IDENTIFICATION OF NEW SIDE CHAIN OXIDIZED STEROLS AS NOVEL LIVER X RECEPTOR AGONISTS WITH THERAPEUTIC POTENTIAL IN THE TREATMENT OF CARDIOVASCULAR AND NEURODEGENERATIVE DISEASES

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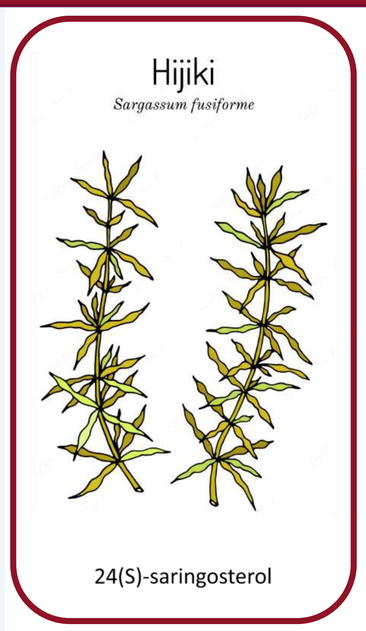
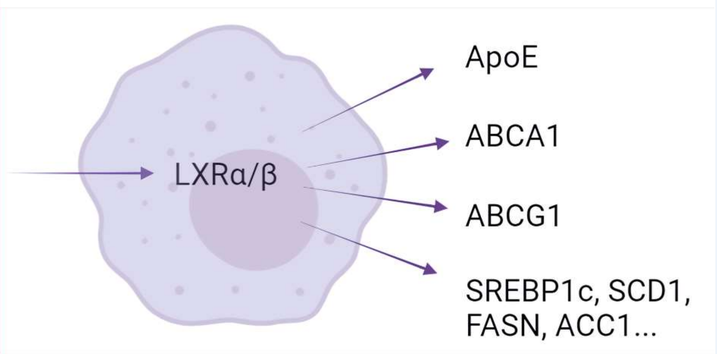




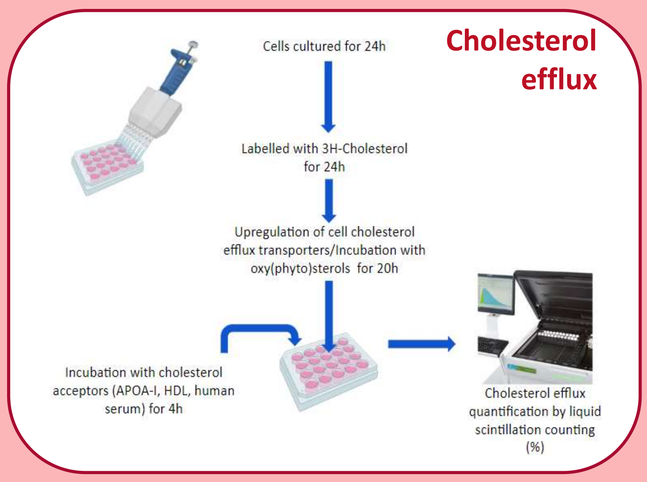
Background

The **nuclear liver X receptors** (LXR) α and β may be potential **therapeutic targets** in cardiovascular and neurodegenerative diseases because of their key role in the regulation of lipid homeostasis and inflammatory processes.

Among the mechanisms involved in the maintenance of macrophage cholesterol homeostasis, **cholesterol efflux** plays a crucial role.

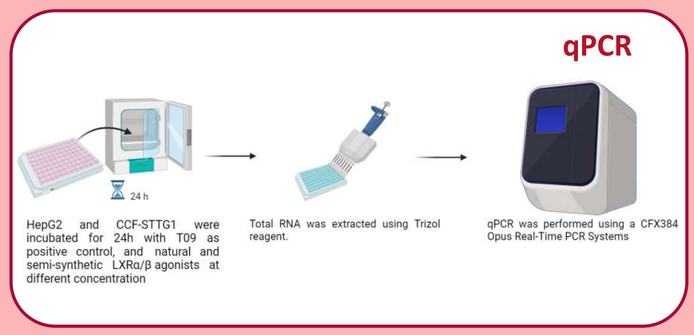
Specific **oxy(phyto)sterols** differentially modulate the transcriptional activity of **LXRs**, providing opportunities to develop **new therapies**. However, this development is precluded by unwanted side effects, such as hypertriglyceridemia and hepatic steatosis due to hepatic LXRα activation.

The aim of this study was to investigate the effect of newly isolated oxyphytosterols from ***Sargassum Fusiforme*** and new synthesized side chain oxidized sterols analogs on cholesterol homeostasis, and in particular on cholesterol efflux, and on genes involved in these processes.



Methods

Cellular cholesterol efflux was evaluated with a radioisotopic cell-based assay on human hepatocellular carcinoma cell line (HepG2) after treatment with LXRα/β agonists (n=6) at different concentrations. Gene expression was assessed in HepG2 and in human astrocytoma cells (CCF-STTG1) by qPCR. The commercial LXR agonist T0901317 1µM was used as positive control.



Results

Among the 6 natural and semi-synthetic 24-oxidized sterols with high potency for LXRα/β activation (**Table 1**), we identified two synthetic side chain compounds (**S2** and **S6**) that also regulate cholesterol homeostasis in different cell models.

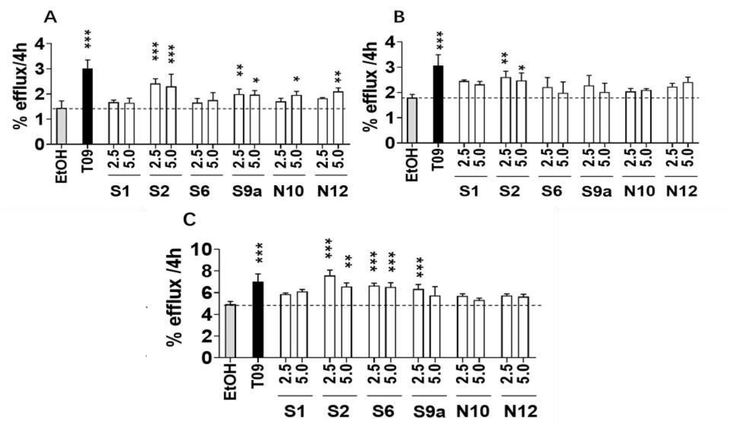
Table 1

Cell line	HepG2	
LXR	α	β
Compounds	5.0µM	
S1	2.56	2.33
S2	2.40	2.55
S3	1.05	1.39
S4	0.82	0.80
S5	0.67	0.71
S6	3.22	2.73
S7	0.83	1.45
S8a	2.64	3.70
S8b	1.54	2.01
S9a	2.34	2.75
S9b	1.37	1.14
N10	2.88	4.15
N11	1.87	2.30
N12	1.38	4.23
N13	0.37	0.59

Fold change
3.5 < X
3.0 < X ≤ 3.5
2.5 < X ≤ 3.0
2.0 < X ≤ 2.5
1.5 < X ≤ 2.0
1.0 < X ≤ 1.5
≤1.0

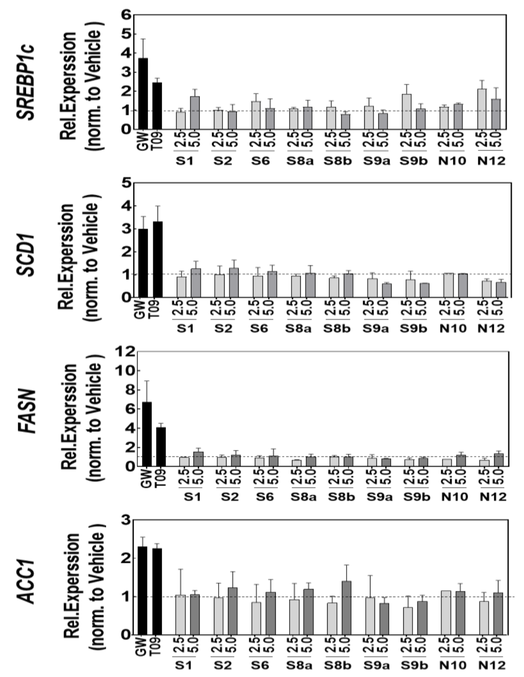
In detail, **S2** increased cholesterol efflux from HepG2 by 54.3%, 15.2%, and 24.5% using isolated APOA-I (**A**) and HDL (**B**), and human serum (**C**) as cholesterol acceptors, respectively. **S6** increased cholesterol efflux by 6.7% only in presence of human serum (**C**) (**Figure 1**).

Figure 1

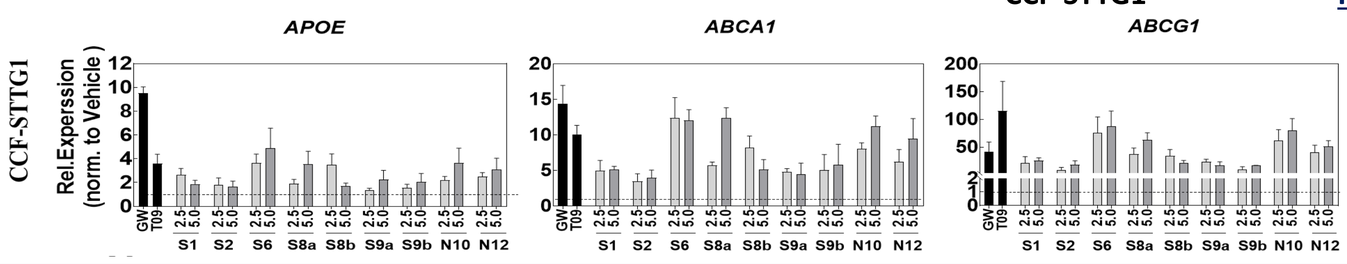


Regarding the impact of these compounds on cholesterol transporters gene expression, in HepG2 cells **S2** and **S6** did not upregulate the expression of **ABCA1** and **ABCG1** (**data not shown**), in addition they did not affect the expression of **SREBP1c**, **SCD1**, **FASN,** or **ACC1** (**Figure 2**), responsible for the hepatic side effects which are usual for synthetic pan-LXR agonist. In astrocytes, **S2** and **S6** slightly increased **APOE**, **ABCA1**, and **ABCG1** mRNA levels (**Figure 3**).

HepG2 **Figure 2**



CCF-STTG1 **Figure 3**



In summary we identified two new synthetic side chain LXRs agonists, which increased cholesterol efflux in human hepatocyte cell model, and upregulated the expression of genes involved in this process in human astrocytes, without affecting the expression of genes potentially responsible for hepatic side effects, typical of commercially synthetic pan-LXR agonists.

These results put the premises to identify and develop novel LXR-activating 24-oxidized sterols as potential therapeutic options in cardiovascular and neurodegenerative diseases.

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