Computational Discovery of Promising Drug Candidates Targeting Alzheimer's Disease Pathways

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by memory loss, cognitive decline, and behavioral changes, with no definitive cure currently available. Existing therapeutic options offer only symptomatic relief and are associated with significant side effects. Given the complexity of AD pathology and the involvement of multiple molecular mechanisms, there is a critical need for the discovery of novel drug candidates. In this study, we employed an integrative in silico approach to identify potential inhibitors targeting TDP-43, a protein increasingly recognized for its involvement in AD and other neurodegenerative diseases. A virtual screening (VS) workflow was carried out using a curated library of over 100 structurally diverse compounds from the PubChem database. Molecular docking studies were conducted using AutoDock 4.2, focusing on the interaction of these compounds with the TDP-43 active site. The top ten compounds were shortlisted based on binding affinity and interaction profiles. Among them, the compound with PubChem CID 46241754 demonstrated the highest binding affinity of −8.2 kcal/mol, indicating a strong and stable interaction with TDP-43. Structural analysis suggested favorable hydrogen bonding and hydrophobic interactions, supporting its potential as a lead compound. This computational study lays the groundwork for further experimental validation and highlights the promise of targeting TDP-43 in the development of new therapeutic agents for Alzheimer's disease.

Keywords- Alzheimer's disease, TDP-43, virtual screening, molecular docking, AutoDock, PubChem, drug discovery, neurodegeneration

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to progressive cognitive decline, memory impairment, and behavioral changes, particularly in aging individuals. It is the most common neurodegenerative disease, affecting over 55 million people worldwide. Current treatment options are limited to Donepezil, an acetylcholinesterase (AChE) inhibitor, and Memantine Hydrochloride, an N-methyl-D-aspartate (NMDA) receptor antagonist. However, these drugs only provide symptomatic relief and may cause adverse effects with prolonged use. Consequently, there is an urgent need for novel therapeutic strategies to address AD more effectively. The progression of AD follows with cognitive and behavioral impairment, ultimately leading to dementia. Despite extensive research, no cure for AD currently exists due to its complex pathology, which involves multiple biological mechanisms. Although different biomarkers like Aβ, tau, and neuroinflammatory markers have been explored as potential therapeutic targets, only a limited number of drugs aimed at these pathways have gained clinical approval. The failure of many experimental treatments in clinical trials is largely due to an incomplete understanding of AD's complex mechanisms, challenges in drug delivery to the brain, and shortcomings in trial design. Thus, a deeper investigation into AD pathophysiology is crucial for developing more effective therapeutic strategies.

TDP-43, a protein associated with several neurodegenerative diseases, has drawn considerable attention in research. Initially identified in the neuronal inclusions of individuals with frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), TDP-43 has since been recognized as a critical factor in disease progression. In ALS, abnormal cytoplasmic accumulation of TDP-43 occurs in over 95% of cases, while up to 50% of FTD patients exhibit similar pathology. The TARDBP gene, located on chromosome 1, encodes TDP-43, which contains functional domains such as a nuclear localization signal (NLS), two RNA recognition motifs (RRM1 and RRM2), a nuclear export signal (NES), and a glycine-rich region. Although TDP-43 is primarily localized in the nucleus, it can translocate to the cytoplasm, where its accumulation is linked to neurodegenerative disorders. Structural studies have faced challenges due to TDP-43's poor solubility and aggregation tendencies, but high-resolution analyses have shed light on its functional domains. TDP-43 plays a key role in mRNA transport, translation

regulation, and interactions with proteins involved in RNA processing and gene expression. Investigating these mechanisms is crucial for understanding disease progression and developing targeted treatments to counteract the toxic effects of TDP-43 aggregation.

This study aims to identify novel TDP-43 inhibitors as potential AD therapeutics using an integrative computational approach. We utilize virtual screening (VS), molecular docking, and structural analysis to examine ligand interactions with Donepezil-bound crystal structures. Accordingly, this research focuses on discovering innovative inhibitors targeting human TDP-43 through a comprehensive strategy involving virtual screening, molecular docking, and MD simulations. The ultimate goal is to contribute to the development of more effective treatments for AD and other neurodegenerative diseases.

Methodology

Preparation of protein TDP-43

The three-dimensional structure of the TDP-43 protein, which is implicated in various neurodegenerative disorders including Alzheimer's disease, was retrieved from the Protein Data Bank (PDB) using the ID 6T4B. To ensure accurate molecular docking and simulation studies, the protein structure underwent a series of meticulous preparatory steps. Initially, all non-essential components such as bound ligands, cofactors, and crystallographic water molecules were removed to isolate the target protein in its native form. This step helped eliminate any interfering interactions that could distort docking results. Subsequently, structural refinement was carried out to ensure completeness and accuracy. Missing atoms and residues were identified and modeled, particularly in loop regions or disordered segments, using homology-based prediction and energy minimization techniques. Hydrogen atoms were added to the structure to maintain proper valency and enhance stereochemical precision, with specific attention paid to preserving the correct chirality of amino acid side chains. Furthermore, steric clashes and inconsistencies in bond geometry were resolved by energy minimization and structural validation tools. Disulfide bonds, if present, were checked and optimized to reflect their biological relevance. These refinement steps collectively ensured the protein was in an energetically favorable and biologically accurate conformation, making it suitable for docking simulations.

Virtual Screening

To identify novel small-molecule inhibitors potentially capable of modulating TDP-43 activity and thereby contributing to Alzheimer's disease therapeutics, a comprehensive virtual screening (VS) protocol was employed. A structurally diverse and pharmacologically relevant compound library comprising over 100 small molecules was curated from the PubChem database. The compounds were pre-filtered based on drug-likeness criteria, including Lipinski's Rule of Five, to ensure oral bioavailability and favorable pharmacokinetics. Each compound was prepared for docking by converting its structure to the appropriate file format (PDBQT), optimizing geometry, and assigning partial atomic charges. The virtual screening was conducted using molecular docking algorithms that evaluated the binding affinity of each compound to the active site of the TDP-43 protein. Binding interactions were assessed based on predicted docking scores, orientation within the binding pocket, and key molecular interactions such as hydrogen bonds, hydrophobic contacts, and π-π stacking. Top-ranking compounds, exhibiting the most favorable binding energies and interaction profiles, were shortlisted for further investigation. These hits serve as promising leads for future in vitro and in vivo validation as potential therapeutic agents against Alzheimer's disease.

Molecular docking

To ensure the reliability of docking predictions, the docking protocol was benchmarked using the known binding energy and orientation of the co-crystallized ligand (CA), which served as a reference for evaluating newly screened ligands. Molecular docking simulations were carried out using the Lamarckian Genetic Algorithm (LGA), a robust and widely validated approach that combines genetic algorithms with local search optimization. This method excels in predicting accurate ligand conformations by simulating biological evolution processes such as selection, crossover, and mutation, while also performing energy minimization to refine poses. The receptor protein was preprocessed by assigning Kollman united atom charges and adding polar hydrogen atoms, ensuring optimal electrostatic and hydrogen bonding interactions with potential ligands. Ligands were prepared by converting their structures into PDBQT format, a requirement for AutoDock, which incorporates information about atomic types, torsional degrees of freedom, and partial charges. In the docking setup, the protein structure was kept rigid to preserve its native conformation, while ligands were allowed full torsional flexibility, enabling exhaustive exploration of conformational space within the active site. To accurately

target the binding region, a grid box was defined around the TDP-43 active site with dimensions of 80 × 80 × 80 Å and a grid spacing of 0.475 Å. This configuration ensured comprehensive coverage of the binding pocket and adjacent areas, thereby increasing the likelihood of identifying energetically favorable ligand poses. This setup provided a high-resolution search space for evaluating molecular interactions critical to the inhibition of TDP-43 function.

Results and Discussions-

Protein structural information

Human TAR DNA-binding protein 43 (TDP-43) plays a critical role in several neurodegenerative disorders, including Alzheimer's disease (AD), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). It is increasingly recognized as a pathological hallmark, particularly due to its aberrant aggregation and mislocalization in affected neurons. TDP-43 is a multifunctional RNA-binding protein involved in mRNA splicing, transport, translation, and stability, making it essential for normal cellular homeostasis. However, like many other proteins with intrinsically disordered regions (IDRs) or low-complexity domains, TDP-43 presents substantial challenges in structural biology. Its tendency to aggregate, misfold, and form insoluble inclusions severely limits the ability to produce full-length recombinant TDP-43 in sufficient quantities and with the necessary stability for detailed X-ray crystallography or NMR-based structural studies. These difficulties stem from the protein's inherent biochemical instability, poor solubility, and its propensity to self-associate under physiological conditions. In particular, the C-terminal glycine-rich domain is prone to aggregation and is often truncated or omitted in recombinant constructs, making it difficult to capture the protein in its native, full-length form. As a result, much of the existing structural information on TDP-43 is limited to isolated domains or truncated versions, which may not fully represent its in vivo conformation or interaction potential. Despite these challenges, recent advancements in cryo-electron microscopy (cryo-EM), molecular modeling, and computational simulations have begun to shed light on the structural behavior of TDP-43, providing valuable insights into its role in neurodegenerative disease pathogenesis.

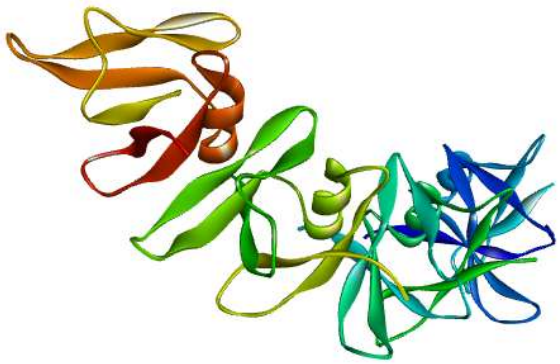

Figure 1- Three-dimensional structure of Human protein TDP-43 visualized through Chimera Tool

Virtual Screening

Virtual Screening for Potential Inhibitors of Alzheimer's Disease Targets
To identify novel therapeutic candidates for the treatment of Alzheimer's disease (AD), a comprehensive virtual screening (VS) process was conducted. This involved the use of a curated chemical library comprising more than 100 bioactive and structurally diverse compounds obtained from the PubChem database. These compounds were selected based on their reported or predicted pharmacological relevance and drug-like properties. Using molecular docking techniques, each compound was virtually screened against a key target protein associated with the pathogenesis of Alzheimer's disease—such as acetylcholinesterase (AChE), beta-secretase (BACE1), or amyloid-beta (Aβ) aggregating sites—depending on the specific therapeutic strategy under investigation. The compounds were ranked based on their binding affinities, docking scores, and interaction profiles with the target's active or allosteric sites. From this screening, the top ten compounds exhibiting the most favorable binding energies and predicted interaction patterns were selected for further in silico evaluation, including molecular dynamics simulation, pharmacokinetic (ADMET) profiling, and structure-activity relationship (SAR) analysis. These selected hits serve as promising leads for future experimental validation and potential development into anti-Alzheimer drug candidates.

Table 1- Screened compounds through PubChem database against Alzeimer's disease

Compound	Molecular Weight	H-bond donor	H-bond acceptor	Docking score
3152	379.5	0	4	-5.9
53394849	377.5	0	4	-6.7
46889660	351.4	0	4	-6.6
46241976	390.6	0	4	-7.3
46241973	394.6	0	4	-7.3
46241866	385.5	0	4	-8.1
46241865	383.5	0	4	-8.1
46241754	392.6	0	4	-8.2
46241752	390.6	0	4	-8.1
46241244	380.5	0	4	-8.1
46241242	392.6	0	4	-8.1

3.3 Molecular docking score studies

Molecular docking analysis was performed using AutoDock 4.2 to evaluate the binding interaction between the target protein TDP-43 and the compound with PubChem CID 46241754. Among all the docking conformations analyzed, the most favorable binding pose exhibited a lowest binding energy of −8.2 kcal/mol, indicating a strong and stable interaction between the ligand and the active site of TDP-43. This high binding affinity suggests that compound 46241754 forms significant molecular interactions—possibly involving hydrogen bonding, hydrophobic contacts, and van der Waals forces—with key amino acid residues in the binding pocket of TDP-43. The docking score of −8.2 kcal/mol places this compound as a promising lead candidate for further structural optimization and biological evaluation as a potential modulator of TDP-43-related pathologies, such as neurodegenerative diseases including ALS (amyotrophic lateral sclerosis) and frontotemporal dementia.

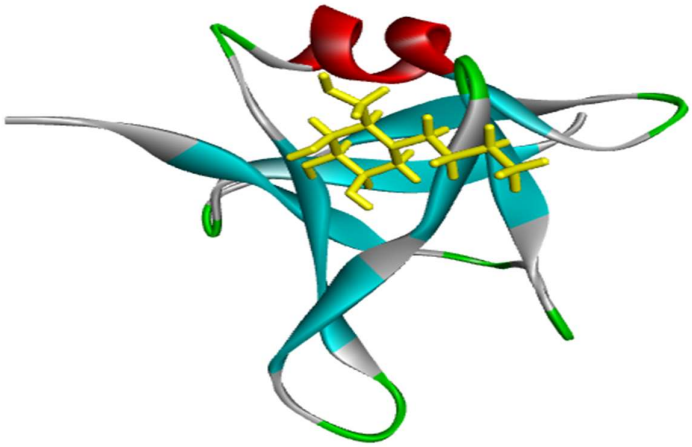

Figure 2- Docked complex of the best compound 46241754 in complex with TDP-43 through AutoDock 4. 2 tool

Conclusion

The present study highlights the potential of a computational drug discovery approach in identifying novel therapeutic candidates for Alzheimer's disease by targeting the TDP-43 protein, a critical player in the pathogenesis of neurodegenerative disorders. Through an integrated workflow involving protein preparation, virtual screening, and molecular docking, we identified compound PubChem CID 46241754 as a lead molecule with the highest binding affinity (−8.2 kcal/mol) to TDP-43. This compound demonstrated favorable interactions within the binding pocket, suggesting strong potential as a TDP-43 modulator. These findings provide a solid foundation for further in vitro and in vivo investigations to validate the efficacy and safety of the identified compound. Overall, this study supports the promise of in silico methods in accelerating the early stages of neurotherapeutic drug development and encourages future efforts in targeting TDP-43 for the treatment of Alzheimer's disease and related neurodegenerative conditions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for this study

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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