ORIGINAL ARTICLE

Molecular Docking Analysis of D-Glucosamine and Rivastigmine Tartrate Targeting Alzheimer's Disease-Associated Proteins: An *In Silico* Approach

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Abstract

Aims: The primary aim of this study is to investigate the potential interactions of D-glucosamine and rivastigmine tartrate with key receptors associated with Alzheimer's disease (AD) using in silico docking analysis. By utilizing the Glide software, the study seeks to explore the binding affinities of these compounds with targets such as acetylcholinesterase (AChE), microglia, astrocytes, and the sigma-1 receptor, aiming to identify potential therapeutic implications. **Objectives:** The objectives of this study are to conduct a comprehensive molecular docking analysis of D-glucosamine and rivastigmine tartrate using the Glide software to evaluate their binding interactions with key Alzheimer's disease (AD)-associated receptors. Specifically, the study aims to assess the binding affinities of these compounds with acetylcholinesterase (AChE), microglia, astrocytes, and the sigma-1 receptor. By comparing the docking scores, the research seeks to determine the relative binding strengths of D-glucosamine and rivastigmine tartrate with each target receptor. Additionally, the study aims to analyze the versatility and potential therapeutic relevance of these compounds in interacting with diverse AD-related receptors, providing insights into their potential roles in AD treatment. **Conclusion:** The in silico docking analysis revealed that rivastigmine tartrate exhibits stronger binding to the primary target enzyme AChE compared to D-glucosamine. Both compounds showed comparable binding potential for microglia. D-glucosamine demonstrated lower docking scores for astrocytes, while rivastigmine tartrate showed higher affinity for the sigma-1 receptor. These findings highlight the diverse interactions of D-glucosamine and rivastigmine tartrate with AD-associated receptors, suggesting the need for further in vitro and in vivo studies to validate these results and explore their potential therapeutic applications in Alzheimer's disease treatment.

Key words: Acetylcholinesterase, Alzheimer's disease, astrocytes, d-glucosamine, *in silico* docking, microglia, rivastigmine tartrate, sigma-1 receptor

INTRODUCTION

Alzheimer's disease (AD) stands as one of the most prevalent neurodegenerative disorders globally, characterized by progressive cognitive decline, memory impairment, and other debilitating neurological symptoms. As populations age, the prevalence of AD continues to rise, presenting a significant and growing health-care challenge worldwide. The disease not only affects individuals directly but also places a considerable burden on caregivers, families, and health-care systems.[1,2]

A hallmark feature of AD pathology is the accumulation of abnormal protein aggregates, such as beta-amyloid plaques and tau tangles, in the brain. These pathological changes lead to synaptic dysfunction, neuronal loss, and ultimately, cognitive decline.[3] Among various therapeutic strategies explored for AD, acetylcholinesterase (AChE) inhibition has long been a

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cornerstone approach. AChE is an enzyme responsible for the breakdown of the neurotransmitter acetylcholine, crucial for cognitive function. By inhibiting AChE activity, drugs like rivastigmine tartrate aim to increase acetylcholine levels in the brain, providing symptomatic relief for AD patients.[4,5]

Rivastigmine tartrate is a drug commonly used in the management of AD. It acts as an AChE inhibitor, helping to alleviate cognitive symptoms associated with AD by enhancing cholinergic neurotransmission in the brain. By inhibiting AChE, rivastigmine tartrate increases the availability of acetylcholine, a neurotransmitter involved in cognitive function. This mechanism of action provides symptomatic relief for AD patients, improving memory, language skills, and executive function. Rivastigmine tartrate is available in various formulations, including oral capsules, oral solution, and transdermal patches, offering flexibility in administration and allowing patients to choose the most suitable option based on their preferences and tolerability.[6,7]

Rivastigmine tartrate also demonstrates favorable tolerability and safety profiles, with common side effects typically related to gastrointestinal symptoms such as nausea, vomiting, and diarrhea. While these side effects may occur, they are often transient and tend to diminish over time. The availability of different dosage forms, including the transdermal patch, provides additional benefits by offering a convenient and well-tolerated alternative for patients who may experience gastrointestinal discomfort with oral administration. Moreover, the transdermal patch formulation ensures continuous drug delivery, maintaining consistent therapeutic levels of rivastigmine tartrate throughout the day. This steady drug delivery may contribute to improved symptom management and enhanced patient adherence to treatment regimens.[8,9]

However, despite the availability of AChE inhibitors, the current treatments for AD offer limited efficacy in halting or reversing disease progression. Hence, there is a pressing need to explore alternative and synergistic therapeutic approaches to improve AD management. In recent years, attention has turned to the potential neuroprotective properties of D-glucosamine, a naturally occurring amino sugar.[10,11]

D-glucosamine has garnered interest due to its purported ability to modulate various cellular pathways involved in neuroprotection, inflammation, and oxidative stress, all of which play critical roles in AD pathogenesis. Preclinical studies have suggested that D-glucosamine may exert neuroprotective effects through mechanisms such as anti-inflammatory and antioxidant activity, modulation of protein aggregation, and promotion of neuronal survival.[12,13]

Considering the promising attributes of both rivastigmine tartrate and D-glucosamine in AD therapy, there is a compelling rationale to investigate their interaction potential with the AChE receptor using computational methods such as *in silico* docking. This computational approach allows for the exploration of ligand-receptor binding modes, affinity predictions, and identification of key molecular interactions, providing valuable insights into their potential efficacy as AD therapeutics.

By elucidating the molecular mechanisms underlying the interactions between rivastigmine tartrate, D-glucosamine, and the AChE receptor, this research holds the potential to inform future drug development efforts and therapeutic strategies for AD. Ultimately, such endeavors aim to address the unmet medical needs of AD patients and improve their quality of life by offering more effective and targeted treatment options.[14,15]

The rationale behind this study lies in the need to explore alternative and synergistic AChE inhibition strategies for AD treatment. Rivastigmine tartrate's established role in AChE inhibition and D-glucosamine's emerging neuroprotective potential make them intriguing candidates. The primary objective is to employ *in silico* docking to predict the binding modes and affinities of both molecules with the AChE receptor. It is aimed to compare and contrast their interactions with the receptor, uncovering potential similarities or unique binding patterns. Ultimately, this study seeks to provide computational evidence supporting the investigation of these molecules, either individually or in combination, as potential therapeutic agents for AD.

The crystal structure of AChE, the primary target enzyme in our study, is represented by 1EEA. This structure serves as the foundation for understanding the interactions between AChE and our ligands of interest. In addition, various cell types relevant to AD are represented by the following codes: 1WY9, 5HK1, 2KVD, 2KVE, and 2W51. Specifically, 1WY9 corresponds to microglia, which are involved in the immune response within the brain. The structure coded as 5HK1 represents the sigma-1 receptor, implicated in various neurological processes. Both 2KVD and 2KVE represent astrocytes, which are key supportive cells in the brain. Moreover, 2W51 represents another astrocyte structure, potentially examined for comparative analysis with the other structures. These diverse structures provide a comprehensive framework for exploring the interactions of our ligands with different cell types involved in AD pathology.

At present, molecular docking has emerged as a crucial component of computer-aided drug development. This methodology involves predicting how a small molecule interacts with a protein at the atomic level. By doing so, researchers gain insights into the behavior of small molecules, such as peptide, within a protein's binding site, shedding light on the underlying biochemical processes. Molecular docking relies on high-resolution 3D representations of target proteins, typically obtained through techniques such as X-ray crystallography, nuclear magnetic resonance spectroscopy, or cryo-electron microscopy. Numerous computational tools and

algorithms exist for molecular docking, both commercially and freely available, serving drug research and academic endeavors alike.[16,17]

Commonly utilized 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. Among these, AutoDock Vina, Glide, AutoDock GOLD, and Schrodinger Maestro stand out as top-ranking choices. The computational electrostatics of ligand-receptor complexes are evaluated, screened, and predicted through docking studies, typically involving two main steps: Sampling ligand conformations according to the protein's active site and ranking these conformations using a scoring function. This dry laboratory approach offers significant advantages over traditional *in vivo* studies in terms of resource and time efficiency. It enables the prediction of ligand orientations within complex structures formed by the ligand and proteins or enzymes, quantifying their shape and electrostatic interactions. While molecular docking has long been recognized for its utility in drug discovery and design, there is a recent surge of interest in its application in pharmaceutical science. Specifically, it is being increasingly utilized to authenticate molecular targets of peptides in disease management. Molecular docking studies provide crucial information in pharmaceutical research, offering insights that inform subsequent *in vitro* investigations. This research aims to leverage molecular docking to evaluate potential lead peptides for Alzheimer's treatment, with the ultimate goal of identifying the most promising candidates.

MATERIALS AND METHODS

Docking study using glide module of schrödinger software

The Glide module of the Schrödinger software facilitates docking studies by aiming to identify favorable interactions between a receptor molecule, typically a protein, and one or more ligand molecules. It is essential to note that each ligand must be a single molecule, while the receptor can comprise 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. Each ligand pose represents the convergence of its position, orientation, and conformation concerning the receptor. A series of hierarchical filters is applied to assess the ligand's interactions with the receptor. Initially, grid-based methodologies employing the empirical ChemScore function evaluate the spatial compatibility of the ligand with the designated active site and analyze the complement arities of ligand-receptor interactions.[18,19]

Ligand poses that pass these initial checks progress to the final stage, involving the evaluation and minimization of a grid approximation of the non-bonded ligand-receptor interaction energy based on the OPLS_3e model. Positions with the lowest energy levels are prioritized. Scoring of poses is conducted using the Glide Score multi-ligand scoring mechanism developed by Schrödinger by default. A composite model score is then used to rank the poses of each ligand, combining the Glide Score, non-bonded interaction energy, and, in the case of flexible docking, the additional internal energy of the generated ligand conformation. 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.[20,21] Table 1 represents docking scores of rivastigmine tartrate and D-glucosamine on various receptors such as AChE, microglia, astrocytes, and Sigma-1 receptor, respectively.

Protein preparation

Protein preparation is a crucial step in ensuring the accuracy of Glide results, as it directly impacts the integrity of initial protein structures. Schrödinger offers a comprehensive tool, the protein preparation Wizard, specifically designed to ensure chemical precision and optimize protein structures for compatibility with Glide and other associated products. In addition, Schrödinger provides LigPrep, a counterpart facility serving 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 achieve optimal results.[22,23]

To incorporate a ligand/protein co-crystallized structure into Maestro, import can be facilitated from the protein data bank (PDB). For enhanced computational efficiency in Glide, especially for multimeric complexes, it is advisable to retain a single ligand-receptor subunit. However, if the active site requires two identical chains, both should be retained. Decisions regarding the retention or removal of water molecules are crucial, with the general practice being the elimination of water, except those coordinated with metals. Waters connecting the ligand and protein may be retained based on specific considerations.[24,25]

Adjustments to cofactors, metal ions, and the protein structure are necessary during this process. Repairs are warranted for structures lacking residues in proximity to the active site. In addition, careful adjustment of formal charges and ligand bond orders, especially concerning bonds between the ligand or a cofactor and a protein metal in complex structures, is required. Caution is advised during protein structure minimization, which is governed by a user-selected root mean square deviation tolerance, ensuring constrained minimization relative to the input protein coordinates. Finally, a thorough review of the resulting structures is imperative. Verification should include confirming the correct orientation

Table 1: Docking scores of rivastigmine tartrate and D-glucosamine on various receptors such as acetylcholinesterase, microglia, astrocytes, and sigma-1 receptor, respectively

S. No.	PDB codes	Ligand	Receptors/proteins	Drug	Docking score
1	1EEA	D-glucosamine	Acetylcholinesterase	-	6.8
2	1EEA	D-glucosamine	Acetylcholinesterase	Rivastigmine tartrate	7.7
3	1WY9	D-glucosamine	Microglia	-	5
4	1WY9	D-glucosamine	Microglia	Rivastigmine tartrate	5.5
5	2KVD	D-glucosamine	Astrocytes	-	4.7
6	2KVD	D-glucosamine	Astrocytes	Rivastigmine tartrate	5.7
7	2KVE	D-glucosamine	Astrocytes	-	4.5
8	2KVE	D-glucosamine	Astrocytes	Rivastigmine tartrate	8.8
9	2W51	D-glucosamine	Astrocytes	-	4.6
10	2W51	D-glucosamine	Astrocytes	Rivastigmine tartrate	4.7
11	5HK1	D-glucosamine	Sigma-1 receptor	-	5.7
12	5HK1	D-glucosamine	Sigma-1 receptor	Rivastigmine tartrate	7.7

of water molecules, resolving steric conflicts, and addressing any hydrogen-bonding issues to ensure the structural integrity and reliability of the prepared systems.[26,27]

Ligand preparation

Ensuring the fidelity of docked structures is essential to yield accurate results reflecting authentic ligand configurations within protein-ligand complexes. Schrödinger's LigPrep, compatible with 2D or 3D structures in SDF formats, adeptly generates high-quality, all-atom 3D structures for a diverse range of drug-like compounds. The LigPrep protocol encompasses a series of procedures aimed at data transformation, structural rectification, introduction of structural variations, elimination of extraneous structures, and optimization of molecular configurations. Several of these steps are discretionary and can be tailored using command-line arguments or preferences in the LigPrep panel. The sequential steps include converting input structures to a compatible format, selecting pertinent structures for processing, introducing hydrogen atoms to attain appropriate protonation states, eliminating undesired molecular entities, balancing charged functional groups, determining ionization states for the molecules, creating tautomeric forms to account for flexibility, applying filters to refine structure selection, introducing alternative chirality where applicable, producing energetically favorable ring conformations, eliminating structures causing computational issues, performing geometric optimization for structural refinement, and transforming the final output file into the desired format. It is noteworthy that LigPrep's flexibility enables users to tailor these steps to meet specific requirements, ensuring the generation of accurate and realistic ligand structures for subsequent docking simulations.

Receptor grid generation

The process of receptor grid generation involves utilizing multiple sets of fields to represent the shape and characteristics of the receptor on a grid, which in turn provides increasingly refined scoring for ligand poses. The receptor grid generation panel plays a crucial role in generating and configuring this grid, a step essential before initiating any ligand docking task. It is important to start with a "prepared" structure, which indicates an all-atom structure with correct bond ordering and formal charges. The OPLS 2005 force field is employed for grid generation, offering a wide range of defined atom types and ensuring precise treatment of metals. The receptor grid generation panel consists of five tabs, each dedicated to specifying settings for the receptor grid generation task. These tabs are as follows: Receptor, site, constraints, rotatable groups, and excluded volumes.[28]

Receptor tab: This tab allows users to define 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 option to utilize 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 used to articulate Glide constraints for the generation of receptor grids. Glide constraints represent receptor-ligand interactions considered crucial to the binding mode based on structural or biochemical data. Implementing 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: Certain 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 example, 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.[29,30]

Ligand docking

Ligand docking tasks in Glide require a predefined set of receptor grids and one or more ligand structures. If a correct Lewis structure cannot be determined for a ligand, or if the ligand contains unparametrized elements like tin or atom types not supported by the OPLS force fields, such as explicit lone pair "atoms," it is automatically excluded from the docking process. The ligand docking panel consists of several tabs, each serving specific functions: Ligands, settings, core, constraints, torsional constraints, and output. It is important to note that if a ligand fails to generate a correct Lewis structure or contains elements unsupported by the force fields, Glide systematically omits it during the docking procedure. Molecular modeling investigations utilizing the Glide module of Schrödinger were conducted to explore potential interactions between the most potent derivative and the protein of interest.[31]

Docking study

Molecular docking investigations involving receptor proteins such as AChE (PDB ID: 1EEA), Microglia (PDB ID: 1WY9), Astrocytes (PDB ID: 2KVD, 2KVE, 2W51), and sigma-1 receptor (PDB ID: 5HK1). The Glide module software within Schrödinger Maestro v13.5 was utilized for these docking studies, with protein structures sourced from the PDB. The obtained protein structures underwent further refinement through the "protein preparation workflow" within Maestro Wizard v13.5. This workflow involved generating states and refinement steps to enhance the protein structure, including optimization of hydrogen-bonded groups, dehydration processes, and restrained minimization using the default force field OPLS_3e. The resultant minimized protein structure was used to generate a grid surrounding the ligand molecule. The docking results revealed diverse conformations of docked ligands, 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. Figure 1a-l represents 3D and 2D interactions along with the docking scores of D-glucosamine and rivastigmine tartrate with different proteins.

Validation of docking procedures

To validate the accuracy of the docking procedure, a verification process was conducted utilizing AutoDock Vina software. Before docking the compounds within the datasets, a crucial step involved the extraction of the cocrystallized ligand situated within the binding site of the protein of interest. Subsequently, this extracted ligand was subjected to redocking within the same binding site of the protein.

This validation step ensured that the docking procedure was capable of reproducing the known binding interactions between the protein and its native ligand. By comparing the predicted binding poses of the redocked ligand with its original conformation in the crystal structure, the reliability and precision of the docking methodology could be assessed. Any significant disparities between the predicted and experimental binding modes would indicate potential limitations or inaccuracies in the docking procedure, prompting further refinement or adjustment of the parameters to enhance its predictive capability.

Overall, this validation process served as a critical quality control measure, providing confidence in the subsequent docking results obtained with the compounds of interest.

RESULTS AND DISCUSSION

The results of a molecular docking study aimed at uncovering potential interactions between a series of potent ligands and a target protein, using the Glide module within the Schrödinger software, are presented. The findings of the *in silico* docking interactions suggest that both D-glucosamine and rivastigmine tartrate have the potential to bind to a variety of receptors relevant to AD. In the case of AChE, the primary target enzyme, rivastigmine tartrate exhibited a higher docking score (7.7) compared to D-glucosamine (6.8), indicating a potentially stronger binding affinity. In microglia, both molecules showed similar docking scores (around 5), suggesting comparable binding potential.

However, in astrocytes, D-glucosamine consistently displayed lower docking scores (4.7, 4.5, 4.6) across different astrocyte structures (2KVD, 2KVE, and 2W51, respectively) compared to rivastigmine tartrate, which had docking scores (5.7, 8.8, 4.7) across the same structures, respectively. Interestingly, for the sigma-1 receptor, rivastigmine tartrate again exhibited a higher docking score (7.7) than D-glucosamine (5.7). These results highlight the potential of both D-glucosamine and rivastigmine tartrate to interact with various receptors in the context of AD. While rivastigmine tartrate generally demonstrated stronger binding to the target enzyme (AChE), further investigation into D-glucosamine's interaction with other receptors, particularly astrocytes, is warranted.

It is important to note that these are *in silico* predictions, and further, *in vitro* and *in vivo* studies are needed to validate these findings and assess their potential therapeutic implications. Utilizing molecular docking is a valuable approach to pinpointing the molecular targets of compounds

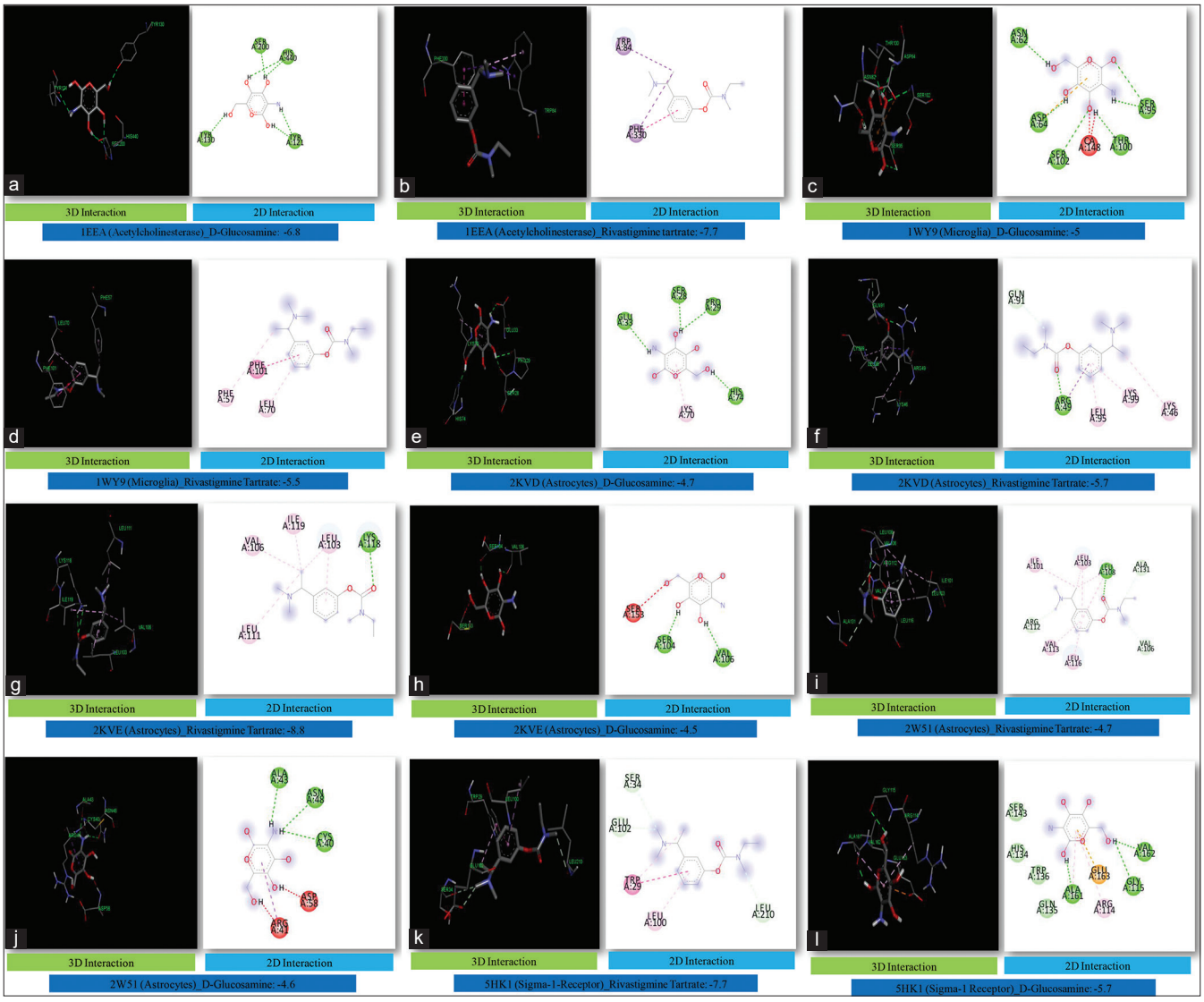

Figure 1: (a-l) 3D and 2D interactions along with the docking scores of D-glucosamine and rivastigmine tartrate with different proteins

for illness treatment. Predicting the binding affinity and conformation of compounds with target proteins aids in identifying potential treatment targets. The availability of databases and advancements in computational tools have elevated molecular docking to a pivotal role in the drug discovery process. Its utilization has significantly enhanced the efficiency and efficacy of drug discovery by reducing the time and costs associated with conventional experimental procedures. Hence, employing molecular docking in research holds substantial promise for identifying novel therapeutic targets and developing safe and effective treatments for diseases.

CONCLUSION

The research conducted utilizing molecular docking techniques within the Schrödinger software has provided valuable insights into the potential interactions between D-glucosamine and rivastigmine tartrate, two compounds of interest in the context of AD, with various receptor proteins. Through meticulous protein and ligand preparation, followed by receptor grid generation and docking studies, we have identified potential binding affinities between these compounds and key proteins associated with the disease.

The results indicate that while rivastigmine tartrate demonstrated stronger binding affinity to AChE, a primary target enzyme in AD, D-glucosamine exhibited comparable or even stronger interactions with other receptors such as those in astrocytes. These findings suggest a multifaceted potential for both compounds in modulating various pathways implicated in AD progression.

It is important to note that these findings are based on *in silico* predictions and further validation through *in vitro* and *in vivo* studies is necessary to confirm their therapeutic relevance. Moreover, the efficiency and cost-effectiveness

of computational tools in drug discovery underscore their significance in accelerating research efforts toward developing safe and effective interventions for various health conditions. Thus, continued exploration of molecular docking holds promise for advancing our understanding of disease mechanisms and facilitating the development of targeted therapeutic interventions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

No animals or humans were used in this study.

AVAILABILITY OF DATA AND MATERIAL

The data will be available from the corresponding author, on request.

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CONSENT TO PARTICIPATE

Sunny Rathee 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. Sanjay K. Jain contributed to the conceptualization of the study, designed the methodology, validated the results, conducted the investigation, performed the formal analysis, and supervised the project.

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