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Computational 3D structure prediction followed by molecular docking to reveal the novel drug targets against ADA

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Abstract

Adenosine deaminase (ADA) is a functional enzyme that transforms deoxyadenosine and adenosine into deoxyinosine and inosine respectively. ADA deficiency causes toxic purine degradation byproducts to build up in the body, which has a particularly negative impact on lymphocytes and results in adenosine deaminase-deficient severe combined immunodeficiency. Different *in silico* techniques including threading, *ab initio* and homology modeling for 3D structure prediction were applied for the prediction of ADA structures. Following the three-dimensional structure prediction analyses, an extensive computational assessment of all predicted structures for reliability was performed. The overall quality factor of the predicted ADA structures was observed 62.45% in the predicted 3D models. A Ramachandran plot was created, and 94.80% of the residues were found in the allowed and favored regions of the protein structure plot. The molecular docking analyses were performed in order to identify the potential therapeutic medication targets against ADA. The virtually examined molecules through a virtually high throughput screening may have the ability the regulation the ADA activity. The least binding energy was calculated through the molecular docking analyses and the energy values were observed -8.7 Kcal/mol. The binding residues (Lys-367, Glu-424, Asp-422, Phe-381, Ile-377, Ser-430 and Glu-374) were conserved in all the interactional analyses of the docked complexes. Finding the effective binding domain in a protein three-dimensional structure is crucial for understanding of its structural makeup and determining its functions.

Introduction

The catalytic enzyme adenosine deaminase (ADA) transforms the adenosine and deoxyadenosine into inosine and deoxyinosine respectively. The adenosine deaminase deficiency leads to the adenosine deaminase-deficient severe combined immunodeficiency where the toxic purine breakdown byproducts accumulate in the body and have a detrimental effect on the lymphocytes. Even while the effects on lymphocytes are the most noticeable, there are other manifestations as well, including skeletal abnormalities, effects on neurodevelopment, and pulmonary signs related to pulmonary-alveolar proteinosis [1]. It has been discovered that a vast range of bacteria, plants, and invertebrates have this universal enzyme. Additionally, it may be present in all mammalian cells, which are necessary for the lymphoid system to grow. Nevertheless, despite a number of studies that have been finished so far, it is still unknown what physiological purpose ADA plays in the different tissues. In severe mixed immunodeficiency disorder (ADA-SCID), which is brought on by inherited ADA deficiency, the development of both T-cells and B-cells is compromised [2]. ADA-SCID was the first disease to be treated with gene therapy utilizing the polyethylene glycol-modified bovine ADA (PEG-ADA). On the contrary, the quantity of ADA can be elevated in a number of disorders. Many ADA inhibitors, classified as transition-state as well as ground-state inhibitors have been designed and produced [3]. In lymphoproliferative illnesses and immunosuppressive therapies, they can be used to resemble the genetic deficiencies of the enzyme, to enhance the effects of antileukemic and antiviral nucleosides, and in combination with adenosine kinase, to reduce the adenosine breakdown in inflammatory diseases, hypertension, and ischemic injury.

The hydrolysis of the deaminates adenosine and 2-deoxyadenosine is essential for the metabolism of purines and the equilibrium of adenosine. This mechanism indirectly participates in the cellular signaling events by modifying the signaling through extracellular adenosine through binding, and functions as a positive regulator of T-cell coactivation [4]. Its interaction with DPP4 controls the adherence of lymphocytes to epithelial cells. By affecting the synthesis of dendritic cell stimulant molecules and their discharge of cytokines and chemokines, the enhanced immunogenicity of dendritic cells is achieved. ADA promotes CD4+ T cell development and expansion. ADA enhances the ligand affinity of the adenosine receptors ADORA1 and ADORA2A, acting as a positive modulator of these receptors [5]. The plasminogen activation is stimulated and contributes to male fertility. ADA has a protective role in the early postimplantation development of the embryo [2].

The achievement of immuno-informatics research and computerized drug design has significantly increased during the preceding fifteen years. The *in silico*, bioinformatics, and immunoinformatic techniques have been used to answer numerous biological problems [6-14]. The molecular docking analyses were used in the current *in silico* effort to identify the novel molecules against ADA. The 3D structure of ADA was predicted and novel molecular ligands with notable structural properties were screened. The Protein Data Bank (PDB) does not contain the 3D experimentally resolved structure of the selected protein (ADA) as determined by Nuclear Magnetic Resonance (NMR) and X-ray crystallography. For the subsequent molecular docking investigations, the chosen model's crystal structures might be used, and the reliable 3D projected structure of ADA was predicted.

Materials and Methods

From the UniProt Knowledgebase Database (UniProtKB), the canonical sequence of amino acid of ADA was retrieved in the acceptable format of FASTA style. In the present study, the 3D structure prediction approaches and molecular docking techniques were used to investigate the structural insights and international studies of the 3D predicted structure of the target protein ADA. The selected protein ADA sequences were obtained in the recommended FASTA format through the protein sequence databases and processed to protein-protein BLAST in order to hunt out the PDB templates that would work best for the target protein structure prediction through the homology modeling approach. The target protein structures were predicted by applying the automated program of homology modeling named MODELLER 9.21 [15] for 3D structure prediction of the proteins. Additionally, the structure prediction for a reliable structure was satisfied along with the spatial constraints. The proper three-dimensional templates for structure prediction using homology modeling techniques were utilized to evaluate the three-dimensional structures of the protein ADA, which was chosen for *in silico*

investigations. Additionally, the *ab initio* and threading methods were used to determine the 3D structure of the selected protein ADA. Utilizing a range of tools for assessing the accuracy of the 3D predictions, including Rampage, Anolea, ERRAT and ProCheck, the geometrical values of the anticipated structure were examined. Additionally, the MolProbity [16] evaluation toold was also used to assess the predicted 3Dstructures for the target protein ADA.

The high-throughput virtual screening of the ADA binding domain and potential therapeutic drug targets was conducted by using the ZINC commercial database and FDA chemical library was selected for virtual screening. For molecular docking investigations of protein-ligand analyses, AutoDock tools [17] and AutoDockVina were used. The total number of rotatable bonds, the total number of H-bond recipients, and the quantity of H-bond contributors were all estimated by using the PubChem program. Using the mCule, the Lipinski's rule of five was calculated by using the target ligand molecules and ChEMBL databases. To lessen the hazards of mutagenesis and carcinogenesis, the carcinogenicity and mutagenesis assessments of the screened ligands were examined. The ADA binding region was evaluated in comparison to the screened ligands in order to discover the novel therapeutic targets. Additionally, the molecular docking anayses were investigated. The geometric optimization was carried out by utilizing the ChemDraw software and UCSC Chimera 10.0 over the intended ligands and the predicted 3D structures. The docked protein and ligand complexes were looked at and evaluated by using UCSC Chimera 10.0. All of the selected ligands were further evaluated for ADMET (absorption, distribution, metabolism, elimination and toxicity) characteristics were investigated by using admetSAR.

Results and Discussion

The present effort was associated with the link of immunodeficiency against ADA. The *in silico* analyses to identify the novel targets for ADA. The templates were scrutinized through PDB for the structure prediction of ADA having satisfying similarity between the query and template along with the query coverage and accession number. The selected templates were utilized for homology modeling of the selected target protein ADA (**Table 1**).

For the purpose of predicting the 3D structure of the target protein ADA, the screened templates were chosen on the basis of query coverage and identity. With the aid of *ab initio*, threading and homology modeling methods, several 3D structures of ADA were generated, and reliable models for ADA were selected for further analyses. The 3D models of ADA were predicted by employing the x-ray crystallographic solved structure of the selected templates.

Various tools of 3D computational evaluation were utilized to verify the reliability of the predicted models of ADA along with the efficacy of the predicted models (**Fig. 1**).

Through the generation of the Ramachandran plot, the outlier region, allowed region, and favoured region were evaluated for the predicted structure of ADA. It was observed that 94.80% of the residues in the selected ADA structure were present in the allowed and favored regions of Ramachandran plot (**Fig. 2**). However, only the outlier region of the chosen principal component of the generated Ramachandran plot was observed 1.26% of the ADA predicted structure and few residues were found in the outlier area of Ramachandran plot (**Fig. 2**).

Table 1: The three-dimensional structure forecasting of ADA using the selected templates and protein BLAST

Description	Query coverage (%)	Total score	Identity (%)	Accession	E-Value
Crystal Structure of Adenosine Deaminase from Salmonella typhimurium	99	604	88.79%	6N9M	0.0
Crystal Structure of Adenosine Deaminase from Vibrio cholera	96	479	68.88%	6N91	9e-171
Crystal Structure of bovine adenosine deaminase	96	164	32.25%	1KRM	2e-47
Crystal Structure of dipeptidyl peptidease IV	96	164	32.25%	1W1I	2e-47
HIV-1 Tat potein derived N-terminal nonpeptide	96	164	32.25%	2BGN	3e-47
Crystal Structure of Adenosine Deaminase complexed with FR117016	96	163	32.25%	1NDV	8e-47

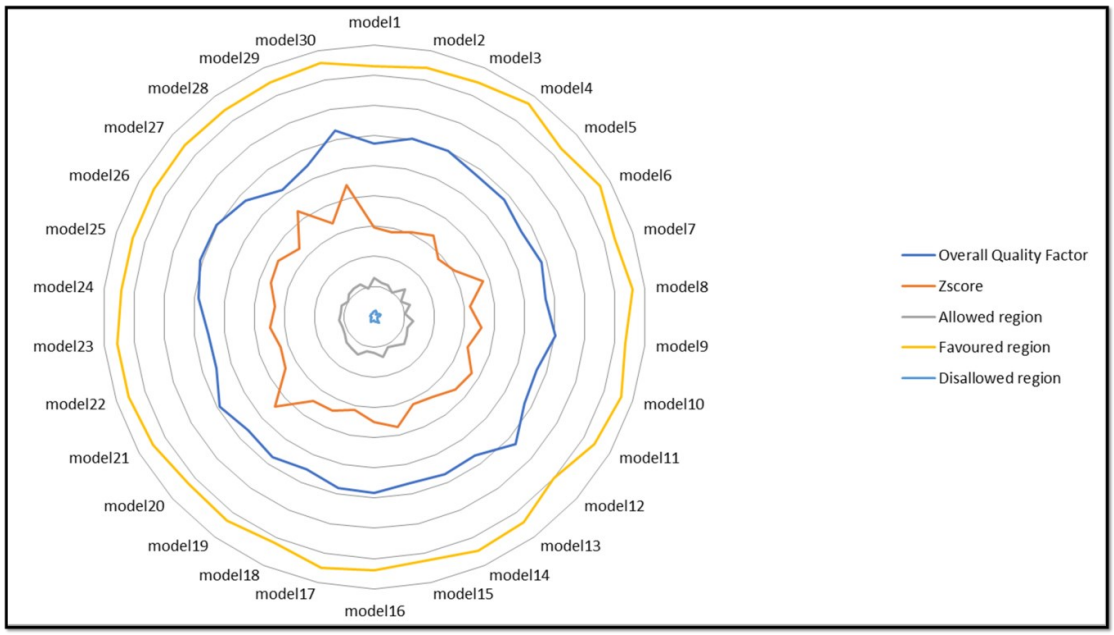

Fig. 1: Comparison structure assessment graph displayed the values of the overall quality factor, Z-score, allowed region, favoured region, and outlier regions of each of the selected ADA structures.

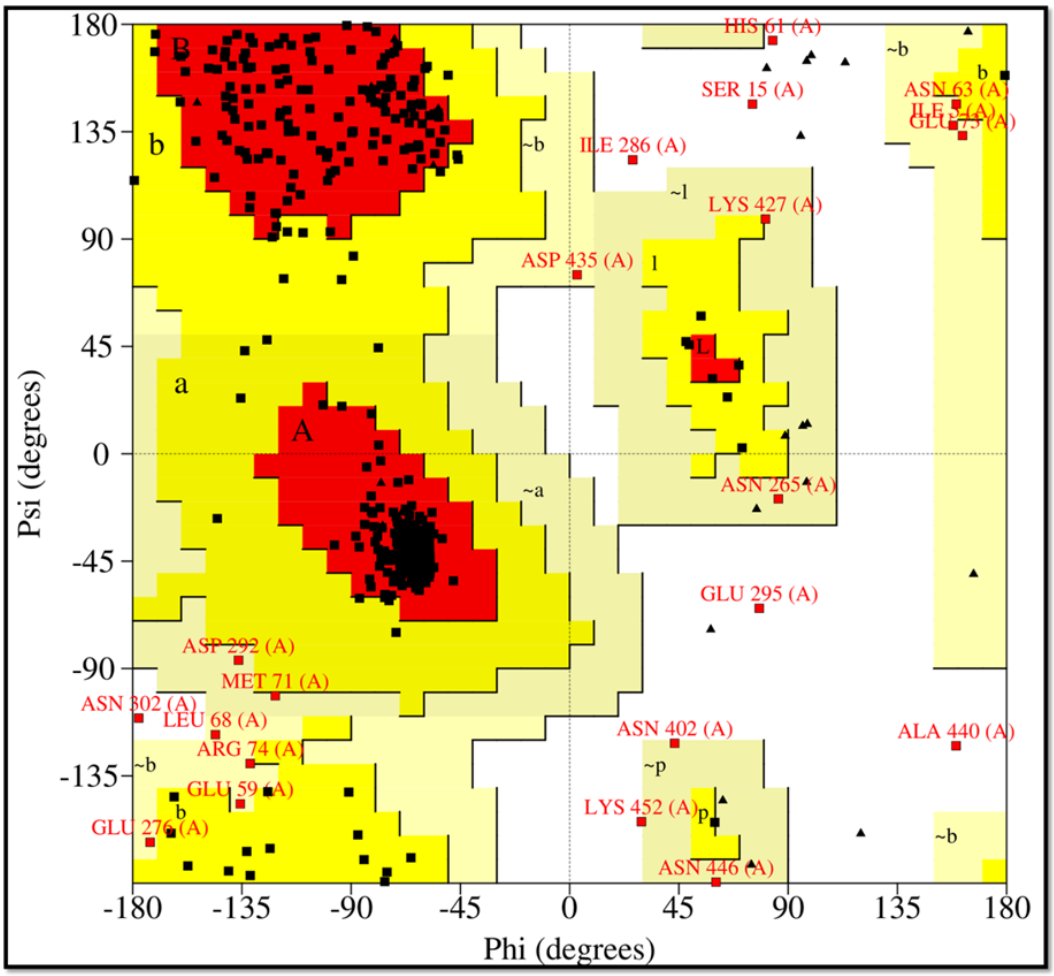

Fig. 2: Ramachangran plot showed the allowed region, favoured region, and outlier regions of ADA structure.

In silico evaluations were conducted to analyze the overall quality factor of the selected projected structure, and 94.45% of the overall quality factor was observed (**Fig. 3A**) and z-score of the selected structure (**Fig. 3B**).

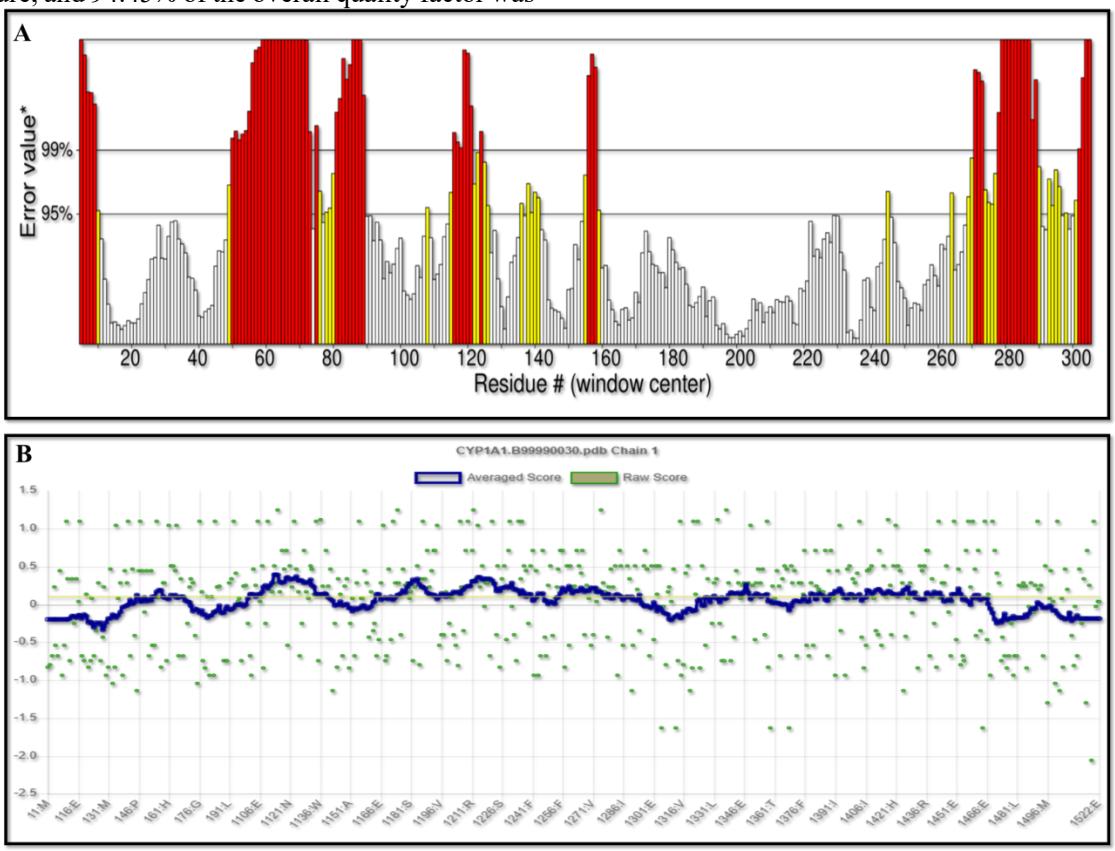

Fig. 3: Model evaluation of the selected structures **A)** Comparison structure assessment showed overall quality factor **B)** Z-score of the selected ADA structures

To test the new compounds against ADA, the FDA database of the ZINC commercialized library was used for a high through put virtual screening. The ADA was docked against all of the chemicals from the FDA library of the ZINC database for new therapeutic targets. The molecular docking experiments were carried out over the FDA library and the screened ligands to examine the variation of the detected binding energy values. For the docking studies, twenty-three different stances were used, and eighty-two trials for every stance were retained for interactional analyses of the chemicals used and the selected protein ADA. Among all the ligand production procedures, the most successful poses of the tested ligands had the lowest binding energy. It was also interestingly noted that the screened compounds showed effective ADA binding affinity (**Table 2**).

The complexes have been assessed based on the binding energy that was lowest, the most efficient pharmacological properties of each screened molecule, and the highest binding affinity in computerized docking studies. After analyzing all the docked ligands, the selected ligand was identified as a cyclic molecule with significant biological capabilities that act as a powerful chemical (**Fig. 4**) and may be evaluated as an anti hypertension drug against ADA.

Table 2: The effectiveness of the described ligand, the conserved ADA binding residues, and computerized studies on molecular docking

Properties	ADA
Estimated free energy of binding (kcal/mol)	-8.7
Final intermolecular energy (kcal/mol)	-8.47
Unbound system's energy (kcal/mol)	-0.49
Torsional free energy (kcal/mol)	3.63
Ligand efficiency	-0.43
Estimated inhibition constant, Ki (μM)	35.92
Binding residues	Lys-367, Glu-424, Asp-422, Phe-381, Ile-377, Ser-430, Glu-374

Table 3: The drug effectiveness and ADMET properties of ZINC509

Ligand properties	ZINC509
Hydrogen bond donor	02
Caco2 permeability (probability)	0.8430
Molecular weight (g/mol)	267.376
Aqueous solubility (LogS)	-1.990
Human intestinal absorption (HIA) (probability)	0.7000
cLogP	-0.95
Hydrogen bond acceptor	03
CYP450 2D6 inhibitor (probability)	0.9000
Rotatable bonds	00
Acute oral toxicity (probability)	0.5100
Fish toxicity (LC50, mg/L)	0.6810
Honey bee toxicity (HBT) (probability)	0.8030
Blood–brain barrier (BBB) (probability)	0.8900
Carcinogens (probability)	0.8900

Using computerized molecular docking techniques, the most highly ranked complexes of the docking studies with the lowest binding energies for ADA were investigated. The binding energies of all docked molecules of the specified protein of interest varied. It was fascinating to see that the screened ligand molecule toward the target protein ADA met all of the set filters and parameters. In-depth computational molecular docking analyses using a chemical library were also used to study the conserved ADA binding area. The observed conserved ADA binding region by molecular docking investigations was indicated by thorough bioinformatics analyses, and it may give rise to mutagenesis studies for additional research analyses [18].

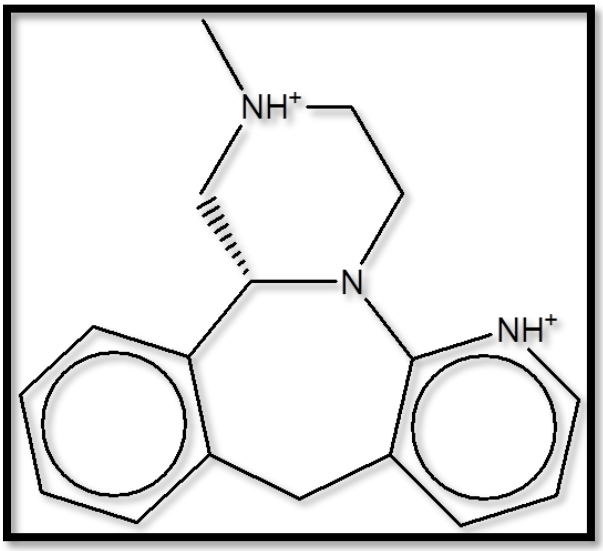

Fig. 4: The 2D structure of ZINC509 selected after a high-throughput virtual screening.

The traditional drug design procedure is costly and time-consuming. As a result, numerous biological computation and bioinformatics approaches have been employed in the development of new compounds that are effective against a wide range of targets. The development of bioinformatics and computational biology tools demonstrated a considerable reduction in the amount of time required for conventional drug design combined with minimal side effects of the compounds disclosed [19].

The high throughput virtual screening chemical was thoroughly evaluated for the purpose of therapy, as well as its oral bioavailability and effectiveness. The ADMET properties of the selected compounds were determined as well. Various toxicity assessments for the examined substances were also computed (**Table 3**). Through a variety of computed toxicities, the contaminants, intermediates, and metabolites were assessed [20].

Less LogP values were noticed as a result of Lipinki's rule of five, which was used to evaluate the LogP values for the screened compounds [21]. Furthermore, it was determined through various carcinogenicity and toxicity risk assessment evaluations that the examined compounds were neither carcinogenic or mutant. The drug targeted should have a binding energy that is lowest, the most efficacious pharmacological properties, and the highest affinity for binding, according to extensive computational studies and literature review [22]. It was discovered by adhering to the intended requirements that the screened chemical may have the ability to fight against hypertension by targeting ADA. The binding residues Lys-367, Glu-424, Asp-422, Phe-381, Ile-377, Ser-430 and Glu-374 (**Fig. 5**) were essential, according to computational docking analyses and drug-like investigations.

Conclusion

Finally, utilizing the methods of homology modeling, threading, and *ab initio* approaches, the three-dimensional structure of ADA was predicted. The predicted structures were then assessed by using a variety of evaluation tools. The high throughput virtual screening method was used to screen the molecule revealed that it was effective against hypertension by specifically targeting ADA and using extensive bioinformatics analyses. The conserved residues Lys-367, Glu-424, Asp-422, Phe-381, Ile-377, Ser-430 and Glu-374 were noted, and the molecule reported may be essential for site-directed mutagenesis. The *in silico* studies indicated that the further hypertension research would be hindered by

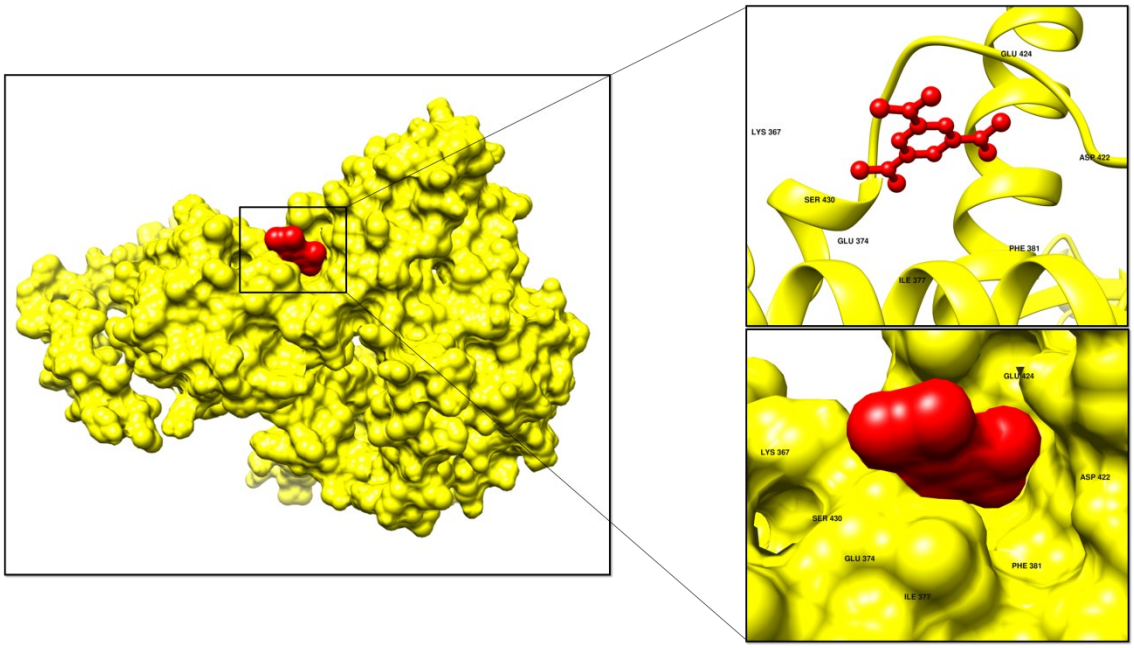

Fig. 5: The conserved ADA residues along with interacting residues with the complex of the selected chemical as a possible target for therapy.

the discovered binding domain. The generated data indicated that the reported chemical might be a lead compound and might stabilize ADA.

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Conflict of interest

The authors declare no conflict of interest.

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