FXR, A Therapeutic Target for Bile Acid and Lipid Disorders

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Abstract: The farnesoid X receptor (FXR) is a nuclear receptor expressed in tissues exposed to high concentrations of bile acids such as the liver, kidney and intestine and functions as a bile acid sensor. FXR regulates the expression of various transport proteins and biosynthetic enzymes crucial to the physiological maintenance of lipids, cholesterol and bile acid homeostasis. The concept of reverse endocrinology, whereby the receptor is identified first, followed by the identification of ligands and the sequential elucidation of the physiological role of the receptor has been widely used for a number of orphan nuclear receptors. The design of synthetic high affinity ligands acting *via* these receptors not only helps to decipher the function of the receptor, but also should lead to the development of novel and highly specific drugs. The bile acid receptor FXR is a perfect example where this strategy helped with understanding the role of this receptor in cholesterol and bile acid homeostasis. Regulation of FXR through small-molecule drugs represents a promising therapy for diseases resulting from lipid, cholesterol and bile acid abnormalities.

Keywords: Farnesoid x receptor, bile acid receptor, nuclear receptor, bile acids, FXR ligands, small molecule drugs, hyperlipidemia, cholestasis.

INTRODUCTION

Nuclear hormone receptors belong to a superfamily of ligand-activated transcription factors that play critical roles in diverse aspects of development and homeostasis by activating or repressing the transcription of specific genes in response to binding small molecular weight ligands [1]. These receptors were first characterized as mediators of steroid hormone signaling linking transcriptional regulation to physiological responses. The signature structural motifs that defines the nuclear receptor family are represented by an N-terminal region that harbors a ligand-independent activation function (AF-1), a core DNA binding domain (DBD), and a ligand binding domain (LBD) that encompasses ligand binding, dimerization and a ligand-dependent activation function (AF-2) (Fig. **1A**). The conserved DBDs as well as LBDs enabled the identification of several novel proteins termed orphan nuclear receptors, because their ligands are unknown (Fig. **1B**).

Characterization of these orphan receptors has led to the concept of reverse endocrinology in which the receptors have been used to identify previously unknown hormone signaling pathways. Several orphan receptors including receptors for fatty acids (peroxisome proliferator activated receptor; PPAR), oxysterols (liver X receptor; LXR), and bile acids (FXR) have recently been shown to constitute a family of so called lipid sensing receptors (Fig. **2**). While nuclear receptor ligands have been used for many years in the treatment of inflammation, cancer, and endocrine disorders, more recently, ligands for several superfamily members have emerged as drugs for the treatment of metabolic disease. For instance, the thiazolidinediones that are now widely prescribed for the treatment of type II diabetes are ligands that bind to and increase the transcriptional activity of PPARγ [2].

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The ability of nuclear receptors to regulate lipid metabolism and glucose utilization suggests that these receptors have significant potential as drug targets for treating metabolic disease. Although FXR (NR1H4) was initially shown to be activated by high concentrations of farnesol and related metabolites necessary for cholesterol synthesis [3], bile acids were subsequently identified as the physiologically relevant ligands for FXR [4-6]. Bile acids play an integral role in lipid homeostasis by facilitating the solubilization and absorption of lipids. Additionally, bile acids are end products of cholesterol catabolism and thus provide a major pathway by which cholesterol is excreted.

Hyperlipidemia, which includes elevated levels of cholesterol and triglycerides, is among the leading factors for the development of cardiovascular disease and atherosclerosis. According to recent statistics, about 100 million American adults have total blood cholesterol levels above 200 mg/dl, which puts them at significant risk for developing atherosclerosis. Elucidation of the cholesterol biosynthetic pathway has led to the development of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (statins), a potent class of cholesterol lowering drugs proven to significantly reduce cardiovascular mortality in hypercholesterolemic patients. Nevertheless, the available statins are not sufficient to fully prevent the progression of coronary heart disease in many susceptible individuals. Thus, the development of drugs that target alternative pathways is greatly needed. The critical role of FXR in controlling bile acid, cholesterol and lipid homeostasis makes this receptor a potentially attractive target for the development of drugs for the treatment of lipid and liver disorders.

Identification of FXR as a Bile Acid Receptor

FXR was discovered by using nuclear receptor homology screens and by searching for proteins that interact with the retinoid X receptor (RXR), a heterodimeric partner for several nuclear receptors [3, 7]. The close homology of FXR

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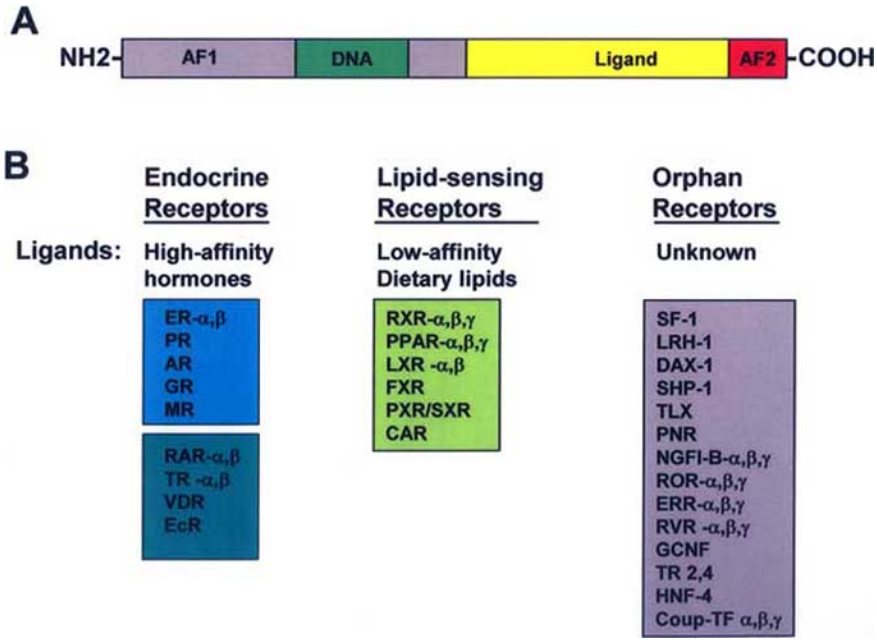

Fig. (1). The nuclear receptor superfamily. (A) Schematic structure of a typical nuclear receptor. **(B)** Nuclear receptors can be divided into three or four groups depending on the source and type of their ligands. Receptors with known physiological ligand are shown in color, current orphan receptors are shown in gray.

to the insect nuclear receptor for ecdysone (EcR) helped to decipher its DNA binding properties and also provided clues as to the possible ligands for this receptor. Farnesol was initially shown to activate FXR, hence the name farnesoid X receptor. However, the concentrations of farnesol needed to activate FXR were considered non-physiological. In addition, evidence for direct binding of farnesol to FXR was not demonstrated. Also, species differences in activation of FXR by farnesol suggested that true physiological FXR ligands remained to be discovered. More recently, three groups independently reported that bile acids are endogenous ligands for FXR. In one study, bile extracts was used to show that the biliary component chenodeoxycholic acid (CDCA) selectively activates FXR [5]. Another group speculated that whereas the conversion of cholesterol into bile acids is regulated through feed-forward activation by LXR, the feedback repression by bile acids may be mediated by FXR. FXR was an ideal candidate for a bile acid receptor since it is expressed in tissues, such as liver, intestine and kidney, normally exposed to high concentrations of bile acids. [6] The third report described a slightly different approach to directly test whether CDCA activated a group of orphan receptor chimeric constructs. This group also found that CDCA selectively activates FXR [4].

Further evidence that FXR is indeed a bile acid receptor came from targeted disruption of the FXR gene in mice [8]. $FXR^{-/-}$ mice are healthy and fertile under normal conditions. Upon dietary challenge with bile acids, however, $FXR^{-/-}$ mice rapidly lose adipose tissue and body weight, and later develop hepatotoxicity from excess accumulation of bile salts. Additionally, $FXR^{-/-}$ mice have significant increases

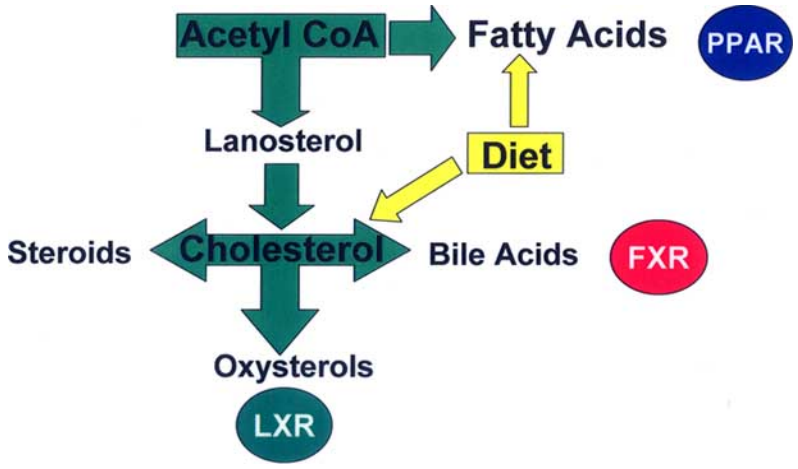

Fig. (2). The lipid sensors of the nuclear receptor superfamily. Metabolic pathways for PPAR, LXR and FXR. With the exception of thyroid hormones and some xenobiotics, all nuclear receptor ligands are derived from acetyl coenzyme A. For simplicity several intermediary steps have been condensed and are not shown. Receptors are shown next to their ligand.

Table I. Structure of Bile Acids

Bile Acid	R_3	R_6	R_7	R_{12}	Conjugate	X
CDCA	OH	H	OH	H	Free Acid	OH
DCA	OH	H	H	OH	Glyco	$NHCH_2CO_2H$
LCA	OH	H	H	H	Tauro	$NHCH_2CH_2CO_2H$
3-deoxyCDCA	H	H	OH	H		
6-EDCA	OH	Et	OH	H		

in serum triglycerides, cholesterol, and bile acids, the latter being secondary to impaired bile acid secretion from the liver and decreased excretion of bile salts into feces. Associated with these pathological changes are alterations in the expression of genes involved in the synthesis, secretion and transport of bile acids.

Bile Acids as FXR Agonists

Bile acids are believed to be the physiological ligands that activate FXR (Fig. **3** and Table **1**) [4]. The most potent is CDCA (EC_{50} ~10 μM), a primary bile acid, although secondary bile acids such as lithocholic acid (LCA) and deoxycholic acid (DCA) can also activate FXR [4-6]. Bile acids are synthesized in the liver from cholesterol and conjugated to glycine or taurine before they are secreted into bile canaliculi [9, 10]. The primary bile acids in humans, cholic acid and chenodeoxycholic acid, are synthesized *via* the concerted action of enzymes located in the endoplasmic reticulum, cytosol, mitochondria and peroxisomes [10]. In humans, conjugated bile acids are the major solutes in bile whereas unconjugated bile acids are almost nondetectable. Importantly, conjugated bile acids are less toxic and are more efficient promoters of intestinal absorption of dietary lipid than unconjugated bile acids [11]. Despite the relatively bulky taurine and glycine side chain at the C24 carboxylate, amidated bile acids still bind and activate FXR without major changes in binding affinity and activation efficacy [4-6]. The recent FXR structures provided an explanation for how conjugated bile acids bind and activate FXR. The structure shows that the carboxylate moiety of CDCA hydrogen bonds with the guanidine group of Arg328 side chain located at the entry point of the pocket. The proximity of the carboxylate on the ligand to the solvent suggests that conjugated amino acids would be positioned completely out of the pocket and solvent exposed, thus not impacting bile acid binding affinity and receptor activation [12, 13].

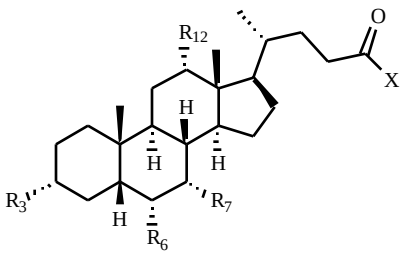

Fig. (3). Structures of natural and synthetic bile acids.

A synthetic bile acid derivative6-ethyl-chenodeoxycholic acid (6-ECDCA, Fig. **3**) [14] was shown to be a very potent (EC_{50} = 99 nM in fluorescence resonance energy transfer assay (FRET)) and selective FXR agonist with anticholeretic activity in a rat model of cholestasis [15]. The X-ray crystal structure of FXR LBD in the presence of a GRIP-1 co-activator peptide with 6-ECDCA and 3-deoxyCDCA reveals that naturally occurring ligands do not entirely fill cavities in the ligand binding pocket, accounting for their lower affinity [12]. The structure further shows that bile acids bind in a very different "reverse" manner than other steroids and their receptors, and that the 3-hydroxy group is not necessary for FXR activation. The postulated activation state of the receptor is through direct contact of the bile acid *cis*-oriented A-ring and a π-cation interaction between Trp466 and His444.

Regulation of Gene Expression by FXR Ligands

Earlier studies clearly established that FXR regulates target gene expression through a heterodimer with the retinoid X receptor, a nuclear receptor for 9-cis retinoic acid and an obligate partner for several nuclear receptors [3, 7]. The high homology (81 %) of FXR and ecdysone receptor (EcR) within their DNA binding domains suggested that they bind to similar response elements. In fact, FXR/RXR heterodimers binds to an EcR/usp (usp; ultraspiracle is a *Drosophila* homolog of RXR) response element present in the *Drosophila* hsp27 gene. This DNA element consists of two imperfect core-binding sites of the sequence AGGTCA arranged as inverted repeats separated by 1 nucleotide (IR-1). The IR-1 DNA response element has been verified in several FXR targets genes, although different FXR binding sites have been identified.

Studies have shown that bile acids repress their own synthesis by inhibiting transcription of the gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the conversion of cholesterol to bile acids [16]. The discovery that FXR is a bile acid sensor allowed identification of the gene encoding a repressor of CYP7A1 transcription, the small heterodimer partner (SHP), as an FXR target gene. In a recently proposed model (Fig. **4**), increased levels of bile acids activates FXR resulting in an induction of SHP, which in turn represses CYP7A1 [17, 18]. A prediction of this regulatory cascade is that loss of SHP would result in abrogation of negative feedback. Two groups directly tested this hypothesis by generating SHP null mice [19, 20]. The consensus from these two studies was that while bile acids still repress CYP7A1 in the

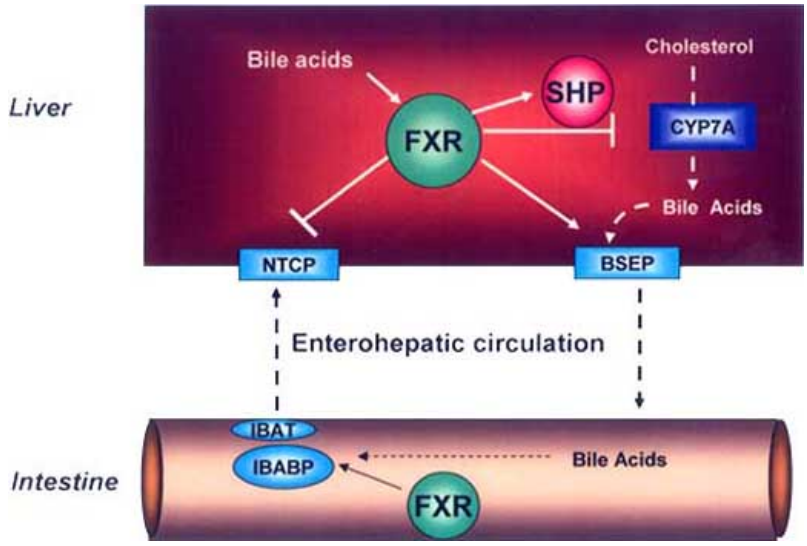

Fig. (4). Schematic representation of FXR action. Unbroken arrows represent activation; bars represent repression and broken arrows represent flux. Adapted from [57].

absence of SHP, a synthetic FXR agonist does not, indicating that redundant pathways of negative feedback regulation of bile acid synthesis exist. Additional pathways for bile acid repression include activation of the xenobiotic pregnane X receptor (PXR) and activation of stress kinase signaling pathways [20]. Furthermore, the recent identification of a G protein-coupled receptor for bile acids suggest that at least two independent pathways exist for bile acids; a membrane mediated signaling cascade and a nuclear receptor mediated response [21, 22]. This should be kept in mind when comparing gene expression profiles of natural versus synthetic FXR ligands. In fact, studies have shown that distinct genetic networks are regulated by CDCA compared to synthetic FXR ligands, although some overlap is evident [13, 23].

Along with SHP, FXR also up-regulates the bile salt export pump (BSEP, ABCB11) [24], a canalicular bile acid export protein, and indirectly down-regulates sodium taurocholate cotransporting polypeptide (NTCP, SLC10A1) [25], a sinusoidal transport protein involved in bile acid uptake in the liver. Thus, activation of FXR in the liver results in an inhibition of bile acid synthesis and a net flux of bile from the liver to the intestine (Fig. **4**). Not surprisingly, in the intestine, bile acids increases expression of the intestinal bile acid binding protein (I-BABP), a cytoplasmic protein implicated in cellular uptake and trafficking of bile acids [26, 27].

Studies in rodents and humans have shown that FXR ligands play a role in regulating plasma triglyceride levels. One suggested mechanism is that FXR induces apolipoprotein CII (ApoCII), an obligate cofactor for lipoprotein lipase (LPL), which hydrolyzes triglycerides in chylomicrons and very low density lipoproteins (VLDL). The ApoCII induction is mediated by an IR-1 sequence in the hepatic control region of the gene [28]. Variants of the prototypic IR-1 motif were identified as functional FXR/RXR binding sites in the promoters of SHP, BSEP, phospholipid transfer protein (PLTP), carnitine palmitoyltransferase-II(CPT-II) and phenylethanolamine *N*-methyltransferase (PNMT) [17, 18, 24, 29]. FXR also regulates the expression of genes required for bile acid conjugation to amino acids. Two genes encoding enzymes involved in conjugation of bile acids to taurine and glycine, namely bile acid CoA synthetase (BACS) and bile acid-CoA: amino acid N-acetyltransferase (BAT) are induced by FXR in rat liver. Analysis of the human BACS and BAT genes revealed functional response elements in the proximal promoter of BACS and in the intronic region between exons 1 and 2 of the BAT gene. The response elements resemble the consensus IR-1 FXR binding site [23].

Atypical FXR binding sites have been identified in the genes for multidrug resistance associated protein 2 (MRP2), dehydroepiandrosterone sulfotransferase (STD) encoding a sulfo-conjugating enzyme, and PPARα [28, 30]. Recently, syndecan-1 (SDC-1), a transmembrane heparan sulfate proteoglycan, which participates in the binding and internalization of extra-cellular ligands, was identified in a screen to isolate genes that are regulated by FXR [31]. The increased expression of SDC-1 by FXR ligands may account in part for the lowering of triglyceride effects observed after administration of CDCA to humans. Another mechanism for triglyceride lowering was suggested from a recent study in which FXR ligands induced the expression of PPARα mRNA [32]. PPARα ligands, like fatty acid derivatives and fibrates, are known regulators of lipid metabolism, but also appears to influence bile acid metabolism, suggesting that PPARα and FXR may modulate common metabolic pathways. Molecular cross-talk between FXR and PPARα pathways was also suggested from a recent report where the apolipoprotein AV (ApoAV) gene, an important regulator of plasma triglycerides, was implicated to be a target for both receptors [33]. Although no increase in ApoAV mRNA by FXR ligands was demonstrated in this study, a putative FXR binding site was identified in the human ApoAV proximal promoter. In conclusion, FXR demonstrates some degree of promiscuity in terms of DNA binding elements. This in turn indicates that overlapping functions between FXR and other nuclear receptors may provide a mechanism for controlling common genetic pathways.

FXR Isoforms and Identification of FXRβ

Early studies by Seol *et al*. indicated the presence of splice variants of FXR in mice resulting in isoforms that differ in their N-terminal domains and by an insertion in the hinge region between the DNA-binding and ligand binding domains [7]. Cloning of hamster FXR confirmed the existence of four FXR isoforms derived from a single gene by the use of an alternative promoter and alternative splice donor/acceptor sites [34]. These isoforms are present not only in hamster and mice but also in humans. Distribution of the various isoforms indicated that the main tissues expressing FXR were liver, small intestine, kidney and adrenal gland although stomach, heart, lung and fat contained measurable levels of each isoform [35]. The IBABP gene is differentially induced by the FXR isoforms lacking the four amino acid insertion in the hinge region. On the other hand, all FXR isoforms activate the SHP and BSEP genes equally well. Since the ligand binding domain is identical in all four isoforms, bile acids as well as synthetic FXR ligands would be expected to bind with equal affinity to all isoforms. However, since the insertion in the hinge region affects the ability to bind certain FXRE, the isoforms are predicted to differentially regulate target genes in various tissues.

The recent discovery of FXRβ [36], by mining human and mouse genomic sequences, warrants a name change for FXR into FXRα1 and the isoforms derived from the same gene into FXRα2-4. The FXR orthologs recently isolated from *Xenopus laevis*, FOR1 and –2 [37], show the highest homology to mouse FXRβ. FXRβ is derived from a unique gene and constitutes a functional nuclear hormone receptor in all mammalian species investigated except primates and humans. Therefore, drugs developed towards FXR would not be expected to be influencing FXRβ in humans since it is a non-functional protein. Nevertheless, the discovery of a functional FXRβ in rodents may have implications for the interpretation of genetic and pharmacological studies governing FXR directed endocrine physiology and drug discovery programs. FXRβ does not appear to be activated by bile acids, but instead by lanosterol, an intermediate metabolite of cholesterol synthesis. This may not be surprising since the homology between ligand binding domains of FXR and FXRβ is less than 30 %. This low homology also implies that synthetic ligands developed for FXR may not necessarily cross-react with FXRβ. Nevertheless, GW4064, a synthetic FXR ligand, was shown to activate FXRβ albeit at high concentrations. The fact that both FXRα and FXRβ bind to the same DNA binding element suggests an overlap in regulation of target genes. The existence of several FXR iso-types should be kept in mind when interpreting data from pharmacological studies and compound testing in nonprimate mammals, which in part is used to deduce metabolic pathways of lipids and cholesterol in humans.

FXR X-Ray Crystal Structure

Recently, two X-ray structures of the FXR LBD with ligands have been reported[12, 13]. One consists of rat FXR LBD with a glucocorticoid receptor interacting protein-1 (GRIP-1) coactivator peptide and the bile acids 6-ECDCA and 3-deoxyCDCA at 2.5 Å and 2.9 Å resolutions respectively. The other structure consists of human FXR LBD with the synthetic ligand fexaramine at 1.78 Å resolution. The binding pocket is mostly hydrophobic and has a volume of 726 $Å^3$ which is average for nuclear receptors. The broad specificity receptors such as the steroid xenobiotic receptor (SXR) have a bigger binding pocket (1150 $Å^3$) while the more specific hormone signaling receptors, such as RXRα (439 $Å^3$), have a smaller pocket size [38, 39]. The FXR secondary structure consists of the typical 12 α-helices of nuclear receptors but lacks the usual β-turn between α5 and α6. Finally, unlike other receptors, FXR may be able to bind two of the three LXXLL motifs present in coactivators [12, 40].

Synthetic FXR Ligands

GW4064 was the first synthetic FXR ligand to be reported and identified (Fig. **5**) [41]. The lead compound was obtained from a combinatorial library of 9900 stilbene carboxylic acids and was optimized through the synthesis of a 600-membered isoxazole focused library. Although this compound displays poor bioavailability (10%) and a short half-life (3.5 h), studies in rodents have demonstrated its utility as a chemical tool for the elucidation of FXR agonist physiological activities. Oral treatment twice daily for 7 days affords a significant reduction in plasma triglycerides (~45% at 100 mg/kg) with an ED_{50} of 20 mg/kg [42]. This process is believed to be mediated *via* the up-regulation of ApoC-II [28], a necessary cofactor for LPL mediated hydrolysis of triglycerides to fatty acids and glycerol. The *trans*-stilbene moiety of GW4064 is not required for good activity since the shorter *trans*-cinnamic acid compound LN6772 (Fig. **5**) displays a 50 nM EC_{50} in a FRET assay [43]. A new triaryl motif, as shown in LN7260 (Fig. **5**), was also identified as being a good pharmacophore and several compounds with 3 different center heterocycles were shown to have 100-200 nM EC_{50} in a FRET assay [43].

The structure of human FXR LBD was solved using a small molecule ligand termed fexaramine (Fig. **5**) [13]. This 25 nM compound was optimized through iterative combinatorial chemistry libraries from an initial 10,000-membered benzopyran library [44]. The molecule occupies about 2/3 of the binding pocket volume of 726 $Å^3$ and is tethered by two H-bonds from the Nε2 proton of His298 and the hydroxyl moiety of Ser336 to the amide oxygen of fexaramine. The remainder of the binding affinity comes from hydrophobic and van der Waals contacts. A gene array study with fexaramine, GW4064 and CDCA was also reported in this paper and shows significant differences between the 3 compounds suggesting the possibility that different compounds displaying different gene selectivities may become important for the design of selective drugs.

Retinoid and Analog Ligands

Since FXR is a permissive heterodimer partner, RXR activation leads to the activation of FXR target genes. Fig. (**6**) shows some of the RXR ligands that have been used to study FXR, namely 9-*cis*-retinoic acid (9-*cis*-RA) and LG1069. The retinoic acid receptor (RAR) ligands TTNPB ((E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalen-yl)-1-propenyl] benzoic acid) and all-*trans*-retinoic acid (all-

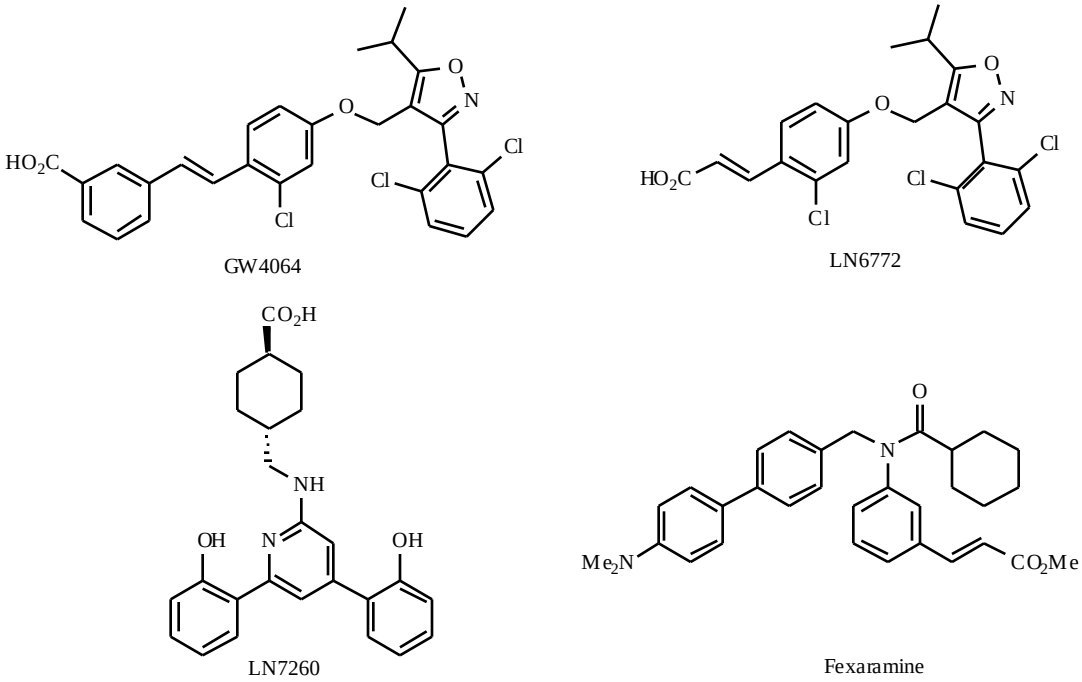

Fig. (5). Structures of synthetic FXR ligands GW4064, LN6772, LN7260 and fexaramine.

trans-RA) have been shown to activate FXR at high concentrations but it is suggested that it may not be direct binding and that a metabolite is possibly the active ligand [45]. AGN29 and AGN31 are synthetic analogs with ~2 μM potency and their efficacy was about half that of CDCA [46]. Although these compounds also show activity on RXR, they where shown through mutation experiments to act directly *via* FXR.

FXR Antagonists

The resin extract of the guggul tree *Commiphora mukul*, called guggulipid, has been used for thousands of years in traditional Indian medicine for the treatment of obesity and lipid disorders and was recently approved in India to treat hyperlipidemia. The naturally occurring plant sterol *guggulsterone* (Fig. **7**) has been shown to be the active ingredient. *Guggulsterone* was shown to be a FXR

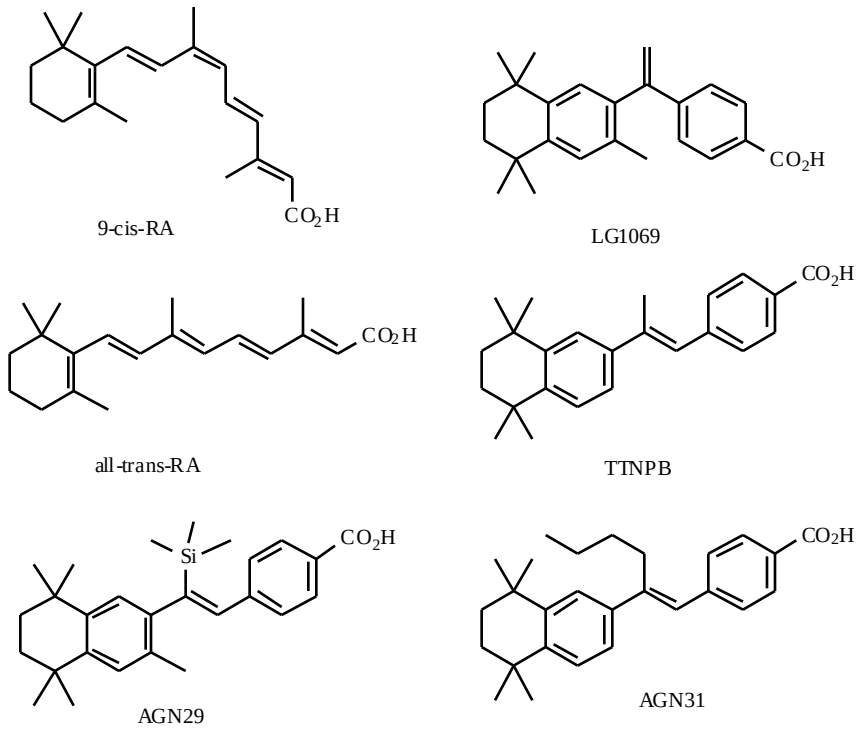

Fig. (6). Structures of retinoid and analog ligands 9-*cis*-RA, LG1069, all-*trans*-RA, TTNPB, AGN29 and AGN31.

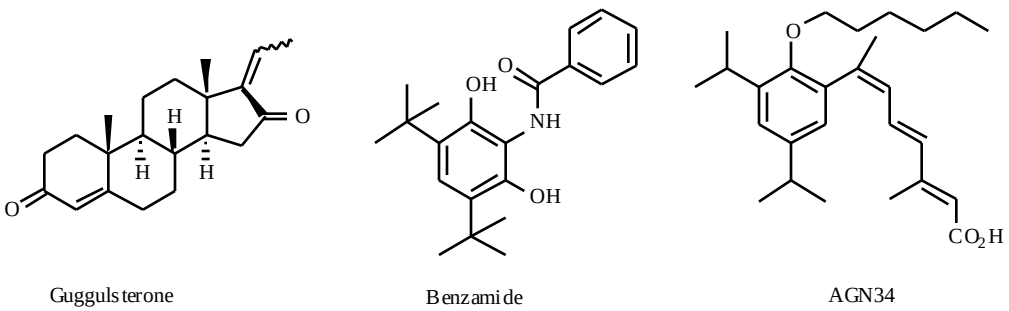

Fig. (7). Structures of E/Z-guggulsterone, N-(3,5-di-t-butyl-2,6-dihydroxyphenyl)benzamide and AGN34.

antagonist *in vitro*, and on promoters and endogenous target genes in cell based assays [47, 48]. The link to FXR was also demonstrated through *in vivo* efficacy at lowering hepatic cholesterol levels in wild-type mice fed a high cholesterol diet while no activity was seen in FXR knockout mice [47]. Recent reports propose that guggulsterone may act as a selective bile acid receptor modulator [49] and that this compound may produce some of its efficacy through the xenobiotic receptor PXR/SXR [50].

More recently, a synthetic benzamide FXR antagonist shown in Fig. (**7**) was reported to have an IC_{50} of about 1 μM in FRET and cell based gene reporter assays [51]. The compound was also efficacious with regard to repression of endogenous target genes *in vitro* and *in vivo*. Finally, the benzamide was shown to lower serum cholesterol by 28% and 32% in hamsters fed a high fat diet with oral administration of 30 and 100 mg/kg respectively for 14 days. AGN34 is a FXR antagonist in a transient cell-based reporter assay but acts in a gene selective manner *in vivo*, representing yet another supporting evidence for the possible design of selective drugs [46].

FXR as a Drug Target for Cholestasis

The ability of FXR agonists to promote the flux of bile from the liver to the intestine suggests that FXR agonists may be effective in the treatment of cholestasis [42]. Cholestasis is defined as an impairment of bile flow by any cause that may lie from the biliary canaliculi to the duodenum [52]. The sources of cholestasis are varied and include viral and bacterial infections, drug induced cholestasis (eg: alcohol, steroids and many known drugs), cirrhosis, mechanical blocks (stones, mucus plugs, tumors) and other. Cholestasis is also a secondary complication in other diseases, for example cystic fibrosis. Cholestasis has been linked to mutations in hepatic transporters. Progressive familiar intra-hepatic cholestasis (PFIC-1, 2, 3) is a disease related to hepatic transporter dysfunction. Mutations in FIC1, a P-type ATPase, are responsible for PFIC-1. Mutations of the bile salt export pump (BSEP), a liver specific ATP binding cassette transporter (ABCB11), are responsible for PFIC-2. Mutations in class III multidrug resistance P-glycoprotein (MDR3), an ATP-binding cassette transporter (ABCB4), are responsible for PFIC-3.

FXR agonists should prevent cholestasis by up-regulating BSEP, down-regulating NTCP and down-regulating CYP7A1. Intra-hepatic cholestasis may benefit from NTCP, CYP7A1 down-regulation and BSEP, Mrp2 up-regulation whereas extra-hepatic cholestasis may benefit only from NTCP and CYP7A1 down-regulation. Downregulation of CYP7A1 should reduce the hydrophobic bile acid content thus preventing membrane damage while NTCP and BSEP will regulate the in and out flow of hepatic bile acids respectively. Importantly, as mentioned above, a synthetic CDCA analog (6-ECDCA) improves bile flow in an animal model of cholestasis [15]. 6-ECDCA prevented reduction in bile flow and liver necrosis induced by lithocholic acid (LCA). The secondary bile acid ursodeoxycholic acid (UDCA) is currently used to treat cholestasis but its mechanism of action is uncertain. A current hypothesis is that this more hydrophilic bile acid effectively reduces the overall hydrophobicity of the bile thus preventing cellular membrane damage [53].

FXR as a Drug Target for Lipid Disorders and Metabolic Disease

Abnormal plasma levels of cholesterol and triglycerides are the major markers of hyperlipidemia. Numerous population-based studies have documented an association between elevated cholesterol levels and increase in the risk and prevalence of coronary artery disease (CAD), including atherosclerosis. Recently, conclusive evidence has established that lowering cholesterol level reduces cardiovascular disease incidence and mortality. Direct links have also been established between lipoprotein levels and the onset of CAD. The key unmet needs in the treatment of hyperlipidemia are for mixed dyslipidemia, particularly for cases of triglyceride and HDL cholesterol abnormalities. It is projected that combination therapies or monotherapies that both lower LDL cholesterol and triglycerides, concomitant with a raise in HDL cholesterol, will gain wide acceptance.

Recent epidemiological studies suggest that elevated triglyceride levels are an independent risk factor for coronary heart disease. According to the NCEP ATP III guidelines issued by the National Institutes of Health, over 40 million adults in the U.S. have high triglyceride levels (>250 mg/dL) and are candidates for lipid-lowering therapy. High triglycerides are an important factor in diabetic dyslipidemia where the most common pattern in type 2 diabetic patients is elevated triglyceride levels and decreased HDL cholesterol levels. Metabolic Syndrome, also known as syndrome X, is a set of concurrent and interrelated symptoms including dyslipidemia, insulin resistance, hypertension, and abdominal obesity, with increased risk for cardiovascular disease (CVD) and type 2 diabetes. Current treatments for the above diseases include statins, cholesterol absorption

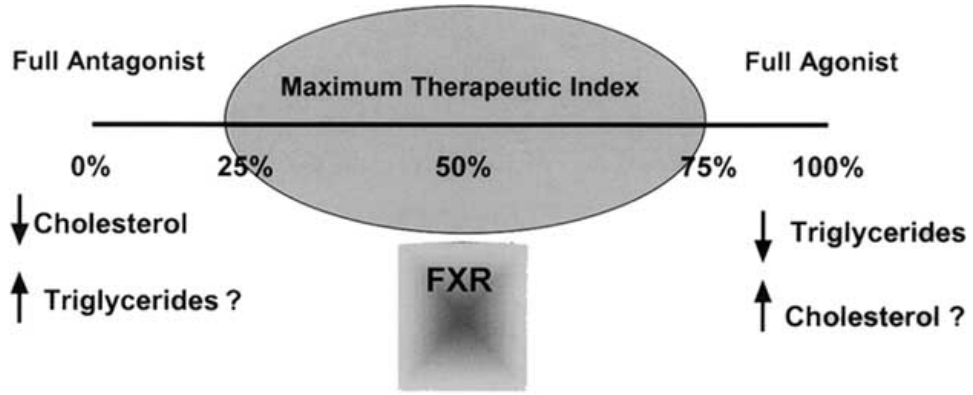

Fig. (8). Ideal profile of an FXR modulator.

inhibitors, fibrates (PPARα ligands), niacin and bile acid sequestrants. Although some of these treatments are very effective, a significantly large patient population does not reach their lipid goals and new therapies are needed.

Based upon the observations described above it has been suggested that an FXR antagonist would stimulate the catabolism of cholesterol by blocking the bile acid-dependent repression of CYP7A1. Indeed guggulsterone, a naturally occurring plant sterol with cholesterol lowering activity, has been shown to be an FXR antagonist [47, 48]. Additionally, bile acid sequestrants act by a similar mechanism (i.e. blocking the feedback repression of CYP7A1) and have been used in people to effectively lower serum cholesterol levels. Nevertheless, the therapeutic potential of FXR antagonists have been called into question by the finding that FXR-independent pathways exist for bile acid mediated repression of CYP7A1.

Although the *in vivo* activities of FXR antagonists remain in question, both natural (bile acids) and synthetic FXR agonists have been studied in various models. In humans and in rodents supplemented with bile acids a significant decrease in serum triglyceride levels have been observed and the use of synthetic FXR agonists have shown, at least in rodents, that this effect is mediated by FXR [42, 54]. In support of the conclusion that FXR regulates triglyceride metabolism, serum triglyceride levels are elevated in $FXR^{-/-}$ mice [8, 55]. The observation that FXR agonists induce expression of apolipoprotein C-II (apoC-II) which functions as a cofactor for lipoprotein lipase-dependent hydrolysis of triglycerides in chylomicrons and very low density lipoprotein suggests one possible mechanism for the triglyceride lowering activity of FXR [28]. Furthermore, recent analysis of $FXR^{-/-}$ mice indicating that this receptor positively regulates genes involved in high density lipoprotein cholesterol (HDL) transport and metabolism further highlights an additional beneficial activity of FXR agonists [55].

Profile of FXR Modulators

Our current understanding of the physiological role of FXR clearly indicates that this nuclear receptor is an important regulator of lipid and cholesterol metabolism. As illustrated in Fig. (**8**), FXR antagonists are predicted to lower cholesterol levels *via* up-regulation of CYP7A1 and promoting cholesterol catabolism. On the other hand, FXR agonists have been shown to significantly lower serum triglyceride levels. Since there are potential benefits to both FXR agonists and antagonists, the maximum therapeutic potential for small molecule FXR ligands may lie with partial agonists, a concept well established within the nuclear receptor field. For instance, the estrogen receptor ligands tamoxifen and raloxifene are partial agonists that mediate a subset of the activities inherent in full estrogen agonists such as estradiol [56]. Similarly, activators of PPARγ are effective insulin sensitizers, however, the most efficacious PPARγ agonists also result in weight gain and edema [2].

CONCLUSION

FXR can now be added to the constellation of nuclear receptors that are lipid sensors and involved in the regulation of metabolism, such as PPAR and LXR, and hence offer a potential avenue into novel therapeutics. FXR shows great promise for the treatment of cholestasis and metabolic diseases such as mixed hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, metabolic syndrome X and diabetic dislipidemia. All these diseases have a significant number of patients with key unmet needs. FXR represents a new avenue to treat different aspects and risk factors of the above diseases *via* a new mechanism of action. There are an increasing number of novel synthetic FXR modulators emerging from the pharmaceutical industry and this is just the beginning of this new and exciting area of research. The success of these new drugs will undoubtedly be associated with displaying the right receptor profile and gene selectivities.

REFERENCES

[1] Mangelsdorf, D.J.;Thummel, C.;Beato, M.;Herrlich, P.;Schutz, G.;Umesono, K.;Blumberg, B.;Kastner, P.;Mark, M.;Chambon, P.;Evans, R.M. *Cell*, **1995**, *83*, 835.

[2] Lee, C.-H.;Evans, R.M. *Trends. Endocrinol. Metab.*, **2002**, *13*, 331.

[3] 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. *Cell*, **1995**, *81*, 687.

[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.;Lehmann, J.M. *Science*, **1999**, *284*, 1365.

[5] Wang, H.;Chen, J.;Hollister, K.;Sowers, L.C.;Forman, B.M. *Mol. Cell*, **1999**, *3*, 543.

[6] 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. *Science*, **1999**, *284*, 1362.

[7] Seol, W.;Choi, H.-S.;Moore, D.D. *Mol. Endocrinol.*, **1995**, *9*, 72.

[8] Sinal, C.J.;Tohkin, M.;Miyata, M.;Ward, J.M.;Lambert, G.;Gonzalez, F.J. *Cell*, **2000**, *102*, 731.

[9] Bahar, R.J.;Stolz, A. *Gastroenterol. Clin. N. Am.*, **1999**, *28*, 27.

[10] Vlahcevic, Z.R.;Pandak, W.M.;Stravitz, R.T. *Gastroenterol. Clin. N. Am.*, **1999**, *28*, 1.

[11] Vessey, D.A.;Crissey, M.H.;Zakim, D. *Biochem. J.*, **1977**, *163*, 181.

[12] Mi, L.Z.;Devarakonda, S.;Harp, J.M.;Han, Q.;Pellicciari, R.;Willson, T.M.;Khorasanizadeh, S.;Rastinejad, F. *Mol. Cell*, **2003**, *11*, 1093.

[13] 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. *Mol. Cell*, **2003**, *11*, 1079.

[14] Pellicciari, R. (2002) *in* WO02072598(A1), pp. 24.

[15] Pellicciari, R.;Fiorucci, S.;Camaioni, E.;Clerici, C.;Costantino, G.;Maloney, P.R.;Morelli, A.;Parks, D.J.;Willson, T.M. *J. Med. Chem.*, **2002**, *45*, 3569.

[16] Russell, D.W.;Setchell, K.D.R. *Biochemistry*, **1992**, *31*, 4737.

[17] 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. *Mol. Cell*, **2000**, *6*, 517.

[18] Lu, T.T.;Makishima, M.;Repa, J.J.;Schoonjans, K.;Kerr, T.A.;Auwerx, J.;Mangelsdorf, D.J. *Mol. Cell*, **2000**, *6*, 507.

[19] Kerr, T.A.;Saeki, S.;Schneider, M.;Schaefer, K.;Berdy, S.;Redder, T.;Shan, B.;Russell, D.W.;Schwarz, M. *Dev. Cell*, **2002**, *2*, 713.

[20] Wang, L.;Lee, Y.K.;Bundman, D.;Han, Y.;Thevananther, S.;Kim, C.S.;Chua, S.S.;Wei, P.;Heyman, R.A.;Karin, M.;Moore, D.D. *Dev. Cell*, **2002**, *2*, 721.

[21] Kawamata, Y.;Fujii, R.;Hosoya, M.;Harada, M.;Yoshida, H.;Miwa, M.;Fukusumi, S.;Habata, Y.;Itoh, T.;Shintani, Y.;Hinuma, S.;Fujisawa, Y.;Fujino, M. *J. Biol. Chem.*, **2003**, *278*, 9435.

[22] Maruyama, T.;Miyamoto, Y.;Nakamura, T.;Tamai, Y.;Okada, H.;Sugiyama, E.;Itadani, H.;Tanaka, K. *Biochem. Biophys. Res. Commun.*, **2002**, *298*, 714.

[23] Pircher, P.C.;Kitto, J.L.;Petrowski, M.L.;Tangirala, R.K.;Bischoff, E.D.;Schulman, I.G.;Westin, S.K. *J. Biol. Chem.*, **2003**, *278*, 27703.

[24] Ananthanarayanan, M.;Balasubramanian, N.;Makishima, M.;Mangelsdorf, D.J.;Suchy, F.J. *J. Biol. Chem.*, **2001**, *276*, 28857.

[25] Denson, L.A.;Sturm, E.; Echevarria, W.; Zimmerman, T.L.; Makishima, M.; Mangelsdorf, D.J.; Karpen, S.J. *Gastroenterology*, **2001**, *121*, 140.

[26] Kanda, T.;Foucand, L.;Nakamura, Y.;Niot, I.;Besnard, P.;Fujita, M.;Sakai, Y.;Hatakeyama, K.;Ono, T.;Fujii, H. *Biochem. J.*, **1998**, *330*, 261.

[27] Grober, J.;Zaghini, I.;Fujii, H.;Jones, S.A.;Kliewer, S.A.;Willson, T.M.;Ono, T.;Besnard, P. *J. Biol. Chem.*, **1999**, *274*, 29749.

[28] 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. *Mol. Endocrinol.*, **2001**, *15*, 1720.

[29] Laffitte, B.A.;Kast, H.R.;Nguyen, C.M.;Zavacki, A.M.;Moore, D.D.;Edwards, P.A. *J. Biol. Chem.*, **2000**, *275*, 10638.

[30] Song, C.S.;Echchgadda, I.;Baek, B.S.;Ahn, S.C.;Oh, T.;Roy, A.K.;Chatterjee, B. *J. Biol. Chem.*, **2001**, *276*, 42549.

[31] Anisfeld, A.M.;Kast-Woelbern, H.R.;Meyer, M.E.;Jones, S.A.;Zhang, Y.;Williams, K.J.;Willson, T.;Edwards, P.A. *J. Biol. Chem.*, **2003**, *278*, 20420.

[32] Pineda Torra, I.;Claudel, T.;Duval, C.;Kosykh, V.;Fruchart, J.C.;Staels, B. *Mol. Endocrinol.*, **2003**, *17*, 259.

[33] Prieur, X.;Coste, H.;Rodriguez, J.C. *J. Biol. Chem.*, **2003**, *278*, 25468.

[34] Huber, R.M.;Murphy, K.;Miao, B.;Link, J.R.;Cunningham, M.R.;Rupar, M.J.;Gunyuzlu, P.L.;Haws, T.F.;Kassam, A.;Powell, F.;Hollis, G.F.;Young, P.R.;Mukherjee, R.;Burn, T.C. *Gene*, **2002**, *290*, 35.

[35] Zhang, Y.;Kast-Woelbern, H.R.;Edwards, P.A. *J. Biol. Chem.*, **2003**, *278*, 104.

[36] 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. *Mol. Cell. Biol.*, **2003**, *23*, 864.

[37] 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. *J. Biol. Chem.*, **2002**, *277*, 17836.

[38] Watkins, R.E.;Wisely, G.B.;Moore, L.B.;Collins, J.L.;Lambert, M.H.;Williams, S.P.;Willson, T.M.;Kliewer, S.A.;Redinbo, M.R. *Science*, **2001**, *292*, 2329.

[39] Egea, P.F.;Mitschler, A.;Rochel, N.;Ruff, M.;Chambon, P.;Moras, D. *Embo J.*, **2000**, *19*, 2592.

[40] Nettles, K.W.;Greene, G.L. *Mol. Cell*, **2003**, *11*, 850.

[41] Blanchard, S.G.;Kliewer, A.;Lehmann, J.;Parks, D.J.;Stimmel, J.B.;Willson, T.M. (2000) *in* WO00037077(A1), pp. 62.

[42] 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. *J. Med. Chem.*, **2000**, *43*, 2971.

[43] Bauer, U.;Cheruvallath, Z.;Deuschle, U.;Dneprovskaia, E.;Gahman, T.;Giegrich, K.;Hanecak, R.;Hébert, N.;Kiely, J.;Kober, I.;Kögl, M.;Kranz, H.;Kremoser, C.;Lee, M.;Otte, K.;Sage, C.;Sud, M. (2003) *in* WO03015771(A1), WO03015777 (A1), WO03016280(A1), WO03016288(A1), pp. 53.

[44] Nicolaou, K.C.;Evans, R.M.;Roecker, A.J.;Hughes, R.;Downes, M.;A., P.J. *Org. Biomol. Chem.*, **2003**, *1*, 908.

[45] Zavacki, A.M.;Lehmann, J.M.;Seol, W.;Willson, T.M.;Kliewer, S.A.;Moore, D.D. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 7909.

[46] Dussault, I.;Beard, R.;Lin, M.;Hollister, K.;Chen, J.;Xiao, J.H.;Chandraratna, R.;Forman, B.M. *J. Biol. Chem.*, **2003**, *278*, 7027.

[47] 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. *Science*, **2002**, *296*, 1703.

[48] Wu, J.;Xia, C.;Meier, J.;Li, S.;Hu, X.;Lala, D.S. *Mol. Endocrinol.*, **2002**, *16*, 1590.

[49] Cui, J.;Huang, L.;Zhao, A.;Lew, J.L.;Yu, J.;Sahoo, S.;Meinke, P.T.;Royo, I.;Pelaez, F.;Wright, S.D. *J. Biol. Chem.*, **2003**, *278*, 10214.

[50] Owsley, E.;Chiang, J.Y. *Biochem. Biophys. Res. Commun.*, **2003**, *304*, 191.

[51] Tojo, S.;Nita, M.;Nishimura, T.;Shan, B. (2002) *in* WO02064125 (A2), pp. 35.

[52] Hofmann, A.F. *Arch. Intern. Med.*, **1999**, *159*, 2647.

[53] Beuers, U.;Boyer, J.L.;Paumgartner, G. *Hepatology*, **1998**, *28*, 1449.

[54] Iser, J.H.;Sali, A. *Drugs*, **1981**, *21*, 90.

[55] Lambert, G.;Amar, M.J.;Guo, G.;Brewer, H.B., Jr.;Gonzalez, F.J.;Sinal, C.J. *J. Biol. Chem.*, **2002**,

[56] Smith, C.L.;O'Malley, B.W. *Trends. Endocrinol. Metab.*, **1999**, *10*, 299.

[57] Sinal, C.;Gonzalez, F. *Trends Endocrinol Metab*, **2002**, *13*, 275.