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Molecular Interactions of Kasugamycin with ALS-Associated Neuroinflammatory Proteins: An Integrated in Silico Investigation

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ABSTRACT: Amyotrophic lateral sclerosis (ALS) is associated with complex neuroimmune and inflammatory dysregulations where there is still room for new molecular targets and compounds of interest. Although Kasugamycin is an aminoglycoside antibiotic with several biological functions beyond its use as an antimicrobial drug, its interaction with proteins involved in ALS pathogenesis has not yet been adequately addressed. In this research, we used an integrated computational approach to investigate the possible interaction of kasugamycin with RAGE (AGER), Toll-like receptor 4 (TLR4), chitinase-3-like protein 1 (CHI3L1), and IL-13-related protein via molecular docking, followed by molecular dynamics (MD) simulation of the RAGE-kasugamycin complex. The docking analysis yielded binding energies of −7.75, −5.29, −4.01, and −3.75 kcal/mol for RAGE, CHI3L1, TLR4, and the IL-13-related protein, respectively. RAGE was the most promising interaction in the present computational study. Therefore, the RAGE-kasugamycin complex underwent a 100 ns MD simulation to evaluate its conformational stability and interaction capability. This information provides the rationale for considering the role of RAGE in future experiments on kasugamycin in neuroinflammatory diseases associated with ALS. However, it should be pointed out that the results have not shown any evidence of binding or target modulation, efficacy against inflammation, neuroprotection, and therapeutical effects. Overall, the study suggests that RAGE is a potential target for kasugamycin. Additionally, the comparative analysis of targets revealed that the interaction depends on specific targets.

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized primarily by the degeneration of upper and lower motor neurons, leading to progressive muscle weakness, impaired voluntary movement, paralysis, and ultimately respiratory failure [1]. Although ALS is predominantly considered a motor system disorder, cognitive and behavioral alterations may also occur in a subset of patients. Most cases are sporadic, whereas a smaller proportion are familial and

associated with genetic variants involving genes such as *SOD1*, *C9ORF72*, *TARDBP*, and *FUS* [2]. Despite advances in understanding ALS-associated molecular abnormalities, available pharmacological interventions provide limited effects on disease progression, highlighting the need to investigate additional molecular pathways and potential therapeutic candidates [3]. The pathobiology of ALS is complex and involves the interaction of multiple cellular and molecular processes, including protein misfolding and aggregation, glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, impaired axonal transport, endoplasmic reticulum stress, altered RNA processing, autophagy dysfunction, and disruption of protein homeostasis [4].

Increasing evidence also supports an important contribution of neuroimmune alterations to ALS pathology. Activation of microglia and astrocytes can be accompanied by increased production of inflammatory mediators, reactive oxygen species, and other signaling molecules that may contribute to an inflammatory environment within the central nervous system [5]. Thus, inflammatory signaling is an important component of the multifactorial molecular landscape of ALS, although its precise contribution may vary across disease stages and pathological contexts.

Several innate immune and inflammation-associated receptors have been investigated in relation to ALS and neuroinflammatory processes [4, 6, 7]. Among these, the receptor for advanced glycation end products (RAGE), encoded by *AGER*, is a multiligand receptor expressed by several cell types in the nervous system and is capable of responding to endogenous molecules associated with cellular stress and damage [8].

RAGE activation has been linked to intracellular signaling pathways involving NF-κB, MAPK, and NADPH oxidase, providing a mechanistic connection between extracellular danger signals, inflammatory signaling, and oxidative stress [9]. Alterations in RAGE and its endogenous ligands have also been reported in ALS-related pathological settings, supporting continued investigation of the RAGE signaling axis in neuroinflammatory disease [10] [11].

Toll-like receptor 4 (TLR4) represents another important component of innate immune signaling. Recognition of endogenous damage-associated molecular patterns by TLR4 can activate MyD88- and TRIF-dependent signaling pathways and promote downstream inflammatory responses, including NF-κB-associated cytokine production [12]. n addition, chitinase-3-like protein 1 (CHI3L1/YKL-40) has been associated with reactive glial responses and neuroinflammation, with altered levels reported in patients with ALS and linked to disease-related clinical features [13]. IL-13Rα2 has also been implicated in immune regulation and extracellular matrix-related signaling, including processes involving TGF-β-associated responses [14]. Collectively, these molecules represent distinct components of the neuroimmune environment associated with ALS and provide candidate targets for investigating potential ligand–protein interactions relevant to inflammatory signaling.

Drug repurposing provides an alternative strategy for identifying new applications for compounds with established pharmacological or clinical backgrounds. Computational approaches, including molecular docking and molecular dynamics simulation, can be used at an early stage to examine the structural compatibility of candidate compounds with selected molecular targets and to prioritize interactions for subsequent experimental investigation [15, 16]. Such approaches do not establish biological efficacy or target inhibition; rather, they provide structural and computational evidence that can contribute to the formulation of testable mechanistic hypotheses [17]. Kasugamycin is an aminoglycoside antibiotic originally developed for antimicrobial applications and has also attracted interest because of reported biological effects beyond its antibacterial activity. Given the involvement of inflammatory and innate immune signaling in ALS-associated pathology, the interaction of Kasugamycin with selected neuroimmune proteins may provide a basis for exploring whether this compound warrants further investigation in the context of neuroinflammatory mechanisms.

Accordingly, the present study employed a computational approach to investigate the predicted molecular interactions of Kasugamycin with four proteins associated with inflammatory and neuroimmune processes relevant to ALS: RAGE (*AGER*), TLR4, CHI3L1, and IL-13Rα2. Molecular docking was used to compare the predicted binding characteristics of Kasugamycin across these targets, while molecular dynamics simulation was subsequently performed for the RAGE–Kasugamycin complex to examine its conformational behavior and interaction stability over a 100-ns simulation period. The study was therefore designed to provide structural insights into the potential interaction of Kasugamycin with these selected targets and to identify molecular interactions that may serve as a basis for subsequent biochemical and experimental validation.

2. MATERIALS AND METHODS

2.1. Selection and Preparation of Target Proteins

RAGE/AGER, TLR4, CHI3L1, and IL-13Rα2 were selected because they are associated with inflammatory and neuroimmune

processes relevant to ALS. RAGE and TLR4 are involved in innate immune signaling, whereas CHI3L1 and IL-13Rα2 have been linked to glial responses and inflammatory regulation. Therefore, these proteins were chosen to examine the potential interaction profile of Kasugamycin across different inflammation-associated targets using a computational approach [18].

The three-dimensional crystal structures of the shortlisted disease-related target receptors like RAGE/AGER, TLR4, CHI3L1, and IL-13Rα2, have been obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank [19]. The choice of receptor proteins for docking was made based on crystal resolution, structural completeness, and the presence of co-crystallized ligands to verify the docking protocol.

Each receptor protein was prepared for molecular docking by removal of the bound ligand from the downloaded receptor complex obtained from the PDB database using UCSF Chimera (v. 1.17.3) software [20]. All the concerned receptors were prepared by removing redundant water molecules, ions, and heteroatoms not involved in ligand binding, followed by addition of polar hydrogen atoms and the assignment of Gasteiger charges and ADT atom types. The nonpolar hydrogen atoms were merged and the receptor structures were saved in the default PDBQT format of AutoDock Tools (v. 1.5.7) software [21].

2.2. Ligand Preparation

The three-dimensional chemical structure of Kasugamycin was retrieved from the PubChem Compound database [22] in the Structure Data File (SDF) format. The ligand structure was then converted to the pdb file format by energy minimization using the MM2 forcefield of Chem3D software [23]. The prepared three-dimensional structure model of Kasugamycin was further optimized for docking analysis by assigning rotatable bonds to maximize flexibility, without altering chemically restrained bonds, and was saved in PDBQT format.

2.3. Binding Site Identification

Coordinates of the co-crystallized ligand in each of the considered receptors' crystal structures served in the identification of the active binding residues present within active binding pockets of the specified target receptor by using Discovery Studio Visualizer (BIOVIA, Dassault Systèmes) [24, 25]. Grid boxes were positioned in the vicinity of the binding pockets in order to cover all the involved residues in the process of ligand recognition. Grid dimensions were chosen so that the whole pocket would be included and enough space would be left for conformational changes of the ligand during docking studies.

2.4. Molecular Docking Simulation

Molecular docking simulations were performed using AutoDock 4.2 software, which uses Lamarckian Genetic Algorithm (LGA) as a genetic approach coupled with local search to find energetically favorable ligand conformations within the receptor's cavity. An imaginary grid box is prepared by covering the extended conformations of the ligand as well as the active binding residues of the macromolecule. For each receptor-ligand pair, a docking parameter file (DPF) was prepared using AutoDock Tools. The docking procedure included 150 genetic algorithm runs with a population size of 150, up to 2,500,000 energy evaluations, 27,000 generations, a 0.02 probability of mutation, and 0.80 probability of crossover. Binding free energy (ΔG, kcal/mol) was evaluated for all docking experiments and docking results were characterized by the lowest value of binding free energy and highest cluster occurrence as the most probable binding conformation [26, 27].

The reliability of the molecular docking protocol was estimated by docking the co-crystallized reference ligand with its concerned receptor's binding site. The reliability of the molecular docking protocol was estimated by determining the root-mean-square deviation (RMSD) between the crystallographic conformation and the docked one. An RMSD lower than 2.0 Å meant the successful replication of the experimental conformation and validation of the docking procedure [28–30].

Moreover, the major interactions of the native ligand with the amino acids were checked for their reproducibility.

Table 1. Target proteins used for molecular docking

Protein	**Gene**	**PDB ID**	**Resolution (Å)**	**Biological relevance in ALS**
RAGE (Receptor for Advanced Glycation End Products)	**AGER**	**3O3U**	**1.50**	Mediates chronic neuroinflammation, oxidative stress, HMGB1/S100 signaling, and NF-κB activation, thereby promoting progressive motor neuron degeneration [31].

Toll-like receptor 4	**TLR4**	**3FXI**	**3.10**	Initiates innate immune signaling through MyD88/TRIF pathways, resulting in microglial activation and production of pro-inflammatory cytokines implicated in ALS progression [32].
Chitinase-3-like protein 1 (YKL-40)	**CHI3L1**	**1HJW**	**2.30**	Biomarker of reactive astrocytes and neuroinflammation; elevated CHI3L1 correlates with disease severity and rapid progression in ALS [33].
Interleukin-13	**IL13**	**5L6Y**	**1.99**	Regulates immune remodeling, extracellular matrix signaling, and TGF-β-associated inflammatory responses that may contribute to neuroinflammation in ALS [34].

2.5. Molecular Dynamics Simulation

Kasugamycin complexed with RAGE receptor was evaluated for their thermodynamic stability by performing molecular dynamics (MD) simulations for 100 ns by using the Desmond module within Schrodinger's Maestro software (v2022.4). This analysis involved a macromolecular complex of the RAGE receptor associated with the ligand Kasugamycin based upon the available literature supporting their pharmacological role in the treatment of inflammatory conditions as well as observed docking outcomes. To ensure accuracy, explicit solvent molecules were included and the system was neutralized with counter ions by using 0.15 N NaCl solution. The system was relaxed using the steepest-descent algorithm to minimize energy to address any steric clashes. The equilibration of the system was achieved throughout the simulation via maintaining low-temperature conditions under constant pressure (NPT) [35]. Throughout the simulation, various parameters, including system energies, atomic positions, and root-mean-square deviations (RMSDs), were carefully monitored to gain insights into the system's dynamics and stability over time.

3. RESULTS

3.1. Selection and Preparation of Target Proteins

RAGE/AGER, TLR4, CHI3L1, and IL-13Rα2 were selected based on their reported association with inflammatory and neuroimmune alterations relevant to ALS pathology. Their involvement in processes such as innate immune signaling, glial activation, and inflammatory regulation provided the biological rationale for evaluating their potential interactions with Kasugamycin in the present computational study.

The three-dimensional structures of RAGE, TLR4, and CHI3L1 retrieved from the RCSB Protein Data Bank, together with the predicted three-dimensional structure of IL-13 obtained from the Protein Data Bank database, were successfully prepared for molecular docking studies. The processed receptor structures were subsequently converted to PDBQT format for molecular docking analysis. The prepared receptor structures retained their native folding architecture and intact ligand-binding cavities, making them suitable for subsequent computational studies.

3.2. Ligand Preparation

The three-dimensional structure of Kasugamycin was successfully prepared by using the SMILES procured from PubChem database and saved in PDBQT format. The final optimized ligand was subsequently utilized for executing molecular docking against all selected receptors. The energy minimized structure of Kasugamycin consists of twelve rotatable bonds and all were kept flexible to impart maximum possible flexibility.

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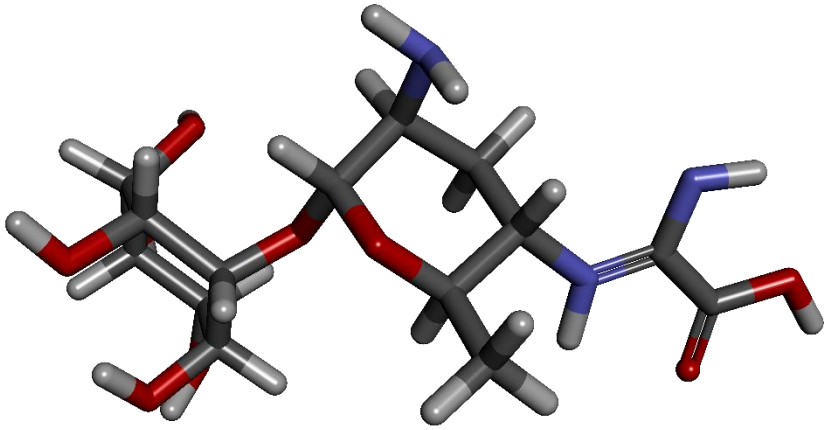

Figure 1. Energy minimized and optimized three-dimensional structure of Kasugamycin.

3.3. Binding Site Identification and Grid Generation

The ligand-binding pockets of all four considered receptors were successfully identified using the coordinates of the co-crystallized ligand. Three-dimensional grid boxes were generated to encompass all active binding residues responsible for ligand recognition at the molecular level. The grid dimensions and center coordinates used for molecular docking of the receptors currently under consideration are summarized in **Table 1**.

Table 2. Grid coordinates used for molecular docking simulations.

Protein	Grid Size (X×Y×Z)	Spacing (Å)	x center	Y center	Z center
RAGE	50*50*50	0.403	21.041	19.877	69.617
TLR4	50*50*50	0.508	202.704	171.447	157.205
CHI3L1	40*40*40	0.403	-26.972	-25.098	37.317
IL-13R	40*40*40	0.447	-13.693	28.843	-20.103

3.4. Docking Parameters and Docked Conformations

The optimized docking parameters were successfully employed for molecular docking simulations of Kasugamycin against all four concerned target proteins. Multiple ligand conformations were generated during docking, and the conformation exhibiting the lowest binding free energy was selected for subsequent interaction analyses. The binding energy obtained for Kasugamycin against each of the considered receptors in the current study are tabulated in **Table 3**. Three-dimensional docked conformation and two-dimensional binding interactions of Kasugamycin within the active binding pockets of RAGE receptor was presented in **Figure 2**.

Table 3. Molecular docking results of Kasugamycin against ALS-associated receptors.

S. No.	Ligand	Binding Energy (kcal/mol)			
		IL13 (pdb: 5l6y)	RAGE (pdb: 5l6y)	TLR4 (pdb: 5l6y)	CHI3LI (pdb: 5l6y)
1	Kasugamycin	-3.75	-7.75	-4.01	-5.29

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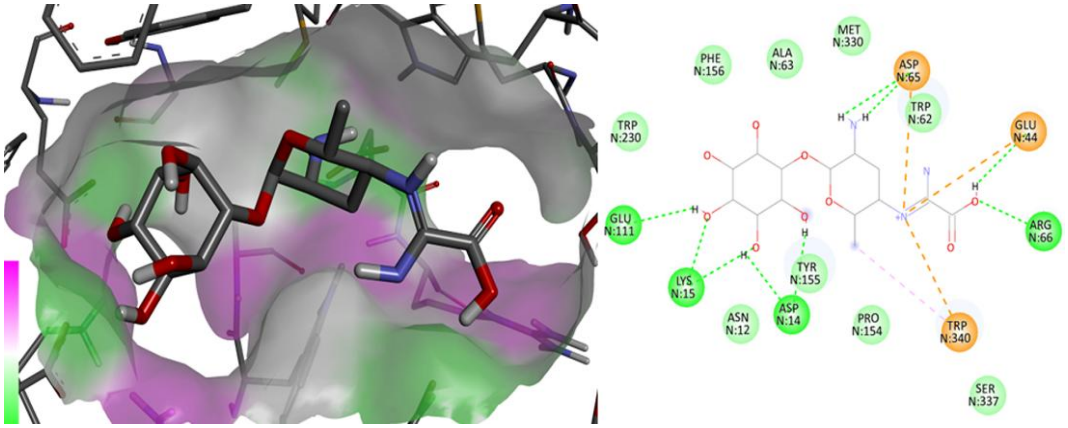

Figure 2. Three-dimensional docked conformation and two-dimensional binding interactions of Kasugamycin within the active binding pockets of RAGE receptor.

3.5 Molecular Dynamics Simulation

The proposed repurposed drug molecule Kasugamycin complexed with the RAGE receptor was further validated for its thermodynamic stability by performing a 100 ns MD simulation using the Desmond module of Schrödinger's Maestro suite. The structural stability of the RAGE–Kasugamycin complex was evaluated by analyzing the root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), protein secondary-structure elements (SSEs), protein–ligand interactions, ligand torsional behavior, and ligand physicochemical properties throughout the simulation. All macromolecular frames were aligned to the protein backbone reference frame to calculate the RMSD of the receptor residues using the selected atoms. The RMSD analysis confirmed the smooth progression of the equilibrated simulation process and demonstrated stable conformational behavior of both the receptor and the bound ligand throughout the simulation period. MD results revealed that the RAGE receptor consists of 580 amino acid residues constituting 4464 heavy atoms out of 8914 atoms in total. The complexed ligand Kasugamycin has 26 heavy atoms out of 51 atoms, with 12 rotatable bonds. The ligand RMSD further indicated Kasugamycin's stability within the receptor's binding cavity in comparison with the receptor's Cα backbone stability during the simulation by aligning the ligand heavy atoms.

The macromolecular RMSD fluctuated between 11 and 13 Å after an initial equilibration period of 20 ns, confirming that the receptor attained a stable conformational state during complex formation with Kasugamycin. The complex ligand Kasugamycin was adjusted within the receptor binding cavity by a couple of conformational changes in the early phase of the simulation to achieve the most stable conformation and maintained the same conformation throughout, with an RMSD ranging from 5.5-6.5 Å, confirming stable binding of Kasugamycin within the active binding pocket of RAGE. The RMSD of the concerned RAGE protein complexed with the Kasugamycin ligand observed during the 100 ns MD simulation was shown in **Figure 3.**

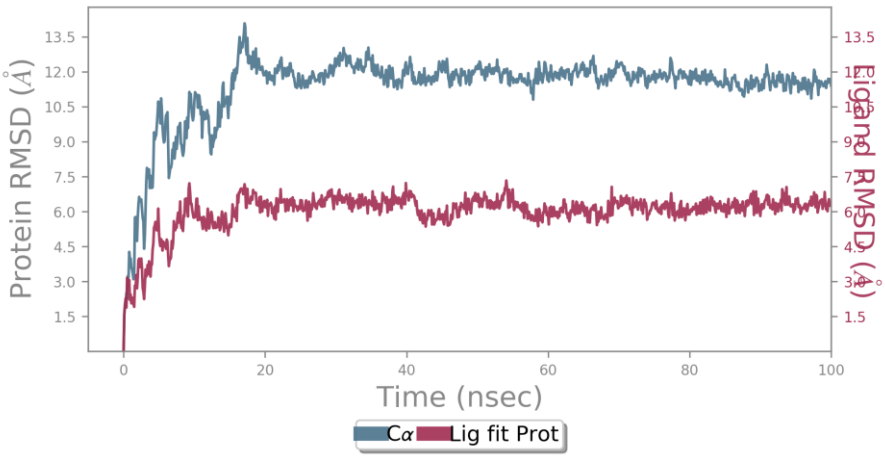

Figure 3: RMSD for the RAGE protein complexed with the Kasugamycin ligand observed during the 100 ns MD simulation.

The RMSF value of the receptor's Cα backbone of RAGE was found to range between 1.5 and 4.5 Å. Similarly, the RMSF observed for the complexed ligand Kasugamycin fluctuated between 1.5 and 2.5 Å throughout the simulation, indicating maintenance of its stabilized conformation and preservation of the structural integrity of the RAGE protein. The RMSF profile of the RAGE protein (a) and the complexed ligand Kasugamycin (b) observed during the 100 ns MD simulation is shown in **Figure 4**.

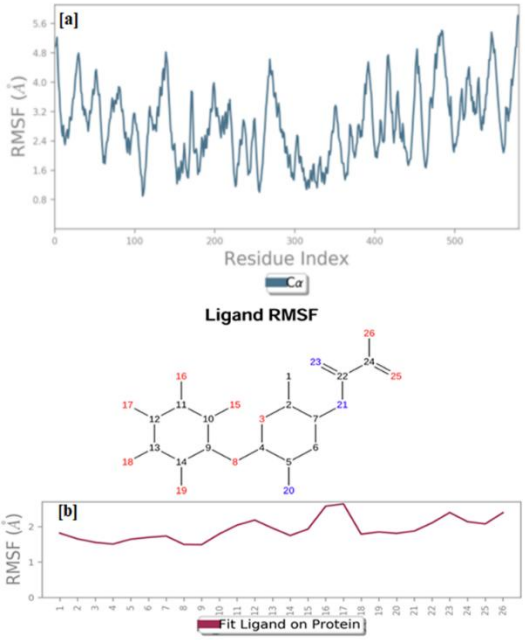

Figure 4: RMSF profile of the RAGE protein (a) and complexed ligand Kasugamycin (b) observed during the 100 ns MD simulation.

The macromolecular–ligand interaction analysis performed throughout the 100 ns simulation revealed that Kasugamycin has been continuously interacting with the target receptor RAGE via formation of hydrogen bonds with Asp14, Glu44, Asp65 and Glu111 residues, while residues Asn12, Asp14, Lys15, Glu44, Asp65, Arg66, Glu111, Ala112, Glu153, Tyr155 via water bridges and residues Trp62, Ala63, Tyr155, and Trp230 via hydrophobic interactions. Overall, more than 10 receptor residues consistently interacted with Kasugamycin throughout the simulation trajectory, indicating stable ligand occupancy within the RAGE binding cavity. The detailed protein–ligand contacts observed throughout the 100 ns molecular dynamics simulation are shown in **Figure 5.**

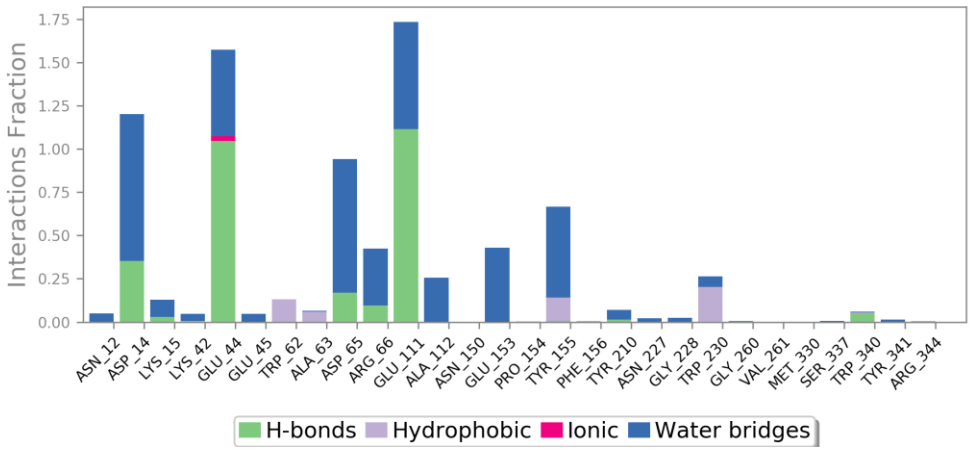

Figure 5: Ligand protein interaction analysis of Kasugamycin against RAGE receptor observed during 100 ns MD simulation.

4. DISCUSSION

The present study used molecular docking and molecular dynamics simulations to investigate the predicted interactions between Kasugamycin and four proteins associated with inflammatory and neuroimmune processes relevant to ALS, namely RAGE, TLR4, CHI3L1, and IL-13Rα2. The docking analysis provided a comparative assessment of the predicted binding energies of Kasugamycin with the selected targets. Among the proteins examined, the lowest predicted binding energy was observed for RAGE (−7.75 kcal/mol), followed by CHI3L1 (−5.29 kcal/mol), TLR4 (−4.01 kcal/mol), and IL-13 (−3.75 kcal/mol). These findings indicate that Kasugamycin showed different predicted interaction strengths across the selected proteins, with the RAGE complex showing the most favorable docking score under the conditions used in this study.

The comparatively favorable docking result obtained with RAGE provided the basis for selecting the RAGE–Kasugamycin complex for molecular dynamics simulation. During the 100-ns simulation, the RAGE–Kasugamycin complex remained associated within the investigated binding region, although conformational fluctuations were observed during the trajectory. The RAGE backbone RMSD fluctuated after the initial equilibration period, while the ligand underwent conformational adjustments during the early phase of the simulation before maintaining a comparatively consistent trajectory. These observations indicate that the docked complex retained its interaction throughout the simulation and provided additional computational insight into the behavior of Kasugamycin within the RAGE binding region.

Analysis of protein–ligand interactions during the simulation further revealed Kasugamycin interactions with several RAGE residues. Hydrogen-bond interactions involving Asp14, Glu44, Asp65, and Glu111 were observed, together with water-mediated and hydrophobic interactions involving additional residues. The persistence of interactions with multiple residues throughout the simulation suggests that Kasugamycin can maintain contacts with the RAGE binding region under the simulated conditions. However, these computational observations should be interpreted as predicted molecular interactions and should not be considered evidence of experimentally confirmed RAGE binding or inhibition.

Kasugamycin also showed predicted interactions with TLR4, CHI3L1, and IL-13-related protein structures in the docking analysis. The differences in predicted binding energies suggest that the interaction profile of Kasugamycin was not identical across the selected targets. However, docking scores alone cannot establish target selectivity, binding affinity, receptor activation or inhibition, or downstream biological effects. Therefore, the docking findings should be considered preliminary structural evidence that may help prioritize targets for subsequent experimental investigation.

The selection of these proteins was based on their reported association with inflammatory and neuroimmune processes relevant to ALS. The computational interaction of Kasugamycin with these proteins therefore provides a basis for considering possible molecular associations within this inflammatory context. Nevertheless, the present study does not demonstrate that Kasugamycin modulates the associated signaling pathways, reduces neuroinflammation, protects motor neurons, or alters ALS progression. Such conclusions would require appropriate biochemical, cellular, and in vivo studies.

An important strength of the present work is the combination of molecular docking with molecular dynamics simulation for the RAGE–Kasugamycin complex. While docking provided an initial assessment of the possible orientation and predicted interaction energy of Kasugamycin within the selected binding site, the subsequent 100-ns simulation enabled examination of the complex's behavior over time and characterization of protein–ligand interactions throughout the trajectory. Together, these approaches provide complementary computational insights into the potential interactions of Kasugamycin with the selected targets.

Several limitations should also be considered. Molecular docking and molecular dynamics simulations are computational approaches and cannot fully reproduce the complexity of a biological system. Predicted binding energies and simulated interaction patterns do not establish experimental binding affinity, pharmacological activity, target inhibition, or therapeutic efficacy. In addition, only the RAGE–Kasugamycin complex was subjected to molecular dynamics simulation in the present study. Therefore, the computational findings require confirmation using appropriate biochemical and cellular experiments, followed, where justified, by suitable preclinical studies. Overall, the present findings provide a computational basis for further investigation of Kasugamycin interactions with RAGE and other selected neuroimmune-associated proteins, with RAGE emerging as the most favorable predicted interaction among the targets examined in this study.

CONCLUSION

In general, from the above computation analysis results, it seems that Kasugamycin can have the possibility of binding with

RAGE among the neuroimmune proteins tested with Kasugamycin-RAGE complex maintaining its structure stability throughout the 100-ns molecular dynamics simulation. This is a preliminary basis from a computational aspect for experimental testing on the possible role of RAGE in the biological activity of Kasugamycin. These findings suggest a hypothesis that could be investigated further for interactions between Kasugamycin and RAGE in neuroinflammatory conditions relevant to ALS. However, it must be noted that the current findings are computational and should not be regarded as any evidence of RAGE binding, target inhibition, anti-inflammatory effects, neuroprotection, or therapeutic benefits.

Competing interests: There are no conflicts of interest.

Credit Author Statement

Conceptualization: Thakur Gurjeet Singh. **Analyzed the data:** Shareen Singh, Thakur Gurjeet Singh; **Wrote the manuscript:** Maneesh Mohan, Somdutt Mujwar, Balbir Singh. **Visualization:** Shareen Singh, Somdutt Mujwar & Thakur Gurjeet Singh. **Editing of the Manuscript:** Shareen Singh, and Thakur Gurjeet Singh. **Critically reviewed the article:** Thakur Gurjeet Singh. **Supervision:** Thakur Gurjeet Singh.

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Consent to Publish: The paper is submitted with the consent of all listed authors.

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Data Availability Statement: All data supporting the findings of this study are included within the manuscript in the form of figures and tables. Additional data can be provided by the corresponding author upon reasonable request.

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