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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Pharmacoinformatics and molecular docking studies reveal potential novel Proline Dehydrogenase (PRODH) compounds for Schizophrenia inhibition

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Abstract Studies on Schizophrenia, so far reveal a complex picture of neurological malfunctioning reported to be strongly associated with proline dehydrogenase (PRODH). This study employs in silico hybrid approach for virtual screening and molecular docking followed by pharmacophore identification and structural modeling. Docking studies revealed critical residues for receptor-ligand interaction. Virtual screening approach coupled with docking energies and drug likeness rules, suggested that of 8 compounds, Clozapine, MCULE-1620364835-0 (Drug Score 88 %) and PB-752728400 (Drug Score 85 % and Binding energy 8.1 kcal/mol) observed as potential inhibitor compounds for targeting PRODH. Energy score of selected compounds were better than the previous listed drug analogs. ALDH4A1, functional partner of PRODH showed strong interactions with PRODH.

Keywords Schizophrenia · Pharmacophore identification · Structure prediction and molecular Docking · PRODH · Virtual screening · Computer-aided drug designing

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Introduction

Schizophrenia (SZ), a severe mental disorder, usually affects 1 % population worldwide and causes lifelong disabilities, suffering and functional decline (Kessel et al. 2008). It is a multifactorial complex psychotic mental disorder characterized by bizarre behavior, in relation to interpersonal interactions, having hallucinations and the like. It affects emotions culminating into dementia and mild impairment of cognitive function. A careful literature review on SZ implicates more than 130 genes that have so far been associated with it. Out of 130 susceptible genes, not a single one showed sufficient level of confidence as a default gene and replicability studies suggest that many of these may be false positives. The prenatal revelation into Lyme disease and tick infestation (Brown 1994), beri-beri or famine (Brown 1994; Hafner and Heiden 2003) rubella and influenza (Murray and Lopez 2006) also increased the risk of SZ.

SZ associated genes can be grouped into PRODH (proline dehydrogenase), SLC1A2, NAALAD2, MTHFR, DAO, and DAOA families, all affecting the availability of glutamate and can be clustered under single group. The PRODH expression level in brain is high and encoded PRODH (Oxidase) that catalyzes proline decomposition. Mutation in PRODH causes probability of SZ and hyperprolinemia type 1 (Kessel et al. 2008; Hafner and Heiden 2003; Murray and Lopez 2006). Evidence suggests that proline plays a major functional role in brain by serving as a metabolic precursor of glutamate in a sub-population of glutamate neurons as well as in the regulation of cortical Ach function (Harrison and Owen 2003; McGuffin et al. 2004). PRODH being a key player gene linked to SZ consists of 15 exons with 23.77 kb span located at 17.3 Mb on chormosome 22q11 centromeric band and its deletion at

centromeric end causes Digorge syndrome/velocardiofacial syndrome (Beate et al. 2006; O'Tuathaigh et al. 2007; Ishiguro et al. 2007; Liu et al. 2000).

PRODH localized to mitochondrial inner membrane and converts proline into D-1-pyroline-5-carboxylate (P5C), which in turn converts to γ-aminobutric acid or glutamate. The final product of P5C reported to significantly involved in schizophrenia pathogenecity (Pearlson 2000) as both are the significant neurotransmitters in the nervous system. Proline catabolism has significant role in establishing mitochondrial redox status (Phang 1985; Phang et al. 2008). The first chemical reaction of proline catabolism catalyzes by considering PRODH, a FAD dependent enzyme that catalyzes the oxidation of L-proline to Δ 1-pyrroline-5-carboxylate (P5C) (Adams and Frank 1980). For the production of ATP, the stored electrons in the flavin are transferred subsequently to the electron transport chain. The equilibrium of P5C depends on its hydrolysis product glutamate γ-semialdehyde (GSA) and oxidized to glutamate by NAD(+)-dependent P5C dehydrogenase, the second enzyme of proline catabolism (Tanner 2008; Singh and Tanner 2012).

Severe SZ symptoms have been reported as results of PRODH mutations that may lower its expression levels and increased blood plasma proline culminating into hyperprolinemia type I. Some evidence also reports its over expression and hyperactivity leading to higher enzyme and glutamate levels coupled with lowered plasma proline (Ishiguro et al. 2007). Proline has functional importance due to its role in brain functionality, where it serves as a glutamate prerequisite metabolite in subgroup of glutamate neurons and adjusting the performance of Ach. Major mutational association of PRODH with respect to schizophrenia relies on the expression of proline oxidize enzyme, the role of proline acting as glutamate transmission receptor in the brain and P5C/proline route in growth suppression affiliated to apoptosis. Mutation can cause increased proline levels in the body and reduction in proline oxidase function (hyperprolinemia), characterized by significant malfunctioning of neurotransmitter and can increase psychiatric disorder risk including SZ (Kessel et al. 2008; Murray and Lopez 2006; Ishiguro et al. 2007; Williams et al. 2002).

PRODH acts as a superoxide producer by contributing in p53 medicated apoptosis and a tumor suppressor protein also known as POX (Proline Oxidase) in humans (Phang et al. 2008; Donald et al. 2001; Hu et al. 2007; Liu et al. 2006, 2009; Pandhare et al. 2006). PRODH itself activates extrinsic and intrinsic apoptotic pathways and its expression is induced by tumor suppressor p53 (Liu et al. 2006). The crucial role of tumor suppressor PRODH is its ability to generate superoxide (Phang et al. 2008; Liu et al. 2006, 2005). Mutations cause hyperprolinemia type I (Phang et al. 2001), which leads to schizophrenia (Willis et al. 2008) by increasing disease susceptibility.

In silico approaches and bioinformatics analyses have shown success in research methodologies to solve the biological problems (Sehgal et al. 2013) and designed numerous novel computer-aided molecules against neurological disorders (Sehgal et al. 2014a; Sehgal et al. 2015; Sehgal et al. 2016) and cancer (Tahir et al. 2013; Sehgal et al. 2014b; Kanwal et al. 2016). This work presents structural and pharmacophore based virtual screening to identifiy novel inhibitors against SZ. Ligand based compound libraries for virtual screening were screened by 2D similarity search against FDA approved and recommended SZ drugs. The novel compounds of common structural features and diverse structural entities were investigated. Experimental validation of PRODH by using NMR and X-ray crystallography has not been reported yet. In order to reveal structural insight, a 3D structure of PRODH was generated by applying numerous bioinformatics approaches. Such a comprehensive, in silico analyses may provide an evidence for a reliable framework which could assist medicinal chemists to design and develop lead drug compounds targeting against SZ.

Materials and methods

This study was planned for structural sequence prediction of PRODH (600 a.a), library screening and molecular docking analyses for its modeling as a strong candidate gene, causing SZ and Hyperprolinemia disease. The sequence was obtained in FASTA format from Uniprot database (accession no. O43272) and was subjected to protein-protein BLAST search against Protein Data Bank (PDB) (Berman et al. 2000) for identification of suitable template structure. [PDB ID: 4F9I] was selected as a suitable template having 27 % identity, 36 % query coverage and E-value 4e-08. The automated protein modeling program MODELLER 9v10 (Eswar et al. 2008) was employed to predict 3D structure of PRODH by satisfying spatial restraints. Threading and ab initio approaches were also utilized for valuable satisfied structure. I-Tasser, Modweb, EsyPred, 3D-JigSaw and SwissModel were also employed for structure prediction. The selected structure was minimized by using UCSF Chimera 1.6 at 1000 steepest and conjugate gradients. The evaluation tools ERRAT (Colovos and Yeates 1993) Anolea (Melo et al. 1997, ProCheck (Laskowski et al. 1993) and Rampage (Lovell et al. 2002) were also applied to assess predicted 3D PRODH model. Predicted structure was further evaluated by MolProbity (Chen et al. 2010) and corrected for poor rotamers and ramachandran outliers by employing WinCoot software (Emsley et al. 2010).

AutoDock, UCSF Chimera 1.6 (Pettersen et al. 2004), VMD, PyMol, Cresset, VegaZZ (Pedretti et al. 2004), Chemdraw (Mendelsohn 2004), mCule, Molinspiration and

Osiris Property Explorer tools were employed to evaluate and design novel molecules, potentially inhibiting PRODH. Similarly, mCule (Kiss et al. 2012), AutoDock tools and AutoDock Vina (Trott and Olson 2010) were used for molecular docking. A number of rotatable bonds, H-bond donors and H-bond acceptors were also analyzed by using Cresset, mCule, Molinspiration, and PubChem (Bolton et al. 2008). The Osiris Property Explorer were utilized to estimate the possible reproductive, mutagenic or tumorigenic risks and to calculate drug like properties of designed molecules. "Lipinski's rule of five" was analyzed by utilizing Cresset and mCule. The drug likeness values calculated by Osiris explorer are positive when fragments of designed molecules frequently found in approved drugs. The mCule, Cresset and Osiris explorer were employed to estimate mutagenesis of novel molecules.

The PRODH was docked in AutoDock Vina, AutoDock Tools and mCule to each of five molecules. Three novel molecules were screened by structure based virtual screening method by utilizing mCule. The aim of molecular docking analyses was to identify binding pattern and relative binding specificities. Properties of known SZ drugs were used for library screening and designing new molecules. There was no ligand reported for PRODH in literature and biological databases. The Molinspiration, Cresset, mCule and Osiris programs (Sander 2001) were employed to test drug like properties of novel designed molecules. The analyses assessed designed molecules solubility, Lipinski's rule of five, number of rotatable bonds, H-bonds donors, H-bond acceptors, molecular mass, log*P* values, mutagenicities, drug score, and toxicities.

The mCule library was also screened for structural virtual screening. The scrutinized drugs 2D structures were used and screened by mCule library. Pharmacophoremic screening of compounds was performed by using LigandScout (Wolber and Langer 2005). Known biomolecules and three compound libraries (ZINC, Drug, and DrugLike) were also screened against PRODH structure. Virtual screening was conducted by using LigandScout screening software. Pharmacophoric sites (positive and negative ionizable groups, hydrogen bond donor, hydrogen bond acceptor, hydrophobic sites, and aromatic ring) were analyzed. To incorporate all associated features of compounds, merge feature model generation and atom overlap scoring function were employed from ligand based module of LigandScout 3.0. The high pharmacophore score compounds were scrutinized and docking analyses were carried out on top hits to reveal their interactions by using AutoDock, which generated screening results in the .dlg format. Grid box was used to define screening site. The docked complexes were visualized in UCSF Chimera 1.6, Discover Studio and Ligplot.

The geometry optimization of designed molecules was performed by utilizing Vega ZZ, UCSF Chimera 1.6, and ChemDraw Ultra. Results were analyzed by using AutoDock tools, UCSF Chimera 1.6, Ligplot (Wallace et al. 1996), Discovery Studio and PyMol. The STRING and STITCH3 servers (Online database for known and predicted protein interactions including direct (physical) and indirect (functional) relationships) were also employed to assess protein interactions for PRODH. Protein docking of PRODH with its interactive protein ALDH4A was simulated by using PatchDock (Franceschini et al. 2013) and Gramm-X (Schneidman-Duhovny et al. 2005). The results were further refined by FireDock. Visual Molecular Dynamics (VMD) (Tovchigrechko and Vakser 2006) software, UCSF Chimera 1.6, Ligplot and PyMol software were used for protein-protein docking analyses.

Results and discussion

The research theme under investigation was based on PRODH association with SZ and its computational analyses for identifying novel inhibitors. Protein BLAST was subjected for the retrieval of suitable template. The five optimal aligned templates with E-value, identity and query coverage were retrieved (Table 1). The five scrutinized templates were employed for 3D structure prediction. Comparatively, template having accession number 4F9I showed 36 % query coverage and showed better evaluation results. The 3D model of PRODH was generated by homology modeling approach. The query coverage and identity were not satisfactory for the generation of 3D structure by comparative modeling approach.

The evaluation tools also validated that the predicted structures have errors and not satisfactory for further analyses. The threading approach and multiple templates comparative modeling approach were utilized for 3D structure prediction of PRODH. To build the 3D structures by employing various web servers (3D-jigsaw, Swiss Model, M4T, I-TASSER) and MODELLER 9.13 were employed. As the result, suitable model tends to propose the

Table 1 Five BLAST aligned templates of PRODH with E-value, query coverage and identity

Accession ID	Total score	Query coverage	E-value	Max-identity
4F9I	56.6	36 %	4e-08	27 %
3HAZ	38.1	42 %	0.019	23 %
ITJ2	36.2	35 %	0.074	26 %
1TIW	36.2	35 %	0.075	26 %
3ITJ	36.2	35 %	0.075	26 %

optimal structure of PRODH, evaluation tools were utilized for the comparison of predicted models. The 3D structure of PRODH (Fig. 1) was predicted by using various tools and selected on the basis of quality factor, spatial restraints, favored region, allowed region, and outlier region. Evaluation tools showed the reliability and efficacy of predicted structure. All the poor rotamers were corrected and outliers were removed for the refinement of predicted structure. Errat evaluation tool showed an overall quality factor of 78.547 % of selected final 3D structure.

Experimental work showed that the FDA approved drugs (Fig. 2) selected in this study have significant value to cure SZ (Leucht et al. 2013; Franza et al. 2013; Stroup et al. 2007). Molecular docking analyses of selected drugs revealed variation in their binding energies (Table 2). Initially, docking analyses were done with 100 runs, 10 poses were saved, out of which the best pose having lowest binding energy was chosen from each compound. The selected four compounds (Clozapine, Perphenazine, Haloperidol, and Chlorpromazine) (Fig. 3) showed high competency to cure the disease pathogencity.

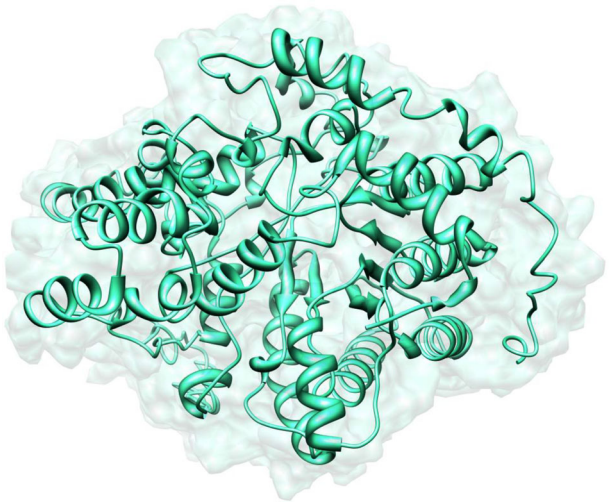

Fig. 1 PRODH predicted structure selected from all the generated structures

Clozapine, Perphenazine, Haloperidol, and Chlorpromazine were analyzed on the basis of their binding energy and drug properties (Table 2). All the selected drugs have tricyclic molecules with significant biological properties and considered as potential anti-schizophrenic agents. Clozapine showed lowest binding energy among all the compounds and showed good ligand properties. mCule library was used to screened the drug compounds. The compounds showed highest similarity score (>0.85) were selected and docked against PRODH. The 30 hits for each compound was retrieved form mCule database of structure sharing structural identity. PRODH was docked by utilizing AutoDock tools, AutoDock Vina, and mCule docking softwares.

The least variation was found among the interacting amino acid residues of PRODH complexes from utilized docking software's. The structurally screened compounds (Fig. 4) having binding energies ranging from −8.7 to −8.6 kcal/mol. The screened compounds (MCULE-1620364835-0, MCULE-5409244446-0 and MCULE-8538288307-0) had lower binding energy than selected drugs and also showed fine drug likeness. The analyzed compounds could be synthesized to cure the SZ as these compounds showed better drug targets as compare to already in used drugs.

Fig. 2 2D structures of selected drugs

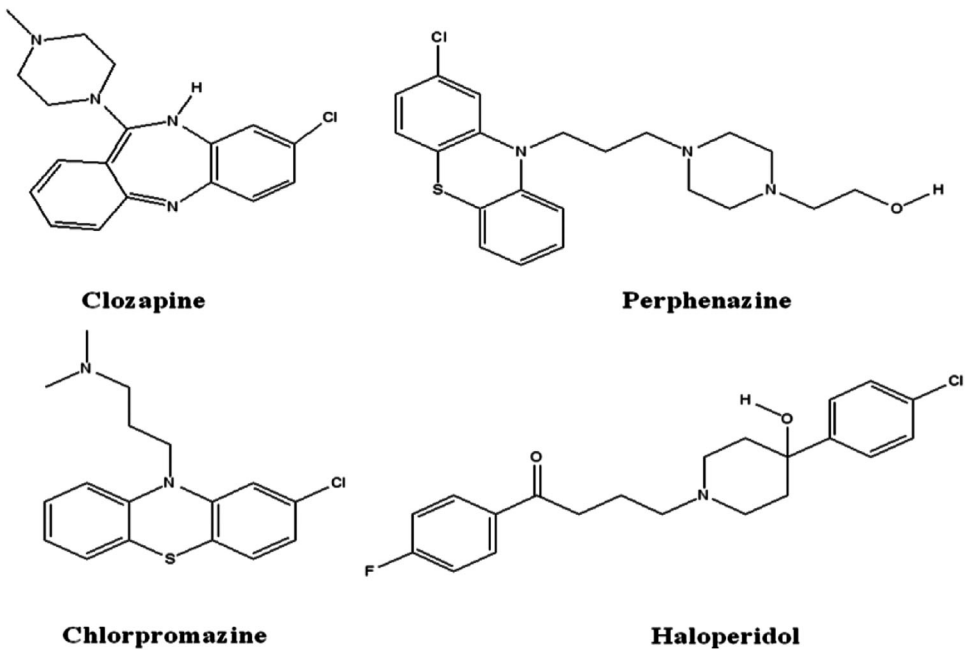

Table 2 Compounds investigating in this study

Ligand properties	Clozapine	Perphenazine	Haloperidol	Chlorpromazine
IUPAC name	8-Chloro-11-(4-methylpiperazin-1-yl)-5H-dibenzo[b,e][1,4]diazepine	2-[4-[3-(2-chloro-10H-phenothiazin-10-yl) propyl] piperazin-1-yl]ethanol	4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one	3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-propan-1-amine
Estimated free energy of binding (kcal/mol) (AutoDock4)	−5.85	−5.41	−5.09	−5.05
Estimated free energy of binding (kcal/mol) (AutoDock-Vina)	−8.5	−7.5	−7.8	−7.7
Estimated inhibition Constant, Ki (μM)	51.79	108.12	184.82	200.32
Final intermolecular Energy (kcal/mol)	−6.15	−7.5	−7.18	−6.24
Ligand efficiency	−0.25	−0.2	−0.2	−0.24
Torsional free energy (kcal/mol)	0.3	2.09	2.09	1.19
Unbound system's energy (kcal/mol)	−0.24	−0.21	−0.61	0.01
Molecular weight	325.83	403.0	375.0	318.0
log*P*	4.15	3.88	3.97	4.95
Hydrogen bond acceptor	3	4	3	2
Hydrogen bond donor	1	1	1	0
Rotatable bonds	1	6	5	4
Rule of five (violation)	0	0	0	0
Clog*P*	3.0	4.55	4.63	5.03
Solubility	−3.74	−4.16	−4.7	−4.8
Drug likeness	8.7	11.84	12.32	8.38
Drug score	79 %	60 %	57 %	55 %
Binding residues (AutoDcok)	Thr-44, Pro-48, Val-50, Phe55, Gly-56, Phe-136, Val-138, Leu-528, Asp-532, Ser-535	Glu-121, Asp-122, Gln-123, Glu-124, Tyr-144, Thr-199, Tyr-200, Glu-205, Gln-246, Phe-247, Val-250, Pro553	Asp-122, Val-194, Thr-199, Tyr-200, Asn-204, Glu-205, Gln-246, Phe-247	Thr-44, Pro-48, Val-50, Phe55, Tyr-133, Phe-136, Ala-262, Glu-264, Leu-528, Phe-536
Binding residues (AutoDcok Vina)	Thr-44, Pro-48, Val-50, Ala-52, Phe-55, Glu-60, Arg-66, Phe-136, His-259, Ala-262, Val-263, Glu-264, Gln-265, As-532, Phe-536	Val-50, Phe-55, Glu-60, His-116, Phe-136, Ala-262, Val263, Glu-264, Asp-532, Phe-536	Asp-122, Val-194, Thr-199, Tyr-200, Asn-204, Glu-205, Phe-247, Val-250, Trp-254	Thr-44, Pro-48, Val-50, Phe55, Gly-56, Phe-136, Val-138, Leu-528, Asp-532, Ser-535, Phe-536

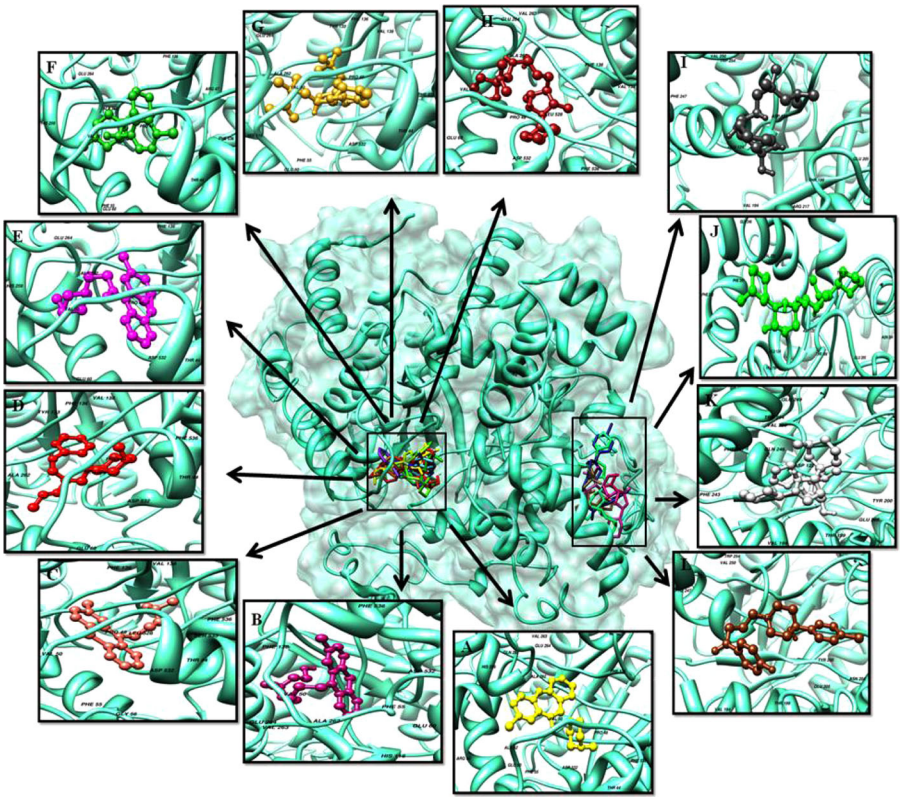

Fig. 3 Binding residues of selected drugs **a** Clozapine **b** Perphenazine **c** Chlorpromazine **d** MCULE-1620364835-0 **e** MCULE-5409244446-0 f MCULE-8538288307-0 **g** PB-236790866 **h** PB-752728400 **i** PB-1162540860 **j** PB-608533672 **k** PB-353794414 **l** Haloperidol, interacting residues targeting with PRODH

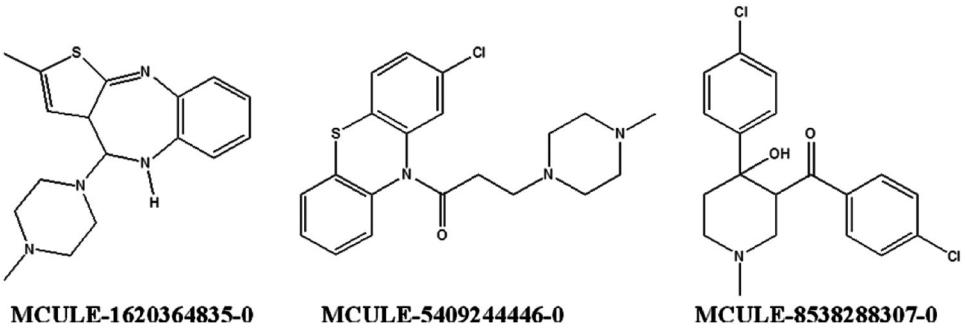

Fig. 4 2D structures of structurally based virtual screening novel compounds

The libraries (ZINC, Drug, and DrugLike) screened by employing LigandScout. The designed pharmacophore have the common properties of selected analyzed drugs. Furthermore, the Drug and DrugLike library query for pharmacologically related compounds yielded 664 and 183 compound hits respectively. After screening selected libraries, the top five compounds having highest pharmacophore score were chosen for docking analyses done by AutoDock tools and AutoDock Vina.

An observation was observed that compounds shared structurally identity had lower binding energies (Table 3) on average than those that were obtained from the pharmacologically based query, whilst the difference in binding energy is not so large. The five lowest binding energy compounds from selected libraries were scrutinized for further analyses. It was also observed that there was slight variation in top five lowest binding energies for compounds in the pharmacologically based analysis with a variation of −1.80 kcal/mol between lowest and highest ranked compounds. Perhaps, the stability of the compounds may be due to the pharmacological properties maintenance, allowing more conserved binding affinities. The drug properties, docking analyses and pertinent information for selected compound (Table 4) have best pharmacophore hits from selected screened libraries.

It was observed that the docked structure of PRODH with other inhibitors showed reliable results. This was the good indication that parameters used in docking analyses had lead to relatively accurate results. In a struggle to investigate the best compounds, top five (Fig. 5) docked

Table 3 Ligand properties and docking analysis of structurally screened compounds

Ligand properties	MCULE-1620364835-0	MCULE-5409244446-0	MCULE-8538288307-0
Estimated free energy of binding (kcal/mol) (AutoDock4)	−6.57	−6.46	−5.27
Estimated free energy of binding (kcal/mol) (AutoDock-Vina)	−8.7	−8.7	−8.6
Estimated inhibition constant, Ki (μM)	15.31	18.52	136.14
Final intermolecular energy (kcal/mol)	−6.87	−7.35	−6.47
Ligand Efficiency	−0.29	−0.25	−0.22
Torsional free energy (kcal/mol)	0.3	0.89	1.19
Unbound system's energy (kcal/mol)	−0.11	−0.53	−0.53
Molecular weight	312.0	387.26	363.0
log*P*	1.19	4.04	3.95
Hydrogen bond acceptor	4	4	3
Hydrogen bond donor	1	0	1
Rotatable bonds	1	4	3
Rule of five (violation)	0	0	0
Clog*P*	2.09	4.67	4.02
Solubility	−2.82	12.06	−4.44
Drug likeness	8.76	11.84	9.06
Drug score	88 %	59 %	65 %
Binding residues (AutoDock4)	Glu-121, Asp-122, Glu-124, Val-194, Glu-205, Phe-247, Glu-249, Val-250, Lys-253, Trp-254, Pro-553	Asp-122, Glu-124, Val-194, Ala-197, Thr-199, Tyr-200, Phe-243, Gln-246, Phe-247, Val-250	Glu-121, Asp-122, Tyr-144, Val-194, Tyr-200, Glu-205, Phe-247, Val-250, Lys-253, Trp-254, Pro553
Binding residues (AutoDock-Vina)	Thr-44, Pro-48, Glu-60, Tyr-133, Phe-136, Val-138, Ala-262, Leu-528, Asp-532, Phe-536	Thr-44, Pro-48, Val-50, Glu-60, Phe-136, Ala-262, Glu-264, His-259, Asp-532	Thr-44, Arg-47, Pro-48, Val-50, Phe-55, Glu-60, Phe-136, His-259, Ala-262, Glu-264, Phe-536

Table 4 Ligand properties and bioinformative detail of top ten screened compounds of present study

Ligand properties	PB-353794414	PB-608533672	PB-236790866	PB-752728400	PB-1162540860
Estimated free energy of binding (kcal/mol) (AutoDock4)	−4.81	−4.25	−3.73	−4.17	−4.71
Estimated free energy of binding (kcal/mol) (AutoDock-Vina)	−7.3	−7.8	−6.8	−8.1	−6.3
Estimated inhibition Constant, Ki (μM)	299.92	764.98	1.85	874.45	352.25
Final intermolecular energy (kcal/mol)	−7.49	−6.94	−7.31	−7.16	−7.69
Ligand efficiency	−0.16	−0.15	−0.14	−0.14	−0.22
Torsional free energy (kcal/mol)	2.68	2.68	3.58	2.98	2.98
Unbound system's energy (kcal/mol)	0.11	−0.55	−0.1	0.33	−0.53
Molecular weight	436.67	414.65	393.63	382.58	295.80
logP	3.62	2.48	4.12	2.94	1.09
Hydrogen bond acceptor	6	6	6	6	6
hydrogen bond donor	3	4	5	3	4
Rotatable bonds	8	7	9	9	6
Rule of five (violation)	0	0	0	0	0
Clog*P*	0.61	0.13	−3.7	0.33	−2.59
Solubility	−3.4	−2.66	−2.5	−2.84	−0.86
Drug likeness	−0.82	2.4	2.05	4.46	3.93
Drug score	50 %	79 %	81 %	85 %	94 %
Binding residues (AutoDock4)	Glu-121, Asp-122, Tyr-200, Asn-204, Glu-205, Val-250, Lys-253, Trp-254, Pro-553, Glu-556, Met-5655	Asp-122, Val-194, Thr-199, Tyr-200, Asn-204, Glu-205, Val-250, Lys-253	Glu-147, Glu-148, Leu-150, Tyr-446, Pro-559, Ser-562, Arg-563, Leu-566	Glu-121, Asp-122, Val-194, Thr-199, Tyr-200, Asn-204, Glu-205, Gln-246, Phe-247, Trp-254	Glu-121, Asp-122 Val-194, Thr-199, Ala-197, Tyr-200, Asn-204, Glu-205, Arg-217, Phe-247, Trp-254, Pro553
Binding residues (AutoDock Vina)	Asp-122, Val-194, Thr-199, Tyr-200, Glu-205, Phe-243, Gln-246, Phe-247, Glu-249, Val-250, Trp-254	Asp-122, Glu-124, Val-194, Thr-199, Tyr-200, Asn-204, Glu-205, Phe-243, Gln-246, Phe-247, Val-250	Thr-44, Pro-48, Val-50, Phe-55, Glu-60, His-116, Tyr-133, Phe-136, Val138, Ala-262, Glu-264, Leu-528, Asp-532, Phe-536	Thr-44, Pro-48, Val-50, Glu-60, Phe-136, Val-138, Ala-262, Val-263, Glu-264, Leu-528, Asp-532, Phe-536	Asp-122 , Glu-124, Val-194, Thr-199, Glu-205, Arg-217, Phe-247, Val-250, Trp-254

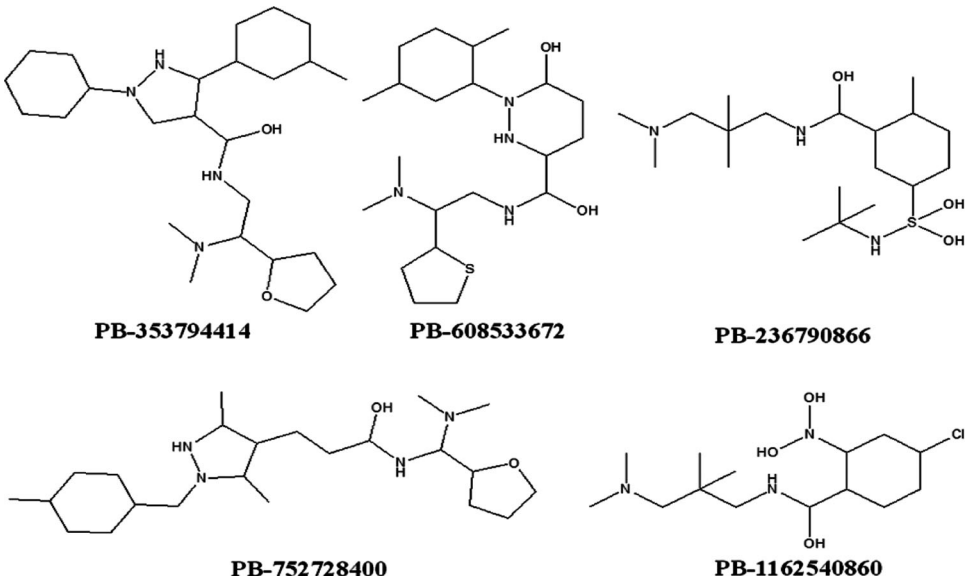

Fig. 5 2D structures of pharmacophore based virtual screening novel compounds

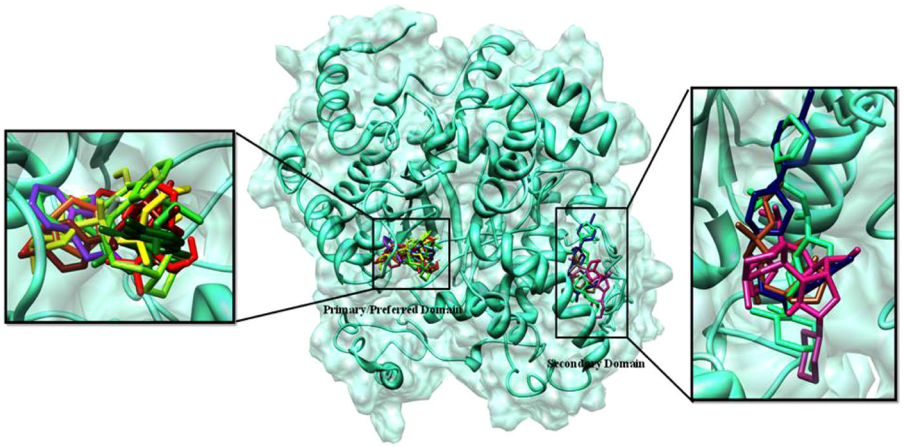

Fig. 6 The ligands (selected drugs, structure-based novel compounds and pharmacophore based) compounds showed binding pocket and interaction on same region

complexes from a combination of selected libraries were explicated (Table 4). It was also analyzed and observed that majority of compounds showed binding between Thr-44 and Phe-536 (Fig. 6).

The analyzed drugs showed interactions at same binding region. The docking analyses were performed by different tools showed that known and novel compounds bind on about same region and revealed the binding pocket. It is also possible that the combination of these binding residues leads to the lower binding energy achieved in this work.

The highest ranked ligand from selected libraries were elucidated, namely MCULE-1620364835-0 from structural based ligand screening and PB-752728400 from pharmacological based screening. Two binding pockets were observed in this experiment and both the pockets showed the potential interacting residues. More ligands showed interaction in primary binding pocket (Thr-44, Pro-48, Val-50, Glu-60, Phe-136, Ala-262, Glu-264, Asp-532 and Phe-536) and one selected drug and pharmacophore based ligands also showed binding in secondary binding pocket (Asp-122, Glu-124, Val-194, Glu-205, Tyr-200, Phe-247, Val-250, Lys-253, Trp-254 and Pro-553). Both the binding domains flaunted effective binding interactions with all the docked ligands.

In a struggle to understand the occurring of better interactions between amino acid residues and ligands in the active site, a plot of ligand-amino acids interactions were generated by employing Ligplot and UCSF Chimera 1.6. The ligplot and UCSF Chimera 1.6 analysis of Clozapine and all the three structural based pharmacophore generated molecules bound to active site presented that the ligand was cradled by numerous amino acids residues.

All the analyzed compounds have no mutagenic, tumorigenic, irritant and reproductive effects. PRODH interacting partners were retrieved from STRING database (Fig. 7). ALDH4A1 showed highest result and closest

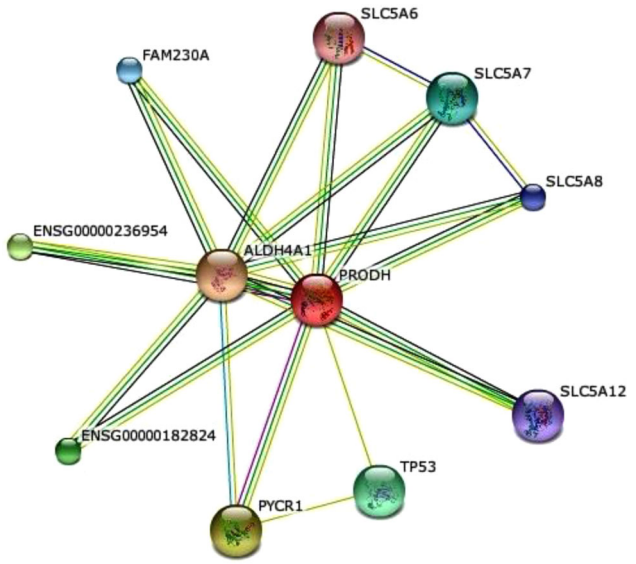

Fig. 7 Interaction partners of PRODH by STRING database. ALDH4A showed highest score of 0.943

interacter of PRODH. The interaction between PRODH and ALDH4A (Table 5) were visualized by Ligplot and UCSF Chimera 1.6 (Fig. 8).

ALDH4A, the interacting partner of PRODH and the protein-protein and the ligand-protein molecular docking analyses were performed separately to check the residual involvement. The docked complex predicts the interacting residues and their importance. The protein-protein docking analyses were performed and analyzed on the basis of approximate interface area of complex and Atomic Contact Energy (ACE) by utilizing PatchDock. The 200 ALDH4A-PRODH complexes were analyzed on the basis of ACE and top 10 complexes having least ACE values were scrutinized for further refinement and analyses by employing FireDock.

The complexes analyzed on the basis of least binding global energy, Attractive and Repulsive VdW (the contribution of the van der Waals forces to the global binding energy), ACE and HB (the contribution of the hydrogen bonds to the global binding energy).

The selected ALDH4A-PRODH complexes showed the least global binding energy and considered reliable for further analyses. The least binding values suggested that ALDH4A and PRODH have effective binding affinity due to which the complex may has capacity to regulate the down expression. The protein-protein also showed the common residues of primary and secondary binding pockets (Fig. 8).

In current research, an integrated approach was used based on structural modeling, pharmacophore identification, docking analyses, and virtual library screening were carried out. The predicted model suggested that structure predicted with good degree of accuracy, especially concentrate at the active site. The docking results satisfied the least docking

Table 5 Tabular form of binding interactions between PRODH and ALDH4A

Receptor protein	Interacting protein	Interactions (Receptor protein→Interacting protein residues)
PRODH	ALDH4A	Thr-305→Leu-30, Glu-304→Leu-30, Arg-47→Ser-28, Thr-305→Lys-31, Glu-304→Ser-28, Pro-38→Arg-23, Gln-540→His-26, Phe-301→Ser-29, Ile-233→Arg-247, Gly-39→Trp-24, Gly-39→Lys-25, Thr-305→Ser-29, Gly-307→Leu-129, Gly-307→Leu-126, Gly-307→Leu-30, Ile-233→Arg-250, Ile-233→Glu-72, Ala-272→Pro-256, Ala-272→Pro-255, Thr-275→Asp-70, Phe-301→Ser-28, Phe-301→Leu-30, Glu-304→Ser-29, Glu-304→Val-178, Arg-47→Ile-121, Arg-47→Thr-27, Gln-265→Leu-118, Gln-265→Glu-115, Ser-309→Asp-133, Ser-309→Leu-129, Ser-309→Lys-130, Phe-136→Asp-123, Phe-136→Pro-120, Arg-240→Glu-72, Arg-240→Glu-71, Leu-306→Val-32, Leu-306→Arg-171, Leu-306→Lys-31, Leu-306→Leu-30, Leu-306→Leu-129, Pro-51→Leu-118, Pro-51→Gln-545, Ala-237→Glu-72, Ala-286→Pro-256, Ala-268→Pro-255, Ala-268→Ala-112, Lys-276→Glu-251, Lys-276→Arg-250, Pro-38→Arg-23, Pro-38→Trp-24, Val-308→Glu-126, Leu-244→Glu-71, Leu-244→Asp-70, Val-50→Leu-118, Ala-35→Trp-24, Val-46→Glu-181, Val-146→Glu-183, Gly-266→Glu-115, Gly-266→Trp-116, Pro-49→Val-196, Pro-49→Tyr-197, Gln-267→Glu-115, Phe-230→Glu-251, Met-273→Gly-253, Ala-271→Pro-256, Ala-271→Asp-70, Gly-269→Pro-255, Ser-42→Lys-25, Gly-310→Asp-133, Val-53→Ile-547, Val-53→Val-196, Ala-43→Lys-25, Pro-48→Pro-120, Ala-541→His-26, Glu-264→Lys-119, Glu-264→Glu-115, Ala-303→Leu-30, Ala-36→Trp-24, Thr-236→Asp-70, Asp-54→Ile-547, Asp-54→Glu-549, Gly-229→Arg-247, Ala-135→Glu-126, Ala-52→Leu-118

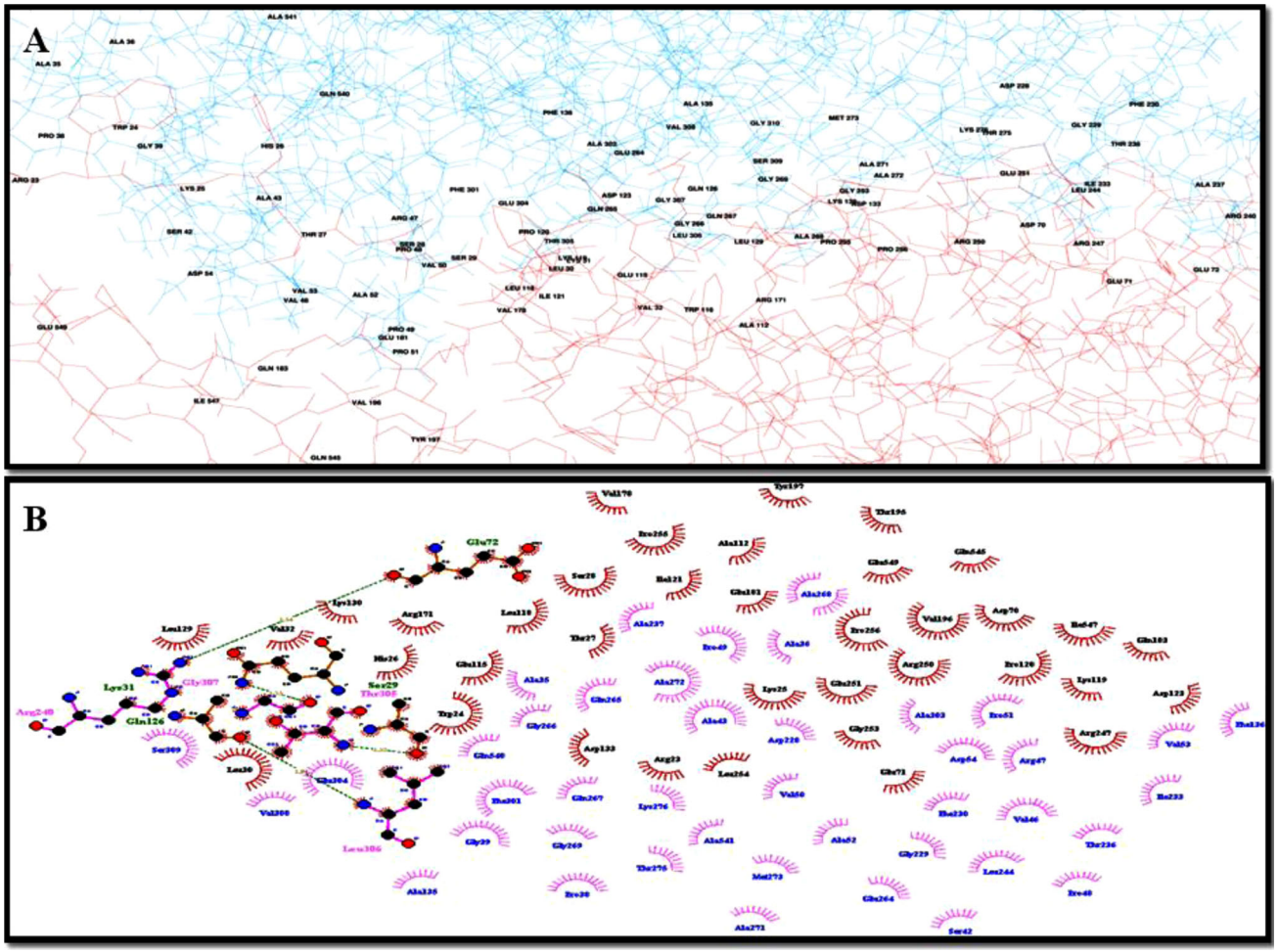

Fig. 8 Interaction results between PRODH and ALDH4A by UCSF Chimera 1.6 and Ligplot

energy, toxicity, drug score Lipinski's rule of five, and suggested that Clozapine (8-Chloro-11-(4-methylpiperazin-1-yl)-5H-dibenzo [b, e][1, 4] diazepine) may proved as a potential drug molecule for SZ treatment by targeting PRODH. An interesting fact was that all scrutinized compounds collectively had lowest binding energy after analysis from selected libraries. The novel identified compounds in current research may have the tendency of good drug candidates for the treatment of SZ. The most interacting amino acid identified through docking may be highly effective in future studies by employing site-directed mutagenesis techniques. The proposed strategy lead to simplify the process of novel drug designing as biological investigation and drug analysis can be carried out on candidates with good results.

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Compliance with ethical standards

Conflict of interest Author has no conflict of interest.

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