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Orphan GPR52 as an emerging neurotherapeutic target

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Abstract

GPR52 is a highly conserved, brain-enriched, $G_{s/olf}$-coupled orphan G protein-coupled receptor (GPCR) that controls various cyclic AMP (cAMP)-dependent physiological and pathological processes. Stimulation of GPR52 activity might be beneficial for the treatment of schizophrenia, psychiatric disorders and other human neurological diseases, whereas inhibition of its activity might provide a potential therapeutic approach for Huntington's disease. Excitingly, HTL0048149 (HTL'149), an orally available GPR52 agonist, has been advanced into phase I human clinical trials for the treatment of schizophrenia. In this concise review, we summarize the current understanding of GPR52 receptor distribution as well as its structure and functions, highlighting the recent advances in drug discovery efforts towards small-molecule GPR52 ligands. The opportunities and challenges presented by targeting GPR52 for novel therapeutics are also briefly discussed.

Keywords

orphan GPCR; GPR52; agonists; central nervous system disorders; schizophrenia; Huntington's disease; drug discovery

Introduction

G protein-coupled receptors (GPCRs) are a superfamily of seven-transmembrane (7TM) protein cell-surface receptors. To date, more than 800 family members have been identified, accounting for approximately 4% of the human genome.(p1) The GPCR superfamily is divided into five subfamilies: rhodopsin-like receptors (class A), secretin receptors (class B), metabotropic glutamate receptors (class C), adhesion receptors and Frizzled/Taste2 receptors.(p2) GPCRs are involved in numerous cellular functions and pathological

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processes, responding to various external stimulants (e.g., heat, light, tastants and viruses) and endogenous signaling ligands (e.g., transmitters, ions, small-molecules, peptide ligands, proteins, lipids and nucleotides).[p3] For decades, GPCRs have been explored as small-molecule drug targets for the treatment of human diseases, such as central nervous system (CNS) disorders, metabolic disorders, infections, inflammation and cancer.[p3],[p4],[p5],[p6] GPCRs are the most successful and promising drug targets: approximately 35% of drugs launched in the market target GPCRs,[p7] and these drugs have a sales share of more than 27% of the global market.[p6],[p8] Currently, 103 members of this family, less than 13% of GPCRs, have been successfully exploited by FDA-approved drugs.[p9] Additionally, only half of the verified, druggable GPCRs possess endogenous ligands.[p10] More than 400 GPCRs are potentially druggable targets for the possible therapeutic treatment of human diseases.[p9],[p11] Approximately 120 are orphan GPCRs (oGPCRs), the endogenous ligands of which are unknown, and their functional roles remain to be elucidated.[p5],[p12],[p13] oGPCRs have distinct tissue-specific distribution and are found enriched in the brain as well as in metabolism- and immunity-related tissues.[p10],[p14] The tissue-specific distribution of oGPCRs leads to specialized functions in tissues, thereby facilitating safety and selectivity in drug development. oGPCRs and their associated downstream proteins mediate a plethora of physiological and pathological processes, including cell growth and survival, metabolism, immune response, allergic reaction, cognition, movement control and reward; however, dysfunctions of known GPCRs and oGPCRs might cause cancer, neuropsychiatric disorders, metabolic disorders, immune diseases and other human disorders.[p5],[p15],[p16] Therefore, the development of small molecules that target oGPCRs represents a rich and untapped area for drug discovery and development.

GPR52 is one of the most promising druggable orphan receptors. Recently, several potent small-molecule GPR52 ligands have been reported, and GPR52 has been attracting great attention from both academia and industry in regards to drug development. The *GPR52* gene was first discovered as a novel human GPCR gene through homology searches of publicly available high-throughput genome databases.[p17] and genome-wide-associated studies have recently identified *GPR52* as a risk gene for schizophrenia.[p18],[p19] GPR52 expression is highly enriched in human striatal brain regions, accounting for about 70% of total GPR52 expression in human tissues (Figure 1a). GPR52 brain and striatum expression findings are similar in both rodent and human studies and were elucidated by profiling a comprehensive anatomical expression of 322 non-odorant GPCR genes in 40 mouse tissues by qPCR,[p10] and by using an informatics approach comparing human mRNA expression in the striatum versus 31 peripheral tissues and 13 brain subregions.[p10],[p20] Replotting the Genotype-Tissue Expression Project (GTEx)[p21] RNA sequencing (RNAseq) results indicates that the highest GPR52 expression is in the human brain, in the nucleus accumbens (NAc), caudate and putamen (Figure 1b). This striatum-selective expression of GPR52 suggests an important function in NAc primary striatal neurons and broader corticostriatal circuitry. GPR52 remains an understudied oGPCR, and no specific review on GPR52 has been published yet. Therefore, this concise review presents the current understanding of the structures and physiological functions of GPR52 and highlights the recent advances in the discovery of small-molecule ligands targeting GPR52.

Structures of GPR52

The GPR52 gene (UniProKB ID: Q9YAT5) is mapped to human chromosome 1q24, and a single exon encodes a 361-amino-acid 7TM spanning protein.[p17] GPR52 contains an extracellular N-terminal domain (amino acid residues 1–39), seven canonical TM domains (TM1–TM7), three extracellular loops (ECLs), three intracellular loops (ICLs) and a C-terminal helix (H8, amino acid residues 327–361) (Figure 2a).[p22] Recently, high-resolution crystal structures of human GPR52 were disclosed in three states: a ligand-free state, a G_s-coupled auto-activation state and a synthetic agonist c17-bound state.[p22] To stabilize GPR52 for crystallography, the ligand-free state crystal structures were determined with a seven-mutation modified GPR52 and two ICL3 fusion-protein partners, rubredoxin (Figure 2b) and flavodoxin (Figure 2c). The configurations of these two GPR52 structures are very similar, indicating that the ICL3 fusion proteins do not alter crystal packing or change the conformation of the receptor. Remarkably, the ECL2 domain of GPR52 occupies and forms multiple interactions with the orthosteric binding pocket, and this ECL2 interaction plays a key part in the basal and agonist activation of the receptor, as evidenced by mutagenesis and cellular functional assays.[p22] Additionally, GPR52 can couple to G_s protein and forms a stable, active, monodispersed GPR52–G protein complex without an agonist (Figure 2d).

The overall configuration of this ligand-free GPR52–mini-G_s–Nb35 complex is highly similar to other class A ligand-bound GPCR–G protein complexes.[p22],[p23],[p24] The intracellular domain of the receptor (GPR52, red, Figure 2d) binds to and forms extensive interactions with the G_s protein (blue, Figure 2d), and the nanobody Nb35 (yellow, Figure 2d) inserts into an area between G_s and G_β (green, Figure 2d) (G_s protein consists of $G_{\alpha s}$, G_β and G_γ). Furthermore, GPR52 can induce a very high level of *in vitro* basal activity in the absence of an agonist.[p25],[p26] This high level of basal activity and the position of the ECL2 in the typical class A GPCR orthosteric binding pocket has led to speculation that GPR52 functions as a self-activated oGPCR[p27] and that the ECL2 acts as an agonist to stimulate the receptor into the active state. However, whether GPR52 is self-activating and highly constitutively active in the brain has not been determined. The co-crystal structure of GPR52 and a surrogate synthetic agonist (agonist 5; c17) has also been reported (Figure 2e).[p22] Agonist **5** occupies a unique side pocket between TM1, TM2, TM7 and ECL2, close to the extracellular surface of the receptor. Compared with the ligand-free GPR52 configuration (Figure 2b,c), the structure of ligand-bound GPR52 (Figure 2e) does not change significantly, including the structure of the ECL2 domain. Although mutagenesis studies suggest that ECL2 is important for both basal activity and agonist **5** activity,[p22] further studies are needed to better understand the molecular basis for how synthetic agonists dynamically bind to and activate GPR52. Excitingly, this co-crystal structure of agonist **5** and GPR52 should enable structure-guided drug discovery to create new selective small-molecule agonists.

Signaling pathway of GPR52

GPR52 is a highly evolutionarily conserved, $G_{s/olf}$-coupled oGPCR, and it shares less than 20% sequence homology with non-orphan GPCRs.[p17],[p22] As a result of its unknown endogenous ligands and low structural similarity to non-orphan GPCRs, the physiological

functions of GPR52 have not been extensively elucidated. However, the functional role of GPR52 in cAMP-dependent cellular responses has become clearer, with information emerging on GPR52′s location and its surrogate ligands, as well as the creation of GPR52-knockout and transgenic animal models. Figure 3 provides a schematic of the cAMP-dependent signaling pathway of GPR52 and crosstalk with other functional proteins in NAc medium spiny neurons (MSNs). GPR52 couples to G_s or G_{olf} and promotes adenylyl cyclase (AC) activity to increase the cAMP level and subsequently induces protein kinase A (PKA) activation and cAMP response element-binding protein (CREB) to regulate cellular responses and gene expression.[p28] In addition, GPR52 can recruit and interact with β-arrestins, which can result in the activation of extracellular signal-regulated kinase (ERK) phosphorylation signaling.[p29],[p30] By contrast, activated dopamine receptors (DARs), such as D_2/D_3 receptors (D_2R/D_3R), couple to $G_{i/o}$ to inhibit AC activity, leading to decreased production of cAMP.[p31] The opposing actions of GPR52 and D_2R in activating and decreasing cAMP signaling, respectively, supports the concept that GPR52 agonists could prevent D_2R signaling, thus providing antipsychotic-like drug effects in neuropsychiatric conditions.[p28]

Owing to the functional crosstalk between GPR52 and D_2R/D_3R and their co-expression in the striatum, GPR52 can modulate dopaminergic signaling pathways. Recently, researchers found that GPR52 promotes the accumulation of huntingtin protein (HTT) via a cAMP-dependent but PKA-independent pathway, leading to a guanine nucleotide exchange factor (GEF) pathway activation of Rab39B[p32] protein-mediated HTT stabilization.[p33] This unique signaling pathway offers a potential mechanism for selective striatal neuron degeneration in Huntington's disease (HD) and provides a possible explanation for why GPR52 stabilizes mutant HTT (mHTT) concentrations and why GPR52 knockout protects striatal neurons *in vitro* and *in vivo*.[p33]

GPR52 functions and CNS diseases

The highest level of GPR52 mRNA expression occurs in the NAc, and anatomical studies have determined that GPR52 is selectively expressed and colocalized with D_2R in NAc D_2MSNs.[p20],[p34] The potential crosstalk of GPR52 with D_2R through the cAMP-dependent signaling pathway (Figure 3) suggests that GPR52 agonists are functionally similar to D_2R antagonists. Common properties can be observed in clinically used antipsychotic drugs, because most of them act as D_2R activity inhibitors.[p35] Recent reports provided additional evidence for this hypothesis. Compared with GPR52 wild-type mice, GPR52-knockout mice show lower expression of D_2R mRNA and enkephalin in the striatum, indicating that GPR52 deletion enhances D_2R signaling in the basal ganglia, reducing the activity of striatopallidal neurons.[p36] Using histological approaches in genetically manipulated mice models, studies have indicated that transgenic mice overexpressing GPR52 exhibit antipsychotic-like behaviors, whereas GPR52-knockout mice display psychosis-related behaviors.[p20] In addition, activation of GPR52 by surrogate agonists increased cAMP levels *in vitro* and showed antipsychotic drug-like activity, inhibiting amphetamine- or methamphetamine-induced hyperlocomotor activity.[p26],[p28],[p37],[p38],[p39],[p40],[p41] Meanwhile, studies of field excitatory postsynaptic potentials (fEPSPs) have suggested dose-dependent potentiation effects following treatment with

GPR52 agonists at synapses in rat cortical slices.[p40] Moreover, episodic memory was significantly increased by GPR52 agonist activation in the rat social recognition model, indicating that targeting GPR52 could improve psychiatric-disorder-related cognitive impairments.[p40] In view of the above, GPR52 has been emerging as a novel promising target for the treatment of psychiatric disorders, including schizophrenia and substance-use disorders.

Schizophrenia

In the medial prefrontal cortex, GPR52 is colocalized with the G_s-coupled D_1R, which is implicated in cognition, memory, attention and reward.[p20],[p42],[p43] rather than with D_2R as in the striatum. Activation of GPR52 potentiates *N*-methyl-D-aspartate receptor (NMDAR)-mediated γ-aminobutyric acid (GABA) release *in vitro* in striatal neurons and also reverses NMDAR inhibitor (MK801)-induced[p44] working and learning memory impairment *in vivo*.[p28] Thus, in the brain cortex, a GPR52 agonist could, in theory, enhance cognitive functions through cortical NMDAR signaling pathways and cAMP signaling, but further study of cortical GPR52 function is needed.[p37] Compared with control mice, GPR52 agonist-treated mice have a higher number of c-fos-positive neurons, and neuronal activation can be observed in cognition-related brain regions using c-fos immunohistochemistry.[p28] Therefore, GPR52 agonists demonstrate preliminary therapeutic potential for cognitive impairment in these preclinical models.

Huntington’s disease

Self-propagating amyloidogenic mHTT-related neurotoxicity is considered as the primary driver of HD.[p45] Studies have revealed that reduction of mHTT expression levels benefits the treatment of HD.[p46],[p47],[p48] Recently, GPR52 was found to have a substantial role in modulating the expression level of both aggregated and soluble mHTT, and inhibition of GPR52 led to dramatically reduced levels of mHTT in striatal neurons.[p33],[p49] Excitingly, further experiments revealed that GPR52 expression could be controlled by taking advantage of genetic techniques in HD model mice. Both aggregated and soluble mHTT expression levels were decreased in GPR52-knockout mice, whereas the concentration of mHTT was restored in GPR52 transgenic mice.[p49] In addition, several HD biomarkers (e.g., DARPP-32, GFAP and Iba1) were detected in this GPR52-knockout HD mouse model, and changes in their expression level were observed.[p49] Moreover, in HD fruit fly and mouse models, short hairpin RNA knockdown of GPR52 rescued HD-related movement and cognitive impairments,[p33],[p49] and decreased expression levels of the GPR52 in induced pluripotent stem cell (iPSC)-derived striatal neurons from patients with HD displayed significant neuronal protection effects.[p33] Collectively, these studies suggest that GPR52 represents a promising neurotherapeutic target for HD.

Small-molecule ligands targeting GPR52

Given the key role of GPR52 in various brain and behavioral functions, modulation of GPR52 activity with potent and selective small-molecule ligands could benefit the treatment of psychiatric and neurological disorders, such as schizophrenia, substance-use disorders and HD. Therefore, there is a crucial need to develop GPR52 ligands as chemical probes

to determine the functional roles of GPR52 or as potential drug candidates to ful-fill unmet medical needs. However, owing to the lack of known endogenous ligands and the low sequence similarity to any non-orphan GPCRs, the development of potent and selective GPR52 modulators poses challenges. Currently, there are no FDA-approved GPR52 agonists or antagonists. However, several small-molecules that act as surrogate GPR52 ligands have been identified to be agonists and antagonists. Herein, we provide a summary on the progress of small-molecule drug discovery efforts targeting GPR52.

GPR52 agonists

The vast majority of currently identified GPR52 ligands are agonists. Although GPR52 exhibits potential self-activation and high constitutive activity,[p22),(p25),(p26),(p27)] from a CNS perspective, agonists that further stimulate GPR52 would lead to increased $G_{s/olf}$-mediated cAMP signaling. This agonist activity to increase cAMP level would be predicted to positively modulate neurons in key brain circuits where GPR52 is expressed, such as in the frontal cortex and the striatum. Compound **1** in Figure 4a was identified as a GPR52 agonist hit with poor potency (EC_{50} > 10 lM) by Takeda Pharmaceuticals through high-throughput screening (HTS).[p37] Early modifications of hit **1** led to compounds **2** and **3**, with EC_{50} values of 0.6 μM and 0.3 mM, respectively.[p37] Optimization of compound **3** led to a series of bicyclic compounds, and compound **4** (Figure 4a) was found to be the best, with an EC_{50} of 30 nM and good efficacy (E_{max} = 79.8% relative to compound **15**).[p37] Compound **4** displayed good pharmacokinetic (PK) properties in mice (C_{max} = 1 08.1 ng/mL; AUC_{0-1} = 613.7 ng•h/mL; F = 73%) at the doses of 0.1 mg/kg taken intravenously (iv) and 1 mg/kg of oral administration (po). Compound **4** also exhibited good brain penetration (brain/plasma (B/P) = 0.94) in mice at the dose of 30 mg/kg (po). Additionally, compound **4** showed good target selectivity and no significant activities against other GPCRs, including D_1R, D_2R, 5-HT_2R, AMPAR and NMDAR.[p37] In an *in vivo* study, compound **4** significantly inhibited methamphetamine-induced hyperlocomotion in mice at a dose of 3 mg/kg (po). Furthermore, compound **4** displayed no strong cataleptic responses at dose ranges from 10 to 100 mg/kg in mice, indicating low risk of extrapyramidal side effects.[p37] Nevertheless, compound **4** has not been advanced into human clinical trials, probably owing to its poor aqueous solubility (<0.05 μg/mL at pH = 6.8 aqueous buffer).[p39]

To circumvent the issues associated with compound **4**, a series of 1-(benzothiophen-7-yl)-1*H*-pyrazole derivatives were designed, synthesized and evaluated.[p39] The 1-(benzothiophen-7-yl)-1*H*-pyrazole derivative compound **5** showed aqueous solubility of 21 mg/mL (at pH = 6.8 aqueous buffer), approximately 420-fold better than the aqueous solubility of compound **4**. Moreover, compound **5** demonstrated high potency (EC_{50} of 21 nM), excellent efficacy (E_{max} = 103% relative to compound **15**) and an acceptable oral PK profile ($AUC_{0-\infty}$= 405.5 ng•h/mL, F = 29.9%).[p39] In an *in vivo* study, compound **5** displayed similar methamphetamine-induced hyperlocomotion inhibition effects to compound **4**. Recently, the co-crystal structure of compound **5** in complex with GPR52 was disclosed, providing a clear binding model for ligand–receptor interactions.[p22] The co-crystal structure revealed that the *N*-(2-hydroxyethyl)formamide side chain and hydroxyl group on the pyrazole moiety form a hydrogen network with residue Cys40

of TM1 and residues of ECL2, including Glu191, Ile189 and Asp188 (Figure 4b,c). The benzothiophene moiety interacts with Phe300 of TM7 through a π-π stacking interaction. The 3-(trifluoromethyl)phenyl group points to a small hydrophobic pocket and forms hydrophobic interactions with residues Ile47, Phe117, Ile307, Ttp304 and Thr303.

A structure-based modification of hit **6** (EC_{50} = 470 nM, E_{max} = 56% relative to compound **15**) led to compound **7** (EC_{50} = 75 nM, E_{max} = 122% relative to compound **15**) (Figure 4a).[p38] Compound **7** displayed good target specificity because it showed no significant activity against other GPCRs, ion channels and enzymes.[p28] Furthermore, compound **7** demonstrated a good *in vivo* PK profile in rats, including high $AUC_{0-\infty}$ of 383.9 ng•h/mL at a dose of 1 mg/kg (po), moderate clearance of 1,409 mL/h/kg (at 0.1 mg/kg, iv), and good bioavailability (F = 53.8%). In addition, compound **7** possessed good metabolic stability, with microsomal clearance values ranging from 9 to 24 μL/min/mg. It also showed good brain penetration, with B/P of 1.53 and 1.68 at doses of 10 mg/kg and 30 mg/kg in mice, respectively. In an *in vivo* study, compound **7** dose-dependently suppressed methamphetamine-induced hyperlocomotion and MK-801-induced cognitive impairment without cataleptogenic side effects.[p28],[p38] *In vitro* induced neural activation and *in vivo* novel object recognition and radial arm maze studies revealed that compound **7** can improve cognitive functions, indicating therapeutic potential for schizophrenia.[p28]

Arena Pharmaceuticals reported a series of 1-heteroaryl-indo line-4-carboxamide analogues as GPR52 agonists.[p50] The representative compound **8** (Figure 4a) showed potent GPR52 agonist activity with an EC_{50} of 12.6 nM in a homogeneous time-resolved fluorescence cAMP assay. Compound **8** showed a B/P at the 1 h time point of 3.51 at a single dose of 3 mg/kg (po) in mice, and it exhibited strong basal locomotor activity inhibition in mice, with an ED_{50} of 2.7 mg/kg. In an *in vivo* study, compound **8** also dose-dependently suppressed amphetamine-stimulated hyperlocomotion.

Optimization of compound **8** led to an additional series of indoline-carboxamide scaffolds with GPR52 agonist activity.[p26] In the GloSensor luciferase-based cAMP assay, compound **9** was similar in potency to compound **8** (EC_{50} of 135 nM and 119 nM, respectively), but with enhanced efficacy (E_{max} of 136% relative to compound **8**). Additionally, compound **9** improved on aqueous solubility and showed no activity in a broad-panel counter screening against numerous GPCRs, transporters and ion channels. Compound **9** also had a favorable PK profile in rats, with a high $AUC_{0-\infty}$ of 13,749 ng•h/mL at 20 mg/kg (po) and 9,030 ng•h/mL at 10 mg/kg (iv), an acceptable half-life of 2.5 h (po) and 1.0 h (iv), moderate clearance of 1.5 L/h/kg (po) and 1.1 L/h/kg (iv), and good bioavailability (F = 76%). Compound **9** showed good brain penetration with a B/P ratio of 0.39 after 1 h at 10 mg/kg (iv). In an *in vivo* mouse study, compound **9** dose-dependently reduced amphetamine-induced hyperlocomotor behavior, indicating antipsychotic-like activity in accordance with previous studies of GPR52 agonists.

Compound **10** was reported by Sosei Heptares as a GPR52 agonist with an EC_{50} of 27.5 nM (Figure 4a).[p51] *In vivo* PK studies of compound **10** in male Sprague-Dawley rats demonstrated low plasma clearance (16 mL/min/kg, ~22% of hepatic blood flow), a moderate volume of distribution (V_{ss}) of 1.0 L/kg and a plasma half-life of 0.8 h at the dose

of 1 mg/kg (iv). Compound **10** showed good oral bioavailability (F = 40%) and apparent oral absorption (F_{abs} = 52%) at 3 mg/kg (po), and it showed good brain penetration with an unbound B/P of 0.35 at 1 mg/kg (iv). *In vivo* PK profiling of compound **10** in CD-1 mice and cynomolgus monkey displayed very low total plasma clearance (~5% of hepatic blood flow), moderate V_{ss} (~1.5 L/kg), and excellent oral bioavailability (F = >80%). In addition, compound **10** showed no significant off-target activity in the Eurofins Safety 47 panel (IC_{50} > 3 mM against all targets), low cardiovascular toxicity risk (hERG and hNaV1.5 IC_{50} > 10 μM), no significant inhibition of any major CYP enzymes (all IC_{50} > 25 μM), no formation of reactive metabolite in a glutathione trapping assay, and no detectable genotoxicity in Ames and *in vitro* micronucleus tests. In *in vivo* antipsychotic studies in a classical psychosis model, compound **10** dose-dependently reduced *d*-amphetamine stimulated hyperlocomotion. Notably, a ^{3}H-labeled version ([3H]-HTL45725) of one of the compounds of this series has been used as a radioligand[p52] and HTL45725 as a pharmacological tool.[p53]

Only one first-in-class GPR52 agonist, HTL'149 (chemical structure not disclosed), is currently under study in phase I human clinical trials.[p54] HTL'149 was developed by Sosei Heptares as a once-daily, orally available small-molecule drug candidate for the treatment of positive symptoms (e.g., hallucinations, delusions and psychosis), negative symptoms (e.g., social withdrawal) and cognitive impairment (e.g., attention, working memory and executive function) of schizophrenia without the adverse effects typically associated with currently available antipsychotic medications. The phase I trial is a randomized, double-blind, placebo-controlled, single- and multiple-ascending dose study to evaluate the pharmacokinetics, pharmacodynamics and safety of oral HTL'149 in healthy volunteers aged 18–55 years. The trial is being conducted in the United Kingdom and the first data are expected in 12–18 months.

GPR52 antagonists

Currently, there is little information on GPR52 inhibitors, and only a few have been reported. The sesquiterpene lactone compound **11** (named compound E7 in published reports), reversed compound **5** (100 nM)-promoted cAMP accumulation in HEK293 cells with an IC_{50} of 12.0 mM; however, it did not affect forskolin-induced cAMP production, suggesting that compound **11** can modulate cAMP concentration by targeting GPR52.[p49] When compound **11** was used to treat striatal neurons derived from the iPSCs of patients with HD, it significantly decreased the expression of mHTT in these stem cells, reaching maximum reduction at 3.75 μM. Moreover, further *in vivo* studies demonstrated that compound **11** reduced mHTT levels and rescued HD-relevant abnormal behaviors in fruit fly and mouse models.[p49] However, studies demonstrated that compound **11** was covalently linked to an intracellular residue (C156) of GPR52, indicating a unique binding site, but it is also likely that the inhibitor displays non-selective covalent linkage to cysteine residues at other proteins.[p55] Compound **11** demonstrated a relatively low potency with multiple Michael acceptors, which might cause off-target effects.[p56] Therefore, additional validating studies of compound **11** activity and selectivity for GPR52 inhibition are needed.

A series of compounds have been reported as GPR52 antagonists.[p57] Hit F11 [(*E*)-1,7-diphenylhept-4-en-3-one] (IC_{50} = 5 μM) was identified through HTS. Modification of F11 led to compound **12**,[p57] which identified with better GPR52 antagonistic activity (IC_{50} = 0.63 μM) than F11 (Figure 4a). Compound **12** dose-dependently decreased mHTT levels in mouse primary striatal neurons derived from HD mice and protected against apoptosis from mHTT toxicity in primary striatal neurons. Moreover, compound **12** reduced levels of soluble mHTT as well as its aggregates in HD mice at a concentration of 0.5 mM administered intracerebroventricularly. Compound **12** crossed the blood–brain barrier and reached the brain at concentrations of 20–60 nM at 5 mg/kg (iv). When compound **12** at 5 mg/kg was intraperitoneally injected into HD mice, it reduced motor deficits and lowered the levels of both soluble and aggregated mHTT in the striatum, but not in the cortex.

The cannabinoid ligands compound **13** (cannabidiol, CBD) and compound **14** (O-1918) were identified by Sosei Heptares as GPR52 inverse agonists with approximate pIC_{50} of 5.61 and 5.45, respectively (Figure 4a).[p58] Both compounds antagonize the response of the selective GPR52 agonist **4**. In addition, they diminished cAMP to below basal levels, indicating they are inverse agonists. However, compounds **13**, **14** and structurally related cannabinoid compounds interact with many GPCRs and ion channels, and more selective GPR52 antagonists from this series are needed. It is intriguing to speculate that some of the CNS psychoactive or therapeutic actions of CBD might be due to GPR52 antagonism, but further investigations are needed.

Concluding remarks and future perspectives

Over the past two decades, significant improvement in the understanding of GPR52 has been achieved by both academia and the pharmaceutical industry through the use of expression profiling and genetic knockout or knockdown strategies. However, the lack of a known endogenous ligand and poor sequence homology to known GPCRs has barricaded the full potential for understanding and targeting GPR52. The lack of clearly identified, relevant disease biomarkers for evaluating the effects of GPR52 regulators also limits the applications of targeting GPR52 in various diseases. Now, the application of sensitive and versatile *in vitro* cAMP assays has accelerated the discovery of potent small-molecule GPR52 agonists through HTS, and has provided scalable screening assays to advance hit-to-lead studies to create selective GPR52 modulators. GPR52 has been emerging as a novel and promising therapeutic target for the treatment of various CNS diseases, including schizophrenia, stimulant-use disorders, HD and other human psychiatric and neurological diseases. Although traditional HTS is a useful approach for the initial discovery of small-molecule GPR52 modulators, it is relatively time-consuming, expensive and risky.[p59] Luckily, the disclosure of the co-crystal structure of GPR52 protein in complex with a tool surrogate agonist is likely to accelerate the identification of more potent and specific small-molecule GPR52 ligands through rational structure-based drug design and fragment-based drug design strategies.[p59],[p60],[p61],[p62],[p63] Moreover, with the GPR52 structure in hand, receptor-based virtual screening might also provide an efficient approach for the identification of novel GPR52 ligands with the aid of computational methods.[p64],[p65]

More extensive studies using the available potent and selective drug-like GPR52 agonists in various animal models of human diseases are anticipated to expand the potential clinical applications of drug candidates targeting GPR52. In addition, the development of potent and selective radiolabeled (e.g., fluorine-18-radiolabeled[(p66)]) GPR52 ligands for positron emission tomography imaging will offer great opportunities and insightful approaches for potential diagnostic tools and neurotherapeutics.

Novel paradigm-shifting approaches in drug discovery, such as proteolysis targeting chimeras (PROTACs),[(p67),(p68),(p69)] might also be beneficial for the identification of efficient GPR52 degraders as pharmacological tools and potential HD therapeutics. Such GPR52 degraders could lead to the degradation and reduction of soluble and aggregated mHTT protein in the striatum, thereby reducing neuronal loss, promoting neuronal survival and improving motor functions *in vivo*, functionally like the reported GPR52 antagonists.[(p57),(p70)] However, achieving good brain penetration with these degraders without off-target effects might be challenging.[(p71),(p72),(p73)]

The currently reported GPR52 antagonists and inverse agonists only show low potency and limited drug-like properties with potential off-target effects. The development of potent and target-selective GPR52 inhibitors or signaling-biased ligands is an area for future drug discovery that could provide powerful pharmacological probes for this emerging target. Taken together, a deeper understanding of the function of GPR52 in the brain, the identification of more potent and specific GPR52 ligands and the further study of GPR52 modulation in animal models of CNS diseases using drug-like ligands are anticipated to promote the understanding of GPR52 physiological function and further validate this novel drug target. Inspired by the successful advancement of one GPR52 agonist into human clinical trials, such efforts could soon pave the way for the development of GPR52-targeted neurotherapeutics and other potential medications.

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Conflicts of interest

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Data availability

No original data were used for the research described in the article. The results shown in Figure 1a,b are derived from publicly accessible RNAseq GPR52 human tissue expression results from the GTEx project.

References

1. Pierce KL et al. Seven-transmembrane receptors. Nat Rev Mol Cell Biol. 2002;3:639–650. [PubMed: 12209124]

2. Lee Y et al. Recent advances in structure-based drug design targeting class A G protein-coupled receptors utilizing crystal structures and computational simulations. J Med Chem. 2018;61:1–46. [PubMed: 28657745]
3. Allen JA, Roth BL. Strategies to discover unexpected targets for drugs active at G protein-coupled receptors. Annu Rev Pharmacol Toxicol. 2011;51:117–144. [PubMed: 20868273]
4. Lappano R, Maggiolini M. G protein-coupled receptors: novel targets for drug discovery in cancer. Nat Rev Drug Discov. 2011;10:47–60. [PubMed: 21193867]
5. Ye N et al. Orphan receptor GPR88 as an emerging neurotherapeutic target. ACS Chem Neurosci. 2019;10:190–200. [PubMed: 30540906]
6. Wold EA et al. Allosteric modulation of class A GPCRs: targets, agents, and emerging concepts. J Med Chem. 2019;62:88–127. [PubMed: 30106578]
7. Sriram K, Insel PA. G protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? Mol Pharmacol. 2018;93:251–258. [PubMed: 29298813]
8. Hauser AS et al. Pharmacogenomics of GPCR drug targets. Cell. 2018;172:41–54. [PubMed: 29249361]
9. Congreve M et al. Impact of GPCR structures on drug discovery. Cell. 2020;181:81–91. [PubMed: 32243800]
10. Yang RY et al. A systematic survey of human tissue-specific gene expression and splicing reveals new opportunities for therapeutic target identification and evaluation. bioRxiv. 2018. 10.1101/311563.
11. Davenport AP et al. Advances in therapeutic peptides targeting G protein-coupled receptors. Nat Rev Drug Discov. 2020;19:389–413. [PubMed: 32494050]
12. Wang P et al. Opportunities and challenges in drug discovery targeting the orphan receptor GPR12. Drug Discov Today. 2023;28 103698. [PubMed: 37422169]
13. Bolinger AA et al. Orphan G protein-coupled receptor GPR37 as an emerging therapeutic target. ACS Chem Neurosci. 2023;14:3318–3334. [PubMed: 37676000]
14. Morri M et al. Optical functionalization of human Class A orphan G-protein-coupled receptors. Nat Commun. 2018;9:1950. [PubMed: 29769519]
15. Tang XL et al. Orphan G protein-coupled receptors (GPCRs): biological functions and potential drug targets. Acta Pharmacol Sin. 2012;33:363–371. [PubMed: 22367282]
16. Alavi MS et al. Orphan G protein-coupled receptors: the role in CNS disorders. Biomed Pharmacother. 2018;98:222–232. [PubMed: 29268243]
17. Sawzdargo M et al. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, ΨGPR53 and GPR55: GPR55 is extensively expressed in human brain. Mol Brain Res. 1999;64:193–198. [PubMed: 9931487]
18. Kelly K Investigating the role of schizophrenia-associated gene expression in the developing human brain using Machine Learning Thesis. Technological Univ. Dublin; 2021 10.21427/04VG-8P95.
19. Pardiñas AF et al. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. Nat Genet. 2018;50:381–389. [PubMed: 29483656]
20. Komatsu H et al. Anatomical transcriptome of G protein-coupled receptors leads to the identification of a novel therapeutic candidate GPR52 for psychiatric disorders. PloS One. 2014;9:e90134. [PubMed: 24587241]
21. Lonsdale J et al. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013;45:580–585. [PubMed: 23715323]
22. Lin X et al. Structural basis of ligand recognition and self-activation of orphan GPR52. Nature. 2020;579:152–157. [PubMed: 32076264]
23. Rasmussen SG et al. Crystal structure of the β2 adrenergic receptor–G_s protein complex. Nature. 2011;477:549–555. [PubMed: 21772288]
24. Garcia-Nafria J et al. Cryo-EM structure of the adenosine A2A receptor coupled to an engineered heterotrimeric G protein. eLife. 2018;7:e35946. [PubMed: 29726815]
25. Martin AL et al. Constitutive activity among orphan Class-A G protein coupled receptors. PloS One. 2015;10:e0138463. [PubMed: 26384023]

26. Wang P et al. Discovery of potent and brain-penetrant GPR52 agonist that suppresses psychostimulant behavior. J Med Chem. 2020;63:13951–13972. [PubMed: 33198466]
27. Krumm B, Roth BL. A self-activating orphan receptor. Nature. 2020;579:35–36. [PubMed: 32123359]
28. Nishiyama K et al. FTBMT, a novel and selective GPR52 agonist, demonstrates antipsychotic-like and procognitive effects in rodents, revealing a potential therapeutic agent for schizophrenia. J Pharmacol Exp Ther. 2017;363:253–264. [PubMed: 28851764]
29. Hatzipantelis CJ et al. β-Arrestin-2-dependent mechanism of GPR52 signaling in frontal cortical neurons. ACS Chem Neurosci. 2020;11:2077–2084. [PubMed: 32519845]
30. Power ME et al. The non-nutritive sweetener sucralose increases β-arrestin signaling at the constitutively active orphan G protein-coupled receptor GPR52. Can J Physiol Pharmacol. 2024;102:116–127. [PubMed: 37748201]
31. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev. 2011;63:182–217. [PubMed: 21303898]
32. Li G, Marlin MC. Rab family of GTPases. Methods Mol Biol. 2015;1298:1–15. [PubMed: 25800828]
33. Yao Y et al. A striatal-enriched intronic GPCR modulates huntingtin levels and toxicity. eLife. 2015;4:e05449. [PubMed: 25738228]
34. Spark DL et al. In the loop: extrastriatal regulation of spiny projection neurons by GPR52. ACS Chem Neurosci. 2020;11:2066–2076. [PubMed: 32519838]
35. Sakuma K et al. Temporal and spatial transcriptional fingerprints by antipsychotic or propsychotic drugs in mouse brain. PloS One. 2015;10: e0118510. [PubMed: 25693194]
36. Nishiyama K et al. Genetic deletion of GPR52 enhances the locomotor-stimulating effect of an adenosine A2A receptor antagonist in mice: a potential role of GPR52 in the function of striatopallidal neurons. Brain Res. 2017;1670:24–31. [PubMed: 28583861]
37. Setoh M et al. Discovery of the first potent and orally available agonist of the orphan G-protein-coupled receptor 52. J Med Chem. 2014;57:5226–5237. [PubMed: 24884590]
38. Tokumaru K et al. Design, synthesis, and pharmacological evaluation of 4-azolylbenzamide derivatives as novel GPR52 agonists. Bioorg Med Chem. 2017;25:3098–3115. [PubMed: 28433511]
39. Nakahata T et al. Design and synthesis of 1-(1-benzothiophen-7-yl)-1H-pyrazole, a novel series of G protein-coupled receptor 52 (GPR52) agonists. Bioorg Med Chem. 2018;26:1598–1608. [PubMed: 29478803]
40. Grottick AJ et al. GPR52 agonists represent a novel approach to treat psychotic disorders and improve cognitive function. Eur Neuropsychopharmacol. 2017;27: S924.
41. Grottick A et al. GPR52 agonists represent a novel approach to treat cognitive deficits associated with schizophrenia. Biol Psychiatry. 2017;81. S271, Abst. 669.
42. Felsing DE et al. Advances in dopamine D1 receptor ligands for neurotherapeutics. Curr Top Med Chem. 2019;19:1365–1380. [PubMed: 31553283]
43. Surmeier DJ et al. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci. 2007;30:228–235. [PubMed: 17408758]
44. Enomoto T et al. Lurasidone reverses MK-801-induced impairment of learning and memory in the Morris water maze and radial-arm maze tests in rats. Behav Brain Res. 2008;186:197–207. [PubMed: 17881065]
45. Ast A et al. mHTT seeding activity: a marker of disease progression and neurotoxicity in models of Huntington's disease. Mol Cell. 2018;71:675–688.e6. [PubMed: 30193095]
46. Kordasiewicz HB et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012;74:1031–1044. [PubMed: 22726834]
47. Yang S et al. CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. J Clin Invest. 2017;127:2719–2724. [PubMed: 28628038]
48. Lu B, Palacino J. A novel human embryonic stem cell-derived Huntington's disease neuronal model exhibits mutant huntingtin (mHTT) aggregates and soluble mHTT-dependent neurodegeneration. FASEB J. 2013;27:1820–1829. [PubMed: 23325320]

49. Song H et al. Targeting Gpr52 lowers mutant HTT levels and rescues Huntington's disease-associated phenotypes. Brain. 2018;141:1782–1798. [PubMed: 29608652]
50. Xiong YG et al. 1-Heteroaryl-indoline-4-carboxamides as modulators of GPR52 useful for the treatment or prevention of disorders related thereto. Patent. 2016. WO2016176571A1.
51. Poulter S et al. The identification of GPR52 agonist HTL0041178, a potential therapy for schizophrenia and related psychiatric disorders. ACS Med Chem Lett. 2023;14:499–505. [PubMed: 37077397]
52. Stott LA et al. Characterisation of [3H]-HTL45725, a novel radioligand for the orphan receptor GPR52 (Poster 18). Presented at Drug Discovery 2021, Liverpool, UK, 19 20 October 2021. Available at https://www.myeventflo.com/event-lecture.aspx?m=4&evID=2374&lectID=24150
53. Sergeev E et al. Effect of HTL0045725, a selective agonist of the orphan receptor GPR52, on kinase and transcription factor phosphorylation in striatal neurons. Neurosci Applied. 2022;1 100412.
54. Heptares S Sosei Heptares doses first subject in phase I trial with HTL0048149, a first-in-class GPR52 agonist for schizophrenia. Sosei Heptares. 2023 https://soseiheptares.com/news/851/129/Sosei-Heptares-Doses-First-Subject-in-Phase-I-Trial-with-HTL0048149-a-First-in-Class-GPR52-Agonist-for-Schizophrenia.html.
55. Ma M et al. Targeted proteomics combined with affinity mass spectrometry analysis reveals antagonist E7 acts as an intracellular covalent ligand of orphan receptor GPR52. ACS Chem Biol. 2020;15:3275–3284. [PubMed: 33258587]
56. Lagoutte R et al. Divergent synthesis and identification of the cellular targets of deoxyelephantopins. Nat Commun. 2016;7:12470. [PubMed: 27539788]
57. Wang C et al. GPR52 antagonist reduces Huntingtin levels and ameliorates Huntington's disease-related phenotypes. J Med Chem. 2021;64:941–957. [PubMed: 33185430]
58. Stott LA et al. Characterisation of inverse agonism of the orphan-G protein-coupled receptor GPR52 by cannabinoid ligands Cannabidiol and O-1918. Heliyon. 2021;7 e07201. [PubMed: 34189291]
59. Chen H et al. Evolutions in fragment-based drug design: the deconstruction-reconstruction approach. Drug Discov Today. 2015;20:105–113. [PubMed: 25263697]
60. Wang X et al. Structure-based drug design strategies and challenges. Curr Top Med Chem. 2018;18:998–1006. [PubMed: 30101712]
61. Kashyap A et al. Counting on fragment based drug design approach for drug discovery. Curr Top Med Chem. 2018;18:2284–2293. [PubMed: 30499406]
62. Chen H et al. Fragment-based drug design and identification of HJC0123, a novel orally bioavailable STAT3 inhibitor for cancer therapy. Eur J Med Chem. 2013;62:498–507. [PubMed: 23416191]
63. Liu Z et al. Discovery of orally bioavailable chromone derivatives as potent and selective BRD4 inhibitors: scaffold hopping, optimization, and pharmacological evaluation. J Med Chem. 2020;63:5242–5256. [PubMed: 32255647]
64. Cerqueira NM et al. Receptor-based virtual screening protocol for drug discovery. Arch Biochem Biophys. 2015;582:56–67. [PubMed: 26045247]
65. Lyne PD. Structure-based virtual screening: an overview. Drug Discov Today. 2002;7:1047–1055. [PubMed: 12546894]
66. Ali S, Zhou J. Highlights on U.S. FDA-approved fluorinated drugs over the past five years (2018–2022). Eur J Med Chem. 2023;256 115476. [PubMed: 37207534]
67. Wang P, Zhou J. Proteolysis targeting chimera (PROTAC): a paradigm-shifting approach in small molecule drug discovery. Curr Top Med Chem. 2018;18:1354–1356. [PubMed: 30306871]
68. Pettersson M, Crews CM. Proteolysis targeting chimeras (PROTACs) - past, present and future. Drug Discov Today Technol. 2019;31:15–27. [PubMed: 31200855]
69. Xue Y et al. Novel approaches to targeted protein degradation technologies in drug discovery. Expert Opin Drug Discov. 2023;18:467–483. [PubMed: 36895136]
70. Komatsu H Discovery of the first druggable GPR52 antagonist to treat Huntington's disease. J Med Chem. 2021;64:938–940. [PubMed: 33443413]

71. Lebraud H et al. Protein degradation by in-cell self-assembly of proteolysis targeting chimeras. ACS Cent Sci. 2016;2:927–934. [PubMed: 28058282]

72. Wager TT et al. Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. ACS Chem Neurosci. 2010;1:435–449. [PubMed: 22778837]

73. Ghose AK et al. Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery. ACS Chem Neurosci. 2012;3:50–68. [PubMed: 22267984]

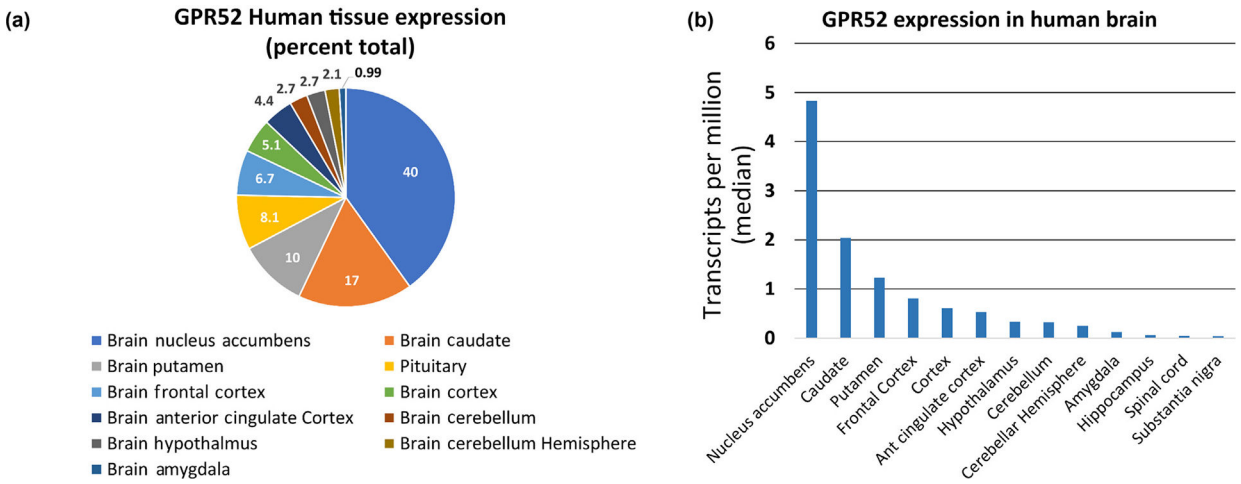

FIGURE 1.

GPR52 mRNA transcript expression in human tissues and brain, generated from the GTEx RNAseq results and online database (GTEx data at: https://www.gtexportal.org/home/gene/GPR52). **(a)** The total percentage of GPR52 mRNA expression in human tissues shows highly enriched expression in the brain. **(b)** GPR52 median transcripts per million mRNA expression in human brain subregions determined by GTEx.

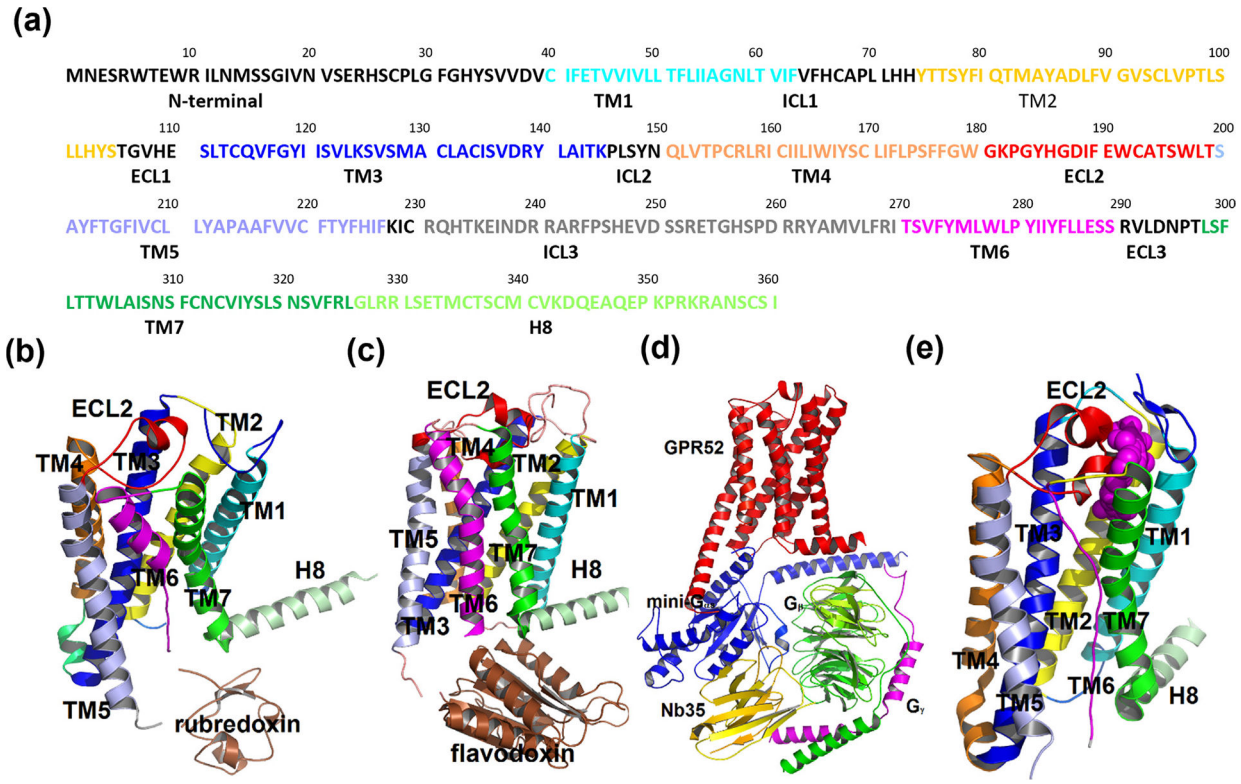

FIGURE 2.

Sequence and structures of GPR52. **(a)** The sequence of GPR52, colored to represent different domains: TM1 (cyan), TM2 (yellow), TM3 (blue), TM4 (orange), TM5 (light blue), TM6 (magenta), TM7 (green), ECL2 (red), ICL3 (gray) and H8 (light green). **(b)** The crystal structures of GPR52 [displayed in the same colors as its sequences in (a)] and its ICL3 fusion-protein partner (rubredoxin, brown) (PDB code: 6LI2). **(c)** The crystal structures of GPR52 [displayed in the same colors as in (a)] and its ICL3 fusion-protein partner (flavodoxin, brown) (PDB code: 6LI1). **(d)** The co-crystal structure of the GPR52–mini-G_s–Nb35 complex (GPR52, red; mini-$G_{\alpha s}$, blue; G_β, green; G_γ, purple; Nb35 nanobody, yellow) (PDB code: 6LI3). **(e)** The co-crystal structure of GPR52 [displayed in the same colors as in (a)] and an agonist c17 (compound 5, purple sphere) in the complex (PDB code: 6LI0).

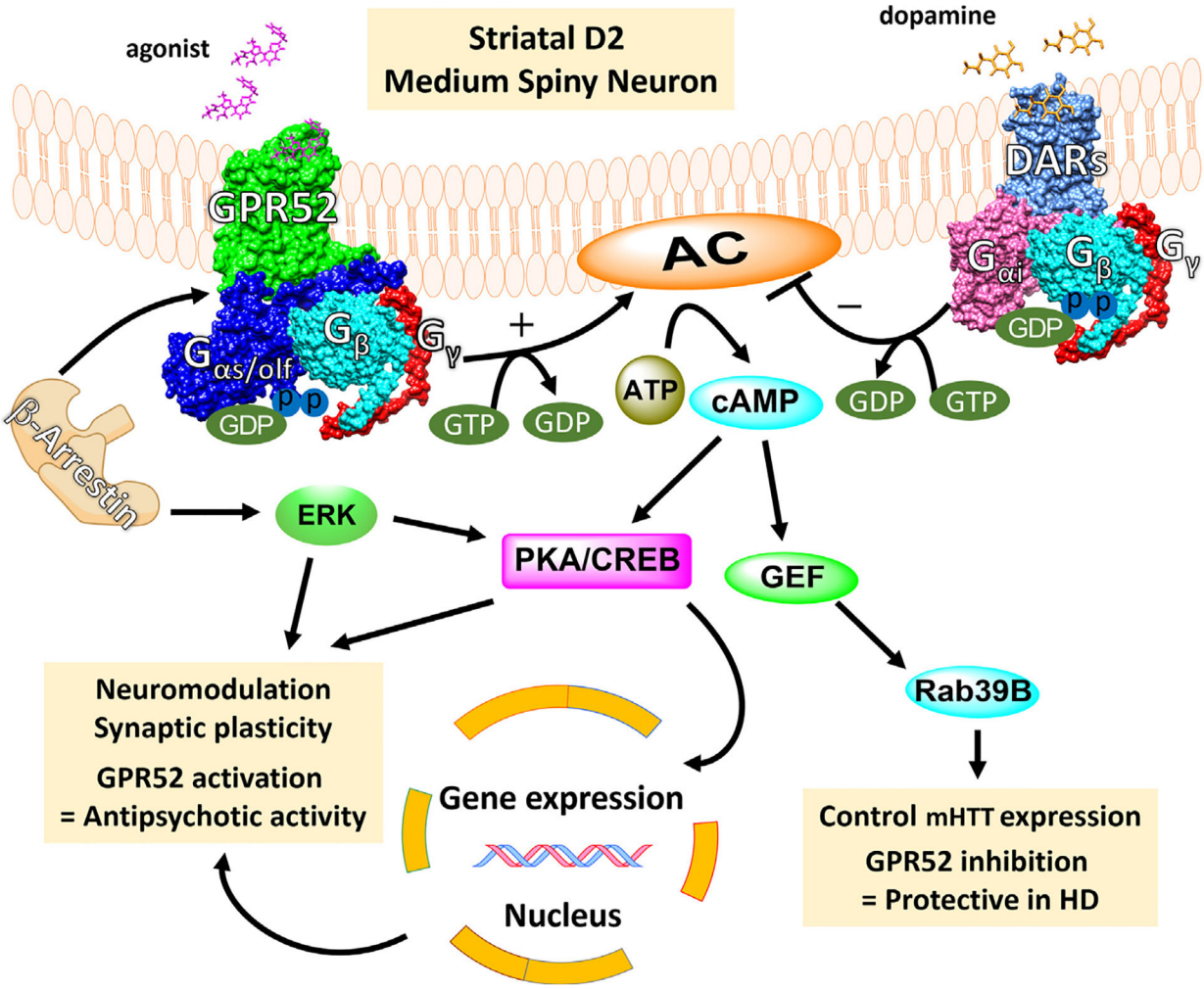

FIGURE 3.

A brief schematic of GPR52 signaling pathways in MSNs and potential interactions with DAR signaling, such as with D_2R/D_3R. GPR52 couples to G_s or G_{olf} (Gs/olf proteins consist of $G_{\alpha s}$ or $G_{\alpha olf}$, G_β, and G_γ) and promotes AC conversion of ATP to cAMP. cAMP regulates various cellular responses through PKA directly, or PKA can result in further phosphorylation of CREB to regulate gene expression and mediate cellular responses. Activated GPR52 promotes the accumulation of cAMP, thereby increasing the activity of an unknown GEF protein and activating Rab39B protein-mediated cellular functions such as HTT expression. In addition, GPR52 can recruit and couple to β-arrestins to activate ERK signaling. D_2R and D_3R couple to G_i (G_i protein consists of $G_{\alpha i}$, G_β and G_γ) to inhibit the activity of AC and reduce cAMP signaling. The convergence of the signaling between GPR52 and the D_2R has supported the general concept that GPR52 agonists could resemble D_2R antagonists.

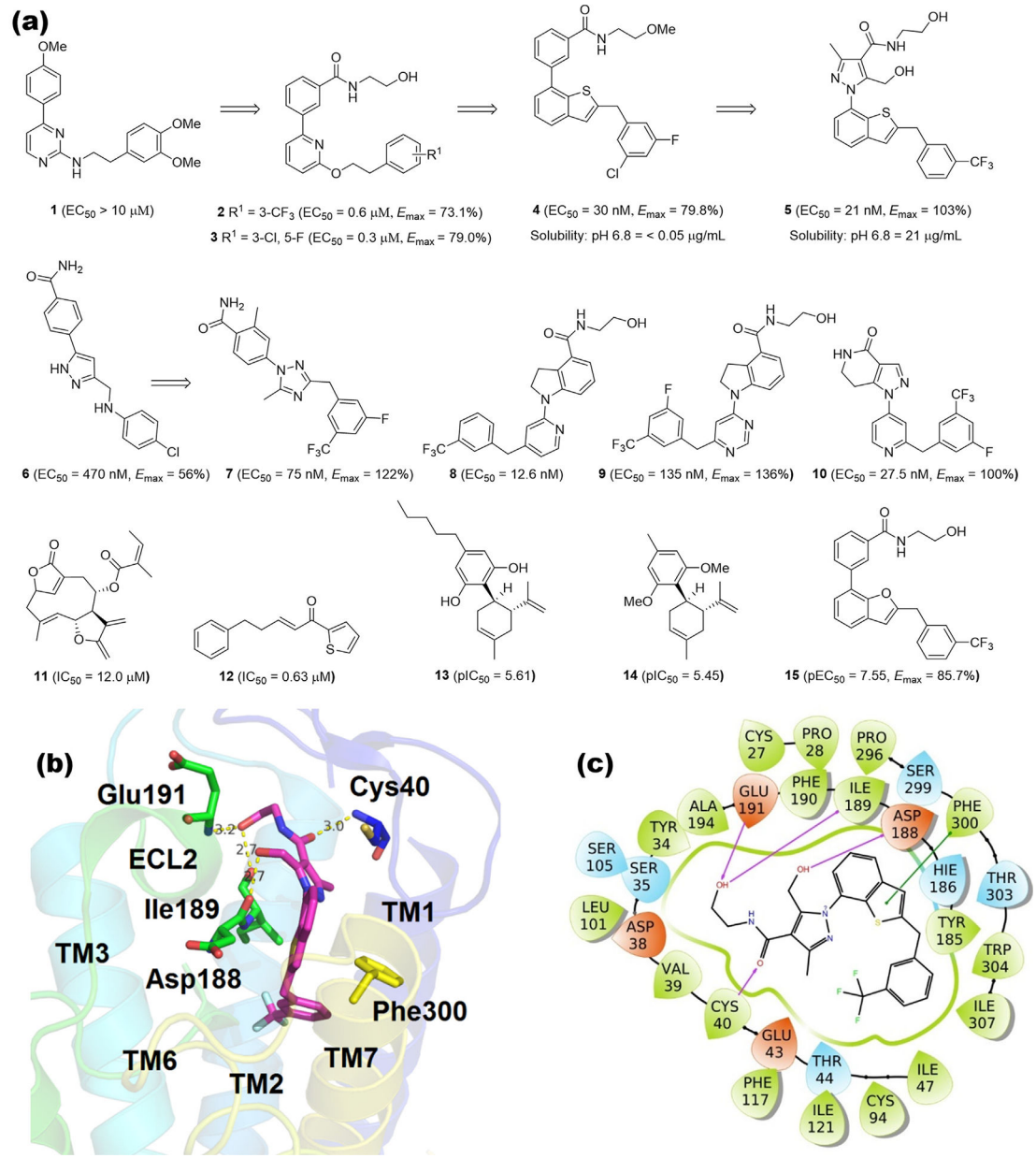

FIGURE 4.

Small-molecule GPR52 ligands and co-crystal structures (PDB code: 6LI0) of compound 5 in complex with GPR52. **(a)** Chemical structures of reported representative small-molecule GPR52 regulators. **(b)** The binding pocket of the co-crystal structure with compound 5 (purple) and GPR52. Important residues are drawn as sticks, and hydrogen bonds are shown as dashed yellow lines. **(c)** The co-crystal structure of compound 5 and GPR52 in 2D binding pocket view. Hydrogen bonds are shown as purple lines and the p-p interaction is shown as a green line.