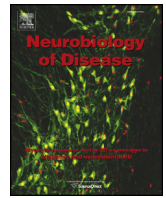




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## Review

## Development of allosteric modulators of GPCRs for treatment of CNS disorders

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## ABSTRACT

The discovery of allosteric modulators of G protein-coupled receptors (GPCRs) provides a promising new strategy with potential for developing novel treatments for a variety of central nervous system (CNS) disorders. Traditional drug discovery efforts targeting GPCRs have focused on developing ligands for orthosteric sites which bind endogenous ligands. Allosteric modulators target a site separate from the orthosteric site to modulate receptor function. These allosteric agents can either potentiate (positive allosteric modulator, PAM) or inhibit (negative allosteric modulator, NAM) the receptor response and often provide much greater subtype selectivity than orthosteric ligands do for the same receptors. Experimental evidence has revealed more nuanced pharmacological modes of action of allosteric modulators, with some PAMs showing allosteric agonism in combination with positive allosteric modulation in response to endogenous ligand (ago-potentiators) as well as “bitopic” ligands that interact with both the allosteric and orthosteric sites. Drugs targeting the allosteric site allow for increased drug selectivity and potentially decreased adverse side effects. Promising evidence has demonstrated potential utility of a number of allosteric modulators of GPCRs in multiple CNS disorders, including neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease, as well as psychiatric or neurobehavioral diseases such as anxiety, schizophrenia, and addiction.

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**Abbreviations:** 5MPEP, 5-methyl-6-(phenylethynyl)-pyridine; 6-OHDA, 6-hydroxydopamine; 7TMR, seven transmembrane receptor; 77-LH-28-1, 1-[3-(4-butyl-1-piperidinyl)propyl]-3,4-dihydro-2(1H)-quinolinone; AC-42, 4-n-butyl-1-[4-(2-methylphenyl)-4-oxo-1-butyl]-piperidine; AChE, acetylcholinesterase; ACPT-1, (1S,3R,4S)-1-aminocyclopentane-1,3,4-tricarboxylic acid; AD, Alzheimer’s disease; ADX71743, (+)-6-(2,4-dimethylphenyl)-2-ethyl-6,7-dihydrobenzo[d]oxazol-4(5H)-one; AFQ056, (3a,5S,7aR)-methyl 5-hydroxy-5-(m-tolylethynyl)octahydro-1H-indole-1-carboxylate; APP, amyloid precursor protein; BINA, potassium 30-[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5yl)oxy]methyl)biphenyl 1-4-carboxylate; BQCA, benzylquinolone carboxylic acid; CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; CFMMC, 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4H-chromen-4-one; CNS, central nervous system; CPPHA, N-[4-chloro-2[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl]-2-hydrobenzamide; CTEP, 2-chloro-4-[(2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl]pyridine; DA, dopamine; DFB, [(3-fluorophenyl)methylene]hydrazone-3-fluorobenzaldehyde; DHPG, dihydroxyphenylglycine; ERK1/2, extracellular signal-regulated kinase 1/2; FMRP, fragile X mental retardation protein; FTIDC, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydro-2H-pyridine-1(2H)-carboxamide; FXS, Fragile X syndrome; GABA,  $\gamma$ -aminobutyric acid; JNJ16259685, (3,4-dihydro-2H-pyrano[2,3-b]quinolin-7-yl)(cis-4-methoxycyclohexyl)methanone; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; L-DOPA, L-3,4-dihydroxyphenylalanine; Lu AF21934, (1S,2S)-N<sup>1</sup>-(3,4-dichlorophenyl)cyclohexane-1,2-dicarboxamide; Lu AF32615, 4-((E)-styryl)-pyrimidin-2-ylamine; mGlu, metabotropic glutamate receptor; M-5MPEP, 2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-c]pyridin-4(5H)-one; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTEP, 3[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine; NAM, negative allosteric modulator; NMDA, N-methyl-D-aspartate; PAM, positive allosteric modulator; PCP, phencyclidine; PD, Parkinson’s disease; PD-LID, Parkinson’s disease levodopa-induced dyskinesia; PET, positron emission tomography; PHCCC, N-phenyl-7-(hydroxylimino)cyclopropa[b]chromen-1a-carboxamide; PQCA, (1-(4-cyano-4-(pyridine-2-yl)piperidine-1-yl)methyl-4-oxo-4 H-quinolizine-3-carboxylic acid); SAM, silent allosteric modulator; SIB-1757, 6-methyl-2-(phenylazo)-3-pyridinol; SIB-1893, 2-methyl-6-(2-phenylethynyl)pyridine; TBPB, 1-(1’-(2-methylbenzyl)-1,4’-bipiperidin-4-yl)-1H-benzod[imidazol-2(3H)-one.

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## Q8 Introduction

There is an urgent need for the development of new therapeutic agents for the treatment of debilitating CNS diseases, which affect millions of people worldwide. While the discovery and development of new therapeutic agents are challenging for all therapeutic areas, CNS drug discovery efforts have been especially challenging and have a very high attrition rate (Kola and Landis, 2004). The GPCRs have been among the most fruitful targets for developing drugs for the treatment of CNS disorders, as well as range of other human disease states. Many current clinical therapeutic agents act by targeting this important receptor class and downstream signaling pathways (Allen and Roth, 2011; Fang et al., 2003; Melancon et al., 2012). However, some major subfamilies of GPCRs have proven intractable in drug discovery efforts because of a difficulty in achieving high subtype selectivity and drug-like properties, including high CNS exposure, that are critical for advancing novel agents for the treatment of neurological and psychiatric disorders. Historically, drug discovery efforts targeting GPCRs have focused on the development of traditional agonists and antagonists that interact with the orthosteric neurotransmitter binding site to either mimic or block the action of the endogenous neurotransmitter or agonist. While this has been fruitful, there are many instances where the high conservation of the orthosteric binding site across related receptors prevents the development of subtype selective agents. Also, developing drug candidates based on the chemical scaffolds of the endogenous ligand may raise challenges in establishing appropriate profiles in terms of pharmacokinetic properties or brain exposure.

In recent years, advances in the development of allosteric modulators of GPCRs have emerged as a promising new approach for developing therapeutic agents that may be useful for the treatment of CNS disorders. Allosteric modulators of GPCRs bind to sites that are separate from the orthosteric binding site of the endogenous ligand and are often less highly conserved than the orthosteric site (Conn et al., 2009a). For some GPCRs, this has allowed optimization of allosteric modulators that achieve much greater subtype selectivity than is possible with traditional orthosteric ligands. In addition, allosteric modulators have other potential advantages, including ability to develop agents that have functional selectivity, allowing for potential targeting of select downstream signaling pathways, and a greater diversity of chemical scaffolds that can facilitate efforts to optimize pharmacokinetic and other drug-like properties of potential drug candidates. The surge in the development of allosteric agents has revealed a varied repertoire of drug activities, including PAMs and NAMs as well as agents with combined allosteric agonist and PAM activity and neutral ligands, termed silent allosteric modulators (SAMs) that bind to the allosteric site but do not potentiate or inhibit responses to the endogenous agonist (see Conn et al., 2009a; Melancon et al., 2012; Niswender and Conn, 2010 for reviews). In addition, allosteric agonists with a bitopic binding

mode (binds to both the allosteric site and the orthosteric site) have been identified (Digby et al., 2012a; Lebon et al., 2009; Spalding et al., 2002). These varied modes of action provide tools for experimental investigation into GPCR structure and function. To date, there is a wide variety of allosteric modulators that are showing promise for potential treatment of CNS diseases (Conn et al., 2009d). Among these, some of the most advanced and well understood include allosteric modulators of the metabotropic glutamate (mGlu) receptors and the muscarinic acetylcholine receptors (mAChRs). For instance, allosteric modulators of specific subtypes of mGlu receptors have potential utility in the treatment of schizophrenia, autistic spectrum disorders, and Parkinson's disease (Morin et al., 2013b). Positive allosteric modulators of the M<sub>1</sub> and M<sub>4</sub> muscarinic receptors show promising applications in both Alzheimer's disease and schizophrenia. This is an exciting time in CNS drug discovery with several allosteric modulator candidates moving from preclinical models into clinical development.

## Allosteric modulators of GPCRs

GPCRs, also called seven transmembrane spanning receptors (7TMRs), represent the largest family of cell surface receptors and are the targets of intense drug discovery efforts. While a number of available drugs on the market target GPCR signaling pathways, overall less than 20% of GPCRs are targeted (Allen and Roth, 2011). Ubiquitous receptors, these seven transmembrane-spanning proteins transduce extracellular signals for ligands as diverse as ions, photons of light, odorants and peptides into intracellular signaling cascades. Over 800 human GPCRs have been identified to date with five major families (and multiple subfamilies) based on their amino acid sequences (Katritch et al., 2013). Despite intense drug discovery and development efforts, clinically useful drugs do not exist for the large majority of these receptors. As noted above, the orthosteric binding site within GPCR subfamilies is often highly conserved, making the development of subtype specific ligands difficult. Of the orthosteric ligands developed, many of those with the highest subtype selectivity are antagonists.

Discovery and optimization of novel highly selective allosteric modulators of GPCRs have opened exciting new opportunities for the development of highly selective drug candidates for specific GPCR subtypes that were intractable using traditional approaches. While the major advances in the discovery of allosteric modulators of GPCRs have only occurred over the past decade, the principle of targeting allosteric sites on neurotransmitter receptors that act as ligand-gated ion channels has a long history and has been highly successful in developing agents for the treatment of CNS disorders (Melancon et al., 2012). The classic example of an allosteric modulator is the benzodiazepine class, which are positive allosteric modulators at the GABA<sub>A</sub> receptors (Mohler et al., 2002). These agents provide effective treatment of anxiety, sleep, and seizure disorders without inducing the adverse side effects

that would be observed with direct-acting GABA<sub>A</sub> receptor agonists. Allosteric modulators have been discovered for GPCRs, enzymes, and other ion channels (Bogoyevitch and Fairlie, 2007; Burford et al., 2013; Conn et al., 2009a; Hogg et al., 2005; Kenakin and Miller, 2010; Lewis et al., 2008; Scott et al., 2009).

As for the ligand-gated ion channels, GPCRs occupied by allosteric ligands can be modulated in a positive or negative manner (Conn et al., 2009a). The interaction of the receptor with an allosteric modulator can result in multiple pharmacological effects: *affinity modulation*, impacting the association–dissociation rate of the orthosteric ligand; *efficacy modulation*, affecting orthosteric ligand-induced downstream signaling responses; and *agonism/inverse agonism*, affecting receptor signaling in a positive or negative manner either in the presence or absence of orthosteric ligand. Ternary complex models that use cooperativity to quantify the allosteric effects are useful conceptually, but have been difficult to fit to experimental data. Using the operational model of allosterism, it is possible to quantify allosteric drug properties for use in drug development efforts. The advantages of allosteric modulators include: receptor selectivity with subtype selectivity and selective cooperativity at a given subtype as well as receptor activity dependence which maintains spatial and temporal activity dependence of endogenous signaling for those ligands which show efficacy only in the presence of the endogenous orthosteric ligand (Conn et al., 2009a; Lewis et al., 2008).

Allosteric agents have the potential to show differential effects on downstream signaling pathways, termed functional selectivity (biased agonism, stimulus trafficking). For example, mGlu<sub>5</sub> activates both intracellular calcium mobilization and extracellular signal-regulated kinase 1/2 (ERK1/2) signaling in rat cortical astrocytes. An early example of functional selectivity of allosteric modulators of GPCRs came with studies comparing the effects of the mGlu<sub>5</sub> PAMs DFB and CPPHA in native rat cortical astrocytes. While both showed similar positive modulatory effects on DHPG-induced intracellular calcium transients, their effects on ERK1/2 signaling differed (Zhang et al., 2005). There are now multiple examples in which allosteric modulators have been identified that have differential effects on coupling of the GPCR to different signaling pathways (Digby et al., 2012a; Kenakin, 2010; Maj et al., 2003; Mathiesen et al., 2005; Niswender et al., 2010; Noetzel et al., 2013; Sachpatzidis et al., 2003; Sheffler and Conn, 2008; Wei et al., 2003). Leveraging the functional selectivity of allosteric modulators of GPCRs provides a potential opportunity to develop agents that selectively target GPCR signaling pathways critical for therapeutic efficacy without modulating signaling pathways that lead to adverse effects.

## Metabotropic glutamate receptors

Metabotropic glutamate (mGlu) receptors represent promising drug targets for a variety of psychiatric and neurodegenerative CNS disorders. Glutamate, the major excitatory neurotransmitter in the CNS, signals through both ionotropic and metabotropic glutamate receptors. Metabotropic glutamate receptors modulate cell excitability and synaptic transmission, as opposed to eliciting fast synaptic responses, which are mediated by ionotropic glutamate receptors. The metabotropic glutamate (mGlu) receptor family, which couple to intracellular second messengers through heterotrimeric G-proteins, includes eight members that serve neuromodulatory roles within the CNS. Members of family 3 (or Class C) GPCRs, mGlu receptors are characterized by a large extracellular “venus flytrap” N-terminal region, which serves as the glutamate (orthosteric) binding site. mGlu receptors are divided into three subgroups according to agonist binding, signaling transduction pathways, and sequence homology. Group I, which includes mGlu<sub>1</sub> and mGlu<sub>5</sub> receptors, are coupled to G<sub>q/11</sub> and mediate IP<sub>3</sub>/Ca<sup>2+</sup> signal transduction (Abe et al., 1992). Group II (mGlu<sub>2,3</sub>) and Group III (mGlu<sub>4,6,7,8</sub>) negatively couple to adenylyl cyclase and other effector systems through G<sub>i/o</sub> proteins. While there is abundant sequence homology within receptor subgroups at the orthosteric binding site, allosteric ligands bind to a

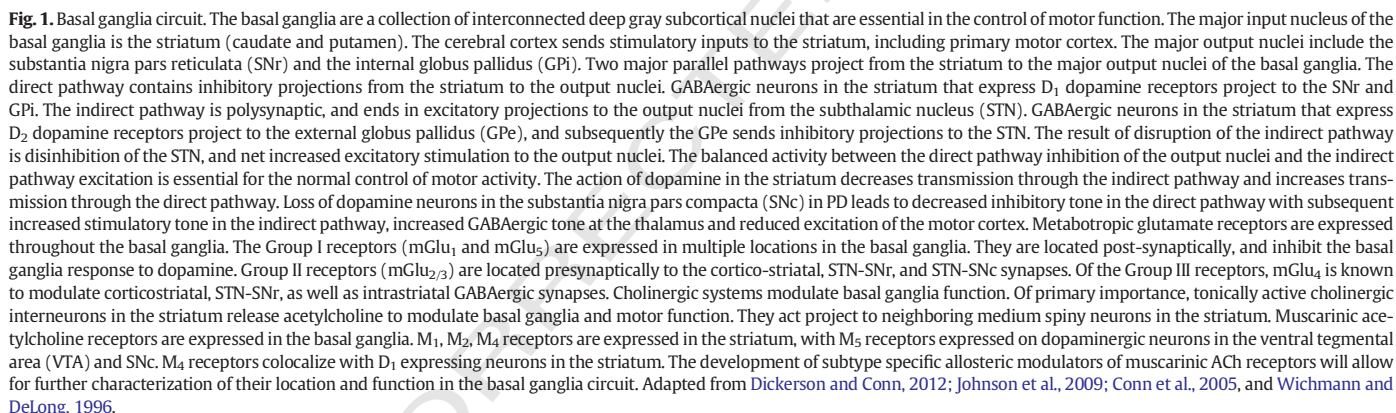
unique topographically distinct site within the transmembrane domain. The allosteric site contains a higher level of sequence diversity between receptor subtypes as compared to the orthosteric site, allowing for greater subtype selectivity of allosteric ligands (Christopoulos and Kenakin, 2002; Conn et al., 2009a). mGlu receptors are expressed in neurons and glial cells, including astrocytes, oligodendrocytes, and microglia. In neurons, mGlu<sub>1</sub> and mGlu<sub>5</sub> are expressed postsynaptically, modulating cell excitability and post-synaptic efficacy whereas mGlu<sub>2,3,4,7,8</sub> are predominately expressed presynaptically, where they can regulate neurotransmitter release (Conn and Pin, 1997). The CNS therapeutic targets for mGlu that have received the most attention include Parkinson's disease, Fragile X syndrome/autism spectrum disorders, schizophrenia, cognition, addiction, depression, anxiety and pain.

## Parkinson's disease

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by motor symptoms of rigidity, bradykinesia, tremor, rigidity, postural instability and gait disturbance (Jankovic, 2008). Associated non-motor symptoms include cognitive decline, mood and sleep disturbance (Chaudhuri et al., 2006). The incidence of PD in patient's older than 55 years is approximately 1% worldwide, creating a substantial disease burden in the aging world population. The pathology observed in PD patients includes progressive degeneration of dopamine neurons in the substantia nigra pars compacta (SNc) with resulting dysfunction of the basal ganglia-thalamocortical motor circuit (Fig. 1) (Dickerson and Conn, 2012; Johnson et al., 2009; Wichmann and DeLong, 1996; Zhang et al., 2005). Currently available pharmacological treatments for PD are aimed at dopamine replacement using L-DOPA or dopamine receptor agonists. While initially effective, dopamine-replacement strategies have undesired side effects including dyskinesia and have decreased efficacy over time as the disease progresses (Prashanth et al., 2011). The basal ganglia deep brain nuclei are central to the control of motor function, and the group III mGlu, including mGlu<sub>4</sub> (Bradley et al., 1999), are expressed in neurons of different basal ganglia nuclei. Dopamine depletion associated with PD in the nigrostriatal pathway leads to hyperactivity of inhibitory projections from the striatum to the globus pallidus, the first synapse in the basal ganglia “indirect pathway” (Hirsch et al., 2000). This glutamatergic overactivity of the indirect pathway is thought to contribute to the motor dysfunction (Blandini et al., 2000) and DA neuronal loss (Greenamyre and O'Brien, 1991; Przedborski, 2005) in PD patients. Clinical and preclinical studies in PD patients and animal models of PD suggest that decreasing the pathologic overactivity of this indirect pathway may be beneficial in reducing the motor symptoms associated with PD. The Group III mGlu (mGlu<sub>4</sub>, mGlu<sub>7</sub>, mGlu<sub>8</sub>) are expressed presynaptically at different synapses in the basal ganglia circuit and are promising targets for PD (Conn et al., 2005). Of particular relevance, mGlu<sub>4</sub> is expressed presynaptically at the striato-pallidal synapse and reduces GABAergic transmission, which is overactive in PD patients following loss of dopamine neurons. The action of mGlu<sub>4</sub> PAMs are hypothesized to act by reducing activity within the indirect pathway (Johnson et al., 2009). Interestingly, the mGlu<sub>4</sub> subtype of mGlu receptor is expressed in presynaptic terminals of striato-pallidal projections and activation of mGlu<sub>4</sub> with the group III selective agonist L-AP4 decreases transmission at the striato-pallidal synapse by inhibiting neurotransmitter release from presynaptic terminals (Bogenpohl et al., 2013; Matsui and Kita, 2003; Valenti et al., 2003b, 2005). This effect was absent in mGlu<sub>4</sub> knockout mice, confirming a critical role for mGlu<sub>4</sub>. Additionally, L-AP4 and other mGlu<sub>4</sub> receptor agonists reverse motor symptoms in preclinical rodent models of PD (Konieczny et al., 2007; MacInnes et al., 2004; Sibille et al., 2007; Valenti et al., 2003b).

The discovery of the mGlu<sub>4</sub>-selective PAMs, including PHCCC (Maj et al., 2003; Marino et al., 2003) and multiple more highly optimized agents (Bennouar et al., 2013; Celanire and Campo, 2012; East et al., 2010; Jimenez et al., 2012; Jones et al., 2011, 2012a; Niswender et al.,





et al., 2006). While the precise mechanism of this neuroprotective effect is not clear, it could be mediated by a combination of reduced excitation of DA neurons (Valenti et al., 2003a), anti-inflammatory effects of mGlu<sub>4</sub> activation (Fallarino et al., 2010; Taylor et al., 2003), and other potential neuroprotective actions on DA neurons (Battaglia et al., 2006; Bruno et al., 2000). Additionally, the mGlu<sub>4</sub> PAM PHCC as well as the Group III agonist ACPT-1 show a decrease in rodent models of neuropathic pain (Goudet et al., 2008), which may be effective for the non-motor symptoms of PD. Thus, mGlu<sub>4</sub> PAMs have potential for both symptomatic and disease-modifying treatment for PD patients. While mGlu<sub>4</sub> PAMs have not advanced to clinical testing, multiple pharmaceutical companies and academic/industry partnerships are making exciting progress that may allow direct testing of this hypothesis in clinical studies (Robichaud et al., 2011).

PD patients chronically treated with L-DOPA require increasing doses to maintain efficacy and progressively develop L-DOPA-induced dyskinesia (LID) (Fabbrini et al., 2007; Jenner, 2008; Marsden and Parkes, 1977; Meissner et al., 2011). Amantadine, an NMDAR antagonist, is currently the only drug to show clinical efficacy in LID, and the

adverse effects include cognitive impairment. Although the exact mechanism resulting in LID is not established, with decreased dopamine in the basal ganglia in PD, mGlu<sub>5</sub> is hypothesized to be involved in compensatory mechanisms and L-DOPA-induced motor complications. mGlu<sub>5</sub> is expressed in the striatum and basal ganglia (Shigemoto et al., 1993), and basal ganglia levels are increased in 6-OHDA lesioned rats (Pellegrino et al., 2007), as well as in parkinsonian primates with LID (Ouattara et al., 2010; Samadi et al., 2008). Preclinical rodent models of PD show efficacy with mGlu<sub>5</sub> NAMs to decrease LID (Dekundy et al., 2006; Levandis et al., 2008; Mela et al., 2007; Rylander et al., 2009). Fenobam, an mGlu<sub>5</sub> NAM, also decreased LID in both rodent and primate models of PD (Rylander et al., 2010). The addition of MPEP to L-DOPA treatment in parkinsonian MPTP lesioned non-human primates substantially decreased LID both in dyskinetic (Morin et al., 2010) and de novo lesioned animals (Morin et al., 2013a). Additionally, basal ganglia [<sup>3</sup>H]ABP688 specific binding (mGlu<sub>5</sub>) was significantly less in primates treated with MPEP combined with L-DOPA compared to L-DOPA treated animals (Morin et al., 2013b). These studies suggest that mGlu<sub>5</sub> NAMs may be useful as adjunct treatments to L-DOPA for PD. Currently, the mGlu<sub>5</sub> NAMs AFQ056 (Mavoglurant) and ADX48621 (Dipraglurant) (Rylander et al., 2010) are in phase IIa clinical studies for the treatment of LID in PD. Other possible therapeutic targets for mGlu<sub>5</sub> NAMs in addition to FXS/autism spectrum disorders and LID, include gastroesophageal reflux disease (GERD) (Keywood et al., 2009; Zerbib et al., 2010), migraine, and anxiety/stress disorders (Swanson et al., 2005).

It is important to note that the administration of mGlu<sub>5</sub> NAMs may be associated with adverse effects. For instance, the mGlu<sub>5</sub> NAM MPEP exacerbates PCP-induced psychotomimetic and cognition impairment in animal models (Brody et al., 2004a; Campbell et al., 2004) and early clinical studies suggest the possibility that mGlu<sub>5</sub> NAMs could have psychotomimetic effects in humans (Friedmann CTH et al., 1980; Itil TM et al., 1978; Pecknold et al., 1982). This may be mediated by inhibition of mGlu<sub>5</sub>-induced regulation of the NMDA subtype of glutamate receptor (Awad et al., 2000; Doherty et al., 2000; Henry et al., 2002; Kinney et al., 2003; Pisani et al., 2001) and the established psychotomimetic effect of manipulations that inhibit NMDA receptor function (Lahti et al., 1995; Malhotra et al., 1997). Interestingly, most mGlu<sub>5</sub> NAMs have inverse agonist activity, which may contribute to this side effect profile (Porter et al., 2005). However, recent studies have shown that it is possible to develop mGlu<sub>5</sub> NAMs with weak negative cooperativity that only partially block glutamate activation of mGlu<sub>5</sub> with full occupancy of the receptor (Rodriguez et al., 2005). While in vivo studies with these partial allosteric antagonists have not been performed, it is possible that these agents could provide clinical efficacy while minimizing adverse effects associated with full blockade or inverse agonist activity at mGlu<sub>5</sub>.

Of interest, A<sub>2A</sub> adenosine receptors are also expressed in the striatopallidal neurons and form oligomers with the D<sub>2</sub> dopamine receptor. A<sub>2A</sub> receptor antagonists are pro-dopaminergic, and therefore have the potential to reduce the symptoms associated with dopamine depletion in PD (Kulisevsky and Poyurovsky, 2012). The A<sub>2A</sub> receptor antagonist preladenant (SCH412384) delays haloperidol-induced extrapyramidal symptom onset in non-human primates (Varty et al., 2008). Therefore, the development of A<sub>2A</sub> NAMs would provide a valuable tool for the study of dyskinesia associated with PD and movement disorders.

In addition to mGlu<sub>4</sub> PAMs and mGlu<sub>5</sub> NAMs, the development of mGlu<sub>2</sub> and mGlu<sub>8</sub> PAMs may be useful for Parkinson's disease therapy. The Group II mGlu<sub>s</sub> are located presynaptically on glutamatergic axon terminals in the substantia nigra pars reticulata (SNr), potentially modulating excitatory neurotransmission (Bradley et al., 2000). Administration of group II agonists, by either the intracerebroventricular or the intranigral route, results in a reversal of akinesia in reserpine-treated rats (Dawson et al., 2000; Murray et al., 2002). Treatment of rat mid-brain slices with the selective agonist LY379268 leads to long-term

depression (LTD) of excitatory postsynaptic current (EPSC) amplitude in GABAergic SNr neurons. This effect was absent in mGlu<sub>2</sub> but not mGlu<sub>3</sub> knockout mice, indicating that activation of mGlu<sub>2</sub> is essential for induction of LTD in the SNr, with possible application of mGlu<sub>2</sub> agonism for the treatment of the motor symptoms of PD (Johnson et al., 2011). Non-selective group III agonists are effective in preclinical PD models. The mGlu<sub>8</sub> agonist DCPG (Thomas et al., 2001), administered by intracerebroventricular route, showed robust reversal of prolonged, but not acute, haloperidol-induced catalepsy and reserpine-induced akinesia (Johnson et al., 2013). Further, DCPG administration decreased forelimb use asymmetry in unilateral 6-OHDA lesioned rats. This evidence supports a role for mGlu<sub>8</sub> agonism in potential PD treatment. Therefore, the development of mGlu<sub>2</sub> and mGlu<sub>8</sub> PAMs may provide therapeutic benefit in PD.

### Fragile X syndrome and autism spectrum disorders

Fragile X syndrome (FXS) is an X-linked monogenic disorder, and is the most common form of human inherited intellectual disability and inherited cause of autism (Santoro et al., 2012). The brains of patient's with FXS appear normal on gross examination, yet microscopically the dendritic spines demonstrate an elongate immature phenotype. Patients with FXS have poor motor coordination, tactile hypersensitivity, loose bowel movements, and an increased incidence of epilepsy. These individuals have mental disability that includes attention deficit hyperactivity, obsessive-compulsive behaviors and labile mood. FXS is most often caused by a trinucleotide repeat expansion (CGG) in 5' untranslated region of the Fragile X mental retardation 1 (FMR1) gene, leading to hypermethylation and vastly decreasing or silencing the expression of the fragile X mental retardation protein (FMRP). FMRP represses translation of specific mRNAs (Bear et al., 2004) and is located in the postsynaptic region of glutamatergic synapses. Interestingly, FMRP inhibits translation of key proteins in the CNS that are stimulated by mGlu<sub>1</sub> and mGlu<sub>5</sub> (Bhakar et al., 2012). FMRP plays a critical role in long-term depression and other forms of synaptic plasticity. The absence of FMRP expression results in increased constitutive mGlu<sub>5</sub> signaling and subsequent "excessive" mGlu<sub>5</sub>-mediated protein synthesis in post-synaptic dendrites with resulting dysregulation of synaptic function. Generation of *Fmr1* knockout mice with decreased mGlu<sub>5</sub> expression (50%) showed improvement in many rodent phenotypes associated with Fragile X, including rescue of neuronal spine density, supporting increased activity of mGlu<sub>5</sub> as a key component in disease development (Dolen et al., 2007). The mammalian target of rapamycin (mTOR) pathway and ERK pathway is implicated in the coupling of mGlu<sub>5</sub> to the translational complex (Bhakar et al., 2012). The mGlu<sub>5</sub> NAMs MPEP and fenobam improve fragile X phenotypes in animal models of FXS. Furthermore, chronic pharmacological mGlu<sub>5</sub> inhibition with CTEP in the *Fmr1* knockout mouse corrected many features of fragile X in adult mice (Michalon et al., 2012). These findings highlight the importance of mGlu<sub>5</sub> in FXS, and raise the possibility that constitutive activity of mGlu<sub>5</sub> may be important in FXS. Therefore, the inverse agonist activity of mGlu<sub>5</sub> NAMs, such as is observed for MPEP, may be important for mGlu<sub>5</sub> NAM efficacy in this disease. Further studies comparing compounds with and without inverse agonist activity will determine the importance of mGlu<sub>5</sub> constitutive activity in FXS. These findings show promise for chronic mGlu<sub>5</sub> NAM treatment of patients with the FXS phenotype. A number of mGlu<sub>5</sub> NAMs are now being investigated in clinical studies for efficacy in treatment of FXS as well as other indications (Bhakar et al., 2012; Emmite, 2013). In a small clinical trial of 30 fragile X patients, the mGlu<sub>5</sub> NAM AFQ056 (Novartis) showed improvement in patients with full methylation of the *FMR1* promoter region, demonstrating that epigenetic modification of the promoter may determine responsiveness to mGlu<sub>5</sub> NAMs (Jacquemont et al., 2011; van Bon et al., 2011). Using lymphoblastoid cell lines from FXS patients, treatment with AFQ056 did not induce demethylation of the

*FMR1* gene promoter and levels of *FMR1* mRNA remained constant (Tabolacci et al., 2012).

In addition to potential utility in the treatment of FXS, recent studies raise the possibility that mGlu<sub>5</sub> NAMs may also be useful for the treatment of a broader range of autistic spectrum disorders, including idiopathic autism (Silverman et al., 2012). However, preclinical studies in mice bearing mutations that lead to tuberous sclerosis, another developmental autistic spectrum disorder suggest that mGlu<sub>5</sub> NAMs could exacerbate symptoms and that mGlu<sub>5</sub> PAMs could have therapeutic effects. Interestingly, tuberous sclerosis complex (TSC) patients often have associated symptoms similar to Fragile X patients, including epilepsy, autism spectrum disorders, and mental disability (Tsai and Sahin, 2011). TSC mice treated with an mGlu<sub>5</sub> PAM showed reversal of cognitive defects, supporting a potential role for mGlu<sub>5</sub> in TSC (Auerbach et al., 2011). Thus, it will be critical to carefully consider specific patient populations and develop a more complete understanding of the potential impact of mGlu<sub>5</sub> modulators in different childhood developmental disorders. FXS and TSC serve as valuable models to understand the neurobiology behind genetically complex developmental brain disorders the potential impact of different genotypes on therapeutic response to mGlu<sub>5</sub> modulators (Krueger and Bear, 2011).

In addition to potential utility of mGlu<sub>5</sub> modulators, it is important to note that the early studies suggest that the signaling by both mGlu<sub>5</sub> and the closely related mGlu<sub>1</sub>, are equally impacted by mutations that lead to FXS (Bhakar et al., 2012). Thus, mGlu<sub>1</sub> NAMs could also provide efficacy in FXS patients. While mGlu<sub>1</sub> has received less attention than mGlu<sub>5</sub> as a potential target for the treatment of FXS, Thomas et al. (Thomas et al., 2012) recently reported that selective mGlu<sub>1</sub> NAMs have robust efficacy in reversing multiple symptoms in FXS mice. Thus, it will be important to understand the potential for selective NAMs for both subtypes in treatment of FXS and related disorders.

#### Schizophrenia and anxiety disorders

Schizophrenia is a debilitating psychiatric disorder affecting approximately 1% of the population across the globe. The manifestation of this disorder includes a triad of symptom clusters: positive symptoms, negative symptoms, and cognitive impairment (Kim et al., 2009). Current therapies for the treatment of psychosis (positive symptoms) in schizophrenia patients focus on blockade of the D<sub>2</sub> dopamine receptors, and are severely limited by poor efficacy as well as adverse side effects (extrapyramidal motor symptoms and metabolic syndrome and sexual dysfunction). These combined effects limit the successful therapeutic window for the D<sub>2</sub> antagonists, with patients frequently switching drugs to maintain effective treatment of symptoms. Additionally, some D<sub>2</sub> antagonists only show partial efficacy in some patients. The finding that NMDA receptor channel blockers, including PCP, ketamine, and dizocilpine (MK-801), induce psychosis in human volunteers, led to the hypoglutamatergic theory of schizophrenia (Javitt, 1987). In addition, the administration of NMDA receptor antagonists to schizophrenic patients exacerbates both cognitive and psychotic symptoms (Lahti et al., 1995; Malhotra et al., 1997). Clinical trials using glycine co-agonists, which enhance NMDA receptor function, in combination with standard antipsychotic therapy show efficacy in decreasing negative symptoms and increasing cognition in schizophrenic patients (Coyle and Tsai, 2004).

There is a large body of evidence that Group II (mGlu<sub>2</sub> and mGlu<sub>3</sub>) agonists or mGlu<sub>2</sub> PAMs provide effective action against the positive symptoms, while mGlu<sub>5</sub> PAMs may have efficacy in reducing all symptoms clusters schizophrenia patients (Herman et al., 2012). The mGlu<sub>2/3</sub> agonists LY354740 and LY379268 have robust efficacy in multiple rodent models of antipsychotic-like activity (Chaki et al., 2013) for both schizophrenia (Conn et al., 2008; Schoepp and Marek, 2002) and anxiety disorders (Schoepp et al., 2003; Swanson et al., 2005). The antipsychotic-like activity is likely to be mediated, at least in part by reduced glutamate release from presynaptic terminals on projections

from the thalamus to the prefrontal cortex in rodents (Cartmell et al., 1999; Marek, 2010; Moghaddam, 2004). Further, an early clinical study with a selective mGlu<sub>2/3</sub> agonist showed promising effects in a phase II clinical trial in patients with schizophrenia (Patil et al., 2007). Unfortunately, this efficacy has not been reliably observed in subsequent clinical studies (Adams et al., 2013; Hopkins, 2013; Kinon et al., 2011). Experiments using mGlu<sub>2</sub> and mGlu<sub>3</sub> knockout mice provide strong evidence that the antipsychotic-like effects of mGlu<sub>2/3</sub> agonists in rodent models are mediated by the activation of mGlu<sub>2</sub> (Fell et al., 2008). However, it has not been possible to develop orthosteric agonists that are highly selective for mGlu<sub>2</sub> relative to mGlu<sub>3</sub>.

Efforts to develop selective mGlu<sub>2</sub> PAMs have been highly successful and multiple studies reveal that mGlu<sub>2</sub>-selective PAMs have robust efficacy in rodent models of antipsychotic activity that are similar to those observed with mGlu<sub>2/3</sub> agonists (Benneyworth et al., 2007; Cartmell et al., 1999; Galici et al., 2006; Lorrain et al., 2003; Moghaddam and Adams, 1998). ADX71149, a highly selective mGlu<sub>2</sub> PAM that is now in clinical development for the treatment of schizophrenia and anxiety depression, met the primary objectives of safety and tolerability in phase I studies and advanced to phase IIa clinical testing. While the results of this important study have not yet been published, preliminary reports from part B of the Phase IIa study suggest that ADX71149 may have efficacy in reducing negative symptoms in a subgroup of schizophrenia patients (<http://www.addextherapeutics.com/investors/press-releases/news-details/article/addex-reports-top-line-data-from-a-successful-phase-2a-clinical-study-with-adx71149-in-schizophrenia/>).

Activation of mGlu<sub>4</sub> with positive allosteric modulators may also provide a promising therapeutic avenue for schizophrenia and the development of antipsychotic agents. The Group III mGlu<sub>4</sub>-preferring orthosteric agonist LSP1-2111 showed dose-dependent inhibition of amphetamine-induced hyperactivity (Wieronska et al., 2012) and reversal of MK-801 induced deficits in novel object recognition in rats (Wieronska et al., 2013), indicating potential effects on both the positive and cognitive symptoms of schizophrenia. Further, recent studies with mGlu<sub>4</sub> PAMs show promising results in animal models that are used to predict antipsychotic effects (Slawinska et al., 2013a,b). For instance, the brain-penetrant mGlu<sub>4</sub> PAMs Lu AF21934 and Lu AF32615 showed dose-dependent reduction of amphetamine-induced hyperactivity and antagonism of 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head twitch tests in wild type but not mGlu<sub>4</sub><sup>−/−</sup> mice, supporting a key role for mGlu<sub>4</sub> in brain circuits involved in these behavioral models (Slawinska et al., 2013a). The mechanism of action for mGlu<sub>4</sub> in rodent models of antipsychotic activity are not known but are likely to be unrelated to the antiparkinsonian effects of mGlu<sub>4</sub> PAMs. Recent studies suggest that antipsychotic actions of mGlu<sub>4</sub> PAMs may parallel the effects of mGlu<sub>2/3</sub> receptor agonists and mGlu<sub>2</sub> PAMs. For instance, activation of mGlu<sub>4</sub> reduces transmission at the same thalamo-cortical terminals in the prefrontal cortex that are modulated by activation of mGlu<sub>2</sub> (Zhang and Marek, 2007). In addition, activation of mGlu<sub>4</sub> reduces excitatory transmission in midbrain dopamine neurons (Valenti et al., 2005) and this could contribute to the antipsychotic-like effects of mGlu<sub>4</sub> agonists or PAMs.

In addition to mGlu<sub>2</sub>-selective PAMs, recent efforts suggest that mGlu<sub>5</sub>-selective PAMs may have potential as a novel approach for the treatment of schizophrenia. Activation of mGlu<sub>5</sub> receptors is known to enhance NMDA receptor function in multiple cell populations and has excitatory effects that may work in concert with NMDA receptors to increase activity in forebrain circuits that are thought to be important for the psychotomimetic effects of NMDA receptor antagonists (Brody et al., 2004b; Kinney et al., 2003; Lecourtier et al., 2007). Additionally, mGlu<sub>5</sub> receptors interact physically with NMDA receptors through intracellular scaffolding proteins (Niswender and Conn, 2010). As noted above, antagonists of NMDA receptors induce positive and negative symptoms, as well as deficits in cognitive function in humans that are similar to those observed in schizophrenia patients (Adler et al., 1998; Halberstadt, 1995; Krystal et al., 1994; Malhotra et al., 1996; 583



Newcomer et al., 1999; Parwani et al., 2005; Rowland, 2005). A large body of clinical and preclinical studies have led to the hypothesis that reduced activity of NMDA subtypes of glutamate receptors or brain circuits that are regulated by NMDA receptors may play an important role in the pathophysiology underlying schizophrenia (Conn et al., 2009d; Field et al., 2011; Nicoletti et al., 2011). Furthermore, multiple studies suggest that agents that enhancing NMDA receptor signaling could provide efficacy in reducing the symptoms associated with schizophrenia (Coyle, 2006; Lindsley et al., 2006). Based on this, and the clear role of mGlu<sub>5</sub> acting in tandem with NMDA receptors to regulate transmission through glutamatergic circuits in forebrain regions, selective activators of mGlu<sub>5</sub> have been raised as a potential novel treatment strategy for schizophrenia that may have efficacy in reducing both psychotic and negative symptoms as well as providing pro-cognitive activity (Conn et al., 2009c). Consistent with this, mGlu<sub>5</sub> KO mice show disrupted prepulse inhibition (PPI), a model of sensory motor gating shown to be disrupted in schizophrenic patients (Brody et al., 2004a; Kinney et al., 2003). In addition, the mGlu<sub>5</sub> NAM MPEP potentiates PCP-induced psychotomimetic and cognition impairment in animal models (Brody et al., 2004a; Campbell et al., 2004). Based on these important findings, major efforts were launched to develop highly selective mGlu<sub>5</sub> PAMs. These efforts have yielded a large number of structurally diverse highly selective mGlu<sub>5</sub> PAMs and multiple studies have demonstrated that mGlu<sub>5</sub>-selective PAMs have robust efficacy in rodent models of schizophrenia that predict efficacy in reducing both positive and negative symptoms (Conn et al., 2009d; Liu et al., 2008; Schlumberger et al., 2009, 2010). In addition, mGlu<sub>5</sub> PAMs potentiate multiple forms of synaptic plasticity that are thought to underlie specific aspects of learning and memory (Ayala et al., 2009; Liu et al., 2008; Rosenbrock et al., 2010; Stefani and Moghaddam, 2010), and improve cognitive function in multiple animal models (Darrah et al., 2008; Stefani and Moghaddam, 2010; Vardigan et al., 2010). These exciting studies suggest that mGlu<sub>5</sub> PAMs have the potential to provide a fundamental advance in schizophrenia therapy that could have efficacy in the treatment of all major symptom clusters of the disease.

While the more subtle approach to modulation of glutamatergic function using mGlu<sub>5</sub> PAMs has the potential to provide less adverse effect liability than would be observed with direct agonists of mGlu<sub>5</sub> or NMDA receptors, recent studies reveal that some mGlu<sub>5</sub> PAMs induce severe seizure activity (Rook et al., 2012) and excitotoxicity leading to cell death in the auditory cortex, hippocampus, and other forebrain regions (Parmentier-Batteur et al., 2013). However, other mGlu<sub>5</sub> PAMs are well tolerated and it is clear that all mGlu<sub>5</sub> PAMs do not have the same adverse effect liability (Rook et al., 2013). This suggests that mGlu<sub>5</sub> PAMs likely differ in their effects on mGlu<sub>5</sub> signaling and understanding these differences and the mechanistic underpinnings of mGlu<sub>5</sub> PAM-mediated toxicity will be critical for fully developing mGlu<sub>5</sub> PAMs as potential therapeutic agents. One property that has been shown to play an important role in determining whether specific mGlu<sub>5</sub> PAMs will induce seizures and behavioral convulsions is the presence or absence of allosteric agonist activity (Rook et al., 2013). Of note, allosteric agonism is context dependent and can be influenced by differences in receptor expression. For example, Rook et al. (2013a,b) recently reported that the mGlu<sub>5</sub> PAM VU0424465 showed intrinsic agonist activity both in cell lines and in native systems, while a group of closely related compounds displayed agonist activity only in overexpressing cell lines and others showed no detectable allosteric agonist activity in any cell lines or native systems examined. Interestingly, VU0424465 showed adverse effects, including epileptiform activity, while the related compounds that were devoid of allosteric agonist activity in native systems did not (Rook et al., 2013). These adverse effects are dose-dependent and showed increased severity over time. These findings highlight the importance of selecting mGlu<sub>5</sub> PAMs lacking detectable intrinsic (glutamate-independent) agonist activity to avoid glutamate-independent receptor activity and the associated side effects. Also, as discussed

above, mGlu<sub>5</sub> PAMs and other GPCR allosteric modulators can regulate specific aspects of mGlu<sub>5</sub> signaling without affecting others (Noetzel et al., 2013; Zhang et al., 2005). It is possible that some mGlu<sub>5</sub> PAMs have greater effects on signaling pathways that are involved in the adverse effect liability and that those that are biased towards other pathways could provide efficacy without the severe adverse effect liability. In addition to these different properties, recent studies suggest that hepatic metabolism of some mGlu<sub>5</sub> PAMs can yield metabolites that have robust activities that differ from activity of the parent compound and can contribute to the adverse effect liability (Bridges et al., 2013). In future studies, it will be critical to gain a clear understanding of the mechanisms underlying the different safety profiles of different mGlu<sub>5</sub> PAMs. Interestingly, in a developmental model of schizophrenia (neonatal PCP-induced cognition impairment), administration of mGlu<sub>5</sub> PAMs in adolescence prevented the appearance of delayed cognitive deficits in adult rats. Further, mGlu<sub>5</sub> PAM administration reversed the delay-induced impairment in adult rats, as evaluated by social novelty discrimination (Clifton et al., 2013). These findings suggest the exciting possibility of a preventative role for mGlu<sub>5</sub> PAM treatment in the development of schizophrenia, and further work will be important to evaluate these findings.

#### Addiction

Glutamatergic neurotransmission is hypothesized to play a key role in both the establishment and maintenance of drug addiction (Nicoletti et al., 2011). Negative allosteric modulators of Group I (mGlu<sub>1</sub> and mGlu<sub>5</sub>) mGlu receptors have potential as therapeutic agents for the treatment of addictive disorders (Achat-Mendes et al., 2012; Bird and Lawrence, 2009). The mGlu<sub>5</sub> receptors are highly expressed in the mesolimbic areas, regions central to the brain reward system. Mutant mGlu<sub>5</sub> null mice do not self-administer cocaine or exhibit locomotor-stimulating effects, despite normal levels of dopamine in the nucleus accumbens, supporting a role for mGlu<sub>5</sub> in addiction (Chiamulera et al., 2001). MPEP and MTEP treatment (mGlu<sub>5</sub> NAMs) show efficacy in cocaine abuse rodent and non-human primate models (Kenny et al., 2005; Kumaresan et al., 2009; Lee et al., 2005; Platt et al., 2008). Additionally, the novel mGlu<sub>5</sub> NAM VU0463841 shows activity in a rat model of cocaine addiction, with dose-dependent reduction of cocaine place preference and cocaine self-administration (Amato et al., 2013). Recently, mGlu<sub>1</sub> antagonist (mGlu<sub>1</sub> antagonist JNJ16259685) was reported to inhibit cocaine-induced conditioned place preference (CPP) through inhibition of protein synthesis in the ventral tegmental areas (VTA) (Yu et al., 2013).

In addition to Group I antagonists, experimental evidence suggests a potential role for mGlu<sub>2</sub> in addictive disorders. Cocaine reinforcement is elevated in mGlu<sub>2</sub> knockout mice, supporting the concept that mGlu<sub>2</sub> negatively regulates the drug reward system (Morishima et al., 2005). mGlu<sub>2/3</sub> agonism is associated with decreased nicotine and cocaine self-administration (Adewale et al., 2006; Liechti and Markou, 2007), providing opportunity for the development of mGlu<sub>2/3</sub> (likely mGlu<sub>2</sub>-selective) PAMs for addiction.

#### Major depressive disorder (MDD)

Major depressive disorder (MDD) represents one of the most common forms of mental illness and is a significant financial and social burden (Chaki et al., 2013). Monoamine oxidase inhibitors and tricyclic antidepressants are the current mainstays of therapy for depression and show slow onset of action as well as poor efficacy. A subgroup of patients are resistant to therapy, termed treatment resistant depression (TRD). Ketamine, a noncompetitive (orthosteric) NMDA receptor antagonist, has shown considerable efficacy for treatment-resistant depression in a double blind placebo controlled trial (Zarate et al., 2006). Additional evidence of the role of hyperfunction of the glutamatergic

**Table 1**

Potential application of metabotropic (mGlu) and muscarinic (mACh) allosteric modulators in CNS diseases.

Receptor	MOA	CNS disease applications	Compounds
<b>Q2</b> mGlu1	NAM	Neuropathic pain, FXS, anxiety/stress disorders, addiction	CPCCOEt (Annoura H et al., 1996), JNJ16259685 (Lavreysen et al., 2004; Thomas et al., 2012)
	PAM		Ro67-7476 (Knoflach et al., 2001), VU71 (Hemstapat et al., 2006), Ro67-4853 (Wichmann et al., 2002), VU48 (Hemstapat et al., 2006)
mGlu5	NAM	Addiction, anxiety, chronic pain, depression, FXS (autism spectrum disorders), migraine, PD-LID,	MPEP (Gasparini et al., 1999), MTEP (Cosford et al., 2003), CTEP (Lindemann et al., 2011), Fenobam (Porter et al., 2005), AFQ056 (Jacquemont et al., 2011); M-5MPEP (partial NAM), Br-5MPEPy (partial NAM) (Rodriguez et al., 2005); GRN-529 (Hughes et al., 2013), VU046381 (Amato et al., 2013), RG7090 (RO4917523, antagonist, in clinical trials, FXS, <a href="http://clinicaltrials.gov/ct2/results?term=RO4917523">http://clinicaltrials.gov/ct2/results?term=RO4917523</a> )
	PAM	Anxiety disorders, Huntington's disease, schizophrenia, TSC	ADX47273 (Liu et al., 2008), VU0360172 (Rodriguez et al., 2010), VU29 (Ayala et al., 2009), LSN2463359 (Gastambide et al., 2013; Gilmour et al., 2013)
mGlu2/3	NAM	Depression	RO4432717 (Goeldner et al., 2013), MNI-137 (Hemstapat et al., 2007)
mGlu2-selective	PAM	Addiction, AD, anxiety disorders, depression, schizophrenia	BINA (Benneyworth et al., 2007; Galici et al., 2006), LY487379 (Johnson et al., 2003), ADX71149 (Hashimoto et al., 2013)
mGlu3-selective	NAM	Depression	MNI-167, RO4988546, RO5488608, RO4491533, RO4491533
mGlu4	NAM	Depression	VU0463597/ML-289 (Sheffler et al., 2012)
	PAM	Neuroinflammation, neuroprotection, PD, schizophrenia	PHCCC (Maj et al., 2003; Marino et al., 2003), VU0155041 (PAM/allosteric agonist) (Niswender et al., 2008), VU0364770 (Jones et al., 2012a), ADX88178 (Celanire and Campo, 2012), Lu AF21934 (Slawinska et al., 2013a,b), Lu AF32615 (East et al., 2010)
<b>Q3</b> mGlu7	NAM	Anxiety, depression	MMPIP (Hikichi et al., 2010; Niswender et al., 2010) and ADX71743 (Kalinichev et al., 2013)
	Allosteric agonist	Anxiety, depression, PD	AMN082 (Ugolini et al., 2008)
mGlu8	Agonist	Parkinson's disease, anxiety	DCPG (Thomas et al., 2001)
M1	PAM	AD, addiction, movement disorders, neuropathic pain, PD, schizophrenia	Brucine (Lazareno et al., 1999), BQCA (Shirey et al., 2009), PQCA (Uslaner et al., 2013), ML-137 (Bridges et al., 2010c), ML-169/VU0405652 (Bridges et al., 2010e)
	Allosteric agonist	AD, movement disorders	AC-42 (Spalding et al., 2002), N-desmethyloclozapine (Sur et al., 2003), TBPB (Jones, Brady et al., 2008), 77-LH-28-1 (Langmead et al., 2008a), VU0184670, VU0357017 (Digby et al., 2012a; Lebois et al., 2010), VU0364572 (Digby et al., 2012b)
M4	M4-selective antagonist	Dystonia, PD	Tropicamide (Betz et al., 2007)
	PAM	AD, addiction, movement disorders, neuropathic pain, OCD, PD, schizophrenia	Thiochrome (Lazareno et al., 2004), VU152099 (Brady et al., 2008), VU152100 (Brady et al., 2008), LY2033928 (Chan et al., 2008), ML173 (Bridges et al., 2010d), ML293 (Sheffler et al., 2012)
M5	NAM	Addiction, anxiety disorders, schizophrenia	N/A
	PAM	Anxiety disorders, ADHA, PD, schizophrenia,	VU0238429 (Bridges et al., 2009) (Bridges et al., 2010b)



system in depression stems from the observation that glutamate levels in plasma and limbic brains areas are elevated in depressed patients (Sanacora et al., 2004). Modulation of glutamatergic transmission, particularly with mGlu<sub>2/3</sub> agonists (Feinberg et al., 2002; Fell et al., 2011), mGlu<sub>2/3</sub> antagonists (Campo et al., 2011; Chaki et al., 2004; Palucha-Poniewiera et al., 2010), and mGlu<sub>5</sub> antagonists (Belozertseva et al., 2007; Campo et al., 2011; Chaki et al., 2013; Li et al., 2006; Palucha et al., 2005; Pilc et al., 2013) shows promising effects as potential treatments for depression. Recently, the novel mGlu<sub>5</sub> NAM GRN-529 showed efficacy in multiple rodent models of depression, including those relevant to anxiety and pain, symptoms often associated with treatment resistant depression (Hughes et al., 2013). Furthermore, fenobam had efficacy in reducing anxiety in a clinical proof-of-concept study (Pecknold et al., 1982; Porter et al., 2005). Currently, the mGlu<sub>5</sub> antagonist RG7090 is in a phase II study for adjunctive therapy in patients with major depressive disorder (MDD) ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)).

#### Additional CNS therapeutic areas for mGlu allosteric modulators

The allosteric modulators of mGlu receptors have many other potential uses including pain, stress/anxiety disorders, and movement disorders (see Table 1) (Nicoletti et al., 2011). Neuropathic pain is a chronic condition that leads to allodynia and hyperalgesia (Schkeryantz et al., 2007). The mGlu<sub>1</sub> receptor is expressed in CNS regions essential to nociceptive processing as well as in afferent nociceptive nerve terminals (Martin et al., 1992). mGlu<sub>1</sub> knockout mice show decreased pain sensitivity (Schkeryantz et al., 2007) and administration of the mGlu<sub>1</sub>-selective NAM JNJ16259685 (Lavreysen et al., 2004) is reported to show efficacy in a rodent neuropathic pain model (formalin hyperalgesia) (Mabire et al., 2005). Additionally, mGlu<sub>1</sub>-selective antagonists showed *in vivo* activity in the spinal nerve ligation test (Bennett et al., 2012). Taken together, the development of mGlu<sub>1</sub>-selective NAMs (Annoura H et al., 1996; Mabire et al., 2005) and efficacy in preclinical models are promising for the potential treatment of neuropathic pain. mGlu<sub>5</sub> NAMs show analgesic efficacy in preclinical models of neuropathic pain (Kumar et al., 2010; Montana et al., 2009). Agonists of mGlu<sub>2/3</sub> receptors also show analgesic effects in models of chronic and neuropathic pain, but show tolerance after chronic treatment (Jones et al., 2005).

In addition to mGlu<sub>4</sub>, subtype-selective allosteric modulators for the other Group III mGlu receptors (mGlu<sub>7</sub> and mGlu<sub>8</sub>) may also have potential for the treatment of CNS disorders. Recently, the mGlu<sub>7</sub> NAMs MPMIP (Hikichi et al., 2010) and ADX71743 (Kalinichev et al., 2013) have shown potential efficacy in animal models for the treatment of anxiety and depression, and provide excellent tool compounds to further elucidate the role of mGlu<sub>7</sub> in CNS diseases. As described above, the mGlu<sub>8</sub> agonist DCPG (Thomas et al., 2001) showed efficacy in preclinical models of PD (Johnson et al., 2013), and the development of mGlu<sub>8</sub> selective PAMs may provide novel therapeutics for PD patients. Recently, mGlu<sub>5</sub> PAMs showed neuroprotective effects in the BACHD mouse model of Huntington's disease (Doria et al., 2013). The continued development of mGlu allosteric modulators provides promising tools for the investigation of the role of individual receptor subtypes in CNS disease and the creation of novel therapeutics for an array of CNS disorders.

#### Muscarinic acetylcholine receptors

Allosteric modulators of muscarinic receptors show promise as potential therapies for a number of CNS disorders, including Alzheimer's disease and schizophrenia. Other target areas of benefit may include neuropathic and chronic pain, epilepsy, sleep disorders, Parkinson's disease, and movement disorders (Conn et al., 2009b). Acetylcholine signals through both muscarinic and nicotinic receptors. Muscarinic acetylcholine receptors are widely expressed in the CNS and include five subtypes (M<sub>1</sub>–M<sub>5</sub>) (see Langmead et al., 2008c for review). These

Family A GPCRs respond to the endogenous agonist acetylcholine and are further subdivided into two groups based on signaling pathways. The M<sub>1</sub>, M<sub>3</sub>, and M<sub>5</sub> receptors signal through G<sub>q/11</sub>, activating PLCβ leading to increased intracellular calcium. The M<sub>2</sub> and M<sub>4</sub> receptors signal through G<sub>i/o</sub> proteins and inhibit adenylate cyclase. Also, M<sub>2</sub> and M<sub>4</sub> signaling through G<sub>iβγ</sub> subunits modulates ion channel activity. Muscarinic acetylcholine receptors (mAChRs) are expressed throughout the CNS, including targets for cholinergic interneurons in the striatum as well as targets of projections from the medial septum and hindbrain nuclei in the midbrain, neocortex and limbic areas important for learning and memory. Both the M<sub>1</sub> and M<sub>4</sub> receptors are associated with learning, memory and cognition (Hasselmo, 2006; Hasselmo and Giocomo, 2006), and drugs specific for these subtypes may be useful in treatment of the cognitive symptoms associated with schizophrenia and Alzheimer's disease. The orthosteric acetylcholine (ACh) binding site is highly conserved, thwarting attempts to create an orthosteric drug with true subtype selectivity. Drugs developed for the orthosteric site lack subtype specificity and result in dose-dependent adverse side effects (bradycardia, sweating, salivation and gastrointestinal distress) from activation of the peripheral M<sub>2</sub> and M<sub>3</sub> mAChR subtypes (Heinrich et al., 2009).

#### Alzheimer's disease

Alzheimer's disease represents a major public health problem as the world population ages in the coming decades. The features of this progressive neurodegenerative disease include neuronal loss, behavioral and cognitive changes and decreased cerebral blood flow (Bartus et al., 1982; Hanyu et al., 2003). Acetylcholine receptor agonists and acetylcholinesterase (AChE) inhibitors improve the cognitive symptoms in Alzheimer disease patients (Feldman, 2002; Grossberg, 2002) and AChE inhibitors are among the few medications available for the treatment of AD. These cholinergic agents have limited efficacy and induce peripheral side effects, which limit their use (Lockhart et al., 2009). Studies have suggested that activation of muscarinic acetylcholine receptors may prove useful in the treatment of multiple symptoms in the spectrum of both Alzheimer's disease and schizophrenia (Langmead et al., 2008b). The muscarinic receptor orthosteric agonist xanomeline (M<sub>1</sub> and M<sub>4</sub> selective) has been shown to be effective in reducing the psychotic symptoms in patients with both schizophrenia and AD. A phase III placebo-controlled clinical trial showed that xanomeline robustly decreased psychotic symptoms in patients with Alzheimer's disease (Bodick et al., 1997a,b). While improved cognition was observed for those patients who completed the trial (high dose vs. placebo), the end-point analysis was not statistically significant for cognitive improvement. The main limiting side effect of xanomeline involved gastrointestinal associated symptoms (Bodick et al., 1997b). This study demonstrates that muscarinic agonists have potential in treating both the cognitive and behavioral aspects of Alzheimer's disease.

Both M<sub>1</sub> and M<sub>4</sub> allosteric agonists and PAMs would be desirable drug candidates for therapeutic development. The M<sub>1</sub> receptor is expressed in high levels in the CNS, particularly the cortex, hippocampus, and striatum (Levey et al., 1991, 1995). Efforts have focused on the development of M<sub>1</sub> specific ligands for AD, as M<sub>1</sub> is thought to be the critical subtype for cognition, attention, and sensory processing (Fisher, 2008; Langmead et al., 2008b; Robinson et al., 2011). Additionally, M<sub>1</sub> knockout mice show specific cognitive deficits (Anagnostaras et al., 2003; Miyakawa et al., 2001). For M<sub>1</sub> selective orthosteric agonists, the lack of true selectivity for M<sub>1</sub>, coupled with high receptor reserve of M<sub>2</sub> and M<sub>4</sub> in native tissues, results in functional activity at multiple receptor subtypes, with unacceptable side effect profiles. For example, the orthosteric agonist AF267, thought to have subtype specificity for M<sub>1</sub>, showed activity at both M<sub>3</sub> and M<sub>5</sub> receptors (Jones et al., 2008). Current M<sub>1</sub>/M<sub>4</sub> selective muscarinic orthosteric ligands show intolerable side effects related to their lack of muscarinic receptor subtype selectivity. The development of muscarinic allosteric modulators has

opened a therapeutic window for treating CNS disorders with the potential to minimize peripheral muscarinic side effects.

Brucine, the first M<sub>1</sub> PAM identified, provided proof-of-concept for the development of M<sub>1</sub> subtype specific ligands (Lazareno et al., 1998). A functional screening approach identified novel M<sub>1</sub> PAMs, including VU0090157 and VU0029767, that showed pure PAM activity (Marlo et al., 2009) and could differentially regulate coupling to different signaling pathways. The systemically active M<sub>1</sub> PAM BQCA induces an increase in activation of the prefrontal cortex (PFC) and improves in PFC-dependent cognitive function in a transgenic mouse model of PD (Shirey et al., 2009). In addition, BQCA has activity in other models that suggest possible efficacy in improving cognitive function and reducing psychotic symptoms (Ma et al., 2009). Recently, a newer M<sub>1</sub> PAM, PQCA was shown to improve cognitive function in non-human primates, including improvements in the object retrieval detour task in rhesus macaques. Further, PQCA treatment increased frontal cortical cerebral blood flow at parallel drug concentrations, providing a potential translational biomarker for future studies (Uslaner et al., 2013). The continued development and optimization of M<sub>1</sub> PAMs, such as ML137, is providing exciting tools for investigative efforts into the role of M<sub>1</sub> in CNS disorders (Melancon et al., 2013; Poslusney et al., 2013).

Additionally, M<sub>1</sub> selective allosteric agonists have been developed, including AC-42 (Spalding et al., 2002), N-desmethyldiazepam (Sur et al., 2003), TBPB (Jones et al., 2008) and 77-LH-28-1 (Langmead et al., 2008a). The M<sub>1</sub> allosteric agonist AC260584 shows antipsychotic activity in preclinical models (Vanover et al., 2008), but also is active at the 5-HT<sub>2A</sub> serotonin receptor, D<sub>2</sub> dopamine receptor and  $\alpha_{1A}$  adrenergic receptor (Heinrich et al., 2009). The recently developed selective allosteric agonists VU0357017 and VU0364572 showed robust efficacy in a rodent hippocampal dependent learning paradigm (Lebois et al., 2010). A number of M<sub>1</sub> allosteric agonists show bitopic binding (binds to both an allosteric site and the orthosteric site), including 77-LH-28-1 (Lebon et al., 2009), AC-42 (Spalding et al., 2002), and VU0364572 (Digby et al., 2012b). These allosteric agonists provided valuable tools for the investigation of M<sub>1</sub> in AD, but are limited by activity at other GPCRs and show complicated pharmacology with their bitopic mode of action. Furthermore, selectivity of these compounds is based on functional selectivity and issues with high receptor reserve have made it difficult to maintain high selectivity while optimizing for maximal M<sub>1</sub> potency and efficacy while achieving drug-like properties of highly selective M<sub>1</sub> allosteric agonists (Digby et al., 2012b).

In addition to acute actions on cognitive function, activation of M<sub>1</sub> reduces the pathological processing of amyloid precursor protein (APP) associated with Alzheimer's disease. TBPB, a systemically active M<sub>1</sub> allosteric agonist, shows decreased production of  $\beta$ APP (Jones et al., 2008) and the M<sub>1</sub> agonist AF267B shifts APP towards the non-amyloidogenic pathway (Caccamo et al., 2006; Jones et al., 2008). Additionally, M<sub>1</sub> agonists show evidence of decreasing CSF A $\beta$ 42 levels in AD patients (Fisher, 2008; Heinrich et al., 2009). Therefore, drugs that activate AChRs may have disease modifying properties in AD as well as improve cognitive function.

## Schizophrenia

The finding that the M<sub>1</sub>/M<sub>4</sub> preferring orthosteric agonist xanomeline has antipsychotic-like effects in AD patients raises the question of whether M<sub>1</sub> and/or M<sub>4</sub> activation could also have efficacy in reducing psychotic symptoms in patients suffering from schizophrenia. The surprising finding of antipsychotic efficacy of xanomeline in AD patients prompted a Phase II clinical trial to evaluate antipsychotic efficacy of xanomeline in schizophrenia patients (Shekhar et al., 2008). Interestingly, xanomeline improved positive, negative, and cognitive symptoms in patients suffering from schizophrenia (Shekhar et al., 2008). This finding is especially interesting in light of previous studies showing that muscarinic receptor antagonists worsen symptoms in schizophrenic patients (Tandon et al., 1991) and produce

psychotic symptoms in some individuals not suffering from schizophrenia or related disorders (Osterholm and Camoriano, 1982). Furthermore, analysis of postmortem brain samples from schizophrenic patients show decreased levels of M<sub>1</sub>/M<sub>4</sub> receptor binding in key brain regions implicated in the disease, including the prefrontal cortex, superior temporal gyrus, hippocampus, and dorsal striatum (Crook et al., 2000; Dean et al., 2002; Deng and Huang, 2005; Zavitsanou et al., 2004). Taken together, these data raise the exciting possibility that M<sub>1</sub> and/or M<sub>4</sub> PAMs may also have potential utility in treatment of schizophrenia.

The M<sub>4</sub> receptor is expressed in numerous areas of the brain including the cortex, striatum, and hippocampus (Levey et al., 1995). M<sub>4</sub> colocalizes with the D<sub>1</sub> receptor in the striatum (Ince et al., 1997), suggesting a balance between cholinergic and dopaminergic neurotransmission. Mutant mice with knockout of M<sub>4</sub> in D<sub>1</sub> dopamine expressing cells (D1-M4-KO) do not show an antipsychotic response to xanomeline in rodent models of schizophrenia, highlighting the potential importance of M<sub>4</sub> receptors in schizophrenia (Dencker et al., 2011). Xanomeline treatment attenuates amphetamine-induced hyperactivity in M<sub>1</sub> knockout mice, and completely inhibits the effects of amphetamine in M<sub>4</sub> knockout mice, suggesting a greater role for M<sub>4</sub> in this process (Woolley et al., 2009). Development of the initial M<sub>4</sub> PAM thiochrome showed proof-of-concept for designing compounds with M<sub>4</sub> subtype selectivity (Lazareno et al., 2004). The discovery of a highly selective M<sub>4</sub> PAM, VU0010010 (Shirey et al., 2008) and subsequent development of centrally active M<sub>4</sub> PAMs (VU0152099, VU0152100, and VU0448088) demonstrated reversal of amphetamine-induced hyperlocomotor activity in rats (Brady et al., 2008; Le et al., 2013). The M<sub>4</sub> PAM LY2033298 also showed an effect in rodent models of antipsychotic efficacy, including conditioned avoidance responding and prepulse inhibition (Chan et al., 2008). LY2033298 potentiated the effect of oxotremorine (nonselective muscarinic agonist)-mediated inhibition of conditioned avoidance responding, indicative of antipsychotic properties (Leach et al., 2010). The effect was reduced in M<sub>4</sub> knockout mice, supporting a potential role for M<sub>4</sub> in models of schizophrenia. Interestingly, LY2033298 can have allosteric agonist activity under specific conditions as well (Chan et al., 2008; Nawaratne et al., 2010). These data provide strong support for the hypothesis that the antipsychotic effects of xanomeline are in part mediated by activation of M<sub>4</sub> and that highly selective M<sub>4</sub> PAMs may provide a novel approach to development of antipsychotic agents. In addition to potential efficacy in patients suffering from schizophrenia, M<sub>4</sub> PAMs could also provide efficacy in reducing the psychotic symptoms observed in patients suffering from AD and other neurodegenerative disorders.

Based on the discussion of M<sub>1</sub> PAM actions in models of AD above, it is possible that M<sub>1</sub> PAMs could also provide efficacy in improving cognitive function in schizophrenia patients. Interestingly, schizophrenic patients with an M<sub>1</sub> genetic polymorphism (*CHRM1*) had more correct responses with less perseverative errors in the Wisconsin Card Sorting Test (Liao et al., 2003). In total, the data generated thus far favors a prominent role of M<sub>4</sub> in psychotic symptoms and M<sub>1</sub> as a major contributor to specific domains of cognitive function. However, further studies are needed to develop a full understanding of the respective roles of M<sub>1</sub> and M<sub>4</sub> in the clinical efficacy of xanomeline and it is likely that M<sub>1</sub> activity also contributes to the antipsychotic efficacy and M<sub>4</sub> to the cognition-enhancing effects. The development of highly selective positive allosteric modulators for M<sub>1</sub> and M<sub>4</sub> receptors provides the tools needed to develop this understanding. Furthermore, M<sub>1</sub> and M<sub>4</sub> PAMs provide exciting new therapeutic opportunities to achieve subtype specificity with minimal peripheral muscarinic side effects and shows promise for the identification of novel therapeutics for both Alzheimer's disease and schizophrenia. Multiple companies are now focusing effort on discovery and development of selective M<sub>1</sub> PAMs for the treatment of schizophrenia and Alzheimer's disease. The Vanderbilt Center for Neuroscience Drug Discovery has now partnered with AstraZeneca to advance M<sub>4</sub> PAMs into clinical development (Jones et al., 2012b).



Hopefully, these and efforts by other companies will provide clinical assessment of the potential utility of M<sub>1</sub> and M<sub>4</sub> PAMs in AD and schizophrenia in the coming years.

M<sub>1</sub>/M<sub>4</sub> agonists also have potential therapeutic applications in addiction and chronic neuropathic pain. The muscarinic receptors in the brain play a key role in the development of addiction (Sofuoglu and Mooney, 2009; Williams and Adinoff, 2008). M<sub>1</sub>/M<sub>4</sub> muscarinic agonists mediate the attenuation of cocaine self-administration and cocaine discriminative stimulus, which is abolished in M<sub>1</sub>/M<sub>4</sub> knockout mice (Thomsen et al., 2010, 2012). Therefore, M<sub>1</sub> and/or M<sub>4</sub> agonism is under investigation in the treatment of drug dependence and addiction. M<sub>1</sub>/M<sub>4</sub> agonists show efficacy in chronic inflammatory and neuropathic pain, with xanomeline showing analgesic effects in a chronic inflammatory neuropathic pain rodent model (Martino et al., 2011).

## M5 modulators

The M<sub>5</sub> muscarinic receptor subtype comprises less than 2% of the CNS muscarinic receptors, and is expressed in both the cerebrovascular system and midbrain dopamine neurons (Weiner et al., 1990). The M<sub>5</sub> receptor is the sole muscarinic subtype detected in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (Vilaro et al., 1990; Weiner et al., 1990), where it is coexpressed with D<sub>2</sub> dopamine receptors, suggesting a role for M<sub>5</sub> in the modulation of dopaminergic transmission (Weiner et al., 1990). Modulation of dopaminergic function using M<sub>5</sub> PAMs or NAMs has been raised as having potential utility in the treatment of addiction (Raffa, 2009) and could also have utility for the treatment of other disorders that involved changes in dopaminergic function, including schizophrenia, and attention-deficit hyperactivity disorder (ADHD). The combination of the genes for M<sub>5</sub> (CHRM5) and α7 nicotinic acetylcholine receptor show linkage with schizophrenia susceptibility in humans (De Luca et al., 2004). Additionally, the M<sub>5</sub> knockout mouse shows decreased amphetamine-induced hyperlocomotion (Wang et al., 2004). The localization of the M<sub>5</sub> receptor to dopaminergic midbrain neurons and association with a preclinical model of schizophrenia is encouraging for research into the role of M<sub>5</sub> in schizophrenia. Studies of M<sub>5</sub> knockout mice suggest that M<sub>5</sub> may be target in AD due to cerebrovascular tone regulation (Yamada et al., 2001) as well as cognitive function. Until recently, no selective M<sub>5</sub> ligands existed, making it difficult to fully evaluate the potential utility of M<sub>5</sub> modulators in animal models related to these disorders. However, the recent discovery of highly selective M<sub>5</sub> PAMs (Bridges et al., 2009, 2010a) provides novel tools for the investigation of the role of M<sub>5</sub> in CNS disorders.

## Potential utility of M<sub>4</sub> NAMs in treatment of Parkinson's disease and dystonia

Historically anticholinergic agents were used as treatments in PD, beginning with the deadly nightshade *Atropa belladonna*. The concept of striatal dopaminergic–cholinergic antagonism developed with findings that with decreased dopamine in the striatum there is evidence of increased acetylcholine levels in PD (Salamone et al., 2001). Anticholinergics in PD act through muscarinic receptors, and acetylcholinesterase inhibitors (such as physostigmine) are known to worsen PD symptoms (Bourke and Druckenbrod, 1998; Ott and Lannon, 1992). Drug-induced tremulous jaw movements serves as a recent model for the resting tremor associated with Parkinson's disease. Tremulous jaw movements induced by the muscarinic antagonist pilocarpine are robustly decreased in M<sub>4</sub> knockout mice, suggesting that the M<sub>4</sub> subtype plays a key role (Salamone et al., 2001). Additionally, the M<sub>4</sub>-preferring antagonist tropicamide inhibits tremulous jaw movements in a rodent model of Parkinson's disease (Betz et al., 2007). Therefore, there is therapeutic potential for use of M<sub>4</sub> NAMs to control tremor associated with PD. M<sub>4</sub>-targeted therapies have potential applications in other movement disorders, including dystonia and Huntington's disease, where there is evidence of cholinergic dysfunction (Pisani et al.,

2007; Smith et al., 2006). Also, muscarinic antagonists are among the few agents that can provide efficacy in treatment of generalized dystonia (Jankovic, 2006; Martella et al., 2009). It is possible that highly selective NAMs for M<sub>4</sub> or another mAChR subtype could provide antidystonic efficacy without the adverse effects observed with non-selective mAChR antagonists.

## Optimization of allosteric modulators as drug candidates

Development and optimization of allosteric modulators of GPCRs as drug candidates present multiple challenges (Conn et al., 2012; Klein et al., 2013; Melancon et al., 2012). The success of lead compound development for a number of allosteric modulators of GPCRs has established a set of drug optimization strategies for this class. With the nonlinear or “flat/shallow” nature of SAR for many members of this drug class, designing libraries around a central core by focusing on “islands” of constituents has led to successful lead optimization (Nawaratne et al., 2010; Wood et al., 2011). Also, it is critical to focus attention on both SAR of potency and cooperativity of allosteric modulators as well as differential effects on different aspects of receptor signaling (Wootten et al., 2013). Attention to principals emerging from medicinal chemistry optimization of allosteric modulators increases the opportunities for advancing successful drug candidates in this class. Once a hit compound is identified via high throughput screening, chemical tractability should be established through the synthesis of appropriate focused iterative compound libraries to generate lead compounds. The use of systematic fluorine substitution strategies has been successful for identifying fluorinated cores tolerant of change, and proved successful for M<sub>1</sub> PAM development (Bridges et al., 2010d; Reid et al., 2011; Yang et al., 2010). Therefore, fluorine substitution may prove a good first tier strategy for the optimization of hit compounds. Selecting hit compounds with the combination of both tractable SAR and encouraging physicochemical properties has proven successful (Kenakin and Miller, 2010). It is essential to fully characterize lead compounds using radioligand binding to establish in vitro receptor interaction. Running multiple (secondary/parallel) functional screens (such as GTPγS if primary screening performed was FLIPR calcium assay) will help to avoid stimulus bias in cases where biased ligands are not desired. In addition, allosteric modulators may show differential signaling depending on the cellular context used for screening (Niswender et al., 2010). The use of in vitro native systems, such as brain slice electrophysiology, supports biological relevance of lead compounds. It is also important to note that the choice of orthosteric compound is critical for allosteric modulator screening and optimization efforts, as the cooperativity between the allosteric modulator and orthosteric site can change depending on the orthosteric probe ligand, a concept termed “probe dependence” (Kenakin, 2005; May and Christopoulos, 2003). Use of the endogenous orthosteric ligand increases the likelihood of physiological relevance. Screening assays using non-native agonists should be interpreted with the caveat of probe-dependent pharmacology. Additionally, allosteric sites vary across species and may lead to differences between in vitro screens and in vivo animal model pharmacology. While cell line systems that allow relatively high throughput are critical for screening compounds in a chemistry program, it is critical to me mindful that there are instances in which there is a disconnect between effects of allosteric modulators in cell based assays and in biologically relevant native systems and in vivo assays. The differences in pharmacology observed in cell based assays as compared to native systems can stem from undetected stimulus bias, or context-dependence of allosteric modulator action and can make allosteric modulator optimization especially challenging. This can be further complicated by “molecular switches” in which subtle changes to the structure of a compound changes the mode of pharmacology of allosteric modulators (i.e. mode switching with PAM to NAM/SAM conversion with subtle structural changes) and/or changes the receptor subtype selectivity can confound lead compound development (Bhagwanth et al., 2012; Gregory et al., 2013; Melancon et al., 2012; Utey et al., 2011;



Wood et al., 2011). The screening assays used to identify lead compounds have a limited ability to detect weak or partial allosteric modulators, which may prove to be useful compounds for further development (Christopoulos and Kenakin, 2002). This is especially important when metabolites of administered allosteric modulators have pharmacological activity that is different from that of the parent compound and thereby confound interpretation of in vivo effects of systemically administered allosteric modulators (Bridges et al., 2013). It is recommended to use compounds of the same chemotype and demonstrate competitive interactions with test compounds for both ex vivo occupancy studies and in vivo imaging studies. In summary, the drug discovery effort for allosteric modulators has provided valuable insight into optimal methods for hit-to-lead development. While the complex pharmacology of mode switching, functional bias and bitopic ligand activity complicate the discovery process for allosteric ligands, they provide novel insight into the function of GPCRs. In fact, functional selectivity may become an asset for allosteric modulators targeting disease states linked to a specific signaling pathway.

## Future opportunities

Allosteric modulators of GPCRs represent exciting drug candidates for the treatment of an array of CNS disorders. In addition to the metabotropic and muscarinic allosteric modulators detailed above, drugs have been developed targeting a number of other GPCRs (see Conn et al., 2009a for review and AlloStereic database (<http://mdl.shsmu.edu.cn/ASD/>)) (Huang et al., 2011). Several drugs have successfully entered the marketplace, providing proof of concept for allosteric modulators as clinical therapeutics. Cinacalcet (Sensipar), a PAM for the calcium sensing receptor (CaSR), is currently used to treat patients with hyperparathyroidism (Davey et al., 2012; Nemeth et al., 2004). Maraviroc (Selzentry), a NAM of chemokine receptor 5 (CCR5) (Garcia-Perez et al., 2011), prevents HIV-1 cellular entry. In addition to GPCRs, allosteric modulators have also been developed for enzymes including allosteric kinase (Akt) inhibitors (Lindsley et al., 2005) and phospholipase D (PLD) (Lavieri et al., 2010; Scott et al., 2009).

Advantages of allosteric modulators of GPCRs include subtype selectivity and functional selectivity. With the emerging literature on signaling bias, it may be possible to tailor allosteric development to target specific downstream receptor pathways, avoiding undesirable side effects elicited by parallel signaling pathways. Due to the functional selectivity possible with allosteric ligands, it is crucial to incorporate multiple functional assays into drug screening paradigms. Probing multiple pathways will allow for early detection of pathway dependent allosteric modulation (Conn et al., 2009a). The development and utilization of the operational model of allosterism allows for characterization and quantification of allosteric drug properties. In addition, it may be possible to utilize partial or silent allosteric modulators to avoid adverse effect liability. PAM ligands may also exhibit allosteric agonist activity, termed ago-potentiators, a feature that may prove advantageous in certain CNS diseases. As a part of drug development in the neurodegenerative and psychiatric area, there is increased emphasis on early demonstration of in vivo target engagement using microPET imaging in non-human primate models, with development of radioactive ligands critical to this effort (Lee and Farde, 2006; Marik J et al., 2011). This PET data is especially important in the absence of specific disease biomarkers, and can help with in vivo dose selection to avoid under or overdosing. The surge in efforts to develop allosteric modulators of GPCRs and the collaboration between industry and academics paints a promising picture for the effort to develop effective treatments for CNS diseases.

## Disclosures

P.J. Conn is a consultant for Karuna Pharmaceuticals and receives research support from Bristol-Meyers Squibb and AstraZeneca. P.J. Conn is

an inventor on multiple composition of matter patents protecting allosteric modulators of GPCRs.

## Uncited reference

Jones et al., 2011

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## References

- Abe, T., et al., 1992. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca<sup>2+</sup> signal transduction. *J. Biol. Chem.* 267, 13361–13368.
- Achat-Mendes, C., et al., 2012. Antagonism of metabotropic glutamate 1 receptors attenuates behavioral effects of cocaine and methamphetamine in squirrel monkeys. *J. Pharmacol. Exp. Ther.* 343, 214–224.
- Adams, D.H., et al., 2013. A long-term, phase 2, multicenter, randomized, open-label, comparative safety study of pomaglumetad methionil (LY2140023 monohydrate) versus atypical antipsychotic standard of care in patients with schizophrenia. *BMC Psychiatry* 13, 143.
- Adeyale, A.S., et al., 2006. Pharmacological stimulation of group ii metabotropic glutamate receptors reduces cocaine self-administration and cocaine-induced reinstatement of drug seeking in squirrel monkeys. *J. Pharmacol. Exp. Ther.* 318, 922–931.
- Adler, C.M., et al., 1998. Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. *Biol. Psychiatry* 43, 811–816.
- Allen, J.A., Roth, B.L., 2011. Strategies to discover unexpected targets for drugs active at G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* 51, 117–144.
- Amato, R.J., Felts, A.S., et al., 2013. Substituted 1-Phenyl-3-(pyridin-2-yl)urea Negative Allosteric Modulators of mGlu: Discovery of a New Tool Compound VU0463841 with Activity in Rat Models of Cocaine Addiction. *ACS Chem. Neurosci.*
- Anagnostaras, S.G., et al., 2003. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat. Neurosci.* 6, 51–58.
- Annoura, H.F.A., Uesugi, M., Tatsuoka, T., Horikawa, Y., 1996. A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxymino)cyclopropylchromen-1a-carboxylates. *Bioorg. Med. Chem. Lett.* 6 (7), 763–766.
- Auerbach, B.D., et al., 2011. Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature* 480, 63–68.
- Awad, H., et al., 2000. Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J. Neurosci.* 20, 7871–7879.
- Ayala, J.E., Chen, Y., et al., 2009. mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* 34 (9), 2057–2071.
- Bartus, R.T., et al., 1982. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217, 408–414.
- Battaglia, G., et al., 2006. Pharmacological activation of mGlu4 metabotropic glutamate receptors reduces nigrostriatal degeneration in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J. Neurosci.* 26, 7222–7229.
- Bear, M.F., et al., 2004. The mGluR theory of fragile X mental retardation. *Trends Neurosci.* 27, 370–377.
- Belozertseva, I.V., et al., 2007. Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. *Eur. Neuropsychopharmacol.* 17, 172–179.
- Bennett, C.E., et al., 2012. Fused tricyclic mGluR1 antagonists for the treatment of neuropathic pain. *Bioorg. Med. Chem. Lett.* 22, 1575–1578.
- Benneyworth, M.A., Xiang, Z., et al., 2007. A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. *Mol. Pharmacol.* 72 (2), 477–484.
- Bennouar, K.E., et al., 2013. Synergy between L-DOPA and a novel positive allosteric modulator of metabotropic glutamate receptor 4: implications for Parkinson's disease treatment and dyskinesia. *Neuropharmacology* 66, 158–169.
- Betz, A.J., McLaughlin, P.J., et al., 2007. The muscarinic receptor antagonist tropicamide suppresses tremulous jaw movements in a rodent model of parkinsonian tremor: possible role of M4 receptors. *Psychopharmacology (Berl)* 194 (3), 347–359.
- Bhagwanth, S., et al., 2012. Transformation of Pro-Leu-Gly-NH(2) peptidomimetic positive allosteric modulators of the dopamine D(2) receptor into negative modulators. *ACS Chem. Neurosci.* 3, 274–284.
- Bhakar, A.L., et al., 2012. The pathophysiology of fragile X (and what it teaches us about synapses). *Annu. Rev. Neurosci.* 35, 417–443.
- Bird, M.K., Lawrence, A.J., 2009. Group I metabotropic glutamate receptors: involvement in drug-seeking and drug-induced plasticity. *Curr. Mol. Pharmacol.* 2, 83–94.
- Blandini, F., et al., 2000. Functional changes of the basal ganglia circuitry in Parkinson's disease. *Prog. Neurobiol.* 62, 63–88.

- Bodick, N.C., et al., 1997a. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. *Arch. Neurol.* 54, 465–473.
- Bodick, N.C., et al., 1997b. The selective muscarinic agonist xanomeline improves both the cognitive deficits and behavioral symptoms of Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* 11 (Suppl. 4), S16–S22.
- Bogenpohl, J., et al., 2013. Metabotropic glutamate receptor 4 in the basal ganglia of parkinsonian monkeys: ultrastructural localization and electrophysiological effects of activation in the striatopallidal complex. *Neuropharmacology* 66, 242–252.
- Bogoyevitch, M.A., Fairlie, D.P., 2007. A new paradigm for protein kinase inhibition: blocking phosphorylation without directly targeting ATP binding. *Drug Discov. Today* 12, 622–633.
- Bourke, D., Druckenbrod, R.W., 1998. Possible association between donepezil and worsening Parkinson's disease. *Ann. Pharmacother.* 32, 610–611.
- Bradley, S.R., et al., 1999. Immunohistochemical localization of subtype 4a metabotropic glutamate receptors in the rat and mouse basal ganglia. *J. Comp. Neurol.* 407, 33–46.
- Bradley, S.R., et al., 2000. Activation of group II metabotropic glutamate receptors inhibits synaptic excitation of the substantia nigra pars reticulata. *J. Neurosci.* 20, 3085–3094.
- Brady, A.E., Jones, C.K., et al., 2008. Centrally active allosteric potentiators of the M4 muscarinic acetylcholine receptor reverse amphetamine-induced hyperlocomotor activity in rats. *J. Pharmacol. Exp. Ther.* 327 (3), 941–953.
- Bridges, T.M., Marlo, J.E., et al., 2009. Discovery of the first highly M5-preferring muscarinic acetylcholine receptor ligand, an M5 positive allosteric modulator derived from a series of 5-trifluoromethoxy N-benzyl isatins. *J. Med. Chem.* 52 (11), 3445–3448.
- Bridges, T.M., et al., 2010a. Chemical lead optimization of a pan Gq mAChR M1, M3, M5 positive allosteric modulator (PAM) lead. Part II: development of a potent and highly selective M1 PAM. *Bioorg. Med. Chem. Lett.* 20, 1972–1975.
- Bridges, T.M., LeBois, E.P., et al., 2010b. The antipsychotic potential of muscarinic allosteric modulation. *Drug News Perspect.* 23 (4), 229–240.
- Bridges, T.M., Lewis, L.M., et al., 2010c. Discovery and development of the a highly selective M1 Positive Allosteric Modulator (PAM). Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD).
- Bridges, T.M., Niswender, C.M., et al., 2010d. Discovery of a Highly Selective in vitro and in vivo M4 Positive Allosteric Modulator (PAM) Series with Greatly Improved Human Receptor Activity. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD).
- Bridges, T.M., Reid, P.R., et al., 2010e. Discovery and development of a second highly selective M1 Positive Allosteric Modulator (PAM). Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD).
- Bridges, T.M., et al., 2013. Biotransformation of a novel positive allosteric modulator of mGlu5 contributes to seizures in rats involving a receptor agonism-dependent mechanism. *Drug Metab. Dispos.*
- Brody, S.A., et al., 2004a. Effect of antipsychotic treatment on the prepulse inhibition deficit of mGluR5 knockout mice. *Psychopharmacology (Berl)* 172, 187–195.
- Brody, S.A., et al., 2004b. Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. *Mol. Psychiatry* 9, 35–41.
- Bruno, V., et al., 2000. Selective activation of mGlu4 metabotropic glutamate receptors is protective against excitotoxic neuronal death. *J. Neurosci.* 20, 6413–6420.
- Burford, N.T., et al., 2013. Discovery of positive allosteric modulators and silent allosteric modulators of the mu-opioid receptor. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10830–10835.
- Caccamo, A., et al., 2006. M1 receptors play a central role in modulating AD-like pathology in transgenic mice. *Neuron* 49, 671–682.
- Campbell, U.C., et al., 2004. The mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates PCP-induced cognitive deficits in rats. *Psychopharmacology (Berl)* 175, 310–318.
- Campo, B., et al., 2011. Characterization of an mGluR2/3 negative allosteric modulator in rodent models of depression. *J. Neurogenet.* 25, 152–166.
- Cartmell, J., et al., 1999. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. *J. Pharmacol. Exp. Ther.* 291, 161–170.
- Celanire, S., Campo, B., 2012. Recent advances in the drug discovery of metabotropic glutamate receptor 4 (mGluR4) activators for the treatment of CNS and non-CNS disorders. *Expert Opin. Drug Discov.* 7 (3), 261–280.
- Chaki, S., et al., 2004. MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* 46, 457–467.
- Chaki, S., et al., 2013. mGlu2/3 and mGlu5 receptors: potential targets for novel antidepressants. *Neuropharmacology* 66, 40–52.
- Chan, W.Y., McKinzie, D.L., et al., 2008. Allosteric modulation of the muscarinic M4 receptor as an approach to treating schizophrenia. *Proc. Natl. Acad. Sci. U. S. A.* 105 (31), 10978–10983.
- Chaudhuri, K.R., et al., 2006. Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol.* 5, 235–245.
- Chiamulera, C., et al., 2001. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat. Neurosci.* 4, 873–874.
- Christopoulos, A., Kenakin, T., 2002. G protein-coupled receptor allostereism and complexing. *Pharmacol. Rev.* 54, 323–374.
- Clifton, N.E., et al., 2013. Enhancement of social novelty discrimination by positive allosteric modulators at metabotropic glutamate 5 receptors: adolescent administration prevents adult-onset deficits induced by neonatal treatment with phencyclidine. *Psychopharmacology* 225, 579–594.
- Conn, P.J., Pin, J.P., 1997. Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* 37, 205–237.
- Conn, P.J., et al., 2005. Metabotropic glutamate receptors in the basal ganglia motor circuit. *Nat. Rev. Neurosci.* 6, 787–798.
- Conn, P.J., et al., 2008. Schizophrenia: moving beyond monoamine antagonists. *Mol. Interv.* 8, 99–107.
- Conn, P.J., et al., 2009a. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discov.* 8, 41–54.
- Conn, P.J., et al., 2009b. Subtype-selective allosteric modulators of muscarinic receptors for the treatment of CNS disorders. *Trends Pharmacol. Sci.* 30, 148–155.
- Conn, P.J., et al., 2009c. Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol. Sci.* 30, 25–31.
- Conn, P.J., Kuduk, S.D., Doller, D., 2012. Drug Design Strategies for GPCR Allosteric Modulators. In: Desai, I.M.C. (Ed.), *Annual Reports in Medicinal Chemistry*. Academic Press, Burlington, pp. 441–457.
- Cosford, N.D., Tehrani, L., et al., 2003. 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J. Med. Chem.* 46 (2), 204–206.
- Coyle, J.T., 2006. Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell. Mol. Neurobiol.* 26, 365–384.
- Coyle, J.T., Tsai, G., 2004. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. *Psychopharmacology* 174, 32–38.
- Crook, J.M., et al., 2000. Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. *Biol. Psychiatry* 48, 381–388.
- Darrah, J.M., et al., 2008. Interaction of N-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm. *Behav. Pharmacol.* 19, 225–234.
- Davey, A.E., et al., 2012. Positive and negative allosteric modulators promote biased signaling at the calcium-sensing receptor. *Endocrinology* 153, 1232–1241.
- Dawson, L., et al., 2000. The group II metabotropic glutamate receptor agonist, DCG-IV, alleviates akinesia following intranigral or intraventricular administration in the reserpine-treated rat. *Br. J. Pharmacol.* 129, 541–546.
- De Luca, V., et al., 2004. Linkage of M5 muscarinic and alpha7-nicotinic receptor genes on 15q13 to schizophrenia. *Neuropsychobiology* 50, 124–127.
- Dean, B., et al., 2002. Decreased muscarinic1 receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol. Psychiatry* 7, 1083–1091.
- Dekundy, A., et al., 2006. Effects of group I metabotropic glutamate receptors blockade in experimental models of Parkinson's disease. *Brain Res. Bull.* 69, 318–326.
- Dencker, D., et al., 2011. Involvement of a subpopulation of neuronal M4 muscarinic acetylcholine receptors in the antipsychotic-like effects of the M1/M4 preferring muscarinic receptor agonist xanomeline. *J. Neurosci.* 31, 5905–5908.
- Deng, C., Huang, X.F., 2005. Decreased density of muscarinic receptors in the superior temporal gyrus in schizophrenia. *J. Neurosci. Res.* 81, 883–890.
- Dickerson, J.W., Conn, P.J., 2012. Therapeutic potential of targeting metabotropic glutamate receptors for Parkinson's disease. *Neurodegener. Dis. Manag.* 2, 221–232.
- Digby, G.J., Noetzel, M.J., et al., 2012a. Novel allosteric agonists of M1 muscarinic acetylcholine receptors induce brain region-specific responses that correspond with behavioral effects in animal models. *J. Neurosci.* 32 (25), 8532–8544.
- Digby, G.J., Utley, T.J., et al., 2012b. Chemical modification of the M(1) agonist VU0364572 reveals molecular switches in pharmacology and a bitopic binding mode. *ACS Chem. Neurosci.* 3 (12), 1025–1036.
- Doherty, A.J., et al., 2000. A novel, competitive mGlu(5) receptor antagonist (LY344545) blocks DHPG-induced potentiation of NMDA responses but not the induction of LTP in rat hippocampal slices. *Br. J. Pharmacol.* 131, 239–244.
- Dolen, G., et al., 2007. Correction of fragile X syndrome in mice. *Neuron* 56, 955–962.
- Doria, J., et al., 2013. Metabotropic glutamate receptor 5 positive allosteric modulators are neuroprotective in a mouse model of Huntington's disease. *Br. J. Pharmacol.* 169, 909–921.
- East, S.P., Bamford, S., et al., 2010. An orally bioavailable positive allosteric modulator of the mGlu4 receptor with efficacy in an animal model of motor dysfunction. *Bioorg. Med. Chem. Lett.* 20 (16), 4901–4905.
- Emmitte, K.A., 2013. mGlu5 negative allosteric modulators: a patent review (2010–2012). *Expert Opin. Ther. Pat.* 23, 393–408.
- Engers, D.W., et al., 2009. Synthesis and evaluation of a series of heterobiaryl amides that are centrally penetrating metabotropic glutamate receptor 4 (mGluR4) positive allosteric modulators (PAMs). *J. Med. Chem.* 52, 4115–4118.
- Engers, D.W., et al., 2010. Discovery of a novel metabotropic glutamate receptor 4 (mGlu4) positive allosteric modulator (PAM) extended probe: characterization of ML292, a potent and selective mGlu4 PAM which produces efficacy alone or in combination with L-DOPA in preclinical rodent models of Parkinson's disease. Probe Reports from the NIH Molecular Libraries Program, Bethesda (MD).
- Fabbri, G., et al., 2007. Levodopa-induced dyskinesias. *Mov. Disord.* 22, 1379–1389 (quiz 1523).
- Fallarino, F., et al., 2010. Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. *Nat. Med.* 16, 897–902.
- Fang, Y., et al., 2003. G protein-coupled receptor microarrays for drug discovery. *Drug Discov. Today* 8, 755–761.
- Feinberg, I., et al., 2002. The selective group mGlu2/3 receptor agonist LY379268 suppresses REM sleep and fast EEG in the rat. *Pharmacol. Biochem. Behav.* 73, 467–474.
- Feldman, H., 2002. Treating Alzheimer's disease with cholinesterase inhibitors: what have we learned so far? *Int. Psychogeriatr.* 14 (Suppl. 1), 3–5.
- Fell, M.J., et al., 2008. Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist (–)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039). *J. Pharmacol. Exp. Ther.* 326, 209–217.
- Fell, M.J., et al., 2011. N-(4-((2-trifluoromethyl)-3-hydroxy-4-(isobutyl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant



- properties: in vivo profiling suggests a link between behavioral and central nervous system neurochemical changes. *J. Pharmacol. Exp. Ther.* 336, 165–177.
- Field, J.R., et al., 2011. Targeting glutamate synapses in schizophrenia. *Trends Mol. Med.* 17, 689–698.
- Fisher, A., 2008. M1 muscarinic agonists target major hallmarks of Alzheimer's disease—the pivotal role of brain M1 receptors. *Neurodegener. Dis.* 5, 237–240.
- Friedmann CTH, D.L., Ciccone, P.E., Rubin, R.T., 1980. Phase II double blind controlled study of a new anxiolytic, fenobam (McN-3377) vs placebo. *Curr. Ther. Res.* 27, 144–151.
- Galici, R., Jones, C.K., et al., 2006. Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. *J. Pharmacol. Exp. Ther.* 318 (1), 173–185.
- Garcia-Perez, J., et al., 2011. Allosteric model of maraviroc binding to CC chemokine receptor 5 (CCR5). *J. Biol. Chem.* 286, 33409–33421.
- Gasparini, F., Lingenhoehl, K., et al., 1999. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* 38 (10), 1493–1503.
- Gastambide, F., Gilmour, G., et al., 2013. The mGlu(5) positive allosteric modulator LSN2463359 differentially modulates motor, instrumental and cognitive effects of NMDA receptor antagonists in the rat. *Neuropharmacology* 64, 240–247.
- Gilmour, G., Broad, L.M., et al., 2013. In vitro characterisation of the novel positive allosteric modulators of the mGlu(5) receptor, LSN2463359 and LSN2814617, and their effects on sleep architecture and operant responding in the rat. *Neuropharmacology* 64, 224–239.
- Goeldner, C., Ballard, T.M., et al., 2013. Cognitive impairment in major depression and the mGlu2 receptor as a therapeutic target. *Neuropharmacology* 64, 337–346.
- Goudet, C., et al., 2008. Group III metabotropic glutamate receptors inhibit hyperalgesia in animal models of inflammation and neuropathic pain. *Pain* 137, 112–124.
- Greenamyre, J.T., O'Brien, C.F., 1991. N-methyl-D-aspartate antagonists in the treatment of Parkinson's disease. *Arch. Neurol.* 48, 977–981.
- Gregory, K.J., et al., 2013. Probing the metabotropic glutamate receptor 5 (mGlu(5)) positive allosteric modulator (PAM) binding pocket: discovery of point mutations that engender a “molecular switch” in PAM pharmacology. *Mol. Pharmacol.* 83, 991–1006.
- Grossberg, G.T., 2002. The ABC of Alzheimer's disease: behavioral symptoms and their treatment. *Int. Psychogeriatr.* 14 (Suppl. 1), 27–49.
- Halberstadt, A.L., 1995. The phencyclidine-glutamate model of schizophrenia. *Clin. Neuropharmacol.* 18, 237–249.
- Hanyu, H., et al., 2003. Effect of age on regional cerebral blood flow patterns in Alzheimer's disease patients. *J. Neurol. Sci.* 209, 25–30.
- Hashimoto, K., Malchow, B., et al., 2013. Glutamate modulators as potential therapeutic drugs in schizophrenia and affective disorders. *Eur. Arch. Psychiatry Clin. Neurosci.*
- Hasselmo, M.E., 2006. The role of acetylcholine in learning and memory. *Curr. Opin. Neurobiol.* 16, 710–715.
- Hasselmo, M.E., Giocomo, L.M., 2006. Cholinergic modulation of cortical function. *J. Mol. Neurosci.* 30, 133–135.
- Heinrich, J.N., et al., 2009. Pharmacological comparison of muscarinic ligands: historical versus more recent muscarinic M1-preferring receptor agonists. *Eur. J. Pharmacol.* 605, 53–56.
- Hemstapat, K., de Paulis, T., et al., 2006. A novel class of positive allosteric modulators of metabotropic glutamate receptor subtype 1 interact with a site distinct from that of negative allosteric modulators. *Mol. Pharmacol.* 70 (2), 616–626.
- Hemstapat, K., Da Costa, H., et al., 2007. A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors. *J. Pharmacol. Exp. Ther.* 322 (1), 254–264.
- Henry, S.A., et al., 2002. The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology* 43, 1199–1209.
- Herman, E.J., et al., 2012. Metabotropic glutamate receptors for new treatments in schizophrenia. *Handb. Exp. Pharmacol.* 297–365.
- Hikichi, H., Murai, T., et al., 2010. Effects of a novel metabotropic glutamate receptor 7 negative allosteric modulator, 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo [4,5-c]pyridin-4(5H)-one (MMPIP), on the central nervous system in rodents. *Eur. J. Pharmacol.* 639 (1–3), 106–114.
- Hirsch, E.C., et al., 2000. Metabolic effects of nigrostriatal denervation in basal ganglia. *Trends Neurosci.* 23, S78–S85.
- Hogg, R.C., et al., 2005. Allosteric modulation of ligand-gated ion channels. *Biochem. Pharmacol.* 70, 1267–1276.
- Hopkins, K.R., 2013. Is there a path forward for mGlu(2) positive allosteric modulators for the treatment of schizophrenia? *ACS Chem. Neurosci.* 4, 211–213.
- Hopkins, C.R., et al., 2009. mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. *Future Med. Chem.* 1, 501–513.
- Huang, Z., et al., 2011. ASD: a comprehensive database of allosteric proteins and modulators. *Nucleic Acids Res.* 39, D663–D669.
- Hughes, Z.A., Neal, S.J., et al., 2013. Negative allosteric modulation of metabotropic glutamate receptor 5 results in broad spectrum activity relevant to treatment resistant depression. *Neuropharmacology* 66, 202–214.
- Ince, E., et al., 1997. Differential expression of D1 and D2 dopamine and m4 muscarinic acetylcholine receptor proteins in identified striatonigral neurons. *Synapse* 27, 357–366.
- Itil T.M., S.B., Huque, M., Mukhopadhyay, S., Blasucci, D.N.K.T., Ciccone, P.E., 1978. The clinical and quantitative EEG effects and plasma levels of fenobam (McN-3377) in subjects with anxiety: an open rising dose tolerance and efficacy study. *Curr. Ther. Res.* 708–724.
- Jacquemont, S., Curie, A., et al., 2011. Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci. Transl. Med.* 3 (64), 64ra1.
- Jankovic, J., 2006. Treatment of dystonia. *Lancet Neurol.* 5, 864–872.
- Jankovic, J., 2008. Parkinson's disease: clinical features and diagnosis. *J. Neurol. Neurosurg. Psychiatry* 79, 368–376.
- Javitt, D.C., 1987. Negative schizophrenic symptomatology and the PCP (phencyclidine) model of schizophrenia. *Hillside J. Clin. Psychiatry* 9, 12–35.
- Jenner, P., 2008. Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat. Rev. Neurosci.* 9, 665–677.
- Jimenez, H.N., et al., 2012. 4-(1-Phenyl-1H-pyrazol-4-yl)quinolines as novel, selective and brain penetrant metabotropic glutamate receptor 4 positive allosteric modulators. *Bioorg. Med. Chem. Lett.* 22, 3235–3239.
- Johnson, M.P., Baez, M., et al., 2003. Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of N-(4-(2-methoxyphenoxy)phenyl)-N-(2,2,2-trifluoroethylsulfonfyl)pyrid-3-ylmethylamine. *J. Med. Chem.* 46 (15), 3189–3192.
- Johnson, K.A., et al., 2009. Glutamate receptors as therapeutic targets for Parkinson's disease. *CNS Neurol. Disord. Drug Targets* 8, 475–491.
- Johnson, K.A., et al., 2011. Activation of group II metabotropic glutamate receptors induces long-term depression of excitatory synaptic transmission in the substantia nigra pars reticulata. *Neurosci. Lett.* 504, 102–106.
- Johnson, K.A., et al., 2013. The metabotropic glutamate receptor 8 agonist (S)-3,4-DCPG reverses motor deficits in prolonged but not acute models of Parkinson's disease. *Neuropharmacology* 66, 187–195.
- Jones, C.K., et al., 2005. Analgesic effects of the selective group II (mGlu2/3) metabotropic glutamate receptor agonists LY379268 and LY389795 in persistent and inflammatory pain models after acute and repeated dosing. *Neuropharmacology* 49 (Suppl. 1), 206–218.
- Jones, C.K., Brady, A.E., et al., 2008. Novel selective allosteric activator of the M1 muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats. *J. Neurosci.* 28 (41), 10422–10433.
- Jones, C.K., et al., 2011. Discovery, synthesis, and structure–activity relationship development of a series of N-4-(2,5-dioxopyrrolidin-1-yl)phenylpicolinamides (VU0400195, ML182): characterization of a novel positive allosteric modulator of the metabotropic glutamate receptor 4 (mGlu(4)) with oral efficacy in an antiparkinsonian animal model. *J. Med. Chem.* 54, 7639–7647.
- Jones, C.K., Bubs, M., et al., 2012a. The metabotropic glutamate receptor 4-positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine 2A antagonist in preclinical rodent models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* 340 (2), 404–421.
- Jones, C.K., et al., 2012b. Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacology* 37, 16–42.
- Kalinichev, M., Rouillier, M., et al., 2013. ADX71743, a potent and selective negative allosteric modulator of metabotropic glutamate receptor 7: in vitro and in vivo characterization. *J. Pharmacol. Exp. Ther.* 344 (3), 624–636.
- Katritch, V., et al., 2013. Structure–function of the G protein-coupled receptor superfamily. *Annu. Rev. Pharmacol. Toxicol.* 53, 531–556.
- Kenakin, T., 2005. New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nat. Rev. Drug Discov.* 4, 919–927.
- Kenakin, T., 2010. G protein coupled receptors as allosteric proteins and the role of allosteric modulators. *J. Recept. Signal Transduct. Res.* 30, 313–321.
- Kenakin, T., Miller, L.J., 2010. Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacol. Rev.* 62, 265–304.
- Kenny, P.J., et al., 2005. Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology* 179, 247–254.
- Keywood, C., et al., 2009. A proof-of-concept study evaluating the effect of ADX10059, a metabotropic glutamate receptor-5 negative allosteric modulator, on acid exposure and symptoms in gastro-oesophageal reflux disease. *Gut* 58, 1192–1199.
- Kim, D.H., et al., 2009. Building a better antipsychotic: receptor targets for the treatment of multiple symptom dimensions of schizophrenia. *Neurotherapeutics* 6, 78–85.
- Kinney, G.C., et al., 2003. Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. *J. Pharmacol. Exp. Ther.* 306, 116–123.
- Kinon, B.J., et al., 2011. A multicenter, inpatient, phase 2, double-blind, placebo-controlled dose-ranging study of LY2140023 monohydrate in patients with DSM-IV schizophrenia. *J. Clin. Psychopharmacol.* 31, 349–355.
- Klein, M.T., et al., 2013. Approaches for probing allosteric interactions at 7 transmembrane spanning receptors. *Prog. Mol. Biol. Transl. Sci.* 115, 1–59.
- Knoflach, F., Mutel, V., et al., 2001. Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. *Proc. Natl. Acad. Sci. U. S. A.* 98 (23), 13402–13407.
- Kola, I., Landis, J., 2004. Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3, 711–715.
- Koniczny, J., et al., 2007. *c. Neurosci* 145, 611–620.
- Krueger, J.D., Bear, M.F., 2011. Toward fulfilling the promise of molecular medicine in fragile X syndrome. *Annu. Rev. Med.* 62, 411–429.
- Krystal, J.H., et al., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch. Gen. Psychiatry* 51, 199–214.
- Kulisevsky, J., Poyurovsky, M., 2012. Adenosine A2A-receptor antagonism and pathophysiology of Parkinson's disease and drug-induced movement disorders. *Eur. Neurol.* 67, 4–11.
- Kumar, N., et al., 2010. Metabotropic glutamate receptors (mGluRs) regulate noxious stimulus-induced glutamate release in the spinal cord dorsal horn of rats with neuropathic and inflammatory pain. *J. Neurochem.* 114, 281–290.
- Kumaresan, V., et al., 2009. Metabotropic glutamate receptor 5 (mGluR5) antagonists attenuate cocaine priming- and cue-induced reinstatement of cocaine seeking. *Behav. Brain Res.* 202, 238–244.



- Lahti, A.C., et al., 1995. Ketamine activates psychosis and alters limbic blood flow in schizophrenia. *Neuroreport* 6, 869–872.
- Langmead, C.J., Austin, N.E., et al., 2008a. Characterization of a CNS penetrant, selective M1 muscarinic receptor agonist, 77-LH-28-1. *Br. J. Pharmacol.* 154 (5), 1104–1115.
- Langmead, C.J., et al., 2008b. Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacol. Ther.* 117, 232–243.
- Lavie, R.R., et al., 2010. Design, synthesis, and biological evaluation of halogenated N-(2-(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decan-8-yl)ethyl)benzamides: discovery of an isoform-selective small molecule phospholipase D2 inhibitor. *J. Med. Chem.* 53, 6706–6719.
- Lavreysen, H., Pereira, S.N., et al., 2004. Metabotropic glutamate 1 receptor distribution and occupancy in the rat brain: a quantitative autoradiographic study using [3H] R214127. *Neuropharmacology* 46 (5), 609–619.
- Lazareno, S., et al., 1998. Subtype-selective positive cooperative interactions between brucine analogues and acetylcholine at muscarinic receptors: radioligand binding studies. *Mol. Pharmacol.* 53, 573–589.
- Lazareno, S., Birdsall, B., et al., 1999. Allosteric effects of four stereoisomers of a fused indole ring system with 3H-N-methylscopolamine and acetylcholine at M1–M4 muscarinic receptors. *Life Sci.* 64 (6–7), 519–526.
- Lazareno, S., Dolezal, V., et al., 2004. Thiochrome enhances acetylcholine affinity at muscarinic M4 receptors: receptor subtype selectivity via cooperativity rather than affinity. *Mol. Pharmacol.* 65 (1), 257–266.
- Le Poul, E., et al., 2012. A potent and selective metabotropic glutamate receptor 4 positive allosteric modulator improves movement in rodent models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* 343, 167–177.
- Le, U., et al., 2013. Discovery of a selective M(4) positive allosteric modulator based on the 3-amino-thieno[2,3-b]pyridine-2-carboxamide scaffold: development of ML253, a potent and brain penetrant compound that is active in a preclinical model of schizophrenia. *Bioorg. Med. Chem. Lett.* 23, 346–350.
- Leach, K., et al., 2010. Molecular mechanisms of action and in vivo validation of an M4 muscarinic acetylcholine receptor allosteric modulator with potential antipsychotic properties. *Neuropsychopharmacology* 35, 855–869.
- Lebois, E.P., Bridges, T.M., et al., 2010. Discovery and characterization of novel subtype-selective allosteric agonists for the investigation of M(1) receptor function in the central nervous system. *ACS Chem. Neurosci.* 1 (2), 104–121.
- Lebon, G., et al., 2009. Mutagenic mapping suggests a novel binding mode for selective agonists of M1 muscarinic acetylcholine receptors. *Mol. Pharmacol.* 75, 331–341.
- Lecourtier, L., et al., 2007. Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses N-Methyl-D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. *Biol. Psychiatry* 62, 739–746.
- Lee, C.M., Farde, L., 2006. Using positron emission tomography to facilitate CNS drug development. *Trends Pharmacol. Sci.* 27, 310–316.
- Lee, B., et al., 2005. Attenuation of behavioral effects of cocaine by the Metabotropic Glutamate Receptor 5 Antagonist 2-Methyl-6-(phenylethynyl)-pyridine in squirrel monkeys: comparison with dizocilpine. *J. Pharmacol. Exp. Ther.* 312, 1232–1240.
- Levandis, G., et al., 2008. Systemic administration of an mGluR5 antagonist, but not unilateral subthalamic lesion, counteracts L-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. *Neurobiol. Dis.* 29, 161–168.
- Levey, A.I., et al., 1991. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* 11, 3218–3226.
- Levey, A.I., et al., 1995. Expression of m1–m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. *J. Neurosci.* 15, 4077–4092.
- Lewis, J.A., et al., 2008. Allosteric modulation of kinases and GPCRs: design principles and structural diversity. *Curr. Opin. Chem. Biol.* 12, 269–280.
- Li, X., et al., 2006. Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. *J. Pharmacol. Exp. Ther.* 319, 254–259.
- Liao, D.L., et al., 2003. Association of muscarinic m1 receptor genetic polymorphisms with psychiatric symptoms and cognitive function in schizophrenic patients. *Neuropsychobiology* 48, 72–76.
- Liechti, M.E., Markou, A., 2007. Interactive effects of the mGlu5 receptor antagonist MPEP and the mGlu2/3 receptor antagonist LY341495 on nicotine self-administration and reward deficits associated with nicotine withdrawal in rats. *Eur. J. Pharmacol.* 554, 164–174.
- Lindemann, L., Jaeschke, G., et al., 2011. CTEP: a novel, potent, long-acting, and orally bioavailable metabotropic glutamate receptor 5 inhibitor. *J. Pharmacol. Exp. Ther.* 339 (2), 474–486.
- Lindsley, C.W., et al., 2005. Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors. *Bioorg. Med. Chem. Lett.* 15, 761–764.
- Lindsley, C.W., et al., 2006. Progress towards validating the NMDA receptor hypofunction hypothesis of schizophrenia. *Curr. Top. Med. Chem.* 6, 771–785.
- Liu, F., Grauer, S., et al., 2008. ADX47273 [S-(4-fluoro-phenyl)-3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl]-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities. *J. Pharmacol. Exp. Ther.* 327 (3), 827–839.
- Lockhart, I.A., et al., 2009. Safety and tolerability of donepezil, rivastigmine and galantamine for patients with Alzheimer's disease: systematic review of the 'real-world' evidence. *Dement. Geriatr. Cogn. Disord.* 28, 389–403.
- Lopez, S., et al., 2007. Targeting group III metabotropic glutamate receptors produces complex behavioral effects in rodent models of Parkinson's disease. *J. Neurosci.* 27, 6701–6711.
- Lorrain, D.S., et al., 2003. Group II mGlu receptor activation suppresses norepinephrine release in the ventral hippocampus and locomotor responses to acute ketamine challenge. *Neuropsychopharmacology* 28, 1622–1632.
- Ma, L., et al., 2009. Selective activation of the M1 muscarinic acetylcholine receptor achieved by allosteric potentiation. *Proc. Natl. Acad. Sci. U. S. A.* 106, 15950–15955.
- Mabire, D., et al., 2005. Synthesis, structure–activity relationship, and receptor pharmacology of a new series of quinoline derivatives acting as selective, noncompetitive mGlu1 antagonists. *J. Med. Chem.* 48, 2134–2153.
- Madlne, N., et al., 2004. Activation of group III metabotropic glutamate receptors in selected regions of the basal ganglia alleviates akinesia in the reserpine-treated rat. *Br. J. Pharmacol.* 141, 15–22.
- Maj, M., Bruno, V., et al., 2003. (–)-PHCCC, a positive allosteric modulator of mGluR4: characterization, mechanism of action, and neuroprotection. *Neuropharmacology* 45 (7), 895–906.
- Malhotra, A.K., et al., 1996. NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. *Neuropsychopharmacology* 14, 301–307.
- Malhotra, A.K., et al., 1997. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* 17, 141–150.
- Marek, G.J., 2010. Metabotropic glutamate 2/3 (mGlu2/3) receptors, schizophrenia and cognition. *Eur. J. Pharmacol.* 639, 81–90.
- Marik, J.S.B., Williams, S.-P., van Bruggen, N., 2011. New imaging paradigms in drug development: the PET imaging approach. In: V. B. Beckmann, N., Lam, K., Timmerman, H. (Eds.), *Drug Discovery Today: Technologies*. Elsevier, p. 2011.
- Marino, M.J., Williams Jr., D.L., et al., 2003. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. *Proc. Natl. Acad. Sci. U. S. A.* 100 (23), 13668–13673.
- Marlo, J.E., et al., 2009. Discovery and characterization of novel allosteric potentiators of M1 muscarinic receptors reveals multiple modes of activity. *Mol. Pharmacol.* 75, 577–588.
- Marsden, C.D., Parkes, J.D., 1977. Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet* 1, 345–349.
- Martella, G., et al., 2009. Impairment of bidirectional synaptic plasticity in the striatum of a mouse model of DYT1 dystonia: role of endogenous acetylcholine. *Brain* 132, 2336–2349.
- Martin, L.J., et al., 1992. Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron* 9, 259–270.
- Martino, G., et al., 2011. The M1/M4 preferring agonist xanomeline is analgesic in rodent models of chronic inflammatory and neuropathic pain via central site of action. *Pain* 152, 2852–2860.
- Mathiesen, J.M., et al., 2005. Identification of indole derivatives exclusively interfering with a G protein-independent signaling pathway of the prostaglandin D2 receptor CRTH2. *Mol. Pharmacol.* 68, 393–402.
- Matsui, T., Kita, H., 2003. Activation of group III metabotropic glutamate receptors presynaptically reduces both GABAergic and glutamatergic transmission in the rat globus pallidus. *Neuroscience* 122, 727–737.
- May, L.T., Christopoulos, A., 2003. Allosteric modulators of G-protein-coupled receptors. *Curr. Opin. Pharmacol.* 3, 551–556.
- Meissner, W.G., et al., 2011. Priorities in Parkinson's disease research. *Nat. Rev. Drug Discov.* 10, 377–393.
- Mela, F., et al., 2007. Antagonism of metabotropic glutamate receptor type 5 attenuates L-DOPA-induced dyskinesia and its molecular and neurochemical correlates in a rat model of Parkinson's disease. *J. Neurochem.* 101, 483–497.
- Melancon, B.J., et al., 2012. Allosteric modulation of seven transmembrane spanning receptors: theory, practice, and opportunities for central nervous system drug discovery. *J. Med. Chem.* 55, 1445–1464.
- Melancon, B.J., et al., 2013. Isatin replacements applied to the highly selective, muscarinic M1 PAM ML137: continued optimization of an MLPCN probe molecule. *Bioorg. Med. Chem. Lett.* 23, 412–416.
- Michalon, A., et al., 2012. Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron* 74, 49–56.
- Miyakawa, T., et al., 2001. Hyperactivity and intact hippocampus-dependent learning in mice lacking the M1 muscarinic acetylcholine receptor. *J. Neurosci.* 21, 5239–5250.
- Moghaddam, B., 2004. Targeting metabotropic glutamate receptors for treatment of the cognitive symptoms of schizophrenia. *Psychopharmacology (Berl)* 174, 39–44.
- Moghaddam, B., Adams, B.W., 1998. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* 281, 1349–1352.
- Mohler, H., et al., 2002. A new benzodiazepine pharmacology. *J. Pharmacol. Exp. Ther.* 300, 2–8.
- Montana, M.C., et al., 2009. The metabotropic glutamate receptor subtype 5 antagonist fenobam is analgesic and has improved in vivo selectivity compared with the prototypical antagonist 2-methyl-6-(phenylethynyl)-pyridine. *J. Pharmacol. Exp. Ther.* 330, 834–843.
- Morin, N., et al., 2010. Effect of the metabotropic glutamate receptor type 5 antagonists MPEP and MTEP in parkinsonian monkeys. *Neuropharmacology* 58, 981–986.
- Morin, N., et al., 2013a. MPEP, an mGlu5 receptor antagonist, reduces the development of L-DOPA-induced motor complications in de novo parkinsonian monkeys: biochemical correlates. *Neuropharmacology* 66, 355–364.
- Morin, N., et al., 2013b. Chronic treatment with MPEP, an mGlu5 receptor antagonist, normalizes basal ganglia glutamate neurotransmission in L-DOPA-treated parkinsonian monkeys. *Neuropharmacology* 73C, 216–231.
- Morishima, Y., et al., 2005. Enhanced cocaine responsiveness and impaired motor coordination in metabotropic glutamate receptor subtype 2 knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4170–4175.
- Murray, T.K., et al., 2002. Evaluation of the mGluR2/3 agonist LY379268 in rodent models of Parkinson's disease. *Pharmacol. Biochem. Behav.* 73, 455–466.
- Nawaratne, V., et al., 2010. Structural determinants of allosteric agonism and modulation at the M4 muscarinic acetylcholine receptor: identification of ligand-specific and global activation mechanisms. *J. Biol. Chem.* 285, 19012–19021.
- Nemeth, E.F., et al., 2004. Pharmacodynamics of the type II calcimimetic compound cinacalcet HCl. *J. Pharmacol. Exp. Ther.* 308, 627–635.

- Newcomer, J.W., et al., 1999. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. *Neuropsychopharmacology* 20, 106–118.
- Nicoletti, F., et al., 2011. Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology* 60, 1017–1041.
- Niswender, C.M., Conn, P.J., 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* 50, 295–322.
- Niswender, C.M., Johnson, K.A., et al., 2008. Discovery, characterization, and antiparkinsonian effect of novel positive allosteric modulators of metabotropic glutamate receptor 4. *Mol. Pharmacol.* 74 (5), 1345–1358.
- Niswender, C.M., Johnson, K.A., et al., 2010. Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol. Pharmacol.* 77 (3), 459–468.
- Noetzel, M.J., et al., 2013. A novel metabotropic glutamate receptor 5 positive allosteric modulator acts at a unique site and confers stimulus bias to mGlu5 signaling. *Mol. Pharmacol.* 83, 835–847.
- Osterholm, R.K., Camoriano, J.K., 1982. Transdermal scopolamine psychosis. *JAMA* 247, 3081.
- Ott, B.R., Lannon, M.C., 1992. Exacerbation of parkinsonism by tacrine. *Clin. Neuropharmacol.* 15, 322–325.
- Ouattara, B., et al., 2010. Effect of L-Dopa on metabotropic glutamate receptor 5 in the brain of parkinsonian monkeys. *J. Neurochem.* 113, 715–724.
- Palucha, A., et al., 2005. Potential antidepressant-like effect of MTEP, a potent and highly selective mGluR5 antagonist. *Pharmacol. Biochem. Behav.* 81, 901–906.
- Palucha-Poniewiera, A., et al., 2010. On the mechanism of the antidepressant-like action of group II mGlu receptor antagonist, MGS0039. *Psychopharmacology (Berl)* 212, 523–535.
- Parmentier-Batteur, S., et al., 2013. Mechanism based neurotoxicity of mGlu5 positive allosteric modulators — development challenges for a promising novel antipsychotic target. *Neuropharmacology*.
- Parwani, A., et al., 2005. The effects of a subanesthetic dose of ketamine on verbal memory in normal volunteers. *Psychopharmacology (Berl)* 183, 265–274.
- Patil, S.T., et al., 2007. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nat. Med.* 13, 1102–1107.
- Pecknold, J.C., et al., 1982. Treatment of anxiety using fenobam (a nonbenzodiazepine) in a double-blind standard (diazepam) placebo-controlled study. *J. Clin. Psychopharmacol.* 2, 129–133.
- Pellegrino, D., et al., 2007. Modulation of dopaminergic and glutamatergic brain function: PET studies on parkinsonian rats. *J. Nucl. Med.* 48, 1147–1153.
- Pilc, A., et al., 2013. Glutamate-based antidepressants: preclinical psychopharmacology. *Biol. Psychiatry* 73, 1125–1132.
- Pisani, A., et al., 2001. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience* 106, 579–587.
- Pisani, A., et al., 2007. Re-emergence of striatal cholinergic interneurons in movement disorders. *Trends Neurosci.* 30, 545–553.
- Platt, D.M., et al., 2008. Attenuation of cocaine self-administration in squirrel monkeys following repeated administration of the mGluR5 antagonist MPEP: comparison with diazepam. *Psychopharmacology* 200, 167–176.
- Porter, R.H., Jaeschke, G., et al., 2005. Fenobam: a clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J. Pharmacol. Exp. Ther.* 315 (2), 711–721.
- Poslusney, M.S., et al., 2013. Spirocyclic replacements for the isatin in the highly selective, muscarinic M1 PAM ML137: the continued optimization of an MLPCN probe molecule. *Bioorg. Med. Chem. Lett.* 23, 1860–1864.
- Prashanth, L.K., et al., 2011. L-Dopa-induced dyskinesia-clinical presentation, genetics, and treatment. *Int. Rev. Neurobiol.* 98, 31–54.
- Przedborski, S., 2005. Pathogenesis of nigral cell death in Parkinson's disease. *Parkinsonism Relat. Disord.* 11 (Suppl. 1), S3–S7.
- Raffa, R.B., 2009. The M5 muscarinic receptor as possible target for treatment of drug abuse. *J. Clin. Pharm. Ther.* 34, 623–629.
- Reid, P.R., et al., 2011. Discovery and optimization of a novel, selective and brain penetrant M1 positive allosteric modulator (PAM): the development of ML169, an MLPCN probe. *Bioorg. Med. Chem. Lett.* 21, 2697–2701.
- Robichaud, A.J., et al., 2011. Recent progress on the identification of metabotropic glutamate 4 receptor ligands and their potential utility as CNS therapeutics. *ACS Chem. Neurosci.* 2, 433–449.
- Robinson, L., et al., 2011. Involvement of the cholinergic system in conditioning and perceptual memory. *Behav. Brain Res.* 221, 443–465.
- Rodriguez, A.L., Nong, Y., et al., 2005. A close structural analog of 2-methyl-6-(phenylethynyl)-pyridine acts as a neutral allosteric site ligand on metabotropic glutamate receptor subtype 5 and blocks the effects of multiple allosteric modulators. *Mol. Pharmacol.* 68 (6), 1793–1802.
- Rodriguez, A.L., Grier, M.D., et al., 2010. Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. *Mol. Pharmacol.* 78 (6), 1105–1123.
- Rook, J.M., et al., 2013. Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor subtype 5 determine differences in in vivo activity. *Biol. Psychiatry* 73, 501–509.
- Rosenbrock, H., et al., 2010. Functional interaction of metabotropic glutamate receptor 5 and NMDA-receptor by a metabotropic glutamate receptor 5 positive allosteric modulator. *Eur. J. Pharmacol.* 639, 40–46.
- Rowland, L.M., 2005. Subanesthetic ketamine: how it alters physiology and behavior in humans. *Aviat. Space Environ. Med.* 76, C52–C58.
- Rylander, D., et al., 2009. Pharmacological modulation of glutamate transmission in a rat model of L-DOPA-induced dyskinesia: effects on motor behavior and striatal nuclear signaling. *J. Pharmacol. Exp. Ther.* 330, 227–235.
- Rylander, D., et al., 2010. A mGluR5 antagonist under clinical development improves L-DOPA-induced dyskinesia in parkinsonian rats and monkeys. *Neurobiol. Dis.* 39, 352–361.
- Sachpatzidis, A., et al., 2003. Identification of allosteric peptide agonists of CXCR4. *J. Biol. Chem.* 278, 896–907.
- Salamone, J.D., et al., 2001. Neostriatal muscarinic receptor subtypes involved in the generation of tremulous jaw movements in rodents implications for cholinergic involvement in parkinsonism. *Life Sci.* 68, 2579–2584.
- Samadi, P., et al., 2008. mGluR5 metabotropic glutamate receptors and dyskinesias in MPTP monkeys. *Neurobiol. Aging* 29, 1040–1051.
- Sanacora, G., et al., 2004. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch. Gen. Psychiatry* 61, 705–713.
- Santoro, M.R., et al., 2012. Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annu. Rev. Pathol.* 7, 219–245.
- Schkeryantz, J.M., et al., 2007. Prospects for metabotropic glutamate 1 receptor antagonists in the treatment of neuropathic pain. *J. Med. Chem.* 50, 2563–2568.
- Schlumberger, C., et al., 2009. Comparison of the mGlu(5) receptor positive allosteric modulator ADX47273 and the mGlu(2/3) receptor agonist LY354740 in tests for antipsychotic-like activity. *Eur. J. Pharmacol.* 623, 73–83.
- Schlumberger, C., et al., 2010. Effects of a positive allosteric modulator of mGluR5 ADX47273 on conditioned avoidance response and PCP-induced hyperlocomotion in the rat as models for schizophrenia. *Pharmacol. Biochem. Behav.* 95, 23–30.
- Schoepp, D.D., Marek, G.J., 2002. Preclinical pharmacology of mGlu2/3 receptor agonists: novel agents for schizophrenia? *Curr. Drug Targets CNS Neurol. Disord.* 1, 215–225.
- Schoepp, D.D., et al., 2003. LY354740, an mGlu2/3 receptor agonist as a novel approach to treat anxiety/stress. *Stress* 6, 189–197.
- Scott, S.A., et al., 2009. Design of isoform-selective phospholipase D inhibitors that modulate cancer cell invasiveness. *Nat. Chem. Biol.* 5, 108–117.
- Sheffler, D.J., Conn, P.J., 2008. Allosteric potentiators of metabotropic glutamate receptor subtype 1 differentially modulate independent signaling pathways in baby hamster kidney cells. *Neuropharmacology* 55, 419–427.
- Sheffler, D.J., Wenthur, C.J., et al., 2012. Development of a novel, CNS-penetrant, metabotropic glutamate receptor 3 (mGlu3) NAM probe (ML289) derived from a closely related mGlu5 PAM. *Bioorg. Med. Chem. Lett.* 22 (12), 3921–3925.
- Shekhar, A., et al., 2008. Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am. J. Psychiatry* 165, 1033–1039.
- Shigemoto, R., et al., 1993. Immunohistochemical localization of a metabotropic glutamate receptor, mGluR5, in the rat brain. *Neurosci. Lett.* 163, 53–57.
- Shirey, J.K., et al., 2008. An allosteric potentiator of M4 mAChR modulates hippocampal synaptic transmission. *Nat. Chem. Biol.* 4, 42–50.
- Shirey, J.K., Brady, A.E., et al., 2009. A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. *J. Neurosci.* 29 (45), 14271–14286.
- Sibille, P., et al., 2007. Synthesis and biological evaluation of 1-amino-2-phosphonomethylcyclopropanecarboxylic acids, new group III metabotropic glutamate receptor agonists. *J. Med. Chem.* 50, 3585–3595.
- Silverman, J.L., et al., 2012. Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. *Sci. Transl. Med.* 4 (131ra51).
- Slawinska, A., Wieronska, J.M., et al., 2013a. The antipsychotic-like effects of mGlu4 receptor positive allosteric modulators in rodents. *Br. J. Pharmacol.*
- Slawinska, A., Wieronska, J.M., et al., 2013b. Anxiolytic- but not antidepressant-like activity of Lu AF21934, a novel, selective positive allosteric modulator of the mGlu(4) receptor. *Neuropharmacology* 66, 225–235.
- Smith, R., et al., 2006. Cholinergic neuronal defect without cell loss in Huntington's disease. *Hum. Mol. Genet.* 15, 3119–3131.
- Sofuoglu, M., Mooney, M., 2009. Cholinergic functioning in stimulant addiction: implications for medications development. *CNS Drugs* 23, 939–952.
- Spalding, T.A., Trotter, C., et al., 2002. Discovery of an ectopic activation site on the M(1) muscarinic receptor. *Mol. Pharmacol.* 61 (6), 1297–1302.
- Stefani, M.R., Moghaddam, B., 2010. Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. *Eur. J. Pharmacol.* 639, 26–32.
- Sur, C., Mallorga, P.J., et al., 2003. N-desmethyloclozapine, an allosteric agonist at muscarinic 1 receptor, potentiates N-methyl-D-aspartate receptor activity. *Proc. Natl. Acad. Sci. U. S. A.* 100 (23), 13674–13679.
- Swanson, C.J., et al., 2005. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. *Nat. Rev. Drug Discov.* 4, 131–144.
- Tabolacci, E., et al., 2012. The mGluR5 antagonist AFQ056 does not affect methylation and transcription of the mutant FMR1 gene in vitro. *BMC Med. Genet.* 13, 13.
- Tandon, R., et al., 1991. Muscarinic cholinergic hyperactivity in schizophrenia. Relationship to positive and negative symptoms. *Schizophr. Res.* 4, 23–30.
- Taylor, D.L., et al., 2003. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. *J. Neurosci.* 23, 2150–2160.
- Thomas, N.K., Wright, R.A., et al., 2001. (S)-3,4-DCPG, a potent and selective mGlu8a receptor agonist, activates metabotropic glutamate receptors on primary afferent terminals in the neonatal rat spinal cord. *Neuropharmacology* 40 (3), 311–318.
- Thomas, A.M., Bui, N., et al., 2012. Group I metabotropic glutamate receptor antagonists alter select behaviors in a mouse model for fragile X syndrome. *Psychopharmacology* 219 (1), 47–58.
- Thomsen, M., et al., 2010. Attenuation of cocaine's reinforcing and discriminative stimulus effects via muscarinic M1 acetylcholine receptor stimulation. *J. Pharmacol. Exp. Ther.* 332, 959–969.
- Thomsen, M., et al., 2012. Contribution of both M1 and M4 receptors to muscarinic agonist-mediated attenuation of the cocaine discriminative stimulus in mice. *Psychopharmacology (Berl)* 220, 673–685.

- 1917 Tsai, P., Sahin, M., 2011. Mechanisms of neurocognitive dysfunction and therapeutic con-  
1918 siderations in tuberous sclerosis complex. *Curr. Opin. Neurol.* 24, 106–113.
- 1919 Ugolini, A., Large, C.H., et al., 2008. AMN082, an allosteric mGluR7 agonist that inhibits af-  
1920 ferent glutamatergic transmission in rat basolateral amygdala. *Neuropharmacology*  
1921 55 (4), 532–536.
- 1922 Uslaner, J.M., Eddins, D., et al., 2013. The muscarinic M1 receptor positive allosteric mod-  
1923 ulator PQCA improves cognitive measures in rat, cynomolgus macaque, and rhesus  
1924 macaque. *Psychopharmacology (Berl)* 225 (1), 21–30.
- 1925 Utley, T., et al., 2011. Synthesis and SAR of a novel metabotropic glutamate receptor 4  
1926 (mGlu4) antagonist: unexpected 'molecular switch' from a closely related mGlu4  
1927 positive allosteric modulator. *Bioorg. Med. Chem. Lett.* 21, 6955–6959.
- 1928 Valenti, O., et al., 2003a. Modulation of excitatory transmission onto midbrain dopaminer-  
1929 gic neurons of the rat by activation of group III metabotropic glutamate receptors.  
1930 *Ann. N. Y. Acad. Sci.* 1003, 479–480.
- 1931 Valenti, O., et al., 2003b. Group III metabotropic glutamate receptor-mediated modulation  
1932 of the striatopallidal synapse. *J. Neurosci.* 23, 7218–7226.
- 1933 Valenti, O., et al., 2005. Group III metabotropic glutamate-receptor-mediated modulation  
1934 of excitatory transmission in rodent substantia nigra pars compacta dopamine neu-  
1935 rons. *J. Pharmacol. Exp. Ther.* 313, 1296–1304.
- 1936 van Bon, B.W., et al., 2011. The phenotype of recurrent 10q22q23 deletions and duplica-  
1937 tions. *Eur. J. Hum. Genet.* 19, 400–408.
- 1938 Vanover, K.E., et al., 2008. Antipsychotic-like behavioral effects and cognitive enhance-  
1939 ment by a potent and selective muscarinic M-sub-1 receptor agonist, AC-260584.  
1940 *Behav. Neurosci.* 122, 570–575.
- 1941 Vardigan, J.D., et al., 2010. MK-801 produces a deficit in sucrose preference that is  
1942 reversed by clozapine, D-serine, and the metabotropic glutamate 5 receptor positive  
1943 allosteric modulator CDPBB: relevance to negative symptoms associated with schizo-  
1944 phrenia? *Pharmacol. Biochem. Behav.* 95, 223–229.
- 1945 Varty, G.B., et al., 2008. The effects of adenosine A2A receptor antagonists on  
1946 haloperidol-induced movement disorders in primates. *Psychopharmacology*  
1947 200, 393–401.
- 1948 Vilaro, M.T., et al., 1990. Localization of m5 muscarinic receptor mRNA in rat brain exam-  
1949 ined by in situ hybridization histochemistry. *Neurosci. Lett.* 114, 154–159.
- 1950 Wang, H., et al., 2004. Decreased amphetamine-induced locomotion and improved latent  
1951 inhibition in mice mutant for the M5 muscarinic receptor gene found in the human  
1952 15q schizophrenia region. *Neuropsychopharmacology* 29, 2126–2139.
- 1953 Wei, H., et al., 2003. Independent beta-arrestin 2 and G protein-mediated pathways for  
1954 angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc. Natl.*  
1955 *Acad. Sci. U. S. A.* 100, 10782–10787.
- 1956 Weiner, D.M., et al., 1990. Expression of muscarinic acetylcholine and dopamine receptor  
1957 mRNAs in rat basal ganglia. *Proc. Natl. Acad. Sci. U. S. A.* 87, 7050–7054.
- 1958 Wichmann, T., DeLong, M.R., 1996. Functional and pathophysiological models of the basal  
1959 ganglia. *Curr. Opin. Neurobiol.* 6, 751–758.
- 1960 Wichmann, J., Bleicher, K., et al., 2002. Alkyl diphenylacetyl, 9H-xanthene- and 9H-  
1961 thioxanthene-carbonyl carbamates as positive allosteric modulators of mGlu1 recep-  
1962 tors. *Farmacol.* 57 (12), 989–992.
- 1963 Wieronska, J.M., et al., 2012. Opposing efficacy of group III mGlu receptor activators, LSP1-  
1964 2111 and AMN082, in animal models of positive symptoms of schizophrenia. *Psycho-*  
1965 *pharmacology (Berl)* 220, 481–494.
- 1966 Wieronska, J.M., et al., 2013. The antipsychotic-like effects of the mGlu group III  
1967 orthosteric agonist, LSP1-2111, involves 5-HT(1)A signalling. *Psychopharmacology*  
1968 (Berl) 227, 711–725.
- 1969 Williams, M.J., Adinoff, B., 2008. The role of acetylcholine in cocaine addiction. *Neuro-*  
1970 *psychopharmacology* 33, 1779–1797.
- 1971 Wood, M.R., et al., 2011. "Molecular switches" on mGluR allosteric ligands that modulate  
1972 modes of pharmacology. *Biochemistry* 50, 2403–2410.
- 1973 Woolley, M.L., et al., 2009. Attenuation of amphetamine-induced activity by the non-  
1974 selective muscarinic receptor agonist, xanomeline, is absent in muscarinic M4 recep-  
1975 tor knockout mice and attenuated in muscarinic M1 receptor knockout mice. *Eur.*  
1976 *J. Pharmacol.* 603, 147–149.
- 1977 Wootten, D., et al., 2013. Emerging paradigms in GPCR allostery: implications for drug  
1978 discovery. *Nat. Rev. Drug Discov.* 12, 630–644.
- 1979 Yamada, M., et al., 2001. Cholinergic dilation of cerebral blood vessels is abolished in M(5)  
1980 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 98,  
1981 14096–14101.
- 1982 Yang, F.V., et al., 2010. Parallel synthesis of N-biaryl quinolone carboxylic acids as selective  
1983 M(1) positive allosteric modulators. *Bioorg. Med. Chem. Lett.* 20, 531–536.
- 1984 Yu, F., et al., 2013. Metabotropic glutamate receptor 1 (mGluR1) antagonism impairs  
1985 cocaine-induced conditioned place preference via inhibition of protein synthesis.  
1986 *Neuropsychopharmacology* 38, 1308–1321.
- 1987 Zarate Jr., C.A., et al., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in  
1988 treatment-resistant major depression. *Arch. Gen. Psychiatry* 63, 856–864.
- 1989 Zavitsanou, K., et al., 2004. Investigation of m1/m4 muscarinic receptors in the anterior  
1990 cingulate cortex in schizophrenia, bipolar disorder, and major depression disorder.  
1991 *Neuropsychopharmacology* 29, 619–625.
- 1992 Zerbib, F., et al., 2010. Efficacy, tolerability and pharmacokinetics of a modified release for-  
1993 mulation of ADX10059, a negative allosteric modulator of metabotropic glutamate re-  
1994 ceptor 5: an esophageal pH-impedance study in healthy subjects. *Neurogastroenterol.*  
1995 *Motil.* 22 (859–65), e231.
- 1996 Zhang, Y., et al., 2005. Allosteric potentiators of metabotropic glutamate receptor subtype  
1997 5 have differential effects on different signaling pathways in cortical astrocytes.  
1998 *J. Pharmacol. Exp. Ther.* 315, 1212–1219.