

***In silico* Identification of Potential Hepatitis B Virus HBx Inhibitors Through Molecular Docking Analysis**

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Abstract

Hepatitis B virus (HBV) remains a major contributor to chronic liver disease worldwide and represents a significant global health challenge. The HBx protein plays an important role in HBV replication and viral pathogenesis and is therefore considered a promising therapeutic target. In the present study, an *in silico* molecular docking approach was employed to identify potential HBx inhibitors from a selected group of fourteen compounds. The three-dimensional structure of the HBx protein was predicted using the I-TASSER server, and the selected compounds were subjected to molecular docking using PyRx and AutoDock Vina. The docking results revealed that several compounds exhibited favorable predicted binding affinities toward HBx, with SC75741 showing the most favorable docking score (−9.8 kcal/mol), followed by Rutin (−9.1 kcal/mol), Cynarin (−8.6 kcal/mol), and Ledipasvir (−8.4 kcal/mol). Notably, Cynarin, which is not currently an approved anti-HBV drug, demonstrated a favorable docking score and interactions with several important HBx residues, highlighting its potential as a candidate lead compound for further investigation. Overall, the findings suggest that the identified compounds, particularly SC75741, Rutin, Cynarin, and Ledipasvir, may serve as potential candidates for HBx-targeted anti-HBV drug discovery.

Keywords: Hepatitis; Bioinformatics; HBV; HBx; Computational Drug Design

1. Introduction

Hepatitis B virus (HBV) is a member of the *Hepadnaviridae* family of enveloped DNA viruses (Hamadalnil & Altayb, 2024). Acute hepatitis caused by HBV infection can develop into chronic hepatitis B, liver fibrosis, cirrhosis, and hepatocellular carcinoma (Saeed et al., 2023). Chronic HBV infections increase the risk of liver cancer and cirrhosis and are therefore considered a silent

killer disease (Ahmadi et al., 2024). HBV is a partially double-stranded DNA virus. Nine genotypes, A to I, and a potential tenth genotype, J, have been identified; full-genome analysis indicates that these genotypes differ by more than 8% (Velkov et al., 2018). According to a worldwide genotypic study, genotypes C (26%), D (22%), E (18%), A (14%), and B (11%) are responsible for 96% of chronic HBV infections (Velkov et al., 2018).

Four copies of the viral mRNA are translated into seven proteins: three surface proteins (S, M, and L), polymerase (P), core protein or capsid (C), additional protein (e), and transactivator X protein (Mohebbi et al., 2023). Of the four HBV open reading frames that partially overlap, the HBx gene is the smallest. It encodes a regulatory protein of 154 amino acids and a molecular mass of 17 kDa and is composed of 452 nucleotides (Pathak et al., 2014). HBV can remain undetectable for long periods, resulting in progressive immune-mediated liver damage (Baei et al., 2025). Since HBV can progress slowly over decades, not everyone infected with the virus develops symptoms. Some individuals may experience nausea, vomiting, fever, dark urine, tremors, low appetite, jaundice, blood in the stool, and diarrhea (Gholizadeh et al., 2023).

Hepatitis B spreads when the blood or seminal fluid of an infected person enters another person's body (Rizzo et al., 2022). HBV is mostly transmitted through contact with contaminated blood or body fluids, breastfeeding by an infected person, travel to regions with high HBV infection rates, sharing personal items, sharing needles, and exposure among drug users and medical staff (Tufon et al., 2019). During delivery, HBV can also be transferred from an infected mother to a newborn (Naderi et al., 2023). According to the World Health Organization's (WHO) 2024 Global Hepatitis Report, there are 254 million people living with HBV worldwide, with an estimated 1.2 million new HBV infections occurring annually (Ugbaja et al., 2025). Iran is one of the countries where HBV prevalence is low to medium (2.2%). Currently, Pakistan and Egypt account for almost 80% of the worldwide hepatitis burden.

The entire HBV virion consists of a nucleocapsid containing relaxed circular DNA (rcDNA) and an outer membrane layer. Heparan sulfate proteoglycans and sodium taurocholate co-transporting polypeptide (NTCP) interact sequentially with HBV to facilitate infection of hepatocytes (Ko et al., 2018). Following fusion of the viral and cellular membranes, the viral particle is internalized through endocytosis. The viral capsid is released into the cytoplasm and transported through endosomal regions and cytosol by the nuclear pore (Hu et al., 2019). The HBV X protein (HBx) is necessary for HBV replication (Tsukuda & Watashi, 2020). Cell invasion and proliferation are influenced by several signaling pathways that are affected by HBx. HBx contributes to oncogenesis in addition to chromosomal instability and viral replication. It regulates oxidative stress, migration, angiogenesis, oncogenesis, and DNA methylation (Shaikh et al., 2022).

Unfortunately, most approved anti-HBV medications have certain therapeutic limitations despite their efficacy (Martyn et al., 2023). Long-term use of HBV polymerase inhibitors and nucleotide analogs (lamivudine, adefovir, entecavir) can lead to drug resistance with mutations in the Pol "Tyr-Met-Asp-Asp (YMDD)" motif; some patients experience adverse effects from interferon (pegIFN- α) treatment (Parvez et al., 2019).

2. Materials and Methods

2.1. Sequence Retrieval

The FASTA sequence of the target protein was retrieved from UniProtKB using the accession number Q69027, corresponding to the HBV protein X of genotype C subtype ayr (UniProt Consortium, 2021). The protein consists of 154 amino acids. After downloading the sequence in FASTA format, it was submitted to the I-TASSER server for structure prediction.

2.2. Structure Prediction

The three-dimensional (3D) structure of the HBx protein was predicted using I-TASSER, which predicts stable and accurate protein structure (Yang et al., 2015). The FASTA sequence was provided as the input. Among the five candidate models generated by the server, the model with the highest C-score was selected as the best-predicted structure. The protein was subsequently processed using SWISS-PDB Viewer to clean the structure, and the cleaned protein structure was saved for molecular docking analysis.

2.3. Ligand Preparation

The selected natural compounds were retrieved from the PubChem database (Kim et al., 2023), an open repository maintained by the National Center for Biotechnology Information (NCBI) that provides comprehensive information on chemical structures and their biological activities (Schoch et al., 2020).

To generate 3D structures, the canonical SMILES (Simplified Molecular Input Line Entry System) notation of each compound was obtained from PubChem and entered into ChemDraw software. ChemDraw was used to generate the corresponding 2D structure and 3D conformations for further analysis. The energy of each ligand was minimized using the Molecular Mechanics 2 (MM2) force field to optimize bond angles, torsional angles, and bond lengths, thereby obtaining a low-energy and more stable molecular conformation. The energy-minimized structures were then saved in PDB format. Subsequently, the energy-minimized 3D structures were imported into PyRx, where the Open Babel module was used to further prepare the ligands and convert them into PDBQT format for molecular docking (Dallakyan & Olson, 2015).

2.4. Molecular Docking

Molecular docking was performed after preparing of the protein and ligand structures to identify compounds with the strongest binding affinity towards the target protein. PyRx, an open-source virtual screening platform that utilizes AutoDock Vina, was used for the docking analysis (Che et al., 2023; Kanwal et al., 2017). The prepared HBx structure was used as the receptor, while the selected compounds were used as ligands. Blind docking was subsequently performed by setting a grid box that surrounded the entire HBx protein structure. This approach ensured that all potential binding pockets were explored during docking, allowing potential active sites to be identified. The best docking pose for each ligand was selected based on the lowest binding energy (kcal/mol) score. Lowest binding energy values indicate stronger predicted interactions between the ligand and target protein.

The docking results were further analyzed and visualized using Discovery Studio Visualizer to characterize the interactions between the ligand and the protein binding site (Baroroh et al., 2023). Molecular interactions, including hydrogen bonds, hydrophobic interactions, and van der Waals forces, were determined to analyze the binding pose and predicted stability of the ligand-protein complexes.

3. Results

3D structure of HBx was predicted using the I-TASSER server. The final protein structure obtained after cleaning was used for evaluating the binding affinities of the selected antiviral compounds against the HBx protein (Figure 1).

The predicted structure of the protein was validated by using multiple structural assessment tools, like ERRAT, Verify3D, and PROCHECK, to evaluate the overall quality and consistency (Figure 2 (a,b) (Patil et al., 2025). These methods of assessment assess the non-bonded atomic interactions, sequence-structure compatibility, and chiral fidelity of the model, confirming its relevance for ensuing computational analysis (Table 1) (Rasool et al., 2018). The Ramachandran plot analysis showed that 98% of residues were located in favored regions and only 30 residues were in disallowed regions.

A total of fourteen ligands were selected for molecular docking analysis against the HBx protein (Figure 3). The optimized ligand structures were later used for molecular docking to evaluate their binding affinity. The selected fourteen compounds were subjected to molecular docking against the HBx protein using PyRx. The binding affinities of the docked complexes were analyzed, and the compounds were ranked according to their docking scores from strongest to weakest binding. Among the screened compounds, SC75741 showed the strongest binding affinity towards the HBx protein. The binding energies of docked compounds are displayed in Table 2.

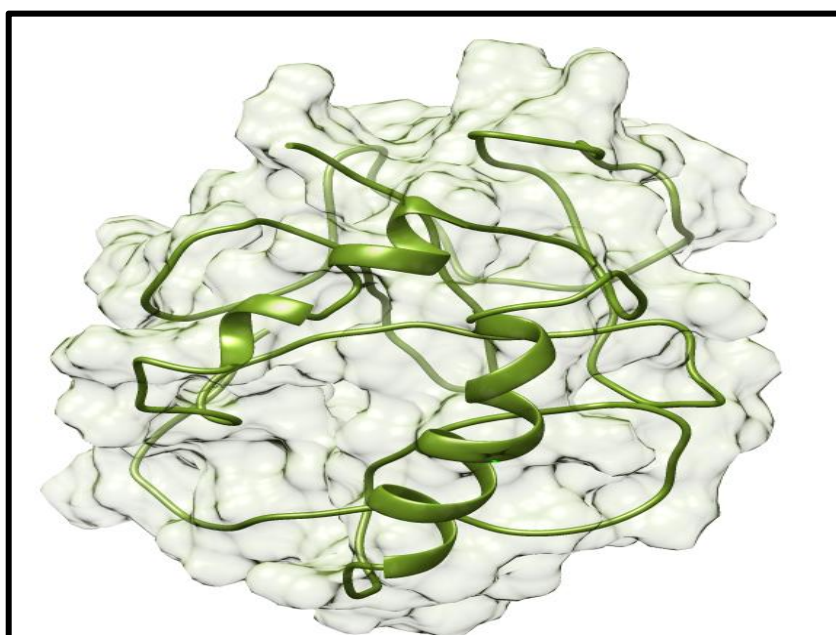


Figure 1. 3D predicted structure of HBx protein

Table 1. Results of structure validation

Analyses	Values
Overall quality factor	60.6897%
Compatibility	89.61%
Allowed region	89.7%

Table 2. Binding energies of docked compounds against HBx

Rank	Compounds	Binding energy (Kcal/mol)	classification
1	Sc75741	-9.8	Strong binder
2	Bilobetin	-9.6	Strong binder
3	Rutin	-9.1	Strong binder
4	Cynarin	-8.6	Strong binder
5	Ledipasvir	-8.4	Strong binder
6	Oleanolic acid	-8.2	Strong binder
7	Luteoline	-8.2	Strong binder
8	Betulinic acid	-7.6	Moderate binder
9	Ellagic acid	-7.3	Moderate binder
10	Resveratrol	-7.1	Moderate binder
11	Curcumin	-6.8	Moderate binder
12	Turmerone	-6.4	Moderate binder
13	Lamivudine	-5.4	Weak binder
14	1,8-cineole (Eucalyptol)	-5.2	Weak binder

The docking analysis demonstrated differences in the predicted binding affinities among the fourteen compounds, indicating variation in their interactions with the HBx binding region. Compounds exhibiting more favorable docking scores were considered to have stronger predicted binding interactions with the target protein and were therefore prioritized for further interaction analysis. The docked complexes of all fourteen compounds were imported into BIOVIA Discovery Studio Visualizer for detailed interaction profiling. Visualization of each complex in both three-dimensional (3D) and two-dimensional (2D) interaction modes revealed the specific amino acid residues of HBx involved in hydrogen bonding, hydrophobic contacts, π - π stacking, and van der Waals interactions with each ligand (Table 3; Figure 4).

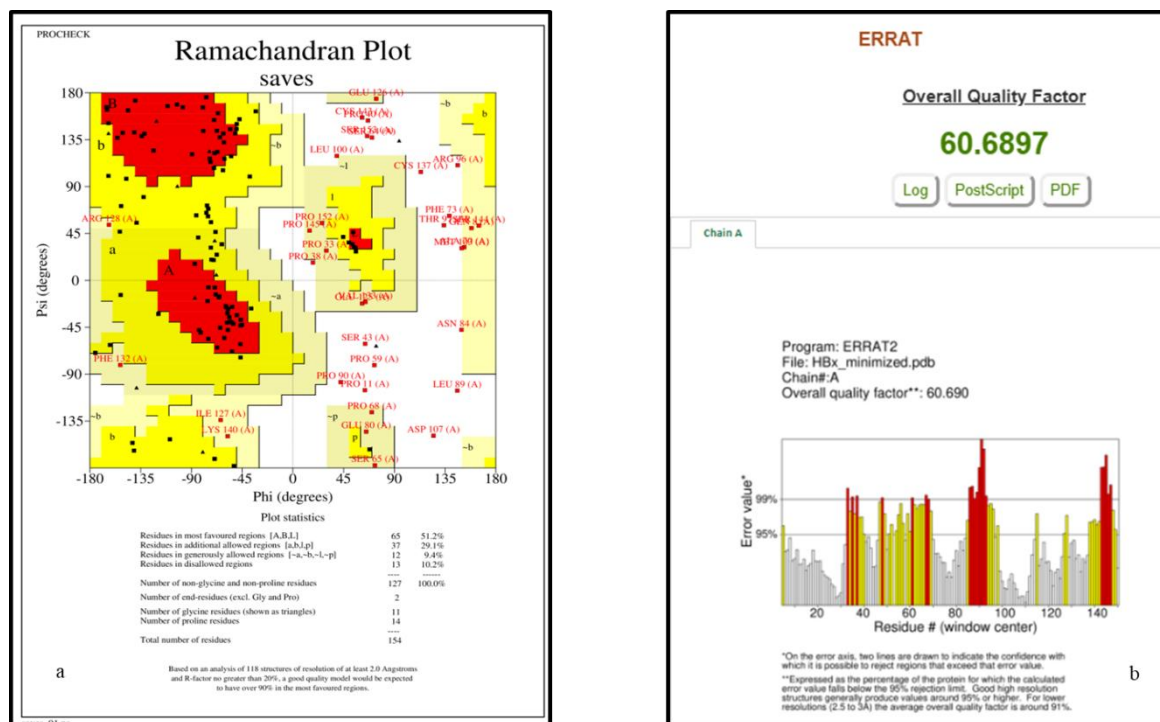


Figure 2. a) Ramachandran plot and b) ERRAT graph of predicted structure

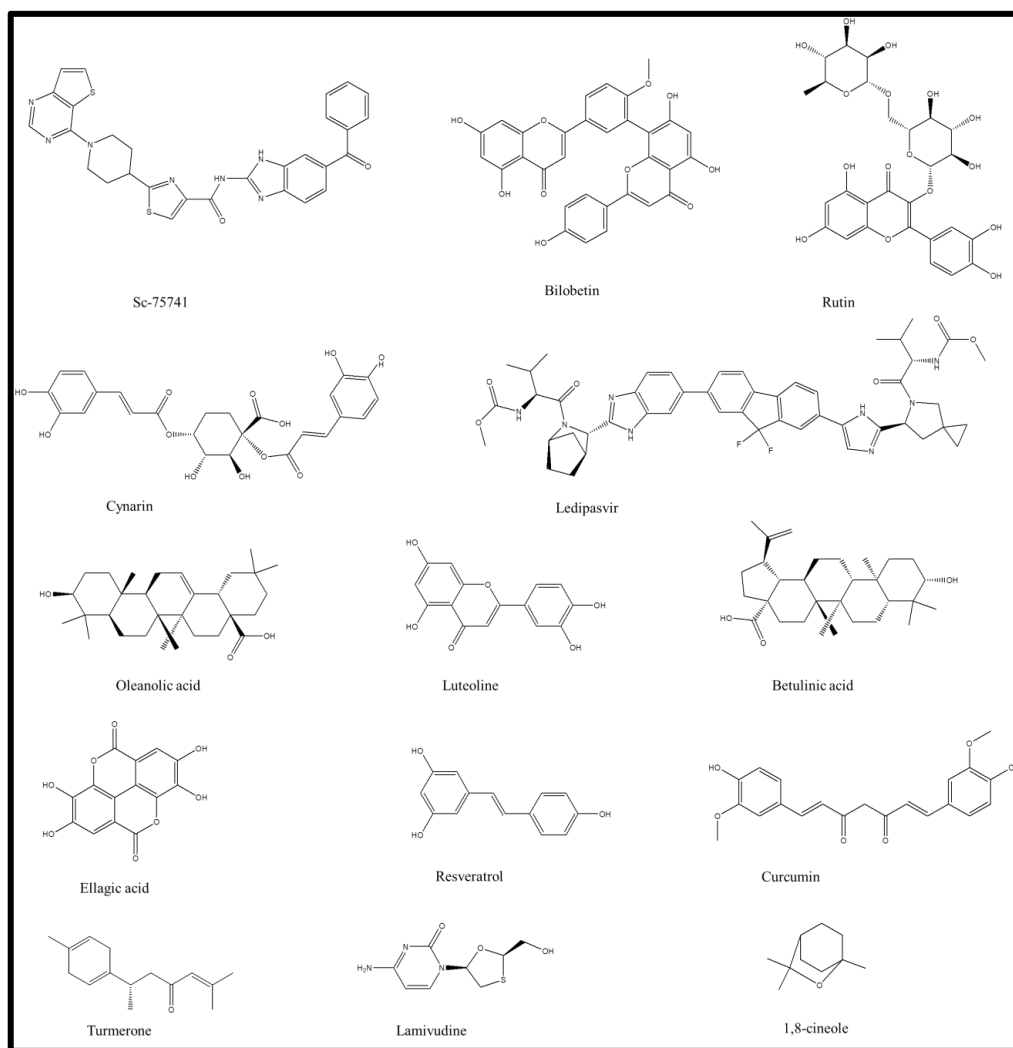


Figure 3. Two-dimensional chemical structures of the fourteen ligands

The interaction analysis provided additional structural insights into the predicted binding modes of the selected compounds. The identification of multiple types of non-covalent interactions helped characterize the ligand-binding patterns and provided a basis for comparing the binding modes of the fourteen compounds. In particular, the interaction profile of SC75741 was further examined to identify the residues and intermolecular interactions potentially contributing to its favorable docking score. Interestingly, it was observed that all the screened compounds bound at a similar binding site (Table 3).

Table 3. Interacting residues of HBx for all complexes

Rank	Compounds	Residues
1	Sc 75741	Leu-9, Asp-10, Arg-13, Val-15, Thr-74, Ala-767, Ser-78, Ile-127, Arg-128, Leu-129, Pro-147, Cys-148, Pro-152, Ser-153
2	Bilobetin	Cys-7, Gln-8, Leu-9, Arg-13, Val-15, Cys-17, Thr-74, Ser-75, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Cys-143, Ser-144, Pro-145, Phe-150, Phe-151, Pro-152,
3	Rutin	Leu-9, Asp-10, Arg-13, Arg-72, Thr-74, Ala-76, Arg-77, Ser-78, Ile-127, Arg-128, Leu-129, Lys-130, Ser-144, Pro-145, Pro-152
4	Cynarin	Leu-9, Asp-10, Arg-13, Val-15, Ala-62, Phe-63, Ser-64, Arg-72, Thr-74, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Lys-130, Phe-132, Arg-138, His-139, Ser-144,
5	Ledipasvir	Leu-9, Asp-10, Pro-11, Ala-12, Arg-13, Val-15, Pro-36, Pro-38, Thr-74, Ser-75, Ala-76, Ile-127, Arg-128, Leu-129, Lys-130, Ser-144, Pro-145, Pro-152, Ser-153
6	Oleanolic acid	Leu-9, Arg-13 Val-15, Thr-74, Ala-76, Ile-127, Arg-128, Leu-129, Pro-152, Ser-153
7	Luteoline	Leu-9, Arg-13 Val-15, Arg-72, Thr-74, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Lys-130, Ser-144, Pro-145, Pro-152
8	Buettulinic acid	Leu-9, Asp-10, Arg-13 Val-15, Thr-74, Ser-75, Ala-76, Ile-127, Arg-128, Leu-129, Pro-147, Cys-148, Pro-152, Ser-153
9	Ellagic acid	Cys-7, Leu-9, Asp-10, Arg-13, Val-15, Cys-17, Thr-74, Ser-75, Ala-76, Arg-128, Pro-152, Ser-153
10	Resveratrol	Leu-9, Arg-13, Val-15, Arg-72, Thr-74, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Lys-130, Ser-144, Pro-145, Cys-148, Pro-152
11	Curcumin	Leu-9, Arg-13, Val-15, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Cys-143, Ser-144, Pro-145, Cys-148, Pro-152
12	Turmerone	Leu-9, Thr-74, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Lys-130, Ser-144, Pro-145, Pro-152
13	Lamivudine	Arg-13, Val-15, Ala-76, Ser-78, Ile-127, Arg-128, Leu-129, Ser-144, Pro-145, Ala-146, Pro-147, Cys-148, Pro-152
14	1,8-cineole (Eucalyptol)	Cys-7, Leu-9, Arg-13, Val-15, Thr-74, Ser-75, Ala-76, Leu-129, Ser-153

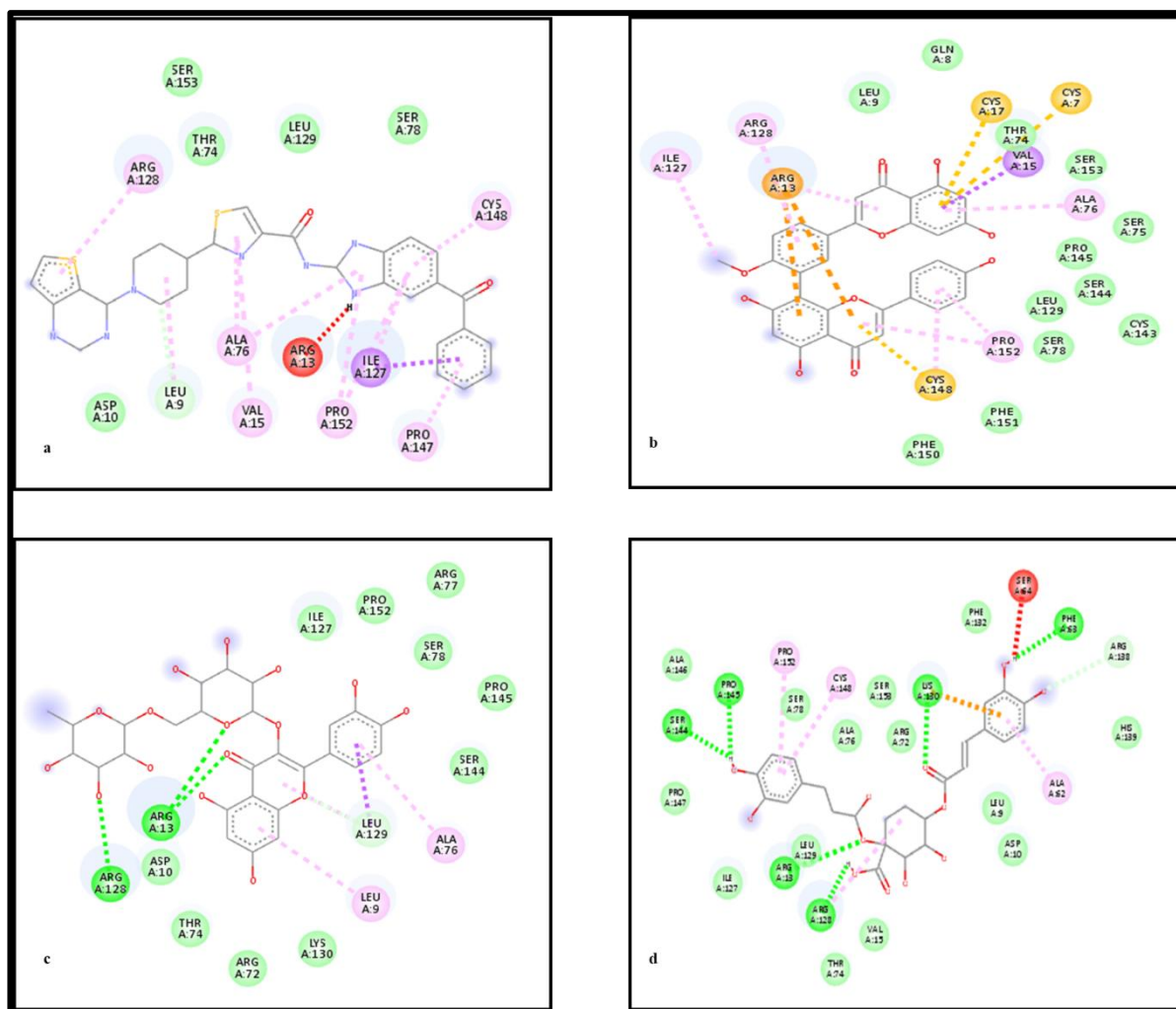


Figure 4. Two-dimensional interactions of a) Sc757241 – HBx complex, b) Bilobetin – HBx complex, c) Rutin – HBx complex and d) Cynarin – HBx complex.

4. Discussion

The present study investigates the inhibitory potential of selected natural compounds against the HBx protein using molecular docking. HBx plays a key role in HBV replication and is a multifunctional regulatory protein involved in the progression of hepatocellular carcinoma and viral chronicity, making it a high-value candidate for therapeutic targeting. Molecular docking showed that SC75741 (-9.8 Kcal/mol), Bilobetin (-9.6 Kcal/mol), Rutin (-9.1 Kcal/mol), Cynarin (-8.6 Kcal/mol), and Ledipasvir (-8.4 Kcal/mol) exhibited the strongest binding affinities towards the HBx protein. These compounds were therefore classified as strong binders to HBX. Lamivudine (-5.4 Kcal/mol), 1,8-cineole (-5.2 Kcal/mol) showed the weakest binding affinities. Lower binding energy values indicated more favorable predicted protein-ligand interactions.

The protein-ligand interaction analysis further supported the docking results. Several common amino acid residues were identified among the top-ranked compounds, including Leu-9, Arg-13, Val-15, Thr-74, Ala-76, Ile-127, Arg-128, Leu-129, Pro-152, and Ser-153. These residues may play key roles in ligand binding within the binding pockets of HBx. The repeated occurrence of these residues in the binding profiles of the top-ranked ligands suggests that they may represent an important binding region within the HBx protein. The existence of hydrophobic interactions

and hydrogen bonds may contribute to the stability of the ligand-protein complexes and potentially explain the favorable binding energies noted for the leading compounds.

Among the selected compounds, Cynarin displayed distinct binding capability, with a favorable binding energy of -8.6 Kcal/mol. It interacted with many key residues participating in binding interactions, including Leu-9, Arg-13, Thr-74, Arg-128, Leu-129, Pro-152, and Ser-153. Cynarin is not currently an approved drug for the treatment of HBV infection; its binding energy and interaction profile suggest that it could serve as a promising lead compound for the development of novel HBx inhibitors.

The findings of the present study are consistent with previous computational studies that have identified HBx as an attractive molecular target for anti-HBV drug discovery. The identification of favorable binding interactions between the selected compounds and HBx provides a basis for further computational and experimental investigations aimed at developing effective HBx-targeted therapies for HBV infection.

5. Conclusion

This study identified potential inhibitors of the HBx protein through molecular docking analysis. Among the fourteen compounds evaluated, SC75741, Bilobetin, Rutin, Cynarin, and Ledipasvir exhibited the strongest binding affinities and favorable interactions with key HBx residues. Cynarin showed promising binding characteristics despite not being an approved anti-HBV drug, highlighting its potential as a novel lead compound. These findings provide a basis for further computational and experimental studies to validate the inhibitory activity and therapeutic potential of these compounds against the HBx protein.

Author Contributions:

Conceptualization, M.A.; methodology, B. H.; resources, M.A.; data curation, B. H.; writing—original draft preparation, M.A. and B. H.; writing—review and editing, M.A.; visualization, B. H.; supervision, M.A.; project administration, M.A.; funding acquisition, M.A. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest:

The authors declare no conflict of interest.

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