

Molecular Modelling to Predict and Understand Chemical Toxicity in the AOP Framework – Case Study: MoA from LXR Activation to Liver Steatosis



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Introduction and Aims

In the mode of action / adverse outcome pathway (MoA/AOP) framework addressing repeated dose toxicity, the **MoA from liver X receptor (LXR) activation to liver steatosis** is one of the toxicological process associated with repeated dose target organ toxicity chosen by SEURAT-1 to be defined and documented as a first step in building a "prototype" safety assessment framework¹. The molecular initiating event identified in this MoA is the binding to LXR and activation by appropriate ligands.

COSMOS supports the development and promotion of adverse outcome pathways (AOPs) by organising the chemistry involved in the processes. One of the approaches selected within COSMOS to understand the chemical toxicity in the MoA/AOP framework is molecular modelling to study the binding of small molecules (the toxicants) to biological macromolecules (biological receptors / targets). This is applicable when the MoA of interest includes receptor binding as a key event.

Here we present the results of a molecular modelling study of the binding of selected ligands to LXR. This will be a useful element in efforts to verify the postulated MoA for LXR-activated liver steatosis.

Moreover this study addresses the challenge of tailoring tools developed for drug discovery to the prediction of the potential toxicity of chemicals.

Methods

Molecular modelling has been widely used in pharmaceutical discovery for more than 50 years. Applying tools developed for drug discovery to the problem of predicting the potential toxicity of chemicals requires an optimisation of the methods that takes into account the differences between the two frameworks. Here these differences are analysed in terms of goals and chemical space. Moreover the purposes of computational chemistry in drug discovery and toxicology are compared.

Drug Discovery	Toxicology
Goals	
Explore the entire chemical space in order to extract a molecule with a given activity and desired properties	Evaluate the effects of a specific molecule on biological systems
Chemical space	
Drugs have a strong interaction with a specific target, have good bioavailability, and are readily metabolised to inactive compounds and cleared from the system, compounds that have specific ADMET profiles and prescribed chemical properties	Chemicals span a considerably larger chemical space and tread into "undesirable" property space from an ADMET perspective (too small, too insoluble, too reactive, etc). They can also elicit adverse biologic effects from both strong and weak interactions with targets
Purposes of computational chemistry methods	
<ul style="list-style-type: none"> - Hit Identification - Lead Identification - Lead Optimisation - ADMET Optimisation - Screening for drug candidates: identification of most potent chemicals; minimise false positives 	<ul style="list-style-type: none"> - Assist evaluation of existing test data - Priority setting - Mechanistic information - Fill data gap - Screening of industrial chemicals: minimise false negatives

The aim of this molecular modelling study performed in three steps is to compare LXR α and LXR β .

1. Retrieval² and analysis of available 3D structures of the ligand binding domain (LBD) of LXR α and LXR β
2. Comparison of the ligand-binding domains (LBD);
3. Docking studies and binding energy calculations on **T0901317** (the LXR agonist proposed as the reference chemical for liver steatosis by the SEURAT Gold Compound Working Group³).

Results

1. Analysis of available 3D structures of the LBD of LXR α and LXR β

Receptor and source	PDB code (Co-crystallised ligand)
LXR α (mus musculus) 3 structures	2ACL (synthetic agonist SB313987), 3FAL (synthetic agonist SB786875)
LXR α (homo sapiens) 4 structures	3IPQ (synthetic agonist GW3965), 1UHL (synthetic agonist T0901317), 3IPS (synthetic agonist), 3IPU (synthetic agonist)
LXR β (homo sapiens) 10 structures	1UPV, 1UPW, 1PQC, 1PQ9 (synthetic agonist T0901317); 1PQ6 (synthetic agonist GW3965), 3LOE (modulator), 3KFC (synthetic agonist), 1P8D (sterol agonist), 4DK7 (full agonist), 4DK8 (partial agonist)

2. Comparison of the ligand-binding domains (LBD) of LXR α and LXR β

LXR α /LXR β sequences were aligned and analysed with BLAST⁴. The two proteins have high homology with 62%, 78% and 100% sequence identity considering respectively the whole sequence, the LBD and the ligand binding pocket. The structures of LXR α /LXR β co-crystallised with **T0901317** were then superimposed⁵. Despite a different binding pose of the ligand (**Figure 1b**), the receptors structures are identical (**Figure 1a**) above all in the ligand binding pocket. The details of the interactions between the receptors and the ligand are represented in **Figure 1c**. Further analysis (results not shown) on the available structures confirmed that LXR α /LXR β have no fundamental differences in the ligand binding pocket.

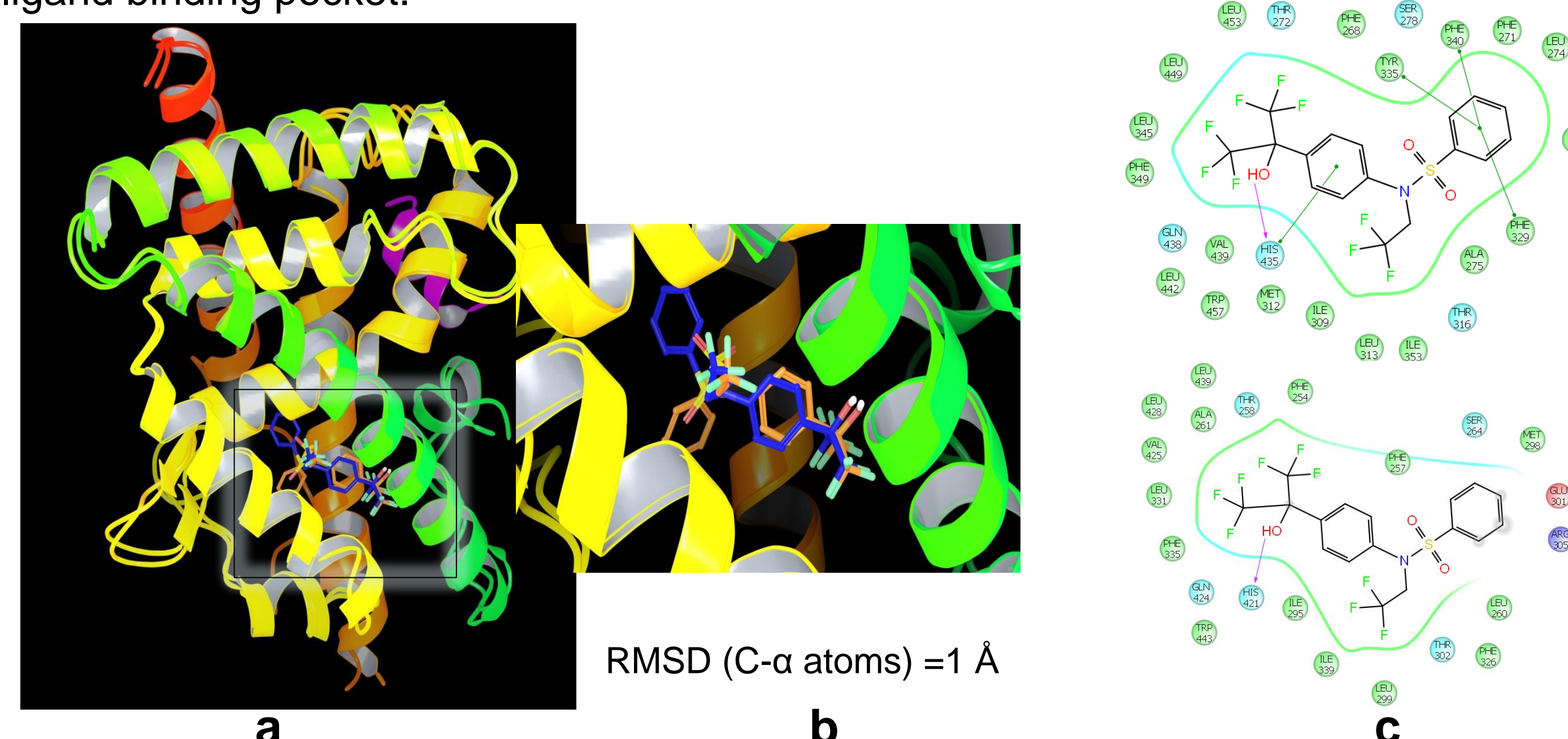


Figure 1. a) Alignment of LXR α /LXR β LBD complexed with T0901317; b) ligand binding pocket of T0901317 (blue/orange LXR α /LXR β); c) ligand interaction diagrams of T0901317 in the ligand binding pocket of LXR α /LXR β (above/below).

3. Docking studies and binding energy calculations on T0901317

A docking study⁶ was carried out to study the binding pose of **T0901317**. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The results showed that the most stable binding pose of **T0901317** in both α and β forms is the one retrieved in LXR β (**Figure 1b**, orange). Prime/MM-GBSA⁷ calculations of the binding energy confirmed these results.

Crystal complex structure	Docking (Glide score)	ΔG binding (Kcals/mol)
LXR α -T0901317	-7,5 / -9 (binding pose A / B)	-86 / -90 (binding pose A / B)
LXR β -T0901317	-10,3 (binding pose B)	-100 (binding pose B)

Conclusions

- Molecular modelling methods offer one of several complementary approaches to evaluate the risk to human health.
- Molecular modelling approach can be applied to the MoA for LXR-activated liver steatosis and the docking studies showed that LXR complexes are suitable for such studies.
- LXR α /LXR β have no fundamental differences in the ligand binding pocket.
- The binding pocket of LXR is large, mainly hydrophobic and flexible, in fact multiple binding poses are allowed.

References

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