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Unlocking ADAMTS-5: *In Silico* insights into TMJ proteomics and docking dynamics

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Abstract

BACKGROUND: Temporomandibular joint (TMJ) disorder refers to a condition involving dysfunction or pain in the jaw joint and the muscles that control jaw movement. It can affect one or both sides of the jaw and can cause various symptoms, including Jaw pain or tenderness; Difficulty or discomfort when chewing; Clicking, popping, or grating sounds in the jaw joint; Jaw locking or limited movement; Earache or pain around the ear; Headaches or migraines; Neck and shoulder pain; Swelling on the side of the face. TMJ disorder can have various causes, including injury to the jaw joint, teeth grinding or clenching (bruxism), arthritis, stress, misalignment of the jaw or teeth, and excessive gum chewing. Computer-aided drug design (CADD) comprises a range of theoretical and computational strategies employed in contemporary drug discovery. Molecular docking stands out as a key technique within CADD, aiding in the comprehension of drug-molecule interactions for rational drug design, mechanistic investigations, and the creation of stable complexes with heightened specificity and potential effectiveness. Through the docking process, valuable information regarding binding energy, free energy, and predictions of complex stability is obtained, offering significant insights into drug development endeavors.

AIM: The objective of this research was to employ docking methodology to identify potential ADAMTS-5 protein for TMJ. Four ADAMTS-5 protein inhibitors previously reported in the literature were selected, and their compound structures were obtained from the Zinc15 database. The ADAMTS-5 protein was designated as the target and optimized utilizing the RCSB Protein Data Bank. Following pharmacophore modeling, 20 novel compounds were identified, and SwissDock was utilized to dock these compounds with the target protein. A comparison was made between the binding energies of the newly discovered compounds and those of previously published molecules with the target.

RESULTS: The results indicated that among the 20 ZINC1846088 and ZINC33606904 exhibited the highest binding energy and displayed superior properties compared to the other molecules.

CONCLUSION: The study concluded that ZINC1846088 and ZINC33606904 exhibited greater binding affinity than the reported inhibitors of ADAMTS-5 protein. Therefore, these two molecules can be used as a potential and promising lead for the treatment of TMJ and could be employed in targeted drug therapy.

CATEGORIES: Dentistry, TMJ.

Keywords:

3D modeling, ADAMTS-5 protein inhibitors, CADD, docking

Introduction

Temporomandibular Disorder (TMD) presents a challenging medical condition characterized by dysfunction and pain in the

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jaw joint and associated muscles, affecting millions of individuals worldwide. The diverse array of symptoms includes jaw pain, difficulty chewing, clicking or popping sounds in the joint, limited movement, and associated ear and head discomfort.[1] TMJ disorder can arise from various factors such as trauma, bruxism (teeth grinding), arthritis, stress, and structural abnormalities of the jaw or teeth. In addition, genetic factors are implicated in the development of chronic pain conditions, likely influencing underlying processes such as nociceptive sensitivity, psychological well-being, inflammation, and autonomic response, in the context of TMD.[1,2] Despite its prevalence and impact on quality of life, effective therapeutic interventions for TMJ disorder remain elusive.

ADAMTS (A Disintegrin-like And Metalloproteinase with ThromboSpondin motifs) proteins are a family of enzymes involved in various biological processes, including extracellular matrix (ECM) metabolism. Among them, ADAMTS5 stands out due to its significant role in joint health and pathology including, TMD.[3] In the context of TMD, ADAMTS-5 plays a critical role in joint homeostasis and pathology.[3,4] This enzyme is particularly involved in the degradation of cartilage within the temporomandibular joint, which is essential for maintaining joint function and resilience. However, when ADAMTS-5 activity becomes dysregulated, such as in cases of TMD, it can lead to excessive degradation of cartilage, contributing to joint degeneration, inflammation, and associated symptoms like pain and restricted jaw movement[5] Understanding the molecular mechanisms underlying ADAMTS-5 involvement in TMD pathology is crucial for developing targeted therapeutic strategies. Research endeavors, such as utilizing molecular docking methodologies, aim to identify potential inhibitors that can modulate ADAMTS-5 activity, thereby mitigating cartilage degradation and alleviating symptoms associated with TMD.

In recent years, computational approaches have emerged as promising tools in drug discovery, offering novel insights and accelerating the identification of potential therapeutic agents. Among these methods, molecular docking, a key component of computer-aided drug design (CADD), plays a crucial role in elucidating the interactions between small molecules and target proteins.[6] By predicting binding affinities and providing structural insights into drug-protein complexes, docking analysis facilitates the rational design and optimization of candidate compounds for various diseases, including TMD.[7]

The objective of this research was to utilize molecular docking methodology to identify potential inhibitors targeting the ADAMTS-5 protein implicated in TMJ disorder. Through a systematic approach involving pharmacophore modeling and docking simulations, novel compounds were screened against ADAMTS-5 to identify promising therapeutic candidates.

Materials and Methods

The research was conducted after obtaining approval from the Scientific Review Board (SRB/SDC/FACULTY/ORTHO) of the Department of Orthodontics, Saveetha Dental College and Hospitals. This study focused on the ADAMTS-5 protein.

Four potential inhibitors were identified through databases like Zinc20, Google Scholar, and PubMed: ZINC1846088, ZINC33606904, ZINC575436534, and ZINC1857603334. Each of these inhibitors has distinct clinical applications and functions. The protein used in the docking analysis was obtained through homology modeling. Therefore, utilizing the chemical structures of these five molecules as a template for the novel compounds, this study aims to assess the binding affinity of these medications with the targeted ADAMTS-5 protein in the form of binding patterns.

Multiple sequence alignment (MSA) by ClustalW

It presents a computational challenge, particularly for large datasets, where dynamic programming becomes impractical due to the sheer number of possible combinations. Therefore, to handle larger problems within practical time frames, heuristic methods are employed. These methods leverage evolutionary relationships among homologous sequences and utilize a phylogenetic tree to guide the alignment process.[8] Beginning with the most closely related sequences, the alignment gradually incorporates more distant ones, allowing for the alignment of virtually any size data set [Figure 1]. While this approach typically yields high-quality alignments for ADAMTS-5, especially evident in simple cases where corresponding domains from sequences with known structures are accurately aligned, more complex scenarios may require additional refinement through automatic or manual methods.

CLUSTALW stands out as one of the most widely used software tools for computing Multiple Sequence Alignments (MSAs).[8,9] Additionally, several other freely accessible web services offer CLUSTALW, such as http://toolkit.tuebingen.mpg.de/sections/alignment. The ADAMTS-5 crystallized structure (PDB_ID: 2RJQ) is proposed as the optimal template for comparative modeling.[8-10] By examining sequence color conservation/change, highly conserved amino acid regions, likely implicated in conformational changes, and semi-conserved amino acid regions,

potentially involved in substrate specificity, can be highlighted.[11] Notably, differences in amino acid composition among Mammalia, Arthropoda, and plant sequences offer insights for further investigation into potential variations in substrate specificities. Various color schemes are available for interpreting MSAs, with the ClustalX standard color table reflecting the physicochemical properties of amino acids.[11,12] Modifications to the color code aid in identifying conserved residues and may provide insights into conserved amino acid substitutions. Alternative color schemes reflect diverse amino acid properties, such as hydrophobicity, helix, strand-, and turn-propensity, steric hindrance, and physicochemical similarities between residues (e.g. Jalview color schemes).[11-13] The accuracy of an MSA can be significantly influenced by distantly related members within a protein family.[14] A thorough understanding of evolutionary and structural relationships within the protein family is crucial to avoid misleading interpretations of an MSA.

Virtual screening

The generated models underwent screening as 3D queries within the ZINC20 database search, targeting the purchasable ZINC subsection. Hits were selected based on shared structural moieties with the training set and subsequently filtered according to Lipinski's Rule of Five properties to identify potential new drug-like candidates.

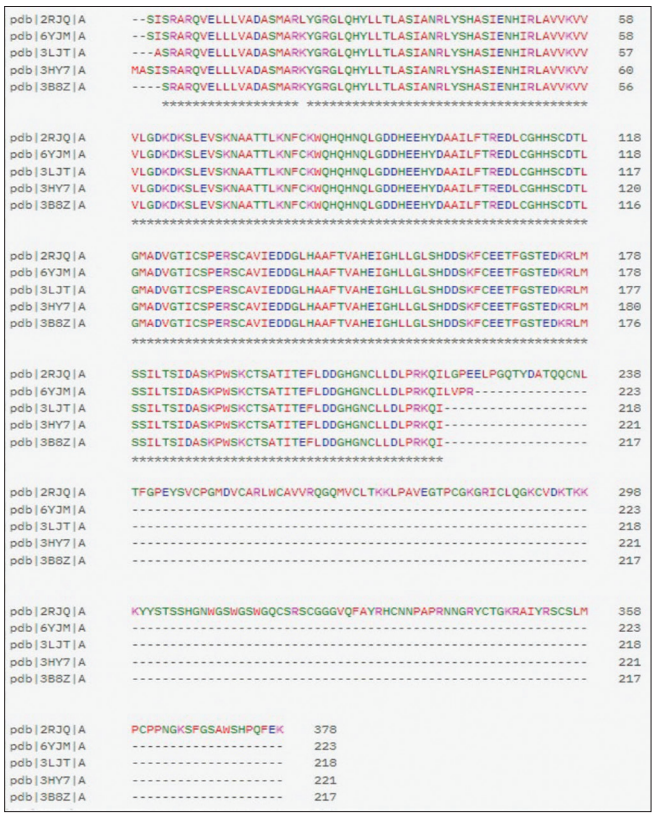

Figure 1: Multiple sequence alignment of 2RJQ using ClustalW

Ligand selection

The study commenced by selecting the most promising compounds from a pool of 100 molecules known for their ADAMTS-5 genetic properties. This selection process relied on the compounds' structural attributes and in-silico data regarding their interaction with ADAMTS-5, including molecular docking properties and the prediction of ADMET properties. Furthermore, the 3D protein structure of human ADAMTS-5 (PDB ID: 2RJQ) was retrieved from the Protein Data Bank, featuring an atomic resolution of 2.60 Å and comprising a total Structure Weight of 42.84 kDa within a single protein chain, complexed with zotepine. Subsequently, the computational 3D protein structure was prepared for protein-ligand docking analyses using Discovery Studio [Figure 2a]. The modeled structural validation was done in the Ramachandran plot server (http://molprobity.biochem.duke.edu/) [Figure 2b].

Molecular docking

In the pursuit of bolstering the reliability and robustness of our computational models, a meticulous approach was adopted, necessitating the execution of a redocking procedure on the top 100 hit molecules.[14,15] This meticulous task was carried out leveraging the advanced capabilities of the BIOVIA Discovery Studio Client 2021, a sophisticated software renowned for its precision in molecular modeling and simulation. The overarching objective of this endeavor was twofold: firstly, to discern and eliminate any potential false positive hits that might have arisen during the initial screening process, thus ensuring the accuracy of our findings; and secondly, to gain deeper insights into the intricate interactions between the identified ligands and the active site of the ADAMTS-5 receptor. The hits obtained from the redocking exercise were meticulously scrutinized and ranked based on their respective binding energies, a pivotal parameter indicative of the strength of ligand-receptor interactions. To further elucidate the nature of these interactions at the atomic level, advanced visualization tools such as PMV (Python Molecular Viewer) and PyMol were employed. These cutting-edge software applications enabled us to dissect and analyze the atomic-level details of ligand binding, offering invaluable insights into the structural

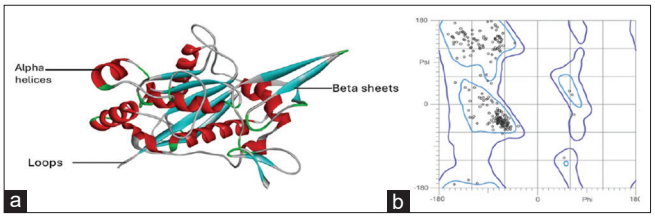

Figure 2: (a) 3D homology model of Human ADAMTS-5 (PDB ID: 2RJQ) and (b) Ramachandran plot

determinants governing ligand affinity and specificity towards the ADAMTS-5 receptor. By integrating computational redocking with state-of-the-art visualization techniques, we not only enhanced the reliability of our model predictions but also enriched our understanding of the molecular mechanisms underpinning ligand recognition and binding in the context of ADAMTS-5 receptor pharmacology. This comprehensive approach underscores our commitment to advancing computational drug discovery methodologies and holds immense promise in the rational design of novel therapeutics targeting the serotonin receptor system.

ADMET study

The prediction of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties was facilitated using the pkCSM web server. This platform integrates data from both approved FDA drugs and experimental compounds, allowing for the anticipation of critical pharmacokinetic attributes. These attributes include, among others, central nervous system (CNS) and blood-brain barrier (BBB) permeability, human intestinal absorption, the likelihood of hepatotoxicity, and assessment of whether a compound acts as an inhibitor or substrate of P-glycoprotein. This comprehensive analysis serves as a crucial tool in the early stages of drug development, assisting researchers in identifying potentially harmful pharmaceutical agents.

Results

The ThorDock molecular docking analysis discovered two novel compounds (ZINC1846088 and ZINC33606904) with much stronger binding patterns (-8.097 and -7.408) compared to the other inhibitors [Figure 3]. In [Figure 4]. 1,4.2,4.3,4.4 depicts 3D and 2D structures of docked receptors and ligands. The properties of all the molecules were compared; these two novel compounds demonstrated superior properties by the criteria required for a pharmaceutical substance, including Lipinski (Rule no 1), Ghose (Rule no 2), Veber (Rule no 3), Egan (Rule no 4), and Muegge (Rule no 5) [Table 1]. Additional factors were evaluated in addition to bioavailability, log P, and TPSA. A comparison was made between the properties of each molecule and the two novel compounds, which had superior properties that complied with all the established criteria [Table 2]. In addition to bioavailability, Log P, and Topological Polar Surface Area (TPSA), additional parameters were compared [Table 3].

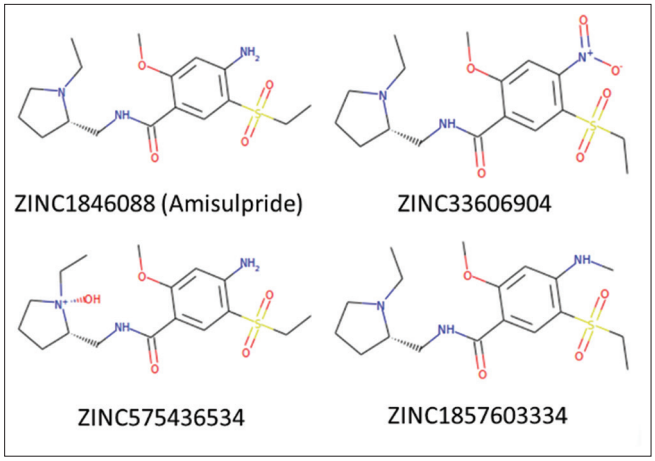

Figure 3: 2D chemical structures of ligands

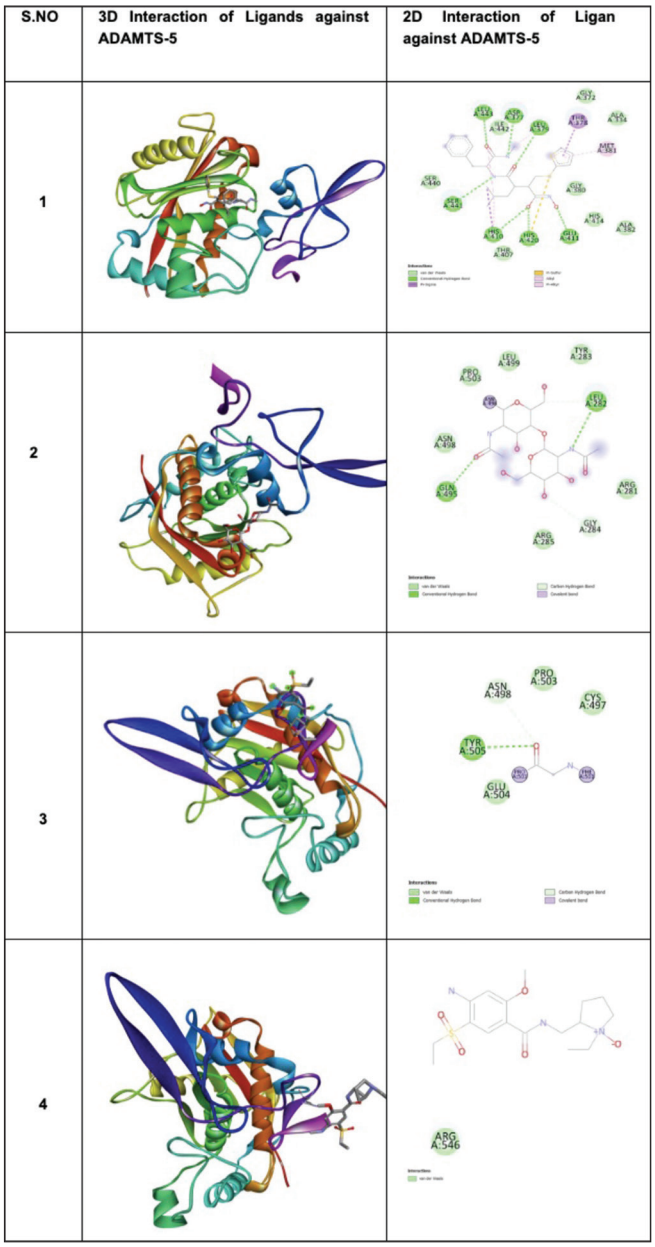

Figure 4: 3D and 2D structures of docked receptor and ligands

Discussion

The primary cause of temporomandibular joint (TMJ) dysfunction is a disruption in the coordinated function of associated muscles, often linked to malocclusion.[16] Psychosocial factors further exacerbate this condition, leading to neuromuscular stress.[17,18] Temporomandibular joint pain dysfunction syndrome (TMJPDS) is a prevalent condition characterized by regional muscle pain, localized tenderness, and discomfort in tense muscle bands. This syndrome is a well-recognized source of persistent localized pain. Extensive evidence underscores the intricate relationship between mind and body in pain management, emphasizing the importance of establishing rapport with patients and fostering a positive attitude towards therapy for long-term improvement.[19]

The main objective of molecular docking is to predict interactions between small drug-like compounds and target proteins. Many diseases stem from protein dysfunction, necessitating therapeutic interventions that modulate target proteins through inhibition or activation.[20] In our study, we employed the free energy concept (δG) to evaluate the binding affinity of protein-ligand complexes using the ThorDock tool. A low or negative δG value indicates strong binding affinity, suggesting a favorable ligand conformation.[21] We screened ZINC1846088, ZINC33606904, ZINC575436534 and ZINC1857603334 against the human ADAMTS-5 target protein to identify potential pharmaceuticals.

Table 1: Binding affinity of potential ligands against ADAMTS-5

Ligand	Affinity	Total Energy	vdW Energy	Elec. Energy
ZINC1846088	−8.097	62.870	−17.361	−23.069
ZINC33606904	−7.408	133.092	−16.530	−24.469
ZINC575436534	−6.884	72.719	−7.839	−33.322
ZINC1857603334	−6.837	80.816	−5.778	−25.305

Pharmacophore modeling was performed for four molecules and validated using PharmaGist software. Subsequently, 20 lead molecules were selected and screened against the ADAMTS-5 protein[22]. The binding energy of these new molecules was compared to that of the four selected compounds. Among the 20 molecules, ZINC1846088 and ZINC33606904 exhibited the highest binding energy and met all five drug-likeness criteria. These identified molecules show promise in targeting ADAMTS-5 protein and hold potential for drug discovery.

Limitations

The present study did not explore the molecular dynamics of the drugs or resulting molecules. Future studies should also investigate other ADAMTS-5-related targets and potential drugs.

Conclusion

Recent advancements in docking and scoring techniques have significantly enhanced their utility in drug discovery. Through a comparative analysis, we evaluated the predictive capabilities of various docking and scoring algorithms. Our findings suggest that the docking programs assessed in this study perform satisfactorily and are poised to contribute significantly to drug development. Specifically, our ligand docking analysis identified ZINC1846088 and ZINC33606904 as exhibiting the highest binding energy among the tested ligands. Overall, our research has identified a highly potent lead compound with promising potential for the development of a novel pharmaceutical agent targeting TMJ while minimizing toxicity.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Table 2: Receptor-ligand interaction on ADAMTS-5 gene

Ligands	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log *P*	Consensus Log *P*
ZINC1846088	2.63	2.74	2.1	2.22	2.68	2.4
ZINC33606904	2.59	2.24	2.13	1.53	2.51	2.41
ZINC575436534	2.34	2.79	2.18	1.2	2.79	2.27
ZINC1857603334	0.89	1.65	1.34	0.44	1.13	0.52

Table 3: Drug likeness prediction to ligand molecules

Ligands	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score
ZINC1846088	0	0	0	0	0	0.55
ZINC33606904	0	0	0	0	0	0.55
ZINC575436534	0	0	0	0	0	0.55
ZINC1857603334	0	0	0	0	0	0.55

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