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Molecular Docking Studies of Squalen Synthase Inhibitors as Potential Anti Cardiovascular Disease Drugs: Insights into Drug-Protein Interaction Discovery

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ABSTRACT

Background: Squalene synthase (E.C, 2.5.1.21) catalyzes the reductive dimerization of two molecules of farnesyl diphosphate to squalene, so that it is involved in the first step in cholesterol biosynthesis. Inhibition of squalene synthase is under consideration as an approach of decreasing cholesterol levels in the prevention of cardiovascular disease. Therefore squalene synthase is attractive object for the treatment of hypercholesterolemia, hypertriglyceridemia and coronary heart disease. Understanding the interaction of squalene synthase binding site with inhibitors is crucial for the development of pharmaceutical agents. At this aim, computer aided drug design is an applicable method that can study theses interactions and describe significant characteristics for squalene synthase binding site recognition. ***Methods:*** In the present work, we applied the molecular docking approach for 9 inhibitors of squalene synthase. Autodock Tools 4.2 was used in order to prepare the docking runs. After the docking runs, the final binding modes of the inhibitors were selected on the basis of the highest score or in other words best fit corresponding to the complex with the lowest free energy of binding. ***Results:*** The docking results indicate that all inhibitors bind to squalene synthase active site. Our results clearly show that non polar interactions play a significant role in determining the binding free energy. The results also demonstrated that the inhibitor binding site is a hydrophobic pocket that completely is surrounded by the hydrophobic residue. Also, it was found that the inhibitor 6 (E5700) have the lowest binding free energy (-9.01 kcal/mol). ***Conclusion:*** The docking results will be useful for the structure-based drug design and the development of the pharmaceutical agents to treatment of coronary heart disease.

Introduction

It has been demonstrated that, elevated plasma cholesterol is one of the fundamental risk factors for cardiovascular disease. The common therapeutic agents for the treatment of hypercholesterolemia is the use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that can decrease plasma cholesterol and then morbidity among patients with cardiovascular disease.[1,2] HMG-CoA reductase catalyzes the biosynthesis of mevalonic acid (Figure 1). HMG-CoA reductase inhibitors or statins are a category of drugs applied to lower cholesterol levels by inhibiting the HMG-CoA reductase, which plays a key role in the production of cholesterol. Statins act by competitively inhibiting HMG-CoA reductase. Since statins are similar to HMG-CoA on a molecular level, they take the place of HMG-CoA in the enzyme and decrease the rate by which it is able to produce mevalonate (Figure 1), this eventually decreases cholesterol.[3,4] Increased cholesterol levels have been related with cardiovascular disorder and accordingly statins are applied in the prevention of these disorder.[4,5] HMG-CoA reductase inhibitors or statins are applied for the treatment of patients with hypercholesterolemia extensively, however they have demonstrated small serious toxicity such as skeletal myopathy or hepatotoxicity.[6,7] The most common adverse side effects are elevated liver enzymes and muscle disorder. In randomized clinical tests, described adverse effects are low; but they are higher in studies of real world use, and more variegated.[8] Other potential adverse effects include cognitive loss, neuropathy, pancreatic and hepatic dysfunction, myalgias, muscle cramps.[8] Therefore, an agent that could reduce plasma cholesterol and triglyceride levels with less adverse effects would be extremely desirable.

Squalene synthase (EC 2.5.1.21) or farnesyl-diphosphate farnesyltransferase catalyzes the conversion of farnesyl pyrophosphate into squalene by

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reductive dimerization. It is particular enzyme of cholesterol biosynthesis that involved in the first step in cholesterol biosynthesis pathway (Figure 1).[9,10] Inhibition of squalene synthase consider as an approach of decreasing cholesterol levels in the prevention of cardiovascular disease.[1,11] Squalene synthase inhibition, bring about a decrease in cholesterol biosynthesis directly and therefore to a fall in plasma cholesterol levels; so that it has been suggested that squalene synthase inhibitors may not have the toxicity of HMG-CoA reductase inhibitors. So that squalene synthase is an unique enzyme and locates in important place in cholesterol pathway, therefore squalene synthase is an attractive object for the treatment of hypercholesterolemia and coronary heart disease and as well as develop the pharmaceutical agents.[2,7]

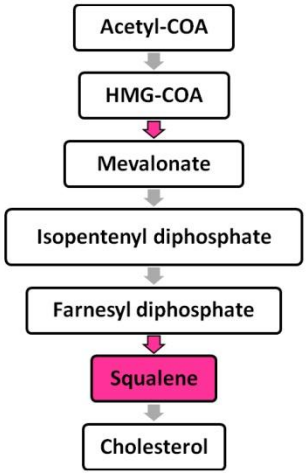

Figure 1. Cholesterol biosynthesis pathway. The purple arrows indicate HMG CoA reductase and squalene synthase, respectively.

Materials and Methods

Understanding the interaction of squalene synthase binding site with inhibitors is crucial for the development of pharmaceutical agents. At this aim, computer aided drug design is an applicable method that can study these interactions and describe significant characteristics for squalene synthase binding site recognition. Automated docking is widely applied for approximation of bio molecular complex and in order to analyze the structure-function processes and the bio molecular design. Drugs design is the other application of docking. The precise interaction of agents or candidate molecules with their targets is crucial in the development procedure.[12,13]

Ligand structure

Due to the special characteristics of squalene synthase, the researchers have focused on various aspects of it and in the several articles, the squalene synthase inhibitors were considered. Since, some of these were the effective inhibitors against the squalene synthase, it may be a potential therapeutic agent for hypercholesterolemia. Therefore, we select some of the putative inhibitors for the docking studies against squalene synthase, so that they are potent and most considered. Figure 2 shows the structure of inhibitors.[10,14-16]

In the present study, molecular modeling of the inhibitors was carried out using Hyperchem 7 software. Hyperchem 7 was employed to draw and optimize the structure of inhibitors.[17]

For all initial structures was performed the geometry optimization calculations by use of molecular mechanics and afterward the lowest energy conformers were optimized using the semiempirical PM3 method and the conjugate gradient and steepest descent algorithm. At the end these structures converted to .pdb format by Hyperchem 7 software. Optimized inhibitor structure was used as input file for docking.

Protein structure

In the current study, the protein X-ray crystal structure of human squalene synthase with 3ASX code and X-ray diffraction at 2.00 Å resolution was received from the Protein Data Bank and was used as the receptor starting structure. The enzyme structure was monomer and comprised a co-crystallized inhibitor codenamed D99 (3R)-1-{4-[{4-chloro-2-[(s)-(2-chlorophenyl)(hydroxy)methyl] phenyl}(2,2-dimethylpropyl) amino]-4-oxobutanoyl}piperidine-3-carboxylic acid) and several water molecules (Figure 3-A). The co-crystallized inhibitor was removed for the docking studies.[7] We applied Autodock tools 4, in order to set up the docking runs and predict the inhibitors binding free energy.[18-20]

Docking protocol

AutoDockTools 4.2 was employed to docking process of inhibitors to squalene synthase. Initially, all of the polar hydrogen was added to the inhibitors and Gasteiger-Marsili atomic partial charges were set for them, and all the inhibitors swirling bonds were adjusted in fewest atoms. The final ligand structures were saved in .pdbqt format. Then polar hydrogen was added to the protein crystal structure and the kollman atomic partial charge was set for squalene synthase. The final protein structure was saved in .pdbqt format. An extended pdb format, called pdbqt, is applied for coordinate files, which includes atomic partial charges and atom types; pdbqt files as well include data on the torsional degrees of freedom. Grid box was created by Autogrid 4 with 90 × 90 × 90 points in x, y and z directions with 0.375 Å spacing and center of box was located on the active site according to co-crystallized inhibitor coordination. The squalene synthase active site was easily distinguished as the hydrophobic cavity comprising the co-crystallized ligand D99. The genetic algorithm was used to determine the probable accommodate for each inhibitor to squalene synthase. Docking was performed with Lamarckian genetic algorithm with population size of 150. Squalene synthase kept rigid in docking process. The ligand

structures were attributed flexible. In other words all the inhibitors rotatable bonds were adjusted in fewest atoms; note also that cyclic rotatable bonds are excluded. The other parameters were used as default docking parameters.[7] Finally, by setting all the parameters, inhibitors were docked to the squalene synthase.

AutoDockTools includes a number of methods for considering the results of docking simulations, including tools for clustering results by conformational resemblance, visualizing conformations, visualizing interactions between ligands and proteins.[19] At the end of a docking process, AutoDock writes the data on clustering and binding energies to the log file.[21,22] The docking results were clustered with 2 Å root mean square deviation and were ranked according to the estimated binding free energy. The structure with proportional lower binding free energy and the most conformation in cluster was selected for the optimum docking conformation.[12,19,23]

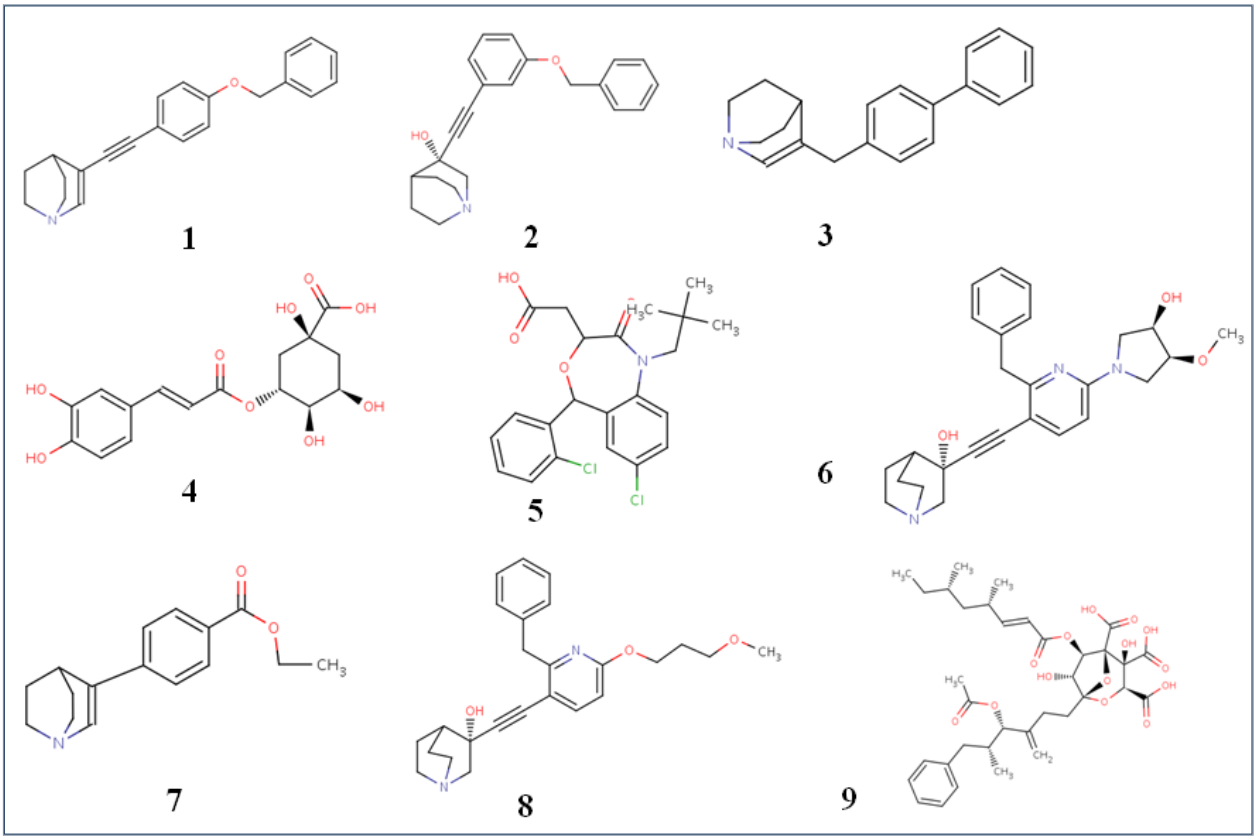

Figure 2. Structure of inhibitors. **[1]**, 3-[[4-(benzyloxy)phenyl]ethynyl]-1-azabicyclo[2.2.2]oct-2-ene; **[2]**, (3R)-3-[[3-(benzyloxy)phenyl]ethynyl]-1-azabicyclo[2.2.2]octan-3-ol; **[3]**, 3-(biphenyl-4-ylmethyl)-1-azabicyclo[2.2.2]oct-2-ene; **[4]**, chlorogenic acid; **[5]**, CP-294838; **[6]**, E5700; **[7]**, ethyl 4-(1-azabicyclo[2.2.2]oct-2-en-3-yl)benzoate; **[8]**, ER119884, **[9]**, zaragozic acid A.

Results and Discussion

Molecular docking was applied to describe and find out the binding sites in squalene synthase. The final product of molecular docking was clustered to specify the binding free energy and optimal docking energy conformation that is investigated as the best docked structure. As well as we consider the molecular docking results to elucidate their binding mode in the squalene synthase.

The AutoDock 4.2 force field is contrived to estimate the binding free energy of inhibitors to protein. It includes an updated charge-based desolvation term, advances in the directionality of hydrogen bonds, and various improved models of the unbound state. AutoDock 4.2 applies a semi-empirical free energy force field and grid-based docking to assess conformations during docking process. Equation1 represent the docking binding free energy, this formula automatically was computed by AutoDock 4.2.[19,22]

Equation 1 $$\Delta G_{binding} = [\Delta G_{intermolecular} + \Delta G_{internal} + \Delta G_{tors}] - [\Delta G_{unbound}]$$

In the above formula, the final intermolecular energy is calculated with equation 2, so that the final intermolecular energy involves in van der Waals, hydrogen bonding, desolvation and electrostatic contribution between the inhibitor and the protein binding site.

Equation 2 $$\Delta G_{intermolecular} = [\Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{desolv}] + \Delta G_{elec}$$

Table 1 summarizes the docking results. In this study also was determined the inhibition constant (Ki) and the RMSD value for drug-like molecules. Negative values of predicted free energies of binding show that all inhibitors correctly docked to the crystal structure of the squalene synthase. Docking results also indicate that the contribution of van der Waals interactions is greater than electrostatic interactions so that, it can be concluded that all of the inhibitors attached to a hydrophobic binding site in squalene synthase. Figure 4 shows the comparison between the van der Waals and electrostatic contribution of all compounds. It is clear that, the van der Waals contributions are more prominent. Among the molecules tested, E5700 demonstrated the lowest binding free energy (-9.01 kcal/mol). In other words, E5700 has the highest interaction and the more potential binding affinity for the enzyme binding site. According to the Table 1, it was demonstrated that E5700 had the lowest binding free energy and also the lowest inhibition constant (0.24 µ M) and subsequently the most experimental affinity.

Table 1. Autodock's binding free energy derived from the docking studies on squalen synthase.

Compound	$\Delta G_{binding}$	Ki	ΔG_{vdW}	ΔG_{ele}	ΔG_{inter}	ΔG_{tors}	$\Delta G_{unbound}$	RMSD	IC50	Refrence
1	-7.80	1.93	-7.70	-1.59	-0.52	+1.49	-0.52	56.771	0.00083	(14)
2	-8.86	0.32	-10.70	+0.06	-0.51	+1.79	-0.51	62.840	0.0015	(14)
3	-8.38	0.71	-9.30	+0.02	-0.66	+0.89	-0.66	55.599	0.00073	(14)
4	-5.46	98.82	-6.88	-1.57	-1.69	+2.98	-1.69	69.229	0.0001	(15)
5	-6.40	20.21	-6.80	-0.80	-0.55	+1.19	-0.55	65.506	0.00013	(16)
6	**-9.01**	**0.24**	-9.71	-0.49	-0.70	+1.19	-0.70	79.663	**8.4e-07**	(14)
7	-7.84	1.78	-8.89	-0.44	-0.47	+1.49	-0.47	38.185	5e-05	(14)
8	-7.59	2.72	-9.60	-0.68	-0.82	+2.68	-0.82	34.455	3.52e-06	(14)
9	-4.78	314.7	-8.01	-3.33	-1.22	+6.56	-1.22	78.286	9.55e-05	(10)

Abbreviations: $\Delta G_{binding}$, Estimated Free Energy of Binding (kcal/mol); ΔG_{vdw}, vander Waals or Lennard–Jones potential factor of binding free energy (kcal/mol); ΔG_{elec}, electrostatic factor of binding free energy (kcal/mol); ΔG_{inter}, Gibbs free energy of binding (kcal/mol); ΔG_{tors}, torsional energy of binding (kcal/mol); $\Delta G_{unbound}$, unbound System's energy (kcal/mol); Ki, inhibition constant (µM); RMSD, reference root mean square deviation; IC50 refers to the experimental predicted activity (mM).

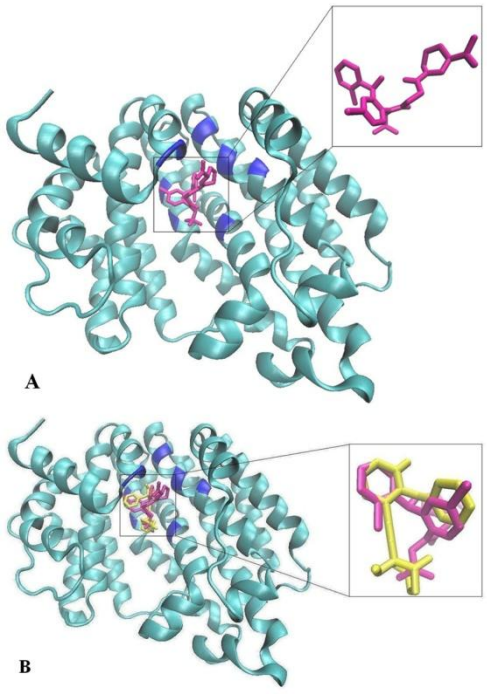

Figure 3. Best virtual docking pose of E5700. (A) X-ray crystal structure of squalene synthase in complex with inhibitor D99, (B) Superimposition of E5700 (purple) and D99 (yellow).

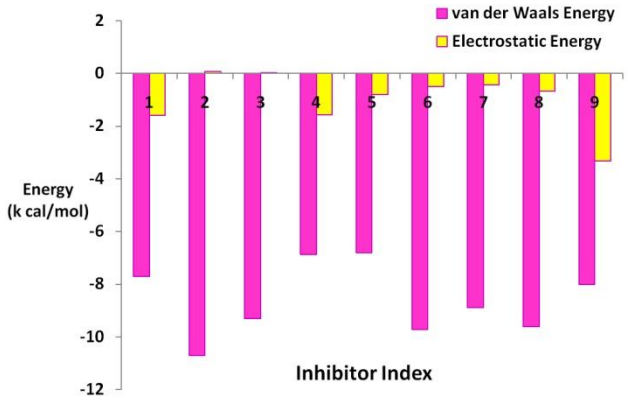

Figure 4. Comparison between the van der Waals and electrostatic contribution of compounds 1–9.

The docking results indicate that all inhibitors bind to squalene synthase active site. The binding manners and geometrical orientation of all compounds in binding site were nearly identical, hence proposing that all the inhibitors have the same interactions with enzyme and occupied a common cavity in the receptor. Figure 3 shows the best virtual docking pose of E5700 and the superimposition of E5700 and D99.

The results also demonstrated that the inhibitor binding site is a hydrophobic pocket that completely was surrounded by the hydrophobic residue. The squalene synthase binding site is a hydrophobic cavity that located at the end of a central channel running through the squalene synthase. This cavity is encompassed by hydrophobic residues that contain the aspartate-rich sequence. The aminoacids that be a part of the squalene synthase aspartate-rich sequence are regarded to play an crucial role in the catalytic reaction mechanism.[24] According to Pandit et al studies this aspartate-rich sequence convergences with a same one in the structural superimposition of squalene synthase, so that it's similar to the class I of isoprenoid biosynthetic enzymes.[25,26] Other interactions proposed by the docking consequences were the hydrophobic interactions of the hydrophobic groups of inhibitors 1-9 as they were observed oriented towards the co-crystallized ligand D99, so that they have similar hydrophobic interactions. Hydrophobic cavity of binding site comprising the hydrophobic residues such as Ser53 ،Phe54 ،Val175, Ala176, Val 179, Leu 211, Gln212, Phe288 ،Pro292 ،Met295 and Leu183, constituting the inner cavity of the active site. Figure 5 shows the docking result of E5700 with squalene synthase. In this figure, the lowest energy configuration of E5700-squalene synthase complex was demonstrated by using VMD (A) and Ligplot (B) presentations.[27-29]

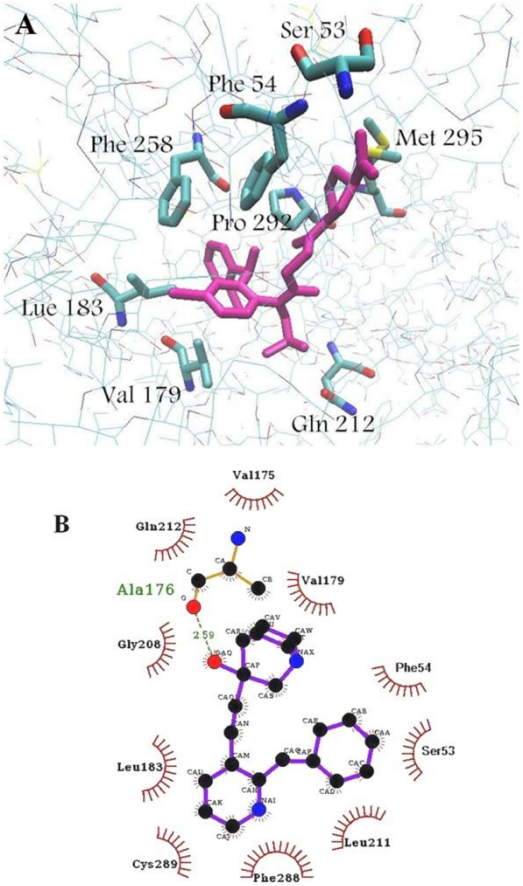

Figure 5. Docking result of E5700 with squalene synthase. The lowest energy configuration of E5700-squalene synthase complex is demonstrated in VMD(A) and Ligplot (B) presentations. Residues appointed in Ligplot are marked and demonstrated in VMD as sticks along with E5700 (purple). In Ligplot presentations (B), carbons are in black, nitrogens in blue and oxygens in red.

Conclusion

In the resent study, we employed computational approaches, such as molecular docking to estimate the binding free energy of 9 inhibitors with squalene synthase. Compare with the van der Waals and electrostatic energies for these components showed a significant share of the van der Waals energies. In other words, our results clearly show that non polar interactions play a significant role in determining the binding free energy. Our results demonstrated that, prediction of the most potent inhibitor is in agreement with highest affinity. The results also indicated that the inhibitor binding site is a hydrophobic pocket that completely is surrounded by the hydrophobic residue. It was illustrated that, all inhibitors bind to squalene synthase active site and subsequently inhibit it. So that they have potent affinity to the squalene synthase and thus they can behave like as the pharmaceutical agents. Understanding, an atomic-level of the catalytic and inhibition mechanisms of squalene synthase could assist to search for rationally-designed inhibitors of squalen synthase, and would be of significant importance squalen synthase activity. The docking results will be useful for the structure-based drug design and the development of the pharmaceutical agents to treatment of coronary heart disease.

References

1. Griebenow N, Buchmueller A, Kolkhof P, Schamberger J, Bischoff H. Identification of 4H,6H-[2]benzoxepino[4,5-c][1,2]oxazoles as novel squalene synthase inhibitors. *Bioorg Med Chem Lett* 2011;21(12):3648-53.
2. Ishihara T, Kakuta H, Moritani H, Ugawa T, Sakamoto S, Tsukamoto S, et al. Syntheses of 3-ethylidenequinuclidine derivatives as squalene synthase inhibitors. Part 2: enzyme inhibition and effects on plasma lipid levels. *Bioorg Med Chem* 2003;11(17):3735-45.
3. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001;108(3):391-7.
4. Hunninghake DB. HMG CoA reductase inhibitors. *Curr Opin Lipidol* 1992;3(1):22-8.
5. Prospective Studies C, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, et al. Blood cholesterol and vascular mortality by age, sex, and

blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 2007;370(9602):1829-39.
6. Nishimoto T, Tozawa R, Amano Y, Wada T, Imura Y, Sugiyama Y. Comparing myotoxic effects of squalene synthase inhibitor, T-91485, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in human myocytes. *Biochem Pharmacol* 2003;66(11):2133-9.
7. Kourounakis AP, Matralis AN, Nikitakis A. Design of more potent squalene synthase inhibitors with multiple activities. *Bioorg Med Chem* 2010;18(21):7402-12.
8. Golomb BA, Evans MA. Statin adverse effects : a review of the literature and evidence for a mitochondrial mechanism. *Am J Cardiovasc Drugs* 2008;8(6):373-418.
9. Soltis DA, Mcmahon G, Caplan SL, Dudas DA, Chamberlin HA, Vattay A, et al. Expression, purification, and characterization of the human squalene synthase: use of yeast and baculoviral systems. *Arch Biochem Biophys* 1995;316(2):713-23.
10. Thompson JF, Danley DE, Mazzalupo S, Milos PM, Lira ME, Harwood HJ. Truncation of human squalene synthase yields active, crystallizable protein. *Arch Biochem Biophys* 1998;350(2):283-90.
11. Alberts AW, Berger GD, Bergstrom JD. Squalene synthetase inhibitors. Google Patents; 1992.
12. Cavasotto CN, Orry AJ. Ligand docking and structure-based virtual screening in drug discovery. *Curr Top Med Chem* 2007;7(10):1006-14.
13. Morris GM, Lim-Wilby M. Molecular docking. *Methods Mol Boil* 2008;443:365-82.
14. Sealey-Cardona M, Cammerer S, Jones S, Ruiz-Perez LM, Brun R, Gilbert IH, et al. Kinetic characterization of squalene synthase from Trypanosoma cruzi: selective inhibition by quinuclidine derivatives. *Antimicrob Agents Chemother* 2007;51(6):2123-9.
15. Choi SW, Hur NY, Ahn SC, Kim DS, Lee JK, Kim DO, et al. Isolation and structural determination of squalene synthase inhibitor from Prunus mume fruit. *J Microbiol Biotechnol* 2007;17(12):1970-5.
16. Lee S, Poulter CD. Cloning, solubilization, and characterization of squalene synthase from Thermosynechococcus elongatus BP-1. *J Bacteriol* 2008;190(11):3808-16.
17. Laxmi D, Priyadarshy S. HyperChem 7. Biotech Software and Internet Report: The Computer Software Journal for Scientists. 2002;3(1):5-9.
18. Lindstrom W, Morris GM, Weber C, Huey R. Using AutoDock for virtual screening. La Jolla, CA: The Scripps Research Institute Molecular Graphics Laboratory; 2006.
19. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* 2009;30(16):2785-91.
20. Morris GM, Huey R, Olson AJ. Using AutoDock for ligand-receptor docking. *Curr Protoc Bioinformatics* 2008;Chapter 8:Unit 8 14.
21. Huey R, Morris GM. Using AutoDock with AutoDockTools: A Tutorial. La Jolla, CA: The Scripps Research Institute, Molecular Graphics Laboratory; 2003.
22. Goodsell DS. Computational docking of biomolecular complexes with AutoDock. *Cold Spring Harb Protoc* 2009;2009(5):pdb prot5200.
23. Saladin A, Fiorucci S, Poulain P, Prevost C, Zacharias M. PTools: an opensource molecular docking library. *BMC struct boil* 2009;9:27.
24. Choi SW, Hur NY, Ahn SC, Kim DS, Lee JK, Kim DO, et al. Isolation and structural determination of squalene synthase inhibitor from Prunus mume fruit. *J Microbiol Biotechnol* 2007;17(12):1970-5.
25. Scheer M, Grote A, Chang A, Schomburg I, Munaretto C, Rother M, et al. BRENDA, The Comprehensive Enzyme Information System in 2011. *Nucleic Acids Res* 2011;39:D670-7.
26. Pandit J, Danley DE, Schulte GK, Mazzalupo S, Pauly TA, Hayward CM, et al. Crystal structure of human squalene synthase. A key enzyme in cholesterol biosynthesis. *J Biol Chem* 2000;275(39):30610-7.
27. Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng* 1995;8(2):127-34.
28. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. *J Mol Graph* 1996;14(1):33-8, 27-8.
29. William H, Andrew D, Klaus S. VMD: visual molecular dynamics. *J Mol Grap* 1996;14:33-8.