Send Orders of Reprints at reprints@benthamscience.net

14 *Current Topics in Medicinal Chemistry,* **2013,** *13,* **14-25**

The Therapeutic Potential of Allosteric Ligands for Free Fatty Acid Sensitive GPCRs

Brian D. Hudson[1], Trond Ulven[2] and Graeme Milligan[1,*]

[1]*Molecular Pharmacology Group, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom (BDH, GM);* [2]*Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark (TU)*

Abstract: G protein coupled receptors (GPCRs) are the most historically successful therapeutic targets. Despite this success there are many important aspects of GPCR pharmacology and function that have yet to be exploited to their full therapeutic potential. One in particular that has been gaining attention in recent times is that of GPCR ligands that bind to allosteric sites on the receptor distinct from the orthosteric site of the endogenous ligand. As therapeutics, allosteric ligands possess many theoretical advantages over their orthosteric counterparts, including more complex modes of action, improved safety, more physiologically appropriate responses, better target selectivity, and reduced likelihood of desensitisation and tachyphylaxis. Despite these advantages, the development of allosteric ligands is often difficult from a medicinal chemistry standpoint due to the more complex challenge of identifying allosteric leads and their often flat or confusing SAR. The present review will consider the advantages and challenges associated with allosteric GPCR ligands, and examine how the particular properties of these ligands may be exploited to uncover the therapeutic potential for free fatty acid sensitive GPCRs.

Keywords: Allosteric ligand, FFA1-3, free fatty acid, GPCR**,** GPR84, GPR120 orthosteric ligand

INTRODUCTION

G protein-coupled receptors (GPCRs) comprise the largest family of membrane bound signal transduction proteins. These receptors are defined by their 7 transmembrane structure and ability to transmit intracellular signaling responses, primarily through the activation of guanine nucleotide binding G proteins, to a wide range of extracellular stimuli ranging from photons of light, to peptides, lipids, neurotransmitters and hormones. It is this ability to generate intracellular responses to extracellular stimuli that has made GPCRs the most historically successful drug targets, with recent estimates suggesting at least 30% of currently available therapeutics act via this family of receptor. Despite their success as drug targets, only a small fraction of known GPCRs are currently targeted, suggesting that GPCRs remain a largely untapped therapeutic resource.

In recent times it has become clear that a number of metabolic intermediates, previously believed to exert their biological effects via intracellular targets, are also capable of activating GPCRs. Of particular interest is a group of GPCRs found to respond to free fatty acids. These include a family of three closely related receptors originally named GPR40, GPR43 and GPR41 [1], which have now been grouped into the free fatty acid (FFA) family and renamed FFA1, FFA2 and FFA3 respectively [2], as well as at least two additional receptors officially still classified as orphans, GPR120 and GPR84. Although these receptors all respond to free fatty acid ligands, the specific fatty acids able to activate each receptor varies greatly depending on both chain length and extent of saturation. For example, among the FFA family members, FFA1 is activated by medium and long chain (between 6 and 23 carbon) saturated and unsaturated fatty acids [3,4], while both FFA2 and FFA3 respond only to short chain fatty acids (SCFAs) (up to 6 carbon) [5-7]. GPR120 responds primarily to long chain (16-22 carbon) unsaturated fatty acids [8], with a preference sometimes reported for n-3 fatty acids [9], while GPR84 responds to medium chain length (8-12 carbon) saturated fatty acids [10].

Each of these receptors has generated some interest in drug development programmes, most commonly for the treatment of either metabolic or inflammatory conditions [11-17]. To date, FFA1 has received the most attention, specifically for the treatment of type 2 diabetes, due to its expression in pancreatic β cells and ability to enhance glucose-stimulated insulin secretion (GSIS) [4]. Indeed, at least two FFA1 agonists have progressed into clinical trials for the treatment of type 2 diabetes (Fig. (**1**)); TAK-875 (**1**) [18] and AMG 837 (**2**) [19], with phase II results for **1** showing great promise [20].

Although FFA1 has received the most attention, substantial interest in the other free fatty acid sensitive GPCRs is also evident. Despite such interest, several experimental challenges associated with these receptors have slowed their progression through the drug development process [14]. These issues have included difficulties in identifying potent and selective ligands, and species orthologue differences in ligand pharmacology and, potentially, expression pattern. Moreover, concerns over receptor desensitisation in

*Address correspondence to this author at the Wolfson Link Building 253, University of Glasgow, Glasgow G12 8QQ, Scotland, UK; Tel +44 141 330 5557; Fax: +44 141 330 5481; E-mail: Graeme.Milligan@glasgow.ac.uk

1873-5294/13 $58.00+.00

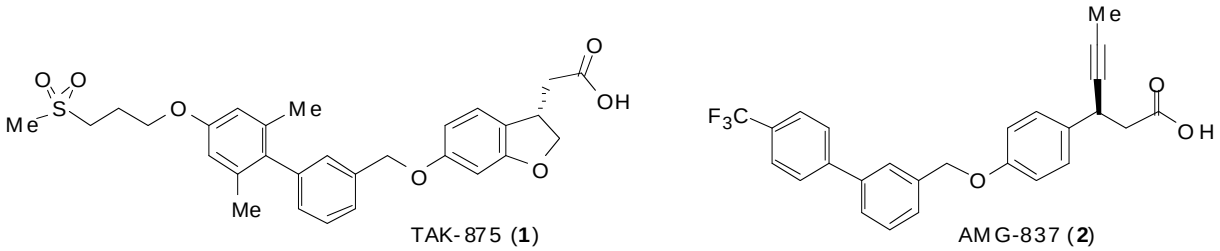


Fig. (1). FFA1 agonists in clinical trials.

situations in which agonism would appear to be the preferred mode of action have been discussed, whilst there are other situations where basic and pre-clinical studies have failed to define clearly whether agonism or antagonism might be the most effective strategy. One drug development approach that may be considered to address and potentially overcome many of these issues is the development of ligands that instead of binding to the orthosteric site of the endogenous ligand(s), interact with a distinct allosteric site. In the present review we will consider the advantages and disadvantages to such an approach to drug development at GPCRs, and examine how this specifically might apply to the receptors for free fatty acids.

ALLOSTERIC MODULATION OF GPCRs

The concept that a molecule binding to a distinct site on a protein may be capable of altering the conformation of that protein so as to modulate function was first described as 'allosterism' in the 1960's [21, 22]. This concept has proven very useful in drug development, particularly for therapeutics targeting ion channels. In contrast, identification and understanding of allosteric modulation of GPCRs has been slower to develop. At least historically, in large part, this has been due to prevalent use of competitive radioligand binding assays to screen for and characterise novel GPCR ligands, an approach not well suited to identifying allosteric compounds [23, 24]. However, the interest in allosteric modulators of GPCRs has received increased attention in recent years [23-28], and indeed at least two GPCR allosteric modulators are now approved for clinical use [24].

Although the classic view of allosterism holds that a ligand binding at one site will result in a change in conformation that alters the function or affinity of a ligand at the second site, this definition is generally modified slightly when used in the context of GPCRs. In this case, a ligand is termed to be 'allosteric' so long as it binds to a site on the receptor distinct from that of the endogenous ligand, the 'orthosteric' binding site. Under this definition allosteric ligands may exert effects through one of two mechanisms (Fig. (**2**)): 1) The classic view of allosteric modulation where binding of the allosteric ligand modulates the properties of the orthosteric site; or 2) by directly altering receptor function independent of the orthosteric site. An allosteric ligand may modulate the orthosteric binding site of a GPCR in several ways, specifically through alteration of either affinity or efficacy. In cases where an allosteric ligand enhances affinity/efficacy it is described as a positive allosteric modulator (PAM), while in cases where the allosteric ligand decreases affinity/efficacy it is referred to as a negative allosteric modulator (NAM). Allosteric ligands that directly activate the receptor (independent of ligand binding at the orthosteric site) are described as allosteric agonists. Further complicating the issue, a single allosteric ligand may possess any combination of these properties, with one common example being the so-called 'ago-allosteric' modulators, which are both allosteric agonists and PAMs. However, this does not represent the only possible scenario and indeed allosteric ligands have been described that at the same time act as both a PAM of affinity and NAM of efficacy [29]. Although this complexity may make defining the properties of allosteric ligands challenging, it is also potentially one of the key advantages that make allosteric ligands attractive in drug discovery.

PHARMACOLOGICAL ADVANTAGES OF ALLOSTERIC GPCR LIGANDS

There are several attributes of allosteric ligands that make them particularly appealing as therapeutics. The first is, as noted above, the varying detail of their potential effects. At least conceptually, an allosteric ligand with a complex mode of action has the potential to produce a more finely tailored biological response than a typical orthosteric agonist or antagonist and, therefore, could be used to develop therapeutics with both improved efficacy and reduced toxicity. Identifying ligands that produce unique functional responses has generated substantial interest lately, although more commonly as it relates to the concept of GPCR functional selectivity or ligand bias [30, 31]. It has become apparent that GPCRs may adopt several different active conformations each capable of producing its own unique set of signaling outcomes. Ligands capable of selectively stabilising one specific active state over the others will therefore direct receptor signaling through the pathway(s) associated with this active state, thus leading to a unique functional response [32, 33]. Conceptually, this could be the basis for a ligand that activates pathways associated with therapeutic benefit, but not pathways associated with adverse side effects. Although this concept applies also to orthosteric ligands, and indeed many functionally selective orthosteric ligands have been described [31, 34, 35]; the complex behavior of allosteric ligands, along with the fact that they may bind to many different sites, suggests that functional selectivity will likely be much more common among allosteric ligands.

The second major advantage of developing GPCR allosteric ligands as therapeutics is that many only produce an effect in a physiological context when an endogenous orthosteric agonist is also present (with allosteric agonists being the obvious exception). Particularly in cases where agonism is preferred this property provides numerous theoretical

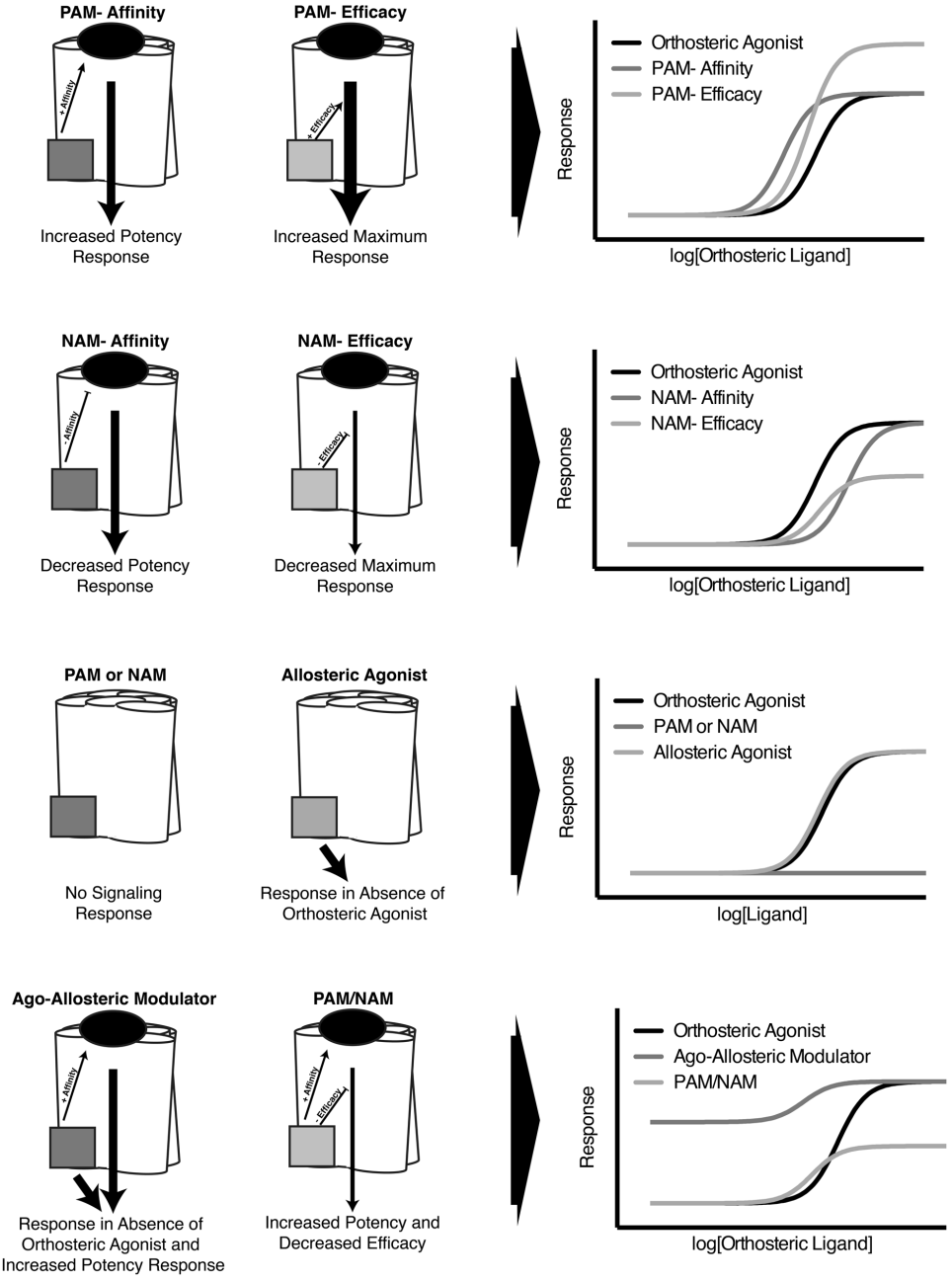


Fig. (2). Complex pharmacological properties of allosteric ligands. A) The effect of a PAM of affinity (dark grey) or efficacy (light grey) on the orthosteric ligand response at a representative GPCR. The PAM binding to a distinct site from the orthosteric agonist (black) alters the receptor conformation so as to increase affinity or efficacy. The resulting concentration-response for the orthosteric agonist in the presence of a single concentration of each PAM is shown. A shift to higher potency for the PAM of affinity and an increased maximal response for the PAM of efficacy are apparent. **B)** The effect of a NAM of affinity (dark grey) or efficacy (light grey) on the orthosteric ligand response at a representative GPCR. The NAM binds to a distinct site to decrease the affinity or efficacy of the orthosteric agonist. The resulting concentration-response to the orthosteric agonist in the presence of single concentration of each NAM shows a shift to lower potency for the NAM of affinity, and a decreased maximal response for the NAM of efficacy. **C)** The effect of an allosteric modulator (either a PAM or NAM; dark grey) compared with a full allosteric agonist (light grey) on the GPCR in the absence of orthosteric ligand. While no response is observed with the modulator, the allosteric agonist produces a full signaling response even in the absence of an orthosteric agonist. In this example the potency of the allosteric agonist is approximately equivalent to the potency of the orthosteric agonist. **D)** Examples of more complex modes of action for allosteric ligands of GPCRs. The ago-allosteric modulator (dark grey) binds to a distinct site and activates the receptor, but also enhances the affinity for the orthosteric agonist. The resulting concentration-response to the orthosteric ligand shows an elevated basal signal due to the allosteric agonism properties of the ligand, combined with a shift to higher orthosteric ligand potency resulting from the PAM property. An example of a ligand that is a PAM of affinity, but a NAM of efficacy is also shown (light grey). In this case, the concentration response to the orthosteric ligand is shifted to lower concentrations due to the PAM effect, while the maximal response is decreased as a result of the NAM properties of the ligand.

benefits including improved safety, decreased risk of desensitisation, and more physiologically appropriate temporal and special patterns of receptor activation [25, 26, 28].

One of the key distinguishing properties of a PAM or NAM compound is that because it binds to a distinct site, and therefore is not directly competitive with the endogenous ligand, its effect are expected to be saturable. This provides a built-in maximum response to the ligand, thus reducing the likelihood of overdose. This has, for example, been critical to developing safe potentiators of the $GABA_A$ receptors [36] and although this example is a ligand-gated ion channel, the same principles should apply to developing allosteric modulators of GPCRs where overdose may be a concern. A second advantage of the fact that allosteric modulators only produce an effect in the presence of an orthosteric ligand is that PAMs have a decreased likelihood of inducing receptor desensitisation. Developing agonist ligands for GPCRs is often challenging since most are rapidly phosphorylated and trafficked away from the cell surface after activation by high efficacy agonists. This is not a general concern for the action of endogenous agonist ligands that are normally released on demand and are present in the vicinity of the GPCR only for a short time. In contrast, a pharmaceutical agent is typically maintained at a concentration sufficiently high to occupy a substantial proportion of the GPCR, and as a result, desensitisation and internalisation could easily lead to drug tolerance or tachyphylaxis. The development of PAMs is an obvious solution to this problem since they should not maintain this constant high level of receptor activation. A closely related advantage of PAMs is that, again because they require the presence of the endogenous ligand to produce response, the temporal and special aspects of their effects should be more physiologically appropriate. Perhaps this is of most value within the central nervous system, where being able to potentiate a neurotransmitter response only at active synapses has obvious advantages over globally activating the neurotransmitter's receptor [26]; however, there are many other systems where this property of a PAM would make it an appealing option over a classic orthosteric agonist as well.

The third major advantage of allosteric ligands for GPCRs is that they provide an alternate means to develop selective ligands for closely related receptors activated by a common endogenous ligand. In these cases, due to the evolutionary pressure to maintain response to the common endogenous ligand, it is not surprising that often the orthosteric binding sites among the individual GPCRs of such families are very similar. As a result, developing selective orthosteric ligands within these families has often been challenging. In contrast, since there is no evidence that allosteric binding sites would be subjected to similar evolutionary pressures, unless they are integral to protein folding and shape, it should be much easier to identify allosteric ligands that will distinguish between closely related receptors. Such has been the case in developing selective allosteric agonists for individual subtypes of both muscarinic [37] and metabotropic glutamate receptors [38]. Finally, since a GPCR presumably has only one orthosteric binding site, yet may have multiple allosteric sites as evidenced by detailed studies of for example muscarinic acetylcholine [37] and FFA1 receptors [39], developing allosteric ligands also represents a viable strategy in situations where the orthosteric site is simply not amenable to drug development.

Challenges Associated with Allosteric GPCR Ligands

While there are many theoretical advantages of allosteric ligands of GPCRs, there are also a number of challenges associated with their development. These begin with the earliest stages of lead identification, a process typically carried out through either structure- or ligand-based screening approaches. Historically, structure-based screening has been challenging at GPCRs, due to the difficulty in obtaining sufficiently detailed structural information at these receptors. Although atomic level crystal structures of bovine rhodopsin have been available for some time [40], it is only recently that detailed crystal structures of additional class A rhodopsin-like GPCRs have started to become available [41-48] and indeed it is even more recent that the first NMR structure of a GPCR within a phospholipid bilayer has been solved [48]. Although the publication of these structures has opened the door to increased structure-based drug development through homology models [50, 51], this is much more challenging for allosteric ligands, as a suitable allosteric site must first be identified. Further complicating this issue, there are now examples of allosteric ligands that interact with both extracellular loop 2 (ECL2) [52, 53], and the intracellular carboxyl terminal tail [54, 55] of GPCRs, two regions that are poorly conserved and therefore difficult to predict through homology models. Considering these factors, at present it is clear that structure-based design approaches are often not the best suited to identifying novel allosteric leads.

As a result of these challenges to structure-based screening, the majority of allosteric ligands have instead been identified through cell-based ligand screening. However, the complexity of effects allosteric ligands may have on their receptors means that even screening approaches are significantly more challenging for allosteric ligands. While radioligand competition assays have long been the standard approach to identify orthosteric ligands at GPCRs, these are poorly suited to identifying allosteric ligands. As a result, most screening for allosteric ligands instead utilizes cell-based functional assays. Complicating the issue, if the desired compound is an allosteric modulator (either PAM or NAM) the screening must be conducted in the presence of an orthosteric agonist. Since it is now recognised that allosteric modulators do not necessary produce the same effect on all orthosteric agonists, a phenomena described as 'probe dependence' [25, 56], the particular orthosteric ligand chosen to use in the screen is critical, though in most cases the endogenous ligand itself is the obvious choice. If instead the goal is to identify allosteric agonists, it is important to confirm any identified 'hits' are actually allosteric in nature through follow-up studies.

Once a lead has been identified, allosteric ligands also present significant challenges to the lead optimisation phase of drug development. Again, due to limitations in homology modeling and, frequently, in defining the location of allosteric binding sites, ligand-based structure-activity relationship (SAR) approaches are generally preferred to structure-based options. Unfortunately, allosteric sites on GPCRs commonly display confusing SAR, making lead optimisation

difficult [57, 58]. A classic example of an allosteric site at which structural alterations to the lead produced little insight derived from efforts to identify PAMs for the metabotropic mGluR5 glutamate receptor. In this study only 4.5% of 985 analogues tested were found to be active, despite the fact that an iterative approach to ligand optimisation was employed [59]. Complicating the issue further, it is not uncommon for very small chemical changes in allosteric ligands to result in profound differences to the pharmacological properties of the ligand, for example switching from a PAM to a NAM. This has been explored in greatest detail for mGluR allosteric ligands and, indeed, it has been proposed that 'molecular-switches' present within allosteric binding sites facilitate these vast differences in function in response to minor chemical alterations [60].

If the issues in lead identification and optimisation can be overcome, there are still additional challenges presented by allosteric ligands. As mentioned above, allosteric binding sites are not believed to be under the same evolutionary pressures as orthosteric sites. While this may be an advantage in developing selective ligands for closely related GPCRs, such a lack of evolutionary pressure may also present a number of significant issues. The first of these is that it could result in much greater variation in the response to an allosteric ligand between species orthologues of a receptor [28]. Although this is likely to complicate the preclinical phase of drug development, where animal models are critical, it does not necessarily preclude the clinical utility of a compound. In contrast, a potentially much more serious problem, that is currently almost completely unexplored, is that the reduced evolutionary pressure may also lead to increased polymorphisms within the human population affecting allosteric ligand function [28], potentially resulting in substantial populations for which a developed allosteric drug may not be effective.

Taken together, it is clear that while allosteric ligands of GPCRs possess many theoretical advantages from the standpoint of pharmacology, they also present a unique, and particularly challenging, problem to the medicinal chemist. Despite these challenges, it is likely that the benefits will outweigh the drawbacks, and the number of therapeutics acting allosterically at GPCRs will almost certainly increase in the coming years. Considering this, the question remains, how such ligands may solve the issues currently associated with drug development at receptors for free fatty acids.

ALLOSTERIC LIGANDS OF FREE FATTY ACID RECEPTORS AS THERAPEUTICS

All five of the GPCRs currently recognised to respond to free fatty acids have received at least some interest as therapeutic targets. This has been directed primarily towards metabolic and inflammatory conditions, and perhaps not surprisingly given its prevalence, type 2 diabetes has received the greatest attention. The development of therapeutics targeting these receptors has, however, been slowed by a number of specific challenges, not least the development of suitably selective ligands [14]. While this, and other challenges may be addressed with allosteric ligands, there has so far only been a limited effort aimed at identifying and characterising the therapeutic potential of allosteric ligands at the free fatty acid receptors.

Allosteric Ligands for FFA1

Of the free fatty acid sensitive GPCRs, FFA1 has received by far the most attention. This is primarily due to the fact that FFA1 is expressed in pancreatic β cells and enhances GSIS [4]. An important feature of this action is that FFA1 agonists only stimulate increased insulin secretion in the presence of glucose, a clear advantage over traditional insulin secretagogues that stimulate insulin secretion regardless of the prevailing concentration of glucose and, as a result, are prone to inducing hypoglycemic episodes in patients [61]. Interestingly, this means that an orthosteric FFA1 agonist produces a functional response in β cells that is in many ways analogous to an allosteric modulator in which glucose is analogous to the endogenous orthosteric ligand. Thus, an FFA1 orthosteric agonist is likely to possess many of the benefits typically associated with allosteric modulators, including producing physiologically appropriate temporal responses (only in the presence of elevated glucose), a decreased likelihood of desensitisation, and reduced incidence of adverse effects (hypoglycemia). As a result, the desire to develop allosteric modulators of FFA1 may not be as strong as for other receptors and, indeed, at least within publically available information, drug development programmes at FFA1 have focused entirely on finding potent and selective orthosteric agonists. These efforts have identified many different classes of FFA1 agonist, many with relatively good potency (Fig. (**3**)) [19, 62-68], all of which have been assumed to be orthosteric. However, a recent study has demonstrated that at least two of these ligands are in fact allosteric [39], drawing into question the previously assumed orthosteric mode of action of many other FFA1 agonists.

The work to define the orthosteric binding pocket of FFA1 focused on the fact that both the endogenous fatty acid ligands and most of the known synthetic ligands possessed a carboxylate head group and could be considered as synthetic fatty acids. As a result, early modeling and mutagenesis studies explored key positively charged residues that might coordinate this carboxylate [69, 70]. These studies identified two arginine residues (R5.39 and R7.35) and one asparagine (N6.55) that when mutated resulted in a receptor with greatly reduced function for the synthetic agonist GW9508 (**3**). These mutations also reduced function for the endogenous fatty acid linoleic acid (though to a lesser degree). It was hence concluded that these amino acids coordinated the binding of both **3** and linoleic acid [70]. Although the vast majority of FFA1 agonist ligands possess such a carboxylate, notable exceptions are the thiazolidinedione compounds including: the PPARγ activator rosiglitazone (**5**) [71], and an FFA1 selective compound developed by Merck (**6**) [64]. Interestingly, although the thiazolidinedione compounds do not possess a carboxylate, they were found to lose function at mutants of the key arginine and asparagine residues and it was therefore concluded that the thiazolidinedione was acting as a carboxylic acid bioisostere and that these compounds were acting orthosterically [72].

This orthosteric nature of synthetic FFA1 agonists was not drawn into question until very recently when it was shown that **2**, a carboxylate containing agonist of FFA1, did not bind competitively with the fatty acid docosahexaenoic acid (**7**) [39]. Even more interestingly, another class of carboxylate containing FFA1 agonist represented by AMG

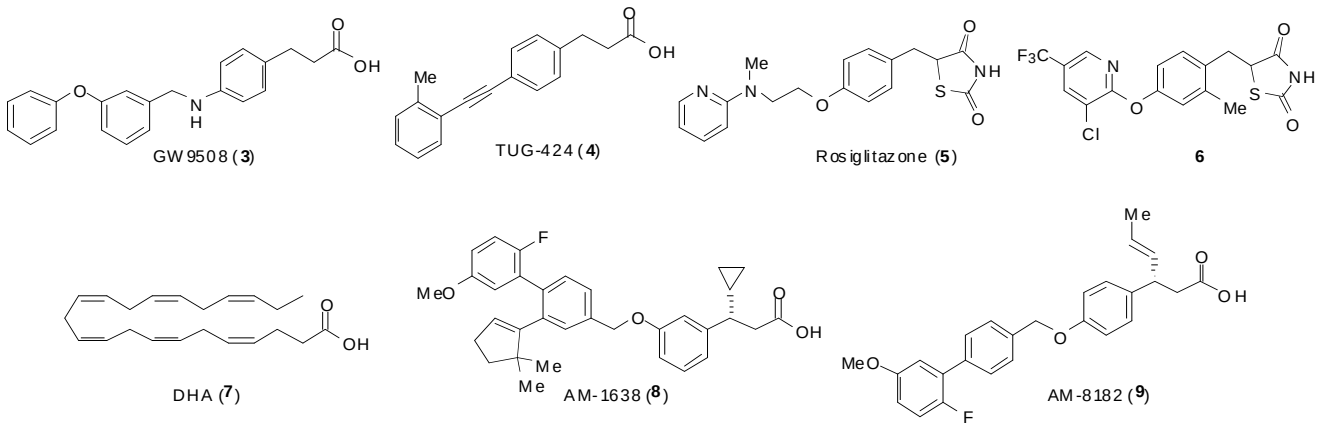


Fig. (3). FFA1 ligands.

1638 (**8**), did not bind competitively with either **2** or **7**. Furthermore, both **2** and **8** showed positive cooperativity with each other as well as with **7** and therefore appear to be ago-allosteric modulators of FFA1 [39]. This work indicates there are at least two allosteric binding sites on FFA1, each able to bind carboxylate containing ligands. A third related synthetic FFA1 agonist (**9**) binds to a site distinct from the allosteric sites of **2** and **8**, and appears to be an orthosteric ligand. In most cases, the structures of allosteric ligands for a receptor are unrelated to its orthosteric ligand. However, for FFA1 this clearly does not appear to be the case, as the structures of **2**, **8** and **9** must be regarded as closely related. This opens the possibility that other carboxylate containing FFA1 agonists might also act as allosteric ligands. Mutation of R5.39 or R7.35 to alanine eliminated the response to **2**, while it had only a small effect on the responses to **6**, **8** and **9** [39]. This is similar to what was observed by others with complete loss of function for **3**, and only a small reduction reported for linoleic acid [69, 70], suggesting that **3** most likely binds to the same allosteric site as **2**. Clearly much more work needs to be done to determine exactly which FFA1 agonists are binding to which sites and, as described by Lin *et al.* [39], the availability of radiolabelled ligands from different chemical series will likely be instrumental in defining this.

With the recognition that at least some FFA1 agonists are allosteric, it raises the question as to what therapeutic implications this might have. Interestingly, it has been noted that several FFA1 agonists, including **2**, **3** and **4**, are in fact partial agonists compared with the endogenous fatty acids [62, 63, 39, 73]. Given that **2** (and possibly **3** and **4**) is allosteric, this may suggest that allosteric agonists of FFA1 produce different functional outcomes than their orthosteric counterparts. In addition to the well-established enhancement of GSIS, FFA1 has also been shown to stimulate the release of glucagon-like peptide-1 (GLP-1) [74], another promising property for the treatment of type 2 diabetes. Interestingly, a recent study demonstrated that while full agonists of FFA1 were capable of both enhancing GSIS and stimulating GLP-1 secretion *in vivo*; the partial agonist, **2**, only produced the GSIS effect, with no observed GLP-1 secretion [73]. Such an observation may suggest orthosteric full agonists are likely to be better therapeutics than allosteric partial agonists.

Another factor that has complicated the development of FFA1 as a therapeutic target is the fact that although short-term fatty acid treatment enhances GSIS [75], chronic fatty acid exposure actually results in β cell death and reduced insulin secretion [76]. Early studies in FFA1$^{-/-}$ mice suggested that both effects were mediated by FFA1 [77], while conflicting subsequent work indicates that only the positive effects on GSIS are FFA1-mediated [78, 79]. These conflicting results led to significant controversy and confusion over whether agonists or antagonists of FFA1 would actually be most beneficial for long-term treatment. This uncertainty appeared to be resolved when it was demonstrated that an FFA1 selective agonist, **6**, produced only the positive effects on GSIS, and did not result in toxic effects on β cells [64]. However, given the findings that some FFA1 agonists are allosteric [39] and that there are clear differences in functional outcomes between orthosteric and allosteric FFA1 agonists [73], it is at least conceivable that the toxic effects of fatty acids on β cells could still be FFA1 mediated, but only occur in response to full agonists, or ligands binding to the orthosteric site. It is plausible, therefore, that allosteric agonists of FFA1 may prove to be the best therapeutic option. Indeed, given that the most advanced FFA1 agonist in clinical trials, **1**, has significant structural similarity to the allosteric compound **2**, including the entire 4-(3-phenylbenzyloxy)dihydrocinnamic acid scaffold and the same stereochemistry at the β-carbon, it is entirely possible that **1** is in fact also allosteric. Considering the recently published promising clinical trial results with **1** [20, 80], this compound may, in the relatively near future, represent a therapeutically approved allosteric agonist of FFA1.

Allosteric Ligands for FFA2 and FFA3

The SCFA receptors FFA2 and FFA3 have also generated interest as therapeutic targets to treat both metabolic and inflammatory conditions. However, the paucity of selective ligands capable of distinguishing between the two has presented a significant challenge in fully defining their function and biology [14]. Although there are differences in the rank order of potency of endogenous SCFAs at FFA2 compared with FFA3 [5, 6], the potency and selectivity of the endogenous ligands is low [81]. As FFA2 and FFA3 are often co-expressed [6, 78], this has made interrogating the function of one over the other difficult in the absence of receptor knockdown or knockout strategies. Further complicating the issue is the recent observation that there is significant species

orthologue variation in both the potency and selectivity of the endogenous SCFAs for these receptors [82, 83], further highlighting the need for selective synthetic ligands that can distinguish between FFA2 and FFA3.

An early attempt to identify selective orthosteric ligands for these receptors screened a library of small carboxylic acids (SCAs) and successfully identified compounds with reasonable selectivity both for FFA2 (Fig. (**4**)), tiglic acid (**10**), and FFA3, 1-methylcyclopropanecarboxylic acid (**11**), [81]. However, although these compounds are selective, their potency is also very low due to the small size and low binding energy of SCAs. Not surprisingly given the relatedness of FFA1-3, efforts to define the orthosteric binding site of FFA2 and FFA3 indicated that an ionic interaction between the key residues conserved across all three FFA family members: R5.39, R7.35 and H6.55 (N6.55 in FFA1) and the carboxylate of the ligand is the primary basis of ligand-receptor interaction [84]. The small size of both the SCFAs and SCAs has led to suggestions that the orthosteric binding pocket of these receptors may be too small to facilitate the development of more potent molecules, however, the recent description of carboxylate containing FFA2-selective agonists that appear likely to be orthosteric (**12**) [85, 17], as well as an orthosteric FFA2 inverse agonist (**13**) [83, 86], indicates that at least for FFA2, potent orthosteric compounds can be identified and potentially developed.

Challenges in identifying selective orthosteric ligands for FFA2 and FFA3 have resulted in some efforts towards identifying and characterising allosteric compounds for these receptors. In particular, (*S*)-4-chloro-α-(1-methylethyl)-*N*-2-thiazolylbenzeneacetamide (**14**) was identified in a high throughput screen and found to be a selective ago-allosteric modulator of FFA2 [87]. As is often the case with allosteric ligands, subsequent attempts to explore the SAR of this series were unable to improve on its modest potency despite modification of all parts of the structure [88, 89]. Defining the allosteric site where **14** binds has also presented a substantial challenge, with several modeling and mutagenesis studies unable to determine a precise mode of binding [88-91]. Somewhat surprisingly, although significant species variation has been observed among the orthosteric SCFAs at FFA2, **14** appears to show little variation in potency and activity between human, rodent [83] and bovine (unpublished observation) orthologues of FFA2. There is, however, also some indication that **14** may produce different signaling responses at FFA2 than the SCFAs, specifically **14** appears to be a full agonist in some pathways but only a partial agonist in others [89], perhaps suggesting some degree of functional selectivity. This, however, needs to be more clearly defined in future studies.

At present there are far fewer reports of FFA3 selective ligands. Currently only a single series has been disclosed through a patent by Arena Pharmaceuticals, including compounds reported as FFA3 agonists (**15**) and antagonists (**16**) [92]. Although it is unclear if these compounds are orthosteric or allosteric, the fact that they do not contain carboxylates, or obvious carboxylate bioisosteres, suggests that they may be allosteric in nature. Small chemical changes between **15** and **16** result in transposition from agonism to antagonism, an observation similar to the molecular switches seen with allosteric modulators of other GPCRs [60]. Although indirect this may also hint that these ligands are allosteric modulators of FFA3.

Although the limited availability of selective ligands has made it challenging to validate FFA2 or FFA3 as therapeutic targets, FFA2 in particular has still attracted significant in-

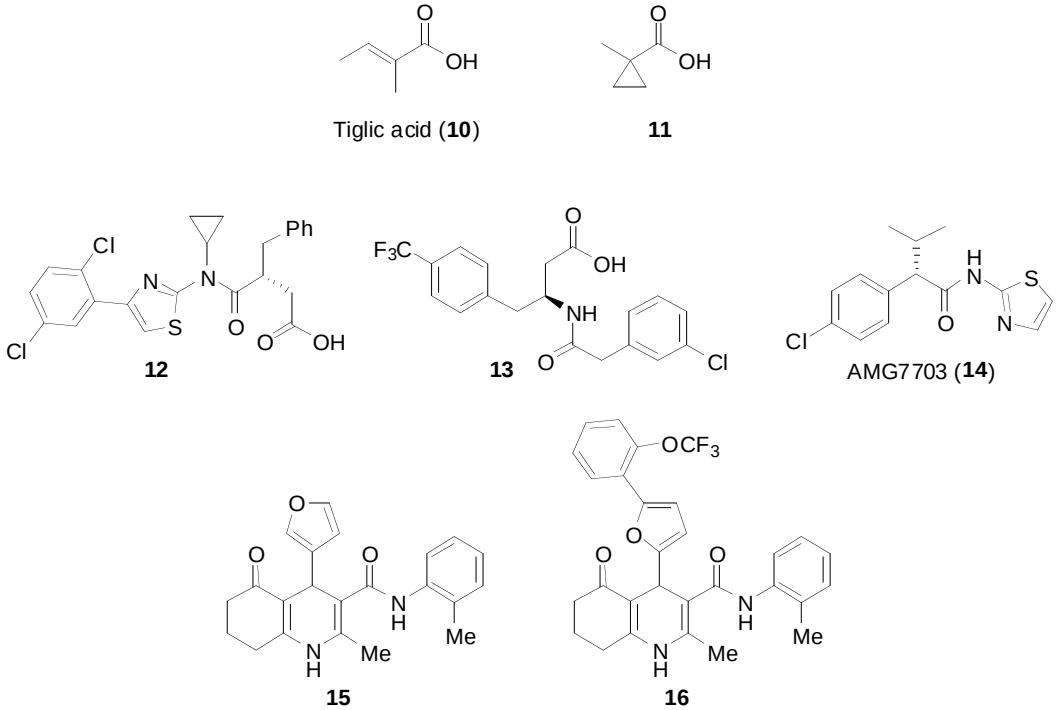


Fig. (4). FFA2 and FFA3 ligands.

terest for the treatment of metabolic and inflammatory conditions [17, 93, 94]. FFA2 agonists are reported to inhibit lipolysis and regulate plasma free fatty acid levels [95], enhance glucose uptake in adipocytes [85] and stimulate GLP-1 release [96]; all properties indicating that agonism of FFA2 may be beneficial for metabolic conditions. However, FFA2 agonists, including the allosteric compound **14**, also promote neutrophil chemotaxis [93, 97], a property potentially detrimental in inflammatory conditions. Indeed, $FFA2^{-/-}$ mice were protected against tissue damage in a model of colitis [93], suggesting FFA2 antagonists, and not agonists, might be useful therapeutically for inflammation. These competing considerations may greatly complicate the development of FFA2 ligands and the receptor as a therapeutic target. Indeed, this may suggest that allosteric ligands of FFA2 with more complex or functionally selective modes of action may be a good option for future development of therapeutics targeting this receptor.

Allosteric Ligands and GPR120

Interest in GPR120 as a therapeutic target for type 2 diabetes was piqued with the observation that it is expressed in enteroendocrine cells and may mediate fatty acid-stimulated GLP-1 secretion [8]. The subsequent finding that GPR120 is also responsible for anti-inflammatory and insulin sensitizing effects of n-3 fatty acids only increased the level of interest [9]. Recent genetic data has further strengthened the case for GPR120 as a therapeutic target in that $GPR120^{-/-}$ mice are more prone to obesity than wild type mice and that a poorly functional polymorphism of GPR120 has been linked to obesity in a human population [98]. Finally, a systems genetics approach identified GPR120 as one of the top 20 genes whose expression level in human islets was linked to type 2 diabetes. Specifically, GPR120 expression was decreased in islets isolated from the cadavers of diabetics compared to healthy controls [99]. Together, these findings suggest that GPR120 agonists might be very promising for the treatment of type 2 diabetes.

Despite the interest in GPR120, validation has been slowed by a lack of potent and selective ligands. Although having only limited sequence similarity, GPR120 responds to the same long chain fatty acids as FFA1. To date, only two academic papers have reported synthetic agonists selective for GPR120 over FFA1 (Fig. (**5**)). The first of these reported a low potency ligand (**17**) with poor selectivity based on carboxylic acid derivatives of PPARγ agonists [100], while the second described a significantly more potent and selective ligand (**18**) derived from a previously described series of FFA1 agonists [101]. Given the lack of sequence similarity between GPR120 and FFA1 it is perhaps surprising that a wider range of selective and potent compounds has not been described. This may reflect a particular challenge associated with developing ligands for the orthosteric binding site of GPR120 and that developing allosteric agonists for GPR120 may prove a useful approach to obtaining better selectivity in the future.

The orthosteric binding pocket of GPR120 has been examined in some detail, primarily through homology modeling [100, 101, 102]. Interestingly, none of the key positively charged amino acids associated with fatty acid binding to the FFA1-3 family members are conserved in GPR120. Instead, each of the models identified one alternate arginine at position 2.64 as being responsible for a key ionic interaction with the carboxylate head group of the ligand [100, 101, 102]. The importance of R2.64 to the binding and/or function of both endogenous fatty acids [103] and **18** [101] has now been verified by mutagenesis. In addition to the endogenous fatty acids, **17** and **18**, a number of other ligands have been described as agonists of GPR120 [14, 16]. These include several disclosed in recent patents **19** [104], **20** [105], **21** [106]; the natural product grifolic acid (**22**) [107]; and several FFA1 agonists, including **3** [62] and **4** [101], that besides relatively potent activity on FFA1 also exhibit micromolar potency on GR120. Structural examination of these GPR120 agonists reveals that the majority are elongated lipophilic compounds containing a carboxylate, and therefore may be regarded as fatty acid analogues and be expected to act at the orthosteric site. However, given the recent data on the non-equivalence of the mode of binding of apparently similar FFA1 ligands [39], this should be considered tentative in the absence of further information. The two obvious exceptions are **19** and **21**, compounds described in patents from Banyu Pharmaceutical [104, 106]. The 3-hydroxyisoxazole moiety of **21** is a carboxylic acid bioisostere, and the compound therefore may very well be an orthosteric agonist. Compound **19,** however, contains no

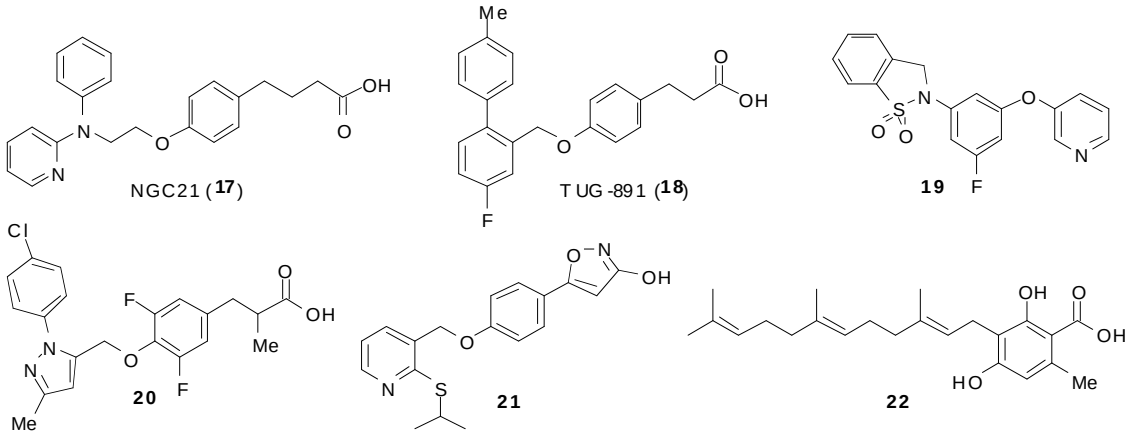


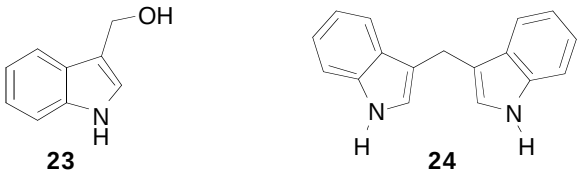
Fig. (5). GPR120 ligands.

thosteric agonist. Compound **19,** however, contains no acidic functional group and therefore is perhaps more likely to be an allosteric agonist of GPR120.

Finally, although no allosteric modulators have been reported for GPR120, there may be a strong argument for their development. Agonist stimulation of GPR120 has been shown to cause rapid receptor phosphorylation [108], strong interaction with β-arrestin-2 [9, 101, 103] and internalisation of the receptor [8, 103]. Given that agonism clearly appears to be the preferred mode of action for therapeutically useful GPR120 ligands, these properties indicate that desensitisation could present a major challenge to the future development of such therapeutics. Considering this, a strong case could be made that a PAM would be the best approach for targeting GPR120 in order to avoid these concerns of receptor desensitisation.

Allosteric Ligands and GPR84

The function of GPR84 remains largely unknown, with very few studies published on this receptor. GPR84 appears to be expressed primarily in immune cells [109] and, as a result, most work has focused on a potential role in inflammation [110]. A very interesting recent model cell study has indicated that GPR84 expression may be markedly up regulated in adipose tissue in response to macrophage infiltration, suggesting that GPR84 may play a role in the link between adiposity and diabetes [111]. However, the lack of potent and selective ligands for GPR84 has made studying this in more detail challenging. The endogenous medium chain fatty acids have modest potency and selectivity and no studies aiming to identify novel synthetic ligands have been published. Currently the only described synthetic ligands for GPR84 are indol-3-calbinol (**23**), and 3,3'-diindolylmethane (**24**) (Fig. (**6**)), two compounds that were identified as surrogate agonists of GPR84 even before it was recognized as a receptor for free fatty acids [112]. Interestingly, while the majority of orthosteric agonists for the other free fatty acid receptors contain a carboxylate moiety, neither **23** nor **24** do, suggesting that these compounds may in fact be allosteric agonists, and future work should consider this possibility.



23 **24**

Fig. (6). GPR84 ligands.

CONCLUSION

Despite presenting significant technical challenges to the drug development process, allosteric ligands for GPCRs provide numerous theoretical advantages over their orthosteric counterparts. Allosteric ligands have already been described for two of the free fatty acid sensitive GPCRs, with potentially important therapeutic implications. It also appears likely that several additional ligands already described for these receptors are in fact also allosteric, and indeed allosteric agonists and modulators appear destined to play a critical role in future drug development at fatty acid-sensitive GPCRs.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Danish Council for Strategic Research 11-116196 (to TU and GM), the Strategic Partnership on Food & Drink Science, Scottish Government Programme of Research 2011-2016/Ref UGW 854/11 (to GM) and the Canadian Institutes of Health Research (fellowship to BDH). Wellcome Trust grant number 089600/Z/09/Z (to GM).

REFERENCES

[1] Sawzdargo, M.; George, S.R.; Nguyen, T.; Xu, S.; Kolakowski, L.F.; O'Dowd, B.F. A cluster of four novel human G protein-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1. *Biochem. Biophys. Res. Commun.*, **1997**, *239*(2), 543-547.

[2] Stoddart, L.A.; Smith, N.J.; Milligan, G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. *Pharmacol. Rev.*, **2008**, *60*(4), 405-417.

[3] Briscoe, C.P.; Tadayyon, M.; Andrews, J.L.; Benson, W.G.; Chambers, J.K.; Eilert, M.M.; Ellis, C.E.; Elshourbagy, N.A.; Goetz, A.S.; Minnick, D.T.; Murdock, P.R.; Sauls, H.R. Jr.; Shabon, U.; Spinage, L.D.; Strum, J.C.; Szekers, P.G.; Tan, K.B.; Way, J.M.; Ignar, D.M.; Wilson, S.; Muir, A.I. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.*, **2003**, *278*(13), 11303-11311.

[4] Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; Tanaka, H.; Maruyama, M.; Satoh, R.; Okubo, S.; Kizawa, H.; Komatsu, H.; Matsumura, F.; Noguchi, Y.; Shinohara, T.; Hinuma, S.; Fujisawa, Y.; Fujino, M. Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. *Nature*, **2003**, *422*(6928), 173-176.

[5] Brown, A.J.; Goldsworthy, S.M.; Barnes, A.A.; Eilert, M.M.; Tcheang, L.; Daniels, D.; Muir, A.I.; Wigglesworth, M.J.; Kinghorn, I.; Fraser, N.J.; Pike, N.B.; Strum, J.C.; Steplewski, K.M.; Murdock, P.R.; Holder, J.C.; Marshall, F.H.; Szekeres, P.G.; Wilson, S.; Ignar, D.M.; Foord, S.M.; Wise, A.; Dowell, S.J. The Orphan G Protein-coupled Receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* **2003**, *278*(13), 11312-11319.

[6] Le Poul, E.; Loison, C.; Struyf, S.; Springael, J.Y.; Lannoy, V.; Decobecq, M.E.; Brezillon, S.; Dupriez, V.; Vassart, G.; Van Damme, J.; Parmentier, M.; Detheux, M. Functional characterization of human receptors for Short Chain fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* **2003**, *278*(28), 25481-25489.

[7] Nilsson, NE.; Kotarsky, K.; Owman, C.; Olde, B. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem. Biophys. Res. Commun.* **2003**, *303*(4), 1047-1052.

[8] Hirasawa, A.; Tsumaya, K.; Awaji, T.; Katsuma, S.; Adachi, T.; Tamada, M.; Sugimoto, Y.; Miyazaki, S.; Tsujimoto, G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.*, **2005**, *11*(1), 90-94.

[9] Oh, D.Y.; Talukdar, S.; Bae, E.J.; Imamura, T.; Morinaga, H.; Fan, W.; Li, P.; Lu, W.J.; Watkins, S.M.; Olefsky, J.M. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* **2010**, *142*(5), 687-698.

[10] Wang, J.; Wu, X.; Simonavicius, N.; Tian, H.; Ling, L. Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. *J. Biol. Chem.*, **2006**, *281*(45), 34457-34464.

[11] Costanzi, S.; Neumann, S.; Gershengorn, M.C. Seven transmembrane spanning receptors for free fatty acids as therapeutic targets for diabetes mellitus: pharmacological, phylogenetic and drug discovery aspects. *J. Biol. Chem*. **2008**, *283*(24), 16269-16273.

[12] Vangaveti, V.; Shashidhar, V.; Jarrod, G.; Baune, B.T.; Kennedy, R.L. Free fatty acid receptors: emerging targets for treatment of diabetes and its complications. *Ther. Adv. Endocrinol. Metab*., **2010**, *1*(4), 165-175

[13] Hara, T.; Hirasawa, A.; Ichimura, A.; Kimura, I.; Tsujimoto, G. Free fatty acid receptors FFAR1 and GPR120 as novel therapeutic targets for metabolic disorders. *J. Pharm. Sci.*, **2011**, *100*(9), 3594-3601.

[14] Hudson, B.D.; Smith, N.J.; Milligan, G. Experimental challenges to targeting poorly characterized GPCRs: Uncovering the therapeutic potential for free fatty acid receptors. *Adv. Pharmacol*., **2011**, *62*, 175-218.

[15] Talukdar, S.; Olefsky, J.M.; Osborn, O. Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases. *Trends Pharmacol. Sci*., **2011**, *32*(9), 543-50.

[16] Holliday, N.D.; Watson, S.-J.; Brown, A.J.H. Drug discovery opportunities and challenges at G protein coupled receptors for long chain free fatty acids. *Front. Endocrinol*. 2:112.

[17] Ulven, T. Short-chain free fatty acid receptors FFA2/GPR43 and FFA3/GPR41 as new potential therapeutic targets. *Front. Endocrinol.* **2012**, *3*, 111.

[18] Tsujiha, Y.; Ito, R.; Suzuki, M.; Harada, A.; Negoro, N.; Yasuma, T.; Momose, Y.; Takeuchi, K. TAK-875, an orally available G protein-coupled receptor 40/free fatty acid receptor 1 agonist, enhances glucose-dependent insulin secretion and improves both postprandial and fasting hyperglycemia in type 2 diabetic rats. *J. Pharmacol. Exp. Ther*., **2011**, *339*(1), 228-37.

[19] Lin, D.C.; Zhang, J.; Zhuang, R.; Li, F.; Nguyen, K.; Chen, M.; Tran, T.; Lopez, E.; Lu, J.Y.; Li, X.N.; Tang, L.; Tonn, G.R.; Swaminath, G.; Reagan, J.D.; Chen, J.L.; Tian, H.; Lin, Y.J.; Houze, J.B.; Luo, J. AMG 837: A Novel GPR40/FFA1 agonist that enhances insulin secretion and lowers glucose levels in rodents. *PLoS One*., **2011**, *6*(11), e27270.

[20] Burant, C.F.; Viswanathan, P.; Marcinak, J.; Cao, C.; Vakilynejad, M.; Xie, B.; Leifke, E. TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2., randomized.; double-blind.; placebo-controlled trial. *Lancet.*, **2012**, *6736*(11), 61879-61875.

[21] Monod, J.; Changeux, J.P.; Jacob, F. Allosteric proteins and cellular control systems. *J. Mol. Biol*., **1963**, *6*, 306-329.

[22] Monod, J.; Wyman, J.; Changeux, J.P. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **1965**, *12*, 88-118.

[23] Christopoulos, A.; Kenakin, T. G protein-coupled receptor allosterism and complexing. *Parmacol. Rev*., **2002**, *54*(2), 323-374.

[24] Milligan, G.; Smith, N.J. Allosteric modulation of heterodimeric G-protein-coupled receptors. *Trends Pharmacol. Sci.,* **2007**, *28*(12), 615-620.

[25] May, L.T.; Leach, K.; Sexton, P.M.; Christopoulos, A. Allosteric modulation of G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol*., **2007**, *47*, 1-51.

[26] Conn, P.J.; Christopoulos, A.; Lindsley, C.W. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discov*. **2009**, *8*(1), 41-54.

[27] Smith, N.J; Milligan, G. Allostery at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. *Pharmacol. Rev*., **2010**, *62*(4), 701-725.

[28] Müller, C.E.; Schiedel, A.C.; Baqi, Y. Allosteric modulators of rhodopsin-like G protein-coupled receptors: opportunities in drug development. *Pharmacol. Ther*., **2012**, *135*(3), 292-315.

[29] Price, M.R.; Baillie, G.L.; Thomas, A.; Stevenson, L.A.; Easson, M.; Goodwin, R.; McLean, A.; McIntosh, L.; Goodwin, G.; Walker, G.; Westwood, P.; Marrs, J.; Thomson, F.; Cowley, P.; Christopoulos, A.; Pertwee, R.G.; Ross, R.A. Allosteric modulation of the cannabinoid CB1 receptor. *Mol. Pharmacol*., **2005**, *68*(5), 1484-1495.

[30] Mailman, R.B. GPCR functional selectivity has therapeutic impact. *Trends Pharmacol. Sci*., **2007**, *28*(8), 390-396.

[31] Hudson, B.D.; Hébert, T.E.; Kelly, M.E.M. Ligand- and heterodimer-directed signaling of the CB(1) cannabinoid receptor. *Mol. Pharmacol.*, **2010**, *77*(1), 1-9.

[32] Kenakin, T. Agonist-receptor efficacy. II. Agonist trafficking of receptor signals. *Trends Pharmacol. Sci*., **1995**, *16*(7), 232–238

[33] Kenakin, T. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J*., **2001**, *15*(3), 598–611.

[34] Smith, N.J.; Bennett, K.A.; Milligan, G. When simple agonism is not enough: emerging modalities of GPCR ligands. *Mol. Cell. Endocrinol*., **2011**, *331*(2), 241-247.

[35] Raehal, K.M.; Schmid, C.L.; Groer, C.E.; Bohn, L.M. Functional selectivity at the μ-opioid receptor: implications for understanding opioid analgesia and tolerance. *Pharmacol. Rev*. **2011**, *63*(4), 1001-1019.

[36] Sigel, E.; Buhr, A. The benzodiazepine binding site of $GABA_A$ recepors. *Trends Pharmacol. Sci*., **1997**, *18*, 425-429.

[37] Gregory, K.J.; Sexton, P.M.; Christopoulos A. Allosteric modulation of muscarinic acetylcholine receptors. *Curr. Neuropharmacol*., **2007**, *5*(3), 157-167.

[38] Sheffler, D.J.; Gregory, K.J.; Rook, J.M.; Conn, P.J. Allosteric modulation of metabotropic glutamate receptors. *Adv. Pharmacol*. **2011**, *62*, 37-77.

[39] Lin, D.C.; Guo, Q.; Luo, J.; Zhang, J.; Nguyen, K.; Chen, M.; Tran, T.; Dransfield, P.J.; Brown, S.P.; Houze, J.; Vimolratana, M.; Jiao, X.Y.; Wang, Y.; Birdsall, N.J.; Swaminath, G. Identification and pharmacological characterization of multiple allosteric binding sites on the free Fatty Acid 1 receptor. *Mol. Pharmacol*., **2012**, *82*(5), 843-859.

[40] Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C.A.; Motoshima, H.; Fox, B.A.; Le Trong, I.; Teller, D.C.; Okada, T.; Stenkamp, R.E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*. **2000**, *289*(5480), 739-745.

[41] Rasmussen, S.G.; Choi, H.J.; Rosenbaum, D.M.; Kobilka, T.S.; Thian, F.S.; Edwards, P.C.; Burghammer, M.; Ratnala, V.R.; Sanishvili, R.; Fischetti, R.F.; Schertler, G.F.; Weis, W.I.; Kobilka, B.K. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature*, **2007**, *450*(7168), 383-287.

[42] Warne, T.; Serrano-Vega, M.J.; Baker, J.G.; Moukhametzianov, R.; Edwards, P.C.; Henderson, R.; Leslie, A.G.; Tate, C.G.; Schertler, G.F. Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature*, **2008**, *454*(7203), 486-491.

[43] Jaakola, V.P.; Griffith, M.T.; Hanson, M.A.; Cherezov, V.; Chien, E.Y.; Lane, J.R.; Ijzerman, A.P.; Stevens, R.C. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science*, **2008**, *322*(5905), 1211-1217.

[44] Rasmussen, S.G.; DeVree, B.T.; Zou, Y.; Kruse, A.C.; Chung, K.Y.; Kobilka, T.S.; Thian, F.S.; Chae, P.S.; Pardon, E.; Calinski, D.; Mathiesen, J.M.; Shah, S.T.; Lyons, J.A.; Caffrey, M.; Gellman, S.H.; Steyaert, J.; Skiniotis, G.; Weis, W.I.; Sunahara, R.K.; Kobilka, B.K. Crystal structure of the β2 adrenergic receptor-Gs protein complex. *Nature*, **2011**, *477*(7366), 549-555.

[45] Granier, S.; Manglik, A.; Kruse, AC.; Kobilka, T.S.; Thian, F.S.; Weis, W.I.; Kobilka, B.K. Structure of the δ-opioid receptor bound to naltrindole. *Nature*. **2012**, *485*(7398), 400-404.

[46] Hanson, M.A.; Roth, C.B.; Jo, E.; Griffith, M.T.; Scott, F.L.; Reinhart, G.; Desale, H.; Clemons, B.; Cahalan, S.M.; Schuerer, S.C.; Sanna, M.G.; Han, G.W.; Kuhn, P.; Rosen, H.; Stevens, R.C. Crystal structure of a lipid G protein-coupled receptor. *Science*, **2012**, *335*(6070), 851-855.

[47] Manglik, A.; Kruse, A.C.; Kobilka, T.S.; Thian, F.S.; Mathiesen, J.M.; Sunahara, R.K.; Pardo, L.; Weis, W.I.; Kobilka, B.K.; Granier, S. Crystal structure of the μ-opioid receptor bound to a morphinan antagonist. *Nature*., **2012**, *485*(7398), 321-326.

[48] Park, S.H.; Das, B.B.; Casagrande, F.; Tian, Y.; Nothnagel, H.J.; Chu, M.; Kiefer, H.; Maier, K.; De Angelis, A.A.; Marassi, F.M.; Opella, S.J. Structure of the chemokine receptor CXCR1 in phospholipid bilayers. *Nature,* **2012**, *491*(7426), 779-783.

[49] Thompson, A.A; Liu, W.; Chun, E.; Katritch, V.; Wu, H.; Vardy, E.; Huang, X.P.; Trapella, C.; Guerrini, R.; Calo, G.; Roth, B.L.; Cherezov, V.; Stevens, R.C. Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. *Nature*, **2012**, *485*(7398), 395-399.

[50] Costanzi, S. Homology modeling of class a G protein-coupled receptors. *Methods Mol. Biol*. **2012**, *857*, 259-279.

[51] Levit, A.; Barak, D.; Behrens, M.; Meyerhof, W.; Niv, MY. Homology model-assisted elucidation of binding sites in GPCRs. *Methods Mol. Biol*., **2012**, *914*, 179-205.

[52] Avlani, V.A.; Gregory, K.J.; Morton, C.J.; Parker, M.W.; Sexton, P.M.; Cristopoulos, A. Critical role for the second extracellular loop in the binding of both orthosteric and allosteric G protein-coupled receptors ligands. *J. Biol. Chem*., **2007**, *282*, 25677-25686.

[53] Peeters, M.C.; Wisse, L.E.; Dinaj, A.; Vroling, B.; Vriend, G.; Ijzerman, A.P. The role of the second and third extracellular loops of the adenosine A1 receptor in activation and allosteric modulation. *Biochem. Pharmacol*., **2012**, *84*(1), 76-87.

[54] Andrews, G.; Jones, C.; Wreggett, K.A. An intracellular allosteric site for a specific class of antagonists of the CC chemokine G protein-coupled receptors CCR4 and CCR5. *Mol. Pharmacol*., **2008**, *73*, 855-867.

[55] Dowal, L.; Sim, D.S.; Dilks, J.R.; Blair, P.; Beaudry, S.; Denker, B.M.; Koukos, G.; Kuliopulos, A.; Flaumenhaft, R. Identification of an antithrombotic allosteric modulator that acts through helix 8 of PAR1. *Proc. Nat. Acad. Sci. U.S.A.*, **2011**, *108*(7), 2951-2956.

[56] Valant, C.; Felder, C.C.; Sexton, P.M.; Christopoulos, A. Probe dependence in the allosteric modulation of a G protein-coupled receptor: implications for detection and validation of allosteric ligand effects. *Mol. Pharmacol*., **2012**, *81*(1), 41-52.

[57] Urwyler, S. Allosteric modulation of family C G-protein-coupled receptors: from molecular insights to therapeutic perspectives. *Pharmacol. Rev*., **2011**, *63*(1), 59-126.

[58] Decker, M.; Holzgrabe, U. M1 muscarinic acetylcholine receptor allosteric modulators as potential therapeutics opportunities for treating Alzheimer's disease. *Med. Chem. Commun*., **2012**, *3*(7), 752-762.

[59] Zhao, Z.; Wisnoski, D.D.; O'Brien, J.A.; Lemaire, W.; Williams, D.L, Jr.; Jacobson, M.A.; Wittman, M.; Ha, S.N.; Schaffhauser, H.; Sur, C.; Pettibone, D.J.; Duggan, M.E.; Conn, P.J.; Hartman, G.D.; Lindsley, C.W. Challenges in the development of mGluR5 positive allosteric modulators: the discovery of CPPHA. *Bioorg. Med. Chem. Lett*., **2007**, *17*(5), 1386-1391.

[60] Wood, M.R.; Hopkins, C.R.; Brogan, J.T.; Conn, P.J.; Lindsley, C.W. "Molecular switches" on mGluR allosteric ligands that modulate modes of pharmacology. *Biochemistry*. **2011**, 50(13), 2403-10.

[61] Barnett, A.H.; Cradock, S.; Fisher, M.; Hall, G.; Hughes, E.; Middleton, A. Key considerations around the risks and consequences of hypoglycaemia in people with type 2 diabetes. *Int. J. Clin. Pract.* **2010**, *64*(8), 1121-1129.

[62] Briscoe, C.P.; Peat, A.J.; McKeown, S.C.; Corbett, D.F.; Goetz, A.S.; Littleton, T.R.; McCoy, D.C.; Kenakin, T.P.; Andrews, J.L.; Ammala, C.; Fornwald, J.A.; Ignar, D.M.; Jenkinson, S. Pharmacolgical regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. *Br. J. Pharmacol*., **2006**, *148*(5), 619-628.

[63] Christiansen, E.; Urban, C.; Merten, N.; Liebscher, K.; Karlsen, K.K.; Hamacher, A.; Spinrath, A.; Bond, A.D.; Drewke, C.; Ullrich, S.; Kassack, M.U.; Kostenis, E.; Ulven, T. Discovery of potent and selective agonists for the free fatty acid receptor 1 (FFA1/GPR40), a potential target for the treatment of type II diabetes. *J. Med. Chem*., **2008**, *51*(22), 7061-7064.

[64] Tan, C.P.; Feng, Y.; Zhou, Y.P.; Eiermann, G.J.; Petrov, A.; Zhou, C.; Lin, S.; Salituro, G.; Meinke, P.; Mosley, R.; Akiyama, T.E.; Einstein, M.; Kumar, S.; Berger, J.P.; Mills, S.G.; Thornberry, N.A.; Yang, L.; Howard, A.D. Selective small-molecule agonists of G protein-coupled receptor 40 promote glucose-dependent insulin secretion and reduce blood glucose in mice. *Diabetes*, **2008**, *57*(8), 2211-2219.

[65] Bharate, S.B.; Nemmani, K.V.S.; Vishwakarma, R.A. Progress in the discovery and development of small-molecule modulators of G-protein-coupled receptor 40 (GPR40/FFA1/FFAR1): an emerging target for type 2 diabetes. *Expert Opin. Ther. Patents*., **2009**, *19*(2), 237-264.

[66] Negoro, N.; Sasaki, S.; Mikami, S.; Ito, M.; Suzuki,M.; Tsujihata, Y.; Ito, R.; Harada, A.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Kogame, A.; Matsunaga, S.; Yasuma, T.; Momose, Y. Discovery of TAK-875: a potent, selective and orally bioavailable GPR40 agonist. *Med. Chem. Lett.*, **2010**, *1*, 290-294.

[67] Christiansen, E.; Urban, C.; Grundmann, M.; Due-Hansen, M.E.; Hagesaether, E.; Schmidt, J.; Pardo, L.; Ullrich, S.; Kostenis, E.; Kassack, M.: Ulven, T. Identification of a potent and selective free fatty acid rReceptor 1 (FFA1/GPR40) agonist with favorable physicochemical and *in vitro* ADME Properties. *J. Med. Chem*., **2011**, *54*(19), 6691-6703.

[68] Christiansen, E.; Due-Hansen, M.E.; Urban, C.; Grundmann, M.; Schröder, R.; Hudson, B.D.; Milligan, G.; Cawthorne, M.A.; Kostenis, E.; Kassack, M.U.; Ulven, T. Free fatty acid receptor 1 (FFA1/GPR40) agonists: mesylpropoxy appendage lowers lipophilicity and improves ADME properties. *J. Med. Chem*. **2012**, *55*(14), 6624-6628.

[69] Tikhonova, I.G.; Sum, C.S.; Neumann, S.; Thomas, C.J.; Raaka, B.M.; Costanzi, S.; Gershengorn, M.C. Bidirectional.; iterative approach to the structural delineation of the functional "chemoprint" in GPR40 for agonist recognition. *J. Med. Chem*., **2007**, *50*(13), 2981-2989.

[70] Sum, C.S.; Tikhonova, I.G.; Neumann, S.; Engel, S.; Raaka, B.M.; Costanzi, S.; Gershengorn, M.C. Identification of residues important for agonist recognition and activation in GPR40. *J. Biol. Chem*., **2007**, *282*(40), 29248-29255.

[71] Kotarsky, K.; Nilsson, N.E.; Flodgren, E.; Owman, C.; Olde, B. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. *Biochem. Biophys. Res. Commun*., **2003**, *301*(2), 406-410.

[72] Smith, N.J.; Stoddart, L.A.; Devine, N.M.; Jenkins, L.; Milligan, G. The action and mode of binding of thiazolidinedione ligands at free fatty acid receptor 1. *J. Biol. Chem*., **2009**, *284*(26), 17527-17539.

[73] Luo, J.; Swaminath, G.; Brown, S.P.; Zhang, J.; Guo, Q.; Chen, M.; Nguyen, K.; Tran, T.; Miao, L.; Dransfield, P.J.; Vimolratana, M.; Houze, J.B.; Wong, S.; Toteva, M.; Shan, B.; Li, F.; Zhuang, R.; Lin, D.C. A Potent Class of GPR40 Full Agonists Engages the EnteroInsular Axis to Promote Glucose Control in Rodents. *PLoS One*, **2012**, *7*(10), e46300.

[74] Edfalk, S.; Steneberg, P.; Edlund, H. GPR40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes*, **2008**, *57*(9), 2280-2287.

[75] Greenough, W.B.; Crespin, S.R.; Steinberg, D. Hypoglycaemia and hyperinsulinaemia in response to raised free-fatty-acid levels. *Lancet*, **1967**, *2*(7530), 1334–1336.

[76] El-Assaad, W.; Buteau, J.; Peyot, M.L.; Nolan, C.; Roduit, R.; Hardy, S.; Joly, E.; Dbaibo, G.; Rosenberg, L.; Prentki, M. Saturated Fatty Acids Synergize with Elevated Glucose to Cause Pancreatic β-Cell Death. *Endocrinology*, **2003**, *144*(9), 4154-4163.

[77] Steneberg, P.; Rubins, N.; Bartoov-Shifman, R.; Walker, M.D.; Edlund, H. The FFA receptor GPR40 links hyperinsulinemia.; hepatic steatosis.; and impaired glucose homeostasis in mouse. *Cell Metab*., **2005**, *1*(4), 245-258.

[78] Kebede, M.A.; Alquier, T.; Latour, M.G.; Poitout, V. Lipid receptors and islet function: therapeutic implicaitons? *Diabetes Obes. Metab*., **2009**, *11*(Suppl 4), 10-20.

[79] Lan, H.; Hoos, L.M.; Liu, L.; Tetzloff, G.; Hu, W.; Abbondanzo, S.J.; Vassileva, G.; Gustafson, E.L.; Hedrick, J.A.; Davis, H.R. Lack of FFAR1/GPR40 does not protect mice from high-fat diet-induced metabolic disease. *Diabetes*, **2008**, *57*(11), 2999-3006.

[80] Kaku, K.; Araki, T.; Yoshinaka, R. Randomized, double-blind, dose-ranging study of TAK-875, a novel GPR40 agonist, in Japanese patients with inadequately controlled type 2 diabetes. *Diabetes Care*, **2013**, *36*(2), 245-250.

[81] Schmidt, J.; Smith, N.J.; Christiansen, E.; Tikhonova, I.G.; Grundmann, M.; Hudson, B.D.; Ward, R.J.; Drewke, C.; Milligan, G.; Kostenis, E.; Ulven, T. Selective orthosteric free fatty acid receptor 2 (FFA2) agonists: identification of the structural and chemical requirements for selective activation of FFA2 versus FFA3. *J Biol Chem.*, **2011**, *286*(12), 10628-10640.

[82] Hudson, B.D.; Christiansen, E.; Tikhonova, I.G.; Grundmann, M.; Kostenis, E.; Adams, D.R.; Ulven, T.; Milligan, G. Chemically engineering ligand selectivity at the free fatty acid receptor 2 based on pharmacological variation between species orthologs. *FASEB J*., **2012**, *26*(12), 4951-4965.

[83] Hudson, B.D.; Tikhonova, I.G.; Pandey, S.K.; Ulven, T.; Milligan, G. Extracellular ionic locks determine variation in constitutive activity and ligand potency between species orthologs of the free fatty acid receptors FFA2 and FFA3. *J. Biol. Chem.*, **2012**, *287*(49), 41195-411209.

[84] Stoddart, L.A.; Smith, N.J.; Jenkins, L.; Brown, A.J.; Milligan, G. Conserved polar residues in transmembrane domains V.; VI.; and VII of free fatty acid receptor 2 and free fatty acid receptor 3 are required for the binding and function of short chain fatty acids. *J. Biol. Chem.*, **2008**, *283*(47), 32913-32924.

[85] Hoveyda, H.; Brantis, C.E.; Dutheuil, G.; Zoute, L.; Schils, D.; Bernard, J. Compounds, pharmaceutical composition and methods for use in treating metabolic disorders. International patent Application, WO 2010/066682, June 17, **2010**.

[86] Brantis, C.E.; Ooms, F.; Bernard, J. Novel amino acid derivatives and their use as GPR43 receptor modulators. International patent application, WO/2011/092284, August 4, **2011**.

[87] Lee, T.; Schwandner, R.; Swaminath, G.; Weiszmann, J.; Cardozo, M.; Greenberg, J.; Jaeckel, P.; Ge, H.; Wang, Y.; Jiao, X.; Liu, J.; Kayser, F.; Tian, H.; Li, Y. Identification and functional characterization of allosteric agonists for the G protein-coupled receptor FFA2. *Mol. Pharmacol*., **2008**, *74*(6), 1599-1609.

[88] Wang, Y.; Jiao, X.; Kayser, F.; Liu, J.; Wang, Z.; Wanska, M.; Greenberg, J.; Weiszmann, J.; Ge, H.; Tian, H.; Wong, S.; Schwandner, R.; Lee, T.; Li, Y. The first synthetic agonists of FFA2: Discovery and SAR of phenylacetamides as allosteric modulators. *Bioorg. Med. Chem. Lett.*, **2010**, *20*(2), 493-498.

[89] Smith, N.J.; Ward, R.J.; Stoddart, L.A.; Hudson, B.D.; Kostenis, E.; Ulven, T.; Morris, J.C.; Tränkle, C.; Tikhonova, I.G.; Adams, D.R.; Milligan G. Extracellular loop 2 of the free fatty acid receptor 2 mediates allosterism of a phenylacetamide ago-allosteric modulator. *Mol. Pharmacol*., **2011**, *80*(1), 163-173.

[90] Milligan, G.; Stoddart, L.A.; Smith, N.J. Agonism and allosterism: the pharmacology of the free fatty acid receptors FFA2 and FFA3. *Br. J. Pharmacol*., **2009**, *158*(1), 146-153.

[91] Swaminath, G.; Jaeckel, P.; Guo, Q.; Cardozo, M.; Weiszmann, J.; Lindberg, R.; Wang, Y.; Schwandner, R.; Li, Y. Mutational analysis of G-protein coupled receptor-FFA2. *Biochem. Biophys. Res. Commun*., **2011**, *405*(1), 122-127.

[92] Leonard, J.N.; Chu,Z.L.; Bruce, M.A.; Boatman, P.D. GPR41 and modulators thereof for the treatment of insulin-related disorders. International patent application, WO/2006/052566, May 18, **2006**.

[93] Sina, C.; Gavrilova, O.; Förster, M.; Till, A.; Derer, S.; Hildebrand, F.; Raabe, B.; Chalaris, A.; Scheller, J.; Rehmann, A.; Franke, A.; Ott, S.; Häsler, R.; Nikolaus, S.; Fölsch, U.R.; Rose-John, S.; Jiang, H.P.; Li, J.; Schreiber, S.; Rosenstiel, P. G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. *J. Immunol*., **2009**, *183*(11), 7514-7522.

[94] Maslowski, K.M.; Vieira, A.T.; Ng, A.; Kranich, J.; Sierro, F.; Yu, D.; Schilter, H.C.; Rolph, M.S.; Mackay, F.; Artis, D.; Xavier, R.J.; Teixeira, M.M.; Mackay, C.R. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, **2009**, *461*(7268), 1282-1286.

[95] Ge, H.; Li, X.; Weiszmann, J.; Wang, P.; Baribault, H.; Chen, J.-L.; Tian, H.; Li, Y. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology*, **2008**, *149*(9), 4519-4526.

[96] Tolhurst, G.; Heffron, H.; Lam, YS.; Parker, HE.; Habib, AM.; Diakogiannaki, E.; Cameron, J.; Grosse, J.; Reimann, F.; Gribble, F.M. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes*, **2012**, *61*(2), 364-371.

[97] Vinolo, M.A.; Ferguson, G.J.; Kulkarni, S.; Damoulakis, G.; Anderson, K.; Bohlooly,-Y.M.; Stephens, L.; Hawkins, P.T.; Curi, R. SCFAs induce mouse neutrophil chemotaxis through the GPR43 receptor. *PLoS One*, **2011**, *6*(6), e21205.

[98] Ichimura, A.; Hirasawa, A.; Poulain-Godefroy, O.; Bonnefond, A.; Hara, T.; Yengo, L.; Kimura, I.; Leloire, A.; Liu, N.; Iida, K.; Choquet, H.; Besnard, P.; Lecoeur, C.; Vivequin, S.; Ayukawa, K.; Takeuchi, M.; Ozawa, K.; Tauber, M.; Maffeis, C.; Morandi, A.; Buzzetti, R.; Elliott, P.; Pouta, A.; Jarvelin, M.R.; Körner, A.; Kiess, W.; Pigeyre, M.; Caiazzo, R.; Van Hul, W.; Van Gaal, L.; Horber, F.; Balkau, B.; Lévy-Marchal, C.; Rouskas, K.; Kouvatsi, A.; Hebebrand, J.; Hinney, A.; Scherag, A.; Pattou, F.; Meyre, D.; Koshimizu, T.A.; Wolowczuk, I.; Tsujimoto, G.; Froguel, P. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature*, **2012**, *483*(7389), 350-354.

[99] Taneera, J.; Lang, S.; Sharma, A.; Fadista, J.; Zhou, Y.; Ahlqvist, E.; Jonsson, A.; Lyssenko, V.; Vikman, P.; Hansson, O.; Parikh, H.; Korsgren, O.; Soni, A.; Krus, U.; Zhang, E.; Jing, X.J.; Esguerra, J.L.; Wollheim, C.B.; Salehi, A.; Rosengren, A.; Renström, E.; Groop, L. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. *Cell Metab*., **2012**, *16*(1), 122-134.

[100] Suzuki, T.; Igari, S.; Hirasawa, A.; Hata, M.; Ishiguro, M.; Fujieda, H.; Itoh, Y.; Hirano, T.; Nakagawa, H.; Ogura, M.; Makishima, M.; Tsujimoto, G.; Miyata, N. Identification of G protein-coupled receptor 120-selective agonists derived from PPARgamma agonists. *J. Med. Chem*., **2008**, *51*(23), 7640-7644.

[101] Shimpukade, B.; Hudson, B.D.; Hovgaard, C.K.; Milligan, G.; Ulven, T. Discovery of a potent and selective GPR120 agonist. *J. Med. Chem*., **2012**, *55*(9), 4511-4515.

[102] Sun, Q.; Hirasawa, A.; Hara, T.; Kimura, I.; Adachi, T.; Awaji, T.; Ishiguro, M.; Suzuki, T.; Miyata, N.; Tsujimoto, G. Structure-activity relationships of GPR120 agonists based on a docking simulation. *Mol. Pharmacol*., **2010**, *78*(5), 804-810.

[103] Watson, S.J.; Brown, A.J.; Holliday, N.D. Differential signaling by splice variants of the human free fatty acid receptor GPR120. *Mol. Pharmacol*., **2012**, *81*(5), 631-642.

[104] Arkawa, K.; Nishimura, T.; Sugimoto, Y.; Takahashi, H. Novel isoindolin-1-one derivative. International patent application, WO 2010/104195, September *16*, **2010.**

[105] Ma, J.; Novack, A.; Nashashibi, I.; Pham, P.; Rabbat, C.J.; Song, J.; Shi, D.F.; Zhao, Z.; Choi, Y.-J.; Chen, X. Aryl GPR120 receptor agonists and uses therof. International patent application, WO 2010/048207, April 29, **2010**.

[106] Hashimoto, N.; Sasaki, Y.; Nakama, C.; Ishikawa, M. Novel phenyl-isoxayol-3-ol derative. United States patent application, US 2010/0130559, May 27, **2010**.

[107] Hara, T.; Hirasawa, A.; Sun, Q.; Sadakane, K.; Itsubo, C.; Iga, T.; Adachi, T.; Koshimizu, T.; Hashimoto, T.; Asakawa, Y.; Tsujimoto, G. Novel selective ligands for free fatty acid receptors GPR120 and GPR40. *Naunyn Schmiedebergs Arch. Pharmacol*., **2009**, *380*(3), 247-255.

[108] Burns, R.N.; Moniri, N.H. Agonsim with the omega-3 faty acids α-linolenic acid and docosahexaenoic acid mediates phosphorylation of both the short and long isoforms of the human GPR120 receptor. *Biochem. Biophys. Res. Commun*., **2010**, *396*(4), 1030-1035.

[109] Yousefi, S.; Cooper, P.R.; Potter, S.L.; Mueck, B.; Jarai, G. Cloning and expression analysis of a novel G-protein-coupled receptor selectively expressed on granulocytes. *J. Leukoc. Biol*., **2001**, *69*(6), 1045-1052.

[110] Bouchard C.; Pagé, J.; Bédard, A.; Tremblay, P.; Vallières, L. G protein-coupled receptor 84, a microglia-associated protein expressed in neuroinflammatory conditions. *Glia*, **2007**, 55, 790-800.

[111] Nagasaki, H.; Kondo, T.; Fuchigami, M.; Hashimoto, H.; Sugimura, Y.; Ozaki, N.; Arima, H.; Ota, A.; Oiso, Y.; Hamada, Y. Inflammatory changes in adipose tissue enhance expression of GPR84; a medium-chain fatty acid receptor: TNFα enhances GPR84 expression in adipocytes. *FEBS Lett.*, **2012**, *586*(4), 368-372.

[112] Takeda, S.; Yamamoto, A.; Okada, T.; Matsumura, E.; Nose, E.; Kogure, K.; Kojima, S.; Haga, T. Identification of surrogate ligands for orphan G protein-coupled receptors. *Life Sci*., **2003**, *74*(2-3), 367-377.

Received: November 16, 2012 Revised: November 29, 2012 Accepted: November 30, 2012

PMID: 23409763