

Article

Potential Hydrophobic Pocket of Squalene Synthase: An In Silico Analysis

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Abstract. Cardiovascular disease cases increase due to consumption cholesterol dietary habit. It is well-known that squalene synthase (SQS) is the first committed enzyme for cholesterol synthesis. Therefore, SQS become target of anti-cholesterol. This paper aims to determine the potential binding pocket of SQS (PDB ID: 1EZP). Dogsitescorer, siteFinder, and DEPTH were used for binding pocket prediction and MOE 2009.10 was performed for molecular docking. We found that there are five out of 37 pockets which have druggability score above 0.8. Pocket_5 is the highest drugability and favorable for hydrophobic interaction, yet lower number of hydrogen bond with the ligand. However, Pocket_2, and Pocket_3 are suitable for hydrogen bond formation of ligand-protein. Molecular docking study showed that TAK-475, D99, and Cynarin inhibitors were embedded on the P_2 and P_3 of SQS, showing that P2 and P3 are promising binding pocket for ligand interactions. These results show a promising alternative to design anti-cholesterol using these potential pocket in silico.

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1. Introduction

High levels of cholesterol in dietary increases the risks of heart disease and stroke [1]. It has been reported by WHO that around 17 million people die by heart disease and stroke [2]. The prevalence of cholesterol cases increasing is closely related to the country's economy. WHO reports that cholesterol cases in high-income countries increase more than 50% of adults, whereas, in low-income countries, it is only 25% of adults [2].

The current hypercholesterol treatments include low cholesterol diet, exercise, inhibitors of cholesterol adsorbtion, and inhibitors of cholesterol synthesis [3]. The most advanced anti-cholesterol drug against HMG-CoA reductase, statin family, showed undesirable side effects. Statins produce statin-induced myotoxicity (SIM) manifest with severe muscle weakness, muscle pain, and muscle tenderness [4]. In addition, HMG-CoA reductase inhibition prevents mevalonate synthesis which is imporant precursor for dolichol, ubiquinone, and RAS [5].

Currently, a promising strategy for blocking cholesterol synthesis is the development of inhibitor for Squalene synthase (SQS). SQS plays an important role as an enzyme for cholesterol biosynthesis which catalyzes the first step of the steroid synthesis pathway [6]. Clinical studies have shown that inhibition of SQS are effective in lowering serum cholesterol concentrations and LDL-C without interrupting isoprenoid production [7][8]. Moreover, SQS inhibitors have have fewer secondary effects than HMG-CoA reductase inhibitors[9] Due to its main role and strategic location in cholesterol synthesis, SQS is potential drug target for the treatment of cholesterol disease.

In order to design the new SQS inhibitors, in silico strategies is considered as the first step of drug development strategy. Huang et.al (2019) reported that three out of 373,782 inhibitors were identified as potential inhibitors of SQS using in silico screening and in vitro studies[6]. Other studies on traditional chinese medicine showed that cynarin, D99, and TAK-475 inhibitors had strong binding in the dynamic system with SQS protein (PDB ID: 3ASX) [7]. Most of these docking studies utilized blind docking. Blind docking, a method for ligand-protein binding prediction, can be used for mapping of drug development. A key advantage of blind docking is the capability to predict the binding without any prior knowledge of the target pocket [10]. However, limitations of blind docking are the unknown number of trials and energy evaluations. It is recommended that more than 100 times of trial, and 10 million energy evaluations [11]. Due to these limitation, therefore, in the in silico drug discovery, the identification of ligand-protein binding pocket is the first stage, followed by hit identification, lead optimization, and ADMET properties calculation [12][13]. Recently, docking protocol using known binding pocket increases accuracy and efficiency than blind docking only with the lower time of trial [14]. Thus, the present study aimed to determine the most potential pocket of squalene synthase for cholesterol synthesis inhibition.

2. Method

2.1. Hardware, Software and Webserver

Personal laptop HP model 14-ck0011TU Intel® Celeron® N4000 CPU 1.1GHz, RAM 4.0 GB, Microsoft Windows 10 Pro 64-bit with internet connection. MOE 2009.10 software (Chemical Computing Group ULC). Webserver <http://www.rcsb.org/pdb/>, <https://proteins.plus/>, and <http://cospi.iiserpune.ac.in/depth>.

2.2. Material

Human squalene synthase (PDB ID: 1EZF) was obtained from the Protein Data Bank (PDB) database (<http://www.rcsb.org/pdb/>). Ligands structure of TAK-475, D99, and cynarine were retrieved from Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>) with the CID : 9874248, 54669582, and 5281769, respectively.

2.3. SQS Binding Pocket Analysis

To predict the binding pocket of human squalene synthase, we have performed the binding pocket analysis. Dogsite scorer server (<https://proteins.plus/>) [15], Site Finder MOE2009.10 software

[16], and Depth server (<http://cospi.iiserpune.ac.in/depth>) [17] were used to measure the volume, druggability, and polarity of the pockets.

2.4. Molecular Docking and Binding Analysis

To further identify the potential binding pocket for inhibitors of human squalene synthase, we have docked 1EZf with TAK-475, D99, and cynarine. Ligands were optimized using MOE 2009.10 [18]. Preparation of Human squalene synthase was carried out using MOE 2009.10 software. The native ligands and water molecules were separated from the human SQS structure (PDB ID: 1EZf) and optimized using current forcefield, adjust hydrogen and lone pairs, gradient 0,05, and forcefield partial charges calculation [19]. The docking study was performed by MOE 2009.10 software, which uses; triangle matcher with 2500000 iterations as placement, one-time rescoring, London dG, 100 repetitions for the first retain, and force field refinement [18] [19].

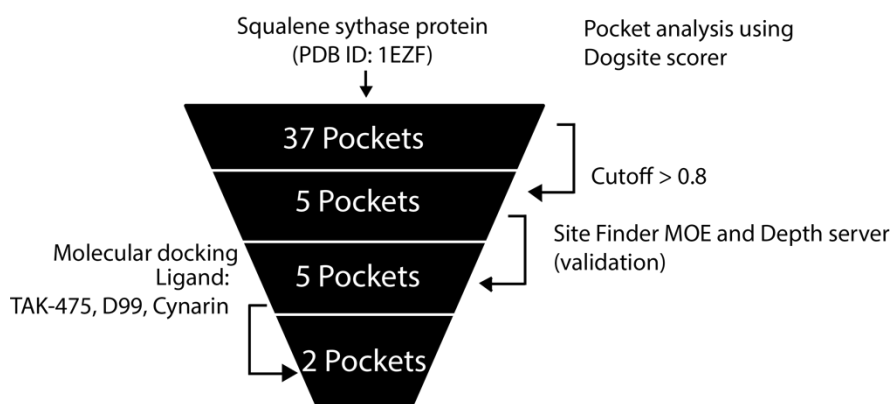


Figure 1. The protocol Utilized in this study for discovering potential pockets

3. Results and Discussion

Binding pocket determination is important to increase accuracy and efficiency in molecular docking. In this study, we analysed the druggability, volume, and polarity of pockets, and ligand-protein interaction to predict the potential binding pocket. SQS structure (PDB ID: 1EZf) was utilized to analyse the druggability, volume, and polarity of pockets. The cutoff of 0.7 was used for druggability score as the based on the average score [20].

Dogsitescorer result showed that there are seven pockets out of fifteen which have the druggable score > 0.70 and also have a high number of volume (Table 1), indicating discerning between the druggable and non-druggable pockets. The pocket volume increase with the increase of druggability (Table 1). Volume is important variable for druggable prediction, which is individually calculated using the number of volume grid points and grid spacing [21]. However, pocket 5 (P_5) having low pocket volume reached the highest druggability score. It might be due to the percentage of hydrophobic (nonpolar) area is higher than hydrophilic area, but not too different. A similar phenomenon in which SARS-Cov-2 S-glycoprotein binding pocket prediction has been reported previously [22]. Previous report highlighted that water molecules trapped inside the binding site is crucial for hydrophobicity and hydrophilicity of the binding site. Therefore, maintaining the water balance is important to ligand recognition.

Table 1. Potential binding pockets of Squalene synthase (PDB ID: 1EZF) based on Drugable score analysed by Dogscorer server

| Pocket ^a | Drugable score | Volume (Å ³) | Nonpolar (%) | Polar (%) | Charge (%) |
|---------------------|----------------|--------------------------|--------------|-----------|------------|
| P_5 | 0.84 | 536.59 | 46 | 40 | 14 |
| P_0 | 0.83 | 768.73 | 48 | 22 | 30 |
| P_1 | 0.83 | 731.62 | 45 | 24 | 31 |
| P_2 | 0.81 | 609.07 | 64 | 28 | 8 |
| P_3 | 0.80 | 560.22 | 58 | 35 | 8 |
| P_4 | 0.79 | 541.14 | 60 | 32 | 8 |
| P_6 | 0.75 | 523.64 | 37 | 19 | 45 |
| P_7 | 0.66 | 306.72 | 35 | 40 | 25 |
| P_12 | 0.63 | 211.83 | 62 | 31 | 8 |
| P_11 | 0.62 | 237.40 | 32 | 42 | 26 |
| P_9 | 0.60 | 278.89 | 23 | 69 | 8 |
| P_10 | 0.60 | 266.81 | 69 | 31 | 0 |
| P_8 | 0.59 | 298.49 | 65 | 29 | 6 |
| P_13 | 0.54 | 211.48 | 33 | 47 | 20 |
| P_14 | 0.43 | 206.58 | 33 | 33 | 34 |

^aPocket 1, Pocket 2, etc are shown as P_1, P_2, etc

In order to provide better discerning between the druggable and non-druggable pockets, we next utilized the pockets which have score ≥ 0.8 . Binding pocket analysis were confirmed using sitefinder MOE, and Depth. Table 2 showed that all the pockets have more hydrophobic residues rather than hydrophilic. Unlike P_0, P_1 and P_5, dogscorer and sitefinder analysis result on P_2 and P_3 were similar. Previous study revealed that the similarity results of the dogscore and sitefinder analysis result in better complex ligand and protein interaction [22].

To confirm the capability of pockets as binding pocket, we docked all five pocket with TAK-475, D99, dan cynarin ligand. H-bond residues for SQS were provided in Tables 3. We showed that there are more hydrogen bonds than hydrophobic interaction. Consistently, Ligand-OppA protein interaction was achieved by utilizing the hydrogen bonds and the electrostatic forces[23]. Moreover, other findings revealed that hydrogen bonds are considered as the main facilitators of protein-ligand interaction [24]. The present study showed that TAK-475 has the highest number of hydrogen bonds in P_3, while cynarin has high number of hydrogen bonds in P_1 and P_5. Taken together, these results suggest that P_2 and P_3 are potential as binding pocket. As the ligands are completely embedded within the pocket protein Figure 1, physicochemical properties of ligand are important to consider ligand-protein interaction. Both TAK-475 and cynarin have high number of hydrogen acceptors and donors, suggesting strong protein-ligand interactions due to physicochemical properties (Table 4). Our result is supported by a recent study showing that hydrogen acceptor and donor of the ligands are the main facilitators of ligand-protein interaction[22]. Therefore, physicochemical properties of ligand and pocket hydrophobicities are suggested as the main factor for the drug design of cholesterol disease.

Table 2. Residues of Amino Acid in binding pockets of SQS

| Pocket ^a | Hydrophobicity | | Charges | | Special charges | |
|---------------------|-----------------------|-----------------------|----------|----------|-----------------|---------------|
| | Hydrophobic | Hydrophilic | (+) es | (-) es | | |
| P_0 | MET_B154 ^b | ILE_B217 ^c | GLN_B212 | LYS_B160 | GLU_B83 | PRO_B232 |
| | PHE_B157 | TYR_B220 | ASN_B215 | HIS_B161 | ASP_B84 | |
| | LEU_B158 | PHE_B230 ^d | GLN_B224 | ARG_B22 | ASP_B219 | |
| | VAL_B162 | TRP_B231 | GLN_B233 | 8 | ASP_B223 | |
| | TYR_B171 | TRP_B236 | | | GLU_B22 | |
| | VAL_B175 | LEU_B243 | | | 9 | |
| | ILE_B216 | | | | | |
| P_1 | MET_A86 | VAL_A175 | GLN_A212 | LYS_A160 | ASP_A80 | GLY_A227 |
| | MET_A150 | ILE_A216 | ASN_A215 | HIS_A16 | GLU_A83 | PRO_A232 |
| | MET_A154 | TYR_A220 | GLN_A224 | 1 | ASP_A84 | |
| | PHE_A157 | | GLN_A233 | ARG_A2 | ASP_A21 | |
| | LEU_A158 | PHE_A230 | | 28 | 9 | |
| | VAL_A162 | TRP_A231 | | | ASP_A22 | |
| | TYR_A171 | TRP_A236 | | | 3 | |
| | LEU_A243 | | | GLU_A22 | | |
| | | | | 9 | | |
| P_2 | PHE_C54 | VAL_C179 | SER_C184 | ARG_C77 | ASP_C80 | TYR_C180GLY_C |
| | ILE_C58 | LEU_C183 | GLN_C293 | | | C208CYS_C |
| | VAL_C69 | PHE_C187 | | | | 289PRO_C2 |
| | PHE_C72 | ALA_C204 | | | | 92 |
| | TYR_C73 | MET_C207 | | | | |
| | LEU_C76 | LEU_C211 | | | | |
| | MET_C150 | TYR_C276 | | | | |
| VAL_C175 | PHE_C288 | | | | | |
| ALA_C176 | | | | | | |
| P_3 | PHE_A54 | VAL_A179 | SER_A184 | ARG_A77 | ASP_A80 | TYR_A177GLY_A |
| | ILE_A58 | LEU_A183 | GLN_A212 | | | A180GLY_A |
| | VAL_A69 | PHE_A187 | GLN_A293 | | | A208CYS_A |
| | PHE_A72 | ALA_A204 | | | | 289PRO_A2 |
| | TYR_A73 | MET_A207 | | | | 92 |
| | LEU_A76 | LEU_A211 | | | | |
| | VAL_A175 | TYR_A276 | | | | |
| ALA_A176 | PHE_A288 | | | | | |
| P_5 | PHE_A54 | ILE_A291 | ASN_A48 | ARG_A52 | GLU_B343 | PRO_B332, |
| | ALA_A55 | ALA_B333 | GLN_A49 | ARG_B36 | | |
| | ALA_A56 | ALA_B336 | SER_A51 | 7 | | |
| | VAL_A57 | ILE_B337 | SER_A53 | HIS_B347 | | |
| | ILE_A58 | TYR_B339 | GLN_A283 | | | |
| | GLN_A59 | MET_B342 | SER_A284 | | | |
| | ALA_A60 | TYR_B346 | ASN_A287 | | | |
| | LEU_A61 | ILE_B363 | THR_A329 | | | |
| | PHE_A288 | ILE_B366 | ASN_B330 | | | |
| | | GLN_B340 | | | | |
| | | SER_B364 | | | | |

^aPocket 1, Pocket 2, etc are shown as P_1, P_2, etc

^bresidues with red colour = predicted by dogsitescorer and sitefinder moe

^cresidues with black colour = predicted by dogsitescorer only

^dresidues with yellow highlight = predicted by dogsitescorer, sitefinder moe, and DEPTH server

Table 3. Binding energy and residues involved in ligands interaction

| Pocket | Ligand | Binding energy | Residues involved | |
|--------|---------|----------------|---|--|
| | | | Hydrogen bond (distance Å ⁰) | Hydrophobic interaction (distance Å ⁰) |
| P_0 | TAK-475 | -17.1669 | TYR_B171 (3.37) ARG_B228 (1.99) | ARG_B77 (3.00) |
| | D99 | -15.5290 | TYR_B171 (3.06) ARG_B228 (2.05) | |
| | Cynarin | -16.1079 | GLU_B116 (1.99) ASP_B219 (1.99) | PHE_B230 |
| P_1 | TAK-475 | -15.0187 | ARG_A52 (2.02) ARG_A77 (2.04) LYS_A117 (2.57) GLN_A212 (1.84) | - |
| | D99 | -12.6647 | ARG_A77 (2.03) ASP_A219 (1.67) | PHE_A230 |
| | Cynarin | -16.2647 | THR_A81 (3.31) ASP_A118 (2.14) ARG_A228 (2.57) GLN_A212 (2.71)(2.54) GLU_A83 (2.23) | - |
| P_2 | TAK-475 | -17.3370 | ARG_C52 (1.78) LYS_C117 (2.1) | - |
| | D99 | -15.8893 | ASN_C215 (2.71) LYS_C117 (1.97) | ARG_C52 (2.66)(3.16) |
| | Cynarin | -16.3325 | ARG_C52 (1.99) VAL_C69 (1.97) | - |
| P_3 | TAK-475 | -14.8776 | ASN_A215 (2.59) SER_A51 (3.41) PHE_A54 (1.83) SER_A53 (2.05) ARG_A52 (1.92) LYS_A117 (2.43) | ARG_A218 |
| | D99 | -13.5409 | ARG_A52 (1.93) LYS_A117 (2.2) | - |
| | Cynarin | -16.0648 | SER_A53 (2.29) TYR_A73 (3.46) ARG_A77 (2.38) | - |
| P_5 | TAK-475 | -12.3923 | SER_A53 (3.35) | ARG_A52 (3.01) |
| | D99 | -12.7368 | ARG_A52 (1.89) ARG_A218 (2.29) LYS_A117 (2.32) | ARG_A218 |
| | Cynarin | -14.7047 | LYS_A117 (4.98)(2.19) SER_A51 (3.56) TYR_A73 (2.13) | ARG_A52, ARG_A77 |

Table 4. Physiochemical properties of ligan

| No | Ligan | Properties | | |
|----|---------|------------|------------|---------|
| | | MW | H Acceptor | H Donor |
| 1 | TAK-475 | 645.1 | 9 | 1 |
| 2 | D99 | 549.5 | 5 | 2 |
| 3 | Cynarin | 516.4 | 12 | 7 |

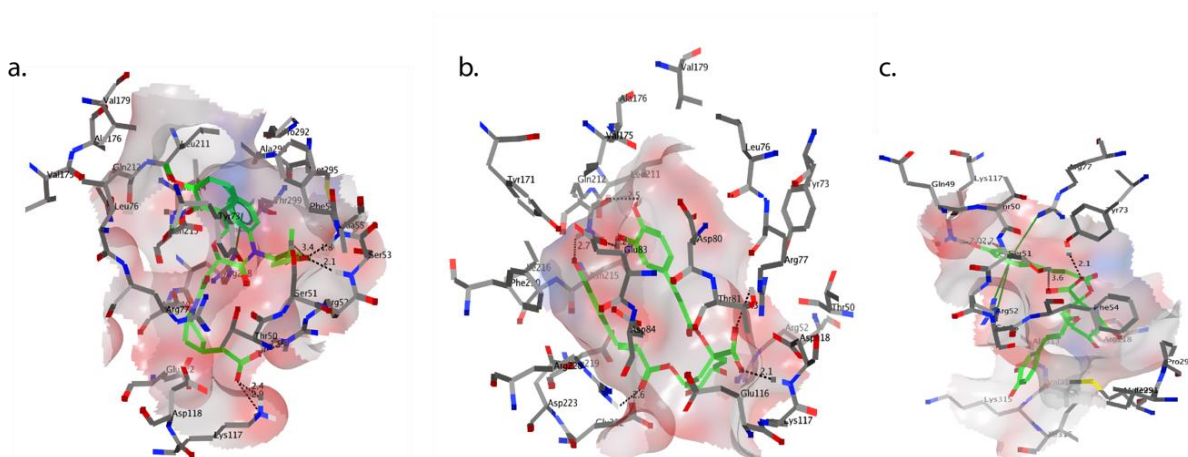


Figure 2. SQS Local Structure of the Docking Complexes: a) TAK-475 with P_3, Cynarin with b) P_1 and c) P_5

4. Conclusion

In conclusion, we have identified two SQS hydrophobic pocket (P_2, and P_3) as potential binding pocket. The findings from molecular docking demonstrated that TAK-475, D99, and Cynarin inhibitors are embedded on the P_2 and P_3 of SQS. Complex of inhibitors and P_3 has lower binding energy and higher hydrogen bond than that and P_2 of SQS. These findings provide promising alternative to design anti-cholesterol.

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