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Review

Farnesoid X receptor (FXR): Structures and ligands

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ABSTRACT

Farnesoid X receptor (FXR) is a bile acid activated nuclear receptor (BAR) and is mainly expressed in the liver and intestine. Upon ligand binding, FXR regulates key genes involved in the metabolic process of bile acid synthesis, transport and reabsorption and is also involved in the metabolism of carbohydrates and lipids. Because of its important functions, FXR is considered as a promising drug target for the therapy of bile acid-related liver diseases. With the approval of obeticholic acid (OCA) as the first small molecule to target FXR, many other small molecules are being evaluated in clinical trials. This review summarizes the structures of FXR, especially its ligand binding domain, and the development of small molecules (including agonists and antagonists) targeting FXR.

Contents

1. Introduction . . . 2148
2. Organization of FXR . . . 2149
3. FXR DNA binding properties . . . 2150
4. Ligand-binding properties of FXR . . . 2152
4.1. Analysis of FXR-LBD structures . . . 2152
4.2. Endogenous FXR ligands . . . 2154
4.3. Synthetic FXR agonists . . . 2154
4.3.1. Steroidal FXR agonists . . . 2154
4.3.2. Nonsteroidal FXR agonists . . . 2154
4.3.3. Partial FXR agonists . . . 2155
4.3.4. Dual FXR and GPBAR1 agonists . . . 2155
4.4. FXR antagonists . . . 2156
4.5. Side effects of FXR agonists . . . 2156
5. Summary and outlook . . . 2157
CRediT authorship contribution statement . . . 2157
Acknowledgments . . . 2157
References . . . 2157

1. Introduction

Farnesoid X receptor (FXR) is a bile acid activated receptor (BAR) with two members in mammals: FXRα and FXRβ [1]. FXRβ is a pseudogene in humans and primates, but encodes a functional receptor in other species [2]. FXRα gene encodes four isoforms:

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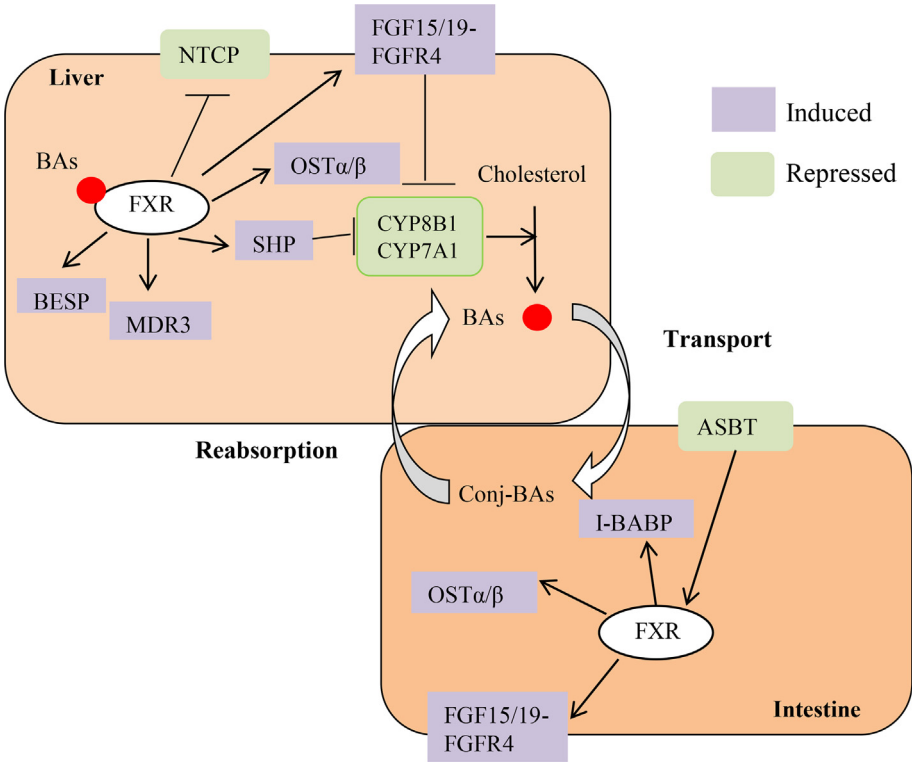

Fig. 1. The importance of FXR in the enterohepatic circulation of bile acids. FXR represses the transcriptional activity of hepatic Cyp7a1 and Cyp8b1 by upregulating the expression of SHP. FXR stimulates the synthesis of the FGF-15/19-FGFR4 pathways to inhibit CYP7A1 and CYP8B1 expression. FXR regulates key genes involved in BA transport, reabsorption, conjugation and detoxification, such as NTCP, BSEP, MDR3 and OSTα/β.

FXRα1-α4 [3,4]. These four isoforms are expressed in a tissue-dependent manner: FXRα1 and FXRα2 are expressed moderately in the ileum and adrenal glands, FXRα3 and FXRα4 are abundantly expressed in the ileum and moderately expressed in the kidney [4]. FXR regulates the metabolism of bile acids, carbohydrates and lipids [5,6].

Upon activation, FXR binds heterodimerically to with retinoid X receptor (RXR), and induces the expression of small heterodimer partner (SHP) gene, leading to transcriptional repression of the rate-limiting enzymes cholesterol 7α-monooxygenase (CYP7A1) and liver receptor homolog 1 (LRH-1) [7,8]. FXR also stimulates the synthesis of fibroblast growth factor-19 (FGF-19) to inhibit CYP7A1 and sterol 12α-hydroxylase (CYP8B1) expression through the fibroblast growth factor receptor 4 (FGFR4) pathway in the hepatocytes [9–11]. The FXR/SHP and FXR/FGF19/FGFR4 pathways constitute the major negative regulators of bile acid synthesis. FXR inhibits sodium taurocholate cotransporting polypeptide (NTCP) through an SHP-dependent mechanism, thereby repressing the uptake of bile acids by the liver [12]. FXR upregulates the gene expression of bile salt export pump (BSEP) and multidrug resistance protein-3 (MDR3) and increases BA efflux from the liver to the canalicular lumen [13,14]. FXR also increases the expression of organic solute transporter alpha/beta (OSTα/β) expression which enhances BA efflux from the liver to the portal vein [15]. In addition, FXR regulates key enzymes involved in BA conjugation and detoxification [13]. In a summary, FXR is intimately involved in the entire metabolic process of bile acid synthesis, transport and reabsorption (Fig. 1) [16,17]. Homozygous loss of FXR function due to NR1H4 mutations (p.R176*, Tyr139_Asn140insLys) causes severe progressive familial intrahepatic cholestasis (PFIC) with a low gamma-glutamyl transferase (GGT) form [18]. In both extra- and intrahepatic models of cholestasis, FXR activation leads to the amelioration of cholestasis to protect the liver from the high cytotoxicity of bile acids [19]. FXR induces the synthesis of FGF15/19 and up-regulates the FGF15/19-FGFR4 signaling, which may promote the risk of HCC [20], while FXR activation has been reported to display potential anti-tumor activity in colorectal cancer [21], HCC [22] and cholangiocarcinoma [23].

Since the approval of obeticholic acid (OCA) by the U.S. FDA in 2016 as a second-line treatment for primary biliary cholangitis (PBC), more and more small molecules targeting FXR have been developed and entered into clinical trials [24]. The complex structures of FXR with some ligands have been determined and yield extensive insights into the understanding of activation or repression of FXR, which has important implications for the treatment of related diseases.

2. Organization of FXR

FXR shares a classic nuclear receptor (NR) organization (Fig. 2A): a ligand-independent transcriptional activation domain (AF1), a core DNA-binding domain (DBD), a hinge region, a C-terminal ligand-binding domain (LBD) and a ligand-dependent activation function domain (AF2) [25].

The AF1 domain is a highly disordered domain that can interact with coregulator proteins. Alternative splicing has given rise to multiple AF1 domain isoforms. FXRα3 and FXRα4 possess an extended N-terminus compared with that of FXRα1 and FXRα2 (Fig. 2B) [1,26]. The FXR-DBD establishes base-specific interactions with DNA, which enables the recognition of specific DNA sequences. The DBD region is highly conserved and contains two α helices (H1 and H2) and two four-cysteine/zinc nucleated modules (Fig. 2C) [27]. The hinge domain is a short, flexible linker with little sequence or size conservation. FXRα1 and FXRα3 each have an insert of four amino acids (MYTG) in this region (Fig. 2B) [4].

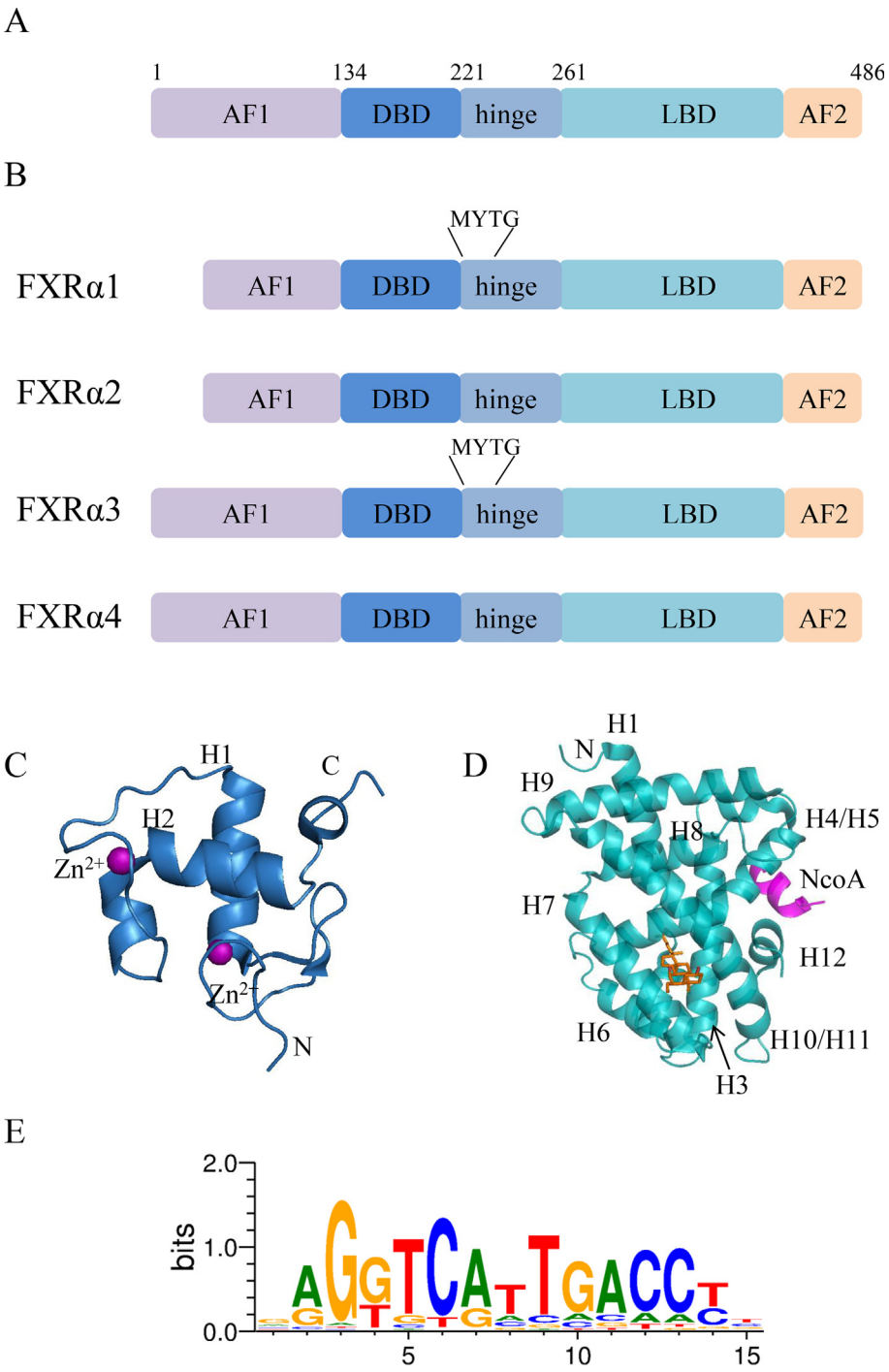

Fig. 2. Schematic diagram and structure of FXR. (A) Organization of FXR. (B) Schematic diagram of four FXRα protein isoforms. (C) Model structure of FXR-DBD. The EcR-DBD structure (PDB ID: 1R0O) is used to represent the FXR-DBD. (D) Crystal structure of the FXR-LBD/OCA complex (PDB ID 1OSV). The FXR-LBD is shown in greencyan, OCA is in orange and the NcoA peptide is colored magenta. (E) The typical IR1 element. The IR1 sequence is found in the footprintDB database. The IR1 schematic diagram is generated by WebLogo. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The FXR-LBD binds to its ligands and interacts with coregulator proteins. This domain consists of 12 α-helices that fold into three parallel layers to form an alpha helical sandwich and contains a hydrophobic ligand-binding pocket (LBP) at the base of the receptor to accommodate its ligands [28] (Fig. 2D). AF2 is in the LBD and includes H12. Similar to other nuclear receptors, H12 of FXR has been shown to undergo dynamic conformation changes upon binding of different ligands, and changes in AF2 orientation facilitate interactions with different regulatory proteins [29]. This mechanism will be discussed further.

3. FXR DNA binding properties

FXR can regulate gene expression by binding DNA as a monomer or as a heterodimer with RXR [30,31]. The DNA motifs specifically recognized by the FXR-DBD are named FXR response elements (FXREs). According to genome-wide ChIP-seq studies, diverse FXREs architectures have been found [31]. Classical FXREs contain two monomeric sites of the consensus half-site 5′-AGGTCA-3′ in palindromes or direct repeats with various numbers of nucleotides in the spacer. The most well-known and highest

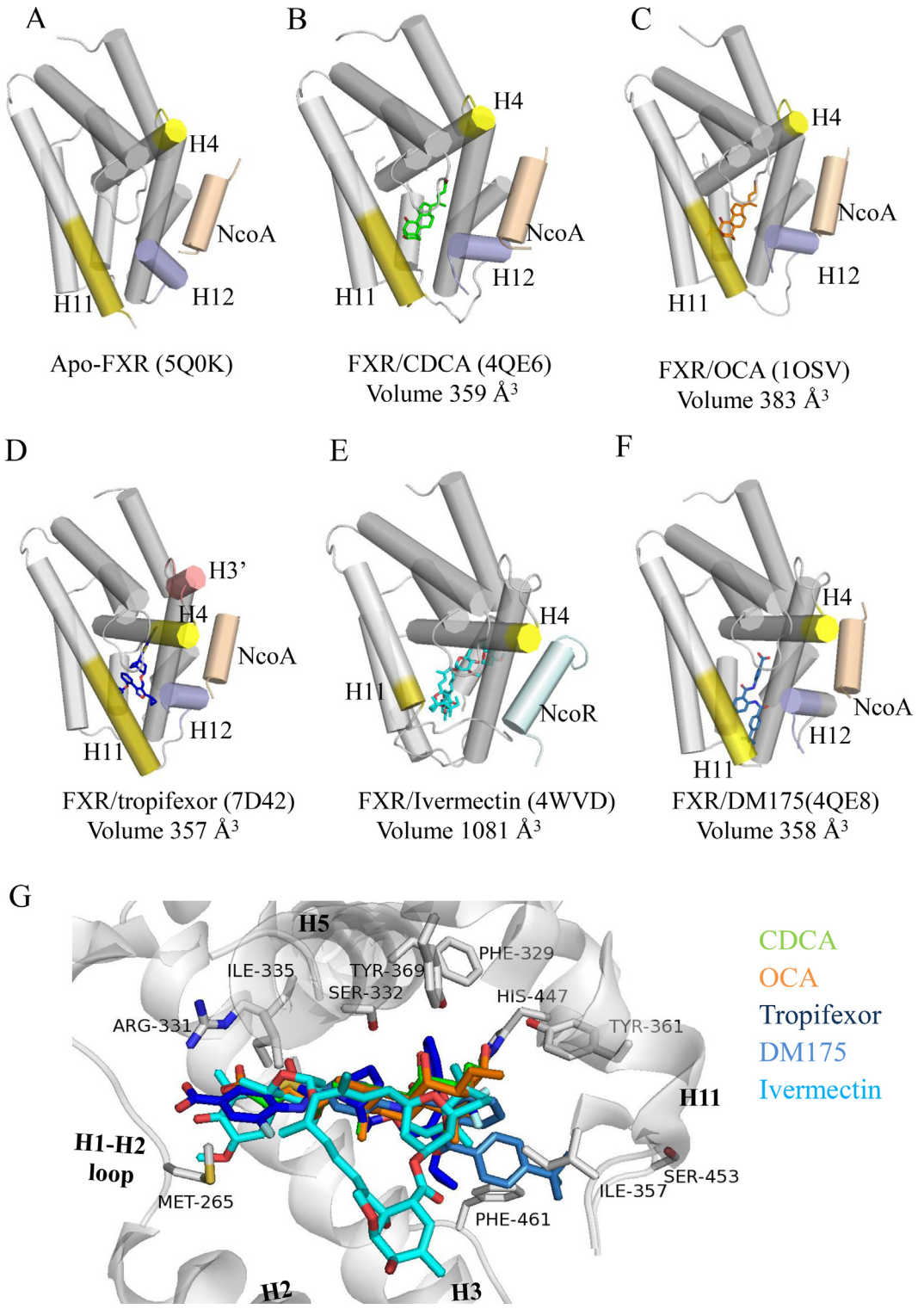

Fig. 3. Overall structures of FXR in different states. (A) The apo-FXR structure (PDB ID 5Q0K). (B) FXR/CDCA (4QE6). (C) FXR/OCA (1OSV). (D) FXR/Tropifexor (7D42). (E) FXR/Ivermectin (4WVD) (F) FXR/DM175 (4QE8). FXR is colored gray. The regions discussed are colored as follows: H4 (yellow), H11 (olive), H3′ (violet), H12 (lightblue), NcoA (lightpink), NcoR (palecyan). The LBP pocket volumes of FXR are calculated using POCASA. (G) Superposition of different ligand binding patterns. Key protein regions affected by ligand binding are highlighted. The critical polar residues and main hydrophobic residues in the pocket are labeled. The ligands are shown in different colors: CDCA (green), OCA (orange), 7D42 (blue), ivermectin (cyan), DM175 (skyblue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

affinity FXRE motif is an inverted repeat of two AGGTCA consensus sequence separated by one nucleotide (IR1) (Fig. 2E) [29]. The FXR/RXR heterodimer also binds to FXREs, such as IR0, or everted repeats with two or eight nucleotide spacers (ER2 or ER8) and direct repeats separated by one nucleotide (DR1) [31–33]. The coactivator-binding site (H10/11) of FXR undergoes allosteric conformational changes induced by the dimerization with RXR, and these changes may enhance the transcriptional activity of FXR to

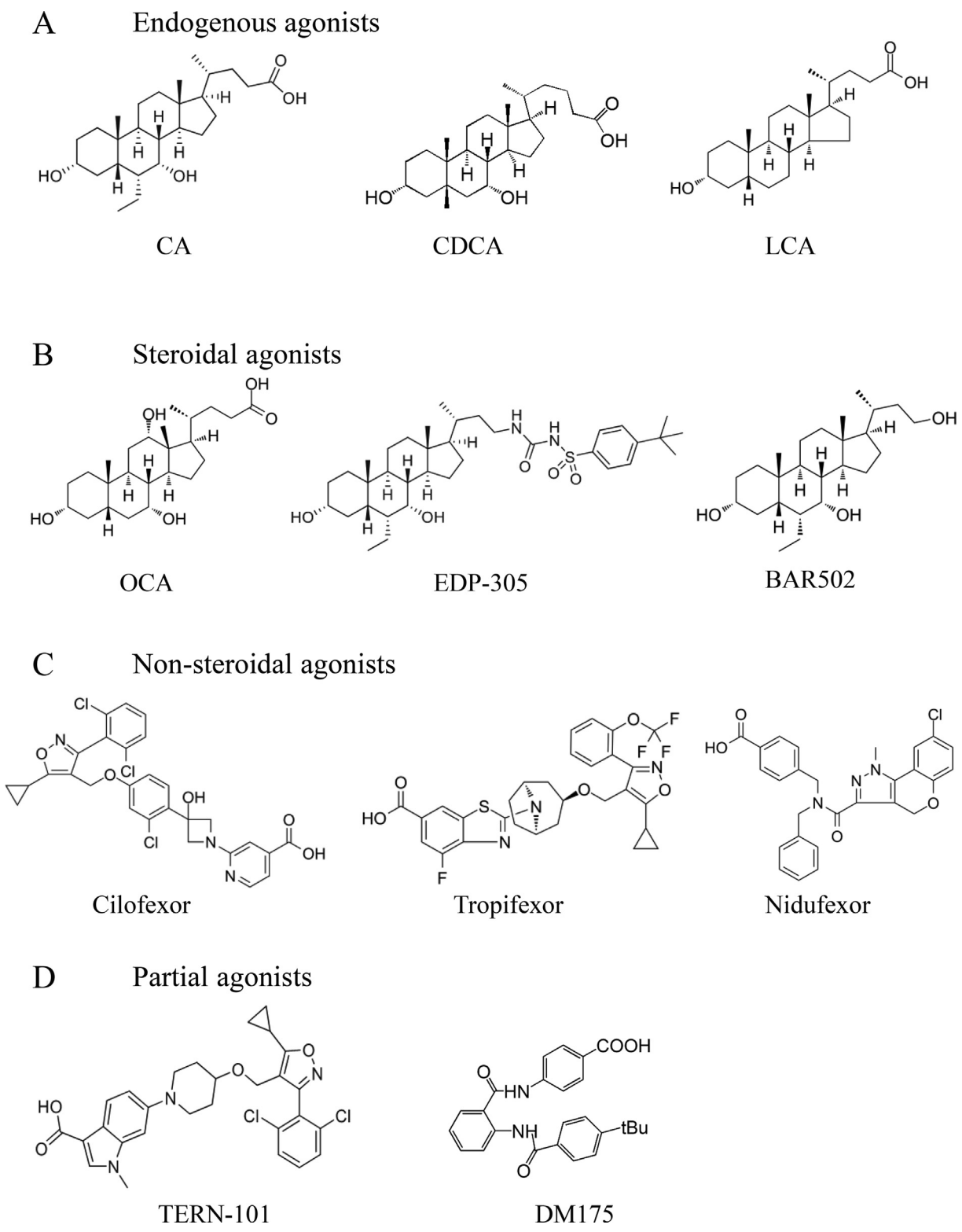

Fig. 4. Chemical structure of FXR agonists.

bind the FXREs [28,34]. The dimerization mechanism between FXR-DBD and RXR-DBD is still unclear. Negative FXREs have also been found in a few FXR target genes, such as ApoA1, to which FXR binds as a monomer or homodimer and represses ApoA1 expression [30]. Diverse FXREs are localized in promoter, intergenic and intron regions of many genes. FXR binds to the FXREs to exert its various biological functions such as metabolism, transport, kinase signaling and glycolysis.

4. Ligand-binding properties of FXR

4.1. Analysis of FXR-LBD structures

The FXR-LBD domain contains the classic NR LBP for ligands binding (Fig. 3). The LBP pocket volume tends to be adjusted by the bound ligand [35]. The chemical structures of the typical FXR ligands are shown in Figs. 4 and 5. The LBP calculated using

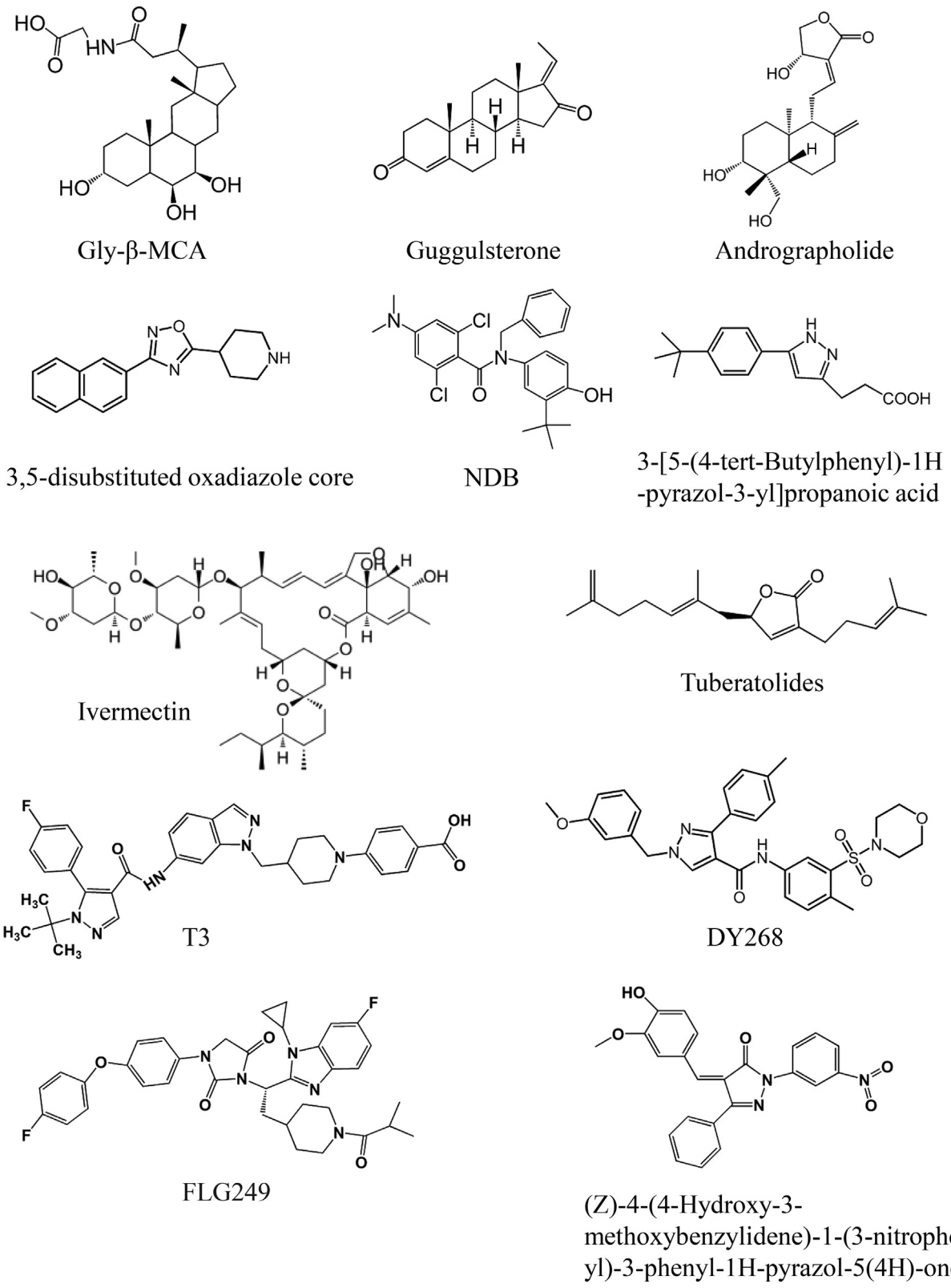

Fig. 5. Chemical structure of FXR antagonists.

POCASA are usually about 300–400 Å^3 in some FXR/ligand structures, while it is about 1081 Å^3 in the FXR/Ivermectin structure [36] (Fig. 3A-F). The critical polar residues (Arg331, His447 and so on) in the pocket establish hydrogen bond interactions with the ligands to position the ligands in the correct orientation, and hydrophobic residues (Ile335, Phe329, Phe461 and so on) establish hydrophobic interactions with ligands to stabilize the whole structure [35,37,38] (Fig. 3G).

Previous studies revealed that the positions of H12 of NRs were different in liganded and unliganded (apo) states. H12 was thought

to be positioned away from the core LBD in apo-state (inactive state), while H12 altered its position to be bound to the core LBD when bound with agonists (active state), which is the model of a classic mouse-trap mechanism for NRs activation [39]. Later studies [40,41] suggested that the terminal LBD region is dynamic in apo-state and that H12 is only formed upon agonist binding.

Merk et al [38] have recently reported the mechanism for FXR activation that also explains partial agonism by crystallization and NMR experiments. The unliganded FXR-LBD recruit corepressor (e.g., NcoR) in solution. Agonistic ligands disrupt the interaction between FXR-LBD and corepressor, leading to partial dissociation of the corepressor from FXR. Agonistic ligands promote the stabilization of H12 and binding to the core LBD. Then coactivator (e.g., NcoA) is recruited to FXR-LBD. In contrast, antagonists stabilize the interaction of FXR-LBD and corepressor in an inactive state with the unordered AF-2 binding to corepressor. Partial agonists weaken the interaction between FXR-LBD and corepressor, induce conformational changes in FXR-LBD that is capable of recruiting both corepressors and coactivators.

The FXR-LBD/ligand complex structures deposited in the PDB database were mainly divided into three categories: apo-FXR-LBD, agonist-FXR-LBD and antagonist-FXR-LBD. The overall structure of apo-FXR-LBD (5Q0K [42] and 6HL0 [38]) contains 12 α-helices and an unordered state of the H11 and L: H3-H4 (loop-linked helix H3 and H4) regions (Fig. 3A). An NcoA peptide is shown in the apo-FXR-LBD structure, which indicates that the FXR-LBD without a ligand is able to recruit coregulators.

In the agonistic-FXR-LBD structure (4QE6 (Fig. 3B), 1OSV (Fig. 3C) [35] and 7D42 (Fig. 3D) [37]), the overall structure of FXR-LBD is similar to that of apo-FXR-LBD, in which the coactivator (NcoA) binds to AF-2 with two conserved ionic interactions. Minor conformational changes are found. H11 is ordered and a 3_{10} helix in the loop region between H3 and H4 (L: H3-H4) is formed (Fig. 3D). H4 is located close to the core of the LBP, leading to a tight LBP bound with nonsteroidal ligands. These conformational changes may stabilize the overall structure of FXR-LBD and promote its affinity for coactivator binding. In addition, a second binding induced, hydrophobic pocket was reported in the crystal structures of FXR-terpenoid (PDB 5IAW and 5ICK) [43]. The first ligand occupies the binding site with similar other ligands [43]. H6 is shifted outward and H2 is also distorted to induce a second pocket to accommodate the extra terpenoid ligand perpendicular to the first one, resulting in the substantial expansion of the pocket size [43].

In the antagonist-FXR structure (4WVD (Fig. 3E) [44] and 4OIV [45]), carboxy terminal AF2 helix (H12 and L: H11-H12) is invisible or changing to a β strand, which suggests that AF2 is a highly flexible and dynamic helix induced by antagonist binding. Ivermectin is a drug approved for use against a variety of nematode and arthropod parasites, and is also a highly selective FXR antagonist [44]. The structure of ivermectin is much larger than other ligands. Therefore, H2 and H6 in the LBP are shifted outward and distorted in the FXR-LBD/ivermectin structure to make extra space to accommodate ivermectin [44]. Besides that, H11 is disorder and a NcoR peptide is observed in the structure (Fig. 3E) [44]. In the FXR/NBD structure, H11 changes to a β-strand and forms a dimerization interface with the β-strand in another FXR molecule [45].

Compared to the three states previously described, the partial agonistic conformation of FXR-LBD has both agonistic and antagonistic conformation characteristics [38]. DM175 promotes H11 formation as FXR agonists but to a lesser extent and does not stabilize the L: H3-H4 in a helical state (Fig. 3F) [38]. Besides, DM175 induces outward movement of W454 in the LBP and causes a shifted position of H12 [38]. Thus, the partial agonistic conformation of FXR-LBD could recruit both corepressors and coactivators.

In summary, the FXR-LBD conformation is affected by the characteristics of ligands and coregulator peptides. Different FXR ligands occupy close to different regions and bind different residues of the FXR LBP, leading to minor conformational changes in the FXR LBP (Fig. 3G). However, the mechanism by which FXR ligands enter and exit the LBPs remains unclear. When bound to different ligands, FXR recruits different coregulator proteins to activate or repress the transcription of target genes.

4.2. Endogenous FXR ligands

FXR is a nuclear receptor for bile acids which are a family of atypical steroids generated in the liver. Two main families of bile acids can be identified in humans. The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are generated from cholesterol. The secondary bile acids lithocholic acid (LCA) and deoxycholic acid (DCA) are generated from CA and CDCA, respectively [46]. The potency of bile acids in activating FXR is ranked as: CDCA > DCA > LCA > CA [47]. Bile acids may function as signaling molecules to regulate their own synthesis and affect diverse biological and pathophysiological processes, such as liver regeneration, proapoptotic and proinflammatory actions [48,49].

4.3. Synthetic FXR agonists

Given the potential of FXR ligands to be an effective approach to treat bile acid-related liver disorders, many more synthetic FXR agonists are currently being developed for liver diseases such as nonalcoholic steatohepatitis (NASH) and PBC [50]. Synthetic FXR agonists include steroidal and nonsteroidal ligands. The FXR ligands in clinical trials or approved by the FDA are summarized in Table 1.

4.3.1. Steroidal FXR agonists

OCA, a semisynthetic derivative of CDCA, is also known as 6-ethyl-CDCA and INT-747 [35]. This drug was developed for the treatment of various liver diseases, including biliary atresia, PBC, NASH and primary sclerosing cholangitis (PSC). The 18-month interim analysis of a phase 3 trial for its use in the treatment of NASH and fibrosis patients showed that 25 mg of OCA significantly attenuated fibrosis and NASH disease activity in participants [51]. OCA might be the first drug approved to treat NASH and fibrosis patients. However, the risk of some side effects, such as pruritus, gallstones and acute cholecystitis, was increased in patients treated with OCA compared with patients who received a placebo [51,52]. The phase 3 trial is still ongoing with patients expected to have a follow-up time of at least 4 years to evaluate the long-term clinical benefits of OCA treatment.

EDP-305 is a steroidal FXR agonist that is developed for the treatment of NASH and PBC [65]. EDP-305 is currently being evaluated in a phase 2b randomized, double-blind, placebo-controlled, multicenter study to evaluate its safety and efficacy in NASH patients (NCT04378010). In the subjects across all studies exposed to EDP-305, the majority of treatment related adverse events are mild to moderate [66]. EDP-305 treatment reduces C4 and high GGT levels and increases FGF-19 and ALP levels in Enanta Pharmaceuticals, Inc homopage.

4.3.2. Nonsteroidal FXR agonists

Most steroidal FXR agonists exhibit poor aqueous solubility and bioavailability. The steroid nucleus and side chain structure endow them with partial GPBAR1 agonistic properties [67,68]. This property can increase the therapeutic potential of steroidal FXR agonists, but also can induce some side effects which might be induced by GPBAR1 [24,69]. To reduce side effects and improve

Table 1
FXR ligands.

Agonists:	EC_{50}/IC_{50} (nM)	PDB ID	Clinical Trial Phase	NCT identifiers	Indication	Reference
Obeticholic acid	99	1OSV	FDA approved phase 2 phase 3	NCT02308111 NCT01585025NCT02548351	PBC PSC, BADNASH	[35,51,52]
EDP-305	n/a	n/a	Phase 2	NCT03394924, NCT04378010	PBC, NASH	[53]
GW4064	65	3DCT	n/a	n/a	n/a	[54]
MET409	16	n/a	Phase 2	NCT04702490	NASH, T2DM	Metacrine
TERN-101	n/a	n/a	Phase 2	NCT04328077	NASH	Terns [55]
EDP-297	n/a	n/a	Phase 1	NCT04559126	n/a	Enanta
XL335	4	3FLI	Phase 1	NCT00499629	n/a	[56]
Cilofexor	43	n/a	Phase 3 Phase 2	NCT03890120 NCT02781584	PBC, PSC, NASH	[57,58]
Tropifexor	0.2	7D42	Phase 2	NCT02516605 NCT04065841	PBC, BAD, NASH	[37,59,60]
Antagonists	EC50/IC50 (nM)	PDB ID	Clinical Trial Phase	NCT Number	Indication	Reference
Nidufexor	7	n/a	Phase 2	NCT03804879	Diabetic Nephropathy	[61]
MCA	40,000	n/a	n/a	n/a	n/a	[62]
Guggulsterone	17,000	n/a	n/a	NCT01492998 (terminated)	HCV	[63,64]
Ivermectin	200	1OSH	n/a	n/a	n/a	[44]
NDB	3400	4OIV	n/a	n/a	n/a	[45]

therapeutic effects, the pharmaceutical industry and academic institutes have been exploring the synthesis of nonsteroidal FXR agonists. Compared to steroidal agonists, nonsteroidal agonists can theoretically preserve the full therapeutic potential of FXR induction and avoid some of its undesirable pharmacokinetic and kinetic properties.

GW4064 is a highly effective and selective nonsteroidal agonist of FXR. GW4064 was found to raise HDL cholesterol levels and decreased triglycerides in various animal species [70]. Due to some limitations, such as solubility limitation, potentially toxicity and UV instability, GW4064 is not a good drug candidate. It is now usually used as a tool compound for investigating the physiological functions of FXR [71]. Many nonsteroidal FXR agonists are developed based on the structure of GW4064. Here we will introduce several promising nonsteroidal agonists.

Cilofexor, also known as GS-9674, is a developed synthetic derivative of GW4064 by Gilead Sciences [72]. Cilofexor is being evaluated in a phase 3 study in noncirrhotic subjects with primary sclerosing cholangitis (PSC) (NCT03890120). Cilofexor is also in a phase 2 study to evaluate the safety, tolerability and efficacy of regimens in subjects with NASH (NCT02781584). A clinical trial demonstrated that cilofexor was well tolerated in patients with PSC and improved the markers of cholestasis, liver biochemistry, C4 and serum bile acids [73]. In addition, cilofexor is combined with selonsertib and firsocostat in a phase 2 trial to circumvent its weak efficacy and dose-dependent development of pruritus observed in the mono treatment (NCT03449446), and is also currently tested in combination with fenofibrate and Vascepa (NCT02781584).

Tropifexor, previously known as LJN452, is another nonsteroidal FXR agonist developed by the Novartis internal drug discovery program [59]. As one of the most advanced synthetic FXR agonists in the clinic, tropifexor has been evaluated for its efficacy in NASH and PBC patients (NCT04065841 and NCT02516605, respectively). Tropifexor is a highly selective and highly potent FXR agonist. In the first-in-human study, tropifexor was well tolerated at pharmacologically active doses and no drug induced pruritus was observed [74]. In addition, tropifexor has completed the investigation in combination with cenicriviroc, a CCR2 antagonist in patients with NASH and fibrosis (the TANDEM study, NCT03517540) in 2020 [60].

Nidufexor (LMB763), a compound based on a tricyclic dihydrochromenopyrazole core, is a partial and highly selective FXR agonists [61]. Nidufexor did not activate GPBAR1 with an EC_{50} value greater than 80 μM, and also did not activate other nuclear receptors *in vivo* (EC_{50} > 10 μM) or *in vitro* (EC_{50} > 30 μM) [61]. Nidufexor was well-tolerated in healthy volunteers and is being investigated in phase 2 trials in patients with NASH and diabetic nephropathy (NCT02913105 and NCT03804879, respectively). Discouragingly, trial NCT02913105, which investigated the safety and efficacy of Nidufexor in NASH, has been terminated due to an increased incidence of major adverse event - pruritus - compared to the placebo group: 54.05% (20/37) in the LMB763 100 mg group, 29.55% (13/44) in the LMB763 50 mg group, and 15.00% (6/40) in the placebo group, respectively.

(E)-3-[1-(4-tert-Butylphenyl)-2,5-dimethyl-1H-pyrrol-3-yl]acrylic acid (**18**) [75], a compound derived from a FXR/PPAR agonist [76], is a selective FXR agonist. **18** activates FXR with EC_{50} = 1.4 μM, and Kd = 4 μM [75]. **18** exhibits favorable metabolic stability and is non-cytotoxic *in vivo* [75].

4.3.3. Partial FXR agonists

Partial FXR agonists may be a valuable strategy to avoid mechanism-based side effects induced by complete FXR activation [38]. Complete FXR activation blocks bile acid biosynthesis and hinders metabolic cholesterol degradation, leading to elevated cholesterol levels in OCA clinical trials [52]. Partial agonists induce conformational changes in FXR-LBD compared to full agonists (Fig. 3) [38]. These conformational changes may induce activation of FXR and reduce efficacy [77]. However, the molecular mechanism of FXR binds to corepressors in the partial agonism remains unknown and needs to be further investigated. Several partial FXRs agonists have been reported [38,55,78]. TERN-101 (LY2562175) is a potent partial FXR agonist with an EC_{50} value of 193 nM [55]. In the insulin resistant female ZDF rat model, LY2562175 significantly reduced triglycerides and elevated high-density lipoprotein (HDLc) by up to 95% [55]. DM175, another partial FXRs agonist, exhibits a partial agonistic/antagonistic profile [38]. DM175 activated FXR with an EC_{50} value of 350 nM in a transactivation assay [38]. DM175 also showed partial FXR antagonistic potency (IC_{50} = 10.9 μM) and repressed GW4064-induced FXR activity to 26 ± 2% activation [38].

4.3.4. Dual FXR and GPBAR1 agonists

Dual FXR and G protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5) agonists appear to be a strategy for treatment

of related diseases. The dual FXR and GPBAR1 agonist not only regulates pathways from both single agonism but may also provide additional mechanisms to the prevention and treatment for related diseases [79]. BAR502 and INT767 are both non-bile acid steroidal, dual agonists for FXR and GPBAR1 [80,81]. In a NASH model, BAR502 reversed the development of NASH like features in mice. BAR502 attenuated body weight gain and increased BMI index caused by feeding mice with a high fat diet and fructose (HFD-F) [82]. BAR502 reduced liver fat accumulation, inflammation and liver fibrosis. BAR502 attenuated liver damage in non-obstructive cholestasis animal models without inducing pruritus [83,84].

4.4. FXR antagonists

Much research interest has been devoted into the identification and discovery of FXR agonists. In fact, some groups aimed at the unexplored field of FXR antagonists (Fig. 5) [62,65,85,86]. FXR antagonists have proven beneficial in animal models of cholestasis and hypercholesterolemia, as well as in pancreatic and colon cancers [87–89]. FXR antagonists may be used for the treatment of type 2 diabetes (T2DM) or other metabolic diseases [90,91]. Various types of FXR antagonists have been reported [92] including natural antagonists [62,93], NDB [85], trisubstituted-pyrazolone derivatives [94], T3 [95], N-phenylbenzamide analogs [96], trisubstituted-pyrazol carboxamide analogs [97], oxadiazole analogs [98] and so on. But none is successful in clinical trials so far. Some FXR antagonists have been shown in Fig. 5.

α- and β-Muricholic acids (MCA), generated in the liver from CDCA, are primary bile acids and antagonists of FXR [62]. Increased tauro-β-MCA inhibits FXR signaling FGF15 expression and ceramide synthesis [99]. Gly-MCA is a potential candidate to treat metabolic disease owing to its selective inhibition of the intestine FXR rather than the hepatic FXR [100].

Guggulsterone is the first example of nonselective natural FXR antagonist [93]. Guggulsterone decreased CDCA-induced FXR activation with IC_{50} values of 15–17 mM [101]. Guggulsterone was once considered as a potentially effective treatment for patients with HCV genotype 1 who did not respond well to first-line therapy (NCT01492998) [64]. However, the trial was terminated.

Ivermectin, a drug approved for nematode and arthropod parasites, is a highly selective FXR antagonist [44]. Ivermectin shows antidiabetic activity by enhancing insulin sensitivity in an FXR-dependent way [44]. As a treatment for human filarial infections, ivermectin is safe and well tolerated in humans and is a safe compound on the basis of which novel FXR antagonists can be designed for the treatment of metabolic diseases.

NDB is identified as a selective FXR antagonist [85]. In primary mouse hepatocytes, NDB treatment reduces the GW4064-stimulated FXR/RXR interaction and represses the expression of FXR target genes, including SHP and BSEP [45]. NDB may be an anti-diabetes agent by decreasing the expression of glycogen genes.

(Z)-4-(4-Hydroxy-3-methoxybenzylidene)-1-(3-nitrophenyl)-3-phenyl-1H-pyrazol-5(4H)-one (**12u**) strongly suppresses the expression of FXR target genes [94]. **12u** lower the triglyceride and cholesterol levels in HepG2 cells and in the cholesterol-fed C57BL/6 mice [94]. T3, a FXR antagonist, significantly decreases the plasma levels of non-HDL cholesterol and apolipoprotein B in a dose-dependent manner in cynomolgus monkeys receiving HFD [95]. FLG249, a nonsteroidal FXR antagonist, regulates the expression of three FXR target genes, FGF15, apical sodium-dependent bile acid transporter (ASBT), and SHP, in the mouse ileum [102]. Noteworthy, Helmstädter et al [75] reported a novel chemotype that bound to FXR and was tunable in its activity type between agonism and antagonism with central heterocycle and side chain saturation acting as switches. The antagonist 3-[5-(4-tert-Butylphenyl)-1H-pyrazol-3-yl]propanoic acid (**24**) robustly decreases CDCA-induced activation with an IC_{50} values of 0.06 μM [75]. **24** binds directly to FXR-LBD with Kd = 0.3 μM [75].

Other compounds have also been identified to exhibit antagonistic activity on FXR include andrographolide (representative bioactive constituent of *Andrographis paniculata Nees*) [103], tuberatolides (from the Korean marine tunicate *Botryllus tuberatus*) [104] 3,5-disubstituted oxadiazole core [98], DY268 [97] and so on.

4.5. Side effects of FXR agonists

The results of clinical trials of FXR agonists indicate that FXR agonists may be a promising strategy for treating liver disease, but is associated with some side effects [105]. Pruritus is a common and dose-dependent symptom among FXR agonists-related side effects [106]. The incidence of pruritus in PBC patients treated with 25 mg OCA is up to 51%, which is even higher than that in patients treated with placebo [106]. Recent reports have suggested that GPBAR1 activation may increase gallbladder weight and activate itching receptors in the skin [68,107]. The similar chemical structure of FXR agonists endows them with the potency to active GPBAR1, which might exacerbate certain side effects and limit their use in clinics. Previous studies measured the potency and selectivity of several FXR agonists. We summarized the EC_{50} values of these FXR agonists against FXR and GPBAR1 in Table 2. Based on the fact that the potency of Nidufexor against FXR is much greater than GPBAR1 [61], Nidufexor should not have GPBAR1-related side effects such as pruritus. However, Nidufexor was shown to induce pruritus in clinical trials, which may be due to unknown mechanisms. This needs further study in the future.

Yang et al recently reported the structure of the INT-777/$GPBAR_{GS}$ complex, which provides the structural basis for GPBAR1 activation and bile acid recognition (Fig. 6A) [111]. In the structural analysis of the complex, INT-777 interacts with F96 and Y240 at the bottom of the orthosteric site of GPBAR1 [111]. OCA is an FXR agonist and shows a high binding affinity for GPBAR1. OCA forms hydrogen bonds with FXR [35] (Fig. 6B). When superposed into the INT-777/GPBAR1 structure, OCA appears to accommodate

Table 2
Agonist Activities to FXR and GPBAR1.

Compound	FXR EC_{50} (μM)	GPBAR1 EC_{50} (μM)	FXR selectivity index, EC_{50} ratio (GPBAR1/FXR)*	References
LCA	20	0.58	0.03	[108]
BAR502	2	0.4	0.2	[84]
CDCA	8.3	6.71	0.81	[108,109]
OCA	0.099	0.918	9.3	[59]
INT-767	0.033	0.670	20	[110]
Nidufexor	0.007	>80	>11,000	[61]
Tropifexor	0.0002	>10	>50,000	[59]

*The high ratio value represents the high selectivity of compound for FXR.

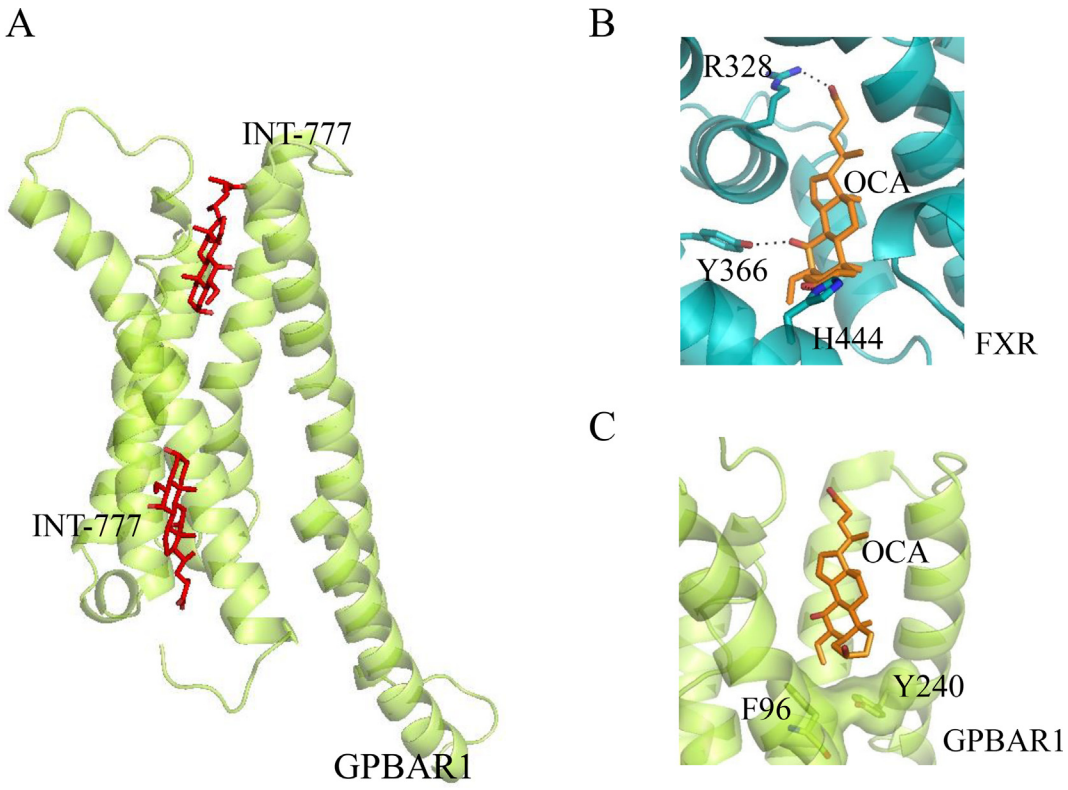

Fig. 6. Structural superposition of OCA with GPBAR1. (A) Structure of INT-777/GPBAR1 complex (7CFN). GPBAR is shown in limon and INT-777 is colored red. (B) Structure of OCA/FXR complex. The hydrogen bonds between OCA (orange) and FXR-LBD (greencyan) are shown as black dashed line. (C) Structural superposition of OCA with GPBAR1. Residues involved in the interactions are labeled and shown as sticks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

well into the binding sites of GPBAR1 [37] (Fig. 6C), a finding consistent with the biochemical assay. A second binding pocket also exists around the receptor surface and is bound with bile acids and their derivatives [111]. Thus, OCA may also be able to bind with this second pocket of GPBAR1 to regulate receptor activity. Nevertheless, the OCA/GPBAR1 structure has not yet been solved and further investigation is needed to clarify the mechanism of pruritus is induced by FXR agonist treatment.

The dual FXR and GPBAR1 agonists may provide additional mechanisms to the treatment for related diseases compared to single agonist [79]. However, GPBAR1 activation may lead to some potential side effects, such as pruritus and increased gallbladder volume [68,107]. Furthermore, to avoid these side effects, partial agonists and combination therapy (cilofexor and selonsertib, tropifexor and cenicriviroc et al) appear the next step in drug development to avoid these side effects.

5. Summary and outlook

Increasing evidence indicates that FXR signaling is critical for metabolism. Currently, the highly selective FXR agonists and partial FXR agonists provide a potent and effective strategy for the treatment of liver diseases, and some of these compounds have been or are still in the process of being evaluated in clinical trials. OCA has been approved by the FDA for PBC treatment and, if approved, might be the first drug to treat the NASH patients with advanced fibrosis worldwide. However, several challenges still exist in the field of FXR agonist development, such as the side effects. Based on the structural differences between FXR and GPBAR1, structural modification of FXR agonists may be a strategy to improve the efficacy, selectivity and safety of drugs used for liver disease. FXR antagonists might be a promising strategy for the treatment of type 2 diabetes or other metabolic diseases.

CRediT authorship contribution statement

Longying Jiang: Writing - original draft, Visualization. **Huajun Zhang:** Writing - review & editing. **Desheng Xiao:** Writing - review & editing. **Hudie Wei:** Writing - review & editing, Funding acquisition. **Yongheng Chen:** Project administration, Supervision, Funding acquisition, Writing - review & editing.

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