Ligands at Free Fatty Acid Receptor 1 (GPR40)

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Contents

Abstract

FFA1 is a G protein-coupled receptor activated by medium- to long-chain fatty acids. FFA1 plays important roles in various physiological processes such as insulin secretion and energy metabolism. FFA1 expressed on pancreatic β-cells and intestine contributes to insulin and incretin secretion, respectively. These physiological functions of FFA1 are interesting as an attractive drug target for type II diabetes and metabolic disorders. A number of synthetic FFA1 ligands have been developed and they have contributed to our current understanding of the physiological and pathophysiological functions of FFA1 both in in vitro and

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in vivo studies. In addition, these synthetic ligands also provided information on the structure–activity relationships of FFA1 ligands. Further, FFA1 protein crystallized with one of the high affinity agonist leads provided useful insights for the development of more effective ligands. Among FFA1 ligands, several compounds have been further investigated in the clinical trials. Thus, FFA1 ligands have great potential as drug candidates. In this section, recent progress about FFA1 ligands and the possibility of their clinical use are described.

Keywords
Diabetes • Fatty acids • FFA1 ligand • Free fatty acid receptor 1 (FFA1) • G protein-coupled receptor • Metabolic disorder • Structure–activity relationships

1 Introduction

G protein-coupled receptors (GPCR) are the major target for approved clinical medicines of various diseases. The human genome project revealed that a large number of GPCRs are encoded in the genome; however, there are still a significant number of orphan GPCRs that are considered as attractive drug targets (Civelli et al. 2013).

Deorphanization of GPCRs identified a group of GPCRs activated by free fatty acids (Briscoe et al. 2003; Hirasawa et al. 2005; Itoh et al. 2003) that are now defined as free fatty acid receptors (FFARs) (Stoddart et al. 2007; Davenport et al. 2013). To date, four FFARs are characterized and defined by differences of the carbon chain length of fatty acid ligands. FFA1 and FFA4 are activated by medium- to long-chain fatty acids, while FFA2 and FFA3 are activated by short-chain fatty acids. FFARs therefore act as sensors for fatty acids.

FFA1 is highly expressed in intestine and pancreatic β-cells. FFA1 activation induces incretin and insulin secretion from intestinal endocrine cells and pancreatic β-cells, respectively. Therefore, synthetic compounds that can interact with FFA1 selectively are considered as potential drug candidates for the treatment of metabolic disorder such as type 2 diabetes. To date, a number of synthetic compounds have been developed and structure–activity relationships of these FFA1 agonists have also been investigated (Defossa and Wagner 2014). In addition, FFA1 crystal structure with the selective agonist TAK-875 ([(3S)-6-({2′,6′-dimethyl-4-′-[3-(methylsulfonyl)propoxy]biphe-nyl-3-yl}meth-oxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid hemi-hydrate) has been reported (Negoro et al. 2010), which provided great insights into the process of developing the ligands more efficiently in terms of the selectivity of the ligands and of pharmacodynamics and pharmacokinetics. Several ligands developed by pharmaceutical companies have entered clinical trials. In this chapter, FFA1 ligands and their therapeutic utility are described together with recent progress in this area.

2 Natural Ligands

Several groups reported that medium- to long-chain fatty acids activated $[Ca^{2+}]_i$ responses in FFA1 expressing cells (Briscoe et al. 2003; Itoh et al. 2003; Kotarsky et al. 2003). Since FFAs are known to have numerous biological effects on intracellular signaling, high-throughput screening using inducible or stable expression of the receptor in cell lines was applied to evaluate FFA1 specific responses.

Agonistic activity of fatty acid ligands of FFA1 was measured by $[Ca^{2+}]_i$ responses. The rank order of the agonistic activities of fatty acid ligands are as follows: docosahexaenoic acid (DHA, C22:6) > α-linolenic acid (α-LA) (C18:3) > oleic acid (C18:1) > palmitic acid (C16) > lauric acid (C12) > capric acid (C10) > caprylic acid (C8) (Christiansen et al. 2015; Itoh et al. 2003). Almost all fatty acids ligands can activate FFA1 in the submicromolar range (Christiansen et al. 2015).

On the other hand, short-chain fatty acids including acetic acid (C2), butyric acid (C4), caproic acid (C6), and methyl linoleate could not activate FFA1 signaling, which indicated that the carbon chain length and carboxylate group in the fatty acid structure would be critical for agonistic activity at FFA1. Several compounds which have different structures from fatty acids have also been reported as natural ligands, for example, conjugated linoleic acid (Schmidt et al. 2011) known as a dietary component associated with anticarcinogenic effects, showed agonistic activity in FFA1 expressing cells. Since fatty acid ligands were considered to exhibit their biological function via some other signaling pathways, including fatty acid binding proteins (Furuhashi and Hotamisligil 2008), FFA1 signaling could provide a better explanation for some of the biological processes of FFAs.

3 Synthetic Ligands

3.1 Agonists

To develop high affinity and selective ligands is of great interest not only for exploring pharmacological functions of FFA1 but also for developing potential candidates for clinical use, because physiological functions of FFA1 are strongly related to glucose homeostasis and energy metabolic processes such as secretion of insulin and incretins and therefore regulation of blood glucose levels. Several research groups including pharmaceutical companies have developed various synthetic compounds (Fig. 1) and characterized them as novel FFA1 ligands by using in vitro and in vivo studies (Garrido et al. 2006; Humphries et al. 2009; Krasavin et al. 2016; Li et al. 2016; Tikhonova et al. 2008; Yang et al. 2016). Various studies have used the synthetic compound GW9508 (4-[[(3-Phenoxyphenyl)methyl]amino] benzenepropanoic acid) as a reference compound. GW9508 also activated FFA4 signaling, although it showed approximately 100-fold selectivity compared to FFA4 (Briscoe et al. 2006; Tikhonova et al. 2008). Some studies have also utilized the FFA1 antagonist GW1100. GW1100 showed inhibitory effects on glucose-stimulated insulin secretion (GSIS) from the mouse insulinoma cell line MIN6

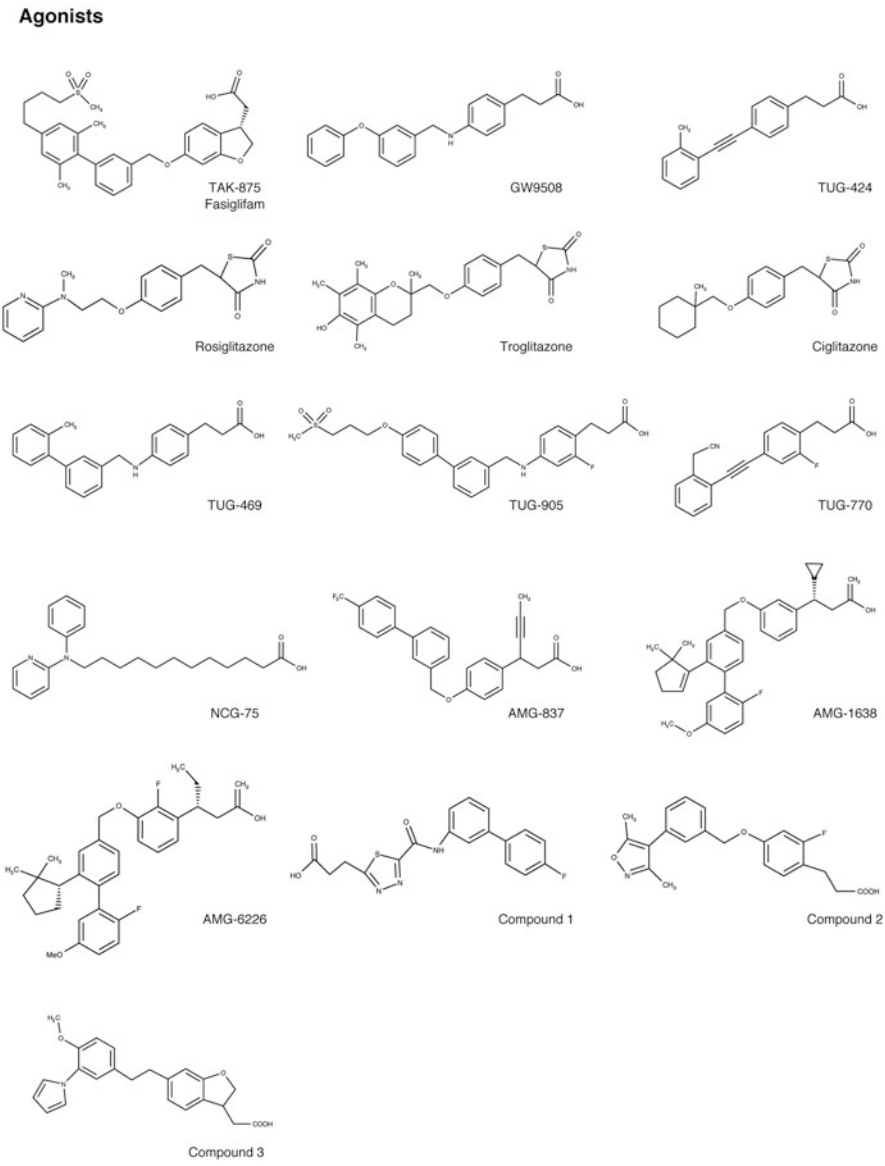

Fig. 1 Representative FFA1 ligands. The structures of representative FFA1 selective ligands are shown

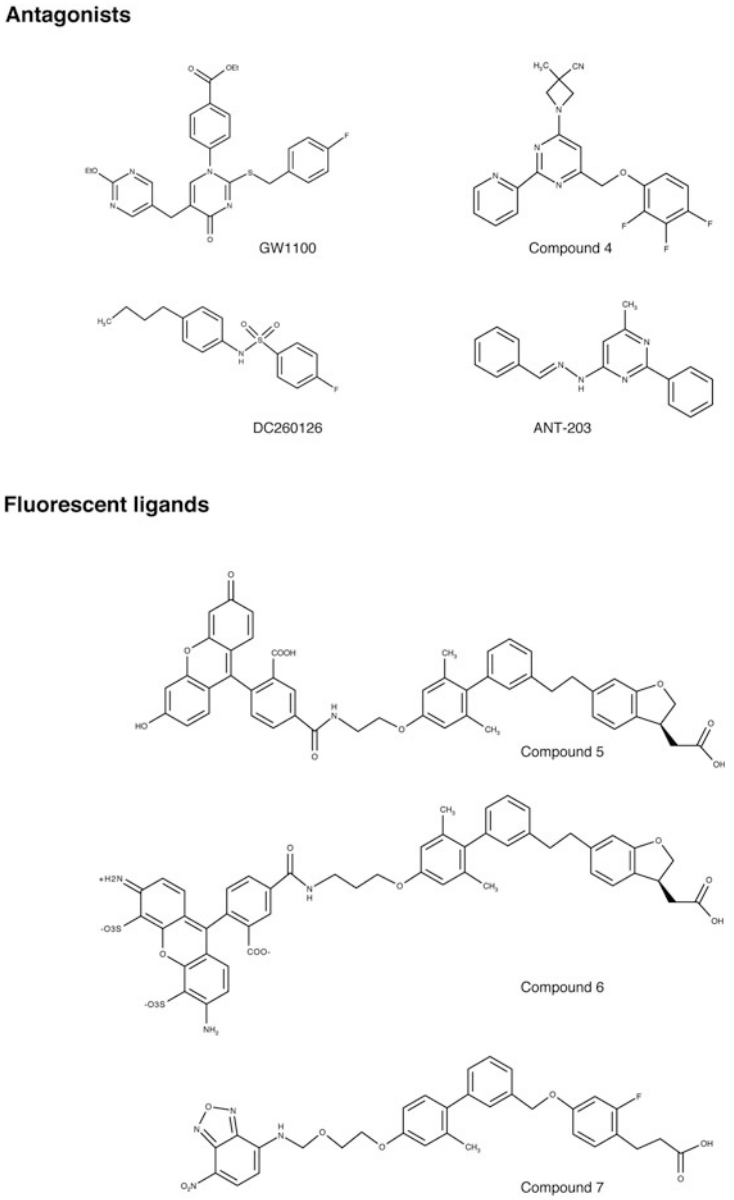

Fig. 1 (continued)

induced by GW9508 (Briscoe et al. 2006). Some research groups reported that thiazolidinediones including peroxisome proliferator-activated receptor-γ (PPARγ) agonists: rosiglitazone, troglitazone, and ciglitazone, those are known as anti-diabetic compounds, showed agonistic activity at FFA1 (Kotarsky et al. 2003; Smith et al. 2009; Stoddart et al. 2007). A series of 4-phenethynyldihydrocinnamic acids showed agonistic activity at FFA1. Among these compounds, TUG-424 showed GSIS in INS-1 cell line and pancreatic islets isolated from wild type mice, but not from FFA1 knock out mice. TUG-770, which was developed based on the structure of TUG-424 and has improved metabolic stability and short plasma half-life, showed a potent effect on glucose tolerance in diet-induced obesity mice, a situation that was sustained after 29 days of chronic dosing (Christiansen et al. 2008, 2013). Further structural optimizations were to lower lipophilicity and increase metabolic stability of the ligand. Combining features of TAK-875 and TUG-469 was explored and resulted in TUG-905, which showed lower lipophilicity and higher metabolic stability while preserving potency to activate FFA1 (Christiansen et al. 2010, 2012).

TAK-875 was developed based on phenylpropanoic acid derivatives. Although these compounds appeared to be susceptible to β-oxidation at the phenylpropanoic acid moiety, cyclization of the phenylpropanoic acid moiety, which produced a series of fused phenylalkanoic acids, showed favourable pharmacokinetic profiles (Negoro et al. 2010). Although prolonged exposure of FFA ligands showed lipotoxicity in the INS-1 cell line and primary pancreatic β-cells, prolonged treatment of TAK-875 did not impair GSIS and insulin content (Tsujihata et al. 2011; Yashiro et al. 2012). TAK-875 showed high selectivity for FFA1 compared to other FFARs including FFA4 ($EC_{50} = 14$ nm in human FFA1 and >10 μm in human FFA4) (Negoro et al. 2010; Srivastava et al. 2014). In addition, TAK-875 acts as partial agonist that binds to an allosteric binding site of FFA1 and increases the agonistic activity of endogenous fatty acid ligands (Srivastava et al. 2014).

Our group developed a synthetic ligand, NCG75, using *in silico* docking simulations of FFA1. NCG75 showed potent agonistic activity in ERK1/2 and $[Ca^{2+}]_i$ assays. NCG75 promoted insulin secretion from mouse insulinoma MIN6 cell line, which expresses FFA1 endogenously (Takeuchi et al. 2013). AMG-837 that was developed by the modification of a series of β-substituted phenylpropanoic acids was identified and characterized as an FFA1 partial agonist (EC_{50} = approximately 0.1 μm) (Houze et al. 2012; Lin et al. 2011; Yazaki et al. 2011). Although both TAK-875 and AMG-837 exhibited anti-hyperglycemic effects, these two compounds did not increase incretin levels in in vivo experiments. AM-1638 and AM-6226 which were designed by modification of AMG-837 showed potent effects on both insulin and incretin secretion (Luo et al. 2012).

In 2016, some further synthetic compounds have been reported as FFA1 agonists. Krasavin et al. reported that Compound 1, containing 1,3,4-thiadiazole-2-carboxamide group showed excellent plasma and metabolic stability with FFA1 selectivity compared to other FFARs. Yang et al. reported that Compound 2 containing 3,5-dimethylisoxazole moiety showed agonistic activity with EC_{50}

value of 15.9 nm and exhibited glucose excursion to approximately 25% at 30 mg/kg of oral administration. Most FFA1 agonists bearing a common biphenyl scaffold had low water-solubility and metabolic toxicity (Takano et al. 2014). In order to improve these properties, Li et al. explored compounds with a non-biphenyl scaffold and developed Compound 3 that showed potent and orally bioavailable agonistic activity without the risk of hypoglycaemia.

3.2 Antagonists

Some compounds have been reported as FFA1 antagonists. GW1100 was first reported as an FFA1 antagonist that inhibited GW9508 and linoleic acid-induced FFA1 signals (Briscoe et al. 2006). DC260126 containing a sulfonamide structure showed inhibitory effects on $[Ca^{2+}]_i$ responses induced by FFA ligands in FFA1 expressing CHO cells (Hu et al. 2009). DC260126 was examined in in vivo experiments with diabetic model mice and rats. Eight weeks treatments of DC260126 decreased insulin levels and improved insulin tolerance in obese Zucker rats (Zhang et al. 2010). Three weeks treatments of DC260126 significantly inhibited GSIS and serum insulin levels in db/db mice. In addition, DC260126 also reduced the apoptotic rate of pancreatic β-cells (Sun et al. 2013).

The pyrimidinylhydrazone ANT-203 was identified in high-throughput screening and showed anti-apoptotic effect on MIN6 cell line (Kristinsson et al. 2013). A series of 2-(pyridinyl)pyrimidines were reported as potent antagonists of FFA1. Among the series of compounds, Compound 4 showed moderate antagonistic activity ($pIC_{50} = 6.2$) in FFA1 stably transfected cells and reduced plasma insulin level which was elevated by β_3-agonist-induced plasma non-esterified fatty acids in Zucker fa/fa rats (Waring et al. 2015). Further studies might reveal the precise binding mode of these antagonists in FFA1.

3.3 Fluorescent Ligands

To assess the pharmacology of FFA1, some fluorescent FFA1 ligands have been reported. Although fluorescent-labeled FFAs containing the BODIPY structure are commercially available, their high lipophilicity and high EC_{50} value (submicromolar) limited their use in studying interactions with FFA1 (Hara et al. 2009). To overcome these problems, compound 5, 6 and 7 were developed based on either TAK-875 or TUG-905 (Bertrand et al. 2016; Christiansen et al. 2016; Ren et al. 2016). Especially, Compound 7 was demonstrated as a useful tracer for bioluminescence resonance energy transfer (BRET)-based binding assays, which allowed for the characterization of binding affinities of various known FFA1 agonists (Christiansen et al. 2016). Hence, these compounds are useful pharmacological tools not only to examine the binding mode of known FFA1 ligands but also to conduct high-throughput screening.

4 Structure–Activity Relationships

FFA1 is involved in the activation of insulin release from pancreatic β-cells and, as such, is considered an attractive therapeutic target for the treatment of diabetes. Many groups have tried to develop compounds which can activate FFA1 efficiently and selectively. To understand ligand recognition in FFA1 protein and how the receptor transduces its signals is helpful for the development and rational design of efficient ligands with high affinity and selectivity. Using *in silico* docking simulations with FFA1 homology models based on identified protein structure of GPCRs and amino acid sequence of FFA1, some research groups, including pharmaceutical companies, have attempted to develop novel FFA1 ligands. Information about the chemical structure and the recognition mechanism of the compound is important to evaluate binding affinity of ligand. In certain class A GPCRs, ionic locks at the extracellular surface of the receptor are considered key components in the activation of the receptor; however, how the ionic lock is involved in detail in ligand binding remains unclear. FFA1 has two such ionic locks between residues of the second extracellular loop and one of the transmembrane domains (Sum et al. 2009). In addition to the importance of such ionic locks, aromatic, hydrophilic and hydrophobic amino acid residues of FFA1 contribute to the ligand binding is defined in mutagenesis studies. Such a mutagenesis study revealed that Arg183, Asn244 and Arg258 were involved in the interaction with the carboxylate group of ligand, whilst His86, Tyr91 and His137 were involved in the interaction with aromatic or hydrophobic properties of the ligand. In addition to these amino acid residues, the binding mode of NCG75 analysed by *in silico* docking simulations showed that Val141, Ala146 and Ala173 in FFA1 might stabilize ligand interactions through hydrophobic interactions (Takeuchi et al. 2013). These findings therefore indicated that amino acid residues, which were expected to be essential for ligand interactions, were different in each ligand binding to FFA1. Since an FFA1 structure crystallized with TAK-875 has been reported recently (Srivastava et al. 2014), the results of *in silico* docking simulations combined with the information obtained from the crystal structure analysis provide great opportunity for further development of potential drug candidates (see Chapter "Homology Modeling of FFA Receptors" organized by Dr. Tikhonova IG).

5 Crystal Structure of FFA1 with TAK-875

Srivastava et al. reported a high-resolution structure of human FFA1 receptor bound to the allosteric agonist TAK-875 (Srivastava et al. 2014). The crystal structure was analysed at 2.3 Å resolution. TAK-875 showed a unique binding property, which suggested that the binding site of TAK-875 was different from that of fatty acid ligands. The binding pocket of TAK-875 that was identified by the crystal structure analysis is located in between helix3 and helix4 (Fig. 2a, b). TAK-875 might enter

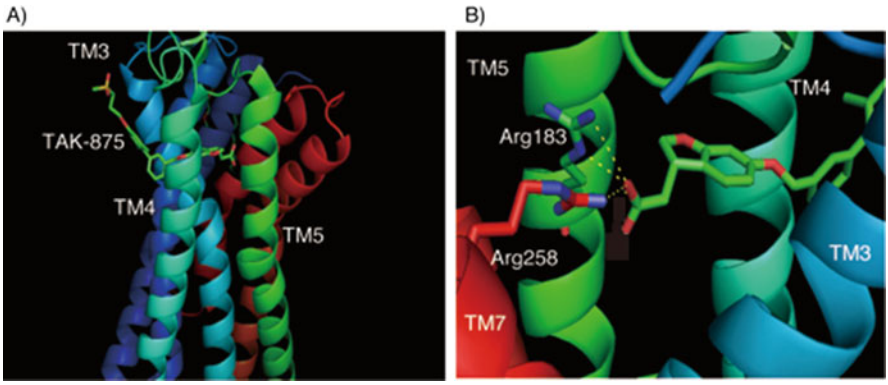

Fig. 2 Docking mode of TAK-875 in a human FFA1 crystal structure. (**a**) Overview of FFA1 structure crystallized with TAK-875. TAK-875 binds to an allosteric binding site located between TM3 and TM4. (**b**) Expansion of the binding mode of TAK-875 in the binding pocket. The interactions between the carboxylate element in TAK-875 with Arg183 and Arg258 are shown as *yellow dotted lines*. Structural information of FFA1 crystallized with TAK-875 (10.2210/pdb4phu/pdb) was derived from PDB database (http://www.rcsb.org.com). The structural data was analysed by MacPyMOL software

this binding pocket via the lipid bilayer from the extracellular side. In addition to the binding pocket for TAK-875, another potential binding pocket located between helix 1 and helix 7 was also predicted. Ligand binding assays with mutated FFA1 protein supported this model. Further, biological assays monitoring Ca^{2+} flux indicated that the effect of γ-linoleic acid was enhanced in a positively cooperative manner by addition of TAK-875 and mutagenesis studies confirmed different amino acid residues required for Ca^{2+} flux via FFA1 (Ito et al. 2013; Lin et al. 2012). These reports supported that more than one binding pocket would be present in FFA1. The FFA1 structure with TAK-875 bound was potentially an inactive state. Thus, FFA1 signaling induced with the ligand binding to the receptor might be modified by another allosteric ligand. Further analysis would be useful to examine if both of the expected binding pockets can be occupied with distinct ligands simultaneously. It will also be interesting to know if both binding pockets are occupied simultaneously if this alters the structure of the receptor and which signaling pathways are engaged.

6 Biased Agonism of FFA1 Ligand

FFA1 is reported to signal mainly via Gq/11 and Gs pathways which are mediated by IP3 and Ca^{2+} modulations and cAMP elevation, respectively. Hauge et al. reported that FFA1 signals were produced via both Gq and Gs upon binding of certain but not all agonists (Hauge et al. 2015). They evaluated the pharmacological profile of FFA1 agonists by measuring IP3 and cAMP levels, and found that

several ligands increased both IP3 and cAMP levels. Interestingly, in receptor binding assays with Gq-only (L358) and both Gs and Gq (Gs + Gq) agonists (AM-1638) showed that L358 binding to FFA1 was increased with co-incubation of the Gs + Gq agonist, and AM-1638 binding also increased in the presence of the Gq selective ligand. However, in silico docking simulations suggested that both the Gq specific and the Gq + Gs ligand could be docked into the same ligand binding site as TAK-875. Further work is therefore required to understand the basis of this differential engagement with G proteins. In vitro studies using primary intestinal cells showed that both Gq specific and the Gq + Gs ligands-induced incretin secretion. However, the effect of Gq + Gs ligands on incretin secretion was higher than that of Gs specific ligands. A similar tendency of these agonists was also shown in in vivo experiment measuring incretin levels.

Manicini et al. reported that FFA1 can be coupled to both G protein and β-arrestin signaling pathways (Mancini et al. 2015). These two pathways were both associated with regulation of insulin secretion. β-arrestin pathways are considered as essential for receptor internalization and desensitization; however, a number of recent studies have revealed that β-arrestin pathways are also key signaling routes for GPCRs (DeWire et al. 2007). Among the FFARs, FFA4, also known as GPR120, has been reported to engage both Gprotein and β-arrestin pathways and that both play important roles in the physiological functions of FFA4 (Ichimura et al. 2012; Oh and Walenta 2014; Oh et al. 2010, 2014). FFA4 expressed on adipocytes was coupled via Gq-protein pathways and contributed to glucose uptake. On the other hand, FFA4 expressed on macrophages is mainly coupled via β-arrestin pathways and contributes to regulation of inflammatory responses. Hence, biased ligands, which can selectively activate only one of the signaling pathways, will be useful to evaluate precise receptor functions, not only in in vitro but also in in vivo studies.

Ligand-induced β-arrestin interactions with FFA1 were evaluated in BRET-based studies (Mancini et al. 2015). TAK-875 produced a potent effect on the recruitment of β-arrestin compared to endogenous FFA ligands, while this compound showed only partial agonistic activity on Gq/11 pathways. Although FFAs-induced insulin secretion was inhibited by treatment with the highly selective Gq/11 inhibitor UBO-QIC, TAK-875-induced insulin secretion was only weakly attenuated by β-arrestin siRNAs. FFA1 is therefore able to engage at least three signaling pathways (Gq/11, Gs and β-arrestin). Further studies of biased agonism at FFA1 might address the specific contribution of each pathway to the physiological functions of FFA1 and assist in tailoring these components to optimize ligands for pre-clinical and clinical assessment.

7 Clinical Trials

A small number of FFA1 ligands have entered clinical trials (Table 1). TAK-875, developed by Takeda progressed to phase III clinical trials. In a phase I, placebo-controlled, double blind trial with healthy subjects, oral administration of TAK-875

Table 1 FFA1 agonists subjected to clinical trials

Compounds	Phase of clinical trials	Companies
TAK-875 (Fasiglifam)	Phase III (-2013) (discontinue due to liver toxicity)	Takeda
JTT-851	Phase II (ongoing)	Japan Tobacco
P11187	Phase I (-2013) (completed → no further information)	Piramal Enterprises
AMG-837	Phase I (-2013) (completed → discontinue)	Amgen
LY2881835	Phase I (-2011) (completed → discontinue)	Eli Lilly
ASP5034	Phase I (-2012) (discontinue)	Astellas Pharma

was examined in terms of pharmacodynamics and safety (Naik et al. 2012). With a single oral administration, TAK-875 was safe and well tolerated. Phase II, randomized, double-blind, multicenter parallel group studies were conducted in type 2 diabetes patients whose symptoms showed tolerability to diet or metformin treatment. Two weeks oral administration of TAK-875 showed efficacy and tolerability (Araki et al. 2012; Kaku et al. 2015). In addition, once daily oral administration for 12 weeks showed an anti-hyperglycemic effect with low risk of hypoglycemia. However, a subsequent phase III trial was discontinued because of the potential of liver injury (Kaku et al. 2015). Li et al. have reported that TAK-875 inhibited the efflux transporter multidrug resistance-associated protein 2 (Mrp2), bile acid transporters, Na(+)/taurocholate co-transporting polypeptide (Ntcp) and the organic anion transporter protein (OATP), which may cause hyperbilirubinemia and hepatotoxicity (Li et al. 2015). Although the mechanism of this adverse effect of TAK-875 remains to be reported in detail, early stage analysis of potential FFA1 ligand effects on such transporters will clearly be essential for progress.

Eli Lilly completed a phase I trial of LY2881835 in 2011 (Defossa and Wagner 2014; Watterson et al. 2014). LY2881835 showed agonistic activity on human FFA1 with moderate potency ($EC_{50} = 230$ nm); however, significant side effects were observed in participants in a phase I trial, resulting in no further development of this compound.

ASP5034 was developed by Astellas. The chemical structure, which was undisclosed, was based on oxadiazolidinediones. In 2012, a phase I clinical trial of ASP5034 for type 2 diabetes was initiated; however, this was stopped from further evaluation based on a comprehensive review of the results and the competitive landscape (Astellas Pharma 2013).

P11187 developed by Piramal Enterprise Ltd. entered a phase I trial (Mancini and Poitout 2015; Watterson et al. 2014). Although the chemical structure has not been disclosed (Defossa and Wagner 2014) and some patent documents indicate the compound likely to be phenyloxetanylacetic acid-based. AMG-837 was developed by Amgen. In a phase I clinical trial, AMG-837 increased plasma insulin level, but did not lower glucose levels (Mancini and Poitout 2015). Further development of this compound has been abandoned for undisclosed reasons (Oh and Olefsky 2016).

JTT-851, also with undisclosed chemical structure, was developed by Japan Tobacco. A phase II trial for type 2 diabetes has progressed in Japan and USA (Japan Tobacco 2013) but further information is lacking at this time.

8 Conclusions

More than a decade has passed since FFA1 was identified as a receptor for medium- to long-chain fatty acids. As discussed elsewhere, identification of cell membrane G-protein coupled receptors for free fatty acids including FFA2 and FFA3 for short-chain fatty acid receptors, and FFA4 for medium- to long-chain fatty acids has resulted in a paradigm shift for fatty acid biology towards drug development. Even though the overlapping endogenous ligand spectrum of FFA1 and FFA4 has caused difficulties defining the specific function of FFA1, development of ligands with high affinity and selectivity has successfully overcome this problem. Structural information of FFA1 ligand interactions provided by both structure–activity relationships and crystal structure analysis have assisted further development of new ligands. A small number of compounds have entered clinical trials; however, almost all of these compounds were terminated due to either publically acknowledged adverse effects or in internal company reviews. Questions remain about the precise binding mode of distinct FFA1 ligand classes, and better knowledge of this is required for structure-based, rational drug design, and to understand more fully the relationships between signaling pathways activated and the biological functions of FFA1.

Taken together, as FFA1 remains a potential drug target, further comprehensive studies of FFA1 may address these issues and lead to the identification of therapeutic candidates for the treatment of type II diabetes and metabolic disorders.

Conflicts of Interest The author declares no conflict of interest associated with this manuscript.

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