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Computational prediction of ADMET properties of ACAT inhibitors for synthesis and pharmacological screening

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***Abstract*---**As we know ACAT enzyme is responsible for the catalysis of cholesterol into cholesteryl esters leading to Hyperlipidemia which is directly linked with cardiac problems. Throughout the world cardiac problems are causing increased mortality. This study aims to synthesize and screen pharmacologically active compound from the best five compounds docked having good binding affinity found in our earlier research against ACAT enzyme. The compounds were detected for their ADME value by SwissADME software and checked the toxicity level via ProTox-II server showing the antihyperlipidemic effect against ACAT enzyme. For pharmacological screening rats were fed with High Fat Diet and then evaluated by Atorvastatin (10 mg/kg) which was used as standard drug and the test compound. Blood samples were collected at 28th day. Serum was separated and then analyzed for lipid profile, using standard diagnostic reagent kits. The results demonstrated that the synthetic drug C10 raised the concentration of high-density lipoprotein in serum while lowering the total cholesterol, triglycerides, and low-density lipoprotein. C10 compound possess antihyperlipidemic activity.

***Keywords*---**Chylomicrons, toxicity, physicochemical, lipoprotein, pharmacokinetic.

Introduction

As in our previous study we discussed that all mammalian cells [1] have the lipid molecule cholesterol in their membranes, which is essential for the growth and survival of those cells. In human macrophages, the enzyme ACAT; a 56-kDa

protein [2] catalyses the storage of excess cellular cholesterol as cholesteryl esters (CE) and this CE serves as the cholesterol reservoir for producing steroid hormones.

Atherosclerosis is brought on by high blood cholesterol levels; the continuous buildup of CE in macrophages makes these cells seem foamy and is a defining feature of early stages of atherosclerosis, a major contributor to cardiovascular issues. In the world's poor and underdeveloped nations, this increases the risk of coronary heart disease and other types of life-threatening cardiovascular and cerebrovascular damage, including deadly attacks. And thus it is the main cause for increased mortality rate also [2, 3, 4, 5, 6, 7]. The adoption of a sedentary lifestyle as well as a rise in the consumption of high-fat foods has a significant impact on its prevalence. [5,8] By means of cholesterol manufacturing, dietary cholesterol absorption, removal from the circulation, and cholesterol elimination through faeces and bile, the normal level of cholesterol in plasma can be maintained. Increased levels of "low-density lipoprotein (LDL), total cholesterol (TC), very low-density lipoprotein (VLDL), and decreased levels of high-density lipoprotein (HDL)" in serum are key risk factors for hyperlipidemia. Steatosis, or fatty liver, is the initial result of such excessive lipid accumulation in the liver. Hepatocytes are destroyed during the chronic stages. [3] ACAT has been regarded a pharmacological target for therapeutic treatment over cholesterol as well as other pathological conditions in an effort to slow down the atherogenic pathway for the purposes mentioned above. [7] Statins, or antihyperlipidemic medications, are often used to lower elevated levels of lipids in the bloodstream. They are thought to achieve this by hitting hepatocytes and blocking HMG-CoA reductase, an enzyme that transforms 3-hydroxy-3-methylglutaryl CoA into mevalonic acid, a precursor to cholesterol. The negative effects of these medications, however, are numerous and include nerve damage, hyperuricemia, erythema, liver cirrhosis, dry skin, gastrointestinal disruption, abdominal discomfort, and bloating. [3, 5]

An important category of heterocyclic compounds with a variety of biological actions, such as "anticancer, antiviral, anti-inflammatory, anti-hypertensive, antihistaminic and antibacterial properties" are aryl-substituted benzoxazoles and benzothiazoles. [9]

New analysis approaches are needed to address new synthesis problems. Past few years have seen enormous advancements in computer and protein crystallography, which has sparked a search for unique chemical substances and innovative structural scaffolds. Although computerized study does not actually replace experimental research, it has become abundantly evident that a successful interaction in between two is crucial for assisting aspiring researchers in the efficient formulation and identification of drugs. Combining random check and rational design has been demonstrated to be a significant advancement in the drug discovery process that results in the identification of compounds. These attempts are anticipated to eventually create molecules with increased potency and effectiveness. Finding the target protein and active site alone is frequently insufficient for a process of drug discovery to come to a rational conclusion. Additionally, it is crucial to examine pharmacokinetics and toxicological profiles in vivo, which can be done by using screening techniques to determine whether a

chemical is active or not. As a result, a thorough computer analysis will not always yield a conclusive result. [10]

Virtual Screening

Virtual screening has emerged as an effective and profitable tool for the pharma company over the past decade for searching for novel chemicals that resemble drugs, or so-called hits. Through *insilico* approach we got five best docked compounds in earlier research C10, C3, C9, C8S, C7S, as antihyperlipidemic agent against ACAT enzyme Table 1. These were subjected to SwissADME software for pharmacokinetic study and toxicity by Protox II software. [11]

Ligand name	**Binding energy** (kcal/mol)	**Full Fitness** (kcal/mol)	**Estimated ΔG** (kcal/mol)
C10	-9.88	57.74	-11.66
C3	-9.79	66.28	-11.88
C9	-9.66	82.5	-11.16
C8S	-9.37	134.62	-11.76
C7S	-8.96	268.55	-11.05

Table 1: Binding energy, full fitness, and estimated ΔG values predicted for ligands docked with the target ACAT enzyme by AutoDock Tools

Ligand Preparation and Drug-Likeness Prediction

The structures drawn on BIOVIA Draw 2021 were saved in SMILES and PDB formats. The compounds were uploaded to the SwissADME webpage in the SMILES type (http://www.swissadme.ch) Table 2 and Table 3 ProTox-II servers. Physicochemical properties like 'molar mass, hydrogen donor, acceptor, log P value, pharmacokinetic properties (gastrointestinal) GI absorption, water solubility, and topological polar surface area" are among the filters and properties checked by the SwissADME server to determine whether a compound has the potential to be drug-like (TPSA). Computational toxicity estimations are performed on the ProTox II server, which are not only quicker than determining harmful doses in animals but may also help to cut down on the number of animal trials. It is a free *insilico* toxicity predictor which predicts the lethal dose 50 (LD50) value in mg/kg body weight, according to which the server has classified the six classes into which the drug can fall depending on its predicted LD50 value. Class 6 is nontoxic and safe for consumption. LD50 is the dose at which 50% or half of the test population will die upon exposure to a compound. The server accepts input in SMILES format and also by drawing through its embedded structure drawing plugin on the site. After submission, the results are returned as the predicted LD50 value in mg/kg weight, the prediction accuracy in percentage, and the similarity of the input compound with other similar toxic compounds from the dataset with known rodent oral toxicity values.

The BOILED-Egg assessment, one among SwissADME's enhanced extras, forecasts GI absorption (HIA) & P-glycoprotein permeability (Pgp). Additionally, it

is possible to anticipate (CYP 450) enzymatic ligand reduction as well as blood-brain barrier (BBB) absorption [12]. The BOILED-Egg outcome is displayed in Fig. 1.

Ligand	Chemical Formula	Swiss ADME Filters							
		Molar mass (g/mol)	H bond acceptor	H bond donor	iLogP	Water Solubility mg/ml	GI absorption	TPSA (Å2)	Drug-like ness
C10	$C_{18}H_{28}N_4O$	310.39	3	2	3.55	8.61(Moderate)	High	53.33	Yes
C3	$C_{17}H_{25}N_5O_3$	341.36	5	2	2.64	1.72(Moderate)	High	99.15	Yes
C9	$C_{17}H_{20}N_4O$	296.37	3	2	3.06	1.4(Moderate)	High	53.33	Yes
C8S	$C_{20}H_{32}N_4S$	354.51	2	2	3.74	1.02(Moderate)	High	68.43	Yes
C7S	$C_{19}H_{24}N_4S$	340.49	2	2	3.44	1.68(Moderate)	High	68.43	Yes

Table 2: Physico-chemical Properties Hydrogen donor, hydrogen acceptor, molar mass, water solubility, log P, gastrointestinal absorption, topological polar surface area, drug-likeness predicted by SwissADME for ligands

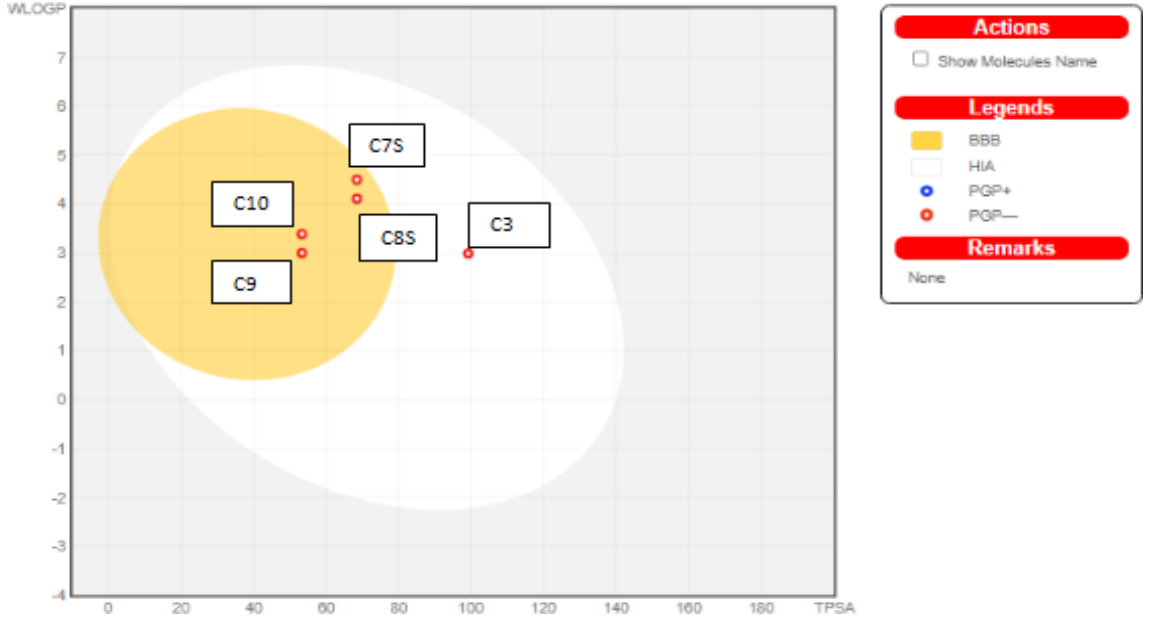


Fig.1. Compounds docked with best affinity are shown for GI absorption and Blood Brain Barrier penetration in the using BOILED-Egg (WLOGP-versus-TPSA).

Ligand name	LD50 mg/kg	Toxicity class
C10	600	4
C3	1000	4
C9	600	4
C8S	1070	4
C7S	1070	4

Table 3: LD50 and toxicity class predicted by Protox-II for the ligands.

Synthesis of Compound

From the above compounds C10 was synthesized [13, 14].

Method

Step I

KS_2COEt 16gm and substituted 2-Amino phenol (1) 11gm were combined with pyridine and refluxed for two hours. It was brought to room temperature before being poured into an ice water and concentrated HCl solution. The product was gathered, cleaned with water, placed in the hood for a few hours at 45°C, and then ground into a beige powder. By combining ethanol, potassium hydroxide, and carbon disulfide, KS_2COEt can be made separately and in advance.

Step II

20 ml of Thionyl chloride was added drop wise to the solution of 20gm of substituted benzoxazole-2-thione (2) in DMF and refluxed for half hour at 65-70^o C. Solvent was removed to get the desired product of substituted 2-chloro-benzoxazole (3).

Step III

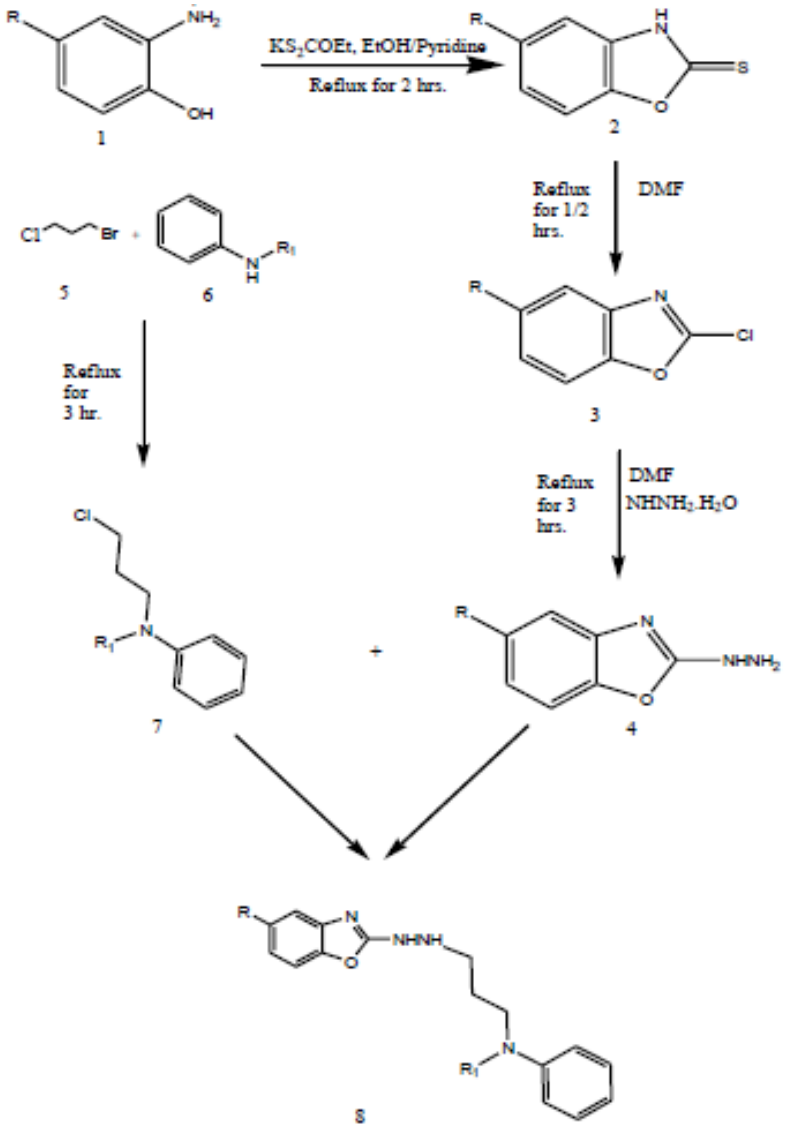
15 gm of substituted 2-chloro benzoxazole (3) in 7ml of DMF was placed in mixture of 5 ml $NHNH_2.H_2O$ and refluxed for 3 hr. The reaction mixture was extracted with ethyl acetate (3 times) and evaporated the collected organic layer to get the desired product substituted (benzoxazole-2-yl)-hydrazine (4). The yield of compound was found to be (90%).The characterization by TLC of the compound was done in methanol: DCM (5% methanol) and Rf value was found to be 0.45.

Step IV

15 ml of 1-Bromo-3-chloro- propane(5) and 10 ml of substituted phenyl-amine(6) was mixed by stirring and refluxed for 3 hr. Reaction mixture was extracted with diethyl ether; organic layer was separated and evaporated to get the desired product substituted (3-chloro-propyl) phenyl-amine(7). Hexane:Ethyl Acetate, 9.5:0.5, was used as the solvent system for TLC monitoring of the reaction.

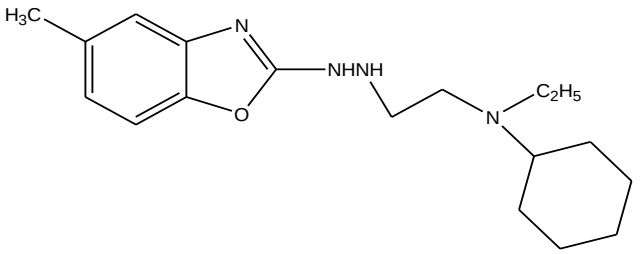
Step V

17 ml of (4) and 8 ml of (7) is refluxed in 5ml of benzene using 8 gm of sodium bicarbonate as a base for 5 hr. Solvent was evaporated to get the desired product (8). TLC was used to detect the reaction's completion using the solvent system (DCM: methanol=9.5:0.5).



Where R=-CH_3; R1=-C_2H_5

Fig2. Schematic representation of the synthesis of compounds



Cyclohexyl-ethyl-{2-[*N*'-(5-methyl-benzooxazol-2-yl)-hydrazino]-ethyl}-amine

Compound C10

Pharmacological Screening

Wister albino adult male rats weighing 150-170g were housed in groups in polyacrylic cages with a 12-hour light/dark cycle at a temperature of 25 ± 0.5 °C, and they were kept under conventional laboratory conditions. The study was conducted in accordance with the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Induction of Hyperlipidemia in rats:

Rats were taken and maintained on normal pellet diet for one week before commencement of the experiment. Rats were fed a high fat diet for 28 days to induce Hyperlipidemia. Rats were separated into 10 groups of five at random after one week [7]. The following four primary reference groups (I–IV) were assignedto rats:

Group I. Normal Controls (NC) group comprised 5 normal rats fed with a normal pellet diet and left intact without any treatment,

Group II. High Fat Diet (HFD) group consisted of 5 normal rats fed with daily High Fat Diet.

Group III. Standard group consisted of 5 normal rats fed a High Fat Diet for a period of 28 days and Atorvastatin from 8th day to 28th day 10mg/kg/day p.o.

Group IV was assigned to the test compound.

The treatment protocol for test compounds is as same as standard drug Atorvastatin i.e. from 8th day to 28th day according to the dose described from toxicity studies orally.

Blood samples were taken from the retro-orbital venous plexus under light ether anaesthesia using a glass capillary tube on 29th day and serum was immediately centrifuged at 3000 rpm for 20 minutes. Serum was separated and biochemical estimations were carried out through commercial reagents for:

- Total cholesterol (TC)
- High density lipoprotein (HDL)
- Low density lipoprotein (LDL)
- Triglycerides

Atherogenic index of plasma (AIP) will be calculated using the formulae:

AIP = Log (Triglycerides/HDL Cholesterol)

Ratio of **LDL/HDL** was also calculated.

Statistical Analysis

Graph Pad Prism software, version 9.4.1, was used to conduct a two-way ANOVA as well as descriptive statistical analysis of the data. Values were presented as Mean ± S.D., with P values 0.05 vs. Statistics were determined to be significant for the control group. [1]

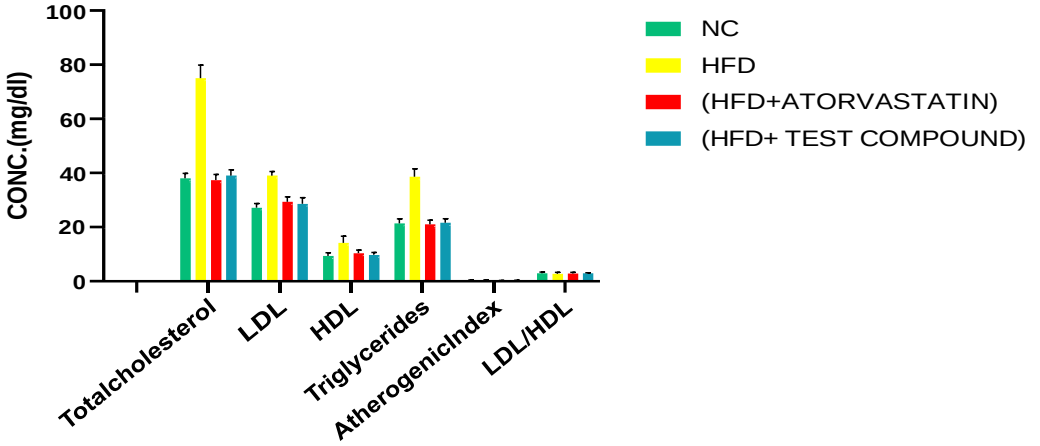


Fig.3. Results of cholesterol levels (mg/dl) analysis for various groups

Table Analyzed	Row statistics of XY: Entering replicate data		
Two-way ANOVA	Ordinary		
Alpha	0.05		
Source of Variation	% of total variation	P value	Significant?
Row Factor	82.54	<0.0001	Yes
Column Factor	7.413	<0.0001	Yes
Number of rows (Row Factor)	6		
Number of values	120		

Table 4. Two- Way ANOVA

Groups	Atherogenic Index	LDL/HDL
1. NC	0.3586 ± 0.0743	2.9338 ± 0.4520
2. HFD	0.438 ± 0.0779	2.8090 ± 0.4735
3. HFD+ATORVASTATIN	0.3062 ± 0.0707	2.8641 ± 0.4419
4. HFD+TEST COMPOUND	0.3436 ± 0.0536	2.9258 ± 0.2324

Values expressed as Mean ± SD of 5 animals P<0.005

Table 5. Effect of Drugs on AIP and LDL/HDL ratio

Discussion

As we have already known that the ACAT enzyme is involved in the production of cholesterol. The goal of our investigation was to create the most effective chemical entity from our previous docking study to act as an antihyperlipidemic drug against ACAT. We created compound C10 from a group of five compounds that displayed the best efficacy after subjecting it to SwissADME pharmacokinetic analysis and ProTox-II toxicity prediction. Pharmacological testing of additional substance for levels of total cholesterol was performed. The triglycerides/HDL ratio was used to calculate AIP.

The conventional approach to thinking about pharmacokinetics (i.e., what happens to a medicinal chemical in the body) is to separate the numerous effects that affect the target's access into separate parameters. These ADME parameters, which stand for Absorption, Distribution, Metabolism, and Excretion, can then each is assessed independently using specialized techniques. It has been shown that early ADME calculation during the discovery phase significantly lowers the percentage of clinical failures attributable to pharmacokinetics. In the early stages, when there are many researched chemical structures but few available compounds, computer models have been promoted as a viable alternative to experimental approaches for the prediction of ADME.

Compound 10 shows that benzoxazole derivative is more active than benzthiazole derivative. Physiochemical studies showed that i log p value was below 5 and water solubility if found to be moderate which indicates that compound is having good GI absorption with drug likeness. After predicting dose toxicity by web server we got to know that it comes in class IV category and can be toxic over 600 mg/kg of dose if given. The BOILED-Egg is depicted in Figure1. Compound C10 correctly lies inside BOILED-Egg's yolk which predicts the passive gastrointestinal absorption and brain access of C10.

On behalf of previous docking study and computational study we proceeded with pharmacological screening of synthesized compound.

Following administration of the test substance, there was a significant dependent drop in plasma total cholesterol [6], triglycerides, and LDL cholesterol levels, together with a sizable increase in levels of HDL cholesterol. According to the data analysis result obtained in Fig. 1, Table 4, and 5. The benchmark used was Atorvastatin. In the case of group 4, the Atherogenic Index was dramatically reduced as compared to healthy controls, reflecting the antihyperlipidemic action.

The balance between risk and protective lipoprotein factors is thought to be reflected by the relationship between triglycerides and HDL cholesterol in the straightforward ratio known as the atherogenic index of plasma (AIP). AIP was previously mentioned as being a reliable indicator of myocardial infarction. The LDL/HDL ratio has been shown in numerous epidemiological and clinical studies to be a highly accurate predictor of HD risk and a highly accurate indicator of the efficacy of lipid-lowering treatments. Additionally, the LDL/HDL ratio exhibited a higher predictive value than just LDL or HDL.

Conclusion

In recent years, scientists have been examining the use of "smart" methods for determining which molecules have the necessary biological activity. When screening a vast and diverse group of samples, cutting-edge computational approaches created to transform raw data into relevant chemical information are needed. Poor pharmacokinetics and bioavailability are blamed for many drug development failures in addition to efficacy and toxicity. It saves time and chemicals to determine the ADMET before manufacturing the molecules. These computational predictors aid in the early stages of drug discovery by selecting chemical libraries and evaluating potential therapeutic candidates. At various stages of the drug development processes, it is critical to estimate two pharmacokinetic behaviors: intestine absorption and brain access. It is suggested that (BOILED-Egg) is a reliable predictor. These features reduce cost and time for production of a chemical entity and thus lead to less sacrifice of animals too.

Acknowledgement

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Conflict Of Interest

The authors declare that they have no conflict of interests.

Abbreviations

GI- Gastrointestinal

TPSA- Topological polar surface area

ACAT - Acyl-Coenzyme A (CoA): Cholesterol Acyltransferase

BOILED-Egg - Brain Or Intestinal Estimated permeation method HFD – High Fat Diet

AIP - Atherogenic Index of Plasma

ANOVA - Analysis of Variance

HDL - High Density Lipoprotein

LDL - Low Density Lipoprotein

mg/dL - milligram/ deciliter

mg/kg - milligram/ kilogram

SD - Standard Deviation

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