Topics in Medicinal Chemistry 13

Sylvain Celanire
Sonia Poli *Editors*

# Small Molecule Therapeutics for Schizophrenia

Springer

# 13
# Topics in Medicinal Chemistry

## Aims and Scope

Drug research requires interdisciplinary team-work at the interface between chemistry, biology and medicine. Therefore, the new topic-related series *Topics in Medicinal Chemistry* will cover all relevant aspects of drug research, e.g. pathobiochemistry of diseases, identification and validation of (emerging) drug targets, structural biology, drugability of targets, drug design approaches, chemogenomics, synthetic chemistry including combinatorial methods, bioorganic chemistry, natural compounds, high-throughput screening, pharmacological in vitro and in vivo investigations, drug-receptor interactions on the molecular level, structure-activity relationships, drug absorption, distribution, metabolism, elimination, toxicology and pharmacogenomics.

In general, special volumes are edited by well known guest editors.

In references *Topics in Medicinal Chemistry* is abbreviated *Top Med Chem* and is cited as a journal.

More information about this series at
http://www.springer.com/series/7355

Sylvain Celanire • Sonia Poli
Editors

# Small Molecule Therapeutics for Schizophrenia

With contributions by

A.M. Cesura · J.M. Cid · L.A. Dawson · B.A. Ellenbroek · T. Enomoto · M.A. Jacobson · M. Kalinichev · B.L. Kormos · H. Lavreysen · A. Mazurov · R.A. Porter · J.-P. Rocher · T. Sumiyoshi · A.A. Trabanco · J.B. Tuttle · T. Williams · D. Yohannes

Springer

*Editors*
Sylvain Celanire
Reigner
France

Sonia Poli
Addex Therapeutics
Geneva
Switzerland

ISSN 1862-2461 ISSN 1862-247X (electronic)
ISBN 978-3-319-11501-6 ISBN 978-3-319-11502-3 (eBook)
DOI 10.1007/978-3-319-11502-3
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014947829



Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

# Foreword

*"If you think this Universe is bad, you should see some of the others."*

—Philip K. Dick

Schizophrenia was first identified as a mental disease in 1887 by Dr. Emile Kraepelin and the word "schizophrenia" is less than 100 years old. However the disease is thought to have affected mankind throughout history. The pathophysiology and the underlying neurobiological dysfunctions which lead to schizophrenia are still far to be completely understood, despite major advances in the understanding of the role of neurotransmitters and their imbalance in the disease. Schizophrenia remains a chronic, severe and disabling brain disorder which affects approximately 1% of the population and for which a treatment is still desperately needed.

Schizophrenia is a complex disease with a complex manifestation that causes profound disruption not only to those suffering from the conditions but also to the people around them. Positive, negative and cognitive symptoms cannot be treated with a single stand-alone therapy, but rather a combination of pharmacological treatments seem to have a better chance to be successful in treating the spectrum of symptoms which can be very different across patients.

While antipsychotic treatments for positive symptoms are available since a few decades, they come with major side effects and have no influence on the negative symptoms. Negative and cognitive symptoms represent the most debilitating feature of the disease and are still a major unmet need and they are often the focus of drug discovery programs.

In this volume of *Topic in Medicinal Chemistry* focused on Schizophrenia, the authors have covered the most promising and advanced therapeutic approaches, discussing the biological rational for the selected targets and the wide diversity of chemotypes, discussed the detailed characterization of their in vitro and in vivo pharmacology, pharmacokinetic profile and preclinical safety when available and last but not least summarized the clinical status of the most advanced candidates. The way drugs act at the selected target have also been described, including in vitro and in vivo target engagement using modern brain imaging technologies, in particular Positron Emission Tomography (PET) tracers. Kinetics of target binding, fine tuning of functional activity (orthosteric agonist versus positive allosteric

modulators) and clarification of biased signalling are routinely part of modern drug discovery programs, and these considerations found ample space in this volume.

Chapter "Antipsychotics and the Dopamine–Serotonin Connection" is nicely introducing the disease and its ethiology, then moving to a comprehensive review of the first and second generation of marketed antipsychotic drugs acting at the dopamine and serotonine receptors. The glutamate hypothesis in schizophrenia is covered by Chapters "GlyT-1 Inhibitors: From Hits to Clinical Candidates", "Metabotropic Glutamate Receptor 2 Activators" and "Activation of the $mGlu_5$ Receptor for the Treatment of Schizophrenia and Cognitive-Deficit-Associated Disorders" which describe the inhibition of Glycine transporter type 1 (GlyT-1) and the activation of metabotropic glutamate receptor type 2 (mGlu2) and type 5 (mGlu5) to achieve therapeutic benefit. Chapters "Muscarinic Acetylcholine Receptor Activators" and "Nicotinic Acetylcholine Receptor Modulators" highlight the drug discovery and development efforts in targeting the acetycholine hypothesis and describe the activation of muscarinic (mACh) and nicotinic receptors (nACh), in particular m1 and m4 ACh, as well as $\alpha4\beta2$ and $\alpha7$ nACh receptor subtypes. Finally, the last chapter is describing the emergence of phosphodiesterase subtypes 10 and 9 inhibitors (PDE10, PDE9) as interesting targets for schizophrenia and cognitive impairment associated with schizophrenia (CIAS).

Despite strong pharmacological rational and preclinical evidence, still several targets have shown limited or disappointing clinical benefit, demonstrating how difficult it is to translate new concepts into useful medicines for schizophrenia. However we believe that clinical testing of new hypothesis is the key to understand the complex disease, to validate preclinical translation approaches and to find the right population that might benefit from a specific treatment.

We would like to deeply thank all the contributing authors to this special volume, providing to the readers and their peers an excellent and compelling overview of the current progresses, challenges and therapeutics opportunities targeting such complex, chronic and debilitating psychiatric illness.

Reigner, France — Sylvain Celanire
Geneva, Switzerland — Sonia Poli

# Contents

Top Med Chem (2015) 13: 1–50
DOI: 10.1007/7355_2014_51

Published online: 11 July 2014

# Antipsychotics and the Dopamine–Serotonin Connection

**Bart A. Ellenbroek and Andrea M. Cesura**

**Abstract** Schizophrenia is a severe psychiatric illness that afflicts around 1% of the population worldwide. Although recognized for over 100 years, the etiology and pathophysiology, as well as the underlying neurobiological substrate are still a matter of intense research and controversy. In the early 1950s of the previous century, a major revolution took place in the treatment of schizophrenia: the introduction of chlorpromazine (**1**) and in its wake a series of more potent antipsychotics drugs. These drugs led to a major improvement on the quality of life of patients with schizophrenia. However, throughout the years of clinical experience it became clear that antipsychotics only significantly improved the psychotic symptoms but had little influence on the negative or cognitive deficits and led to parkinsonian-like side effects. The introduction of the so-called second generation antipsychotics only slightly improved this situation. The vast majority of antipsychotics bind to dopamine as well as serotonin receptors, and it has been suggested that the ratio between the affinities for these receptors may be crucial for the therapeutic properties. The aim of the present review is to evaluate the role of dopamine and serotonin receptors in the antipsychotic potential of known as well as potential novel antipsychotics.

**Keywords** Antipsychotics, Benzamides, Butyrophenones, Clozapine, Cognitive symptoms, Dopamine, Environmental factors, Extrapyramidal side effects, First generation, Gene–environment interactions, Genetic factors, Negative symptoms, Phenothiazines, Positive symptoms, Second generation, Serotonin

B.A. Ellenbroek (✉)
School of Psychology, Victoria University of Wellington, P.O. Box 600, Wellington 6041, New Zealand
e-mail: bart.ellenbroek@vuw.ac.nz

A.M. Cesura
A.M.Cesura Pharma Consulting, Crans-près-Céligny, Switzerland
e-mail: a.cesura@bluewin.ch

## Contents



## Abbreviations

| | |
|---|---|
| 5-HT | 5-hydroxytryptamine |
| BDNF | Brain-derived neurotrophic factor |
| CAFE | Comparison of atypicals in first episode |
| cAMP | Cyclic adenosine monophosphate |
| CATIE | Clinical antipsychotic trials of intervention effectiveness |
| COMT | Catechol-O-Methyl transferase |
| CNV | Copy number variations |
| CUtLASS | Cost utility of the latest antipsychotic drugs in schizophrenia study |
| DA | Dopamine |
| DR | Dorsal raphe |
| DNA | Deoxyribonucleic acid |
| EPS | Extra pyramidal side-effects |
| EUFEST | European first episode schizophrenia trial |
| FDA | Food and drug administration |
| GWAS | Genome wide association studies |
| LSD | Lysergic acid diethylamide |
| Mb | Megabase |
| Mr | Median raphe |
| NA | Noradrenaline |
| NMDA | N-methyl-D-aspartate |
| OR | Odds ratio |
| PET | Positron emission tomography |
| PLC | Phospholipase C |
| SN | Substantia nigra |

| | |
|---|---|
| SNc | Substantia nigra pars compacta |
| SNP | Single nucleotide polymorphism |
| SOHO | Schizophrenia outpatients health outcomes |
| SPECT | Single photon emission computed tomography |
| VTA | Ventral tegmental area |

## 1 Schizophrenia

First described by Emil Kraeplin as dementia praecox, and later renamed by Eugen Bleuler, schizophrenia is one of the most severe mental illnesses. Although the lifetime prevalence is estimated at about 0.4–0.7%, conservative estimates have calculated the direct medical costs at about 35 billion Euros in Europe [1]. However, the total costs including indirect costs are much higher. Indeed a study in England showed that whereas the direct medical costs in 2004/2005 were about two billion pounds, the indirect costs were estimated at almost five billion [2] and the most recent pan-European study calculated the total costs for schizophrenia at around 94 billion Euros, making it the third most expensive brain disorder after dementia and mood disorders [3]. These exceptionally high costs are in part due to the fact that the symptoms typically become apparent at a relatively young age (generally between 18 and 30 years [4]) and are usually so severe that most patients fail to obtain an educational degree. Moreover, as will be discussed extensively below, a significant proportion of patients do not respond adequately to treatment, with a need for constant care, and even among those who do respond to therapy, a substantial proportion cannot contribute to the economic process, leading to very high indirect costs, such as loss of productivity.

The symptoms of schizophrenia are generally subdivided into three different domains: positive, negative and cognitive symptoms.

1. *Positive symptoms* are features that are normally not present in healthy control subjects but occur in patients as a result of the disease process. In schizophrenia these symptoms include predominantly hallucinations, delusions and thought disorder. With respect to hallucination, those in the auditory domain (especially hearing voices) are the most prominent, though visual and tactile hallucinations also occur. Delusions usually take on a negative form, such as thought withdrawal and broadcast and paranoid delusions. Delusions of grandeur (often present in bipolar disorders) are less prevalent in schizophrenia.
2. *Negative symptoms* include social withdrawal, apathy, poverty of speech and anhedonia. While these traits may also be present to some extent in normal subjects they are greatly exaggerated in schizophrenic patients.
3. *Cognitive symptoms* are deficits in learning and memory as well as other aspects within the cognitive domain. Although studies in schizophrenic patients have shown that virtually all domains of cognition are affected, the deficit seems to particularly severe in executive function, attention, working memory and verbal learning [5, 6].

## *1.1 The Etiology of Schizophrenia*

Both adoption and twin studies have provided clear evidence that schizophrenia has a strong heritability. The average estimated risk of developing schizophrenia if one of a monozygotic twin pairs has already developed schizophrenia is roughly 50–70% [7]. Although this is significantly higher than the risk in dizygotic twins (9–18%), it is at the same time much less than the 100% one would expect for a disorder with a pure genetic origin, indicating that genes increase the risk of developing schizophrenia, but do not, by themselves cause the disease.

### 1.1.1 Genetic Factors

With the advent of molecular genetics, there was hope of identifying the gene (or genes) responsible for this increased susceptibility. However, the results of linkage, candidate gene and genome wide association studies have identified an enormous number of genes, most of which have only a small odds ratio (OR: 1.1–2.0). The 2009 release of the schizophrenia gene resource database listed 7,855 genes that were somehow involved in the etiology of schizophrenia [8]. In line with this, genome wide association studies (GWAS) have often led to very few strongly positive results and have often failed to replicate previous GWAS studies [9–11]. While a recent paper highlights the polygenic nature of schizophrenia [12] given the large number of potential genes we will not discuss any individual genes here. However, we do want to point to two interesting new developments: copy number variations and de novo mutations. Copy number variations (CNVs) are insertions/deletions of significant parts of a chromosome ranging from roughly 1 kilobase (Kb) to several megabases (Mb). They occur naturally and are thought to make up about 12% of the normal DNA. Interest in CNVs in schizophrenia was triggered by the finding that individuals with a specific deletion on chromosome 22 (22q11.2) involving roughly 3 Mb and about 45 different genes have a significantly higher risk of developing schizophrenia [13]. Although much less investigated than single nucleotide polymorphism (SNPs), CNVs seem to be rarer but infer higher risks. Thus the 22a11.2 CNV has been reported to have an OR of 21, while others have ORs of between 11 and 17 [10], thus making these genetic variations much more interesting, especially given the new technologies for detecting CNVs. The second novel approach, looking for de novo mutations, makes use of the occurrence of novel case of schizophrenia (i.e. where neither the father nor the mothers is afflicted by the disorder). By comparing the DNA from the patient and the parents (and ideally non-affected siblings) it may be possible to identify genetic mutations that are specific for the affected sibling. This approach was recently successfully applied to autism [14] and schizophrenia [15–17]. In addition to identifying new and potentially causative genetic factors in schizophrenia, these analyses have also led to the discovery that de novo

mutations appear more common in schizophrenia than would be expected, suggesting that the genetic make-up of schizophrenic patients is more vulnerable than that of healthy controls.

### 1.1.2 Environmental Factors

In addition to the search for the genetic factors, researches have also tried to identify non-genetic (environmental) factors that contribute to the development of schizophrenia. However, this search has met with considerable more technical problems. First of all, many of the environmental risk factors are considered to impinge very early in life (either prenatally or in the first years after birth). This implies that many studies are forced to use a retrospective design which, if not backed up by objective data (such as hospital records), can lead to a subjective bias or false memories. A second confounding factor has been the use of the so-called *ecological design*, as exemplified by the 1957 influenza pandemic. The first results of this study showed that children born shortly after this pandemic had a significantly increased risk of developing schizophrenia (as compared to children born in 1956 of 1958 during the same period of the year [18]). However, later studies were unable to confirm this. It has been suggested that this may be due to the design of the study. Thus, although many more individuals suffered from influenza in 1957 than in the year before or after, the total incidence of influenza even at its peak was considered to be around 25%. In other words, 75% of pregnant mothers even during the peak of the pandemic were influenza free, leading to a misclassification in the majority of cases. Designs based on birth cohort studies (using hospital records to confirm the presence or absence of an infection in individual patients) showed much more robust results [19]. A third factor is that, in contrast to genetic mutations, the nature of the environmental factor is much less well circumscribed and verifiable. For instance, immigration has often been found to increase the risk of schizophrenia (see below). However, which aspect of immigration (i.e. stress of a foreign culture, foreign pathogens, foreign foods, etc.) mediates this risk is unclear. Finally, the timing of the environmental factor may be a highly relevant factor in determining the risk of developing a disorder. For instance, the offspring of pregnant females exposed to the Dutch hunger winter have an increased risk of developing schizophrenia when exposed in the first trimester but an increased risk of developing depression when exposed in the second or third trimester [20].

In spite of these inherent difficulties, a range of different environmental risk factors for schizophrenia have been identified (see Table 1), including several prenatal (infection, malnutrition and stress), perinatal (obstetric complications) and later onset (urbanicity, migration, cannabis use). The risk factors shown in Table 1 by no means pretend to be complete as many other environmental factors have been linked to the development of schizophrenia including vitamin D deficiency, prenatal lead exposure, and others [7, 21]. When inspecting these risk factors however, it is clear that several different periods of vulnerability exist

**Table 1** Environmental risk factors in the development of schizophrenia

| Risk factor | Odds ratio |
|---|---|
| Urbanicity | 2–3 |
| Migration | 2–3 |
| Winter birth | 1.1 |
| Maternal infection | 2–3 |
| Prenatal malnutrition | 2–3 |
| Prenatal stress | 1–2 |
| Obstetric complications | 2–3 |
| Advanced paternal age | 1.5–3 |
| Childhood trauma | 2–3 |
| Cannabis use | 2–3 |

during which adverse life events enhance the risk for the development of schizophrenia. In addition to the prenatal period, especially the time around puberty seems to be a particularly vulnerable period and stress, infections or cannabis use during this period seem to enhance the risk. Given the multitude of changes happening within the brain during this period, this is not at all surprising [22] and indeed does not seem to be limited to schizophrenia either. Another interesting characteristic of these environmental risk factors is that although they are very different, all ORs seem fairly similar, reminiscent of the very similar ORs of the various SNPs that also all seem to be fairly similar to irrespective of the individual gene involved.

### 1.1.3 Gene–Environment Interactions

Although the ORs for the environmental risk-factor (2.0–3.0) appear somewhat higher than for the genetic factors (1.1–2.0), they are nonetheless fairly small, indicating again that multiple factors are likely to interact. Indeed, there is now a fair degree of consensus that schizophrenia ultimately results from an interaction between genetic and environmental factors. How many and the exact nature of these factors, however, still eludes us. Nonetheless, the research into the role of genes and environment interactions in psychiatry in general and schizophrenia in particular is gaining tremendous momentum.

Although it would be beyond the scope of this review to discuss these in any great detail, especially as none of the treatments currently available or in development aim to specifically influence these gene–environment interactions, we would like to just name a few examples. In one of the first large-scale gene–environment interaction studies Caspi and his colleagues [23] showed that while adolescent cannabis use was positively associated with the diagnosis of schizophreniform disorder in individuals with the COMT Val/Val genotype (OR 10.5), it had no effect in individuals with the Met/Met genotype (OR 1.1). Interestingly, they found a gene-dosage effect as the OR increased to 2.5 for the heterozygous individuals (Val/Met). In a similar study, the influence of cannabis use on schizophrenia was also found to be moderated by an SNP in the *Akt* gene (rs2494732 C/T) [24], a finding recently replicated in an independent cohort of first

episode patients with schizophrenia [25]. This latter study also illustrated a very important aspect of etiological studies in schizophrenia. The authors found that although there was a highly significant interaction, neither the AKT SNP nor the use of cannabis by itself was significantly correlated with the diagnosis. In other words, if the authors had focussed on only one of the factors, they had concluded that neither was a risk factor for the development of schizophrenia. Moreover, close inspection of the data presented in the paper provide an explanation for this lack of effect. Thus while patients with the c/c genotype who smoked cannabis daily had a significantly increased OR (7.3), those with the c/c genotype who did not smoke cannabis at all had a much lower OR (0.53). In other words, in non-cannabis smokers the c/c genotype was actually a protective factor. Such genes are often referred to as "plasticity genes" (as opposed to vulnerability genes) as they seem to increase the susceptibility for environmental factors "for better or for worse" [26, 27]. The concept that one and the same gene can be both protective and deleterious for schizophrenia (or diseases in general) has important implications not only for the aetiology of a disease (i.e. the number of genetic factors may in fact even be a lot higher) but also for our efforts in developing novel animal models with improved predictability, as such models are nowadays still largely designed to mimic either a genetic or an environmental factor [28, 29]. The COMT $Val^{148}Met$ SNP has also recently been studied in relation to childhood trauma [30]. In this study, the severity of positive symptoms was greater in Met carriers who had experienced physical abuse, but the negative symptoms were more severe in Met carriers who had experienced social neglect. In addition the authors also looked at the cognitive performance and found, surprisingly that in the Val carriers (which by themselves have poorer performance), physical abuse was actually associated with a better performance in executive functioning tasks. The effects of childhood trauma on cognition also appear to be moderated by the BDNF $Val^{66}Met$ SNP: the meth carriers exposed to childhood trauma had significantly poorer cognitive performance compared to the Val/Val carriers exposed to trauma, especially physical abuse and emotional neglect, with a much smaller effect for sexual abuse. However, there was a significant interaction between the BDNF $Val^{66}Met$ SNP and sexual abuse in hippocampal and ventricular size.

Although the research into gene–environment interactions is still in its infancy, it has already led to a number of very interesting findings. Moreover, it has led to the realization that the original idea that genes are vulnerability factors is too simplistic. In fact genes may increase the overall vulnerability of an individual for environmental effects. This offers the possibility of identifying protective environments which may actually decrease the risk of developing schizophrenia. Although at present this is only theory, it may in time offer a more promising approach to reducing the burden of schizophrenia.

## *1.2 The Pathology of Schizophrenia*

As with the etiology, the pathology of schizophrenia is still largely unknown and highly variable, emphasizing the idea that schizophrenia in itself is a heterogeneous disorder. Systematic reviews and meta-analyses of structural magnetic resonance imaging studies show a reduction in total brain and gray matter volume, as well as an increased ventricular space [31]. More detailed analyses also showed reductions in the temporal lobe (especially the hippocampus and amygdala), prefrontal cortex, thalamus anterior cingulate and corpus callosum [32].

An important question with respect to the pathology of schizophrenia is whether schizophrenia is a neurodevelopmental or a neurodegenerative disorder, i.e. if the pathology is due to disturbance in the normal development or occurs later on in life and progresses as the disease continues. An evaluation of the current literature suggests that schizophrenia likely has components of both. Thus, there are clear indications of a disturbance in neurodevelopment, such as misplaced cells in the temporal cortex [33, 34], low brain and body weight at birth [35, 36], and developmental disturbances in dermatoglyphic features of the hand [37]. In addition, many studies have identified developmental changes in children who grow up developing schizophrenia, such as a retardation of development milestones [38–40] emphasizing that schizophrenia has a neurodevelopmental component [41, 42].

With respect to a potential neurodegenerative component the literature is less consistent. The virtually complete lack of gliosis (a hallmark of neurodegeneration) has suggested to many that the pathology in schizophrenia is not progressive [43]. However, longitudinal studies using structural magnetic resonance imaging (MRI) or computer tomography find clear evidence for changes in brain region volume over time [44–47]. A recent meta-analysis of over 18,000 patients showed that gray matter volume was significantly and negatively correlated with duration of illness [48]. On the other hand, gray matter volume was also negatively correlated with dose of antipsychotic drugs at time of the scan, suggesting that these drugs may contribute to total volume loss.

### 1.2.1 Neurotransmitter Changes in Schizophrenia

The dopamine (DA) hypothesis has been without a doubt the most influential neurochemical theory in schizophrenia and has had a major impact on the development on antipsychotic drugs. It was already recognized in 1958 that high doses or chronic use of the DA releasing drug amphetamine can induce a psychosis very similar to that observed in schizophrenia [49]. In addition a number of psychotomimetic drugs were shown to act as agonists at DA receptors in addition to receptors for serotonin (5-hydroxy-tryptamine, 5-HT). However, for a long time it appeared technically difficult to reliably quantify parameters of dopaminergic transmission in vivo. It has only been through the use of sophisticated protocols

using positron emission tomography (PET) or single photon emission computed tomography (SPECT) that we have been able to unequivocally show changes in basal dopaminergic neurotransmission. These studies showed that the basal release of DA is significantly enhanced in patients with schizophrenia [50, 51]. Subsequent studies also showed an enhanced DA release after an amphetamine challenge [52, 53], and both appear tightly correlated with each other [54]. This increase in DA release seen after amphetamine was correlated with positive but not negative symptoms and could also be observed in drug-naïve first episode patients, but not during remission [55]. In several recent studies, it was shown that the enhanced DA release is even seen in ultra-high risk patients during the prodromal phase [56] with a further increase upon onset of florid psychotic symptoms [57]. Although schizophrenia is no doubt much too complex to be associated with only one neurotransmitter, these data nonetheless clearly show that the positive psychotic symptoms appear to be related to changes in the presynaptic control of the dopaminergic system [58]. Further improvement of the spatial resolution has pointed to the associative anterodorsal striatum, rather than the often implied ventral striatum as the site for the enhanced DA (re)activity [56, 59]. Indeed DA release in the ventral striatum (including the nucleus accumbens) seems to be more related to euphoria [60].

Besides DA, dysfunction in 5-HT neurotransmission has also been implicated in the etiology of schizophrenia. As for DA, this was triggered by the fact that the psychotomimetic drug lysergic acid diethylamide (LSD) as well as many other hallucinogenic drugs [61] acts at 5-HT receptors. It is today believed that hallucinogens are agonists at the $5\text{-HT}_{2A}$ receptor subtype, especially those expressed on neocortical pyramidal cells (see, e.g., [62] for a review). The interest in the "serotoninergic" hypothesis for schizophrenia has somewhat declined over the last 30 years and its discussion is beyond the scope of this review and well covered in previous ones. Yet the fact that several antipsychotic drugs, in particular of the second generation (atypical antipsychotics, see below) bind to certain 5-HT receptors subtypes, in particular the $5\text{-HT}_{2A}$, underscores the importance of 5-HT neurotransmission in schizophrenia.

In addition to the dopaminergic theory, many other neurotransmitters have also been implicated in the neurobiology of schizophrenia, but the evidence is less conclusive. While many of these hypotheses will be discussed in later chapters, we will only briefly discuss the glutamate hypothesis which currently appears to be the most attractive one also in terms of antipsychotic drug development. As with the DA hypothesis, the glutamate hypothesis is partly based on the finding that specific drugs such as phencyclidine and ketamine can induce schizophrenia-like symptoms in otherwise healthy individuals [63, 64]. Interestingly, and perhaps in contrast to amphetamine, these drugs seem to induce both positive and negative symptoms [65, 66]. As these drugs block glutamatergic N-methyl-D-aspartate (NMDA) receptors [67] this has led to the idea that glutamatergic neurotransmission is compromised in schizophrenia [68–71]. However, it has proven elusive to identify glutamatergic deficits in particular in drug-naïve schizophrenic patients. This is certainly in part due to methodological difficulties (i.e. the lack of imaging PET ligands selectively binding to NMDA receptors suitable for use in vivo) [72].

Nonetheless deficits in glutamatergic neurotransmission may indeed underlie some of the symptoms of schizophrenia and may explain a phenomenon that has received a lot of attention in recent years, namely functional dysconnectivity [73]. This theory states that the neurobiological deficit in schizophrenia is not primarily due to a single dysfunctional structure (as is, for instance, the case in Parkinson's disease) but rather due to dysfunctional communication between different structures within a network. Using different techniques, it was found that the normal synchrony observed between specific brain regions is significantly altered in patients with schizophrenia (for a recent review, see [74]).

## 2 The Treatment of Schizophrenia

While compounds like insulin or cardiazol had been used to treat schizophrenia [75] before the advent of modern psychopharmacology, it is safe to say that the pharmacological treatment of schizophrenia started with the introduction of chlorpromazine (**1**) through the seminal observations of Delay and Deniker [76, 77]. Searching for a novel (antihistaminic) sedative drug they started using chlorpromazine (**1**, RP4560) after Henri Laborit had used the drug as an anesthetic booster in a clinical trial [78]. It soon became clear, however, that chlorpromazine (**1**) was much more than a sedative and that it was capable of selectively reducing the psychotic symptoms in schizophrenic patients.

The introduction of chlorpromazine (**1**) was rapidly followed by a large number of other drugs originally referred to as neuroleptic drugs (a term coined by Delay and Deniker) but now commonly referred to as antipsychotic drugs. As Fig. 1 indicates the number of approved antipsychotics steadily climbed until around 1975 culminating with the introduction of clozapine (**5**). This period was followed by a long plateau phase, marked by the withdrawal of clozapine for safety reasons which will be explained below, followed by its reintroduction in the market. After this, in 1994, risperidone (**8**) was introduced followed by a series of novel antipsychotics, that last of which being lurasidone (not shown), which was approved by the FDA in 2010. The antipsychotics are generally subdivided into two categories: first generation (classical) and second generation (atypical) antipsychotics, with clozapine (**5**) marking the transition between the two classes. In some papers aripiprazole (**9**) has been sometime referred to as the third generation drug as it has some unique mechanism of action (see below). However, as aripiprazole (**9**) is only one of the antipsychotics among several drugs with a similar mode of action which did not reach the market, we conservatively propose classifying this drug still as a second generation drug, also because its differentiation in terms of mechanism of action does not justify, in our opinion, its classification into an additional category.

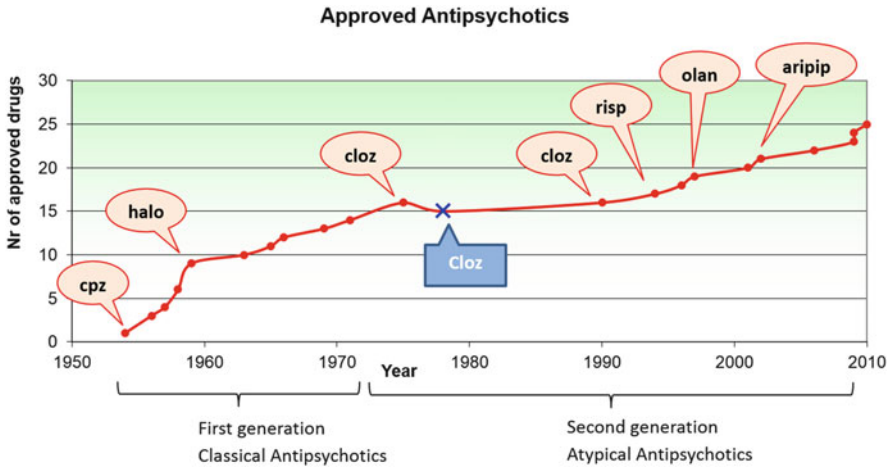



**Fig. 1** The development of antipsychotic registration with some highlights depicted. *cpz* chlorpromazine, *hal* haloperidol, *cloz* clozapine, *risp* risperidone, *olan* olanzapine, *aripip* aripiprazole

## *2.1 The First Generation of Antipsychotics*

Most of the first generation antipsychotics generally belong to four different chemical classes: the tricyclic phenothiazines and thioxanthenes and the butyrophenones and the diphenylbutylamines (see Fig. 2).

After the successful introduction of chlorpromazine (**1**) thousands of variations in the basic structure of the thioridazines have been tested in animal models for antipsychotic activity – mostly inhibition of motor behaviour or conditioned avoidance response [79]. As a result, the medicinal chemistry characteristics of tricyclic compounds are very well known. Here we will only briefly touch upon them. For a more detailed analysis the reader is referred to the excellent chapter of Altar et al. in Burger's textbook on medicinal chemistry and drug discovery [80].

Within the class of the tricyclics the distance between the –N– of the side chain and the tricyclic structure should optimally be a three carbon-chain. Shorter chains decrease the antipsychotic potency while at the same time increasing the antihistaminic and anticholinergic properties. With respect to the nature of the amino-group, it has been shown that a tertiary amino group is optimal, with mono- and desmethylchlorpromazine being several times less active.

Within the tricyclic ring, electron-withdrawing substituents (i.e. –Cl or $–CF_3$) in the $R_1$-position greatly enhance the antipsychotic potential. Indeed, it was long thought that this was essential for antipsychotic activity and, until the introduction of olanzapine (**6**) (see below) all tricyclics contained an electron-withdrawing group in the $R_1$-position. While keeping the diaryl nature of the tricyclic rings, a variety of alterations have been evaluated within the central ring. Changing the –S– into a –O– or a –C– leads to a reduction in the antipsychotic efficacy. Interestingly,

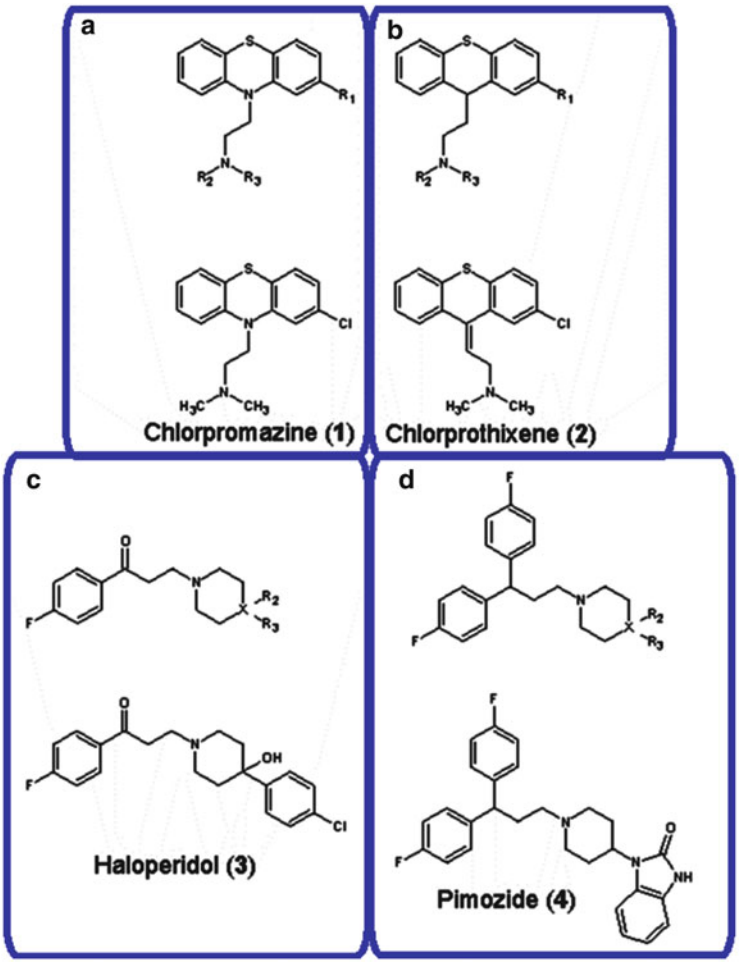



**Fig. 2** The general structure of the major classes of first generation antipsychotics (*top*) and a representative example (*bottom*). (**a**) Phenothiazines; (**b**) thiothixenes; (**c**) butyrophenones; (**d**) diphenylbutylamines

a change into a $–CH_2–CH_2–$ (i.e. changing the six-membered ring into a seven-membered ring, while reducing the antipsychotic efficacy) increases the antidepressant activity (especially when the –N– is changed into a –C as well). A final change in the central ring is the replacement of the –N– for a –C– leading to the group of the thiotixenes, which are also highly potent antipsychotics and include drugs such as thiotixene (**2**), chlorprotixene and flupenthixol.

The butyrophenones were discovered in the laboratory of Paul Janssen in Beerse, Belgium in a search for meperidine-like analgesics. By increasing the distance between the aryl group and the tertiary amine and by introducing an electron-withdrawing group in the 4-position of the aryl group, the analgesic properties

significantly decreased, while at the same time the antipsychotic efficacy increased. Indeed some of the butyrophenones are among the most potent antipsychotics (such as trifluperidol, spiroperidol and haloperidol (**3**)).

The medicinal chemical properties of butyrophenones have been much less extensively studied, compared to those of the tricyclics discussed above. Most substitutions have been on the tertiary amino side, including the X, $R_1$ and $R_2$ position (see Fig. 2c). In most cases, X represents a C–, but replacing it with an N– can also lead to potent antipsychotics. Related to the butyrophenones are the diphenylbutylamines, in which the ketone group is replaced by a phenyl group (again with an F– in the 4-position). This change led to several potent long-acting antipsychotics, such as fluspirilene and pimozide (**4**).

In addition to these four classes of drugs a large number of other antipsychotics have been developed that do not really fit into any other structural class, including butaclamol, molindone, oxypertine and several others.

## *2.2 The Second Generation of Antipsychotics*

As mentioned above, the introduction of clozapine (**5**) in the early 1970s, and especially its re-introduction in 1989 marked a new era of antipsychotic development. Especially the very low frequency of extra-pyramidal (parkinsonian-like) symptoms (EPS, a major concern with first generation antipsychotics), accompanied also by a lower incidence of tardive dyskinesia were considered unique and earned it the name "atypical antipsychotic" [79]. Actually, the finding that some antipsychotics had less EPS than others was far from new, and was already established in the early 1960 in large studies [79]. Unfortunately, clozapine (**5**) suffers from a number of serious and even fatal side effects, most notably agranulocytosis, a potentially deadly reduction in white blood cells and the reason why it was voluntarily withdrawn from the market in the first place [81]. While clozapine (**5**) was reintroduced in 1989 after studies demonstrated its effectiveness especially in treatment-resistant schizophrenia, its use is strictly controlled, with several "black box" warnings and currently, in spite of its obvious benefits (see below), clozapine (**5**) is reserved almost exclusively for therapy-resistant patients. Also the introduction of newer and safer second generation antipsychotic drugs has further limited its use.

However, the unique characteristics of clozapine (**5**) sparked the development of a whole series of novel molecules, including risperidone (**8**), olanzapine (**6**), quetiapine (**7**) and aripiprazole (**9**) (Fig. 3). Most of these drugs have quite unique chemical structures and therefore only relatively limited medical chemical information is available. As can be seen in Fig. 3, both olanzapine and quetiapine are clearly based on the tricyclic structure of clozapine. Interestingly, whereas clozapine retained the electron-withdrawing halogen on the phenyl ring, it is notably absent in both olanzapine (**6**) and quetiapine (**7**). Although this may actually have reduced the overall antipsychotic efficacy [82], it seems to have contributed to the "atypical" character.

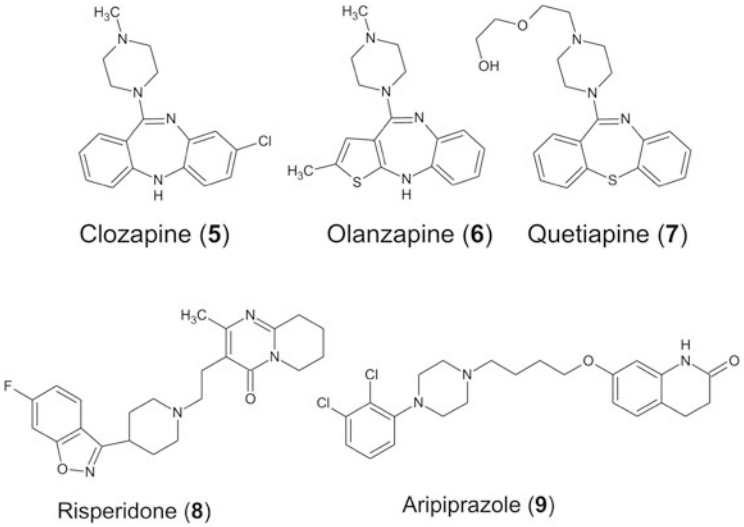



**Fig. 3** The chemical structure of several second generation antipsychotics. (**a**) Clozapine, (**b**) olanzapine, (**c**) quetiapine, (**d**) risperidone, (**e**) aripiprazole

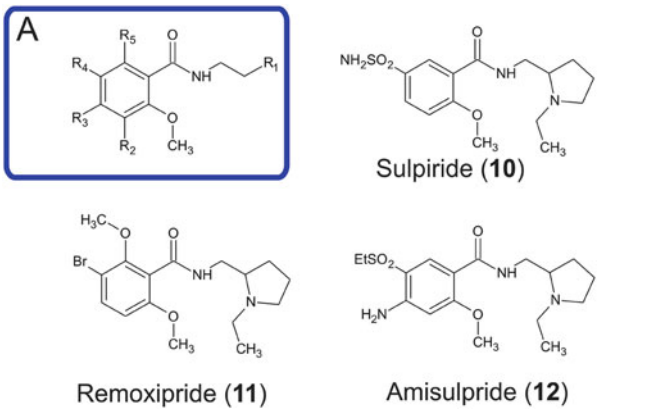



**Fig. 4** The structure of the benzamides (**a**) with some representative examples: (**b**) sulpiride, (**c**) remoxipride, (**d**) amisulpride

A last class on antipsychotics that is worth mentioning in this respect is the benzamides (see Fig. 4). This class also contains a large series of drugs including the anti-emetic drug metoclopramide, the first generation antipsychotic sulpiride (**10**) and clebopride and the second generation remoxipride (**11**), raclopride and amisulpride (**12**). The general structure is given in Fig. 4 and medicinal chemical approaches rapidly established the essential nature of the 2-$OCH_3$ group on the phenyl ring. Further substitutions on the phenyl ring (especially Br– substitution on

the X-position) greatly enhanced the potency leading to remoxipride (**11**), which was briefly introduced in clinical practice, but was withdrawn after reports of aplastic anemia. The latest addition to the benzamides is amisulpride (**12**), a drug which is registered in several countries is Europe, but not in the USA. It seems to have a clinical profile similar to remoxipride (**11**), although it does not induce the aplastic anemia.

## *2.3 The Clinical Features of Antipsychotic Drugs*

The existence of so many antipsychotics warrants the questions whether there are (fundamental) differences between them. For many years it has been common practice to subdivide the antipsychotics into classical and atypical drugs, based on their potency to induce EPS [83–85]. However, this distinction seems to be more quantitative than qualitative. Thus, when given in high enough doses virtually all antipsychotics induce to a certain extent EPS of higher or lower severity. Atypical antipsychotics may be different from the more traditional one in that their therapeutic width is (slightly) larger. Thus careful titration might lead to less EPS with these drugs at doses that produce sufficient antipsychotic activity. The relevance of this distinction for everyday clinical practice becomes therefore less evident. Moreover, even in animal research the distinction between classical and atypical antipsychotics has not always been clear. For instance, two papers published in 1983 studied the effects of repeated antipsychotic administration of the firing properties of the dopaminergic cells in the midbrain [85, 86]. Both found that atypical antipsychotics such as clozapine (**5**) differentiated between the A9 and the A10 cell groups. Interestingly, both groups also studied the effects of sulpiride (**10**) with some authors [85] classifying it as a classical antipsychotic and others [86] three as an atypical antipsychotic. Likewise, whereas Meltzer and colleagues classified thioridazine as an atypical antipsychotic in one paper in 1989 [87], in another paper in the same year they classified it as an atypical antipsychotic [84]. Based on these and other findings that the clinical and neurochemical difference between these two classes of drugs may be less conspicuous than originally thought, we and others prefer the terms first and second generation antipsychotics.

In order to determine whether there are meaningful differences between the individual antipsychotics, two approaches have been used: meta-analyses and large-scale multicentre studies. Several meta-analyses have been published mainly comparing first and second generation antipsychotics [88–91]. In addition, several large-scale studies have been conducted to investigate potential differences in the treatment of schizophrenia (see Table 2). Although both types of studies have their advantages and limitations (see also [92]), including different read-out parameters, poor comparability in terms of dosing regimens and patient selection, the conclusions are remarkably similar.

**Table 2** Large-scale multicentre trials

| Acronym | Title | Primary outcome |
|---|---|---|
| CATIE | Clinical antipsychotic trials of intervention effectiveness | Discontinuation of medication |
| CUtLASS | Cost utility of the latest antipsychotic drugs in schizophrenia study | Quality of life and costs |
| SOHO | Schizophrenia outpatients health outcomes | Two years symptoms recovery |
| CAFE | Comparison of atypicals in first episode | Discontinuation of medication |
| EUFEST | European first episode schizophrenia trial | Discontinuation of medication |

Overall, all first and second generation antipsychotics show a clear therapeutic effect on the positive symptoms, while being much less effective in treating the negative or the cognitive symptoms. For instance in the CATIE study, a 35% reduction in positive symptoms was seen with all drugs evaluated after about 12 months, while reductions in negative and cognitive symptoms were less than 15% [93–95]. In addition the CATIE study showed that compliance rates were generally very poor with 60 and 75% of patients discontinuing their treatment within 12 and 18 months, respectively. This is remarkably similar to the 68.4, 70.9 and 71.4% discontinuation seen after 1 year with olanzapine (**6**), quetiapine (**7**) and risperidone (**8**) in the CAFE study [96]. The discontinuation was lower in the EUFEST study (ranging from 33% for amisulpride (**12**) to 72% for haloperidol (**3**) after 1 year) which may be due to the fact that this study only included first episode patients. Interestingly, although there were differences in compliance rates between the different antipsychotics (with haloperidol being the least well tolerated), the therapeutic effects were virtually identical for all antipsychotics involved [97].

In addition to small differences between different drugs with respect to the therapeutic effects, larger differences have been reported with respect to the side effects. Although the second generation antipsychotics are certainly not without motor (EPS) side effects, the incidence seems somewhat lower than with the first generation antipsychotics. For instance in the EUFEST study, haloperidol induced EPS in a significantly higher incidence (34% of the patients) than after treatment with olanzapine (**6**) (6%), quetiapine (**7**) (11%), ziprasidone (16%) and amisulpride (**12**) (17%) [97]. A similar pattern was found with respect to akathisia (although in this case ziprasidone was no different from haloperidol). This small advantage of second generation antipsychotics was also observed in the CATIE study, with EPS observed in 8% of the patients treated with the first generation drug perphenazine, vs. 2–4% for the second generation drugs olanzapine (**6**), risperidone (**8**), ziprasidone (not shown), and quetiapine (**7**) [98]. On the other hand, the second generation drugs seem to induce more metabolic side effects especially increases in body weight. Thus, whereas the average weight gain over a 1 year treatment was 7.6 kg for haloperidol, it was estimated at 9.7 kg for amisulpride (**12**), 10.5 kg for quetiapine (**7**) and 13.9 kg for olanzapine (**6**) [97]. Again, the data are similar to

those of the CATIE study, showing an increased risk for obesity, especially for olanzapine (with 30% of the patients gaining more than 7% in body weight). Interestingly, in both studies ziprasidone was the least likely to induce weight gain [98].

Overall then the conclusion of the comparative studies shows that although subtle differences exist between individual antipsychotics, they do not show great superiority for the second generation. Moreover, none of the current drugs seem particularly effective in the treatment of negative and cognitive symptoms, and overall therapy compliance is low, especially in more chronic patients. In other words, there is a clear unmet medical need for drugs with an improved profile, in terms of both better therapeutic efficacy and reduced side effect liability.

Before discussing the therapeutic mechanisms of antipsychotics, it is important to mention the special position of clozapine. As discussed before, clozapine was re-introduced in 1989 in spite of the fatal risk of agranulocytosis. The reason for this was clozapine's unique therapeutic profile. Indeed clozapine seems to induce very few if any parkinsonian-like side effects. Moreover, clozapine seems unique in that it shows efficacy in therapy-resistant patients. This was the primary outcomes of the landmark study that led to the re-introduction of clozapine [99] and has since been replicated in several studies, including the CATIE study [94, 100, 101]. Although not all studies have confirmed clozapine's superiority over other drugs [90], it is fair to say that clozapine represents a unique antipsychotic drug and it does indicate that it is possible to develop drugs that are more effective than most of the currently available therapies.

## *2.4 The Mechanisms of Action of Antipsychotic Drugs*

Given that the differences between the antipsychotics are marginal, especially in relation to the reduction in positive symptoms, it is not surprising that all antipsychotic drugs are thought to have a common mechanism of action (with the possible exception of clozapine). Indeed as early as the beginning of the 1960s it was proposed that all antipsychotics act through an interaction with catecholaminergic receptors as they all led to increases in metabolic products of DA and noradrenaline (NA), respectively [102]. It was Jacques van Rossum who in 1966 proposed that all antipsychotics were antagonists of the DA receptor (at that time only one DA receptor was known) as they all reversed the behavioural effects of L-Dopa [103]. However, it took another 10 years before two groups independently showed that indeed all antipsychotics bind to DA receptors, and that there was a linear correlation between the binding to the receptor and the average daily dose used in the clinic [104, 105], while there was no such correlation with other receptors [106]. Final confirmation about the relevance of the DA $D_2$ receptor subtype for the therapeutic effect came from brain imaging studies, using PET and SPECT ligands to visualize $D_2$ occupancy

of antipsychotic drugs in clinically relevant doses. These data clearly show that for the vast majority of antipsychotic drugs therapeutically relevant doses lead to a 70–80% occupation of the $D_2$ receptor [107–112]. It is important to realize that this level of occupancy was observed for both first (haloperidol, chlorpromazine, flupenthixol, molindone, etc.) and second (olanzapine, remoxipride, amisulpride, lurasidone and ziprasidone). So far only two antipsychotics appear to require a lower occupancy rate: clozapine (**5**) and quetiapine (**7**). Both drugs appear therapeutically active in doses which occupy only 20–40% of the $D_2$ receptors. With respect to clozapine, given its effectiveness in therapy-resistant patients, it is tempting to speculate that properties additional to the $D_2$ blocking effects may be responsible for this unique effect. This is unlikely the case for quetiapine (**7**), as its effect in therapy-resistant patients is not different from other antipsychotics. However, it is important to note that the half-life of quetiapine (**7**) is short, and thus receptor occupancy as assessed using brain imaging may underestimate the true binding. An additional finding of the brain imaging studies showed that there is a small but important therapeutic window between the therapeutic effect (roughly 60–80% occupancy) and the development of EPS (above 80–85%). Again, there appears to be an exception to this rule. Aripiprazole (**9**) generally occupies over 90% of the $D_2$ receptors, yet does not induce significantly more EPS. In spite of this, this drug does not induce more EPS side effects than the other antipsychotics. A likely explanation is that whereas all other antipsychotic drugs are antagonists, aripiprazole (**9**) is a partial agonist at $D_2$ receptors whose efficacy in vitro relative to that of DA varies depending on the presence of spare receptors/receptor reserve for DA [113]. The partial agonist properties of aripiprazole may represent a feature possibly protecting against severe EPS side effects.

## *2.5 The Binding Profile of Antipsychotic Drugs*

Although all antipsychotics bind to the $D_2$ receptor and this effect seems directly related to the therapeutic effect (on the positive symptoms), it is important to realize that virtually all antipsychotics are dirty drugs (or as some have referred to "have a rich pharmacology"). In fact with the exception of the benzamides, all antipsychotics show appreciable affinity for 5-HT, adrenoceptors, muscarinic and or histaminergic receptors. Tables 3 and 4 list the $K_i$ values for 7 first and 12 second generation antipsychotics, obtained from the PDSP database (http://pdsp.med.unc.edu/indexR.html). Given that there are large differences in doses used for each antipsychotic, the absolute $K_i$ values are only of limited use. A better representation can be obtained if we look at the affinities relative to the $D_2$ receptor, as antipsychotics are usually titrated to reach about 80% $D_2$ occupancy as discussed above. This is illustrated for the 5-HT receptors for several representative examples of first and second generation antipsychotics in Fig. 5. Data are converted to p*K*i, implying that a score of +1 means a tenfold lower affinity for the receptor compared to the $D_2$ receptor, a score of +2 means 100-fold, etc. Negative scores imply that the

**Table 3** Binding of antipsychotics to human dopamine and serotonin receptors

| Drug | D1 | D2 | D3 | D4 | D5 | 5-HT1A | 5-HT1B | 5-HT1D | 5-HT1E | 5-HT2A | 5-HT2B | 5-HT2C | 5-HT3 | 5-HT5A | 5-HT6 | 5-HT7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chlorpromazine | 112.0 | 2.0 | 3.0 | 24.0 | 133.0 | 2,115.5 | 1,489.0 | 452.0 | 344.0 | 3.3 | 6.0 | 12.4 | 977.0 | 118.0 | 17.0 | 21.0 |
| Fluphenazine | 24.0 | 0.5 | 0.7 | 36.0 | 12.0 | 145.4 | 334.0 | 334.0 | 540.0 | 21.0 | | 982.5 | >10,000 | 145.0 | 34.7 | 8.0 |
| Haloperidol | 83.0 | 2.0 | 5.8 | 15.0 | 147.0 | 1,202.0 | 165.0 | 7,606.0 | >10,000 | 107.2 | 1,203.9 | 6,026.9 | >10,000 | 2,247.0 | 5,306.4 | 378.0 |
| Loxapine | 54.0 | 10.0 | 23.3 | 12.0 | 75.0 | 2,456.0 | 388.0 | 3,468.0 | 1,399.0 | 4.4 | | 13.3 | 190.0 | 776.0 | 33.0 | 88.0 |
| Spiperone | 220.0 | 0.1 | 0.3 | 1.9 | 4,500.0 | 177.1 | >10,000 | 3,090.3 | 5,051.0 | 8.3 | 1,114.2 | 820.4 | | | | 110.0 |
| Thioridazine | 89.0 | 10.0 | 3.1 | 17.0 | 216.0 | 108.0 | 109.0 | 579.0 | 194.0 | 20.4 | | 53.0 | >10,000 | 364.0 | 57.0 | 99.0 |
| Thiothixzene | 51.0 | 1.4 | 0.4 | 203.0 | 261.0 | 410.0 | 151.0 | 659.0 | >10,000 | 50.0 | | 1,355.5 | 1,863.0 | 361.0 | 208.0 | 15.0 |
| Amisulpride | >10,000 | 3.0 | 2.4 | 2,369.0 | >10,000 | >10,000 | 1,744.0 | 1,341.0 | >10,000 | 8,304.0 | 13.0 | >10,000 | >10,000 | >10,000 | 4,154.0 | 73.5 |
| Aripirpazole | 387.0 | 1.0 | 4.6 | 514.0 | 1,676.0 | 5.6 | 833.0 | 63.0 | 8,000.0 | 8.7 | 0.4 | 22.4 | 628.0 | 1,241.0 | 642.4 | 10.0 |
| Asenapine | 2.9 | 1.4 | 1.8 | 1.8 | 22.7 | 53.6 | 5.2 | 10.2 | 37.4 | 0.3 | | 0.2 | 447.6 | | 1.4 | 0.9 |
| Clozapine | 189.0 | 431.0 | 283.1 | 39.0 | 235.0 | 372.7 | 398.0 | 2,132.0 | 966.0 | 9.3 | 8.4 | 13.4 | 241.0 | 3,857.0 | 13.5 | 18.0 |
| Iloperidone | 42.7 | 0.4 | 11.0 | 13.5 | 319.0 | 20.7 | 89.0 | 10,000.0 | | 1.7 | | 9.6 | >10,000 | | 63.0 | 112.0 |
| Olanzapine | 58.0 | 72.0 | 37.4 | 19.0 | 90.0 | 2,063.0 | 509.0 | 540.0 | 2,408.0 | 3.0 | 11.9 | 13.2 | 202.0 | 1,212.0 | 6.0 | 105.0 |
| Paliperidone | 41.0 | 9.4 | 3.2 | 54.3 | 29.0 | 542.0 | 108.7 | 15.0 | >10,000 | 1.9 | 61.9 | 48.0 | >10,000 | 277.9 | 2,414.0 | 2.7 |
| Quetiapine | 712.0 | 567.0 | 403.5 | 1,202.0 | 1,738.0 | 431.0 | 1,109.0 | >10,000 | 2,402.0 | 470.7 | | 1,840.2 | >10,000 | 3,120.0 | 1,864.0 | 308.0 |
| Risperidone | 60.6 | 4.9 | 55.9 | 18.6 | | 427.0 | 53.0 | 29.2 | >10,000 | 0.9 | 61.9 | 31.1 | >10,000 | 205.8 | 2,241.0 | 6.6 |
| Sertindole | | 4.1 | 5.8 | 9.3 | 16.0 | 536.7 | 60.0 | 96.0 | 430.0 | 0.4 | | 3.5 | | | 5.4 | 28.0 |
| Ziprasidone | 30.0 | 4.0 | 7.4 | 105.0 | 152.0 | 76.0 | 4.0 | 9.0 | 1,279.0 | 0.5 | 27.2 | 4.5 | >10,000 | 291.0 | 61.0 | 6.0 |
| Zotepine | 71.0 | 25.0 | 6.4 | 18.0 | 248.0 | 407.0 | 67.0 | 175.0 | 1,080.0 | 2.7 | | 3.2 | 472.0 | 29.0 | 6.0 | 12.0 |

Values are $K_i$ in nM for cloned human receptors. Data were obtained from the PDSP database (http://pdsp.med.unc.edu/indexR.html). Values are either PDSP certified data or the average of all data in the database

**Table 4** Binding of antipsychotics to human adrenergic, muscarinic and histaminergic receptors

| | Noradrenaline | | | | | Acetylcholine | | | | | Histamine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Drug | α1A | α1B | α2A | α2B | α2C | m1 | m2 | m3 | m4 | m5 | H1 | H2 |
| Chlorpromazine | 0.3 | 0.8 | 74.0 | 28.0 | 46.0 | 47.0 | 433.0 | 47.0 | 151.0 | 18.0 | 0.2 | 174.0 |
| Fluphenazine | 6.4 | 13.0 | 314.0 | 82.0 | 29.0 | 1,095.0 | 7,163.0 | 1,441.0 | 5,321.0 | 357.0 | 7.3 | 560.0 |
| Haloperidol | 12.0 | 8.0 | 1,130.0 | 480.0 | 550.0 | >10,000 | >10,000 | >10,000 | >10,000 | 657.0 | 3,002.0 | 1,003.0 |
| Loxapine | 31.0 | 53.0 | 151.0 | 108.0 | 80.0 | 175.0 | 590.0 | 122.0 | 2,232.0 | 91.0 | 2.8 | 208.0 |
| Spiperone | 20.4 | 3.1 | | | 450.0 | | | | | | 272.0 | |
| Thioridazine | 1.3 | 2.4 | 134.0 | 342.0 | 75.0 | 33.0 | 831.0 | 43.0 | 913.0 | 12.0 | 14.0 | 136.0 |
| Thiothixzene | 12.0 | 35.0 | 80.0 | 50.0 | 52.0 | >10,000 | >10,000 | >10,000 | >10,000 | 5,370.0 | 12.0 | 411.0 |
| Amisulpride | >10,000 | >10,000 | 1,114.0 | | 1,540.0 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 |
| Aripirpazole | 25.0 | 34.0 | 74.0 | 102.0 | 38.0 | 6,776.0 | 3,507.0 | 4,677.0 | 1,521.0 | 2,327.0 | 29.0 | >10,000 |
| Asenapine | 4.4 | 3.9 | 10.5 | 10.7 | 17.8 | 24.3 | 79.1 | 38.7 | >10,000 | 9.5 | 0.2 | 2.3 |
| Clozapine | 1.6 | 7.0 | 142.0 | 27.0 | 34.0 | 14.0 | 14.0 | 25.0 | 39.0 | 94.0 | 2.0 | 153.0 |
| Iloperidone | 31.0 | | 162.0 | 162.0 | 16.2 | 4,898.0 | 3,311.0 | >10,000 | 8,381.0 | >10,000 | 12.3 | |
| Olanzapine | 109.0 | 263.0 | 314.0 | 82.0 | 29.0 | 24.0 | 79.0 | 51.0 | 998.0 | 9.0 | 4.9 | 44.0 |
| Paliperidone | 2.5 | 0.7 | 4.7 | 57.0 | 3.7 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | 5.6 | 121.2 |
| Quetiapine | 22.0 | 39.0 | 3,630.0 | 747.0 | 29.0 | 858.0 | 1,339.0 | 1,943.0 | 542.0 | 1,942.0 | 7.5 | >10,000 |
| Risperidone | 5.0 | 9.0 | 151.0 | 108.0 | | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | 5.2 | 120.0 |
| Sertindole | 1.8 | | 640.0 | 450.0 | 1.3 | | | | | | 130.0 | |
| Ziprasidone | 18.0 | 9.0 | 160.0 | 48.0 | 77.0 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | 130.0 | 3,500.0 |
| Zotepine | 7.0 | 5.0 | 236.0 | 5.0 | 62.0 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | 5.8 | 500.0 |

Values are $K_i$ in nM for cloned human receptors. Data were obtained from the PDSP database (http://pdsp.med.unc.edu/indexR.html). Values are either PDSP certified data or the average of all data in the database

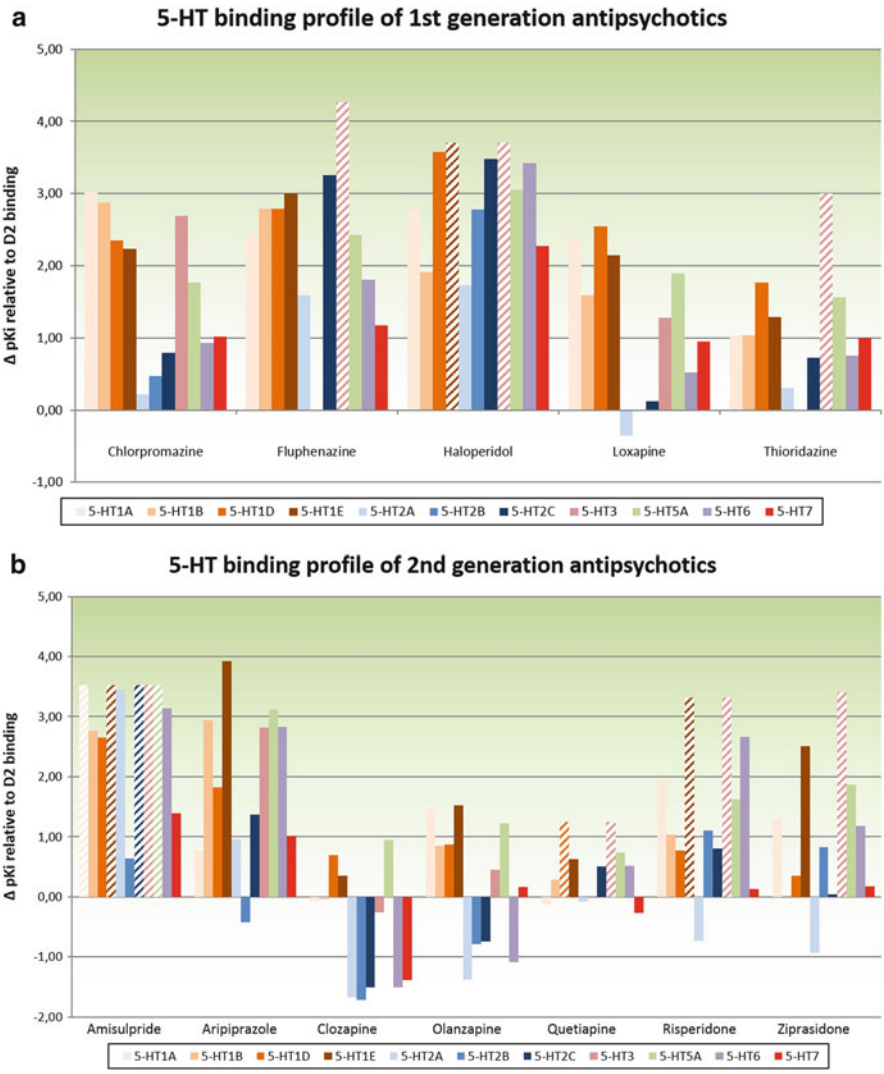



**Fig. 5** The affinities of several first and second generation antipsychotics for the 5-HT receptors, relative to their affinity for the $D_2$ receptor ($pK_i$, see text for explanation). The *hatched bars* indicate minimal values (i.e. the $K_i$ are >10,000 nM)

antipsychotic has a higher affinity for that receptor than for the $D_2$ receptor. From the figure it becomes automatically clear that there are marked differences in binding profiles between the first and second generation antipsychotics. Whereas the first generation antipsychotics bind almost without exception more potently to the $D_2$ receptor, many of the second-generation antipsychotics have higher affinity for 5-HT receptors, most notably 5-$HT_{2A}$ where they act as antagonists. The figure,

**Table 5** The involvement of neurotransmitter receptors in the side effects of antipsychotic drugs [114–116]

| Receptor | Side effects |
|---|---|
| 5-$HT_{1A}$ | Weight gain |
| | Cognitive impairment (?) |
| 5-$HT_{2A}$ | Sexual dysfunction |
| 5-$HT_{2C}$ | Weight gain, diabetes |
| | Sexual disturbances |
| α1 | Orthostatic hypertension |
| | Dizziness, reflex tachycardia |
| $D_2$ receptor | Extrapyramidal side effects |
| | Endocrine (Prolactin elevation) |
| $H_1$ | Sedation, weight gain, diabetes |
| m1 | Dry mouth, blurred vision, constipation |
| | Cognitive impairment |

however, also indicates that the second generation antipsychotics are a much more heterogeneous group of drugs. For instance, amisulpride (**12**) and aripiprazole (**9**) have a profile very similar to that of the first generation type antipsychotics, while clozapine (**4**) and olanzapine (**5**) show a very different profile.

In addition to binding to DA and 5-HT receptors, most first and second generation antipsychotics also have appreciable affinity for adrenergic $\alpha_1$ and histaminergic $H_1$ receptors. In addition, some drugs also show significant binding to some of the muscarinic receptors, especially $m_1$, $m_3$ and $m_5$ receptors. The differences in receptor affinities are likely to underlie some of the clear differences especially in terms of side effects between the individual antipsychotic drugs (see Table 5).

## 3 The Dopamine–Serotonin Interaction

As Fig. 5 and Tables 3 and 4 illustrate, the binding of most antipsychotics is complex, but a clear distinction between first and second generation drugs on the basis of the binding profile alone seems impossible. Nonetheless, it has been suggested that especially the binding to the 5-$HT_2$ receptors is an important distinguishing factor between the two groups of drugs. Herbert Meltzer was one of the first who suggested that second generation antipsychotics could be distinguished from first generation antipsychotics by their more potent 5-$HT_{2A}$ blockade (relative to $D_2$ blockade [84, 117, 118]). Indeed inspection of Fig. 5 shows that most first generation antipsychotics are less potent on 5-$HT_{2A}$ and 5-$HT_{2C}$ receptor (with the exception of loxapine and possibly chlorpromazine (**1**)), while most second generation antipsychotics are more potent (with the exception of amisulpride (**12**)). In spite of the lack of a clear therapeutic difference between the two groups of antipsychotics, this has led to the idea that drugs that

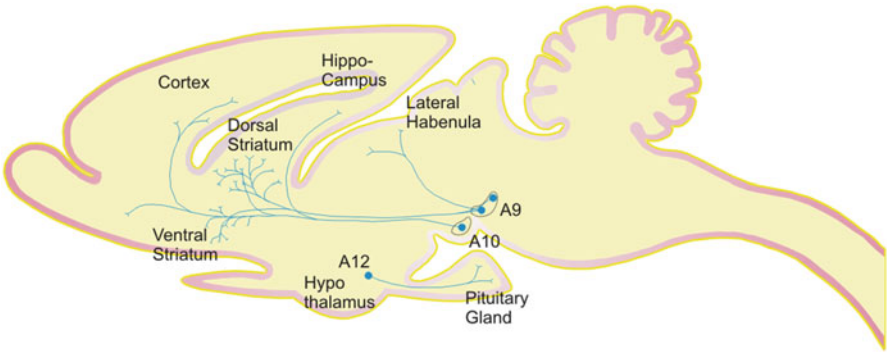



**Fig. 6** The anatomy of the dopaminergic system in a sagittal section of the rat brain

interact with both DA and 5-HT receptors might be clinically superior to drugs that are more selective. In the remainder of this chapter, we will therefore focus specifically on this dopamine–serotonin interaction and what the potential consequences of these interactions for the clinical profile of antipsychotics drugs could be.

## *3.1 The Dopamine System*

The dopaminergic system consists of several clusters of cells (generally referred to as A8 to A15) located in the midbrain and the hypothalamus [119]. The major cell clusters are the A9 (or substantia nigra pars compacta, SNc) which sends fibres predominantly to the dorsal striatum, the A10 (or ventral tegmental area, VTA) which sends fibres to the ventral striatum and prefrontal cortex and the A12 (in the periventricular and arcuate nucleus of the hypothalamus) which sends fibres to the median eminence and the posterior lobe of the pituitary gland (see Fig. 6).

DA interacts with five different receptors, conveniently labeled $D_1$ to $D_5$, which are subdivided into two families: the $D_1$ family (comprising of the $D_1$ and $D_5$ receptor) and the $D_2$ family (comprising of the $D_2$, $D_3$ and $D_4$ receptor). All DA receptors are G-protein-coupled receptors (GPCRs), with the $D_1$ family coupled to $G_s$ (and possibly $G_q$) and the $D_2$ family to $G_i$. Most of the dopaminergic receptors are concentrated within the basal ganglia, especially the dorsal and ventral striatum, with the $D_1$ family also abundant in the prefrontal cortex. In addition the $D_3$ is found in the island of Calleja, the $D_4$ receptor in the amygdala and the $D_5$ receptor in the hippocampal formation [120–122]. However, the lack of selective ligands for the individual subtypes has greatly hampered our understanding of the role of each of these receptors in any detail.

DA is probably one of the most studied neurotransmitters and it is known to play a prominent role in motor coordination and initiation, motivation and reward as well as

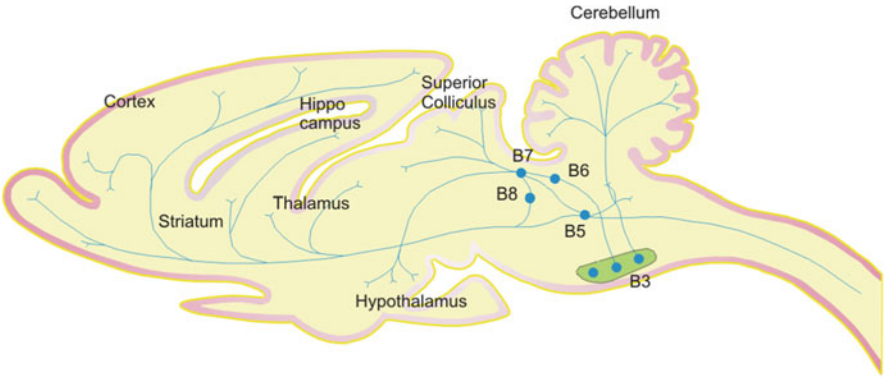



**Fig. 7** The anatomy of the serotonergic system in a sagittal section of the rat brain

in cognition. It is therefore not surprising that alterations in the dopaminergic system have been implicated in several major disorders, such as Parkinson's disease, drug dependence and addiction and schizophrenia [123–125].

## *3.2 The Serotonergic System*

Similar to the organization of the dopaminergic system, serotonergic cell bodies are also located in several clusters located in the midbrain. In their classical study Dahlstron and Fuxe labeled these cell clusters B1–B9 [119]. Figure 7 shows the major serotonergic cell clusters: B3 (Raphe magnus), B5 (medial raphe, MR), B6, B7 (both parts of the dorsal raphe, DR) and B8 (median raphe). Serotonergic fibres run both caudally (from B1 to B4 into the brainstem and spinal cord) and rostrally (from B5 to B9) innervating almost all forebrain region [126]. The vast majority of these ascending serotonergic fibres arise in the dorsal and median raphe and are organized into a dorsal and ventral system [127].

The pharmacology of the serotonergic system is highly complex as 5-HT is known to interact with thirteen different receptors (with a putative fourteenth receptor ($5\text{-HT}_{5B}$) identified in rodents) belonging to seven different classes ($5\text{-HT}_1$ to $5\text{-HT}_7$) [128]. Moreover, some receptors occur in different isoforms, due to posttranslational modification. For instance, at least 14 functional isoforms of the $5\text{-HT}_{2C}$ and nine of the $5\text{-HT}_4$ receptors have so far been identified [129]. With the exception of the $5\text{-HT}_3$ family, all these receptors are GPCRs. The $5\text{-HT}_1$ and $5\text{-HT}_5$ receptor families are coupled to $G_{i/o}$, whereas the $5\text{-HT}_4$, $5\text{-HT}_6$ and $5\text{-HT}_7$ are coupled to $G_s$. Finally the $5\text{-HT}_2$ receptors are coupled to $G_{q/11}$. All the receptors are widespread across the brain with many localized in key areas related to schizophrenia, such as the basal ganglia and the cortex.

5-HT is one of the evolutionary oldest neurotransmitters and plays a key role in emotions, social behaviour, cognition and impulsivity. Hence it has been implicated in major mood disorders such as depression, disorders and anxiety disorders, as well as obsessive compulsive disorders and autism [130].

### 3.3 *Functional Interaction Between the DA and 5-HT Neurotransmission*

Given the both DA and 5-HT innervate (at least in part) similar areas of the brain and the receptors from both are highly concentrated in the basal ganglia, the midbrain and the prefrontal cortex, it is not surprising that these neurotransmitters intimately interact with each other. However, the interactions are highly complex given the multiple receptors of both neurotransmitters that often have opposite intracellular effects. Especially electrophysiological studies have yielded complex interactions between DA and 5-HT [131]. This is in part due to the fact that most studies have been done under general anaesthesia, which influences the electrophysiological properties of neuronal cells. However, a more important limitation is the above-mentioned plethora of receptors involved in (especially) serotonergic signaling. Moreover, there is a reciprocal connection between the dopaminergic cells and the serotonergic cells. Thus, the DR sends projections to SN and VTA, whereas the MR only sends projections to the VTA but not the SN [132]. Likewise there the SNc and VTA send dopaminergic projections to the DR [133, 134]. In line with this, serotonergic cells within the DR express both $D_2$ and $D_3$ receptors [135, 136].

Studies using microdialysis especially in combination with specific serotonergic receptor (ant)agonists have provided more consistent data. Although 5-HT can also inhibit DA release, its major effect, as observed with in vivo microdialysis is an increase in striatal DA release [137]. 5-$HT_{1A}$ agonists increase DA release in the hippocampus and prefrontal cortex, but not in the dorsal or ventral striatum. Although antagonists by themselves have no effect, they do prevent the effects of 5-$HT_{1A}$ agonists [138]. This effect is likely due to postsynaptic 5-$HT_{1A}$ receptors as the effect is still present after denervation of the 5-HT fibres. Moreover, there is a dense 5-$HT_{1A}$ receptor population in the medial prefrontal cortex and hippocampus, while the receptors are scarce in the dorsal and ventral striatum [139]. 5-$HT_{1B}$ receptors also enhance DA release, but in contrast to the 5-$HT_{1A}$ receptors, this seems to be a more general effect, observed in the dorsal and ventral striatum as well as in the medial prefrontal cortex. This effect could be mediated via an indirect effect at the level of the cell bodies. 5-$HT_{1B}$ receptors are located on GABAergic terminals within the VTA and SNc and 5-$HT_{1B}$ agonists reduce GABA outflow, thereby disinhibiting the DAergic cells [140].

Probably the most convincing serotonergic influence of dopaminergic neurotransmission is mediated via the 5-$HT_2$ receptor family, with the 5-$HT_{2A}$ and 5-$HT_{2C}$ having opposite effects. Thus, the non-selective 5-$HT_{2A}$ agonists DOI enhance basal DA release in the prefrontal cortex and nucleus accumbens, an effect that is blocked by the selective 5-$HT_{2A}$ antagonist MDL100907 [140]. Interestingly, while 5-$HT_{2A}$ antagonists inhibit psychostimulant-induced DA release, they do not influence the dopamine release induced by morphine. This is likely due to the fact that the psychostimulant-induced DA release is impulse independent, while the morphine-induced increase depends on increased firing of the dopaminergic cells

[141]. However, the role of 5-$HT_{2A}$ receptors is more complex as evidenced by studies that showed that local application of DOI into the striatum increased DA release via 5-$HT_{2C}$ but not 5-$HT_{2A}$ receptors [137]. Moreover, within the prefrontal cortex, it has been shown that 5-$HT_{2A}$ antagonists can increase DA release when combined with first generation antipsychotics such as sulpiride and haloperidol [142]. These latter authors suggest that this effect is actually due to an indirect effect on release 5-HT on 5-$HT_{1A}$ receptors, as the effects are selectively blocked by the 5-$HT_{1A}$ antagonist WAY100635.

5-$HT_{2C}$ receptors also influence dopaminergic neurotransmission with antagonists increase DA cell firing, burst cell activity and DA release in the medial prefrontal cortex and striatum [140].

5-$HT_3$ and 5-$HT_4$ receptors are also involved in the release of DA albeit in a slightly different manner. Whereas systemic application of 5-$HT_3$ agonists enhances DA release, systemic application of 5-$HT_4$ agonists has no effect [140]. On the other hand, antagonists of both receptors block the morphine-induced increase in DA release, while not affecting the amphetamine-induced increase, suggesting that these receptors only affect impulse-dependent DA release [143, 144].

Much less is known with respect to the other 5-HT receptors, although a recent study showed that the selective 5-$HT_6$ agonist ST1936 enhances DA release in the medial prefrontal cortex and the ventral striatum, especially the nucleus accumbens shell; an effect blocked by selective 5-$HT_6$ antagonists [145].

In addition to microdialysis, behavioural studies have highlighted the interactions between dopaminergic and serotonergic drugs. DA agonists, especially indirect agonists such as amphetamine and cocaine are well known to increase locomotor activity. This hyperactivity is blocked by, among others, 5-$HT_{1A}$ agonists [146], 5-$HT_{1B}$ antagonists [147], 5-$HT_{2A}$ antagonists [148], 5-$HT_{2C}$ agonists [149], 5-$HT_3$ antagonists [150], 5-$HT_4$ antagonists [151] and 5-$HT_7$ antagonist [152, 153] but not 5-$HT_6$ antagonists [154, 155]. One issue of concern with these pharmacological studies is the selectivity of the effect, i.e. whether the doses used to reduce drug-induced hyperactivity are ineffective in blocking spontaneous activity. Other behavioural studies have specifically looked at the interaction between 5-HT receptors and antipsychotic drugs, especially in relation to the side effects. Thus, 5-$HT_{1A}$ agonists [156], 5-$HT_3$ antagonist [157] and 5-$HT_6$ antagonists [157] reverse haloperidol-induced catalepsy, while 5-$HT_4$ [158], 5-$HT_5$ and 5-$HT_7$ antagonists [157] are without effect. The influence of 5-$HT_{2A}$ antagonists is less clear with some reporting an inhibitory effect [159], while others found no effect [160]. The effects of 5-$HT_2$ receptors on antipsychotic-induced motor inhibition were also investigated and found that the interaction is highly complex and depends on the antipsychotic [161]. It is interesting, however, to see that a number of the drugs that reverse the DA agonist induced hyperactivity, also reverse the DA antagonist induced catalepsy in rodents. On the one hand this emphasizes the complex pharmacological interaction between DA and 5-HT, and, on the other hand, suggest that such ligands may possess antipsychotic properties (as they reverse DA-induced hyperactivity) while exhibiting less EPS (as they reverse catalepsy).

**Table 6** Examples of recent *DA-specific* and *5HT-specific drugs* that have failed in different stages of development for schizophrenia

| Drug | Company | MOA | Remarks |
|---|---|---|---|
| CEE-03-310 | Addex | D1 ↓ | Development ceased during/after phase IIa trials |
| SDZ-HDC-912 | Novartis | D2 ↓ | Development ceased during/after phase II trials |
| Aplindore | Pfizer | D2/D3 ↑ | In phase II for PD |
| Sonepiprazole | Pfizer | D4 ↓ | Development ceased during/after phase II trials |
| AV-965 | Avera Pharma | 5-HT1A ↓ | Development ceased during/after phase I trials |
| Adatanserin | Pfizer | 5-HT1A ↑/5-HT2A ↓ | Development ceased during/after phase II trials |
| Volinanserin | Sanofi | 5-HT2A ↓ | Not superior to placebo in phase III trial |
| Eplivanserin | Sanofi | 5-HT2A/5-HT2B ↓ | Development ceased during/after phase IIb trials |
| Vabicaserin | Pfizer | 5-HT2C ↑/5-HT2B ↓ | Development ceased during/after phase II trials |
| Zacopride | Sanofi | 5-HT3 ↓ | Development ceased during/after phase II trials |
| Ricasetron | GlaxoSmithKline | 5-HT3 ↓ | The drug was in a phase II trial for anxiety |
| PRX-07034 | Epix | 5-HT6 ↓ | Phase II trial was planned |
| LY-483518 | Lundbeck | 5-HT6 ↓ | Development ceased during/after phase II trials |
| Cerlapirdine | Pfizer | 5-HT6 ↓ | Development ceased during/after phase I trials |
| ABT-354 | Abbott | 5-HT6 ↓ | Development ceased after phase I |
| Lu-AE-58054 | Lundbeck | 5-HT6 ↓ | Positive phase III data reported for AD |

Data were obtained from the Citeline® database (www.citeline.com)
↑ refers to agonistic activity
↓ refers to antagonistic (inhibitory) activity

## 4 The Future DA/5-HT Antipsychotics

As discussed at length already, most antipsychotics possess both DA and 5-HT receptor blocking properties. This is especially true for the second generation antipsychotics. Moreover, as indicated in Fig. 5, most of these interact with several different receptors. The question that remains is whether a truly innovative antipsychotic with a binding profile focusing on DA, 5-HT or an interaction between them is still possible. In the remainder of this chapter we will discuss the theoretical and practical strategies used to answer this question. As we will see, most of these strategies are based on (are at least inspired by) the binding profile of clozapine, still considered the most effective antipsychotic treatment. Tables 6, 7, 8, 9, and 10 give an overview of the different drugs that have at least led to clinical trials. As is evident, however, the development of most drugs has either been

**Table 7** Examples of recent *mixed DA/5-HT drugs* that have failed in different stages of development for schizophrenia

| Drug | Company | MOA | Remarks |
|---|---|---|---|
| SLV-314 | Abbott | D2 ↓/5-HT1A ↑ | Development ceased during/after phase II trials |
| Adoprazine | Abbott | D2 ↓, 5-HT1A ↑ | Development ceased during/after phase II trials |
| PF-217830 | Pfizer | D2/5-HT2A ↓, 5-HT1A ↑ | Development ceased during/after phase II trials |
| Lu-35-138 | Lundbeck | D4/5-HT2 ↓ | Development ceased during/after phase II trials |
| Fananserin | Sanofi | D4/5-HT2A ↓ | Development ceased during/after phase II trials |
| Belaperidone | Abbott | D4/5-HT2 ↓ | Development ceased during/after phase II trials |
| Roxindole | Merck KGaA | D2, SERT ↓/5-HT1A ↑ | No efficacy in phase II trial |

Data were obtained from the Citeline® database (www.citeline.com)
↑ refers to agonistic activity
↓ refers to antagonistic (inhibitory) activity

discontinued (Tables 6 and 7) or the developmental status is unknown (Tables 8 and 9), meaning in most cases also that the drug is no longer actively pursued.

## *4.1 Selective Dopaminergic Ligands*

As DA interacts with five different receptors, while most antipsychotics have high affinity for only one or do not clearly distinguish between the different receptors (especially between $D_1$ and $D_5$, and between $D_2$, $D_3$ and $D_4$), strategies have been developed to design more selective drugs. As discussed above, the effects of antipsychotic drugs on the positive symptoms seem mediated through the $D_2$ receptor, leading to the question whether the other four receptors also may have a role in the therapy of schizophrenia.

### 4.1.1 $D_1$-Family Specific Ligands

Given the strong homology between the $D_1$ and the $D_5$ receptors it has so far been impossible to develop ligands with any pharmacologically relevant selectivity for one of these two receptors. We will therefore refer to them as $D_{1/5}$ ligands. The first of these were the benzazepines SKF 38393 (**13**) [162] and Sch 23390 (**14**) [163] a (partial) agonist, resp., antagonist (see Fig. 8). Following the recognition that SKF38393 is only a partial agonist, additional full agonists were developed using the same general scaffold. Interestingly, both agonists and antagonists have been suggested to be beneficial in schizophrenia. We found that, like clozapine (**5**) and quetiapine (**7**), SCH23390 selectively reversed the amphetamine-induced social

**Table 8** Examples of recent *DA-specific* and *5HT-specific drugs* for which the developmental status for schizophrenia is unclear

| Drug | Company | Mechanism of action | Remarks |
|---|---|---|---|
| Uridine | Polifarma | DA ↑ | No development after start of phase II trials |
| DAR-0100 | BioValve Technology | D1 ↑ | No development after start of phase II trials |
| TSR-1938 | Paion | D1 ↓ | No development after start of phase I trials |
| YKP-1358 | SK Holdings | D2 ↓ | No development after start of phase II trials |
| JNJ-37822681 | Johnson & Johnson | D2 ↓ | No development after start of phase II trials |
| AVE-5997EF | Sanofi | D2/D3 (↑) | No development after start of phase I trials |
| BP4.897 | Bioprojet | D3 ↑ | No development after start of phase II trials |
| ABT-925 | Abbott | D3 ↓ | Phase II trial was terminated |
| NGD 94-1 | Merck & Co | D4 ↓ | No development after start of phase I trials |
| Sulamserod | Hoffmann-La Roche | 5-HT4 ↑ | No development after start of phase I trials |
| SYN-114 | Hoffmann-La Roche | 5-HT6 ↓ | No development after phase I trials |
| SB-271046 | GlaxoSmithKline | 5-HT6 ↓ | No development after start of phase I trials |
| PNU-22394 | Pfizer | 5-HT2A/2B/2C ↓ | No development after start of phase II trials |

Data were obtained from the Citeline® database (www.citeline.com)
↑ refers to agonistic activity
↓ refers to antagonistic (inhibitory) activity
(↑) refers to partial agonistic activity

isolation in Java monkeys [164, 165]. Although some beneficial effect was found in earlier studies [166] poor oral bioavailability and rapid tolerance severely limited the usefulness of these drugs [167]. Additional trials with selective $D_{1/5}$ antagonists including CEE-03-310 and TSR-1938 were equally ineffective.

More recently, a potential role for $D_{1/5}$ agonists has been proposed, based on findings in animal and human studies that increasing $D_{1/5}$ tone, especially in the prefrontal cortex, could improve cognitive performance [168, 169]. Recently, this hypothesis was tested using the polycyclic compound dihydrexidine (**16**, DAR-0100). The results showed that the drug was well tolerated and at a dose of 20 mg increased indeed prefrontal perfusion [170, 171]. Larger scale clinical trials have, however, not yet been performed. DAR-0100 is currently in clinical testing as cognitive enhancer in schizotypal personality disorder (ClinicalTrials.gov Identifier: NCT01466205) while a trial in neuropsychiatric disorders has been completed (ClinicalTrials.gov Identifier: NCT01519557) but no results have been published to the best of our knowledge.

**Table 9** Examples of recent *mixed DA/5HT drugs* for which the developmental status for schizophrenia is unclear

| Drug | Company | Mechanism of action | Remarks |
|---|---|---|---|
| ZD-3638 | AstraZeneca | D1/D2/5-HT2A ↓ | Phase II data reported |
| TGOF02N | Fabre-Kramer | D1-D4/5-HT1A/5-HT2A ↓ | No development after start of phase II trials |
| SSR-181507 | Sanofi | D2 ↓/5-HT1A ↑ | No development after start of phase I trials |
| Bifeprunox | Abbott | D2/5-HT1A (↑) | No development after start of phase III trials |
| SDZ-MAR-327 | Novarts | D2/5-HT1A (↑) | No development after start of phase II trials |
| GSK-773812 | GlaxoSmithKline | D2/5-HT2 ↓ | No development after start of phase II trials |
| Abaperidone | Ferrer | D2/5-HT2 ↓, 5-HT1A (↑) | No development after start of phase I trials |
| SLV-310 | Abbott | D2, SERT ↓ | No development after start of phase II trials |
| SEL-73 | Selvita | D2/5-HT2A/5-HT6/5-HT7 ↓ | Clinical trials were expected in 2010 |
| BTS-79018 | Abbott | D3 ↓/5-HT1A ↑ | No development after start of phase I trials |
| SL-91.0177 | Sanofi | D4/5-HT2A ↓ | No development after start of phase III trials |

Data were obtained from the Citeline® database (www.citeline.com)
↑ refers to agonistic activity
↓ refers to antagonistic (inhibitory) activity
(↑) refers to partial agonistic activity

An interesting compound as potential cognitive enhancer in schizophrenia is stepholidine (**17**) [172–174], which was originally isolated from the roots of the plant Stephania intermedia and acts as a partial $D_1$ agonist and full $D_2$ antagonist. In animal research this drug showed an interesting pharmacological profile [175, 176]. Unfortunately, few informative clinical studies have been performed with this drug, so its potential cannot be assessed.

Although originally described as a classical $D_{1/5}$ agonist, SKF 83959 (**15**, Fig. 8) was found to have several unique characteristic. Biochemically, rather than increasing cAMP, it activates phospholipase C (PLC) stimulating the hydrolysis of $PIP_2$ into $IP_3$ and DAG. Behaviourally it was shown to have antiparkinsonian-like properties in both rat and monkey models [177–179] and several other unique characteristics. Given that there is only one $D_1$ and one $D_5$ receptor gene, neither of which seems to have any functional isoforms, the question is how SKF83959 can activate PLC whereas other agonists activate adenylate cyclase. One possibility that has been suggested by several studies is that when $D_1$ receptors form heterodimers with $D_2$ receptors, the intracellular machinery changes from a $G_s$ (activating adenylate cyclase) to a $G_q$ (activating PLC) protein [180], with SKF83959 occupying a specific binding pocket in the dimer. The potential relevance for the treatment of schizophrenia has yet to be determined [181, 182].

**Table 10** Examples of recent DA/5-HT drugs that are (presumably) still in development for schizophrenia

| Drug | Company | Mechanism of action | Remarks |
|---|---|---|---|
| Seridopidine | Neurosearch | DA ↑↓ | In phase I for PD and Tourette syndrome |
| Brexpiprazole | Otsuka | D2/D3 ↑ | Also in phase III for AD and depression |
| Eltoprazine | Abbott | 5-HT1A (↑)/5-HT1B ↑ | Positive phase II data reported for ADHD |
| MT210/CYR101 | Mitsubishi Tanabe | 5-HT2A/sigma ↓ | Phase IIa trials in 100 patients was positive |
| MG01C1 | Alcobra | 5-HT2B ↓ | In phase II for ADHD |
| Pimavanserin | Acadia | 5-HT2A ↓ | In phase III for PD |
| SUVN-502 | Suven Life Sciences | 5-HT6 ↓ | In phase I for AD |
| GSK-742457 | GlaxoSmithKline | 5-HT6 ↓ | Positive phase II data reported for AD |
| AVN-211 | AviNeuro | 5-HT6 ↓ | Positive phase II data reported |
| RP-5063 | Reviva Pharm | broad spectrum ↓ | Phase III trials scheduled for early 2014 |
| ITI-007 | Bristol Myers Squibb | D2 ↑/5-HT2A ↓ | Phase III trials planned for second half 2014 |
| Zicronapine | Lundbeck | D1/D2/5-HT2A/5-HT2C ↓ | |

Data were obtained from the Citeline® database (www.citeline.com)

### 4.1.2 $D_2$-Family Specific Ligands

Without exception all the antipsychotics in clinical use today block the $D_2$ receptor (with aripiprazole (**9**) being a partial agonist). Moreover, the clinical effects of the highly selective benzamides have shown that $D_2$ blockade is by itself sufficient for antipsychotic activity. Nonetheless, it should be remembered that almost without exception these drugs also affect the $D_3$ and $D_4$ receptor (see Table 3). Given the overall sequence homology between these receptors (which is between 73 and 90% for the transmembrane domains), this is not surprising. Many of the (non)-selective DA antagonists (such as not only the butyrophenones and the diphenylbutylamines, Fig. 2, but also the second generation antipsychotics risperidone (**8**) and aripiprazole (**9**), Fig. 3) belong to the basic 1,4-disubstituted aromatic piperazines and piperidines. As discussed in a recent paper [183] this structure has four basic components as illustrated in Fig. 9, with the linker between the amine unit and the lipophilic terminal region being responsible for the subtype selectivity. Thus, a long linker shows more $D_3$ selectivity, a short linker has more $D_4$ selectivity, while an intermediate linker is more $D_2$ selective. It has therefore been possible to develop more selective $D_3$ and $D_4$ ligands. Moreover, the recent publication of the crystal structure of the $D_3$ receptor might lead to a further improvement of the selectivity of the $D_2$ family ligands.

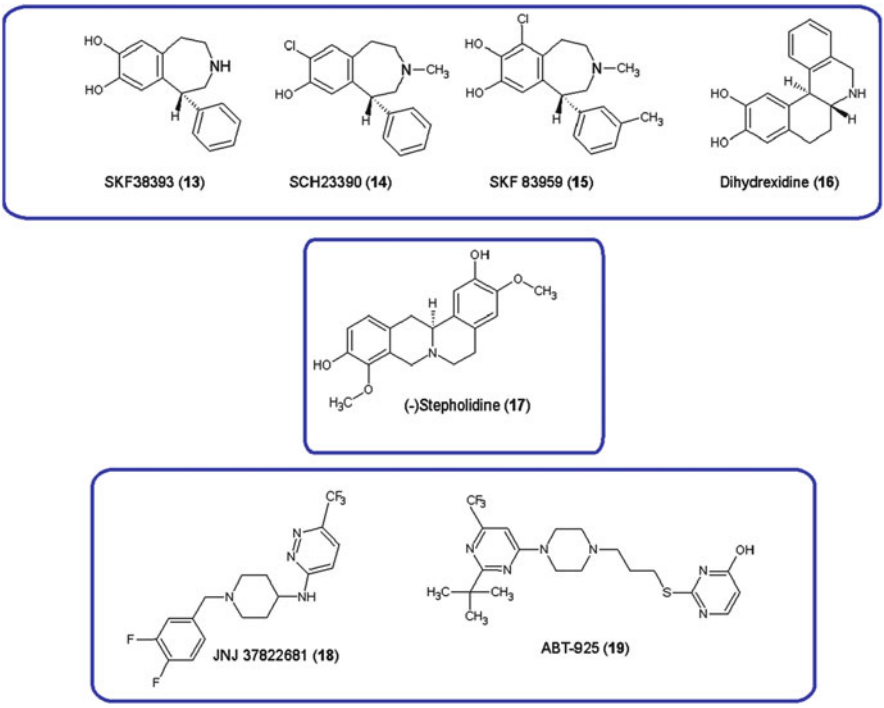



**Fig. 8** The structure of representative selective dopamine receptor ligands. (**a**) $D_1$ ligands, (**b**) a $D_1$ agonist/$D_2$ antagonist, (**c**) a fast dissociating $D_2$ antagonists, (**d**) a selective $D_3$ antagonist

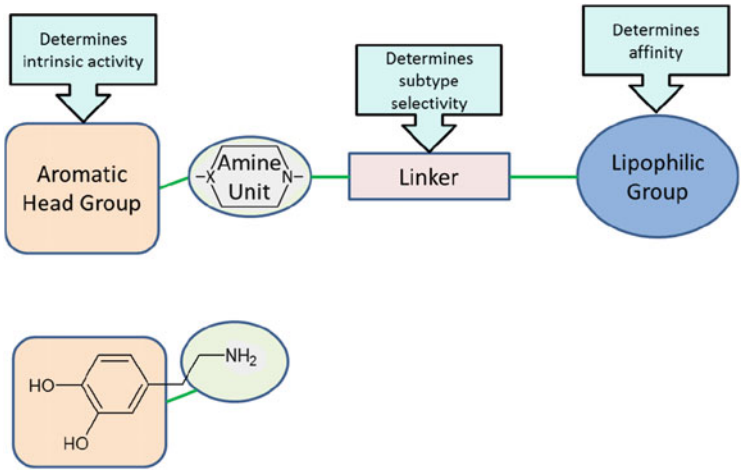



**Fig. 9** The basic structure of dopaminergic 1,4 disubstituted aromatic piperazines and piperidines

Recent research has focused on developing drugs with selectivity for $D_3$ or $D_4$ receptors. One possible exception currently pursued by Johnson & Johnson is the development of a fast dissociating non-selective $D_2$ antagonist (JNJ-37822681, **18**, see Fig. 8). The idea behind this is that rapid dissociation might decrease the risk of side effects [184] and in a recent study JNJ-37822681 (at least at a low dose of 10 mg bid) had a slightly better side effect profile than olanzapine (similar EPS but less weight gain,)[185]. It was even suggested that the drug might also have a faster onset of action [186]. While JNJ-37822681 completed phase II studies, no information on its development status is available (see Table 8).

The DA $D_3$ receptor was the third receptor to be identified in rodents and humans [187] and, although much less numerous than the $D_1$ and the $D_2$ receptor, their location especially in more limbic regions, in addition to the fact that most antipsychotic drugs also potently inhibit the $D_3$ receptor has led to the hypothesis that selective $D_3$ receptor antagonist may be beneficial in the treatment of schizophrenia [188]. Interestingly, selective $D_3$ antagonists fail in most classical models for antipsychotics, such as DA agonist-induced locomotor activity and conditioned avoidance response. This could indicate that these drugs may not have much effect on the positive symptoms. On the other hand, it may be argued that these models were predominantly developed to pick up DA $D_2$ receptor antagonists, rather than antipsychotics per se [188]. $D_3$ antagonists are, however, effective in a variety of models assessing cognitive functioning [189–191]. Whether this translates to improved cognition in patients with schizophrenia remains to be investigated. So far only one $D_3$ antagonist has been studied in patients (ABT-925 (**19**) see Fig. 8), with doses up to 150 mg q.d. for 6 weeks being ineffective on positive and negative symptoms [192]. Unfortunately, due to side effects higher doses could not be tested. Given that the 150 mg dose blocked less than 40% of the $D_3$ receptors [193], the potential usefulness of $D_3$ antagonists for the treatment of schizophrenia remains to be determined. Cariprazine (not shown) is a mixed $D_3/D_2$ partial agonist with about a tenfold selectivity for the $D_3$ receptor [194]. Clinical trials have shown doses between 1.5 and 4.5 mg/day to be effective in reducing the symptoms of an acute exacerbation, similar to 4 mg/day risperidone, with a slightly better side effect profile (Durgam et al. 2013). However the FDA, though acknowledging that cariprazine demonstrated effectiveness in the treatment of schizophrenia and mania associated with bipolar disorder, has declined approval, requesting additional trials data and possibly new clinical trials. Sanofi also tested a partial $D_{2/3}$ agonist (AVE-5997EF) but no development was reported after phase I trials.

Like the $D_3$ receptors, the density of $D_4$ receptors is significantly lower than $D_1$ or $D_2$ receptors, but interest in the potential role of the $D_4$ receptor in schizophrenia was sparked by the finding that clozapine has high affinity for the $D_4$ receptor [195], see also Table 3. Moreover, $D_4$ receptors reduce NMDA signaling on the prefrontal cortex, suggesting that $D_4$ antagonists might alleviate prefrontal hypofunctioning and thus cognitive deficits [196]. However, clinical trials with $D_4$ selective drugs (including sonepiprazole) have failed to produce a strong antipsychotic effect [197] and work in this area seems to have come to a standstill after the initial enthusiasm

for $D_4$ receptors. An interesting recent study actually found evidence that $D_4$ agonists might be relevant to the treatment of the cognitive symptoms of schizophrenia [198]. In this study, the pro-cognitive effects of lurasidone in marmoset monkeys were blocked by the addition of a $D_4$ antagonist, while a $D_4$ agonist improved cognition.

## *4.2 Selective Serotonergic Ligands*

In an attempt to develop non-dopaminergic antipsychotics several companies have looked towards selective serotonergic drugs given their close interaction with the dopaminergic system (see Sect. 3.3; also refer to Table 8). Although so far, these strategies have not led to successful drugs, we will nonetheless briefly discuss them.

### 4.2.1 5-$HT_{2A}$ Receptor Ligands

Most attention has been paid to selective 5-$HT_{2A}$ receptor antagonists, inspired predominantly by the theory put forward by Meltzer and his colleagues that an increase in 5-$HT_{2A}$ binding over $D_2$ binding leads to less EPS (see Sect. 3.3). However, although several ligands (with varying degree of selectivity over the 5-$HT_{2C}$ receptor) have been tried in schizophrenic patients, none have been found particularly effective in treating positive, negative or cognitive symptoms. Although these studies using inverse agonists such as pimavanserin (**20**, ACP-103) and nelotanserin (**21**, APD-125), or antagonists such as eplivanserin (**22**, SR-46349), volinanserin (**23**, MDL 100,907) and CYR-101 were generally found to be superior to placebo, they were invariably less effective than standard antipsychotics [199]. As Fig. 10 shows the two inverse agonists are structurally quite similar with two phenyl rings interspersed with a urea (N–CO–N) linker. Volinaserin (**23**) and ketanserin (**24**) show structural similarity to the butyrophenones and ritanserin (**25**) to the diphenylbutylamines, although the distance between the aromatic group and the nitrogen of the piperidine is larger in the selective 5-$HT_{2A}$ antagonists.

In addition to 5-$HT_{2A}$ antagonists, several companies have also evaluated 5-$HT_{2C}$ agonists. As discussed above, 5-$HT_{2C}$ receptors are intimately related to the dopaminergic cells within the midbrain and 5-$HT_{2C}$ agonists have been found to reduce dopaminergic cell firing. Moreover, given that one of the major side effects of second generation antipsychotics (weight gain) seems at least in part mediated via blockade of 5-$HT_{2C}$ receptors, these 5-$HT_{2C}$ agonists were thought to be devoid of this risk. Lorcaserin (**26**), a benzazepine 5-$HT_{2C}$ agonist (see Fig. 10) has been approved for the treatment of obesity by the FDA in 2012 but was subsequently classified as a schedule IV drug in 2013 because of the occurrence of psychomimetic effect (especially hallucinations). Vabicaserin (**27**, a benzodiazepine derivative), a 5-$HT_{2C}$ agonist and 5-$HT_{2B}$ antagonist was tested in patients with schizophrenia but a

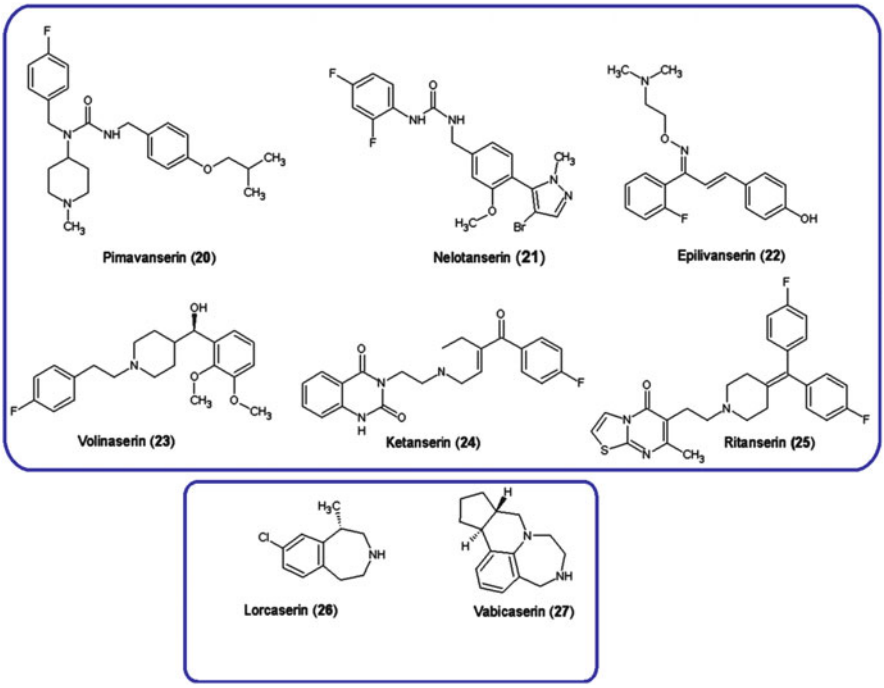



**Fig. 10** The structure of representative selective serotonergic receptor ligands proposed as antipsychotic drugs

recent study evaluating vabicaserin in patients with acute exacerbations was withdrawn in 2012 and its development discontinued. As Table 10 indicates, several more or less selective 5-$HT_{2A}$ ligands appear to be still in development, such as MT201/CYR101 (a 5-$HT_{2A}$ and sigma receptor antagonist) which showed improvement in cognitive and negative symptoms in a double blind placebo controlled phase IIa study in 100 patients. Likewise, pimavanserin is still in development (phase III) for Alzheimer's disease.

In addition to these 5-$HT_2$ ligands as alternatives to antipsychotic drugs, several 5-HT selective ligands have been proposed as add-on to existing antipsychotics, either as a means to reduce the side effect or to improve the therapeutic effects, mainly focusing on the negative and/or cognitive effects.

The 5-$HT_{1A}$ receptor has been implicated in the treatment of depression especially in relation to neurogenesis, an expanding field whose occurrence outside the hippocampus and relevance if any to humans in psychiatric and cognitive disorders remains to be assessed (see, e.g., [200]). As negative symptoms show at least at face value similarity to depressive symptoms and neurogenesis has also been implicated in cognition, selective 5-$HT_{1A}$ receptor agonists have been thought to improve the therapeutic effect as add-on to existing antipsychotic medication [201]. Moreover, as discussed above, 5-$HT_{1A}$ agonists reduce the antipsychotic

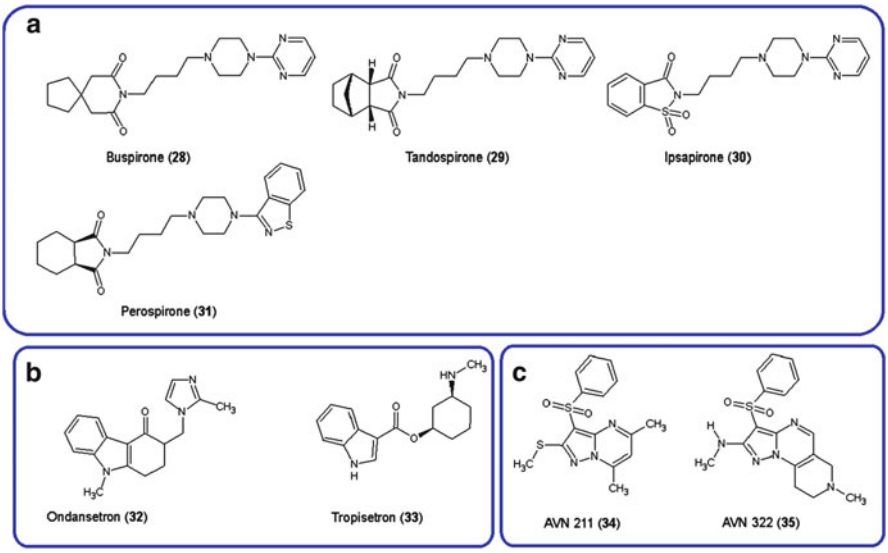



**Fig. 11** The structure of representative selective serotonergic receptor ligands proposed as add-on to existing antipsychotic treatment. (**a**) partial 5-$HT_{1A}$ agonists, (**b**) 5-$HT_3$ antagonist, (**c**) 5-$HT_6$ antagonists

induced catalepsy, generally regarded as an animal model for EPS, suggesting that adding a 5-$HT_{1A}$ agonist might also reduce the side effect liability of antipsychotic medication. Although several 5-$HT_{1A}$ ligands are currently on the market as anxiolytic drugs, all of these are only partial agonists, with varying degrees of selectivity over the 5-$HT_{2A}$, $\alpha_1$ and $\alpha_2$ receptor. These drugs all belong to the azapirones, including buspirone (**28**), tandospirone (**29**) and ipsapirone (**30**, see Fig. 11). Several of the azapirones actually have $D_2$ blocking properties and one of them (perospirone, **31**) is marketed as an antipsychotic in Japan. A recent meta-analysis, however, showed it to be less efficacious than other second generation antipsychotics [202]. In line with this, a recent meta-analysis of 5-$HT_{1A}$ add-ons (especially buspirone and tandosprione) showed no significant improvement negative or positive symptoms or discontinuation rates. Only the overall psychopathology improved significantly with additional treatment with a 5-$HT_{1A}$ agonist [203]. One important aspect to keep in mind here is the fact that most azapirones are only partial agonist of the 5-$HT_{1A}$ receptor and only when full agonists become available we will be able to assess the potential benefits of 5-$HT_{1A}$ stimulation in schizophrenia. Eltoprazine, a partial agonist for the 5-$HT_{1A}$ and a full agonist for the 5-$HT_{1B}$ receptor seems to be still in development and at least with respect to Alzheimer's disease, positive phase II results have been published.

The second class of potentially interesting add-ons is the selective 5-$HT_3$ antagonists, currently in use for the treatment of nausea especially in relation to chemotherapy. In recent years, several studies have evaluated 5-$HT_3$ antagonists, including ondansetron (**32**) and tropisetron (**33**) as add-on to existing antipsychotic

medication in patients with schizophrenia [204–206]. These studies all showed that the addition of the 5-$HT_3$ antagonist led to an additional reduction in positive symptoms, as well as a reduction in EPS. Perhaps more interesting all studies also showed a significant improvement in the negative symptoms. As the 5-$HT_3$ receptor is the only ionotropic 5-HT receptor, the development of selective ligands is significantly easier. The pharmacophore is thought to consist of three components: a carbonyl-containing linking moiety, a (hetero)aromatic ring and a basic centre. Although several different chemical classes of 5-$HT_3$ antagonists have been developed (ondansetron is a carbazole and tropisetron an indole for instance), they all seem to fit reasonably within the pharmacophore [207]. It should be kept in mind that some of the 5-$HT_3$ receptor antagonists also have affinity for the nicotinic acetylcholine receptor (which, like the 5-$HT_3$ receptor belongs to the class of the cys-loop ionotropic receptors) (see [208], this book). However, the actions of 5-$HT_3$ antagonists on the nicotinic receptors are complex with tropisetron stimulating and ondansetron inhibiting the receptor. It is therefore likely that the therapeutic effects of the various setrons in schizophrenia are indeed due to their 5-$HT_3$ blocking effect, though a role for the nicotinic receptors cannot be completely excluded at this point.

The last 5-HT receptor that has been suggested to be beneficial to the therapeutic effects of antipsychotics is the 5-$HT_6$ receptor. Especially 5-$HT_6$ receptor antagonists have been found to show cognitive enhancing effects in rodent models [209–211]. As a result, the potential cognitive enhancing properties of 5-$HT_6$ antagonists are now being evaluated in phase II/III clinical studies in Alzheimer's disease (AVN322 (**35**), SUVN-502, LuAE58054, GSK742457, PF05212377 and SYN-120). Although a number of these drugs were also evaluated in schizophrenia, currently, to the best of our knowledge only Avineuro's AVN-211 (**34**) is still in phase II development for schizophrenia. The development of several other 5-$HT_6$ antagonists seems to have ceased, including SYN-114, SB-271046 and ABT-354 (see Tables 6, 7, 8 and 9).

Significant progress has been made in the development of selective 5-$HT_6$ receptor antagonists and drugs from many different chemical classes have shown affinity for the 5-$HT_6$ receptor [212]. Earlier drugs, such as the pyrido-indole dimebon showed a multireceptor profile, with appreciable affinity for the histamine $H_1$ and $H_2$ receptors. Removal of the N-side chain and subsequently increasing the size of the phenyl-substituent (with, for instance, a benzenesulfonyl group) significantly improved selectivity, although many of these drugs still showed appreciable affinity for several other 5-HT receptors [213]. Additional changes of the three-cyclic pyrido-indole structure to a two-ring structure led to the pyrrolo [2,3-b]pyridine and -pyrazolo[1,5-a]pyrimidines, which are among the most potent 5-$HT_6$ antagonists, including AVN 211, which seems quite selective (50-fold over the nearest other receptor, the 5-$HT_{2B}$). Interestingly, some of the 5-$HT_6$ ligands also show appreciable affinity of butyrylcholinesterase which may provide additional benefits for the treatment of Alzheimer's disease, although whether it provides any benefits or liability for the treatment of schizophrenia is largely unknown.

### *4.3 Mixed Dopaminergic/Serotonergic Ligands*

Since all strategies that have focused on 5-HT receptors alone have so far not led to successful marketing, many drug companies are developing additional multi-receptor drugs. However, as all second generation antipsychotics interact with multiple dopaminergic and serotonergic receptors (with the possible exception of amisulpride (**12**)), it is becoming increasingly difficult to provide develop drugs that are superior to the existing drugs, and thus warrant marketing. Indeed several drugs seem to have failed mainly because they did not provide additional benefits over existing medication. Tables 7 and 9 list a number of multi-receptor drugs that have been developed for the treatment of schizophrenia and that have since been discontinued or for which no development has been reported.

Given the scarcity of data from pharmaceutical companies, this list is by no means complete, but it does show that a large number of approaches has been tried and failed. These approaches range from very broad spectrum antagonists (similar to clozapine and asenapine) such as SEL-70 to more selective (mostly dual) receptor ligands. Moreover, various combinations of dual receptor ligands have been studied, including $D_2$ antagonists/5-$HT_{1A}$ agonists, $D_4$/5-$HT_{2A}$ antagonists and $D_3$ antagonist/5-$HT_{1A}$ agonists. The reason for these failures is not always clear with some likely due to drug-specific side effects, while others to corporate management strategic decisions. Another important reason for failure is the difficulty in developing what we might call selective dual receptor ligands. As discussed above, there are at least theoretical reasons why specific combinations (D2 antagonism plus 5-$HT_{1A}$ agonism, $D_2$ plus 5-$HT_{2A}$ antagonism, $D_2$ plus 5-$HT_6$ antagonism, etc.) might be more effective than existing second generation antipsychotics. However, many of the existing drugs as well as those listed in Tables 6 and 7 affect more than just two receptors. Although important progress is being made in defining the characteristics of the pharmacophore for several 5-HT receptors, the fairly large homology between receptors, especially in the transmembrane domain remains a significant obstacle for developing highly selective compounds. This is highlighted in a recent review on chain arylpiperazines as ligands for the 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_7$ receptor [214].

Finally, there is the problem of balance. As discussed above, it has been hypothesized that the ratio between $D_2$ and 5-$HT_{2A}$ antagonism determines the EPS liability. Likewise, it was recently suggested that the ratio amount of 5-$HT_{1A}$ agonism is important [201], with too much agonism limiting the therapeutic efficacy and too little having no benefit.

## 5 Summary

In this chapter, we have tried to give an overview of the current status of antipsychotic treatment, highlighting the enormous validity of the current therapeutic arsenal, which has significantly reduced the burden of millions of patients. However, from

our survey it is also clear that there is still room for significant improvement, especially in relation to the cognitive and negative symptoms, as well as overall compliance. As virtually all antipsychotics affect both DA and 5-HT receptors, we next discussed these two neurotransmitters and their interaction in some detail. Finally, we looked at potential future antipsychotics. Although virtually all second generation antipsychotics (with amisulpride being the only exception) have considerable affinity for several DA and 5-HT receptors, the role of virtually all these individual receptors in the clinical effects is still under debate. In fact there is only strong evidence for a role of the $D_2$ receptor in reducing the positive symptoms. Fortunately, medicinal chemical knowledge of the requirements for the different DA and 5-HT receptors is increasing thus leading to more and more selective drugs. Although the history of the development of antipsychotics suggests that such selective drugs may not necessarily be very effective antipsychotics, they may provide us with tools for probing the role of the various receptors. This knowledge can then help us to find the right combination of receptors to successfully treat the entire complex of schizophrenic symptomatology. However, it is likely that the road to this "holy grail" of psychopharmacology is still long. The several recent disappointments and the threat of a major disinvestment by the pharmaceutical industry in making new drugs for brain disorders do not presently cast a good omen. Yet, we are deeply convinced that it is journey well worth it. This is in the hope to alleviate suffering from neuropsychiatric disorders which are increasingly recognized as a major burden to humanity [215].

## References

1. Andlin-Sobocki P, Jonsson B, Wittchen HU et al (2005) Cost of disorders of the brain in Europe. Eur J Neurol 12:1–27
2. Mangalore R, Knapp M (2007) Cost of schizophrenia in England. J Ment Health Policy Econ 10(1):23–41
3. Olesen J, Gustavsson A, Svensson M et al (2012) The economic cost of brain disorders in Europe. Eur J Neurol 19(1):155–162
4. Hafner H, Maurer K, Loffler W et al (1993) The influence of age and sex on the onset and early course of schizophrenia. Br J Psychiatry 162(1):80–86
5. Hill SK, Keshavan MS, Thase ME et al (2004) Neuropsychological dysfunction in anti psychotic-naive first-episode unipolar psychotic depression. Am J Psychiatr 161(6):996–1003
6. Saykin AJ, Shtasel D, Gur RE et al (1994) Neuropsychological deficits in neuroleptic naive patients with first-episode schizophrenia. Arch Gen Psychiatry 51:124–131
7. Tandon R, Keshavan MS, Nasrallah HA (2008) Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr Res 102(1–3):1–18
8. Sun J, Kuo PH, Riley BP et al (2008) Candidate genes for schizophrenia: a survey of association studies and gene ranking. Am J Med Genet B Neuropsychiatr Genet 147B(7):1173–1181
9. Girard SL, Dion PA, Rouleau GA (2012) Schizophrenia genetics: putting all the pieces together. Curr Neurol Neurosci Rep 12(3):261–266

10. Girard SL, Xiong L, Dion PA et al (2011) Where are the missing pieces of the schizophrenia genetics puzzle? Curr Opin Genet Dev 21(3):310–316
11. Owen MJ (2012) Implications of genetic findings for understanding schizophrenia. Schizophr Bull 38(5):904–907
12. Purcell SM, Moran JL, Fromer M et al (2014) A polygenic burden of rare disruptive mutations in schizophrenia. Nature 506:185–190
13. Karayiorgou M, Morris MA, Morrow B et al (1995) Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. Proc Natl Acad Sci U S A 92(17):7612–7616
14. Sanders SJ, Murtha MT, Gupta AR et al (2012) De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485(7397):237–241
15. Girard SL, Gauthier J, Noreau A et al (2011) Increased exonic de novo mutation rate in individuals with schizophrenia. Nat Genet 43(9):860–863
16. Xu B, Roos JL, Dexheimer P et al (2011) Exome sequencing supports a de novo mutational paradigm for schizophrenia. Nat Genet 43(9):864–868
17. Fromer M, Pocklington AJ, Kavanagh DH et al (2014) De novo mutations in schizophrenia implicate synaptic networks. Nature 506:179–184
18. Mednick SA, Machon RA, Huttunen MO et al (1988) Adult schizophrenia following prenatal exposure to an influenza epidemic. Arch Gen Psychiatry 45(2):189–192
19. Brown AS, Derkits EJ (2010) Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am J Psychiatry 167(3):261–280
20. Kyle UG, Pichard C (2006) The Dutch famine of 1944–1945: a pathophysiological model of long-term consequences of wasting disease. Curr Opin Clin Nutr Metab Care 9(4):388–394
21. Brown AS (2011) The environment and susceptibility to schizophrenia. Prog Neurobiol 93(1):23–58
22. Paus T, Keshavan M, Giedd JN (2008) Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9(12):947–957
23. Caspi A, Moffitt TE, Cannon M et al (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57(10):1117–1127
24. van Winkel R, van Beveren NJ, Simons C (2011) AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. Neuropsychopharmacology 36(12):2529–2537
25. Di Forti M, Iyegbe C, Sallis H et al (2012) Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. Biol Psychiatry 72(10):811–816
26. Belsky J, Pluess M (2009) Beyond diathesis stress: differential susceptibility to environmental influences. Psychol Bull 135(6):885–908
27. Belsky J, Jonassaint C, Pluess M et al (2009) Vulnerability genes or plasticity genes? Mol Psychiatry 14(8):746–754
28. Ellenbroek BA (2003) Animal models in the genomic era: possibilities and limitations with special emphasis on schizophrenia. Behav Pharmacol 14(5–6):409–417
29. Ellenbroek BA (2010) Schizophrenia. In: Koob GF, Le Moal M, Thompson RF (eds) Encyclopedia of behavioral neuroscience, vol 3. Academic, Oxford, pp 188–195
30. Green MJ, Chia TY, Cairns MJ et al (2013) Catechol-O-methyltransferase (COMT) genotype moderates the effects of childhood trauma on cognition and symptoms in schizophrenia. J Psychiatr Res 49:43–50
31. Wright IC, Rabe HS, Woodruff PR et al (2000) Meta-analysis of regional brain volumes in schizophrenia. Am J Psychiatr 157(1):16–25
32. Keshavan MS, Tandon R, Boutros NN et al (2008) Schizophrenia, "just the facts": what we know in 2008 part 3: neurobiology. Schizophr Res 106(2–3):89–107
33. Kovelman JA, Scheibel AB (1984) A neurohistological correlate of schizophrenia. Biol Psychiatry 19:1601–1621

34. Jakob J, Beckmann H (1986) Prenatal developmental disturbances in the limbic allocortex in schizophrenia. J Neural Transm 65:303–326
35. Nielsen PR, Mortensen PB, Dalman C et al (2013) Fetal growth and schizophrenia: a nested case–control and case-sibling study. Schizophr Bull 39(6):1337–1342
36. Fineberg AM, Ellman LM, Buka S et al (2013) Decreased birth weight in psychosis: influence of prenatal exposure to serologically determined influenza and hypoxia. Schizophr Bull 39(5):1037–1044
37. Shamir EZ, Cassan SM, Levy A et al (2013) Biometric parameters of the hand as an index of schizophrenia-a preliminary study. Psychiatry Res 210(3):716–720
38. Isohanni M, Jones PB, Moilanen K et al (2001) Early developmental milestones in adult schizophrenia and other psychoses. A 31-year follow-up of the northern Finland 1966 birth cohort. Schizophr Res 52(1–2):1–19
39. Jones P, Rodgers B, Murray R et al (1994) Child development risk factors for adult schizophrenia in the British 1946 birth cohort. Lancet 344(8934):1398–1402
40. Walker EF, Savoie T, Davis D (1994) Neuromotor precursors of schizophrenia. Schizophr Bull 20(3):441–451
41. Rapoport JL, Addington AM, Frangou S et al (2005) The neurodevelopmental model of schizophrenia: update 2005. Mol Psychiatry 10(5):434–449
42. Rapoport JL, Giedd JN, Gogtay N (2012) Neurodevelopmental model of schizophrenia: update 2012. Mol Psychiatry 17(12):1228–1238
43. Harrison PJ (2008) Neuropathology of schizophrenia. Psychiatry 7(10):421–424
44. Pantelis C, Yucel M, Wood SJ et al (2005) Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. Schizophr Bull 31(3):672–696
45. van Haren NEM, Hulshoff Pol HE, Schnack HG et al (2006) Gray matter changes in schizophrenia across the course of the illness: a five-year study. Schizophr Res 81:23
46. Cahn W, Pol HEH, van Haren NE et al (2005) Schizophrenia and progressive gray matter volume decrease during the first five years of illness. Schizophr Bull 31(2):384–385
47. van Haren NE, Cahn W, Hulshoff Pol HE et al (2008) Schizophrenia as a progressive brain disease. Eur Psychiatry 23(4):245–254
48. Haijma SV, Van Haren N, Cahn W et al (2013) Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. Schizophr Bull 39(5):1129–1138
49. Connell PH (1958) Amphetamine psychosis. Maudsley monographs. Oxford University Press, London
50. Reith J, Benkelfat C, Sherwin A et al (1994) Elevated dopa decarboxylase activity in living brain of patients with psychosis. Proc Natl Acad Sci U S A 91(24):11651–11654
51. Hietala J, Syvalahti E, Vuorio K, Rakkolainen V et al (1995) Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. Lancet 346(8983):1130–1131
52. Breier A, Su TP, Saunders R et al (1997) Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A 94(6):2569–2574
53. Laruelle M, Abi-Dargham A, van Dyck CH et al (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci U S A 93(17):9235–9240
54. Abi-Dargham A, van de Giessen E, Slifstein M et al (2009) Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. Biol Psychiatry 65(12):1091–1093
55. Laruelle M, Abi-Dargham A, Gil R et al (1999) Increased dopamine transmission in schizophrenia: relationship to illness phases. Biol Psychiatry 46(1):56–72
56. Howes OD, Bose SK, Turkheimer F et al (2011) Dopamine synthesis capacity before onset of psychosis: a prospective [18F]-DOPA PET imaging study. Am J Psychiatry 168(12):1311–1317

57. Howes O, Bose S, Turkheimer F et al (2011) Progressive increase in striatal dopamine synthesis capacity as patients develop psychosis: a PET study. Mol Psychiatry 16(9):885–886
58. Howes OD, Kambeitz J, Kim E et al (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment: meta-analysis of imaging studies. Arch Gen Psychiatry 69:776–786
59. Kegeles LS, Abi-Dargham A, Frankle WG et al (2010) Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. Arch Gen Psychiatry 67(3):231–239
60. Drevets WC, Gautier C, Price JC et al (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry 49:81–96
61. Pletscher A, Ladewig D (1994) Fifty years of LSD: current status and perspectives of hallucinogens. The Parthenon Publishing Group, New York
62. Nichols DE (2004) Hallucinogens. Pharmacol Ther 101(2):131–181
63. Greifenstein FE, Yoshitake J, DeValut M et al (1958) A study of l-aryl cyclohexyl amine for anesthesia. Anesth Analg 37:283–294
64. Luby ED, Cohen BD, Rosenbaum G et al (1959) Study of a new schizophrenomimetic drug - sernyl. AMA Arch Neurol Psychiatry 81:363–369
65. Krystal JH, Karper LP, Seibyl JP et al (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine in humans. Arch Gen Psychiatry 51:199–214
66. Malhotra AK, Pinals DA, Adler CM et al (1997) Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology 17(3):141–150
67. Contreras PC, Monahan JB, Lanthorn TH et al (1987) Phencyclidine. Physiological actions, interactions with excitatory amino acids and endogenous ligands. Mol Neurobiol 1 (3):191–211
68. Kim JS, Kornhuber HH, Schmid-Burgk W et al (1980) Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci Lett 20(3):379–382
69. Jentsch JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20(3):201–225
70. Moghaddam B, Krystal JH (2012) Capturing the angel in "angel dust": twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. Schizophr Bull 38(5):942–949
71. Moghaddam B, Javitt D (2012) From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology 37(1):4–15
72. Marsman A, van den Heuvel MP, Klomp DW et al (2013) Glutamate in schizophrenia: a focused review and meta-analysis of (1)H-MRS studies. Schizophr Bull 39(1):120–129
73. Brennan AM, Harris AW, Williams LM (2013) Functional dysconnectivity in schizophrenia and its relationship to neural synchrony. Expert Rev Neurother 13(7):755–765
74. Jiang T, Zhou Y, Liu B et al (2013) Brainnetome-wide association studies in schizophrenia: the advances and future. Neurosci Biobehav Rev 37(10 Pt 2):2818–2835
75. Cook LC (1938) Cardiazol convulsion therapy in schizophrenia: (section of psychiatry). Proc R Soc Med 31(6):567–577
76. Deniker P (1983) Discovery of the clinical use of neuroleptics. In: Parnham MJ, Bruinvels J (eds) Psycho- and neuropharmacology, vol 1, Discoveries in pharmacology. Elsevier, Amsterdam, pp 163–180
77. Delay J, Deniker P, Harl JM (1952) Therapeutic use in psychiatry of phenothiazine of central elective action (4560 RP). Ann Med Psychol (Paris) 110(2 1):112–117
78. Laborit H, Huguenard P, Alluaume R (1952) A new vegetative stabilizer; 4560 R.P. Presse Med 60(10):206–208
79. Ellenbroek BA (1993) Treatment of schizophrenia: a clinical and preclinical evaluation of neuroleptic drugs. Pharmacol Ther 57(1):1–78
80. Altar CA, Martin AR, Thurkauf A (2003) Antipsychotic agents. In: Abraham DJ (ed) Burger's medicinal chemistry and drug discovery, vol 6. Wiley, Hoboken, NJ, pp 599–672

81. Idanpaan-Hiekkila J, Alhave E, Olkinuora M et al (1977) Agranulocytosis during treatment with clozapine. Eur J Clin Pharmacol 11:193–198
82. Chakrabarti JK, Horsman L, Hotten TM et al (1980) 4-piperazinyl-10H-thieno[2,3-b][1,5] benzodiazepines as potential neuroleptics. J Med Chem 23(8):878–884
83. Ellenbroek BA, Cools AR (2000) Atypical antipsychotics. Milestones in drug therapy. Birkhauser, Basel
84. Meltzer HY, Matsubara S, Lee JC (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. J Pharmacol Exp Ther 251(1):238–246
85. Chiodo LA, Bunney BS (1983) Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. J Neurosci 3:1607–1619
86. White FJ, Wang RY (1983) Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopamine neurons. Science 211:1054–1056
87. Gudelsky GA, Meltzer HY (1989) Activation of tuberoinfundibular dopamine neurons following the acute administration of atypical antipsychotics. Neuropsychopharmacology 2(1):45–51
88. Davis JM, Chen N, Glick ID (2003) A meta-analysis of the efficacy of second-generation antipsychotics. Arch Gen Psychiatry 60(6):553–564
89. Rummel-Kluge C, Komossa K, Schwarz S et al (2010) Second-generation antipsychotic drugs and extrapyramidal side effects: a systematic review and meta-analysis of head-to-head comparisons. Schizophr Bull 38:167–177
90. Leucht S, Komossa K, Rummel-Kluge C et al (2009) A meta-analysis of head-to-head comparisons of second-generation antipsychotics in the treatment of schizophrenia. Am J Psychiatry 166(2):152–163
91. Hartling L, Abou-Setta AM, Dursun S et al (2012) Antipsychotics in adults with schizophrenia: comparative effectiveness of first-generation versus second-generation medications: a systematic review and meta-analysis. Ann Intern Med 157:498–511
92. Ellenbroek BA (2012) Psychopharmacological treatment of schizophrenia: what do we have and what could we get? Neuropharmacology 62:1371–1380
93. Keefe R, Bilder RM, Davis SM et al (2006) Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. Neuropsychopharmacology 31:S244–S245
94. McEvoy JP, Lieberman JA, Stroup TS et al (2006) Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. Am J Psychiatr 163(4):600–610
95. Lieberman JA, Stroup TS, McEvoy JP et al (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia: primary efficacy and safety outcomes of the clinical antipsychotic trials of intervention effectiveness (CATIE) schizophrenia trial. Neuropsychopharmacology 30:S32
96. McEvoy JP, Lieberman JA, Perkins DO et al (2007) Efficacy and tolerability of olanzapine, quetiapine, and risperidone in the treatment of early psychosis: a randomized, double-blind 52-week comparison. Am J Psychiatry 164:1050–1060
97. Kahn RS, Fleischhacker WW, Boter H et al (2008) Effectiveness of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: an open randomised clinical trial. Lancet 371(9618):1085–1097
98. Lieberman JA, Stroup TS, McEvoy JP et al (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 353(12):1209–1223
99. Kane JM, Honigfeld G, Singer J et al (1988) Clozapine for the treatment-resistant schizophrenic: a double-blind comparison with chlorpromazine. Arch Gen Psychiatry 45:789–796
100. Sacchetti E, Galluzzo A, Valsecchi P et al (2009) Ziprasidone vs clozapine in schizophrenia patients refractory to multiple antipsychotic treatments: the MOZART study. Schizophr Res 113(1):112–121

101. Schletema Beduin AA, Baken BCM, Van Os S et al (2012) Clozapine, an overview of clinical pharmacology, efficacy and safety. Minerva Psichiatr 53:197–215
102. Carlsson A, Lindqvist M (1963) Effects of chlorpromazine or haloperidol on formation of 3-methoxytyramin and normetanephrine in mouse brain. Acta Pharmacol Toxicol 20:140–144
103. van Rossum J (1966) The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. Arch Int Pharmacodyn Ther 160:492–494
104. Creese I, Burt D, Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potenties of antischizophrenic drugs. Science 192:481–483
105. Seeman P, Lee T, Choa-Wong M et al (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717–719
106. Peroutka SJ, Synder SH (1980) Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potency. Am J Psychiatry 137(12):1518–1522
107. Nord M, Farde L (2011) Antipsychotic occupancy of dopamine receptors in schizophrenia. CNS Neurosci Ther 17(2):97–103
108. Farde L, Wiesel FA, Nordstrom AL et al (1989) D1 and D2 dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology (Berl) 99:S28–S31
109. Wong DF, Kuwabara H, Brasic JR et al (2013) Determination of dopamine D-2 receptor occupancy by lurasidone using positron emission tomography in healthy male subjects. Psychopharmacology (Berl) 229(2):245–252
110. Yilmaz Z, Zai CC, Hwang R, Mann S et al (2012) Antipsychotics, dopamine D(2) receptor occupancy and clinical improvement in schizophrenia: a meta-analysis. Schizophr Res 140(1–3):214–220
111. Lako IM, van den Heuvel ER, Knegtering H et al (2013) Estimating dopamine D(2) receptor occupancy for doses of 8 antipsychotics: a meta-analysis. J Clin Psychopharmacol 33(5):675–681
112. Farde L, Wiesel FA, Halldin C et al (1988) Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry 45(1):71–76
113. Burris KD, Molski TF, Xu C et al (2002) Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. J Pharm Exp Ther 302(1):381–389
114. Richelson E (1999) Receptor pharmacology of neuroleptics: relation to clinical effects. J Clin Psychiatry 60(Suppl 10):5–14
115. Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227
116. Knegtering H, van der Moolen AEGM, Castelein S, Kluiter H, van den Bosch RJ (2003) What are the effects of antipsychotics on sexual dysfunctions and endocrine functioning? Psychoneuroendocrinology 28(Suppl 2):109–123
117. Meltzer HY (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. Psychopharmacology (Berl) 99 Suppl:S18–S27
118. Meltzer HY (2013) Update on typical and atypical antipsychotic drugs. Annu Rev Med 64:393–406
119. Dahlstrom A, Fuxe K (1964) Evidence for existence of monoamine-containing neurons in central nervous system.I. Demonstration of monoamines in cell bodies of brain stem neurons. Acta Physiologica Scandinavica 232:1–55
120. Undieh AS (2010) Pharmacology of signaling induced by dopamine D1-like receptor activation. Pharmacol Ther 128(1):37–60
121. Joyce JN, Meador WJH (1997) Linking the family of D2 receptors to neuronal circuits in human brain: insights into schizophrenia. Neuropsychopharmacology 16(6):375–384
122. Rommelfanger KS, Wichmann T (2010) Extrastriatal dopaminergic circuits of the basal ganglia. Front Neuroanat 4:139

123. Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35(1):217–238
124. Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5(6):483–494
125. Wise RA, Rompre PP (1989) Brain dopamine and reward. Annu Rev Psychol 40:191–225
126. Consolazione A, Cuello AC (1982) CNS serotonergic pathways. In: Osborne NN (ed) Biology of serotonergic transmission. Wiley, Chichester
127. Nieuwenhuys R (1985) Chemoarchitecture of the brain. Springer, Berlin
128. Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38(8):1083–1152
129. Filip M, Bader M (2009) Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. Pharmacol Rep 61(5):761–777
130. Murphy DL, Andrews AM, Wichems CH et al (1998) Brain serotonin neurotransmission: an overview and update with an emphasis on serotonin subsystem heterogeneity, multiple receptors, interactions with other neurotransmitter systems, and consequent implications for understanding the actions of serotonergic drugs. J Clin Psychiatry 59(Suppl 15):4–12
131. Di Giovanni G, Di Matteo V, Pierucci M et al (2008) Serotonin-dopamine interaction: electrophysiological evidence. Prog Brain Res 172:45–71
132. Steinbusch HW (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6(4):557–618
133. Beckstead RM, Domesick VB, Nauta WJ (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res 175(2):191–217
134. Kitahama K, Nagatsu I, Geffard M et al (2000) Distribution of dopamine-immunoreactive fibers in the rat brainstem. J Chem Neuroanat 18(1–2):1–9
135. Mansour A, Meador WJH, Bunzow JR et al (1990) Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. J Neurosci 10(8):2587–2600
136. Suzuki M, Hurd YL, Sokoloff P et al (1998) D3 dopamine receptor mRNA is widely expressed in the human brain. Brain Res 779(1–2):58–74
137. Gareeva AE, Zakirov DF, Valinurov RG et al (2013) Polymorphism of RGS2 gene as genetic marker of schizophrenia risk and pharmacogenetic markers of the efficiency of typical neuroleptics. Mol Biol 47(6):814–820
138. Staring ABP, Van der Gaag M, Mulder CL (2013) Schizophrenia and antipsychotic medication - better adherence, better outcomes? Schizophr Res 151(1–3):296–297
139. Seki Y, Kato TA, Monji A et al (2013) Pretreatment of aripiprazole and minocycline, but not haloperidol, suppresses oligodendrocyte damage from interferon-gamma-stimulated microglia in co-culture model. Schizophr Res 151(1–3):20–28
140. Di Matteo V, Di Giovanni G, Pierucci M et al (2008) Serotonin control of central dopaminergic function: focus on in vivo microdialysis studies. Prog Brain Res 172:7–44
141. Resnick B, Galik E (2013) Antipsychotic use and the impact on function and behavior in moderate to severely cognitively impaired assisted living and nursing home residents. Gerontologist 53:121
142. Ichikawa J, Ishii H, Bonaccorso S et al (2001) 5-HT2A and D-2 receptor blockade increases cortical DA release via 5-HT1A receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. J Neurochem 76(5):1521–1531
143. Herrmann N, Lanctot KL, Hogan DB (2013) Pharmacological recommendations for the symptomatic treatment of dementia: the Canadian Consensus Conference on the Diagnosis and Treatment of Dementia 2012. Alzheimers Res Ther 5:S5
144. Ludwin B, Meeks S (2013) Facility- level predictors of antipsychotic medication use in nursing home residents. Gerontologist 53:445
145. Channon S, Crawford S, Orlowska D et al (2014) Mentalising and social problem solving in adults with Asperger's syndrome. Cogn Neuropsychiatry 19(2):149–163
146. Przegalinski E, Filip M (1997) Stimulation of serotonin (5-HT)1A receptors attenuates the locomotor, but not the discriminative, effects of amphetamine and cocaine in rats. Behav Pharmacol 8(8):699–706

147. Castanon N, Scearce-Levie K, Lucas JJ et al (2000) Modulation of the effects of cocaine by 5-HT1B receptors: a comparison of knockouts and antagonists. Pharmacol Biochem Behav 67(3):559–566
148. McMahon LR, Cunningham KA (2001) Antagonism of 5-hydroxytryptamine(2a) receptors attenuates the behavioral effects of cocaine in rats. J Pharmacol Exp Ther 297(1):357–363
149. Devroye C, Filip M, Przegalinski E et al (2013) Serotonin2C receptors and drug addiction: focus on cocaine. Exp Brain Res 230(4):537–545
150. Costall B, Naylor RJ, Tyers MB (1990) The psychopharmacology of 5-HT3 receptors. Pharmacol Ther 47(2):181–202
151. McMahon LR, Cunningham KA (1999) Antagonism of 5-hydroxytryptamine(4) receptors attenuates hyperactivity induced by cocaine: putative role for 5-hydroxytryptamine(4) receptors in the nucleus accumbens shell. J Pharmacol Exp Ther 291(1):300–307
152. Galici R, Boggs JD, Miller KL et al (2008) Effects of SB-269970, a 5-HT7 receptor antagonist, in mouse models predictive of antipsychotic-like activity. Behav Pharmacol 19(2):153–159
153. Pouzet B, Didriksen M, Arnt J (2002) Effects of the 5-HT(7) receptor antagonist SB-258741 in animal models for schizophrenia. Pharmacol Biochem Behav 71(4):655–665
154. Frantz KJ, Hansson KJ, Stouffer DG et al (2002) 5-HT6 receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. Neuropharmacology 42(2):170–180
155. Pouzet B, Didriksen M, Arnt J (2002) Effects of the 5-HT(6) receptor antagonist, SB-271046, in animal models for schizophrenia. Pharmacol Biochem Behav 71(4):635–643
156. Broekkamp CLE, Oosterloo SK, Berendsen HHG et al (1988) Effect of metergoline, fenfluramine, and 8-OHDPAT on catalepsy induced by haloperidol or morphine. Naunyn Schmiedebergs Arch Pharmacol 338(2):191–195
157. Ohno Y, Imaki J, Mae Y et al (2011) Serotonergic modulation of extrapyramidal motor disorders in mice and rats: role of striatal 5-HT3 and 5-HT6 receptors. Neuropharmacology 60(2–3):201–208
158. de Deurwaerdere P, Cervo L, Stinus L et al (2002) Central 5-HT4 receptors and dopamine-dependent motor behaviours: searching for a functional role. Pharmacol Biochem Behav 71:627–633
159. Neal-Beliveau BS, Joyce JN, Lucki I (1993) Serotonergic involvement in haloperidol-induced catalepsy. J Pharmacol Exp Ther 265(1):207–217
160. Creed-Carson M, Oraha A, Nobrega JN (2011) Effects of 5-HT(2A) and 5-HT(2C) receptor antagonists on acute and chronic dyskinetic effects induced by haloperidol in rats. Behav Brain Res 219(2):273–279
161. Ellenbroek BA, Prinssen EP, Cools AR (1994) The role of serotonin receptor subtypes in the behavioural effects of neuroleptic drugs. A paw test study in rats. Eur J Neurosci 6(1):1–8
162. Setler PE, Sarau HM, Zirkle CL et al (1978) The central effects of a novel dopamine agonist. Eur J Pharmacol 50(4):419–430
163. Iorio LC, Barnett A, Leitz FH et al (1983) Sch-23390, a potential benzazepine anti-psychotic with unique interactions on dopaminergic systems. J Pharmacol Exp Ther 226(2):462–468
164. Ellenbroek BA, Lubbers LJ, Cools AR (1996) Activity of "seroquel" (ICI 204,636) in animal models for atypical properties of antipsychotics: a comparison with clozapine. Neuropsychopharmacology 15(4):406–416
165. Ellenbroek BA, Willemen AP, Cools AR (1989) Are antagonists of dopamine D1 receptors drugs that attenuate both positive and negative symptoms of schizophrenia? A pilot study in java monkeys. Neuropsychopharmacology 2(3):191–199
166. den Boer JA, Korf J (2000) Dopamine receptor subtypes and schizophrenia: a clinical perspective. In: Ellenbroek BA, Cools AR (eds) Atypical antipsychotics. Milestones in drug therapy. Birkhäuser Verlag, Basel, pp 163–190
167. Zhang J, Xiong B, Zhen X et al (2009) Dopamine D1 receptor ligands: where are we now and where are we going. Med Res Rev 29(2):272–294

168. Arnsten AFT, Cai JX, Murphy BL et al (1994) Dopamine D-1 receptor mechanisms in the cognitive performance of young-adult and aged monkeys. Psychopharmacology (Berl) 116(2):143–151
169. Goldman-Rakic PS, Castner SA et al (2004) Targeting the dopamine D-1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology (Berl) 174(1):3–16
170. Mu Q, Johnson K, Morgan PS et al (2007) A single 20 mg dose of the full D1 dopamine agonist dihydrexidine (DAR-0100) increases prefrontal perfusion in schizophrenia. Schizophr Res 94(1–3):332–341
171. George MS, Molnar CE, Grenesko EL et al (2007) A single 20 mg dose of dihydrexidine (DAR-0100), a full dopamine D1 agonist, is safe and tolerated in patients with schizophrenia. Schizophr Res 93(1–3):42–50
172. Sun BC, Zhang XX, Jin GZ (1996) (−)-Stepholidine acts as a D1 partial agonist on firing activity of substantia nigra pars reticulata neurons in 6-hydroxydopamine-lesioned rats. Life Sci 59(4):299–306
173. Huang KX, Sun BC, Jin GZ (1992) (−)-Stepholidine: a dopamine receptor antagonist shows agonistic effect on rotational behavior in 6-hydroxydopamine-lesioned rats. Zhongguo Yao Li Xue Bao 13(1):17–22
174. Sun BC, Jin GZ (1992) Effects of (−)-Stepholidine on firing activity of dopamine rneurons in ventral tegmental area of the rats. Acta Pharmacol Sin 13:395–399
175. Ellenbroek BA, Zhang XX, Jin GZ (2006) Effects of (−)stepholidine in animal models for schizophrenia. Acta Pharmacol Sin 27(9):1111–1118
176. Natesan S, Reckless GE, Barlow KB et al (2008) The antipsychotic potential of l-stepholidine–a naturally occurring dopamine receptor D1 agonist and D2 antagonist. Psychopharmacology (Berl) 199(2):275–289
177. Cools AR, Lubbers L, van Oosten RV et al (2002) SKF 83959 is an antagonist of dopamine D1-like receptors in the prefrontal cortex and nucleus accumbens: a key to its antiparkinsonian effect in animals? Neuropharmacology 42(2):237–245
178. Andringa G, Drukarch B, Leysen JE et al (1999) The alleged dopamine D1 receptor agonist SKF 83959 is a dopamine D1 receptor antagonist in primate cells and interacts with other receptors. Eur J Pharmacol 364(1):33–41
179. Andringa G, Stoof JC, Cools AR (1999) Sub-chronic administration of the dopamine D(1) antagonist SKF 83959 in bilaterally MPTP-treated rhesus monkeys: stable therapeutic effects and wearing-off dyskinesia. Psychopharmacology (Berl) 146(3):328–334
180. Rashid AJ, So CH, Kong MMC et al (2007) D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci U S A 104(2):654–659
181. Perreault ML, O'Dowd BF, George SR (2011) Dopamine receptor homooligomers and heterooligomers in schizophrenia. CNS Neurosci Ther 17(1):52–57
182. Faron-Gorecka A, Gorecki A, Kusmider M et al (2008) The role of D1-D2 receptor heterodimerization in the mechanism of action of clozapine. Eur Neuropsychopharmacol 18(9):682–691
183. Lober S, Hubner H, Tschammer N et al (2011) Recent advances in the search for D3- and D4-selective drugs: probes, models and candidates. Trends Pharmacol Sci 32(3):148–157
184. Kapur S, Seeman P (2001) Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics?: a new hypothesis. Am J Psychiatry 158(3):360–369
185. Schmidt ME, Kent JM, Daly E et al (2012) A double-blind, randomized, placebo-controlled study with JNJ-37822681, a novel, highly selective, fast dissociating D(2) receptor antagonist in the treatment of acute exacerbation of schizophrenia. Eur Neuropsychopharmacol 22(10):721–733
186. Anghelescu IG, Janssens L, Kent J et al (2013) Does early improvement predict response to the fast-dissociating D-2 receptor antagonist JNJ-37822681 in patients with acute schizophrenia? Eur Neuropsychopharmacol 23(9):1043–1050

187. Sokoloff P, Giros B, Martres MP et al (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347:146–151
188. Gross G, Wicke K, Drescher KU (2013) Dopamine D(3) receptor antagonism–still a therapeutic option for the treatment of schizophrenia. Naunyn Schmiedebergs Arch Pharmacol 386(2):155–166
189. Millan MJ, Brocco M (2008) Cognitive impairment in schizophrenia: a review of developmental and genetic models, and pro-cognitive profile of the optimised D-3 > D-2 antagonist, S33138. Therapie 63(3):187–229
190. Millan MJ, Buccafusco JJ, Loiseau F et al (2010) The dopamine D3 receptor antagonist, S33138, counters cognitive impairment in a range of rodent and primate procedures. Int J Neuropsychopharmacol 13(8):1035–1051
191. Millan MJ, Di Cara B, Dekeyne A et al (2007) Selective blockade of dopamine D(3) versus D(2) receptors enhances frontocortical cholinergic transmission and social memory in rats: a parallel neurochemical and behavioural analysis. J Neurochem 100(4):1047–1061
192. Redden L, Rendenbach-Mueller B, Abi-Saab WM et al (2011) A double-blind, randomized, placebo-controlled study of the dopamine D(3) receptor antagonist ABT-925 in patients with acute schizophrenia. J Clin Psychopharmacol 31(2):221–225
193. Graff-Guerrero A, Redden L, Abi-Saab W et al (2010) Blockade of [11C](+)-PHNO binding in human subjects by the dopamine D3 receptor antagonist ABT-925. Int J Neuropsychopharmacol 13(3):273–287
194. Kiss B, Horvath A, Nemethy Z et al (2010) Cariprazine (RGH-188), a dopamine D(3) -receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. J Pharmacol Exp Ther 333(1):328–340
195. Seeman P, Corbett R, Van THH (1997) Atypical neuroleptics have low affinity for dopamine D2 receptors or are selective for D4 receptors. Neuropsychopharmacology 16(2):93–110
196. Wang X, Zhong P, Gu Z, Yan Z (2003) Regulation of NMDA receptors by dopamine D4 signaling in prefrontal cortex. J Neurosci 23(30):9852–9861
197. Corrigan MH, Gallen CC, Bonura ML et al (2004) Effectiveness of the selective D-4 antagonist sonepiprazole in schizophrenia: a placebo-controlled trial. Biol Psychiatry 55(5):445–451
198. Murai T, Nakako T, Ikeda K et al (2013) Lack of dopamine D receptor affinity contributes to the procognitive effect of lurasidone. Behav Brain Res 261C:26–30
199. Mestre TA, Zurowski M, Fox SH (2013) 5-Hydroxytryptamine 2A receptor antagonists as potential treatment for psychiatric disorders. Expert Opin Investig Drugs 22(4):411–421
200. Spalding KL, Bergmann O, Alkass K et al (2013) Dynamics of hippocampal neurogenesis in adult humans. Cell 153(6):1219–1227
201. Schreiber R, Newman-Tancredi A (2014) Improving cognition in schizophrenia with antipsychotics that elicit neurogenesis through 5-HT receptor activation. Neurobiol Learn Mem
202. Kishi T, Iwata N (2013) Efficacy and tolerability of perospirone in schizophrenia: a systematic review and meta-analysis of randomized controlled trials. CNS Drug 27(9):731–741
203. Kishi T, Meltzer HY, Iwata N (2013) Augmentation of antipsychotic drug action by azapirone 5-HT1A receptor partial agonists: a meta-analysis. Int J Neuropsychopharmacol 16(6):1259–1266
204. Akhondzadeh S, Mohammadi N, Noroozian M et al (2009) Added ondansetron for stable schizophrenia: a double blind, placebo controlled trial. Schizophr Res 107(2–3):206–212
205. Zhang ZJ, Kang WH, Li Q et al (2006) Beneficial effects of ondansetron as an adjunct to haloperidol for chronic, treatment-resistant schizophrenia: a double-blind, randomized, placebo-controlled study. Schizophr Res 88(1–3):102–110
206. Noroozian M, Ghasemi S, Hosseini SM et al (2013) A placebo-controlled study of tropisetron added to risperidone for the treatment of negative symptoms in chronic and stable schizophrenia. Psychopharmacology (Berl) 228(4):595–602

207. Thompson AJ, Lummis SC (2006) 5-HT3 receptors. Curr Pharm Des 12(28):3615–3630
208. Mazurov A, Yohannes D (2014) Nicotinic acetylcholine receptor modulators. Top Med Chem. doi:10.1007/7355_2014_56
209. Rogers DC, Hagan JJ (2001) 5-HT6 receptor antagonists enhance retention of a water maze task in the rat. Psychopharmacology (Berl) 158(2):114–119
210. Rodefer JS, Nguyen TN, Karlsson JJ et al (2008) Reversal of subchronic PCP-induced deficits in attentional set shifting in rats by sertindole and a 5-HT6 receptor antagonist: comparison among antipsychotics. Neuropsychopharmacology 33(11):2657–2666
211. de Bruin NM, van Drimmelen M, Kops M et al (2013) Effects of risperidone, clozapine and the 5-HT6 antagonist GSK-742457 on PCP-induced deficits in reversal learning in the two-lever operant task in male Sprague Dawley rats. Behav Brain Res 244:15–28
212. Ivachtchenko AV, Ivanenkov YA, Tkachenko SE (2010) 5-Hydroxytryptamine subtype 6 receptor modulators: a patent survey. Expert Opin Ther Pat 20(9):1171–1196
213. Ivachtchenko AV, Ivanenkov YA (2013) Small molecule 5-HT6R ligands: a comprehensive insight into their selectivity and activity. Curr Bioact Compd 9(1):64–100
214. Bielenica A, Koziol AE, Struga M (2013) Computational methods in determination of pharmacophore models of 5-HT(1A), 5-HT(2A) and 5-HT(7) receptors. Mini Rev Med Chem 13(7):933–951
215. Nutt DJ (2011) The full cost and burden of disorders of the brain in Europe exposed for the first time. Eur Neuropsychopharmacol 21(10):715–717

Top Med Chem (2015) 13: 51–100
DOI: 10.1007/7355_2014_53

Published online: 9 August 2014

# GlyT-1 Inhibitors: From Hits to Clinical Candidates

**Roderick A. Porter and Lee A. Dawson**

**Abstract** The treatment of schizophrenia has long been dominated by aminergic receptor antagonist-based therapeutics largely founded on the dopamine hypothesis of schizophrenia. More recently the glutamatergic theory has come to the fore which may potentially address some of the deficiencies of current therapies. While there are many approaches to manipulating the glutamatergic system, the most advanced approach is to increase synaptic concentrations of the NMDA receptor co-agonist glycine via inhibition of the glycine transporter 1 (GlyT-1). Here we will describe the background biological rationale for this approach and review the diverse classes of compounds which have been identified as GlyT-1 inhibitors with particular focus on the identification of those molecules which have entered the clinical stages of development. The role of target kinetics in drug action, a review of the rich vein of PET ligand development and their use in clinical development and the status of clinical-stage compounds will be addressed. Finally there is a discussion of some of the issues that have arisen with the discovery and development of GlyT-1 inhibitors and the prospects for the future of this mechanistic approach.

**Keywords** Bitopertin, Glutamate, Glycine transporter, GlyT-1, NMDA receptor, Schizophrenia

## Contents



R.A. Porter (✉)
Rod Porter Consultancy, Ashwell, Herts, UK
e-mail: rod.porter@rodporterconsultancy.com

L.A. Dawson
Eisai Limited, European Knowledge Centre, Mosquito Way, Hatfield, Hertfordshire AL10 9SN, UK
e-mail: Lee_Dawson@eisai.net

## Abbreviations

| | |
|---|---|
| ADMET | Adsorption, distribution, metabolism, excretion, toxicity |
| ASST | Attentional set-shifting task in rats |
| CADSS | Clinician administered dissociative symptoms scale |
| CAR | Conditioned avoidance response |
| CBT | Cognitive-behavioural therapy |
| CSF | Cerebrospinal fluid |
| DAAO | D-Amino acid oxidase |
| DAT | Dopamine transporter |
| EEG | Electroencephalogram |
| EPSCs | Excitatory postsynaptic currents |
| ErbB4 | Receptor tyrosine-protein kinase erbB-4 |
| Gly | Glycine |
| GlyB | Strychnine-insensitive glycine-B subunit |
| GlyR | Glycine receptor |
| GlyT-1 | Glycine transporter-1 |
| GlyT-2 | Glycine transporter-2 |
| h | Hour(s) |
| hERG | Human ether-à-go-go-related gene |
| HTS | High-throughput screening |
| i.v. | Intravenous |
| kg | Kilogram |
| LeuT | Leucine transporter |

| | |
|---|---|
| LeuTAa | Leucine transporter from *Aquifex aeolicus* |
| LTP | Long-term potentiation |
| mPFC | Medial prefrontal cortex |
| MED | Minimum effective dose |
| MEST | Maximal electroshock test |
| NMDA | *N*-Methyl-D-aspartic acid |
| NR1 | NMDA receptor subunit-1 |
| NRG-1 | Neuregulin receptor 1 |
| ORD | Object retrieval–detour |
| p.o. | Per os |
| PANNS | Positive and negative syndrome scale |
| PCP | Phencyclidine |
| PDSS | Panic Disorder Severity Scale |
| PET | Positron emission tomography |
| PFC | Prefrontal cortex |
| P-gp | P-glycoprotein |
| PK | Pharmacokinetics |
| PPB | Plasma protein binding |
| s.c. | Subcutaneous |
| SAR | Structure activity relationship |
| SSRI | Selective serotonin reuptake inhibitor |
| SRR | Serine racemase |
| TCA | Tricyclic antidepressant |
| TM | Transmembrane helix |
| WT | Wild type |

## 1 Introduction

The NMDA receptor (and more specifically a dysfunction of NMDA-mediated neurotransmission) has gained significant interest as the mediator of the dysfunction seen in schizophrenia. This hypothesis has been derived from a number of observations: firstly, both of the NMDA receptor blockers phencyclidine (PCP) and ketamine exacerbate psychotic symptoms in schizophrenic individuals, reinstate schizophrenic-like symptoms in remitting patients [1] and induce schizophrenic-like psychotic states and impairments in cognitive performance in healthy individuals [1–3] and secondly the ever-increasing genetic observations which are continuing to cluster around the NMDA receptor [4–6]. For example, neuregulin-1 (NRG-1)-mediated suppression of NMDA receptor function, possibly via enhanced activation of ErbB4 [7], has been demonstrated in postmortem prefrontal cortex from schizophrenic subjects. Moreover, mice with mutations in NRG-1 transmembrane regions produced selective imbalances in glutamatergic and dopaminergic neurotransmission [8]. G72 and G30 were identified in a segment from chromosome 13q34 that has been shown to be genetically linked to schizophrenia [9]. The G72 gene product was found

to increase the activity of D-amino acid oxidase (DAAO), the enzyme responsible for the breakdown of the NMDA co-agonists D-serine and D-alanine ([9]; for reviews see [10, 11]). Burnet and colleagues reported that DAAO activity and expression were upregulated in tissue from schizophrenic patients [12, 13]. Furthermore, there is evidence that serine racemase (SRR), which is responsible for the catalytic conversion of L-serine to D-serine [14], may also be a susceptibility gene [15, 16] and its expression may also be altered in the disorder [13, 17]. Thus, there is increasing evidence of a variety of potential subtle dysfunctions around the NMDA receptor synapse which may increase susceptibility to progression into schizophrenia and be an underlying substrate for the symptoms of the disease. Targeting the NMDA receptor synapse has, therefore, become an obvious focus for novel therapeutic approaches to treat schizophrenia. Direct activation of the NMDA receptor by an "orthosteric agonist" or elevation of the endogenous agonist, glutamate, can result in overexcitation, potentially leading to seizurogenesis and/or excitotoxicity and as such is not a viable approach. However, the NMDA receptor is unique in that it requires the presence of both an agonist (i.e. glutamate) and an obligatory co-agonist (i.e. glycine (Gly), D-serine and/or D-alanine) which bind at a distinct site, known as the strychnine-insensitive glycine-B (GlyB) site located on the NR1 subunit. It is only in the presence of both agonists that the ligand-gated ion channel opens. Therefore, one such approach for enhancing NMDA receptor function has been to increase occupancy and activity at this co-agonist/regulatory GlyB site.

There are now a number of clinical observations that at least partially provide credence to this approach. Javitt and colleagues have carried out a series of double-blind placebo-controlled studies of high-dose Gly given adjunctively to current antipsychotics [18–21]. The general outcomes were a significant reduction in negative symptoms and improvements in cognition and general psychopathology but with no effect on positive symptom domains. Similarly, a double-blind placebo-controlled trial of D-serine (30 mg/kg/day) in chronic schizophrenia patients who were poorly responsive to neuroleptics (for 6 weeks) produced significant reductions in negative and cognitive symptoms and, in this case, also positive symptoms [22]. Gly and D-serine are not suitable drugs for long-term therapy due to the requirement for very high doses (due to their poor pharmacokinetics (PK) and low brain penetration). In addition, D-serine has shown some evidence of toxicity [23]. However, taken together these studies do substantiate the hypothesis that co-agonism at the GlyB site may modulate NMDA receptor function and thus have therapeutic potential in schizophrenia. Of course the alternative strategy, and perhaps more viable approach, would be to elevate the various endogenous co-agonists within the synapse.

## 2 Glycine Transporters

Extracellular Gly levels are regulated by two specific sodium-dependent solute carrier family 6 (SLC6) transporters present either in the plasma membrane of the presynaptic nerve terminals or in astrocytes [24]. The two transporters, Gly transporter 1 (GlyT-1 [25]) and Gly transporter 2 (GlyT-2 [26]), share ~50% amino acid

sequence homology but can display quite different pharmacology and function [27]. There are currently five identified variants of GlyT-1 (GlyT-1a, GlyT-1b, GlyT-1c, GlyT-1d and GlyT-1e) and three of GlyT-2 (GlyT-2a, GlyT-2b and GlyT-2c). These originate from differing promoter controls and/or splice variants (for review see [28]). Unfortunately to date the relative CNS distribution of the various variants have not been systematically reported, with the possible exception of GlyT-1e which appears to be largely retinal [29]. Thus, the exact functional consequence of this diversity is not clear; however, functional consequences have been demonstrated, e.g. GlyT-2b does not appear to transport Gly [30].

In situ hybridisation studies have revealed that GlyT-1 expression appears to be localised in astrocytes and widely expressed across the brain, while GlyT-2 is predominantly expressed in glycinergic nerve terminals co-localised with Gly receptors (GlyR) [24]. This differential cellular localisation leads to differing functional roles. Glial GlyT-1 ensures the removal of Gly from the synaptic cleft leading to the termination of Gly-mediated neurotransmission; GlyT-1's presence in glutamatergic synapse and co-localisation with NMDA receptors [24, 31] suggests a critical role in regulating excitatory neurotransmission. GlyT-1 also mediates the clearance of Gly from the synaptic cleft of inhibitory synapses and concurrently participates in the regulation of Gly concentrations at excitatory synapses and thus plays a key role in balancing excitatory vs. inhibitory activity. In contrast, GlyT-2 ensures the refilling of presynaptic vesicles of glycinergic neurons [32, 33]. It is worth noting that data from knockout mouse models have shown that constitutive disruption of GlyT-1 or GlyT-2 is lethal [32, 33], presumably as a result of excessive or deficient glycinergic inhibition, respectively. Heterozygous GlyT-1 knockdowns are, however, viable and show ~50% reductions in tissue uptake of Gly, an increased NMDA receptor function and subsequent behavioural improvements in cognitive and "psychosis" models [34–36]. Furthermore, this delineated localisation and function may not be as clear-cut as originally thought [37, 38], and the dual excitatory vs. inhibitory role of GlyT-1 may lead to unwanted outcomes or limitations in tolerance to pharmacological intervention (see Sect. 8).

## 3 Molecular Structure of GlyT-1

The $Na^+Cl^-$ coupled solute carrier SLC6 gene family includes monoamine (dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT)) and GABA transporters (GAT1-4) alongside GlyT-1 and GlyT-2. GlyT-1's stoichiometry is bidirectional transport of Gly which involves a two $Na^+$, one $Cl^-$ symporter dependency. This stoichiometry is however somewhat different for GlyT-2 which is unidirectional in its transport of Gly [28]. An excellent review [39] covers early structural work on SLC6 neurotransmitter transporters; however, until recently there has been only limited crystallographic data for this family class.

This section will briefly review more recent crystallographic data for SLC6 family members and its relevance to GlyT-1, alongside some results of mutagenesis studies.

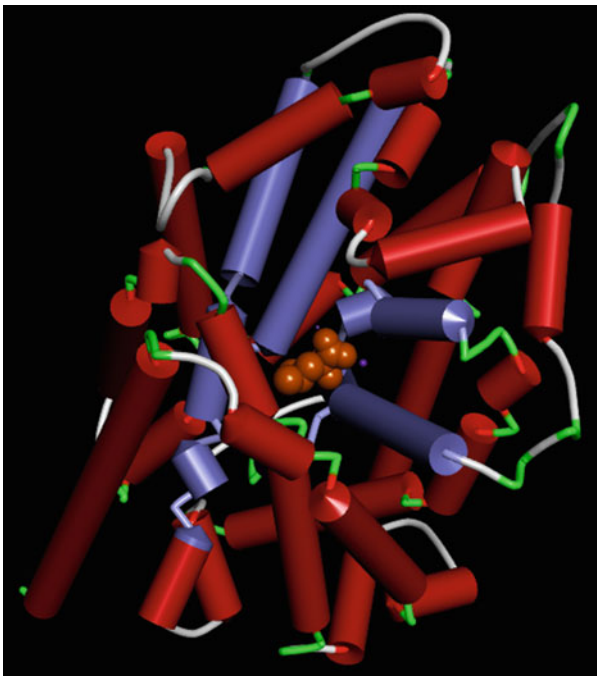


**Fig. 1** A side on view of leucine (*orange*) bound in LeuTAa with helices 1, 3, 6 and 8 highlighted in *magenta* from pdb 2A65 [40]

## *3.1 Crystallography*

Several structures of SLC6 family member proteins have now been reported:

- The bacterial $Na^+/Cl^-$-dependent leucine transporter from *Aquifex aeolicus* (LeuTAa; Fig. 1 [40])
- A dopamine transporter from *Drosophila melanogaster* [41] with nortriptyline bound
- Multiple structures of LeuTAa with bound tryptophan (a competitive inhibitor [42]), selective serotonin reuptake inhibitors (SSRIs [43]) and tricyclic antidepressants (TCAs [44, 45])
- Structures of LeuTAa Δ13 mutants in which key residues have been changed to those corresponding to SERT, which recapitulates the pharmacology of SSRIs with several different SSRIs bound (e.g. fluoxetine (**1**) [46])

These crystal structures have confirmed a 12 transmembrane (TM) helical arrangement of this family with corresponding intracellular and extracellular loops and intracellular N- and C-termini (Fig. 1). TM1 and TM6 are partially unwound to give TM1a, TM1b and TM6a, TM6b and, in the LeuTAa, provide the majority of interactions with bound leucine along with TM3 and TM8 residues, including the highly conserved TM3 Tyr108 (=Tyr 128 GlyT-1, Tyr289 GlyT-2a). GlyT-2a Tyr289 has been implicated in substrate binding and ion coupling [47]. Analysis of residues involved in binding leucine to LeuTAa and equivalent

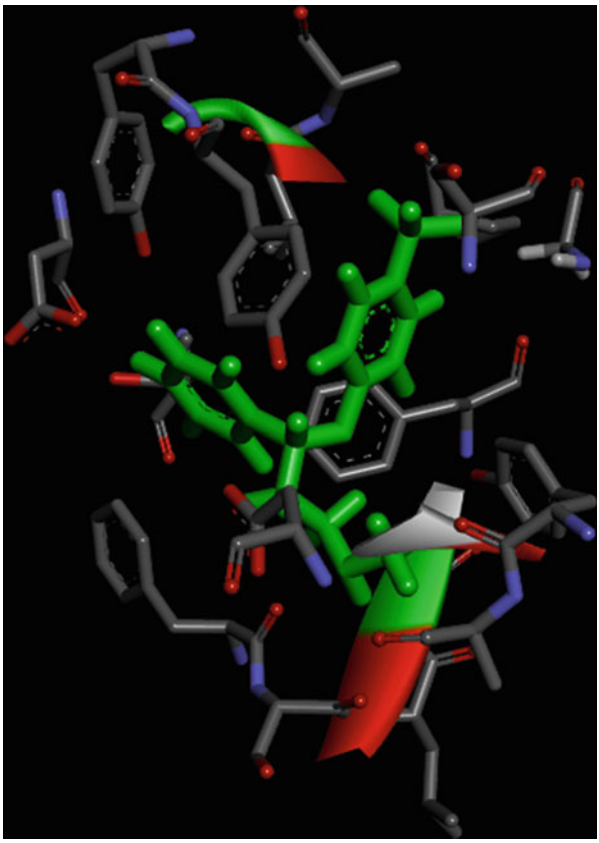


**Fig. 2** Fluoxetine (**1**) (*green*) bound to LeuTAa Δ13 showing residues within 4A from pdb 4MM8 [46]

residues in GlyT-1 highlights the more bulky side chains of the latter. It has been postulated that the change from the less sterically demanding Gly305 (hGlyT-1b) to the corresponding bulkier Ser481 (GlyT-2) accounts for the selectivity of sarcosine (**2**) for GlyT-1 vs. GlyT-2 [48]. Furthermore, sarcosine (**2**) can be transported by a Ser481Gly mutant of GlyT-2 [49].

Nortriptyline is bound in an outward-open transporter conformation in the DAT, blocking isomerisation to an inward facing conformation and engaging with residues from TMs 1, 3, 6 and 8 [41]. Also present in this structure is a molecule of cholesterol which has been previously proposed [50] to regulate neurotransmitter transporters and specifically for DAT stabilisation of an outward-open state [51]. Both GlyT-1 [52] and GlyT-2 [53] have also been reported to associate with cholesterol-rich lipid rafts which affects transporter activity.

As fluoxetine **1** (Fig. 3) was used as an initial design motif for early sarcosine (**2**)-based GlyT-1 inhibitors (see Sect. 4.1), the LeuTAa mutant/fluoxetine (**1**) complex is of particular interest (Fig. 2) ([46]; pdb accession code 4MM8). However, the well-documented non-competitive nature of these sarcosine analogues differs from the competitive nature of SSRI binding to SERT, a difference which

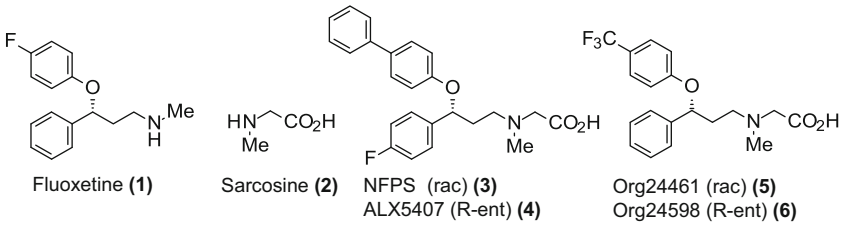



**Fig. 3** NFPS/ALX5407, Org24598/Org24461 and their progenitor ligands

remains to be explained. It is noteworthy that in the structures discussed here $Na^+$ is bound, while NFPS (**3**) (Sect. 4.1) binding has been reported to be $Na^+$ independent. Transporter inhibitors, being substantially larger than the endogenous amino acid or bioamine cargoes, interact with additional residues, in particular those within TM10.

Consistent with these crystallographic findings, data have been generated demonstrating that TM domains 1 and 3 of GlyT-1 are determinants of NFPS (**3**) (Sect. 4.1) activity. When GlyT-1 TM3 or particularly TM1 were replaced with the equivalent helices from GlyT-2, the activity of NFPS (**3**) was substantially reduced. Conversely, a chimeric GlyT-2 receptor with TM1 and TM3 from GlyT-1 showed sensitivity to NFPS (**3**). While a GlyT-1/TM1/GlyT-2 chimera retained non-competitive kinetics for NFPS (**3**), the GlyT-1/TM3/GlyT-2 chimera had mixed kinetics [54]. TM-1 has also been implicated in the binding of Gly and sarcosine (**2**) [55].

GlyT-1 exists in N-terminal (pre-helix 1) splice variants that are reported to effectively show no variation in ligand pharmacology [48]. This is readily understood as no residue outside the 12TM bundle is invoked in ligand (or ion) binding from the reported structural studies. However, these splice variants may still influence biological function due to differences in cell tracking or protein–protein associations.

## *3.2 Structure-Based Design*

While homology models have been built based on crystallographic data, for use in virtual screening [56], there have been few reports on homology models of GlyT-1 [48]. Furthermore, the detail of conformational changes involved in function of SLC6 class neurotransmitter transporters is not immediately clear from the crystallographic studies reported thus far, with structures tending to only show inhibitors or substrates bound in a common "open-out" conformation. Accelerated molecular dynamics simulations of the LeuT +/− leucine and $Na^+$ [57] suggest that there are seven unique conformations, only two of which have been seen in crystallography studies. A further challenge is the uncertainty over the binding mode needed to give preferred target kinetics for optimal efficacy and safety (Sects. 5 and 8).

# 4 Reported Inhibitors: Structural Classes and Pharmacology

Many companies have pursued GlyT-1 inhibitors resulting in a diverse range of structures and mechanisms of action. These have been identified from high-throughput screening (HTS) activities and structural analogy with other SLC transport inhibitors (e.g. SERT and DAT inhibitors) and more recently via pharmacophore-based rational design. This section will discuss the development of the various classes of GlyT-1 inhibitor and their biological profiles, focussing on those classes that have ultimately resulted in molecules which have progressed into the clinic. Broadly the discussion will be categorised based on ionisation state of the molecules, namely, zwitterionic sarcosine-derived inhibitors, basic compounds and non-physiologically ionisable compounds; although the reader will note that significant overlaps between these categories are emerging. For more detail several reviews on GlyT-1 inhibitors have been published [58–68].

## *4.1 Sarcosine and Sarcosine Analogues*

### 4.1.1 Aryloxyphenylpropyl Substituted Glycines

Sarcosine (**2**) is a very weak competitive substrate/inhibitor of GlyT-1 ($IC_{50}$ 91 μM [69]) but with selectivity over GlyT-2. Despite this, the compound is undergoing clinical evaluation (Table 10). By analogy with fluoxetine (**1**), hydrophobic sarcosine *N*-substituents gave high affinity non-substrate inhibitors (Fig. 3). The earliest examples of this approach are NFPS (racemate) (**3**) or ALX5407 (single enantiomer) (**4**) [70] and Org24461 (racemate) (**5**) and Org24598 (single enantiomer) (**6**) [71] (Fig. 3). ALX5407 (**4**) showed low nM activity ($IC_{50} = 3$ nM) and excellent selectivity over GlyT-2 ($IC_{50} > 75$ μM), other glycine binding proteins and other transporters including SERT.

[$^3$H]NFPS has been shown to have saturable rapid binding to rat forebrain membranes Kd 7.1 nM, Bmax 3.14 pmol/mg protein and a dissociation $t_{1/2}$ of 28 min [72].

Functional studies revealed that NFPS (**3**) was a non-competitive inhibitor of [$^3$H]Gly uptake and did not interact with $Na^+$ and $Cl^-$ binding sites of GlyT-1 [72] but inhibited uptake of [$^3$H]Gly in hippocampal synaptosomes ($IC_{50}$ of ~20 nM [73]). In vivo NFPS (**3**) increased cerebrospinal fluid (CSF) levels of Gly and elevated, transiently, extracellular levels of Gly in the prefrontal cortex (PFC) of rats at 10 mg/kg p.o. [73]; interestingly a concurrent and much more sustained increase was seen in the cerebellum of rats [74]. Functionally NFPS (**3**) potentiated NMDA receptor-mediated responses and long-term potentiation (LTP, a marker of glutamate-induced synaptic plasticity) both in vitro [75–77] and in vivo [76, 78]. These neurophysiological changes resulted in behavioural reversal of

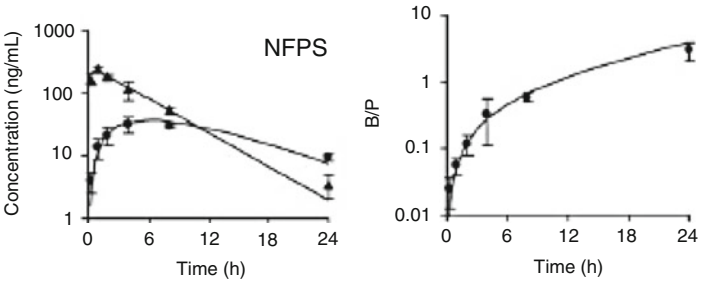



**Fig. 4** Brain and plasma concentration-time profiles and brain/plasma (B/P) concentration profile for NFPS (**3**). *Triangle* and *circle symbols* are observed plasma and brain concentrations (mean +/− S.D. $n = 3$–4), respectively. Liu et al. [89] published with permission

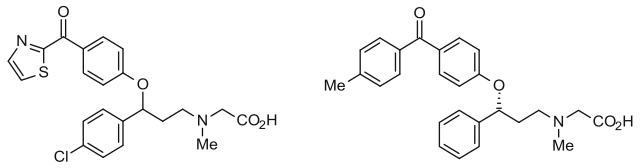



**Fig. 5** Pfizer sarcosine analogues

NMDA receptor antagonist-induced impairments in models of cognition (such as novel object recognition [79, 80] and social working memory [81]), significantly reversed PCP-induced alterations in striatal dopamine efflux [82–84] and inhibited PCP and D-amphetamine-induced hypermotility in mice [73]. Taken together these data support the proof of mechanism of NFPS (**3**) (i.e. elevations in synaptic Gly, enhancement of NMDA function) and supports the hypothesis that GlyT-1 inhibition may have therapeutic utility across a number of schizophrenic symptom domains. Unfortunately, NFPS (**3**) was generally poorly tolerated in vivo, potentially due to its irreversible GlyT-1 inhibition [74, 85]. Other sarcosine-based structures such as Org24461 (**5**), CP-802,079 (**7**) (Fig. 5) and LY2365109 (**9**) (Fig. 6) produced largely similar profiles in these types of model [73, 86, 87]. Org24461 (**5**) has also been shown to prevent chronic D2 receptor antagonist-mediated increases in striatal dopamine when co-administered with risperidone, possibly indicating that adjunctive GlyT-1 inhibition may also attenuate classic antipsychotic-induced motor side effects [88].

Structure activity relationship (SAR) studies have been published for Org24598 (**6**) [71]. Key observations from these studies are (1) extending the *N*-methyl substituent (to, e.g. ethyl) results in a tenfold reduction in activity, (2) the corresponding 3-propionic acid was essentially inactive, (3) the phenoxy ether can be replaced with a methylene with only a threefold loss in activity and (4) *ortho*-ring substitution was poorly tolerated with an electron-withdrawing

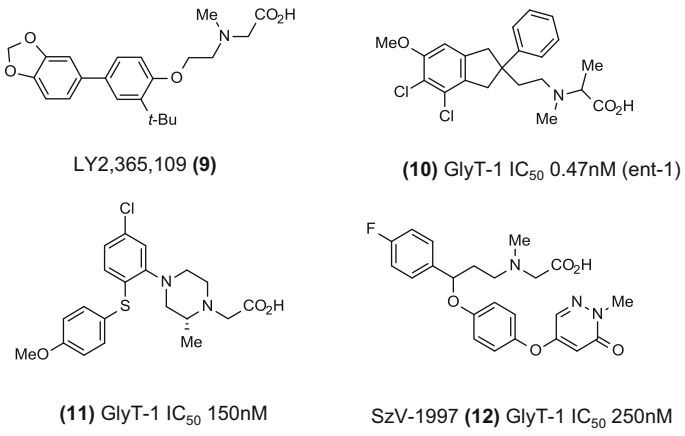



**Fig. 6** Miscellaneous sarcosine analogues

*para*-substituent preferred for target activity in the phenoxy ring. Finally the R(−) enantiomer was most active with a eudismic ratio of approximately tenfold and resulted in Org24598 (**6**) which had a $pIC_{50}$ 6.9 in a Gly uptake assay (GlyT-1 expressing CHO cell line) and excellent selectivity over GlyT-2 ($pIC_{50} < 4.0$).

In a detailed study of "time to reach brain equilibrium" in the rat [89], it was shown that NFPS (**3**) took ~6 h to reach brain $C_{max}$, while the brain plasma ratio was still rising at 24 h post dose (Fig. 4). Noteworthy is that brain levels at 24 h are comparable to brain concentrations at around the 1 h time-point.

In a binding assay, with cloned human GlyT-1c expressed in HEK293 cells, [$^3$H]-(*R*)-NPTS (**8**) (Fig. 5) had a Kd 1 nM [90] making it a useful tool to be used to support HTS studies. A close analogue of (*R*)-NPTS (**8**), CP-802,079 (**7**) ($IC_{50} = 16$ nM in a rat synaptosomal uptake assay [86]), gave a concentration-dependent increase in extracellular Gly levels when administered to rats via reverse microdialysis (0.1 or 1 μM). At the concentration estimated as $IC_{50}$ (based on an assumed 10–20% efficiency at crossing the microdialysis membrane), a 59% increase in Gly was observed. Effects on LTP in the CA1 region of rat hippocampal slices were determined using 25 nM CP-802,079 (**7**) and 750 μM sarcosine (**2**). Increases in amplitude of response, following induction of LTP, were 195 and 213% above baseline, respectively.

### 4.1.2 Other Research Stage Sarcosine Analogues

More structurally diverse sarcosine-based GlyT-1 inhibitors have also been described (Fig. 6). Examples include the work from Lilly reporting 1,2,4-trisubstituted aromatics leading to LY2365109 (**9**) [74, 91, 92]. NFPS (**3**) (0.1–10 mg/kg p.o.) and LY2365109 (0.3–30 mg/kg p.o.) gave comparable increases in CSF Gly.

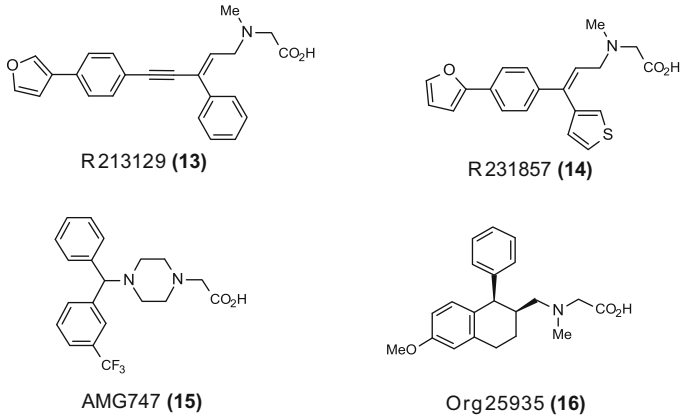



**Fig. 7** Clinical-stage sarcosine analogues

Indandiones, e.g. (**10**) reported by Merck [93], give excellent target activity. This template evolved from in-house screening and derivatisation of published inhibitors, although no specifics were disclosed – unusually it was noted that an α-substituent was well tolerated.

The Lundbeck team have reported arylthio-substituted analogues such as (**11**) (Fig. 6 [94]). Compounds showed good permeability, although in some cases P-glycoprotein (P-gp) substrate activity was evident [95]. Pharmacokinetics of (**11**) in the rat were also encouraging with 100% oral bioavailability (dosed as a solution) and low clearance (0.4 L/h/kg) and a terminal $t_{½}$ of 4 h. In freely moving mice a dose-dependent increase in Gly was seen with an increase to 140% of baseline levels 60 min post 4.6 mg/kg s.c.

While few polar-substituted sarcosine analogues have been reported, one exception was the arylpyridazinone SzV-1997 (**12**) [48]. SzV-1997 (**12**) showed modest potency in a rat cortical synaptosomal [$^3$H]Gly uptake assay ($IC_{50}$ 250 nM cf. NFPS (**3**) $IC_{50}$ 12 nM), but despite this a 10 mg/kg i.p. dose produced a robust increase in striatal Gly at 2 h post dose, while an equivalent dose of NFPS (**3**) took 4 h to achieve a significant increase. In contrast, in a mouse hyperlocomotion model, NFPS (**3**) gave profound locomotor and behavioural changes at 10 mg/kg i.p. [74], while the same dose of SzV-1997 (**12**) was without effect. The authors suggested that the substantially increased polarity of SzV-1997 (**12**) (cLog*P* 2.55 unionised species, cLog$D_{7.4}$ − 0.18), relative to NFPS (**3**) (cLog*P* 4.95 unionised species, cLog$D_{7.4}$ 2.21), may be contributing to a change in target kinetics and a consequent change in side-effect profile (see Sect. 5); however, neither exposure nor kinetic data were presented.

### 4.1.3 Sarcosine Analogues and the Clinic

Several sarcosine-derived compounds have progressed to the clinic with little preclinical data published (Fig. 7). Early clinical data has been reported for the

Allelix/Johnson and Johnson compound R213129 (JNJ-17305600) (**13**) [96, 97] and R231857 (**14**) [98].

Benzhydryl piperazines from Amgen (e.g. AMG747 (**15**)) have shown excellent target activity [99] and this compound has been reported to be in PhII clinical studies. AMG747 (**15**) has approximate $IC_{50}$s of 75, 79 and 205 nM against human, rat and dog GlyT-1 with >100-fold selectivity over GlyT-2 and reportedly good PK. Rat CSF Gly is elevated with an MED of 0.3 mg/kg and AMG747 (**15**) is active in a model of object recognition in naïve rats (MED 0.3 mg/kg p.o.) and in a subchronic PCP-induced model of cognitive impairment also in rats (MED 0.1 mg/kg, p.o.) [204].

Org25935 (**16**) (renamed as SCH900435 a conformationally constrained analogue of Org24598 (**6**)) is thought to be the sarcosine analogue that has been advanced furthest into the clinic (see Sect. 7). Org25935 (**16**) is known to be a selective inhibitor of GlyT-1 vs. GlyT-2 and monoamine transporters. Scientists at Organon Laboratories Ltd. have demonstrated high radioligand binding of Org25935 (**16**) in several brain regions [101]. Functionally Org25935 (**16**) has been demonstrated to specifically increase Gly levels in rat frontal cortex (for up to 4 h [101]) and in the nucleus accumbens and striatum [102] using in vivo microdialysis in the rat. No concurrent change in extracellular levels of taurine or β-alanine (both GlyR agonists) was observed [100], suggestive of at least some selective action on the Gly uptake mechanism. Furthermore, an increase in Gly can also be demonstrated in the CSF of both rats and primates [101]. Org25935 (**16**) has also been shown electrophysiologically to enhance NMDA-induced currents in pyramidal cells of the rat mPFC neurons [103]. Further neurochemical evaluation demonstrated that, like previous sarcosine-like molecules, Org25935 (**16**) produced increases in nucleus accumbens dopamine levels while attenuating PCP- [104] and ethanol-induced increases in subcortical dopamine [100, 105]. In a conditioned avoidance response (CAR) paradigm in rats, Org25935 (**16**) (1, 3 and 6 mg/kg s.c.) produced a very small but statistically significant suppression of CAR at 6 mg/kg. Org25935 (**16**) (6 mg/kg) adjunctively produced a small augmentation of haloperidol but not olanzapine- or risperidone-induced suppression of CAR. Finally, Org25935 (**16**) (0.1, 0.3 and 1 mg/kg, p.o.) produced a significant attenuation of the scopolamine-induced impairment in a primate object retrieval task, but a higher dose (3 mg/kg) was ineffectual [106]. The predicted GlyT-1 occupancies of Org25935 (**16**) at effective doses ranged from 16 to 80% [106]. These data suggest that GlyT-1 inhibitors have the potential to improve performance in PFC-dependent tests, but that efficacy is lost when higher occupancies are achieved. This "bell-shaped" dose phenomenon was also seen with bitopertin (**58**) (Sect. 4.3.3), the Roche clinical GlyT-1 molecule, in this task and seems to have been borne out by the clinical findings with this molecule (Sect. 7).

## *4.2 Basic GlyT-1 Inhibitors*

Several series of basic GlyT-1 inhibitors have been reported but here particular focus will be on work resulting in clinical candidates.

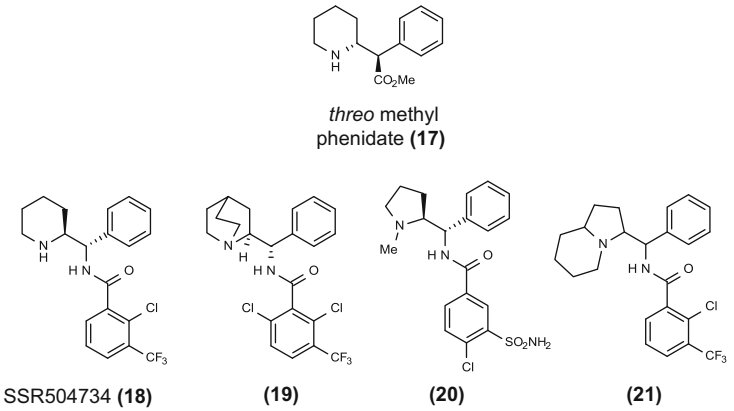



**Fig. 8** Examples of Sanofi aminophenethylbenzamides

### 4.2.1 Aminophenethylbenzamides SSR504734, SSR103800 and GSK1018921

This class of compound has generated intense interest from several companies and resulted in at least three molecules being progressed to clinical evaluation. The initial template can be seen to emerge from the DAT inhibitor methylphenidate (**17**) from which Sanofi researchers identified SSR504734 (**18**) [107] (Fig. 8) and subsequently SSR103800. While the structure of SSR103800 has not been disclosed, a recent patent from Sanofi ([108] also see [109]) discloses novel polymorphic forms of (*S*,*S*)azabicyclooctane (**19**) (Fig. 8).

SSR504734 (**18**) is a selective (vs. 120 different receptors, ion channels, enzymes or transporters including GlyT-2) and reversible inhibitor at human, rat and mouse GlyT-1 ($IC_{50}$'s of 18, 15 and 38 nM, respectively). SSR504734 (**18**) blocked Gly uptake ex vivo and produced an increase in extracellular Gly and dopamine levels in the rat PFC (MED 3 mg/kg i.p.). SSR504734 (**18**) potentiated NMDA-mediated excitatory postsynaptic currents (EPSCs) in rat hippocampal slices and increase electroencephalography (EEG) spectral power in mice and rats. SSR504734 (**18**) prevented ketamine-induced metabolic activation in mice and reversed MK-801-induced hyperactivity in rats and mice. Additionally, it normalised the endogenous deficit in pre-pulse inhibition seen in DBA/2 mice (MED, 15 mg/kg i.p.) and reversed *d*-amphetamine-induced hyperlocomotion [110]. This compound also showed additional activity in chronic mild stress in mice and rat pup maternal separation models of anxiety [110]. In the attentional set-shifting task in rats (ASST), a model of cognitive flexibility/executive function, SSR-504734 (**18**)-treated animals required significantly less trials to criteria during the extra-dimensional shift (EDs) phase of the ASST, an effect completely prevented by the Gly/NMDAR site antagonist, L-687,414 [111], demonstrating that this molecule can enhance executive function performance via activation of

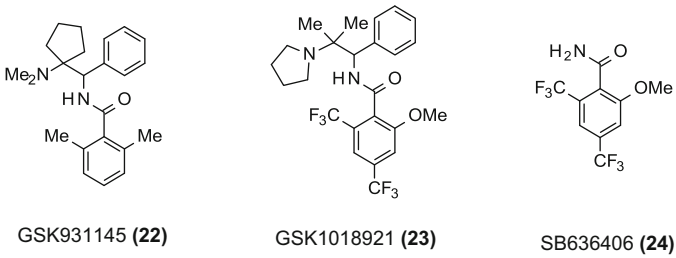


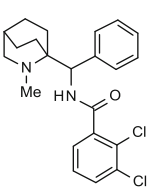

**Fig. 9** Examples of GlaxoSmithKline *gem*-dialkylaminophenethylbenzamides

the Gly site of the NMDA receptor. Similar enhancements of activity have been seen in other models of cognitive performance [112–114].

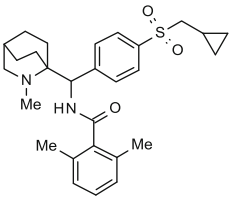
SSR103800 behaved in a similar manner to previous Sanofi molecules with human, rat and mouse $IC_{50}$ values of 1.9, 5.3 and 6.8 nM, respectively and showing reversible inhibition of Gly uptake in mouse cortical homogenates. Using in vivo microdialysis SSR130800 increased extracellular levels of Gly in the rat PFC and potentiated NMDA-mediated EPSCs in rat hippocampal slices. SSR103800 (30 mg/kg, p.o.) attenuated MK-801- and PCP-induced hyperlocomotor behaviour and neonatal PCP-induced deficits in social recognition in rats. SSR103800 (30 mg/kg, i.p.) also normalised the pre-pulse inhibition of the startle reflex seen in DBA/1 J mice. In addition, in putative models of anxiety SSR103800 decreased defensive- and despair-related behaviours' in the gerbil tonic immobility test (10 and 30 mg/kg, p.o.) and in the rat forced-swimming test (1 and 3 mg/kg, p.o.) suggestive of anxiolytic behaviour [115, 116]. Both SSR504734 (**18**) and SSR103800 have been reported to have progressed to the clinic for schizophrenia, although interestingly not anxiety.

Sanofi has disclosed other aminophenethylbenzamide templates (Fig. 8) such as pyrrolidines, e.g. (**20**) [117], and pyrrolizine; indolizine, e.g. (**21**); and quinolizines [118] with low nM activity. *Gem*-dialkylaminophenethylbenzamides have been disclosed by GlaxoSmithKline (GSK; Fig. 9), including GSK931145 (**22**) (see Sect. 6.1 [119]) and GSK1018921 (**23**) (see Sect. 7 [120]). Preclinical data on these compounds are sparse; however, detailed metabolism of GSK1018921 (**23**) in both human and preclinical species has been disclosed. Notable was the extensive metabolism in humans relative to preclinical species and in particular the high level of the primary amide metabolite SB636406 (**24**). Other metabolites generated included *N*,*N*-debutylated and *O*-demethylated analogues and various permutations of oxidation and glucuronidation [121].

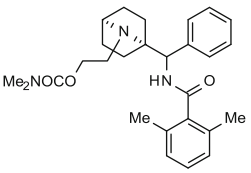
Numerous patent disclosures from other companies have emerged exploring the aminophenethylbenzamide motif. AstraZeneca (Fig. 10) has investigated further basic bicyclic motifs such as (**25**) [122] and notably successful approaches to introduce polarity either via an arylsulphone, e.g. (**26**) [123], or via an *N*-alkyl substitution such as urethane (**27**) [124]. Nonbasic cyclohexylsulphones will be discussed in Sect. 4.3.1.



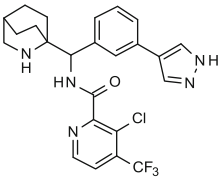

**(25)** AstraZeneca
GlyT-1 $IC_{50}$ 1nM




**(26)** AstraZeneca
GlyT-1 $IC_{50}$ 9nM

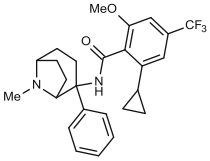



**(27)** AstraZeneca
GlyT-1 $IC_{50}$ 1nM

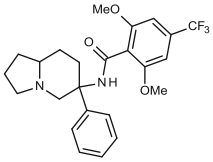



**(28)** Taisho
GlyT-1 $IC_{50}$ 0.2nM

**Fig. 10** Examples of AstraZeneca and Taisho aminophenethylbenzamides




**(29)** GlyT-1 $IC_{50}$ 4nM




**(30)** GlyT-1 $IC_{50}$ 13nM

**Fig. 11** Examples of templates from Roche with a quaternary carbon adjacent to the benzamide

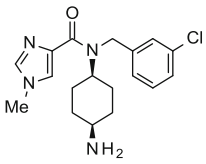
In a further approach to increase polarity, Taisho [125] have identified heterobiaryls (**28**) with excellent target activity (Fig. 10) by retaining the core 3-azabicyclo[2.2.2]octan-4-yl of (**25**) but now introducing a polar pyrazole heterocycle and nicotinamide.

While all these templates retained a secondary carbon adjacent to the benzamide, Roche have reported templates with a corresponding quaternary carbon centre (Fig. 11). Particularly potent were 4-substituted 8-azabicyclo[3.2.1]octanes such as (**29**) [126]. Structures based on a 6-substituted 2,3,5,7,8,8a-hexahydro-1H-indolizine core (**30**) also retained good target activity [127].

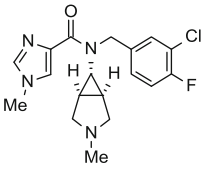
Little literature data has appeared describing the SAR of aminophenethylbenzamides; however, some general trends can be discerned from analysis of the data and claims disclosed in patents in this field, i.e.:

- A two-carbon amide/amine spacer is maintained in all the examples described.
- Preferred benzamide substitution, at least for target activity, appears to be 2,3-, 2,6- or 2,4,6- and is generally hydrophobic. Exceptions to this include the

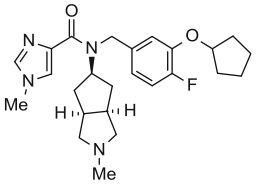
**Table 1** Pfizer amines



**(31)**



PF3463275 **(32)**



**(33)**

| Compound | GlyT-1 Ki (nM) | GlyT-2 Ki (nM) | HLM % $t_{1/2}$ (min) | MDCK AB-C[a] | CSF Gly $ED_{200}$[b] |
|---|---|---|---|---|---|
| (**31**) | 2 | 1,150 | >120 | 0.5 | 3.9 |
| PF3463275 (**32**) | 12 | >10,000 | 80 | 13.2 | 3.5 |
| (**33**) | 4 | >10,000 | 78 | 10.4 | 181% at 1 mg/kg |

Data from [128, 129]

[a]MDCK AB-C apparent permeability through MDCK cell membrane units $10^{-6}$ cm/s

[b]Dose of drug (in mg/kg) that doubles the endogenous CSF Gly concentration (90 min post dose)

2-nicotinamides such as (**28**) (Fig. 10) and the unusual 3-sulphonamide of the 3,4-disubstituted benzamide (**20**) (Fig. 8). However, exemplification of this pattern is sparse, suggesting it is generally disfavoured.

- A secondary benzamide is present in almost all molecules disclosed suggesting a key role for this motif.
- Despite SSR504734 (**18**) being a secondary amine, the majority of compounds disclosed are tertiary amines and considerable effort has gone into identifying a range of tertiary amine templates.
- The phenyl ring is not obligate (see Sect. 4.2.3 for an example of an ethylenediamine benzamide) but is present in the great majority of published patents and is generally unsubstituted. However, examples of polar substituents have been reported, e.g. compounds (**26**) and (**28**) (Fig. 10).
- Substitution of the basic nitrogen has generally not been widely explored, with only one report identified (**27**) (Fig. 10) in which polar substitution was reported to give robust target activity.

### 4.2.2 4-Aminocyclohexanes/Piperidines and Isosteres, PF-03463275

An HTS at Pfizer identified the 4-substituted aminocyclohexane (**31**) (Table 1 [128]). Compound (**31**) had a very encouraging overall profile with significant in vivo activity, as determined by increases in CSF Gly following oral dosing, and no hERG (patch clamp) or cytochrome P4502D6 or 3A4 liabilities. However, permeability was poor; thus, effort was focussed on preparing metabolically stable tertiary amines and modulating aryl ring substitutions. This resulted in the tetrahydrocyclopropyl[c]pyrrole, PF-03463275 (**32**), which has progressed to the clinic, and the octahydro-cyclopenta[c]pyrrole (**33**) [129]. PF-03463275 showed

good target affinity and selectivity and good in vivo activity coupled with improved permeability. A kilogramme scale synthesis of PF-03463275 (**32**) has been reported [130].

PF-03463275 (**32**) has been shown [131], in the non-human primate, to alleviate the deficit in spatial working memory induced by ketamine at all doses tested (0.01–0.17 mg/kg s.c.). However, ketamine-induced hallucinatory effects were not reversed. PF-03463275 (**32**) is primarily metabolised by cytochrome P450 3A4 and 2D6 and may be effluxed by P-gp ([132]).

### 4.2.3 Other Basic GlyT-1 Inhibitors

In this section a miscellaneous selection of basic GlyT-1 inhibitors will be briefly surveyed demonstrating the diversity of structures with activity at the target.

Ethylenediamines

Roche have reported a range of cyclo(hetero)alkylethylenediamine benzamides such as (**34**) [133]. Noteworthy again is the *ortho*-substituted benzamide.

In an alternative approach to hit identification, a virtual screening approach was adopted by a group from AstraZeneca [134]. Compounds with pharmacophoric similarities to literature GlyT-1 inhibitors were identified using 2D topological fingerprints to encode pharmacophoric features. Compounds that met fingerprint criteria were clustered and sample members from each cluster filtered by CNS drug-like properties (e.g. molecular weight and lipophilicity). After this process 15,000 compounds were assayed leading, after optimisation, to (**35**) that demonstrated modest CNS exposure (brain 61 ng/g 1 h post 0.99 mg/kg s.c.) but a reasonable brain/plasma ratio of 2.1 (Fig. 12).

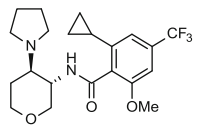
Ethylenediamine Piperazinesulphonamides

In a design strategy invoking a tethered benzamide and propyl sulphonamide held in appropriate proximity to reflect pharmacophoric similarities to other GlyT-1 inhibitors, a team at AMRI [135] identified metabolically labile spirosubstituted ethylenediamines such as (**36**) (Table 2). As previously observed *ortho*-substitution of the benzamide was found to be essential for good target activity. Introduction of a difluorocyclohexane and heterocyclic sulphonamides (see Sect. 4.3.1) leads to compounds such as (**37**) with excellent target activity and selectivity; (**37**) gave prolonged in vivo activity increasing CSF Gly levels. An example from this series (structure unknown) has been reported [136] to bind competitively with Gly to GlyT-1.

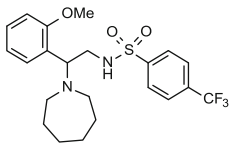
Spiropiperidines

Optimising a spiropiperidine HTS hit [137] that had multiple developability issues led to compounds such as RO4543338 (**38**) (Fig. 13). Issues that were addressed, with some success, during the optimisation included increasing target activity,

**Fig. 12** Roche and AZ ethylenediamines

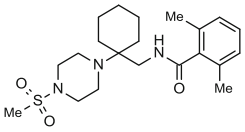


**(34)** GlyT-1 $IC_{50}$ = 8nM

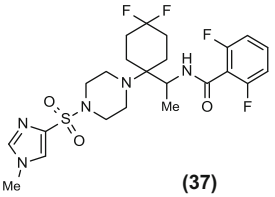


**(35)** GlyT-1 $IC_{50}$ = 37nM

**Table 2** AMRI ethylenediamine piperazinesulphonamides



**(36)**



**(37)**

| | GlyT-1 Ki (nM) | GlyT-2 Ki (nM) | HLM % remaining 60 min | PTT (min) | Dose (mg/kg) | % increase CSF glycine |
|---|---|---|---|---|---|---|
| (**36**) | 15 | >75,000 | 29 | | | |
| (**37**) | 1 | >75,000 | – | 360 | 3 | 227 |
| | | | | | 10 | 339 |

Data from [135]

selectivity over the μ-opioid receptor, metabolic stability and reducing hERG channel interaction [138–141]. While substantial progress was made in addressing multiple issues, it remained challenging to retain good target activity while maintaining acceptable adsorption, distribution, metabolism, excretion, toxicity (ADMET) and PK properties. Despite this RO4543338 (**38**) was shown to have activity in models of cocaine-seeking behaviours, albeit at relatively high doses (i.e. 30 or 45 mg/kg i.v. [142]).

## Phenethylamines

Abbott/Abbvie has reported potent phenethylamines (Fig. 13) such as compounds (**39**) [143] and (**40**) [144]. The nonbasic carboxamide (**41**) was only marginally active indicating the key importance of the basic centre. Heteroarylsulphonamides are particularly preferred for target activity, e.g. *N*-methylimidazoles and *N*-methylpyrazoles, while scope for substituting the pendant phenyl ring appeared limited. Some compounds, e.g. (**40**), showed evidence of being effluxed from the brain.

## Arylsulphonamides

Amongst the earliest non-sarcosine-based GlyT-1 inhibitors were 1,4-disubstituted piperidine arylsulphonamides reported by NPS/Allelix, e.g. (**42**) (Fig. 14 [145]) and can be seen as an early template of series of more recent interest.

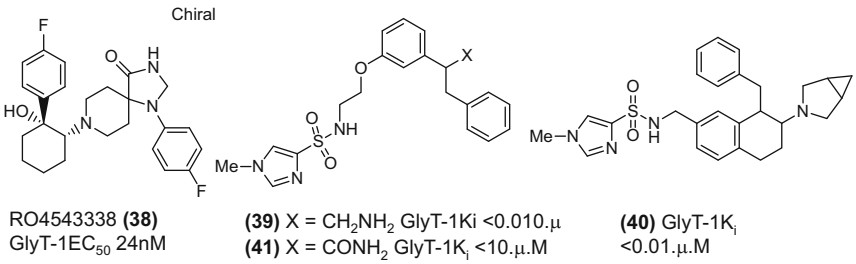



**Fig. 13** Roche spiropiperidines and Abbvie phenethylamines

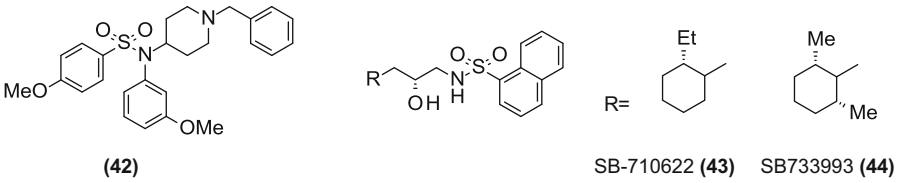



**Fig. 14** Basic arylsulphonamides – NFPS/Allelix and GSK

Diaminopropan-2-ol sulphonamides were identified from an HTS campaign of which SB710622 (**43**) (GlyT-1 Ki 100 nM) was an optimised example [146] albeit still retaining multiple ADMET issues. Despite modest exposure (brain 564 ng/g 1 h post 5 mg/kg s.c. with a brain/plasma ratio of 0.4) SB710622 (**43**) demonstrated in vivo efficacy following s.c. dosing in the maximal electroshock threshold test (MEST [147]). An analogue, SB733993 (**44**), was reported to have a Kd of 2 nM in HEK293 membranes expressing human GlyT-1 [148].

## *4.3 Nonbasic GlyT-1 Inhibitors*

A diverse range of nonbasic GlyT-1 inhibitors have been disclosed and have led to several clinical candidates, including the most advanced inhibitor bitopertin (**58**) which is currently in phase III (January 2014).

### 4.3.1 1,4-Disubstituted Piperidines/Cyclohexanes: DCCCyB (48)

In an extensive optimisation campaign by Merck, the HTS hit (**45**) led initially to the more potent competitive [149] inhibitor ACPPB (46) (Fig. 15). ACPPB (46) (1, 3 and 10 mg/kg i.v.) selectively increased Gly levels in rat PFC. ACPPB (46) (3–100 mg/kg s.c.) also significantly increased prepulse inhibition in DBA/2 mice without affecting basal startle amplitude [66, 150]. However, ACPPB

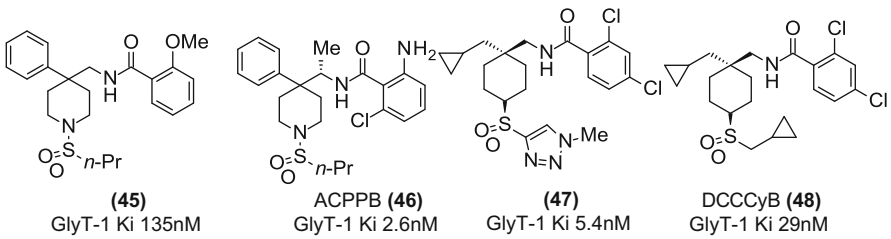



**Fig. 15** GlyT-1 inhibitory data for selected (Merck) 1,4-disubstituted piperidines/cyclohexanes

**Table 3** Pharmacokinetic data for (Merck) 1,4-disubstituted piperidines/cyclohexanes

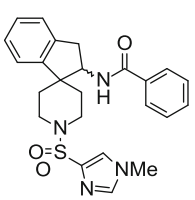
| Compound | Species | CL[a] | Vdss (L/kg) | $T_{1/2}$ (h) | %Fpo | $C_{max}$ (μM) |
|---|---|---|---|---|---|---|
| (**47**) | Rat | 34 | 2.6 | 1.1 | | |
| DCCCyB (**48**) | Rat | 36 | 4.2 | 2.4 | 65 | 0.14 |
| | Rhesus | 24 | 2.3 | 1.5 | 2 | 0.04 |
| | Dog | 4.9 | 3.1 | 10 | 48 | 1.39 |

Data from [153, 154]
[a]Clearance in mL/min/kg

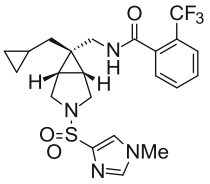
(46) suffered from time-dependent cytochrome P450 inhibitory activity and poor water solubility, limiting further progression. In a series of papers, the Merck team outlined issues addressed, and new ones identified, as they progressed the structural class to a clinical candidate. Issues solved included time-dependent inhibition of cytochrome P450s [151], P-gp substrate and subsequent poor CNS exposure [152] and poor oral bioavailability [153–155]. *N*-Dealkylation of *N*-alkylheterocycle sulphonamides resulted in potent P450 inhibitors and piperidine ring oxidation [154]. This work led to the identification of (**47**).

Exemplar (**47**) had a good rat PK profile (Table 3) resulting in good receptor occupancy ($Occ_{50}$ of 3.4 mg/kg p.o. [154]). However, (**47**) was a P-gp substrate, giving a low brain/plasma ratio (0.16), and had potential to form *N*-demethylated NH triazole metabolites which were shown to be potent cytochrome P4502C9 inhibitors ($IC_{50}$ 10 nM [153]). Returning to an alkylsulphonamide gave the clinical candidate DCCCyB (**48**). This ligand represented the best compromise of target activity and ADMET properties with good rat and dog PK. Removal of the P-gp liability resulted in a rat blood/plasma ratio of ~2.3. In vivo 3 mg/kg p.o. increased Gly levels in rat frontal cortex. In the rhesus monkey, using the PET ligand [$^{18}$F]MK-6577 (**76**) (Sect. 6.2), DCCCyB (**48**) gave an estimated plasma $EC_{50}$ of 120 nM which was comparable to that seen in the rat (plasma $EC_{50}$ of 350 nM at 3 mg/kg p.o.).

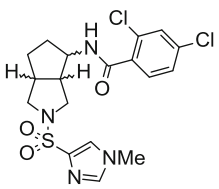
Further scaffold hopping led to novel nonbasic piperidine bioisosteres sulphonamides (Fig. 16), e.g. (**49**) [149], (**50**) [156] and (**51**) [157]. Examples showed excellent selectivity, significant free fraction, CNS exposure and activity in pre-pulse inhibition in mice. It is however unclear if examples of these *N*-methylheterocycle sulphonamides suffer from *N*-dealkylation to potent P450 inhibitors identified by the Merck team vide supra.



**(49)** GlyT-1 $IC_{50}$ 15nM

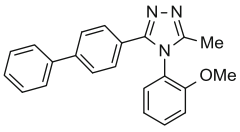


**(50)** GlyT-1 $IC_{50}$ 5nM

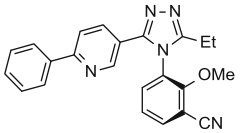


**(51)** GlyT-1 $IC_{50}$ 25nM

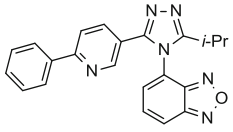
**Fig. 16** Vanderbilt University GlyT-1 inhibitors



**(52)** GlyT-1 $IC_{50}$ 1.8uM
sel. GlyT-2 0.9 fold



**(53)** GlyT-1 $IC_{50}$ 0.064uM
sel. GlyT-2 >450 fold



ASP2535 **(54)** GlyT-1 $IC_{50}$ 0.09uM
sel. GlyT-2 50 fold

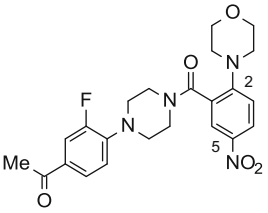
**Fig. 17** Astellas triazoles

### 4.3.2 Biaryl Triazoles ASP2535 (54)

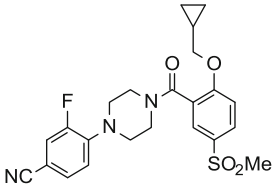
A series of biaryl triazoles (Fig. 17) have been identified by the Astellas group, optimised from their HTS hit (**52**) [158, 159]. (**52**) lacked selectivity over GlyT-2, but optimisation resulted in (**53**) identified as the more active (*R*)-atropisomer (the (*S*)-atropisomer was ~100-fold less active [160]). Target activity was much improved as was the selectivity over GlyT-2. Despite moderate oral bioavailability (32% in mouse and 23% in monkey) the molecule showed activity in vivo. The synthesis of the atropisomers has also been reported [161]. From the same series of compounds, ASP2535 (**54**) [162] progressed into phase 1.

ASP2535 (**54**) showed minimal affinity for 54 receptors, 7 ion channels and 4 transporters with the closest cross-reactivity being to the μ-opioid receptor ($IC_{50}$ 1.83 μM). It inhibited ex vivo [$^3$H]-Gly uptake in mouse cortical homogenates with an MED of 10 mg/kg p.o. and attenuated an MK-801-induced working memory deficits in mice and visual learning deficits in a mouse neonatal PCP model (0.3–3 mg/kg, p.o. and 0.3–1 mg/kg, p.o., respectively). ASP2535 (**54**) also improved the PCP-induced deficit in pre-pulse inhibition in rats (1–3 mg/kg, p.o.) and scopolamine-induced deficits in working memory in mice and spatial learning deficits in aged rats (0.1–3 mg/kg, p.o. and 0.1 mg/kg, p.o. respectively [162]). Interestingly in all of these models, there was evidence of the bell-shaped dose response that appears to be common across GlyT-1 inhibitor compounds.

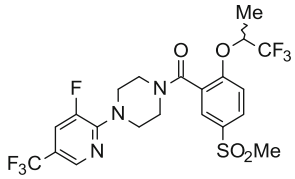
**Table 4** Roche benzoylpiperazines and the invention of RG1678 (bitopertin) (**58**)




**(55)**




**(56)**




**(57)** = (R)
RG1678 = (S) **(58)**

| Compound | $EC_{50}$ GlyT-1 (μM) | hERG (μM) | $ID_{50}$ (mg/kg)[a] | PK[b] | Cl[c] | Fpo (%) | $T_{1/2}$ (h) | B/P |
|---|---|---|---|---|---|---|---|---|
| (**55**) | 0.015 | | | | | | | |
| (**56**) | 0.016 | 0.6 | 3.0 | m | 9.5 | 100 | 5.8 | 0.1 |
| (**57**) | 0.057 | 28 | 3.0 | | | | | |
| RG1678 (**58**) | 0.03 | 17 | 0.5 | m | | | | 0.5 |
| | | | | r | 4.3 | 78 | 5.8 | 0.7 |
| | | | | mk | 3.6 | 56 | 6.4 | – |

Data from [164]
[a]Reversal of L-687,414-induced hyperlocomotion in mouse (p.o.)
[b]PK species
[c]Clearance in mL/min/kg
*m* mouse, *r* rat, *mk* monkey

### 4.3.3 Benzoylpiperazines: Bitopertin (RO4917838/RG1678) (58)

Benzoylpiperazines initially identified through HTS (Table 4) have received intense scrutiny with the most clinically advanced compound, bitopertin (**58**) (aka RO4917838 or RG1678), deriving from this work. The primary hit (**55**) was potent, selective (vs. GlyT-2 > 320-fold) but metabolically labile [163]; the 5-nitro group was seen as a potential liability and important to remove. Preliminary SAR studies showed that the 5-nitro substituent could be effectively replaced by primary or *N*-methylsubstituted sulphonamides and methylsulphones, which gave the best balance of target activity vs. polarity and metabolic stability. The 2-substituent morpholine could be replaced by a range of *N*-, *S*-, *O*- or *C*-linked substituents, but at least 4 heavy atoms were required to maintain target activity. Electron-donating arylpiperazine aromatic ring substituents were generally not tolerated, while electron-withdrawing 4-substituents proved to be beneficial for target activity. Exploration of this SAR resulted in the lead compound (**56**) which showed a good balance of target activity, excellent selectivity (over GlyT-2; $IC_{50} > 30$ μM) and physicochemical properties/solubility with negligible cytochrome P450 activity ($IC_{50} > 29$ μM), modest (88%) plasma protein binding (PPB) and an encouraging PK profile (Table 4). This profile gave in vivo proof of mechanism with increased striatal Gly levels following a 10 mg/kg p.o. dose. Unfortunately (**56**) had unacceptable hERG activity, attributed to direct interaction of the polar cyano substituent with the hERG channel, and only modest brain exposure.

An optimisation campaign to address these issues led to the identification of the clinical candidate RG1678, redesignated bitopertin (**58**) and its less active enantiomer (**57**) [164]. Bitopertin had no interactions with cytochrome P450s ($IC_{50} > 24$ μM) and was inactive in Ames and micronucleus genotoxicity assays.

Bitopertin (**58**) non-competitively inhibited Gly uptake at human recombinant GlyT-1 with an $IC_{50}$ of 25 nM while having no affinity for GlyT-2 (up to 30 μM). In hippocampal CA1 pyramidal neurons, bitopertin (**58**) enhanced NMDA-dependent LTP at 100 nM but interestingly not at 300 nM. A dose-dependent increase in both CSF and striatal extracellular levels of Gly were observed in vivo with a plateau effect reached at 10 mg/kg p.o. (as measured using microdialysis in rats). Bitopertin (**58**) attenuated D-amphetamine or L-687,414-induced hyperlocomotion in both rats and mice [165]. Bitopertin (**58**) was also characterised in the object retrieval–detour (ORD) task in scopolamine-impaired rhesus monkeys and concurrently assessed using PET [106]. Low doses of bitopertin (**58**) (0.3 and 1.0 mg/kg, p.o.) significantly attenuated the scopolamine-induced impairments, while the highest dose tested (1.8 mg/kg) was ineffectual. The predicted GlyT-1 occupancies at these effective doses were ~10 and 30%, respectively. Interestingly these are the approximately equivalent to those occupancies [166] which resulted in clinical efficacy in the proof of concept study (see Sect. 7 [167]).

### 4.3.4 Isoindolines

Further exploration of benzoylpiperazines particularly focussed on the structural diversity tolerated by the left-hand side arylpiperazine. This led to the identification of a series of potent isoindolines (Table 5). The initial chloroisoindoline lead (**59**) [168] had good target activity and over 200-fold selectivity over GlyT-2, had moderate to low in vitro clearance in mouse and human liver microsomes (23 and 2 μL/min/mg, respectively) and, despite poor solubility (<1 μg/mL), was active in vivo. Introduction of a pyran, alongside manipulation of the 2-substituent, gave RO5013853 (**60**) (solubility 76 μg/mL) and RO5013852 (**61**) (solubility 191 μg/mL). Both RO5013853 (**60**) and RO5013852 (**61**) had good rat PK profiles and elevated extracellular Gly levels in rat striatum at 10 mg/kg p.o. Both RO5013853 (**60**) and RO5013852 (**61**) have been investigated as PET ligands (Sect. 6.3).

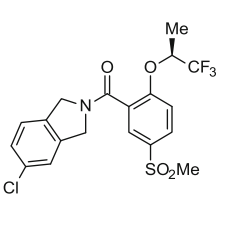
### 4.3.5 Miscellaneous Nonbasic GlyT-1 Inhibitors

Further diverse structures have been reported as GlyT-1 inhibitors, although none of the following classes have apparently progressed beyond research leads and tools.

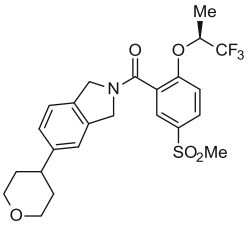
#### Heterocycles

Several heterocyclic structures (Fig. 18) have been described. These include benzodiazepine-2-ones, e.g. (**62**) [169], and aminoimidazolinones, e.g. (**63**) [170], both described by Roche. Imidazolinones, e.g. (**64**) (GSK [171]), have also been

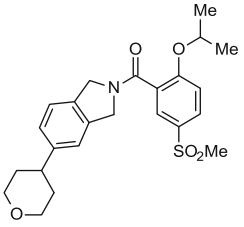
**Table 5** Roche benzoylisoindolines




**(59)**




RO5013853 **(60)**




RO5013852 **(61)**

| Compound | $EC_{50}$ GlyT-1 (μM) | $ID_{50}$ (mg/kg)[a] | Cl[b] | Vss (L/kg) | Fpo (%) | $T_{1/2}$ (h) | B/P |
|---|---|---|---|---|---|---|---|
| (**59**) | 0.135 | 20 | | | | | |
| RO5013853(**60**) | 0.014 | 0.5 | 21 | 3.7 | 65 | 3.2 | 0.6 |
| RO5013852(**61**) | 0.028 | 0.5 | 22 | 2.0 | 40 | 1.6 | 0.6 |

Data from [168]
[a]Reversal of L-687,414-induced hyperlocomotion in mouse (p.o.)
[b]Clearance in mL/min/kg

extensively exemplified in the patent literature along with unusual difluorinated benzoxazinones disclosed by GSK, e.g. (**65**) [172].

### Linear Amides

Linear amides (Fig. 19) have been generated from these heterocyclic systems by Roche, e.g. (**66**) from (**62**) [169], while GSK have described linear tertiary amides such as (**67**) from (**64**) [173].

From the limited data disclosed, particularly for structures such as (**66**) and (**67**), it is evident that there are a number of developability issues including solubility and high metabolic clearance. Following the amide theme cyclic tetrapeptides showing nM inhibitory activity and 100-fold selectivity over GlyT-2 (Fig. 19) e.g. (**68**) have been isolated from *Nonomuraea sp.* TA-0426 [174].

## *4.4 Marketed Drugs as GlyT-1 Inhibitors*

Some marketed drugs have been reported to show marginal GlyT-1 inhibitory activity and debatably may achieve sufficient total brain concentrations to be pharmacologically relevant.

The "typical" antipsychotics chlorpromazine (**69**) and haloperidol (**70**) (Fig. 20) are reported to have weak GlyT-1 inhibitory activity with IC50s between 9 and 21 μM but were nonselective with respect to GlyT-2 [175]. Clozapine (**71**) an "atypical" antipsychotic ($IC_{50}$ 100 μM [83]) has been suggested to be able to achieve up to a modest but arguably clinically relevant [166] 30% GlyT-1 occupancy (see Sect. 7) at clinical exposures.

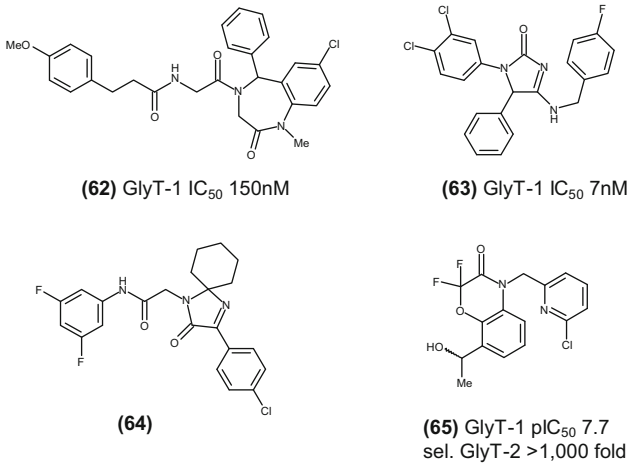



**Fig. 18** Miscellaneous heterocycles

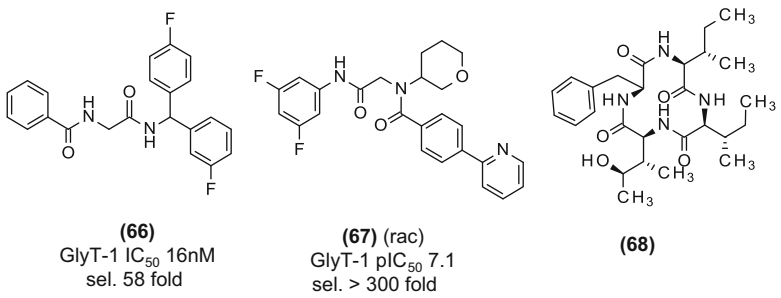



**Fig. 19** Linear amides – Roche and GSK and cyclic tetrapeptides

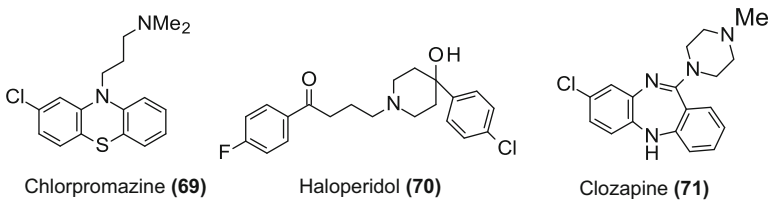
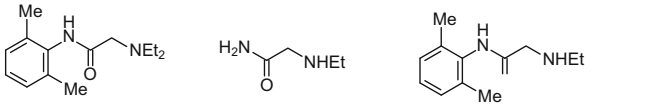



**Fig. 20** Marketed antipsychotics as GlyT-1 inhibitors

From the anaesthetic lidocaine (**72**) (Fig. 21), the metabolite *N*-ethylglycine (**73**) was found to be a GlyT-1 substrate, while monoethylglycinexylidide (**74**), also a lidocaine metabolite, was shown to inhibit GlyT-1 activity both in primary astrocytes and in GlyT-1-expressing *Xenopus laevis* oocytes [176]. While these effects appear modest (i.e. μM), a cocktail of lidocaine (**72**) and its metabolites at clinically relevant concentrations gave comparable inhibition of Gly uptake in vitro to that achieved by NFPS (**3**) at 200 nM.




Lidocaine **(72)** N-Ethylglycine **(73)** Monoethylglycinexylidide **(74)**

**Fig. 21** Anaesthetic metabolites as GlyT-1 inhibitors

**Table 6** Calculated physicochemical properties of clinical candidates

| Clinical compounds | Mol (Wt) | $pIC_{50}$ | cLog*P* | cLog$D_{7.4}$ | LLE[a] | PSA $A^2$ |
|---|---|---|---|---|---|---|
| Sarcosine analogues | | | | | | |
| Alx5407 (**4**) | 393 | 8.5 | 4.95[b] | 2.21 | 3.55 | 54 |
| R231857 (**14**) | 353 | | 3.92[b] | 1.20 | | 58 |
| R213129 (**13**) | 371 | | 4.70[b] | 1.96 | | 58 |
| Org25935 (**16**) | 339 | | 3.80[b] | 1.07 | | 54 |
| AMG747 (**15**) | 378 | 7.2 | 3.90[b] | 1.15 | 3.3 | 48 |
| Basic inhibitors | | | | | | |
| GSK1018921 (**23**) | 488 | | 5.55 | 4.26 | | 43 |
| SSR504734 (**18**) | 397 | 7.8 | 4.86 | 2.97 | 2.94 | 46 |
| PF3463275 (**32**) | 363 | 7.9 | 1.87 | 0.76 | 6.03 | 43 |
| Nonbasic inhibitors | | | | | | |
| Bitopertin (**58**) | 543 | 7.5 | 3.46 | 3.46 | 4.04 | 80 |
| DCCCyB (**48**) | 494 | 7.5 | 4.53 | 4.53 | 2.97 | 63 |
| ASP2535 (**54**) | 382 | 7.1 | 4.01 | 4.01 | 3.09 | 83 |

[a]*LLE* lipophilic ligand efficiency $pIC_{50}$−cLog*D*, all calculations using the ChemAxon calculator plug-in
[b]cLog*P* nonionic species

## *4.5 Physicochemical Properties of Clinical GlyT-1 Inhibitors*

Despite wide structural diversity and substantial lead optimisation, clinical-stage GlyT-1 inhibitors are often showing somewhat unfavourable physicochemical properties (Table 6). This is perhaps reflected in the multiple developability challenges that have had to be overcome to identify these clinical-stage compounds. Reducing lipophilicity has often been hampered by the presence of lipophilic-substituted benzamides important for target affinity and to achieve effective CNS exposures/avoiding efflux transporter activity. Also it has been suggested that higher lipophilicity may be influencing kinetics at the target and in particular target off-rates (see Sect. 5); however, data is too limited to draw definitive conclusions at this stage.

**Table 7** Compilation of structures with their target kinetics

| Compound | Clinical analogue | Gly Comp.[a] | $Na^+$ dependent | Reference |
|---|---|---|---|---|
| Sarcosine (**2**) | Sarcosine (**2**) | C | Yes | Herdon et al. [148] |
| NFPS (**3**) | Org25935 (**16**) | NC | No | Herdon et al. [148] |
| Org25935 (**16**) | Alx5407 (**4**)<br>R231857 (**14**)<br>R213129 (**13**)<br>AMG747 (**15**) | | | Mallorga et al. [72] |
| GSK931145 (**22**) | GSK1018921 (**23**) | C | No | Herdon et al. [148] |
| SSR504734 (**18**) | SSR504734 (**18**)<br>SSR103800 | C | | Deportère et al. [110] |
| SB733993 (**44**) | – | C | Yes | Herdon et al. [148] |
| ACPPB (**46**) | DCCCyB (**48**) | C | Yes | Lindsley et al. [149] |
| (**55**) | Bitopertin (**58**) | NC | | Alberati et al. [165] |
| RO5013853 (**60**) | | | | |
| (**49**) | – | C | | Lindsley et al. [149] |
| (**37**) | – | C | | Mhyre et al. [136] |
| Clozapine (**71**) | Clozapine (**71**) | NC | No | Williams et al. [175] |

[a]Gly Comp., competitive with glycine (C) or non-competitive (NC)

## 5 Target Kinetics

Kinetics of inhibition of GlyT-1 are complex with possible differences in competitiveness with endogenous ligand and dependencies on $Na^+$ and $Cl^-$ concentrations coupled with differences in $k_{off}$. These differences for selected inhibitors are summarised with the relevant tool compound and the structurally closest clinical compound (Table 7). Less clear are the pharmacological consequences of these variations. The clinically most advanced GlyT-1 inhibitor bitopertin (**58**) is non-competitive with respect to Gly and competitive with respect to the sarcosine analogue [$^3$H]ORG24598 (**6**), However, other non-competitive and competitive compounds alike have all been terminated at the early clinical stages of development. Although reasons for the various terminations are largely unknown, it would appear that simple competitive vs. non-competitive pharmacology is not a differentiator with respect to clinical progression at least.

While the competitive vs. non-competitive nature of drug target interaction has not been directly implicated in issues with compounds, it has been suggested [177] that GlyT-1 inhibitors with a short residence time at the target are less likely to induce the unwanted hyperlocomotion effects which have been observed in preclinical species (Table 8). Interestingly from the limited available data, reducing lipophilicity tends to give faster $K_{off}$-rates which may be something which can be optimised in future pharmacophore-based virtual screening campaigns.

**Table 8** Summary of effects of target kinetics on hyperlocomotion and pre-pulse inhibition

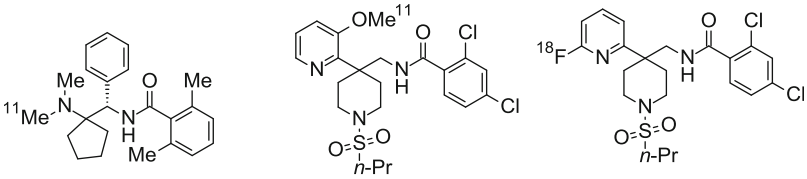
| Compound | $K_i$ nM | Functional $k_{off\ min}$ | Comp/non-comp | OP[a] | cLog*P* (cLog*D*)[b] |
|---|---|---|---|---|---|
| Sarcosine (**2**) | 39,000 | <10 | C | N | −0.8 (−2.8) |
| ALX-5407(**4**) | 0.9 | 294 | NC | Y | 4.95 (2.2) |
| ACPPB (**46**) | 3.8 | 103 | C | Y | 4.2 |
| (**55**) | 25 | <10 | NC | N | 2.5 (2.5) |
| SB733933 (**44**) | 2 | 5[c] | C | – | 3.0 (2.9) |
| GSK931145 (**22**) | 2 | 40[c] | C | – | 5.3 (3.6) |

Data from [148, 177]

[a]OP – hyperlocomotion – obstinate progression observed at doses efficacious in increasing prepulse inhibition

[b]cLog*P*/Log*D* calculated using ChemAxon calculator plug-in

[c]$K_{off}$




[11C]GSK931145 **(22)** [11C]CMPyPB **(75)** [18F]MK-6577 **(76)**

**Fig. 22** Early GlyT-1 PET ligands – GSK and Merck

## 6 Positron Emission Tomography (PET) Ligands

One feature of the development of clinical GlyT-1 inhibitors has been the parallel development of several PET ligands. This has been of particular utility as, with all targets, there is the importance of demonstrating target engagement vs. exposure relationships, but of particular concern for GlyT-1 is the need to more fully understand the occupancy required for efficacy vs. potential adverse effects/tolerability.

Of particular advantage for GlyT-1 is the high Bmax in the cortex, with one estimate of 3,000 fmol/mg protein in rat cortical membranes [148] which reduces the necessity for very high affinity ligands.

Synthesis of the sarcosine-based [11C]NFPS (**3**) has been reported [178], but characterisation data is sparse, probably reflecting slow brain uptake with this class of inhibitor. However, several non-sarcosine-derived ligands have been reported.

### *6.1 [11C]GSK931145*

[11C]GSK931145 (**22**) (Fig. 22) was selected based on brain penetration, heterogeneous distribution consistent with known GlyT-1 distribution and the level of specific binding as determined in the pig [179, 180]. Biomathematical modelling also identified [11C]GSK931145 (**22**) as a preferred ligand from a set of four

**Table 9** Comparison of PET ligand tracer characteristics

| Compound | $hIC_{50}$ (nM) | Species[a] | $K_1$[b] | $V_T$ thal | $V_T$ pons | $V_T$ cereb | BP thal | BP pons | BP cereb |
|---|---|---|---|---|---|---|---|---|---|
| GSK931145 (**22**) | 3 | p | 0.07 | 4.8 | | 3.46 | 1.71 | | 0.97 |
| | | bab | 0.13 | 4.55 | | 4.34 | 2.83 | | 2.53 |
| | | h | 0.03 | 0.75 | | 0.7 | 1.74 | | 1.55 |
| MK-6577 (**76**) | 2 | rh | | 4.9 | 6.79 | 6.53 | | | |
| | | h | | 5 | | 6 | | | |
| RO5013853 (**60**)[c] | 1.7 ($K_d$) | bab | | 1.66 | 1.80 | 1.36 | 1.48 | 1.78 | 1.01 |
| | | h | 0.04 | ~2.1 | 2.59 | 1.86 | 1.43 | 1.93 | 1.26 |

$V_T$ observed distribution vol mL $cm^{-3}$ BPND $V_T$/fp distribution normalised to free tracer concentration

[a]species: *p* pig, *bab* baboon, *h* human, *rh* rhesus

[b]$K_1$ tracer delivery mL $cm^{-3}$ $min^{-1}$

[c]Data from two-tissue 5-parameter model

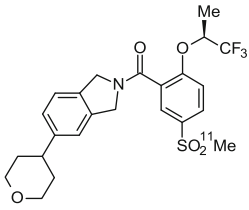
analogues [181]. [$^{11}$C]GSK931145 (**22**) has been dosed to baboons and to humans; the limiting organ with highest radiation absorbed dose was the liver in both species [182, 183].

In a study of 13 healthy volunteers, radioactivity was taken up rapidly into the cerebellum, brainstem and thalamus which was similar to that seen previously in the baboon [184]. Plasma $EC_{50}$ for target occupancy by GSK1018921 (**23**) in the baboon and human were 22.5 and 45.7 ng/mL, respectively. One caveat to the use of [$^{11}$C]GSK931145 (**22**) is that it shows unusually large differences in plasma protein binding across species [184] with 8% fraction unbound in primate but only 0.8% fraction unbound in human which translates to a reduced tracer delivery $K_1$ and reduced $V_T$ of the ligand in humans (Table 9). Furthermore, test/retest reproducibility was poor with [$^{11}$C]GSK931145 (**22**).

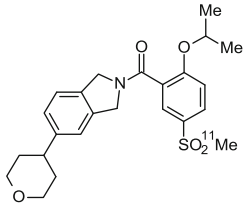
## 6.2 *Disubstituted Piperidine Sulphonamides [$^{11}$C]CMPyPB and [$^{18}$F]MK-6577*

Both [$^{11}$C]CMPyPB (**75**) and [$^{18}$F]MK-6577 ([$^{18}$F]CFpyPB) (**76**) [185] (Fig. 22) showed good uptake in rhesus monkey brain with, as expected, highest uptake in the pons, thalamus and cerebellum and lowest uptake in the striatum and grey matter of the cerebral cortex. However, [$^{18}$F]MK-6577 (**76**) showed higher brain uptake than [$^{11}$C]CMPyPB (**75**) (1.5 vs. 1.0 SUV in the pons) and a higher ratio of tracer uptake in the pons relative to the striatum (2.2 vs. 1.6). In both rhesus monkey and human brain slices, [$^{18}$F]MK-6577 (**76**) showed high binding to cerebellum, thalamus and white matter of the frontal cortex with low binding in the caudate and grey matter of the frontal cortex. Two more polar metabolites of [$^{18}$F]MK-6577 (**76**) were observed in the rhesus monkey with 68% of radioactivity due to parent 90 min post dose.

**Fig. 23** Roche PET ligands




[$^{11}$C]RO5013853 **(60)**




[$^{11}$C]RO5013852 **(61)**

Detailed in vivo kinetics have been established [186] in the rhesus monkey for [$^{18}$F]MK-6577 (**76**). Human studies have been undertaken with [$^{18}$F]MK-6577 (**76**) ([$^{18}$F]CFpyPB [187]) revealing highest exposures in the lower intestine, ovaries and liver. Rapid brain penetration was achieved with the highest uptake in brainstem ($V_T = 7$) with cerebellum, thalamus and midbrain also showing good exposure. As expected low binding was seen in cortical grey matter ($V_T = 2$)

In a head-to-head comparison of [$^{11}$C]GSK931145 (**22**) and [$^{18}$F]MK-6577 (**76**) in anaesthetised baboons [188], [$^{18}$F]MK-6577 (**76**) gave more reliable measurement of binding parameters. It showed higher baboon plasma free fraction 12.8 vs. 5.6% for [$^{11}$C]GSK931145 (**22**), higher brain uptake SUV ~4 vs. ~2 and faster kinetics. Mean distribution volumes were at least twofold higher in relevant brain regions for [$^{18}$F]MK-6577 (**76**) vs. [$^{11}$C]GSK931145 (**22**).

## *6.3 Benzoylindolines [$^{11}$C]RO5013853 and [$^{11}$C]RO5013852*

Roche have disclosed two [$^{11}$C] PET ligands, [$^{11}$C]RO5013853 (**60**) and [$^{11}$C] RO5013852 (**61**) (Fig. 23), which have been evaluated in baboons [189] and the latter in humans [190]. In the baboon, tracer uptake of both compounds was rapid and high in the thalamus, cerebellum and pons with the lowest level seen in the occipital cortex which was used as the reference brain region; kinetics appeared to be reversible. Pretreatment with unlabelled RO5013853 (**60**) or bitopertin (**58**) almost completely blocked binding of both ligands. In the baboon [$^{11}$C] RO5013853 (**60**) did show slightly higher retention and slower clearance and demonstrated higher contrast between regions with high and low levels of GlyT-1 than those seen with [$^{11}$C]RO5013852. [$^{11}$C]RO5013853 (**60**) was 30–35% metabolised in the baboon over the course of a 90 min experiment. [$^{11}$C] RO5013853 (**60**) was safe and well tolerated in humans [190, 191]. Using a two-tissue 5-parameter model, as expected, volumes of distribution were higher in the cerebellum, pons and thalamus (1.99–2.59 mL/mL) and lower in putamen, caudate and cortical areas (0.86–1.13 mL/mL) with test/retest showing good reproducibility with $< 10\%$ difference between scans. As in the baboon bitopertin (**58**) effectively blocked tracer binding.

Comparing [$^{11}$C]RO5013853 (**60**) with [$^{11}$C]GSK931145 (**22**) and [$^{18}$F]MK-6577 (**76**), the authors conclude that while brain uptake of [$^{11}$C]RO5013853 (**60**)

may be lower than the other ligands, this was compensated for by several other factors. These are as follows: (1) a lower dose of radiation was required for [$^{11}$C]RO5013853 (**60**) (0.013 rem/mCi) compared with (0.015 rem/mCi) and (0.095 rem/mCi) for [$^{11}$C]GSK931145 (**22**) and [$^{18}$F]MK-6577 (**76**), respectively; (2) $V_T$ test/retest reliability was substantially greater for [$^{11}$C]RO5013853 (**60**) relative to [$^{11}$C]GSK931145 (**22**); and (3) synthesis of [$^{11}$C]RO5013853 (**60**) via methylation of the sulphinate was efficient [192] and high specific activity could be achieved (> 49GBq/umol) along with high chemical purity (100%).

Cortical GlyT-1 occupancy studies in the baboon, using [$^{11}$C]RO5013853 (**60**) [189] and bitopertin (**58**), gave 50% occupancy at plasma concentrations of 150–300 ng/mL. This level of occupancy is considered to be an efficacious level in a range of preclinical models [165, 193, 194] and subsequently in man [166].

### *6.4 PET Ligand Comparison*

Some of the comparative characteristics of the various PET ligands have been summarised in Table 9.

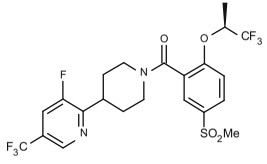
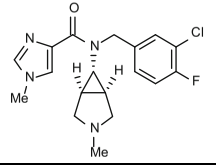
## 7 Clinical Evaluation of GlyT-1 Modulators

A number of synthetic GlyT-1 inhibitors have been assessed in schizophrenic patients (Table 10), but Roche's bitopertin (**58**) (also known as RG1678 or RO4917838) is the most advanced in development.

### *7.1 Bitopertin*

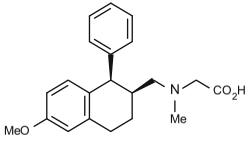
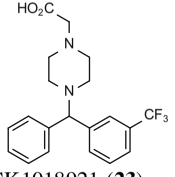
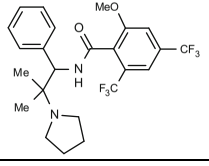
In a proof-of-mechanism study in healthy volunteers, once-daily oral doses of 3–60 mg bitopertin (**58**) for 10 days resulted in a dose-dependent increase in CSF Gly levels [195]. Steady state (10–12 days dosing) transporter occupancy was also evaluated using PET and [$^{11}$C]RO5013853 (**60**) in healthy male volunteers [166]. At steady state, occupancy amounted to 30, 40, 60, 74 and 85% after 5, 15, 30, 60 and 175 mg doses, respectively; Emax was 92%, with an ED50 of 15 mg which equated to a plasma EC50 of 190 ng/mL. Subsequently, a 320 patient phase II proof-of-concept study (NCT0616798) evaluated the efficacy of bitopertin (**58**) (10, 30 or 60 mg daily) in the treatment of negative symptoms in patients with schizophrenia whose symptoms were stabilised by an antipsychotic medication. The molecule was well tolerated at all doses. Improvements were seen in negative symptoms and patients' personal and social performance, reaching statistical significance on primary and secondary end points at the 10 mg dose group; interestingly, in comparison efficacy was reduced at the 30 mg dose and absent in the group receiving 60 mg [167]. It would therefore appear that the mechanism seems to have therapeutically relevant efficacy at transporter occupancy levels as low as 30–40%, with the caveat that this is extrapolated from the earlier study. As mentioned

**Table 10** Compounds with reported clinical trial data

| Compound | Study | Dosing regimen | Measures and outcome | Reference/sponsor |
|---|---|---|---|---|
| Bitopertin (**58**) (aka RO4917838 or RG1678)  | **Phase III** (ongoing)<br>Evaluating the efficacy and safety of adjunctive bitopertin (58) | 5, 10 or 20 mg daily, 52 weeks followed by an optional 3 year | Efficacy and safety of bitopertin (58) in antipsychotic – stabilised patients with persistent, predominant negative symptoms of schizophrenia (ongoing) | NCT01192867, NCT01192906, NCT01192880, NCT01235520, NCT01235559<br>Hoffmann-La Roche |
| | Evaluated the efficacy of adjunctive bitopertin (58) | 10, 30 or 60 mg daily; 3 months | Efficacy and safety of bitopertin (58) in antipsychotic – stabilised patients with persistent, predominant negative symptoms of schizophrenia<br>Efficacy consistently improved at 10 mg, to a lesser extent in the 30 mg and no efficacy in the 60 mg dose group | NCT00616798<br>Hoffmann-La Roche |
| | **Phase II** (ongoing)<br>Effect of adjunctive bitopertin (58) | 10 mg once daily; 6 weeks | Changes in biomarkers of cognitive dysfunction in schizophrenia patients stabilised on antipsychotic medication (study ongoing) | NCT01116830<br>Hoffmann-La Roche |
| Sarcosine (**2**)  | **Phase II** (ongoing)<br>Evaluating the efficacy of adjunctive sarcosine (2) | 2 g once daily; 6 months | Positive and negative symptom, quality of life and cognitive and sexual function measures in patients with schizophrenia stabilised on antipsychotic medication (ongoing) | NCT01503359<br>Medical University of Lodz |
| | **Phase II** (ongoing)<br>Evaluating the efficacy and safety of adjunctive sarcosine (2) | 2 g once daily; 12 weeks | Positive, negative and cognitive symptom measures in schizophrenia patients stabilised on antipsychotic medication (ongoing) | NCT01047592<br>Chang-Hua Hospital |
| PF03463275 (**32**)  | **Phase II**<br>Effects of adjunctive PF03463275 | 30 mg twice daily; 12 weeks | Add-on therapy in outpatients with persistent negative symptoms of schizophrenia (study terminated September 2010; reasons unknown) | NCT00977522<br>Pfizer |

(continued)

**Table 10** (continued)

| Compound | Study | Dosing regimen | Measures and outcome | Reference/sponsor |
|---|---|---|---|---|
| Org25935 (**16**) (aka SCH 900435)  | Phase II | 4–16 mg twice daily; 12 weeks | Effects on negative symptoms in schizophrenia patients stabilised on second generation antipsychotics (completed 2008; no data reported) | NCT00725075 |
| | Effects of adjunctive Org25935 (16) | | Study was withdrawn prior to recruitment (reasons unknown) | NCT00988728 Merck Sharp & Dohme |
| AMG747 (**15**)  | Phase II Effects of adjunctive AMG747 | 3 doses (not specified) once daily; 12 weeks | Effects on negative symptoms in schizophrenia patients stabilised on antipsychotics (study terminated June 2013) | NCT01568229 NCT01568216 Amgen |
| GSK1018921 (**23**)  | Phase I | Not specified | Assess safety, tolerability, pharmacokinetics, pharmacodynamics in healthy volunteers and patients with schizophrenia and to evaluate its effect on PK of midazolam (study terminated June 2009) | NCT00929370 GlaxoSmithKline |

previously these occupancies appear to be equivalent to those which have been shown to be efficacious in preclinical models.

A subsequent study has been carried out in patients experiencing acute exacerbations of schizophrenic symptoms (NCT01234779). Here 10 and 30 mg doses were dosed for 4 weeks (followed by a 4-week follow-up) and compared to the positive comparator, olanzapine (15 mg). Positive and negative PANNS scores were used as the primary end point. This study has completed but no data have been released to date (as of December 2013). However, several phase III studies have commenced: NCT01192867, NCT01192906 and NCT01192880 are phase III, 24-week, double-blind placebo-controlled studies to evaluate efficacy and safety of RO4917838 in stable patients with persistent, predominant negative symptoms of schizophrenia treated with antipsychotics, followed by a 28-week, double-blind treatment period; NCT01235520 and NCT01235559 are phase III, 12-week placebo-controlled studies to evaluate the efficacy and safety of bitopertin (**58**) in patients with suboptimally controlled (with antipsychotics) symptoms of schizophrenia followed by a 40-week double-blind, placebo-controlled treatment period. No dose information is currently available and none of these studies appear to have a positive comparator component. Early phase III studies are reporting out as this document is completing; unfortunately initial results have not proved encouraging [196]. All studies will complete towards the end of 2014/early 2015 with full data expected in 2015.

## *7.2 Sarcosine*

Interestingly, based on its likely very poor pharmacokinetics/CNS penetration and the subsequent need for very high doses, the only other molecule currently undergoing clinical evaluation is sarcosine (**2**). Two separate studies are evaluating 2 g doses for 12 weeks (NCT01503359) and 6 months (NCT01047592) in antipsychotic-stabilised schizophrenic patients. Positive and negative symptom, quality of life and cognitive and sexual function measures are all being used as assessment end points.

## *7.3 Other GlyT-1 Inhibitors*

A number of other small molecule GlyT-1 inhibitors have been assessed in the clinic for schizophrenia (Table 10) but have all generally failed to progress beyond phase I. On the whole the reasons for this have not been released, but one may speculate that this is due to adverse events/tolerability of the compounds.

### 7.3.1 Org25935 (SCH900435)

Early stage clinical evaluation of Org25935 (**16**) (0.5–30 mg) was examined for its observable central nervous system effects and pharmacokinetic profile in healthy

male volunteers [197]. Pharmacokinetics were dose-linear over the dose range studied and peak concentrations of Org25935 (**16**) (1,170 ng/mL at 30 mg) were reached between 30 and 50 min after dosing. EEG measures demonstrated small statistically significant reductions in alpha2 power spectra (10.5–12.5 Hz) suggestive of central penetration and pharmacodynamic activity. The compound was generally well tolerated, although some increases in dizziness were reported and again dose-related visual disturbances were clearly evident.

Org25935 (**16**) has been assessed for its effects on ketamine-induced schizophrenia-like psychotic symptoms and perceptual alterations in 12 healthy male subjects [198]. Ketamine produced behavioural, subjective and cognitive effects consistent with its NMDA receptor antagonist activity. Org 25935 (**16**) reduced the ketamine-induced increases in psychosis (PANSS positive and negative) and perceptual alterations (Clinician Administered Dissociative Symptoms Scale (CADSS)). None of the behavioural effects of ketamine were increased/augmented by Org25935 (**16**). These data fundamentally support the mechanism of action of GlyT-1 and demonstrate the interaction at the level of the NMDA receptor in human subjects.

In addition to its assessment in schizophrenia, Org25935 (**16**) was also assessed for its efficacy in panic disorder as an augmentation strategy to cognitive-behavioural therapy (CBT; NCT00725725 [199]). Patients ($n = 40$) diagnosed (DSM-IV) with panic disorder receive either a dose of Org 25935 (4 or 12 mg) or placebo 2 h prior to an assessment. The primary end point was symptomatic change as measured by the Panic Disorder Severity Scale (PDSS) vs. CBT alone. Although mean PDSS total scores did decrease significantly from baseline, no statistically significant benefit vs. placebo was observed for either dose of Org 25935 (**16**). Org 25935 (**16**) showed no major safety issues at either dose but was much better tolerated at the 4 mg cf. 12 mg.

### 7.3.2 R213129 and R231857

R213129 (**13**) and R231857 (**14**) have been assessed for their effects on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. Despite scopolamine producing the predicted and reproducible anticholinergic CNS impairments, R231857 lacked any consistent dose-related effects, probably due to a low CNS penetration and exposure at the doses tested [97, 98]. Both compounds are believed to have been discontinued [67].

### 7.3.3 GSK1018921

A single oral dose PhI study with GSK1018921 (**23**) (0.5–280 mg) showed a dose proportional pharmacokinetic profile and a dose-dependent increases in reported frequency of dizziness format doses from 70 to 280 mg. Despite this the authors predicted that receptor occupancy up to 80% would be well tolerated [200].

## 8 The Safety vs. Efficacy Dilemma

GlyT-1 knockout animals provided some of the early indications that this target may have some safety concerns. Knockout animals showed deficits in locomotor behaviour and respiratory function prior to postnatal death [201]. Heterozygote animals are however viable and show the characteristic profile [201] seen with most of the small molecule inhibitors describe here. Although the reported adverse events with higher doses of GlyT-1 inhibitors vary across research groups and compounds, they generally agree with the findings in the knockout animals, i.e. behavioural disturbances, locomotor deficits and ultimately respiratory issues [61, 74, 177].

Recent preclinical and clinical assessments of the efficacy vs. occupancy relationship has revealed that low levels of transporter occupancy (10–40%) are actually required to gain efficacy both in preclinical models ([106] and in the Roche clinical studies [166, 167]. Furthermore, high occupancy levels (>50%) appear to be detrimental to efficacy both clinically [166, 167] and across a wide range of preclinical models of function and behaviour [106, 162, 165].

Based on the NMDA receptor hypofunction hypothesis of schizophrenia, it is presumed that blockade of GlyT-1 transport in the forebrain, and perhaps more specifically prefrontal regions, results in enhanced NMDA function and hence efficacy. However GlyT-1 is expressed across the brain and particularly in the hindbrain regions, such as the brainstem and cerebellum. Here NMDA receptor function is more restricted and the glycinergic system prevails; thus, concurrent blockade of GlyT-1 here will increase glycinergic overflow onto inhibitory strychnine-sensitive GlyRs. This hypothesis is partially substantiated by the observations that the respiratory depression seen in the knockout animals could be attenuated by strychnine. Perry et al. [74] demonstrated concurrent elevations in Gly in the cerebellum of animals treated with NFPS (**3**) (Fig. 3) and with LY2365109 (**9**) (Fig. 6). These increases appeared to be of a greater magnitude than those seen in the PFC and correlated with the onset of the locomotor and respiratory side effects. Again locomotor effects of LY2365109 (**9**) were attenuated by strychnine [74]. Behavioural effects were observed including hunched back posture and a tendency to walk on their toes, such that animals were unable to perform on the rotarod and showed laboured, shallow breathing, lateral recumbency, tremors, chromodacryorrhoea, hypothermia and tachycardia [74]. Interestingly, Kalinichev et al. [147] reported anticonvulsant activity of a range of GlyT-1 inhibitors in MEST. It is somewhat counterintuitive that this effect is mediated by forebrain NMDA function and doses which produced robust efficacy were on the high side. Thus, these anticonvulsant effects may also be mediated via the glycinergic receptor system.

A further potential issue is the now multiple reports of visual disturbances with different GlyT-1 inhibitors in clinical trials [197, 200]. Similar effects have also been observed preclinically using PF-03463275 (1, 3 or 10 mg/kg s.c) which produced significant alterations in the electroretinogram of albino rats [202]. Gly is certainly one of the essential neurotransmitters which appear to modulate visual

signals in the retina [203]. It is not clear if GlyT-1 per se is the modulator of retinal Gly levels, but the dose ranges and time course of effects in the two clinical studies are definitely commensurate with an acute pharmacological effect [197, 200]. Thus, this may be another safety concern which needs consideration for this target, although to date no visual disturbances have been reported with bitopertin (**58**). Liem-Moolenaar et al. [197] did suggest early evidence of tolerance to these visual disturbances, so perhaps this may be the reason.

## 9 Issues and Challenges in Inventing Clinical GlyT-1 Inhibitors

From the lack of successful clinical outcomes with GlyT-1 inhibitors, it is clear that potential mechanistic liabilities are complicating the development of molecules targeting this approach. However, clearly the phase II clinical observations with the Roche compound have suggested that a therapeutic window is achievable and therapeutic benefit can be delivered, although within a relatively narrow dose range. Why this molecule has been able to achieve this when so many of the others have failed is not clear, perhaps the differences in chemotype have resulted in subtle differences in binding kinetics vs. other molecules? The community obviously hopes for positive phase III data with bitopertin (**58**) and a novel therapeutic for schizophrenic suffers; however, we should be cognisant that efficacy and long-term tolerability have not yet been proven. Early indications released during the writing of this chapter are unfortunately not encouraging.

The complexity of target kinetics has until recently hampered the successful use of rational or pharmacophore-based design strategies, although there are some examples (Sect. 4). The increasing availability of structural information for SLC6 transporters may suggest that alternative routes to defining new chemotypes are available, but caveats still remain with respect to the interpretation of these (Sect. 3.2).

The relatively high lipophilicity of many clinical agents and frequent reports of efflux liabilities reflects the difficulty in achieving a balance between effective CNS exposure and manageable physicochemical properties. It has been noted [177] that functional $K_{off}$ of some of these inhibitors may be able to limit at least the motor function deficits seen with GlyT-1 inhibitors and as such may be a strategy to enhance the therapeutic window, a hypothesis yet to be proven. If it is confirmed that target off-rates are an important indicator of clinical efficacy and safety, then there will be a need to rationally design compounds with more rapid $K_{off}$-rates. Reducing lipophilicity may be a useful guide (Sect. 5), but again this awaits confirmation.

Finally, both preclinical and now clinical data are suggesting that the level of transporter occupancy required to achieve efficacy is relatively low. However, the apparent bell-shaped dose relationships in efficacy and potential adverse events suggest that the optimal pharmacokinetic/occupancy level is within a quite narrow

range. This puts further constraints on defining an acceptable pharmacokinetic profile with a predictable low peak-trough inhibitor ratio or that appropriate controlled release formulations may be required.

There is now an extensive amount of information available to help the medicinal chemist to design new GlyT-1 inhibitors. If close attention is paid to physicochemical properties, target kinetics and pharmacokinetic profile, there is still scope to potentially differentiate the next generation of GlyT-1 inhibitors from existing compounds. However, the concern must now be that with the extensive effort already dedicated to this target and the paucity of clinical success to date that, despite the major unmet medical need in schizophrenia, it may be difficult for the community to justify significant new research investment in this target.

## References

1. Lahti AC, Weiler MA, Tamara Michaelidis BA et al (2001) Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacol 5:455–467
2. Javitt DC (1987) Negative schizophrenic symptomatology and the PCP (phencyclidine) model of schizophrenia. Hillside J Clin Psychiatry 9:12–35
3. Krystal JH, Karper LP, Seibyl JP 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
4. Harrison PJ, Owen MJ (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361:417–419
5. Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10:40–68
6. Moghaddam B (2003) Bringing order to the glutamate chaos in schizophrenia. Neuron 40: 881–884
7. Hahn CG, Wang HY, Cho DS et al (2006) Altered neuregulin 1-ErbB4 signalling contributes to NMDA receptor hypofunction in schizophrenia. Nat Med 12:824–828
8. Newell KA, Karl T, Huang XF (2013) A neuregulin 1 transmembrane domain mutation causes imbalanced glutamatergic and dopaminergic receptor expression in mice. Neurosci 248:670–680
9. Chumakov I, Blumenfeld M, Guerassimenko O et al (2002) Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci U S A 99:13675–13680
10. Abou Jamra R, Schmael C, Cichon S et al (2006) Genes and Schizophrenia. The G72/G30 gene locus in psychiatric disorders: a challenge to diagnostic boundaries? Schizophr Bull 32: 599–608
11. Detera-Wadleigh SD, McMahon FJ (2006) G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. Biol Psychiatry 60:106–114
12. Burnet PJ, Hutchinson L, von Hesling M et al (2008) Expression of D-serine and glycine transporters in the prefrontal cortex and cerebellum in schizophrenia. Schizophr Res 102: 283–294
13. Verrall L, Walker M, Rawlings N et al (2007) d-Amino acid oxidase and serine racemase in human brain: normal distribution and altered expression in schizophrenia. Eur J Neurosci 26:1657–1669
14. Wolosker H, Blackshaw S, Snyder SH (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission. Proc Natl Acad Sci U S A 96:13409–13414

15. Goltsov AY, Loseva JG, Andreeva TV et al (2006) Polymorphism in the 5′-promoter region of serine racemase gene in schizophrenia. Mol Psychiatry 11:325–326
16. Morita Y, Ujike H, Tanaka Y et al (2007) A genetic variant of the serine racemase gene is associated with schizophrenia. Biol Psychiatry 61:1200–1203
17. Steffek AE, Haroutunian V, Meador-Woodruff JH (2006) Serine racemase protein expression in cortex and hippocampus in schizophrenia. Neuroreport 17:1181–1185
18. Heresco-Levy U, Javitt DC, Ermilov M et al (1996) Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. Br J Psychiatry 169:610–617
19. Heresco-Levy U, Javitt DC, Ermilov M et al (1999) Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry 56:29–36
20. Heresco-Levy U, Javitt DC (2004) Comparative effects of glycine and D-cycloserine on persistent negative symptoms in schizophrenia: a retrospective analysis. Schizophr Res 66: 89–96
21. Javitt DC, Zylberman I, Zukin SR et al (1994) Amelioration of negative symptoms in schizophrenia by glycine. Am J Psychiatry 151:1234–1236
22. Tsai G, Yang P, Chung LC et al (1998) D-serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 44:1081–1089
23. Carone FA, Ganote CE (1975) D-serine nephrotoxicity. The nature of proteinuria, glucosuria, and aminoaciduria in acute tubular necrosis. Arch Pathol 99:658–662
24. Eulenburg V, Armsen W, Betz H et al (2005) Glycine transporters: essential regulators of neurotransmission. Trends Biochem Sci 30:325–333
25. Guastella J, Brecha N, Weigmann C et al (1992) Cloning, expression, and localization of a rat brain high-affinity glycine transporter. Proc Natl Acad Sci U S A 89:7189–7193
26. Liu QR, Nelson H, Mandiyan S, López-Corcuera B et al (1992) Cloning and expression of a glycine transporter from mouse brain. FEBS Lett 305:110–114
27. Dohi T, Morita K, Kitayama T et al (2009) Glycine transporter inhibitors as a novel drug discovery strategy for neuropathic pain. Pharmacol Ther 123:54–79
28. Supplisson S, Roux MJ (2002) Why glycine transporters have different stoichiometries. FEBS Lett 529:93–101
29. Hanley JG, Jones EMC, Moss SJ (2000) GABA receptor ρ1 subunit interacts with a novel splice variant of the glycine transporter, GLYT-1. J Biol Chem 275:840–846
30. Ponce J, Poyatos I, Aragón C et al (1998) Characterization of the 5′region of the rat brain glycine transporter GLYT2 gene: identification of a novel isoform. Neurosci Lett 242:25–28
31. Betz H, Laube B (2006) Glycine receptors: recent insights into their structural organization and functional diversity. J Neurochem 97:1600–1610
32. Gomeza J, Hülsmann S, Ohno K et al (2003) Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. Neuron 40:785–796
33. Gomeza J, Ohno K, Hülsmann S et al (2003) Deletion of the mouse glycine transporter 2 results in a hyperekplexia phenotype and postnatal lethality. Neuron 40:797–806
34. Gabernet L, Pauly-Evers M, Schwerdel C et al (2005) Enhancement of the NMDA receptor function by reduction of glycine transporter-1 expression. Neurosci Lett 373:79–84
35. Gomeza J, Armsen W, Betz H et al (2006) Lessons from the knocked-out glycine transporters. Handb Exp Pharmacol 175:457–483
36. Yee BK, Balic E, Singer P et al (2006) Disruption of glycine transporter 1 restricted to forebrain neurons is associated with a procognitive and antipsychotic phenotypic profile. J Neurosci 26:3169–3181
37. Aroeira RI, Sebastião AM, Valente CA (2013) GlyT1 and GlyT2 in brain astrocytes: expression, distribution and function. Brain Struct Funct 219:817–830
38. Raiteri L, Stigliani S, Usai C et al (2008) Functional expression of release-regulating glycine transporters GLYT1 on GABAergic neurons and GLYT2 on astrocytes in mouse spinal cord. Neurochem Int 52:103–112

39. Kristensen AS, Andersen J, Jørgensen TN et al (2011) SLC6 Neurotransmitter transporters: structure, function, and regulation. Pharmacol Rev 63:585–640
40. Yamashita A, Singh SK, Kawate T et al (2005) Crystal structure of a bacterial homologue of Na+/Cl–dependent neurotransmitter transporters. Nature 437:215–223
41. Penmatsa A, Wang KH, Gouaux E (2013) X-ray structure of dopamine transporter elucidates antidepressant mechanism. Nature 503:85–95
42. Singh SK, Piscitelli CL, Yamashita A et al (2008) A competitive inhibitor traps LeuT in an open-to-out conformation. Science 322:1655–1661
43. Zhou Z, Zhen J, Karpowich NK et al (2009) Antidepressant specificity of serotonin transporter suggested by three LeuT-SSRI structures. Nat Struct Mol Biol 16:652–657
44. Singh SK, Yamashita A, Gouaux E (2007) Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. Nature 448:952–956
45. Zhou Z, Zhen J, Karpowich NK et al (2007) LeuT-desipramine structure suggests how antidepressants human neurotransmitter transporters. Science 317:1390–1393
46. Wang H, Goehring A, Wang KH et al (2013) Structural basis for action by diverse antidepressants on biogenic amine transporters. Nature 503:141–145
47. Ponce J, Biton B, Benavides J et al (2007) Transmembrane domain III plays an important role in ion binding and permeation in the glycine transporter GLYT2. J Biol Chem 275: 13856–13862
48. Harsing LG, Zsilla G, Matyus P et al (2012) Interactions between glycine transporter type 1 (GlyT-1) and some inhibitor molecules - glycine transporter type 1 and its inhibitors (review). Acta Physiol Hung 99(1):1–17
49. Vandenberg RJ, Shaddick K, Ju P (2007) Molecular basis for substrate discrimination by glycine transporters. J Biol Chem 282:14447–14453
50. Scanlon SM, Williams DC, Schloss P (2001) Membrane cholesterol modulates serotonin transporter activity. Biochem 40:10507–10513
51. Hong WC, Amara SG (2010) Membrane cholesterol modulates the outward facing conformation of the dopamine transporter and alters cocaine binding. J Biol Chem 285: 32616–32626
52. Liu X, Mitrovic AD, Vandenberg RJ (2009) Glycine transporter 1 associates with cholesterol-rich membrane raft microdomains. Biochem Biophys Res Commun 384:530–534
53. Núñez E, Alonso-Torres P, Fornés A et al (2008) The neuronal glycine transporter GLYT2 associates with membrane rafts: functional modulation by lipid environment. J Neurochem 105:2080–2090
54. Nunez E, Martinez-Maza R, Geerlings A et al (2005) Transmembrane domains 1 and 3 of the glycine transporter GLYT1 contain structural determinants of *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)-propyl]sarcosine specificity. Neuropharmacol 49:922–934
55. Vandenberg RJ (2006) Mutational analysis of glutamate transporters. Handb Exp Pharmacol 175:113–135
56. Gabrielsen M, Sylte I, Dahl SG et al (2011) A short update on the structure of drug binding sites on neurotransmitter transporters. BMC Res Notes 4(Proc Suppl):559
57. Thomas JR, Gedeon PC, Grant BJ et al (2012) LeuT conformational sampling utilizing accelerated molecular dynamics and principal component analysis. Biophys J 103:L01–L03
58. Bridges TM, Williams R, Lindsley CW (2008) Design of potent GlyT1 inhibitors: *in vitro* and *in vivo* profiles. Curr Opin Mol Ther 10:591–601
59. Cioffi CL, Liu S, Wolf M (2010) In: Recent developments in glycine transporter-1 inhibitors. In: Macor JE (ed) Annual Reports in Medicinal Chemistry, vol 45. Academic Press, pp 19–35
60. Gilfillan R, Kerr J, Walker G et al (2009) Glycine transporters and their inhibitors. In: Napier S, Bingham M (eds) Transporters as targets for drugs, vol 4, Top Med Chem. Springer, Heidelberg, pp 223–248
61. Harsing LG, Juranyi Z, Gacsalyi I et al (2006) Glycine transporter type-1 and its inhibitors. Curr Med Chem 13:1017–1044

62. Harsing LG (2013) An overview on GlyT-1 inhibitors under evaluation for the treatment of schizophrenia. Drugs Future 38:555–568
63. Harvey RJ, Yee BJ (2013) Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. Nat Rev Drug Disc 12:866–885
64. Hashimoto K (2006) Glycine transporter inhibitors as therapeutic agents for schizophrenia. Recent Pat CNS Drug Discov 1:43–53
65. Lechner SM (2006) Glutamate-based therapeutic approaches: inhibitors of glycine transport. Curr Opin Pharmacol 6:75–81
66. Lindsley CW, Wolkenberg SE, Kinney GG (2006) Progress in the preparation and testing of glycine transporter type-1 (GlyT1) inhibitors. Curr Top Med Chem 6:1883–1896
67. Thomsen C (2006) Glycine transporter inhibitors as novel antipsychotics. Drug Discov Today Ther Strateg 3:539–545
68. Wolkenberg SE, Sur C (2010) Recent progress in the discovery of non-sarcosine based GlyT1 inhibitors. Curr Top Med Chem 10:170–186
69. Herdon HJ, Godfrey FM, Brown AM et al (2001) Pharmacological assessment of the role of the glycine transporter GlyT-1 in mediating high-affinity glycine uptake by rat cerebral cortex and cerebellum synaptosomes. Neuropharmacol 41:88–96
70. Atkinson BN, Bell SC, De Vivo M et al (2001) ALX 5407: a potent, selective inhibitor of the hGlyT1 Glycine transporter. Mol Pharmacol 60:1414–1420
71. Brown A, Carlyle I, Clark J et al (2001) Discovery and SAR of Org 24598-a selective glycine uptake inhibitor. Bioorg Med Chem Lett 11:2007–2009
72. Mallorga PJ, Williams JB, Jacobson M et al (2003) Pharmacology and expression analysis of glycine transporter GlyT1 with [$^3$H]-(*N*-[3-(4′-fluorophenyl)-3-(4′phenylphenoxy)propyl]) sarcosine. Neuropharmacol 45:585–593
73. Harsing LG Jr, Gacsalyi I, Szabo G et al (2003) The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav 74:811–825
74. Perry KW, Falcone JF, Fell MJ et al (2008) Neurochemical and behavioral profiling of the selective GlyT1 inhibitors ALX5407 and LY2365109 indicate a preferential action in caudal vs. cortical brain areas. Neuropharmacol 55:743–754
75. Bergeron R, Meyer TM, Coyle JT et al (1998) Modulation of *N*-methyl-D-aspartate receptor function by glycine transport. Proc Natl Acad Sci U S A 95:15730–15734
76. Chen L, Muhlhauser M, Yang CR (2003) Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons *in vitro* and *in vivo*. J Neurophysiol 89: 691–703
77. Kinney GG, Sur C, Burno M et al (2003) The glycine transporter type 1 inhibitor *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine potentiates NMDA receptor-mediated responses *in vivo* and produces an antipsychotic profile in rodent behaviour. J Neurosci 23:7586–7591
78. Manahan-Vaughan D, Wildförster V, Thomsen C (2008) Rescue of hippocampal LTP and learning deficits in a rat model of psychosis by inhibition of glycine transporter-1 (GlyT1). Eur J Neurosci 28:1342–1350
79. Hashimoto K, Fujita Y, Ishima T et al (2008) Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the glycine transporter-1 inhibitor NFPS and D-serine. Eur Neuropsychopharmacol 18:414–421
80. Karasawa J, Hashimoto K, Chaki S (2008) D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. Behav Brain Res 186:78–83
81. Shimazaki T, Kaku A, Chaki S (2010) D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. Psychopharmacol (Berl) 209:263–270
82. Bennett S, Gronier B (2005) Modulation of striatal dopamine release *in vitro* by agonists of the glycine B site of NMDA receptors; interaction with antipsychotics. Eur J Pharmacol 527:52–59

83. Javitt DC, Duncan L, Balla A et al (2005) Inhibition of System A-mediated glycine transport in cortical synaptosomes by therapeutic concentrations of clozapine: implications for mechanisms of action. Mol Psychiatry 10:275–286
84. Javitt DC, Balla A, Burch S et al (2004) Reversal of phencyclidine-induced dopaminergic dysregulation by *N*-methyl-D-aspartate receptor/glycine-site agonists. Neuropsychopharmacol 29:300–307
85. Aubrey KR, Vandenberg RJ (2001) *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl] sarcosine (NFPS) is a selective persistent inhibitor of glycine transport. Br J Pharmacol 134:1429–1436
86. Martina M, Gorfinkel Y, Halman S et al (2004) Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. NPTS analogue CP802,079. J Physiol 557:489–500
87. Papp A, Juranyi Z, Nagymajtenyi L et al (2008) The synaptic and nonsynaptic glycine transporter type-1 inhibitors Org-24461 and NFPS alter single neuron firing rate in the rat dorsal raphe nucleus. Further evidence for a glutamatergic-serotonergic interaction and its role in antipsychotic action. Neurochem Int 52:130–134
88. Nagy K, Marko B, Zsilla G et al (2010) Alterations in brain extracellular dopamine and glycine levels following combined administration of the glycine transporter type-1 inhibitor Org-24461 and risperidone. Neurochem Res 35:2096–2106
89. Liu X, Smith BJ, Chen C et al (2005) Use of a physiologically based pharmacokinetic model to study the time to reach brain equilibrium: an experimental analysis of the role of blood-brain barrier permeability, plasma protein binding, and brain tissue binding. J Pharmacol Exp Ther 313:1254–1262
90. Lowe JA, Drozda SE, Fisher K et al (2003) [$^3$H]-(R)-NPTS, a radioligand for the type 1 glycine transporter. Bioorg Med Chem Lett 13:1291–1292
91. Man T, Milot G, Porter WJ et al (2005) 2-aryloxyethyl glycine derivatives and their use as glycine transport inhibitors Eli Lilly Patent application WO2005/100301-A1, 27 Oct 2005
92. Walter MW, Hoffman BJ, Gordon K et al (2007) Discovery and SAR studies of novel GlyT1 inhibitors. Bioorg Med Chem Lett 17:5233–5238
93. Thomson CG, Duncan K, Fletcher SR et al (2006) Sarcosine based indandione hGlyT1 inhibitors. Bioorg Med Chem Lett 16(5):1388–1391
94. Smith G, Ruhland T, Mikkelsen G et al (2004) The synthesis and SAR of 2-arylsulfanyl-phenyl piperazinyl acetic acids as glyT-1 inhibitors. Bioorg Med Chem Lett 14:4027–4030
95. Smith G, Mikkelsen G, Eskildsen J, Bundgaard C (2006) The synthesis and SAR of 2-arylsulfanylphenyl-1-oxyalkylamino acids as GlyT-1 inhibitors. Bioorg Med Chem Lett 16:3981–3984
96. Egle I, Frey J, Isaac M (2001a) Diaryl-enynes WO 2001/032602-A1, 10 May 2001
97. Liem-Moolenaar M, Zoethout RWM, de Boer P et al (2010) The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. J Psychopharmacol 24:1671–1676
98. Liem-Moolenaar M, Zoethout RW, de Boer P et al (2010) The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. J Psychopharmacol 24:1681–1687
99. Hitchcock S, Amagadzie A, Qian W et al (2008) Glycine transporter-1 inhibitors WO2008/002583-A1, 3 Jan 2008
100. Lidö HH, Stomberg R, Fagerberg A et al (2009) The glycine reuptake inhibitor org 25935 interacts with basal and ethanol-induced dopamine release in rat nucleus accumbens. Alcohol Clin Exp Res 33:1151–1157
101. Andrews N, Ge J, Walker G et al (2007) Effect of the selective glycine re-uptake (GlyT-1) inhibitor Org 25935 on glycine levels in CSF and dialysates. Eur Neuropsychopharmacol 17 (Suppl 4):S497–S498

102. Ge J, Hamilton M, Shahid D et al (2001) The effects of Org 25935 on the extracellular levels of glycine in brain regions of freely moving rats using microdialysis. Br J Pharmacol 133(Proc Suppl):135
103. Jardemark K, Marcus MM, Malmerfelt A et al (2012) Differential effects of AMPA receptor potentiators and glycine reuptake inhibitors on antipsychotic efficacy and prefrontal glutamatergic transmission. Psychopharmacol (Berl) 221:115–131
104. Balla A, Schneider S, Sershen H et al (2012) Effects of novel, high affinity glycine transport inhibitors on frontostriatal dopamine release in a rodent model of schizophrenia. Eur Neuropsychopharmacol 22:902–910
105. Molander A, Söderpalm B (2005) Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. Alcohol Clin Exp Res 29:27–37
106. Eddins D, Hamill TG, Puri V et al (2014) The relationship between glycine transporter 1 occupancy and the effects of the glycine transporter 1 inhibitor RG1678 or ORG25935 on object retrieval performance in scopolamine impaired rhesus monkey. Psychopharmacol (Berl) 231:511–519
107. Dargazanli G, Estenne-Bouhtou G, Magat P et al (2003). Preparation of *N*-[phenyl(piperidin-2-yl)methyl]benzamides as specific inhibitors of glycine transporters GlyT1 and/or GlyT2. Patent WO2003/089411-A1, 30 Oct 2003
108. Zlotinikov E, Wu X-D, Lieberman H et al (2010) Novel polymorphic forms of an azabicyclo-trifluoromethylbenzamide WO 2010/065701-A1, 10 June 2010
109. Darganzali G, Estenne-Bouhtou G, Magat P et al (2005) Use of tricyclic compounds as glycine transport inhibitors WO2005/037783-A228 April 2005
110. Depoortère R, Dargazanli G, Estenne-Bouhtou G et al (2005) Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacol 30:1963–1985
111. Nikiforuk A, Kos T, Rafa D et al (2011) Blockade of glycine transporter 1 by SSR-504734 promotes cognitive flexibility in glycine/NMDA receptor-dependent manner. Neuropharmacol 61:262–267
112. Black MD, Varty GB, Arad M et al (2009) Procognitive and antipsychotic efficacy of glycine transport 1 inhibitors (GlyT1) in acute and neurodevelopmental models of schizophrenia: latent inhibition studies in the rat. Psychopharmacol (Berl) 202:385–396
113. Nishikawa H, Inoue T, Izumi T et al (2010) SSR504734, a glycine transporter-1 inhibitor, attenuates acquisition and expression of contextual conditioned fear in rats. Behav Pharmacol 21:576–579
114. Singer P, Feldon J, Yee BK (2009) The glycine transporter 1 inhibitor SSR504734 enhances working memory performance in a continuous delayed alternation task in C57BL/6 mice. Psychopharmacol (Berl) 202:371–384
115. Boulay D, Pichat P, Dargazanli G et al (2008) Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia. Pharmacol Biochem Behav 91:47–58
116. Boulay D, Bergis O, Avenet P et al (2010) The glycine transporter-1 inhibitor SSR103800 displays a selective and specific antipsychotic-like profile in normal and transgenic mice. Neuropsychopharmacol 35:416–427
117. Dargazanli G, Estenne-Bouhtou G, Medaisko F et al (2005b) Derivatives of *N*-[phenyl (pyrrolidine-2-yl)methyl]benzamide and *N*-[(azepan-2-yl)phenylmethyl]benzamide, preparation method thereof and application of same in therapeutics WO2005/037785-A2, 28 April 2005
118. Dargazanli G, Estenne-Bouhtou G, Medaisko F et al (2008) Derivatives of pyrrolizine, indolizine and quinolizine, preparation thereof and therapeutic use thereof WO2008/037881-A2, 3 April 2008
119. Gentile G, Herdon HJ, Passchier J et al (2007) Radiolabelled ligand for the glycine 1 transporter WO2007/147838-A1, 27 Dec 2007

120. Bradley DM, Branch CL, Chan WN et al (2006) Glycine transport inhibitors WO2006/067423-A1, 29 June 2006
121. Griffini P, James AD, Roberts AD et al (2010) Metabolites in safety testing: issues and approaches to the safety evaluation of human metabolites in a drug that is extensively metabolized. J Drug Metabol Toxicol 1:102
122. Albert JS, Alhambra C, Brugel TA et al (2009) 2-Azabicyclo(2.2.2)octane derivatives as modulators of the glycine transporter-1 receptor WO2009/013535-A1, 29 Jan 2009
123. Albert JS, Alhambra C, Brugel TA (2010a) 2-Azabicyclo[2.2.2]octane compounds and uses thereof WO2010/087761-A1, 5 Aug 2010
124. Albert JS, Alhambra C, Brugel TA et al (2010b) 2-Aza-bicyclo[2.2.1]heptane compounds and uses thereof WO2010/087762-A1, 5 Aug 2010
125. Sekiguchi Y, Okubo T, Shibata T et al (2008) Glycine transporter inhibitor WO2008/018639, 14 Feb 2008
126. Kolczewski S, Pinard E (2011) Amido-tropane derivatives F. Hoffmann-La Roche AG WO2011/161006-A1, 29 Dec 2011
127. Kolczewski S, Pinard E (2011) Quinolizidine and indolizidine derivatives F. Hoffmann-La Roche AG WO2011/161008-A1, 29 Dec 2011
128. Lowe JA, Hou X, Schmidt C et al (2009) The discovery of a structurally novel class of inhibitors of the type 1 glycine transporter. Bioorg Med Chem Lett 19:2974–2976
129. Lowe JA III, DeNinno S, Drozda SE et al (2010) An octahydro-cyclopenta[c]pyrrole series of inhibitors of the type 1 glycine transporter. Bioorg Med Chem Lett 20:907–911
130. Berliner MA, Dubant SPA, Makowski T et al (2011) Use of an iridium-catalyzed redox-neutral alcohol-amine coupling on kilogram scale for the synthesis of a GlyT1 inhibitor. Org Process Res Dev 15:1052–1062
131. Roberts BM, Shaffer CL, Seymour PA et al (2010) Glycine transporter inhibition reverses ketamine-induced working memory deficits. Neuroreport 21:390–394
132. Pfizer Inc. PF-03463275. http://www.ncats.nih.gov/files/PF-03463275.pdf. Accessed 22 Jan 2014
133. Kolczewski S, Pinard E (2011) Tetrahydro-pyran derivatives against neurological illnesses F. Hoffmann-La Roche AG WO2011/095434-A1, 11 Aug 2011
134. Varnes JG, Forst JM, Hoerter TN et al (2010) Identification of *N*-(2-(azepan-1-yl)-2-phenylethyl)-benzenesulfonamides as novel inhibitors of GlyT1. Bioorg Med Chem Lett 20:4878–4881
135. Cioffi CL, Wolf MA, Guzzo PR et al (2013) Design, synthesis, and SAR of *N*-((1-(4-(propylsulfonyl)piperazin-1-yl)cycloalkyl)methyl)benzamide inhibitors of glycine transporter-1. Bioorg Med Chem Lett 23:1257–1261
136. Mhyre AJ, Cioffi CL, Beebe AJ et al (2011) A novel series of GlyT-1 inhibitors for treating schizophrenia: is binding competitively with glycine important? Abstr Soc Neurosci 899:10
137. Pinard E, Ceccarelli SM, Stalder H et al (2006) Discovery of N-(2-aryl-cyclohexyl) substituted spiropiperidines as a novel class of GlyT1 inhibitors. Bioorg Med Chem Lett 16:349–353
138. Alberati D, Ceccarelli SM, Jolidon S et al (2006) Design and synthesis of 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as a novel class of GlyT1 inhibitors: achieving selectivity against the mu opioid and nociceptin/orphanin FQ peptide (NOP) receptors. Bioorg Med Chem Lett 16:4305–4310
139. Alberati D, Hainzl D, Jolidon S et al (2006) Discovery of 4-substituted-8-(2-hydroxy-2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as a novel class of highly selective GlyT1 inhibitors with improved metabolic stability. Bioorg Med Chem Lett 16:4311–4315
140. Alberati D, Hainzl D, Jolidon S et al (2006) 4-Substituted-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as a novel class of highly selective GlyT1 inhibitors with superior pharmacological and pharmacokinetic parameters. Bioorg Med Chem Lett 16:4321–4325

141. Ceccarelli SM, Pinard E, Stalder H et al (2006) Discovery of *N*-(2-hydroxy-2-aryl-cyclohexyl) substituted spiropiperidines as GlyT1 antagonists with improved pharmacological profile. Bioorg Med Chem Lett 16:354–357
142. Nic Dhonnchadha BÁ, Pinard E, Alberati D et al (2012) Inhibiting glycine transporter-1 facilitates cocaine-cue extinction and attenuates reacquisition of cocaine-seeking behavior. Drug Alcohol Depend 122:119–126
143. Amberg W, Ochse M, Lange U et al (2012) Phenalkylamine derivatives, pharmaceutical compositions containing them, and their use in therapy WO2012/020130-A1, 16 Feb 2012
144. Amberg W, Lange U, Pohlki F et al (2013) *N*-substituted aminobenzocycloheptene, aminotetraline, aminoindane and phenalkylamine derivatives, pharmaceutical compositions containing them, and their use in therapy WO2013/072520-A1, 23 May 2013
145. Egle IR, Frey J, Isaac MB et al (2001) Aminopiperidines WO2001/081308-A2, 1 Nov 2001
146. Rahman SS, Coulton S, Herdon HJ et al (2007) 1,3-Diaminopropan-2-ol sulfonamides as potent and selective inhibitors of the glycine transporter type 1. Bioorg Med Chem Lett 17: 1741–1745
147. Kalinichev M, Starr KR, Teague S et al (2010) Glycine transporter 1 (GlyT1) inhibitors exhibit anticonvulsant properties in the rat maximal electroshock threshold (MEST) test. Brain Res 1331:105–113
148. Herdon HJ, Roberts JC, Coulton S et al (2010) Pharmacological characterisation of the GlyT-1 glycine transporter using two novel radioligands. Neuropharmacol 59:558–565
149. Lindsley CW, Conn JP, Williams R et al (2010) Vanderbilt University Alkylsulfonyl-2,3-dihydrospiro[indene-1,4′-piperidine] analogs as glyt1 inhibitors, methods for making same, and use of same in treating psychiatric disorders WO2010/102003-A2, 9 Oct 2010
150. Lindsley CW, Zhao Z, Leister WH et al (2006) Design, synthesis, and *in vivo* efficacy of glycine transporter-1 (GlyT1) inhibitors derived from a series of [4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl benzamides. Chem Med Chem 1:807–811
151. Zhao Z, O'Brien JA, Lemaire W et al (2006) Synthesis and SAR of GlyT1 inhibitors derived from a series of *N*-((4-(morpholine-4-carbonyl)-1-(propylsulfonyl)piperidin-4-yl)methyl) benzamides. Bioorg Med Chem Lett 16:5968–5972
152. Zhao Z, Leister WH, O'Brien JA et al (2009) Discovery of *N*-{[1-(propylsulfonyl)-4-pyridin-2-ylpiperidin-4-yl]methyl}benzamides as novel, selective and potent GlyT1 inhibitors. Bioorg Med Chem Lett 19:1488–1491
153. Blackaby WP, Lewis RT, Thomson JL et al (2010) Identification of an orally bioavailable, potent, and selective inhibitor of GlyT1. ACS Med Chem Lett 1:350–354
154. Thomson JL, Blackaby WP, Jennings AS et al (2009) Optimisation of a series of potent, selective and orally bioavailable GlyT1 inhibitors. Bioorg Med Chem Lett 19:2235–2239
155. Wolkenberg SE, Zhao Z, Wisnoski DD et al (2009) Discovery of GlyT1 inhibitors with improved pharmacokinetic properties. Bioorg Med Chem Lett 19:1492–1495
156. Jones CK, Sheffler DJ, Williams R et al (2014) Novel GlyT1 inhibitor chemotypes by scaffold hopping. Part 1: development of a potent and CNS penetrant [3.1.0]-based lead. Bioorg Med Chem Lett 24:1067
157. Sheffler DJ, Nedelovych MT, Williams R et al (2014) Novel GlyT1 inhibitor chemotypes by scaffold hopping. Part 2: development of a [3.3.0]-based series and other piperidine bioisosteres. Bioorg Med Chem Lett 24:1062–1066
158. Sugane T, Tobe T, Hamaguchi W et al (2011) Synthesis and biological evaluation of 3-biphenyl-4-yl-4-phenyl-4H-1,2,4-triazoles as novel glycine transporter 1 inhibitors. J Med Chem 54:387–391
159. Sugane T, Tobe T, Hamaguchi W et al (2012) Synthesis and biological evaluation of (4*H*-1,2,4-triazol-4-yl)isoquinoline derivatives as selective glycine transporter 1 inhibitors. Bioorg Med Chem 20:34–41
160. Sugane T, Tobe T, Hamaguchi W et al (2013) Atropisomeric 4-phenyl-4*H*-1,2,4-triazoles as selective glycine transporter 1 inhibitors. J Med Chem 56:5744–5756

161. Sugane T, Hamada N, Tobe T et al (2012) Practical and efficient synthesis of the (*R*)-atropisomer of a 4-phenyl 1,2,4-triazole derivative as a selective GlyT1 inhibitor. Tetrahedron Asymmetry 23:1528–1533
162. Harada K, Nakato K, Yarimizu J et al (2012) A novel glycine transporter-1 (GlyT1) inhibitor, ASP2535 (4-[3-isopropyl-5-(6-phenyl-3-pyridyl)-4H-1,2,4-triazol-4-yl]-2,1,3-benzoxadiazole), improves cognition in animal models of cognitive impairment in schizophrenia and Alzheimer's disease. Eur J Pharmacol 685:59–69
163. Pinard E, Alberati D, Borroni E et al (2008) Discovery of benzoylpiperazines as a novel class of potent and selective GlyT1 inhibitors. Bioorg Med Chem Lett 18:5134–5139
164. Pinard E, Alanine A, Alberati D et al (2010) Selective GlyT1 inhibitors: Discovery of [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a promising novel medicine to treat schizophrenia. J Med Chem 53:4603–4614
165. Alberati D, Moreau JL, Lengyel J et al (2012) Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. Neuropharmacol 62:1152–1161
166. Martin-Facklam M, Pizzagalli F, Zhou Y et al (2013) Glycine transporter type 1 occupancy by bitopertin: a positron emission tomography study in healthy volunteers. Neuropsychopharmacol 38:504–512
167. Umbricht D, Yoo K, Youssef E et al (2010) Glycine transporter type 1 (GLYT1) inhibitor RG1678: positive results of the proof-of-concept study for the treatment of negative symptoms in schizophrenia. Neuropsychopharmacol 35:S320–S321
168. Pinard E, Alberati D, Bender M et al (2010) Discovery of benzoylisoindolines as a novel class of potent, selective and orally active GlyT1 inhibitors. Bioorg Med Chem Lett 20:6960–6965
169. Jolidon S, Alberati D, Dowle A et al (2008) Design, synthesis and structure-activity relationship of simple bis-amides as potent inhibitors of GlyT1. Bioorg Med Chem Lett 18:5533–5536
170. Jolidon S, Narquizian R, Norcross R et al (2007) 4-amino-1,5-substituted 1,5-dihydro-imidazol-2-ones Hoffmann-La Roche Ag patent application WO2007/101802-A1, 13 Sept 2007
171. Coulton S, Marshall H, Nash DJ et al (2007) *N*-phenyl-2-0x0-1,4-diazaspiro [4.5] dec-3-en-1-yl acetamide derivatives and their use as glycine transporter inhibitors WO2007/104776-A1, 20 Sep 2007
172. Blunt R, Eatherton AJ, Garzya V et al (2011a) Benzoxazinone derivatives for the treatment of Glytl mediated disorders Glaxo Group Ltd WO2011/012622-A1, 3 Feb 2011
173. Blunt R, Porter R, Johns A et al (2011) Acylglycinamides as inhibitors of glycine transporter type 1. Bioorg Med Chem Lett 21:6176–6179
174. Terui Y, Chu YW, Li JY et al (2008) New cyclic tetrapeptides from Nonomuraea sp. TA-0426 that inhibit glycine transporter type 1 (GlyT1). Bioorg Med Chem Lett 18: 6321–6323
175. Williams JB, Mallorga PJ, Conn PJ et al (2004) Effects of typical and atypical antipsychotics on human glycine transporters. Schizophr Res 71:103–112
176. Werdehausen R, Kremer D, Brandenburger T et al (2012) Lidocaine metabolites inhibit GlyT-1: A novel mechanism for the analgesic action of Lidocaine. Anesthesiol 116:147–158
177. Kopec K, Flood DG, Gasior M et al (2010) Glycine transporter (GlyT1) inhibitors with reduced residence time increase prepulse inhibition without inducing hyperlocomotion in DBA/2 mice. Biochem Pharmacol 80:1407–1417
178. Ravert HT, Mathews WB, Klitenick MA et al (2001) Radiosynthesis of a ligand for studying the glycine transporter: [$^{11}$C]ALX-5407. J Label Compd Radiopharm 44:241–246
179. Murthy NV, Passchier J, Gunn RN et al (2008) [$^{11}$C]GSK931145: A new PET ligand for glycine transporter 1. Neuroimage 41(Suppl 2):T21
180. Passchier J, Gentile G, Porter R et al (2010) Identification and evaluation of [$^{11}$C]GSK931145 as a novel ligand for imaging the type 1 glycine transporter with positron emission tomography. Synapse 64:542–549

181. Guo Q, Brady M, Gunn RN (2009) A biomathematical modeling approach to central nervous system radioligand discovery and development. J Nucl Med 50:1715–1723
182. Bullich S, Slifstein M, Passchier J et al (2011) Biodistribution and radiation dosimetry of the glycine transporter-1 ligand [$^{11}$C]-GSK931145 determined from primate and human whole-body PET. Mol Imaging Biol 13:776–784
183. Passchier J, Murthy V, Catafau AM et al (2008) Development and evaluation of [$^{11}$C] GSK931145, a new PET ligand for imaging type 1 glycine transporters (GlyT1) in the living human brain. J Nucl Med 49:129P
184. Gunn RN, Murthy V, Catafau AM et al (2011) Translational characterization of [$^{11}$C] GSK931145, a PET ligand for the glycine transporter type 1. Synapse 65:1319–1332
185. Hamill TG, Eng W, Jennings A et al (2011) The synthesis and preclinical evaluation in rhesus monkey of [$^{18}$F]MK-6577 and [$^{11}$C]CMPyPB glycine transporter 1 positron emission tomography radiotracers. Synapse 65:261–270
186. Sanabria-Bohórquez SM, Joshi AD, Holahan M et al (2012) Quantification of the glycine transporter 1 in rhesus monkey brain using [$^{18}$F]MK-6577 and a model-based input function. Neuroimage 59:2589–2599
187. Sanabria-Bohorquez S, Van Laere K, Hamill T et al (2009) Evaluation of the novel glycine transporter 1 (GlyT1) tracer [$^{18}$F]CFpyPB: dosimetry and brain quantification in human. J Nucl Med 50:S1211
188. Zheng M-Q, Holden D, Hamill T et al (2013) Comparative study of two glycine transporter 1 radiotracers [$^{11}$C]GSK931145 and [$^{18}$F]MK6577 in baboons. J Nucl Med 54(Suppl 2):414
189. Borroni E, Zhou Y, Ostrowitzki S et al (2013) Pre-clinical characterization of [$^{11}$C] R05013853 as a novel radiotracer for imaging of the glycine transporter type 1 by positron emission tomography. Neuroimage 75:291–300
190. Wong DF, Ostrowitzki S, Zhou Y et al (2013) Characterization of [$^{11}$C]RO5013853, a novel PET tracer for the glycine transporter type 1 (GlyT1) in humans. Neuroimage 75:282–290
191. Wong D, Borroni E, Ostrowitzki S et al (2012) Imaging biomarkers for the glycine transporter type 1. J Nucl Med 53(Suppl 1):199
192. Pinard E, Burner S, Cueni P et al (2011) Radiosynthesis of [5-[$^{11}$C]methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-[5-(tetrahydro-pyran-4-yl)-1,3-dihydro-isoindol-2-yl]-methanone ([$^{11}$C]RO5013853), a novel PET tracer for the glycine transporter type I (GlyT1). J Label Compd Radiopharm 54:702–705
193. Alberati D, Borroni E, Moreau J et al (2011) Partial occupancy of the glycine transporter type 1 in rat by RG1678 leads to efficacy in models relevant to schizophrenia. Schizophr Bull 37 (Suppl 1):286
194. Borroni E, Wong DF, Alberati D et al (2011) Partial occupancy of the glycine transporter type 1 in monkey by RG1678 leads to efficacy in a model of prefrontal cortical function. Schizophr Bull 37(Supplement 1):296–297
195. Hofmann C, Alberati D, Banken L et al (2011) Glycine transporter type 1 (GlyT1) inhibitor RG1678: proof of mechanism of action in healthy volunteers. Schizophr Bull 37(Suppl 1):306
196. Hayes E (2014) The Pink Sheet Jan 21, 2014. http://www.elsevierbi.com/publications/the-pink-sheet-daily/2014/1/21/bitopertins-schizophrenia-failure-stirs-doubt-about-roches-cns-plans. Accessed 25 Jan 2014
197. Liem-Moolenaar M, Peeters P, Kamerling M et al (2013) Early stage development of the glycine-1 re-uptake inhibitor SCH 900435: central nervous system effects compared with placebo in healthy men. Br J Clin Pharmacol 75:1455–1467
198. D'Souza DC, Singh N, Elander J et al (2012) Glycine transporter inhibitor attenuates the psychotomimetic effects of ketamine in healthy males: preliminary evidence. Neuropsychopharmacol 37:1036–1046
199. Nations KR, Smits JA, Tolin DF et al (2012) Evaluation of the glycine transporter inhibitor Org 25935 as augmentation to cognitive-behavioral therapy for panic disorder: a multicenter, randomized, double-blind, placebo-controlled trial. J Clin Psychiatry 73:647–653

200. Ouellet D, Sutherland S, Wang T et al (2011) First-time-in-human study with GSK1018921, a selective GlyT-1 inhibitor: relationship between exposure and dizziness. Clin Pharmacol Ther 90:597–604
201. Tsai G, Ralph-Williams RJ, Martina M et al (2004) Gene knockout of glycine transporter 1: characterization of the behavioral phenotype. Proc Natl Acad Sci U S A 101:8485–8490
202. Liu C-N, Pettersen B, Seitis G et al (2013) GlyT1 inhibitor reduces oscillatory potentials of the electroretinogram in rats. Cutan Ocul Toxicol. doi:10.3109/15569527.2013.833937. Posted online on October 22, 2013
203. Shen W, Jiang Z (2007) Characterization of glycinergic synapses in vertebrate retinas. J Biomed Sci 14:5–13
204. Treanor J, Chen H, Murphy O et al (2013) AMG 747, a novel glycine transporter type-1 (GlyT1) inhibitor with cognition-enhancing and antipsychotic-like effects in preclinical rodent models of schizophrenia Poster 152.08/W9. Society for Neurosciences San Diego Nov 9th–13th 2013, Neuroscience 2013

Top Med Chem (2015) 13: 101–142
DOI: 10.1007/7355_2014_48

Published online: 16 April 2014

# Metabotropic Glutamate Receptor 2 Activators

**José M. Cid, Andrés A. Trabanco, and Hilde Lavreysen**

**Abstract** Schizophrenia is a common and severe, often disabling psychiatric illness of unknown aetiology that affects approximately 24 million people worldwide. The illness is characterized by symptomatology comprising positive symptoms (hallucinations and delusional behaviours), negative symptoms (anhedonia, social withdrawal and apathy) and cognitive dysfunction (diminished capacity for learning, memory and executive function). Current pharmacological treatments are effective at alleviating positive symptoms but have limited impact on negative symptoms and cognitive deficits. Furthermore, the extrapyramidal symptoms, hyperprolactinemia and metabolic syndrome, including substantial weight gain, are typical side effects limiting the value of many of these drugs for patients. Thus, drugs that better serve the patient population by effectively treating all symptoms with improved safety and tolerability remain a critical unmet need. Modulation of the metabotropic glutamate type 2 (mGlu2) receptor has emerged as a promising mechanism for the treatment of CNS diseases, with the potential to provide a new and more effective avenue for the treatment of schizophrenia.

**Keywords** Metabotropic glutamate receptor 2, mGlu2, PAM, Positive allosteric modulator, Potentiator

J.M. Cid (✉) and A.A. Trabanco
Neuroscience Medicinal Chemistry, Janssen Research and Development, Janssen-Cilag S.A., Jarama 75, 45007 Toledo, Spain
e-mail: jcid@its.jnj.com

H. Lavreysen
Neuroscience Biology, Janssen Research and Development, Turnhoutseweg 30, 2340 Beerse, Belgium

## Contents



## Abbreviations

| | |
|---|---|
| Ar | Aryl |
| B/P | Brain-to-plasma ratio |
| BINA | Biphenyl indanone A |
| $CF_3O$ | Trifluoromethoxy |
| Clp | Clearance |
| CNS | Central nervous system |
| F% | % Oral bioavailability |
| hERG | Human ether-a-go-go-related gene |
| HTS | High-throughput screening |
| MDD | Major depressive disorders |
| MeO | Methoxy |
| mGlu | Metabotropic glutamate |
| NMDA | *N*-methyl-D-aspartate |
| PAM | Positive allosteric modulator |
| PCP | Phencyclidine |
| PhO | Phenoxy |
| PK | Pharmacokinetics |
| REM | Rapid eye movement |
| SAR | Structure–activity relationship |
| Sw-EEG | Sleep-wake electroencephalogram |
| $t_{1/2}$ | Half-life |
| μ | Micro |

## 1 Introduction

Schizophrenia is a common and severe, often disabling psychiatric illness of unknown aetiology that affects approximately 1% of the world population [1]. Schizophrenia is characterized by three groups of symptoms: (1) positive symptoms that appear to reflect an excess or distortion of normal functions, including auditory hallucinations, disorganized or bizarre thoughts, delusions and irrational fear; (2) negative symptoms that reflect a diminution or loss of normal functions, including social withdrawal, loss of will or drive, poverty of speech, apathy and lack of energy; and (3) cognitive dysfunction ranging from impaired attention to abnormal executive function, as well as memory impairment, depression and/or anxiety. Although not continuously, all patients with schizophrenia experience positive symptoms; approximately one-third of all patients experience negative symptoms. Cognitive dysfunction may present prior to the emergence of positive and negative symptoms and can change over time. Given the extensive heterogeneity of symptoms between individual patients, schizophrenia may be considered a clinical syndrome rather than a single disease entity.

Current treatments involve direct modulation of the $D_2$ receptor and their associated signalling pathways [2]. While the treatment and maintenance of the positive symptoms of schizophrenia are largely addressed with these medications, the major challenge for researchers nowadays is to identify compounds showing clinically significant improvements in the treatment of negative symptoms and cognitive dysfunction [3–5]. In addition, a major issue with most of the currently prescribed antipsychotic drugs is the side-effect liabilities of weight gain, metabolic abnormalities, diabetes and potential cardiovascular safety concerns, and so significant improvements in safety and tolerability still need to be made [6–8].

Compounds capable of addressing both social and cognitive deficits will fill a significant unmet need in the treatment and management of the schizophrenia.

## 2 Glutamate and Schizophrenia

The dopaminergic hypothesis of schizophrenia reposes on the fact that some compounds effective in the treatment of mainly the positive symptoms are dopaminergic antagonists. Also, there is a direct correlation between plasma homovanillic acid (metabolite of dopamine) concentration and the severity of schizophrenic illness. Moreover, some of the symptoms of schizophrenia are recapitulated by abuse drugs like amphetamines, which are known to increase the dopaminergic tone. More recent findings also suggest a dysregulation of glutamatergic neurotransmission in schizophrenia through the so-called NMDA receptor hypofunction hypothesis. This hypothesis originates from clinical observations that non-competitive antagonists of NMDA receptors, such as phencyclidine (PCP) and ketamine, are able to induce psychotic symptoms in healthy

volunteers and exacerbate either positive, negative or cognitive symptoms in patients with schizophrenia [9]. Also molecular studies using human post-mortem brain tissue and animal models of schizophrenia support the hypothesis that reduced signalling at postsynaptic NMDA receptors is linked to schizophrenia [10–12].

The microcircuitry underlying the NMDA receptor hypofunction is thought to involve a reduction of NMDA receptor function on midbrain inhibitory GABAergic neurons resulting in a disinhibition of glutamatergic projection neurons and excessive glutamate release in the prefrontal cortex. The paradoxal increase in extracellular glutamate levels as a result of NMDA receptor hypofunction was confirmed by studies demonstrating a hyperglutamatergic state in patients with schizophrenia by in vivo magnetic resonance spectroscopy [13]. In patients with stable schizophrenia treated with antipsychotic drugs, however, a relative glutamate hypofunction in the left anterior cingulate cortex was found [14]. Indeed, recent meta-analysis of $^{1}$H-MRS studies [15] revealed that in patients with schizophrenia, glutamate and glutamine concentrations decreased at a faster rate with age as compared with healthy controls. Hence, it is not yet completely understood how glutamatergic systems in schizophrenia are altered and it may well depend on the disease stage (acute versus stable) as well as the nature of the symptoms.

The fact that glutamatergic signalling may be disrupted in patients with schizophrenia has been reinforced with the discovery of several susceptibility genes related to glutamatergic pathways [16, 17]. While thought to be more proximal to the root causes of schizophrenia, the glutamate hypothesis does not negate hypothesis, and the two may in fact ultimately be brought together by circuit-based models [18].

Notably, in addition to the dopamine and glutamate hypothesis, several factors can likely contribute to the development of schizophrenia including (1) biological factors arising from physiology, biochemistry, genetic make-up and physical constitution; (2) psychological factors including childhood experiences, emotional trauma and interactions with people; and (3) social factors associated with lifestyle and sociocultural influences.

## 3 The Glutamate Receptors

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) of vertebrates. Glutamate elicits and modulates synaptic responses by activating two families of receptors, ligand-gated cation channels termed ionotropic glutamate (*i*Glu) receptors and G protein-coupled glutamate receptors termed metabotropic glutamate (mGlu) receptors [19]. In general, mGlu receptors bind glutamate and function to modulate or 'fine-tune' excitatory and inhibitory transmission by presynaptic, postsynaptic and glial mechanisms. mGlu receptors are characterized by a large N-terminal extracellular domain of ~560 amino acids that contains the glutamate-binding domain and confers selectivity for agonists; specific

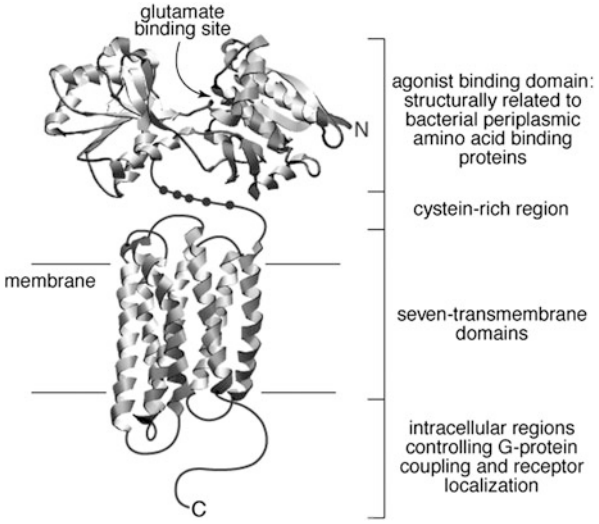



**Fig. 1** General structure of mGlu receptors

amino acids downstream from the first transmembrane domain might also influence agonist affinity and potency. In contrast to other G protein-coupled receptors where the third intracellular loop appears critical for G protein coupling, in mGlu receptors the second intracellular loop serves for this function and is furthermore important for determining the intracellular transduction mechanism (Fig. 1).

To date, eight mGlu receptor subtypes and multiple splice variants have been cloned and classified into three groups based upon sequence homology, pharmacological profile and preferential signal transduction pathway. Group I mGlu receptors (mGlu1 and mGlu5) are coupled to the stimulation of phospholipase C when expressed in heterologous systems, whereas group II (mGlu2 and mGlu3) and III receptors (mGlu4, mGlu6, mGlu7 and mGlu8) are negatively coupled to adenylate cyclase activation [20]. Group I receptors are primarily expressed postsynaptically, whereas both group II and III are mainly found presynaptically where they reduce glutamate release. Pharmacological studies with group-specific agonists and antagonists suggest that, in general, activation of group I mGlu receptors increases the excitability of neurons, whereas group II and group III receptor activation suppresses neuronal excitability. The receptors and mechanisms by which group-specific agonists enhance or suppress glutamatergic excitability is currently an active area of pharmacological research.

Group II mGlu receptors reduce transmission at glutamatergic synapses in brain regions where excessive glutamatergic transmission may be implicated in the pathophysiology of anxiety and schizophrenia, such as the prefrontal cortex and hippocampus [21, 22]. It is therefore hypothesized that activation of group II mGlu receptors may provide anxiolytic and/or antipsychotic effects [22]. mGlu2/mGlu3 receptor agonists have demonstrated therapeutic potential in animal models of anxiety, psychosis, epilepsy and addiction. Initial human data have suggested potential therapeutic benefit of mGlu2/3 receptor agonists in schizophrenia and

generalized anxiety disorder although more recent studies warrant further investigation of effects dependent on disease stage and subpopulation of patients.

The individual contributions of mGlu2 and mGlu3 receptors as targets for the treatment of psychiatric disorders have not been extensively elucidated because of the dearth of subtype-selective agents, though knockout studies suggest that the antipsychotic effects of mGlu2/mGlu3 mixed receptor agonists may be mediated by the mGlu2 receptor [23, 24].

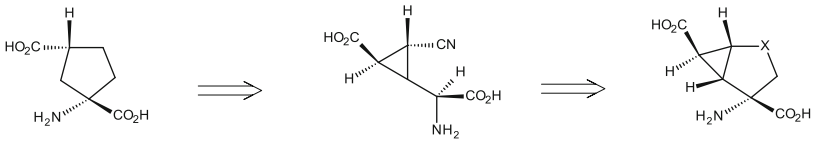
## 4 mGlu2/mGlu3 Receptor Agonists

Pharmacological studies in humans and animals suggest that PCP administration to test species represents a useful model of psychosis and serves as the basis for the "glutamate hypothesis of schizophrenia." PCP produces schizophrenia-like symptoms in healthy subjects and worsens psychosis in patients with schizophrenia. PCP has multiple behavioural effects in animals, such as increased motor behaviours, that have been linked to enhanced release of neurotransmitters, including glutamate.

Use of mixed mGlu2/mGlu3 receptor agonists in animal studies with PCP provided the first clues that mGlu2 receptor may represent a novel target for the treatment of schizophrenia. The first generation of molecules capable of activating the mGlu2 receptor were conformationally restricted analogues of the endogenous ligand glutamate. Initial prototypes (**1**, Fig. 2) lacked selectivity and were moderately potent, acting as orthosteric agonists at both mGlu2 and mGlu3 receptors [25]. Relevant improvements in both potency and selectivity were later reported in series of cyclopropanes (**2**, Fig. 2) [26, 27], where substitution at $C_3$ seems to be the most sensitive position to modulate both potency and selectivity and even to turn agonism into antagonism. Compound **2** was reported to have the best balance between potency and selectivity in this series. The next optimization step on these constrained glutamate chemotypes was achieved by hybridation of the cyclopentane (**1**) and cyclopropane (**2**) series leading to bicyclic analogues (**3-6**, Fig. 2). LY354740 (**3**) was one of the first analogues reported that displayed excellent activity at both mGlu2 and mGlu3 receptor subtypes, showed good selectivity over other glutamate receptors and was the first group II mGlu receptor agonist that showed activity in vivo [22, 25, 28].

Further evolution of the bicyclic series is illustrated by compounds LY379268 (**4**), LY389795 (**5**) or LY404039 (**6**) resulting from the replacement of $X{=}CH_2$ (**3**) by O, S or $SO_2$, respectively. All these novel bicyclic subclasses delivered excellent pharmacological tools that were extensively characterized over the years and that proved to be active in many different animal models of anxiety and schizophrenia [23, 29–31].

Initial clinical data strengthened the rationale for group II receptor intervention as a treatment for schizophrenia. LY2140023 (**7**, Fig. 3) (termed pomaglumetad methionil), the oral prodrug of the mGlu2/mGlu3 receptor agonist LY404039,

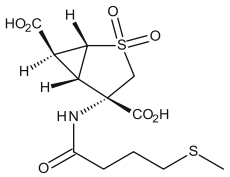



**1**, trans-ACPD
mGlu2/3 $EC_{50}$ (cAMP) = 15/10 μM

**2**
mGlu2/3 $EC_{50}$ (cAMP) = 0.19/0.064 μM

**3** (LY354740), X = $CH_2$
mGlu2/3 $EC_{50}$ (cAMP) = 0.011/0.038 μM
**4** (LY379268), X = O
mGlu2/3 EC50 (cAMP) = 0.003/0.005 μM
**5** (LY389795), X = S
mGlu2/3 $EC_{50}$ (cAMP) = 0.004/0.008 μM
**6** (LY404039) X = $SO_2$
mGlu2/3 $EC_{50}$ (cAMP) = 0.023/0.048 μM

**Fig. 2** Evolution of orthosteric mGlu2/mGlu3 mixed receptor agonists




**7**, LY2140023

**Fig. 3** Structure of the Eli Lilly's mGlu2/mGlu3 receptor agonist **7** (LY2140023)

showed improvement of both positive and negative symptoms in schizophrenia in a double-blind placebo-controlled study in patients with schizophrenia [28, 32, 33]. Importantly, treatment with LY2140023 was safe and well tolerated, did not affect prolactin levels nor did it induce extrapyramidal symptoms or weight gain, in contrast to many of the current $D_2$ receptor antagonist antipsychotics. These positive data could however not be confirmed in follow-up studies [34, 35] and, very recently, Eli Lilly and co. announced the decision to stop the phase III clinical trials investigating LY2140023 for the treatment of patients suffering from schizophrenia [36].

## 5 mGlu2 Receptor Positive Allosteric Modulators

It has been suggested that the mGlu2 and not the mGlu3 receptor mediates the actions of the mGlu2/mGlu3 receptor agonists LY379268 (**4**) and LY404039 (**6**) in mouse models predictive of antipsychotic activity [24]. All agonists identified so far, however, lack mGlu2 receptor subtype selectivity and also act on the mGlu3 receptor. Moreover, agonists hold a risk of tolerance development [37]. As an alternative to mGlu2/mGlu3 receptor agonists, mGlu2 receptor positive allosteric modulators (PAMs) may therefore provide a promising alternative. mGlu2 receptor

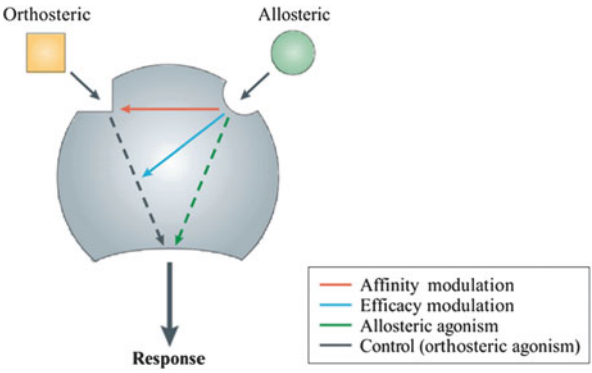



**Fig. 4** Modes of action of allosteric modulators. *Source*: Conn et al. [38]

PAMs act selectively on the mGlu2 receptor, and since they merely potentiate the response to the endogenous agonist glutamate without affecting the receptor on its own, they preserve the temporal pattern of receptor signalling, with less risk of overactivation or desensitization/tolerance. Allosteric modulators bind to G protein-coupled receptors (GPCR) at sites that are topographically distinct from the orthosteric site, leading to a change in receptor conformation [38]. As a consequence, the interactive properties of the GPCR, with orthosteric ligands as well as the cellular host environment, can be modified in either a positive (PAM) or negative direction (NAM). PAMs exhibit one or more of the following pharmacological properties (Fig. 4):

- Affinity modulation: the resulting conformational change increases the binding affinity of the orthosteric ligand.
- Efficacy modulation: the allosteric effect can change intracellular responses, thus leading to increased signalling capacity (or intrinsic efficacy) of an orthosteric ligand.
- Agonism: the allosteric modulator can also act as a direct agonist.

In essence, PAMs provide an approach to increase mGlu2 receptor signalling, with greater selectivity compared to agonists, by maintaining activity based on transient and dynamic release of glutamate and without inducing overactivation or desensitization.

LY487379 (**8**, Fig. 5) was identified as the first mGlu2 receptor-specific PAM [39]. LY487379 was active in animal models predictive of anxiolytic or antipsychotic activity [37, 40], thus providing preclinical proof of concept that PAMs can mimic the effects of orthosteric agonists. Minimal efficacy at low doses as well as short duration of action limited the use of the LY487379 series for further development. Rodriguez et al. [41] reported on a new series of mGlu2 receptor potentiators. One of these compounds, named BINA (**9**, Fig. 5), has been extensively characterized as a selective and potent mGlu2 receptor PAM with long-lasting activity in some animal models used to predict potential antipsychotic and anxiolytic activity [42, 43].

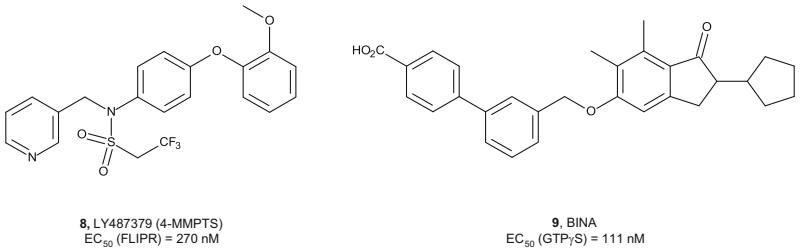



**Fig. 5** First reported mGlu2 receptor PAMs

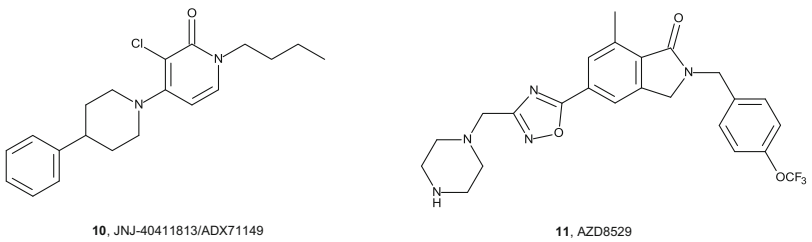



**Fig. 6** Structures of the Janssen/Addex and AstraZeneca clinical leads

Since the discovery of LY487379 and BINA and up to early 2009, a variety of structurally diverse chemotypes were published in both patent applications and research journals. Thus, shortly after the publication of LY487379, series such as acetophenones [44–47], indoles [48], 1,5-pyridones [49], 1,4-pyridones [50], isoindolones [51, 52], pyrazolones [53], thienopyrimidines [54], oxazolidinones [55, 56], benzimidazoles [57–59] and oxazolopyrimidones [60], were reported as novel mGlu2 receptor PAM chemotypes. For detailed background information, we refer to the reviews by Sheffler [61], Rudd and McCauley [62], Fraley [63], Marek [64] and Trabanco [65, 66], which cover literature and patent applications.

To date, two mGlu2 receptor PAM molecules have advanced into clinical trials. AZD8529 from AstraZeneca (**11**, Fig. 6) and JNJ-40411813 (**10**, also known as ADX71149, Fig. 6) from Janssen Pharmaceuticals, Inc. (Janssen) and Addex Therapeutics (Addex). AZD8529 completed phase I [67] and advanced into phase II clinical trial in patients with schizophrenia in June 2009 [68, 69]. On 27 January 2011, AstraZeneca announced the discontinuation of AZD8529 phase IIa POC study in schizophrenia (http://www.astrazeneca.com/Investors/financial-information/Financial-results/2010-Financial-results). The details of the failed POC recently disclosed showed no effect of AZD8529 (tested at a dose of 40 mg QOD, as monotherapy), while active control (risperidone) showed good activity ($p < 0.001$) on the primary outcome – change in PANSS total score from baseline to week 4 [70].

In an exploratory phase IIa study in schizophrenia [71], JNJ-40411813/ADX71149 met the primary objectives of safety and tolerability. Moreover, patients treated with antipsychotics who experience residual negative symptoms were identified as the subgroup of patients who may potentially benefit from add-on treatment with JNJ-40411813, although this is yet to be established in a formal proof-of-concept study [72; http://www.addextherapeutics.com/investors/press-releases/news-details/article/addex-reports-topline-data-from-a-successful Phase-IIa-clinical-study-with-adx71149-in-schizophrenia/].

In addition, a second phase IIa study with JNJ-40411813 as adjunctive therapy in patients with major depressive disorder (MDD) with significant anxiety symptoms was conducted [73], and top-line data was very recently announced. Overall, JNJ-40411813 was well tolerated and treatment emergent adverse events reported were similar to those seen in previous clinical studies. Based on a preliminary analysis of the primary efficacy end point, the 6-Item Hamilton Anxiety Subscale, (HAM-A6), JNJ-40411813 did not meet the criterion for efficacy signal detection vs. placebo. Despite a lack of signal on the primary outcome measure, treatment with JNJ-40411813 showed efficacy signals on several anxiety measures (HDRS17 anxiety somatization factor, IDS-C30 anxiety subscale) and on all depression measures (HDRS17, HAM-D6 and IDS-C30) (http://www.addextherapeutics.com/investors/press-releases/news-details/article/addex-reports-top-line-data-from adx71149-phase-2a-study-in-patients-with-major-depressive-disorder/). Although efficacy signals were evident, overall the data does not support the further development of JNJ-40411813 in anxious depression. Further exploration of JNJ-40411813 in other indications remains of potential interest.

In this section we describe in more detail the different chemotypes of mGlu2 receptor PAM that have been disclosed so far, which include the following sections.

## *5.1* N-*Arylsulfonamides*

Scientists at Eli Lilly pioneered the field of mGlu2 receptor PAMs in the early 2000s with the report of series of *N*-arylsulfonamides (Fig. 7) [39, 74]. The initial hit LY181837 (**12**), identified by high-throughput screening (HTS), showed moderate in vitro activity in the micromolar range. Subsequent optimization led to analogues with remarkable improvements in potency. Some representative examples are LY487379 (**8**) and the 2,2,2-TEMPS (**13**) [75]. Mutagenesis studies have demonstrated that 2,2,2-TEMPS binds to an allosteric site in regions IV/V of the transmembrane domain instead of the agonist-binding pocket in the extracellular domain of the receptor [76]. Both leads demonstrated to have behavioural effects similar to mGlu2/mGlu3 receptor agonists proving efficacy in a wide range of models of psychiatric disorders providing preclinical proof of concept for PAMs [37, 40, 41, 77, 78].

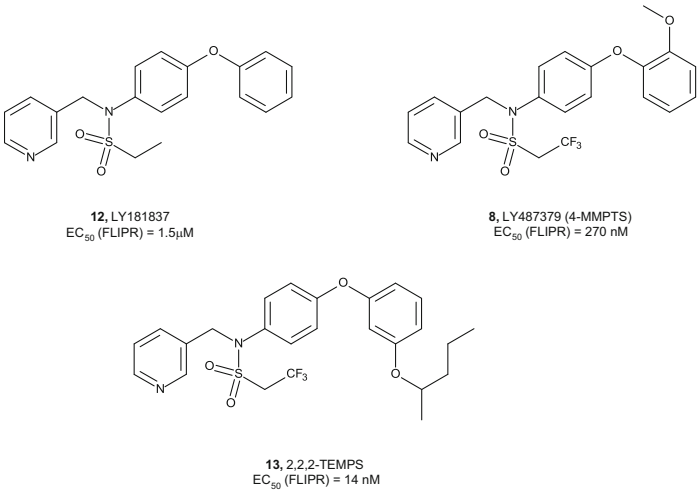



**Fig. 7** Lead optimization of the *N*-arylsulfonamides disclosed by Eli Lilly

## *5.2 Acetophenones*

Acetophenones were identified by scientists at Merck & Co., Inc. (Merck) in the early 2000s as one of the first mGlu2 receptor PAM series with activities in the nanomolar range. Compound **14** (Fig. 8) was the first reported mGlu2 receptor potentiator showing moderate mGlu2 receptor potency and good selectivity against other mGlu receptor subtypes. Furthermore, compound **14** exhibited efficacy in rodent models with relevance for schizophrenia, attenuating ketamine-induced norepinephrine release and ketamine-induced hyperactivity [44]. However, **14** exhibited a poor PK profile in rats ($t_{1/2}$ = 0.2 h; Clp = 33 mL/min/kg) and was not significantly brain penetrant (B/P = <0.03). Further potency increase was achieved with subtle modifications (**15**) [45]. In subsequent explorations, Merck addressed the poor brain penetration with a series of compounds that replaced the phenyl-tetrazolyl group by a 4-thiopyridine group (**16**) [47].

More recently, work by Eli Lilly has resulted in the identification of a group of acetophenone derivatives containing an imidazole carboxamide function as potent mGlu2 receptor potentiators with $EC_{50}$ values below 150 nM in a FLIPR assay [79]. Representative compound, **17** (THIIC) shown in Fig. 8, has an $EC_{50}$ of 22.5 nM and was found to be active in vivo in models predictive for antidepressant activity (e.g. mouse forced swim test; rat differential reinforcement of low rate 72-s assay and the rat dominant-submissive test) and anxiolytic activity (e.g. rat stress-induced hyperthermia and the mouse stress-induced elevation of cerebellar cyclic guanosine monophosphate (cGMP) assay and marble-burying model) [80]. Recent data have shown that THIIC is also able to prevent lactate-induced panic-like response in panic-vulnerable rats with an efficacy similar to alprazolam [81].

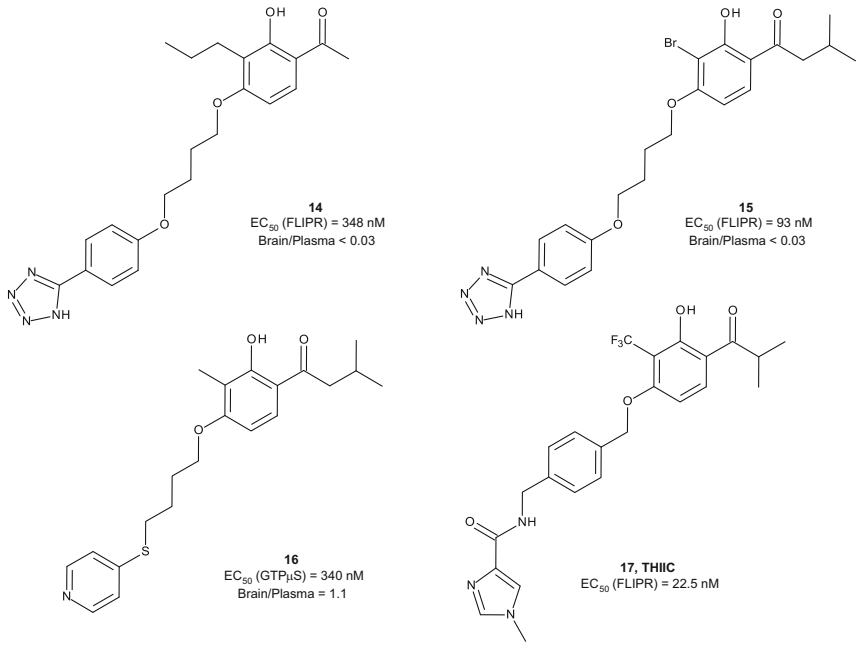



**Fig. 8** Representative mGlu2 receptor PAM acetophenones from Merck (**14–16**) and Eli Lilly (**17**)

## *5.3 Indanones*

Merck also reported a family of bicyclic ketones identified through HTS [44, 82–84]. One of these compounds, identified as BINA (**9**, Fig. 9), has been extensively characterized as a selective and potent mGlu2 receptor PAM with activity in a number of animal models used to predict potential antipsychotic and anxiolytic activity [37, 42, 43].

This series is structurally similar to the acetophenone series. The ketone functionality present in the acetophenone series is now part of a bicyclic moiety (indanone). Phenyl-tetrazolyl indanone (**18**, Fig. 9) was the first indanone described as a selective mGlu2 receptor PAM [84]. Compound (**18**) is a weak mGlu2 receptor potentiator ($EC_{50}$ = 600 nM) with poor rat PK properties characterized by high plasma clearance (Clp = 502 mL/min/kg) and a short half-life ($t_{1/2}$ = 0.3 h). Given the analogy with previously reported acetophenones, similar approaches were investigated to improve the potency, brain penetration and biological activity. Those efforts converged on the identification of the thiopyridine analogue (**19**, Fig. 9), which showed a large improvement on the brain-to-plasma ratio (B/P = 1.08), although the absolute brain levels were low. Compound **19** was active in the ketamine-induced hyperactivity model, a rodent model for schizophrenia [85].

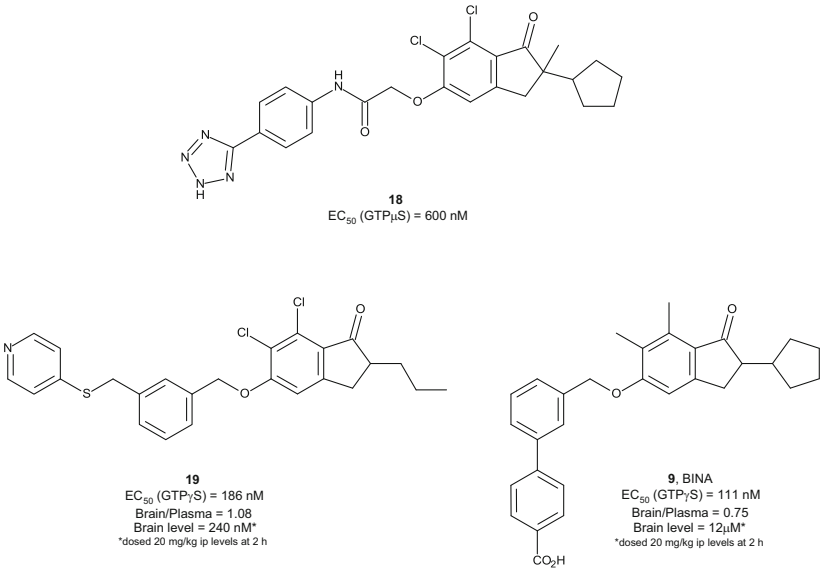
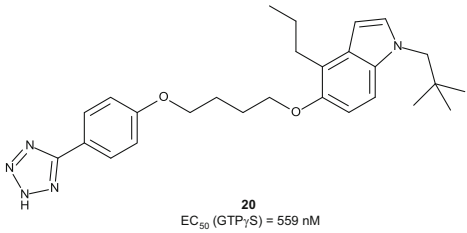



**Fig. 9** Representative mGlu2 receptor PAM indanones **9**, **18** and **19** from Merck

Additional improvements on PK properties were achieved with the biphenyl carboxylic acid moiety as bioisosteric replacement of the tetrazole ring (BINA). BINA exhibited a more favourable PK profile, with improved plasma and brain exposures. BINA [42, 43] remains the most attractive representative in the series and is, to date, one of the most extensively characterized mGlu2 receptor PAM compounds that has been used to predict antipsychotic and anxiolytic-like activity [43, 86–89].

## *5.4 Indoles*

The indole series was developed by Merck from previous acetophenones and indanones trying to improve the PK properties of previous chemotypes by masking the hydroxyketone motif. Most of the structure-activity relationship (SAR) [48, 90] was built keeping compound **14** (Fig. 8), as template. Among the different heterocyclic replacements of the indanone core tried (indoles, indazoles, benzotriazoles and benzisoxazoles), indole turned out to be the most attractive. Compound **20** (Fig. 10) was the best analogue reported with acceptable rat PK but limited central penetration (B/P = 0.16). Nevertheless, compound **20** reduced ketamine-induced hyperactivity in rats, at the dose of 25 mg/kg s.c., suggesting potential efficacy in schizophrenia.




**20**
$EC_{50}$ ($GTP\gamma S$) = 559 nM

**Fig. 10** Representative mGlu2 receptor PAM indole from Merck

## *5.5 Isoindolones*

This series, structurally very similar to the indanone series, was initially disclosed by AstraZeneca with their collaborator NPS Pharmaceuticals in 2006 [51]. Since then, numerous patent applications around this chemotype have been published with over 1200 examples disclosed. However, to our knowledge, no publications reporting on the biological characterization of this indolone family are available. In the first patent application, most of the examples show the *N*-benzyl substitution, preferably substituted in the para position with groups such as $CF_3O$, MeO or PhO. Position $C_7$ was explored preferentially with groups such as methyl, chloro or MeO. The data presented suggest that lipophilicity might be beneficial for activity as the most potent compounds contain either methyl or chloro substituents. The $C_5$ position of the isoindolone core was extensively explored. It appears that a wide variety of substitutions were tolerated for good potency as illustrated with compounds **21**–**24** (Fig. 11).

Subsequent work delivered compounds **25** [52], **26** or **27** [91], with improved properties over compounds **23**, **22** and **24**, respectively (Fig. 12).

Follow-up work around compounds **24** and **27** led to compounds claimed to have favourable solubility and low capacity to block the hERG channel (Fig. 13) [92, 93]. Some examples are compounds **28** ($EC_{50}$ = 48 nM) and **29** ($EC_{50}$ = 154 nM). Relevant difference between both **28** and **29** is the relative orientation of the oxadiazole which, seems to have an impact on hERG and aqueous solubility. In addition, introduction of (*S*)-methyl substitution in the benzylic substituent at position $N_2$ seemed to be well tolerated for the in vitro potency (**28**). Additional subtle modification within this template led to the identification of compound **11** (AZD8529), as previously mentioned, the clinical lead [71]. Interestingly, in 2011, AstraZeneca filed a patent application claiming the preparation of polymorphs of compound **11** [94, 95], exemplified in previous patents [96, 97].

On a continuing effort to optimize the series, AstraZeneca reported on a group of analogues where the terminal basic group was replaced by an amide function (**31** and **32**, Fig. 14) [98]. In these compounds, the nature of the amide proved to be very important to tune potency, solubility and hERG profile. Compound **31** was the most potent analogue exemplified from this exploration, with an $EC_{50}$ of 37 nM but also the least soluble (3.57 μM). Hydrophobic residues on the terminal amide appeared to be optimal for in vitro activity; however, they were detrimental for aqueous solubility. Conversely, the introduction of hydrophilic substituents, such as

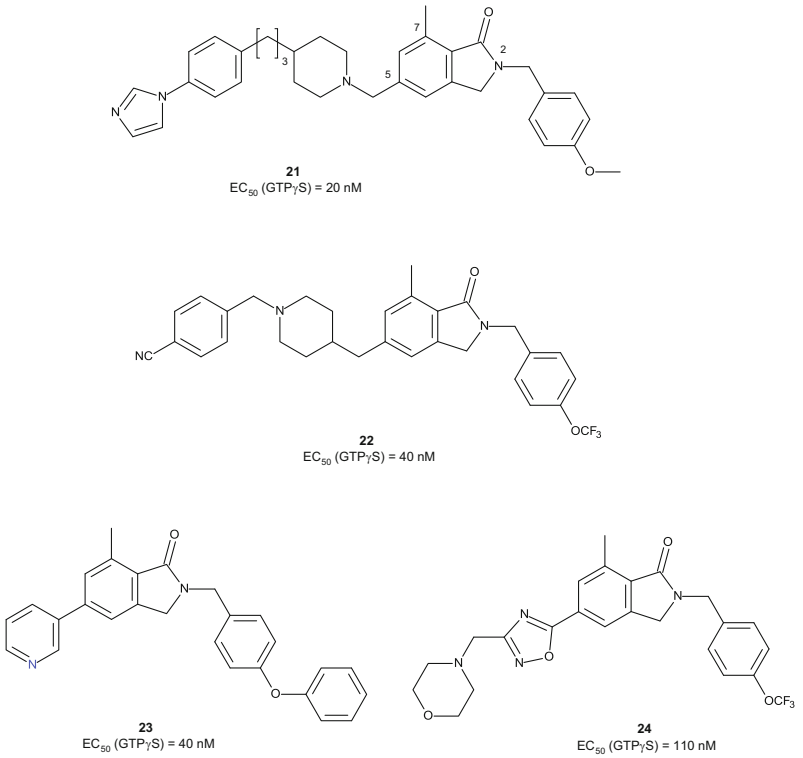



**Fig. 11** First representative isoindolones from AstraZeneca/NPS

the hydroxyethyl group in compound **32**, proved to be beneficial for solubility; however, primary mGlu2 receptor PAM activity was decreased.

Isoindolone SAR has been transferred to novel series of aza-isoindolones (**33**, Fig. 15) [99] and cyclic hydrazide derivatives (**34**, Fig. 15) [100], containing the arylsulfonamide substituent at $C_5$ position as preferred substitution. Interestingly high in vitro activity was reported for several hydrazide derivatives such as **34** with a remarkable mGlu2 receptor PAM $EC_{50}$ of 24 nM.

Abbott has also reported a similar series of isoindolones with mGlu2 receptor PAM $EC_{50}$ values equal to or lower than 10 μM (Fig. 16) [101–103]. These compounds are reported to show antagonistic activity towards 5-$HT_{2A}$ receptors with $IC_{50}$ of ~100 nM values in some cases. These isoindolone analogues differ from previously described isoindolones in the substitution at position $C_5$, where a pyrazole fragment, as shown in compound **35** or other heterocycles such as isoxazole (**36**) or pyridine (**37**, **38**), is used as spacers between the isoindolone core and the distal aryl ring. Interestingly and in contrast to previously reported isoindolones, no substituents at position $C_7$ have been exemplified.

In May 2011, Organon published novel SAR on series of isoindolones and isoquinolones as mGlu2 receptor PAMs [104]. The main difference with previously

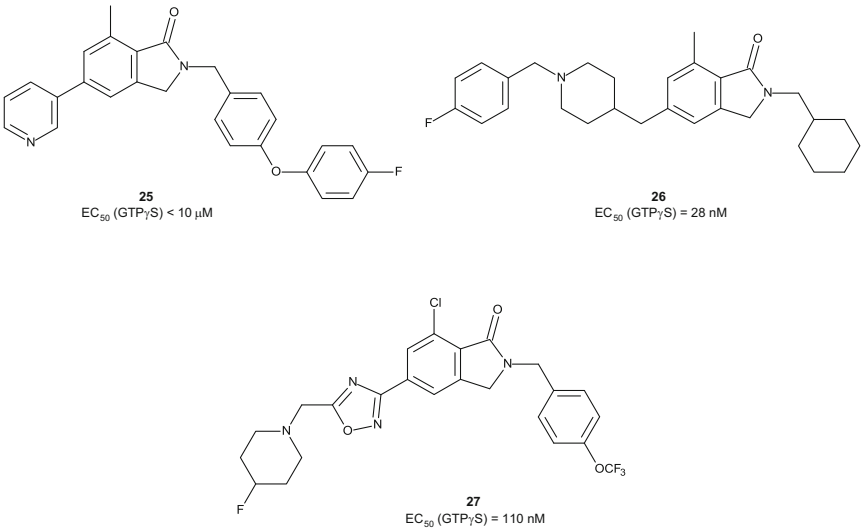



**Fig. 12** Isoindolones from AstraZeneca/NPS with improved properties

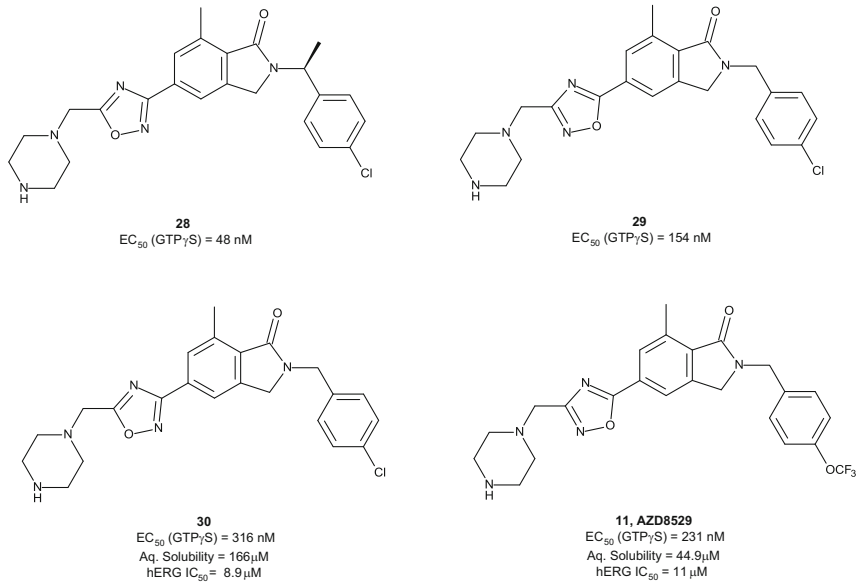
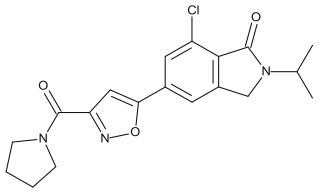
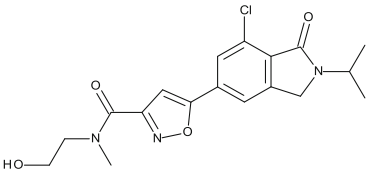



**Fig. 13** Representative isoindolones from AstraZeneca claiming favourable solubility and hERG including the clinical compound AZD8529

reported isoindoles is an imidazolidinone or imidazolidindione substituent on the aromatic ring at the $C_5$ position of the isoindolone core. The disclosed compounds are claimed to show activity in potentiating the mGlu2 receptor in a FLIPR TETRA

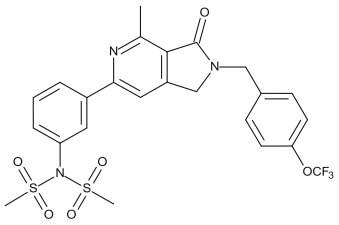


**31**
$EC_{50}$ (GTPγS) = 37 nM
Aq. Solubility = 3.57 μM

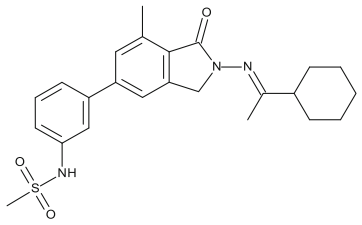


**32**
$EC_{50}$ (GTPγS) = 618 nM
Aq. Solubility = 54.1 μM

**Fig. 14** Representative isoindolones from AstraZeneca having terminal amide function on the left-hand side

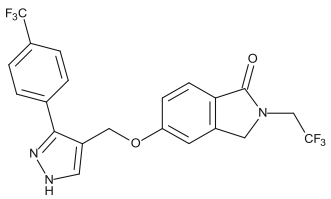


**33**
$EC_{50}$ (GTPγS) = 160 nM

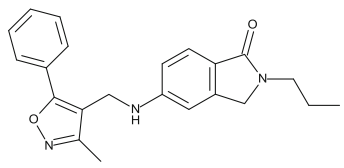


**34**
$EC_{50}$ (GTPγS) = 24 nM

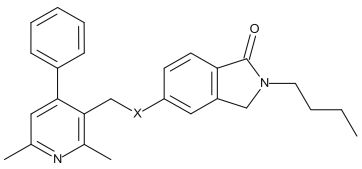
**Fig. 15** Aza-isoindolones **33** and **34** cyclic hydrazide isoindolones from AstraZeneca



**35**
$EC_{50}$ (GTPγS) < 10 μM



**36**
$EC_{50}$ (GTPγS) < 10 μM



**37**; X = O, $EC_{50}$ (FLIPR) < 0.5μM
**38**; X = $NH_2$, $EC_{50}$ (FLIPR) < 0.5μM

**Fig. 16** Representative isoindolones from Abbott

or FlexStation assay with $EC_{50}$ values < 10 μM, with preferred examples possessing $EC_{50}$ values < 1 μM. Two representative compounds from this application (**39** and **40**) are shown in Fig. 17.

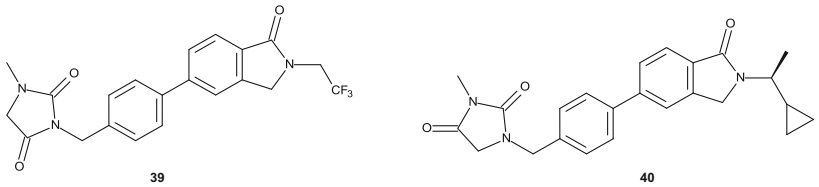



**Fig. 17** mGlu2 receptor PAM isoindolones from Organon

Scientists at the Sanford-Burham Medical Research Institute have followed up on previous work on indanones, in particular BINA (**9**) with the aim of improving its potency as well as PK properties. Their exploration was focused on the incorporation of heteroatoms, thus leading to novel series of isoindolinones (**41**, Fig. 18), tetrahydroisoquinolones (**42**, Fig. 18), benzisothiazolones (**43**, Fig. 18) and benzoisoxazolones (**44**, Fig. 18) [105–107]. This approach delivered highly selective compounds for the mGlu2 receptor with significantly better PK profiles.

In particular, the benzisothiazolone **43** has been shown to possess excellent brain penetration (B/P = 4.8) and oral bioavailability (86%). Compound **43** has showed activity in a model of intravenous cocaine self-administration in rats at an oral dose of 40 mg/kg, providing evidence that mGlu2 receptor PAMs could be useful for the treatment of addiction.

## *5.6 Isoquinolones*

Isoquinolones were identified by Addex and Janssen from screening of the Addex compound collection. Relevant SAR information for this chemical series of compounds was later published [108–110]. Compounds **45** and **46** (Fig. 19) were described as the most interesting examples.

## *5.7 Benzimidazoles*

Benzimidazole derivatives were first disclosed in 2007 and have become one of the most widely exemplified series of mGlu2 receptor PAMs. AstraZeneca/NPS [111, 112], Pfizer [57, 59, 113–116] and GSK [58] have exemplified over 2000 compounds in patent applications and scientific publications. Benzimidazoles were identified by Pfizer and GSK via an mGlu2 receptor PAM FLIPR HTS, with compounds **47**–**52** being among the preferred ones (Fig. 20). Scientists at Pfizer have reported their attempts to replace the benzimidazole core with benzothiazole, imidazopyridine or quinoline heterocycles; however, all these replacements were found to be detrimental for activity.

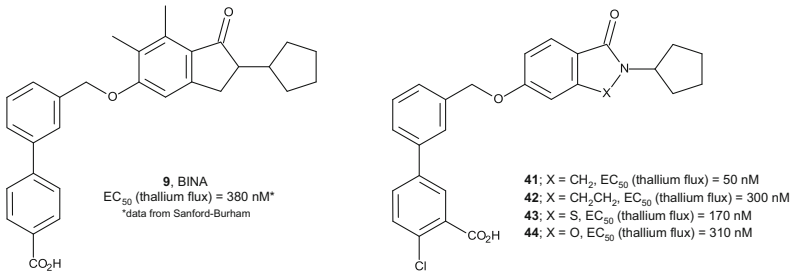



**Fig. 18** Isoindolones and analogues from Sanford-Burham Medical Research Institute

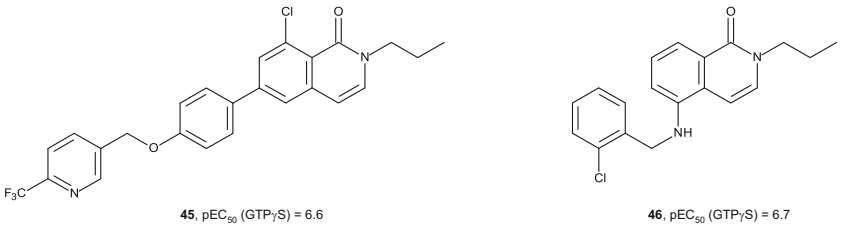



**Fig. 19** Isoindolones from Janssen/Addex

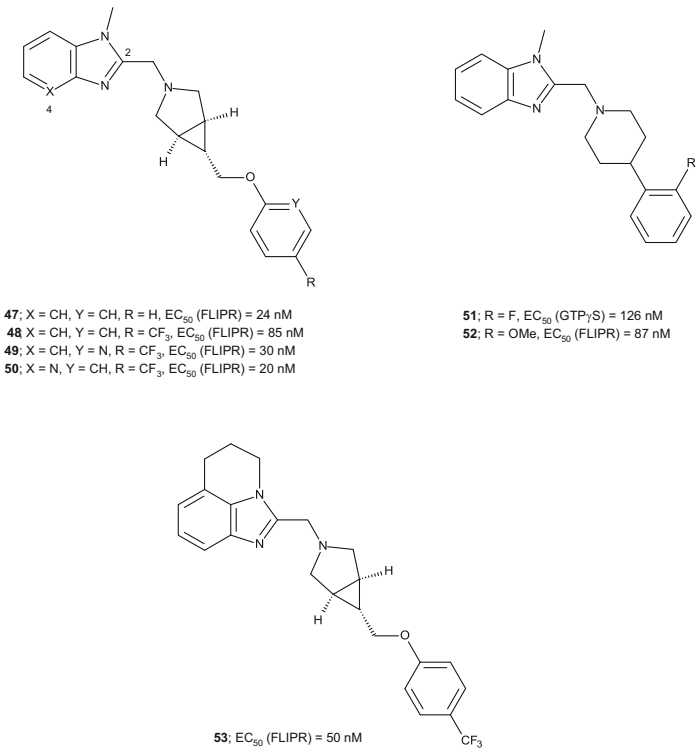



**Fig. 20** Benzimidazoles from Pfizer (**47**–**50**), GSK (**51**, **52**) and AstraZeneca/NPS (**53**)

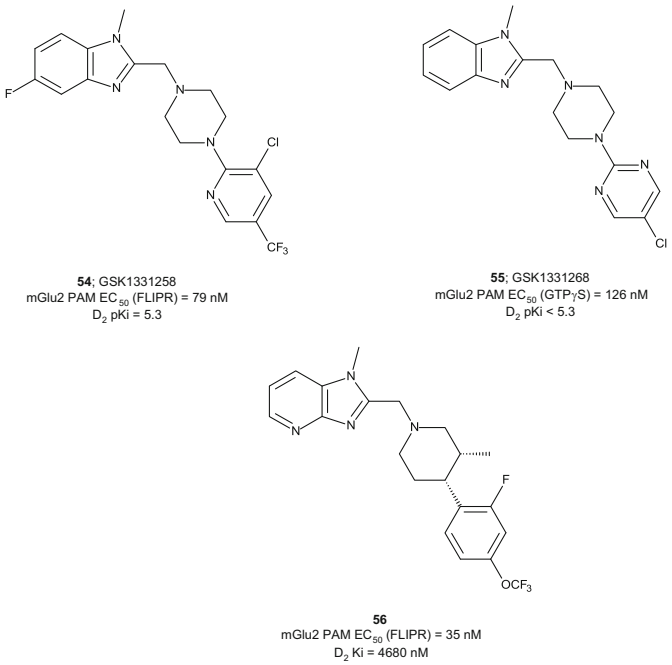



**Fig. 21** Benzimidazoles from GSK (**55**, **55**) and Pfizer (**56**) with reduced dopaminergic activity

The high clearance and low oral bioavailability found with the original Pfizer lead **47** were successfully addressed by the introduction of para substituents (**48**) in the distal aromatic ring as well as by the incorporation of pyridyl nitrogens (**49**). Introduction of the nitrogen into the benzofused ring to yield an aza-benzimidazole core was well tolerated for activity with many examples reported, the $C_4$ position being the more favourable for potency as illustrated with compound **50**.

In addition, a subseries of tricyclic benzimidazoles, where a cycloalkyl ring is fused from the imidazole nitrogen back to the benzo ring, was reported by AstraZeneca/NPS (**53**). Modification of the aliphatic ring seemed to be tolerated although in most examples this was found detrimental for activity.

More recently, scientists at GSK reported on their progress in the optimization of their HTS hit **51**. The initial exploration was focused on reducing off-target dopaminergic activity, as compound **51** acted on the $D_2$ receptor with an $IC_{50}$ of 40 nM. SAR studies showed that replacement of the piperidine ring by piperazine could, in some cases, reduce interaction with $D_2$ and $D_3$ receptors while maintaining acceptable mGlu2 receptor PAM activity. GSK1331258 (**54**, Fig. 21) and GSK1331268 (**55**, Fig. 21) were identified as the preferred leads. An in vivo rat PK study with a 1 mg/kg oral dose revealed a reasonable PK profile for **54** – low clearance (18.0 mL/min/kg), half-life of 4.1 h, B/P of 3.2 with $C_{max}$ in brain of 147 ng/g and an oral bioavailability of 58%. Compound **55** was shown to induce a three-fold increase in mGlu2 receptor agonist (LY354740, **3**) induced inhibition of

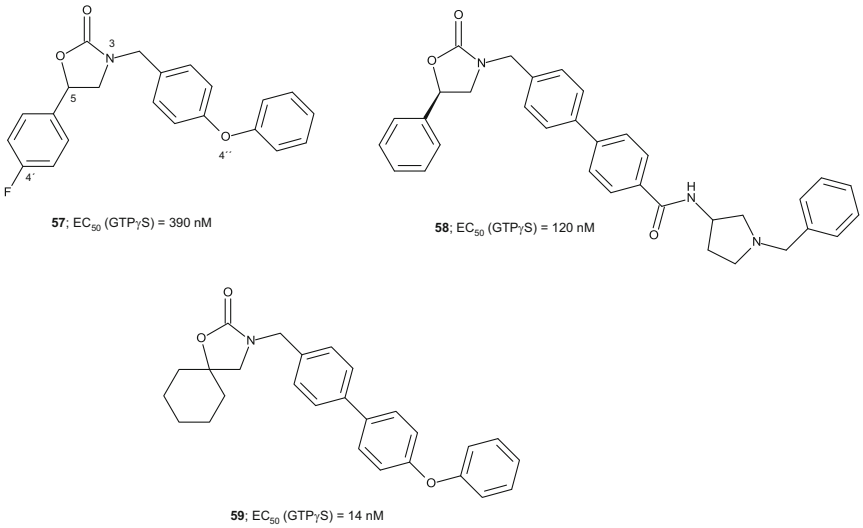



**Fig. 22** Set of representative mGlu2 receptor PAM oxazolidinones from AstraZeneca/NPS (**57**, **58**) and AstraZeneca (**59**)

synaptic transmission in rat hippocampal slices. Similar off-target dopaminergic activity was addressed by scientists at Pfizer by inducing conformational changes of the terminal phenyl ring in compound **52**. Thus, **56** (Fig. 21) was identified as an advanced lead having a good balance between potency, selectivity and PK properties. In addition, compound **56** demonstrated dose-dependent inhibition of methamphetamine-induced hyperactivity and mescaline-induced scratching in mice, models predictive for antipsychotic activity.

## *5.8 Cyclic Carbamates*

AstraZeneca/NPS pioneered the exploration of a series of cyclic carbamates, disclosing the discovery of a group of C5 phenyl *N*-benzyloxazolidinones, with mGlu2 receptor PAM $EC_{50}$ values $< 10$ μM in a GTPγS-binding assay (**57** and **58**, Fig. 22) [117]. Substitution on the pendant $C_5$ phenyl ring is limited to mainly small lipophilic groups and halogens, with the $C_4'$ position being the most modified within the provided examples. The absolute *R* configuration at the $C_5$ asymmetric centre seems to be preferred. The broad exemplified exploration of groups on the *N*-benzyl substituent may suggest that activity and ADMET properties of this series could be modulated in this region of the molecules. Consequently, a subset of compounds with an extra phenyl substituent at position $C_4''$ was extensively explored, indicating that highly lipophilic groups in this region may be beneficial for achieving good levels of activity. An effort to include basic/solubilizing

residues that may address the unfavourable solubility properties of the lipophilic biaryl group was also carried out. Compound **58**, showing an $EC_{50}$ value of 120 nM, was the most potent example from this series. Later on, a subseries of spirocyclic oxazolidinones was also reported [118]. In this case, a spirocyclic cyclohexyl group at position $C_5$ of the cyclic carbamate is always present. Specific data is provided only for four compounds in the patent application, of which **59** is the most potent example with an $EC_{50}$ of 14 nM in a GTPγS functional assay.

Scientists at Pfizer have also reported the synthesis and lead optimization of a family of potent *N*-benzyl 5- and 6-membered cyclic carbamates (Fig. 23) [119]. A hit-to-lead optimization program from hit **60** (FLIPR $EC_{50}$ 117 nM) [55] resulted in the identification of the 5*R*-methyloxazolidinone core as the optimal scaffold (**61** and **62**). Suboptimal metabolic turnover of these compounds was addressed by compounds **63** and **64** possessing a biaryl substituent on the carbamate nitrogen. Compound **63** reduced methamphetamine-induced hyperlocomotion (predictive for antipsychotic activity) in mice at 10 mg/kg s.c. Despite these encouraging results, the reduction of the high overall lipophilicity of the molecules, likely to be responsible for the high plasma protein binding observed with this family of compounds, remained a challenge.

Additional compounds from this cyclic carbamate series has been disclosed by scientists at Merck in two patent applications [120, 121]. Compounds are claimed to have FLIPR $EC_{50}$ activities lower than 10 μM, with preferred examples having activities lower than 1 μM in both FLIPR and GTPγS assays. Detailed SAR has been reported in a subsequent publication [58] describing the optimization process from the HTS hit **65**, focusing on improving potency as well as reducing clearance. The explorations carried out at both N and $C_5$ positions converged on the identification of **66**, a compound with improved potency and PK. Compound **66** was found to be active in a rat ketamine-induced hyperlocomotion model. Some relevant SAR shows that the (*R*)-configuration of $C_5$ position is crucial for activity, which was also the case for previously described oxazolidinone series. Interestingly, substitution at the ortho position of the phenyl ring is not permitted for activity, which suggests that co-planarity between the aryl ring and the oxazolidinone core is beneficial for achieving good activity. This hypothesis was later validated by the preparation of conformationally restricted compounds such as the novel oxazolobenzimidazole chemotype that according to Merck scientists successfully addressed the inherent limitations of the oxazolidinone core (Fig. 24).

## *5.9 Oxazolobenzimidazoles*

A series of oxazolobenzimidazoles was disclosed by Merck researchers in 2009 [122, 123]. As previously described, compounds in this series (**B**) can be seen as a conformationally restricted analogues of *N*-phenyloxazolidinones (**A**) by cyclization of the phenyl ring onto the $C_2$ position of the oxazolidinone core. This

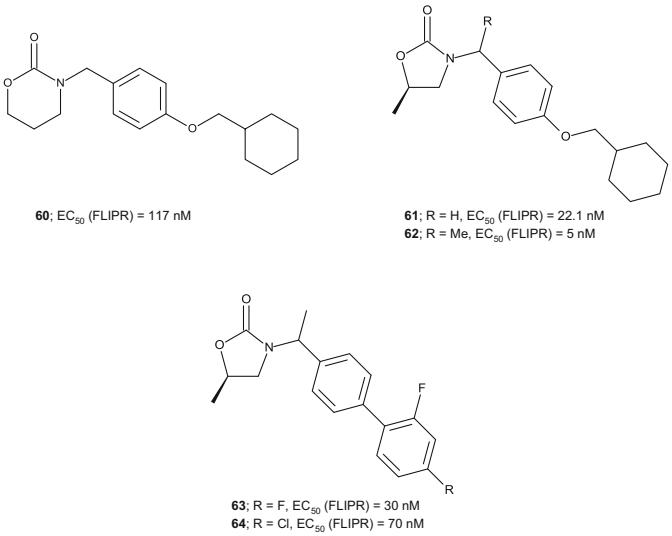



**Fig. 23** Set of representative mGlu2 receptor PAM oxazolidinones from Pfizer

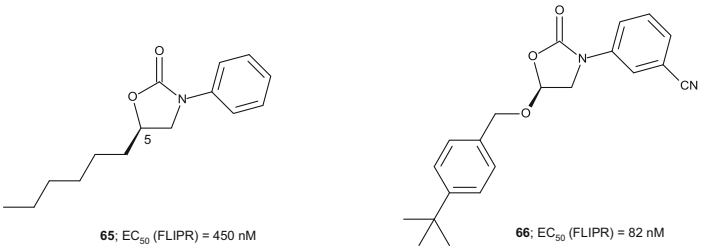



**Fig. 24** Set of representative mGlu2 receptor PAM oxazolidinones from Merck

cyclization locks a co-planar conformation which is suggested to be preferred for mGlu2 receptor PAM activity (Fig. 25) [124].

From the data presented in Merck's patent application, it can be deduced that aryloxymethyl- or heretoaryloxymethyl-groups are largely preferred as substituents at $C_2$, with the absolute (*S*)-configuration at the $C_2$ stereogenic centre in most cases. Decoration at the level of the $C_2$ pendant ring has been limited mainly to halogens, lipophilic alkyl groups and aromatic rings, para substitution being preferred (**67**, Fig. 26). An attempt to introduce some polarity and partial basicity in this area of the molecules resulted in the preparation of a subset of pyridyl analogues [124]. The most interesting compound, TBPCOB (**68**, Fig. 26), showed robust efficacy in inhibiting PCP-induced locomotor activity, a model for schizophrenia, in rats after oral administration at 100 mg/kg. Substitution on the benzofused aromatic ring was explored with small groups (halogen, cyano, trifluoromethyl, methoxy) mainly at positions $C_7$ and $C_8$, with the $C_8$ cyano- substituent being the

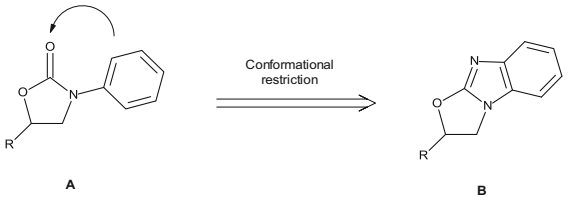



**Fig. 25** Resemblance between *N*-phenyloxazolidinones **A** and oxazolobenzimidazoles **B**

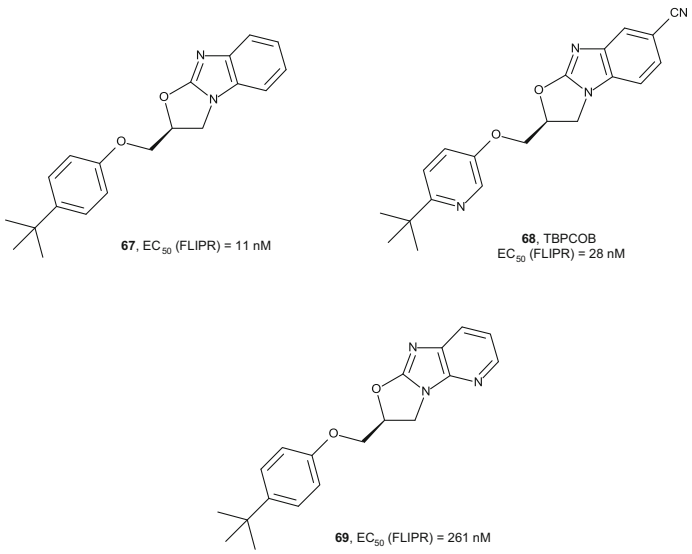



**Fig. 26** Set of representative mGlu2 receptor PAM oxazolobenzimidazoles from Merck

most exemplified substitution pattern. A small set of oxazoloazabenzimidazoles was also prepared, however the introduction of the additional nitrogen atom seemed to be somewhat detrimental for the activity (**69**, Fig. 26).

In a third patent application, scientists at Merck exemplified different substituents at $C_2$, aliphatic groups being extensively used for this exercise. A representative example for this patent application is compound **70** (Fig. 27), with an mGlu2 receptor PAM $EC_{50}$ value of 11 nM.

## *5.10 Oxazolopyrimidinones*

Scientists at Sanofi-Aventis have reported on oxazolopyrimidinones as a novel mGlu2 receptor PAM chemotype. This novel scaffold can be seen as another conformational restriction around the *N*-phenyloxazolidinone series (**A**, Fig. 28),

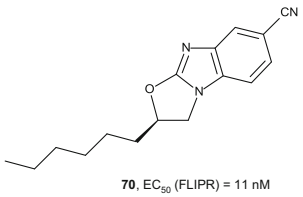



**Fig. 27** Chemical structure of compound **70** from Merck

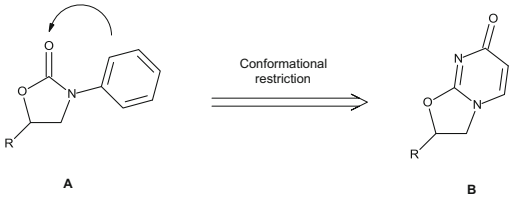



**Fig. 28** Resemblance between *N*-phenyloxazolidinones **A** and oxazolopyrimidinones **B**

where the acceptor properties of the oxazolidinone carbonyl group are now embedded into a pyrimidone amide-like function (**B**, Fig. 28) [125–129].

Most of the compounds described maintain the substituents and substitution patterns previously described in the oxazolobenzimidazole series. Thus a pendant aryloxymethyl group at position $C_2$ of the oxazolopyrimidone core, with the (*S*)-absolute configuration at the $C_2$ asymmetric centre seeming to be largely preferred. A short exploration with small substituents at both $C_5$ and $C_6$ suggests that substitution at $C_5$ by small lipophilic groups (methyl, ethyl, cyclopropyl, fluoromethyl) may be beneficial in terms of in vitro activity (**72**, Fig. 29). Cyclization through positions $C_5$ and $C_6$ also seems to be tolerated in terms of activity, with compounds such as **73** (Fig. 29) claimed to have mGlu2 receptor PAM activity with $EC_{50}$ values between 0.5 nM and 3 μM.

## *5.11 Pyridones*

Series of pyridones were identified by Janssen/Addex after an HTS (Fig. 30) of the Addex compound collection. Representative hits were *N*-alkylpyridones substituted at either the $C_5$ (**74**) or $C_4$ (**75**) with an aromatic substituent [130]. Compound **76** [49], resulting from the optimization of hit **74**, proved to be active in vivo in the PCP-locomotor activity model, suggesting antipsychotic-like activity.

Longer-lasting efforts were made on the optimization of $C_4$-substituted pyridone (**75**). Exploration at the $C_4$ position led to the identification of a diverse set of substituents that essentially can be grouped into three main different subclasses: (1) biarylethers (**77**) [50, 131], (2) pyranylarylethers (**78**) [50, 132] and

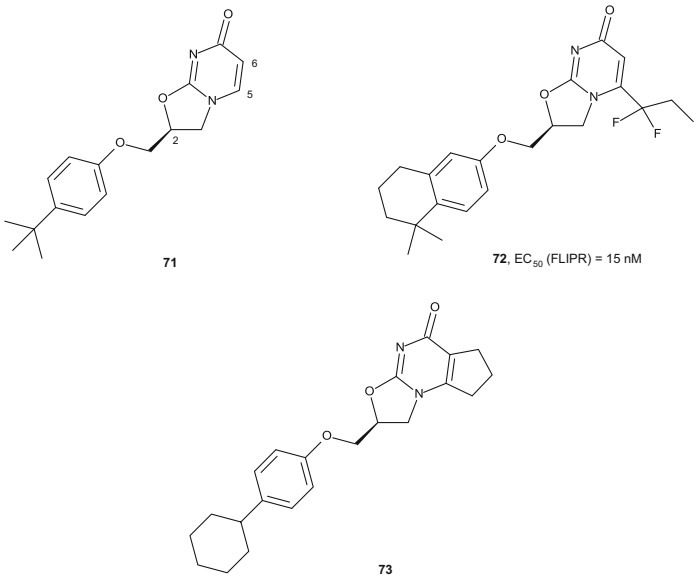



**Fig. 29** mGlu2 receptor PAM oxazolopyrimidinones from Sanofi-Aventis

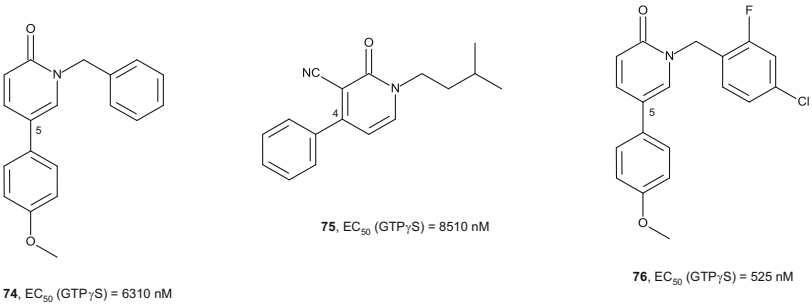



**Fig. 30** mGlu2 receptor PAM pyridone initial prototypes from Janssen/Addex

(3) arylpiperidine/piperazines (JNJ-40068782, **79**) [133] (Fig. 31). Extensive in vitro and in vivo characterization has been recently reported for JNJ-40068782 [134]. For instance, JNJ-40068782 influenced rat sleep-wake organization measured using sw-EEG by decreasing REM sleep at an oral dose of 3 mg/kg. In addition, JNJ-40068782 reversed PCP-induced hyperlocomotion in mice with an $ED_{50}$ of 5.7 mg/kg s.c.

Further progress made on the three different subclasses involved the replacement of the cyano function at position $C_3$ of the pyridone by alternative groups such as small alkyls, $CF_3$ or Cl, the latter one being the most exemplified. These SAR studies resulted in the disclosure of compounds with overall improvements [135–137]. Representative compounds are **80**, **81** (Fig. 32) and compound JNJ-40411813/

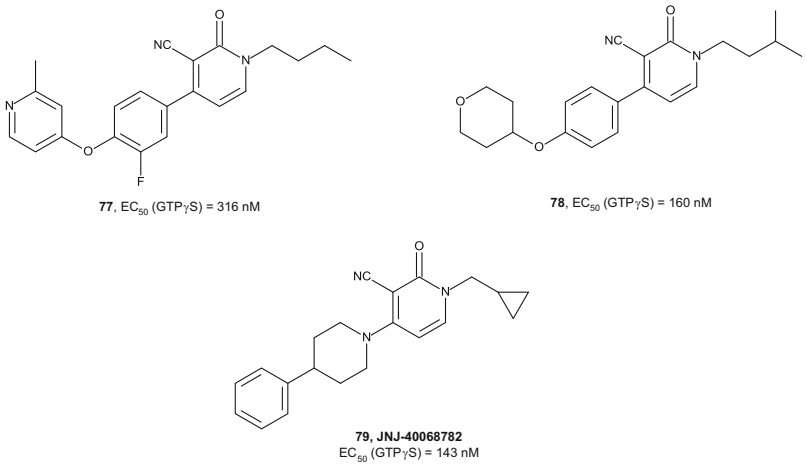



**Fig. 31** Representative mGlu2 receptor PAM 1,4-cyanopyridones from Janssen/Addex

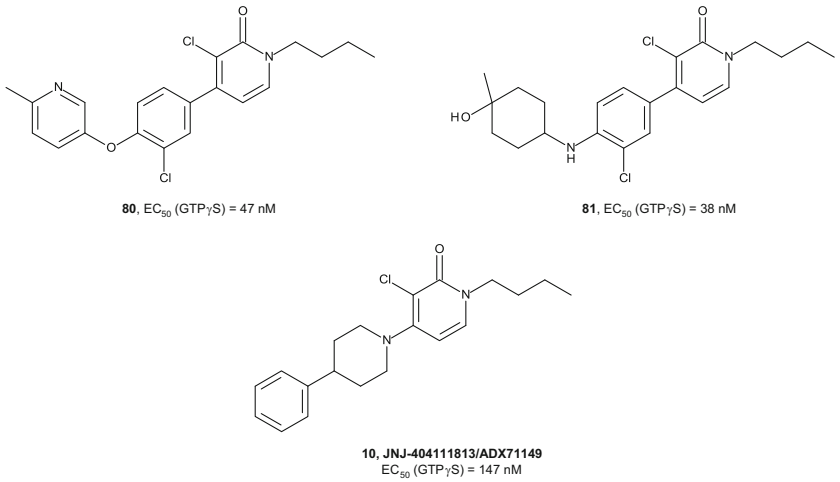
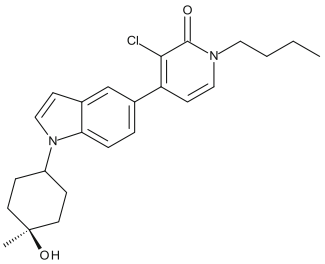


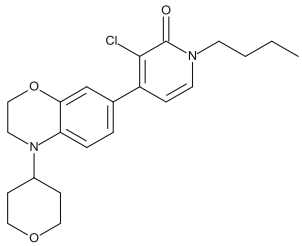

**Fig. 32** Representative mGlu2 receptor PAM 1,4-chloropyridones from Janssen/Addex

ADX71149 (**10**, Fig. 32), which is, as previously mentioned, to date, the most advanced mGlu2 receptor PAM in clinical studies.

Further modifications around **78** and **81** are represented in analogues **82** and **83** (Fig. 33), where a more restricted conformation of the $C_4$ substituent has been explored by means of indole and benzomorpholine heterocycles [138]. In vitro data are provided for all examples in the patent application, illustrating that substitution of the pyridone core with an indole radical is preferred over the substitution with the benzomorpholine group.




**82**, $EC_{50}$ (GTPγS) = 76 nM




**83**, $EC_{50}$ (GTPγS) = 589 nM

**Fig. 33** Representative mGlu2 receptor PAM indole and benzomorpholine pyridones from Janssen/Addex

## *5.12 Imidazopyridines*

Janssen/Addex have identified a class of imidazo[1,2-a]pyridine derivatives as PAMs of mGlu2 receptor [139, 140]. This novel imidazopyridine scaffold (**B**, Fig. 34) was designed via medicinal chemistry-driven SAR and computational techniques based on 3D-shape and electrostatic similarity. The imidazopyridine scaffold provided a similar spatial distribution of the pendent substituents as well as comparable electrostatic surface potential around the central core compared to the corresponding $C_4$-substituted pyridone (**A**, Fig. 34).

Like in the pyridone series, $R_2$ are preferably small substituents, with cyano and chloro present in most reported examples. Based on $R_3$ substitution, exemplified compounds can be grouped into three main subfamilies: (1) aminoimidazopyridines (**84**, **85**, Fig. 35), (2) arylimidazopyridines (**86**, **87**, Fig. 35) and (3) indolylimidazopyridines (**88**, Fig. 35) [141], compound **84**, being one of the most potent mGlu2 receptor PAM in the series ($EC_{50}$ = 10 nM). Lipophilic substituents at $R_1$ are beneficial for activity, with aliphatic alkyl chains largely exemplified and the trifluoroethyl being the most recurrent. This trifluoroethyl group has been reported to provide compounds combining acceptable potency and metabolic stability. Compound **88** has been highlighted in a recent publication [142] as one of the most interesting ones within the series. Compound **88** has been shown to reduce REM sleep in rats as measured with sw-EEG at an oral dose of 10 mg/kg.

## *5.13 Triazolopyridines*

Scientists at Janssen/Addex have reported the discovery of the 1,2,4-triazolopyridine core (Fig. 36) as a suitable replacement for the imidazopyridine scaffold which provides improved metabolic stability to the compounds while keeping or slightly improving the primary activity.

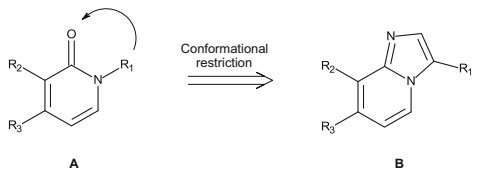



**Fig. 34** Rational for the discovery of the imidazopyridine series

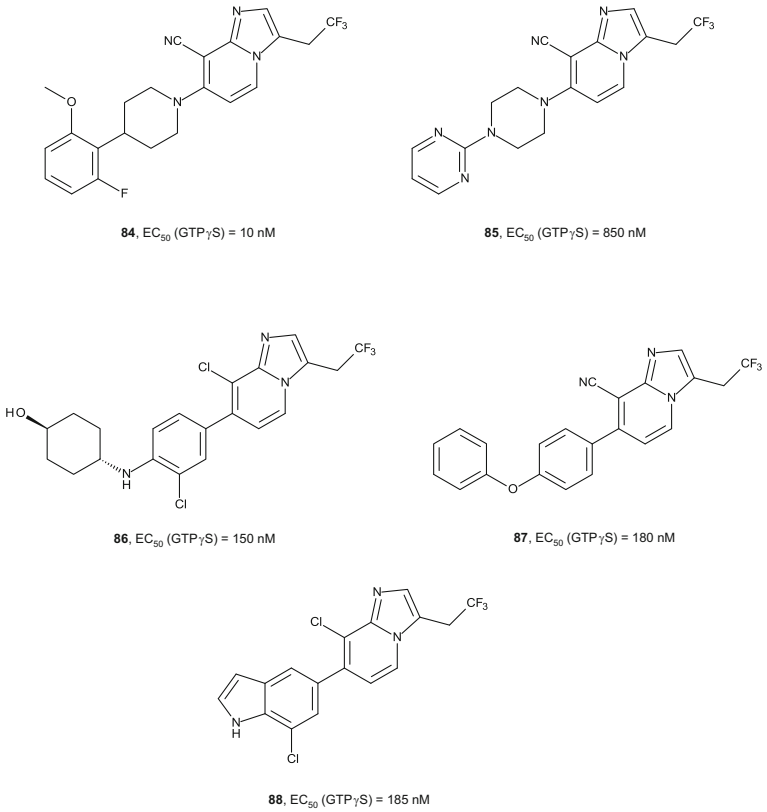



**Fig. 35** Set of representative mGlu2 receptor PAM imidazopyridines from Janssen/Addex

Very potent compounds are disclosed in a patent application claiming series of triazolopyridines containing arylpiperidine or piperazine groups at $R_3$ [143]. Thus, the recently published JNJ-42153605 (**89**, Fig. 37) [144] displays excellent mGlu2 receptor PAM activity ($EC_{50}$ = 17 nM) and remarkable selectivity for the mGlu2 receptor versus other mGlu receptors (>50-fold). Likewise, JNJ-42153605 is reported to have an acceptable PK profile in rodent and non-rodent species, combined with in vivo central activity in sw-EEG, showing suppressed REM

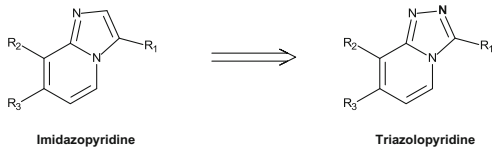



**Fig. 36** Design of the triazolopyridine series

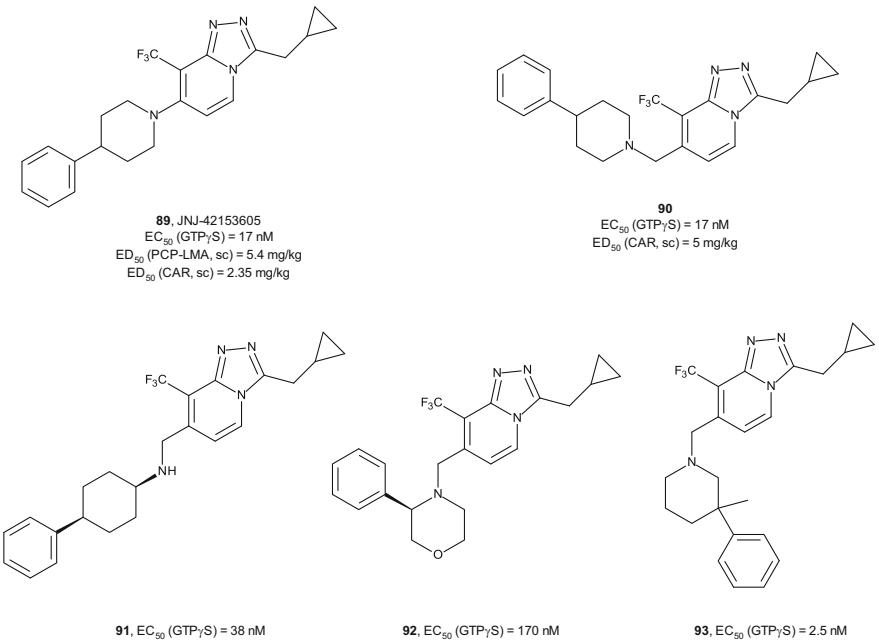


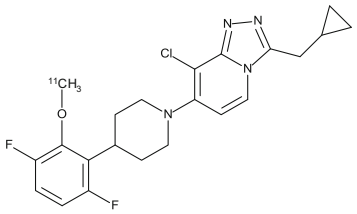

**Fig. 37** Representative mGlu2 receptor PAM triazolopyridines containing arylpiperidine-type of substitutions from Janssen/Addex (**89**, **90**) and Janssen (**91**-**93**)

sleep duration after administration of an oral dose of 3 mg/kg. Elongation of the phenylpiperidine moiety by the introduction of a methylene spacer (**90**, Fig. 37), as well as changes on the geometry around the piperidine ring, was permitted for potency (**91** [145], **92** [146], **93** [147], Fig. 37).

Interestingly, this series served as template for developing potential radiolabeled mGlu2 receptor PAM PET ligands [148]. Thus, [$^{11}$C] JNJ-42491293 (**94**, Fig. 38) has been reported as PET tracer for measuring mGlu2 receptor availability in humans that may be suitable for assessment of occupancy by mGlu2 receptor PAM drug candidates [149].

Two additional subfamilies have also been broadly explored: pyridyloxyphenyl-triazolopyridines (**95**, Fig. 39) [150] and heterobicyclic-triazolopyridines (**96**, **97**, Fig. 39) [151].

Both subfamilies delivered very potent and selective mGlu2 receptor PAM compounds. Interestingly, some examples in these patent applications are described




**94,** [$^{11}$C] JNJ-42491293
$IC_{50}$ = 9.2 nM

**Fig. 38** Representative mGlu2 receptor PAM PET ligand from Janssen

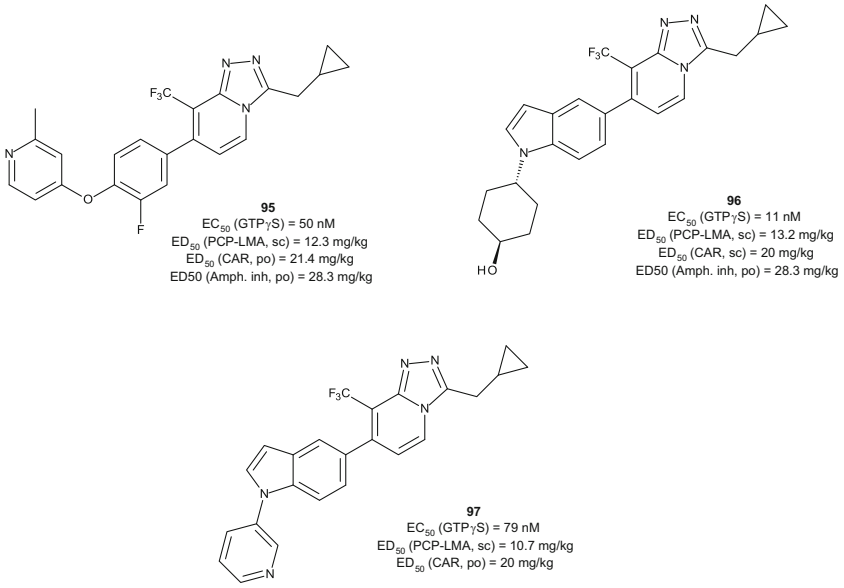
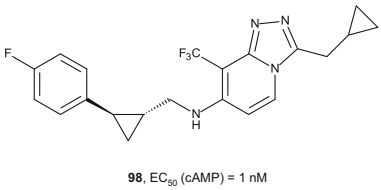



**Fig. 39** Representative mGlu2 receptor PAM aryl-substituted triazolopyridines from Janssen/Addex

to be active in models predictive for antipsychotic activity. Thus, compounds **96** and **97** inhibited PCP-induced hyperlocomotion in mice after subcutaneous administration, with ED50 values of 13.2 and 10.7 mg/kg, respectively. Compounds **96** and **97** were also shown to be active in the conditioned avoidance response (CAR) test in rats, with $ED_{50}$ values of 20 mg/kg after s.c. administration for **97** and p.o. dose for **98**. Compound **98** was also mentioned to dose-dependently decrease REM sleep (lowest active dose 10 mg/kg, p.o.) in a sw-EEG test in rats.

Finally, Bristol–Myers Squibb has very recently disclosed similar 1,2,4-triazolopyridines having secondary amines at the $C_7$ position leading to molecules with nanomolar activity in a cAMP biological assay, such as compound **98** (Fig. 40) [152].




**98**, $EC_{50}$ (cAMP) = 1 nM

**Fig. 40** Representative mGlu2 receptor PAM triazolopyridines substituted at $C_7$ with aminoalkyl radicals from Bristol–Myers Squibb

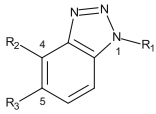



**Fig. 41** General formula of mGlu2 receptor PAM benzotriazoles from Merck

## *5.14 Benzotriazoles*

A family of 1,2,3-benzotriazoles was disclosed as potent mGlu2 receptor PAMs in a series of eight patent applications from Merck published between 2010 and 2012 [153–160].

This benzotriazole series is closely related to a series of benzazole mGlu2 receptor potentiators also disclosed by Merck in 2006. In this series Br, Cl or $CF_3$ at the $C_4$ position and a pendant lipophilic alkyl chain from $N_1$ are preferred groups (Fig. 41). A large variety of diverse $R_3$substituents is tolerated at position $C_5$.

## *5.15 (Aza)benzimidazolones*

More recently, Merck published a series of patent applications detailing series of benzimidazolone and aza-benzimidazolone derivatives as mGlu2 receptor PAMs (Fig. 42). As in similar chemotypes published by Merck (e.g. 1,2,3-benzotriazoles), substituents at $N_1$ are lipophilic groups with the *tert*-butylmethyl group largely preferred and most of the structural diversity coming from the exploration of substituents at the $C_5$ position. Some representative examples of $C_5$ substitutions are 3-substituted piperidines (**99**) [161, 162], aryl- or heteroaryl groups, which in most examples are additionally substituted at the meta position by a methylene-spaced cyclic amine (**100**) [163–165].

1,3-Dihydro-2,1,3-benzothiadiazole-2,2-dioxides have also been reported suitable scaffolds for attaching the preferred decorations identified in the previous (aza)benzimidazolones(**101**, Fig. 42) [166]. Preferred examples in the corresponding patent applications were very potent in both FLIPR and GTPγS.

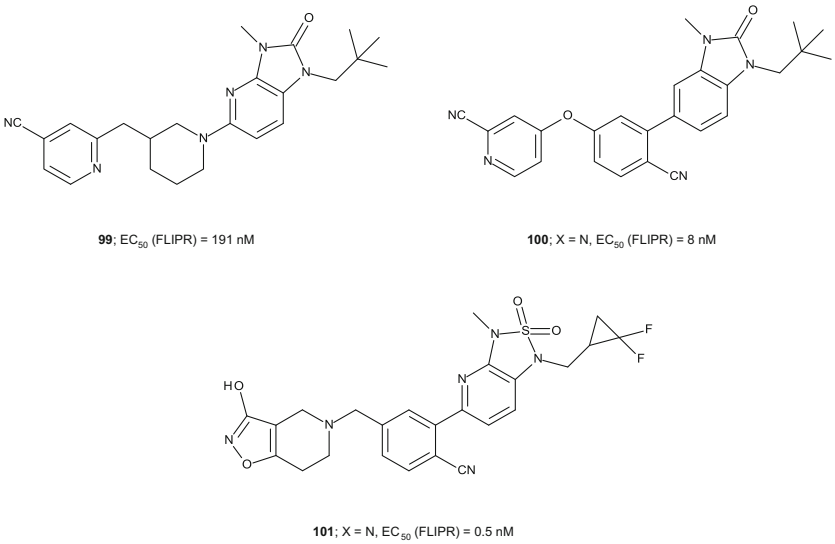



**Fig. 42** mGlu2 receptor PAM (aza)-benzimidazolones and benzothiadiazole-2,2-dioxides from Merck

## 6 Summary

Current antipsychotic therapies are largely efficacious for positive symptoms of schizophrenia; however, compounds with improved efficacy for negative symptoms and cognitive dysfunction associated with the disease are awaited. High rates of weight gain and metabolic syndrome associated with the atypical antipsychotics highlight the need for treatments with improved safety and tolerability profiles. The first decade of the twenty-first century has seen a significant discovery and early development effort around modulation of the mGlu2 receptor, in the hope of identifying safer and more effective antipsychotics. The first generation of mGlu2 receptor activators were conformationally constrained analogues of glutamate acting as agonists at both mGlu2 and mGlu3 receptors. These orthosteric mGlu2/mGlu3 receptor agonists have produced some very encouraging results that served to accumulate evidence to support potential utility of the target for the treatment of schizophrenia and other CNS disorders. Targeting the mGlu2 receptor with a PAM may offer advantages over orthosteric ligands such as improved selectivity, increased chemical tractability and better tolerability. Thus, the emerging field of positive allosteric modulation of mGlu2 receptor offers a very selective avenue for therapeutic intervention in neurological and psychiatric disorders. A huge effort in medicinal chemistry throughout the pharmaceutical industry has resulted in the identification of multiple structurally distinct mGlu2 receptor PAM chemotypes. Some analogues have served to provide preclinical proof of concept that PAMs can mimic the effects of orthosteric agonists. Disappointedly, clinical proof-of-concept

studies conducted with LY2140023, the prodrug of an orthosteric mixed mGlu2/mGlu3 agonist and AZD8529, an mGlu2 receptor PAM, failed to show conclusive evidence of efficacy in patients with schizophrenia, although the compounds were well tolerated. In an exploratory phase IIa study in schizophrenia, JNJ-40411813, however, met the primary objectives of safety and tolerability, and patients with residual negative symptoms were identified as the subgroup of patients that may serve best from add-on treatment with an mGlu2 PAM; future proof-of-concept studies are awaited to confirm these findings.

All in all, the question for the right patient population that may be benefit from treatment with an mGlu2 modulator is still open.

Further studies will likely contribute to the fundamental understanding of the pharmacology of mGlu2 selective receptor activation and, more importantly, to assess the potential of the mGlu2 receptor as a viable target for the treatment of CNS diseases.

## References

1. Rossler W, Joachim SH, van Os J et al (2005) Size of burden of schizophrenia and psychotic disorders. Eur Neuropsychopharmacol 15:399–409
2. Macdonald GJ, Bartolomé JM (2010) A decade of progress in the discovery and development of 'Atypical' antipsychotics. Prog Med Chem 49:37–80
3. Green MF (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry 153:321–330
4. Davidson M, Galderisi S, Weiser M et al (2009) Cognitive effects of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: a randomized, open-lab clinical trial (EUFEST). Am J Psychiatry 166:675–682
5. Green MF, Kern RS, Heaton RK et al (2004) Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophr Res 72:41–51
6. Carpenter WT Jr, Buchanan RW, Conley RR (1998) Pharmacotherapy of schizophrenia. In: Pharmacological management of neurological and psychiatric disorders. McGraw-Hill, New York, pp 27–51
7. Newcomer JW (2005) Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 19(Suppl 1):1
8. Miyamoto S, Duncan GE, Marx CE et al (2005) Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol Psychiatry 10:79–104
9. Lahti AC, Weiler MA, Tamara Michaelidis BA et al (2001) Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 25:455–467
10. Conn PJ, Lindsley CW, Jones CK (2008) Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. Trends Pharmacol Sci 30:25–31
11. Moghaddam B (2004) Targeting metabotropic glutamate receptors for treatment of the cognitive symptoms of schizophrenia. Psychopharmacology 174:39–44
12. Vinson PN, Conn PJ (2012) Metabotropic glutamate receptors as therapeutic targets for schizophrenia. Neuropharmacology 62:1461–1472
13. Theberge J, Bartha R, Drost DJ et al (2002) Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. Am J Psychiatry 159:1944–1946

14. Theberge J, Al-Semann Y, Williamson PC et al (2003) Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. Am J Psychiatry 160:2231–2233
15. Marsman A, van den Heuvel MP, Klomp DWJ et al (2013) Glutamate in schizophrenia: a focused review and meta-analysis of 1H-MRS studies. Schizophr Bull 39:120–129
16. Schosser A, Aschauer HN (2004) In search of susceptibility genes for schizophrenia. Wiener Klinische Wochenschrift 116:27–833
17. Makino C, Shibata H, Ninomiya H et al (2005) Identification of single-nucleotide polymorphisms in the human *N*-methyl-D-aspartate receptor subunit NR2D gene, GRIN2D, and association study with schizophrenia. Psychiatr Genet 15:215–221
18. Lisman JE, Coyle JT, Green RW et al (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci 31:234–242
19. Kew JN, Kemp JA (2005) Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology 179:4–29
20. Brauner-Osborne H, Wellendorph P, Jensen AA (2007) Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. Curr Drug Targets 8:169–184
21. Schoepp DD, Jane DE, Monn JA (1999) Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 38:1431–1476
22. Swanson CJ, Bures M, Johnson MP et al (2005) Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov 4:131–144
23. Fell MJ, Svensson KA, Johnson BG et al (2008) Evidence for the role of mGlu2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist LY404039. J Pharm Exp Ther 326:209–217
24. Woolley ML, Pemberton DJ, Bate S et al (2008) The mGlu2 but not the mGlu3 receptor mediates the actions of the mGlu2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. Psychopharmacology 196:431–440
25. Monn JA, Valli MJ, Massey SM et al (1997) Design, synthesis, and pharmacological characterization of (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740): a potent, selective, and orally active group 2 metabotropic glutamate receptor agonist possessing anticonvulsant and anxiolytic properties. J Med Chem 40:528–537
26. Pellicciari R, Marinozzi M, Natalini B et al (1996) Synthesis and pharmacological characterization of all sixteen stereoisomers of 2-(2′-carboxy-3′-phenylcyclopropyl)glycine. Focus on (2S,1′S,2′S,3′R)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine, a novel and selective group II metabotropic glutamate receptors. J Med Chem 39:2259–2269
27. Pellicciari R, Costantino G, Marinozzi M et al (2001) Design, synthesis and preliminary evaluation of novel 3′-substituted carboxycyclopropylglicines as antagonists at group 2 metabotropic glutamate receptors. Bioorg Med Chem Lett 11:3179–3182
28. Fell MJ, McKinzie DL, Monn JA et al (2012) Group II metabotropic glutamate receptor agonists and positive allosteric modulators as novel treatments for schizophrenia. Neuropharmacology 62:1473–1483
29. Moghaddam B, Adams BW (1998) Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 281:1349–1352
30. Cartmell J, Schoepp DD (2000) Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75:889–907
31. Imre G (2007) The preclinical properties of a novel group II metabotropic glutamate receptor agonist LY379268. CNS Drug Rev 13:444–464
32. Patil ST, Zhang L, Martenyi F et al (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase II clinical trial. Nature Med 13:1102–1107
33. Kinon BJ, Gómez JC (2013) Clinical development of pomaglumetad methionil: A non-dopaminergic treatment for schizophrenia. Neuropharmacology 66:82–86

34. Kinon BJ, Zhang L, Millen BA et al (2011) A multicenter, inpatient, Phase II, double-blind, placebo-controlled dose-ranging study of LY2140023 monohydrate in patients with DSM-IV schizophrenia. J Clin Psychopharmacol 31:349–355
35. Stauffer VL, Millen BA, Andersen S et al (2013) Pomaglumetad methionil: no significant difference as an adjunctive treatment for patients with prominent negative symptoms of schizophrenia compared to placebo. Schizophr Res 150:434–441
36. Eli Lilly and Co. (2012) Lilly Stops Phase III development of pomaglumetad methionil for the treatment of Schizophrenia based on efficacy results. https://investor.Lilly.com/releaseDetail.cfm ReleaseID=703018
37. Galici R, Echemendia NG, Rodriguez AL et al (2005) A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. J Pharm Exp Ther 315:1181–1187
38. Conn PJ, Christopoulos A, Lindsley CW (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat Rev Drug Discov 8:41–54
39. Johnson MP, Baez M, Jagdmann GE 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-trifluoroethylsulfonyl)pyrid-3-yl-methylamine. J Med Chem 46:3189–3192
40. Johnson MP, Barda D, Britton TC et al (2005) Metabotropic glutamate 2 receptor potentiators: receptor modulation, frequency-dependent synaptic activity, and efficacy in preclinical anxiety and psychosis model(s). Psychopharmacology 179:271–283
41. Rodriguez D (2004) Attenuation of ketamine-induced hyperactivity responses in rats following administration of a novel metabotropic glutamate receptor 2 selective positive modulator. In: Proceedings of the annual meeting of the society for neuroscience; Oct 23–27; Abstract number 798.8, Dallas, TX. Society for Neuroscience, Washington, DC
42. Galici R, Jonas CK, Hemstapat 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 Pharm Exp Ther 318:173–185
43. Bonnefous C, Vernier JM, Hutchinson JH et al (2005) Biphenyl-indanones: allosteric potentiators of metabotropic glutamate subtype 2 receptor. Bioorg Med Chem Lett 15:4354–4358
44. Pinkerton AB, Vernier JM, Schaffhauser H et al (2004) Phenyl-tetrazolyl acetophenones: discovery of allosteric potentiators for the metabotropic glutamate 2 receptor. J Med Chem 47:4595–4599
45. Pinkerton AB, Cube RV, Hutchinson JH et al (2004) Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 1: identification and synthesis of phenyl-tetrazolyl acetophenones. Bioorg Med Chem Lett 14:5329–5332
46. Pinkerton AB, Cube RV, Hutchinson JH et al (2004) Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 2: 4-thiopyridyl acetophenones as non-tetrazole-containing mGlu2 receptor potentiators. Bioorg Med Chem Lett 14:5867–5872
47. Cube RV, Vernier JM, Hutchinson JH et al (2005) 3-(2-ethoxy-4-{4-[3-hydroxy-2-methyl-4-(3-methylbutanoyl)phenoxy] butoxy}phenylpropanoic acid: a brain penetrant allosteric potentiator at the metabotropic glutamate receptor 2 (mGluR2). Bioorg Med Chem Lett 15:2389–2393
48. Govek SP, Bonnefous C, Hutchinson JH et al (2005) Benzazoles as allosteric potentiators of metabotropic glutamate receptor 2 (mGluR2): efficacy in animal model for schizophrenia. Bioorg Med Chem Lett 15:4068–4072
49. Cid JM, Duvey G, Cluzeau P et al (2010) Discovery of 1,5-disubstituted pyridines: a new class of positive allosteric modulators of the metabotropic glutamate 2 receptor. ACS Chem Neurosci 1:788–795
50. Cid JM, Duvey G, Tresadern G et al (2012) Discovery of 1,4-disubstituted 3-cyano-2-pyridones: a new class of positive allosteric modulators of the metabotropic glutamate 2 receptor. J Med Chem 55:2388–2405

51. Clayton J, Ma F, Van Wagenen B et al (2006) Preparation of isoindolones as metabotropic glutamate receptor potentiators. WO 2006/020,879
52. Van Wagenen B, Ukkiramapandian R, Clayton J et al (2007) Preparation of metabotropic glutamate-receptor-potentiating isoindolones. WO 2007/021,308
53. Balestra M, Bunting H, Chen D et al Preparation of pyrazolones as metabotropic glutamate receptor agonists for the treatment of neurological and psychiatric disorders. WO 2006/071730, 6 July 2006.
54. Imogai H, Duvey GAJ, Cid-Núñez JM et al Novel thieno-pyridine and thieno-pyrimidine derivatives and their use as positive allosteric modulators of mGlu2-receptors. WO 2006/030031, 23 Mar 2006.
55. Duplantier AJ, Efremov I, Candler J et al (2009) 3-Benzyl-1,3-oxazolidin-2-ones as mGluR2 positive allosteric modulators: hit to lead and lead optimization. Bioorg Med Chem Lett 19:2524–2529
56. Brnardic EJ, Fraley ME, Garbaccio RM et al (2010) 3-Aryl-5-phenoxymethyl-1,3-oxazolidin-2-ones as positive allosteric modulators of mGluR2 for the treatment of schizophrenia: hit-to-lead efforts. Bioorg Med Chem Lett 20:3129–3133
57. Zhang L, Rogers BN, Duplantier AJ et al (2008) 3-(Imidazolyl methyl)-3-aza-bicyclo [3.1.0] hexan-6-yl)methyl ethers: a novel series of mGluR2 positive allosteric modulators. Bioorg Med Chem Lett 18:5493–5496
58. D'Alessandro PL, Corti C, Roth A et al (2010) The identification of structurally novel, selective, orally bioavailable positive allosteric modulators of mGluR2. Bioorg Med Chem Lett 20:759–762
59. Zhang L, Brodney MA, Candler J et al (2011) 1-[(1-Methyl-1H-imidazol-2-yl)methyl]4-phenylpiperidines as mGluR2 positive allosteric modulators for the treatment of psychosis. J Med Chem 54:1724–1739
60. Cao B, Gurunian V, Kongsamut S et al (2008) Substituted dihydro- and tetrahydrooxazolopyrimidones, preparation and use as metabotropic glutamate receptor modulators. WO 2008/112,483
61. Sheffler DJ, Pinkerton AB, Dahl R et al (2011) Recent progress in the synthesis and characterization of group II metabotropic glutamate receptor allosteric modulators. ACS Chem Neurosci 2:382–393
62. Rudd MT, McCauley JA (2005) Positive allosteric modulators of the metabotropic glutamate receptor subtype 2 (mGluR2). Curr Top Med Chem 5:869–884
63. Fraley ME (2009) Positive allosteric modulators of the metabotropic glutamate receptor 2 for the treatment of schizophrenia. Expert Opin Ther Patents 19:1259–1275
64. Marek, GJ, Kinon BJ, McKinzie DL et al (2012) Metabotropic glutamate 2/3 receptor agonists and positive allosteric modulators of metabotropic glutamate receptor 2 as novel agents for the treatment of schizophrenia. Targets and Emerging Therapies for Schizophrenia 143–185
65. Trabanco AA, Cid JM, Lavreysen H et al (2011) Progress in the development of positive allosteric modulators of the metabotropic glutamate receptor 2. Curr Med Chem 18:47–68
66. Trabanco AA, Cid JM (2013) mGluR2 positive allosteric modulators (PAMs): a patent review (2009 - present). Expert Opin Ther Patents 23:629–647
67. ClinicalTrials.gov (2008) ADZ8529 single ascending dose study (SAD). http://clinicaltrials.gov/show/ NCT00755378
68. ClinicalTrials.gov (2009) Study to assess the efficacy, safety, and tolerability of ADZ8529 in adult schizophrenia patients. http://clinicaltrials.gov/ show/NCT00921804
69. The effects ADZ8529 on cognition and negative symptoms in schizophrenics (2009). http://clinicaltrials.gov/ show/NCT00986531
70. Litman RE (2013) AZD8529, A positive allosteric modulator at the mGluR2 receptor, does not improve symptoms in schizophrenia: a proof of principle study NCDEU An Annual Meeting of the ASCP Westin Diplomat, Hollywood Florida, US May 28–31

71. Study to explore the safety, tolerability and potential clinical efficacy of JNJ-40411813 in schizophrenic patients (2011). http://clinicaltrials.gov/show/NCT01323205
72. Lavreysen H (2013) Discovery and early clinical development of novel mGlu2 receptor PAMs. American College of Neuropsychopharmacology (ACNP) 52nd Annual Meeting, Hollywood, Florida, US, December 10
73. Study to evaluate the efficacy and overall safety and tolerability of treatment with adjunctive JNJ 40411813 compared to placebo in patients with MDD with anxiety symptoms being treated with an antidepressant. http://clinicaltrials.gov/show/NCT01582815
74. Britton T, Barda DA, Hornback WJ et al (2002). Selective, non-amino acid allosteric potentiators of mGlu2 receptors. 223rd ACS National Meeting, Orlando, Florida, US, April 7-11.
75. Barda DA, Wang Z-Q, Britton TC, Henry SS et al (2004) SAR study of a subtype selective allosteric potentiator of metabotropic glutamate 2 receptor, N-(4-phenoxyphenyl)-N-(3-pyridinylmethyl)ethanesulfonamide. Bioorg Med Chem Lett 14:3099–3102
76. Schaffhauser H, Rowe BA, Morales S et al (2003) Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. Mol Pharmacol 64:798–810
77. Harich S, Gross G, Bespalov A (2007) Stimulation of the metabotropic glutamate 2/3 receptor attenuates social novelty discrimination deficits induced by neonatal phencyclidine treatment. Psychopharmacology 192:511–519
78. Nikiforuk A, Popik P, Drescher KU et al (2010) Effects of a positive allosteric modulator of group II metabotropic glutamate receptors, LY487379, on cognitive flexibility and impulsive-like responding in rats. J Pharm Exp Ther 335:665–673
79. Khilevich A, Liu B, Mayhugh DR et al (2010) Imidazolecarboxamide derivatives as mGlu2 receptor potentiators and their preparation, pharmaceutical compositions and use in the treatment of depression. WO 2010/009,062
80. Fell MJ, Witkin JM, Falcone JF et al (2011) N-(4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyryl)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 Pharm Exp Ther 336:165–177
81. Johnson PL, Fitz SD, Engleman EA et al (2013) Group II metabotropic glutamate receptor type 2 allosteric potentiators prevent sodium lactate-induced panic-like response in panic-vulnerable rats. J Psychopharmacol 27:152–161
82. Pinkerton AB, Vernier JM, Cube RV et al (2006) Preparation of indanone potentiators of metabotropic glutamate receptors for use against neurol. and psychiatric disorders. WO 2006/015,158
83. Pinkerton, AB, Vernier JM, Cube RV et al (2006) Preparation of heterocyclic indanone potentiators of metabotropic glutamate receptors for the treatment of neurological and psychiatric disorders. WO 2006/047,237
84. Pinkerton AB, Cube RV, Hutchinson JH et al (2005) Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 3: identification and biological activity of indanone containing mGlu2 receptor potentiators. Bioorg Med Chem Lett 15:1565–1571
85. Lorrain DS, Schaffhauser H, Campbell UC 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
86. Benneyworth MA, Xiang Z, Smith RL et al (2007) A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. Mol Pharmacol 72:477–484
87. Ahnaou A, Dautzenberg FM, Geys H et al (2009) Modulation of group II metabotropic glutamate receptor (mGlu2) elicits common changes in rat and mice sleep-wake architecture. Eur J Pharmacol 603:62–72

88. Hackler EA, Byun NE, Jones CK et al (2010) Selective potentiation of the metabotropic glutamate receptor subtype 2 blocks phencyclidine-induced hyperlocomotion and brain activation. Neuroscience 168:209–218
89. Hikichi H, Kaku A, Karasawa J et al (2013) Stimulation of metabotropic glutamate (mGlu) 2 receptor and blockade of mGlu1 receptor improve social memory impairment elicited by MK-801 in rats. J Pharm Sci 122:10–16
90. Govek SP, Vernier JM, Kamenecka T et al (2006) Preparation of benzazole potentiators of metabotropic glutamate receptors. WO 2006/091,496
91. Van Wagenen B, Ukkiramapandian R, Clayton J et al (2007) Substituted isoindolones as metabotropic glutamate receptor potentiators, their preparation, pharmaceutical compositions, and use in therapy. WO 2007/021,309
92. Clayton J, Egle I, Empfield J et al (2008) Preparation of metabotropic glutamate receptor oxadiazole ligands and their use as potentiators. WO 2008/150,232
93. Clayton J, Egle I, Empfield J et al (2008) Preparation of oxadiazole derivatives as metabotropic glutamate receptor potentiators. WO 2008/150,233
94. Blade H, Cosgrove SD (2011) Polymorphs of metabotropic glutamate receptor positive allosteric modulator. WO 2011/136,723
95. Boyd A, Fielding MR, Ford JG et al (2011) Process for preparing 7-methyl-5-(3-piperazin-1-ylmethyl-[1,2,4]oxadiazol-5-yl)-2-(4-trifluoromethoxybenzyl)-2,3-dihydroisoindol-1-one, a metabotropic glutamate receptor positive allosteric modulator. WO 2011/084,098
96. Clayton J, Empfield J, Folmer J et al (2008) Preparation of oxadiazole derivatives and their use as metabotropic glutamate receptor potentiators. US 2008/0,306,077
97. Van Wagenen B, Ukkiramapandian R, Clayton J et al (2011) Preparation of substituted isoindolones as metabotropic glutamate receptor potentiators and useful in the treatment of schizophrenia. US 2011/7,868,008
98. Cacciola J, Empfield J, Folmer J et al (2009) Preparation of isoxazole derivatives and their use as metabotropic glutamate receptor potentiators. WO 2009/148,403
99. Slassi A, Isaac M, Clayton J et al (2008) Preparation of aza-isoindolones as metabotropic glutamate receptor potentiators. WO 2008/100,715
100. Egle I, Slassi A, Isaac M et al (2008) Preparation of hydrazides as metabotropic glutamate receptor potentiators. WO 2008/130,853
101. Geneste H, Sauer D, Braje W et al (2008) Preparation of heterocyclic compounds, in particular pyrazole derivatives as positive modulators of metabotropic glutamate receptor 2 (mGlu2 receptor). WO 2008/145,616
102. Braje W, Jantos K, Geneste H et al Novel small molecule potentiators of metabotropic glutamate receptors. US 2011/0,245,232
103. Braje W, Jantos K, Geneste H et al (2011) Novel small molecule potentiators of metabotropic glutamate receptors. US 2011/0,245,247
104. Adam J, Gillespie J, Laats S et al (2011) Preparation of isoindolinylbenzylimidazolidine-2,4-dione derivatives as mGlu2 receptor modulators. WO 2011/051,490
105. Cosford NDP, Panickar DR, Sidique S (2011) Preparation of cycloalkyloxoisoindolinylbiphenylcarboxylic acid derivatives and analogs for use as metabotropic glutamate subtype 2 receptor positive allosteric modulators. WO 2011/116,356
106. Dhanya R-P, Sidique S, Sheffler DL et al (2011) Design and synthesis of an orally active metabotropic glutamate receptor subtype-2 (mGluR2) positive allosteric modulator (PAM) that decreases cocaine self-administration in rats. J Med Chem 54:342–353
107. Sidique S, Dhanya R-P, Sheffler DL et al (2012) Orally active metabotropic glutamate subtype 2 receptor positive allosteric modulators: structure-activity relationships and assessment in a rat model of nicotine dependence. J Med Chem 55:9434–9445
108. Imogai H, Cid-Núñez JM, Duvey GAJ et al (2006) Novel pyridinone derivatives and their preparation, pharmaceutical compositions, and use as positive allosteric modulators of mGlu2 receptors for treatment of various neurological and psychiatric disorders. WO 2006/030,032

109. Trabanco AA, Duvey G, Cid JM et al (2011) New positive allosteric modulators of the metabotropic glutamate receptor 2 (mGluR2): Identification and synthesis of N-propyl-8-chloro-6-substituted isoquinolones. Bioorg Med Chem Lett 21:971–976
110. Trabanco AA, Duvey G, Cid JM et al (2011) New positive allosteric modulators of the metabotropic glutamate receptor 2 (mGluR2) Identification and synthesis of *N*-propyl-5-substituted isoquinolones. Med Chem Commun 2:132–139
111. Isaac M, Slasi A, Egle I et al (2007) Preparation of tricyclic benzimidazoles and their use as metabotropic glutamate receptor modulators. US 2007/0,032,469
112. Egle I, Isaac M, Urbanek R et al (2007) Preparation of benzimidazole derivatives and their use as metabotropic glutamate receptor potentiators. WO 2007/115,077
113. Berke H, Duplantier AJ, Efremov I et al (2007) Benzimidazolyl compounds as mGluR2 agonists and their preparation, pharmaceutical compositions and use in the treatment of diseases. WO 2007/135,527
114. Efremov IV, Rogers BN, Duplantier AJ et al (2008) Azabenzimidazolyl compounds as potentiators of mGlu2 subtype of glutamate receptor and their preparation, pharmaceutical compositions and use in the treatment of diseases. WO 2008/012,622
115. Efremov IV, Rogers BN, Duplantier AJ et al (2008) Benzimidazolyl compounds as potentiators of mGlu2 subtype of glutamate receptor and their preparation, pharmaceutical compositions and use in the treatment of diseases. WO 2008/012,623
116. Berke H, Duplantier AJ, Efremov IV et al (2007) Preparation of azabenzimidazoles as glutamate mGluR2 agonists. WO 2007/135,529
117. Slassi A, Joseph B, Ma F et al (2007) Oxazolidinone compounds and their use as metabotropic glutamate receptor potentiators in the treatment of neurological and psychiatric disorders and preparation. WO 2007/078,523
118. Isaac M, Slassi A, Egle I et al (2008) Preparation of spiro-oxazolidinone compounds and their use as metabotropic glutamate receptor potentiators. WO 2008/032,191
119. Duplantier AJ, Efremov IV, Zhang L et al (2009) Preparation of *N*-Benzyl oxazolidinones and related heterocycleic compounds as potentiators of glutamate receptors. WO 2009/004,430
120. Brnardic EJ, Fraley M, Garbaccio R et al (2009) Preparation of 3,5-substituted-1,3-oxazolidin-2-one derivatives as potentiators of metabotropic glutamate receptors. WO 2009/094,265
121. Brnardic EJ, Fraley M, Layton M et al (2010) Preparation of 3,5-substituted-1,3-oxazolidin-2-one derivatives as potentiators of metabotropic glutamate receptors. US 2010/0,292,241
122. Brnardic EJ, Converso A, Fraley M et al (2009) Preparation of oxazolobenzimidazole derivatives as potentiators of metabotropic glutamate receptors. WO 2009/140,163
123. Brnardic EJ, Fraley M, Garbaccio RM (2009) Oxazolobenzimidazole derivatives as mGluR inhibitors and their preparation, pharmaceutical compositions and use in the treatment of diseases. WO 2009/140,166
124. Garbaccio RM, Brnardic EJ, Fraley ME et al (2010) Discovery of oxazolobenzimidazoles as positive allosteric modulators for the mGlu2 receptor ACS Med Chem Lett 1:406–410
125. Cao B, Gurunian V, Kongsamut S et al (2008) Substituted dihydro- and tetrahydro oxazolopyrimidinones, preparation and use as metabotropic glutamate receptor modulator. WO 2008/112,483
126. Kosley RW Jr., Sher R (2009) Cycloalkyloxazolopyrimidinone derivatives as metabotropic glutamate receptors modulators and their preparation, pharmaceutical compositions and use in the treatment of central nervous system disorders. WO 2009/110,901
127. George P, Hall D, Hartung R et al (2011) Preparation of biphenyloxymethyldihydrooxazolopyrimidone derivatives for use as metabotropic glutamate receptor modulators. WO 2011/034,828
128. Kosley RW Jr., Sher R (2011) Preparation of dihydrobenzocycloalkyloxymethyloxazolopyrimidinone derivatives for use as metabotropic glutamate receptor modulators. WO 2011/034,830

129. Kosley RW Jr., Sher R, Neuenschwander KW et al (2011) Preparation of phenoxymethyldihydrooxazolopyrimidinone derivatives for use as metabotropic glutamate receptor modulators. WO 2011/034,832
130. Imogai HJ, Cid-Núñez JM, Andrés-Gil JI et al (2007) 1,4-Disubstituted 3-cyanopyridone derivatives and their use as positive allosteric modulators of mGlu2-receptors and their preparation. WO 2007/104,783
131. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ et al (2008) Preparation of 1,4-disubstituted 3-cyanopyridone derivatives as positive mGlu2 receptor modulators. WO 2008/107,480
132. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ et al (2008) Preparation of 3-cyano-4-(4-tetrahydropyranphenyl)pyridin-2-one derivatives as positive mGlu2 receptor modulators. WO 2008/107,481
133. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ et al (2008) Preparation of 3-cyano-4-(4-phenylpiperidin-1-yl)-pyridin-2-one derivatives for treating and preventing neurol. and psychiatric diseases associated with glutamate dysfunction. WO 2008/107,479
134. Lavreysen H, Langlois X, Ahnaou A et al (2013) Pharmacological characterization of JNJ-40068782, a new potent, selective, and systemically active positive allosteric modulator of the mGlu2 receptor and its radioligand [$^3$H]JNJ-40068782. J Pharmacol Exp Ther 346:514–527
135. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ et al (2009) Preparation of disubstituted pyridin-2-ones as positive allosteric modulators of mGluR2 for treating neurological and psychiatric disorders. WO 2009/033,702
136. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ et al (2009) Preparation of 1,3-disubstituted-4-phenyl-1H-pyridin-2-ones as positive allosteric modulators of mGluR2 for treating neurological and psychiatric disorders. WO 2009/033,703
137. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ et al (2009) Preparation of bipyridinones as positive allosteric modulators of mGluR2 for treating neurological and psychiatric disorders. WO 2009/033,704
138. Cid-Núñez JM, Trabanco-Suárez AA (2010) Preparation of indole and benzomorpholine derivatives as modulators of metabotropic glutamate receptors for treating neurological and psychiatric disorders. WO 2010/043,396
139. Trabanco-Suárez AA, Tresadern GJ, Vega-Ramiro JA et al (2009) Preparation of imidazopyridine derivatives for use as mGlu2 receptor modulators. WO 2009/062,676
140. Tresadern G, Cid JM, Macdonald GJ et al (2010) Scaffold hopping from pyridines to imidazo [1,2-a]pyridines. New positive allosteric modulators of metabotropic glutamate 2 receptor. Bioorg Med Chem Lett 20:175–179
141. Cid-Núñez JM, Trabanco-Suárez AA, Macdonald GJ (2010) Preparation of indole and benzoxazine derivatives as modulators of metabotropic glutamate receptors for treating neurological and psychiatric disorders. WO 2010/060,589
142. Trabanco AA, Tresadern G, Macdonald GJ et al (2012) Imidazo[1,2-a]pyridines: orally active positive allosteric modulators of the metabotropic glutamate receptor 2. J Med Chem 55:2688–2701
143. Cid-Núñez JM, Oehlrich D, Trabanco-Suárez AA et al (2010) Preparation of 1,2,4- triazolo [4,3-a]pyridines for the treatment or prevention of neurological and psychiatric disorders. WO 2010/130,424
144. Cid JM, Tresadern G, Vega JA et al (2012) Discovery of 3-cyclopropylmethyl-7-(4- phenylpiperidin-1-yl)-8-trifluoromethyl[1,2,4]triazolo[4,3a] pyridine (JNJ-42153605): a positive allosteric modulator of the metabotropic glutamate 2 receptor. J Med Chem 55:8770–8789
145. Cid-Núñez JM, Trabanco-Suárez AA, Oehlrich D et al (2012) Preparation of 1,2,4-triazolo [4,3-a]pyridine derivatives and their use as positive allosteric modulators of mGlu2 receptors for treating neurological disorders and psychiatric disorders. WO 2012/062,750

146. Cid-Núñez JM, Trabanco-Suárez AA, Vega-Ramiro JA et al (2012) Preparation of 1,2,4-triazolo[4,3-a]pyridine derivatives and their use as positive allosteric modulators of mGlu2 receptors for treating neurological disorders and psychiatric disorders. WO 2012/062,751
147. Cid-Núñez JM, Trabanco-Suárez AA, Vega-Ramiro JA et al (2012) Preparation of 1,2,4-triazolo[4,3-a]pyridine derivatives and their use as positive allosteric modulators of mGlu2 receptors for treating neurological disorders and psychiatric disorders. WO 2012/062,759
148. Andrés JI, Alcazar J, Cid JM et al (2012) Synthesis, evaluation, and radiolabeling of new potent positive allosteric modulators of the metabotropic glutamate receptor 2 as potential tracers for positron emission tomography imaging. J Med Chem 55:8685–8699
149. Schmidt M (2012) Human PET imaging with [$^{11}$C]JNJ-42491293, a tracer for the mGluR2 positive allosteric site. J Cereb Blood Flow Metab 32:S13–S40. doi:10.1038/jcbfm.2012.77
150. Cid-Núñez JM, De Lucas-Olivares AI, Trabanco-Suárez AA et al (2010) 7-Aryl-1,2,4-triazolo[4,3-a]pyridine derivatives as positive allosteric modulators of mGlu2 receptors and their preparation, pharmaceutical compositions and use in the treatment of CNS disorders. WO 2010/130,423
151. Cid-Núñez JM, De Lucas-Olivares AI, Trabanco-Suárez AA et al (2010) 1,2,4- triazolo [4,3-a]pyridine derivatives as mGlu2 modulators and their preparation and use for the treatment of CNS diseases. WO 2010/130,422
152. Mattson RJ, Meng Z (2013) Preparation of triazolo[4,3-a]pyridinamine compounds as positive allosteric modulators of mGluR2 for the treatment of neurological and psychiatric diseases. WO 2013/138,687
153. Dudkin V, Fraley M, Wang C et al (2010) Preparation of aminobenzotriazole derivatives for use as metabotropic glutamate receptor modulators. WO 2010/114,726
154. Beshore DC, Dudkin V, Kuduk S et al (2010) Preparation of biaryl benzotriazole derivatives for use as metabotropic glutamate receptor potentiators and useful in treatment of neurological and psychiatric disorders associated with glutamate dysfunction. WO 2010/141,360
155. Dudkin VY, Fraley ME, Wang C et al (2011) Preparation of ether benzotriazole derivatives for use as metabotropic glutamate receptor potentiators and useful in treatment of neurological and psychiatric disorders associated with glutamate dysfunction. WO 2011/022,312
156. Beshore DC, Dudkin V, Garbaccio RM et al (2012) Preparation of ether benzotriazole derivatives as potentiators of metabotropic glutamate receptors. US 2012/0135,977
157. Beshore DC, Garbaccio RM, Kuduk SD et al (2012) Preparation of aminomethyl biaryl benzotriazole derivatives as potentiators of mGluR2. WO 2012/151,136
158. Beshore DC, Kuduk SD (2012) Preparation of hydroxymethyl biaryl benzotriazole derivatives as potentiators of metabotropic glutamate receptors. WO 2012/151,140
159. Beshore DC, Kuduk SD (2012) Preparation of cyclohexene benzotriazole derivatives for treatment or prevention of neurological and psychiatric disorders. WO 2012/151,138
160. Kuduk SD, Skudlarek JW (2012) Preparation of alkyne benzotriazole derivatives as metabotropic glutamate receptors. WO 2012/151,139
161. Layton ME, Pero JE, Rodzinak KJ et al (2011) Preparation of imidazopyridin-2-one derivatives as potentiators of metabotropic glutamate receptors. WO 2011/034,741
162. Fiji HD, Kelly MJ, Kern JC et al (2012) Preparation of imidazopyridin-2-one derivatives as potentiators of metabotropic glutamate receptors. WO 2012/174,199
163. Arrington KL, Dudkin V, Layton ME et al (2011) Preparation of 5-substituted 1,3-dihydro-2H-imidazo[4,5-b]pyridine-2-one derivatives as positive allosteric modulators of mGluR2 therapy. WO 2011/156,245
164. Dudkin V, Fraley ME, Arrington K et al (2012) Benzimidazolone derivatives as positive allosteric modulators of mGluR2 and their preparation and use for the treatment of neurological and psychiatric disorders. WO 2012/021,382
165. Layton ME, Kelly MJ (2011) Substituted 1,3-benzothiazol-2(3H)-ones and [1,3]thiazolo [5,4-b]pyridine-2(1H)-ones as positive allosteric modulators in mGluR2. WO 2011/137,046
166. Layton ME, Kelly MJ, Hartingh TJ (2011) Preparation of benzothiadiazole and thiadiazolopyridine dioxides as positive allosteric modulators of mGluR2. WO 2011/109,277

Top Med Chem (2015) 13: 143–182
DOI: 10.1007/7355_2014_57

Published online: 29 June 2014

# Activation of the $mGlu_5$ Receptor for the Treatment of Schizophrenia and Cognitive-Deficit-Associated Disorders

**Theresa Williams, Marlene A. Jacobson, Mikhail Kalinichev, and Jean-Philippe Rocher**

**Abstract** $mGlu_5$ receptors have been implicated in the pathophysiology of schizophrenia and cognitive dysfunction, and activation of these receptors has been proposed as a potential therapeutic strategy for treating these diseases. This chapter describes the recent progress in the discovery and development of $mGlu_5$ positive allosteric modulators (PAMs) as a novel therapeutic approach which does not involve a direct interaction with dopamine receptors. This new class of molecules is structurally very diverse and includes several drug-like chemical series. The pharmacological activity shown by these molecules in preclinical studies confirms their therapeutic potential in all domains of schizophrenia, including positive and negative symptoms and cognitive abnormalities. These data support the development of $mGlu_5$ PAMs as novel antipsychotic drugs that resonate with the glutamatergic hypofunction hypothesis of schizophrenia. The fine tuning of the functional activity and the clarification of biased signaling pathways in different chemical series may provide clues to achieve efficacy and a good safety profile for novel pharmacotherapies.

**Keywords** Class C GPCR, Cognition, Glutamate, Metabotropic glutamate receptor 5, $mGlu_5$ PAM, Positive allosteric modulator, Schizophrenia

T. Williams
Harleysville, PA 19438, USA
e-mail: williamst6824@comcast.net

M.A. Jacobson (✉)
School of Pharmacy, Temple University, Philadelphia, PA 19140, USA
e-mail: marlene.jacobson@temple.edu

M. Kalinichev
H. Lundbeck A/S, Valby 2500, Denmark
e-mail: MIAL@lundbeck.com

J.-P. Rocher (✉)
Pierre Fabre Research Institute, 81106 Castres, France
e-mail: jean.philippe.rocher@pierre-fabre.com

**Contents**



## Abbreviations

| | |
|---|---|
| Ago-PAM | Agonist and positive allosteric modulator |
| $hmGlu_5$ | Human metabotropic glutamate receptor 5 |
| IP | Intraperitoneally |
| LLE | Ligand lipophilic efficiency |
| MED | Minimum effective dose |
| MPEP | 2-Methyl-6-(phenylethynyl)pyridine |
| MTEP | 3-((2-Methyl-1,3-thiazol-4-yl)ethynyl)pyridine |
| NAM | Negative allosteric modulator |
| PAM | Positive allosteric modulator |
| PO | Per os (by mouth; orally) |
| PPI | Prepulse inhibition |
| $rmGlu_5$ | Rat metabotropic glutamate receptor 5 |
| SAM | Silent allosteric modulator |
| SC | Subcutaneous |

## 1 Introduction

Schizophrenia is a complex, chronic, and debilitating psychiatric illness which affects around 1% of the world's population. Its symptoms have been clustered into three domains, termed positive, negative, and cognitive. Positive symptoms, also termed psychosis, include hallucinations, delusions, suspiciousness, and

paranoia, reflecting features that are absent in the general population. Negative symptoms include blunted affect, anhedonia, apathy, social withdrawal, and paucity of speech, reflecting features that are reduced in comparison to those seen in healthy individuals. The cognitive abnormalities include deficits in attention, executive function, learning, and short- and long-term memory.

Since the fortuitous discovery of antipsychotic properties of chlorpromazine, the pharmacological management of schizophrenia has been based on antagonists or partial agonists of dopamine $D_2$ receptor. This approach gave rise to "The dopamine hypothesis of schizophrenia." According to this hypothesis, symptoms of schizophrenia are mediated by dysregulated dopaminergic neurotransmission, including increased activity in the mesolimbic pathway and reduced activity in the mesocortical pathway. While this hypothesis offers a conceptual framework for positive symptoms of schizophrenia, it leaves the negative symptoms and cognitive abnormalities largely unexplained. Indeed, although current pharmacological intervention offers relieve from positive symptoms in most patients, improvements in negative and cognitive symptoms are largely absent, despite the fact that these symptoms are recognized as core features of schizophrenia. In fact, negative symptoms and cognitive abnormalities precede the onset of psychosis and remain the key predictors of functional disability and poor quality of life of schizophrenic patients. Also, it needs to be indicated that many schizophrenia patients do not respond to current treatments, while those that show certain improvements have to suffer from a range of adverse side effects, including sedation, weight gain, metabolic abnormalities, sexual dysfunction, and motor disturbances. Thus, there is urgent need for development of novel "antischizophrenia" medication that can offer relief in all three domains of the disease, fewer, less-severe side effects, and improved quality of life.

Even though the underlying pathophysiology of schizophrenia is still unknown, accumulating evidence from psychopharmacological, postmortem, and genetic linkage studies implicates glutamatergic neurotransmission in etiology and pathophysiology of schizophrenia. "The glutamatergic hypothesis of schizophrenia" suggests that reduced activation of the NMDA subtype of ionotropic glutamate receptors results in compensatory increase in glutamate release and neurotoxicity in the cortical areas as well as dysregulation of glutamate-dopamine interaction, offering a conceptual framework for all domains of the disease. According to this hypothesis, increases in activity at the NMDA receptor can offer therapeutic benefit to schizophrenic patients. Since glutamate is the key excitatory neurotransmitter in the mammalian brain and the NMDA receptor is ubiquitously expressed in nearly all subtypes of neurons mediating fast excitatory neurotransmission, a direct pharmacological manipulation of this receptor can produce a profound disruption in brain function and severe side effects. Therefore, search for alternative targets that can potentiate activity of the NMDA receptor indirectly and thus offer an alternative approach in novel treatment of schizophrenia has been underway. Within the recent years, the metabotropic glutamate receptor 5 ($mGlu_5$), a closely associated signaling partner of the NMDA receptor, emerged as a promising target for the treatment of schizophrenia.

## 2 Biology and Molecular Pharmacology of Metabotropic Glutamate (mGlu) Receptor 5

### 2.1 Structure and Functions

The metabotropic glutamate (mGlu) receptors are G protein-coupled receptors that share a common structural feature of seven transmembrane (7TM) spanning domains, with an extracellular N-terminus and intracellular C-terminus. mGlu receptors are members of the family C G protein-coupled receptors and possess a large extracellular domain (~560 amino acids) in the amino-terminal portion of the receptor containing the endogenous glutamate agonist binding site [1]. The extracellular domain consists of two lobes termed the Venus flytrap (VFT) with the glutamate binding site residing between the two lobes. The VFT is linked to the 7TM via a cysteine-rich region. The large extracellular binding domain of family C GPCRs is distinguished from other GPCR families, specifically family A rhodopsin-like GPCRs, where in comparison, the agonist binding sites are associated within the seven transmembrane region or within the extracellular loops connect the transmembrane spanning α-helices.

Eight subtypes of the metabotropic glutamate receptors, $mGlu_1$–$mGlu_8$, have been identified by molecular cloning [2, 3]. mGlu receptor subtypes are classified into three subgroups on the basis of their preferred coupling to intracellular signaling pathways, pharmacological profile, and overall sequence homology. Group I mGlu receptors, $mGlu_1$ and $mGlu_5$, couple to $Gq/G_{11}$ G proteins. Activation results in phospholipase C-mediated signaling and production of inositol 1,4,5-triphosphate via inositol phosphate hydrolysis and calcium release from intracellular stores. Activation of protein kinase C (PKC) and downstream effectors including mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinases (ERK), and cAMP response element-binding protein (CREB) has been reported to be associated with $mGlu_5$ activation [4].

### 2.2 Distribution

The $mGlu_5$ receptor is primarily localized to postsynaptic terminals with moderate to high levels of expression found in the cerebral cortex, hippocampus, striatum, lateral septum, and spinal nucleus of the trigeminal nerve [5]. $mGlu_5$ receptors are also expressed on nonneuronal cells including astrocytes, microglia, and oligodendrocytes [6]. Within the cortex, $mGlu_5$ is abundantly expressed postsynaptically on pyramidal cells and interneurons [7].

In comparison, Group II ($mGlu_2$ and $mGlu_3$) and Group III ($mGlu_4$, $_6$, $_7$, and $_8$) couple to $G_{i/o}$ G proteins. Activation of Group II and III mGlu receptors results in a decrease in intracellular cAMP levels through inhibition of adenylyl cyclase

activity. Group II and III mGlu receptors are primarily localized to presynaptic terminals and can act as autoreceptors to inhibit activity on glutamate release [8].

There are two splice variants of $mGlu_5$ which produce different translated forms of the receptor: $mGlu_{5a}$ and $mGlu_{5b}$. $mGlu_{5a}$ is the predominant form during early postnatal period and is thought to play a role in development. In comparison, $mGlu_{5b}$ contains an additional 32-amino-acid segment in the C-terminal cytoplasmic tail and is the predominant form found in the adult [5].

## 2.3 NMDA Receptor Interaction

$mGlu_5$ and NMDA receptors are co-localized in a number of brain regions including the hippocampus, striatum, and cortex [9]. $mGlu_5$ receptors are physically coupled to synaptic scaffolding proteins, PSD-95, Shank, and Homer and biochemically coupled to the NMDA receptor via protein kinase C [10]. Activation of $mGlu_5$ receptors, by either orthosteric agonists or positive allosteric modulators (PAMs; or potentiators), has been shown to potentiate NMDA receptor signaling [11, 12]. Studies in primary neuronal cultures measuring NMDA-mediated calcium influx and in hippocampal slice preparations measuring enhancement of NMDA-receptor-dependent long-term potentiation have provided evidence for a functional interaction of $mGlu_5$ and NMDA receptors [13, 14]. The $mGlu_5$-mediated increase of NMDA receptor activity produces an enhancement in synaptic plasticity, learning and memory, and cognition.

## 2.4 Rationale for Allosteric Modulators

Due to the high degree of homology in the orthosteric site of mGlu receptors, the availability of subtype selective ligands is limited. Orthosteric agonists for $mGluR_5$, (S)-3,5-dihydroxyphenylglycine (DHPG), and quisqualate (L)-(+)-α-amino-3,5-dioxo-1,2,4-oxadiazolidine-2-propanoic acid) are amino acid analogs derived from glutamate and exhibit selectivity for Group I over Group II and III mGlu receptors; however they are nonselective between $mGlu_5$ and $mGlu_1$ receptors. CHPG (2-chloro-5-hydroxyphenylglycine) was reported to be selective for $mGlu_5$ over $mGlu_1$; however it has relatively weak potency and efficacy which limits its utility; moreover a recent report demonstrated that it is not selective and activates both $mGlu_1$ and $mGlu_5$ [15].

An alternative approach to achieve subtype selective ligands for mGlu receptors is to develop allosteric modulators (AMs) which bind to a site topographically distinct from the orthosteric site. This strategy assumes that allosteric sites are less conserved between subtypes within a receptor family and therefore increases the likelihood of achieving subtype selectivity. This approach has proven highly successful for mGlu receptors as allosteric modulators have been described for

nearly all mGlu subtypes [16, 17]. Positive allosteric modulators enhance the affinity and/or responsiveness of the orthosteric agonist. Negative allosteric modulators (NAMs) decrease the affinity and/or the responsiveness of the orthosteric agonist. Neutral allosteric ligands, previously termed silent allosteric modulators or SAMs, bind to the receptor but have no effect on affinity or efficacy of the orthosteric ligand. Ago-potentiators (ago-PAMs) have intrinsic activity alone to directly activate the receptor in addition to their activity to potentiate the orthosteric agonist. Pure PAMs have no intrinsic activity alone and function to increase the sensitivity of the receptor for the endogenous agonist by shifting the concentration response curve of the orthosteric agonist to the left. The potency of PAMs is reported as $EC_{50}$ values measured in the presence of a subthreshold concentration of the orthosteric agonist, usually an $EC_{20}$, and a fold shift of the agonist dose–response curve (glutamate shift) when measured in the presence of increasing PAM concentrations. Negative allosteric modulators, NAMs, act as antagonists and shift the potency of endogenous agonist to the right. The allosteric modulator activities are dependent on the presence of agonist and would be expected to be pharmacologically silent in the absence of agonist. Allosteric modulators have the advantage over orthosteric agonists in their selective ability to exhibit temporal and spatial control of receptor activity. Since glutamate is released in a temporal and spatial manner, the activity of PAMs would be realized only during the normal physiological process. Another benefit of allosteric modulators is that receptors are not subject to desensitization with chronic exposure as observed with orthosteric agonists [18].

## 2.5 *mGlu$_5$ Allosteric Modulator Activity*

Small modifications within a compound series have been reported to dramatically affect the pharmacology profile of $mGlu_5$ allosteric modulators, a result termed "molecular switching." This has led to the discovery of modulators with similar structures exhibiting either PAM or NAM activities. For example, Sharma et al. reported methyl substitutions of an $mGlu_5$ partial antagonist that led to the discovery of either a full antagonist (3-methyl group substitution) or PAM (2-methyl group substitution) [19]. This phenomenon has been observed in multiple $mGlu_5$ allosteric ligand series as well as with other mGlu receptors, and detailed information will be provided in Sect. 3.

Allosteric modulators can be characterized by determining their effects on orthosteric agonist affinity and efficacy. The interaction between the orthosteric and allosteric site is reciprocal, so that the ligand in one site modulates the ligand in the other site to the same extent and in the same way. These effects can be quantified using an operational model of allosterism through the determination of an affinity cooperativity factor $\alpha$ and efficacy cooperativity factor $\beta$ for a particular ligand, GPCR, and signaling pathway [17]. A positive cooperativity factor is $>1$, a negative cooperativity factor is $<1$ but greater than 0, and a neutral cooperativity

factor = 1. Bradley et al. evaluated the effects of the orthosteric agonist quisqualate on the affinity and efficacy of several $mGlu_5$ PAMs in rat astroglia. Quisqualate increased affinity for DFB **1**, CDPBB **4**, and ADX47273 **13** at the $mGlu_5$ NAM MPEP **21** binding site, with $\alpha = 1.5$, 11, and 3.2, respectively, but it did not increase the affinity of CPPHA **2** for this site. Analysis of data from potentiation of quisqualate-induced [$^3$H]inositol phosphate accumulation in rat astrocytes gave $\beta = 1.4$, 2.3, and 9.4 for DFB **1**, CDPBB **4**, and ADX47273 **13**, respectively. Thus CDPBB **4** achieved positive modulation of $mGlu_5$ primarily through effects on orthosteric agonist affinity, and ADX47273 **13** achieved its effects primarily through efficacy modulation [20]. Gregory et al. characterized eight PAMs and two ago-PAMs in terms of their effects on glutamate affinity and efficacy on expressed rat $mGlu_5$ receptors. In this study PAM affinities were unchanged in the presence of glutamate, which would correspond to $\alpha = 1$. The PAMs exerted their effects on glutamate-mediated calcium mobilization through efficacy modulation, with $\beta$ values >1.

An interesting observation was that the value of $\beta$ depended on the expression level of $mGlu_5$ for some PAMs. Higher values of $\beta$ at high receptor expression levels (compared to low levels) reached statistical significance for two PAMs (VU0405398 **40**, VU0405386 **42**), while other PAMs had similar values of $\beta$ at low and high receptor levels (e.g., CPPHA **2**, CDPBB **4**, VU29 **5**, VU0092273 **35**, VU0360172 **37**, VU0415051 **41**, VU0357121 **60**, VU0364289 **72**) (vide supra) [21].

## 2.6 *$mGlu_5$-Biased Signaling*

Activation of GPCRs can differentially couple to different signaling pathways and ultimately functional responses. This observation is referred to as biased signaling or functional selectivity and was first defined for GPCR receptor activation through orthosteric ligands [22, 23]. Recently, $mGlu_5$ allosteric modulators have been reported to show preferences for signaling pathways. In astrocytes, DFB showed similar activity to potentiate glutamate-mediated calcium increase and ERK1/2 phosphorylation. In comparison, CPPHA exhibited a different pharmacological profile than DFB where inhibition of ERK1/2 phosphorylation was observed with high concentrations of agonists and a calcium response similar to DFB [24]. Recently, NCFP **3**, (*N*-(4-chloro-2-((4-fluoro-1,3-dioxoisindolin-2-yl)methyl) phenyl)picolinamide) [25], a structural analog of CPPHA **2**, was reported to display a similar profile as CPPHA in potentiating $mGlu_5$-mediated responses to glutamate in recombinant and native cell preparations and in the DHPG-mediated potentiation of STN neuron depolarization in brain slices. However, unlike other $mGlu_5$ PAMs, NCFP **3** did not potentiate DHPG-induced LTD in hippocampal Schaffer collateral-CA1 synapse in the hippocampus [26]. These findings support the hypothesis that $mGlu_5$ PAMs may induce differential responses as a result of bias signaling and could have different physiological outcomes.

Furthermore, evidence has supported CPPHA **2** and NCFP **3** binding to non-MPEP allosteric sites on $mGlu_5$. Since these $mGlu_5$ PAMs display bias signaling and have been suggested to bind to different allosteric sites in comparison with other $mGlu_5$ PAMs, it has been postulated that the response observed may be site specific. If this is true, this raises the possibility of targeting one allosteric site over another on $mGlu_5$ to achieve a specific response through a desired pathway.

## 3 Overview of the Discovery of $mGlu_5$ Positive Allosteric Modulators

The first allosteric potentiators of mGlu receptors were $mGlu_1$ PAMs in 1998. Then in 2003 the first $mGlu_5$ PAM, DFB **1**, was identified. Since that time, many chemotypes of $mGlu_5$ PAMs with better drug likeness have been disclosed. Recent reviews have discussed the historical and main series within the $mGlu_5$ PAM field [27, 28]. The present article focuses on the structural characteristics of the different classes of $mGlu_5$ PAMs and which mainly include:

DFB and CPPHA
Diphenyl pyrazoles
*N*-Acyl 3-piperidinyl oxadiazole and related templates
Alkynes and related isosters
*N*-Aryl piperazine
*N*-Aryl glycine sulfonamide and pyridyloxybenzyl isoindoline

### *3.1 DFB and CPPHA*

DFB (**1**, Fig. 1) was the first $mGlu_5$ PAM discovered ($EC_{50} = 2.7$ μM, Fig. 2), and close analogs showed a continuum of functional activity; the replacement of the fluorine substituents by methoxy groups switched the activity to NAM (3,3′-dimethoxybenzaldazine, DOMeB), but replacement of fluorine by chlorine produced the SAM DCB (3,3′-dichlorobenzaldazine). These molecules were competitive with the common $mGlu_5$ NAM MPEP binding site [27, 28 and references therein].

CPPHA (**2**, Fig. 1) was discovered by the Merck team and characterized. CPPHA ($EC_{50} = 370$ nM) interacted with a separate allosteric site than DFB; a potent and efficacious analog, NCFP (**3**, Fig. 1) ($EC_{50} = 214$ nM), has also been described. CPPHA and NCFP do not compete with MPEP and activate different signaling pathways of functional responses, a phenomenon referred as biased signaling (see Sect. 2.6) [26].

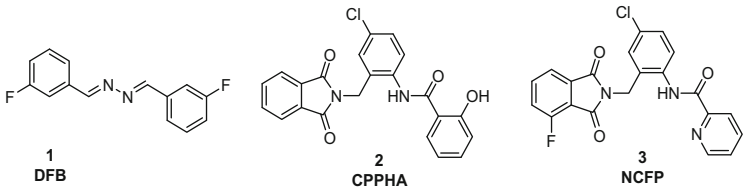



**Fig. 1** Chemical structures in two historical $mGlu_5$ PAM series

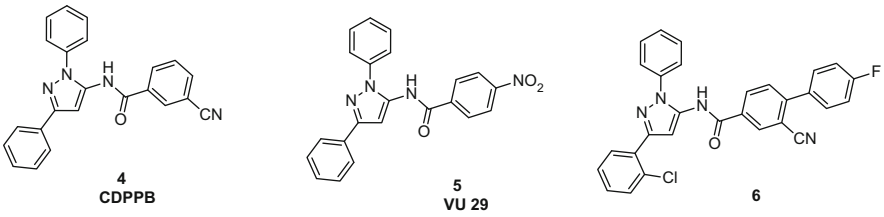



**Fig. 2** Chemical structures of diphenyl pyrazole benzamide derivatives

## *3.2 Diphenyl Pyrazoles*

CDPPB (3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide, Fig. 2) **4** was the third $mGlu_5$ PAM discovered after DFB **1** and CPPHA **2**. It had improved potency, drug likeness, and brain permeability. The series was identified following the design of a fragment library based on the picolinoyl amide moiety of CPPHA **2** [29]. This modulator has demonstrated high selectivity, and it has shown potent activity in vitro ($EC_{50} = 27$ nM in human CHO $mGlu_5$ transfected cells), with 7.7 fold glutamate potentiation shift (glutamate shift). Studies demonstrated its competition with the MPEP binding site ([$^3$H]methoxyPEPy $K_i = 2.6$ μM). Its preferential brain distribution turned this compound into a very interesting tool. It has shown in vivo activity in various rodent models, in particular a dose-dependent reversal of amphetamine-induced locomotor activity at 1, 3, and 10 mg/kg SC and in the reversal of the amphetamine-induced disruption of PPI model at 10 and 30 mg/kg SC [30]. The exploration of the SAR in the series led to the identification of potent MPEP-competitive binders such as compound VU29 (**5**, Fig. 2) [31, 32], with [$^3$H]methoxyPEPy $K_i = 250$ nM. It also showed an improved functional activity in vitro in rat astrocytes ($EC_{50} = 10$ nM) compared to CDPPB ($EC_{50} = 77$ nM). The effect of aromatic substitution was explored further by analogy with the alkyne class of $mGlu_5$ NAMs [33]. Highly lipophilic CDPPB derivatives with high MPEP binding affinity such as (**6**, Fig. 2) were disclosed ([$^3$H] MPEP $K_i = 23$ nM; $rmGlu_5$ $EC_{50} = 2{,}430$ nM (68%)). These new biphenyl derivatives showed partial functional activity.

A functional switch has been observed in this series when the phenyl substituent was moved from the 1,3 position (PAM) to the 1,4 position (NAM) on the pyrazole

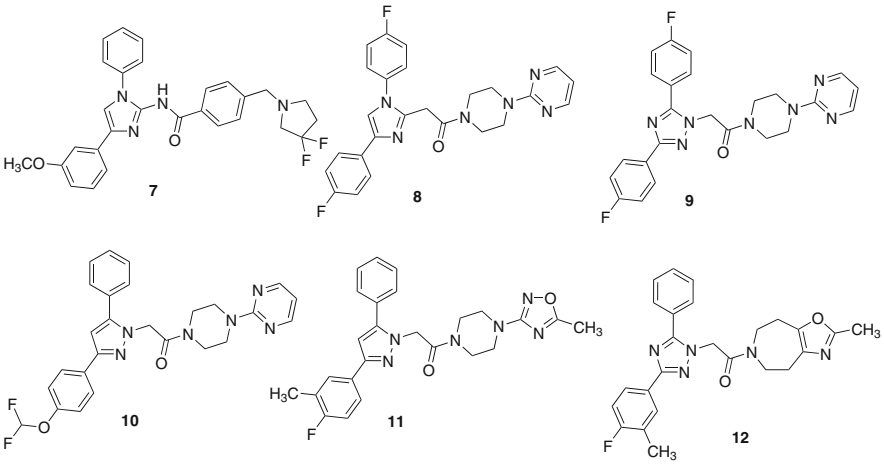



**Fig. 3** Chemical structures of new diphenyl-5-membered heteroaryl compounds

ring. When the diphenyl pyrazole moiety of **6** was replaced by the 2-methylpyridine MPEP moiety, the combined compound became a potent $mGlu_5$ NAM [31].

CDPPB **4** has been evaluated in numerous animal models for effects on learning and memory processes [34], cognitive disorders induced by MK801 [35], and negative symptoms of schizophrenia, through enhancing NMDA receptor function [36, 37]. The results reinforce interest in $mGlu_5$ PAMs as a new target to address unmet therapeutic needs in schizophrenia.

Taisho Pharmaceuticals further explored the CDPPB series, as disclosed in a patent application [38], where pyrazole is replaced by imidazole. Compound **7** was described as a subnanomolar compound active on the rat $mGlu_5$ CHO clone ($EC_{50} = 0.62$ nM; Fig. 3). The diphenyl-5-membered heteroaryl core can be considered as a privileged structure in $mGlu_5$ ligands.

In 2013 Boehringer Ingelheim scientists published new classes of potent PAM compounds which kept a similar core on the left-hand side (Fig. 3) such as bisphenyl-substituted 1*H*-imidazol-2-yl **8** ($hmGlu_5$ $EC_{50} = 43$ nM), (1,2,4)triazol-1-yl **9** ($hmGlu_5$ $EC_{50} = 24$ nM), or 3,5-diphenyl-substituted pyrazol-1-yl **10** ($hmGlu_5$ $EC_{50} = 4$ nM) [39–44]. The CDPPB carboxamido linker was replaced by a ketomethylene linker, which allowed the right-hand side (RHS) to be an heteroaryl piperazine. In a variation of the inventions, an heteroaryl RHS such as a methyl oxadiazolyl residue **11** ($hmGlu_5$ $EC_{50} = 2$ nM) can be fused to the piperazinyl ring system or the like and maintain PAM activity for **12** ($hmGlu_5$ $EC_{50} = 28$ nM). We can notice that these new molecules are slightly less lipophilic (clog*P* ~4.0) than the classical CDPPB derivatives. These patent applications do not report any in vivo activity.

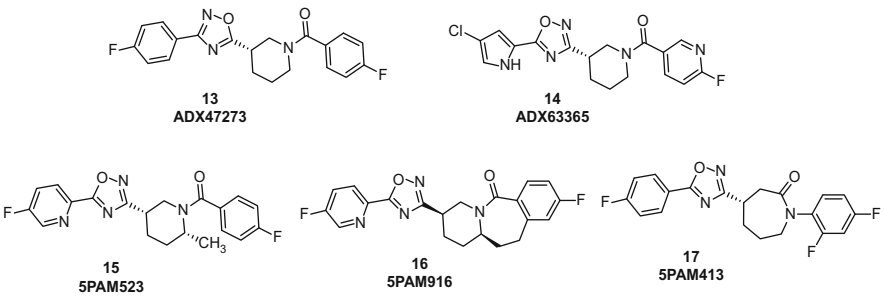



**Fig. 4** Chemical structures of piperidinyl oxadiazole $mGlu_5$ PAM series and analogs

## *3.3* N-*Acyl-3-Piperidinyl Oxadiazole*

Addex researchers reported the discovery and profiling of ADX47273 (**13**, Fig. 4), a potent and systemically active $mGlu_5$ PAM [45]. This compound has been the prototype for several chemical variations, leading to the subsequent discovery of various active analogs. The basic structure-activity relationships were described by Engers et al. [46]. They confirmed the enantioselective activity in the series and identified a functional switch in pharmacology. ADX47273 was a moderately potent $mGlu_5$ PAM ($hmGlu_5$ $EC_{50} = 170$ nM) and showed a high glutamate shift (10–13 times) [45]. Addex Therapeutics filed several patent applications around the scaffold, illustrating the large scope of the series by varying the core oxadiazole ring. The oxadiazole can be replaced by various 5-membered heteroaryl ring like triazole and tetrazole, or even a carbamate group [27].

Another observation in this highly chemically tractable series was that discrete structural features led to opposite functional activity. The work performed by Gedeon Richter scientists detailed the chemical variations of the piperidinyl ring system and the RHS amido groups which contribute to NAM activity [47]. Potent NAMs usually combine a 2-piperidinyl or a 2-pyrrolidinyl core acylated by a small cycloalkyl or 5-membered heteroaryl ring. Lamb et al. presented a structural exploration of SAR focused mainly on cycloalkylamido derivatives in the 3-piperidinyl, 2-piperidinyl, and 2-pyrrolidinyl series [48]. The results are in good agreement with the previous study. They disclosed a continuum in this series, including finding an ago-PAM and partial antagonist [49]. Similarly to ADX47273, the selected molecules **14**, **15**, **16**, and **17** (Fig. 4) showed in vitro potency for $mGlu_5$ receptor (human $mGlu_5$ $EC_{50} = 92$, 201, 17, and 125 nM, respectively, with glutamate shift = 14, 13, 5.5, and 23) and in vivo efficacy in attenuation of amphetamine-induced locomotor activity in rats. Most of the compounds in this series are moderate binders of the MPEP binding site ($K_i$ ~1–3 μM range) and may overlap with the MPEP binding site [50].

The introduction of an angular methyl on the piperidine ring in 5PAM523 **15** contributed to an improved plasma clearance compared to the unsubstituted piperidino analogs. The tricyclic ring compound 5PAM916 **16**, where the angular

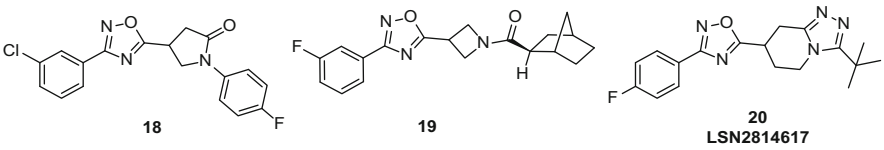



**Fig. 5** Novel generation of $mGlu_5$ PAM series related to ADX47273 series

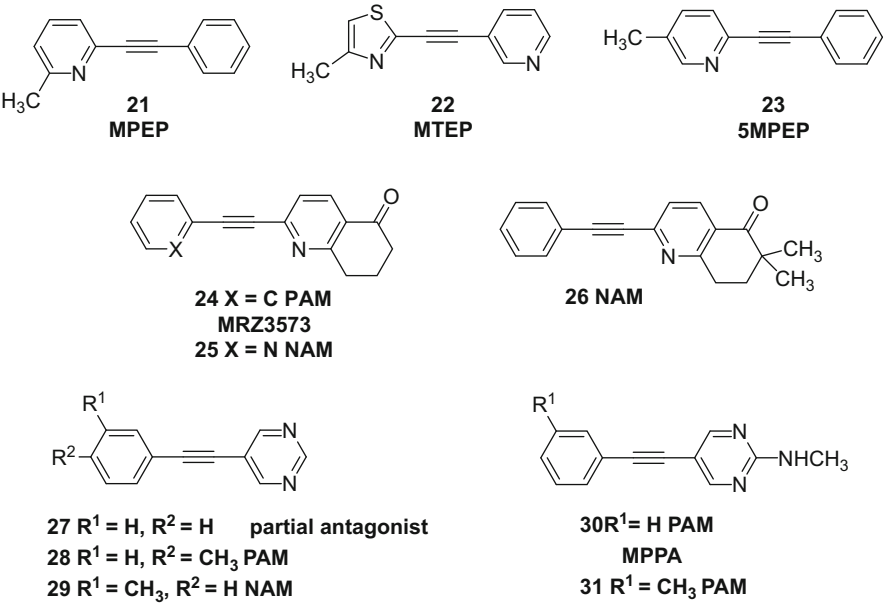



**Fig. 6** First generation of $mGlu_5$ AMs in the pyridine alkyne series

methyl of 5PAM523 is constrained with the 4-fluorobenzamide, is 12 times more potent than **15** and showed a longer half-life (12.3 h and 5 h, respectively, in rats) and good brain penetration (0.1 and 0.045 CSF/plasma value in rats, respectively). We can notice that the 4-chloro-pyrrole is a good bioisosteric replacement of the 4-fluorophenyl group in **14**. The caprolactam 5PAM413 **17** showed a very efficient glutamate shift (23 fold).

A related oxadiazolyl-butyrolactam series (**18**, Fig. 5) was discovered at Lundbeck (Fig. 6); **18** included a 3-chlorophenyl group on the LHS. This privileged fragment is found in numerous $mGlu_5$ NAMs in the MPEP and similar series [47]. However, **18** is a potent $mGlu_5$ PAM ($hmGlu_5$ $EC_{50} = 10.7$ nM) which showed partial efficacy (55%) [51]. This pyrrolidinone series was widely explored, and the SAR revealed that a subtle pattern of substitution on the RHS with simple substituents (F, $CH_3$, Cl) could switch the PAM activity to NAM. Molecules in this series suffer from poor solubility, which has limited their development. An attempt to improve the physiochemical properties of **18** consisted of replacing the

pyrrolidinone ring by an azetidine ring and extruding the carbonyl group. This optimization resulted in the azetidinyl oxadiazole series illustrated by **19** (Fig. 5), a potent ($hmGlu_5$ $EC_{50} = 72$ nM) and brain penetrant compound with improved solubility and lipophilicity (clog$P = 2.1$, LLE = 5.1) [52].

The triazolo tetrahydropyridine derivative LSN2814617 (**20**, Fig. 5) is a potent and selective $mGlu_5$ PAM ($hmGlu_5$ $EC_{50} = 52$ nM). Compared to ADX47273 **13** and CDPPB **4**, it showed excellent receptor occupancy at 10 mg/kg (62%) compared to 5% and 6%, respectively, for CDPPB and ADX47273 administered at 100 mg/kg. This molecule shows a robust dose-dependent increase in wakefulness in rat at 3 mg/kg PO [53].

A comparative in vivo study of structurally distinct $mGlu_5$ PAMs derived from ADX47273 (PAMs **15**, **16**, and **17**) was performed by Merck and revealed a potential mechanism-based neurotoxicity [50]. Orthosteric $mGlu_5$ agonists have previously been reported to produce seizures in rodents [54, 55], and the potential for similar adverse events occurring through allosteric modulation of $mGlu_5$ has only been recently evaluated as the result of the availability of $mGlu_5$ PAMs with properties suitable for in vivo studies. Parmentier-Batteur et al. observed convulsions and neuronal death in the auditory cortex and hippocampus after dosing $mGlu_5$ PAM **15**, **16**, and **17** in rats [50]. All three $mGlu_5$ PAMs were devoid of intrinsic agonist activity. These regions are associated with high $mGlu_5$ receptor expression. The association of mGluR PAM mechanism-based neurotoxicity was further established in studies with $mGlu_5$ KO and wild-type mice. $mGlu_5$ wild-type mice treated with **15** showed physical signs and a similar pattern of neuronal loss observed in rats. In contrast, dosing in $mGlu_5$ KO mice lacked any physical signs and no neuronal loss was observed by histological staining. These results confirmed $mGlu_5$ involvement in the neurotoxicity observed with **15**. It is important to note that the three $mGlu_5$ PAMs evaluated in the in vivo toxicology studies exhibited different therapeutic windows and the results suggest that $mGlu_5$ PAMs with lower cooperatively (smaller glutamate shifts) may have improved safety margins.

### *3.4 Alkynes*

Allosteric modulators of $mGlu_5$ were first identified in 1999 by scientists at Sibia Neurosciences using high-throughput screening [HTS] to measure effects on intracellular calcium levels in cells expressing human $mGlu_5$ [56]. These compounds displayed noncompetitive inhibition of glutamate-mediated increases in calcium levels and thus were negative allosteric modulators or NAMs. Further studies led to the discovery of MPEP **21** and MTEP **22** (Fig. 6), $mGlu_5$ NAMs with an unusual alkyne structure that have since been used extensively in in vitro and in vivo studies [57, 58]. Two $mGlu_5$ NAM radioligands were synthesized to facilitate receptor binding and characterization, [$^3$H]-methoxymethyl-MTEP and [$^3$H]-methoxyPEPy [59]. Since the discovery of alkynylpyridine $mGlu_5$ NAMs, a range of pharmacological effects have been observed for related structures binding at or near the

MPEP site. This series has also produced PAMs as well as "neutral" SAMs such as 5MPEP **23**. By themselves SAMs have no effect on glutamate receptor activation, but they do block the effects of NAMs and PAMs [60].

The first PAM within the alkyne series was MRX3573 **24**, discovered at Merz Pharmaceuticals (Fig. 6) [61]. While constructing a pharmacophore model to screen for novel $mGlu_1$ NAMs, Merz scientists reasoned that the model might also identify allosteric modulators of $mGlu_5$, since the two receptors have a high degree of structural similarity in the transmembrane region where allosteric ligands bind [62]. Using the pharmacophore model, a small library was purchased and screened for both $mGlu_1$ and $mGlu_5$ activity. Follow-up of an initial weak $mGlu_1$ NAM screening hit led to selective, potent $mGlu_1$ NAMs and the $mGlu_5$ PAM **24** ($rmGlu_5$ $EC_{50} = 34$ nM, glutamate shift = 2). However, small structural changes to **24** were sufficient to switch the pharmacology from positive to negative modulation. Replacing phenyl with 2-pyridyl produced the weak NAM **25** ($rmGlu_5$ $IC_{50} = 60$ μM), wherein the 2-pyridyl group acted as a molecular switch for NAM activity. A hydrogen bond acceptor, such as the carbonyl group in MRX3573 **24** or a hydroxyl group in the same position, was important for PAM activity. NAMs were produced following complete reduction to the tetrahydroquinoline or introduction of a gem-dimethyl group adjacent to the carbonyl (**26**, $rmGlu_5$ $IC_{50} = 23$ nM).

In the process of identifying NAMs acting as partial antagonists of $mGlu_5$ based on MPEP, researchers at the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) found that adding a methyl group to the partial antagonist **27** ($rmGlu_5$ $IC_{50} = 480$ nM) produced weak PAM **28** ($rmGlu_5$ $EC_{50} = 3.3$ μM, $Glu_{max} = 99\%$, glutamate shift = 4.2) [63]. Small substituents such as a methyl group at the 4 position of the phenyl group proved to be a molecular switch for PAM activity. However, moving the methyl group from the 4 to the 3 position gave a full antagonist (**29**, $rmGlu_5$ $IC_{50} = 7$ nM). Further SAR studies to optimize PAM activity were unable to exploit the 4-methyl molecular switch, instead showing flat SAR. In this instance, changes to the pyrimidine group led to the potent 2-aminomethylpyrimidine PAM **30** (MPPA) ($rmGlu_5$ $EC_{50} = 14$ nM, glutamate shift = 15) [19]. Activity was not observed in the absence of glutamate, indicating **30** did not possess intrinsic agonist activity. In rats, **30** reversed amphetamine-induced hyperlocomotion at a dose of 30 mg/kg IP. This compound was insensitive to the 3-methyl molecular switch identified earlier, instead maintaining robust PAM activity for **31** ($rmGlu_5$ $EC_{50} = 21$ nM, glutamate shift = 5.9).

A modest $mGlu_5$ PAM was identified at Lundbeck in a HTS campaign utilizing a functional calcium flux assay ($hmGlu_5$ $EC_{50} = 0.97$ μM, $Glu_{max} = 73\%$) [64]. Noting the similarity of **24** and MPEP **21**, these investigators postulated that one of the aminal nitrogens of **32** (Fig. 7) might serve as a hydrogen bond acceptor mapping to the carbonyl molecular switch group of **24**. Replacing the aminal with an amide group indeed preserved PAM activity and increased potency 37-fold for cyclopentyl amide **33** ($hmGlu_5$ $EC_{50} = 30$ nM, glutamate shift = 5.5 at 1 μM). Compound **33** displaced [$^3$H]MPEP with a $hmGlu_5$ $K_i = 1.8$ μM, consistent with binding to the MPEP binding site on the receptor. Compound **33** was orally

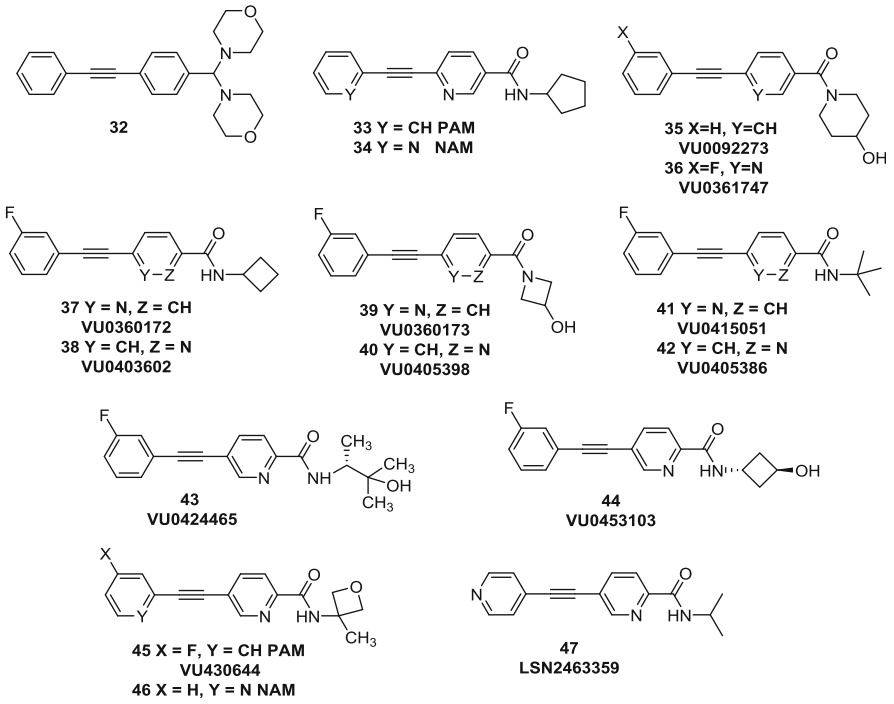



**Fig. 7** Alkyne $mGlu_5$ PAM series: aminal and amido series

bioavailable in rats and mice, with elimination half-lives of 1 and 4 h and brain/plasma ratios of 0.5 and 0.3, respectively, in these species. Later studies by the same group found that slight changes in structure could change the mode of pharmacology from positive to either negative or silent modulation. For example, substituting 2-pyridyl for phenyl gave **34**, a moderately active NAM ($hmGlu_5$ $IC_{50} = 227$ nM), consistent with the switch in pharmacology observed between **24** and **25** [65].

A similar series of compounds was concurrently discovered in a functional screen at VCNDD, designed to identify $mGlu_5$ agonists, antagonists, and potentiators in a single assay (Fig. 7) [66]. A screening hit, **35** (VU0092273; $rmGlu_5$ $EC_{50} = 10$ nM, $Glu_{max} = 72\%$), was further optimized to **37** ($rmGlu_5$ $EC_{50}$=16 nM, $Glu_{max} = 87\%$), wherein a modestly basic pyridine was incorporated with little loss of potency, in order to facilitate salt formation and formulation for in vivo studies.

Nicotinamide **37** (VU0360172) showed competitive binding with [$^3$H] methoxyPEPy, with rat $K_i = 195$ nM. Compound **37** had rat plasma free fraction = 1.1%, and a 10 mg/kg oral dose in rats gave plasma $C_{max} = 21$ μM at 1 h, and brain $C_{max} = 2$ μM. Compound **37** significantly reduced amphetamine-induced hyperlocomotion in rats at oral doses of 56.6 and 100 mg/kg, formulated in 20% hydroxypropyl β-cyclodextrin.

As some $mGlu_5$ PAMs had direct agonist activity (i.e., were ago-PAMs), the question arose whether agonist activity contributed to efficacy in animal models of

schizophrenia. Investigation into the agonist properties of PAMs led researchers at VCNDD to discover that the level of $mGlu_5$ receptor expression, while having little effect on PAM activity, could influence agonist behavior in cell culture. This study found that **35** and **37** behaved as ago-PAMs at high receptor expression levels but as pure PAMs at low expression levels and in native systems (**35**, $rmGlu_5$ PAM $EC_{50} = 35$ nM, agonist $EC_{50} = 1.3$ μM; **37**, $rmGlu_5$ PAM $EC_{50} = 13$ nM, agonist $EC_{50} = 220$ nM) [67]. In addition, **36** was found to be a pure PAM regardless of the level of receptor expression ($rmGlu_5$ $EC_{50} = 126$ nM). Unlike the orthosteric agonist DHPG, none of the compounds by themselves induced LTD at the hippocampal Schaffer collateral CA1 synapse, but all potentiated the effects of DHPG. All three compounds decreased amphetamine-induced locomotor hyperactivity in rats. This led to the conclusion that the physiological and behavioral responses to an ago-PAM and a pure PAM were similar, with the caveat that agonist activity might still be differentiated in other behavioral models.

Continued optimization led to the discovery of ago-PAM **43** (Fig. 7), which had agonist activity in both high and low receptor expression systems and in native tissues ($rmGlu_5$ PAM $EC_{50} = 2$ nM, $Glu_{max} = 88\%$, glutamate shift = 6.6; agonist $EC_{50} = 171$ nM, $Glu_{max} = 65\%$) [68]. **43** was selective for $mGlu_5$ vs other mGlu subtypes. Evidence that agonist activity was mediated through the allosteric binding site included displacement of [$^3$H]methoxyPEPy ($rmGlu_5$ $K_i = 11.8$ nM), right shifting of the **43** dose–response curve with no change in the maximum response by the allosteric SAM 5MPEP but not by an orthosteric antagonist, and agonist activity on the F585I but not A809V $rmGlu_5$ mutant receptor (vide infra). Compound **43** had effects similar to the orthosteric agonist DHPG in vitro and in vivo. It triggered $Ca^{2+}$ mobilization in rat cortical astrocytes and induced significant LTD at the SC-CA1 synapse in rat hippocampus. It also caused robust epileptiform activity in rat hippocampus area CA3 and dose-dependent seizures in rats from 0.1 to 10 mg/kg IP, as monitored by behavioral response and (at the highest dose) cortical surface electrode EEG. In the EEG study, the unbound brain levels of **43** were 1.3 times the rat agonist $EC_{50}$ and 146 times the rat PAM $EC_{50}$ ($[brain]_{unbound} = 220$ nM at 10 mg/kg IP). The epileptiform and seizure activities were blocked by the allosteric antagonist MTEP, supporting the involvement of $mGlu_5$. In contrast, the pure PAM **36** had no in vitro effects in rat hippocampal slices and in vivo caused no behavioral or EEG response in rats, despite unbound brain levels 8.7 times the PAM $EC_{50}$ ($[brain]_{unbound} = 1{,}107$ nM at 56.6 mg/kg IP). Given that it is well established that orthosteric $mGlu_5$ agonists can induce seizures, it was concluded that $mGlu_5$ allosteric agonists (or more precisely, ago-PAMs) can induce seizure activity and that it is possible for a pure $mGlu_5$ PAM to avoid these adverse effects [54, 55].

Other studies at VCNDD highlighted the importance of monitoring the $mGlu_5$ activity and brain penetration of metabolites. While demonstrating efficacy for ago-PAM **38** in the amphetamine-induced rat hyperlocomotion assay, marked adverse effects including seizures were noted [69]. It was discovered that the primary oxidative metabolite **44** was also an ago-PAM (**38**: $rmGlu_5$ PAM $EC_{50} = 4$ nM, $Glu_{max} = 100\%$, glutamate shift = 9, agonist $EC_{50} = 31$ nM, $Glu_{max} = 49\%$; **44**: $rmGlu_5$ PAM $EC_{50} = 17$ nM, $Glu_{max} = 94\%$, glutamate

shift = 8.2, agonist $EC_{50}$ = 400 nM, $Glu_{max}$ = 78%). Significant brain penetration was observed for the metabolite **44**. Both parent and metabolite were selective for $mGlu_5$ over other mGlu subtypes. While both **38** and **44** demonstrated agonist properties in rat hippocampal slices, the adverse effects appeared to be mediated through $mGlu_5$ primarily by the metabolite **44**, as coadministration of either MPEP or the pan-P450 inactivator 1-aminobenzotriazole (ABT) with **38** blocked its effects. The ABT-treated rats showed a substantial decrease in **44** $C_{max}$ in both plasma (11-fold) and brain (13-fold), while brain exposure to **38** did not change very much relative to ABT-untreated rats. Finally, direct administration of **44** to rats produced the same set of behavioral adverse effects. Given that small changes in structure can have significant effects on both $mGlu_5$ pharmacology, this study illustrated the importance of characterizing the activity of metabolites.

As mentioned above, intrinsic agonist activity (ago-PAMs) and/or high glutamate cooperativity (glutamate shift) have been implicated in the neurotoxicity of some PAMs [50, 68]. Therefore the research effort at VCNDD sought to obtain a compound in this series that lacked agonist activity and had low glutamate fold-shift associated with PAM activity. After extensive SAR studies, the picolinamide **45** (VU430644, Fig. 7), which has a 3-methyl-substituted oxetane as the eastern amide group, was found to fulfill both criteria ($rmGlu_5$ $EC_{50}$ = 9.3 nM, $Glu_{max}$ = 45%, glutamate shift = 2.8) [70]. No agonist activity was observed in the low expression cell line as well as in two native systems. The glutamate shift was a modest 2.8 fold. In this series, a 2-pyridyl group as the western aryl substituent was found to abrogate agonist activity in many ago-PAMs. Most interestingly, incorporating a 2-pyridyl group into a pure PAM like **45** converted it to an antagonist (**46**, $rmGlu_5$ $IC_{50}$ = 731 nM, $Glu_{min}$ = 16.7%), similar to SAR observed at Merz for **24** and at Lundbeck for **33** [61, 65]. Compound **45** was selective for $mGlu_5$ compared to $mGlu_{1–4}$ and $_{6–8}$ and did not show off-target activity in a panel of 68 GPCRs, ion channels, and transporters. In vitro studies predicted low hepatic clearance in rat and human (1.6 and 0.2 mL/min/kg, respectively). The free fraction in human and rat plasma was 3.5% and 3.6%, and the free fraction in rat brain homogenate was 1.6%. Compound **45** inhibited CYP1A2 ($IC_{50}$ = 5.3 μM) but had no other CYP liability. A single high-dose administered IP was reported to reverse amphetamine-induced hyperlocomotion in rats, although no details were provided [70, 71]. Preliminary stability studies in acidic media showed hydrolytic ring opening of the oxetane ring which might complicate oral dosing [71].

Researchers at Lilly reported a compound in the same series, **47** (LSN2463359, Fig. 7), that also was free of intrinsic agonist activity and had a low glutamate fold-shift. Similar levels of potentiation were observed for **47** in cell culture with expressed human $mGlu_5$ and in rat primary cortical neurons ($hmGlu_5$ $EC_{50}$ = 33 nM, $Glu_{max}$ = 65%) ($rmGlu_5$ $EC_{50}$ = 24 nM, $E_{max}$ = 64%, agonist shift = 1.9) [53]. In rat brain membranes, **47** displaced 95% [$^3$H]-MPEP binding with $K_i$ = 377 nM. No activity was observed on $mGlu_{1–4}$, $mGlu_8$, or $GABA_B$ receptors, and less than 50% activity at 10 μM was observed in binding studies with 25 other CNS targets. Direct target engagement was assessed by receptor

occupancy (RO) studies with **47** and unlabeled methoxyPEPy using LCMS detection. Oral dosing of **47** to rats 1 hour prior to methoxyPEPy dosing (IV) elicited dose-dependent RO with $ED_{50} = 6.9$ mg/kg and 87% maximum occupancy. Based on an unbound brain fraction of 0.063, the total brain concentration (1,582 nM) at the $ED_{50}$ dose yielded an unbound brain concentration of 99 nM, which is within 4-fold of the in vitro $K_i$ determination. Compound **47** had little to no effect on drug-induced locomotor hyperactivity in rats. Oral doses of **47** up to 10 mg/kg in amphetamine-treated rats and up to 30 mg/kg in PCP or SDZ220, 581 treated rats, were ineffective in attenuating hyperactivity. From separate in vivo studies, the unbound brain levels 80 min post-dose can be estimated as 125 nM at 10 mg/kg and 257 nM at 30 mg/kg, both values higher than the in vitro $EC_{50}$ but less than the $K_i$ [53, 72]. In the absence of additional data, this leaves open the question of whether a higher dose would have worked in this assay. Compound **47** up to 10 mg/kg did not much affect baseline performance in the delayed-matching-to-position (DMTP) test. However, **47** was found to improve performance in the variable interval 30 s task and the DMTP task in rats treated with the competitive NMDA receptor antagonist SDZ 220,581, albeit with a U-shaped dose–response curve in the 1–10 mg/kg dose range. As well, **47** attenuated deficits in cognitive flexibility in the neurodevelopmental MAM model of schizophrenia, but not in the acute PCP model [73]. It was also found that 2.5, 5, and 10 mg/kg doses significantly increased wakefulness in rats, as determined by EEG monitoring. The clinical significance of these results is much anticipated.

Conformational constraints were used to confine the rotation of the eastern secondary amide in many of the above series. Synthesis of a constrained analog from the global energy minimum conformation of **33** gave **48** (Fig. 8), leading to a 3-fold increase in potency ($hmGlu_5$ $EC_{50} = 5.9$ nM, $Glu_{max} = 109\%$) [65]. Similar to SAR for **33** and in the series mentioned above, changing the western phenyl group to 2-pyridyl engendered a pharmacology switch to a functional NAM. The isoindolone **50**, with a western 3-fluorophenyl group and without an N-substituent, retained good activity. Unlike its *des*-fluoro analog, **50** did not have agonist activity (rat $EC_{50} = 66$ nM, $Glu_{max} = 71\%$) [74]. Efficacy switching was observed with small structural changes in this series.

Scientists at AstraZeneca made changes to the eastern bicyclic heterocycle to obtain azabenzimidazole **51** ($hmGlu_5$ $EC_{50} = 17.4$ nM) and aza-oxazine **52** ($hmGlu_5$ $EC_{50} = 30.2$ nM), both of which maintained reasonable potency [75]. Although **51** had better solubility at pH 7.4 and improved log P compared to **52** (42 μM and 3.1 vs 4 μM and 4.5), **52** proved more amenable to alkyne replacement (Sect. 3.5).

Fused thiazoles were also optimized by Lundbeck to obtain potent PAMs. A potent early lead **53** (LuAF11205, $hmGlu_5$ $EC_{50} = 6.1$ nM, $Glu_{max} = 167\%$, Fig. 8) showed efficacy in the novel object recognition (NOR) assay as well as in the phencyclidine disrupted NOR assay in rats [76]. However, in vitro metabolism of **53** produced a significant amount of glutathione adducts. To reduce nucleophilic reactivity and maintain potency, a strategy was adopted that included introducing a cyclic constraint and repositioning the carbonyl group. The fused *N*-methyl lactam

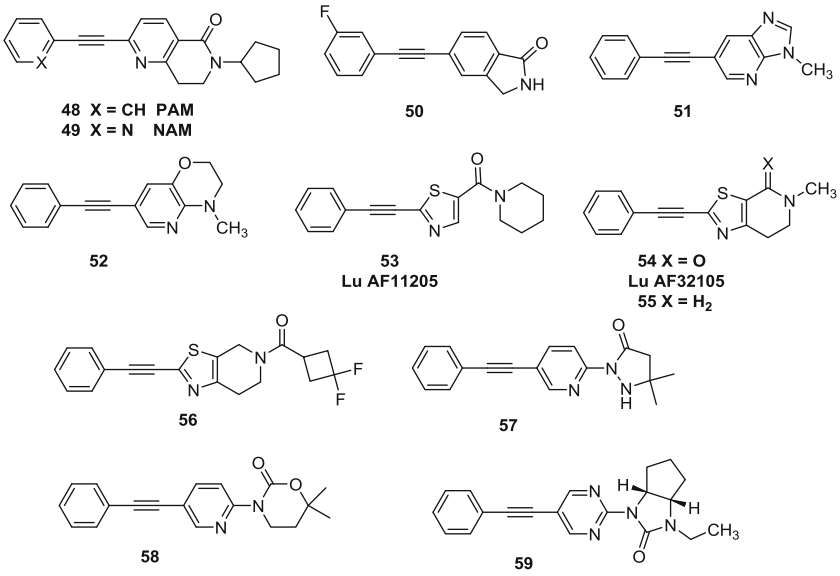



**Fig. 8** New generation of alkyne $mGlu_5$ PAM series

thiazole **54** (Lu AF32105) maintained excellent potency ($hmGlu_5$ $EC_{50} = 2.4$ nM, $Glu_{max} = 180\%$) and was selective vs other mGlu receptors but still showed significant reactivity with glutathione in human microsomes. Removing (**55**) or relocating the amide carbonyl so that it was exocyclic (**56**) reduced glutathione adduct formation. Compound **55** had good potency ($hmGlu_5$ $EC_{50} = 34$ nM, $Glu_{max} = 110\%$) and improved solubility relative to **54**. Compound **56** was slightly more potent ($hmGlu_5$ $EC_{50} = 18$ nM, $Glu_{max} = 89\%$) but had poor solubility. The intrinsic clearance was significantly lower for **56** compared to **54** and **55** (Fig. 8). Efficacy switching from PAM to NAM was observed with small structural changes within the fused thiazole series.

A patent application from Roche [77], broadly claimed $mGlu_5$ positive allosteric modulators with a glutamate shift less than 3 (at 10 μM), in order to ensure high tolerability and safety. Previously disclosed specific examples included compounds with a low glutamate shift such as **57** ($EC_{50} = 13$ nM, $Glu_{max} = 33\%$, glutamate shift $hmGlu_5 = 1.75$, glutamate shift $rmGlu_5 = 1.44$) and, for comparison, compounds with higher glutamate shift like **58** ($EC_{50} = 10$ nM, $Glu_{max} = 80\%$, $hmGlu_5$ glutamate shift = 9.73, $rmGlu_5$ glutamate shift = 7.67). Using 17 examples, it was disclosed that compounds with glutamate shifts less than 3 were well tolerated when dosed to rats (e.g., **57** at 100 mg/kg, route not specified), but compounds with higher glutamate shifts caused seizures (e.g., **58** at 3 mg/kg). An interesting in vivo test for $mGlu_5$ PAMs in rats was described as correlating well with results from pharmacological models with known relevance to schizophrenia, such as the amphetamine-induced hyperlocomotion assay. Based on the well-studied body-temperature-lowering effects of $mGlu_5$ NAMs in rats, $mGlu_5$ PAMs were found

to increase body temperature in a dose-dependent manner. In this assay **57** had MED = 0.3 mg/kg and **58** MED = 0.1 mg/kg (dosed by an injection route), so while **57** and **58** had similar activity in the body temperature assay, **57** was much better tolerated at higher doses. In a following Roche patent application [78], the Michael-addition-type reactivity of the molecules was evaluated in the presence of 5 mM glutathione, and the potentially chemically reactive drugs were flagged. The alkynyl pyrimidine derivative **59** (Fig. 8) while showing a potent activity PAM ($EC_{50}$ = 9.1 nM) did not show a flag in the disclosed glutathione addition assay.

## *3.5 Alkyne Replacements*

Replacement of the alkyne group in the aforementioned PAM series was also investigated, and indeed other two-atom linkers produced active compounds. These included amides as in VU0357121 **60** ($EC_{50}$ = 33 nM, $Glu_{max}$ = 92%, glutamate shift = 2.6; Fig. 9) [79]. Initially **60** did not appear to bind to the MPEP site as it failed to displace [$^3$H]-methoxyPEPy, similar to earlier observations with CPPHA. However unlike CPPHA, **60** was susceptible to mutations in rat $mGlu_5$ that affected MPEP and VU29 binding. Since **60** did not behave like CPPHA in several other studies, it appeared **60** might be binding at a third distinct allosteric site on $mGlu_5$. Subsequently it was discovered that **60** was able to partially displace (~35%) [$^3$H]-methoxyPEPy binding at 30 μM [80]. Selectivity was observed for **60** relative to known mGlu receptors. Poor solubility and metabolic stability prevented **60** from being evaluated in vivo [81]. The series in general was characterized by shallow SAR and so far has not yielded a NAM. Compound **61** (VU0365396) however was found to be a SAM.

Methylene-oxy replacements for the alkyne are seen in compounds **62–64** (Fig. 9) and were reported by AstraZeneca and VCNDD/Janssen Pharmaceutical team.

Oxazine **62** (an analog of alkyne **52**) had good potency but high log P and modest solubility ($hmGlu_5$ $EC_{50}$ = 50.1 nM, log*P* = 3.5) [75]. Compound **63** (VU404251) had sufficient potency ($rmGlu_5$ $EC_{50}$ = 7.2 nM, $Glu_{max}$ = 83%, glutamate shift = 8.2), selectivity vs other mGlu subtypes, and pharmacokinetics to allow in vivo efficacy studies [72]. At 100 mg/kg PO, **63** reversed amphetamine-induced hyperlocomotion in rats. Evidence supported **63** binding at the MPEP site, as it fully displaced [$^3$H]-methoxyPEPy with a $rmGlu_5$ $K_i$ = 153 nM [82]. Compound **64** was a weak PAM in terms of $EC_{50}$ but had a moderate 4.4 glutamate shift at 10 μM ($hmGlu_5$ $EC_{50}$ = 1,195 nM, $Glu_{max}$ = 89%, glutamate shift = 4.4; $rmGlu_5$ $EC_{50}$ = 3,408 nM, $Glu_{max}$ = 62%). For **64**, mGlu 1–4 and 6–8 had $EC_{50}$ > 10 μM, but given the micromolar potency vs $mGlu_5$, selectivity was not well established. Surprisingly, **64** was active in the rat amphetamine-induced hyperlocomotion assay at 10 to 100 mg/kg PO. In separate pharmacokinetic studies, 10 mg/kg **64** gave brain $C_{max}$ = 522 nM, well below the rat $EC_{50}$ value. These series are typically partial PAMs as shown in several patent applications from VCNDD. In particular compound **65** had $pEC_{50}$ = 7.59 ($EC_{50}$ = 26 nM), $Glu_{max}$ = 61% on $hmGlu_5$ cloned HEK cells; this

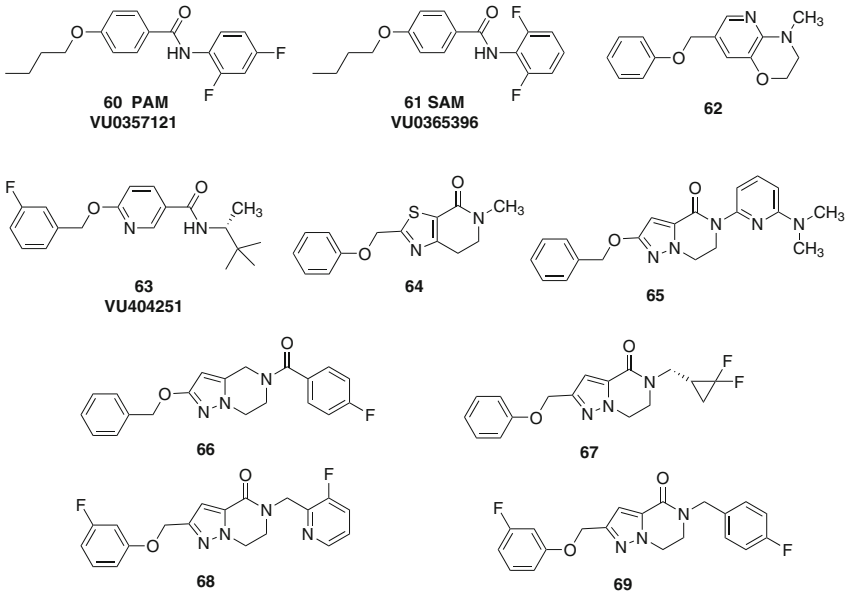



**Fig. 9** Carboxamido and ether $mGlu_5$ PAM alkyne isosters

molecule reversed hyperlocomotor activity induced by amphetamine (1 mg/kg SC) in rats at the dose of 10 mg/kg PO (32% reversal of effect) [83]. It should be noted that the piperazinone ring can be expanded to a [1,4]diazepinone while keeping PAM activity. Extrusion of the carbonyl led to **66** ($hmGlu_5$ $pEC_{50} = 6.78$ ($EC_{50} = 166$ nM) $Glu_{max} = 68\%$); this molecule demonstrated 44% reversal at 30 mg/kg PO in the amphetamine test [84]. Compound **67** shows a glutamate shift enantioselectivity. While being very similar in terms of potency and efficacy, the S isomer **67** ($hmGlu_5$ $pEC_{50} = 6.63$ ($EC_{50} = 234$ nM) $Glu_{max} = 55\%$) had a shift of 3.9 (10 μM), while the R isomer ($hmGlu_5$ $pEC_{50} = 6.42$ ($EC_{50} = 380$ nM) $Glu_{max} = 65\%$) had a shift of 10.4 (10 μM) [85]. Another class of derivatives like **68** has been described in a patent application [86]; **68** was still a partial PAM ($hmGlu_5$ $pEC_{50} = 6.26$ ($EC_{50} = 550$ nM), $Glu_{max} = 65\%$) and was active in the amphetamine hyperlocomotion rat model at 10 mg/kg PO. A parent compound, 5-(4-fluorobenzyl)-2-((3-fluorophenoxy)methyl)-4,5,6,7-tetrahydropyrazolo[1,5-α]pyrazine **69** (VU0448187), has been shown to specifically activate the midazolam hydroxylase activity of CYP3A4 [87] leading to the occurrence of potential drug-drug interaction. This compound is moderately potent ($hmGlu_5$ $EC_{50} = 1{,}900$ nM).

## *3.6* N-*Aryl Piperazines*

Related *N*-aryl piperazine $mGlu_5$ PAMs were independently discovered by AstraZeneca, GSK, and VCNDD (Fig. 10). Compound **70** (CPPZ) resulted from

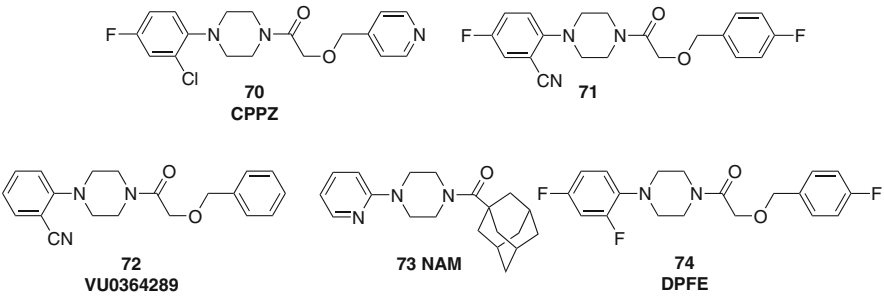



**Fig. 10** Chemical structures of *N*-aryl piperazine $mGlu_5$ modulators

SAR studies of substituted acetamide 4-aryl piperazine and piperidine HTS leads. It had moderate PAM activity (human $EC_{50} = 550$ nM), with similar potency observed for the related 4-aryl piperidine analog [88]. On human $mGlu_5$, the maximum glutamate response in the presence of **70** was 80% that of glutamate alone, and no agonist activity was observed. Compound **70** had no detectable activity on mGlu 1–4 and 6–8 up to 25 μM.

In rat primary cortical neurons, **70** potentiated the response to 3,5-DHPG by 2.6-fold at 3 μM and increased the maximal response by 24% [89]. In radioligand binding studies, **70** completely displaced [$^3$H]MPEP with a $K_i$ of 2.37 μM, consistent with binding at the MPEP site. Pharmacokinetic studies in rats showed high plasma clearance (55 mL/min/kg), moderate volume of distribution (4.8 L/kg), 41% oral bioavailability, and a 7.3-hour terminal half-life. Pharmacokinetic properties trended similarly in dogs ($t_{1/2} = 2.9$ h, $F = 37\%$). Plasma protein binding was highest in dog and lowest in human plasma (21% and 47% free, respectively). A high brain/plasma ratio in rats (4.6) reflected excellent in vitro permeability and lack of P-gp-mediated efflux. LTP induction in rat hippocampal slices was potentiated 17% following a subthreshold stimulus train in the presence of **70** (100 nM). MK-801-induced hyperlocomotion in mice was significantly reduced with 10 μmol/kg SC (the minimum effective dose) and 30 μmol/kg SC of **70**. The estimated unbound concentrations in brain were 300 and 900 nM, indicating that brain levels above one half of the $EC_{50}$ were required for activity. The same pharmacodynamic conclusion was reached in a rat conditioned avoidance response (CAR) test, where **70** showed a dose-dependent decrease in avoidance responding at estimated unbound brain levels between 330 and 650 nM.

A similar compound, *N*-aryl piperazine **71**, was disclosed in a patent application from GSK [90]. Published information about **71** is restricted to the patent application, which reported that the human $mGlu_5$ PAM $pEC_{50}$ was greater than 6.0 ($EC_{50} < 1$ μM).

The *N*-aryl piperazine **72** (VU0364289) was derived from a structurally related $mGlu_5$ NAM. While exploring the SAR of piperazine adamantyl amide $mGlu_5$ NAM HTS lead **73** ($IC_{50} = 0.99$ μM, 3.75% $Glu_{max}$), it was discovered that a benzyloxy acetamide analog was a weak PAM and that benzyloxy acetamide was a molecular switch in this series. This discovery was pursued further to obtain PAM **72** (initial

$rmGlu_5$ $EC_{50} = 1.6$ μM) [91, 92]. Extensive studies at VCNDD compared **72**–**74** (Fig. 10), a compound originally disclosed in an AstraZeneca patent application [93]. Functional rat $mGlu_5$ activity was determined in a $Ca^{2+}$ mobilization assay for **72** and **74** ($EC_{50} = 450$, 120 nM; $Glu_{max} = 100\%$, glutamate shift = 7.3, 17.2 fold at 30 μM, respectively) [94]. Partial agonism of $mGlu_5$ was observed at 100 μM (**72**) and 30 μM (**74**), and effects on potentiation of other mGlu receptors were not detected up to 10 μM. Evaluation of progressive glutamate fold-shift data using an operational model of allosterism showed that both **72** and **74** exerted their effects mainly by efficacy modulation ($\beta = 7.9$, 13.8, respectively) rather than glutamate affinity modulation ($K_B = 6.6$, 4.8 μM). Binding to the MPEP site was supported by several studies: [$^3$H]-methoxyPEPy competition binding results (**72** $K_i = 15$ μM, **74** $K_i = 8$ μM), effects of 5MPEP on concentration response curves, and the impact of point mutations A809V and F585I on potentiation (decrease and no effect, respectively) [21, 94]. Potentiation of glutamate-induced pERK1/2 and analysis of the fold-shift data using an operational model of allosterism revealed higher affinity estimates for **72** and **74** ($K_B = 513$, 135 nM) and lower cooperativity ($\beta = 1.4$,1.3) compared to effects on calcium mobilization. Of note, both **72** and **74** (3 μM) acted as agonists to induce phosphorylation of ERK1/2 with greater efficacy than glutamate.

Pharmacokinetic studies demonstrated that micromolar plasma levels could be achieved in rats with 10 mg/kg IP doses of **72** and **74** ($C_{max} = 3.8$, 3.0 μM, $T_{max} = 0.15$ h). Concentration-time plots from time course studies showed that **74** maintained higher brain and plasma levels through 6 hours (≥270 nM) compared to **72** (~30 nM at 3 h). Both **72** and **74** had good brain/plasma ratios (1.09, 1.26) and free fraction (0.10, 0.04).

In rats, **72** and **74** dose-dependently reversed amphetamine-induced hyperlocomotion, at minimum effective doses of 56.6 and 30 mg/kg IP. Activity was also observed in the NR1KD transgenic mouse model of NMDAR hypofunction, where **74** (30 and 56.6 mg/kg IP) reversed hyperlocomotion. In this same mouse model, **74** had no effect at 10 mg/kg IP on cognitive performance (y-maze test) or social interactions. In another cognition test, **74** was active in contextual fear conditioning at a dose much lower than those required to reverse hyperlocomotion (0.56 mg/kg IP). However **74** did not reverse apomorphine-induced disruption of prepulse inhibition of the acoustic startle reflex even at 30–100 mg/kg IP. In rat sleep-wake architecture studies, acute oral doses of **74** at 30 and 100 mg/kg primarily increased passive wakefulness. Monoamine release in the rat prefrontal cortex (PFC) was dose dependent; at 10 mg/kg PO norepinephrine increased 200% over control, while at 30 and 100 mg/kg PO, there was a 300–500% increase in norepinephrine, dopamine, and serotonin levels.

## *3.7 Miscellaneous*

The *N*-aryl glycine sulfonamide **75** (VU400100, Fig. 11) is an $mGlu_5$ PAM that does not appear to bind to the MPEP site, based on radioligand studies with

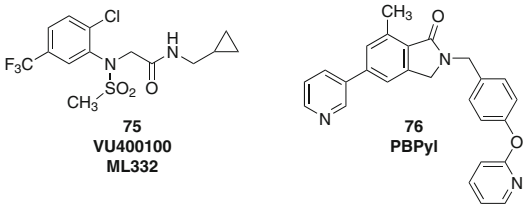



**Fig. 11** Chemical structures of *N*-aryl glycine sulfonamide and pyridyloxybenzyl isoindoline $mGlu_5$ PAM series

[$^3$H]-methoxyPEPy ($K_i > 30$ μM) [79, 95]. It had modest activity on rat and human receptors (rm$Glu_5$ $EC_{50} = 398$ nM, $Glu_{max} = 61\%$, hm$Glu_5$ $EC_{50} = 4.5$ μM, $Glu_{max} = 70\%$). Extensive first-pass hepatic metabolism occurred after oral dosing in rats. Better exposure was obtained with IP or SC administration, which allowed a determination that **75** was brain penetrant (brain/plasma AUC ratio = 0.4). Like CPPHA, SAR studies with **75** showed steep SAR, and no molecular switches have been identified to date. Low-energy shape-based alignment of **75** with other $mGlu_5$ modulators showed a preferred fit for CPPHA **2** relative to MPEP-based modulators; however data was not presented to support the implication that **75** was binding at the CPPHA site.

## *3.8 Radiolabelled Agents*

A radioligand analog of the $mGlu_5$ antagonist, MPEP, [$^3$H]-methoxyPEPy ([$^3$H]-3-methoxy-5-(pyridine-2-ylethynyl)pyridine), has provided a valuable tool for mapping $mGlu_5$ allosteric binding sites. The ability of $mGlu_5$ PAMs with diverse structures to compete for binding of [$^3$H]-methoxyPEPy has been used to determine whether multiple allosteric sites exist on $mGlu_5$. The $mGlu_5$ PAMs DFB **1**, CDPPB **4**, ADX47273 **13**, VU29 **5**, and Lundbeck alkyne **33** competed with [$^3$H]-methoxyPEPy binding. VU0357121 **60** shares a functional interaction with the MPEP site [79]. In contrast, CPPHA **2** and VU400100 **75** did not displace [$^3$H]-methoxyPEPy binding [96]. VU400100 **75** is structurally unrelated to CPPHA **2**, and there is no published data regarding its interaction with that site, so its binding site is currently unknown. Overall, these studies have resulted in clustering $mGluR_5$ PAMs as binding to MPEP and non-MPEP sites.

A dedicated effort at AstraZeneca sought suitable $mGlu_5$ PAM ligands for radiolabelling and imaging studies and identified **76** (PBPyl) as a good candidate (Fig. 11). In a FLIPR assay, it had similar activity on human recombinant $mGlu_5$ ($EC_{50} = 87$ nM, $Glu_{max} = 89\%$) and rat cortical neurons ($EC_{50} = 81$ nM, $E_{max} = 42\%$), so that activity in the overexpressing human cell line might translate to a native preparation [97]. It should be noticed that only $mGlu_2$ showed selectivity for PBPyl ($EC_{50} = 430$ nM). A single tritium was introduced to **76** in one step using Ir-catalyzed tritium hydrogen exchange with Crabtree™ catalyst, to give [$^3$H]-PBPyl. In recombinant hm$Glu_5$ membranes, [$^3$H]-PBPyl demonstrated saturable binding and $K_d = 18.6$ nM, $B_{max} = 8{,}634$ fmol/mg. Reference $mGlu_5$ NAM

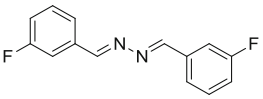
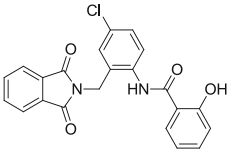
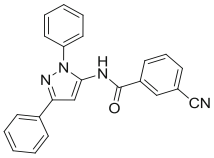
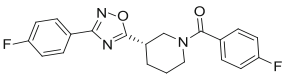
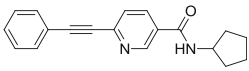
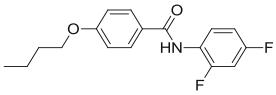
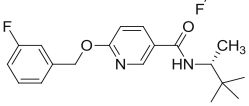
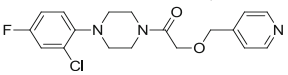
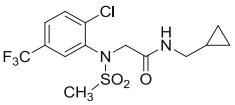
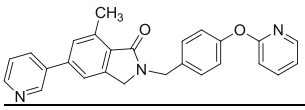
and PAM compounds displaced [$^3$H]-PBPyl in a concentration-dependent manner: MPEP $K_i = 1.42$ nM, VU29 **5** $K_i = 144$ nM, and ADX47273 **13** $K_i = 5{,}800$ nM. PBPyl had reasonable brain penetration (brain/plasma ratio = 0.35). However low solubility, high lipophilicity (clog$P = 4.26$), large volume of distribution, and high plasma protein binding (99.4%) made it unsuitable for in vivo studies. As the first $mGlu_5$ PAM radiolabel, it will prove useful for in vitro studies.

## 4 Mutagenesis Studies and Mapping of $mGlu_5$ Allosteric Sites

Table 1 summarizes the functional SAR and putative binding sites of the various classes of $mGlu_5$ PAMs presented in this article. The functional PAM/NAM continuum means that there are chemical variations in the same series leading to molecules with opposite functional activity. The mapping of allosteric sites on $mGlu_5$ has been pursued through mutagenesis studies of amino acid residues to determine whether $mGlu_5$ PAMs bind to the same or different sites than the allosteric antagonist, MPEP **21**. Previously, amino acid residues within TM3, TM5, TM6, and TM7 were identified to contribute to the MPEP antagonist allosteric site. Specifically, amino acid residues Ala-810 in TM7, and Pro-655 and Ser-658 in TM3, were identified as essential residues in human $mGlu_5$ to support methoxyPEPy binding [62]. Of the equivalent residues in rat $mGlu_5$, it was found that Ala-809 and Pro-654 were important for MPEP binding and activity, but not Ser-657. The residues Thr-780, Trp-784, Phe-787, and Tyr-791 in TM6, Leu-743 in TM5, and Tyr-658 in TM3 were also found to be important for MPEP binding and activity [63]. The structurally unrelated NAM fenobam was found to depend on the same rat $mGlu_5$ residues as MPEP and methoxyPEPy, with the exception of Leu-743 in TM3 (no effect on binding) and Arg-647 in TM3 (important for binding) [98]. Thus these NAMs share some of the same binding site. A different study identified two additional mutations in human $mGlu_5$ that significantly decreased MPEP activity, Ser-809 in TM7 and Ile-651 in TM3 [99].

Site-directed mutagenesis of these amino acid was subsequently explored to determine if they were also essential for $mGlu_5$ PAM activity. Mutations in the rat receptor that disrupted the increase in functional response to DFB **1** were observed for Ser-657, Thr-780, and Met-801. Interestingly, an increase in DFB potentiation of quisqualate $EC_{50}$ and $E_{max}$ occurred with mutations at Leu-743 and Trp-784, while Phe-787 substituted with Ala caused DFB to become a weak partial antagonist. Except for Met-801 in TM7, which has not been much studied, all of these residues were found to affect one or more NAMs above, indicating overlap in the binding site with DFB **1** [100]. In other studies, substitution of Ala-810 with valine in human $mGlu_5$ resulted in a significant reduction on the functional potency of PAMs CDPPB **4**, VU29 **5**, ADX47273 **13**, and Lundbeck alkyne **33**, but had no effect on the functional potency of CPPHA **2** [Jacobson, unpublished results].

**Table 1** Properties of $mGlu_5$ PAM ligands

| Compound | Name, chemical class | PAM/ NAM continuum | MPEP site competitive | References |
|---|---|---|---|---|
|  | DFB, **1** Benzaldazine | Yes | Yes | [101] |
|  | CPPHA, **2** Dioxoisoindolinyl-methylphenyl benzamide | No | No | [96, 102] |
|  | CDPPB, **4** Diphenyl pyrazole | Yes | Partial overlap | [41] |
|  | ADX47273, **13** Piperidinyl oxadiazole | Yes | Partial overlap | [49] |
|  | Lundbeck cpd, **33** Diarylalkyne | Yes | Yes | [65] |
|  | VU0357121, **60** Diacrylamide | PAM/ SAM | Partial overlap | [79] |
|  | VU404251, **63** Diarylether | unknown | Yes | [82] |
|  | CPPZ, **70** *N*-aryl piperazine | Yes | Yes | [88] |
|  | VU400100, **75** *N*-arylglycine sulfonamide | No | No (unknown for CPPHA) | [96] |
|  | PBPyl, **76** Pyridyloxybenzyl isoindolinone | Unknown | Yes | [97] |

A small decrease in potency was observed for DFB **1** with substitution of Ala-810 with valine. A decrease in functional potency was also observed with substitution of Pro-655 with serine for ADX47273 **13**, Lundbeck alkyne **33**, and MPEP **21**; however, no effect on the functional potency of CDPPB **4**, VU29 **5**, DFB **1**, and CPPHA **2** was observed [Jacobson, unpublished results].

These results suggested that the binding sites for ADX47273 **13**, Lundbeck alkyne **33**, and MPEP **21** on $mGlu_5$ might be in common, whereas CDPPB **4**,

VU54, and DFB **1** may have some overlap with the MPEP binding site. In contrast, the functional activity of CPPHA **2** was not affected by the amino acid substitutions suggesting that CPPHA may not require the same amino acid contacts as MPEP and might bind to another non-MPEP site [101]. This is consistent with results where modification of a residue in TM1, F585 to isoleucine, led to loss of CPPHA **2** and NCFP **3** potentiation of $mGlu_5$ [26, 102].

Studies on a group of PAMs structurally related to **33** expand on the results above. Picolinamides VU0403602 **38**, VU0405398 **40**, and VU0405386 **42** had about 10-fold higher affinity for $mGlu_5$ than their nicotinamide analogs VU0360172 **37**, VU0367173 **39**, and VU0415051 **41** (Fig. 7). These six $mGlu_5$ PAMs were tested on mutant rat $mGlu_5$ receptors to assess the effect of various point mutations on affinity and cooperativity, using an operational model of allosterism [80]. Affinity for all six compounds was significantly impacted by mutations at three conserved residues (Tyr-658, Thr-780, Ser-808) and two nonconserved residues (Pro-654, Ala-809), so that these amino acids were deemed critical for PAM affinity within this series. Point mutations that altered cooperativity, sometimes drastically, were also identified. Mutation of Tyr-658 to valine switched picolinamide VU0405398 **40** from a PAM to a weak NAM, while the other five compounds lost all potentiation. Mutation of Thr-780 to alanine in TM6 converted nicotinamide VU0415051 **41** to a weak NAM and led to loss of potentiation for the other two nicotinamides, VU0360172 **37** and VU0367173 **39**, without significantly affecting the picolinamides. The largest effect was seen for Ser-808 substituted with alanine in TM7, which converted VU0405398 **40** from a weak PAM to a full NAM, and VU0405386 **42** from a PAM to a SAM.

In the absence of structural information, it is difficult to conclude with certainty that $mGluR_5$ PAMs bind at distinct or overlapping sites, or whether they interact indirectly through an allosteric mechanism. Since a crystal structure of the transmembrane region of a family C GPCR has yet to be determined, modeling studies of ligands with the receptor have relied on the generation of homology models of $mGlu_5$. Differences in construction and ligand modeling have produced a number of homology models of NAM and PAM binding [62, 80, 98–100, 103, 104].

A homology model for the human $mGlu_5$ receptor was generated using a combination of published sequence alignments [62, 98, 102] and the rhodopsin crystal structure, 1L9H. Ten models were built with MOE (molecular operating environment) using default values, and the final model was minimized using Merck molecular force field 94. Ligands were docked using FLOG (flexible ligand oriented on grid), and essential points of contact were derived from point mutation data to orient the ligands in the transmembrane domains. The potential overlap of binding sites for MPEP and the $mGlu_5$ PAMs, ADX47273 **13**, CDPPB **4**, and Lundbeck **33** is illustrated in the homology model shown in Fig. 12. The docking results shown were guided by the change in functional potencies resulting from the point mutagenesis. The model supports Lundbeck **33** sharing the most overlap with the MPEP binding site. ADX47273 **13** was found to partially overlap with MPEP with positioning in the interface of TM3, TM6, and TM7. In comparison, CDPPB partially overlaps with the MPEP binding site. However, the ligand is

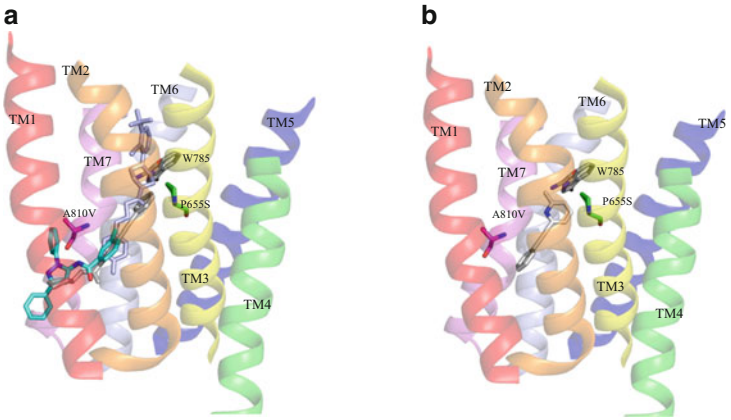



**Fig. 12** A hmGlu$_5$ receptor homology model with binding poses for PAMs (**a**) CDPPB **4** (*cyan*), ADX47273 **13** (*purple*), Lundbeck **33** (*gray*) and (**b**) MPEP **21** (NAM). Amino acid residues Ala-810 Pro-655 and Trp-785 side chains shown

positioned further from the Pro-655 in comparison with MPEP and the other PAMs modeled.

A model proposing a common allosteric binding site on mGlu$_5$ shared by PAMs of diverse scaffolds is further supported by a recent report by researchers at VCNDD and Monash University [104]. Using the rat mGlu$_5$ as a template, the effect of site-directed mutagenesis of residues previously identified as affinity determinants of mGlu5 PAMS and NAMs (P654, Y658, T780, W784, S808, and A809) was assessed with diverse scaffolds. Similar to the model developed for the human mGlu$_5$, a common allosteric binding site with the long axis of the scaffolds positioned parallel to TM3 and TM7 was postulated. The contribution of W784 in TM6 of rat mGlu$_5$ (human W785, Fig. 12) is a key determinant of NAM cooperativity and is responsible for the molecular switch of NAM to PAM activity for two NAM scaffolds, VU366248 and VU366249.

## 5 mGlu$_5$ PAMs as a Novel Treatment for Schizophrenia: In Vivo Validation

There is growing in vivo evidence supporting the notion that positive allosteric modulators of the mGlu$_5$ receptor can offer therapeutic benefit in treatment of patients with schizophrenia. Thus far, the preclinical research efforts have been focused on evaluation of efficacy of mGlu$_5$ PAMs in rodent models of positive symptoms and cognitive abnormalities of schizophrenia, while those linked to negative symptoms of the disease received less attention.

Amphetamine-induced hyperactivity in rodents has been traditionally used as one of the first screening assays for evaluation of antipsychotic-like efficacy of

novel compounds. The hyperactivity is believed to be mediated by mesolimbic dopamine pathway and is sensitive to typical and atypical antipsychotic drugs [105]. A wide range of $mGlu_5$ PAMs with distinct chemical scaffolds, CDPPB **4**, ADX47273 **13**, 5PAM523 **15**, VU0360172 **37**, VU0364289 **66**, and DPFE **68**, caused robust reductions in amphetamine-induced hyperactivity in rodents [4, 50, 66, 89, 94, 96, 106], consistent with a potential role for $mGlu_5$ PAMs in treatment of positive symptoms of schizophrenia. Evaluation of $mGlu_5$ PAMs in other pharmacological models of psychosis involving indirect (amphetamine) or direct (apomorphine) dopamine agonists revealed similar results. For example, ADX47273 **13** reduced apomorphine-induced hyperactivity as well as apomorphine-induced climbing in mice [4], revealing a profile that is also seen in mice treated with an atypical antipsychotic drug clozapine [107]. The effect of ADX47273 in the climbing assay appeared to be $mGlu_5$ receptor specific, as its antagonists, MPEP and MTEP, markedly attenuated the effect of ADX47273 [4]. The effects of $mGlu_5$ PAMs in models involving behaviors stimulated by dopamine agonists could, in part, be mediated by the reduction of dopamine neurotransmission, as ADX47273 reduced extracellular concentrations of dopamine in the nucleus accumbens, the projection target of the mesolimbic dopamine pathway, in awake, freely moving rats [108]. It is important to note that ADX47273 had only minimal effects on apomorphine-induced stereotypy, believed to be mediated by nigrostriatal dopamine pathway and did not alter concentrations of dopamine in the striatum of freely moving rats [4]. Such selective reduction in mesolimbic relative to nigrostriatal dopamine has been also observed in animals treated with antipsychotic drugs [107].

In another standard screening model for antipsychotic efficacy, the conditioned avoidance response (CAR) test in the rat [109], ADX47273, dose-dependently decreased avoidance responding, without increasing the number of no-response trials [4]. Similar effects have been seen in animals treated with typical and atypical antipsychotic drugs [109]. As seen in apomorphine-induced climbing assay, the effect of ADX47273 in the CAR was significantly attenuated by pretreatment of animals with $mGlu_5$ antagonists MPEP or MTEP confirming the target specificity of the effect [4].

Further, in a model relevant to the sensory-motor deficits observed in schizophrenic patients, ADX47273 reversed amphetamine-induced disruption of the prepulse inhibition (PPI) of the acoustic startle test in the rat [89]. In accord with these findings, CDPPB and an $mGlu_5$ receptor agonist CHPG reversed amphetamine-induced disruption of PPI in rats [30, 109]. These effects were specific to the PPI deficit, as the compound did not impact the baseline PPI performance in drug-naïve animals. The effects of $mGlu_5$ activators in models involving pharmacological disruption of PPI are well aligned with the evidence that $mGlu_5$ knock-out mice exhibit consistent deficits in PPI in comparison to their wild-type controls [109, 110].

As there is a close association between the $mGlu_5$ and NMDA receptors, evaluation of $mGlu_5$ PAMs in a set of pharmacological models of psychosis involving behavioral responses induced by NMDA receptor antagonists is particularly important. ADX47273 reduced PCP-induced hyperactivity in mice [4] and

ketamine-induced hyperactivity in rats [14], providing additional evidence supporting its antipsychotic-like profile. Also, a genetic model of NMDA receptor hypofunction, DPFE **68** reduced locomotor hyperactivity exhibited by NR1KD mice [111]. These animals have approximately 90% reduction in functional NMDA receptors in limbic and forebrain regions and exhibit a series of behavioral alterations believed to be relevant to symptoms of schizophrenia [111].

While the majority of studies involving evaluation of $mGlu_5$ PAMs in models of positive symptoms of schizophrenia showed a clear efficacy of these compounds, few reported lack of efficacy or only marginal activity. For example, [112] was unable to detect efficacy of ADX47273 in PCP-induced hyperactivity in the rat. Also, in the amphetamine-induced hyperactivity test in the rat, Gilmour et al. [53] detected only negligible activity of CDPPB **4** and ADX47273 **13** and recently characterized $mGlu_5$ PAMs, LSN2814617 **20**, and LSN2463359 **47**. Currently, the reasons of this discrepancy are not understood, but one can speculate that the choice of the pharmacological agent used, the experimental variables (e.g., the strain of rats used), or aspects of pharmacological characteristics of various groups of PAMs (e.g., affinity vs cooperativity in the PAM/glutamate interaction [94]) may have an impact on the behavioral outcome.

There is a growing body of evidence suggesting that $mGlu_5$ PAMs show pro-cognitive effects in drug-naïve, intact animals as well as in models of impaired cognitive functioning, together supporting the notion that in addition to positive symptoms, $mGlu_5$ PAMs can improve cognitive functioning in patients with schizophrenia. For example, in the novel object recognition (NOR) test performed in drug-naïve rats, ADX47273 dose-dependently reduced natural forgetting, improving recall following a 48-h delay [4]. Previously, it has been reported that activation of Group I mGlu receptors in the perirhinal cortex is necessary for the acquisition, but not consolidation or retrieval of object recognition memory [113]. ADX47273 **13** also reduced natural forgetting assessed in the NOR test in mice [114]. In the five-choice serial reaction time (5-CSRT) test performed in drug-naive rats, ADX47273 reduced premature responding, indicative of inhibition of impulsivity, albeit without improving percentage of correct responding. The 5-CSRT test in the rat is believed to represent a preclinical analog of the continuous performance test, a widely used cognitive test for evaluation of impulsivity and attention in humans [115]. The ability of ADX47273 to reduce premature responding in rats suggests that allosteric activation of $mGlu_5$ may reduce impulsivity observed in schizophrenic patients [116].

In a model of cognitive functioning, involving assessment of hippocampus-dependent learning task in drug-naïve animals, the contextual fear conditioning test in mice [117], DPFE **68** enhanced acquisition of conditioning [94]. Interestingly, DPFE also increased concentration of monoamines and serotonin in the prefrontal cortex of freely moving rats [94], consistent with previously reported profile of an atypical antipsychotic drug, risperidone [118, 119]. In another hippocampus-dependent spatial learning task performed in drug-naïve animals, the Morris water maze (MWM) test in rats, ADX47273 and CDPPB **4** reduced

the number of days required to reach the acquisition criteria, indicative of cognitive enhancement [120].

Signs of perseveration and lack of cognitive flexibility have been commonly described in schizophrenia patients. Several studies provided evidence that $mGlu_5$ PAMs can improve cognitive flexibility in these patients. For example, in the MWM test in drug-naïve mice, ADX47273 administered before the reversal training decreased the latency to learn the position of the platform, indicative of the enhancement of cognitive flexibility [121]. These results are well aligned with those indicating that $mGlu_5$ KO mice show impairment in reversal learning in the MWM test [122]. Also, in a model of pharmacologically disrupted cognitive flexibility, the set-shifting test disrupted by MK-801 in the rat, CDPPB **4**, improved cognitive flexibility [123]. Also, in another study, $mGlu_5$ PAMs reversed NMDA-induced deficit in the set-shifting test in the rat [35]. The set-shifting test in the rat is believed to represent a preclinical analog of the Wisconsin card sorting test, a widely used cognitive test for evaluation of cognitive flexibility in humans. It has been shown that schizophrenia patients exhibit marked deficits in the performance of the Wisconsin card sorting test. Thus, improvement of cognitive flexibility in drug-naïve animals together with reversal of the pharmacologically induced deficit in cognitive flexibility following treatment of $mGlu_5$ PAMs suggests that these compounds have a potential in reversing deficits in cognitive flexibility exhibited by schizophrenia patients.

While ADX47273, CDPPB **4**, and DPFE **68** have shown pro-cognitive effects in several tests across number of laboratories, effects of newly characterized PAMs, LSN2814617 **20**, and LSN2463359 **47** provided conflicting results. On the one hand, in a neurodevelopmental model of schizophrenia based on the disruption of neurogenesis on gestational day 17 with MAM (methyl azoxy methanol E17 model; [124, 125]), LSN246339 **20** selectively attenuated reversal learning deficits [73]. On another hand, LSN246339 failed to attenuate reversal learning deficit induced by PCP [73]. The lack of effect in the latter test might be related to the pharmacological mechanism of learning deficit, as LSN246339 attenuated reversal learning deficit associated with the competitive NMDA antagonist SDZ 220,581 [73]. In fact, in this experiment LSN246339 reduced perseverative deficits and reduced the number of regressive errors [73]. Previously, it has been shown that lesions in the orbitofrontal cortex and infralimbic cortex in the rat results in impairment in reversal learning in the form of increased perseverative and "new learning" errors, respectively [126]. It can be hypothesized that activation of $mGlu_5$ receptors located in these regions with a PAM can eliminate cognitive deficits in learning and cognitive flexibility observed in schizophrenia patients.

There is indirect, electrophysiological evidence supporting the role of $mGlu_5$ PAMs in cognition. For example, $mGlu_5$ receptor activation has been found to facilitate NMDA currents [127], long-term potentiation (LTP) [120, 128, 129] and long-term depression (LTD ) [120, 130, 131]. The LTP and LTD are thought to play an important role in learning and memory [132, 133]. In accord with these findings, $mGlu_5$ KO mice showed deficits in NMDA currents, NMDA-mediated synaptic plasticity and learning [134, 135]. Also, an $mGlu_5$ PAM, CPPHA increased

phosphorylation of ERK and CREB in hippocampal slices [136], two signaling molecules that are believed to play an important role in learning and memory. A follow-up study showed increases in phosphorylation of ERK and CREB in the hippocampus and prefrontal cortex following treatment with ADX47273 **13** [4].

There is accumulating evidence suggesting that $mGlu_5$ PAMs can elicit significant changes in sleep/wake architecture, which may further augment pro-cognitive effects of these compounds. Specifically, DPFE increased wakefulness in the rat [94]. Increases in wakefulness and light sleep have been reported in rats treated with CDPPB [50]. Also, the novel $mGlu_5$ PAMs, LSN2463359 **47** and LSN2814617 **20**, that showed only marginal effects in models of positive symptoms of schizophrenia, showed robust increases in wakefulness in the rat [53].

So far there has been only one study which evaluated effects of $mGlu_5$ PAMs in animal models of negative symptoms. According to [37] acute administration of CDPPB eliminated deficit in sucrose consumption caused in rats by MK-801, in a manner similar to that seen with acute D-serine or subchronic clozapine, but not subchronic haloperidol. MK-801-induced reduction in sucrose consumption in the rat is believed to mimic features of anhedonia (reduced experience of pleasure), which is one of the core component of negative symptoms of schizophrenia [137, 138]. This is the first evidence that activation of $mGlu_5$ with a PAM can offer therapeutic benefit in treatment of negative symptoms as well as positive symptoms and cognitive abnormalities in patients with schizophrenia.

## 6 Conclusion

In the time since this first report of the discovery of a $mGlu_5$ positive allosteric modulator a decade ago, our understanding of the medicinal chemistry and pharmacology of $mGlu_5$ PAMs has been advanced through the significant contributions of academic and pharmaceutical research groups. Several structural series have emerged from these efforts, many with demonstration of robust in vivo efficacy in rodent models of schizophrenia symptomatology. The availability of diverse analogs has provided valuable probes to facilitate studies to explore questions of whether these analogs share binding sites and/or overlap with the binding site for the $mGlu_5$ NAM, MPEP. The discoveries of new $mGlu_5$ PAMs have enabled studies to address the potential for selective activation of signaling pathways and biased ligands have been identified. Characterization of $mGlu_5$ PAM activity in native preparations has revealed that PAMs may have differences in functional activity in CNS circuits. Recently, the relationship between positive allosteric modulation of $mGlu_5$ receptors and neurotoxicity has been reported with structural diverse $mGlu_5$ PAMs. These results have led some to question whether $mGlu_5$ PAMs are a viable therapeutic path and if safe treatments could be developed. Based on the recent findings, the safety margin for $mGlu_5$ PAMs might be narrow; however the challenge remains to identify and develop $mGlu_5$ PAMs devoid of intrinsic agonist activity with low to moderate glutamate shifts while maintaining

in vivo activity in animal models predictive of therapeutic efficacy. Optimizing PAMs with high affinity would also aid in the discovery of radiolabelled $mGlu_5$ PAMs for in vitro and in vivo studies. With the availability of robust and predictive assays to characterize the in vitro pharmacological profile of $mGlu_5$ PAMs, and a better understanding of desired properties to translate into in vivo models to define PK/PD correlations, it is highly likely that $mGlu_5$ PAMs will be discovered and advanced into development of a new non-dopaminergic approach for the treatment of schizophrenia.

**Acknowledgments** The authors acknowledge Georgia McGaughey for the $mGlu_5$ homology model and ligand docking generated at Merck Research Labs.

## References

1. Pin JP, Glavez T, Prezeau L (2003) Evolution, structure and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther 98:325–354
2. Schoepp DD, Jane JE, Monn JA (1999) Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 38:1431–1476
3. Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Ann Rev Pharmacol Toxicol 37:205–237
4. Liu F, Grauer S, Kelley C 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 327:827–839
5. Ferraguti F.and Shigemoto R (2006) Metabotropic glutamate receptors. Cell Tissue Res 326:483–504
6. Luyt K, Varadi A, Molnar E (2003) Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells. J Neurochem 84:1452–1464
7. Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. Ann Rev Pharm Tox 50:295–322
8. Schoepp DD (2001) Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther 299:12–20
9. Alagarsamy S, Rouse ST, Junge C et al (2002) NMDA-induced phosphorylation and regulation of mGluR5. Pharmacol Biochem Behav 73:299–306
10. Tu JC, Xiao B, Naisbitt S et al (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. Neuron 23:583–592
11. Kotecha SA, Jackson MF, Al-Mahrouki A et al (2003) Co-stimulation of mGluR5 and *N*-methyl-D-aspartate receptors is required for potentiation of excitatory synaptic transmission in hippocampal neurons. J Biol Chem 278:27742–27749
12. O'Brien JA, Lemaire W, Wittmann M et al (2004) A novel selective allosteric modulator potentiates the activity of native metabotropic glutamate receptor subtype 5 in rat forebrain. J Pharmacol Exp Ther 309:568–577
13. Lüscher C, Huber KM (2010) Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron 65:445–459
14. Rosenbrock H, Kramer G, Hobson S 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 639:40–45
15. Kammermeier PJ (2012) The orthosteric agonist 2-chloro-5-hydroxyphenylglycine activates mGluR5 and mGluR1 with similar efficacy and potency. BMC Pharmacol 12:6

16. Sheffler DJ, Gregory KJ, Rook JM et al (2011) Allosteric modulation of metabotropic glutamate receptors. Adv Pharmacol 62:37–77
17. Gregory KJ, Dong EN, Meiler J et al (2011) Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential. Neuropharmacology 60:66–68
18. May LT, Leach K, Sexton PM, Christopoulos A (2007) Allosteric modulation of G protein-coupled receptors. Annu Rev Pharmacol Toxicol 47:1–51
19. Sharma S, Kedrowski J, Rook JM et al (2009) Discovery of molecular switches that modulate modes of metabotropic glutamate receptor subtype 5 ($mGlu_5$) pharmacology in vitro and in vivo within a series of functionalized regioisomeric 2- and 5-(phenylethynyl)pyrimidines. J Med Chem 52:4103–4106
20. Bradley SJ, Langmead CJ, Watson JM, Challiss RAJ (2011) Quantitative analysis reveals multiple mechanisms of allosteric modulation of the mGlu5 receptor in rat astroglia. Mol Pharmacol 79:874–885
21. Gregory KJ, Noetzel MJ, Rook JM et al (2012) Investigating metabotropic glutamate receptor 5 allosteric modulator cooperativity, affinity, and agonist: enriching structure-function studies and structure–activity relationships. Mol Pharm 82:860–875
22. Rajagopal S, Rajagopal K, Lefkowitz RJ (2010) Teaching old receptors new tricks: biasing seven-transmembrane receptors. Nat Rev Drug Discov 9:373–386
23. Wootten D, Christopoulos A, Sexton PM (2012) Emerging paradigms in GPCR allostery: implications for drug discovery. Nat Rev Drug Discov 12:630–644
24. Zhang Y, Rodriguez AL, Conn JP (2005) Allosteric potentiators of metabotropic glutamate receptor subtype 5 have differential effects on different signaling pathways in cortical astrocytes. J Pharmacol Exp Ther 315:1212–1219
25. Zhao Z, Wisnoski DD, O'Brien JA et al (2007) Challenges in the development of mGluR5 positive allosteric modulators: the discovery of CPPHA. Bioorg Med Chem Lett 17:1386–1389
26. Noetzel MJ, Gregory KJ, Vinson PN 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 Pharm 83:835–847
27. Lindsley CW, Stauffer SR (2013) Metabotropic glutamate receptor 5-positive allosteric modulators for the treatment of schizophrenia (2004–2012). Pharm Pat Analyst 2(1):93–108
28. Stauffer RS (2011) Progress toward positive allosteric modulators of the metabotropic glutamate receptor subtype 5 (mGluR5). ACS Chem Neurosci 2(8):450–470
29. Lindsley CW, Wisnoski DD, Leister WH et al (2004) Discovery of positive allosteric modulators for the metabotropic glutamate receptor subtype 5 from a series of *N*-(1,3-Diphenyl-1H-pyrazol-5-yl) benzamides that potentiate receptor function in vivo. J Med Chem 47:5825–5828
30. Kinney GK, O'Brien JA, Lemaire W et al (2005) A novel selective allosteric modulator of metabotropic glutamate receptor subtype 5 (mGluR5) has an antipsychotic profile in rat behavioral models. J Pharmacol Exp Therapeut 313:199–208
31. De Paulis T, Hemstapat K, Chen Y et al (2006) Substituent effects of *N*-(1,3-diphenyl-1H-pyrazol-5-yl)benzamides on positive allosteric modulation of the metabotropic glutamate-5 receptor in rat cortical astrocytes. J Med Chem 49(11):3332–3344
32. Chen Y, Nong Y, Goudet C et al (2007) Interaction of novel positive allosteric modulators of metabotropic glutamate receptor 5 with the negative allosteric antagonist site is required for potentiation of receptor responses. Mol Pharm 71:1389–1398
33. Zou M-F, Cao J, Rodriguez AL et al (2011) Design and synthesis of substituted *N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamides as positive allosteric modulators of the metabotropic glutamate receptor subtype 5. Bioorg Med Chem Lett 21(9):2650–2654
34. Fowler SW, Walker JM, Klakotskaia et al (2013) Effects of a metabotropic glutamate receptor 5 positive allosteric modulator, CDPPB, on spatial learning task performance in rodents. Neurobiol Learn 99:25–31

35. Stefani MR, Moghaddan B (2010) Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. Eur J Pharmacol 639(1–3):26–32
36. Uslaner JM, Parmentier-Batteur S, Flick RB et al (2009) Dose-dependent effect of CDPPB, the mGluR5 positive allosteric modulator, on recognition memory is associated with GluR1 and CREB phosphorylation in the prefrontal cortex and hippocampus. Neuropharmacol 57 (5–6):531–538
37. Vardigan JD, Huszar SL, McNaughton CH et al (2010) MK-801 produces a deficit in sucrose preference that is reversed by clozapine, D-serine, and the metabotropic glutamate 5 receptor positive allosteric modulator CDPPB: relevance to negative symptoms associated with schizophrenia? Pharmacol Biochem Behav 95(2):223–229
38. Yasuhara A, Sun XM, Sakagami K et al (2009) Amino imidazole derivative. WO 2009/099,177, 13 Aug 2009
39. Grauert M, Bischoff D, Dahmann G et al (2013) Substituted triazoles and their use for treatment and/or prevention neurological and psychiatric disorders. WO 2013/107,761, 25 July 2013
40. Rudolf K, Bischoff D, Dahmann G et al (2013) Piperazine derivatives and their use as positive allosteric modulators of mGlu5 receptors. WO 2013/08,780,620, June 2013
41. Rudolf K, Bischoff D, Dahmann G et al (2013) 4-Aryl imidazole derivatives as mGlu5 positive allosteric modulators. WO 2013/087,807, 20 June 2013
42. Grauert M, Bischoff D, Dahmann G et al (2013) 1,2,4-triazoles as allosteric modulators of mGlu5 receptor activity for the treatment of schizophrenia and dementia. WO 2013/083,741, 13 June 2013
43. Grauert M, Bischoff D, Dahmann G et al (2013) Novel compounds. US 2013/0,137,688, 30 May 2013
44. Grauert M, Bischoff D, Dahmann G et al (2013) Novel compounds. US 2013/0,143,870, 6 June 2013
45. Le Poul E, Bessis AS, Lütjens R et al (2005) In vitro pharmacological characterisation of selective mGluR5 positive allosteric modulators. Poster presented at the 5th international metabotropic glutamate receptors meeting, Taormina, Sicily, Italy, 18–23 September 2005
46. Engers DW, Rodriguez AL, Williams R et al (2009) Synthesis, SAR and unanticipated pharmacological profiles of analogues of the mGluR5 ago-potentiator ADX-47273. ChemMedChem 4:505–511
47. Wagner G, Weber C, Nyeki O et al (2010) Hit-to-lead optimization of disubstituted oxadiazoles and tetrazoles as mGluR5 NAMs. Bioorg Med Chem Lett 20:3737–3741
48. Lamb JA, Engers DW, Niswender CM et al (2011) Discovery of molecular switches within the ADX-47273 mGlu5 PAM scaffold that modulate modes of pharmacology to afford potent mGlu5 NAMs, PAMs, and partial antagonists. Bioorg Med Chem Lett 21:2711–2714
49. Wood MR, Hopkins CR, Brogan JT et al (2011) 'Molecular switches' on allosteric ligands that modulate modes of pharmacology. Biochemistry 50:2403–2410
50. Parmentier-Batteur S, Hutson P, Menzel K et al (2014) Mechanism based neurotoxicity of mGlu5 positive allosteric modulators – development challenges for a promising novel antipsychotic target. Neuropharmacology 82:161–173
51. Packiarajan M, Mazza Ferreira CG, Hong SP et al (2012) *N*-aryl pyrrolidinonyloxadiazoles as potent mGluR5 positive allosteric modulators. Bioorg Med Chem Lett 22:5658–5662
52. Packiarajan M, Mazza Ferreira CG, Hong SP et al (2012) Azetidinyl oxadiazoles as potent mGluR5 positive allosteric modulators. Bioorg Med Chem Lett 22:6469–6474
53. Gilmour G, Broad LM, Wafford KA et al (2013) In vitro characterisation of the novel positive allosteric modulators of the mGlu5 receptor, LSN2463359 and LSN2814617, and their effects on sleep architecture and operant responding in the rat. Neuropharmacology 64:224–239
54. Tizzano JP, Griffey KI, Schoepp DD (1995) Receptor subtypes linked to metabotropic glutamate receptor agonist-mediated limbic seizures in mice. Ann N Y Acad Sci 765:230–235

55. Wong RK, Bianchi R, Chuang SC et al (2005) Group 1 mGluR-induced epileptogenesis: Distinct and overlapping roles of mGluR1 and mGluR5 and implications for antiepileptic drug design. Epilepsy Curr 5:63–68
56. Varney MA, Cosford NDP, Jachec C et al (1999) SIB-1757 and SIB-1893: selective, non-competitive antagonists of metabotropic glutamate receptor type 5. J Pharmacol Exp Ther 290:170–181
57. Gasparini F, Lingenhöhl K, Stoehr N et al (1999) 2-Methyl-6-(phenylethynyl)pyridine (MPEP), a potent selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 38:1493–1503
58. Cosford ND, Tehrani L, Roppe J et al (2003) 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate receptor subtype 5 receptor antagonist with anxiolytic activity. J Med Chem 46:204–206
59. Cosford ND, Roppe J, Tehrani L et al (2003) [3H]-methoxymethyl-MTEP and [3H]-methoxy-PEPy: potent and selective radioligands for the metabotropic glutamate subtype 5 (mGlu5) receptor. Bioorg Med Chem Lett 13:351–354
60. Rodriguez AL, Nong Y, Sekaran NK et al (2005) A close structural analog of 2-methyl-6-(phenylethynyl)pyridine acts as a neutral allosteric site ligand on metabotropic receptor subtype 5 and blocks the effects of multiple allosteric modulators. Mol Pharmacol 68:1793–1802
61. Vanejevs M, Jatzke C, Renner S et al (2008) Positive and negative modulation of group I metabotropic glutamate receptors. J Med Chem 51:634–647
62. Pagano A, Rüegg D, Litschig S et al (2000) The non-competitive antagonists 2-methyl-6-(phenylethynyl)pyridine and 7-hydeoxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester interact with overlapping binding pockets in the transmembrane region of Group 1 metabotropic glutamate receptors. J Biol Chem 275:33750–33758
63. Sharma S, Rodriguez AL, Conn PJ et al (2008) Synthesis and SAR of a $mGlu_5$ allosteric partial antagonist lead: unexpected modulation of pharmacology with slight structural modifications to a 5-(phenylethynyl)pyrimidine scaffold. Bioorg Med Chem Lett 18:4098–5101
64. Ritzén A, Sindet R, Hentzer M et al (2009) Discovery of a potent and brain penetrant mGluR5 positive allosteric modulator. Bioorg Med Chem Lett 19:3275–3278
65. Sams AG, Mikkelsen GK, Brodbeck RM et al (2011) Efficacy Switching SAR of mGluR5 allosteric modulators: Highly potent positive and negative modulators from one chemotype. Bioorg Med Chem Lett 21:3407–3410
66. Rodriguez AL, Grier MD, Jones CK 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 78:1105–1123
67. Noetzel MJ, Rook JM, Vinson PN et al (2012) Functional impact of allosteric agonist activity of selective positive allosteric modulators of metabotropic glutamate receptor subtype 5 in regulating central nervous system function. Mol Pharmacol 81:120–133
68. Rook JM, Noetzel MJ, Pouliot WA et al (2013) Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor 5 determine differences in in vivo activity. Biol Psychiatry 73:501–509
69. Bridges TM, Rook JM, Noetzel MJ et al (2013) Biotransformation of a novel positive allosteric modulator of metabotropic glutamate receptor subtype 5 contributes to seizure-like adverse events in rats involving a receptor agonism-dependent mechanism. Drug Metab Dispos 41:1703–1714
70. Turlington M, Noetzel MJ, Chun A et al (2013) Exploration of allosteric agonism structure-activity relationships within an acetylene series of metabotropic glutamate receptor 5 ($mGlu_5$) positive allosteric modulators (PAMs): discovery of 5-((3-fluorophenyl)ethynyl)-*N*-(3-methoxetan-3-yl)picolinamide (ML254). J Med Chem 56:7976–7996

71. Zhou Y, Chun A, Gogliotti RD et al (2011) Pure positive allosteric modulators (PAMs) of mGlu5 with competitive MPEP-site inter-action. Probe Reports from the NIH Molecular Libraries Program http://www.ncbi.nlm.nih.gov/pubmed/23762950. Accessed 12 Nov 2013
72. Gastambide F, Gilmour G, Robbins TW et al (2013) The $mGlu_5$ positive allosteric modulator LSN2463359 differentially modulates motor, instrumental and cognitive affects of NMDA receptor antagonists in the rat. Neuropharmacology 64:240–247
73. Gastambide F, Cotel MC, Gilmour G et al (2012) Selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator. Neuropharmacology 37:1057–1066
74. Williams R, Manka JT, Rodriguez AL et al (2011) Synthesis and SAR of centrally active $mGlu_5$ positive allosteric modulators based on an aryl acetylenic bicyclic lactam scaffold. Bioorg Med Chem Lett 21:1350–1353
75. Varnes JG, Marcus AP, Mauger RC et al (2011) Discovery of novel positive allosteric modulators of the metabotropic glutamate receptor 5 ($mGlu_5$). Bioorg Med Chem Lett 21:1402–1406
76. Packiarajan M, Grenon M, Zorn S et al (2013) Fused thiazole alkynes as potent mGlu5 receptor positive allosteric modulators. Bioorg Med Chem Lett 23:4037–4043
77. Biemans B, Jaeschke G, Lindemann L et al (2013) Pharmaceutically acceptable mGluR5 positive allosteric modulators and their methods of identification. WO 2013/045,380, 04 April 2013
78. Jaeschke G, Lindemann L, Stadler H et al (2014) Arylethynyl pyrimidines. WO 2014/026,880, 20 February 2014
79. Hammond AS, Rodriguez AL, Townsend SD et al (2010) Discovery of a novel chemical class of mGlu(5) allosteric ligands with distinct modes of pharmacology. ACS Chem Neurosci 2010(1):702–716
80. Gregory KJ, Nguyen ED, Reiff SD et al (2013) Probing the metabotropic glutamate receptor 5 (mGlu5) positive allosteric modulator (PAM) binding pocket: discovery of point mutations that engender a "molecular switch" in PAM pharmacology. Mol Pharm 83:991–1006
81. Manka JT, Vinson PN, Gregory KJ et al (2012) Optimization of an ether series of $mGlu_5$ positive allosteric modulators: molecular determinants of MPEP-site interactions. Bioorg Med Chem Lett 22:6481–6485
82. Bartolomé-Nebreda JM, Conde-Ceide S, Delgado F et al (2013) Dihydrothiazolopyridone derivatives as a novel family of positive allosteric modulators of the metabotropic glutamate 5 ($mGlu_5$) receptor. J Med Chem 56:7243–7259
83. Conn JP, Lindsley CW, Stauffer SR et al (2013) Substituted bicyclic alkoxy pyrazole analogs as allosteric modulators of mGluR5 receptors. WO 2013/192,343, 27 December 2013
84. Conn JP, Lindsley CW, Stauffer SR et al, Substituted bicyclic alkoxy pyrazole analogs as allosteric modulators of mGluR5 receptors. WO 2013/192,346, 27 December 2013
85. Conn JP, Lindsley CW, Stauffer SR et al (2013) Substituted bicyclic cycloalkyl pyrazole lactam analogs as allosteric modulators of mGluR5 receptors. WO 2013/192,347, 27 December 2013
86. Conn JP, Lindsley CW, Stauffer SR et al, Substituted bicyclic aralkyl pyrazole lactam analogs as allosteric modulators of mGluR5 receptors. WO 2013/192350, 27 December 2013
87. Blobaum AL, Bridges TM, Byers FW et al (2013) Heterotropic activation of the midazolam hydroxylase activity of CYP3A by a positive allosteric modulator of $mGlu_5$: in vitro to in vivo translation and potential impact on clinically relevant drug-drug interactions. Drug Metab Dispos 41:2066–2075
88. Xiong H, Brugel TA, Balestra M et al (2010) 4-Aryl piperazine and piperidine amides as novel mGluR5 positive allosteric modulators. Bioorg Med Chem Lett 20:7381–7384
89. Spear N, Gadient RA, Wilkins DE et al (2011) Preclinical profile of a novel metabotropic glutamate receptor 5 positive allosteric modulator. Eur J Pharmacol 659:146–154
90. Artioli A, Lovell PJ Novel compounds. WO 2008/152090, 18 December 2008

91. Rodriguez AL, Williams R, Zhou Y et al (2009) Discovery and SAR of novel mGluR5 non-competitive antagonists not based on an MPEP chemotype. Bioorg Med Chem Lett 19:3209–3213
92. Zhou Y, Manka JT, Rodriguez AL et al (2010) Discovery of *N*-aryl piperazines as selective mGlu5 potentiators with efficacy in a rodent model predictive of anti-psychotic activity. ACS Med Chem Lett 1:433–438
93. Slassi A, Isaac M, Arora J et al (2007) Piperazines and piperidines as mGluR5 potentiators. WO 2007/087,135, 2 August 2007
94. Gregory KJ, Herman EJ, Ramsey AJ et al (2013) *N*-Aryl piperazine metabotropic glutamate receptor 5 positive allosteric modulators possess efficacy in preclinical models of NMDA hypofunction and cognitive enhancement. J Pharmacol Exp Ther 347:438–457
95. Rodriguez AL, Tarr JC, Zhou Y et al (2012) Identification of a glycine sulfonamide based non-MPEP site positive allosteric potentiator (PAM) of mGlu5. Probe reports from the NIH Molecular Libraries Program; http://www.ncbi.nlm.nih.gov/books/NBK154499/
96. Rodriguez AL, Zhou Y, Williams R et al (2012) Discovery and SAR of a novel series of non-MPEP site $mGlu_5$ PAMs based on an aryl glycine sulfonamide scaffold. Bioorg Med Chem Lett 22:7388–7393
97. Zysk JR, Spear N, Fieles W et al (2013) In vitro binding of a radiolabeled positive allosteric modulator for metabotropic glutamate receptor subtype 5. Synapse 67:135–144
98. Malherbe P, Kratochwil N, Mühlemann A et al (2006) Comparison of the binding pockets of two chemically unrelated allosteric antagonists of the mGlu5 receptor and identification of crucial residues involved in the inverse agonism of MPEP. J Neurochem 98:601–615
99. Mølck C, Harpsøe K, Gloriam DE et al (2012) Pharmacological characterization and Modeling of the binding sites of novel 1,3-bis(pyridinylethynyl)-benzenes as metabotropic glutamate receptor 5-selective negative allosteric modulators. Mol Pharm 82:929–937
100. Mühlemann A, Ward NA, Kratochwil N et al (2006) Determination of key amino acids implicated in the actions of allosteric modulation by 3,3′-defluorobenzaldazine on rat mGlu5 receptors. Eur J Pharmacol 529:95–104
101. McGaughey GB, Anthony NJ, Finger EN et al (2010) mGluR5 positive allosteric modulators. In: Keystone symposium: computer aided drug design, Whistler, Canada
102. Chen Y, Goudet C, Pin JP, Conn PJ (2008) N-{4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) acts through a novel site as a positive allosteric modulator of group 1 metabotropic glutamate receptors. Mol Pharmacol 73:909–918
103. Malherbe P, Kratochwil N, Zenner MT et al (2003) Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor negative modulator 2-methyl-6-(phenylethynyl)-pyridine. Mol Pharmacol 64:823–832
104. Gregory KJ, Nguyen ED, Malosh C et al (2014) Identification of specific ligand-receptor interactions that govern binding and cooperativity of diverse modulators to a common metabotropic glutamate receptor 5 allosteric site. ACS Chem Neurosci 5:282–295
105. Kinney GG, Burno M, Campbell UC et al (2003) Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. J Pharmacol Exp Ther 306:116–123
106. Schlumberger C, Pietraszek M, Gravius A 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
107. Marquis KL, Sabb AL, Logue SF et al (2007) WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-Octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole]: a Novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. J Pharmacol Exp Ther 320:486–496
108. Costall B, Naylor RJ, Nohria V (1980) On the importance of mesolimbic mechanisms for the control of apomorphine induced climbing behavior in the mouse [proceedings]. Br J Pharmacol 68:175P–176P

109. Wadenberg M-LG (2010) Conditioned avoidance response in the development of new antipsychotics. Curr Pharm Des 16:358–370
110. Geyer MA, Dulawa SC, Ralph RJ et al (2000) Startle habituation and prepulse inhibition studies in mutant mice. Biol Psychiatry 47:124S
111. Ramsey AJ (2009) NR1 knockdown mice as a representative model of the glutamate hypothesis of schizophrenia. Prog Brain Res 179:51–58
112. Schlumberger C, Pietraszek M, Gravius A 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
113. Barker GR, Bashir ZI, Brown MW et al (2006) A temporally distinct role of group I and group II metabotropic glutamate receptor in object recognition memory. Learn Mem 13:178–186
114. Epping-Jordan MP, Nayak S, Derouet F et al (2005) In vivo characterization of mGluR5 positive allosteric modulators as novel treatments for schizophrenia and cognitive dysfunction. Neuropharmacology 49(Suppl 1):243
115. Robbins TW (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology 163:362–380
116. Moeller FG, Barratt ES, Dougherty DM et al (2001) Psychiatric aspects of impulsivity. Am J Psychiatry 158:1783–1793
117. Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285
118. Gessa GL, Deveto P, Diana M et al (2000) Dissociation of haloperidol, clozapine, and olanzapine effects on electrical activity of mesocortical dopamine neurons and dopamine release in the prefrontal cortex. Neuropsychopharmacology 22:642–649
119. Ohoyama K, Yamamura S, Hamaguchi T et al (2011) Effect of novel atypical antipsychotic, blonanserin, on extracellular neurotransmitter level in rat prefrontal cortex. Eur J Pharmacol 653:47–57
120. Ayala JE, Chen Y, Banko JL et al (2009) mGlu5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. Neuropsychopharmacol 34:2057–2071
121. Xu J, Zhu Y, Kraniotis S et al (2013) Potentiating mGluR5 function with a positive allosteric modulator enhances adaptive learning. Learn Mem 20:438–445
122. Xu J, Zhu Y, Contractor A, Heinemann SF (2009) mGluR5 has a critical role in inhibitory learning. J Neurosci 29:3676–3684
123. Darrah JM, Stefani MR, Moghaddam B (2008) Interaction of *N*-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioural flexibility using a novel operant set-shift paradigm. Behav Pharmacol 19:225–234
124. Flagstad P, Mørk A, Glenthøj BY et al (2004) Disruption of neurogenesis on gestational day 17 in the rat causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens. Neropsychopharmacology 29:2052–2064
125. Moore H, Jentsch JD, Ghajarnia M et al (2006) A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. Biol Psychiatry 60:253–264
126. Chudasama Y, Robbins T (2003) Dissociative contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. J Neurosci 23:8771–8780
127. Mannaioni G, Marino MJ, Valenti O (2001) Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. J Neurosci 21:5925–5934
128. Francesconi W, Cammalleri M, Sanna PP (2004) The metabotropic glutamate receptor 5 is necessary for late-phase long-term potentiation in the hippocampal CA1 region. Brain Res 1022:12–18

129. Manahan-Vaughan D, Braunewell KH (2005) The metabotropic glutamate receptor, mGluR5, is a key determinant of good and bad spatial learning performance and hippocampal synaptic plasticity. Cereb Cortex 15:1703–1713
130. Bellone C, Luscher C, Mameli M (2008) Mechanisms of synaptic depression triggered by metabotropic glutamate receptors. Cell Mol Life Sci 65:2913–2923
131. Gladding CM, Fitzjohn SM, Molnar E (2009) Metabotropic glutamate receptor-mediated long-term depression: molecular mechanisms. Pharmacol Rev 61:395–412
132. Riedel G, Platt B, Micheau J (2003) Glutamate receptor function in learning and memory. Behav Brain Res 140:1–47
133. Simonyi A, Schachtman TR, Christoffersen GR (2005) The role of metabotropic glutamate receptor 5 in learning and memory process. Drug News Perspect 18:353–361
134. Lu YM, Jia Z, Janus C et al (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 17:5196–5205
135. Jia Z, Lu Y, Henderson J et al (1998) Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. Learn Mem 5:331–343
136. Liu F, Zhang G, Hornby G et al (2006) The effect of mGlu5 receptor positive allosteric modulators on signalling molecules in brain slices. Eur J Pharmacol 536:262–268
137. Loas G, Noisette C, Legrand A et al (1996) Anhedonia, depression and the deficit syndrome of schizophrenia. Acta Psychiatr Scand 94:477–479
138. Park IH, Kim JJ, Chun J et al (2009) Medial prefrontal default-mode hypoactivity affecting trait physical anhedonia in schizophrenia. Psychiatry Res 171:155–165

Top Med Chem (2015) 13: 183–212
DOI: 10.1007/7355_2014_47

Published online: 27 April 2014

# Muscarinic Acetylcholine Receptor Activators

**Takaaki Sumiyoshi and Takeshi Enomoto**

**Abstract** Modulation of muscarinic acetylcholine receptors (mAChRs) is one of the most attractive therapeutic strategies for the treatment of schizophrenia. Pilot clinical studies of the $M_1/M_4$ mAChR-preferring agonist xanomeline as well as animal studies using $M_1$–$M_5$ mAChR knockout mice suggest that selective activation of $M_1$ and/or $M_4$ mAChRs is a key concept in the treatment of psychosis and cognitive deficits in patients with schizophrenia. However, over the past two decades, clinical development of mAChR agonists has not been successful mainly due to these agents' narrow safety margin caused by the lack of true subtype selectivity. However, recent advances in medicinal chemistry might enable researchers to overcome the hurdles that earlier mAChR agonists failed to pass. Here, we describe recent advances in the development of subtype-selective mAChR activators for treatment of schizophrenia.

**Keywords** Allosteric agonists, $M_1$ muscarinic acetylcholine receptor, $M_4$ muscarinic acetylcholine receptor, Positive allosteric modulators, Subtype selectivity

T. Sumiyoshi (✉)
Drug Research Division, Dainippon Sumitomo Pharma Co., Ltd., 3-1-98 Kasugade-naka, Konohana-ku, Osaka 554-0022, Japan

Department of Life Science and Biotechnology, Faculty of Chemistry, Materials and Bioengineering, Kansai University, 3-3-35 Yamate-cho, Suita, Osaka 564-8680, Japan
e-mail: t-sumiyo@kansai-u.ac.jp

T. Enomoto
Medical Affairs, Drug Development division, Dainippon Sumitomo Pharma Co., Ltd., 13-1 Kyobashi 1-Chome, Chuo-ku, Tokyo 104-8356, Japan

Drug Development Research Laboratories, Drug Research Division, Dainippon Sumitomo Pharma Co., Ltd., 33-94 Enoki-cho, Suita, Osaka 564-0053, Japan
e-mail: takeshi-enomoto@ds-pharma.co.jp

## Contents



## Abbreviations

| | |
|---|---|
| [$^{123}$I]-IQNB | [$^{123}$I]-iodoquinuclidinyl benzilate |
| ACh | Acetylcholine |
| AD | Alzheimer's disease |
| BA | Bioavailability |
| BQCA | Benzylquinolone carboxylic acid |
| BuTAC | [5*R*-(*exo*)]-6-[4-butylthio-1,2,5-thiadiazol-3-yl]-1-azabicyclo-[3.2.1]-octane |
| CNS | Central nervous system |
| EPS | Extrapyramidal side effects |
| Et | Ethyl |
| GPCR | G-protein-coupled receptor |
| hERG | Human ether-a-go-go related gene |
| HTS | High throughput screening |
| IA | Intrinsic activity |
| mAChR | Muscarinic acetylcholine receptor |
| Me | Methyl |
| NAM | Negative allosteric modulator |
| NDMC | *N*-desmethylclozapine |
| NMDA | *N*-methyl-D-aspartate |
| PAM | Positive allosteric modulator |
| PANSS | Positive and negative syndrome scale |
| PK | Pharmacokinetic |
| PPI | Prepulse inhibition |
| TBPB | 1-(1′-(2-tolyl)-1,4′-bipedidin-4-yl)-1*H*-benzo[*d*]imidazol-2-(3*H*)-one |

## 1 Introduction

Schizophrenia is a heterogeneous disease with a diverse symptomatology that includes three major types of symptoms: (1) positive symptoms (e.g., hallucinations, delusions, and excitement), (2) negative symptoms (e.g., flattened affect, apathy, and social withdrawal), and (3) cognitive deficits (e.g., deficit in working memory, executive function, attentional processing, and memory) [1, 2]. Dopamine $D_2$ receptor antagonists are currently used to treat schizophrenia, particularly its positive symptoms. However, there is an ongoing need for alternative approaches, considering the dose limitation imposed on $D_2$ receptor antagonists due to their extrapyramidal side effects (EPS) [3] and the increasing number of non-responders to $D_2$ receptor antagonists [4]. Another disadvantage of current $D_2$ antagonists is their poor efficacy on schizophrenia negative symptoms and cognitive dysfunction [5, 6].

The muscarinic hypothesis of schizophrenia postulates that disturbance in the muscarinic acetylcholine (ACh) system plays a crucial role in the pathophysiology of schizophrenia [7]. Multiple lines of evidence from neuroimaging, postmortem, and preclinical and clinical pharmacology studies support this hypothesis. In fact, the clinical trial of xanomeline, the $M_1/M_4$ muscarinic ACh receptors (mAChRs)-preferring agonist, in patients with schizophrenia provides the proof of concept that activation of $M_1/M_4$ mAChRs is effective for the positive, negative, and cognitive symptoms of schizophrenia [8]. In addition, recent findings from behavioral studies using $M_1$ or $M_4$ mAChR-deficit mice revealed that activation of the $M_1$ mAChR might be effective mainly for certain types of cognitive deficits, whereas stimulation of the $M_4$ mAChR may provide benefits for psychosis associated with schizophrenia [9]. In this article, we summarize the medicinal chemistry of mAChRs activators as new candidates for treatment of schizophrenia symptoms.

## 2 Involvement of mAChRs in Schizophrenia

Many important physiological actions of ACh are mediated by mAChRs. The mAChRs are members of the rhodopsin-like G-protein-coupled receptors (GPCRs) and have five mAChR subtypes ($M_1$–$M_5$) [10–13]. The $M_1$, $M_3$, and $M_5$ mAChRs couple to $G_{q/11}$ proteins, whereas the $M_2$ and $M_4$ mAChRs couple to $G_{i/o}$ proteins. Each mAChRs subtype shows a different pattern of distribution. The $M_1$, $M_4$, and $M_5$ mAChRs are predominantly distributed in the central nervous system (CNS), whereas the $M_2$ and $M_3$ mAChRs are distributed in both the CNS and peripheral systems [9, 12].

A neuroimaging study has shown that subjects with schizophrenia exhibit abnormalities of brain mAChRs [14]. Single-photon emission computed tomography using a nonselective muscarinic ligand [$^{123}$I]-iodoquinuclidinyl benzilate ([$^{123}$I]-I-QNB) revealed lower mAChRs availability in the cortex and basal ganglia of unmediated subjects with schizophrenia than in those of healthy subjects. More importantly, the severity of positive symptoms in these patients negatively correlated with mAChRs availability.

Information on schizophrenia-associated alterations in each mAChR subtype comes from postmortem studies [7]. Binding levels of [$^3$H]-pirenzepine, a $M_1$ and $M_4$ mAChRs-preferring ligand, are reduced in the prefrontal cortex and hippocampus of patients with schizophrenia [15–17]. Expression levels of mRNA for $M_1$ and $M_4$ mAChRs are also decreased in the prefrontal cortex and hippocampus of these patients, respectively [16, 18]. However, no alterations in mRNA expression levels for $M_2$ and $M_3$ mAChRs are detected in the prefrontal cortex of patients with schizophrenia [19]. Interestingly, a recent study has shown that the schizophrenia cohort can be divided into two distinct populations based on cortical [$^3$H]-pirenzepine binding density [20]. The first subpopulation of patients with schizophrenia (26% of all subjects) show marked reduction of cortical [$^3$H]-pirenzepine binging and were accordingly termed by Scarr and colleagues "muscarinic receptor-deficit schizophrenia." On the other hand, there is no difference in [$^3$H]-pirenzepine binding between the second subgroup of patients and control subjects. Since mAChRs-deficit in this subgroup was found in postmortem studies, it remains to be investigated how mAChRs-deficiency, particularly dysfunction of $M_1$ and $M_4$ subtypes, contributes to schizophrenia psychotic and cognitive symptoms, and what would be the outcome of appropriate treatment.

## 3 Phenotypes of $M_1$, $M_4$, or $M_5$ mAChR-Deficient Mice

Studies with $M_1$–$M_5$ mAChRs knockout mice have provided important information on the potential roles of individual mAChR subtypes in the pathophysiology of schizophrenia [9]. It is well known that nonselective mAChR antagonists, such as scoplomanine, impair a wide range of cognitive functions [21]. However, $M_1$ mAChR knockout mice have been found to exhibit no impairment of learning and memory in the Morris water maze and contextual fear conditioning tests [22, 23]. Interestingly, $M_1$ mAChR-deficient mice showed working memory deficit in the win-shift task, which requires interaction between the cortex and hippocampus [23]. Mice lacking the $M_1$ mAChR exhibit increased perseverative response in the five-choice serial reaction time task compared to wild-type mice [24]. Since working memory deficit and perseverative response are also observed in patients with schizophrenia [25, 26], it is believed that certain types of schizophrenia-associated cognitive deficit might arise from $M_1$ mAChR deficiency. Two distinct lines of $M_1$ mAChR knockout mice exhibit locomotor hyperactivity associated with enhanced dopamine transmission in the striatum [22, 27]. In addition, amphetamine-induced locomotor hyperactivity is enhanced in $M_1$ mAChR knockout mice compared to wild-type mice [27]. These findings suggest that $M_1$ mAChR dysfunction contributes to not only cognitive deficit but also psychosis in schizophrenia.

Mice lacking the $M_4$ mAChR also exhibit locomotor hyperactivity [28, 29]. Tzavara and colleagues have reported that the $M_4$ mAChR acts as an autoreceptor in midbrain cholinergic afferents and that $M_4$ mAChR-deficiency elevates basal ACh transmission in the midbrain [30]. In their hypothesis, loss of $M_4$ mAChR control would lead to hyperexcitability of midbrain dopamine neurons.

$M_4$ mAChR-deficient mice actually show increased basal dopamine level in the nucleus accumbens in microdialysis experiments. In addition, dopamine response to amphetamine or the *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine is also elevated in the nucleus accumbens of $M_4$ mAChR knockout mice. These findings suggest that dysfunction in $M_4$ mAChR contributes to the psychosis associated with schizophrenia. As mice lacking the $M_4$ mAChR also exhibit abnormal social behavior reminiscent of the negative symptoms of schizophrenia [29], it is worth assessing whether $M_4$ mAChR deficiency is involved in schizophrenia negative symptoms. Only a few studies have investigated cognition in $M_4$ mAChR knockout mice [29, 31]. These studies showed that $M_4$ mAChR-deficit does not affect learning and memory in the passive avoidance and Morris water maze tests [29, 31]. As schizophrenics show multiple affected cognitive domains, the cognitive profile of $M_4$ mAChR-deficient mice need to be re-investigated using comprehensive rodent cognitive batteries [32].

In this review, we focus on $M_1$ and $M_4$ mAChRs, which are considered to be the most important mAChR targets for treatment for schizophrenia. However, it is worth to mention the phenotype of $M_5$ mAChR knockout mice. $M_5$ mAChR is the only mAChR subtype that is localized in the cell bodies of dopaminergic neurons in the midbrain [12, 33]. Recent studies with $M_5$ mAChR knockout mice show that activation of $M_5$ mAChRs in the ventral tegmental area enhances the dopamine release in the nucleus accumbens [33]. Wang and colleagues have shown that amphetamine-induced locomotor hyperactivity is decreased in $M_5$ mAChR-deficient mice compared to wild-type mice [34]. Furthermore, $M_5$ mAChR-deficient mice, like antipsychotics-treated animals, show the enhancement of latent inhibition compared to wild-type mice [34, 35]. These results indicate that deletion of $M_5$ mAChRs leads to reduction of dopaminergic transmission in the nucleus accumbens, and the blockade of $M_5$ mAChR might be effective on the psychosis in schizophrenia.

In summary, studies of $M_1$ and $M_4$ mAChRs mutant mice indicate that dysfunction of the $M_1$ mAChR would contribute to schizophrenia cognitive impairment and psychosis, whereas deficit in $M_4$ mAChR function would contribute mainly to psychosis. Thus, activation of $M_1$ and $M_4$ mAChRs would be an attractive therapeutic strategy for schizophrenia-associated psychosis and cognitive impairment.

## 4 Preclinical and Clinical Studies of Classical mAChRs Activators

Over the past two decades, considerable efforts have been focused on developing $M_1$ and/or $M_4$ mAChR-preferring agonists, especially for treatment of Alzheimer's disease (AD) [36, 37]. However, no $M_1$ and/or $M_4$ mAChR agonist has been approved for this disorder, probably due to these activators' low safety profiles and/or marginal cognitive efficacy. Among the developed mAChRs agonists, xanomeline exhibited the most promising efficacy in a large scale of placebo-controlled double-blind clinical study (Fig. 1) [38]. Xanomeline improved

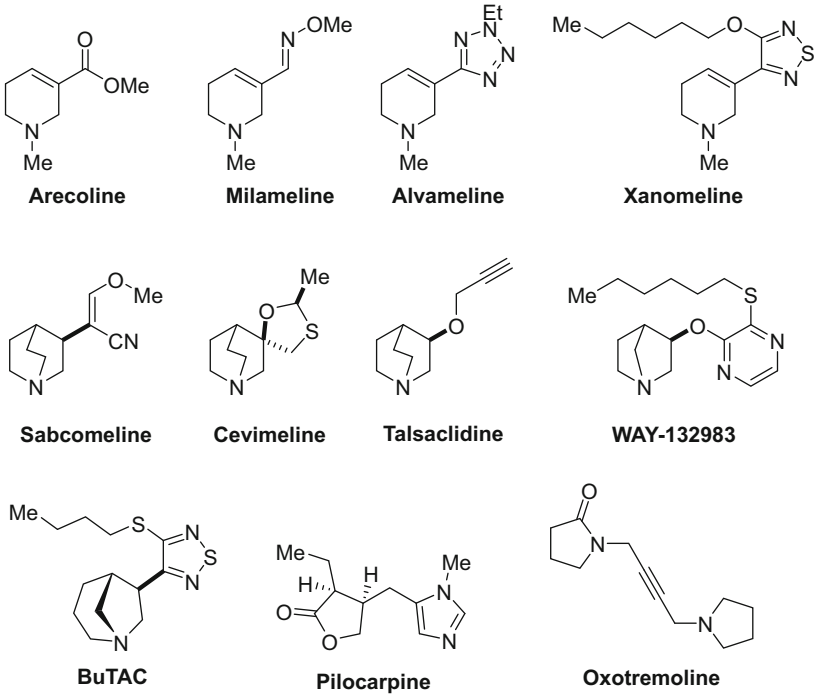



**Fig. 1** Structures of orthosteric mAChR agonists

cognition and behavioral symptoms in patients with Alzheimer disease, although it dose-dependently induced gastrointestinal adverse effects. The low selectivity of this agent for $M_1$ and $M_4$ mAChRs versus $M_2$ and $M_3$ mAChRs might have hampered its further development.

One of the most important findings with xanomeline is that it improved positive, negative, and cognitive symptoms in patients with schizophrenia [8, 39]. This provides the proof of concept that activation of $M_1/M_4$ mAChR could be beneficial in the treatment of schizophrenia. Interestingly, patients who took part in the study of xanomeline for schizophrenia were mainly those who had exhibited either poor response or worsening symptoms under treatment with $D_2$ receptor antagonists. Therefore, activation of $M_1$ and $M_4$ mAChRs might be an attractive approach for patients resistant to current $D_2$ receptor antagonists. However, it should be noted that the study of xanomeline for schizophrenia was a pilot clinical study with only 20 patients. Therefore, the efficacy of xanomeline or other $M_1/M_4$ mAChR agonists for schizophrenia remains to be investigated in large-scale clinical studies.

While information on the clinical efficacy of $M_1/M_4$ mAChR agonists remains limited, many preclinical studies have been published in this field. Consistent with its clinical antipsychotic efficacy, xanomeline has been shown to have robust antipsychotic-like efficacy in several rodent behavioral tasks. Xanomeline inhibits conditioned avoidance response, apomorphine-induced climbing and prepulse inhibition (PPI) deficit, and amphetamine-induced locomotor hyperactivity in rats or

mice [40–42]. The beneficial antipsychotic-like effects were also observed with other mAChR agonists, such as sabcomeline, milameline, and talsaclidine [38]. Although xanomeline acts not only on mAChRs but also on other off-targets, including the serotonergic receptors [43, 44], the findings that several different mAChR agonists also show antipsychotic-like efficacy indicate that xanomeline's antipsychotic-like effects arise from activation of mAChRs. Furthermore, several studies in $M_1$ or $M_4$ mAChR knockout mice provide clear evidence of the roles of individual mAChR subtypes in the antipsychotic-like efficacy of xanomeline. Woolley and colleagues have shown that the effects of xanomeline on amphetamine-induced locomotor hyperactivity are abolished in $M_4$ mAChR knockout mice, but are only marginally attenuated in $M_1$ mAChR knockout mice [45]. Dencker and colleagues have also found that xanomeline exerts no antipsychotic-like effects in mice lacking the $M_4$ mAChR in $D_1$ dopamine receptor-expressing cells [46]. Taken together, these findings indicate that antipsychotic-like efficacy of xanomeline might be predominantly mediated via the $M_4$ mAChR. This assumption may be supported by a recent paper showing that the potency of [5R-(*exo*)]-6-[4-butylthio-1,2,5-thiadiazol-3-yl]-1-azabicyclo-[3.2.1]-octane (BuTAC), another mAChR agonist, in the conditioned avoidance test is right-shifted in $M_4$ mAChR knockout mice [47].

It has been hypothesized that dysfunctions in the prefrontal cortex are involved in the pathophysiology of schizophrenia and that the efficacy of atypical antipsychotics on this disorder negative and cognitive symptoms might be attributed to their ability to increase dopamine and ACh efflux in the prefrontal cortex [48]. In a rat microdialysis study, xanomeline potently increased extracellular dopamine level in the prefrontal cortex [49]. In addition, it is reported that xanomeline and sabcomeline increase not only dopamine efflux, but also ACh efflux in the rat prefrontal cortex and that both agents potentiate risperidone-induced dopamine or ACh efflux [48]. It is therefore believed that the effects of mAChR agonists in the prefrontal cortex are relevant to treatment of schizophrenia negative and cognitive symptoms as monotherapy or adjunctive therapy with atypical antipsychotics.

The efficacy of mAChR agonists for cognitive impairment has been well investigated in rodents and primates [21, 37]. Although beyond the scope of this review, mAChR agonists, including xanomeline, have been shown to be effective in a number of studies using normal, cholinergic-manipulated, transgenic, or aged animals. However, most of these models reflect normal cognition or cognitive deficit associated with AD rather than schizophrenia. It is therefore necessary to evaluate the efficacy of mAChR agonists in animal models of schizophrenia, such as glutamatergic-manipulated animals or neurodevelopmental models [32, 50, 51]. Another consideration is that patients with schizophrenia exhibit cognitive impairment in multiple domains of cognition, including attention, working memory, reasoning, and problem solving. Recently, preclinical batteries of behavioral tasks have been developed to investigate effects of putative cognitive enhancers on distinct domains of cognitive functions [32, 52, 53]. Therefore, it is important to identify the cognition domains responsive to $M_1$ and $M_4$ mAChRs activation using validated behavioral tasks and animal models of schizophrenia.

## 5 Medicinal Chemistry of Selective mAChR Activators

The purpose of early drug discovery studies is to identify centrally active, potent orthosteric mAChR agonists. For that, two well-known scaffolds, dihydropyridines and quinuclidines (Fig. 1), are used. The clinical trials of arecoline, alvameline, sabcomeline, and xanomeline for the treatment of AD were all discontinued mainly due to the dose-limiting adverse effects attributed to these agents activation of the peripheral $M_2$ and/or $M_3$ mAChR [36–38, 43, 54, 55]. In fact, we have shown in a calcium mobilization assay that xanomeline and sabcomeline, both of which were in clinical development for treatment of schizophrenia, exhibited the subtype nonselective activation of mAChRs in Chinese hamster ovary cell expressing human $M_1$–$M_5$ mAChRs [56].

In order to overcome mAChR agonists' lack of selectivity and avoid cholinergic side effects, medicinal chemists have turned their attention to new scaffolds. Although this strategy led to the discovery of $M_1$ mAChR-selective agonists and positive allosteric modulators (PAMs), the fact that xanomeline was found to improve all three symptoms of schizophrenia turned medicinal chemists' attentions to find selective $M_4$ mAChR activators and dual $M_1$ and $M_4$ mAChRs activators. In this review, we summarize recent reports describing $M_1$ mAChR allosteric agonists, $M_1$ mAChR PAMs, $M_4$ mAChR PAMs, and $M_1$ and $M_4$ mAChRs-dual agonists.

### *5.1 $M_1$ mAChR Agonists*

It is believed that activation of $M_1$ mAChR in the forebrain potentiates NMDA receptor currents, which might play important role in the treatment of schizophrenia based on this disorder glutamatergic hypothesis [57, 58]. In fact, $M_1$ mAChR is co-localized with the NR1 subunit of NMDA receptors in CA1 pyramidal cells in the hippocampus and other cortical regions [58]. In addition, *N*-desmethylclozapine (NDMC, Fig. 2), which is a major metabolite of clozapine, was found to have allosteric $M_1$ mAChR agonistic activity that potentiates NMDA receptor currents in CA1 pyramidal cells in the hippocampus [59–61]. However, the clinical development was discontinued because this agent showed poor efficacy for psychosis as measured by the Positive and Negative Syndrome Scale (PANSS) [62].

Development of selective $M_1$ mAChR agonists [63] started with discovery of the $M_1$ mAChR allosteric agonist AC-42 [64] (Fig. 3). AC-42 shows the high selectivity for the $M_1$ mAChR ($hM_1$ $pEC_{50} = 6.54$ in a functional assay) over other subtypes through binding to the allosteric site of this receptor. However, AC-42 is not an ideal tool compound because it has potent binding affinity to the dopamine $D_2$ and serotonin 5-$HT_{2B}$ receptors [43]. AC-260584 ($hM_1$ $EC_{50} = 41$ nM), an analogue of AC-42, shows cognitive enhancement and antipsychotic-like effects in rodents [65]. However, AC-260584 also has the binding affinity for several non-muscarinic off-targets including dopamine $D_2$, adrenergic $\alpha_{1A}$, and

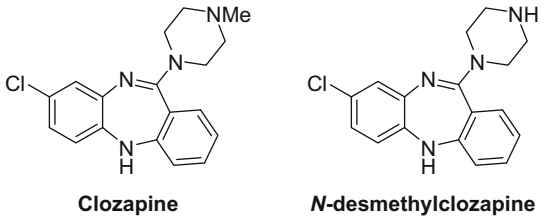



**Fig. 2** Structures of clozapine and NDMC

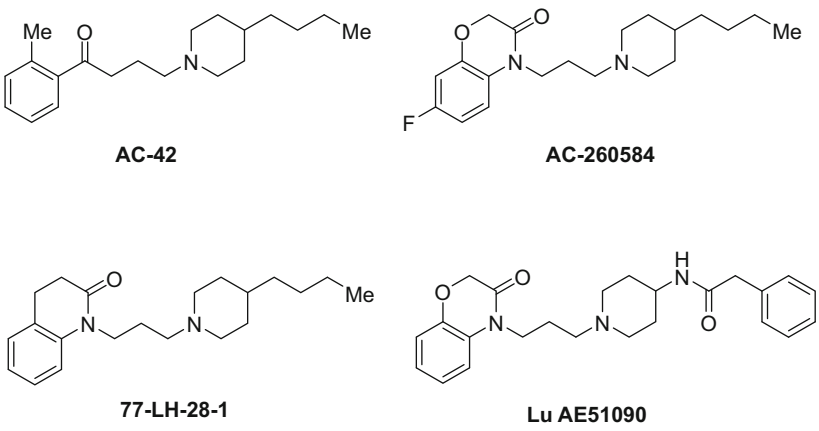



**Fig. 3** $M_1$ mAChR agonists with piperidine and *N*-propylene linker

serotonergic 5-$HT_{2B}$ receptors [43, 66]. 77-LH-28-1, another AC-42 analogue, was found to weakly activate the $M_3$ mAChR ($hM_1$ $pEC_{50} = 8.1 \pm 0.3$, $hM_2$ $pEC_{50} < 5$, $hM_3$ $pEC_{50} = 5.6 \pm 0.2$, $hM_4$ $pEC_{50} < 5$, $hM_5$ $pEC_{50} < 5$ in calcium mobilization assays) because its binding site overlaps with the orthosteric site [43, 67, 68]. Lu AE51090, another analogue of AC-42, showed high selectivity for the $M_1$ mAChR [$hM_1$ $EC_{50} = 61$ nM, intrinsic activity (IA) = 83%] over other subtypes ($hM_{2–5} < 10\%$ activation at 10 μM in calcium mobilization assays) with moderate brain exposure (brain/plasma ratio = 0.20) and low bioavailability (BA) in rats (4%, 2 mg/kg, po) [69].

Another scaffold for affinity toward the allosteric site of $M_1$ mAChR was suggested following discovery of the $M_1$ mAChR agonist [1-(1′-(2-tolyl)-1, 4′-bipedidin-4-yl)-1*H*-benzo[*d*]imidazol-2-(3*H*)-one] (TBPB, $M_1$ $EC_{50} = 289$ nM, Fig. 4) [70]. Point mutation in the ACh orthosteric binding site of $M_1$ mAChR produced no change in TBPB agonistic activity. However, TBPB is also known to bind to not only an allosteric site but also the orthosteric site of $M_1$ mAChR, because TBPB inhibited, in a concentration-dependent manner, the binding of [$^3$H]*N*-methylscopolamine from $M_1$ mAChR [71]. In addition, TBPB has low

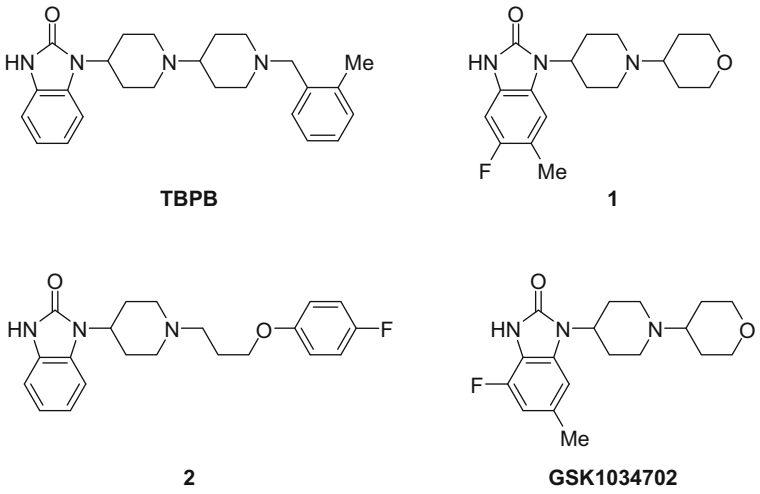
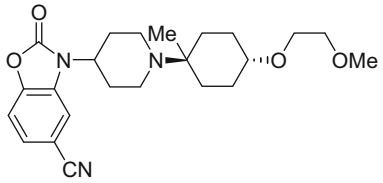



**Fig. 4** $M_1$ mAChR agonists with benzimidazolone scaffold

binding affinity for dopamine $D_2$ receptors ($K_i$ = 1.4 μM) [70]. In the search for more selective $M_1$ mAChR agonists, GSK researchers found compound **1** as a highly selective $M_1$ mAChR agonist ($M_1$ $pEC_{50}$ = 8.1, $M_2$ $pEC_{50}$ = 6.2, $M_3$ $pEC_{50}$ = 5.6, $M_4$ $pEC_{50}$ = 6.3, and $M_5$ $pEC_{50}$ = 6.1) [72]. Modification of phenoxypropyl moiety of virtual screening hit compound **2** led to potentiate $M_1$ mAChR agonistic activity, increase subtype selectivity, and decrease affinity to off-targets. Compound **1** showed high BA (49%) and brain penetrating ability (brain/blood = 0.6) in rats. In rodents, compound **1** increased cell firing in the CA1 region of the hippocampus (1 mg/kg, iv) and reversed scopolamine-induced learning impairment in the passive avoidance test in a dose-dependent manner (1–10 mg/kg, po) [72]. As far as we know, GSK1034702 [73], an isomer of compound **1**, is the first allosteric $M_1$ mAChR agonist to enter clinical development. GSK1034702 showed signs of cognitive improvement in healthy smokers using the nicotinic abstinence model of cognitive dysfunction, although this model might not be the best for cognitive deficits in schizophrenia [74].

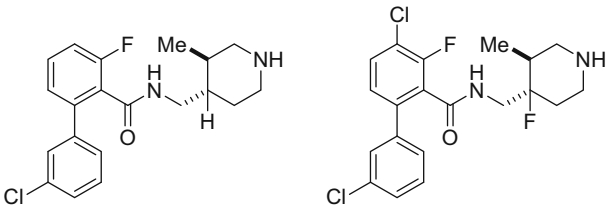
To improve the modest brain penetration of compound **1**, Johnson and co-workers replaced the benzimidazolone scaffold by an oxindole and a benzoxazolinone (Fig. 5) [75]. The obtained compound **3** showed selective $M_1$ mAChR agonistic activity over $M_{2-5}$ mAChRs ($M_1$ $pEC_{50}$ = 8.0, $M_2$ $pEC_{50}$ = 5.6, $M_3$ $pEC_{50}$ = 5.4, $M_4$ $pEC_{50}$ = 6.1, and $M_5$ $pEC_{50}$ = 6.0 in calcium mobilization assays) and high brain penetration ($C_{free,\ brain}$ = 261 nM, $C_{free,\ blood}$ = 265 nM) [75]. Compound **3** (1 mg/kg) reversed temporal-induced memory deficits in the rat novel object recognition test.

GSK researchers have disclosed another scaffold for selective allosteric $M_1$ mAChR agonists identified through HTS of their libraries (Fig. 6). HTS picked up compounds originally designed for the $M_3$ mAChR antagonist program as initial




3

**Fig. 5** $M_1$ mAChR agonists with benzoxazolone scaffold




4 5

**Fig. 6** $M_1$ mAChR agonists with biphenyl amide scaffold

hit compounds with biphenyl amide structure. Potencies of the optimized compounds **4** and **5** increased to $pEC_{50} = 9.1$ and 8.0 in calcium mobilization assays, respectively [76]. These compounds showed subtype selectivity against $M_{2–5}$ mAChRs (compound **4**: $M_2$ $pEC_{50} = 6.7$, $M_3$ $pEC_{50} = 7.4$, $M_4$ $pEC_{50} = 6.8$, $M_5$ $pEC_{50} = 6.3$ and compound **5**: $M_2$ $pEC_{50} = 5.6$, $M_3$ $pEC_{50} = 5.3$, $M_4$ $pEC_{50} = 5.5$, $M_5$ $pEC_{50} = 5.2$ in calcium mobilization assays) and possessed good oral bioavailability in rats. In addition, compound **4** exhibits no affinity for other off-targets in a CEREP panel. Brain blood ratios of compounds **4** and **5** were 0.9 and 0.3 in rats, respectively.

The researchers at Vanderbilt University disclosed the compounds with *N*-carbethoxy piperidine scaffold (Fig. 7). The HTS campaign through 65,000 compounds picked up VU0177548 and VU0207811. Modification of aryl moiety led to identify VU0184670 and VU0357017. VU0357017 is the recently proposed allosteric $M_1$ mAChR agonists ($M_1$ $EC_{50} = 198$ nM, IA = 81% in a calcium mobilization assay) [77]. VU0357017 could penetrate the brain (brain/plasma ratio = 4:1) and was able to reverse scopolamine-induced disruption of contextual fear conditioning response in rats. This series of $M_1$ mAChR allosteric agonists, such as VU0184670 and VU0357017, is reported to bind to the third extracellular loop of $M_1$ mAChRs which is different from other $M_1$ mAChR allosteric agonists such as AC-42, 77-LH-28-1, and TBPB. Introduction of central piperidine ring led to VU0364572 ($M_1$ $EC_{50} = 110$ nM, IA = 71%) [78].

Replacement of the *N*-carbethoxy piperidine of VU0364572 by a tropane ring afforded compound VU0409066 as a potent $M_1$ mAChR agonist (Fig. 8, $hM_1$ $EC_{50} = 59$ nM in a calcium mobilization assay) [79]. VU0415371 with its *exo* tropane scaffold ($hM_1$ $EC_{50} = 110$ nM) was more potent than VU0413144 having

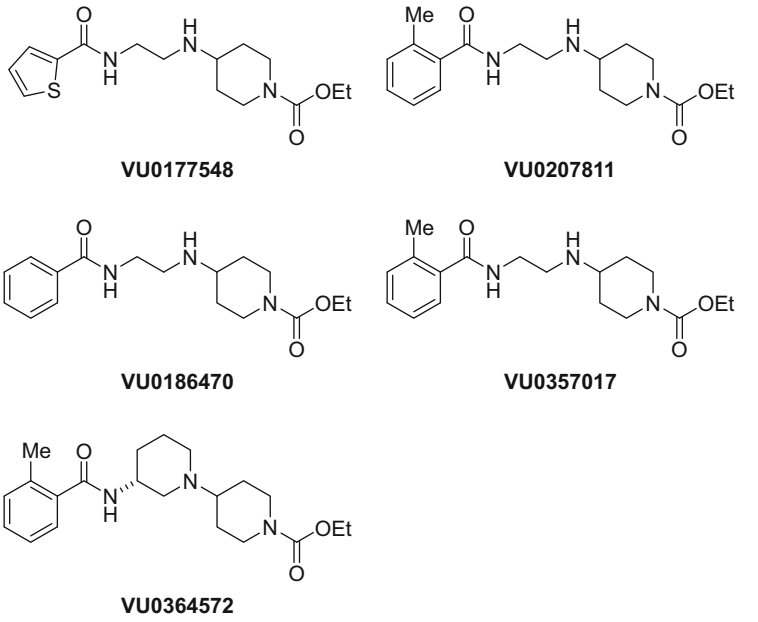



**Fig. 7** $M_1$ mAChR agonists with *N*-carbethoxy piperidine scaffold

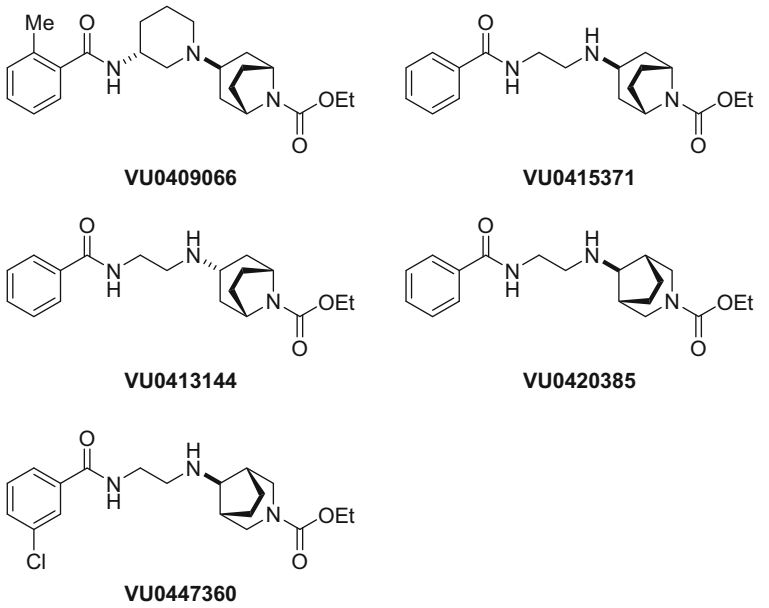



**Fig. 8** $M_1$ mAChR agonists with *N*-carbethoxytropane scaffold

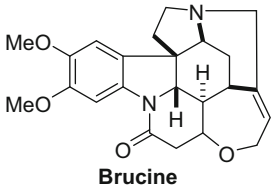



**Fig. 9** Structure of Brucine

the *endo* tropane ($hM_1$ $EC_{50}$ = 970 nM). Moving the bicyclic bridge of tropane led to more potent compounds, i.e. VU0420385 ($hM_1$ $EC_{50}$ = 92 nM) and VU 0447360 ($hM_1$ $EC_{50}$ = 47 nM).

## 5.2 *$M_1$ mAChR Positive Allosteric Modulators*

Positive allosteric modulators (PAMs) are generally believed to be useful strategy for high selective activation of receptors [80]. Attempts to identify high selective $M_1$ mAChR agonists were not successful because of high conservation of the orthosteric binding site of mAChRs. To overcome this hurdle, drug discovery of $M_1$ mAChR PAMs have attracted attentions of many researchers [63]. Reported PAMs showed greater subtype selectivity which has not observed with traditional mAChR agonists.

Lazareno and co-workers reported the natural product brucine as the first generation $M_1$ mAChR PAM (Fig. 9) [81]. Although PAM activity was weak (<2-fold enhancement of ACh activity), the discovery of brucine has led to drug discovery researches for selective activation of $M_1$ mAChR with PAM compounds.

Merck researchers identified benzylquinolone carboxylic acid (BQCA) as a $M_1$ mAChR PAM through a HTS campaign (Fig. 10) [82]. BQCA selectively potentiated $M_1$ mAChR ($M_1$ inflection point = 845 nM in a calcium mobilization assay) and showed no potentiation, agonism, or antagonism for other mAChRs [82, 83]. Although potency of BQCA for $M_1$ mAChRs ($EC_{50}$ = 267 nM in the calcium mobilization assay) and its brain permeability are low in rats, BQCA (15 and 20 mg/kg, ip) reversed scopolamine-induced learning impairments in the mice contextual fear conditioning test. Modification of the methoxyphenyl to a biaryl led to increased potency (compound **6**, $M_1$ inflection point = 61 nM), but has poor CNS exposure [84]. Further modification of the biaryl moiety led to compound **7** with high potency ($M_1$ inflection point = 171 nM) and good free fraction in the presence of rat and human serum [85]. Although compound **7** showed limited brain exposure, it reversed scopolamine-induced memory impairment in the mouse contextual fear conditioning test at low plasma levels relative to the previously reported quinolone carboxylic acids. Kuduk and co-workers reported SAR study following replacement of the phenyl ring by a heterocyclic ring in the quinolone moiety [86]. The reported compound **8** had a modestly potentiated affinity for $M_1$ mAChR ($M_1$ inflection point = 0.52 μM) and exhibited good pharmacokinetics (PK) profile (BA: 33%).

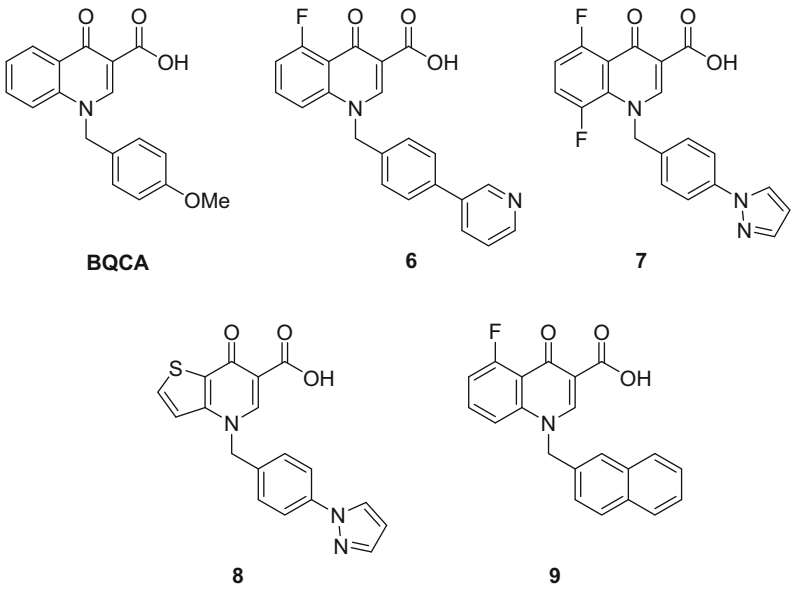



**Fig. 10** $M_1$ mAChR PAMs with quinolone scaffold

To find a less lipophilic linker, the biaryl moiety was replaced by a naphthyl moiety, giving compound **9**, which showed potentiated affinity for $M_1$ mAChR ($M_1$ inflection point = 69 nM) with good PK profile (BA: 33%) [87].

Kuduk and co-workers also reported replacement of the quinolone by a quinolizidinone (Fig. 11). Their efforts to find an alternative scaffold to increase CNS exposure led to compound **10** as a potent $M_1$ mAChR PAM ($M_1$ inflection point = 0.52 μM) with improved bioavailability (23%) and brain penetration in rats ($CSF/U_{plasma}$ = 0.42) [88]. Replacement of the piperadine by a cyano inserted piperidine afforded the potent compound **11** [89]. Compound **11** increased the potency for $M_1$ mAChR ($M_1$ inflection point = 135 nM) and exhibited a good PK profile in rats (BA: 68%, $CSF/U_{plasma}$ = 0.3). Compound **11** had no activities for any of the other 140 tested targets even at 30 μM. To overcome carboxylic acid moiety limited brain exposure, Kuduk and co-workers examined amide formation at the carboxylic acid moiety [90]. The identified compound **12** exhibited decreased protein binding, but good PK profile. When evaluated in vivo, compound **10** (30 mg/kg, ip), compound **11** (10 and 30 mg/kg, ip), and compound **12** (10 and 30 mg/kg, ip) reversed scopolamine-induced memory impairment in the mouse contextual memory fear conditioning test [88–90]. Another quinolone scaffold was disclosed by the researchers at Takeda [91]. Discovered compound **13** (Fig. 11) showed $M_1$ mAChR PAM activity ($M_1$ inflection point = 55 nM) and brain exposure ($K_p$ = 0.33). Further modification of fused ring led to compounds **14** ($M_1$ inflection point = 121 nM) and **15** ($M_1$ inflection point = 23 nM) with methoxynaphthalene scaffold (Fig. 11) [92]. Compounds **14** (B/P: 0.21, $CSF/U_{plasma}$ = 0.9) and **15** (B/P: 0.2, $CSF/U_{plasma}$ = 0.38) showed brain penetration.

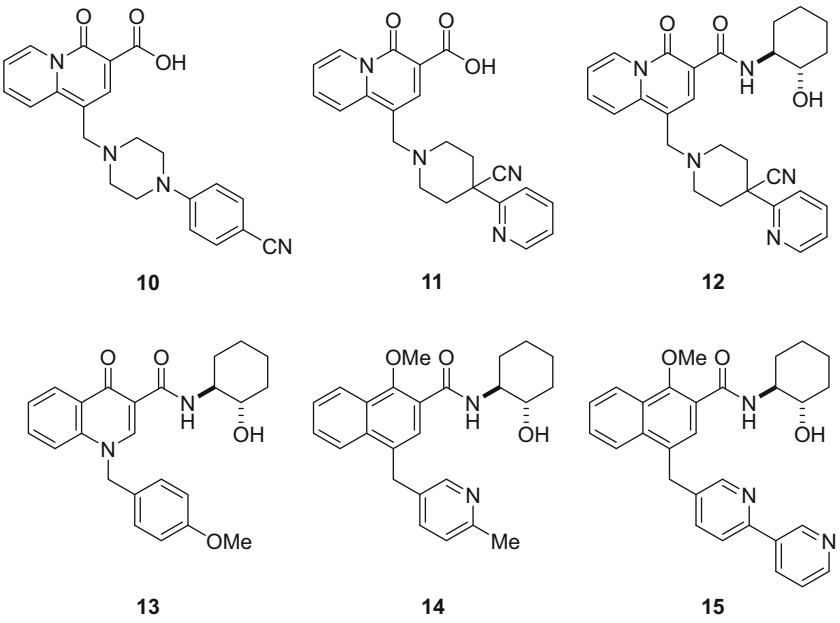



**Fig. 11** $M_1$ mAChR PAMs with quinolizidinone, naphthalene, and quinolone scaffolds

Other attempts to modify the core ring were summarized in Fig. 12 and the review [93]. As far as we know, the strategies for modification that core ring was replaced by quinolone [94] and that tetrahydroquinoline [95] and formation of tri-cyclic rings [96–104] were disclosed by patent applications. Among them, compound **16** (Fig. 13) with naphthyl lactam scaffold showed $M_1$ PAM activity ($M_1$ inflection point = 15 nM) and CNS exposure ($CSF/U_{plasma} = 0.51$) [105]. Administration of compound **16** significantly reversed scopolamine-induced deficit in the mouse contextual memory fear conditioning test (1 mg/kg, ip).

Another tricyclic scaffold was disclosed by the researchers at Asceneuron. They discovered $M_1$ mAChR PAMs with tetraaza-cyclopenta[a]indenyl scaffold [106]. Although chemical structure was not disclosed, researchers at Asceneuron reported the development of ASN-51 and its analog [107]. Interesting point of their presentation is that Y179 in $M_1$ mAChR protein is an essential structural determinant of the $M_1$PAM mechanism of action [107]. Recently other scaffolds have been reported by researchers at Vanderbilt University (Fig. 14). Lindsley and co-workers reported VU0119498 [108] as an $M_1$, $M_3$, and $M_5$ mAChRs PAM with indoline-2,3-dione. Replacement of Br by an aryl led to VU0366369 as a selective $M_1$ mAChR PAM [109]. Replacement of isatin by oxindole decreased potency ($hM_1$ $EC_{50}$ = 2.4 μM). HTS campaign also picked up compound **17** with indole scaffold. As a result of further modifications, Wood and co-workers reported VU0405652 ($hM_1$ $EC_{50}$ = 1.38 μM) [110] and VU0456940 ($hM_1$ $EC_{50}$ = 310 nM) [111] as potent $M_1$ mAChR PAMs with 3-sulfonyl indole scaffold. Isoindoline-1-one compounds were disclosed as another chemotype in the patent application [112].

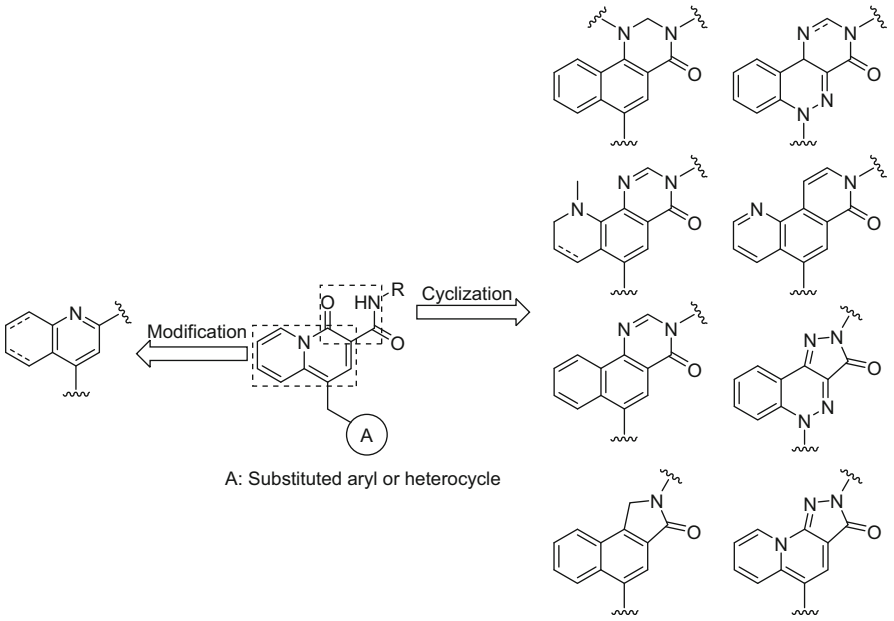
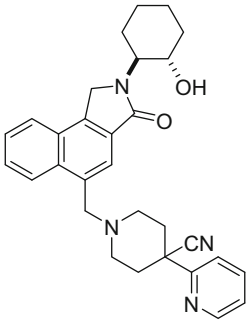



**Fig. 12** Further modification of central core of $M_1$ mAChR PAMs




**16**

**Fig. 13** Chemical structure of compound **16**

## *5.3 $M_1$ and $M_4$ mAChRs Agonists*

The $M_1$ and $M_4$ mAChR-preferring xanomeline improve the three major symptoms of schizophrenia in a pilot clinical trial. Since activation of $M_1$ and $M_4$ mAChRs might contribute to different aspect of therapeutic efficacy for schizophrenia [113], we believed that development of $M_1$ and $M_4$ mAChR-dual agonists represents the most promising strategy for treatment of a wide range of symptoms in schizophrenia. However, identification of highly selective $M_1$ and $M_4$ mAChR agonists is

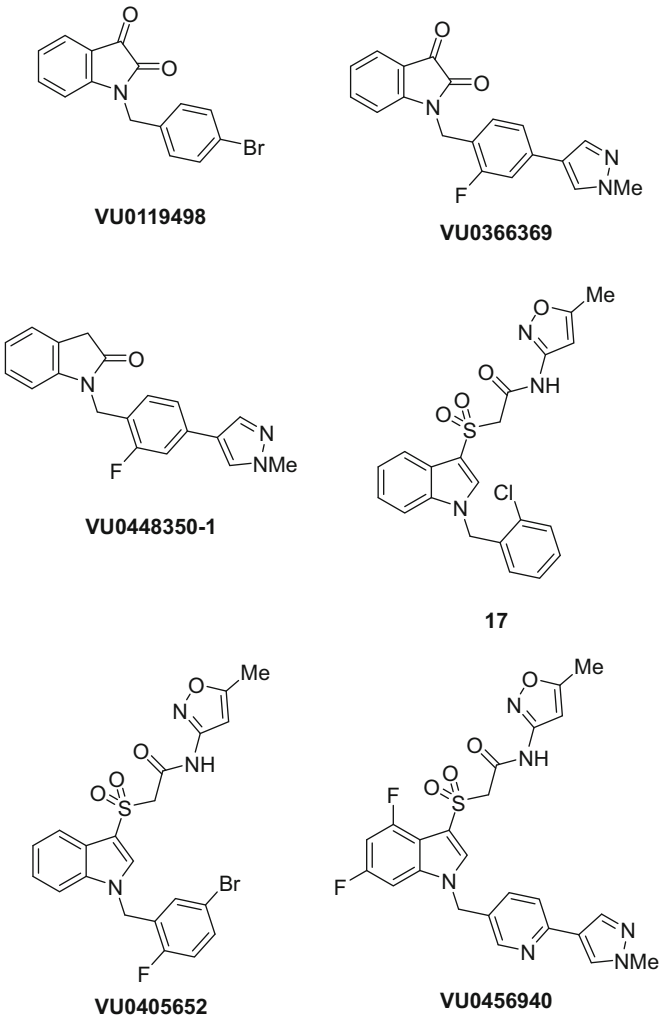



**Fig. 14** $M_1$ mAChR PAMs discovered by Vanderbilt University groups

challenging, because of the high sequence homology and conservation of the orthosteric ACh binding site among mAChR subtypes.

In our drug discovery program for selective $M_1$ and $M_4$ mAChRs agonists, we found 8-azaquinolone as a promising orally active candidate [114]. First, we screened our chemical library and found compound **18** as hit compound. Based on information in Vertex pharmaceuticals [115] and Banyu [116] patent applications, we hypothesized the pharmacophore of allosteric $M_4$ mAChR agonists (Fig. 15).

To satisfy the pharmacophore of allosteric $M_4$ mAChR agonists (Fig. 16), we introduced *N*-carbethoxy piperidine moiety to our hit compound **18**. As expected, the prepared compound **19** showed relatively stronger agonistic activity for $M_1$ and

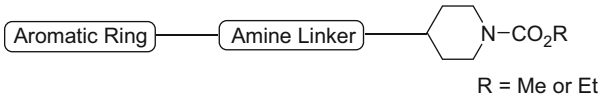



**Fig. 15** Plausible pharmacophore of allosteric $M_4$ mAChR agonists

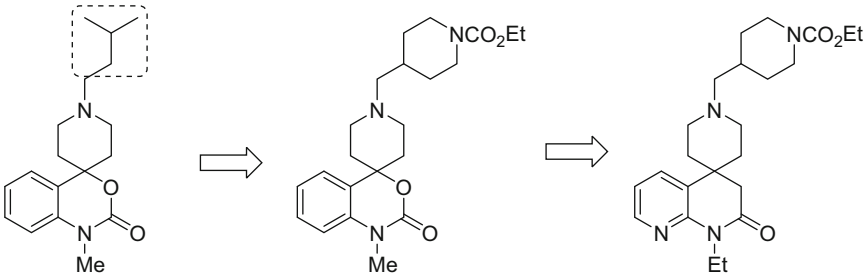



**Fig. 16** $M_1$ and $M_4$ mAChRs agonists

$M_4$ mAChRs. However, compound **19** still showed the activation of $M_3$ mAChR. As a result of further modification, we found that introduction of a nitrogen atom to the benzene ring decreased $M_3$ mAChR agonistic activity. Further optimization of substituents of the nitrogen atom afforded the selective $M_1$ and $M_4$ mAChRs agonist **20**. In a calcium-mobilization assay, compound **20** showed potent activation of $M_1$ (91% at 1 μM) and $M_4$ mAChRs (134% at 1 μM) with weak or negligible activation of $M_2$ (27% at 10 μM), $M_3$, (3% at 10 μM) and $M_5$ mAChRs (3% at 10 μM). In addition, compound **20** exhibited weak human ether-a-go-go related gene (hERG) inhibition ($IC_{50}$ = 2.9 μM). These encouraging results led us to investigate the antipsychotic-like efficacy of compound **20**. Oral administration of compound **20** reversed methamphetamine-induced locomotor hyperactivity in rats ($ED_{50}$ = 0.21 mg/kg). Further, radioligand binding assays revealed that compound **20**, even at 8 μM, only weakly inhibited the binding of [$^3$H]-spiperone to human dopamine $D_2$ receptor (23%).

We also disclosed *N*-substituted oxindole derivatives as $M_1$ and $M_4$ mAChRs partial agonists (Fig. 17) [56]. In our HTS screening for $M_4$ mAChR agonists, oxindole compound **21** was identified as a hit compound. To increase $M_4$ mAChR agonistic activity, we replaced the dimethyl olefin unit by the *N*-carbethoxy piperidine, a pharmacophore of $M_4$ mAChR agonists. Further modification of ring size of amine linker afforded compound **22** with good selectivity for $M_1$ and $M_4$ over $M_2$, $M_3$, and $M_5$ mAChRs. Replacement of piperidine of compound **22** by bulky amine ring led to the identification of compound **23** as a selective $M_1$ and $M_4$ mAChRs partial agonist.

In our calcium mobilization assay, compound **23** selectively activated $M_1$ ($EC_{50}$ = 12 nM, IA = 60%) and $M_4$ mAChRs ($EC_{50}$ = 29 nM, IA = 42%) and

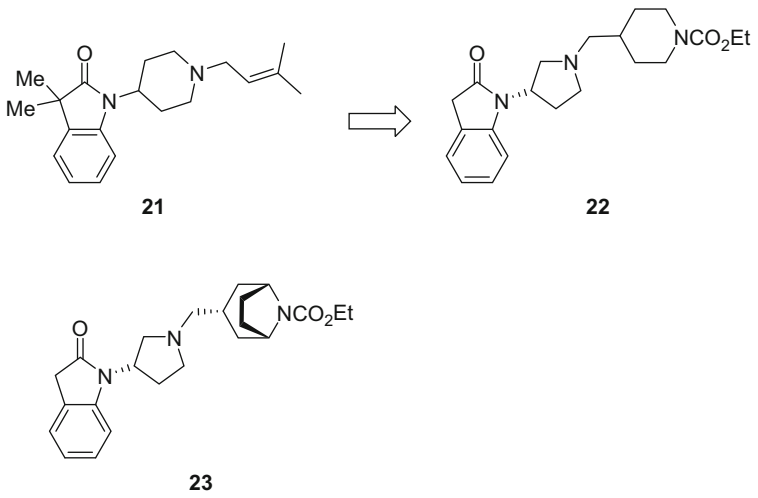



**Fig. 17** $M_1$ and $M_4$ mAChRs agonists with *N*-substituted oxindole scaffold

showed very weak or negligible activation of $M_2$, $M_3$, and $M_5$ even at 30 μM. Compound **23** at 3 μM showed no binding inhibition against dopamine $D_2$ receptor and other 67 off-targets (except for sigma receptors) in a panel assay including a broad range of G-protein coupled receptors, ion channels, enzymes, and transporters. In addition, compound **23** showed good CNS penetration (brain/blood ratio = 2.0) in a rat pharmacokinetic study and produced the antipsychotic-like efficacy in the several rodent behavioral tasks. Subcutaneous administration of compound **23** (1, 3, and 10 mg/kg) significantly reversed methamphetamine-induced locomotor hyperactivity in rats. Apomorphine-induced climbing behavior and PPI deficit were also improved by treatment with compound **23**. Moreover, compound **23** at a sub-effective dose (0.6 mg/kg, sc) enhanced the efficacy of risperidone (0.6 mg/kg, po) in methamphetamine-induced locomotor hyperactivity test in rats. On the other hand, compound **23**, given alone, (10 and 30 mg/kg, sc) did not induce catalepsy and did not worsen risperidone (10 mg/kg)-induced catalepsy in rats. These results indicate that compound **23**, used alone or combination with antipsychotics, has a good potential in the treatment of schizophrenia. The high selectivity of compound **23** for $M_1$ and $M_4$ mAChRs helped avoid salivation in mice. Unlike xanomeline (1 and 3 mg/kg, sc), administration of compound **23** (1.5 and 6 mg/kg, sc) did not increase mean blood pressure in telemetered cynomolgus monkeys. Furthermore, compound **23** (1.5 and 6 mg/kg, sc) did not induce vomiting and drowsiness, although these side effects were observed with xanomeline (1 and 3 mg/kg, sc) in monkeys. Taken together, these findings support compound **23** as a promising candidate for monotherapy or adjunctive therapy of schizophrenia.

## 5.4 $M_4$ mAChR Positive Allosteric Modulators

The first generation $M_4$ mAChR PAMs LY2033298 and VU010010 were reported by researchers at Vanderbilt University [117] and Eli Lilly [118] (Fig. 18). Chan and co-workers described LY2033298 as a potent $M_4$ mAChR PAM. Further pharmacological studies showed that LY2033298 enhances both the binding affinity and efficacy of ACh at the $M_4$ mAChR. This finding indicates that subtype selectivity, which can potentially limit side effects, can be attained via an allosteric mechanism. Shirey and co-workers identified VU010010 as another $M_4$ mAChR PAM. VU010010 left-shifted the concentration-response curve of ACh for $M_4$ mAChRs, but showed no effects for the other mAChR subtypes. However, VU010010 showed poor efficacy in vivo because of its physicochemical properties such as poor solubility, *P*-glycoprotein efflux, and high log*P* value. Screening for $M_4$ mAChR PAM and optimization of the amine moiety of VU010010 led to the discovery of VU0152099 (rat $M_4$ $EC_{50} = 0.40$ μM) and VU0152100 (rat $M_4$ $EC_{50} = 0.38$ μM) with improved log*P* values [119]. Pharmacokinetic studies of these two compounds revealed that VU0152100 ability to penetrate the CNS is better than that of VU0152099. In a rat model, VU0152100 (56.6 mg/kg, ip) reversed amphetamine-induced locomotor hyperactivity without causing sedation. Both VU0152099 and VU0152100 have recently been modified to produce ML173 ($hM_4$ $EC_{50} = 0.5$ μM) [120], VU0448088 [121] and compound **24** ($M_4$ $EC_{50} = 0.10$ μM) [122].

Hopkins and co-workers discovered VU0232776 as another $M_4$ mAChR PAM with the benzothiazole scaffold (Fig. 19) [123]. Further optimization of this compound led to the identification of VU0409524. Evaluation of VU0409524 showed high selectivity, but modest activation of human $M_4$ mAChR ($EC_{50} = 1.3$ μM), good in vivo PK properties in rats, and excellent brain exposure (brain/plasma = 0.85).

## 5.5 $M_5$ mAChR Antagonists and Negative Allosteric Modulators

$M_5$ mAChR is localized in the cell bodies of dopaminergic neurons in the ventral tegmental area, and activation of $M_5$ mAChR leads to enhancement of dopamine release in the nucleus accumbens, as described above [12, 33, 34]. In addition, Grant and El-Fakahany have reported that xanomeline acts as a $M_5$ mAChR partial agonist and antagonizes a mAChR full agonist carbacol-induced phosphoinositide hydrolysis in CHO cells expressing human $M_5$ mAChR [124]. Therefore, $M_5$ mAChR antagonists or negative allosteric modulators (NAM) might be an attractive approach for the treatment of psychosis in schizophrenia. Zheng and colleagues have recently reported $M_5$ mAChR antagonist **25** (Fig. 20, $hM_1$ $K_i = 25.3$ μM, $hM_2$ $K_i > 100$ μM, $hM_3$ $K_i > 100$ μM, $hM_4$ $K_i > 100$ μM, $hM_5$ $K_i = 2.24$ μM)

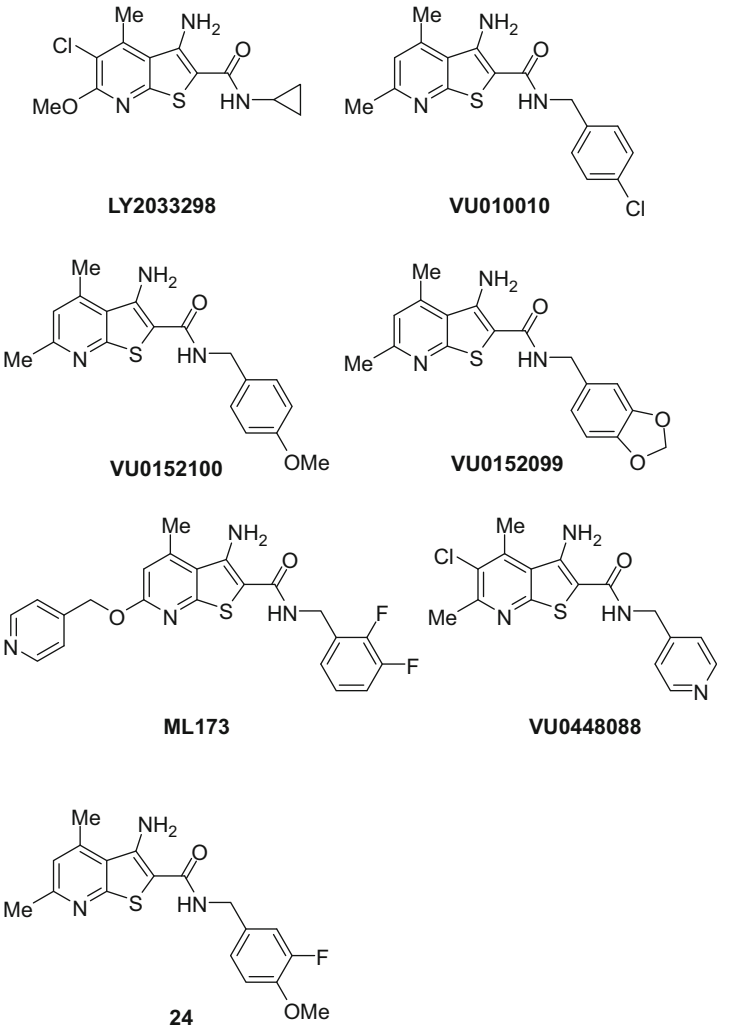



**Fig. 18** $M_4$ mAChR PAMs with the thienopyridine scaffold

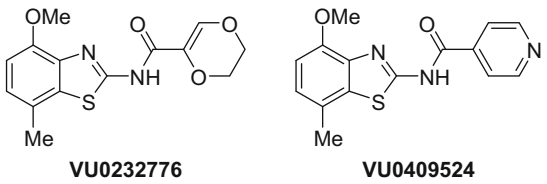



**Fig. 19** $M_4$ mAChR PAMs with the benzothiazole scaffold

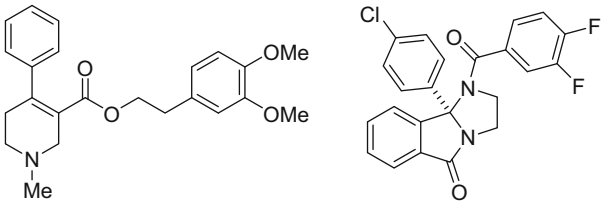



**Fig. 20** Structures of $M_5$ mAChR antagonist and NAM

[125]. Gently and colleagues have discovered the first $M_5$ mAChR negative allosteric modulator (NAM) ML375 (Fig. 20, $hM_5$ $IC_{50} = 300$ nM, $hM_1$–$M_4$ $IC_{50} > 30$ μM in calcium mobilization assays) [126]. ML375 shows high oral bioavailability (80%) and CNS penetration in rats. However, ML375 displays slightly weaker potency for rat $M_5$ mACh ($IC_{50} = 790$ nM) compared to human $M_5$ mACh and might not be suitable for in vivo experiments in rodents. Further works are necessary for demonstration of the proof of concept of $M_5$ mAChR negative regulators because in vivo efficacy of these compounds has been still unclear.

## 6 Perspective

As summarized above, recent advances in pharmacological and medicinal chemistry research support the muscarinic approach as one of the most promising strategies in drug discovery for schizophrenia treatment. The search for potent activators of $M_1$ and/or $M_4$ mAChRs has led to the discovery of new scaffolds with true subtype selectivity. However, no selective mAChR activator has so far succeeded in clinical development. This is probably because a balance between subtype selectivity ($M_1$ and/or $M_4$ mAChR), efficacy, potency of mAChR activation, and pharmacokinetic profile could not be achieved. Therefore, it is important to develop new compounds or scaffolds that can lead to druggable candidates. Recently, the concept of "bitopic agonist" is reported as a new strategy to accomplish both high potency and good selectivity [127, 128]. This strategy can be applied to the discovery of highly selective and potent $M_1$ and $M_4$ mAChR agonists. In addition, recent developments allowing stabilization of GPCR proteins by point mutation [129] as well as crystallization of $M_2$ and $M_3$ mAChRs [130, 131] have been found to be very useful tools for rational drug design. In fact, Heptares therapeutics identified HTL9936 as a selective $M_1$ mAChR agonist, using a structure-based drug design that makes use of point mutation technology. They have recently initiated a clinical study for the development of this compound [132]. It is expected that future studies would yield selective and druggable $M_1$ and/or $M_4$ mAChR activators good enough to be used as novel treatment for schizophrenia.

## References


1. Andreasen NC, Flaum M, Swayze VW et al (1990) Positive and negative symptoms in schizophrenia. A critical reappraisal. Arch Gen Psychiatry 47:615–621
2. Meltzer HY (1999) Treatment of schizophrenia and spectrum disorders: pharmacotherapy, psychosocial treatments, and neurotransmitter interactions. Biol Psychiatry 46:1321–1327
3. Casey DE (1996) Extrapyramidal syndromes and new antipsychotic drugs: findings in patients and non-human primate models. Br J Psychiatry Suppl 29:32–39
4. Hirsch S, Barnes TRE (1995) The clinical treatment of schizophrenia with antipsychotic medication. In: Hirsch SR, Weinberger DR (eds) Schizophrenia. Blackwell, Oxford
5. Leucht S, Corves C, Arbter D et al (2009) Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 373:31–41
6. Woodward ND, Purdon SE, Meltzer HY et al (2005) A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. Int J Neuropsychopharmacol 8:457–472
7. Raedler TJ, Bymaster FP, Tandon R et al (2007) Towards a muscarinic hypothesis of schizophrenia. Mol Psychiatry 12:232–246
8. Shekhar A, Potter WZ, Lightfoot J et al (2008) Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. Am J Psychiatry 165: 1033–1039
9. Wess J, Eglen RM, Gautam D (2007) Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat Rev Drug Discov 6:721–733
10. Caulfield MP, Birdsall NJ (1998) International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 50:279–290
11. Eglen RM (2005) Muscarinic receptor subtype pharmacology and physiology. Prog Med Chem 43:105–136
12. Eglen RM (2012) Overview of muscarinic receptor subtypes. Handb Exp Pharmacol 208: 3–28
13. Hulme EC, Birdsall NJ, Buckley NJ (1990) Muscarinic receptor subtypes. Annu Rev Pharmacol Toxicol 30:633–673
14. Raedler TJ, Knable MB, Jones DW et al (2003) In vivo determination of muscarinic acetylcholine receptor availability in schizophrenia. Am J Psychiatry 160:118–127
15. Crook JM, Tomaskovic-Crook E, Copolov DL et al (2000) Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. Biol Psychiatry 48:381–388
16. Dean B, McLeod M, Keriakous D et al (2002) Decreased muscarinic$_1$ receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol Psychiatry 7:1083–1091
17. Zavitsanou K, Katsifis A, Mattner F et al (2004) Investigation of M1/M4 muscarinic receptors in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression disorder. Neuropsychopharmacology 29:619–625
18. Scarr E, Sundram S, Keriakous D et al (2007) Altered hippocampal muscarinic M4, but not M1, receptor expression from subjects with schizophrenia. Biol Psychiatry 61:1161–1170
19. Scarr E, Keriakous D, Crossland N et al (2006) No change in cortical muscarinic M2, M3 receptors or [$^{35}$S]GTPγS binding in schizophrenia. Life Sci 78:1231–1237
20. Scarr E, Cowie TF, Kanellakis S et al (2009) Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. Mol Psychiatry 14:1017–1023
21. Bubser M, Byun N, Wood MR et al (2012) Muscarinic receptor pharmacology and circuitry for the modulation of cognition. Handb Exp Pharmacol 208:121–166
22. Miyakawa T, Yamada M, Duttaroy A et al (2001) Hyperactivity and intact hippocampus-dependent learning in mice lacking the $M_1$ muscarinic acetylcholine receptor. J Neurosci 21:5239–5250
23. Anagnostaras SG, Murphy GG, Hamilton SE et al (2003) Selective cognitive dysfunction in acetylcholine $M_1$ muscarinic receptor mutant mice. Nat Neuroscience 6:51–58

24. Bartko SJ, Romberg C, White B et al (2011) Intact attentional processing but abnormal responding in $M_1$ muscarinic receptor-deficient mice using an automated touchscreen method. Neuropharmacology 61:1366–1378
25. Keefe RSE (2000) Working memory dysfunction and its relevance to schizophrenia. In: Sharma T, Harvey P (eds) Cognition in schizophrenia. Oxford University Press, Oxford, pp 16–50
26. Chudasama Y, Robbins TW (2006) Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. Biol Psychol 73:19–38
27. Gerber DJ, Sotnikova TD, Gainetdinov RR et al (2001) Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. Proc Natl Acad Sci USA 98:15312–15317
28. Gomeza J, Zhang L, Kostenis E et al (1999) Enhancement of D1 dopamine receptor-mediated locomotor stimulation in $M_4$ muscarinic acetylcholine receptor knockout mice. Proc Natl Acad Sci USA 96:10483–10488
29. Koshimizu H, Leiter LM, Miyakawa T (2012) $M_4$ muscarinic receptor knockout mice display abnormal social behavior and decreased prepulse inhibition. Mol Brain 5:10
30. Tzavara ET, Bymaster FP, Davis RJ et al (2004) $M_4$ muscarinic receptors regulate the dynamics of cholinergic and dopaminergic neurotransmission: relevance to the pathophysiology and treatment of related central nervous system pathologies. FASEB J 18:1410–1412
31. Tzavara ET, Bymaster FP, Felder CC et al (2003) Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in M2, M4 and M2/M4 muscarinic receptor knockout mice. Mol Psychiatry 8:673–679
32. Floresco SB, Geyer MA, Gold LH et al (2005) Developing predictive animal models and establishing a preclinical trials network for assessing treatment effects on cognition in schizophrenia. Schizophr Bull 31:888–894
33. Forster GL, Yeomans JS, Takeuchi J et al (2001) M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. J Neurosci 21:RC190
34. Wang H, Ng K, Hayes D et al (2004) Decreased amphetamine-induced locomotion and improved latent inhibition in mice mutant for the $M_5$ muscarinic receptor gene found in the human 15q schizophrenia region. Neuropsychopharmacology 29:2126–2139
35. Weiner I (2003) The "two-headed" latent inhibition model of schizophrenia: modeling positive and negative symptoms and their treatment. Psychopharmacology 169:257–297
36. Fisher A, Pittel Z, Haring R et al (2003) $M_1$ muscarinic agonists can modulate some of the hallmarks in Alzheimer's disease: implications in future therapy. J Mol Neurosci 20:349–356
37. McArthur RA, Gray J, Schreiber R (2010) Cognitive effects of muscarinic M1 functional agonists in non-human primates and clinical trials. Curr Opin Investig Drugs 11:740–760
38. Bodick NC, Offen WW, Levey AI et al (1997) Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. Arch Neurol 54:465–473
39. Bolbecker AR, Shekhar A (2012) Muscarinic agonists and antagonists in schizophrenia: recent therapeutic advances and future directions. Handb Exp Pharmacol 208:167–190
40. Shannon HE, Hart JC, Bymaster FP et al (1999) Muscarinic receptor agonists, like dopamine receptor antagonist antipsychotics, inhibit conditioned avoidance response in rats. J Pharmacol Exp Ther 290:901–907
41. Shannon HE, Rasmussen K, Bymaster FP et al (2000) Xanomeline, an $M_1/M_4$ preferring muscarinic cholinergic receptor agonist, produces antipsychotic-like activity in rats and mice. Schizophr Res 42:249–259
42. Stanhope KJ, Mirza NR, Bickerdike MJ et al (2001) The muscarinic receptor agonist xanomeline has an antipsychotic-like profile in the rat. J Pharmacol Exp Ther 299:782–792

43. Heinrich JN, Butera JA, Carrick T et al (2009) Pharmacological comparison of muscarinic ligands: historical versus more recent muscarinic $M_1$-preferring receptor agonists. Eur J Pharmacol 605:53–56
44. Watson J, Brough S, Coldwell MC et al (1998) Functional effects of the muscarinic receptor agonist, xanomeline, at 5-$HT_1$ and 5-$HT_2$ receptors. Br J Pharmacol 125:1413–1420
45. Woolley ML, Carter HJ, Gartlon JE et al (2009) Attenuation of amphetamine-induced activity by the non-selective muscarinic receptor agonist, xanomeline, is absent in muscarinic $M_4$ receptor knockout mice and attenuated in muscarinic $M_1$ receptor knockout mice. Eur J Pharmacol 603:147–149
46. Dencker D, Wörtwein G, Weikop P et al (2011) Involvement of a subpopulation of neuronal $M_4$ muscarinic acetylcholine receptors in the antipsychotic-like effects of the $M_1/M_4$ preferring muscarinic receptor agonist xanomeline. J Neurosci 31:5905–5908
47. Watt ML, Rorick-Kehn L, Shaw DB et al (2013) The muscarinic acetylcholine receptor agonist BuTAC mediates antipsychotic-like effects via the M4 subtype. Neuropsychopharmacology 38:2717–2726
48. Li Z, Snigdha S, Roseman AS et al (2008) Effect of muscarinic receptor agonists xanomeline and sabcomeline on acetylcholine and dopamine efflux in the rat brain; comparison with effects of 4-[3-(4-butylpiperidin-1-yl)-propyl]-7-fluoro-4*H*-benzo[1,4]oxazin-3-one (AC260584) and *N*-desmethylclozapine. Eur J Pharmacol 596:89–97
49. Perry KW, Nisenbaum LK, George CA et al (2001) The muscarinic agonist xanomeline increases monoamine release and immediate early gene expression in the rat prefrontal cortex. Biol Psychiatry 49:716–725
50. Enomoto T, Noda Y, Nabeshima T (2007) Phencyclidine and genetic animal models of schizophrenia developed in relation to the glutamate hypothesis. Methods Find Exp Clin Pharmacol 29:291–301
51. Tseng KY, Chambers RA, Lipska BK (2009) The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav Brain Res 204:295–305
52. Enomoto T, Floresco SB (2009) Disruptions in spatial working memory, but not short-term memory, induced by repeated ketamine exposure. Prog Neuropsychopharmacol Biol Psychiatry 33:668–675
53. Floresco SB, Zhang Y, Enomoto T (2009) Neural circuits subserving behavioral flexibility and their relevance to schizophrenia. Behav Brain Res 204:396–409
54. Sedman AJ, Bockbrader H, Schwarz RD (1995) Preclinical and phase 1 clinical characterization of CI-979/RU35926, a novel muscarinic agonist for the treatment of Alzheimer's disease. Life Sci 56:877–882
55. Thal LJ, Forrest M, Loft H et al (2000) Lu 25-109, a muscarinic agonist, fails to improve cognition in Alzheimer's disease. Lu25-109 Study Group. Neurology 54:421–426
56. Sumiyoshi T, Enomoto T, Takai K et al (2013) Discovery of *N*-substituted oxindoles as selective $M_1$ and $M_4$ muscarinic acetylcholine receptors partial agonists. ACS Med Chem Lett 4:244–248
57. Carlsson A, Hansson LO, Waters N et al (1999) A glutamatergic deficiency model of schizophrenia. Br J Psychiatry Suppl 37:2–6
58. Marino MJ, Rouse ST, Levey AI et al (1998) Activation of the genetically defined m1 muscarinic receptor potentiates *N*-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells. Proc Natl Acad Sci USA 95:11465–11470
59. Davies MA, Compton-Toth BA, Hufeisen SJ et al (2005) The highly efficacious actions of *N*-desmethylclozapine at muscarinic receptors are unique and not a common property of either typical or atypical antipsychotic drugs: is $M_1$ agonism a pre-requisite for mimicking clozapine's actions? Psychopharmacology 178:451–460
60. Sur C, Mallorga PJ, Wittmann M et al (2003) *N*-desmethylclozapine, an allosteric agonist at muscarinic 1 receptor, potentiates *N*-methyl-D-aspartate receptor activity. Proc Natl Acad Sci USA 100:13674–13679

61. Weiner DM, Meltzer HY, Veinbergs I et al (2004) The role of M1 muscarinic receptor agonism of *N*-desmethylclozapine in the unique clinical effects of clozapine. Psychopharmacology 177:207–216
62. ACADIA Pharmaceuticals (2008) ACADIA pharmaceuticals announces results from ACP-104 phase IIb schizophrenia trial. http://www.acadia-pharm.com/. Accessed 8 March 2014
63. Melancon BJ, Tarr JC, Panarese JD et al (2013) Allosteric modulation of the $M_1$ muscarinic acetylcholine receptor: improving cognition and a potential treatment for schizophrenia and Alzheimer's disease. Drug Discovery Today 18:1185–1199
64. Spalding TA, Trotter C, Skjærbæk N et al (2002) Discovery of an ectopic activation site on the $M_1$ muscarinic receptor. Mol Pharmacol 61:1297–1302
65. Spalding TA, Ma J-N, Ott TR et al (2006) Structural requirements of transmenbrane domain 3 for activation by the $M_1$ muscarinic receptor agonists, AC-42, AC-260584, clozapine, and *N*-desmethylclozapine: evidence for three distinct models of receptor activation. Mol Pharmacol 70:1974–1983
66. Bradley SR, Lameh J, Ohrmund L et al (2010) AC-260584, an orally bioavailable $M_1$ muscarinic receptor allosteric agonist, improves cognitive performance in an animal model. Neuropharmacology 58:365–373
67. Langmead CJ, Austin NE, Branch CL et al (2008) Characterization of a CNS penetrant, selective $M_1$ muscarinic receptor agonist, 77-LH-28-1. Br J Pharmacol 154:1104–1115
68. Avlani VA, Langmead CJ, Guida E et al (2010) Orthosteric and allosteric modes of interaction of novel selective agonists of the $M_1$ muscarinic acetylcholine receptor. Mol Pharmacol 78:94–104
69. Sams AG, Hentzer M, Mikkelsen GK et al (2010) Discovery of *N*-{1-[3-(3-Oxo-2,3-dihydrobenzo[1,4]oxazin-4-yl)propyl]piperidin-4-yl}-2-phenylacetamide (Lu AE51090): an allosteric muscarinic $M_1$ receptor agonist with unprecedented selectivity and procognitive potential. J Med Chem 53:6386–6397
70. Jones CK, Brady AE, Davis AA et al (2008) Novel selective allosteric activator of the $M_1$ muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats. J Neurosci 28:10422–10433
71. Keov P, Valant C, Devine SM et al (2013) Reverse engineering of the selective agonist TBPB unveils both orthosteric and allosteric modes of action at the $M_1$ muscarinic acetylcholine receptor. Mol Pharmacol 84:425–437
72. Budzik B, Garzya V, Shi D et al (2010) Novel *N*-substituted benzimidazolones as potent, selective, CNS-penetrant, and orally active $M_1$ mAChR agonists. ACS Med Chem Lett 1: 244–248
73. Huiban M, Pampols-Maso S, Passchier J (2011) Fully automated synthesis of the $M_1$ receptor agonist [$^{11}$C] GSK1034702 for clinical use on an Eckert & Ziegler Modular Lab system. Appl Radiat Isotopes 69:1390–1394
74. Nathan PJ, Watson J, Lund J et al (2013) The potent $M_1$ receptor allosteric agonist GSK1034702 improves episodic memory in humans in the nicotine abstinence model of cognitive dysfunction. Int J Neuropsychopharmacol 16:721–731
75. Johnson DJ, Forbes IT, Watson SP et al (2010) The discovery of a series of N-substituted 3-(4-piperidinyl)-1,3-benzoxazolinones and oxindoles as highly brain penetrant, selective muscarinic $M_1$ agonists. Bioorg Med Chem Lett 20:5434–5438
76. Budzik B, Garzya V, Shi D et al (2010) Biaryl amides as novel and subtype selective $M_1$ agonists. Part II: Further optimization and profiling. Bioorg Med Chem Lett 20:3545–3549
77. Lebois EP, Bridges TM, Lewis LM 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 1:104–121
78. Lebois EP, Digby GJ, Sheffler DJ et al (2011) Development of a highly selective, orally bioavailable and CNS penetrant $M_1$ agonist derived from the MLPCN probe ML071. Bioorg Med Chem Lett 21:6451–6455

79. Melancon BJ, Gogliotti RD, Tarr JC et al (2012) Continued optimizationn of the MLPCN probe ML071 into highly potent agonists of the $hM_1$ muscarinic acetylcholine receptor. Bioorg Med Chem Lett 22:3467–3472
80. Conn PJ, Christopoulos A, Lindsley CW (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat Rev Drug Discov 8:41–54
81. Birdsall NJM, Farries T, Gharagozloo P et al (1997) Selective allosteric enhancement of the binding and actions of acetylcholine at muscarinic receptor subtypes. Life Sci 60:1047–1052
82. Ma L, Seager MA, Wittman M et al (2009) Selective activation of the $M_1$ muscarinic acetylcholine receptor achieved by allosteric potentiation Proc Natl Acad Sci USA 106: 15950–15955
83. Shirey JK, Brady AE, Jones PJ et al (2009) A selective allosteric potentiator of the $M_1$ muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. J Neurosci 29:14271–14286
84. Yang FV, Shipe WD, Bunda JL et al (2010) Parallel synthesis of *N*-biaryl quinolone carboxylic acids as selective $M_1$ positive allosteric modulators. Bioorg Med Chem Lett 20: 531–536
85. Kuduk SD, Di Marco CN, Cofre V et al (2010) N-Heterocyclic derived $M_1$ positive allosteric modulators. Bioorg Med Chem Lett 20:1334–1337
86. Kuduk SD, Di Marco CN, Chang RK et al (2010) Heterocyclic fused pyridone carboxylic acid $M_1$ positive allosteric modulators. Bioorg Med Chem Lett 20:2533–2537
87. Kuduk SD, Di Marco CN, Cofre V et al (2011) Fused heterocyclic $M_1$ positive allosteric modulators. Bioorg Med Chem Lett 21:2769–2772
88. Kuduk SD, Chang RK, Di Marco CN et al (2010) Quinolizidinone carboxylic acids as CNS penetrant, selective $M_1$ allosteric muscarinic receptor modulators. ACS Med Chem Lett 1:263–267
89. Kuduk SD, Chang RK, Di Marco CN et al (2011) Discovery of a selective allosteric $M_1$ receptor modulator with suitable development properties based on a quinolizidinone carboxylic acid scaffold. J Med Chem 54:4773–4780
90. Kuduk SD, Chang RK, Greshock TJ et al (2012) Dentification of amides as carboxylic acid surrogates for quinolizidinone-based $M_1$ positive allosteric modulators. ACS Med Chem Lett 3:1070–1074
91. Sakamoto H, Sugimoto T (2013) WO2013/129,622
92. Kuduk SD, Di Marco CN, Saffold JR et al (2014) Identification of a methoxynaphthalene scaffold as a core replacement in quinolizidinone amide $M_1$ positive allosteric modulators. Bioorg Med Chem Lett 24:1417–1420
93. Kuduk SD, Beshore DC (2012) Novel $M_1$ allosteric ligands: a patent review. Expert Opin Ther Patents 22:1385–1398
94. Kuduk SD, Di Marco CN,Yang ZQ et al (2012) WO2012/158,474
95. Kuduk SD, Gilvert KF (2011) WO2011/159,554
96. Kuduk SD, Chang RK, Greshock TJ (2012) WO2012/047,702
97. Beshore DC, Kuduk SD (2010) WO2010/096,338
98. Kuduk SD, Di Marco CN, Beshore DC (2012) WO2012/158,473
99. Kuduk SD, Beshore DC, Yang Z et al (2012) WO2012/003,147
100. Kuduk SD, Skudlarek JW (2012) WO2011/159,553
101. Kuduk SD, Beshore DC, Yang ZQ et al (2012) WO2012/158,475
102. Kuduk SD, Beshore DC, Di Marco CN et al (2011) WO2011/084,371
103. Beshore DC, Kuduk SD (2011) WO2011/137,049
104. Kuduk SD, Beshore DC, Di Marco CN (2010) WO2010/059,773
105. Yang ZQ, Shu Y, Ma L et al (2014) Discovery of naphthyl-fused 5-member lactams as a new class of $M_1$ positive allosteric modulators. ACS Med Chem Lett. doi:10.1021/ml500055h
106. Swinnen D, Montagne C, Pomel V et al (2013) WO2013/091,773

107. Beher D, Quattropani A, Osen-Sand A et al (2013) Potent M1 muscarinic acetylcholine receptor positive allosteric modulators as potential therapeutics for Alzheimer's disease. Soc Neurosci Abst 41:02
108. Marlo JE, Niswender CM, Days EL et al (2009) Discovery and characterization of novel allosteric potentiators of $M_1$ muscarinic receptors reveals multiple modes of activity. Mol Pharmacol 75:577–588
109. Bridges TM, Kennedy JP, Noetzel MJ et al (2010) Chemical lead optimization of a pan $G_q$ mAChR $M_1$, $M_3$, $M_5$ positive allosteric modulator (PAM) lead. Part II: development of a potent and highly selective $M_1$ PAM. Bioorg Med Chem Lett 20:1972–1975
110. Reid PR, Bridges TM, Sheffler DJ et al (2011) Discovery and optimization of a novel, selective and brain penetrant $M_1$ positive allosteric modulator (PAM): the development of ML169, an MLPCN probe. Bioorg Med Chem Lett 21:2697–2701
111. Tarr JC, Turlington ML, Reid PR et al (2012) Targeting selective activation of $M_1$ for the treatment of Alzheimer's disease: further chemical optimization and pharmacological characterization of the $M_1$ positive allosteric modulator ML169. ACS Chem Neurosci 3:884–895
112. Lindsley CW, Conn PJ, Wood MR et al (2013) WO2013/063,549
113. Foster DJ, Choi DL, Conn PJ et al (2014) Activation of $M_1$ and $M_4$ muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia. Neuropsychiatr Dis Treat 10: 183–191
114. Takai K, Inoue Y, Konishi Y et al (2013) Identification of *N*-substituted 8-azatetrahydroquinolone derivatives as selective and orally active $M_1$ and $M_4$ muscarinic acetylcholine receptors agonists. Bioorg Med Chem Lett 23:4644–4647
115. Makings L, Garcia-Guzman Blanco M, Hurley DJ et al (2006) WO 2006/023,852
116. Yamakawa T, Ando M, Ohwaki K et al (2001) WO 01/27,104
117. Shirey JK, Xiang Z, Orton D et al (2008) An allosteric potentiator of $M_4$ mAChR modulates hippocampal synaptic transmission. Nat Chem Biol 4:42–50
118. Chan WY, McKinzie DL, Bose S et al (2008) Allosteric modulation of the muscarinic $M_4$ receptor as an approach to treating schizophrenia. Proc Natl Acad Sci USA 105:10978–10983
119. Brady AE, Jones CK, Bridges TM et al (2008) Centrally active allosteric potentiators of the $M_4$ muscarinic acetylcholine receptor reverse amphetamine-induced hyperlocomotor activity in rats. J Pharm Exp Ther 327:941−953
120. Kennedy JP, Bridges TM, Gentry PR et al (2009) Synthesis and structure–activity relationships of allosteric potentiators of the $M_4$ muscarinic acetylcholine receptor. Chem Med Chem 4:1600–1607
121. Le U, Melancon BJ, Bridges TM 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
122. Huynh T, Valant C, Crosby IT et al (2013) Probing structural requirements of positive allosteric modulators of the $M_4$ muscarinic receptor. J Med Chem 56:8196–8200
123. Salovich JM, Vinson PN, Sheffler DJ et al (2012) Discovery of *N*-(4-methoxy-7-methylbenzo [*d*]thiazol-2-yl)isonicatinamide, ML293, as a novel, selective and brain penetrant positive allosteric modulator of the muscarinic 4 ($M_4$) receptor. Bioorg Med Chem Lett 22:5084–5088
124. Grant MK, El-Fakahany EE (2013) Persistent binding and functional antagonism by xanomeline at the muscarinic $M_5$ receptor. J Pharmacol Exp Ther 315:313–319
125. Zheng G, Smith AM, Huang X et al (2013) Structural modifications to tetrahydropyridine-3-carboxylate esters en route to the discovery of $M_5$-preferring muscarinic receptor orthosteric antagonists. J Med Chem 56:1693–1703
126. Gentry PR, Kokubo M, Bridges TM et al (2013) Discovery of the first $M_5$-selective and CNS penetrant negative allosteric modulator (NAM) of a muscarinic acetylcholine receptor: (*S*)-9b-(4-chlorophenyl)-1-(3,4-difluorobenzoyl)-2,3-dihydro-1*H*-imidazo[2,1-*a*]isoindol-5 (9b*H*)-one (ML375). J Med Chem 56:9351–9355

127. Mohr K, Schmitz J, Schrage R et al (2013) Molecular alliance–from orthosteric and allosteric ligands to dualsteric/bitopic agonists at G protein coupled receptors. Angew Chem Int Ed 52: 508–516
128. Davie BJ, Christopolos A, Scammells PJ (2013) Development of $M_1$ mAChR allosteric and bitopic ligands: prospective therapeutics for the treatment of cognitive deficits. ACS Chem Nerosci 4:1026–1048
129. Robertson N, Jazayeri A, Errey J et al (2011) The properties of thermostabilised G protein-coupled receptors (StaRs) and their use in drug discovery. Neuropharmacology 60:36–44
130. Haga K, Kruse AC, Asada H et al (2012) Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. Nature 482:547–552
131. Kruse AC, Hu J, Pan AC et al (2012) Structure and dynamics of the M3 muscarinic acetylcholine receptor. Nature 482:552–559
132. Heptares Therapeutics (2013) Heptares initiates clinical study with first selective muscarinic M1 receptor agonist for improving cognition in patients with Alzheimer's disease. http://www.heptares.com/. Accessed 8 March 2014

Top Med Chem (2015) 13: 213–254
DOI: 10.1007/7355_2014_56

Published online: 20 July 2014

# Nicotinic Acetylcholine Receptor Modulators

**Anatoly Mazurov and Daniel Yohannes**

**Abstract** Nicotine and its pharmacology have long been associated with schizophrenia and its epidemiology and treatment. Schizophrenics use nicotine in its major delivery form of cigarettes at a significantly higher incidence than the general population (80–90% vs. 25–30% of the general population) and even than those diagnosed with other psychiatric diseases. Various studies have demonstrated that cigarette smoking transiently restores cognitive and sensory deficits in patients with schizophrenia/schizoaffective disorders. Conversely, smoking cessation appears to exacerbate the symptoms of the disease. A disturbance of nicotinic receptor expression, affecting the α7 and α4β2 subunits, in various cerebral areas has been revealed in postmortem binding studies. Genetic linkage studies have also demonstrated that the α7 subunit is involved in the pathology of schizophrenia. Much of the drug discovery and development efforts in the nAChR field have focused on α7 and α4β2 nAChR subtypes. These include studies using nicotine and varenicline (the smoking cessation drug marketed as Chantix or Champix) as well as numerous other small-molecule therapeutics targeting these receptor subtypes. While some early studies included α4β2 modulators, the large majority of drug development of nAChR ligands for schizophrenia have been α7 agonists and partial agonists. Such therapeutics are in late-stage development and may constitute a breakthrough for the treatment of schizophrenia with safe and effective medicines.

**Keywords** ABT-126, Alpha4beta2, Alpha7, AQW-051, EVP-6124, nAChR, Nicotinic, Nicotinic acetylcholine receptor, Nicotinic agonist, Positive allosteric modulator, Schizophrenia, TC-5619, α4β2, α7

A. Mazurov
Asinex, Corp., One Technology Place, Winston-Salem, NC 27101, USA

D. Yohannes (✉)
Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA
e-mail: dyohanne@wakehealth.edu

## Contents



## Abbreviations

| | |
|---|---|
| 5-CSRTT | 5-Choice Serial Reaction Time Task |
| 5HT3R | 5-Hydroxytryptamine (serotonin) receptor 3 |
| ACh | Acetylcholine |
| AD | Alzheimer's disease |
| ADAS-Cog | Alzheimer's Disease Assessment Scale – Cognitive |
| CANTAB | Cambridge Neuropsychological Test Automated Battery |
| CDA | Cognitive dysfunction in schizophrenia |
| CGI-I | Clinical Global Impression – Global Improvement |
| CGI-S | GGI – Severity |
| CHRNA | Cholinergic receptor, neuronal nicotinic, alpha |
| CIAS | Cognitive Impairment Associated with Schizophrenia |
| CSB | CogState Schizophrenia Battery |
| DA | Dopamine |
| DMTS | Delayed matching to sample |
| DNMT1 | DNA methyltransferase 1 |
| $EC_{50}$ | Effective concentration 50% of maximal |
| FDA | Food and Drug Administration |
| GABA | Gamma-aminobutyric acid |
| GAD | Glutamic acid decarboxylase |
| GMLT | Groton Maze Learning Task |
| hERG | Human Ether-à-go-go-Related Gene |
| MATRICS | Measurement and Treatment Research to Improve Cognition in Schizophrenia |
| MCCB | MATRICS Consensus Cognitive Battery |
| MLA | Methyllycaconitine |
| nAChR | Nicotinic acetylcholine receptor |
| NMDA | *N*-methyl-D-aspartic acid |
| NRT | Nicotine replacement therapy |

| | |
|---|---|
| OCI | Overall cognitive index |
| ORT | Object recognition task |
| PAM | Positive allosteric modulators |
| PANSS | Positive and Negative Symptom Scale for Schizophrenia |
| PCP | Phencyclidine |
| PoC | Proof of concept |
| RBANS | Repeatable Battery for Assessment of Neuropsychological Status |
| SANS | Scale for Assessment of Negative Symptoms in Schizophrenia |
| SAPS | Scale for the Assessment of Positive Symptoms |
| SCoRS | Schizophrenia Cognition Rating Scale |
| SENCAR | Sensitive to carcinogens |
| SHR | Spontaneously hypertensive rat |
| SN | Substantia nigra |
| SNc | Substantia nigra pars compacta |
| TURNS | Treatment Units for Research of Neurocognition in Schizophrenia |
| VTA | Ventral tegmental area |
| α-BTX/ α-Bgt | α-Bungarotoxin |

## 1 Introduction

Numerous international studies show a strong association between schizophrenia and the use of tobacco products (smoking) [1]. The fact that nicotine from smoking can result in cognition enhancement in people has been known for several decades [2].

There are two important considerations to be made on the association between schizophrenia and smoking. On one hand, as in the general population, smoking can bring harmful consequences to those schizophrenic patients who smoke, including increased rates of mortality and cardiovascular disease [3, 4], lower therapeutic efficacy of treatments currently available, and greater financial burden for the society [5, 6]. However, the neurobiological consequences of nicotine intake through smoking in schizophrenics suggest potential new therapeutic development avenues to treat the disease. Investigation of nAChR's role in schizophrenia was originated largely by epidemiologic reports showing an increased rate of smoking among schizophrenics compared with the general population and suggesting an attempt at self-medication with nicotine [7]. The development and application of medicines based on the pharmacology of nicotinic acetylcholine receptors is important not only to reduce and eliminate smoking in schizophrenic patients but also to address the symptoms and perhaps causes of the disease.

In healthy volunteers, behaviors and processes related to attention and working memory are mainly responsive to improvement by nicotine [8]. Given the adverse events related to the use of nicotine itself (dizziness, nausea/emesis, locomotor effects, seizures, etc.) [9], improvement of cognitive function through modulation of nicotinic receptors requires therapeutics that selectively target the appropriate

nicotinic receptor subtypes while at the same time not interacting with the receptor subtypes responsible for the adverse events. Drugs based on nAChR pharmacology include nicotine and varenicline, as well as numerous experimental agents which are in clinical trials or in preclinical evaluation. These agents agonize both the α4β2 and α7 nAChRs, which are distributed throughout the brain, whereas the adverse effects of nicotine and nonselective nicotinics are mediated by the α3β4 nAChR subtype, which is primarily expressed in the autonomic ganglia [10]. Thus, both agents are suboptimal therapeutics for the treatment of schizophrenia.

Symptoms associated with schizophrenia are divided into three categories: (1) positive (hallucinations, delusions, thought disorder), (2) negative (anhedonia, poverty of speech, lack of motivation), and (3) cognitive (attention, memory, executive function). Current antipsychotic medications for the treatment of schizophrenia address the positive symptoms of schizophrenia well, but many patients with negative symptoms and disturbances of memory and attention do not achieve full functional recovery [11]. Research conducted over the past decade supports the potential of the α7 nAChR as a therapeutic target for cognitive deficits and negative symptoms of schizophrenia [12]. The α7 nicotinic acetylcholine receptor (nAChR α7) has been genetically linked with schizophrenia, and its expression is reduced in the hippocampi of schizophrenic patients. Agents targeting nAChR α7-mediated signaling have shown broad efficacy in animal cognition models.

This chapter will start with a review of our understanding of the neuropathophysiology of nicotine in schizophrenia and will then walk the reader through current new therapeutics being developed based on the pharmacology of nicotinic receptors.

## 2 Epidemiology of Nicotine Use in Schizophrenia

Each year more than 440,000 people in the United States die of illness related to smoking, and close to half a billion dollars in health-related economic losses is directly attributable to smoking [13, 14]. Over the past three decades, cigarette smoking has been the single largest source of preventable morbidity and mortality in the United States, and the global mortality rate of smoking (5 million/year) is increasing [15]. On average, smokers die 10 years earlier than those who have never smoked, and smoking cessation unmistakably reduces morbidity and mortality. Although cigarette smoking from early adult life triples age-specific mortality rates at middle age (43% vs. 15%), ceasing smoking at age 50 cuts the risk in half, and cessation at age 40 virtually eliminates the elevated risk [16]. Almost 45% of all cigarettes sold in the United States are sold to people with mental illness: those with major mental illness are more likely than those without psychiatric illness to be heavy smokers [17].

Mentally ill patients with schizophrenia smoke more than those with a diagnosis other than schizophrenia (85% vs. 67%, respectively) [18]. Indeed, smoking is very common among individuals with schizophrenia [19]. Between 72% and 90% of

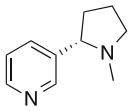
schizophrenic patients smoke cigarettes compared with 24% of the general population [20]. Patients with schizophrenia smoke many more cigarettes on average per day [21], and these patients have higher serum levels of cotinine (the primary metabolite of nicotine) [22], extract more nicotine per cigarette than smokers without psychiatric illness [23], and often spend one third of their weekly income on cigarettes [24]. Mortality from diseases related to smoking, such as cardiovascular and pulmonary diseases, is two-fold to six-fold higher in patients with schizophrenia than in age-matched nonpsychiatric controls [25].

Many factors contribute to the increased smoking rate in those diagnosed with schizophrenia. Perhaps the simplest explanation might be the use of smoking as a "behavioral filler" to alleviate boredom during chronic hospitalization. While this explanation may account for the maintenance of smoking behavior to some extent, institutionalization may not be as strong a primary contributing factor to the high prevalence rates of smoking as previously thought. High rates of smoking are not limited to inpatients or the chronically ill schizophrenic; elevated rates of smoking are found consistently among both inpatients and outpatients with schizophrenia. Other factors thought to cause the increase in smoking among schizophrenics include the positive effects of nicotine on cognition and neurotransmitter systems involved in schizophrenia, nicotine's mitigation of the side effects of antipsychotic and neuroleptic medicines, increased nicotine withdrawal symptoms in patients with schizophrenia, and social factors such as lower income and educational attainment [26–29]. Finally, symptoms of schizophrenia include auditory hallucinations, paranoia, delusions, and disorganized thinking, and these symptoms are understood to be caused by the inability of the brains of schizophrenics to focus on, sort, and differentiate the array of stimuli that exist around us [30]. Thus, the effects of cigarette smoking and nicotine are understood to help schizophrenics address some of their symptoms through the known selective attentional effects of nicotine [31].

## 3 Clinical Studies of Nicotine and NRTs

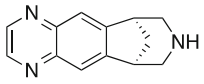
Nicotine (**1**, Fig. 1) and varenicline (**2**, Fig. 2) remain the only marketed therapeutics with a mechanism which primarily invokes nicotinic acetylcholine receptor (nAChR) pharmacology. Nicotine has been used in the marketplace predominantly as nicotine replacement therapy (NRT) for the treatment of nicotine addiction. Prior to the introduction of varenicline to the market, NRTs were also established to have efficacy in helping schizophrenic smokers stay tobacco-free. Extrapolating beyond smoking cessation, nicotine replacement pharmacotherapy approaches have recently been clinically pursued for the treatment of many of the symptoms of schizophrenia that receive benefit from cigarette smoking. For example, NRT may reduce agitation in schizophrenia, and a recent study confirmed that smokers admitted to a psychiatric emergency service with a diagnosis of schizophrenia showed significantly lower agitation scores at 4 and 24 h when randomized to nicotine replacement patches compared with placebo [32]. Several studies have

**Fig. 1** Structure of nicotine



**1** nicotine

**Fig. 2** Structure of varenicline (Chantix®, Champix®)



**2** varenicline

also shown broader benefits of NRT in patients with schizophrenia. NRT gums have been shown to improve performance on attentional tasks [33]. A high-dose version of the nicotine patch has shown to improve impulse response on attentional tasks with respect to control in nonsmokers with schizophrenia [34], and it has also shown to normalize working memory performance in schizophrenics [35].

Given the very common comorbidity of nicotine dependence and schizophrenia, and the great difficulty associated with ceasing smoking behavior and maintaining avoidance of nicotine-seeking behavior in schizophrenics, the potential for the utility of novel NRT delivery systems like e-cigarettes to provide symptomatic relief for schizophrenic patients appears attractive. The appeal for the e-cigarette stems in part from drawbacks with currently approved smoking cessation medications. Bupropion has side effects which may exacerbate psychosis and preclude its use by those susceptible to seizures and those who take monoamine oxidase inhibitors. And varenicline has been associated with aggressive behavior and suicidal ideation. As a result, recent studies have evaluated the use of e-cigarettes to replace cigarette consumption and address the adverse symptoms of schizophrenia and have shown that the use of e-cigarette substantially decreased cigarette consumption without causing significant side effects in chronic schizophrenic patients who smoke and do not intend to quit [36]. This was accomplished without serious adverse impact on the symptoms of schizophrenia as assessed by SAPS and SANS symptoms scales.

## 4 α4β2 nAChR Pharmacotherapy for Schizophrenia

### *4.1 α4β2 nAChRs: Function and Regulation in Schizophrenia*

The nicotinic acetylcholine receptor system has been strongly implicated in the pathophysiology of schizophrenia. The expression and function of high-affinity α4β2 and low-affinity (relative to nicotine) α7 nAChRs are known to be downregulated in the hippocampus and other regions of the brains of schizophrenic

patients independent of whether they smoke or not [37]. Some rationale for reduced expression of α7 receptors in schizophrenia can be taken from polymorphisms in the promoter regions of the α7 nAChR gene that are known to result in reduced transcription of those receptors [38].

Also, it is known that repeated nicotine administration to animals increases the expression of high-affinity nAChRs in the brain [39, 40]. Given the heavy use of tobacco in the schizophrenic population, and that nicotine is a major component of tobacco smoke, it has long been believed that the use of cigarettes by schizophrenics is an attempt to correct that cholinergic neurotransmitter deficit at nicotinic receptors. Because an upregulation of α4β2 receptors has been observed due to nicotine intake, the positive effects of nicotine known in patients with schizophrenia may be partly due to a compensation for a decrease in α4β2 nicotinic acetylcholine receptors. This correction has been described as "self-medication" with cigarettes, because the nicotine in tobacco (as a result of the restoration of nicotinic receptor density that accompanies smoking) helps address negative symptoms and, even more so, cognitive deficits as well as neurophysiological abnormalities associated with schizophrenia [41].

High-affinity nicotinic receptors in the brain are genetically controlled mainly by CHRNA4 and CHRNB2 genes coding for the α4 and β2 subunits, respectively [42]. In addition to the decrease in receptor density in high-affinity nAChRs in schizophrenia, it has also been proposed that the nACh α4 receptor subunit function is specifically lost in schizophrenics, which may in turn account for the differential pattern of gene expression and brain activation found in those patients [43].

In addition to the aforementioned decrease of high- and low-affinity nAChR subtypes in the hippocampus and other brain regions, a postmortem comparison of brains from schizophrenic versus nonpsychiatric patients reveals a GABAergic neuropathology. Accumulating evidence suggests a critical role for altered DNA methylation processes on GABAergic neurons in the pathogenesis of schizophrenia and related psychiatric disorders. The overexpression of DNA methyltransferase 1 (DNMT1) in telencephalic GABAergic neurons has been shown to be responsible for the epigenetic hypermethylation of specific GABAergic gene promoters, including $GAD_{67}$ [44, 45]. Nicotine and varenicline have been reported to decrease cortical DNMT1 mRNA by 30–40% and increase expression of $GAD_{67}$ mRNA and protein in hippocampal (but not striatal) GABAergic neurons [46]. Given the history of tobacco (nicotine) use among schizophrenics and the abovementioned effects of nicotine and varenicline, the use of α4β2 nicotinic receptor agonists was posited to have therapeutic effects in schizophrenics (the vast majority of whom are smokers) as a pharmacological strategy to reduce the hypermethylation of GABAergic promoter [47]. These effects of nicotine are blocked by mecamylamine but not by hexamethonium. The data above support the notion that agonists active at central α4β2 nAChRs may represent an effective pharmacological mechanism to correct the epigenetic GABAergic neuropathology present in schizophrenic patients.

## 4.2 α4β2 nAChR Agonists and Partial Agonists for Schizophrenia

The use of nicotine for the treatment of cognitive deficits associated with schizophrenia is limited by its aversive effects manifested at high doses and tachyphylaxis upon prolonged treatment. Therefore, given the role of nicotine in the epidemiology of schizophrenia, there has been and remains ample opportunity to develop nAChR therapies that retain the benefits of nicotine in that population while providing improved toxicity and tolerability profiles. The first of a new generation of neuronal nicotinic acetylcholine receptor FDA-approved therapeutics emerged from Pfizer in 2006, and it was called varenicline (**2**, Champix®, Chantix®, Fig. 2).

Varenicline, the only approved smoking cessation treatment (aside from nicotine) with a primarily nAChR mechanism, can be effective in the general smoking population, but it was unclear until recently whether or not it would worsen symptoms in smoking schizophrenic patients who attempt to quit smoking. A review published last year evaluating the question suggested that varenicline treatment is not associated with worsening of psychiatric symptoms for most patients with schizophrenia or schizoaffective disorders who are well monitored [48]. Further, varenicline has been found to be a safe and effective therapy for smoking cessation in individuals with schizophrenia/schizoaffective disorder [49]. The study compared smokers (all with diagnosed but clinically stable schizophrenia/schizoaffective disorder) who were randomized into either a placebo or varenicline-treated group and found that, at the end of the 12-week treatment period, 19% of participants receiving varenicline had a significantly higher abstinence rate from smoking compared to 4.7% in the placebo-controlled group.

The effectiveness in smoking cessation and the reasonable tolerability of varenicline [50–52], combined with its α7 full agonist properties, have led to the question of whether the drug may have a dual utility for treating nicotine dependence in schizophrenic patients and acting as a cognitive rectifier in that population. It has been shown that varenicline reverses nicotine withdrawal-induced cognitive deficits in an animal model, suggesting that varenicline may be effective in treating nicotine withdrawal-associated deficits in learning and memory [53]. A subsequent study in humans provided clinical evidence that varenicline can improve working memory-related brain function following nicotine abstinence (particularly at high levels of task difficulty) with associated enhancements in cognitive performance among highly dependent smokers [54]. In a randomized, double-blind, placebo-controlled 8-week trial with 120 outpatients with chronic schizophrenia (60 smokers and 60 nonsmokers), one recent study found beneficial effects of adjunctive varenicline treatment with antipsychotics for some cognitive impairments in subjects with schizophrenia [55]. Another recently published double-blind trial found that varenicline significantly reduced P50 sensory gating deficits in nonsmokers and reduced startle reactivity and improved executive function regardless of smoking status [56]. Thus, varenicline may bring a therapeutic benefit on core schizophrenia-related symptoms while addressing this population's high rate of nicotine dependence.

The majority of nAChR agonists and partial agonists acting at the orthosteric binding site (where nicotine interacts with its receptor) which are being clinically studied are those acting at the α7 nAChRs, and they will be reviewed in a later section in this chapter. However, given nicotine and varenicline's lead in the marketplace, there have been a few other α4β2 ligands with potential for advancement in the treatment of schizophrenia and other cognitive deficits that deserve a quick review.

Compounds activating α4β2 nAChRs have shown beneficial effects on attention, in line with the observation that these receptors are reported to play a role in attention and memory. Altinicline (**3**, SIB-1765F, SIB-1508Y, Fig. 3), an α4β2 nAChR-selective agonist [57], was shown to significantly improve attentional performance in rats [58]. Sazetidine-A (**4**), a selective α4β2 nAChR desensitizing agent and partial agonist, originally developed for the treatment of nicotine addiction [59], was found (when administered acutely) to significantly improve attention by reversing impairments caused by the muscarinic cholinergic antagonist scopolamine and the NMDA glutamate antagonist dizocilpine [60]. Administration of the α4β2 nAChR agonist ABT-418 (**5**) resulted in enhanced attention in the 5-CSRTT behavioral model in unperturbed rats [61]. In septal-lesioned rats, ABT-089 administered acutely was associated with marginal improvement in performance on a water maze test of spatial discrimination [62]. However, there was greater improvement, with 45% error reduction on the last training day, when ABT-089 (**6**) was given continuously by subcutaneous osmotic pumps at a minimum effective dose of 1.3 μmol/kg/day [63]. Short-term administration of ABT-089 in monkeys modestly improved a delayed memory task in young adult monkeys, with greater improvements in aged monkeys. Similarly, improvements in accuracy in a delayed recall task in both aged and younger adult monkeys after administration of ABT-418 have been reported [64]. Two small PoC clinical studies have demonstrated improvement in attention after administration of the α4β2-selective agonists ABT-089 and ABT-418 in adults with attention deficit disorder [65, 66]. This was followed up by a more robust randomized, double-blind, placebo-controlled, crossover study of ABT-418 with 236 patients, where the compound demonstrated significant improvement on the primary outcome compared with placebo, as well as in several secondary outcome measures [67]. AZD1446 (**7**, TC-6683) is a highly selective agonist of central α4β2 and α2β2 neuronal nicotinic receptors [68]. The compound, which is in phase II, has been shown to improve cognition in multiple animal models and has demonstrated potential for treatment of cognitive disorders, including Alzheimer's disease (AD) and schizophrenia. Consistent with the above clinical results for ABT-089 and ABT-418, repeated administration of the α4β2-selective agonist AZD3480 (**8**, TC-1734) both preclinically [69] and clinically improved attention in healthy adults compared with placebo [70]. In line with the aggregate preclinical and clinical evidence, it has further been proposed that selective α4β2 receptor agonists should have superior pro-attentional efficacy compared with nicotine [71].

Thus, based on the above body of evidence, it is clear that agonists acting at α4β2 nAChRs have potential to alleviate attentional and working memory deficits seen in schizophrenia. In what appears to be the only example of an α4β2-selective nAChR agonist (not nicotine or varenicline) investigated in schizophrenic subjects, AZD3480 was assessed for effects on cognitive function in 440 patients with

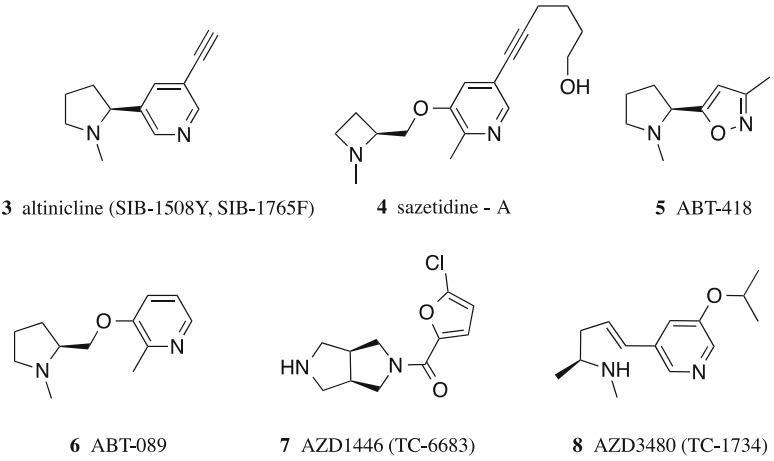



**Fig. 3** Structures of α4β2-selective nAChR agonists and partial agonists

schizophrenia who were taking a single atypical antipsychotic medication and who were active smokers. Disappointingly, while it was well tolerated in the population studied, it displayed little improvement relative to placebo in the primary endpoint, which was a change in cognitive function from baseline to week 12 as measured by IntegNeuro computerized test battery of cognitive function scores [72].

Several clinical studies are in progress which evaluate the pro-cognitive effects of α4β2 nAChR agonists in indications other than schizophrenia. However, with the exception of the AZD3480 study mentioned above, relatively few other studies have evaluated compounds acting solely on the α4β2 nAChR subtype in animal models of schizophrenia or in schizophrenic patients. The above body of evidence suggests that additional studies are warranted which extend the evaluation of α4β2 nAChR-selective compounds in animal models of schizophrenia and patients with schizophrenia. Such compounds additionally need to be evaluated in smokers with schizophrenia. Therapeutics for schizophrenia targeting the α4β2 nAChR receptor, however, have the added challenge of having to displace nicotine, present in smoking schizophrenics, from the α4β2 nAChR for which nicotine itself has a high affinity.

## *4.3 α4β2 nAChR-Positive Allosteric Modulators for Schizophrenia*

As an alternative to activation of a receptor with an exogenous orthosteric agonist, positive allosteric modulators (PAMs) enhance receptor function elicited by the endogenous ligand without directly activating or desensitizing the target receptor [73].

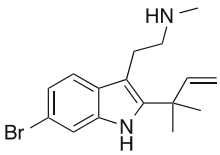
Because PAMs typically enhance acetylcholine agonist responses without activating receptors, synaptic currents remain linked to endogenous neurotransmitter release. As such, PAMs represent an attractive alternative pharmacological treatment approach.

An α4β2 nAChR-selective PAM, which activates those receptors only in the presence of ACh, might find application in the treatment of various diseases of cognitive impairment such as Alzheimer's disease or cognitive dysfunction in schizophrenia (CDS). However, while several chemical classes of allosteric modulators that are selective for α7 nicotinic receptors have been characterized (discussed in the following section), selective potentiators for the most widely expressed α4β2 nicotinic receptor subtype are fewer in number and less advanced, likely due to the difficulty in discriminating α4β2 from other heteromeric nAChR subtypes to produce selective potentiation of responses. We will limit our discussion in this section below to potentiators which are claimed to be selective for α4β2 nAChRs.

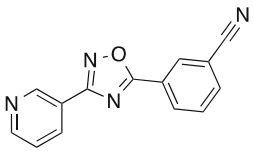
Desformylflustrabromine (**9**, dFBr, Fig. 4) is a tryptamine derivative which was first isolated as an active metabolite of the marine bryozoan *Flustra foliacea*, commonly found in the North Sea [74, 75]. It was identified as selective potentiator of α4β2 receptors (vs. α7 and α3β4) by using two-electrode voltage clamp whole-cell recordings [76]. In addition, dFBr was reported to activate desensitized receptors [77]. Several deconstructed analogs of dFBr have been synthesized and similarly possess a potentiating effect on α4β2 receptors [78]. NS9283 (**10**) has also been described as a PAM acting at α4β2 receptors [79]. NS9283 was further shown, via electrophysiological techniques, to increase agonist-evoked response amplitude particularly of low-sensitivity $(\alpha4)_3(\beta2)_2$ nACh receptors without similar effects at high-sensitivity $(\alpha4)_2(\beta2)_3$ or α3-containing nAChRs [80].

PAMs acting at α4β2 receptors that have demonstrated in vivo outcomes in cognitive enhancement or negative/positive symptoms of schizophrenia are limited to NS9283. NS9283 has demonstrated cognition improvement in models of AD and ADHD. In rodent models of short-term episodic memory (social recognition model) and long-term special memory (Morris water maze), as well as in sustained attention model (5-choice serial reaction time), NS9283 has demonstrated cognition enhancement superior to nicotine. Particularly relevant to schizophrenia, NS9283 had desirable effects on sensory information processing, as shown by reversal of PCP-disrupted prepulse inhibition [81]. The P300 (P3) event-related potential (ERP) is a neurophysiological signal believed to reflect cognitive processing of salient cues and is thus used as a measure of attention and working memory. Pharmacological modulation of the rat P3-like ERP in cortical and subcortical regions by NS9283 has been demonstrated [82]. Data from the phase II clinical trials of ABT-894 suggested that higher doses might have increased efficacy in ADHD. Alternatively combining ABT-894 and an α4β2 PAM (NS9283, in this case) allowed tonic stimulation as well as upregulation of endogenous signaling with limited side effects liability [83]. NS9283 appears to be the only α4β2 nAChR-selective PAM disclosed with in vivo data supporting its potential in schizophrenia. PAMs selective for α4β2 have yet to be clinically tested.

**Fig. 4** Structures of α4β2 positive allosteric modulators




**9** desformylflustrabromine (dFBr)




**10** NS9283

# 5 α7 nAChR Pharmacotherapy for Schizophrenia

## *5.1 α7 nAChRs: Function and Regulation in Schizophrenia*

The α7 nAChR subtype is a well-characterized member of the ligand-gated ion channel superfamily [84]. In neurons, α7 protein subunits assemble as a pentameric complex. The human α7 subunit is approximately 50 kD in size and composed of 502 amino acids, including a 22 amino acid N-terminal signal peptide. The molecule can be subdivided into an N-terminal domain and a transmembrane domain. The extracellular N-terminal domain (approximately 200 amino acids) forms ten β strands and is involved in forming the ligand-binding domain. The transmembrane domain is composed of four alpha helices that cross the lipid bilayer and form the ion channel. The subunits interact at multiple sites. In the extracellular domain, the positive face of one subunit interacts with the negative face of an adjacent subunit to form the ligand-binding pocket [85]. The binding of ligands at the junctions between subunits initiates conformational changes of the receptor that lead to opening of an ion-conducting channel across the plasma membrane. Although nAChRs were originally described as sodium channels, the α7 homopentameric complex is also highly permeable to calcium [86]. The α7 receptor complex is known to desensitize and become inactive (<100 ms) after interaction with two molecules of agonists [87]. Although rapid desensitization has been a challenge for studying the α7 nAChR involvement in CNS function, it is a sophisticated system that has evolved to control cholinergic signaling, shape synaptic plasticity, and possibly prevent excitotoxic cell death mediated through calcium signaling [88]. However, the kinetics of activation/desensitization of α7 pentameric receptor complex in vivo and its consequence in the modulation of sensory gating and numerous other α7-mediated processes remain to be elucidated. Therefore, identification of molecules with optimal kinetics of the receptor interaction (activation, recovery rates from inactivation) might be crucial for the selection of clinical trial candidate.

The mRNA encoding the α7 subunit is found throughout the brain, with particularly high levels in the hippocampus and cortex and very low expression in the thalamus. Approximately 50% of the cells in the ventral tegmental area and substantia nigra also express α7 mRNA [89]. The α7 nAChR modulates key neurotransmitters, including glutamate, γ-aminobutyric acid (GABA), and dopamine (DA). The α7 nAChR is found on midbrain DA cell bodies of the ventral

tegmental area (VTA) and SNc (pars compacta of substantia nigra); on presynaptic DA terminal regions in the striatum, nucleus accumbens, and frontal cortex; on glutamate and GABA neurons that project onto DA somatodendritic regions and DA terminals and serotonin neurons that synapse onto DA cell bodies and terminals; and on thalamic and hippocampal neurons that relay signals from the striatum and frontal cortex [90–92]. These systems contribute to either modulation of DA release or regulation of DA effects throughout the brain. Pharmacological activation of α7 nAChRs increases the firing rates of midbrain VTA DA neurons resulting in elevated DA release in the frontal cortex and improvements in cognitive tasks [93, 94]. It was hypothesized that the firing of DA cells is under hierarchal control by nAChRs so that activation of α4β2 nAChRs brings cells from a resting to an excited state, whereas activation of α7 nAChRs fine-tunes the release of DA once the cells have been turned on [95].

α7 nAChRs are involved in glutamatergic pathways as well. NMDA receptors are expressed on GABAergic neurons of the reticular thalamic nucleus [96] as are α7 nAChRs, and their blockade leads to inhibition of reticular thalamic neurons with a reduction in the release of GABA on thalamocortical cells. Thus, activation of α7 nAChRs may compensate for a lack of NMDA receptor function by increasing GABA release on thalamocortical cells [97]. The α7 nAChR is also extensively expressed on glutamatergic neurons that modulate DA neuronal output [98]. Nicotine-induced DA release is attenuated by blocking NMDA receptors and α7 nAChRs indicating that α7 nAChRs have similar effect as NMDA receptors in modulating DA release in the striatum. The α7 nAChR modulation of glutamate release may also be involved in the precognitive activity of nicotine by influencing glutamate output in the cerebral cortex [99]. A reduction in glutamatergic-induced tonic DA release and accompanying loss of autoreceptor stimulation could drive pathological potentiation of DA cell burst firing in response to phasic DA release [100].

Activation of α7 nAChRs may also increase long-term potentiation in hippocampal cells that are associated with enhanced cognitive function [101]. α7 nAChRs on GABAergic interneurons of the hippocampus modulate inhibitory synaptic transmission and impaired function of these interneurons. In part because of decreased α7 nAChR expression, they may contribute into the manifestation of negative and cognitive symptoms of schizophrenia [102]. Application of selective α7 nAChR agonist to rat hippocampal slices increased activation of GABA inhibitory neurons and corrected amphetamine-induced sensory gating deficits in rats.

α7 nAChRs are involved in various mechanistic pathways associated with the etiology and symptomatology of schizophrenia. Postmortem samples of brain tissue from schizophrenic patients show a significant decrease in the density of α7 nAChRs in hippocampus and cortex [103, 104]. Sensory inhibition deficits and familial schizophrenia have been linked to the 15q13-q14 region on chromosome 15. The gene for α7 nAChR (CHRNA7) is located in this region, and polymorphisms in the promoter region of the gene have been identified [105].

Investigation into the functional consequences of α7 nAChR modulation using animal models has provided additional support for their therapeutic potential in the

treatment of schizophrenia. In DBA/2 mice, a naturally occurring reduction in α7 nAChR is observed along with deficits in sensorimotor gating, which has made this an appropriate schizophrenia-like model for studying the effects of potential α7 nAChR therapies [106]. α7 nAChR agonists normalize the sensory gating deficits in DBA/2 mice, an effect that is blocked with the α7 nAChR antagonist, α-BTX, but not with the nonselective nAChR antagonist mecamylamine [107].

Most schizophrenic patients exhibit cognitive impairment, including attention disorders and deficits in executive function [108]. Cognitive dysfunction in schizophrenia (CDS) affects multiple domains (e.g., attention, problem solving, learning/memory), is a major predictor of functional outcome, and continues to be a large unmet medical need [109]. In the early part of the 2000s, the National Institute of Mental Health initiative, Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS), was designed as a multi-sector collaboration between the government, industry and academia to enable the discovery and development of new treatments for CDS. In particular, the goals of the MATRICS initiative were to establish (1) how to measure cognition in schizophrenia, (2) promising pharmacological approaches, (3) clinical trial design for putative treatments, and (4) regulatory approval for new drugs. From the MATRICS initiative, three primary drug mechanisms of interest were identified: cholinergic, dopaminergic, and glutamatergic. Recent consensus meetings (the MATRICS/TURNS initiative) have identified cognitive dysfunction in schizophrenia as a core feature of the illness that contributes significantly to the lack of functionality of patients [110]. In the absence of a therapy to treat cognitive deficits in schizophrenia, the MATRICS initiative endorsed the α7 nAChR as a primary therapeutic target relevant to CDS.

The first α7 nAChR agonists were discovered among extensively explored 5-$HT_3$ receptor (5-$HT_3$R) ligands [111]. Previously reported potent α7 nAChR agonists [112] lacked selectivity versus 5-$HT_3$R [113, 114], and antagonist activity at 5-$HT_3$R often translated into agonism at α7 nAChR. The crossover in affinity might be explained by common pharmacophoric elements for both 5-$HT_3$R and α7 nAChR: a basic amine (protonated at physiological pH) provides for a cation-π interaction; a hydrogen bond acceptor, e.g., a carbonyl group, forms a hydrogen bond; and aromatic moieties participate in π–π interactions [115, 116]. The 5-$HT_3$R and nAChRs are both part of the Cys-loop superfamily of ligand-gated ion channels. Further, there is significant sequence homology between 5-$HT_3$R and α7 nAChR in the ligand recognition domain [117]. In view of reported side effects, i.e., constipation, asymptomatic electrocardiogram changes, and arrhythmias, associated with 5-$HT_3$R antagonists [118–121], efforts of research groups were focused on the design of ligands specifically interacting with only the α7 nAChR receptor to maximize the therapeutic effect and minimize the adverse effects. Over the past ten years, drug discovery efforts significantly expanded the quantity and quality of selective α7 nAChR ligands. Those have been summarized in several reviews [122–124]. Below are highlighted the most advanced and characterized α7 nAChR orthosteric and allosteric α7 nAChR modulators.

## 5.2 α7 nAChR Agonists for Schizophrenia

The conformationally restricted carbamate AR-R17779 (**11**) [125, 126] (Fig. 5) was the first agonist with selective affinity for α7 nAChRs described in the literature: α7 $K_i = 92$ nM ([125I]α-bungarotoxin binding assay), $EC_{50} = 21$ μM, and $E_{max} = 96\%$ at the rat α7 nAChR subtype in oocytes; $K_i = 420$ nM ([$^3$H]MLA radioligand binding assay), $EC_{50} = 420$ nM, and $E_{max} = 93\%$ in human recombinant nAChR subunit combinations expressed in GH4 cells [127]. Despite a less than desirable pharmacokinetic profile and insufficient efficacy, AR-R17779 served as an invaluable tool for the in vivo assessment of α7 nAChR function, validating a link between its activation and a variety of biological effects. AR-R17779 has been shown to enhance learning and memory function in rats in a radial arm maze model [128] and social recognition test [129] but has failed to improve performance in a model of attention [58] and had no effect on auditory gating [130] using a prepulse inhibition of the acoustic startle response (relevant to sensory deficits in schizophrenic patients [131].

A derivative of AR-R17779 called W-56203 (**12**) enhanced cognition in auditory sensory gating and reversed the scopolamine-induced impairment of cognitive performance in an eight-arm radial maze task in rat models [132]. Given its moderate pharmacokinetic profile, W-56203 was reported as a promising drug candidate for the treatment of cognitive impairment associated with neurological disorders. Since this compound significantly increased levels of extracellular dopamine in the medial prefrontal cortex, it was proposed that dopaminergic hypofunction in the prefrontal cortex is responsible for functional abnormalities in schizophrenia.

AZD0328 (**13**) was designed to circumvent the poor pharmacokinetic profile of AR-R17779 by replacement of the hydrogen bond acceptor carbonyl moiety with a pyridine nitrogen [133]. 3-Pyridyloxyquinuclidines are known as $5HT_3R$ antagonists [134], and possessing a rigid quinuclidinyl pyridyl ether structure, AZD0328 maintained $5HT_3R$ antagonism. AZD0328 improved novel object recognition in mice over an incredibly broad range of doses (0.00178–1.78 mg/kg) and working memory (spatial delayed response performance) in rhesus monkeys [135]. The behavioral effects were blocked by pharmacological antagonism of α7 nAChR function or genetic deletion of the gene encoding the α7 subunit, demonstrating that these actions of AZD0328 are most likely mediated through α7 nAChRs. Interaction with α7 nAChRs by AZD0328 selectively elevates midbrain dopaminergic neuronal activity, causing an enhancement of cortical dopamine levels; these neurochemical changes might underlie the positive behavioral responses observed in the animal models [136].

Quinuclidine amides are well-explored ligands for nAChR and $5HT_3$ receptors. The α7 nAChR, unlike the $5HT_3R$, appears to stereospecifically prefer (R)-3-aminoquinuclidine aromatic amides for binding. PNU-282987 (**14**) was identified as a potent selective agonist for α7 nAChRs with low affinity to $5HT_3R$ and demonstrated in vivo efficacy in animal models associated with cognitive dysfunction in schizophrenia [137]. However, the PNU-282987 was not further developed

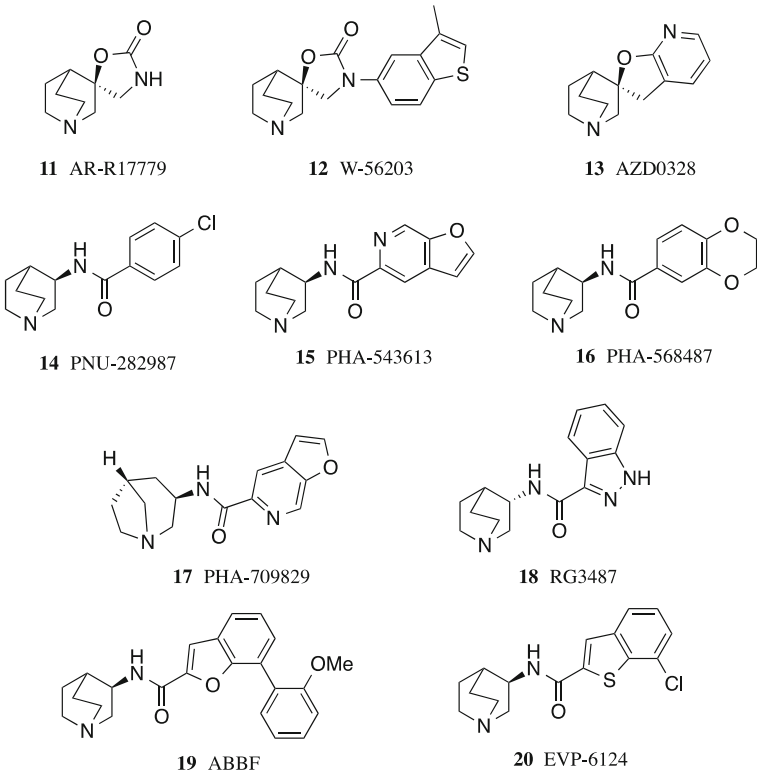



**Fig. 5** Structures of α7 nAChR agonists – part 1

because of significant hERG potassium channel inhibition (11% and 57% at 2 and 20 μM, respectively). Fused heterocyclic analogs preserve α7 nAChR functional potency, providing prospects for improvement of the safety profile. Furopyridine PHA-543613 (**15**) [138] demonstrated efficacy in the reversal of an amphetamine-induced P50 gating deficit and improved performance in a novel object recognition test. The benzodioxane PHA-568487 (**16**) [139] was highly efficacious at 1 mg/kg in the reversal of an amphetamine-induced P50 gating deficit. Both phase I clinical candidates PHA-543613 and PHA-568487 were discontinued because of cardiovascular findings of non-sustained ventricular tachycardia. Modification of PHA-543613 by replacement of (3R)-1-azabicyclo[2.2.2]-octane with (3R,5R)-1-azabicyclo [3.2.1]octane resulted in PHA-709829 [140]. PHA-709829 (**17**) is two-fold more potent than PHA-543613 in an amphetamine-induced gating model (MEDs are 0.1 and 0.24 mg/kg, respectively), and it is efficacious over a range of doses (0.1–1.0 mg/kg). In the cardiovascular safety study on dogs, the "no observable adverse effect level" for PHA-709829 was established at a dose of 40 mg/kg ($C_{max} = 22$ μM), which is five-fold higher than for PHA-543613 ($C_{max} = 3.8$ μM). Taken together, the efficacy and safety data represent a ten-fold improvement in

cardiovascular therapeutic index for PHA-709829 compared to PHA-543613. Additionally, it was shown that PHA-709829 remains efficacious after chronic administration.

RG3487 (**18**) [141] (acquired by Roche from Memory Pharmaceuticals and formerly known as MEM3454) displayed high affinity at rat α7 nAChRs with a $K_i$ of 10 nM, as demonstrated by binding to [$^3$H]MLA-labeled sites in rat brain membrane preparation. Assessment of affinity at 5-HT$_3$Rs revealed a slight binding preference for this target, with a $K_i$ of 2 nM. The intrinsic activity profile of RG3487 ($EC_{50} = 0.4$ μM, $E_{max} = 67\%$) was determined by whole-cell patch-clamp recordings using a cell line stably expressing the monkey wild-type α7 nAChR. RG3487 displayed minimal affinities/efficacies at other nicotinic receptors (α4β2, α3β, and α1β1δγ) and showed selectivity against other neurotransmitter and hormone receptor sites in CEREP profiling. Studies using in vitro rat hippocampal CA1 slices demonstrated that RG3487 (1–10 μM) enhanced 40 Hz long-term potentiation, which was significantly blocked by MLA, thereby demonstrating nicotinic α7 receptor specificity [142]. RG3487 binds potently to the human α7 nAChR ($K_i = 6$ nM), in which it acts as a partial agonist (63–69% of acetylcholine) as assessed by whole-cell patch-clamp recordings in both oocytes and QM7 cell lines, respectively [143]. RG3487 also activates human α7 nAChRs with $EC_{50}$ of 0.8 μM (oocytes) and 7.7 μM (QM7 cells). RG3487 additionally exhibits antagonist properties at the 5-HT$_3$R, $IC_{50} = 2.8$ nM (oocytes) and 32.7 nM (N1E-115 cells). Pro-cognitive effects of RG3487 were demonstrated in behavioral models representing multiple cognitive domains (e.g., episodic, spatial reference, and working memories) [144] with a MED of 1.0 mg/kg p.o. (low-nanomolar range at brain and plasma concentrations) in a novel object recognition test and in models predictive of cognitive and sensory gating restoration in schizophrenia [145] with a MED of 0.03 mg/kg i.p. in a PPI test and phencyclidine-induced impairment of young and aged rats. RG3487 showed a significant dose effect on percent hit accuracy performance in rats without any significant alteration on percent correct rejection performance [146]. In a time-dependent test, RG3487 significantly increased the percent hit accuracy performance when animals were injected 60 min before the start of an attentional task. Administration of galantamine (an acetylcholinesterase inhibitor/α7 nAChR allosteric-positive modulator) failed to significantly increase percent hit accuracy performance, and increasing the dose of galantamine produced a decrease in percent correct rejection performance. In 2007, Memory Pharmaceuticals reported positive results from a phase IIa trial of 20 individuals for the treatment of Alzheimer's disease, but RG3487 did not reverse cognitive impairment in schizophrenics using the MATRICS scale [147].

A compound with the abbreviation ABBF (**19**) was developed by Bayer Healthcare [148] and showed equal affinity to α7 nAChRs in rat brain membranes ($K_i = 62$ nM) and to recombinant human 5-HT$_3$Rs ($K_i = 60$ nM). ABBF was a potent agonist at the recombinant rat and human α7 nAChR expressed in *Xenopus* oocytes ($EC_{50}$ of 3 and 5.5 μM, respectively), but it did not show agonist activity at other nAChR subtypes. ABBF acted as an antagonist of the 5-HT$_3$R and α3β4, α4β2, and muscle nAChRs (at higher concentrations). ABBF can improve performance in several learning and memory tests in both rats and mice without

producing nicotine-like discriminative stimulus effects. This compound also improved social recognition memory in rats (0.3–1 mg/kg p.o.), and this effect was blocked by intracerebroventricular administration of MLA at 10 μg, indicating that it is mediated by α7 nAChR agonism. In addition, ABBF improved working memory of aged rats in a water maze repeated acquisition paradigm (1 mg/kg p.o.) and object recognition memory in mice (0.3–1 mg/kg p.o.).

In November of 2004, EnVivo Pharmaceuticals licensed from Bayer Healthcare the exclusive rights to its α7 agonist program and identified EVP-6124 (**20**) as a clinical candidate [149]. EVP-6124 was evaluated in vitro and in vivo [150]. In binding ($K_i = 9.98$ nM in rat brain homogenates) and functional experiments ($EC_{50} = 0.16$ μM, partial agonist at human α7 nAChRs expressed in *Xenopus* oocytes), EVP-6124 showed selectivity for α7 nAChRs and did not activate or inhibit heteromeric α4β2 nAChRs, although it is a potent $5HT_3R$ antagonist. EVP-6124 had good brain penetration and an adequate exposure time. EVP-6124 (0.3 mg/kg p.o.) significantly restored memory function in scopolamine-treated rats (0.1 mg/kg i.p.) in an object recognition task (ORT). Although donepezil at 0.1 mg/kg p.o. or EVP-6124 at 0.03 mg/kg p.o. did not improve memory in this task, coadministration of these sub-efficacious doses fully restored memory. In a natural forgetting test, an ORT with a 24-h retention time, EVP-6124 improved memory at 0.3 mg/kg p.o. This improvement was blocked by the selective α7 nAChR antagonist methyllycaconitine (0.3 mg/kg i.p. or 10 μg i.c.v.). In co-application experiments of EVP-6124 with acetylcholine, sustained exposure to the EVP compound in functional investigations in oocytes caused desensitization at concentrations greater than 3 nM, while lower concentrations (0.3–1 nM) caused an increase in the acetylcholine-evoked response. These actions were interpreted as representing a co-agonist activity of EVP-6124 with acetylcholine on α7 nAChRs. The concentrations of the EVP compound that resulted in physiological potentiation were consistent with the free drug concentrations in brain that improved memory performance in the ORT. These data suggest that the selective partial agonist EVP-6124 improves memory performance by potentiating the acetylcholine response of α7 nAChRs.

Highly potent and selective α7 nAChR agonist TC-5619 (**21**) [151] (Fig. 6) binds to the α7 nAChR both in rat hippocampal membranes and in a HEK293 cell line co-expressing human α7 and RIC3 cDNAs with a $K_i$ of 1 nM. In a broad receptor selectivity battery (NovaScreen), TC-5619 showed positive interactions in a nonselective opioid receptor assay (58% inhibition) and at the sodium site 2 (79% inhibition). Dose–response assessments of these interactions showed that the $K_i$ values for the opioid site and for the sodium site 2 were both 13 μM, providing a greater than 1,000-fold separation from the binding affinity at the α7 nAChR. Binding of TC-5619 (10 μM) to the $5HT_3R$ displayed 59% inhibition of radioligand binding at the mouse receptor and 25% inhibition at the human receptor. An investigation of functional activation at the human $5HT_3R$ suggested minimal to no activation; a maximal response of 15% was obtained at 100 μM. At human α7 nAChRs transiently expressed in *Xenopus* oocytes, TC-5619 displayed an $EC_{50}$ of 33 nM and an $E_{max}$ of 100% relative to ACh. Compound TC-5619 demonstrated

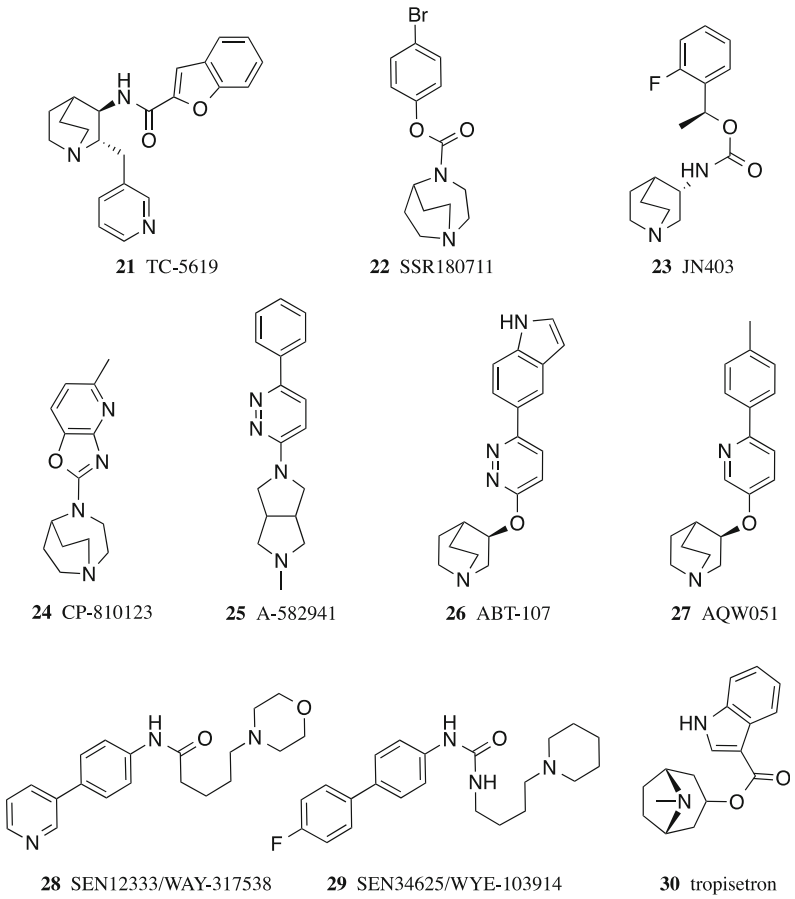



**Fig. 6** Structures of α7 nAChR agonists – part 2

statistically significant enhancement of short-term working memory in the novel object recognition paradigm (MED = 0.3 mg/kg p.o.), and these effects on memory were seen up to 18 h following oral administration, suggesting a positive effect on long-term memory consolidation as well. TC-5619 also reversed apomorphine-induced prepulse inhibition (MED 0.3 mg/kg p.o.) in both mice and rats, which may suggest a benefit against the positive symptoms associated with schizophrenia. The effect of TC-5619 in the social withdrawal model in mice suggests that the compound also has the potential to target negative symptoms of the disease. Taken together, these findings indicate that TC-5619 may have potential to impact all of the primary domains of schizophrenia, positive symptoms, negative symptoms, and cognitive dysfunction. Another unique feature of this compound is that its effects appear to be additive or possibly synergistic with those of antipsychotics, further supporting the therapeutic potential of α7 nAChR-selective compounds not only as monotherapy but also together with existing drugs [152]. The transgenic

*th(tk-)/th(tk-)* mouse model expresses dopaminergic dysfunction similar to that in schizophrenia and reflects many of the developmental, anatomical, and biochemical aspects of the disease. Although clozapine or 0.1 mg/kg of TC-5619 separately had no effect on investigation time, treatment with the TC compound and clozapine together increased the investigation time of a female stimulus mice in control and homozygous transgenic *th(tk-)/th(tk-)*mice; also, treatment with TC-5619 (0.1 mg/kg) and clozapine together increased the investigation time of a male stimulus animal in *th(tk-)/th(tk-)* but not in control mice.

SSR180711 (**22**) [153–156] displayed high affinity for rat and human α7 nAChRs ($K_i$ of 22 and 14 nM, respectively). In functional studies performed with human α7 nAChRs expressed in *Xenopus* oocytes or GH4C1 cells with ACh as normalizing reference, the compound showed partial agonist effects (intrinsic activity 51% and 36%, $EC_{50}$ of 4.4 and 0.9 μM, respectively). In rat-cultured hippocampal neurons, SSR180711 induced large GABA-mediated inhibitory postsynaptic currents and small α-Bgt-sensitive currents through the activation of presynaptic and somatodendritic α7 nAChRs, respectively. In mouse hippocampal slices, the compound increased the amplitude of both glutamatergic (EPSCs) and GABAergic (IPSCs) postsynaptic currents evoked in CA1 pyramidal cells. In rat and mouse hippocampal slices, 0.3 μM of SSR180711 increased long-term potentiation (LTP) in the CA1 field. Null mutation of the α7 nAChR gene totally abolished SSR180711-induced modulation of EPSCs, IPSCs, and LTP in mice. The SSR compound produced a dose- and time-dependent increase in the expression of Arc mRNA in the prefrontal cortex and the ventral orbital cortex. The protein Arc encoded by the effector immediate-early gene arc or arg3.1 has been shown to be strongly implicated in long-term memory function. Thus, α7 nAChR activates a subset of neurons in the rat prefrontal cortex and this activation likely is important for the attentional effects of this new class of drugs [157]. SSR180711 enhanced episodic memory in the object recognition task in rats and mice and reversed MK-801-induced deficits in retention of episodic memory in rats. It reversed selective attention impaired by neonatal phencyclidine (PCP) treatment and restored MK-801- or PCP-induced memory deficits in the Morris or linear maze. In neurochemical and electrophysiological correlates of antipsychotic drug action, SSR180711 increased extracellular levels of dopamine in the prefrontal cortex and enhanced spontaneous firing of retrosplenial cortex neurons in rats. SSR180711was proposed to be beneficial not only for the treatment of cognitive symptoms in schizophrenia but also for the positive symptoms as demonstrated in pharmacological and neurodevelopmental latent inhibition models of schizophrenia; the antidepressant-like properties of the SSR compound are of added interest, considering the high prevalence of depressive symptoms in schizophrenic patients. However, in 2007, Sanofi-Aventis suspended the development of SSR180711 for the treatment of mild Alzheimer's disease because of an insufficient expected benefit/risk ratio (http://clinicaltrials.gov/ct2/show/NCT00602680).

JN403 (**23**) was evaluated by Novartis in a number of in vitro systems of different species, at recombinant receptors using radioligand binding, signal transduction, and electrophysiological studies [158]. When using [$^{125}$I]α-Bgt as a

radioligand, JN403 has high affinity for human recombinant α7 nAChR ($pK_D = 6.7$, $K_D = 200$ nM). Functionally, JN403 is a partial and potent agonist at human α7 nAChR. The compound stimulates calcium influx in GH3cells recombinantly expressing the human nAChR with a $pEC_{50}$ of 7.0 ($EC_{50}$ 100 nM) and an $E_{max}$ of 85% (compared to the full agonist epibatidine). In *Xenopus* oocytes expressing human α7 nAChR, JN403 induces inward currents with a $pEC_{50}$ of 5.7 ($EC_{50} = 2$ μM) and an $E_{max}$ of 55%. In functional ion-flux assays, JN403 shows a 200-fold selectivity over other nAChRs like α3β4 or α4β2 as well as $5HT_3$Rs. Similarly, JN403 showed low binding activity at a wide panel of neurotransmitter receptors. JN403 enhances learning and memory performance in the social recognition test in mice, reverses the auditory gating deficit in anesthetized and awake DBA/2 mice, and is effective in animal models of persistent inflammatory and neuropathic pain [159].

CP-810123 (**24**) was discovered by bioisosteric replacement of quinuclidine aryl carbamate with a benzoxazole ring followed by optimization of the heteroaromatic ring [160]. CP-810123 is a partial α7 nAChR agonist ($K_i = 10.8$ nM, $EC_{50} = 244$ nM, $E_{max} = 46\%$) and $5HT_3$R antagonist ($K_i = 269$ nM, $IC_{50} = 5$ nM). The authors postulated that the risk associated with $5HT_3$R antagonism was minimal, since clinical studies suggest that the $5HT_3$R antagonist ondansetron is well tolerated in patients with schizophrenia [161]. On the basis of evaluation of in vitro potency and selectivity profiles at α7 nAChR, $5HT_3$R, hERG, and genetic toxicity, CP-810123 was selected for evaluation in in vivo efficacy models. It demonstrated efficacy in an amphetamine-induced P50 gating deficit model and improved performance in the novel object recognition test. CP-810123 was introduced by Pfizer as a third-generation α7 nAChR agonist for the treatment of cognitive deficits associated with schizophrenia [162]; to date, there has been no report of its clinical advancement.

An elegant approach that converted an α4β2 nAChR agonist into a selective α7 nAChR agonist was demonstrated by Abbott's scientists in the case of A-582941 (**25**) [163]. While a π-cation interaction has been established as an essential pharmacophore for binding to both α4β2 and α7 nAChRs, the α7 binding site is more lipophilic and presents a less negative electrostatic surface than that for the α4β2 receptor. A-582941 exhibited high-affinity binding and partial agonism at α7 nAChRs. Similar affinities were observed in rat brain membranes ($K_i = 10.8$ nM) and in membranes from human frontal cortex ($K_i = 17$ nM), indicating that cross-species differences appear to be minimal for this α7 ligand. A-582941 also displaced the α7-selective antagonist [$^3$H]methyllycaconitine from rat brain membranes with a $K_i$ of 88 nM. It was screened for activity across a panel of 78 receptor targets including G-protein-coupled receptors, ligand- and voltage-gated ion channels, and neurotransmitter uptake sites. Significant affinity was observed only at the $5HT_3$R ($K_i = 150$ nM), representing approximately 15-fold selectivity for the human α7 nAChR relative to the 5HT3R. Metabolic conversion of A-582941 by liver microsomes or hepatocytes in vitro was highly species dependent, with the rate of turnover fastest in dog, intermediate in rodent and monkey, and slowest in human. A-582941 exhibits acceptable pharmacokinetic behavior in rodents, dog,

and monkey. In mouse and rat, the compound is efficiently absorbed following oral administration, as evidenced by the very high oral bioavailability (100% and 90%, respectively). The much lower bioavailability (22%) in dog is in accord with a much higher rate of metabolism in this species and likely reflects a larger contribution of first-pass metabolism. Consistent with its lipophilic character, A-582941 is characterized by very large volumes of distribution (4–11 L/kg) and correspondingly high clearance values. A-582941 was evaluated across a battery of behavioral assays in rodent and nonhuman primate models that assess cognitive performance: social recognition in rats, delayed matching-to-sample (DMTS) test in primates, inhibitory avoidance (one trial) in mice, sensory gating in rats and DBA2 mice, and five-trial passive avoidance in spontaneously hypertensive rat (SHR) pups. These studies demonstrate that A-582941 exhibits broad-spectrum efficacy across various domains of cognition including working memory, short-term recognition memory, long-term memory consolidation, and pre-attentional sensory gating. With respect to cognitive domains, A-582941 appears to be especially effective in tasks that involve learning and memory (monkey DMTS at long delay, rat social recognition, and mouse inhibitory avoidance). The pro-cognitive effects of A-582941 in these models were manifested at similar plasma exposures, approximately 3–6 ng/mL (10–20 nM) across several models and species. These plasma levels correspond closely to the binding affinity for A-582941 at the α7 nAChR and are approximately 200-fold lower than the $EC_{50}$ for ion channel opening in vitro, indicating that signaling can occur at agonist concentrations well below those required for a macroscopic current in oocytes, measured as the synchronous opening of many channels elicited by rapid application of agonist. It was suggested that low concentrations of agonist can elicit a small but sustained current by stimulating the opening of a small percentage of the α7 nAChR. Individual receptors may be stimulated, desensitized, and recovered with normal dynamics, while the size of the pool of open channels remains relatively stable at any one time. Secondary pharmacodynamic and tolerability profiles of A-582941 were assessed in a battery of assays of cardiovascular, gastrointestinal, and CNS function. In animal experiments, A-582941 was well tolerated at exposures greater than those required for efficacy in cognition models. In rats, single oral dosages of 400 mg/kg (1,400 μmol/kg) induced clinical signs indicating CNS activity, such as tremor and spasms. A dose of 800 mg/kg p.o. was lethal within 10 min. To explore the potential of A-582941 to promote tumor growth, the compound was applied to shaved skin patches on the backs of SENCAR (sensitive to carcinogens) mice every 2 days for a period of 2 weeks. A-582941 produced only a mild irritation of the skin, with no histologic evidence of epidermal hyperplasia or increased proliferation of basal keratinocytes as assessed by immunohistochemical detection of bromodeoxyuridine reagent. Comprehensive detailed characterization of A-582941 in combination with its broad-spectrum efficacy, favorable tolerability, and relatively low potential for toxicity has made it a valuable tool compound for evaluation of the α7 nAChR platform and as a candidate for potential drug development.

Clinical drug candidate ABT-107 (**26**) [164, 165] was introduced to address the potential issues that are likely to be encountered in the clinical development of an α7

nAChR agonist for the treatment of Alzheimer's disease. Those issues may include drug–drug interactions in patients already receiving other AD therapeutics, dosing constraints defined by pharmacokinetic–pharmacodynamic limitations, and adverse events, in particular nicotine-like abuse liability. ABT-107 displayed high-affinity binding to α7 nAChRs [rat or human cortex, [$^3$H](1S,4S)-2,2-dimethyl-5-(6-phenylpyridazin-3-yl)-5-aza-2-azoniabicyclo[2.2.1]heptane (A-585539) $K_i = 0.2$-0.6 nM, or [$^3$H]MLA, 7 nM] that was at least 100-fold selective versus non-α7 nAChRs and other receptors. Functionally, ABT-107 did not evoke detectible currents in *Xenopus* oocytes expressing human or nonhuman α3β4, chimeric (α6/α3)β4, or 5-$HT_3$A receptors and weak or negligible $Ca^{2+}$ responses in human neuroblastoma IMR-32 cells (α3* function) and human α4β2 and α4β4 nAChRs expressed in human embryonic kidney 293 cells. ABT-107 potently evoked human and rat α7 nAChR current responses in oocytes ($EC_{50}$ of 50–90 nM total charge, ~80% normalized to acetylcholine) and enhanced spontaneous inhibitory postsynaptic current activity in dentate gyrus granule cells. Both effects were augmented by the positive allosteric modulator 4-[5-(4-chlorophenyl)-2-methyl-3-propionylpyrrol-1-yl]benzenesulfonamide (A-867744). In rat hippocampus, ABT-107 evoked α7-like currents in rat hippocampus, which were inhibited by the α7 antagonist MLA. ABT-107 was also effective in protecting rat cortical cultures against glutamate-induced toxicity. During in vivo experiments, the ABT compound improved cognition in monkey delayed matching to sample, rat social recognition, and mouse two-trial inhibitory avoidance and continued to improve cognitive performance at times when exposure levels continued to decline. Rats concurrently infused with ABT-107 and donepezil at steady-state levels consistent with clinical exposure showed improved short-term recognition memory. Compared with nicotine, ABT-107 did not produce behavioral sensitization in rats or exhibit psychomotor stimulant activity in mice. Repeated (3 days) daily dosing of ABT-107 increased extracellular cortical acetylcholine in rats, whereas acute administration increased cortical extracellular signal-regulated kinase and cAMP response element-binding protein phosphorylation in mice, neurochemical and biochemical events relevant to cognitive function. ABT-107 increased cortical phosphorylation of the inhibitory residue (Ser9) of glycogen synthase kinase-3, a primary tau kinase associated with Alzheimer's disease pathology. In addition, continuous infusion of the ABT compound in tau/amyloid precursor protein transgenic AD mice reduced spinal tau hyperphosphorylation. ABT-107 exhibited a preclinical efficacy profile that included (1) PD-PK discordance consistent with prolonged biochemical signaling germane to synaptic plasticity and enhanced cognition, (2) maintenance of efficacy with concurrent use of an AChEI, and (3) lack of nicotine-like abuse liability and CNS stimulatory activity. These findings show that targeting α7 nAChRs may have potential utility for the treatment of cognitive disorders. In clinical trials, ABT-107 was well tolerated over the tested single (1–100 mg) and multiple (2–15 mg once daily for 7 days) dose range; however, it exhibited nonlinear pharmacokinetics in humans [166], and its development was discontinued in 2009.

AQW051 (**27**), a compound similar in structure to ABT-107, was advanced into the clinic not long ago for the treatment of cognitive impairment associated with

schizophrenia. Its structure and preclinical data were recently disclosed for the first time at an American Chemical Society conference [167]. AQW051 demonstrates high affinity for nAChR α7 ($Ki = 27 \pm 0.9$ nM in an [125I]α-bungarotoxin binding assay) and acts as a potent agonist at the receptor ($pEC_{50} = 7.41 \pm 0.09$ nM in a calcium flux assay; $E_{max} = 73 \pm 4.1\%$). No stimulation of α4β2, α3β4, and α1β1γδ nicotinic subtypes and the 5-HT3 receptor was seen up to a concentration of 100 μM. The 4-methyl group was credited with removing 5HT3 activity in the compound. AQW051 was found to have good drug disposition in mice, with a free fraction of 13–19%, a half-life ($T_{1/2}$) of ~0.8 h, and rapid permeation into the blood–brain barrier (brain/plasma ratios of 10 to 80 at 0.08 to 7 h in mice). It significantly improves memory function in rodent cognition paradigms, e.g., in the mouse object recognition test (0.03–0.3 mg/kg) and rat Morris water maze (3 mg/kg), and MLA treatment reversed the cognitive improvements. Thus, AQW051 appears to possess the requisite preclinical rationale as a therapeutic agent for cognition indications.

SEN12333/WAY-317538 (**28**) was evaluated in in vivo efficacy models based on its generally good in vitro profile (α7 nAChR $K_i = 260$ nM, $EC_{50} = 1.65$ μM), ease of synthesis, and selectivity on a related panel of nicotinic (α1*,α3*,α4β2) and highly homologous $5HT_3$ARs [168]. When the compound was tested at 10 μM in a panel of ~70 binding sites including all major classes of neurotransmitter, growth factor, and peptide receptors, no significant activity was observed except at the histamine $H_3$ receptor, where binding was observed leading to receptor antagonism. In vivo, SEN12333 improved performance in rodent behavioral assays of cognitive function and perceptual processing, producing enhancement of normal memory performance. The ability to attenuate pharmacologically induced deficits via either the glutamatergic or cholinergic system was demonstrated as well. Treatment with SEN12333 (3 mg/kg i.p.) minimized spontaneous decay of episodic memory in a novel object recognition task in rats; the compound was able to reverse both a scopolamine and MK-801-induced deficit in the pharmacological models for these recognition memory tests [169].

Further improvement of both potency and selectivity of SEN12333 resulted in SEN34625/WYE-103914 (**29**) [170]. This compound demonstrated good $\alpha3^*/\alpha7$ selectivity (7.3) and in vitro potency (α7 nAChR $K_i = 44$ nM, $EC_{50} = 0.49$ μM) although relatively low hERG $IC_{50} = 1.3$ μM.

SEN34625 displayed efficacy in assays of cognitive function and perceptual processing, showing an ability to attenuate pharmacologically induced deficits via the glutamatergic system. It reversed MK-801-induced deficits in both the novel object recognition and prepulse inhibition models with a minimum efficacious dose of 3 mg/kg in spite of the relatively moderate brain to plasma ratio 0.3. Unlike SEN12333, for which the efficacy was observed after i.p. administration, the activity of SEN34625 was observed in both models after oral administration.

Tropisetron (**30**), the $5HT_3$R antagonist ($K_i = 5.3$ nM), which is available in Europe for the treatment of emesis, is a potent and selective partial agonist ($K_i = 6.9$ nM, $EC_{50} = 1.3$ μM, $E_{max} = 36\%$) for the α7 nAChR [171]. Its unremarkable safety profile can be interpreted as demonstrating the safety of both $5HT_3$R antagonist and α7 nAChR partial agonist mechanistic approaches. The

discovery that tropisetron is also a potent partial agonist for α7 nAChRs gives cause for the reexamination of both clinical and preclinical findings with this compound, particularly when it displayed pharmacological effects different from other $5HT_3R$ antagonists. It was reported that the $5HT_3R$ antagonists tropisetron and ondansetron had qualitatively different results in learning and memory paradigms in rats [172]; tropisetron, but not the selective 5-$HT_3R$ antagonist ondansetron, attenuated PCP-induced cognitive deficits in mice, and this effect of tropisetron was blocked by coadministration of the selective α7 nAChR antagonist MLA [173]. By the use of [$^{11}$C]CHIBA-1001 and PET imaging techniques, it was found that tropisetron, but not ondansetron, after a single oral administration (5, 10, or 20 mg), binds to α7 nAChRs in the intact human brain in a dose-dependent manner. All of these results substantiated a clinical trial of tropisetron in patients with cognitive deficits in schizophrenia [174]. The compound (10 mg/day for 8 weeks), but not placebo, significantly improved auditory sensory gating P50 deficits in patients with schizophrenia and had a significant impact on the sustained visual attention in nonsmoking patients. Overall, the clinical study suggests that tropisetron might be a potential therapeutic drug for cognitive deficits in schizophrenia.

Over the past decade and beyond, a considerable number of α7 agonists have been preclinically evaluated within a variety of different companies (Table 1). These numbers in part reflect the influence of the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative and the genetic validation established for the α7 receptor in schizophrenia [175]. Some of these compounds have attendant activity at 5HT3 receptors. Many of these compounds possessed sufficiently strong preclinical rationale that has propelled them into the clinic for evaluation in indications of cognitive impairment, including schizophrenia and Alzheimer's disease. A discussion of the outcomes of the compounds that advanced into human testing is presented in Sect. 6 of this chapter.

## *5.3 α7 nAChR-Positive Allosteric Modulators for Schizophrenia*

There are differences in the pharmacological profiles of positive allosteric modulators of the α7 nAChRs versus orthosteric modulators [176]. Type I PAMs increase the apparent peak amplitude agonist-evoked responses with minor effects on current desensitization/deactivation. For type II PAMs, peak current is dramatically increased and there is a significant prolongation of the current decay, suggesting attenuated desensitization/deactivation. The ramification on safety profiles of chronic treatment with agonists (orthosteric modulators) affecting α7 nAChRs is not yet clear; the benefit of this approach may be suboptimal because of the unknown long-term impact of sustained activation and desensitization of the nAChRs. In this context, allosteric modulation of the α7 nAChR was introduced as a novel therapeutic principle for treating cognitive dysfunction associated with various forms of

**Table 1** Preclinical in vitro comparison of α7 agonists ($5HT_3$ function is provided where affinity is unavailable)

| Compound | # | α7 $K_i$ (nM) | α7 $EC_{50}$ (nM) | α7 $E_{max}$ (%) | $5HT_3$ $K_i$ (nM) | Preclinical efficacy in rodent model |
|---|---|---|---|---|---|---|
| AR-R17779[a] | **11** | 92 | 21,000 | 96 | 2.5% at 10 μM | Radial arm maze |
| | | | | | | Social recognition test |
| W-56203 | **12** | 3 | | | 10 | Auditory sensory gating |
| | | | | | | Radial arm maze |
| AZD0328 | **13** | 3 | 470 | 40 | 12 | Novel object recognition |
| | | | | | | Spatial delayed response performance |
| PNU-282987 | **14** | 24 | 128 | | 1,662 | Reversal of an amphetamine-induced P50 gating deficit |
| | | | | | | Novel object recognition |
| PHA-543613 | **15** | 8.8 | 65 | | 511 | Novel object recognition |
| | | | | | | Reversal of an amphetamine-induced P50 gating deficit |
| PHA-568487 | **16** | 44 | 258 | | 2,800 | Novel object recognition |
| | | | | | | Reversal of an amphetamine-induced P50 gating deficit |
| PHA-709829 | **17** | 3.5 | 46 | | 350 | Reversal of an amphetamine-induced P50 gating deficit |
| RG3487 | **18** | 10 | 400 | 67 | 2 | Novel object recognition |
| | | | | | | Auditory sensory gating |
| ABBF | **19** | 62 | 3000 | | 60 | Social recognition memory |
| | | | | | | Novel object recognition |
| | | | | | | Water maze |
| EVP-6124 | **20** | 4.3 | | | 299 ($IC_{50}$) | Novel object recognition |
| | | | | | | Auditory sensory gating |
| TC-5619 | **21** | 1 | 33 | 100 | 25% at 10 μM | Novel object recognition |
| | | | | | | Apomorphine-induced prepulse inhibition |

| | | | | | | |
|---|---|---|---|---|---|---|
| SSR180711 | **22** | 14 | 900 | 36 | <50% at 10 μM | Novel object recognition<br>Morris water maze |
| JN403 | **23** | 200 ($K_d$) | 100 | 85 | 13,000 ($K_d$) | Social recognition test<br>Auditory sensory gating |
| CP-810123 | **24** | 13.5 | 244 | 46 | 269 | Novel object recognition<br>Reversal of an amphetamine-induced P50 gating deficit |
| A-582941 | **25** | 10.8 | | | 150 | Social recognition test<br>Auditory sensory gating |
| ABT-107 | **26** | 7 | 50 | 80 | 4% at 10 μM | Social recognition test<br>Auditory sensory gating |
| AQW051 | **27** | 27 | 7 | 73 | 0% at 100 μM | Novel object recognition<br>Morris water maze |
| SEN12333/WAY-317538 | **28** | 260 | | | <50% at 10 μM | Novel object recognition |
| SEN34625/WAY-103914 | **29** | 44 | 490 | 70 | <50% at 10 μM | Novel object recognition<br>Prepulse inhibition |
| Tropisetron | **30** | 6.9 | 1,300 | 36 | 5.3 | Auditory sensory gating |

[a]AR-R17779 was found to be inactive in the models of attention and of auditory sensory gating

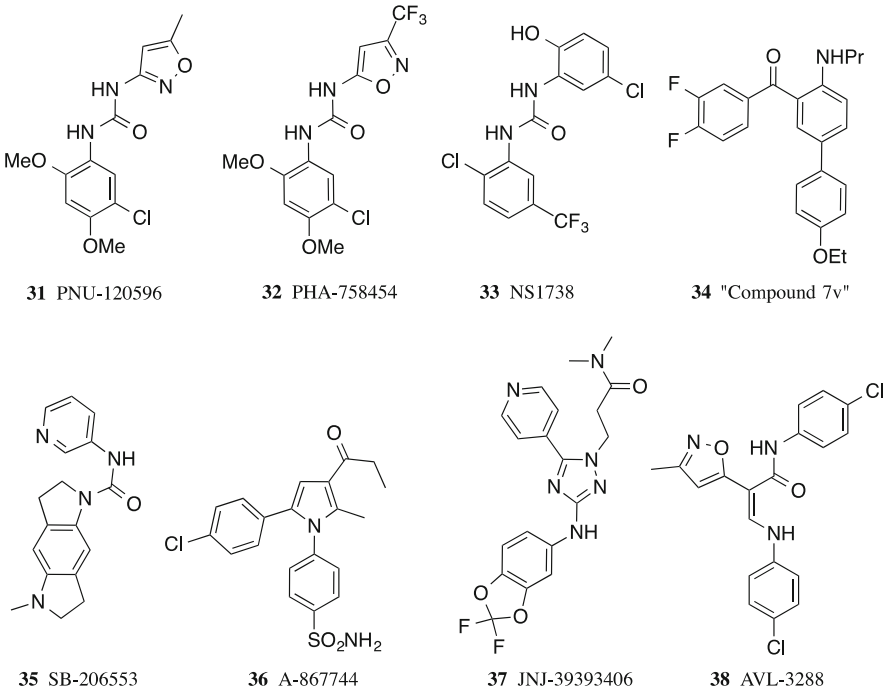



**Fig. 7** Structures of α7-positive allosteric modulators

dementia and schizophrenia without the potential downside of sustained orthosteric agonism of nicotinic receptors. However, according to recent studies [177], repeated administration of α7 nAChR orthosteric agonists leads to significantly increased [$^{125}$I]Bgt binding, not only in the prefrontal regions as seen with acute treatment but also in the parietal cortex and hippocampus. Such upregulation observed with orthosteric agonists of the α7 nAChRs does not occur with PAMs, several of which are being evaluated preclinically for further advancement (Fig. 7).

PNU-120596 (**31**) increased peak agonist-evoked currents mediated by human wild-type receptors expressed in *Xenopus* oocytes and in rat hippocampal neurons [178]. It also demonstrated a pronounced prolongation of the evoked response in the continued presence of agonist. PNU-120596 produced no detectable change in currents mediated by α4β2, α3β4, and α9α10 nAChRs. PNU-120596 increased the channel mean open time of α7 nAChRs but had no effect on ion selectivity and relatively little, if any, effect on unitary conductance. Subsequent application of PNU-120596 on desensitized receptors in the continued presence of nicotine resulted in a reactivation of the agonist-bound receptor. Co-treatment of neurons with both PNU-120596 and the selective α7 nAChR antagonist MLA (10 nM) abolished the ACh-evoked current, indicating that the PNU compound was acting at α7-containing nAChRs. When applied to acute hippocampal slices, PNU-120596

increased the frequency of ACh-evoked GABAergic postsynaptic currents measured in pyramidal neurons; this effect was suppressed by tetrodotoxin, suggesting that PNU-120596 modulated the function of α7 nAChRs located on the somatodendritic membrane of hippocampal interneurons. Systemic administration of the PNU compound to rats improved the auditory gating deficit caused by amphetamine. Consistent with its classification as a type II PAM, PNU-120596 affects desensitization and prolongs excitatory synaptic events that could have excitotoxic consequences. PNU-120596 was reported to be neurotoxic in an in vitro model, although in vivo toxicity studies have not been reported for the compound.

PHA-758454 (**32**) is an optimized analog of PNU-120596 with enhanced potency and improved physical and ADME properties [179]. In the presence of Ach, PHA-758454 enhanced evoked calcium currents in rat hippocampus. In a rat model of impaired sensory gating, PHA-758454 reversed amphetamine-induced disruption of hippocampal auditory gating at 0.1 and 0.3 mg/kg.

NS1738 (**33**) [180], a type I PAM, increased the peak amplitude of ACh-evoked currents at all concentrations with only marginal effects on the desensitization kinetics of α7 nAChRs. Mechanistically, NS1738 modulates the activity of the α7 nAChR by facilitating the energetic coupling between agonist binding and ion channel gating, thereby increasing the receptor open probability at all ACh concentrations. However, this enhanced efficiency of the binding-gating coupling is dissociated from the energetics of the desensitization process, which proceeds with nearly unaltered kinetics. NS1738 (10 and 30 mg/kg) improved performance to the same extent as (−)-nicotine in the social recognition test, a model of short-term memory, and it reversed the cognitive impairment induced by (−)-scopolamine of acquisition of a water maze learning task (at 30 mg/kg), a model of long-term spatial memory.

"Compound **7v**" (**34**) (as it is described in its disclosure) was developed on the hypothesis that the $GABA_A$ and α7 nAChR exhibit sufficient homology to allow the discovery of compounds that simultaneously modulate both receptors [181]. Structure–activity relationship studies resulted in the identification of compound **7v** as a potent and efficacious type I PAM with maximum modulation of a nicotine $EC_{50}$ response of 1,200% and $EC_{50} = 0.18$ μM. Compound **7v** crossed the blood–brain barrier when dosed intraperitoneally in mice and was active in reversing the effect of scopolamine in the novel object recognition paradigm with a minimum effective i.p. dose of 1.0 mg/kg (2.7 μmol/kg). This effect was blocked by the selective α7 nAChR antagonist MLA. The compound is a potent allosteric modulator of α7 nAChRs that may have therapeutic value in restoring impaired sensory gating and cognitive deficits in schizophrenia.

The $5\text{-}HT_{2B/C}$ receptor antagonist SB-206553 (**35**) [182] was identified as a PAM of α7 nAChRs [183] in a randomized screening effort and was further confirmed and characterized using different functional assays of α7 nAChR activation, both in a recombinant cell line and natively expressed receptors in hippocampal slices. SB-206553 produced an eight-fold potentiation of the evoked calcium signal in the presence of an $EC_{20}$ concentration of nicotine and a

corresponding $EC_{50}$ of 1.5 μM for potentiation of $EC_{20}$ nicotine responses in GH4C1 cells expressing the α7 receptor. SB-206553 was devoid of direct α7 receptor agonist activity and selective against other nicotinic receptors. Native nicotinic receptors in CA1 stratum radiatum interneurons of rat hippocampal slices were activated by ACh (200 μM), an effect that was entirely blocked by the α7-selective antagonist MLA. SB-206553 does not share the profound prolongation of desensitization kinetics with PNU-120596 and is closer to a type I PAM. In behavioral assays, SB-206553 reversed MK-801-disrupted prepulse inhibition, indicating that other α7 nAChR PAMs may be active in a model of sensorimotor gating relevant to schizophrenia pathology. Subsequently, it was demonstrated that the reversal of MK-801-disrupted prepulse inhibition by SB-206553 was attenuated by MLA. This supports the notion that the in vivo pharmacological activity of SB-206553 is driven by its α7 PAM profile and not its 5-$HT_{2B/C}$ receptor antagonism profile.

A-867744 (**36**), in oocytes expressing α7 nAChR, potentiated ACh-evoked currents with an $EC_{50}$ of 1 μM [184]. In the presence of A-867744, ACh concentration responses were potentiated by increases in potency, Hill slope, and maximal efficacy. The compound, as type II PAM, not only enhances the ACh-evoked currents but also slows the desensitization profile of agonist responses [185]. When examined in rat hippocampus CA1 stratum radiatum interneurons or dentate gyrus granule cells, A-867744 (10 μM) increased ACh-evoked α7 currents and recovery from inhibition/desensitization and enhanced spontaneous inhibitory postsynaptic current activity. A-867744 did not displace the binding of [$^3$H]MLA to rat cortex α7* nAChRs but displaced the binding of the agonist A-582941 in rat cortex, with a $K_i$ of 23 nM. A-867744 neither increased agonist-evoked responses nor displaced the binding of A-582941 in an α7/5-HT3 chimera, suggesting an interaction distinct from the α7 N-terminus or M2-M3 loop. In a panel of receptor binding assays for >70 diverse neurotransmitter receptor and ion channel sites (CEREP Inc.), no significant interaction at any of the targets (>50% displacement of test ligand) was observed at the screening concentration of 10 μM. Since A-867744 demonstrated acceptable animal pharmacokinetics and safety profiles, it was tested in vivo in the DBA/2 mouse. In a paired auditory stimulus paradigm, A-867744 (0.1–10.0 μmol/kg i.p.) improved sensory gating deficits, reducing the ratio of the response to test stimulus relative to the preceding response to conditioning stimulus (T/C ratio).

ABT-779, a type II PAM whose structure has not yet been disclosed [186], potentiated peak current amplitude and attenuated desensitization responses to ACh at human and rat α7 nAChRs with potencies ($EC_{50}$ values: 80–200 nM) highest among known α7 PAMs. At much higher concentrations (≥3 μM), ABT-779 alone directly evoked MLA-sensitive weakly desensitizing current. In a cell line endogenously expressing human α7 nAChRs (IMR-32), ABT-779 activated $Ca^{2+}$ flux responses in the presence of an exogenously added agonist ($EC_{50} = 80$ nM) but with about a four-fold weaker maximum efficacy than modulators A-867744 and PNU-120596. ABT-779 also directly evoked $Ca^{2+}$ signals at ≥3 μM. No potentiation of agonist-evoked responses mediated by α4β2, α3β4* (IMR-32 cell line), 5-$HT_{3A}$,

and chimeric human α7-5-$HT_{3A}$ receptors was observed with ABT-779; instead weak inhibition of $Ca^{2+}$ flux, membrane potential imaging, and current responses were observed ($IC_{50} > 450$ nM) or no effect (5-$HT_{3A}$). ABT-779 (10 μM) showed a clean profile in the CEREP radioligand binding panel across various receptors, ion channels, and transporters, showing no inhibition greater than 80% across all targets tested. ABT-779 (10 μM) also did not show inhibition of human acetylcholinesterase activity. In rat brain hippocampal slices (CA1 region), current responses evoked by choline were amplified in the presence of ABT-779; these responses were inhibited by MLA. In vivo administration of ABT-779 enhanced ACh release from prefrontal cortex and hippocampus in a dose-dependent manner, with significant effects observed at doses of 0.1 and 1 μmol/kg. These studies demonstrate that ABT-779 can modulate native α7 nAChRs controlling synaptic activity and important for cognitive processes. The structure of ABT-779 has not yet been published.

JNJ-39393406 (**37**) [187] is a positive allosteric modulator at the nicotinic α7 receptor that is now a research tool available in the National Center for Advancing Translational Sciences (NCATS) program at the NIH (http://www.ncats.nih.gov/research/reengineering/rescue-repurpose/therapeutic-uses/directory.html. Accessed 23 Apr 2014). From the NCATS site for compound **37** (http://www.ncats.nih.gov/files/JNJ-39393406.pdf. Accessed 23 Apr 2013): "In vitro, it potentiates a 100 μM choline-induced rise in intracellular $Ca^{2+}$ mediated by human α7 channels expressed in GH4C1 cells, with an EC50 of 660 nM (fluorimetric measurements). The concentration response curves of choline, acetylcholine and nicotine are shifted to the left (10, 10, and 20-fold) and upwards (20, 17, and 17-fold), indicating that JNJ-39393406 increases both the potency and efficacy of these agonists. The compound is selective for the α7 receptor and does not act on α4β2, α3β4 or 5-HT3A channels, and does not interact with a panel of 62 receptors and enzymes. JNJ-39393406 has shown bell-shaped dose–response activity in two out-sourced animal models: the auditory evoked potential (AEP) in DBA2 mice (0.63–5 mg/kg s.c., lower and higher doses were inactive), a model for sensory gating and the attentional set-shifting in rat (1.2 and 5 mg/kg s.c., partial effect at 0.3 mg/kg, not tested at doses higher than 5 mg/kg).

In phase 1 clinical trials of JNJ-39393406, no significant safety signals were observed with good oral bioavailability when dosed orally as a nanosuspension. Exposure in plasma and CSF well exceeded levels applied in preclinical testing. In a multicenter, double-blind, placebo-controlled, randomized, four way cross-over proof-of-mechanism study, JNJ-39393406 was tested in 39 regularly smoking male patients with schizophrenia in a set of electrophysiology measures. No indication was found that JNJ-3939406 has the potential to reverse basic deficits of information processing in schizophrenia (sensory P50 gating) or have a significant effect on other tested electrophysiological markers (MMN, P300 and quantitative resting EEG). Sensitivity analyses including severity of disease, baseline P50 gating, medication and gene variants of the CHRNA7 gene did not reveal any subgroups with consistent significant effects. CSF levels of the compound were measured in a separate trial and considered adequate. As mentioned above this study was only testing regularly smoking male patients with stable schizophrenia. Other

populations or detailed cognitive measures were not tested." As a result of its inefficacy in schizophrenic patients, compound **37** has been discontinued in the clinic.

Perhaps the most advanced α7 PAM still being actively pursued in the clinic may be AVL-3288 (**38**) [188]. AVL-3288 (a type I PAM) is readily brain penetrable and possesses a chemical structure that most medicinal chemists would say is not the most druggable. Nevertheless, AVL-3288 improves cognitive behavior in animal models [189] and is currently in human phase I trials for cognitive dysfunction in schizophrenia. It has been advanced through the aid of a grant awarded by the National Institute of Mental Health (NIMH) to support studies through to proof-of-concept phase II.

## 6 Clinical Proof-of-Concept (PoC) Studies of α7 Agonists

Given the favorable preclinical findings of multiple α7 agonists, including enhanced cognitive function and antipsychotic effects, and positive safety profile in phase I clinical trials, several α7 nAChR agonists were advanced into phase II clinical trials for schizophrenia.

Administration of the α7 nAChR partial agonist (and the α4β2 nAChR antagonist) GTS-21 to nonsmoking schizophrenic patients significantly improved cognitive performance in the Repeatable Battery for Assessment of Neuropsychological Status (RBANS) test, and it normalized the P50 auditory-evoked potential [190]. However, GTS-21 did not reverse cognitive impairments in schizophrenic patients using the MATRICS scale in a small crossover design study [191].

A multiple ascending dose (MAD) study of the very potent AZD0328 established a maximum tolerated dose of 1 mg for patients in the intended indication (http://www.ncats.nih.gov/files/AZD0328.pdf. Accessed 12 Dec 2013). In a 14-day phase IIa clinical trial, a dose-related incidence of nausea was apparent; however, in the likely optimal dose range, the incidence was approximately 5%. In that same phase IIa clinical trial, which was conducted in patients with schizophrenia who were concurrently taking an additional antipsychotic drug, no statistically significant improvement in cognition or other secondary endpoints was observed. Since AZD0328 was unlikely to meet AstraZeneca's target product profile, further its development was discontinued in 2008 (http://www.astrazenecaclinicaltrials.com/other-drug-products/discontinued-products/AZD0328/. Accessed 10 Jan 2009).

Although RG3487, the α7 nAChR partial agonist and the $5HT_3R$ antagonist, exhibited significant effects on episodic and working memory scales in patients with mild-to-moderate Alzheimer's disease, it failed to demonstrate cognitive improvement in schizophrenic patients in a 215 patient double-blind, placebo-controlled adjunctive to antipsychotics study (including smokers) [192].

Another α7 nAChR partial agonist and $5HT_3R$ antagonist, EVP-6124, showed a clinically meaningful and statistically significant impact on patients' overall cognition – the trial's prespecified primary endpoint – when taken in combination with second-generation antipsychotics and as measured by the full CogState overall

cognitive index, or "OCI" ($p = 0.05$ for all patients treated with EVP-6124 vs. placebo) This positive effect on the OCI was supported by a strong positive trend for improved cognition on the MCCB of cognition tests, which were conducted on all US patients in the trial. Additionally, EVP-6124 demonstrated positive effects in key secondary endpoints: improvement in clinical function (as assessed by the Schizophrenia Cognition Rating Scale) and reduction of the negative symptoms of schizophrenia (as measured by the Negative Symptom Scale of the Positive and Negative Symptoms Scale). In a separate clinical study, EVP-6124 did not delay cardiac repolarization in healthy subjects at supra-therapeutic exposures. EVP-6124 was well tolerated in 52 subjects of study, when administered as a 2-day regimen (8 mg (day 1) and 80 mg (day 2)) in cranberry juice [193]. In February 2013, EnVivo Pharmaceuticals announced the initiation of its phase III clinical trial program for EVP-6124. This program includes two 26-week multinational trials which are designed to enroll approximately 700 patients each and evaluate the safety and efficacy of two doses of once-daily treatment with EVP-6124 as a pro-cognitive treatment compared to placebo when added to chronic, stable, atypical antipsychotic therapy in people with schizophrenia. The primary endpoints of the trials include effect on cognitive function as measured by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS™) Consensus Cognitive Battery (MCCB™) overall cognitive index and effect on clinical function as measured by the interview-based Schizophrenia Cognition Rating Scale (SCoRS). Secondary endpoints of the trial include the clinical efficacy of the two doses of EVP-6124 as measured by negative subscale of the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression – Severity (CGI-S), Clinical Global Impression – Change Scale (CGI-C), and quality of life, using the EuroQoL-5D™ (EQ-5D) [194]. EnVivo continues to pursue the development of EVP-6124 in phase III clinical trials, maintaining the focus on improving cognition in schizophrenia [195].

The selective α7 nAChR agonist TC-5619, lacking in $5HT_3R$ interaction, was announced to have positive results for both negative symptoms and cognition in a 12-week exploratory phase IIa trial [196]. That trial was conducted to test the effects of TC-5619 on cognitive dysfunction and negative symptoms in subjects with schizophrenia. In the United States and India, 185 outpatients (18–60 years, male 69%, 46% tobacco users) with schizophrenia treated with quetiapine or risperidone monotherapy were randomized to 12 weeks of placebo ($n = 91$) or TC-5619 ($n = 94$; orally once daily 1 mg day 1 to week 4, 5 mg week 4 to 8, and 25 mg week 8 to 12). The primary efficacy outcome measure was the Groton Maze Learning Task (executive function) of the CogState Schizophrenia Battery (CSB). Secondary outcome measures included CSB composite score; Scale for Assessment of Negative Symptoms (SANS); Clinical Global Impression – Global Improvement (CGI-I); CGI – Severity (CGI-S); and Subject Global Impression – Cognition. GMLT statistically favored TC-5619 ($P = 0.036$) in this exploratory trial. SANS also statistically favored TC-5619 ($P = 0.030$). No other secondary outcome measure demonstrated a drug effect in the total population; there was a statistically significant drug effect on working memory in tobacco users. The results were

typically stronger in favor of TC-5619 in tobacco users and occasionally better in the United States than in India. TC-5619 was generally well tolerated with no clinically noteworthy safety findings.

TC-5619 is currently completing a phase IIb study that is evaluating the clinical drug candidate as a treatment for negative symptoms and cognitive dysfunction in schizophrenia at sites in the United States and Eastern Europe (http://www.targacept.com/therapeutic-pipeline/TC-5619.cfm). This trial is a double-blind, placebo-controlled, parallel group study in schizophrenic patients with stable psychosis taking a fixed dose of an atypical antipsychotic. Following a four-week screening period, patients receive either one of two doses of TC-5619 (5 mg or 50 mg) or placebo, randomized in a 2:1:1 ratio (placebo: TC-5619 5 mg: TC-5619 50 mg). The primary outcome measure is change from baseline on the Scale for Assessment of Negative Symptoms (SANS). Key secondary outcome measures include the composite score on the CogState Schizophrenia Battery and the University of California, San Diego, Performance-Based Skills Assessment brief version.

At the time of the completion of this review, top-line results were released from the abovementioned phase IIb clinical trial of TC-5619 as an augmentation therapy for the treatment of negative symptoms of schizophrenia [197]. TC-5619 did not meet the primary outcome measure, change from baseline on the SANS after 24 weeks versus placebo in that trial. In addition, TC-5619 did not demonstrate improvement on the key secondary measures of cognitive function. TC-5619 exhibited a benign safety and tolerability profile in the study. The future of this α7 agonist is unclear at this time.

In November of 2009, Abbott initiated a phase I trial to assess the safety, tolerability, and pharmacokinetics of the α7 nAChR agonist, ABT-126, in stable subjects with schizophrenia. This study investigated how ABT-126 is absorbed, distributed, metabolized, and eliminated by the body in stable schizophrenic volunteers receiving treatment with an atypical antipsychotic. Information regarding the results of this trial is scarce. However, following the successful completion of the phase I trials in January 2010 and a phase II trial in 2011, Abbott initiated a phase II study of ABT-126 for CIAS (adjunctive to background antipsychotics) with an estimated 430 patients enrolled (http://clinicaltrials.gov/ct2/show/NCT01655680?term=ABT-126&rank=3 ). Although MATRICS is the primary outcome measure, CANTAB for cognitive assessment and scales to assess positive and negative symptoms in schizophrenia were included. In August 2013, it was announced at the Alzheimer's Association International Conference that ABT-126 demonstrated cognitive benefits similar to those seen with donepezil in a phase II trial of patients with mild-to-moderate Alzheimer's disease (http://www.internalmedicinenews.com/index.php?id=2049&type=98&tx_ttnews[tt_news]=215191&cHash=da03e20e36). ABT-126 had an adequate safety profile in subjects with mild-to-moderate Alzheimer's dementia. High-dose ABT-126 improved ADAS-Cog scores comparable to donepezil, and the ADAS-Cog improvement correlated significantly with ABT-126 plasma exposure.

Finally, AQW-051 is an orally active α7 nAChR agonist in clinical trials for the cognitive deficits in schizophrenia (as well as for Alzheimer's and Parkinson's

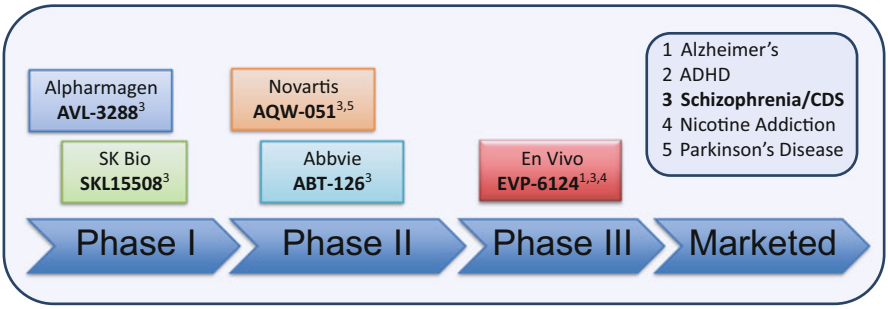



**Fig. 8** α7-based therapeutics in development for CNS disorders, including schizophrenia or CDS. AVL-3288, unlike the other products, is an α7 PAM

diseases). Early clinical studies showed that AQW051 is safe and well tolerated in young and elderly healthy volunteers. Its pharmacokinetic properties make it suitable for once-daily oral administration, and a study to evaluate the effects of once-daily doses of AQW-051 on cognition (147 patients) in stable schizophrenic patients started recruiting patients in September of 2012 [198]. A previous 57-patient clinical trial on cognitive function in patients with chronic stable schizophrenia was completed in September of 2011 [199]. Very little news flow has emerged this compound in recent years.

The initial successful clinical proof-of-concept trials of EVP-6124 and TC-5619 has generated optimism that α7 nAChR agonists might achieve their potential as promising adjunctive treatment paradigms to improve cognitive impairments and negative symptoms in schizophrenia. The highly successful phase IIb results for EVP-6124 further underscored the hope placed in the α7 mechanism to treat schizophrenia (as well as AD), and this hope is reflected in the entry of new α7-based therapies, including SKL-15508 and the α7 PAM AVL-3288 (Fig. 8). However, that optimism is tempered by the dearth of clinical success for α7 agonists in the treatment of cognition impairment, including the earlier failed trials of GTS-21, AZD0328, and RG3487, and recently underscored by the unsuccessful phase IIb outcome for TC-5619 reported at the end of 2013 (Table 2). The industry waits, not without some anxiety, for the results of the phase II clinical trials of AQW-051 (Novartis) and ABT-126 (AbbVie), as well as the phase III studies on EVP-6124 (Forum Pharmaceuticals).

## 7 Perspective

Given the relatively modest benefit and better tolerability (relative to neurological side effects) of current antipsychotic medicines, there is still an urgent need for improved therapeutics to treat schizophrenic patients. Considering the relatively poor predictive validity of the currently available animal models, and inefficiency

**Table 2** Status of selected clinical stage of α7 agonists

| Compound | Indication | Development stage |
|---|---|---|
| EVP-6124 | Cognitive impairment associated with schizophrenia | Phase 3 |
| EVP-6124 | Alzheimer's disease | Phase 3 |
| ABT-126 | Cognitive deficits in schizophrenia | Phase 2 |
| AQW051 | Cognitive impairment associated with schizophrenia | status uncertain[a] |
| GTS21 | Cognitive impairment associated with schizophrenia | Status uncertain[a] |
| ABT-126 | Alzheimer's disease | Discontinued |
| BMS-933043 | Cognitive deficits in schizophrenia | Discontinued |
| TC-5619 | Negative symptoms in schizophrenia | Discontinued |
| AZD0328 | Schizophrenia | Discontinued |
| TC-5619 | Negative symptoms in schizophrenia | Discontinued |
| RG3487 | Alzheimer's disease | Discontinued |
| RG3487 | Cognitive impairment associated with schizophrenia | Discontinued |
| SSR180711 | Alzheimer's disease | Discontinued |

[a]This compound has been in phase II trials without progression to phase III trials

of the drug discovery process, it is difficult to predict which of the current pharmacological approaches will ultimately translate to good medicines available for treating schizophrenic patients [200]. It is possible that some of the problems of translation of the animal models for CIAS may relate to whether or not the animal models used to justify progression of compounds into the clinic employ cognition-impaired animals versus cognition-normal animals; presumably, cognition-impaired animal models more closely mimic the human condition than non-impaired animal models and are thus more translationally relevant.

Studies to date, nevertheless, do support a role of nAChRs in the treatment of cognitive impairment associated with schizophrenia. These patients have not responded fully to treatment with antipsychotics. Nicotine replacement therapies and varenicline (Chantix®/Champix®) have leveraged the epidemiology of nicotine "self-medication" as first-generation nAChR medicines that are sometimes used to treat schizophrenia. Preclinical studies of positive allosteric modulators (PAMs) of the nAChRs have not yet resulted in a meaningful clinical response to those experimental drugs, but the first of this type of nicotinic cholinergic pharmacology is just now being tested in the clinic. However, as a result of almost a decade of research since varenicline entered the market, several clinical studies of orthosteric nAChR ligands (in particular of α7-selective agonists) were being conducted at the time of the writing of this chapter (Fig. 8).

Indeed, a recent article reported that the α7 nAChR is one of the pharmaceutical targets with the most number of compounds in clinical development [201]. These multiple clinical trials will hopefully result in a breakthrough effective and well-tolerated neuronal nicotinic receptor therapeutic that will improve cognitive function in subjects suffering from schizophrenia. The development of medicines for schizophrenia has been difficult, and already two companies with pipeline products for this indication late in 2013 are no longer advancing their α7 products in the clinic (Targacept and BMS). However, we have reason to be optimistic that a safe

and effective treatment for schizophrenic patients can emerge from the ample pipeline of α7 nicotinic cholinergic drug candidates being evaluated in the clinic, particularly of the compounds in late-stage clinical trials by at least three different companies (EnVivo, AbbVie, Novartis), most of which will read out in 2014 or early 2015. And opportunities for even more improved nAChR therapeutics for schizophrenia will come with the maturation of PAM therapeutics.

## References


1. De Leon J, Diaz FJ (2005) Schizophr Res 76(2–3):135
2. Newhouse PA, Potter A, Singh A (2004) Curr Opin Pharmacol 4:36
3. Keltner NL, Grant JS (2006) Perspect Psychiatr Care 42(4):256
4. Goff DC, Sullivan LM, McEvoy JP et al (2005) Schizophr Res 80(1):45
5. McCloughen A (2003) Int J Ment Health Nurs 12(2):119
6. Steinberg ML, Williams JM, Ziedonis DM (2004) Tobacco Control 13(2):206
7. Leonard S, Adler LE, Benhammou K (2001) Pharmacol Biochem Behav 70:561
8. Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J, Rose JE (1998) Psychopharmacology (Berl) 140:135
9. Wilens TE, Decker MW (2007) Biochem Pharmacol 74:1212
10. Martin LF, Kem WR, Freedman R (2004) Psychopharmacology (Berlin). 174(1):54
11. Green MF, Kern RS, Braff DL et al (2000) Schizophr Bull 26:119
12. Lippiello PM, Bencherif M, Hauser TA et al (2007) Expert Opin Drug Discov 2:1185
13. Center for Disease Control and Prevention (2002) Annual smoking-attributable mortality, years of potential life lost, and economic costs—United States, 1995–1999. MMWR 51:300
14. Reducing the Health Consequences of Smoking: 25 Years of Progress (2008) A Report of the Surgeon General. http://wonder.cdc.gov/wonder/prevguid/p0000061/p0000061.asp. Accessed 29 Jan 2008
15. McGinnis JM, Foege WH (1993) JAMA 270:2207
16. Doll R, Peto R, Boreham J et al (2004) BMJ 328:1519
17. Lasser K, Boyd JW, Woolhandler S, Himmelstein DU et al (2000) JAMA 284:2606
18. De Leon J, Dadvand M, Canuso C et al (1995) Am J Psychiatry 152:453
19. McCloughen A (2003) Int J Mental Health Nurs 12(2):119
20. Hughes J (1986) Am J Psychiatry 143:993
21. McCreadie RG, Kelly C (2000) Br J Psychiatry 176:109
22. Olincy A, Young DA, Freedman R (1997) Biol Psychiatry 42:1
23. Williams JM, Ziedonis DM, Abanyie F et al (2005) Schizophr Res 79:323
24. McDonald C (2000) Br J Psychiatry 176:596
25. Goff DC, Cather C, Evins AE et al (2005) J Clin Psychiatry 66:183
26. Goff DC, Henderson DC, Amico E (1992) Am J Psychiatry 149:1189
27. Lohr JB, Flynn K (1992) Schizophr Res 8:93
28. Pomerleau OF (1992) Am J Med 93:1A–7S
29. Ziedonis DM, Kosten TR, Glazer WM et al (1994) Hosp Community Psych 45:204
30. Adler LE, Hoffer LD, Wiser A, Freedman R (1993) Am J Psychiatry 150:1856
31. Eysenck HJ (1973) In: Dunn WL (ed) Smoking behaviour: motives and incentives. Winston, Washington
32. Allen MH, Debanné M, Lazignac C et al (2010) Am J Psychiatry 168:395
33. Harris JG, Kongs S, Allensworth D et al (2004) Neuropsychopharmacology 29:1378
34. Barr RS, Culhane MA, Jubelt LE et al (2008) Neuropsychopharmacology 33:480
35. Jacobsen LK, D'Souza DC, Mencl WE et al (2004) Biol Psychiatry 55:850

36. Caponnetto P, Auditore R, Russo C et al (2013) Int J Environ Res Public Health 10:446
37. Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009) Physiol Rev 89:73
38. Leonard S, Gault J, Hopkins J et al (2002) Arch Gen Psychiatry 59:1085
39. Auta J, Longone P, Guidotti A et al (1999) J Mol Neurosci 13:31
40. Breese CR, Lee MJ, Adams CE et al (2000) Neuropsychopharmacology 23:351
41. Glassman AH (1993) Am J Psychiatry 150:546
42. Leonard S, Bertrand D (2001) Nicotine Tob Res 3(3):203
43. Winterer G (2010) Curr Opin in Psychiatr 23:112
44. Veldic M, Kadriu B, Maloku E et al (2007) Schizophr Res 91(1–3):51
45. Ruzicka WB, Zhubi A, Veldic M et al (2007) Mol Psychiatry 12(4):385
46. Satta R, Maloku E, Zhubi A et al (2008) Proc Natl Acad Sci USA 105(42):16356
47. Maloku E, Kadriu B, Zhubi A et al (2011) Neuropsychopharmacology 36:1366
48. Cerimele JM, Durango A (2012) J Clin Psychiatry 73(8):e1039
49. Williams JM, Anthenelli RM, Morris CD et al (2012) J Clin Psychiatry 73(5):654
50. Gonzales D, Rennard SI, Nides M et al (2006) JAMA 296:47
51. Jorenby DE, Hays JT, Rigotti NA et al (2006) JAMA 296:56
52. Tonstad S, Tonnesen P, Hajek P et al (2006) JAMA 296:64
53. Raybuck JD, Portugal GS, Lerman C et al (2008) Behav Neurosci 122:1166
54. Loughead J, Ray R, Wileyto EP et al (2010) Biol Psychiat 67(8):715
55. Shim JC, Jung DU, Jung SS et al (2012) Neuropsychopharmacology 37(3):660
56. Hong LE, Thaker GK, McMahon RP et al (2011) Arch Gen Psychiatry 68(12):1195
57. Cosford ND, Bleicher L, Vernier JM et al (2000) Pharmaceutica Acta Helvetiae 74(2–3):125
58. Grottick AJ, Higgins GA (2000) Behav Brain Res 117:197
59. Levin ED, Rezvani AH, Xiao Y et al (2010) J Pharmacol Exp Ther 332:933
60. Rezvani AH, Cauley M, Xiao Y et al (2013) Psychopharmacology 226:35
61. Hahn B, Sharples CG, Wonnacott S et al (2003) Neuropharmacology 44:1054
62. Decker MW, Bannon AW, Curzon P et al (1997) J Pharmacol Exp Ther 283:247
63. Rueter LE, Anderson DJ, Briggs CA et al (2004) CNS Drug Rev 10(2):167
64. Prendergast MA, Terry AV Jr, Jackson WJ et al (1997) Psychopharmacology (Berl) 130:276
65. Wilens TE, Biederman J, Spencer TJ et al (1999) Am J Psychiatry 156:1931
66. Wilens TE, Verlinden MH, Adler LA et al (2006) Biol Psychiatry 59:1065
67. Bain EE, Robieson W, Pritchett Y et al (2013) Neuropsychopharmacology 38(3):405
68. Mazurov AA, Miao L, Bhatti BS et al (2012) J Med Chem 55:9181
69. Rezvani AH, Cauley MC, Johnson EC et al (2012) Psychopharmacology (Berl) 223(3):251
70. Dunbar G, Boeijinga PH, Demazieres A et al (2007) Psychopharmacology (Berl) 191:919
71. Howe WM, Ji J, Parikh V et al (2010) Neuropsychopharmacology 35:1391
72. Velligan D, Brenner R, Sicuro F et al (2012) Schizophr Res 134(1):59
73. Lightfoot AP, Kew JNC, Skidmore J (2008) Progr Med Chem 46:131
74. Peters L, Wright AD, Kehraus S et al (2004) Planta Med 70(10):883
75. Lysek N, Rachor E, Lindel T (2002) Z Naturforsch. C J Biosci 57(11–12):1056
76. Kim J-S, Padnya A, Weltzin M et al (2007) Bioorg Med Chem Lett 17(17):4855
77. Weltzin MM, Schulte MK (2010) J Pharmacol Exp Ther 334:917
78. German N, Kim J-S, Jain J et al (2011) J Med Chem 54(20):7259
79. Malysz J, Dyhring PK, Ahring GM et al (2009) Biochem Pharmacol 78:919
80. Grupe M, Jensen AA, Ahring PK et al (2013) Br J Pharmacol 168(8):2000
81. Timmermann DB, Sandager-Nielsen K, Dyhring T et al (2012) Br J Pharmacol 167(1):164
82. Grupe M, Grunnet M, Laursen B et al (2013) Neuropharmacology. doi:10.1016/j.neuropharm.2013.12.008
83. Lee CH, Zhu C, Malysz J et al (2011) Biochem Pharmacol 82(8):959
84. Lippiello PM, Mazurov A, Bencherif M (2011) The a7 nicotinic acetylcholine receptor in health and disease. In: Pharmacology of nicotinic acetylcholine receptors from the basic and therapeutic perspectives, Ed Hugo R Arias pp 101–150
85. Mazurov A, Hauser T, Miller CH (2006) Curr Med Chem 13:1567

86. Shen JX, Yakel JL (2009) Acta Pharmacol Sin 30:673
87. Adler LE, Olincy A, Waldo M et al (1998) Schizophr Bull 24:189
88. Giniatullin R, Nistri A, Yakel JL (2005) Trends Neurosci 28:371
89. Klink R, de Kerchove DA, Zoli M et al (2001) J Neurosci 21:1452
90. Dani JA, Bertrand D (2007) Annu Rev Pharmacol Toxicol 47:699
91. Behrendt RP (2006) J Psychopharmacol 20:356
92. Quick M, Polonskaya Y, Gillespie A et al (2000) J Comp Neurol 425:58
93. Sydserff S, Sutton EJ, Song D et al (2009) Biochem Pharmacol 78:880
94. Pichat P, Bergis OE, Terranova JP et al (2007) Neuropsychopharmacology 32:17
95. Mameli-Engvall M, Evrard A, Pons S et al (2006) Neuron 50:911
96. Jones EG, Tighilet B, Tran BV et al (1998) J Comp Neurol 397:371
97. Hajos M, Hurst RS, Hoffman WE et al (2005) J Pharmacol Exp Ther 312:1213
98. Carlsson A, Waters N, Holm-Waters S et al (2001) Annu Rev Pharmacol Toxicol 41:237
99. Levin ED, Simon BB (1998) Psychopharmacology (Berlin) 138:217
100. Thompson JL, Pogue-Geile MF, Grace AA (2004) Schizophr Bull 30:875
101. Matsuyama S, Matsumoto A, Enomoto T et al (2000) Eur J Neurosci 12:3741
102. Freedman R, Adams CE, Leonard S (2000) J Chem Neuroanat 20:299
103. Guan ZZ, Zhang X, Blennow K et al (1999) Neuroreport 10:1779
104. Freedman R, Hall M, Adler LE et al (1995) Biol Psychiatry 38:22
105. Leonard S, Freedman R (2006) Biol Psychiatry 60:115
106. Olivier B, Leahy C, Mullen T et al (2001) Psychopharmacology (Berl) 156:284
107. Stevens KE, Kem WR, Mahnir VM et al (1998) Psychopharmacology (Berl) 136:320
108. Walker E, Kestler L, Bollini A et al (2004) Annu Rev Psychol 55:401
109. Wallace TL, Porter RHP (2011) Biochem Pharmacol 82:891
110. Fenton WS, Stover EL, Insel TR (2003) Psychopharmacology (Berl) 169:365
111. Gurley DA, Lanthorn TH (1998) Neurosci Lett 247:107
112. Phillips E, Mack R, Macor J et al (1999) WO Patent 9,903,859
113. Baker SR, Boot J, Brunavs M et al (2005) Bioorg Med Chem Lett 15:4727
114. Macor JE, Gurley D, LanthornT et al (2001) Bioorg Med Chem Lett 11:319
115. Thompson AJ, Lummis SCR (2006) Curr Pharm Des 12:3615
116. Cappelli A, Anzini M, Vomero S et al (2002) Curr Topics Med Chem 2:599
117. Karlin A, Akabas MH (1995) Neuron 15:1231
118. Aventis Pharmaceuticals, Inc (2005) Anzemet [package insert]. Aventis Pharmaceuticals, Inc. Kansas City
119. Roche Laboratories, Inc (2005) Kytril [package insert]. Roche Laboratories, Inc., Nutley
120. GlaxoSmithKline (2004) Zofran [package insert]. GlaxoSmithKline, Blagrove
121. Lisi DM (2002) Arch Intern Med 162:101
122. Hajós M, Rogers BN (2010) Curr Pharm Des 16:538
123. Lightfood AP, Kew JNC, Skidmore J (2008) Progr Med Chem 46:131
124. Mazurov AA, Speake JD, Yohannes D (2011) J Med Chem 54:7943
125. Gordon JC, Griffith RC, Murray RJ et al (1999) US Pat 5,902,814
126. Mullen G, Napier J, Balestra M et al (2000) J Med Chem 43:4045
127. Astles PC, Baker SR, Boot JR et al (2002) Curr Drug Targets CNS Neurologic Disord 1:337
128. Levin ED, Bettegowda C, Blosser J et al (1999) J Behav Pharmacol 10:675
129. Van Kampen M, Selbach K, Schneider R et al (2004) Psychopharmacology 172:375
130. Schreiber R, Dalmus M, De Vry J (2002) Psychopharmacology 159:248
131. Griffith JM, O'Neill JE, Petty F et al (1998) Biol Psychiatry 44:98
132. Tatsumi R, Fujio M, Takanashi S et al (2006) J Med Chem 49:4374
133. Phillips E, Mack R, Macor J et al (2004) US Pat 6,706,878
134. John Wyeth and Brother Limited (1990) US Pat 4,921,860
135. Morrow JA, Collie IT, Dunbar DR et al (1998) FEBS Lett 439:334
136. Sydserff S, Sutton EJ, Song D et al (2009) Biochem Pharm 78:880
137. Bodnar AL, Cortes-Burgos LA, Cook KK et al (2005) J Med Chem 48:905

138. Wishka DG, Walker DP, Yates KM et al (2006) J Med Chem 49:4425
139. Wall TM, Hurst RS, Wong EHF et al (2006) Bioorg Med Chem 14:8219
140. Acker BA, Jacobsen EJ, Rogers BN et al (2008) Bioorg Med Chem Lett 18:3611
141. Xie W, Herbert B, Schumacher RA et al (2004) WO 2004,029,050
142. Wang S, Michael T, Dragan S et al (2006) Soc Neurosci 163:2/Z8
143. Wallace TL, Callahan PM, Tehim A et al (2011) J Pharmacol Exp Ther 336:242
144. Callahan PM, Rowe WB, Xie W et al (2006) Soc Neurosci 163:5/Z11
145. Rowe WB, Callahan PM, Xie W et al (2006) Soc Neurosci 163:4/Z10
146. Rezvani AH, Kholdebarin E, Bricato FH et al (2009) Prog Neuropsychopharmacol Biol Psych 33:269
147. Umbricht DMS, Lowe D, Yoo K et al (2009) 48th Annual meeting of American college of neuropsychopharmacology. Hollywood, Florida
148. Boess FG, De Vry J, Erb C et al (2007) J Pharmacol Exp Ther 321:716
149. Hilt D, Safirstein B, Hassman D et al (2009) Presented at ICAD 2009, Vienna, Austria
150. Prickaerts J, van Goethem NP, Chesworth R et al (2012) Neuropharmacology 62:1099
151. Mazurov AA, Kombo DC, Hauser TA et al (2012) J Med Chem 55:9793
152. Hauser TA, Kucinski A, Jordan KG et al (2009) Biochem Pharmacol 78:803
153. Hashimoto K, Ishima T, Fujita Y et al (2008) Biol Psychiatry 63:92
154. Barak S, Arad M, De Levie A et al (2009) Neuropsychopharmacology 34:1753
155. Biton B, Bergis O, Galli F et al (2007) Neuropsychopharmacology 32:1
156. Pichat P, Bergis OE, Terranova JP et al (2007) Neuropsychopharmacology 32:17
157. Kristensen SE, Thomsen MS, Hansen HH et al (2007) Neurosci Lett 418:154
158. Feuerbach D, Lingenhohl K, Dobbins P et al (2007) Neurosci Lett 416:61
159. Feuerbach D, Lingenhohl K, Olpe HR et al (2009) Neuropharmacology 56:254
160. O'Donnell CJ, Rogers BN, Bronk BS et al (2010) J Med Chem 53:1222
161. Sirota P, Mosheva T, Shabtay H et al (2000) Am J Psychiatry 157:287
162. O'Donnell CJ (2009) CP-810,123, a third generation alpha-7 nAChR agonist for treatment of cognitive deficits associated with schizophrenia. nicotinic acetylcholine receptor-based therapeutics: emerging frontiers in basic research & clinical science. Presented at the satellite meeting to the Society for Neuroscience, Chicago
163. Tietje KR, Anderson DJ, Bitner RS et al (2008) CNS Neurosci 14:65
164. Malysz J, Anderson DJ, Grønlien JH et al (2010) J Pharm Exp Ther 334:863
165. Bitner RS, Bunnelle WH, Decker MW et al (2010) J Pharm Exp Ther 334:875
166. Othman AA, Lenz RA, Zhang J, Li J, Awni WM, Dutta S (2011) J Clin Pharmacol 51:512
167. Hurth K (2014) In: 247th National meeting of the American chemical society, Dallas, 16–20 Mar 2014, American Chemical Society, Washington, DC, p 222
168. Haydar SN, Ghiron C, Bettinetti L et al (2009) Bioorg Med Chem 17:5247
169. Roncarati R, Scali C, Comery TA et al (2009) J Pharmacol Exp Ther 329:459
170. Ghiron C, Haydar SN, Aschmies S et al (2010) J Med Chem 53:4379
171. Macor JE, Gurley D, Lanthorn T et al (2001) Bioorg Med Chem Lett 11:319
172. Pitsikas N, Borsini F (1997) Pharmacol Biochem Behav 56:571
173. Hashimoto K, Fujita Y, Ishima T et al (2006) Eur J Pharmacol 553:191
174. Shiina A, Shirayama Y, Niitsu T et al (2010) Ann Gen Psychiatry 9:27
175. Marder SR, Fenton W (2004) Schizophr Res 72:5
176. Grønlien JH, Håkerud M, Ween H et al (2004) Mol Pharm 72:715
177. Christensen DZ, Mikkelsen JD, Hansen HH et al (2010) J Neurochem 114:1205
178. Hurst RS, Hajos M, Raggenbass M et al (2005) J Neurosci 25:4396
179. Rogers BN (2008) In: American chemical society meeting, Philadelphia
180. Timmermann DB, Grønlien JH, Kohlhaas KL et al (2007) J Pharmacol Exp Ther 323:294
181. Hogenkamp DJ, Ford-Hutchinson TA, Li WY et al (2013) J Med Chem 56:8352
182. Kennett GA, Wood MD, Bright F et al (1996) Br J Pharmacol 117:427
183. Dunlop J, Lock T, Jow B et al (2009) J Pharmacol Exp Ther 328:766
184. Faghih R, Gopalakrishnan SM, Grønlien JH et al (2009) J Med Chem 52:3377

185. Malysz J, Grønlien JH, Anderson DJ et al (2009) J Pharmacol Exp Ther 330:257
186. Malysz J, Gronlien JH, Briggs CA et al (2011) Biochem Pharm 82:1030
187. Winterer G, Gallinat J, Brinkmeyer J et al (2013) Neuropharmacology 64:197
188. Ng HJ, Whittemore ER, Tran MB et al (2007) Proc Natl Acad Sci USA 104:8059
189. Pandya AA, Yakel JL (2013) Biochem Pharmacol 86:1054
190. Olincy A, Harris JG, Johnson LL et al (2006) Arch Gen Psychiatry 63:630
191. Freedman R, Olincy A, Buchanan RW et al (2008) Am J Psychiatry 165:1040
192. Umbricht D, Murray SR, Lowe DA et al (2009) The effect of the partial nicotinic alpha7 receptor agonist R3487 on cognitive deficits in schizophrenia. In: Proceedings of the 48th annual meeting of the American college of neuropsychopharmacology. American College of Neuropsychopharmacology, Nashville
193. Loewen G, Cawryl M, Schutz R et al (2013) Alzheimer's Dement 9:P660
194. EnVivo (2013) Pharmaceuticals Announces Initiation of Phase 3 Clinical Trial Program of EVP-6124 in Schizophrenia. Available from: http://www.envivopharma.com/news-item.php?id=45. Accessed 25 Nov 2013
195. En Vivo (2013) Nicotinic alpha7 Agonist Program. Available from http://www.envivopharma.com/pipeline-nicotinic.php Accessed 20 Dec 2013
196. Lieberman JA, Dunbar G, Segreti AC et al (2013) Neuropsychopharmacology 38:968
197. Targacept (2013) Targacept press release, 16 Dec 2013 http://www.targacept.com/newsroom/index.cfm?nid=Targacept%20Announces%20Negative%20Top%2DLine%20Results%20from%20Phase%202b%20Clinical%20Trial%20of%20TC%2D5619%20in%20Schizophrenia&newsyear=2013
198. Novartis Pharmaceuticals (2013) A study to evaluate the effects of once daily doses of AQW051 on cognition, in stable schizophrenia patients In: ClinicalTrials.gov [Internet]. National Library of Medicine (US), Bethesda.. http://clinicaltrials.gov/ct2/show/NCT01730768?term=Aqw051&rank=5 NLM Identifier: NCT01730768
199. Novartis Pharmaceuticals (2013) Effect of AQW051 on Cognitive Function in Patients With Chronic Stable Schizophrenia. In: ClinicalTrials.gov [Internet]. National Library of Medicine (US), Bethesda http://clinicaltrials.gov/ct2/show/NCT01163227?term=Aqw051&rank=2 NLM Identifier: NCT01163227
200. Ellenbroek BA (2012) Neuropharmacology 62:1371
201. Agarwal P, Sanseau P, Cardon LR (2013) Nat Rev Drug Discov 12:575

Top Med Chem (2015) 13: 255–316
DOI: 10.1007/7355_2014_54

Published online: 9 August 2014

# The Use of PDE10A and PDE9 Inhibitors for Treating Schizophrenia

**Jamison B. Tuttle and Bethany L. Kormos**

**Abstract** Schizophrenia (Scz) is a chronic and debilitating neurological disorder that afflicts approximately 1% of the general population with an increased incidence within families. Signs of this disorder typically appear between the ages of 16–30 although rare cases of childhood (<13) onset exist. Diagnosis of scz requires symptoms that persist for a duration of 1 month over a 6-month time period. The broad spectrum of symptoms are typically split into three categories: positive, negative, and cognitive. Implicit within these categories is the difficulty for stand-alone treatment methods, suggesting a combination of pharmacological and psychological treatment strategies is needed to manage this disease. Furthermore, while current pharmaceuticals are moderately effective at managing positive symptoms, the severe side effects lower patient compliance. Additionally, drugs used to treat negative and cognitive symptoms only have modest benefits, at best. Due to these limitations, there is a clear need for novel pharmaceuticals that modulate dysfunctional neurological pathways in scz patients. As such, both academic and pharmaceutical researchers are focused on identifying enzymes that can attenuate neurological signaling in disease-relevant brain regions. Specifically, this chapter will provide an in-depth review of current drug discovery efforts focused on inhibiting phosphodiesterase 10 and 9 (PDE10A, PDE9A, respectively).

**Keywords** Basal ganglia, BAY 73-6691, cAMP, cGMP, Clinical trials, Cognition, Competitive landscape, Long-term potentiation, MP-10, PET ligands, PF-04447943, Phosphodiesterase 10A, Phosphodiesterase 9A, Positive and negative symptoms, Positron Emission Tomography, Schizophrenia, Striatum, Synaptic plasticity, THPP-1, TP-10

J.B. Tuttle (✉) and B.L. Kormos
Neuroscience Medicinal Chemistry, Pfizer Inc., 610 Main St, Cambridge, MA 02139, USA
e-mail: jamison.b.tuttle@pfizer.com; bethany.kormos@pfizer.com

## Contents



## Abbreviations

| | |
|---|---|
| 5′ AMP | 5′ Adenosine monophosphate |
| 5′ GMP | 5′ Guanosine monophosphate |
| AC | Adenylate cyclase |
| AKAP | A-kinase anchoring protein |
| ATP | Adenosine triphosphate |
| BID | Bis in die (twice a day) |
| cAMP | Cyclic adenosine monophosphate |
| cGMP | Cyclic guanosine monophosphate |
| CNGA2 | Cyclic nucleotide-gated channel alpha 2 |
| CNS | Central nervous system |
| CSF | Cerebral spinal fluid |
| DG | 2-Deoxy-D-glucose |
| D1 | Dopamine 1 |
| D2 | Dopamine 2 |
| eNOS | Endothelial NOS |
| EPS | Extrapyramidal side effects |
| ERK | Extracellular receptor kinase |
| FDG-PET | Fluorodeoxyglucose positron emission tomography |
| GAF | cGMP phosphodiesterases *Anabaena* adenylyl cyclases, and *Escherichia coli* Fh1A |
| GC | Guanylate cyclase |
| GP | Globus pallidus |
| GPCR | G-protein coupled receptors |
| h | Hour |
| HB | Hydrogen bond |
| IP | Intraperitoneal |

| | |
|---|---|
| KO | Knock-out |
| LH | Lateral habenula |
| LipE | Lipophilic binding efficiency |
| LTP | Long-term potentiation |
| mRNA | Messenger ribonucleic acid |
| NHP | Nonhuman primate |
| nM | Nanomolar |
| NMDA | *N*-methyl-D-aspartate |
| nNOS | Neuronal nitric oxide synthase |
| NO | Nitric oxide |
| NOR | Novel object recognition |
| NOS | Nitric oxide synthase |
| NR | Not reported |
| NT | Neurotensin |
| ORD | Object retrieval detour |
| PDE | Phosphodiesterase |
| PET | Positron emission tomography |
| PK | Pharmacokinetic |
| PKA | Protein kinase A |
| PKG | Protein kinase G |
| PO | Per os |
| PPI | Pre-pulse inhibition |
| QD | Quaque die (once a day) |
| SAR | Structure–activity relationship |
| scz | Schizophrenia |
| sGC | Soluble guanylate cyclase |
| SNpr | Substantia Nigra pars reticulata |
| THP | Tetrahydropyran |
| THPP-1 | Tetrahydropyridopyrimidine |
| VDW | Van der Waals |
| VTA | Ventral tegmented area |

## 1 Introduction

Schizophrenia (scz) is a chronic neurological disorder that afflicts 1% of the general population worldwide, the symptoms of which typically appear in early adulthood. While ancient historical accounts exist, the disease was first defined by Dr. Emile Kraepelin in 1887 as dementia praecox and then schizophrenia in 1908 by Dr. Eugene Bleuler, both of whom are considered the "fathers" of this disorder [1]. In fact, the basis for current psychiatric diagnostics derived from DSM-IV [2] for this heterogeneous disorder is rooted in their early observations. The disease has been divided into five behavioral categories that are paranoid, disorganized, catatonic, undifferentiated, and residual. Within each of these subtypes exists a myriad of symptoms which have been simplified into three representatative categories: positive,

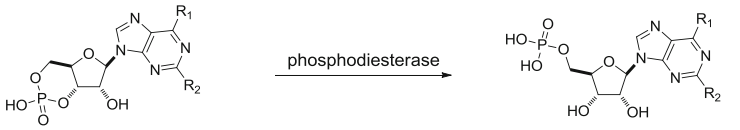
negative, and cognitive. One, positive symptoms include a range of psychoses such as hallucinations, delusions, thought disorders, and movement disorders. Two, negative symptoms include symptoms such as alogia, anhedonia, and affective flattening. Three, cognitive deficits, which lead to poor executive functioning, working memory issues, and focus problems. These disparate symptoms illustrate the implicit challenges associated with finding a remedy for this disease.

Historically, highly invasive techniques were used to treat patients and, only recently, have evolved to include pharmaceuticals. Prior to the 1970s, patients were committed to long-term hospitalizations and underwent severe procedures that included hydrotherapy, insulin-induced comas, frontal lobotomies, and electroshock therapy [1]. Although some of these techniques are still utilized, the benchmark introduction of the first pharmaceutical intervention, clozapine, in the 1950s had a major impact. It helped to manage positive symptoms, mainly hallucinations and delusions, leading to widespread dehospitalization, and provided an in-road for the scientific community to discover neurological mechanisms underpinning scz [3, 4]. Further research into clozapine's mechanism of action has shown aberrant dopamine signaling in brain regions associated with scz and provided the foundation for the widely recognized dopamine hypothesis of scz. Moreover, investigation into this circuitry has led to the development of additional neuroleptics, termed atypical or second generation antipsychotics, and clinical trials continue to assess the efficacy of novel compounds that attenuate dopaminergic circuitry for treating scz [5].

Despite the efficacy of marketed antipsychotics for treating positive symptoms, there remains a considerable socioeconomic impact associated with this disease whereby estimates of indirect and direct costs in the USA are $62 billion/year [6]. This is largely attributed to marketed drugs having undesirable side effects and lack of efficacy for negative and cognitive symptoms leading to low compliance. As such, significant research efforts continue to focus on developing drugs that (1) treat positive symptoms while minimizing undesired side effects, (2) treat negative symptoms, and/or (3) treat cognitive deficits associated with this disorder [7]. Towards these ends, one enzyme family that has been the focus of recent research efforts is the phosphodiesterases (PDEs), specifically PDE10A and PDE9A. Due to the unique expression of PDE10A in brain regions associated with scz and PDE9A having the lowest measured $K_m$ for cyclic guanosine monophosphate (cGMP), which is reported to be decreased in the cerebral spinal fluid (CSF) of scz patients, these phosphodiesterases have been highly studied and are the focus of this review. The goal of this chapter is to provide a brief introduction to these PDEs, correlate their mechanism of action with potential disorders found in scz, review the patent and clinical landscapes, then conclude with the future potential of these PDE inhibitors as treatments for this disease.

## 2 Introduction to Phosphodiesterases

Phosphodiesterases are zinc- and magnesium-dependent hydrolases that consist of 11 families encoded by 21 genes. These enzymes are widely distributed throughout the body and play a central role in cellular function by metabolizing cyclic




**1**, cAMP; $R_1 = NH_2$, $R_2 = H$
**2**, cGMP; $R_1 = OH$, $R_2 = NH_2$
Active Form

**3**, 5' AMP; $R_1 = NH_2$, $R_2 = H$
**4**, 5' GMP; $R_1 = OH$, $R_2 = NH_2$
Inactive Form

**Fig. 1** Structure and hydrolysis product of cAMP/cGMP regulated by phosphodiesterases

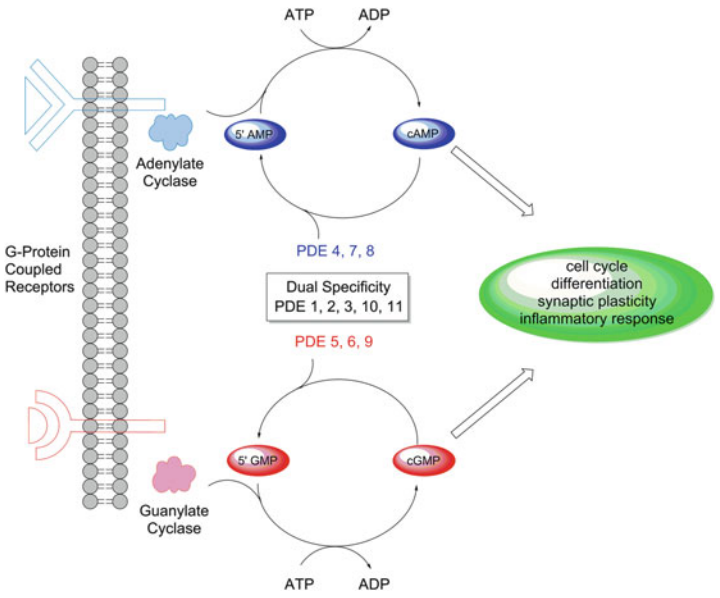



**Fig. 2** Cyclic nucleotide signaling cascade and catalytic cycle

guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) to their inactive forms, 5′ guanosine monophosphate (5′ GMP) and 5′ adenosine monophosphate (5′ AMP), respectively (Fig. 1) [8, 9].

The cyclic nucleotides, cGMP/cAMP, act as "secondary messengers" and have a central role in cellular signaling cascades that are initiated by non-cell penetrant molecules such as hormones and neurotransmitters. In a typical signaling event, extracellular molecules activate G-protein coupled receptors (GPCRs) which subsequently induce adenylate cyclase (AC) or guanylate cyclase (GC), enzymes that utilize adenosine triphosphate (ATP), to biosynthesize cAMP/cGMP. These nucleotides, in turn, propagate signaling cascades by activating protein kinase A (PKA) and protein kinase G (PKG) which then phosphorylate a number of downstream substrates that include ion channels and transcription factors. These go on to regulate a broad range of physiological functions that include cell cycle, differentiation, synaptic plasticity, and inflammatory responses (Fig. 2) [10].

Due to their importance in key cellular processes, the local tissue distribution and cellular concentrations of cGMP/cAMP are strictly regulated by cyclases and, conversely, by PDEs which hydrolyze these nucleotides to their inactive 5′AMP/5′GMP forms (Fig. 2). Interestingly, recent studies found cyclic nucleotides are highly compartmentalized within cells allowing for highly controlled temporal and spatial signaling events [11]. This local regulation is dependent on A-kinase anchoring protein (AKAP) mediated scaffolding of PDEs, which acts to abolish secondary signaling, and PKA, which propagates this secondary signaling [12]. It is believed that PDEs play the key role in maintaining this balance and several techniques have been developed to study this phenomenon [13–15].

Phosphodisterases are classified according to their cyclic nucleotide specificity. For instance, PDEs 4, 7, and 8 hydrolyze cAMP whereas PDEs 5, 6, and 9 hydrolyze cGMP. The remaining PDEs, 1, 2, 3, 10, and 11 are cAMP and cGMP dual hydrolases. In addition to this nucleotide substrate classification, PDEs are further divided into two additional categories, genes transcribing the enzyme and splice variants, that broaden the PDE family to 60 unique isoforms. This additional complexity at the transcription level is due to the control of structurally complex coding sequences by multiple promotors that, coupled with the aforementioned alternate splicing, suggests well over 100 unique transcripts may be expressed throughout the body [16]. Of all tissues, the brain has the highest abundance and variety of PDEs of any other organ [17]. As such, this has led to a garnering interest in the role PDEs play in neurological function whereby several family members have been targeted for treating brain disorders and/or enhancing cognition [18].

## 3 PDE10A

Phophodiesterase 10A is a dual cAMP/cGMP hydrolase independently discovered in 1999 by three research groups and is unique amongst the other phosphodiesterase family members in its localization [19–22]. It is highly expressed as a single isoform and only found in medium spiny neurons which comprise 90–95% of the corpus striatum. Brain protein levels of PDE10A are similarly restricted to the putamen and caudate nucleus regions of the striatum across several mammalian species that include mouse, rat, dog, cynomolgus macaque, and humans [23], which is consistent with reported mRNA levels [17]. This brain region, which is a component of the basal ganglia system, is believed to play a role in executive function, response to external stimuli, and cognition. Because PDE10A is highly expressed in this region which governs behaviors that are impaired in several neurological diorders, a large body of research has been devoted to understanding the role PDE10A plays in striatal function. This is especially relevant to this topic as abnormal neurological signaling within this brain region is one of the key tenets of the aforementioned dopamine hypothesis of scz and shown to have region-specific activation for patients displaying positive and negative behaviors [24]. This circuitry and the role PDE10A has in its function is discussed in Sect. 3.4.

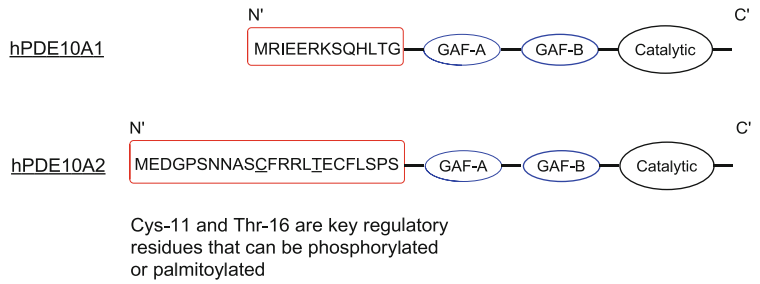



**Fig. 3** Schematic depicting the key differences between human PDE10A1 and PDE10A2

## *3.1 PDE10A Splice Variants*

In humans, PDE10A is expressed as two splice variants, PDE10A1 and PDE10A2 [25, 26]. Phosphodiesterase 10A1 contains 779 amino acids while PDE10A2 has an additional ten amino acids situated on the N-terminus (Fig. 3).

Whereas A1 is cytosolic, the additional residues of A2 contain regulatory amino acids that allow for intracellular trafficking between the cytosol and cell membrane [27]. Specifically, Cys-11 and Thr-16 are palmitoylated or phosphorylated, respectively, depending on intracellular levels of cAMP. When local cAMP levels are low, A2 is palmitoylated on Cys-11, causing association with transport vesicles that traffic the enzyme to neuronal cell membranes. Conversely, when cytosolic cAMP levels are high, PKA is activated and PDE10A2 is phosphorylated on Thr-16 which prevents palimitoylation, restricts the enzyme to the cytosol then leads to the reduction of local cAMP levels. This process, along with AKAP-mediated scaffolding, helps finely tune the propagation of neuronal signaling by regulating active and inactive pools of cyclic nucleotides.

## *3.2 GAF Domains*

Phosphodiesterase 10A contains two tandem regions known as cGMP phosphodiesterases, *Anabaena* adenylyl cyclases, and *Escherichia coli* Fh1A (GAF) domains termed GAF-A and GAF-B. These domains are present in all living organisms and allosterically regulate enzymatic activity by small molecule or protein–protein interactions. The PDE10A GAF domain crystal structures have been solved and are well characterized [28, 29]. The regulatory role these domains have on PDE10A function remains controversial but is proposed to be dependent on cAMP and not cGMP. An early account utilizing a PDE10A GAF domain/cyanobacterial adenylyl cylase chimera to assess allosteric regulatory effects found cAMP increased PDE10A catalytic activity nine-fold over control ($EC_{50} = 19.8$ μM) [30]. In contrast to these studies, experiments using a PDE10A GAFB domain binding assay found that, although cAMP bound with a $K_d$ of 48 nM, there was no stimulation of

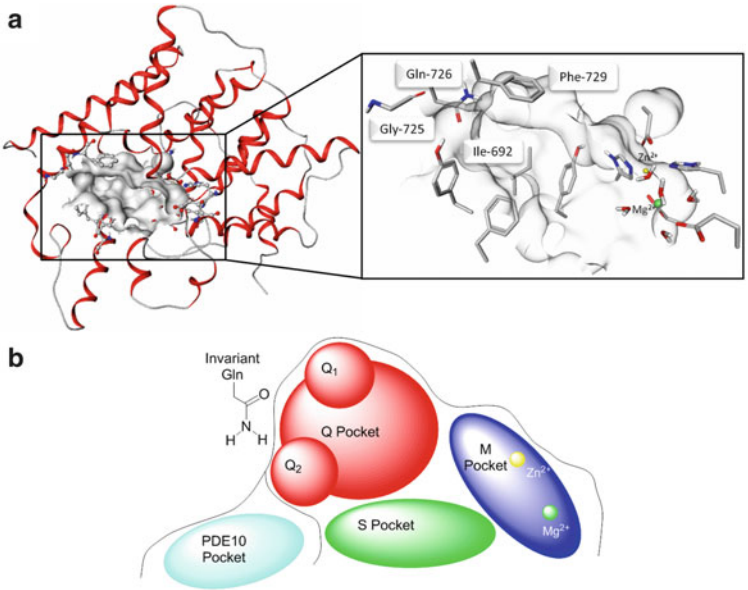



**Fig. 4** PDE10A catalytic domain and binding site (**a**) X-ray crystal structure of human PDE10A (PDB ID: 2OUP) with binding site surface and key residues highlighted (*gray*). *Inset*: close-up of PDE10A binding site (**b**) Schematic of the binding site and location of pockets in PDE10A, PDE9A, and common to most PDEs, and location of the PDE10A-specific pocket

catalytic activity [31]. Recently, new data suggests a more complicated role of cAMP in PDE10A function. It was found to stimulate PDE10A catalytic activity by threefold but, interestingly, at high [cAMP], cGMP turnover is inhibited. Conversely, at low [cAMP] there is a 25% increase in cGMP turnover [32]. These studies do not address phosphorylation or palmitoylation effects on PDE10A catalytic activity which are needed to best understand in vivo enzyme kinetics.

## *3.3 Catalytic Domain*

There are many published X-ray crystal structures of the PDE10A catalytic domain that provide insight into substrate binding modes. For this section, the X-ray crystal structure of human PDE10A (PDB ID: 2OUP) [33] is used to highlight binding site residues and regions that are important for substrate binding to this enzyme and PDEs in general (Fig. 4a): (1) Gln-726, the invariant glutamine residue found in all PDEs, forms hydrogen bond (HB) interactions with the adenine or guanine ring, (2) Phe-729 and Ile-692 form the hydrophobic clamp (P clamp), stabilizing the flat, aromatic adenine or guanine ring through hydrophobic interactions, and (3) two divalent metals in the metal binding pocket (M pocket), $Zn^{2+}$ and $Mg^{2+}$, coordinate the phosphate group for hydrolysis. The pocket containing the P clamp and the

invariant glutamine is referred to as the Q pocket, which contains two subpockets, $Q_1$ and $Q_2$, as defined by Card et al. [34]. A third pocket called the solvent pocket (S pocket) involves the residues at the entrance to the binding pocket. These features and pockets, highlighted in Fig. 4b, are common amongst the catalytic domains of most PDEs [34]. The PDE10A pocket, also highlighted in Fig. 4b, is a unique pocket that is accessible to PDE10A inhibitors. It is hypothesized that this pocket is only found in PDE10A due to a unique Gly at position 725 [35]. The endogenous substrates, cAMP and cGMP, do not interact with the residues in this pocket.

## *3.4 PDE10A in Schizophrenia*

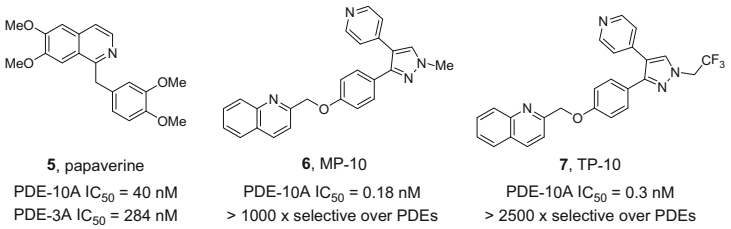
### 3.4.1 Circuitry

In order to understand the importance of PDE10A for treating scz, it is important to review the striatal circuitry where the enzyme is expressed. The striatum occupies a central role in signal attenuation throughout the basal ganglia circuitry that is involved in cognition, emotional processes, and motor responses. Several recent review articles detail the intricacies of this brain region [36–40]. It has been hypothesized that two main circuits signal from the striatum: (1) the striatopallidal/indirect pathway and (2) the striatonigral/direct pathway. The striatopallidal pathway acts to filter out irrelevant background information. The striatonigral pathway acts to attenuate the strength of inputs encoding behaviors that are adaptive for a desired response. It is generally believed that these two pathways are antagonistic, although recent research found there may be synergy with respect to controlling movement [41].

From a circuitry perspective, the direct and indirect pathways in the basal ganglia are hypothesized to function independently and are dependent on dopaminergic signaling [42]. Evidence for this was discovered using D2 antagonists in a 2-deoxy-D-glucose (DG) autoradiography study [43]. In the event, a D2 antagonist, haloperidol (indirect pathway antagonism), demonstrated a dose-dependent uptake of 2-DG in the lateral habenula (indirect pathway) but no effect in the globus pallidus (direct pathway). Also, D1 agonists (direct pathway activation), CY 208–243 and D-amphetamine, increased Fos-like immunoreactivity in basal ganglia regions regulated by striatalnigral (direct pathway) outputs. In the same study, D2 antagonists, haloperidol and clozapine, elevated Fos-like immunoreactivity in circuits associated with the striatopallidal (indirect) pathway [44].

Evidence for PDE10A having a regulatory role in the striatonigral direct pathway signaling was demonstrated using papaverine **5** for an ex vivo patch clamp study. The results of this study show PDE10A inhibition enhances D1 receptor agonist-evoked GABAergic currents in the Substantia Nigra pars reticulata (SNpr), a region involved with the striatal nigral (direct) pathway. Importantly, mild AC stimulation is necessary because basal levels of AC activity are low in these tissue preparations [45].

Contradictory evidence for these pathways functioning independently was shown by measuring cerebral glucose uptake after intrastriatal injection of D1 agonists and D2 antagonists. In the study, glucose uptake was decreased in nuclei




**5**, papaverine
PDE-10A $IC_{50}$ = 40 nM
PDE-3A $IC_{50}$ = 284 nM

**6**, MP-10
PDE-10A $IC_{50}$ = 0.18 nM
> 1000 x selective over PDEs

**7**, TP-10
PDE-10A $IC_{50}$ = 0.3 nM
> 2500 x selective over PDEs

**Fig. 5** Examples of PDE10A inhibitors used to assess striatal function

found in both the direct and indirect pathways for both treatments [46]. Based on these results, it is clear more experimentation is needed to develop an understanding of how dopaminergic inputs affect synaptic transmission in these pathways.

Phosphodiesterase 10 inhibition may provide a unique opportunity to understand how these pathways function clinically. Because both D1- and D2-dependent neurons require cAMP/cGMP messaging to propagate neurochemical inputs, PDE10A inhibitors provide a novel opportunity to test the dual activation of these pathways; the proposed effect has been discussed [9]. Evidence for this was found using a PDE10A inhibitor, MP-10 **6**, in a fluorodeoxyglucose positron emission tomography (FDG-PET) study in mice [47]. At a minimally effective dose this inhibitor showed region-specific increases of 2-DG uptake in the globus pallidus (GP) and the lateral habenula (LH). Both pathways influence the basal ganglia circuitry where the GP (direct pathway) is involved with motor control [48] and the LH (indirect pathway) is involved in processing reward stimuli, avoidance learning, pain and stress [49]. This circuitry has been proposed to be involved with scz pathophysiology [50]. Interestingly, whereas 2-DG uptake in the LH correlated with a 100% D2 receptor occupancy by haloperidol, a similar effect was observed for **6**, also known as Pfizer clinical candidate PF-02545920, at 10–40% occupancy. This suggests PDE10A inhibitors, including compounds **5**–**7**, may be more effective at influencing this circuitry than D2 antagonists (Fig. 5).

### 3.4.2 Biochemical and Behavioral Effects of Tool Compounds

This section reviews the biochemical and behavioral effects of two PDE10A selective tool compounds, MP-10 **6** and THPP-1 **8**, that show inhibiting PDE10A effectively improves preclinical models of schizophrenic symptoms. Whereas a brief introdution to MP-10 has been discussed in light of an FDG-PET, further details about the mechanism of action is discussed below.

#### MP-10

The discovery of MP-10, **6**, was reported by Pfizer in 2009 [35] and is the most widely profiled of all PDE10A inhibitors. Pharmacologically, this compound is highly potent and selective with respect to PDEs and CEREP panel. The X-ray

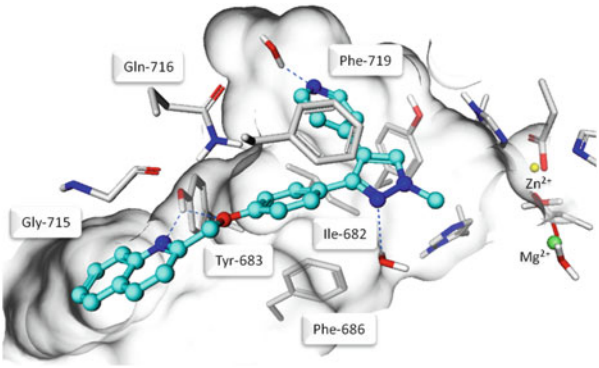



**Fig. 6** X-ray crystal structure of **6** (*cyan*) bound to PDE10A (PDB ID: 3HR1). Key residues and binding site pocket are highlighted (*gray*) and HB interactions are depicted by *blue dashed lines*

crystal structure of **6** bound to rat PDE10A (PDB ID: 3HR1) highlights key interactions (Fig. 6) [35]. This structure reveals that **6** does not make a HB interaction with the conserved glutamine, Gln-716 (residue numbering for the rat PDE10A construct is slightly different from the human PDE10A construct), unlike most PDE inhibitors. In addition, **6** does not have the planar, aromatic ring system that typically interacts with the P clamp (Phe-719 and Ile-682). Rather, the phenyl ring of **6** makes an amino-π interaction with Gln-716 and an edge-to-face interaction with Phe-686. The pyrazole and pyridyl nitrogens form HB interactions with water molecules that are involved in HB networks with the protein. The selectivity of **6** over other PDEs is likely due to interactions of the quinoline group with the PDE10A pocket and a HB interaction with Tyr-683 (Fig. 6).

In preclinical pharmacokinetic experiments, **6** is 30% orally bioavailable in rats and 68–100% in dogs with low to moderate clearance (Fig. 7). Importantly, **6** has good brain exposure with a brain to plasma ratio of 0.86 making it a candidate for measuring central effects. Indeed, this was demonstrated in the striatum where **6** caused a dose-dependent increase of proteins involved in neurological function using 0.1–5 mg/kg MP-10 in male CF mice dosed intraperitoneally (IP). The upregulated proteins include cGMP, CREB S133 phosphorylation, DARPP32 phospho-T34, phospho GluR1, and small, but significant, increases in total GluR2/3 and phospho NR2B 1472 [51]. Importantly, these effects were not observed in **6** treated PDE10A knock-out (KO) mice, strongly suggesting PDE10A plays a direct role in the regulation of these biochemical events.

The effect of PDE10A inhibition was assessed for transcription effects. Several neurotrophic mRNA transcripts that include neurotensin (NT), a regulator of dopamine pathways; cFOS, a marker for neuronal activation; enkaphalin, an indirect pathway marker; and substance P, a direct pathway marker were increased after inhibiting PDE10A at a 3 mg/kg, i.p. dose. [52]. Of relevance to this outcome, marketed D2 antagonists produce a similar increase of immediate early genes, cFOS and NT [44].

An MP-10-related PDE10A inhibitor, TP-10 **7**, was utilized for in vivo electrophysiology experiments in freely moving rats. In these studies, PDE10A inhibition led to an increased responsiveness of medium spinal neurons to corticalstriatal input [53]. Additionally, the data indicates that striatopallidal MSNs are more responsive to depolarizing currents, suggesting a larger effect on the direct basal ganglia pathway. Electrophysiology experiments using MP-10 suggest PDE10A

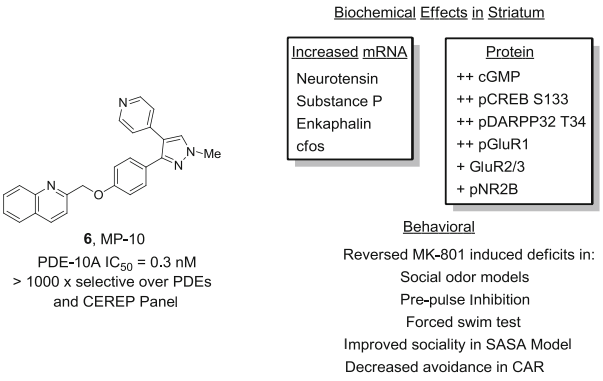



**Fig. 7** MP-10 structure, biochemical and behavior effects

inhibition attenuates dopaminergic neuron firing in the ventral tegmented area (VTA), a brain region associated with emotions and reward, following a high dose of D-amphetamine [54]. A caveat to these results is that there was no inhibition at a low dose of D-amphetamine suggesting a D1-regulated feedback control may be required for the effect seen with PDE10A inhibition.

These biochemical effects provided the impetus to broadly assess the impact **6** has in models predictive of antipsychotic activity and extrapyramidal side effects (EPS). In the case of antipsychotic activity, **6** was shown to attenuate an *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, induced deficits in a pre-pulse inhibition study (PPI) at 3 and 10 mg/kg doses but had no effect on startle response in this assay. In terms of negative symptoms, this inhibitor improved sociality in a rodent social approach/social avoidance model in the 0.1 and 0.3 mg/kg groups. Interestingly, preclinical cognition models provided mixed results. Inhibitor **6** reversed MK-801-induced deficits in social odor recognition model at 3 mg/kg po but not in a novel object recognition model at any dose. This lack of efficacy in the NOR model may be compound specific as other PDE10A inhibitors have shown positive effects.

In terms of locomotor effects, **6** produced a minimal effect in a mouse catalepsy model with the peak cataleptogenic potential observed at 47% at the 3 mg/kg i.p. dose 90 min after dosing. In an apomorphine-induced climbing model that measures EPS liability MP-10 decreased induced climbing with an ID50 of 0.375 mg/kg i.p. and induced stereotypy with an ID50 of 25 mg/kg. Both the catalepsy and climbing model results suggest that **6** behaves like an atypical antipsychotic with minimal EPS effects. Inhibitor **6** decreased avoidance in the conditioned avoidance response assay which is proposed to be predictive of antipsychotic activity with an ID50 of $1.07 \pm 0.167$ mg/kg i.p. [55]. Additionally, PDE10A inhibition led to reversal of MK-801-induced immobility in a forced swim test model, proposed to mimic negative symptoms in scz, using mice at 1 mg/kg po [56]. Interestingly, a recent report showed **6** induced significant akathisia-like behavior in nhp at 3 mg/kg but not 1 mg/kg (Fig. 7) [57]. Derivative **7** was subjected to a similar battery of tests and demonstrated a nearly exact outcome in these assays compared to **6** [58].

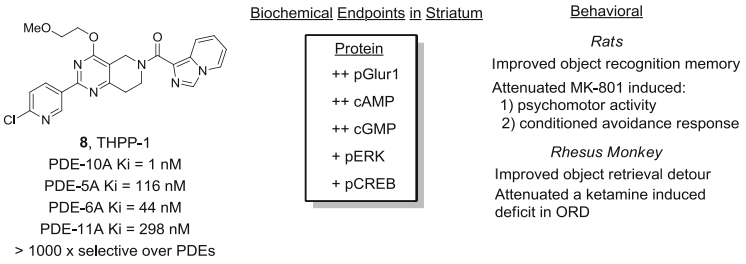



**Fig. 8** THPP-1 structure, biochemical and behavior effects

THPP-1

Tetrahydropyridopyrimidine (THPP-1) **8** is a potent and selective PDE10A inhibitor having a favorable selectivity profile against other PDEs (Fig. 8) [59]. It has an excellent pharmacokinetic (PK) profile with low clearance $<8$ mL/min and oral bioavailability $>31\%$ across preclinical species. Inhibitor **8** showed a dose-dependent increase in rat CSF suggesting it is brain penetrant and, therefore, suitable for use in measuring central effects and behavioral tasks. Following oral administration, **8** demonstrated a dose-dependent increase of cAMP, cGMP, pGlur1 S845 and, to a lesser but significant extent, phospho extracellular receptor kinase (ERK) and phospho CREB in rat striatal tissue. These latter two enzymes play a role in signaling pathways that modulate glutamate and dopamine signaling in the striatum [60–62]. These protein changes were not observed in other brain regions, which is consistent with the expression pattern of PDE10A.

Having demonstrated excellent neuropharmacokinetics and increases of proteins involved in synaptic plasticity, **8** was utilized for behavioral tasks in both rodents and nonhuman primates. In rats, **8** attenuated MK-801-induced locomotion in both acute and, importantly, 14-day subchronic dosing at 3 mg/kg po. This suggests that PDE10A inhibition will not diminish in efficacy due to toleration. Similar to **6**, inhibitor **8** decreased avoidance response at 1 and 3 mg/kg po. In the novel object recognition (NOR) cognition task, a 1 mg/kg po administration of **8** significantly improved the ability of a rat to identify new items after 48 h post initial dose. An additional cognition experiment was reported for nonhuman primates (NHP) where **8** improved difficult task success and reversed ketamine-induced deficits in an object retrieval detour (ORD) task at 10 mg/kg. This is the only report of PDE10A inhibition improving cognition in nonhuman primates (Fig. 8).

## *3.5 PET Ligands*

The use of positron emission tomography (PET) to detect radioactive decay from isotopically labelled compounds has become an integral tool for neuroscientists. This noninvasive technique allows for the observation of drug action in the brain,

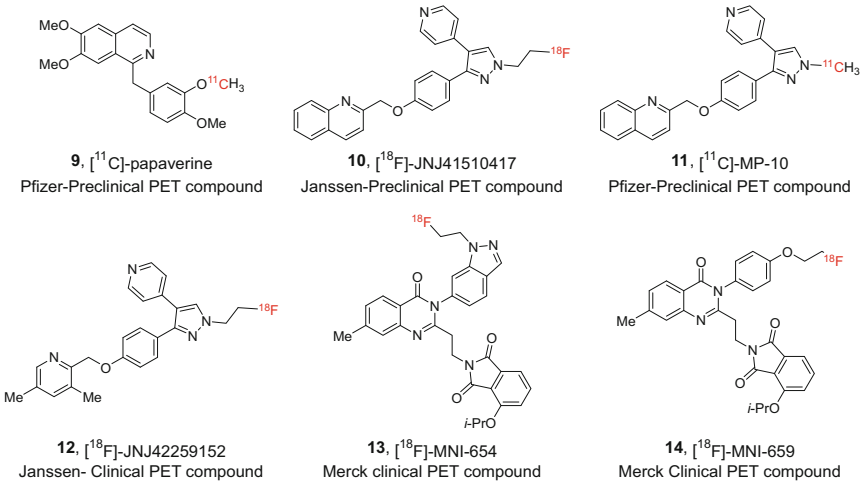



**Fig. 9** PDE10A PET ligands

helps to validate target engagement, and informs future development decisions. Because PDE10A inhibition has shown promise for treating scz, several efforts for developing radioactive PET ligands have been reported (Fig. 9).

The first PET tracer was [$^{11}$C]-papaverine **9** [63]. Consistent with earlier reports, this radioligand exclusively labelled the striatum in ex vivo rat brain slices. While in vivo studies showed **9** did initially accumulate in PDE10A-rich regions of the brain in rat and nonhuman primates, rapid clearance and modest potency prevents this compound from being a useful PET tracer in the clinic due to a rapid decrease in signal.

The high potency/selectivity and favorable in vivo profile of **6** renders this compound an attractive candidate for PET studies. As such, radioligands that incorporate $^{18}$F and $^{11}$C radioisotopes have been profiled in vivo. In one account, JNJ41510417 **10** was measured for tissue distribution and latency times in mice and rats [64]. It was found to bind specifically and reversibly to PDE10A in the striatum as shown in wild-type and KO animals. However, two issues were reported. One, its relatively slow kinetics necessitate prohibitively longer acquisition times in the clinic and, two, the presence of a brain-penetrant radiometabolite may complicate the quantifying of PDE10A in the striatum. An alternative radioligand was prepared as [$^{11}$C]MP-10, compound **11**. This ligand had a similar striatal distribution as that reported for **10** in both nonhuman primate and rodent brains [65]. Unfortunately, a time-dependent accumulation of radioactivity was observed in both the striatum and cerebellum that was attributed to a brain-penetrant metabolite. As before, the presence of this metabolite complicated accurate quantifying of PDE10A receptor engagement.

An improved radioligand, [$^{18}$F]-JNJ42259152 **12**, has recently been reported [66]. A thorough preclinical evaluation indicated this tracer binds specifically to

PDE10A and has a good latency period. Despite the presence of a brain-penetrant metabolite, the researchers utilized a reference tissue model to quantify the binding potential for this radioligand. Having found an accurate quantifying method, **12** was utilized as the first PDE10A radioligand in the clinic to measure biodistribution and dosimetry [67]. The results show **12** is safe and well tolerated with good striatal uptake and good washout. Interestingly, in contrast to reports of PDE10A mRNA expression in the testes and spermatozoa [20, 68], no radioactivity was measured in this region. These results are consistent with an immunohistochemical experiment that did not find PDE10A protein in the testes [23]. This is one of the two advanced PDE10A radioligands and these promising initial results warrant further clinical analysis for use in disease characterization and PDE10A binding kinetics in the brain (Fig. 9).

Recently, PET data using two Merck chemotypes, MNI-654 **13** and MNI-659 **14** were reported. In nonhuman primates both tracers displayed good signal in the putamen and GP and were blocked from binding to this region by MP-10 in a dose-dependent manner. Receptor occupancy for MNI-659 was 85% at 1.8 mg/kg; these data were not reported for MNI-654. Subsequently these compounds were progressed into the clinic with healthy volunteers. In the event, MNI-654 showed variability after repeat dosing. In contrast, MNI-659 showed consistent signal and is currently being used to assess PDE10A expression levels in Huntington's disease patient populations [69–71].

One last disclosure was reported from Immanova using IMA106 and IMA107, structures unknown, along with MP-10 as a comparator in a PET study using baboon and pigs. In both species these compounds localized to brain regions consistent with high levels of PDE10A expression and their specificity for this receptor was demonstrated by competition with an unidentified inhibitor. Additional information regarding use of these PET ligands for future studies were not discussed [72].

### *3.6 Competitive Landscape*

Due to the compelling biological dataset that correlates PDE10A inhibition with positive benefits in psychiatric and neurodegenerative disorders there has been broad interest in developing small molecule treatments. This section provides a general survey of the reported patentscape. Exemplars were chosen based on potency and to give a general sense of the diverse range of chemotypes developed to inhibit PDE10A. In the cases where potency data were unavailable, a representative example was chosen. In this crowded field, many patents have been filed that cover a broad spectrum of chemical matter, and the authors refer the reader to the original patents to build a deeper understanding of SAR and key structural interactions. Additional off-target potency data has been incorporated when reported.

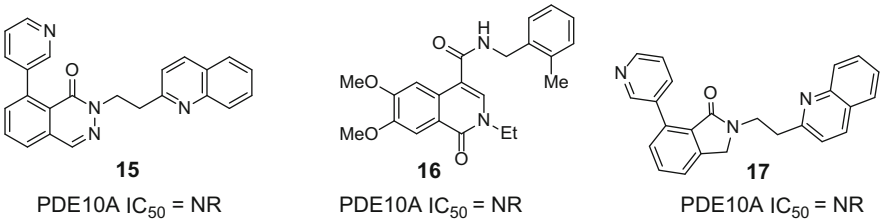



**Fig. 10** Representative chemotypes disclosed by AbbVie/Abbott

### 3.6.1 AbbVie/Abbot

AbbVie reported on a series of 6–6 fused heterocyclic cores that include phthalazinone and isoquinolone derivatives **15** and **16** [73, 74]. No potency data was provided in these patents (Fig. 10). Abbott published a recent patent of which indolinone **17** was identified as a representative chemotype [75]. No potency data was provided in this patent.

### 3.6.2 Allergan

Allergan has filed two patents [76, 77], one of which is a selection invention for retinal diseases containing the MP-10 chemotype **6**. Another example contains an isoquinolinone isostere of the papaverine core as shown in **18**. Both compounds are highly potent derivatives that bind to PDE10A with $IC_{50} < 1$ nM and extend the use of PDE10A inhibitors to retinal diseases (Fig. 11).

### 3.6.3 Altana Pharma AG

Altana has filed two patents claiming a range of substitution patterns around a pyrrolodihydroisoquinoline core **19** and **20** [78, 79]. In their first patent, their matter is utilized to treat cancer that, while outside of the scope of this review, represents another opportunity for PDE10A inhibitors (Fig. 12).

### 3.6.4 Amgen/Memory

Amgen/Memory have reported on a range of different chemotypes having nM to sub nM binding potencies to PDE10A [80–83]. Early chemotypes were focused on papaverine type analogs substituted with a variety of disubstituted pyridines, two potent examples bearing 2-amino groups such as compounds **21** and **22**. Finally, cyclobutanes and azetidines, such as **23** and **24**, were reported in a series of more recent patents demonstrating the successful use of phenyl bioisosteres to improve the ligand efficiency (Fig. 13).

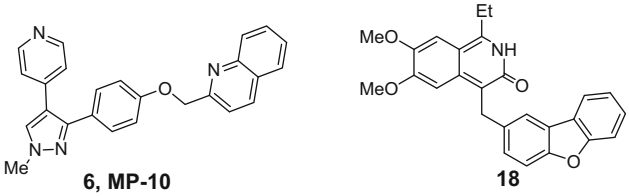



**Fig. 11** Representative chemotypes disclosed by Allergan

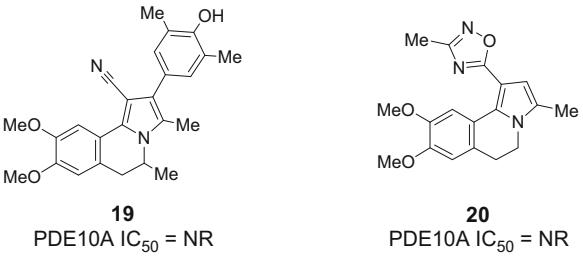



**Fig. 12** Representative chemotypes disclosed by Altana

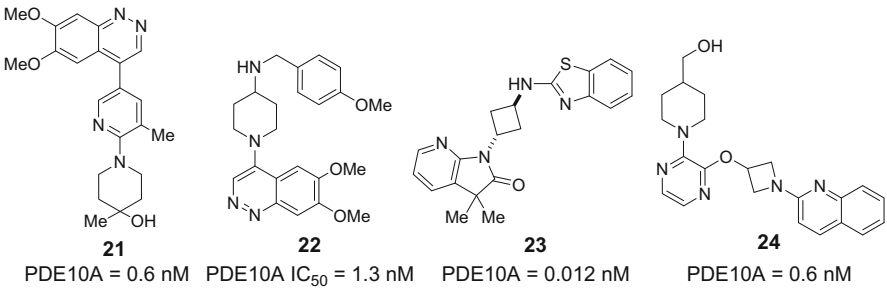


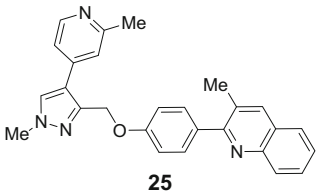

**Fig. 13** Representative chemotypes disclosed by Amgen/Memory

### 3.6.5 Astellas

Astellas has filed a single patent on a regioisomer related to **6** [84]. In this case, the ether linker is repositioned from the phenyl and quinazoline to between the pyrazole and phenyl as shown by **25**. This alternative configuration has sub-nanomolar potency (Fig. 14).

### 3.6.6 Bayer

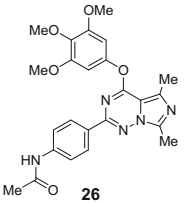
Bayer has filed several patents on PDE10A inhibitors for neurological disorders, cancer [85–88], and diabetes [89]. For central nervous system (CNS) disorders,




**25**

PDE10A $IC_{50}$ = 0.4 nM

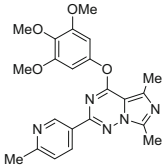
**Fig. 14** Representative chemotype disclosed by Astellas




**26**

PDE10A $IC_{50}$ = NR

Single Compound in Patent




**27**

PDE10A $IC_{50}$ = NR

**Fig. 15** Representative chemotypes disclosed by Bayer

the chemotypes are largely based on imidazotriazine cores, such as **26** and **27**, with small changes being introduced in the 2-position [90, 91]. Potency data was not reported for this series of compounds (Fig. 15).

### 3.6.7 Biotie/Wyeth/Elbion

A collaboration between Wyeth/ Biotie and, by extension, Elbion, produced a range of fused heterocyclic tricycles that included fused imidazo[1,5-A]-pyrazines, **28**, pyrido[3,2-E]pyrazines **29**, and imidazotriazine, **30** [92–94]. In general, these are single digit nM binders to PDE10A and were reported to have good PDE2 selectivity in the case of **30** (Fig. 16).

### 3.6.8 Bristol Myers Squibb

BMS has reported on compounds bearing a triazolopyrazine bicycle connected to fused, **31**, and unfused, **32**, bicyclic cores that bind strongly to PDE10A (Fig. 17) [95, 96].

### 3.6.9 EnVivo Pharmaceuticals

EnVivo has developed novel chemotypes for their inhibitors; two chemotypes are shown below [97, 98]. Their patents cover hydrazone linkers, such as **33** and biphenyl ethers derivatives exemplified by **34**. No potency data was reported in these patents (Fig. 18).

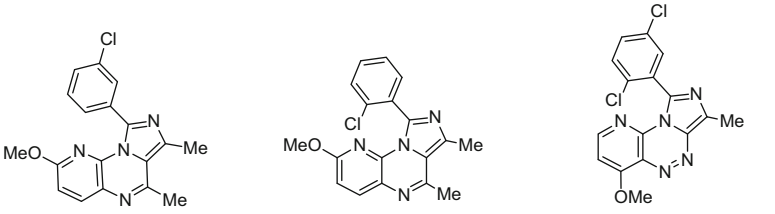



**Fig. 16** Representative chemotypes disclosed by Biotie/Wyeth/Elbion

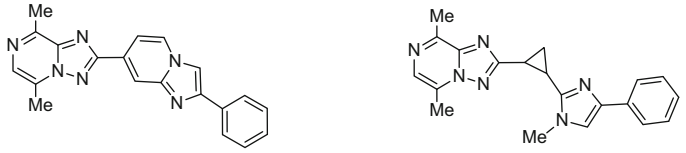



**Fig. 17** Representative chemotypes disclosed by BMS

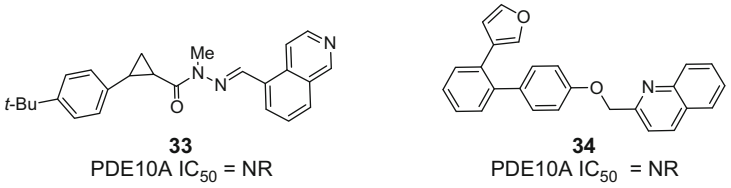


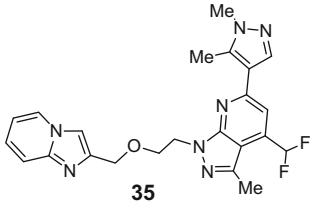

**Fig. 18** Representative chemotypes disclosed by EnVivo

### 3.6.10 Evotec LTD

Evotec has reported on a fused pyrazolopyridine core **35** bearing a conserved substituted pyrazole as PDE10A inhibitors [99]. This is a potent series of compounds where the exemplar $IC_{50} = 7$ nM (Fig. 19).

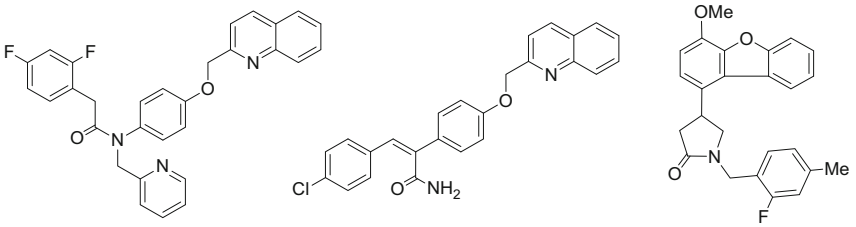
### 3.6.11 Glenmark

Glenmark has filed three recent patents that describe two related chemotypes bearing heterocycles linked to aryl ether cores, such as amide **36** and acrylamide **37**, and heterocyclic cores, such as pyrollidinone **38** shown below [100–102]. Potency data was not provided (Fig. 20).




**35**
PDE10A $IC_{50}$ = 7 nM

**Fig. 19** Representative chemotype disclosed by Evotec

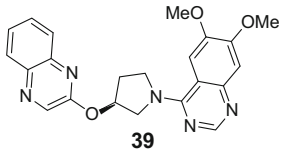



**36**
PDE10A $IC_{50}$ = NR

**37**
PDE10A $IC_{50}$ = NR

**38**
PDE10A $IC_{50}$ = NR

**Fig. 20** Representative chemotypes disclosed by Glenmark




**39**
PDE10A $IC_{50}$ = NR

**Fig. 21** Representative chemotype disclosed by Helmholtz/Leipzig

### 3.6.12 Helmholtz-Zentrum Dresden/University of Leipzig

This patent describes the use of novel papaverine analogs as PDE10A inhibitors, exemplified by **39** [103]. Potency data was not reported (Fig. 21).

### 3.6.13 Hoffman-LaRoche

Hoffman-LaRoche has published many patents in this arena that report inhibitors which bind to PDE10A with sub-nanomolar potencies, a few recent examples are shown below [104–106]. These include amide substituted amino pyridines **40**, diamide substituted N-alkyl pyrazoles, exemplified by **41**, and triazolopyrimidines, **42**, as potent PDE10A inhibitors (Fig. 22).

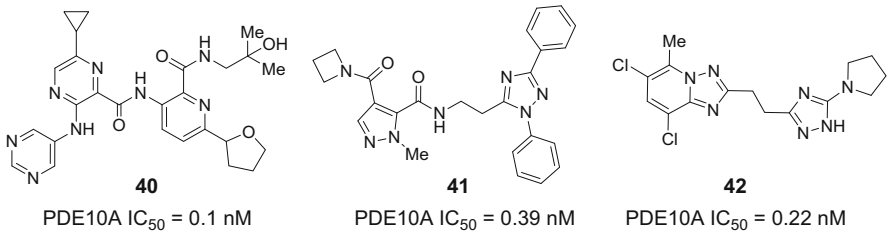



**Fig. 22** Representative chemotypes disclosed by Hoffman-LaRoche

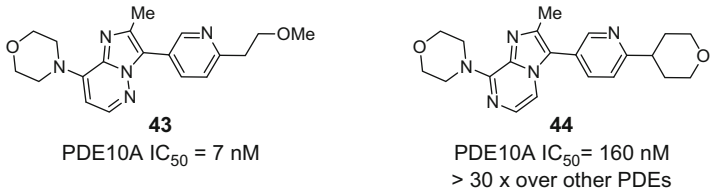



**Fig. 23** Representative chemotypes disclosed by Janssen

### 3.6.14 Janssen Pharmaceuticals

Janssen filed three recent patents around imidazo[1,2-b]pyridazine derivatives **43** and imidazo[1,2-a]pyrazine analogs **44**, the exemplified compound of which was covered as a single compound [107–109]. In addition to these, Janssen has filed two additional patents on the previously discussed PET ligands (Fig. 23) [110, 111].

### 3.6.15 Kyorin Pharmaceuticals

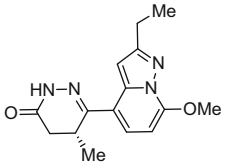
Kyorin has developed pyrazolopyridine-4-yl pyridazinone derivatives, exemplified by **45**, as PDE10A inhibitors [112]. No potency data was reported in this patent (Fig. 24).

### 3.6.16 Kyowa, Hakko, Kogyo

In this patent, biaryl substituted quinolines, exemplified by **46**, are reported to inhibit PDE10A [113]. Potency data was unavailable (Fig. 25).

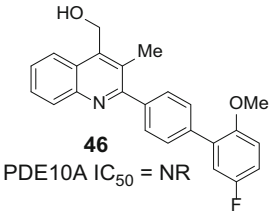
### 3.6.17 Lundbeck

Lundbeck has reported several chemotypes as PDE10A inhibitors (Fig. 26). Several cases describe closely related phenotypes exemplified by aryl substituted



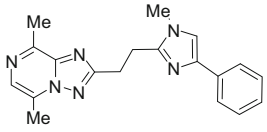

**45**
PDE10A $IC_{50}$ = NR

**Fig. 24** Representative chemotype disclosed by Kyorin

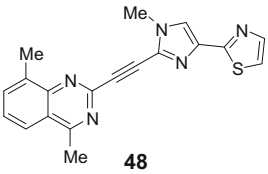



**46**
PDE10A $IC_{50}$ = NR

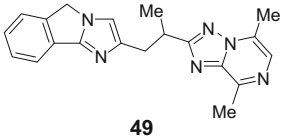
**Fig. 25** Representative chemotype disclosed by Kyowa, Hakko, Kogyo




**47**
PDE10A $IC_{50}$ = 2 nM




**48**
PDE10A $IC_{50}$ = 0.007 nM




**49**
PDE10A $IC_{50}$ = 7.7 nM

**Fig. 26** Representative chemotypes disclosed by Lundbeck

imidazoles or triazoles are connected to fused heterobicycles via small aliphatic linker, such as compounds **47** and **48**, and are potent nanomolar inhibitors [114, 115]. In a recent series of filings tricyclic heterocycles such as **49** were reported having single digit nanomolar binding affinities to PDE10A [116].

### 3.6.18 Merck

Merck has been active in this field and has published on a diverse set of chemotypes (Fig. 27). In several embodiments, disubstituted cyclopropyl linkers bearing fused heterocycles such as **50** bind to PDE10A with $IC_{50} < 1$ nM [117]. Recent embodiments describe a number of potent compounds containing a range of heterocyclic functionality as shown by compounds **51** and **52** [118, 119]. A patent covering their aforementioned PET ligand has also been filed (Fig. 32) [120].

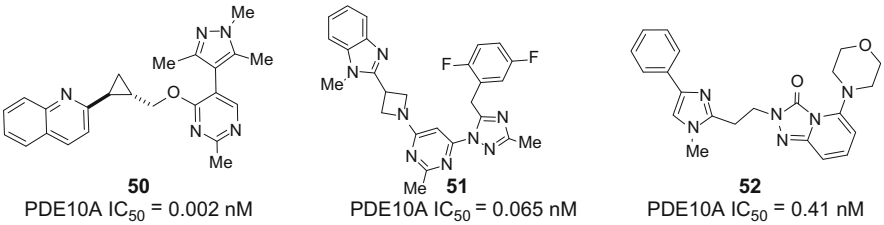



**Fig. 27** Representative chemotypes disclosed by Merck

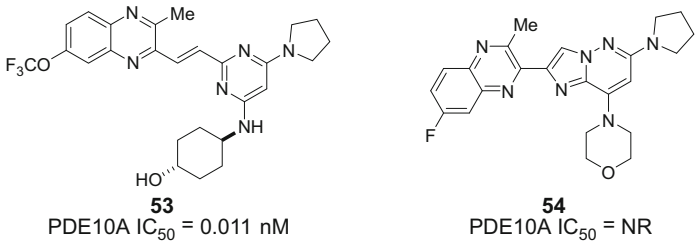



**Fig. 28** Representative chemotypes disclosed by Mitsubishi Tanabe

### 3.6.19 Mitsubishi Tanabe Pharma

Mitsubishi Tanabe has discovered a number of aminopyrimidine containing PDE10A inhibitors such as **53** as are highly potent, subnanomolar inhibitors of the enzyme [121]. In one recent patent trisubstituted heterocyclic cores such as fused imidazopyridazines **54** were disclosed [122]. These compounds are highly potent, subnanomolar inhibitors of the enzyme (Fig. 28).

### 3.6.20 Mochida Pharmaceutical Co Ltd

Mochida has reported alkene linked heterocycles, exemplified by **55** and **56**, as PDE10A inhibitors but does not provide potency values (Fig. 29) [123, 124].

### 3.6.21 Omeros Corporation

Omeros has reported on two distinct chemotypes. The first is disclosed in a series of three patents and describe a range of heteroaryl, **57**, and aryl **58** substitutions on a hydrazone linker [125, 126]. Potency data for the exemplars were not reported (Fig. 30).

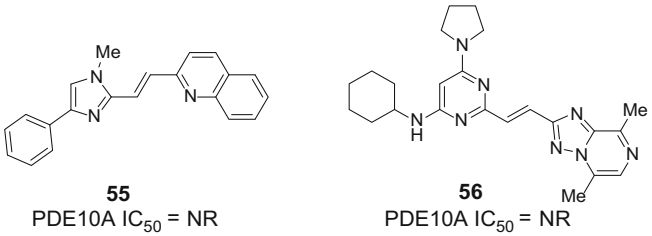



**Fig. 29** Representative chemotypes disclosed by Mochida

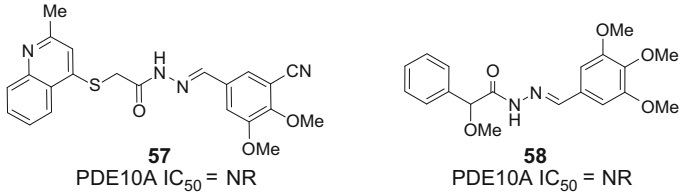



**Fig. 30** Representative chemotypes disclosed by Omeros

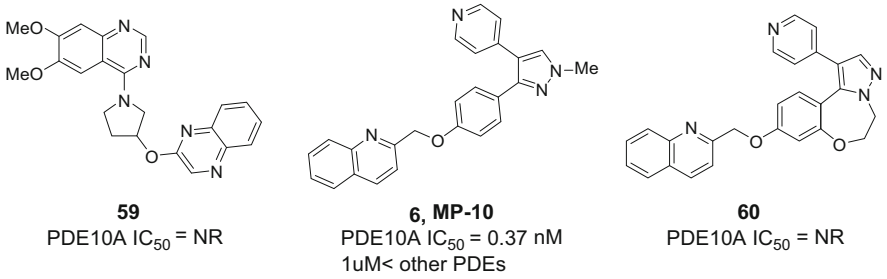



**Fig. 31** Representative chemotypes disclosed by Pfizer

### 3.6.22 Pfizer

Pfizer has published several patents detailing compounds for use as PDE10A inhibitors (Fig. 31). One series, exemplified by **59**, was based on the papaverine pharmacophore bearing substituted heterocycles such as pyrrolidines and piperidines [127]. A second series of patents describe the use of disubstituted pyrazole phenyl ethers, such as **6**, and cyclized versions, such as **60**, as inhibitors [128, 129]. One exemplified compound **6** is the aforementioned clinical candidate, MP-10.

### 3.6.23 Schering Corporation

Prior to the merger with Merck, Schering released two patents [130, 131]. One embodiment covers a number of substituted triazolopyridines **61** and pyrazoloquinazoline **62** derivatives. Additional heterocylic fused papaverine analogs, exemplified

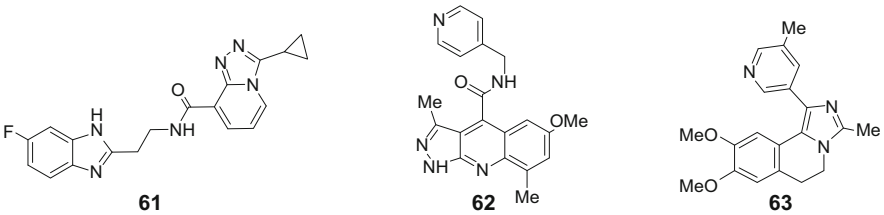



**Fig. 32** Representative chemotypes disclosed by Schering

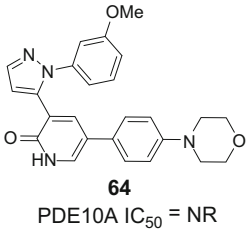



**Fig. 33** Representative chemotype disclosed by Taisho

by **63**, were discovered in a joint Schering/Organon collaboration [132]. Potency values were not reported (Fig. 32).

### 3.6.24 Taisho Pharmaceutical Co.

Taisho has described pyrazolylpyridone derivatives, such as **64**, but does not include potency data in their patent (Fig. 33) [133].

### 3.6.25 Takeda Pharmaceutical Co. Ltd.

Takeda has filed several patents around three primary chemotypes (Fig. 34). One series of patents share a common pyridazinone pyrazole bicycle substituted with a variety of aryl, heteroaryl, and alkyl substituents exemplified by **65** [134] . While potency data was typically unavailable, compound **65** was reported to be a very potent and selective PDE10A inhibitor. Additionally, several patents claim the use of heteroaryl substituted phenyl ethers, exemplified by **66** PDE10A inhibitors [135]. Finally, one recent embodiment describes the use of biarylether indolinones for inhibiting PDE10A, **67** is one such example [136].

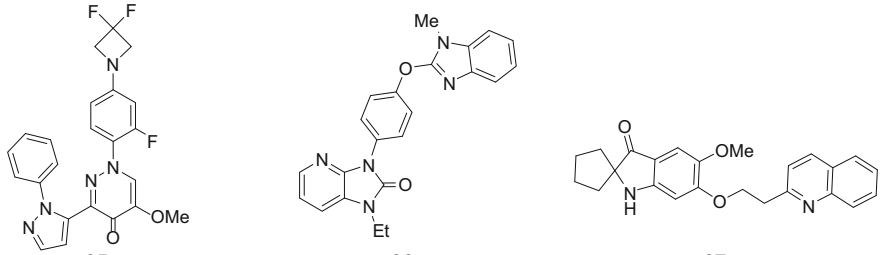



**Fig. 34** Representative chemotypes disclosed by Takeda

## *3.7 Clinical Trials*

Several PDE10A inhibitors have progressed to the clinic and are mostly at the Phase I stage with two candidates actively recruiting for Phase II [137]. In general, most trials utilize low (<20 mg) doses of drug candidates suggesting these are potent compounds having good central exposures. To date, no data has been published from completed trials, although in a recent poster, Pfizer reported no differentiation over control in a clinical study using **6** [138]. One interesting hypothesis that has emerged from this failed clinical trial postulates there may be an undesired balancing out of both direct and indirect pathway signaling in the basal ganglia. This co-stimulation may interfere with the desired antipsychotic effects attributed to indirect pathway activation [139]. Indeed, this lack of efficacy has spurned a series of recent activity aimed at explaining the gaps and issues with PDE10A biology [140, 141].

Several studies are currently recruiting patients to assess safety, tolerability, and effect of PDE10A inhibitors in stable scz patients and as an adjunct with current standard of care. Recently, Omeros reported results from a Phase 2a trial in which scz patients receiving standard of care treatment were dosed with their candidate at a concentration that exceeded a 66% PDE10A receptor occupancy without EPS which is a first for the field. In light of these results, Omeros is recruiting for a Phase 2 trial using OMS824 to treat psychiatrically stable scz patients [142]. Also, ongoing PET studies are in the recruitment phase indicating companies are seeking to develop a deeper understanding of PDE10A engagement in the brain and inhibitor binding kinetics (Table 1). In recent news, PDE10A inhibitors are being utilized in Huntington's disease trials.

**Table 1** Clinical landscape of PDE10A inhibitors[a]

| Company | Compound | Clinical dose | Title | Phase | NCT Number | Status |
|---|---|---|---|---|---|---|
| Amgen | AMG579 | NR | First-in-Human Study to Evaluate AMG 579 in Healthy Subjects and Patients with Stable Schizophrenia | 2 | 1568203 | Terminated |
| CHDI Foundation | [$^{18}$F] MNI-659 | <10 μg | [PETDE10] Imaging of PDE10A Enzyme Levels in Huntington's Disease Gene Expansion Carriers and Healthy Controls With PET | 0 | 02061722 | Recruiting |
| EnVivo | EVP-6308 | NR | Study to Assess the Safety, Tolerability, Pharmacokinetic (PK) and Pharmacodynamic (PD) Effects of EVP-6308 and the Potential of EVP-6308 to Affect the PK Properties of the Antipsychotic Regimen in Subjects with Schizophrenia Currently Receiving Stable Treatment with up to 2 Atypical Antipsychotics | 1 | 02037074 | Not yet recruiting |
| EnVivo | EVP-6308 | NR | Study of EVP-6308 to Assess the Dose- and Concentration-dependent Displacement of [$^{18}$F] MNI-659 by EVP-6308 | 1 | 2001389 | Recruiting |
| Hoffman-La Roche | RO5545965 | 4-6 mg, QD, oral | A Study of the Safety, Tolerability and Pharmacokinetics of RO5545965 in Patients with Scz on Risperidone | 1 | 2019329 | Recruiting |
| Hoffman-La Roche | RO5545965 | Single dose, amount NR | A PET Study with RO5545965 in Healthy Male Volunteers | 1 | 1923025 | Completed |

(continued)

**Table 1** (continued)

| Company | Compound | Clinical dose | Title | Phase | NCT Number | Status |
|---|---|---|---|---|---|---|
| Hoffman-La Roche | RO5545965 | NR | A Study of Safety, Pharmacokinetics (Including Food Effect) and Pharmacodynamics of RO5545965 in Healthy Volunteers | 1 | 1711801 | Completed |
| Hoffman-La Roche | RO5545965 | NR | A Study of Safety, Pharmacokinetics and Pharmacodynamics of RO5545965 in Healthy Volunteers | 1 | 1864226 | Completed |
| Omeros | OMS824 / OMS6433762 | NR | Safety, Tolerability, and Pharmacokinetics of OMS643762 in Psychiatrically Stable Schizophrenia Subjects | 2 | 1952332 | Recruiting |
| Omeros | OMS824 / OMS6433762 | NR | Safety and Efficacy of OMS643762 in Subjects with Huntington's Disease | 2 | 2074410 | Recruiting |
| Takeda | TAK-063 | NR | Effects of TAK-063 on Preventing Ketamine-Induced Brain Activity Changes as Well as Psychotic-Like Symptoms in Healthy Male Adults | 1 | 01892189 | Recruiting |
| Takeda | TAK-063 | NR | Safety, Tolerability and Pharmacokinetic Study of Multiple Rising Doses of TAK-063 in Participants with Stable Schizophrenia and Healthy Participants | 1 | 01879722 | Recruiting |
| Pfizer | PF-02545920 | 10 mg, single dose | Effects of PF-02545920 on Ketamine-Induced Abnormal Prefrontal Brain Response to Associative Learning in Healthy Subjects | 1 | 01244880 | Terminated |
| Pfizer | PF-02545920 | 2 mg BID × 7 days, then 5 mg BID until week 12; 5 mg BID × 7 days, then | An Outpatient Study of the Efficacy, Safety, and Tolerability of PF-02545920 in the Adjunctive Treatment of Sub-Optimally Controlled Symptoms of Schizophrenia | 2 | 01939548 | Recruiting |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 10 mg × 7 days, then 15 mg BID until week 12 | | | | |
| Pfizer | PF-02545920 | 15 mg BID for 21 days | Double Blind, Randomized, 3 Week Inpatient Study to Evaluate the Safety and Efficacy of PF-02545920 Compared with Placebo | 2 | 00570063 | Terminated |
| Pfizer | PF-02545920 | 15 mg (2 mg × 2 days, 5 mg × 2 days, 8 mg × 3 days, then 15 mg) Q12 h<br>15 mg (5 mg × 2 days, 10 mg × 2 days, then 15 mg) Q12 h<br>15 mg (5 mg BID for 7 days 10 mg BID for 7 days, then 15 mg BID for 4 days) Q12 h | A Study to Examine the Safety, Tolerability and Pharmacokinetics of PF-02545920 in Psychiatrically Stable Subjects with Schizophrenia | 1 | 01829048 | Completed |
| Pfizer | PF-02545920 | NR | Study Investigating the Safety and Tolerability of Multiple Doses of PF-02545920 in Subjects with Schizophrenia | 1 | 00463372 | Completed |
| Pfizer | PF-02545920 | 1,2,5,10, 30 mg | A Study to Evaluate the PDE10A Enzyme Occupancy Following a Single Dose of PF-02545920 in Healthy Male Volunteers (PET) | 1 | 01918202 | Not yet recruiting |

(continued)

**Table 1** (continued)

| Company | Compound | Clinical dose | Title | Phase | NCT Number | Status |
|---|---|---|---|---|---|---|
| Pfizer | PF-02545920 | 5 mg, 15 mg BID, 28 days | An Inpatient Study of the Efficacy, Safety, and Tolerability of PF-02545920 in the Treatment of Acute Exacerbation of Schizophrenia | 1 | 01175135 | Completed |
| Pfizer | PF-02545920 | 3, 6, 15 mg single dose | Evaluation of Striatal Glucose Metabolism with Positron Emission Tomography Following PF-02545920 in Healthy Subjects | 1 | 01103726 | Completed |
| Pfizer | PF-02545920 | 20 mg titrated and 5 mg dose | Study Evaluating the Safety, Tolerability and Brain Function of 2 Doses of PF-0254920 in Subjects with Early Huntington's Disease | 2 | 01806896 | Recruiting |

[a]The data in this table was last updated 23 March 2014

## 4 PDE9A

Phosphodiesterase 9A is a high-affinity, cGMP-specific PDE, as demonstrated by its relative difference in $K_m$ for cGMP (0.07–0.17 μM) compared to cAMP (230 μM) [143, 144]. It has one of the highest substrate affinities of all the known PDEs, which suggests that it may be functional at cGMP concentrations lower than that of any other isozyme [144]. Patients with scz have been shown to have decreased levels of cGMP in the CSF compared to healthy controls [145, 146]. This observation, along with preclinical research implicating cGMP signaling pathways in cognitive processing (vide infra), suggests that PDE9A inhibition could be a therapeutic strategy for the treatment of cognitive deficits associated with scz.

### *4.1 Splice Variants and Localization*

Human PDE9A is encoded by a single gene for which over 20 splice variants have been identified [147, 148]. Only two of the splice variants have been expressed and characterized: PDE9A1, which was found to be localized in the nucleus, and PDE9A6 (originally called PDE9A5), which was reported to be cytosolic [149]. Contrary to most other PDEs, little is known about the regulation of PDE9A and no regulatory sequences have been identified on the N-terminal domain [150].

Phosphodiesterase 9A mRNA is widely expressed throughout the body with the highest transcript levels found in kidney, brain, spleen, gastrointestinal, and prostate tissues [17, 143, 144, 147–149, 151]. In mammalian brains, PDE9A mRNA is expressed in multiple regions, including the hippocampus, striatum, cerebellum, amygdala, cortex, olfactory bulb, thalamus, and hypothalamus [17, 152–156]. Mainly expressed in neurons, high levels of PDE9A mRNA have been found in hippocampal granule and pyramidal cells and cerebellar Purkinje cells. Several of these brain regions, including the hippocampus, cerebellum, amygdala and cortex, are known to be relevant for cognition and memory [157–162]. As such, PDE9A is a promising target to test the hypothesis that cognitive deficits in scz can be treated by elevating cGMP in these brain regions through its inhibition.

### *4.2 Catalytic Domain*

Several X-ray crystal structures of inhibitors bound to the catalytic domain of PDE9A have been published, providing insight into substrate and inhibitor binding modes and isozyme selectivity profiles [163–168]. A mechanism for cGMP hydrolysis was proposed based on PDE9A X-ray crystal structures obtained by a freeze trapping technique [163]. The X-ray crystal structure of PDE9A with cGMP (PDB ID: 3DYL) reveals: (1) the flat, aromatic guanine ring is stabilized between Phe-456 and

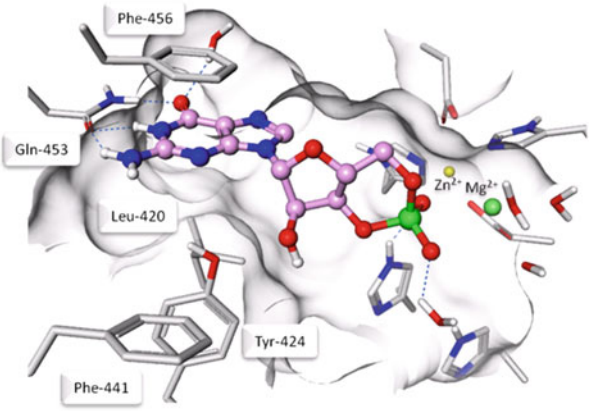



**Fig. 35** X-ray crystal structure of cGMP (*pink*) bound to human PDE9A (PDB ID: 3DYL). Key residues and binding site pocket are highlighted (*gray*) and HB interactions are depicted by *blue dashed lines*

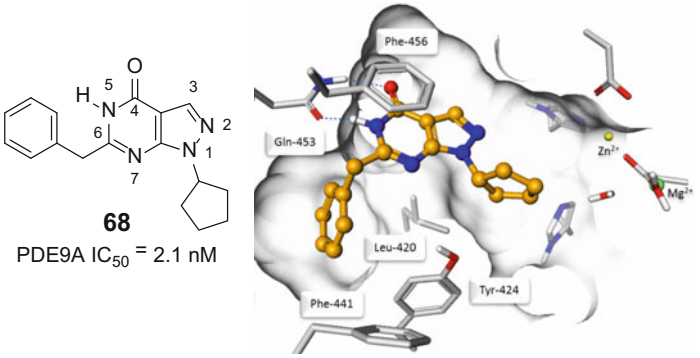



**Fig. 36** X-ray crystal structure of **68** (*orange*) bound to human PDE9A (PDB ID: 3SJI). Key residues and binding site pocket are highlighted (*gray*) and HB interactions are depicted by *blue dashed lines*

Leu-420, which form the P clamp, (2) HB interactions between the guanine ring and the invariant glutamine residue, Gln-453, and (3) interactions between the phosphate group and the divalent metals in the M pocket (Fig. 35).

In general, the small molecule inhibitors that have been co-crystallized with PDE9A have common structural motifs, suggesting there are key interactions necessary for PDE9A binding. The X-ray crystal structure of **68** with PDE9A (Fig. 36) [169] is used to highlight the characteristics of the binding pocket and these key inhibitor interactions (PDB ID: 3JSI). The bicyclic pyrazolopyrimidinone core is sandwiched between the P clamp residues, which form favorable hydrophobic interactions to hold the inhibitor in position. The pyrimidinone motif in the bicyclic core of **68** forms two HBs to the invariant Gln-453. Phosphodiesterase 9A

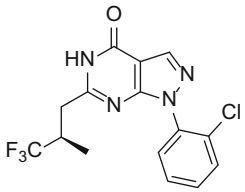
mutagenesis studies have validated the importance of the Gln-453 residue, demonstrating that mutation to Ala caused ~500-fold loss in potency of BAY 73-6691 **69** [164] and mutation to Glu caused the $K_m$ for cGMP to increase ~25-fold [165]. The cyclopentyl group of **68** is in the M pocket and forms hydrophobic interactions with the protein rather than interacting with the metal ions or the tightly bound water molecules surrounding them. Finally, the benzyl group fills the $Q_2$ subpocket; some substituents off of this position also extend into the S pocket in PDE9A inhibitors. Strategies that have been used to gain PDE9A selectivity focus on filling the hydrophobic $Q_2$ subpocket and interactions with the unconserved Tyr-424 and Phe-441 residues in the S pocket. An analysis of PDE9A inhibitors suggested that PDE9A is much more specific for the chemotypes that it binds compared to PDE2 and PDE10A and that PDE9A inhibitors are significantly more polar than those of PDE2 and PDE10A [170].

## *4.3 PDE9A in Schizophrenia*

### 4.3.1 Circuitry

It is believed that the second messenger cGMP plays a major role in synaptic plasticity through the NO/sGC/cGMP/PKG signaling cascade in the hippocampus, cerebellum, amygdala, and other brain regions involved with cognition [159, 160, 162]. The balance between cGMP synthesis by GC and degradation by PDEs determines its intracellular concentration levels. Regulation of cGMP in the brain and the role of cGMP in neuronal signaling have been thoroughly reviewed by Kleppisch and Feil [160]. Briefly, $Ca^{2+}$ influx mediated by NMDA receptors on postsynaptic neurons activates neuronal nitric oxide synthase (nNOS) leading to the production of nitric oxide (NO). This induces cGMP synthesis through activation of NO-dependent soluble GC (sGC) that, in turn, activates signaling cascades including (1) activation of PKG to initiate the cGMP/PKG/CREB pathway, which is thought to influence the synthesis of proteins that are important for LTP and memory consolidation [171], and (2) activation of cyclic nucleotide-gated ion channels which attenuate synaptic plasticity [159].

There is a substantial amount of evidence supporting the role of cGMP in cognitive processing through this pathway. For example, nNOS KO mice demonstrated impaired cognitive performance [172], hippocampal slices from doubly mutant ($nNOS^-$/endothelial NOS $(eNOS)^-$) mice had reduced LTP [173], and eNOS KO mice exhibited a deficiency in LTP [174]. In addition, there is evidence that NO levels increase with LTP-inducing electrophysiological stimulation [175], while NOS inhibitors impair hippocampal LTP [171, 176] and cognition [177]. The importance of GC in LTP was shown by NO-GC KO mice, in which LTP was abolished [178], and by GC inhibitors, which were shown to impair LTP [171, 179–182]. Intrahippocampal infusion of cGMP analogs into the rat brain demonstrated this nucleotide is involved in the early stages of memory consolidation [183–186], and injection of cGMP or cGMP analogs into cultured neurons or brain slices enhanced LTP




**69**, BAY 73-6691
PDE9A $IC_{50}$ = 55 nM

PDE1C $IC_{50}$ = 1.4 uM
PDE2A $IC_{50}$ > 4 uM
PDE3B $IC_{50}$ > 4 uM
PDE5A $IC_{50}$ > 4 uM
PDE7B $IC_{50}$ > 4 uM
PDE8A $IC_{50}$ > 4 uM
PDE10A $IC_{50}$ > 4 uM
PDE11A $IC_{50}$ = 2.6 uM

**Fig. 37** BAY 73-6691 structure and potency data

[179, 180]. These studies suggest that increased levels of cGMP enhance LTP and contribute to improved synaptic plasticity, which is hypothesized to have a positive effect on cognition.

### 4.3.2 Biochemical and Behavioral Effects of Tool Compounds

Cyclic GMP-specific PDE inhibitors are important tools to test the hypothesis that increasing cGMP levels can improve cognition through enhancing LTP and synaptic plasticity. Indeed, a number of preclinical studies have demonstrated that inhibiting cGMP-specific PDEs leads to positive cognitive effects, especially in information processing, attention, memory, and executive functioning [162, 187–194]. To date, there are only a handful of brain-penetrant, selective PDE9A inhibitors reported in the literature that have been used to probe the effects of PDE9A inhibition.

#### BAY 73-6691

Bayer was the first company to characterize and publish a potent, selective PDE9A inhibitor, BAY 73-6691 **69** (Fig. 37) [195], which has been widely profiled in biochemical assays and behavioral models. Wunder et al. showed that **69** inhibits human PDE9A activity in vitro with an $IC_{50} = 55$ nM and has good selectivity over other PDEs, with weak activity against PDE1C and PDE11A (1,400 and 2,600 nM, respectively). Compound **69** was shown to be cell penetrant and potentiated cGMP levels in a PDE9A reporter cell line under sGC stimulated conditions. It was also shown to increase cGMP levels in rodent hippocampus and amygdala [196], as well as increase basal synaptic transmission and enhance early and late LTP in rat hippocampal slices [197, 198].

Compound **69** has also been used to build an understanding of the effects of PDE9A inhibition on learning, memory, and cognition through its use in rodent behavioral models (Table 2) [197]. It was shown to improve memory consolidation in rodent models of cognition, including social recognition tasks and novel object recognition tasks in unimpaired rodents. **69** also reversed MK-801-induced short-term memory deficits in the T-maze alternation task and attenuated scopolamine-induced retention deficits in a passive avoidance task.

An X-ray crystal structure of **69** (PDB ID: 3K3E) bound to PDE9A reveals the expected binding mode [164], with the pyrazolopyrimidinone core forming two

**Table 2** Summary of effects of PDE9A tool compounds in rodent cognition models

| Cognitive process | Compound | Model | Species | Impairment | Dose | Results | References |
|---|---|---|---|---|---|---|---|
| Episodic memory | BAY 73-6691 | Novel object recognition | Rat | Unimpaired | 0.1, 0.3, 1 or 3 mg/kg p.o. 30 min before T1 | 0.1 and 0.3 mg/kg improved memory performance | [197] |
| | PF-04447943 | Novel object recognition | Rat | Scopolamine (0 or 1 mg/kg i.p. 30 min before test) | 0, 1, 3 or 10 mg/kg p.o. 60 min before test | 3 mg/kg improved scopolamine-induced deficits in episodic memory | [199] |
| | | Novel object recognition | Rat | Scopolamine (0 or 0.2 mg/kg s.c. 30 min before test) | 0, 0.32, 1 or 3.2 mg/kg s.c. 60 min before test | 1 and 3.2 mg/kg improved scopolamine-induced deficits in episodic memory | [200] |
| Spatial memory | BAY 73-6691 | T-maze alternation | Mouse | MK-801 (0 or 0.06 mg/kg s.c. 30 min before test) | 0, 1, 3 or 10 mg/kg p.o. 60 min before test | 10 mg/kg improved MK-801-induced deficits in spatial memory | [197] |
| | PF-04447943 | Y-maze spatial recognition | Mouse | Unimpaired | 0, 1 or 3 mg/kg p.o. 30 min before test | 1 and 3 mg/kg improved spatial memory | [199] |
| | | Morris water maze | Rat | Scopolamine (0 or 0.32 mg/kg s.c. 30 min before test) | 0, 3.2 or 10 mg/kg s.c. 60 min before test | 3.2 and 10 mg/kg improved scopolamine-induced deficits in spatial memory | [200] |
| Working memory | BAY 73-6691 | Passive avoidance | Rat | Scopolamine (0 or 0.03 mg/kg s.c. 30 min before test) | 0, 0.3, 1 or 3 mg/kg p.o. 60 min before test | 1 and 3 mg/kg attenuated scopolamine-induced retention deficits | [197] |
| | PF-04447943 | Radial arm maze | Rat | Ketamine (0 or 10 mg/kg | 0, 0.032, 0.1, 0.32, 1, 3.2 or 10 mg/kg | | [200] |

(continued)

**Table 2** (continued)

| Cognitive process | Compound | Model | Species | Impairment | Dose | Results | References |
|---|---|---|---|---|---|---|---|
| | | | | s.c. 30 min before test) | s.c. 60 min before test | 1 mg/kg improved ketamine-induced deficits in working memory | |
| Social Memory | BAY 73-6691 | Social recognition | Rat | Unimpaired | 0, 0.03, 0.3 or 3 mg/kg p.o. 60 min before T1 | 0.3 and 3 mg/kg improved memory performance with a familiar juvenile | [197] |
| | BAY 73-6691 | Social recognition | Mouse | Unimpaired | 0, 0.03, 0.3 or 3 mg/kg p.o. 30 min before T1 | 0.3 and 3 mg/kg improved memory performance with a familiar juvenile | [197] |
| | BAY 73-6691 | Social recognition | Rat | Unimpaired | 0, 0.03, 0.3 or 3 mg/kg p.o. immediately after T1 or 60 min before T2 | 0.03, 0.3, and 3 mg/kg improved memory performance with a familiar juvenile | [197] |
| | BAY 73-6691 | Social recognition | Rat | Unimpaired | 0 or 1 mg/kg p.o. 60 min before T1 | 1 mg/kg improved memory performance with a familiar juvenile and not with a novel juvenile | [197] |
| | PF-04447943 | Social recognition | Mouse | Unimpaired | 0, 1, 3 or 10 mg/kg p.o. 30 min before T1 | 1 mg/kg improved memory performance with a familiar female | [199] |
| Attention | PF-04447943 | Conditioned avoidance attention | Rat | Scopolamine (0 or 0.13 mg/kg s.c. 30 min before test) | 0, 0.1, 0.3, 1 or 3 mg/kg i.p. 30 min before test | 1 mg/kg improved scopolamine-induced attention deficits at 1 and 3 second trials | [201] |
| Sensori-motor processing | PF-04447943 | Auditory gating | Rat | D-amphetamine sulfate (1 mg/kg i.v.) | 0, 0.1, 0.32, 1 or 10 mg/kg s.c. | 0.32, 1 and 10 mg/kg restored amphetamine-induced deficits in auditory gating | [200] |
| | PF-04449613 | Auditory gating | Rat | | 1 mg/kg s.c. | 1 mg/kg restored amphetamine-induced | [200] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | D-amphetamine sulfate (1 mg/kg i.v.) | | deficits in auditory gating | |
| PF-04447943 | Prepulse inhibition of acoustic startle response | Mouse | MK-801 (0 or 0.178 mg/kg s.c. 30 min before test) | 0, 1, 3.2, 10 or 32 mg/kg s.c. 30 min before test + 0 or 0.32 mg/kg s.c. risperidone | 3.2, 10 and 32 mg/kg + risperidone increased PPI; no effect on PPI alone or reversing MK-801-induced deficits | [200] |
| PF-04447943 | Mescaline-induced scratching | Mouse | Mescaline (0 or 30 mg/kg p.o. 15 min before test) | 0, 1, 3.2 or 10 mg/kg s.c. 35 min before test | 10 mg/kg reversed mescaline-induced scratching | [200] |

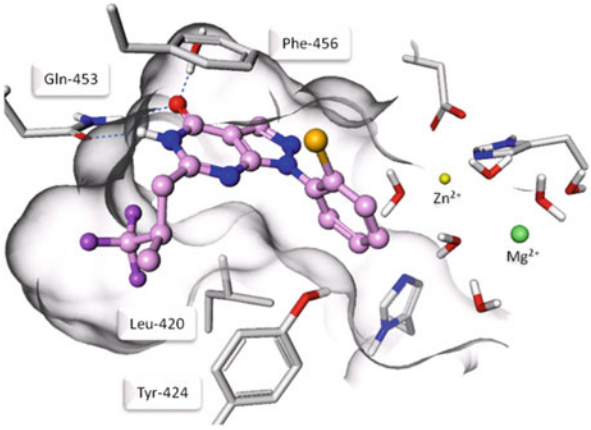



**Fig. 38** X-ray crystal structure of **69** (*pink*) bound to human PDE9A (PDB ID: 3K3E). Key residues and binding site pocket are highlighted (*gray*) and HB interactions are depicted by *blue dashed lines*

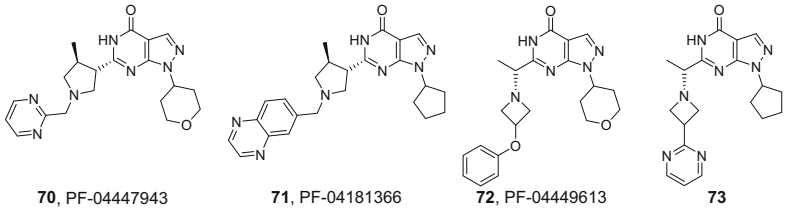



**Fig. 39** PF-04447943, PF-04181366, PF-04449613 and preclinical candidate **73** structures and potency data

HBs with Gln-453 and sandwiched between the P clamp residues, the *ortho*-Cl phenyl forming hydrophobic interactions in the M pocket, and the 3,3,3-trifluoro-2-methylpropyl substituent occupying $Q_2$ (Fig. 38). A rationale for the difference in potency between the two enantiomers is proposed based on the interactions made in the $Q_2$ pocket by the $CF_3$ in the case of **69** ($IC_{50} = 22$ nM) and the methyl group in the case of its enantiomer ($IC_{50} = 88$ nM) (PDB ID: 3K3H).

## PF-04447943 and Related Tools

Pfizer has published several papers describing the discovery of the PDE9A clinical candidate, PF-04447943, **70** ($IC_{50} = 8.3$ nM) [167], related PDE9A inhibitors, PF-04181366 **71** ($IC_{50} = 1.8$ nM) [169] and PF-04449613 **72** ($IC_{50} = 14$ nM) [166, 200], and preclinical PDE9A candidate **73** ($IC_{50} = 32.4$ nM) [166] (Fig. 39).

Inhibition of PDE9A with these selective tools was shown to produce a dose-dependent increase of cGMP in the CSF, striatum, hippocampus, cortex and/or cerebellum in rodents, NHPs, and humans [167, 169, 199, 200, 202, 203]. Consistent with these results, PDE9A KO mice were reported to have elevated levels of cGMP in the same brain regions and CSF, while no changes in cAMP were observed [202]. Compound **70** was also shown to enhance early LTP as well as increase neurite outgrowth and synapse formation in cultured hippocampal neurons [199].

These inhibitors have also been used in rodent behavioral models to build an understanding of the effects of PDE9A inhibition on learning, memory, and cognition (Table 2). Compound **70** was shown to improve cognitive performance in rodent models of cognition, including the mouse Y-maze spatial recognition and mouse social recognition tasks, and attenuated a scopolamine-induced deficit in a rat novel object recognition task [199, 200] and a rat conditioned avoidance attention task [201]. In addition, **70** and **72** have shown efficacy in a number of rodent models dependent on hippocampal cholinergic function and sensory gating, including reversal of ketamine-induced working memory disruption, scopolamine-induced spatial memory disruptions, amphetamine-induced deficits in auditory gating and mescaline-induced scratching, and potentiation of improvements in sensorimotor gating induced by risperidone [200].

Compound **70** was reported to have aligned in vitro ADME properties and an excellent lipophilic binding efficiency (LipE) of 9.58 [167]. Its brain/plasma ratio ranges from 0.59 to 0.88 in rats and mice, respectively. The free brain/free plasma ratio was reported to be 0.32 and 0.52 for rats and mice, respectively, and the free CSF/free plasma ratio was 0.19 in rat. This disequilibrium suggested some brain impairment for compound **70**, which was determined to be rodent-specific. Its bioavailability ranged from 73% to 91% in rats and dogs, respectively. Compound **71** was reported to have a brain/plasma ratio of 1.4 in rat with free drug exposure equal to the CSF exposure [169]. Striatal cGMP was shown to increase 145% in mice with a 3.2 mg/kg dose of **71** and a 215% elevation occurred at 10 mg/kg. Physiological effects were seen at ~5x the PDE9A IC50. Compound **73** has a brain/plasma ratio of 1.4 and free brain/free plasma and free CSF/free plasma ratios of 1.4, indicating no brain impairment [166]. It has an oral bioavailability of 63% in rat and 71% in dog and was predicted to be 80% bioavailable in human.

X-ray crystal structures of **70** (PDB ID: 4E90, Fig. 40) and **73** (PDB ID: 4G2L) bound to PDE9A reveal the expected binding modes [166, 167], with the pyrazolopyrimidinone core sandwiched between the P clamp residues and forming two HBs with Gln-453. The N1 4-tetrahydropyran (4-THP) substituent interacts with residues in the M pocket and the C6 substituent accesses the $Q_2$ and S pockets with the pyrrolidine methyl and the pyrimidine, respectively. The basic amine appears to form a water-mediated (**70**) or direct (**73**) HB interaction with Tyr-424 and the pyrimidine ring forms a π-stacking interaction with Phe-441 and an edge-to-face interaction with Phe-456. These interactions and an increase in the polarity of the N1 substituent were hypothesized to improve selectivity for PDE9A over other PDEs, especially PDE1C, which has a more lipophilic Phe residue at the PDE9A Tyr-424 position.

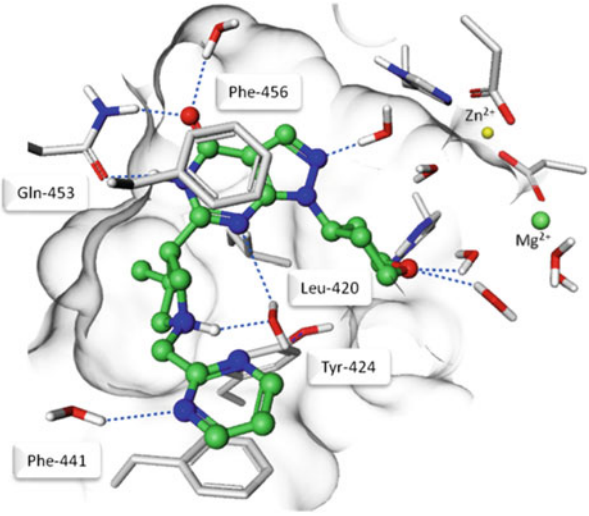



**Fig. 40** X-ray crystal structure of **70** (*green*) bound to human PDE9A (PDB ID: 4E90). Key residues and binding site pocket are highlighted (*gray*) and HB interactions are depicted by *blue dashed lines*

Together, studies with these tool compounds have built a strong case linking PDE9A inhibition and the subsequent increase in cGMP to improvements in memory, sensory, auditory gating, and cognitive processes known to be dysfunctional in scz [204–212].

## *4.4 Competitive Landscape*

As described herein, there is strong evidence that increasing cGMP levels through the use of potent, selective, brain-penetrant PDE9A inhibitors may be an effective mechanism to treat cognitive deficits associated with scz. Several companies have reported PDE9A inhibitors with these attributes. The first PDE9A inhibitors were patented by Pfizer in 2003 for the treatment of metabolic diseases. Since then, approximately nine other companies have patented chemical matter for both CNS and non-CNS indications. Highlighted in this section is the patented PDE9A chemical matter that is focused on treating CNS disorders, implying the compounds described are intended to be brain penetrant. Patents are listed in chronological order by company. Exemplars were chosen based on potency and SAR, and selectivity data is also reported when available.

### 4.4.1 Bayer

In a series of five patents [213–217], Bayer claimed **69** and a number of related compounds containing a pyrazolopyrimidinone core or variations thereof. The compounds are claimed to improve cognitive processes, demonstrated by the

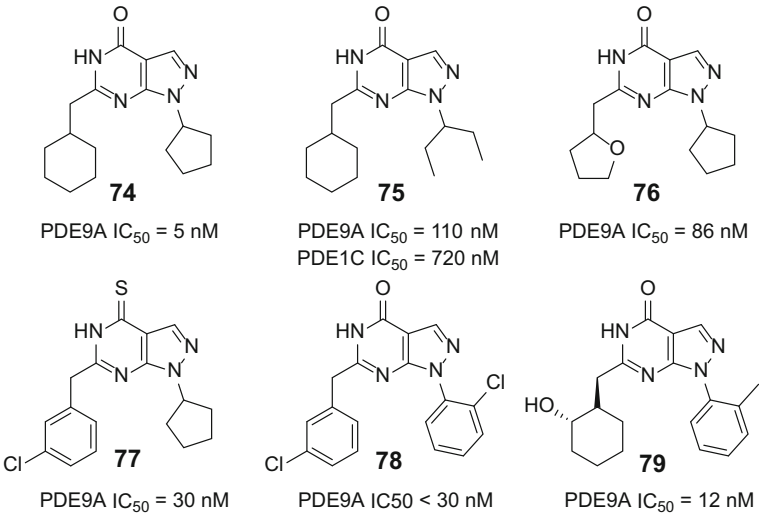



**Fig. 41** Representative pyrazolopyrimidinones disclosed by Bayer

increase of cGMP levels in a neuronal cell line, an increase in LTP in rodent hippocampal slices, and activity in a rodent social recognition model. All of the compounds in these patents would be expected to bind to PDE9A in a similar manner to **69** (Fig. 38).

The first patent, DE10238722 A1, exemplifies two compounds, **74** and **75** (Fig. 41). These compounds show the importance of filling the M pocket with the N1 substituent, as the change from the cyclopentyl group (**74**, $IC_{50} = 5$ nM) to a 3-pentyl group (**75**, $IC_{50} = 110$ nM) has a large impact on potency. The subsequent patents maintain the pyrazolopyrimidinone core and vary the N1 and C6 substituents. The reported data suggest that alkyl, carbon-linked cycloalkyl, and benzyl substituents are tolerated at the C6 position (**74**–**79**). A 2-tetrahydrofuranyl group in the $Q_2$ region causes a loss in potency (**76**, $IC_{50} = 86$ nM), while a HB donor is tolerated (**79**, $IC_{50} = 12$ nM) in that region. It is demonstrated that the carbonyl in the core can be substituted by a thioketone (**77**, $IC_{50} = 30$ nM) without a large effect on potency. As for the N1 substituent, aryl groups are tolerated in addition to alkyl and cycloalkyl substituents (**78**–**79**). Nearly all of the aryl groups at the N1 position are substituted with a small substituent at the *ortho* position, which would cause the ring to be orthogonal to the plane of the core and improve van der Waals (VDW) interactions in the M pocket.

In addition to the patents described above, Bayer has published two patents describing cyanopyrimidinones claimed for improving concentration, learning capacity, memory capacity, and other cognitive functions [218, 219]. These compounds also increased cGMP levels in a neuronal cell line, increased LTP in rodent hippocampal slices, and improved outcomes in a rodent social recognition model. The compounds in these patents maintain a pyrimidin-4-one core with a nitrile group

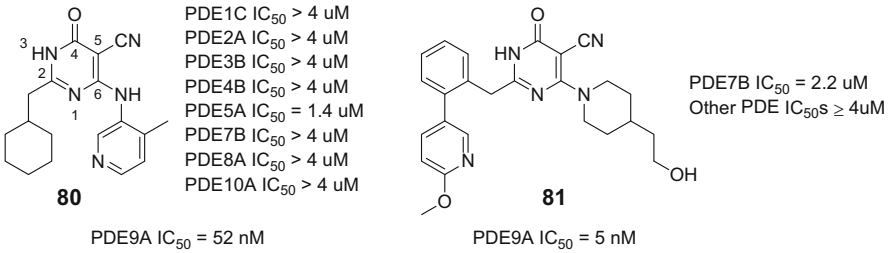



**Fig. 42** Representative cyanopyrimidinones disclosed by Bayer

at position 5 (**80**–**81**, Fig. 42) and would be expected to bind to PDE9A in a similar manner to **69** with the cyano group pointing toward the metal binding pocket. The compounds in WO04113306 A1 maintain carbon-linked cycloalkyl and branched alkane chains at the C2 position, which likely access the $Q_2$ region, while the majority of the C6 substituents are N-linked *ortho*-substituted aryl groups, which likely interact with residues in the M pocket (**80**). As with the pyrazolopyrimidinones, the *ortho* substituent would cause the aryl ring to turn orthogonal to the plane of the core, improving VDW interactions with residues in the M pocket. In the WO05068436 A1 patent, the C6 substituents are primarily N-linked alkyl groups or cycloalkanes or cyclic amines, while substituted benzyl, biaryl benzyl or carbon-linked thiophene substituents are exemplified at the C2 position (**81**). The aromatic C2 substituent may interact with Phe-441 to provide some selectivity over other PDEs.

### 4.4.2 Pfizer

Pfizer has published two patents to date claiming chemical matter for the inhibition of PDE9A. Analogs **70** and **71** are exemplified in the WO08139293 A1 patent [220], which claims PDE9A inhibitors for the treatment of neurodegenerative and cognitive disorders. This patent exemplifies mainly cyclopentyl and 4-THP at the N1 position, along with other aliphatic, cycloalkyl, and aryl groups. The data suggests that substitution of a 4-THP group (**82**, $IC_{50} = 0.9$ nM) for cyclopentyl (**71**, $IC_{50} = 1.8$ nM) at the N1 position maintains or improves PDE9A potency, and again shows the importance of filling the M pocket to drive potency through VDW interactions (Fig. 43). A methyl-, ethyl-, cyclopropyl or $CF_3$-substituted pyrrolidine is maintained at the C6 position for the compounds in this patent. A chain of varying length attaches a substituent to the pyrrolidine nitrogen. As this substituent was hypothesized to be important for selectivity due to π-stacking and HB interactions with the protein, most of the exemplified compounds contain an aryl at this position, though alkyl and cycloalkyl groups are also exemplified. The data suggests that the

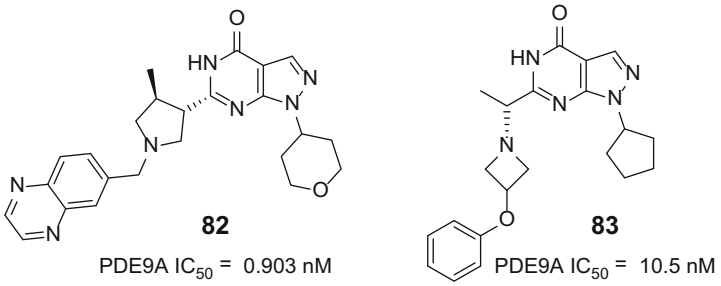



**Fig. 43** Representative chemotypes disclosed by Pfizer

larger ethyl substituent on the pyrrolidine in place of a methyl can maintain potency and likely fills the $Q_2$ subpocket.

Compounds **72** and **73** are exemplified in the WO10084438 A1 patent [221], which also claims PDE9A inhibitors for the treatment of neurodegenerative and cognitive disorders. Most exemplars contain cyclopentyl and 4-THP at the N1 position, as well as isopropyl and cyclobutyl. A 4-THP at the N1 position maintains or improves potency compared to a cyclopentyl group in this series of compounds. The C6 substituents are primarily exemplified as $CH_2$-linked azetidines or pyrrolidines, where the linker has a methyl or ethyl group oriented toward the $Q_2$ subpocket. The basic nitrogen of the azetidines and pyrrolidines are oriented to interact with Tyr-424. An aryl substituent either directly attached or ether-linked to the azetidine or pyrrolidine is found in most of the exemplified compounds, which is oriented to interact with Phe-441 and/or Phe-456 to obtain selectivity over other PDEs, evident in the X-ray crystal structures of **73** and **83** (PDB IDs: 4G2L and 4G2J) [166].

### 4.4.3 Boehringer Ingelheim

Boehringer Ingelheim has published six patents to date claiming PDE9A inhibitors for the treatment of CNS disorders including improvement of perception, concentration, learning and/or memory [222, 223], and Alzheimer's disease [224, 225] and/or scz [226, 227]. All of the PDE9A inhibitors exemplified in these patents contain a pyrazolopyrimidinone core, and would be expected to adopt the same binding mode as described for the other compounds containing this core. The patents WO09068617 A1 [222], WO09121919 A1 [224], and WO10026214 A1 [223] exemplify compounds with broad N1 substituents that occupy the M pocket, including *ortho*-substituted aryl groups and substituted cycloalkyl and heterocycloalkyl groups. The C6 position is also broadly substituted with substituted benzyl substituents, alkyl and $CH_2$-linked cycloalkyl groups, which interact with the $Q_2$ and S pockets, likely providing some selectivity over other PDEs. The data presented in these patents is not very specific, with only percent inhibition of PDE9A at 10 μM and ranges of PDE9A $IC_{50}$ potency data provided (**84–86**, Fig. 44).

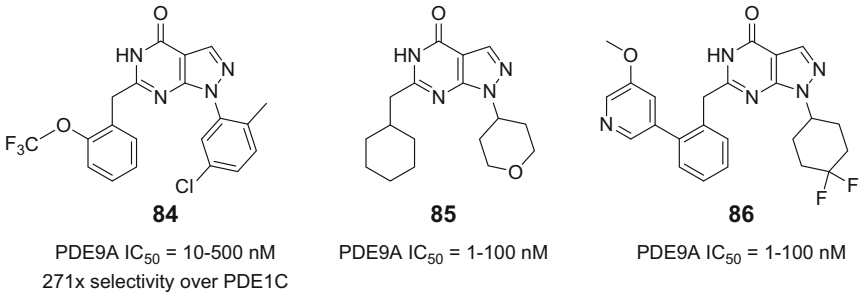



**Fig. 44** Representative chemotypes disclosed by Boehringer Ingelheim

The final three patents from Boehringer Ingelheim, WO11018495 A1 [225], WO12020022 A1 [226], and WO12110441 A1 [227], substitute a cycloalkyl group for the linker at the C6 substituent. The C6 substituent most often contains a cyclobutyl linker, though cyclopropyl, cyclopentyl, and cyclohexyl linkers are also exemplified. The cycloalkyl group is usually substituted with another group, often an amide (**87**), or a heterocycle (**88**–**89**) (Fig. 45). The cyclobutyl linker likely fills the $Q_2$ subpocket while orienting the attached aryl group for interactions with the residues in the S pocket that are important for selectivity, enabling a π-stacking interaction with Phe-441 and a HB interaction with Tyr-424. The N1 substituents exemplified in these patents include 4-THP, 4,4-difluorocyclohexyl, *ortho*-substituted aryl groups, cycloalkyl and alkyl groups which occupy the M pocket.

### 4.4.4 Sun Yat-Sen University

Sun Yat-Sen University published a patent claiming PDE9A inhibitors in CN102260266 A [228] which contain the pyrazolopyrimidinone core. The majority of exemplified compounds have *ortho*-Cl phenyl at N1. The importance of the *ortho* phenyl substituent is evident based on the >300-fold gain in PDE9A potency reported for **90** ($IC_{50} = 42$ nM) compared to **91** ($IC_{50} = 13{,}301$ nM), though the improvement in potency can vary depending on the C6 substituent [168]. The exemplified compounds contain a substituted aryl or benzyl group attached to the C6 position through an amine (**90**–**92**, Fig. 46). In the X-ray crystal structure of **90** bound to PDE9A, the C6 substituent interacts with residues in the $Q_2$ and S pockets (PDB ID: 4GH6) [168]. The amide oxygen forms a HB interaction with Tyr-424, which may confer PDE9A selectivity for this series of compounds over other PDEs. The *para*-methoxy phenyl group is not optimally oriented to form a π-stacking interaction with Phe-441, but was rather designed to occupy a region in the S pocket [168].

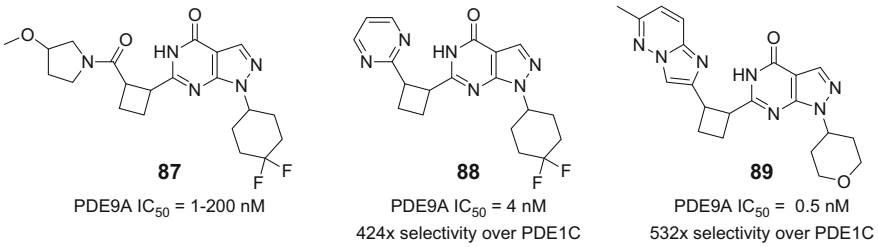



**Fig. 45** Representative chemotypes disclosed by Boehringer Ingelheim

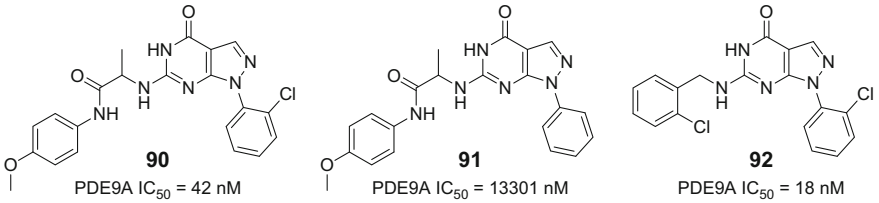



**Fig. 46** Representative chemotypes disclosed by Sun Yat-Sen University

### 4.4.5 EnVivo Pharmaceuticals

EnVivo published two patents that claim PDE9A inhibitors for CNS or neurodegeneration disorders [229, 230]. The compounds claimed in WO12040230 A1 and WO13142269 A1 are differentiated from compounds described in previous patents due to an imidazotriazinone core in place of a pyrazolopyrimidinone core (**93**–**94**, Fig. 47). Based on structural similarity and the SAR and selectivity data provided in the patents, it is likely that the imidazotriazinones have a similar PDE9A binding mode to the pyrazolopyrimidinones. The compounds exemplified in these patents all contain a 4-methyl or 4-heterocycloalkyl pyrrolidine at the C2 position, which would interact with residues in the $Q_2$ pocket. The pyrrolidine is further N-substituted with benzyl, aryl, or heterocycloalkyl groups, which interact with residues in the $Q_2$ and S pockets. The aryl groups would be positioned to interact with Tyr-424 and Phe-441 to provide selectivity for PDE9A over other PDEs. The C7 position in the exemplified compounds is substituted with heterocycloalkyl, cycloalkyl, and ortho-substituted phenyl groups, including 4-THP, 4,4-difluorocyclohexane and *ortho*-methyl phenyl, which interact with residues in the M pocket.

### 4.4.6 Eisai

Eisai filed a patent claiming a series of PDE9A inhibitors for improvement of learning and memory to treat cognitive dysfunction in Alzheimer's disease [231]

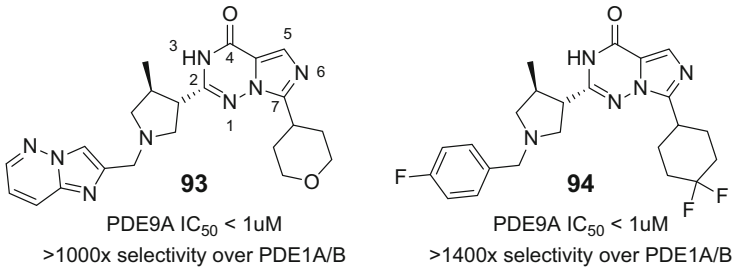



**Fig. 47** Representative chemotypes disclosed by EnVivo

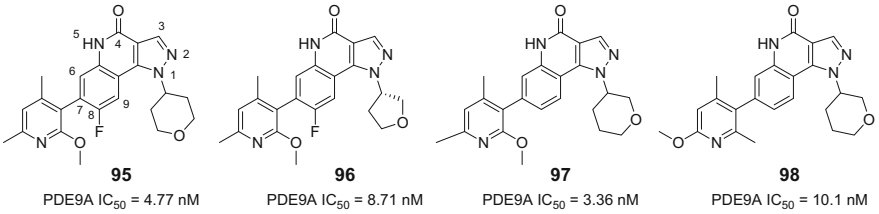



**Fig. 48** Representative chemotypes disclosed by Eisai

demonstrated by PDE9A inhibition and an increase in cGMP in rodent CSF and hippocampus. The compounds claimed in WO13051639 A1 are differentiated from other PDE9A compounds by a tricyclic pyrazoloquinolinone core (**95**–**98**, Fig. 48). Based on structural similarity and the SAR data provided in the patents, it is likely that the pyrazoloquinolinones have a similar PDE9A binding mode to the pyrazolopyrimidinones and imidazotriazinones. The N1 substituent is most often a heterocycloalkyl group in the exemplified compounds, which would interact with residues in the M pocket. The change in N1 from a 4-THP (**95**, $IC_{50} = 4.77$ nM) to a 3-tetrahydrofuranyl (3-THF) (**96**, $IC_{50} = 8.71$ nM) group does not greatly affect PDE9A potency, though the larger THP group may fill the M pocket better for slightly improved potency. The exemplified compounds are substituted at C7 by a substituted pyridyl group, which may interact with Phe-441 for selectivity over other PDEs. The substitution pattern on the pyridyl ring affects potency (**97**, $IC_{50} = 3.36$ nM vs. **98**, $IC_{50} = 10.1$ nM), likely due to optimal VDW interactions in the $Q_2$ and S pockets.

### 4.4.7 Lundbeck

Lundbeck filed two patents claiming PDE9A inhibitors for the treatment of neurodegenerative and psychiatric disorders [232, 233]. In the first patent, WO13053690 A1, a series of compounds containing an imidazopyrazinone core are claimed as PDE9A inhibitors (**99**–**100**, Fig. 49). Based on structural similarity and the SAR

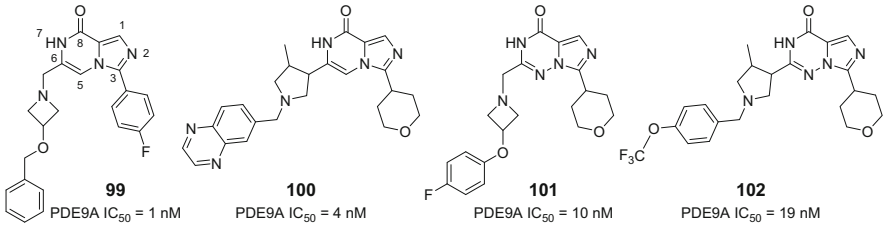



**Fig. 49** Representative chemotypes disclosed by Lundbeck

data in the patent, it is likely that the imidazopyrazinone compounds have a similar PDE9A binding mode to the pyrazolopyrimidinones. The C3 substituent is most often a substituted phenyl, cycloalkyl, or heterocycloalkyl group in the exemplified compounds, which would interact with residues in the M pocket. The C6 position is broadly claimed by substituted heterocycloalkyl groups that are attached either directly or through a carbon linker, which would be positioned to interact with residues in the $Q_2$ and S pockets for potency and selectivity.

The second patent, WO13110768 A1, claims PDE9A inhibitors that contain an imidazotriazinone core (**101**–**102**, Fig. 49). The C7 substituent is claimed as a substituted aryl, cycloalkyl, or heterocycloalkyl group. It is most often 4-THP in the exemplified compounds, interacting with residues in the M pocket. The C2 position is claimed broadly as a substituted heterocycloalkyl group that is attached either directly or through a carbon linker. Most of the exemplified compounds contain either a $CH_2$-linked azetidine (**101**) or a pyrrolidine (**102**) at C2, which are typically further substituted with aromatic groups that likely interact with residues in the $Q_2$ and S pockets for potency and selectivity.

## *4.5 Clinical Trials*

Compound **70** is the only PDE9A inhibitor taken into clinical trials to date. It increased cGMP levels in the CSF of healthy human volunteers [203], demonstrating inhibition of PDE9A expressed in the CNS. In Phase I studies, it was found to be well tolerated with high exposure and a half-life that ranges between 19 and 31 h [234]. A 40 mg dose elevated CSF cGMP by 300%. However, in a randomized 12-week Phase II clinical trial of patients with mild to moderate Alzheimer's disease, **70** did not improve cognition and behavior over placebo [235]. Additional data generated with **70** may provide a rationale for why it did not meet the Phase II primary endpoint as well as provide alternative ways to test the hypothesis that PDE9A inhibition can improve cognition, such as testing a lower dose [199–201], performing a longer duration trial, or using a different patient population [200]. There have been no reports of utilizing PDE9A inhibitors for treating scz.

## 5 Summary, Outlook, Conclusions

The strength of PDE10A and PDE9A neurobiology positions these as high-value drug targets. Phosphodiesterase 10 is solely expressed in the striatum; a brain region hypothesized to be impaired in scz, and which plays a central role in attenuating synaptic plasticity by regulating the cellular concentrations of the signaling nucleotides, cGMP and cAMP. In pre-clinical settings, potent/selective and brain-penetrant compounds have shown positive pharmacokinetic/pharmacodynamic effects in vivo. In addition, PDE10A inhibitors have been effective at improving behavior in a number of preclinical animal models used to assess positive, negative, and cognitive symptoms of scz. As such, several have been progressed into the clinic as monotherapies and, recently, as adjuncts to marketed anti-psychotics. Currently, no positive clinical data has been reported. Because of this, the outcome of the PET studies will be critical to provide deeper insights into the target occupancy in the brain and inform future dose selections which should improve the probability of having a positive clinical effect. While outside the scope of this review, recently clinical trials utilizing PDE10A inhibitors for Huntington's disease have been initiated.

In addition, the evidence presented herein suggests that PDE9A inhibition by a safe, selective, brain-penetrant inhibitor should elevate cGMP levels in brain regions that are important for cognition, which may be an effective treatment for cognitive deficits associated with schizophrenia. That said, some key gaps need to be addressed to obtain a more complete understanding of PDE9A function in vivo and its link to scz. Only two of more than 20 human PDE9A splice variants have been studied extensively [149]. A full characterization of all of the PDE9A splice variants and their localization in different brain regions, especially hippocampus, cerebellum, amygdala and cortex, is needed for a more complete picture of their neurological effects. In addition, a better understanding of cGMP-specific PDE localization in the brain is required to understand compensatory effects that these PDEs may have upon inhibition of PDE9A. It is also important to gain a better understanding of how age and different disease states affect both human and rodent central PDE9A expression. These data will help to inform patient population selection criteria as well as understand translation of clinical results to animal models and vice versa. Indeed, several preliminary studies have been reported that address these gaps. Phosphodiesterase 9A inhibition by **69** demonstrated an increase in basal synaptic transmission and enhanced LTP better in older rats compared to younger rats [197]. This is likely due to age-related decreases in cGMP levels [159, 236] rather than changes in PDE9A expression levels, which have been shown to change during developmental stages [154], but do not significantly differ between young and old rats [237]. Additionally, expression of PDE9A in post-mortem brain tissue of patients with Alzheimer's disease shows the same distribution as in healthy brain tissues of comparable age [156]. It would be useful to understand the expression of PDE9A in the brains of schizophrenic patients by performing a similar study or through the development of a PET ligand. Though more work needs to be done to understand the

role of PDE9A in scz, the evidence from preclinical in vivo cognition models coupled with the low level of cGMP in patients with this disease strongly suggests this hypothesis should continue to be tested in the clinic.

## References


1. Beck AT, Rector NA, Stolar N, Grant P (2009) Schizophrenia cognitive theory, research, and therapy, vol 1. Guilford, New York
2. American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders: DSM-IV-TR. American Psychiatric Association, Washington, DC
3. Seeman P, Lee T (1975) Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 188(4194):1217–1219
4. Seeman P, Lee T, Chau-Wong M, Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261(5562):717–719
5. Rao NP, Remington G (2013) Investigational drugs for schizophrenia targeting the dopamine receptor: Phase II trials. Expet Opin Investig Drugs 22(7):881–894. doi:10.1517/13543784.2013.795945
6. Wu EQ, Birnbaum HG, Shi L, Ball DE, Kessler RC, Moulis M, Aggarwal J (2005) The economic burden of schizophrenia in the United States in 2002. J Clin Psychiatr 66(9):1122–1129
7. Bray NJ, Leweke FM, Kapur S, Meyer-Lindenberg A (2010) The neurobiology of schizophrenia: new leads and avenues for treatment. Curr Opin Neurobiol 20(6):810–815. doi:10.1016/j.conb.2010.09.008
8. Francis SH, Blount MA, Corbin JD (2011) Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. Physiol Rev 91(2):651–690. doi:10.1152/physrev.00030.2010
9. Chappie T, Humphrey J, Menniti F, Schmidt C (2009) PDE10A inhibitors: an assessment of the current CNS drug discovery landscape. Curr Opin Drug Discov Dev 12(4):458–467
10. Jeon YH, Heo YS, Kim CM, Hyun YL, Lee TG, Ro S, Cho JM (2005) Phosphodiesterase: overview of protein structures, potential therapeutic applications and recent progress in drug development. Cell Mol Life Sci 62(11):1198–1220. doi:10.1007/s00018-005-4533-5
11. Baillie GS (2009) Compartmentalized signalling: spatial regulation of cAMP by the action of compartmentalized phosphodiesterases. FEBS J 276(7):1790–1799. doi:10.1111/j.1742-4658.2009.06926.x
12. Houslay MD (2010) Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. Trends Biochem Sci 35(2):91–100. doi:10.1016/j.tibs.2009.09.007
13. Aye-Han NN, Zhang J (2014) A multiparameter live cell imaging approach to monitor cyclic AMP and protein kinase A dynamics in parallel. Meth Mol Biol 1071:207–215. doi:10.1007/978-1-62703-622-1_16
14. Klarenbeek J, Jalink K (2014) Detecting cAMP with an EPAC-based FRET sensor in single living cells. Meth Mol Biol 1071:49–58. doi:10.1007/978-1-62703-622-1_4
15. Stangherlin A, Koschinski A, Terrin A, Zoccarato A, Jiang H, Fields LA, Zaccolo M (2014) Analysis of compartmentalized cAMP: a method to compare signals from differently targeted FRET reporters. Meth Mol Biol 1071:59–71. doi:10.1007/978-1-62703-622-1_5
16. Conti M, Beavo J (2007) Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. Annu Rev Biochem 76:481–511. doi:10.1146/annurev.biochem.76.060305.150444
17. Lakics V, Karran EH, Boess FG (2010) Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. Neuropharmacology 59(6):367–374. doi:10.1016/j.neuropharm.2010.05.004

18. DeNinno MP (2012) Future directions in phosphodiesterase drug discovery. Bioorg Med Chem Lett 22(22):6794–6800.
19. Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S-I, Okumura K, Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J Biol Chem 274(26):18438–18445. doi:10.1074/jbc.274.26.18438
20. Fujishige K, Kotera J, Omori K (1999) Striatum- and testis-specific phosphodiesterase PDE10A: isolation and characterization of a rat PDE10A. Eur J Biochem 266(3):1118–1127. doi:10.1046/j.1432-1327.1999.00963.x
21. Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999) Isolation and characterization of PDE10A, a novel human 3', 5'-cyclic nucleotide phosphodiesterase. Gene 234(1):109–117. doi:10.1016/S0378-1119(99)00171-7
22. Soderling SH, Bayuga SJ, Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. Proc Natl Acad Sci U S A 96(12):7071–7076. doi:10.1073/pnas.96.12.7071
23. Coskran TM, Morton D, Menniti FS, Adamowicz WO, Kleiman RJ, Ryan AM, Strick CA, Schmidt CJ, Stephenson DT (2006) Immunohistochemical localization of phosphodiesterase 10A in multiple mammalian species. J Histochem Cytochem 54(11):1205–1213. doi:10.1369/jhc.6A6930.2006
24. Sorg C, Manoliu A, Neufang S, Myers N, Peters H, Schwerthoffer D, Scherr M, Muhlau M, Zimmer C, Drzezga A, Forstl H, Bauml J, Eichele T, Wohlschlager AM, Riedl V (2013) Increased intrinsic brain activity in the striatum reflects symptom dimensions in schizophrenia. Schizophr Bull 39(2):387–395. doi:10.1093/schbul/sbr184
25. Kotera J, Fujishige K, Yuasa K, Omori K (1999) Characterization and phosphorylation of PDE10A2, a novel alternative splice variant of human phosphodiesterase that hydrolyzes cAMP and cGMP. Biochem Biophys Res Comm 261(3):551–557. doi:10.1006/bbrc.1999.1013
26. Kotera J, Sasaki T, Kobayashi T, Fujishige K, Yamashita Y, Omori K (2004) Subcellular localization of cyclic nucleotide phosphodiesterase type 10A variants, and alteration of the localization by cAMP-dependent protein kinase-dependent phosphorylation. J Biol Chem 279(6):4366–4375. doi:10.1074/jbc.M308471200
27. Charych EI, Jiang LX, Lo F, Sullivan K, Brandon NJ (2010) Interplay of palmitoylation and phosphorylation in the trafficking and localization of phosphodiesterase 10A: implications for the treatment of schizophrenia. J Neurosci 30(27):9027–9037. doi:10.1523/JNEUROSCI.1635-10.2010
28. Handa N, Mizohata E, Kishishita S, Toyama M, Morita S, Uchikubo-Kamo T, Akasaka R, Omori K, Kotera J, Terada T, Shirouzu M, Yokoyama S (2008) Crystal structure of the GAF-B domain from human phosphodiesterase 10A complexed with its ligand, cAMP. J Biol Chem 283(28):19657–19664. doi:10.1074/jbc.M800595200
29. Heikaus CC, Pandit J, Klevit RE (2009) Cyclic nucleotide binding GAF domains from phosphodiesterases: structural and mechanistic insights. Structure 17(12):1551–1557. doi:10.1016/j.str.2009.07.019
30. Gross-Langenhoff M, Hofbauer K, Weber J, Schultz A, Schultz JE (2006) cAMP is a ligand for the tandem GAF domain of human phosphodiesterase 10 and cGMP for the tandem GAF domain of phosphodiesterase 11. J Biol Chem 281(5):2841–2846. doi:10.1074/jbc.M511468200
31. Matthiesen K, Nielsen J (2009) Binding of cyclic nucleotides to phosphodiesterase 10A and 11A GAF domains does not stimulate catalytic activity. Biochem J 423(3):401–409. doi:10.1042/BJ20090982
32. Jager R, Russwurm C, Schwede F, Genieser HG, Koesling D, Russwurm M (2012) Activation of PDE10 and PDE11 phosphodiesterases. J Biol Chem 287(2):1210–1219. doi:10.1074/jbc.M111.263806

33. Wang H, Liu Y, Hou J, Zheng M, Robinson H, Ke H (2007) Structural insight into substrate specificity of phosphodiesterase 10. Proc Natl Acad Sci U S A 104(14):5782–5787. doi:10.1073/pnas.0700279104
34. Card GL, England BP, Suzuki Y, Fong D, Powell B, Lee B, Luu C, Tabrizizad M, Gillette S, Ibrahim PN, Artis DR, Bollag G, Milburn MV, Kim S-H, Schlessinger J, Zhang KYJ (2004) Structural basis for the activity of drugs that inhibit phosphodiesterases. Structure 12 (12):2233–2247
35. Verhoest PR, Chapin DS, Corman M, Fonseca K, Harms JF, Hou X, Marr ES, Menniti FS, Nelson F, O'Connor R, Pandit J, Proulx-LaFrance C, Schmidt AW, Schmidt CJ, Suiciak JA, Liras S (2009) Discovery of a novel class of phosphodiesterase 10A inhibitors and identification of clinical candidate 2-[4-(1-Methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)-phenoxymethyl]-quinoline (PF-2545920)(I) for the treatment of schizophrenia. J Med Chem 52(16):5188–5196. doi:10.1021/jm900521k
36. Buot A, Yelnik J (2012) Functional anatomy of the basal ganglia: limbic aspects. Rev Neurol 168(8–9):569–575. doi:10.1016/j.neurol.2012.06.015
37. Da Cunha C, Gomez AA, Blaha CD (2012) The role of the basal ganglia in motivated behavior. Rev Neurosci 23(5–6):747–767. doi:10.1515/revneuro-2012-0063
38. Liljeholm M, O'Doherty JP (2012) Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cognit Sci 16(9):467–475. doi:10.1016/j.tics.2012.07.007
39. Leisman G, Melillo R (2013) The basal ganglia: motor and cognitive relationships in a clinical neurobehavioral context. Rev Neurosci 24(1):9–25. doi:10.1515/revneuro-2012-0067
40. Shepherd GM (2013) Corticostriatal connectivity and its role in disease. Nat Rev Neurosci 14 (4):278–291. doi:10.1038/nrn3469
41. Cui G, Jun SB, Jin X, Pham MD, Vogel SS, Lovinger DM, Costa RM (2013) Concurrent activation of striatal direct and indirect pathways during action initiation. Nature 494 (7436):238–242
42. Gerfen CR, Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. Annu Rev Neurosci 34:441–466. doi:10.1146/annurev-neuro-061010-113641
43. Dedeurwaerdere S, Wintmolders C, Vanhoof G, Langlois X (2011) Patterns of brain glucose metabolism induced by phosphodiesterase 10A inhibitors in the mouse: a potential translational biomarker. J Pharmacol Exp Ther 339(1):210–217. doi:10.1124/jpet.111.182766
44. Robertson GS, Jian M (1995) D1 and D2 dopamine receptors differentially increase Fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. Neuroscience 64(4):1019–1034
45. Mango D, Bonito-Oliva A, Ledonne A, Nistico R, Castelli V, Giorgi M, Sancesario G, Fisone G, Berretta N, Mercuri NB (2014) Phosphodiesterase 10A controls D1-mediated facilitation of GABA release from striato-nigral projections under normal and dopamine-depleted conditions. Neuropharmacology 76(Part A):127–136
46. Conti G, Blandini F, Tassorelli C, Giubilei F, Fornai F, Zocchi A, Orzi F (2001) Intrastriatal injection of D1 or D2 dopamine agonists affects glucose utilization in both the direct and indirect pathways of the rat basal ganglia. Neurosci Lett 309(3):161–164
47. Dedeurwaerdere S, Wintmolders C, Vanhoof G, Langlois X (2011) Patterns of brain glucose metabolism induced by phosphodiesterase 10A inhibitors in the mouse: a potential translational biomarker. J Pharmacol Exp Therapeut 339(1):210–217. doi:10.1124/jpet.111.182766
48. Kita H (2007) Globus pallidus external segment. Progr Brain Res 160:111–133. doi:10.1016/S0079-6123(06)60007-1
49. Kimura M, Satoh T, Matsumoto N (2007) What does the habenula tell dopamine neurons? Nat Neurosci 10(6):677–678. doi:10.1038/nn0607-677
50. Hikosaka O (2010) The habenula: from stress evasion to value-based decision-making. Nat Rev Neurosci 11(7):503–513. doi:10.1038/nrn2866

51. Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, Langen B, Logue S, Brennan J, Jiang L, Charych E, Egerland U, Liu F, Marquis KL, Malamas M, Hage T, Comery TA, Brandon NJ (2009) Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J Pharmacol Exp Therapeut 331(2):574–590. doi:10.1124/jpet.109.155994
52. Strick CA, James LC, Fox CB, Seeger TF, Menniti FS, Schmidt CJ (2010) Alterations in gene regulation following inhibition of the striatum-enriched phosphodiesterase, PDE10A. Neuropharmacology 58(2):444–451. doi:10.1016/j.neuropharm.2009.09.008
53. Threlfell S, Sammut S, Menniti FS, Schmidt CJ, West AR (2009) Inhibition of Phosphodiesterase 10A Increases the Responsiveness of Striatal Projection Neurons to Cortical Stimulation. J Pharmacol Exp Therapeut 328(3):785–795
54. Sotty F, Montezinho LP, Steiniger-Brach B, Nielsen J (2009) Phosphodiesterase 10A inhibition modulates the sensitivity of the mesolimbic dopaminergic system to D-amphetamine: involvement of the D1-regulated feedback control of midbrain dopamine neurons. J Neurochem 109(3):766–775. doi:10.1111/j.1471-4159.2009.06004.x
55. Arnt J (1982) Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacol Toxicol 51(4):321–329. doi:10.1111/j.1600-0773.1982.tb01032.x
56. Langen B, Dost R, Egerland U, Stange H, Hoefgen N (2012) Effect of PDE10A inhibitors on MK-801-induced immobility in the forced swim test. Psychopharmacology 221(2):249–259. doi:10.1007/s00213-011-2567-y
57. Bleickardt CJ, Kazdoba TM, Jones NT, Hunter JC, Hodgson RA Antagonism of the adenosine A2A receptor attenuates akathisia-like behavior induced with MP-10 or aripiprazole in a novel non-human primate model. Pharmacol Biochem Behav doi:10.1016/j.pbb.2013.10.030
58. Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, Hoffman WE, Lebel LA, McCarthy SA, Nelson FR, Proulx-LaFrance C, Majchrzak MJ, Ramirez AD, Schmidt K, Seymour PA, Siuciak JA, Tingley FD 3rd, Williams RD, Verhoest PR, Menniti FS (2008) Preclinical characterization of selective phosphodiesterase 10A inhibitors: a new therapeutic approach to the treatment of schizophrenia. J Pharmacol Exp Therapeut 325 (2):681–690. doi:10.1124/jpet.107.132910
59. Raheem IT, Breslin MJ, Fandozzi C, Fuerst J, Hill N, Huszar S, Kandebo M, Kim SH, Ma B, McGaughey G, Renger JJ, Schreier JD, Sharma S, Smith S, Uslaner J, Yan Y, Coleman PJ, Cox CD (2012) Discovery of tetrahydropyridopyrimidine phosphodiesterase 10A inhibitors for the treatment of schizophrenia. Bioorg Med Chem Lett 22(18):5903–5908. doi:10.1016/j.bmcl.2012.07.072
60. Girault JA (2012) Signaling in striatal neurons: the phosphoproteins of reward, addiction, and dyskinesia. Progr Mol Biol Translat Sci 106:33–62. doi:10.1016/b978-0-12-396456-4.00006-7
61. Nestler EJ, Carlezon WA Jr (2006) The mesolimbic dopamine reward circuit in depression. Biol Psychiatr 59(12):1151–1159. doi:10.1016/j.biopsych.2005.09.018
62. Pozzi L, Hakansson K, Usiello A, Borgkvist A, Lindskog M, Greengard P, Fisone G (2003) Opposite regulation by typical and atypical anti-psychotics of ERK1/2, CREB and Elk-1 phosphorylation in mouse dorsal striatum. J Neurochem 86(2):451–459
63. Tu Z, Xu J, Jones LA, Li S, Mach RH (2010) Carbon-11 labeled papaverine as a PET tracer for imaging PDE10A: radiosynthesis, in vitro and in vivo evaluation. Nucl Med Biol 37 (4):509–516. doi:10.1016/j.nucmedbio.2009.12.012
64. Celen S, Koole M, De AM, Sannen I, Chitneni SK, Alcazar J, Dedeurwaerdere S, Moechars D, Schmidt M, Verbruggen A, Langlois X, Van LK, Andres JI, Bormans G (2010) Preclinical evaluation of 18F-JNJ41510417 as a radioligand for PET imaging of phosphodiesterase-10A in the brain. J Nucl Med 51(10):1584–1591. doi:10.2967/jnumed.110.077040
65. Tu Z, Fan J, Li S, Jones LA, Cui J, Padakanti PK, Xu J, Zeng D, Shoghi KI, Perlmutter JS, Mach RH (2011) Radiosynthesis and in vivo evaluation of [11C]MP-10 as a PET probe for

imaging PDE10A in rodent and non-human primate brain. Bioorg Med Chem 19(5):1666–1673. doi:10.1016/j.bmc.2011.01.032
66. Celen S, Koole M, Ooms M, De AM, Sannen I, Cornelis J, Alcazar J, Schmidt M, Verbruggen A, Langlois X, Van LK, Andres JI, Bormans G (2013) Preclinical evaluation of [(18)F]JNJ42259152 as a PET tracer for PDE10A. Neuroimage 82:13–22
67. Laere K, Ahmad RU, Hudyana H, Celen S, Dubois K, Schmidt ME, Bormans G, Koole M (2013) Human biodistribution and dosimetry of 18F-JNJ42259152, a radioligand for phosphodiesterase 10A imaging. Eur J Nucl Med Mol Imag 40(2):254–261. doi:10.1007/s00259-012-2270-1
68. Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, Lanfear J, Ryan AM, Schmidt CJ, Strick CA, Varghese AH, Williams RD, Wylie PG, Menniti FS (2003) Immunohistochemical localization of PDE10A in the rat brain. Brain Res 985(2):113–126. doi:10.1016/S0006-8993(03)02754-9
69. Barret O, Thomae D, Alagille D, Lee H, Papin C, Baldwin R, Jennings D, Marek K, Seibyl J, Tamagnan G (2012) First in vivo assessment of two PDE10 tracers [18F]MNI654 and [18F]MNI659. J Nucl Med Meet Abstr 53(1):361
70. Marek K, Tamagnan G (2013) In vivo assessment of a novel PDE10 tracer: [18F]MNI659. In: NIMH. http://mli.nih.gov/mli/download/1360. Accessed 25 Jan 2013
71. Tamagnan G (2012) Use of exploratory IND for early phase clinical trials using PET or SPECT imaging. In: University of British Columbia, TRIUMF. http://7isr.triumf.ca/talks/Sunday/Session II/Tamagnan.pdf. Accessed 16 Sept 2012
72. Rabiner EA (2013) Imaging the PDE-10 enzyme. In: NIH. http://mli.nih.gov/mli/download/1356. Accessed 25 Jan 2013
73. Geneste H, Ochse M, Drescher K, Turner S, Behl B, Laplanche L, Dinges J, Jakob C, Black L (2013) Heterocyclic fused pyridines and related compounds as inhibitor compounds of phosphodiesterase type 10A and their preparation and use in the treatment of neurological and psychiatric disorders. US20130116233 A1
74. Geneste H, Ochse M, Drescher K, Dinges J, Jakob C (2013) Dihydroisoquinolines and related as phosphodiesterase type 10a and their preparation and use in the treatment of neurological and psychiatric disorders. US20130116229 A1
75. Geneste H, Ochse M, Drescher K, Behl B, Laplanche L, Dinges J, Jakob C (2013) Preparation of isoindole carboxamides, pyrrolopyridine carboxamides, and similar compounds as inhibitors of phosphodiesterase type 10A for treating neurological and psychiatric disorders. WO2013000994 A1
76. Donello JE, Yang R, Viswanath V, Leblond B, Beausoleil E, Pando MP, Desire LJR, Casagrande A-S (2012) Methods using phosphodiesterase-10 inhibitors (PDE10) inhibitors for treatment of retina diseases. WO2012112918 A1
77. Donello JE, Yang R, Leblond B, Beausoleil E, Casagrande A-S, Desire LJR, Pando MP, Chauvignac C, Taverne T (2012) Substituted 6,7-dialkoxy-3-isoquinolinol derivatives as inhibitors of phosphodiesterase 10 (PDE10A) and their preparation. WO2012112946 A1
78. Vennemann M, Baer T, Braunger J, Ciapetti P, Contreras J-M, Wermuth CG (2006) Preparation of pyrrolodihydroisoquinolines as phosphodiesterase 10 (PDE10) inhibitors. WO2006089815 A1
79. Maier T, Graedler U, Gimmnich P, Quintini G, Ciapetti P, Contreras J-M, Wermuth CG, Vennemann M, Baer T, Braunger J (2005) Preparation of pyrrolodihydroisoquinolines as cyclic 3',5'-nucleotide phosphodiesterase (PDE10) inhibitors. WO2005003129 A1
80. Allen J, Horne DB, Hu E, Kaller MR, Monenschein H, Nguyen TT, Reichelt A, Rzasa RM (2011) Preparation of heteroaryloxyheterocyclyl and heteroarylaminoheterocyclyl compounds as phosphodiesterase 10 (PDE10) inhibitors. WO2011143495 A1
81. Allen JR, Amegadzie A, Andrews KL, Brown J, Chen JJ, Chen N, Harrington EH, Liu Q, Nguyen TT, Pickrell AJ, Qian W, Rumfelt S, Rzasa RM, Yuan CC, Zhong W (2013) Heterobicyclic compounds as PDE10 inhibitors and their preparation. US20130225552 A1

82. Hitchcock SA, Liu R, Arrington MP, Hopper AT, Conticello RD, Nguyen TM, Danca MD (2007) Preparation of cinnoline derivatives as phosphodiesterase 10 inhibitors. WO2007098214 A1
83. Hu E, Kunz R, Chen N, Nixey T, Hitchcock S (2009) Preparation of quinoline derivatives and their aza analogs as phosphodiesterase 10 inhibitors. WO2009025823 A1
84. Masuda N, Miyamoto S, Kikuchi S, Samizu K, Sato F, Shiina Y, Hamaguchi W, Seo R, Mihara T (2012) Preparation of pyrazole compounds as inhibitors of phosphodiesterase 10A. WO2012133607 A1
85. Niewoehner U, Bauser M, Ergueden J-K, Flubacher D, Naab P, Repp T-O, Stoltefuss J, Burkhardt N, Sewing A, Schauer M, Schlemmer K-H, Weber O, Boyer SJ, Miglarese M (2002) Preparation of pyrrolo[2,1-a]isoquinolines as phosphodiesterase 10a inhibitors. WO2002048144 A1
86. Niewoehner U, Bauser M, Ergueden J-K, Flubacher D, Naab P, Repp T-O, Stoltefuss J, Burkhardt N, Sewing A, Schauer M, Schlemmer K-H, Weber O, Boyer SJ, Miglarese M, Ying S (2003) Preparation of pyrrolo[2,1-a]isoquinoline-1-carboxylates as phosphodiesterase 10a inhibitors for treatment of cancer. WO2003014117 A1
87. Niewoehner U, Bauser M, Ergueden J-K, Flubacher D, Naab P, Repp T-O, Stoltefuss J, Burkhardt N, Sewing A, Schauer M, Weber O, Schlemmer K-H, Boyer JS, Miglarese M (2003) Preparation of pyrrolo[2,1-a]isoquinolinecarboxylates as phosphodiesterase 10a inhibitors for treating cancer. WO2003014115 A1
88. Niewohner U, Bauser M, Ergueden J-K, Flubacher D, Naab P, Repp T-O, Stoltefuss J, Burkhardt N, Sewing A, Schauer M, Schlemmer K-H, Weber O, Boyer SJ, Miglarese M, Fan J, Phillips B, Raudenbush BC, Wang Y (2003) Preparation of pyrrolo[2,1-a]isoquinoline-1-carboxylates as phosphodiesterase 10a inhibitors. WO2003014116 A1
89. Sweet L (2005) PDE10a inhibitors for treating diabetes and related disorders. WO2005012485 A2
90. Niewoehner U, Hendrix M, Brueckner D, Friedl A, Gerlach I, Hinz V, Keldenich J, Mauler F, Schauss D, Schlemmer K-h, Tersteegen A, Yalkinoglu O (2004) Preparation of imidazotriazines as selective phosphodiesterase-10a inhibitors for the treatment of cancer and neurodegenerative diseases. WO2004005291 A1
91. Niewohner U, Erguden J-K, Bauser M, Burkhardt N, Flubacher D, Friedl A, Gerlach I, Hinz V, Jork R, Naab P, Repp T-O, Schlemmer K-H, Stoltefuss J (2003) Phosphodiesterase (PDE) 10A inhibitor use for the treatment and/or prophylaxis of neurodegenerative disease. WO2003000269 A2
92. Hoefgen N, Stange H, Langen B, Egerland U, Schindler R, Pfeifer T, Rundfeldt C (2007) Preparation of imidazo[1,5-a]pyrido[3,2-e]pyrazines as inhibitors of phosphodiesterase 10. WO2007137820 A1
93. Malamas MS, Ni Y, Erdei JJ, Stange H, Schindler R, Hoefgen N, Egerland U, Langen B (2009) Pyrido[3,2-e]pyrazines, process for preparing the same, and their use as inhibitors of phosphodiesterase 10 for treating nervous system disorders, obesity, and metabolic disorders. WO2009070583 A1
94. Stange H, Langen B, Egerland U, Hoefgen N, Priebs M, Malamas MS, Erdei JJ, Ni Y (2010) Preparation of triazine derivatives as inhibitors of phosphodiesterases for treating CNS disorders, metabolic disorders, and other diseases. WO2010054253 A1
95. Chaturvedula PV, Kimura SR (2012) Preparation of phenylimidazolylcycloproylheteroaryl derivatives for use as PDE10 inhibitors. WO2012177738 A1
96. Schmitz WD, Debenedetto MV, Kimura SR (2013) Preparation of substituted 2-(imidazo[1,2-a] pyridin-7-yl)-[1,2,4]triazolo[1,5-a]pyrazines as inhibitors of PDE10. WO2013003298 A2
97. Chesworth R, Shapiro G, Ripka A (2009) Di-substituted phenyl compounds as phosphodiesterase 10 inhibitors and their preparation useful in the treatment of CNS disorders and disorders affected by CNS function. WO2009158467 A2
98. Ripka A, Shapiro G, Chesworth R (2010) Preparation of vicinal substituted cyclopropyl compounds as Pde-10 inhibitors. WO2010006130 A2
99. Davenport T, Vile J, Pal S (2012) Pyrazolopyridine compounds as PDE10a inhibitors. WO2012019954 A1

100. Gharat LA, Gajera JM, Khairatkar-Joshi N, Bajpai M (2011) Tricycle compounds as phosphodiesterase-10 inhibitors and their preparation. WO2011132051 A2
101. Gharat LA, Narayana L, Yadav PS, Khairatkar-Joshi N, Bajpai M (2011) Preparation of heteroaryl compounds as PDE10A inhibitors. WO2011132048 A1
102. Lingam PRVS, Thomas A, Khairatkar-Joshi N, Bajpai M, Gullapalli S, Dahale DH, Mindhe AS, Rathi VE (2011) Preparation of aryl substituted olefinic compounds as phosphodiesterase 10A (PDE10A) inhibitors. WO2011138657 A1
103. Nieber K, Erdmann S, Briel D, Schwan G, Kubicova L, Barbar AG, Straeter N, Zahn M, Funke U, Scheunemann M, Fischer S, Brust P (2012) Novel alkoxyquinazolines as PDE10A modulators, and their preparation and use in the treatment of neurological and neuropsychiatric diseases. WO2012052556 A1
104. Bachmann S, Flohr A, Zbinden KG, Koerner M, Kuhn B, Peters J-U, Rudolph M (2013) Preparation of substituted pyrazolecarboxamides as PDE10 modulators. US20130059833 A1
105. Bleicher K, Flohr A, Groebke ZK, Gruber F, Koerner M, Kuhn B, Peters J-U, Rodriguez SRM (2011) Nitrogen containing heteroaryl compounds as PDE10A inhibitors and their preparation and use in the treatment of diseases. WO2011154327 A1
106. Flohr A, Groebke ZK, Kuhn B, Lerner C, Rudolph M, Schaffhauser H (2013) Triazolo compounds as pde10 inhibitors. WO2013178572 A1
107. Bartolome-Nebreda JM, Conde-Ceide S, MacDonald GJ, Pastor-Fernandez J, Van GMLM, Martin-Martin ML, Vanhoof GCP (2011) Preparation of imidazo[1,2-a]pyrazine derivatives and their use for the prevention or treatment of neurological, psychiatric and metabolic disorders and diseases. WO2011110545 A1
108. Pastor-Fernandez J, Bartolome-Nebreda JM, MacDonald GJ, Conde-Ceide S, Delgado-Gonzalez O, Vanhoof GCP, Van GMLM, Martin-Martin ML, Alonso-De DS-A (2011) Imidazo[1,2-b]pyridazine derivatives as PDE10 inhibitors and their preparation, pharmaceutical compositions and use in the treatment of CNS diseases. WO2011051342 A1
109. Swinney KA, Wuyts S (2012) Salts of an imidazo[1,2-b]pyridazine derivative as PDE10 inhibitors for treatment of CNS and metabolic disorders. WO2012146644 A1
110. Andres-Gil JI, Bormans GMR, De AM, Celen SJL (2010) Radiolabelled PDE10 ligands. WO2010097367 A1
111. Andres-Gil JI, De AM, Bormans GMR, Celen SJL (2011) Radiolabelled pde10 ligands. WO2011051324 A1
112. Kohno Y, Adams DR, Ando N (2006) Preparation of 6-(pyrazolo[1,5-a]pyridin-4-yl)-3(2H)-pyridazinone derivatives and addition salts thereof as phosphodiesterase inhibitors. WO2006095666 A1
113. Osakada N, Haruoka M, Ikeda K, Toki S, Miyaji H, Shimada J (2004) Preparation of quinoline derivatives as phosphodiesterase 10A inhibitors. WO2004002484 A1
114. Kehler J, Kilburn JP, Nielsen J, Pueschl A, Langgaard M, Jessing M, Marigo M (2013) Preparation of quinazoline derivatives for use as PDE10A enzyme inhibitors. WO2013050527 A1
115. Ritzen A, Kehler J, Langgaard M, Nielsen J, Kilburn JP, Farah MM (2009) Novel phenylimidazole derivatives as PDE10A enzyme inhibitors for treating neurodegenerative disorder, psychiatric disorder, or drug addiction. WO2009152825 A1
116. Marigo M, Kilburn JP, Nielsen J, Pueschl A, Langgaard M, Jessing M, Kehler J (2013) Preparation of quinazoline linked heteroaromatic tricycle derivatives as PDE10A enzyme inhibitors. WO2013045607 A1
117. Breslin MJ, Cox CD, Hartingh TJ, Pero J, Raheem IT, Rossi M, Vassallo L (2012) Preparation of aryloxymethylcyclopropane derivatives as PDE10 inhibitors. WO2012162213 A1
118. Barrow JC, Cox CD, Nolt MB, Shipe WD (2013) Triazolopyridinones as PDE10 inhibitors. WO2013074390 A1
119. Cox CD, Beshore DC, Kim JJ, Kuduk SD, McVean C, Reger T, Sheen J (2013) Preparation of 1,3-substituted azetidine PDE10 inhibitors for treatment of CNS disorders. WO2013052395 A1

120. Hostetler E, Cox CD, Fan H (2010) Radiolabeled pde10 inhibitors. WO2010138577 A1
121. Kawanishi E, Matsumura T (2010) Preparation of trisubstituted pyrimidines as phosphodiesterase PDE10 inhibitors. WO2010027097 A1
122. Kawanishi E, Tanaka Y, Matsumura T, Kado Y, Taniuchi H (2013) Fused heterocyclic compounds, method for inhibiting PDE10 by them, and pharmaceutical compositions containing them. WO2013027794 A1
123. Ishikawa M, Haitani S, Kosuga N (2012) Preparation of novel azole derivatives as inhibitors of phosphodiesterase 10 (PDE 10). WO2012147890 A1
124. Miyazaki Y, Haitani S, Kudo T, Ishikawa M (2013) Preparation of nitrogenated heterocyclic compounds as PDE10 inhibitors. WO2013005798 A1
125. Cutshall NS, Gage JL (2009) Hydrazides as PDE10 inhibitors and related compositions, preparation and use in the treatment of diseases. WO2009143178 A2
126. Cutshall NS, Gage JL, Wheeler NT (2010) Preparation of (hetero)arylacetohydrazides as PDE10 phosphodiesterase inhibitors. WO2010017236 A1
127. Chappie TA, Humphrey JM (2006) Heteroaryl pyrrolidine derivatives as phosphodiesterase inhibitors, their preparation, pharmaceutical compositions, and use in therapy. WO2006070284 A1
128. Verhoes PR (2008) Tricyclic heteroaryl compounds as selective PDE10 inhibitors, their preparation, pharmaceutical compositions, and use in therapy. WO2008001182 A1
129. Verhoest PR, Helal CJ, Hoover DJ, Humphrey JM (2006) Heteroaromatic quinoline compounds as phosphodiesterase inhibitors, their preparation, pharmaceutical compositions, and use in therapy. WO2006072828 A2
130. Ho GD, Smith EM, Kiselgof EY, Basu K, Tan Z, McKittrick B, Tulshian D (2010) Preparation of substituted triazolopyridines and analogs as phosphodiesterase 10 (PDE10) inhibitors. WO2010117926 A1
131. Ho GD, Yang S-W, Smith EM, McElroy WT, Basu K, Smotryski J, Tan Z, McKittrick BA, Tulshian DB (2010) Preparation of substituted pyrazoloquinolines and derivatives thereof. WO2010062559 A1
132. Ho GD, Seganish WM, Tulshian DB, Timmers CM, Rijn RDV, Loozen HJJ (2011) 5,6-Dihydroimidazo[5,1-a]isoquinoline derivatives as PDE10 inhibitors and their preparation and use for the treatment of PDE10-modulated diseases. WO2011008597 A1
133. Kanuma K, Amada H, Miyakoshi N, Tamita T, Kashiwa S (2012) Preparation of 3-pyrazolyl-2-pyridone derivatives as PDE10A inhibitors. WO2012029326 A1
134. Taniguchi T, Miura S, Hasui T, Halldin C, Stepanov V, Takano A (2013) Preparation of radiolabeled (3-pyrazol-5-yl)pyridazin-4(1H)-one derivatives as radiotracers useful for quantitative imaging of phosphodiesterase (PDE10A) in mammals. WO2013027845 A1
135. Taniguchi T, Yoshikawa M, Miura K, Hasui T, Honda E, Imamura K, Kamata M, Kamisaki H, Quinn JF, Raker J, Camara F, Wang Y (2011) Preparation of fused heterocyclic compounds as phosphodiesterase 10A inhibitors for preventing and treating schizophrenia. WO2011163355 A1
136. Fukushi H, Taniguchi T (2013) Preparation of fused heterocyclic compounds as antipsychotic drugs. WO2013035826 A1
137. clinicaltrials.gov. (2006) ClinicalTrials.gov [Internet]. National Library of Medicine, Bathesda. http://clinicaltrials.gov/
138. DeMartinis N, Banerjee A, Kumar V, Boyer S, Schmidt C, Arroyo S (2012) Results of a Phase 2A Proof-Of-Concept Trial with a PDE10A Inhibitor in the Treatment of Acute Exacerbation of Schizophrenia. Paper presented at the 3rd Biennieal Schizophrenia international research conference. http://mli.nih.gov/mli/download/1355. Florence, Italy
139. Schmidt C (2013) Inhibition of PDE10A for the treatment of schizophrenia: preclinical rationale (what we think we know). In: NIMH. http://mli.nih.gov/mli/download/1355. Accessed 25 Jan 2013
140. Brandon N (2013) What are some of the unknowns around PDE10A biology? In: NIMH. http://mli.nih.gov/mli/download/1354. Accessed 25 Jan 2013

141. Lilly E (2013) Effects of MP-10 in preclinical assays-the lilly experience. In: NIMH. http://mli.nih.gov/mli/download/1357. Accessed 25 Jan 2013
142. Omeros (2014) http://investor.omeros.com/phoenix.zhtml?c=219263&p=irol-newsArticle_Print&ID=1906687&highlight=;http://investor.omeros.com/phoenix.zhtml?c=219263&p=irol-newsArticle_Print&ID=1894589&highlight
143. Soderling SH, Bayuga SJ, Beavo JA (1998) Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. J Biol Chem 273(25):15553–15558
144. Fisher DA, Smith JF, Pillar JS, Denis SHS, Cheng JB (1998) Isolation and characterization of PDE9A, a novel human cgmp-specific phosphodiesterase. J Biol Chem 273 (25):15559–15564
145. Gattaz WF, Cramer H, Beckmann H (1983) Low CSF concentrations of cyclic GMP in schizophrenia. Br J Psychiatr 142(3):288–291
146. Beckmann H, Gattaz WF (2002) Multidimensional analysis of the concentrations of 17 substances in the CSF of schizophrenics and controls. J Neural Transm 109(5–6):931–938. doi:10.1007/s007020200076
147. Guipponi M, Scott HS, Kudoh J, Kawasaki K, Shibuya K, Shintani A, Asakawa S, Chen H, Lalioti MD, Rossier C, Minoshima S, Shimizu N, Antonarakis SE (1998) Identification and characterization of a novel cyclic nucleotide phosphodiesterase gene (PDE9A) that maps to 21q22.3: alternative splicing of mRNA transcripts, genomic structure and sequence. Hum Genet 103(4):386–392. doi:10.1007/s004390050838
148. Rentero C, Monfort A, Puigdomènech P (2003) Identification and distribution of different mRNA variants produced by differential splicing in the human phosphodiesterase 9A gene. Biochem Biophys Res Commun 301(3):686–692. doi:10.1016/S0006-291X(03)00021-4
149. Wang P, Wu P, Egan RW, Billah MM (2003) Identification and characterization of a new human type 9 cGMP-specific phosphodiesterase splice variant (PDE9A5): Differential tissue distribution and subcellular localization of PDE9A variants. Gene 314:15–27. doi:10.1016/S0378-1119(03)00733-9
150. Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev 58(3):488–520. doi:10.1124/pr.58.3.5
151. van Staveren WCG, Glick J, Markerink-van Ittersum M, Shimizu M, Beavo JA, Steinbusch HWM, de Vente J (2003) Cloning and localization of the cGMP-specific phosphodiesterase type 9 in the rat brain. J Neurocytol 31(8/9):729–741
152. Andreeva SG, Dikkes P, Epstein PM, Rosenberg PA (2001) Expression of cGMP-specific phosphodiesterase 9A mRNA in the rat brain. J Neurosci 21(22):9068–9076
153. van Staveren WCG, Glick J, Markerink-van Ittersum M, Shimizu M, Beavo JA, Steinbusch HWM, de Vente J (2002) Cloning and localization of the cGMP-specific phosphodiesterase type 9 in the rat brain. J Neurocytol 31(8–9):729–741. doi:10.1023/A:1025704031210
154. Van Staveren WCG, Steinbusch HWM, Markerink-Van Ittersum M, Repaske DR, Goy MF, Kotera J, Omori K, Beavo JA, De Vente J (2003) mRNA expression patterns of the cGMP-hydrolyzing phosphodiesterases types 2, 5, and 9 during development of the rat brain. J Comp Neurol 467(4):566–580. doi:10.1002/cne.10955
155. Van Staveren WCG, Steinbusch HWM, Markerink-van Ittersum M, Behrends S, De Vente J (2004) Species differences in the localization of cGMP-producing and NO-responsive elements in the mouse and rat hippocampus using cGMP immunocytochemistry. Eur J Neurosci 19(8):2155–2168. doi:10.1111/j.0953-816X.2004.03327.x
156. Reyes-Irisarri E, Markerink-Van Ittersum M, Mengod G, de Vente J (2007) Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. Eur J Neurosci 25(11):3332–3338
157. Bear MF, Kirkwood A (1993) Neocortical long-term potentiation. Curr Opin Neurobiol 3 (2):197–202. doi:10.1016/0959-4388(93)90210-P
158. Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361(6407):31–39

159. Domek-Lopacinska KU, Strosznajder JB (2010) Cyclic GMP and nitric oxide synthase in aging and Alzheimer's Disease. Mol Neurobiol 41(2–3):129–137. doi:10.1007/s12035-010-8104-x
160. Kleppisch T, Feil R (2009) cGMP signalling in the mammalian brain: role in synaptic plasticity and behaviour. Handbook Exp Pharmacol 191:549–579
161. Otani S, Daniel H, Roisin M-P, Crepel F (2003) Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. Cerebr Cortex 13(11):1251–1256
162. Sanderson TM, Sher E (2013) The role of phosphodiesterases in hippocampal synaptic plasticity. Neuropharmacology 74:86–95. doi:10.1016/j.neuropharm.2013.01.011
163. Liu S, Mansour MN, Dillman KS, Perez JR, Danley DE, Aeed PA, Simons SP, LeMotte PK, Menniti FS (2008) Structural basis for the catalytic mechanism of human phosphodiesterase 9. Proc Natl Acad Sci U S A 105(36):13309–13314. doi:10.1073/pnas.0708850105, S13309/13301-S13309/13314
164. Wang H, Luo X, Ye M, Hou J, Robinson H, Ke H (2010) Insight into binding of phosphodiesterase-9A selective inhibitors by crystal structures and mutagenesis. J Med Chem 53(4):1726–1731. doi:10.1021/jm901519f
165. Hou J, Xu J, Liu M, Zhao R, Luo H-B, Ke H (2011) Structural asymmetry of phosphodiesterase-9, potential protonation of a glutamic acid, and role of the invariant glutamine. PLoS One 6(3):e18092. doi:10.1371/journal.pone.0018092
166. Claffey MM, Helal CJ, Verhoest PR, Kang Z, Bundesmann MW, Hou X, Lui S, Kleiman RJ, Vanase-Frawley M, Schmidt AW, Menniti F, Schmidt CJ, Hoffman WE, Hajos M, McDowell L, O'Connor RE, MacDougall-Murphy M, Fonseca KR, Becker SL, Nelson FR, Liras S (2012) Application of structure-based drug design and parallel chemistry to identify selective, brain penetrant, in vivo active phosphodiesterase 9A inhibitors. J Med Chem 55 (21):9055–9068. doi:10.1021/jm3009635
167. Verhoest PR, Fonseca KR, Hou X, Proulx-LaFrance C, Corman M, Helal CJ, Claffey MM, Tuttle JB, Coffman KJ, Liu S, Nelson F, Kleiman RJ, Menniti FS, Schmidt CJ, Vanase-Frawley M, Liras S (2012) Design and discovery of 6-[(3S,4S)-4-Methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d] pyrimidin-4-one (PF-04447943), a selective brain penetrant pde9a inhibitor for the treatment of cognitive disorders. J Med Chem 55(21):9045–9054. doi:10.1021/jm3007799
168. Meng F, Hou J, Shao Y-X, Wu P-Y, Huang M, Zhu X, Cai Y, Li Z, Xu J, Liu P, Luo H-B, Wan Y, Ke H (2012) Structure-based discovery of highly selective phosphodiesterase-9A inhibitors and implications for inhibitor design. J Med Chem 55(19):8549–8558. doi:10.1021/jm301189c
169. Verhoest PR, Proulx-Lafrance C, Corman M, Chenard L, Helal CJ, Hou X, Kleiman R, Liu S, Marr E, Menniti FS, Schmidt CJ, Vanase-Frawley M, Schmidt AW, Williams RD, Nelson FR, Fonseca KR, Liras S (2009) identification of a brain penetrant PDE9A inhibitor utilizing prospective design and chemical enablement as a rapid lead optimization strategy. J Med Chem 52(24):7946–7949. doi:10.1021/jm9015334
170. Jorgensen M, Kehler J, Langgard M, Svenstrup N, Tagmose L (2013) Selective Inhibitors of PDE2, PDE9, and PDE10: modulators of activity of the central nervous system. Annu Rep Med Chem 48:37–55
171. Lu Y-F, Kandel ER, Hawkins RD (1999) Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. J Neurosci 19(23):10250–10261
172. Weitzdoerfer R, Hoeger H, Engidawork E, Engelmann M, Singewald N, Lubec G, Lubec B (2004) Neuronal nitric oxide synthase knock-out mice show impaired cognitive performance. Nitric Oxide 10(3):130–140. doi:10.1016/j.niox.2004.03.007
173. Son H, Hawkins RD, Martin K, Kiebler M, Huang PL, Fishman MC, Kandel ER (1996) Long-term potentiation is reduced in mice that are doubly mutant in endothelial and neuronal nitric oxide synthase. Cell 87(6):1015–1023
174. Hopper RA, Garthwaite J (2006) Tonic and phasic nitric oxide signals in hippocampal long-term potentiation. J Neurosci 26(45):11513–11521

175. Chetkovich DM, Klann E, Sweatt JD (1993) Nitric oxide synthase-independent long-term potentiation in area CA1 of hippocampus. NeuroReport 4(7):919–922
176. Bon CLM, Garthwaite J (2003) On the role of nitric oxide in hippocampal long-term potentiation. J Neurosci 23(5):1941–1948
177. Yamada K, Hiramatsu M, Noda Y, Mamiya T, Murai M, Kameyama T, Komori Y, Nikai T, Sugihara H, Nabeshima T (1996) Role of nitric oxide and cyclic GMP in the dizocilpine-induced impairment of spontaneous alternation behavior in mice. Neuroscience 74(2):365–374. doi:10.1016/0306-4522(96)00161-3
178. Taqatqeh F, Mergia E, Neitz A, Eysel UT, Koesling D, Mittmann T (2009) More than a retrograde messenger: nitric oxide needs two cGMP pathways to induce hippocampal long-term potentiation. J Neurosci 29(29):9344–9350
179. Zhuo M, Hu Y, Schultz C, Kandel ER, Hawkins RD (1994) Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. Nature 368:635–639
180. Arancio O, Kandel ER, Hawkins RD (1995) Activity-dependent long-term enhancement of transmitter release by presynaptic 3′,5′-cyclic GMP in cultured hippocampal neurons. Nature 376:74–80
181. Boulton CL, Southam E, Garthwaite J (1995) Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. Neuroscience 69(3):699–703. doi:10.1016/0306-4522(95)00349-N
182. Monfort P, Muñoz Ma-D, Kosenko E, Llansola M, Sánchez-Pérez A, Cauli O, Felipo V (2004) Sequential activation of soluble guanylate cyclase, protein kinase G and cGMP-degrading phosphodiesterase is necessary for proper induction of long-term potentiation in CA1 of hippocampus: Alterations in hyperammonemia. Neurochem Int 45(6):895–901. doi:10.1016/j.neuint.2004.03.020
183. Bernabeu R, Schmitz P, Faillace MP, Izquierdo I, Medina JH (1996) Hippocampal cGMP and cAMP are differentially involved in memory processing of inhibitory avoidance learning. NeuroReport 7(2):585–588
184. Bernabeu R, Schroder N, Quevedo J, Cammarota M, Izquierdo I, Medina JH (1997) Further evidence for the involvement of a hippocampal cGMP/cGMP-dependent protein kinase cascade in memory consolidation. NeuroReport 8(9):2221–2224
185. Prickaerts J, de Vente J, Honig W, Steinbusch HWM, Blokland A (2002) cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. Eur J Pharmacol 436(1–2):83–87. doi:10.1016/S0014-2999(01)01614-4
186. Rubin MA, Jurach A, da Costa Júnior EM, Lima TT, Jiménez-Bernal RE, Begnini J, Souza DO, de Mello CF (1996) GMP reverses the facilitatory effect of glutamate on inhibitory avoidance task in rats. NeuroReport 7(13):2078–2080
187. Blokland A, Schreiber R, Prickaerts J (2006) Improving memory: a role for phosphodiesterases. Curr Pharm Des 12(20):2511–2523. doi:10.2174/138161206777698855
188. Menniti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. Nat Rev Drug Discov 5(8):660–670. doi:10.1038/nrd2058
189. Halene TB, Siegel SJ (2007) PDE inhibitors in psychiatry-future options for dementia, depression and schizophrenia? Drug Discov Today 12(19&20):870–878. doi:10.1016/j.drudis.2007.07.023
190. Reneerkens OAH, Rutten K, Steinbusch HWM, Blokland A, Prickaerts J (2009) Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. Psychopharmacology (Berlin, Ger) 202(1–3):419–443. doi:10.1007/s00213-008-1273-x
191. Bales K, Plath N, Svenstrup N, Menniti F (2010) Phosphodiesterase inhibition to target the synaptic dysfunction in Alzheimer's disease. In: Dominguez C (ed) Neurodegenerative diseases, vol 6. Topics in Medicinal Chemistry. Springer, Berlin Heidelberg, pp 57–90. doi:10.1007/7355_2010_8
192. Schmidt CJ (2010) Phosphodiesterase inhibitors as potential cognition enhancing agents. Curr Top Med Chem (Sharjah, UAE) 10(2):222–230. doi:10.2174/156802610790411009

193. Xu Y, Zhang H-T, O'Donnell JM (2011) Phosphodiesterases in the central nervous system: implications in mood and cognitive disorders. Handb Exp Pharmacol 204:447–485. doi:10.1007/978-3-642-17969-3_19
194. Rodefer JS, Saland SK, Eckrich SJ (2012) Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. Neuropharmacology 62(3):1182–1190. doi:10.1016/j.neuropharm.2011.08.008
195. Wunder F, Tersteegen A, Rebmann A, Erb C, Fahrig T, Hendrix M (2005) Characterization of the first potent and selective PDE9 inhibitor using a cGMP reporter cell line. Mol Pharmacol 68(6):1775–1781. doi:10.1124/mol.105.017608
196. Liddie S, Anderson Karen L, Paz A, Itzhak Y (2012) The effect of phosphodiesterase inhibitors on the extinction of cocaine-induced conditioned place preference in mice. J Psychopharmacol 26(10):1375–1382
197. van der Staay FJ, Rutten K, Baerfacker L, DeVry J, Erb C, Heckroth H, Karthaus D, Tersteegen A, van Kampen M, Blokland A, Prickaerts J, Reymann KG, Schroeder UH, Hendrix M (2008) The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. Neuropharmacology 55(5):908–918. doi:10.1016/j.neuropharm.2008.07.005
198. Kroker KS, Rast G, Giovannini R, Marti A, Dorner-Ciossek C, Rosenbrock H (2012) Inhibition of acetylcholinesterase and phosphodiesterase-9A has differential effects on hippocampal early and late LTP. Neuropharmacology 62(5–6):1964–1974. doi:10.1016/j.neuropharm.2011.12.021
199. Hutson PH, Finger EN, Magliaro BC, Smith SM, Converso A, Sanderson PE, Mullins D, Hyde LA, Eschle BK, Turnbull Z, Sloan H, Guzzi M, Zhang X, Wang A, Rindgen D, Mazzola R, Vivian JA, Eddins D, Uslaner JM, Bednar R, Gambone C, Le-Mair W, Marino MJ, Sachs N, Xu G, Parmentier-Batteur S (2011) The selective phosphodiesterase 9 (PDE9) inhibitor PF-04447943 (6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) enhances synaptic plasticity and cognitive function in rodents. Neuropharmacology 61(4):665–676. doi:10.1016/j.neuropharm.2011.05.009
200. Kleiman RJ, Chapin DS, Christoffersen C, Freeman J, Fonseca KR, Geoghegan KF, Grimwood S, Guanowsky V, Hajos M, Harms JF, Helal CJ, Hoffmann WE, Kocan GP, Majchrzak MJ, McGinnis D, McLean S, Menniti FS, Nelson F, Roof R, Schmidt AW, Seymour PA, Stephenson DT, Tingley FD, Vanase-Frawley M, Verhoest PR, Schmidt CJ (2012) Phosphodiesterase 9A regulates central cGMP and modulates responses to cholinergic and monoaminergic perturbation in vivo. J Pharmacol Exp Ther 341(2):396–409. doi:10.1124/jpet.111.191353
201. Vardigan JD, Converso A, Hutson PH, Uslaner JM (2011) The Selective Phosphodiesterase 9 (PDE9) Inhibitor PF-04447943 Attenuates a Scopolamine-Induced Deficit in a Novel Rodent Attention Task. J Neurogenet 25(4):120–126. doi:10.3109/01677063.2011.630494
202. Schmidt CJ, Harms JF, Tingley FD, Schmidt K, Adamowicz WO, Romegialli A, Kleiman RJ, Barry CJ, Coskran TM, O'Neill SM, Stephenson DT, Menniti FS (2009) PDE9A-mediated regulation of cGMP: developing a biomarker for a novel therapy for Alzheimer's disease. Alzheimers Dement 5(4):P331
203. Nicholas T, Evans R, Styren S, Qiu R, Wang EQ, Nelson F, Le V, Grimwood S, Christoffersen C, Banerjee S, Corrigan B, Kocan G, Geoghegan K, Carrieri C, Raha N, Verhoest P, Soares H (2009) PF-04447943, a novel PDE9A inhibitor, increases cGMP levels in cerebrospinal fluid: translation from non-clinical species to healthy human volunteers. Alzheimers Dement 5(4):P330–P331
204. Aleman A, Hijman R, de Haan EHF, Kahn RS (1999) Memory Impairment in Schizophrenia: A Meta-Analysis. Am J Psych 156(9):1358–1366
205. Brown C, Cromwell RL, Filion D, Dunn W, Tollefson N (2002) Sensory processing in schizophrenia: missing and avoiding information. Schizophr Res 55(1–2):187–195. doi:10.1016/S0920-9964(01)00255-9

206. Javitt DC (2009) Sensory processing in schizophrenia: neither simple nor intact. Schizophr Bull 35(6):1059–1064
207. Siegel C, Waldo M, Mizner G, Adler LE, Freedman R (1984) Deficits in sensory gating in schizophrenic patients and their relatives: evidence obtained with auditory evoked responses. Arch Gen Psychiatr 41(6):607–612. doi:10.1001/archpsyc.1984.01790170081009
208. Patterson JV, Hetrick WP, Boutros NN, Jin Y, Sandman C, Stern H, Potkin S, Bunney WE (2008) P50 sensory gating ratios in schizophrenics and controls: a review and data analysis. Psychiatr Res 158(2):226–247
209. Sánchez-Morla EM, García-Jiménez MA, Barabash A, Martínez-Vizcaíno V, Mena J, Cabranes-Díaz JA, Baca-Baldomero E, Santos JL (2008) P50 sensory gating deficit is a common marker of vulnerability to bipolar disorder and schizophrenia. Acta Psychiatr Scand 117(4):313–318. doi:10.1111/j.1600-0447.2007.01141.x
210. O'Carroll R (2000) Cognitive impairment in schizophrenia. Adv Psychiatr Treat 6(3):161–168
211. Kuperberg G, Heckers S (2000) Schizophrenia and cognitive function. Curr Opin Neurobiol 10(2):205–210. doi:10.1016/S0959-4388(00)00068-4
212. Keefe RE, Harvey P (2012) Cognitive impairment in schizophrenia. In: Geyer MA, Gross G (eds) Novel antischizophrenia treatments, vol 213. Handbook of experimental pharmacology. Springer, Berlin Heidelberg, pp 11–37. doi:10.1007/978-3-642-25758-2_2
213. Boss F-G, Erb C, Hendrix M, Van KM, Wunder F (2004) Selective phosphodiesterase 9A inhibitors for the improvement of cognitive processes. DE10238722 A1
214. Hendrix M, Boess F-G, Burkhardt N, Erb C, Tersteegen A, Van KM (2004) Preparation of pyrazolopyrimidines as phosphodiesterase PDE9A inhibitors. DE10238724 A1
215. Hendrix M, Boess F-G, Burkhardt N, Erb C, Tersteegen A, Van KM (2004) Preparation of 6-benzylpyrazolo[3,4-d]pyrimidin-4-ones as phosphodiesterase-9A (PDE9A) inhibitors. WO2004018474 A1
216. Hendrix M, Baerfacker L, Erb C, Hafner F-T, Heckroth H, Schauss D, Tersteegen A, Van DSF-J, Van KM (2004) Preparation of 6-arylmethylpyrazolopyrimidines as PDE9A inhibitors for the treatment of Alzheimer's disease. WO2004099210 A1
217. Hendrix M, Baerfacker L, Erb C, Hafner F-T, Heckroth H, Schauss D, Tersteegen A, Van DSF-J, Van KM (2004) Preparation of pyrazolopyrimidinones as phosphodiesterase 9A inhibitors useful as nootropics. WO2004099211 A1
218. Hendrix M, Baerfacker L, Beyreuther B, Ebert U, Erb C, Hafner F-T, Heckroth H, Liu Y-H, Schauss D, Tersteegen A, Van DSF-J, Van KM (2004) Preparation of 6-arylamino-5-cyano-4-pyrimidinones as PDE9A inhibitors for the treatment of Alzheimer's disease. WO2004113306 A1
219. Hendrix M, Baerfacker L, Heckroth H, Karthaus D, Tersteegen A (2005) Preparation of 6-amino-5-cyano-pyrimidine-4-ones as phosphodiesterase 9A inhibitors for improving, perception. WO2005068436 A1
220. Verhoest PR, Proulx-Lafrance C (2008) Amino-heterocyclic compounds as PDE9 inhibitors and their preparation, pharmaceutical compositions and use in the treatment of, neurodegenerative diseases. WO2008139293 A1
221. Claffey MM, Helal CJ, Verhoest PR (2010) Preparation of dihydropyrazolopyrimidinone derivatives for use as PDE9 inhibitors. WO2010084438 A1
222. Eickmeier C, Doerner-Ciossek C, Fiegen D, Fox T, Fuchs K, Giovannini R, Heine N, Hendrix M, Rosenbrock H, Schaenzle G (2009) Preparation of 1,5-dihydropyrazolo[3,4-d] pyrimidin-4-one derivatives as PDE9A modulators for the treatment of CNS disorders. WO2009068617 A1
223. Fuchs K, Dorner-Ciossek C, Eickmeier C, Fiegen D, Fox T, Giovannini R, Heine N, Hendrix M, Rosenbrock H, Schaenzle G (2010) Preparation of pyrazolopyrimidinones and their use for the treatment of CNS disorders. WO2010026214 A1
224. Giovannini R, Doerner-Ciossek C, Eickmeier C, Fiegen D, Fox T, Fuchs K, Heine N, Rosenbrock H, Schaenzle G (2009) Preparation of heterocyclyldihydropyrazolopyrimidinone derivatives for use as PDE9A modulators. WO2009121919 A1

225. Heine N, Dorner-Ciossek C, Eickmeier C, Fiegen D, Fox T, Fuchs K, Giovannini R, Rosenbrock H, Schaenzle G, Baerfacker L (2011) 1,5-Dihydropyrazolo[3,4-d]pyrimidin-4-one derivatives as PDE9 inhibitors and their preparation and use for the treatment of CNS diseases. WO2011018495 A1
226. Heine N, Eickmeier C, Ferrara M, Giovannini R, Rosenbrock H, Schaenzle G (2012) preparation of 6-cycloalkyl-1,5-dihydropyrazolo[3,4-d]pyrimidin-4-one derivatives as PDE9 inhibitors. WO2012020022 A1
227. Heine N, Giovannini R (2012) Preparation of cycloalkylpyrazolopyrimidinone derivatives for use as PDE9 inhibitors for treatment and prevention of CNS diseases. WO2012110441 A1
228. Wan Y, Luo H, Meng F, Liu P, Zhu X, Wen D, Shao Y, Zhang C, Huang J (2011) Preparation of pyrazolo[3,4-d]pyrimidone derivatives as phosphodiesterase IX inhibitors. CN102260266 A
229. Ripka A, Shapiro G, McRiner A (2012) Preparation of imidazotriazinone derivatives for use as phosphodiesterase 9 inhibitors. WO2012040230 A1
230. Ripka A, Shapiro G, McRiner AJ, Bursavich MG (2013) Imidazotriazinone compounds as PDE9 inhibitors and their preparation. WO2013142269 A1
231. Norimine Y, Takeda K, Hagiwara K, Suzuki Y, Ishihara Y, Sato N (2013) Pyrazoloquinoline derivative as PDE9 inhibitor, pharmaceutical composition containing it. WO2013051639 A1
232. Svenstrup N, Simonsen KB, Rasmussen LK, Juhl K, Langgaard M, Wen K, Wang Y (2013) Preparation of imidazotriazinone derivatives for use as PDE9 inhibitors. WO2013110768 A1
233. Svenstrup N, Simonsen KB, Rasmussen LK, Juhl K, Langgaard M, Wen K, Wang Y (2013) Preparation of PDE9 inhibitors with imidazopyrazinone backbone. WO2013053690 A1
234. Evans RM, Nicholas T, Le V, Qiu R, Martin W, Martin D, Styren S, Fullerton T, Schwam E (2010) Safety and pharmacokinetics of PF-04447943, a PDEA inhibitor, in single and multiple dose Phase 1 studies in healthy volunteers. Alzheimers Dement 6(4):S135
235. Schwam E, Evans R, Nicholas T, Chew R, Davidson W, Ambrose D, Altstiel L (2011) PF-04447943: a phase II controlled clinical trial of a selective PDE9A inhbitor in Alzheimer's disease. Alzheimers Dement 7(4):S695
236. Kawamoto E, Vasconcelos A, Degaspari S, Böhmer A, Scavone C, Marcourakis T (2013) Age-related changes in nitric oxide activity, cyclic GMP, and TBARS levels in platelets and erythrocytes reflect the oxidative status in central nervous system. AGE 35(2):331–342. doi:10.1007/s11357-011-9365-7
237. Kelly MP, Adamowicz W, Bove S, Hartman AJ, Mariga A, Pathak G, Reinhart V, Romegialli A, Kleiman RJ (2014) Select 3′,5′-cyclic nucleotide phosphodiesterases exhibit altered expression in the aged rodent brain. Cell Signal 26(2):383–397. doi:10.1016/j.cellsig.2013.10.007

# Index