



In Silico Mining of Potential Anti-cancer Liver X Receptor β Agonists

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Abstract: Cancer continues to be a leading cause of mortality worldwide, with the most common treatments such as chemotherapy and radiotherapy producing severe off-target effects. This study aims to identify potential therapeutic compounds by screening the Natural Products Atlas (NPAtlas) for potential agonists of the Liver X Receptor Beta (LXR- β). LXR- β is a nuclear receptor involved in lipid metabolism and is known to induce apoptosis in cancer cells when acted upon by an agonist. Screening of 36,310 compounds from the NPAtlas database was performed using molecular docking, pharmacokinetic filtering, absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling, and confirmatory molecular dynamics simulations. From the initial dataset, 9 candidates passed the docking and ADMET criteria, and molecular dynamics simulations successfully validated 7 of them. Among these, Cis-veramycin E (NPA033420) and Solanapyrone E (NPA010954) emerged as top candidates. Cis-veramycin E demonstrated a high docking score (-9.31 kcal/mol), low root mean square deviation (RMSD) of <1.0 nm, minimal root mean square fluctuation (RMSF) of <0.3 nm, and a favorable ADMET profile, including Ames safety, good absorption, and moderate clearance. Protein-ligand interaction profiling revealed a binding profile similar to known LXR- β agonists, including hydrogen bonding with Ser278, π - π stacking with Phe340, and hydrophobic contacts across core residues. Solanapyrone E exhibited comparable binding stability and engaged the critical His435-Trp457 electrostatic switch via a salt bridge, but was flagged as Ames toxic, indicating a need for structural optimization. The results support the recommendation to identify the compounds as lead candidates for LXR- β -targeted cancer therapy.

Keywords: Induced apoptosis; cancer treatment; in silico mining

1. Introduction

Cancer continues to be a leading global issue, with approximately 20 million new diagnoses and 9.7 million deaths worldwide in 2022 alone. It is projected to reach 35 million by 2050, based on estimated population growth [1]. Drug discovery in cancer research has shifted towards the molecular targeting approach that focuses on potential therapeutic targets [2]. As cancer is characterized by uncontrolled cell division and unlimited proliferation, identifying potential targets with anti-proliferative effects is the main goal of therapeutic discovery. One such molecular target involved in cell fate determination is the Liver X Receptor (LXR).

LXRs are members of the nuclear receptor superfamily that is composed of important ligand-inducible transcription factors [3]. LXRs are involved in glucose and lipid metabolism, cholesterol transport and metabolism, and in inflammatory responses [4]. With increased demand for cancer cell resources, activation of LXR with synthetic agonists induces degradation of low-density lipoprotein receptors via the Inducible Degradation of the LDL receptor (IDOL) pathway and promotes cholesterol efflux, reducing the intracellular cholesterol pool [5]. This deprives cancer cells of materials necessary for biogenesis and cell membrane maintenance. This shows that LXR activation can induce cell stress and ultimately lead to apoptosis, highlighting the potential of LXR agonists as promising therapeutic targets for cancer treatment. In breast cancer, estrogen metabolism is a key determinant, and LXR exerts some control over estrogen homeostasis, with estrogen sulfotransferase (EST) being a transcriptional target of LXR [6]. It was demonstrated that LXR inhibits estrogen-dependent cancer cell proliferation by regulating hepatic EST. In addition, LXR agonists were observed to decrease the proliferation of breast cancer cell lines without affecting lipid biosynthesis through the decreased expression of specific cyclins and increased expression of p53 at the protein level [7].

In a study by Pencheva et al., ApoE was identified as a suppressor of melanoma metastasis, with the activation of LXRs enhancing ApoE transcription and inhibiting melanoma progression [8]. Knockdown experiments showed that LXR- β , but not LXR- α , is necessary for the suppression of melanoma invasion and endothelial recruitment by LXR agonists. Oral administration of LXR agonists GW3965 and T0901317 significantly suppressed tumor growth in mouse melanoma models, including various human melanoma cell lines, in a dose-dependent manner, with higher doses leading to greater suppression. The study also showed that LXR agonists reduced the number and size of endothelial vessels in tumors, leading to decreased tumor angiogenesis and modest reductions in tumor cell proliferation. While LXR- α and LXR- β share high structural similarity in their ligand-binding domains, they differ significantly in their tissue distribution and functional roles. LXR- α is primarily expressed in tissues with high metabolic activity, such as the liver, intestine, and adipose tissue, where it plays a vital role in lipid homeostasis and cholesterol transport [9]. In contrast, LXR- β is expressed ubiquitously across virtually all tissues, making it a more broadly applicable cancer therapeutic target. More importantly, simultaneous activation of both LXR isoforms results in significant hepatotoxicity and hypertriglyceridemia due to LXR- α -mediated upregulation of hepatic lipogenic genes (e.g., SREBP-1c, FAS) [9]. Thus, selective targeting of LXR- β offers a more promising anticancer strategy, potentially maintaining efficacy while minimizing hepatic side effects of LXR agonists.

Uno et al. also showed that LXR agonists inhibit Wnt signaling activation by reducing β -catenin's transactivation activity [10]. In HCT116 cells, LXR agonists reduce mRNA expression of β -catenin target genes, including the MYC proto-oncogene, matrix metalloproteinase 7 (MMP-7), and bone morphogenetic protein 4 (BMP-4). MYC is well studied for its critical role in cell growth, proliferation, and survival, and is frequently overexpressed in cancers [11]. MMP-7 is involved in the breakdown of the extracellular matrix, facilitating cancer cell invasion and metastasis [12]. BMPs have been shown to induce epithelial-to-mesenchymal transition, during which cells acquire migratory capability, making them invasive [13]. Furthermore, Fukuchi et al. [14] showed that LXR agonist T0901317 inhibits the proliferation of human prostate LNCaP cells. It achieves this by increasing the expression of the cyclin-dependent kinase inhibitor p27, inducing cell cycle arrest at the G1/S transition. Additional studies showed that T0901317 leads to downregulation of the protein B kinase (Akt), which plays a key role in promoting survival, growth, and proliferation in many types of cancer [15].

In the search for novel drugs, *in silico* methods have become indispensable tools in pharmacokinetic and pharmacochemical research, offering efficient and cost-effective alternatives to traditional experimental approaches [16]. These methods assess crucial factors such as drug absorption routes, distribution mechanisms, and potential risks and safety profile of candidate compounds. They streamline the drug discovery process, allowing researchers to rapidly and effectively identify promising compounds for further development. Our study aims to investigate potential anticancer compounds that target the LXR- β pathway of tumor suppression, using protein docking simulations and multiple *in silico* methods. For example, the synthetic LXR agonists GW3965 and T0901317 have demonstrated potent anticancer activity in preclinical models. However, their clinical application has been severely limited due to dose-limiting hepatotoxicity and

hypertriglyceridemia arising from non-selective LXR- α activation in hepatic tissue [17, 18]. These adverse effects underscore the need for novel LXR- β -selective agonists with improved therapeutic windows. Natural products offer a rich and structurally diverse chemical space that has historically yielded selective bioactive compounds with better tolerability profiles, making microbially derived natural product libraries particularly attractive for the discovery of next-generation LXR- β agonists. Structure-based virtual screening is particularly well-suited for targeting LXR- β , as high-resolution crystal structures of the LXR- β ligand-binding domain in complex with known agonists are publicly available, such as in the Protein Data Bank (PDB), enabling accurate modeling of ligand-receptor interactions. Previous in silico studies targeting LXRs and related receptors have successfully identified novel agonists using computational screening [19, 20, 21]. That is, these examples of successful computational screening approaches support the validity and efficiency of this methodology for nuclear receptor drug discovery. However, unlike prior work, this study differs in several key aspects. First, whereas most prior studies investigate the effects of a limited number of synthetic LXR agonists on specific cancer cell lines, this work performs a large-scale virtual screen of over 36,000 structurally diverse microbially derived natural products against LXR- β . Additionally, integrating molecular docking, multi-parameter ADMET profiling, and molecular dynamics simulation provides a multi-stage computational validation pipeline. Finally, the specific focus on selective LXR- β agonists from natural products represents a novel angle that has not been well explored in existing literature.

2. Materials and Methods

The study used the following software: PyRx, AutoDock Tools (ADT), VMD, PyMOL, OpenBabel, pkCSM, and GROMACS. PyRx uses AutoDock Vina to simulate and automate protein docking [22, 23, 24, 25]. pkCSM through Deep-PK was used to remove candidates based on comprehensive ADMET guidelines, while GROMACS was used to perform the molecular dynamics (MD) simulations [26, 27]. The computational load was processed on a Ryzen 5 3600 processor with 16 GB RAM, running Ubuntu 22.04 LTS on Windows Subsystem for Linux 2.

2.1 Target Protein Preparation

The LXR- β structure was downloaded from the Protein Data Bank. The crystal structure of human Liver X Receptor beta (LXR- β), selected as the template for all in silico estimations, was obtained from the Protein Data Bank (PDB) under PDB ID 6S4U, with an X-ray diffraction structure resolved at 2.81 Å. The structure corresponds to the ligand-binding domain (LBD) of Oxysterols receptor LXR- β in complex with a small-molecule inhibitor (KVK), comprising three protein chains (A, B, and C) with approximately 233–236 residues per chain. The co-crystallized ligand and water molecules were removed during receptor preparation before docking simulations in PyMOL, after which the receptor was transferred to ADT. ADT prepared the receptor and ligand files, converting them to PDBQT format that includes atomic coordinates, partial charges, and AutoDock atom types. The protonation state of the receptors was then assigned using the H++ server, which estimates pKas to allow the addition of missing H+ to macromolecules [28].

2.2 Sample Proteins Preparation

The Natural Products Atlas (NPAtlas) database is a comprehensive, open-access repository containing >36,300 microbially derived natural product structures [29]. The database was downloaded in SDF format, and a preliminary screening was performed using Open Babel and the International Chemical Identifier (InChI) to remove duplicates [30, 31]. As the files are in their 2D conformation, they were all minimized in PyRx using the uff94 force field to obtain an approximation of their 3D structures. Among these, those with extremely high energy values (>1000) were removed, as they indicate high steric strain and low stability, making them unsuitable for docking, as the stable 3D conformation cannot be determined reliably. The ligands were then partitioned into batches of 1000 for automated large-volume molecular docking screening in PyRx.

2.3 Molecular Docking Simulations

AutoDock Vina was used to perform docking computations, automated with PyRx. Docking grid dimensions are set to the full size of the LXR- β receptor, 99.5 x 93 x 74 Å. Docking parameters such as the

number of runs and population size were set to exhaustiveness levels of 10 and 50, respectively, for fast and extensive docking predictions. ADT and PyMOL were then used to visualize the docking results and evaluate the binding affinity of the ligand and the receptors. PyRx displayed the binding affinity values in kcal/mol, with lower (more negative) values indicating stronger binding. For drug discovery, a binding affinity of -6.0 to -7.0 kcal/mol or lower is considered promising [32, 33]. To validate the docking protocol, Beta-sitosterol (SIT), a known LXR- β agonist, was included as a positive control in docking simulations. The control ligand exhibited an average binding affinity of -8.33 kcal/mol under the established docking parameters, which served as the benchmark for evaluating candidate compounds. Ligands demonstrating binding affinities equal to or more negative than that of SIT were retained for subsequent pharmacokinetic screening and molecular dynamics confirmatory testing. Although explicit redocking of the attached ligand inhibitor was not performed, Beta-sitosterol (SIT) is a known agonist, and the Vina docking software has been widely used in previous studies as a standard for *in silico* molecular docking experiments [32].

2.4 ADMET Pharmacokinetics Screening

Lipinski's Rule of 5 was applied manually by checking the molecular weight, log P, and the number of hydrogen bond donors and acceptors for each candidate. After this, the SMILES sequences of the remaining candidates were uploaded to the Deep-PK web server for further screening using their comprehensive library of ADMET criteria [26]. The following quantitative thresholds and classification criteria were applied during ADMET screening using Deep-PK. For absorption, Caco-2 permeability (log Papp, log cm/s) was assessed, where values greater than 0.90 (corresponding to Papp > 8×10^{-6} cm/s) indicate high permeability; compounds were additionally evaluated for Human Intestinal Absorption (HIA), with those absorbing less than 30% considered poorly absorbed and excluded. Distribution was assessed via blood-brain barrier (BBB) permeability, where compounds with logPS ≥ -2 were classified as CNS-penetrant (CNSp+) and those with logPS ≤ -3 as non-penetrant (CNSp-). Metabolism was assessed through CYP3A4 inhibitor and substrate classifications: a compound is considered a CYP3A4 inhibitor (Class 1) if the IC₅₀ is less than 10 μ M, and a substrate (Class 1) if its pharmacokinetics are largely driven by CYP3A4-mediated metabolism. For excretion, total clearance (CL_{tot}) was predicted in mL/min/kg; values between 5 and 15 mL/min/kg were considered moderate and preferred. For toxicity, Ames mutagenicity was used as the primary genotoxicity filter; a positive Ames result indicates mutagenic potential and possible carcinogenicity, and all Ames-positive compounds were flagged. Any compound classified as "Unsafe" by Deep-PK across any of their parameter (e.g., Biodegradation, Ecotoxicity, Animal Toxicity, etc.) was excluded from further analysis.

2.5 Molecular Dynamics Simulations

To confirm binding stability, MD simulations were performed using GROMACS. The protein and ligand were combined and were energy-minimized using the steepest descent algorithm with a convergence criterion of Fmax < 1000 kJ/mol/nm. The CHARMM36 force field was used for the protein, while ligand parameters were generated using ACPYPE based on the General AMBER Force Field. After minimization, the system was equilibrated using a two-step protocol. First, NVT (number, volume, and temperature) equilibration was performed for 100 ps at 300 K using the V-rescale thermostat, with position restraints on hydrogen bond donors and acceptors to preserve key interactions during thermal equilibration. This was followed by NPT (number, pressure, and temperature) equilibration for 100 ps at 1 bar, employing the Parrinello-Rahman barostat to stabilize system pressure and density. A production MD run of 2,500,000 steps was then conducted in vacuum (i.e., without explicit solvent), using a time step of 2 fs for a total simulation time of 5 ns. Electrostatics were calculated using a cutoff of 1.0 nm. Constraints were applied to all hydrogen bonds using the LINCS algorithm to maintain bond lengths, eliminating drift. Structures that could not be minimized due to steric strain were excluded from testing. All simulations were run with 6 OpenMP threads on a 6-core virtual machine. Trajectory data were recorded every 10 ps and were used for subsequent structural and energetic analyses. While this may not fully capture the interaction over time, 5-ns simulations are sufficient to confirm binding stability and identify ligands for further studies [34]. Post-dynamics analysis includes the Root Mean Square Deviation (RMSD) and the Root Mean Square Fluctuation (RMSF). RMSD confirms whether the ligand remains stably associated with LXR β during the MD simulation, reinforcing the

initial docking prediction [35]. RMSF quantifies the average positional deviation of each residue over the course of the simulation, providing insight into local flexibility and structural stabilization. This was performed on the alpha carbons of each amino acid in the protein backbone [36, 37]. Molecular dynamics simulations were conducted in vacuum as a computationally efficient initial screening strategy to assess the relative binding stability of candidate compounds and prioritize lead candidates for further study [38]. While explicit solvent MD simulations more accurately represent physiological conditions, vacuum approaches have been employed in prior computational screening studies as a rapid confirmatory step before more resource-intensive solvated systems [39, 40, 41]. The absence of explicit solvent is acknowledged as a limitation of this study, and future work should include fully solvated, longer-timescale MD simulations to validate these findings under physiologically relevant conditions. A simulation duration of 5 ns was selected as a practical and computationally feasible timescale for initial stability screening, consistent with prior virtual screening studies that have employed short MD runs (1–10 ns) to filter ligand candidates based on binding stability before committing to resource-intensive extended simulations. Although 5 ns is insufficient to capture slow conformational dynamics or unbinding events, this was used to prioritize computational throughput to navigate vast chemical libraries. Based on previous studies, it is adequate for detecting gross conformational instabilities and ranking the relative stability of protein-ligand complexes at an early screening stage [38, 42, 43]. Future studies should employ longer simulation timescales (100 ns or beyond) with explicit solvent to more rigorously characterize the binding dynamics of the top lead compounds.

2.6 Receptor-Ligand Complex Interactions

To further characterize the binding of the two lead compounds, protein–ligand interaction profiling of the docked complexes was performed using the Protein–Ligand Interaction Profiler (PLIP) [44], a widely used automated platform for detecting and visualizing non-covalent protein–ligand interactions from three-dimensional structural data. Using the best-binding pose from the molecular docking process for the final lead targets, their structures were combined with the cleaned LXR β structure and uploaded to the PLIP platform.

3. Results and Discussion

3.1. Nine (9) candidate compounds were identified after molecular docking and pharmacokinetic screening.

The NPAtlas Database was screened for LXR- β using the fast docking protocol. Based on the predicted docking scores, 1,385 compounds of the original 36,310 displayed docking scores lower than that of SIT. More exhaustive docking parameters further reduced the number of compounds to 1,371. Among these, only 9 ligands met the criteria defined by the comprehensive Deep-PK ADMET guidelines and screening using Lipinski's Rule of 5. These compounds were identified as pharmacologically viable and not classified as "unsafe" by Deep-PK's advanced in silico screening, which integrates absorption, distribution, metabolism, excretion, and toxicity parameters into a unified drug-likeness filter. The average exhaustive docking scores and 2D chemical structures of the 9 compounds are listed in Table 1, while Table 2 presents their comprehensive ADMET predictions.

Table 1. Two-dimensional chemical structures and average fast and exhaustive Vina docking scores (in kcal/mol) for the top 9 potential NPAAtlas compounds against the LXR- β protein compared to SIT.

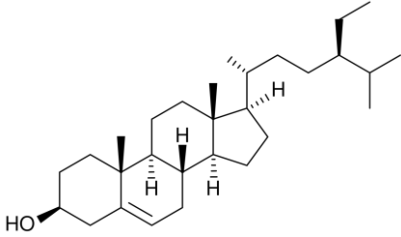
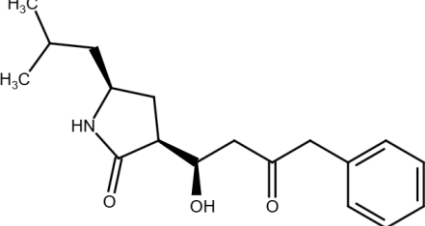
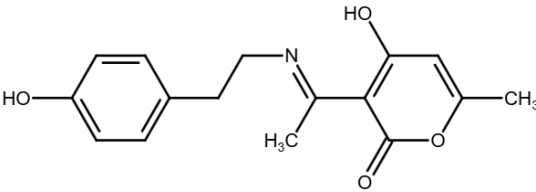
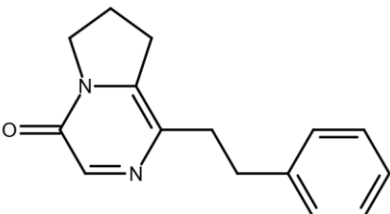
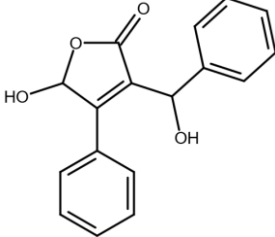
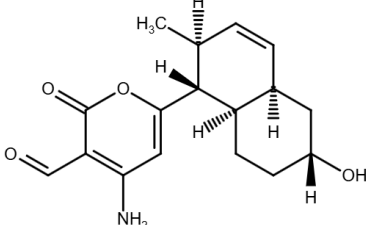
Compound Name NPAAtlas Code	Two-Dimensional Chemical Structure	Average Predicted Docking Score (kcal/mol)	
		Fast	Exhaustive
Beta-Sitosterol		-8.33	-8.33
NPA004934 (Berkeleyamide A)		-9.4	-9.62
NPA006504 (Fusamine)		-8.4	-8.6
NPA008173 (Nannoazinone A)		-8.7	-9.2
NPA008573 (Ralfuranone J)		-8.6	-9.26
NPA010954 (Solanapyrone E)		-8.4	-8.43

Table 1. Two-dimensional chemical structures and average fast and exhaustive Vina docking scores (in kcal/mol) for the top 9 potential NPAAtlas compounds against the LXR- β protein compared to SIT. (continue)

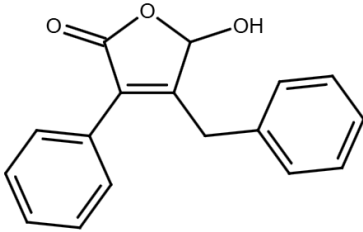
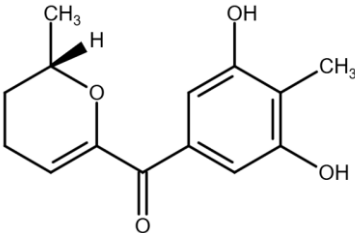
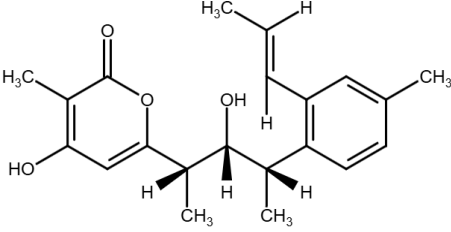
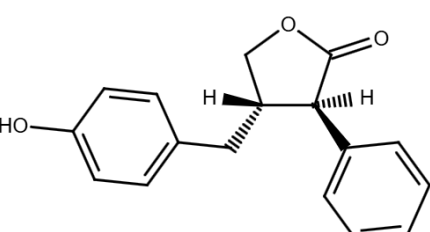
Compound Name NPAAtlas Code	Two-Dimensional Chemical Structure	Average Predicted Docking Score (kcal/mol)	
		Fast	Exhaustive
NPA016720 (EQ-4)		-9.2	-9.52
NPA023365 (Raistrickione E)		-8.54	-8.67
NPA033420 (Cis-veramycin E)		-8.9	-9.31
NPA035593 (Aspergulfuranone B)		-8.7	-8.82

Table 2. Highlighted ADMET Criteria from Predictions of the Final 9 Lead Compounds Selected from 1,371 Docking Candidates via Deep-PK Screening

Compound Name/Code	Absorption (A)		Distribution (D)	Metabolism (M)	Excretion (E)	Toxicity (T)
	Caco-2 Permeability (log Paap)	Human Intestinal Absorption (HIA)	Blood-Brain Barrier	CYP3A4 Inhibitor/Substrate	Total Clearance (ml/min/kg)	Ames Toxicity
NPA004934 (Berkeleyamide A)	-4.73	Absorbed	Penetrable	Non-Inhibitor; Non-Substrate	6.72	Safe
NPA006504 (Fusamine)	-4.83	Absorbed	Non-Penetrable	Inhibitor; Substrate	6.11	Safe
NPA008173 (Nannoizinone A)	-4.96	Absorbed	Penetrable	Non-Inhibitor; Substrate	6.61	Safe
NPA008573 (Ralfuranone J)	-4.43	Absorbed	Non-Penetrable	Non-Inhibitor; Non-Substrate	3.42	Safe
NPA010954 (Solanapyrone E)	-4.78	Absorbed	Penetrable	Inhibitor; Non-substrate	5.34	Toxic
NPA016720 (EQ-4)	-4.55	Absorbed	Penetrable	Non-Inhibitor; Non-Substrate	5.42	Safe
NPA023365 (Raistrickione E)	-4.64	Absorbed	Penetrable	Inhibitor; Non-Substrate	10.5	Safe
NPA033420 (Cis-veramycin E)	-4.81	Absorbed	Penetrable	Inhibitor; Substrate	5.17	Safe
NPA035593 (Aspergulfuranone B)	-4.82	Absorbed	Penetrable	Inhibitor; Non-Substrate	6.66	Safe

Based on the docking results, Berkeleyamide A (NPA004934) appears to be the most promising candidate with an exhaustive docking score of -9.62 kcal/mol. Its structure includes a fused bicyclic core and a flexible polar tail featuring hydroxyl and amide groups, which could contribute to its strong binding affinity and potential bioavailability. EQ-4 (NPA016720) also demonstrated excellent binding potential (-9.52 kcal/mol). The molecule features a central lactone ring conjugated to two aromatic rings, a scaffold known to support π - π stacking and hydrogen bonding [45]. This planar geometry and electron distribution could facilitate strong interactions with key LXR- β residues involved in ligand recognition. Cis-veramycin E (NPA033420), with a docking score of -9.31 kcal/mol, presents multiple hydroxyl and carbonyl groups, suggesting a high capacity for hydrogen bonding and potentially stabilizing its conformation for LXR- β agonist activity. Other compounds such as Fusamine (NPA006504) and Solanapyrone E (NPA010954), despite having slightly lower docking scores, still showed sufficient receptor affinity (-8.6 to -8.82 kcal/mol) and

passed all ADMET filters. These molecules are characterized by conjugated hydroxyl groups, which, according to Charlton et al., are among the most common pharmacophore features in antioxidants [46].

In Table 2, each compound is annotated across the five major ADMET categories that serve as core indicators of oral bioavailability, systemic exposure, safety, and therapeutic viability. Absorption was assessed through two key parameters: Caco-2 permeability (log Paap) and human intestinal absorption (HIA). Caco-2 cells are used in vitro to simulate intestinal epithelial permeability, and their log Paap values provide insight into a compound's ability to pass through the gut wall via passive diffusion [47]. Although all 9 compounds demonstrated moderate Caco-2 permeability values ranging from -4.43 to -4.96 , each was still predicted to be "absorbed" in the HIA model. This implies that, despite some variation in in vitro transport potential, the compounds possess physicochemical properties compatible with effective in vivo absorption [48]. The blood–brain barrier (BBB) permeability was included as a core distribution trait to indicate whether each compound is likely to penetrate the central nervous system (CNS) [26]. Among the selected compounds, 7 were classified as BBB-penetrant, suggesting they could reach neural tissues. This could be advantageous for CNS-specific applications but may also raise concerns about neurotoxicity or off-target neurological effects. Conversely, compounds such as Fusamine and Ralfuranone J were predicted to be non-penetrant, thereby reducing CNS-associated risks.

The metabolic liability of each compound was evaluated through predicted interactions with cytochrome P450 3A4 (CYP3A4), the most prominent metabolic enzyme in the human liver [49]. Compounds may act as inhibitors that block the enzyme, as substrates that are metabolized by it, or as non-interactors. Inhibitors can cause harmful drug–drug interactions, while substrates may be rapidly metabolized and eliminated. Among the 9 compounds, Berkeleyamide A and EQ-4 were predicted to be neither inhibitors nor substrates, indicating a minimal risk for metabolic interference. In contrast, compounds like Fusamine and Cis-veramycin E were identified as both inhibitors and substrates, which could complicate dosing strategies and require further studies to optimize their potential negative effects. Total clearance, expressed as mL/min/kg, was used to estimate the rate of elimination of a compound from the body via renal and hepatic routes. Clearance affects dosing frequency, therapeutic window, and potential for drug accumulation. According to Xiong et al., a good clearance value would be between 5 and 15 mL/min/kg, as these are considered moderate [50]. The 9 compounds exhibited a wide range of predicted clearance values, but all fell within the aforementioned range. Raistrickione E showed the highest clearance relative to the other compounds, suggesting it may be eliminated quickly and require more frequent administration. Ralfuranone J showed the lowest clearance, potentially increasing its systemic retention compared with the other candidates. Compounds with moderate clearance are generally ideal, as they remain in systemic circulation long enough to be effective without overaccumulating or causing toxicity [26, 50]. For toxicity predictions, Ames mutagenicity is a common in silico test for DNA damage and carcinogenic potential [51]. Among the 9 candidates, only Solanapyrone E was classified as "Toxic", raising concerns regarding its safety in therapeutic contexts. All other compounds were labeled "Safe", indicating a low risk of genotoxicity.

3.2 Cis-veramycin E (NPA033420) and Solanapyrone E (NPA010954) are lead compounds.

Among the 9 candidates, MD simulations could not be performed for EQ-4 and Raistrickione E. Based on the LINCS algorithm, they were too sterically strained, with bonds rotating by more than 30 degrees during simulation. The RMSD values for the seven remaining candidates over the 5-ns production time were plotted as a function of time in Figure 1. Among the candidates, Berkeleyamide A, Fusamine, Nannoizinone A, and Aspergulfuranone B exhibited consistent RMSD values of 4.8–4.9 nm throughout the simulation. Ralfuranone J exhibited a low RMSD value of 0.43–0.52 nm, but with severe spikes around 4–5 nm throughout the simulation. Solanapyrone E and Cis-veramycin E had the lowest stable RMSD values, all stabilizing below 1.0 nm.

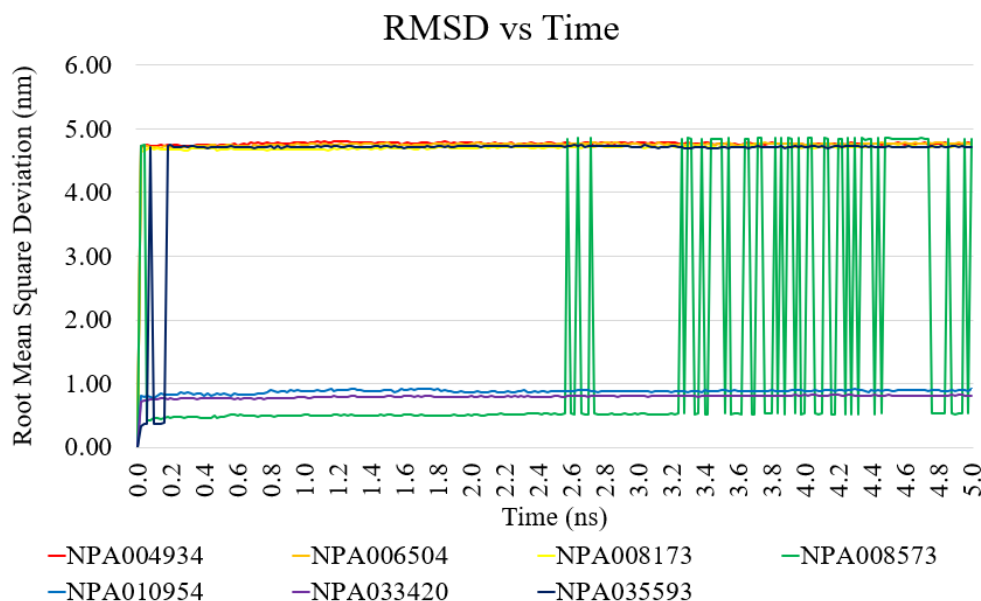


Figure 1. Root Mean Square Deviation (RMSD, nm) of the protein backbone $\text{C}\alpha$ atoms as a function of simulation time (ns) over a 5 ns in vacuo molecular dynamics simulation for each of the seven candidate protein–ligand complexes. Lower and more stable RMSD values throughout the simulation indicate greater structural stability of the complex. Compounds with RMSD values consistently below 1.0 nm are considered stable, while those with values in the 4–5 nm range indicate significant structural deviation and were deprioritized.”

High RMSD suggests considerable movement or structural deviations in the ligand relative to the binding site, or larger possible conformational changes in the LXR- β structure. These may indicate instability in the binding. However, large deviations could also be due to induced fit of the ligand and warrant further study by longer simulations [52].

As such, compounds exhibiting RMSD values in the 4–5 nm range (Berkeleyamide A, Fusamine, Nannoizinone A, and Aspergilluranone B) are not considered acceptable leads in this study. The high RMSD values serve as the primary quantitative criterion for their deprioritization and exclusion from the final lead set. These high RMSD values, particularly in the context of vacuum MD simulations, where solvent absence may amplify protein conformational drift, indicate that these complexes do not maintain stable binding configurations over the 5-ns timescale and therefore cannot be confidently proposed as LXR- β binders [53]. In contrast, the complexes containing Solanapyrone E and Cis-veramycin E showed low and stable RMSD values, fluctuating around 0.5–0.9 nm, suggesting that these ligands do not significantly alter the protein structure, indicating stable binding [54]. Ralfuranone J showed an observably low RMSD but pronounced, frequent spikes throughout the simulation. This may indicate unstable or transient ligand–receptor binding, making it an unreliable candidate. Interestingly, almost all of the candidates equilibrated quickly, with RMSD values stabilizing within 300 to 500 picoseconds of the simulation. This suggests that the protein–ligand complex can achieve stable conformations quickly, supporting efficient agonist activity, which is desirable for activating the LXR- β pathway. However, RMSD analysis does not fully capture long-term conformational adaptations or possible unbinding events [34].

RMSF values for the different ligands bound to LXR- β are shown in Figures 2 and 3. Cis-veramycin E (NPA033420) and Solanapyrone E (NPA010954) exhibited the lowest overall fluctuations and the lowest values, suggesting stability of the protein backbone, which is beneficial in the context of therapeutic targeting [55]. This aligns with their favorable docking scores and supports their potential as LXR- β agonist candidates. Berkeleyamide A (NPA004934) and Nannoizinone A (NPA008173) exhibited moderate RMSF profiles with peaks at around 0.5 nm. This indicates that while the ligand can support binding, it may have weaker contact that may not necessarily compromise binding stability, but should be subject to further MD testing [56].

Fusamine (NPA006504) displayed increased RMSF values, particularly with peaks hovering near the 0.6 nm range. This could imply looser binding or induced strain in receptor regions. Aspergilfurane B (NPA035593) showed even more pronounced RMSF peaks (>1.2 nm) and sustained high flexibility across both chains, which may reflect suboptimal stabilization and could impair consistent receptor activation [57]. Ralfuranone J (NPA008573) exhibited greater fluctuations, exceeding 3.0 nm, indicating excessive flexibility. This suggests structural instability or unfavorable binding interactions, making it a weaker candidate for LXR- β agonist activity. Overall, RMSF values were consistent with expected structural behavior, with the highest values observed at the N- and C-terminal regions across all systems, reflecting their inherent flexibility [55].

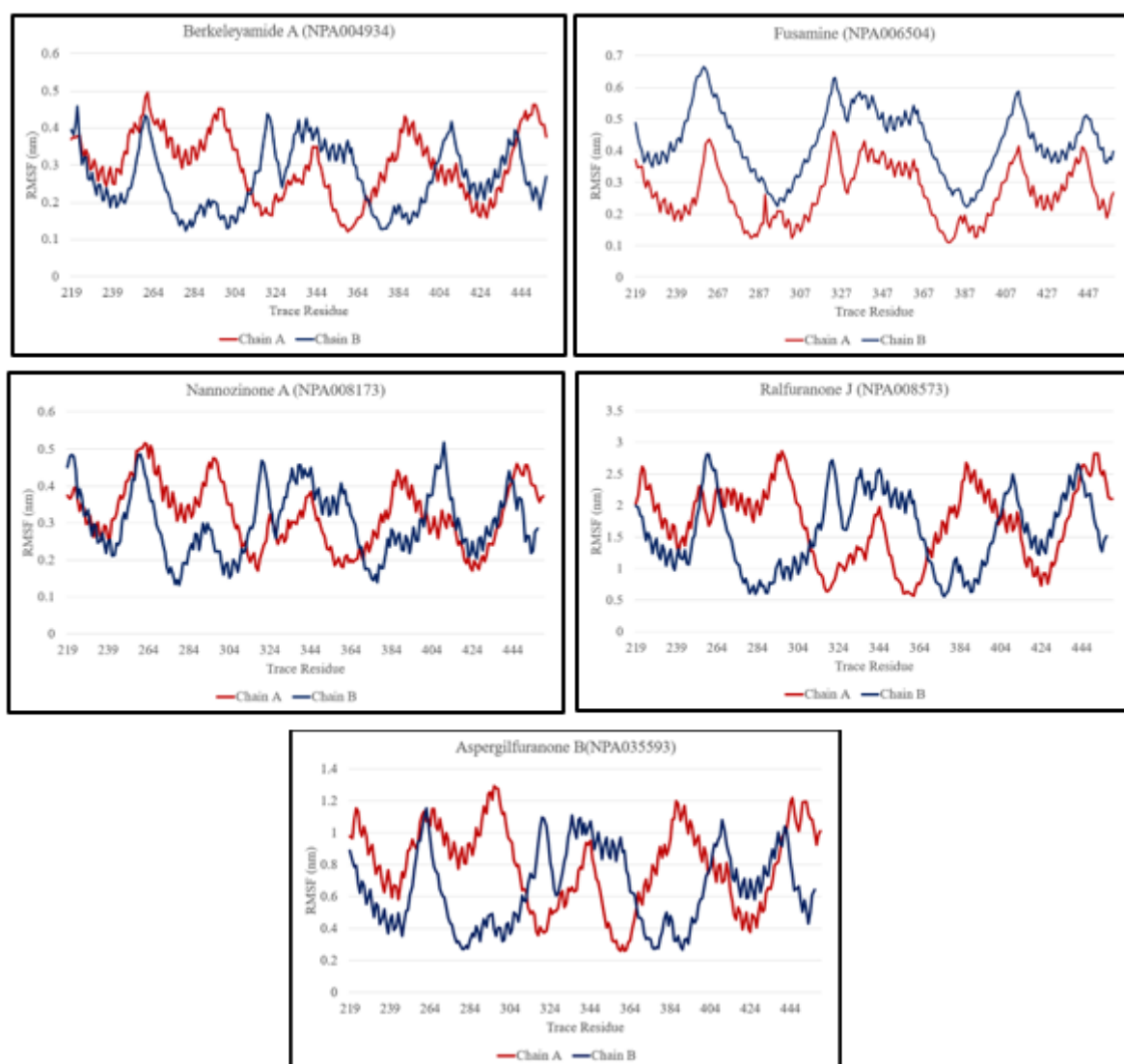


Figure 2. Root Mean Square Fluctuation (RMSF, nm) of the protein backbone C α atoms per residue for LXR- β in complex with Berkeleyamide A, Fusamine, Nannozone A, Ralfuranone J, and Aspergilfurane B during a 5 ns vacuum molecular dynamics simulation. Moderate per-residue fluctuations and elevated RMSF values across chains A and B indicate increased local flexibility within the protein–ligand complexes.

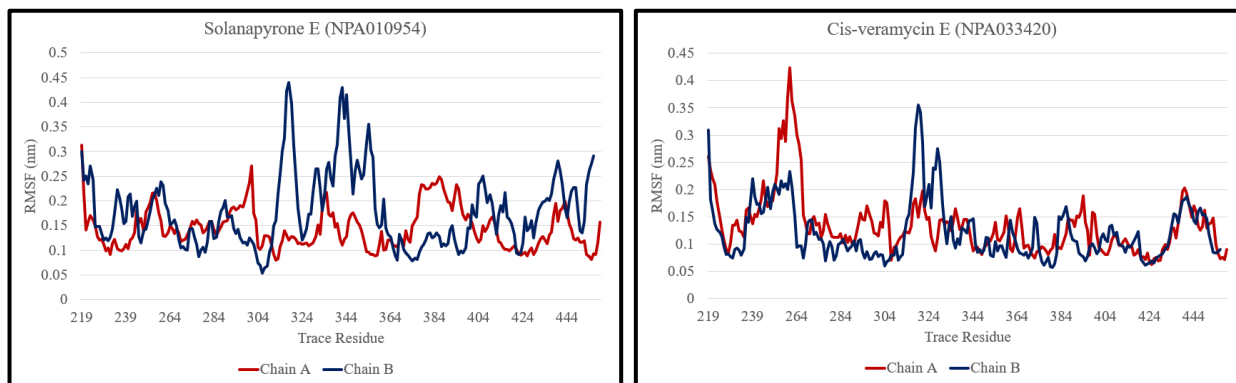


Figure 3. Root Mean Square Fluctuation (RMSF, nm) of the protein backbone C α atoms per residue for LXR- β in complex with Solanapyrone E and Cis-veramycin E during a 5 ns in vacuo molecular dynamics simulation. Lower per-residue fluctuations across chains A and B indicate greater structural rigidity and stability of the protein–ligand complexes.

A summary of the molecular interactions for Cis-veramycin E (NPA033420) and Solanapyrone E (NPA010954) within the ligand-binding domain (LBD) of LXR- β is presented in Table 3. Within the LBD, Cis-veramycin E established a single hydrogen bond involving the backbone nitrogen of Ser278 at a distance of 4.06 Å, complemented by a parallel π – π stacking interaction with Phe340 (distance: 4.16 Å; angle: 2.14°). Furthermore, ten hydrophobic contacts were observed with residues Phe271, Ala275, Leu313, Thr316, Phe340, Leu345, Ile350, Ile353 (two interactions), and Phe354, which were primarily localized around the hydrophobic core. Although no formal PLIP interactions were assigned, mechanistically significant residues His435 and Trp457 were located in proximity at 5.8 Å and 6.3 Å, respectively. In contrast, Solanapyrone E (NPA010954) displayed a unique interaction profile within the same pocket, characterized by the absence of classical hydrogen bonds. Instead, it formed a salt bridge with His435 (distance: 4.73 Å) through its carboxylate moiety, alongside a parallel π – π stack with Phe340 (distance: 3.94 Å; angle: 7.49°). It also maintained nine hydrophobic contacts with Ala275, Thr316, Phe329, Leu345 (two interactions), Phe349, Ile350, and Ile353 (two interactions). The ionic engagement of His435 is highly significant, as this residue serves as a pivotal anchor for the His435–Trp457 switch that stabilizes helix 12 (AF-2) into its active state [32]. Both lead compounds exhibited convergent occupation of the LXR- β hydrophobic core, sharing interactions with Ala275, Thr316, Leu345, Ile350, and Ile353, while utilizing Phe340 for π – π stacking. These interaction profiles align with the binding stability confirmed via MD simulations, providing evidence for their roles as LXR- β ligands.

Table 3. Protein–ligand interactions of Cis-veramycin E and Solanapyrone E with the ligand-binding domain of liver X receptor beta (LXR- β)

Compound	Interaction Type	Residues	Distance (Å)
Solanapyrone E (NPA010954)	Salt Bridge	His435	4.73
	π – π stacking (parallel)	Phe340	3.94 (centroid)
	Hydrophobic contacts	Ala275, Thr316, Phe329, Leu345, Phe349, Ile350, Ile353	3.23–3.80
Cis-veramycin E (NPA033420)	Hydrogen bond	Ser278	4.06 (D–A)
	π – π stacking (parallel)	Phe340	4.16 (centroid)
	Hydrophobic contacts	Phe271, Ala275, Leu313, Thr316, Phe340, Leu345, Ile350, Ile353, Phe354	3.34–3.81

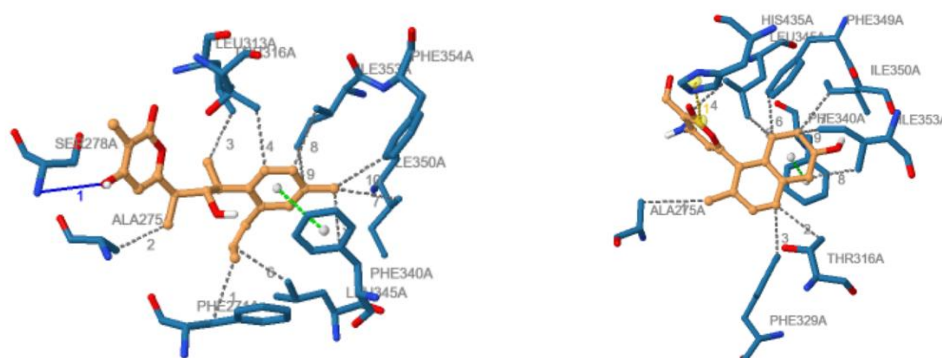


Figure 4. Protein–ligand interaction profiles of Cis-veramycin E (left) and Solanapyrone E (right) in complex with the ligand-binding domain of liver X receptor beta (LXR- β)

The binding profiles of Cis-veramycin E and Solanapyrone E can be meaningfully contextualized with those of well-characterized synthetic LXR agonists such as T0901317 and GW3965, which have served as the primary pharmacological tools for validating LXR- β as an anticancer target. T0901317 has demonstrated anti-cancer activity across multiple tumor types, including inhibition of lung cancer cell migration and invasion through suppression of the ERK/MAPK signaling pathway and downregulation of MMP9 [58]. At the same time, GW3965 has shown therapeutic benefit in preclinical models through ABCA1-mediated apoE lipidation and cholesterol efflux [59]. However, both agonists are limited clinically by hepatic fat accumulation and hypertriglyceridemia arising from upregulation of SREBP-1c and downstream lipogenic genes [60]. Crystallographically, both reference agonists stabilize the LXR- β AF-2 helix through the His435–Trp457 electrostatic switch and engage a shared hydrophobic core — including Phe340, Leu345, Ile350, and Ile353 [61, 62]. The lead compounds in this study show convergent binding features: Solanapyrone E directly engages His435 via a salt bridge (4.73 Å) with Trp457 in proximity (3.7 Å), while Cis-veramycin E engages the same hydrophobic core residues through π – π stacking with Phe340 and contacts with Leu345, Ile350, and Ile353.

Apart from their shared structural characteristics with established agonists, the distinct interaction profiles of these lead candidates offer insights into the mode of activation of LXR- β and its subsequent anti-tumorigenic properties. For Solanapyrone E, the formation of a direct salt bridge with His435 (4.73 Å) aligns with the structural requirements for the His435–Trp457 electrostatic switch that stabilizes helix 12 into its active state to facilitate co-activator recruitment and downstream transcriptional regulation [62]. Although Cis-veramycin E engages His435 more distally at 5.8 Å, its array of hydroxyl and carbonyl groups supports hydrogen bonding while maintaining essential contacts with the hydrophobic core, which have been implicated in the indirect stabilization of the AF-2 domain for diverse LXR- β ligands [63]. Successful LXR- β activation by these compounds is anticipated to modulate several key regulatory axes, including the upregulation of ABCA1, ABCG1, and ApoE for enhanced cholesterol efflux, the inhibition of Wnt/ β -catenin-driven expression of MYC and MMP-7, and the induction of programmed cell death via p27-dependent cell cycle arrest and AKT signaling suppression [5, 9, 14, 15]. Such pathways highlight the recognized mechanism for LXR- β -targeted oncology. As microbially derived natural products with favorable ADMET profiles, both leads share key features with established LXR- β agonists while potentially offering an improved safety window. Thus, further validation through LXR- β assays will be essential to determine if the structural stability predicted in this *in silico* study correlates with functional biological activity.

4. Conclusions

This study performed a large-scale *in silico* screen of 36,310 microbially derived natural products from NPAtlas against LXR- β , a nuclear receptor preferentially targeted over LXR- α due to its ubiquitous tissue distribution and reduced hepatotoxicity risk. Using a multi-stage pipeline (molecular docking against PDB ID 6S4U (2.81 Å), ADMET profiling via Deep-PK, and 5-ns *in vacuo* MD simulations), nine compounds passed

initial screening, of which seven were validated by MD simulation. Cis-veramycin E (NPA033420) emerged as the top lead, with a docking score of -9.31 kcal/mol, stable RMSD below 1.0 nm, minimal RMSF below 0.3 nm, a favorable ADMET profile, and a binding mode similar to established LXR- β agonists. Solanapyrone E (NPA010954) is a secondary lead with strong binding stability and direct engagement of the His435–Trp457 electrostatic switch, but requires structural optimization to address Ames mutagenicity.

Key limitations include simulations performed in a vacuum and a short 5-ns timescale, both adopted to maintain computational efficiency consistent with prior virtual screening studies. Future work should prioritize solvated extended MD simulations (>100 ns), *in vitro* LXR- β binding experimentation, and structural optimization of Solanapyrone E to eliminate genotoxic liability. Overall, our findings suggest Cis-veramycin E (NPA033420) and Solanapyrone E (NPA010954) are promising lead candidates for future *in vitro* and *in vivo* studies targeting LXR- β -mediated anti-cancer pathways.

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