GPR35: from enigma to therapeutic target

Graeme Milligan*

School of Molecular Biosciences

College of Medical, Veterinary and Life Sciences

University of Glasgow

Glasgow G12 8QQ

Scotland, United Kingdom

*Correspondence: Graeme.Milligan@glasgow.ac.uk (G.Milligan)

Keywords:

G protein-coupled receptor, orphan receptor, kynurenic acid, fatty liver disease, inflammatory bowel diseases, digestive system cancers

Abstract:

GPR35 is a poorly characterised, orphan G protein-coupled receptor that is attracting considerable interest as a therapeutic target. Marked differences in pharmacology between human and rodent orthologues of the receptor and a dearth of antagonists with affinity for mouse and rat GPR35 have previously restricted use of pre-clinical disease models. The development of improved ligands, novel transgenic knock-in mouse lines and detailed analysis of the disease relevance of single nucleotide polymorphisms has greatly enhanced understanding of key roles of GPR35 and stimulated efforts towards disease-targeted proof-of-concept studies. In this Opinion, new information on the biology of the receptor is considered, whilst insight into how GPR35 is currently being assessed for therapeutic utility in areas ranging from inflammatory bowel diseases to non-alcoholic steatohepatitis and various cancers is also provided.

Main text

GPR35 is a poorly understood receptor

First identified more than 20 years ago, and nominally identified in 2006 as a receptor for the tryptophan metabolite kynurenic acid [1], **GPR35** (see **Glossary**) officially remains an '**orphan**' (**Box 1**) G protein-coupled receptor (**GPCR**) [2], and the nature of the endogenous ligand(s) that stimulate GPR35 remains a highly active area of research. Marked variation in the potency of many synthetic agonists between human GPR35 and both the rat and mouse orthologues [3] (**Box 2**), and the exceptionally limited range of available antagonist ligands, has greatly restricted progress in understanding the functions and regulation of this receptor. Moreover, the currently described antagonists are 'human specific', displaying negligible affinity at rat and mouse GPR35 [3-5]. Recently the first atomic level cryo-EM structure of a human GPR35-G protein complex was published with global resolution reported to be to 3.2 Å [6]. As with other GPCRs this identified the canonical transmembrane domain of seven transmembrane helices, three extracellular loops, three intracellular loops, and an amphipathic helix at the cytoplasmic interface. Although of considerable interest and including a range of mutational studies that largely supported earlier efforts to understand the nature of ligand binding selectivity [7-8], this study concentrated more on the role of potential allosteric cations than considering species orthologue differences in pharmacology. It did, however, re-iterate the importance of positively charged residues around the ligand binding pocket [6] that reflects that many agonist ligands contain a carboxylate moiety or are di-acids [3].

Whilst species orthologues of GPR35 can each interact effectively with **arrestin** adapter proteins in an agonist-dependent manner [e.g., 9] it remains uncertain which G protein subsets are the primary transducers of GPR35-mediated signals. In various settings results favour either pertussis toxin-sensitive G_i-family G proteins [10-13] or the less well studied, pertussis toxin-insensitive G_{12}/G_{13} G protein family [5, 12, 14-17]. Indeed, the reported cryo-EM structure incorporated a chimeric G protein that was based significantly on the sequence of $G\alpha_{13}$ [6]. Whether G protein selection is tissue or cell type specific also remains a question for future research.

Despite such issues there has been growing interest in the potential to target GPR35 in a range of conditions. This Opinion will therefore also consider therapeutic opportunities currently attracting the greatest interest with a focus on inflammatory bowel diseases, the spectrum of fatty-liver diseases and stomach and lower-gut tumours.

What is/are the endogenous activator(s) of GPR35?

Kynurenic acid can activate GPR35

As noted earlier kynurenic acid [18-19] was the first ligand reported to activate GPR35 [1, 3]. The basic observations have been repeated and confirmed in numerous *in vitro* and *ex vivo* assays but, as with other GPR35 activators, there is variation in potency of kynurenic acid between human, mouse and rat orthologues [1, 14]. Indeed, the very modest potency of kynurenic acid reported by many groups at, particularly human, GPR35 has led to doubts if this is the key endogenous agonist [13-14]. Kynurenic acid has a range of other known molecular targets [20-21] and in recent studies was shown to be some 20-fold more potent in activating the GPCR hydroxycarboxylic acid receptor (HCAR) 3 than GPR35 [22]. In addition, it can act as a **negative allosteric modulator** (NAM) (and hence blocker) of the adenosine A_{2B} receptor [22]. Despite these illustrations that simple small molecules that derive from intermediary metabolism (and many such molecules are indeed carboxylic acids) can have multiple targets, kynurenic acid has been used extensively in both *ex vivo* and *in vivo* studies with the anticipation that effects will reflect activation of GPR35. These include early studies that indicated that at concentrations lower than might be expected (from *in vitro* studies) to be able to occupy GPR35 to a substantial degree kynurenic acid was able to induce interactions between monocytes and intercellular adhesion molecule (ICAM)-1 expressing human umbilical vein endothelial cells (HUVECs) [10].

Use of kynurenic acid in rodent models

Clearly the lack of both receptor knock-out lines and cross-species active antagonist ligands means that *in vivo* studies performed with kynurenic acid in rat are difficult to interpret [e.g., 23]. By contrast, studies in mice have the potential, and now indeed expectation, to use GPR35 knock-out animals as controls to provide greater confidence of reported effects being '**on-target**'. For example**,** Agudelo et al., [24] used wild type and GPR35 knock-out mice to assess the effect of intraperitoneal (ip) injection of kynurenic acid on adipose tissue energy homeostasis and inflammation and to define a role for GPR35. However, whilst related studies have integrated siRNA-based knock-down of GPR35 [25], in such settings effects are still difficult to attribute specifically to the receptor. This is also the case in studies in which GPR35 'antagonists' have been employed in cells and tissue from species in which these ligands have been shown to lack significant affinity. For example, in a study exploring effects of kynurenic acid to limit lipopolysaccharide-induced endometritis in

mouse Wang et al., [26] reported that ML194 (better known as CID2745687, 1-(2,4-difluorophenyl)-5-[[2-[[(1,1-dimethylehyl)amino]thioxomethyl]hydrazinylidene]methyl]-1*H*-pyrazole-4-carboxylic acid methyl ester) prevented anti-inflammatory effects of kynurenic acid. However, this compound has no measurable effect at mouse GPR35 in defined cell lines expressing this orthologue [5], indicating that the reported effect cannot reflect blockade of GPR35. The same issue raises concerns over the use of ip-injected CID2745687 in a study in which it was reported to block effects of kynurenic acid on bone mineral loss in ovariectomized female mice [27]. This must be an '**off-target**' effect. There is clearly a need for the identification of cross-species GPR35 antagonist ligands, with well characterised pharmacology at rodent orthologues of the receptor, to help better define effects that are mediated unequivocally by GPR35.

A much more intriguing study has recently suggested that protective effects of kynurenic acid on ischaemia in mice is produced via GPR35 [12]. Here, using both GPR35 knock-out mice and the re-introduction of a kynurenic acid-defective binding site mutant (Arg ^{151}Ala) of GPR35 into induced pluripotent stem cell-derived cardiomyocytes, the direct role of GPR35 was defined unambiguously. As part of well performed studies, a fascinating (but surprising) feature, however, was that activation of GPR35 was associated with its translocation to mitochondria. This study has also received additional commentary [28]. How the trans-plasma membrane receptor is then able to regulate the ATP synthase inhibitory factor 1 present on the mitochondrial cristae (and therefore on the mitochondrial inner membrane) remains to be established. In the context of the NLR family pyrin-domain-containing 3 (NLRP3) inflammasome, use of bone marrow-derived macrophages from wild type and GPR35 knock-out mice has shown that kynurenic acid, in a GPR35-dependent manner, can also limit mitochondrial damage by suppressing mitochondrial production of reactive oxygen species [29]. Whether this may be related to the release of oxidised DNA fragments [30-31] is uncertain but may warrant investigation. Most recently, use of wild type and GPR35 knock-out mice has indicated that kynurenic acid can limit the development of high-fat-diet induced **non-alcoholic steatohepatitis** (NASH) [32]. Although this once more does not define that kynurenic acid is the only or indeed most important endogenous activator of GPR35, these studies are consistent with the potential of agonists of GPR35 to be useful in treating fatty liver diseases, including NASH [33] (**Figure 1A**). The therapeutic potential of agonists of GPR35 will be discussed in a subsequent section.

Publication of negative data can be just as useful in defining or eliminating roles for GPR35 and kynurenic acid. For example, by employing a bone marrow transplantation

strategy from wild type and GPR35 knock-out mice, Baumgartner et al., [34] suggested that GPR35 expression does not play a direct role in macrophage activation, vascular inflammation, or the development of atherosclerosis. This study indicates that regulation of GPR35 is unlikely to provide an effective means to limit the development of atherosclerosis.

CXCL17 is not an agonist of GPR35

In 2015 Maravillas-Montero et al., [35] suggested that the molecule **CXCL17** was a high potency activator of GPR35. The evidence to support this was indirect, and although the study used β-arrestin interaction studies to show that CXCL17 did not activate either of the true chemokine receptors CXCR2 or CCR5, they did not assess whether CXCL17 promoted interactions of GPR35 with an arrestin. This seemed a surprising omission as β-arrestin interaction studies have been widely used, over many years, to identify and characterise a wide range of ligands and compounds with agonist activity at GPR35 [e.g., 9]. Subsequent reports found no evidence to support the CXCL17-GPR35 pairing [36-37] and no other direct evidence has been forthcoming to confirm a role for CXCL17. As such this must be considered as one of many efforts at de-orphanisation of poorly characterised GPCRs that have not been widely replicated and failed to stand up to scrutiny. Unfortunately, the initial report [35] suggested renaming GPR35 as an additional chemokine receptor '**CXCR8**', even though systematic naming of receptors is the perogative of the International Union of Pharmacology (IUPHAR) (see **Resources I**). This has resulted in various publications using the 'CXCR8' terminology, particularly those focused on expression patterns of GPR35 and CXCL17 and their potential association with early cancer diagnosis and predictions of prognosis [38-40] (**Figure 1B**). Although these are useful studies, it is important to note that CXCL17 should not be directly linked to GPR35, but rather they should be considered as two independent markers.

5-hydroxyindoleacetic acid as a putative activator of GPR35

The most recent suggestion of a high potency endogenous activator of GPR35 is the serotonin metabolite 5-hydroxyindoleacetic acid (**5-HIAA**) [13]. Given that serotonin is present in high levels in the intestine and colon, that 5-HIAA is a major metabolite, and that expression of GPR35 is also highest in the lower gut, this is an enticing possibility. De Giovanni et al., [13] presented clear data that both kynurenic acid and the high potency synthetic GPR35 agonist lodoxamide (*N*,*N*′-(2-chloro-5-cyano-1,3-phenylene)dioxamic acid)

[6-7] were able to promote chemotaxis of murine WEHI-231 B lymphoma cells that had been virally transduced to express a GFP-tagged form of GPR35. By contrast application of various lysophosphatidic acids (LPA) did not produce this effect, despite a limited number of studies having suggested previously that at least certain species of LPA can activate GPR35 [41-42]. Consistent with the concept that 5-HIAA may directly activate GPR35, although serotonin was unable to promote migration of GPR35-GFP transduced WEHI-231 cells, 5-HIAA was able to do so in a concentration-dependent manner with a clear peak between 10-100 nM, whilst in neutrophil attachment assays 5-HIAA promoted this for cells from wild type but not GPR35 knock-out mice. Moreover, returning to the WEHI-231 cell model 1 μM 5-HIAA was shown to be as effective as 10 μM lodoxamide [13], suggesting it to act as a high-efficacy and potency agonist. Such effects were observed for both human and mouse GPR35. This study [13] represents perhaps the most comprehensive analysis of the likely pairing of a new endogenous ligand with GPR35 and provides strong support for the relevance of 5-HIAA. One element lacking from the study was, however, direct measures of activation of each of human and rodent orthologues of GPR35 after their transfection into, and confirmed expression in, more standard cell lines that are used widely for pharmacological and functional characterisation of GPCRs. As such, although the results of [13] are exciting and appear compelling, independent validation is still awaited.

Synthetic ligands for GPR35 research

Agonists of GPR35 to interrogate GPR35 function

The first substantially characterised synthetic ligand that acts as an agonist at GPR35 was zaprinast (5-(2-propoxyphenyl)-1*H*-[1,2,3]triazolo[4,5-d]pyrimidin-7(4*H*)-one). It displays moderate potency at GPR35 that is not dissimilar to its potency as an inhibitor of cGMP-phosphodiesterase subtypes. However, although of little use in specifically defining GPR35-mediated effects *in vivo*, zaprinast remains a widely utilised tool compound for *in vitro* and even *ex vivo* studies [3]. This reflects that it shows relatively similar potency at human, rat and mouse GPR35, with rank order rat > mouse > human. Subsequent years resulted in the identification of a substantial number of both synthetic and naturally produced (but not endogenously generated) compounds that displayed modest potency as agonists [see 3, 18 for reviews]. Recognition that cromolyn (5,5′-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4-oxo-4*H*-chromene-2-carboxylic acid) disodium is able to activate GPR35

with rather higher potency at human than rodent forms [43] was the initial indication that some clinically employed medicines might mediate at least some of their effects via GPR35. A major surprise was the recognition that pamoate salts of various drugs activated (at least human) GPR35 and that this congener was the common feature of a disparate group of medicines that apparently activated the receptor [44]. Pamoate is also markedly selective for human GPR35 and in many settings acts as a partial agonist. Starting with the discovery that compounds from library screens could be developed via medicinal chemistry to produce ligands with mid-nM and higher potency [45] substantial progress has been made [46-48]. However, serendipity has also played a part in the identification of ligands such as lodoxamide [7] that display excellent potency at human GPR35 and have become widely employed tool compounds [6]. As highlighted earlier, variation in potency of agonist ligands between rat, mouse and human GPR35 can be very marked. For example, lodoxamide, which is a mast cell stabiliser used to treat allergic conjunctivitis, is more than 100-fold less potent at mouse GPR35 than at either rat or human [49]. This makes it a poor choice of ligand to assess the function of GPR35 in wild type mice. In contrast, although pemirolast (9-methyl-3-(1*H*-tetrazol-5-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one) is also a mast cell stabiliser and anti-allergy medicine and is a relatively potent activator of rat and mouse GPR35, this ligand has no significant potency at human GPR35 [49]. Thus, whilst lodoxamide might find use as a 're-purposed' GPR35-targeting medicine, pemirolast will not. However, in rat, equivalent results produced by lodoxamide, pemirolast and zaprinast might be consistent with a GPR35-mediated end point, even in the absence of suitable antagonists or knock-out models. Overall, considerable thought needs to be given to the choice of ligands used to assess GPR35 function in different species because, although more information is becoming available, it is not uncommon for detailed pharmacology to be available only for human GPR35.

Antagonists of GPR35 and species selectivity

As highlighted, there are few characterised GPR35 antagonists, with CID2745687 [50] and ML145 (CID2286812) (2-hydroxy-4-[4-[(5Z)-5-[(E)-2-methyl-3-phenylprop-2-enylidene]-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl]butanoylamino]benzoic acid) [51] being the only ones used in a significant number of studies in human-derived cells and tissues [e.g., 52-53]. However, as they both lack any significant affinity at either rat or mouse GPR35 [54] they clearly cannot be used to define roles of GPR35 in rat or in wild type mice. Some studies have reported effects, and these have been reviewed and considered elsewhere [3]. To overcome this issue, Lin et al., [33] recently generated and employed tissue from a

transgenic knock-in mouse line in which mouse GPR35 was replaced with the equivalent human isoform. Here, ML145 was able to reverse, in a concentration-dependent manner, and with IC_{50} consistent with its known binding affinity at human GPR35, the ability of lodoxamide to prevent triglyceride accumulation in hepatocytes of these animals. The ability of current antagonists to block human GPR35 suggest these transgenic mice may find use in a range of disease models where contributions of GPR35 are being assessed [3].

GPR35 and disease:

Inflammatory bowel disease

Despite the challenges of defining roles of GPR35, the strong genetic association between **single nucleotide polymorphisms** (SNPs) within the *GPR35* gene [3] and both inflammatory bowel diseases and autoimmune liver diseases, including primary sclerotic cholangitis [55-58] has provided clear line-of-sight to targeting this receptor. **Dextran sulphate sodium** (DSS)-induced colitis in mice has been the most used animal model to assess contribution of GPR35 and potential for therapeutic intervention. Farooq et al., [59] illustrated the greater extent and degree of damage induced by exposure to DSS in GPR35 knock-out compared to wild type mice. This built on earlier studies of Tsukahara et al., [11] that reached broadly similar conclusions and also incorporated a number of pharmacological interventions. These, however, included some surprising outcomes. These included that the human orthologue specific GPR35 antagonist CID2745687 was able to prevent effects of various GPR35 activators in studies conducted in either murine young adult colon epithelium or rat small intestinal epithelial cells [11]. This is incompatible with the orthologue selectivity of CID2745687 discussed earlier. DSS treatment in mice resulted in marked shortening of the colon, and sustained treatment with pamoic acid as a potential GPR35 agonist (at 100mg/kg but not 30mg/kg, injected subcutaneously) both reversed this feature and all but eliminated expression of the inflammatory markers TNFα, IL1 and IL6. Hence, despite the earlier noted very low potency of pamoic acid at murine GPR35, and the lack of potential 'off-target' controls in the mouse studies, aspects of this report were also supportive of the potential of GPR35 agonism. Additionally, specific deletion of GPR35 from $CX3CR1^{+}$ macrophages is reported to aggravate DSS-induced colitis, suggesting a specific mechanism [37]. However, by contrast Yansen et al., [60] reported 'lower' susceptibility to DSS in GPR35 knock-out than wild type animals while Schneditz et al., [61] did not observe a significant difference.

Based in part on accumulating evidence that predominantly indicates that lack of GPR35 increases susceptibility to DSS-induced colitis, GlaxoSmithKline and Sosei-Heptares have announced a programme of work to assess small molecule agonists of GPR35 in such conditions (see **Resources II)** (**Figure 1C**). This likely reflects that although there are current treatments including aminosalicylates, glucocorticoids and increasingly anti-tumour necrosis factor-targeting biologicals, for ulcerative colitis and Crohn's disease, which are the major forms of inflammatory bowel disease, there remains a substantial number of patients for whom such treatments are ineffective. In addition, Melhem et al., [62] have shown recently that cell-type-specific deletion of GPR35 in epithelial cells, but not in macrophages, results in goblet cell depletion and dysbiosis, rendering animals more susceptible to *Citrobacter rodentium* infection (**Figure 1C**).

Fatty liver disease

Initially founded on studies that showed that lodoxamide was able to limit lipid accumulation induced by exposure to a liver X-receptor activator in human Hep3B hepatoma cells [63] there has been growing interest in the idea that agonism of GPR35 may be a useful approach to treat diseases linked to 'fatty liver' [32-33]. Surprisingly, however, Nam et al., [63] also showed that low concentrations of lodoxamide were able to replicate this effect in primary hepatocytes from wild type mice in a manner that was prevented, in a concentration-dependent fashion, by the human GPR35 specific antagonist CID2745687. Moreover, in these studies oral delivery of lodoxamide at only 1mg/kg, for the final 7 days of a 7 week diet-induced obesity model of fat accumulation in the liver, was reported to suppress both lipid levels and reduce the amount of the key lipogenic transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) [63]. Although fascinating, as noted earlier, lodoxamide is at least 100-fold less potent at mouse GPR35 than at either the human or rat orthologues [49] and little is known about either bio-availability or pharmacokinetic and pharmacodynamic characteristics of this ligand. As such, it is unclear if this dose would be sufficient to occupy mouse GPR35 to a substantial level. Based on these studies however, Lin et al., [33] confirmed the ability of lodoxamide to prevent liver X-receptor-mediated lipid accumulation in the more widely used human HepG2 hepatoma cell line. Encouraged by this, they used genome-editing to produce HepG2 clones lacking expression of GPR35 and noted both higher basal lipid levels in these, and now, a lack of effect of lodoxamide until human GPR35a was transiently re-introduced. Although the GPR35 agonist bufrolin (6-butyl-4,10-dioxo-1,7-dihydro-1,7-phenanthroline-2,8-dicarboxylic acid), which has moderate potency at

mouse GPR35 [49], was able to reduce liver X-receptor-induced lipid accumulation in primary hepatocytes from wild type mice [33], the lack of antagonists with affinity at mouse GPR35 led this group to generate a transgenic mouse line in which human GPR35a replaced endogenous mouse GPR35. Using primary hepatocytes from these animals, concentrations of lodoxamide anticipated to occupy human GPR35a were able to limit lipid accumulation, and this was prevented by one of the human specific GPR35 antagonists ML145 [33]. Moreover, addition of lodoxamide post-initiation of fat accumulation was able to reverse this feature [33]. Although limited to *ex vivo* studies these outcomes are certainly encouraging that direct *in vivo* delivery of GPR35 agonists with suitable drug-like characteristics might be effective in reducing fat accumulation in hepatocytes (**Figure 1A**). Further support for this idea was recently provides by Wei et al., [32]. Here, in mouse models, knock-out of GPR35 exacerbated diet-induced steatohepatitis, whilst simple overexpression of GPR35 was able to restrict this. Moreover, provision of kynurenic acid (5 mg/kg, ip) was able to restore many of the baseline characteristics, potentially by regulating cholesterol homeostasis.

GPR35 as a target in oncology

There have been more publications alluding to potential links between GPR35 and cancer than to any other disease set. These range from correlations in expression of GPR35 with progression and stage of various cancers [38], analysis to suggest that high level expression of the long GPR35b isoform (**Box 3**) in lymph nodes of colon cancer patients is associated with poor prognosis [64-65], or maybe that expression of GPR35 is positively linked to survival [39]. Mice lacking GPR35 develop less intestinal tumours in spontaneous and inflammation-induced cancer models [61]. Moreover, although macrophage-specific knock-out of GPR35 decreases tumour size it appears to do so by creating a tumour suppressive micro-environment whereas wild type macrophages secrete substantially more angiogenic mediators [66]. These studies point towards GPR35 as a tumour-promoting protein. Although a little difficult to unravel, the well-appreciated high-level expression of GPR35 in the intestine, colon and, indeed, in the stomach, where expression levels have been associated with gastric cancers [67] and with poor prognosis in such cancers [68], and also in non-small-cell lung cancer [68] may indicate opportunities to target this receptor in such disease settings (**Figure 1B**). This has recently been reviewed [69]. The literature does not provide immediately obvious guidance as to whether activation or blockade of GPR35 might be more effective. Clearly the identification and use of novel, drug-like antagonists of GPR35 will be integral to unravelling such questions.

Concluding Remarks and Future Perspectives

After years of relative disinterest, recent times have seen great strides taken in our understanding of the function of the enigmatic receptor GPR35. These include the effective use of both constitutive and, increasingly, tissue-specific knock-out mouse lines and the first reports on knock-in mouse lines in which a human GPR35 isoform replaced the mouse orthologue. This will hopefully start to allow challenges about the use of human selective pharmacological ligands to be unravelled and whether effects they are reported to produce in wild type rodents reflect genuine 'on-target' functions (see **Outstanding Questions**). Potential efforts at drug-repurposing to target GPR35 are being rapidly bolstered by new chemistry programmes designed to develop a larger group of drug-like agonists and, hopefully also, antagonist ligands which will likely validate GPR35 in therapeutics areas ranging from ulcerative colitis to cancer.

Outstanding Questions

- Will kynurenic acid or 5-hydroxyindoleacetic acid (or some other endogenously produced ligand) be defined as the true ligand partner for GPR35?
- Will it be possible to identify high affinity cross-species antagonists of GPR35 to assist with target validation in rodent models of disease?
- What will additional either x-ray crystallography and/or cryo-EM tell us about the details of ligand binding to GPR35 and will this promote effective structure-based drug design of novel antagonist and agonist ligands?
- Will efforts to develop GPR35 agonists for the treatment of lower gut inflammation be successful in a clinical setting?
- Will activators of GPR35 gain a place in the wider pharmacopeia currently being directed towards non-alcoholic fatty liver diseases, including NASH?
- Will potential effects of GPR35 ligands on blood pressure and other aspects of cardiovascular biology limit systematic use of GPR35 directed ligands?

Acknowledgements and Funding

I thank Li-Chiung Lin and Tezz Quon for assistance with Figure production.

This work is supported by grants from UKRI, Medical Research Council (grant number MR/X008827/1) and Biotechnology and Biosciences Research Council (grant number BB/P000649/1) to GM.

Declaration of interests

No interests are declared.

Resources and References

Resources

(I) IUPHAR/BPS Guide to Pharmacology (https://www.guidetopharmacology.org/).

(II) Information on GlaxoSmithKline/Sosei-Heptares collaboration (https://www.prnewswire.com/news-releases/sosei-heptares-and-gsk-enter-global-collaboration-and-license-agreement-targeting-immune-disorders-of-the-digestive-system-301196419.html)

References

1. Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, and Ling L. (2006) Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem. 281(31):22021-22028
2. Alexander SP, Christopoulos A, Davenport AP, Kelly E, Mathie A, Peters JA, Veale EL et al. (2021) THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: G protein-coupled receptors. Br J Pharmacol. 176 Suppl 1:S27-S156
3. Quon T, Lin LC, Ganguly A, Tobin AB, and Milligan G. (2020) Therapeutic Opportunities and Challenges in Targeting the Orphan G Protein-Coupled Receptor GPR35. ACS Pharmacol Transl Sci. 3(5):801-812
4. Jenkins L, Harries N, Lappin JE, MacKenzie AE, Neetoo-Isseljee Z, Southern C, McIver EG, Nicklin SA, Taylor DL, and Milligan G. (2012) Antagonists of GPR35 display high species ortholog selectivity and varying modes of action. J Pharmacol Exp Ther. 343(3):683-695
5. Mackenzie AE, Quon T, Lin LC, Hauser AS, Jenkins L, Inoue A, Tobin AB, Gloriam DE, Hudson BD, and Milligan G. (2019) Receptor selectivity between the G proteins $G\alpha_{12}$ and $G\alpha_{13}$ is defined by a single leucine-to-isoleucine variation. FASEB J 33(4):5005-5017
6. Duan J, Liu Q, Yuan Q, Ji Y, Zhu S, Tan Y, He X, Xu Y, Shi J, Cheng X, Jiang H, Xu H E, Jiang Y. (2022) Insights into divalent cation regulation and G_{13}-coupling of orphan receptor GPR35. Cell Discov. 8(1):135

7. MacKenzie AE, Caltabiano G, Kent TC, Jenkins L, McCallum JE, Hudson BD, Nicklin SA, Fawcett L, Markwick R, Charlton SJ, and Milligan G. (2014) The antiallergic mast cell stabilizers lodoxamide and bufrolin as the first high and equipotent agonists of human and rat GPR35. Mol Pharmacol. 85(1):91-104
8. Zhao P, Lane TR, Gao HG, Hurst DP, Kotsikorou E, Le L, Brailoiu E, Reggio PH, and Abood ME. (2014) Crucial positively charged residues for ligand activation of the GPR35 receptor. J Biol Chem. 289(6):3625-3638
9. Divorty N, Jenkins L, Ganguly A, Butcher AJ, Hudson BD, Schulz S, Tobin AB, Nicklin SA, and Milligan G. (2022) Agonist-induced phosphorylation of orthologues of the orphan receptor GPR35 functions as an activation sensor. J Biol Chem. 298(3):101655
10. Barth MC, Ahluwalia N, Anderson TJ, Hardy GJ, Sinha S, Alvarez-Cardona JA, Pruitt IE, Rhee EP, Colvin RA, Gerszten RE. (2009) Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. J Biol Chem. 284(29):19189-19195
11. Tsukahara T, Hamouda N, Utsumi D, Matsumoto K, Amagase K, Kato S. (2017) G protein-coupled receptor 35 contributes to mucosal repair in mice via migration of colonic epithelial cells. Pharmacol Res. 123:27-39
12. Wyant GA, Yu W, Doulamis IP, Nomoto RS, Saeed MY, Duignan T, McCully JD, Kaelin WG Jr. (2022) Mitochondrial remodeling and ischemic protection by G protein-coupled receptor 35 agonists. Science 377(6606):621-629
13. De Giovanni M, Tam H, Valet C, Xu Y, Looney MR, Cyster JG. (2022) GPR35 promotes neutrophil recruitment in response to serotonin metabolite 5-HIAA. Cell. 185(5):815-830.e19
14. Jenkins L, Alvarez-Curto E, Campbell K, de Munnik S, Canals M, Schlyer S, and Milligan G. (2011) Agonist activation of the G protein-coupled receptor GPR35 involves transmembrane domain III and is transduced via $G\alpha_{13}$ and β-arrestin-2. Br J Pharmacol. 2011 Feb;162(3):733-748
15. Jenkins L, Brea J, Smith NJ, Hudson BD, Reilly G, Bryant NJ, Castro M, Loza MI, Milligan G. (2010) Identification of novel species-selective agonists of the G-protein-coupled receptor GPR35 that promote recruitment of β-arrestin-2 and activate Gα13. Biochem J. 2010 Dec 15;432(3):451-459
16. Schihada H, Klompstra TM, Humphrys LJ, Cervenka I, Dadvar S, Kolb P, Ruas JL, Schulte G.J (2022) Isoforms of GPR35 have distinct extracellular N-termini that

allosterically modify receptor-transducer coupling and mediate intracellular pathway bias. J, Biol Chem. 2022 Sep;298(9):102328.

17. Hu H, Deng H, Fang Y. (2012) Label-free phenotypic profiling identified D-luciferin as a GPR35 agonist. PLoS One. 2012;7(4):e34934

18. Mackenzie AE, Milligan G. (2017) The emerging pharmacology and function of GPR35 in the nervous system. Neuropharmacology113(Pt B):661-671

19. Pires AS, Sundaram G, Heng B, Krishnamurthy S, Brew BJ, Guillemin GJ (2022) Recent advances in clinical trials targeting the kynurenine pathway. Pharmacol Ther. 236:108055

20. Joisten N, Ruas JL, Braidy N, Guillemin GJ, Zimmer P. (2021) The kynurenine pathway in chronic diseases: a compensatory mechanism or a driving force? Trends Mol Med. 27(10):946-954

21. Roth W, Zadeh K, Vekariya R, Ge Y, Mohamadzadeh M. (2021) Tryptophan Metabolism and Gut-Brain Homeostasis. Int J Mol Sci. 22(6):297

22. Kapolka NJ, Taghon GJ, Rowe JB, Morgan WM, Enten JF, Lambert NA, Isom DG. (2020) DCyFIR: a high-throughput CRISPR platform for multiplexed G protein-coupled receptor profiling and ligand discovery. Proc Natl Acad Sci U S A. 117(23):13117-13126

23. Resta F, Masi A, Sili M, Laurino A, Moroni F, Mannaioni G. (2016) Kynurenic acid and zaprinast induce analgesia by modulating HCN channels through GPR35 activation. Neuropharmacology. 108:136-143.

24. Agudelo LZ, Ferreira DMS, Cervenka I, Bryzgalova G, Dadvar S, Jannig PR, Pettersson-Klein AT, Lakshmikanth T, Sustarsic EG, Porsmyr-Palmertz M, Correia JC, Izadi M, Martínez-Redondo V, Ueland PM, Midttun Ø, Gerhart-Hines Z, Brodin P, Pereira T, Berggren PO, Ruas JL. (2018) Kynurenic Acid and Gpr35 Regulate Adipose Tissue Energy Homeostasis and Inflammation. Cell Metab. 27(2):378-392.e5

25. Jung TW, Park J, Sun JL, Ahn SH, Abd El-Aty AM, Hacimuftuoglu A, Kim HC, Shim JH, Shin S, Jeong JH. (2020) Administration of kynurenic acid reduces hyperlipidemia-induced inflammation and insulin resistance in skeletal muscle and adipocytes. Mol Cell Endocrinol. 518:11092

26. Wang Y, Liu Z, Shen P, Zhao C, Liu B, Shu C, Hu X, Fu Y. (2022) Kynurenic acid ameliorates lipopolysaccharide-induced endometritis by regulating the GRP35/NF-κB signaling pathway. Toxicol Appl Pharmacol. 438:115907

27. Shi T, Shi Y, Gao H, Ma Y, Wang Q, Shen S, Shao X, Gong W, Chen X, Qin J, Wu J, Jiang Q, Xue B (2022) Exercised accelerated the production of muscle-derived kynurenic acid in skeletal muscle and alleviated the postmenopausal osteoporosis through the Gpr35/NFκB p65 pathway. J Orthop Translat. 35:1-12
28. Nesci S. (2022) GPR35, ally of the anti-ischemic ATPIF1-ATP synthase interaction. Trends Pharmacol Sci. 43(11):891-893
29. Sun T, Xie R, He H, Xie Q, Zhao X, Kang G, Cheng C, Yin W, Cong J, Li J, Wang X. (2022) Kynurenic acid ameliorates NLRP3 inflammasome activation by blocking calcium mobilization *via* GPR35. Front Immunol. 2022 Oct 13;13:1019365
30. Xian H, Watari K, Sanchez-Lopez E, Offenberger J, Onyuru J, Sampath H, Ying W, Hoffman HM, Shadel GS, Karin M. (2022) Oxidized DNA fragments exit mitochondria via mPTP- and VDAC-dependent channels to activate NLRP3 inflammasome and interferon signaling. Immunity. 55(8):1370-1385.e8
31. Xian H, Karin M. (2023) Oxidized mitochondrial DNA: a protective signal gone awry. Trends Immunol. 2023 Feb 2:S1471-4906(23)00017-0
32. Wei et al., (2022) G protein-coupled receptor 35 attenuates non-alcoholic steatohepatitis by reprogramming cholesterol homeostasis in hepatocytes. Acta Pharmaceutica Sinica B (in press)
33. Lin LC, Quon T, Engberg S, Mackenzie AE, Tobin AB, Milligan G. (2021) G Protein-Coupled Receptor GPR35 Suppresses Lipid Accumulation in Hepatocytes. ACS Pharmacol Transl Sci. 4(6):1835-1848
34. Baumgartner R, Casagrande FB, Mikkelsen RB, Berg M, Polyzos KA, Forteza MJ, Arora A, Schwartz TW, Hjorth SA, Ketelhuth DFJ. (2021) Disruption of GPR35 Signaling in Bone Marrow-Derived Cells Does Not Influence Vascular Inflammation and Atherosclerosis in Hyperlipidemic Mice. Metabolites. 11(7):411
35. Maravillas-Montero JL, Burkhardt AM, Hevezi PA, Carnevale CD, Smit MJ, Zlotnik A. (2015) Cutting edge: GPR35/CXCR8 is the receptor of the mucosal chemokine CXCL17. J. Immunol. 194(1):29-33
36. Park SJ, Lee SJ, Nam SY, Im DS. (2018) GPR35 mediates lodoxamide-induced migration inhibitory response but not CXCL17-induced migration stimulatory response in THP-1 cells; is GPR35 a receptor for CXCL17? Br J Pharmacol. 2018 Jan;175(1):154-161

37. Binti Mohd Amir NAS, Mackenzie AE, Jenkins L, Boustani K, Hillier MC, Tsuchiya T, Milligan G, Pease JE. (2018) Evidence for the Existence of a CXCL17 Receptor Distinct from GPR35. J Immunol. 201(2):714-724
38. Guo YJ, Zhou YJ, Yang XL, Shao ZM, Ou ZL. (2017) The role and clinical significance of the CXCL17-CXCR8 (GPR35) axis in breast cancer. Biochem Biophys Res Commun. 493(3):1159-1167
39. Yao H, Lv Y, Bai X, Yu Z, Liu X. (2020) Prognostic value of CXCL17 and CXCR8 expression in patients with colon cancer. Oncol Lett. 20(3):2711-2720
40. Hao J, Gao X, Wang YP, Liu Q, Zhu H, Zhao SJ, Qin QH, Meng J, Li LL, Lin SC, Song Z, Li H. (2022) Expression and clinical significance of CXCL17 and GPR35 in endometrial carcinoma. Anticancer Drugs. 33(5):467-477
41. Oka S, Ota R, Shima M, Yamashita A, Sugiura T. (2010) GPR35 is a novel lysophosphatidic acid receptor. Biochem Biophys Res Commun. 395(2):232-237
42. Kaya B, Doñas C, Wuggenig P, Diaz OE, Morales RA, Melhem H; Swiss IBD. Cohort Investigators, Hernández PP, Kaymak T, Das S, Hruz P, Franc Y, Geier F, Ayata CK, Villablanca EJ, Niess JH. (2020)Lysophosphatidic Acid-Mediated GPCR Signaling in CX3CR1^{+} Macrophages Regulates Intestinal Homeostasis. Cell Rep. 32(5):10797
43. Yang Y, Lu JY, Wu X, Summer S, Whoriskey J, Saris C, Reagan JD. (2010) G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. Pharmacology. 86(1):1-5
44. Neubig RR. (2010 Mind your salts: when the inactive constituent isn't. Mol Pharmacol. 78(4):558-559
45. Deng H, Hu H, He M, Hu J, Niu W, Ferrie AM, Fang Y. (2011) Discovery of 2-(4-methylfuran-2(5H)-ylidene)malononitrile and thieno[3,2-b]thiophene-2-carboxylic acid derivatives as G protein-coupled receptor 35 (GPR35) agonists. J Med Chem. 54(20):7385-7389
46. Funke M, Thimm D, Schiedel AC, Müller CE. (2013) 8-Benzamidochromen-4-one-2-carboxylic acids: potent and selective agonists for the orphan G protein-coupled receptor GPR35. J Med Chem. 56(12):5182-5197
47. Wei L, Wang J, Zhang X, Wang P, Zhao Y, Li J, Hou T, Qu L, Shi L, Liang X, Fang Y. (2017) Discovery of 2H-Chromen-2-one Derivatives as G Protein-Coupled Receptor-35 Agonists. J Med Chem. 60(1):362-372

48. Wei L, Hou T, Li J, Zhang X, Zhou H, Wang Z, Cheng J, Xiang K, Wang J, Zhao Y, Liang X. (2021) Structure-Activity Relationship Studies of Coumarin-like Diacid Derivatives as Human G Protein-Coupled Receptor-35 (hGPR35) Agonists and a Consequent New Design Principle. J Med Chem. 64(5):2634-2647
49. Mackenzie AE (2015) An investigation of the molecular pharmacology of G protein-coupled receptor GPR35. PhD thesis, University of Glasgow

50. Zhao P, Sharir H, Kapur A, Cowan A, Geller EB, Adler MW, Seltzman HH, Reggio PH, Heynen-Genel S, Sauer M, Chung TD, Bai Y, Chen W, Caron MG, Barak LS, Abood ME. (2010) Targeting of the orphan receptor GPR35 by pamoic acid: a potent activator of extracellular signal-regulated kinase and β-arrestin2 with antinociceptive activity. Mol Pharmacol. 78(4):560-568
51. Heynen-Genel S, Dahl R, Shi S, Sauer M, Hariharan S, Sergienko E, Dad S, Chung TDY, Stonich D, Su Y, Caron M, Zhao P, Abood ME, Barak LS. (2010) Feb 28 [updated 2010 Oct 4]. Selective GPR35 Antagonists - Probes 1 & 2. In: Probe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2010–.PMID: 21433393
52. McCallum JE, Mackenzie AE, Divorty N, Clarke C, Delles C, Milligan G, Nicklin SA. (2015) G-Protein-Coupled Receptor 35 Mediates Human Saphenous Vein Vascular Smooth Muscle Cell Migration and Endothelial Cell Proliferation. J Vasc Res. 52(6):383-395
53. Boleij A, Fathi P, Dalton W, Park B, Wu X, Huso D, Allen J, Besharati S, Anders RA, Housseau F, Mackenzie AE, Jenkins L, Milligan G, Wu S, Sears CL. (2021) G-protein coupled receptor 35 (GPR35) regulates the colonic epithelial cell response to enterotoxigenic Bacteroides fragilis. Commun Biol. 4(1):585
54. Jenkins L, Harries N, Lappin JE, MacKenzie AE, Neetoo-Isseljee Z, Southern C, McIver EG, Nicklin SA, Taylor DL, Milligan G. (2012) Antagonists of GPR35 display high species ortholog selectivity and varying modes of action. J Pharmacol Exp Ther. 343(3):683-695
55. Li X, Shen J, Ran Z. (2017) Crosstalk between the gut and the liver via susceptibility loci: Novel advances in inflammatory bowel disease and autoimmune liver disease. Clin Immunol. 175:115-123
56. Kaya B, Melhem H, Niess JH. (2021) GPR35 in Intestinal Diseases: From Risk Gene to Function. Front Immunol. 12:717392

57. Freudenberg JM, Dunham I, Sanseau P, Rajpal DK. (2018) Uncovering new disease indications for G-protein coupled receptors and their endogenous ligands. BMC Bioinformatics. 19(1):345.
58. Li Y, Liu N, Ge Y, Yang Y, Ren F, Wu Z. (2022) Tryptophan and the innate intestinal immunity: Crosstalk between metabolites, host innate immune cells, and microbiota. Eur J Immunol. 52(6):856-868
59. Farooq SM, Hou Y, Li H, O'Meara M, Wang Y, Li C, Wang JM. (2018) Disruption of GPR35 Exacerbates Dextran Sulfate Sodium-Induced Colitis in Mice. Dig Dis Sci. 63(11):2910-2922
60. Yansen Z, Lingang Z, Dali L, Mingyao L. (2021) Inflammatory bowel disease susceptible gene GPR35 promotes bowel inflammation in mice. Yi Chuan. 43(2):169-181
61. Schneditz G, Elias JE, Pagano E, Zaeem Cader M, Saveljeva S, Long K, Mukhopadhyay S, Arasteh M, Lawley TD, Dougan G, Bassett A, Karlsen TH, Kaser A, Kaneider NC. (2019) GPR35 promotes glycolysis, proliferation, and oncogenic signaling by engaging with the sodium potassium pump. Sci Signal. 12(562):eaau9048
62. Melhem H, Kaya B, Kaymak T, Wuggenig P, Flint E, Roux J, Oost KC, Cavelti-Weder C, Balmer ML, Walser JC, Morales RA, Riedel CU, Liberali P, Villablanca EJ, Niess JH. (2022) Epithelial GPR35 protects from Citrobacter rodentium infection by preserving goblet cells and mucosal barrier integrity. Mucosal Immunol. 15(3):443-458
63. Nam SY, Park SJ, Im DS. (2019) Protective effect of lodoxamide on hepatic steatosis through GPR35. Cell Signal. 53:190-200
64. Ali H, AbdelMageed M, Olsson L, Israelsson A, Lindmark G, Hammarström ML, Hammarström S, Sitohy B. (2019) Utility of G protein-coupled receptor 35 expression for predicting outcome in colon cancer. Tumour Biol. 41(6):1010428319858885
65. Mackiewicz T, Jacenik D, Talar M, Fichna J. (2022) The GPR35 expression pattern is associated with overall survival in male patients with colorectal cancer. Pharmacol Rep. 74(4):709-717
66. Pagano E, Elias JE, Schneditz G, Saveljeva S, Holland LM, Borrelli F, Karlsen TH, Kaser A, Kaneider NC. (2022) Activation of the GPR35 pathway drives angiogenesis in the tumour microenvironment. Gut. 71(3):509-520

67. Shu C, Wang C, Chen S, Huang X, Cui J, Li W, Xu B. (2022) ERR activated GPR35 promotes immune infiltration level of macrophages in gastric cancer tissues. Cell Death Discov. 8(1):444
68. Wang W, Han T, Tong W, Zhao J, Qiu X. (2018) Overexpression of GPR35 confers drug resistance in NSCLC cells by β-arrestin/Akt signaling. Onco Targets Ther. 11:6249-6257
69. Hashemi SF, Khorramdelazad H. (2022) The cryptic role of CXCL17/CXCR8 axis in the pathogenesis of cancers: a review of the latest evidence. J Cell Commun Signal. doi: 10.1007/s12079-022-00699
70. Milligan G. (2011) Orthologue selectivity and ligand bias: translating the pharmacology of GPR35. Trends Pharmacol Sci. 32(5):317-325
71. Deng H, Hu J, Hu H, He M, Fang Y. (2012) Thieno[3,2-b]thiophene-2-carboxylic acid derivatives as GPR35 agonists. Bioorg Med Chem Lett. 22(12):4148-52
72. Neetoo-Isseljee Z, MacKenzie AE, Southern C, Jerman J, McIver EG, Harries N, Taylor DL, Milligan G. (2013) High-throughput identification and characterization of novel, species-selective GPR35 agonists. J Pharmacol Exp Ther. 344(3):568-578
73. Marti-Solano M, Crilly SE, Malinverni D, Munk C, Harris M, Pearce A, Quon T, Mackenzie AE, Wang X, Peng J, Tobin AB, Ladds G, Milligan G., Gloriam DE, Puthenveedu MA, Babu MM. (2020) Combinatorial expression of GPCR isoforms affects signalling and drug responses. Nature. 587(7835):650-656
74.

Glossary

Arrestin: A member of a small group of cytosolic proteins that can interact with agonist-occupied GPCRs to 'arrest' and prevent interactions of the receptor with G proteins.

Dextran sulphate sodium: (DSS) a sulphated polysaccharide widely used to induce a disease model of colitis in mice.

CXCL17: A molecule termed CXCL17, although lacking a chemokine fold, has been described as an activator of GPR35. Although this is incorrect, it is also sometimes described as an 'orphan' ligand because its cognate receptor has yet to be defined.

'CXCR8': a term sometimes used to describe GPR35 as a member of the chemokine receptor family. This is based on a single publication which has not been replicated.

GPR35: A member of the GPCR superfamily which has been suggested to be the cognate receptor for each of kynurenic acid, 5-hydroxyindoleacetic acid and the ligand CXCL17. Still officially designated as an 'orphan'.

GPCR: G protein-coupled receptor. A member of a superfamily of trans-plasma membrane proteins with seven transmembrane domain architecture and which mediates a range of its actions by facilitated interaction with, and activation of, members of the family of heterotrimeric G proteins.

Negative allosteric modulator: A compound that blocks the function of a receptor protein in a non-competitive manner and does so by binding to an allosteric site, spatially separate from the orthosteric binding cavity.

Non-alcoholic steatohepatitis (NASH): A condition within the spectrum of diseases related to accumulation of triglycerides and lipids within the liver. NASH is associated with liver inflammation and fibrosis.

Orphan receptor: a receptor protein, for example a GPCR, for which the cognate activating ligand(s) remains unidentified or is (are) not fully accepted.

On-target: a biological effect of a ligand, drug or medicine which is produced unambiguously by interaction with a specified protein or other receptor species.

Off-target: a biological effect produced by a ligand, drug or medicine but by means that do not reflect regulation of the specifically defined target receptor or other protein.

Pertussis toxin-sensitive: Pertussis toxin is produced by the bacterium *Bordetalla pertussis*. By causing ADP-ribosylation of a cysteine reside that is present in all the widely expressed 'G_i'-members of the family of heterotrimeric G proteins it prevents their interaction with

GPCRs. Hence a signalling pathway that is 'Pertussis toxin-sensitive' is concluded to be transduced by one or other members of the 'G_i' G protein group.

Single nucleotide polymorphism (SNP): A single alteration in the nucleotide sequence of a gene that alters directly or indirectly the function of the anticipated encoded protein.

Text Boxes

Box 1. Orphan G protein-coupled receptors and their potential endogenous activators

Despite extensive efforts over many years, clear understanding of the identity of ligands that are the endogenous activators of a significant number of GPCRs remains either uncertain or completely unknown. Such GPCRs are designated as 'orphans'. A key resource for many aspects of the function and ligand regulation of all GPCRs, including orphans, is provided by the IUPHAR/BPS Guide to Pharmacology [2] (see **Resources I**). In many cases, including GPR35, GPCRs remain orphans despite a range of publications supporting the ability of specific ligands to activate the receptor. In the case of GPR35 a substantial number of reports have shown that kynurenic acid can certainly activate the receptor and this is now well established. However, particularly for the human orthologue the potency of kynurenic acid is low, and this has resulted in discussions as to whether the concentration of kynurenic acid may be too low in many settings to occupy the receptor to a significant degree. By contrast, the suggestion that ligand CXCL17 is the key endogenous agonist of GPR35 was based on a single publication [35], that other reports subsequently refuted [36-37]. Most recently, a single report to date [13] has provided evidence that 5-HIAA is a potent activator of GPR35, as least in the context of neutrophil function. Across the GPCR field there are many reports of the pairing of new and distinct ligands with orphan GPCRs. In many cases these have been validated by further work by the research community, but in a substantial number of other cases reproduction of initial findings have not been forthcoming. The IUPHAR/BPS Guide to Pharmacology (noted above) plays an important role in recording these developments.

Box 2. Pharmacology differences between GPR35 orthologues

One of the greatest challenges in understanding the function(s) of GPR35 and the opportunities to target the receptor therapeutically has been, and remains, the complex differences in ligand pharmacology between human and rodent orthologues of the receptor. Moreover, differences in GPR35 pharmacology between rat and mouse are frequently as marked as between either of these and human. Challenges posed by this were highlighted more than 10 years ago [70] but still remain. Unless there are unappreciated aspects of the mode of action and ligand binding to GPR35 in native systems that are simply not replicated in simple heterologous cell lines, then none of the very limited set of currently reported

'GPR35 antagonists' have useful affinity at either rat or mouse GPR35. As such, and as highlighted in the main text, reports in which CID2745687 or ML145 have been used in mouse or rat tissues or in cell lines derived from these species cannot reflect blockade of GPR35 and hence must be 'off-target'. Even for studies on the human receptor more 'drug-like' GPR35 selective antagonists are greatly needed to allow better therapeutic validation of GPR35 and, once more as discussed in the main text, may provide stating points for therapeutic intervention. Whilst agonist ligands display a gamut of characteristics across species, careful reading of the literature, allows selection of molecules with at least moderate potency at each of human, mouse and rat. There are potentially compounds that display levels of 'bias' between signal pathways [71-72], but this topic has been assessed in a less rigorous and systematic manner than for many other GPCRs. This may reflect the challenges for many years in establishing the G protein-coupling pattern of GPR35 and suitable assays to measure these.

Box 3. Isoform variation in human GPR35

Unlike both rat and mouse that express a single isoform of GPR35, human differentially expresses two isoforms across tissues [3,73]. The shorter GPR35a isoform corresponds to the single rat and mouse isoform in amino acid number. This is the reason that Lin et al., [33] selected to replace mouse GPR35 with the shorter human GPR35a isoform when generating the transgenic knock-in mouse line described in the main text. Compared to GPR35a, the long GPR35b isoform has an additional N-terminal 31 amino acid extension [3, 71]. The functional significance of this additional isoform is uncertain. No distinct pharmacology has been reported for the two forms after expression in heterologous cells. However, Schihada et al., [16] have reported differences in the ability of the long and short isoforms to mediate interactions with G proteins versus β-arrestin-2. This was not observed, however, by Marti-Solano et al., [73] who noted rather that the GPR35b isoform was less effective in promoting both G protein activation and β-arrestin-2 recruitment in response to various agonists than the GPR35a isoform. As such, although rather little focus has been directed to the GPR35b isoform there are clearly contradictions in reports of their functions that remain to be clarified.

Figure Legends

Figure 1. Therapeutic opportunities in targeting GPR35

Recent work has highlighted a number of disease areas in which activation or blockade of GPR35 may prove to be therapeutically useful.

Figure 1A. GPR35 activation reduces liver steatosis

GPR35 can be activated by ligands such as lodoxamide or kynurenic acid, and ultimately inhibits liver steatosis. Potential mechanisms involved in this inhibitory effect of GPR35 include SREBP1-induced lipogenesis or CREB-regulated cholesterol homeostasis. Lodoxamide-activated GPR35 may act through a p38 MAPK/JNK signaling pathway to reduce SREBP1 protein expression, and hence block the lipid accumulation in human hepatocellular carcinoma cells [63]. Kynurenic acid also activates GPR35 in mouse primary hepatocytes. Although which G protein is regulated in this system by GPR35 activation has not been identified (and therefore is designated G_x), this may up-regulate ERK-CREB signaling pathways and further increase the expression of STARD4, which maintains cholesterol homeostasis [33].

CRE = cAMP response element, CREB = cAMP response element-binding protein KYNA = kynurenic acid, SRE = sterol regulatory element, SREBP1 = sterol regulatory element-binding protein 1, STARD4 = steroidogenic acute regulatory protein 4.

Figure 1B. GPR35 as a prognostic and potential therapeutic in intestinal cancers

Links between GPR35 levels and outcomes in intestinal and other cancers have been established with prognosis and survival inversely correlated with expression levels [38-40, 64-65]. Higher levels of GPR35 may promote hyperplasia with subsequent angiogenesis [61, 66] and metastasis. GPR35 antagonists/inverse agonists may be worth assessing as a novel therapy.

Figure 1C. Roles of lower gut expressed GPR35 in irritable bowel disease, ulcerative colitis and *Citrobacter rodentium* infection

GPR35 is present in the lower gut, with high expression in both immune cells and epithelial [3]. A T108M variation is strongly associated with susceptibility to inflammatory bowel diseases including ulecerative colitis and Crohn's disease. In the majority of studies in mice global elimination of GPR35 worsens the severity of Dextran Sodium Sulphate (DSS)-induced colitis [e.g. 59] resulting in epithelial damage, alterations in colon length, diarrhoea and bleeding. This may relate to contributions of macrophage-expressed GPR35 as targeted knock-out produces similar outcomes. Studies are underway to assess the potential effectiveness of GPR35 agonists for treatment of colitis in clinical settings (see Resources II). In the case of epithelial specific knock-out of GPR35 this is associated with reduced goblet cell number and greater susceptibility to *Citrobacter rodentium* infection [62]