Pharmacology & Therapeutics 191 (2018) 162–177

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Progress and challenges of selective Farnesoid X Receptor modulation

Vittoria Massafra [a], Roberto Pellicciari [b], Antimo Gioiello [c,*,1], Saskia W.C. van Mil [a,d,**,1]

[a] Center for Molecular Medicine, UMC Utrecht, Utrecht University, Utrecht, the Netherlands
[b] TES Pharma S.r.l., Corciano, Perugia, Italy
[c] Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[d] Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, the Netherlands

ARTICLE INFO

Available online 20 June 2018

Keywords:
FXR
SBARM
Dissociated ligand
Tissue-specific
NASH
IBD

ABSTRACT

Bile acids are amphipathic molecules that were previously known to serve as fat solubilizers in the intestine in postprandial conditions. In the last two decades, bile acids have been recognized as signaling molecules regulating energy metabolism pathways via, amongst others, the farnesoid X receptor (FXR). Upon bile acid activation, FXR controls expression of genes involved in bile acid, lipid, glucose and amino acid metabolism. In addition, FXR activation has been shown to limit the inflammatory response. The central role of FXR in various aspects of metabolism and inflammation makes FXR an attractive drug target for several diseases, such as obesity, metabolic syndrome, non-alcoholic steatohepatitis, cholestasis and chronic inflammatory diseases of the liver and intestine. However, most of the currently available compounds impact on all discovered FXR-mediated functions and may have, on top of beneficial effects, undesired biological actions depending on the disease. Therefore, research efforts are increasingly focused on the development of selective FXR modulators, i.e. selective bile acid receptor modulators (SBARMs), aimed at limiting the potential side-effects of conventional full FXR agonists upon chronic treatment.

Here, we review the rationale for the design of SBARMs comprising dissociation between metabolic and inflammatory signaling, gene-selective and tissue-specific targeting. We discuss the potential structural mechanisms underlying the binding properties of dissociating ligands of FXR in light of ongoing efforts on the generation of dissociated ligands for otxher nuclear receptors, as well as their pharmacological and therapeutic potential.

Contents

Abbreviations: AF1, activation function 1 (domain); BSEP, bile salt export pump; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; FXR, farnesoid x receptor; GR, glucocorticoid receptor; GS, guggulsterone; HCC, hepatocellular carcinoma (HCC); IBD, inflammatory bowel disease; LBD, ligand-binding domain; MF, mometasone furoate; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor kappa b; NR, nuclear receptor; OCA, obeticholic acid; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PK, pharmacokinetic; PUFA, polyunsaturated fatty acid; RXR, retinoid x receptor; SAR, structure-activity relationship; SBARM, selective bile acid receptor modulator.

* Correspondence to: A. Gioiello, Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06122, Perugia, Italy.
** Correspondence to: S.W.C. van Mil, Center for Molecular Medicine, UMC Utrecht, Utrecht University, HP STR3.217, PO box 85060, 3508 AB, Utrecht, the Netherlands.
E-mail addresses: antimo.gioiello@unipg.it (A. Gioiello), S.W.C.vanMil@umcutrecht.nl (S.W.C. van Mil).
[1] These authors contributed equally to this work

https://doi.org/10.1016/j.pharmthera.2018.06.009

1. Introduction: FXR as a therapeutic target

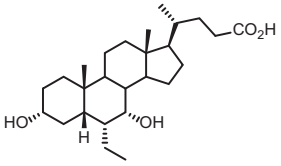
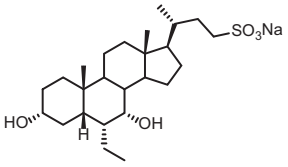
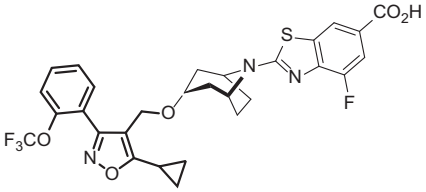
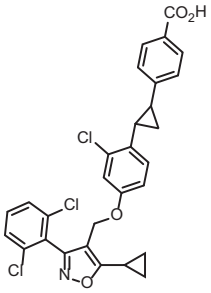
The farnesoid X receptor (FXR; Gene symbol: NR1H4) is a bile acid-binding transcription factor belonging to the superfamily of nuclear receptors (NRs) (Makishima et al., 1999; Parks et al., 1999; Wang, Chen, Hollister, Sowers, & Forman, 1999). FXR functions as an enterohepatic regulator of bile acid homeostasis, lipid (Sinal et al., 2000), glucose (Ma, Saha, Chan, & Moore, 2006) and amino acid metabolism (Massafra et al., 2017), and inflammation (Gadaleta, van Erpecum, et al., 2011). Several pharmacological modulators of FXR activity have been tested in clinical trials for primary biliary cholangitis (PBC) (Bowlus, 2016), type 2 diabetes (Mudaliar et al., 2013), and non-alcoholic steatohepatitis (NASH) (Neuschwander-Tetri et al., 2015). Studies in mice also suggest that FXR activation could be beneficial for gallstone disease (Moschetta, Bookout, & Mangelsdorf, 2004) and inflammatory bowel disease (IBD) (Gadaleta, van Erpecum, et al., 2011).

The first FXR agonist that has recently reached clinical practice is obeticholic acid (OCA, OCALIVA™) (Table 1). OCA was approved by the US Food and Drug Administration for the treatment of PBC in adults with an inadequate response to ursodeoxycholic acid (UDCA) or as monotherapy in adults unable to tolerate UDCA. OCA is currently being evaluated in additional clinical trials, e.g. for NASH (ClinicalTrials.gov identifier: NCT01265498) and primary sclerosing cholangitis (PSC) (ClinicalTrials.gov identifier: NCT02177136). Next to bile acid derivatives (Gioiello et al., 2014; Halilbasic, Fuchs, Traussnigg, & Trauner, 2016), several non-bile acid modulators of FXR have also been discovered, as reviewed elsewhere (Carotti et al., 2014). They are classified as steroidal or non-steroidal, based on their chemical structure, and are derived either from natural sources (steroids, triterpenes, polyphenols) or synthetic chemical libraries. Many such ligands have been employed as chemical tools to understand FXR signaling and transcriptional control. While this has validated FXR as a target for drug development, many compounds failed in preclinical and clinical settings due to toxicity and/or pharmacokinetic (PK) issues, with relatively few compounds entering clinical trials (Table 1) (Carotti et al., 2014).

In addition to FXR, multiple other NRs, including the pregnane X receptor (PXR), vitamin D receptor (VDR), constitutive androstane receptor (CAR) and liver X receptor (LXR), may be activated by bile acids or their precursors and metabolites (Goodwin et al., 2003; Makishima et al., 2002; Staudinger et al., 2001; Xie et al., 2001). While the genomic actions of bile acids rely on the interaction with these receptors, non-genomic functions may arise from their ability to modulate muscarinic receptors (Raufman, Chen, Zimniak, & Cheng, 2002; Raufman, Zimniak, & Bartoszko-Malik, 1998), to inhibit the activity of formyl-peptide receptors (FPRs) (Chen et al., 2000), and to activate the G-protein coupled receptor, TGR5 (Kawamata et al., 2003; Maruyama et al., 2002). TGR5 is an important regulator of metabolism and energy homeostasis in skeletal muscle and adipose tissue (Watanabe et al., 2006), and inhibits inflammation in Kupffer cells and macrophages (Pols et al., 2011; Schaap, Trauner, & Jansen, 2014). In addition, the sphingosine-1-phosphate receptor, S1PR, responds to conjugated bile acids to regulate hepatic lipid metabolism (Kwong, Li, Hylemon, & Zhou, 2015; Nagahashi et al., 2015). Bile acids are therefore important regulatory molecules acting on multiple receptors in different tissues.

The central role of FXR in the various aspects of metabolism and inflammation makes FXR an attractive drug target. With respect to treatment of NASH, diabetes and PBC, beneficial effects of FXR ligands include improvement of bile acid, glucose and lipid metabolism and reduced inflammation (Chow, Lee, & Guo, 2017). However, long-term clinical outcomes and safety issues have been raised. These may include

Table 1
FXR ligands currently in clinical trials (www.clinicaltrials.gov).

Compound name	Structure	Source	Primary indications	Development phase
OCA		Intercept Pharmaceuticals	PBC NASH PSC Biliary Atresia	Approved Phase III Phase II Phase I
INT-767		Intercept Pharmaceuticals	Hepatic fibrosis	Phase I
Tropifexor (LNJ-452)		Novartis	PBC NASH	Phase II Phase II
GS-9674 (Px-104)		Phenex	PBC PSC NASH	Phase II Phase II Phase II
EDP-305	Unknown (steroidal)	Enanta	NASH	Phase I

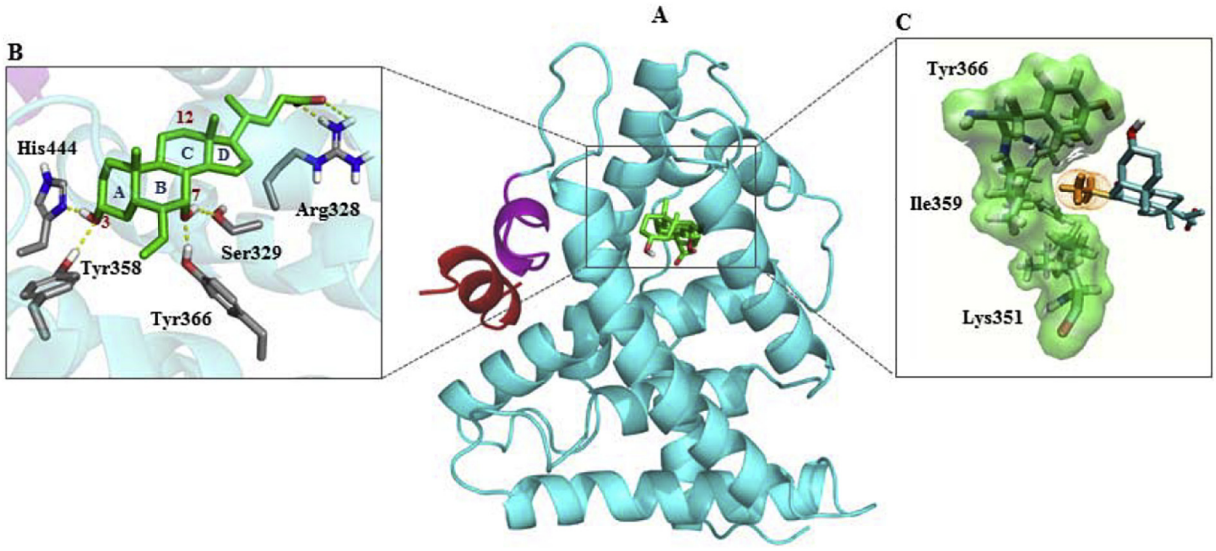


Fig. 1. (A) Overall view of the crystal *rat* FXR complexed with OCA (Mi et al., 2003): H12 is shown in purple, GRIP-1 peptides in red, and OCA in green. Two relevant sets of interactions between OCA and FXR are shown in more detail in B and C. (B) Key hydrogen bond interactions between OCA and amino acid residues within the canonical S1 pocket of the FXR LBD. (C) The ethyl group (orange) in C6α position of OCA (blue) positions within a hydrophobic cavity in FXR ligand binding pocket (green).

an unfavorable serum lipid profile with increased total cholesterol and low-density lipoprotein cholesterol and a decline in high-density lipoprotein cholesterol and severe itching (Mudaliar et al., 2013; Neuschwander-Tetri et al., 2015). FXR preserves the intestinal barrier function and prevents bacterial translocation, therefore FXR is an attractive target for the treatment of IBD (Ding, Yang, Wang, & Huang, 2015). Yet, the benefits of these enteroprotective FXR ligands are expected to be compromised by accompanying hepatic metabolic alterations. Similarly, harnessing the therapeutic potential of FXR modulators as cholesterol-lowering agents for treatment of atherosclerosis will be challenging because of the reduction in plasma HDL (Moris, Giaginis, Tsourouflis, & Theocharis, 2017). Therefore, research efforts are increasingly focused on developing selective FXR modulators, also known as "selective bile acid receptor modulators" (SBARMs), which should activate or repress specific FXR functions, to reduce side effects upon chronic treatment. The ultimate goal of this approach would be to tailor FXR activation to specific, desirable, functions in tissues relevant to the treatment of different diseases.

This review focuses on the state of the art in the development of SBARMs aimed at the selective modulation of FXR function. We distinguish SBARMs that selectively regulate the expression of specific subsets of FXR targets, without affecting others, i.e. gene-selective FXR modulators, from those SBARMs, which restrain the action of FXR to specific tissues, i.e. tissue-specific FXR modulators. We provide biological and chemical standpoints on the strengths and the limitations of current FXR modulators and discuss molecular mechanisms relevant for achieving selectivity, with a view to driving rational design of drugs with an improved therapeutic index.

2. FXR agonism and antagonism

In common with other NRs, the FXR protein exhibits a modular structure with different regions corresponding to autonomous functional domains, including a N-terminal activation function (AF) 1 domain and a highly conserved DNA binding domain (DBD) that is connected to the ligand binding domain (LBD) by a flexible hinge region (Glass, 1994; Mangelsdorf & Evans, 1995). The LBD contains two well-conserved regions: a signature motif and the AF2 motif located at the carboxy-terminal end of the domain. The AF2 motif is responsible for the ligand-dependent transactivation function (Zavacki et al., 1997). As for other NRs, all the so far determined crystal structures of FXR LBD complexed with agonist show a canonical transcriptionally active conformation where helix H12 folds back against the LBD core, seals the LBD to entrap the ligand ('mousetrap' model) and contributes to the formation of the coactivator binding groove (Mi et al., 2003). This conformational change in the LBD allows the release of corepressors and binding to coactivators to promote transcriptional initiation. Stabilization of H12 in this position is probably due to direct contacts between ligand and helix, and/or via ligand-dependent stabilization of contacts between the helix and the LBD core.

Co-crystallization of OCA with *rat* FXR provided insight into the molecular basis of bile acid binding, recognition and activation, and revealed unprecedented interactions between a NR and its ligand (Fig. 1A) (Mi et al., 2003). Indeed, the ring A of the bile acid scaffold faces the C-terminal H12, or AF2, in contrast with previously reported active steroids, such as progesterone, estrogen, testosterone, and glucocorticoids, that are all oriented in the opposite direction with their ring D facing H12 of their respective receptors. The relative affinities of natural bile acids are dictated by the specific pattern of hydroxyl groups at the C7 and C12 positions (Fig. 1B). Essential for high-affinity binding to FXR are the hydrogen bonds between the 7α-hydroxyl group and Tyr366/Ser329, together with the hydrophobic interactions of the ring core to the hydrophobic pocket of FXR (Mi et al., 2003). The bile acid side chain adopts an extended disposition, enabling the carboxylic group to interact with the guanido group of Arg328, thus approaching the entry pocket from the back (Fig. 1B). As suggested by studies on OCA, the introduction of an ethyl moiety at the B ring of a bile acid scaffold bestows high potency, by fitting a hydrophobic cavity in the FXR LBD corresponding to the C6α position of the steroid nucleus (Fig. 1C) (Mi et al., 2003; Pellicciari et al., 2002).

The agonist function of bile acids relies on the stabilization of FXR in an active conformation, by the interaction between a tyrosine-histidine-tryptophan (Tyr358/His444/Trp466) triad ("activation trigger") and the ring A of the steroid bile acid backbone (Fig. 2) (Gioiello et al., 2014; Mi et al., 2003). Other natural FXR ligands, including sesterterpenes and ophiuroid sterols, adopt a similar binding mode to bile acids, while non-steroidal compounds share a common binding cavity, but engage with different key residues, potentially resulting in diverse activation mechanisms and functional effects (Fig. 2). In fact, the LBD is inherently plastic, since potent FXR agonists, such as MFA-1, adopt an orientation opposite to that of bile acids and activate FXR through a different mechanism compared to bile acid-mediated activation (Soisson et al., 2008).

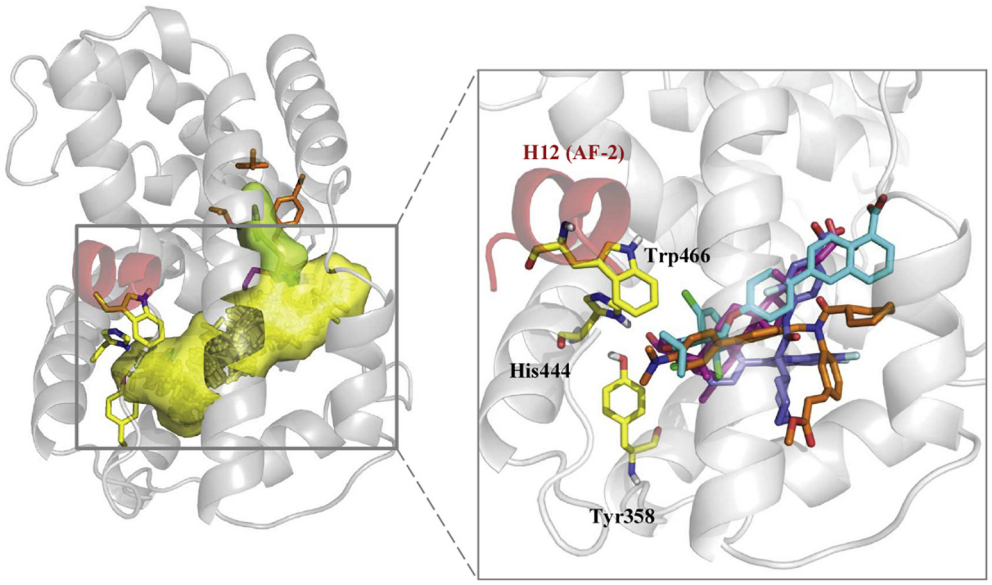


Fig. 2. The canonical binding site (S1) of the FXR LBD is inherently plastic. Superimposition of a bile acid (OCA, purple) and non-steroidal FXR ligands, fexaramine (orange), benzimidazole derivative (blue) and GSN8062 (light blue) in S1 of the FXR LBD.

While FXR agonists stabilize the interaction of FXR to its coactivators, FXR antagonists either destabilize the receptor-coactivator complex (active antagonists) or stabilize the receptor-corepressor complex (passive antagonists), resulting in gene silencing. 'Active antagonists' are generally more sterically demanding and bulkier than agonists or cognate hormone ligands. Their binding to the NR prevents the initiation of transcription, by inducing a different positioning of H12 to sterically hinder binding of coactivators to the coactivator NR box (LXXLL motif binding site). The first evidence for this notion came from the structural determination of the estrogen receptor α (ERα)-LBD in complex with the selective anti-estrogens raloxifene (Brzozowski et al., 1997). On the other hand, the passive antagonist, suvanine, impairs the crucial cation-π interaction between His444 and Trp466 within FXR, resulting in an inability to release the corepressor (Di Leva et al., 2013). Recently also another non-classical form of antagonism of FXR function was described (Xu et al., 2015). N-benzyl-N-(3-(tert-butyl)-4-hydroxyphenyl)-2,6-dichloro-4-(dimethylamino) benzamide (NDB) causes an unusual rearrangement of H11 and H12, stabilizing the homodimerization of FXR (Xu et al., 2015). Only few target genes, for example, GLUT4 and UGT2B4, have been described as transcriptionally regulated by FXR homodimers (Barbier et al., 2003; Shen et al., 2008). In contrast, transcription of most FXR target genes is described as being activated by FXR/RXR heterodimers (Ananthanarayanan, Balasubramanian, Makishima, Mangelsdorf, & Suchy, 2001), and NDB was consequently postulated as an antagonist of FXR/RXR heterodimeric actions.

Although the H12 structure-function model derived from NR LBD crystal structures provides insights into the relationships between the two major and opposing functional states (active state/agonism and inactive state/antagonism), the mechanisms for partial agonism are less easily explained. Partial agonism, also called intermediate agonism or graded activation, is thought to produce dynamic switching of H12 between active and inactive structural conformations. This may occur via ligand binding in two different orientations, one associated with the active and one associated with the inactive conformational state of the NR (Bruning et al., 2010; Hughes et al., 2012). In addition, a sub-optimal alignment of the transactivation H12 with the rest of the LBD has been described as contributing to partial agonism (Pike et al., 1999; Pike, Brzozowski, & Hubbard, 2000). This intermediate/quasi-antagonist conformation may result in impaired establishment of robust coregulator interactions and could thereby decrease the transactivation response. Unfortunately, crystallizing partial agonists to NR LBDs has proved difficult and this limits the mechanistic understanding of partial agonism. Furthermore, most of our current understanding on partial agonism relies on ER crystallography studies and may not universally apply to other NRs.

As an alternative mechanism, the allosteric binding of ligands to pockets distinct from the canonical LBD binding site (Site-1, S1), as shown in Figs. 1 and 2, may result in partial agonism and/or potential selective modulation. In this respect, the identification of a potential second binding pocket (Site-2, S2) in the FXR-LBD close to the H1-H2 loop may be of great relevance for the design of selective FXR modulators and is discussed in Section 3.2.4.

3. Gene-selective FXR modulators

3.1. The rationale for gene-selective FXR modulation

The rationale to develop gene-selective FXR modulators stems from the multitasking role of FXR as a regulator of genes involved in various metabolic and inflammatory processes. The pleiotropic effects of FXR activity, next to providing beneficial effects, also induce side-effects and by selectively targeting FXR functions, the aim is to generate effective therapies for diverse diseases, while minimalizing side effects. FXR, as most NRs, is characterized by a multi-domain architecture that allows the regulation of gene expression through the binding of compounds and coregulator proteins to the LBD, which, in turn, facilitates binding of the DBD to target genes. The wide range of cellular actions regulated by FXR, combined with the subtle structural variation found in the conformation of the FXR LBD and ligands, make it challenging to understand how gene-selectivity could be induced in a way to provide significant benefits for particular diseases. In the following paragraphs, we describe four potential strategies by which gene-selectivity may be accomplished. This involves separating target gene activation based on: i) differential cofactor binding; ii) differential DNA binding; iii) transactivation versus transrepression; and, iv) binding of ligands to allosteric pockets (Fig. 3). These strategies should not be considered as mutually exclusive. For example, differential cofactor binding could prepare FXR for differential DNA binding. Similarly, different DNA motifs could favor the specific recruitment of coregulatory factors. Model compounds for these strategies will be discussed.

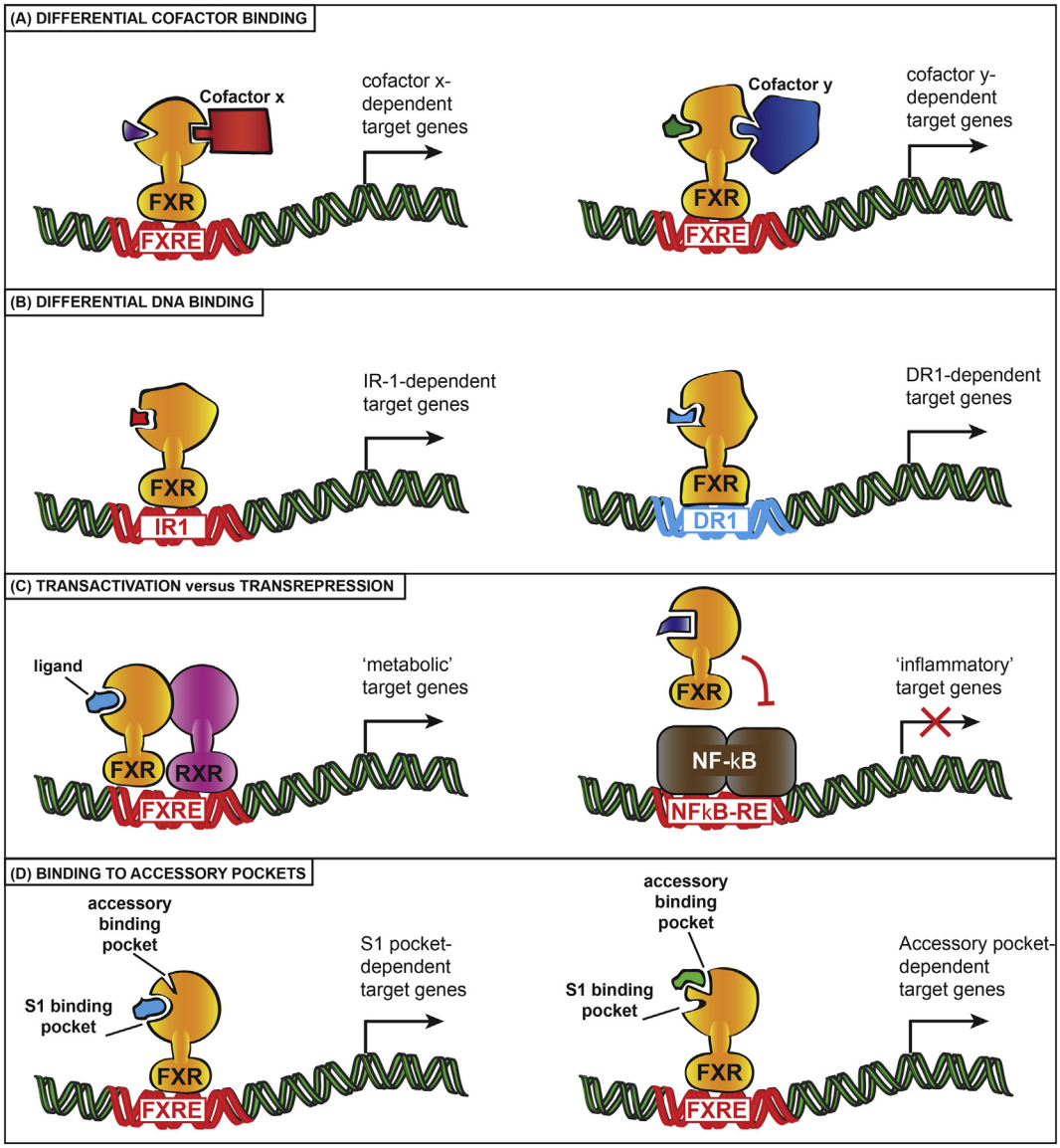


Fig. 3. Potential modalities to achieve ligand-induced gene-selective modulation of FXR activity. (A) Differential interactions between FXR and co-regulatory proteins may yield different transcriptional programs. (B) Binding of FXR to different DNA motifs may result in regulated expression of alternative subsets of genes. Upon ligand binding, FXR transactivates the expression of several genes involved in metabolism, in collaboration with its heterodimeric partner RXR. (C) Activated FXR inhibits the expression of inflammatory genes, by tethering transrepression of NF-κB. (D) Ligand binding may occur in accessory pockets, thus yielding allosteric effects on regulation of FXR activity. As a result, different subsets of genes are induced in comparison with those regulated by ligand access to the main ligand-binding pocket.

3.2. Mechanisms and strategies underlying gene-selective FXR modulation

3.2.1. Differential cofactor binding

Conformational rearrangement of FXR following binding to an agonistic ligand allows the dissociation of co-repressors and the recruitment of co-activators. Different cofactors cooperate with NRs to generate specific expression programs (Petta et al., 2016; Rosenfeld, Lunyak, & Glass, 2006). Some cofactors, such as conjugases and ligases, are NR-specific (Ghisletti et al., 2007), thus, targeting of the receptor with structurally distinct ligands may differentially modulate association with coregulator proteins and may produce FXR target gene-selectivity (Fig. 3A).

Many cofactors decorate NRs with an array of post-translational modifications (PTMs), such as phosphorylation, acetylation and sumoylation, and such effects are often interdependent. These PTMs orchestrate the transcriptional activity of FXR at many levels, including subcellular localization, protein–protein interactions, sequence-specificity of DNA binding, transcriptional regulatory activity, and protein stability. For example, constitutive FXR acetylation at K217 in diet-induced obese mice promotes hepatic inflammatory responses, which was associated with liver steatosis and impaired insulin signaling (Kim et al., 2015). Mechanistically, this acetylation was shown to prevent FXR sumoylation at K277, which promotes the interaction between FXR and NF-κB at the expense of the FXR/RXR interaction. Thus, sumoylated FXR is selectively recruited to repress inflammatory genes without inducing expression of FXR/RXRα target genes (Kim et al., 2015). This supports the therapeutic rationale for controlling the post-translational status of FXR to enable targeting of particular subsets of FXR-regulated genes.

Other than acetylation and sumoylation, FXR is a target of several other post-translational modifications, such as phosphorylation (Gineste et al., 2008; Hashiguchi et al., 2016), O-GlcNAcylation (Berrabah et al., 2014) and methylation (Balasubramaniyan, Ananthanarayanan, & Suchy, 2012); however, the effects of these modifications on target gene selectivity have yet to be addressed. Nevertheless, the above observations suggest that gene-selective modulation by

FXR could be achieved by differential sumoylation or acetylation. Screening for compounds that favor FXR sumoylation of K277 over acetylation at K217 might yield novel FXR ligands that selectively repress the inflammatory response.

3.2.2. Differential DNA binding

Ligand-dependent gene selectivity of FXR activity may also be achieved by differential binding of FXR to different DNA motifs (Fig. 3B). Indeed, FXR binds preferentially to the DNA response element IR-1 (inverted hexameric repeat spaced by 1 nucleotide) (Thomas et al., 2010), but may also bind to alternative motifs, such as DR-1 (direct hexameric repeat spaced by 1 nucleotide). Binding to DR-1 type motifs results in suppression of apolipoproteins ApoA and ApoCIII (Chennamsetty et al., 2011; Claudel et al., 2003) and of autophagy genes (Lee, 2016). In addition, FXR binds to an ER-8 (an everted repeat in which the two core half-motifs display a tail-to-tail orientation and are spaced by 8 nucleotides) motif, thereby regulating ABCC2 expression (Kast et al., 2002). These observations support differential DNA binding as a modality for gene-selectivity, although it remains to be addressed whether different ligands can drive differential DNA binding. In any case, the effects of different DNA motifs on FXR target gene-selectivity are not yet comprehensively described and it is therefore unclear whether targeting the binding of FXR to different DNA motifs would be a useful therapeutic strategy.

As noted above, interaction of FXR with DNA response elements and coregulators should not be seen as mutually exclusive mechanisms to elicit gene-expression specificity. Indeed it is suggested that individual glucocorticoid responsive regions may use particular coregulators to control gene expression (Clark & Belvisi, 2012), and if true for FXR-mediated transcriptional control, pharmacological modulation of coregulator recruitment and/or response element binding may hold promise for selective modulation of the FXR–driven gene expression profile.

3.2.3. Gene selectivity based on separation of transactivation and transrepression mechanisms

Activation of intestinal and hepatic FXR results in suppression of bile acid synthesis via FGF19- and SHP-mediated repression of CYP7A1 expression (Kong et al., 2012; Lin, Wang, Blackmore, & Desnoyers, 2007; Sinal et al., 2000). Additionally, activation of hepatic FXR promotes bile acid efflux to the canalicular lumen through upregulation of bile salt export pump (ABCB11) and phosphatidylcholine translocator (ABCB4) (Ananthanarayanan et al., 2001; Liu et al., 2003) and to the apical membrane by inducing expression of organic solute transporter α/β (SLC51A/B) (Landrier, Eloranta, Vavricka, & Kullak-Ublick, 2006). Aside from regulating bile acid homeostasis, FXR reduces hepatic fatty acid accumulation and gluconeogenesis (Ma et al., 2006), promotes amino acid catabolism and ammonium detoxification (Massafra et al., 2017), and suppresses autophagy (Lee et al., 2014; Seok et al., 2014), thereby acting as a gatekeeper of liver energy homeostasis. Most of the above-mentioned effects are believed to involve FXR activity as a direct transcriptional regulator, or "transactivator", by binding to the DNA and enhancing in trans the expression of target genes.

In addition, FXR may repress inflammation via an alternative molecular mechanism. FXR activation by OCA ameliorated symptoms of colitis in wild type, but not FXR null mice, by preserving the intestinal barrier function and reversing gut inflammation induced in experimental colitis models (Gadaleta, van Erpecum, et al., 2011; Massafra et al., 2016). Extensive evidence also exists for anti-inflammatory effects of FXR in liver cells and mouse models, as reviewed elsewhere (Adorini, Pruzanski, & Shapiro, 2012). It has been suggested that an important mechanism by which FXR mediates repression of inflammation is via FXR binding to the transcription factor, NF-κB, and thereby interfering with NF-κB-mediated induction of pro-inflammatory cytokines (Fig. 3C). This model is supported by the fact that: i) FXR interacts with the NF-κB subunits p50 and p65 in GST-pull down assays (Gadaleta, Oldenburg, et al., 2011); ii) FXR activation reduces NF-κB reporter activity; iii) FXR inhibits p65 recruitment to pro-inflammatory gene promoters (Bijsmans et al., 2015); and, iv) FXR decreases pro-inflammatory cytokine expression in intestinal and liver cells (Bijsmans et al., 2015; Gadaleta, Oldenburg, et al., 2011). The inhibition of NF-κB by FXR is a form of transrepression, where a NR binds, or tethers, to and thereby represses the activity of a target transcription factor, such as NF-κB (Hollman, Milona, van Erpecum, & van Mil, 2012; Ratman et al., 2013).

Targeting either transactivation or transrepression was postulated to contribute to the dissociation of metabolic and anti-inflammatory FXR functions (Fig. 3C). The idea of dissociating transactivation and transrepression is not new in the NR field (See also Fig. 4). A landmark finding has been the discovery that mice carrying a mutant version of glucocorticoid receptor (GR) unable to homodimerize (GR^{dim} mice) retained their anti-inflammatory effects on PMA-induced skin inflammation, but did not activate the expression of gluconeogenic genes (Tuckermann et al., 1999). However, as the GR^{dim} mutation fails to abolish all transactivation by GR, the relative contributions of transactivation and transrepression remain unclear (Newton & Holden, 2007). Nevertheless, an apparently dissociated GR compound (CpdA) has been shown to downregulate the expression of NF-κB-driven genes via GR binding, while not inducing hyperglycemia (De Bosscher et al., 2005) (Fig. 4). Thus, following the description of anti-inflammatory effects by FXR, such studies of dissociating GR ligands fueled research into the development of drugs retaining the anti-inflammatory effects of FXR ligands, without the induction of transactivation. While such drugs are predicted to have therapeutic potential for the treatment of IBD, and hepatic and biliary inflammatory diseases (Chignard & Poupon, 2009), careful analysis of the relative roles for transactivation and repression is warranted.

We have recently generated evidence to support pharmacological dissociation of transactivation from tethering transrepression by FXR (Bijsmans et al., 2015). A high-throughput luciferase reporter assay was set up to screen for compounds that decrease NF-κB activity. Subsequent analyses were performed to assess whether "hit" compounds yielded anti-inflammatory effects in an FXR-dependent manner and whether such compounds failed to trigger FXR-dependent metabolic activity. Mometasone furoate (MF, Table 2) suppressed NF-κB activity, and displayed low, or absent, activity on promoters of classical FXR metabolic targets, including small heterodimeric partner (SHP) and ileal bile acid binding protein (IBABP). MF abolished TNFα-mediated induction of pro-inflammatory genes IL8, CXCL2 and MCP1 in HepG2 cells overexpressing FXR, with minor effects on FXR metabolic targets SHP, FGF19, SCD1 and ICAM1. Furthermore, in small intestinal organoids derived from wild type, but not $FXR^{-/-}$ mice, MF reduced Tnfα and Cxcl2 expression and revealed only minor effects on Shp, Fgf15 and Ibabp expression. Mechanistically, MF reduced p65 recruitment to pro-inflammatory gene promoters in an FXR-dependent manner and this is consistent with MF separating between FXR tethering transrepression

Fig. 4. Other NR modulators: what we can learn and apply to FXR. (A) AL-438 promotes the binding of GR to GRIP1 rather than to PGC1, thereby favoring GR-mediated anti-inflammatory activity (Caplan et al., 2017; Fardet and Feve, 2014; Coghlan et al., 2003; Kassel et al., 2004). A similar mechanism could underpin the separation of FXR-mediated transactivation and transrepression, suggesting that compounds promoting the interaction to GRIP1, rather than to PGC1, may act as SBARMs ((Ananthanarayanan et al., 2004; Savkur et al., 2005; Burris et al., 2013). (B) CpdA may support a conformational change in GR that prohibits homodimerization (De Bosscher et al., 2005). As with GR, modulating the preference of FXR for a heterodimeric or monomeric state may differentially activate gene expression programs (Hollman et al., 2012). (C) Compared to other LXR ligands, 25-hydroxy cholesterol (25HC) increases Abca1 expression, but does not repress iNOS. This may be due to a failure of 25HC to induce LXR sumoylation and consequently impaired clearance of NF-κB by the proteasome machinery (Ghisletti et al., 2007; Pascual et al., 2005; Hua, Ganti and Chambon, 2016; Hua, Paulen and Chambon, 2016). In the context of FXR, a ligand that promoted the sumoylation-dependent transrepression pathway, but failed to activate expression of metabolic genes, might represent an effective and selective anti-inflammatory drug (Kim et al., 2015).

Box 1. Mechanisms of gene-selectivity: insights from other NRs

Efforts aimed at selectively modulating NR function are not novel in the field of NRs (Burris et al., 2013). Glucocorticoids, as ligands for GR, are the most potent anti-inflammatory agents currently available and they are used to treat a wide variety of allergic and inflammatory diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis (Caplan et al., 2017). However, chronic use has been associated with severe side effects, including osteoporosis, muscle wasting, cardiovascular events and disorders of glucose and lipid metabolism (Caplan et al., 2017; Fardet and Feve, 2014). There is therefore a strong incentive to develop selective GR ligands that preserve the beneficial anti-inflammatory activity, yet minimize the side-effect profile. Mechanistically, differential ligand-binding may result in an alternative conformation of the helices in the NR LBD leading to differential coregulator binding. For example, a gene-selective GR modulator, AL-438, was shown to induce the interaction between GR and coactivator GRIP1, which is necessary for AP-1-mediated tethering transrepression mechanisms (Coghlan et al., 2003). Alternatively, the mechanism of AP-1 repression may be via GR-mediated activation of for example DUSP1, as discussed before. AL-438 disabled the interaction of GR with PGC1, a cofactor implicated in hepatic glucose metabolism (Coghlan et al., 2003) (Figure 4A). AL-438 preserved anti-inflammatory efficacy comparably to classic agonists, such as prednisolone, and showed less elevation in blood glucose levels and reduced osteoporosis compared to prednisolone (Coghlan et al., 2003; Kassel et al., 2004). Intriguingly, FXR also binds PGC1α (Savkur et al., 2005) and GRIP1 (Ananthanarayanan et al., 2004), thus similar differential modulatory mechanisms could exist for FXR (Figure 4A). NR dimerization has also caught the attention to selectively modulate NRs. The GR selective modulator CpdA prohibits GR dimerization, thereby favoring a monomeric conformation (Figure 4B). Although CpdA actions may not be solely dependent on GR, the lack of dimerization of GR allows tethering to downregulate NF-κB-driven genes, without inducing hyperglycemic effects due to transactivation via dimerized GR (De Bosscher et al., 2005). Interestingly, both monomeric and heterodimeric actions have also been described for FXR (Hollman et al., 2012) and ligand-dependent differentiation between the homodimeric and heterodimeric actions of FXR can potentially be achieved similarly. Finally, post-translational modification of NRs may contribute towards selective modulation of target gene expression. For example, PPARγ and LXR repressed inflammatory gene expression in a sumoylation-dependent manner that involves inhibiting the clearance of corepressors at the promoters of inflammatory genes (Ghisletti et al., 2007; Pascual et al., 2005). PPARγ and LXR are conjugated to different SUMO proteins (SUMO 1 and SUMO2/3, respectively) by different E3 ligases (PIAS1 for PPARγ and HDAC4 for LXR) and regulate different pro-inflammatory genes, including IL1β and TNFα, respectively (Ghisletti et al., 2007; Pascual et al., 2005). Some endogenous LXR ligands were shown to selectively promote transactivation, but not transrepression; 25- and 27-hydroxycholesterol promote transactivation of ABCA1, but do not repress iNOS gene expression. This is in contrast to other endogenous ligands (e.g. 22R-hydroxycholesterol), that induce ABCA1 expression and repress iNOS expression in a sumoylation-dependent manner (Ghisletti et al., 2007) (Figure 4C). Likewise, sumoylation of GR was shown to be indispensable for both NF-κB/AP1-mediated glucocorticoid-induced tethered indirect transrepression (Hua et al., 2016a) and for direct transrepression via binding to an inverted repeated negative GC response element (IR nGRE) (Hua et al., 2016b). Similar sumoylation mechanisms may also account for FXR-mediated transrepression, as was recently described (Kim et al., 2015).

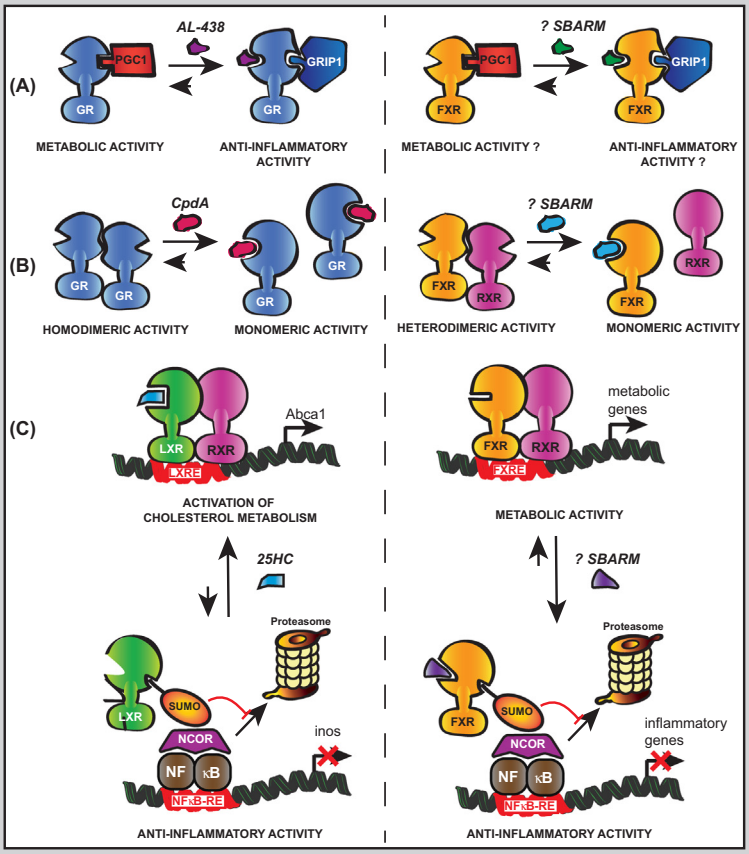
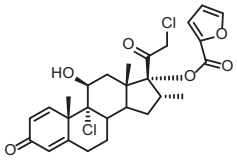
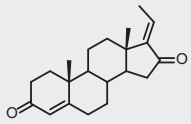
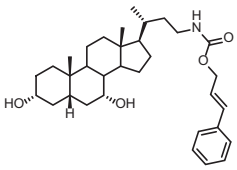
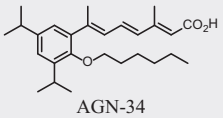
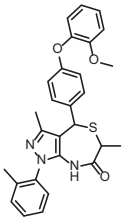
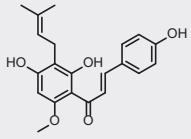
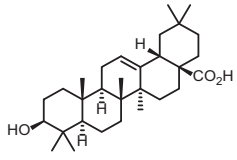


Figure 4. Gene-selective modulation of other NRs: what we can learn and apply to FXR. See text for details.

Table 2
Model compounds for gene-selective FXR modulation.

Compound	Gene-selective modulation	Receptor selectivity	References
 Mometasone furoate	✓ Counteracts TNFα-induced expression of pro-inflammatory genes (IL8, MCP-1, and CXCL2) in an FXR-dependent manner in HepG2 cells. ✓ Reduces p65 recruitment to pro-inflammatory gene promoters (CXCL2 and IL8) in an FXR-dependent manner in HepG2 cells. ✗ Does not induce FXR transactivation of SHP and IBABP promoters, nor upregulates the expression of FXR targets SHP, FGF19, KNG1, SCD1 and ICAM1 in HepG2 cells, compared to GW4064.	FXR GR	(Bijsmans et al., 2015)
 Guggulsterone	✓ Enhances CDCA-mediated induction of BSEP in HepG2 cells. ✗ Inhibits CDCA-induced expression of IBABP in Caco-2 cells ✗ Inhibits CDCA-induced expression of SHP in HepG2 cells	PR AR ER GR MR PXR FXR	(Burris et al., 2005; Cui et al., 2003; Urizar et al., 2002; Wu et al., 2002)
 UPF-838	❖ Promotes the stabilization of helix H3, thus potentially determining a gene-expression profile different than OCA.	FXR	(Gioiello et al., 2011)
 AGN-34	✓ Enhances CDCA-dependent repression of CYP7A1 expression in HepG2 cells. ✗ Counteracts CDCA-dependent induction of IBABP expression in Caco-2 cells. ✗ No effect on SHP expression compared to CDCA, both in Caco-2 and HepG2 cells.	RXR/FXR	(Dussault et al., 2003)
 Pyrazole[3,4-e][1,4]thiazepin-7-one	✓ Induces OSTβ and repress CYP7A1 expression in HepG2 cells. ✗ No effect on BSEP expression compared to OCA, in HepG2 cells.	FXR	(Marinozzi et al., 2012)
 Xanthohumol	✓ Decreases the expression of Srebp1c, Scd1, Pepck and G6pase in KK-A^y mice, similarly to CDCA. ✗ Represses Shp and induces Cyp7a1 expression in KK-A^y mice, in contrast to CDCA.	FXR CAR GABAA	(Chang et al., 2016; Nozawa, 2005; Yao et al., 2011)
 Oleanoic acid	✓ Suppresses CDCA-dependent induction of BSEP expression in HepG2 cells. ✗ No effect on expression of SHP and OSTβ in HepG2 cells, compared to CDCA.	FXR CAR PXR PPARα	(Liu et al., 2013; Liu and Wong, 2010; Lu et al., 2013)

✓ Gene regulatory effects similar to those induced by conventional full agonists.
✗ Gene regulatory effects distinct from those induced by conventional full agonists.
❖ Undefined gene regulatory effects.

and transactivation mechanisms. While the above observations suggest that dissociation of transactivation and transrepression may yield, at least partial, gene-selectivity, uncoupling transrepression and transrepression may, as was found for GR (Clark & Belvisi, 2012; Newton & Holden, 2007; Newton, Shah, Altonsy, & Gerber, 2017), not fully dissociate anti-inflammatory from metabolic effects. GR^{dim} mice were not protected from all the side effects of glucocorticoids that were associated with the transactivation mechanism, such as glucocorticoid-induced muscle atrophy (Waddell et al., 2008) or osteoporosis (Rauch et al., 2010). In addition, and unlike wild type mice with a fully functional GR, GR^{dim} mice proved to be insensitive to the therapeutic effects of glucocorticoids in experimental models of contact allergy (Tuckermann et al., 2007) and arthritis (Baschant et al., 2011). From these data, it was concluded that transactivation by GR is also important for anti-inflammatory functions. Such statements are consistent with prior findings that the ability of glucocorticoids to repress the expression of inflammatory gene expression may be prevented by inhibitors of transcription and translation (Clark & Belvisi, 2012; Newton & Holden, 2007). For example, glucocorticoid-mediated induction of the dual specificity phosphatase, Dusp1, is essential to improve symptoms of sepsis (Wang, Nelin, et al., 2008) and acute local inflammation (Li et al., 2011). Similarly, transactivation mechanisms are likely to participate in the anti-inflammatory effects of FXR. In support of this, the onset of inflammation in steatotic livers involves the disruption of FXR-regulated bile acid, glucose and lipid homeostasis (Chow et al., 2017). This suggests that anti-inflammatory actions of FXR may depend indirectly on transactivation of genes suppressing hepatic fat accumulation, reducing bile acid synthesis, and enhancing hepatic bile acid efflux.

In conclusion, full separation between anti-inflammatory and metabolic effects of FXR is unlikely to be feasible. However, data with MF illustrates that some separation between FXR target gene activation and repression may be possible. This may limit the array of target genes modulated by FXR activation, thereby potentially reducing side effects.

3.2.4. Gene selectivity via binding of ligands to canonical and allosteric pockets

From a chemical standpoint, the history of selective GR modulators supports the concept that minor changes in ligand structure may provoke dramatic differences in resultant gene expression profiles (De Bosscher, 2010). The pivotal mechanism has been attributed to the flexibility of the C-terminal H12 that undergoes a conformational change upon agonist binding to form the AF-2 site for coactivator recruitment. Notably, many conformations of H12 have been observed in different crystal structures suggesting that the ligand does not induce one particular receptor conformation, but instead changes the dynamic equilibrium of H12 that results in several conformational states.

Next to screening ligands for their ability to dissociate FXR functions, rational design of selective ligands may potentially be achieved in the future. However, in-depth studies are needed to address how the structural features of FXR ligands may affect the conformation of FXR in complex with cofactors and the DNA. In addition, it is necessary to ascertain which receptor conformations drive the desired FXR-selective response. The structure of MF may provide the first clues in this respect. Computational simulations suggested that MF adopts a similar binding mode as bile acids to the 'canonical' S1 ligand binding pocket, but engaged with different amino acid residues compared to ligands such as CDCA (Fig. 5A). The most favorable binding mode of MF to FXR orients the furoate group in a region between helices H11 and H12, with several hydrophobic and hydrophilic contacts stabilizing the interaction. Although other binding modes cannot be excluded, it is likely that the hydroxy group at the C11β position forms hydrogen bonds with residues of Ser329 and His291 (Fig. 5A) (Bijsmans et al., 2015). The carbonyl groups at C17 position may interact with Tyr358 and His444 side chains, and π-π interactions are established between the furoate group and the aromatic side chain of Phe281, Trp451 and Trp466 (Fig. 5A) (Bijsmans et al., 2015). This ligand binding mode supports FXR-dependent repressive effects, but seems not to rely on the interaction with the classical FXR coactivator, SRC1. Indeed, MF has only an EC_{50} of 10.9 μM and an efficacy of 12% in recruiting the coactivator protein SRC1, when compared to the endogenous FXR ligand chenodeoxycholic acid (CDCA). Presumably, MF binds to the canonical S1 FXR LBD as a partial agonist and determines a different conformational rearrangement of the receptor compared to current non-selective agonists. The mechanisms that link this binding mode to gene-selective regulation remain to be uncovered.

Besides the canonical binding site, alternate pockets, often referred to as 'allosteric sites', have been identified in NRs (Burris et al., 2013). Interestingly, conformational changes that are induced by binding to allosteric sites are sought to alter the behavior of a structurally-coupled, but distinctly located canonical active site, resulting in a conformational state redistribution and, in turn, in different biological outcomes (Mackinnon, Gallastegui, Osguthorpe, Hagler, & Estebanez-Perpina, 2014). Thus, although the pursuit of NR allosteric modulators remains a nascent field of drug discovery, selective targeting of NR regulated genes may be achieved by non-canonical or allosteric ligand binding.

In the case of FXR, computational modelling (Meyer, Costantino, Macchiarulo, & Pellicciari, 2005) and, more recently, amide hydrogen/deuterium exchange coupled with mass spectrometry (Yang, Broderick, Jiang, Hsu, & Maier, 2014) showed the presence of a non-canonical binding site, also known as S2 or the 'back door', which faces the loop region between helices H1 and H2 of the LBD (Fig. 5B)

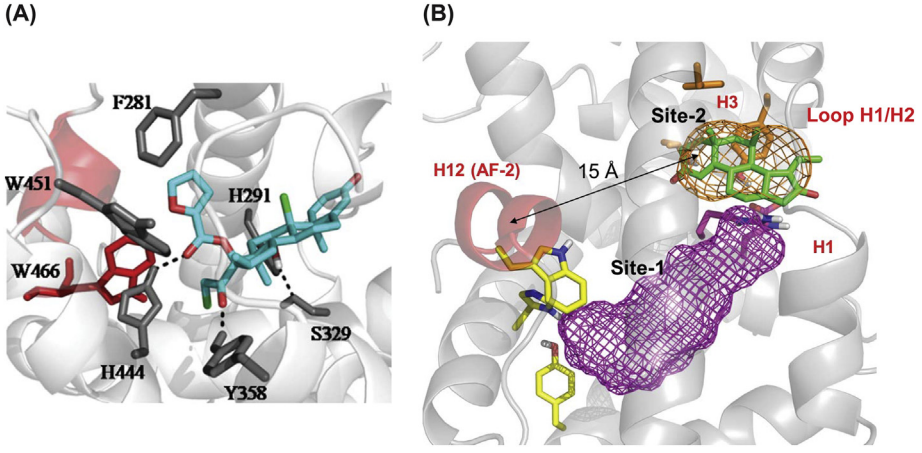


Fig. 5. Binding modes of putative gene-selective compounds binding canonical or allosteric pockets in FXR. (A) Putative binding mode of MF to FXR (Bijsmans et al., 2015); (B) GS binds to a non-canonical FXR binding site (S-2) delineated by helices H1 and H3 (Meyer et al., 2005; Yang et al., 2016).

close to the coactivator peptide binding cleft. In particular, it was found that guggulsterone (GS, Table 2), originally identified as a FXR antagonist in vitro (Cui et al., 2003) and later as a SBARM (Urizar et al., 2002; Wu et al., 2002), occupied the allosteric S2 pocket in the FXR LBD with a more favorable binding energy than for the canonical S1 pocket. However, GS binds to multiple endocrine NRs, including progesterone receptor (PR), androgen receptor (AR), ER, GR, mineralocorticoid receptor (MR), PXR and FXR (Burris et al., 2005), and using GS as a template for generating FXR-selective SBARMs could therefore be challenging. Nevertheless, the finding that GS binds to the S2 site provides clues for understanding the antagonistic properties of GS in FXR. This delineates the intriguing possibility of modulating FXR transactivation properties via this allosteric site. In this respect, chemical manipulation of bile acids to attain high potency FXR agonism (Gioiello et al., 2014), together with the identification of the FXR accessory S2 binding cleft, encouraged the design of bile acid derivatives with a long side chain extending towards the S2 'back door' pocket. In particular, substitution of the carboxylic tail of CDCA by carbamate moieties resulted in an array of derivatives showing a broad range of FXR functional profiles due probably to differential perturbations in the helixes around the S2 site (Pellicciari et al., 2006). These derivatives (e.g. UPF-838, Table 2) displayed properties either as agonists, antagonists or partial agonists, potentially able to regulate the transcription of only selected target genes. Docking experiments and molecular dynamic simulations revealed that the bile acid core of the carbamate derivatives occupied the canonical S1 binding site, whereas the long side chain occupied the allosteric S2 site of the receptor (Fig. 6) (Pellicciari et al., 2006). Furthermore, in agreement with previous studies reporting that PPARγ partial agonists stabilize helix H3 (Montanari et al., 2008), it was demonstrated that carbamates with extended side chains promote stabilization of helices H3 and H12 in FXR. While the bile acid part of the ligand binds to S1 and stabilizes H12 by hydrophobic contacts, the extended side chain part of the ligand occupies the S2 and favors the packing of H3 to the binding side by hydrophobic interactions (Fig. 6). Surprisingly, attempts to increase the potency of carbamates, and specifically of the binding affinity of UPF-838 by insertion of the ethyl group at the C6α position, disrupted the packing of H3 to the steroid binding site (S1), while promoting the packing of H12 and the full activation of the receptor (Gioiello et al., 2011). This suggests that, as for PPARγ (Montanari et al., 2008), partial FXR agonists seem to act through the stabilization of H3.

Taken together, selective modulation of FXR may be achieved via interaction of compounds with the S2 pocket, thereby altering the conformation of the binding cleft for coregulators without an apparent impact on the conformation of either H12 or H3. If true, then the receptor 'back door' or S2 pocket may be exploited to achieve a broad range of FXR modulation (agonism, partial agonism and antagonism). On the one hand, these findings pave the way for the identification of novel steroidal SBARMs. On the other hand, virtual screening may be used to identify novel small molecules that could fit into the S2 pocket, and for which putative selective effects could subsequently be investigated at the transcriptome level.

3.3. Natural and synthetic gene-selective FXR modulators

Only a few examples of potential gene-selective FXR modulators are described in literature (Table 2). A retinoic acid derivative, AGN-34, was reported by Forman and colleagues as an antagonist of FXR/RXR heterodimer functions (Dussault et al., 2003). However, AGN-34 was shown to bind to RXR, thereby 'trans-antagonizing' coactivator recruitment to the FXR-RXR heterodimer in vitro. Although the dependency on FXR was not analyzed, AGN-34 antagonized CDCA-mediated induction of IBABP expression in Caco-2 cells, and enhanced CDCA-mediated repression of CYP7A1 in HepG2 cells, while having no effect on SHP gene expression in both Caco-2 and HepG2 (Dussault et al., 2003). Thus AGN-34 may be considered as a gene-selective modulator of FXR-dependent gene expression by virtue of RXR antagonism. Similarly, the RXR agonist LG100268 has been shown to antagonize BSEP expression mediated by endogenous and synthetic FXR ligands (Kassam, Miao, Young, & Mukherjee, 2003). This form of agonism/antagonism in which binding to one partner of a heterodimeric transcription factor results in a linked conformational change in the second partner in the heterodimer has been termed the "phantom ligand effect" (Schulman, Li, Schwabe, & Evans, 1997) or "permissive agonism" (Aranda & Pascual, 2001). However, structural and biochemical assays showed that the "phantom ligand effect" of RXR modulators, such as LG100754, may be due to binding of the ligand to RAR (Sato et al., 2010). Thus, the design of selective FXR modulators via binding to RXR is compromised by pharmacological uncertainty.

Recently, pyrazole[3,4-e][1,4]thiazepin-7-ones (Table 2), derived from a virtual screening and hit optimization approach, were found to activate FXR at low micromolar concentrations (Marinozzi et al., 2012). Like OCA, these compounds repressed CYP7A1 and induced OSTβ, but unlike OCA, they did not affect the expression of BSEP in HepG2 cells (Marinozzi et al., 2012). Assuming that these effects are FXR-dependent would suggest gene-selective modulation by these compounds. For membrane-bound receptors, such as G-protein-coupled receptor, 'functional selectivity', or more correctly, biased agonism, has been described, whereby a ligand confers selective activation via one transduction pathway in preference to another pathway (Rankovic, Brust, & Bohn, 2016). Similarly, it may be that differential ligand binding to FXR could lead to differential conformational changes

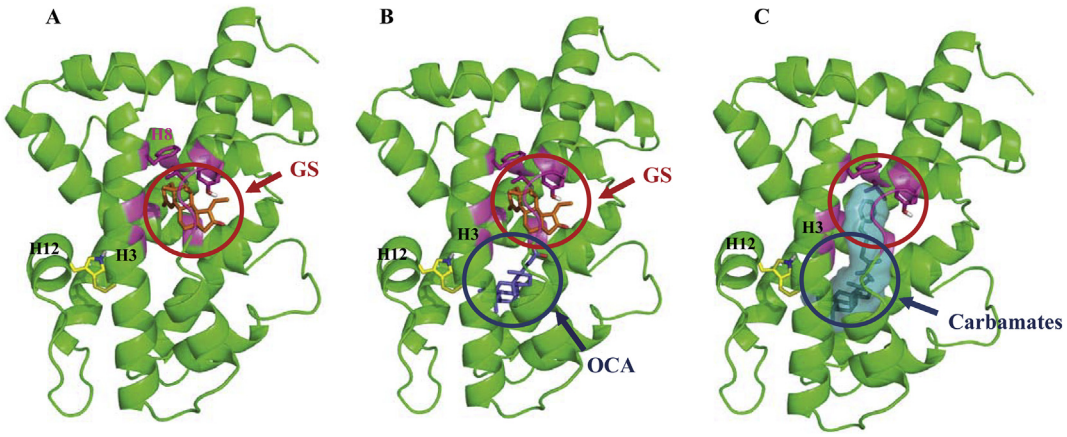


Fig. 6. The side chain of extended bile acid derivatives fits in a receptor cavity corresponding to the guggulsterone 'noncanonical' binding site (S2). A) Binding of GS to S2 (Meyer et al., 2005; Yang et al., 2016); B) binding of OCA and GS to S1 and S2, respectively; C) the extended side chain of both carbamates occupies the non-canonical S2 FXR binding (receptor's 'back door') (Pellicciari et al., 2006).

that in turn promote particular interactions between FXR and specific DNA-elements, co-factors, or indeed, other transcription factors, to differentially modulate gene expression.

Although a number of naturally-occurring FXR ligands have been described with SBARM-like profiles, most of them also activate other NRs. Xanthohumol (Table 2), the main prenylated chalcone from beer hops *Humulus lupulus* L., lowered plasma triglycerides and glucose concentration in a mouse model of obesity and type 2 diabetes mellitus (Nozawa, 2005). Xanthohumol increased BSEP-promoter driven reporter activity in HepG2 cells, although the dependency on FXR was not tested. Of note, xanthohumol decreased the expression of lipogenic genes Srebp1c and Scd1 and gluconeogenic genes Pepck and G6pase in mice, similarly to CDCA. In contrast to CDCA, xanthohumol repressed Shp expression and induced Cyp7a1 expression (Nozawa, 2005). Unlike guggulsterones, xanthohumol and related prenylflavonoids bind to the FXR canonical binding site, as determined by hydrogen/deuterium exchange-mass spectrometry, fluorescence titration and molecular docking studies (Yang et al., 2016). However, xanthohumol also binds to CAR, GABAA and other receptors, and is therefore not FXR-specific (Chang et al., 2016; Yao et al., 2011).

Finally, oleanolic acid (Table 2) is a widely occurring plant pentacyclic triterpenoid that may protect against type 2 diabetes and chronic liver diseases via activation of TGR5 (Genet et al., 2010; Sato et al., 2007) and FXR (Liu & Wong, 2010), respectively. Oleanolic acid concentration-dependently suppressed FXR activity by binding to its LBD and blocking interaction with the coactivator, SRC-3. This defines oleanolic acid as a FXR antagonist. However, since oleanolic acid suppressed CDCA-dependent induction of BSEP expression in HepG2 cells, while not affecting CDCA-induced regulation of other FXR target genes, oleanolic acid may be a gene-, or function-, selective ligand for FXR (Liu & Wong, 2010).

Taken together, targeting FXR in a gene-selective manner seems a feasible and potentially attractive way to limit the pleiotropic effects of FXR activation. However, gene-selectivity has so far been studied on only a few FXR target genes and most of the compounds thus far described have promiscuous effects on other receptors, thereby limiting their suitability for further investigations.

4. Tissue-specific FXR modulators

4.1. The rationale for tissue-specific FXR modulation

FXR is expressed in diverse tissues, including the adrenal gland, kidney, stomach, duodenum, jejunum, ileum, colon, gallbladder, liver and macrophages, as well as in white and brown adipose depots (Forman et al., 1995), and in bone marrow cells (Cho et al., 2013). Therapeutic benefits of pharmacological FXR modulation may therefore increase by restricting FXR activity to specific tissues. Indeed, the modulation of FXR in different tissues may have diverse, even conflicting, effects in the pathogenesis or treatment of a specific disorder. For example, selective activation of FXR in the intestines of IBD patients would be expected to reduce inflammation in the intestine, without interfering with the metabolic function of FXR in the liver.

Since FXR signaling provides cross-talk between the intestine and the liver, for instance via activating the expression of the enterokine FGF15/19, therapeutic indications for the use of intestinal-specific FXR modulators may also extended to liver disorders. In fact, intestinal-specific overexpression of a constitutively active form of FXR reduced liver toxicity, bile acid pool size, and inflammatory infiltrates in mouse models of obstructive extrahepatic cholestasis (bile duct ligation), and intrahepatic cholestasis (alpha-naphthylisothiocyanate -induced chemical damage and Mdr2$^{-/-}$ mice) (Modica et al., 2012). Mechanistically, activation of intestinal FXR was shown to increase FGF15 expression, which via binding to FGFR4 abrogated bile acid synthesis, thereby reducing hepatic bile acid overload (Modica et al., 2012). In addition, FXR null mice in which FXR was constitutively activated (via VP16 fusion) in the intestine were protected against spontaneous development of hepatocellular carcinoma (Degirolamo et al., 2015). Constitutively active intestinal FXR improved bile acid homeostasis and reduced cellular proliferation, hepatic inflammation and fibrosis in young FXR null mice (Degirolamo et al., 2015). The development of intestinal-specific FXR agonists may therefore offer potential for therapeutic interventions in liver cholestasis disorders and in the prevention of hepatocellular carcinoma in for example NASH patients, who have a four-fold increased risk of developing hepatocellular carcinoma (Ekstedt et al., 2006; Soderberg et al., 2010).

Conversely, the antioxidant tempol, most probably by modifying the gut microbiome, resulted in the inhibition of intestinal FXR and protected control mice, but not intestinal-specific FXR null mice, against obesity (Li et al., 2013). Similarly, intestine-specific FXR null mice were protected against high fat diet-induced NAFLD (Jiang, Xie, Li, et al., 2015), potentially because of reduced hepatic lipid accumulation, improved mitochondrial function and reduced ceramide synthesis (Jiang, Xie, Li, et al., 2015; Jiang, Xie, Lv, et al., 2015). These observations suggest a therapeutic advantage for intestinal-specific FXR antagonists in the treatment of metabolic syndrome.

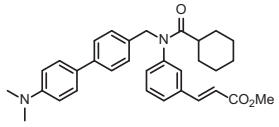
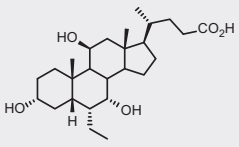
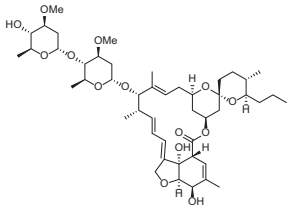
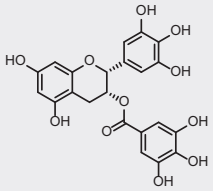
Finally, liver-specific modulation of FXR may be therapeutically advantageous, as compared to whole-body targeting of FXR, in the context of cholestasis, hyperammonemia, metabolic syndrome, diabetes and (non-)alcoholic liver disease. Activation of hepatic FXR decreased lipogenesis in diabetic mice (Watanabe et al., 2004), promoted glycogen synthesis in mice upon fasting and refeeding (Duran-Sandoval et al., 2005) and reduced markers of liver inflammation in cell and mouse models (Adorini et al., 2012; Wang, Chen, et al., 2008). Selective hepatic FXR modulation may therefore be pursued as a therapeutic intervention for NAFLD/NASH. In addition, agonism of hepatic FXR promoted ammonium clearance via ureagenesis and glutamine synthesis (Massafra et al., 2017). This encourages possible pharmacological targeting of hepatic FXR aimed at preventing hyperammonemia in patients with liver disorders, without interfering with kidney metabolism.

4.2. Mechanisms underlying tissue-specific FXR modulation

Tissue-specific FXR modulators may be developed by refining the physicochemical properties and pharmacokinetic profile of FXR ligands. Indeed, since a compound with an appropriate pharmacokinetic profile can be made to reach therapeutically effective concentration at a specific site pharmacokinetic studies may be considered as important as those relating to ligand specificity and selectivity. Aside from ligand-based modulation, regulation of NR activity in different tissues may involve tissue-specific expression of NR gene subtypes or isoforms, and/or differential expression of co-regulatory proteins. However, as this remains to be explored for FXR, we will therefore further focus on methods to achieve tissue specificity.

One additional point to be considered is the importance of transporters in the tissue-specific uptake of endogenous bile acid and bile acid-based FXR ligands (Halilbasic, Claudel, & Trauner, 2013). Bile acid transporters are of critical importance for the maintenance of bile acid homeostasis and enterohepatic circulation. Bile acid transporters not only have different transport affinities for various bile acid species, but also for other endogenous and exogenous compounds, including drugs and toxins. Hereditary and acquired defects of bile acid transporters contribute to the manifestation of several hepatobiliary diseases, such as cholestasis, gallstones, fatty liver disease and liver cancer, but also are determinant factors for intestinal and metabolic disorders (Halilbasic et al., 2013). Targeting hepatobiliary transporters could therefore open new therapeutic avenues for the regulation of NR function in the context of a broad range of diseases of the liver and beyond. In this respect, Rao. et al. recently showed that blocking apical sodium-dependent bile acid transporter (ASBT), the uptake transport protein for bile acids in the intestine, using a gut lumen- restricted ASBT-inhibitor, improved both hepatic and whole body aspects of NAFLD (Rao et al., 2016).

Table 3
FXR ligands claimed as tissue specific FXR modulators.

Compound	Tissue-specific effects and SAR features	Receptor selectivity	References
 Fexaramine	✓ Activates FXR target genes Shp, Fgf19, Fabp6 (Ibabp), Slc51a/b (Ostα/β) in the intestine. ✗ Does not activate FXR targets Shp, Abcb11 (Bsep), Slc51b (Ostβ) in the liver and kidney. ❖ Reduces diet-induced body weight. ❖ Reduces hepatic glucose production. ❖ Enhances adipose tissue browning and energy expenditure. ❖ Induces production of FGF15.	FXR	(Fang et al., 2015)
 TC-100	✓ Increases intestinal expression of FXR targets Fgf15, Shp and Ang1 in mice subjected to obstructive cholestasis. ✗ Has low hepatic residence, due to its high maximum biliary secretion and hydrophilic profile.	FXR	(Pellicciari et al., 2016)
 Ivermectin	✓ Upregulates intestinal FXR targets more robustly than GW4064. ✗ Upregulates FXR liver targets less robustly than GW4064. ❖ Lowers serum glucose and cholesterol levels in wild type and KK-A^{y} mice. ❖ Reduces hepatic lipid accumulation and body weight of obese diabetic mice.	FXR CAR LXRα PXR	(Jin et al., 2015)
 ECGC	✓ Activates Shp and FGF19, but not Ibabp in the ileum (no comparison with broad-spectrum agonist) ✗ Is rapidly glucuronidated in the liver and eliminated. ❖ Inhibits GW4064-dependent induction of SHP, OSTα /β , BSEP in HepG2 cells. ❖ Inhibits recruitment of SRC-2 (GRIP-1) to FXR by GW4064.	FXR	(Li et al., 2012)

✓ Effects on targeted tissues.
✗ Effects on untargeted tissues.
❖ Other key SAR features.

4.3. Intestinal-specific FXR modulators

Fexaramine (Table 3) was the first described intestinal-specific FXR modulator. It was derived from a screening of a 10,000-membered compound library constructed around the 2,2-dimethylbenzopyran scaffold by Nicolaou and coworkers in 2003 (Nicolaou et al., 2003). From a structure-activity point of view, fexaramine has been shown to establish two sets of interactions within the FXR LBD. The first set includes van der Waals contacts between the hexyl ring and Ile339 and Leu344 (H5), hydrophobic interactions of fexaramine's central nitrogen and the benzyl group with Phe333 (H5), and Met369 and Phe370 (H7) (Downes et al., 2003). Additionally, the methyl ester aliphatic chain makes van der Waals contacts with Met294 (H3), Leu352, and Ile356 (H6). The second group of interactions stabilizes the biaryl rings and the dimethyl amine moiety of fexaramine, due to van der Waals contacts with 15 residues. Comparative analyses revealed that fexaramine is a more potent agonist than CDCA, due to both the higher number of contacts established by the methyl ester group with H3 and the greater length of fexaramine, that more effectively fill the ligand binding pocket (Downes et al., 2003). When orally administered, fexaramine is poorly absorbed into the circulation and therefore preferentially activates FXR target genes in the intestine compared to the liver and kidneys (Fang et al., 2015). Chemical cues underlying the intestinal specific profile of fexaramine have not been clarified yet. Unlike systemic agonism, fexaramine reduces diet-induced body weight gain and hepatic glucose production. In addition, fexaramine enhances adipose tissue browning and energy expenditure in brown adipose tissue in a mouse model of obesity (Fang et al., 2015). The systemic metabolic effects are at least partly coordinated via the induced production of FGF15, which not only increased metabolic rate and improved glucose and lipid homeostasis, but also altered bile acid composition. A reduction in hepatic CYP7A1, accompanied by an increase in CYP7B1 expression, shifted

bile acid synthesis away from cholic acid (CA), towards CDCA and lithocholic acid (LCA). These findings suggest that intestinal-restricted FXR activation is potentially safer than systemic FXR agonism in the treatment of insulin resistance and metabolic syndrome.

Tissue selectivity may also be achieved by reducing the residence time of a compound in a given tissue. As an example, TC-100 (Table 3) was recently reported as the first, and sole bile acid derivative with a potent and selective activity for FXR (no binding/activation of TGR5 and other bile acid-responsive NRs) (Pellicciari et al., 2016). TC-100 is a semisynthetic bile acid derivative characterized by the insertion of a hydroxyl group at the C11β position of the OCA scaffold. This apparently minor chemical modification was the key structural motif responsible for the selectivity at FXR ($EC_{50} = 0.14\ \mu M$) over TGR5 and 13 other NRs. While the C11β-OH group hydroxyl derivative ensured an additional hydrogen bond with the carbonyl group of Leu284 at the canonical FXR binding site, most importantly, it was found to strongly affect the physicochemical properties, PK and biodistribution of the compound. TC-100 is indeed endowed with high water solubility, poor detergency and absence of cytotoxicity, and a lower lipophilicity with respect to natural bile acids and OCA. Accordingly, the intestinal absorption by passive diffusion of TC-100 was relatively high. In a bile duct ligation study, TC-100 was rapidly converted into the corresponding tauro-conjugate (50%) with preferential localization in the small intestine and rapid hepatic release within the enterohepatic circulation (Pellicciari et al., 2016). Moreover, following intravenous administration, the hepatic uptake of TC-100 was highly efficient and this is associated with an efficient biliary secretion of the unmodified parent compound and its taurine conjugate. TC-100 treatment increased intestinal expression of FXR targets *Fgf15*, *Shp* and *Ang1* even more efficiently than OCA and CDCA in C57BL/3 mice subjected to obstructive cholestasis. In light of the above observations, TC100 has been suggested as a promising therapeutic agent for the treatment of enterohepatic disorders, including cholestasis, IBD and NASH (Pellicciari et al., 2016).

Another FXR modulator with potential tissue-specific activity is ivermectin (Table 3). Ivermectin was identified as a partial FXR agonist ($EC_{50} = 200$ nM) with unique properties in modulating coregulator recruitment (Jin et al., 2013). In particular, ivermectin enhanced the interaction of FXR with LXXLL motifs of various coactivators and induced recruitment of the corepressor, NCOR2, to FXR. The crystal structure of the ivermectin/FXR complex revealed a highly dynamic AF2 helix, which is critical for NRs to interact with coregulators. Ivermectin was highly selective for FXR in both cell-based reporter assays and mammalian two-hybrid assays (Jin et al., 2013), although a recent study reported antagonistic effects of ivermectin on CAR, LXRα and PXR (Hsu et al., 2014). Compared to the conventional full agonist GW4064, ivermectin upregulated FXR target genes more strongly in the intestine and less strongly in the liver, thus suggesting that ivermectin may preferentially be targeting the intestine (Jin et al., 2015). Ivermectin lowered serum glucose and cholesterol levels (Jin et al., 2013) and reduced hepatic lipid accumulation and body weight (Jin et al., 2015) in diabetic mice. It remains to be clarified whether the differential cofactor usage and/or pharmacokinetic profile of ivermectin contribute to the tissue-selectivity and the beneficial effects of ivermectin on metabolism.

Epigallocatechin-3-gallate (EGCG) (Table 3), a natural component from green tea, was described to function as a tissue-specific FXR modulator (Li et al., 2012). Although EGCG alone concentration-dependently increased expression of the FXR targets, SHP, OSTα/β and BSEP in HepG2 cells, it antagonized the induction of the same targets by CDCA and GW4064. In addition, EGCG inhibited the GW4064-dependent recruitment of SRC2 (GRIP1) to FXR. The modulation of FXR activity by EGCG was claimed to be tissue- and gene-selective since EGCG was not able to induce FXR targets in the liver and regulated Shp and Fgf19, but not Ibabp, in mouse small intestine. However, this in vivo study lacked comparative analysis with conventional full agonists. The specificity was ascribed to rapid hepatic metabolism and elimination by glucuronidation (Lu et al., 2003).

In conclusion, the development of tissue-selective FXR modulators is expected to increasingly involve the refinement of the PK profile, in favor of increased residence in a given tissue.

5. Expert opinion and final remarks

Pharmacological targeting of FXR is anticipated to be successful as a therapeutic intervention for cholestatic liver disorders, NASH, obesity, metabolic syndrome and IBD. However, due to the pleiotropic functions of FXR, there is a need to generate FXR-specific, but also gene- and tissue-selective ligands in order to limit associated side effects.

Although mechanistic understanding of the interplay between FXR and coregulatory proteins, FXR post-translational modifications, and ligand binding to canonical and allosteric sites in FXR has dramatically increased, the field of gene-selective FXR modulators is still in its infancy. As we attempt to twist FXR activity in favor of regulation of a given subset of target genes, it is worth remembering that full dissociation of FXR functions is unlikely to be achieved, and a comprehensive investigation of the transcriptional, proteomic and metabolomic landscape regulated by FXR is needed for a robust assessment of drug-induced regulation of selective FXR functions.

Further characterization of ligand binding to accessory binding sites, such as the described S2 pocket, may open up novel ways of gene-selective targeting of FXR. Future efforts should be directed towards the understanding of the dynamic processes involved in ligand binding and coregulator recruitment to different FXR conformations. A mechanistic understanding of these processes will ultimately provide insights into gene/tissue-selective pharmacological outcomes and drive the design of novel gene- and tissue-selective FXR compounds.

To date, over 60 FXR structures have been uploaded to the Protein Data Bank (PDB). While most of these are generated by X-ray crystallography, and have been instrumental to define FXR structure and the molecular basis of activation, this technique only draws a static picture of the different FXR structural conformations. The use of other biophysical methods, such as hydrogen/deuterium exchange (HDX), nuclear magnetic resonance, and small angle X-ray scattering is therefore particularly sought to gain new experimental insights into the dynamics of FXR function. In this respect, the unravelling of key details that allow allosteric modulation of FXR function may provide a rational basis for virtual screening to discover compounds able to induce gene-selectivity by targeting allosteric sites in FXR.

The current dogma that FXR activation occurs via a 'mousetrap mechanism', in which H12 functions as a gate locking the ligand inside the LBD, is based on structure function relationships within the FXR-LBD and ignores allosteric modulation, for example, by the S2 site. Therefore, the impact of allosteric control in FXR requires investigation in the context of the full-length receptor. We recommend a multidisciplinary approach that combines the use of chemical probes, high-resolution protein crystallographic studies, biophysical and biochemical techniques, and molecular dynamic simulations. This strategy will unravel the molecular changes in FXR structure that may lead to differential signaling and thus facilitate future drug discovery programs aimed at developing novel selective FXR modulators.

Selective targeting of FXR in specific tissues also seems a promising strategy to increase the therapeutic index of FXR modulators. Studies reporting the therapeutic potential of intestinal-selective regulation of FXR activity have provided a rationale to refine the pharmacokinetic and physiochemical properties of FXR ligands. Such efforts are predicted to lead to tissue-specific therapeutic actions, which promise reduced side effects compared to whole-body targeting of FXR. Thus, while the development of predictive models for the biodistribution of FXR ligands becomes increasingly imperative, it is critical to take into account the possible effects of bile acid transporter expression and function.

In conclusion, improved integration of medicinal chemistry and FXR biology is crucial in the pursuit of selective FXR ligands. By iterating between design/generation of chemical probes and testing their effects in diverse biological assays and disease models, it will be possible to improve understanding of the FXR structure-function relationship. Ultimately, such multidisciplinary approaches will set the stage for the development of a novel generation of FXR-targeting drugs with improved pharmacological actions and reduced adverse effects.

Conflict of interest statement

AG and RP are co-founders of TES Pharma (www.tespharma.com).

Grant support

S.W.C.v.M. is supported by the Netherlands Organization for Scientific Research (NWO) Project VIDI (917.11.365), FP7 Marie Curie Actions IAPP (FXR-IBD, 611979).

References

Adorini, L., Pruzanski, M., & Shapiro, D. (2012). Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discovery Today 17*, 988–997.

Ananthanarayanan, M., Balasubramanian, N., Makishima, M., Mangelsdorf, D. J., & Suchy, F. J. (2001). Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *The Journal of Biological Chemistry 276*, 28857–28865.

Ananthanarayanan, M., Li, S., Balasubramaniyan, N., Suchy, F. J., & Walsh, M. J. (2004). Ligand-dependent activation of the farnesoid X-receptor directs arginine methylation of histone H3 by CARM1. *The Journal of Biological Chemistry 279*, 54348–54357.

Aranda, A., & Pascual, A. (2001). Nuclear hormone receptors and gene expression. *Physiological Reviews 81*, 1269–1304.

Balasubramaniyan, N., Ananthanarayanan, M., & Suchy, F. J. (2012). Direct methylation of FXR by Set7/9, a lysine methyltransferase, regulates the expression of FXR target genes. *American Journal of Physiology. Gastrointestinal and Liver Physiology 302*, G937–G947.

Barbier, O., Torra, I. P., Sirvent, A., Claudel, T., Blanquart, C., Duran-Sandoval, D., ... Staels, B. (2003). FXR induces the UGT2B4 enzyme in hepatocytes: A potential mechanism of negative feedback control of FXR activity. *Gastroenterology 124*, 1926–1940.

Baschant, U., Frappart, L., Rauchhaus, U., Bruns, L., Reichardt, H. M., Kamradt, T., ... Tuckermann, J. P. (2011). Glucocorticoid therapy of antigen-induced arthritis depends on the dimerized glucocorticoid receptor in T cells. *Proceedings of the National Academy of Sciences of the United States of America 108*, 19317–19322.

Berrabah, W., Aumercier, P., Gheeraert, C., Dehondt, H., Bouchaert, E., Alexandre, J., ... Lefebvre, P. (2014). Glucose sensing O-GlcNAcylation pathway regulates the nuclear bile acid receptor farnesoid X receptor (FXR). *Hepatology 59*, 2022–2033.

Bijsmans, I. T., Guercini, C., Ramos Pittol, J. M., Omta, W., Milona, A., Lelieveld, D., ... van Mil, S. W. (2015). The glucocorticoid mometasone furoate is a novel FXR ligand that decreases inflammatory but not metabolic gene expression. *Scientific Reports 5*, 14086.

Bowlus, C. L. (2016). Obeticholic acid for the treatment of primary biliary cholangitis in adult patients: Clinical utility and patient selection. *Hepatic Medicine 8*, 89–95.

Bruning, J. B., Parent, A. A., Gil, G., Zhao, M., Nowak, J., Pace, M. C., ... Nettles, K. W. (2010). Coupling of receptor conformation and ligand orientation determine graded activity. *Nature Chemical Biology 6*, 837–843.

Brzozowski, A. M., Pike, A. C., Dauter, Z., Hubbard, R. E., Bonn, T., Engstrom, O., ... Carlquist, M. (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature 389*, 753–758.

Burris, T. P., Montrose, C., Houck, K. A., Osborne, H. E., Bocchinfuso, W. P., Yaden, B. C., ... Stayrook, K. R. (2005). The hypolipidemic natural product guggulsterone is a promiscuous steroid receptor ligand. *Molecular Pharmacology 67*, 948–954.

Burris, T. P., Solt, L. A., Wang, Y., Crumbley, C., Banerjee, S., Griffett, K., ... Kojetin, D. J. (2013). Nuclear receptors and their selective pharmacologic modulators. *Pharmacological Reviews 65*, 710–778.

Caplan, A., Fett, N., Rosenbach, M., Werth, V. P., & Micheletti, R. G. (2017). Prevention and management of glucocorticoid-induced side effects: A comprehensive review: A review of glucocorticoid pharmacology and bone health. *Journal of the American Academy of Dermatology 76*, 1–9.

Carotti, A., Marinozzi, M., Custodi, C., Cerra, B., Pellicciari, R., Gioiello, A., & Macchiarulo, A. (2014). Beyond bile acids: Targeting Farnesoid X Receptor (FXR) with natural and synthetic ligands. *Current Topics in Medicinal Chemistry 14*, 2129–2142.

Chang, Y., Lin, T. Y., Lu, C. W., Huang, S. K., Wang, Y. C., & Wang, S. J. (2016). Xanthohumol-induced presynaptic reduction of glutamate release in the rat hippocampus. *Food & Function 7*, 212–226.

Chen, X., Yang, D., Shen, W., Dong, H. F., Wang, J. M., Oppenheim, J. J., & Howard, M. Z. (2000). Characterization of chenodeoxycholic acid as an endogenous antagonist of the G-coupled formyl peptide receptors. *Inflammation Research 49*, 744–755.

Chennamsetty, I., Claudel, T., Kostner, K. M., Baghdasaryan, A., Kratky, D., Levak-Frank, S., ... Kostner, G. M. (2011). Farnesoid X receptor represses hepatic human APOA gene expression. *The Journal of Clinical Investigation 121*, 3724–3734.

Chignard, N., & Poupon, R. (2009). Targeting farnesoid x receptor in hepatic and biliary inflammatory diseases. *Gastroenterology 137*, 734–735 (discussion 736).

Cho, S. W., An, J. H., Park, H., Yang, J. Y., Choi, H. J., Kim, S. W., ... Shin, C. S. (2013). Positive regulation of osteogenesis by bile acid through FXR. *Journal of Bone and Mineral Research 28*, 2109–2121.

Chow, M. D., Lee, Y. H., & Guo, G. L. (2017). The role of bile acids in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Molecular Aspects of Medicine 56*, 34–44.

Clark, A. R., & Belvisi, M. G. (2012). Maps and legends: The quest for dissociated ligands of the glucocorticoid receptor. *Pharmacology & Therapeutics 134*, 54–67.

Claudel, T., Inoue, Y., Barbier, O., Duran-Sandoval, D., Kosykh, V., Fruchart, J., ... Staels, B. (2003). Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. *Gastroenterology 125*, 544–555.

Coghlan, M. J., Jacobson, P. B., Lane, B., Nakane, M., Lin, C. W., Elmore, S. W., ... Miner, J. N. (2003). A novel antiinflammatory maintains glucocorticoid efficacy with reduced side effects. *Molecular Endocrinology 17*, 860–869.

Cui, J., Huang, L., Zhao, A., Lew, J. L., Yu, J., Sahoo, S., ... Wright, S. D. (2003). Guggulsterone is a farnesoid X receptor antagonist in coactivator association assays but acts to enhance transcription of bile salt export pump. *The Journal of Biological Chemistry 278*, 10214–10220.

De Bosscher, K. (2010). Selective Glucocorticoid Receptor modulators. *The Journal of Steroid Biochemistry and Molecular Biology 120*, 96–104.

De Bosscher, K., Vanden Berghe, W., Beck, I. M., Van Molle, W., Hennuyer, N., Hapgood, J., ... Haegeman, G. (2005). A fully dissociated compound of plant origin for inflammatory gene repression. *Proceedings of the National Academy of Sciences of the United States of America 102*, 15827–15832.

Degirolamo, C., Modica, S., Vacca, M., Di Tullio, G., Morgano, A., D'Orazio, A., ... Moschetta, A. (2015). Prevention of spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice by intestinal-specific farnesoid X receptor reactivation. *Hepatology 61*, 161–170.

Di Leva, F. S., Festa, C., D'Amore, C., De Marino, S., Renga, B., D'Auria, M. V., ... Fiorucci, S. (2013). Binding mechanism of the farnesoid X receptor marine antagonist suvanine reveals a strategy to forestall drug modulation on nuclear receptors. Design, synthesis, and biological evaluation of novel ligands. *Journal of Medicinal Chemistry 56*, 4701–4717.

Ding, L., Yang, L., Wang, Z., & Huang, W. (2015). Bile acid nuclear receptor FXR and digestive system diseases. *Acta Pharmaceutica Sinica B 5*, 135–144.

Downes, M., Verdecia, M. A., Roecker, A. J., Hughes, R., Hogenesch, J. B., Kast-Woelbern, H. R., ... Evans, R. M. (2003). A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Molecular Cell 11*, 1079–1092.

Duran-Sandoval, D., Cariou, B., Percevault, F., Hennuyer, N., Grefhorst, A., van Dijk, T. H., ... Staels, B. (2005). The farnesoid X receptor modulates hepatic carbohydrate metabolism during the fasting-refeeding transition. *The Journal of Biological Chemistry 280*, 29971–29979.

Dussault, I., Beard, R., Lin, M., Hollister, K., Chen, J., Xiao, J. H., ... Forman, B. M. (2003). Identification of gene-selective modulators of the bile acid receptor FXR. *The Journal of Biological Chemistry 278*, 7027–7033.

Ekstedt, M., Franzen, L. E., Mathiesen, U. L., Thorelius, L., Holmqvist, M., Bodemar, G., & Kechagias, S. (2006). Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology 44*, 865–873.

Fang, S., Suh, J. M., Reilly, S. M., Yu, E., Osborn, O., Lackey, D., ... Evans, R. M. (2015). Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nature Medicine 21*, 159–165.

Fardet, L., & Feve, B. (2014). Systemic glucocorticoid therapy: A review of its metabolic and cardiovascular adverse events. *Drugs 74*, 1731–1745.

Forman, B. M., Goode, E., Chen, J., Oro, A. E., Bradley, D. J., Perlmann, T., ... Weinberger, C. (1995). Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell 81*, 687–693.

Gadaleta, R. M., Oldenburg, B., Willemsen, E. C., Spit, M., Murzilli, S., Salvatore, L., ... van Mil, S. W. (2011). Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-kappaB signaling in the intestine. *Biochimica et Biophysica Acta 1812*, 851–858.

Gadaleta, R. M., van Erpecum, K. J., Oldenburg, B., Willemsen, E. C., Renooij, W., Murzilli, S., ... van Mil, S. W. (2011). Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut 60*, 463–472.

Genet, C., Strehle, A., Schmidt, C., Boudjelal, G., Lobstein, A., Schoonjans, K., ... Wagner, A. (2010). Structure-activity relationship study of betulinic acid, a novel and selective TGR5 agonist, and its synthetic derivatives: Potential impact in diabetes. *Journal of Medicinal Chemistry 53*, 178–190.

Ghisletti, S., Huang, W., Ogawa, S., Pascual, G., Lin, M. E., Willson, T. M., ... Glass, C. K. (2007). Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. *Molecular Cell 25*, 57–70.

Gineste, R., Sirvent, A., Paumelle, R., Helleboid, S., Aquilina, A., Darteil, R., ... Staels, B. (2008). Phosphorylation of farnesoid X receptor by protein kinase C promotes its transcriptional activity. *Molecular Endocrinology 22*, 2433–2447.

Gioiello, A., Cerra, B., Mostarda, S., Guercini, C., Pellicciari, R., & Macchiarulo, A. (2014). Bile acid derivatives as ligands of the farnesoid x receptor: Molecular determinants for bile acid binding and receptor modulation. *Current Topics in Medicinal Chemistry 14*, 2159–2174.

Gioiello, A., Macchiarulo, A., Carotti, A., Filipponi, P., Costantino, G., Rizzo, G., ... Pellicciari, R. (2011). Extending SAR of bile acids as FXR ligands: Discovery of 23-N-(carbocinnamyloxy)-3alpha,7alpha-dihydroxy-6alpha-ethyl-24-nor-5beta-cholan-23-amine. *Bioorganic & Medicinal Chemistry 19*, 2650–2658.

Glass, C. K. (1994). Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocrine Reviews 15*, 391–407.

Goodwin, B., Gauthier, K. C., Umetani, M., Watson, M. A., Lochansky, M. I., Collins, J. L., ... Repa, J. J. (2003). Identification of bile acid precursors as endogenous ligands for

the nuclear xenobiotic pregnane X receptor. *Proceedings of the National Academy of Sciences of the United States of America 100*, 223–228.
Halilbasic, E., Claudel, T., & Trauner, M. (2013). Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *Journal of Hepatology 58*, 155–168.
Halilbasic, E., Fuchs, C., Traussnigg, S., & Trauner, M. (2016). Farnesoid X receptor agonists and other bile acid signaling strategies for treatment of liver disease. *Digestive Diseases 34*, 580–588.
Hashiguchi, T., Arakawa, S., Takahashi, S., Gonzalez, F. J., Sueyoshi, T., & Negishi, M. (2016). Phosphorylation of Farnesoid X receptor at serine 154 links ligand activation with degradation. *Molecular Endocrinology 30*, 1070–1080.
Hollman, D. A., Milona, A., van Erpecum, K. J., & van Mil, S. W. (2012). Anti-inflammatory and metabolic actions of FXR: Insights into molecular mechanisms. *Biochimica et Biophysica Acta 1821*, 1443–1452.
Hsu, C. W., Zhao, J., Huang, R., Hsieh, J. H., Hamm, J., Chang, X., ... Xia, M. (2014). Quantitative high-throughput profiling of environmental chemicals and drugs that modulate farnesoid X receptor. *Scientific Reports 4*, 6437.
Hua, G., Ganti, K. P., & Chambon, P. (2016). Glucocorticoid-induced tethered transrepression requires SUMOylation of GR and formation of a SUMO-SMRT/NCoR1-HDAC3 repressing complex. *Proceedings of the National Academy of Sciences of the United States of America 113*, E635–E643.
Hua, G., Paulen, L., & Chambon, P. (2016). GR SUMOylation and formation of an SUMO-SMRT/NCoR1-HDAC3 repressing complex is mandatory for GC-induced IR nGRE-mediated transrepression. *Proceedings of the National Academy of Sciences of the United States of America 113*, E626–E634.
Hughes, T. S., Chalmers, M. J., Novick, S., Kuruvilla, D. S., Chang, M. R., Kamenecka, T. M., ... Kojetin, D. J. (2012). Ligand and receptor dynamics contribute to the mechanism of graded PPARgamma agonism. *Structure 20*, 139–150.
Jiang, C., Xie, C., Li, F., Zhang, L., Nichols, R. G., Krausz, K. W., ... Gonzalez, F. J. (2015). Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *The Journal of Clinical Investigation 125*, 386–402.
Jiang, C., Xie, C., Lv, Y., Li, J., Krausz, K. W., Shi, J., ... Gonzalez, F. J. (2015). Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nature Communications 6*, 10166.
Jin, L., Feng, X., Rong, H., Pan, Z., Inaba, Y., Qiu, L., ... Li, Y. (2013). The antiparasitic drug ivermectin is a novel FXR ligand that regulates metabolism. *Nature Communications 4*, 1937.
Jin, L., Wang, R., Zhu, Y., Zheng, W., Han, Y., Guo, F., ... Li, Y. (2015). Selective targeting of nuclear receptor FXR by avermectin analogues with therapeutic effects on nonalcoholic fatty liver disease. *Scientific Reports 5*, 17288.
Kassam, A., Miao, B., Young, P. R., & Mukherjee, R. (2003). Retinoid X receptor (RXR) agonist-induced antagonism of farnesoid X receptor (FXR) activity due to absence of coactivator recruitment and decreased DNA binding. *The Journal of Biological Chemistry 278*, 10028–10032.
Kassel, O., Schneider, S., Heilbock, C., Litfin, M., Gottlicher, M., & Herrlich, P. (2004). A nuclear isoform of the focal adhesion LIM-domain protein Trip6 integrates activating and repressing signals at AP-1- and NF-kappaB-regulated promoters. *Genes & Development 18*, 2518–2528.
Kast, H. R., Goodwin, B., Tarr, P. T., Jones, S. A., Anisfeld, A. M., Stoltz, C. M., ... Edwards, P. A. (2002). Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *The Journal of Biological Chemistry 277*, 2908–2915.
Kawamata, Y., Fujii, R., Hosoya, M., Harada, M., Yoshida, H., Miwa, M., ... Fujino, M. (2003). A G protein-coupled receptor responsive to bile acids. *The Journal of Biological Chemistry 278*, 9435–9440.
Kim, D. H., Xiao, Z., Kwon, S., Sun, X., Ryerson, D., Tkac, D., ... Kemper, J. K. (2015). A dysregulated acetyl/SUMO switch of FXR promotes hepatic inflammation in obesity. *The EMBO Journal 34*, 184–199.
Kong, B., Wang, L., Chiang, J. Y., Zhang, Y., Klaassen, C. D., & Guo, G. L. (2012). Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology 56*, 1034–1043.
Kwong, E., Li, Y., Hylemon, P. B., & Zhou, H. (2015). Bile acids and sphingosine-1-phosphate receptor 2 in hepatic lipid metabolism. *Acta Pharmaceutica Sinica B 5*, 151–157.
Landrier, J. F., Eloranta, J. J., Vavricka, S. R., & Kullak-Ublick, G. A. (2006). The nuclear receptor for bile acids, FXR, transactivates human organic solute transporter-alpha and -beta genes. *American Journal of Physiology. Gastrointestinal and Liver Physiology 290*, G476–G485.
Lee, J. M. (2016). Transcriptional coordination of hepatic autophagy by nutrient-sensing nuclear receptor PPARalpha and FXR. *Annals of Pediatric Endocrinology & Metabolism https://e-apem.org/ 21*, 193–198.
Lee, J. M., Wagner, M., Xiao, R., Kim, K. H., Feng, D., Lazar, M. A., & Moore, D. D. (2014). Nutrient-sensing nuclear receptors coordinate autophagy. *Nature 516*, 112–115.
Li, F., Jiang, C., Krausz, K. W., Li, Y., Albert, I., Hao, H., ... Gonzalez, F. J. (2013). Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nature Communications 4*, 2384.
Li, F., Zhang, M., Hussain, F., Triantaphyllopoulos, K., Clark, A. R., Bhavsar, P. K., ... Chung, K. F. (2011). Inhibition of p38 MAPK-dependent bronchial contraction after ozone by corticosteroids. *The European Respiratory Journal 37*, 933–942.
Li, G., Lin, W., Araya, J. J., Chen, T., Timmermann, B. N., & Guo, G. L. (2012). A tea catechin, epigallocatechin-3-gallate, is a unique modulator of the farnesoid X receptor. *Toxicology and Applied Pharmacology 258*, 268–274.
Lin, B. C., Wang, M., Blackmore, C., & Desnoyers, L. R. (2007). Liver-specific activities of FGF19 require Klotho beta. *The Journal of Biological Chemistry 282*, 27277–27284.
Liu, W., & Wong, C. (2010). Oleanolic acid is a selective farnesoid X receptor modulator. *Phytotherapy Research 24*, 369–373.
Liu, Y., Binz, J., Numerick, M. J., Dennis, S., Luo, G., Desai, B., ... Jones, S. A. (2003). Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis. *The Journal of Clinical Investigation 112*, 1678–1687.
Lu, H., Meng, X., Li, C., Sang, S., Patten, C., Sheng, S., ... Yang, C. S. (2003). Glucuronides of tea catechins: Enzymology of biosynthesis and biological activities. *Drug Metabolism and Disposition 31*, 452–461.
Ma, K., Saha, P. K., Chan, L., & Moore, D. D. (2006). Farnesoid X receptor is essential for normal glucose homeostasis. *The Journal of Clinical Investigation 116*, 1102–1109.
Mackinnon, J. A., Gallastegui, N., Osguthorpe, D. J., Hagler, A. T., & Estebanez-Perpina, E. (2014). Allosteric mechanisms of nuclear receptors: Insights from computational simulations. *Molecular and Cellular Endocrinology 393*, 75–82.
Makishima, M., Lu, T. T., Xie, W., Whitfield, G. K., Domoto, H., Evans, R. M., ... Mangelsdorf, D. J. (2002). Vitamin D receptor as an intestinal bile acid sensor. *Science 296*, 1313–1316.
Makishima, M., Okamoto, A. Y., Repa, J. J., Tu, H., Learned, R. M., Luk, A., ... Shan, B. (1999). Identification of a nuclear receptor for bile acids. *Science 284*, 1362–1365.
Mangelsdorf, D. J., & Evans, R. M. (1995). The RXR heterodimers and orphan receptors. *Cell 83*, 841–850.
Marinozzi, M., Carotti, A., Sansone, E., Macchiarulo, A., Rosatelli, E., Sardella, R., ... Pellicciari, R. (2012). Pyrazole[3,4-e][1,4]thiazepin-7-one derivatives as a novel class of Farnesoid X Receptor (FXR) agonists. *Bioorganic & Medicinal Chemistry 20*, 3429–3445.
Maruyama, T., Miyamoto, Y., Nakamura, T., Tamai, Y., Okada, H., Sugiyama, E., ... Tanaka, K. (2002). Identification of membrane-type receptor for bile acids (M-BAR). *Biochemical and Biophysical Research Communications 298*, 714–719.
Massafra, V., Ijssennagger, N., Plantinga, M., Milona, A., Ramos Pittol, J. M., Boes, M., & van Mil, S. W. (2016). Splenic dendritic cell involvement in FXR-mediated amelioration of DSS colitis. *Biochimica et Biophysica Acta 1862*, 166–173.
Massafra, V., Milona, A., Vos, H. R., Ramos, R. J., Gerrits, J., Willemsen, E. C., ... van Mil, S. W. (2017). Farnesoid X receptor activation promotes hepatic amino acid catabolism and ammonium clearance in mice. *Gastroenterology 152*(6), 1462–1476.e10.
Meyer, U., Costantino, G., Macchiarulo, A., & Pellicciari, R. (2005). Is antagonism of E/Z-guggulsterone at the farnesoid X receptor mediated by a noncanonical binding site? A molecular modeling study. *Journal of Medicinal Chemistry 48*, 6948–6955.
Mi, L. Z., Devarakonda, S., Harp, J. M., Han, Q., Pellicciari, R., Willson, T. M., ... Rastinejad, F. (2003). Structural basis for bile acid binding and activation of the nuclear receptor FXR. *Molecular Cell 11*, 1093–1100.
Modica, S., Petruzzelli, M., Bellafante, E., Murzilli, S., Salvatore, L., Celli, N., ... Moschetta, A. (2012). Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. *Gastroenterology 142*(355–365), e351–e354.
Montanari, R., Saccoccia, F., Scotti, E., Crestani, M., Godio, C., Gilardi, F., ... Pochetti, G. (2008). Crystal structure of the peroxisome proliferator-activated receptor gamma (PPARgamma) ligand binding domain complexed with a novel partial agonist: A new region of the hydrophobic pocket could be exploited for drug design. *Journal of Medicinal Chemistry 51*, 7768–7776.
Moris, D., Giaginis, C., Tsourouflis, G., & Theocharis, S. (2017). Farnesoid-X Receptor (FXR) as a promising pharmaceutical target in atherosclerosis. *Current Medicinal Chemistry 24*(11), 1147–1157.
Moschetta, A., Bookout, A. L., & Mangelsdorf, D. J. (2004). Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. *Nature Medicine 10*, 1352–1358.
Mudaliar, S., Henry, R. R., Sanyal, A. J., Morrow, L., Marschall, H. U., Kipnes, M., ... Shapiro, D. (2013). Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology 145*(574–582), e571.
Nagahashi, M., Takabe, K., Liu, R., Peng, K., Wang, X., Wang, Y., ... Zhou, H. (2015). Conjugated bile acid-activated S1P receptor 2 is a key regulator of sphingosine kinase 2 and hepatic gene expression. *Hepatology 61*, 1216–1226.
Neuschwander-Tetri, B. A., Loomba, R., Sanyal, A. J., Lavine, J. E., Van Natta, M. L., Abdelmalek, M. F., ... Network, N. C. R. (2015). Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *Lancet 385*, 956–965.
Newton, R., & Holden, N. S. (2007). Separating transrepression and transactivation: A distressing divorce for the glucocorticoid receptor? *Molecular Pharmacology 72*, 799–809.
Newton, R., Shah, S., Altonsy, M. O., & Gerber, A. N. (2017). Glucocorticoid and cytokine crosstalk: Feedback, feedforward, and co-regulatory interactions determine repression or resistance. *The Journal of Biological Chemistry 292*, 7163–7172.
Nicolaou, K. C., Evans, R. M., Roecker, A. J., Hughes, R., Downes, M., & Pfefferkorn, J. A. (2003). Discovery and optimization of non-steroidal FXR agonists from natural product-like libraries. *Organic & Biomolecular Chemistry 1*, 908–920.
Nozawa, H. (2005). Xanthohumol, the chalcone from beer hops (*Humulus lupulus* L.), is the ligand for farnesoid X receptor and ameliorates lipid and glucose metabolism in KK-A(y) mice. *Biochemical and Biophysical Research Communications 336*, 754–761.
Parks, D. J., Blanchard, S. G., Bledsoe, R. K., Chandra, G., Consler, T. G., Kliewer, S. A., ... Lehmann, J. M. (1999). Bile acids: Natural ligands for an orphan nuclear receptor. *Science 284*, 1365–1368.
Pascual, G., Fong, A. L., Ogawa, S., Gamliel, A., Li, A. C., Perissi, V., ... Glass, C. K. (2005). A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature 437*, 759–763.
Pellicciari, R., Fiorucci, S., Camaioni, E., Clerici, C., Costantino, G., Maloney, P. R., ... Willson, T. M. (2002). 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *Journal of Medicinal Chemistry 45*, 3569–3572.
Pellicciari, R., Gioiello, A., Costantino, G., Sadeghpour, B. M., Rizzo, G., Meyer, U., ... Fiorucci, S. (2006). Back door modulation of the farnesoid X receptor: Design, synthesis, and biological evaluation of a series of side chain modified chenodeoxycholic acid derivatives. *Journal of Medicinal Chemistry 49*, 4208–4215.

Pellicciari, R., Passeri, D., De Franco, F., Mostarda, S., Filipponi, P., Colliva, C., ... Gioiello, A. (2016). Discovery of 3alpha,7alpha,11beta-Trihydroxy-6alpha-ethyl-5beta-cholan-24-oic acid (TC-100), a novel bile acid as potent and highly selective FXR agonist for enterohepatic disorders. *Journal of Medicinal Chemistry 59*(19), 9201–9214.

Petta, I., Dejager, L., Ballegeer, M., Lievens, S., Tavernier, J., De Bosscher, K., & Libert, C. (2016). The Interactome of the glucocorticoid receptor and its influence on the actions of glucocorticoids in combatting inflammatory and infectious diseases. *Microbiology and Molecular Biology Reviews 80*, 495–522.

Pike, A. C., Brzozowski, A. M., & Hubbard, R. E. (2000). A structural biologist's view of the oestrogen receptor. *The Journal of Steroid Biochemistry and Molecular Biology 74*, 261–268.

Pike, A. C., Brzozowski, A. M., Hubbard, R. E., Bonn, T., Thorsell, A. G., Engstrom, O., ... Carlquist, M. (1999). Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *The EMBO Journal 18*, 4608–4618.

Pols, T. W., Nomura, M., Harach, T., Lo Sasso, G., Oosterveer, M. H., Thomas, C., ... Schoonjans, K. (2011). TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metabolism 14*, 747–757.

Rankovic, Z., Brust, T. F., & Bohn, L. M. (2016). Biased agonism: An emerging paradigm in GPCR drug discovery. *Bioorganic & Medicinal Chemistry Letters 26*, 241–250.

Rao, A., Kosters, A., Mells, J. E., Zhang, W., Setchell, K. D., Amanso, A. M., ... Karpen, S. J. (2016). Inhibition of ileal bile acid uptake protects against nonalcoholic fatty liver disease in high-fat diet-fed mice. *Science Translational Medicine 8*, 357ra122.

Ratman, D., Vanden Berghe, W., Dejager, L., Libert, C., Tavernier, J., Beck, I. M., & De Bosscher, K. (2013). How glucocorticoid receptors modulate the activity of other transcription factors: A scope beyond tethering. *Molecular and Cellular Endocrinology 380*, 41–54.

Rauch, A., Seitz, S., Baschant, U., Schilling, A. F., Illing, A., Stride, B., ... Tuckermann, J. P. (2010). Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor. *Cell Metabolism 11*, 517–531.

Raufman, J. P., Chen, Y., Zimniak, P., & Cheng, K. (2002). Deoxycholic acid conjugates are muscarinic cholinergic receptor antagonists. *Pharmacology 65*, 215–221.

Raufman, J. P., Zimniak, P., & Bartoszko-Malik, A. (1998). Lithocholyltaurine interacts with cholinergic receptors on dispersed chief cells from guinea pig stomach. *The American Journal of Physiology 274*, G997–1004.

Rosenfeld, M. G., Lunyak, V. V., & Glass, C. K. (2006). Sensors and signals: A coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes & Development 20*, 1405–1428.

Sato, H., Genet, C., Strehle, A., Thomas, C., Lobstein, A., Wagner, A., ... Saladin, R. (2007). Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. *Biochemical and Biophysical Research Communications 362*, 793–798.

Sato, Y., Ramalanjaona, N., Huet, T., Potier, N., Osz, J., Antony, P., ... Rochel, N. (2010). The "phantom effect" of the Rexinoid LG100754: Structural and functional insights. *PLoS One 5*, e15119.

Savkur, R. S., Thomas, J. S., Bramlett, K. S., Gao, Y., Michael, L. F., & Burris, T. P. (2005). Ligand-dependent coactivation of the human bile acid receptor FXR by the peroxisome proliferator-activated receptor gamma coactivator-1alpha. *The Journal of Pharmacology and Experimental Therapeutics 312*, 170–178.

Schaap, F. G., Trauner, M., & Jansen, P. L. (2014). Bile acid receptors as targets for drug development. *Nature Reviews. Gastroenterology & Hepatology 11*, 55–67.

Schulman, I. G., Li, C., Schwabe, J. W., & Evans, R. M. (1997). The phantom ligand effect: Allosteric control of transcription by the retinoid X receptor. *Genes & Development 11*, 299–308.

Seok, S., Fu, T., Choi, S. E., Li, Y., Zhu, R., Kumar, S., ... Kemper, J. K. (2014). Transcriptional regulation of autophagy by an FXR-CREB axis. *Nature 516*, 108–111.

Shen, H., Zhang, Y., Ding, H., Wang, X., Chen, L., Jiang, H., & Shen, X. (2008). Farnesoid X receptor induces GLUT4 expression through FXR response element in the GLUT4 promoter. *Cellular Physiology and Biochemistry 22*, 1–14.

Sinal, C. J., Tohkin, M., Miyata, M., Ward, J. M., Lambert, G., & Gonzalez, F. J. (2000). Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell 102*, 731–744.

Soderberg, C., Stal, P., Askling, J., Glaumann, H., Lindberg, G., Marmur, J., & Hultcrantz, R. (2010). Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology 51*, 595–602.

Soisson, S. M., Parthasarathy, G., Adams, A. D., Sahoo, S., Sitlani, A., Sparrow, C., ... Becker, J. W. (2008). Identification of a potent synthetic FXR agonist with an unexpected mode of binding and activation. *Proceedings of the National Academy of Sciences of the United States of America 105*, 5337–5342.

Staudinger, J. L., Goodwin, B., Jones, S. A., Hawkins-Brown, D., Mackenzie, K. I., Latour, A., ... Kliewer, S. A. (2001). The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proceedings of the National Academy of Sciences of the United States of America 98*, 3369–3374.

Thomas, A. M., Hart, S. N., Kong, B., Fang, J., Zhong, X. B., & Guo, G. L. (2010). Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. *Hepatology 51*, 1410–1419.

Tuckermann, J. P., Kleiman, A., Moriggl, R., Spanbroek, R., Neumann, A., Illing, A., ... Schutz, G. (2007). Macrophages and neutrophils are the targets for immune suppression by glucocorticoids in contact allergy. *The Journal of Clinical Investigation 117*, 1381–1390.

Tuckermann, J. P., Reichardt, H. M., Arribas, R., Richter, K. H., Schutz, G., & Angel, P. (1999). The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1-dependent genes in skin. *The Journal of Cell Biology 147*, 1365–1370.

Urizar, N. L., Liverman, A. B., Dodds, D. T., Silva, F. V., Ordentlich, P., Yan, Y., ... Moore, D. D. (2002). A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science 296*, 1703–1706.

Waddell, D. S., Baehr, L. M., van den Brandt, J., Johnsen, S. A., Reichardt, H. M., Furlow, J. D., & Bodine, S. C. (2008). The glucocorticoid receptor and FOXO1 synergistically activate the skeletal muscle atrophy-associated MuRF1 gene. *American Journal of Physiology. Endocrinology and Metabolism 295*, E785–E797.

Wang, H., Chen, J., Hollister, K., Sowers, L. C., & Forman, B. M. (1999). Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Molecular Cell 3*, 543–553.

Wang, X., Nelin, L. D., Kuhlman, J. R., Meng, X., Welty, S. E., & Liu, Y. (2008). The role of MAP kinase phosphatase-1 in the protective mechanism of dexamethasone against endotoxemia. *Life Sciences 83*, 671–680.

Wang, Y. D., Chen, W. D., Wang, M., Yu, D., Forman, B. M., & Huang, W. (2008). Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology 48*, 1632–1643.

Watanabe, M., Houten, S. M., Mataki, C., Christoffolete, M. A., Kim, B. W., Sato, H., ... Auwerx, J. (2006). Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature 439*, 484–489.

Watanabe, M., Houten, S. M., Wang, L., Moschetta, A., Mangelsdorf, D. J., Heyman, R. A., ... Auwerx, J. (2004). Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *The Journal of Clinical Investigation 113*, 1408–1418.

Wu, J., Xia, C., Meier, J., Li, S., Hu, X., & Lala, D. S. (2002). The hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor. *Molecular Endocrinology 16*, 1590–1597.

Xie, W., Radominska-Pandya, A., Shi, Y., Simon, C. M., Nelson, M. C., Ong, E. S., ... Evans, R. M. (2001). An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proceedings of the National Academy of Sciences of the United States of America 98*, 3375–3380.

Xu, X., Xu, X., Liu, P., Zhu, Z. Y., Chen, J., Fu, H. A., ... Shen, X. (2015). Structural basis for small molecule NDB (N-benzyl-N-(3-(tert-butyl)-4-hydroxyphenyl)-2,6-dichloro-4-(dimethylamino) benzamide) as a selective antagonist of farnesoid X receptor alpha (FXRalpha) in stabilizing the homodimerization of the receptor. *The Journal of Biological Chemistry 290*, 19888–19899.

Yang, L., Broderick, D., Campbell, Y., Gombart, A. F., Stevens, J. F., Jiang, Y., ... Maier, C. S. (2016). Conformational modulation of the farnesoid X receptor by prenylflavonoids: Insights from hydrogen deuterium exchange mass spectrometry (HDX-MS), fluorescence titration and molecular docking studies. *Biochimica et Biophysica Acta 1864*, 1667–1677.

Yang, L., Broderick, D., Jiang, Y., Hsu, V., & Maier, C. S. (2014). Conformational dynamics of human FXR-LBD ligand interactions studied by hydrogen/deuterium exchange mass spectrometry: Insights into the antagonism of the hypolipidemic agent Z-guggulsterone. *Biochimica et Biophysica Acta 1844*, 1684–1693.

Yao, R., Yasuoka, A., Kamei, A., Kitagawa, Y., Rogi, T., Taieishi, N., ... Abe, K. (2011). Polyphenols in alcoholic beverages activating constitutive androstane receptor CAR. *Bioscience, Biotechnology, and Biochemistry 75*, 1635–1637.

Zavacki, A. M., Lehmann, J. M., Seol, W., Willson, T. M., Kliewer, S. A., & Moore, D. D. (1997). Activation of the orphan receptor RIP14 by retinoids. *Proceedings of the National Academy of Sciences of the United States of America 94*, 7909–7914.