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***Corresponding Author**
Syed Atta-ul-Mubeen Shah

E-mail
syedattaulmubeenshah@gmail.com

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Special Issue: Computational drug designing and molecular docking analyses

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Molecular docking studies elucidate potential hydroxyproline dehydrogenase compounds for schizophrenia inhibition

Syed Atta-ul-Mubeen Shah[1,*], Jonathan Javid[1], Haseeb Akram[2]

[1]Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Pakistan
[2] District Head Quarter Hospital, Chiniot, Pakistan

Abstract

Hydroxyl Proline dehydrogenase HYPDH is an enzyme used in the dehydrogenation of proline and is involved in the conversion of trans-4hydroxy proline to Δ-1- pyrroline-3-hydroxy-5-carboxylate oxidized to glutamate. PRODH2 is involved schizophrenia through microdeletion results in the loss of gene sequence. Consequently, the production of glutamate effected lead to a neurodegenerative disorder called schizophrenia which affects 1% of the population and is characterized by several behavioral abnormalities and lack of social behavior. Bioinformatics techniques were used for the prediction of 3D structures and protein-protein molecular docking studies of PRODH2. Threading and homology modeling approaches were employed for 3D structure prediction of PRODH2. STRING database was used and ALDH4A1 was observed as the interacting partner of PRODH2. Protein-protein docking studies of PRODH2 against ALDH4A1 were done to analyze the interacting potential residues. Virtual screening was performed against FDA library from ZINC database and molecular docking was done by AutoDock Vina. It was observed that ZINC000016 molecule has least binding energy of -12.2 kcal/mol. The scrutinized compound may have potential for further drug discovery processes. The observed results of this research may help in designing the novel therapeutic targets against PRODH2.

Introduction

Hydroxyproline dehydrogenase (HYPDH) is a protein encoded by proline dehydrogenase 2 (PRODH2) and also known as HSPOX 2. HYPDH belongs to the proline oxidase family [1, 2]. HYPDH performs numerous functions including use of flavin-adenine-dinucleotide to build a new electron transfer pathway and proline dehydrogenation. It involves the conversion of trans-4hydroxy proline to Δ-1-pyrroline-3-hydroxy-5-carboxylate oxidized to glutamate [3]. Glutamate is a wonderful neurotransmitter involved in signaling among neural cells [4, 5].

PRODH2 is known to express in many tissues however it has common expression in brain. It is a mitochondrial protein and involved in the transfer of redox potential. PRODH2 is present in the centromeric region of 22q11 and contains 14 exons. The 22q11 region of chromosome undergoes microdeletion and known as a velocardiofacial syndrome (VCFS) [6, 7]. VCSF is characterized by distinct facial features and frequent infections. It has also been reported that the growth and development were also affected.

The microdeletions occur at 22q11 leads to affect HYPDH results in a deficiency of the enzyme proline oxidase known as hyperprolinemic type 1 characterized by high level of proline in the blood circulation [8]. Therefore, the production of glutamate reduced to perform the required function of neurotransmission as glutamate is the product of proline degradation pathway [9].

The deficiency of neurotransmitter results in various disorders as seizures and intellectual disabilities. It has reported that a high level of proline in blood circulation is associated with a low level of glutamate in the regions of brain that was weekly associated with schizophrenia a neurological disorder characterized by intellectual disabilities including hallucination [10].

Schizophrenia is a neurodegenerative disorder and is known to effect 1% of the population worldwide. It is a severe mental illness and also affects the family psychologically [11]. It is characterized by severe mental illness including delusions, disrupt speech, out of order thinking and lack of social behavior [12]. Schizophrenia is mainly caused by various genetic factors. Variations in many genes or deletions in many parts of the chromosomes results in the loss of information needed for a metabolism or pathway. The degradation of proline occurs and results in the low or completely lost expression of specific genes. Additionally, with genetic factors there are many epigenetic and environmental aspects which lead towards schizophrenia [13].

It has reported that schizophrenia is associated with VCSF. The deletion of chromosome 22 undergoes microdeletions leads to genes lost. The results are severe and affect numerous metabolisms. The centromeric region of chromosome 22 encodes an enzyme called hydroxyl proline dehydrogenase takes part in proline degradation [11]. The microdeletion results in the loss of gene sequence and affects the production of glutamate results in weak neurotransmission and ultimately leads towards schizophrenia [14].

Computational drug designing have shown success in computational research methodologies to solve different biological problems [15] and computationally designed various molecules against cancer [16, 17] and neurological disorders [18-23]. The current effort has structural and virtual screening to identify novel compound against schizophrenia. The NMR and X-ray crystallographic structure of PRODH2 has not been reported yet. To predict the structural insight, a 3D structure of PRODH2 was predicted by utilizing numerous computational approaches.

Materials and methods

PRODH2 has one described isoform under the accession number of Q9UF12 in Uniport knowledge base and has six splice variants. In this study, sequence analyses, 3D structure prediction, virtual screening and comparative molecular docking studies have been performed.

The amino acid sequence of PRODH2 was retrieved from Uniport KB (http://www.uniprot.org/) and subjected to blastp for the selection of suitable templates against Protein Data Bank (PDB) [24] for structure prediction. The MODELLER 9.20 [25] was employed to predict the three-dimensional (3D) structure of PRODH2. The comparative modeling is also performed by CPHmodels3.2 [26] which recognize templates based on profile-profile alignment and SWISS-MODEL [27]. Threading approach based programs including I-TASSER [28] and SPARKS-X [29] were also utilized. Some other structure prediction tools including Phyre2 [30], M4t [31] and Robetta [32] were also used to predict the structure of PRODH2. The 3D structure of PRODH2 isoforms were visualized and minimized through UCSF Chimera 1.13 [33]. Anolea [34], ERRAT [35], Rampage [36] and Verify3D [37] evaluation tools

were used for the critical assessment of the structures and overall quality of the models. The Ramachandran plots were generated to evaluate φ and ψ angles which showed the distribution of non-Glycine, non-proline residues.

STRING (Search Tool for the Retrieval of Interacting Genes/Protein) database [38] was employed to analyze the interacting protein against PRODH2. The crystalline structure was obtained from PDB (PDB ID: 4EO5). Gramm-X [39] online server was applied for protein-protein docking studies of PRODH2 isoform against 4EO5. PatchDock [40] was applied for further protein-protein interaction assessment. The hydrophobic and electrostatic interactions were mapped by using ligPlot [41].

Pyrx tool was used for the molecular docking analyses by optimizing AutoDock [42]. The interaction between the protein and ligand was employed for orientation and conformation. The FDA library of ZINC database was used for virtually screening of target protein against the library molecules.

Results and Discussions

Structural bioinformatics is one of the milestones archived by computational biologists. This dimension of biology aims to justify the macromolecular anatomy and physiology in a well-organized manner. The structural biology provides many disciplines in the field of pharmacoinformatics, percussion medicine which helps to understand the potential of schizophrenia by analyzing various proteins or defective enzymes involved in schizophrenia susceptibility. Besides, all the researches holding in all over the World on exploring the effective remedies of schizophrenia and many researchers involved in it around the Globe. PRODH2 is involved in schizophrenia as the dehydrogenation of proline by help in oxidization of L-proline to delta-1 pyrroline-5-carboxylate. It was found that the variation in PRODH2 locus affects the availability of glutamate and mice with induced mutations exhibits abnormalities in their behaviors leads towards schizophrenia. In genome wide association studies, it was reported that the schizophrenia susceptibility locus was present at chromosome 22q11 region and microdeletion occurs leads towards schizophrenia.

Sequence Analyses

Extensive literature review and exploring biological databases revealed significant information about the sequence of PRODH2. UCSC genome browser was used to identify the exact location of PRODH2 in human genome. It was present on the q arm of chromosome 19 (**Fig. 1**).

Clustal omega and NCBI blast [43] was used to get the MSA of the PRODH2 family to reveal the conserved regions for further analyses. Query coverage and percentage identity was analyzed having a word size of 3 and BLOSSOM62 matrix was utilized having a threshold of 10.

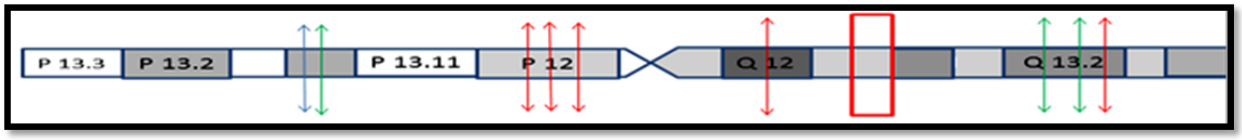

Fig. 1: Location of human PRODH2 protein on chromosome 19.

NCBI blast, ENSEMBLE and UCSC Genome Browser BLAT [44] were used for MSA. Clustal Omega was used for MSA of the sequences. Amino acids composition was calculated through Expesy ProtParam tool [45]. Numerous parameters were calculated including theoretical pI, which is 8.94, number of positively charged residues are 46 and negatively charged residues are 56 with the aliphatic index of 93.38 and the respective composition of amino acids were calculated (**Fig. 2**).

Structure prediction

Comparative modeling [46] and threading techniques were utilized for 3D structure prediction of PRODH2 as the structure was not solved through X-ray crystallography and nuclear magnetic resonance (NMR). The sequence was retrieved in FASTA format from Uniport KB [47, 48] and subjected to blastp [49] to search for suitable templates against PDB for structure prediction. Three different templates having high query coverage, identity and E-value were selected as suitable templates for homology modeling having PDB ID 5UR2, 4NM9

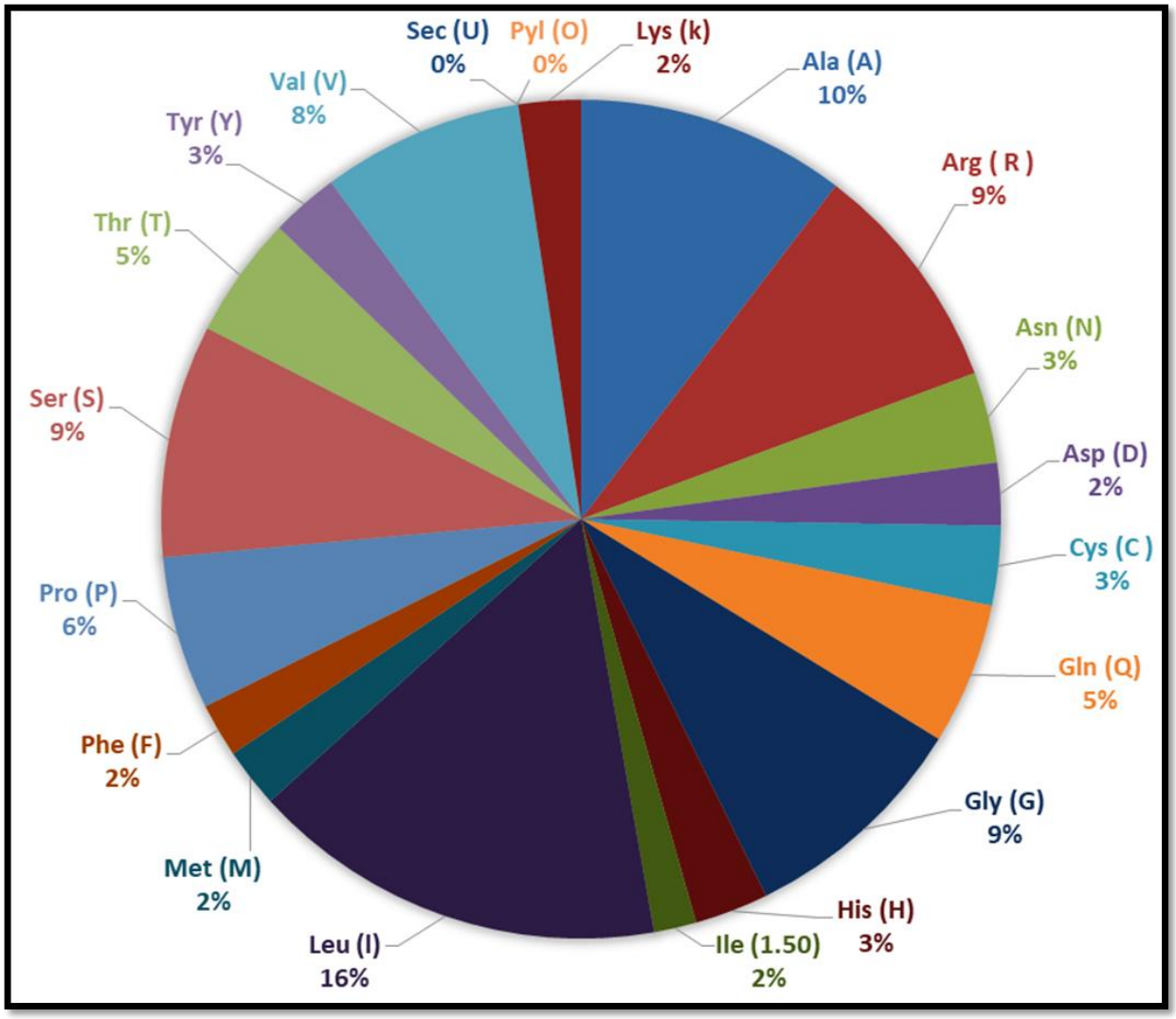

Fig. 2: The composition of the amino acid of PRODH2 calculated from ProtParam tool.

and 5UX5 (**Table 1**). Modeller was used for homology modeling approach and models were generated against all the selected templates. It was observed that the maximum query coverage of the templates was 29% so threading approach it was utilized to lower down the error rate.

Numerous structure predictions tolls including I-TASSER, SWISS-MODEL, M4T, phyre2, CPHmodels, SPARKS-X, Raptor-X and Robetta were used for implementing homology modeling and

Table 1: The best three templates

Description	Max Score	Query Cover	E Value	Per. Ident	Accession
Chain A, Bifunctional protein PutA [Bdellovibrio bacteriovorus HD100]	67.4	29%	3e-11	30.38%	5UR2
Chain A, Proline Dehydrogenase And Delta-1-pyrroline-5-carboxylate Dehydrogenase [Geobacter sulfurreducens PCA]	52.0	24%	2e-06	29.23%	4NM9
Chain A, BIFUNCTIONAL PROTEIN Proline utilization A (PutA) [Corynebacterium freiburgense]	43.1	29%	0.001	26.99%	5UX5

Note: Employed from the BLASTp

threading approaches against PRODH2. The predicted structures were further evaluated through various evaluation tools. Rampage tool was utilized to check the stereochemical parameters of the predicted structures. The phi and psi torsion angles were also calculated. The favored region allowed region and outliers of the structures were calculated and the structures were further evaluated through ERRATE used to calculate the overall quality factor of the predicted models [56] (**Fig. 4**).

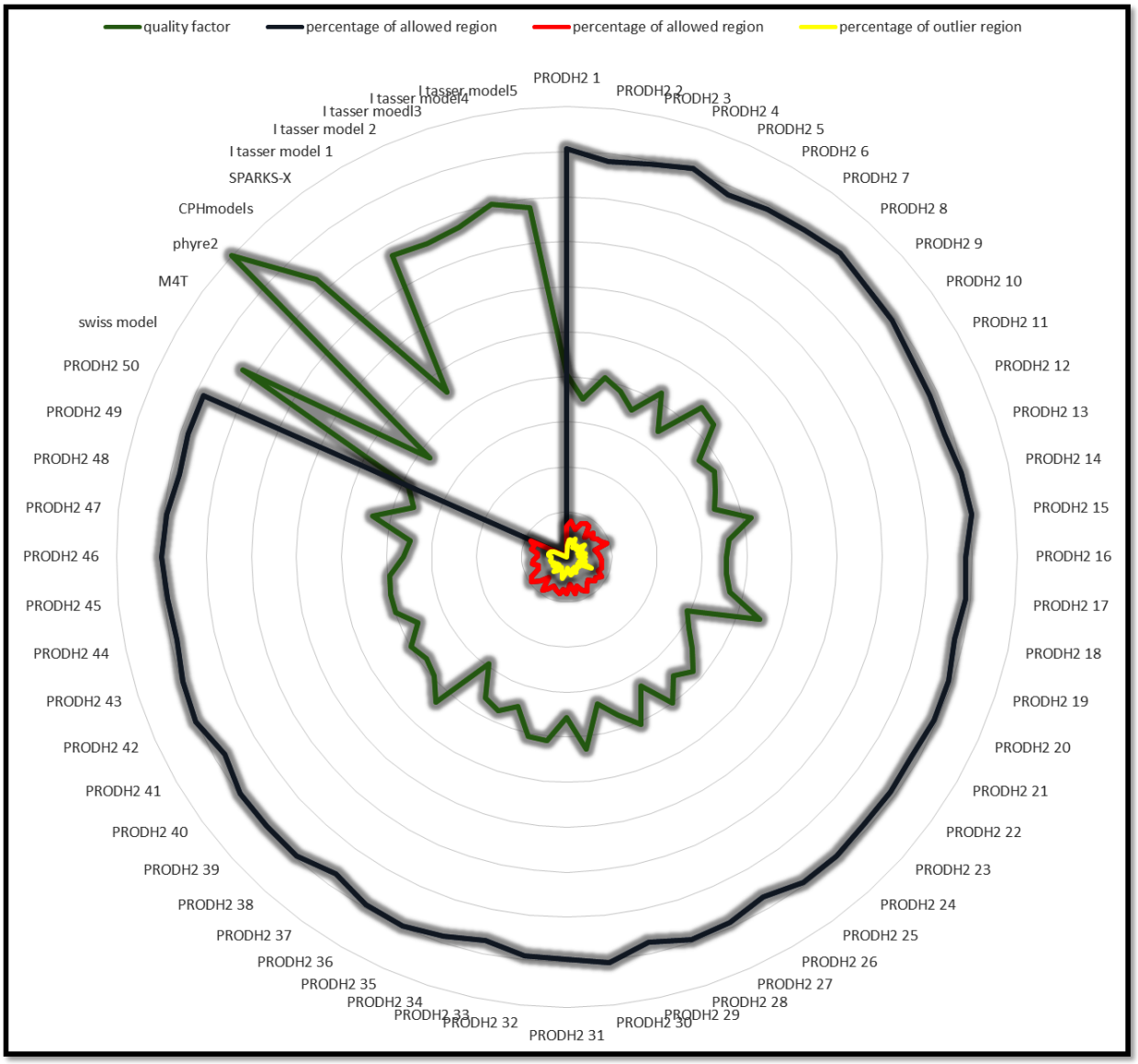

Fig. 3: Graph of quality factor, favored region, allowed region and outliers of the PRODH2 structures evaluated by using ERRAT and Rampage structure evaluations tools.

All the predicted structures were evaluated on the values of their favored region, allowed region, outliers and overall quality factors. The comparative graph was generated from the evaluated values of the predicted structures. The most reliable structure was selected from the graphs for further analyses. Interestingly, it was observed that the highest overall quality factor was 100%. The minimization of the selected structure was performed by using UCSF Chimera 1.13 to improve the stereochemistry and optimization of the model through 1000 steepest and 1000 conjugate steps.

Protein-protein docking analyses

String database was used to find the interacting partner of PRODH2, and it was observed that the

crystalline structure having PDB ID:4OE5 was the interacting partner of PRODH2. The protein-protein docking studies were carried out by using GrammX online server and cross verify the results through PatchDock and FireDock. The interacting residues of the complexes were visualized and analyzed by using UCSF Chimera and ligplot.

The interaction between PRODH2 and ALDH4A1 (**Table 2**) were visualized and analyzed through UCSF Chimera (**Fig. 6**). ALDH4A1 was observed as the interacting partner of PRODH2 leads to perform the protein-protein molecular docking analyses to analyze the residual interactions. The docked complex predicts the interacting. The protein-protein molecular docking studies were analyzed on the basis of least binding energy values. Numerous complexes were analyzed and top ranked complexes having least energy values for further refinement. The selected complex showed the least global binding energy and suggested that PRODH2 and ALDH4A1 have efficient binding affinity.

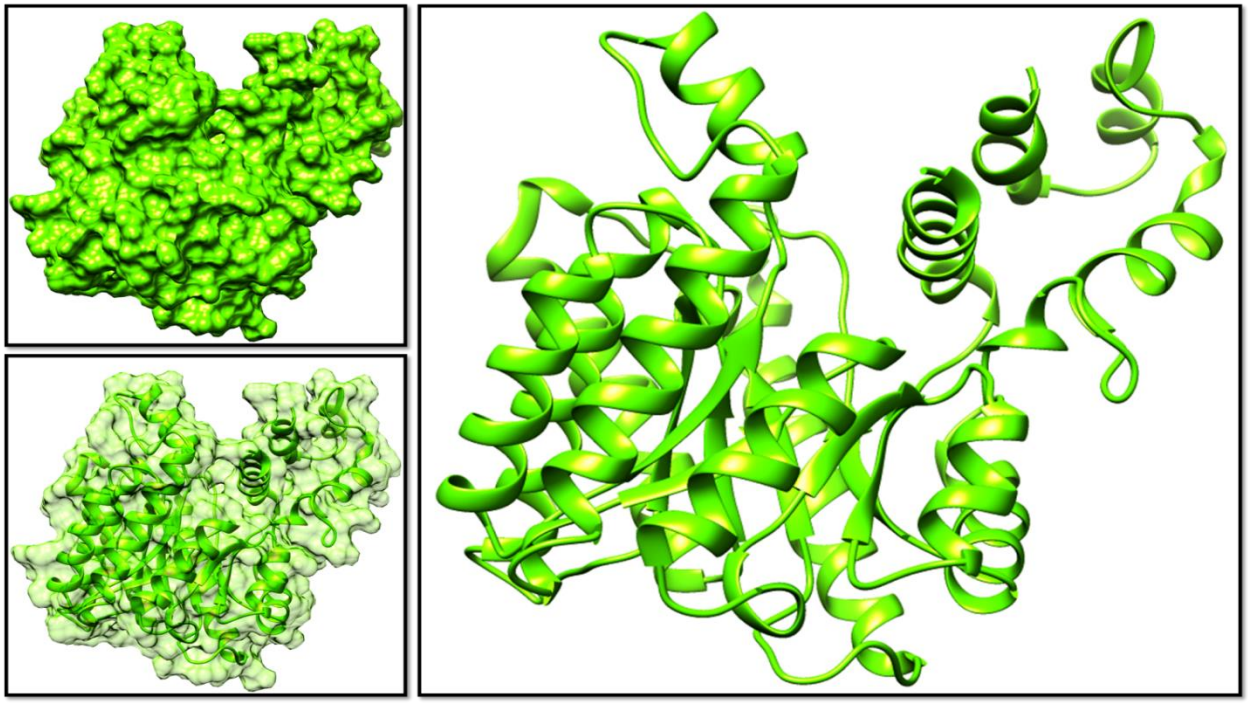

Fig. 4: 3D structure of PRODH2 predicted model.

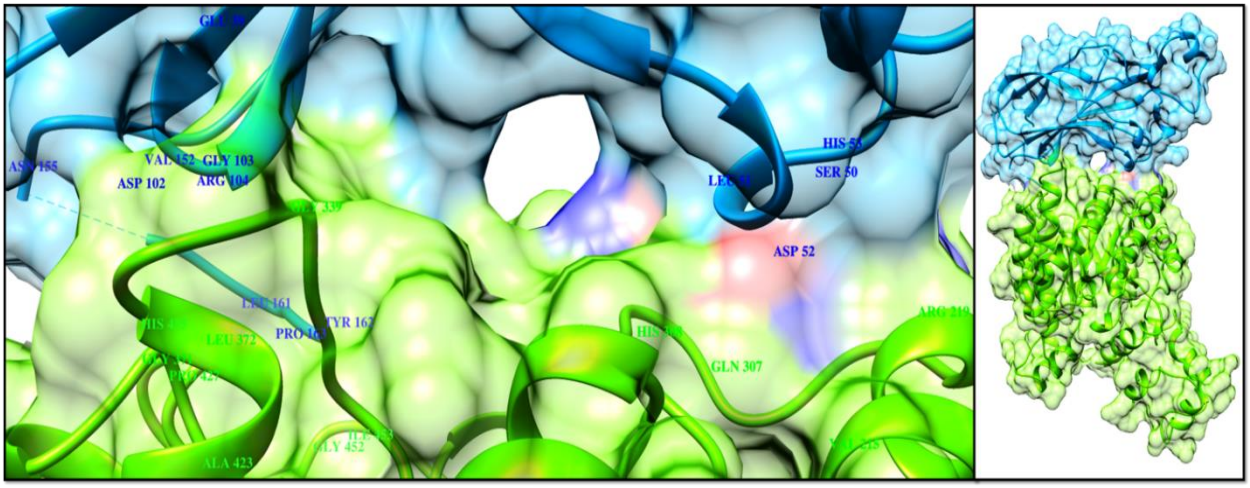

Fig. 5: Protein-protein docking analyses of PRODH2 and ALDH4A1.

Comparative molecular docking

Virtual screening was performed for comparative molecular docking analyses of PRODH2 (Figure 7). The FDA library of ZINC database was screened by utilizing AutoDock Vina. Moreover, the FDA library query for virtual screening yielded 134 compound hits. The molecular docking analyses were done by AutoDock Vina. The top ranked least binding energy molecule was scrutinized for further analyses. The slight variation in least binding energy was observed (**Table 3**). It was observed that the docked structure of PRODH showed reliable docking results. All the docked complexes showed similar binding domain. The top ranked ligand from selected library was elucidated, namely ZINC0000016 (**Fig. 7**) and the observed interacting residues were GLU504, ARG500, ARU380, GLY381, ALA382, HIS436, VAL379, TYR497, LYS236, SER435, ALA434, GLN463, LYS377, LEU464, SER485, GLN234, ASP314 (**Fig. 8**).

Table 2: Interacting residues of the target protein PRODH2 and Interacting protein ALDH4A1.

Target protein	Target protein Residues	Interacting protein	Interacting protein residues
PRODH2	GLY 103, LEU 373, ASP 52, VAL 215, TYR 162, GLY 452	ALDH4A1	HIS53, SER50, ASP52, LEU51, GLN307, HIS308, ASN155, VAL152, ASP102, GLY103, ARG104, LEU161, GLY339, TYR162, PRO163, LEU372, PRO427, GLY371, ALA423, GLY452, ILE453, HIS415, GLU39, ARG219, VAL215.

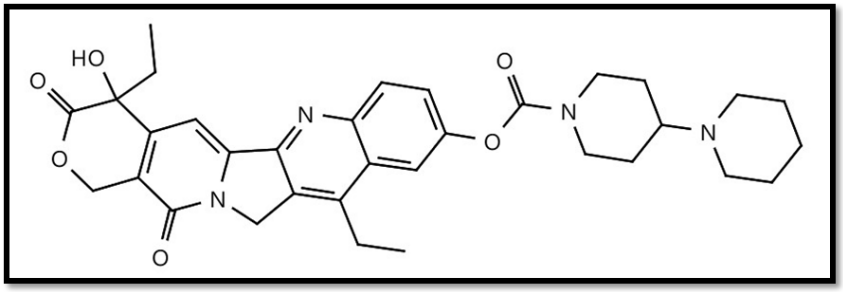

Fig. 7: 2D structure of scrutinized compound ZINC000016.

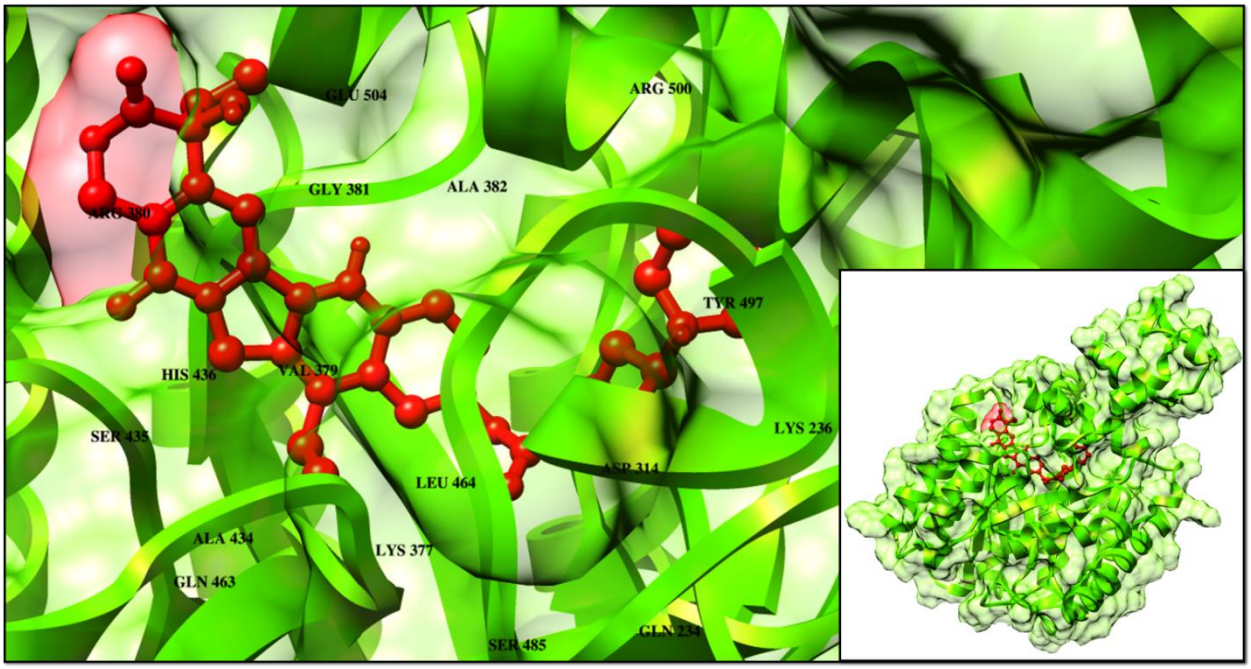

Fig. 8: Molecular docking analyses of PRODH2 and top ranked scrutinized ligand ZINC000016.

Table 3: Top ranked four compounds having least binding energy

Compound name	Binding affinity (kcl/mol)	RMSD (lower)	RMSD (upper)
ZINC000016	-12.2	0.0	0.0
ZINC000059	-11.3	0.0	0.0
ZINC000076	-10.8	0.0	0.0
ZINC000060	-10.9	4.116	4.586

Conclusion

Schizophrenia is a complex disease that affects 1% of the population. In conclusion, *in silico* approaches were used based on structural modeling, virtual screening and molecular docking analyses. The predicted 3D structure suggested the reliability of the structure with good degree of accuracy. The molecular docking results satisfied the least binding energy and suggested that ZINC000016 may prove as a potent molecule for SZ by targeting PRODH2. The applied methodology leads to simplify the process of novel drug designing.

Conflict of interest

The authors declare no conflicts of interest.

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References

[1] Hui Liu SCH, Christina Sobin, J Louw Roos, Brandi L Galke, Maude L Blundell, Marge Lenane, Brian Robertson, Ellen M Wijsman, Judith L Rapoport, Joseph A Gogos, Maria Karayiorgou. Genetic Variation at the 22q11 PRODH2/DGCR6 Locus Presents an Unusual Pattern and Increases Susceptibility to Schizophrenia. Proceedings of the National Academy of Science 2002;99(6):3717-3722.

[2] Lowther CBSLCJTJJDPRPHWT. Proline dehydrogenase 2 (PRODH2) is a hydroxyproline dehydrogenase (HYPDH) and molecular target for treating primary hyperoxaluria. BioChemical Journal 2015;466(2):273-281.

[3] Kawakami R, Satomura T, Sakuraba H, Ohshima T. L-proline dehydrogenases in hyperthermophilic archaea: distribution, function, structure, and application. Appl Microbiol Biotechnol 2012;93(1):83-93.

[4] Fonnum F. Glutamate: a neurotransmitter in mammalian brain. J Neurochem 1984;42(1):1-11.

[5] McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JA, Zackai EH, Emanuel BS, Vermeesch JR, Morrow BE, Scambler PJ, Bassett AS. 22q11.2 deletion syndrome. Nat Rev Dis Primers 2015;1:15071.

[6] Modinos G, Iyegbe C, Prata D, Rivera M, Kempton MJ, Valmaggia LR, Sham PC, van Os J, McGuire P. Molecular genetic gene-environment studies using candidate genes in schizophrenia: a systematic review. Schizophr Res 2013;150(2-3):356-65.

[7] Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT. Neurobiology of schizophrenia. Neuron 2006;52(1):139-53.

[8] Andreasen NC. Negative symptoms in schizophrenia. Definition and reliability. Arch Gen Psychiatry 1982;39(7):784-8.

[9] Roth TL, Lubin FD, Sodhi M, Kleinman JE. Epigenetic mechanisms in schizophrenia. Biochim Biophys Acta 2009;1790(9):869-77.

[10] J P. Does a Diagnosis of Velocardiofacial Syndrome Mean Schizophrenia? In: Hauptman A, Salpekar J (eds) Pediatric Neuropsychiatry 2019.

[11] Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 2009;373(9659):234-9.

[12] Cummings JL. Organic delusions: phenomenology, anatomical correlations, and review. Br J Psychiatry 1985;146:184-97.

[13] Petronis A, Paterson AD, Kennedy JL. Schizophrenia: an epigenetic puzzle? Schizophr Bull 1999;25(4):639-55.

[14] MariaCarlsson A. Interactions between glutamatergic and monoaminergic systems within the basal ganglia-implications for schizophrenia and Parkinson's disease. Trends in Neurosciences 1990;13(7).

[15] Sehgal SA, Khattak NA, Mir A. Structural, phylogenetic and docking studies of D-amino acid oxidase activator (DAOA), a candidate schizophrenia gene. Theor Biol Med Model 2013;10:3.

[16] Tahir RA, Sehgal SA, Khattak NA, Khan Khattak JZ, Mir A. Tumor necrosis factor receptor superfamily 10B (TNFRSF10B): an insight from structure modeling to virtual screening for designing drug against head and neck cancer. Theor Biol Med Model 2013;10:38.

[17] Kanwal S, Jamil F, Ali A, Sehgal SA. Comparative Modeling, Molecular Docking, and Revealing of Potential Binding Pockets of RASSF2; a Candidate Cancer Gene. Interdiscip Sci 2017;9(2):214-223.

[18] Sehgal SA, Mannan S, Kanwal S, Naveed I, Mir A. Adaptive evolution and elucidating the potential inhibitor against schizophrenia to target DAOA (G72) isoforms. Drug Des Devel Ther 2015;9:3471-80.

[19] Sehgal SA, Mannan S, Ali S. Pharmacoinformatic and molecular docking studies reveal potential novel antidepressants against neurodegenerative disorders by

targeting HSPB8. Drug Des Devel Ther 2016;10:1605-18.
[20] Sehgal SA. Pharmacoinformatics and molecular docking studies reveal potential novel Proline Dehydrogenase (PRODH) compounds for Schizophrenia inhibition. Medicinal Chemistry Research 2017;26:314-326.
[21] Sehgal SA. Pharmacoinformatics, Adaptive Evolution, and Elucidation of Six Novel Compounds for Schizophrenia Treatment by Targeting DAOA (G72) Isoforms. Biomed Res Int 2017;2017:5925714.
[22] Tahir RA, Sehgal SA. Pharmacoinformatics and Molecular Docking Studies Reveal Potential Novel Compounds Against Schizophrenia by Target SYN II. Comb Chem High Throughput Screen 2018;21(3):175-181.
[23] Tahir RA, Wu H, Javed N, Khalique A, Khan SAF, Mir A, Ahmed MS, Barreto GE, Qing H, Ashraf GM, Sehgal SA. Pharmacoinformatics and molecular docking reveal potential drug candidates against Schizophrenia to target TAAR6. J Cell Physiol 2019;234(8):13263-13276.
[24] Rose PW, Prlic A, Bi C, Bluhm WF, Christie CH, Dutta S, Green RK, Goodsell DS, Westbrook JD, Woo J, Young J, Zardecki C, Berman HM, Bourne PE, Burley SK. The RCSB Protein Data Bank: views of structural biology for basic and applied research and education. Nucleic Acids Res 2015;43(Database issue):D345-56.
[25] Webb B, Sali A. Protein Structure Modeling with MODELLER. Methods Mol Biol 2017;1654:39-54.
[26] Nielsen M, Lundegaard C, Lund O, Petersen TN. CPHmodels-3.0--remote homology modeling using structure-guided sequence profiles. Nucleic Acids Res 2010;38(Web Server issue):W576-81.
[27] Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46(W1):W296-W303.
[28] Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. Nat Methods 2015;12(1):7-8.
[29] Yang Y. SPARKS-X: Protein fold recognition. Laboratory of Structural Bioinformatics Jan 15, 2020.
[30] Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 2015;10(6):845-58.
[31] Fernandez-Fuentes N, Madrid-Aliste CJ, Rai BK, Fajardo JE, Fiser A. M4T: a comparative protein structure modeling server. Nucleic Acids Res 2007;35(Web Server issue):W363-8.
[32] Kim DE, Chivian D, Baker D. Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 2004;32(Web Server issue):W526-31.
[33] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem 2004;25(13):1605-12.
[34] Melo F, Devos D, Depiereux E, Feytmans E. ANOLEA: a www server to assess protein structures. Proc Int Conf Intell Syst Mol Biol 1997;5:187-90.
[35] Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 1993;2(9):1511-9.
[36] Lovell SC, Davis IW, Arendall WB, 3rd, de Bakker PI, Word JM, Prisant MG, Richardson JS, Richardson DC. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins 2003;50(3):437-50.
[37] Eisenberg D, Luthy R, Bowie JU. VERIFY3D: assessment of protein models with three-dimensional profiles. Methods Enzymol 1997;277:396-404.
[38] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ, von Mering C. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45(D1):D362-D368.
[39] Tovchigrechko A, Vakser IA. GRAMM-X public web server for protein-protein docking. Nucleic Acids Res 2006;34(Web Server issue):W310-4.
[40] Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res 2005;33(Web Server issue):W363-7.
[41] Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51(10):2778-86.
[42] Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol 2015;1263:243-50.
[43] Sievers F, Higgins DG. Clustal Omega for making accurate alignments of many protein sequences. Protein Sci 2018;27(1):135-145.
[44] Haeussler M, Zweig AS, Tyner C, Speir ML, Rosenbloom KR, Raney BJ, Lee CM, Lee BT, Hinrichs AS, Gonzalez JN, Gibson D, Diekhans M, Clawson H, Casper J, Barber GP, Haussler D, Kuhn RM, Kent WJ. The UCSC Genome Browser database: 2019 update. Nucleic Acids Res 2019;47(D1):D853-D858.
[45] Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, Appel RD, Hochstrasser DF. Protein identification and analysis tools in the ExPASy server. Methods Mol Biol 1999;112:531-52.
[46] Eswar N, John B, Mirkovic N, Fiser A, Ilyin VA, Pieper U, Stuart AC, Marti-Renom MA, Madhusudhan MS, Yerkovich B, Sali A. Tools for comparative protein structure modeling and analysis. Nucleic Acids Res 2003;31(13):3375-80.
[47] UniProt C. The Universal Protein Resource (UniProt) in 2010. Nucleic Acids Res 2010;38(Database issue):D142-8.
[48] Magrane M, UniProt C. UniProt Knowledgebase: a hub of integrated protein data. Database (Oxford) 2011;2011:bar009.
[49] Hung JH, Weng Z. Sequence Alignment and Homology Search with BLAST and ClustalW. Cold Spring Harb Protoc 2016;2016(11).