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Research Article

In silico Elucidation of Potent Therapeutic Targets against Alzheimer's by Targeting CAPNS2

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Abstract

Alzheimer's disease (AD) is considered one of the common types of dementia in which brain cells gradually deteriorate, leading to memory loss. Approximately 50 million people are afflicted by AD. There is no permanent solution for this malady; however, early detection may help slow down the disease prognosis. In the current study, a hybrid approach of molecular docking studies and virtual screening is taken to test compounds from the 'Zinc database library' that may be effective against AD by targeting CAPNS2. The top-ranked four compounds were reported by molecular docking analysis on the basis of their binding affinity by targeting CAPNS2. Molecular docking analyses revealed that Lys-5, Ala-4, Leu-3, Tyr-56, Ile-48, Ala-52, Gln-55, Cys-56, Ala-152, Ala-54, and Ala-53 are the interacting residues from the selected target protein. In addition, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) analyses were performed to assess the drug-like properties of the selected compounds. After extensive *in silico* analyses, the present study deduced that the top four scrutinized compounds might be potent against AD by targeting CAPNS2.

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Keywords: Bioinformatics, Alzheimer's disease, computational drug design, CAPNS2, virtual screening

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder marked by two neuropathological indicators: extracellular deposition of amyloid plaques and intracellular neurofibrillary tangles (Bush 2003, It and KNOW 1986, Rasool et al. 2018). AD is one of the main reasons for dementia. The prognosis of AD is grim but a timely diagnosis can help slow down its progression (Breijyeh and Karaman 2020, Vaz and Silvestre 2020, Behl et al. 2021). Dementia is a common disease characterized by memory loss, leading to a performance decline in the daily life functions of a person. There are about 50 million people suffering from dementia (Organization 2019, Guesmi, Salah, and Ayed 2023). In a study, the brain of AD patients exhibits noticeable decay in which expansion of sulci and diminution of gyri occurs. In most cases, major or every part of the cerebral cortex is involved (Singh et al. 2016). AD is considered to be a multistage disorder with higher elements of risk including genetic elements, vascular diseases, increasing age, infections, and head injuries are considered the major risk factors (A. Armstrong 2019). The symptoms of AD may vary from person to person, while in most cases it manifests with textbook symptoms such as

memory loss, depression, aphasia, aggression, and psychosis (Webb and Sali 2014, Muzammil et al. 2021).

Calapin small subunit 2(CAPNS2), a novel human subunit is encoded by the gene *capns2,* having no intron in its sequence, located at chromosome number 16 (SCHÁD et al. 2002). There are 14 family members of the Calapin protease family; whereas, Calapin 1 and Calapin 2, including their small subunits, showed overwrought connections with neurological disorders, including AD. (Huang and Wang 2001). Calapin protein relies on the cytoplasm calcium for its regulation. Calapin was found to be involved in numerous cell processes that require calcium for their regulation, like membrane fusion, cell propagation, cell cycle advancement, divergence, signal transduction, platelet stimulation, remodeling of cytoskeleton, and apoptosis (Goll et al. 2003). The Calapin small subunit may act as a tissue-specific companion of the larger subunits and also help with the correct folding for normal activity (Suzuki et al. 2004, Saido, Sorimachi, and Suzuki 1994, Huang and Wang 2001). It was revealed that calapin is present in the cytoplasm, cell membrane, and dense perinuclear region (Hood et al. 2003).

Bioinformatics is an interdisciplinary science that solves biological problems by applying mathematical and statistical tools and using computational power (Malik et al. 2023). This subject mainly instrumentalizes molecular biology, genetics, computer science, mathematics, and statistics (Jones and Pevzner 2004). It is a modern approach to studying biology involving the collection, storage, analysis, distribution, and retrieval of biological data extracted functions and sequences analyses. This computational biology contains enormous amounts of information on genes, their protein sequences, structures, and functions, which can be retrieved by using available bioinformatics tools (Sehgal, Mirza, et al. 2018, Sehgal, Hammad, et al. 2018b, Iqbal et al. 2023). Bioinformatics is a vast field that contains copious amounts of sorted data, which aids researchers with easily retrieving available information, and introducing new records, as they are investigated; moreover, it provides different assistance tools that facilitate data analysis and comparing large datasets (Gibas and Jambeck 2001, Tahir et al. 2020).

CAPNS2 is involved in AD development, and no drug has been reported to target it specifically. In the following studies, extensive *in silico* analyses were performed, including molecular docking, virtual screening, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) analyses were performed. The Food and Drug Association (FDA) approved compounds were used to treat AD by targeting CAPNS2.

2. Materials and Methods

CAPNS2 (target protein) has 248 amino acids, and their sequence was retrieved from the 'UniProt Knowledgebase database' in the FASTA format with Uniprot accession number Q96L46. In this research article, threading, *ab initio,* and homology modeling techniques for the prediction of 3D structures of the selected target protein were done. The predicted structure was further processed by employing molecular docking approach for high through-put virtual screening. The canonical sequence of CAPNS2 was submitted to screen the acceptable hits of the templates to protein BLASTp through 3D structural database named 'Protein Data Bank (PDB)' (Berman et al. 2000). The most cited command based and an automated structure prediction program MODELLER 10.4 (Eswar et al. 2008), was applied for the 3D structure prediction of the target protein's structure. The

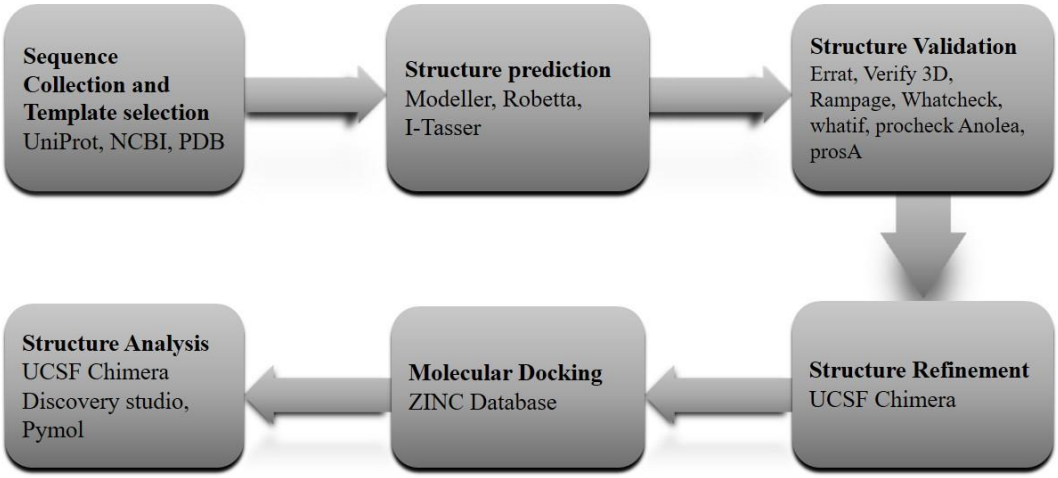

Figure 1: Methodology followed for the elucidation of novel compounds against AD.

template hits from protein BLASTp were retrieved and analyzed, and the top five templates were scrutinized on the basis of percentage identity, maximum score, query coverage, and resemblance. The selected five templates were further employed to predict the 3D structure of CAPNS2 (Sehgal, Mirza, et al. 2018). Various evaluation tools including ERRAT (Laskowski et al. 1993), Anolea (Melo et al. 1997), prosA (Wiederstein and Sippl 2007), and ProCheck (Laskowski et al. 1993) were used to evaluate the predicted 3D structures of the protein and to cross verify the efficacy and reliability of CAPNS2 predicted structures.

ZINC commercial database of compounds was used to retrieve the FDA compounds library for high throughput virtual screening. This library, with numerous biological compounds, was utilized to assess, evaluate, and scrutinize the interacting residues of CAPNS2. The minimization and bond angles optimization and the bond length of all the molecules from the selected FDA library were performed by using ChemDraw (Mendelsohn 2004). The predicted 3D structure of CAPNS2 was also performed by using UCSC Chimera 1.9 (Pettersen et al. 2004). All of the compounds present in the selected dataset for high throughput virtual screening were minimized for the optimization of the geometry of all compounds. All the compounds were used for molecular docking analyses through AutoDock, Vina, and AutoDock tools of comparative molecular docking studies (Trott and Olson 2010). While performing molecular docking studies, the grid was set on the protein values as x = 15.34, y = -6.09, and z = -8.58. All the selected compounds were utilized to calculate the drug properties, including H-bond acceptors, H-bond donors, and the number of rotatable bonds present in the compounds via PubChem (Hahnke, Kim, and Bolton 2018). mCule (Kiss, Sandor, and Szalai 2012) and Molinspiration (Jarrahpour et al. 2012) were used to measure the efficacy and reliability of the selected molecules, in addition to Lipinski's rule of five. The mutagenesis analysis of the chosen compounds, toxicity calculation, and carcinogenicity of all the scrutinized top

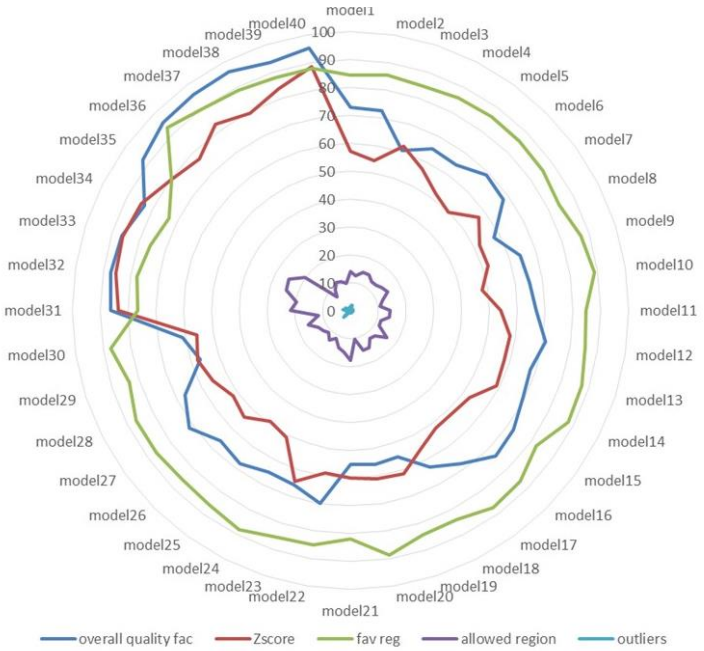

Figure 2: The graph was generated for the detailed comparative model assessment of all the 3D predicted structures of CAPNS2. The favored region, allowed region, outliers, Z-score, and the overall quality factor observed in the 3D predicted structures were analyzed.

ranked compounds were measured. All the docked complexes of the target protein, as well as the top-ranked screened compounds, were analyzed for the conserved binding regions; moreover, the docked complexes were visualized by ligplot (Wallace, Laskowski, and Thornton 1995), and UCSC Chimera 1.9. ADMET properties of all the top-ranked screened compounds were calculated to verify the key role and reliability of said compounds by using the admetSAR online server (Cheng et al. 2012). The utilized methodology (Figure 1) showed reliable results (Waqas et al. 2021, Tahir et al. 2018b, Zaka et al. 2017b, Tahir et al. 2019, Sehgal et al. 2014, Arshad et al. 2023, Ghaffar et al. 2020b, Ahmed et al. 2019, Khattak et al. 2017).

3. Results and Discussion

The objectives and aims of the current project were to conduct *in silico* analyses on the selected target protein, CAPNS2, against AD. Moreover, an extensive literature review was performed to study the *in silico* relationship between CAPNS2 and AD. *In silico* analyses were performed in detail regarding computer-aided drug design (CADD) to examine, identify, and evaluate the novel bioactive molecules against AD by targeting CAPNS2.

To investigate the structural insights and structural features of CAPNS2, 3D structure prediction was performed using threading, comparative modeling, and *ab initio* approaches of 3D prediction of the structures. The canonical sequence of CAPNS2 was used to retrieve the acceptable templates for comparative modeling. Moreover, numerous templates were analyzed and the top five significantly aligned 3D templates with the total score, PDB accession number, maximum score, percentage identity, the query coverage of the templates, the targets, and the E-values were selected for the 3D homology modeling of the CAPNS2 (Table 1). All the scrutinized template structures were employed to predict the 3D model of the CAPNS2. However, the crystal structure of

Table 1: The selected aligned template for the prediction of 3D structures for CAPNS2 with e-value, accession number of Uniprot ID, percentage identity, query coverage, and maximum score.

Accession ID	Total score	Query coverage	Maximum Identity	E-value
1KFU	297	73%	73%	7e-103
1DF0	289	73 %	71%	9e-100
1ALV	285	69 %	76%	2e-98
1DVI	286	73 %	70%	2e-98
4PHJ	283	69 %	75%	3e-97

human m-Calpain form II, having accession number 1KFU with 2.50 Å, was used for homology modeling. The chosen template showed reliable assessment results for the 3D structure of CAPNS2. Moreover, threading and *ab initio* techniques were also utilized to check the efficacy and reliability of the predicted structure. The query coverage and percentage identity of the selected templates and the target sequence of CAPNS2 were considered sufficient for the projection of the 3D structure of the CAPNS2.

Furthermore, the *in silico* approaches of 3D structure prediction, such as threading and *ab initio*, were used to cross-verify the predicted models of CAPNS2 for more reliability. Consequently, forty different 3D structures were predicted for CAPNS2. Additionally, numerous 3D tools for the evaluation of the predicted structure were used to assess its effectuality, and the optimally projected 3D structure of CAPNS2, with fewer errors, was selected for further experiments.

The evaluation tools showed reliable values of all the predicted structures (Supplementary File 1). A graph was generated to select the most suitable structure of CAPNS2 from all the predicted structures. The results indicated the efficiency, efficacy, and stability of the predicted 3D structure of CAPNS2 with an overall quality factor of 96.05%. The selected 3D structure of CAPNS2 showed 88.8% residues in the favored region of the generated Ramachandran plot for structural evaluation, 11.2% residues in the allowed region of the generated plot, and 0% residues from the predicted structure were observed in the outlier region (Figure 2). Interestingly, no amino acid was detected in the outlier region.

The predicted 3D structures were analyzed and visualized to check the efficacy of the predicted structures (Figure 3). The 3D predicted models were minimized for the removal and to fix the torsion angles and steric collisions to relax the utilized system.

The selected FDA compounds library from the freely available ZINC commercial database was utilized for the high throughput virtual screening and also to perform molecular docking studies.

The molecular docking results and the docked complexes demonstrated reliable values and the deduced conclusion from the generated docked complexes of all the compounds available in the FDA library against CAPNS2. The efforts were initiated to reveal the novel lead compounds for the target protein and the top-ranked four compounds were selected from the FDA library for further analyses (Table 2). The interactional analyses of all the docked compounds were performed and it was noticed that all the compounds from the selected compound library bound at similar interactional sites of CAPNS2 (Figure 4).

Table 2: Molecular docking studies revealing the insights and the binding residues of the top four docked compounds.

Ligand	Binding affinity (kcal/mol)	Interacting residues
ZINC27990463	-11.7	Lys5, Ala4, Leu3, Met1, Phe2, Tyr56, Ile48, Ala52, Gln55
ZINC3932831	-11.6	Lys5, Phe2, Met1, Gln55, Ile48, ALa4, Leu3
ZINC538658	-10.9	Lys5, Phe2, Met1, Cys156, Ala52, Ile48, Leu3, Ala4
ZINC43200832	-10.8	Lys5, Leu8, Phe2, Met1, Leu3, Ala54, Ala53, Ala52

The top four selected molecules from the molecular docking studies with the lowest binding energy were revealed to have ZINC ID (ZINC43200832, ZINC27990463, ZINC3932831 and ZINC538658) (Figure 3). The observed interacted residues were Lys-5, Ala-4, Leu-3, Tyr-56, Ile-48, Ala-52, Gln-55, Cys-56, Ala-152, Ala-54, and Ala-53 from the target protein CAPNS2, showed maximum binding affinity (Table 3). The interactions of the top-ranked 4 screened compounds were analyzed through molecular docking studies against CAPNS2 and the interacting residues were visualized (Figure 5).

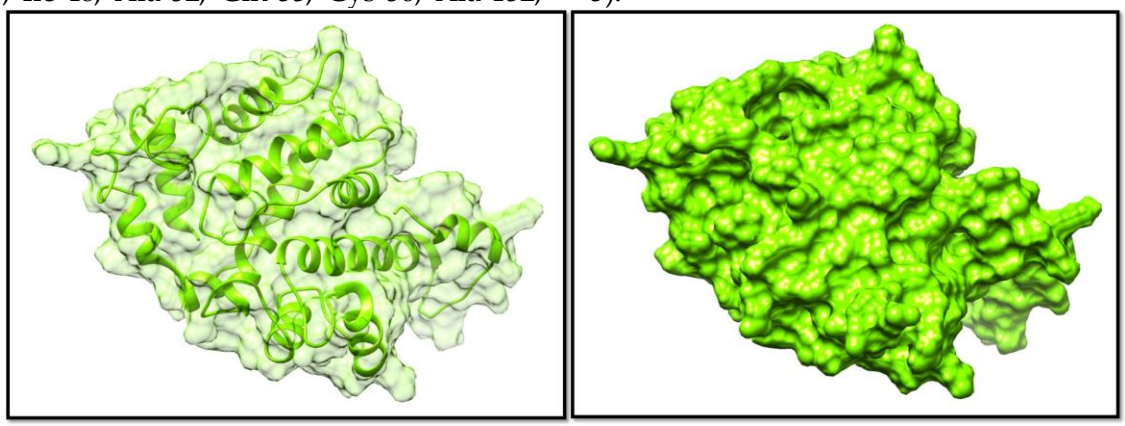

Figure 3: The 3D predicted structure of CAPNS2 selected from the generated graph.

The selection of the top-ranked 4 docked complexes was based on their highest binding affinities, calculated post-molecular docking studies (Figure 6). ADMET analyses were performed for the selected top-ranked four compounds (Table 4). The observed results of the molecular docking analyses gave reliable results, and the conclusion was drawn, from the docked complexes of the screened compounds, by targeting the CAPNS2.

CAPNS2 is involved in AD and is considered a therapeutic target to treat AD. The *in silico* approaches for computational drug design helped screen the molecules against CAPNS2. The 3D predicted structure of CAPNS2 and the structural insights of CAPNS2 may lead to a deeper understanding of functional behavior and therapeutic targets of the selected protein. Computational biology and bioinformatics approaches and techniques are being used to

Table 3: ADMET and drug-like properties of the top-ranked selected compounds.

	ZINC538658	ZINC3932831	ZINC27990463	ZINC43200832
Blood-Brain Barrier	0.8429	0.9884	0.9731	0.8354
Human Intestinal Absorption	0.9610	1.0000	1.0000	0.7938
AMES Toxicity	0.7247	0.6677	0.6983	0.5895
Carcinogens	0.8623	0.9149	0.8527	0.8859
Fish Toxicity(mg/l)	1.1082	0.9946	1.3643	1.3640
Honey Bee Toxicity	0.9103	0.8333	0.8826	0.8808
Acute Oral Toxicity	0.6408	0.4420	0.5197	0.6294
Carcinogenicity	0.5551	0.4507	0.6560	0.5942
Aqueous solubility(LogS)	-4.2805	-4.3409	-4.0968	-3.5789
Caco-2 Permeability(cm/s)	1.2308	1.3301	0.7460	0.4644
Rat Acute Toxicity(mol/kg)	2.2269	2.6885	2.7151	2.6233
Tetrahymena Pyriformis Toxicity(ug/l)	0.4955	0.7573	0.5661	0.6342
Net Charge	0	0	1	0
H-bond donors	2	2	3	3
H-bond acceptors	3	2	2	8
Tpsa	69	58	62	136
Rotatable bonds	3	2	10	5
Apolar desolvation	10.3	11.07	19.63	3.78
Polar desolvation	-10.89	-13.6	-47.63	-25.53

find suitable solutions for the complications being faced by biological sciences, with the help of computational analyses, in addition to mathematical and statistical approaches (Ghaffar et al. 2020a, Sehgal, Kanwal, et al. 2018). Bioinformatics analysis has proven to be helpful in solving immunoinformatics problems (Tahir et al. 2018a), (Sehgal, Hammad, et al. 2018a) and vaccine design against various viral diseases including SARS-CoV-II (Waqas et al. 2020, Waqas et al. 2021, Sajid et al. 2022). The traditional methods of drug design are not only resource-extensive but also time-consuming. However, numerous *in silico* approaches and methodologies were employed in the present effort (Zaka et al. 2017a, Rasool et al. 2018, Marriam et al. 2023, Sehgal, Hammad, et al. 2018b).

In this paper, *in silico* analyses were performed, followed by the 3D structure prediction of CAPNS2. The predicted 3D structures showed higher accuracy and reliability, additionally, the binding site of CAPNS2 was also analyzed. The molecular docking studies were done to examine the interacting residues between the target protein CAPNS2 and the screened molecules. The screened molecules elucidated potent binding interactions, at the similar binding site of the protein, with all the docked compounds and the critical interacting binding residues (Lys-5, Ala-4, Leu-3, Tyr-56, Ile-48, Ala-52, Gln-55, Cys-56, Ala-152, Ala-54, and Ala-53) were analyzed by molecular docking studies (Figure 4). The binding pocket was also subjected to observation. Through molecular

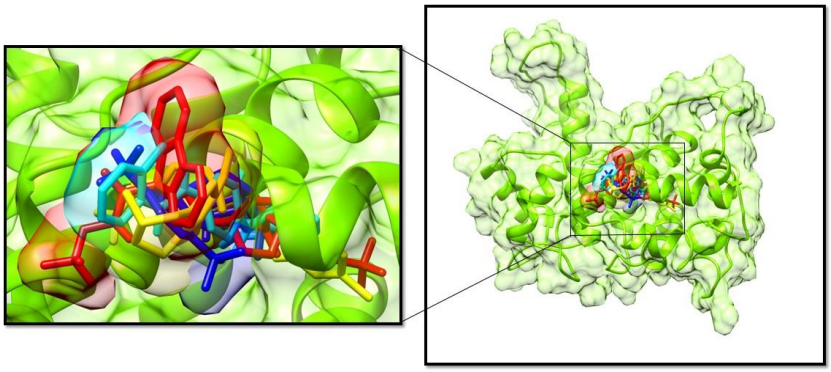

Figure 4: Binding site of the target protein observed by molecular docking analysis.

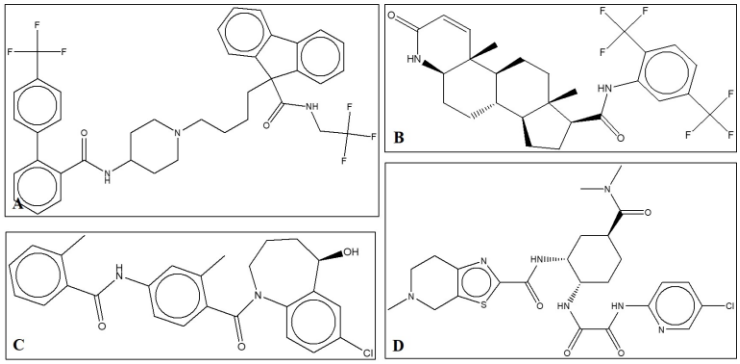

Figure 5: Top four compounds screened out from molecular docking studies.

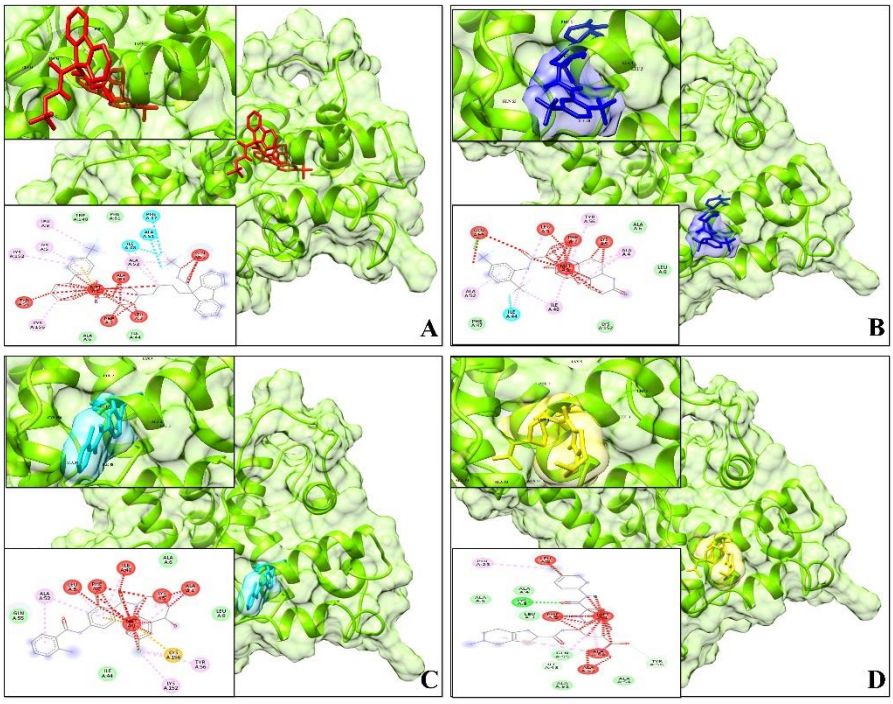

Figure 6: 2D diagram of the four selected compounds having the least binding energy A) ZINC27990463, B) ZINC3932831, C) ZINC538658), D) ZINC43200832.

docking analysis, the screened (top-ranked 4) molecules showed the least binding energy score. The ADMET analyses suggested that the screened molecules fit the bill of a therapeutic agent due to their proven drug-like properties. Based on the comprehensive *in silico* analyses, it is safe to conclude that the suggested compounds (ZINC43200832, ZINC27990463, ZINC3932831, and ZINC538658) may be potent against AD by targeting CAPNS2.

4. Conclusions

After conducting the *in silico* experiments, it can be suggested that the screened bioactive molecules have the potential to be used against AD by targeting CAPNS2. However, it is important to note that there may be notable differences between clinical studies and computational drug design methods, despite the satisfactory results obtained from in silico-based analyses of anti-AD bioactive compounds. In the future, more comprehensive studies and chemical synthesis of these compounds, considering the conditions of the current study, may expect similar results.

Conflict of Interest

The authors declare that they have no competing interests.

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There was no external or internal funding received for this research project.

Study Approval

This study was approved by the departmental review board of the University of Okara, Pakistan.

Consent Forms

NA.

Data Availability

All the raw data related to this study is available with the authors.

Author's Contribution

SN conceptualized the study, MM, MTR, and MK performed the experiments, AR, AA, and MSA analyzed the results, and SN, and HBW wrote the initial manuscript. All the authors contributed to the final manuscript.

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Reference

A. Armstrong, Richard. 2019. "Risk factors for Alzheimer's disease." *Folia Neuropathologica* no. 57 (2):87-105. doi: 10.5114/fn.2019.85929.

Ahmed, Muhammad Saad, Sheikh Arslan Sehgal, Rana Adnan Tahir, and Chun Li. 2019. "Identification of effective membrane efflux transporters against β-amyrin through molecular docking approach." *Journal of Chemical Technology & Biotechnology* no. 94 (9):2869-2875.

Arshad, Ihtesham, Muhammad Ahsan, Imran Zafar, Muhammad Sajid, Sheikh Arslan Sehgal, Waqas Yousaf, Amna Noor, Summya Rashid, Somenath Garai, and Meivelu Moovendhan. 2023. "Bioinformatics approaches in upgrading microalgal oil for advanced biofuel production through hybrid ORF protein construction." *Biomass Conversion and Biorefinery*:1-21.

Behl, Tapan, Dapinder Kaur, Aayush Sehgal, Sukhbir Singh, Neelam Sharma, Gokhan Zengin, Felicia Liana Andronie-Cioara, Mirela Marioara

Toma, Simona Bungau, and Adrian Gheorghe Bumbu. 2021. "Role of monoamine oxidase activity in Alzheimer's disease: an insight into the therapeutic potential of inhibitors." *Molecules* no. 26 (12):3724.

Berman, H. M., T. N. Bhat, P. E. Bourne, Z. Feng, G. Gilliland, H. Weissig, and J. Westbrook. 2000. "The Protein Data Bank and the challenge of structural genomics." *Nat Struct Biol* no. 7 Suppl:957-9. doi: 10.1038/80734.

Breijyeh, Zeinab, and Rafik Karaman. 2020. "Comprehensive Review on Alzheimer's Disease: Causes and Treatment." *Molecules* no. 25 (24):5789.

Bush, Ashley I. 2003. "The metallobiology of Alzheimer's disease." *Trends in neurosciences* no. 26 (4):207-214.

Cheng, Feixiong, Weihua Li, Yadi Zhou, Jie Shen, Zengrui Wu, Guixia Liu, Philip W Lee, and Yun Tang. 2012. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. ACS Publications.

Eswar, N., D. Eramian, B. Webb, M. Y. Shen, and A. Sali. 2008. "Protein structure modeling with MODELLER." *Methods Mol Biol* no. 426:145-59. doi: 10.1007/978-1-60327-058-8_8.

Ghaffar, A., S. A. Sehgal, R. Fatima, R. Batool, U. Aimen, S. Awan, S. Batool, F. Ahmad, and S. M. Nurulain. 2020a. "Molecular docking analyses of CYP450 monooxygenases of Tribolium castaneum (Herbst) reveal synergism of quercetin with paraoxon and tetraethyl pyrophosphate: in vivo and in silico studies." *Toxicol Res (Camb)* no. 9 (3):212-221. doi: 10.1093/toxres/tfaa023.

Ghaffar, Ammarah, Sheikh Arslan Sehgal, Rida Fatima, Roya Batool, Ume Aimen, Sliha Awan, Sajida Batool, Faheem Ahmad, and Syed M Nurulain. 2020b. "Molecular docking analyses of CYP450 monooxygenases of Tribolium castaneum (Herbst) reveal synergism of quercetin with paraoxon and tetraethyl pyrophosphate: in vivo and in silico studies." *Toxicology Research* no. 9 (3):212-221.

Gibas, Cynthia, and Per Jambeck. 2001. *Developing bioinformatics computer skills*: " O'Reilly Media, Inc.".

Goll, Darrel E, Valery F Thompson, Hongqi Li, WEI Wei, and Jinyang Cong. 2003. "The calpain system." *Physiological reviews*.

Guesmi, Dhouha, Fatma Salah, and Yassine Ben Ayed. 2023. Recognition of Alzheimer's Disease Based on Transfer Learning Approach Using Brain MR Images with Regularization. Paper read at Advances in Computational Collective Intelligence, 2023//, at Cham.

Hahnke, V. D., S. Kim, and E. E. Bolton. 2018. "PubChem chemical structure standardization." *J Cheminform* no. 10 (1):36. doi: 10.1186/s13321-018-0293-8.

Hood, Joshua L., Barbara B. Logan, Anthony P. Sinai, William H. Brooks, and Thomas L. Roszman. 2003. "Association of the calpain/calpastatin network with subcellular organelles." *Biochemical and Biophysical Research Communications* no. 310 (4):1200-1212.

doi: https://doi.org/10.1016/j.bbrc.2003.09.142.

Huang, Yuanhui, and Kevin KW Wang. 2001. "The calpain family and human disease." *Trends in molecular medicine* no. 7 (8):355-362.

Iqbal, Azhar, Faisal Ali, Moawaz Aziz, Asad Ullah Shakil, Shanza Choudhary, Adiba Qayyum, Fiza Arshad, Sarah Ashraf, Sheikh Arslan Sehgal, and Momina Hussain. 2023. "In Silico Analysis of Drug Off-Target Effects on Diverse Isoforms of Cervical Cancer for Enhanced Therapeutic Strategies." *bioRxiv*:2023.09.09.556929.

It, Katzman, and WHAT DO WE KNOW. 1986. "What Is Alzheimer's Disease?" *N Engl] Med* no. 314:964-973.

Jarrahpour, Aliasghar, Jihane Fathi, Mostafa Mimouni, Taibi Ben Hadda, Javed Sheikh, Zahid Chohan, and Ali Parvez. 2012. "Petra, Osiris and Molinspiration (POM) together as a successful support in drug design: antibacterial activity and biopharmaceutical characterization of some azo Schiff bases." *Medicinal Chemistry Research* no. 21:1984-1990.

Jones, Neil C, and Pavel A Pevzner. 2004. *An introduction to bioinformatics algorithms*: MIT press.

Khattak, Naureen Aslam, Sheikh Arslan Sehgal, Yongsheng Bai, and Youping Deng. 2017. Structure modeling and molecular docking studies of schizophrenia candidate genes, synapsins 2 (SYN2) and trace amino acid receptor (TAAR6). Paper read at Bioinformatics Research and Applications: 13th International Symposium, ISBRA 2017, Honolulu, HI, USA, May 29–June 2, 2017, Proceedings 13.

Kiss, Robert, Mark Sandor, and Ferenc A Szalai. 2012. "http://Mcule. com: a public web service for drug discovery." *Journal of cheminformatics* no. 4 (1):1-1.

Laskowski, Roman A, Malcolm W MacArthur, David S Moss, and Janet M Thornton. 1993. "PROCHECK: a program to check the stereochemical quality of protein structures." *Journal of applied crystallography* no. 26 (2):283-291.

Malik, A., M. N. Iqbal, S. Ashraf, M. S. Khan, S. Shahzadi, M. F. Shafique, Z. Sajid, M. Sajid, and S. A. Sehgal. 2023. "In silico elucidation of potential drug targets against oxygenase domain of Human eNOS Dysfunction." *PLoS One* no. 18 (4):e0284993. doi: 10.1371/journal.pone.0284993.

Marriam, Saigha, Muhammad Sher Afghan, Mazhar Nadeem, Muhammad Sajid, Muhammad Ahsan, Abdul Basit, Muhammad Wajid, Sabeen Sabri, Imran Zafar, and Summya Rashid. 2023. "Elucidation of novel compounds and epitope-based peptide vaccine design against C30 endopeptidase regions of SARS-CoV-2 using immunoinformatics approaches." *Frontiers in Cellular and Infection Microbiology* no. 13:568.

Melo, Francisco, Damien Devos, Eric Depiereux, and Ernest Feytmans. 1997. ANOLEA: a www server to assess protein structures. Paper read at Ismb.

Mendelsohn, Loren D. 2004. "ChemDraw 8 ultra, windows and macintosh versions." *Journal of chemical*

information and computer sciences no. 44 (6):2225-2226.

Muzammil, Aqsa, Muhammad Waqas, Ahitsham Umar, Muhammad Sufyan, Abdur Rehman, Ali Haider, Haseeb Akram, Seemab AF Khan, Muhammad Afzal, and Muhammad Wajid. 2021. "Anti-aging natural compounds and their role in the regulation of metabolic pathways leading to longevity." *Mini Reviews in Medicinal Chemistry* no. 21 (18):2630-2656.

Organization, World Health. 2019. "Risk reduction of cognitive decline and dementia: WHO guidelines."

Pettersen, Eric F, Thomas D Goddard, Conrad C Huang, Gregory S Couch, Daniel M Greenblatt, Elaine C Meng, and Thomas E Ferrin. 2004. "UCSF Chimera—a visualization system for exploratory research and analysis." *Journal of computational chemistry* no. 25 (13):1605-1612.

Rasool, Mahmood, Arif Malik, Sulayman Waquar, Qura Tul-Ain, Tassadaq H Jafar, Rabia Rasool, Aasia Kalsoom, Muhammad A Ghafoor, Sheikh A Sehgal, and Kalamegam Gauthaman. 2018. "In-silico characterization and in-vivo validation of Albiziasaponin-A, Iso-Orientin, and Salvadorin using a rat model of alzheimer's disease." *Frontiers in Pharmacology* no. 9:730.

Saido, Takaomi C, Hiroyuki Sorimachi, and Koichi Suzuki. 1994. "Calpain: new perspectives in molecular diversity and physiological-pathological involvement." *The FASEB Journal* no. 8 (11):814-822.

Sajid, Muhammad, Saigha Marriam, Hamid Mukhtar, Summar Sohail, Muhammad Sajid, and Sheikh Arslan Sehgal. 2022. "Epitope-based peptide vaccine design and elucidation of novel compounds against 3C like protein of SARS-CoV-2." *PLoS One* no. 17 (3):e0264700.

SCHÁD, Éva, Attila FARKAS, Gáspár JÉKELY, Peter TOMPA, and Peter FRIEDRICH. 2002. "A novel human small subunit of calpains." *Biochemical Journal* no. 362 (2):383-388. doi: 10.1042/bj3620383.

Sehgal, S. A., M. A. Hammad, R. A. Tahir, H. N. Akram, and F. Ahmad. 2018a. "Current Therapeutic Molecules and Targets in Neurodegenerative Diseases Based on in silico Drug Design." *Curr Neuropharmacol* no. 16 (6):649-663. doi: 10.2174/1570159X16666180315142137.

Sehgal, SA, RA Tahir, S Shafique, M Hassan, and S Rashid. 2014. "Molecular modeling and docking analysis of CYP1A1 associated with head and neck cancer to explore its binding regions." *J Theor Comput Sci* no. 1 (112):2.

Sehgal, Sheikh A, Mirza A Hammad, Rana Adnan Tahir, Hafiza Nisha Akram, and Faheem Ahmad. 2018b. "Current therapeutic molecules and targets in neurodegenerative diseases based on in silico drug design." *Current neuropharmacology* no. 16 (6):649-663.

Sehgal, Sheikh Arslan, Sonia Kanwal, Rana Adnan Tahir, Zunera Khalid, and Mirza A Hammad. 2018. "In silico elucidation of potential drug target sites of the Thumb Index Fold Protein, Wnt-8b." *Tropical Journal of Pharmaceutical Research* no. 17 (3):491-497.

Sehgal, Sheikh Arslan, A Hammad Mirza, Rana Adnan Tahir, and Asif Mir. 2018. *Quick guideline for computational drug design*: Bentham Science Publishers.

Singh, Sandeep Kumar, Saurabh Srivastav, Amarish Kumar Yadav, Saripella Srikrishna, and George Perry. 2016. "Overview of Alzheimer's Disease and Some Therapeutic Approaches Targeting Aβ by Using Several Synthetic and Herbal Compounds." *Oxidative Medicine and Cellular Longevity* no. 2016:7361613. doi: 10.1155/2016/7361613.

Suzuki, Koichi, Shoji Hata, Yukiko Kawabata, and Hiroyuki Sorimachi. 2004. "Structure, Activation, and Biology of Calpain." *Diabetes* no. 53 (suppl_1):S12-S18. doi: 10.2337/diabetes.53.2007.S12.

Tahir, R. A., H. Wu, M. A. Rizwan, T. H. Jafar, S. Saleem, and S. A. Sehgal. 2018a. "Immunoinformatics and molecular docking studies reveal potential epitope-based peptide vaccine against DENV-NS3 protein." *J Theor Biol* no. 459:162-170. doi: 10.1016/j.jtbi.2018.10.005.

Tahir, Rana Adnan, Afsheen Bashir, Muhammad Noaman Yousaf, Azka Ahmed, Yasmine Dali, Sanaullah Khan, and Sheikh Arslan Sehgal. 2020. "In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1." *PloS one* no. 15 (2):e0228265.

Tahir, Rana Adnan, Hao Wu, Naima Javed, Anila Khalique, Seemab Amjad Fateh Khan, Asif Mir, Muhammad Saad Ahmed, George E Barreto, Hong Qing, and Ghulam Md Ashraf. 2019. "Pharmacoinformatics and molecular docking reveal potential drug candidates against Schizophrenia to target TAAR6." *Journal of cellular physiology* no. 234 (8):13263-13276.

Tahir, Rana Adnan, Hao Wu, Muhammad Ahmad Rizwan, Tassadaq Hussain Jafar, Shahzad Saleem, and Sheikh Arslan Sehgal. 2018b. "Immunoinformatics and molecular docking studies reveal potential epitope-based peptide vaccine against DENV-NS3 protein." *Journal of theoretical biology* no. 459:162-170.

Trott, O., and A. J. Olson. 2010. "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading." *J Comput Chem* no. 31 (2):455-61. doi: 10.1002/jcc.21334.

Vaz, Miguel, and Samuel Silvestre. 2020. "Alzheimer's disease: Recent treatment strategies." *European Journal of Pharmacology* no. 887:173554. doi: https://doi.org/10.1016/j.ejphar.2020.173554.

Wallace, Andrew C, Roman A Laskowski, and Janet M Thornton. 1995. "LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions." *Protein engineering, design and selection* no. 8 (2):127-134.

Waqas, Muhammad, Ali Haider, Abdur Rehman, Muhammad Qasim, Ahitsham Umar, Muhammad Sufyan, Hafiza Nisha Akram, Asif Mir, Roha Razzaq, and Danish Rasool. 2021. "Immunoinformatics and molecular docking studies predicted potential multiepitope-based peptide vaccine and novel compounds against novel SARS-

CoV-2 through Virtual screening." *BioMed research international* no. 2021.

Waqas, Muhammad, Ali Haider, Muhammad Sufyan, Sami Siraj, and Sheikh Arslan Sehgal. 2020. "Determine the potential epitope based peptide vaccine against novel SARS-CoV-2 targeting structural proteins using immunoinformatics approaches." *Frontiers in molecular biosciences* no. 7:227.

Webb, B., and A. Sali. 2014. "Protein structure modeling with MODELLER." *Methods Mol Biol* no. 1137:1-15. doi: 10.1007/978-1-4939-0366-5_1.

Wiederstein, Markus, and Manfred J Sippl. 2007. "ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins." *Nucleic acids research* no. 35 (suppl_2):W407-W410.

Zaka, M., S. A. Sehgal, S. Shafique, and B. H. Abbasi. 2017a. "Comparative in silico analyses of Cannabis sativa, Prunella vulgaris and Withania somnifera compounds elucidating the medicinal properties against rheumatoid arthritis." *J Mol Graph Model* no. 74:296-304. doi: 10.1016/j.jmgm.2017.04.013.

Zaka, Mehreen, Sheikh Arslan Sehgal, Shagufta Shafique, and Bilal Haider Abbasi. 2017b. "Comparative in silico analyses of Cannabis sativa, Prunella vulgaris and Withania somnifera compounds elucidating the medicinal properties against rheumatoid arthritis." *Journal of Molecular Graphics and Modelling* no. 74:296-304.