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MINIREVIEW

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# Pharmacology and function of the orphan GPR139 G protein-coupled receptor

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**Abstract**

G protein-coupled receptors (GPCRs) constitute the largest family of receptors and membrane proteins in the human genome with ~800 members of which half are olfactory. GPCRs are activated by a very broad range of endogenous signalling molecules and are involved in a plethora of physiological functions. All GPCRs contain a transmembrane domain, consisting of a bundle of seven α-helices spanning the cell membrane, and forming the majority of the known ortho- or allosteric ligand binding sites. Due to their many physiological functions and the accessible and druggable transmembrane pocket, GPCRs constitute the largest family of drug targets mediating the actions of 34% of currently marketed drugs. GPCRs activate one or more of the four G protein families ($G_{q/11}$, $G_{i/o}$, $G_s$ and $G_{12/13}$) and/or ß-arrestin. About a third of the non-olfactory GPCRs are referred to as orphan receptors which means that their endogenous agonist(s) have not yet been found or firmly established. In this MiniReview, we focus on the orphan GPR139 receptor, for which the aromatic amino acids L-Trp and L-Phe as well as ACTH/α-MSH-related peptides have been proposed as endogenous agonists. GPR139 has been reported to activate several G protein pathways of which $G_{q/11}$ is the primary one. The receptor shows the highest expression in the striatum, thalamus, hypothalamus, pituitary and habenula of the human, rat and mouse CNS. We review the surrogate agonists and antagonists that have been published as well as the agonist pharmacophore and binding site. Finally, the putative physiological functions and therapeutic potential are outlined.

## 1 | G PROTEIN-COUPLED RECEPTORS

G protein-coupled receptors (GPCRs) mediate many of the physiological responses to endogenous ligands such as neurotransmitters, hormones, metabolites, ions and sensory signals.[1] Although their ligands are structurally very diverse, GPCRs share a common molecular structure of seven transmembrane-spanning α-helices connected by three intracellular and three extracellular loops, with an extracellular N terminus and intracellular C terminus.[2] Amongst all of the drugs approved by the Food and Drug Administration (FDA), 34% target GPCRs [3] making this the largest class of drug targets. However, despite this, only 27% of all non-olfactory GPCRs are presently drug targets. Based on previous success with targeting this protein family and strong

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unexploited disease associations, GPCRs remain highly pursued targets for basic research and drug discovery.[3]

## 2 | GPCR SIGNAL TRANSDUCTION

In the human genome, there are 16 Gα subunits, 5 Gβ subunits and 12 Gγ subunits, which couple to GPCRs as heterotrimeric Gαβγ proteins.[4] The Gα subunits are divided into four classes based on structural and functional similarities termed $G\alpha_{q/11}$ (constituted by $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_{15}$), $G\alpha_{12/13}$ (constituted by $G\alpha_{12}$ and $G\alpha_{13}$), $G\alpha_s$ (constituted by $G\alpha_s$ and $G\alpha_{olf}$), and $G\alpha_{i/o}$ (constituted by $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_z$, $G\alpha_{gust}$, $G\alpha_{t1}$ and $G\alpha_{t2}$).[5]

$G\alpha_{q/11}$ activate phospholipase C β isoforms ($PLC_\beta$) resulting in the hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) into inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ then induces the release of intracellular $Ca^{2+}$ from the endoplasmic reticulum, and DAG activates protein kinase C (PKC) (Figure 1A). Both $Ca^{2+}$ and PKC participate in diverse signalling to induce different cellular events.[6,7]

Activation of $G\alpha_s$ and $G\alpha_{i/o}$ stimulates and inhibits adenylyl cyclase, respectively,[5] which subsequently converts adenosine 5′-triphosphate (ATP) to 3′,5′-cyclic adenosine monophosphate (cAMP). cAMP then activates protein kinase A (PKA), which phosphorylates further downstream effectors (Figure 1C, D).[5]

$G\alpha_{12/13}$ is the least characterized G protein signalling pathway. $G\alpha_{12/13}$ activates Rho-guanine nucleotide exchange factors (RhoGEFs) causing a GDP-GTP exchange in the monomeric GTPase RhoA (Figure 1B).[8] No second messenger assay exists for the quantification of the $G\alpha_{12/13}$ activation, but this can be measured by monitoring G protein activation by bioluminescence resonance energy transfer (BRET) [9] or by Corning Epic dynamic mass redistribution assay.[10]

The dimeric Gβγ complex can modulate the activity of various downstream effector molecules, like PLCβ, mitogen-activated protein kinase (MAPK) and different types of adenylyl cyclase.[11]

GPCRs also signal through G protein-independent signalling pathways.[12] Until recently, it was assumed that the recruitment of β-arrestin caused termination of G protein signalling by steric hindrance of G proteins and initiation of receptor internalization into cytosolic endosomes.[12] However, it has now been

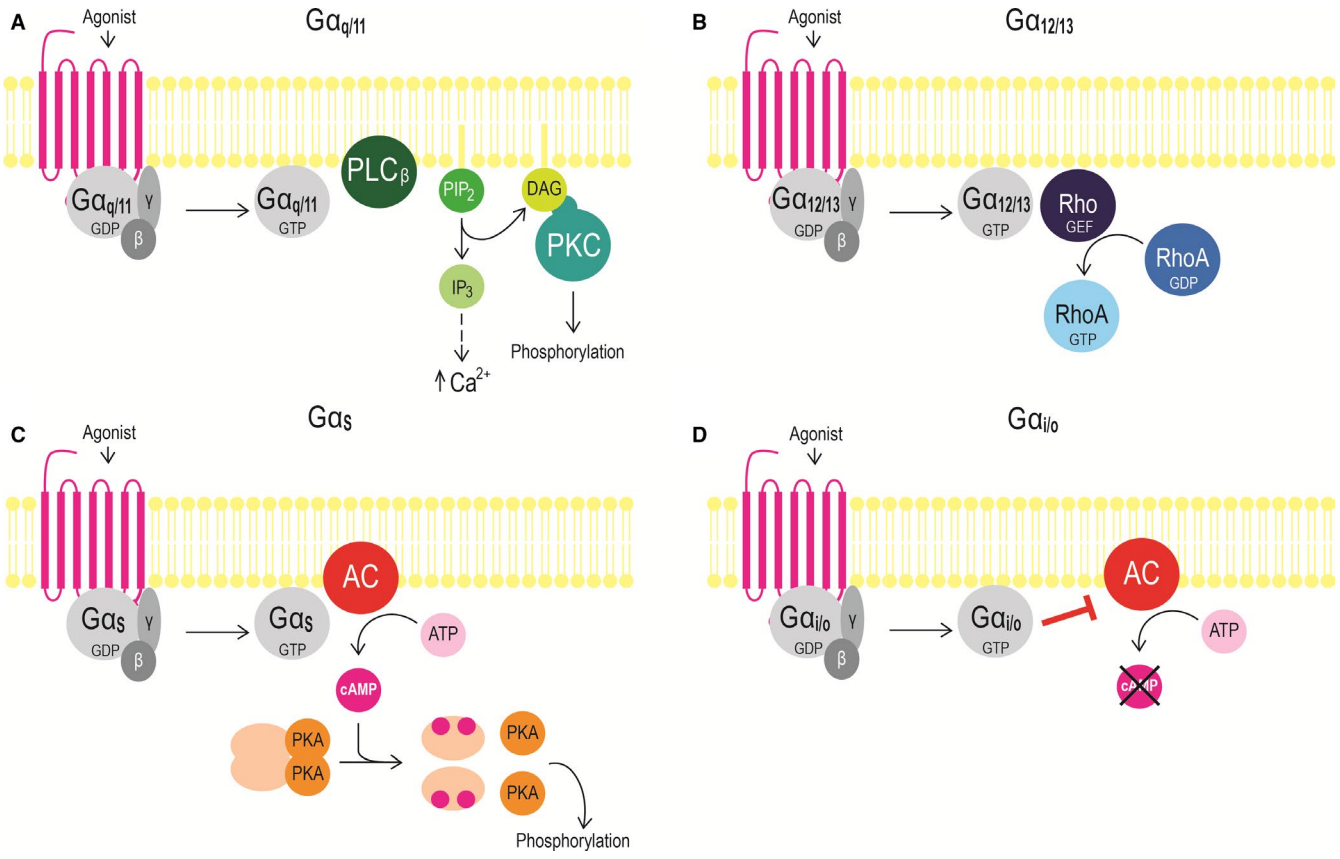


**FIGURE 1** A schematically simplified overview of (A) $G\alpha_{q/11}$, (B) $G\alpha_{12/13}$, (C) $G\alpha_s$, and (D) $G\alpha_{i/o}$ signalling. Abbreviations: AC, adenylyl cyclase; ATP, adenosine 5′-triphosphate; cAMP, 3′,5′-cyclic adenosine monophosphate; DAG, diacylglycerol; GDP, guanosine 5′-diphosphate; GTP, guanosine 5′-triphosphate; $IP_3$, inositol 1,4,5-trisphosphate; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLCβ, phospholipase C-β; Rho-GEF, Rho-guanine nucleotide exchange factor. Adapted from Wettshureck et al[5]

recognized that β-arrestins can initiate downstream signalling, including activation of extracellular signal-regulated kinase 1/2 (ERK1/2) [12]). Additionally, some GPCRs can continue to signalling after they have been internalized.[13-16]

## 3 | ORPHAN GPCRS

An orphan GPCR is a receptor for which an endogenous ligand has not yet been identified. There are presently 121 non-sensory orphan GPCRs, including GPR139.[17] The naming and classification of orphan GPCRs upon pairing with an endogenous ligand is done by the International Union of Basic and Clinical Pharmacology Committee of Receptor Nomenclature and Drug Classification (NC-IUPHAR), which maintains a database of all non-sensory human GPCRs (www.guidetopharmacology.org).[18] To be considered "deorphanized," the proposed receptor-ligand pairing must meet the following criteria (a) have been reported by at least two independent research groups in peer-reviewed papers and (b) be present in sufficient concentration in the tissue(s) where the receptor is expressed. Additionally, there are two further recommendations: (a) selective antagonists and agonists should block and mimic the action of the putative endogenous ligand on the receptor, respectively, and (b) radioligand binding and functional assays should be performed both in vitro and in native tissue.[19]

## 4 | DISCOVERY OF GPR139

A partial, one-exon GPR139 gene, located on chromosome 16 in human beings, was first assigned as a homologue to the thyrotropin-releasing hormone receptor in 2002.[20] In 2005, Gloriam et al[21] identified and curated the full-length GPR139 (mRNA NM_001002911.3 and protein NP_001002911.1) by searches in genome databases. GPR139 has also had a number of aliases, including GPCR12,[20] PGR3,[22] KOR3L [23] and GPRg1,[24] but is now exclusively termed GPR139.[17,19] Basic local alignment search tool (BLAST) searches and phylogenetic analyses showed that GPR139 belongs to the rhodopsin class of GPCRs (also termed class A) and is closest related to another orphan receptor, GPR142.[21] Moreover, Gloriam et al found that the full GPR139 gene has two exons and is highly conserved across species, as the amino acid sequence of the human GPR139 protein is 96%, 92% and 70% identical to the mouse, chicken and zebrafish orthologs, respectively.

**TABLE 1** Regions with highest GPR139 mRNA expression in humans, rat and mouse

| Area of expression | | Human beings | Rat | Mouse |
|---|---|---|---|---|
| CNS | Brainstem | Wang, 2019 | | Wang, 2019 |
| | CA1 area of hippocampus | | Dvorak, 2014 | Süsen, 2006 |
| | CA3 area of hippocampus | | Dvorak, 2014 | |
| | Dentate gyrus of hippocampus | | Dvorak, 2014 | Süsen, 2006 |
| | Habenula (lateral) | | Wagner, 2016 | |
| | Habenula (medial) | | Dvorak, 2014; Wagner, 2016 | Matsuo, 2005; Süsen, 2006 |
| | Hypothalamus | Liu, 2015; Matsuo, 2005; Wang, 2019 | Liu, 2015; Wang, 2019 | Wang, 2019 |
| | Insular cortex | Liu, 2015 | | |
| | Medulla | Süsen, 2006; Wang, 2019 | | Wang, 2019 |
| | Olfactory | Wang, 2019 | | Wang, 2019 |
| | Pituitary | Liu, 2015; Matsuo, 2005 | Liu, 2015; Wang, 2019 | |
| | Spinal cord | Wang, 2019 | | |
| | Striatum | Liu, 2015; Matsuo, 2005; Süsen, 2006; Wang 2019 | Liu, 2015; Dvorak, 2014; Wang, 2019 | Matsuo, 2005; Wang, 2019 |
| | Substantia nigra | Wang 2019 | Liu, 2015 | |
| | Thalamus | Liu, 2015; Wang, 2019 | Liu, 2015; Wagner, 2016; Wang, 2019 | Wang, 2019 |
| | Zona incerta | | | Matsuo, 2005 |
| Foetus | | Matsuo, 2005 | | Süsen, 2006[a] |

mRNA expression was determined using in situ hybridization by Dvorak et al, 2014[25] and Wagner et al, 2016,[28] whereas Liu et al, 2015[26] used qPCR, Matsuo et al, 2005[24] used qPCR and in situ hybridization, Süsens et al, 2006[27] used Northern blot, and Wang et al, 2019[29] used RNA sequencing. Blank spots in the table indicate that GPR139 expression in these areas has not yet been determined.

[a]Matsuo et al[24] were not able to confirm this finding with PCR.

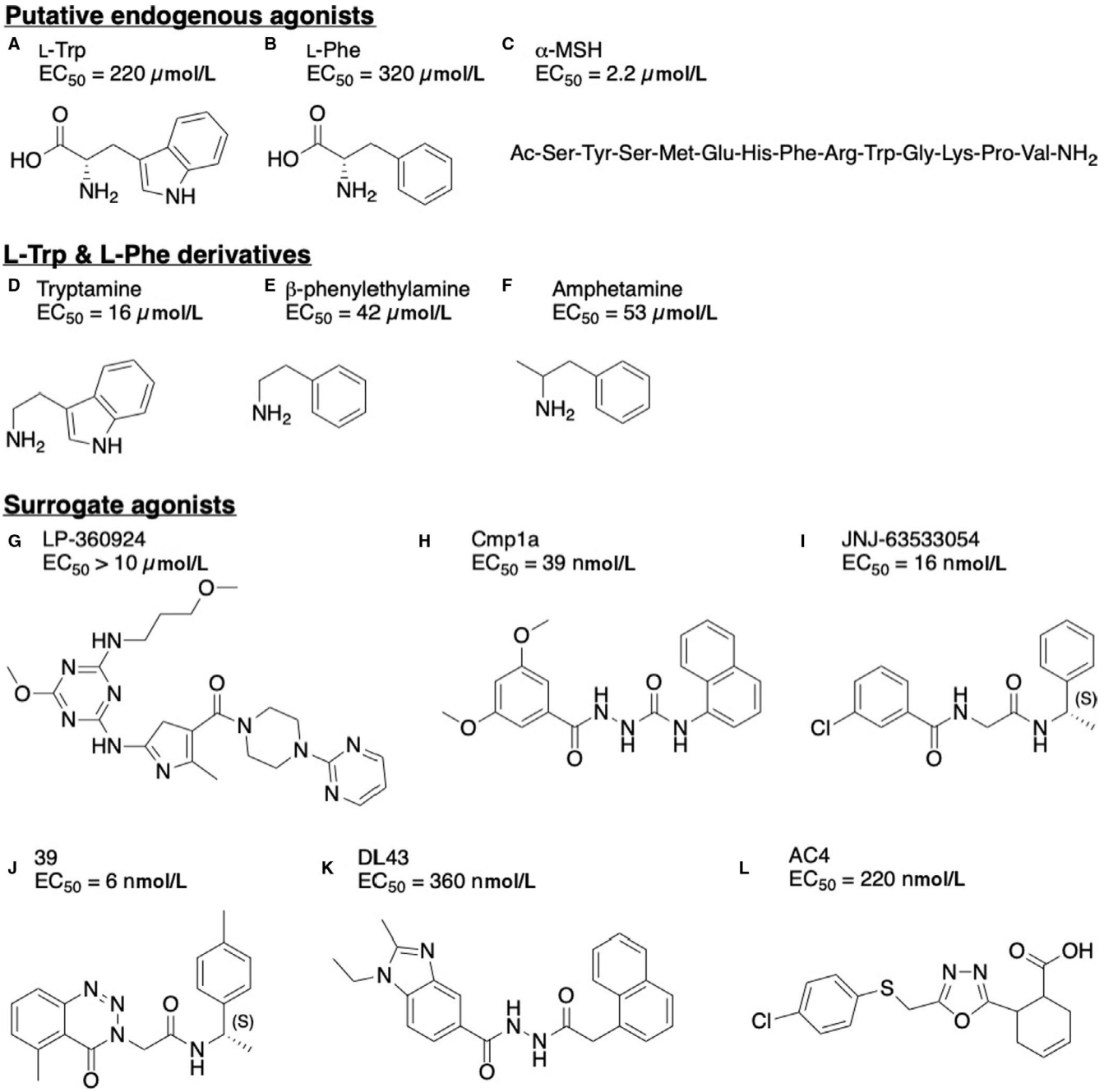


**FIGURE 2** Structures of selected GPR139 agonists (A) L-tryptophan,[26,32] (B) L-phenylalanine,[26,32] (C) α-MSH,[35] (D) tryptamine,[25,26] (E) β-phenylethylamine,[25,26] (F) amphetamine,[25,26] (G) LP-360924 by Lexicon Pharmaceuticals,[37] (H) cmp1a by H. Lundbeck A/S,[38] (I) JNJ-63533054 (also known as 7c) by Janssen Research and Development,[26,39] (J) 39 by Takeda Pharmaceutical Company Limited,[40] (K) DL43 a bioisostere of cmp1a [41] and (L) AC4 [43]

## 5 | GPR139 EXPRESSION

GPR139 expression studies have mainly been based on mRNA levels and depending on species found to have the highest transcript expression in the striatum, thalamus, hypothalamus, pituitary and habenula of the CNS.[24-29] Profiling of mRNA expression in a broad range of CNS and peripheral tissues have revealed that GPR139 expression is restricted to the CNS in human beings, rat and mouse,[29,30] suggesting that the receptor does not have a peripheral function. An overview of the GPR139 mRNA expression in human, rat and mouse tissue is provided in Table 1. Additionally, GPR139 protein expression has been studied in mice by Liu et al,[26] who showed GPR139 antibody immunoreactivity in the habenula in wild-type mice, but importantly not in knockout mice. Additionally, they confirmed expression of β-galactosidase in the same brain area in the GPR139 knockout mouse (originating from a LacZ

gene replacing the GPR139 gene in the knockout mouse). These findings correlate well with the expression of GPR139 mRNA in the habenula (Table 1). Radiolabelled GPR139 ligands have been developed and used in autoradiography studies on wild-type vs. GPR139 knockout mouse brains, but no specific binding was detected, most likely due to a too low endogenous receptor expression level or to a too high level of background radioligand binding.[26,31]

## 6 | PUTATIVE ENDOGENOUS GPR139 RECEPTOR AGONISTS

In 2014, Isberg et al[32] developed a pharmacophore model based on the surrogate agonists from Shi et al (see next section) that led to the identification of four aromatic-containing dipeptides with pharmacological agonist activity at the human GPR139 receptor. The authors continued with a subsequent pharmacological evaluation of each individual amino acid. They assayed all L-α-amino acids and found the highest potencies for L-tryptophan (L-Trp, $EC_{50}$ = 220 μmol/L) and L-phenylalanine (L-Phe, $EC_{50}$ = 320 μmol/L) (Figure 2A, B), which were proposed as endogenous agonists for GPR139.[32] The activity of L-Trp and L-Phe was subsequently confirmed by Liu et al[26] at Janssen Research and Development, who also reported that a range of derivatives including tryptamine (Figure 2D), β-phenylethylamine (Figure 2E) and amphetamine (Figure 2F) activate GPR139.[33,34] Interestingly, Liu et al[26] found that the aromatic amino acids were slightly more potent at human GPR139 in their assays (L-Trp, $EC_{50}$ = 49 μmol/L; L-Phe, $EC_{50}$ = 60 μmol/L) and moreover reported that brain and serum fractions that activated GPR139 contained L-Trp or L-Phe. Liu et al[26] also reported that rat plasma contains concentrations of L-Trp (125 μmol/L fasted and 188 μmol/L fed) and L-Phe (121 μmol/L fasted and 155 μmol/L fed), which is within the dynamic range of the GPR139 receptor. Collectively, these results support that the aromatic amino acids could be the endogenous signalling molecules for GPR139, but evidence of a closer link between the ligands, the receptor and a physiological function is needed to fully prove it.

Recently, Nøhr et al reported similarities between the ligand binding pocket residues of GPR139 and the melanocortin 4 receptor (MC4R). The authors subsequently demonstrated GPR139 activity of three known endogenous MC4R agonists: neuropeptides adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH) (Figure 2C) and β-melanocyte-stimulating hormone (β-MSH), as well as their conserved common motif HFRW.[35] All three peptides activated GPR139 in the high nanomolar to low micromolar range.[35] Another noteworthy finding was that a predicted cleavage product ($\alpha$-$MSH_{1\text{-}10}$) of the pre-pro-protein pro-opiomelanocortin (POMC) activated the receptor in the submicromolar range ($EC_{50}$ = 318 nmol/L), thus qualifying the peptide as the most potent and selective endogenous agonist of GPR139 identified to date.[35] Given that the potency of the peptides is higher than L-Trp and L-Phe, the authors proposed that GPR139 is a peptide-binding GPCR, albeit the endogenous peptide agonist might be another more potent peptide (derivative) than tested in the study.[35] However, as detailed below, Nepomuceno et al (Janssen Research and Development) found the ACTH, α-MSH and β-MSH peptides to be less potent, but interestingly, the peptide agonists shifted the signalling pathway of the cognate melanocortin receptors when co-expressed with GPR139.[36] Collectively, these results suggest that GPR139 could also play a role as peptide receptor, either directly or indirectly via heterodimerization. However, more studies are required to elucidate the physiological endogenous agonist(s) for GPR139.

## 7 | SURROGATE GPR139 RECEPTOR AGONIST LIGANDS, PHARMACOPHORE AND STRUCTURE-ACTIVITY RELATIONSHIPS

Shortly after the discovery of GPR139, the first two series of small-molecule surrogate agonists were reported by Hu et al (exemplified by LP-360924, Figure 2G)[37] and Shi et al (exemplified by cmp1a, Figure 2H).[38] The compounds were selective towards GPR139 compared to $\beta_2$AR and GPR142 and a selectivity panel consisting of 90 different receptors, ion channels and transporters. Furthermore, Janssen Research and Development identified a potent and selective (tested against a panel consisting of 50 different GPCRs, ion channels and transporters, including GPR142) surrogate small-molecule agonist (JNJ-63533054, also known as compound 7c) (Figure 2I) that crosses the rat blood-brain barrier, reaching brain levels in the micromolar range after oral administration.[26,39] Subsequently, several groups have reported GPR139 surrogate agonist for GPR139, including Hitchcock et al (compound 39, Figure 2J [40]), Shehata et al (DL43, Figure 2K [41]) and Nøhr et al (AC4, Figure 2L).[42]

Shehata et al reported an updated agonist pharmacophore model (Figure 3) highlighting ligand features important for GPR139 agonism.[41] The model spans six features: Two terminal aromatic systems (R1 and R2) separated by a linker consisting of two hydrogen bond acceptors (A3 and A4); one hydrogen bond donor (D5); and a dual hydrophobic/halogen or hydrogen bond acceptor element (H6/A2) placed at position 3 of R1. The model matches known GPR139 agonists and thus represents a typical GPR139 agonist. The authors

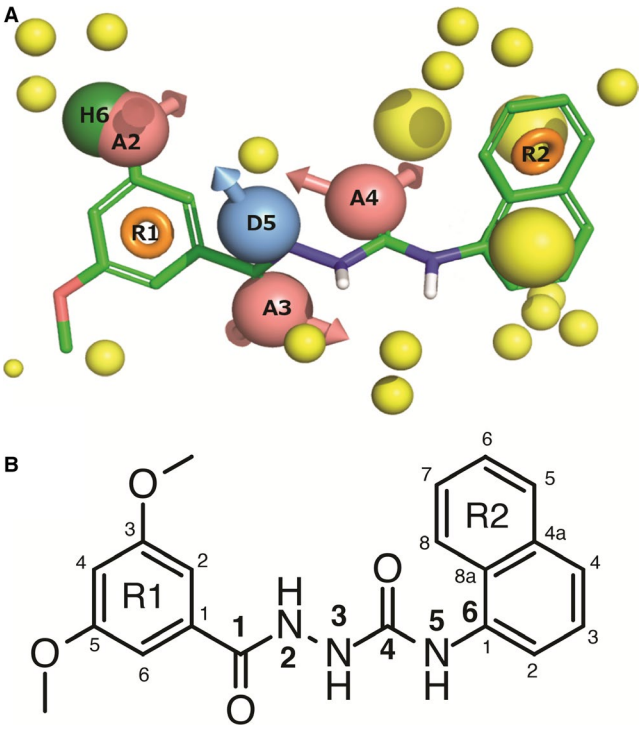


**FIGURE 3** A, Agonist pharmacophore model showing cmp1a with green carbons and the following pharmacophore features: red: hydrogen bond acceptor, blue: hydrogen bond donor, green: hydrophobic element, orange: aromatic system and yellow: exclusion volume. B, Chemical structure of cmp1a with atom numbering used to discuss the structure-activity-relationship. From [41] with permission under Creative Commons Attribution 4.0 International License

also represented a combined structure-activity relationship (SAR) of published GPR139 agonists [41] and reported that a reduction of the linker length results in loss of activity, while compounds with linkers longer than 6-atoms only had reduced activity. The 1-carbonyl oxygen atom represents a critical hydrogen bond acceptor, and removal leads to loss of activity. Methylation or carbon substitution of the 2-nitrogen atom leads to a reduced potency, indicating the importance of a hydrogen bond donor on this position. Contrary to this, carbon substitution of the 3-nitrogen has minor effect on potency. The 4-carbonyl oxygen atom represents another important hydrogen bond acceptor, and removal results in loss of activity. Nitrogen to carbon substitution on the 5-position only causes a minor shift in potency. Locking the linker 1-2 atoms and/or the aromatic ring in a favourable conformation generally improves potency, as opposed to 5-6-cyclization. The R1 aromatic ring is present in all active surrogate agonists and most likely contributes to ligand potency. Both L-Trp and L-Phe overlay with R1 and R2, respectively, as well as with several linker characteristics, indicating that they also accommodate the pharmacophore and the same binding site. Interestingly, the most recently published surrogate agonist, AC4 (Figure 2L), contains a novel scaffold, which does not fully fit the pharmacophore as it lacks the second terminal ring. However, AC4 still possesses nanomolar potency and binds to the receptor in the same binding site as other GPR139 agonists (cf. section "GPR139 receptor agonist binding site" below).[42]

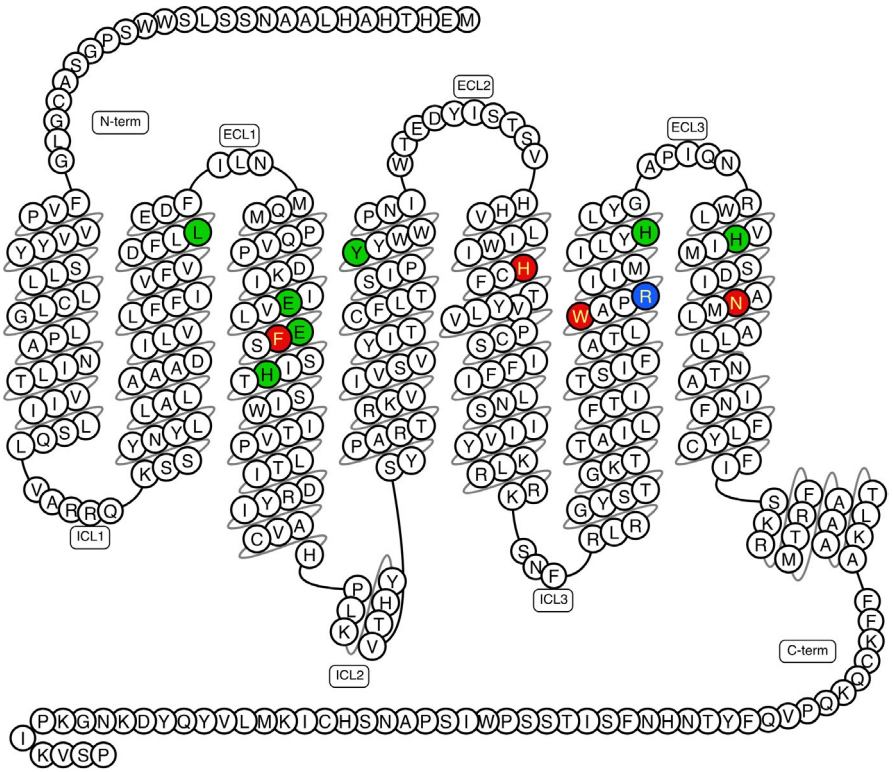


**FIGURE 4** Snake plot diagram of GPR139 agonist binding site showing 12 mutated residues in red (hot spot), green (no effect) and blue (very low expression). Drawn in GPCRdb [61] based on data from.[43] N-term: amino-terminal, C-term: carboxy-terminal, ECL: extracellular loop and ICL: intracellular loop

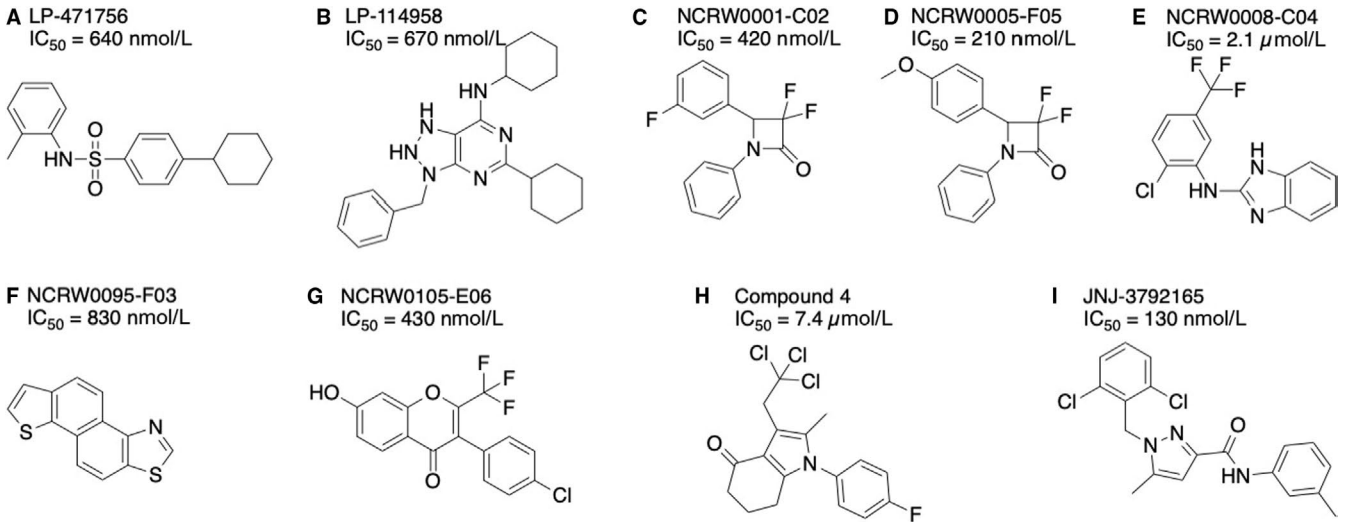


**FIGURE 5** GPR139 antagonist structures. A, LP-471756, (B) LP-114958 by Lexicon Pharmaceuticals,[37] (C) NCRW0001-C02, (D) NCRW0005-F05, (E) NCRW0008-C04, (F) NCRW0095-F03, (G) NCRW0105-E06 by the National Center for Drug Screening, CAS, Shanghai,[45] (H) compound 4 by H. Lundbeck A/S [46] and (I) JNJ-3792165 by Janssen Research and Development [36]

## 8 | GPR139 RECEPTOR AGONIST BINDING SITE

Nøhr et al elegantly confirmed that the endogenous L-Trp and L-Phe share a common binding site with the surrogate agonists cmp1a, JNJ-63533054 and AC4.[42,43] By combining in silico and in vitro mutagenesis, they identified a common binding pocket consisting of a hydrophobic region deeply buried between F109$^{3x33}$, H187$^{5x43}$ and W241$^{6x48}$, and a proximal polar region enclosed by N271$^{7x38}$, R244$^{6x51}$ and E108$^{3x32}$ (Figure 4).[43] The binding site was confirmed by Wang et al, who also identified a less buried hydrophobic binding site defined by V76$^{2x73}$, F79$^{2x56}$, I104$^{3x28}$, V83$^{2x60}$ and I80$^{2x57}$ shared by larger GPR139 agonists.[44] They furthermore identified several series of human GPR139 mutations resulting in gain, reduction or loss of function. The two first sets of mutations were located in the common binding pocket and near the G protein binding site on helix 5 and 6, which are known to move upon G protein binding. Another set of mutations impact the transmembrane helices structure and thus might change the common ligand binding site or the ability to bind G proteins. The authors speculate that the last two set of mutations have a structural impact on GPR139 function.[44]

## 9 | GPR139 RECEPTOR ANTAGONISTS

Finding antagonists for GPR139 has proven to be more challenging. However, Hu et al have reported two antagonists LP-471756 and LP-114958 (Figure 5A, B),[37] that are selective towards the GPR139 receptor, compared to vector and $\beta_2$AR cells. Wang et al identified five small-molecule antagonists (NCRW0001-C02, NCRW0005-F05, NCRW0008-C04, NCRW0095-F03 and NCRW0105-E06) representing four different scaffolds but reported no selectivity data (Figure 5C-G).[45] Lastly, Andersen et al report a GPR139 antagonist compound 4 (Figure 5H).[46] Overall, these antagonists have relatively low potency and unknown receptor selectivity severely limiting their usability as pharmacological tools to study function and physiology of the GPR139 receptor. However, Janssen Research and Development recently reported the discovery of the GPR139 antagonist JNJ-3792165 (Figure 5I), which is fairly potent ($pK_b$ = 7.4 in [$^{35}$S]GTPγS assay and $pK_b$ = 6.9 in calcium mobilization assay) and selective compared to 50 neurotransmitter and neuropeptide receptors.[36]

## 10 | GPR139 SIGNALLING PATHWAYS

There is some discrepancy in the literature reports of GPR139 signalling pathways. Of note, the signalling pathways have only been studied in transfected cells, because a cell line or primary cells endogenously expressing GPR139 have not yet been identified. All the reported results on GPR139 signalling pathways are summarized in Figure 6 and described briefly below.

Matsuo et al report constitutive activity of GPR139 expressed in 293-EBNA cells (HEK293 cell line expressing Epstein-Barr virus). They were able to block this constitutive activity with a $G_{q/11}$ inhibitor (YM-254890) and partly inhibit it with a $G_{12/13}$ pathway inhibitor (Y-27632 binding to Rho-associated protein kinase), suggesting coupling to $G_{q/11}$ and $G_{12/13.}$[24] Shi et al, Wang et al and Isberg et al have all used the same stable transfected CHO-GPR139 cell line

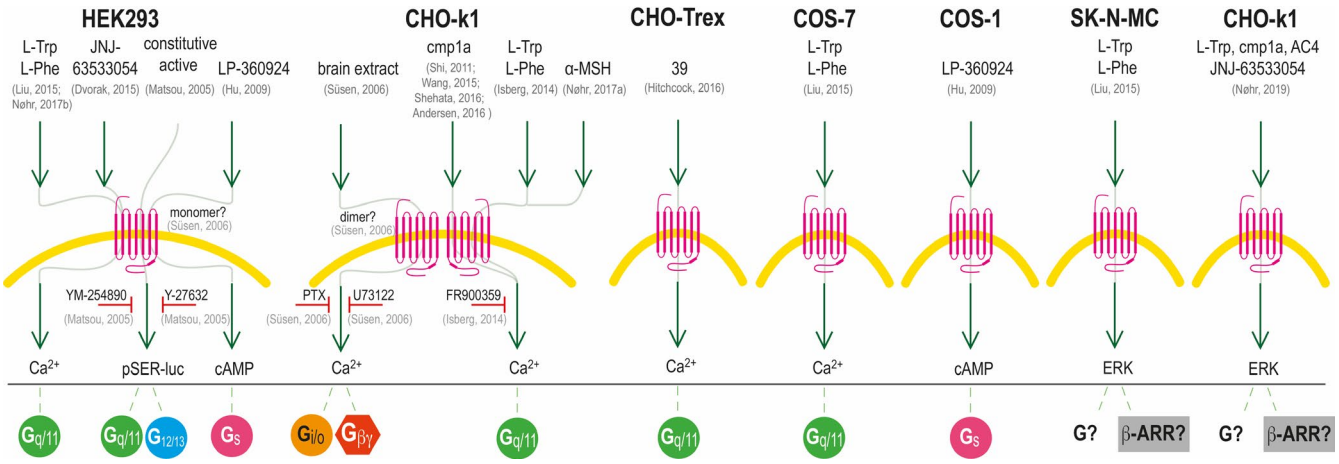


**FIGURE 6** Schematic drawing of the reported GPR139 signalling in different cell types. GPR139 signalling has been reported in HEK293, CHO-k1, COS-1, COS-7, SK-N-MC and CHO-Trex cells. Green arrows indicate an effect on GPR139, and red horizontal T-bars indicate inhibition of the signalling pathways by different pathway inhibitors: YM-254890 is a $G_{q/11}$ inhibitor; Y-27632 is a ROCK inhibitor that inhibits the $G_{12/13}$ signalling pathway; PTX, pertussis toxin, is a $G_{i/o}$ inhibitor; U73122 is a PLC inhibitor, and FR900359 is a $G_{q/11}$ inhibitor. At the bottom of the drawing, the association with the different G proteins and ß-arrestin (ß-ARR) is given. The references are listed in light grey: Andersen et al, 2016[46]; Dvorak et al, 2015[39]; Hitchcock et al, 2016[39]; Hu et al, 2009[37]; Isberg et al, 2014[37]; Liu et al, 2015[26]; Matsuo et al, 2005[26]; Nøhr et al, 2017a,[35] Nøhr et al 2017b[35]; Nøhr et al 2019[42]; Shehata et al, 2016[43]; Shi et al, 2011[38]; Süsens et al, 2006[38]; Wang et al, 2015[45]

and have consistently shown that cmp1a activates a $Ca^{2+}$ response [32,38,45] that can be inhibited by the $G_{q/11}$ inhibitor FR900359, suggesting $G_{q/11}$ coupling.[32] Furthermore, Nøhr et al and Liu et al show that HEK293 cells transiently transfected with GPR139 give a $Ca^{2+}$ response upon stimulation with L-Trp and L-Phe.[26,43] Additionally, Hitchcock et al show $Ca^{2+}$ signalling in CHO-TREx cells expressing GPR139 and Dvorak et al show a $Ca^{2+}$ response in stably expressing HEK293-GPR139 cells upon stimulation with the agonist JNJ-63533054.[39,40] Altogether, there is strong evidence of $G_{q/11}$ as the primary transducer of GPR139 signalling.

A few studies have also suggested other signalling pathways. Hu et al observed a cAMP stimulation response in HEK293 and COS-1 cells expressing GPR139 upon stimulation with the agonist LP-360924 indicative of $G_s$ coupling,[37] whereas Süsen et al via indirect measurements suggested that GPR139 expressed in CHO-k1 cells couples to $G_{i/o}$ upon stimulation with brain extracts.[27] However, Shehata et al have been unable to detect $G_s$ or $G_{i/o}$-mediated responses from cmp1a, JNJ-63533054, DL43, L-Trp or L-Phe in CHO-GPR139 cells.[41] This does not rule out the possibility that GPR139 might be able to activate non-$G_{q/11}$-mediated signalling, as the cellular background could influence the preferred signalling pathway depending on G protein and β-arrestin subtype expression. Another possibility is that distinct ligands could induce signalling bias.[47,48] More studies are thus needed to delineate the role of the non-$G_{q/11}$ pathways in GPR139 receptor signalling.

Interestingly, Nøhr et al recently demonstrated a robust GPR139-mediated ERK phosphorylation response in stably transfected CHO-GPR139 cells,[42] which further elaborated initial observations by Liu et al.[26] All the tested compounds (incl. L-Trp, cmp1a, JNJ-63533054 and AC4) displayed a concentration-dependent response, with potencies and potency rank order similar to what had previously been observed in the calcium mobilization assay.[42] More studies are needed to elucidate whether the ERK is mediated via $G_{q/11}$, ß-arrestin or a third signalling pathway.

## 11 | PUTATIVE GPR139 HETERODIMERIZATION

As described above, Nøhr et al have reported that high nanomolar to low micromolar concentrations of the peptides ACTH, α-MSH and variants hereof activated the GPR139 receptor stably expressed in a CHO cell line, albeit with generally relatively lower potencies than reported at the canonical targets; melanocortin receptors 1-5 ($MC_{1\text{-}5}$).[35] When reproducing this, Nepomuceno et al [36] observed GPR139 receptor activity of these peptides at the highest tested concentrations in some cell lines and pharmacological assays, but obtained lower potencies.[35] The reason for this discrepancy is unclear but it is well known that GPCR and/or G protein expression levels can vary between studies and affect agonist potencies.[47] However, in both studies, the potencies of the peptides were likely too low to be physiologically relevant albeit peptide variants of different length or with post-translational modifications might be more potent and thus endogenous agonists.[35]

Interestingly, Nepomuceno et al [36] observed that co-expression of GPR139 with $MC_3$ or $MC_5$ enabled robust and potent intracellular calcium mobilization by ACTH, α-MSH and ß-MSH. $MC_3$ and $MC_5$ are $G_s$ coupled

receptors which usually do not couple to intracellular calcium mobilization when expressed alone, so this study suggests that the GPR139 receptor can heterodimerize with other GPCRs and modulate their signalling. Wang et al subsequently performed a study where they observed that mRNA expression of the GPR139 and dopamine D2 receptor correlated in a broad range of CNS regions, which led the authors to hypothesize that they might functionally interact.[29] Interestingly, co-expression of the two receptors in HEK293 cells enabled the dopamine D2 receptor to increase intracellular calcium, while the D2 receptor did not respond in this pathway when expressed alone. Moreover, the calcium response from the co-expressed receptors could be antagonized by either a D2 or GPR139 receptor antagonist. Collectively, these results suggest that the receptors interact functionally and that GPR139 modulate D2 receptor signalling.[29] Further studies on cells or tissues with native GPR139 receptor expression are required to fully elucidate the role of the GPR139 receptor on ACTH/α-MSH/ß-MSH pharmacology and function and on the role of GPR139 in modulating melanocortin and dopamine D2 receptor signalling.

## 12 | PHYSIOLOGICAL FUNCTION AND THERAPEUTIC POTENTIAL OF GPR139

GPR139 is still classified as an orphan receptor, as its endogenous ligand and function remain to be firmly established according to the NC-IUPHAR guidelines (cf. section "Orphan GPCRs" above). However, based on the expression pattern, putative endogenous ligands and preliminary animal studies, four major hypotheses about the physiological function and therapeutic potential of the receptor have been developed.

### 12.1 | Hypothesis I: Locomotor activity

The consistent cross-species expression of GPR139 mRNA in the striatum (Table 1) suggests that GPR139 may have a role in the control of locomotor activity (movement). Parkinson's disease (PD) is a major movement disorder. Its aetiology/cause is still largely unknown; however, patients with PD suffer from degeneration of dopaminergic (DA) neurons in substantia nigra, which gives input to the striatum. The striatum consists of caudate nucleus and putamen and is part of the basal ganglia.[33] There is a direct motor loop through the basal ganglia that facilitates the initiation of willed movements, which is disrupted in patients with PD. More than 6 million people worldwide have PD [49] and presently only the symptoms can be treated; hence, novel treatment strategies are acutely needed.[50] Besides GPR139 mRNA expression in the striatum, there are three studies pointing towards a role in locomotor activity for the receptor:

1. A patent from Regeneron Pharmaceuticals Inc in 2004 describes GPR139 knockout mouse phenotypes.[23] Interestingly, they showed that in a rotarod performance test, which is a test known to evaluate balance and motor co-ordination, the mean latency time to fall off the rod was significantly lower for GPR139 knockout than wild-type mice. Furthermore, when their GPR139 knockout mice were placed on a 2-cm-thick beam, they fell off at rates that were significantly faster than their wild-type littermates. However, other tests for balance and motor control like a gait analysis and a thin 5-mm beam balance test showed no difference between wild-type mice and their GPR139 knockout littermates.[23] Nonetheless, all together these tests suggest impairment in balance and in sensory motor integration in their GPR139 knockout mice, but further behavioural studies are needed to clarify this.
2. Recently, Liu et al[26] published a potent GPR139 agonist JNJ-63533054 (Figure 2I). They dosed male Sprague Dawley rats with this compound and observed significantly decreased locomotor activity compared to vehicle and to rats dosed with the less active enantiomer of JNJ-63533054. However, future work is needed to elucidate whether the mechanism behind this locomotor effect was mediated by GPR139, as the compound could potentially have sedative effects that could partly explain the observed decrease in locomotor activity.[26]
3. Lastly, Andersen et al[46] showed that GPR139 agonists (including cmp1a) protected primary dopaminergic (DA) neurons against the toxin 1-methyl-4-phenylpyridinium ($MPP^+$) in vitro. $MPP^+$produces similar neuropathological defects as observed in PD patients by degenerating DA neurons [51] and is therefore often used in animal models for PD.[52] However, GPR139 agonists did not show any neuroprotective activity upon treatment with rotenone,[46] which is another toxin used in animal models of PD.[52] Despite the inclusive results, Andersen and coworkers suggest that GPR139 could be a potential target for neuroprotection in PD.[46]

### 12.2 | Hypothesis II: Metabolism

GPR139 mRNA is expressed in the hypothalamus and pituitary in humans and rat, and both GPR139 mRNA and proteon have been found in the habenula in rodents (Table 1). On this basis, GPR139 has been postulated to regulate food consumption and/or energy expenditure.[28]

The close GPR139 homologue GPR142 shares the activation by L-Trp and L-Phe, but is mainly expressed in the pancreas and gut, where it is involved in the regulation of insulin and incretin secretion, respectively, making GPR142 a potential target in type II diabetes.[53,54] As L-Trp and L-Phe are both also putative endogenous ligands of GPR139,[26,32] it has been suggested that GPR139 is a nutrient-sensing receptor.[26] Thus, based on the expression pattern and nature of the putative endogenous agonists, GPR139 could be involved in metabolism-related disorders like type II diabetes.

Furthermore, in the non-peer-reviewed patent from Regeneron Pharmaceuticals, it is stated that the GPR139 knockout mice had increased lean body mass and decreased body fat compared to wild-type mice.[23] Hence, the patent supports the hypothesis that GPR139 plays a role in energy homeostasis. Additionally, as described above, it has been shown that the appetite-regulating hormones ACTH, α-MSH and β-MSH [55] can activate GPR139 at relatively high concentrations [35] or that GPR139 modulate the signalling the canonical melanocortin receptors,[36] which further support a role of GPR139 in energy homeostasis. In conclusion, GPR139 could be a potential target for the treatment of metabolism-related disorders like obesity and type II diabetes.

### 12.3 | Hypothesis III: Alcohol addiction and hyperalgesia

The habenula is also known as a brain region involved in addiction.[56] Interestingly, a recent study has shown that systemic or habenula-specific injection of the GPR139 selective agonist JNJ-63533054 reduced alcohol self-administration in a subgroup of alcohol-dependent rats that exhibited a compulsive-like phenotype. Moreover, the GPR139 agonist also reduced withdrawal-induced hyperalgesia.[34] Importantly, these effects were not observed in non-dependent rats and the agonist did not affect water or saccharin intake. It was thus concluded that GPR139 is a potential new drug target for treatment of alcohol misuse disorders and potentially also other drugs of abuse.

### 12.4 | Hypothesis IV: Phenylketonuria (PKU)

Phenylketonuria (PKU) is an autosomal recessive genetic disorder, which is associated with intellectual disabilities when untreated. Patients suffering from PKU have a non-functional phenylalanine hydroxylase enzyme, which is a hepatic enzyme that metabolizes L-Phe to L-Tyr. In the absence of this enzyme, L-Phe accumulates in the body to a neurotoxic level (reviewed in [57]). Since it has been shown that L-Phe is an agonist for GPR139,[26,32] it has been suggested that the abnormally high levels of L-Phe in PKU patients cause overstimulation of GPR139 in the brain which could be one of the reasons for the intellectual disabilities associated with PKU.[25] Inhibiting GPR139 in PKU patients might therefore limit the intellectual disabilities seen in these patients.

### 12.5 | Other potential (patho) physiological roles of GPR139

Genetic variations in the *GPR139* locus have been linked to schizophrenia[58] and symptoms of inattention in attention deficit hyperactivity disorder (ADHD).[59] GPR139 has also been proposed to have a role in depression[40] and foetal development.[27] Recently, Janssen Research and Development showed that the brain penetrant agonist JNJ-63533054 had no or only minor effects in a range of rodent mood/anxiety models questioning the GPR139 receptor role in mood/anxiety.[60]

## 13 | CONCLUSION AND PERSPECTIVES

Recent years have brought a number of significant tools in the studies of the GPR139 receptor including a range of potent and selective GPR139 agonists of which some have been shown to penetrate the blood-brain barrier and be active in animal models. The amino acids L-Trp and L-Phe as well as derivatives of the peptide hormones ACTH and α-MSH have been suggested as potential endogenous GPR139 receptor agonists, but remain to be fully validated. A specific radioligand and antibody have been published but are not commercially available limiting application. Likewise, only limited information has been drawn from published GPR139 knockout mice. The main tool remaining to be developed to study the GPR139 receptor is an even more potent and selective antagonist, preferably blood-brain barrier permeable and metabolic stable. The lower number of reported antagonists is striking, given the many potent and selective agonists that have been published. It would also be of great interest to investigate the full range of signalling pathways employed by GPR139 in cells with native receptor expression and whether some of the many agonists developed show signalling bias, which could translate into different physiological/therapeutic effects. Finally, it would also be very valuable to develop the first allosteric modulators, which might have therapeutic benefits compared to the orthosteric ligand. Nevertheless, the tools available will undoubtedly become very valuable in future years to further characterize the pharmacology, physiological function and therapeutic potential of the GPR139 receptor.

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