Specific Ligand-Residue Interactions that Lead to Liver X Receptor Isoform Selectivity

LA-SiGMA Louisiana Alliance for Simulation-Guided Materials Applications

Kathryn M. Hardin[1], Susannah L. Davis[2], Kevin E. Riley, PhD[3], Jayalakshmi Sridhar, PhD[3]

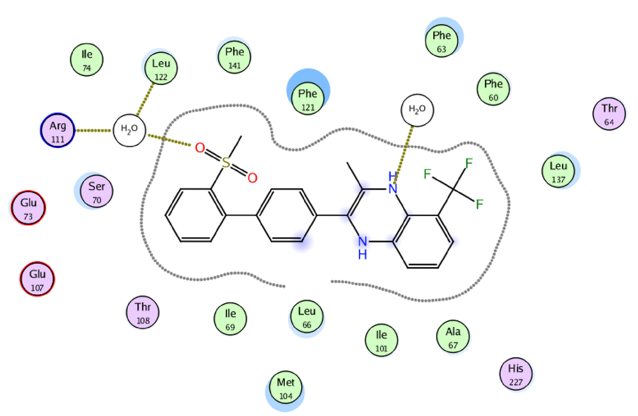
[1] Department of Biomedical Engineering, Boston University, Boston, MA 02215
[2] Department of Engineering, Smith College, Northampton, MA 01063
[3] Department of Chemistry, Xavier University, New Orleans, LA 70125

Abstract

Though very similar in structure, the alpha and beta isoforms of nuclear receptor LXR affect the body in different ways when activated. Through isoform selective ligands, the potential exists to treat a variety of human diseases, including hormone-dependent cancer, atherosclerosis, and Alzheimer's disease. While the structural components that determine alpha selectivity are partially understood, this is not the case for beta selectivity. To better understand LXR isoform binding, four ligands with varying selectivity were analyzed. Important features of beta selective ligand wye-672 were identified through glycine scanning and used to generate novel ligands in a scaffold replacement search. These ligands will be evaluated for their potential to be synthesized for clinical use.

Introduction

LXR proteins belong to the nuclear receptor superfamily. These ligand-activated transcription factors are involved in the metabolism of cholesterol and fatty acid, glucose homeostasis, inflammation, and neurological homeostasis. LXR exists in two isoforms, LXRα and LXRβ. LXRβ is widespread throughout the body, while LXRα is predominately found in the liver, small intestine, and macrophage.

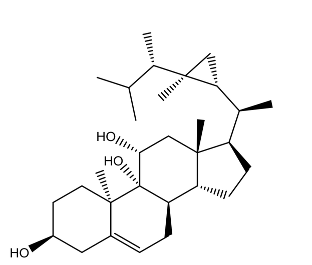


Ligand interaction diagram for wye-672 on 1PQ6 (LXRβ).

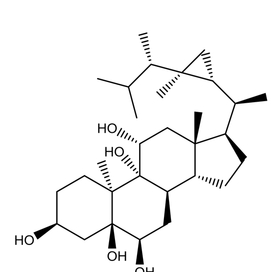
The role of LXR in regulating cholesterol makes it a promising target for pharmacological treatments of hormone-dependent cancers, skin disorders, Alzheimer's disease, atherosclerosis, inflammation, and diabetes. Though both LXRα and LXRβ reduce levels of plasma cholesterol when activated, LXRα also undesirably increases levels of hepatic triglyceride (TG). The development of beta selective ligands is therefore particularly desirable, especially considering most natural ligands are non-selective. The first beta selective ligand to reach human clinical trials, LXR-623, had adverse side affects on the central nervous system. Given LXRβ agonists' potential for the treatment of disease, more investigation is needed to find isoform selective ligands without these side effects.

The structural features that make a ligand beta selective are still not well understood. Examining binding interactions through computational modeling can provide insight into this process and save time and money by screening ligands before synthesis.

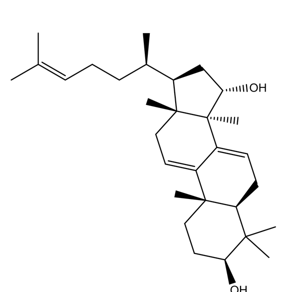
The ligands used in our investigation were gorgost-5-ene-3β,9α,11α-triol (gorgost-5); gorgostane-3β,9α,5α, 6β, 11α-tetrol (gorgostane); polycarpol; and wye-672; pictured below.



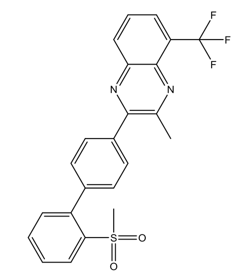
gorgost-5-ene-3β,9α,11α -triol
Non-isoform selective



gorgostane-3β,9α,5α, 6β, 11α-tetrol
LXRα isoform selective



polycarpol
LXRα isoform selective



wye-672
LXRβ isoform selective

Methods

MOLECULAR OPERATING ENVIRONMENT (MOE)

Glycine Scanning

- 3IPQ (LXRα) and 1PQ6 (LXRβ) proteins were downloaded from RCSB Protein Data Bank.
- Protein structures were completed using Homology Modeling.
- 24s, 25-Epoxycholesterol was docked into completed proteins to replace crystal structure ligand (GW3965).
- Gorgost-5, gorgostane, polycarpol, and wye-672 were docked using induced fit protocol, dummy atoms as the site, and a layer solvent of margin 4.0 Å.
- The pose with the lowest S score and a reasonable orientation was minimized and rescored for each ligand. Poses in which each end of the ligand was positioned near the area of the pocket with corresponding polarity were considered reasonable.
- Glycine scanning was performed on the minimized poses based on the ligand interaction diagrams.

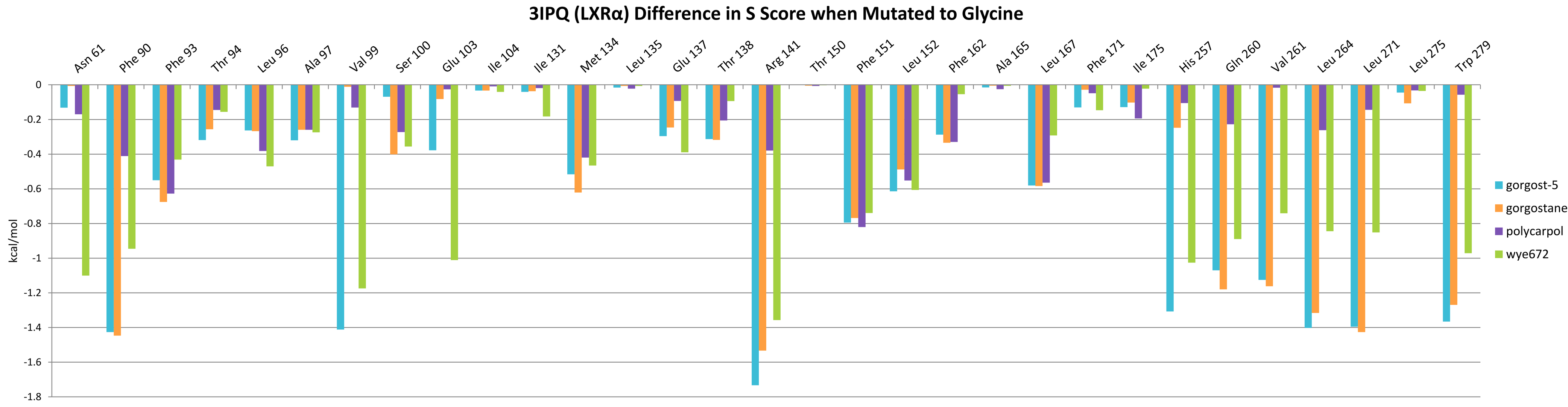
Scaffold Replacement

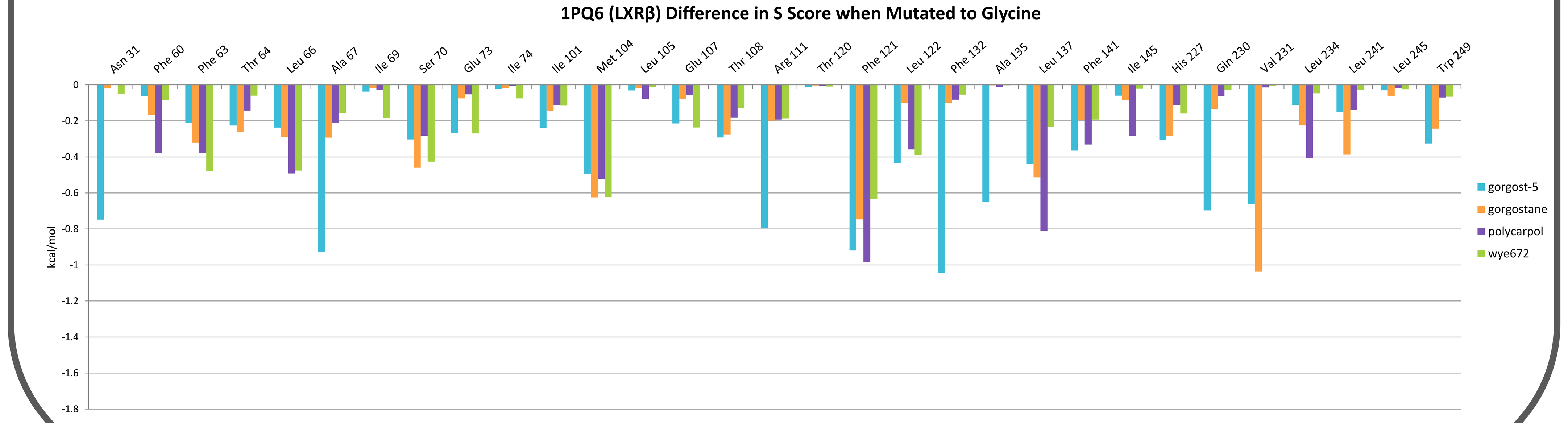
- Glycine scan results were used to identify structural features of wye-672 critical for ligand binding to LXRβ.
- Scaffold replacement was performed to find alternate ligand structures with these features for their potential in synthetic development.

Results and Discussion

A glycine scan of four different ligands of varying selectivity on LXRα and LXRβ indicated the relative importance of several amino acids for ligand binding. The data showed that different amino acids are important for binding in each protein, but did not show a trend based on ligand selectivity. For example, Phe 90/60, Arg 141/111, Leu 264/234, Leu 271/241, and Trp 279/249 all showed greater importance for LXRα than LXRβ regardless of ligand selectivity, but the same amino acids showed a greater difference for non-selective and alpha selective ligands (gorgost-5 and gorgostane) than the beta selective ligand (wye-672) on both proteins. Some amino acids did not play a large role in binding for either protein, such as Ile 104/174, Leu 135/105, Thr 150/120, and Leu 275/245.

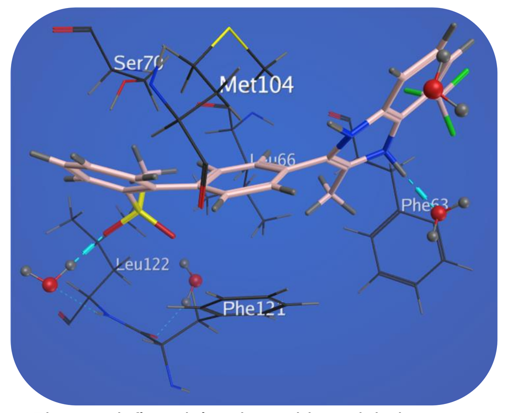
In general, alpha selective ligand polycarpol seemed to interact to the same degree on both LXRα and LXRβ for each amino acid. This unexpected behavior disrupted any possible trends. In contrast, amino acids differed in importance between LXRα and LXRβ for gorgostane, the other alpha selective ligand. Polycarpol's behavior also differed from that of the non-selective ligand, gorgost-5, which demonstrated distinct amino acid interactions from one protein to another.





Results and Conclusion

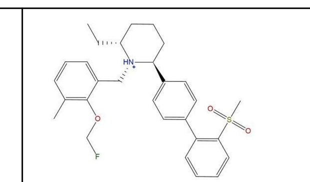
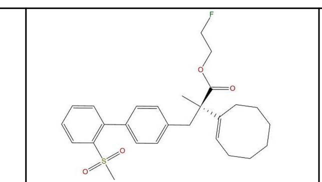
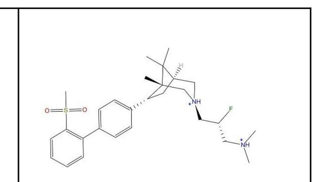
Wye-672—Important Interactions on LXRβ



Six most influential amino acids and their interactions with wye-672.

Amino Acid	Interaction
Phe 121	pi-pi
Met 104	hydrophobic
Phe 63	pi-methyl; hydrophobic
Leu 66	hydrophobic
Ser 70	pi-OH
Leu 122	H-bond bridged by H_2O

Though no selectivity trends appeared, this analysis was useful for identifying features on specific ligands. Glycine scanning revealed the six most important amino acids for wye-672 binding to LXRβ. Key structural features of the ligand were identified by examining these interactions. The hydrophilic sulfonyl group and two adjacent aromatic rings, along with the methyl substituent, were found significant to ligand binding and were used as queries during scaffold replacement. Sixty-two ligand structures with these features were generated and docked into LXRβ. The three best performing structures are detailed below and will be further evaluated for possible synthetic development.

Scaffold Replacement Results			
S Score (kcal/mol)	-12.4994	-11.9174	-11.4165

Future Work

- Evaluate scaffold replacement results for viability as LXRβ selective ligands.
- Perform glycine scanning and scaffold replacement using other beta selective ligands.
- Corroborate results by repeating procedure with alternate starting crystal structures for LXRα and LXRβ.
- Perform molecular dynamics simulation to obtain snapshots for further analysis.

References

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Acknowledgements

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