SRI VENKATESWARA INTERNSHIP PROGRAM
FOR RESEARCH IN ACADEMICS
(SRI-VIPRA)
Student Internship

SRI-VIPRA

Project Report of 2025: SVP-2502

"Exploring the Bioactivity of Synthesized Heterocycles through Serum Protein Binding Studies"

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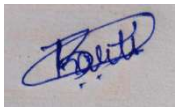
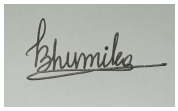
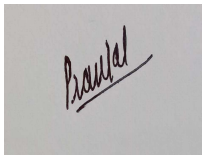
SRIVIPRA PROJECT 2025

Title: Exploring the Bioactivity of Synthesized Heterocycles through Serum Protein Binding Studies

Name of Mentor: Dr. POOJA **Name of Department: Chemistry** **Designation: Assistant Professor**	

List of students under the SRIVIPRA Project

S.No	Photo	Name of the student	Roll number	Course	Signature
1		Bharti Kaswan	1323019	B.Sc. (H) Biological sciences	
2		Bhumika	1123091	B.Sc. Life Sciences	
3		Pranjal Sharma	1524022	B.Sc. (H) Chemistry	

Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2502 titled "**Exploring the Bioactivity of Synthesized Heterocycles through Serum Protein Binding Studies"**. The participants have carried out the research project work under my guidance and supervision from 1st July, 2025 to 30th September 2025. The work carried out is original and carried out in an online/offline/hybrid mode.

Signature of Mentor

Acknowledgements

We would like to begin by expressing our sincere gratitude to **Dr. Pooja** for her unwavering support, guidance, and expertise, which were fundamental to the success of this research on **"Exploring the Bioactivity of Synthesized Heterocycles through Serum Protein Binding Studies".** Her insights into bio-organic research and critical contributions were essentially helpful to explore synthesis. We are deeply thankful to Sri Venkateswara College for providing us with the incredible opportunity to be part of the SRIVIPRA-2025 research initiative. The platform, resources, and academic environment fostered by the institution were crucial in enabling us to explore new ideas and achieve our research goals. We would also like to extend our gratitude to the SRIVIPRA team for their continuous support and encouragement throughout this project. This project has been a true team effort, and we owe its success to the dedication and hard work of all students involved in this project. Their collaboration and insights have driven this research forward, making this journey an enriching and rewarding experience. Additionally, we express our appreciation to the lab staff for their consistent support and technical assistance, which was invaluable throughout the research process. Finally, we are profoundly grateful to God for blessing us with the strength, wisdom, and perseverance needed to undertake this research. His guidance has been a constant source of inspiration, giving us the resilience to overcome obstacles and remain committed to our work. Together, with the combined efforts of our mentor, team, and the blessings of God, we have been able to accomplish what we set out to do. Thank you all for your contributions.

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Introduction

The most prevalent protein in human plasma is human serum albumin (HSA), which makes up between 55 and 60 percent of the total protein in plasma. Numerous endogenous and exogenous substances, such as fatty acids, hormones, drugs, and xenobiotics, can be transported by it in a variety of ways. Because it affects how drugs are absorbed, distributed, metabolized, and excreted, HSA is essential to pharmacokinetics. The presence of two main drug-binding regions—Sudlow's site I in subdomain IIA and Sudlow's site II in subdomain IIIA—as well as structural flexibility and multiple ligand-binding sites contribute to its high binding capacity. These locations are abundant in hydrophobic residues like tyrosine and tryptophan, which allow for a variety of interactions, such as hydrogen bonds, π–π stacking, and hydrophobic forces. HSA is essential to comprehending drug bioavailability, toxicity, and possible drug–drug interactions because of its broad range of interactions. Examining how heterocyclic compounds bind to HSA offers crucial information about their pharmacological transport and therapeutic effectiveness.

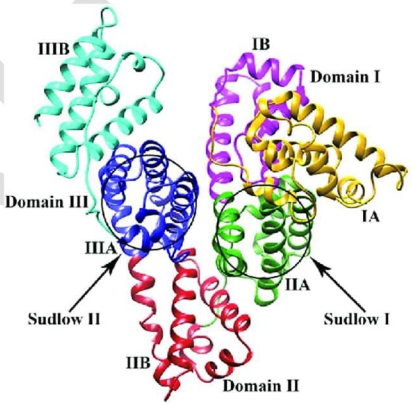

Figure 1: HSA protein structure

Strong and specific binding to HSA is exhibited by heterocyclic compounds, frequently with binding constants in the micromolar to nanomolar range. Nitrogen-containing heterocycles, including derivatives of acridine, quinoline, indole, and pyridine, exhibit high affinity (up

to 10^6 M^{-1}), mostly at Sudlow's site I, where hydrophobic pockets promote hydrogen bonding and π–π stacking. Hydrophobic and van der Waals forces are the primary means of interaction between oxadiazole derivatives, and substitution patterns have a major impact on binding affinity and fluorescence quenching behaviour. Even weaker ligands can change the secondary structure of a protein, as evidenced by the moderate binding of coumarin derivatives like 7-hydroxycoumarin and warfarin, which can cause conformational changes in HSA. Schiff bases made from mercaptobenzimidazole combine with HSA to form stable complexes; fluorescence studies show static quenching, and important hydrogen bonds are verified. Strong and specific binding to HSA is exhibited by heterocyclic compounds, frequently exhibiting binding constants in the micromolar to nanomolar range. Nitrogen-containing heterocycles, including derivatives of acridine, quinoline, indole, and pyridine, exhibit high affinity (up to 10^6 M^{-1}), mostly at Sudlow's site I, where hydrophobic pockets promote hydrogen bonding and π–π stacking. Likewise, the nitrogen heterocyclic alkaloid huperzine A forms stable HSA complexes, suggesting that albumin binding is necessary for its transport. The variety of interactions is demonstrated by metal–heterocyclic complexes, like β-carboline silver compounds, where the heterocyclic core and counterions both affect binding strength through hydrogen bonding and hydrophobic interactions.

Methodology

Spectroscopic methods, thermodynamic analysis, molecular docking, molecular dynamics simulations, and Binding Properties of Heterocyclic Compounds competition experiments are frequently combined in studies on HSA–heterocyclic binding. Quantitative binding data and structural alterations in HSA are revealed by fluorescence quenching, UV-Vis absorption, and circular dichroism. Vant Hoff plots are frequently used in thermodynamic analyses to identify the main interaction forces, which are typically hydrogen bonds and hydrophobic interactions. Stability over time, interacting residues, and ligand binding sites are mapped using molecular docking and dynamics simulations. Site

specificity and possible drug-drug interaction risks can be identified through competition experiments with reference ligands like ibuprofen (site II) and warfarin (site I).

Pharmacological Implications and Binding Insights

HSA's crucial role in drug transport is confirmed by its high binding capacity. Binding affinity and site preference are significantly influenced by the presence and arrangement of heteroatoms, aromatic substitutions, and metal complexes. HSA's capacity to carry extra molecules may be impacted by conformational changes brought on by ligand binding. Understanding HSA interactions is crucial for drug design and safety assessment because, although high-affinity binding can increase a drug's half-life, it may also decrease the free fraction of co-administered medications. HSA also binds strongly to fatty acids, hormones, and metabolites like bilirubin and thyroxine, which affects the pharmacokinetics of therapeutic agents and HSA's ability to bind ligands.

Natural and Endogenous Substances

Hormones and fatty acids are examples of endogenous ligands that alter the structural conformation and ligand-binding behaviour of HSA. Alkaloids (Huperzine A and β-carbolines) and flavonoids are examples of natural compounds that bind through hydrogen bonds and hydrophobic interactions to form stable complexes that are verified by molecular dynamics and spectroscopy. Conformational changes brought on by even mild binders, such as coumarins, can impact how well other medications bind.

Xenobiotics and Synthetic Heterocyclic Compounds

With constants ranging from 10^4 to 10^6 M^{-1}, synthetic heterocycles such as quinoline, indole, pyridine derivatives, oxadiazoles, and Schiff bases exhibit strong and frequently site-

specific binding to HSA. Cannabinoids, synthetic cathinones, drugs of abuse, and environmental pollutants all have strong interactions and often compete with classical ligands at Sudlow's sites I and II. Silver β-carbolines and other metal–heterocyclic complexes show how hydrophobicity and counterions affect HSA binding and can cause secondary structural alterations.

The Binding Mechanism of Coumarin and Imidazole Derivatives

Due to hydrogen bonding and hydrophobic interactions, coumarins, including 7-hydroxycoumarin and warfarin, mainly bind to Sudlow's site I of human serum albumin (HSA) with moderate-to-high affinity (10^4–10^5 M^{-1}). Warfarin's >97% plasma binding emphasizes the clinical significance of site-specific interactions, while hydroxyl substitutions improve stability and could cause conformational changes. Static quenching indicates stable complexes. Imidazole derivatives, such as Schiff bases and antifungal agents, bind strongly (10^4–10^6 M^{-1}) at sites I and II through hydrogen bonding and π–π stacking. Hydrophobic insertion, hydrogen bonding, and electrostatic forces control binding; competition studies with ibuprofen and warfarin validate site selectivity. While metal–heterocyclic complexes may introduce extra interactions and cause slight structural changes, fatty acids and endogenous ligands can allosterically modulate these sites. All things considered, coumarins and imidazole's exhibit robust, site-specific binding that increases circulation half-life but may change the free fraction of medications taken together, highlighting the significance of HSA in pharmacokinetics and medication safety.

Living organisms naturally generate reactive oxygen species (ROS) during cellular metabolism, but their excessive accumulation can overwhelm antioxidant defence and cause oxidative stress. This imbalance damages proteins, lipids, and DNA, contributing to the onset and progression of major chronic and degenerative diseases such as cancer,

neurodegenerative disorders, and cardiovascular conditions [https://doi.org/10.7314/APJCP.2014.15.11.4405].

Binding Properties of Heterocyclic Compounds

Because of this central role in pathology, antioxidants that neutralize ROS are of great therapeutic interest, with heterocyclic scaffolds being especially promising due to their structural ability to stabilize free radicals.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable nitrogen-centered free radical widely used to evaluate the radical scavenging ability of potential antioxidants. In solution, DPPH exhibits a deep violet color with a strong absorbance at 517 nm, which decreases when it accepts an electron or hydrogen atom from an antioxidant, leading to a visible color change. This simple, rapid, and reproducible assay is highly popular for screening natural and synthetic compounds.

Imidazo[4,5-b] pyridines are a well-studied class of nitrogen-containing heterocycles known for their wide pharmacological profile. Literature reports show their antioxidant and anti-glycation activity. Some novely synthesized imidazopyridine compounds studied showed a possible role in helping cure oxidative stress diseases and diabetic complication. [https://doi.org/10.1016/j.arabjc.2015.08.004]. The fusion of imidazole and pyridine rings provides electron-rich nitrogen sites and structural versatility, which not only contribute to their broad biological activity but also suggest potential as radical scavengers, making them suitable candidates for antioxidant evaluation.

Pyranopyrans are bicyclic heterocyclic frameworks formed by the fusion of two pyran rings, and they are frequently encountered in a variety of natural products. These scaffolds are considered “privileged structures” in medicinal chemistry because their conjugated systems and heteroatoms allow diverse biological interactions. Compounds bearing pyranopyran

cores are reported to exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer activities, with their antioxidant potential often attributed to their ability to stabilize free radicals through electron delocalization. Owing to this versatility, pyranopyran derivatives continue to attract significant interest as potential therapeutic agents.

Conclusion

Human serum albumin (HSA), the most abundant plasma protein, is essential for transporting various endogenous and exogenous compounds. Its flexible structure and multiple binding sites allow strong and selective interactions with heterocyclic ligands through hydrophobic forces, hydrogen bonding, and π–π stacking, influencing drug distribution, metabolism, and potential drug–drug interactions. Heterocyclic compounds such as coumarins, imidazoles, quinolines, and oxadiazoles show moderate to strong affinity for HSA, with substitution patterns and metal coordination affecting binding strength and site specificity. Beyond binding, these heterocycles also exhibit notable antioxidant activity, effectively assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which measures radical scavenging through decreased absorbance at 517 nm. Compounds like imidazo[4,5-b]pyridines and pyranopyrans display strong DPPH activity due to their conjugated systems and electron-delocalizing heteroatoms. Overall, combined HSA-binding and DPPH analyses highlight the therapeutic potential of heterocyclic scaffolds in drug development and oxidative stress-related disease management.

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