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Subtype-selective allosteric modulators of muscarinic receptors for the treatment of CNS disorders

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Abstract

Muscarinic acetylcholine receptors (mAChRs) have long been viewed as viable targets for novel therapeutic agents for the treatment of Alzheimer's disease (AD) and other disorders involving impaired cognitive function. More recent evidence indicates that mAChR activators might also have utility in treating psychosis and other symptoms associated with schizophrenia and other central nervous system (CNS) disorders. Efforts to develop mAChR subtype-selective agonists have been hampered by difficulty in achieving high selectivity for individual mAChR subtypes important for CNS function (M₁ and M₄) and adverse effects due to activation of peripheral mAChRs (especially M₂ and M₃). Major advances have now been achieved in the discovery of allosteric agonists and positive allosteric modulators of M₁ and M₄ that show greater selectivity for individual mAChR subtypes than do previous mAChR agonists. Early studies indicate that these allosteric mAChR activators have properties needed for optimization as potential clinical candidates and have robust effects in animal models that predict efficacy in the treatment of AD, schizophrenia and related disorders.

Introduction

Acetylcholine (ACh)-containing neurons originating in the medial septum and the diagonal band of Broca provide one of the most crucial neuromodulatory inputs to the forebrain in humans and other mammals [1]. These cholinergic projections make synaptic contact with widespread but highly specific targets in a variety of forebrain regions including the basal ganglia, cortex and hippocampus. In addition, there are several other crucial cholinergic systems in the central nervous system (CNS) including cholinergic interneurons in the striatum, nucleus accumbens, olfactory tubercle and Islands of Calleja, in addition to cholinergic projection neurons in the substantia innominata, magnocellular preoptic field and nucleus basalis [1–4]. These nuclei send projections throughout the neocortex and limbic areas and have a crucial role in information processing and multiple forms of learning

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and memory [3,5,6]. In addition, cholinergic projections have been implicated in a variety of other CNS functions including nociception, regulation of sleep–wake cycles, motor control and arousal. Based on this broad influence of cholinergic pathways in the CNS, agents that regulate cholinergic transmission have been proposed to have potential efficacy in a wide variety of CNS disorders including chronic and neuropathic pain, sleep disorders, epilepsy, schizophrenia, Alzheimer's disease (AD), Parkinson's disease and other movement disorders [5–9].

Therapeutic potential of M₁ for the treatment of AD Based on the broad influence of cholinergic systems in the CNS, it is surprising that there have not been greater advances in the development of therapeutic agents that target cholinergic signaling. Efforts to develop agents that enhance cholinergic transmission for ameliorating the loss of cognitive function in patients with AD and other memory disorders have been partially successful, and clinical trials with tacrine and other acetylcholinesterase (AChE) inhibitors have established dose-related improvements in measures of cognitive performance and quality of life [3,10]. However, efficacy of these drugs in enhancing cognition is limited, in part because of central and peripheral side effects that are due to generalized activation of multiple cholinergic systems and receptor subtypes.

Evidence indicates that cholinergic transmission in the forebrain regions and cholinergic involvement in learning and memory are mediated primarily by muscarinic ACh receptors (mAChRs) [1,6]. It is possible that selective agonists of mAChR subtypes that are most crucial for regulation of processes involved in cognitive function could provide an advantage to AChE inhibitors for the treatment of AD and related disorders. The mAChRs are members of the family A G-protein-coupled receptors (GPCRs) and include five subtypes termed M₁–M₅. M₁, M₃ and M₅ couple to G_q and activate phospholipase (PL)C, whereas M₂ and M₄ couple to G_{i/o} and associated effector systems (Figure 1) such as ion channels and adenylyl cyclase [6,11]. Evidence indicates that the most prominent adverse effects of AChE inhibitors and other cholinergic agents are mediated by activation of peripheral M₂ and M₃ mAChRs and include bradycardia, gastrointestinal distress, excessive salivation and sweating [7,9]. By contrast, M₁ has been viewed as the most likely subtype for mediating the effects on cognition, attention mechanisms and sensory processing. Because of this, considerable effort has been focused on developing selective M₁ agonists for the treatment of AD [5,6]. Unfortunately, these efforts have been largely unsuccessful because of an inability to develop compounds that are highly selective for the M₁ mAChR. Because of this, mAChR agonists that have been tested in clinical studies induce the same adverse effects of AChE inhibitors by activation of peripheral mAChRs. To fully understand the physiological roles of individual mAChR subtypes and to further explore the therapeutic utility of mAChR ligands in AD and other disorders, it will be crucial to develop molecules that are highly selective activators of M₁ and other individual mAChR subtypes.

Discovery of subtype-selective allosteric modulators of mAChRs

Previous attempts to develop agonists that are highly selective for individual mAChR subtypes have failed because of the high conservation of the orthosteric ACh-binding site and difficulty developing compounds that are truly subtype specific. Although there have been reports of subtype-selective M₁ agonists in the patent and primary literature, subsequent studies across multiple systems revealed that previous orthosteric agonists are not highly selective when evaluated across multiple systems. A reason for this is that selectivity of these agents is based on functional selectivity or selectivity based on efficacy rather than selective binding to individual mAChR subtypes. Because of this, a weak partial agonist at multiple mAChR subtypes might seem to be selective in cell lines in which there is little or no receptor reserve but might have robust agonist activity at these same mAChR

subtypes in systems that have high receptor reserve. Because high receptor reserve is common for mAChRs in native tissues, these agents often activate multiple mAChR subtypes in animal models and humans [6,9,11]. A more recent example, AF267, was suggested to have selective agonist activity at M₁ but has similar profiles to previous orthosteric agonists and activates multiple mAChR subtypes (i.e. M₁, M₃ and M₅) [12]. These studies do not rule out the possibility that highly selective orthosteric agonists of individual mAChR subtypes will eventually be developed but do raise a need for also considering other strategies for selectively activating members of this receptor family.

An alternative approach to targeting the highly conserved orthosteric ACh site is to develop compounds that act at allosteric sites on mAChRs that are removed from the orthosteric site and might be less highly conserved. This approach is proving to be highly successful in developing selective ligands for multiple GPCR subtypes (for reviews, see Refs [13,14]). In the case of mAChRs, a major goal has been to develop allosteric ligands that selectively increase the activity of M₁ or M₄. Allosteric activators can include allosteric agonists, which act at a site removed from the orthosteric site to directly activate the receptor in the absence of ACh, in addition to positive allosteric modulators (PAMs), which do not activate the receptor directly but potentiate activation of the receptor by the endogenous orthosteric agonist ACh. Also, it is possible for a single molecule to have both allosteric potentiator and allosteric agonist activity [13,14].

Early work by several laboratories [15–18] identified a diverse array of chemotypes that possess PAM activity at the M₁ and M₄ mAChR subtypes (Figure 2). The first mAChR PAM reported was brucine (structure 1; Figure 2) [15,18], which acts as an allosteric potentiator at M₁ and induces a relatively small increase in the affinity of M₁ for carbachol (CCh) or ACh. Brucine is a weak M₁ PAM requiring high micromolar concentrations for activity and induces only a twofold increase in ACh affinity at the M₁ receptor. However, brucine is relatively selective for M₁ and this provided an important proof of concept that it will be possible to develop selective PAMs for this important mAChR subtype. Interestingly, the corresponding N-oxide of brucine (structure 2 in Figure 2) increased ACh affinity

1.5-fold at the M₄ receptor. Equilibrium and nonequilibrium radioligand-binding studies with brucine, the mAChR antagonist radioligand [³H]-NMS and unlabeled ACh were consistent with a ternary allosteric model in which both the orthosteric and allosteric ligands bind to the receptor simultaneously and modify the affinities of each other.

Discovery of brucine was followed by the discovery of thiochrome (structure 3; Figure 2) as an M₄ PAM [17] and SCH-202676 (structure 4; Figure 2) as a PAM of multiple GPCR subtypes including M₁ [16,19]. Thiochrome was especially interesting in that it provided M₄ subtype selectivity. This compound increases the affinity of ACh three- to fivefold at M₄ and increased ACh potency in activating M₄ in functional assays but has no effect on ACh affinity at M₁, M₂ or M₅ [17]. Interestingly, thiochrome has no effect on the equilibrium binding of [³H]-NMS to the five human mAChRs (M₁–M₅) but inhibits the [³H]-NMS dissociation from M₁–M₄ [17]. This indicates that the selectivity of thiochrome for M₄ is due to selective cooperativity rather than selective interactions with this single mAChR subtype.

In addition to allosteric potentiators, early efforts led to discovery of highly selective allosteric agonists of M₁. For instance, AC-42 (structure 5; Figure 2) [20] was identified as the first M₁ agonist that seems to act by binding to an allosteric site rather than the orthosteric ACh site. AC-42 fully activates M₁ and is highly selective for M₁ relative to other mAChR subtypes (Figure 2). As with allosteric potentiators, this selectivity is likely

to be achieved by targeting a site distinct from the ACh-binding site. Thus, mutations that render M_1 insensitive to ACh do not alter the activity of AC-42, but the activity of AC-42 can be eliminated by mutations in transmembrane domains one and seven that do not alter activation by ACh [20]. N-desmethylozapine (structure 6; Figure 2) is another M_1 agonist that demonstrates a mode of interaction with the M_1 receptor that differs from that of classic orthosteric ligands and might be mechanistically similar to AC-42 [21].

Unfortunately, brucine, AC-42 and other early mAChR allosteric agonists and PAMs lacked the pharmacological profiles and physicochemical properties required to be useful tools to probe the effect of allosteric activation of mAChRs in more complex native systems. For instance, these compounds have off-target activities at other receptors and have relatively low potencies at M_1 or M_4 . This, combined with poor solubility in physiological buffer systems, prevented their use for studies in tissue or animal model systems. However, discovery of these early compounds provided an important advance in establishing the potential of developing more selective activators of M_1 and other mAChR subtypes by targeting allosteric sites.

Discovery of highly selective and systemically active M_1 PAMs

Over the past two years, several novel selective M_1 agonists and allosteric potentiators have been identified. These compounds are providing important new tools to evaluate the potential utility of selective activators of M_1 for treatment of AD and other CNS disorders. For instance, TBPB (structure 7; Figure 3) [12,22,23] and 77-LH-28-1 (structure 8; Figure 3) [24,25] have been reported as highly selective agonists of M_1 relative to other mAChR subtypes (Figure 3). Both of these compounds are systemically active and are proving to be useful in *in vivo* studies of M_1 activation. TBPB selectively activates M_1 in cell lines and has no agonist activity at any other mAChR subtypes. Mutations that reduce the activity of orthosteric agonists have no effect on the response to TBPB, and Schild analysis of the blockade of TBPB effects with the orthosteric antagonist atropine reveals that TBPB does not interact with the orthosteric site in a competitive manner. These data are consistent with the predictions of an allosteric ternary complex model for the actions of two molecules that interact with distinct sites [13,14] and are consistent with the hypothesis that TBPB acts as an allosteric M_1 agonist [12]. However, extensive molecular pharmacology and mutagenesis studies will be required to fully establish the mechanism of TBPB action and verify that this compound activates M_1 by actions at an allosteric site that is completely distinct from the orthosteric site.

Interestingly, TBPB induces M_1 -dependent potentiation of N-methyl-D-aspartic acid (NMDA)-receptor currents in hippocampal pyramidal cells, an action that is thought to be important for the cognition-enhancing effects of mAChR activation. By contrast, TBPB does not reduce inhibitory or excitatory synaptic transmission in these neurons; these are thought to be mediated by M_2 and M_4 mAChRs, respectively [12]. These findings verify the ability of TBPB to activate M_1 and the selectivity of TBPB for M_1 relative to other mAChR subtypes in a native system. Interestingly, early studies in cell lines indicate that TBPB induces an increase in processing of the amyloid precursor protein towards the non-amyloidogenic pathway and decreased amyloid β production *in vitro* [12]. This is consistent with previous extensive studies indicating that mAChR activation has favorable effects on amyloid precursor protein processing in animal models and perhaps in humans [5,26] and provides further support to the hypothesis that selective activation of M_1 might have disease-modifying effects in the treatment of AD in addition to its potential efficacy in acutely enhancing cognition in patients suffering from this disorder. TBPB is systemically active and crosses the blood-brain barrier, making this a useful tool for studies of cognition-enhancing effects of M_1 -selective agonists. However, TBPB did not have any of the

peripheral adverse effects seen with other less selective mAChR agonists when evaluated in a modified Irwin battery in rodents and compared with oxotremorine; this indicates that the in vitro mAChR selectivity profile is mirrored in vivo [12]. These encouraging results provide strong support for the utility of M₁ allosteric agonists in activating this crucial mAChR subtype in the CNS without inducing the adverse effects associated with less selective mAChR agonists.

A second systemically active M₁ agonist, 77-LH-28-1 was discovered as a structural analog of AC-42 [24,25]. 77-LH-28-1 is highly selective for M₁ relative to other mAChR subtypes but does have weak agonist activity at M₃ [24]. Interestingly, in contrast to effects of atropine on the response to TBPB, the orthosteric antagonist scopolamine induces parallel rightward shifts in the 77-LH-28-1 concentration–response relationship [24]. Consistent with this, recent radioligand-binding studies reveal that 77-LH-28-1 binds to the orthosteric site in a competitive manner [27]. However, extensive mutagenesis studies combined with functional studies and analysis of the effects of 77-LH-28-1 on binding of an orthosteric antagonist ligand are consistent with an allosteric mode of agonist action of this compound. These studies also raise the possibility that 77-LH-28-1 might be what has been referred to as a ‘bi-topic’ ligand that binds to a site that overlaps with the orthosteric site but also includes an allosteric site that modulates orthosteric-site affinity [27]. Electrophysiological studies indicate that 77-LH-28-1 increases hippocampal activity as assessed by increases in single-unit firing of hippocampal CA1 pyramidal cells in vitro and in vivo and induction of synchronous network activity in the gamma and/or theta frequency bands in the hippocampus [24]. Interestingly, 77-LH-28-1 selectively activates the coupling of M₁ to G_{α_q} and G_{α_s} signaling without activating the coupling of M₁ to G_{α_i} in Chinese hamster ovary cells [25]; this indicates that this compound might differentially activate different responses to M₁ activation. Such differential effects of allosteric agonists on different responses to M₁ activation could prove to be crucially important in determining the in vivo and ultimately therapeutic potential of allosteric M₁ agonists and raise the need to fully evaluate these novel models in animal models and ultimately in clinical studies.

Findings that TBPB and 77-LH-28-1 might differentially alter activities of orthosteric site ligands indicate that these compounds might act at by different mechanisms to activate M₁. Mutagenesis and binding studies also indicate that AC-42 and 77-LH-28-1 interact with the receptor in a similar manner [27]. This is interesting in light of the structural similarities between AC-42 and 77-LH-28-1 and the fact that these compounds are structurally distinct from TBPB. On the surface, these data might indicate that multiple allosteric sites are present on M₁ (as was previously demonstrated for class C GPCRs such as metabotropic glutamate mGlu₅ receptors [13,14,28]). However, it is also possible that different allosteric agonists interact with overlapping sites and that there is no strict allosteric ‘pharmacophore’ that is clearly related to selective interaction at this site. The latter point can be addressed within the TBPB series, in which subtle structural changes have been shown to erode mAChR subtype selectivity to provide non-selective mAChR agonists; this indicates that close structural similarity does not ensure selective allosteric-site interactions [29,30].

In addition to the discovery of novel M₁-selective allosteric agonists, exciting progress has been made in the discovery of novel M₁ PAMs that serve as allosteric potentiators of this receptor. For instance, multiple M₁ allosteric potentiators (structures 9–12; Figure 4) have recently been identified in a high-throughput functional-screening campaign [31]. These molecules belong to several structurally distinct chemical scaffolds and include compounds that are selective for M₁ relative to other mAChR subtypes. None of the compounds identified had agonist activity but each behaved as a pure PAM and induced parallel leftward shifts in the ACh concentration–response relationships. None of the novel M₁ PAMs competes for binding at the orthosteric ACh-binding site. The two most selective

compounds, VU0090157 (structure 11; Figure 4) and VU0029767 (structure 12; Figure 4), were studied in detail and induced progressive shifts in ACh affinity at M_1 that are consistent with their effects in a functional assay; this indicates that they mechanistically enhance M_1 activity by increasing ACh affinity. However, these compounds were strikingly different in their ability to potentiate responses at a mutant M_1 receptor that exhibits decreased affinity for ACh and in their ability to affect responses of the allosteric M_1 agonist TBPB. Furthermore, VU0090157 induced similar potentiation in M_1 activation of PLC and PLD activity, whereas VU0029767 potentiated activation of PLC but not PLD signaling. This provides another example of an ability of novel M_1 PAMs to differentially regulate coupling of the receptor to different signaling pathways. Based on this, it is possible that different M_1 PAMs will have different actions on different responses to M_1 activation in native systems.

In a preliminary report, researchers reported an encouraging advance in the discovery of benzyl quinolone carboxylic acid (BQCA; structure 13; Figure 4) as a highly selective and efficacious allosteric potentiator for M_1 [32,33]. As with VU0090157 and VU0029767, BQCA has no direct agonist activity but induces a robust leftward shift of the concentration–response relationship of ACh at activating M_1 . BQCA has no activity at M_2 , M_3 or M_4 . Interestingly, BQCA is systemically active and fully reverses the cognitive impairment induced by scopolamine in a contextual fear-conditioning model of episodic-like memory [32]. In addition, BQCA induces changes in electroencephalography oscillations in a manner that is consistent with a potential cognition-enhancing effect [33]. These preliminary findings provide exciting new data in support of the hypothesis that it will be possible to develop highly selective M_1 PAMs that have potential for cognition-enhancing effects in animal models.

It is important to note that, at present, the mechanisms underlying the selectivities of M_1 -selective allosteric agonists are not fully established. The initial rationale driving this approach is that allosteric ligands bind to evolutionarily less-conserved allosteric sites on the M_1 receptor, as opposed to the highly conserved orthosteric (ACh) binding site and might, therefore, bind selectively to allosteric sites on individual mAChR subtypes. This hypothesis has been clearly established for metabotropic glutamate receptors where selective binding of allosteric ligands to individual subtypes has been clearly established as the basis for the selectivity of some ligands [13]. Also, the finding that M_1 PAMs do not bind to the orthosteric site on M_1 or any other mAChR subtypes and selectively increase ACh affinity at the orthosteric site of M_1 but not M_2 – M_5 is consistent with this hypothesis. However, this does not rule out the possibility that these compounds bind to allosteric sites on multiple mAChR subtypes with similar affinities but that selectivity is conferred by selective cooperativity with orthosteric-site agonists [13,14], as has been suggested for selective PAM activity of thiochrome on M_4 [17]. If this is the case, it is possible that selectivity of these compounds will vary depending on the system in which their effects are measured. Thus, it will be crucial to develop a clear understanding of the molecular basis for the observed selectivity in future studies. Optimally, this should be addressed with radioligands that act at the allosteric but not the orthosteric sites. However, studies involving detailed analysis of effects on binding of orthosteric-site ligands in addition to mutagenesis studies can also shed light on this important issue.

Allosteric modulators of mAChRs have efficacy as novel antipsychotic agents of the orthosteric mAChR agonists that have been developed in clinical trials for the treatment of AD, an M_1/M_4 agonist termed xanomeline advanced furthest in clinical development [34,35]. Although potential cognition-enhancing effects of this compound in Phase III trials were encouraging, the most surprising finding in the xanomeline trial was that this M_1/M_4 agonist had robust therapeutic effects on psychotic symptoms and behavioral

disturbances associated with AD [34,35]. Xanomeline induced robust dose-dependent reductions in vocal outbursts, suspiciousness, delusions, hallucinations, mood swings and other behavioral disturbances. This is consistent with a growing body of clinical evidence that AChE inhibitors have efficacy in treating behavioral disturbances and psychotic symptoms in patients suffering from AD in addition to Lewy body dementia, Parkinson's disease dementia, and vascular dementia [36–42]. More recently, xanomeline [43] was shown to induce a robust improvement in positive and negative symptoms and also provide improvements in some measures of cognitive function in schizophrenic patients. Interestingly, the response was superior to that seen with traditional antipsychotics, and therapeutic effects were seen in less than one week, as opposed to the multi-week delay for traditional anti-psychotics. Together, these studies provide strong clinical validation of mAChR agonists as novel therapeutic agents used for the treatment of psychosis and behavioral disturbances in patients suffering from a broad range of disorders including schizophrenia, AD and other neurodegenerative disorders.

Although the exact mAChR subtype responsible for the effects of xanomeline in schizophrenia patients is not certain, xanomeline is an M₁/M₄-preferring agonist and multiple animal studies indicate that one or both of these mAChR subtypes is likely to be responsible for the clinical efficacy of this compound [6,7,44–49]. Thus, it will be important to rigorously evaluate the potential roles of both of these receptor subtypes in animal models that predict efficacy in the different symptom clusters associated with schizophrenia (i.e. positive, negative and cognitive symptoms). Interestingly, the M₁-selective allosteric agonist TBPB has activity in multiple animal models used to predict the efficacy in treatment of positive symptoms of schizophrenia [12]. These include the reversal of amphetamine-induced hyperlocomotor activity and disruption of prepulse inhibition, in addition to changes in c-fos expression that are almost identical to changes induced by atypical antipsychotic agents. These data indicate that selective activators of M₁ might mimic some of the effects of xanomeline in animal models that are relevant to the clinical efficacy of the less selective mAChR agonist.

An important breakthrough for selective activation of M₄ occurred with the discovery of VU0010010 (structure 14; Figure 5) as a highly selective M₄ PAM with no activity at any other mAChR subtype [50]. VU0010010 is an allosteric potentiator and does not activate M₄ directly but dramatically potentiates the response of the receptor to ACh. Extensive in vitro pharmacological characterization reveals that VU0010010 binds to an allosteric site to increase the affinity of M₄ for ACh and increase the efficiency of coupling of M₄ to G proteins, and that it does not bind to, or functionally modulate, any other mAChR subtype. VU0010010 induces a selective potentiation of M₄-mediated depression of transmission at excitatory synapses in hippocampal CA1 pyramidal cells, but it has no effect on responses that are thought to be mediated by M₁ or M₂. More recently, two related compounds were reported, VU0152099 and VU0152100 (structures 15 and 16; Figure 5), that are also highly selective for M₄, readily cross the blood–brain barrier, and have pharmacokinetic properties making them highly suitable for behavioral studies [51]. Interestingly, VU0152100 and VU0152099 reverse amphetamine-induced hyperlocomotion in rats [51], which indicates that M₄ PAMs have efficacy in at least one model used to predict antipsychotic efficacy. Chan and colleagues [52] reported the discovery of a similar, but structurally distinct, M₄ PAM termed LY2033298 (structure 17; Figure 5). This compound exhibits highly selective M₄ potentiator activity similar to that of VU0010010. Interestingly, LY2033298 has also been shown to possess in vivo efficacy in reducing the conditioned avoidance response, which is another rodent paradigm predictive of antipsychotic drug efficacy. Collectively, the studies with M₁ and M₄ allosteric modulators clearly demonstrate the potential of targeting allosteric sites for developing highly selective activators of individual mAChR subtypes and indicate that both M₁ and M₄ might provide viable targets for the development of novel anti-

psychotic agents. Because rat preclinical models are employed, an important point with respect to these M₄ PAMs concerns species differences. Uniformly VU0152099, VU0152100 and LY2033298 are approximately tenfold more potent on human M₄ than rat M₄ with fold-shifts of comparable or greater magnitude, and mAChR subtype-selectivity remains intact.

A final point that is interesting to note is that, to date, there have been no reports of negative allosteric modulators of M₁ or M₄, which could potentially provide a novel therapeutic approach for the treatment of disorders such as Parkinson's disease, dystonia or epileptic disorders. Moreover, unlike the metabotropic glutamate receptors [53,54], there have not yet been any reports of mAChR PAMs being converted into mAChR negative allosteric modulators (NAMs) or neutral modulators. However, based on successes in discovery of NAMs for other GPCR subtypes, it is likely that allosteric modulators might also provide a viable approach to the discovery of selective allosteric antagonists for individual mAChR subtypes.

Conclusions

Exciting new data from animal and clinical studies indicate that ligands at mAChR subtypes might provide a novel approach to the treatment of multiple CNS disorders. This includes compelling evidence that selective increases in the activity of M₁ and M₄ could provide a novel approach to the treatment of AD and schizophrenia and perhaps have efficacy in treating psychosis and cognitive impairment in other neurodegenerative diseases. Major advances have been made in establishing selective allosteric agonists and PAMs of both M₁ and M₄ as an alternative approach to orthosteric agonists for the development of selective receptor activators. These molecules have now been optimized for use in animal models, and early studies indicate that they have effects in animal models that predict efficacy in the treatment of these disorders. In addition to potential clinical utility of selective mAChR activators, abundant clinical and animal studies indicate that highly selective antagonists of specific mAChR subtypes might have utility in the treatment of other CNS disorders including dystonia, Parkinson's disease, epilepsy and others [5–9]. In each case, subtype selectivity will be a key to achieving clinical efficacy in the absence of adverse effects. For other GPCRs, it has been possible to discover allosteric modulators that serve as either activators or inhibitors (i.e. NAMs) of receptor function [12]. Thus, it might be possible to discover selective allosteric antagonists of mAChR subtypes. Indeed, a recent high-throughput screening campaign that focused on M₁ PAMs was also successful in identifying NAMs of mAChR subtypes [31]. These exciting advances are providing a fundamental advance in our approaches to regulating mAChRs as drug targets and might provide a novel approach to treatment of AD and schizophrenia in addition to other CNS disorders.

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Figure 1.

Schematic illustration of structure and effector systems of the mAChR subtypes. M₁, M₃ and M₅ typically couple to G_q and associated effector systems such as PLC. M₂ and M₄ typically couple to G_{i/o} and associated effector systems such as inhibition of adenylyl cyclase (AC) and ion channels, although each receptor subtype can also couple to other signaling pathways. The orthosteric binding site for ACh has been well characterized and resides in the seven transmembrane domain of the receptor. Allosteric ligands bind to other sites on the receptor to either activate (allosteric agonists) or modulate (positive and negative allosteric-site ligands) receptor function. Abbreviations: IP₃, inositol (1,4,5)-trisphosphate; ? denotes that exact allosteric binding sites have not been defined.

Figure 2.
Early PAMs and allosteric agonists of the mAChRs.

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Figure 3.
M₁ allosteric agonists.

Figure 4.
 M_1 positive allosteric modulators.

Figure 5.
M₄ positive allosteric modulators.