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Synthesis and initial biological evaluation of new mimics of the LXR-modulator 22(*S*)-hydroxycholesterol

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ABSTRACT

The generic, synthetic oxysterol 22(*S*)-hydroxycholesterol (22SHC) has shown antagonistic effects towards liver X receptor (LXR) in vitro and promising effects on plasma triacylglycerol level and body weight-gain in animal studies.[1,2] On the contrary, the endogenic LXR agonist 22(*R*)-hydroxycholesterol (22RHC) and synthetic LXR agonists convincingly have shown agonistic effects on genes involved in lipogenesis, and inhibitory effects on cell proliferation in vitro and in vivo.[3] We hypothesized that the carbon side chain containing the hydroxyl group at the 22-position was a pharmacophore affecting these opposite effects on LXR. This prompted us to initiate a rational drug design incorporating the 22-hydroxylated 20–27 cholesterol moiety into cholesterol-mimicking building blocks. The two enantiomers of the 22-hydroxylated 20–27 cholesterol moiety were synthesized with an excellent enantiomeric excess and the stereochemistry are supported by X-ray crystallography. Molecular modelling of the new compounds showed promising LXR selectivity (LXRβ over LXRα) and initial in vitro biological evaluation in human myotubes showed that compound **16b** had agonistic effects on the gene expression of SCD1 and increased lipogenesis.

1. Introduction

An increasing number of reports document a repertoire of biological roles of the liver X receptor (LXR) within major disease areas.[1–6] The biological effects of steroidal-as well as synthetic LXR modulators now establish LXR as a promising target for several new therapeutic opportunities. The two isoforms LXRα and LXRβ are relatively new drug targets within the nuclear receptor super family and they are important because they are key players in cholesterol and lipid metabolism, and also influence glucose metabolism.[7,8] LXR agonists have been developed as potential drugs for treatment of metabolic and cardiovascular diseases regulate inflammatory responses and immunity, skin diseases and are effective for treatment of murine models of atherosclerosis, diabetes, and Alzheimer's disease.[9–11] The agonists also display anti-inflammatory activities[12–14] and inhibit cell proliferation in a number of major cancer forms.[9,10] However, lack of selectivity between the two isoforms LXRα and LXRβ hampers most of the agents so far approaching clinical use. Another complexing factor concerns the very flexible ligand-binding pocket (LBP) of the LXRs. Their LBP allows binding of compounds of highly different structures such as T0901317, GW3965 and oxygenated sterols (Fig. 1).

The endogenic LXR agonist 22(*R*)-hydroxycholesterol (22RHC) and synthetic LXR agonists convincingly have shown inhibitory effects on cell proliferation in vitro and in vivo.[15] LXR agonists have earlier been shown to have anti proliferative effects on LNCaP human prostate cancer cells and activation of LXR also inhibited the proliferation of different other prostate and breast cancer cell lines.[16] In the same study, oral administration of the LXR agonist T0901317 inhibited the growth of LNCaP tumours in athymic nude mice.[16] Oxysterols have also been shown to induce apoptosis in a wide range of models such as human leukaemia cell lines[15] and pancreatic beta-cells.[17] Likewise, pharmacological activation of LXRs leads to increase apoptosis of LNCaP xenografted prostate cancer cells.[3] In another study, oxygenated steroids and nonsteroidal LXRα agonists at low micro molar concentrations inhibited proliferation of breast carcinoma cell lines in culture.[18] LXRα agonists are described to show promising results in hormone-dependent cancers such as prostate,[3,19] ovarian,[20] certain types of breast cancer,[18,21] benign prostate hyperplasia[11] and LXR agonists have been reported to reduce cell proliferation in colon cancer.[22] These results have also

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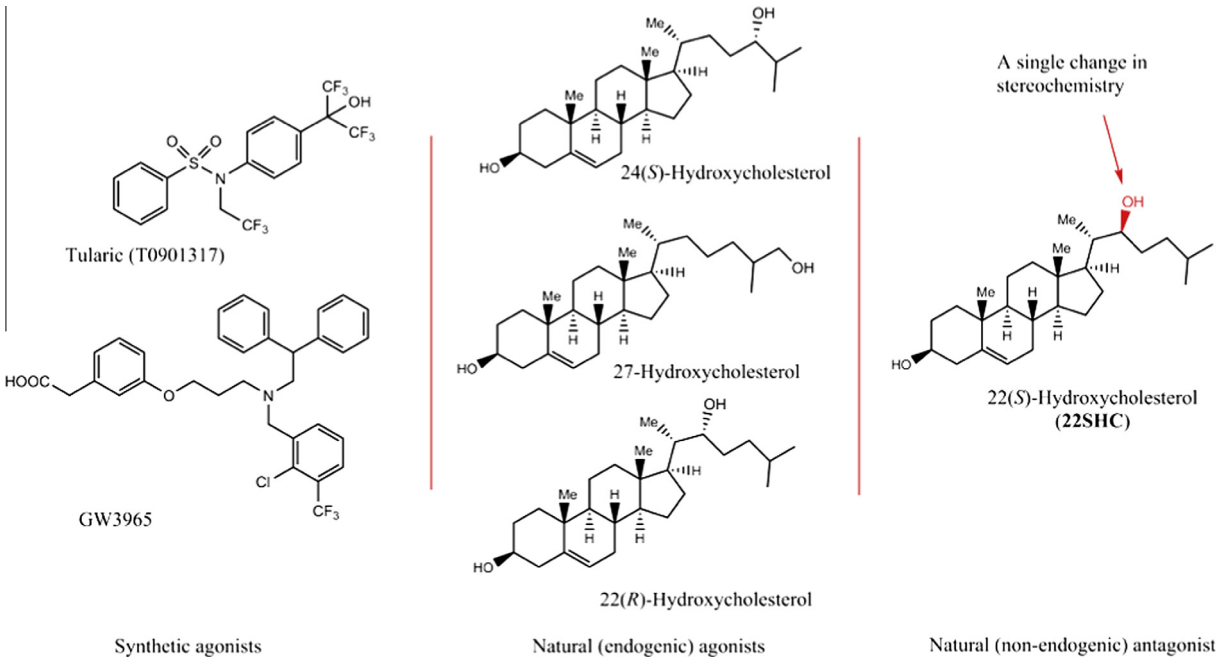


Figure 1. Overview of known modulators of the liver X receptor.

been supported by others[15,23,24] and recent reviews broadly document the key role of LXR in cancer and other diseases.[9–11]

It has recently been shown that the synthetic oxysterol 22SHC behaved like an LXR antagonist in skeletal muscle cells (human myotubes), SGBS (preadipocytes), HepG2 (human hepatoblastoma cell line) and CaCo-2 (human epithelial colorectal adenocarcinoma cells), in contrast to its diastereomer, the agonistic 22RHC (Fig. 1).[1,2] 22SHC repressed genes involved in lipogenesis (i.e. SREBP, FAS, ASCL1, SCD1) and lipid handling that resulted in reduced synthesis of complex lipids even when compared to untreated myotubes.[25] It was shown that exposure to a synthetic LXR agonist (T0901317, Fig. 1) increased lipogenesis more in myotubes from type 2 diabetic subjects than in myotubes from lean subjects,[1,2] and that these effects could be counteracted by 22SHC. Further, 22SHC reduced *de novo* lipogenesis below basal, reduced fatty acid uptake and oxidation at the same time as glucose uptake and oxidation were increased.[2] Thus, the two stereoisomers 22SHC (synthetic antagonist) and 22RHC (endogenous agonist) seem to have opposite physiological effects.

Based on the different effect the change in stereochemistry at the 22-position of 22-hydroxycholesterol afforded, it was assumed that the stereochemistry is an essential part of the pharmacophore for LXR regulators. This has also been supported by molecular modelling.[1] In order to mimic the structure and binding of 22SHC, while keeping the pharmacophore intact, a series of potential modulators were generated (Fig. 2) and subjected to in vitro evaluation.

2. Results and discussion

2.1. Synthesis

The synthetic pathway to the key building block **5** and all tested compounds are given in Scheme 1. The moderate yield in the PCC oxidation of **1** to **2** was related to loss of **2** because of solvent azeotropic effects during distillation. The diol **5** was then used in the synthesis of the screening compounds (**7**, **9**, **13** and **16a**–**d**). Building blocks comprising substituted aromatic or alicyclic groups

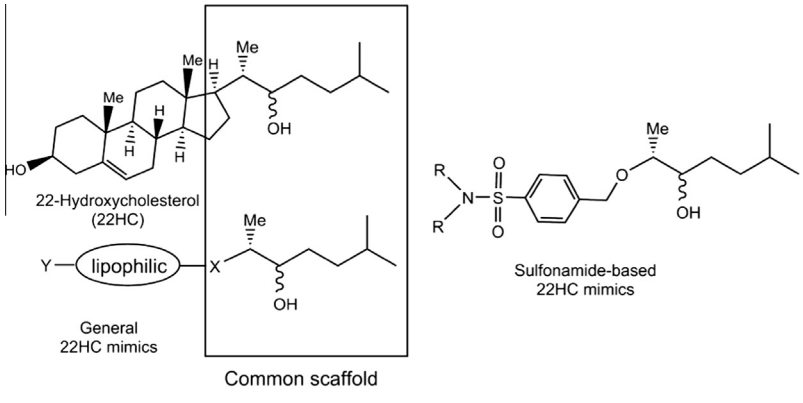
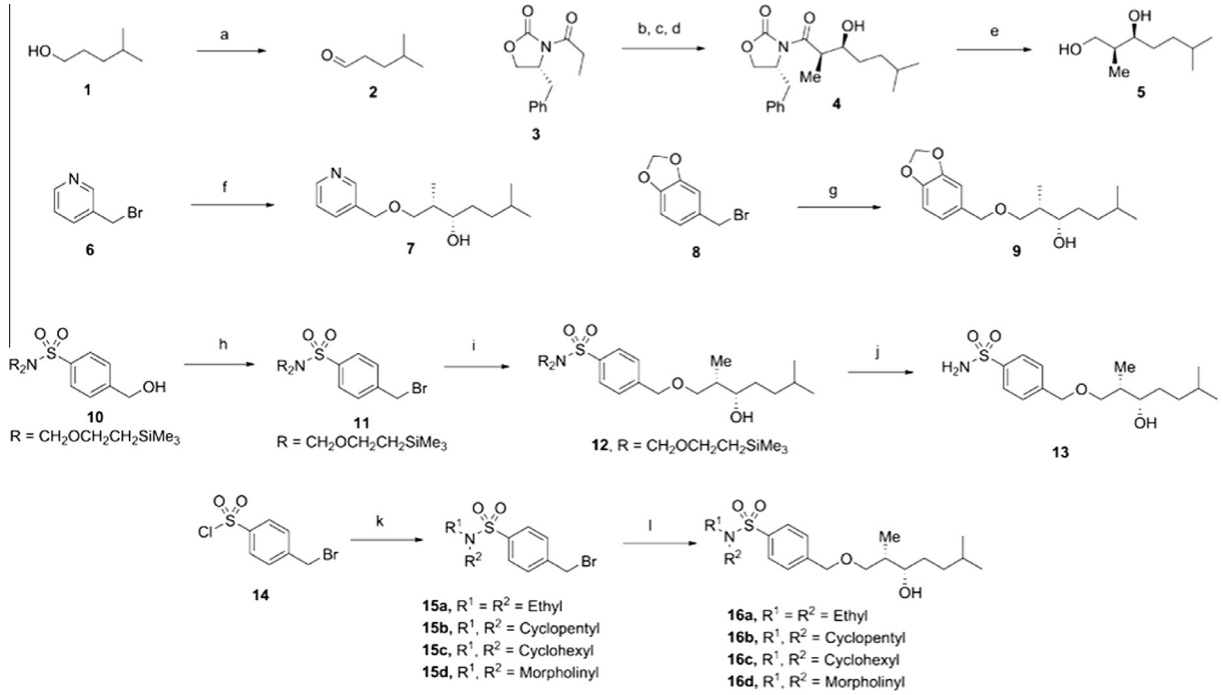


Figure 2. Pharmacophore analysis based on 22-hydroxycholesterol.



Scheme 1. Experimental conditions: (a) PCC, CH_2Cl_2, rt 60%. (b) nBu_2BOTf, DIPEA, CH_2Cl_2, 0 °C. (c) Compound **2**, CH_2Cl_2, −78 °C, (d) MeOH, H_2O_2, rt, 62%. (e) $LiBH_4$, Et_2O, 0 °C, 67%. (f) NaH, THF, **5**, 0 °C–rt, 28%. (g) NaH, THF, **5**, 0 °C–rt, 8%. (h) PPh_3, CBr_4, CH_2Cl_2, 0 °C, 48%. (i) NaH, THF, **5**, 0 °C–rt, 18%. (j) AcOH, H_2O, 0 °C, 44%. (k) HNR^1R^2, TEA, CH_2Cl_2, 0 °C, 20–50%. (l) Compound **5**, NaH, THF, DMF, 0 °C–rt, 10–50%.

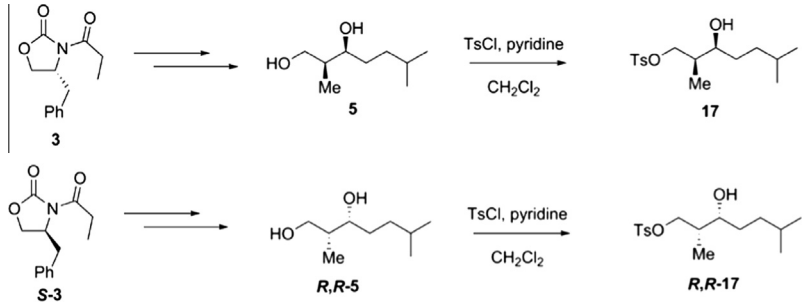
were identified in molecular modelling as synthesis candidates with respect to mimicking the cholesterol A and B rings in 22SHC. Direct alkylation of **5** with benzyl bromides NaH in THF/DMF at 0 °C gave good regioselectivity for primary hydroxyl group alkylation with minimal alkylation of the secondary hydroxyl group, in low to moderate yields. This method was sufficient for the purpose and not optimized further at the present stage.

No metabolism or degradation studies have been conducted for the new modulators.

Using the auxiliary (*R*)-4-benzyl-3-propionyloxazolidin-2-one (**3**), the protocol conveniently gave the enantiomer **5** in good yield with excellent enantiomeric excess shown by chiral HPLC of the tosylates **17** and ***R,R*-17**, see Scheme 2. Correspondingly, the tosylate ***R,R*-17** was prepared in similar yields from (*S*)-4-benzyl-3-propionyloxazolidin-2-one (***S*-3**). The absolute stereochemistry was supported by single crystal X-ray structure analysis of **4** and **5** (Fig. 3).[26]

2.2. Molecular modelling

The binding affinities for the new LXR receptors were predicted in silico using the Internal Coordinate Mechanics (ICM) program.



Scheme 2. Preparation of **17** and ***R,R*-17** for HPCL evaluation of **5**.

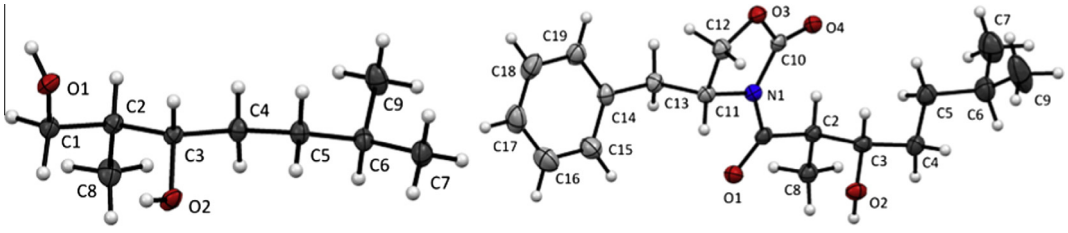
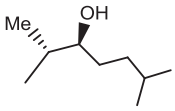
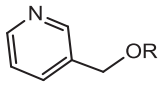
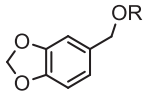
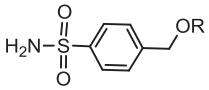
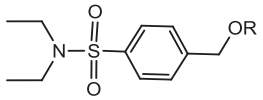
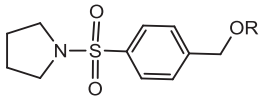
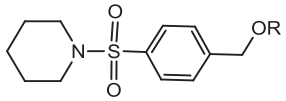
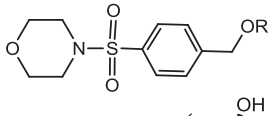
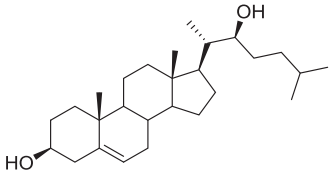


Figure 3. X-ray structures of compounds **4** (right) and **5** (left).

Table 1
Structures and docking scores of the compounds presented

Compound number	Chemical structure R = 	Docking score LXRα	LXRβ
7		−11.68	−14.94
9		−13.83	−19.81
13		−20.18	−28.98
16a		−16.76	−22.99
16b		−18.31	−19.74
16c		−10.35	−22.61
16d		−14.68	−24.75
22SHC	 **22SHC**	−17.5	−29.2

The results are found in Table 1 and the best docking pose of the ligands docked into the ligand binding pocket of LXRβ is seen in Figure 4. All compounds fit well inside the defined ligand binding pocket, however the docking scores show that the binding affinity is generally low towards both LXRα and LXRβ, see Table 1. (A strong binder needs a docking score of <−32). Only compound **13** and **22SHC** show good docking score towards LXRβ.

The X-ray crystal structures of the LXRα ligand binding domain (PDB entry: 1UHL) and LXRβ ligand binding domain (PDB entry: 1PQ6) were used as targets for docking. The docking scores were then calculated using the ICM Virtual Ligand Screening add-on and the docking results are the best of three docking runs. The processes were identical for both LXRα and LXRβ.

2.3. Gene expression studies and lipogenesis

In an indicative first study, the compounds were compared to the characteristic gene expression pattern of 22SHC and T0901317 on stearyl-CoA desaturase 1 (SCD1) (Hessvik et al., 2012). SCD1 is a direct LXR target and an important enzyme in lipid metabolism and lipogenesis. The results confirmed previous findings (Hessvik et al., 2012), showing that the synthetic LXR-agonist T0901317 up-regulated SCD1, while 22SHC tended to down-regulate ($p = 0.1$) (Fig. 5). Only compound **16b** seemed to behave as an agonist of LXR and up-regulated gene expression of SCD1. To test if **16a** had any functional effects, we tested its effect on lipogenesis. Both T0901317 and compound **16b** increased lipogenesis significantly, while T0901317 and compound **16a** in combination increased lipogenesis even more. 22SHC did not alter lipogenesis itself, but seemed to abolish the T0901317-induced effect. These results show that the new compound **16b** acted as an LXR agonist in these biological assays, similar to the known LXR agonist T0901317. Extended biological evaluation should be performed in other test systems, like cancer cell assays.

3. Conclusion

Seven new synthetic compounds with potential LXR modulator properties were prepared in decent overall yield and the stereochemical configuration of the key intermediate **5** was confirmed by X-ray analysis. The molecular modelling data suggests that the new modulators will bind weakly to the LXR binding pocket, and based on in vitro testing on SCD1, only small changes are observed in the gene expression. Of the new modulators tested, **16b** showed significant agonistic behaviour in SCD1 gene expression experiments and this was also confirmed in lipogenesis assays in myotubes. The lipogenesis was more than two-fold increased when treated with **16b** alone as compared to control. The effect of **16b** co-administered with T0901317 also seemed to give an additional increase in lipogenesis as compared to T0901317 alone.

4. Experimental protocols

4.1. General

All reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. For column chromatography and filtration trough a pad of silica Merck silica 60 mesh (35–70 μm) is used unless noted otherwise. ^{1}H and ^{13}C NMR were recorded on Bruker DPX 300 and Bruker AVII 400 instrument equipped with a BACS-60 and a BACS-120 automatic sample changer, respectively. All experiments were performed at 25 °C in DMSO-d_6 and $CDCl_3$.

4.2. Synthesis

4.2.1. 4-Methylpentanal (2)

A solution of 4-methylpentanol (**1**, 4.0 g, 39.15 mmol) in CH_2Cl_2 (133 ml) under nitrogen atmosphere was added PCC (12.67 g, 58.78 mmol) in portions and the mixture was stirred for 24 h at room temperature. The reaction mixture was added celite, filtered through a pad of silica and concentrated in vacuo at room temperature. Distillation yielded the title compound as a colorless oil. The organic layer was dried ($MgSO_4$) and distilled. Distillation gave 2.35 g (60%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.77 (t, $J = 1.9$ Hz, 1H), 2.52–2.23 (m, 2H), 1.71–1.46 (m, 3H), 0.91 (d, $J = 6.2$ Hz, 6H), which are in consensus with published data.[5]

4.2.2. 4-(*R*)-Benzyl-3-(3-(*S*)-hydroxy-2(*S*),6-dimethylheptanoyl)-oxazolidin-2-one (4)

To a cooled (0 °C) stirred solution of (*R*)-(−)-4-benzyl-3-propionyl-2-oxazolidinone (**3**, 1.17 g, 5 mmol) in dry CH_2Cl_2 (10 ml) was added slowly di-*n*-butylbortriflate (1 M in CH_2Cl_2, 5.5 ml, 5.5 mmol) followed by *N*,*N*-diisopropylethylamine (1.05 ml, 6 mmol). The reaction mixture was stirred at 0 °C for 30 min and cooled to −78 °C. Freshly distilled 4-methylpentanal (**2**, 0.55 g, 5.5 mmol) was added in one portion. The reaction mixture kept at −78 °C for 30 min, allowed to reach room temperature and stirred for 2 h. The reaction mixture was quenched by addition of 4 ml

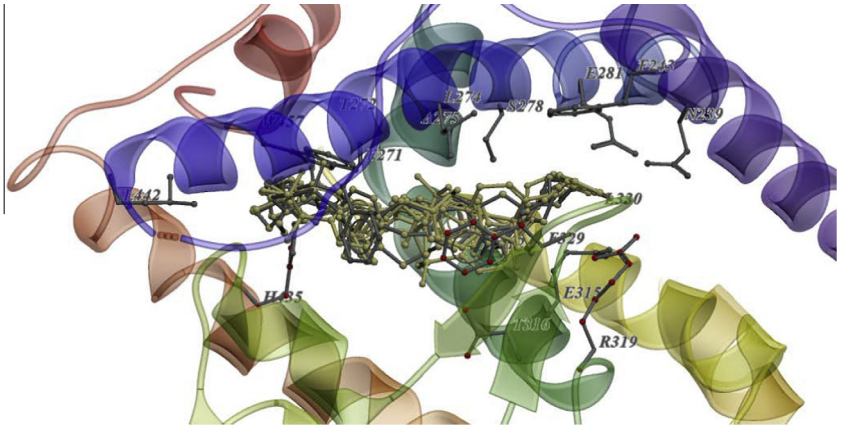


Figure 4. Molecular docking into the ligand binding pocket of LXRβ. All new modulators were docked and the best confirmations were superimposed in the binding pocket of LXRβ (PDB: 1PQ6).

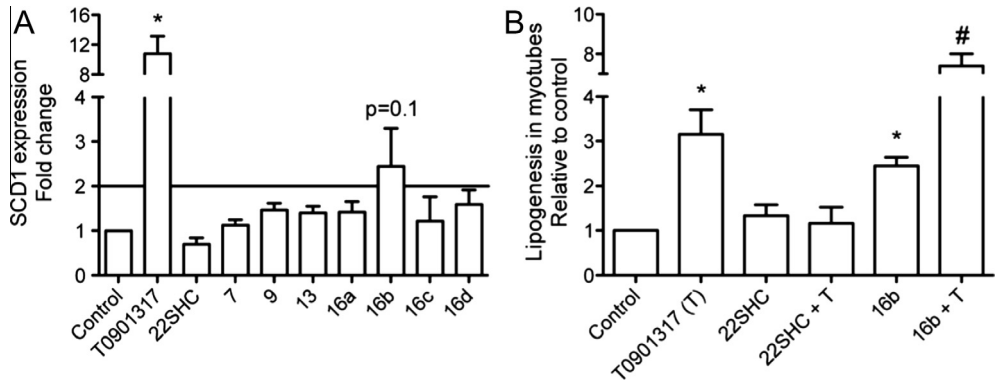


Figure 5. Biological effects on gene expression and lipogenesis. (A) Myotubes were treated with DMSO (0.1%), 1 μM T0901317, 10 μM 22SHC, compounds **9**, **14**, **15**, **21**, **16a**, **16b** and **16c** for 4 days. Total RNA was isolated from the cells and analyzed by qPCR as described in Materials and Methods. Gene expressions were normalized to 36B4. Values represent fold change relative to control given as mean ± SEM (n = 3–6). SCD1, stearoyl-CoA desaturase **1**. (B) Myotubes were treated with DMSO (0.1%), 10 μM 22SHC and compounds **22** ± 1 μM T0901317 for 4 days. The cells were incubated with [1-^{14}C]acetate (1 μCi/ml, 100 μM) for 24 h before lipids were isolated by filtration through hydrophobic MultiScreen® HTS plates. The levels of lipids were determined by scintillation counting. Values represent fold change relative to control for total lipids synthesized from acetate given as means ± SEM (n = 3–5). $^{*}P < 0.05$ versus control (DMSO) and $^{\#}P < 0.05$ for all other treatments.

phosphate buffer (pH 7.2) and methanol (12 ml). This solution was treated with 12 ml methanol: 30% H_2O_2 (2:1) and stirred for 1 h at room temperature. Water was added and the aqueous phase was extracted with CH_2Cl_2 (×3). The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane/EtOAc (70:30) yielded 1.04 g (62%) of the title compound as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (s, 3H), 0.92 (s, 3H), 1.15–1.34 (m, 4H), 1.34–1.50 (m, 2H), 1.50–1.62 (m, 2H), 2.81 (dd, J = 13.4 Hz, 9.4 Hz, 1H), 2.92 (s, 1H), 3.27 (dd, J = 13.5 Hz, 3.2 Hz, 1H), 3.63–3.87 (m, 1H), 3.89–4.03 (m, 1H), 4.09–4.32 (m, 2H), 4.68–4.75 (m, 1H), 7.21–7.23 (m, 2H), 7.26–7.37 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.4, 22.6, 22.7, 28.1, 31.8, 35.2, 37.9, 42.1, 55.2, 66.3, 71.9, 127.5, 129.1, 129.5, 135.1, 153.1, 177.7 HRMS (EI) calcd for $C_{19}H_{27}NO_4$ 333.1940, found 333.1942.

4.2.3. (2*S*,3*S*)-2,6-Dimethylheptane-1,3-diol (5)

To a cooled (0 °C) solution of 4-(*R*)-benzyl-3-(3-(*S*)-hydroxy-2(*S*),6-dimethyl-heptanoyl)-oxazolidin-2-one (**4**, 0.99 g, 2.97 mmol) in dry diethyl ether (60 ml) was added water (1 ml) followed by $LiBH_4$ (0.17 g, 7.75 mmol). The reaction mixture was stirred at 0 °C and then 1 h at room temperature. Water was added and the water phase was extracted with diethyl ether. The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane/EtOAc (90:10)–(80:20)–(70:30)–(50:50) yielded 0.32 g (67%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82–0.86 (m, 9H), 1.02–1.17 (m, 1H), 1.25–1.57 (m, 4H), 1.66–1.75 (m, 1H), 3.31 (br s, 1H), 3.60 (d, J = 6 Hz, 2H), 3.74 (bs, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.1, 22.7 (2× CH_3), 28.2, 31.8, 35.5, 39.0, 66.7, 74.3. HRMS (EI) calcd for $C_9H_{18}O$ $[M-H_2O]^+$ 142.1358, found 142.1347.

4.2.4. (2*S*,3*S*)-2,6-Dimethyl-1-(pyridin-3-ylmethoxy)heptan-3-ol (7)

A suspension of NaH dispersion in mineral oil (0.053 g, 1.33 mmol) in dry THF (14 ml) was cooled to 0 °C under N_2-atmosphere before a solution of **5** (0.202 g, 1.26 mmol) in dry THF (3 ml) was added. The reaction mixture was stirred at 0 °C for 30 min. A suspension of NaH dispersion in mineral oil (0.048 g, 1.20 mmol) in dry THF (14 ml) was cooled to 0 °C under N_2-atmosphere before commercially available 3-(bromomethyl)-pyridine hydrobromide (**6**, 0.285 g, 1.13 mmol) was added. The mixture was stirred for 30 min and then added to the solution of **5**. The cooling bath was removed and the mixture was stirred for 71 h. Water (10 ml) was added, the mixture was extracted with EtOAc (50 ml × 2), dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane—heptane/EtOAc (90:10)–(50:50) yielded 0.080 g (28%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 8.52 (s, 1H),

8.46 (d, J = 4.2 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.43 (dd, J = 7.7, 4.9 Hz, 1H), 4.56 (s, 2H), 3.64 (td, J = 6.4, 3.6 Hz, 1H), 3.55 (dd, J = 9.1, 6.9 Hz, 1H), 3.40 (dd, J = 9.1, 6.2 Hz, 1H), 1.83 (ddd, J = 13.3, 6.8, 3.6 Hz, 1H), 1.05–1.65 (m, 6H), 0.79–0.96 (m, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 149.28, 149.13, 137.61, 136.54, 125.16, 74.85, 72.98, 71.25, 39.83, 36.65, 33.43, 29.30, 23.12, 22.98, 11.26. MS (electrospray) (pos): 252 $(M+H)^+$/274 $(M+Na)^+$. HRMS (EI) calcd for $C_{15}H_{25}NO_2$, 251.1885, found 251.1891.

4.2.5. (2*S*,3*S*)-1-(Benzo[*d*][1,3]dioxol-5-ylmethoxy)-2,6-dimethyl heptan-3-ol (9)

A suspension of NaH 60% dispersion in mineral oil (0.146 g, 3.66 mmol) in dry THF (20 ml) and dry DMF (20 ml) was cooled to 0 °C under N_2-atmosphere before a solution **5** (0.551 g, 3.44 mmol) in dry THF (3 ml) was added drop wise. After 30 min at 0 °C, 5-(bromomethyl)benzo[*d*][1,3]dioxole[27] (**8**, 0.665 g, 3.09 mmol) in dry THF (3 ml) was added. The cooling bath was removed and the reaction mixture was stirred for 130 min. NH_4Cl (aq) satd (25 ml) was added, the mixture was extracted with EtOAc (50 ml), dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane—heptane/EtOAc (95:5) yielded 0.534 g (53%) of the title compound as a colorless oil as well as 0.079 g (8%) of the regioisomer 3(*S*)-(1,3-dihydroisobenzofuran-5-ylmethoxy)-2(*S*),6-dimethyl-heptan-1-ol (**9a**) as colorless oils. Compound **9a** was not used in the further work. **9**: ^{1}H NMR (300 MHz, CD_3OD) δ 6.73–6.86 (m, 3H), 5.93 (s, 2H), 4.29–4.48 (m, 2H), 3.60 (td, J = 6.4, 3.7 Hz, 1H), 3.47 (dd, J = 9.2, 6.7 Hz, 1H), 3.32–3.38 (m, 1H), 1.78 (ddd, J = 13.3, 6.7, 3.7 Hz, 1H), 1.27–1.62 (m, 4H), 0.99–1.25 (m, 1H), 0.81–0.97 (m, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 149.18, 148.57, 133.73, 122.45, 109.37, 108.85, 102.31, 74.25, 73.96, 73.30, 39.76, 36.62, 33.39, 29.31, 23.13, 22.98, 11.42. MS (electrospray) (pos): 317 $(M+Na)^+$. HRMS (EI) calcd for $C_{17}H_{26}O_4$, 294.1831, found 294.1826.

4.2.6. 4-(((2*S*,3*S*)-3-Hydroxy-2,6-dimethylheptyl)oxy)methyl)benzenesulfonamide (13)

4.2.6.1. (a) 4-(Bromomethyl)-*N*,*N*-bis((2-(trimethylsilyl)-ethoxy)-methyl)-benzenesulfonamide (11). 4-(Hydroxymethyl)-*N*,*N*-bis((2-(trimethylsilyl)-ethoxy)-methyl)-benzenesulfonamide (**10**, prepared according to literature,[28]) was dissolved in dry CH_2Cl_2 (60 ml) was cooled to 0 °C under N_2-atmosphere before PPh_3 (2.173 g, 8.29 mmol) and CBr_4 (2.023 g, 6.10 mmol) was added. The mixture was stirred at 0 °C for 2 h and then diluted with 100 mL CH_2Cl_2, washed with 50 mL water, dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane/EtOAc (90:10) yielded 1.35 g (48%) of the title compound as a sticky, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 4.77 (s, 4H), 4.48 (s, 2H), 3.38–3.55 (m, 4H), 0.77–0.89 (m, 4H), −0.07–0.00 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.50, 141.28, 129.54, 127.91, 76.41, 65.90, 31.68, 17.94, −1.25. MS (electrospray) (pos): 532/533/534/535/536/537 $(M+Na)^+$.

4.2.6.2. (b) 4-((((2*S*,3*S*)-3-Hydroxy-2,6-dimethylheptyl)oxy) methyl)-*N*,*N*-bis((2-(trimethylsilyl)-ethoxy)methyl)-benzenesulfonamide (12). A suspension of NaH dispersion in mineral oil (0.124 g, 3.10 mmol) in dry THF (20 ml) and dry DMF (20 ml) was cooled to 0 °C under N_2-atmosphere before a solution of **5** (0.472 g, 2.94 mmol) in dry THF (3 ml) was added drop wise. The reaction mixture was stirred at 0 °C for 35 min before a solution of **11** (1.35 g, 2.64 mmol) in dry THF (3 ml) was added. The cooling bath was removed and the reaction mixture was stirred for 3 h. NH_4Cl (aq) satd (25 ml) was added, the mixture was extracted with EtOAc (50 ml), dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane–heptane/EtOAc (98:2)–(95:5) yielded 0.306 g (18%) of the title compound as a colorless oil. ^{13}C NMR (300 MHz, CD_3OD) δ 7.86 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 4.79 (s, 4H), 4.53–4.63 (m, 2H), 3.63–3.69 (m, 1H), 3.56 (dd, J = 9.1, 6.9 Hz, 1H), 3.35–3.50 (m, 5H), 1.80–1.88 (m, 1H), 1.14–1.65 (m, 5H), 0.87–0.97 (m, 10H), 0.72–0.86 (m, 4H), −0.04–0.02 (m, 18H). ^{13}C NMR (75 MHz, CD_3OD) δ 145.76, 141.61, 128.58, 128.45, 77.93, 74.97, 73.02, 72.99, 66.79, 39.88, 36.68, 33.48, 29.33, 23.15, 23.02, 18.69, 11.34, −1.31. MS (electrospray) (pos): 612/613/614 $(M+Na)^+$. HRMS (EI) calcd for $C_{17}H_{26}O_4Na$, 612.3187, found 612.3202.

4.2.6.3. (c) 4-((((2*S*,3*S*)-3-Hydroxy-2,6-dimethylheptyl)oxy)methyl) benzenesulfonamide (13). A mixture of **12** (0.221 g, 0.38 mmol) in CH_3CO_2H (6.8 ml) and water (3.4 ml) was stirred at 70 °C under N_2-atmosphere for 3 h. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. MeOH (15 ml) was added and the mixture was evaporated under reduced pressure, this was repeated once. Flash chromatography on silica gel eluting with heptane–heptane/EtOAc (50:50) yielded 0.055 g (44%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 7.88 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 4.52–4.66 (m, 2H), 3.63–3.68 (m, 1H), 3.49–3.60 (m, 1H), 3.35–3.43 (m, 1H), 1.80–1.88 (m, 1H), 1.04–1.67 (m, 6H), 0.82–0.97 (m, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 144.69, 143.93, 128.70, 127.15, 74.81, 73.06 (2C), 39.76, 36.61, 33.38, 29.25, 23.13, 22.99, 11.28. MS (electrospray) (pos): 352/353/354 $(M+Na)^+$. HRMS (EI) calcd for $C_{16}H_{27}NO_4SNa$, 352.1559, found 352.1568.

4.2.7. General procedure for condensation products between (2*S*,3*S*)-2,6-dimethylheptane-1,3-diol (5) and the building blocks with benzylbromide functionality1-((4-(bromomethyl)-phenyl)sulfonamides (15a–d)

4.2.7.1. *General procedure part I:* 1-((4-(Bromomethyl)phenyl) sulfonamides (15a–d). To a solution of commercially available 4-(bromomethyl)benzenesulfonyl chloride (**14**, 5 mmol) in dry CH_2Cl_2 (10 ml) under argon at 0 °C was added triethylamine (10 mmol) followed by the amine (5 mmol) with stirring. After 75 min at 0 °C, 2 M HCl was added and the aqueous phase extracted with CH_2Cl_2 (×2). The combined organic phase was washed with 2 M HCl, brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. This allowed the isolation of the sulfonamides in low to moderate yields (20–50%) as pale yellow to white solids, used without further purification or reaction optimization.

4.2.7.2. *General procedure part II:*. Condensation products (**16a**–**d**) between (2*S*,3*S*)-2,6-dimethylheptane-1,3-diol (**5**) and the building blocks with benzylbromide functionality1-((4-(bromomethyl)-phenyl)sulfonamides (**15a**–**d**)

To a solution of **5** (1 mmol) in THF/DMF (4 mL, 50:50) under argon at 0 °C was added sodium hydride (1.15 mmol). After stirring at 0 °C for 20 min, the respective sulfonamide **15a**–**d** (1 mmol) in THF (2 mL) was added. The reaction mixture was allowed to reach room temperature and stirred overnight. NH_4Cl (satd) was added and the aqueous phase extracted with ether (×3). The combined organic extract was washed with brine, dried (Na_2SO_4), filtered and concentrated. Purification by flash chromatography (heptane/EtOAc 70:30) afforded the title compounds in low yields, that were subjected to analysis and biological evaluation without further method optimization.

4.2.8. 4-(Bromomethyl)-*N*,*N*-diethylbenzenesulfonamide (16a)

^{1}H NMR (300 MHz, chloroform-*d*) δ 7.73 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 4.53 (s, 2H), 3.70 (ddd, J = 7.9, 4.6, 2.7 Hz, 1H), 3.64–3.42 (m, 2H), 3.19 (q, J = 7.1 Hz, 4H), 2.49 (s, 1H), 1.96–1.75 (m, 1H), 1.61–1.00 (m, 12H), 0.99–0.75 (m, 9H). ^{13}C NMR

(75 MHz, chloroform-*d*) δ 143.0, 139.5, 127.6, 127.2, 75.2, 73.9, 72.4, 42.1, 38.0, 35.5, 32.1, 28.1, 22.7, 22.6, 14.2, 10.6. HRMS (EI) calcd for $C_{20}H_{35}NO_4S$, 385.2287, found 385.2276.

4.2.9. 1-((4-(Bromomethyl)phenyl)sulfonyl)piperidine (16b)

^{1}H NMR (300 MHz, chloroform-*d*) δ 7.77 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 4.55 (s, 2H), 3.72 (ddd, *J* = 7.9, 4.7, 2.7 Hz, 1H), 3.65–3.43 (m, 2H), 3.20 (t, *J* = 6.8 Hz, 4H), 2.71 (s, 1H), 1.96–1.81 (m, 1H), 1.80–1.64 (m, 4H), 1.62–0.99 (m, 6H), 0.92 (d, *J* = 7.1 Hz, 3H), 0.86 (dd, *J* = 6.6, 1.3 Hz, 6H). ^{13}C NMR (75 MHz, chloroform-*d*) δ 143.7, 136.4, 128.0, 128.0, 75.7, 74.4, 72.8, 48.3, 38.3, 35.8, 32.4, 28.5, 25.6, 23.0, 23.0, 11.0. HRMS (EI) calcd for $C_{20}H_{33}NO_4S$, 383.2134, found 383.2126.

4.2.10. (2*S*,3*S*)-2,6-Dimethyl-1-((4-(morpholinosulfonyl)benzyl)oxy)heptan-3-ol (16c)

^{1}H NMR (300 MHz, chloroform-*d*) δ 7.69 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 4.55 (s, 2H), 3.72 (ddd, *J* = 7.9, 4.6, 2.7 Hz, 1H), 3.64–3.46 (m, 2H), 3.04–2.84 (m, 4H), 2.43 (s, 1H), 1.89 (dd, *J* = 4.8, 1.6 Hz, 1H), 1.74–0.99 (m, 12H), 0.99–0.79 (m, 9H). ^{13}C NMR (75 MHz, chloroform-*d*) δ 143.4, 135.4, 127.9, 127.6, 75.4, 74.0, 72.5, 47.0, 38.0, 35.5, 32.1, 28.2, 25.2, 23.5, 22.7, 22.7, 10.64. HRMS (EI) calcd for $C_{21}H_{35}NO_4S$, 397.2287, found 397.2291.

4.2.11. 4-((4-(Bromomethyl)phenyl)sulfonyl)morpholine (16d)

^{1}H NMR (300 MHz, chloroform-*d*) δ 7.70 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 2H), 4.57 (s, 2H), 3.71 (q, *J* = 4.8 Hz, 5H), 3.54 (qd, *J* = 9.0, 5.7 Hz, 2H), 3.06–2.87 (m, 4H), 2.42 (s, 1H), 1.89 (dd, *J* = 4.9, 1.9 Hz, 1H), 1.68–1.01 (m, 5H), 1.01–0.77 (m, 9H). ^{13}C NMR (75 MHz, chloroform-*d*) δ 144.1, 134.2, 128.1, 127.7, 75.3, 73.9, 72.4, 66.1, 46.0, 38.0, 35.5, 32.1, 28.2, 22.7, 22.7, 10.6. HRMS (EI) calcd for $C_{20}H_{33}NO_5SNa$, 422.1978, found 422.1986.

4.2.12. (2*S*,3*S*)-3-Hydroxy-2,6-dimethylheptyl 4-methylbenzenesulfonate (17)

To a cooled (0 °C) solution of 2-(*S*)-6-dimethyl-heptane-1,3-(*S*)-diol (**5**, 1.76 g, 11 mmol) in CH_2Cl_2 (80 ml) was added pyridine (3.55 ml, 44 mmol) followed by tosyl chloride (2.30 g, 12.1 mmol). The reaction mixture was stirred at room temperature for 2 days. The reaction mixture was quenched with water and the aqueous phase was extracted with diethyl ether (×2). The combined organic phase was washed with 2 M HCl and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography on silica gel eluting with heptane/EtOAc (80:20)–(70:30) yielded 2.59 g (75%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82–0.88 (m, 9H), 1.07–1.19 (m, 1H), 1.23–1.45 (m, 3H), 1.48–1.54 (m, 1H), 1.84–1.92 (m, 1H), 2.43 (s, 3H), 3.62–3.67 (m, 1H), 3.88 (dd, *J* = 9.6 Hz, 6.0 Hz, 1H), 4.07 (dd, *J* = 9.6 Hz, 7.8 Hz, 1H), 7.33 (dd, *J* = 8.6 Hz, 0.6 Hz, 2H), 7.73–7.80 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.5, 21.7, 22.62, 22.64, 28.1, 32.3, 35.3, 37.8, 70.9, 73.0, 127.9, 130.0, 133.1, 144.9.

(2*R*,3*R*)-3-Hydroxy-2,6-dimethylheptyl 4-methylbenzenesulfonate (***R*,*R*-17**) was prepared from 4-(*S*)-benzyl-3-(3-(*S*)-hydroxy-2(*S*),6-dimethyl-heptanoyl)-oxazolidin-2-one using the same protocol as for **17**, and was used for analytical purposes only in the present work.

4.3. Determination of enantiomeric excess by chiral HPLC

The enantiomeric excess of compound **5** was determined based on analysis of the corresponding tosylate **17** using a CHIRAPAK AS-H column with 0.6 mL/min 9:1 iso-hexane/2-propanol at room temperature equipped with a UV detector. The UV absorption was measured at 254 nm and the enantiomeric excess was determined by comparing the integral of the peaks with retention time 16.5 and 20.1 min respectively. The opposite diastereomer and the racemic mixture were also analysed using identical conditions. The results are uncorrected.

4.4. Culturing of human myotubes

Satellite cells were isolated as previously described (Gaster M, Kristensen SR, Beck-Nielsen H, Schroder HD (2001) A cellular model system of differentiated human myotubes. APMIS 109:735–744) from the M. obliquus internus abdominis from healthy volunteers. The biopsies were obtained with informed written consent and approval by the National Committee for Research Ethics (Oslo, Norway). The research performed in this study was approved, as a part of a larger project, by the National Committee for Research Ethics (Oslo, Norway). The cells were cultured in DMEM-Glutamax-I with 5.5 mM glucose supplemented 2% foetal bovine serum, 2% Ultroser G, penicillin (100 units/ml) and streptomycin (100 μg/ml), amphotericin B (1.25 μg/ml) and 5.5 mM sodium pyruvate for proliferation. At 70–80% confluence the growth medium was replaced by DMEM-Glutamax-I with 5.5 mM glucose supplemented with 2% foetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), amphotericin B (1.25 μg/ml), 5.5 mM sodium pyruvate and insulin (25 pM) to induce differentiation. The cells were cultured in humidified 5% CO_2 atmosphere at 37 °C, and the medium was changed every 2–3 days. Experiments were performed after 7 days of differentiation.

4.5. RNA isolation and analysis of gene expression by qPCR

After proliferation and differentiation in DMEM-media, cells were harvested and total RNA was isolated by RNeasy Mini kit (Qiagen Sciences, Oslo, Norway) according to the supplier's total RNA isolation protocol. Equal amount of RNA obtained from myotubes from different donors were reversely transcribed with a High Capacity cDNA Archive Kit. Total RNA (1 μg/μl) was reversely transcribed with hexamere primers using a PerkinElmer Thermal Cycler 9600 (25 °C for 10 min, 37 °C for 1 h, 99 °C for 5 min) and a TaqMan reverse-transcription reagents kit (Applied Biosystems). Primers (36B4 and SCD1) were designed using Primer Express® (Applied Biosystems). Primer sequences are available upon request. Each target gene were quantified in triplicates and carried out in a 25 μl reaction volume according to the supplier's protocol. All assays were run for 40 cycles (95 °C for 12 s followed by 60 °C for 60 s). The transcription levels were normalized to the reference control gene 36B4.

4.6. Lipogenesis

For lipogenesis, cells were incubated in DMEM-media with [U-^{14}C]glucose (1 μCi/ml, 200 μM) for 24 h and harvested in dH_2O, assayed for protein (Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72: 248–254), and total lipids were isolated by filtration of the cell lysate through hydrophobic MultiScreen® HTS plates (Millipore, Billerica, MA, USA). The amount of lipids was determined by liquid scintillation counting, and lipogenesis was related to cell protein content and given as nmol/mg protein.

4.7. Presentation of data and statistical analysis

Data in text and figures are given as mean (±SEM) from *n* = number of separate experiments, all performed on muscle cells established from separate cell donors. At least 3 parallels were included in each experiment. For lipogenesis, comparisons of different treatments were evaluated by two-tailed, paired, Student's *t*-test, and $P < 0.05$ was considered significant. For gene expression

studies, more than a 2-fold change was considered an up-regulation.

4.8. Molecular modelling

The Internal Coordinate Mechanics program[29] was used for docking of 22SHC and the newly synthesized compounds into the ligand binding domain of the human LXR receptors. The X-ray crystal structures of the LXRα ligand binding domain (PDB entry: 1UHL) and LXRβ ligand binding domain (PDB entry: 1PQ6) were used as targets for docking.

A virtual library of **22SHC** and the newly synthesized compounds was created using the ICM Chemist package. The crystal structure was loaded, converted to an ICM object, all water molecules removed and all hydrogen atoms optimized using the default conditions set by the program. One of the dimers of the loaded crystal structure was removed prior to the ligand binding pocket was identified. The virtual binding pocket was created by selecting the co-crystallized ligand and creating a ligand pocket that extends 5 Å around the ligand. The ligand was then removed and a virtual docking of the new compounds was performed using default conditions. The docking scores were then calculated using the ICM Virtual Ligand Screening add-on and the docking results are the best of three docking runs. The processes were identical for both LXRα and LXRβ.

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