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Pharmacological Characterization of GPR55, A Putative Cannabinoid Receptor

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Abstract

GPR55 has recently attracted much attention as another member of the cannabinoid family, potentially explaining physiological effects that are non-CB1/CB2 mediated. However, the data gathered so far are conflicting with respect to its pharmacology. We review the primary literature to date on GPR55, describing its discovery, structure, pharmacology and potential physiological functions. The CB1 receptor antagonist/inverse agonist AM251 has been shown to be a GPR55 agonist in all reports in which it was evaluated, as has the lysophospholipid, lysophosphatidylinositol (LPI). Whether GPR55 responds to the endocannabinoid ligands anandamide and 2-arachidonylglycerol and the phytocannabinoids, delta-9-tetrahydrocannabidiol and cannabidiol, is cell-type and tissue-dependent. GPR55 has been shown to utilize G_q, G_{12}, or G_{13} for signal transduction; RhoA and phospholipase C are activated. Experiments with mice in which GPR55 has been inactivated reveal a role for this receptor in neuropathic and inflammatory pain as well as in bone physiology. Thus delineating the pharmacology of this receptor and the discovery of selective agonists and antagonists merits further study and could lead to new therapeutics.

Keywords

G protein coupled receptor; cannabinoid; GPR55; lysophosphophatidylinositol

1. Introduction

Marijuana remains the most widely used illegal drug (Murray et al., 2007), and its validated targets include plasma membrane cannabinoid receptors, many of which are found in the central nervous system. The diverse physiological effects produced by marijuana and cannabinoid ligands suggest the possibility that several receptors are responsible for their activity. Yet to date, only two receptor subtypes, CB1 and CB2, have convincingly been confirmed as cannabinoid targets. However, in support of the notion that other cannabinoid receptors remain to be identified, the complex pharmacological properties of exogenous cannabinoids and endocannabinoids are not fully explained by CB1 and CB2 signal transduction. Recently, the orphan G protein coupled receptor, GPR55, was presented as one of the missing candidate cannabinoid receptor subtypes (Johns et al., 2007; Ryberg et al., 2007), but the validity of this

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assignment is under debate. In particular, Oka et al (2007) reported that while cannabinoids did not appear to activate GPR55, lysophosphatidyinositol (LPI) derivatives resulted in robust stimulation of the receptor. Thus, the chemical space of GPR55 agonists remains ill defined. As a consequence of the identification, whether correct or incorrect, that GPR55 is a target for cannabinoid binding, GPR55 now shoulders a potentially important but un-defined role in the paradigm of drug addiction. It thus becomes incumbent to identify GPR55-selective ligands in order to substantiate GPR55 pharmacology and to characterize its biology.

GPR55 was initially identified as a candidate cannabinoid receptor in patent applications from GlaxoSmithKline and AstraZeneca (Brown and Wise, 2001; Drmota et al., 2004). The ability of GPR55 to recognize cannabinoids was first described in a yeast expression system in the GlaxoSmithKline patent, where the CB1 antagonists AM251 and SR141716A acted as agonists at micromolar concentrations (Brown and Wise, 2001; Brown and Hiley, 2009) (Please see figure 1 for structures). In contrast, the AstraZeneca group reported that when GPR55 was expressed in HEK293 cells, nanomolar concentrations of many cannabinoid agonists stimulated GTPγS binding (Drmota et al., 2004; Ryberg et al., 2007). Most of the endocannabinoids, including anandamide, 2-arachidonylglycerol (2-AG), virodhamine, noladin ether, oleoylethanolamide and palmitoylethanolamide as well as the several agonists including CP55,950 and Δ^9-THC, stimulated GTPγS binding, which was not antagonized by AM281, but was blocked with 450 nM cannabidiol (CBD) (Drmota et al., 2004; Ryberg et al., 2007). AM251 produced an agonist response in HEK293 cells, similar to that found in the yeast expression system (Ryberg et al., 2007). Lauckner et al (2008) reported that GPR55 was a cannabinoid receptor, based on their data that Δ^9-THC, anandamide and JWH-015, increased intracellular calcium in transfected cells and also in large dorsal root ganglion neurons. In contrast to these results, Oka et al (2007) reported that GPR55 is not a typical cannabinoid receptor as numerous endogenous and synthetic cannabinoids, including many mentioned above, had no effect on GPR55 activity. Instead, their data suggests that the endogenous lipid LPI and its 2-arachidonyl analogs are agonists at GPR55 as a result of their abilities to phosphorylate extracellular regulated kinase and induce calcium signaling (Oka et al., 2007; Oka et al., 2009c). Thus GPR55 may recognize cannabinoids, but has a unique response profile differing from CB1 and CB2.

Several recent reviews have highlighted the enigmatic pharmacology of GPR55 (Brown and Hiley, 2009; De Petrocellis and Di Marzo, 2009; Godlewski et al., 2009; Kreitzer and Stella, 2009; Ross, 2009). Here we review the primary literature and include papers and abstracts not previously cited.

2. Discovery of GPR55

Human GPR55 (hGPR55) was originally isolated in 1999 as an orphan GPCR with high levels of expression in human striatum (Sawzdargo et al., 1999)(Genbank accession # NM_005683.3). Initial characterization of human GPR55 identified it as a potential member of the purinergic or chemokine receptor family based on amino acid homology; it shares 29% identity with the P2Y5 purinergic receptor (NM_005767.4), 30% identity with GPR23 (NM_005296.2) , 27% identity with GPR35 (NM_005301.2) and 23% identity with the CCR4 chemokine receptor (NM_005508.4) (Sawzdargo et al., 1999). Relevant to later discussion, GPR23 has been classified as the LPAR4 receptor, although LPA activates at high micromolar concentrations (Yin et al., 2009) and P2Y5 also responds to LPA and has been classified as LPAR6 (Pasternack et al., 2008). In contrast, hGPR55 exhibits low amino acid identity to CB1 (13.5%) or CB2 (14.4%) receptors. hGPR55 is clearly a member of the Class A (Rhodopsin) family of GPCRs based on sequence similarities with rhodopsin (see below). Drmota et al (2004) have also isolated a variant of hGPR55, hGPR55a, which contains three amino acid substitutions (F3.33(102)L, G5.52(195)S, C7.47(281)R (Baker et al., 2006). Sequences for rat

and mouse GPR55 have also been reported (Ryberg et al., 2007) as has a putative chimpanzee sequence (Baker et al., 2006). Interestingly, orthologs for GPR55 have primarily been reported in mammals with a somewhat similar sequence in marsupials (opossum, XM_001373864) (Baker et al., 2006). A subsequent study found orthologs of GPR55 in zebrafish and puffer fish (McPartland et al., 2007), suggesting an early chordate phylogenic origin of GPR55.

hGPR55 was mapped to human chromosome 2q37, and in the human CNS it is predominantly localized to the caudate, putamen, and striatum (Sawzdargo et al., 1999). In rats, in situ hybridization indicated expression in hippocampus, thalamus and regions of the midbrain (Sawzdargo et al., 1999). Ryberg et al (2007) reported mRNA expression levels in the mouse. They found the level of expression to be highest in the adrenals > frontal cortex > striatum which was similar in expression to jejunem and ileum > hypothalamus, brainstem > hippocampus, cerebellum, spleen > spinal cord >> lung, liver, uterus, bladder, stomach, kidney > esophagus > adipose (ibid). Thus GPR55 mRNA is found in a number of tissues outside the CNS, where it is broadly expressed, albeit at levels significantly lower than those of CB1, perhaps the most highly expressed GPCR in the CNS (Howlett et al., 2002; Ryberg et al., 2007).

3. Structure of GPR55

hGPR55 (Sawzdargo et al., 1999) is a 319 amino acid protein which belongs to the Class A GPCRs. It shares many similarities with rhodopsin. Figure 2 shows a model of hGPR55. Among the highly conserved residues typically used in sequence alignments with rhodopsin, GPR55 has the conserved patterns in TMH1, 2, 4 and 5 (i.e., N1.50, D2.50, W4.50 and P5.50). In this, hGPR55 differs from CB1 and CB2, as these latter receptors lack the highly conserved Pro in TMH5. In the conserved TMH3 E/DRY motif, hGPR55 has the conservative substitution DRF. In TMH6, the highly conserved CWXP motif found in rhodopsin and CB1/CB2 is conservatively substituted with SFLP in hGPR55. The greatest divergence from the rhodopsin sequence (and from CB1/CB2) appears in TMH7 of hGPR55, here the highly conserved NPXXY motif is replaced with DVFCY (GPR55). Like rhodopsin, hGPR55 has an F in the intracellular extension of TMH7 (called Hx8) at position 7.60. In rhodopsin, there is an aromatic interaction between Y7.53(306) and F7.60(313) which has been proposed to provide structural constraints that rearrange in response to photoisomerization (Fritze et al., 2003). In hGPR55, the analogous relationship between Y7.53 and F7.60 can be established. Interestingly, no such interaction is possible in CB1/CB2 as position 7.60 is a Leu in CB1 and Ile in CB2.

hGPR55 also potentially has another significant similarity to rhodopsin in its EC-2 loop structures (figure 2). In rhodopsin, the EC-2 loop dips down into the binding pocket to form a disulfide bridge between an EC-2 Cys residue and C3.25. It then loops back over itself to make its connection with the top of TMH5. hGPR55 also has a Cys at 3.25 and a Cys residue in the EC-2 loop that could potentially form a disulfide bond. So, it is likely that the EC-2 loop structure of hGPR55 will differ from that of CB1 and CB2.

Pharmacology of GPR55

4.1 Pharmacology in vitro- Transfected Cells

GPR55 has been examined in transfected HEK293 cells with a number of cannabinoid ligands. The results of these studies are quite mixed (please see Table 1 for a summary of compounds reported in multiple studies). Ryberg et al (2007) used a GTPγS functional assay and found that hGPR55 stably transfected in HEK293s cells was activated by nanomolar concentrations of the endocannabinoids 2-arachidonylglycerol (2-AG), virodhamine (O-arachidonoyl ethanolamine), noladin ether (2-arachidonoyl glyceryl ether), oleoylethanolamide and

palmitoylethanolamide (PEA). PEA had originally been suggested to be an endogenous ligand for the CB2 receptor (Facci et al., 1995), but subsequent studies found it had little affinity for CB2 (Griffin et al., 2000;Showalter et al., 1996). PEA is a potent anti-inflammatory, anti-excitotoxic and anti-hyperalgesic compound (Jaggar et al., 1998;Skaper et al., 1996), so finding the receptor(s) involved in its activity is of interest. Ryberg et al (Ryberg et al., 2007) also reported that the phytocannabinoid compound Δ^9-THC as well as the structurally related compounds CP55940, HU210, O-1602 and abnormal-cannabidiol (abn-CBD) stimulated GTPγS binding. In addition, AM251, a pyrazole CB1 antagonist/inverse agonist, but not AM281, a highly related compound, acted as an agonist at GPR55 (Ryberg et al., 2007). CBD antagonized the effects of CP55,940 and anandamide with an IC50 of 440 nM. Cannabinol had no activity at GPR55, nor did WIN 55,212-2 (Ryberg et al., 2007).

Ryberg et al (2007) investigated downstream signaling pathways for hGPR55. The G protein involved was not pertussis toxin sensitive nor did they find evidence of Gq coupling using a FLIPR assay. Instead, they found using peptides and/or antibodies directed against the C-terminus of Gα subunits that Gα13 was responsible for the GTPγS activation produced by 1 μM O-1602, a maximally active concentration of this compound. Co-transfection with Gα13 augmented the signal produced. Consistent with a Gα12/13-mediated response, RhoA was activated, as was rac1 and cdc42, by 1 μM O-1602 or 1 μM anandamide; this effect was blocked by 10 μM cannabidiol.

Subsequent studies have reproduced only some of these data reported by (Ryberg et al., 2007). Johns et al (2007) reported that nanomolar concentrations of abn-CBD and O-1602 but not WIN 55,212-2 stimulated GTPγS binding in HEK293T cells transiently transfected with hGPR55. No other compounds were reported in this study, but the authors generated GPR55 KO animals in which the vasodilator effects of abn-CBD were retained and antagonized by O-1918. An “abn-CBD receptor” had previously been documented in several studies (reviewed in (Godlewski et al., 2009)); thus GPR55 does not appear to be that site.

In contrast, Oka et al (2007) found that numerous cannabinoid compounds (including 2-AG, anandamide, PEA, oleoylethanolamide, virodhamine, CP55940, HU-210, WIN55,212-2, THC, abn-CBD and SR141716A) had no effect in HEK293 cells stably transfected with a tetracycline-inducible human construct. Instead, lysophosphatidylinositol (LPI) induced phosphorylation of ERK1/2 in hGPR55-expressing cells in a concentration-dependent manner with an EC50 of 200 nM. LPI also induced a rapid Ca^{2+} transient in hGPR55-expressing cells with a similar EC50; this was attenuated by siRNA treatment. LPI stimulated GTPγS binding in a concentration-dependent manner; this EC50 appears to be in the high micromolar range (Oka et al., 2007). As LPI can be a mixture of fatty acids, a subsequent study by this group identified 2-arachidonoyl-sn-glycero-3-phosphoinositol (2-AGPI) as the most potent lipid activator of hGPR55 (Oka et al., 2009c). Very recently, they reported that LPI rapidly induced p38 MAP kinase with an EC50 value of ~300 nM and also the phosphorylation of activating transcription factor-2 (Oka et al., 2009a; Oka et al., 2009b).

Lauckner et al (2008) reported that 3–5 μM concentrations of Δ^9-THC, the anandamide analog methanandamide, and the CB2 agonist JWH-015, increased intracellular calcium in HEK293 cells transiently transfected with hGPR55 as well as in large dorsal root ganglion (DRG) neurons from mice. HEK293 cells transiently transfected with mouse GPR55 produced a similar profile of responses, consistent with the data obtained from the DRG. Anandamide was a partial agonist, producing a 45 nM rise in intracellular calcium, as compared with a ~100 nM increase produced by THC, methananandamide and JWH-015. 3 μM LPI increased intracellular calcium in DRG neurons to a similar extent as the cannabinoid compounds. Co-addition (co-perfusion) of LPI with the CB1 receptor antagonist/inverse agonist SR141716A reduced the responses (in both hGPR55-HEK293 cells and mouse DRG) without having an

effect on its own. Several other cannabinoids were evaluated and found to have negligible agonist activity in hGPR55-expressing HEK293 cells, including 2-AG, CP55940, PEA, virodhamine, abn-CBD, CBD and WIN55,212-2. Using a combination of inhibitors and dominant negative constructs, the calcium response was found be mediated by both Gq- and G12- mediated pathways and was dependent on an intact actin cytoskeleton. Activation of hGPR55 by 5 μM THC resulted in a 38% inhibition of a (Gq and PIP2-requiring) M-type potassium current; interestingly, 5 μM THC caused a small (11%) inhibition of this current in cells that were not transfected with hGPR55. These results differ from Ryberg et al, both in terms of the ligands that activate hGPR55 as well as the Gq coupling observed; however, both report RhoA-dependent pathways (Lauckner et al., 2008; Ryberg et al., 2007).

Other studies indicate that LPI and the rimonabant-like CB_1 inverse agonist AM251 induce oscillatory Ca^{2+} release through $G_{\alpha 12/13}$ and RhoA in HEK293 cells stably transfected with hGPR55 (Henstridge et al., 2009b). In contrast to the studies cited above (Lauckner et al., 2008; Oka et al., 2007; Oka et al., 2009c), where single Ca^{2+} transients were measured, Henstridge et al (2009b) report a concentration-dependent induction of oscillatory Ca^{2+} transients by LPI which persisted for up to 45 min after agonist removal. The EC50 of LPI was calculated to be 49 nM, and 1 μM LPI increased intracellular Ca^{2+} levels by 929 nM above basal levels. This signaling was shown to be mediated by Gα12/13 through a RhoA-Rho kinase (ROCK)-phospholipase C-dependent release of intracellular Ca^{2+} from the endoplasmic reticulum, which subsequently activated NFAT and led to its nuclear translocation. 1 μM LPI also induced internalization and translocation into intracellular vesicles. While the endocannabinoids anandamide and 2-AG increased intracellular Ca^{2+} levels, this was a GPR55-independent effect; i.e., the response to 3–30 μM of these compounds was the same in untransfected and in hGPR55-expressing cells. However, a concentration-dependent, hGPR55-mediated increase in intracellular Ca^{2+} was observed with the CB1 antagonist/inverse agonist AM251 with an EC50 of 612 nM and a lower maximal Ca^{2+} response (586 nM) as compared with LPI. CP55,940 did not activate hGPR55, but shifted the concentration-response curve for LPI to the right, indicating that CP55,940 acts as a competitive antagonist in this assay.

These reports were all performed in HEK 293 cells, yet each documented a distinct and conflicting chemical space of agonists that recognized GPR55. To attempt to resolve these inconsistencies in classification, Kapur et al (2009) employed an alternative approach for identifying GPR55 ligands using β-arrestin recruitment. β-arrestins are intracellular proteins that bind and desensitize activated GPCRs and in the process form stable receptor/arrestin signaling complexes (Gurevich and Gurevich, 2006; Shenoy and Lefkowitz, 2005). β-arrestin-green fluorescent chimeras can make this process attractive to monitor by forming remarkably sensitive and specific probes of GPCR activation that are independent of downstream G protein mediated signaling (Barak et al., 1997; Marion et al., 2006; McGuinness et al., 2009). Kapur et al (2009) determined hGPR55 responsiveness to a representative panel of cannabinoid ligands and LPI in the presence (and absence) of a β-arrestin2-green fluorescent protein (βarr2-GFP) biosensor in U2OS cells stably transfected with hGPR55E and in HEK293 cells transiently transfected with hGPR55E or hGPR55. The hGPR55E construct used in these studies contains a serine enhanced C-terminus to increase receptor affinity for β-arrestin without changing its response profile to ligands (Kapur et al., 2009; Oakley et al., 1999). Out of numerous cannabinoid compounds tested only two unambiguously activated hGPR55 in addition to the lysophospholipid LPI (Table 1). These compounds, LPI, SR141716A and AM251 had a rank order of potency of LPI> SR141716A> AM251 (1.2, 3.9 and 9.6 μM) and comparable efficacies. SR141716A and AM251 are cannabinoid receptor inverse agonist/antagonists (Kapur et al., 2008; Lan et al., 1999) whereas the one hGPR55 receptor antagonist identified, CP55,940 (K_i ~200 nM), is a cannabinoid receptor agonist. In contrast, AM281, which is structurally related to biarylpyrazole analogs (SR141716A and AM251), failed to

activate hGPR55 at concentrations up to 30 μM (in agreement with Ryberg et al, 2007). LPI did not activate CB1 receptors in a CB1-βarr2-GFP-expressing cell line, whereas CP55,940 produced the expected response (Kapur et al., 2009). In addition to measuring βarr2-GFP trafficking, agonist-induced internalization, ERK phosphorylation and activation of PKCβII-GFP, a G-protein dependent response, were assessed (Kapur et al., 2009). These studies confirmed that the endogenous compound LPI is unequivocally a GPR55 agonist at low micromolar concentrations causing β-arrestin activation, receptor internalization, activation of PKCβII and ERK1/2 phosphorylation.

JWH015 has been shown to increase intracellular Ca^{2+} (Lauckner et al., 2008). However, Kapur et al. (2009) found that JWH015 (CB_2 receptor agonist), SR144528 (CB_2 receptor antagonist), the classical CB_1 agonists (HU210 and THC), the endocannabinoids (anandamide and 2-AG), and cannabidiol had no effect on their own or on LPI-induced βarr2 trafficking in hGPR55 U2OS cells. Kapur et al (2009) also demonstrated that the endocannabinoids (anandamide and 2-AG) and atypical cannabinoids (abn-CBD, O-1602 and O-1918) failed to evoke hGPR55-modulated βarr2-GFP redistribution. These compounds had previously been reported to activate GTPγS binding (Johns et al., 2007; Ryberg et al., 2007). Lauckner et al (2008) reported that SR141716A is a GPR55 antagonist at 2 μM in a calcium signaling assay. In contrast, Kapur et al (2009) showed that 10–30 μM SR141716A produces robust activation and internalization of hGPR55. This discrepancy may be a reflection of the different range of doses and efficacy of the compounds that were utilized. Henstridge et al (2009a) reported that SR14716A was an agonist at GPR55 at 1 μM. That SR141716A (rimonabant) activates GPR55 at μM concentrations may be clinically relevant, as peak plasma levels or rimonabant were typically in the ~500 nM range. Rimonabant had been marketed for the treatment of obesity and off-target effects of this and related compounds may be manifest at GPR55.

The profile of ligands inducing agonist-induced internalization confirmed those for βarr2-GFP trafficking (Kapur et al., 2009). CP55,940 also antagonized agonist-induced internalization. Furthermore, LPI, AM251 and SR141716A were agonists in the G-protein dependent response, activation of PKCβII-GFP. Within 60 seconds of addition of agonists, membrane rearrangements began to occur followed by protrusions and blebbing. Cytoskeletal changes were also observed in the β-arrestin trafficking assay. However, CP55,940 acted as a partial agonist in the PKCβII-GFP response, inducing some recruitment of PKCβII-GFP. There was also some degree of constitutive activity in this assay, whereby HEK293 cells co-transfected with hGPR55 and PKCβII-GFP displayed some membrane fluorescence as compared to cells transfected with PKCβII-GFP alone. Thus CP55,940 acts as a partial agonist/antagonist at hGPR55 in these studies (Kapur et al., 2009).

A similar set of ligands that activate hGPR55 stably transfected in HEK293 cells to that of Kapur et al (2009) was recently reported at the 2009 International Cannabinoid Research Symposium (Balenga et al., 2009; Henstridge et al., 2009a). Henstridge et al (2009a) showed that AM251 and SR141716A stimulate Ca^{2+} release with EC50‘s of ~1 μM and receptor internalization; similar to what they had previously reported with LPI (Henstridge et al., 2009b). However, in these cells, AM281 produced agonist responses, albeit at concentrations 10X higher than those for AM251, SR141716A and LPI. Furthermore, they found robust ERK1/2 MAPK activation with LPI whereas AM251, SR141716A and AM281 produced much less activation of ERK1/2. Another report used the same cell line to examine transcription factor activation and showed that LPI and AM251 were efficacious activators of NFAT and NFκB at μM concentrations, whereas AM281 was most efficacious for CREB activation (Balenga et al., 2009), suggesting biased agonism for activation of downstream pathways.

Another recently published study characterizing GPR55 activation using a related technology (β-arrestin, PathHunter) demonstrated responses to AM251, SR141716A and LPI in a HEK293

cell line transiently expressing hGPR55 (Yin et al., 2009). The PathHunter technology uses enzyme (β-galactosidase) complementation of a tagged receptor with β-arrestin2 to measure β-arrestin2 recruitment (McGuinness et al., 2009). Yin et al (2009), from the GPCR Platform, Genomics Institute of the Novartis Research Foundation, also used a GPR55 reporter gene assay to evaluate potential ligands. The pGL3 basic vector was modified to have three repeats of multiple response element consensus sequence (MRE), a cAMP-response element from a vasoactive intestinal peptide gene promoter (CRE), a serum-response element promoter (SRE) before the luciferase reporter gene (Yin et al., 2009). The pGL3-CRE-MRE-SRE-luciferase reporter gene was transiently transfected into a HEK cell line stably transfected with hGPR55 and test compounds applied for 16–24 hours. The same general ligand profile for agonist activation of hGPR55 was seen with the luciferase reporter gene and β-arrestin assays. AM251 was the most potent and efficacious compound tested, with an EC50 of ~ 3 μM. LPI had similar potency (EC50 of 3.6 μM) and slightly less efficacy. SR141716A was less potent (EC50 of 10.9 μM) and also slightly less efficacious than AM251. Several cannabinoid compounds tested exhibited no agonist activity in either assay including CP55,940, WIN55,212-2, HU210, SR144528, AM630, Abn-CBD and O-1602. THC and anandamide showed a small amount of activity (12%) in the β-arrestin assay and 2-AG showed some activity in the reporter gene assay, consistent with Ryberg et al (2007). The authors state that the effects of THC and anandamide were enhanced to a more significant level when 5 μM AM251 was co-applied, so rather than acting as partial agonists they may have allosteric interactions in this system (Yin et al., 2009). β-arrestin redistribution assays can recognize agonists, antagonists, and allosteric modulators as demonstrated in a recent study with the CB1 cannabinoid receptor (van der Lee et al., 2009).

4.2 Pharmacology in vitro-endogenous cell lines and tissue

Most of the studies published have tried to characterize the pharmacological pattern of GPR55 in HEK293 transfected cells. To get a better understanding of the pharmacological and functional identity of GPR55, it is important to examine its functionality in its “natural” environment, i.e., in endogenous systems.

Endothelial cells—One example comes from the identification of GPR55 in endothelial cells. Evidence suggests the presence of an atypical cannabinoid receptor in endothelial cells (Brown and Hiley, 2009; Jarai et al., 1999; Offertaler et al., 2003). The former is responsible for some of the effects mediated by anandamide on non CB1/CB2 receptors and was given the name e-aR, (i.e. endothelial anandamide receptor). Waldeck-Weiermair et al. (2008) have suggested that the previously characterized e-aR is GPR55. In their study they have shown that the signaling pathway initiated following anandamide application is dependent upon the presence or absence of extracellular Ca^{2+}, therefore leading to a cross-talk between the endogenously expressed CB1R and GPR55. They show that in the presence of extracellular Ca^{2+} (2 μM), anandamide (10 μM), induced a very small $Ca^{2+}{}_i$ elevation. However, in the absence of extracellular Ca^{2+}, a significant rise in $Ca^{2+}{}_i$ was observed with anandamide. Interestingly, application of anandamide in the presence of the CB1 antagonist AM251 (10 μM) resulted in $Ca^{2+}{}_i$ rise even in the presence of extracellular Ca^{2+}. Moreover, HU-210 (10 μM) failed to induce $Ca^{2+}{}_i$ rise either in the presence or absence of extracellular calcium. Notably the application of O1602 (10 μM) resulted in a robust $Ca^{2+}{}_i$ rise even in the presence of extracellular Ca^{2+}, while O1918 (10 μM), prevented anandamide-induced calcium signaling in the absence of extracellular Ca^{2+}. However, O1602 failed to elicit the $Ca^{2+}{}_i$ rise in the presence of the CB1R agonist HU-210, but was retrieved when the latter was washed from the bathing solution. These data, therefore, suggest a possible cross-talk between the CB1R and the e-aR, which was molecularly identified to be GPR55 using RT-PCR to detect mRNA expression as well as modulation of the anandamide response by over-expression or siRNA knockdown of GPR55.

The dependence upon anandamide responsiveness was further attributed to integrin clustering and was supported by the ability of other divalent cations (i.e, Sr^{2+} and Ba^{2+}) to modify anandamide-induced $Ca^{2+}{}_i$. Moreover, when Mn^{2+} (70 μM) a potent modulator of integrin clustering was introduced, anandamide-induced $Ca^{2+}{}_i$ was observed even in the presence of extracellular Ca^{2+}. To further establish the role of integrin clustering on anandamide responsiveness, the authors inhibited RhoA associated kinases 1 and 2 (ROCK1 and ROCK2), involved in integrin clustering with Y27632 and successfully abolished the anandamide-induced $Ca^{2+}{}_i$ response in the absence of extracellular Ca^{2+}. The integrins involved were shown to be αvβ3 and α5β1 using functional antibody inhibition of these proteins. Two different signaling pathways were suggested dependent on integrin clustering; when unclustered, (i.e. in the presence of extracellular Ca^{2+}), anandamide binds to CB1R, resulting in a Gi-mediated activation of spleen tyrosine kinase (Syk) and accumulation of NFκB. Moreover, under these condition, Syk also inhibits PI3K, therefore preventing the signaling pathway initiated by GPR55. When clustered, anandamide induces detachment of CB1R from β1 integrin, therefore enforcing the signaling pathway via GPR55, which leads to the activation of PI3K-Bmx-PLCγ pathway, resulting in the release of Ca^{2+} from intracellular stores and subsequent accumulation of NFAT. Notably, under both conditions (i.e., clustered and unclustered integrins) the ERK1/2 pathway is intact (Waldeck-Weiermair et al., 2008).

Neuronal cells—As previously described, Lauckner et al. (2008) reported that application of THC (5 μM), JWH015 (3 μM) and LPI (3 μM) increased intracellular calcium, that was antagonized by low micromolar concentrations of SR141716A not only in hGPR55-HEK293 cells, but also in large DRG in mice, where it is abundantly expressed. Whereas previous studies had documented endogenous GPR55 expression by measuring mRNA levels, Lauckner et al (2008) generated a specific C-terminal antibody to GPR55 that allowed immunohistochemical detection of receptor. The authors exclude the possibility of cannabinoids acting at TRP channels because the intracellular calcium rise and immunostaining was evident only in large diameter neurons whereas TRP channels are present in small, medium and large DRG (Lauckner et al., 2008).

Microglial cells—Pietr at al (2009), have recently reported the expression of GPR55 mRNA in mouse microglial primary culture and in the murine microglial cells line BV-2. GPR55 mRNA levels of primary microglial cells were significantly reduced upon treatment with IFNγ (200 U/ml) and LPS (100 ng/ml). Treatment with LPS also caused a concentration-dependent decrease in GPR55 mRNA levels, with the largest decrease observed upon treatment with 100 ng/ml LPS, while the addition of IFNγ resulted in a concentration and time-dependent up-regulation of GPR55 mRNA levels. The authors further demonstrate that the up-regulation of mRNA GPR55 upon stimulation with IFNγ can affect ERK1/2 phosphorylation. Indeed, up-regulated BV-2 cells exhibit even higher levels of ERK1/2 phosphorylation following treatment with LPI, (1, 5 and 10 μM) compared to the phosphorylation levels obtained upon LPI treatment in non-stimulated cells. The elevated GPR55 mRNA levels corresponding with increased ERK1/2 phosphorylation have to be further established because LPS, the ligand shown in this study to down regulate GPR55 mRNA levels, induced high levels of ERK1/2 phosphorylation on its own.

The presence of GPR55 in BV-2 cells along with some pharmacology was recently reported by Eldeeb et al (2009). This study assessed $Ca^{2+}{}_i$ release (using a microfluorimeter) by LPI and a panel of cannabinoid ligands in BV-2 cells. While 10 μM LPI produced an increase in $Ca^{2+}{}_i$ release (51–57%), most of the cannabinoids tested (including anandamide, 2-AG, HU210, CP55940, WIN55212-2 and SR141716A) did not produce agonist responses up to 10 μM (Eldeeb et al., 2009).

The CB2 receptor is known to exhibit an anti-inflammatory phenotype in microglial cells (Cabral et al., 2008; Romero-Sandoval et al., 2009; Walter et al., 2003). Therefore, the similarity in regulation pattern of GPR55 and CB2R in both mouse microglial primary culture and BV-2 cells suggests that not only CB2 has a prominent role in CNS immunity, but so does GPR55 (Pietr et al., 2009).

Osteoclasts and osteoblasts—Recently Whyte et al (2009) have demonstrated the physiological relevance of GPR55 in bone metabolism. Significant levels of GPR55 mRNA were detected in human osteoclasts generated from macrophage colony-stimulating factor-dependent monocytes, in multinucleated human and mouse osteoclasts, in human and mouse primary osteoblasts and in human TE85 osteoblast-like cells. They first measured the effects of different GPR55 ligands on osteoclast formation (quantified by staining for the vitronectin receptor). Application of varying concentrations (1 nM-10μM) of O-1602, previously shown to be an agonist at GPR55, did not affect total human osteoclast number. Interestingly, application of CBD (500 nM), previously shown to be an antagonist at GPR55, significantly increased osteoclast formation. In contrast, an inhibitory effect of O-1602 was apparent in the formation of multinucleated osteoclasts from mouse bone marrow, and was retrieved in the presence of 500 nM CBD. Mouse osteoclast formation was also inhibited upon treatment with LPI (1 nM-1 μM), which in most studies is so far considered to be the most potent GPR55 ligand. The inhibitory effect of O-1602 or LPI was not observed in osteoclast generated from $GPR55^{-/-}$ bone marrow macrophages.

The authors further sought to investigate the ability of O-1602 and CBD in stimulating osteoclast polarization and function. Treatment of human osteoclasts with O-1602 resulted in an increase of the proportion of polarized, resorbing osteoclasts with F-actin rings, and the area of the resorption pits. Application of CBD (500 nM) following O-1602 (50 nM) treatment significantly inhibited both the increase in resorption area and the F- actin ring number. Higher concentrations of CBD (1 μM) alone significantly inhibited both polarization and resorption. The same effect of increase in proportion of osteoclast with F- actin rings and resorption area, was evident in mouse osteoclasts. An increase in F- actin rings and resorption area was also evident following treatment with LPI, with the higher increase observed at 100nM.

The authors further demonstrate significant increase of Rho A, known to have a role in osteoclast formation and bone resorption, following treatment with O-1602 and LPI, that is abolished following pretreatment with 1 μM CBD. LPI treatment significantly increased ERK1/ 2 phosphorylation and was abolished by pretreatment with 1 μM CBD, again the same trend was evident upon treatment with O-1602. Activation of the Rho A pathway following O-1602 or LPI was evident in mouse osteoclasts, but was absent in $GPR55^{-/-}$ osteoclasts. The author's in vivo studies with GPR55 KO and WT mice further confirm a role of GPR55 in bone remodeling (see below).

4.3 Pharmacology in vivo

To date, the conflicting data regarding GPR55 agonists and antagonists makes it difficult to conduct in vivo pharmacological studies. However, studies from GPR55 knockout mice may shed some light on its physiological relevance in vivo.

Whyte et al (2009) found that GPR55 affects osteoclast and osteoblast differentiation to influence bone mass. In this study, receptor knockout mice were generated by deletion of the entire coding region of GPR55 by homologous recombination. The $GPR55^{-/-}$ mice were backcrossed for 6 generations onto the C57Bl/6 background; background differences have previously been shown to be important for bone morphological studies with the CB_1 receptor (Bab and Zimmer, 2008). Male $GPR55^{-/-}$ mice have increased numbers of morphologically inactive osteoclasts resulting in a significant increase in the volume and thickness of trabecular

bone and the presence of unresorbed cartilage. Treatment of male mice for 8 weeks with 10 mg/kg CBD (3 times per week) significantly decreased the level of serum type 1 collagen C-terminal telopeptide fragments, a biochemical marker of bone resorption, by 18%. Microtomographic analysis of the proximal tibiae also revealed a trend toward increased bone volume/tissue volume (+ 10%) and trabecular number (+ 10%), with a decrease in trabecular separation (− 7%), trabecular pattern factor (− 5%), and structure modulus index (− 8%) in the tibia of the CBD-treated mice relative to control. These findings were consistent with a decrease in bone resorption in CBD-treated mice, although these changes were not statistically significant over this treatment schedule (Whyte et al., 2009). CBD acted as a GPR55 antagonist in osteoclast cultures, although the effects of CBD on osteoclasts prepared from GPR55$^{-/-}$ mice were not reported. Nonetheless, these studies indicate a role for GPR55 in bone resorption, and indicate that CBD and GPR55 antagonists may be useful for the treatment of osteoporosis.

Staton et al (2008) reported that GPR55 $^{-/-}$ mice (also backcrossed to a C57Bl/6 background), possessed no overt phenotype, but were protected in models of inflammatory and neuropathic pain. The strategy for targeting the gene in these animals left intact the first 118 bp of GPR55 as well as the distal portion of the sequence (beyond lysine 281). As this latter sequence contains several methionines, it is possible that if it is transcribed it will be translated. Staton et al (2008) reported that no GPR55 mRNA could be detected by RT-PCR in the animals. Also the levels of CB1 and CB2 mRNA were unchanged in the brain and spleen, respectively, indicating that compensatory changes in these two receptors did not occur in the mice. Female GPR55$^{-/-}$ did display a reduction in withdrawal latency in an acute pain model, the hot water tail flick latency test, when tested at 50 °C, but not at higher temperatures (52.5 °C, 55 °C. A more robust difference was seen in two models of hyperalgesia. In mechanical hyperalgesia induced by intraplantar administration of Freund's complete adjuvant (FCA), inflammatory mechanical hyperalgesia was completely absent in both male GPR55 KO mice up to 14 days post-injection. Female GPR55$^{-/-}$ mice were protected for one day post-FCA. In the partial nerve ligation model of neuropathic hypersensitivity, GPR55$^{-/-}$ mice of both sexes failed to develop mechanical hyperalgesia up to 28 days post-ligation.

Cytokine profiling experiments showed increased levels of IL-4, IL-10, IFN γ and GM-CSF in paws from the FCA-injected GPR55$^{-/-}$ mice when compared with the FCA-injected GPR55 wild-type mice (at 1 and 14 days post-injection for the female and male mice, respectively). This suggests that GPR55 signaling can influence the regulation of certain cytokines and this may contribute to the lack of inflammatory mechanical hyperalgesia in the GPR55 $^{-/-}$ mice. These data suggest that GPR55 antagonists may have therapeutic potential in the treatment of both inflammatory and neuropathic pain.

5. Possible physiological functions of the GPR55 receptor

The signaling pathways initiated by activation of GPR55 have been shown to have important physiological roles in other GPCRs (Dorsam and Gutkind, 2007; Luttrell and Luttrell, 2003; Rozengurt, 2007). Receptor activation of MAPK signaling (discussed below), elevated calcium levels and the production of transcription factors all have physiological roles which need to be further evaluated for GPR55.

Communication between the plasma membrane and regulatory targets in various intracellular compartments is mediated via intracellular signaling cascades. A key pathway involved in mitogenic signaling induced by GPCRs is the extracellular-regulated protein kinase (ERK), which belongs to the mitogen–activated protein kinase (MAPK) signaling cascades.

While the main core phosphorylation chain of the cascade includes Raf kinases, MEK1/2, ERK1/2 (ERKs) and RSKs, other alternatively spliced forms and distinct components exist in the different tiers, and participate in ERK signaling under specific conditions. These

components enhance the complexity of the ERK cascade and thereby enable the wide variety of functions that are regulated by it. Other factors that have to be accounted for are the multiplicity of the cascade's substrates, which include transcription factors, protein kinases and phosphatases, cytoskeletal elements, regulators of apoptosis, and a variety of other signaling-related molecules, which increase the complexity. These factors therefore contribute to the distinct, and even opposing cellular processes that are regulated by the ERK cascade (Shaul and Seger, 2007; Yoon and Seger, 2006).

Waldeck-Weiermair et al. (2008) demonstrated that in endothelial cells, the activation of GPR55 by application of 10 μM anandamide resulted in a marked increase of ERK1/2 phosphorylation that was evident 2 min following drug activation and was sustained for up to 3 hr. Another example comes from our unpublished observations in hGPR55E-U2OS cells where treatment with 10 μM LPI resulted in a marked increase of ERK1/2 phosphorylation that was evident already 5 min following drug treatment and was sustained for at least 2 hr. The outcome of this nature of ERK1/2 phosphorylation has to be further investigated since, as previously reported, it can be indicative of either a precursor of cell death or a survival signal, suppressing apoptosis (Correa et al., 2005; Galve-Roperh et al., 2008; He et al., 2004; Sarfaraz et al., 2006)..

Several studies have reported morphological changes following activation of GPR55 (Kapur et al., 2009; Oka et al., 2009b). In cells co-transfected with hGPR55 and PKCβII-GFP within 60 seconds of addition of agonists, membrane rearrangements began to occur followed by protrusions and blebbing (Kapur et al., 2009). Cytoskeletal changes were also observed in the β-arrestin trafficking assay upon addition of agonist (Kapur et al., 2009). Further, the necessity of an intact actin cytoskeleton was demonstrated to have a role in the release of calcium from intracellular stores following activation of the receptor, and was demonstrated to be induced by RhoA (Lauckner et al., 2008). In agreement with Lauckner et al (2009), Oka et al. (2009b) have recently reported that the morphological changes induced by 2-AG LPI can be abrogated by Y-27632, a specific inhibitor of ROCK, or C3 toxin, an inhibitor of RhoA. Thus activation of GPR55 links the Rho-ROCK pathway to the morphological changes, evident by the formation of actin stress fiber formation.

6. Potential mechanisms underlying the discrepant pharmacology of GPR55

Allostery/Biased Agonism/Functional Selectivity

The most parsimonious explanation of this apparent disagreement between reports (Johns et al., 2007; Kapur et al., 2009; Lauckner et al., 2008; Oka et al., 2007; Ryberg et al., 2007; Yin et al., 2009) is that distinct conformations of the receptor resulting from the binding of different ligands might couple differentially and/or to multiple downstream effectors (Hudson et al., 2009; Kenakin, 2009; Ross, 2009; Violin and Lefkowitz, 2007). Indeed, the primary action of drugs on GPCRs is to promote a conformational change in tertiary conformation, an obviously complex outcome (Kenakin, 2009). Although functional selectivity (biased agonism) is treated separately from allosterism, it can be seen as a vectorial manisfestation of the general allosteric nature of GPCRs (Kenakin, 2009).

For GPR55, a common final pathway that has been reported in most but not all papers is the activation of MAPK (ERK1/2). GPCR activation can result in ERK1/2 phosphorylation by G-protein and G-protein-independent pathways (Lefkowitz and Shenoy, 2005). The observation that LPI alone induces a significant activation of ERK1/2 in cells expressing hGPR55 while cannabinoids (AM251, SR141716A, anandamide and 2-AG) either produced no (Kapur et al., 2009) or little (Henstridge et al., 2009a) response are mostly in agreement with Oka et al (2007). However, this divergence in efficacy between signaling pathways (βarrestin trafficking, Ca^{2+} signaling, etc., vs. ERK activation) suggests that LPI and cannabinoids

display functional selectivity (Gonzalez-Maeso and Sealfon, 2009; Hudson et al., 2009; Kenakin, 2009; Violin and Lefkowitz, 2007).

Biased agonism is also suggested by the studies assessing transcription factor activation, where AM281 was the most efficacious agent activating CREB, whereas LPI and AM251 were the most efficacious at activating NFAT (and NFκB) (Balenga et al., 2009). Biased agonism may also explain the ability of CP55940 to act as a partial agonist in PKCβII-GFP activation and an antagonist in the βarr2-GFP trafficking assays (Kapur et al., 2009), although partial agonists can act as antagonists depending on the signal strength (Kenakin, 2007).

Cell line and tissue heterogeneity

It is well known that cell lines present inconsistent phenotype over time. For example, Dubi et al. (2008) have recently demonstrated that the androgen insensitive PC-3 cell line exhibited two sublines that showed distinct receptor activation. Moreover, the clonal background of HEK293 cells can differ markedly between laboratories (Henstridge et al., 2009b). In addition, Ryberg et al (2007) used a HEK293s cell line, Johns et al (2007) used a HEK293T cell line, and the HEK293 cells in other papers were from different laboratories (Henstridge et al., 2009b; Kapur et al., 2009; Lauckner et al., 2008; Oka et al., 2007). Indeed, although HEK293 cells are referred to as “human embryonic kidney” cells, a study on the origin of the line suggests they may instead be derived by adenoviral transformation of a neuronal precursor present in the human embryonic kidney cell cultures (Shaw et al., 2002).

The response and signaling pathways, initiated by the same agonist, might differ among different tissues. One example comes from GPR39, which also belongs to the Class A GPCRs, recently identified as an extracellular zinc-sensing receptor (Yasuda et al., 2007). Activation of the zinc-sensing receptor leads to intracellular Ca^{2+} release with K_m values of 55 μM, 146 μM and 200 μM in salivary gland, brain and prostate cells, respectively (Besser et al., 2009; Dubi et al., 2008; Sharir and Hershfinkel, 2005). Moreover, extracellular Ca^{2+} had been demonstrated to allosterically modulate the receptor’s affinity (Sharir and Hershfinkel, 2005). Activation of the zinc-sensing receptor also results in phosphorylation of ERK1/2. In PC-3 cells activation of the receptor resulted in sustained ERK1/2 phosphorylation, for at least 3 hr (Dubi et al., 2008). In HT-29 cells, however, ERK1/2 phosphorylation peaked at 30 min following ligand application, and approached resting levels after 2 hr (Azriel-Tamir et al., 2004).

Receptor over-expression

Many of the in vitro studies using transfected cells were over-expressing GPR55 (Henstridge et al., 2009b; Johns et al., 2007; Kapur et al., 2009; Lauckner et al., 2008). If overexpression of the receptor induces constitutive activity, this can lead to altered behavior of ligands (Kenakin, 2001). An additional difference that arises when considering transient versus stable expression systems is that in a cell stably expressing a protein it is necessary that the protein be expressed at levels where it is not detrimental to the health and division of the cell (otherwise the cell does not survive the selection process). This might involve lower levels of expression, or a matching of expression of proteins that might ameliorate some of the toxicity of the expressed proteins. Additionally, there may also be investigator-initiated selection bias in stable lines where a line exhibiting a more reproducible response is chosen for future studies. These factors may also explain differences between the results of studies conducted using stable versus transient expression.

Modulation of the GPR55 response by other proteins or factors in the cell/cell media

Because of cell line and tissue heterogeneity, there may be proteins in the various cell lines that modify the response of GPR55. The differential signaling of anandamide due to integrin

clustering in endothelial cells and CB1 activation is one published example (Waldeck-Weiermair et al., 2008). Another example is that interaction with other GPCRs alters the pharmacology of CB1 cannabinoid receptors (recently reviewed by (Hudson et al., 2009)).

The differential response of GPR55 in different laboratories may also be dependent on cell culture conditions. The presence of endocannabinoids in serum has been documented (Valk et al., 1997) and other growth factors are present as well. In addition, HEK293 and other cells can synthesize endocannabinoids and this may alter the measured response (Turu et al., 2009).

Phospholipase C Activation

LPI is synthesized by phospholipase A-mediated removal of acyl moieties of phosphatidylinostol. In this regard, it is similar to 2-AG which can be generated by phospholipase C or diacylglycerol lipase (PLC & DAG). One hypothesis that has been put forward is that cannabinoids acting at CB1 or CB2 receptors or a “receptor X” generate LPI that then acts at GPR55 (De Petrocellis and Di Marzo, 2009). One would have to invoke the “other receptor” to explain the CB1 receptor antagonists/inverse agonists SR141716A and AM251’s agonist activity at GPR55 and the antagonism of LPI’s effects by CP55,940. Thus, it seems more likely that these are direct actions at GPR55. However, since near micromolar concentrations of these compounds are generally reported for these compounds it is not possible to conduct radioligand binding assays. Thus, this possibility cannot be eliminated at this time.

CONCLUSIONS

The classification of GPR55 as a cannabinoid receptor at this time is problematic, due to the conflicting reports on the ligands with which it interacts. Until this controversy is resolved, GPR55 can be regarded as an “atypical” cannabinoid receptor. A consensus among the papers published on GPR55 is that LPI is an agonist for this receptor. In addition, several compounds that are regarded as cannabinoids are agonists, partial agonists and antagonists at GPR55. Another agreement among reports is that the aminoalkylindole, WIN55212-2, a potent CB_1 and CB_2 receptor agonist, does not activate GPR55. An anandamide- and WIN-sensitive GPCR present in CB_1 knockout animals had previously been described (Breivogel et al., 2001; Di Marzo et al., 2000; Monory et al., 2002); clearly GPR55 is not this receptor. Therefore, additional cannabinoid receptors remain to be discovered.

GPR55 has been shown to utilize G_q and $G_{12}/_{13}$ for signal transduction; PLC and RhoA are activated (Henstridge et al., 2009b; Kapur et al., 2009; Lauckner et al., 2008). This signaling mode is associated with temporal changes in cytoplasmic calcium, membrane-bound diacylglycerol, and plasma membrane topology. LPI, SR141716A and AM251 recruit PKCβII-GFP and cause widespread plasma membrane remodeling upon GPR55 activation (Kapur et al., 2009). Involvement of the actin cytoskeleton was also reported by Lauckner et al (2008) and Sugiura et al (2009). A summary of the intracellular signaling pathways initiated by GPR55 is presented in figure 3. One should note that although several Gα subunits have been implicated in signal initiation, it appears that activation of GPR55 results in the ultimate generation of the same intracellular molecules, leading to the activation of the MAPK pathways and transcription factors. Future studies determining the outcome of the suggested downstream signaling pathways will be important to delineate the role of GPR55 in cell growth, differentiation and cell death.

GPR55 has been implicated in neuropathic and inflammatory pain (Staton et al., 2008) and bone remodeling (Whyte et al., 2009). Thus delineating the pharmacology of this receptor and the discovery of selective agonists and antagonists merits further study and could lead to new therapeutics.

Abbreviations

2-AG	2-arachidonylglycerol
2-AGPI	2-arachidonoyl-sn-glycero-3-phosphoinositol
e-aR	anandamide endothelial receptor
abn-CBD	abnormal cannabidiol
βarr2-GFP	β-arrestin2-green fluorescent protein
CBD	cannabidiol
Δ^9-THC	delta-9-tetrahydrocannabidiol
DRG	dorsal root ganglion
ERK	extracellular signal-regulated kinase
LPI	lysophosphatidylinositol
PEA	palmitoylethanolamide
PKCβII-GFP	Protein kinase C betaII-green fluorescent protein
ROCK	Rho A-associated kinase

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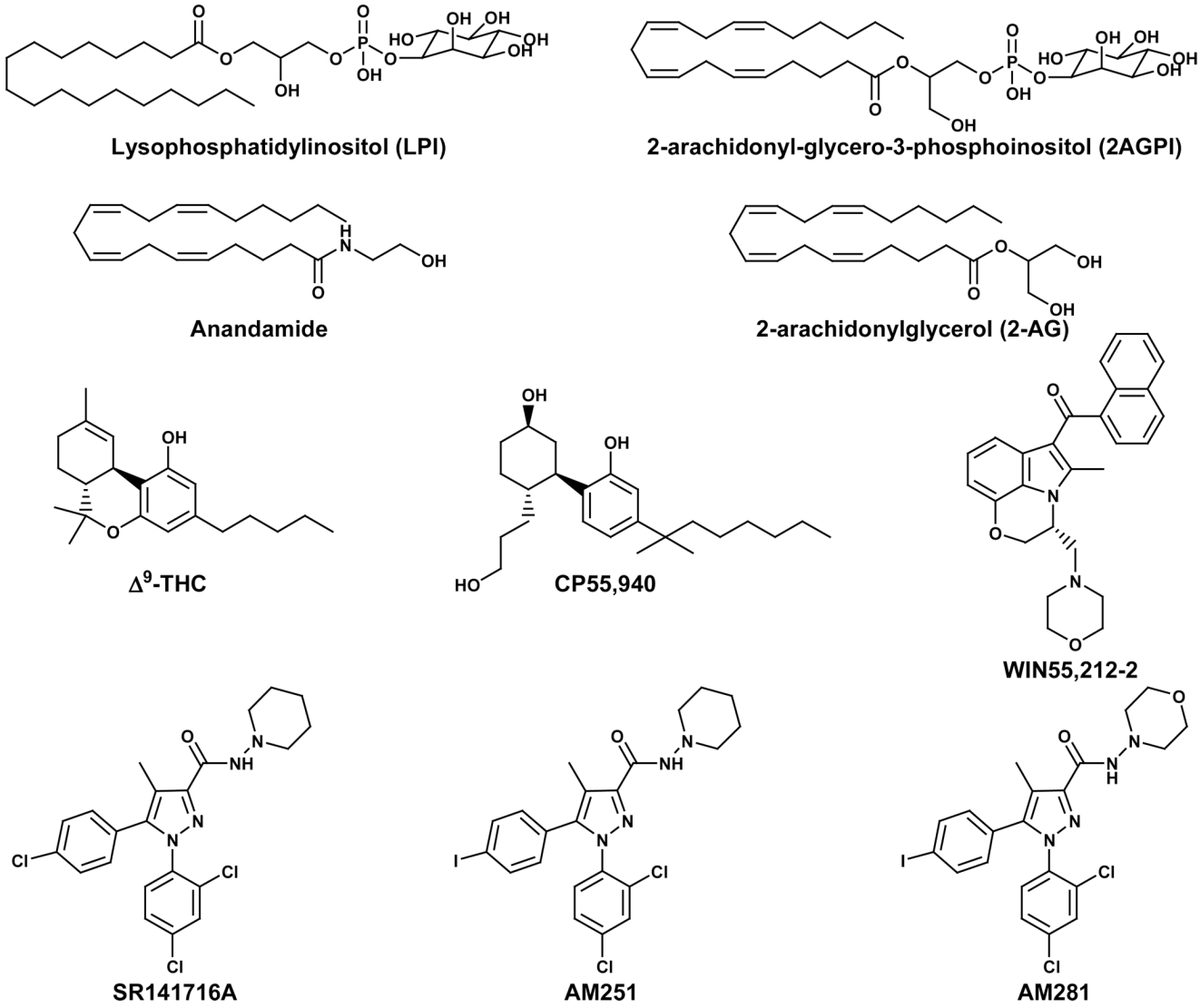

Figure 1.

The structures of several compounds studied as GPR55 ligands. Lysophosphatidylinositol (LPI) and 2-arachidonyl-glycero-3-phosphoinositol (2AGPI) are lyosphospholipid agonists of GPR55. Anandamide and 2-arachidonylglycerol (2-AG) are endocannabinoids. THC is a phytocannabinoid and CP55,940 the prototypical non-classic cannabinoid agonist. WIN55,212-2 is the prototypical aminoalkylindole compound. SR141716A, AM251 and AM281 are pyrazole CB1 receptor antagonists/inverse agonists.

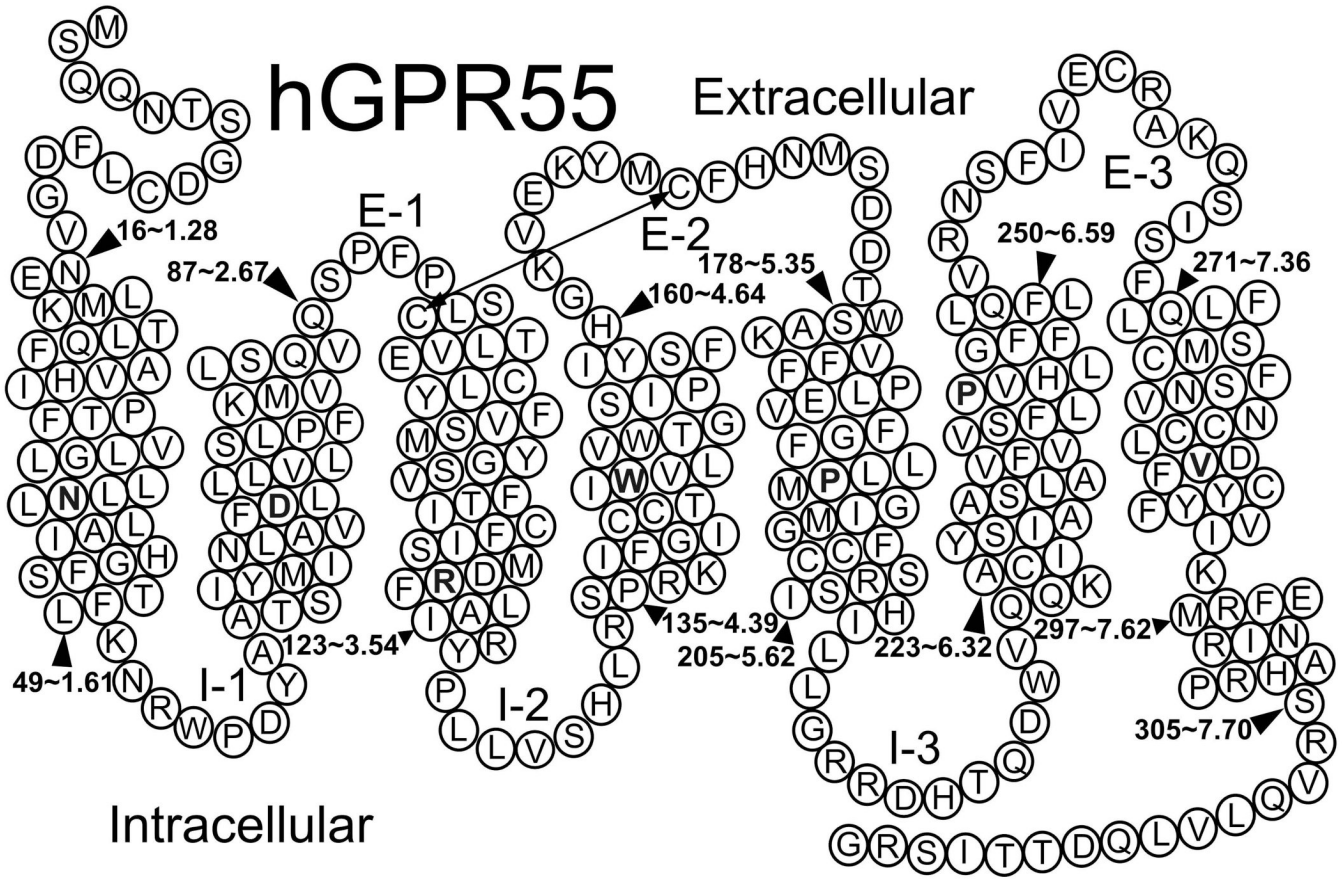

Figure 2.
Helix net representation of human GPR55. The residues shaded in black represent highly conserved residues in rhodopsin, the prototypical Class A GPCR.

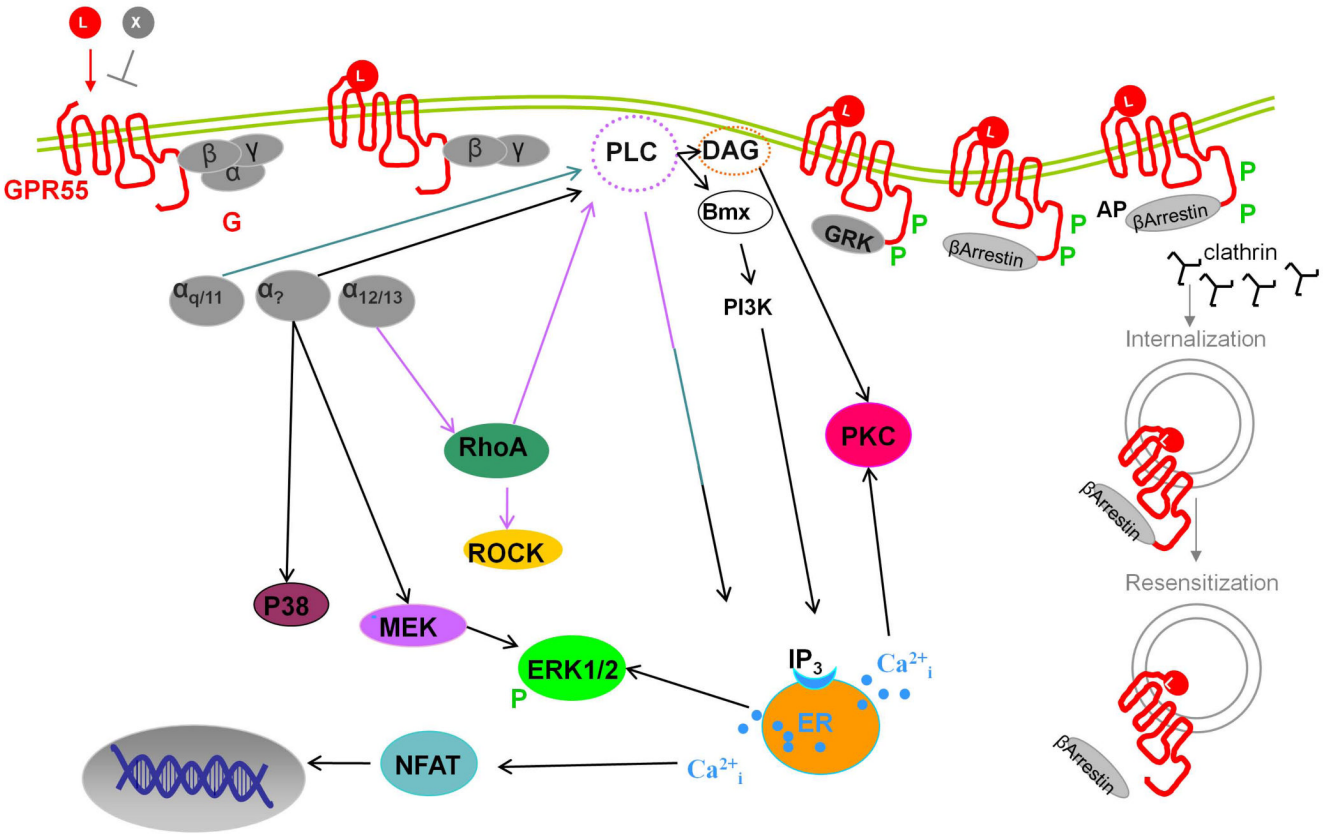

Figure 3.
A summary of the signaling pathways initiated by GPR55.

Table 1

List of agonists and antagonists reported in multiple studies on GPR55. For a complete list of compounds see text.

	Cell Type	Read out							
		[^{s}35]GTPγS	**ERK1/2**	**[Ca^{2+}]$_i$ mobilization**	**βArrestin**	**Internalization**	**Rho**	**NFAT**	**PKCβ**
LPI	hGPR55-HEK293	EC_{50}=1 μM (Oka et al., 2007)	EC_{50}=200 nM (Oka et al., 2007)	≥30 nM (Oka et al., 2007), 3 μM (Lauckner et al., 2008),EC_{50}= 49 nM (Henstridge et al, 2009b)	EC_{50}=3.6 μM (Yin et al., 2009)	1 μM agonist (Henstridge et al., 2009b)	1μM agonist (Henstridge et al., 2009b), agonist (Sugiura et al., 2009)	EC_0= 1.4 μM (Henstridge et al., 2009b) (Balenga et al., 2009)	Not tested
	hGPR55E-HEK293	Not tested	Not tested	Not tested	Not tested	3 μM agonist (Kapur et al., 2009)	Not tested	Not tested	Not tested
	hGPR55-PKCβII-GFP-HEK293	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	10 μM agonist (Kapur et al., 2009)
	hGPR55E-U2OS	Not tested	10 μM agonist (Kapur et al., 2009)	Not tested	EC_{50}=1.2 μM (Kapur et al., 2009)	3 μM agonist (Kapur et al., 2009)	Not tested	Not tested	Not tested
	Mouse DRG	Not tested	Not tested	3 μM agonist (Lauckner et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	EA.hy926	Not tested	Not tested	10 μM agonist (Waldeck-Weiermair et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	Human osteoclasts	Not tested	1 μM agonist (Whyte et al., 2009)	Not tested	Not tested	Not tested	1 μM agonist (Whyte et al., 2009)	Not tested	Not tested
	Mouse osteoclasts	Not tested	Not tested	Not tested	Not tested	Not tested	1 μM agonist (Whyte et al., 2009)	Not tested	Not tested
	BV-2	Not tested	1 μM agonist (Pietr et al., 2009)	EC_{50}~14 μM (Eldeeb et al., 2009)	Not tested	Not tested	Not tested	Not tested	Not tested
AEA	hGPR55-HEK293	EC_{50}=18 nM (Ryberg et al., 2007)	No effect (Oka et al., 2007; Oka et al., 2009)	5 μM agonist (Lauckner et al., 2008)	Very weak (Yin et al., 2009)	Not tested	1 μM agonist (Ryberg et al., 2007)	Not tested	Not tested
	hGPR55E-HEK293	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	30μM no effect (Kapur et al., 2009)	Not tested	Not tested	Not tested

	Cell Type	Read out							
		[^{35}]GTPγS	**ERK1/2**	**[Ca^{2+}]$_i$ mobilization**	**βArrestin**	**Internalization**	**Rho**	**NFAT**	**PKCβ**
	hGPR55E-U2OS	Not tested	No effect (Kapur et al., 2009)	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested
	EA.hy926	Not tested	10 μM agonist (Waldeck-Weiermair et al., 2008)	EC_{50}=7.3 μM (Waldeck-Weiermair et al., 2008)	Not tested	Not tested	Not tested	10 μM agonist (Waldeck-Weiermair et al., 2008)	Not tested
	BV-2			10 μM no effect (Eldeeb et al., 2009)					
2-AG	hGPR55-HEK293	Ec_{50}=3 nM (Ryberg et al., 2007)	No effect (Oka et al., 2007; Oka et al., 2009)	5 μM no effect (Lauckner et al., 2008),3–30 μM (Henstridge et al, 2009b)	No effect (Yin et al., 2009)	Not tested	Not tested	Not tested	Not tested
	hGPR55E-HEK293	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested
	hGPR55E-U2OS	Not tested	No effect (Kapur et al., 2009)	Not tested	No effect (Kapur et al., 2009)	No effect	Not tested	Not tested	Not tested
	BV-2	Not tested	Not tested	10 μM no effect (Eldeeb et al., 2009)	Not tested	Not tested	Not tested	Not tested	Not tested
Δ^9-THC	hGPR55-HEK293	EC_{50}= 8 nM (Ryberg et al., 2007)	1 μM no effect (Oka et al., 2007), 5 μM no effect (Lauckner et al., 2008)	5 μM agonist (Lauckner et al., 2008)	Very weak agonist (Yin et al., 2009)	Not tested	5 μM agonist (Lauckner et al., 2008)	Not tested	Not tested
	mGPR55-HEK293	Not tested	Not tested	5 μM agonist (Lauckner et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	hGPR55E-HEK293	Not tested	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested
	hGPR55E-U2OS	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested
	Mouse DRG	Not tested	Not tested	5 μM agonist (Lauckner et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	BV-2	Not tested	Not tested	Modest increase	Not tested	Not tested	Not tested	Not tested	Not tested

	Cell Type	Read out							
		[^{35}]GTPγS	**ERK1/2**	**[Ca^{2+}]$_i$ mobilization**	**βArrestin**	**Internalization**	**Rho**	**NFAT**	**PKCβ**
				(Eldeeb et al., 2009)					
CBD	hGPR55-HEK293	IC_{50}=350 nM (Ryberg et al., 2007)		3 μM no effect (Lauckner et al., 2008)	No effect (Yin et al., 2009)	Not tested	10 μM antagonist (Ryberg et al., 2007)	Not tested	Note tested
	hGPR55E-U2OS	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested
	BV-2	Not tested	Not tested	Modest increase (Eldeeb et al., 2009)	Not tested	Not tested	Not tested	Not tested	Not tested
	Human osteoclasts	Not tested	1 μM antagonist (Whyte et al., 2009)	Not tested	Not tested	Not tested	1 μM antagonist (Whyte et al., 2009)	Not tested	Not tested
Abn-CBD	hGPR55-HEK293	EC_{50}= 2.5 μM (Ryberg et al., 2007), EC_{50}= 2.5 nM (Johns et al., 2007)	1 μM no Effect (Oka et al., 2007)	3 μM no effect (Lauckner et al., 2008)	No effect (Yin et al., 2009)	Not tested	Not tested	Not tested	Not tested
	hGPR55E-U2OS	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested
O-1602	hGPR55-HEK293	EC_{50}= 13 nM (Ryberg et al., 2007), EC_{50}= 1.4 nM (Johns et al., 2007)	Not tested	1 μM no effect (Oka et al., 2009)	No effect (Yin et al., 2009)	Not tested	1 μM agonist (Ryberg et al., 2007)	Not tested	Not tested
	hGPR55E-U2OS	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested
	EA.hy926	Not tested	Not tested	10 μM agonist (Waldeck-Weiermair et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	human osteoclasts	Not tested	1 μM agonist (Whyte et al., 2009)	Not tested	Not tested	Not tested	1 μM agonist (Whyte et al., 2009)	Not tested	Not tested

	Cell Type	Read out							
		[^{35}]GTPγS	**ERK1/2**	**[Ca^{2+}]$_i$ mobilization**	**βArrestin**	**Internalization**	**Rho**	**NFAT**	**PKCβ**
	Mouse osteoclasts	Not tested	Not tested	Not tested	Not tested	Not tested	1 μM agonist (Whyte et al., 2009)	Not tested	Not tested
CP55940	hGPR55-HEK293	EC_{50}=5 nM (Ryberg et al., 2007)	1 μM no effect (Oka et al., 2007; Oka et al., 2009)	No effect (Lauckner et al., 2008; Oka et al., 2009) 3 μM antagonist (Henstridge et al., 2009b)	No effect (Yin et al., 2009)	Not tested	Not tested	Not tested	Not Tested
	hGPR55E-HEK293	Not tested	Not tested	Not tested	Not Tested	10 μM no effect (Kapur et al, 2009)	Not tested	Not tested	Not tested
	hGPR55-PKCβII-GFP-HEK293	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	10 μM partial agonist (Kapur et al., 2009)
	hGPR55E-U2OS	Not tested	10 μM antagonist (Kapur et al., 2009)	Not tested	Ki ~200 nM (Kapur et al., 2009)	Ki ~200 nM (Kapur et al., 2009)	Not tested	Not tested	Not tested
	BV-2	Not tested	Not tested	10 μM no effect (Eldeeb et al., 2009)	Not tested	Not tested	Not tested	Not tested	Not tested
JWH015	hGPR55-HEK293	Not tested	5 μM no effect (Lauckner et al, 2008)	3 μM agonist (Lauckner et al, 2008)	Not tested	Not tested	3 μM agonist (Lauckner et al., 2008)	Not tested	Not tested
	mGPR55-HEK293	Not tested	Not tested	3 μM agonist (Lauckner et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	Mouse DRG			3 μM agonist (Lauckner et al, 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	hGPR55E-U2OS	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested
SR141716A	hGPR55-HEK293	Not tested	1 μM no effect (Oka et al., 2007) Agonist (Henstridge et al., 2009a)	2 μM antagonist (Lauckner et al., 2008) 100nM-3μM agonist (Henstridge et al., 2009a)	EC_{50}=9.3 μM (Yin et al, 2009)	100nM-3μM Agonist (Henstridge et al., 2009a)	Not tested	Agonist (Balenga et al., 2009)	Not tested

	Cell Type	Read out							
		[^{s}35]GTPγS	**ERK1/2**	**[Ca^{2+}]$_i$ mobilization**	**βArrestin**	**Internalization**	**Rho**	**NFAT**	**PKCβ**
	hGPR55E-HEK293	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	30 μM Agonist (Kapur et al, 2009)	Not tested	Not tested	Not tested
	hGPR55-PKCβII-GFP-HEK293	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	10 μM agonist (Kapur et al., 2009)
	hGPR55E-U2OS	Not tested	No effect (Kapur et al., 2009)	Not tested	EC_{50}=3.9 μM (Kapur et al., 2009)	30 μM Agonist (Kapur et al, 2009)	Not tested	Not tested	Not tested
	EA.hy926	Not tested	Not tested	1 μM antagonist (Waldeck-Weiermair et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	Mouse DRG	Not tested	Not tested	2 μM antagonist (Lauckner et al., 2008)	Not tested	Not tested	Not tested	Not tested	Not tested
	BV-2	Not tested	Not tested	10 μM no effect (Eldeeb et al., 2009)	Not tested	Not tested	Not tested	Not tested	Not tested
AM251	hGPR55-HEK293	EC_{50}=39 nM (Ryberg et al., 2007)		EC_{50}~ 2 μM (Henstridge et al., 2009b)	EC_{50}~3 μM (Yin et al., 2009)	100 nM - 3 μM Agonist (Henstridge et al., 2009b)		Agonist (Balenga et al., 2009)	
	hGPR55E-HEK293	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	30 μM Agonist (Kapur et al, 2009)	Not tested	Not tested	Not tested
	hGPR55-PKCβII-GFP-HEK293	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	10 μM Agonist (Kapur et al., 2009)
	hGPR55E-U2OS	Not tested	No effect (Kapur et al., 2009)	Not tested	EC_{50}=9.6 μM (Kapur et al., 2009)	30 μM agonist (Kapur et al, 2009)	Not tested	Not tested	Not tested
AM281	hGPR55-HEK293	EC_{50}>30 μM (Ryberg et al., 2007)	Not tested	3 μM – 30 μM (Henstridge et al., 2009a)	Not tested	3 μM – 30 μM (Henstridge et al., 2009a)	Not tested	Agonist (Balenga et al., 2009)	Not tested
	hGPR55E-U2OS	Not tested	Not tested	Not tested	No effect (Kapur et al., 2009)	Not tested	Not tested	Not tested	Not tested