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Synthesis, in vitro and in vivo biological evaluation of new oxysterols as modulators of the liver X receptors

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ABSTRACT

Liver X Receptor (LXR) modulators have shown potential as drugs since they target genes affecting metabolism and fatty acid synthesis. LXR antagonists are of particular interest since they are able to reduce the synthesis of complex fatty acids and glucose uptake. Based on molecular modeling, five new cholesterol mimics were synthesized, where four contained a hydroxyl group in the 22-S-position. The new compounds were screened in vitro against several genes affecting lipid metabolism. The compound that performed best in vitro was a dimethylamide derivative of 22(*S*)-hydroxycholesterol and it was chosen for in vivo testing. However, the blood plasma analysis from the in vivo tests revealed a concentration lower than needed to give any response, indicating either rapid metabolism or low bioavailability.

1. Introduction

The Liver X Receptors (LXRs) belong to the nuclear receptor superfamily. LXRs have been identified as promising drug targets due to their involvement in regulation of cholesterol, lipid metabolism and glucose metabolism [1,2]. LXR consists of two isoforms, LXRα and LXRβ [3]. LXRα is the main isoform found in the liver, but the receptor is also found in adipose tissue, skeletal muscle, macrophages, kidney and the small intestine. LXRβ is, however, found throughout the body [4]. The endogenous ligands for LXRs are oxysterols and bile acids[5–8].

LXR agonists have been developed as potential treatments for e.g. metabolic and cardiovascular disorders, and have shown promising regarding treatment of atherosclerosis and diabetes [9–11].

Several studies have shown that LXR activation over a prolonged period of time results in elevated uptake of glucose and fatty acids, leading to increased storage of fatty acids [12]. LXRs are also suggested to be involved in the pathogenesis of type 2 diabetes. [12] Specific LXR antagonists could reduce the synthesis of complex fatty acids, an underlying part of the pathogenesis of type 2 diabetes. However, only a few compounds have been described as LXR antagonists [13–16], e.g. 5α,6α-Epoxycholesterol (and derivatives), 22(*S*)-hydrocycholesterol (**22SHC**, **1**) and GSK2033 [13,17,18].

A selective LXR antagonist may have beneficial effects on glucose uptake and lipid metabolism, two processes of importance for obesity and type 2 diabetes [1,2]. LXR target genes stearoyl–CoA desaturase 1 (SCD1) and fatty acid synthase (FAS) code for enzymes that are key regulators in lipid metabolism; compounds repressing these genes could be lead drug candidates for treatment of type 2 diabetes and metabolic syndrome [18]. A small modification in the stereochemistry of an endogenous LXR agonist (22(*R*)-hydroxycholesterol) resulted in a compound (**22SHC**, Fig. 1-1) with selective antagonistic properties on lipogenesis, reducing or

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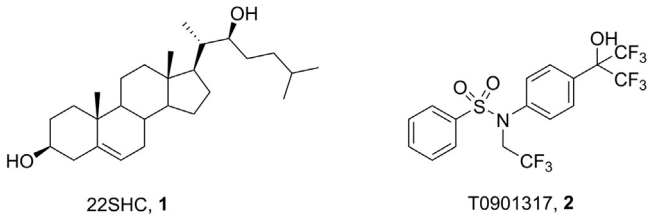


Fig. 1. Structure of the known LXR modulators **22SHC** (**1**) and LXR agonist **T0901317** (**2**).

abolishing the effect of the potent LXR agonist **T0901317** (Fig. 1-2). [18] This indicated that synthetic modulators could alter gene expressions and increase the lipid metabolism and glucose uptake in human cells. Thus, the main focus in this work was to continue our search for new LXR modulators [19,20], and explore whether newly synthesized derivatives of **22SHC** (**1**), based on molecular modelling showed similar or more potent effects on lipid and glucose metabolism both in vitro and in vivo than the parent compound. Such compounds would have great potential as new clinical candidates if intellectual patent rights can be secured.

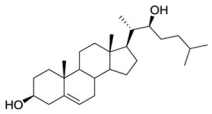
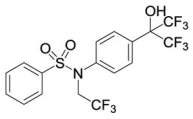
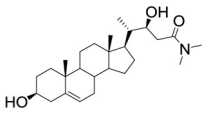
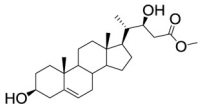
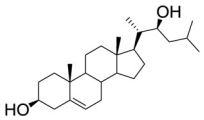
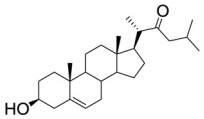
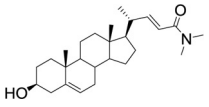
2. Results and discussion

Since LXRβ is found throughout the whole body, we focused our molecular modelling efforts on this isoform. In addition, the structure of 24(*S*),25-epoxycholesterol in complex with LXRβ is known X-ray crystallography (PDB code: 1P8D) and could be used as guide in the evaluation of the docked complexes. A series of steroid based compounds were constructed in silico and the compounds were docked into LXRβ structure after removal of 24 (*S*),25-epoxycholesterol. Compound **10** (one of the compounds with highest docking score,) can be seen docked into the ligand binding pocket of LXRβ in Fig. 2. Some of the highest scoring compounds (docking score below −35) from the docking are listed in Table 1.

Some of the compounds with the best results from the molecular modeling were chosen for synthesis, as these compounds were not described in the literature. The commercially available Fernholtz acid (**3**) was used as a synthetic starting point, which conveniently could be converted to the key aldehyde intermediate (**8**).[21] Lithium enolate addition to **8** provided the protected amide **9** in good yield and 2:1 d.r. of 22*S* and 22*R*, which after recrystallization could be obtained as a single diastereomer. Treatment of **9** with TBAF afforded compound **10** in good yield. A Mukaiyama aldol reaction gave the β-hydroxy esters **13** and **14** directly, although in poor yield (reaction not optimized). A Grignard-reaction provided compound **15**, which after deprotection afforded nor-22SHC (**16**). Compound **15** could also be oxidized to ketone **17**, which after deprotection gave the β-keto-alcohol **18**. Dimethylamide **21**, an unsaturated analogue of **10**, which lack the 22(*S*)-hydroxy group, was prepared according to Scheme 3 in a slightly different way starting from the ester **19**. The synthesis of all new modulators is shown in Schemes 1–3 and the detailed experimental procedures can be found in the supporting information.

We have previously shown that **22SHC** (**1**) behaved as an antagonist in skeletal muscle cells and HepG2 cells giving reduced lipogenesis and increased glucose uptake. [22] In this study, we have synthesized cholesterol mimetics of **22SHC** (**1**) and tested them in the same cell systems. The results confirm that **22SHC** (**1**)

Table 1
Docking scores of selected modulators to LXRβ (PDB-code 1PQ6). The results are the best score obtained during 3 simulations.

Compound number	Chemical structure	Docking score LXRβ
22SHC, 1		−36.73
T0901317, 2		−22.60
10		−42.00
14		−35,36
16		−36.11
18		−37.73
21		−42.61

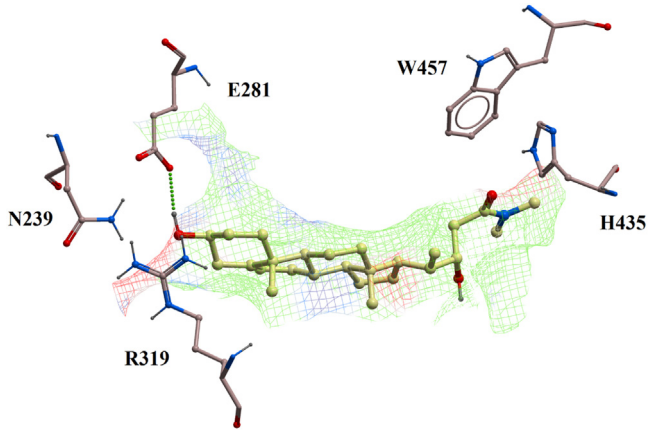


Fig. 2. Compound **10** docked to the ligand binding pocket of LXRβ including relevant amino acid side chains. The Ligand Surface (mesh) will be displayed colored by binding property – white = neutral surface, green = hydrophobic surface, blue = hydrogen bonding acceptor potential, and red = hydrogen bond donor potential.

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