Perspective

Farnesoid X Receptor: From Structure to Potential Clinical Applications

Roberto Pellicciari, Gabriele Costantino, and Stefano Fiorucci

J. Med. Chem., **2005**, 48 (17), 5383-5403• DOI: 10.1021/jm0582221 • Publication Date (Web): 02 August 2005

Journal of
Medicinal
Chemistry

Volume 48, Number 17 August 25, 2005

Perspective

Farnesoid X Receptor: From Structure to Potential Clinical Applications

Roberto Pellicciari,*,‡ Gabriele Costantino,‡ and Stefano Fiorucci§

Dipartimento di Chimica e Tecnologia del Farmaco, Università di Perugia, Via del Liceo 1, I-06123 Perugia, Italy, and Dipartimento di Medicina Clinica e Sperimentale, Università di Perugia, Via del Giochetto, I-06122 Perugia, Italy

Received April 20, 2005

1. Introduction

The human nuclear receptor (NR) family consists of 48 transcription factors divided into seven classes (Table 1) and activated by a large variety of ligands such as steroids, retinoids, vitamin D, thyroid hormone, prostanoids, and cholesterol metabolites such as oxysterols and bile acids. NRs play a key role in a broad spectrum of physiological responses, and some of them, usually referred to as "endocrine" NRs, are clinically exploited drug targets for the treatment of significant diseases such as prostate and breast cancer, liver and coronary heart disease, osteoporosis, and diabetes. Other members of this family are incompletely understood in terms of both the physiological role and the activating ligands and are referred to as "orphan" NRs. There is a high probability that these "orphan receptors" will also make good targets for drug development. In this regard, the unravelling of the biological role of the orphan receptors has relied on "reverse endocrinology",[1] a strategy in which the initial step is the discovery of potent small-molecule ligands that are then used to identify the receptor target genes and their products. The "reverse endocrinology" strategy has resulted in the discovery of unanticipated nuclear signaling pathways for retinoids, fatty acids, eicosanoids, and steroids. Thus, the deorphanization of LXR[2] and PPARs (α, γ, and δ)[3] had led to the recognition of their role as physiological sensors for lipids and cholesterol metabolites. Other former orphan nuclear receptors such as PXR[4] and CAR[5] have been characterized as specialized xenobiotic sensors involved in the detoxification of the organism through activation of the cytochrome P family of enzymes. Far from being fully understood, the ligand-dependent activation of "metabolic" (as opposite to "endocrine") nuclear receptors is emerging as a tangled process where many known and still unknown cofactors enter the game, eventually leading to a selective gene transcription that ultimately governs the homeostasis of lipids in higher vertebrates.

The farnesoid X receptor (FXR) is a well-characterized member of the "metabolic" subfamily of NRs. In 1999 FXR was recognized to be a transcriptional sensor for bile acids which are therefore witnessing their "renaissance" as signaling molecules after more than 20 years of research activity culminating with the clinical use of chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) in the treatment of gallbladder stones and cholestatic liver diseases. Bile acids and oxysterols and cholestanoic acids are distinct classes of steroidal molecules derived from cholesterol (Figure 1). All of them act as signaling molecules and participate in an intricate network of interactions that ultimately govern lipid, steroid, and cholesterol homeostasis and are involved in processes such as glucose utilization, inflammation, and cancer.

This Perspective will focus on the steps that led to the discovery and deorphanization of FXR and to the development of steroidal and nonsteroidal modulators, with a special emphasis given to the increasing therapeutic opportunities associated with FXR modulation.

2. Nuclear Receptor Superfamily at Glance

NRs are evolutionarily derived from a common ancestor, and the analysis of the complete human genome

* To whom correspondence should be addressed. Phone: +39 075 585 5128. Fax: +39 075 585 5165. E-mail: rp@unipg.it.
‡ Dipartimento di Chimica e Tecnologia del Farmaco.
§ Dipartimento di Medicina Clinica e Sperimentale.

10.1021/jm0582221 CCC: $30.25
Published on Web 08/02/2005

Table 1.

classification[a]	class and gene ID[b]	denomination	ligand
		Endocrine Receptors	
ERα,β	II (NR3A1,A2)	estrogen	estradiol
PR	III (NR3C3)	progesterone	progesterone
AR	III (NR3C4)	androgen	dihydrotestosterone
GR	III (NR3C1)	glucocorticoid	cortisone
MR	III (NR3C3)	mineralcorticoid	aldosterone
RARα,β,γ	I (NR1B1, B2,B3)	retinoic acid	*trans*-retinoic acid
TRα,β	I (NR1A1, A2)	tyroid hormone	T_3
VDR	I (NR1l1)	vitamin D	1,25-dihydroxyVit-D_3
		Adopted Orphan Receptors (Metabolic Receptors)	
RXRα,β,γ	II (NR2B1,B2,B3)	retinoid X	9-*cis*-retinoic acid
PPARα,γ,δ	I (NR1C1, C2, C3)	peroxisome proliferator activated	eicosapentanoic adic
LXRα,β	I (NR1H2,H3)	liver X	24(*S*)-hydroxycholesterol
FXR	I (NR1H4)	farnesoid X	chenodeoxycholic acid
PXR	I (NR1I2)	pregane X	5β-pregane-3,20 dione
CAR	I (NR1I3)	constitutive androstan	3α,5α-androstanol
EERα,β,γ	III (NR3B1,B2,B3)	estrogen related	estradiol
HNF-4α,β	II (NR2A1, A2)	hepatocyte nuclear factor 4	palmitic acid
RORα,β,γ	I (NR1F1,F2,F3)	retinoic acid orphan related	stearic acid
		Orphan Receptors	
COUPα,β,γ	II (NR2F1,F2,F3)	chicken ovalbumin upstream promoter transcription factor	unknown unknown
DAX	0 (NR0B1)	dosage-sensitive sex reversal	unknown
GCNF	VI (NR6A1)	germ cell nuclear factor	unknown
LRH-1	V (NR5A2)	liver-related homologue-1	unknown
NGFI-Bα,β,γ	IV (NR4A1,A2,A3)	NGF-induced clone B	unknown
PNR	II (NR2E3)	photoreceptor-specific	unknown
RevErbAα,β	I (NR1D1,D2)	reversal ErbA	unknown
SF-1	V (NR5A1)	steroidogenic factor 1	unknown
SHP	0 (NR0B2)	small heterodimer partner	unknown
TLX	II (NR2E1)	tailles-related receptor	unknown
TR2α,β	II (NR2C1, C2)	testis receptor	unknown

[a] Classification based on biochemical data. Endocrine receptors bind with high-affinity "hormonal" lipids and steroid. Metabolic receptors bind with low-affinity "dietary" and "metabolic" lipids and steroids. [b] Systematic nomenclature based on the evolution of the DNA binding domain and of the ligand binding domain and adopted by the Nuclear Receptors Committee (*Cell* **1999**, *97*, 161−163).

sequence showed that the NR superfamily is a relatively small group, consisting of only 48 members (Table 1). This family includes not only the classic endocrine receptors that mediate the actions of steroid hormones, thyroid hormones, and the fat-soluble vitamins A and D but also a subset of so-called *orphan* NRs. Nuclear receptors share a modular structure made up of a variable modular region, a conserved DNA binding domain (DBD), and hormone or ligand binding domain (LBD) (Figure 2).

The transcriptional activity of NRs resides in the ability of the highly conserved DBD to recognize specific nucleotide sequences, called hormone response elements (HREs). The physical binding of DBD to DNA depends on various levels of regulation, such as compartmentalization (although most NRs are thought to be constitutively located into the nucleus), covalent modification, protein−protein interactions, and DNA conformation.[6] In addition, DNA binding is only one of several mechanisms by which NRs mediate transcription; once bound to DNA, the receptor may be inactive, may repress transcription, or may up-regulate transcription. Whether the ligand is bound or not and whether the ligand is an agonist or an antagonist provide another possible level of control for DNA-bound receptors.

The LBD contains the ligand-dependent activation function 2 (AF2) and is by far the most complex and functionally important domain that, in addition to the specific control through the binding of lipophilic molecules,[7] is also involved in protein−protein interactions, nuclear localization, and transactivation function.[8] The LBDs contain two well-conserved regions: a *signature motif* or Ti, and the AF2 motif located at the carboxy terminal end of the domain, which is related to the ligand-dependent transactivation function. Many of the traits relative to LBD functioning have been elucidated through resolution of the crystal structures of either apo, holo, or antagonist-bound LBDs for many NRs. These features will be thoroughly described below.

The binding of NRs to HRE of DNA usually requires the formation of a dimer, either a homodimer or a heterodimer, although some orphan NRs can bind DNA as monomers. Classical steroid receptors bind almost exclusively as homodimers to the HREs, and the dimerization interfaces have been identified in both LBD and DBD. The majority of metabolic receptors bind to their HREs preferentially as heterodimers with retinoic X receptor (RXR). Theoretically, four different states of heterodimer occupancy can be predicted: both receptors unoccupied, only RXR occupied, only the partner receptor occupied, and both receptors occupied. However, only three types of heterodimeric complexes exist: (i) unoccupied heterodimers, (ii) nonpermissive heterodimers that can be activated only by the partner's ligand but not by an RXR ligand alone,[9] and (iii) permissive heterodimers that can be activated by ligands of either RXR or its partner receptor and are synergistically activated in the presence of both ligands.[10] In nonpermissive heterodimers, including RXR/TR or RXR/VDR, the ligand-induced transcriptional activities for RXR are suppressed when complexed with the partner. In this case, when the formation of the heterodimer precludes binding of ligands to RXR, the RXR receptor is said to be a *silent partner*.

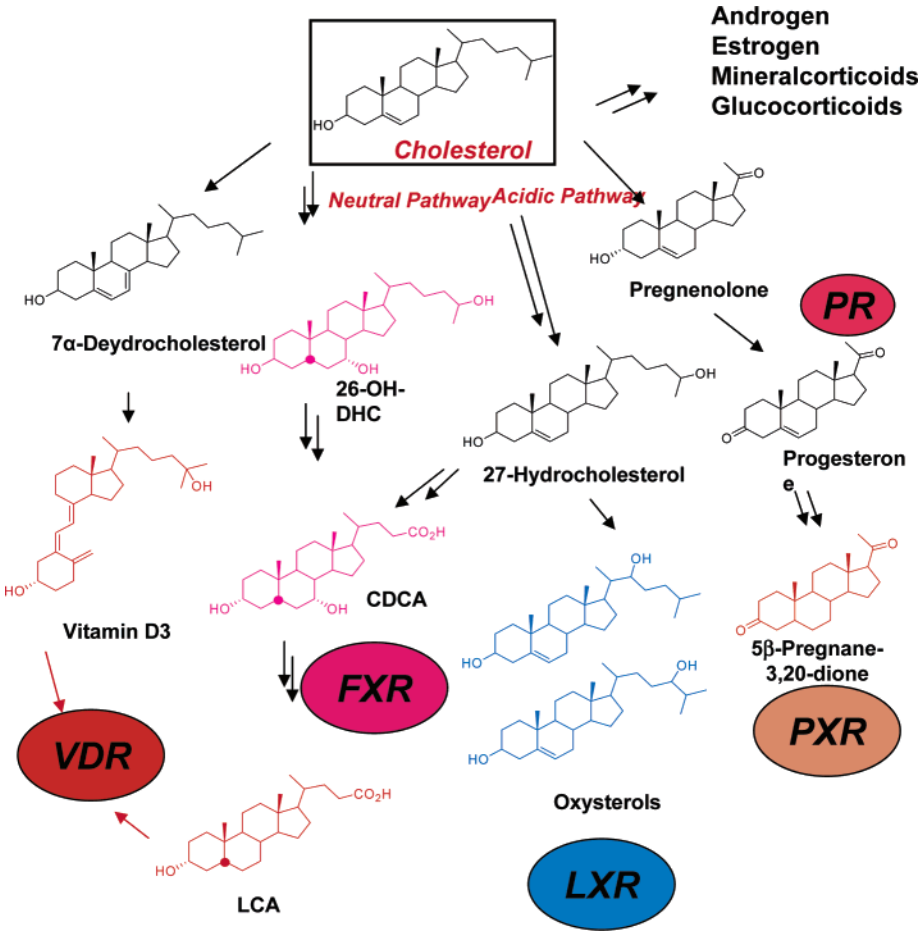


Figure 1. Several cholesterol metabolites act as signaling molecules regulating transcriptional activity of both endocrine and metabolic nuclear receptors. Cholesterol metabolites acting at VDR (red pathway), FXR (purple pathway), LXR (blue pathway), and PXR (orange pathway) are represented.

3. Discovery of Farnesoid X Receptot (FXR): A Bile Acid Nuclear Receptor

In 1995, using a genetic yeast system, Seol et al. identified a number of cDNAs whose products interacted with the ligand binding domain of RXRα.[11] Of these, some were recognized as already known NRs, like RAR (retinoic acid receptor) and PPAR (peroxisome proliferator-activated receptor). Others, identified as RIP14 and RIP15 (RIP = RXR interacting protein), were unique in their interaction with RXR. Northern blot analysis indicated, in particular, that RIP14 is expressed specifically in liver and kidney and binds as heterodimer with RXR to the retinoic acid (RA) response element (RARE) from the promoter of the RARβ2 isoform and also binds the ecdysone response element from the Drosophila heat shock protein 27 promoter. In cotransfection, RIP14 was unable to transactivate a reporter containing multiple copies of the β RARE under any conditions, suggesting that its activity was dependent on the binding of yet unidentified ligands. In the same year, Forman et al. were able to clonate a rat receptor by using a polymerase chain reaction (PCR) and degenerated primers corresponding to the DNA binding domain of NRs.[12] This receptor was recognized to bear a 95% sequence identity with the RIP14 protein and was named FXR (farnesoid X receptor) after the observation that farnesol (**1**, Chart 1), a metabolic isoprenoid element along the HMG reductase pathway, was able to activate the receptor at supraphysiological concentrations. It should be noted, however, that a direct interaction of farnesol with FXR has never been demonstrated.

The HRE to which the dimer FXR/RXR binds, originally referred to as FXRE, contains a consensus sequence consisting of an inverted repeat of the sequence AGGTCA with a one base pair spacing (IR-1).[13] This sequence was shown to be a high-affinity binding site for FXR/RXR in vitro and to confer ligand-dependent transcriptional activation by FXR/RXR to a heterologous promoter. The identification of the IR-1 and related elements as high-affinity binding sites and functional response elements for FXR/RXR has greatly assisted the susbsequent identification of genes regulated by activation of the FXR/RXR dimer.

Deorphanization of FXR took place in 1999, when three groups independently reported that bile acids are the endogenous ligands for FXR, which can therefore be defined as a nuclear bile acid receptor.[14−16] Indeed, bile acids, oxidized products of cholesterol metabolism, were recognized early (1957) as being involved in the feedback regulation of their own biosynthesis,[17] and in the 1990s several studies reported that the regulatory ability of hydrophobic bile acids was associated with transcriptional activity.[18] The orphan NR FXR (or RIP14) was selected as a suitable candidate as the transcriptional sensor for bile products. Indeed, FXR is abundantly expressed in the liver, intestine, and kidney; the cytochrome CYP7A promoter contains FXRE ele-

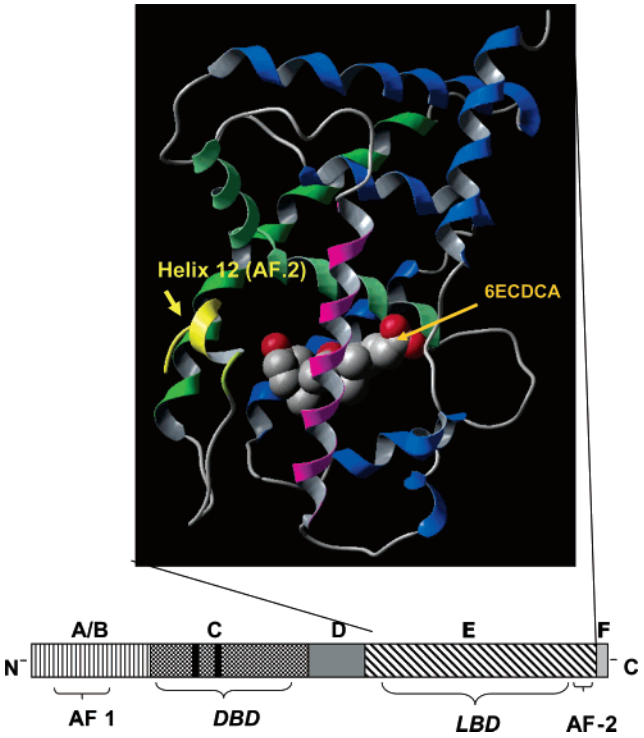
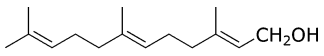


Figure 2. Modular structure of NRs (from left to right): N-terminal domain; modular domain A/B that encompasses the ligand-independent activation function-1 (AF-1); DNA binding domain (DBD) containing a highly conserved Zn-finger apparatus; DBD is involved in both physical association with the hormone response element and in dimerization; hinge region (D); ligand binding domain (LBD), which contains the ligand-dependent activation function-2 (AF2); C-terminal domain. The ligand binding domain of rFXR (pdb code: 1OSV) cocrystallized with 6ECDCA (**38**) is evident. Helix-12 (AF-2) is shown in purple, H3 is shown in yellow, and H11 is shown in green.

Chart 1

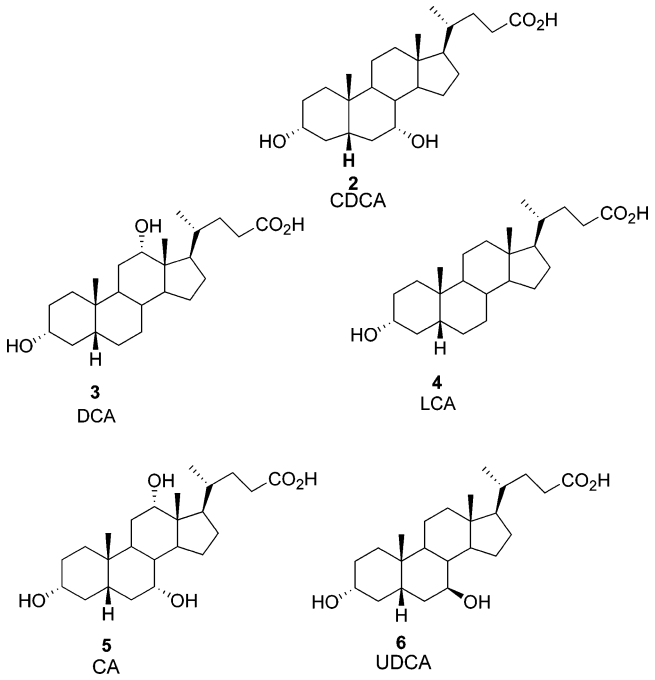


Farnesol, **1**

ments, and importantly, another NR, the LXR receptor that senses other cholesterol metabolites, was recognized to feedforward-regulate the synthesis of bile acids.[19] Since FXR/RIP14 is highly homologous to LXR and, similarly to other metabolic NRs, heterodimerizes with RXR, it was hypothesized that bile products could activate FXR. Thus, a series of bile acid metabolites were screened against cells transfected with rat, mouse, or human FXR plasmids, and FXR was strongly activated by the primary bile acid chenodeoxycholic acid (CDCA, **2**, Chart 2) and, to a lesser extent, by deoxycholic acid (DCA, **3**) and lithocholic acid (LCA, **4**).[14−16] Other steroids, cholesterol metabolites, and different bile acids were unable to activate FXR up to 100 μM. FXR fully responded to the physiological concentration of **2** (EC_{50} = 10−30 μM), and together with the observation that **2** could promote the physical association of FXR with the steroid receptor coactivator-1 (SRC-1) peptide, a known coactivator for NRs, this result was a confirmation that a subset of naturally occurring bile acids are the physiological ligands of FXR.

3.1. Bile Acids Are Ligands for the FXR Receptor. Bile acids (BAs), steroid end-products of cholesterol

Chart 2

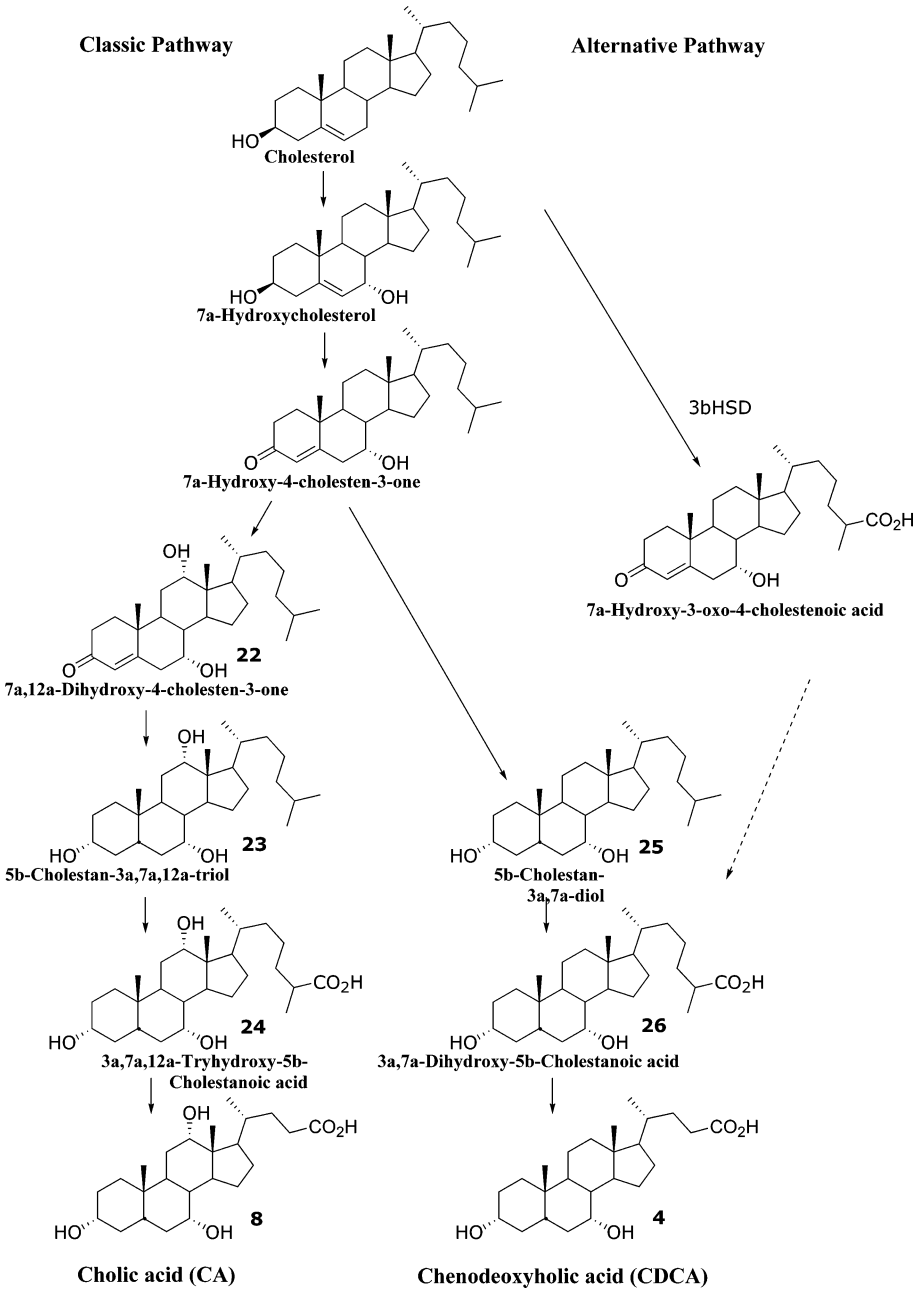


metabolism, are the major constituents of bile, where they play fundamental roles in lipid and vitamin absorption, in cholesterol homeostasis, and in the regulation of the bile flow. For many years, the physiological effects of bile acids as well as their potential therapeutic applications have been interpreted on the basis of their peculiar physicochemical features, which provide an optimal hydrophobic/hydrophilic balance and the ability to form fat-solubilizing micelles at physiological concentration.[20] Naturally occurring BAs are categorized into (i) primary BAs that are synthesized from cholesterol in the liver, (ii) secondary BAs that are produced from primary BAs by intestinal bacteria, and (iii) tertiary BAs resulting from absorption of secondary BAs from the liver and conversion on passing through the liver. Bile acids are synthesized from cholesterol into the liver through two pathways, namely, the classic (neutral) pathway and the alternative (acidic) pathway (Scheme 1).[21]

In the classic pathway, modification of the steroid nucleus precedes oxidative cleavage of the side chain, whereas in the alternative pathway side chain oxidation precedes steroid ring modification. In humans, the first and rate-limiting step of the classic pathway is the modification of the cholesterol ring structure involving the introduction of a hydroxyl group in the axial (α) configuration at position C-7. This reaction is catalyzed by a unique cytochrome P-450 enzyme, cholesterol 7α-hydroxylase (CYP7A1), a microsomal enzyme and one of the three cytochrome P-450 enzymes that participate in the BA biosynthesis.

In the alternative (or acidic) pathway, the rate-limiting enzyme is sterol 27-hydroxylase (CYP27A1), a mitochondrial P450 enzyme that catalyzes the oxidation and cleavage of the steroid side chain. In peripheral tissues, CYP27A1 converts cholesterol to 27-hydroxycholesterol and cholestenoic acids. The acidic pathway can be considered as a reverse cholesterol transport process for removing excess oxidized cholesterol from peripheral tissues to the liver.

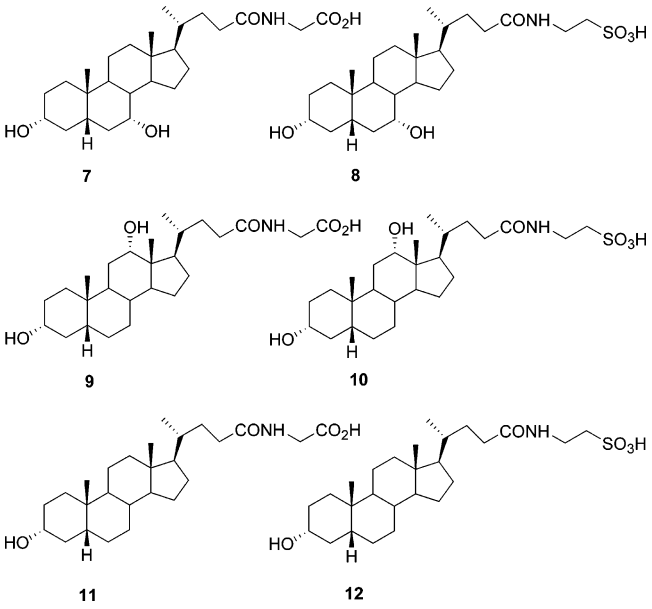
Scheme 1



Although early reports associated **2** with a rather high selectivity in activating FXR over other naturally occurring BA,[14−16] more recent data suggest that structurally diverse bile acids are in fact able to interact with the LBD of FXR and, even more interestingly, that minor structural modifications may be able to modulate both the gene selectivity and the pharmacological character of the bile acid. Thus, when human or rat full-length FXR was cotransfected with RXRα into CV-1 cells,[14,15] the secondary bile acids **3** and **4** activated the receptor with a lower efficacy than CDCA, while cholic acid (CA, **5**) and ursodeoxycholic acid (UDCA, **6**) were essentially inactive. In the same transativation system, the glyco- and tauroconjugates of **2**, **3**, and **4** (**7**−**12**, Chart 3) were inactive as well. However, coexpression of the ileum specific bile acid transport (ISBAT), which is required for efficient uptake of these relatively hydrophilic compounds, resulted in a strong activation of FXR/RXR by these conjugated bile acids. Interestingly, the presence of the ISBAT transporter also made **5** an efficacious activator of FXR/RXR.[14]

Recruitment of the glucocorticoid receptor interacting protein-1 (GRIP-1) peptide (or its closely related SRC-1 peptide) in a cell-free assay is an accepted indicator of the ligand binding activity. Thus, **2** (and its tauro- and glycoconjugates **7** and **8**, respectively) is able to recruit a peptide containing the LxxLL motif from the SRC-1 coactivator with an EC_{50} of 3.5 μM. Compounds **3**−**5** did not enhance coactivator recruitment up to 1000 μM (100 for **3**); however, they (and their conjugates) disrupted the interaction between FXR, SRC-1, and **2** (50 μM) in a dose-dependent manner, thus indicating that they behave as partial antagonists in this assay.[15] In particular, **4** was proposed as a bona fide FXR antagonist on the basis of its ability to antagonize the CDCA-promoted association of FXR with SRC-1 by homogeneous time-resolved fluorescence spectroscopy.[22] The LCA antagonism in the cell-free fluorescence resonance

Chart 3



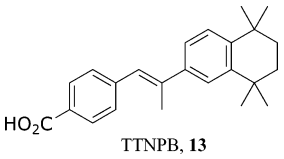
energy transfer (FRET) assay was correlated with the antagonist activity of **4** in FXR transactivation in HepG2 cells and with the partial induction of BSEP in the same cell line. Interestingly, **6** showed activity as an FXR agonist (albeit with reduced potency and efficacy) in a transactivation assay in the same HepG2 cell line. This activity correlated with the increased induction of bile salt export pump (BSEP) expression or reduced expression of CYP7A1.[23]

3.2. Ligands for the FXR Receptor. Deorphanization of FXR, in 1999, pushed several laboratories from both academics and industry to search for potent, selective, and pharmacokinetically suitable ligands to be used for the pharmacological characterization of the receptor, on route toward clinically useful agents. The search for FXR ligands was directed into three different areas, namely, non-steroid ligands, semisynthetic bile acid derivatives, and naturally occurring steroid compounds. Each of these approaches provided individual compounds endowed with peculiar properties in terms of potency, bioavailabilty, and selective target gene modulation.

3.2.1. Non-Steroid Ligands. As already mentioned, **1** was the first compound proposed to be the endogenous activator of FXR, but its direct interaction with the LBD has never been demonstrated. Nevertheless, the idea that there exists a common synthetic pathway for endogenous ligands of the nuclear receptor superfamily has gained interest over the past years. Indeed, steroids, retinoids, and farnesoids are all derived from the same biosynthetic pathway that involves as a rate-limiting step the HMG-CoA reductase. Thus, retinoids were also proposed as ligands for FXR before its deorphanization as a bile acid receptor. In particular, the synthetic RXR agonist TTNBP (**13**, Chart 4) was shown to also activate the RIP14 component of the RXR/RIP heterodimer.[24]

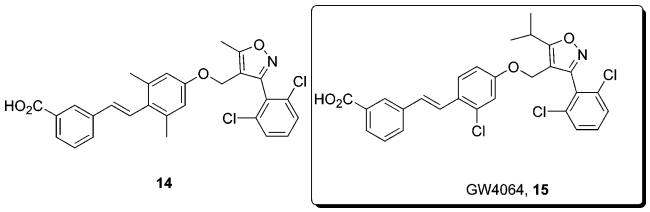
Although active only at very high concentration, the activity of the stilbene derivative **13** at FXR should not be overlooked because it was the basis for the development of pharmacologically relevant ligands (vide infra). Indeed, the breakthrough in the search for potent FXR ligands took place in 2000, when the group of Timothy

Chart 4



TTNPB, **13**

Chart 5



Willson at GSK (Research Triangle Park, NC) generated a combinatorial library of 9900 stilbene derivatives whose screening led to the identification of **14** (Chart 5) as a moderately potent FXR partial agonist.[28] A focused three-component library of isoxazoles was then synthesized to explore the structural requirements for FXR activity with the aim of increasing both potency and efficacy (Chart 6). In particular, condensation of four vinyl-substituted acids with five halo-substituted phenols led to the corresponding 20 stilbene carboxylic acids, which were loaded onto Sarsin resin, deprotected, and coupled with a series of 4-hydroxymethylisoxazols. Cleavage of the final products from the resin with TFA gave 600 compounds in discrete quantities. Screening of the library against FXR allowed the identification of GW4064 (**15**) as a highly potent and efficacious FXR agonist with an EC_{50} of 70 nM. Since then, because of its high potency and selectivity against other NRs or because of the lack of nonreceptor-mediated action on the biliary pool, **15** became the standard non-steroid agonist for probing the physiopathological roles of FXR.

A second class of potent FXR agonists was developed by Nicolaou and co-workers.[25,26] The initial 10 000 members of the benozopyran-based library of general formula **I** (Chart 7) was screened against FXR to give a number of screening hits, here represented by general structure **II**, characterized by a benzopyran moiety linked to an aromatic amide via a methylene bridge. A series of solid- and solution-phase libraries prepared by systematically modifying areas A, B, and C in **II** allowed the definition of the SAR profile of this class of compounds, finally leading to a series of highly potent and selective compounds, among which are fexaramine (**16**), fexarine (**17**), and fexarene (**18**) with EC_{50} = 255, 222, and 255 nM, respectively, in a FRET bases assay.

Although less employed as pharmacological tools with respect to **15** or **16**, other retinoid-related compounds were identified as FXR ligands. Thus, Dussault et al. identified **19**−**21** (Chart 8) as novel non-steroid FXR modulators unrelated to both **15** and **16**.[27] In particular, the two stilbene derivatives **19** and **20** were able to potently activate the RXR/FXR heterodimer (91- and 85-fold at 5 μM, respectively).[27]

Unlike another potentiator shown to activate the RXR/FXR, namely, **22**,[28,29] the action of **19** and **20** is directed toward FXR, as confirmed by the ability to recruit coactivators in vitro. Since specific mutations on the AF2 of FXR abolished the activity of **19** and **20** but

Chart 6

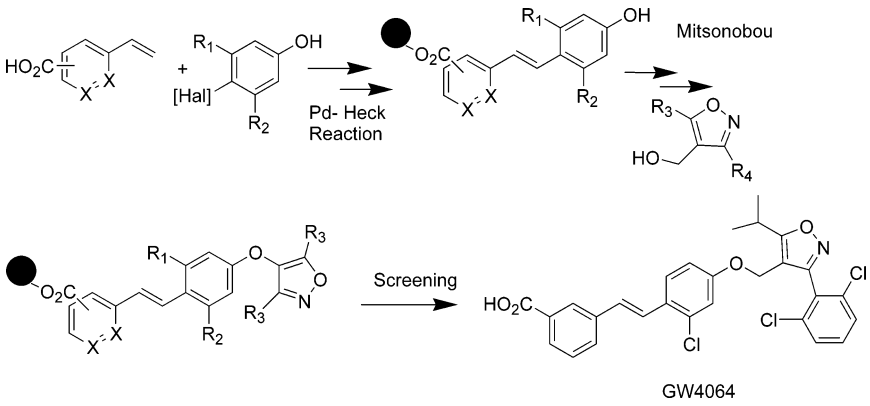


Chart 7

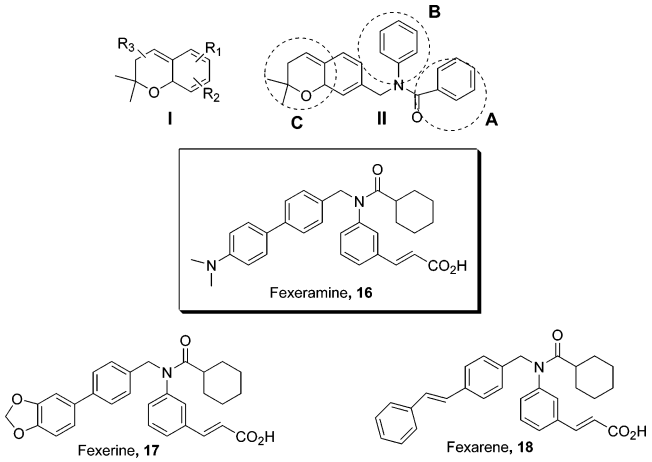
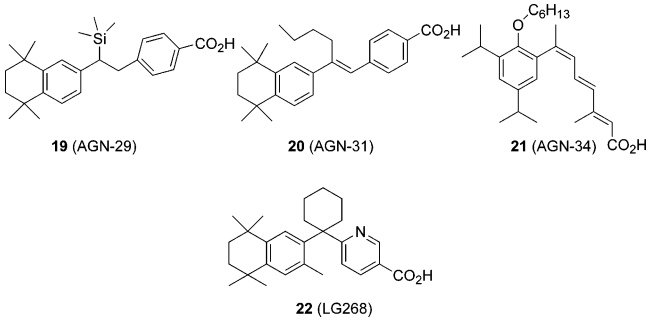


Chart 8



not that of **22** and since mutations on the AF2 of RXR did the opposite, it is established that the molecules targeted different receptors. In the same paper, the structurally diverse molecule **21** displayed an interesting profile of trans antagonist properties of the RXR/FXR heterodimer acting at RXR.[27] Indeed, **21** was able to antagonize the heterodimer with $IC_{50} < 10$ nM. The biphasic behavior of the dose−response curve, however, does not rule out the possibility of a residual activity of **21** at FXR. Interestingly, **21** enhances the expression of CYP7A, is an antagonist on BSEP, and is neutral on SHP. These findings are further confirmation that FXR modulators can be developed to regulate transcription in a gene-specific fashion.

Phenex Pharmaceuticals AG reported a patent with a series of 2,4,6-trisubstituted pyridines (**23**, Chart 9) as FXR modulators.[30]

Chart 9

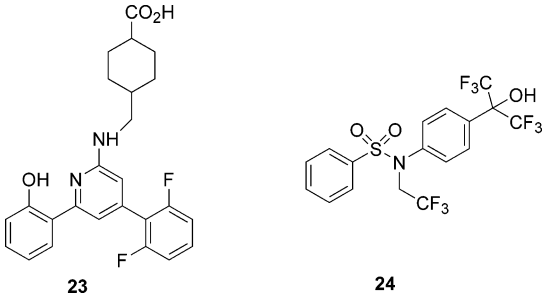
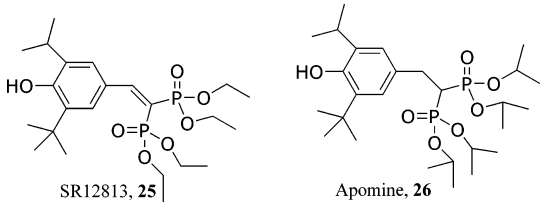


Chart 10



Finally, it is also worth noticing the activity of T0901317 (**24**) as an FXR agonist,[31] recently reported by the group of Mangelsdorf as a potent LXR agonist.[32]

This observation is particularly relevant not only for the understanding of the common molecular basis for FXR/LXR receptor recognition encoded in the structure of **24**[33] but also for the therapeutic potential offered by a dual LXR/FXR agonist. This issue will be addressed more thoroughly below.

It should finally be noted that a class of 1,1,-biphosphonate esters, exemplified by the structure of **25** (Chart 10)) and apomine (**26**), were also proposed to strongly activate RP14/FXR, with a potency comparable with that of **2**.[34] It should be mentioned, however, that these compounds have not been further characterized as FXR ligands, and **26** in particular, seems to act by reducing the levels of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR),[35] the rate-limiting enzyme in the mevalonate pathway. The observed effect on regulation of gene expression should therefore be ascribed to the reduction in the synthesis of endogenous ligands regulating transcriptional activity.

3.2.2. Bile Acid Based FXR Ligands. Compelling evidence is accumulating that indicates that the steroid bile acid scaffold, consisting of a unique cis fusion between rings A and B, has been employed as a privileged structure for NR recognition during evolution. Thus, besides the potentiality for a wide FXR modula-

Chart 11

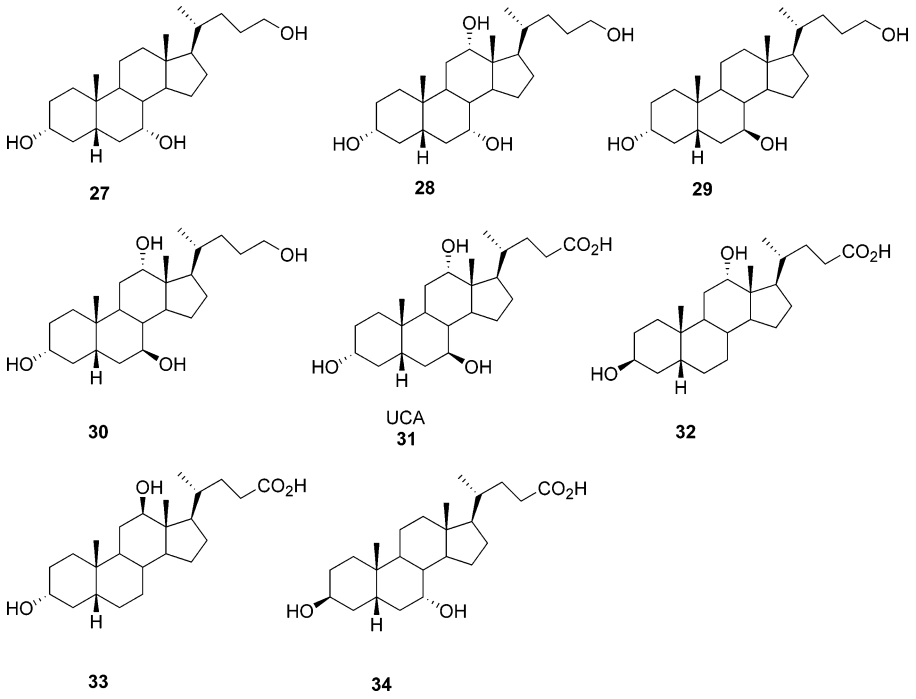
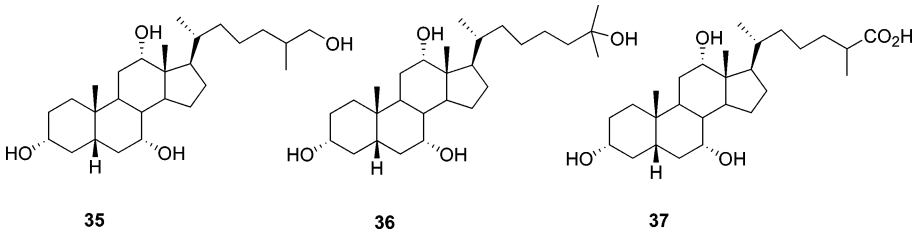


Chart 12



tion endowed with chemical elaboration of the CDCA nucleus that will be described below, modified bile acid structures are found with interesting properties against metabolic NRs. Thus, LCA has been shown to be a PXR ligand[36] and its acetate derivative is a moderately potent vitamin D receptor agonist.[37] In a recent study, Fujino T. et al.[38] investigated the effect of the conversion of the 24-carboxyl group of endogenous BAs to the corresponding alcohol and of the introduction of alkyl groups at the 3 and 7 positions of **2**. When tested on a cell-based luciferase assay, alcohols **27** and **28** (Chart 11), derived from **2** and **5**, respectively, exhibited activities as FXR ligands comparable to that of CDCA **2**. In this regard, it should be mentioned that while **5** has been shown to activate the receptor only if the transporter is expressed,[15] its derivative **28** efficiently activated FXR in the absence of the transporter, thus suggesting that the conversion of the carboxyl group to an alcohol may facilitate its transport into cells by passive diffusion. In contrast to **27** and **28**, their 7β epimers **29** and **30** derived from UDCA (**6**) and ursocholic (UCA, **31**), respectively, were still inactive as the original BAs.

In the same study, the importance of the orientation of the hydroxyl group at the C-3 and C-12 positions was also studied. The 3β and 12β epimers of **3** and **4** (compounds **32** and **33**, respectively) were completely inactive in a cell-based luciferase reporter assay. Similar results were obtained when a nonpolar (hydrophobic) alkyl group was introduced at the 3β and 7β positions of **2**. It is, however, interesting to note that 3β,7α-dihydroxy-3α-methyl-5β-cholan-24-oic acid (**34**) exhibited activity comparable to that of **2**, thus indicating that the 3α-hydroxyl group of **2** is not responsible for FXR activation.

Nishimaki-Mogami et al.[39] have also reported the evaluation, as potential FXR activators, of early intermediates along the bile acid biosynthetic pathway from cholesterol. Thus, in a cell-based reporter assay and coactivator recruitment assays in vitro, intermediates possessing an intact cholesterol side chain were inactive, whereas 26- or 25-hydroxylated bile alcohols (**35**, **36**, Chart 12) and cholestanoic acid **37** were highly efficacious ligands for FXR at a level comparable to that of **2** Since derivatives **35**−**37** are known to be evolutionary precursors of bile acids in mammals, these findings suggest that human FXR may have retained affinity for these precursors during evolution.

3.2.3. Semisynthetic Bile Acid Derivatives as FXR Ligands. Discovery of 6ECDCA (INT-747), a Highly Potent FXR Agonist. The potential embedded with a tuned chemical manipulation of the steroid bile acid skeleton is exemplified by the discovery of 6α-ethyl-chenodeoxycholic acid (6ECDCA INT-747, **38**, Chart 13) as a nanomolar (EC_{50} = 99 nM) potent FXR agonist.[40] Thus, the original identification of CDCA as the plausible endogenous FXR ligand prompted us to re-evaluate a small library of bile acid derivatives previously synthesized for optimization of their physicochemical

Chart 13

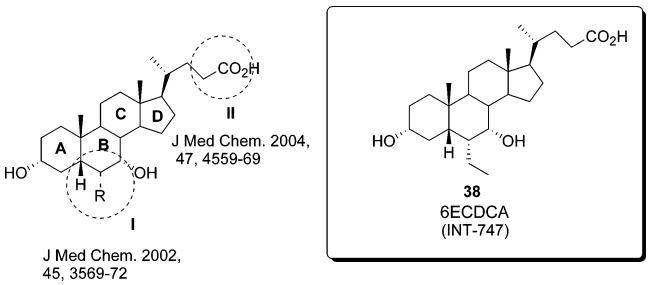
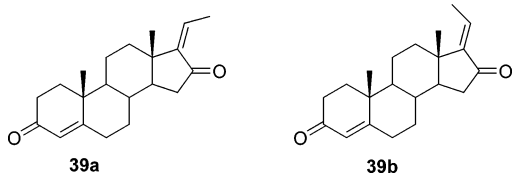


Chart 14



properties/activity relationships. Two different areas, identified as I and II in Chart 13, were investigated, namely, ring B and the acidic side chain. Among the studied derivatives, the 6α-methyl derivative of **2** showed a 10-fold increase in the activity as FXR agonist compared to the parent CDCA derivative, thus suggesting the existence of a potential hydrophobic pocket in the FXR ligand binding domain in correspondence with the 6α position of bile acid. To confirm this hypothesis, a series of 6α-alkyl derivatives, including 6α-ethyl, *n*-propyl, and *n*-butyl derivatives, was synthesized and tested for their ability to recruit the SRC-1 peptide in a FRET assay. Among these, the 6α-ethyl derivative (**38**) was the most potent derivative, with $EC_{50} = 99$ nM and with the lengthening of the alkyl chain producing a decrease in the activity. The SAR of compound **38** will be further described in the X-ray section below. It is also worth mentioning that a variety of other chemical modifications have been introduced in the bile acid skeleton either in ring B or in the side chain, giving rise to an array of derivatives some of which displayed interesting properties as FXR modulators, although none of them had an overall profile superior to that of **38**.[41] It is also pointed out that a full exploitation of the structure−activity relationship encoded in these data will require a still partially missing understanding of the relationships between affinity of the ligand for the LBD, ability to promote coactivator association, and selective gene transactivation.

3.2.4. FXR Antagonists: Guggulsterone. The gum resin of *Commiphora mukul* was known since 600 B.C. in ayurvedic medicine for its antinflammatory, antiseptic, and antirheumatic properties.[42,43] More recently, the use of the extract of the resin, known as guggulipid, has been associated with antihyperlipidemic effects such as reduction of the serum level of the total cholesterol, of the LDL cholesterol, and of triglycerides. This important action of guggulipid has been attributed to its active principle, termed guggulsterone and consisting of a mixture of *Z*- and *E*-4,17(20)-pregnadiene-3,16-dione (**39a** and **39b**, respectively; Chart 14).

Several studies have indicated that many of the observed properties of guggulipid can be ascribed to the interaction of its active principle, guggulsterone, with an array of nuclear receptors (NRs).[44] Thus, both *Z*- and *E*-guggulsterone have been shown to interact with the members of the subfamily of endocrine NRs, such as the α-isoform of the ER, the PR, the AR, the GR, and the MR.[45] Furthermore, guggulsterones have also been found to interact with members of the subfamily of the formerly orphan metabolic NRsm, such as the PXR,[46] and the bile acid receptor FXR.[47−49]

The activity of guggulsterone (**39**) at FXR is particularly intriguing. In fact, both *Z*- and *E*-guggulsterone (**39a**, **39b**) failed to activate FXR in a cell-based transactivation assay but inhibited CDCA activation in a dose-dependent manner. This antagonist property of **39** at FXR has been confirmed in a cell-free coactivator association assay, where guggulsterone was unable to recruit a synthetic peptide corresponding to the GRIP-1 region of SRC-1 coactivator protein and reverted, in a dose-dependent manner, to the recruitment of the peptide by **2**. Despite this clear antagonist-like properties, **39** was shown to be able to enhance (rather than decrease, as would be expected) the **2** or the **15** stimulated transcription of the BSEP.[48] Thus, **39** turned out to be the first example of a selective nuclear receptor modulator (SNuRM) for the FXR receptor. Indeed, with the exception of the semisynthetic 3β-hydroxy-5,16-pregnadien-20-one and some polyunsaturated fatty acids, **39** is the only example of a direct FXR antagonist in a coactivator association assay. From a molecular point of view, the explanation of the antagonist action of **39** at FXR is not intuitive. Nuclear receptor's antagonists are usually larger than agonists, and this led to the canonical mechanism for NR antagonism that relies on the ability of antagonists to displace H12 from its active disposition, thus preventing coactivator association (or promoting corepressor association). Guggulsterone hardly fits this scheme because it is significantly smaller than bile acids and does not embed chemical functionalities that may account for H12 displacement. Thus, the mechanism of antagonism of **39** is unclear and may be related to the SNuRM activity also exhibited by this molecule.

4. FXR: Structure and Function

4.1. Ligand Binding Domain of FXR. The FXR receptor possesses the classical modular architecture (Figure 2) observed for all known ligand dependent NRs.[50] The amino terminal domain encompasses the ligand-independent activation function 1 (AF1) and is the less conserved domain across species and other NRs. The highly conserved Zn-finger domain is responsible for DNA binding and is followed by the distinctive carboxy terminal domain, which contains the ligand binding domain (LBD) and the ligand-dependent activation function 2 (AF2). It is well-known that the LBD of NRs behaves as a molecular switch that responds to the binding of the small lipophilic ligands by inducing conformational changes that results in the dissociation of corepressor peptides and recruitment of coactivators to the sensible DNA element, a process that culminates in the gene transcription.[51] Thus, many features that are essential to the understanding of the ligand-induced NR activation can be analyzed in terms of the ligand ability to recognize and to "activate" the ligand binding domain. The availability of highly potent steroid and non-steroid FXR ligands, coupled with the ability to engineer the soluble LBD of the rat or human FXR from

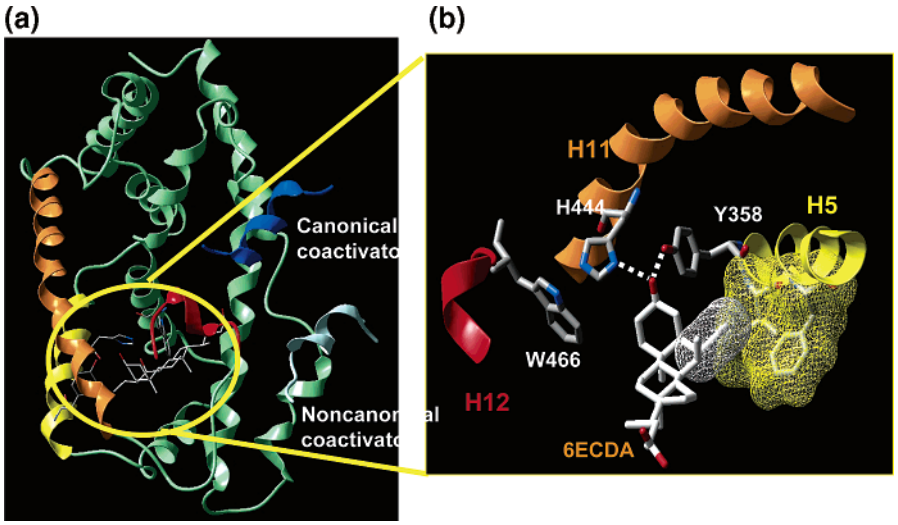


Figure 3. (a) Chain B of crystallized LBD of rFXR (pdb code: 1OSV). Two GRIP-1 peptides are cocrystallized. The blue one is on the "canonical" cleft secured by the charge clamp involving helix-12 (red). The cyan one is the "noncanonical" peptide. The ligand pocket is encompassed by helices 12 (red), 11 (orange), and 5 (yellow). Ring A of 6ECDCA is directed toward helices 11 and 12. (b) Detailed view of the canonical binding pocket. The cis junction between rings A and B of 6ECDCA can be appreciated. The 3-OH group of 6ECDCA interacts with H444 and Y358, giving rise to the trigger activation of W466 in H12. The 6α-ethyl moiety of 6ECDCA perfectly fits in a hydrophobic cavity made up of residues on helix 5. The van der Waals volume of 6α-ethyl is shown as white dots.

E.coli, has allowed the determination of the crystal structure of both human and rat FXR-LBD at high resolution.[52,25] Thus, the LBD of rFXR could be crystallized in the presence of the potent agonist **38** or in the presence of 3-deoxy-CDCA and the structures could be determined at 2.5 and 2.9 Å resolution, respectively.[55] Both structures were solved in the presence of a GRIP peptide containing the LxxLL sequence of coactivator proteins.[67] The LBD of hFRX could be crystallized in the presence of **16** and the structure solved at 1.78 Å resolution.[25] Inspection of the 3D structures revealed a common architecture (Figure 2) entirely similar to that observed for all the other NR LBD so far determined.

Thus, the LBD of both rat and human FXR consists of a bundle of three layers of α-helices that encompass the ligand binding pocket, lined up by helices 3, 5, 10, and 12. This structure is conserved, with minor differences, in PPARα, PPARγ, RORα, RAR, RXR. The three structures so far available for FXR-LBD represent *holo* conformations trapped in the activated states. Thus, the AF2 helix (H12) is secured in the active disposition by agonist binding, is packed against helices 3, 4, and 10, and makes room for the binding of the coactivator peptide. Despite the overall similarity with the structure of the LBD of other metabolic NRs, the LBD of both human and rat FXR showed peculiar features and differences that deserve comments and that can be exploited for a deeper understanding of the molecular basis for FXR activation by a small ligand. Examination of the 3D structure of the LBD of rFXR (Figure 3a), in particular, revealed three noteworthy features. (i) The bile acid ligand (either **38** or 3-deoxy-CDCA) bound the LBD with ring A facing H12, while the carboxy tail approaches the entry pocket at the back. This disposition, predicted by us in a homology modeling study,[53] is different from that adopted by other cholesterol metabolites that bind to their cognate receptors with the oxidized tail toward H12. (ii) The H12 helix is stabilized in the active disposition through a triad of residues W466 (H12), H444 (H11), and Y358 (H10)

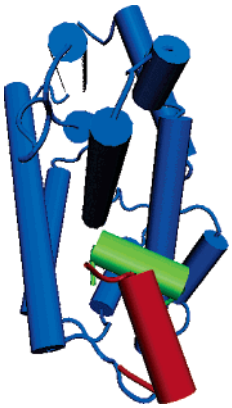


Figure 4. Helix 12 is unstable in the absence of agonist. The crystal structure of LBD of FXR, with H12 in the canonical "active" disposition (green), is compared with the last frame of a 2.6 ns molecular dynamics simulation in the absence of the agonist molecule (red). A significant shift of H12 from the active disposition can be appreciated. The structure is reprinted with permission from *Journal of Medicinal Chemistry*.[54]

(rFXR numbering) (Figure 3b). A desolved π−cation interaction between W466 and H444 is proposed to play an important role in maintaining this conformation. Intriguingly, the bile acid agonists did not directly interact with the triad but rather provided the correct disposition of the partner residues through steric restriction of His mobility. Molecular dynamics experiments (Figure 4) clearly demonstrated, however, that the triad is unable to maintain H12 in the active form unless the bile acid or the coactivator peptide are bound to LBD.[54] (iii) The crystallographic cell of rFXR-LBD complexed with **38** contained two copies of the LBD, named chain A and chain B, respectively. Inspection of the two chains revealed that chain B is a quaternary complex, where the LBD is bound to ECDA and to *two* LxxLL peptides (Figure 3a).

The first peptide occupied the canonical crevice, secured by the highly conserved charge clamp between

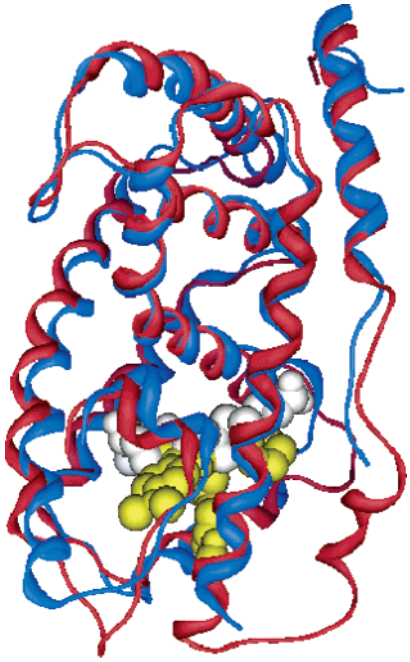


Figure 5. Superimposition of the ligand binding domains of rFXR (red, pdb code 1OSV) and hFXR (blue, pdb code 1OSH) cocrystallized with 6ECDCA (white) and fexeramine (yellow), respectively.

E464 and K300. The second peptide was found in a unprecedented crevice formed along loop H1−H2. Although it cannot be ruled out that the binding of the second peptide is the consequence of a crystallographic artifact, intriguing speculation can also be made. In particular, it has been proposed that the second crevice represents an "on deck" site for the coactivator,[55] and computational studies brought support to this hypothesis.[54] If experimentally confirmed, the presence of an additional site for coactivator peptides, unprecedented in the NR field, may provide the molecular basis for understanding the enhanced coactivator's recruitment by FXR agonists and, importantly, may offer medicinal chemists with another site for modulating the receptor's activity. The X-ray structure of the LBD-rFRX complexed with **38** flanked by molecular modeling studies provided an explanation for the enhanced activity of **38** with respect to the parent **2**. Thus, the gain in potency of **38** compared to the parent **2** can be ascribed to the effect of the directional 6α-ethyl chain that filled almost perfectly a small cavity in the LBD, bridging together helix 3 and helix 8 (Figure 3b). Filling up this cavity not only provides additional affinity but is likely to favor the positioning of the coactivator peptides by compacting the core structure of the LBD.

Very interesting observations also came from the comparison of the 3D structure of the LBD of rat and human FXR complexed with 6ECDCA or fexaramine (Figure 5).

First, the two structures differ slightly but significantly in some domains. These differences might certainly be due to the different resolution of the two crystal structures and to the different experimental conditions, but they can also reflect the ability of diverse chemotypes to induce different conformational states of the LBD, thus providing the molecular basis for the selective gene transcription. The following points are of particular relevance. (i) Although very similar, the structures of the holo-, agonist-bound LBD of hFXR and rFXR are not identical, being the root-mean-square deviation (rmsd) calculated over the whole backbone structure is 1.34 Å. (ii) Although both structures clearly showed helix H12 in the active disposition, packed against helices 3, 4, and 10, the ligand occupancy of the binding pocket is rather diverse. In particular, the nonsteroid ligand **16** is larger than steroidal **38** and occupies a region of the receptor not accessed by **38**. Furthermore, only a few residues that contact the ligand are in common with one another. How this would affect a differential H12 stabilization or coactivator recruitment is not apparent, but it has been proposed that steroid and nonsteroidal agonists would affect FXR's transactivation ability in very different ways. In particular, the activation of diverse gene subsets by **16**, **15**, or **2** can be attributed to their individual association with the LBD of FXR achieving specificity by aggregating with distinct coactivator complexes.[25] (iii) The loop between helices H1 and H2 is disordered in hFXR-LBD while present in rFXR. Since **38** is in contact with that loop whereas **16** is not, this observation may have substantial importance for understanding the molecular basis of FXR activation.

4.2. Conservation of FXR Sequence across Species and FXR Isoforms. The FXR receptor has been identified in a number of species, thus supporting the notion of its implication in fundamental processes related to lipid homoeostasis. So far, FXR receptors have been cloned from humans, hamster, rats, mice, chicken, and fugu.[56] Furthermore, the Drosophila ecdysone receptor,[57] which recognizes the metamorphosis steroid ecdysone as ligand, belongs to the NR1H subfamily of NRs together with FXR. Coherently with known species difference related to cholesterol metabolism, sensitivity to dietary cholesterol, and composition of the biliary pool, one could expect differences in FXR response to chemically different bile acids. Although quite a high sequence similarity does exist in the ligand binding domain of FXR, especially from mammals, there are point mutations that can explain the different sensitivity to ligands. For example, it is known that bile acid signaling pathway differs considerably between humans and mice.[58] Human CYP7A is more sensitive to bile acid feedback inhibition than the murine orthologue, and cholesterol feeding increases CYP7A expression much more in mice than in humans. This species difference has been related to the presence of a LXR response element that is present in the human CYP7A promoter and not in the murine one.[59,60] However, murine FXR-LBD is intrinsically less susceptible to **2** than its human counterpart, and this effect has been associated with two point mutations present in helix 7 and helix 8.[61] Remarkably, the double replacement of Lys366 and Val384 in mFXR with Asn366 and Ile384 reconstructed a fully active "humanized" receptor. Thus, these two residues are critical for **2** (and likely other bile acid derivatives) recognition. It is intriguing to note, however, that both amino acids are not in direct contact with the BA binding pocket, and the reason for their involvement in CDCA recognition is not immediately apparent.[61]

Table 2. FXR Target Genes

gene	reg.	description	function
ABCC2	+	multidrug-resistance-associated protein 2	mediates the efflux of several conjugated compounds across the apical membrane of the hepatocyte into the bile canaliculi
Apo A-1	−	apolipoprotein A-I	partially mediates the antiatherogenic effect of HDL
Apo AV	+	apolipoprotein AV	regulates plasma trygliceride levels
Apo CII	+	apolipoprotein CII	regulates plasma trygliceride levels
Apo CIII	−	apolipoprotein C III	regulates tryglyceride metabilism and plasma levels
ApoE	+	apolipoprotein E	regulates lipid transport and affects atherogenesis
BACS	+	bile acid−CoA synthetase	conjugation of bile acids with glycine and taurine
BSEP		bile salt excretory pump	ATP-dependent transport of BA across the hepatocyte canalicular membrane
CYP7A1	−	cytochrome 7α	rate-limiting enzyme of BA synthesis
CYP8B1	−	cytochrome 8	key enzyme of BA synthesis
FGF-19	+	fibroblast growth factor 19	secreted growth factor negatively affecting expression of CYP7A1
I-BABP	+	intestinal bile acid binding protein	cytosolic protein binding bile acids with high affinity
NTCP	−	Na(+)/taurocholate cotransporting peptide	principal hepatic bile acid transporter
KNG	+	human kininogen	antiadhesion, antiplatelet aggregation, antithrombosis
PLTP	+	phospholipid transfer protein	essential in the transfer of VLDL phospholipids into HDL
SHP	+	small heterodimer partner	promoter-specific repressor of gene transcription
STD	+	dehydroepiandrosterone sulfoltransferase	hydroxysteroid sulfo-conjugating enzyme
SDC1	+	syndecan-1	transmembrane heparan sulfate proteoglycan that participates in the binding and internalization of extracellular ligands
UGT2b4	+	human uridine 5′-diphosphate glucuronosyltransferase 2B4	converts hydrophobic bile acids into more hydrophilic glucuronide derivatives

Genomic analysis has allowed us to identify, across species, the existence of a molecularly distinct FXR isoforms that have been termed FXR2 in fugu,[56] FOR2 in frog,[62] and FXRβ in other species. In particular, four variants of FXR were determined in mouse, termed FXRα1, FXRα2, FXRβ1, and FXRβ2.[63] FXRβ has been identified, in addition to primates, in other mammals such as rabbit, dog, mouse, and rat.[64] While in primates, including humans, FXRβ has been classified as a ubiquitously distributed pseudogene, and it has functional relevance in other mammals. For instance, murine FXRβ heterodimerizes with RXR and stimulates transcription through the binding to specific DNA response elements. Murine FXRβ1 and FXRα1 have a four amino acid insertion in the hinge region compared to FXRα2 and FXRβ2, and this is associated with a weaker affinity for several FXREs.[63]

Interestingly, lanosterol, an intermediate through the cholesterol biosynthetic pathway, has been proposed as a putative endogenous ligand for FXRβ, thus suggesting a direct involvement of FXRβ in the control of cholesterol biosynthesis, at least in nonprimates.[64]

The increasing awareness of the species differences in the regulation of the cholesterol and lipid metabolism through the FXR receptors is likely to prompt soon a revisitation of the pharmacological data gathered so far with nonprimate models. Indeed, both ligand affinity and gene transcription efficacy/selectivity assessed in nonprimate models or nonprimate receptors might not be predictive of the behavior in man. On the other hand, it should also be considered that comparative pharmacology across species can provide valuable information as specific molecular requirements for the selective interaction of individual ligands with the LBDs and thus accelerate the process of drug discovery.

4.3. FXR Tissue Expression. Very high levels of mRNA for FXR were found in the liver and in the gastrointestinal tract,[9] and this is consistent with the role of FXR as a bile acid receptor. However, high levels of mRNA were also found in the kidney and in the adrenal gland, which are organs generally not considered targets for bile acids. Furthermore, low levels of mRNA for FXR were found in a variety of tissues, including heart, ovary, thymus, eye, spleen, and testes.[50,62] More recently, it has been discovered that in man FXR is highly expressed in the vascular smooth muscle of normal and atherosclerotic blood vessels.[65] The discovery of FXR expression and activity in tissues that are not "classical" targets for bile acids raises the question of whether bile acids are the endogenous activator of FXR in all tissues. While this question still waits for answers, it can be anticipated that the wider than expected expression of FXR in man can broaden the potential for its modulation, and many effects previously ascribed to bile acids should be retrospectively analyzed as possibly mediated by FXR.

4.4. FXR-Null Mouse. Sinal et al., generated FXR−/− mice.[66] The FXR-null animals were viable and outwardly identical to wild-type mice. Null mice were characterized by elevated serum bile acid, cholesterol, and triglycerides, increased hepatic cholesterol and triglycerides, and a proatherogenic serum lipoprotein profile. Furthermore, a reduced bile acid pool and reduced fecal excretion were observed, consistent with a reduced expression of the major hepatic canalicular bile acid transport protein.[66] The importance of the critical role of FXR in lipid homeostasis was further demonstrated by the analysis of double-null FXR/PXR mice, which showed severe impairments in bile acid, cholesterol, and lipid homeostasis.[67,74]

4.5. FXR Target Genes. The action of bile acids as signaling molecules is mediated by the FXR receptor whose activation perturbs the expression of a variety of target genes. Most of the target genes so far identified are related to control of the bile acid's own biosynthesis and transport, and many of them, once activated, promote the decrease of bile acid's concentration within the hepatocyte. The systematic search for novel FXR target genes carried out over the past 2 years has, however, allowed the identification of a number of genes, activated by FXR, that regulated the overall lipid homeostasis (Table 2).

Understanding the interconnection between bile acid signaling, mediated by FXR, and the expression (or

Table 3. Interconnection between Nutrients, NRs, and Transcrption Factors

NR	nutrient	primary physiological role	ligand (drug)
PPARα	fatty acids	homeostasis of FA, TG, and lipids	fibrates
PPARγ	fatty acids	glucose and energy metabolism	thiazolidinones
PPARδ	fatty acids	homeostasis of FA, TG, and lipids	
LXR	cholesterol metabolites	reverse cholesterol transport and absoption	24,25-epoxycholesterol 22R-OH-cholesterol
FXR	cholesterol metabolites	bile acid metabolism and transport	CDCA, GW4064, 6ECDCA
VDR	vitamin D	mineral metabolism and bone growth	1,25-dihydroxycholecalciferol
RXR RAR	vitamin A	cellular development and body growth, vision	retinoic acid
PXR	vitamin E	antioxidant	α-tocopherol
SREBP	carbohydrates	induction of lipogenic genes	
ChREBP		after carbohydrate intake	
USF			

repression) or genes involved in lipid control and homeostasis will certainly extend the scope of novel FXR modulators. Thus, it has been shown, in particular, that FXR controls bile acid synthesis by a feedback repression of CYP7A1[68] and CYP8B1 genes,[69] encoding for cholesterol 7α-hydroxylase and sterol 12α-hydroxylase, enzymes central to the synthesis of bile acids from cholesterol. Interestingly, when regulation of CYP7A1 by **2** was studied in rats, deletion analysis identified an FXR response element between nucleotides 148 and 128, but RXRα/FXR did not bind to this sequence. These results first suggested that bile acid activated FXR exerts its inhibitory effect on CYP7A1 transcription by an indirect mechanism.[70] The molecular effector of this indirect mechanism was found to be an atypical orphan receptor that lacks the DNA binding domain, termed small heterodimer partner (SHP).[71,72] SHP represses expression of CYP7A1 by inhibiting the activity of liver receptor homologue 1 (LRH-1), an orphan nuclear receptor that is known to regulate CYP7A1 expression positively.[71] The indirect mechanism of feedback regulation of CYP7A1 by FXR prompted us to study whether the same mechanism could be effective in the case of CYP8B1. Surprisingly, feeding of rats with CDCA led to a decrease of mRNA expression of CYP8B1 but not to a decrease of SHP mRNA. Transient transfection assay of promoter/reporter genes coupled with mutational analysis identified an FXR response element that has an HNF4α binding site embedded in two overlapping α-fetoprotein transcription factor (FTF) binding sites. Mutation of the HNF4α binding site markedly reduced basal promoter activity and its repression by bile acids. Thus, FTF and HNF4α not only play critical roles in CYP8B1 gene transcription but also mediate bile acid feedback inhibition.[69]

Bile acid disposal is further controlled by bile acids themselves through FXR by regulating a variety of other genes. In particular, the bile acid is transported by regulating the expression of the critical hepatic bile Na^+/taurocholate cotransporting polypeptide[73] and the BSEP.[74] FXR also controls the expression of the IBABP,[75] the apolipoproteins A-I,[76] C-II,[77] and CIII[78] and was also reported to activate the expression of human kininogen gene, the products of which have crucial roles in vasodilatation and anticoagulation.[79] Finally, Table 3 provides a list of other genes identified to be transactivated (or transrepressed) by FXR,[80−88] which are likely to have relevance in the control of lipid and glucose homeostasis and which will be discussed below.

4.6. FRX Assays. Identification of novel, chemically diverse FXR modulators requires setting up efficient screening procedures. As for classical endocrine NRs, a variety of assays can be designed by exploiting properties of either the DNA binding domain or the ligand binding domain. In particular, for the identification of novel ligands, agonists, antagonists, or partial agonists, both biochemical (or "cell-free") assays and cell-based assays can be set up around the properties of the LBD. These assays are based on the general concept of receptor/coregulator interactions and ligand−receptor interactions.[89]

The ligand inducible recruitment of an LxxLL peptide by LBD is at the basis of the well-established time-resolved fluorescence resonance energy transfer (TR-FRET) assay.[90] This technique exploits the ability of two fluorophores (the donor and the acceptor) with overlapping emission/absorption spectra to transfer their excited-state energy when they are spaced within ∼50 Å of each other with their transition dipoles appropriately oriented. In the standard setup, the presence of an FXR ligand promotes the recruitment of biotinylated peptide containing an SRC-1 LxxLL receptor interaction motif to the glutathione-*S*-transferase (GST) linked to the FXR-LBD, bringing into proximity an anti-GST antibody conjugated to a europium chelate (Eu). Upon excitation of the reaction at 340 nm, the europium chelate emits at 615 nm, thus exciting the Sa-APC with a resulting emission at 665 nm. The reading at 665 nm gives an indication of the strength of the protein−protein interaction. This assay is especially designed to identify agonists, i.e., molecules able to promote the recruitment of the coactivator peptide. Since no known ligand is required to carry out the experiment, this technique has been and is the technique of choice to identify ligands for orphan receptors.

The fluorescence polarization (FP) is another cell-free technique that measures the ligand-induced direct interaction between a nuclear receptor and a synthetic peptide.[91] A free LxxLL-containing peptide labeled with a fluorophore emits depolarized light as a consequence of its high speed of rotation. When the ligand is added, the recruitment of the peptide to the LBD is promoted, and the resulting ternary complex, much larger, produces polarized light. The difference between nonpolarized and polarized light is used as a measure of ligand binding. As for the FRET experiment, FP is suitable for identifying agonists and can be used for screening of orphan receptors.

Among the cell-based assay, the transactivation reporter assay is highly employed. In a typical setup, a reporter gene can be responsive to either a full-length NR (in this case the reporter gene contains the hormone response element for that NR) or the yeast transcription factor GAL4. The LBD of the studied receptor is fused

to the DNA binding domain of Gal4, which binds the upstream activation sequences. Interaction of the ligand with the LBD induces reporter gene activation.[92]

The two-hybrid assay is based on the discovery that the yeast Gal4 transcription factor is endowed with a modular DNA binding domain and modular transcriptional activation domains that interact with the transcription machinery, thus increasing the transcriptional activity. The most used assay is the mammalian two-hybrid assay evolved from the original yeast two-hybrid assay.[93] In a mammalian two-hybrid assay, an LxxLL-containing peptide is fused to the DNA binding domain of Gal4 and the LBD of the NR is expressed as VP16 activation domain fusion protein. The interaction between the ligand and the LBD is then detected by using a reporter gene, usually luciferase.

4.7. Gene-Selective FXR Modulators. The plethora of genes activated by FXR makes problematic the direct association between a molecular event eventually detected by an in vitro assay, such as coactivator recruitment, and the resulting transcriptional effect in vivo, which is linked to the ability of predicting a given therapeutic opportunity for FXR modulators. This is a well recognized, but still poorly understood, problem that is common to all the nuclear receptors, where classical pharmacological concepts such as antagonism, agonism, partial agonism need to be deeply revisited and reinterpreted. In particular, a given chemotype can facilitate transcription of an individual gene while repressing another one or being neutral on a third part. That chemotype will be considered as an agonist or an antagonist depending on the final transcriptional activity regardless of its action in the first stages of interaction with LBD and coactivators. This concept is commonly adopted in the case of the estrogen receptor modulators, which may have estrogenic or antiestrogenic properties depending on the tissue.[94] These modulators are referred to as selective estrogen receptor modulators (SERMs).

Although SERMs have been exploited in therapy for 20 years, the molecular basis for their mechanism of action is only recently appreciated.[95,96] The same concept of selective modulation can be applied to FXR ligands. In a simplified view, for example, an FXR antagonist should be endowed with cholesterol-lowering activity, since it should repress the feedback inhibition of the bile acid synthesis, thus depleting the cholesterol pool, and this is related to the effect of FXR on CYP7A1 activity. Actually, antagonism of FXR is likely to do much more, like elevation of triglycerides levels and lowering of bile acid transport because of the effect on apolipoproteins or bile acid transport systems. Thus, there is a quest for the discovery of a selective FXR modulator (referred to as selective bile acid receptor modulator (SBAR))[48] able to activate/repress individual gene without affecting other gene subsets. As has been described above, selective FXR modulators are already available, such as AG34[27] or guggulsterone itself, but the molecular mechanism of the selective modulation is still poorly understood. Thus, the search for an SBAR will benefit from understanding the molecular basis of the crucial steps leading to activation/repression of the transcriptional machinery.

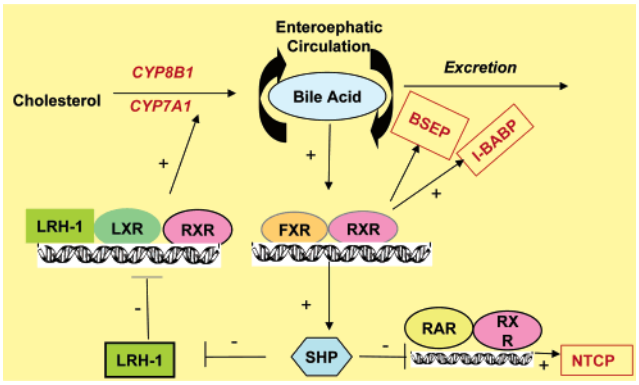


Figure 6. FXR activation controls bile acid homeostasis through a coordinated action involving transactivation of the orphan nuclear receptor SHP, repression of LRH-1, and regulation of bile acid transporters BSEP, I-BABP, and NTCP.

5. Potential Therapeutic Opportunities Associated with FXR Modulation

5.1. FXR Agonists and Liver Diseases. FXR is highly expressed in the liver and intestine, target organs for bile acids, and FRX activation transactivates (or transrepresses) a variety of genes involved in the regulation of bile acids homeostasis. (Figure 6). It was therefore expected that the first therapeutic indications for FXR modulators would be associated with liver diseases. The availability of potent agonists, **38** and **15** in particular, has facilitated the understanding of the role of FXR in liver diseases including cholestasis and liver fibrosis, which can now be considered validated targets for FXR modulators.

5.1.1. FXR and Liver Fibrosis. Hepatic fibrosis is a scarring process of the liver that includes components of both increased and altered deposition of extracellular matrix (ECM) and wound contraction.[97] In chronic liver disease (chronic hepatitis and liver cirrhosis) of viral and nonviral etiology, hepatic stellate cells (HSCs), the major source of ECM in the liver, undergo a progressive process of transdifferentiation from a resting, fat-storing, phenotype toward a myofibroblast-like phenotype characterized by increased expression of fibroblastic cell markers such as α-smooth muscle actin (α-SMA).[97] Through increased secretion of ECM proteins and the tissue inhibitor of metalloproteinases-1 and -2 (TIMP-1 and -2), activated HSCs are responsible for deposition and accumulation of the majority of the excess ECM in the fibrotic liver.[97]

We have recently shown that FXR is expressed by HSCs and that FXR activation provides a counter-regulatory mechanism that limits the activation of myofibroblast-like cells. In vitro exposure of HSCs to natural and synthetic FXR ligands such as **38**, **15**, and **2** reduces expression of α-SMA and α1 collagen mRNA (two markers of HSCs activation). These in vitro effects are translated in in vivo models where activation of FXR with 3−5 mg/kg **38** reduces α-SMA, α1-collagen, and TGFβ_1 gene expression in the liver.[98] In addition **38** increased the liver expression of SHP, BSEP, MDR-2, and MRP-2 (Table 3) while it decreased NTCP, CYP7A1, and CYP8B1 mRNA, indicating that 6-ECDCA is a full FXR agonist in vivo. Liver histology in these models confirms the potent antifibrotic activity of the FXR ligand (Figure 7). Supporting the concept that FXR

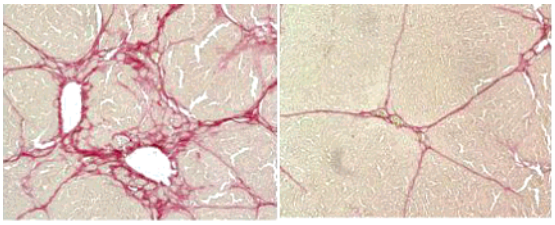


Figure 7. 6ECDCA (right) causes a reduction of liver collagen accumulation after porcine serum administration in HSC. Left: microphotograph of liver obtained from a rat treated with porcine serum to induce liver fibrosis. Shown is the Sirius red staining of liver collagen. Note the continuous mesh of collagen bridging the portal−portal and the portal−central spaces. Right: microphotograph of liver obtained from a rat treated with 6-ECDCA in combination with porcine serum. Note the marked reduction of the collagen mesh.

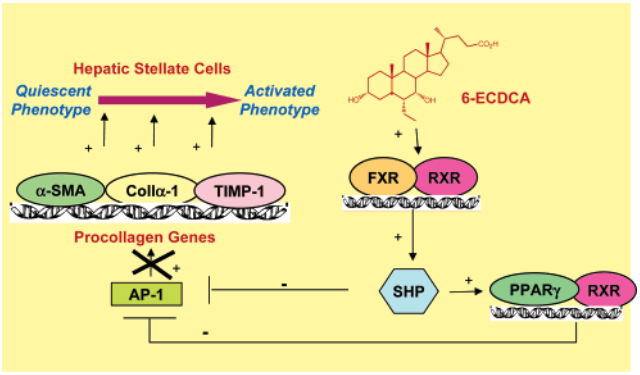


Figure 8. The suggested mechanism for the FXR-induced reduction in collagen deposition involves a FXR−SHP−PPARγ regulatory cascade.

ligand limits HSCs activation, we found that genetic ablation of FXR in mice results in accelerated fibrosis in response to profibrogenetic agents (unpublished). The antifibrotic activity of the FXR ligand appears to be SHP-mediated and is lost in cells that are rendered SHP deficient by small interference RNA.[98]

In addition to this mechanism, FXR might exert its antifibrotic activity by modulating the expression/activity of other nuclear receptors. In this context it is particularly intriguing that SHP (a known FXR target) modulates the activity of PPAR-γ. There is evidence that forced expression of PPAR-γ reduces HSCs activation in vitro,[99] and PPAR-γ ligands exert antifibrotic activities when administered in vivo to rats rendered cirrhotic by bile duct ligation or carbon tetrachloride administration.[100] We have recently shown that FXR increases PPAR-γ expression/activity in an SHP-dependent fashion, raising the possibility that a FXR−SHP−PPAR-γ regulatory cascade mediates some of the counter-regulatory effects FXR ligands exert on collagen deposition (Figure 8). Liver fibrosis is a common feature in chronic liver disease (chronic hepatitis, nonalcoholic steatohepatitis (NASH) and liver cirrhosis). These diseases represent a major cause of death worldwide, and liver fibrosis represents a recognized therapeutic target. Prevention of liver fibrosis or its reversal will contribute to reduce or prevent portal hypertension, a major cause of death in patients with chronic liver diseases. Several drugs are currently being evaluated for their antifibrotic potential. The demonstration of a potent antifibrotic activity in preclinical studies adds FXR ligands to this growing list. The development of selective FXR modulators (FXRMs) that enhances SHP/PPAR-γ without interfering with bile acid transporters might be useful for the treatment of liver fibrosis in addition to anti-inflammatory and metabolic effects of these ligands.

5.1.2. FXR and Cholestasis. Cholestasis is a common feature in chronic liver diseases. It is clinically characterized by elevated plasma concentrations of biliary constituents resulting in jaundice and liver damage. Irrespective of the primary cause of cholestasis, an important consequence for the hepatocyte is the altered expression and function of transport proteins located at basolateral and canalicular domains of the hepatocyte. Adaptation of the hepatocyte during cholestasis aims at limiting the accumulation of potentially toxic biliary constituents within these cells.[101] These changes include (1) the down-regulation of expression or function of the bile acid basolateral transporters OATP1 and NTCP, which are transcriptionally down-regulated in adult and pediatric patients with extrahepatic cholestasis[101,102] and (2) the up-regulation of basolateral and apical transporters including MRP-1 and MRP-3, while MRP-2, MDR-2, and BSEP are unchanged.[101,102] Potent FXR ligands have been proposed to be beneficial in the treatment of cholestatic disorders.[103,104] The potential for anticholestatic effects of FXR ligands has been tested in rodent models of cholestasis. Bile duct ligation (BDL) represents a severe and complete disruption of the enterohepatic circulation that mimics the obstructive cholestasis observed in patients with complete bile duct obstruction. BDL leads to damage of hepatocyte integrity, altered bile duct morphology,[102−104] and increased liver expression of proinflammatory cytokines.[101,102] Administering rats with **15** lowered biochemical markers of hepatocyte damage but did not decrease serum bile acids or bilirubin levels in this model. Liver histology of GW4064-treated rats showed decreased bile duct proliferation and fewer signs of hepatocellular damage such as mitosis, fatty degeneration, and necrosis. **15** had no effect on basolateral bile transporters but induced MRP-2 and MDR-2, whereas MRP-3 increased only after BDL. In summary, biochemical and histological criteria indicate that FXR activation may protect the hepatocyte during bile accumulation induced by extrahepatic obstruction. In addition to the BDL model, GW4064(15) reverts cholestasis in other rodent models of cholestasis, including cholestasis induced by α-naphthyl isothiocyanate (ANIT) and estrogen administration. These animal models present features of intrahepatic cholestasis and mimic cholestasis not due to mechanical obstruction. In these models **15** and **38** reversed ductular proliferation and necrosis and induced basolateral and apical transporters including BSEP, MDR-2, and MRP-2. MRP-3 was not up-regulated, while NTCP and OATP-1 expression was slightly raised compared with ANIT/vehicle-treated rats. In addition, in the model of cholestasis induced by LCA where a disseminated necrosis of liver cells is observed, **38** fully reversed the impairment of bile flow in and transiently protected against liver necrosis. Administration of **38** in estrogen-induced cholestasis resulted in increased liver expression of SHP, BSEP, MRP2, and MDR2 while reducing CYP7A1, CYP8B1, and NTCP mRNA.[104] Taken to-

gether, FXR ligand treatment in animal models of intrahepatic cholestasis induces the expression of genes encoding proteins involved in the secretion of biliary constituents into the canalicular system and decreasing bile acid uptake and de novo bile acid synthesis. The deleterious accumulation of bile acids within the hepatocytes may thus be reduced or prevented. This is reflected by the suppression of activity of biochemical markers and histological signs of hepatocellular damage. Because the net effect of FXR activation is to protect hepatocytes from a bile overload, it is tempting to speculate that FXR agonists will be of potential use as new therapeutic agents to treat cholestasis diseases (i.e., primary biliary cirrhosis and sclerosing cholangitis). While both diseases cause significant morbidity and mortality and represent a leading cause of liver transplant worldwide, no effective therapies are available. However, because FXR also controls the UDP-glucuronosyltransferase-2B4 (UGT2B4), sehydroepiandrosterone sulfotransferase (SULT2A1), and bile acid amino conjugation detoxification pathways, which also participate in the metabolism of other compounds, it should be considered that FXR agonists could also influence drug metabolism by acting on these pathways. Moreover, since FXR is also involved in lipid metabolism, an ideal FXRM should be able to selectively activate the protective network (i.e., proteins involved in transport and detoxification of bile acids and repression of their endogenous synthesis) without affecting lipid metabolism.

5.2. FXR and Metabolic Diseases. The advent of the reverse endocrinology and the deorphanization of the "metabolic nuclear receptors" has allowed their identification as key players in lipid and energy metabolism and homeostasis. Thus, LXR and LRH-1 have been involved in the reverse cholesterol transport and absorption and in bile acid metabolism. FXR has been shown to regulate bile acid biosynthesis, cholesterol disposal, and triglyceride homeostasis. PPARγ has been implicated in the control of glucose and energy metabolism, and the three PPAR isoforms (α, γ, δ) are involved in the control of the homeostasis of fatty acids, lipids, and triglycerides. Some of these receptors are already targets for clinically used drugs. Others, like LXR or PPARδ, are still being validated as potential drug targets. There is also an increasing awareness that cross-talk does exist between nuclear receptors that regulate lipid metabolism (cholesterol and triglycerides), bile acid metabolism, glucose utilization, and energy balance. Indeed, phenotype-based epidemiologic studies have shown a positive correlation between elevated levels of triglycerides and the incidence of gallbladder stones, a gallbladder-related pathology that is highly prevalent in metabolic syndrome and type 2 diabetic patients, and treatment of gallstone patients with bile acids is associated with reduction of plasma tryglycerides.[105] This phenotype-based evidence has led to the notion that in genotype-sensible backgrounds, the interaction of nutrients and metabolic intermediates with nuclear receptors may contribute to the development of the typical features of the metabolic syndrome (obesity, insulin resistance, and vascular inflammation), a highly prevalent disease in Western countries. Central in this scenario is the role of FXR.

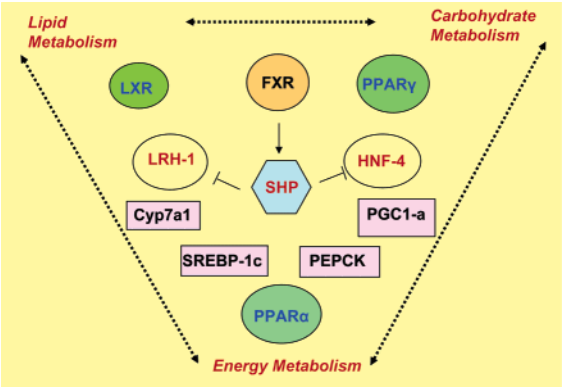


Figure 9. FXR plays a central role in regulating the homeostat of lipid, carbohydrate, and energy metabolism. This control is achieved at a molecular level through a coordinated cross-talk between FXR, other NRs such as LXR and PPARs, orphan NRs, and specific target genes.

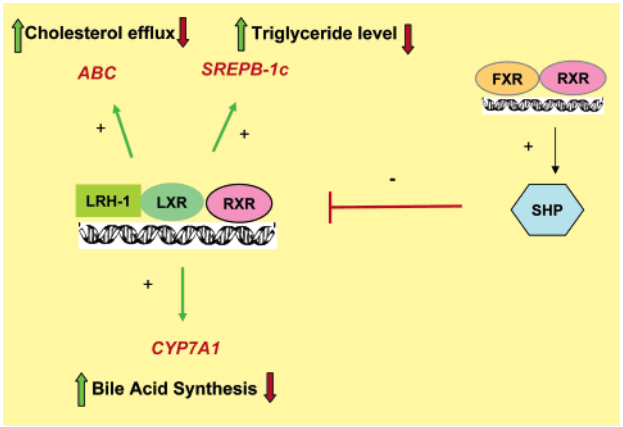


Figure 10. Cross-talk between LXR and FXR in regulating the lipid metabolism and the serum levels of TG and cholesterol. Green arrows indicate the action of LXR in the absence of FXR stimulation, whereas red arrows are the results of the cross-talk mediated by SHP.

5.2.1. FXR and Lipid Metabolism. The molecular cross-talk between FXR and LXR has been recognized early (Figures 9 and 10). In particular, LXR feedforward-regulates the acid synthesis. Once activated by oxysterols, the permissive LXR/RXR heterodimer binds and activates an LXRE on CYP7A1, thus promoting the synthesis of bile acids. This process is facilitated by interaction with the orphan NR LRH-1. The conversion of cholesterol into bile acids then leads to activation of FXR and formation of the FXR/RXR heterodimer, which induces the expression of the orphan NR SHP, which in turn blocks LRH-1 and limits the action of LXR/RXR.[106] In addition to this mechanism, it has also been suggested that FXR activation leads to an SHP-dependent down-regulation of SREBP-1c (sterol response element binding protein-1c) in the liver. SREPB-1c is a master gene regulator that increases liver expression/activity of a number of genes involved in fatty acids and trygleride synthesis, including acetyl-CoA carboxylase (ACC), acetyl-CoA synthetase (AceCS), and fatty acid synthase (FAS). Inhibition of SREPB-1c lowers circulating levels of triglycerides and VLDL and might be responsible for the lowering triglyceride effect observed in animals administered natural and synthetic FXR ligands. In contrast, SREBP-1c is up-regulated by LXR ligands, which accounts for the rise in triglyceride level

observed in response to LXR activation.[107] The observation that LXR is up-regulated by insulin secretion provides another link between triglyceride metabolism, FXR, and glucose utilization. Reduction of VLDL might also be of potential interest in the prevention of atherosclerosis.

Because of its involvement in the control of cholesterol and lipid homeostasis as well as macrophage inflammatory gene expression, LXR has been proposed as a potential target for the treatment of hypercholesterolemia and other diseases associated with dislipidemic states, such as atherosclerosis. The introduction of LXR-activating agents in the treatment of these diseases, however, has been hampered by the negative and concomitant rise in triglyceride levels induced by LXR ligands.[108] FXR may offer alternative approaches to the treatment of these diseases. First, a dual LXR/FRX agonist such as T0901317 (**24**)[31] may be endowed with compensatory activity, favoring reverse cholesterol transport from liver to bile without altering triglyceride levels. However, it is also possible FXR modulators might also exert a direct antiatherosclerotic activity. Thus, both the human and mouse Apo A-I genes were found to be negatively regulated by the FXR, which binds as a monomer to a negative response element in the Apo A-I promoter.[76] Consistent with these findings, HDL cholesterol levels are higher in FXR-deficient mice than in wild-type mice.[66] In addition, FXR ligands also induce the expression of the phospholipid transfer protein (PLTP),[85] an enzyme involved in HDL remodeling. On the other hand, high triglyceride levels constitute an independent risk factor for atherosclerosis. Because Apo C-III is of major importance in triglyceride metabolism and constitutes an independent predictor of coronary events and because FXR activation decreases triglyceride levels and Apo C-III gene expression,[78] it could be of interest to test FXR agonists for treatment of hypertriglyceridaemic states. Thus, a selective FXR modulator able to antagonize the effect on Apo A-I and to positively modulate Apo C-III should be endowed with an optimal profile for the treatment of atherosclerosis.

5.2.2. FXR and Carbohydrate Metabolism. The phenotype-based evidence that patients with type 2 diabetes or insulin resistance have an increased prevalence of hypertriglyceridemia provides the clue that FXR expression may be altered by disruption of glucose homeostasis.[109] In primary rat hepatocytes, D-glucose increased FXR mRNA, whereas insulin counteracted this effect. Furthermore, expression of the FXR target genes, SHP and apolipoprotein C-III, was additively regulated by D-glucose and FXR ligands, thus demonstrating that FXR is decreased in animal models of diabetes.[110]

In addition to its role in bile acid metabolism and transport, as well as in lipid metabolism, FXR has been assigned a potentially relevant role in carbohydrate metabolism. Thus, FXR agonists induce the expression of phosphoenolpyruvate carboxykinase (PEPCK) mRNA and augment the glucose output in a way comparable with glucocorticoid agonists.[111] The molecular link between FXR and carbohydrate metabolism involves the induction of PPARα mRNA expression. Also of great relevance is the notion that FXR regulates the expression of PDK4 (pyruvate dehydrogenase kinase-4), which inactivates the pyruvate dehydrogenase complex (PDC), thus influencing the utilization of fat versus carbohydrate as a source of energy. Expression of PDK4 mRNA, known to be facilitated by glucocorticoid and PPARα agonists, is also induced by FXR ligands in rat hepatoma cells and in human primary hepatocytes. Concomitant to the rise in PDK4 expression, a marked reduction of serum triglycerides is observed in mice treated with FXR agonists, suggesting an additional pathway for the FXR-mediated reduction in triglyceride levels by increased fatty acid oxidation via a pathway that involves induction in the expression of PPARα and PDK4.[112]

The regulation of carbohydrate metabolism by FXR activation and the regulation of FXR expression by altering glucose homeostasis provide clear evidence of a tight cross-talk between FXR and PPARs, considered key players in the homeostatic control of lipids and cholesterol as well as in the energy and glucose metabolism. Indeed, FXR induces PPARα expression in a species-specific manner (i.e., in humans but not in mice) via death receptor-5 (DR-5) FXRE.[113]

PPARγ coactivator 1α (PGC1α), a nuclear receptor cofactor and a key player in the control of adaptive thermogenesis as well as glucose metabolism, induces the expression of the FXRβ isoform.[114] Moreover, PGC1α was identified as a potential FXR cofactor mediating the increase of triglyceride clearance and the decrease of triglyceride synthesis in response to FXR ligands.

Taken together, these data suggest an important role for FXR in both lipid and carbohydrate metabolism and homeostasis through a coordinated cross-talk with other NRs and transcription factors. However, the precise role and the possible druggability of each individual player still need a much deeper understanding. For example, the role of FXR in glucose metabolism can be profoundly different under fasting or feeding conditions, where the amount of bile acid circulating is different as well as the insulin stimulation.[115] Understanding these links will be eventually helped by a closer integration between reverse endocrinology and "nutriomics", the availability of potent and selective small molecules as NR modulators being crucial for the dissection of specific roles played by individual targets.

5.2.3. FXR and Cardiovascular Diseases. Tightly involved in lipid metabolism and homestasis, FXR has been implicated in the pathogenesis and progression of cardiovascular diseases for which altered lipid levels are very well assessed risk factors. The recent discovery that FXR is expressed in the vasculature supports the idea that FXR may be a direct target for cardiovascular diseases such as atherosclerosis, characterized by narrowing of the large blood vessels.[116] In particular, when stimulated by agonists in smooth muscle cells, FXR induces the expression of SHP and of PLTP, a secreted protein responsible for the regulation of the reverse cholesterol transport and the size and composition of the vascular protective HDL.[65] It is finally mentioned that while bile acids are circulating in the blood vessels, albeit bound to albumin, their role as "endogenous" FXR activator in the vasculature and in the vascular smooth muscle cells remains to be determined.

6. Conclusions and Outlook

From the selected examples mentioned in this review, it appears that FXR plays a key role in the transcriptional regulation of genes involved in bile acid metabolism and lipid/cholesterol and glucose homeostasis. The regulation of these interactions is highly complex and contains multiple feedback loops to self-regulate the transcriptional circuits. The overlapping range of agonistic and antagonistic ligands, as well as of target genes shared by FXR with other metabolic nuclear receptors including PPARs and LXR, may serve as a redundant safety mechanism to elicit a protective response so that even when one pathway is compromised, a salvage pathway takes over. Crucial to the complexity of putative convergent and divergent functions of the metabolic nuclear receptors are their transcriptional coactivators and corepressors, our detailed knowledge of which is still limited. Thus, future directions of research in the FXR field will be the definition of cofactors involved in repression and activation of this NR as well as the identification of their network of interactions with other metabolic NRs (PPARs and LRH1) and xenobiotic sensors (PXR and CAR, among others). In addition to the foreseeable application of FXR ligands in treating inflammatory, cholestatic, and fibrotic liver disorders, FXR ligands might have application in the treatment of metabolic disorders including hypertriglyceridemic and hypercholesterolemic states and, by extension, atherosclerosis and its complications. In conclusion, FXR is emerging as a particularly intriguing therapeutic target, not only for the promising application associated with its modulation but also for its peculiar mechanism of ligand recognition and gene activation.

There is therefore a large room for medicinal chemists in the field, with a quest for both agonists and antagonists optimized for their affinity, selectivity of receptor recognition, and selectivity of target gene modulation, and it is expected that many of the future developments in the FXR field will reside on the availability of such tools.

Acknowledgment. The authors are grateful to Dr. Mark Pruzanski (Intercept Pharmaceuticals) for financial support and helpful discussions.

Biographies

Roberto Pellicciari is Professor of Medicinal Chemistry in the Department of Chemistry and Technology of Drugs of the University of Perugia (Italy), Adjunct Professor at MPRC, University of Maryland, and Coordinator of the International Ph.D. Degree Program in Chemistry and Technology of Drugs of the University of Perugia. He is past President of the Division of Medicinal Chemistry of the Società Chimica Italiana and President-Elect of the European Federation of Medicinal Chemistry (EFMC). In 1999 he was awarded with the "Domenico Marotta" prize of the "Accademia Nazionale delle Scienze" and, in 2001, with the Mentzer prize of the French "Société de Chimie Thérapeutique". Author of more than 230 papers and patents, Prof. Pellicciari's research interests have shifted through years from the development of new synthetic methodologies and natural products chemistry to the design and synthesis of CNS acting molecules. Recent years have seen a deep interest in the design and synthesis of modulators for metabolic nuclear receptors and FXR receptor in particular.

Gabriele Costantino is Associate Professor of Medicinal Chemistry in the Department of Chemistry and Technology of Drugs of the University of Perugia (Italy). Prof. Costantino is author of more than 70 scientific papers, and his scientific interests cover the development and application of computational chemistry and molecular modeling techniques to the understanding of ligand−biotarget interactions. Prof. Costantino was awarded with the "Best Young Researcher" award from Società Chimica Italiana and Famindustria (2003) and has been the recipient of XVI "Merz Award". Prof. Costantino is a member of the Scientific Committee of the European School of Medicinal Chemistry (ESMEC) of the Società Chimica Italiana.

Stefano Fiorucci is Associate Professor of Gastroenterology in the Department of Internal Medicine of University of Perugia (Italy). He has authored more than 140 scientific papers in leading journals. He has also edited seven scientific books and is a member of several scientific associations, including the American Gastroenterological Association. His scientific interests cover liver, gastrointestinal, and cardiovascular diseases ranging from pathogenesis to diagnosis and treatment, and his strong interests are now focused on translation of molecular biology into medicine. In recent years he has been actively involved in the FXR field and development of FXR ligands.

References

(1) Kliewer, S. A.; Lehmann, J. M.; Willson, T. M. Orphan nuclear receptors: shifting endocrinology into reverse. *Science* **1999**, *284*, 757−760.

(2) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J. L.; Sundseth, S. S.; Winegar, D. A.; Blanchard, D. E.; Spencer, T. A.; Willson, T. M. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **1997**, *272*, 3137−3144.

(3) (a) Green, S.; Wahli, W. Peroxisome proliferator-activated receptors: finding the orphan a home. *Mol. Cell. Endocrinol*. **1994**, *100*, 149−153. (b) Kliewer, S. A.; Sundseth, S. S.; Jones, S. A.; Brown, P. J.; Wisely, G. B.; Koble, C. S.; Devchand, P.; Wahli, W.; Willson, T. M.; Lenhard, J. M.; Lehmann, J. M. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors *a* and y. *Proc. Nat. Acad. Sci. U.S.A.* **1997**, *94*, 4318−4323.

(4) Kliewer, S. A.; Moore, J. T.; Wade, L.; Staudinger, J. L.; Watson, M. A.; Jones, S. A.; McKee, D. D.; Oliver, B. B.; Willson, T. M.; Zetterstrom, R. H.; Perlmann, T.; Lehmann, J. M. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell*. **1998**, *92*, 73−82.

(5) Forman, B. M.; Tzameli, I.; Choi, H. S.; Chen, J.; Simha, D.; Seol, W.; Evans, R. M.; Moore, D. D. Androstane metabolites bind to and deactivate the nuclear receptor CAR-beta. *Nature* **1998**, *395*, 612−615.

(6) (a) Maglich, J. M.; Sluder, A.; Guan, X.; Shi, Y.; McKee, D. D.; Carrick, K.; Kamdar, K.; Willson, T. M.; Moore, J. T. Comparison of complete nuclear receptor sets from the human, *Caenorhabditis elegans* and Drosophila genomes. *Genome Biol.* **2001**, *2*, 0029.1−0029.7. (b) Aranda, A.; Pascual, A. Nuclear hormone receptors and gene expression. *Pharm. Rev.* **2001**, *83* (1), 1269−1299.

(7) Wurtz, J. M.; Bourguet, W.; Renaud, J. P.; Vivat, V.; Chambon, P.; Moras, D.; Gronemeyer, H. A canonical structure for the ligand-binding domain of nuclear receptors. *Nat. Struct. Biol.* **1996**, *3*, 206.

(8) Giguere, V. Orphan nuclear receptors: from gene to function. *Endocr. Rev.* **1999**, *20*, 689−725.

(9) Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell* **1995**, *81*, 541−550.

(10) (a) Janowski, B. A.; Willy, P. J.; Devi, T. R.; Falck, J. R.; Mangelsdorf, D. J. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* **1996**, *383*, 728−731. (b) Kliewer, S. A.; Umesono, K.; Mangelsdorf, D. J.; Evans, R. M. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. *Nature* **1992**, *355*, 446−449.

(11) Seol, W.; Choi, H. S.; Moore, D. D. Isolation of proteins that interact specifically with the retinoid X receptor: two novel orphan receptors. *Mol. Endocrinol*. **1995**, *9*, 72−85.

(12) Forman, B. M.; Goode, E.; Chen, J.; Oro, A. E.; Bradley, D. J.; Perlmann, T.; Noonan, D. J.; Burka, L. T.; McMorris, T.; Lamph, W. W.; et al. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell*. **1995**, *81* (5), 687−693.

(13) Laffitte, B. A.; Kast, H. R.; Nguyen, C. M.; Zavacki, A. M.; Moore, D. D.; Edwards, P. A. Identification of the DNA binding specificity and potential target genes for the farnesoid X-activated receptor. *J. Biol. Chem.* **2000**, *275*, 10638−10647.

(14) Wang, H.; Chen, J.; Hollister, K.; Sowers, L. C.; Forman, B. M. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell* **1999**, *3*, 543−553.
(15) Parks, D. J.; Blanchard, S. G.; Bledsoe, R. K.; Chandra, G.; Consler, T. G.; Kliewer, S. A.; Stimmel, J. B.; Willson, T. M.; Zavacki, A. M.; Moore, D. D.; Lehmann, J. M. Bile acids: natural ligands for an orphan nuclear receptor. *Science* **1999**, *284*, 1365−1368.
(16) Makishima, M.; Okamoto, A. Y.; Repa, J. J.; Tu, H.; Learned, R. M.; Luk, A.; Hull, M. V.; Lustig, K. D.; Mangelsdorf, D. J.; Shan, B. Identification of a nuclear receptor for bile acids. *Science* **1999**, *284*, 1362−1365.
(17) Bauer, U.; Cheruvallath, Z.; Deuschle, U.; Dneprovskaia, E.; Gahman, T.; Giegrich, K.; Hanecak; R.; Hebert, N.; Kieley, M. WO 016280, 2003.
(18) (a) Duckworth, P. F.; Vlahcevic, Z. R.; Studer, E. J.; Gurley, E. C.; Heuman, D. M.; Beg, Z. H.; Hylemon, P. B. Effect of hydrophobic bile acids on 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and mRNA levels in the rat. *J. Biol. Chem.* **1991**, *266*, 9413−9418. (b) Twisk, J.; Lehmann, E. M.; Princen, H. M. Differential feedback regulation of cholesterol 7 alpha-hydroxylase mRNA and transcriptional activity by rat bile acids in primary monolayer cultures of rat hepatocytes. *Biochem. J.* **1993**, *290*, 685−691. (c) Vlahcevic, Z. R.; Jairath, S. K.; Heuman, D. M.; Stravitz, R. T.; Hylemon, P. B.; Avadhani, N. G.; Pandak, W. M. Transcriptional regulation of hepatic sterol 27-hydroxylase by bile acids. *Am. J. Physiol*. **1996**, *270*, G646−G652. (d) Agellon, L. B.; Cheema, S. K. The 3′-untranslated region of the mouse cholesterol 7alpha-hydroxylase mRNA contains elements responsive to post-transcriptional regulation by bile acids. *Biochem. J*. **1997**, *328*, 393−399.
(19) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J. L.; Sundseth, S. S.; Winegar, D. A.; Blanchard, D. E.; Spencer, T. A.; Willson, T. M. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **1997**, *272*, 3137−3140.
(20) Hofmann, A. F.; Roda, A. Physicochemical properties of bile acids and their relationship to biological properties: an overview of the problem. *J. Lipid Res*. **1984**, *25*, 1477−1489.
(21) Chiang, J. Y. Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *J. Hepatol*. **2004**, *40*, 539−545.
(22) Yu, J.; Lo, J. L.; Huang, L.; Zhao, A.; Metzger, E.; Adams, A.; Meinke, P. T.; Wright, S. D.; Cui, J. Lithocholic acid decreases expression of bile salt export pump through farnesoid X receptor antagonist activity. *J. Biol. Chem.* **2002**, *277*, 31441−31447.
(23) Lew, J. L.; Zhao, A.; Yu, J.; Huang, L.; De Pedro, N.; Pelaez, F.; Wright, S. D.; Cui, J. The farnesoid X receptor controls gene expression in a ligand- and promoter-selective fashion *J. Biol. Chem.* **2004**, *279*, 8856−8866.
(24) Zavacki, A. M.; Lehmann, J. M.; Seol, W.; Willson, T. M.; Kliewer, S. A.; Moore, D. D. Activation of the orphan receptor RIP14 by retinoids. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 7909−7911.
(25) Downes, M.; Verdecia, M. A.; Roecker, A. J.; Hughes, R.; Hogenesch, J. B.; Kast-Woelbern, H. R.; Bowman, M. E.; Ferrer, J. L.; Anisfeld, A. M.; Edwards, P. A.; Rosenfeld, J. M.; Alvarez, J. G.; Noel, J. P.; Nicolaou, K. C.; Evans, R. M. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol. Cell* **2003**, *11*, 1079−1092.
(26) Nicolaou, K. C.; Evans, R. M.; Roecker, A. J.; Hughes, R.; Downes, M.; Pfefferkorn, J. A.; Discovery and optimization of non-steroidal FXR agonists from natural product-like libraries. *Org. Biomol. Chem*. **2003**, *1*, 908−920.
(27) Dussault, I.; Beard, R.; Lin, M.; Hollister, K.; Chen, J.; Xiao, J. H.; Chandraratna, R.; Forman, B. M. Identification of gene-selective modulators of the bile acid receptor FXR. *J. Biol. Chem.* **2003**, *278*, 7027−7033.
(28) Love, J. D.; Gooch, J. T.; Benko, S.; Li, C.; Nagy, L.; Chatterjee, V. K.; Evans, R. M.; Schwabe, J. W. The structural basis for the specificity of retinoid-X receptor-selective agonists: new insights into the role of helix H12. *J. Biol. Chem.* **2002**, *277*, 11385−11391.
(29) Boehm, M. F.; Zhang, L.; Zhi, L.; McClurg, M. R.; Berger, E.; Wagoner, M.; Mais, D. E.; Suto, C. M.; Davies, J. A.; Heyman, R. A. Design and synthesis of potent retinoid X receptor selective ligands that induce apoptosis in leukemia cells. *J. Med. Chem.* **1995**, *38*, 3146−3155.
(30) Bauer, U.; Cheruvallath, Z.; Deuschle, U.; Dneprovskaia, E.; Gahman, T.; Giegrich, K.; Hanecak, R.; Hebert, N.; Kiely, J.; Kober, I.; Koegl, M.; Kranz, H.; Kremoser, C.; Lee, M.; Otte, K.; Sage, C.; Sud, M. FXR (NR1H4 nuclear receptor)-binding compounds. WO 2003015771, 2003.
(31) Houck, K. A.; Borchert, K. M.; Hepler, C. D.; Thomas, J. S.; Bramlett, K. S.; Michael, L. F.; Burris, T. P. T0901317 is a dual LXR/FXR agonist. *Mol. Genet. Metab*. **2004**, *83*, 184−187.
(32) Schultz, J. R.; Tu, H.; Luk, A.; Repa, J. J.; Medina, J. C.; Li, L.; Schwendner, S.; Wang, S.; Thoolen, M.; Mangelsdorf, D. J.; Lustig, K. D.; Shan, B. Role of LXRs in control of lipogenesis. *Genes Dev*. **2000**, *14*, 2831−2838.
(33) Hoerer, S.; Schmid, A.; Heckel, A.; Budzinski, R. M.; Nar, H. Crystal structure of the human liver X receptor beta ligand-binding domain in complex with a synthetic agonist. *J. Mol. Biol*. **2003**, *334*, 853−861.
(34) Niesor, E. J.; Flach, J.; Lopes-Antoni, I.; Perez, A.; Bentzen, C. L. The nuclear receptors FXR and LXRalpha: potential targets for the development of drugs affecting lipid metabolism and neoplastic diseases. *Curr. Pharm. Des*. **2001**, *7*, 231−255.
(35) Roitelman, J.; Masson, D.; Avner, R.; Ammon-Zufferey, C.; Perez, A.; Guyon-Gellin, Y.; Bentzen, C. L.; Niesor, E. J. Apomine, a novel hypocholesterolemic agent, accelerates degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and stimulates low density lipoprotein receptor activity. *J. Biol. Chem.* **2004**, *279*, 6465−6473.
(36) Staudinger, J. L.; Goodwin, B.; Jones, S. A.; Hawkins-Brown, D.; MacKenzie, K. I.; LaTour, A.; Liu, Y.; Klaassen, C. D.; Brown, K. K.; Reinhard, J.; Willson, T. M.; Koller, B. H.; Kliewer, S. A. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci. U.S.A*. **2001**, *98*, 3369−3374.
(37) Adachi, R.; Honma, Y.; Masuno, H.; Kawana, K.; Shimomura, I.; Yamada, S.; Makishima, M. Selective activation of vitamin D receptor by lithocholic acid acetate, a bile acid derivative. *J. Lipid Res*. **2005**, *46*, 46−57.
(38) Fujino, T.; Une, M.; Imanaka, T.; Inoue, K.; Nishimaki-Mogami, T. Structure−activity relationship of bile acids and bile acid analogs in regard to FXR activation. *J. Lipid Res*. **2004**, *45*, 132−138.
(39) Nishimaki-Mogami, T.; Une, M.; Fujino, T.; Sato, Y.; Tamehiro, N.; Kawahara, Y.; Shudo, K.; Inoue, K. Identification of intermediates in the bile acid synthetic pathway as ligands for the farnesoid X receptor. *J. Lipid Res*. **2004**, *45*, 1538−1545.
(40) Pellicciari, R.; Fiorucci, S.; Camaioni, E.; Clerici, C.; Costantino, G.; Maloney, P. R.; Morelli, A.; Parks, D. J.; Willson, T. M. 6α-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J. Med. Chem.* **2002**, *45*, 3569−3572.
(41) Pellicciari, R.; Costantino, G.; Camaioni, E.; Sadeghpour, B. M.; Entrena, A.; Willson, T. M.; Fiorucci, S.; Clerici, C.; Gioiello, A. Bile acid derivatives as ligands of the farnesoid X receptor. Synthesis, evaluation, and structure−activity relationship of a series of body and side chain modified analogues of chenodeoxycholic acid. *J. Med. Chem.* **2004**, *47*, 4559−4569.
(42) Singh, R. B.; Niaz, M. A.; Ghosh, S. Hypolipidemic and antioxidant effects of Commiphora mukul as an adjunct to dietary therapy in patients with hypercholesterolemia. *Cardiovasc. Drugs Ther*. **1994**, *4*, 659−664.
(43) Sinal, C. J.; Gonzalez, F. J. Guggulsterone: an old approach to a new problem. *Trends Endocrinol. Metab*. **2002**, *13*, 275−276.
(44) Urizar, N. L.; Moore, D. D. Guggulipid: a natural cholesterol-lowering agent. *Annu. Rev. Nutr*. **2003**, *23*, 303−313.
(45) Burris, T. P.; Montrose, C.; Houck, K. A.; Osborne, H. E.; Bocchinfuso, W. P.; Yaden, B. C.; Cheng, C. C.; Zink, R. W.; Barr, R. J.; Hepler, C. D.; Krishnan, V.; Bullock, H. A.; Burris, L. L.; Galvin, R. J.; Bramlett, K. S.; Stayrook, K. R. The hypolipidemic natural product guggulsterone is a promiscuous steroid receptor ligand. *Mol. Pharmacol*. **2005**, *67*, 948−952.
(46) Broobst, D. E.; Ding, X.; Creech, K. L.; Goodwin, B.; Kelley, B.; Staudinger, J. L. Guggulsterone activates multiple nuclear receptors and induce CYP3A gene expression through the pregnane X receptor. *J. Pharmacol. Exp. Ther*. **2004**, *310*, 528−535.
(47) Urizar, N. L.; Liverman, A. B.; Dodds, D. T.; Silva, F. V.; Ordentlich, P.; Yan, Y. A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science* **2002**, *296*, 1703−1706.
(48) Cui, J.; Huang, L.; Zhao, A.; Lew, J. L.; Sahoo, S.; Meinke, P. T.; Royo, I.; Pelaez, F.; Wright, S. D. Guggulsterone is a farnesoid X receptor antagonist in coactivator association assays but acts to enhance transcription of bile salt export pump. *J. Biol. Chem*. **2003**, *278*, 10214−10220.
(49) Wu, J.; Xia, C.; Meier, J.; Li, S.; Hu, X.; Lala, D. S. The hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor. *Mol. Endocrinol*. **2002**, *16*, 1590−1597.
(50) Carson-Jurica, M. A.; Schrader, W. T.; O'Malley, B. W. Steroid receptor family: structure and functions. *Endocr. Rev*. **1990**, *11*, 201−202.
(51) Wurtz, J. M.; Bourguet, W.; Renaud, J. P.; Vivat, V.; Chambon, P.; Moras, D.; Gronemeyer, H. A canonical structure for the ligand-binding domain of nuclear receptors. *Nat. Struct. Biol*. **1996**, *3*, 87−94.

(52) Mi, L. Z.; Devarakonda, S.; Harp, J. M.; Han, Q.; Pellicciari, R.; Willson, T. M.; Khorasanizadeh, S.; Rastinejad, F. Structural basis for bile acid binding and activation of the nuclear receptor FXR. *Mol Cell*. **2003**, *11*, 1093−1100.
(53) Costantino, G.; Macchiarulo, A.; Entrena-Guadix, A.; Camaioni, E.; Pellicciari, R. Binding mode of 6ECDCA, a potent bile acid agonist of the farnesoid X receptor (FXR). *Bioorg. Med. Chem. Lett*. **2003**, *13*, 1865−1868.
(54) Costantino, G.; Entrena-Guadix, A.; Macchiarulo, A.; Gioiello, A.; Pellicciari, R. Molecular dynamics simulation of the ligand binding domain of farnesoid x receptor (FXR). Insights into helix-12 stability and coactivator peptide stabilization in response to agonist binding. *J. Med. Chem*. **2005**, *48*, 3251−3259.
(55) Nettles, K. W.; Greene, G. L. Nuclear receptor ligands and cofactor recruitment: is there a coactivator "on deck"? *Mol. Cell* **2003**, *11*, 850−851.
(56) Maglich, J. M.; Caravella, J. A.; Lambert, M. H.; Willson, T. M.; Moore, J. T.; Ramamurthy, L. The first completed genome sequence from a teleost fish (Fugu rubripes) adds significant diversity to the nuclear receptor superfamily. *Nucleic Acids Res*. **2003**, *31*, 4051−4058.
(57) Maglich, J. M.; Sluder, A.; Guan, X.; Shi, Y.; McKee, D. D.; Carrick, K.; Kamdar, K.; Willson, T. M.; Moore, J. T. Comparison of complete nuclear receptor sets from the human, Caenorhabditis elegans and Drosophila genomes. *Genome Biol*. **2001**, *2*.
(58) Chen, W.; Owsley, E.; Yang, Y.; Stroup, D.; Chiang, J. Y. L. Nuclear receptor-mediated repression of human cholesterol 7-hydroxylase gene transcription by bile acids. *J. Lipid Res.* **2001**, *42*, 1402−1412.
(59) Chiang, J. Y.; Kimmel, R.; Stroup, D. Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). *Gene* **2001**, *262*, 257−265.
(60) Peet, D. J.; Turley, S. D.; Ma, W.; Janowski, B. A.; Lobaccaro, J. M.; Hammer, R. E.; Mangelsdorf, D. J. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* **1998**, *93*, 693−704.
(61) Cui, J.; Heard, T. S.; Yu, J.; Lo, J.-L.; Huang, L.; Li, Y.; Schaeffer, J. M.; Wright, S. D. The amino acid residues asparagine 354 and isoleucine 372 of human farnesoid x receptor confer the receptor with high sensitivity to chenodeoxycholate *J. Biol. Chem.* **2002**, *277*, 25963−25969.
(62) Seo, Y. W.; Sanyal, S.; Kim, H. J.; Won, D. H.; An, J. Y.; Amano, T.; Zavacki, A. M.; Kwon, H. B.; Shi, Y. B.; Kim, W. S.; Kang, H.; Moore, D. D.; Choi, H. S. FOR, a novel orphan nuclear receptor related to farnesoid X receptor. *J. Biol. Chem.* **2002**, *277*, 17836−17844.
(63) Zhang, Y.; Kast-Woelbern, H. R.; Edwards, P. A. Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation. *J. Biol. Chem.* **2003**, *278*, 104−110.
(64) Otte, K.; Kranz, H.; Kober, I.; Thompson, P.; Hoefer, M.; Haubold, B.; Remmel, B.; Voss, H.; Kaiser, C.; Albers, M.; Cheruvallath, Z.; Jackson, D.; Casari, G.; Koegl, M.; Paabo, S.; Mous, J.; Kremoser, C.; Deuschle, U. Identification of farnesoid X receptor beta as a novel mammalian nuclear receptor sensing lanosterol. *Mol. Cell. Biol*. **2003**, *23* (3), 864−872.
(65) Bishop-Bailey, D.; Walsh, D. T.; Warner, T. D. Expression and activation of the farnesoid X receptor in the vasculature. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3668−3673.
(66) Sinal, C. J.; Tohkin, M.; Miyata, M.; Ward, J. M.; Lambert, G.; Gonzalez, F. J. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* **2000**, *102*, 731−734.
(67) Guo, G. L.; Lambert, G.; Negishi, M.; Ward, J. M.; Brewer, H. B., Jr.; Kliewer, S. A.; Gonzalez, F. J.; Sinal, C. J. Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. *J. Biol. Chem.* **2003**, *278*, 45062−45071.
(68) Repa, J. J.; Turley, S. D.; Lobaccaro, J. A.; Medina, J.; Li, L.; Lustig, K.; Shan, B.; Heyman, R. A.; Dietschy, J. M.; Mangelsdorf, D. J. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* **2000**, *289*, 1524−1529.
(69) Yang, Y.; Zhang, M.; Eggertsen, G.; Chiang, J. Y. On the mechanism of bile acid inhibition of rat sterol 12alpha-hydroxylase gene (CYP8B1) transcription: roles of alpha-fetoprotein transcription factor and hepatocyte nuclear factor 4alpha. *Biochim. Biophys. Acta* **2002**, *1583*, 63−73.
(70) Chiang, J. Y.; Kimmel, R.; Weinberger, C.; Stroup, D. Farnesoid X receptor responds to bile acids and represses cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription. *J. Biol. Chem.* **2000**, *275*, 10918−10924.
(71) Goodwin, B.; Jones, S. A.; Price, R. R.; Watson, M. A.; McKee, D. D.; Moore, L. B.; Galardi, C.; Wilson, J. G.; Lewis, M. C.; Roth, M. E.; Maloney, P. R.; Willson, T. M.; Kliewer, S. A. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol. Cell* **2000**, *6*, 517−526.
(72) Lu, T. T.; Makishima, M.; Repa, J. J.; Schoonjans, K.; Kerr, T. A.; Auwerx, J.; Mangelsdorf, D. J. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell* **2000**, *6*, 507−515.
(73) Denson, L. A.; Sturm, E.; Echevarria, W.; Zimmerman, T. L.; Makishima, M.; Mangelsdorf, D. J.; Karpen, S. J. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* **2001**, *121*, 140−147.
(74) Ananthanarayanan, M.; Balasubramanian, N.; Makishima, M.; Mangelsdorf, D. J.; Suchy, F. J. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J. Biol. Chem.* **2001**, *276*, 28857−28865.
(75) Grober, J.; Zaghini, I.; Fujii, H.; Jones, S. A.; Kliewer, S. A.; Willson, T. M.; Ono, T.; Besnard, P. Identification of a bile acid-responsive element in the human ileal bile acid-binding protein gene. Involvement of the farnesoid X receptor/9-cis-retinoic acid receptor heterodimer. *J. Biol. Chem.* **1999**, *274*, 29749−29754.
(76) Claudel, T.; Sturm, E.; Duez, H.; Torra, I. P.; Sirvent, A.; Kosykh, V.; Fruchart, J. C.; Dallongeville, J.; Hum, D. W.; Kuipers, F.; Staels, B. Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. *J. Clin. Invest*. **2002**, *109*, 961−971.
(77) Kast, H. R.; Nguyen, C. M.; Sinal, C. J.; Jones, S. A.; Laffitte, B. A.; Reue, K.; Gonzalez, F. J.; Willson, T. M.; Edwards, P. A. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. *Mol. Endocrinol*. **2001**, *15*, 1720−1728.
(78) Claudel, T.; Inoue, Y.; Barbier, O.; Duran-Sandoval, D.; Kosykh, V.; Fruchart, J.; Fruchart, J. C.; Gonzalez, F. J.; Staels, B. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. *Gastroenterology* **2003**, *125*, 544−555.
(79) Zhao, A.; Lew, J. L.; Huang, L.; Yu, J.; Zhang, T.; Hrywna, Y.; Thompson, J. R.; de Pedro, N.; Blevins, R. A.; Pelaez, F.; Wright, S. D.; Cui, J. Human kininogen gene is transactivated by the farnesoid X receptor. *J. Biol. Chem.* **2003**, *278*, 28765−28770.
(80) Prieur, X.; Coste, H.; Rodriguez, J. C. The human apolipoprotein AV gene is regulated by peroxisome proliferator-activated receptor-alpha and contains a novel farnesoid X-activated receptor response element. *J. Biol. Chem.* **2003**, *278*, 25468−25480.
(81) Mak, P. A.; Kast-Woelbern, H. R.; Anisfeld, A. M.; Edwards, P. A. Identification of PLTP as an LXR target gene and apoE as an FXR target gene reveals overlapping targets for the two nuclear receptors. *J. Lipid Res*. **2002**, *43*, 2037−2041.
(82) Pircher, P. C.; Kitto, J. L.; Petrowski, M. L.; Tangirala, R. K.; Bischoff, E. D.; Schulman, I. G.; Westin, S. K. Farnesoid X receptor regulates bile acid−amino acid conjugation. *J. Biol. Chem.* **2003**, *278*, 27703−27711.
(83) Holt, J. A.; Luo, G.; Billin, A. N.; Bisi, J.; McNeill, Y. Y.; Kozarsky, K. F.; Donahee, M.; Wang, Y.; Mansfield, T. A.; Kliewer, S. A.; Goodwin, B.; Jones, S. A. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev*. **2003**, *17*, 1581−1591.
(84) Huang, L.; Zhao, A.; Lew, J. L.; Zhang, T.; Hrywna, Y.; Thompson, J. R.; de Pedro, N.; Royo, I.; Blevins, R. A.; Pelaez, F.; Wright, S. D.; Cui, J. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. *J. Biol. Chem.* **2003**, *278*, 51085−51090.
(85) Urizar, N. L.; Dowhan, D. H.; Moore, D. D. The farnesoid X-activated receptor mediates bile acid activation of phospholipid transfer protein gene expression. *J. Biol. Chem.* **2000**, *275*, 39313−39317.
(86) Song, C. S.; Echchgadda, I.; Baek, B. S.; Ahn, S. C.; Oh, T.; Roy, A. K.; Chatterjee, B. Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. *J. Biol. Chem.* **2001**, *276*, 42549−42556.
(87) Anisfeld, A. M.; Kast-Woelbern, H. R.; Meyer, M. E.; Jones, S. A.; Zhang, Y.; Williams, K. J.; Willson, T.; Edwards, P. A. Syndecan-1 expression is regulated in an isoform-specific manner by the farnesoid-X receptor. *J. Biol. Chem.* **2003**, *278*, 20420−20428.
(88) Barbier, O.; Torra, I. P.; Sirvent, A.; Claudel, T.; Blanquart, C.; Duran-Sandoval, D.; Kuipers, F.; Kosykh, V.; Fruchart, J. C.; Staels, B. FXR induces the UGT2B4 enzyme in hepatocytes: a potential mechanism of negative feedback control of FXR activity. *Gastroenterology* **2003**, *124*, 1926−1940.
(89) Chen, T.; Xie, W.; Agler, M.; Banks, M. Coactivators in assay design for nuclear hormone receptor drug discovery. *Assay Drug Dev. Technol*. **2003**, *1*, 835−842.
(90) Jones, S. A.; Parks, D. J.; Kliewer, S. A. Cell-free ligand binding assays for nuclear receptors. *Methods Enzymol*. **2003**, *364*, 53−71.
(91) Burke, T. J.; Loniello, K. R.; Beebe, J. A.; Ervin, K. M. Development and application of fluorescence polarization assays in drug discovery. *Comb. Chem. High Throughput Screening* **2003**, *6*, 183−194.

(92) Webster, N. J.; Green, S.; Jin, J. R.; Chambon, P. The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. *Cell* **1988**, *54*, 199−207.
(93) Fields, S.; Song, O. A novel genetic system to detect protein−protein interactions. *Nature* **1989**, *340*, 245−246.
(94) McDonnell, D. P.; Condor, C. E.; Wijayartne, A.; Chang, C.-Y.; Norris, J. D. Definition of the molecular and cellular mechanisms underlying the tissue-selective agonist/antagonist activities of selective estrogen receptor modulators. *Recent Prog. Horm. Res*. **2002**, *57*, 295−316.
(95) McDonnell, D. P.; Wijayaratne, A.; Chang, C. Y.; Norris, J. D. Elucidation of the molecular mechanism of action of selective estrogen receptor modulators. *Am. J. Cardiol*. **2002**, *3* (90), 35F−43F.
(96) Dutertre, M.; Smith, C. L. Molecular mechanisms of selective estrogen receptor modulator (SERM) action. *J. Pharmacol. Exp. Ther*. **2000**, *295*, 431−437.
(97) Friedman, S. L. Liver fibrosis—from bench to bedside. *J. Hepatol*. **2003**, *38*, S38−S53.
(98) Fiorucci, S.; Antonelli, E.; Rizzo, G.; Renga, B.; Mencarelli, A.; Riccardi, L.; Orlandi, S.; Pellicciari, R.; Morelli, A. The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. *Gastroenterology* **2004**, *127*, 1497−1512.
(99) Miyahara, T.; Schrum, L.; Rippe, R.; Xiong, S.; Yee, H. F., Jr.; Motomura, K.; Anania, F. A.; Willson, T. M.; Tsukamoto, H. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. *J. Biol. Chem.* **2000**, *275*, 35715−35722.
(100) Galli, A.; Crabb, D. W.; Ceni, E.; Salzano, R.; Mello, T.; Svegliati-Baroni, G.; Ridolfi, F.; Trozzi, L.; Surreni, C.; Casini, A. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. *Gastroenterology* **2002**, *122*, 1924−1940.
(101) Trauner, M.; Meier, P. J.; Boyer, J. L. Molecular pathogenesis of cholestasis. *N. Engl. J. Med*. **1998**, *339*, 1217−1227.
(102) Trauner, M.; Boyer, J. L. Bile salt transporters: molecular characterization, function, and regulation. *Physiol. Rev*. **2003**, *83*, 633−671.
(103) Liu, Y.; Binz, J.; Numerick, M. J.; Dennis, S.; Luo, G.; Desai, B.; MacKenzie, K. I.; Mansfield, T. A.; Kliewer, S. A.; Goodwin, B.; Jones, S. A. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis. *J. Clin. Invest*. **2003**, *112*, 1678−1687.
(104) Fiorucci, S.; Clerici, C.; Antonelli, E.; Orlandi, S.; Goodwin, B.; Sadeghpour, B.; Sabatino, G.; Russo, G.; Castellani, D.; Willson, T. M.; Pellicciari, R.; Morelli, A. Protective effects of 6-ethyl chenodeoxycholic acid, a farnesoid X receptor (FXR) ligand, in estrogen induced cholestasis. *J. Pharmacol. Exp. Ther*. **2005**, January 11.
(105) Watanabe, M.; Houten, S. M.; Wang, L.; Moschetta, A.; Mangelsdorf, D. J.; Heyman, R. A.; Moore, D. D.; Auwerx, J. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J. Clin. Invest*. **2004**, *113*, 1408−1418.
(106) Zhang, Y.; Mangelsdorf, D. J. LuXuRies of lipid homeostasis: the unity of nuclear hormone receptors, transcription regulation, and cholesterol sensing. *Mol. Interventions* **2002**, *2*, 78−87.
(107) Repa, J. J.; Liang, G.; Ou, J.; Bashmakov, Y.; Lobaccaro, J. M.; Shimomura, I.; Shan, B.; Brown, M. S.; Goldstein, J. L.; Mangelsdorf, D. J. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev*. **2000**, *14*, 2819−2830.
(108) Grefhorst, A.; Elzinga, B. M.; Voshol, P. J.; Plosch, T.; Kok, T.; Bloks, V. W.; van der Sluijs, F. H.; Havekes, L. M.; Romijn, J. A.; Verkade, H. J.; Kuipers, F. Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low-density lipoprotein particles. *J. Biol. Chem.* **2002**, *277*, 34182−34190.
(109) Boland, L. L.; Folsom, A. R.; Rosamond, W. D. Hyperinsulinemia, dyslipidemia, and obesity as risk factors for hospitalized gallbladder disease: a prospective study. *Ann. Epidemiol*. **2002**, *12*, 131−140.
(110) Duran-Sandoval, D.; Mautino, G.; Martin, G.; Percevault, F.; Barbier, O.; Fruchart, J. C.; Kuipers, F.; Staels, B. Glucose regulates the expression of the farnesoid X receptor in liver. *Diabetes* **2004**, *53*, 890−898.
(111) Stayrook, K. R.; Bramlett, K. S.; Savkur, R. S.; Ficorilli, J.; Cook, T.; Christe, M. E.; Michael, L. F.; Burris, T. P. Regulation of carbohydrate metabolism by the farnesoid X receptor. *Endocrinology* **2005**, *146*, 984−991.
(112) Savkur, R. S.; Bramlett, K. S.; Michael, L. F.; Burris, T. P. Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor. *Biochem. Biophys. Res. Commun*. **2005**, *329*, 391−396.
(113) Pineda Torra, I.; Claudel, T.; Duval, C.; Kosykh, V.; Fruchart, J. C.; Staels, B. Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. *Mol. Endocrinol*. **2003**, *17*, 259−272.
(114) Zhang, Y.; Castellani, L. W.; Sinal, C. J.; Gonzalez, F. J.; Edwards, P. A. Peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) regulates triglyceride metabolism by activation of the nuclear receptor FXR. *Genes Dev*. **2004**, *18*, 157−169.
(115) Duran-Sandoval, D.; Cariou, B.; Fruchart, J. C.; Staels, B. Potential regulatory role of the farnesoid X receptor in the metabolic syndrome. *Biochimie* **2005**, *87*, 93−98.
(116) Bishop-Bailey, D. FXR as a novel therapeutic target for vascular disease. *Drug News Perspect*. **2004**, *17*, 499−504.

JM0582221