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Toxicity and Binding Assessment of Pararosaniline with Biological Proteins: Myoglobin and Hemoglobin

Ritika Singh[1], Nibedita Mahato[2], Dr. Prateek Pandya[3]

[1,2]Student, Amity Institute of Forensic Sciences Amity University, Noida, Uttar Pradesh
[3]Professor, Amity Institute of Forensic Sciences Amity University, Noida, Uttar Pradesh

Abstract
This study explores the intricate molecular interactions between pararosaniline (BR9) and crucial proteins, myoglobin (MB) and hemoglobin (HB), employing advanced computational docking methodologies. The study prepares a protein structure by removing the solvent molecules and irrelevant ligands, ensuring a refined environment for accurate docking stimulations. Additionally, the ligand structure of pararosaniline undergoes optimization to facilitate compatibility with docking software utilizing CB Dock and Seam Dock software. Docking simulations are conducted carefully, with defined parameters allowing for the exploration of various binding modes and prediction of binding affinities. The comparative analysis of two docking platforms serves to enhance the reliability of the results. Furthermore, the study integrates the toxicity predictions of pararosaniline using computational tools to discern potential biological implications. By intervening in structural biology, computational chemistry, and toxicology, this comprehensive approach offers profound insight into intricate molecular dynamics and potential toxicological ramifications of pararosaniline interactions with myoglobin and hemoglobin.

Keywords: Pararosaniline, Hemoglobin, Myoglobin, Docking Interaction.

1. INTRODUCTION

Concerns have been raised about the detrimental impact of food dyes on human health. Artificial food colors, which are commonly employed to improve the aesthetic appeal of foods, have been linked to potential human toxicity [1]. Excessive consumption of food dyes including pollutants has been linked to health risks, with some dyes being carcinogenic and toxic. Furthermore, artificial food dye use, particularly among children, has been associated to hyperactivity in sensitive people and allergic reactions. Furthermore, evidence suggests that certain synthetic food dyes may aggravate indication ofADHD in children [2]. Furthermore, studies have shown that food additive dyes can increase protein unfolding and aggregation, such as myoglobin, indicating their potential molecular toxicity [2]. As a result, studying the binding of food dyes to proteins such as myoglobin is critical for understanding their potential health effects and toxicological profiles [2][3].

Pararosaniline which is also known as also known as C.I Basic Red 9 (BR9), a dye that is prohibited due to is carcinogenic and toxic properties [4]. Pararosaniline is a salt of colored organic base containing amino acid and imino groups. Its relevance lies in its historical use as a food dye. Pararosaniline

continues to be used in other applications such as coloring, paper, leather, and natural and artificial fibers [6].

Myoglobin is a globular protein composed of a single polypeptide chain that contains 153 amino acidresidues [7]. It folds into eight α - helices which are joined by a loop, resulting in a compact and approximately oblate form. Myoglobin contains a heme group, which is a porphyrin ring with an iron atom at its center. The iron atom can bind to the oxygen molecule, enabling myoglobin to store and release oxygen within muscle cells [8]. Additionally, Myoglobin first facilitates oxygen diffusion andserves as a buffer of intracellular oxygen concentration and an oxygen reservoir in muscle.

HB protein found in red blood cells has a major character in delivering oxygen from lungs to body tissue and while returning carbon dioxide from the tissues to the lungs for elimination [9]. Hb protein consists of four Protein chains two alpha chains and two beta chains [10]. Each has a ring-like heme group which contains an iron atom. The alpha and beta chains present in HB are similar to the Myoglobin protein. The Heam group Present in HB is responsible for the binding of oxygen, which allows the transportation of oxygen from the lungs to various bodily regions [11]. Approximately arranged in a tetrahedral pattern, hemoglobin's four subunits make up its quaternary structure [12]. The quaternary structure is different. In Oxy and deoxyhemoglobin [9]. With each Subunit having a molecular weight of roughly 16,000 Daltons, the tetramer's total molecular weight is approximately 64,000 Daltons [12]. The subunits are physically Identical and almost the same size. Hemoglobin serves as a respiratory pigment that helps carry oxygen from the lungs as oxyhemoglobin. HB also helps carry some carbon dioxide back to the body as carbaminohemoglobin [11].

1.1. Objective:

The main intention of the study is to investigate the docking interactions between pararosaniline and the proteins myoglobin and hemoglobin, assessing their binding modes and affinity. The study will use docking websites such as CB-Dock and Seam-Dock to gain insights into specific Interactions between pararosaniline and proteins. Additionally, to access the potential toxicity of pararosaniline utilising a web- server-based toxicity analysis tool. The study is relevant because pararosaniline is a hazardous substance that has been harmed for use in food due to its Carcinogenic and Toxic Properties [6]. Understanding the interactions between them can provide insights into the potential health risks associated with exposure to pararosaniline and inform regulation on its use in other applications.

1.2. Aim of study: potential applications of studying these interactions:

Studying the docking interaction between pararosaniline and proteins such as hemoglobin and myoglobin holds significant implications across various domains, encompassing both scientific research and practical applications, here are some potential applications and areas of interest:

Structural biology: Research on this docking interaction can contribute to a finer understanding of how pararosaniline may act on biological processes and lead to the progress of targeted therapies or inventions. Molecular docking as a powerful approach for structure-based drug discovery allows for the prediction of interaction between molecules and biologicaltargets, providing insight into the binding mechanism of compounds like BR9 with specific proteins [13][15]. By Studying the docking interactions researchers can gain valuable information on how pararosaniline interacts with the protein involved in essential biological functions such as oxygen, transport, and storage, which are crucial for understanding its potential impact on biological processes. This can help to identify potential therapeutic targets or interventions to migrate any adverse effects caused by this compound by elucidating the binding mechanism and effects of BR9 on these proteins. Researchers can explore the development of targeted therapies that specifically modulate these interactions to either enhance desired defects or reduce harmful impacts on biological processes. This research can

pave the way for the design of novel drugs or interventions, that can effectively counteract the negative effects of pararosaniline on biological systems based drug discovery allows for the prediction of interaction between molecules and biological targets, providing insight into the binding mechanism of compounds like BR9 with specific proteins [13][15]. By Studying the docking interactions researchers can gain valuable information on how pararosaniline interacts with the protein involved in essential biological functions such as oxygen, transport, and storage, which are crucial for understanding its potential impact on biological processes. This can help to identify potential therapeutic targets or interventions to migrate any adverse effects caused by this compound by elucidating the binding mechanism and effects of BR9 on these proteins. Researchers can explore the development of targeted therapies that specifically modulate these interactions to either enhance desired defects or reduce harmful impacts on biological processes. This research can pave the way for the design of novel drugs or interventions, that can effectively counteract the negative effects of pararosaniline on biological systems

Drug development: Understanding the binding mechanism of pararosaniline with hemoglobinand Myoglobin can aid in designing safer drugs by predicting potential interaction and side effects early in the drug development process. This is achieved through molecular docking, which helps estimate the interaction happening between molecules and biological targets. It is done by first anticipating the molecular orientation of the ligand enclosed in a receptor and then conjecturing their complementary with the employment of the scoring function. This has been an important implement for drug discovery and evolution. Molecular docking has been used to spot structural elements obligatory for ligand-receptor binding, develop accurate docking methods, and design and optimize compounds with therapeutic interests. However, the implementation of docking in drug design is restricted to biological targets whose crystal structures are known, further work is being done in order to get some of its inherent drawbacks. These restrictions incorporate the use of the approximated scoring system and a sample of the ligand and receptor confirmations in posture. Molecular dynamics has also been frequently employed in silico approach and investigates the conformational space of the ligands, targets, and ligand-target complexes [13]

Toxicity assessment: A study of docking interaction between pararosaniline and proteins can provide insight into the potential toxic effect of kerosene on these proteins, which could impact oxygen transport and storage in the body [14]. In addition, acute toxicity and genetic toxicity have been linked to pararosaniline. It is unclear if iron released during the production of red blood cells or methemoglobin is accountable for this. poisonous tissue changes and alterationsand scars caused. Kidneys are also a concern prior to the origination of malignancies in the spleen and liver. Increased incidences of hematopoietic system tumors in female myths have also been associated with exposure. These potential toxic effects are crucial for accessing the safety of pararosaniline and related compounds.

Environmental impact assessment: Interactions like this can aid in accessing the environmental influence of these compounds, especially in cases where they are available in the environment, household, Industrial, or other sources. BR9 is a hazardous substance that can cause genetic toxicity and acute toxic effects, but it is not clear whether it is regulated by iron released during the formation of methemoglobin or red blood cell turnover and stress associatedwith this process [13].1 BR9 is also toxic to aquatic organisms and can cause long- term adverse effects in the aquatic environment, making it important to consider its environmental impact.

2. REVIEW OF LITERATURE:

The literature review on the docking of various compounds with hemoglobin revealed several studies

that have investigated the interaction between HB and synthetic dyes, malachite, green and hemin.
A study found that bovine HB can form multiple interactions with synthetic dyes such as pararosaniline, which was done using spectroscopic techniques and molecular docking [15]. The study suggested that the interaction between hemoglobin and synthetic dyes bear significant components of their safety profile.
In another study, it was found that malachite green can bind to the central cavity of HB with high affinity. This was determined by molecular docking and molecular spectroscopy [11]. Therefore, the study suggests that Malachite green may have implications for the safety profile by binding to HB.
In the third study, it was found that malachite green oxalate MGO can brain to human serum albumin (HSA) with an estimated free energy of -32.93 KJ/mole, as determined by molecular dynamics simulations and spectroscopic approaches [16]. The study suggests that the binding of MGO to HSA may have implications for the safety profile of MGO.
A review of literature on hemopexin, a plasma protein that binds free heme, found that hemopexin can form covalent bonds with the heme group of hemoglobin and that this cross- link constitutes an irrefutable biomarker for the peroxidation action of holo-myoglobin [15]. The review also discusses the role of haptoglobin in free HB metabolism and the toxicity of free heme.
The literature review on the docking of various compounds with myoglobin reveals several studies that investigated the interaction between myoglobin and Lactate, Pyruvate, and additives.
According to a study, lactate (LAC) can attach itself to Oxy-MB close to the O2 binding site and its residue, as established by research utilizing molecular docking [17]. According to the study, LAC may also liberate oxygen from myoglobin, which may have consequences for the bioenergetics of cells while they are at rest versus during periods of high exertion (hypoxia) conditions.
Pyruvate (PYR) was spotted to interact with both Oxy and deoxy MB with a 1:1 stoichiometry in another work using circular dichroism, isothermal titration calorimetry, and O2-Kinetic studies [18]. The study also revealed that, in contrast to LAC, which favors Oxy-MB, PYR has a stronger affinity for deoxy MB. Additionally, PYR interaction with Oxy MB was found to release a notably less amount of O2 than LAC.
A survey of the literature on molecular dynamics simulation and docking revealed that the binding mechanisms between myosin, myoglobin, and additives can be found using molecular docking. The binding mechanisms of these interactions can be followed up by molecular dynamics simulations [19]. A literature review on the exchange of hydrogen and deuterium found the application of mass spectrometry to epitome mapping revealed that the identification of antibody binding sites on myoglobin molecules can be executed through computational docking on myoglobin utilizing peptides obtained from the anti-myoglobin [20].
In summary, the study suggests that soluble substances found in the body can interact with MB and HB in a variety of ways and that the binding of these compounds to the proteins can have implications for their safety profiles.

3. MATERIALS AND METHODS:

Two different software tools were used to investigate the potential binding conformation of BR9 with HB and MB, which includes the CB-Dock and Seam Dock online servers.
Every docking result that was received was evaluated using the Biovia Discovery Studio program was used to evaluate [21].

3.1. Development of the protein target:

To ensure the accuracy and dependability of the docking simulation, a number of crucial actions must be followed while designing protein structures for molecular docking studies [2][3]. The first step is to obtain myoglobin (PDB ID 1azi) and hemoglobin (PDB ID 2D60) from a reliable protein structure, database like Protein Data Bank (PDB) [3]. Second, in order to leave only the protein chains, protein structures should be pre-processed using molecular visualization tools such as Biovia Discovery Studio to diminish cofactors, water molecules, and other heteroatoms [23]. Thirdly, the processed protein structures should be Transformed into the proper file format for docking, like the PDB format. Lastly, it is important to validate the protein structures through comparison with published research and experimental findings [24].

3.2. Development of ligand:

Get the 3D structure of the ligand pararosaniline from a reliable source, such as PubChem, in order to prepare it for molecular docking studies. After checking the structure for any missing atoms or bonds, it should be optimized for docking studies utilizing the relevant web servers [23]. The pararosaniline three-dimensional conformer was obtained in SDF file format from PubChem databases. The Biovia discovery studio was then used to convert the ligand into a MOL2 file format. Lastly, the structure is validated by competent research and experimental data [24]

3.3. Docking mechanism

3.3.1. CB Dock:

Instead of considering the entire protein surface, CB Dock focuses on focused docking, more precisely on the predicted binding area [25]. Cavity discovery is the initial step in locating potential binding sites. Cavity sorting is the process of selecting larger cavities for further analysis, which often correspond to ligand binding sites [26]. Next, the dimensions of the docking box are adjusted, and the docking center is established. When utilizing AutoDock Vina for molecular docking, the centre ,and size are important factors that must be considered. When docking is complete, the resulting bound poses are reranked based on the docking score. The matching site represents the optimum binding location, and the best binding pose is the conformation that scores the greatest.

3.3.2. Seam Dock:

Seam Dock is a collaborative web docking tool specially designed for compact compound molecular docking [27]. In the first instance, Pararosaniline was uploaded in MOL2 format and HB was supplied in PDB format as the main input. The HB molecule was loaded and processed, and then the docking box's settings were established. Giving the docking box's 3D coordinates was necessary for this, and the size coordinates had to be adjusted to 20 Å, 20 Å, and 20 Å. After that, AutoDock Vina was utilized to perform the molecular docking approach with a 1.0 Å spacing and certain settings. The exhaustiveness parameter was established to 8, showing that the search process was comprehensive, and the mode number was adjusted to 2, representing the number of wanted docking modes.

In the second case, pararosaniline was uploaded in MOL2 format as well and myoglobin was supplied in PDB format. Following the molecular loading, the docking box’s settings were defined as, the size coordinates were established to 20 Å, 20 Å, and 20 Å. The exhaustiveness parameter was adjusted to 8 and the mode number was adjusted to 2.

3.4. Toxicity prediction:

The STopTox web-based server was utilized to explore BR9's toxicological properties. It is a useful, dependable, and user-friendly instrument that is used for determining if a chemical has the potential to be acutely hazardous [28]. The molecular structure of pararosaniline was drawn and submitted to the STopTox server for analysis. The output generated by the STopTox server provided insights into pararosaniline, guiding further toxicity assessments.

4. Results and discussion:

4.1. Binding energy assessment:

A number of molecular docking programs were used to look at the manner and interactions of binding between BR9 and the proteins, MB and HB. A variety of binding affinities were found byanalyzing docking results (Table 1). In the matter of binding affinities, binding modes, and interaction strength, different software may use different algorithms and energy-scoring methods. Remarkably, VAL68, ILE107, ALA71, LEU89, and LEU72 were consistently determined to be important amino acid residues in the binding process during docking simulations performed by bothsoftware. This puts forward that these residues actively contribute to the hydrophobic contacts that BR9 created inside the MB protein. In general, hydrophobic interactions have been the primary means of supporting the establishment of the BR9-MB complex (Fig. 2). The hydrophobic domains of the MB protein and BR9 are what elucidates these interactions, enabling them to bind and fortify the complex structure firmly. Whereas, in the case of the BR9-HB complex it's interesting to note that different amino acids were required in the binding process of docking calculations in both the software. It was seen that hydrophobic interactions were primarily responsible for the formation and stabilization of the BR9-HB complex. Therefore, in every docking program, the hydrophobic interactions and van der Waals forces made a substantial contribution to the overall stability and specificity of the BR9- MB and BR9-HB complex formation, making it easier for the BR9 and, MB and HB to recognize and attach.

Table 1: Binding affinities observed in the BR9-MB complex and the BR9-HB complex.

s.no	Protein	Different docking tools	Binding affinity (kcal/mol)
1.	Myoglobin	CB- dock	-6.9
		Seam dock	-7.2
2.	Hemoglobin	CB- dock	-9.2
		Seam dock	-7.5

4.2. Interaction analysis:

4.2.1. BR9-MB Complex:

4.2.1.1. CB-Dock:

Molecular Interaction investments have been carried out to investigate the possible binding of BR9 tothe MB protein. The reverberation of the research showed that BR9 interacted with the MB protein central cavity with the only chain which is α which certain MB protein residue BR9 created five hydrophobic contacts (figure 2). It is stabilized by pi-sigma contact at a distance of 3.64 Å with α- VAL68 Å and a distance of 3.79 with α-ILE107. Furthermore, it had pi- alkyl interaction with α- ALA71, α-LEU89, α-ILE107, and α-LEU72 at a distance of 4.98 Å, 5.39 Å, 4.82 Å,5.09 Å. The binding of BR9 in the MB protein core cavity was further facilitated by these interactions. The main interaction found between

BR9, and MB protein was hydrophobic interaction. The intricate interaction between the ligand and the protein is highlighted by the particular interactions that have been found which shed light on the binding and acknowledgment between BR9 and MB. Moreover, the Vanderwaals interaction also contributed predominantly to the stability and uniqueness of the complex.

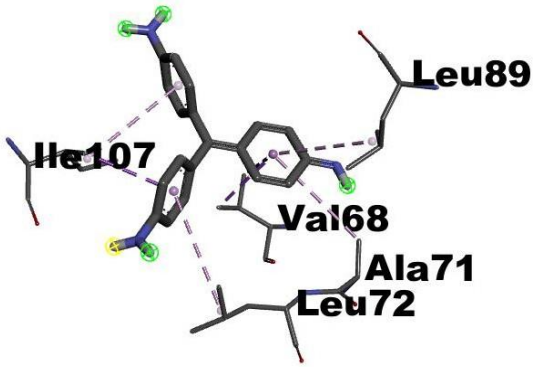

Fig 1: Interactions between BR9 and MB using CB- Dock.

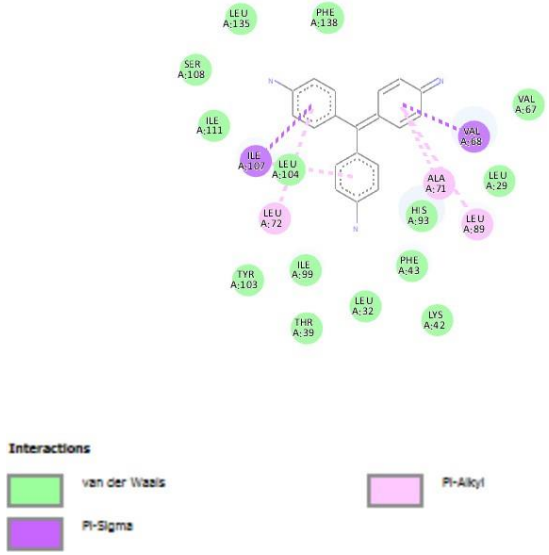

Fig 2: 2-D Interaction between BR9 and MB using CB- Dock.

4.2.1.2. *Seam Dock:*

It was discovered that BR9 prefers to bind in contact with the central cavity of MB. Six hydrophobic contacts were created by BR9 during this binding process (Figure 3). The groups in BR9 participatedin a number of distinct pi-alkyl interactions with α-VAL68 and α-ILE107 at a distance of 3.64 Å and 3.82 Å respectively. Furthermore, it established pi-alkyl interactions with α- ALA71, α-LEU89, α-ILE107 α-LEU72 at a distance of 5.01 Å, 5.44 Å, 4.83 Å, and 5.1 Å respectively. Additionally, Vander Waals's forces also helped in stabilizing the complex (figure 4). Therefore, the hydrophobic interactions and the Vanderwaals interactions collectively aided the complex to become stable.

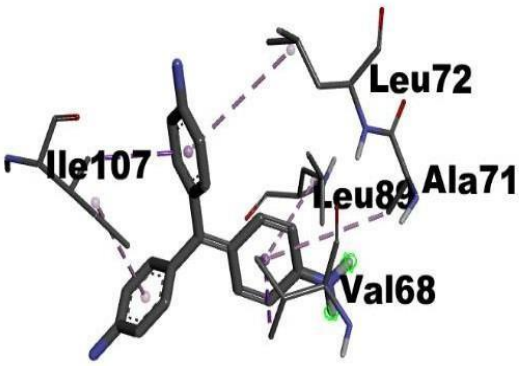

Fig 3: Interactions between BR9 and MB using Seam Dock.

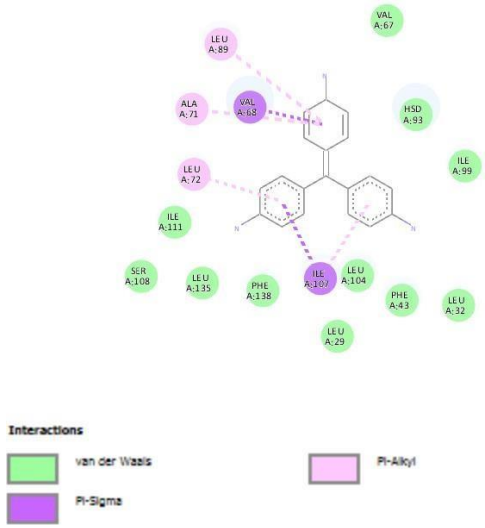

Fig 4: 2-D Interactions between BR9 and MB using Seam Dock.

Table 2: Binding factors in BR9-MB complex.

Docking software	Binding affinity	Hydropho bic	Vander Waals
CB Dock	-6.9	α- VAL68, α- ILE107, α- ALA71, α- LEU89, α-LEU72	α- VAL67, α- LEU29, α-HIS93, α- PHE138, α- LYS42, α- LEU32, α- THR39, α-ILE99, α- TYR103, α- ILE111, α- SER108, α- LEU135, α- PHE138
Seam Dock	-7.2	α- VAL68, α- ILE107, α- ALA71, α- LEU89, α-LEU72	α- VAL67, α- HSD93, α-ILE99, α- LEU32, α- PHE43, α- LEU104, α- LEU29,

			α- PHE138 α- LEU135, α- SER108, α- ILE111

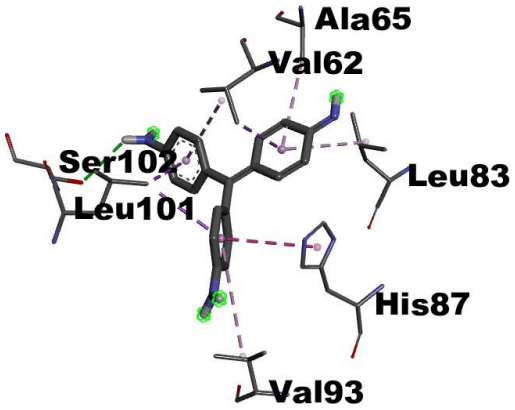

Fig 5: Forces responsible for stabilizing BR9-MB complex

4.2.2. BR9-HB Complex:

4.2.2.1. *CB-Dock:*

The study finds that BR9 interacted with HB, where it built interaction with only one chain. Inside this complex, BR9 set up one hydrogen bond and six hydrophobic interactions which stabilized the complex. BR9 established a conventional hydrogen bond with α1-SER102 with an interspace of 3.03Å. In addition, it formed a hydrophobic connection with α1-VAL62, α1-LEU101, α1-HIS87, α1- ALA65, α1-LEU83, α1-
VAL93 at a distance of 3.97 Å, 3.7 Å, 5.1 Å, 4.73 Å, 4.98 Å, 5.39 Å
respectively. Furthermore, it established pi- sigma interaction with α1-VAL62, α1- LEU101, pi-pi stacked interaction with α1- HIS87, and pi-alkyl interactions with α1- ALA65, α1-LEU83, α1-VAL93 (figure 5).

Overall, the complex was stabilized by conventional hydrogen bonds, Vanderwaal bonds, and hydrophobic interactions.

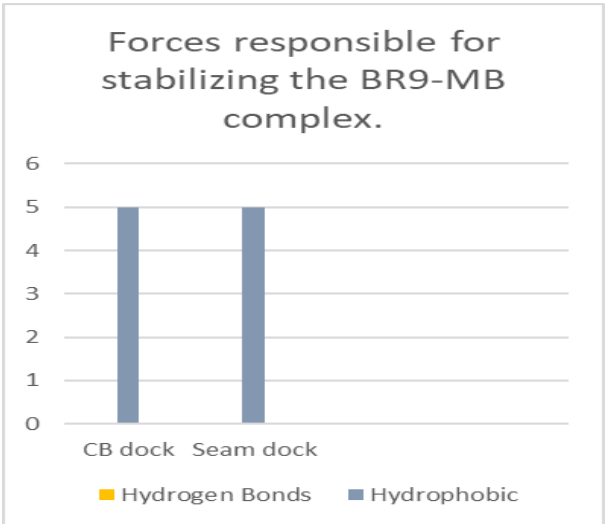

Fig 6: Interactions between BR9 and HB using CB- Dock

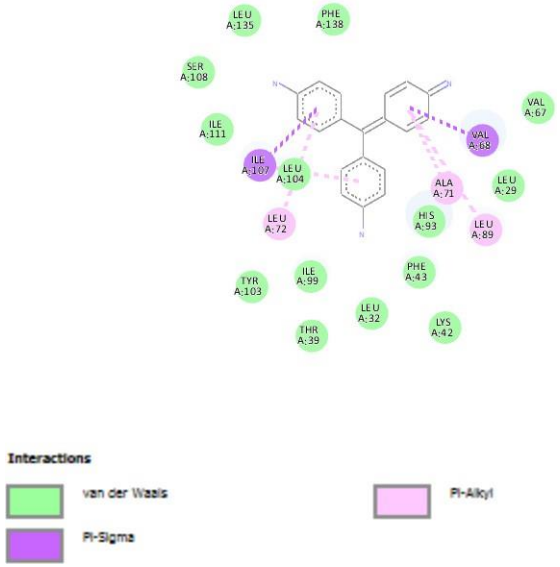

Fig 7: 2-D Interaction between BR9 and HB using CB- Dock

4.2.2.2. *Seam Dock:*

Van der Waals forces, hydrogen bonds, and hydrophobic interactions were principally accountable for the stability of BR9-HB complex formation. Remarkably, BR9 exhibited a predilection for settling inside the middle cavity of HB, where it interacted with the α1, β1, and α2 chains (Fig. 9). BR9 had four pi-alkyl interactions inside the complex one with β1-LEU105, one with α1-LYS99, two with α2-PRO95, at distances of 5.42 Å,
4.73 Å, 4.97 Å and 4.26 Å, respectively (fig. 8). The complex structure's anchoring and positioning of BR9 was probably caused by these interactions. Furthermore, the residueof HB was involved in pi-sigma interaction with BR9 at distances of 3.88 Å, and 3.54 Å. These exchanges additionally aided in the stabilization of the BR9-HB complex.

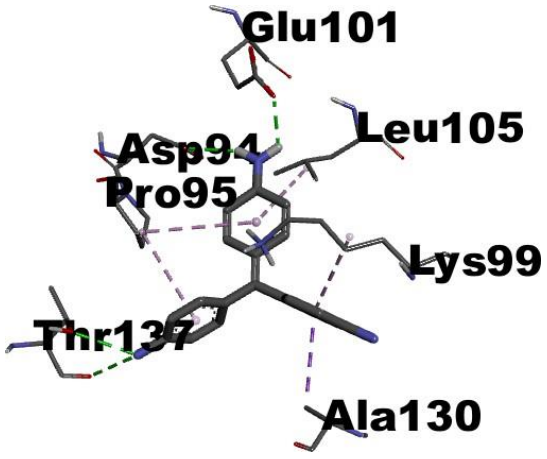

Fig 8: Interactions between BR9 and HB using Seam Dock

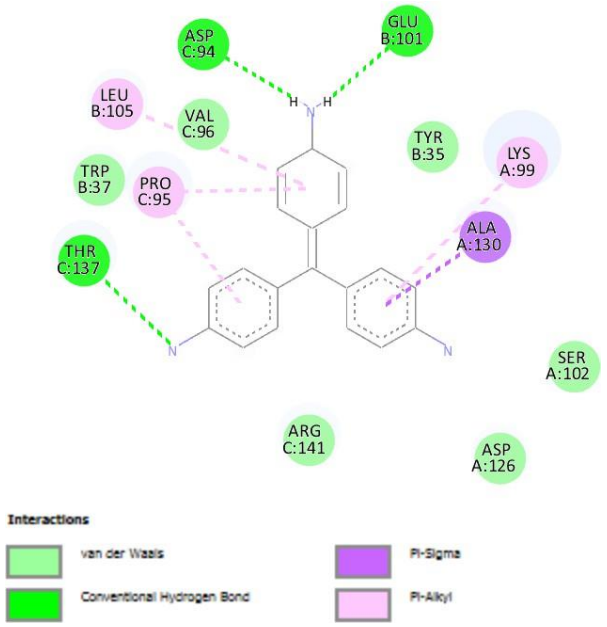

Fig 9: 2-D Interaction between BR9 and HB using Seam Dock

Table 3: Binding factors in BR9-HB complex.

Docking software	Binding affinity	Hydrogen Bonds	Hydroph obic	Vander Waals
CB Dock	-9.2	α1- SER102	α1- VAL62, α1- LEU101, α1- HIS87, α1- ALA65, α1- LEU83, α1- VAL93	α1- LYS61, α1- HIS58, α1- LEU29, α1- MET32 , α1- TYR42 , α1- PHE43, α1- ASN97 , α1- PHE98, α1- SER13 3, α1- VAL13 2, α1- LEU12 9, α1- LEU66, α1- LEU13 6, α1- LEU10 5
Seam Dock	-7.5	α2-ASP94 β1- GLU101 α2- THR137	α1- ALA130, β1- LEU105, α2- PRO95, α1- LYS99	α1- SER10 2, α1- ASP12 6, β1- TYR35 , β1- TRP37, α2- ARG14 1, α2-

				VAL96

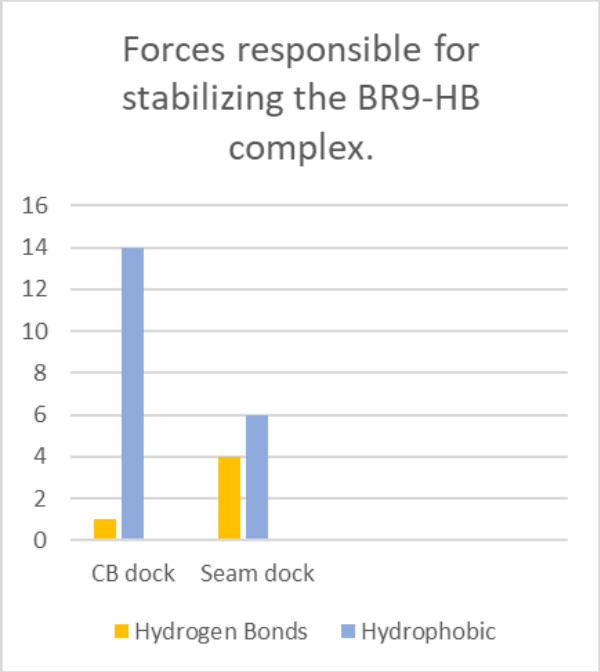

Fig 10: Forces responsible for stabilizing BR9-HB complex.

4.3. Binding position:

4.3.1. BR9 with MB:

The binding position in molecular docking refers to a specific location within a protein where the Ligand binds. The molecular docking method is exerted to speculate the binding orientation, affinity,and interaction of a ligand in the binding site of a protein [14][28]. A molecular docking sampling algorithm generates an enormous number of possible binding modes between two molecules ranked via a scoring function. The scoring function should ideally mark the experimental binding mode as the best solution. The primary binding site in CB- Dock was identified as α-VAL68, α-ILE107, α-ALA71,α-LEU89, and α-LEU72 with the calculated binding affinity of –6.9 Kcal/mol. Whereas in Seam Dock, it predicted as α1- ALA130, β1-LEU105, α2-PRO95, α1-
LYS99 which has a binding affinity of –7.2 Kcal/mol. Here the binding positions of myoglobin with pararosaniline are predicted using webserverssuch as CB-Dock (figure 3) and Seam Dock (figure 4). The binding position is a crucial aspect of molecular docking as it determines the orientation and the confirmation of the ligand within a proteinbinding site, which in turn affects the binding affinity and interaction between ligand and protein.

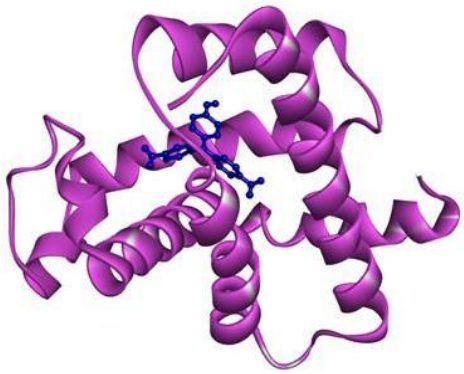

Fig 11: Interaction between pararosaniline and myoglobin using CB-Dock.

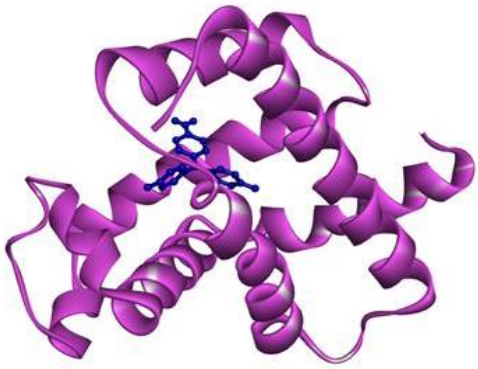

Fig 12: Interaction between pararosaniline and myoglobin using Seam Dock.

4.3.2. BR9 with HB:

The binding positions of BR9 with HB were explored through computational simulations using CB-Dock and Seam Dock. The primary binding site in CB-Dock was identified as α1- SER102, α1-VAL62, α1-LEU101, α1- HIS87, α1-ALA65, α1-LEU83, α1-VAL93
with a calculated binding affinity of –9.2 Kcal/mol. Whereas in Seam Dock, it predicted a distinct binding profile compared to CB-Dock. The primary binding site was identified as α2-ASP94, β1-GLU101, α2- THR137, α1-ALA130, β1-LEU105, α2-
PRO95, α1-LYS99 with a binding affinity of –7.5 Kcal/mol indicating stronger interaction observed with Seam Dock. Here the binding positions of hemoglobin with pararosaniline is predictedusing webservers such as CB- Dock (figure 5) and Seam Dock (figure 6). Therefore, a notable difference is projected in binding positions and affinity comparing between the two docking servers. Additionally, the secondary and tertiary binding site differs between the two methods, highlighting the importance of considering multiple docking approaches for comprehensive analysis. While both the tools offer useful predictions, discrepancies in results emphasize the need for cautious interpretation and validation through experimental studies.

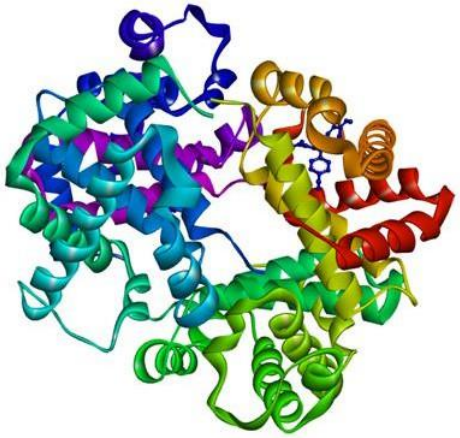

Fig 13: Interaction between pararosaniline and hemoglobin using CB-Dock.

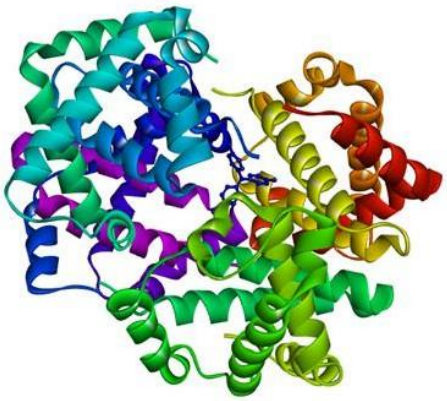

Fig 14: Interaction between pararosaniline and hemoglobin using Seam Dock

4.4 Prediction of toxicity:

The toxicological parameters of pararosaniline were evaluated utilizing the STopTox Web-based Server, an efficient, dependent, and user-friendly implement for determining a chemical's capability to induce acute toxicity to analyze the toxicological characteristics of pararosaniline. This method effectively identifies potential hazards. The finding demonstrated that pararosaniline can have an acutely harmful effect on the respiratory system and the oral system. It also indicated that pararosaniline can cause acute dermal toxicity and is also toxic to the skin. It can also induce irritation and corrosion upon coming in contact with the eyes.

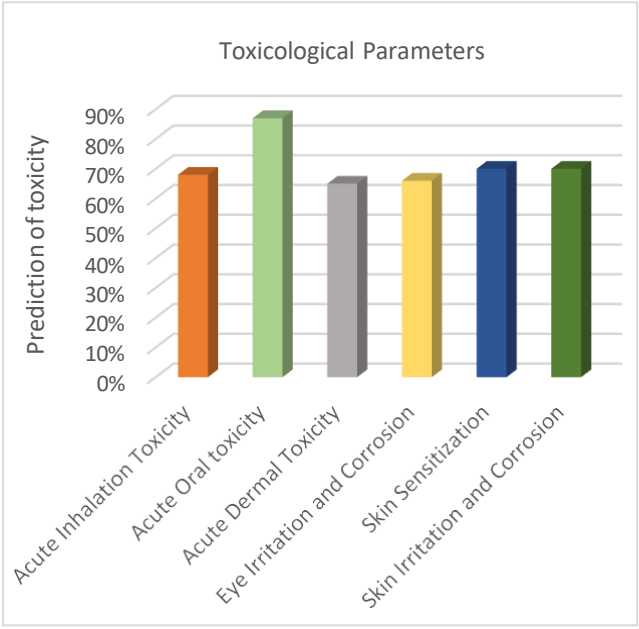

Fig 15: Toxicological parameters of Pararosaniline

5. Conclusion

Our docking studies provided valuable insights into the interaction between pararosaniline and Important proteins, myoglobin, and hemoglobin using CB Dock and Seam Dock. The favorable binding interaction suggests that Pararosaniline has the potential to modulate the function of these proteins which could have implications for human health and food safety. To check the reliability of our studies two different software were used to predict the BR9-MB complex and the BR9-HB complex. Throughout different binding affinities of BR9 are seen with different molecules in each software. Remarkably, hydrophobic interaction was shown to stabilize the complexes made by BR9. Additionally, the STopTox web server calculated the toxic parameters of BR9 which revealed its acute inhalation and oral toxicity prediction, also revealing the risk of eye irritation, acute dermal toxicity, and skin sensitization. Overall our study underscores the importance of computational docking techniques in elucidating the molecular mechanism underlying the interactions between food dye and biological macromolecules, contributing to a broader understanding of their safety and regulatory implications. The findings of our study also highlight the potential health risks associated with pararosaniline exposure and emphasize the importance of continued research efforts to elucidate the toxicological effects. This conclusion emphasizes the importance of computational tools like STop Tox. In early hazard identification and risk assessment, while also acknowledging the necessity of experimental validation to confirm and expand upon the predicted toxicological effects.

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