RESEARCH ARTICLE

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The Inhibition Mechanism of Pancreatic Ductal Adenocarcinoma via LXR Receptors: A Multifaceted Approach Integrating Molecular Docking, Molecular Dynamics and Post-MD Inter-Molecular Contact Analysis

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Abstract

Objective: Pancreatic ductal adenocarcinoma (PDAC) has an unfavorable outlook due to its aggressive characteristics, delayed diagnosis, and limited effective treatment options for advanced stages of the disease. The significant mortality rate has prompted investigations into additional factors that could aid in managing this type of cancer. Liver X receptors, specifically LXRα and LXRβ, are nuclear receptors that oversee the expression of genes related to cholesterol, glucose, lipid metabolism, and inflammatory responses. LXRs have also emerged as potential targets for addressing PDAC, and recent findings have demonstrated that LXR ligands can impede cell proliferation in various cancer forms, notably pancreatic cancer. This comprehensive computational research study involving oncological in silico mechanism discovery explored inhibitory ligands for Liver X receptors (LXRα and LXRβ), which are believed to have prognostic significance in PDAC. **Methods:** The study utilized Baicalein, Beta-Sitosterol, Polydatin ligands in molecular docking and dynamics and post-molecular Hydrogen bonding contact analyses dynamics to characterize receptor inhibition. **Result:** The outcomes suggest that Baicalein exhibits versatile inhibitory effects on both receptors, while Beta-Sitosterol emerges as a highly effective inhibitor of LXRβ. **Conclusion:** Further *in vitro* and *in vivo* investigations will be beneficial and would shed light onto the mechanism to decipher the suppression of PDAC evaluating the potential of Baicalein, Beta-Sitosterol, Polydatin natural ligand compounds.

Keywords: Molecular Dynamics- *in silico* studies- LXR- Baicalein- Beta-Sitosterol- Polydatin

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Introduction

PDAC cancer stands as one of the malignancies associated with elevated mortality and morbidity rates, ranking as the eighth leading cause of cancer-related fatalities on a global scale (Siegel et al., 2014). PDAC, characterized by its aggressive behavior, delayed detection, and inadequate therapeutic solutions for advanced stages, carries an unfavorable prognosis. The significant mortality rates have prompted investigations into supplementary factors that could aid in its treatment. Adipose tissue serves as an endocrine organ, synthesizing and secreting a range of factors with diverse effects (Wozniak et al., 2009). Recently, the focus has shifted to the role of adipocytokines originating from visceral adipose tissue, such as tumor necrosis factor (TNF)-alpha, interleukin (IL-6), type 1 plasminogen activator inhibitor, hepatocyte growth factor, Adiponectin, Leptin, Resistin, Visfatin, and Apelin, in the development of metabolic syndrome and carcinogenesis (Booth et al., 2015).

Recent progress in the field of cancer metabolism has unveiled numerous potential targets for therapeutic intervention in cancer treatment. Metabolic reprogramming, an adaptive strategy employed by cancer cells to support rapid growth, has emerged as a novel hallmark of cancer (Ward & Thompson, 2012). In-depth investigations have demonstrated heightened glycolysis, glutaminolysis, nucleotide synthesis, and lipid production within cancer cells (Son et al., 2013).

Functioning as a regulator of cholesterol levels, the nuclear receptor known as liver X receptor plays a significant role in maintaining cholesterol homeostasis (Guo et al., 2018). LXRs, specifically LXRα and LXRβ, are nuclear receptors responsible for governing the transcription of genes associated with cholesterol, glucose, lipid metabolism, and inflammatory reactions (Jakobsson et al., 2012). These LXRs have also gained attention as potential targets for PDAC, with LXR ligands

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demonstrating anti-proliferative effects across various cancer types, particularly in pancreatic cancer (Candelaria et al., 2014; Lin et al., 2016). The assessment of Farnesoid X receptors (FXR) receptor expression in neoplastic hepatic abnormalities aims to harness the diagnostic potential while also uncovering noteworthy findings that could yield valuable insights into the development of hepatocellular carcinoma. FXR expression found to be a downregulater in hepatocellular carcinomacases where it was highly expressed in all non-neoplastic hepatic lesions with a highly significant differences. Liver's X receptors, and have inhibiting LXR activity has been observed to curb cell proliferation and enhance responsiveness to chemotherapy. (Salama et al., 2023; Srivastava et al., 2020).

Baicalein, a notable flavonoid sourced from the roots of *Scutellaria baicalensis*, holds a prominent place in traditional Chinese medicine, often incorporated into herbal tea formulations. Its diverse biological effects, encompassing antioxidant, anti-inflammatory, anti-hepatotoxic, antiviral, and anti-tumor attributes, have prompted thorough investigation (Chandrashekar & Pandi, 2022). Mechanistically, it has been implicated in impacting cell proliferation, metastasis, apoptosis, and autophagy. The scrutiny of baicalein's potential spans a range of cancers, including breast, lung, hepatocellular, and colorectal cancer (Bie et al., 2017; Chen et al., 2021; Yan et al., 2018; Yu et al., 2017).

In a convergent perspective, Ma et al. have highlighted the pivotal role of baicalein in suppressing pancreatic tumorigenesis, both in vitro and in vivo for PDAC (Ma et al., 2021). Their study underscores baicalein's potency as a pancreatic cancer inhibitor, achieved through apoptosis modulation by regulating miR-139-3p or miR-196b-5p expression. Exploration into Baicalein's effects on PDAC has suggested its involvement in the modulation of the sonic Hedgehog pathway, inhibition of Akt and ERK signaling pathways, induction of S-phase cell cycle arrest, hindrance of cell migration and invasion, activation of caspases, and provocation of mitochondrial-dependent apoptosis (Song et al., 2018; Zhang et al., 2020; Zhou et al., 2017). In our investigation, we propose that one of these mechanisms could potentially involve the inhibition of LXRα and LXRβ, pivotal players in cholesterol metabolism.

β-sitosterol is a compound present in a diverse range of plant sources, including vegetable oils, nuts, and avocados. Its influence extends across various cellular signaling pathways, encompassing activities such as regulating the cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, inflammation, and exhibiting effects that are anticancer, antioxidant, hepatoprotective, cardioprotective, and antidiabetic (Khan et al., 2022). The anticancer potential of β-sitosterol has been demonstrated in diverse cancer types, including fibrosarcoma (Srivastava et al., 2020), colon (Chandrashekar & Pandi, 2022; Lin et al., 2016), breast (Yan et al., 2018; Yu et al., 2017), and prostate cancer (A. Awad et al., 2003; A. B. Awad et al., 2005; Bie et al., 2017; Choi et al., 2003; Moon et al., 2007).

Numerous investigations have linked its anticancer effects to apoptosis induction through the interception of multiple cell signaling mechanisms (Bin Sayeed & Ameen, 2015). Notably, Cao et al. discovered that combining β-sitosterol with gemcitabine resulted in notable growth inhibition of PC xenografts (Cao et al., 2019). Despite being relatively underexplored in the context of PDAC, variants like liposomal forms of β-sitosterol could hold promise for efficacy in PDAC.

Polydatin, a stilbenoid component derived from *Polygonum cuspidatum* (Polygonaceae), has a well-established role in traditional Chinese medicine. Its multifaceted activities span antioxidant, anti-inflammatory, anticancer, hepatoprotective, neuroprotective, and immunostimulatory effects (Shah et al., 2022). Polydatin's anticancer prowess is attributed to several mechanisms, encompassing control of reactive oxygen species (ROS) and suppression of the PI3K/AKT pathway (Frisch et al., 2016; Pan et al., 2017; Quan et al., 2010). Although its influence on PDAC remains relatively uncharted, its efficacy within PDAC cell lines has been demonstrated (Boukes & Van De Venter, 2016).

In the area of medicinal/pharmaceutical chemistry, the investigation of binding affinities holds paramount importance, often involving the synthesis and evaluation of numerous drug candidates. The potential for computational approaches to rapidly and dependably ascertain binding affinities is of considerable interest. We hypothesize that Baicalein, β-sitosterol, and Polydatin possess substantial potential as novel PDAC treatment options.

This comprehensive research delves into the detailed exploration of LXRα and LXRβ inhibitory ligands—Baicalein, β-Sitosterol, and Polydatin—anticipated to bear prognostic significance in PDAC. Employing an array of in silico methodologies, including molecular docking, molecular dynamics, and post-molecular dynamics characterizations, the study elucidates that Baicalein and β-Sitosterol hold promise as natural agents for pancreatic cancer treatment.

Materials and Methods

Geometric Optimization

To comprehend the active sites of a molecule and explore its interactions with enzymes, achieving precise geometric optimization is pivotal. In our ongoing investigation, identification of suitable ligands for inhibiting LXRα and LXRβ was guided by existing scientific literature. These ligands, alongside their most stable molecular geometries, underwent processing using the Gaussian 09 program (Frisch et al., 2016), employing density functional theory (DFT)/B3LYP functional (Becke, 1992) and the 6-31G(d,p) principle. The resulting most stable molecular structures of Baicalein, β-Sitosterol, and Polydatin were selected for further computational and simulation research (depicted in Figure 1). Input files for DFT calculations and post-processing of output files were prepared using Gauss View 5.0 and Avogadro software programs (Dennington et al., 2019).

Molecular Docking Simulations

Employing AutoDock Vina 1.1.2, molecular docking simulations were conducted with 100 poses, yielding a

total of 1200 poses. Ligands Baicalein, β-Sitosterol, and Polydatin were obtained from the Zinc Database (with IDs 3871633, 4095717, and 4098633, respectively), optimized through Gaussview and Avogadro. These simulations illustrated interactions and bindings of the drugs with receptor fragments of LRX-α and LRX-β, utilizing AF-C9JTS4-F1 and AF-MOR3A7-F1 IDs from the AlphaFold Database. Docking scores were expressed in kcal/mol, representing Gibbs free binding energy. Optimal binding energy poses within the best-clustered data were selected as initial structures for subsequent Molecular Dynamics (MD) simulations for each drug.

Molecular Dynamics (MD) Simulations

Ligands underwent Molecular Dynamics (MD) simulations using the Desmond Program (Bergdorf et al., 2016), with 50 ns intervals, each comprising 5000 poses at 10 ps intervals. The MD simulations were executed thrice with distinct seed numbers to ensure accurate simulation parameters and protein-bound ligand complex structures (Cheraghi et al., 2023; Şenel et al., 2020, 2022). The study evaluated the dynamic traits of the ligand-receptor complexes over time. The system's grid box was established at 110 × 110 × 110 $Å^3$ with a 0.5 Å spacing for protein receptors. TIP3P-type water molecules were added, along with 0.15 M NaCl ions to neutralize the system. Initial structures for MD simulations were derived from the most favorably binding docking poses. Temperature and pressure parameters included NPT at 310 K with Nose-Hoover temperature coupling (Li et al., 1998) and constant pressure of 1.01 bar via Martyna Tobias−Klein pressure coupling (Evans & Holian, 1985). No system constraints were imposed, and default initial velocity values for forcefield calculations were used. The study encompassed MD simulations of various drug-receptor complexes and non-ligand-bound protein fragments. Trajectory analyses yielded RMSD curves, depicting the drug-receptor complex dynamics. Interaction dynamics between drug variants and receptors, including hydrogen bonding with amino acids of LRX-α and LRX-β chains, were investigated based on these curves.

Results

Molecular Docking/Dynamics Simulations and Post-Molecular Dynamics Characterizations

The Post-MD Evaluations

Table 1 and Figure 1 yield some valuable data regarding the affinities and regioselective tendencies of the compounds before going into further results of MD poses that will be mentioned later.

One can easily observe that Baicalein's chemical structure is the best fit for suppressing and inhibiting both the LRX-α and LRX-β since its inhibition constant drops to 1.0 μM levels for LRX-β and it possesses quite stabilized complex structure in the RMSD post-MD graphic as illustrated. This makes Baicalein the fittest structure in terms of versatility among all of the compounds.

In the case of the Beta-Sitosterol - LRX-β complex, Beta-Sitosterol reaches a very high intercalation MD binding score of -15.7 kcal/mol, where it shuts down the active site of LRX-β completely, making it the best drug of interest for LRX-β, inhibiting the receptor protein with high precision and efficiency. However, this cannot be said for its binding efficiency for LRX-α. For its efficient binding on LRX-β, it should be stated that its inhibitory concentration is quite low (around 7.4 μM) as well proving its ability to suppress the protein efficiently once more while maintaining a very significant intercalation feature causing being embedded into the active site.

Polydatin is observed to possess the least affinity and efficiency in the inhibition role of the proteins due to its

Table 1. The Post-MD Characterizations of Drug-Receptor Complexes.

Compound	Molecular Dynamics Ligand-Receptor Binding Data	
	Binding Energy Δ(ΔG) kcal/mol	Inhibition Constant (μM)
Baicalein - LRX-α	-13.8	178.9
Beta-Sitosterol - LRX-α	-13.0	277.9
Polydatin - LRX-α	-13.9	164.7
Baicalein - LRX-β	-12.6	1.0
Beta-Sitosterol - LRX-β	-15.7	164.7
Polydatin - LRX-β	-11.9	498.5
Beta-Sitosterol - LRX-β	-15.7	7.4
Polydatin - LRX-β	-11.9	498.5

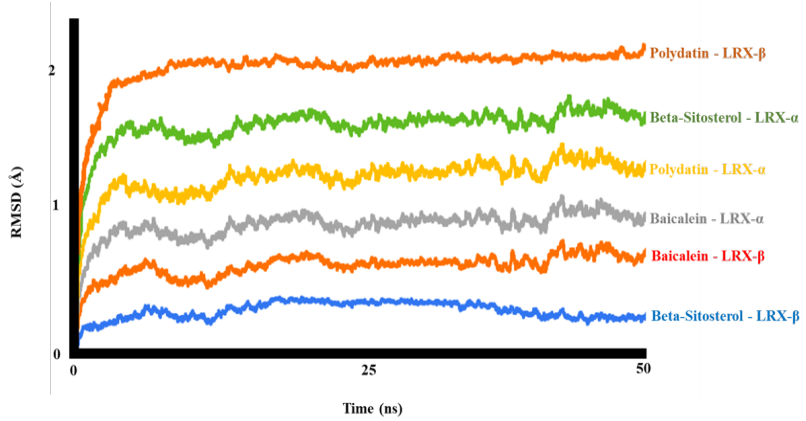

Figure 1. The Post-MD Analysis under OPLS 3.0 Forcefield for 50 ns RMSD Plot of All Drugs with the Receptors.

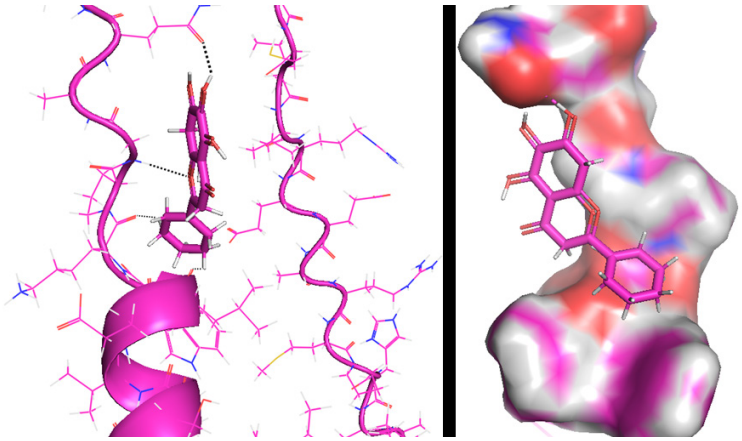

Figure 2. The Best Stabilized MD Poses of Baicalein-LRX-α Complex in Schrodinger's Desmond under OPLS 3.0 Forcefield

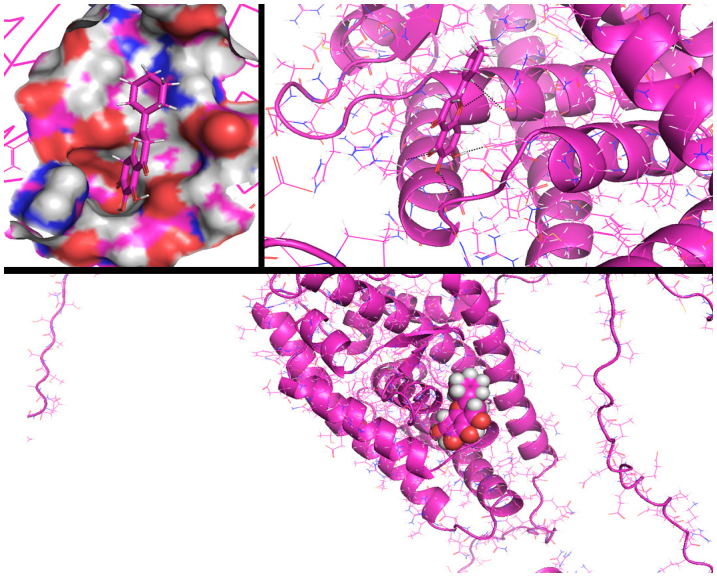

Figure 3. The Best Stabilized MD Poses of Baicalein-LRX-β in Schrodinger's Desmond under OPLS 3.0 Forcefield

bulky chemical structure causing high inhibition constant which means that the efficiency of true inhibitory binding is worse than the others.

The MD Results of Baicalein

As illustrated in Figure 2, the MD pose after the 2,500th frame where the complex stabilization occurs can be observed. The MD results of Baicalein vindicate what was told in its molecular docking binding to the amino acids of LRX-α. It has strong Hydrogen bonds where its Δ(ΔG) binding score converges around -13.8 kcal/mol which is a clear indication that protein inhibitions are regarded as true suppression if only it achieves to pass -10 to -11 kcal/mol as well known in the scientific literature.

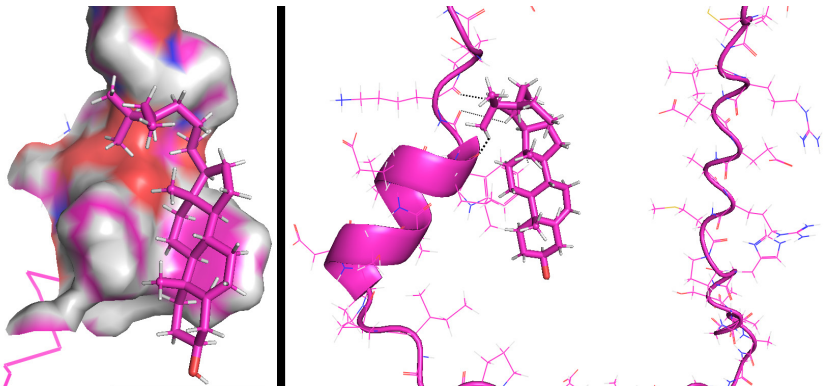

Figure 4. The Best Stabilized MD Poses of Beta-Sitosterol-LRX-α Complex in Schrodinger's Desmond under OPLS 3.0 Forcefield.

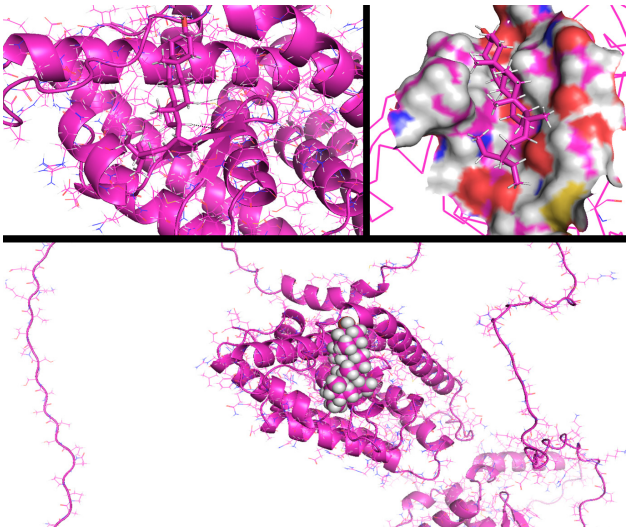

Figure 5. The Best Stabilized MD Poses of Beta-Sitosterol-LRX-β in Schrodinger's Desmond under OPLS 3.0 Forcefield.

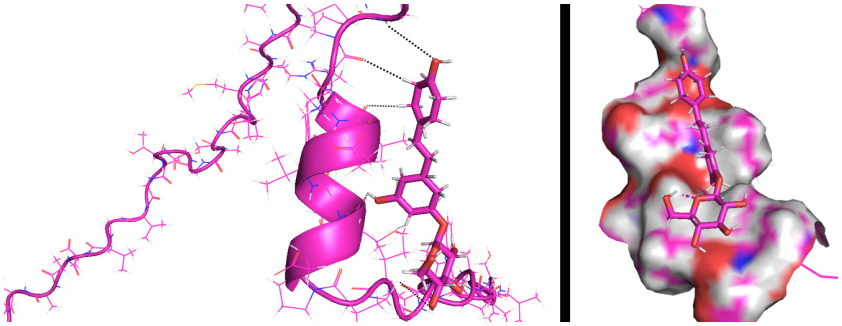

Figure 6. The Best Stabilized MD Poses of Polydatin-LRX-α complex in Schrodinger's Desmond under OPLS 3.0 Forcefield.

Therefore, inhibition from the active site amino acids was observed in the MD frames. The drug Baicalein fits the active site of the protein to effectively inhibit its activity, exhibiting a versatile possible drug potential for both of the proteins as can be seen from its stabilized complex structures mentioned in Figure 1.

In Figure 3, one can easily observe how effective the inhibition of the alpha helices is since the inhibition constant value is 1.0 μM and the MD frames of the stabilized complex illustrate the intercalation type embedding of the drug into the grooves of alpha helices. The whole charge distribution and the 3D morphology of

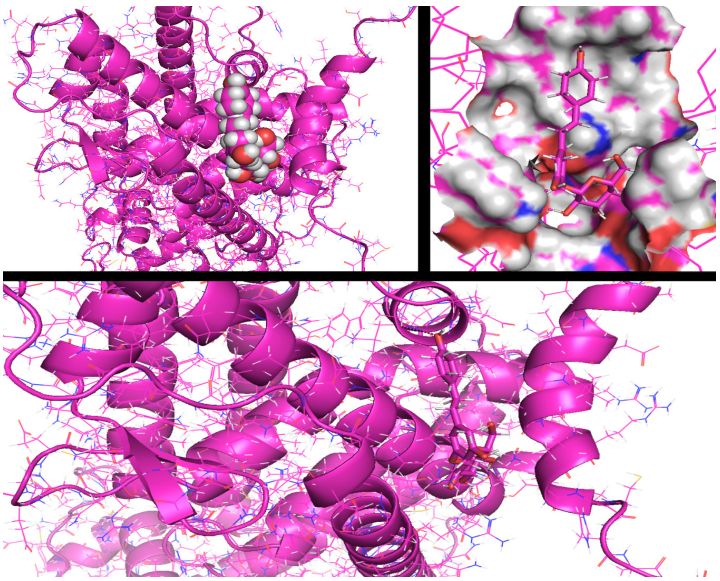

Figure 7. The Best Stabilized MD Poses of Polydatin-LRX-β in Schrodinger's Desmond under OPLS 3.0 Forcefield.

the alpha helices alter drastically, meaning that there is a very strong inhibition in the protein through its active site.

The MD Results of Beta-Sitosterol

The MD results of Beta-Sitosterol, illustrated in Table 1, Figures 1, 4, and 5, revealed an intriguing fact regarding its mechanism and indication efficiency. Although it seemed to be a lengthy molecule compared to Baicalein which is a pretty significant issue in terms of steric hindrance according to the laws of organic chemistry and pharmaceutical chemistry, the polar charge distribution within its chemical structure due to a polar oxygen at the tail domain makes it a more suitable inhibitory drug for LRX-β compared to LRX-α. Its significantly high Δ(ΔG) binding scores (-13.0 and -15.7 kcal/mol) to these proteins respectively with a polar molecule dipole effect most probably cause a highly stabilized structure within a bulky medium of the active site in the LRX-β. However, since there is more space for the drug in LRX-α compared to LRX-β, the torsional and rotational free energies cause less binding energy to the structure of protein and less inhibition effect by increasing its inhibition constant from 7.4 to 277.9 μM.

The RMSD results of Beta-Sitosterol once more prove this fact where the drug-receptor complex is quite stable for the case of LRX-β proving that it is the right structure for the protein inhibition where the drug positions and aligns itself into the active site groove of amino acids aforementioned in the molecular dockings part of this paper. However, in the case of LRX-α, it has a reduced indication efficiency due to less binding energy, less alpha-helix morphology inhibition, and higher inhibition constant compared to the dramatically successful affinity of Beta-Sitosterol to LRX-β.

The MD Results of Polydatin

According to Figures 6 and 7, although the binding energies of Polydatin to the proteins have quite significant values (-13.9 and -11.9 kcal/mol), the simulated medium yields the data of higher binding constants of 164.7 and 498.5 μM for LRX-α and LRX-β which are not as efficient as the aforementioned drugs but certainly does not mean that it cannot be a drug of choice. It certainly follows what molecular docking results have found in the MD results as well in terms of amino acid regioselectivity and thus positions itself into the active site of these proteins correctly.

Discussion

In concusion, the current study elucidates the molecular structural and electrostatic attributes of Baicalein, Beta-sitosterol, and Polydatin molecules, computed using the DFT/B3LYP/6-31G(d,p) theoretical approach. The objective of this investigation was to comprehensively understand the interaction mechanisms between Baicalein, Beta-sitosterol, and Polydatin ligands with the LXRα and LXRβ receptors. This was achieved through a combination of molecular docking, molecular dynamics simulations, and subsequent characterization methods, aimed at illuminating the precise binding modes between the ligands and receptors.

The results unveiled Baicalein as the optimal candidate for inhibiting both LXRα and LXRβ molecules, exhibiting highly stable suppression complexes with the receptors. Meanwhile, Beta-sitosterol demonstrated remarkable specificity as a potent inhibitor of LRXβ. While slightly less potent than Baicalein and Beta-sitosterol, Polydatin also exhibited notable potential in restraining both receptors. Depending on these great simulation results, further in vitro and in vivo analyses would definitely shed light onto the suppression of PDAC.

Author Contribution Statement

Soykan Agar: Writing the draft, methodology and in silico simulations; Barbaros Akkurt: Writing the draft, proofreading; Engin Ulukaya: Conceptualization, checking the draft.

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Any conflict of interest

Authors Assistant Professor Soykan AGAR (Ph.D.), Teaching & Research Fellow Barbaros AKKURT (Ph.D.) and Professor Engin Ulukaya (M.D., Ph.D.) declare that there is no conflict of interest for this research paper and its datasets.

How the ethical issue was handled (name the ethical committee that approved the research)

All in silico data was studied and represented with honest work and since it was an in silico study not an experimental study, there was no such need for ethical committee approval for this research paper which is compatible with the laws of national ethical committee.

Availability of data (if apply to your research)

It can be shared with open access in case of journal asks of us.

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