains of learning and memory. However, further in vivo characterization of M<sub>4</sub> is required to elucidate the therapeutic potential of M<sub>4</sub> modulation in treating the cognitive impairments and psychotic symptoms associated with SZ and AD.

## Summary

The recent discovery of novel allosteric agonists and modulators of M<sub>1</sub> and M<sub>4</sub> mAChRs have further validated the approach of targeting these muscarinic subtypes in the treatment of cognitive and behavioral impairments present in AD and SZ. Despite the fact that most discovery efforts are still in the preclinical phase of development, there are now several tool compounds that continue to provide important findings, furthering our fundamental understanding of the role of mAChRs and these debilitating neuropsychiatric diseases. Data from clinical trials demonstrating the efficacy of xanomeline in AD and SZ patients have created intense interest in the pursuit of highly selective M<sub>1</sub> and M<sub>4</sub> activators to ultimately provide novel therapeutic options with minimal adverse side effects.

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