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In Silico Evaluation of Guanfacine Interactions with ALS-Associated Neuroimmune Proteins: Structural and Molecular Dynamics Analysis

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ABSTRACT: Amyotrophic Lateral Sclerosis (ALS) is linked to multiple neuroimmune and inflammatory alterations, where further identification of additional molecular targets and candidate molecules is important. Guanfacine is a selective central α_2A -adrenergic receptor agonist, already used in various clinical applications, as well as a molecule showing interesting properties due to its influence on glial cells and inflammatory processes. Due to the fact that dysregulation of neuroimmune signaling is related to the pathological mechanisms of ALS, the current study utilizes an integrative computational approach to investigate the potential interactions between Guanfacine and four neuroimmune-associated proteins: RAGE (AGER), Toll-like receptor 4 (TLR4), chitinase-3-like protein 1 (CHI3L1), and interleukin-13 receptor α_2 (IL-13R α_2). The molecular docking was conducted for comparing the binding of Guanfacine to these proteins, and then the 100-ns molecular dynamics simulation was conducted for investigating the RAGE-Guanfacine complex. Guanfacine showed docking scores of -7.47, -6.65, -5.21, and -4.28 kcal/mol against RAGE, CHI3L1, TLR4, and IL-13, respectively, and the most promising interaction was shown by RAGE. During the molecular dynamics simulation, the RAGE-Guanfacine complex stayed bound in the studied region, and multiple hydrogen bonds, water bridges, and hydrophobic interactions occurred. This evidence suggests a structure-based rationale for selecting RAGE to conduct future investigations of the interaction between Guanfacine and its neuroinflammatory role in ALS. However, the results obtained from the computational analysis are not sufficient to prove binding affinity, modulation, anti-inflammatory, and neuroprotective effects of Guanfacine. Thus, the paper presents a hypothesis that needs to be proven.

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 (Morris, 2015). 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* (Mohan et al., 2025). 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 (Wolfson et al., 2023). 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 (Rokade et al., 2022).

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 (Mohan et al., 2025b). 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 (Castro-Gomez and Heneka, 2024; Griffiths et al., 2010; Rokade et al., 2022). 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 (Mohan et al., 2025c).

RAGE activation has been linked to intracellular signaling pathways involving NF- κ B, MAPK, and NADPH oxidase, providing a mechanistic connection between extracellular danger signals, inflammatory signaling, and oxidative stress (Sparvero et al., 2009). 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 (Chuah et al., 2013) (Chuong et al., 2009).

Toll-like receptor 4 (TLR4) represents another important component of innate immune signaling. TLR4 recognition of endogenous damage-associated molecular patterns can activate MyD88- and TRIF-dependent signaling pathways and promote downstream inflammatory responses, including NF- κ B-associated cytokine production (Juraneck et al., 2022). In 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 (Connolly et al., 2023). IL-13R α 2 has also been implicated in immune regulation and extracellular matrix-related signaling, including processes involving TGF- β -associated responses (Chiaramonte et al., 2003). 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 (Al Khzem and Wali, 2025; Parvathaneni et al., 2019). 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. Guanfacine is a centrally acting selective α_2 A-adrenergic receptor agonist initially used for its antihypertensive properties and later introduced into clinical practice for the management of attention-deficit/hyperactivity disorder (Martinez-Raga et al., 2015). Guanfacine acts by activating central α_2 A-adrenergic receptors, modulating noradrenergic signaling and cAMP-dependent pathways. In addition to its known applications in psychiatry and cardiology, growing data suggest that the α_2 A-adrenergic signaling pathway may play a role in neuroinflammatory and glial responses (Mei et al., 2024). Guanfacine has been shown to affect astrocyte reactivity, glutamate homeostasis, and microglial responses. Furthermore, the recent findings have demonstrated that the activation of α_2 A-adrenergic receptors could regulate cell recruitment and production of pro-inflammatory cytokines (Castro Hernández et al., 2025). Considering the importance of altered neuroimmune signaling and inflammation in ALS pathology, these pharmacological features make guanfacine a suitable candidate for investigating its

possible interactions with specific neuroimmune targets involved in ALS pathology.

Thus, this study used a computational approach to elucidate the molecular interactions predicted between guanfacine and four neuroimmune-related proteins implicated in ALS: RAGE (AGER), TLR4, CHI3L1, and IL-13R α 2. We used molecular docking for comparative analysis of guanfacine binding to the target molecules, followed by molecular dynamics simulations of the RAGE-guanfacine complex to analyze its conformational properties over a 100-ns simulation time course (Joyce et al., 2025). This study aimed to provide structural insight into the possible interaction of guanfacine with the selected neuroimmune-related targets and to identify molecular interactions for experimental validation in future studies.

2. MATERIALS AND METHODS

2.1. Selection and Preparation of Target Proteins

RAGE/RAGE/AGER, TLR4, CHI3L1, and IL-13R α 2 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-13R α 2 have been linked to glial responses and inflammatory regulation. Therefore, these proteins were chosen to examine the potential interaction profile of Guanfacine across different inflammation-associated targets using a computational approach (Lee et al., 2022).

The three-dimensional crystal structures of the shortlisted disease-related target receptors like RAGE/AGER, TLR4, CHI3L1, and IL-13R α 2, have been obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (wwPDB consortium, 2019). 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 (Pettersen et al., 2004). 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 (Morris et al., 2009).

2.2. Ligand Preparation

The three-dimensional chemical structure of Guanfacine was retrieved from the PubChem Compound database (Kim et al., 2016) 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 (Allinger, 1977). The prepared three-dimensional structure model of Guanfacine 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) (Khan, 2015; Soni et al., 2015). 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 (Gupta et al., 2022; Kaur et al., 2016).

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. Moreover, the major interactions of the native ligand with the amino acids were checked for their reproducibility (Agrawal et al., 2022; Kciuk et al., 2024; Leutcha et al., 2025).

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- κ B activation, thereby promoting progressive motor neuron degeneration (Bank, n.d.).
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 (Bank, n.d.).
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 (Bank, n.d.).
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 (Bank, n.d.).

2.5. Molecular Dynamics Simulation

Guanfacine complexed with the RAGE receptor was evaluated for its thermodynamic stability by performing molecular dynamics (MD) simulations for 100 ns using the Desmond module within Schrödinger's Maestro software (v2022.4). This analysis involved a macromolecular complex of the RAGE receptor associated with the ligand Guanfacine 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) (Abraham et al., 2015). 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-13R α 2 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 Guanfacine 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 Guanfacine was successfully prepared by using the SMILES procured from PubChem database and saved in PDBQT format. The final optimized ligand was then used to perform molecular docking against all selected receptors. The energy minimized structure of Guanfacine consists of twelve rotatable bonds and all were kept flexible to impart maximum possible flexibility.

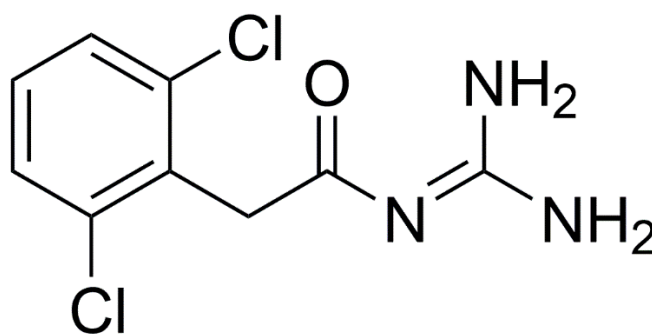


Figure 1. Energy minimized and optimized two-dimensional structure of Guanfacine.

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. **Table 1** summarizes the grid dimensions and center coordinates used for molecular docking of the receptors under consideration.

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 Guanfacine 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 Guanfacine 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 Guanfacine within the active binding pockets of RAGE receptor

was presented in **Figure 2**.

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

S. No.	Ligand	Binding Energy (kcal/mol)			
		IL13 (pdb: 5l6y)	RAGE (pdb: 5l6y)	TLR4 (pdb: 5l6y)	CHI3LI (pdb: 5l6y)
1	Guanfacine	-3.75	-7.75	-4.01	-5.29
1	Guanfacine	-4.28	-7.47	-5.21	-6.65

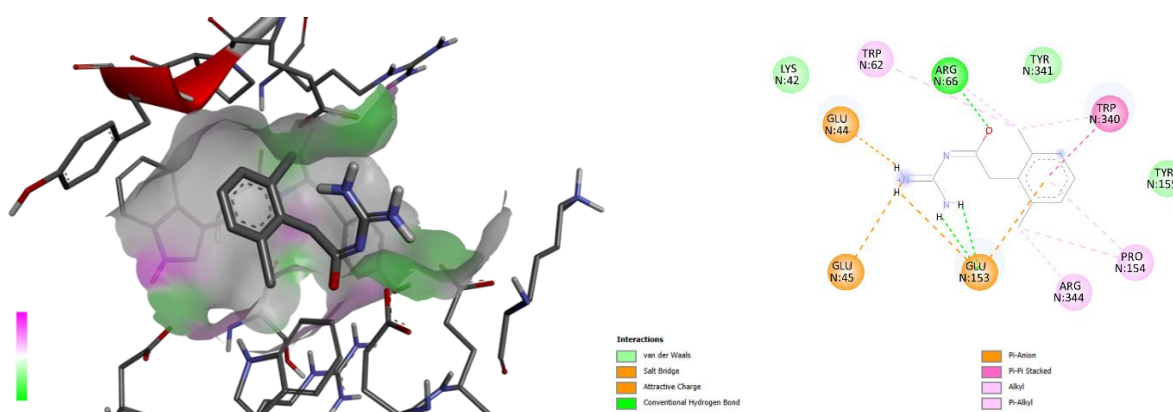


Figure 2. Three-dimensional docked conformation and two-dimensional binding interactions of Guanfacine within the active binding pockets of RAGE receptor.

3.5 Molecular Dynamics Simulation

The proposed repurposed drug molecule Guanfacine 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–Guanfacine 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 Guanfacine has 26 heavy atoms out of 51 atoms, with 12 rotatable bonds. The ligand RMSD further indicated Guanfacine's stability within the receptor's binding cavity in comparison with the receptor's C α 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 Guanfacine. The complex ligand Guanfacine 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 Guanfacine within the active binding pocket of RAGE. The RMSD of the concerned RAGE protein complexed with the Guanfacine ligand observed during the 100 ns MD simulation was shown in **Figure 3**.

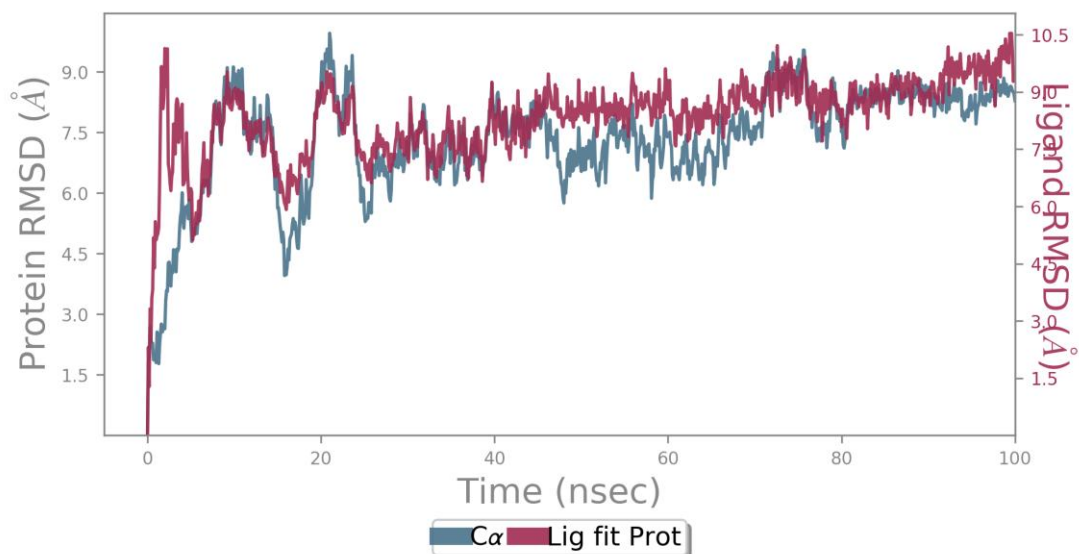
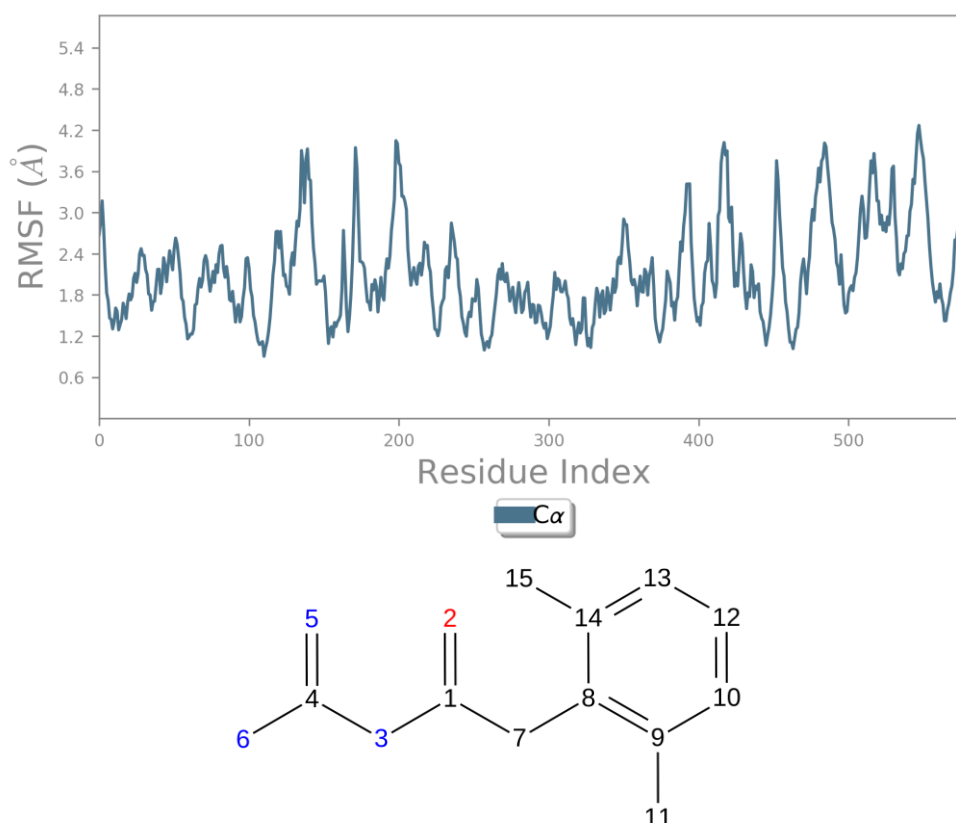


Figure 3: RMSD for the RAGE protein complexed with the Guanfacine ligand observed during the 100 ns MD simulation.

The RMSF value of the receptor's C α backbone of RAGE was found to range between 1.5 and 4.5 Å. Similarly, the RMSF observed for the complexed ligand Guanfacine 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 Guanfacine (b) observed during the 100 ns MD simulation is shown in **Figure 4**.



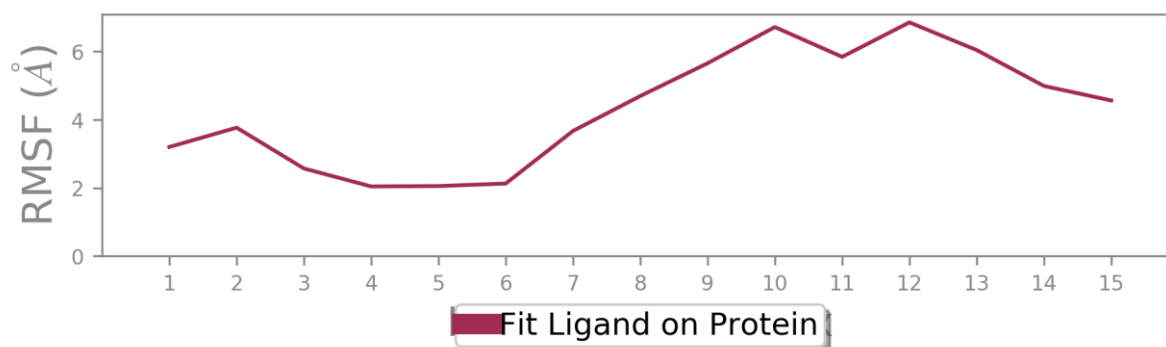


Figure 4: RMSF profile of the RAGE protein (a) and complexed ligand Guanfacine (b) observed during the 100 ns MD simulation.

The macromolecular–ligand interaction analysis performed throughout the 100 ns simulation revealed that Guanfacine 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 Guanfacine 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**.

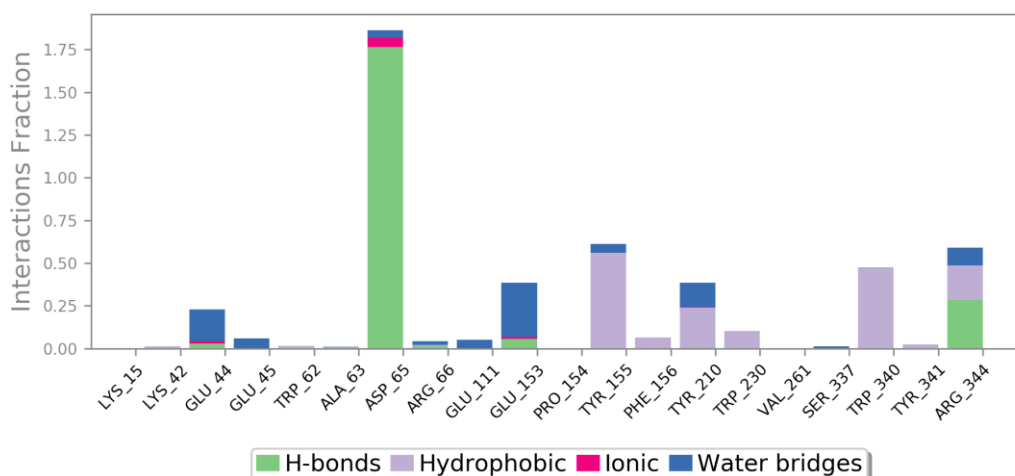


Figure 5: Ligand protein interaction analysis of Guanfacine against RAGE receptor observed during 100 ns MD simulation.

4. DISCUSSION

This study used molecular docking and molecular dynamics simulations to investigate the predicted interactions between Guanfacine and four proteins associated with inflammatory and neuroimmune processes relevant to ALS: RAGE, TLR4, CHI3L1, and IL-13R α 2. The docking analysis provided a comparative assessment of Guanfacine's predicted binding energies with the selected targets. Among the proteins examined, the lowest predicted binding energy was observed for RAGE (−7.47 kcal/mol), followed by CHI3L1 (−6.65 kcal/mol), TLR4 (−5.21 kcal/mol), and IL-13 (−4.28 kcal/mol). These findings indicate that Guanfacine 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–Guanfacine complex for molecular dynamics simulation. During the 100-ns simulation, the RAGE–Guanfacine 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 interactions throughout the simulation and provided additional insight into Guanfacine behavior within the RAGE binding region.

Analysis of protein–ligand interactions during the simulation further revealed Guanfacine 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 Guanfacine 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.

Guanfacine 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 Guanfacine 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 Guanfacine with these proteins therefore provides a basis for considering possible molecular associations within this inflammatory context. Nevertheless, the present study does not demonstrate that Guanfacine 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–Guanfacine complex. While docking provided an initial assessment of the possible orientation and predicted interaction energy of Guanfacine 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 Guanfacine 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–Guanfacine 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 Guanfacine 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

The present computational findings suggest that Guanfacine has the potential to interact with RAGE among the neuroimmune proteins investigated, with the Guanfacine–RAGE complex maintaining interactions throughout the 100-ns molecular dynamics simulation. The observed hydrogen-bond, water-mediated, and hydrophobic contacts provide a structural basis for further experimental investigation of the potential association of Guanfacine with RAGE in the context of ALS-relevant neuroimmune mechanisms. These findings should be regarded as a hypothesis-generating computational framework rather than evidence of confirmed target engagement or therapeutic efficacy. Biochemical binding studies and disease-relevant cellular and in vivo experiments are warranted to determine whether the predicted Guanfacine–RAGE interaction has functional biological significance.

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: Balbir Singh, Somdutt Mujwar, Maneesh Mohan. **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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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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