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In Silico Development For Cholesterol Management-By The Inhibition Of Lanosterol Synthase

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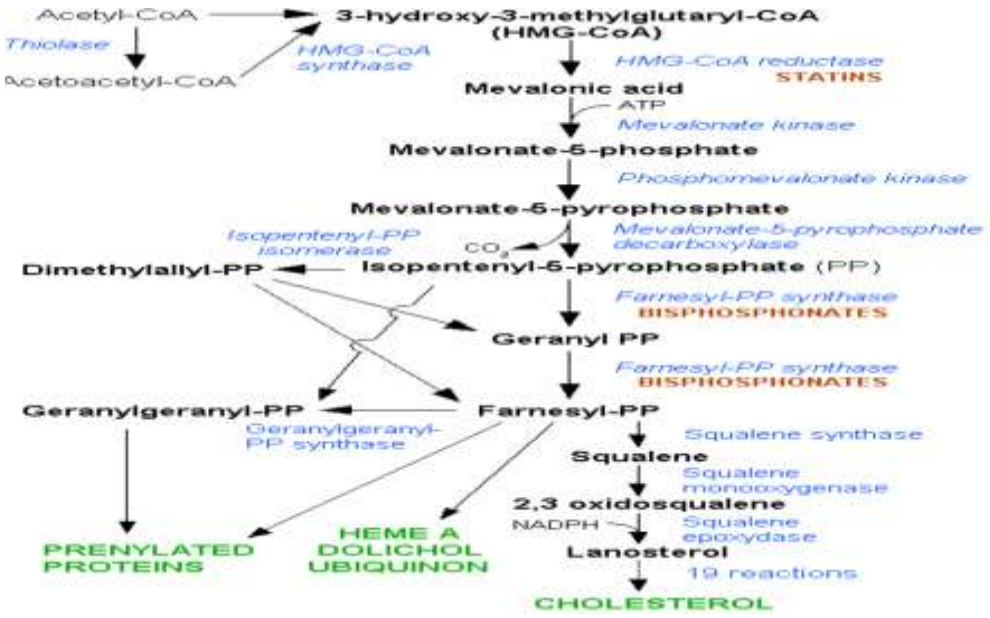
Abstract: *Cardiovascular disease remains the major cause of morbidity and death in developed countries, claiming 17.1 million lives a year. Tobacco use, an unhealthy diet, physical inactivity and harmful use of alcohol increase the risk of heart attacks and strokes .Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is the condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol. Though statins drugs are widely used for the treatment of atherosclerosis it also causes many side effects likes liver failure, rhabdomyolysis kidney failure etc. In this paper, we summarized the* **in silico** *methods used to enhance the understanding of Lanosterol synthase, particularly in its binding site, to design new drug molecules for the inhibition of cholesterol biosynthesis. Several computational methods such as docking approaches, molecular dynamics studies, quantum mechanical studies, among other physicochemical methods that exhibit quantitative structure-activity relationships have been used for this purpose.*

I. Introduction

Cholesterol is a lipid, waxy steroid [1] found in the cell membranes and transported in the blood plasma of all animals. It is an essential component of mammalian cell membranes where it is required to establish proper membrane permeability and fluidity [2]. Cholesterol is the principal sterol synthesized by animals, but small quantities are synthesized in other eukaryotes, such as plants and fungi. It act as the precursor of steroid hormones, bile salts and vitamin D [3]. There are two types of cholesterol HDL and LDL. High density lipoprotein, also known as HDL, is considered the "good" cholesterol. HDL is produced by the liver to carry cholesterol and other lipids (fats) from tissues and organs back to the liver for recycling or degradation. High levels of HDL are a good indicator of a healthy heart low density lipoproteins, or VLDL, are lipoproteins that carry cholesterol from the liver to organs and tissues in the body. They are formed by a combination of cholesterol and triglycerides. VLDLs are heavier than low density lipoproteins, and are also associated with atherosclerosis and heart disease. In human, about 20–25% of total daily cholesterol production occurs in the liver; other sites of cholesterol biosynthesis [4]

1.1 Scheme1: Cholesterol biosynthesis pathway



1.1 Inhibition of cholesterol synthesis:

Biosynthesis of cholesterol can be controlled by inhibiting the activity of enzymes which are involved in the biosynthetic pathway such as HMG Co. A synthase, HMG Co. A reductase, lanosterol synthase etc. Statins (vastatins, pravastatin, simvastatin ,lovastatin etc.,) which are a class of anticholesterol drugs found to be very effective inhibitors of HMG Co.A reductase (Alberts *et al*.,1980). Other possible targets includes apolipoprotein , ATP- binding cassette Protein G(ABCG), angiotensin converting enzyme like protein , c-reactive protein and sterol regulatory element binding protein The gene codes for the protein PCSK9 is a promising new target for the development of a new class of cholesterol lowering drug.[5]

1.3 Lanostrol synthase [oxido squalene cyclases]

Lanosterol synthase [EC5.4.99.7] [PDB id: 1W6J] also has great biological and medicinal significance. Vertebrates synthesize, OSC as the initial sterol precursor to cholesterol, the steroid hormones and vitamin-D. It alters 20 bonds and forms 4 rings and sets seven stereo centers. Sterols are important as structural compounds of plasma membrane [6]. Plants sterols show a wide structural variety and significant structural differences from those of animals. The difference in the biosynthesis of sterols between plants and animals begin at the step of cyclization of 2,3-oxido squalene to lanosterol in animals and to cyclo artenol in plants,in the presence of the enzyme lanosterol synthase.

This enzyme majorly related to fluidity and ion permeability. The study of molecular evolution and phylogenitic utility for the lanosterol synthase gene could contribute to the elucidation of evolutionary histories related to lineages of animals and fungi. Although molecular phylogenitics using DNA sequences have solved many controversial systematic problems.

With 5 protein sequences of lanosterol synthase which are included in animals and fungi, 200 homologous sequences were extracted from the UniProt database. The exclusion of paralogous sequences from the dataset composed of 200 homologs, 5 reference sequences, and an out group finally generated a protein alignment of 25 lanosterol synthase and one cycloartenol synthase with the length of 679 amino-acid residues. From the alignment and its codon-based mRNA aligned dataset, the following major results were derived: 1) All phylogenetic trees supported the bifurcation of animals and fungi. 2) The maximum-likelihood parameter estimates of various evolutionary models indicated that most regions of lanosterol synthase have evolved under purifying selection. 3) The gene encoding lanosterol synthase provided the most sufficient phylogenetic information in comparison to the gene encoding the second largest subunit of RNA polymerase II (RPB2) and the small subunit of ribosomal DNA (rDNA-18S). 4) The lanosterol synthase gene and rDNA-18S were highly compatible to each other with strong congruence.

Lanosterol synthase plays central role in cholesterol and sterols biosynthesis, there is a great interest in the identification of drugs that target this enzyme for anticholesteramic purpose.Histone deacetylase 3[HDAC3] repress transcription from the synthesis promoted. The human lanosterol synthase, gene encodes a 732 aminoacid protein with a predicted molecular mass of 83kPA.In human OSC,There is already five natural.ligands present.They are R71A1733,BOGA1734,C14A1735,BOGA1736,BOGA1737.Two crystal structure of human OSC have been reported.A large active site cavity is located in the centre of the molecule between the two domains.

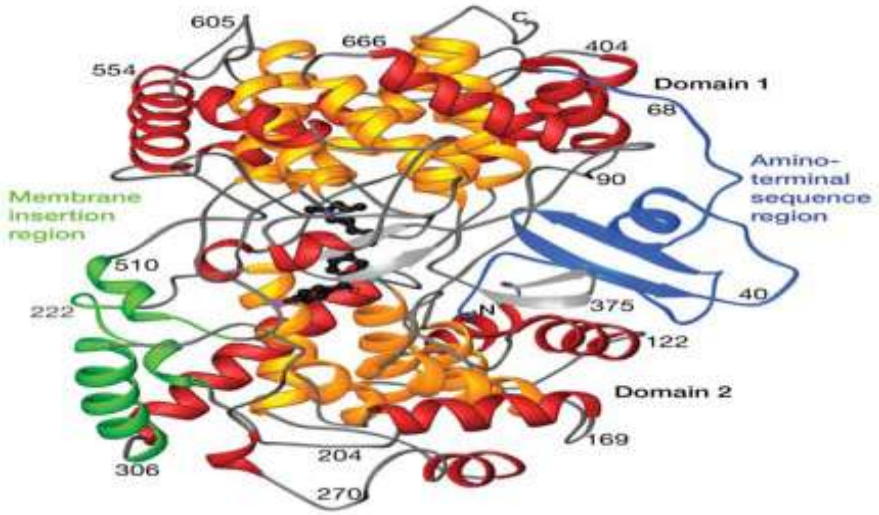


Figure1:Retrieval of structure of human lanosterol synthase from- protein data bank[7]

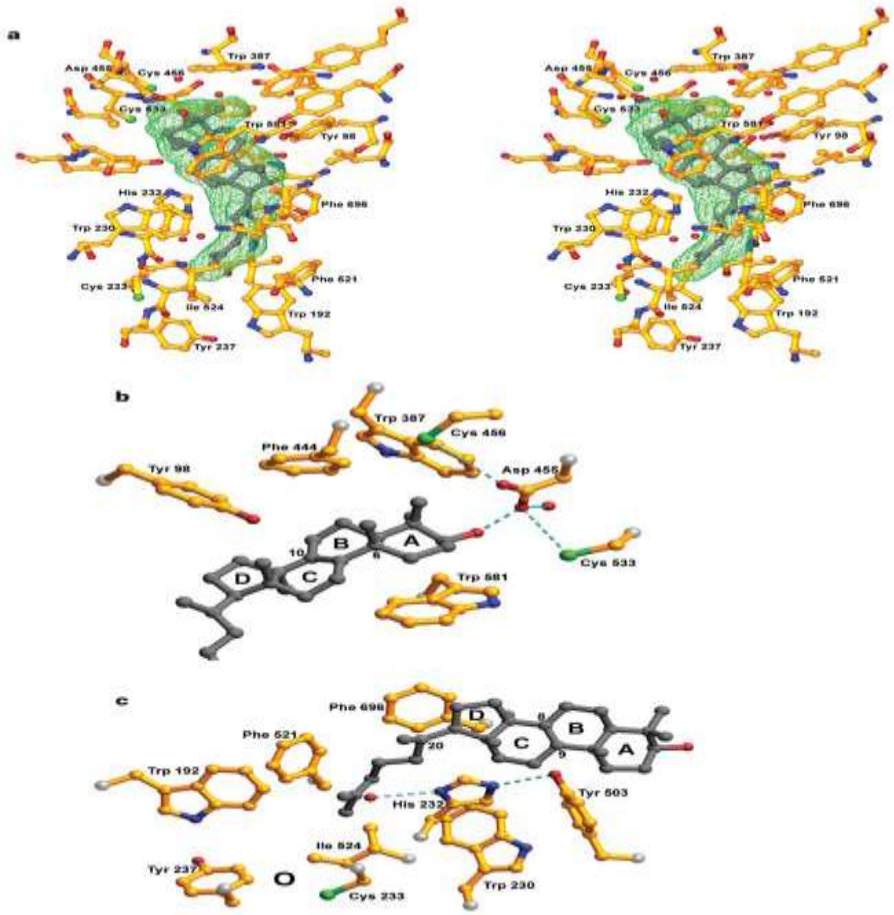


Figure 2: Active site determination[7]

II. Computational Details

On the basis of this active site [7] and pharmacophore prediction, we predicted, which ligand bind to the corresponding active site. This process is known as docking. By knowing the structure of ligand in the active site, by using virtul screen we can produce number of derivatives of that ligand. From this process we got an optimized ligand. *In silico* research in medicine is thought to have the potential to speed the rate of discovery while reducing the need for expensive lab work and clinical trials. One way to achieve this is by producing and screening drug candidates more effectively. In 2010, for example, using the protein docking algorithm EADock (Protein-ligand docking), researchers found potential inhibitors to an enzyme associated with cancer activity *in silico*[8].

ZINC DATA BASE was used for making drug derivatives or ligand derivatives. SCHRODINGER GLIDE-9.0 was used for molecular docking. Through molecular docking we tried to predict the structure of the intermolecular complex formed between two or more constituent molecules. It was also used to predict the binding orientation of small drug candidate with their protein target. [9]

2.1 ADMET-prediction [10]

- ADMET calculation was performed using Qik prop to predict the toxicity and side effect of the ligand.
- Qik prop predict the physically significant descriptors and pharmaceutically relevant properties for organic structures.
- Provides ranges for comparing the particular molecule's properties with those of 95% of known drugs.[11]

2.2 QSAR ANALYSIS

It was done in order to correlate the molecular descriptors [physicochemical properties] with the biological activity, i.e, the docking score by using fast statistics. Using Fast statistics, regression analysis was done and the value coefficient of determination [R squared].

III. Result And Disscussion

Table (a)

Title	Docking sco	mol MW	CNS	SASA	PISA	WPSA	dipole	volume	QPlogHER	QPlogKhs	#ringatom	PSA
ZINC08442127	-10.897925	430.505	0	728.869	331.588	0	5.85	1328.807	-6.318	0.471	24	76.205
ZINC00633914	-9.19	461.375	0	754.744	300.414	102.254	6.713	1305.388	-5.387	0.459	17	76.001
ZINC19867021	-9.120204	388.466	0	681.599	132.587	0	5.989	1221.978	-5.949	0.222	12	109.123
ZINC00633982	-9.34	464.368	-2	799.78	329.229	155.088	6.553	1375.451	-5.807	0.244	17	105.047
ZINC12378847	-8.701834	376.473	-2	720.099	154.832	35.187	8.741	1233.854	-5.95	-0.105	11	107.51
ZINC19990034	-8.23772	285.403	1	562.95	259.863	69.074	4.821	964.831	-5.38	0.424	12	31.671
ZINC08442268	-8.1961	444.486	0	713.146	450.481	0	5.289	1326.812	-6.612	0.611	22	74.596
ZINC06182368	-7.5979	267.299	0	492.371	114.391	28.839	6.406	819.067	-4.345	-0.348	10	81.493
ZINC00633900	6.406	498.387	-2	763.728	267.561	185.505	2.781	1313.36	-6.701	0.411	16	102.917
ZINC00634014	-9.87	437.559	-2	832.564	319.263	21.413	4.28	1440.508	-7.247	0.691	17	97.358
ZINC05921051	-7.6	556.7	-2	935.384	205.138	0	9.833	1755.071	-6.519	1.283	26	115.406
ZINC05556455	-7.04	249.269	-1	505.239	62.713	0	0.945	845.845	-4.159	-0.793	9	92.016
ZINC00633938	-8.73	499.329	0	748.344	264.109	174.855	2.605	1295.07	-6.548	0.596	17	81.317
ZINC08442218	-9.721485	423.486	-2	746.325	187.819	49.194	4.808	1285.256	-4.84	-0.215	18	106.548
ZINC00634001	-9.69	435.945	-2	745.442	304.526	130.403	3.165	1286.453	-6.748	0.347	16	102.521
ZINC00977563	-8.82	452.327	0	693.972	454.641	118.638	10.918	1180.967	-7.283	0.696	21	91.08
ZINC05556455	-7.04	249.269	-1	515.992	69.331	0	3.094	852.747	-4.406	0.005	9	84.626
ZINC00634140	-7.72	447.53	0	772.322	366.314	0	6.132	1428.175	-5.236	0.078	18	66.515
ZINC00667005	-6.54	445.492	-2	791.255	156.433	20.877	5.614	1375.075	-6.23	0.107	15	136.688
ZINC19794473	-9.5187	374.841	-2	636.554	265.827	88.281	2.367	1087.595	-5.98	0.253	17	81.855
ZINC00634140	-7.72	447.53	0	772.322	366.314	0	6.132	1428.175	-5.236	0.078	18	66.515
ZINC00730699	-9.699	435.497	-2	747.069	202.446	0.667	3.017	1304.271	-4.8	0.025	18	125.58
ZINC00666288	-9.98	452.542	-1	770.204	265.779	95.701	4.74	1335.191	-6.661	0.714	24	90.078
ZINC09365179	-8.0192	276.337	0	563.827	395.509	0	5.627	945.438	-6.23	0.383	16	42.001
ZINC00633970	-9.12	454.543	-2	833.394	243.642	26.385	0.869	1448.592	-5.046	0.462	17	121.916
ZINC02124700	-9.13	517.426	0	807.438	256.837	157.647	7.197	1443.152	-6.561	1.374	25	61.293
ZINC00633951	-6.7	464.941	-1	823.068	231.514	135.894	5.833	1405.178	-6.889	0.841	17	80.709
ZINC02258599	-8.8636	340.437	-2	683.571	15.947	30.539	3.845	1150.517	-5.946	0.228	9	103.227
ZINC08442201	-10.45098	434.548	-2	772.891	300.793	114.736	5.28	1316.011	-6.989	0.045	15	115.341
ZINC08442244	-9.748405	547.453	-2	776.255	257.271	68.086	3.803	1437.413	-6.1	0.871	30	111.983
ZINC08442277	-6.490466	462.522	-2	779.243	420.627	1.703	11.047	1373.85	-7.308	0.099	22	107.499

Table (b)

ZINC00633984	-9.37	437.286	0	669.78	281.552	155.488	9.779	1137.29	-6.326	0.326	17	83.348
ZINC00633925	-9.01	497.356	0	765.018	233.123	174.855	1.361	1332.831	-6.436	0.898	17	71.819
ZINC00633953	-10.4	456.389	-1	786.519	131.336	155.176	7.462	1358.318	-4.762	0.246	17	100.717
ZINC08442294	-9.6324	506.55	-2	795.481	434.868	52.908	4.402	1425.057	-7.329	-0.016	27	126.256
ZINC00634138	-6.83	447.53	0	733.581	362.143	0	6.059	1385.96	-4.805	-0.044	18	67.162
ZINC00634115	-9.88	429.312	1	661.519	264.294	76.996	3.421	1167.233	-5.859	0.517	21	43.94
ZINC08442217	-6.256756	451.583	-2	816.732	17.262	20.491	3.831	1463.172	-4.072	-0.475	15	103.911
ZINC00633947	-8.55	458.592	-2	845.191	233.778	70.414	1.687	1455.296	-6.938	0.828	17	80.7
ZINC02124712	-9.16	485.37	0	755.883	342.923	137.736	2.093	1305.039	-6.985	1.159	25	47.258
ZINC00664948	-8.61	430.16	2	664.842	293.819	257.985	4.127	1188.988	-5.904	0.783	17	20.249
ZINC00633997	-9.37	421.919	-2	730.969	307.219	142.153	4.774	1243.097	-6.801	0.275	16	104.361
ZINC08442215	-1.452454	464.523	-2	959.154	297.664	0	10.119	1600.931	-7.967	0.47	12	128.1
ZINC00634008	-9.01	437.559	-2	842.313	299.531	21.411	5.955	1453.346	-7.221	0.723	17	101.233
ZINC00633992	-9.1	421.919	-2	731.203	306.932	140.131	2.933	1242.825	-6.806	0.274	16	104.903
ZINC02124704	-8.05	452.954	0	754.489	285.699	99.87	3.355	1331.367	-6.523	1.036	25	62.468
ZINC08442269	-8.7943	428.487	0	711.504	452.263	0	4.842	1315.471	-6.666	0.776	22	66.119
ZINC08442297	10.1344	421.467	-1	662.722	379.841	1.691	7.262	1201.785	-6.177	-0.063	21	86.487
ZINC02124722	-6.57	470.945	0	775.364	267.126	142.125	2.833	1353.432	-6.638	1.092	25	60.564
ZINC05286115	-8.5117	354.447	1	609.502	373.87	47.426	4.574	1058.12	-6.164	0.182	20	34.243
ZINC00633966	-8.19	478.395	-1	819.754	320.283	153.72	7.363	1418.104	-5.817	0.355	17	98.917
ZINC00666285	-8.85	426.917	0	715.669	223.358	115.716	3.075	1241.499	-6.148	1.012	24	65.079
ZINC08442278	-9.126	430.536	-2	687.096	284.097	1.689	9.961	1256.535	-5.806	0.037	18	92.114
ZINC00634138	-6.83	447.53	0	733.581	362.143	0	6.059	1385.96	-4.805	-0.044	18	67.162
ZINC08442161	-11.273061	421.516	0	702.63	468.654	24.346	4.914	1273.38	-6.864	0.941	27	45.762
ZINC18153302	-5.6654	136.113	-1	297.138	125.716	0	1.983	440.976	-3.055	-0.777	9	82.664
ZINC08442296	-8.6444	540.995	-2	801.41	401.184	101.409	5.3	1455.096	-7.088	0.064	27	126.256
ZINC20030231	-5.084842	209.201	-2	450.154	145.256	0	4.174	706.702	-2.806	-0.611	6	93.577
ZINC02124702	-8.05	529.423	0	801.023	264.167	147.009	7.699	1418.204	-6.641	1.506	25	50.897
ZINC00634006	-7.93	437.559	-2	842.61	299.663	21.412	5.453	1453.813	-7.224	0.724	17	101.315
ZINC02124706	-8.62	457.373	0	762.891	336.882	154.116	2.202	1321.477	-6.967	1.204	25	47.257
ZINC00667005	-6.54	445.492	-2	791.255	156.433	20.877	5.614	1375.075	-6.23	0.107	15	136.688
ZINC02124697	-9.09	485.37	1	685.632	274.443	169.104	2.494	1243.98	-5.82	0.789	25	47.708

The output from in silico development method has been shown in Table[a]and [b].The value of R squared was found to be 0.1474. R Squared has been used in the context of statistical models, whose main purpose is the prediction of future out comes on the basis of other related information. The normal range of R squared is in the between 1 and 0.

Multiple linear Regression analysis, docking score versus #rotor?(no. of non-trivial, non-hindered rotatable bonds),CNS(predicted central nervous system activity on a -2(inactive) to +2(active)) ,mol MW(molecular weight of the molecule),dipole(computed dipole moment of the molecule),SASA(total solvent accessible surface area in square angstroms using a probe with a1.4A^0 radius),FOSA(hydrophobic component of

SASA),PISA(hydrophilic component of SASA), WPSA(weakly polar component of the SASA),volume(total solvent-accessible volume in cubic angstroms using a $1.4A^0$ radius).
Dockingscore=3.7295+.3114*#ROTOR+.9470*CNS+.0432*molmw+.0733*dipole+793.061*SASA+793.083* FOSA+793.059*FISA+793.062*PISA+793.067*WPSA+0.266*Volume

IV. Conclusion

Atherosclerosis is a complex chronic disease characterized by the accumulation of lipids within the arterial walls that eventually go on to from plaques. One well known risk factor in humans in hypercholesterolemia. That is elevated total cholesterol [TC] and [LDL-C]. Statin or HMG CO-A reductase inhibitors are a class of drugs include Pravastin, vastatin, imvastin, lovastatin, fluvastatin, atrovastin etc,that up regulates LDL receptor activity and as a result decrease the plasma cholesterol concentration. Though they are widely used continuous usage of the drugs may causes many serious side effects such as neuropathy, liver damage, kidney failure etc

In the present study[12] large number of ligand were screened to predict their binding affinity with the target Lanosterol Synthase .Some zinc compound like Zinc08442161, Zinc 08442201, Zinc08442127, Zinc019989790, Zinc08442201 and Zinc08442200 gave comparatively high docking score corresponding -11.273,-10.897,-10.407,-10.450 and -10.278 respectively. And their binding affinity can be improved by the ligand optimization that may help in the future development of drug candidate with fewer side effects.

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