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Discovery of Apob Small Inhibitor Molecules by Computer Aided Drug Design to Lower Cholesterol Accumulation Based On Docking Analysis

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Abstract: *Cholesterol accumulation leads to coronary heart disease, artherosclerosis, non-insulin dependent diabetes and various other cardiovascular diseases. Apolipoprotein B-100 (ApoB-100) is an integral part of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low density lipoprotein (LDL), and it plays a key role as a transporter of cholesterol and triacylglycerols to cells throughout the body. Microsomal trigylceride transfer protein (MTP) is a dependent factor as it transfers triglycerides onto lipoprotein particles. The Lipoprotein N-terminal domain (LPD_N)is found in ApoB-100 and MTP. Our study involves the design of suitable small inhibitor molecules to bind to Lipoprotein N-terminal domain and inhibit primarily ApoB-100. A library of designed compounds was virtually screened. 14 compounds have been designed that may potentially paralyze its cholesterol transport function. Molecular docking analysis of the ApoB-100 target and designed small inhibitor molecules provides evidence of effective binding of the compounds and interacting residues have been examined. This study could be promising in the discovery of drugs that reduce cholesterol accumulation and therefore result in potential decrease of cardiovascular diseases.*

Keywords: *Apolipoprotein B, ApoB, Cholesterol, Docking, Drug designing*

I. Introduction

Cholesterol accumulation is an increasing cause for cardiovascular diseases and is currently the major cause of morbidity and mortality. Due the change in lifestyle leading to cholesterol-related diseases such as, atherosclerosis, non-insulin dependent diabetes, obesity, myocardial infarction and coronary heart disease.

ApoB-100 (Apolipoprotein B-100) is part of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and LDL, and it functions as a transporter of cholesterol and triacylglycerols to cells throughout the body[8]. The assembly and secretion of ApoB-containing lipoproteins within the endoplasmic reticulum is strictly dependent on the microsomal trigylceride transfer protein (MTP) which shuttles triglycerides onto the nascent lipoprotein particle [6]. Sequence analysis showed that the amino-terminal 700 amino acids of vitellogenin Vtg and apolipoprotein (apo) B-100 are homologous, although the similarity is limited. Coincidentally, Vtg also binds lipids and transports them into the oocytes. The sequence and functional relationship of these two proteins support the idea that they have a common ancestor [2]. Like ApoB- 100, vitellogenin binds hydrophobic molecules such as phospholipids, triacylglycerols and cholesterol and it transports these molecules to a target cell. Also, like ApoB- 100, vitellogenin binds to a membrane receptor and enters the cell by endocytosis [12].

Computational (*in silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. These *in silico* methods include databases, quantitative structure-activity relationships, similarity searching, pharmacophores, homology models and other molecular modeling, machine learning, data mining, network analysis tools and data analysis tools that use a computer. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization [19].

Our study involves the design of a suitable inhibitor molecule to bind to the vitellogenin domain and inhibit MTP and ApoB thereby resulting in potential decreased risk of cardiovascular diseases. By binding to ApoB may paralyze its cholesterol transport function. Bezafibrate is a drug shown to reduce the plasma cholesterol levels in many studies, similarly Miglustat an imino sugar; its treatment reduces lipid storage. Tea catechins [18, 7, 11] were identified by studies to be responsible for the cholesterol lowering function of green tea and therefore selected for this study. Canadine and berberine, plant alkaloids were identified as cholesterol lowering compounds [17]. Significant phytosterols [15, 21, 10, 4] gugglusterone, stanosterol, stigmastan, campestanol and campesterol have been previously studied and shown to have effects in lowering the level of cholesterol and therefore were selected for the designing of potential inhibitor molecules. Molecular docking studies were

carried out to determine interactionsin order to provide a new insight to design novel molecules that can inhibit the function of the target. The computational analysis was carried out using Discovery Studio (DS) 2.5 (Accelrys Software Inc., San Diego; http://www.accelrys.com).

II. Materials And Methods

Cholesterol-related diseases have caused large number of deaths and therefore, in our study this issue has been addressed by designing potent inhibitors in order to help combat the rising health problems associated with cholesterol accumulation.The target APOB_HUMAN (P04114) was retrieved from UniProt[3] and its domains were analyzed in SMART [8].The Lipoprotein N-terminal domain (LPD_N) was found from position 46-598 with an E-value of 6.97e-140. This represents a conserved region found in several lipid transport proteins, including vitellogenin, microsomal triglyceride transfer protein and Apolipoprotein B-100 [1]. The target ApoB is then modeled by homology modeling using CPHmodels-3.0 [14]with a Z-score = 59.41and validated in DS 2.5.The template chosen was 1LSH- the refined molecular structure of lipovitellin solved at 1.9 Å having an e-value of 5e-05 and 99% query coverage when a similarity search by protein-BLAST[20] against Protein Data Bank (PDB)[16]was conducted.

2.1Receptor – Ligands Preparation:

For the current study, bezafibrate, miglustat, canadine, berberine, catechin gallate, epigallocatechin gallate, guggulsterone, stanosterol, stigmastan, campestanol and campesterol were retrieved from PubChem[5] based on various literatures [15,18,7,11,21,10,4] substantiating their biological functionality. Furthermore, CHEMBL143610, CHEMBL141881, CHEMBL140786 and CHEMBL143821 from ZINC[9]database were retrieved for the corresponding target and were also used as scaffold molecules.The lead molecules were designed using ACD/ChemSketch Freeware. The modifications were not made to their functional group maintaining the molecules basic functionality. The 'General Purposes' protocol was used to calculate the molecular properties of the lead molecules to check if the compounds satisfy Lipinski's 'rule of 5'. The target ApoB-100 modeled structure was obtained and the site sphere (59.8562, 11.7025, 64.5437, 23) was defined for the binding site; typing was carried out by CHARMm forcefield (Momany-Rone parital charges methods) and followed by Conjugate Gradient minimization; until a constant potential energy is obtained. The screened compounds were typed similarly.

2.2 Virtual Screening:

The aforementioned library of compounds (approximately 222 compounds) was then subjected to Toxicity Prediction (TOPKAT) in the 'ADMET' protocol. NTP Carcinogenicity Call (Male Mouse) (v3.2), FDA Carcinogenicity Female Mouse Single vs Mult (v3.1), Developmental Toxicity Potential (DTP) (v3.1), Rat Oral LD50 (v3.1), Skin Irritation (v6.1) and Aerobic Biodegradabilty (v6.1), were the six criteria selected for the toxicity prediction. Further analysis by ADMET Descriptors in the 'ADMET' protocol was carried out to study the lead compounds pharmacokinetic properties.

2.3 Molecular Docking*:*

LibDock, a relatively fast algorithm that conducts 'HotSpots' matching of ligand conformations with rigid binding site's HotSpots map that is well-suited for large sized libraries [13], was used to dock the target and ligands into the binding site. The resulting poses with higher LibDock score were investigated and the interacting residues were examined.

III. Results And Discussion

Out of the 222 lead compounds designed, most of them qualified Lipinski's 'rule of 5' (Molecular properties shown in Table 1) and their toxicities were studied. Male and Female Mouse Carcinogenicity and Aerobic Biodegradability models were primarily considered for the evaluation of toxicity level. Probability values from 0.0 to 0.30 are considered low probabilities,and are likely to produce a negative response in an experimentalassay; whereas probability values greater than 0.70 are consideredhigh, and are likely to produce a positive response in an experimental assay, probabilities greater than 0.30 but less than 0.70 are considered indeterminate (shown in Table 2). 14 lead molecules with low and intermediate probability values were further investigated for pharmacokinetic properties. The ADMET profiles of the 14 lead compounds are shown in Table 3. Based on the drug-likeness and pharmacokinetic properties these lead molecules were selected for docking and the interactions were studied by docking simulations. The LibDock scores and the binding energies are shown in Table 4. The lead SSr133-[(2E)-2-(3-hydroxycyclohexylidene)ethyl]nonanoic acidderived from stanosterol docked with the highest LibDock score of 121.359and CG383-(benzyloxy)-3,4-dihydro-2H-chromene-6-thiol derived from catechin gallate, docked with a score of 109.827. SSr13 (shown in Fig 1a) interacts with residues LYS209, PRO123 within the lipid transport domain by Intermolecular Hydrogen bonds (Fig 2a) and Bumps (Fig 2b), respectively. CG38 (shown in Fig 1b) interacts with GLY93 by Intermolecular Hydrogen bonds (Fig 3). These compounds have good absorption and high brain concentration/blood concentration level indicated in Fig 4a and Fig 4b.

Scaffold	Lead ID	ALogP	Mol. Wt	H-Acceptor	H-Donors	Rotatable Bonds	Aromatic Rings
Bezafibrate	BF3	4.482	393.884	5	2	7	2
Canadine	CD18	1.992	323.407	6	4	7	1
143610	143610-1	2.476	336.384	3	3	4	2
Canadine	CD25	2.476	219.28	3	1	5	1
Canadine	CD26	1.385	235.279	4	2	6	1
Canadine	CD31	2.141	309.381	6	3	6	1
Stanosterol	SSr1	4.034	196.329	1	1	4	0
Stanosterol	SSr12	3.914	240.382	2	2	7	0
Stanosterol	SSr13	4.581	282.418	3	2	9	0
Stanosterol	SSr2	2.352	198.302	2	2	4	0
Stanosterol	SSr3	2.487	198.302	2	2	4	0
Catechin Gallate	CG21	1.644	275.3	5	2	5	1
Catechin Gallate	CG37	4.034	274.742	2	0	3	2
Catechin Gallate	CG38	3.729	272.362	3	1	3	2

Table 1: Molecular properties of the 14 lead molecules; the molecules satisfy Lipinski's 'rule of 5'

Molecule	NTP Carcinogenicity Call (Male Mouse)	FDA Carcinogenicity Female	Developmental Toxicity Potential	Computed Rat Oral LD50	Skin Irritation	Aerobic Biodegradability
143610-1	0.026	0.011	0	4.8g/kg	0	0.001
BF3	0	0	1	1.5g/kg	0	0
CD18	0.036	0	0.999	10g/kg	0.122	0
CD25	0.037	0	1	1.5g/kg	0	0.003
CD26	0	0	1	1.9g/kg	0	0.26
CD31	0	0	1	10g/kg	0.31	0
SSr1	0	0	0	10g/kg	1	0
SSr12	0	0	0	353.3mg/kg	1	0
SSr13	0	0	0	10g/kg	1	0.011
SSr2	0	0	0	10g/kg	1	0
SSr3	0	0	0	10g/kg	1	0
CG21	0.476	0	1	9.1g/kg	1	0.161
CG37	0.522	0.003	0.997	2.3g/kg	0	0.015
CG38	0.004	0.004	1	2.7g/kg	0	0

Table 2: Probability values of the TOPKAT prediction indicate all molecules are non-carcinogenic and have high level of aerobic biodegradability.

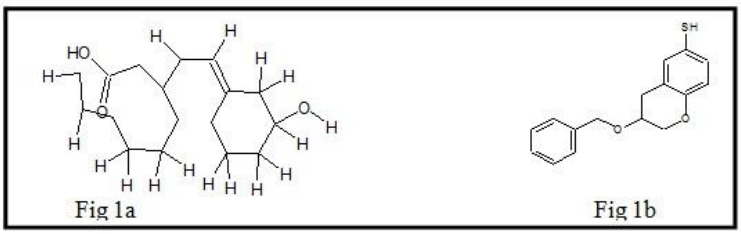
Lead ID	BB level	Absorption level	Hepatotoxicity probability	CYP2D6 Probability	PPB Level	AlogP98
BF3	1	0	0.569	0.356	2	4.482
CD18	3	0	0.463	0.415	2	1.993
143610-1	3	0	0.582	0.504	0	2.476
CD25	1	0	0.205	0.079	2	2.476
CD26	3	0	0.562	0.118	2	1.385
CD31	2	0	0.543	0.425	2	2.53
SSr1	0	0	0.39	0.188	1	4.034
SSr12	1	0	0.582	0.594	0	3.914
SSr13	1	0	0.509	0.455	1	4.581
SSr2	2	0	0.456	0.049	0	2.353

SSr3	2	0	0.543	0.059	0	2.487
CG21	3	0	0.39	0.405	0	1.644
CG37	0	0	0.364	0.702	2	4.034
CG38	0	0	0.337	0.702	2	3.729

Table 3: ADMET Descriptors; indicate that the lead compounds are predicted to be easily absorbed, low probability of causing hepatotoxicity and are non – inhibitors of CYPD26 enzyme

Molecule	Absolute Energy Kcal/mol	LibDock Score	Intermolecular Hbonds	Intermolecular Bumps
143610-1	90.5718	109.343	arg208	phe1, pro123
BF3	62.8112	68.2287	asn110	phe265, lys171
CD18	80.4664	86.3169	-	tyr138, lys171
CD25	59.3112	106.294	-	phe1, ile90
SSr1	36.4553	84.1861	-	phe1, gly93,val121
SSr12	28.3601	108.98	glu92	phe1, cys43, phe45, lys209
SSr13	23.6667	121.359	pro123	phe1, lys209,
SSr2	28.8808	81.3555	leu119	phe1, ile90, gly93
SSr3	26.6484	87.8954	leu119	phe1, pro123, lys209
CG21	29.0228	67.9289	-	ile111, val137
CG37	34.4242	98.921	gly93, lys209	-
CG38	36.1876	112.114	gly93	-
CD26	60.2112	109.827	-	lys209, ile90, pro123
CD31	74.864	82.9614	lys171	lys171

Table 4: LibDock scores and binding energies, indicating the residues participating in the binding between receptor and ligand



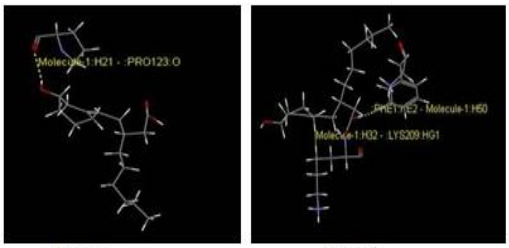


Fig 2a shows Intermolecular Hydrogen bond between SSr13 (H21) and (O-PRO123) target ApoB LPD_N lipid transport domain.
Fig 2b shows Intermolecular Bumps between SSr13 (H32) and (HG1-LYS209) of target ApoB LPD_N lipid transport domain. The interaction with PHE1 is outside the lipid transport domain

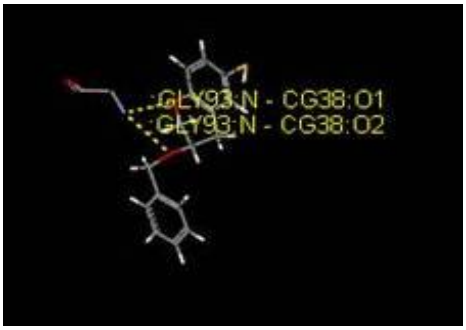


Fig 3 shows Intermolecular Hydrogen bond between CG38 (O1 and O2) and (N-GLY93) target ApoB LPD_N lipid transport domain

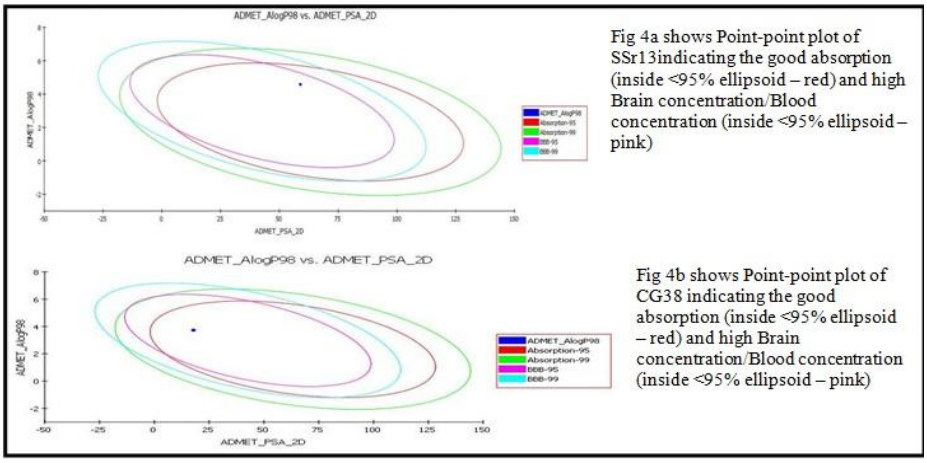


Fig 4a shows Point-point plot of SSr13indicating the good absorption (inside <95% ellipsoid – red) and high Brain concentration/Blood concentration (inside <95% ellipsoid – pink)

Fig 4b shows Point-point plot of CG38 indicating the good absorption (inside <95% ellipsoid – red) and high Brain concentration/Blood concentration (inside <95% ellipsoid – pink)

IV. Discussion

Apolipoprotein B (ApoB) bind to lipid molecules such as cholesterol and triglycerides and transport them and it is primary constituent of chylomicrons and Low Density Lipoproteins that generally contribute to the bad cholesterol in the body leading to various heart diseases. Due this function they play a key role in cholesterol accumulation and have been selected for the current study. The compounds selected as scaffold were phytosterols (guggulsterone, campesterol, campestanol, stanosterol, and stigmastan), tea catechins (cateching gallate and epigallocatechin gallate), alkaloids (canadine and berberine) and FDA approved drug (bezafibrate and miglustat) compounds; all proven to reduce the levels of cholesterol.The lead compounds were predicted as non – carcinogenic, non – hepatotoxic, non – inhibitors of the CYPD26 enzyme with good absorption levels and aerobic biodegradability, thus indicating low risk of possible side effects. Molecular docking studies lead to the observation that SSr13 and CG38 bind with highest docking scores thereby indicating potential inhibition of the target ApoB-100 protein. The interacting residues of target, which form hydrogen bonds and bumps with the compounds are ILE90 , GLU92, GLY93, ASN110,ILE111,VAL121,PRO123, VAL137, TYR138,LYS171,ARG208,LYS209, PHE265, lying within the LPD_N lipid transport domain (46 – 598) of the target responsible for the lipid transport.

V. Conclusion

Drug discovery process is multi-phased and involves preclinical and clinical trials. Computer aided approach is rapid and significant because it selects thelead molecules with good pharmacological and drug-like properties. ApoB-100 has important role in the utilization of cholesterol, triacylglycerols, and other lipids. The ApoB-containing lipoproteins depend on the microsomal tricylceride transfer protein (MTP) for its secretion. 14 small inhibitor molecules have been designed to bind to the LPD_N lipid transport domain.

Out of the 14 designed inhibitors, 3-[(2E)-2-(3-hydroxycyclohexylidene)ethyl] nonanoic acid (SSr13) designed from the scaffold molecule Stanosterol and 3-(benzyloxy)-3,4-dihydro-2H-chromene-6-thiol (CG38) derived from Catechin gallate was found to be the compounds with best docking scores. Furthermore, the interactions between SSr13 and LPD_Ndomain are more in number suggesting stronger and potentially effective binding. The interacting residues are within the lipid transport domain further substantiating the conclusion that SSr13 is the most potent inhibitor molecule designed to inhibit ApoB-100 and may potentially paralyze its cholesterol transport function.

This study could be a platform for facilitating further development of drugs that moderate cholesterol accumulation and thereby result in likelydecrease of the life threatening cardiacdiseases.

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