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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 squalence 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: 1EZF). 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].

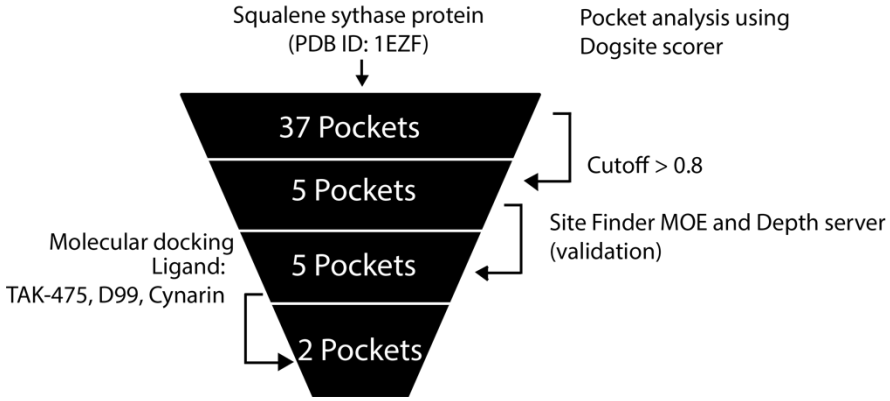

Figure 1. The protocol Utilized in this study for discovering potential pockets

3. Results and Discussion

Binding pocket determination is important to increases accuracy and efficiency in molecular docking. In this study, we analysed the drugability, 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 drugability, 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 drugable 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 drugability (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 drugability 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 Dogsitescorer server

Pocket[a]	Drugable score	Volume (A^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

[a]**Pocket 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, physiochemical 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] PHE_B157 LEU_B158 VAL_B162 TYR_B171 VAL_B175 ILE_B216	ILE_B217[c] TYR_B220 PHE_B230[d] TRP_B231 TRP_B236 LEU_B243	GLN_B212 ASN_B215 GLN_B224 GLN_B233	LYS_B160 HIS_B161 ARG_B228	GLU_B83 ASP_B84 ASP_B219 ASP_B223 GLU_B229	PRO_B232
P_1	MET_A86 MET_A150 MET_A154 PHE_A157 LEU_A158 VAL_A162 TYR_A171	VAL_A175 ILE_A216 TYR_A220 PHE_A230 TRP_A231 TRP_A236 LEU_A243	GLN_A212 ASN_A215 GLN_A224 GLN_A233	LYS_A160 HIS_A161 ARG_A228	ASP_A80 GLU_A83 ASP_A84 ASP_A219 ASP_A223 GLU_A229	GLY_A227 PRO_A232
P_2	PHE_C54 ILE_C58 VAL_C69 PHE_C72 TYR_C73 LEU_C76 MET_C150 VAL_C175 ALA_C176	VAL_C179 LEU_C183 PHE_C187 ALA_C204 MET_C207 LEU_C211 TYR_C276 PHE_C288	SER_C184 GLN_C293	ARG_C77	ASP_C80	,Y_C180GLY_C208CYS_C289PRO_C292
P_3	PHE_A54 ILE_A58 VAL_A69 PHE_A72 TYR_A73 LEU_A76 VAL_A175 ALA_A176	VAL_A179 LEU_A183 PHE_A187 ALA_A204 MET_A207 LEU_A211 TYR_A276 PHE_A288	SER_A184 GLN_A212 GLN_A293	ARG_A77	ASP_A80	,Y_A177GLY_A180GLY_A208CYS_A289PRO_A292
P_5	PHE_A54 ALA_A55 ALA_A56 VAL_A57 ILE_A58 GLN_A59 ALA_A60 LEU_A61 PHE_A288	ILE_A291 ALA_B333 ALA_B336 ILE_B337 TYR_B339 MET_B342 TYR_B346 ILE_B363 ILE_B366	ASN_A48 GLN_A49 SER_A51 SER_A53 GLN_A283 SER_A284 ASN_A287 THR_A329 ASN_B330 GLN_B340 SER_B364	ARG_A52 ARG_B367 HIS_B347	GLU_B343	PRO_B332,

[a]Pocket 1, Pocket 2, etc are shown as P_1, P_2, etc

[b] residues with red colour = predicted by dogsitescorer and sitefindermoe

[c]residues with black colour = predicted by dogsitescorer only

[d]residues 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 A^0)	Hydrophobic interaction (distance A^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) (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

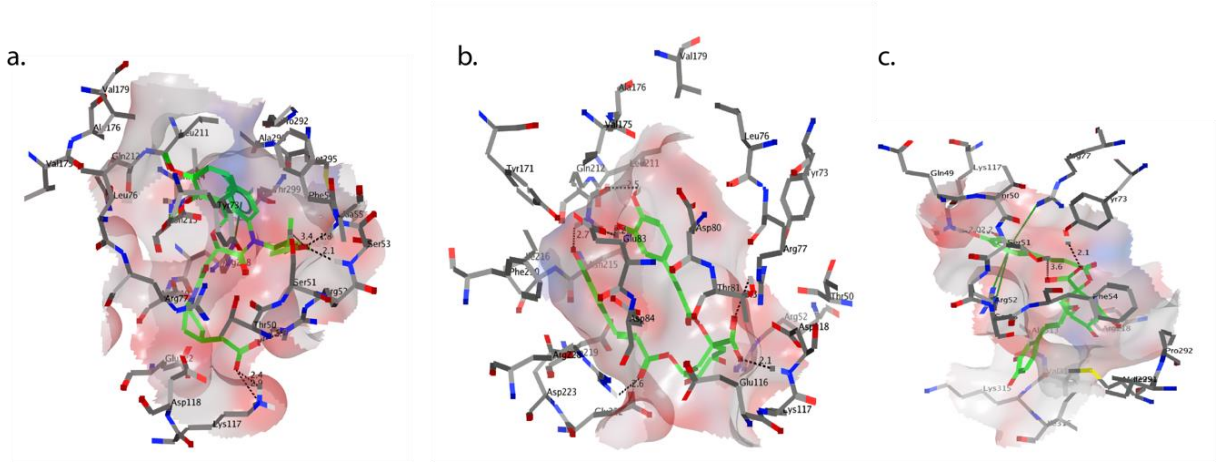

Figure 2. SQS Local Structure of the Docking Complexes: a) TAK-475 with P_3, Cynarin with b) P_1 and c) P_5

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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