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Detection of the Atherosclerotic PCSK9 gene Inhibitors Through *in silico* Method to Improve Targeted Therapy

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Abstract

The PCSK9 is one of the most important marks for the evolution of therapeutic agents for atherosclerosis because its interaction with low-density lipoprotein receptors causes atherosclerosis. Protein-ligand interactions help us to understand the true mechanism of pharmacological action. This study seeks to identify the most powerful suppression options for PCSK9. Initially, the reported ACE inhibitors were included in pharmacophore modeling using PharmaGist. Next, ZINCPHARMER was used to screen the selected model against a ZINC database to identify putative drug candidates docked to the target protein to understand the interactions. The 10 best pharmacological candidates for PCSK9 with a binding energy of 9.8-8.2 kcal mol-1 were identified by molecular docking and their pharmacokinetic properties and oral bioavailability were evaluated. The (S) severalplant obtained chemicals have been discovered, including anti-hypersensitive drugs such as “Canadine, Hesperetin, and Labetalol”. According to Biochemistry, these compounds formed a stable “protein-ligand” complex. The (S) canadine PCSK9 complex had the lowest RMSD and was the most stable. Future in vitro studies could identify (S) canadin as a promising atherosclerosis inhibitor for the evolution of novel PCSK9 inhibitors.

Keywords: PCSK9; Therapeutics; ACE inhibitors; Protein – ligand; Docking.

INTRODUCTION

Highly elevated LDL cholesterol known as “Familial hypercholesterolemia” (FH) is recognized as a hereditable disease. It can lead to an increase of Low Density Lipoprotein (LDL) cholesterol in the blood. This can increase LDL quantity by more than 4 mmol / L (> 180 mg/dL) (Zhao et al., 2019), increasing the threat of cardiovascular diseases (CVDs) such as atherosclerosis, heart failure and myocardial infarction, etc. Patients with genetic modification in the Familial hypercholesterolemia gene, such as Low density lipoprotein receptor (LDLR), Proprotein convertase subtilisin/kexin type 9 (PCSK9), and ApoB, are at increased risk of atherosclerosis (ASCVD) than those without the mutation but with comparable cholesterol levels (Khera et al., 2016). Mutant FH-related genes can cause an increase in low-density lipoprotein cholesterol (LDLC) (Semenova et al., 2020), cause vascular endothelial dysfunction. ASCVD, including CAD, is a well-known disease characterized by endothelial dysfunction. Endothelial dysfunction is the first step in the development of atheroma. Hypercholesterolemia is one of the most common causes of endothelial dysfunction that causes stenosis of the aorta of the heart (Zhang et al., 2020). Cholesterol is required for some physiological activities. Cell membranes are important organelles that require large amounts of cholesterol. In addition, cholesterol is essential for the production of bile acid and steroid hormones. An increase in cholesterol, particularly under oxidizing circumstances, may culminate in atherosclerosis, causing carotid, peripheral artery, and coronary heart disease (Andreadou et al., 2017). One of the leading causes of stroke and heart attack is atherosclerosis. Cholesterol buildup may result in the formation of arterial plaques, leading to atherosclerosis. Elevations in LDLC and apolipoprotein B100 (apoB100) are precisely connected to cardiovascular events such as atherosclerosis. Decreased LDLC levels may reduce the risk of cardiovascular events, as reduction of ApoB on the arterial wall can cause lesion and lead to the formation of atherosclerosis (Mahley, 2016). LDLC deposits can result in fatty streaks accumulation and reduced flow of blood to many organs as a result of inflammatory changes in the arterial wall. PCSK9 interacts with the LDL receptor (LDLR) and causes liposomal degradation that promotes atherosclerosis. Therefore, PCSK9 inhibition is essential for risk halting of CVDs (Latimer et al., 2016). PCSK9 is a protein with 692 amino acids which in mainly expressed in liver and intestine (Horton et al., 2007). PCSK9 is synthesized in the endoplasmic reticulum (ER), and its 73 kDa precursor peptide is zymogen. This zymogen makes many changes before it stick out the cell surface. There are auto-catalytic activity in-between 152nd and 153rd amino acids. The N-terminal pro-domain stays firmly linked with the catalytic domain in the secretory route(Hyock et al., 2008).

PCSK9 can break LDLR with its proteolysis activity when in contact with the extra-cellular sites of LDLR. PCSK9 has a subtilisin-like catalytic domain that can bind to the LDLR-EGFA domain. This domain contains Asp374, and changing to Tyr increases PCSK9's affinity for LDLR (Hyock et al., 2008). Binding of Low density lipoprotein to Low density lipoprotein receptor is important for reducing lipid sedimentation and the threat of CVDs. When PCSK9 binds to LDLR, it protects LDL from attaching to Low density lipoprotein (LDLR) and causes lipid sedimentation. To avoid CAD, you should block the association between PCSK9 and its LDLR (Horton et al., 2009). Natural treatments using mAb and short molecule drug therapies are the two main approaches used in PCSK9 targeted therapies. Unfortunately, certain advances in small molecule drug therapy for PCSK9 inhibition are hampered by the fact that the molecular structure of PCSK9 required for small molecule binding to reduce the activity of this protein has not been identified (Joseph & Robinson, 2015). Computational methods based on bioinformatics and chemoinformatics help identify new binding sites for inhibiting proteins. Bioinformatics and chemoinformatics deal with the creation and manipulation of databases and statistical methods for the management and analysis of biological and chemical data. These devices help to find and study about targets, chemical structures, and mobile sites. These devices can also discover potential medicinal molecules for specific therapeutic targets and quantify their medicinal similarity via molecular docking (Wishart, 2005).

Medicinal candidates are evaluated for their binding affinity and their properties are subject to change as needed. The concept of computationally-based drug discovery is called structural design. Therefore, the *in silico* approach can provide a broad and favorable approach for identifying new target proteins and predicting their biological activity (Ramharack & Soliman, 2018).

The drug components such as, “Captopril, Zophonopril, Enalapril, Ramipril, Quinapril, Perindopril, Lisinopril, Benazepril, Fosinopril, Sirazapril, Moexipril, Trandolapril, Alicin, Teprotide” were used in this incilico study (Attique et al., 2019). Using computational approaches such as the pharmacophore design of existing drugs, new small inhibitors of PCSK9 have been identified. Molecular docking was performed to study the interaction of PCSK9 target proteins with computer-identified new drugs. This study is essential for the discovery of new drugs targeting PCSK9 that can be administered to cure or reduce heart disease and address the increasing challenges associated with excess lipids for the reduction and treatment of atherosclerosis or its symptoms.

MATERIALS AND METHODS

3D structure of the protein of interest (PCSK9)

The 3D structure of the target protein was obtained from the PDB library (https://www.rcsb.org/search) (Burley et al., 2021). The interaction between the EGFA domain of PCSK9 and LDLR occurs at the target site. This interaction (Du et al., 2011) between the pro-domain and C-terminal of PCSK9 increases blood LDLC quantity and thus increases susceptibility to CVD. The PDB ID 6U26 (Petrilli et al., 2020) was determined based on its quality (1.49) after analyzing all known structures in the PDB database, including domains with one active site.

PCSK9 active site characterization

The active site of PCSK9 was found in-between a unique pocket, catalytic and C-terminal domain(Petrilli et al., 2020). LIG PLOT is used to plot this pocket (Figure 1). LIGPLOT provides data on hydrogen bonds and hydrophobic contacts (RA & MB, 2011).

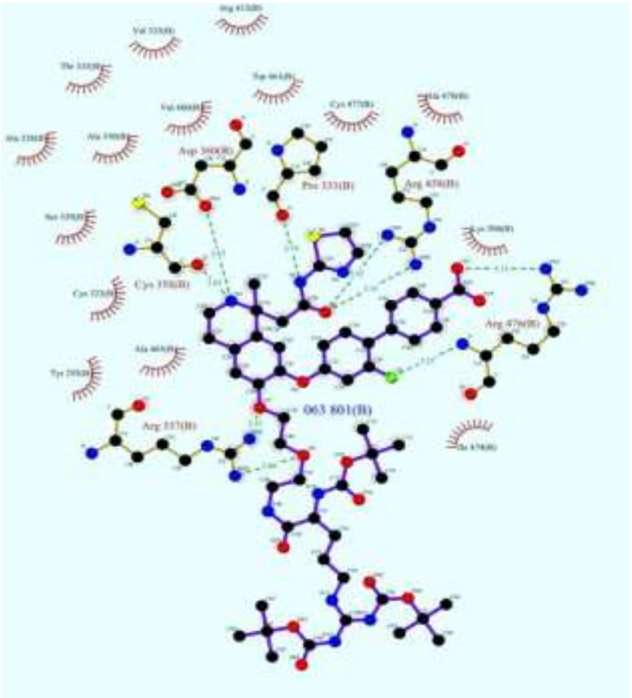

Figure 1. LIGPLOT for 6U26:

(Figure 1) - It shows data on hydrogen bonds and hydrophobic contacts.

Preparation of the protein target and ligand

An autodock tool(Hanwell et al., 2012) was used to purify target protein to remove heteroatom, polar hydrogen, and Colman charges. The 3D structure of the PCSK9 target protein was generated by converting to the PDBQT format. Angiotensin-converting enzyme inhibitor (ACEi) with atherosclerosis activity was found in ligand synthesis (Table S1). Those 2 dimensional structures were taken from the PubChem database(Kim et al., 2021) using individual PubChem IDs and energy was reduced using the ChemAxon Marvin Suite. We used Raccoon software (Hanwell et al., 2012) to convert the energy-optimized structure to PDBQT format.

Table 1. The EI and PI of the Molecules.

Molecule name	PubChem ID	Binding energy	Residues	EI	PI
Captopril/	44093	-7.0	ASP 651 and ARG 545	0.60	0.98
Zofenopri	92400	-9.3	ARG 458 and TRP 461	0.24	0.89
Enalapril	5388962	-8.8	ARG 458	0.28	0.80
Ramipril/	5362129	-9.4	ARG 458	0.33	0.88
Quinapril	54892	-9.9	ARG 458	0.24	0.60
Perindopril	107807	-8.2	ARG 458	0.30	0.93
Lisinopril	5362119	-8.8	ARG 458	0.49	0.92
Benazepri	5362124	-8.3	ARG 458 and ARG 476	0.51	2.03
Fosinopri	55891	-8.7	ARG 357	0.08	0.74
Cilazapril	56330	-9.6	ARG 458	0.09	0.46
Moexipril	91270	-9.4	ARG 458	0.27	0.86
Trandolapril	5484727	-9.1	ARG 458	2.52	2.40
Allicin	65036	-9.9	ARG 458	2.35	2.68
Teprotide	443376	-9.7	ARG 458, ARG 357, ASN 298, TRP 461, CYS 323 and ARG 458	2.77	2.67

(Table 1) - It shows the EI and PI of the molecules with its binding energy.

Molecular docking of recognised ACE inhibitors with PSCK9

The energy-reducing structure (PDBQT format) was docked to the target protein using Autodock Vina (Ukuku et al., 2012). All known molecules with above average binding energies (moexipril, trandolapril, allicin, teprotide, zophenapril, lamapril, quinapril) were selected for the pharmacophore design. Inhibitor selection was based on intracting energy and structural comparison (Table 1).

Pharmacophore designing/modeling

The pharmacophore was designed using PharmaGist (Laskowski et al., 2018), and the pharmacophore was selected predicted on its high range and the number of molecules placed in the design. Pharmacophores are a three-dimensional sequence of properties (chemical bond donors, chemical bond acceptors, anions/cations, aromatic rings, hydro-phobic groups) required for a ligand to communicate with a particular target (Schneidman-Duhovny et al., 2008).

Screening virtually for the pharmacophore

Prior to wet-lab investigations, one of the fundamental levels in drug improvement is digital screening. This manner consists of estimating the binding affinity of a capability medicine candidate to a goal protein. Virtual screening is likewise utilised to evaluate capability binding modalities of the medicinal candidate and different drug-like short molecules throughout interplay with the goal protein. Using HPC “High-Performance Computing” configuration technologies (Jaghoori et al., 2016), the maximum distinguished medicine applicants displaying capability binding affinity in the direction of the goal protein can be eliminated. Through the Virtual screening manner, numerous bioactive compounds that could have interaction with the goal protein can be found (Langer & Hoffmann, 2008). The pharmacophore become absolutely screened towards the ZINC drug database the use of ZINCPHARMER (Koes & Camacho, 2012), and 1033 hits/ compounds have been identified.

Molecular docking for the last molecules screened

SDF-formatted structures (each molecule in a single file) were obtained and used to dock the terminal of screened 1033 compounds (using the desired pharmacophore). I used a Python script to separate and reduce the energy of these structures. The resulting structure was changed to PDBQT format using Vina to dock to the required protein. All compounds were docked to Autodock Vina (Guidi et al., 2006) and the top results (contingent on interaction and affinity energy) were examined using Autodock Tools (Hanwell et al., 2012). The docking grids coordinates were 40.516000 (X), 24.375625 (Y), and 25.098375 (Z), and each grid had dimensions of 25. There were 20 modes in all. Table 2 shows the 10 most important ZINC molecules selected as potential treatment candidates.

Table 2. Amino acid interactions of molecules.

Molecule name	Binding energy	Number of interactions	Amino acid interaction
Apomorphine	-10.8	7	ARG 458, PRO 438, ARG 357, TRP 461 and VAL 460
Oxyphencyclimine	-10	8	CYS 358, PRO 331, ALA 478, VAL 460, ARG 458, VAL 359, THU 459 and PRO 438
Canadine	-10	7	ARG 357, ASP 360, PRO 331, ALA 478, VAL 460 and PRO 438
Naltrexone	-9.8	10	ARG 476, ARG 458, VAL 460, PRO 331, ALA 330, THR 335, ALA 328 and CYS 358
Oxolinic	-9.6	7	LEU 440, PRO 438, TRP 461, VAL 650, THR 437 and THR 459
Norfloxacin	-9.6	9	TRP 461, LEU 436, ILE 416, ALA 649, PRO 438, ARG 458, ARG 357 and ARG 412
Canadine	-9.6	7	ARG 458, ASP 360, PRO 438, ILE 416, LEU 440, THR 459, VAL 460 and ARG 357
Hesperetin	-9.4	9	ARG 412, ALA 330, ARG 357, CYS 358, PRO 331, TRP 461, THR 459 and VAL 460
Labetalol	-9.3	9	TRP 461, CYS 358, THR 437, PRO 438, VAL 650 and ASP 651
Benorilate	-9.2	6	ARG 458, VAL 460, ARG 357, PRO 438 and LEU 436

(Table 2) - The binding energy of top ten molecules are shown with their amino acid interactions.

Final medication candidates' pharmacokinetic characteristics

We used OpenBabel (http://openbabel.org/wiki/Main Page) to create a simplified molecular input system & # 40; SMILES & # 41; AutodockVina For the top 10 compounds identified during virtual screening. The resulting SMILES code is ZINC (Sterling & Irwin, 2015), Molinspiration Cheminformatics' free online service (,Pubchem. Analyzed using a comparison website. , SWISS ADME (Daina et al., 2017) and admet SAR (Yang et al., 2019). SWISSADME was used to calculate the oral bioavailability of each drug (Table 3). For bioactivity prediction and drug similarity from Lipinski's Rule 5, use the Predict Bioactivity tool available to calculate molecular properties and bioactivity scores. Was evaluated by. The LE and LELP parameters were found from the specified results.

Table 3. Bioavailability of drugs.

Molecule	Formula	MW	Ali Log S	Bioavailability Score	Synthetic Accessibility
Apomorphine	C17H18NO2	268.33	-3.88	0.65	4.29
Oxyphencyclimine	C20H28N2O3	344.45	-4.89	0.65	5.08
(S)-Canadine)	C20H22NO4	340.39	-4.65	0.65	4.68
Naltrexone	C20H24NO4	342.41	-3.69	0.65	5.76
Oxolinic acid	C13H11NO5	261.23	-3.16	0.66	3.26
Norfloxacin	C16H19FN3O3	320.34	-1.14	0.65	1.5
(R)-Canadine	C20H22NO4	340.39	-4.65	0.65	4.68
Hesperetin	C16H14O6	302.28	-5.27	0.65	4.22
Labetalol	C19H25N2O3	329.41	-5.86	0.65	4.04
Benorilate	C17H15NO5	313.3	-4.5	0.65	3.16

(Table: 3) - The molecular weight and the bioavailability value of various molecules are given with its synthetic accessibility.

Molecular dynamics of complexes of protein and ligand

The molecular dynamics of the protein-ligand complex was prepared and calculated using GROMACS (Abraham et al., 2015) version 2018.4 on a desktop computer with a 2.80 GHz and NVIDIA Graphics GeForce GTX 1060. We did it with a 6GB card. The GROMACS source code was taken from the website http://www.gromacs.org/ and built using OpenCL version 1.2 of Microsoft Visual Studio Community MSVC19.16.27025.1 and NVIDIA GPU Computing Toolkit CUDA v10.0 for Windows 10. The PDBQT file of the protein-ligand complex obtained by virtual screening at Autodock Vina is used as the first coordinate source for both PSCK9 and the screened virtual inhibitor. Using Open Babel, the coordinates were converted to in PDBQT format, PDB for proteins and MOL2 for ligands. The generated PDBQT file was reconstructed in SWISSMODEL (Waterhouse et al., 2018) using the entire human PCSK9 amino acid sequence (Bateman et al., 2015; Leinonen et al., 2004) and lacked the original 3D structure. I filled in the loop that is. New PDB coordinates were retrieved and utilised by gmx pdb2gmx with the CHARMM36 allatom force field to generate the GRO protein topology (July 2020). In the Avogadro programme (http:// avogadro.cc/) (Hanwell et al., 2012), MOL2 files were updated with all the missing hydrogen atoms. The updated MOL2 files were visually verified and modified for problems. Using sort mol2 bonds, a perl script created by Justin A. Lemkul and provided under the GPL3.0 licence, the bonds in the coordinate files were separated in ascending order.

Such processed MOL2 data was sent to SwissParam (https://swissparam.ch/) to build a ligand topology for retrieving "ITP and PDB" files. The received PDB file was converted to GRO using gmxeditconf and the ITP file was correctly placed in the respective topol.top file for each ligand. Next, we defined the unit cell and filled it with water. gmxeditconf generated a dodecahedron cell from the proteinligandGRO file and the correct topol.top file. Sodium and chloride ions were added to achieve a concentration of 0.155 M with a neutral charge while energy was minimised. The ligands and protein were individually restrained by constructing position restraint topologies, and twophase 100 ps, 310 K, and 1 bar equilibrations were carried out to create first NVT and then NPT outputs. Terminal molecular dynamics production was set for "10 nanoseconds" of simulation at 320 Kelvin and 1 bar of pressure, and a single simulation of a proteinligandcombination on the previously reported computer equipment lasted around 24 hours. For both energy minimization and equilibration, the similar temperature and pressure conditions were employed. Visual Molecular Dynamics (VMD) (Yamada et al., 2017) was used to visualise the results, and GROMACS gmx rms was used to determine the rootmeansquare deviation (RMSD).

RESULTS AND DISCUSSION

Results

Active site 3D structure of the target protein (PCSK9)

Figure 1 shows the LIGPLOT of the structure expressing the residues participated in the interaction. The dashed line shows the hydrogen bond interaction between the amino acids and legends of the target protein. Figure 2 uses Discovery Studio to contrast this interaction and distinguish the active area residues from the rest of the structure.

Figure 2. Active cite pocket.

(Figure 2) - Ball sticks representation of active site pocket

Utilizing molecular docking research to identify known inhibitors for pharmacophore design.

To identify the optimal structure of PCSK9 and its active sites and recognized ACE inhibitors such as, "captopril, zophenopril, enalapril, ramipril, quinapril, perindopril, lisinopril, benazepril, fosinopril, silazapril, moexipril, trandolapril, alicin", etc. We reviewed the literature in (S1 table). All known ACEi were docked to the target protein PCSK9 and the top results were evaluated based on the interaction between binding energy and the active site.

Molecules evaluated on the basis of pharmacophore

In this study, pharmacophore was adopted as a drug discovery strategy and a novel PCSK9 inhibitor was used with established inhibitors such as, "moexipril, trandolapril, alicin, teprotide, zophenopril, ramipril, quinapril" shown in Table S1. The pharmacophore model created by PharmaGist was evaluated and the optimal pharmacophore was selected based on a score of 18.102 and 5 attributes (1 hydrophobicity, 1 negative ion, and 3 hydrogen bond acceptors). rice field. In ZINCPHARMER, pharmacophores were screened along the entire ZINK drug database and 1033 hits / molecule were identified.

Molecular docking to identify the top 10 most promising medicinal candidates

After evaluating all 1033 molecular docking complexes with the required protein, their affinity energies and interactions were saved. The docking results of the top 10 ZINC molecules were sorted based on the interaction of the active sites assessed for lower binding energies calculated by AutoDock (Table 2 and Figure 3a–3j). Lower energies exhibit stronger affinities and sustained interactions. Therefore, the top compounds may be more effective in exerting the expected activity of the PCSK9 and are therefore excellent therapeutic candidates.

Final 10 drug candidates

The drugs such as, “Apomorphine, oxyphencyclimine, (S) canadine, naltrexone, oxolinic acid, norfloxacin, (R) canadine, hesperetin, labetalol, and benolylate” were the top 10 choices. Table S4 shows their PubChemCID, name, structural formula, putative characteristics, and drug similarity according to Lipinski's rule of five. According to PubChem, apomorphine (ZINC00009073) is the first molecule with the least change in docking energy, a non-selective dopamine used to treat Parkinson's disease. Oxyfencyclimin (ZINC00020260) is an oral muscarinic receptor antagonist used to treat gastric ulcers and gastrointestinal problems. The third is (S) canadine which is also called as (S) tetrahydroberberine and xanthopsin (ZINC00033518), which is a benzylisoquinoline alkaloid (BIA) in the protoberberine structural subgroup (Xu et al., 2019) and is found in some poppy and corydalis plants. The next substance (ZINC00001773) is naltrexone, which is commonly used to treat alcohol or opioid addiction. Oxolinicacid, ZINC00001875, is a synthetic antibiotic used for veterinary medicine. The antibiotic ZINC00003742 is norfloxacin, which has broad-spectrum antibacterial activity over the Gram-negative and Gram-positive bacteria. (R) canadine is an enantiomer of (S) canadine. ZINC00039092 is a secondary plant metabolic product, hesperetin, that acts as an antioxidant and antitumor agent. Labetalol is the chemical name for 2 hydroxy 5 [(1S) 1 hydroxy 2 [[(2R) 4 phenylbutane 2 yl] amino] ethyl] benzamide, also known as ZINC00000416. It is a third-generation vasodilator, an antihypertensive drug selective α1 adrenergic blocker and non-selective β-adrenergic blocker. The final product (ZINC00001003) is benorilate, an esterified codrug of aspirin and acetaminophen. It is used as an anti-inflammatory and antipyretic drug(Bass, 1973).

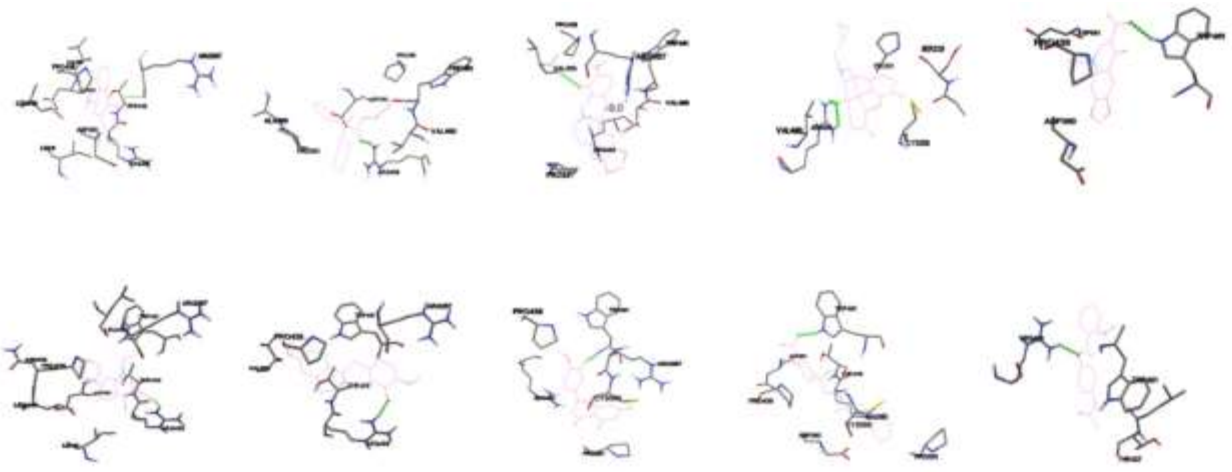

Figure 3. The 10 Drug candidates.

(Figure 3) - It shows the final drugs

Table 4. PubChem ids and names of molecules.

Molecule name	CIDs	LE	LELP	AlogP	MilogP	Drug similarity
Apomorphine	6931310	0.5	8.7	2.43	4.16	Pass
Oxyphencyclimine	667690	0.5	9.9	3.73	4.57	Pass
(S)-Canadine	21171	0.5	9.3	2.67	3.99	Pass
Naltrexone	5360515	0.5	4.9	1.11	2.37	Pass
Oxolinicacid	4628	0.6	2.5	2.42	1.68	Pass
Norfloxacin	4539	0.5	-2.8	1.24	-1.69	Pass
(R)-Canadine	443422	0.4	9.7	2.67	3.99	Pass
Hesperetin	72281	0.5	6.1	3.52	2.94	Pass
Benzamide Labetalol	134045	0.4	9.2	2.11	3.85	Pass
Benorilate	21102	0.5	8.3	3.79	3.61	Pass

(Table 4) - The PubChem ids and the drug similarity report based on the “Lipinski rule” is given in detail

The pharmacokinetic characteristics and bioavailability of the medication will be evaluated.

Using tools, “Molinspiration, KNIME, SWISSADME, and admetSAR, computational structural analysis, drug-likeness, ADME characteristics, oral bioavailability, and toxicity profiling” have been conducted (3 and 4 Tables). Using SMILES codes, the pinnacle molecules have been re-searched towards the ZINC database, Molinspiration, and PubChem, and that they have been all recognized as acknowledged compounds, a number of plant beginning such as (S)-canadine and hesperetin. An in addition antihypertensive agent, labetalol, turned into found (4 Table). Depending at the diploma of nitrogen protonation, numerous chemical substances containing nitrogen atoms may also exist in lots of paperwork. These compounds incorporate numerous PubChem codes, distinct SMILES, however are stated as "discern compounds" on this database. Consequently, it is able to be concluded that they are the equal chemical compounds that tackle numerous paperwork in accordance at the pH. Figure 4 depicts the availability radars of the pinnacle ten nominated compounds.

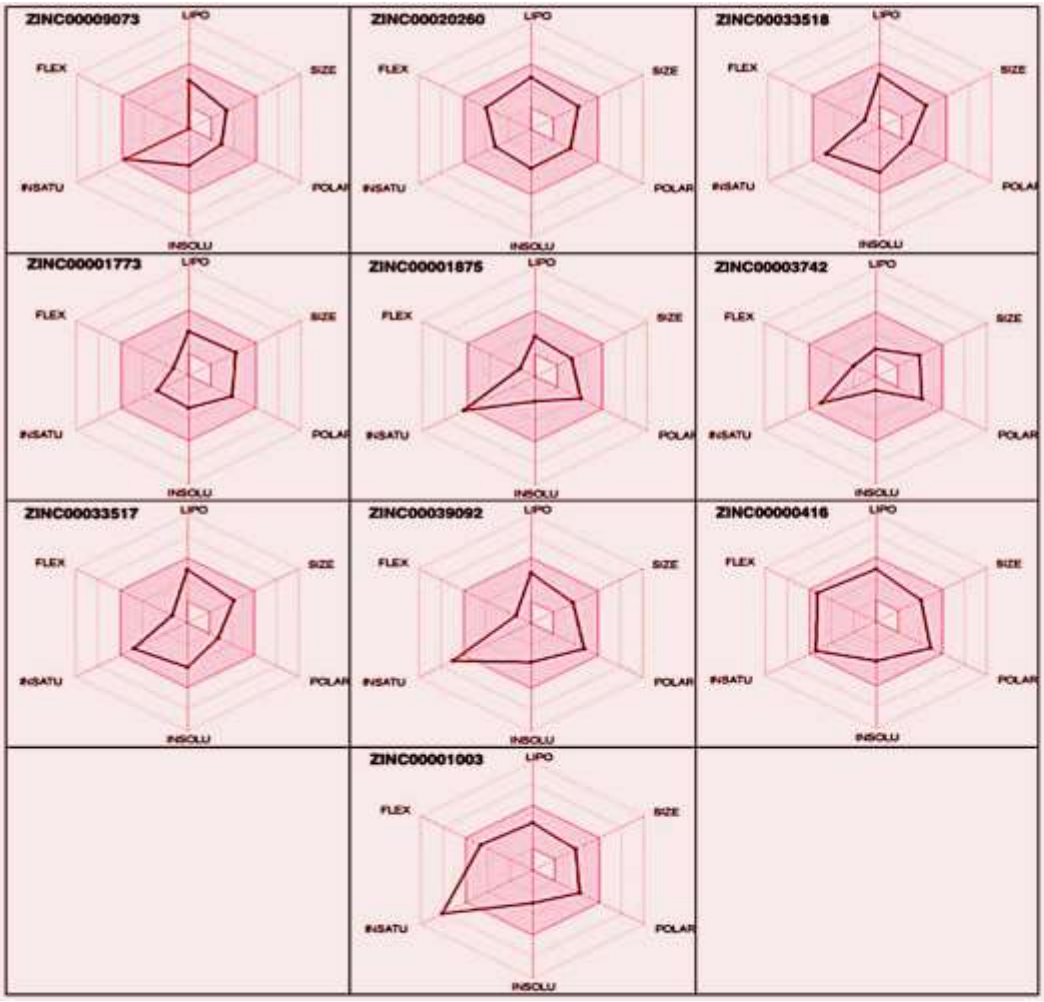

Figure 4. Molecules Radar chart.

(Figure 4) – This shows the molecular radar chart

Evaluation of the stability of the suggested protein-ligand complexes

Upon visual inspection with VMD, (S) canadin, hesperetin, and labetalol produced a strong protein-ligand complex throughout molecular dynamics and remained at the docking targets for at least 10 ns. The RMSD value of the heavy atom of the ligand in the 10ns lasting simulation of the three compounds was - 0.007. 0.13 0.024; 0.13 and 0.035 (mean SD) (Figure 5), respectively, suggesting that the position of the ligand changes only slightly. The value of (S) canadine is 0.1, indicating the excellent stability of this compound. This molecule produced the most stable (S) canadine complex in terms of duration and molecular dynamics, with the lower RMSD (Fig. 5). This compound can be further researched for the discovery of new PCSK9 inhibitors.

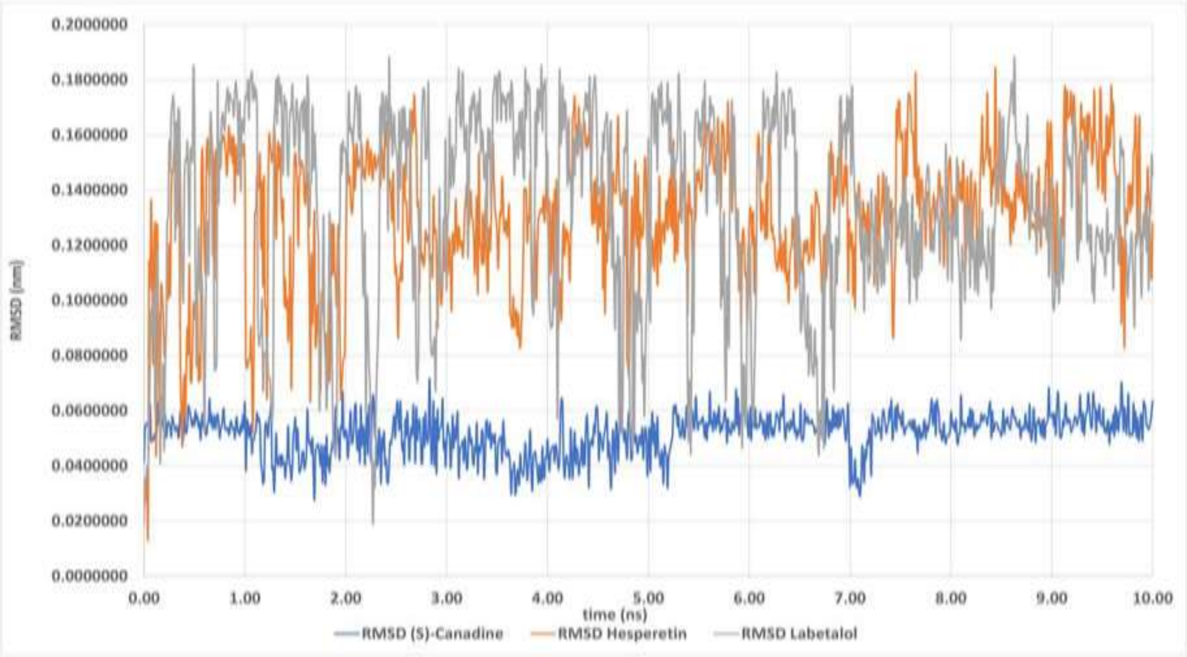

Figure 5. RMSD stimulation.

(Figure 5) – It shows the RMAD stimulation

Discussion

The manufacturing of arterial plaques is one of the main reasons of atherosclerosis, which in the end outcomes in arterial blockage and coronary heart attack. Moreover, better stages of apoB-a hundred and ldl cholesterol are without delay connected to atherosclerosis-associated CVDs. The lipid cycle commonly begins with the liver`s launch of im-mature VLDL. In addition, apoB-a hundred, cholesteryl esters, triglycerides, and ldl cholesterol are present. VLDL broaden and end up a supply of electricity in “adipose tissues, skeletal and cardiac muscular tissues” all through movement withinside the blood (Chaudhary et al., 2017). One of the jobs of VLDL is the trade and elimination of triglycerides, which ends up withinside the conversion of VLDL into intermediate-density lipoproteins (IDL) (Shelness & Sellers, 2001). Some of those IDL can be removed through the liver all through endocytosis, whilst IDL with a more ldl cholesterol content material are transformed to LDL, which additionally consists of apoB-a hundred. This apoB-a hundred attaches to LDLR at its N-terminal area after which passes via the acidic endosome via endocytosis. LDLR in the end breaks from LDL and is reintroduced into the mobileular membrane (Ding et al., 2015). At an acidic pH, structural changes in LDLR might also additionally decorate PCSK9's affinity for LDLR relative to LDL(Horton et al., 2009). Although mutations might also additionally dispose of the catalytic hobby of PCSK9, they do now no longer have an effect on its cappotential to bind to LDLR. A stoppage withinside the protein's manufacturing might also additionally bring about blockading its binding to LDLR. PCSK9 mRNA degradation might also additionally in the end hinder its reference to LDLR, or a tiny drug meant to save you this courting also can do so (McNutt et al., 2007). Atherosclerosis is as a result of the interplay among PCSK9 and LDLR (EGF-A area or epidermal boom factor-like repeats), which inhibits the endocytosis of LDL. The connection among PCSK9 and LDLR can be blocked to halt the buildup of LDL. PCSK9 is produced withinside the ER and transferred to the plasma membrane to be able to have interaction with LDLR. Protein-protein interactions govern a extensive quantity of organic activities. The law of those interactions is a famous and potential drug improvement pathway. To save you the EGF area of LDLR from interacting with PCSK9, it's far important to perceive the interplay's goal location.

It is acknowledged that antihypertensive medications, specifically ACE inhibitors, have a essential position in atherosclerosis (Pitt, 1995). ACEi are putative plaque formation inhibitors (Ferrari et al., 2010). Experimental, epidemiological, and scientific research have proven that ACE inhibitors decorate arterial endothelial feature and, thus, lower the improvement of atherosclerosis (Lonn, 2001). It has been proven that they lower erythropoietin (EPO) stages and hematocrit. ACEi might also additionally modulate a whole lot of bioactive peptides, along with angiotensin 1–7 and substance P. In hematopoiesis, numerous peptides had been located to have a position. Reducing the quantity of angiotensin II might also additionally restrict erythropoiesis. ACEi additionally have an effect on the renin-angiotensin system (Younas et al., 2017).

Several ACE inhibitors, along with "Captopril, Zofenopril, Enalapril, Ramipril, Quinapril, Peri- ndopril, Lisinopril, Benazepril, Fosinopril, Cilazapril, Moexipril, Trandolapril, Allicin, and Teprotide", had been used on this study. Captopril decreases LDL oxidation, which is understood to make contributions to the improvement of atherosclerosis (K.R. et al., 2014). Zofenopril and Enalaprilare ACEi which can lessen vascular harm and enhance endothelial progenitor mobileular movement. They inhibit endothelial harm and the improvement of atherosclerosis (Pines & Fisman, 2003).

Arterial plaque formation is one of the leading causes of atherosclerosis and ultimately causes arterial occlusion and heart attack. In addition, high levels of ApoB100 and LDL cholesterol are directly associated with cardiovascular disease associated with atherosclerosis. The lipid cycle usually begins with the release of immature VLDL by the liver. It also contains apoB100, cholesteryl ester, triglycerides and cholesterol. VLDL develops in adipose tissue, skeletal muscle, and myocardium during circulation in the blood and becomes an energy source (Chaudhary et al., 2017). One of the roles of VLDL is the exchange and removal of triglycerides, converting VLDL to intermediate density lipoprotein (IDL) (Shelness & Sellers, 2001). Some of these IDLs can be eliminated from the liver during endocytosis, but IDLs with high cholesterol content are converted to LDL, which also contains apoB100. This apoB100 binds to LDLR at its N-terminal domain and passes through acidic endosomes by endocytosis. LDLR is eventually cleaved from LDL and reintroduced into the cell membrane (Ding et al., 2015). At acidic pH, changes in LDLR conformation may increase the affinity of PCSK9 for LDLR compared to LDL [10]. Mutations can eliminate the catalytic activity of PCSK9, but do not impair its ability to bind to LDLR. Interfering with protein production, can block binding to the LDLR. Degradation of PCSK9 mRNA can ultimately prevent its association with LDLR. Alternatively, the same can be done with small medicines designed to prevent this relationship (McNutt et al., 2007). Atherosclerosis is caused by the interaction of PCSK9 with LDLR (EGFA domain or epidermal growth factor-like repeat) and inhibits LDL endocytosis. You can block the connection between PCSK9 and LDLR to stop LDL accumulation. PCSK9 is produced in the ER and transcribed into the plasma membrane to interact with the LDLR. Protein-protein interactions control a significant number of biological activities. ACE inhibitors (Table 1) have been shown to play an active role in the prevention of atherosclerosis. Therefore, pharmacophore modeling and screening using the ZINC database has been introduced into the drug development process. The binding affinity of the newly identified chemical (1033) from the ZINC database was assessed using the AutoDock tool. The program calculated several factors, including reduced binding energy, interaction with the active site, and the number of hydrogen bonds. Comparing these criteria yielded a list of the 10 most promising drug candidates for atherosclerosis (Table 2). Some of the molecules virtually screened from the ZINC database showed good interactions, while others showed no or weak interactions. The results show that the top 10 compounds with a binding energy of 9.8-8.2 kcal / mol (Table 2) are similar to the reference compounds (Table 1) and are therefore desirable and viable drug candidates. Candidate target for PCSK9 in atherosclerosis. In other words, in this study, the top-selected compounds showed the highest binding affinity for the PCSK9 target protein for atherosclerosis. In addition, Molinspiration, computational structural analysis of these compounds using SWISS ADME, drug similarity, ADME properties, and oral bioavailability,

Based on these results, (S) canadin, hesperetin, and labetalol contain heavy atoms and have LE and LELP values, which is why further research and possible design of new drugs based on pharmacophore structure is the most. We can conclude that it is a promising candidate. Same range as known ACE inhibitors (Tables 1 and 4). The results of AutoDockVina led to the selection for further molecular dynamics analysis of the complex with PCSK9. During molecular dynamics, all three compounds appeared to form a stable PCSK9-ligand complex, staying at the docking site for at least 10 ns, and their heavy atom RMSD values were minimal. A notable advantage of (S) canadine, also known as (S) tetrahydroberberine, is a significant reduction in RMSD (Figure 5), which promotes the formation of a highly stable complex with PCSK9. This is an interesting finding, as studies suggest that berberine inhibits PSCK9 (Xu et al., 2019). Although the direct inhibitory effect of such compounds is unknown, this study provides insights into (S) canadin and berberine with very similar pharmacophore structures that differ only in the degree of hydrogen atom saturation. increase. Berberine is an aromatic chemical, but (S) canadine is not. Experimental studies in mice have found that berberine delays the onset of atherosclerosis in cholesterol-fed apoE / knockout mice (Wan et al., 2018). In addition, a study of the Coptis chinensis decoction network path, a traditional Chinese healing method, revealed:

Based on these results, (S) canadin, hesperetin, and labetalol are the most promising candidates for further research, and (Seidah et al., 2017) the potential for new drug development based on the study is (S) canadin and berberine. We can conclude that it indicates that. Of the most probable drug for atherosclerosis. In vivo studies conducted with hesperetin to assess its role in LDLR expression showed that hesperetin dose (200M) stimulated LDLR gene expression, increased mRNA levels of transcription factors SREBP1 and SREBP2, and plasma levels. The effect of LDLC on reducing the risk of cardiovascular disease has been suggested to reduce (Bawazeer et al., 2016). In addition, studies suggest that activation of SREBP2 causes co-expression of PCSK9 and LDLR, leading to LDLR depletion and increased LDLC circulation (Maxwell et al., 2003; Urban et al., 2013). As a result, the effects of hesperetin can be counteracted. Studies show that saturation increases with increasing Fsp3 levels (Lovering et al., 2009; Wei et al., 2020). The relationship between Fsp3 and the solubility and melting point attributes of 1000 compounds in the dataset showed a positive correlation between Fsp3 and solubility and a negative correlation with melting point (Lovering et al., 2009). Compounds with a lower melting point show better absorption than compounds with a higher melting point (Chu & Yalkowsky, 2009). Therefore, low chemical saturation (Fsp3) can lead to high melting points, limited drug absorption, and reduced oral bioavailability. Therefore, it is assumed that low levels of hesperetin saturation (Table 3) reduce oral bioavailability. Although various studies have shown that hesperetin has low oral bioavailability (Kanaze et al., 2007; Liu & Chen, 2008), it is possible to increase oral bioavailability by specific chemical approaches [69]. (S) and (R) canadins have unique bioavailability properties due to their ability to efficiently cross the blood-brain barrier (BBB) and reach their targets (Table 3). Their high gastrointestinal absorption and ability to bind to Pgp make these molecules excellent candidates for orally administrable drugs (Laskowski et al., 2018). Based on current research, the pharmacophore structure of (S) canadin may function as an inhibitor of the PCSK9 protein molecule. Using existing ACE inhibitors, pharmacophore was used as a drug discovery method to identify new PCSK9 inhibitors. A literature search was conducted to identify the optimal structure of PCSK9 and the active site of known ACE inhibitors. The results of molecular docking, incorporating a computational and unbiased approach, highlighted the relevance of drug development in atherosclerosis. In this study, the binding affinities were analyzed and the molecules with the highest affinities for the target of atherosclerosis were Zinc000073, ZINC00020260, ZINC00033518, ZINC00001875, ZINC00003742, ZINC0003517, ZINC000390, ZINC000390, ZINC0003900, ZINC0000390, ZINC000390, ZINC000390, ZINC000390, ZINC0003900, ZINC0003900, ZINC0003900, ZINC0003900, ZINC0003900, ZINC0003900, ZINC0003900, ZINC0003900 Lipinski's Rule 5 was applied and passed for these major drug candidates. Several molecules from virtual screening and drug database searches were found as herbal chemicals such as (S) canadine, hesperetin, or the antihypertensive drug labetalol. The formation of stable protein-ligand complexes by these chemicals has been demonstrated using molecular dynamics. For (S) canadine, the complex prepared with this chemical is the most stable in molecular dynamics and has the lowest RMSD.

CONCLUSIONS

The PCSK9 inhibitors are found through the computational methods, these inhibitors can be used as a vital target for the novel drug screening and discovery for atherosclerosis in future. The computational findings should be conformed in future using *invitro* and *invivo* method for further understanding the inhibitors role in atherosclerosis. Hence a depth wet lab research need to be done with is inhibitors as a target for treating atherosclerosis

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REFERENCES

Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindah, E. (2015). Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX, 1–2,* 19–25. https://doi.org/10.1016/j.softx.2015.06.001

Andreadou, I., Iliodromitis, E. K., Lazou, A., Görbe, A., Giricz, Z., Schulz, R., & Ferdinandy, P. (2017). Effect of hypercholesterolaemia on myocardial function, ischaemia–reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. In *British Journal of Pharmacology* (Vol. 174, Issue 12). https://doi.org/10.1111/bph.13704

Attique, S. A., Hassan, M., Usman, M., Atif, R. M., Mahboob, S., Al-Ghanim, K. A., Bilal, M., & Nawaz, M. Z. (2019). A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension. *International Journal of*

Environmental Research and Public Health, *16*(6), 1–17. https://doi.org/10.3390/ijerph16060923

Bass, J. W. (1973). Oral Antipyretic Therapy-Reply. *American Journal of Diseases of Children*, *126*(4), 564. https://doi.org/10.1001/archpedi.1973.02110190459029

Bateman, A., Martin, M. J., O'Donovan, C., Magrane, M., Apweiler, R., Alpi, E., Antunes, R., Arganiska, J., Bely, B., Bingley, M., Bonilla, C., Britto, R., Bursteinas, B., Chavali, G., Cibrian-Uhalte, E., Da Silva, A., De Giorgi, M., Dogan, T., Fazzini, F., … Zhang, J. (2015). UniProt: A hub for protein information. *Nucleic Acids Research*, *43*(D1), D204–D212. https://doi.org/10.1093/nar/gku989

Bawazeer, N. A., Choudhry, H., Zamzami, M. A., Abdulaal, W. H., Middleton, B., & Moselhy, S. S. (2016). Role of hesperetin in LDL-receptor expression in hepatoma HepG2 cells. *BMC Complementary and Alternative Medicine*, *16*(1), 1–7. https://doi.org/10.1186/s12906-016-1165-2

Burley, S. K., Bhikadiya, C., Bi, C., Bittrich, S., Chen, L., Crichlow, G. V., Christie, C. H., Dalenberg, K., Di Costanzo, L., Duarte, J. M., Dutta, S., Feng, Z., Ganesan, S., Goodsell, D. S., Ghosh, S., Green, R. K., Guranovic, V., Guzenko, D., Hudson, B. P., … Zhuravleva, M. (2021). RCSB Protein Data Bank: Powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. *Nucleic Acids Research*, *49*(1), D437–D451. https://doi.org/10.1093/nar/gkaa1038

Chaudhary, R., Garg, J., Shah, N., & Sumner, A. (2017). PCSK9 inhibitors: A new era of lipid lowering therapy. *World Journal of Cardiology*, *9*(2), 76. https://doi.org/10.4330/wjc.v9.i2.76

Chu, K. A., & Yalkowsky, S. H. (2009). An interesting relationship between drug absorption and melting point. *International Journal of Pharmaceutics*, *373*(1–2), 24–40. https://doi.org/10.1016/j.ijpharm.2009.01.026

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(January), 1–13. https://doi.org/10.1038/srep42717

Ding, Z., Liu, S., Wang, X., Deng, X., Fan, Y., Sun, C., Wang, Y., & Mehta, J. L. (2015). Hemodynamic shear stress via ROS modulates PCSK9 expression in human vascular endothelial and smooth muscle cells and along the mouse aorta. *Antioxidants and Redox Signaling*, *22*(9), 760–771. https://doi.org/10.1089/ars.2014.6054

Du, F., Hui, Y., Zhang, M., Linton, M. F., Fazio, S., & Fan, D. (2011). Novel domain interaction regulates secretion of proprotein convertase subtilisin/kexin type 9 (PCSK9) protein. *Journal of Biological Chemistry*, *286*(50), 43054–43061. https://doi.org/10.1074/jbc.M111.273474

Ferrari, R., Guardigli, G., & Ceconi, C. (2010). Secondary prevention of CAD with ACE inhibitors: A struggle between life and death of the endothelium. *Cardiovascular Drugs and Therapy*, *24*(4), 331–339. https://doi.org/10.1007/s10557-010-6244-x

Guidi, I., Galimberti, D., Lonati, S., Novembrino, C., Bamonti, F., Tiriticco, M., Fenoglio, C., Venturelli, E., Baron, P., Bresolin, N., & Scarpini, E. (2006). Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of Aging*, *27*(2), 262–269. https://doi.org/10.1016/j.neurobiolaging.2005.01.001

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeerschd, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, *4*(8). https://doi.org/10.1186/1758-2946-4-17

Horton, J. D., Cohen, J. C., & Hobbs, H. H. (2007). Molecular biology of PCSK9: its role in LDL metabolism. *Trends in Biochemical Sciences*, *32*(2), 71–77. https://doi.org/10.1016/j.tibs.2006.12.008

Horton, J. D., Cohen, J. C., & Hobbs, H. H. (2009). PCSK9: A convertase that coordinates LDL catabolism. *Journal of Lipid Research*, *50*(SUPPL.). https://doi.org/10.1194/jlr.R800091-JLR200

Hyock, J. K., Lagace, T. A., McNutt, M. C., Horton, J. D., & Deisenhofer, J. (2008). Molecular basis for LDL receptor recognition by PCSK9. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(6), 1820–1825. https://doi.org/10.1073/pnas.0712064105

Jaghoori, M. M., Bleijlevens, B., & Olabarriaga, S. D. (2016). 1001 Ways to run AutoDock Vina for virtual screening. *Journal of Computer-Aided Molecular Design*, *30*(3), 237–249. https://doi.org/10.1007/s10822-016-9900-9

Joseph, L., & Robinson, J. G. (2015). Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibition and the Future of Lipid Lowering Therapy. *Progress in Cardiovascular Diseases*, *58*(1), 19–31. https://doi.org/10.1016/j.pcad.2015.04.004

K.R., W., K.R., T., J.A., R., M.P., R., N.J., M., J.F., F., J.D., C., T.-A., N., C.D., F., S.A., T., & M.S., C. (2014). PCSK9 is a critical regulator of the innate immune response and septic shock outcome. *Science Translational Medicine*, *6*(258), 258ra143. http://stm.sciencemag.org/content/6/258/258ra143.full.pdf%0Ahttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed12&NEWS=N&AN=2014858109

Kanaze, F. I., Bounartzi, M. I., Georgarakis, M., & Niopas, I. (2007). Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *European Journal of Clinical Nutrition*, *61*(4), 472–477. https://doi.org/10.1038/sj.ejcn.1602543

Khera, A. V., Won, H. H., Peloso, G. M., Lawson, K. S., Bartz, T. M., Deng, X., van Leeuwen, E. M., Natarajan, P., Emdin, C. A., Bick, A. G., Morrison, A. C., Brody, J. A., Gupta, N., Nomura, A., Kessler, T., Duga, S., Bis, J. C., van Duijn, C. M., Cupples, L. A., … Kathiresan, S. (2016). Diagnostic Yield and Clinical Utility of Sequencing Familial Hypercholesterolemia Genes in Patients With Severe Hypercholesterolemia. In *Journal of the American College of Cardiology* (Vol. 67, Issue 22). Elsevier Ltd. https://doi.org/10.1016/j.jacc.2016.03.520

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2021). PubChem in 2021: New data content and improved web interfaces. *Nucleic Acids Research*, *49*(D1), D1388–D1395. https://doi.org/10.1093/nar/gkaa971

Koes, D. R., & Camacho, C. J. (2012). ZINCPharmer: Pharmacophore search of the ZINC database. *Nucleic Acids Research*, *40*(W1), 409–414. https://doi.org/10.1093/nar/gks378

Langer, T., & Hoffmann, R. D. (2008). Pharmacophore Modelling. *Encyclopedia of Molecular Pharmacology*, 960–960. https://doi.org/10.1007/978-3-540-38918-7_6434

Laskowski, R. A., Jabłońska, J., Pravda, L., Vařeková, R. S., & Thornton, J. M. (2018). PDBsum: Structural summaries of PDB entries. *Protein Science*, *27*(1), 129–134. https://doi.org/10.1002/pro.3289

Latimer, J., Batty, J. A., Neely, R. D. G., & Kunadian, V. (2016). PCSK9 inhibitors in the prevention of cardiovascular disease. *Journal of Thrombosis and Thrombolysis*, *42*(3), 405–419. https://doi.org/10.1007/s11239-016-1364-1

Leinonen, R., Garcia Diez, F., Binns, D., Fleischmann, W., Lopez, R., & Apweiler, R. (2004). UniProt archive. *Bioinformatics*, *20*(17), 3236–3237. https://doi.org/10.1093/bioinformatics/bth191

Liu, L., & Chen, J. (2008). Solubility of hesperetin in various solvents from (288.2 to 323.2) K. *Journal of Chemical and Engineering Data*, *53*(7), 1649–1650. https://doi.org/10.1021/je800078j

Lonn, E. (2001). Antiatherosclerotic effects of ace inhibitors:Where are we now? *American Journal of Cardiovascular Drugs*, *1*(5), 315–320. https://doi.org/10.2165/00129784-200101050-00001

Lovering, F., Bikker, J., & Humblet, C. (2009). Escape from flatland: Increasing saturation as an approach to improving clinical success. *Journal of Medicinal Chemistry*, *52*(21), 6752–6756. https://doi.org/10.1021/jm901241e

Mahley, R. W. (2016). Apolipoprotein E: from cardiovascular disease to neurodegenerative disorders. *Journal of Molecular Medicine*, *94*(7), 739–746. https://doi.org/10.1007/s00109-016-1427-y

Maxwell, K. N., Soccio, R. E., Duncan, E. M., Sehayek, E., & Breslow, J. L. (2003). Novel putative SREBP and LXR target genes identified by microarray analysis in liver of cholesterol-fed mice. *Journal of Lipid Research*, *44*(11), 2109–2119. https://doi.org/10.1194/jlr.M300203-JLR200

McNutt, M. C., Lagace, T. A., & Horton, J. D. (2007). Catalytic activity is not required for secreted PCSK9 to reduce low density lipoprotein receptors in HepG2 cells. *Journal of Biological Chemistry*, *282*(29), 20799–20803. https://doi.org/10.1074/jbc.C700095200

Petrilli, W. L., Adam, G. C., Erdmann, R. S., Abeywickrema, P., Agnani, V., Ai, X., Baysarowich, J., Byrne, N., Caldwell, J. P., Chang, W., DiNunzio, E., Feng, Z., Ford, R., Ha, S., Huang, Y., Hubbard, B., Johnston, J. M., Kavana, M., Lisnock, J. M., … Imbriglio, J. E. (2020). From Screening to Targeted Degradation: Strategies for the Discovery and Optimization of Small Molecule Ligands for PCSK9. *Cell Chemical Biology*, *27*(1), 32-40.e3. https://doi.org/10.1016/j.chembiol.2019.10.002

Pines, A., & Fisman, E. Z. (2003). ACE inhibition with moexipril: A review of potential effects beyond blood pressure control. *American Journal of Cardiovascular Drugs*, *3*(5), 351–360. https://doi.org/10.2165/00129784-200303050-00006

Pitt, B. (1995). Potential role of angiotensin converting enzyme inhibitors in the treatment of atherosclerosis. *European Heart Journal*, *16*(SUPPL. K), 49–54. https://doi.org/10.1093/eurheartj/16.suppl_k.49

RA, L., & MB, S. (2011). LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling*, *51*, 2778–2786.

Ramharack, P., & Soliman, M. E. S. (2018). Bioinformatics-based tools in drug discovery: the cartography from single gene to integrative biological networks. *Drug Discovery Today*, *23*(9), 1658–1665. https://doi.org/10.1016/j.drudis.2018.05.041

Schneidman-Duhovny, D., Dror, O., Inbar, Y., Nussinov, R., & Wolfson, H. J. (2008). Deterministic pharmacophore detection via multiple flexible alignment of drug-like molecules. *Journal of Computational Biology*, *15*(7), 737–754. https://doi.org/10.1089/cmb.2007.0130

Seidah, N. G., Abifadel, M., Prost, S., Boileau, C., & Prat, A. (2017). The proprotein convertases in hypercholesterolemia and cardiovascular diseases: Emphasis on proprotein convertase subtilisin/Kexin 9. *Pharmacological Reviews*, *69*(1), 33–52. https://doi.org/10.1124/pr.116.012989

Semenova, A. E., Sergienko, I. V., García-Giustiniani, D., Monserrat, L., Popova, A. B., Nozadze, D. N., & Ezhov, M. V. (2020). Verification of underlying genetic cause in a cohort of russian patients with familial hypercholesterolemia using targeted next generation sequencing. *Journal of Cardiovascular Development and Disease*, *7*(2). https://doi.org/10.3390/JCDD7020016

Shelness, G. S., & Sellers, J. A. (2001). Very-low-density lipoprotein assembly and secretion. *Current Opinion in Lipidology*, *12*(2), 151–157. https://doi.org/10.1097/00041433-200104000-00008

Sterling, T., & Irwin, J. J. (2015). ZINC 15 - Ligand Discovery for Everyone. *Journal of Chemical Information and Modeling*, *55*(11), 2324–2337. https://doi.org/10.1021/acs.jcim.5b00559

Ukuku, D. O., Bari, L., & Kawamoto, S. (2012). Hydrogen Peroxide. *Decontamination of Fresh and Minimally Processed Produce*, *2*, 197–214. https://doi.org/10.1002/9781118229187.ch11

Urban, D., Pöss, J., Böhm, M., & Laufs, U. (2013). Targeting the proprotein convertase subtilisin/kexin type 9 for the treatment of dyslipidemia and atherosclerosis. *Journal of the American College of Cardiology*, *62*(16), 1401–1408. https://doi.org/10.1016/j.jacc.2013.07.056

Wan, Q., Liu, Z., Yang, Y., & Cui, X. (2018). Suppressive effects of berberine on atherosclerosis via downregulating visfatin expression and attenuating visfatin-induced endothelial dysfunction. *International Journal of Molecular Medicine*, *41*(4), 1939–1948. https://doi.org/10.3892/ijmm.2018.3440

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., De Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., & Schwede, T. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Research*, *46*(W1), W296–W303. https://doi.org/10.1093/nar/gky427

Wei, W., Cherukupalli, S., Jing, L., Liu, X., & Zhan, P. (2020). Fsp3: A new parameter for drug-likeness. *Drug Discovery Today*, *25*(10), 1839–1845. https://doi.org/10.1016/j.drudis.2020.07.017

Wishart, D. S. (2005). Bioinformatics in drug development and assessment. In *Drug Metabolism Reviews* (Vol. 37, Issue 2). https://doi.org/10.1081/DMR-200055225

Xu, S., Luo, S., Zhu, Z., & Xu, J. (2019). Small molecules as inhibitors of PCSK9: Current status and future challenges. *European Journal of Medicinal Chemistry*, *162*, 212–233. https://doi.org/10.1016/j.ejmech.2018.11.011

Yamada, Y., Gohda, S., Abe, K., Togo, T., Shimano, N., Sasaki, T., Tanaka, H., Ono, H., Ohba, T., Kubo, S., Ohkubo, T., & Sato, S. (2017). Carbon materials with controlled edge structures. *Carbon*, *122*(October 1995), 694–701. https://doi.org/10.1016/j.carbon.2017.07.012

Yang, H., Lou, C., Sun, L., Li, J., Cai, Y., Wang, Z., Li, W., Liu, G., & Tang, Y. (2019). AdmetSAR 2.0: Web-service for prediction and optimization of chemical ADMET properties. *Bioinformatics*, *35*(6), 1067–1069. https://doi.org/10.1093/bioinformatics/bty707

Younas, F., Aslam, B., Muhammad, F., Mohsin, M., Raza, A., Faisal, M. N., Shamshud-Ul-Hassan, & Majeed, W. (2017). Haematopoietic effects of angelica sinensis root cap polysaccharides against lisinopril-induced anaemia in albino rats. *Pharmaceutical Biology*, *55*(1), 108–113. https://doi.org/10.1080/13880209.2016.1230635

Zhang, D., Wang, Y., Yi, M., Zhang, S., & Wu, Y. (2020). The Peroxisome Proliferator-Activated Receptor γ Agonist Pioglitazone Protects Vascular Endothelial Function in Hypercholesterolemic Rats by Inhibiting Myeloperoxidase. *Cardiology Research and Practice*, *2020*. https://doi.org/10.1155/2020/1845969

Zhao, P. J., Ban, M. R., Iacocca, M. A., McIntyre, A. D., Wang, J., & Hegele, R. A. (2019). Genetic Determinants of Myocardial Infarction Risk in Familial Hypercholesterolemia. *CJC Open*, *1*(5), 225–230. https://doi.org/10.1016/j.cjco.2019.06.001