FARNESOID X RECEPTOR (FXR): A VERSATILE BILE ACID RESPONSIVE ELEMENT: A MOLECULAR BASIS OF REVIEW

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Abstract:

Bile acids are an end product of cholesterol catabolism produced in the liver. It comprises of Chenodeoxycholic acid (CDCA), Deoxycholic acid (DCA), Lithocholic acid (LCA), Cholic Acid (CA) are act as an endogenous ligand by acting on the FXR receptor belongs to the nuclear receptor family to exert the various biological functions. FXR is considered as a chief regulator of bile acid metabolism as it is involved in all phases of the bio-synthetic pathway. Activation of these nuclear receptors by their respective ligands leads to a reduction in bile acid synthesis, promotion of lipid oxidation, drug metabolism and transport, as well as affecting cholesterol metabolism. Conversely dysregulation of bile acid metabolism has a significant impact on inflammatory and metabolic disorders, such as Non-alcoholic fatty liver disease, diabetes, and obesity. In this review, we have summarized the regulation of bile acid synthesis, transport, absorption, different ligands for FXR and their chemical structure in detail.

Key words: Farnesoid X receptor, nuclear receptor, Deoxycholic acid, Lithocholic acid, Cholic Acid, agonist.

INTRODUCTION:

The farnesoid X receptor (FXR) is a associate of the nuclear receptor super family that is expressed in liver, kidney, intestine and the adrenal gland. **[1]** These proteins bind to cis-acting elements in the promoters of their target genes and modulate gene expression in response to metabolites. **[2]** The FXR were first described in 1995 by Forman and co-workers. **[2]** FXRs were found to be activated by the farnesol derivative, farnesyl pyrophosphate, which is a metabolic intermediate and the last precursor common to all branches of the mevalonic pathway, which leads to the biosynthesis of cholesterol, bile acids (BAs), sterol compounds, porphyrin, dolichol, ubiquinone, carotenoids, retinoids, vitamin D, steroid hormones, and farnesylated proteins. **[3]** FXR is activated by bile acids such as the primary bile acid chenodeoxycholic acid which is the most potent

bile acid ligand for the human FXR. **[4,5]** In addition to bile acids synthetic FXR agonists have also been identified. **[6,7]** In response to ligand- binding, FXR regulates a variety of genes involved in bile acid, cholesterol, triglyceride and lipoprotein metabolism. Targeted disruption of the FXR gene in mice confirmed its critical role in bile acid and lipid metabolism. **[8]** FXR links cholesterol to bile acid metabolism by inhibiting transcription of the cyp7a1 gene **[9],** which encodes the rate-limiting enzyme catalyzing the conversion of cholesterol into bile acids. FXR also regulates the expression of various genes involved in the uptake, intracellular transport and export of bile acids such as intestinal bile acid -binding protein. **[10]** the bile salt export pump **[11]** and the naþ-taurocholate co-transporting polypeptide **[12].** Furthermore, FXR plays an important role in lipoprotein and high density lipoprotein metabolism. FXR positively regulates the phospholipid transfer protein gene **[13],** which encodes a secreted protein that facilitates the transfer of phospholipids between lipoproteins and modulates plasma High density lipoprotein metabolism. In addition, apo ai, the major apolipoprotein component of High density lipoprotein, is down-regulated by FXR. **[14]** FXR also controls plasma triglycerides levels by activating the peroxisome proliferator-activated receptor a gene, another nuclear receptor controlling tg metabolism **[15],** and the apo cii gene **[16],** an obligate cofactor for lipoprotein lipase responsible for the hydrolysis of triglycerides in chylomycrons, and by inhibiting apo ciii **[17],** which plays an important role in the control of triglycerides metabolism.

LOCATION: The location of farnesoid X receptor receptor in body-

- Liver,
- Kidney,
- Intestine
- Adrenal Gland

An Overview and Brief History of Bile Acids:

The first description of a bile acid was made in 1848 when cholic acid (CA) was discovered in ox gall. Subsequent studies in the early 1900s identified additional bile acids that included lithocholic acid (LCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), and muricholic acid (MCA) from ox, goose, bear, and rodents, respectively, as described by Wieland in his 1928 Nobel lecture. **[18]** More sophisticated methodologies subsequently led to the identification of multiple additional species of bile acids, including deoxycholic acid (DCA), that contribute to the ‘‘bile acid pool’’ (2–4 g in humans). The relative concentrations of individual bile acids within the bile acid pool of different mammals can vary significantly and may affect bile acid-dependent signaling. Bile acids are known to play a number of roles in lipid metabolism. First, bile acids are essential for the formation of mixed micelles in the small intestine that facilitate solubilization, digestion, and absorption of dietary lipids and fat-soluble vitamins. Second, the micelles present in the gall bladder serve to solubilize cholesterol in bile, thus impairing cholesterol crystallization and gallstone formation. Third, bile salts induce bile flow from hepatocytes into the bile canaliculi and then gall bladder. Fourth, the hepatic conversion of cholesterol to bile acids and the subsequent excretion of bile acids in the feces represent the major route for cholesterol excretion that is important in whole-body sterol homeostasis. Bile is also thought to have a bacteriostatic function that maintains sterility in the biliary tree. Consistent with these roles, disruption of normal bile acid synthesis and metabolism is associated with cholestasis, gallstones, inflammation, malabsorption of lipids and fat-soluble vitamins, bacterial overgrowth in the small intestine, atherosclerosis, neurological diseases, and various inborn errors such as progressive familial intrahepatic cholestasis types I–III (PFIC I-III). The discovery that specific bile acids differentially activate three nuclear receptors, namely farnesoid X receptor (FXR), pregnane X receptor (PXR), and vitamin D receptor (VDR) and one G protein-coupled receptor (TGR5), identified bile acids as hormones that alter multiple metabolic pathways in many tissues. The synthesis and use of specific agonists for FXR or TGR5 in rodents, together with preliminary clinical findings with FXR agonists, suggest that such agonists may prove useful in the treatment of a number of diseases. **[19-28]**

FXR REGULATION OF BILE ACID SYNTHESIS, TRANSPORT AND ABSORPTION:

FXR plays a central role in regulation of bile acid synthesis and transport. FXR inhibits the CYP7A1 and CYP8B1 genes involved in bile acid synthesis. On the other hand, FXR stimulates bile acid conjugation by inducing BCAS and bile acid CoA: amino acid N-acetyltransferase (BAT). FXR markedly induces bile salt export pump (BSEP, ABCB11) **[29]**, which is the principle bile acid transporter for excretion of bile acid conjugates. FXR also induces multidrug resistance associated protein 2 (MRP2, ABCC2) for transport of sulfate, glutathione or glucuronide conjugated anionic compounds including bile acids. **[30]** Bile acids facilitate the biliary excretion of phosphatidylcholine by inducing multi-drug resistant protein 2 (MDR2), **[31]** and cholesterol by inducing ABCG5 and G8 half transporters [Bile acids are quantitatively reabsorbed in the intestine, mostly in the ileum by an active transport process involving the apical sodium-dependent bile salt transporter (ASBT). **[32]** FXR induces ileum bile acid binding protein (IBABP) **[33]**, which binds bile acids and protects enterocytes for cytotoxic effect of bile acids. Bile acids are excreted into portal circulation, transported back to the liver, and reabsorbed into hepatocytes by sinusoidal Na2þ-dependent taurocholate cotransport peptide (NTCP). FXR inhibits NTCP and may protect hepatocytes from accumulating high levels of toxic bile acids during inflammation. **[34]** FXR also induces organic anion transport protein 2 (OATP2), which takes up bile acids from the sinusoid. FXR induces DHEA-sulfate transferase (SULT2A1), which transfers a sulfate group to the secondary bile acids for rapid excretion into bile via MRP2. Guggulsterone, a FXR antagonist, has been used as a lipid-lowering drug in humans. **[35]** Guggulsterone inhibits IBABP gene in the intestine **[36]**, and BSEP and CYP7A1 gene expression in the liver. **[37–39]** Selective FXR modulators may be useful for lipid-lowering. **[40]**

Synthesis of bile acid:

Bile-acid synthesis is the primary pathway for cholesterol catabolism. Approximately 500 mg of cholesterol is converted into bile acids each day in the adult human liver. Bile-acid biosynthesis involves modification of the ring structure of cholesterol, oxidation and shortening of the side chain, and finally conjugation of the bile acid with an amino acid.**[41]** The intermediates and enzymes of the classical (or neutral pathway) and the alternative (or acidic) bile-acid biosynthetic pathway are displayed in FIG. 1. Cholesterol 7α-hydroxylase, which is encoded by the gene *CYP7A1*, is the first and rate-limiting enzyme of the classical pathway that accounts for the majority of total bile-acid synthesis, as was demonstrated by the ablation of *Cyp7a1* in mice.**[42,43]** It is noteworthy that in these animals, the intestinal absorption of lipids and lipophilic vitamins is severely impaired, resulting in increased perinatal mortality.**[43]** Some mutations identified in the human *CYP7A1* gene are correlated with an increase in the occurrence of hypercholesterolemia and atherosclerosis.**[44,45]** The vital importance of the enzymes that synthesize bile acids is further illustrated by the dramatic consequences observed in humans who have a sterol 27-hydroxylase (*CYP27A1*) deficiency. Mutations in *CYP27A1*, which encodes the
enzyme responsible for the hydroxylation of the cholesterol side chain (FIG. 1), lead to a rare disease referred to as cerebrotendinous xanthomatosis, which is characterized by an accumulation of cholestanol in tissues, early development of atherosclerosis and premature death. **[46,47]**

The most abundant bile acids in humans include the primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), and their respective secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), which are formed by deconjugation and 7a-dehydroxylation by microbial enzymes in the colon (FIG. 2). In the mouse and rat, CDCA is efficiently converted into muricholic acid.**[48]** Before bile acids are transported out of the hepatocytes, most of them are conjugated to either taurine (in the mouse) or glycine (in humans). As most of these bile acids have different chemical properties, they inherently exhibit distinct biological activities (BOX 1). In order to maintain a functional bile-acid pool, bile acids are extensively recycled in the body by an elaborate transport system that is active in the liver, the intestine and the kidney (detailed in FIG. 3). The combined and coordinated action of the several bile-acid transporter proteins ensures the formation of mixed

micelles in bile, as well as efficiently limiting the faecal and urinary loss of bile acids. Under pathophysiological conditions, such as cholestasis, bile-acid transporters exert crucial adaptive functions to minimize the deleterious effects of bile-acid accumulation. Important transporters that are typically activated during bile-acid overload are those located at the basolateral membrane of hepatocytes. These transporters mediate bile-acid efflux, providing important spillover routes for bile acids and bilirubin. Consequently, bile-acid transporters the expressions of which are predominantly controlled at the transcriptional level are vital components in mediating the cycling and homeostasis of bile acids.

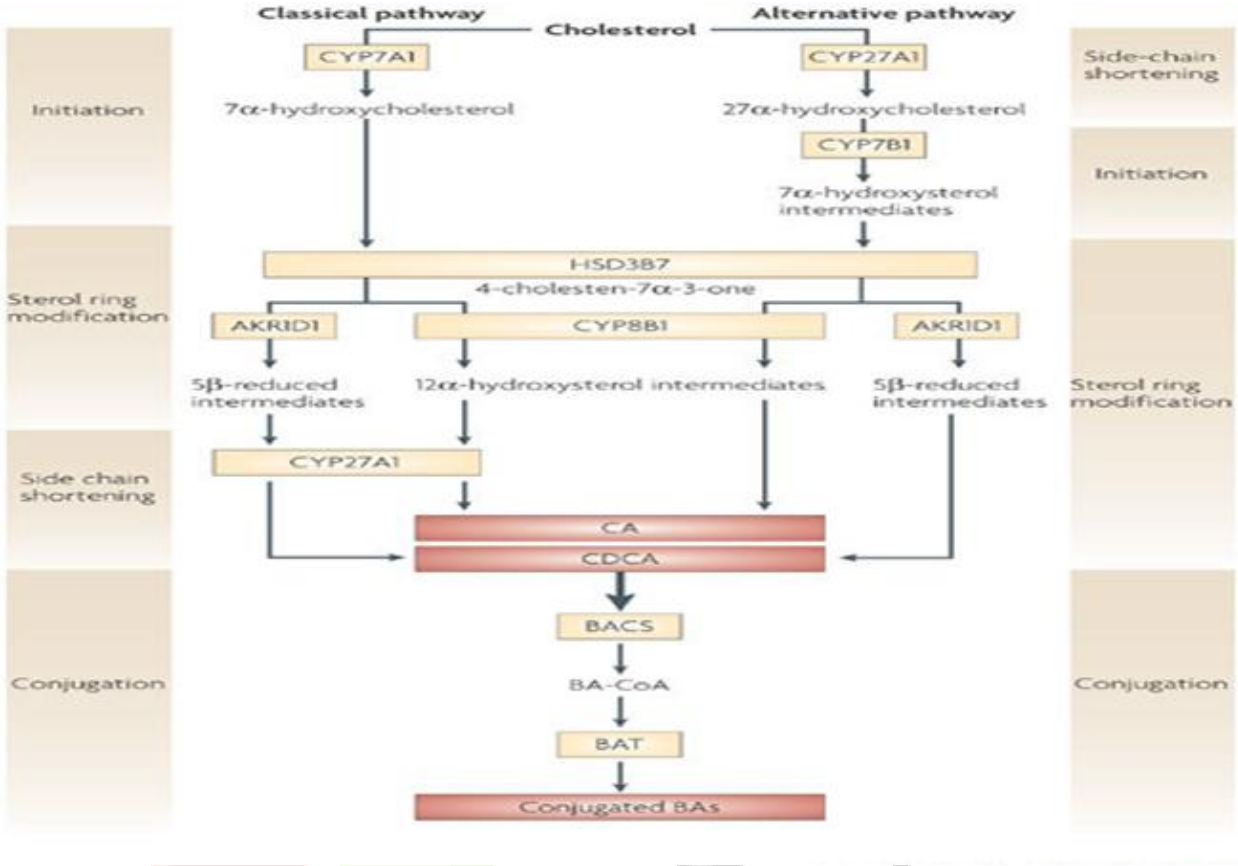

Figure 01: Bile-acid synthesis pathways-

Cholesterol conversion into bile acids (BAs) occurs via two different pathways: the classical (or neutral) pathway and the alternative (or acidic) pathway. The classical pathway accounts for at least 75% of the total bile-acid pool. Cholic acid (CA) and chenodeoxycholic acid (CDCA) represent the two main end products of these pathways. The steps leading to synthesis of primary BAs include initiation (hydroxylation in position 7), modification of the sterol ring, oxidation and shortening of the side chain, and conjugation.**[41]** Three enzymes have major regulatory roles in these two pathways. Cholesterol 7α-hydroxylase (encoded by *CYP7A1*) is the rate-limiting enzyme in the classical pathway, whereas sterol‑27 hydroxylase (encoded by *CYP27A1*) is the first enzyme in the alternative pathway. Sterol 12α-hydroxylase (encoded by *CYP8B1*) introduces a hydroxyl group at position 12 of the steroid nucleus, which leads to the generation of CA. Schematically, CYP7A1 determines the BA pool size, whereas CYP8B1 is crucial for BA pool composition.**[49,50]** Newly synthesized free bile acids will be extensively conjugated (98%) in a two-step process, which starts with the generation of BA–CoA by BA–CoA synthase (BACS).**[51,52]** The enzyme BA-CoA:amino acid *N*‑acyltransferase (BAT), then further amidates BA‑CoA with either a glycine (human) or a taurine (mouse).**[53]** In the intestinal lumen, especially in the colon, gut flora deconjugates, oxidates and dehydroxylates the primary BAs produced in the

liver to generate secondary BAs. Once transported back to the liver, these secondary BAs can be further processed to form tertiary BAs, which represent only a marginal BA species under normal conditions. These synthesis and metabolic pathways allow the generation of more than 18 different BA species, which ensures the perfect solubilization and absorption of a broad range of lipophilic molecules in the intestine and a multitude of signalling activities in the body. AKR1D1, aldo-keto reductase family 1, member D1; HSD3B7, hydroxy-δ-5-steroid dehydrogenase, 3β- and steroid δ-isomerase. **[43]**

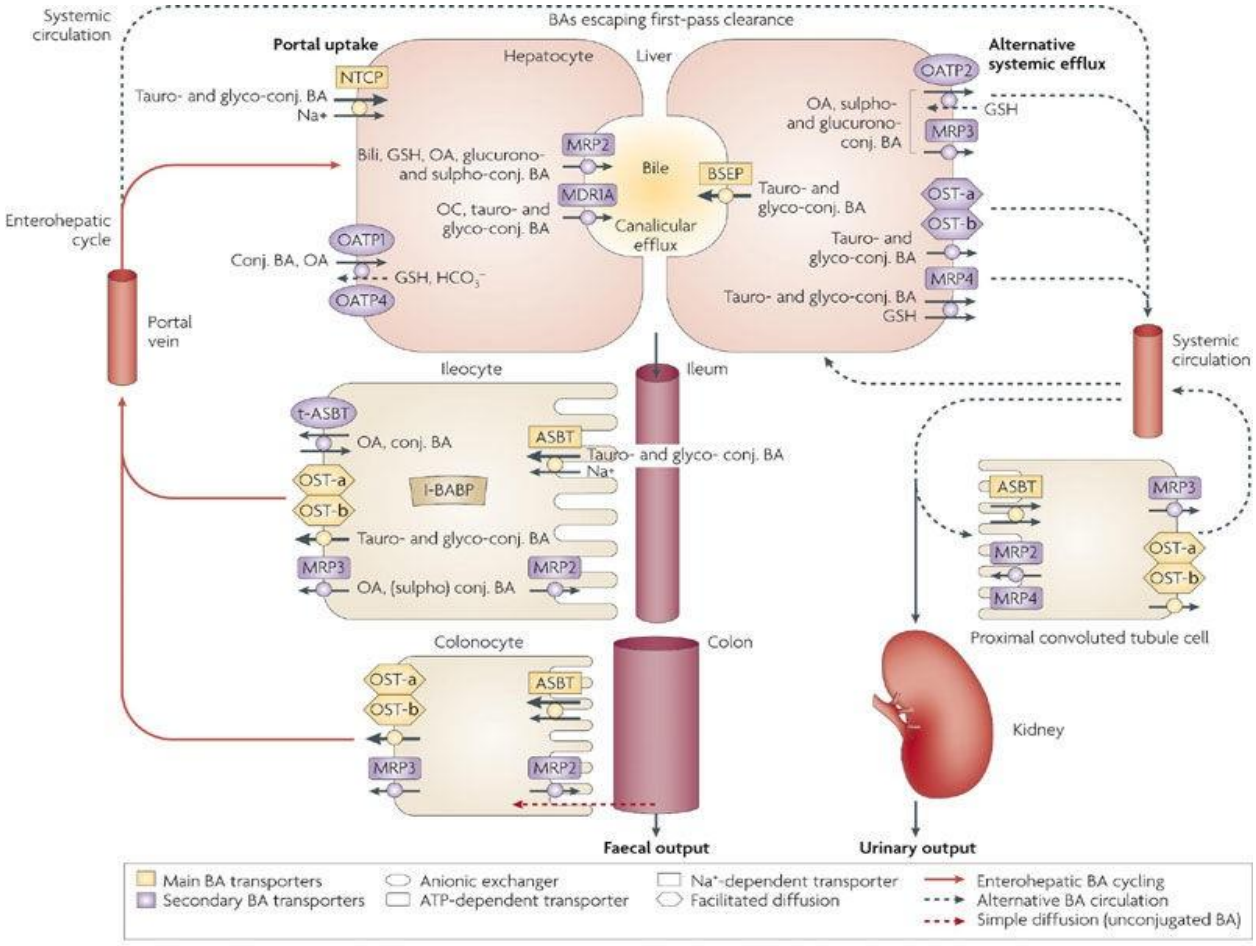

Figure 02: Overview of the bile-acid transport system

Once hepatic bile acid (BA) biosynthesis is completed, monovalent tauro- and glyco-conjugated (conj.) BAs are excreted in the bile canaliculi through the canalicular bile-salt export pump (BSEP).**[54,55]** At the same time, divalent BAs with two negative charges — that is, sulphated (sulpho-) or glucuronidated (glucurono-) BAs amidated with a taurine or a glycine, organic anions (OAs), organic cations (OCs) and reduced glutathione (GSH) — are taken up by the multidrug resistance-associated protein 2 (MRP2, ABCC2) and the multidrug export pump 1a (MDR1A, ABCB1A) to be excreted into the bile along with cholesterol and phospholipids.**[54,55]** At the basolateral membrane of hepatocytes, BA efflux is an important spillover route for BA and bilirubin (bili) that has accumulated during BA overload under cholestatic conditions.**[56]** The most established basolateral export transport systems are mediated by members of the MRP family including MRP3 (ABCC3) and MRP4 (ABCC4), but the organic anion transporting polypeptide 2 (OATP2, SLCO1A4) and the recently identified heteromeric organic solute transporter-α/β (OST-α/β) also provide alternative excretion routes for BAs and other OAs into the systemic circulation.**[54,55]** Under normal conditions, bile is stored in the gall bladder and released into the intestinal lumen upon feeding. BAs ensure the digestion and absorption of ingested lipids and are afterwards extensively reclaimed by the terminal ileum via the apical Na+-dependent

bile-salt transporter (ASBT, SLC10A2) and effluxed by OST-α/β, MRP3 and a truncated form of ASBT (t-ASBT).**[57]** Intracellularly, the ileal bile-acid-binding protein (I-BABP, FABP6) promotes BA flux and protects ileocytes against the deleterious effect of BAs. MRP2 and MRP4, present at the apical membrane of enterocytes and the proximal renal tubules, respectively, may ensure apical excretion of BAs. Similar mechanisms for the reuptake of BAs exist in cholangiocytes (not illustrated on the figure), colonocytes and proximal convoluted renal tubules, thus limiting the loss of BA via faeces and urine.**[54,55]** In the ileum, BAs are recycled to the liver (enterohepatic cycling) where they are mainly taken up by the Na+-taurocholate cotransporting polypeptide (NTCP) and to a lesser extent by organic anion transporter 1 (OATP1, SLCO1A1) and OATP4 (SLCO1B2).**[54,55]** After the first hepatic pass, BAs that have not been cleared (less than 10%) are filtrated by the renal glomerulus and reabsorbed by epithelial cells of the proximal convoluted tubules of the kidney. Together, these transport systems minimize faecal and urinary loss of BAs. Although BAs are virtually absent in the urine of healthy subjects, they become easily detectable upon cholestasis.**[56]** This is due to the decreased renal absorption of systemic BAs in order to promote the urinary excretion of toxic BAs accumulating in the liver, further underscoring the relevance of the adaptive response of BA transporters in conditions of BA overload.

STRUCTURE AND FUNCTION OF FARNESOID-X- ACTIVATED RECEPTOR:

Farnesoid X Receptor

The farnesoid x receptor protein, whose name is derived from its ability to be activated by supra physiological Levels of farnesol **[58]**, belongs to a large family (~100 members) of transcription factors called nuclear receptors. Nuclear receptors share several structural features, including an amino-terminal, highly conserved DNA binding domain and a cooh-terminal ligand binding domain. **[59]** DNA-binding domains direct nuclear receptors to specific DNA sequences [also known as response elements] in the promoters of various target genes, while Ligand binding domains interact directly with small lipophilic molecules and serve as molecular switches. Upon ligand binding, the nuclear receptors can recruit transcriptional coactivators or co-repressors and regulate transcription of the target genes. Because of this intrinsic property of ligand dependency, nuclear receptors are strong candidates for linking physiological, lipophilic signaling molecules with transcriptional responses. Several nuclear receptors have been shown to serve as receptors or sensors of small molecules with great relevance to physiology and metabolism. Two examples of this phenomenon are: (1) peroxisome proliferator- activated receptor gamma, which is involved in energy bile acid lance regulation And is activated by assorted fatty acids and their metabolites **[60]** and (2) liver x receptor which is activated by oxysterols such as 24, 25 epoxycholesterol and plays a role in cholesterol metabolism. **[61, 62, 63]** In the case of FXR, the cognate ligands are bile acids. **[64, 65, 66]** Binding of bile acids to FXR has been demonstrated by two methods.

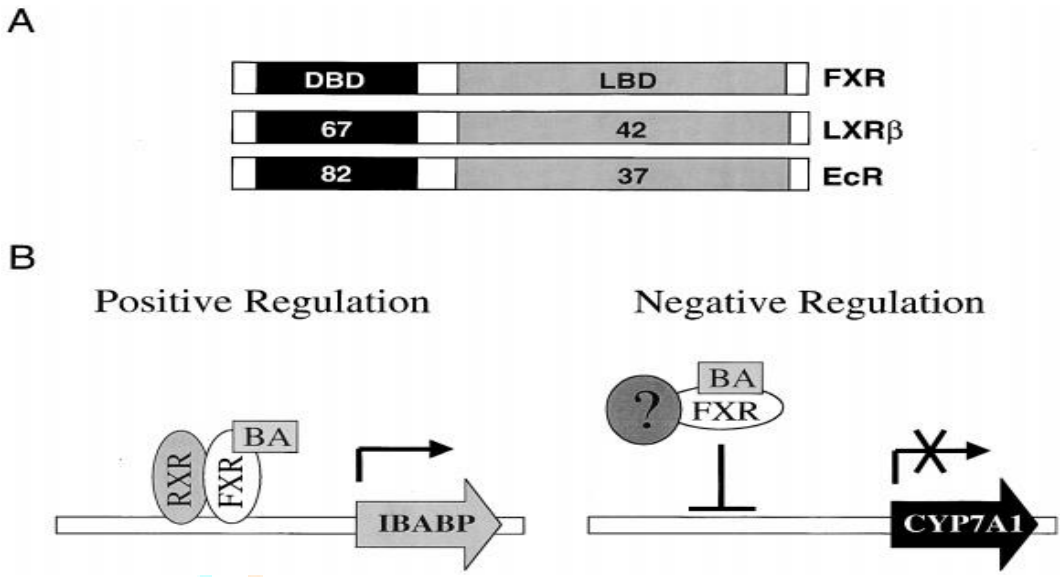

Figure 03 : Structure and function of farnesoid X receptor. (a) Farnesoid x receptor contains an n-terminal DNA binding domain (*black box*) and a c-terminal ligand binding domain. The percent homologies of both the DNA binding domain and ligand binding domain of FXR to the two closest members of the nuclear receptor family, liver x receptor B and the *drosophila* ecdysone receptor, are shown. (b) two modes of transcriptional regulation by FXR are shown. Positive regulation: FXR activates intestinal bile acid binding protein by binding directly to the bile acid BP promoter as a farnesoid x receptor /retinoid x receptor heterodimer. Negative regulation: FXR inhibits *cyp7a1* (*black arrow*) gene expression through an as yet unknown mechanism. Bile acid.

The first was a biochemical approach bile acid based on ligand induced conformational changes, where fluorescent energy transfer could be demonstrated between FXR and a co-activator peptide upon binding of bile acids. **[67, 68]** Such bile acid binding can be confirmed by ELISA **[69]** and electrophoretic mobility shift assays. **[70]** The second approach utilized a cell-bile acid based assay that measured directly the ability of bile acids to stimulate transcription of a reporter gene in the presence of FXR. **[71,72,73]** Taken together, these experiments demonstrated that bile acids are *bona fide* ligands for FXR with EC50 values of about 10–15 μ m, levels well within the physiological range.

SIGNAL TRANSDUCTION MECHANISM OF FARNESOID X RECEPTOR:

Hepatitis B Virus Infection, FXR, and Hepatocellular Carcinoma (HCC):

It has been established that viral infection is the most important causal factor of HCC. **[74-76]** From the initial hepatitis B virus infection and acute hepatitis B to the patients who are already infected and became chronic hepatitis B and developed into hepatic cirrhosis and /or long latency of HCC, the exact molecular bile acid sis for hepatitis B virus to cause HCC remains poorly defined. Two mechanisms have been proposed for hepatitis B virus-induced hepatocarcinogenesis: the theory of chromosomal integration of hepatitis B virus DNA and the theory of transcriptional activation mediated by HBx.**[77,88]** According to the integration theory, the integration of Hepatitis B Virus DNA can influence cellular gene transcription, disturb immune system, cause chronic inflammation, and result in fibrosis, cirrhosis, and HCC. HBx is the central player in the transcriptional activation theory.**[89,80,81]** HBx, the smallest of four ORFs of hepatitis B virus genome that encodes a154-amino acid protein, was considered to be an important etiological factor in hepatitis B virus-associated HCC.**[82–85]** HBx can modulate host cell signal transduction and directly or indirectly affect host and viral gene expression. HBx has been shown to trans activate and upregulate proto- oncogenes Ras, c-Fosand TGF-

beta; to silence the tumor suppressor gene p53, and to affect the NF-kappa B, PKA, pTEN, and WNT signaling pathways.**[86-99]** In HBx transfected cells, HBx is located not only in the cytoplasm, but also to some extent, in the nucleus of transfected cells.**[100]** Intracellular localization change of HBx is consistent with stimulation of transcription by activating nuclear receptor signaling pathways as a co-factor or co-activator. **[101-111]**

It is known that bile acid metabolism is a critical function of the liver, which is often impaired in chronic hepatitis B and cirrhosis. Several lines of evidence strongly suggested that endogenous bile acids are both pro-inflammatory and carcinogenic.**[112**] Long-term exposure to toxic bile acids may trigger continuous inflammation, uncontrolled liver cell proliferation or irregular liver regeneration.**[113,114-118]** Liver regeneration is an important function to repair injury, and accumulation of bile acids has been shown to contribute to liver regeneration in hepatocarcinogenesis. **[119,120,121**] For these reasons, changes in individual serum bile acids levels have been proposed as early indicators of hepatocarcinogenesis. Normal liver regeneration is dependent on FXR signaling pathway. **[122]** reported that treatment of wild type mice with dietary cholic acid accelerated liver regeneration in the partial hepatectomy model. The effect of cholic acid on liver regeneration was attenuated in FXR null mice. Inhibition of NF-kappa B is known to have a protective role in preventing liver injury and promote liver regeneration. Wang and colleagues reported that FXR could selectively inhibit the NF-kappa B mediated hepatic inflammatory responses, whereas activation of NF-kappa B suppressed farnesoid x receptor -mediated gene expression. **[123]** FXR was also found to regulate the activation of liver natural killer (NK) T cells in acute hepatitis and innate immunity. **[124,125]** Consistent with the protective role of FXR , null mice have been reported to have elevated serum and liver bile acid levels, serious inflammation reactions, fibrosis, apoptosis, and spontaneous liver tumors. **[126,127]**

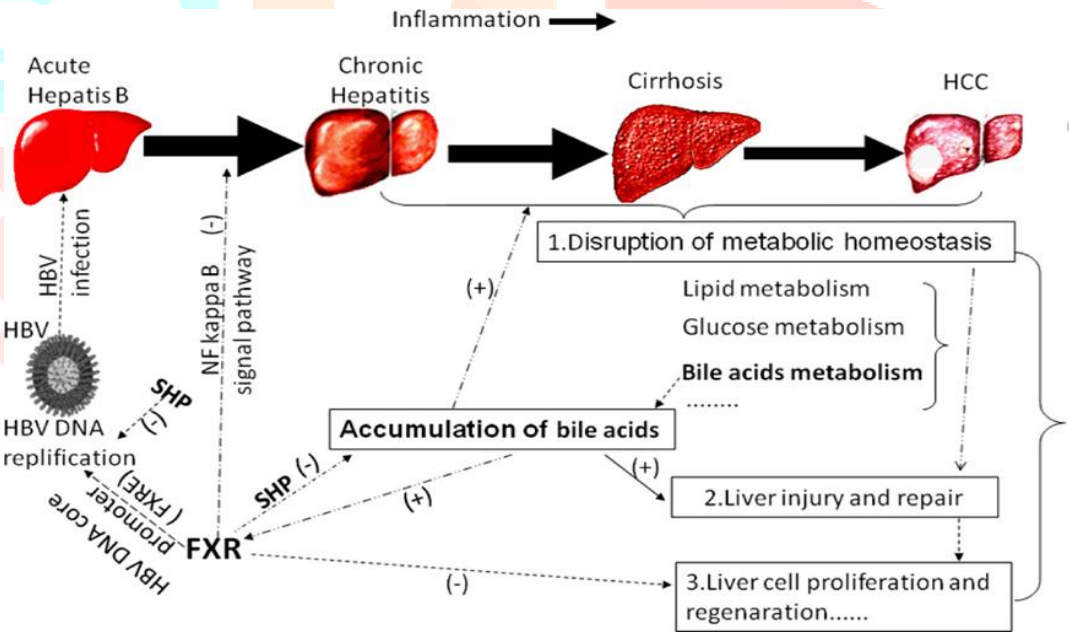

Figure 04 : Model of multiple roles of bile acid-FXR signaling pathway in hepatitis B virus infection associated HCC. In response to the accumulation of bile acids, bile acid-FXR signaling pathway is involved in hepatitis B virus DNA replication, inflammation response, liver cell proliferation, and liver regeneration in hepatocarcinogenesis.

In contrast, activation of FXR Has been found to alleviate age related proliferation in regenerating mouse livers by interacting with the transcription factor forkhead box m1b. **[128]** The interaction between FXR and PPAR was found to play a role in the anti-fibrotic activity of FXR in a rodent model of liver cirrhosis.**[129]** small

heterodimer partner (SHP), as a primary FXR target gene, also plays a role in HCC. Huang and colleagues observed spontaneous liver tumors from adeno carcinomas to carcinomas with massive hepatocyte proliferation in 55% of 15-month old SHP null mice, suggesting that SHP is an anti-oncoprotein. SHP may suppress tumorigenesis by modulating cyclin D1 expression and targeting the mitochondria. **[130,131]**

Table 1: Reported Farnesoid X Receptor Ligands

Year	Function	Compounds	Structure type	Source	References
1995	Agonist	Farnesol Farnesol metabolites	Terpenoid	Nature	Forman et al. (1995)
1999	Agonist	CDCA DCA LCA	Steroids	Nature	Makishima et al. (1999)
		TTNPB	Aromatic	Synthesis	Parks et al. (1999)
1999	Agonist	CDCA CA DCA LCA	Steroid	Nature	Wang et al. (1999)
2000	Agonist	Forskolin	Steroid	Nature	Howard et al. (2000)
2000	Agonist	GW4064	Aromatics	Synthesis	Maloney etal.(2000)
2001	Agonist	GW9047 1,1-Bisphosphonate esters	Aromatics	Synthesis	Niesor et al. (2001)
2002	Agonist	6-ECDCA	Steroid	Semi-synthesis	Pellicciari et al.(2002)
2003	Agonist	Fexaramine	Aromatics	Synthesis	Downes et al. (2003)
2003	Antagonist	Guggulsterone	Steroid	Nature	Urizar et al(2002)
2003	Agonist	Fexaramate Fexarene Fexaramine Fexarine Fexarchloramide	Aromatics	Synthesis	Nicolaou et al.(2003)
2003	Agonist Antagonist	AGN29 AGN 31	Aromatics	Synthesis	Dussault et al. (2003)
2004	Agonist	UDCA	Steroid	Nature	Lew et al. (2004)
2004	Agonist	CDCA derivatives	Steroid	Semi-synthesis	Pellicciari et al. (2004)
2004	Antagonist	Arachidonic acid Fatty acid Docosahexaenoic acid Linolenic acid		Nature	Zhao et al. (2004)
2005	Agonist	Xanthohumol	Aromatics	Nature	Nozawa (2005)
2006	Agonist	22 (R)-hydroxycholesterol	Steroid	Nature	Deng et al. (2006)
2006	Agonist Antagonist	Bile alcohols 5b-cyprinol Bile alcohols 5b-bufol Bile alcohols 5a-cyprinol Bile alcohols 5a-bufol	Steroid	Semi-synthesis	Nishimaki-Mogami etal.(2006)
2006	Agonist	Androsterone	Steroid	Nature	Wang et al. (2006)
2006	Agonist	Diphenylmethane skeleton	Steroid	Synthesis	Kainuma et al. (2006)
2007	Antagonist	GW4064 derivatives	Aromatics	Semi-synthesis	Kainuma et al. (2007)
2007	Antagonist	Stigmasterol	Steroid	Nature	Carter et al. (2007)
2007	Agonist	Cafestol	Terpenoid	Nature	Ricketts et al. (2007)
2008	Agonist	GSK8062	Aromatics	Semi-synthesis	Akwabi-Ameyaw et al. (2008)
2008	Agonist	Pyrazolidine-3,5-dione derivatives	Aromatics	Semi-synthesis	Deng et al. (2008)
2008	Agonist	Coumestrol	Steroid	Nature	Takahashi et al. (2008)
2008	Agonist	Methyl cholate Methyl deoxycholate	Steroid	Semi-synthesis	Suzuki et al. (2008)

		5b-Cholanic acid 5b-Cholanic acid-7a,12a-diol NIHS700 MarchantinA Aromatics Nature Marchantin E			
2009	Agonist	Froglitazone Rosiglitazone Pioglitazone	Alkaloid	Synthesis	Kaimal et al. (2009)
2009	Agonist	WAY-362450	Alkaloid	Synthesis	Flatt et al. (2009)
2009	Agonist	Pyrrole[2,3-d] azepines	Alkaloid	Semi-synthesis	Mehlmann et al. (2009)
2009	Agonist	N-oxide pyridine GW4064	Aromatics		Feng et al. (2009)
2009	Agonist	Bile alcohols	Steroid	Semi-synthesis	Iguchi et al. (2010)
2010	Antagonist	Oleanolic acid Terpenoid	Nature		Shneider 2001
2011	Agonist	GSK2324	Aromatics	Semisynthesis	Bile acid ss et al. (2011)
2011	Antagonist	Sulfated sterols	Steroid	Semi-synthesis	Sepe et al. (2011)
2011	Agonist	6a-Ethyl-24-norcholanyl-23-amine derivate	Steroid	Semi-synthesis	Gioiello et al. (2011)
2011	Antagonist	Tuberatolides	Terpenoid	Nature	Choi et al. (2011)

[132- 167]

Chemical Structures of FXR ligands:

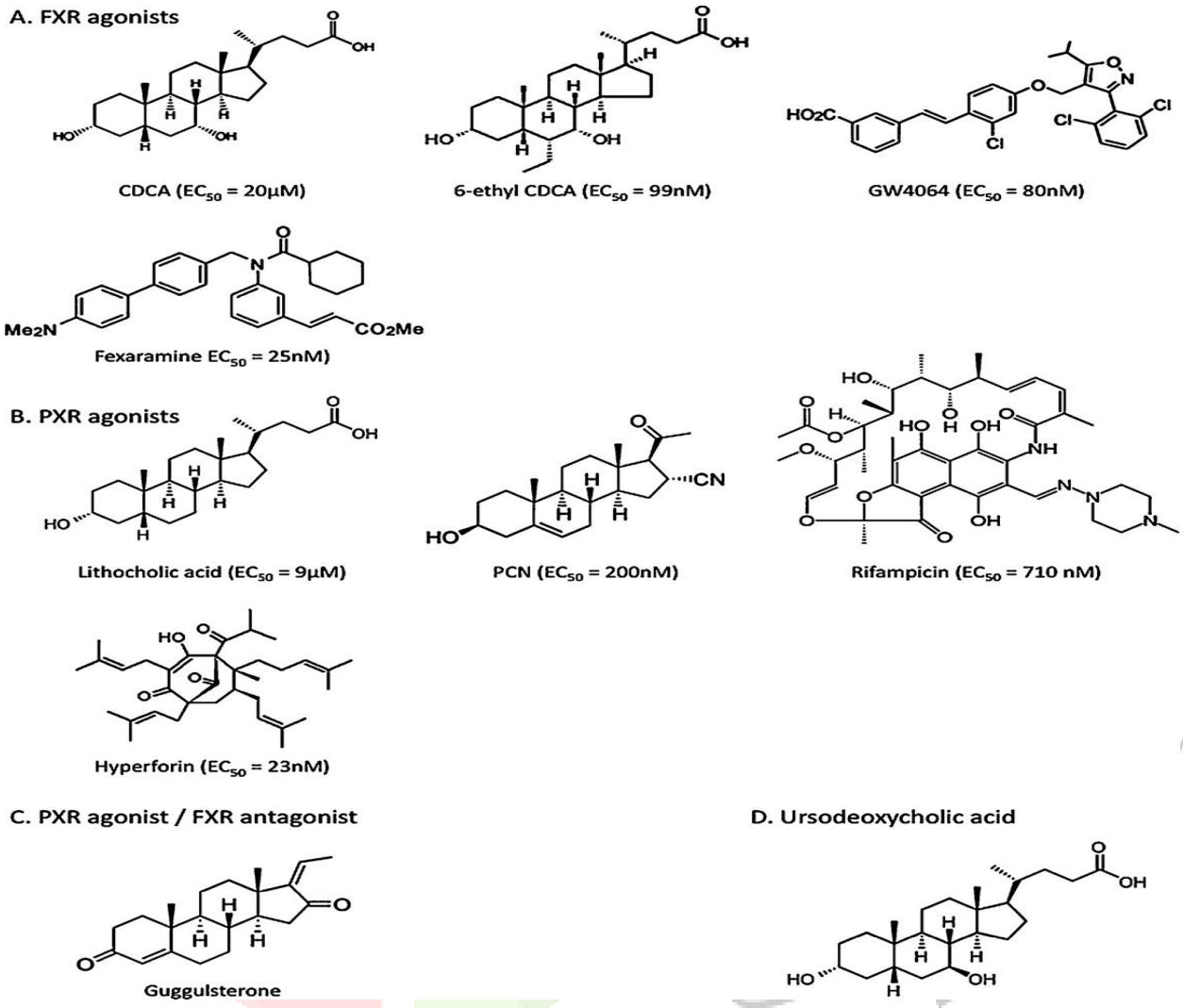

Figure 5 : Structures of ligands that interact with FXR and or PXR. Compounds that are agonists for FXR or PXR are shown in panels (A) and (B), respectively. The effective agonist concentration that elicits 50% of maximum activation of the respective human receptor (EC50) is provided, with the exception of PCN, a selective mouse PXR ligand, where the EC50 for mouse PXR is shown. Guggulsterone, a promiscuous nuclear receptor ligand that acts as both an FXR antagonist and a PXR agonist, is shown in (C). Ursodeoxycholic acid, a naturally occurring epimer of chenodeoxycholic acid that is used in the therapy of some cholestatic liver disorders, but is not thought to significantly interact with nuclear receptors, is shown for comparative purposes in (D). CDCA, chenodeoxycholic acid; PCN, pregnenolone 16α carbonitrile.**[168]**

Table: 2 Main Traditional Chinese Medicine (TCM) plants and their major compounds; **[169]**

Latin name	Plant part	Compound	Nuclear Receptor (NR)
Angelica sinensis	Root	N-butylidenephthalide (BP)	NGFI-B
Ganoderma lucidum	Carpophore	Ergosterol peroxide, ganodermanontriol	FXR
Ginkgo biloba	Seed	Quercetin, kaempferol	CAR, PXR
		Ginkgolides A and B	PXR
Glycyrrhizae uralensis	Root	Liquiritigenin	ER
Inula japonica	Flower	Bigelovin	RXR
Magnolia officinalis	Bark and flower buds	Honokiol	PPAR
Momordica charantia	Fruit	9c, 11t, 13t-conjugated linolenic acid, DMC, 3 beta,7 beta-dihydroxy-25-methoxycucurbita- 5,23-diene-19-al)	PPAR
Panax ginseng	Root, leaves	Ginsenosides (Rg1, Re, Rb1)	ER, GR
Panax notoginseng	Root	Dammarane-type saponins	FXR, LXR
Psoralea corylifolia	Seed	Bakuchiol	ER
Pueraria lobata	Root	Puerarin	ER
Rheum palmatum	Root	Rhein,	LXR, RXR
		Danthron	RXR
Rhizoma drynariae	Root	Naringin, naringenin	ER
Salvia miltiorrhiza	Root	Cryptotanshinone	AR
		Tanshinone IIA	PPAR
		Rosmarinic acid, salvianolic acid B	FXR, LXR
Schisandra chinensis	Fruit seed	Schisandrols A and B, Schisandrins A and B	PXR
Tithonia diversifolia	Leaves	Tirotundin, tagitinin A	PPAR
Tripterygium wilfordii	Root	Celastrol, triptolide	AR
Venenum Bufonis	Venom	Bufalin	VDR

Table : 3 Traditional Chinese Medicine (TCM) Effects (Activation/Inhibition) On Nuclear Receptor;

Name	Pharmacology	Activity	TCM	Active Component
TR	Endocrine/Metabolic	–		
RAR	Endocrine/Metabolic	A	Buzhong Yiqi	n-hexadecanoic acid
PPAR	Metabolic	A	Gynostemma pentaphyllum	Gypenoside XLIX
		A	*Citrus aurantium*	Naringenin
		I	Danshen	Tanshinone IIA
REVERB	Orphan	-		
ROR	Metabolic	-		
LXR	Metabolic	I	Rheum palmatum	Rhein
FXR	Metabolic	A	Ganoderma lucidum	lanostanes triterpenes
		I	*Various TCM*	oleanolic acid
VDR	Endocrine/Metabolic	A	Ch'an Su	Bufalin
PXR	Metabolic	A	Danshen	Tanshinone IIA
CAR	Metabolic	A	Yin Zhi Huang	6,7-dimethylesculetin
HNF4	Metabolic	I	Xiao-Chai-Hu-Tang	Extract of HD-1S
RXR	Metabolic	I	Rhubarb	Danthron
TR2, TR4	Orphan	-		
TLX, PNR	Orphan	-		
COUP-TF	Orphan	-		
ER	Endocrine	A	Rhizoma drynariae	Naringenin
		A	Panax ginseng	Ginsenoside Rg1
		I	Tripterygium wilfordii	Triptolide
ERR	Metabolic	A	Scutellaria baicalensis	Baicalin
GR	Endocrine	A	Panax ginseng	Ginsenoside Rg1
		I	Paeoniae Rubra Radix	PGG, NPF
MR	Endocrine	-		
PR	Endocrine	-		
AR	Endocrine	I	Tripterygium wilfordii Hook	Celastrol, Triptolide
NGFIB	Orphan	A	Antiaris toxicaria	Toxicarioside D
NURR1	Orphan	A	Bushen Huoxue Decoction	Decoction
SF1, LRH1	Orphan	-		
GCNF1	Orphan	-		
DAX1, SHP	Orphan	-		

Table 4 : Bile Acids and FXR Agonists: Current Clinical Status

Compound	Clinical Status	Target	Target Disease	Effect
Bile acid (BA) sequestrant	Approved	Increased BA excretion	Hyperlipidemia Type 2 diabetes	Increased hepatic LDLR Decreased plasma LDL Increased insulin sensitivity
Cholic acid	Approved	Provide bile acids	Inborn errors of BA	Increased lipid

		(BAs) for lipid absorption	metabolism that inhibit BA synthesis	absorption
CDCA	Approved	Replacement BA	Cerebrotendinous xanthomatosis	Improved liver function Reduce endogenous BA synthesis
UDCA	Approved	Gallstone dissolution	Gallstones	Solubilize cholesterol gallstones
UDCA	Approved	Liver	Primary biliary cirrhosis (PBC)	Improved liver function
INT-747 (6-ECDCA; obeticholic acid/OCA)	Phase III (2011)	FXR	Type 2 diabetes	Improved insulin sensitivity
OCA	Phase I (2011)	FXR	Nonalcoholic steatohepatitis (NAFLD)	Improved insulin sensitivity Improved liver function
OCA	Phase III (2011)	FXR	PBC	Improved liver function
PX-102	Phase I (2011)	FXR	Metabolic syndrome NAFLD	Improved insulin sensitivity Improved liver function
nor-UDCA	Phase I (2011)	Liver	Sclerosing cholangitis	Improved liver function

Clinically approved treatments that involve bile acid sequestrants, bile acids, or specific FXR agonists are listed, together with their target disease and real or potential beneficial effect. Where noted, FXR agonists are in phase I–III clinical trials. LDL, low-density lipoprotein; LDLR, LDL receptor. **[170,171]**

Table 5 : Bile acid pool, FXR-α and TGR5 modulators in the clinic [172-185]

Compound	**Primary indication**	**Status**	**Reported impact on metabolism**	**Reference**
FXR- α agonists				
CDCA	Cholelithiasis, cerebrotendinous xanthomatosis	Not marketed in the United States or European Union	Lowers serum TG levels*‡	172,173
GW4064	Cholelithiasis	Preclinical	Improves insulin sensitivity*, lowers serum TG levels*	174,175
INT-747 (6a-ethyl-CDCA)	Primary biliary cirrhosis, non-alcoholic fatty liver disease	Phase II	Reduces hepatosteatosis*	176,177
FXR-450	Hypertriglyceridaemia	Phase I	Lowers hepatic lipids, LDL-C and TG levels*	178
Fexaramine	Hypercholesterolaemia	Preclinical	Lowers serum cholesterol	179,180
FXR-α				

modulators				
AGN-34	Hypercholesterolaemia, colon cancer	Preclinical	Lowers serum cholesterol	181
Guggulsterone	Hypercholesterolaemia	Traditional medicine	Lowers serum cholesterol*	182,183
Bile-acid pool modulators				
Bile acid-binding resins (e.g., cholestyramine)	Hypercholesterolaemia	Approved	Improves insulin sensitivity*, body-weight loss*	184,185,186
ASBT inhibitors (SC-435, S-8921, S-0960)	Hypercholesterolaemia	Preclinical	Lowers serum cholesterol	187,188
Ursodeoxycholic acid	Cholelithiasis, primary biliary cirrhosis	Approved	Improves insulin sensitivity*	189
Cholic acid	3β-Hydroxysteroid oxido-reductase deficiency	Not approved in the European Union	Improves insulin sensitivity*, increases energy expenditure*, lowers serum TG levels*	190,191
TGR5 agonists				
Oleanolic acid	Various cancers (e.g., skin, lung, colon)	Phase II	Improves insulin sensitivity*	192
6α-ethyl,23(*S*)-methyl-CDCA	Metabolic diseases	Preclinical	Not reported	193

*Study performed in rodents. ‡Study performed in humans. ASBT, apical sodium bile-salt transporter; CDCA, chenodeoxycholic acid; FXR-a, farnesoid X receptor-a; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TGR5, also known as G-protein coupled bile acid receptor 1 (GPBAR1). **[186-193]**

REFERENCES:

1. Seol, W., Choi, H.S. and Moore, D.D. (1995) Mol. Endocrinol. 9, 72–85.

2. Forman BM, Goode E, Chen J, et al. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell 1995;81:687-93. [Pub Med]

3. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature 1990;343:425-30. [Pub Med]

4. 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. and Lehmann, J.M. (1999) Science

284, 1365–1368.

5. Makishima, M., Okamoto, A.Y., Repa, J.J., Tu, H., Learned, R.M., Luk, A., Hull, M.V., Lustig, K.D., Mangelsdorf, D.J. and Shan, B. (1999) Science 284, 1362–1365.
6. Maloney, P.R., Parks, D.J., Haffner, C.D., Fivush, A.M., Chandra, G., Plunket, K.D., Creech, K.L., Moore, L.B., Wilson, J.G, Lewis, M.C., Jones, S.A. and Willson, T.M. (2000) J. Med. Chem. 43, 2971–2974.
7. Pellicciari, R., Fiorucci, S., Camaioni, E., Clerici, C., Costantino, G., Maloney, P.R., Morelli, A., Parks, D.J. and Willson, T.M. (2002) J. Med. Chem. 45, 3569–3572.
8. Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G. and Gonzalez, F.J. (2000) Cell 102, 731–744.
9. Davis, R.A., Miyake, J.H., Hui, T.Y. and Spann, N.J. (2002) J. Lipid Res. 43, 533–543.

10. Hwang, S.T., Urizar, N.L., Moore, D.D. and Henning, S.J. (2002) Gastroenterology 122, 1483–1492.
11. Plass, J.R., Mol, O., Heegsma, J., Geuken, M., Faber, K.N., Jansen, P.L. and Muller, M. (2002) Hepatology 35, 589–596.
12. Denson, L.A., Sturm, E., Echevarria, W., Zimmerman, T.L., Makishima, M., Mangelsdorf, D.J. and Karpen, S.J. (2001) Gastroenterology 121, 140–147.
13. Urizar, N.L., Dowhan, D.H. and Moore, D.D. (2000) J. Biol. Chem. 275, 39313–39317.
14. Claudel, T., Sturm, E., Duez, H., Torra, I.P., Sirvent, A., Kosykh, V., Fruchart, J.C., Dallongeville, J., Hum, D.W., Kuipers, F. and Staels, B. (2002) J. Clin. Invest. 109, 961–971.
15. Pineda Torra, I., Claudel, T., Duval, C., Kosykh, V., Fruchart, J.C. and Staels, B. (2003) Mol. Endocrinol. 17, 259–272.
16. Kast, H.R., Nguyen, C.M., Sinal, C.J., Jones, S.A., Laffitte, B.A., Reue, K., Gonzalez, F.J., Willson, T.M. and Edwards, P.A. (2001) Mol. Endocrinol. 15, 1720–1728.
17. Claudel, T., Inoue, Y., Bile acid rbier, O., Duran-Sandoval, D., Kosykh, V., Fruchart, J., Fruchart, J.C., Gonzalez, F.J. and Staels, B. (2003) Gastroenterology 125, 544–555.
18. Wieland, H.O. (1966). Nobel Prize Lecture (1928): the chemistry of the bile acids. In Nobel Lectures, Chemistry 1922–1941 (Amsterdam: Elsevier PublishingCompany).
19. Angelin, B., Larsson, T.E., and Rudling, M. (2012). Circulating fibroblast growth factors as metabolic regulators—a critical appraisal. Cell Metab. 16, 693–705.
20. Chiang, J.Y. (2009). Bile acids: regulation of synthesis. J. Lipid Res. 50, 1955–1966.
21. Keitel, V., and Ha¨ ussinger, D. (2012). Perspective: TGR5 (Gpbar-1) in liver physiology and disease. Clin. Res. Hepatol. Gastroenterol. 36, 412–419.
22. Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., and Staels, B. (2009). Role of bile acids and bile acid receptors in metabolic regulation. Physiol. Rev. 89, 147–191.
23. Matsubara, T., Li, F., and Gonzalez, F.J. (2013). FXR signaling in the enterohepatic system. Mol. Cell. Endocrinol. 368, 17–29.
24. Pols, T.W., Noriega, L.G., Nomura, M., Auwerx, J., and Schoonjans, K.(2011b). The bile acid membrane receptor TGR5: a valuable metabolic target. Dig. Dis. 29, 37–44.
25. Porez, G., Prawitt, J., Gross, B., and Staels, B. (2012). Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. J. Lipid Res. 53, 1723–1737.
26. Russell, D.W. (2003). The enzymes, regulation, and genetics of bile acid synthesis.
Annu. Rev. Biochem. 72, 137–174.
27. Russell, D.W. (2009). Fifty years of advances in bile acid synthesis and metabolism. J. Lipid Res. 50(Suppl), S120–S125.
28. Thomas, C., Pellicciari, R., Pruzanski, M., Auwerx, J., and Schoonjans, K. (2008). Targeting bile-acid signalling for metabolic diseases. Nat. Rev. Drug Discov. 7, 678–693.
29. Ananthanarayanan M, Balasubramanian NV, Makishima M, J.Y.L. Chiang / Journal of Hepatology 40 (2004) 539–551 549 Mangelsdorf DJ, Suchy FJ. Human bile salt export pump (BSEP) promoter is transactivated by the farnesoid X receptor/bile acid receptor (FXR/BAR). J Biol Chem 2001; 276:28857–28865.
30. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, et al. Regulation of Multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane x receptor, farnesoid x-activated receptor, and constitutive androstane receptor. J Biol Chem 2002; 277:2908–2915.

31. Yu L, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, et al. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J Clin Invest 2002;110:671–680.
32. Wong MH, Oelkers P, Craddock AL, Dawson PA. Expression cloning and characterization of the hamster ileal sodium- dependent bile acid transporter. J Biol Chem 1994;269:1340–1347.
33. Grober J, Zaghini I, Fujii H, Jones SA, Kliewer SA, Willson TM, et al. 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.
34. Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, et al. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. Gastroenterology 2001;121:140–147.
35. Urizar NL, Moore DD. A gugulipid: natural cholesterol-lowering agent. Annu Rev Nutr 2003;.
36. Urizar NL, Liverman AB, Dodds DT, Silva FV, Ordentlich P, Yan Y, et al. A natural product that lowers cholesterol as an antagonist ligand for FXR. Science 2002;296:1703–1706.
37. Cui J, Huang L, Zhao A, Lew JL, Yu J, Sahoo S, et al. Guggulsterone is an FXR antagonist in coactivator association assays but acts to enhance transcription of bile salt export pump. J Biol Chem 2003; 278:10214–10220.
38. Owsley E, Chiang JY. Guggulsterone antagonizes farnesoid X receptor induction of bile salt export pump but activates pregnane X receptor to inhibit cholesterol 7a-hydroxylase gene. Biochem Biophys Res Commun 2003;304:191–195.
39. Wu J, Xia C, Meier J, Li S, Hu X, Lala DS. The hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor. Mol Endocrinol 2002;16:1590–1597.
40. Dussault I, Beard R, Lin M, Hollister K, Chen J, Xiao JH, et al. Identification of gene-selective modulators of the bile acid receptor FXR. J Biol Chem 2002.
41. Russell, D. W. The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.* 72, 137–174 (2003).
42. Schwarz, M. *et al.* Disruption of cholesterol 7α-hydroxylase gene in mice. II. Bile acid deficiency is overcome by induction of oxysterol 7α-hydroxylase. *J. Biol. Chem.* 271, 18024–18031 (1996).
43.Ishibashi, S., Schwarz, M., Frykman, P. K., Herz, J. & Russell, D. W. Disruption of cholesterol 7α-hydroxylase gene in mice. I. Postnatal lethality reversed by bile acid and vitamin supplementation. *J. Biol. Chem.* 271, 18017–18023 (1996).

44. Beigneux, A., Hofmann, A. F. & Young, S. G. Human CYP7A1 deficiency: progress and enigmas. *J. Clin. Invest.* 110, 29–31 (2002).
45. Pullinger, C. R. *et al.* Human cholesterol 7α-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J. Clin. Invest.* 110, 109–117 (2002).
46.Salen, G. Cholestanol deposition in cerebrotendinous xanthomatosis. A possible mechanism. *Ann. Intern. Med.* 75, 843–851 (1971).
47. Salen, G. & Grundy, S. M. The metabolism of cholestanol, cholesterol, and bile acids in cerebrotendinous xanthomatosis. *J. Clin. Invest.* 52, 2822–2835 (1973).
48. Botham, K. M. & Boyd, G. S. The metabolism of chenodeoxycholic acid to β-muricholic acid in rat liver. *Eur. J. Biochem.* 134, 191–196 (1983).
49. Pandak, W. M. *et al.* Expression of sterol 12α- hydroxylase alters bile acid pool composition in primary rat hepatocytes and *in vivo*. *Gastroenterology* 120, 1801–1809 (2001).

50. Li-Hawkins, J. *et al.* Cholic acid mediates negative feedback regulation of bile acid synthesis in mice. *J. Clin. Invest.* 110, 1191–1200 (2002).
51. Falany, C. N. *et al.* Molecular cloning and expression of rat liver bile acid CoA ligase. *J. Lipid Res.* 43, 2062–2071 (2002).
52. Shonsey, E. M. *et al.* Bile acid coenzyme A: amino acid *N*-acyltransferase in the amino acid conjugation of bile acids. *Methods Enzymol.* 400, 374–394 (2005).
53. He, D., Barnes, S. & Falany, C. N. Rat liver bile acid CoA:amino acid *N*-acyltransferase: expression, characterization, and peroxisomal localization.
J. Lipid Res. 44, 2242–2249 (2003).
54. Meier, P. J. & Stieger, B. Bile salt transporters. *Annu. Rev. Physiol.* 64, 635–661 (2002).
55. Trauner, M. & Boyer, J. L. Bile salt transporters: molecular characterization, function, and regulation. *Physiol. Rev.* 83, 633–671 (2003).
56.Zollner, G., Marschall, H. U., Wagner, M. & Trauner, M. Role of nuclear receptors in the adaptive response to bile acids and cholestasis: pathogenetic and therapeutic considerations. *Mol. Pharm.* 3, 231–251 (2006).
57. Inoue, Y. *et al.* Regulation of bile acid biosynthesis by hepatocyte nuclear factor 4α. *J. Lipid Res.* 47, 215–227 (2006).
58. Forman B, Goode E, Chen J, et al.: 1995. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell 81:687–693.
59. Mangelsdorf DJ, Thummel C, Beato M, et al.: 1995. The nuclear receptor superfamily: the second decade. Cell 83:835–839.
60. Kliewer SA, Willson TM: 1998. The nuclear receptor PPARgamma bigger than fat. Curr Opin Genet Dev 8:576–581.
61. Janowski BILE ACID , Willy PJ, Devi TR, et al.: 1996. An oxysterol signaling pathway mediated by the nuclear receptor LXR alpha. Nature 383:728–731.
62. Lehmann JM, Kliewer SA, Moore LB, et al.: 1997. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J Biol Chem 272:3137–3140.
63. Peet DJ, Turley SD, Ma W, et al.: 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclearreceptor LXR alpha. Cell 93:693–704.
64. Makishima M, Okamoto A, Repa J, et al.:1999. Identification of a nuclear receptor for bile acids. Science 284:1362–1365.
65. Parks D, Blanchard S, Bledsoe R, et al.: 1999. Bile acids: natural ligands for an orphan uclear receptor. Science 284:1365–1368.
66. Wang H, Chen J, Hollister K, et al.: 1999. Endogenous bile acids are ligands for the nuclear receptor FARNESOID X RECEPTOR /BILE ACID R. Mol Cell 3:543–553.
67. Makishima M, Okamoto A, Repa J, et al.:1999. Identification of a nuclear receptor for bile acids. Science 284:1362–1365.
68. Parks D, Blanchard S, Bledsoe R, et al.: 1999. Bile acids: natural ligands for an orphan nuclear receptor. Science 284:1365–1368.
69. Makishima M, Okamoto A, Repa J, et al.: 1999. Identification of a nuclear receptor for bile acids. Science 284:1362–1365.
70. Wang H, Chen J, Hollister K, et al.: 1999. Endogenous bile acids are ligands for the nuclear receptor FARNESOID X RECEPTOR /BILE ACID R. Mol Cell 3:543–553.

71. Makishima M, Okamoto A, Repa J, et al.:1999. Identification of a nuclear receptor for bile acids. Science 284:1362–1365.
72. Parks D, Blanchard S, Bledsoe R, et al.: 1999. Bile acids: natural ligands for an orphan nuclear receptor. Science 284:1365–1368.
73. Wang H, Chen J, Hollister K, et al.: 1999. Endogenous bile acids are ligands for the nuclear receptor FARNESOID X RECEPTOR /BILE ACID R. Mol Cell 3:543–553.
74. Bile acid r-YishayI, ShaulY, Shlomai A. Hepatocyte metabolic signal-ling pathways and regulation of hepatitis B virus expression. Liver I nt 2011;31:282–90.
75.Yang JD, Roberts LR. Hepato cellular carcinoma: a globile acid l view. Nat Rev Gastroenterol Hepatol 2010;7:448–58.
76. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907–17.
77. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557–76.
78. Murakami Y, Saigo K, Takashima H, Minami M, Okanoue T, Bre´chot C, et al. Larges called analysis of hepatitis B virus(HEPATITIS B VIRUS) DNA integration in HEPATITIS B VIRUS related hepato cellular carcinomas. Gut 2005;54:1162–8.
79. Ramiere C, Scholtes C, Diaz O, I card V, Perrin-Cocon L, Trabile acid ud MA, et al. Transactivation of the hepatitis B virus core promoter by the nuclear receptor FARNESOID X RECEPTOR alpha. J Virol 2008;82:10832–40.
80. Tamori A, Nishiguchi S, Shiomi S, Hayashi T, Kobile acid yashi S, Habu D, et al. Hepatitis B virus DNA integration in hepato cellular carcinoma after interferon-induced disappearance of hepatitis C virus. Am J Gastroenterol 2005;100:1748–53.
81. Shafritz DA. Integration of Hepatitis B Virus DNA into liver and hepato cellular carcinoma cells during persistent Hepatitis B Virus infection. J Cell Biochem 1982;20:303–16.
82. Lara-Pezzi E, Go´mez-Gaviro MV, Ga´ lvez BG, Mira E, In˜ iguez MA, Fresno M, et al. The hepatitis B virus X protein promotes tumor cell invasion by inducing membrane type matrix metalloproteinase-1and cyclooxygenase-2 expression. J Cli Invest 2002;110:1831–8.
83. Feitelson MA, Lee J. Hepatitis B virus integration, fragilesites, and hepato carcinogenesis. Cancer Lett 2007;252:157–70.
84. Birrer RB, Birrer D, Klavins JV. Hepato cellular carcinoma and hepatitis virus. Ann ClinLabSci 2003;33:39–54.
85. Murakami S. Hepatitis B virus X protein: a multifunctional viral regulator. J Gastroenterol 2001;36:651–60.
86. Arbuthnot P, Kew M. Hepatitis B virus and hepatocellular carcinoma. Int JE xp Pathol 2001;82:77–100.
87. Chung TW, Lee YC, Ko JH, Kim CH. Hepatitis Bvirus X protein modulates the expression of PTEN by inhibiting the function of p53, a transcriptional activator in liver cells. Cancer Res 2003;63:3453–8.
88. Tacke F, Liedtke C, Bocklage S, Manns MP, Trautwein C. CREB/PKA sensitive signalling pathways activate and maintain expression levels of the hepatitis B virus pre-S2/ Spromoter. Gut 2005;54:1309–17.
89. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;54:4855–78.
90. Bengochea A, de Souza MM, Lefrancois L, Le Roux E, Galy O, Chemin I, et al. Common dys regulation of Wnt/Frizzled receptor elements in human hepato cellular carcinoma. Br JCancer 2008;99:143–50.
91. Harris CC. Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J Nat Cancer Inst 1996;88:1442–55.

92. Park SG, Min JY, Chung C, Hsieh A, Jung G. Tumor suppressor protein p53 induces degradation of the oncogenic protein HBx. Cancer Lett 2009;282:229–37.
93. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harrism CC. Mutational hot spot in the p53 gene in human hepato cellular carcinomas. Nature 1991;6317:427–8.
94. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepato cellular carcinoma from southern Africa. Nature 1991;6317:429–31.
95. Murakami Y, Hayashi K, Sekiya T. Detection of aberrations of the p53 alleles and the gene transcript in human tumor celllines by single-strand conformation polymorphism analysis. Cancer Res 1991;51:3356–61.
96. Kwon JA, Rho HM. Transcriptional repression of the human p53 gene by hepatitis B viral core protein (HBc) in human liver cells. Bio l Chem 2003;384:203–12.
97. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserve dhomolog, Bile acid x, that accelerates programmed cell death. Cell 1993;74:609–19.
98. Bouchard M, Giannakopoulos S, Wang EH, Tanese N, Schneider RJ. Hepatitis B virus HBx protein activation of cyclin A-cyclin-dependent kinase 2 complexes and G1transit via aSrc kinase pathway. J Virol 2001;75:4247–57.
99. Huo TI, Wang XW, Forgues M, Wu CG, Spillare EA, Giannini C, et al. Hepatitis B virus X mutants derived from human hepatocellular carcinoma retain the ability to abrogate p53- induced apoptosis. Oncogene 2001;20:3620–8.
100. Doria M, Klein N, nLucito R, Schneider RJ. The hepatitis B virus HBx protein is adual specificity cytoplasmic activator of Rasand nuclear activator of transcription factors. EMBO J 1995;14: 4747–57.

101. Han J, Ding L, Yuan B, Yang X, Wang X, LiJ, et al. Hepatitis B virus X protein and the estrogen receptor variant lacking exon 5 inhibit estrogen receptor signaling in hepatoma cells. Nucl Acids Res 2006;34:3095–106.
102. Chiu CM, YehSH, Chen PJ, Kuo TJ, Chang CJ, Chen PJ, et al. Hepatitis B virus X protein enhances androgen receptor responsive gene expression depending on androgen level. Proc Nat Acad Sci USA 2007;104:2571–8.
103. Zheng Y, Chen WL, Ma WL, Chang C, Ou JH. Enhancement of gene transactivation activity of androgen receptor by hepatitis B virus X protein. Virology 2007;363:454–61.
104. Ma WL, Hsu CL, Wu MH, Wu CT, Wu CC, Lai JJ, et al. Androgen receptor ris a new potential therapeutic target for the treatment of hepato cellular carcinoma. Gastroenterology 2008;135: 947–55.
105. Bouchard MJ, Wang L, Schneidern RJ. Activation of focal adhesion kinase by hepatitis B virus HBx protein: multiple functions in viral replication. J Virol 2006;80:4406–14.
106. Wang SH, Yeh SH, Lin WH, Wang HY, Chen DS, Chen PJ. Identification of androgen response elements in the enhancer I of hepatitis B virus: a mechanism for sex disparity in chronic hepatitis B. Hepatology 2009;50:1392–402.
107. Kim K, Kim KH, Kim HH, Cheong J. Hepatitis Bvirus X protein induces lipogenic transcription factor SREBP1 and fatty acid synthase through the activation of nuclear receptor LXR alpha. Biochem J 2008;416:219–30.
108. Na TY, Shin YK, Roh KJ, Kang SA, Hong I, Oh SJ, et al. Liver X receptor mediates hepatitis B virus X protein induced lipogenesis in hepatitis B virus-associated hepatocellular carcinoma. Hepatology 2009;49:1122–31.

109. Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, et al. Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1and PPARgamma. Gastroen-terology 2007;132:1955–67.
110. Lee MO, Kang HJ, Cho H, Shin EC, Park JH, Kim SJ. Hepatitis B virus X protein induced expression of the Nur 77gene. Biochem Biophys Res Commun 2001;288:1162–8.
111. Chen RN, Huang YH, Yeh CT, Liao CH, Lin KH. Thyroid hormone receptor ssuppress pituitary tumor transforming gene1 activity in hepatoma. Cancer Res 2008;68:1697–706.
112. Wang R, Sheps JA, Ling V. ABC transporters, bile acids, and inflammatory stress in liver cancer. Curr Pharm Biotechnol 2011;12:636–46.
113. Bile acid I C, Canfield PJ, Stacey NH. Individual serum bile acids as early indicators of carbontetrachloride induced and chloroform- induced liver injury. Toxicology 1992;75:221–34.
114. Brandsaeter B, Isoniemi H, Broome´ U, OlaussonM, B¨ ackman L, Hansen B, et al. Liver transplantation for primary sclerosing cholangitis; predictors and consequences of hepato biliary malignancy. J Hepatol 2004;40:815–22.
115. Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, et al. A Follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 1994;3:3–10.
116. Wu HC, Wang Q, Wang LW, Yang HI, Ahsan H, Tsai WY, et al.Urinary 8-oxodeoxy guanosine, aflatoxin B1exposure and hepatitis B virus infection and hepato cellular carcinoma in Taiwan. Carcinogenesis 2009;28:995–9.
117. Jansen PL. Endogenous bile acids as carcinogens. J Hepatol 2007;47:4345.
118. Oliver JD, Roberts RA. Receptor mediated hepatocarcinogenesis: role of hepatocyte proliferation and apoptosis. Pharmacol Toxicol 2002;91:1–7.
119. Fiorucci S, Cipriani S, Mencarelli A, Renga B, Distrutti E, Bile acid ldelli F.Counter-regulatory role of bile acid activated receptors in immunity and inflammation. Curr Mol Med 2010;10:579–95.
120. Ueno Y, Moriyama M, Uchida T, Arakawa Y. Irregular regeneration of hepatocytes is an important factor in the hepatocarcinogenesis of liver disease. Hepatology 2001;33:357–62.
121. Shibile acid ta M, Morizane T, Uchida T, Yamagami T, Onozuka Y, Nakano M, et al. Irregular regeneration of hepatocytes and risk of hepatocellular carcinoma in chronic hepatitis and cirrhosis with hepatitis-C virus infection. Lancet 1998;351: 1773–7.
122. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, et al. Nuclear receptor dependent bile acid signaling is required for normal liver regeneration. Science 2006;312:233–6.
123. Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor kappa B in hepatic inflammatory response. Hepatology 2008;48:1632–43.
124. Mencarelli A, Renga B, Migliorati M, Cipriani S, Distrutti E, Santucci L, et al. The bile acid sensor farnesoid X receptor is a modulator of liver immunity in a rodent model of acute hepatitis. J Immunol 2009;183:6657–66.
125. Kim MS, Shigenaga J, Moser A, Feingold K, Grunfeld C. Repression of farnesoid X receptor during the acute phase response. J Biol Chem 2003;278:8988–95.
126. Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid Xreceptor. Cancer Res 2007;67:863–7.
127. Kim I, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. Carcinogenesis 2007;28:940–6.

128. Chen WD, Wang YD, Zhang L, Shiah S, Wang M, Yang F, et al. Farnesoid X receptor alleviates age-related proliferation defects in regenerating mouse livers by activating forkhead boxm1b transcription. Hepatology 2010;51:953–62.
129. Fiorucci S, Rizzo G, Antonelli E, Renga B, Mencarelli A, Riccardi L, et al. Cross talk between farnesoid X receptor (FARNESOID X RECEPTOR) and peroxisome proliferator activated receptor gamma contributes to the antifibrotic activity of FARNESOID X RECEPTOR ligands in rodent models of liver cirrhosis. J Pharmacol Exp Ther 2005;315:58–68.
130. Zhang Y, Xu P, Park K, Choi Y, Moore DD, Wang L. Orphan receptor small hetero dimerpartner suppresses tumorigenesis by modulating cyclin D1 expression and cellular proliferation. Hepatology 2008;48:289–98.
131. Zhang Y, Soto J, Park K, Viswanath G, Kuwada S, Abel ED, et al. Nuclear receptor SHP, a death receptor that targets mitochondria, induces apoptosis and inhibits tumor growth. Mol Cell Biol 2010;30:1341–56.
132. 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., Evans, R.M., Weinberger, C., 1995. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell 81, 687–693.
133. 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., 1999. Identification of a nuclear receptor for bile acids. Science 284, 1362–1365.
134. 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., 1999. Bile acids: natural ligands for an orphan nuclear receptor. Science 284, 1365– 1368.
135. Wang, H., Chen, J., Hollister, K., Sowers, L.C., Forman, B.M., 1999. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol. Cell 3, 543–553.
136. Howard, W.R., Pospisil, J.A., Njolito, E., Noonan, D.J., 2000. Catabolites of cholesterol synthesis pathways and forskolin as activators of the farnesoid X-activated nuclear receptor. Toxicol. Appl. Pharmacol. 163, 195–202.
137. Maloney, P.R., Parks, D.J., Haffner, C.D., Fivush, A.M., Chandra, G., Plunket, K.D., Creech, K.L., Moore, L.B., Wilson, J.G., Lewis, M.C., Jones, S.A., Willson, T.M., 2000. Identification of a chemical tool for the orphan nuclear receptor FXR. J. Med. Chem. 43, 2971–2974.
138. Niesor, E.J., Flach, J., Lopes-Antoni, I., Perez, A., Bentzen, C.L., 2001. The nuclear receptors FXR and LXRalpha: potential targets for the development of drugs affecting lipid metabolism and neoplastic diseases. Curr. Pharm. Des. 7, 231–259.
139. Pellicciari, R., Fiorucci, S., Camaioni, E., Clerici, C., Costantino, G., Maloney, P.R., Morelli, A., Parks, D.J., Willson, T.M., 2002. 6 alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. J. Med. Chem. 45, 3569–3572.
140. 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., 2003. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. Mol. Cell 11, 1079–1092.
141. Urizar, N.L., Liverman, A.B., Dodds, D.T., Silva, F.V., Ordentlich, P., Yan, Y., Gonzalez, F.J., Heyman, R.A., Mangelsdorf, D.J., Moore, D.D., 2002. A natural product that lowers cholesterol as an antagonist ligand for FXR. Science 296, 1703–1706.
142. Nicolaou, K.C., Evans, R.M., Roecker, A.J., Hughes, R., Downes, M., Pfefferkorn, J.A., 2003. Discovery and optimization of non-steroidal FXR agonists from natural product-like libraries. Org. Biomol. Chem. 1, 908–920.

143. Dussault, I., Beard, R., Lin, M., Hollister, K., Chen, J., Xiao, J.H., Chandraratna, R., Forman, B.M., 2003. Identification of gene-selective modulators of the bile acid receptor FXR. J. Biol. Chem. 278, 7027–7033.
144. Lew, J.L., Zhao, A., Yu, J., Huang, L., De Pedro, N., Pelaez, F., Wright, S.D., Cui, J., 2004. The farnesoid X receptor controls gene expression in a ligand- and promoterselective fashion. J. Biol. Chem. 279, 8856–8861.
145. Pellicciari, R., Costantino, G., Camaioni, E., Sadeghpour, B.M., Entrena, A., Willson, T.M., Fiorucci, S., Clerici, C., Gioiello, A., 2004. 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. 47, 4559–4569.
146. Zhao, A., Yu, J., Lew, J.L., Huang, L., Wright, S.D., Cui, J., 2004. Polyunsaturated fatty acids are FXR ligands and differentially regulate expression of FXR targets. DNA Cell Biol. 23, 519–526.
147. Nozawa, H., 2005. Xanthohumol, the chalcone from beer hops (Humulus lupulus L.), is the ligand for farnesoid X receptor and ameliorates lipid and glucose metabolism in KK-A(y) mice. Biochem. Biophys. Res. Commun. 336, 754–761.
148. Deng, R., Yang, D., Yang, J., Yan, B., 2006. Oxysterol 22(R)-hydroxycholesterol induces the expression of the bile salt export pump through nuclear receptor farsenoid X receptor but not liver X receptor. J. Pharmacol. Exp. Ther. 317, 317–325.
149. Wang, S., Lai, K., Moy, F.J., Bhat, A., Hartman, H.B., Evans, M.J., 2006. The nuclear hormone receptor farnesoid X receptor (FXR) is activated by androsterone. Endocrinology 147, 4025–4033.
150. Kainuma, M., Kasuga, J., Hosoda, S., Wakabayashi, K., Tanatani, A., Nagasawa, K., Miyachi, H., Makishima, M., Hashimoto, Y., 2006. Diphenylmethane skeleton as a multi-template for nuclear receptor ligands: preparation of FXR and PPAR ligands. Bioorg. Med. Chem. Lett. 16, 3213–3218.
151. Kainuma, M., Makishima, M., Hashimoto, Y., Miyachi, H., 2007. Design, synthesis, and evaluation of non-steroidal farnesoid X receptor (FXR) antagonist. Bioorg. Med. Chem. 15, 2587–2600.
152. Carter, B.A., Taylor, O.A., Prendergast, D.R., Zimmerman, T.L., Von Furstenberg, R., Moore, D.D., Karpen, S.J., 2007. Stigmasterol, a soy lipid-derived phytosterol, is an antagonist of the bile acid nuclear receptor FXR. Pediatr. Res. 62, 301–306.
153. Ricketts, M.L., Boekschoten, M.V., Kreeft, A.J., Hooiveld, G.J., Moen, C.J., Muller, M., Frants, R.R., Kasanmoentalib, S., Post, S.M., Princen, H.M., Porter, J.G., Katan, M.B., Hofker, M.H., Moore, D.D., 2007. The cholesterol-raising factor from coffee beans, cafestol, as an agonist ligand for the farnesoid and pregnane X receptors. Mol. Endocrinol. 21, 1603–1616.
154. Akwabi-Ameyaw, A., Bass, J.Y., Caldwell, R.D., Caravella, J.A., Chen, L., Creech, K.L., Deaton, D.N., Jones, S.A., Kaldor, I., Liu, Y., Madauss, K.P., Marr, H.B., McFadyen, R.B., Miller, A.B., Iii, F.N., Parks, D.J., Spearing, P.K., Todd, D., Williams, S.P., Wisely, G.B., 2008. Conformationally constrained farnesoid X receptor (FXR) agonists: Naphthoic acid-based analogs of GW 4064. Bioorg. Med. Chem. Lett. 18, 4339–4343.
155. Deng, G., Li, W., Shen, J., Jiang, H., Chen, K., Liu, H., 2008. Pyrazolidine-3,5-dione derivatives as potent non-steroidal agonists of farnesoid X receptor: virtual screening, synthesis, and biological evaluation. Bioorg. Med. Chem. Lett. 18, 5497–5502.
156. Takahashi, M., Kanayama, T., Yashiro, T., Kondo, H., Murase, T., Hase, T., Tokimitsu, I., Nishikawa, J., Sato, R., 2008. Effects of coumestrol on lipid and glucose metabolism as a farnesoid X receptor ligand. Biochem. Biophys. Res. Commun. 372, 395–399.
157. Suzuki, T., Tamehiro, N., Sato, Y., Kobayashi, T., Ishii-Watabe, A., Shinozaki, Y., Nishimaki-Mogami, T., Hashimoto, T., Asakawa, Y., Inoue, K., Ohno, Y., Yamaguchi, T., Kawanishi, T., 2008. The novel compounds that activate farnesoid X receptor: the diversity of their effects on gene expression. J. Pharmacol. Sci. 107, 285–294.

158. Kaimal, R., Song, X., Yan, B., King, R., Deng, R., 2009. Differential modulation of farnesoid X receptor signaling pathway by the thiazolidinediones. J. Pharmacol. Exp. Ther. 330, 125–134.
159. Flatt, B., Martin, R., Wang, T.L., Mahaney, P., Murphy, B., Gu, X.H., Foster, P., Li, J., Pircher, P., Petrowski, M., Schulman, I., Westin, S., Wrobel, J., Yan, G., Bischoff, E., Daige, C., Mohan, R., 2009. Discovery of XL335 (WAY-362450), a highly potent, selective, and orally active agonist of the farnesoid X receptor (FXR). J. Med. Chem. 52, 904–907.
160. Mehlmann, J.F., Crawley, M.L., Lundquist, J.T.T., Unwalla, R.J., Harnish, D.C., Evans, M.J., Kim, C.Y., Wrobel, J.E., Mahaney, P.E., 2009. Pyrrole[2,3-d]azepino compounds as agonists of the farnesoid X receptor (FXR). Bioorg. Med. Chem. Lett. 19, 5289–5292.
161. Feng, S., Yang, M., Zhang, Z., Wang, Z., Hong, D., Richter, H., Benson, G.M., Bleicher, K., Grether, U., Martin, R.E., Plancher, J.M., Kuhn, B., Rudolph, M.G., Chen, L., 2009. Identification of an N-oxide pyridine GW4064 analog as a potent FXR agonist. Bioorg. Med. Chem. Lett. 19, 2595–2598.
162. Iguchi, Y., Yamaguchi, M., Sato, H., Kihira, K., Nishimaki-Mogami, T., Une, M., 2010.Bile alcohols function as the ligands of membrane-type bile acid-activated G protein-coupled receptor. J. Lipid Res. 51, 1432–1441.
163. Shneider, B.L., 2001. Intestinal bile acid transport: biology, physiology, and pathophysiology. J. Pediatr. Gastroenterol. Nutr. 32, 407–417.
164. Bass, J.Y., Caravella, J.A., Chen, L., Creech, K.L., Deaton, D.N., Madauss, K.P., Marr, H.B., McFadyen, R.B., Miller, A.B., Mills, W.Y., Navas 3rd, F., Parks, D.J., Smalley Jr., T.L., Spearing, P.K., Todd, D., Williams, S.P., Wisely, G.B., 2011. Conformationally constrained farnesoid X receptor (FXR) agonists: heteroaryl replacements of the naphthalene. Bioorg. Med. Chem. Lett. 21, 1206–1213.
165. Sepe, V., Bifulco, G., Renga, B., D'Amore, C., Fiorucci, S., Zampella, A., 2011. Discovery of sulfated sterols from marine invertebrates as a new class of marine natural antagonists of farnesoid-X-receptor. J. Med. Chem. 54, 1314–1320.
166. Gioiello, A., Macchiarulo, A., Carotti, A., Filipponi, P., Costantino, G., Rizzo, G., Adorini, L., Pellicciari, R., 2011. Extending SAR of bile acids as FXR ligands: discovery of 23-N-(carbocinnamyloxy)-3alpha,7alpha-dihydroxy-6alpha-ethyl-24-nor-5beta-cholan-23-amine. Bioorg. Med. Chem. 19, 2650–2658.
167. Choi, H., Hwang, H., Chin, J., Kim, E., Lee, J., Nam, S.J., Lee, B.C., Rho, B.J., Kang, H.,2011. Tuberatolides, potent FXR antagonists from the Korean marine tunicate Botryllus tuberatus. J. Nat. Prod. 74, 90–94.
168. Jonker JW, Liddle C, Downes M. FXR and PXR: potential therapeutic targets in cholestasis. The Journal of steroid biochemistry and molecular biology. 2012 Jul 31;130(3):147-58.
169. Li L, Bonneton F, Chen XY, Laudet V. Botanical compounds and their regulation of nuclear receptor action: The case of traditional Chinese medicine. Molecular and cellular endocrinology. 2015 Feb 5;401:221-37.

170. Heubi, J.E., Setchell, K.D., and Bove, K.E. (2007). Inborn errors of bile acid metabolism. Semin. Liver Dis. 27, 282–294.

171. Porez, G., Prawitt, J., Gross, B., and Staels, B. (2012). Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. J. Lipid Res. 53, 1723–1737.

172. Schoenfield, L. J. & Lachin, J. M. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: the National Cooperative Gallstone Study. A controlled trial of efficacy and safety. *Ann. Intern. Med.* **95**, 257–282 (1981).173. Leiss, O. & von Bergmann, K. Different effects of chenodeoxycholic acid and ursodeoxycholic

acid on serum lipoprotein concentrations in patients with radiolucent gallstones. *Scand. J. Gastroenterol.* **17**, 587–592 (1982)

174. Maloney, P. R. *et al.* Identification of a chemical tool for the orphan nuclear receptor FXR. *J. Med. Chem.* **43**, 2971–2974 (2000).

175. Liu, Y. *et al.* Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intraand extrahepatic cholestasis. *J. Clin. Invest.* **112**, 1678–1687 (2003).

176. Pellicciari, R., Costantino, G. & Fiorucci, S. Farnesoid X receptor: from structure to potential clinical applications. *J. Med. Chem.* **48**, 5383–5403 (2005).

177. Pellicciari, R. *et al.* 6α‐ethyl‐chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J. Med. Chem.* **45**, 3569–3572 (2002).

178. Harnish, D. C. *et al.* A synthetic farnesoid X receptor (fxr) agonist protects against diet-induced dyslipidemia. *J. Clin. Lipidol*. **1**, 362 (2007).

179. Downes, M. *et al.* A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol. Cell* **11**, 1079–1092 (2003).

180. Cai, S. Y., Xiong, L., Wray, C. G., Ballatori, N. & Boyer, J. L. The farnesoid X receptor FXRα/NR1H4 acquired ligand specificity for bile salts late in vertebrate evolution. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**, R1400–R1409 (2007).

181. Dussault, I. *et al.* Identification of gene-selective modulators of the bile acid receptor FXR. *J. Biol. Chem.* **278**, 7027–7033 (2003).

182. Urizar, N. L. *et al.* A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science* **296**, 1703–1706 (2002).

183. Szapary, P. O. *et al.* Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial. *Jama* **290**, 765–772 (2003).

184. Kobayashi, M. *et al.* Prevention and treatment of obesity, insulin resistance, and diabetes by bile acid-binding resin. *Diabetes* **56**, 239–247 (2007).

185.Garg, A. & Grundy, S. M. Cholestyramine therapy for dyslipidemia in non‐insulin‐dependent diabetes mellitus. A short-term, double-blind, crossover trial.
Ann. Intern. Med. **121**, 416–422 (1994).

186. Yamakawa, T., Takano, T., Utsunomiya, H., Kadonosono, K. & Okamura, A. Effect of colestimide therapy for glycemic control in type 2 diabetes mellitus with hypercholesterolemia. *Endocr. J.* **54**, 53–58 (2007).

187. Sakamoto, S. *et al.* Glucuronidation converting methyl 1‐(3,4-dimethoxyphenyl)‐3‐(3-ethylvaleryl)‐4‐hydroxy‐6,7,8‐trimethoxy‐2-na phthoate (S‐8921) to a potent apical sodium-dependent bile acid transporter inhibitor, resulting in a hypocholesterolemic action. *J. Pharmacol. Exp. Ther.* **322**, 610–618 (2007).

188. West, K. L., Zern, T. L., Butteiger, D. N., Keller, B. T. & Fernandez, M. L. SC‐435, an ileal apical sodium co-dependent bile acid transporter (ASBT) inhibitor lowers plasma cholesterol and reduces atherosclerosis in guinea pigs. *Atherosclerosis* **171**, 201–210 (2003).

189. Ozcan, U. *et al.* Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* **313**, 1137–1140 (2006).

190. Watanabe, M. *et al.* Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* **439**, 484–489 (2006).

191. Watanabe, M. *et al.* Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J. Clin. Invest.* **113**, 1408–1418 (2004).

192. Sato, H. *et al.* Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea Europea*. *Biochem. Biophys. Res. Commun.* **362**, 793–798 (2007).

193. Pellicciari, R. *et al.* Nongenomic actions of bile acids. Synthesis and preliminary characterization of 23- and 6,23‐alkyl‐substituted bile acid derivatives as selective modulators for the G‐protein coupled receptor TGR5. *J. Med. Chem.* **50**, 4265–4268 (2007).