LXR inverse agonists demonstrate liver lipid lowering effects through multiple mechanisms in rodent models of NASH and in human hepatocytes

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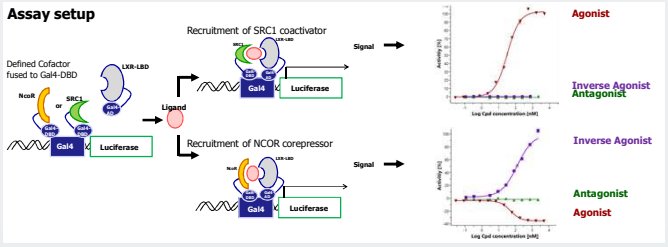
BACKGROUND & AIMs

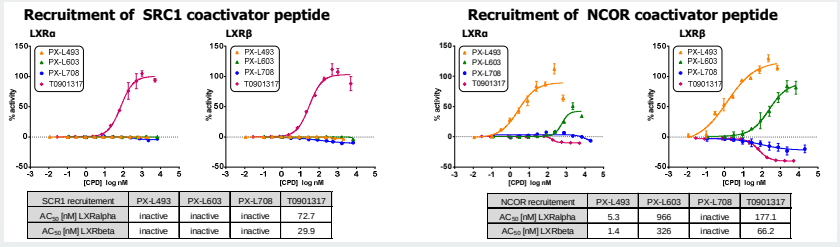
Several mechanisms are currently evaluated as potential pharmacotherapies for the spectrum of non-alcoholic fatty liver disease (NAFLD), including modulators of nuclear receptors such as PPARα/γ/δ or FXR. Activation of Liver X Receptor (LXR) in the liver by potent, synthetic agonists is known to result in severe steatosis and hypertriglyceridemia in various animal models[1,2] and in humans[3]. Thus, we have designed and synthesized LXR inverse agonists with the aim to inhibit LXR´s pro-steatotic transcriptional activity. The pharmacological effects of these LXR inverse agonists were evaluated in human hepatocytes and in a mouse and a rat steatosis model. These first results confirm the findings by another group[4,5], that synthetic LXR inverse agonists can reduce liver fat content which may provide a new mechanism for the treatment of NAFLD / NASH.

IN VITRO ACTIVITIES

Cellular reporter assay that differentiates between agonists, antagonists and inverse agonists of LXRα or LXRβ



Gal4-LXR-2-hybrid assay using a Gal4 driven luciferase. Corepressors or coactivators were transfected as fusions to Gal4 DNA-binding domain, LXR-LBD as fusion to transactivation domain.

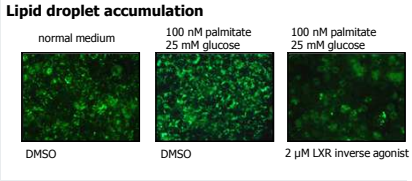


SCR1 recruitement	PX-L493	PX-L603	PX-L708	T0901317
AC_{50} [nM] LXRalpha	inactive	inactive	inactive	72.7
AC_{50} [nM] LXRbeta	inactive	inactive	inactive	29.9

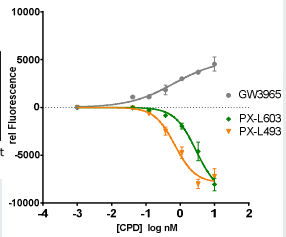
NCOR recruitement	PX-L493	PX-L603	PX-L708	T0901317
AC_{50} [nM] LXRalpha	5.3	966	inactive	177.1
AC_{50} [nM] LXRbeta	1.4	326	inactive	66.2

Compounds PX-L493 and PX-L603 were characterized in cellular reporter assays as inverse agonists of LXRα and LXRβ. Obtained AC_{50} for LXR(α/β) in NCoR recruitment Gal4 2-hybrid assay: PX-L493 (5.3/1.4 nM) ; PX-L603 (966/326 nM)

Anti steatotic effects in primary human hepatocytes

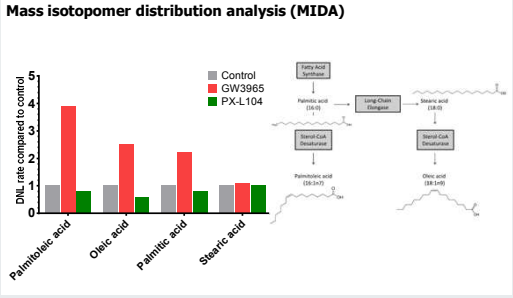


Human primary Upcyte® hepatocytes were cultivated in the presence of 100 nM palmitate and 25 mM glucose for 5 days. The addition of LXR inverse agonists PX-L603 and PX-L493 dose-dependently reduced the hepatocyte lipid load as determined by Bodipy™ staining and fluorescence measurements.



	PX-L493	PX-L603	GW3965
AC_{50} [µM]	0.7	2.9	0.7

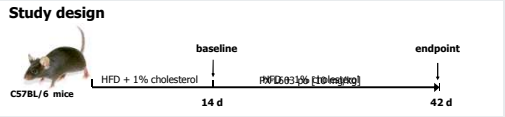
De novo lipogenesis (DNL)



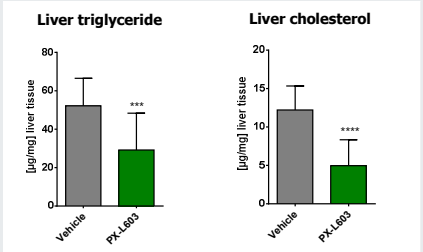
In vitro de novo lipogenesis was assessed using mass isotopomer distribution analysis (MIDA) following [1,2-^{13}C]-acetate treatment of hepatocytes.
Upcyte hepatocytes were cultivated in the presence of 100 mM [1,2-^{13}C]-acetate and LXR Agonist GW3965 (0.5 µM) or LXR inverse Agonist PX-L104 (0.5 µM) (EC50 for LXR(α/β) in NCoR recruitment Gal4 2-hybrid assay 78 nM/ 4,6 nM) for 72 h. To quantify de novo lipid biosynthesis, label-incorporation into palmitic, stearic, plamitoleic and oleic acid was determined using HR-LC/MS after fatty acid saponification and extraction.
% abundance of [^{13}C]- labeled fatty acids in relation to [^{12}C]- species was normalized against control to generate DNL rate.

IN VIVO ACTIVITIES

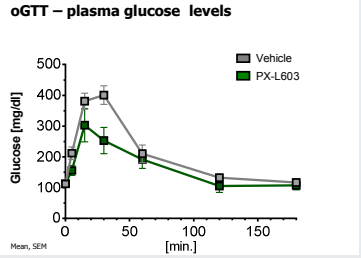
LXR inverse agonists show liver lipid lowering in high fat diet fed mice



C57Bl/6 mice were maintained on a Survit-type high fat diet with 1% cholesterol for two weeks. PX-L603 (10 mg/kg, po) LXR inverse agonists or vehicle were administered for the following 4 weeks on the same diet.
Hepatic triglyceride content was significantly reduced from 52.1(±19.3) µg/mg to 29.1 (±14.4) µg/mg. Total cholesterol in the livers of animals treated with PX-L603 was 5.0 (±3.4) µg/mg compared to 12.2 (±3.1) µg/mg of vehicle treated animals.

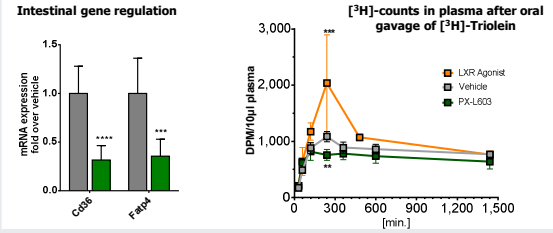


oGTT – plasma glucose levels



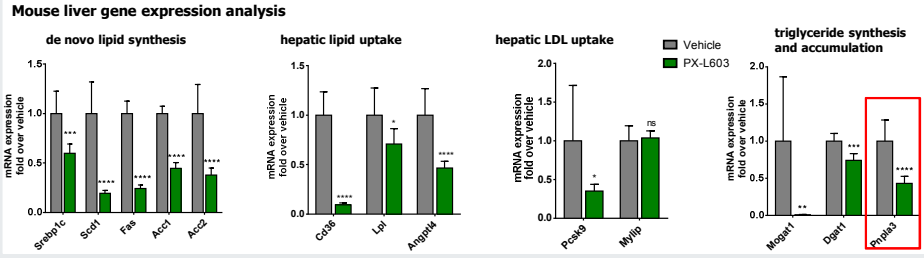
PX-L603 shows lowering in plasma glucose after oral challenge compared to vehicle.

Reduced intestinal fatty acid uptake upon LXR inverse agonism



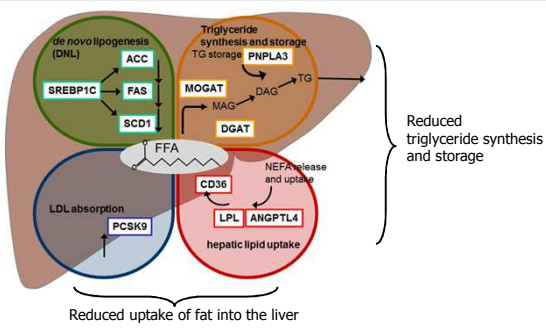
Gene expression analysis of mouse intestinal segments revealed that LXR inverse agonist PX-L603 repressed Cd36 and Fatp4 mRNA expression. Both are genes invovled in intestinal lipid uptake.

Mouse liver gene expression analysis



Gene expression analysis of the liver tissue from the mouse study revealed that LXR inverse agonist PX-L603 repressed known LXR target genes involved in fatty acid synthesis and lipid homeostasis such as Scd1, Fas, Cd36 or Angptl4. It is noteworthy, that PX-L603 treatment reduced the mRNA expression of **Pnpla3** with high significance. Pnpla3 expression is highly associated with human NAFLD development.

CONCLUSION



Inhibition of LXR´s transcriptional activity by synthetic inverse agonists results in:

- Inhibition of *de novo* lipogenesis (DNL)
- Reduction of free fatty acid (FFA) release from chylomicrons and reduced FFA uptake
- Reduced triglyceride synthesis through downregulation of Mogat and Dgat
- Downregulation of Pnpla3 expression, an enzyme with proven clinical significance in NASH patients

→ ultimately resulting in reduced liver fat.

→ **This suggests that inhibition of the LXR pathway in the liver is a useful novel approach for a pharmacotherapy of NAFLD.**

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