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# *In Silico* Evaluation Of *Flacourtia Jangomas* Compounds For Hepatoprotective Activity: A Molecular Docking Study

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***Abstract***

The liver is prone to damage induced by xenobiotics, which significantly contributes to the widespread prevalence of liver diseases. To explore potential therapeutic avenues, this *in silico* study utilizes molecular docking techniques to elucidate interactions between bioactive compounds derived from *Flacourtia jangomas* and their target proteins, particularly the androgen receptor (AR) and acetyl cholinesterase, both implicated in hepatocellular carcinogenesis. While pharmaceutical drugs for liver diseases exist, their limitations necessitate the exploration of alternative options. Plant-derived compounds have garnered attention for their potential beneficial effects, with *Flacourtia jangomas* emerging as a promising candidate due to its known pharmacological activities. Through our investigation, we identified several compounds, including catechin, limonin, jangomolide, rutin hydrate, hydnocarpic acid, and chaulmoogric acid, which exhibited notable interactions with AR and acetyl cholinesterase. Enzalutamide, an AR inhibitor, demonstrated a docking score lower than catechin but higher than other compounds, indicating its potential therapeutic efficacy. Catechin exhibited the highest binding affinity, supported by more favorable scores, signifying strong interactions with AR. Rutin hydrate displayed superior docking parameters against acetyl cholinesterase compared to neostigmine. Considering various scoring parameters such as lipo, ambig, clash, and rot scores, catechin and rutin hydrate emerged as favorable options over enzalutamide and neostigmine, respectively. However, experimental validation is essential to confirm these findings. The compounds identified in this study hold promise for the development of clinically effective hepatoprotective agents.

***Keywords – Androgen receptor; acetyl cholinesterase; binding; liver disease.***

## Introduction:

The liver, playing a pivotal role in energy metabolism, is highly susceptible to damage induced by xenobiotics, contributing significantly to the prevalence of liver diseases[1]. Prolonged exposure to xenobiotics emerges as a leading cause of liver injuries, encompassing conditions such as cirrhosis, liver cancer, acute liver failure, hepatitis B, and hepatitis C[2, 3]. Recent reports indicate a staggering estimate of 1.5 billion individuals worldwide affected by chronic liver disease, irrespective of the severity of the disease stage[4].Hence, identifying effective strategies for addressing this life-threatening condition holds paramount significance. As a pivotal tool in structural molecular biology, molecular docking plays a critical role in elucidating and optimizing the interactions between bioactive compounds and their target proteins[5].

In humans, androgen receptor (AR) and acetyl cholinesterase seem to be involved in hepatocellular carcinogenesis (HCC)[5, 6].Numerous research reports have addressed the relevance of AR expression in liver-related pathologies[7, 8], with particular emphasis on its downregulation associated with the severity of alcoholic liver injury[9]. AR enhances the expression of EZH2 by binding to the EZH2 promoter and activating its transcriptional activity[10]. Furthermore, Yoon et al [11]presented evidence suggesting that androgens may play a role in regulating hepatocarcinogenesis by enhancing the transcription of TGF-β1. This regulatory mechanism is proposed to occur through direct interactions between androgens, AR, and the androgen response element (ARE) in the TGF-β1 gene. AR functions as a pivotal transcription factor, regulates various oncogenic signaling pathways crucial for driving HCC [12-14]. Furthermore, acetyl cholinesterase activity in human liver tumor samples was observed to be lower compared to that in normal tissue[15]. Reported in cases of liver cirrhosis [16] are notable alterations in acetyl cholinesterase at both the protein and mRNA levels, despite the absence of differences in enzymatic activity. These findings may signify shifts in the pathophysiological role of acetyl cholinesterase in this context. Cumulatively, the evidence presented accentuates the significance of AR and acetyl cholinesterase as potential therapeutic targets in strategies aimed at combating HCC. Currently, numerous pharmaceutical drugs, including NAC, GSH, Glycyrrhizin acid preparation, Bicyclol, Polyenephosphatidylcholine, and Silymarin, are accessible for the treatment of liver diseases[17]. However, each of these options has inherent limitations, leading to the occurrence of irreversible side effects.

The utilization of natural products not only allows for the development of improved drug-like analogs but also provides various advantages while occupying a complementary chemical space[18].*Flacourtia jangomas*, belonging to the Flacourtiaceae family, are compact trees, reaching a height of 5–10 meters[19]. Its natural habitat includes the tropical and subtropical regions of Africa and Asia. Existing literature clearly indicates that *F. jangomas* exhibits a broad array of pharmacological activities, as evidenced by its diverse range of chemical constituents. Both phytochemical and pharmacological studies have revealed that extracts from different parts and the main active components of *F. jangomas* demonstrate antimicrobial, antidiabetic, antidiarrheal, and antioxidant properties[20, 21]. Moreover, a recent study noted the hepatoprotective activity of *F. jangomas* in the Paracetamol-induced HepG2 cell line [22].Hence, the objective of this research is to identify a highly selective compound that targets both the androgen receptor and/oracetyl cholinesterase, with the potential to serve as a therapeutic agent for the treatment of liver-related diseases.

## Methods:

### *Compounds database building*

To gather information on compounds present in various extracts of *F. jangomas*, we conducted a literature search on Google Scholar, PubMed, Embase, and Chinese databases, including CNKI (http://new.oversea.cnki.net/), WANFANG data (http://www.wanfangdata.com.cn/), and SinoMed (http://www.sinomed.ac.cn/). The keywords used were "*Flacourtia jangomas*," "liver disease," "ingredient," and "compound." Subsequently, Compound IDs and SMILES identifiers were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and a compound database for *F. jangomas* was established in Microsoft Excel v.2013. Utilizing the SwissTargetPrediction webserver (http://www.swisstargetprediction.ch/), which predicts protein targets by comparing structural similarity, we identified potential active compound targets.

***Molecular Docking Studies***

In this verification process, we utilized key compounds and core targets. Initially, ligands were obtained from the PubChem database in SDF format, and protein receptors were sourced from the RCSB website (http://www.rcsb.org/) in PDB format. Subsequently, OpenBabel v.3.1.1 was employed to convert ligand SDF files to PDB format, while PyMol v.2.3.0 was used to eliminate original co-crystal ligands and water molecules from the receptors[23]. The FlexX 2.3.2 program facilitated the identification of probable binding sites between various ligands and the target protein[24]. FlexX, a docking software, predicts complex shape and estimates binding strength for a given protein-ligand pair. The docking parameters included a maximum allowed overlap volume of 2.5 $Å^3$, a clash factor of 0.6, full score contribution with a threshold of 0.30, and no score contribution with a threshold of 0.70.

**Results:**

***Interaction of the ligands with the receptors***

Upon conducting our initial literature survey, we identified a total of 10 compounds derived from *F. jangomas*. Following a thorough SwissTargetPrediction webserver analysis, it was determined that among these compounds, namely catechin, limonin, jangomolide, rutin hydrate, hydnocarpic, and chaulmoogric acid, exhibited noteworthy interactions with two prominent target genes. Specifically, these target genes were identified as the androgen receptor and acetylcholinesterase. The observed significant interactions between these compounds and the specified target genes suggest their potential relevance in biological activities associated with the androgen receptor and acetyl cholinesterase, warranting further investigation and exploration for potential therapeutic applications in liver-related diseases.

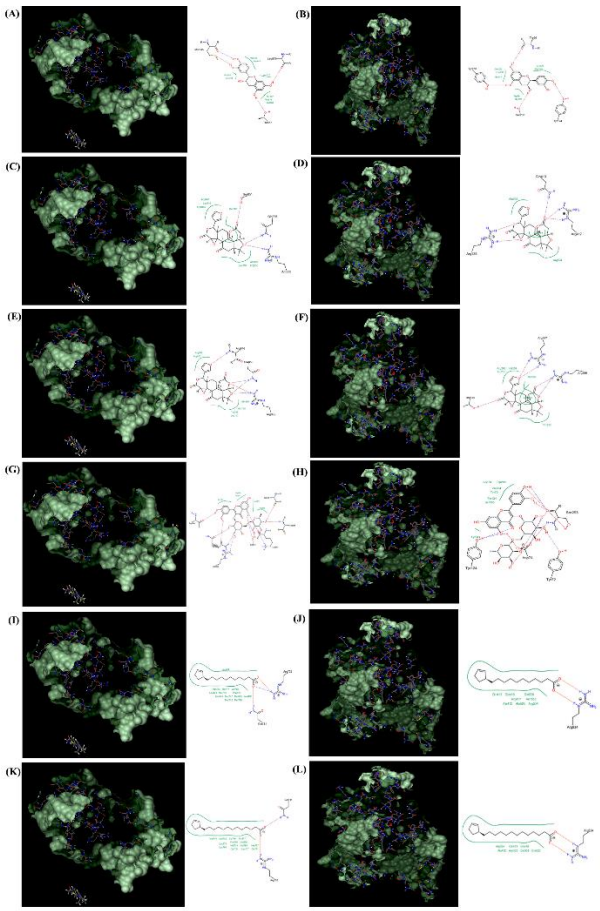


**Figure 1**: The three-dimensional representation and optimal pose view, highlighting the interaction between the androgen receptor and the ligands: (A) catechin; (C) limonin; (E) jangomolide; (G) rutin hydrate; (I) hydnocarpic; and (K) chaulmoogric acid. Similarly, the interactions of acetyl cholinesterase with the ligands are depicted in (B) catechin; (D) limonin; (F) jangomolide; (H) rutin hydrate; (J) hydnocarpic; and (L) chaulmoogric acid.

Additionally, to validate and contextualize our findings, we conducted a comprehensive literature survey to identify the most established ligands associated with the selected receptors. Our investigation revealed that enzalutamide is widely acknowledged as the primary ligand for the androgen receptor, while neostigmine is recognized as the prominent ligand for acetyl cholinesterase. Subsequently, employing the same criteria as those applied in our current study, we performed docking analyses against these targets (Figure 2).

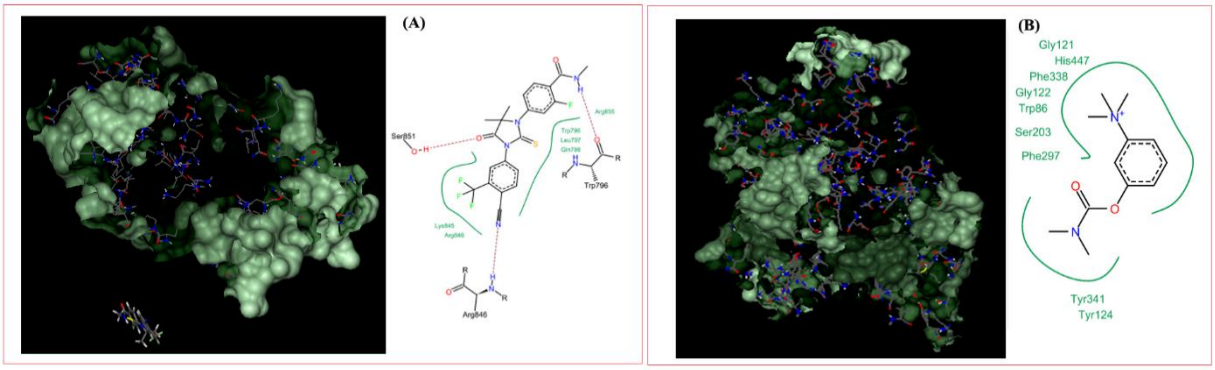


**Figure 2:** Three-dimensional representation and optimal pose view, emphasizing the interaction between (A) the androgen receptor and its most recognized ligand, enzalutamide, and (B) acetylcholinesterase and its principal ligand, neostigmine.

**Inhibition of activities of the ligands**

When a ligand binds to the active catalytic site of a receptor, it physically competes for the active site. Further, more the free energy of binding, i.e., docking score, more is the stability of the ligand-receptor complex. For androgen receptor catechin showed the highest score (-21.7), even more than its known inhibitor enzalutamide (-21.21). Similarly, for acetyl cholinesterase, rutin hydrate (-25.19) showed the highest score, significantly more than its known inhibitor neostigmine (-16.01). A higher match score was also observed for catechin (-19.17) and rutin hydrate (-30.36) with their respective target proteins. Which further indicates a more favorable interaction between the ligand and the target protein, suggesting a potentially stronger binding affinity. Furthermore, it may be noted that as shown in the table 1 with respect to lipo, ambig, clash and rot score given by flex software catechin showed superior interaction with androgen receptor compared to enzalutamide. Similarly, rutin hydrate showed docking properties with acetyl cholinesterase.

**Table 1:** The docking scores were computed for various ligands interacting with their corresponding receptors. Enzalutamide is a recognized inhibitor for the androgen receptor, while neostigmine serves as a known inhibitor for acetyl cholinesterase. The scoring calculations were conducted through the utilization of the FlexX software.

| | **Androgen receptor** | | | | | | **Acetyl cholinesterase** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Score*** | ***Match*** | ***Lipo*** | ***Ambig*** | ***Clash*** | ***Rot*** | ***Score*** | ***Match*** | ***Lipo*** | ***Ambig*** | ***Clash*** | ***Rot*** |
| **Catechin** | -21.7 | -19.78 | -11.26 | -6.603 | 2.18 | 8.4 | -24.62 | -23.44 | -9.87 | -8.43 | 3.32 | 8.4 |
| **Limonin** | -15.02 | -12.64 | -9.93 | -8.96 | 9.72 | 1.4 | -15.84 | -16.7 | -4.33 | -5.78 | 4.19 | 1.4 |
| **Jangomolide** | -20.47 | -18.49 | -7.37 | -8.96 | 7.56 | 1.4 | -17.26 | -15.75 | -7.8 | -6.7 | 6.28 | 1.4 |
| **Rutin hydrate** | -17 | -32.35 | -8.52 | -11.06 | 8.5 | 21 | -25.19 | -30.36 | -14.64 | -12.19 | 5.62 | 21 |
| **Hydnocarpic** | -8.02 | -12.54 | -15.23 | -5.18 | 4.14 | 15.4 | -10.36 | -16.6 | -9.96 | -7.26 | 2.67 | 15.4 |
| **Chaulmoogric acid** | -4.15 | -11.2 | -16.28 | -5.23 | 4.96 | 18.2 | -8.28 | -16.6 | -9.76 | -7.95 | 2.43 | 18.2 |
| **Enzalutamide** | -21.21 | -14.33 | -10.35 | -5.94 | 2.62 | 1.4 | - | - | - | - | - | - |
| **Neostigmine** | - | - | - | - | - | - | -16.01 | -12.58 | -10.51 | -4.86 | 2.35 | 4.2 |

**Discussion:**

Phytochemicals have garnered significant scientific attention in the current landscape due to their myriad health benefits [25]. Numerous studies have highlighted the potent hepatoprotective activities of plant-based flavonoids attributed to their antioxidant and anti-inflammatory properties[26]. This study aims to assess the hepatoprotective potential of various compounds extracted from *F. jangomas* through computational modeling analysis. Earlier investigations have indicated the presence of androgen receptor (AR) expression in the normal liver tissue of both male and female mammals[12]. However, it has been reported that the expression and activation of AR are heightened in tumor tissues and the adjacent liver tissues of individuals diagnosed with Hepatocellular Carcinoma (HCC). This study, therefore, endeavors to conduct molecular docking analyses of

natural compounds against AR, aiming to explore potential therapeutic interactions. Additionally, acetyl cholinesterase was selected as a second target protein, given its recognized role in liver diseases[16, 27].Our findings suggest that various compounds, including catechin, limonin, jangomolide, rutin hydrate, hydnocarpic and chaulmoogric acid exhibit potential inhibitory effects on both the androgen receptor and acetyl cholinesterase, as outlined in Table 1. These compounds are likely to interfere with the active catalytic sites of these receptors, as illustrated in Figure 1.

Enzalutamide falls within the category of medications known as androgen receptor inhibitors[28]. This pharmaceutical agent operates by inhibiting the activity of androgen receptors, exerting its effects within this specific class of drugs. Enzalutamide, an approved inhibitor of the androgen receptor at three key stages, is utilized in the treatment of metastatic castration-resistant prostate cancer[29].In the context of the androgen receptor, Enzalutamide exhibited a docking score of -21.21, which, while lower than that of Catechin (-21.7), surpassed the scores of other compounds subjected to docking against the androgen receptor. This suggests that, within the tested compounds, Catechin demonstrated the highest binding affinity with the androgen receptor, whereas Enzalutamide ranked higher than the remaining compounds in terms of docking scores against the same receptor. The match score provided by the FlexX software relies on a scoring function that considers diverse molecular interactions, encompassing van der Waals forces, hydrogen bonding, electrostatic interactions, and other factors pivotal for the stability of the ligand-protein complex. In the case of Catechin, a docking match score of -19.78 was observed, surpassing the score of Enzalutamide, which recorded -14.33. A higher match score signifies a more favorable and stable interaction between the ligand and the protein[30], suggesting enhanced binding affinity in the case of Catechin compared to Enzalutamide. A more negative lipo score generally indicates a stronger lipophilic interaction[31], suggesting a favorable binding affinity between the ligand and the receptor. In our study, catechin docked with the androgen receptor has a lipo score of -11.26, while enzalutamide has a lipo score of -10.35. The more negative lipo score for catechin (-11.26) compared to enzalutamide (-10.35) suggests that catechin forms a more robust lipophilic interaction with the androgen receptor during the docking process. Further, catechin docked with the androgen receptor, the "ambig" score is -6.6, and for enzalutamide with the androgen receptor, the score is -5.9. A more negative ambig score generally indicates a lower level of ambiguity or uncertainty in the docking solution[32].A lower clash score generally indicates better spatial compatibility between the ligand and the receptor, suggesting a more favorable fit within the binding site[33]. In this context, catechin, with a clash score of 2.18, demonstrates a slightly lower clash or steric hindrance compared to enzalutamide, which has a clash score of 2.62. Catechin docked with the androgen receptor, the rot score is 8.4, implying that Catechin has 8.4 rotatable bonds in its structure. On the other hand, Enzalutamide, when docked with the androgen receptor, has a lower rot score of 1.4, indicating that it possesses fewer rotatable bonds (i.e., is less flexible) compared to Catechin. The rot score can provide insights into the flexibility and conformational adaptability of the ligand within the binding site[34]. Generally, a lower rot score suggests a more rigid molecular structure, which may impact how well the ligand fits into and interacts with the binding site on the receptor. Similarly, Rutin hydrate exhibited more favorable docking parameters when interacting with acetyl cholinesterase, as contrasted with Neostigmine, as illustrated in Table 1. Literature suggests that considering the rot score alongside other docking parameters and experimental validation is crucial for a thorough evaluation of ligand-receptor interactions. In light of these considerations, our findings collectively indicate that Catechin and Rutin hydrate emerges as a more favorable option compared to Enzalutamide and Neostigmine respectively. Therefore, based on our results, we propose that Catechin and Rutin hydrate may serve as novel hepatoprotective agents for the treatment of liver diseases. It is important to note that this proposition awaits further experimental validation.

**Conclusion:**

In summary, this computational study underscores the potential hepatoprotective effects of compounds derived from *F. jangomas*, specifically highlighting the significance of catechin and rutin hydrate. These results offer a molecular rationale for the traditional utilization of *F. jangomas* in liver-related therapies, underscoring the need for subsequent experimental validations. The identified compounds may serve as a foundation for the exploration and development of novel hepatoprotective agents with promising clinical applications.

**References**

1. Galani, B.R., et al., *Hepatoprotective activity of Leptadenia hastata (asclepiadaceae) on acetaminophen-induced toxicity in mice: in vivo study and characterization of bioactive compounds through molecular docking approaches.* BioMed Research International, 2020. **2020**.
2. Friedman, S.L., *Mechanisms of disease: mechanisms of hepatic fibrosis and therapeutic implications.* Nature clinical practice Gastroenterology & hepatology, 2004. **1**(2): p. 98-105.
3. Jaramillo-Morales, O.A., et al., *Hepatoprotective Activity, In Silico Analysis, and Molecular Docking Study of Verbascoside from Leucophyllum frutescens in Rats with Post-Necrotic Liver Damage.* Scientia Pharmaceutica, 2023. **91**(3): p. 40.
4. Cheemerla, S. and M. Balakrishnan, *Global epidemiology of chronic liver disease.* Clinical liver disease, 2021. **17**(5): p. 365.
5. Agu, P., et al., *Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management.* Scientific Reports, 2023. **13**(1): p. 13398.
6. Kanda, T., et al., *Hepatitis C virus core protein augments androgen receptor-mediated signaling.* Journal of virology, 2008. **82**(22): p. 11066-11072.
7. Kanda, T. and O. Yokosuka, *The androgen receptor as an emerging target in hepatocellular carcinoma.* Journal of hepatocellular carcinoma, 2015: p. 91-99.
8. Chiu, C.-M., et al., *Hepatitis B virus X protein enhances androgen receptor-responsive gene expression depending on androgen level.* Proceedings of the National Academy of Sciences, 2007. **104**(8): p. 2571-2578.
9. Zhang, H., et al., *Significance and mechanism of androgen receptor overexpression and androgen receptor/mechanistic target of rapamycin cross-talk in hepatocellular carcinoma.* Hepatology, 2018. **67**(6): p. 2271-2286.
10. Song, H., et al., *Androgen receptor drives hepatocellular carcinogenesis by activating enhancer of zeste homolog 2-mediated Wnt/β-catenin signaling.* EBioMedicine, 2018. **35**: p. 155-166.
11. Yoon, G., et al., *Direct activation of TGF-β1 transcription by androgen and androgen receptor complex in Huh7 human hepatoma cells and its tumor in nude mice.* Journal of cellular biochemistry, 2006. **97**(2): p. 393-411.
12. Ma, W.L., et al., *Androgen receptor is a new potential therapeutic target for the treatment of hepatocellular carcinoma.* Gastroenterology, 2008. **135**(3): p. 947-955. e5.
13. Zender, L. and S. Kubicka, *Androgen receptor and hepatocarcinogenesis: what do we learn from HCC mouse models?* Gastroenterology, 2008. **135**(3): p. 738-740.
14. Yu, Z., et al., *Cell cycle-related kinase mediates viral-host signalling to promote hepatitis B virus-associated hepatocarcinogenesis.* Gut, 2014. **63**(11): p. 1793-1804.
15. Pérez-Aguilar, B., et al., *Acetyl cholinesterase is associated with a decrease in cell proliferation of hepatocellular carcinoma cells.* Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2015. **1852**(7): p. 1380-1387.
16. Garcia-Ayllon, M.-S., et al., *Readthrough acetyl cholinesterase is increased in human liver cirrhosis.* 2012.
17. Li, M., et al., *Pharmacotherapies for drug-induced liver injury: a current literature review.* Frontiers in Pharmacology, 2022. **12**: p. 806249.
18. Harvey, A.L., et al., *Current strategies for drug discovery through natural products.* Expert opinion on drug discovery, 2010. **5**(6): p. 559-568.
19. Singh, A.K. and J. Singh, *Evaluation of anti-diabetic potential of leaves and stem of Flacourtia jangomas in streptozotocin-induced diabetic rats.* Indian Journal of Pharmacology, 2010. **42**(5): p. 301.
20. Rai, A. and T. Mishra, *Ethnomedicinal and therapeutic values of Flacourtia jangomas.* The Journal of Indian Botanical Society, 2020. **100**(3and4): p. 169-176.
21. Sarker, G.C., et al., *Antibacterial activity of Flacourtia jangomas and Flacourtia sepiaria.* International journal of pharmacy & life sciences, 2011. **2**(7).
22. Pai, A. and C. Shenoy, *Hepatoprotective activity of Flacourtia jangomas (Lour.) Raeuschleaves and fruit methanolic extract on paracetamol-induced hepatotoxicity in HepG2 Cells.* Biomedicine, 2021. **41**(3): p. 587-591.
23. O'Boyle, N.M., et al., *Open Babel: An open chemical toolbox.* Journal of cheminformatics, 2011. **3**(1): p. 1-14.
24. Schellhammer, I. and M. Rarey, *FlexX-Scan: Fast, structure-based virtual screening.* PROTEINS: Structure, Function, and Bioinformatics, 2004. **57**(3): p. 504-517.
25. Singh, R., et al., *Phytochemicals in antidiabetic drug discovery.* J. Biomed. Ther. Sci, 2014. **1**(1): p. 1-33.

26. Datta, S., et al., *Hepatoprotective effects of natural drugs: Current trends, scope, relevance and future perspectives.* Phytomedicine, 2023: p. 155100.
27. García-Ayllón, M.S., et al., *Changes in liver and plasma acetyl cholinesterase in rats with cirrhosis induced by bile duct ligation.* Hepatology, 2006. **43**(3): p. 444-453.
28. Sternberg, C.N., *Enzalutamide, an oral androgen receptor inhibitor for treatment of castration-resistant prostate cancer.* Future Oncology, 2019. **15**(13): p. 1437-1457.
29. Krauwinkel, W., et al., *A comparison of the pharmacokinetics and safety of enzalutamide in subjects with hepatic impairment and matched healthy subjects.* Journal of Clinical Pharmacy and Therapeutics, 2017. **42**(3): p. 268-275.
30. Ferrara, P., et al., *Assessing scoring functions for protein− ligand interactions.* Journal of medicinal chemistry, 2004. **47**(12): p. 3032-3047.
31. Testa, B., et al., *Lipophilicity in molecular modeling.* Pharmaceutical research, 1996. **13**: p. 335-343.
32. Schneidman-Duhovny, D., R. Pellarin, and A. Sali, *Uncertainty in integrative structural modeling.* Current opinion in structural biology, 2014. **28**: p. 96-104.
33. Hattotuwagama, C.K., M.N. Davies, and D.R. Flower, *Receptor-ligand binding sites and virtual screening.* Current medicinal chemistry, 2006. **13**(11): p. 1283-1304.
34. Meli, R., G.M. Morris, and P.C. Biggin, *Scoring functions for protein-ligand binding affinity prediction using structure-based deep learning: A review.* Frontiers in bioinformatics, 2022. **2**: p. 57.