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Research Article

IDENTIFICATION OF POTENTIAL INHIBITORS FOR LOWERING CHOLESTEROL LEVEL BY INHIBITING PROPROTEIN CONVERTASE SUBTILISIN KEXIN 9

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ABSTRACT

Objective: Proprotein convertase subtilisin kexin 9 (PCSK9) has medical significance in lowering cholesterol levels. Inhibitors target and inactivate PCSK9 in the liver. Knocking out PCSK9 reduces the amount of harmful low-density lipoprotein cholesterol (LDL-C) circulating in the bloodstream. There are two known inhibitors for treating the cardiovascular disease "Arilocumab" and "Evalocumab." These drugs have many side-effects; therefore, there is a need for new drug with less or no side effect. The current study is to identify natural and synthetic inhibitor using the pharmacophoric feature of the known inhibitor and validating the shortlisted candidates using molecular dynamics (MD) and absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties.

Methods: Known inhibitors for the PCSK9 protein were taken from the BINDING database. Molecular docking was performed for the known inhibitors with the PCSK9 protein. After docking, the best inhibitor was selected, and the docking result was then imported to find the pharmacophoric features.

Results: The pharmacophore model was generated with three features containing 1 hydrogen bond acceptor (A), 1 hydrogen bond donor (B), and 1 aromatic ring. The constructed e-pharmacophore model was screened with more than 20,000 natural compounds. Five compounds were shortlisted. Among them, ZINC85625485 has glide score of −13.03 kcal/mol with glide energy was −57.62 kcal/mol and ZINC85625406 has glide score of −8.1 kcal/mol with glide energy was −39.33 kcal/mol were taken as the best hits.

Conclusion: PCSK9 is known to be a therapeutic agent as it controls the plasma LDL-C levels by post-translational regulation of the LDL receptor. Therefore, up-regulation of PCSK9 can lead to elevated cholesterol level in such case inhibition of PCSK9 will be an effective remedy. In this study, already known inhibitors were taken and pharmacophore feature was generated. Zinc database was screened to find out novel compounds with similar pharmacophore features that can act as potentially active compound against PCSK9. ZINC85625485 and ZINC85625406 were shortlisted as lead compounds with MD simulation and checking the ADMET properties.

Keywords: Proprotein convertase subtilisin kexin 9, Docking, Absorption; distribution; metabolism; excretion; and toxicity, Molecular dynamics.

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INTRODUCTION

Proprotein convertase subtilisin kexin 9 (PCSK9) is the family of proprotein convertases. The main function of PCSK9 is to activate other protein by cleaving the sections of peptide chains. This protein gets processed by an autocatalytic cleavage of its N-terminal prosegment that remains associated with the catalytic domain. PCSK9 contains an N-terminal signal peptide, followed by a pro-domain, catalytic domain (subtilisin-like), and a C-terminal domain. The prodomain serves as a chaperone for folding and as an inhibitor of catalytic activity. The C-terminal domain is predicted to mediate protein-protein interaction [1]. Individuals with PCSK9 down-regulation show low low-density lipoprotein-cholesterol (LDL-C) levels, whereas those with upregulation show increased plasma LDL-C levels [2]. PCSK9 is known to be a therapeutic agent as it controls the plasma LDL-C levels, which is done during post-translational regulation of the LDL receptor (LDLR) [3]. PCSK9 circulates in the blood and conjugates with the extracellular domain of the LDLR, which initiates the receptor degradation. PCSK9 is articulated the maximum level in the liver, small intestine, kidney, and brain and is regulated by the same mechanism that modulates expression of the LDLR [4]. By reducing the level of LDL-C circulating in the bloodstream, it can decrease the risk of cardiovascular disease such as heart attacks. The observation that PCSK9 lowers the hepatic LDLR postulates the hypothesis that PCSK9 is the reason behind LDLR degradation. A study showed that the inactive or dead PCSK9 protein retained the ability to lower LDLR levels [5]. Lower LDL is the main reason for healthier arteries and reducing the rate of heart attacks, strokes, and other problems related to cholesterol-clogged arteries. Many studies have been conducted on lowering cholesterol levels; however, these studies focused on new drugs and did not target PCSK9 [6-8]. Therefore, in this study, we try to inhibit PCSK9, which in turn will lower the cholesterol level.

Here, we focus on identifying the potential inhibitors against the target protein PCSK9. Molecular docking was performed with known inhibitors to identify the pharmacophoric feature. These pharmacophoric features were used for screening of 20000 natural compounds taken from zinc database. High-throughput virtual screening (HTVS) was performed to shortlist the best candidates. The shortlisted lead compounds were taken for molecular dynamics (MD) study and ADME properties. The flowchart for the study is given in Fig. 1.

METHODS

Ligand preparation

For molecular docking study, known inhibitors from binding database were downloaded and are prepared using the "Ligprep" module [9] implemented in the Maestro program of the Schrodinger software. The ligand structures are desalted and are optimized using the OPLS2005 force field [10,11].

Protein preparation

The crystal structure of PCSK9 protein target was obtained from RCSB protein data bank (PDB Id: 2P4E). A chain from crystal structure was

used for protein preparation. Protein was prepared using the protein preparation wizard workflow of Schrodinger 9.2v for the three-dimensional (3D)-structures of proteins by removing water molecules, adding hydrogen's, assigning atom and bond types, and a refinement by completing missing side chains. After implementing necessary corrections to the structure, the protein was minimized using OPLS 2005 force field using heavy atom convergence of 0.5 Å [12].

Active site region
The active site of the model was analyzed to assess the presence of catalytic and conserved substrate binding residues. Active site was identified using SiteMap Module [13,14]. SiteMap was used to estimate the location of the active site, generating hydrophobic and hydrophilic contour maps of the protein, and calculating energy potentials.

Receptor grid generation
After protein preparation and Sitemap, receptor grids were generated by specifying the binding site with a 3D cubic box. Grid files represent physical properties of a volume of the receptor that are searched while performing docking [15]. Enclosing box of x: −39.84, y: −8.58, and z: −2.61 was placed depending on SiteMap prediction. Based on the fact that the binding site is not shallow, the non-polar atoms were removed, by choosing the van der Waals radius scaling factor of 0.75 for non-polar parts, this will help in ligand binding. Rotation of all receptor hydroxyl and thiol groups within the grid was allowed.

Virtual screening of small compounds
HTVS is typically done to check a library of compounds for potential activity against the chosen target. Here, structure-based screening is done to find the binding mode of the protein target. The compounds from the phase dataset are taken and screened with target protein based on Qikprop and Lipinski rule of five. It screened the compounds by considering physiochemical properties and drug-like properties such as MW <500, hydrogen bond donor <5, hydrogen bond acceptor <10, and logP <5. The hit compounds can be marked and ranked according to the fitness score which should be in the range from 0 to 3 and docking score was calculated [16,17].

Prediction ADME properties
The QikProp program was employed to obtain ADME properties of the compounds. Physically significant descriptors such as partition coefficient, van der Waals surface, aqueous solubility, and pharmaceutically relevant properties for small molecules. All of the compounds were neutralized before being used by QikProp. The program was processed in normal mode and predicts physiochemical properties and principal descriptors, along with a detailed analysis of log P (octanol/water), QP%, SASA, % oral absorption, and log HERG. It also checks the acceptability of the compounds based on Lipinski's rule of five, which is crucial for rational drug design [18].

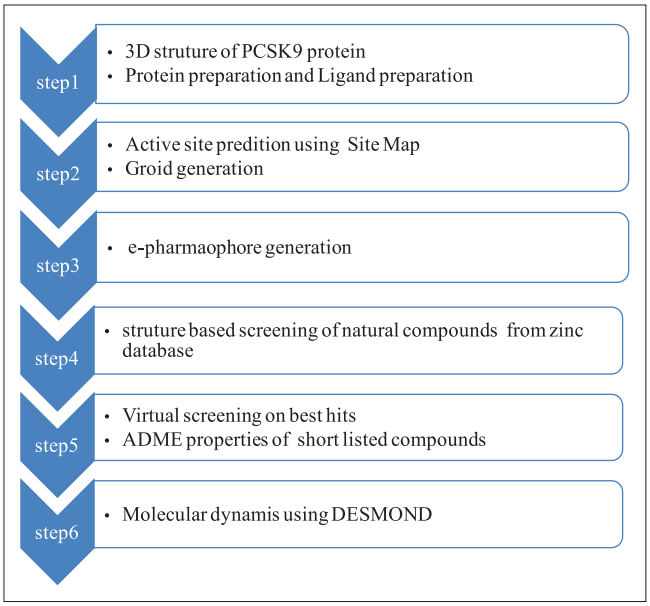

Fig. 1: Flow chart for identifying potential inhibitor against proprotein convertase subtilisin kexin 9

RESULTS AND DISCUSSION

Prediction of active site
The prepared protein was used for to find the possible binding sites of PCSK9 were searched using binding site prediction software SiteMap. This software generates information on the binding site's characteristics using novel search and analytical facilities. 5 sites were generated, and the comparative study revealed that Site 2 has the best score (Table 1). Site 2 had the active site residues shown in Fig. 2. The above-identified active site was chosen as the most favorable site for docking studies. Grid was generated using these amino acid residues.

Molecular docking of binding database
Four known inhibitors were taken from binding DB and docked with PCSK9. Among the four ligands, BDBM50014066 shows better interaction with PCSK9 (Table 2). BDBM50014066 had glide score −7.6 kcal/mol (Fig. 3), and this compound has been taken for e-pharmacophore generation.

Structure-based pharmacophore generation
The binding pose of BDBM50014066 was given as an input for e-pharmacophore generation. Three pharmacophore features selected are 1 acceptor (A1), 1 donor (D6), and 1 ring aromatic (R8). NDR was chosen as the best pharmacophore. These energetically favorable sites encompass the specific interactions of junction peptides and the PCSK9 protein, and this information should prove helpful in the development of new PCSK9 inhibitors. The score, distance, and angle between the pharmacophore sites is given in Table 3 and shown in Fig. 4. The pharmacophoric features were then taken for the screening of known compounds with the ZINC database compounds. The option "find matches to the hypothesis" is used. Thousand hits with pharmacophoric features identical to the known ligand were shortlisted.

Table 1: Active site's predicted by sitemap

Title	Site score	Size	D score	Volume	Exposure
SiteMap_2_Site_2	1.033	313	1.034	590.646	0.468
SiteMap_2_Site_1	1.029	463	0.95	1111.32	0.493
SiteMap_2_Site_3	0.95	80	0.972	232.897	0.649
SiteMap_2_Site_4	0.862	59	0.856	297.724	0.663
SiteMap_2_Site_5	0.706	34	0.666	87.808	0.507

Table 2: Docking results of binding database

Binding DB Id	XP score (Kcal/mol)	Glide energy (Kcal/mol)	XP H-bond
BDBM5028216	−4.96818	−27.1604	−1.14034
BDBM50067889	−5.29299	−31.8286	−0.55042
BDBM50014066	−7.62705	−45.5613	−1.27456
BDBM266996	−6.53684	−45.3158	−1.08332

Table 3: Score and distance between the pharmacophoric features (ADR)

Rank	Feature	Score	Site 1	Site 2	Distance
1	A1	−0.67	A1	D6	9.77
2	D6	−0.55	A1	R8	5.59
3	R8	−0.92	D6	R8	4.73

ADR: Acceptor, Donor and Ring aromatic

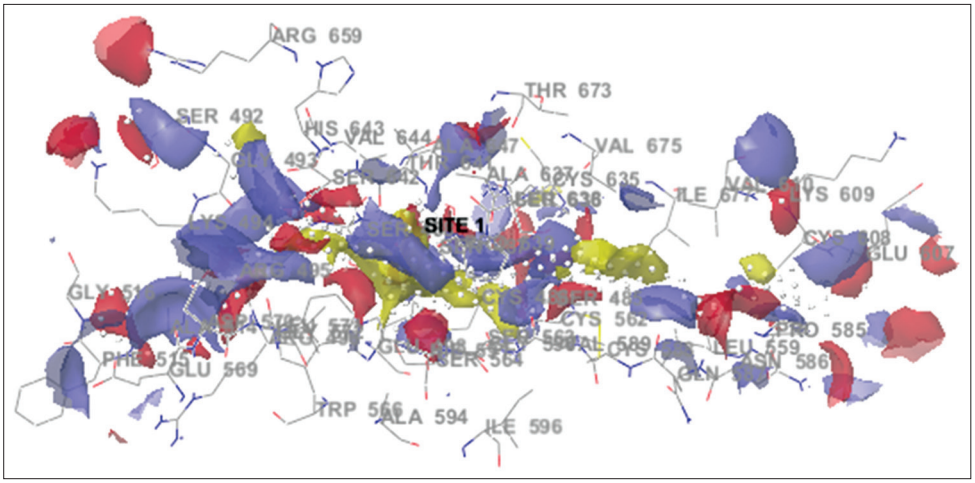

Fig. 2: Active site (Sitemap_site_2) predicted with SiteMap

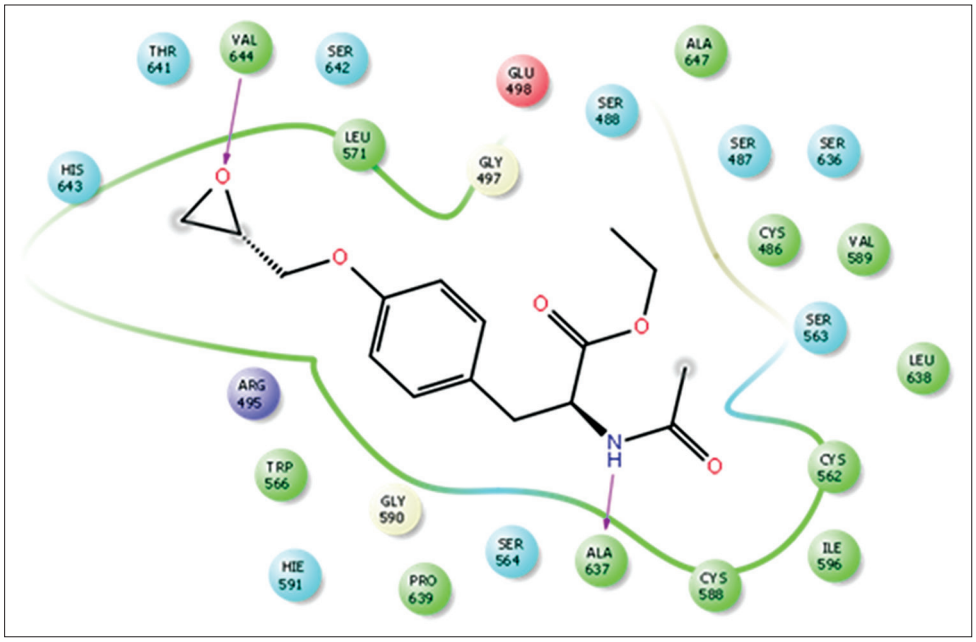

Fig. 3: Docking interactions of BDBM50014066 with the active site of proprotein convertase subtilisin kexin 9 protein

Structure-based screening of natural compounds

Around 20,000 natural compounds from zinc database were screened based on the pharmacophore hypothesis generated (A1, D6, R8). The compounds with fitness score above 1.5 were taken for further docking studies as mentioned in Table 4.

Table 4: Structure-based screening of zinc natural compounds

Title	Fitness score
ZINC34237065	2.30
ZINC01530713	2.26
ZINC20464408	2.20
ZINC71404899	2.19
ZINC20464414	2.16
ZINC20464417	2.16
ZINC20464397	2.15
ZINC20464404	2.15
ZINC20464420	2.14

Virtual screening on best hits

The structurally matched ligands were then taken for the HTVS. The virtual screening workflow option is performed for pre-filtering ligands. The virtual screening options for HTVS, SP, and Glide XP docking were all checked to be executed. A total of 5 ligands were obtained from virtual screening (Table 5).

ZINC85625485 has glide score of −13.03 kcal/mol with glide energy was −57.62 kcal/mol, and ZINC85625406 has glide score of −8.1 kcal/mol with glide energy was −39.33 kcal/mol (Fig. 5).

Analysis of ADME properties

The QikProp program was used to obtain drug-likeness properties of the compounds. It predicts pharmaceutically relevant properties such as physiochemical properties, log P (octanol/water), and the percentage of oral absorption. It also evaluated the acceptability of the compounds based on Lipinski's rule of five, which is essential for rational drug design. Out of 5 shortlisted compounds, two natural compounds ZINC85625485 (42.635%) and ZINC85625406 (35.465%) show higher human oral absorption (Table 6). These two compounds were taken for MD study.

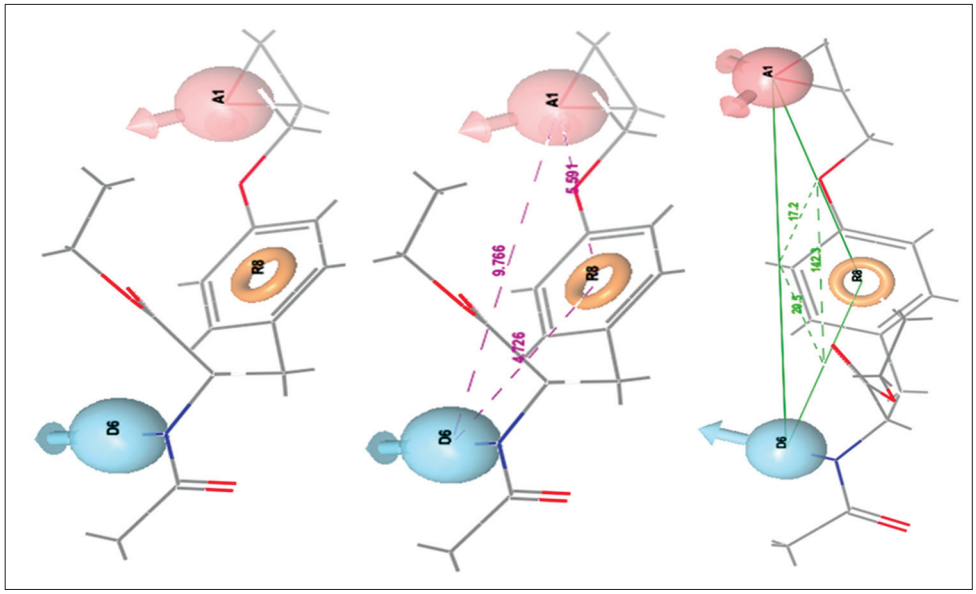

Fig. 4: (a) Three pharmacophore features developed for proprotein convertase subtilisin kexin 9 (PCSK9) (Acceptor, Donor and Ring aromatic [ADR]), (b) distance between the three pharmacophore features developed for PCSK9 (ADR), (c) angle between the three pharmacophore features developed for PCSK9 (ADR)

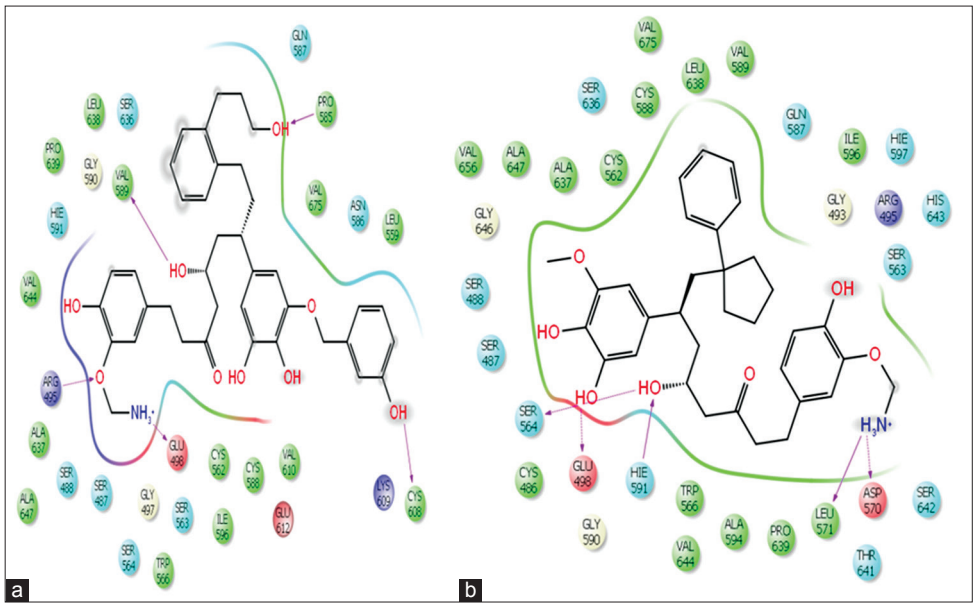

Fig. 5: Molecular interactions of ZINC85625485 (a) and ZINC85625406 (b) with proprotein convertase subtilisin kexin 9

Table 5: Docking results of shortlisted compounds

Title	XP G score (kcal/mol)	Glide energy (kcal/mol)	Glide emodel (kcal/mol)	XP H-bond (kcal/mol)
ZINC85625485	−13.03749	−57.629837	−91.670604	−3.264457
ZINC85625523	−12.925718	−79.264685	−119.027808	−3.629028
ZINC31167448	−12.348004	−72.306324	−89.342669	−5.058737
ZINC85625406	−12.583352	−77.525799	−111.048505	−2.591186
ZINC85625489	−12.428332	−72.79603	−124.659648	−5.78483

MD using DESMOND

MD simulation predicts molecular interactions such as hydrogen bonds between amino acids and substrate. Desmond is used to perform MD. MD simulation results show that the conformation derived from docking is basically consistent with the average structure extracted from MD simulation. When predicting the binding orientation of ligand to the binding site, docking results can provide calculations for predicting the binding affinity of the molecules and to assess the permanence of the predicted interactions involved in binding.

Root-mean-square deviation (RMSD) was calculated for the complex backbone atoms over the 2 ns of MD simulation. 2P4E complex with ZINC85625485 RMSD was stable and was within 0.8-1.6 Å (Fig. 6a).

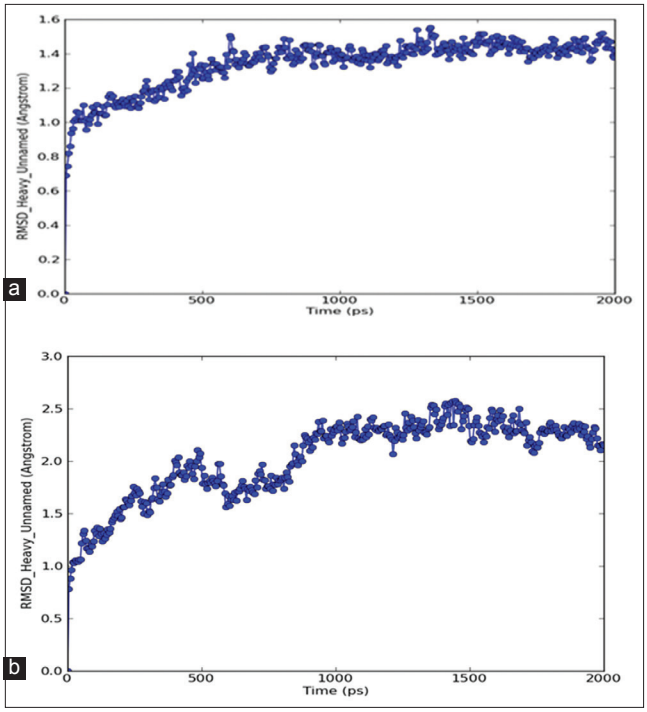

Fig. 6: (a) Root-mean-square deviation (RMSD) graph of 2P4E complex with ZINC85625485, (b) RMSD graph of 2P4E complex with ZINC85625406

Table 6: ADME properties if shortlisted compound

Title	Molecular weight (KDa)	Donor HB	Acceptor HB	% Human oral absorption
ZINC85625485	563.689	5	7.45	42.635
ZINC85625523	463.527	6	9.15	31.978
ZINC31167448	504.935	6	12.3	5.219
ZINC85625406	659.775	7	9.9	35.467
ZINC85625489	497.544	6	8.2	25.394

ADME: Absorption, distribution, metabolism, excretion

2P4E complex with ZINC85625406 shows simulation range of backbone atoms varied from 1 to 2.4 Å (Fig. 6b).

CONCLUSION

PCSK9 regulates the lifespan of the receptor on the liver that clears cholesterol. If the protein is inhibited, lower cholesterol levels are seen in several studies because the protein causes the liver receptor that clears cholesterol to be destroyed and recycled. So, if protein is inhibited, the receptor stays on the liver longer and clears more cholesterol from the body. Many clinical trials show that PCSK9 inhibitors are exceptionally potent cholesterol-lowering agents. Therefore, structure-based pharmacophore modeling and virtual screening were done to screen zinc natural and synthetic compounds. The leads shortlisted in both the approaches were employed with different filtering criteria. In structure-based pharmacophore modeling, the refined common pharmacophore (Adverse drug reaction) model was used as query for screening. Molecular docking was performed to get the best hit. ZINC00652090 had the best glide score of −9.24 kcal\mol and has an H-bond interaction with Zn^{2+}. Binding orientation and protein-ligand interaction have been analyzed. VAL 644 and GLU 450 commonly interact with selected ligand. Pertaining to the docking results, MD simulation and ADME properties, two compounds ZINC85625485 and ZINC85625406 may act as the potential inhibitors for PCSK9.

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