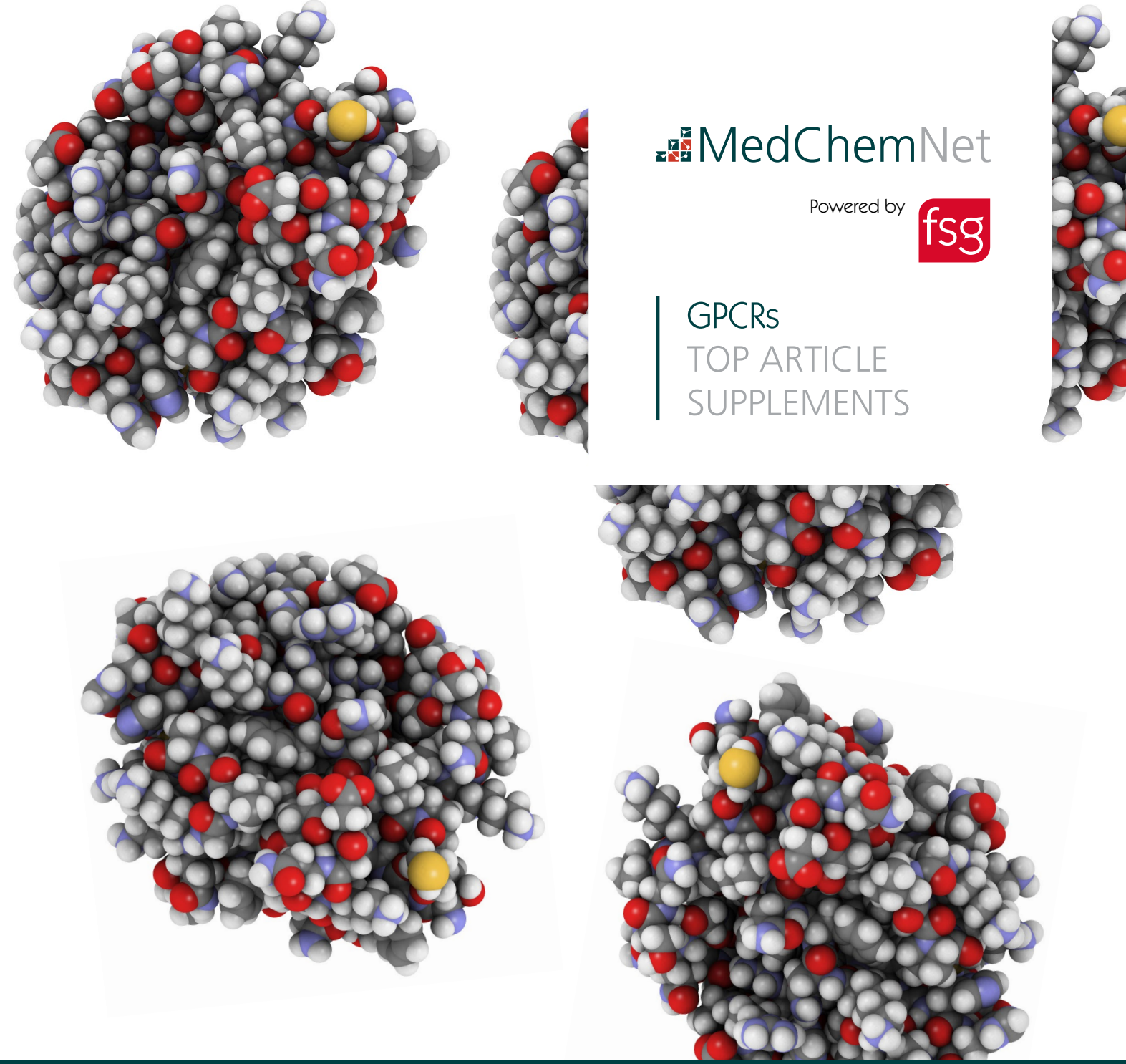

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Patent Review

Pharmaceutical Patent Analyst

FFA4/GPR120 agonists: a survey of the recent patent literature

FFA4/GPR120, a member of the rhodopsin family of G-protein-coupled receptors (GPCRs), is becoming an important target for therapeutic intervention in several areas of disease, including metabolic diseases, inflammation and cancer. In the last few years several patents on original chemotypes have been generated by different companies. In this review an analysis of the patents in the FFA4 agonism field is presented, with an emphasis on the documents published between 2013 and mid-2015. A discussion of the biological methods used in the patents is included. The general interest in this area is growing fast as half of the existing patents on FFA4 agonists have been issued after 2013. There is, however, a need of further diversifying new chemical classes away form the current substrate-like, carboxylic acid-containing agonists.

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Free fatty acids (FFAs) are essential dietary nutrients that act in various metabolic and cellular processes. FFAs exert their function via influence on lipid membrane composition and diverse receptors and pathways including nuclear peroxisome proliferator-activated receptors (PPARs) and a subfamily of transmembrane G-protein-coupled receptors (GPCRs), the FFA receptor family. The receptors FFA2 and FFA3 are activated by short chain fatty acids while receptors FFA1 and FFA4 have been shown to bind polyunsaturated (long-chain) fatty acids (PUFA) with a C16 to C22 backbone such as α-linolenic acid, linoleic acid and palmitoleic acid. In humans, FFA4 is expressed in the intestinal tract (ileum, cecum, enteroendocrine L cells of the colon, rectum), but also in the thymus, lung, spleen, adrenal gland, pancreas and taste buds. Activation of FFA4 present on endoenterocrine cells triggers a rise in intracellular Ca^{++} causing secretion of GLP-1 (a potent incretin hormone that enhances the secretion of insulin from pancreatic β cells) *in vitro* and *in vivo* which leads to a subsequent increase in circulating insulin [1]. A role for FFA4 in adipogenesis has been described as well. FFA4 mRNA is highly expressed in four different adipose tissues (subcutaneous, perirenal, mesenteric, epididymal) and the amount of mRNA is elevated in adipose tissues of mice fed a high fat diet [2]. The level of FFA4 mRNA increased during adipocyte differentiation of 3T3-L1 cells used as an *in vitro* model system for adipogenesis. Similar results were observed in human adipose tissue, human preadipocytes and cultured adipocytes [2]. It has been also shown that dysfunction of the lipid sensor FFA4 leads to obesity in both mouse and human where the nonsynonymous mutation p.R270H (that inhibits FFA4 signaling) increases the risk of obesity in European populations [3].

Interestingly, the potent anti-inflammatory role of omega-3 fatty acids has been recognized for a long time and it was demonstrated that FFA4 expressed on macrophages plays a central role thereby repressing the release of inflammatory cytokines. In particular, FFA4-induced anti-inflammatory effects were demonstrated to be mediated by β-arrestin signaling [4]. Recently, Oh *et al.* [5] showed that a selective high affinity, orally available, small-molecule FFA4 agonist exerted potent anti-inflammatory effects on macrophages *in vitro* and in obese mice *in vivo*. FFA4 agonist treatment of high-fat diet-fed obese mice caused improved glucose

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Defined key terms:

FFA4: Free fatty acid receptor 4; also G-protein-coupled receptor 120 (GPR120); a protein that in humans is encoded by the *FFAR4* gene.

β-arrestin: β-arrestin-2, also known as arrestin-3, is an intracellular protein that in humans is encoded by the *ARRB2* gene.

FLIPR assay: FLIPR® Calcium Assay Kits are the platform of choice for measuring changes in intracellular calcium during drug discovery and research.

ERK: Extracellular signal-regulated kinases (ERKs) or classical MAP kinases are widely expressed protein kinase intracellular signaling molecules that are involved in functions including the regulation of meiosis, mitosis and postmitotic functions in differentiated cells.

pERK: Phosphorylated ERK.

GTT assay: A glucose tolerance test (GTT) is of interest in research on diabetes, obesity, metabolic syndrome or cardiovascular diseases. The glucose tolerance assay or test consists of giving glucose orally (OGTT), intraperitoneally (IPGTT) or intravenously (IVGTT) to fasted animals, in order to analyze the insulin release into blood. The giving of glucose can be associated with the administration of a test substance designed to further increase insulin release.

DIO mice: B6 DIO mice are a model of pre-Type 2 diabetes and obesity with elevated blood glucose and impaired glucose tolerance.

tolerance, decreased hyperinsulinemia, increased insulin sensitivity and decreased hepatic steatosis.

In addition to its involvement in metabolic diseases and inflammatory processes, FFA4 has also recently been linked to cancer. However, it remains unclear what role FFA4 plays in cancer and what possible effects receptor modulators might have as one study showed a role of FFA4 in suppression of cell proliferation in prostate cancer [6] while another group found FFA4 to be tumor-promoting in colorectal cancer [7].

Based on these findings, the identification of FFA4 selective agonists would open new treatment opportunities for diabetes, obesity and inflammatory diseases.

Biological assays reported in the patents: a critical appraisal

Activated FFA4 can exert its effects through different signaling pathways and in the patents reviewed here structure–activity relationship (SAR) analyses of FFA4 agonists are performed using different functional assay systems. While functional assays are valuable for the characterization of ligand-receptor activity, they provide only indirect functional measurements of compound half-maximal effective concentrations (EC_{50}) and not direct detection of binding affinity K_i values. As EC_{50} values depend not only on agonist affinity and efficacy but also on properties of the assay system and cell line used (e.g., on signal amplification of the pathway) comparison of compound potencies across different assays may not be easily possible.

FFA4 in enteroendocrine cells has been shown to utilize the Gαq/calcium pathway [4]. Receptor activation and coupling to Gαq leads to activation of PLCβ, which generates the signaling molecules inositol trisphosphate (IP_3) and diacylglycerol (DAG) through hydrolysis of the membrane lipid phosphatidylinositol bisphosphate (PIP_2). Subsequently, calcium is released from intracellular stores and it can exert various effects through binding to and opening of ion channels, triggering release of neurotransmitters at synapses or release of hormones from endocrine cells, binding to calmodulin and activation of transcription factors. *In vitro*, this pathway can be studied in live, cell-based assays using calcium-sensitive indicators, for example, calcium-sensitive fluorescent dyes. Calcium assays are among the most widely used assays to study GPCRs in general [8] and FFA4 in particular [9] and they were used in the majority of the FFA4 patents reviewed here [10–16]. The major advantages of this type of assay are its usually reliable and very robust performance, providing good signal-to-background ratios and little-to-moderate variability, and its adaptability to different formats and the use of lab automation systems; it is therefore highly compatible with high-throughput screening (HTS) of large compound libraries. A possible shortcoming of this type of assay is that with its reading of maximum calcium response, it is a non-equilibrium assay and measurements might therefore not accurately reflect agonist-binding affinities at the receptor.

If a receptor does not naturally signal through this pathway, so-called promiscuous G-proteins can be co-expressed to artificially couple the receptor to the calcium pathway; with FFA4, Gα16 [15,16] was used. While this forced switch of coupling brings the opportunity to use this type of assay, it also has its liabilities. Ligand-selective and G-protein-selective conformations of receptors have been reported [17]. Therefore, results obtained with the use of promiscuous G-proteins can include false-positives as well as false-negatives and it is advisable to cross-check the performance of identified compounds with orthogonal assays.

FFA4 has been shown to activate the calcium pathway in enteroendocrine cells [1]. It is of importance to note here, however, that in humans, two different splice variants of FFA4 exist: a short isoform (which is homologous to the FFA4 in rodents) and a longer isoform with an insertion of 16 amino acids in the third intracellular loop of the receptor [18]. A recent report described differential signaling by the two splice variants [19]. Specifically, the short isoform of FFA4 was

able to activate the calcium pathway in HEK293 cells in response to both natural and synthetic ligands while the long isoform remained inactive in this system. The patents on FFA4 reviewed here used both the short and long isoforms. However, also different cellular systems were used in the patents, which might express other G-proteins, augmenting differences in signaling. It is therefore always advisable to be aware of and control for the cellular environment, co-factors and receptor variants used when assaying receptor function.

Another type of *in vitro* assay used with FFA4 involves β-arrestins, analyzing a different signaling pathway of the receptor, which has been shown to be active in macrophages and involved in inflammatory response. In classical models of GPCR signaling, receptor activation is terminated by phosphorylation of the receptor and binding to arrestins, leading to desensitization and internalization of the receptor. It is now understood, however, that receptors can also directly signal through arrestins without prior G-protein involvement; this arrestin signaling can lead to activation of the ERK pathway, transactivation of other receptor pathways and transcriptional control [20]. Therefore, β-arrestin assays have the advantage of monitoring receptor activation and potential G-protein-independent signaling. One of the β-arrestin assays described in the patents uses bioluminescent resonance energy transfer (BRET) between fusion proteins of a luminescent donor and β-arrestin on the one hand and a fluorescent acceptor fused FFA4 on the other hand [21,22]. Another β-arrestin assay uses a chemiluminescent enzyme (β-galactosidase) fragment complementation [10–13]. Enzyme fragment complementation assays usually display a high signal to background ratio and are often robust assays and have been used for other receptors in drug discovery. Additionally, being equilibrium assays the determined EC_{50} values are better indicators of compound affinity and efficacy at the receptor. However, the sensitivity of the assay might be less compared with other assay systems because of low signal amplification and limited receptor reserve. Also, the assay time window is considerably larger compared with, for example, the calcium assay, which means that usually single time points after a long incubation period are read; signaling kinetics are usually not recorded.

In addition to aforementioned signaling pathways, FFA4 was also shown to activate the ERK pathway [1,4]. Some patents investigated this FFA4 signaling pathway with an assay on phosphorylation of ERK [23–26]. In this assay, compounds will stimulate the receptor in a living cellular environment, before cells are lysed and pERK is detected with a sandwich immunoassay. These types of assays are usually also HTS compatible but depending on baseline levels of pERK, low signal to background levels might occur and assay optimization might be required.

In addition to these *in vitro* assays, *in vivo* assays have been performed, for example, glucose tolerance tests that monitor blood glucose levels and metabolism [10–13,23–26]. These studies not only involved wild-type mice of specific strains but also included environmental exposure disease models like diet-induced obesity (DIO) mice. This is of importance as in diseases and disease models, individual factors might influence and potentiate each other without recapitulating human physiology; this is especially true for obesity and subsequent complications like Type 2 diabetes. Environmental exposure models like a diet-induced obesity (DIO) model have recently become widely viewed as good disease models mimicking human disease.

Chemical matter

Bristol-Myers Squibb

Bristol-Myers Squibb contributed four patent disclosures in September–October 2014 [23,24], showing a continuing interest in the FFA4 modulation approach, for the treatment of diseases such as diabetes and related conditions, metabolic syndrome, disorders of glucose metabolism, obesity and other related conditions. The four patents differ for the central scaffold, while common decorations are variously substituted diphenyl ethers, and a short chain (alkyl or alkoxy) bearing an acidic function, which is typical of most current FFA4 modulators. In two earlier patent applications [23,24], oxabicyclo [2.2.2] acid derivatives are described, in particular [24] is the international version of [23], with an extension of the claims; in both patents, 120 compounds are described and tested *in vitro* assay using a pERK assay in human FFA4, to assess an FFA4 agonist activity. Many compounds have an EC_{50}< 500 nM, but only four of them show an EC_{50}< 100 nM. Molecules that activated FFA4 with sufficient potency and efficacy in the pERK assay and with desirable pharmacokinetic properties, were also evaluated in an *in vivo* assay (OGTT assay) in mice. Representative examples (**1–3**) of this patent are shown in Figure 1; for example, compound **3** (EC_{50}: 220 nM), exhibited a significant decrease in glucose levels (60%) at 30 mg/Kg, os in the OGTT assay.

In a third patent application [25], bicyclo [2.2.1] heptane derivatives were described; 100 compounds were tested in an *in vitro* pERK assay, among these 19 had EC_{50}< 500 nM; only two compounds (**4, 5;** Figure 1) were tested in an *in vivo* OGTT assay; the best compound was **4** with an EC_{50} of 600 nM (25% decrease in glucose levels).

In a fourth patent application [26], bicyclo [2.2.2] octane derivatives were described; only 15 compounds

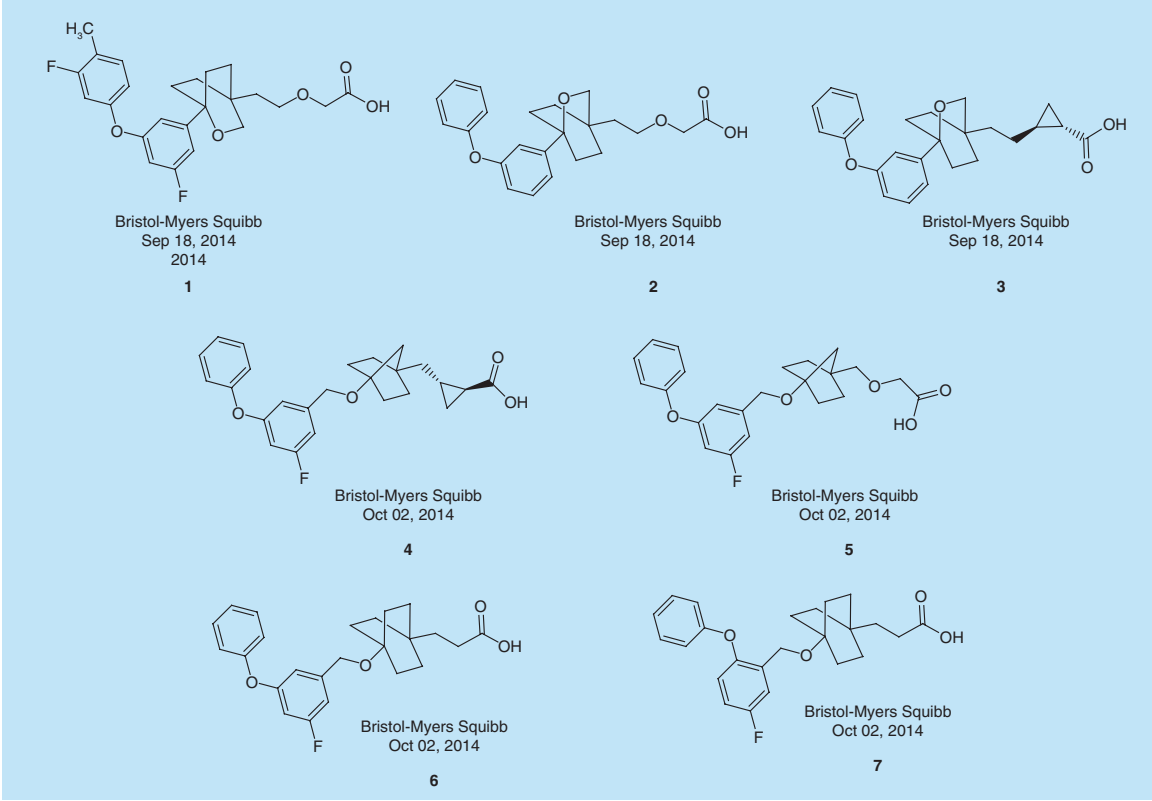

Figure 1. Compounds 1–7 from Bristol-Myers Squibb. Each compound represents a separate patent application. Applicant and priority date are listed beneath each structure [23–26].

were tested *in vitro* pERK assay. Representative examples of this patent are shown in Figure 1 (**6, 7**), the best one being compound **6** with an EC_{50} of 270 nM. No *in vivo* data are reported in this patent case.

In general, selectivity data versus other FFA receptors are not reported in the patents.

Janssen Pharmaceutica

Between September and December 2014, Janssen Pharmaceutica filed four patent applications for different series of chemical compounds, specifically, benzo-fused heterocyclic [10], isothiazole and thiophene derivatives [11], and bicyclic pyrrole derivatives [12,13], as FFA4 agonists. Most of the compounds are characterized by the presence of the acidic function, typical of FFA4 modulators.

In the first patent application [10], 123 benzo-fused heterocyclic derivatives were described and tested in two *in vitro* assays, β-arrestin assay and calcium flux assay, to assess their FFA4 agonist activity. In a β-arrestin assay, six compounds showed an EC_{50}< 100 nM, while in the calcium flux assay 24 compounds showed EC_{50}< 50 nM. The representative compounds of the invention were tested in an *in vivo* assay; in particular ten compounds were tested in DIO mice OGTT screening and 25 compounds were tested in C57B16 mouse IPGTT and OGTT. A representative example from this patent is compound **8** (Figure 2) (β-arrestin assay EC_{50}= 110 nM, calcium flux assay EC_{50}= 39 nM), that was shown to reduce *in vivo* glucose by 70% at 10 mg/kg, os and by 66% at 30 mg/kg, ip.

In a second patent application [11] 251 isothiazole and thiophene derivatives were described and tested in an *in vitro* assay (β-arrestin assay). Thirty-two compounds displayed EC_{50}< 100 nM and, of these, only six had an EC_{50}< 50 nM; in a calcium flux assay 62 compounds had EC_{50}< 100 nM and of these 18 were most potent (EC_{50}< 50 nM). The best compounds, for the *in vitro* activity and for its good pharmacokinetic properties, were also evaluated in an *in vivo* assay (DIO mice OGTT screening and C57B16 mouse IPGTT and OGTT). In DIO mice, OGTT screening nine compounds were tested, while in C57B16 mouse IPGTT and OGTT 12 compounds were assessed. Representative compounds of this patent are shown in Figure 2 (**9,10**), the best one being compound **9** (β-arrestin assay EC_{50}= 179 nM, calcium flux assay EC_{50}= 42 nM). Compound **9** showed a reduction in glucose Area Under the Curve (AUC) of 80% in the OGTT assay.

Again in September 2014, Janssen Pharmaceutica released a further patent application [12]: 70 bicyclic-

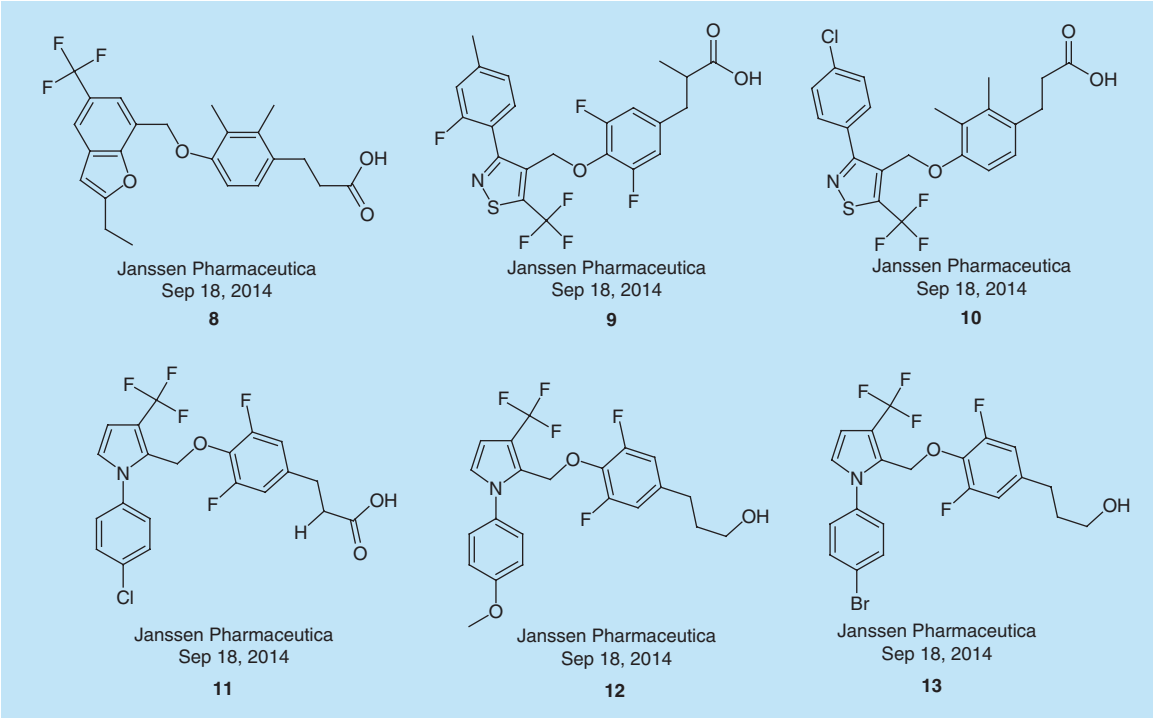

Figure 2. Compounds 16–21 from Janssen Pharmaceutica. Each compound represents a separate patent application. Applicant and priority date are listed beneath each structure [10–13].

pyrrole derivatives were described and tested in an *in vitro* assay. In a β-arrestin assay 12 compounds have shown EC_{50}< 100 nM and four of these had EC_{50}< 50 nM. The most active compounds in this assay were also tested *in vivo* (C57B16 mouse IPGTT and OGTT). In a calcium flux assay 19 compounds have an EC_{50}< 100 nM and of these five reached an EC_{50}< 50 nM; compounds with good activity and good pharmacokinetic properties in this assay were tested *in vivo* (DIO mice OGTT screening). The representative examples of this invention are shown in Figure 2 (**11–13**). Compound **11** (β-arrestin assay: EC_{50} of 69 nM, calcium flux assay EC_{50} of 80 nM), the best one, was tested in DIO mice OGTT screening at different doses; 10, 3, 1, 0.3 and 0.1 mg/kg, giving, respectively, a reduction AUC of glucose amount to -82, -76, -54, -8 and 9%. Compounds **12** and **13** were also tested in C57B16 mouse IPGTT and OGTT assay at three different concentrations, 10, 3 and 1 mg/kg; as an example compound **13** (β-arrestin assay EC_{50} of 61 nM, calcium flux assay EC_{50} of 143 nM) gave a reduction of glucose AUC of -44, -39 and 2% at the three decreasing doses.

In December 2014, Janssen Pharmaceutica released a latest patent application [13]; this is a US patent and is an extension of patent [12] where 18 compounds, bicyclic pyrrole derivatives, were added; but no one of these showed particular activity.

No selectivity data versus other FFA receptors are provided in the above patents.

Merck

In 2014, Merck Sharp & Dohme Corp. reported azaspiro- and oxaazaspiro-piperidinyl compounds as FFA4 modulators for the treatment and/or preventing diabetes, obesity, hyperlipidemia, inflammation and related disorders [14]. The core spiro scaffold is decorated with a substituted phenyl ring at nitrogen, and an acidic function on the opposite side linked by a short alkyl chain. In same patent examples, a single methyl substituent is present on a ring of the spiro core. The FFA4 agonist activity was evaluated by FLIPR calcium ion assay. The most potent compound showed an EC_{50} value of 44.8 nM (compound **14**, Figure 3).

In the same patent application compound **15** was reported, with an EC_{50} value of 790 nM. Da Young Oh *et al.* [5] observed an anti-inflammatory insulin-sensitizing effect after oral administration of compound **15** in mice, emphasizing the potential beneficial effects of FFA4 agonists in the treatment of human insulin resistant states.

Syddansk University

Syddansk University discovered substituted fluorophenyl-methoxy-benzene-carboxylic acid compounds as modulator of FFA4 and, in 2013, two patent applications were published [21,22]. The reported compounds are claimed as useful for the treatment of type II diabetes, hypertension, ketoacidosis, obesity, glucose intolerance and hypercholesterolemia and the associated

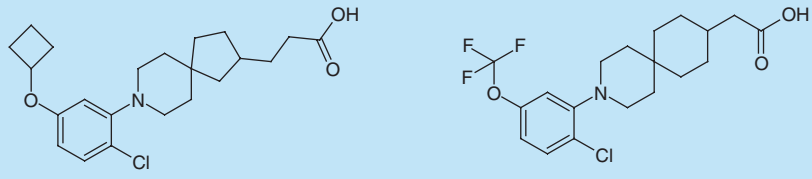

Figure 3. Compounds 14 and 15 from Merck Sharp & Dohme. Each compound represents a separate patent application. Applicant and priority date are listed beneath each structure [14].

disorders. As an example, Figure 4 shows two of the claimed compounds (**16, 17**). In the patent application [21] a series of 2-substituted-5-fluorobenzyl derivatives are reported, while in [22] the fluorine atom is shifted to obtain a 2-substituted-6-fluorobenzyl pattern and the compounds are characterized by the presence of a pyridin-2-yl, pyridin-4-yl, or 4-cyanophenyl moiety instead of 4-methylphenyl group. In both patent applications, the alkyl length of the carboxylic acid function varied from 2 to 3 carbon atoms. The biological activity was evaluated in a BRET β-arrestin 2 interaction assay; the presence of a pyridin-3-yl moiety brings about an EC_{50} less than 1 μM. The replacement of cyano group of **17** with a chlorine leads to less active compound, as well as the replacement of the pyridine ring with hydroxy-substituted phenyl groups.

Compound **16** (TUG-891) was fully characterized by Shimpukade *et al.* [27] and Hudson *et al.* [28]. The compound showed a potent and full agonist activity on hFFA4 receptor (pEC_{50} = 7.36) in the BRET assay, associated with a good selectivity against hGPR40/FFA1 (pEC_{50} = 4.19).

LG Life Sciences

LG Life Sciences, Ltd. reported in 2014 two series of, respectively, biaryl [15] and thioaryl [16] derivatives as FFA4 agonists, promoting GLP-1 formation in the GI tract and improving insulin resistance. The invention defined a method of preparation, a pharmaceutical composition and the use for preventing or treating diabetes, complications of diabetes, inflammation, obesity, nonalcoholic fatty liver steatohepatitis or osteoporosis.

The biological activity was evaluated in a cell-based assay using CHO-K1 cells expressing Gα16 and hFFA4. Some representative examples (**18, 19**) are shown in Figure 5. The most potent compound showed an EC_{50} value of 0.011 and 0.006 μM, respectively, in the two assays. A representative example of the second patent is shown, again in Figure 5, compound **20,** having EC_{50} of 0.019 μM and **21** with an EC_{50} of 0.018 μM.

Each compound represents a separate patent application. Applicant and priority date are listed beneath each structure [15,16]

A synoptic view of the main *in vitro/in vivo* data for the most significant compounds within the reviewed patents is reported in Table 1.

Future perspective

At least 13 new patents have been published in 2013–2014, the same number of patents disclosed before 2013 [29].

Companies entering the FFA4 field with patent disclosures after 2013 are Bristol-Myers Squibb, Merck Sharp&Dohme, Janssen Pharmaceutica and LG life Sciences joining other companies like IRM LLC, Metabolex, Banyu, Kindex and Pharma Frontier which have published several patents in the timeframe 2008–2012 as covered in [29].

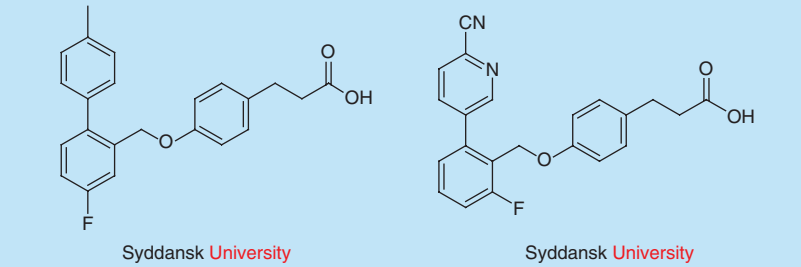

Figure 4. Compounds 16 and 17 from Syddansk University. Each compound represents a separate patent application. Applicant and priority date are listed beneath each structure [21,22].

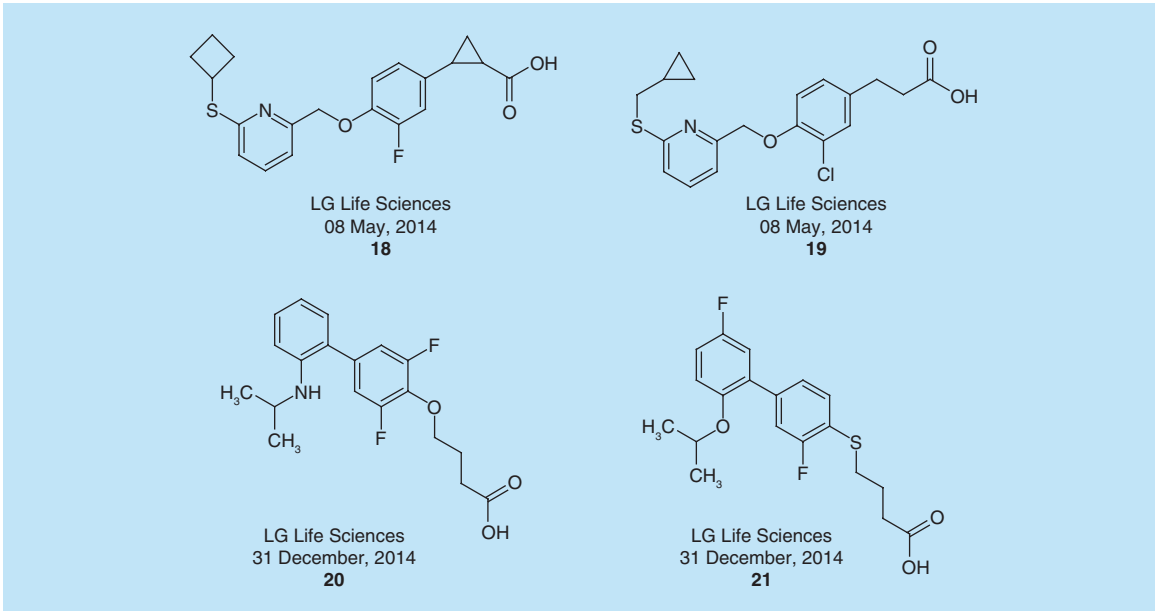

Figure 5. Compounds 18–21 from LG Life Sciences.

Thus, in the last 2 years, the importance of FFA4 as a target for diabetes, obesity and other metabolic conditions has grown consistently. The proof-of-concept in animal models [5] and the demonstration that selectivity toward related human GPRs, such as FFA1/GPR40, is feasible [15,16], is contributing to this surge of interest. The publication in 2014 of at least nine patents from major pharmaceutical companies (BMS, Janssen, MSD) witnesses the fact that FFA4 is becoming a hot target in the metabolic disease arena, although FFA receptors have still to be validated in the clinical setting. Indeed, the FFA1/GPR40 agonist TAK875 (fasiglifam) was halted in Phase III clinical trials for diabetes because of liver toxicity [30]. Most of the current lead compounds (e.g., TUG-891 of [27,28] or MSD Compound A of [5]) are clearly designed based on the

Table 1. Biological activity of selected FFA4 agonists.

Company/institution	Compound entry	Assay type	EC_{50} (nM)
***In vivo* testing**			
Bristol-Myers Squibb	3	pERK	220
Bristol-Myers Squibb	6	pERK	270
Janssen	8	β-arrestin	110
Janssen	8	Calcium flux	39
Janssen	9	β-arrestin	179
Janssen	9	Calcium flux	42
Janssen	13	β-arrestin	61
Janssen	13	Calcium flux	143
Merck Sharp & Dohme	14	Calcium flux	44.8
Syddansk University	16	β-arrestin (BRET)	43.5
LG Life Science	21	hGPR120/CHO-K1 cells	18
***In vivo* testing**			
Bristol-Myers Squibb	3	OGTT mice	60% (30 mg/Kg, os)
Janssen	8	OGTT mice	70% (10 mg/Kg, os)
Janssen	9	OGTT mice	80% (10 mg/Kg, os)
Janssen	13	OGTT mice	44% (10 mg/Kg, ip)

polyunsaturated fatty acid (PUFA) substrates. Notably, some Janssen compounds in [12], as well as former Banyu compounds reviewed in [29], lack the carboxylic acid moiety typical of the PUFA substrates, indicating that an ionic interaction between this acid moiety and Arg99 in the active site of FFA4 is not mandatory or that other possible (e.g., allosteric) interactions may produce potent FFA4 agonists. GSK also reported a series of sulfonamides [31] lacking the acid moiety. The carboxylic acid moiety may, in some cases, bring about undesired effects such as low tissue distribution. Furthermore, a number of carboxylic acid drugs have been associated with adverse reactions, linked to the metabolic activation of the carboxylic acid moiety of the compounds, in other words, formation of acyl-glucuronides and acyl-CoA thioesters [32].

The emerging nonacid compounds may pave the way toward chemotypes different from the current substrate-like agonists. It is anticipated that current screening efforts on the target will produce multiple, differentiated new classes of FFA4 agonists within the next few years [32,33].

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Executive summary

- Free fatty acid (FFA)4 is emerging as an interesting target for finding new therapies in the field of metabolic diseases, obesity, inflammation and cancer.
- The biological machinery behind FFA4 physiology is now rather well understood and several pharmacological tools for discovery programs are available.
- The main companies entering the field with patent disclosures after 2013 are Bristol-Myers Squibb, Merck Sharp & Dohme, Janssen Pharmaceutica and LG life Sciences. Other companies like IRM LLC, Metabolex, Banyu, Kindex and Pharma Frontier have published several patents in the timeframe 2008–2012 as covered in [29]:
- Bristol-Myers Squibb: recent effort on four chemical classes.
- Janssen Pharmaceutica: recent effort on four chemical classes.
- Merck Sharp & Dohme: one new chemical class disclosed and a landmark FFA4 paper.
- Syddansk University: academic leader in the field for patents and publications.
- LG Life Sciences: one patent in 2014.
- There is a need of finding new and diversified chemical classes able to modulate, for example, agonize, FFA4. The present chemotypes show indeed a high degree of similarity due to their substrate-like (PUFA) nature, with the notable exception of some recent Janssen examples and former Banyu compounds. In particular, the presence of a carboxylic acid moiety (or its bioisosters) in these molecules can limit their volume of distribution and tissue permeability, hence the frequent need of adopting prodrug approaches when dealing with carboxylate containing drugs, and/or may cause formation of reactive adducts in Phase II metabolism.
- The interest in the target is growing fast as witnessed by the publication of the same number of patents in the last 2 years (2013–2014) as compared with those published before 2013 and by the entrance in the field of big pharma players. It is anticipated that new, more diversified chemotypes will emerge in the next few years from dedicated HTS efforts.

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Review

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Future Medicinal Chemistry

Biological characteristics and agonists of GPR120 (FFAR4) receptor: the present status of research

GPR120 receptor functions as a receptor for ω-3 fatty acid, involving regulating the secretion of gastrointestinal peptide hormone, adipogenesis, adipogenic differentiation and anti-inflammatory process and the like in the aspect of biological functions. In view that the dysfunction of GPR120 receptor is closely correlated with metabolic disorders, GPR120 may act as a novel potential therapeutic target for the treatment of obesity, insulin resistance, Type 2 diabetes and so on. Therefore, mounting scientists devote themselves to probing the molecular mechanism of the biological function of GPR120 receptor and their ligands for the treatment of impaired metabolic health. Herein, we summarize the mechanisms of signal transduction through GPR120 receptor, and discovery and development of GPR120 agonists thereof.

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Human GPR120, with 10q23.33 chromosomal location, was originally identified by Fredriksson *et al.* by searching databases for rhodopsin-like GPCR [1]. In the transmembrane domains (TMD) of GPR120, Arg 2.64 at the top of TMD2 and Arg 4.65 at the top of TMD4 were identified as active sites, which may produce interactions with carboxylate groups of agonists. The snake-like diagram for the human GPR120 secondary structure is presented in Figure 1. Animal studies displayed that GPR120-deficient mice fed with a high fat diet have a tendency for developing obesity, glucose intolerance and decreasing adipocyte differentiation, as well as lipogenesis. In addition, GPR120 expresses higher in adipose tissue in obese individuals than in lean controls. Furthermore, a deleterious nonsynonymous mutation (p.R270H) increases the obesity risk through inhibition of GPR120 signaling pathway. Consequently, GPR120 notches a significant position in energy balance in both humans and rodents [2].

It has been reported the sequences of the human, mouse, rat and cynomolgus monkey GPR120 receptors in the literature (Figure 2). For human GPR120 receptor, two sequences (Q5NUL3 and Q5NUL3–2 from Uniprot database) have been described. The principal structural distinction between GPR120S (Q5NUL3–2, the short isoform that contains 361 residues) and GPR120L (Q5NUL3, the long isoform that contains 377 residues) is that GPR120L has 16 additional amino acids in the third intracellular loop between positions 231 and 247, which possibly causes differential signaling properties. The third intracellular loop of rhodopsin-like GPCRs is a fundamental region of recognizing G-protein and β-arrestin [4]. While in the mouse, rat and cynomolgus monkey, only one variant is unearthed to hold high similarity with a human counterpart. In 2009, a pharmacological characterization of the human splice variant and a comparison of functional activity between cynomolgus and human GPR120 receptors were presented. They have similar pharmacology that measured intracellular calcium release generated by free fatty acids (FFAs) and small molecule GPR120 agonist GW9508 (**1**, Table 1) in cells expressing these two sequences [5]. Moniri *et al.* compared the signaling of these isoforms and identified that additional gap in the third intracellular loop of the GPR120L isoform contains four phospho-labile serine/threonine residues. Nevertheless, there is no dissimilarity between two variants of the

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Key term

Agonist: Can bind and activate the receptor to produce a biological response, and be divided into endogenous and exogenous agonist.

human GPR120 in degree and rate of phosphorylation following agonism. However, basal phosphorylation of GPR120S without agonist was demonstrated to be more pronounced, which suggested that additional gap in the third loop may cover some constitutive phosphorylation sites [6]. Furthermore, Watson *et al.* studied the signaling and intracellular trafficking of GPR120S and GPR120L receptors by calcium mobilization, dynamic mass redistribution (DMR) assays. The results demonstrated that the third intracellular loop of GPR120L remains the function of coupling with β-arrestin, but mislays the function of leading intracellular calcium and DMR responses.

Interestingly, the signal transduction through GPR120 receptor in the different tissues are distinct: G_q/G_{11} is the dominant pathway in adipose tissue and enteroendocrine cells, while the anti-inflammatory effects of GPR120 activation in the formation of macrophages is engaged through an β-arrestin-generated scaffold. Hence, on the basis of GPR120 differentiational functions, this review will summarize the distinct biological functions and the current stage of its agonists.

GPR120 biological characteristics

It has been reported that GPR120 expresses in many types of tissues and cell lines based on numerous studies *in vitro* and *in vivo*. Stimulation of FFAs, incretin hormones secretion in the mouse, rat and murine enteroendocrine cell line (STC-1) is through GPR120 receptor coupling with Gq family proteins to mediate the $[Ca^{2+}]_i$ responses [21]. Furthermore, FFAs were identified to inhibit serum deprivation-induced apoptosis through GPR120 in STC-1 cells [22]. In addition to Gq-dependent pathways, activated GPR120 can not only enhance glucose uptake in adipocytes but also promote anti-inflammation in macrophages through β-arrestin 2 signaling pathway [23]. Most of the biological characteristics are summarized in Figure 3.

Distribution

Hirasawa and his coworkers studied the GPR120 distribution by antibody-specific examination [24]. The results revealed that GPR120 is abundant in the mouse large intestine, lung, adipose tissue and colon. It should be underlined that this anti-GPR120 antibody provides a high value of the further study of GPR120 function. The GPR120 expression in adipose tissue significantly higher in obese individuals than in lean controls has been proved [2]. Initially, GPR120 mRNA is not detected in the whole pancreas; some recent studies have identified the expression in pancreatic islets of human, mouse and rat. Moreover, it is uncertain that the exact cell types express this receptor [25–27]. Besides, GPR120 were identified to express in stomach, colon and spleen in the human body [1]. Recent studies revealed that

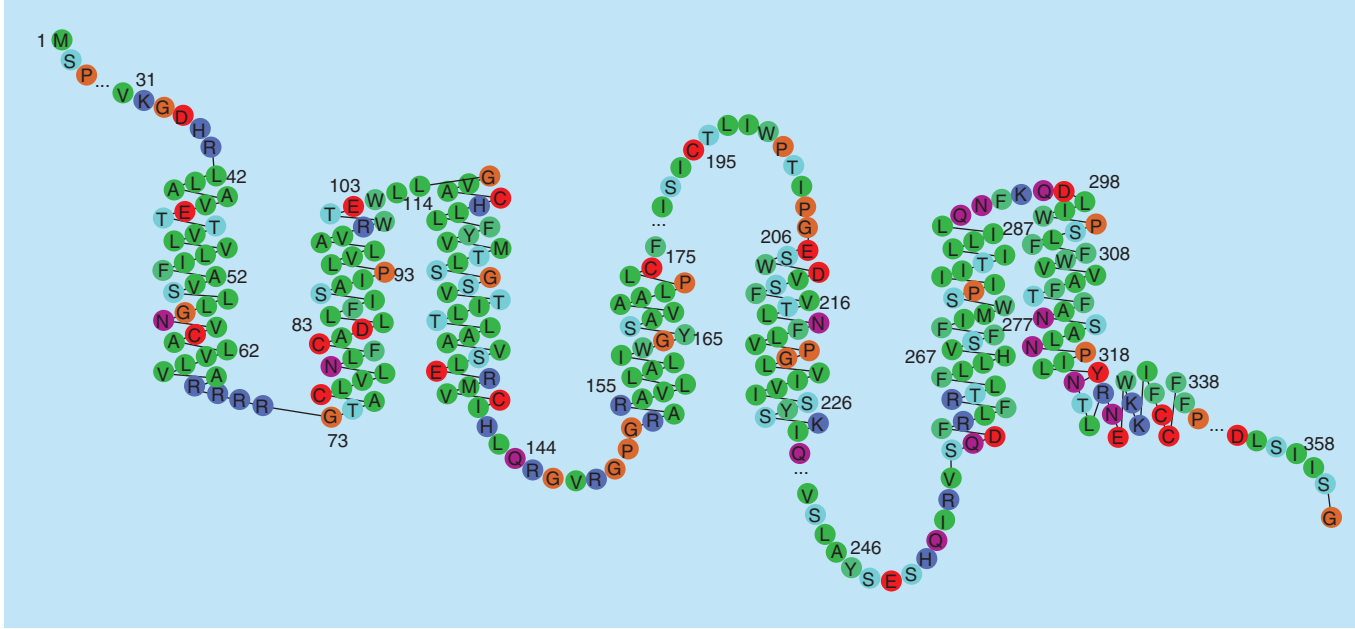

Figure 1. Snake-like topographical representation of human GPR120 receptor depicted by the residue-based diagram generator program [3]. Between positions 231 and 247 in intracellular loop 3, the long isoform (GPR120L) contains 16 additional residues. For rhodopsin members, intracellular loop 3 is critical to recognition both G-protein and β-arrestin.

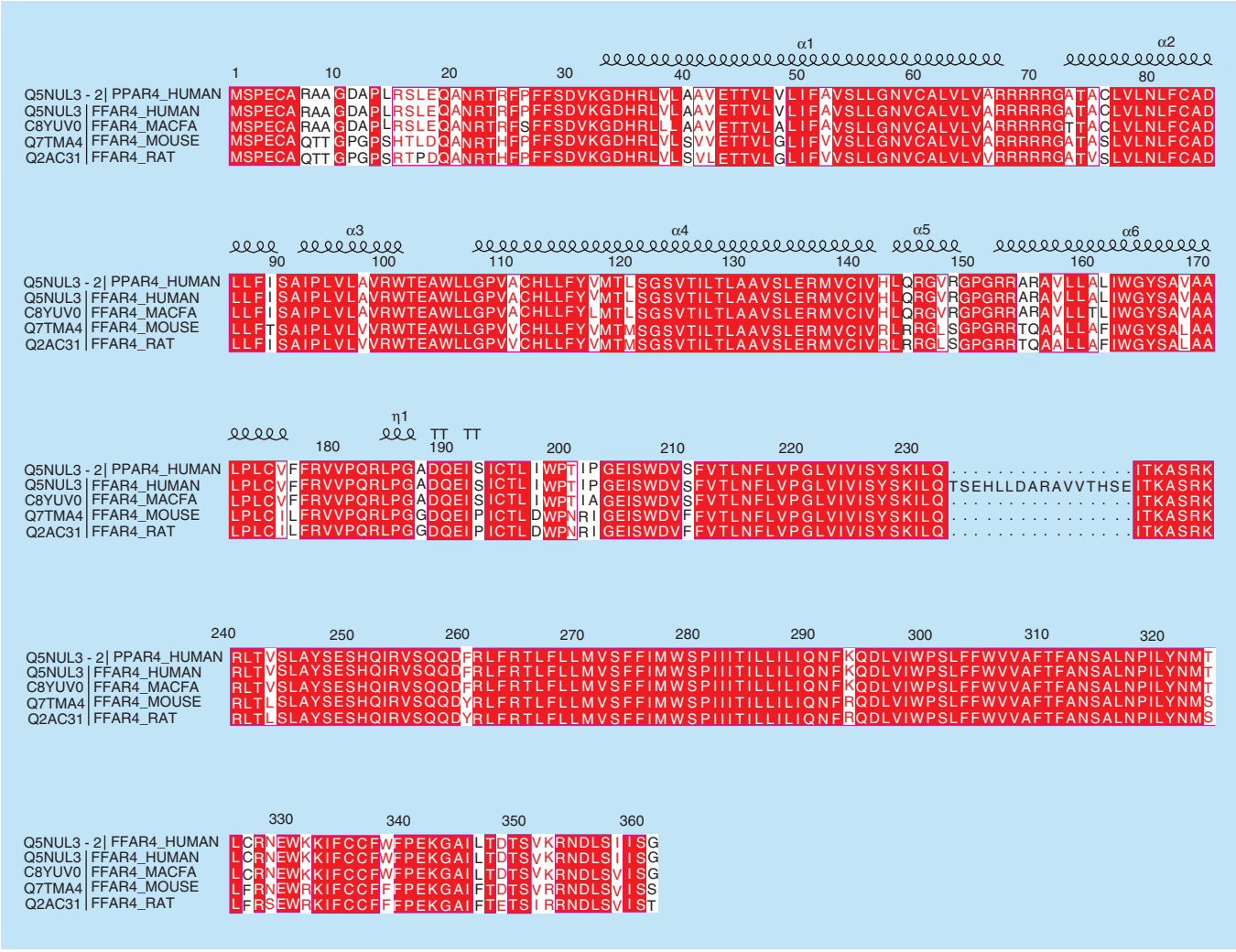

Figure 2. Sequence alignment of human GPR120S (Q5NLU3–2), human GPR120L (Q5NLU3), *Macaca fascicularis* GPR120 (C9YUV0), mouse GPR120 (Q7TMA4) and rat GPR120 receptors (Q2AC31) reproduced by T-Coffee [7] and ESPript programs [8].

GPR120 mRNA is abundantly expressed in various types of cells: taste cells [28], gastric ghrelin cells [29], duodenal enteroendocrine I cells [30], murine K cells [31], adipocyte and macrophage [23], osteoblast and osteocalcin [32]. GPR120 also expresses in numerous cell lines, including mouse GLUTag cells [33], murine enteroendocrine cell line STC-1 [21], ghrelin-producing cell line MGN3–1 [34], human breast cancer cell line MCF-7 [35], islet-α cell line αTC1–6 [36], human RAW 264.7 cells [32], rat β-cell line [37], human MDA-MB-231 breast cancer cells [38], human MCF-7 cells and MCF10A cells [35].

Appetite regulation

In 2007, Matsumura *et al.* identified the expression and localization of GPR120 in the taste bud cells. They detected a high expression of GPR120 mRNA in the epithelium of the circumvallate papillae while GPR40 mRNA was not detected in the sensory papillae [28]. Two years later, they identified that GPR120 expresses in type 2 taste cells in the circumvallate and fungiform papillae, which functions as a sensor for dietary fat. However, it remains ambiguous about the physiological role of GPR120 in taste bud cells [39]. GPR120-knockout mice showed less preference for α-linoleic acid (α-LA, **2**, Table 1) and reduction of taste nerve responses to several fatty acids, compared with wild-type mice [40]. It suggested that GPR120 might take part in the processes from intake to metabolism that may function as the transporter of the ligand to the receptor. Based on expression in the taste cells of the circumvallate papillae, it is a suggested role for GPR120 in sensing dietary fat.

Generated by ghrelin cells in the GI tract, ghrelin is a peptide that generally functions as a neuropeptide in the CNS [41,42]. In order to elucidate the mechanism of the secretion of ghrelin, Lu *et al.* isolated gastric ghrelin cells that were detected a high level of expression of

Table 1. GPR120 agonists.

No.	Structure and drug name	GPR120 EC_{50} (μM)	GPR40 EC_{50} (μM)	Selectivity index†	Material‡	Method	Ref.
1	GW-9508	28.2	1.41	20.0	HEK cells	BRET	[9]
2	Alpha-linolenic	3.23	2.88	1.12	HEK cells	BRET	[9]
3	DHA	3.98	1–3.9	1.02–3.98	HEK293 cells	FLIPR assay	[10]
4	NCG-21	2.40	9.70	0.24	HEK cells	BRET	[9]
5	TUG-891	43.7	64.5	<0.001	HEK cells	BRET	[9]
6	TH-44	25.5	10.8	2.36	Stable cell lines	FLIPR assay	[11]
7	GSK137647A	0.50	>30.2	<0.17	HEK293 cells	FLIPR assay	[12]
8	Phenyl-isoxazole-3-ol series	63	–	NA	CHO cells	FLIPR assay	[13]
9	Isoindolin-1-one series	180	–	NA	CHO cells	FLIPR assay	[14]
10	Pyrazol-phenyl series	<1	–	NA	Stable cell lines	Fluorescence intensities	[15–17]
11	Benzenesulfonic acid series	78	–	NA	HEK293 cells	FLIPR assay	[18]
12	NCG-75	62.8	4.5	13.9	Cells	FLIPR assay	[19]
13	KDT-501	30.3	–	NA	CHO cells	FLIPR assay	[20]

†Calculated as the difference between EC_{50} for GPR120 and GPR40.
‡Transfected with hGPR120 or hGPR40.
NA: Not available.

GPR120. After short-term culturing the gastric ghrelin-expressing green fluorescent protein cells, the long-chain fatty acids (LCFAs) significantly decreased ghrelin secretion. After gastric gavage of LCFA-rich lipid in mice with pylorus ligation, ghrelin levels in serum were suppressed, which indicated that GPR120 might mediate prandial LCFA inhibition of ghrelin secretion *in vivo* [29]. Moreover, Gong *et al.* revealed the possible mechanism of GPR120 suppressing the secretion of ghrelin. In ghrelin-producing cell lines, addition of GW9508 and α-LA suppressed the secretion of ghrelin through ERK activity. It should be noted that such an inhibitory effect can be stopped by a siRNA on the sequence of GPR120, in the meanwhile, a significant decrease of plasma ghrelin levels in mice were observed [43].

Cholecystokinin (CCK) is another kind of gut hormone, which regulating a wide range of intestinal responses concludes inhibition of gastric motility and pancreatic secretion [44]. Before the significant role of GPR120 in FFA-induced CCK secretion in STC-1 cells is discovered, it was only reported that FFA-induced CCK secretion in enteroendocrine cells leads to an increase in intracellular Ca^{2+} concentration [45]. Moreover, a variety of FFA-induced functions have been proved to be regulated by a group of GPCRs [21]. Recently, the results indicating LCFA-induced CCK secretion through GPR120-coupled Ca^{2+} signaling have been described. The CCK secretion in STC-1 cells was abolished when the Ca^{2+} channel blocker was added, or by transfection of GPR120-specific [46]. Further studies indicated that monovalent cation-specific transient receptor potential channel type M5 (TRPM5) plays a key role of LA-induced CCK secretion in STC-1 cells. LA-simulated TRPM5 currents and rise in intracellular calcium and CCK secretion are significantly decreased when the expression of TRPM5 is reduced by RNA interference [47]. In conclusion, FFAs may induce CCK secretion by GPR120/TRPM5/Ca^{2+} signaling pathway.

Proliferation & adipogenesis

One physiological function of GPR120 stimulated by FFAs is inhibition of apoptosis in STC-1 without serum conditions, and the possible mechanism is α-LA-activated ERK and PI3K/Akt pathway in STC-1, because the inhibitors for ERK kinase and PI3K reduced the anti-apoptotic effects caused by α-LA (Figure 4) [22], and the evidence showed that long-term administration of α-LA led the proliferation of pancreatic β cells in the rat, which probably result from the increase of glucagon-like peptide-1 (GLP-1) level [48]. Furthermore, culturing of human islets with eicosapentaenoic acid (EPA) inhibited LA-induced apoptosis, while GPR120 knockdown human islets reduce the ability of EPA preventing from apoptosis [25].

Based on clinical studies aimed at body fat mass being related to bone density and fracture risk, there is possibility that lipids may directly act on bone. Cornish *et al.* assessed the effects of FFAs and chemical agonists on bone metabolism. C_{14}-C_{18} fatty acids inhib-

ited osteoclastogenesis during bone marrow cultures, and GPR120/GPR40 agonist mimicked the inhibitory effects. However, no signal pathway has been found in lipid and bone metabolism; the article provides potential novel targets for treatment of abnormal bone metabolism [32].

Numerous studies have shown that it is possible for FFAs activate adipocyte through GPR120 as its receptor. It has been reported that LCFAs mediate secretion of leptin in rat adipocytes [49]. GW9508 and DHA (**3**, Table 1) simulation of GPR120 in 3T3-L1 adipocytes increased glucose intake through GPR120/$G_{q/11}$/PI3K/Akt/GLUT4 signaling pathway, indicating that GPR120 possibly involves in lipogenesis [23]. In Gotoh's paper, the study indicated the indispensable role of GPR120 in adipogenesis by functioning maturation of adipocyte differentiation *in vitro* [50]. Originally, they observed that during adipocytes differentiation, the level of GPR120 mRNA increased using 3T3-L1 cells and human adipose tissue. Moreover, during the adipogenic differentiation of 3T3-L1 cells, the expression of GPR120 protein was upregulated corresponded with mRNA expression. Moreover, using a small siRNA to downregulate GPR120 mRNA expression led to inhibiting the adipocyte differentiation [24]. Although the precise molecular mechanism of GPR120 in adipogenesis is still unclear, GPR120 is an indispensable part in the process of adipocyte differentiation and maturation.

Key terms

Insulin resistance: Physiological condition in which cells fail to respond to the normal actions of the hormone insulin and result in high blood glucose.

Incretins: Group of hormones that stimulate a decrease in blood glucose levels.

Incretin secretion

Diabetes is a kind of metabolic disease in which body does not produce enough insulin due to pancreatic β-cell loss (Type 1 diabetes) or insulin resistance in metabolic tissues (Type 2 diabetes). Adipocytes play a critical role in obesity-induced insulin resistance. There are two main pathways of the insulin signaling: PI3K/AKT pathway and the ERK pathway [51]. If insulin signaling is restricted at any point in this signaling cascade, insulin resistance will occur.

Some GPR120 agonists, such as α-LA, DHA and GW9508, exhibited the ability of stimulation of insulin secretion [52]. Incretins are a group of gastrointestinal hormones stimulating a decrease glucose level in blood. The two main candidates of incretins are

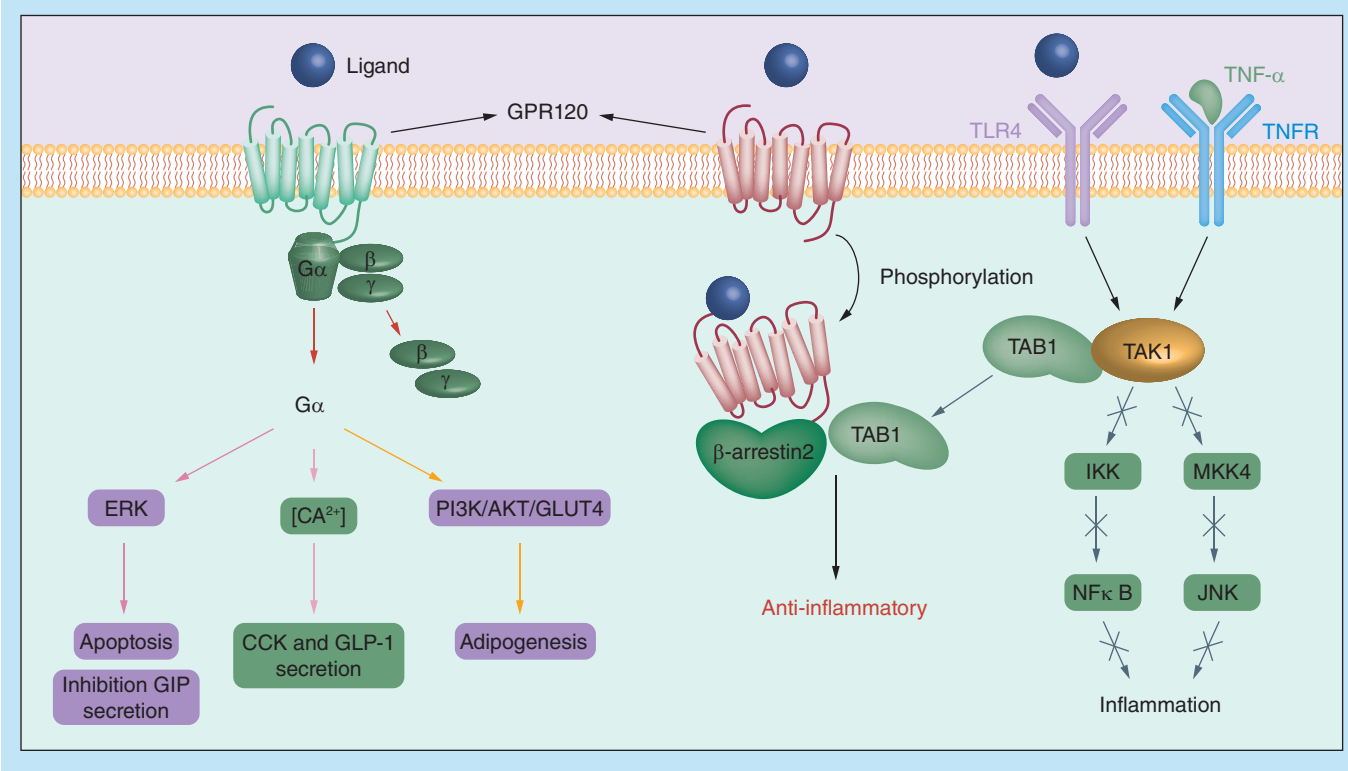

Figure 3. Two distinctive signaling pathways of GPR120. GPR120 couples to β-arrestin 2, after stimulation by ligand, which is followed by receptor endocytosis and inhibition of TAB1-mediated activation of TAK1. Coupled with G-protein, GPR120 receptor regulates the secretion of gastrointestinal peptide hormone, adipogenesis and adipogenic differentiation.

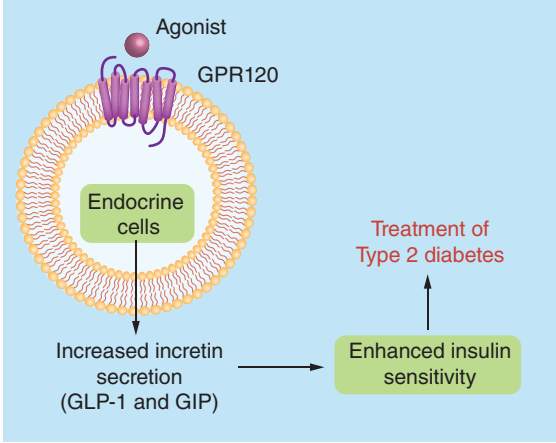

Figure 4. The relationship between GPR120 and Type 2 diabetes. The secretion of GLP-1 and GIP are indirectly regulated by FFAs through GPR120, and increasing of these incretins can result into enhancing insulin sensitivity and GPR120 can be regard as a novel potential target on treatment of Type 2 diabetes.

GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) [46,53]. Both secretions of GLP-1 and GIP can be indirectly regulated by FFAs through GPR120, and increasing of the incretins can result in glucose-dependent insulin secretion. Moreover, this indirect relationship between GPR120 and treatment of Type 2 diabetes is illustrated in Figure 4.

The major source of GLP-1 in the body is the intestinal L cell [54]. With activation of GPR120 by unsaturated fatty acids, this process can be observed in STC-1 [21]. Hirasawa's *et al.* showed that long-term oral administration of α-LA to mice leads raises GLP-1 and insulin concentration in plasma [21]. There is also evidence that administration of α-LA to the rat leads to higher plasma GLP-1 level [48]. Therefore, activation of GPR120 promotes secretion of insulin through secretion of GLP-1 from the GI tract. Because of this function of GPR120 and GLP-1 effecting appetite, the agonists of GPR120 probably benefit the treatment of diabetes. In addition, enhanced incretin secretion can also affect many physiological aspects involving receptors of GLP-1 [54].

Enteroendocrine K cells synthesize GIP, found in the mucosa of the duodenum and the jejunum of the GI tract, and it is now believed that the function of GIP is inducing secretion of insulin [55]. Moreover, the evidence that GPR120 is expressed abundantly in K cells of the upper small intestine and plays a critical role in lipid-induced GIP secretion has been proved [56]. Parker and his coworkers generated the transgenic mice with fluorescently labeled K cells, which enable identify and purify K cells from the isolated tissue. Moreover, in primary cultures, GIP release was detected after stimulation by glucose and α-LA. These transgenic mice and primary culture techniques provide novel opportunities that FFAs regulate GIP secretion through GPR120 [31].

Anti-inflammation

The state of inflammation leads to increase in secretion of local cytokine. Short-chain fatty acids activate an inflammasome activation pathway resulting IL-1β cleavage and release, and IL-1β stimulates production of TNF-α which can interact with TNF receptor [57,58]. And Fetuin-A serves as an adaptor in the Toll-like receptor 4 inflammatory pathway [59]. When Toll-like receptor 4 and TNF receptor are activated, TGF avtivated kinase 1 will bind protein TAB1, which initiates a proinflammatory signaling [60]. Then, ω-3FAs serve as an important role of anti-inflammatory process by decreasing circulating and tissue levels of pro-inflammatory factors including TNF-α, IL-1β and IL-6 by macrophages in mice and human [61,62]. Oh and his colleagues revealed the possible mechanism that the GPR120 functions as the ω-3FAs sensor [23]. The possible signaling pathway is GPR120 couple to β-arrestin 2, after stimulation of ligand, which is followed by receptor endocytosis and inhibition of TAB1-mediated activation of TGF avtivated kinase 1. This mechanism may inhibit both TLR and TNF-α pro-inflammatory signaling pathways as represented in Figure 3. However, the mechanism of the nutrients in the modulation of hypothalamic inflammation is poorly understood. A recently study investigated ω-3FAs have ability to active the same receptor in the hypothalamus in a mouse model of diet-induced obesity [63]. Another paper also demonstrated this transduction pathway using the hypothalamic neuronal model isolated from the rat hypothalamus [64]. Because of inflamed neurons of the CNS causing various metabolic diseases, further investigation is required to figure whether GPR120 is a putative receptor to cure this inflammation in CNS. In recent times, ω-3FAs were identified mediating hepatoprotective effects in Kupffer cells through GPR120. A clinical ω-3FAs formulation, Omegaven®, might eliminate inflammatory response-caused ischemia-reperfusion injury by dampening the NF-κB/JNK-mediated inflammatory response. It is suggested that GPR120 might be a therapeutic target to alleviate inflammation in the liver [65]. In view of that, GPR120 can serve as an important control point in the integration of anti-inflammatory and insulin-sensitizing responses, such a receptor is advantageous in the future development of new therapeutic approaches for the treatment of insulin-resistant diseases.

Colorectal carcinoma

Before found as the ligands of GPR120, the ω-3FAs have been well known because of their health benefits

against many diseases, especially reducing obesity-associated inflammation and insulin resistance. Recently, some evidence has indicated that EPA and DHA have a close relationship with colon cancer [66]. Low plasma concentrations of DHA and EPA among colon cancer patients have high risk of developing colon cancer. After treatment of colon cancer patients with DHA and EPA, cell proliferation led to a significant decrease [67]. However, what role GPR120 plays in the tumor progression is uncertain. Ellies LG *et al.* consider that obesity promotes mammary tumor progression in postmenopausal breast cancer and that ω-3FAs inhibit mammary tumor progression in obese mice, independent of GPR120 [68]. While in colorectal carcinoma, GPR120 functions as a tumor-promoting receptor, and it can be considered as a novel potential target for cancer therapeutics. The data suggest GPR120 signaling promotes angiogenesis *in vitro* and colorectal carcinoma tumor growth *in vivo*, probably through angiogenesis increasing [69]. Therefore, the relationship between GPR120 and cancer need further study.

Free fatty acid receptors

Several GPCRs act as the receptors of FFAs, including GPR119, GPR120 (FFAR4) and GPR40 (FFAR1). Among them GPR119 and GPR120 serve as long-chain fatty acid (14–18 carbons) receptors, and the ligands of GPR119 include lipid amides, and retinoic acid, with oleoylethanolamide being the most potent and efficacious, while GPR40 can be activated by medium-chain fatty acids (6–12 carbons) [70,71]. Based on the similarity of endogenous ligands of GPR120 and GPR40, there is a high possibility that a GPR120 ligand has the capacity to activate GPR40 receptor as well, in which all of them express in endocrine cells of the GI tract and have ability to increase the GLP-1 secretion and significantly improve glucose tolerance [21,72–73]. But GPR40 and GPR120 can stimulate another incretin GIP secretion [56,73]. GPR119 expresses in the pancreas, ileum and colon, and GPR40 preferentially expresses in pancreatic beta cells [74,75]. Additionally, all of the three receptors can activate G-protein while GPR120 can couple with β-arrestin 2 resulting anti-inflammation [22,70,76].

GPR120 agonists

In this review, we classified GPR120 ligands into endogenous ligands, small molecule agonists in articles and patents. Among them, some representative compounds are listed in Table 1. Compared with the increasing reports on investigating the pharmacological and physiological functions of GPR120, a relatively few small molecule agonists with high activity are available so far.

Key terms

Inflammation: Protective immunovascular response to harmful stimuli (e.g., pathogens) and considered as a mechanism of innate immunity.

Polyunsaturated fatty acids: Fatty acids with more than one double bond that are never adjacent. The methyl group at the end of the molecule is termed as the ω-carbon. In unsaturated fatty acids, the position of the first double bond relative to the ω-carbon is also counted.

Endogenous ligands

Fatty acids are hydrocarbon chains with a carboxyl acid group at one terminal and a methyl at the other. They are many varieties not only in the chain length of carbon atoms but the number, location and geometric configurations of unsaturated bonds [60]. Hirasawa and his fellows tested over 1000 chemical compounds on HEK293 cells stably expressing GPR120-EGFP to identify endogenous ligands for GPR120. Furthermore, they use HEK293 cells stably expressing the mouse GPR120-$G_{\alpha 16}$ fusion protein to examine the dose response of compounds on the concentration of intracellular Ca^{2+}. These two experiments both proved that long-chain saturated FFAs, especially with a chain as long as C_{14}–C_{18}, are the specific ligands for GPR120 [21].

Some long-chain ω-3 polyunsaturated fatty acids, such as DHA (C22: 6n-$3^{\Delta 4,7,10,13,16,19}$), have been found as ligands for the GPR120 leading anti-inflammatory properties [23]. However, ω-6 polyunsaturated fatty acids (PUFAs) are also natural GPR120 ligands. Mobraten *et al.* studied the difference of GPR120-mediated signaling events between binding ω-6 PUFAs and ω-3 FUFAs using Caco-2 cells as a model system that expresses GPR120 exclusively. Both ω-3 and ω-6 PUFAs increased the cytosolic concentration of the second messenger Ca^{2+} with the same potency (but different kinetics) and activated MAPK are protein kinases that are specific to the amino acids serine, threonine and tyrosine) ERK1/2 (but different kinetics and intensity) [77].

Chemical agonists

As described above, discovering small-molecule agonists targeting GPR120 is vital to the treatment of Type 2 diabetes and other metabolic diseases. Although ω-3FAs have been used as ligands to understand signaling pathway, no evidence was provided for direct interaction between GPR120 and FFAs. In this decade, several potent GPR120 agonists (EC_{50} were as low as nanomolar concentrations) have been development. Furthermore, even structure–activity relationships suggest that carboxyl group is essential for activity. GW9508 (**1**, Table 1) can activate both

GPR120 and GPR40, but it exhibited 100-fold more selectivity than GPR120 [78]. The evidence appears to be feasible to develop selective ligands for both the receptors. Because of no expression of GPR40 in adipocyte or macrophagocyte, GW9508 is usually used as the probe to study physiological functions and molecule mechanisms of GPR120 [23]. Suzuki *et al.* found a weak and nonselective GPR120 agonist **10** by screening a serious of carboxylic acids from a peroxisome proliferator-activated receptor γ (PPARγ) agonist. Based on the homology model of GPR120, they developed the first GPR120-selective agonist 12 after modification [79]. Hara *et al.* discovered that grifolin derivatives, grifolic acid and grifolic acid methyl ether promoted ERK and $[Ca^{2+}]_i$ responses in GPR120-expressing cells and GLP-1 secretion in STC-1 cells among more than 80 natural compounds, which were identified as selective GPR120 ligands. Moreover, this is the first time of identification of selective GPR120 ligands from natural products [80]. GPR120 agonistic activities of a series of compounds derived from a PPARγ agonist were well examined. Sun *et al.* calculated the hydrogen bonding energies between the candidates and GPR120 homology model derived from the crystal structure of bovine rhodopsin. The compound NCG-21 (**4**, Table 1) not only showed the lowest energy, but the most potent ERK activation, intracellular calcium responses in a cloned GPR120 system and GLP-1 secretion in STC-1 cells. Moreover, NCG-21 was also examined *in vivo*, which directly increased the plasma GLP-1 level in the mouse after administration [81]. Shimpukade *et al.* identified a selective and potent GPR120 agonist TUG-891 (**5**, Table 1) by screening a range of GPR40 agonists. They suggested that the carboxylic acid residue was indispensable, which serves as an electrostatic and double hydrogen bond interaction with Arg99 [9]. Furthermore, TUG-891 was used for the further examination of the function of human GPR120 and mouse GPR120, concluded $[Ca^{2+}]_i$ responses, β-arresstin1 and β-arresstin2 recruitment, ERK phosphorylation and internalization of the receptor. They also revealed that TUG-891 enhanced glucose uptake in 3T3-L1 adipocytes and inhibited the release of proinflammatory mediators from RAW 264.7 macrophages [82]. More recently, Shimpukade *et al.* reported the molecular basis of TUG derivatives interaction at GPR120. They first examined GPR120 binding site in detail with homology modeling [83]. Their efforts paved the way of drug discovery at the GPR120 *in silico*. The conceptional dual-agonist GPR120 and GPR40 for the treatment of diabetes on the basis of their novel glucose-dependent mechanism of action has been put forward, and the compound TH-44 (**6**, Table 1) is a representative one [11]. In recent times, the diarylsulfonamide series of GPR120 agonists has been described. The compound GSK137647A (**7**, Table 1) showed high selectivity and potency but low EC_{50} value. However, this article breaks the inherent structural characterization of GPR120 [12]. The results demonstrate that these small lead compounds can serve as tools for probing the further biology of GPR120 and developing more potent and selective GPR120 agonists.

Till 2015, a number of patent applications for antidiabetic compounds as GPR120 agonists have been disclosed. A series of phenylisoxazol-3-ol derivatives (representative one of them is **8**, Table 1) have been patented primarily by Banyu Pharmaceutical Co. Ltd from Japan, of which with medium activity (0.1 μM < EC_{50} <1 μM) targeting GPR120 using fluorometric imaging plate reader (FLIPR) on Chinese hamster ovary cells transfected with human GPR120 receptor [13]. Furthermore, several isoindolin-1-one derivatives (representative one of them is **9**, Table 1) invented by Banyu Pharmaceutical Co. Ltd were patented in 2010. Most worthy of mention in these patents is that these classes of compounds showed submicromolar potency in the absence of carboxylic acid [14]. Metabolex, Inc. reported a series of pyrazole, imidazole, triazole and dihydrobenzofuran derivatives (representatvie one of them is **10**, Table 1) as GPR120 modulators between 2010 and 2011. The activity of the compounds was examined by FLIPR calcium ion assay with human GPR120 stably expressing cells, and their glucose-lowering effects were evaluated in mice by oral administration of glucose. Mounting chemical compounds in these patents exhibited moderate activities (<1 μM) [15–17]. IRM LLC invented a large number of phenyl thiazole and phenyl oxazole with a carboxylic acid group or sulfonic acid in 2008 and 2010 [84]. Moreover, similar types of thiazole derivatives or tetrazole isosteres with carboxylic acid (representatvie one of them is **11**, Table 1) were synthesized and patented. The compounds were examined on human GPR120-fusing $G_{\alpha 16}$ stable cell line using FLIPR assay, and some potent agonists whose EC_{50} value are <10 μM were claimed [18]. Kyoto University patented a serious of synthesized aliphatic acid compounds with aromatic rings. In the patent, not only GPR120 EC_{50} values of these compounds were tested with Fip-in hGPR120 cells, but also GPR40 with T-Rex-hGPR40 cells. Considering that NCG-75 (**12**, Table 1) bears the high activity for GPR40 and GPR120, it may be a reliable candidate as synergic dual agonist of GPR120 and GPR40 to treat metabolic disorders [19]. In 2013, some fluoro-phenyl-methoxy-benzene carboxylic acid compounds as GPR120 agonists were discovered by Syddansk Universitet. Twenty-five compounds gave low EC_{50} values (<1 μM) using bioluminescence

resonance energy transfer [85,86]. Another compound should be mentioned is +KDT501 (**13**, Table 1) considered initially partial PPARγ agonists, which also activates GPR120 with medium activity with respect to α-LA and DHA. Furthermore, +KDT501 presented a significant reduction of glucose levels compared with metformin control *in vitro*. Therefore, the selective agonists of PPARγ have high possibility to be the candidates targeting GPR120 [20]. Recently, the invention patented by LG Life Sciences Ltd relates to a large number of thioaryl derivatives, among whom several compounds demonstrated superior GPR120 agonistic effects (EC_{50} <0.2 μM) [87].

Conclusion & future perspective

The concept of biased signaling of GPCR defined as an agonist being selective on one signaling pathway (β-arrestin or G-protein) from the same receptor has received a growing interest because of its potential therapeutic effects [88]. For GPR120 receptor, it has been reported that anti-inflammatory effects are mediated by β-arrestin2 and $G_{q/11}$ inducing gastrointestinal peptide hormone secretion, respectively [82]. In consideration of these, the biased ligand targeting GPR120 may show improved efficacy and reduced adverse effects on diabetes treatment or anti-inflammation. Thereby, the possibilities of bias properties of ligands should be increasingly developed in order to exploit these differential coupling mechanisms. The most novel, potent and selective agonist, TUG-891, shows no stimulus bias, though it appears to share similar stimulation efficiency with natural fatty acids [9].

Until now, a few synthetic ligands selectively targeting GPR120 have been developed, leading the result that many studies have been restricted to using various fatty acids showing the modest affinity for the receptor or low selective synthetic ligands. Considering the similar chemical structures of currently published GPR120 ligands, researchers need enough endeavor to increase chemical diversity among GPR120 agonist ligands. As we can see in this review, endogenous ligand and synthetic small molecule of GPR120 both have ability to activate GPR40, and the two receptors share some similar functions like stimulating insulin secretion. Therefore, the conceptional dual-agonist GPR120 and GPR40 for the treatment of diabetes on the basis of their novel glucose-dependent mechanism of action has been put forward [11].

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

Background

- Human GPR120 was originally identified by searching databases for rhodopsin-like guanosine-binding protein-coupled receptors.
- GPR120 functions as a receptor for ω-3 fatty acid, involving in regulating the secretion of gastrointestinal peptide hormone, adipogenesis, adipogenic differentiation and anti-inflammatory process.
- There are two isoforms of GPR120, and GPR120L has 16 additional amino acids in the third intracellular loop between positions 231 and 247.

Main biological characteristics

- GPR120 expresses in many types of tissues and cells.
- GPR120 plays a crucial role in sensing dietary fat and regulating energy balance.
- Both secretions of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide can be indirectly regulated by free fatty acids through GPR120.
- Broad anti-inflammatory effects can be caused after agonists stimulating GPR120.
- The relationship between GPR120 and colorectal carcinoma need further study.
- Endogenous and exogenous agonists.
- Long-chain saturated free fatty acids, especially with a chain as long as C14 to C18, are the specific ligands for GPR120.
- Many patents and literature have reported a lot of novel structures of small molecule agonists of GPR120, among which TUG-891 is the most novel, potent and selective agonist.

Future perspective

- The biased ligand targeting GPR120 may show improved efficacy and reduced adverse effects on diabetes treatment or anti-inflammation.
- Dual-agonist GPR120 and GPR40 may be the treatment of diabetes on the basis of their novel glucose-dependent mechanism of action in the very future.

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Editorial

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Future Medicinal Chemistry

Natural compound renews hope for diabetes and obesity therapeutic target

"...the identification of Gordonoside F as a potent GPR119 agonist and its broad *in vitro* and *in vivo* characterization further supports the potential value of GPR119 as antiobesity and antidiabetes target."

Keywords: diabetes • Gordonoside F • GPR119 • *Hoodia gordonii* • obesity

With 2.3 billion overweight and 700 million adipose adults worldwide in 2015 (WHO) [1], obesity and consequently diabetes mellitus Type 2 still are amongst the biggest global health problems. Though the number of adipose people and diabetes patients is ever growing, however, pharmacological treatment options are still not satisfactory especially for obesity. Recently, the US FDA approved the 5-HT_{2C} agonist lorcaserin as anorectic but its clinical efficacy [2] was not overwhelming and further clinical practice will have to show its value.

Pharmaceutical companies are therefore running expensive development programs for novel therapies to control obesity, and some experimental compounds are in clinical development. These include agents addressing known metabolic targets such as the GLP-1 mimetic liraglutide and combinations of ataractics such as the monoamine reuptake inhibitor bupropion with the opioid naltrexone or the anticonvulsant zonisamide [2]. Moreover, several experimental targets are evaluated with agents in clinical development such as the neuropeptide Y5 receptor antagonist velneperit (S2367) which has completed Phase II trials [2,3]. However, the results, according to what has been published so far, are less promising than anticipated.

The G-protein coupled receptor (GPCR) GPR119 was also considered a very promising target for the treatment of obesity and diabetes a long time [4,5]. The receptor is predominantly expressed on β-cells of pancreas islets and intestinal K and L cells [5,6]. It is a $G_{\alpha s}$-coupled rhodopsin-like seven transmembrane GPCR and it is not closely related to any other GPCR [7]. Physiologically, GPR119 is activated by oleic acid metabolites, especially oleylethanolamine as well as the dietary metabolite 2-monoacylglycerol and thereby the receptor acts as a fat sensor [5]. Interestingly, GPR119 seems to have a high constitutive activity as well but this mechanism must be further studied [7].

In vitro studies on GPR119 indicated a possible role of the receptor in glucose-dependent insulin release in pancreas similar to the activity of sulfonylureas as well as in the liberation of GLP-1 from intestinal cells [4,5]. Pharmacological GPR119 activation might therefore result in a very beneficial dual response for obesity and diabetes treatment by fortifying glucose-dependent insulin release and inducing GLP-1 secretion. Accordingly, GPR119 agonists such as AR231453 and PSN632408 increased plasma insulin levels and levels of GLP-1 *in vivo* and improved oral glucose tolerance tests [8,9]. Moreover, food consumption was reduced in animal models along with lower body weight gain [4].

These promising preclinical observations caused the development of several synthetic GPR119 agonists and several agents entered clinical trials. However, the most advanced compound GSK1292263 though well tolerated was not efficacious and failed to convince in a Phase II trial. This observed tachyphylaxis of GSK1292263 is considered as due to an agonist-induced receptor desensitization or downregulation but might be compound specific since it was not observed for other GPR119 agonists [5]. The disappointing results of GSK1292263 still dampened some

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of the hopes that were placed on GPR119 agonists and the clinical development of PSN821 was discontinued in Phase II leaving MBX-2982 as the last GPR119 agonist in clinical trials (Phase II, completed). So far, the Phase II results of neither PSN821 nor MBX-2982 are available and their efficacy can therefore not be assessed but no further development has been announced yet.

Eventually, GPR119 will gain attention again with recently published results on the popular weight loss herb *Hoodia gordonii* that was shown to exhibit its activity at least in part via the GPCR [10]. The succulent plant from the African desert has been used by bushmen for centuries as anorectic suppressing hunger and thirst in hunting trips. It contains pregnane glycosides of 6-deoxy- and 2,6-dideoxysugars with Hoodigogenin A as most important aglycone. For P57, a diglycoside of Hoodigogenin A, anorectic activity was observed *in vivo* and it was postulated that the agent might increase ATP levels in the hypothalamus but no molecular target of the compound could be identified [11]. Additionally, later animal experiments indicated that P57 does not reach the CNS after all [12].

> "Although the first synthetic GPR119 agonists that have reached clinical development could not provide convincing results, the GPCR might see a renaissance with Gordonoside F as a novel prominent agonist."

Recent experiments have now shown that the Hoodigogenin A triglycoside Gordonoside F, which is also a component of Hoodia gordonii potently activates GPR119. The natural compound showed agonistic activity on the receptor as measured by a CRE-dependent reporter gene assay, detection of intracellular cAMP levels and intracellular calcium mobilization. In these test systems, Gordonoside F had nanomolar to low micromolar EC_{50} values and was similarly potent as synthetic GPR119 agonists such as PSN632408. Gordonoside F furthermore did not activate other GPCRs *in vitro* but induced the phosphorylation of ERK1/2 which is a common pathway of GPCR signaling. However, ERK1/2 phosphorylation was only detectable in GPR119-expressing cells confirming the activity of Gordonoside F on GPR119 and its selectivity. Interestingly, neither the aglycone Hoodigogenin A nor P57 which was believed the most important active component of Hoodia gordonii showed any activity on GPR119 [10].

Stimulation of isolated rat pancreas islets with Gordonoside F resulted in an increased glucose-dependent insulin release but the agent had no effect on pancreas islets from GPR119 knockout mice. *In vivo*, Gordonisde F as well as Hoodia gordonii extract improved oral glucose tolerance and increased the plasma levels of insulin and GLP-1. Most notable, the extract and the isolated natural compound both reduced the cumulative food intake of mice. In GPR119 knockout mice, the beneficial effects on glucose tolerance, insulin and GLP-1 levels and food intake were blocked but in case of the Hoodia extract not completely abolished which indicates that further active components and targets are involved. More experiments are required to understand this further antidiabetic activity and eventually another valuable target can be identified [10].

Overall, the identification of Gordonoside F as a potent GPR119 agonist and its broad *in vitro* and *in vivo* characterization further supports the potential value of GPR119 as antiobesity and antidiabetes target. With these results, a link has been made from the well-known anorectic activity of a traditionally used herb to a molecular target with very promising activity for a number of metabolic diseases. The preclinical observations with Gordonoside F combined with the known activity in men indicate that pharmacologically relevant and efficacious anorectic and antidiabetic effects can be accomplished by GPR119 activation. Although the first synthetic GPR119 agonists that have reached clinical development could not provide convincing results, the GPCR might see a renaissance with Gordonoside F as a novel prominent agonist.

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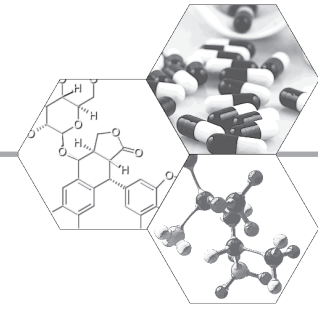

The roles of computational chemistry in the ligand design of G protein-coupled receptors: how far have we come and what should we expect?

"*Given the outstanding interest of both G protein-coupled receptor biology and computational chemistry, we frequently hear the question: how much does computational chemistry count in the characterization of G protein-coupled receptors and the design of new ligands?*"

Keywords: allosteric modulation ■ chemogenomics ■ computational chemistry ■ G protein-coupled receptors ■ virtual screening

G protein-coupled receptors (GPCRs) constitute a family of drug targets of outstanding interest for the biopharmaceutical sector since decades ago. Approximately a third of the current US FDA-approved drugs target a GPCR to any extent, including many blockbusters in the pharmaceutical industry [1]. However, many of these drugs have been in the market even before the first GPCR was cloned, and we can affirm that the drug discovery has in this sense been ahead of the functional and structural characterization of the receptors targeted. The 1980s and 1990s were decades presided by the functional characterization of GPCRs through the advances in molecular biology and pharmacology. With the new century, the structure of many receptors started to be accessible by x-ray crystallography [2,3], allowing the alluring perspective of GPCR structure-based drug design. The significant evolution of the field has been recognized in 2012 with the Nobel Prize in Chemistry awarded to Leifkowitz and Kobilka. Noteworthy, the year after the Nobel Committee highlighted the importance of computational chemistry in the understanding of biochemical processes (2013 Nobel prize in Chemistry to Karplus, Levitt and Warshel). Given the outstanding interest of both GPCR biology and computational chemistry, we frequently hear the question: how much does computational chemistry count in the characterization of GPCRs and the design of new ligands? Indeed, some key findings in the field have come through a proper combination of biochemical studies, pharmacology, medicinal chemistry and computational modeling (reviewed in [4]). Computational studies frequently contribute to the design of new compounds, although this is not always the case and some notes of caution have been written in this respect [5].

With the blossom of crystal structures (at an approximate rate of five structures per year since 2007) it seems relevant to look back and evaluate the performance of computer-aided, structure-based ligand design in this so-called golden era of GPCR structural biology. The 60 crystal structures of GPCR-ligand complexes currently available at the PDB account for 20 unique receptors, which represents slightly more than 5% of the 370 human GPCRs that are potential drug targets [6]. This small subset can be referred to as privileged receptors, since the determination of their crystal structure is usually the ending point of a deep biochemical and pharmacological characterization. In these cases, the mature methods of the modern computational chemistry such as structure-based virtual screening (SBVS) can provide remarkable results in the identification of novel compounds, as it was recently reviewed [7,8]. However, despite the refinement and accuracy of the computational strategies followed in these SBVS campaigns, the relatively high rate of true actives (between 20 and 73%) and the high potency of the confirmed hits (in many cases with sub-μM affinity) can be partially explained by two facts: on one hand, the orthosteric binding site of many GPCRs is highly druggable, with a narrow and mainly hydrophobic cavity with well-defined polar anchoring points (i.e., aminergic receptors or adenosine receptors). On the other hand, the majority of the commercial chemical databases screened are rich in compounds and chemotypes that bind a GPCR, as it was exemplified with the GlaxoSmithKline screening collection [9]. Nevertheless, many of

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the hit compounds revealed novel chemotypes for the receptor targeted, an added value of the rational computational explorations.

A forthcoming question is whether these methods would be equally valid for those receptors of yet unknown structure. An optimistic answer arises from the comparable performance of the SBVS pipeline of the Shoichet laboratory using either a homology-based model, either the crystal structure of the D3 dopamine receptor [10]. Again, one should be reminded that aminergic receptors are particularly advantageous, given the current availability of highly homologous templates and the existence of well-defined anchoring points in their orthosteric binding site. More astonishing are two stories of the pharmaceutical sector, where the computational filtering of chemical databases led to drugs that reached clinical trials. At the time when the only known GPCR structure was bovin rhodopsin, the recently disappeared company Predix (MA, USA) presented the discovery of a novel 5-HT_{1A} agonist following an exclusive *in silico* model-driven and structure-based approach [11]. Computational modeling also presided the optimization for selectivity against the hERG ion channel, producing a compound that reached Phase III clinical trials for the treatment of anxiety and depression. More recently, the GPCR-focused company Heptares (Hertfordshire, UK) demonstrated that a homology-based model of the A_{2A} adenosine receptor could be successfully used in SBVS to identify novel A_{2A} antagonists [12]. The structural model was based on the distal homologue β2 adrenergic receptor but appropriately assessed with site-directed mutagenesis and proprietary biophysical mapping experiments. The subsequent lead optimization, also assessed by computational chemistry, led to a compound currently licensed for clinical development.

> *"In the new era presided by advances in G protein-coupled receptor structural biology, ligand-based and structure-based approaches should optimally go hand-in-hand to deal with current challenges."*

Besides the many successful examples of structure-based hit identification and drug design, computational chemistry plays a key role at other stages along the way to GPCR ligand discovery: the generation of high-quality homology models, the characterization of the conformational equilibrium with molecular dynamics simulations, the construction of GPCR-focused libraries or the determination of selectivity and polypharmacology profiles; all constitute a unique feature of computational studies, alone or in combination with experimental data. In this sense, it is noteworthy that the quality of homology models (and of alternative modeling approaches adapted to GPCRs, such as topology-based or threading) has increased dramatically with the availability of the new structures. Since 2008, the GPCR-dock competitions have measured the ability of current computational methods to predict the binding mode of prototypical antagonists to unknown GPCR structures [13,14]. These evaluations confirmed that, at least in those receptors in which a template with enough homology is available (typically more than 30% sequence ID in the transmembrane region, which encloses ~60% of non-olfactory and non-orphan human GPCRs [8]), experimental ligand binding modes could be predicted with high accuracy. In addition to the previously discussed utility of computer-generated receptor models in drug discovery, a specific take-home message from these competitions is that the most successful predictions were precisely those where experimental data was properly integrated in the computational modeling pipelines.

From the ligand perspective, the ligand-based computational techniques are especially mature in the GPCR field, precisely due to the traditional lack of structural information. Indeed, novel ligand-based computational methods, such as ligand-based virtual screening, are often benchmarked to their ability of identifying novel structures that bind a given receptor. Consequently, GPCR-focused chemical libraries have been computationally designed [1,15] and chemogenomic analyses of annotated chemical libraries have been carried out for GPCRs [16,17].

In the new era presided by advances in GPCR structural biology, ligand-based and structure-based approaches should optimally go hand-in-hand to deal with current challenges. One of the most intricate issues in GPCR-ligand design is the determination of receptor specificity, as it comprises two sides of the same coin that often need to be addressed, namely subtype selectivity and polypharmacology. While the former is usually desired, and to some extent achieved in computational ligand design programs, the fine-tuning of the polypharmacology profile is more complex since it involves simultaneous design of selective and promiscuous profiles.

The paradigmatic case is probably schizophrenia, where despite the elegant computational approaches designed to rationalize the multireceptorial binding-affinity profiles of marketed antipsychotics [18], an example of a prospective study is yet to come. Finally, everything can be turn upside down with the chemogenomic perspective, by which mathematical and computational models arise from joint analyses of chemical and biological spaces allowing the identification of novel bioactivities for known ligands, as well as of expected side effects, due to cross-pharmacology [16]. These strategies are specially suited for the highly demanded discovery of allosteric modulators [17], devoted to a higher efficacy and fewer side effects since they bind to distinct sites than the orthosteric binding site of the natural ligand. Alternatively, molecular simulations seem mature enough to characterize allosteric binding sites of GPCRs [19,20], and might well influence with equal success in the near future the design of allosteric or bitopic ligands (those targeting allosteric and orthosteric sites simultaneously), which are intended to be a breakthrough in the field.

The reported success and the remaining challenges of computational chemistry in GPCR ligand design should always be put in the context of appropriate cross-talk with wet laboratory experiments in a multidisciplinary environment. In this sense, a European research network has been recently established under the name of GLISTEN [101]. It joins together a wide panel of experimental and theoretical researchers with expertise in complementary approaches, coming from both the academia and the industry, aiming to unravel details of the activation mechanism and ligand binding on GPCRs. Computational groups have the chance to access first-hand data to build and enrich models, which shall be used in the design and testing of new compounds with the complicity of medicinal chemists and pharmacologists.

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