RESEARCH ARTICLE

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The Inhibition of RXRα and RXRβ Receptors Provides Valuable Insights for Potential Prostate Cancer Treatment, *in silico* Molecular Docking and Molecular Dynamics Studies

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Abstract

Introduction: Prostate cancer has emerged as a widespread health concern, with systemic inflammation believed to substantially contribute to its development and progression. The presence of systemic inflammatory responses has been established as an independent predictor of unfavorable long-term outcomes in prostate cancer patients. The goal of this study is to inhibit RXRα and RXRβ receptors, which are involved in prostate cancer, with Luteolin, Formononetin, and Kaempferol, with varying success. **Methods:** Retinoid X receptors (RXRs) hold crucial roles within the nuclear receptor (NR) superfamily, and compelling evidence from preclinical studies underscores the therapeutic potential of targeting RXRs for treating neurodegenerative and inflammatory conditions. Consequently, the ability to regulate and modulate RXRs using phytoestrogen ligands, Formononetin, Kaempferol, and Luteolin, assume paramount importance in treatment strategies. **Results:** The comprehensive in silico findings of this study vividly demonstrate the remarkable efficacy of Luteolin in inhibiting and modulating RXRα and RXRβ, while Formononetin emerges as a notably potent suppressor of RXRβ. Kaempferol, as the third compound, also exhibits commendable inhibitory attributes, although its impact is slightly less pronounced compared to the other two. **Discussion:** These findings highlight the notable binding and inhibition capabilities to RXRα and RXRβ, offering valuable insights for potential prostate cancer treatment avenues warranting further exploration through in vitro and in vivo analyses.

Keywords: Prostate Cancer RXR Receptors- in silico molecular docking and molecular dynamics

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Introduction

Prostate cancer ranks among the prevalent malignancies in men worldwide, with approximately 1,600,000 cases and 366,000 annual fatalities [1]. Androgen deprivation therapy (ADT) typically serves as the primary approach for advanced prostate cancer, yet the majority eventually advance despite ADT, leading to castration-resistant prostate cancer (CRPC). The underlying mechanisms propelling the transition from androgen-dependent (hormone-sensitive or castration-sensitive) prostate cancer to CRPC remain largely unresolved, although it is associated with sustained androgen receptor signaling amidst diminished circulating androgens and androgen receptor blockade.

Systemic inflammation is believed to contribute to prostate cancer initiation and progression [2]. The presence of systemic inflammatory responses has independently indicated an adverse long-term prognosis for prostate cancer patients [3].

Retinoid X receptors (RXRs) are pivotal components of the nuclear receptor (NR) superfamily, wielding diverse roles in regulating various physiological processes. RARs govern the expression of numerous genes overseeing cell proliferation, differentiation, survival, and overall bodily homeostasis. RARs comprise three subtypes—RARα (NR1B1), RARβ (NR1B2), and RARγ (NR1B3)—encoded by distinct genes [4]. RXRα is notably expressed in the liver, kidney, epidermis, and spleen; RXRβ is universally present; and RXRγ, the least abundant of the trio, is prominent in muscular and cerebral tissues.

Considering that RXRα is pivotal for maintaining the suppressive function of T regulatory cells (Tregs), RXR agonists could offer dual benefits as a therapeutic approach for managing inflammatory disorders [5]. Substantial preclinical evidence underscores the therapeutic potential of RXRs as drug targets for treating neurodegenerative and inflammatory ailments. However, the exploration of this therapeutic potential has been hampered by the scarcity of safer RXR ligands. Systemic inflammatory responses have been independently linked to unfavorable long-term outcomes in prostate cancer patients. Hence,

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investigating the modulatory and inhibitory interactions of such natural compounds alongside RXRs assumes paramount significance. To this end, phytoestrogenic compounds Farmononetin, Kaempferol, and Luteolin were selected [6].

This study delves into and researches the comprehensive investigation of ligands that inhibit RXRα and RXRβ receptors, pivotal players in the inflammatory response associated with prostate cancer. Notably, Luteolin emerged as the most versatile and efficacious drug for inhibiting both receptors, potentially paving the way for further in vitro and in vivo studies for prostate cancer treatment.

Materials and Methods

Geometric Optimization

In our ongoing research, the selection of precise and accurate ligands for inhibiting LXR-alpha and LXR-beta was guided by scientific literature. Subsequently, these chosen structures, along with their most stable molecular geometries, underwent processing through the Gaussian 09 program [7], employing density functional theory (DFT)/ B3LYP functional [8,9], and utilizing the 6-31G(d,p) principle. This procedure gathered the most stable molecular configurations of Formononetin, Kaempferol, and Luteolin, paving the way for further computational and simulation-based investigations. For input file preparation of DFT calculations and post-processing of output files, Gauss View 6.0 and Avogadro software tools [10] were employed. The study of small molecule interactions, termed ligands, with macromolecules like DNA and proteins, referred to as receptors, bears relevance to anticancer exploration [11–14]. The initial geometries of Farmononetin, Kaempferol, and Luteolin molecules were sourced from PubChem [12,15,16].

The crystal structure of human RXRα and RXRβ receptors, complexed as targets within the context of inflammatory response in prostate cancer, from the AlphaFold protein database [17] (AF_K4MR37_F1_ model_v4, AF-K4MU26-F1-model_v4) were sourced.

Molecular Docking Simulations

Molecular docking simulations were executed using AutoDock Vina 1.1.2. [18], encompassing 100 posed simulations, culminating in 600 poses. The drugs Formononetin, Kaempferol, and Luteolin were graphically represented in Gaussview, optimized using both Gaussview and Avogadro, and then subjected to simulations, illustrating interactions and bindings with receptor fragments of RXRα and RXRβ, utilizing IDs AF_ K4MR37_F1_model_v4 and AF-K4MU26-F1-model_v4 from the AlphaFold Database. The grid box dimensions were set at 40 × 40 × 40 Å3. The docking scores were quantified in kcal/mol, denoting Gibbs free binding energy. From the best-clustered data, docking poses featuring the most accurate and favorable binding energy were selected as initial structures for subsequent MD simulations for each drug. The Gaussian 09 program was employed to determine the most stable geometric structure of these molecules. For Farmononetin, Kaempferol, and Luteolin molecules, optimized geometries were chosen for insertion analysis utilizing Gaussian 09 software [19–21].

Molecular Dynamics (MD) Simulations

Utilizing Schrödinger's Desmond program [20], MD simulations were conducted for all ligands, involving intervals of 50 ns, each comprising 5000 poses at 10 ps intervals. Each molecular dynamics simulation was repeated three times using different seed numbers to ensure accuracy of simulation parameters and protein-bound ligand complex structures. The aim of MD simulations was to assess dynamic attributes of the ligand-receptor complex over time. The grid box dimensions were set at 120 × 120 × 120 Å3, with a 0.5 Å spacing for protein receptors. TIP3P-type water molecules were introduced into the system, and 0.15 M NaCl ions were incorporated to neutralize the setup. The initial structures for MD simulations were derived from the docking poses exhibiting optimal and favorable binding energy based on docking results [22–24]. Temperature and pressure parameters included NPT at 310 K with Nose-Hoover temperature coupling [25], and constant pressure of 1.01 bar via Martyna Tobias−Klein pressure coupling [26]. The systems had no constraints, and default initial velocity values were employed for forcefield calculations. The study encompassed MD simulations of varying drug-receptor complexes as well as non-ligand bound protein fragments. Investigation of hydrogen bonds formed between drugs and amino acid domains of RXRα and RXRβ chains was also undertaken.

Results

The Molecular Dynamics Analysis and Characterizations
The Molecular Dynamics Characterizations of Formononetin-RXRα and Formononetin-RXRβ Complexes

As depicted in Figures 1, 2, and Table 1, the MD poses captured after the 2500th frame out of the total 5000 frames, where complex stabilization takes place, are observable. Formononetin engages with the external active site of the RXRα protein, forming a robust hydrogen bond at a distance of 1.92 Å, accompanied by a binding energy of -13.3 kcal/mol. Meanwhile, its inhibition constant remains approximately 239.5 μM. This suggests Formononetin's effectiveness as a hydrogen bond binder in suppressing RXRα, with its binding energy of -13.3 kcal/ mol indicating substantial protein inhibition, a recognized hallmark in scientific literature, achieved when values should range minimum of -10 to -11 kcal/mol for excellent inhibition [23, 24, 27].

Formononetin displays heightened efficacy in terms of binding mode and inhibition constant when interacting with RXRβ, featuring distances of 1.79 Å and 1.80 Å. Exhibiting an inhibition constant of 2.5 μM, its binding mechanism showcases the modulation of two α-helices, hinting at a more potent suppressor role. By exerting pressure on the two α-helices, it obstructs and reshapes the active site of RXRβ, leading to complete protein shutdown. This mode of blockage proves notably more efficient.

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

	Molecular Dynamics Ligand-Receptor Binding Data		
Complex	Binding Energy Δ(ΔG) kcal/mol	Inhibition Constant μM	Mode of Inhibition
Formononetin-RXRα	-13.3	239.5	Active site inhibitor
Formononetin-RXRβ	-10	2.5	Double α-helix modulator
Kaempferol-RXRα	-13.2	265.7	Single α-helix modulator via intercalation
Kaempferol-RXRβ	-14.1	53.3	Single α-helix modulator
Luteolin-RXRα	-16	6.3	Double α-helix modulator via intercalation
Luteolin-RXRβ	-15.9	11.4	Single α-helix modulator

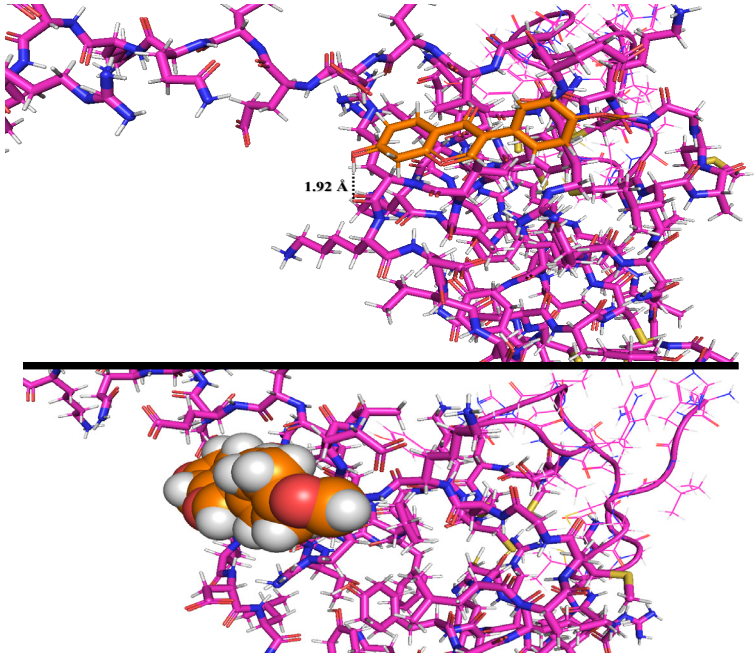

Figure 1. The Best Favored Pose from the Molecular Dynamics Video when the Formononetin-RXRα Complex becomes Stabilized.

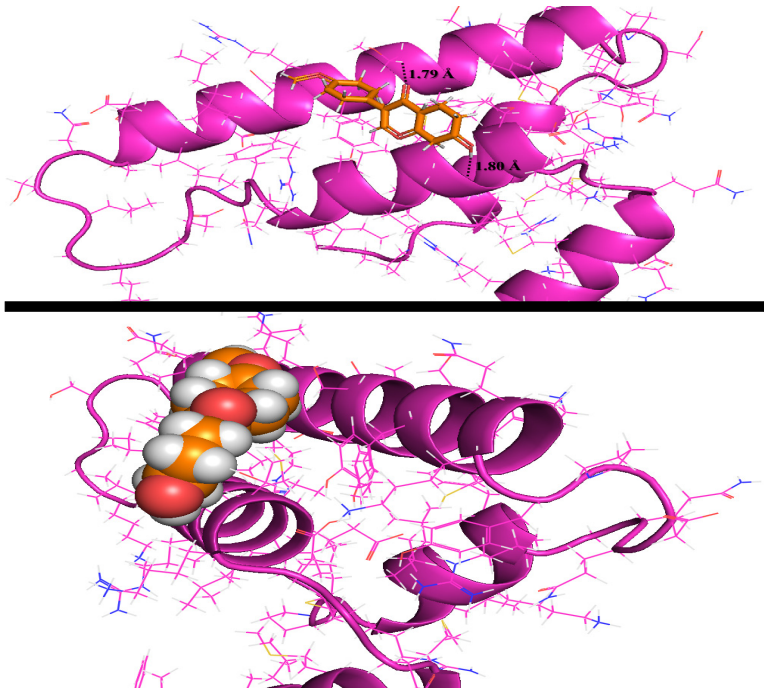

Figure 2. The Best Favored Pose from the Molecular Dynamics Video when the Formononetin-RXRβ Complex becomes Stabilized.

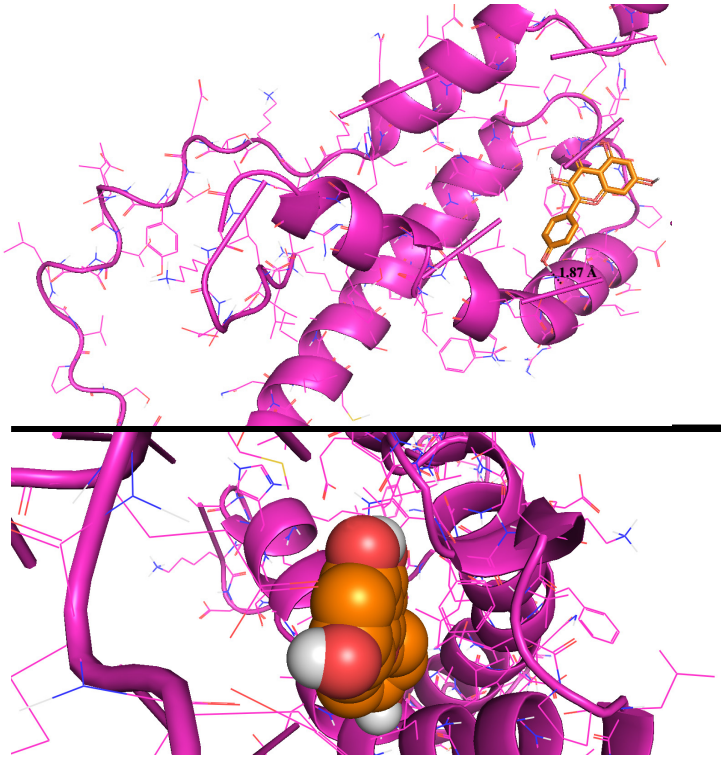

Figure 3. The Best Favored Pose from the Molecular Dynamics Video when the Kaempferol-RXRα Complex becomes Stabilized.

The Molecular Dynamics Characterizations of Kaempferol-RXRα and Kaempferol-RXRβ Complexes

Based on Figures 3, 4, and Table 1, the preferred MD poses are evident after the 2500th frame out of the 5000 frames, reflecting the stabilization of the complexes. When Kaempferol binds to RXRα and RXRβ, it distinctly targets a single α-helix, functioning as a protein modulator that influences the morphology/topology and form.

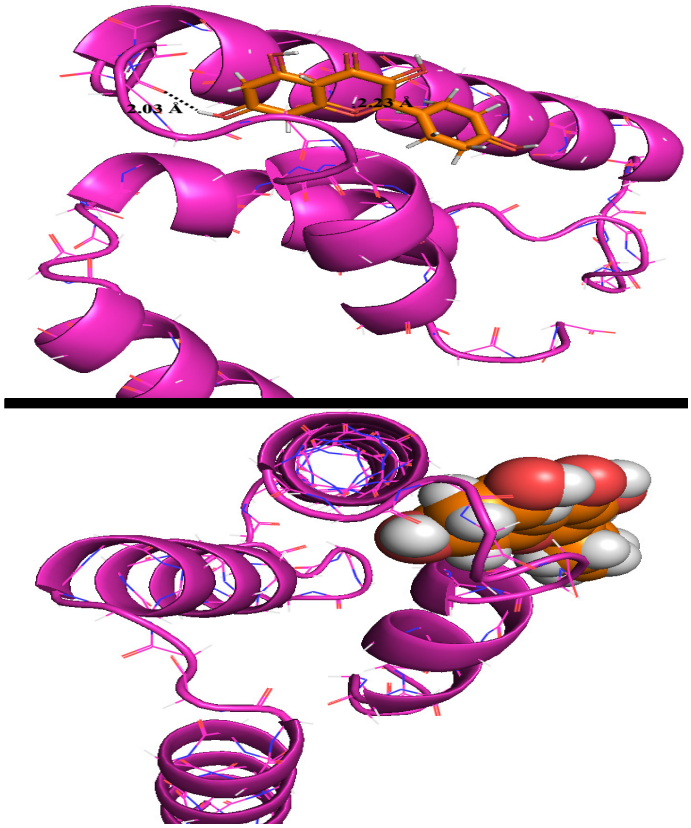

Figure 4. The Best Favored Pose from the Molecular Dynamics Video when the Kaempferol-RXRβ Complex becomes Stabilized.

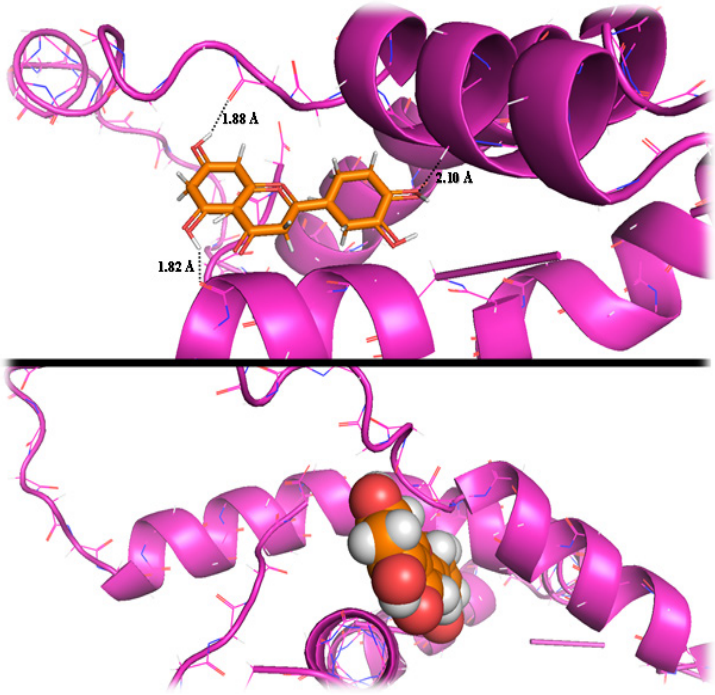

Figure 5. The Best Favored Pose from the Molecular Dynamics Video when the Luteolin-RXRα Complex becomes Stabilized.

With binding energies of -13.2 and 14.1 kcal/mol respectively, it first intercalates with RXRα and subsequently establishes effective inhibition through hydrogen bonds, while exclusively forming hydrogen bonds with RXRβ. In terms of inhibition constants, the RXRβ complex, with a value of 53.3 µM, exhibits significantly greater inhibition efficiency compared to the RXRα complex, which has an inhibition constant of 265.7 µM. This disparity can be attributed to the disruptive impact on protein structure caused by the intercalation mechanism in RXRα. Consequently, a more stabilized and effective complex is less likely to form with later hydrogen bonding, as opposed to the promptly stabilizing Kaempferol-RXRβ complex.

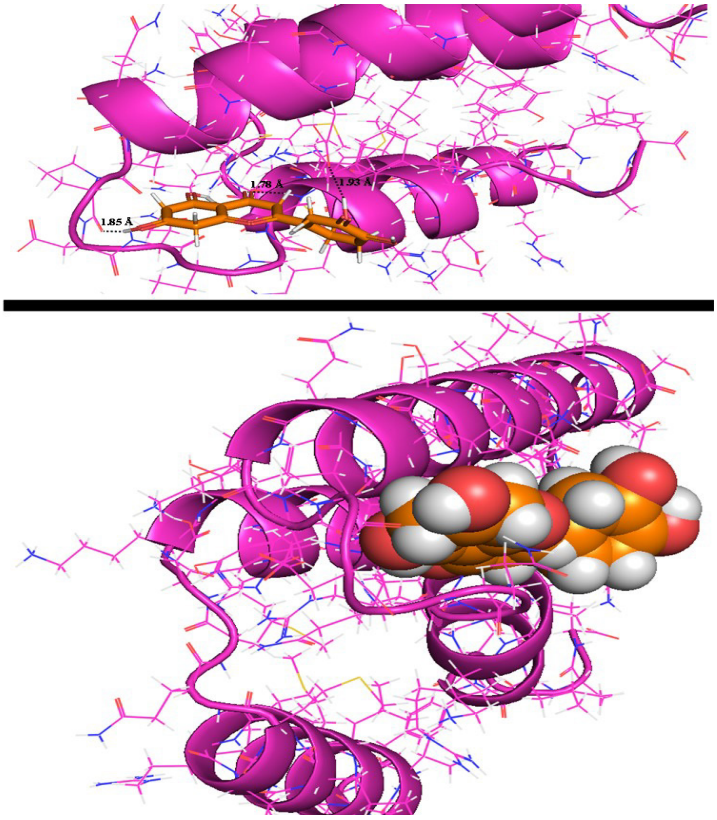

Figure 6. The Best Favored Pose from the Molecular Dynamics video when the Luteolin-RXRβ Complex becomes Stabilized.

The Molecular Dynamics Characterizations of Luteolin-RXRα and Luteolin-RXRβ Complexes

Figures 5, 6, and Table 1 offer intriguing insights into the most favorable MD poses of Luteolin in complex with RXRα and RXRβ. Luteolin's binding energies are remarkably high, with the Luteolin-RXRα complex exhibiting a binding energy of -16.0 kcal/mol. Notably, this complex forms triple hydrogen bonds at distances of 1.82, 1.88, and 2.10 Å, engaging with two α-helices and showcasing a notably favorable inhibition constant of 6.3 μM. This outcome positions Luteolin as the most potent suppressor among the three compounds for RXRα. A similar trend is observed for RXRβ, where its complex demonstrates a binding energy of -15.9 kcal/mol, an inhibition constant of 11.4 μM, and forms hydrogen bonds at distances of 1.85, 1.78, and 1.93 Å with three distinct locations, ultimately modulating an α-helix.

The Post-Molecular Dynamics Evaluation of all complexes

As evident from Table 1, the outcomes after MD simulations clearly demonstrate Luteolin as the most potent inhibitor for both receptors, given its remarkably elevated binding energy along with a notably favorable inhibition constant. The sustained protein inhibition/suppression through α-helical modulation suggests a distinct binding mechanism.

Although the other compounds also exhibit substantial receptor inhibition, Formononetin notably excels as an effective receptor inhibitor, particularly for RXRβ.

Discussion

Based on our investigations involving phytoestrogen compounds, we have ascertained that Luteolin stands out as a highly promising, versatile, and efficacious drug capable of inhibiting both RXRα and RXRβ receptors, thereby holding a great potential for prostate cancer treatment. Formononetin follows closely, particularly showcasing impressive outcomes against the RXRβ receptor. While Kaempferol emerges as a third option for potential in vitro and subsequent in vivo analyses, our in silico findings suggest its comparatively lesser efficacy compared to the other two compounds, despite its substantial binding energies for protein suppression. The outcomes of our study underscore the prospect of utilizing our ligands to target the RXR pathway, presenting an innovative immunomodulatory strategy for addressing prostate cancer.

In Conclusion, prostate cancer continues to pose significant challenges in terms of both mortality and morbidity, despite advancements in diagnostic and treatment modalities. This study aims to target RXRα and RXRβ receptors that cause prostate cancer, using Luteolin, Formononetin, and Kaempferol as drugs, with varying degrees of success. Retinoid X receptors (RXRs) are crucial components of the nuclear receptor superfamily, and preclinical studies strongly suggest the therapeutic potential of targeting RXRs for conditions such as neurodegenerative and inflammatory diseases. Therefore, the ability to regulate RXRs using phytoestrogen ligands/drugs like Formononetin, Kaempferol, and Luteolin is of paramount importance in treatment strategies. The comprehensive computational findings of this study vividly illustrate Luteolin's remarkable efficacy in inhibiting and modulating RXRα and RXRβ, with Formononetin showing notable potency as a suppressor of RXRβ. Kaempferol, the third compound, also demonstrates commendable inhibitory properties, albeit slightly less pronounced compared to the other two. These results underscore the significant binding and inhibition capabilities to RXRα and RXRβ, providing valuable insights for potential avenues in prostate cancer treatment that merit further investigation through both in vitro and in vivo analyses.

Author Contribution Statement

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

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Presence of approval by any scientific body / approval of the student thesis

This work has not been approved by any scientific body nor has been approved as a student's MSc or PhD thesis.

Availability of data (if apply to your research)

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

How the ethical issue was handled

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.

Any conflict of interest

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

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