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Exploring the Energetics and Intermolecular Interactions of Myoglobin Inhibiting Drugs: Bioinformatics

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KEYWORDS

Mb; drugs; DCSP dye; Molecular docking; Hydrogen-Bonding; Binding energy; hydrophobic interactions.

ABSTRACT:

Introduction: Docking techniques were employed to ascertain the nature of interaction existing between Myoglobin (Mb) with drugs that specifically inhibit Mb.

Objectives: Mb as the host and the guest molecules comprises drugs belonging to family of antibiotics (Amoxicillin (AMX), Penicillin G (PenG)), antipyretics (Aspirin (ASA)) and antipsychotics (Promazine (PRZ), Chlorpromazine (CPZ)). 4-dicyanomethylene-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCSP) dye as the competing guest was simultaneously docked to protein-drug complex to explore the binding affinity of protein-drug vs protein-dye complex. The drugs chosen were based on the specific inhibiting action on Mb wherein the protein acts as the host molecule and drug as the guest.

Methods: Molecular Docking (Mol.Dock) studies by AutoDock software version 4.2 as performed for regarding protein- drug-dye system of 10 conformers generated, the stable clusters were selected in the descending order of binding energy (B.E) Mb-drug complex was further docked with DCDAP dye based on Lipinski rule of Five.

Results: The binding energy (B.E) of Mb-drug complex were in the order of AMX > PenG > CPZ > PRZ > ASA. The introduction DCSP dye with the Mb-drug complex resulted in enhanced binding stability. However, simultaneous docking of these drugs to Mb-dye complex resulted in a comprehensive decrease in the affinity of dye towards Mb. The stability and the binding affinity of host-guest complex in the presence of competing guest and vice versa were explored in depth wherein the bimolecular interactions play a significant role.

Conclusions: The study highlight's the importance of Mol.Dock in understanding the biochemical interactions existing between Mb with drugs and dye, providing a significant and valuable link between chemistry and medicine. Both polar and non-polar amino acids contribute to the stability of the complexes in varying aspects.

1. Introduction

Myoglobin (Mb), a globin protein belongs to the superfamily of heme-proteins, consisting of a heme group, an iron atom trapped by a porphyrin ring. The major metabolic function of Mb is responsible for binding, transportation and storage of dioxygen molecule[1]. In vertebrates, the highest concentration of Mb is found in striated muscles which includes both skeletal and cardiac muscles. However, the smooth muscles contain the lowest concentration of Mb[2]. All cells require oxygen, but muscles demand higher oxygen levels for sustained activity. Further, the red colour of muscle depends upon concentration of Mb. Clinically, Mb levels above 91ng/mL in males and 63ng/mL in females indicate muscle damage when detected in blood or urine[3]. Functionally and structurally, Mb is closely related to haemoglobin (Hb).

Hb is a tetrameric protein with four cooperative reversible binding sites for oxygen molecule whereas Mb is a monomeric protein with single non-cooperative reversible binding site[4]. The significance of Mb is that it effectively extracts the oxygen molecule from Hb that transport oxygen molecule through bloodstream. However, both their binding affinities and function are found to be distinct[5,6]. When the body's increased metabolic activities expose the body to oxygen deficient

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state, Mb act as the oxygen source, whereas Hb transports the oxygen from lungs to Mb[7].

Mb is a single polypeptide chain consisting of 153 amino acids (AAs) that form eight alpha helices[8] labelled A-H. These helices constitute approximately 75% of the main chain, with the remaining constituting turns and loops. Interestingly, the sequence of AAs begins and end with glycine (GLY) and exhibits a balanced distribution of 76 polar and 77 non-polar residues. Further, the structure comprising a hydrophobic core and hydrophilic periphery provides an interesting target molecule of study with ligands and drugs[9]. Ongoing research has focused mostly on Mb interaction with various compounds that has been established experimentally by analytical techniques and docking methods. The ligands include phenolic compounds such as gallic acid, tannin and epdigallocatechin-3-gallate (EGCG) as the guest molecules[10-12]. Similarly, studies involving chlorogenic acid, glycerol, lactates, primaquine, nanoparticles, exogenous additives[13-18] with Mb has been well established.

Experimental studies on globular proteins face several challenges mainly due to their solubility in water. X-ray crystallography and NMR spectroscopy have limited resolution, making it difficult to resolve protein structures at the atomic level. Additionally, experimental methods often fail to capture the dynamic behavioural aspects of proteins in solutions that involves protein-ligand interactions and conformational changes accompanying it. Solubility factor poses a significant challenge, as proteins are difficult to solubilize, making it hard to study their behaviour in solution at neutral pH condition. Further conditions like temperature, pH, and ionic strength are significant in optimizing the experimental conditions[19,20] in order to avoid protein denaturation.

To overcome these challenges, molecular simulation (MS) methods offer a more comprehensive and flexible approach. It offers numerous advantages over conventional experimental methods. One of their primary benefits is their cost-effectiveness; significantly reduce the financial burden of research and development involving drug binding with protein. This technique further reduces the time as well as the resources thereby accelerating the drug discovery and development process[21]. Another significant advantage of in-silico methods is their ability to provide atomic-level detail of protein-ligand interactions and allows the researchers to quickly screen large libraries of compounds. This enables researchers to gain a deeper understanding on the binding mechanisms and interactions existing between protein and ligands[22]. Furthermore, an in-silico method provides the binding affinity and specificity of protein-ligand interactions, allowing researchers to design and optimize ligands with improved binding properties[23].

The application of in-silico methods in pharmacokinetics and pharmacodynamics is particularly significant in terms of medicinal chemistry. These methods can predict the Absorption, Distribution, Metabolism and Excretion (ADME) properties of drugs, enabling researchers to identify potential issues early in the development process. The above method optimizes drug design by predicting the binding affinity and specificity of protein-ligand interactions, reducing the risk of adverse effects and improving efficacy. Furthermore, in-silico methods can simulate the pharmacokinetic and pharmacodynamics profiles of drugs, enabling researchers to predict their behaviour in different populations and conditions[24]. These methods presumably identify potential drug targets by analysing protein structures and predicting their interactions with ligands such that the site selective and site-specific nature of the guest with host complex can be ascertained. We have explored binding affinity and energetics of well-established antibiotics norfloxacin with Mb and ligands (Flavonoids)[25,26]. Further, these methods are employed in understanding the effects of mutations and other modifications on protein-ligand interactions to understand the mechanisms underlying the protein function[27,28].

In contemporary, Mol.Dock studies have focused on exploring the interactions of food proteins, egg proteins, and milk proteins with their suitable guest molecules[29-31]. The study extends the application of docking methods to investigate the interactions of heme-proteins with different classes of drug molecules. For comparative purposes, different classes of drug molecules including Antipyretics like Aspirin (ASA), Antibiotics like Amoxicillin (AMX), Penicillin G (PenG) and Antipsychotics like Promazine (PRZ) and Chlorpromazine (CPZ) were chosen, since these drugs inhibit Mb compared to other class of drugs. Additionally, the interaction between host and guest molecules was analysed in the presence of a competing ligand (dye). The binding affinity of the host-guest molecule complex may impact Mb's natural role in storing and transporting oxygen. This study compares the binding affinity and various intermolecular interactions of host-guest interactions existing in different conformers that includes binding affinity and energetics of Mb-drug complex in the presence of dye and vice versa. The structures of guest and the competing guest molecules were provided in **Figure 1**.

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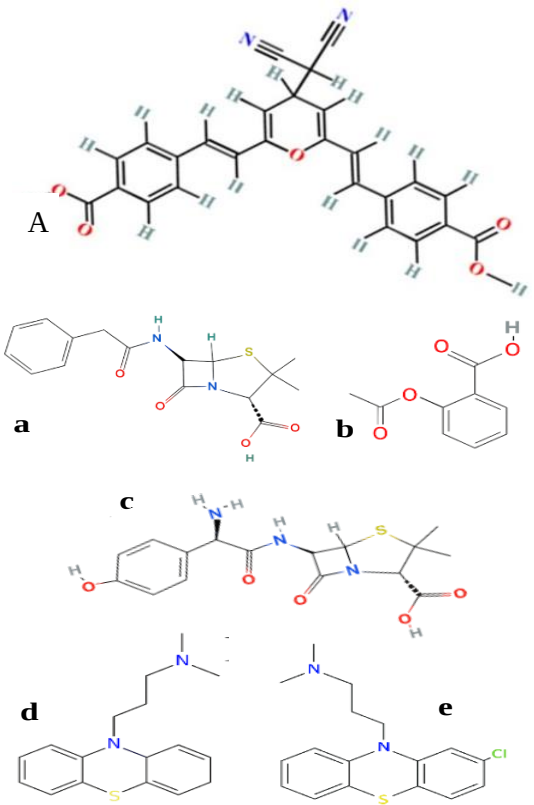

Figure 1: Structure of dye and drugs. A-DCSP dye, a-PEG, b-APN, c-AMX, d-PRZ and e-CPRZ.

2. In-silico methods

These methods utilize computational power to investigate the binding properties and behaviour of large molecules with specific ligand molecules. This approach complements traditional in-vitro and in-vivo studies by providing valuable insights into molecular interactions. Mol.Dock is a key technique employed in in-silico methods[32]. It involves in predicting the binding affinity, active sites and domains of guest into the host molecule. Further, the stabilization/destabilization of the host-guest complex are influenced by the interactions like conventional and non-conventional hydrogen-bonding (H-bonding), hydrophobic, weak force of attraction and unfavourable interactions. On a closer approach on analysis, understanding the underlying mechanisms that govern molecular recognition and binding can be ascertained. The formatting of protein is provided in **Scheme 1**. The three-dimensional crystal structure was retrieved from the Protein Data Bank (PDB)[34,35] using the **PDB ID: 3RGK**, which corresponds to the "Crystal structure of human myoglobin mutant K45R". This structure was experimentally determined using X-ray diffraction studies at a resolution of 1.65Å. The file was downloaded in PDB format and then modified using MGL Tools (Molecular Graphics Laboratory Tools) version 1.5.6. To prepare the protein for docking, water molecules and other ligands, including a sulphate ion and a protoporphyrin ring containing iron were removed. The protein was then formatted to retain only it's A chain and saved it in pdb format, rendering it suitable for docking simulations as in the **Scheme 1**.

A derivative of the dicyanomethylene dimethyl pyran (DDP) dye, known as 4-(Dicyanomethylene)-2,6-diyl(4-carboxystyryl)-4H-pyran (DCSP) dye, was employed as a competing guest molecule. The dye has been involved as the competing guest in studies involving globular proteins[30,31,35]. Moreover, the dye has been utilized as a probe molecule to investigate H-bonding and hydrophobic interactions of protein-guest complexes. Notably, DCSP possesses a greater number of H-bonding acceptors (HB_A) than H-bonding donors (HB_D) compared to the drug as illustrated in **Figure 1**.

To investigate the binding properties of drugs towards Mb, several drug molecules with remarkable binding affinities were selected as the guest. These drugs inhibit Mb, as their binding affinities impact biological functions of Mb[36,37]. For a comparative study, three classes of drugs were explored, which were commonly prescribed drug molecules. The selection of drug molecules was based on Lipinski's Rule of Five, a widely accepted guideline for evaluating the pharmacokinetic properties of drugs[38]. Lipinski's Rule for drug molecules is provided in **Table 1**. The pharmacokinetic properties of the selected drug molecules with Mb were evaluated based on their potential for oral bioavailability.

Molecular Docking

Mol.Dock is a computational technique used to predict the binding affinity and intermolecular interactions between a host molecule (protein) and a guest (ligand). The binding score and nature of interactions determine the stability of the resulting supramolecule. In this study, Auto Dock 4.2, molecular docking software developed by Scripps Research Institute is employed. The docking process[39-41] involved in present study is provided detail in supporting information. The schematic representation of the pathway and methodology employed is provided as a flow chart in **Scheme 1.**

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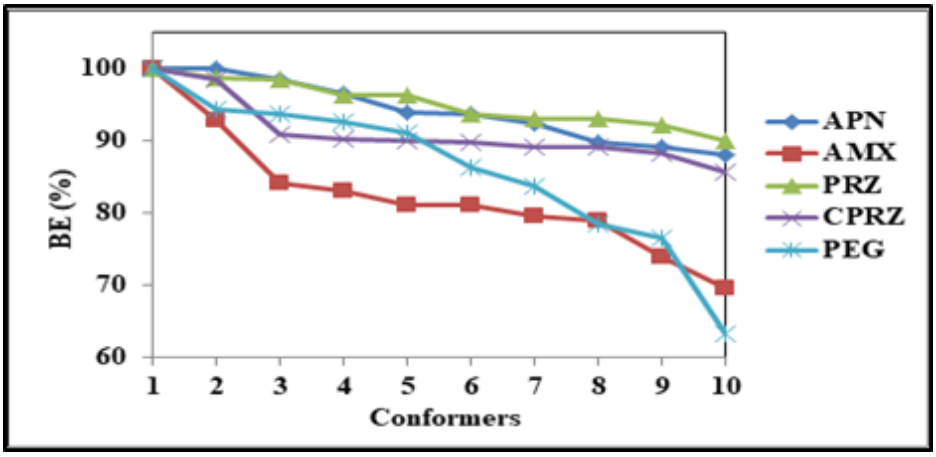

Figure 2: Extent of BE of conformers with Mb-drugs.

Table 1: Lipinski's Rule for drug molecules.

	PEG	AXN	APN	PRZ	CPRZ
Name	Penicillin G	Amoxicillin	Aspirin	Promazine	Chlorpromazine
Type	Antibiotic	Antibiotic	Antibacterial	Antipsychotic	Antipsychotic
Mol. Formula	$C_{16}H_{18}N_2O_4S$	$C_{16}H_{19}N_3O_5S$	$C_9H_8O_4$	$C_{17}H_{20}N_2S$	$C_{17}H_{19}N_2SCl$
Mol. Weight (g/mol)	334.4	365.4	180.2	284.4	318.9
X logP_3 value	1.8	-2	1.2	4.5	5.2
HBD	2	4	1	0	0
HBA	5	7	4	3	3
TPSA (	112	158.0	63.6	31.8	31.8
Rotatable bond	4	4	3	4	2
Heavy atom count	23	25	13	20	21
LIPINSKI's Rule	Obeys	Obeys	Obeys	Obeys	Obeys

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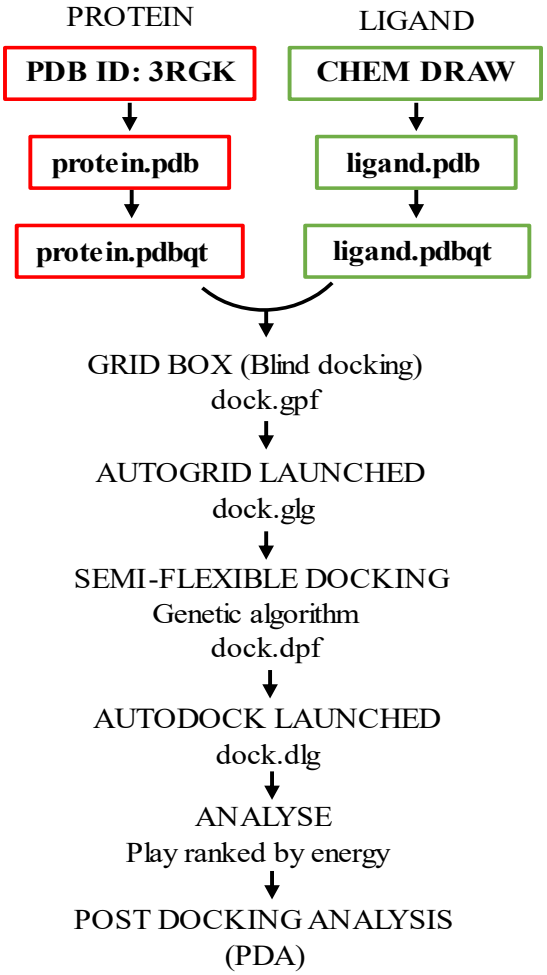

Scheme 1: Work flow in Mol.Dock.

Results and Discussions

Post docking analysis (PDA) was carried out in BIOVIA discovery studio visualizer in which the 3D and 2D structure of the conformers are available for the Mol.Dock studies were performed for Mb with ASA, AMX, PenG, PRZ, and CPZ which forms distinct complexes with supramolecules. For each supramolecule, 10 conformers were generated and based on their docking score, they are ranked according to their inhibitory constant value. The docking score, expressed in kcal/mol, indicated the energetic favourability of each conformer. The first conformer of each supramolecule represented the most energetically favourable binding pose, with the most negative BE value. On the contrary, the energetically least conformer is the last conformer had the positive deviation from the BE value, indicating a less favourable docking score. The 2D and 3D structure of conformers represents the nature and the number of bimolecular interactions with specific AAs.

The ranking of BE values for the fittest conformer is as follows: -7.55, -7.40, -7.16, -6.37 and -6.37 kcal/mol for AMX, PenG, CPZ, PRZ and ASA respectively. The difference in BE values between the extreme conformers determined the probability of their existence as a complex. A plot of BE values for all the 10 conformers with drugs (**Figure 2**) reveal a clear decrement pattern. Notably, antibiotics (AMX and PenG) exhibited a significant deviation from their energetically most stable conformer (~40%), with PenG exhibiting a greater deviation than AMX. In contrast, antipsychotic (PRZ and CPZ) and antipyretic (ASA) drugs displayed relatively lesser deviation (8-12%). This analysis suggested that multiple conformers may coexist in the antipyretic and antipsychotic drug classes, whereas the fittest conformer is more likely to exist in the antibiotic drug class due to its minor deviation in BE values compared with that of

Table 2: BE value of docked complexes ($KcalM^{-1}$).

	Mb+ drug	Mb-drug + dye	Difference	Mb + dye	Mb-dye+ drug	Difference
APN	-4.58	-10.91	6.33	-10.89	-5.11	-5.78
AMX	-7.55	-10.29	2.74	-10.89	-7.32	-3.57
PEG	-7.4	-9.74	2.34	-10.89	-6.49	-4.4
PRZ	-6.37	-10.51	4.14	-10.89	-6.61	-4.28
CPRZ	-7.16	-11.17	4.01	-10.89	-6.67	-4.22

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all the conformers. The BE value of docked complexes is provided in **Table 2**.

To investigate the binding behaviour of the competing guest molecule (DCSP dye) towards Mb-drug complexes, simultaneous docking was performed. The DCSP dye was successfully docked to all the Mb-drug complexes, using the same docking protocol. The resulting complexes, denoted as (Mb-drug) + dye exhibited BE values higher than those of the corresponding Mb-drug complexes (**Figure 3**). The increase in BE value exemplifies that the dye possesses a greater binding affinity towards Mb in the presence of drugs in its active pocket. A comparative analysis of the BE values authenticates that the addition of dye to Mb-drug complex resulted in a significant increase in BE values. The antipyretic, antipsychotic, and antibiotic drugs exhibiting 2.4, 1.7 and 1.4-fold increase respectively.

The BE values of the Mb-drug complexes were normalized to 100%, and that of the (Mb-Drug) + dye complexes were found to be the highest in the case of ASA. The above ranking exemplifies that the dye binds most efficiently to Mb in the presence of ASA. Notably, the increasing trend of BE values for the (Mb-drug) + dye complex and the decreasing order of BE values for the Mb-drug complexes followed a similar sequence of drug

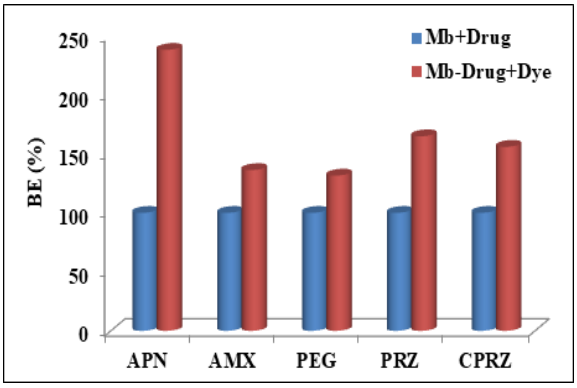

Figure 3: Binding energies of Mb+ Drug and Mb-Drug+ Dye complexes.

complex and the decreasing order of BE values for the Mb-drug complexes followed a similar sequence of drug molecules. This observation suggests a correlation between the binding affinity of dye with specific drug molecule present in the active pocket of Mb. The effective binding of dye to Mb in the presence of drugs would influence the biological functions of Mb, including oxygen storage and transport.

3.1.2 Intermolecular interactions

In addition, to BE, intermolecular interactions play a crucial role in stabilizing the supramolecules. These interactions can be broadly categorized into H-bonding and hydrophobic interactions. H-bonding includes conventional and non-conventional interactions, while hydrophobic encompasses various types, such as pi-alkyl, pi-sigma, pi-anion, and pi-pi stacked interactions. These interactions exist between the guest molecules and specific amino acids (AAs) of Mb. Among the 153 AAs of Mb, a diverse range of AAs from different helices participate in interactions, as the active binding sites vary for different target molecules. **Figure 4** provides a visual representation of the number of polar and non-polar AAs involved in the intermolecular interactions. The represented AAs are a compilation of those involved in interactions across all 10 conformers of each drug complex. The pie charts reveal that the Mb-PRZ and Mb-CPZ complexes exhibit a preference for non-polar AAs over polar AAs, with Mb-PRZ exhibiting a lesser extent than Mb-CPZ. The bimolecular interactions indicate that the antipsychotic drugs in Mb are stabilized primarily through hydrophobic interactions rather than through HB.

Although the top 10 conformers were accounted for, the number of H-bonding and hydrophobic interactions for the energetically favoured conformer of each supramolecule was plotted and analysed in detail. The variation stabilization mechanism is presumably attributed to the unique chemical structures and properties of each drug class.

i. Antipsychotic drugs often possess non-polar, aromatic rings that facilitate hydrophobic interactions with Mb.

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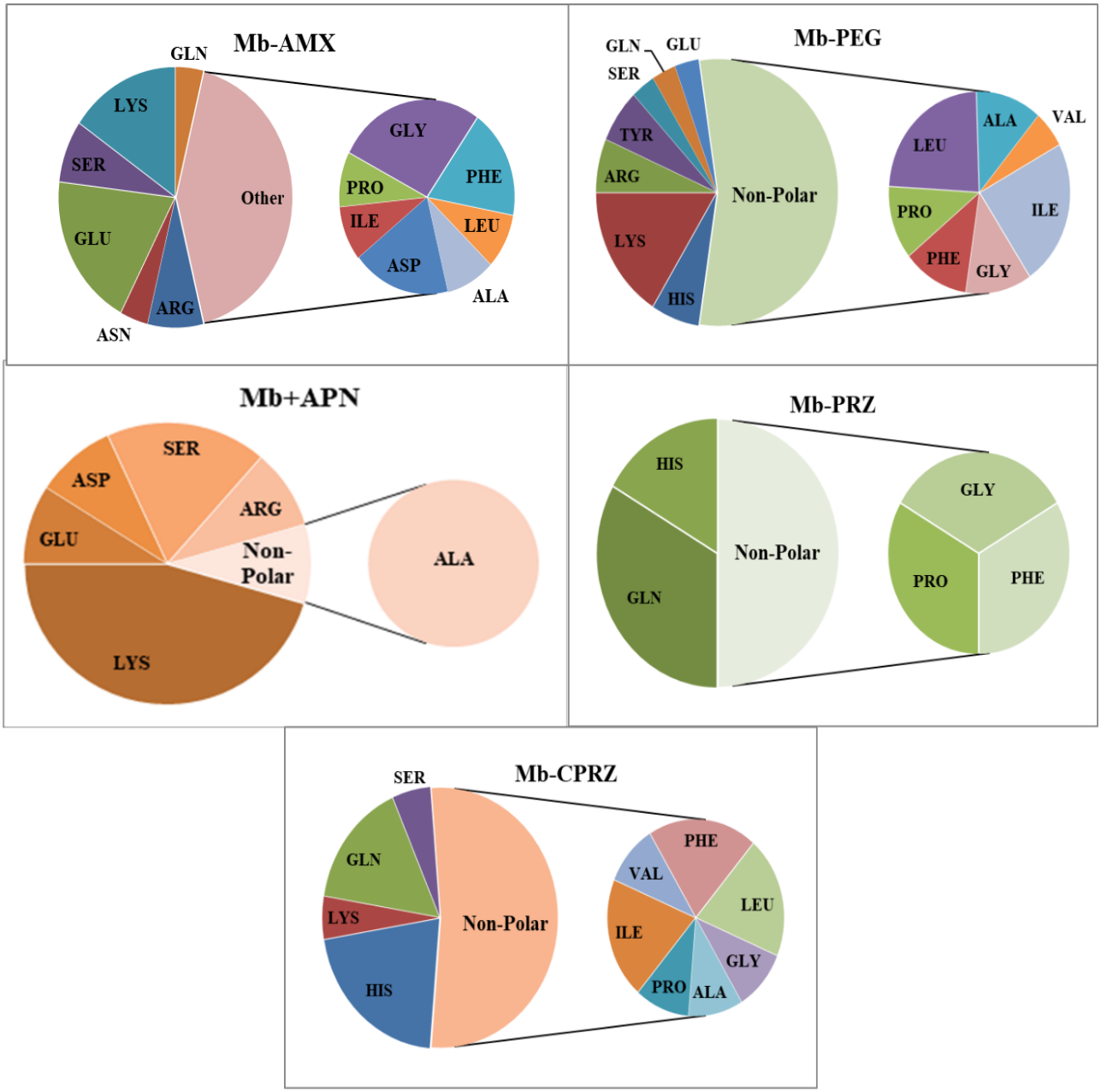

Figure 4: Number of polar and non-polar Amino Acids involved in bimolecular interaction of Mb with antibiotics.

ii. Antipyretic drug (ASA) contains polar functional groups that enable H-bonding interaction with Mb.

iii. Antibiotics, AMX and PenG, have a mix of polar and non-polar functional groups, allowing them to engage in both H-bonding and hydrophobic interactions with Mb.

These findings highlight the importance of considering the specific chemical properties of each drug molecules when investigating their binding mechanisms to Mb.

The intermolecular interactions between Mb and drug molecules were varied considerably in the presence of a competing guest molecule (dye). To investigate the above phenomenon, docking simulations were performed with Mb-dye complex as the host molecule with drug as the guest (Mb-Dye) + drugs. The number of H-bonding and hydrophobic interactions for the fittest

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conformer of each supramolecule were analysed as shown in **Figure 5**. These bars illustrate the contribution of interaction responsible for stabilizing the Mb-dye complex in the presence of various drugs employed. The presence of the dye in the active site of Mb influences the total number of interactions and the contribution of HB and hydrophobic interactions between Mb and drug. Specifically, the total number of

interactions enhanced for Mb-dye complex in the presence CPZ. However, the bimolecular interaction provided a decrease in the case of all other drugs except in ASA. The variation in interaction is presumably attributed to the competing effects of dye and drug molecules for similar binding sites in Mb. The dye, being a planar molecule, presumably occupy a significant portion of the active pocket, influencing the binding mode and interactions of the drug molecules. To further understand the behaviour of guest molecules towards Mb and the effect of the competing guest, a mapping scale of HB and hydrophobicity was employed. This scale provided information on the activity of drug molecules as H-bonding donors (HB_D) or acceptors (HB_A) and their hydrophobicity rates. The mapping scale provided clear variation in the behaviour of different drug molecules towards Mb in complex formation. For instance:

i. The rate of hydrophobicity increased for CPZ in the presence of the dye.
ii. The H-bonding activity of ASA decreased in the presence of the dye.

The mapping scales for Mb-drug complexes and Mb-dye+ drug complexes were compiled in Figure 6, illustrating the role of hydrophobicity, HB_D, and HB_A existing between Mb and drug molecules in the presence and absence of the competing guest.

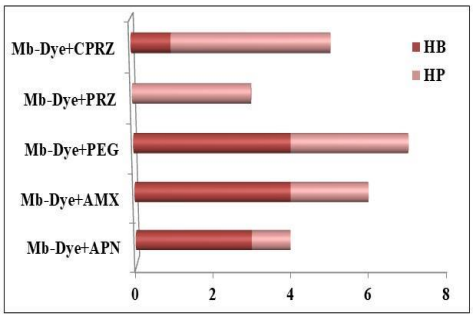

Figure 5: Bimolecular interactions of Mb+ Drug complexes.

The docking simulation between Mb and DCSP dye was performed to predict the direct interaction between the dye and Mb, as well as to identify the active binding pocket of Mb in the presence of dye. The fittest conformer of the Mb-dye complex exhibited BE of -10.89 kcal/mol, indicating a strong interaction between the dye and Mb. To investigate the effect of drug molecules on the Mb-dye complex, the drugs were docked with the most stable conformer of the Mb+ Dye complex. Interestingly, the BE scores of the Mb-Dye in the presence of drug decreased considerably to an extent of 30-40% compared to the Mb-dye complex as shown in the **Figure 6**. This clearly reveals that the drug molecules are less efficient in binding to the Mb-dye complex, likely due to the strong binding of the dye to Mb. Notably, the Mb-Dye + drug complexes exhibited similar BE's, indicating that the Mb-dye complex could not differentiate between the target drug molecules. Regardless of the drug molecule bound to the Mb-dye complex, the decrease in BE scores was found to be similar. In contrast, the Mb-dye + ASA, exhibited a unique behaviour, with a BE roughly half of the Mb-dye complex.

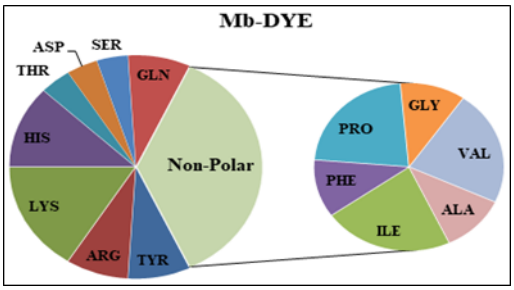

Figure 6: Binding Energies of Mb+ Dye and Mb-Dye+ Drug complexes.

In addition to BE, intermolecular interactions play a crucial role in stabilizing the supramolecule. A collective analysis of the amino acids (AAs) involved in the interactions of the Mb+ Dye complex across all 10 conformers is presented in

Figure 7. Although non-polar AAs contribute to the interactions, polar AAs are primarily responsible for stabilizing the complex. A detailed analysis of the energetically favoured conformer authenticates that hydrophobic interactions, such as pi-pi stacked, pi-sigma, and pi-alkyl, dominate over HB in stabilizing the supramolecule. The dye predominantly acts as an HB_A and, to a lesser extent, as HB_D, with approximately half of the surface are molecule contributing to hydrophobicity. The 2D interaction and mapping scale of HB and hydrophobicity for the fittest conformer of the Mb+ Dye complex are shown in **Figure 8**

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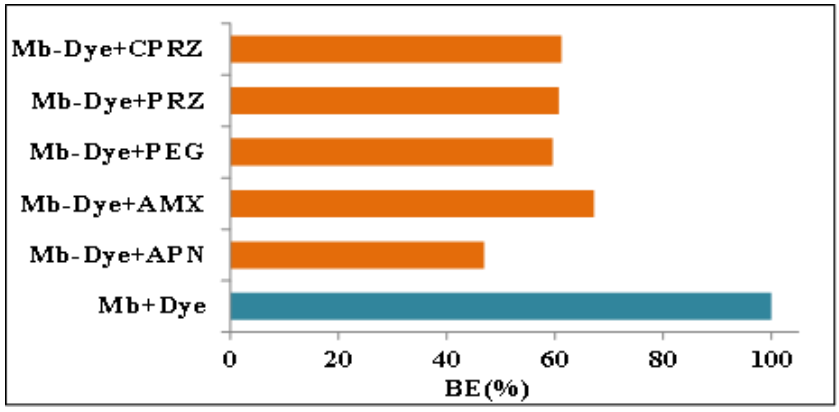

Figure 7: Amino acids involved in bimolecular interaction of Mb+ Dye complex

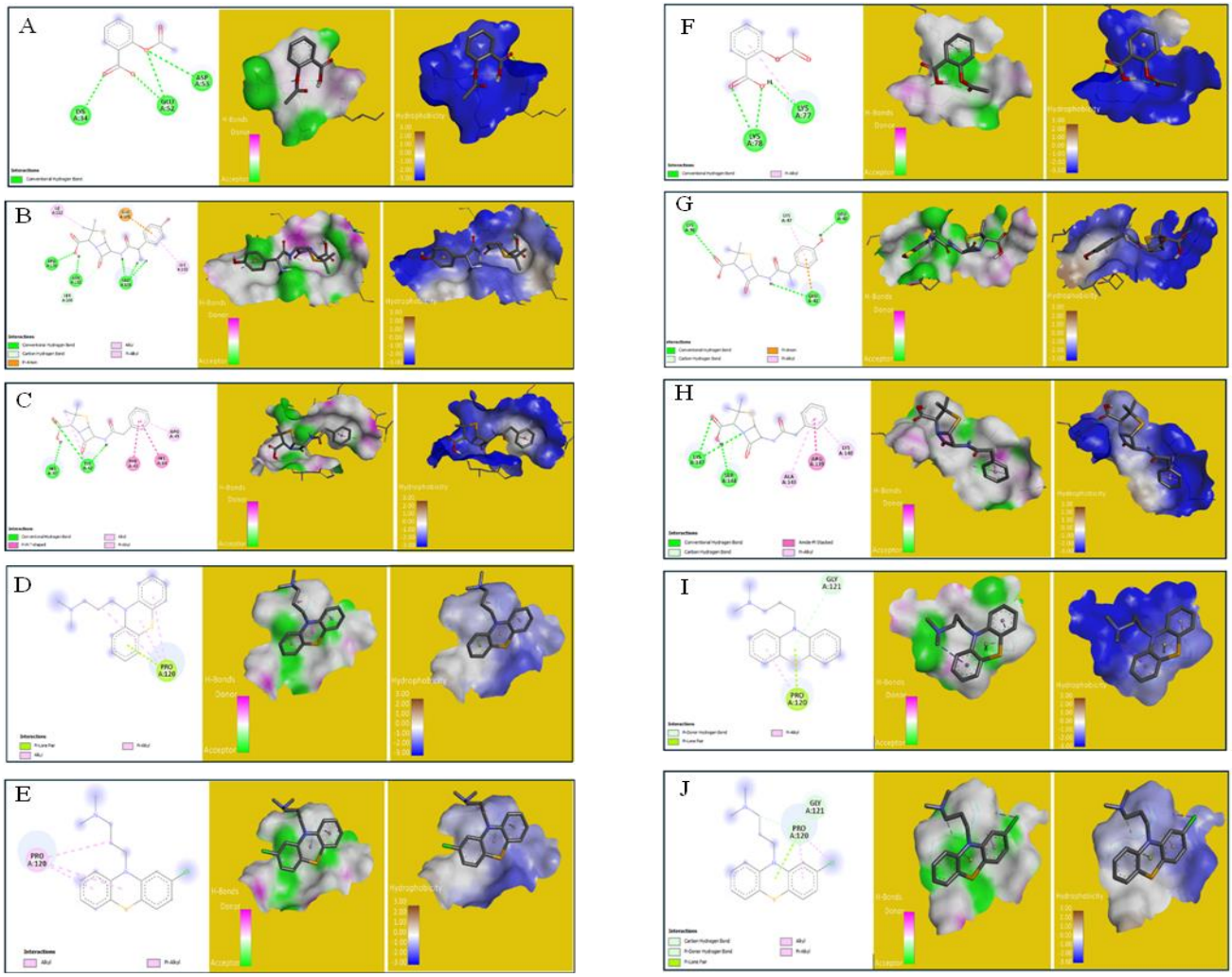

Figure 8: Bimolecular interactions and mapping scales. A to E represent Mb-Drug complexes, whereas F to J represent Mb-Dye complex in the presence of Drugs.

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To investigate how the dye interacts with Mb in the presence of drugs, the bimolecular interaction of the Mb+ Dye complex was compared with Mb-Drug+ Dye complexes. The effects of drug molecules on the interactions were also evaluated.

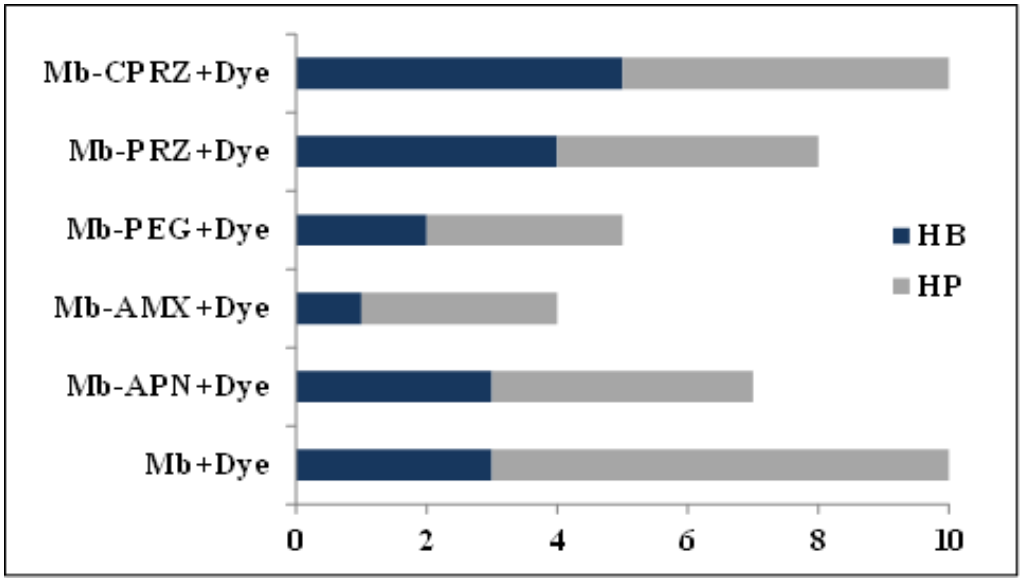

Figure 9 illustrates the HB and hydrophobic interactions of Mb-Drug complex + Dye with the Mb+ Dye complex.

The results depict that, except for antipyretic drugs, HB interactions decreases in the case of antibiotics and increases for antipsychotic drugs while hydrophobic interactions decreased due to the influence of drugs. **Figure 10** illustrates the interactions of the Mb-Drug complex + Dye and the AAs involved in these interactions also the **Figure 9:** Bimolecular interactions of Mb+ Dye and Mb-Drug+ Dye complexesmapping scale of HB and hydrophobicity. A comparison of **Figure 11** reveals, the number and nature of interactions around the dye molecule varies when it is docked directly to Mb (Mb+ Dye) versus to a Mb+ Drug complex (Mb-Drug+ Dye).

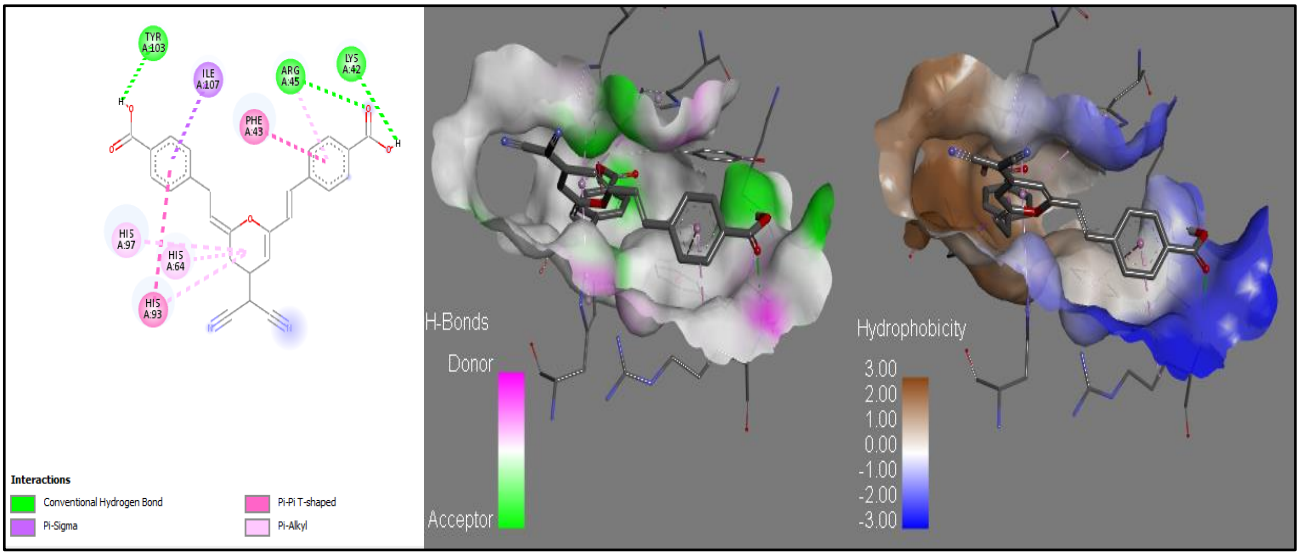

Figure 10: Bimolecular interactions and mapping scales of Mb+ Dye complex.

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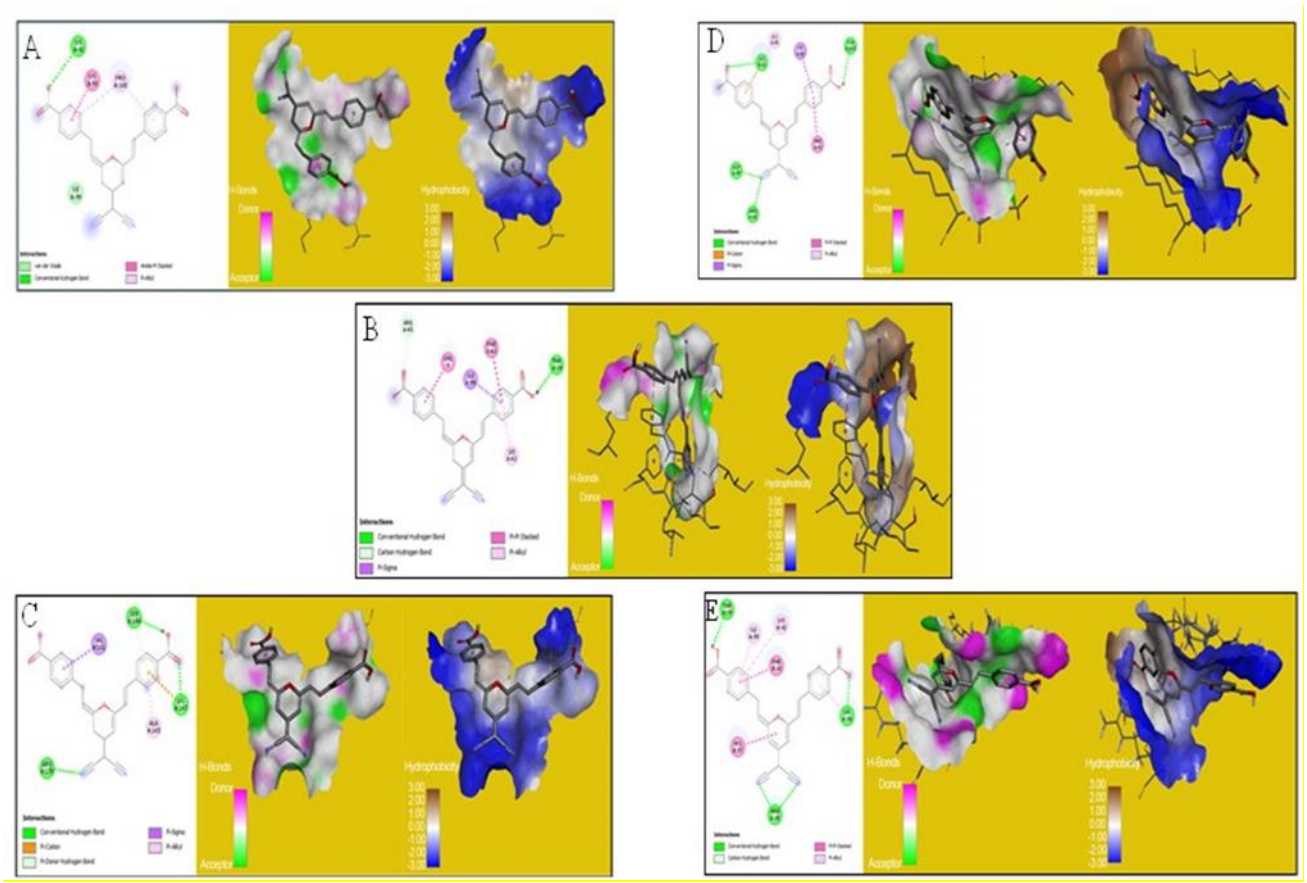

Figure 11: Bimolecular interactions and mapping scales of Mb-Drug+ Dye complexes.

Helical Selectivity

Complex	Mb-Helices
Mb+APN	
Mb-Dye+APN	
Mb+AMX	
Mb-Dye+AMX	
Mb+PEG	
Mb-Dye+PEG	
Mb+PRZ	
Mb-Dye+PRZ	
Mb+CPRZ	
Mb-Dye+CPRZ	
Mb+Dye	
Mb-APN+Dye	
Mb-AMX+Dye	
Mb-PEG+Dye	
Mb-PRZ+Dye	
Mb-CPRZ+Dye	

Helix	Color	Range
Head		1-2
A		3-18
A&B		19-20
B		20-35
C		36-42
C&D		43-50
D		51-57
E		58-76
E&F		77-82
F		83-95
F&G		96-99
G		100-118
G&H		119-123
H		124-149
Tail		150-153

As previously mentioned, Mb's single polypeptide chain contains 8 alpha helices (A-H), connected by turns and loops, with each helix corresponding to a specific range of AAs. **Scheme 2** provides a tabulated representation of the AA ranges for each helix, along with their corresponding colours. Furthermore, the **Scheme 2** illustrates the helices contributing to the active binding sites of the supramolecules under investigation. The scheme also facilitates a comparison of how the active binding site of Mb varies with different guest molecules and the selectivity of these guest molecules in the presence of competing guest molecules. The subsequent discussion elaborates on these findings. The binding site for both Mb+ PRZ and Mb+ CPZ complexes is specifically located in the helices G and H. Notably, the presence of dye has no effect on the binding site of Mb and these antipsychotic drugs.

Scheme 2: Helical selectivity of ligands towards eight helices and non-helical parts of Mb.

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In the Mb+ AMX complex, the primary binding site for AMX is formed by helices G&H. Upon addition of the dye, the site selectivity was significantly reduced. The dye relocates the AMX to helix C and non-helical segments such as C&D and F&G loops. Consequently, the dye diminishes the site selectivity of AMX towards Mb. In the Mb+ ASA complex, the active binding site is primarily formed by helices B and D. However, in presence of the dye, ASA exhibits site-specific binding property towards Mb. Notably, the dye displays ASA from its original helical binding site to non-helical binding site such as turns or loops between the helix E&F. This observation suggests that the dye significantly influences the binding site of ASA.

In the Mb+ PenG complex, PenG's primary binding site includes helices C and E and non-helical parts C&D and F&G. However, in the presence of dye, PenG relocates specifically to the helix H, thereby becoming a site-specific binder to Mb. In the Mb+ Dye complex, the active binding site comprises six regions, including both helical and non-helical segments, indicating a lack of site selectivity. However, in presence of drug molecules, the active binding sites become selective. Notably, all drugs except ASA narrow the binding sites to helix c and non-helical regions C&D and F&G. In contrast, ASA induces site-selective binding of the dye to helices G and H of Mb.

Conclusion

In-silico methods, including AutoDock, PyRx, AutoDock Vina, are employed to investigate the binding properties and interactive behaviour of guest molecules with Mb at the atomic level. Specifically, using the blind docking technique, the molecular interaction reveals the active binding site of guest molecules towards Mb, providing valuable insights into the binding mechanisms and interaction between the amino acid moieties of protein with ligands.

This study provides insights into the energetics and bimolecular interactions of Mb complexes with drugs and dye. The binding energy (BE) of Mb-Drug+ Dye complexes were found to be significantly higher than those of Mb+ Drug complexes, whereas the BE of Mb-Dye+ Drug complexes were lower than those of Mb+ Dye complex. These findings suggest that the presence of a dye can enhance the binding affinity of drugs to Mb. Furthermore, the analysis of bimolecular interactions revealed that antipsychotic drugs exhibit site-specific binding to Mb, both in the presence and absence of a dye. However, the presence of a dye can alters the site-specificity of certain drugs such as AMX.

In contrast, the presence of a drug enhances the dye selectivity of Mb-Drug+ Dye complexes. These findings highlight the crucial role of competing guest in influencing the BE's and bimolecular interactions of Mb complexes. Additionally, this study demonstrates the importance of considering the helical selectivity of Mb when designing drugs or dyes that target the Mb.

Overall, this research study contributes to the better understanding of the pharmacokinetics and pharmacodynamics of drugs that interact with Mb in the concept of drug metabolism and dynamics. The findings of this study can be applied to the development of novel therapeutic agents that target Mb or other globular proteins.

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Conflicts of Interest

The authors declare that there is no conflicts of Interest.

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