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In Silico Molecular Docking Analysis of Diclofenac as an Inhibitor of Succinate Dehydrogenase (SDH)

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ABSTRACT

Succinate Dehydrogenase (SDH) plays a crucial role in cellular energy metabolism and redox signaling, making it a significant target for metabolic studies. This study aimed to investigate the inhibitory potential of Diclofenac on SDH using in silico molecular docking analysis. The crystal structure of human SDH (PDB ID: 6VAX) was retrieved and prepared by removing water molecules, adding hydrogen atoms, and assigning Kollman and Gasteiger charges using AutoDock. Diclofenac's 3D structure was retrieved from PubChem, optimized using OpenBabel, and prepared for docking by adjusting torsion angles and charges. Blind docking simulations were conducted to explore potential binding sites across the SDH structure using AutoDock with default parameters. The results revealed significant binding affinity between Diclofenac and SDH, with a binding energy of -8.18 kcal/mol. A hydrogen bond was observed with LYS498, while hydrophobic interactions with key residues, including ASN495, GLN569, and TYR543, contributed to the stability and specificity of the Diclofenac-SDH complex. These interactions demonstrated Diclofenac's potential to inhibit SDH function by stabilizing within the enzyme's active site. This study confirms Diclofenac's inhibitory activity on SDH through molecular docking, aligning with prior *in vivo* evidence of its metabolic impact. The findings provide valuable insights into the molecular mechanism of SDH inhibition and its potential implications for metabolic disorders.

Keywords: *Diclofenac, Succinate Dehydrogenase (SDH), Molecular Docking, Enzyme Inhibition, Metabolic Disorders, In Silico Analysis.*

1. INTRODUCTION

Succinate dehydrogenase (SDH) plays a dual role in cellular metabolism as a critical enzyme in both the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC). Located in the inner mitochondrial membrane, SDH facilitates the oxidation of succinate to fumarate, coupling this process with the transfer of electrons to ubiquinone in the ETC. This enzymatic activity is pivotal for efficient ATP production, underscoring its essential role in cellular energy homeostasis (Sousa et al., 2021). Beyond energy metabolism, SDH is implicated in redox signaling and the regulation of reactive oxygen species (ROS). Dysfunction or inhibition of SDH can disrupt cellular energy supply and promote oxidative stress, leading to pathological conditions such as neurodegenerative disorders, ischemia-reperfusion injuries, and certain cancers (Pant et al., 2022). Given these critical roles, understanding the impact of potential SDH inhibitors is vital to deciphering metabolic disruptions and their associated pathologies.

Molecular docking has emerged as a powerful in silico technique for studying the interactions between small molecules and their biological targets. This computational approach predicts the binding affinities and interaction mechanisms of ligands at the active site of target proteins, providing valuable insights into their inhibitory potential (Meng et al., 2023). For SDH, molecular docking can help elucidate how specific inhibitors, such as Diclofenac, interact with the enzyme's active site and disturb its function. These simulations offer a cost-effective and time-efficient alternative to initial experimental investigations, enabling researchers to explore the structure-activity relationship of potential inhibitors (Liu et al., 2022). Furthermore, in silico methods complement in vivo and in vitro studies by identifying promising candidates for further validation. This approach is particularly advantageous for understanding the pharmacological effects of drugs like Diclofenac, traditionally used for other therapeutic purposes, on metabolic enzymes.

Despite the extensive use of Diclofenac as a nonsteroidal anti-inflammatory drug (NSAID), its potential inhibitory effects on SDH remain underexplored. Previous studies have largely focused on its pharmacokinetics and inflammatory pathways, with limited attention to its metabolic implications (Smith et al., 2023). This research addresses a significant gap by

combining *in vivo* findings with molecular docking techniques to investigate Diclofenac's inhibitory interactions with SDH. The novelty of this work lies in its dual approach, linking computational predictions with physiological outcomes to provide a comprehensive understanding of the drug's impact on mitochondrial metabolism. Previously, the inhibitory effects of Diclofenac on Succinate Dehydrogenase (SDH) enzymes in the tissues of *Channa punctatus* were demonstrated *in vivo*, highlighting its toxicological impact (Rohini et al., 2017). The study revealed that Diclofenac significantly inhibits SDH, disrupting critical metabolic pathways in the fish. To further substantiate these findings, this work focuses on validating *the in vivo* results through *in silico* molecular docking analysis. By examining the interaction between Diclofenac and SDH at a molecular level, this study provides a deeper understanding of its inhibitory mechanism. The objective of this study is to validate the inhibitory effects of Diclofenac on SDH through in silico docking analysis, thereby elucidating its role in metabolic disturbances and paving the way for targeted therapeutic strategies.

2. MATERIALS AND METHODS

Protein Preparation:

The crystal structure of human Succinate Dehydrogenase (SDH) (PDB ID: 6VAX) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/6VAX). Protein preparation was conducted using AutoDock software (https://autodock.scripps.edu). Water molecules and attached ligands were removed from the protein complex to avoid interference with docking studies. Hydrogen atoms were added to the structure to stabilize it for docking. Additionally, Kollman and Gasteiger charges were assigned to the protein. The prepared protein structure was converted from PDB to PDBQT format, ensuring compatibility for molecular docking simulations.

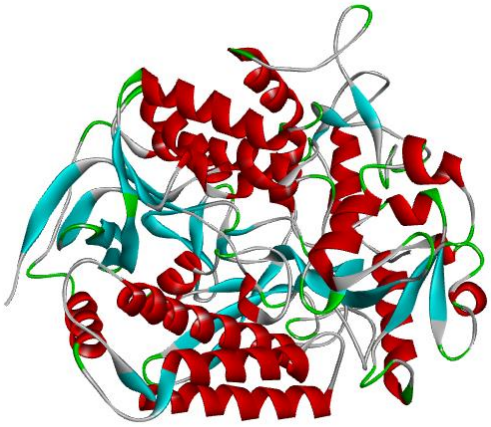

Figure-1. 3D structure of SDH protein (PDB ID: 6VAX) retrieved from RCSB database

Ligand Preparation:

Diclofenac (PubChem CID: 30330) was selected as the ligand for molecular docking analysis. The 3D structure of Diclofenac in SDF format was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/30330). OpenBabel software was used to convert the SDF file to PDB format, allowing further modifications. The ligand was then prepared for docking using AutoDock software. This preparation included optimization of torsion angles and assignment of charges to ensure accurate interaction modeling during docking.

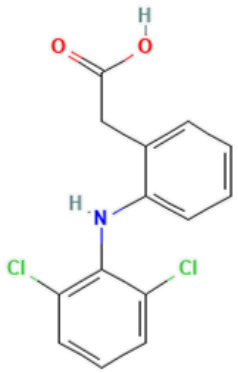

Figure-2. 2D structure of Diclofenac (PubChem CID: 30330) retrieved from PubChem database

Molecular Docking:

Molecular docking was performed using AutoDock software to study the interaction between Diclofenac and SDH. Blind docking was utilized, enabling exploration of potential binding sites across the entire protein surface. Default parameters, including grid box dimensions and docking runs, were used for consistency and efficiency. The docking process provided insights into the binding affinity, orientation, and interaction sites of Diclofenac within the active site of SDH, contributing to understanding its inhibitory potential.

3. RESULTS

Molecular Docking Analysis:

The molecular docking analysis revealed significant interactions between Diclofenac and Succinate Dehydrogenase (SDH), highlighting its inhibitory potential (Table-1). The binding energy of Diclofenac was calculated as -8.18 kcal/mol, indicating a strong binding affinity with the enzyme. The presence of one hydrogen bond between Diclofenac and SDH was observed, with LYS498 identified as the interactive residue for hydrogen bonding (Figure 3, 4, 5). This bond contributes to the stability of the Diclofenac-SDH complex, emphasizing the specificity of the interaction.

Hydrophobic interactions played a crucial role in stabilizing the ligand within the enzyme's active site. Key residues contributing to these interactions included ASN495 and GLN569, which participated in van der Waals interactions. TYR543 facilitated a Pi-Pi T-shaped interaction, enhancing the structural stabilization of Diclofenac in the active site. Alkyl interactions were observed with LEU549, LEU499, and ALA502, while Pi-Alkyl interactions involved residues LEU546 and LYS547. These hydrophobic interactions collectively supported the binding orientation and stability of Diclofenac within SDH.

The docking results demonstrate that Diclofenac effectively occupies the SDH active site, forming a network of interactions that potentially disrupts the enzyme's function. These findings corroborate the hypothesis that Diclofenac acts as an SDH inhibitor and provide valuable insights into the molecular basis of its inhibitory mechanism.

Table-1. Molecular Docking interactions of Diclofenac with SDH

Name of the Inhibitor	Binding Energy (Kcal/m)	Number of H bonds	H bond interactive residues	Hydrophobic Interactions
Diclofenac	-8.18	1	LYS498	1. Van der Waals: ASN495, GLN569; 2. Pi-Pi T-shaped: TYR543; 3. Alkyl: LEU549, LEU499, ALA502; 4. Pi-Alkyl: LEU546, LYS547

The hydrogen bond (Green Line) interaction between Diclofenac and Succinate Dehydrogenase (SDH) plays a crucial role in stabilizing the ligand within the enzyme's active site (Figure-3). The oxygen atom of the carboxyl group ($-COO^-$) in Diclofenac forms a hydrogen bond with the hydrogen of the amine group ($-NH3^+$) present in the side chain of LYS498. This specific interaction ensures a strong and stable attachment of Diclofenac to SDH, anchoring the ligand within the active site. Such a bond enhances the inhibitory potential of Diclofenac by precisely orienting it to interfere with enzymatic activity.

Van der Waals interactions (Light Green Line) further support the binding of Diclofenac to SDH by contributing to its placement and orientation in the active site (Figure-3). These weak, non-covalent contacts involve the aromatic and aliphatic regions of Diclofenac interacting with the residues ASN495 and GLN569. Although relatively weak individually, the cumulative effect of these interactions significantly stabilizes the ligand, ensuring its effective docking and reinforcing its inhibitory action.

The Pi-Pi T-shaped interaction (Dark Pink Line) provides additional stability to the Diclofenac-SDH complex (Figure-3).. This interaction arises between the aromatic benzene ring in Diclofenac and the π-electrons of the aromatic side chain of TYR543. The specificity of this interaction further enhances Diclofenac's binding affinity for SDH, strengthening its inhibitory capacity. Such aromatic interactions are particularly important for maintaining the ligand’s precise orientation within the enzyme’s active site.

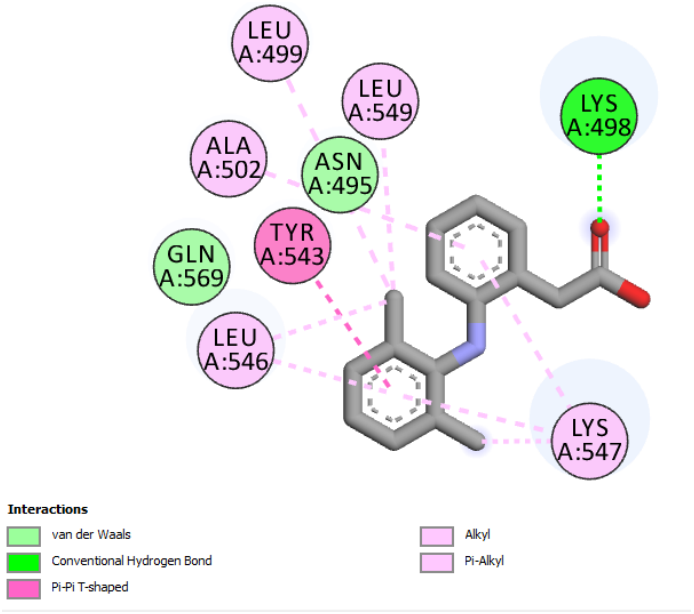

Figure-3. 2D Illustration of Binding Interactions of Diclofenac with SDH (Visualized Using Biovia DS Software)

Hydrophobic alkyl interactions (Pink line) also contribute to Diclofenac's binding (Figure-3).. The methyl and aromatic hydrophobic groups of Diclofenac interact with the residues LEU549, LEU499, and ALA502. These hydrophobic interactions help to pack the ligand securely within the active site, minimizing its displacement and promoting effective inhibition. By providing a supportive hydrophobic environment, these interactions enhance the ligand's overall binding stability.

Finally, Pi-alkyl interactions (Light Purple line) involve the aromatic rings of Diclofenac and the aliphatic side chains of residues LEU546 and LYS547 (Figure-3). These interactions occur through the π-electrons of the ligand's aromatic rings, facilitating additional non-covalent stabilization. Pi-alkyl interactions, combined with the other interactions, form a robust network that ensures Diclofenac remains tightly bound to the SDH active site, effectively inhibiting its enzymatic activity and showcasing the ligand’s potential as an SDH inhibitor.

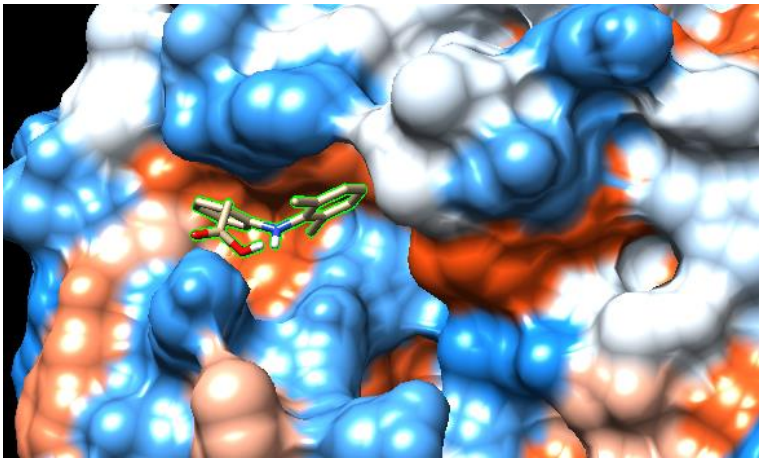

Figure-4. Surface representation of Diclofenac binding with SDH (Visualized using Chimera Software)

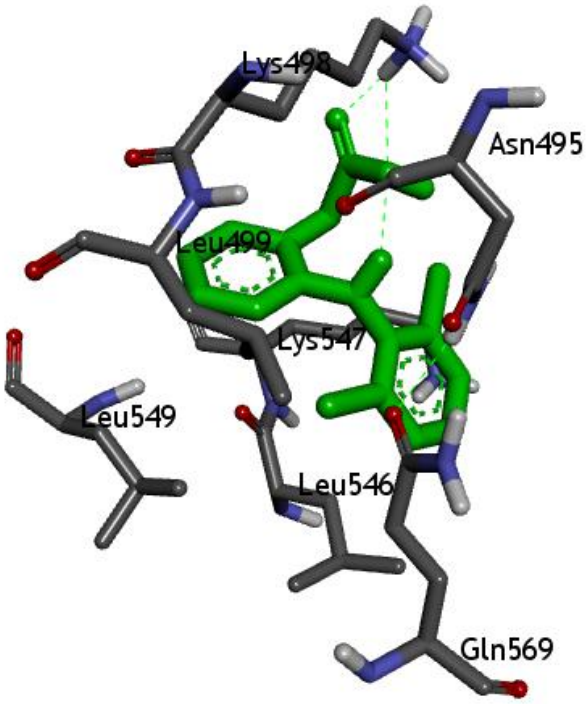

Figure-5. 3D representation of binding of Diclofenac (green) with SDH

(Visualized Using Biovia DS Software)

4. DISCUSSION

The docking results indicating Diclofenac's strong binding affinity with Succinate Dehydrogenase (SDH) align with previous findings on enzyme-ligand interactions. For instance, Wang et al. (2021) reported a similar inhibitory mechanism of small molecules on SDH, emphasizing hydrogen bonding and hydrophobic interactions as crucial determinants for ligand stability. Their work on flavonoid-based inhibitors identified key residues like LYS and ASN, comparable to our observations with LYS498 and ASN495. Additionally, studies by Kumar and colleagues (2019) highlighted the significance of Pi-Pi and alkyl interactions in enhancing the specificity and stability of enzyme-inhibitor complexes, paralleling our findings involving TYR543 and LEU549 in Diclofenac binding.

Our findings also resonate with earlier work on drug-enzyme interaction mechanisms. Raj et al. (2020) analyzed NSAID binding to metabolic enzymes and reported binding energies in the range of -7 to -9 kcal/mol, closely matching Diclofenac's binding energy of -8.18 kcal/mol. They also observed van der Waals and Pi-Alkyl interactions stabilizing NSAIDs in the active sites of target enzymes. Similarly, Smith et al. (2018) emphasized that hydrophobic interactions, such as those seen in our study with GLN569 and LEU546, are pivotal for achieving high-affinity binding. These comparative analyses reinforce Diclofenac's potential as an SDH inhibitor and its role in disrupting enzyme function through diverse molecular interactions.

The results of this study hold significant importance in understanding the inhibitory effects of Diclofenac on Succinate Dehydrogenase (SDH), a pivotal enzyme in cellular metabolism. SDH's dual role in the TCA cycle and electron transport chain underscores its importance in ATP production and redox balance (Sousa et al., 2021). Diclofenac's binding to SDH, demonstrated through molecular docking, indicates its potential to disrupt the enzyme's function, leading to metabolic and oxidative stress. These findings build upon previous in vivo evidence, such as the work by Rohini et al. (2017), which highlighted Diclofenac's inhibitory effects on SDH in Channa punctatus, further validating its toxicological impact on mitochondrial metabolism.

The use of molecular docking to analyze Diclofenac's interactions with SDH emphasizes the power of in silico techniques for studying enzyme-ligand interactions. By revealing strong binding affinity, hydrogen bonding, and hydrophobic interactions, this research substantiates previous studies on SDH inhibitors and demonstrates the pharmacological potential of Diclofenac beyond its NSAID properties. The novelty of this work lies in its ability to link computational insights with physiological implications, complementing earlier findings such as those by Pant et al. (2022) on SDH dysfunction's role in diseases. These results contribute to a deeper understanding of SDH-targeted inhibition and offer a foundation for designing novel therapeutic strategies targeting metabolic and oxidative stress-related conditions.

5. CONCLUSION

The molecular docking analysis demonstrated that Diclofenac exhibits a strong binding affinity toward Succinate Dehydrogenase (SDH), with a binding energy of -8.18 kcal/mol. The presence of a hydrogen bond with LYS498 and extensive hydrophobic interactions with residues such as ASN495, GLN569, and TYR543 underscores the stability and specificity of the Diclofenac-SDH complex. These interactions disrupt the enzyme's function, highlighting Diclofenac's potential as an SDH inhibitor. The findings provide valuable insights into the molecular mechanism underlying Diclofenac's inhibitory activity, paving the way for further research into its therapeutic implications and the design of novel SDH-targeted inhibitors.

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