# Molecular Docking on Bromodomain (Brd4-Bd2) Which is a Potential Drug Target for Prostate Cancer Treatment and Prevention

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**DOI: https://dx.doi.org/10.47772/IJRISS.2025.90400030**

**Received: 26 March 2025; Accepted: 04 April 2025; Published: 26 April 2025**

## ABSTRACT

Molecular docking has revolutionized drug discovery by providing an efficient and cost-effective approach of identifying potential therapeutic candidates. One of the most significant cancer research targets is the bromodomain-containing protein 4 (BRD4-BD2), which controls transcription by detecting acetylated lysine residues on histones. BRD4-BD2, a key epigenetic reader, stimulates gene expression by engaging transcriptional machinery, which affects the course of cell cycles and proliferation. BRD4-BD2's post-translational modifications (PTMs) activate carcinogenic pathways, making it an attractive target in the development of cancer therapeutics, particularly in prostate cancer, where overexpression has been associated with disease progression. However, there are considerable issues in targeting bromodomain for therapeutic development. These include selectivity problems, structural flexibility of the docking site, off-target interactions, and potential drug resistance pathways. However, there are considerable challenges involved with targeting the bromodomain for therapeutic development. These include selectivity problems, the structural flexibility of the docking site, off-target interactions, and putative drug resistance mechanisms. Because different bromodomains are structurally similar, developing highly selective inhibitors is difficult, and may result in unpleasant side effects. Also, the flexibility of the BRD4-BD2 binding pocket complicates the development of long-term inhibitors. Despite these challenges, computational techniques such as molecular docking have emerged as valuable tools for identifying the binding interactions between small molecules and BRD4-BD2, enabling the rational design of novel inhibitors. In this study, AutoDock, a popular molecular docking software, was used to find probable interactions between BRD4-BD2 and potential ligands. This computational approach determines essential molecular interactions, such as hydrogen bonding, hydrophobic interactions, and van der Waals forces that contribute to ligand binding stability. Furthermore, Swiss ADME was utilized to assess the pharmacokinetic properties of the tested drugs utilizing Lipinski's rule of five. Targeting BRD4-BD2 has demonstrated significant potential in prostate cancer prevention and treatment as it can reduce the expression of oncogenic genes that promote tumor formation and progression. The ability of small molecules to bind to BRD4-BD2 and engage with its regulatory function indicates a potential therapy strategy for reducing disease progression. This study underlines the significance of computational approaches like molecular docking, pharmacokinetic screening, and structural analysis in identifying effective and selective BRD4-BD2 inhibitors.

**Keywords:** Molecular Docking, Bromodomain BRD4-BD2, Prostate Cancer, Computational Drug Discovery.

## INTRODUCTION

Cancer accounts for a large share of the worldwide disease burden. Every year, tens of millions of people worldwide are diagnosed with cancer, and more than half of them eventually die as a result [1]. The study of cancer in humans began in 1775 with Sir Percival Pott's discovery of chimney sweeps' cancer, followed by extensive laboratory research. Research over the last 40 years has shown a significant temporal lag between the initial exposure to a carcinogenic stimulus and the development of clinical neoplasia [2]. Cancer is a complex disorder defined by numerous temporal-spatial changes in cell physiology that eventually lead to malignant tumors. The condition's biological goal is aberrant cell growth (neoplasia) [3].

Sustained cell proliferation in an environment containing inflammatory cells, growth factors, active stroma, and DNA-damage-promoting chemicals raises the risk of cancer, even if proliferation itself does not cause cancer [4]. Stromal cells nourish hematopoietic stem cells and their progeny. In vitro, these cells are non-hematopoietic adherent cell components of long-term cultures. In vivo, they produce the microenvironment of hematopoiesis, comprised of non-hematopoietic cells from multiple hematopoietic sites [5].

DNA replication takes place in the S (synthetic) phase of the cell cycle, which precedes the G1 phase. The division of nuclear material occurs in the M (mitosis) phase, which follows the G2 phase. Differentiated cells do not proliferate during the G0 phase, but a growing cell's G1, S, and G2 phases correspond to the period between two successive mitoses. Cyclin-dependent kinase regulates cell progression during the cell cycle in order to prevent the commencement of a new cell cycle before the previous one is complete [6].

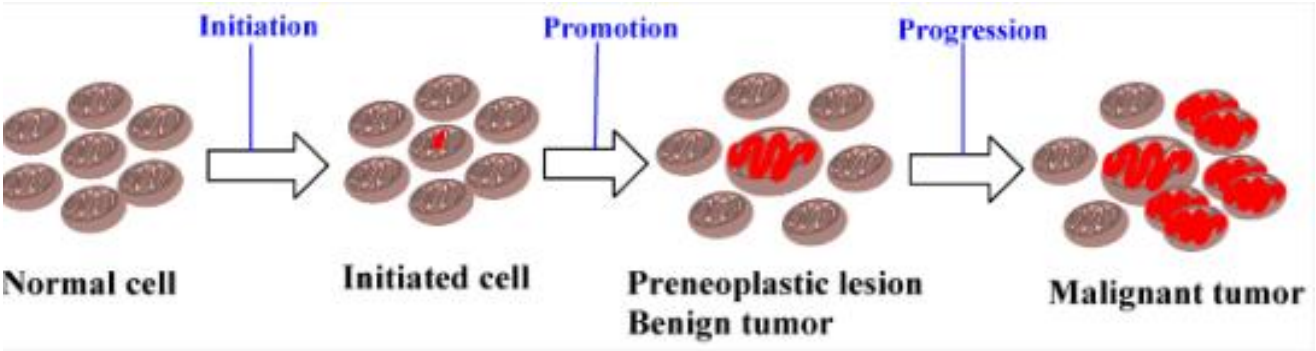


Figure 1. A clear outline of the stages of carcinogenesis, involving initiation, promotion, and progression.

Traditional chemotherapeutics have been shown to have a synergistic effect when platinum and taxane chemicals are combined. Though a number of pathways have already been carefully explored to analyze the course of cancer therapy and improve them. If existing medicines have not had the intended impact on metastatic cancer, other therapy techniques, such as immune-mediated therapies, medications, and biological molecules, have been used [7]. The ideal cancer therapy remained uncertain. The outcomes of surgery after 5 years of monitoring remain dismal, regardless of the kind of surgical intervention. Radiation treatment, chemotherapy, or a combination has not been shown to be better than surgery, but they can provide significant short-term alleviation of symptoms and even full remission in certain individuals [8].

Prostate cancer is the second most frequent malignancy among males worldwide (after lung cancer), accounting for 1,276,106 new cases and 358,989 fatalities (3.8% of all masculine cancer death) in 2018 [9]. Prostate cancer can be asymptomatic in its early stages, with a slow progression that requires little or no therapy. The most common complaint is difficulty urinating and nocturia, which are also signs of prostatic enlargement. As the axial skeleton is the most prevalent location of bone metastatic illness, later stages may show with urine retention and back discomfort [10].

During cancer creation and progression, tumor suppressor genes and oncogenes typically lose function, owing to alterations in cell genomes. Genomic alterations encompass not just gene mutations, those are changes in a gene's sequence that alter its function, but also alterations in gene copy number or dosage [11].

Androgens, working via the androgen receptor (AR), are essential for prostate growth and proper function [12]. The intracellular androgen receptor (AR), which belongs to the ligand-dependent transcription factor superfamily, regulates androgen activity. AR binds to testosterone, promoting transcription of androgen-responsive genes and controlling the development of both healthy and malignant prostate glands [13]. Despite mRNA expression studies conclusively confirming MYC overexpression in the majority of human prostate cancer malignancies, until recently, the stage of the progression of prostate cancer in which MYC protein is created in humans was unknown [14].

Several attempts have been undertaken in recent years to generate dual-target inhibitors based on BRD4 as anticancer treatments, include HDAC/BRD4 dual inhibitors, PLK1/BRD4 dual inhibitors, and PI3K/BRD4 dual inhibitors, among others. Most chemicals have strong anti-tumor properties [15].

Surveillance, prostatectomy, and radiation are the standard treatments for stage I to III patients. Stage IV and high-risk. For stage III individuals, androgen ablation via surgical or pharmacological castration may result in long-term remission. For stage III individuals, androgen ablation via surgical or pharmacological castration may result in long-term remission. Castration resistance, induced by genetic defects in the androgen receptor (AR), develops at stage IV and has a poor prognosis. Docetaxel, the standard treatment for metastatic castration-resistant prostate cancer (CRPC), has an average overall survival improvement of around three months [16].

Molecular docking is a targeted method beneficial in drug development and pharmaceutical research. This computer-assisted drug design approach uses mathematical algorithms to identify the medication's appropriate biological binding arrangement to the target molecule. Indeed, the recommended drug design is based on the molecular structure, making it conceivable to predict and forecast molecular interactions in addition to monitor biochemical processes [17].

**Drug Designing:**

Developing a new treatment can take years, if not decades, and is incredibly expensive. By reducing the requirement for wet-lab trials, computer modeling may conserve time and money in research. Drug design is a method of finding, developing, and optimizing new pharmaceutical compounds to treat diseases. It entails investigating the disease process, discovering biological targets, and developing drugs that communicate effectively with these targets. Molecular docking, structure-based virtual screening (SBVS), and molecular dynamics (MD) constitute some of the most frequently used SBDD strategies because of their diverse applications in the investigation of molecular recognition activities such as binding energies, molecular interactions, and induced conformational changes [18].

**Drug Target and Ligand:**

Proteins are the primary building blocks of life. Examples involve hormones, enzymes, and antibodies. Proteins operate based on their structure. The introduction of ligands to proteins alters their usual action. The therapeutic target here is BRD4 (bromodomain-containing protein 4) BD2. The bromodomain is a conserved region of around 110 amino acids that forms 4 α-helices (αz, αA, αB, and αC) and 2 loops, uniting αz and αA (ZA loop) and αB and αC (BC loop). It might attach to acetyl-lysine residues in histones and several other proteins [19]. Domain interacts with acetylated histones, which are involved in the production of mRNA (transcription control), MYC oncogenes, inflammatory cytokines, cell cycle regulators, and DNA repair proteins in the organism of Homo sapiens.

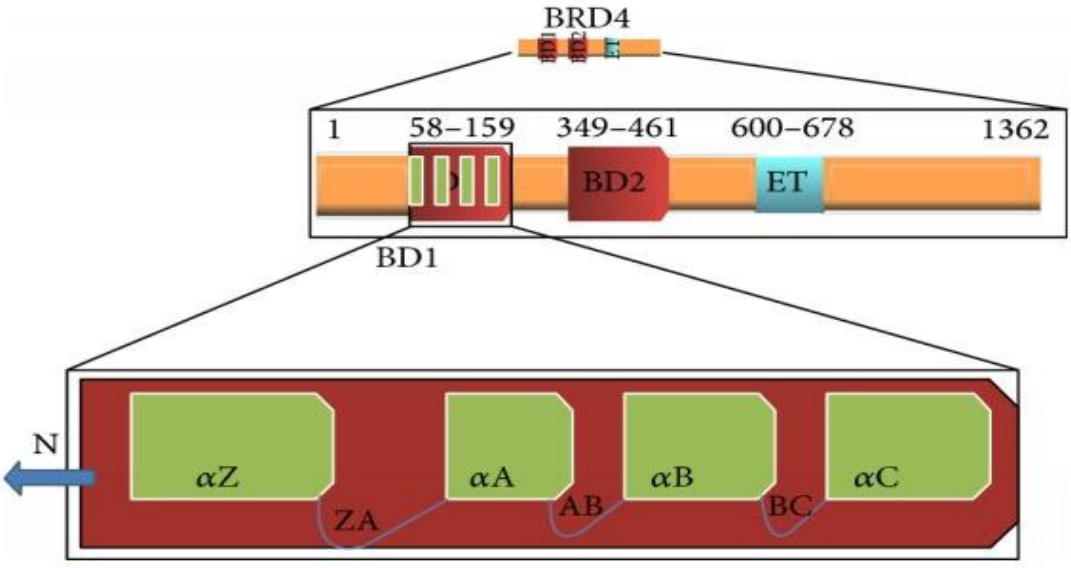


Figure 2. Human BRD4 diagram, exhibiting two N-terminal bromodomains (BD1 and BD2) and an extra terminal (ET) domain. The bromodomain-1 (BD1) highlights the N-terminus, alpha-Z, the ZA loop, alpha-A, the AB loop, alpha-B, the BC loop, and alpha-C [20].

The ligands are enterobactin (a bacterial siderophore), 4-aniline (a quinoxaline derivative), and 4-phenylchalcone oxide (a chalcone epoxidation derivative).

**Sequence And Structure Retrieval of Drug Target:**

The sequence of the drug target is retrieved from Uniprot. (https://www.uniprot.org/)

Uniprot Sequence ID: O60885.

The molecular structure of the drug target is derived from the protein data bank. (https://www.rcsb.org/)

PDB ID: 7USK (A).

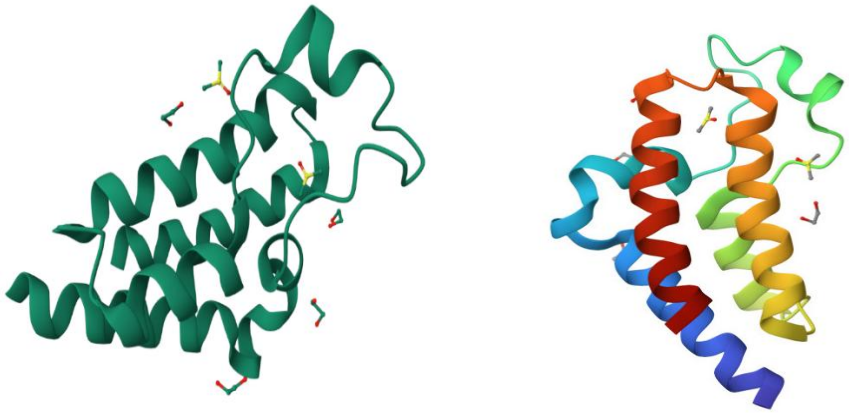

Figure 3. Three-dimensional structure of pharmacological target macromolecule (BD4-BD2)

**Domain Analysis:**

Domain analysis is conducted using Pfam Sanger [21] and includes determining conserved domains and functional features within a particular protein sequence. Pfam is a vast database of protein families, each one represented by multiple sequence alignments and hidden Markov models (HMM). It aids in determining functionally essential areas and evolutionary links within proteins. Domain ranges are 44-168 (BD1), 348-472 (BD2), 581-640 (ET), and 1363-1403 (CTD). (https://pfam.sanger.ac.uk/)

**Active Site Prediction:**

The Q-site finder is a computational method that identifies energetically favorable binding pockets to predict protein active sites and ligand-binding sites. It operates by employing van der Waals probes to identify probable ligand-binding sites. Q-site Finder is used for active site prediction. Pocket binding sites of BRD4-BD2 are Gly12 and Leu34 for Site 1, Asp56 and Phe78 for Site 2, Val102 and Thr125 for Site 3, Ser150 and Ile172 for Site 4, and Met200 and Glu223 for Site 5.

(http://www.modelling.leeds.ac.uk/qsitefinder/)

**Retrieval Of Ligands:**

Lipinski's Rule of Five is a set of criteria for identifying the drug-likeness of a molecule, specifically its oral bioavailability. Christopher A. Lipinski invented it in 1997. Lipinski's criteria is critical for choosing ligands with true therapeutic promise in molecular docking studies. It aids in the screening of promising medication candidates prior to experimental validation. It predicts drug-likeness, ensuring that the ligand has optimal physicochemical characteristics for oral absorption. The ligands are obtained from PDBQT.

Both aniline (a derivative of quinoxaline) and 4-phenylchalcone oxide (a derivative of epoxidized chalcone) adhere to Lipinski's rule of five. However, enterobactin (a bacterial siderophore) does not fall within the Lipinski rule of five.

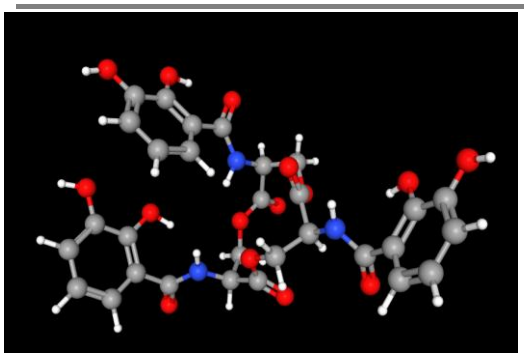

Figure 4. 3D Structure of Enterobactin

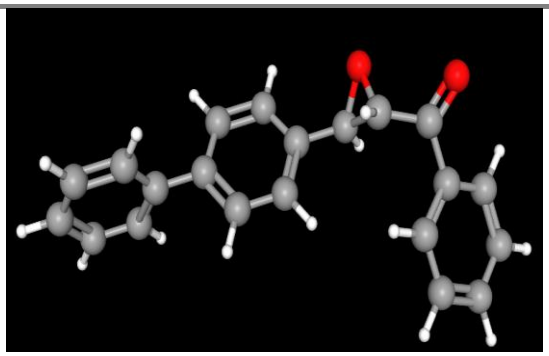

Figure 5. 3D Structure of 4-Phenylchalcone Oxide

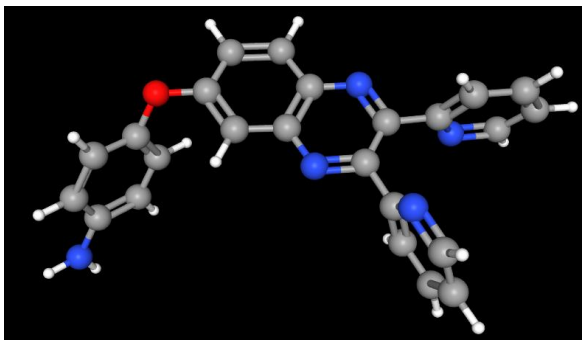

Figure 6. 3D Structure of 4-Aniline

**Auto Docking:**

AutoDock is a typical docking tool that predicts how small molecules (ligands) will interact with a target protein. It's very useful for medication research and virtual screening.

The therapeutic target BRD4-BD2 Ligand Free was docked with the selected ligands Enterobactin, 4-Phenylchalcone oxide, and 4-Aniline using the AutoDock program.(https://autodock.scripps.edu/)

**Docking Results:**

Molecular docking studies provide crucial insights into ligand-protein interactions, allowing researchers to find potential treatment options. Typically, the output includes binding energy, docking scores, and interaction data. Structure-based drug design relies heavily on protein-ligand interactions.

The docking energy of enterobactin with the target is -8.6 kcal/mol, whereas the docking energy of 4-aniline with the target is -7.5 kcal/mol. The docking energy of 4-phenylchalcone oxide with the target is -7.1 kcal/mol.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.6 | 0.000 | 0.000 |
| 2 | -8.5 | 0.849 | 10.075 |
| 3 | -7.8 | 18.833 | 22.544 |
| 4 | -7.6 | 19.180 | 24.280 |
| 5 | -7.6 | 19.477 | 23.529 |
| 6 | -7.6 | 18.456 | 21.951 |
| 7 | -7.4 | 15.400 | 20.550 |
| 8 | -7.3 | 4.676 | 9.181 |
| 9 | -7.3 | 3.622 | 8.535 |
| 10 | -7.2 | 13.085 | 19.653 |

Docking results for Enterobactin:

Figure 7. Results of binding energy with Enterobactin

**Ligand Efficiency (LE)** is calculated as:

$$LE = \frac{\text{Binding Energy (kcal/mol)}}{\text{Number of Heavy Atoms (NHA)}}$$

Figure 8. Ligand Efficiency Calculation Formula

| | |
|---|---|
| **Binding Energy (kcal/mol)** | **-8.6** |
| **Ligand Efficiency** | **-0.179** |
| **Molecular Weight (g/mol)** | **669.5** |
| **Log P** | **2.3** |
| **Hydrogen Bond Donor Count** | **9** |
| **Hydrogen Bond Acceptor Count** | **15** |
| **Rotatable Bond Count** | **6** |
| **Heavy Atom Count** | **48** |
| **Intermolecular Energy (kcal/mol)** | **-9.2** |
| **Total Internal Energy (kcal/mol)** | **-10.1** |
| **Electrostatic Energy (kcal/mol)** | **-2.1** |
| **Torsional Energy (kcal/mol)** | **0.5** |
| **Inhibition Constant (Ki)** | **0.32 µM** |

Table 1. Final Conformation of

Enterobactin with Target Molecule 7USK.

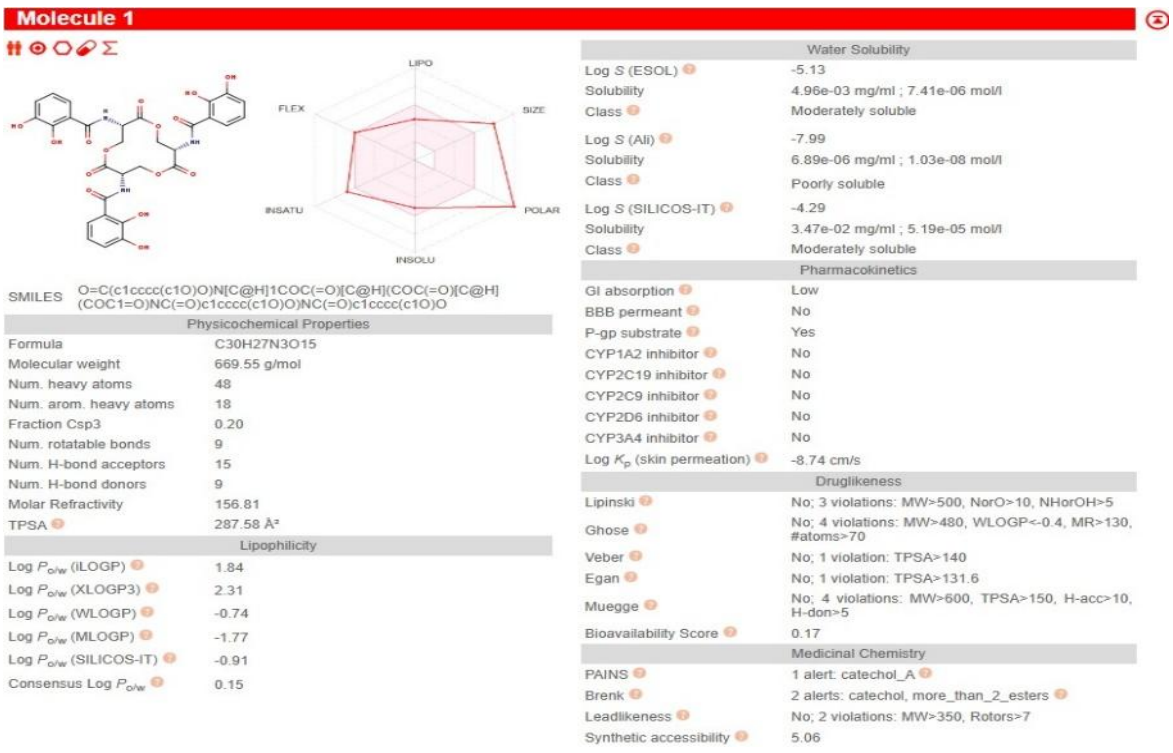


Figure 9. Analysis of Ligand Enterobactin in Swiss ADME

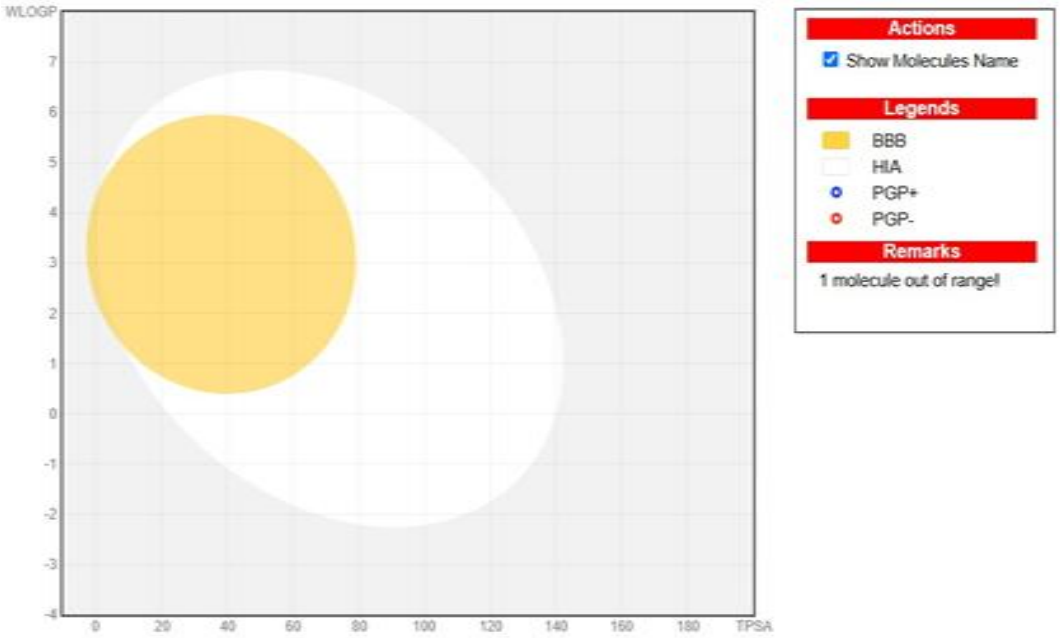


Figure 10. Results from BOILED-Egg Indicates 1

molecule out of range.

- The binding energy for enterobactin, a ligand for the protein target, is -8.6 kcal/mol based on docking data.
- Enterobactin was examined using Swiss ADME; the ligand does not obey the Lipinski rule of five.
- In Swiss ADME, the drug likeness shows 3 violations. So, it has a high binding energy of -8.6 kcal/mol, but it has a violation that is not employed in drug formation.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.5 | 0.000 | 0.000 |
| 2 | -6.2 | 2.453 | 3.710 |
| 3 | -6.0 | 2.644 | 3.754 |
| 4 | -6.0 | 12.261 | 15.547 |
| 5 | -5.9 | 6.355 | 10.860 |
| 6 | -5.9 | 14.927 | 18.151 |
| 7 | -5.7 | 19.558 | 21.282 |
| 8 | -5.6 | 15.339 | 18.083 |
| 9 | -5.6 | 20.411 | 23.187 |
| 10 | -5.5 | 6.260 | 10.689 |

Docking results for 4_Aniline:

Figure 11. Results of binding energy with 4_Aniline.

| | |
|---|---|
| **Binding Energy (kcal/mol)** | **-7.5** |
| **Ligand Efficiency** | **-0.25** |
| **Molecular Weight (g/mol)** | **391.4** |
| **Log P** | **3.3** |
| **Hydrogen Bond Donor Count** | **1** |
| **Hydrogen Bond Acceptor Count** | **6** |

| | |
|---|---|
| **Rotatable Bond Count** | **4** |
| **Heavy Atom Count** | **30** |
| **Intermolecular Energy (kcal/mol)** | **-8.2** |
| **Total Internal Energy (kcal/mol)** | **-9** |
| **Electrostatic Energy (kcal/mol)** | **-1.5** |
| **Torsional Energy (kcal/mol)** | **0.6** |
| **Inhibition Constant (Ki)** | **2.1 µM** |

Table 2. Final Confirmation of4_Aniline with Target Molecule 7USK.

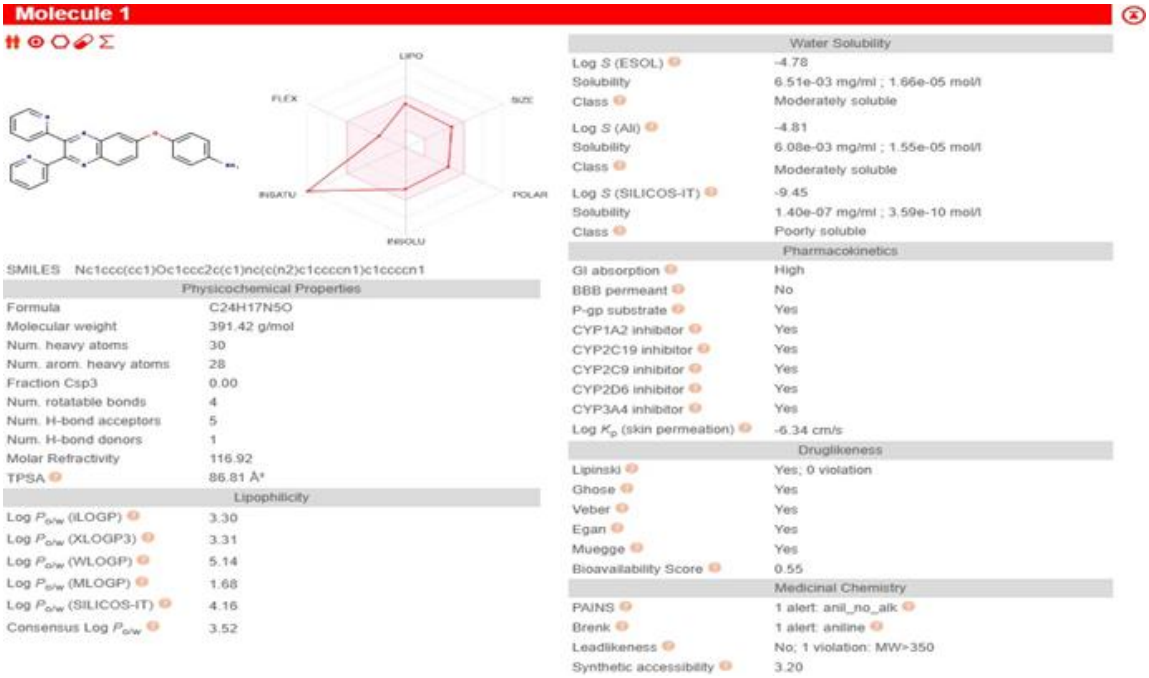


Figure 12. Analysis of Ligand 4_Aniline in Swiss ADME

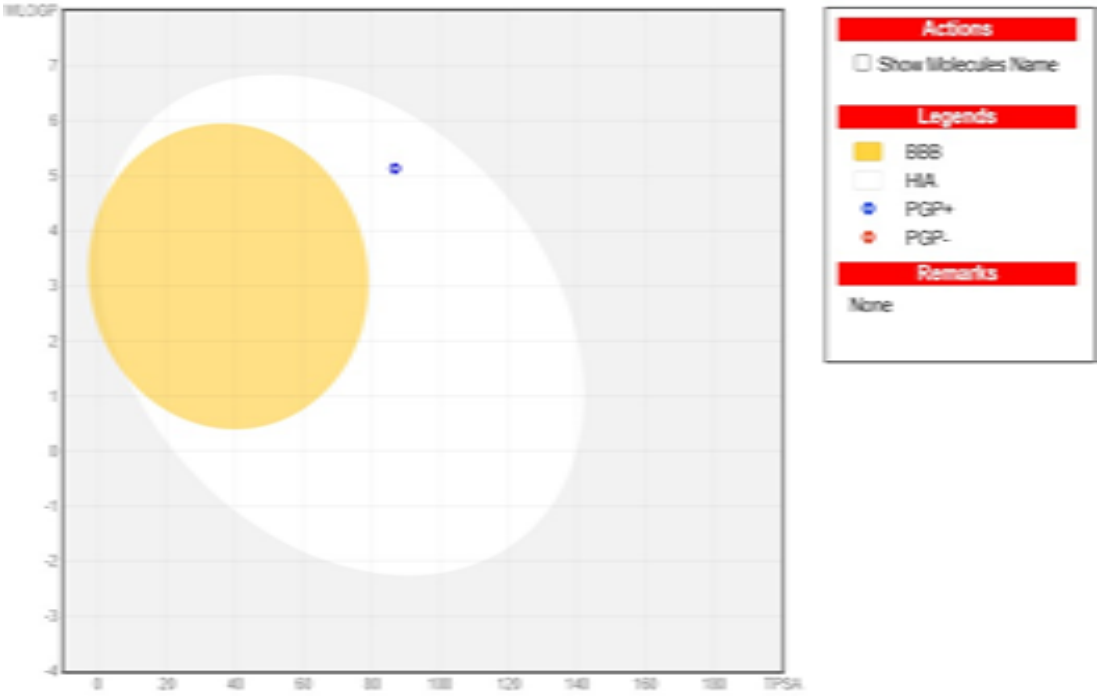


Figure 13. No molecule out of range.

- The binding energy for 4_Aniline, a ligand for the protein target, is -7.5 kcal/mol based on docking data.
- 4_Aniline was examined using Swiss ADME; the ligand obey the Lipinski's rule of five.
- In Swiss ADME, the drug likeness shows 0 violations in drug Likeness. So, it have Binding energy of -7.5 kcal/mol is used in the drug formation.

Docking results for 4_Phenylchalconeoxide:

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.1 | 0.000 | 0.000 |
| 2 | -7.0 | 1.914 | 9.285 |
| 3 | -6.5 | 1.588 | 9.224 |
| 4 | -6.4 | 1.713 | 2.543 |
| 5 | -6.2 | 14.952 | 17.695 |
| 6 | -6.0 | 20.822 | 21.867 |
| 7 | -6.0 | 3.668 | 6.596 |
| 8 | -5.9 | 2.108 | 2.755 |
| 9 | -5.9 | 15.894 | 17.908 |
| 10 | -5.6 | 17.132 | 18.905 |

Figure 14. Results of binding energy with 4_Phenylchalconeoxide

| | |
|---|---|
| **Binding Energy (kcal/mol)** | **-7.1** |
| **Ligand Efficiency** | **-0.309** |
| **Molecular Weight (g/mol)** | **300.3** |
| **Log P** | **4.5** |
| **Hydrogen Bond Donor Count** | **0** |
| **Hydrogen Bond Acceptor Count** | **2** |
| **Rotatable Bond Count** | **4** |
| **Heavy Atom Count** | **23** |
| **Intermolecular Energy (kcal/mol)** | **-7.8** |
| **Total Internal Energy (kcal/mol)** | **-8.5** |
| **Electrostatic Energy (kcal/mol)** | **-1.2** |
| **Torsional Energy (kcal/mol)** | **0.4** |
| **Inhibition Constant (Ki)** | **3.5 µM** |

Table 3. Final Confirmation of 4_Phenylchalcone oxide with Target Molecule 7 USK.

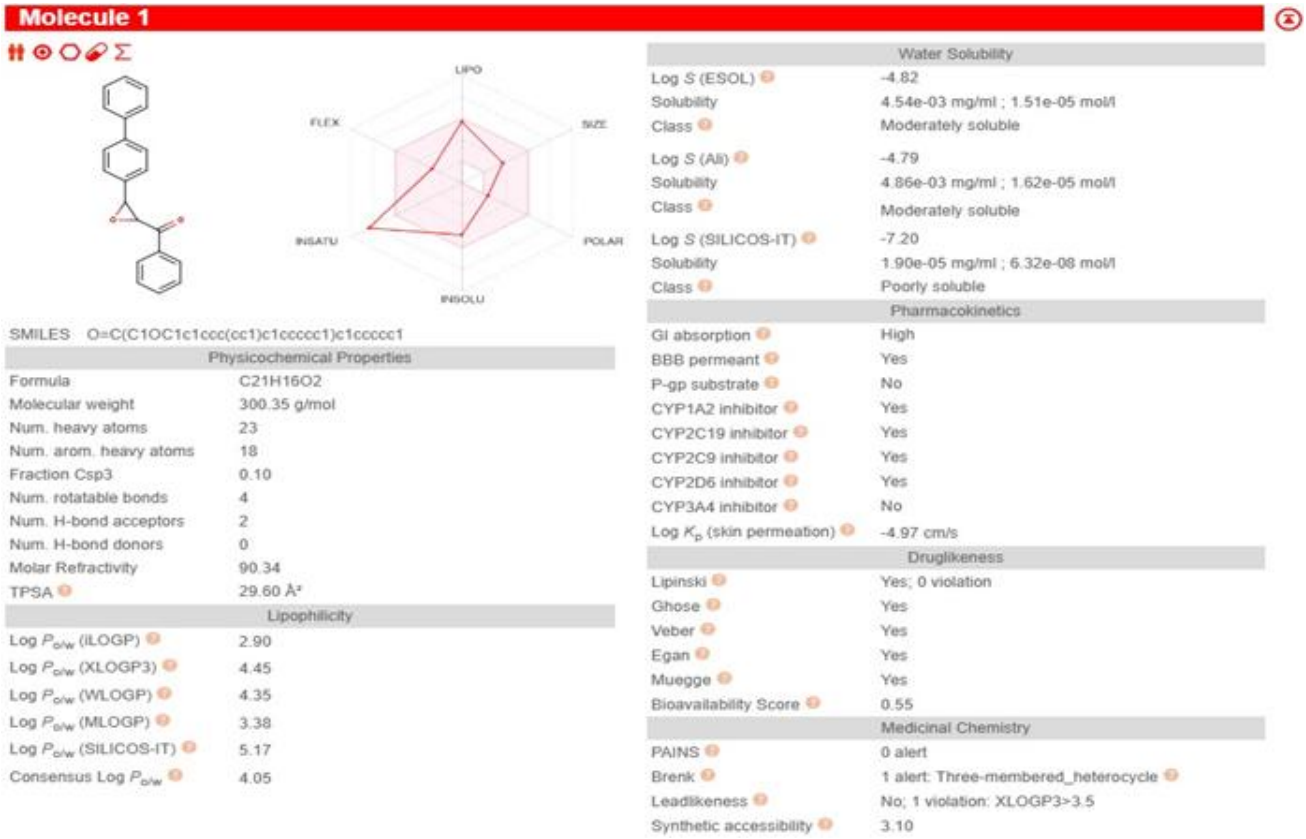


Figure 15. Analysis of Ligand 4_Phenylchalcone Oxide in Swiss ADME

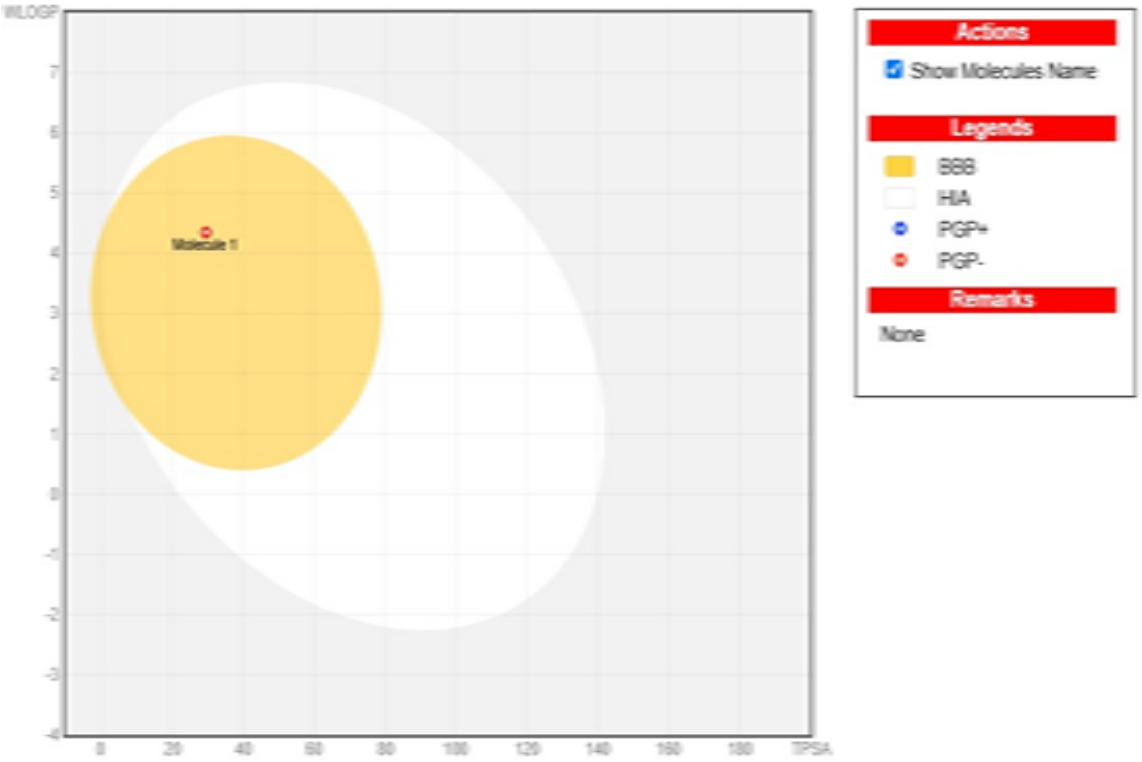


Figure 16. No molecule out of range.

- The binding energy for 4_Phenylchalcone oxide, a ligand for the protein target, is -7.1 kcal/mol based on docking data.
- 4_Phenylchalcone oxide was examined using Swiss ADME; the ligand obey the Lipinski's rule of five.

- In Swiss ADME, the drug likeness shows 0 violations in drug Likeness. So, it have Binding energy of -7.1 kcal/mol is used in the drug formation

## CONCLUSION

In this molecular docking research, the binding affinity and interactions of three ligands—enterobactin, 4-phenylchalcone oxide, and 4-aniline—were tested against the 7usk protein target using AutoDock. The docking data revealed details about their binding strengths, hydrogen bond production, and total interaction energy.

Enterobactin had the highest binding affinity (Δg = -8.6 kcal/mol), suggesting substantial interactions with the protein's active site. However, enterobactin does not meet Lipinski's rule of five, indicating poor drug-likeness features such as limited bioavailability. Because of this restriction, enterobactin was not deemed a feasible therapeutic option, despite its high binding affinity.

On the other hand, 4-phenylchalcone oxide and 4-aniline, adhered to Lipinski's rule of five, making them more promising for medication development. 4-phenylchalcone oxide has a binding energy of -7.1 kcal/mol, indicating considerable stability within the binding site, owing mostly to hydrophobic interactions and hydrogen bonds. 4-aniline had a somewhat greater binding affinity (-7.5 kcal/mol), with important hydrogen bonds and electrostatic interactions helping to maintain its stability.

Based on these data, 4-phenylchalcone oxide and 4-aniline appear as more attractive medication candidates due to their adherence to Lipinski's rule and high docking scores utilizing Auto Dock Tools.

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