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DISCOVERY OF SMALL MOLECULE INHIBITORS OF PCSK9 USING VIRTUAL SCREENING AS POTENTIAL THERAPEUTICS FOR ATHEROSCLEROSIS

Matthew N. Siciliano [1]

[1] Glastonbury High School, Glastonbury, Connecticut, United States of America

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Abstract

High LDL cholesterol can cause heart disease. Current treatments like statins are not always effective, so new therapies are needed. PCSK9 is a protein that reduces the number of LDL receptors in the liver, which keeps cholesterol in the blood, making it a strong target for new drugs. Blocking PCSK9 with small molecules could be a better alternative to current treatments. To find potential inhibitors, I first analyzed the PCSK9 protein to locate possible binding sites using computational methods, which revealed multiple sites that small molecules could target. Then, I created two pharmacophore maps and screened two chemical libraries to find molecules that fit the key interactions. I used molecular docking simulations to estimate how strongly these molecules bind to PCSK9, identifying several with strong predicted binding. Using SwissADME to check drug likeness and absorption properties, we narrowed down the candidates to seven molecules suitable for oral use. Finally, predicted toxicity was analyzed which helped identify the safest and most effective compounds. After comparing binding strength, drug likeness, and toxicity, Z73447142 and Z52103291 are the two most promising drug candidates for PCSK9. These molecules could be further tested using biophysical binding assays and cell-based experiments to confirm their effects, with the goal of eventually developing new oral treatments to lower cholesterol and reduce cardiovascular risk.

Keywords: *PCSK9, cholesterol, virtual screening, molecular docking, drug discovery*

Introduction

Cholesterol is a type of fat that your body needs to build cells and make hormones, but having too much in your blood can be bad and lead to cardiovascular disease (Cui et al., 2025; Guo et al., 2024). It travels through the blood inside particles called lipoproteins. Low density lipoprotein (LDL) carries cholesterol to the body's tissues while high density lipoprotein (HDL) takes extra cholesterol back to the liver to get rid of it (Cui et al., 2025; Guo et al., 2024). The liver controls cholesterol by making it, absorbing it from food, and removing LDL through LDL receptors (Cui et al., 2025; Guo et al., 2024). If this balance is disrupted, LDL can build up in artery walls and cause atherosclerosis, which is when arteries get clogged and can lead to

heart attacks or strokes. Understanding these processes is important before learning about PCSK9 because this protein affects how the liver handles LDL.

Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is a protein made mostly in the liver that controls cholesterol levels by deciding how many LDL receptors are on liver cells (Ajoolabady et al., 2025; Blanchard et al., 2019). It sticks to LDL receptors and makes the liver break them down instead of recycling them to the surface to catch more LDL (Ajoolabady et al., 2025; Blanchard et al., 2019). So when there's too much PCSK9, there aren't enough receptors, and LDL cholesterol stays in the blood. If there's less PCSK9, more LDL gets cleared out (Ajoolabady et al., 2025; Blanchard et al., 2019). Some research also suggests that PCSK9 might influence other parts of metabolism, like blood sugar and inflammation, which could also affect heart health (Ajoolabady et al., 2025). Because of all this, PCSK9 plays a critical role in cholesterol control, and that's why scientists are focusing on it when making new cholesterol lowering drugs.

When PCSK9 is hyperactive, LDL cholesterol levels increase, which raises the risk of cardiovascular events like heart attacks and strokes (Bodapati et al., 2023; Katzmann & Laufs, 2024; Jeswani et al., 2024). Drugs that block PCSK9, like monoclonal antibodies and newer therapeutic approaches, can lower LDL cholesterol and reduce cardiovascular risk significantly (Bodapati et al., 2023; Katzmann & Laufs, 2024; Jeswani et al., 2024; Coppinger et al., 2022). Studies have shown that PCSK9 inhibitors can reduce LDL cholesterol by approximately 50–60% more than what is achieved with statins, which are commonly prescribed drugs that are used to lower LDL cholesterol and triglycerides, which is known to reduce heart attacks and strokes (Bodapati et al., 2023; Katzmann & Laufs, 2024; Jeswani et al., 2024; Coppinger et al., 2022; Chen et al., 2019; Monami et al., 2019). For example, monoclonal antibody drugs like Evolocumab and Alirocumab, which were approved in 2015, were tested in large human outcome trials and showed significant reductions in LDL cholesterol and major cardiovascular events in high risk patients who were already using statin therapy (Rajtar-Salwa et al., 2024). These drugs have been effective in a wide range of patients, including ones who continue to have high LDL levels despite statin use (Bodapati et al., 2023; Katzmann & Laufs, 2024; Jeswani et al., 2024; Coppinger et al., 2022; Chen et al., 2019; Monami et al., 2019). Researchers are also investigating whether PCSK9 inhibitors may benefit patients with diabetes or other metabolic conditions, since PCSK9 appears to influence processes beyond cholesterol metabolism (Monami et al., 2019; Ruhela et al., 2025). Overall, blocking PCSK9 has become one of the most effective strategies for lowering LDL cholesterol and preventing serious cardiovascular disease (Bodapati et al., 2023; Katzmann & Laufs, 2024; Jeswani et al., 2024; Coppinger et al., 2022; Ruhela et al., 2025).

Currently, the main way doctors lower LDL cholesterol is still with statins, which work by reducing how much cholesterol the liver makes. Statins are effective for a lot of people, but there are still patients who either don't reach their LDL goals or can't tolerate higher doses because of side effects like muscle pain (Santulli et al., 2025; Li et al., 2025). Because of this, other drugs such as ezetimibe are sometimes added, which lowers cholesterol absorption in the intestine and gives an extra drop in LDL when statins don't work good enough on their own (Santulli et al., 2025; Liu et al., 2024). Recently, PCSK9 inhibitors have become a major option for patients at high cardiovascular risk, especially those with very high LDL or heart disease (Kao et al., 2025; Garwood et al., 2025). These therapies can lower LDL much more than the traditional treatments, which is why guidelines are starting to recommend them earlier for select high risk patients (Li et al., 2025; Garwood et al., 2025; Nicholls & Nelson, 2025). Even with all these options, managing cholesterol can still be difficult, and many patients need a combination of treatments to successfully get their LDL to safe levels.

PCSK9 is influenced a lot by genetics, which is very important in people with hypercholesterolemia (FH). FH is usually caused by mutations that affect how LDL receptors work, and PCSK9 mutations can make the condition even worse by increasing LDL levels even more (Grejtakova et al., 2025; Kuang et al., 2025). Some genetic variants cause PCSK9 to be overactive, which leads

to very high cholesterol starting at a young age, which increases the risk of early heart disease (Grejtakova et al., 2025; Huh & Kim, 2025). People with a loss of function PCSK9 mutations tend to have much lower LDL levels and a lower risk of cardiovascular events, which helped inspire the development of PCSK9 targeted drugs (Kuang et al., 2025; Huh & Kim, 2025). Because FH is inherited, understanding the genetic role of PCSK9 is important for more than just treatment, but also for screening family members who may be at risk (Grejtakova et al., 2025; Maştaleru et al., 2025). This genetic connection explains why PCSK9 inhibitors work even better in FH patients who do not respond enough to statins alone.

While most PCSK9 inhibitors that are used today are injectable antibodies, researchers are now making small molecule and oral PCSK9 inhibitors, which could make the treatment process easier for patients. The new drugs will block PCSK9 inside cells or interfere with how it is made or functions, instead of neutralizing it in the bloodstream (Ferri & Marodin, 2024; Ho et al., 2025). Early studies show that some of these small molecule approaches can significantly lower LDL cholesterol, although a lot are still in clinical trials and not available globally (Ferri & Marodin, 2024; Ho et al., 2025). An advantage of small molecule inhibitors is that they could be taken as pills, which might improve patient compliance compared to injections (Farhan et al., 2025). However, researchers are still working through challenges like making sure these drugs are specific, safe, and effective in the long term (Ferri & Marodin, 2024; Abdulla et al., 2024). Even so, this area is moving at a fast pace, and if it works, small molecule PCSK9 inhibitors could become a big part of future cholesterol treatment alongside existing therapies (Farhan et al., 2025; Abdulla et al., 2024).

Materials and methods

Analysis of binding sites

Geometric method

First I went to the protein plus website (https://proteins.plus/), I entered the PDB code 6U26, I clicked go, and then I clicked DoGSiteScorer twice, and then I kept all of the settings unchanged and clicked calculate.

PrankWeb

First I went to the PrankWeb website (https://prankweb.cz/). I entered the PDB code 6U26, and then clicked submit.

Identifying Small Molecules

Pharmacophore Map A Using MolPort

First I went to the Pharmit website (https://pharmit.csb.pitt.edu/) and entered the PDB code 6U26 in the "start from PDB" box, and left everything else unchanged and pressed submit. I went into the "Visualization" settings and changed ligand to none, kept results as stick, and changed receptor to none. I put the receptor surface opacity at the minimum and changed the background color to black, which did not have an effect on the results; it was solely for preference. In the Pharmacophore box, I turned on the HydrogenDonors, the first HydrogenAcceptor, and the first Hydrophobic and turned everything else off. I then clicked "Search MolPort" at the top.

Pharmacophore Map A Using Enamine

To use the Enamine library with Map A, I followed all of the steps as in 2.2.1, but instead of clicking "Search MolPort", I clicked the downwards arrow, and selected "Enamine" and then clicked "Search Enamine".

Pharmacophore Map B Using MolPort

Map B has all of the steps as 2.2.1 except for what is turned on and off in the "Pharmacophore" section. For Map B, the HydrogenDonors, the first HydrogenAcceptor, the first Hydrophobic, and the fourth Aromatic were turned on and everything else was off. I clicked "Search MolPort"

Pharmacophore Map B Using Enamine

To use the Enamine library with Map B, I followed the same steps as in 2.2.1 except I clicked the downwards arrow next to "Search MolPort" and selected Enamine in the dropdown menu. Then, I clicked "Search Enamine".

SwissDock Molecular Docking Methods

First I went to the SwissDock website (https://www.swissdock.ch/) to test how the different molecules bind to the PCSK9 protein. I used molecules from the Enamine (https://enaminestore.com/) and Molport (https://www.molport.com/shop/index) websites, and the SMILES codes were also copied from these websites. Once the SMILES was put into the SwissDock website and the green checkmark was showing, then

I clicked "Prepare Ligand". After preparing the ligand, the target protein was set up using the Protein Data Bank (PDB) code 6U26. Only chain A of the protein was used, and all heteroatoms were removed. I selected "Prepare Target" and it was confirmed once the green checkmark appeared. Next, I defined the search space. The search box center was set to 31, 24, 48, and the search box size was set to 31, 34, 47. These values were kept the same for all of the compounds. After setting the search space I selected "Check Parameters". Finally, the docking simulation was started by clicking "Start Docking".

Evaluation of Drug Likeness Using SwissADME

First I went to the SwissADME website (https://www.swissadme.ch/) and I inserted the SMILES of my top 7 compounds from the previous experiment. Once all the SMILES were in, I clicked run. For each compound, I recorded the molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, and the consensus Log P. In addition to those, ESOL solubility and predicted gastrointestinal (GI) absorption were collected.

Identifying Drug Toxicity Using Protox 3

First I went to the Protox 3 website (https://tox.charite.de/protox3/index.php?site=home) and I went to Tox Prediction at the top and clicked it. Then, I put in the SMILES code for the compound I was using and clicked the blue smiles button afterwards. Then I went down to the bottom and clicked the all button to select all pathways. Finally, I clicked Start Tox-Prediction and recorded my results.

Results and discussion

Identifying Potential Binding Sites

Protein Plus/DoGSiteScorer

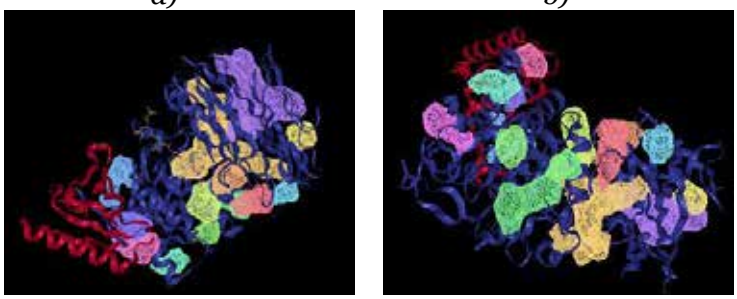
The goal of this experiment was to identify potential binding sites on PCSK9 that could be targeted by small molecule inhibitors. Using DoGSiteScorer, a lot of surface pockets were discovered on the PCSK9 protein (Figure 1). Out of all of them, pockets P_0, P_1, and P_2 showed the most promising characteristics based on size and drug score (Table 1). Pocked P_1 had the highest drug score, .83, and a large pocket volume, 944.38 Å^3, showing that it is a strong option for binding small molecules. Pocket P_0 had the largest volume overall, 1340.9 Å^3, with a high drug score of 0.80, while P_2 also showed a good drug score of 0.90 but had a smaller volume. Smaller pockets like P_10 – P_13 had low drug scores which indicates that they are worse than the others for small molecule binding. Overall, DoGSiteScorer identified multiple binding sites, with P_1 as the best candidate due to its balance of size, surface area, and drug score.

Table 1. *Results from DoGSiteScorer showing the Name, Pocket Volume, Surface Area, Drug Score, and corresponding color in figure 1*

Name	Color	Pocket Volume (Å^3)	Surface Area (Å^2)	Drug Score
P_0	Khaki	1340.9	1533.29	0.8
P_1	Light Purple	944.38	1010.19	0.83
P_10	Lime Green	131.82	298.94	0.3
P_11	Pink	127.54	171.07	0.28
P_12	Cyan	111.72	183.33	0.25
P_13	Yellow	107.87	204.79	0.21
P_2	Light Green	437.89	684.43	0.8
P_3	Red	284.17	612.05	0.49
P_4	Dark Cyan	217.87	233.32	0.48
P_5	Bright Yellow	215.05	414.08	0.49
P_6	Teal	181.6	279.15	0.43
P_7	Orange	146.53	305.24	0.38

Figure 1. *Images from Do G Site Scorer showing potential binding sites in PCSK9 using the geometric method. The blue ribbon represents the structure of the protein and the colored spheres represent the binding sites*

a) *b)*



PrankWeb

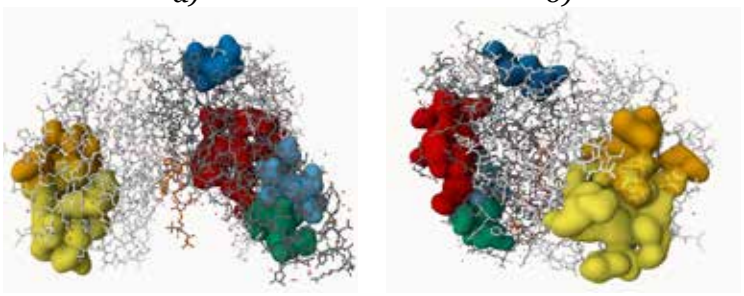
PrankWeb was used to identify and rank potential binding pockets on the PCSK9 protein and to look at the amino acids in each site (Figure 2, Table 2). The highest ranked pocket that PrankWeb identified was pocket 1, which included Gln226, Trp42, and Leu318 residues, all located in the same binding site. The other pockets were ranked lower and were in different areas of the protein's surface. For example, Pocket 2 included Val614, His561, and Gly560, while Pocket 3 included Glu582, Pro609, and Gln557. Other lower ranked pockets involved residues like Pro179, Leu82, Asn177, and Gly46. Overall, PrankWeb found multiple potential binding pockets on PCSK9, with Pocket 1 as the highest ranked.

Table 2. *Results from PrankWeb showing potential pockets in PCSK9 using the machine learning method including the ranking, the score, and the number of residues*

Color	Pocket Ranking	Pocket Score	No. of Residues
Red	1	13.67	19
Yellow	2	7.20	25
Orange	3	3.84	13
Cyan	4	3.10	10
Light Green	5	2.18	8
Blue	6	1.36	8

Figure 2. *Images from PrankWeb showing binding spots on PCSK9. The colored shapes represent the binding sites and the white and gray residues represent the structure of the protein*

a) *b)*



Pharmacophore Based Virtual Screening of PCSK9

Two pharmacophore maps were generated from the predicted PCSK9 binding site and used to identify small molecules with similar interactions (Figure 3). A pharmacophore represents a three dimensional map of interactions that are required for a molecule to bind to a protein, including the type of key interactions such as hydrogen bonding and

hydrophobic contacts. Each pharmacophore map was screened independently against two compound libraries, MolPort and Enamine, using the Pharmit server. These virtual screens evaluated millions of candidate molecules per library and ranked them based on how well they matched the pharmacophore features, measured by RMSD, which measures how closely a compound aligns with the pharmacophore in a three dimensional space. From these screening results, the highest ranking compounds from all four searches (Map A-MolPort, Map A-Enamine, Map B-MolPort, Map B-Enamine) were selected with a total of 17 small molecules. These compounds represent the best overall matches across both pharmacophore maps and both libraries. The final set of 17 compounds included molecules identified using both Map A and Map B, as well as compounds from both MolPort and Enamine. Of the 17 selected compounds, 15 were from the Enamine library while the remaining two were from MolPort. As shown in Table 3, the RMSD values of the selected compounds ranged from 0.019 to 0.034 Å, which means that there is excellent alignment between the compounds and their pharmacophore features. The lowest RMSD value, 0.019 Å, was from MolPort-051–895–715, which was found using pharmacophore map B, which suggests a very strong match.

Figure 3. *Pharmacophore maps A and B. The white spheres represent hydrogen donors, the pink sphere represents aromatic, the green spheres represent hydrophobic, and the orange spheres represent hydrogen acceptors*

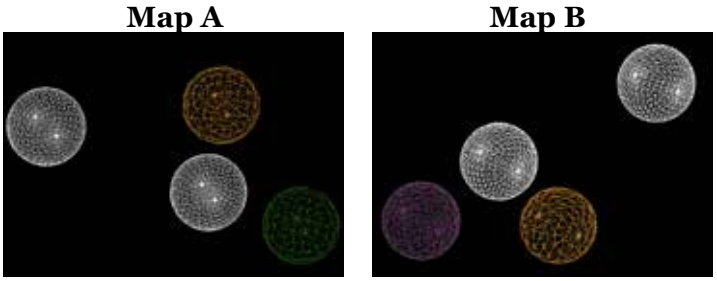
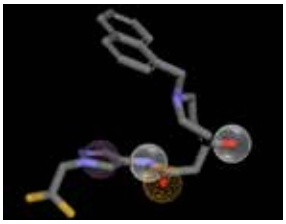
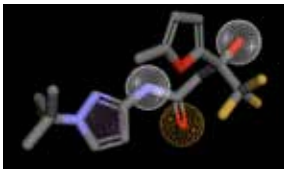
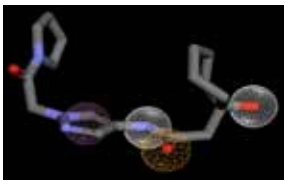
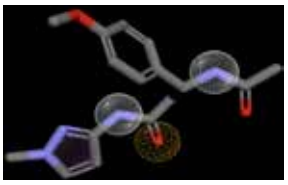
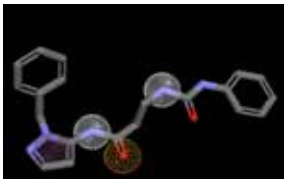
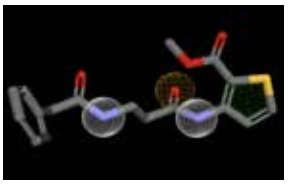
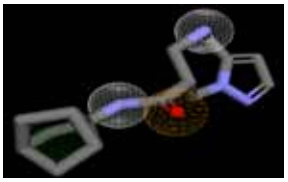
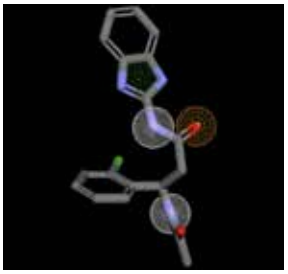
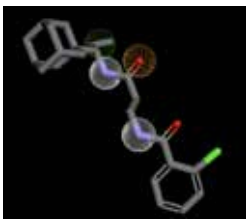
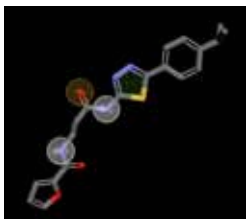
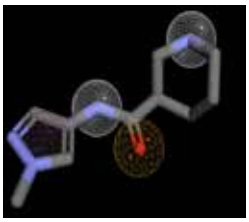
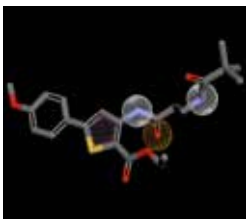
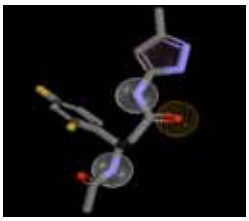
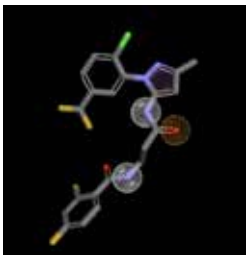


Table 3. *Top 17 potential compounds identified through pharmacophore based virtual screening of PCSK9 using the Pharmit server. Compounds are ranked by RMSD, where lower values indicate better alignment with the pharmacophore features. Two pharmacophore maps (A and B) were used for the screening*

Name	RMSD	Pharmacophore Map	Library	Figure
Molport-051-895-715	0.019	B	Molport	
Z1584010628	0.022	A	Enamine	
Z373772438	0.029	B	Enamine	

Name	RMSD	Pharmacoph-ore Map	Library	Figure
Z1171598708	0.029	B	Enamine	
Molport-020-269-245	0.031	B	Molport	
Z225654462	0.032	B	Enamine	
Z52103291	0.032	B	Enamine	
Z225520308	0.033	A	Enamine	
Z4422192504	0.033	A	Enamine	
Z27664946	0.034	A	Enamine	
Z440626236	0.034	A	Enamine	

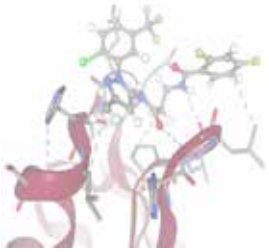
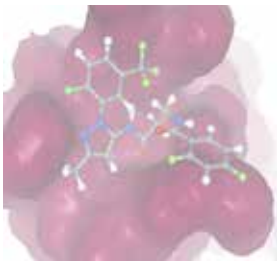
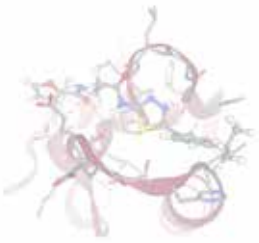
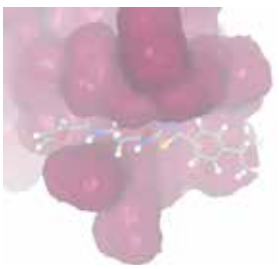
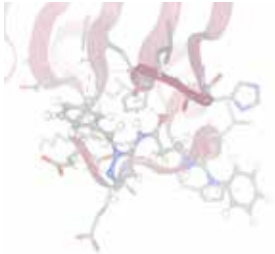
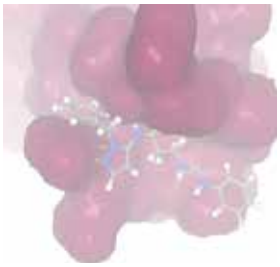
Name	RMSD	Pharmacophore Map	Library	Figure
Z28609494	0.034	A	Enamine	
Z73447142	0.034	A	Enamine	
Z1095263098	0.034	B	Enamine	
Z280289292	0.034	B	Enamine	
Z1279873071	0.034	B	Enamine	
Z764690862	0.034	B	Enamine	

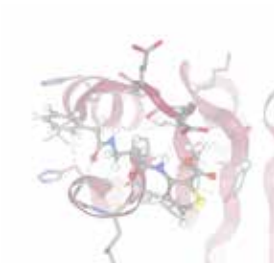
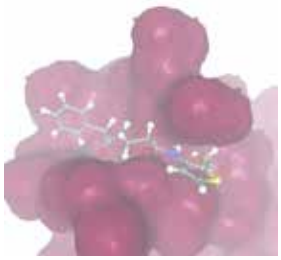
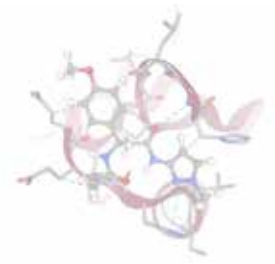
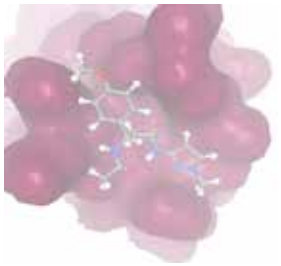
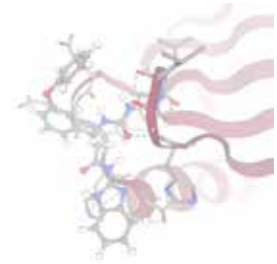
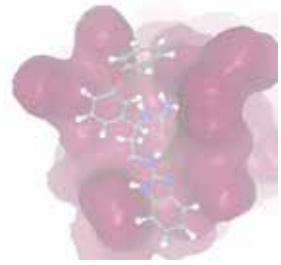
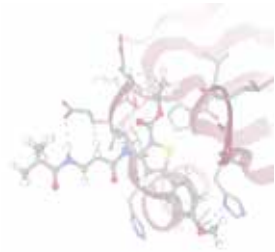
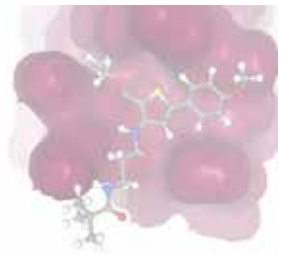
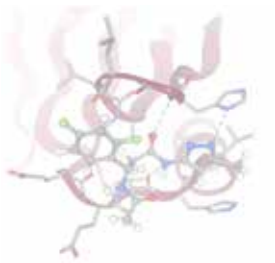
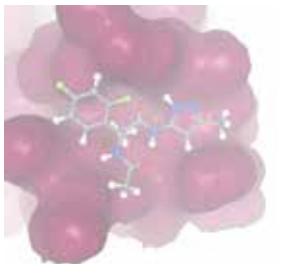
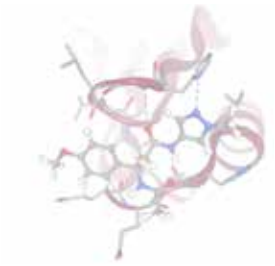
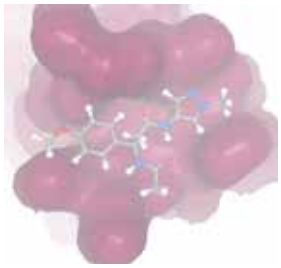
Swiss Dock Virtual Screening Results

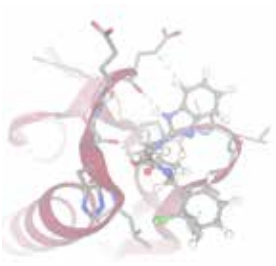
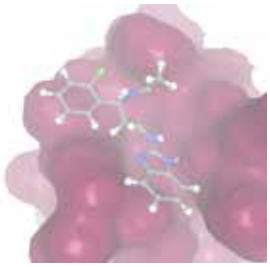
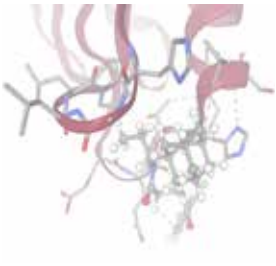
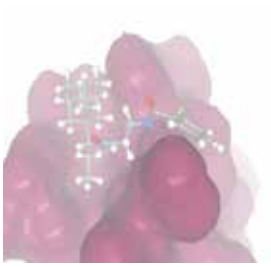
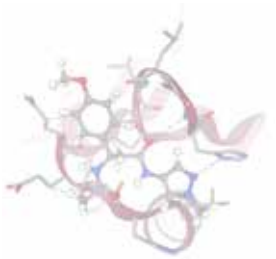
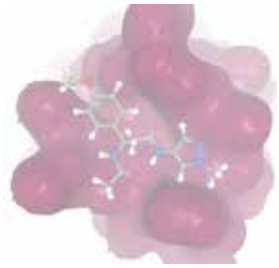
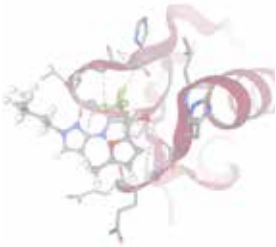
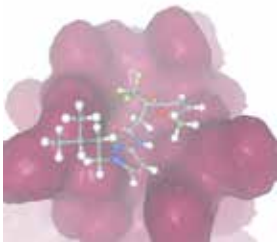
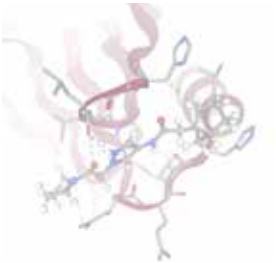
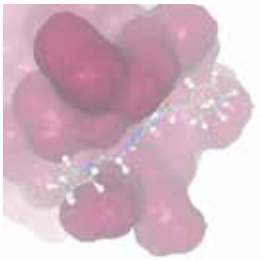
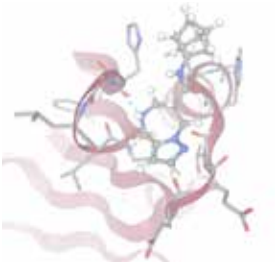
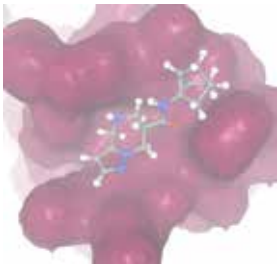
SwissDock was used to test how well each compound might bind to the PCSK9 protein, and SwissParam scores were used to compare their predicted binding strength. Molecular docking is a computational method that predicts how a small molecule, like a lipid, fits into a protein's binding site and estimates the strength of that interaction. The SwissDock scoring provides an estimate of the Gibbs free energy of binding (ΔG), in kilocalories per mol (kcal/mol). Gibbs free energy represents how energetically favorable the interaction is, and more negative ΔG values indicate stronger and more stable binding. Overall,

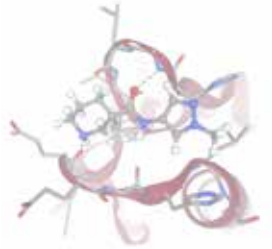
the compounds showed SwissParam scores ranging from around –6.22 kcal/mole to –7.20 kcal/mol, which means that all of the molecules showed at least decent predicted binding to the PCSK9 protein. Most of the compounds had scores between –6.7 kcal/mole and –7.1 kcal/mol, which shows that many of them behaved similarly in the docking simulation. The strongest predicted binder was Z764690862 from the Enamine library, which had the most negative SwissParam score of –7.2042 kcal/mol (Table 4). Two other molecules, Z73447142 and Z52103291, also showed strong predicted binding with SwissParam scores of –7.1420 kcal/mol and –7.1030 kcal/mol respectively. In total, three compounds had SwissParam scores below –7.10 kcal/mol, which indicates that these are the top three molecules for binding. Surprisingly, the molecule with the best SwissParam score also had the worst RMSD value. The fact that the top ΔG scorer had a high RMSD suggests that binding strength and structural stability do not perfectly correlate. Overall, the RMSD and SwissParam scores do not fully agree on which molecule would bind the best.

Table 4. *SwissParam scores obtained from SwissDock virtual screening of selected compounds docked to the target protein. More negative SwissParam scores indicate stronger predicted binding interactions. Compounds are listed with their corresponding chemical library sources. The top figures in each row show the interactions in the molecule and the bottom figure shows the molecule on the protein's surface. Blue represents hydrogen bonds, yellow represents ionic interactions, orange represents Cation-π interactions, the black represents hydrophobic contacts, and the green represents the π-stacking interactions. The red ribbon and blob represent the protein's structure*

Molecule ID	SwissParam Score (Kcal/mol)	Residue Interaction Pose	Protein Surface
Z764690862	–7.2042		
Z73447142	–7.1420		
Z52103291	–7.1030		

Molecule ID	SwissParam Score (Kcal/mol)	Residue Interaction Pose	Protein Surface
Z225520308	–6.9003		
Z225654462	–6.8825		
Z27664946	–6.8772		
Z280289292	–6.8420		
Z1279873071	–6.8089		
Z373772438	–6.8059		

Molecule ID	SwissParam Score (Kcal/mol)	Residue Interaction Pose	Protein Surface
Z440626236	–6.7975		
Z28609494	–6.7942		
Z1171598708	–6.7447		
Z1584010628	–6.5722		
Mol-port-020–269–245	–6.4930		
Z4422192504	–6.3860		

Molecule ID	SwissParam Score (Kcal/mol)	Residue Interaction Pose	Protein Surface
Z1095263098	–6.2196		

Evaluating Drug Likeness with SwissAdme Server

To determine whether the compounds that we have found could function as oral drug candidates, we analyzed the physicochemical and pharmacokinetic properties of the top seven compounds using the SwissADME server. In this experiment, Lipinski's Rule of Five, predicted solubility, and gastrointestinal absorption were analyzed.

Lipinski's Rule of Five is used to estimate whether a compound is likely to be a good oral drug. According to this rule, a molecule should have no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular weight under 500 daltons, and a consensus LogP value under five. If a compound does not meet all of these rules, it is less likely to be absorbed effectively and was not allowed to continue in this study.

As shown in Table 5, all seven molecules met Lipinski's rules with zero violations. Molecular weights ranged from 316.36 Da to 486.82 Daltons, and none exceeded the 500 Da limit (Table 5). The number of hydrogen bond donors ranged from 2 to 4, and hydrogen bond acceptors ranged from 3 to 8, which all meet the criteria (Table 5). Consensus LogP values ranged from 1.18 to 4.68, which means all of the compounds were below the maximum of 5 (Table 5).

Predicted solubility using the ESOL model was different among all of the compounds (Table 5). Molecule 5 was classified as very soluble. Molecules 2, 3, and 4 were classified as soluble, while Molecules 1, 6, and 7 were moderately soluble (Table 5).

GI absorption also helped narrow down the best compounds. Six of the seven molecules were predicted to have high GI absorption. Molecule 1 was the only compound predicted to have low GI absorption (Table 5), which suggests that even though it meets Lipinski's rules, it may not be absorbed efficiently when taken orally.

Overall, based on the data summarized in Table 5, all seven compounds met the required drug likeness scores and were allowed to continue on in the study.

Table 5. *Summary of Lipinski's Rule of Five parameters (molecular weight, hydrogen bond donors, hydrogen bond acceptors, and consensus LogP), ESOL solubility classification, and predicted gastrointestinal (GI) absorption for the seven compounds using the SwissADME server*

Molecular ID	Molecular Weight (Da)	H-Bond Donors	H-Bond Acceptors	Consensus LogP	Lipinski's Violations	ESOL Solubility	GI Absorption
Z764690862	486.82	2	8	4.88	None	Moderately Soluble	Low
Z73447142	384.45	2	5	2.88	None	Soluble	High

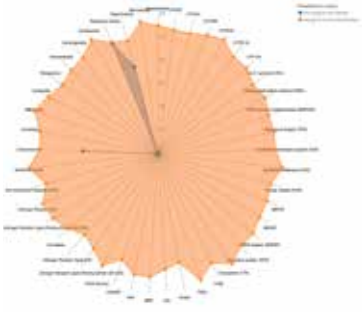
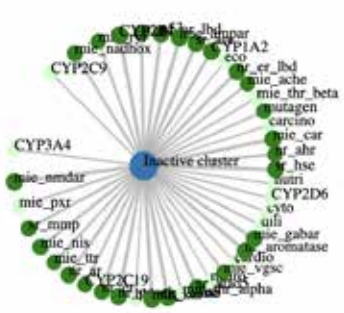
Molecular ID	Molecular Weight (Da)	H-Bond Donors	H-Bond Acceptors	Consensus LogP	Lipinski's Violations	ESOL Solubility	GI Absorption
Z52103291	363.41	3	3	2.11	None	Soluble	High
Z225520308	346.40	2	4	2.37	None	Soluble	High
Z225654462	316.36	2	4	1.18	None	Very Soluble	High
Z27664946	415.44	4	4	2.67	None	Moderately Soluble	High
Z280289292	432.53	2	5	3.80	None	Moderately Soluble	High

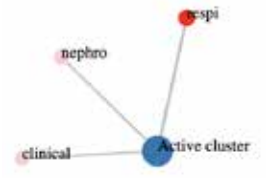
Identifying Drug Toxicity with Protox 3

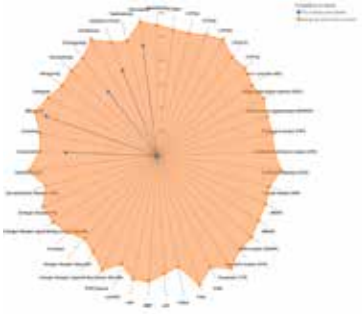
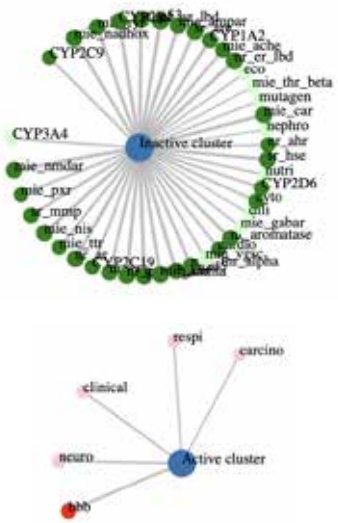
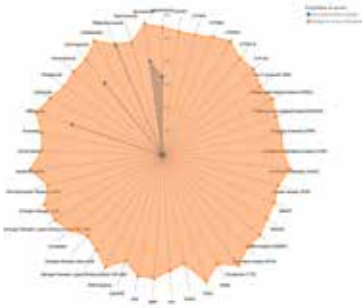
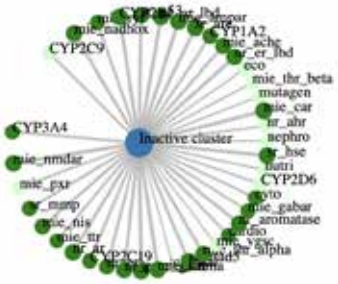
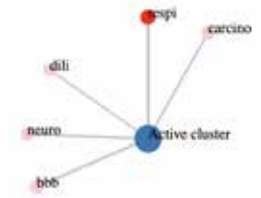
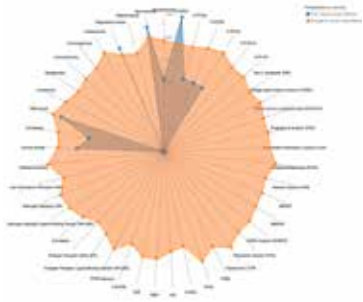
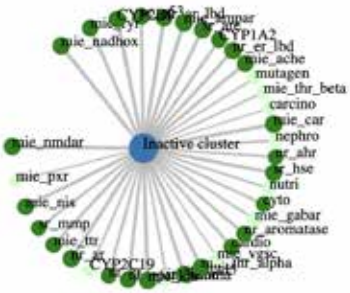
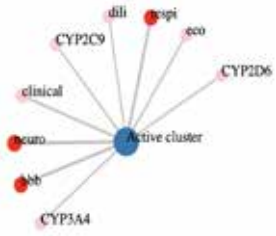
The purpose of this experiment was to evaluate the predicted toxicity of the top compounds using Protox 3 and determine whether their toxicity levels are within the FDA's limits (Table 6). The toxicity was evaluated using predicted LD50 values, toxicity class, and toxicity radar charts. In general, a higher LD50 means the compound is less toxic, and a lower LD50 means it is more toxic.

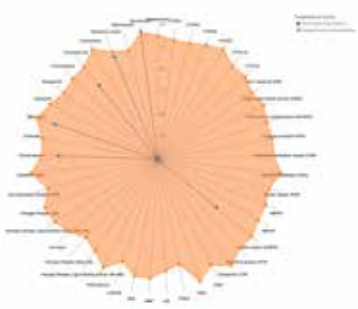
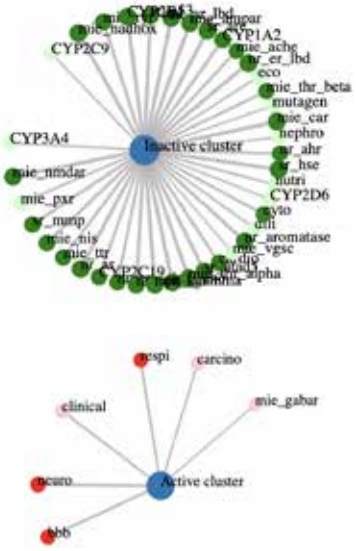
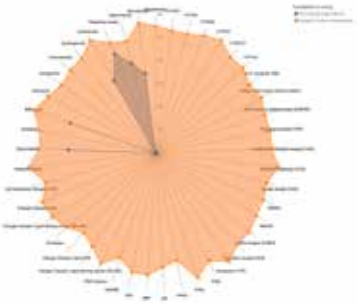
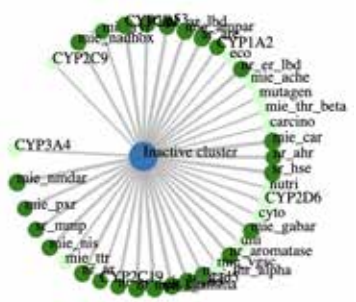
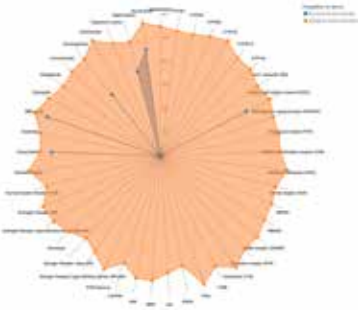
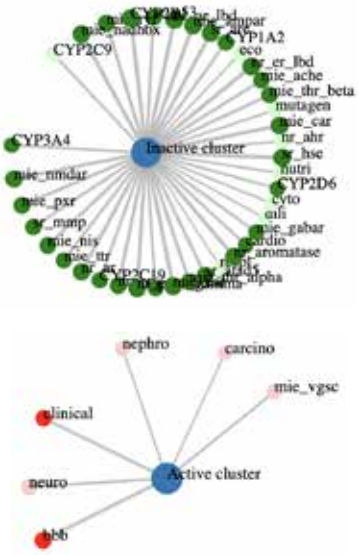
The predicted LD50 values ranged from 300 mg/kg to 2500 mg/kg (Table 6). Z280289292 had the highest LD50, 2500 mg/kg, and had a toxicity class of 5, which means it has the lowest predicted toxicity out of the group. Z225654462 (1500 mg/kg) and four other compounds- Z73447142, Z764690862, Z52103291, and Z225520308 (1000–1100 mg/kg)- were placed in toxicity class 4, which indicates moderate toxicity. Z27664946 had the lowest LD50 value of 300 mg/kg and was classified as toxicity class 3, meaning it is more toxic than the others.

Table 6. *Outcomes from predicted toxicity analysis using Protox 3. LD50 (mg/kg) represents the predicted dose required to cause death in 50% of test subjects, and toxicity class categorizes compounds based on toxicity level. The toxicity radar charts display predicted toxicity endpoints compared to FDA reference thresholds*

Molecule	LD50 (mg/kg)	Predicted Toxicity Class	Toxicity Radar Chart	Active and Inactive Network Chart
Z280289292	2500	5		



Molecule	LD50 (mg/kg)	Predicted Toxicity Class	Toxicity Radar Chart	Active and Inactive Network Chart
Z225654462	1500	4		
Z73447142	1100	4		 
Z764690862	1000	4		 

Molecule	LD50 (mg/kg)	Predicted Toxicity Class	Toxicity Radar Chart	Active and Inactive Network Chart
Z52103291	1000	4		
Z225520308	1000	4		
Z27664946	300	3		

The toxicity radar charts compare each compound's predicted toxicity endpoints to FDA limits. Most of the compounds stayed within the FDA limits across the chart. Z52103291 had one point slightly above the FDA limit, but it was only about 1% higher, so it is still safe. In contrast, Z764690862 had multiple points above the FDA limit, which suggests higher risk across several toxicity categories. Because of this, Z764690862 is the least favorable option from a toxicity standpoint and will not be moving on in the study.

The active and inactive network charts show predicted biological targets related to toxic effects. The active charts represent proteins the compounds may interact with that are connected to toxicity pathways, while the inactive charts represent predicted non-interacting targets.

Overall, most of the compounds showed predicted toxicity levels within the FDA limits.

Conclusion

This study used several computational drug discovery tools to search for small molecules that could block PCSK9, a protein that contributes to high LDL cholesterol. By combining virtual screening, molecular docking, and drug-likeness analysis, two compounds – Z73447142 and Z52103291 – were identified as the strongest potential inhibitors.

Z7344714 had a docking score of –7.1420 kcal/mol and an RMSD of 0.034. This means that it has really stable binding. It has a molecular weight of 384.45 Da, 2 hydrogen bond donors, and 5 hydrogen bond acceptors, and no Lipinski's Rule of Five violations. It also has a consensus LogP of 2.88, was predicted to be soluble, and a high GI absorption. It also had an LD50 of 1100 mg/kg with a predicted toxicity class of 4.

Z52103291 had a docking score of –7.1030 kcal/mol and an RMSD of 0.032. It has a molecular weight of 363.41 Da, 3 hydrogen bond donors, and 3 hydrogen bond acceptors, and no Lipinski's Rule of Five violations. Its consensus LogP was 2.11, it was soluble, and had a high GI absorption. It had an LD50 of 1000 mg/kg with a toxicity class of 4. Although one value on the radar chart was about 1% above the FDA limit, it was only slightly higher so it is still considered safe.

There are a few limitations to this study. First, all experiments were done virtually, so we do not know if the compounds will behave the same way in real cells or in humans. The docking and toxicity predictions might not translate to real biological results. Also, only two chemical libraries were screened, so other potentially better compounds may exist that were not included in this study. Because of this, lab testing has to be done to confirm that the selected compounds actually have the predicted effectiveness from our study.

Overall, Z73447142 is the top compound because it showed slightly stronger binding and a slightly higher LD50 value, which means that it is more stable and safe. This compound should continue for future lab testing and Z52103291 is a good second candidate if Z73447142 isn't effective in a lab setting.

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Contact: matthew.siciliano99@icloud.com