ORIGINAL ARTICLE

In Silico Molecular Docking Studies of Cell-Penetrating Peptide and Doxorubicin toward Multiple Tumor Receptors

Sakshi Soni, Sushil K. Kashaw, Vandana Soni

Department of Pharmaceutical Sciences, Dr. Harisingh Gour University, Sagar, Madhya Pradesh, India

Abstract

Molecular docking, a powerful computational tool, is revolutionizing drug discovery by illuminating the intricate dance between ligands and proteins. This research delves deep into this molecular tango, analyzing how various ligands interact with key disease-linked proteins. The study employed molecular docking to assess the binding affinity and interaction modes of a novel peptide and doxorubicin across diverse target proteins. The peptide emerged as a star performer, exhibiting remarkably strong binding to crucial proteins such as HER3 kinase (−11.906 docking score) and VEGFR (−7.609 docking score). These impressive scores suggest the peptide's potential as a potent inhibitor for these proteins, potentially disrupting critical cancer pathways. In contrast, doxorubicin displayed significantly weaker binding across all targets, highlighting its potential limitations as an inhibitor. The study further explored the influence of ligand structure and chemical properties on their binding specificity, shedding light on the molecular determinants governing these interactions. By harnessing the power of molecular docking, the research ventured into the exciting realm of rational drug design and virtual screening. Identifying key amino acid residues involved in ligand binding paved the way for designing novel ligands with enhanced binding affinities and improved selectivity profiles. This research paves the way for a deeper understanding of drug-protein interactions at the molecular level, ultimately fostering the development of more effective and targeted therapeutic agents for various diseases.

Key words: Binding affinity, doxorubicin, drug discovery, ligand-protein interactions, molecular docking, peptide, receptors (HER3 kinase, TUBULIN-COLCHICINE complex, NRP-1, EGFR kinase domain, VEGFR)

INTRODUCTION

Breast cancer, a formidable foe impacting millions globally, stands as a testament to the need for relentless research and cutting-edge advancements in therapeutic development. Thankfully, the recent surge of *in-silico* drug development techniques has breathed new life into the fight, with molecular docking emerging as a pivotal weapon in our arsenal. This innovative technology allows us to peer into the intricate dance of molecules at the atomic level, offering precious insights into the very molecular interactions that fuel cancer's progression and pave the way for the design of novel therapeutic agents. In this dynamic landscape of cancer therapeutics, the interaction between surface receptors of breast cancer cells and therapeutic agents holds immense significance. Among these agents, two have shown particular promise: Doxorubicin, a widely used chemotherapy drug, and Peptide sequences, known for their ability to facilitate cellular uptake of various cargo molecules. Doxorubicin, a potent anthracycline antibiotic, exerts its anti-cancer effects by intercalating into DNA and inhibiting topoisomerase II, ultimately leading to DNA damage and cell death, structure shown in Figure 1a. Meanwhile, peptide, structure shown in Figure 1b, possesses the unique ability to translocate across cellular membranes, thereby facilitating the intracellular delivery of therapeutic payloads. When these agents are employed in tandem, their synergistic action on breast cancer surface receptors presents a compelling avenue for therapeutic intervention. By leveraging molecular docking techniques, we can elucidate the intricate binding mechanisms of doxorubicin

Address for correspondence:
Prof. Vandana Soni, Department of Pharmaceutical Sciences, Dr. Harisingh Gour University, Sagar, Madhya Pradesh - 470 003, India. Phone: 9425172178.
E-mail: drsonivandana@gmail.com

Received: 09-02-2024
Revised: 22-03-2024
Accepted: 31-03-2024

and peptides to specific surface receptors expressed in breast cancer cells. This precise understanding allows for the rational design of novel therapeutic combinations tailored to target and disrupt the signaling pathways implicated in breast cancer progression.[1]

Molecular docking has emerged as a cornerstone of *in silico* drug discovery, offering researchers a powerful tool to unravel the intricate dance between small molecules (like potential drugs) and target proteins at the atomic level. Imagine a protein with a specific binding site like a lock waiting for its key is shown in Figure 2. Docking software predicts how well a small molecule fits into this site, gauging its potential to influence the protein's function.[2] This "dry lab" approach, meaning within the computer, offers a valuable glimpse into the molecular interactions underlying disease and treatment. Docking delves deep into the binding site of a target protein, revealing the intricate biochemical processes governing its interactions with small molecules. This information is crucial for designing drugs that can bind effectively and exert their desired therapeutic effects. High-resolution 3D representations of these proteins, obtained through techniques such as X-ray crystallography or cryo-electron microscopy, serve as the foundation for docking studies.[3] A wealth of computational tools and algorithms is available for molecular docking, catering to both commercial and academic needs. These programs and tools have been developed and are currently being used in drug research and academic fields. Some of the most commonly used docking programs include AutoDock Vina, Discovery Studio, Surfex, AutoDock GOLD, Glide, MCDock, MOE-Dock, FlexX, DOCK, LeDock, rDock, ICM, Cdcker, LigandFit, FRED, Schrodinger Maestro, and UCSF Dock. Popular programs include AutoDock Vina, Glide, AutoDock GOLD, and Schrodinger Maestro.[4] These tools empower researchers to simulate and predict the formation of ligand-receptor complexes, providing valuable insights into the potential efficacy of various drug candidates. The computational electrostatics of these complexes are meticulously analyzed through a two-step process. First, ligand conformations are sampled based on the active site of the target protein. These conformations are then ranked using a scoring function, allowing researchers to prioritize the most promising drug candidates. This process predicts the ligand's orientation within the binding site, offering a comprehensive understanding of the complex's shape and electrostatic interactions. The dry lab approach of molecular docking offers a significant advantage over traditional *in vivo* laboratory experiments in terms of resource and time investment. This computational method allows researchers to predict the behavior of ligands in complex formations with proteins or enzymes, providing a cost-effective and efficient alternative to experimental studies. While the utility of docking in drug discovery is well established, its application in pharmaceutical science, particularly cancer research, has seen a recent surge of interest. Docking is increasingly used to validate the molecular targets of peptides, offering crucial insights before embarking on time-consuming and resource-intensive *in vitro* investigations. In the context of breast cancer, molecular docking plays a vital role in identifying and validating lead peptides for potential therapeutic interventions. These studies assess the most promising lead peptides for cancer treatment, paving the way for further *in vitro* and *in vivo* investigations. Predicting ligand behavior and interactions at the atomic level provides researchers with a valuable tool to streamline the drug development process, potentially accelerating the discovery of novel and effective treatments for breast cancer.[5]

However, our foe is cunning and multifaceted, as breast cancer's inherent complexity lies in its heterogeneity, with various subtypes harboring distinct molecular and genetic signatures. While early detection and treatment advancements have yielded positive results for many patients, the battle remains far from over. It is precisely here, where traditional approaches may falter, that the innovative power of molecular docking offers its greatest promise, providing us with the tools to develop effective and targeted interventions against this complex adversary. As research

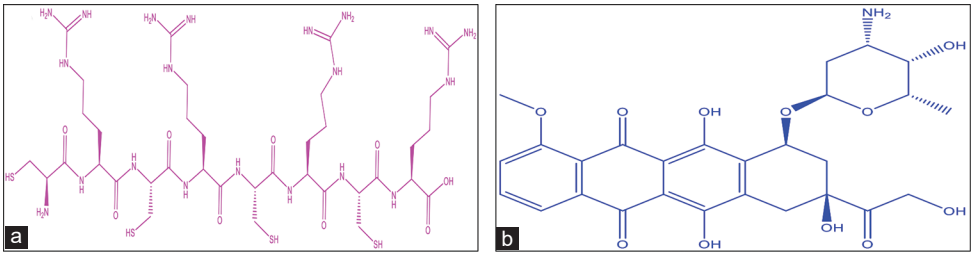

Figure 1: (a and b) Structure of active molecule (peptide) and doxorubicin

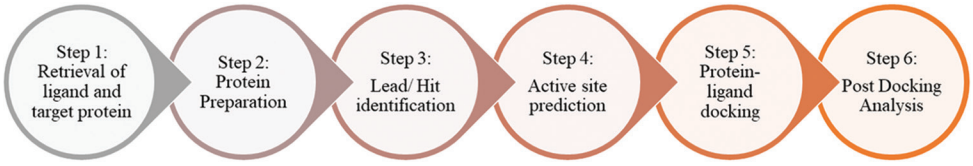

Figure 2: A prototype flow chart of a molecular docking study

continues to evolve, molecular docking is poised to play an even more significant role in the fight against breast cancer.[6] By integrating this powerful technique with other cutting-edge technologies, such as artificial intelligence and machine learning, researchers can gain deeper insights into the complex biology of the disease and develop more targeted and personalized treatment strategies. The future of breast cancer research looks brighter than ever, thanks in part to the power of molecular docking.

MATERIALS AND METHODS

Docking study using glide module of Schrödinger Software

In the context of Glide (Grid-based Ligand Docking with Energetics), the objective is to identify favorable interactions between a receptor molecule, typically a protein, and one or more ligand molecules. It is noteworthy that each ligand must consist of a single molecule, while the receptor can encompass multiple entities, such as a protein and a cofactor. Glide offers two docking modes: rigid and flexible. In flexible docking, the algorithm automatically generates various conformations for each input ligand. A ligand pose in flexible docking represents the convergence of the ligand's position, orientation, and conformation concerning the receptor. A series of hierarchical filters is applied to assess the ligand's interactions with the receptor. The initial filters employ a grid-based methodology, utilizing the empirical ChemScore function to assess the spatial compatibility of the ligand with the designated active site and to analyze the complementarity of ligand-receptor interactions. Poses that successfully pass these initial checks progress to the final stage of the algorithm. This stage involves evaluating and minimizing a grid approximation of the non-bonded ligand-receptor interaction energy, based on the OPLS_3e model. Positions with the lowest energy levels are then prioritized. The scoring of poses is performed by default using the GlideScore multi-ligand scoring mechanism developed by Schrödinger. A composite model score is subsequently utilized to rank the poses of each ligand. This score combines the Glide Score, non-bonded interaction energy, and, in the case of flexible docking, the additional internal energy of the generated ligand conformation. In summary, Glide employs a sophisticated approach to assess and prioritize ligand poses based on spatial fit, interaction complementarity, and energetics. The methodology integrates grid-based filters, empirical scoring functions, and energy minimization to identify and rank the most favorable ligand-receptor interactions.[7]

Protein preparation

The accuracy of Glide results is contingent upon the integrity of the initial protein structures. Schrödinger provides a comprehensive protein preparation tool, the Protein Preparation Wizard, designed to ensure chemical precision and optimize protein structures for compatibility with Glide and other associated products. LigPrep, a counterpart facility by Schrödinger, serves as a complete ligand preparation tool with similar functionalities. It is strongly recommended to utilize these tools for processing both protein and ligand structures to attain optimal results. For incorporating a ligand/protein co-crystallized structure into Maestro, the import can be facilitated from the Protein Data Bank (PDB). To enhance computational efficiency in Glide, particularly for multimeric complexes, it is advisable to retain a single ligand-receptor subunit. However, if the active site necessitates two identical chains, both should be retained. Decisions regarding the retention or removal of water molecules are crucial. In general, waters, except those coordinated with metals, are eliminated. Waters connecting the ligand and protein may be retained based on specific considerations. Adjustments to cofactors, metal ions, and the protein structure are necessary. Repairs are warranted for structures lacking residues in proximity to the active site. In addition, formal charges and ligand bond orders require careful adjustment, particularly regarding bonds between the ligand or a cofactor and a protein metal in complex structures. Caution is advised during protein structure minimization. This process, governed by a user-selected RMSD tolerance, ensures constrained minimization relative to the input protein coordinates. Finally, a thorough review of the resulting structures is imperative. Verification should encompass the correct orientation of water molecules, resolution of steric conflicts, and addressing any hydrogen-bonding issues to ensure the structural integrity and reliability of the prepared systems.[7-9]

Ligand preparation

The fidelity of docked structures is pivotal for producing accurate results that mirror authentic ligand configurations within protein-ligand complexes. Schrödinger's LigPrep, provided with 2D or 3D structures in SDF, formats, proficiently generates high-quality, all-atom 3D structures for a diverse array of drug-like compounds. The LigPrep protocol comprises a series of procedures designed to transform data, rectify structures, introduce structural variations, eliminate extraneous structures, and optimize molecular configurations. Several of these steps are discretionary and can be customized using command-line arguments or by selecting preferences in the LigPrep panel. The sequential steps include as Figure 3: Convert Structure Format: Convert input structures into a compatible format. Select Structures: Choose relevant structures for processing. Add Hydrogen Atoms: Introduce hydrogen atoms to achieve appropriate protonation states. Remove Unwanted Molecules: Eliminate undesired molecular entities. Neutralize Charged Groups: Neutralize charged functional groups. Generate Ionization States: Derive ionization states for the molecules. Generate Tautomer: Generate tautomeric forms for flexibility. Filter Structures: Apply filters to refine the selection of structures. Generate Alternative Chirality: Introduce alternative chirality

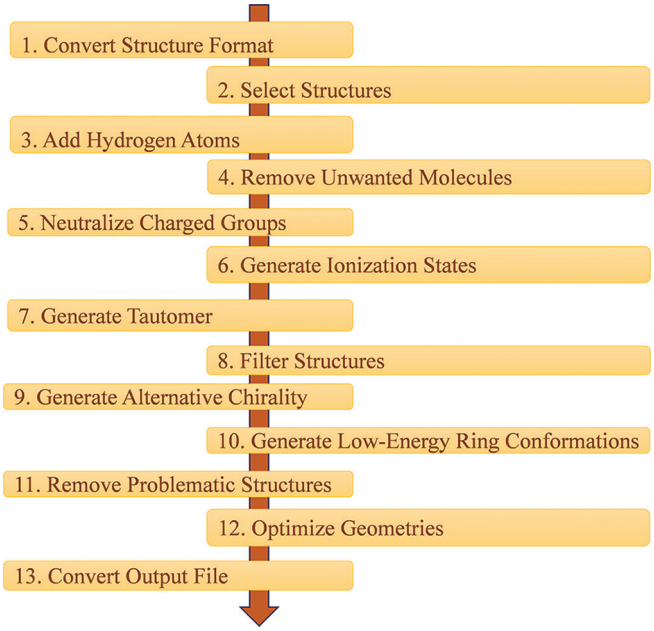

Figure 3: Sequential steps of ligand preparation

where applicable. Generate Low-Energy Ring Conformations: Generate energetically favorable ring conformations. Remove Problematic Structures: Eliminate structures causing computational issues. Optimize Geometries: Conduct geometric optimization for structural refinement. Convert Output File: Convert the final output file into the desired format. It is important to note that the flexibility of LigPrep allows users to tailor these steps based on specific requirements, ensuring the creation of accurate and realistic ligand structures for subsequent docking simulations.[7-9]

Receptor grid generation

Multiple sets of fields are employed to depict the shape and characteristics of the receptor on a grid, providing progressively refined scoring for ligand poses. The Receptor GridGeneration panel is pivotal for generating and configuring the receptor grid. It is imperative to generate receptor grids before initiating a ligand docking task. A "prepared" structure, denoting an all-atom structure with correct bond ordering and formal charges, is requisite for receptor grid formation. The OPLS 2005 force field is employed for grid generation, offering an extensive range of defined atom types and facilitating precise treatment of metals. The Receptor Grid Generation panel comprises five tabs, each serving to specify settings for the receptor grid generation task. These tabs are designated as follows: Receptor, site, constraints, rotatable groups, and excluded volumes.

- Receptor Tab: In this tab, the user defines the portion of the Workspace system for which receptor grids should be computed. In addition, parameters such as scaling receptor atom van der Waals radii can be specified, and the choice of utilizing partial charges from the force field or the input structure is provided.
- Site Tab: Settings within this tab determine the positioning and preparation of scoring grids from the structure in the workspace.
- Constraints Tab: This tab is employed to articulate glide constraints for the generation of receptor grids. Glide constraints represent receptor-ligand interactions deemed crucial to the binding mode based on structural or biochemical data. The implementation of constraints allows glide to eliminate ligands, conformations or poses early in the evaluation process that does not meet these predefined criteria for docking suitability.
- Rotatable Groups Tab: Hydroxyl groups in residues such as Ser, Thr, and Tyr, as well as the thiol group in Cys, can exhibit varied orientations with different ligands. Glide accommodates the flexibility of these groups, allowing them to adopt diverse orientations during ligand docking to optimize interaction outcomes.
- Excluded Volumes Tab: This tab permits the user to restrict ligands from occupying specific spatial regions under defined circumstances. For instance, it enables the prevention of ligands from filling a pocket near the active site if it is known that ligands do not bind there. By configuring this tab, ligands can be prohibited from certain spatial regions during the docking process.[7-9]

Ligand docking

Glide ligand docking tasks necessitate a pre-defined set of receptor grids and one or more ligand structures. In instances where a correct Lewis structure cannot be ascertained for a ligand, the docking job excludes that particular ligand from the process. Glide also autonomously bypasses ligands featuring unparametrized elements, such as tin, or atom types not supported by the OPLS force fields, including explicit lone pair "atoms." The Ligand Docking panel encompasses several tabs, each serving specific functions: ligands, settings, core, constraints, torsional constraints, and output. Notably, if a ligand fails to generate a correct Lewis structure or includes elements unsupported by the force fields, Glide systematically omits it during the docking procedure. Molecular modeling investigations employing the Glide module of Schrödinger were conducted to explore potential interactions between the most potent derivative and the protein of interest.[7-9]

Docking study

Molecular docking investigations involving doxorubicin and (CR) 4 peptide were conducted utilizing the receptor proteins HER3 (PDB 4RIW), TUBULIN (PDB 1SA0), NRP-1 (PDB 4DEQ), VEGFR (PDB 3w7b), EGFR (PDB 4i23), and HER2 (PDB 3ppo), respectively. The glide module software within Schrödinger Maestro v13.5 was employed for these docking studies. Protein structures were sourced from the PDB. The acquired protein structure underwent further refinement through the "protein preparation workflow" within Maestro Wizard version 13.5. This workflow encompassed generating states and refinement steps to enhance the protein structure.

Improvements included the optimization of hydrogen-bonded groups, dehydration processes, and restrained minimization using the default force field OPLS_3e. The resultant minimized protein structure was then utilized to generate a grid surrounding the ligand molecule. Diverse conformations of docked ligands were observed in the docking results, each exhibiting distinctive binding energy scores. Rankings were assigned based on these scores, with higher ranks corresponding to lower scoring conformations. This ranking system was employed to identify and prioritize ligand poses based on their binding affinities.[10,11]

RESULTS AND DISCUSSION OF MOLECULAR DOCKING

A molecular docking study was undertaken to elucidate potential interactions between a series of potent ligands and a target protein using the Glide module within the Schrödinger software. The present study employs molecular docking techniques to investigate potential interactions between a series of potent ligands, namely a peptide (active compound), and doxorubicin (reference compound), with various target proteins implicated in cancer pathways. Utilizing the Glide module within the Schrödinger software, the study aims to elucidate the binding affinities of these ligands toward their respective protein targets, thereby shedding light on their potential as inhibitors of enzyme activity crucial for cancer progression. Docking scores, which serve as a measure of the predicted binding affinity between a ligand and a protein, were calculated for both the peptide and doxorubicin across different proteins of interest.

The docking results presented in the Table 1 demonstrate the predicted binding affinities between different receptors and their respective ligands, as indicated by the docking scores. Lower docking scores suggest stronger interactions between the receptor and ligand molecules. For instance, HER3 kinase exhibited a higher docking score with peptide (−11.906) compared to doxorubicin (−6.458), suggesting a potentially stronger binding affinity of peptide to HER3 Kinase. Similarly, tubulin β colchicine showed a more favorable docking score with peptide (−9.582) compared to doxorubicin (−7.759), implying a stronger interaction with peptide. These trends are consistent across other receptors such as NRP-1, EGFR Kinase, and VEGFR receptors, where peptide generally exhibited lower docking scores compared to doxorubicin. Overall, the docking results validate the hypothesis that Peptide ligands have a higher predicted binding affinity to these receptors compared to doxorubicin, providing valuable insights for further experimental validation and potential drug discovery efforts targeting these receptors.

Table 1: Docking score of ligand receptor interaction

S. no	Receptor name	PDB file number	Ligand name	Docking score
1.	HER3 Kinase	4riw	Peptide	−11.906
2.	HER3 Kinase	4riw	Doxorubicin	−6.458
3.	Tubulin β colchicine	1SA0	Peptide	−9.582
4.	Tubulin β colchicine	1SA0	Doxorubicin	−7.759
5.	NRP-1	4deq	Peptide	−7.695
6.	NRP-1	4deq	Doxorubicin	−5.040
7.	EGFR Kinase	4i23	Peptide	−8.455
8.	EGFR Kinase	4i23	Doxorubicin	−5.274
9.	VEGFR receptors	3w7b	Peptide	−7.609
10.	VEGFR receptors	3w7b	Doxorubicin	−6.145

The molecular docking study revealed varying docking scores for both the peptide and doxorubicin across different target proteins. Notably, the peptide demonstrated strong binding affinity with several proteins, including an impressive docking score of −11.906 with the HER3 kinase (4RIW). Similarly, interactions with TUBULIN-COLCHICINE complex (1SA0), NRP-1 (4DEQ), EGFR kinase domain (4i23), and VEGFR (3w7b) also exhibited favorable docking scores of −9.582, −7.695, −8.455, and −7.609, respectively, as shown in Figure 4a-l. These findings suggest the peptide's potential as an effective inhibitor for these proteins involved in cancer pathways. In contrast, doxorubicin displayed lower docking scores across all proteins compared to the peptide, with the lowest docking score of −5.040 observed for its interaction with NRP-1 (4DEQ). This implies a comparatively weaker binding affinity of doxorubicin toward NRP-1, suggesting its limited effectiveness as an inhibitor for this protein.

The findings of this study highlight the potential of the peptide as a more potent inhibitor of the investigated proteins compared to doxorubicin. Furthermore, molecular docking emerges as a valuable tool for identifying potential treatment targets, enabling the prediction of binding affinities and conformations of ligands with target proteins. The utilization of computational techniques such as molecular docking has revolutionized the drug discovery process, improving efficiency and efficacy by reducing the time and expense required for conventional experimental procedures. Moreover, molecular docking holds considerable promise in research on dietary supplements, offering a means to identify novel therapeutic targets and develop safe and effective supplements for disease treatment. By leveraging the availability of databases and advancements in computational tools, researchers can harness the power of molecular docking to accelerate the discovery of novel therapeutic interventions.

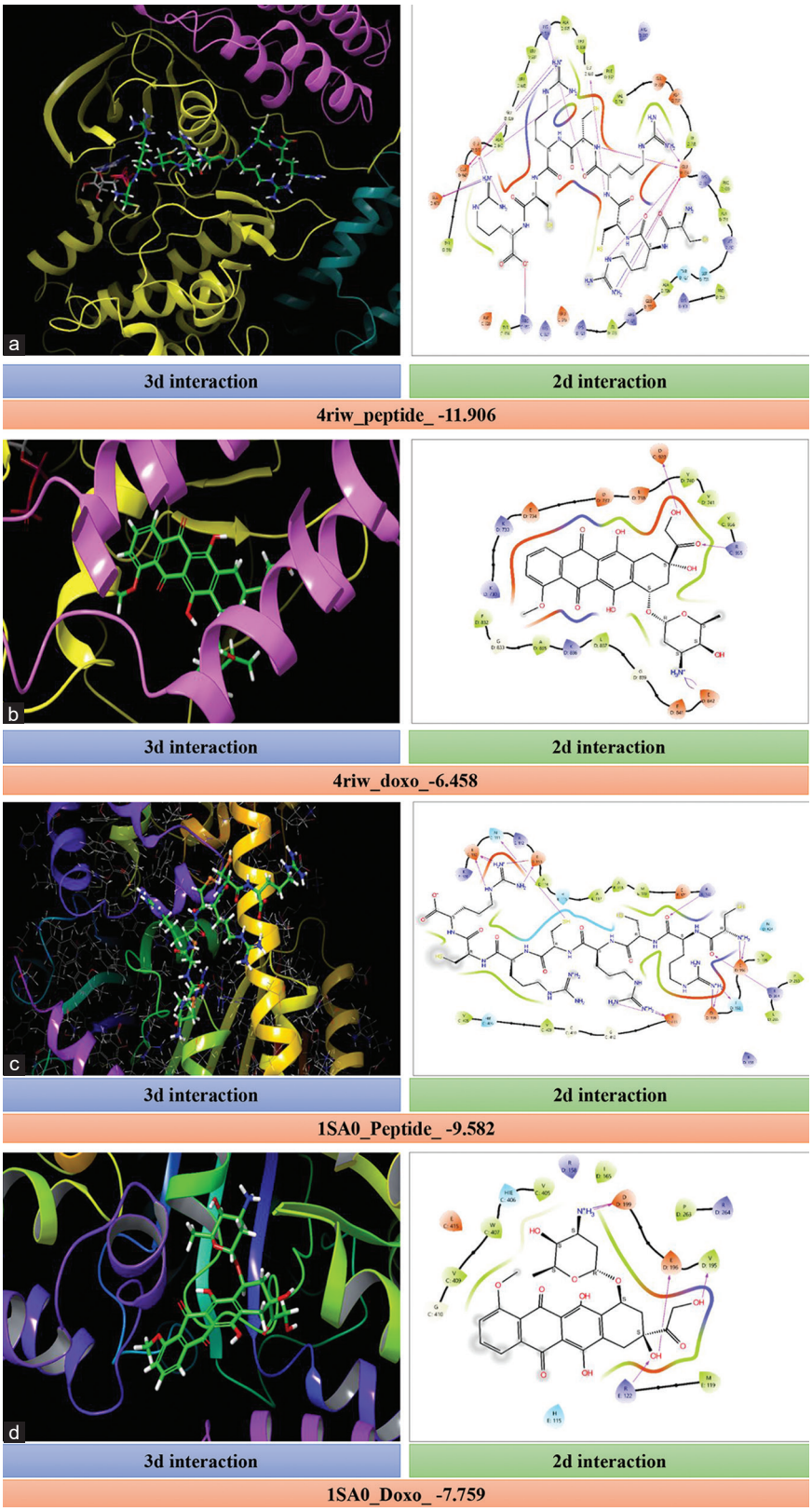

Figure 4: (a) 3D and 2D interaction of PDB 4riw_peptide_−11.906. (b) 3D and 2D interaction of PDB 4riw_doxo_−6.458. (c) 3D and 2D interaction of PDB 1SA0_Peptide_−9.582. (d) 3D and 2D interaction of PDB 1SA0_Doxo_−7.759. (e) 3D and 2D interaction of PDB 4deq_Peptide_−7.695. (f) 3D and 2D interaction of PDB 4deq_doxo_−5.040. (g) 3D and 2D interaction of PDB 4i23_peptide_−8.455. (h) 3D and 2D interaction of PDB 4i23_doxo_−5.274. (i) 3D and 2D interaction of PDB 3w7b_Peptide_−7.609. (j) 3D and 2D interaction of PDB 3w7b_doxo_−6.145. (k) 3D and 2D interaction of PDB 3ppo_peptide_−8.727. (l) 3D and 2D interaction of PDB 3ppo_Doxo_−7.715

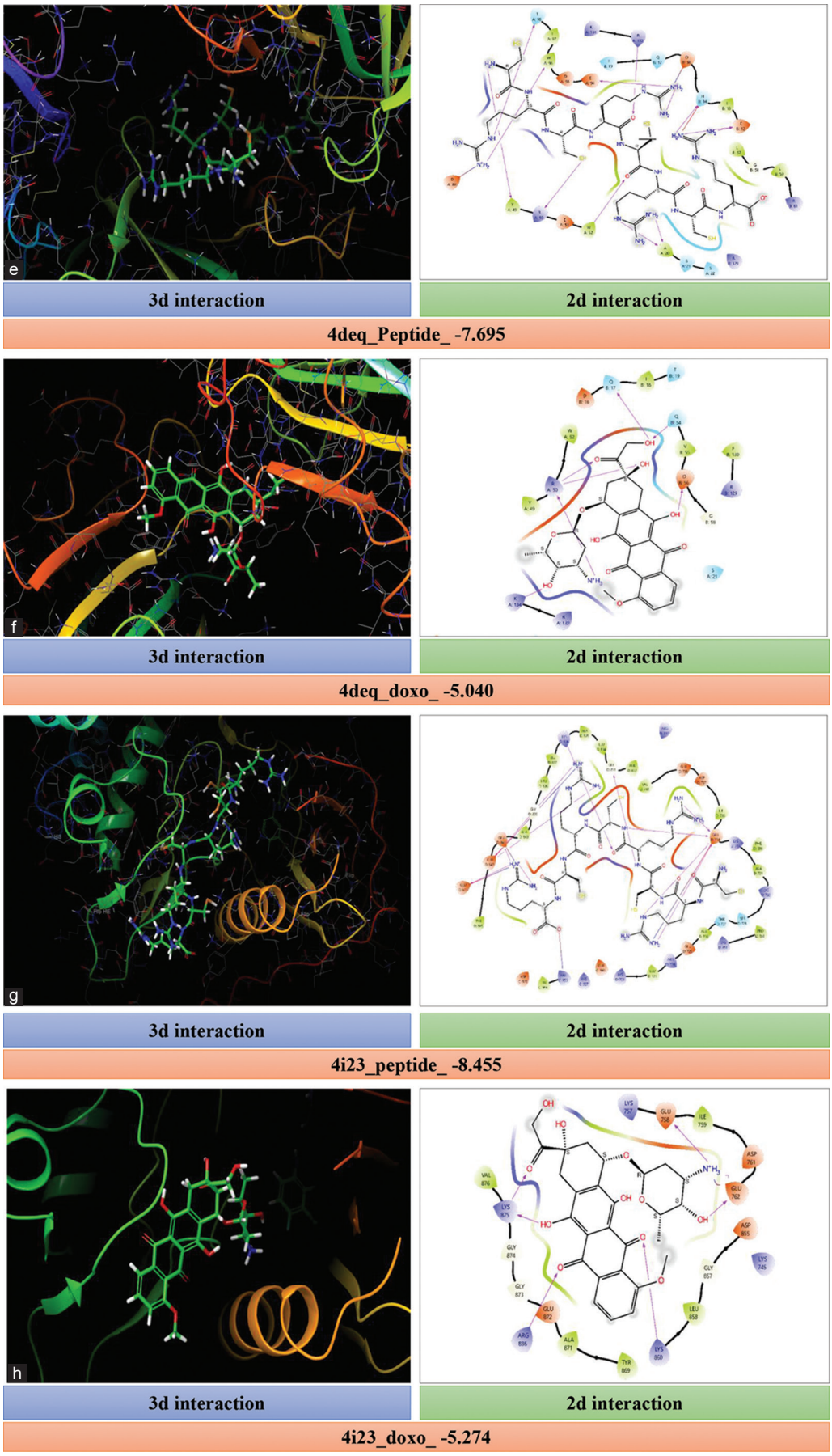

Figure 4: *(Continued)*

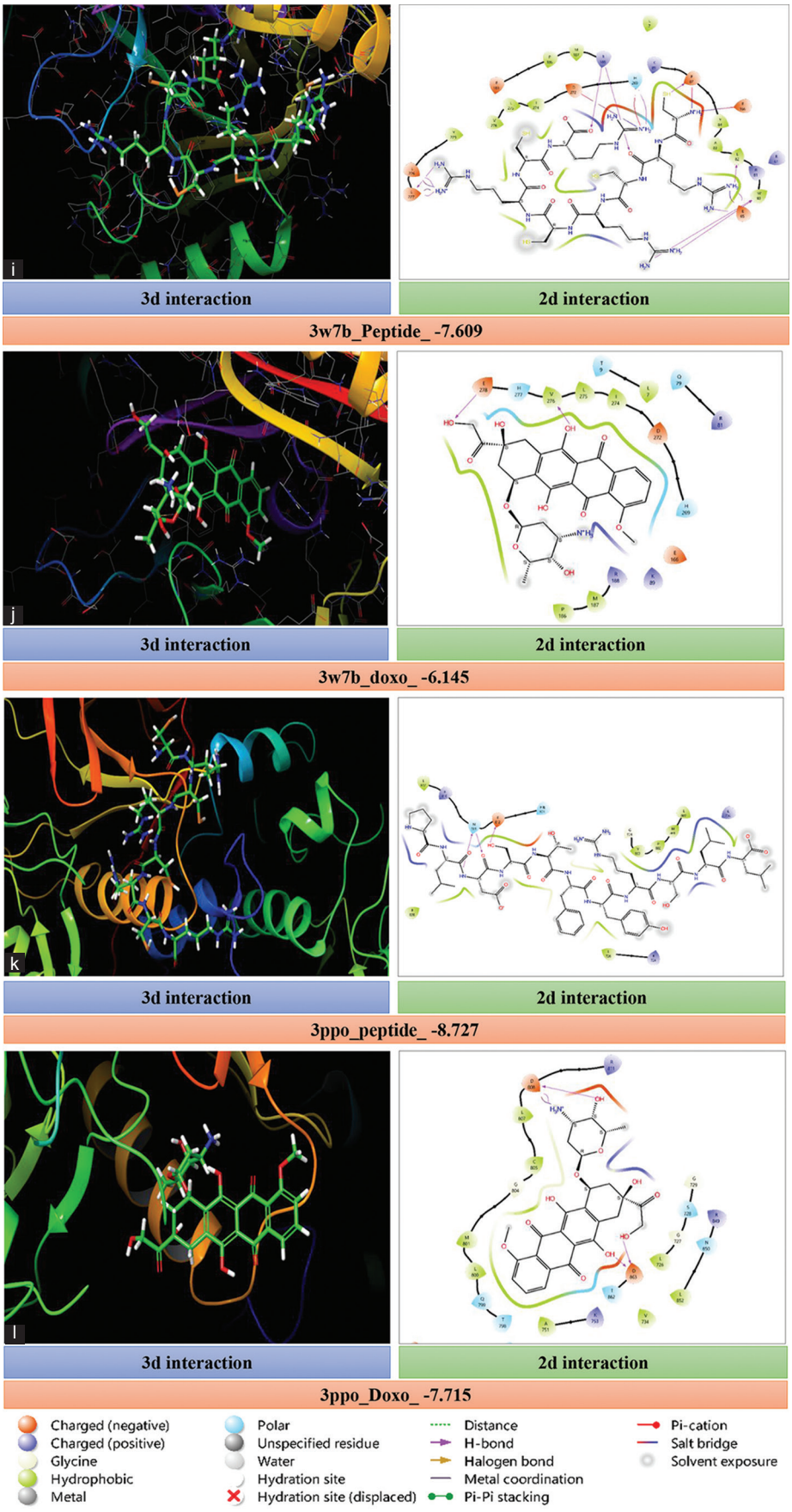

Figure 4: *(Continued)*

CONCLUSION

The results of this study underscore the potential of molecular docking as a valuable approach in drug discovery research, particularly in the identification of potent inhibitors for cancer-related proteins. By elucidating the binding affinities of ligands toward target proteins, molecular docking offers insights that can inform the development of targeted and personalized treatment strategies for cancer and other diseases.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

There is no use of animals.

CONSENT TO PARTICIPATE

Sakshi Soni performed the investigation, conducted the docking study, wrote the original draft, reviewed and edited the manuscript, conceptualized the study, designed the methodology, validated the results, and curated the data. Prof. Vandana Soni and Prof. Sushil K. Kashaw contributed to the conceptualization of the study, designed the methodology, validated the results, conducted the investigation, performed the formal analysis, wrote the original draft, reviewed and edited the manuscript and supervised the project.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Muhammad N, Hanif M, Yang P. Beyond cisplatin: New frontiers in metallodrugs for hard-to-treat triple negative breast cancer. Coord Chem Rev 2024;499:215507.
2. Available from: https://www.eurekalert.org/news-releases/650767 [Last accessed on 2024 Jan 26].
3. López-Cortés A, Cabrera-Andrade A, Vázquez-Naya JM, Pazos A, Gonzáles-Díaz H, Paz-Y-Miño C, *et al*. Prediction of breast cancer proteins involved in immunotherapy, metastasis, and RNA-binding using molecular descriptors and artificial neural networks. Sci Rep 2020;10:8515.
4. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, *et al*. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep 2023;13:13398.
5. Beloor J, Zeller S, Choi CS, Lee SK, Kumar P. Cationic cell-penetrating peptides as vehicles for siRNA delivery. Ther Deliv 2015;6:491-507.
6. Sankaran S, Selvaraj J, Pottabathula SS. *Alpinia galanga* bioactive constituents as multi-target inhibitors of SARS-CoV-2 proteins: A molecular docking, molecular simulation and ADMET analysis. Tradit Med Res 2024;9:24.
7. Dar AM, Mir S. Molecular docking: Approaches, types, applications and basic challenges. J Anal Bioanal Tech 2017;8:356.
8. Sethi A, Joshi K, Sasikala K, Alvala M. Molecular docking in modern drug discovery: Principles and recent applications. In: Drug Discovery and Development - New Advances. London: IntechOpen; 2020.
9. Meza Menchaca T, Juárez-Portilla C, Zepeda RC. Past, present, and future of molecular docking. In: Drug Discovery and Development - New Advances. London: IntechOpen; 2020.
10. Asati V, Bharti SK, Das R, Kashaw V, Kashaw SK. Discovery of novel ALK2 inhibitors of pyrazolo-pyrimidines: A computational study. J Biomol Struct Dyn 2022;40:10422-36.
11. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 2010;31:455-61

Source of Support: Authors acknowledge UGC Non-NET Fellowship for providing funding opportunity to Ms. Sakshi Soni (Registration No. Y20454003).**Conflicts of Interest:** None declared.