The Pharmacology of LXR

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Abstract: Liver X receptors (LXRs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Two LXRs (LXRα and LXRβ) were initially characterized as orphan members of this superfamily with disparate patterns of tissue expression. These two receptors later were recognized as sterol-responsive with the ability to directly bind several oxysterol metabolites. Many LXR target genes have been identified that implicate these receptors in a variety of physiological processes including cholesterol transport and metabolism, glucose metabolism, and inflammation. Synthetic LXR ligands have been designed with the potential to treat disorders such as atherosclerosis and diabetes. In this review, we describe the potential utility of LXR ligands in the treatment of disease.

Keywords: Nuclear receptor, steroid, HDL, LDL, transcription, heart disease, diabetes, inflammation.

INTRODUCTION

In an attempt to identify orphan members of the nuclear hormone receptor superfamily, LXRα (NR1H3) was originally identified in a screen of a human liver cDNA library [1,2]. Tissue expression pattern analysis of LXR confirmed high levels in the liver, which led to the name, liver X receptor. LXRα also can be detected in other tissues that are involved in lipid metabolism, such as adipose tissue, kidney, and intestine [3]. A second LXR subtype, LXRβ (NR1H2), was identified independently by several laboratories during the same timeframe by using two-hybrid screening and traditional cDNA library screening [4-7]. In contrast to LXRα, LXRβ is widely expressed, a fact that yielded one of its original names: UR-1 or ubiquitous receptor 1 [5].

The LXRs belong to the nuclear receptor superfamily of ligand-activated transcription factors that includes receptors for the steroid hormones, retinoids and vitamin D. As illustrated in Figure **1**, these receptors encode conserved domain structures, including a DNA-binding domain (DBD) and a carboxy-terminal ligand binding domain (LBD). These two domains are well conserved between the two LXR subtypes with approximately 78% sequence identity in the DBD and 77% sequence identity within the LBD. In contrast to the steroid hormone receptors that function as homodimers, the LXRs belong to the class II subfamily of nuclear receptors that require heterodimerization with another nuclear receptor known as the retinoid X receptor (RXR). Heterodimerization with RXR is obligatory for LXR function, and interestingly, the LXR/RXR heterodimer is "permissive" in that it will respond to ligands of either receptor of the dimer. In contrast, the thyroid hormone receptor (TR) and the vitamin D receptor (VDR) are "non-permissive" since, despite the fact that they require heterodimerization with RXR for function, they only activate transcription in response to the presence of TR or VDR ligands, respectively, but not to RXR ligands. The LXR/RXR heterodimer preferentially recognizes specific DNA sequences within the promoter regions of target genes. These DNA response elements, known as LXR response elements or LXREs, are characterized by two conserved hexanucleotide sequences arranged in a direct repeat with 4 nucleotides separating the conserved regions. As illustrated in Table **1**, this sequence, which is known as a DR4 element, has been identified in 5'-flanking regions of the target genes that are regulated directly by LXR. The DBD of LXR functions to direct the receptor to target genes *via* recognition of specific LXREs within the target gene promoter, while the function of the LBD is to modulate the transcription of target genes. The 3-dimensional structure of the LBD of LXR has been solved recently and is consistent with the structures of other nuclear receptor LBDs [8]. The LBD is composed of a globular 3-layered α-helical sandwich in which the ligand is buried within the protein structure. Based on observations from a number of LBD structures, binding of the ligand leads to a conformational change that results in relocation of the carboxy-terminal α-helical segment known as helix 12 (H12). Repositioning of H12 upon ligand binding creates a surface on the LBD that is capable of recognizing accessory proteins known as coactivators that mediate transcriptional activation of the target gene through recruitment of additional transcription factors and subsequent modification both of the chromatin structure as well as of other regulatory proteins.

Although LXR was initially identified as an orphan receptor having no identified ligands, addition of organic tissue extracts to cell-based assays led to activation of the receptor, suggesting the existence of natural ligands [9]. Further examination revealed that oxysterols serve as putative ligands for LXR in the cell-based assays [9]. Oxysterols later were confirmed to be *bona fide* LXR ligands using radioligand binding assays [10]. Several oxysterols, including 22(R)-hydroxycholesterol and 24(S),25-epoxycholesterol, are ligands with affinities in the 200-400 nM range both for LXRα and LXRβ [10], which is consistent with the physiological levels of these cholesterol metabolites in target tissues [11]. Characterization of oxidized cholesterol metabolites as LXR ligands, along with the identification of an LXRE in the rat cholesterol 7α-hydroxylase gene (*cyp7a1*) that encodes the rate-limiting

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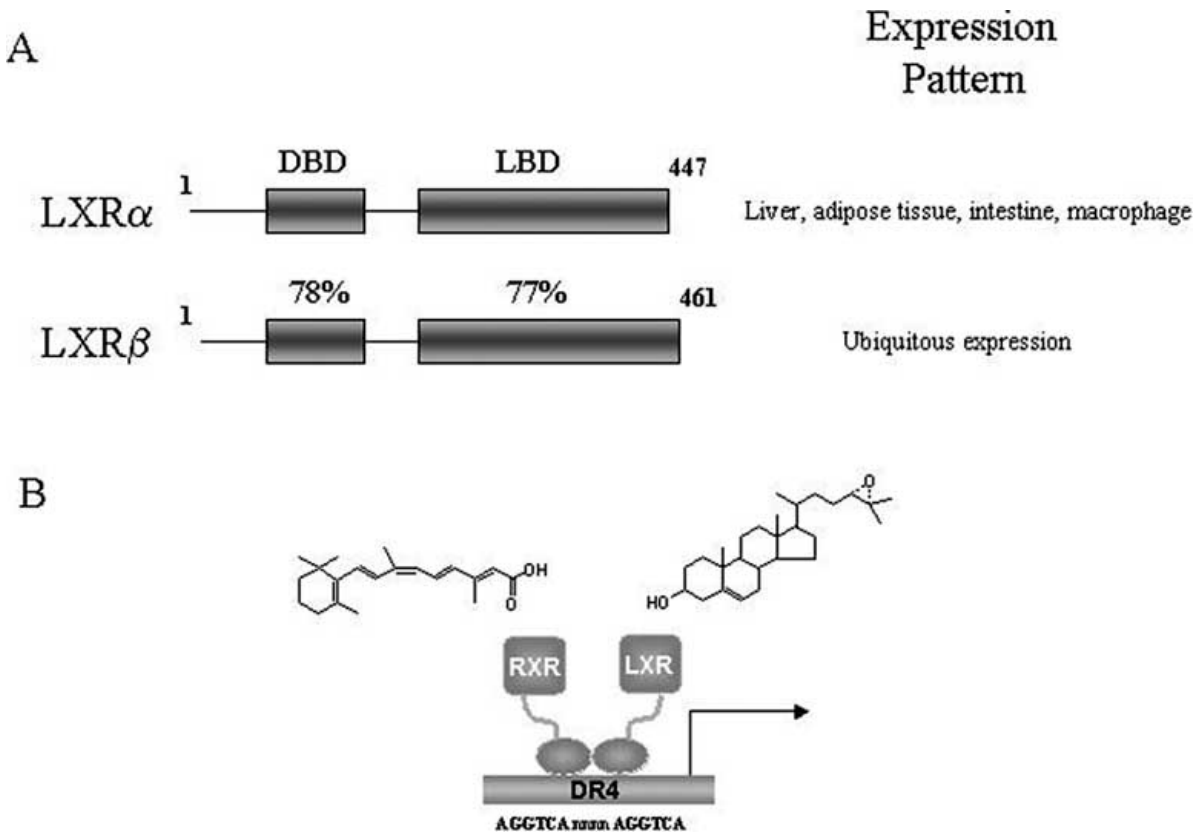

Fig. (1). LXRs are members of the nuclear hormone receptor superfamily. A) Two LXR subtypes exist, LXRα and LXRβ, with very similar DNA binding (DBD) and ligand binding domains (LBD). Expression patterns for the two receptors are distinct as illustrated. B) LXRs function as permissive heterodimers with another nuclear hormone receptor known as the retinoid X receptor (RXR). The heterodimer recognizes a direct repeat element known as an LXRE. The heterodimer may respond to either RXR or LXR ligands. The structures of a RXR ligand (9-*cis* retinoic acid) and a LXR ligand (24,25-epoxycholesterol) are shown.

enzyme for the conversion of cholesterol to bile acids, suggested that LXR plays a key physiological role in regulating cholesterol catabolism [12]. Indeed, the characterization of LXRα null mice underscores this hypothesis since mice lacking the receptor are unable to increase the expression of *cyp7a1* in response to a high cholesterol diet and are thus unable to increase bile acid synthesis in response to this dietary stress [13]. As a result of the lack of ability to catabolize cholesterol, the null mice accumulate significant amounts of hepatic lipid and exhibit elevated LDL [13].

The relevance of this pathway in humans was initially questioned because of the differential response to a cholesterol loading diet in primates as compared to rodents. Although rodents respond to a high cholesterol diet by increasing *cyp7a* expression and by increasing bile acid production, some primates do not respond in this manner. In fact, some primates repress *cyp7a* expression in response to cholesterol loading [14]. In concordance, the LXRE within the rodent *cyp7a* promoter is not present in the human gene [15,16]. Yet, the relative conservation of the rank order of potency and efficacy of various oxysterol ligands for mouse LXR and human LXR suggests that although sequence conservation at the level of *cyp7a* regulation of cholesterol catabolism is lacking, preservation of cholesterol metabolism between the species at some other level is likely.

Development of specific non-sterol ligands for LXR lead to the identification of conserved metabolic pathways. Tularik, Inc. described the first non-sterol agonist of LXRα and LXRβ (T0901317). This agonist has facilitated the identification of a large number of target genes including ABC transporters (ABCA1, ABCG1, ABCG5/G8) that are involved in sterol transport [17-22] (Table **1**). Additional target genes, including PLTP, ApoE, CETP, and LPL, all with the characteristic DR4 LXRE were identified, further illustrating an important role for LXR in cholesterol transport and metabolism [23-29]. SREBP-1c, the 'master regulator' of fatty acid and triglyceride synthesis, also was identified as an LXR target gene [30-31]. Most recently, LXR as been shown to play a role in other metabolic pathways, including glucose metabolism and inflammation [32-34].

THERAPEUTIC APPLICATIONS FOR LXR AGONISTS

Atherosclerosis

Cardiovascular disease encompasses a variety of conditions involving pathological changes in blood vessels, including but not limited to stroke, ischemic heart disease, and coronary heart disease (CHD). The chronic condition common to these diseases is atherosclerosis, which is characterized by a thickening of the inner wall of the artery

Table 1. LXR Target Genes

	Regulation		Response element	Reference
CYP7A1 (murine)	↑	direct	TGGTCA ctca AGTTCA	12
ABCA1	↑	direct	AGGTTA ctat CGGTCA	19
ABCG1	↑	direct	TGGTCA ctca AGTTCA	22
		direct	AGTTTA taat AGTTCA	
CETP	↑	direct	GGGTCA ttgt CGGGCA	28
PLTP	↑	direct	AGGTTA ctag AGTTCA	23,25
ApoE	↑	direct	GGGTCA ctgg CGGTCA	27
LXRα	↑	direct	AGGTTA ctgc TGGTCA	72,74
Angptl3	↑	direct	AGGTTA catt CGTGCA	76
LPL	↑	direct	TGGTCA ccac CGGTCA	29
SREBP-1c	↑	direct	GGGTTA ctgg CGGTCA	30,31
SHP (human)	↑	direct	TGACCT tgtt TATCCA	73
	Regulation		**Mechanism**	**Reference**
ABCG5/G8	↑	unknown	unknown	17,20
PEPCK	↓	unknown	unknown	32
PC	↓	unknown	unknown	32
FBPase	↓	unknown	unknown	32
CYP7A1 (human)	↓	indirect	repression *via* upregulation of SHP, which inactivates FXR	73
MMP-9	↓	indirect	transrepression of NF-kB	75
COX-2	↓	indirect	transrepression of NF-kB	34
iNOS	↓	indirect	transrepression of NF-kB	34

that ultimately reduces the size of the arterial lumen. Accumulation of lipid deposits in the arterial wall typifies the beginning of the atherosclerotic process. High concentrations of lipids derived from plasma lipoproteins exacerbate the promotion and development of arterial lipid deposits because monocytes and T-lymphocytes are recruited to, adhere to and ultimately migrate into the subepithelial space at the site of lipid deposition. Once within the subepithelial space, monocytes differentiate into macrophages that engulf large amounts of lipids, largely oxidized LDL, and ultimately transform into lipid-laden foam cells that are characteristic of an atherosclerotic plaque.

Despite improved clinical care and widespread use of lipid-lowering drugs, such as statins, CHD remains the leading cause of death in the United States. Currently an estimated 12 million persons in the U.S. are living with CHD [35]. Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, are the most effective class of LDL-cholesterol lowering drugs. Although LDL-cholesterol lowering is the current primary target of therapy, effective treatment of CHD necessitates increasing low HDL cholesterol levels [36]. Numerous population studies have illustrated an inverse correlation between plasma HDL levels and risk of CHD. LXR is one promising molecular target for raising HDL cholesterol. Potent LXR agonists activate genes involved not only in HDL accumulation, but also in catabolism of cholesterol to bile acids, in regulation of several genes important for reverse cholesterol transport from peripheral tissues, and in cholesterol excretion into bile or intestinal lumen. The battery of LXR target genes mediating these effects includes Cyp7A1, ABCA1, ABCG1, apolipoprotein E, PLTP, LPL, CETP, ABCG5, and ABCG8 [12, 13, 17, 21, 24, 26-29, 37, 38].

The discovery that the causative genetic defect underlying the development of Tangier disease, a rare recessive disorder typified by low HDL and ApoA1 levels, is a mutation in the ATP-binding cassette transporter, class A1 gene (*Abca1*) infused excitement into the field of HDL metabolism research [39-42]. Further research confirmed that cholesterol efflux from macrophages/foam cells to HDL particles requires the expression of ABCA1 [43-45]. Expression of the *Abca1* gene is controlled, in part, by LXR/RXR heterodimers in macrophages and liver. Because of the critical role ABCA1 plays in reverse cholesterol transport from macrophages, LXR agonists that can augment expression of the *Abca1* gene in macrophages/foam cells may act either to slow the development of atherosclerotic lesions or to regress existing lesions. Proof-of-concept experiments demonstrating the critical role of LXR in the progression of foam cell/atherosclerotic lesion development have been performed in a variety of rodent models. Despite normal plasma cholesterol levels in low-fat fed LXRα/LXRβ null mice, a significant increase in foam cell accumulation occurred in several organs and the aorta as

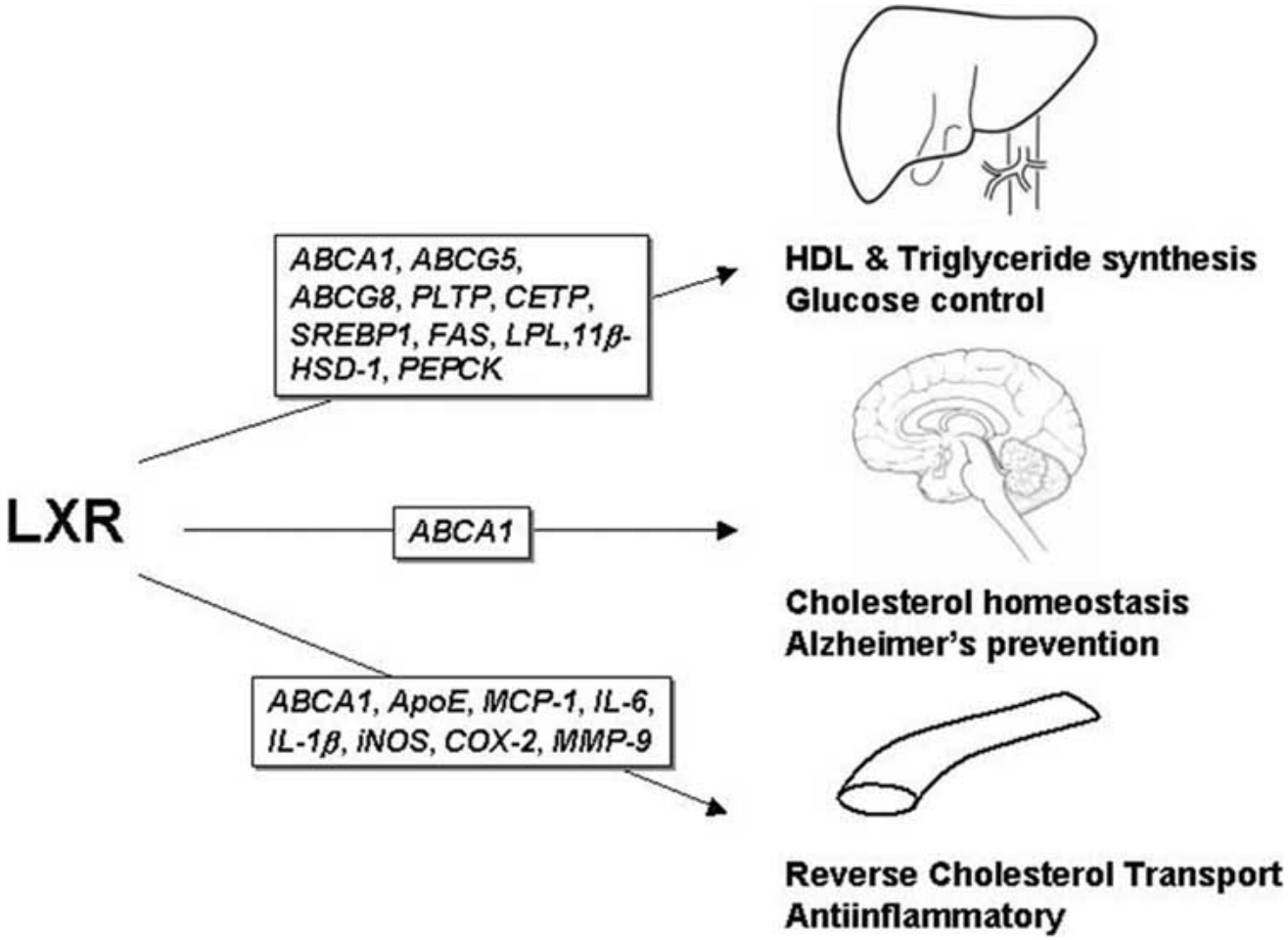

Fig. (2). Mechanisms contributing to LXR pharmacology. In liver, LXR promotes HDL formation by increasing the expression of genes such as ABCA1, ABCG5, ABCG8, PLTP. The master regulator of lipogenesis, SREBP1, is also an LXR target gene. Anti-diabetic activities of LXR include the repression of the PEPCK and 11βHSD genes. In the central nervous system, cellular cholesterol depletion by increasing ABCA1-mediated cholesterol efflux may, in fact, decrease Abeta formation. Anti-inflammatory activities in the vascular wall by the LXR agonists have been noted since agonists reduce expression of a variety of genes involved in innate immunity.

compared to wild-type control mice [46]. Likewise, bone marrow transplantation from LXRα/LXRβ null mice into either LDL receptor (LDLR) or ApoE null mouse models lead to a significant increase in atherosclerosis without causing significant changes in lipoprotein parameters. Results from both studies suggest that the absence of LXR promotes/accelerates the development of atherosclerosis [47]. Conversely, administration of the non-steroidal agonist, GW3965, either to LDLR- or ApoE-null mouse models caused an ~30-50% reduction in lesion area [48]. GW3965 also increased *Abca1* gene expression in the aortas of hyperlipidemic mice [48]. These cumulative results from *in vivo* studies warrant further evaluation of LXR agonists as potential pharmacological interventions for patients with low HDL cholesterol.

Sterol regulatory element-binding proteins (SREBPs) are considered to be the key transcription factors that regulate the synthesis of sterols and unsaturated fatty acids in mammalian cells. Ligand activation of LXR *in vivo* induces expression of SREBP-1c and increases plasma and liver triglyceride levels, findings that firmly establish the SREBP1 gene as a direct target gene of LXR [18, 30, 31]. Fatty acid synthase (FAS), a pivotal enzyme in *de novo* lipogenesis and an established target gene of SREBP-1, also is induced by LXR ligands. Recently, sequences within the 5'-flanking region of FAS gene have been characterized as an LXRE [49]. This finding demonstrates that not only can LXR indirectly increase expression of FAS, but also it can directly increase FAS gene transcription. As synthetic LXR agonists are further developed for therapeutic use, vigilance for induction of *de novo* lipogenesis will be necessary.

METABOLIC SYNDROME/INSULIN RESISTANCE

Metabolic Syndrome is characterized by hyperlipidemia, hypertension, obesity, and insulin resistance. Several research groups who have explored the putative connection between glucose homeostasis and LXR action recognized the close link between lipid and glucose metabolism. This was first demonstrated in rodent models of diet-induced obesity and insulin resistance or genetic rodent models of insulin resistance (*db/db* mice and *fa/fa* rats). LXR action in both liver and adipose tissue exerts some control over glucose homeostasis. Specifically, administration of the synthetic

agonist T0901317 to *db/db* mice and Zucker diabetic fatty rats resulted in a dose-dependent lowering of plasma glucose [32]. Likewise, GW3965 improved glucose tolerance in a C57BL/6 mouse model of diet-induced obesity and insulin resistance as compared to lean mice [50]. The mechanism by which LXR exerts these 'anti-diabetic' activities include the repression of hepatic gluconeogenesis and the augmentation of glucose uptake by adipose tissue. In the liver, both synthetic agonists suppressed the expression of some genes responsible for the gluconeogenic program, such as phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase, and peroxisome proliferators-activatedreceptor γ coactivator-1α. In adipose, GW3965 stimulated the expression of the insulin-sensitive glucose transporter, GLUT4, but had no effect on the expression of GLUT1. Treatment of 3T3-L1 adipocytes with GW3965 caused a dose-dependent increase in insulin-stimulated glucose uptake. Cumulatively, these actions of LXR agonists could limit hepatic glucose production and increase peripheral glucose utilization.

By yet another mechanism, LXR agonists may ameliorate the development of insulin resistance. Obesity is associated with elevated levels of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1), and overexpression of 11β-HSD-1 in mice causes insulin resistance, diabetes, and hyperlipidemia. In contrast, mice deficient in 11β-HSD-1 exhibit improved glucose tolerance, enhanced hepatic insulin sensitivity, and an anti-atherogenic lipid profile. Treatment of mice with T0901317 resulted in ~50% decrease in 11β-HSD-1 mRNA expression both in liver and in brown adipose tissue [51]. Expression of the 11β-HSD-1 gene likewise was regulated by LXR agonists in 3T3-L1 adipocytes. This finding substantiates the fact that LXR agonists could benefit patients with metabolic syndrome/type 2 diabetes who are at risk for cardiovascular disease.

INFLAMMATION

Atherosclerosis is an inflammatory disease. Inflammatory mediators such as MCP-1, IL-6 and IL-1β recruit monocytes to lesions and stimulate smooth muscle proliferation. The matrix metalloproteinase, MMP-9, is expressed in atherosclerotic lesions and plays a role in vascular remodeling and plaque instability. To explore a putative role for LXR in blunting a macrophage-mediated inflammatory response, Joseph *et al*. treated mouse macrophages with GW3965 and challenged them with lipopolysaccharide (LPS) [34]. Expression of a variety of genes involved in innate immunity was inhibited by the LXR agonists, namely, the genes encoding MCP-1, IL-6, IL-1β, iNOS, and COX-2. *Apoe* null mice treated with GW3965 demonstrated a substantial decrease in MMP-9 mRNA in atherosclerotic areas of the aorta. Thus, the potential benefit for developing an LXR agonists explicitly for the treatment of atherosclerosis may lie in the ability of LXR to regulate both lipid homeostasis and anti-inflammatory activities in the vascular wall.

The anti-inflammatory activity of LXR also has been observed in irritant and allergic contact dermatitis models. Irritant dermatitis, as measured by increased ear weight and thickness, is markedly suppressed by topical application of the LXR agonist, GW3965 [33]. Mechanistically, inhibition of the inflammatory response likely occurred, in part, by a repression in the production of the pro-inflammatory cytokines IL-1α and tumor necrosis factor-α. A reduction in edema and inflammatory filtrate by LXR agonists, GW3965 and T0901317, also was described in a similar murine model of irritant contact dermatitis. Hence, LXR agonists may also serve as therapeutic agents for cutaneous inflammatory disorders.

CNS DISORDERS

The importance of LXR in brain physiology was first explored in the LXR null mouse models. Absence of both LXRα and -β in aged mice led to severe disturbances in CNS lipid homeostasis and correlated with age-related neuropathological changes [52]. Specifically, the lateral ventricles were closed and lined with lipid-laden cells, and some brain blood vessels were enlarged. Excessive lipid deposits, proliferation of astrocytes, loss of neurons, and disorganized myelin sheaths were also noted. These findings illustrate that LXRs have an important function in lipid homeostasis in the brain and that loss of these receptors results in neurodegenerative disorders.

The involvement of cerebral cholesterol homeostasis in Alzheimer's disease (AD) progression was first illuminated by the description of the apolipoprotein E (apoE) allele epsilon;4 as a strong risk factor for development AD [53, 54]. Indeed, epidemiological and biochemical studies have further shown that cellular cholesterol levels can regulate the synthesis of amyloid beta-protein (Abeta), which forms plaques in the brains of afflicted individuals. Abeta synthesis occurs through sequential cleavage of the type I integral membrane amyloid precursor protein (APP) by β- and γ-secretase. Once β-secretase cleaves APP extracellularly, γ-secretase yields the Abeta peptide fragment following an intramembranous cleavage. The initial β-secretase-mediated cleavage of APP has been suggested to occur in cholesterol-rich ordered domains of the plasma membrane [55]. Accordingly, two laboratories hypothesized that cellular cholesterol depletion by ABCA1-mediated cholesterol efflux may, in fact, decrease Abeta formation [56, 57]. Treatment of neuron-derived cell lines with LXR agonists T0901317 and 22(R)hydroxycholesterol increased the expression of cellular ABCA1 protein and reduced the level of Abeta secretion [56]. Yet, the effect of ABCA1 to decrease Abeta secretion was independent of cellular lipid efflux. The hypothesis that ABCA1-induced redistribution of cholesterol at the plasma membrane or membranes of the Golgi or endocytic compartments may lead to a decrease in γ-secretase activity has been suggested. Despite the mechanism, LXR-mediated ABCA1 induction may serve a protective role against AD.

MEDICINAL CHEMISTRY AND STRUCTURE ACTIVITY RELATIONSHIPS

Agonists

Oxysterols, a subset of cholesterol derivatives adorned with oxidation in the C(20)-C(27) sidechain, activate LXR

and can be found at concentrations in the requisite tissues that are consistent with their proposed role as endogenous ligands. Since the discovery of oxysterols as activators of LXR a number of groups have disclosed structure activity relationships (SAR) of natural and synthetic oxysterols. Janowski *et al*.[10] have developed a radioligand binding assay for both LXRα and LXRβ and have demonstrated that these ligands bind directly to the receptors. In related studies, Spencer *et al*.[58] and Lehmann *et al*. [12] investigated the activation of LXRα using natural and synthetic oxysterols with a cell-free assay that responds to the ligand dependent recruitment of a coactivator LXXLL peptide derived from SRC1 (ligand sensing assay (LiSA) (See Table **2** and Fig. **3**). Both groups found that 24(S),25-epoxycholesterol (**1**), 24(S)-hydroxycholesterol (**6**), and 22(R)-hydroxycholesterol (**7**) were potent transcriptional activators of LXRα and LXRβ. Janowski *et al*. disclosed that compounds **1**, **6**, and **7** had K_is of 200 nM, 110 nM and 380 nM respectively at LXRα with similar binding affinities also observed for LXRβ. In agreement with this, Spencer *et al*. showed that 24(S),25-EC (**1**), 24(S)-HC (**6**), and 22(R)-HC (**7**) possessed transcriptional potencies of 460 nM, 130 nM, and 325 nM respectively in the SRC1 recruitment assay. That these activities occur at concentrations similar to those reported in liver tissues strengthens the hypothesis that these oxysterols are endogenous ligands for both LXRα and LXRβ. However, the unnatural isomer of 24(S), 25-EC (**1**), *i.e.* 24(R),25-EC (**2**) has an EC_{50} of 670 nM in the LiSA assay which is only slightly less potent than 24(S),25-EC (**1**). This is in contrast to the results of Janowski *et al*. where the binding and co-transfection assays for (**2**) was substantially less potent than for (**1**).

Another significant difference in activities between these studies is revealed in (22(S)-HC) (**8**). Compound (**8**), the unnatural epimer of (22(R)-HC) **7,** possesses similar binding affinities for both LXRα and LXRβ (K_i =150 nM and 160 nM respectively). However, both groups found that 22(S)-

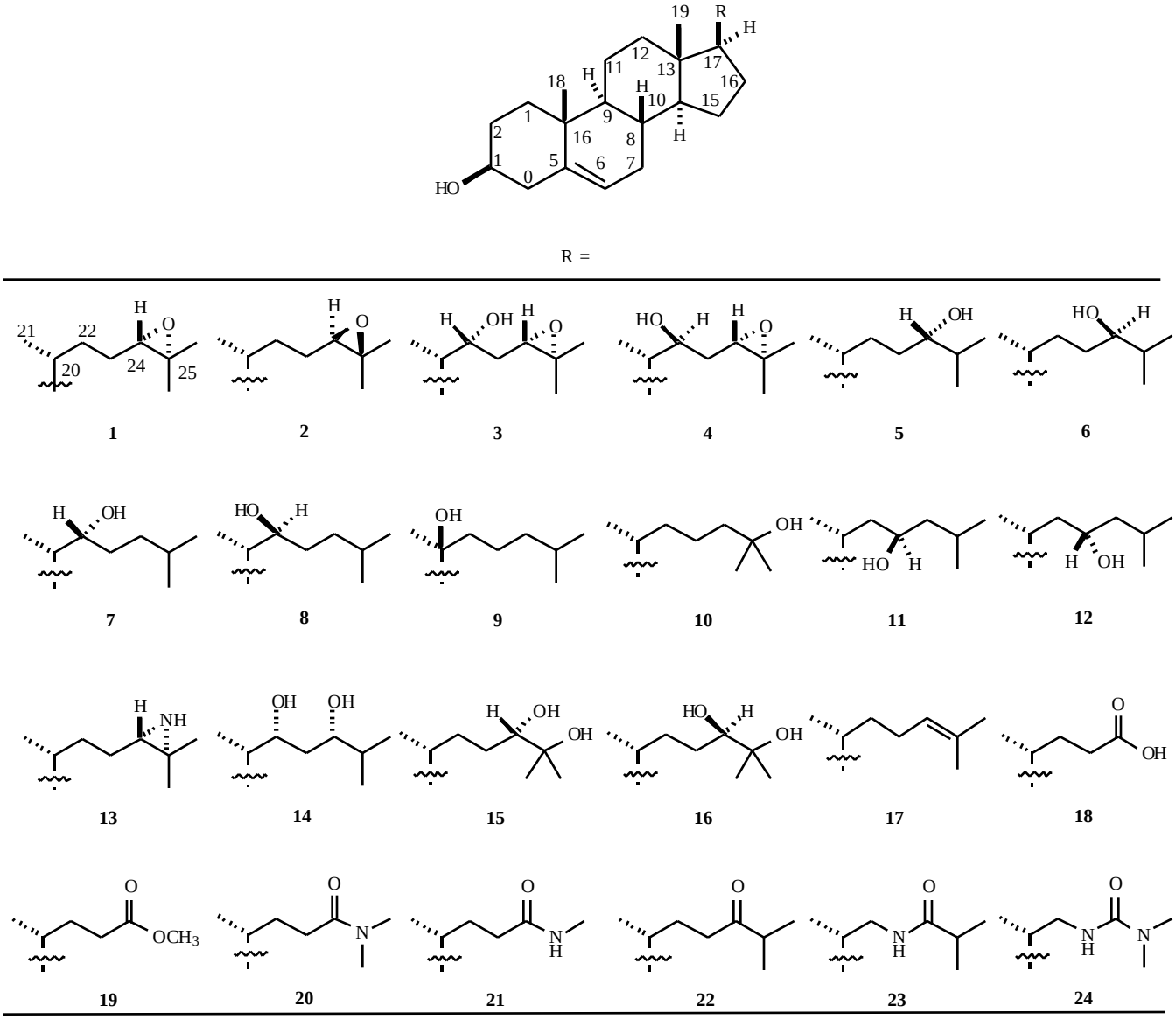

Fig. (3). Sterol Sidechain Structural Modifications. See Table **2** for the corresponding LXR data. Standard steroid numbering is shown.

Table 2. Summary of LXR Data from References 10 and 58 (See Figure 3 for Corresponding Structures)

		Reference 10				Reference 58
		WT LXRα		WT LXRβ		LXRα
	Compound	Ki, nM	EC_{50}, (RE) uM	Ki, nM	EC_{50}, (RE) uM	EC_{50}, uM
1	24(S),25-epoxycholesterol	200	4 (1.0)	200	3 (1.0)	0.46
2	24(R),25-epoxycholesterol	1,200	10 (0.47)	710	10 (0.50)	0.67
3	22(R)-ol-24(S),25-epoxycholesterol	ia	ia	300	ia	NR
4	22(S)-ol-24(S),25-epoxycholesterol	440	ia	920	ia	NR
5	24(R)-hydroxycholesterol	ia	7 (0.20)	ia	4 (0.38)	0.22
6	24(S)-hydroxycholesterol	110	4 (0.40)	100	3 (0.70)	0.13
7	22(R)-hydroxycholesterol	380	5 (0.40)	130	3 (0.57)	0.32
8	22(S)-hydroxycholesterol	150	ia	160	ia	ia
9	20(S)-hydroxycholesterol	NR	NR	NR	NR	0.47
10	25-hydroxycholesterol	180	7 (0.16)	300	ia	1.16
11	23(R)-hydroxycholesterol	NR	NR	NR	NR	ia
12	23(S)-hydroxycholesterol	NR	NR	NR	NR	5.90
13	24(S),25-iminocholesterol (Aziridine)	990	toxic	1000	toxic	NR
14	22(R),24(S)-dihydroxycholesterol	950	ia	710	ia	NR
15	24(R),25-dihydroxycholesterol	ia	ia	1700	ia	NR
16	24(S),25-dihydroxycholesterol	1200	ia	ia	ia	NR
17	24,25-dehydrocholesterol (Alkene)	ia	No sat (0.16)	ia	No sat (0.31)	NR
18	cholenic acid	NR	NR	NR	NR	3.00
19	cholenic acid methyl ester	110	8 (0.74)	170	8 (0.83)	0.10
20	cholenic acid dimethylamide	130	2 (0.60)	100	2 (0.50)	0.17
21	cholenic acid monomethylamide	NR	NR	NR	NR	0.72
22	24-ketocholesterol	NR	NR	NR	NR	0.18
23	isobutyramide	NR	NR	NR	NR	ia
24	dimethylurea	NR	NR	NR	NR	ia

HC (**8**) suffered significant loss of functional activity in whole cells. Spencer *et al*. further characterized (**8**) as an antagonist in the LXRα LiSA and LXRα GAL4 assays. Janowski *et al*. reports that (**8**) is not an antagonist and suggests the lack of antagonist activity is explained as the result of the compound's inability to get to the receptor in the whole-cell assay due to intrinsic pharmacokinetic properties. Although one must proceed with caution when comparing results of studies containing substantially different assays, this difference in reported activities for 22(S)-HC (**8**) requires further clarification.

Incorporating a hydroxyl at C(23) yielded compounds **11** and **12** with significant decreases in potency relative to 24,25-EPC (**1**). In the LiSA assay both the (R) and (S) isomers at C-24, *i.e.* (**5**) and (**6**) were active although the unnatural isomer (**5**) was less efficacious than the natural isomer (**6**). Compound **9**, 20(S)-HC which has oxygenation only at C-20, showed equipotent activity (EC_{50} of 470 nM) to 24(S),25-EC in the LiSA assay. Interestingly, 25-HC (**10**), a compound possessing an achiral hydroxyl group at C-25 exhibited substantial binding potency but much like 22(S)-HC (**8**) lacks transcriptional activity. Compound **10** also showed low efficacy in the LiSA assay (EC_{50} = 1.16 μM, RE = 0.35).

Multiple sidechain oxidation led to compounds exhibiting decreased receptor affinity and functional activity. For instance, incorporating a hydroxyl group at C-22 of 24(S),25 EC (**1**) provided diastereomers **3** and **4,** each possessing a loss in binding and transcriptional potency relative to 24(S),25 EC (**1**). Moreover, compounds possessing a diol functional group (*i.e.* **14**, **15** and **16)** all had significant decreases in receptor binding and functional potencies.

Replacing the epoxide in **1** with an aziridine as in **13** resulted in a considerable loss in binding and functional activity. An expected loss in hydrogen bond acceptor ability of the aziridine nitrogen is predicted to cause the loss in binding since it would be protonated at the pH of the assay. Removing the hydrogen bond acceptor at position-24 as in cholesterol itself (data not shown) or in 24,25-dihydrocholesterol (**17**) led to compounds without binding and functional activity at the receptor.

Compounds **18-24** were synthesized to study the effect of alternative hydrogen bond acceptors at C(24). Ketone **22** showed an increase in potency (EC_{50} = 180 nM) and efficacy (RE = 1.3) relative to **1**. The carboxylic acid **18** was nearly inactive while the corresponding methyl ester **19** showed equi-potency to the ketone **22**. The secondary amide **21** exhibited a four-fold decrease in potency relative to ketone **22** but the dimethyl amide **20** was the most potent and efficacious compound described in both studies. The reverse isobutrylamide **23** and dimethylurea **24** were inactive indicating that a nitrogen group at C(23) was not tolerated. Ultimately, both studies concluded that the hydrogen bond acceptor functionality at C(24) is a necessary pharmacophore for potency and efficacy at LXR. It is notable that the dimethyl amide (**20**) was identified as the most potent and efficatious compound in both studies.

Most of the compounds described in Table **2** had nearly equipotent binding and functional activities towards both LXRα and LXRβ [58]. An example of a selective compound is the bis-epoxide **25**, which shows selectivity as an LXRα agonist (See Fig. **4**). It possesses a K_i of 330 nM at LXRα along with a moderate EC_{50} = 7 μM at LXRα, but it is inactive at LXRβ. It is unclear whether this selectivity is a result of the hydrogen bond acceptor ability of the second epoxide or an induced conformational change of the B ring of the sterol nucleus as a result of the second epoxide.

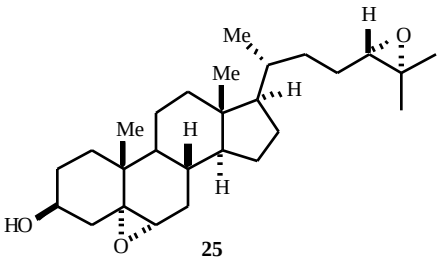

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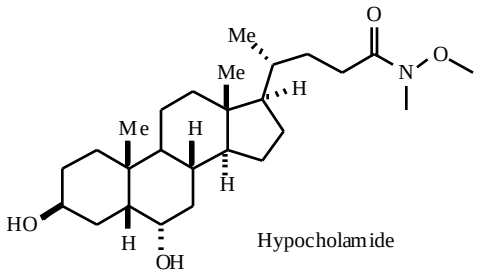
Fig. (4). Structure of 5,6-24(S),25-Bisepoxycholesterol (25).

Bile acids have been reported to be selective agonists of LXRα versus LXRβ [59]. Synthetic analogues of these bile acids also possessed selective activity towards LXRα as exemplified by hypocholamide (See Fig. **5**). Hypocholamide has an ED_{50} of 60 nM on LXRα and an ED_{50} of 300 nM on LXRβ using a natural reporter gene assay. Hypocholamide is ~16 times more active than the previously identified 2,2,2-trifluoroethyl-3α,6α-dihydroxy-5β-cholanamide and was therefore orally dosed in mice.

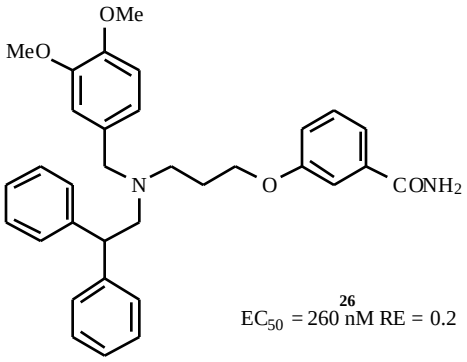
When administered orally to male C57BL/6J mice (mice susceptible to atherosclerosis development), hypocholamide lowered serum cholesterol levels in a dose dependent manner while eliciting no increase in serum triglyceride levels [60]. This is in contrast to compound T0901317 **38** (see below), which increased serum HDL cholesterol and serum triglycerides when administered orally in mice. The reasons for these differences have yet to be determined, but a possible rationale is the selective activation of LXRα by hypocholamide whereas T0901317 **38** activates both LXRα and LXRβ. The deactivation of hypocholamide in the liver through glucuronidation has also been postulated to account for the differences.



Hypocholamide

Fig. (5). Structure of hypocholamide.

A GlaxoSmithKline group recently disclosed a series of LXR agonists identified from a focused library of tertiaryamines [61]. Carboxamide **26** was identified as a lead in the series from a high-throughput screen using the LiSA assay. Amide **26** exhibited an EC_{50} of 260 nM at LXRα with partial efficacy (RE = 0.2) in the LiSA assay relative to 24,25-EPC (**1**).



26

EC_{50} = 260 nM RE = 0.2

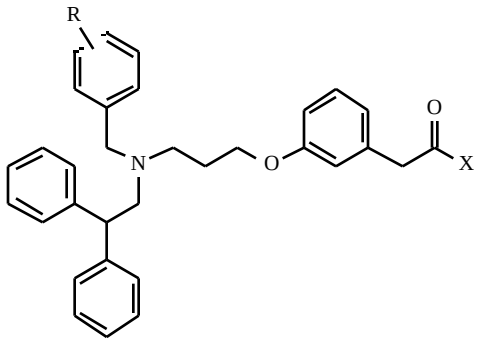
Fig. (6). Structure of tertiary amine lead from reference 61.

SAR intended to simultaneously explore the benzyl amine and benzamide portion of the molecule identified acetamide **27** with a potency of 260 nM at LXRα and increased efficacy in the SRC1 recruitment assay relative to **26**. See Table **3**. Compound **27** lacked potency in the whole-cell Gal4 assay, but the corresponding carboxylic acid **28** showed improved efficacy with an EC_{50} = 8 μM in the Gal4 assay. Continued SAR on the benzyl amine portion of the molecule indentified compounds **29-32** with improved the potency and efficacy both in the LiSA and Gal4 assays. As indicated by compounds **32-35** the 3-trifluoromethyl functionality was identified as a recurring pharmacophore for potency in these assays. Compound **34**, containing the 2-chloro-3-trifluoromethylbenzylamine, was recognized as the most potent molecule in this series possessing an EC_{50} of 45 nM and 425 nM in the LiSA and Gal4 assays, respectively. The relative loss of potency in the Gal4 assay for compound **34** was ameliorated, as before, with the synthesis of carboxylic acid **35**. Carboxylic acid **35** exhibited a potency of 125 nM in the SRC1 recruitment assay and maintained its potency in the Gal4 assay with an EC_{50} of 190 nM. Analogue **35** was tested in a nuclear hormone receptor screen and also showed activity at LXRβ and to a lesser extent, PXR. *In vivo* studies of carboxylic acid **35**, dosing at 10mg/kg *bid* for 14 days in C57BL/6 mice revealed an increase in ABCA1 expression in the small intestine and peripheral macrophages. Levels of HDLc in plasma increased 30% at day three and this level was maintained for the 14-day duration.

A group at Merck has introduced a dimeric compound (APD **36**) with LXR agonist activity ~1000 times more potent than 22(R)-hydroxycholesterol (**7**) in transactivation assays for LXRα and LXRβ [62] (See Fig. **7**). Moreover this compound exhibited six times the maximal stimulation of 22(R)-hydroxycholesterol (**7**) and showed little activity at the other nuclear hormones PPARα, PPARγ, PPARδ and RXR. APD was shown to recruit SRC1 through LXRα and LXRβ and has induced the *abca1* expression in a number of cell-types. *In vivo* data for this compound has yet to be reported.

T0314407 (**37**) and T0901317 (**38**) represent two potent agonists identified by researchers at Tularik. In a rhodamine-labeled LXXLL peptide FPA assay T0314407 (**37**) has an EC_{50} of 100 nM while T0901317 (**38**) possesses a five-fold increase in potency with an EC_{50} of 20 nM at LXRα. Both compounds are at least as efficacious as 24,25-EC (**1**). In a radioligand binding assay these compounds have IC_{50} values

Table 3. Summary of LXR Activity of Tertiary Amines



Compd	X	R	LXRα/ SRC1 LiSA EC_{50}	LXRα/Gal4 EC_{50}
27	NH_2	4-methoxy	260 0.2	>10 uM
28	OH	4-methoxy	860 0.8	8000 0.9
29	NH_2	3,4-dimethoxy	660 0.7	4000 0.6
30	NH_2	3-fluoro-4-methoxy	250 0.7	910 0.5
31	NH_2	2-fluoro-4-methoxy	190 0.9	700 0.4
32	NH_2	3-trifluoromethyl	85 0.7	650 0.3
33	NH_2	4-fluoro-3-trifluoromethyl	85 0.9	945 0.4
34	NH_2	2-chloro-3-trifluoromethyl	45 1.1	425 1.5
35	OH	2-chloro-3-trifluoromethyl	125 1.0	190 1.7

of 100 nM and 20 nM respectively when competing for radiolabled T0314407. They also exhibited comparable values in HEK293 co-transfection assay using WT LXRα. Selectivity for the LXRs was exhibited, as these compounds did not appreciably activate the other nuclear receptors.

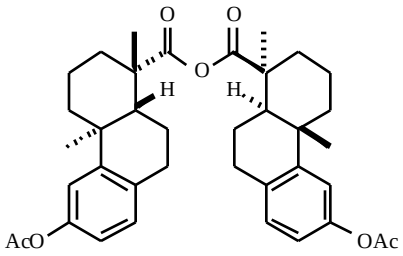
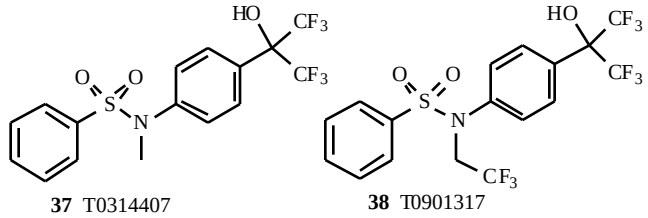


Fig. (7). Structure of APD (**36**).

T0901317 (**38**) was further characterized *in vivo* and up-regulated a number of fatty acid biosynthetic genes including the genes for fatty acid synthase and SREBP-1. T0901317 (**38**) increased plasma and hepatic triglyceride and phospholipid levels in mice when treated for 7 days at 5.0 mg/kg [30]. This study also showed that the increases in lipid changes where an LXR mediated event utilizing LXRα/β-/- mice. Treatment of these knockout mice with T0901317 (**38**) resulted in no appreciable increases in hepatic triglycerides and only minute increases in total plasma triglycerides and plasma VLDL-triglycerides. Treatment of LXRα/β-/- mice with the LXR agonist T0901317 (**38**) has also implicated LXR's in ABCA1 gene expression [18].

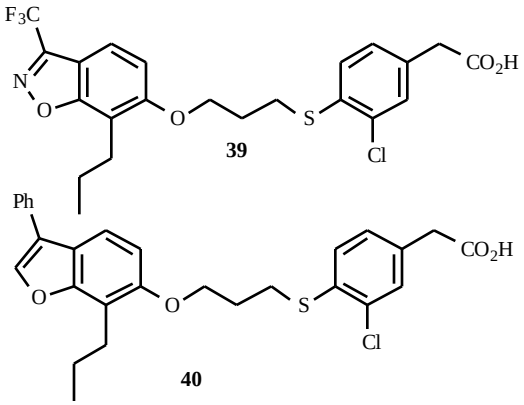
Merck has reported that benzisoxazole **39** is an LXR agonist [63] (See Fig. **9**). It shows activity in both LXRα and LXRβ SRC-1 recruitment assays and possesses binding affinities of 13 nM and 7 nM at LXRα and LXRβ,



37 T0314407 **38** T0901317

Fig. (8). Structure of Tularik's T0314407 and T0901317.

respectively. Compound **39** also shows cell-based transcriptional activity at mouse, rat, hamster and human receptors. Benzisoxazole **39** and the structurally related benzofuran **40** have previously been shown to possess PPAR agonist activity [64] at both mouse and human receptors. Benzofuran **40** was inactive in all of the LXR assays described above and thus was proposed as a negative control for **39** to establish which effects are mediated through LXR.



39 **40**

Fig. (9). Structure of benzisoxazole **39** and benzofuran **40**.

Bramlett *et al*. reported Paxilline (**41**) as the first non-oxysterol natural product ligand for LXR [65] (See Fig. **10**).

Paxilline (**41**), an indole alkaloid fungal metabolite, displaced tritiated 25-hydroxycholesterol with K_i values of 660 nM and 1100 nM in LXRα and LXRβ respectively, but did not bind or activate the other nuclear receptors examined. Co-factor recruitment assays with paxilline **41** using LXRα resulted in an EC_{50} of 1800 nM for SRC1 and 660 nM for TIF2. In comparison 22(R)-HC (**7**) had EC_{50} values of 2600 nM and 1400 nM for SRC1 and TIF2 respectively. Paxilline acts as an agonist as demonstrated through co-tranfection assays with $EC_{50's}$ of ~4000 nM for both receptors, which is equipotent to 22(R)-HC (**7**). Finally, paxilline was shown to induce the expression of the natural target gene ABCA1 in THP-1 cells, exhibiting a 7-fold induction with an EC_{50} = 1300 nM.

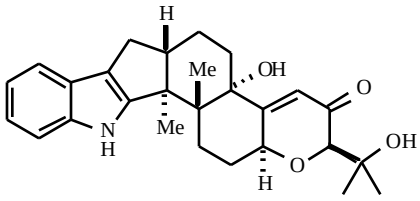


Fig. (10). Structure of paxilline (**41**).

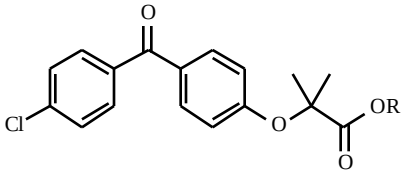
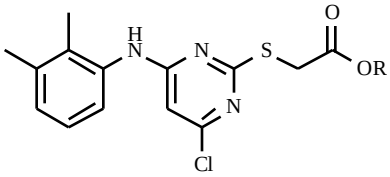
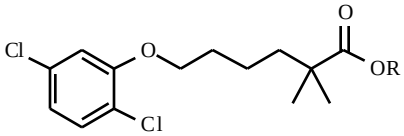
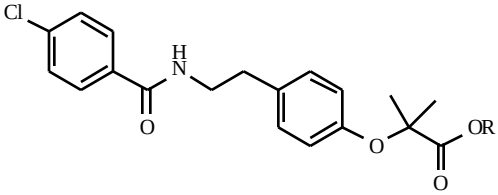
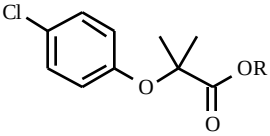
ANTAGONISTS

Unsaturated fatty acids act as competitive antagonists of LXR by inhibiting binding of the known LXR ligands T0901317, 24(S), 25-epoxycholesterol and 22(R)-HC [31, 66]. The inhibitory effects of these fatty acids were not seen at RXR, FXR and ERβ.

5α, 6α-Epoxycholesterol-3-sulfate and 7-ketocholesterol-3-sulfate have been shown to antagonize the effects of N, N-dimethylcholestenamide (CAM) on LXRα and LXRβ with IC_{50}s of 2 μM and 5 μM respectively [60]. The non-sulfated forms of these molecules do not possess an antagonistic effect. Geranylgeranyl pyrophosphate (GGPP), an intermediate in the mevalonate pathway, possesses some antagonistic effects at LXR [66, 68]. Thus GGPP partially reduces the 22(R)-HC mediated expression of ABCA1 and inhibits the interaction of LXRα and LXRβ with the co-factor SRC-1. It is interesting to note that LXR can be modulated in distinct ways from different intermediates of the mevalonate metabolic pathway. Thus oxysterols like 22(R)-HC (**7**) or 24(S), 25-epoxycholesterol (**1**) can act as agonists of LXR while GGPP has some antagonist effects indicating that the regulation of cholesterol and lipid metabolism can be altered by different products in the mevalonate pathway.

A series of unnatural antagonists of LXR have recently been reported [69] (See Table **4**). These iso-propyl esters of fatty acids (exemplified by fenofibrate) repressed the T0901317 activation of LXR in HEK293 cells with various potencies. Fenofibrate also repressed LXR agonist-induced

Table 4. Summary of LXRα, LXRβ and PPARα Data for Select Fatty Acids and their Corresponding *iso*-Propyl Esters

	Structure		LXRα (K_i uM)	LXRβ (K_i uM)	PPARα (K_i uM)
Fenofibric acid and ester		R = *i*-Pr R = H	7 >100	5.0 >100	>100 22
WY14643 acid and ester		R = *i*-Pr R = H	8 >100	5.6 >100	>100 9.4
Gemfibric acid and ester		R = *i*-Pr R = H	12 >100	5.2 >100	>100 5.8
Bezafibric acid and ester		R = *i*-Pr R = H	34 >100	31 >100	>100 >100
Clofibric acid and ester		R = *i*-Pr R = H	98 >100	82 >100	>100 >100

transcription of hepatic lipogenic genes but did not repress the LXR-induced transcription of various ATP-binding cassette transporters. Consistent with the functional potency these esters also showed binding affinities in a radioligand binding assay using tritiated 25-hydroxycholesterol. The K_is ranged from 5-98 μM and were similar for both LXRα and LXRβ. Interestingly, these iso-propyl esters showed no affinity (>100 μM) for PPARα, although the corresponding acids possessed modest affinity in some cases. Moreover, the acids also showed no affinity for LXRα and LXRβ thus demonstrating that the acid/ester exchange can act as a chemical switch determining PPARα *vs.* LXR specificity.

X-RAY CRYSTAL STRUCTURE OF LXRβ LIGAND BINDING DOMAIN

Williams *et al*. has recently disclosed the x-ray crystal structure of an oxsterol (24(S),25-epoxysterol, **1)** and nonoxysterol agonist (T0901317, **38**) bound in the LXRβ ligand binding domain [70]. The asymmetric unit possessed an LXRβ homodimer, where each monomer was occupied by a single ligand in the binding pocket. One of the more exceptional features of the binding pocket was the large ligand-binding domain seen in LXRβ as compared to the other nuclear hormone receptors. As in other x-ray crystal structures of nuclear hormones complexed with ligands, the hydrophobic contacts at the LXRβ ligand-receptor interface account for the majority of the interactions seen, but interesting hydrogen bonding interactions were also observed [71]. The compound 24(S),25-epoxysterol **1**, was bound with the D-ring and epoxide sidechain directed towards the C-terminal end of helix 10. The epoxide moiety interacted as a hydrogen bond acceptor with histidine 435, which aligned the imidazole ring of H435 against the indole ring of W457 thus creating an electrostatic interaction between the two amino acid side chains. It is postulated that this electrostatic interaction holds the AF2 helix in the active position. In an intriguing reversal of binding modalities, the acidic hydroxyl of T0901317 **38** acts as a hydrogen bond donor with histidine 435 setting up a similar, but not identical, electrostatic interaction with tryptophane 457 and again holding the helix 12 (AF2) in the active conformation. Interestingly, T0901317 exhibited different conformations about the tertiary sulfonamide in the two LXRβ subunits. It adopted a *gauche* conformation in one subunit and an *anti* conformation in the other thereby attesting to the large binding pocket exhibited within LXRβ.

LXR has emerged as an attractive target for the development of therapeutics for the prevention and treatment of cardiovascular diseases as well as other important disorders of glucose metabolism and inflammation. While many of the studies described above have showcased new tools for the study of the molecular basis of LXR activation more research is warranted. A major challenge for the pharmaceutical industry will be the development of LXR modulators that address the problem of increased hepatic lipogenesis leading to undesired side effects while maintaining the desired cholesterol homeostasis effects. The recent disclosure of the x-ray crystal structure of the LXRβ binding domain will surely aid in the development of more potent and specific LXR modulators.

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