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THEMED ISSUE REVIEW

Molecular insights into orphan G protein-coupled receptors relevant to schizophrenia

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Abstract

Schizophrenia remains a sizable socio-economic burden that continues to be treated with therapeutics based on 70-year old science. All currently approved therapeutics primarily target the dopamine D_2 receptor to achieve their efficacy. Whilst dopaminergic dysregulation is a key feature in this disorder, the targeting of dopaminergic machinery has yielded limited efficacy and an appreciable side effect burden. Over the recent decades, numerous drugs that engage non-dopaminergic G protein-coupled receptors (GPCRs) have yielded a promise of efficacy without the deleterious side effect profile, yet none have successfully completed clinical studies and progressed to the market. More recently, there has been increased attention around non-dopaminergic GPCR-targeting drugs, which demonstrated efficacy in some schizophrenia symptom domains. This provides renewed hope that effective schizophrenia treatment may lie outside of the dopaminergic space. Despite the potential for muscarinic receptor- (and other well-characterised GPCR families) targeting drugs to treat schizophrenia, they are often plagued with complications such as lack of receptor subtype selectivity and peripheral on-target side effects. Orphan GPCR studies have opened a new avenue of exploration with many demonstrating schizophrenia-relevant mechanisms and a favourable expression profile, thus offering potential for novel drug development. This review discusses centrally expressed orphan GPCRs: GPR3, GPR6, GPR12, GPR52, GPR85, GPR88 and GPR139 and their relationship to schizophrenia. We review their expression, signalling mechanisms and cellular function, in conjunction with small molecule development and structural insights. We seek to provide a snapshot of the growing evidence and development potential of new classes of schizophrenia therapeutics.

KEYWORDS
GPCR, orphan GPCR, schizophrenia, structural biology

Abbreviations: Cryo-EM, cryogenic electron microscopy; ECL, extracellular loop; FDA, Food and Drug Administration; GR, glucocorticoid receptor; MSN, medium spiny neuron; S1P, sphingosine-1-phosphate; SPC, sphingosylphosphorylcholine; SREB, Super conserved Receptor Expressed in Brain.

1 | INTRODUCTION

1.1 | G protein-coupled receptor (GPCR) and schizophrenia overview

GPCRs are seven transmembrane proteins and constitute the largest group of membrane proteins. Approximately 34% of FDA-approved drugs are targeted at GPCRs (Hauser et al., 2018). They are widely expressed in the central nervous system (CNS), and many have been extensively studied for their roles in regulating brain function. GPCRs transmit signals by responding to extracellular stimuli including small molecules, hormones, and lipids; making them tractable targets for many drugs. Whilst many GPCRs have been characterised against their endogenous ligands, there remains a pool of GPCRs for which an endogenous ligand is yet to be assigned—these are orphans GPCRs.

Schizophrenia is a chronic mental disorder that affects ~20 million people worldwide (James et al., 2018). A recent paper has summarised the current understanding of schizophrenia pathology; dopamine and glutamate were highlighted as the main dysregulated neurochemicals in schizophrenia (Jauhar et al., 2022). Currently, all standard-of-care therapeutics primarily target **dopamine D_2 receptors**, despite GPCRs being the target of many failed investigational new drugs.

As a consequence of the varying dysregulation of neurotransmitter systems, there is a heterogeneity of symptoms that includes, but is not limited to, positive (or psychotic) symptoms such as hallucinations and delusions; negative symptoms (e.g., anhedonia); and cognitive deficits such as poor working memory (Owen et al., 2016). The aetiology of schizophrenia remains poorly understood; however, there is a clear impact on brain structure and neurochemistry in regions including the prefrontal cortex and cortico-subcortical circuits (Fallon et al., 2003). Current medications can relieve psychotic symptoms (hallucinations and delusions), but many patients remain refractory to these treatments. More importantly, the negative and cognitive symptoms that have a long-lasting impact on the life quality of schizophrenia patients have yet to be addressed (Carbon & Correll, 2014).

1.2 | Schizophrenia drug discovery landscape

Whilst there are many hypotheses on propagation of dysfunction in schizophrenia, the prevailing hypothesis remains steeped in the dopaminergic system, primarily due to the evidence that blockade of dopamine D_2 receptors generally improves psychotic symptoms. Evidence for dysregulated dopamine neurotransmission has been described in many preclinical animal models and human schizophrenia studies (Howes & Kapur, 2009). Additionally, dysregulated dopaminergic activity resulted in a change in **NMDA receptor** and **GABA receptor** conductance that gave rise to the symptoms (Avery & Krichmar, 2015). Drugs that antagonise hyperactive dopaminergic drive in the striatum have effectively reduced positive symptoms (Patel et al., 2014). Consequently, a large number of D_2 receptor antagonists were developed as drugs, which target the dopamine D_2 receptor with narrow or broad activity at other GPCRs such as serotonin receptors (Patel et al., 2014). While all of them display some efficacy at treating positive symptoms, the side effects are often intolerable and arise from unwanted on-target activity (Li et al., 2016). Therefore, the need for novel, non-dopaminergic drugs remains high.

Well-characterised non-dopaminergic GPCR targets, such as **muscarinic acetylcholine receptors** (M receptors), have also demonstrated promise in schizophrenia clinical trials. **Xanomeline**, an M_1 and M_4 receptor-preferring agonist, improved positive and negative symptoms and mildly improved cognitive function (Shekhar et al., 2008).

In a subsequent phase II clinical trial, the peripheral side effects of xanomeline, such as gastrointestinal symptoms, were addressed by co-administration with trospium, a M receptor antagonist that is peripherally restricted. Subjects with schizophrenia had improved scores in the positive and negative symptoms scales, with fewer adverse events (Brannan et al., 2021). The positive outcomes from these clinical studies are no doubt a quantum leap forward for the field but remind us that there is still work to be done.

In addition to serotonin–dopamine receptor dual antagonism in second-generation antipsychotics, agents that selectively target subtypes of serotonin receptors also have gained attention for their potential as a treatment, particularly in addressing negative symptoms and cognitive deficits (Yang & Tsai, 2017). In clinical trials, these agents displayed either no significant symptomatic improvement or only served as an adjunct treatment (Garay et al., 2016). Overall, the strategy that targets serotonin receptors for the treatment of schizophrenia still remains unclear.

Other CNS-active mediators have been of interest over the years for the development of new therapeutics, such as adenosine and histamine. None of their receptor targets have yielded any real traction, and all investigational new drugs targeting these receptor systems have failed.

1.3 | Probing orphan GPCR structure

Structural biology, specifically cryogenic electron microscopy (cryo-EM), is being widely adopted for use in the drug discovery process—especially for GPCRs. While high-throughput screens coupled to medicinal chemistry programs are still largely employed, structural biology enables the precise characterisation of the interaction between protein macromolecules and novel ligands. Whilst structural biology alone cannot create a drug, understanding the three dimensions of a ligand binding pocket provides vital information for medicinal chemistry. Analysis of interactions could enhance molecule refinements and provide insights in development of new chemotypes.

In this review, we will discuss the potential for orphan GPCRs to be targeted to treat schizophrenia. Orphan GPCRs, which have no characterised endogenous ligand(s), are increasingly of interest in drug discovery (Stockert & Devi, 2015). The understanding of orphan

GPCR biology is greatly enhanced through structural biology, because this approach does not always require a ligand to be bound to solve a structure—thus providing a map of potential ligand binding pockets to take a rational drug design approach.

Many orphan GPCRs are highly enriched in the CNS in regions relevant to schizophrenia (Figure 1), making them an attractive choice for drug discovery through improved tissue specificity. GPR3, GPR6, GPR12, GPR52, GPR85, GPR88 and GPR139 have been reported to be potential targets for intervention or to display schizophrenia-relevant function. Not only are these receptors expressed in brain regions relevant to the disorder, they also share homology with other receptors. Together this may lead to a better understanding of their function or accelerate the deorphanisation process.

This review intends to provide an overview on the potential of orphan GPCRs as targets for treatment of schizophrenia, focusing on their expression profiles, known mechanisms and functions, and small molecule discovery progress (with or without structural biology insights).

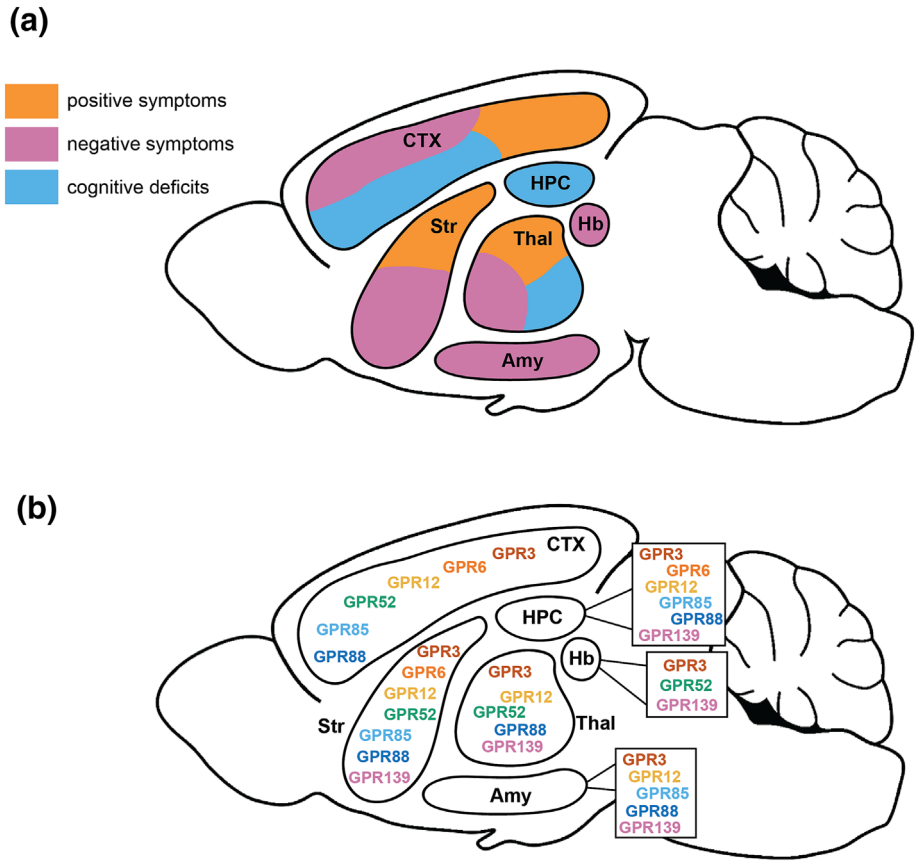

FIGURE 1 A schematic representation of selected orphan GPCR expression in the brain. (a) Key brain regions implicated in schizophrenia symptom pathology and/or presentation. Each brain region is coloured according to the symptom domain in which it is most heavily implicated, where orange = positive symptoms, pink = negative symptoms and blue = cognitive deficits. The cortex (CTx), particularly the prefrontal cortex, elicits top-down control over many subcortical regions that drive schizophrenia-relevant symptoms, including various cognitive functions such as working memory, cognitive flexibility and episodic memory, mood-related functions including motivation and goal-directed behaviours, and positive symptoms including disordered thinking and speaking and hyperactivity. The striatum (Str) is most implicated in positive symptoms of schizophrenia; increased dopaminergic drive underpins these symptoms. The striatum is also heavily involved in reward processing and functions of motivation, and therefore implicated in negative symptoms. The thalamus (Thal) comprises many smaller nuclei, each with specific functions. These thalamic nuclei are consistently functionally connected to and make key neural circuits with other brain regions in a way that underpins behaviours of all symptom domains of schizophrenia including cognitive function—for example, working memory, emotion and mood processing, and disordered thinking. The hippocampus (HPC) is primarily involved in cognitive processes, and it forms key neural circuits with the prefrontal cortex and other brain regions to critically underpin several cognitive functions that are consistently dysfunctional in schizophrenia patients, for example, working memory and episodic memory. The habenular nuclei (Hb) and amygdala (Amy) are brain regions most consistently implicated in emotional processing, motivation and anxiety and fear-related responses. They are therefore most heavily implicated in negative symptoms of schizophrenia; however, they are also involved in emotion- or fear-related cognitive functions. (b) Breakdown of CNS expression/enrichment of the orphan GPCRs; GPR3 (Valverde et al., 2009), GPR6 (Marchese, Cheng, et al., 1994), GPR12 (Ignatov et al., 2003), GPR52 (Komatsu et al., 2014), GPR85 (Hellebrand et al., 2000, 2001; Matsumoto et al., 2005), GPR88 (Ghate et al., 2007; Logue et al., 2009; Mizushima et al., 2000; Van Waes et al., 2011), GPR139 (Gloriam et al., 2005; Matsuo et al., 2005; Süsens et al., 2006).

1.4 | GPR3, GPR6 and GPR12 overview

The identification and cloning of **GPR3**, **GPR6** and **GPR12** in the mid-90s showed that they share around 60% amino acid identity and thus were grouped as one family (Heiber et al., 1995; Marchese, Docherty, et al., 1994; Song et al., 1995). These receptors were proposed to be involved in a range of physiological and disease processes, including oocyte meiosis and a putative role in cancer (Laun et al., 2019). Notably, their predominant CNS expression promoted the exploration of their roles in the brain and brain-related disorders. Recently, a human whole exome sequencing study revealed that genetic variance in *GPR12* was associated with an altered response to the antipsychotic drug, risperidone (Zhao et al., 2022). Herein, we will discuss what is known about GPR3, GPR6 and GPR12 as it pertains to new drug discovery for schizophrenia.

1.5 | Expression of GPR3, GPR6 and GPR12 in the CNS

GPR3 is expressed in several regions of the CNS (Ikawa et al., 2021), including in regions associated with all three schizophrenia-relevant symptom domains; the cerebral cortex, hippocampus, thalamus, striatum, habenula and amygdala (Ikawa et al., 2021; Tanaka et al., 2009). Specifically, enrichment of GPR3 in neurons implicated in all symptom domains of schizophrenia, such as layer 5 neurons of the cortex, is key given they are dysfunctional in schizophrenia patients (Black et al., 2004). Similarly, the medial habenula whose cholinergic neurons regulate motivation and addiction highlights the therapeutic potential of GPR3 as a target for addressing the poorly treated symptom domains.

GPR3, GPR6 and GPR12 are all constitutively active in vitro and an in vivo study of *Gpr3* KO mice confirmed this, showing reduced basal cAMP in hippocampal neurons; resulting in differentially altered dopamine, noradrenaline and serotonin contents in hippocampus, hypothalamus and frontal cortex (Uhlenbrock et al., 2002; Valverde et al., 2009).

The expression pattern of GPR6 is well conserved across mammalian species including humans and rats, with the highest expression associated with the striatum (Marchese, Cheng, et al., 1994). With striatal-enriched GPCRs already representing putative targets of interest for schizophrenia, particularly the alleviation of positive symptoms, conserved GPR6 expression in the frontal cortex and hippocampus also highlights possible roles in regulating cognitive deficits associated with schizophrenia (Marchese, Cheng, et al., 1994). GFP-tagged GPR6 revealed its enrichment in striatopallidal medium spiny neurons (MSNs), specifically co-expressed with dopamine D_2 receptors, but not with **dopamine D_1 receptors** (Lobo et al., 2007). Given that D_2 receptors on striatopallidal MSNs are the primary target of all currently marketed antipsychotic drugs, this expression profile bolsters the potential of GPR6 as a non-dopaminergic target for treating positive symptoms in schizophrenia.

GPR12 also has an interesting and similar expression profile to GPR3 and GPR6. In adult mouse brains, GPR12 levels are highly enriched in the cerebral cortex, hippocampus, nucleus accumbens of the striatum, thalamus and amygdala (Ignatov, Lintzel, Hermans-Borgmeyer, et al., 2003). Again, this enrichment in brain regions specifically implicated in schizophrenia symptomatology implicates GPR12 as a target of interest in the treatment of not only positive symptoms but also of the currently poorly treated negative and cognitive symptoms of schizophrenia.

1.6 | GPR3, GPR6, GPR12—schizophrenia-relevant function and intracellular signalling

While GPR3, GPR6 and GPR12 were shown to be involved in a range of schizophrenia-relevant physiological processes, the mechanisms underlying these processes are only partially resolved.

GPR3 is involved in neuron differentiation, neuron polarity formation, pain sensitivity and drug-induced reward activity (Ruiz-Medina et al., 2011; Tanaka et al., 2009, 2022; Tourino et al., 2012) via its signalling through numerous kinases (Tanaka et al., 2014, 2022). **GRK2** regulates GPR3 surface expression via β-arrestin-2 and plays a part in GPR3-mediated neurite growth (Lowther et al., 2013; Tanaka et al., 2022). Importantly, Tourino et al. (2012) highlighted a sensitivity to cocaine in GPR3 knockout mice, resulting in increased locomotor activity—a standard preclinical test for antipsychotic drugs. This suggests a GPR3 antagonist may suppress locomotor activity—acting as a potential novel antipsychotic target. Moreover, the deletion of GPR3 increased drug seeking behaviour, a behaviour that manifests in schizophrenia patients (Kosten & Ziedonis, 1997). Together, coupled with the role of GPR3 in neurite growth, there is a compelling argument for GPR3 to be a target of interest for schizophrenia.

GPR6-mediated neurite growth could be important in striatal neuron development. Enhanced neurite growth and resistance to growth inhibition were observed in neuronal cultures overexpressing GPR6, whose endogenous expression was promoted by a striatal neuron development transcription factor Sp9 (Tanaka et al., 2007; Zhang et al., 2016). A down-regulation of GPR6 was noted in the central extended amygdala when **the μ opioid receptors** (μ receptors) were chronically stimulated by morphine, suggesting a role of GPR6 in this circuit associated with the μ receptor, availability of which was reduced in people with schizophrenia (Ashok et al., 2019; Befort et al., 2008). In addition to μ receptors, GPR6 is linked to **glucocorticoid receptor** (GR)-mediated activity, where a reduced GR activity achieved by adrenalectomy increased GPR6 expression in the prefrontal cortex (Costin et al., 2013). Given that GR mRNA was found to be reduced in multiple brain regions, including frontal cortex, in schizophrenia patients, understanding the role of GPR6 in GR-mediated activity may provide insight into drug targets (Webster et al., 2002).

Similar to GPR3, genetic ablation of GPR6 increased basal locomotor activity in mice, which was reversed with haloperidol—suggesting an interaction with striatal dopaminergic function (Oeckl et al., 2014). Pharmacological inactivation of GPR6 with an inverse agonist, CVN424 (see below for more information), also increased locomotor activity in mice (Brice et al., 2021), thus corroborating the

genetic evidence. Relevant to the pathophysiology of schizophrenia, Oeckl et al. (2014) demonstrated a modulation of striatal dopamine through genetic ablation of GPR6. Knockout of GPR6 resulted in a modest increase in striatal dopamine, which is known to be elevated in the majority of schizophrenia patients. This evidence suggests that GPR6 activation could not only result in an antipsychotic-like behaviour but could also potentially modify the disease state through the reduction in striatal dopamine.

While GPR12 was also found to be involved in promoting neurite growth, cell proliferation and survival, less is known, but one physiological function of GPR12 may be in the regulation of working memory processes (Hsiao et al., 2020),

1.7 | GPR3, GPR6, GPR12—small molecule and structural studies

Endogenous ligands of this family have been actively sought but are yet to be fully validated. In vitro studies demonstrated that GPR6 was detected mainly in the intracellular compartments of HEK293 cells and striatal neurons, hinting that the endogenous ligand (if one exists) may either be membrane permeable or produced intracellularly, such as lipid-like molecules (Padmanabhan et al., 2009). **Sphingosine 1-phosphate (S1P)** was proposed as a ligand for this family, and showed a stimulatory effect on cAMP production, calcium release and S1P-mediated receptor translocation (Ignatov, Lintzel, Hermans-Borgmeyer, et al., 2003; Ignatov, Lintzel, Kreienkamp, & Schaller, 2003; Uhlenbrock et al., 2002). However, a high level of S1P stimulated response was also observed in the control cells, which created an ambiguity around the interpretation of the finding.

In addition, a structurally related molecule to S1P, **sphingosylphosphorylcholine** (SPC), was also proposed to be a ligand for GPR3, GPR6 and GPR12. SPC increased calcium flux in GPR6 overexpressing cells, which was absent in the control cells, indicating a more specific activation of GPR6 by SPC (Ignatov, Lintzel, Kreienkamp, & Schaller, 2003). It remains unclear whether SPC activates GPR6 in a native environment at physiological receptor concentrations. In addition to GPR3 and GPR6, activity of SPC was also observed at GPR12 (Ignatov, Lintzel, Hermans-Borgmeyer, et al., 2003). Interestingly, S1P and SPC seemed to selectively activate the calcium response; neither was able to recruit β-arrestins to GPR3, GPR6 or GPR12 (Yin et al., 2009). Whilst studies of S1P and SPC activation of GPR3, GPR6 and GPR12 in overexpressing systems provide insights into their pharmacology and putative second messenger signalling profiles, the role of these ligand–receptor pairs in native systems remains to be determined.

Similar to sphingosine ligands, cannabidiol, synthetic cannabinoids and endocannabinoid-like molecules have also been postulated as ligands of this receptor family. GPR3, GPR6 and GPR12 are phylogenetically related to **cannabinoid receptors**, sharing a 35% amino acid identity (Morales & Reggio, 2017; Uhlenbrock et al., 2002). Interestingly, in contrast to S1P and SPC, cannabinoid-related ligands did not regulate cAMP or calcium flux, but rather displayed inverse agonist activity for β-arrestin recruitment (Laun et al., 2018; Laun & Song, 2017; Shrader & Song, 2020). The divergent roles of S1P- and cannabinoid-related molecules on this receptor family still need to be fully understood; one could imagine that this receptor family has multiple binding sites that can be engaged by a range of endogenous molecules that are unrelated and regulate distinct signalling pathways. Further, the activation of this receptor family by cannabinoid-related molecules might not be via a cannabinoid-like binding mode—the important functional residues of cannabinoid receptors are largely absent in GPR3, GPR6 and GPR12. This suggests a divergence in ligand-receptor interactions compared with cannabinoid receptors (McPartland & Glass, 2003).

In addition to proposed endogenous ligands, there are a limited number of published small molecules specifically targeting GPR3, GPR6 or GPR12. **Diphenyleneiodonium chloride** and AF64394 have been shown to be GPR3-specific agonist and inverse agonist tools, respectively, aiding further exploration of GPR3 function (Jensen et al., 2014; Ye et al., 2014). A 3D model and likely binding pockets of GPR3 have been computationally predicted with analysis of potential binding poses of the inverse agonist, AF64394; three potential sites of AF64394 were identified (Bharathi and Roy, 2022). This suggests there is a need for further in vitro characterisation and experimentally determined GPR3 structures to enhance our understanding of GPR3-ligand molecular interactions. Whilst it may not be straightforward to obtain an inverse agonist-bound GPR3, the constitutive activity of GPR3, GPR6 and GPR12 may help with producing a ligand-free structure (Chen et al., 2022; Lin et al., 2020; Uhlenbrock et al., 2002; Valverde et al., 2009).

The small molecule **CVN424** was discovered and optimised as a GPR6-specific inverse agonist through a cAMP-directed high-throughput screen. It was efficacious in vivo in a preclinical Parkinson's disease model, reversing a 6-hydroxydopamine-induced locomotor deficit, and has displayed promising pharmacokinetic properties as a therapeutic candidate (Brice et al., 2021; Sun et al., 2021). It was also well tolerated in phase I human trials and is currently in phase II studies (Margolin et al., 2022). As a first-in-class candidate, it would be useful to further understand the molecular mechanism of CVN424 at GPR6.

In summary, GPR3, GPR6 and GPR12 present compelling targets for the treatment of schizophrenia based on their expression profile and pharmacology. Despite this, their full potential remains to be explored and dissected through the lens of schizophrenia.

1.8 | GPR52 overview

GPR52 is one of the most well-conserved CNS-specific genes, expressed in various regions of the brain (Komatsu et al., 2014; Sawzdargo et al., 1999). GPR52 has been extensively studied as a specific target for schizophrenia partly due to its unique expressing patterns in schizophrenia-relevant brain nuclei.

GPR52 is a G_s-coupled GPCR expressed in key regions of the brain that are dysregulated in schizophrenia. GPR52 was found to be

co-localised with dopamine D_1 receptors in the prefrontal cortex and with dopamine D_2 receptors in medium spiny neurons (MSNs) of the striatum, respectively (Komatsu et al., 2014). A similar striatal expression pattern was also observed for the **adenosine A_{2A} receptor** (A_{2A} receptor), which co-localises with dopamine D_2 receptors in the MSNs. Both are proposed to play a counterbalancing role against hyperdopaminergic activity in schizophrenia (Valle-León et al., 2021). Loss of GPR52 expression potentiated A_{2A} receptor antagonist-mediated hyperlocomotor activity, suggesting the potential of GPR52 to be an effective target in reducing the positive symptoms in schizophrenia through its modulation of striatal activity (Nishiyama, Suzuki, Maruyama, et al., 2017).

1.9 | GPR52 function and mechanisms of action

Numerous preclinical tests suggest that activation of GPR52 would be associated with relieving symptoms of schizophrenia. *Gpr52* KO mice showed a higher sensitivity in the prepulse inhibition test compared with WT mice, who were primed with the psychostimulant, MK-801 (Komatsu et al., 2014). In addition, GPR52 selective small molecule agonists reduce amphetamine-induced hyperlocomotor activity and enhanced cognitive activity in rodents (Nakahata et al., 2018; Nishiyama, Suzuki, Harasawa, et al., 2017; Setoh et al., 2014; Tokumaru et al., 2017). It was noted that GPR52 expressed in recombinant cells was activated by the dopamine-depleting drug, reserpine; however, this is a complicated mechanism to deconvolve in vivo (Komatsu et al., 2014).

Nonetheless, the activation of Gαs protein by GPR52 in the striatopallidal pathway in the striatum may not be its only mechanism of action to counteract striatal hyperactivity. Despite the enriched GPR52 expression in D_2 receptor-expressing MSNs, there is evidence that GPR52 can exert control over the D_1 receptor-expressing population through extrastriatal GPR52 populations (Spark et al., 2020). Cortical GPR52 appears to modulate striatal glutamate transmission via **$mGlu_1$** receptors. Given that corticostriatal circuitry is disturbed in schizophrenia, this suggests that GPR52 may ameliorate aberrant signalling across both major striatal neuronal populations; the consequence of this mechanism points to relief of positive symptoms.

Potential therapeutic benefit of GPR52 agonists is not limited to its activity through G proteins. The receptor stimulates ERK1/2 phosphorylation in a β-arrestin-2-dependent manner in cortical neurons (Hatzipantelis et al., 2020). This is of particular interest given that deep layer frontal cortical neurons are within a nexus of cognitive control (Snelleksz et al., 2022).

1.10 | Small molecule agonist development for GPR52

The first synthetic small molecule agonist series for GPR52 was exemplified by compound 7m (or 3-BTBZ), which has a benzothiophene as the core structure (Setoh et al., 2014; Spark et al., 2020). While 3-BTBZ is a high-nanomolar potent compound, orally bioavailable, selective and displays efficacy in models of positive symptoms in rodents, its high lipophilicity (cLogP > 6) makes it unsuitable as a drug candidate (Van De Waterbeemd et al., 1998). Subsequently, a small molecule, compound 17 (c17; also, a benzothiophene) with improved pharmacokinetic properties was developed and also demonstrated efficacy in preclinical models of positive symptoms (Nakahata et al., 2018). Subsequent to the development of the benzothiophene series, another agonist with a thiazole core structure, FTBMT, was also published; it has much improved physicochemical and pharmacokinetic properties compared with 3-BTBZ. Further, it also showed pro-cognitive function in vivo (Nishiyama, Suzuki, Harasawa, et al., 2017; Tokumaru et al., 2017).

Together, the development of structurally diverse GPR52 agonists has enabled better understanding of GPR52 function and physiology. Most recently, Sosei-Heptares have developed a number of GPR52 agonists around a pyridine core. Whilst cAMP potencies of the series vary greatly, the series exemplar, HTL-0041178, has an excellent pharmacokinetic profile with a brain: plasma ratio of >2, >50% bioavailability across multiple species and long duration of action (>12 hr) (Poulter et al., 2023). Sosei-Heptares plans to enter phase I trials in the first half of 2023.

1.11 | Structural studies of GPR52

Structural studies have advanced the understanding of the molecular mechanisms of GPR52 activation and agonist-receptor interactions. A recent study reported GPR52 in multiple states (apo state, ligand-free bound with heterotrimeric G protein state and small molecule-bound state), which revealed several key features of GPR52 including a putative orthosteric ligand binding pocket, a potential mechanism of GPR52 constitutive activity, and a small molecule binding site (Lin et al., 2020). In the absence of a ligand, a high level of cAMP was observed in GPR52 overexpressing HEK293 cells. The molecular mechanism was explained by the crystal structure of apo GPR52, in which extracellular loop 2 (ECL2) of GPR52 occupies the likely orthosteric binding site to yield constitutive activation.

The key residues that form intra-molecular interactions with the transmembrane bundles are in the upper to middle section of ECL2, ranging from residues 182–190; equivalent regions in other GPCRs are the key points engaged with their ligands. The side chain of tyrosine 185 ($Tyr185^{ECL2}$) on the ECL2 was surrounded by hydrophobic residues on transmembrane 6 (TM6). Lysine 182^{ECL2} and aspartic acid 188^{ECL2} formed a salt bridge that was critical for stabilising the ECL2 motif in the pocket. The cysteine 193^{ECL2} and cysteine $114^{3.25}$ (TM3) formed a disulfide bond that strengthened the stability of ECL2 in this pocket.

Furthermore, GPR52 constitutive activity was also supported by an active GPR52 and G protein complex structure, which was formed in the absence of a ligand. Disruption of each of these key interactions weakened the engagement of ECL2 and had a profound negative impact on GPR52 constitutive activity. Overall, the structural

understanding of GPR52 has revealed a key role of ECL2 being a self-activating mechanism in the receptor. Nonetheless, it does not exclude the existence of endogenous ligands.

In addition to understanding the constitutive activity mechanism, a ligand bound GPR52 structure provided insight into the interactions between the agonist C17, (sharing the same core structure as 3-BTBZ) and GPR52 (Lin et al., 2020). This revealed that c17 is seated in a pocket formed by TM1, TM2, TM7 and ECL2, which is adjacent to the putative orthosteric binding pocket occupied by ECL2 (Figure 2). The key interactions that were revealed included hydrogen

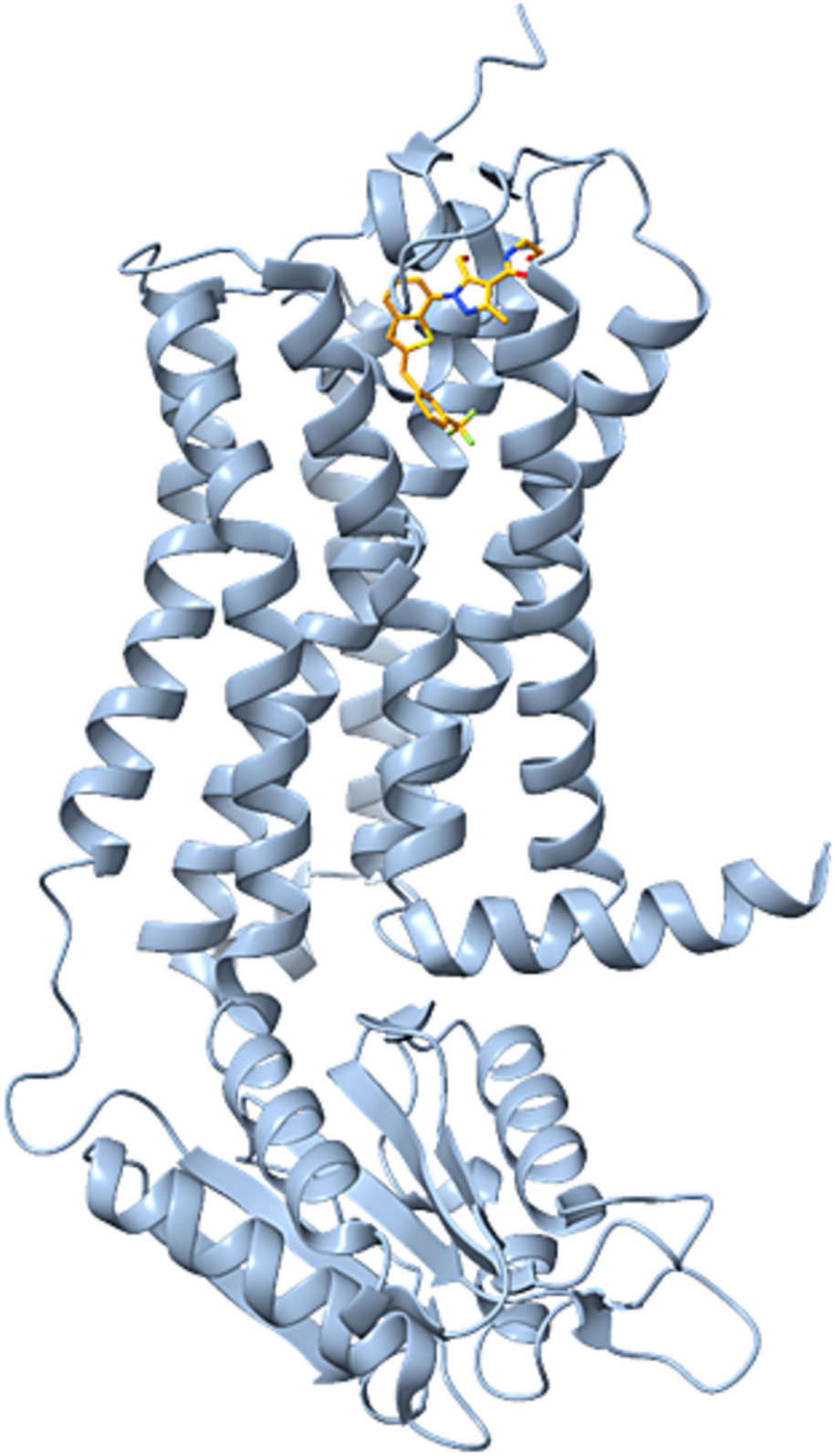

FIGURE 2 An X-ray crystal structure of GPR52 bound with the compound, c17 (PDB: 6LI0, Lin et al., 2020).

bonds with three ECL2 residues Ile189^{ECL2}, Glu191^{ECL2}, Asp188^{ECL2} and one residue on TM1, Cys40$^{1.32}$. Hydrophobic and π-π interactions also helped stabilise c17 in the pocket. Whilst the endogenous ligand (if any) remains to be determined, the synthetic ligand binding site is located in a region typical to Family A GPCRs. It not only enhanced understanding of the small molecule engagement with GPR52 but also provided an opportunity for structure-based drug design for new chemical tools and schizophrenia therapeutics.

1.12 | GPR85 overview

GPR85 is a putative G_s protein-coupled member of the Super conserved Receptor Expressed in Brain (SREB) family, also named SREB2 (Matsumoto et al., 2005). GPR85 is closely related to GPR27 and GPR137, which are also known as SREB1 and SREB3, respectively (Breton et al., 2021). Several pieces of evidence suggest GPR85 may play a part in the mechanism of development of schizophrenia. Genetically, *GPR85* is located at a locus (7q31.1) that is linked (although not primarily) to psychiatric disorders and over transmission of minor alleles of *GPR85* SNPs were observed in individuals with schizophrenia in a Family-Based Association Test (Matsumoto et al., 2008). GPR85 overexpressing transgenic mice shared some phenotypes with those seen in patients with schizophrenia, including increased ventricular volume and smaller size of cortical neurons (Matsumoto et al., 2008). In a brain transcriptome analysis, *Gpr85* transcript was increased in mice overexpressing SHANK3, a genetic variant of which is associated with schizophrenia (Jin, Kang, et al., 2018). Studies on GPR85 in different models are likely to be translatable as this receptor is highly conserved across species, including humans, rodents and fish (Hellebrand et al., 2000; Matsumoto et al., 2005).

1.13 | GPR85—CNS expression, function and signalling

Transcriptional analysis of *GPR85* in the human brain revealed highest enrichment in the thalamus, and further schizophrenia-relevant expression in the cerebral cortex, hippocampus, amygdala and striatum (Hellebrand et al., 2000; Matsumoto et al., 2005). Expression analysis of the mouse brain showed a higher level of GPR85 transcript detected during embryonic development when compared with an adult mouse brain, indicating its putative role in CNS development (Hellebrand et al., 2001). However, the exact physiological role of GPR85 in both the developing and adult brain, both mouse and human, remains to be determined.

Evidence suggested that GPR85 likely plays a role in regulation of neurogenesis and neuroplasticity. GPR85 was negatively associated with neurogenesis; it was shown that the number of proliferating neurons was reduced in GPR85-overexpressing transgenic mice but increased in GPR85 KO mice at adulthood (Chen et al., 2012). Moreover, the expression of GPR85 may also be involved in regulating other gene expression in the same environment where it was observed that increasing GPR85 reduced dentate gyrus enriched genes, which are likely associated with mediating neuroplasticity (Chen et al., 2012). It is likely that GPR85 also plays a tuning role in regulating neuron development. While a study reported that a high level of GPR85 transcripts was detected in several brain regions, especially the cerebral cortex, during the embryonic period, another study found GPR85 transcripts were low in hippocampal neuronal stem cells but high in differentiated cells (Chen et al., 2012; Hellebrand et al., 2001). It is plausible that the expression of GPR85 is spatially and temporally controlled in the neurons so that some neurons continue while halt proliferation at certain times in specific regions. If GPR85 is critically involved in tuning the neuron proliferation and differentiation, the activity of GPR85 needs to be strictly regulated. Interestingly, GPR85 has not been reported to display constitutive activity, unlike many Class A orphan GPCRs (Martin et al., 2015). However, the exact underlying signalling pathway and mechanisms are still unknown. Given the strong neurodevelopmental implications in schizophrenia, GPR85 could play a role in altering disease trajectory or in regulating the maladaptive circuitry in schizophrenia.

1.14 | Small molecule identification for GPR85

GPR85 activity could be beneficial in enhancing neuron proliferation and cognitive function—consequently inverse agonists have been sought. Due to a lack of constitutive activity, small molecules were screened in systems that expressed GPR85-Gαs fusion proteins (Sakai et al., 2022; Yanai et al., 2016). Nonselective inverse agonists of GPR85 were identified in GTPγ^{35}S binding experiments, which were carried out in GPR85-Gαs expressing membranes from Sf9 insect cells (Sakai et al., 2022). Three compounds, 2g, 3h and 3i (Figure 3a), were identified with low micromolar potencies for inverse agonist activity at GPR85 but displayed similar potencies at GPR27 and GPR173. However, low potency, lack of selectivity and poor physicochemical properties complicated further pharmacological probing of these ligands. Optimisation of these chemical targets will certainly assist in providing insight into the therapeutic potential of GPR85 modulators.

1.15 | GPR88 overview

GPR88 is one of the most extensively studied orphan GPCRs in the context of schizophrenia and psychiatric diseases. GPR88 is a class A GPCR that displays constitutive activity for Gαi coupling (Dzierba et al., 2015; Jin et al., 2014; Watkins & Orlandi, 2021) and is greatly enriched in the striatum.

1.16 | GPR88 expression

GPR88 was first identified by the differential display screening method as a novel gene, which was abundantly expressed in the striatum and well conserved in both humans and rodents (Mizushima

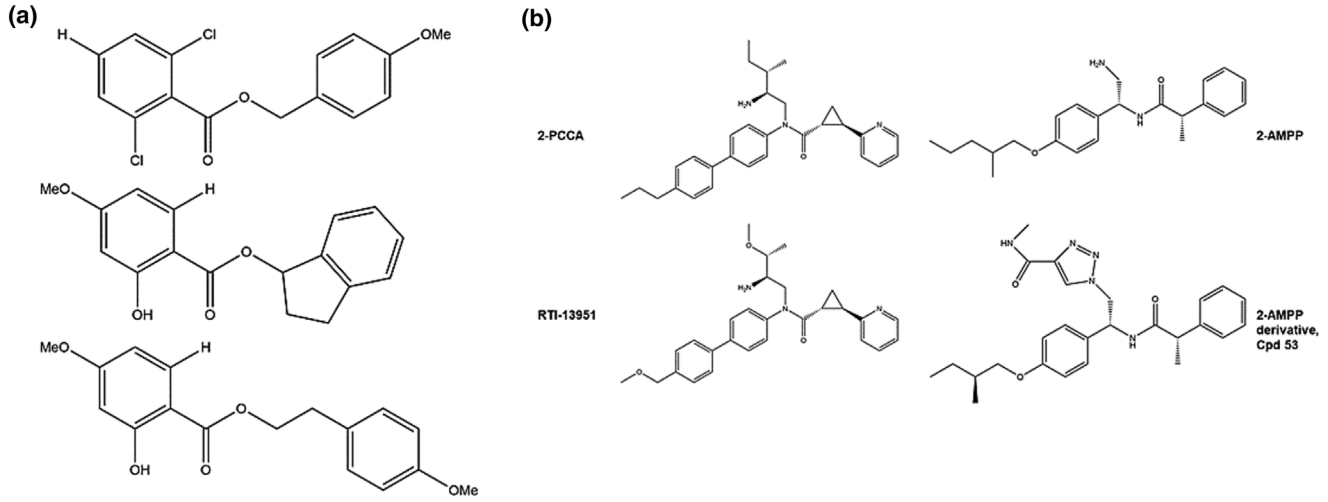

FIGURE 3 (a) GPR85 ligand candidates 2g (top), 3h (middle) and 3i (bottom). (b) Key agonists of GPR88. RTI-13951 is a derivative of 2-PCCA, which has better pharmacokinetic properties. Compound 53 has an improved brain penetration property compared with its parent compound 2-AMPP.

et al., 2000). Striatal dysfunction is linked to the development and symptoms of schizophrenia due to it being a key region that integrates glutamatergic and dopaminergic signalling (McCutcheon et al., 2019). GPR88 expression was also mapped in the mouse and rat brain, confirming that GPR88 is predominantly expressed as a striatal marker. A lower but measurable level of GPR88 was detected in the frontal cortex (Ghate et al., 2007; Van Waes et al., 2011). Regional variations of GPR88 expression were observed in striatum with highest level in the lateral side and moderate level in the medial area (Ghate et al., 2007; Van Waes et al., 2011). To further resolve GPR88 CNS expression, a Venus-tagged GPR88 knock-in study has not only confirmed its expression in the cortex but also detected GPR88 in subcortical areas other than striatum, such as hippocampus, thalamus, hypothalamus and midbrain (Ehrlich et al., 2018).

GPR88 was confirmed to be a neuron-enriched GPCR by in situ hybridisation (Massart et al., 2009; Van Waes et al., 2011) and is found in the striatum on medium spiny neurons (MSNs) in both the striatonigral and striatopallidal pathways. In the dorsal striatal MSNs, GPR88 was detected in the somatodendritic compartments on a postsynaptic location, which was likely in contact with excitatory presynaptic glutamatergic neurons (Massart et al., 2009).

1.17 | Function and molecular mechanisms of GPR88

The GPR88 expression pattern strongly indicates a potential regulatory role in the symptoms of schizophrenia, specifically within components dependent on cortico-striatal circuitry. GPR88 is enriched in both striatonigral and striatopallidal MSNs and MSN-specific deletion of *Gpr88* engendered an increase in basal locomotor activity, poor motor coordination and impaired spatial learning (Meirsman, Le Merrer, et al., 2016; Meirsman, Robé, et al., 2016; Quintana et al., 2012). Interestingly, amphetamine-induced locomotor activity was increased in rats treated with *Gpr88* lentiviral knock-down compared with the vector control rats (Ingallinesi et al., 2015), suggesting the GPR88 tone significantly contributes to motor activity. The cellular mechanisms are not fully understood but the loss of GPR88 in the MSNs likely has a significant impact on the normal GABAergic neuronal activity. Deletion of the GABA-synthesising enzyme, glutamic acid decarboxylase, in GPR88-expressing neurons has shown similar behavioural dysfunction, such as increased motor activity and impaired spatial learning (Zhang et al., 2014).

One postulate is that GPR88 may functionally interact and influence activity of other GPCRs. First, the function of GPR88 may be closely related to opioid receptors. GPR88 was discovered to be a μ receptor-dependent gene, where chronic activation of μ receptors upregulated GPR88 transcript (Befort et al., 2008). It was then found that morphine-induced locomotor activity was enhanced in GPR88 KO mice compared with control mice (Laboute et al., 2020). Further, antagonising the δ opioid receptor reversed motor, spatial learning and emotional deficits that were caused by loss of GPR88, suggesting at minimum a functional communication between these two receptors (Meirsman, Le Merrer, et al., 2016). At a signalling level, GPR88 co-localised with opioid receptors and regulated their activity and trafficking profiles in vitro (Laboute et al., 2020). Second, the activity of GPR88 is interwoven with adenosine A_{2A} receptor (A_{2A} receptor) activity. Specific conditional deletion of GPR88 in A_{2A} receptor-expressing MSNs displayed comparable phenotypes to those seen in the constitutive GPR88 KO mouse, specifically increased locomotor activity and reduced anxiety-like behaviours (Meirsman et al., 2019; Meirsman, Robé, et al., 2016). A_{2A} receptor expression in the CNS is largely restricted to the striatum and it co-localises with dopamine D_2 receptors, recombination of *Gpr88* in A2A receptor-expressing MSNs reduced the GPR88 expression in striatopallidal at the exclusion of striatonigral neurons (Meirsman, Robé, et al., 2016). Mice with a

conditional GPR88 KO in A_{2A} receptor-expressing neurons showed higher locomotor activity when stimulated by the D_2 receptor agonist quinpirole compared with their control mice (Meirsman et al., 2017). Whilst this corroborates and extends the role of GPR88 in locomotion (Logue et al., 2009; Quintana et al., 2012), it highlights the interaction with the most common antipsychotic drug target, the dopamine D_2 receptor.

Interestingly, the anxiolytic phenotype of the constitutive GPR88 KO could be attributed to loss of *Gpr88* expression in the amygdala. However, it is clear that a major component of anxiety processing is regulated by the striatum (Lago et al., 2017), and GPR88 plays a role in anxiety signal processing. Not only is GPR88 associated with the positive symptom domain of schizophrenia, evidence is mounting that GPR88 is critically involved in cognition. Constitutive deletion of *Gpr88* in mice demonstrated a vital role of GPR88 in working memory and cognitive flexibility, with knockout mice consistently underperforming in an n-back task in a radial arm maze and subtly underperforming in a reversal learning task, compared with WT mice (Thomson et al., 2021). GPR88 constitutive knockout mice also displayed reduced accuracy and increased premature response rates compared with WT mice, in a 5-choice serial reaction time task, a measure of attention and impulse control (Ben Hamida, Sengupta, et al., 2022). Together, these findings comprehensively demonstrate that GPR88 plays a pivotal role in modulating schizophrenia-relevant brain regions that manifest in behaviours associated with the disease.

1.18 | Small molecules targeting GPR88

Whilst GPR88 is considered a plasma membrane protein, it may not spend most of its time at the most accessible membrane. Throughout early rat development, GPR88 was largely expressed across the plasma membrane and cytoplasm, nuclear membrane and the nucleoplasm of, at least, cortical and amygdala neurons, a distribution pattern that remains during adulthood (Massart et al., 2016). GPR88 also interacts with nuclear proteins in the cerebral cortex (Rebeillard et al., 2022). HEK293 cells express GPR88 in the perinuclear areas rather than the plasma membrane when cells stably expressed human GPR88 (Massart et al., 2009); these findings suggest that the location of GPR88 is a key consideration when designing small molecules for GPR88.

Two main chemotypes of agonists discovered for GPR88, namely, 2-PCCA-like and 2-AMPP-like derivatives, are lipophilic and lipid-like small molecules (Dzierba et al., 2015; Jin et al., 2014). 2-PCCA has shown potency in the nanomolar range at inhibiting cAMP production via GPR88 in vitro (Jin et al., 2014; Li et al., 2013). 2-PCCA is a useful in vitro tool but is highly lipophilic and not drug-like in its physicochemical properties.

Structure-activity relationships around 2-PCCA to improve potency and pharmacokinetic properties yielded a potent and brain permeable small molecule, RTI-13951 (Jin et al., 2016; Jin, Decker, et al., 2018). However, the activity of this small molecule has not yet been evaluated in animal models directly related to symptoms of schizophrenia, although it was shown to reduce alcohol self-administration in rats (Ben Hamida, Carter, et al., 2022; Jin, Decker, et al., 2018). Whilst substance-use disorders are not explicitly a symptom of schizophrenia, they are one of the most common comorbidities and speak to the underpinning dysfunction in schizophrenia and its linked addiction liability.

2-AMPP has a comparable potency to 2-PCCA but is more amenable to structure-activity relationship modifications (Dzierba et al., 2015), resulting in improvements in the potency of 2-AMPP from triple digit nanomolar down to single-digit nanomolar values (Bi et al., 2015; Dzierba et al., 2015; Jin et al., 2017; Rahman et al., 2020, 2021). The latest modified version of the 2-AMPP derivative has an equivalent activity in GPR88-mediated cAMP inhibition compared with RTI-13951-33 from the 2-PCCA series and improved brain penetration (Kp = 0.34) (Rahman et al., 2021). Notwithstanding, there remains an array of cellular signalling and in vivo studies to fully characterise this compound.

1.19 | Structural studies of GPR88

Recently, unique molecular features of GPR88 and the mechanisms of agonist 2-PCCA binding and activation were revealed in a structural study of GPR88 (Chen et al., 2022). While the endogenous ligand of GPR88 remains unknown, its existence has perhaps already been identified in the GPR88-Gαi1 protein complex from two cryo-EM structures. This study has reported two GPR88-Gαi1 structures, in the presence and absence of a 2-PCCA agonist, where *both* structures revealed a density at the same location surrounded by TM3, TM4, TM5 and TM7.

In the 2-PCCA-bound GPR88 structure, an additional density at the cytoplasmic side of TM5, TM6 and seated next to the adjacent membrane was observed, unambiguously assigned to 2-PCCA, and shown by site-directed mutagenesis to be the functionally relevant ligand binding site. The authors speculate that the unassigned density (that was present in both structures) was possibly occupied by an agent present during the protein purification.

While this is exciting and intriguing with regard to GPR88 deorphanisation, as the purification was conducted in a highly isolated in vitro system, it is unknown whether the density is an artefact of a molecule, such as a lipid, binding or it is an endogenous molecule that has physiological activity. In addition to the identification of a potential orthosteric binding pocket, the structural study has provided valuable insights into the molecular interactions between 2-PCCA and GPR88. Specifically, it uncovered the important role of each moiety in 2-PCCA in forming either hydrogen bonds or hydrophobic interactions with residues on GPR88.

The different chemotypes of agonists 2-PCCA and 2-AMPP share a similar pharmacophore (Figure 3b). Thus, when assuming 2-AMPP and 2-PCCA bind at the same putative allosteric pocket, this allows a certain level of confidence in predicting the binding mode of 2-AMPP from the insight of the 2-PCCA bound GPR88 structure. Interestingly,

the nitrogen from the arylcyclopropyl moiety of 2-PCCA, which has been inserted into a region created by TM5 and TM6, formed a hydrogen bond with G283$^{6.34}$ of GPR88. However, the equivalent moiety in 2-AMPP lacks the nitrogen atom, which would potentially affect any hydrogen bond formation and an overall strength of interactions. Moreover, the biaryl moiety in 2-PCCA was placed at a hydrophobic pore that suited the shape of the moiety well, whereas the equivalent moiety in 2-AMPP was also hydrophobic but less bulky with only one phenyl group. It is not known whether 2-AMPP would fit into the region as well as 2-PCCA and if it would not, 2-AMPP may bind at a different region or have a different binding pose. The nitrogen atom from the primary amine of aminoalkyl moiety of 2-PCCA formed a hydrogen bond with residue S282$^{6.33}$ of GPR88. There is also a nitrogen atom at the equivalent position in 2-AMPP, suggesting that this interaction may also exist between 2-AMPP and GPR88 on the assumption that 2-AMPP binds and poses the same way as 2-PCCA.

Collectively, the putative endogenous ligand density and the transmembrane binding site of 2-PCCA suggest a lipid-like ligand is favoured for GPR88. Moreover, the structural findings have been well correlated with thestructure-activity relationships studies of 2-PCCA, where reduction in potency due to moiety modifications largely resulted from disrupting the agonist and receptor interactions that were revealed in the structure.

The binding of 2-PCCA to its intracellular site contributed to a hydrophobic network formed at the interface between the α5 helix of Gαi1 and GPR88, which potentially has a stabilisation role in the GPR88-Gαi1 complex. This structural study may also provide insight into the potential mechanism of GPR88 genetic association with schizophrenia. A polymorphism (V190) located on TM5 of GPR88 was detected in the study of schizophrenia triads in the South Africa population (Del Zompo et al., 2014). The residue, at the top of TM5, is in close proximity to potential ligand density and thus may play a role in regulating the interaction between a potential endogenous ligand and GPR88.

1.20 | GPR139 overview

GPR139 is an orphan GPCR and is highly enriched in the CNS; and the gene is well conserved across species, for example, human, rat, mouse and chicken (Gloriam et al., 2005; Süsens et al., 2006; Vanti et al., 2003). Genetic evidence has suggested that copy number variation in the *GPR139* gene may play a role in developing schizophrenia (Castellani et al., 2014).

1.21 | Expression of GPR139 in the CNS

CNS enrichment of GPR139 makes it a target of interest for psychiatric disorders, and its specific distribution in key brain regions relevant to schizophrenia symptoms further embeds its place in schizophrenia drug discovery. In the human brain, GPR139 is specifically enriched in the striatum, with lower expression in the habenula, hippocampus, thalamus and pituitary gland. In mouse studies (Matsuo et al., 2005; Süsens et al., 2006), its specific expression in the parafascicular thalamic nucleus is particularly interesting for schizophrenia as this locus forms a distinct cortical-thalamic-striatal circuit that may underlie several schizophrenia symptom-relevant processes (Jiang et al., 2021; Mandelbaum et al., 2019).

1.22 | GPR139 signalling, ligands and structural biology insights on molecular mechanisms

GPR139 is likely coupled to multiple Gα proteins, preferentially coupling to Gαq/11, and potentially Gαs and Gαi. GPR139 mediated a robust increase of SRE luciferase activity in the reporter assay, believed to be Gαq/11 dependent, while a small increase of CRE activity suggested a Gαs coupling to a lesser extent (Matsuo et al., 2005). GPR139 activation in brain extract was inhibited by pertussis toxin (PTX), suggesting a role for Gαi coupling in the GPR139 signalling profile. Among these Gα proteins, Gαq is likely the primary cognate protein at GPR139; all proposed endogenous ligands activated a calcium response via Gαq. L-tryptophan (L-Trp) and L-phenylalanine (L-Phe) were identified as two endogenous ligands in multiple studies but were of low potency,220 μM and 320 μM, respectively, in stimulating intracellular calcium release (Isberg et al., 2014; Liu et al., 2015; Nøhr et al., 2017). It was postulated that GPR139 is a small peptide receptor based on its calcium responses to di-peptides such as TrpTrp and TrpPhe (Isberg et al., 2014; Nøhr et al., 2017). However, there is still debate on this topic. While the binding cavity sequence alignments suggested, and calcium assay supported, that GPR139 shared the same peptide ligands as the **melanocortin 4 receptor** (MC_4 receptor) - adrenocorticotropic hormone (ACTH) and α- and β-melanocyte stimulating hormone (α-MSH and β-MSH), this was not reproduced in other studies. Binding and activation of GPR139 by these peptides was not achieved at the physiological relevant concentrations of ACTH, α-MSH and β-MSH (Nepomuceno et al., 2018). Despite this, it is plausible that GPR139 responds to peptide ligands via a direct or indirect interaction with melanocortin receptors. Co-expressing GPR139 with one of the melanocortin receptors has produced relatively potent calcium responses stimulated by ACTH, α-MSH and β-MSH, and these responses were not observed when GPR139 and melanocortin receptors were expressed individually (Nepomuceno et al., 2018).

1.23 | Identification of GPR139 small molecules by HTS

From a 200,000 small-molecule library, the compound LP-360924 was identified as a GPR139 agonist for cAMP production with good specificity when screened against β2AR and GPR142 (Hu et al., 2009). However, LP-360924 stimulated GPR139 seemed to be Gαs selective as there was no observed calcium response. In addition, LP-471756

and LP-114958 were identified as two GPR139 antagonists, which specifically inhibited LP-360924 stimulated cAMP in a dose-dependent manner. This provides insight into the propensity of GPR139 to selectively signal to discrete G protein pathways and potentially generate safe and more targeted drugs.

Notwithstanding, screening small molecules in calcium mobilisation assays has yielded more GPR139 candidate compounds, which are structurally distinct from those screened in cAMP assays. Discovery of the first potent small molecule, 1a, at GPR139 in the calcium mobilisation pathway has revealed a particular importance of the central linker in the molecule, which is a hydrazinecarboxamide. Modifications on the central linker region can greatly affect ligand activity (Shi et al., 2011). Despite a relatively high potency (EC_{50} = 39 nM), 1a has poor pharmacokinetic properties, which limited its progression into in vivo studies. Further exploration has discovered a more potent small molecule 7c, also named as JNJ-63533054, with variation of the central linker and peripheral substituents (Figure 4; Dvorak et al., 2015). With a good pharmacokinetic profile, 7c was moved forward for in vivo characterisation. However, 7c-mediated activity such as neuronal activation was not observed in the medial habenula, a region in which GPR139 is abundantly expressed; nor did 7c display an apparent effect in the behavioural tests (Shoblock et al., 2019). A small molecule discovered by the Takeda pharmaceutical company, named TAK-041 (Figure 4), containing a distinct peripheral benzotriazine moiety, has almost an equal potency (EC_{50} = 22 nM) as 7c as well as a favourable pharmacokinetic profile. Moreover, the parental tool compound of TAK-041, compound 21, was examined for its cellular activity and in vivo efficacy—it demonstrated neuronal activation in the medial habenula of WT mice, an effect not observed in GPR139 KO mice after administration of compound 21. It also improved social interaction behaviour in transgenic mice that are naturally low in sociability, suggesting a beneficial role in reversing negative symptoms of schizophrenia (Reichard et al., 2021). Recently, it was reported that TAK-041 was well tolerated in a phase I study in both control volunteers and adults with schizophrenia (Yin et al., 2022). TAK-041 (relabelled as NBI-1065846 at Neurocrine) originally entered trials for negative symptoms of schizophrenia, but has recently been allocated to treat anhedonia in major depressive disorder (Neurocrine pipeline; https://www.neurocrine.com/pipeline). Notwithstanding, GPR139 expression across schizophrenia-relevant circuits tethers GPR139 as a potentially clinically useful target to treat schizophrenia beyond positive symptoms.

1.24 | Structural insights on GPR139 small molecules

Structural biology and molecular modelling have advanced the understanding of the relationship between GPR139 small molecules and the receptor.

A recent publication on 7c bound GPR139 $G_{s/q}$ and 7c bound GPR139 G_{i1} complex structures revealed residues of GPR139 that are involved in binding with 7c and its different binding poses (Figure 5; Zhou et al., 2022). Residues that are important for forming hydrogen bonds and hydrophobic interactions with 7c were mapped on GPR139. Molecular docking of TAK-041 revealed the same binding site and similar interactions with GPR139 as those of 7c. The exception was that 7c formed critical interactions with tryptophan 166 and tryptophan 241, but these mutations only minimally affected TAK-041 activity. Interestingly, in the chimeric $G_{s/q}$ complex, 7c displayed an unambiguous binding pose that is upright with the phenyl ring placed at the bottom deep in a hydrophobic environment formed by the transmembrane bundle; and the chlorophenyl group of 7c was at the top also in a hydrophobic pocket—closer to the extracellular region. However, in the G_{i1} complex, two potential poses were observed. Pose 1 was similar to that in the $G_{s/q}$ complex but pose 2 has a flipped amide bond resulting in direction change of chloride and carbonyl oxygen. This dynamic binding was supported by molecular dynamic simulations, which showed that a tryptophan in the extracellular loop 2 was able to adopt multiple conformations. Overall,

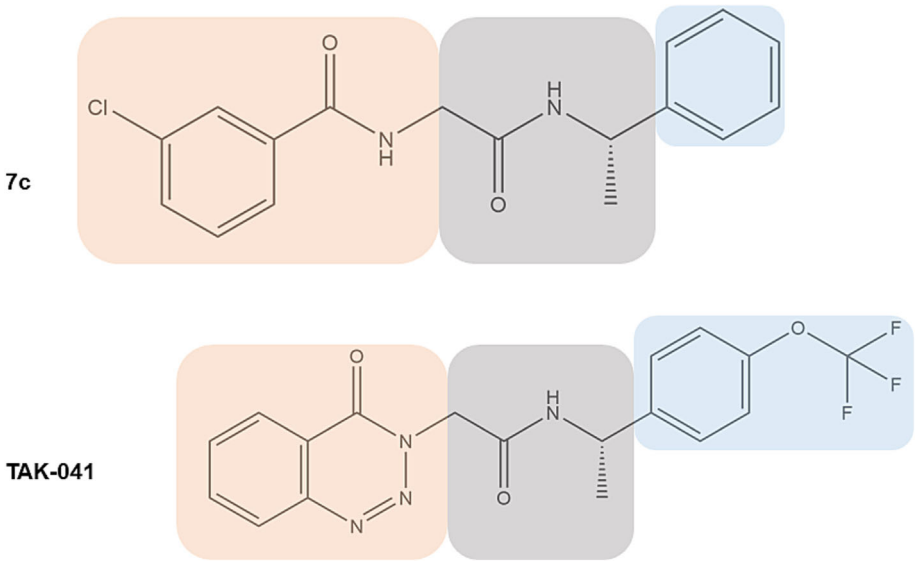

FIGURE 4 Two-dimensional chemical structure comparison of GPR139 small molecule 7c and TAK-041.

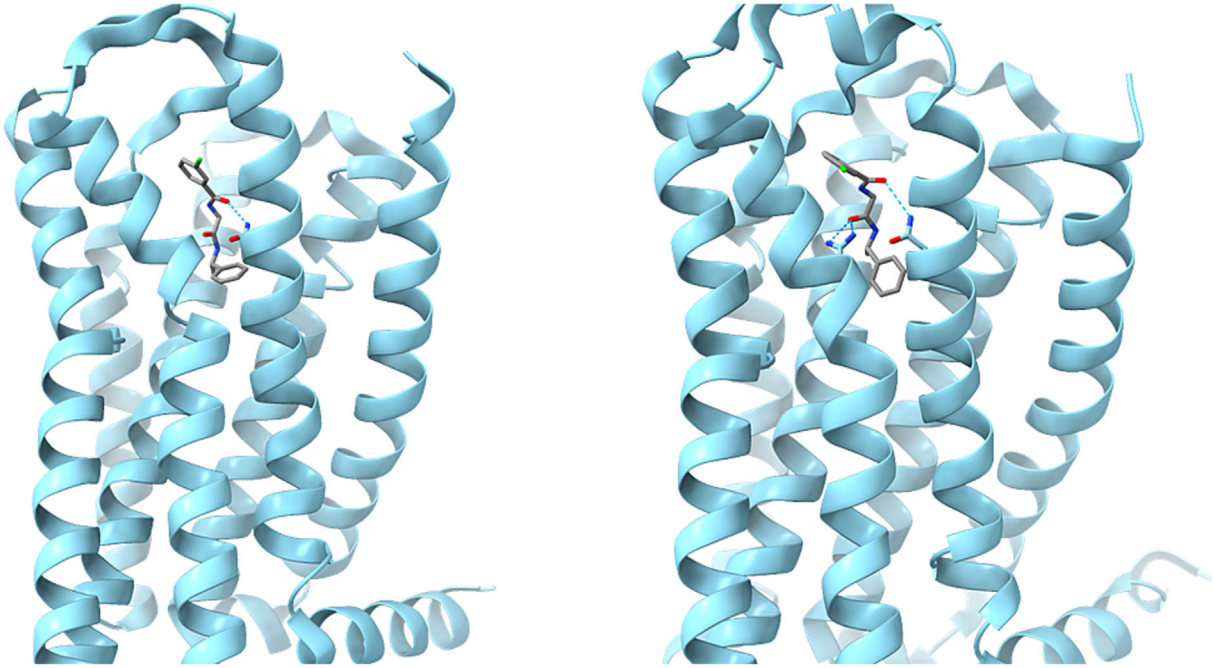

FIGURE 5 7c bound with GPR139 that is complexed with either Gαi (left, PDB: 7VUG) or Gs/q (7VUH). In the Gαi complex, 7c formed a H-bond with Asn 271 whereas, in the Gs/q complex, 7c formed H-bonds with Arg 244 and Asn 271.

these findings helped understanding of the similarity and differences of tool compounds and investigational new drugs in terms of their molecular interactions with GPR139. The difference between the 7c binding poses between $G_{s/q}$ and G_{i1} complexes causes pause for thought on identifying tool compounds using a different G protein pathway readout, which potentially selects for ligands with a distinct binding mode and interactions with GPR139, and therefore potentially tailored clinical sequelae. However, the physiological relevance of a selective activation of discrete G proteins by GPR139 remains to be studied.

2 | CONCLUDING REMARKS

Schizophrenia is one of the more difficult diseases to target in the psychiatry therapeutic space. This is owing to the heterogeneity of disease, complex aetiology and lack of bona fide biomarkers. Consequently, schizophrenia drug discovery history is littered with clinical failures, resulting in limited advances in novel therapies over the past decades. Recent pharmaceutical programs have breathed new life into schizophrenia drug discovery and provide hope for novel therapies. Whilst promising inroads have been made, there remains a wealth of untapped target resources in the orphan GPCR space. Many CNS-expressed orphan GPCRs are enriched in the CNS with minimal peripheral expression, which can reduce on-side side effect liability. A major challenge that remains with many orphan GPCR programs is the lack of insight into potential endogenous ligand binding interactions that could generate unforeseeable treatment sequelae. This stresses the importance of early in vivo target engagement assays when generating new chemical series. Despite the challenges in targeting orphan GPCRs, more recent technologies, such as cryo-EM and CRISPR gene editing, allow for detailed interrogation of the receptor's physical landscape and physiological function, respectively. Leveraging these tools allows faster and more precise development on novel chemical entities for the treatment of schizophrenia and beyond.

2.1 | Nomenclature of targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos et al., 2021; Alexander, Cidlowski et al., 2021; Alexander, Fabbro et al., 2021; Alexander, Mathie et al., 2021).

AUTHOR CONTRIBUTIONS

Yao Lu: Conceptualization (supporting); writing—original draft (lead). **Cassandra Hatzipantelis:** Conceptualization (supporting); writing—original draft (supporting). **Christopher J. Langmead:** Conceptualization (equal); funding acquisition (equal); supervision (equal); writing—review and editing (equal). **Gregory Stewart:** Conceptualization (lead); funding acquisition (equal); supervision (equal); writing—review and editing (equal).

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