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*Original Research Article*

# COMPUTER AIDED DRUG DESIGN AND DOCKING ANALYSIS OF SOME NOVEL ACAT INHIBITORS

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## ABSTRACT

In the present paper we carried out computational drug designing and docking studies of benzoxazole derivatives on ACAT domain (1WL5) with very low energies.

**Key words:** Docking, Hyperlipidaemia, Scaffold, Hydrophobic group, Statins.

## INTRODUCTION

Hyperlipidaemia[1] a broad term, also called hyperlipoproteinemia, is a common disorder in developed countries and is the major cause of coronary heart disease. It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins. The consequence of hyperlipidaemia is that with time it can cause atherosclerosis, and thus the risk of coronary heart disease and stroke is increased. LDL is strongly associated with a higher risk, and HDL is associated with a lower risk, of coronary heart disease (CHD). Lowering lipids through dietary or pharmacological therapy has been shown to decrease the incidence of atherosclerotic events. Since lipid levels have been observed to track into adulthood, adolescents with hyperlipidaemia are also at greater CHD risk. The extent of abnormal lipids and other cardiovascular risk factors during childhood and adolescence is related to the severity of atherosclerosis seen in autopsies of young adults.

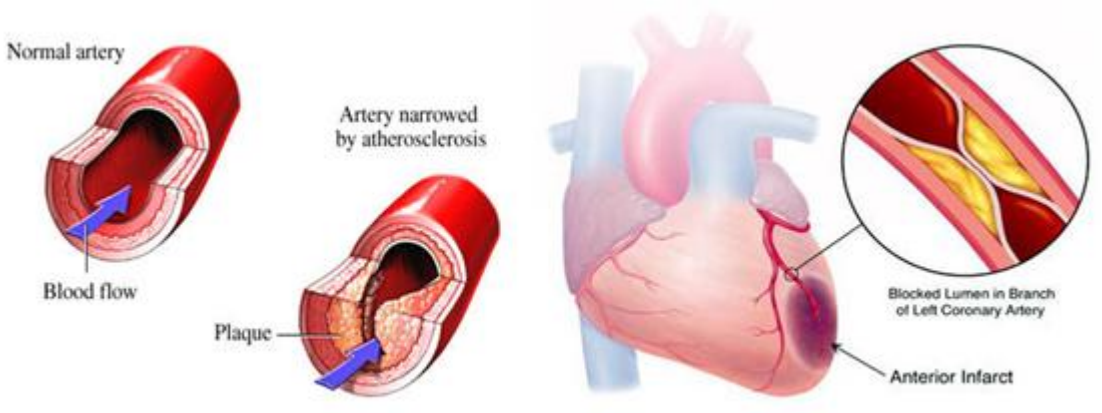


**Fig.1.Hyperlipidaemia is typically asymptomatic and is frequently detected during routine screening**

**Atherosclerosis[2]** represents one of the leading causes of cardiovascular morbidity and mortality in which plaque disruption and thrombus formation play a pivotal role. Itis known that thrombus formation often leads to the disruptionof the lipid-rich plaque, so that the core of the plaque comes into contact with blood cells. Once plaques are deposited in arteries, heart muscles can become deprived of oxygen and angina may occur. If the arteries are completely blocked, the region of the heart maintained by the arteries can die, eventually leading to heart failure.[3] Heart disease is a major health problem worldwide. It has been established that hyperlipidaemia is a risk factor in the onset of these diseases. Several pharmacological strategies have been used to develop plasma cholesterol lowering agents. One of those is the inhibition of acyl-CoA cholesterol: acyltransferase (ACAT, EC 2.3.1.26). ACAT is an endoplasmic reticulum-bound enzyme that catalyses the

formation of cholesteryl esters from cholesterol and long chain fatty acids in a wide variety of cells. ACAT plays a major role in cellular cholesterol homeostasis[4]. Cholesteryl esters are stored as cytoplasmic storage droplets or, in lipoprotein secreting cells, can be packaged in the hydrophobic core of lipoproteins for transport. In early atherogenesis, macrophages and smooth muscle cells accumulate large quantities of cholesteryl ester, a process catalyzed by ACAT. The cholesterol is derived from atherogenic lipoproteins present in the arterial intima. Intestinal and hepatic ACAT synthesize the majority of cholesteryl esters transported in lipoproteins with atherogenic potential, namely, chylomicron remnants, VLDL remnants and LDL. Thus, there is considerable interest in the potential for ACAT inhibitors to prevent atherogenesis[5].

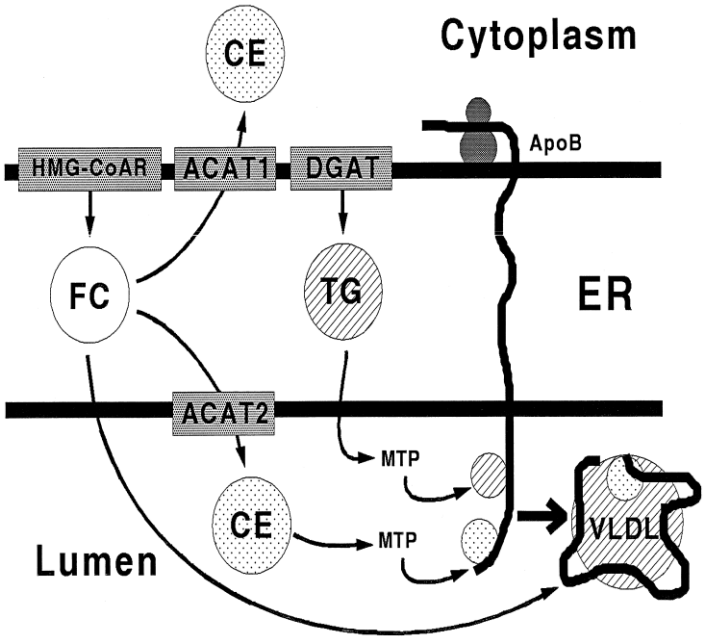


**Fig.2.A simplified schematic of the proposed role of acyl coenzyme A: cholesterol acyltransferase (ACAT) in hepatic apolipoprotein B-containing lipoprotein assembly and secretion**

Very low density lipoprotein (VLDL) secretion requires the coordinated synthesis and assembly of apoB, triglyceride (TG), free cholesterol (FC), cholesteryl ester (CE) and phospholipids.

**This process involves:**

(1)apoB mRNA transcription and translation

(2)Translocation of apoB across the endoplasmic reticulum (ER) membrane,

(3) i) Synthesis of cholesterol [3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) pathway],

ii) CE (ACAT) pathway,

iii) phospholipid and TG [acyl coenzyme A: diacylglycerolacyltransferase (DGAT)

pathway] and iv) Transfer to apoB via microsomal triglyceride transfer protein (MTP),

(4) Intracellular degradation,

(5) Transport through the secretory pathway and secretion into plasma.

Each step may be regulated by ACAT inhibition.

The shaded rectangles represent integral membrane proteins. It has been postulated that ACAT1 may represent the ER enzyme responsible for generating CE's for storage whereas, ACAT2 mayrepresent the enzyme responsible for generating CE's destined for lipoprotein assembly and secretion.

On the horizon is the possibility of combining Acyl coenzyme A: cholesterol acyltransferase (ACAT) inhibitor a drug that is designed to reduce lipid uptake by the macrophage[6] with statins. In theory, ACAT inhibitors may have a beneficial effect on earlier stages of atherosclerosis – fatty streak formation. Second, ACAT inhibitors may have effect on lesions characterized by smooth muscle cells (e.g. in restenosis), as these cells seem resistant to the toxicity of free cholesterol[7,8]. An exciting third avenue is application in Alzheimer's disease. ACAT inhibitors have been shown to decrease the generation of Amyloid beta peptide in mice[9].

**Molecular modeling:**

In recent years, the field of computer-aided drug design (CADD) has grown rapidly, enhancing our understanding of complex biological processes and protein-ligand interactions. CADD can predict experimental results with reasonable accuracy and reduced time, cost and equipment. CADD continuously enhances the progress of drug discovery and refinement of therapeutic agents with many successful examples.[10]

Computational drug design has been widely used in the pharmaceutical industry to either identify new compounds or optimize lead compounds that show significant inhibitory activity against a target biological receptor. A small number of examples of these uses are included in Table 1. It is known that chemicals can bind to biological receptors and produce a specific therapeutic response. Drug design is often targeted against receptor molecules which are proteins. The ability of a ligand to bind to a specific protein is related to molecular structure, orientation and conformation. During the binding process, there are enthalpy and entropy changes in the protein-ligand system, associated with alteration of both intra- and inter-molecular structures of protein and ligand. These conformational changes allow the ligand to bind to the protein active site in a more stable manner. In general, protein-ligand interactions of pharmaceutical interest principally involve non-covalent interactions, including hydrogen bonds, ionic interactions, hydrophobic interactions, $\pi$-$\pi$ interactions and cation-π interactions. Computational molecular modeling methods attempt to predict these interactions and thus the binding affinities and conformation of protein-ligand complexes.[10]

**Table.1.Examples of Marketed Drugs Involving the Use of Structure-based Drug Design**

| Year | Generic Name | Brand Name | Manufacturer | Against / Inhibits |
|---|---|---|---|---|
| 1989 | Zanamivir (vonltzstein *et al.*, 1996) | Relenza | GlaxoSmithKline | Neuraminidase |
| 1997 | Nelfinavir (Kaldor *et al.*, 1997) | Viracept | Hoffman-La Roche | HIV protease |
| 1998 | Raltitrexed (Blackledge, 1998) | Tomudex | AstraZeneca | Thymidylate |
| 1999 | Amprenavir (Adkins & Faulds, 1998) | Agenerase | GlaxoSmithKline | HIV protease |
| 2007 | Raltegravir (Schames *et al.*, 2004) | Isentress | Merck | HIV integrase |

In modern drug discovery, computational methods are generally involved in identifying and modifying lead compounds. For lead discovery and lead optimization, 3D structural information on the ligand, the protein receptor, or both, is highly desirable. A commonly-used method in 3D computer-aided drug design is molecular docking[10]. In molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions[11,12]. The protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components, the search algorithm and the scoring function[13].Molecular docking algorithms fit molecules together in complementary fashions. The technique has attracted increasing attention as a way to predict the geometries of bimolecular complexes[14]. Most of docking programs in use account for a flexible ligand, and several are attempting to model a flexible protein receptor. Each snapshot of the pair is referred to as a pose[15]. Stochastic search, incremental construction, and multi-conformer docking are three ways to classify these algorithms. AutoDock, Internal Coordinate Mechanics (ICM), Genetic Optimization for Ligand Docking (GOLD), etc are the representatives for stochastic search algorithm. These algorithms are based on genetic algorithms and Monte Carlo-simulated annealing[16,17]. Docking procedures aim to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, docking describes a process by which two molecules fit together in three-dimensional space.[18] One main motivation in drug discovery is the identification of innovative small molecular scaffolds exhibiting high binding affinity and selectivity for the target together with a reasonable ADME (absorption, distribution, metabolism,

excretion) profile, lead and/or drug likeness. Such chemical entities are likely to be able to enter higher phases in the further drug development process. Molecular docking, compared to the fast and successful method of three-dimensional pharmacophore modeling is a rather complex and computer-intensive approach to find new compounds by virtual screening.[18] During our study, we designed 36 ligands having potential as inhibitor to ACAT enzyme. This inhibitory action of the ligand could inhibit the ACAT enzyme to prevent atherogenesis. All the ligands were screened for Lipinski's Rule of 5, and later on docking was done. These ligands and receptor were also energetically minimized during those processes. Majority of our work was performed using Accelrys Discovery studio 3.5.

**Molecular modelling of ACAT inhibitors**

Computer-assisted drug design (CADD) approach has contributed to the successful discovery of several novel antihyperlipidaemic agents. Molecular Docking continues to hold great promise in the fieldof computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. Number of reports citing successful application of CADD indeveloping specific drugs in different therapeutic areas is expanding rapidly.

The present studyhas been carried out to screen antihyperlipidaemic agents using AutoDock with the objective to find potential drug targets against ACAT. Application of force field to the database proved to be more promising, reducing the number of docked conformations significantly. Inspection of the highest scoring inhibitor conformations, and their relative orientations, after docking of the database, showed only limited specificity for the inhibitor.

**EXPERIMENTAL SECTION**

- Disease selection
- Receptor identification
- Structure modelling
- Structure validation
- Scaffold identification
- Lead library design
- Virtual screening
- Molecular docking
- Pharmacophore analysis

***Target identification and validation*:**

ACAT receptor protein PDB id:**1WL5** was downloaded from protein data base website (http://www.rcsb.org/pdb/explore/explore.do?structureId=1WL5)

***Scaffold Selection*:** Literature review was done on various ACAT inhibitors and after studying the structure activity relationship of the compounds we found that benzoxazole derivative can be used as scaffold for the inhibition of ACAT enzyme having antihyperlipidaemic activity substantiating their biological functionality.

***Lead library Designing*:** Lead library was designed based on Lipinski's rule of five. Lead design was performed with ChemDraw Ultra 7.0. Care was taken not to include

heavy atoms or carcinogenic atoms to the molecule.

***Virtual screening:***

Ligand based virtual screening was carried for the lead library that bind to the ACAT receptor (1WL5) to find new scaffolds followed by an evaluation of the docked conformation with a binding-score function.

***Receptor and Ligand Preparation:***

The validated model was then determined for largest binding site using Accelrys DiscoveryStudio 3.5. The sphere was defined for the binding site; typing was carried out by CHARMm force field (Momany-Roneparital charges methods). Minimization was carried out in Accelrys Discovery Studio 3.5 using 1400 cycle's of conjugate gradient; a constant potential energy of -22322.84878 kcal/mol was obtained.

The screened compounds were typed similarly using CHARMm for partial charges set up and minimized by Conjugate Gradient until a constant potential energy was obtained.

**Preparation of ligand:-**

A small Library of ligands was prepared. Ligand structure was drawn on **Discovery Studio**[19]and saved in MOL 2 and PDB file format (file format required for Autodock vina). PDB file was loaded in ADT software which automatically calculated number of rotatable bonds and calculated charges per atom and merged polar Hydrogen. Now save ligand in PDBQT file format.

**Preparation of protein:-**

Protein (PDB id: 1WL5) was downloaded from protein data base. It was bound with ligand so it was first prepared by removing water and ligand using Discovery Studio software and hydrogen was added to the protein structure and only protein devoid of water and bound ligand was saved as PDB file (protein) loaded in Autodock Tool (ADT TOOL) software which automatically added charges and merged polar hydrogen. Now the file was saved in PDBQT format.

***Receptor-Ligand Docking:***

The minimised receptor and ligand was docked with AutoDock, The Receptor-Ligand complex is studied to determine the potentiality of the molecules docked.

**DOCKING**:

Docking of all ligand against protein (PDB id: 1WL5) was done by **PyRx**[20]software (PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets). The entire ligand library was loaded in the PyRx at once and the receptor protein (PDB id: 1WL5) was also loaded.

Grid box was set on the region of binding pocket (grid dimensions are as follows)

**center_x = 10.1232254999;**
**center_y = 39.4152206065;**
**center_z = 26.3980596464;**
**size_x = 25.0; size_y = 25.0; size_z = 25.0**

Exhaustiveness parameter was set to 8 and next; docking program (**Vina**[21]) was executed.

***Pharmacophore Analysis:***

The ligands were analysed for pharmacophore using the common purpose pharmacophore in Pharmacophore protocol available in Discovery studio.Pharmacophore analysis include aromatic group, donor molecule, positive and negative ionizing group ,hydrophobic group and hydrophilic group.

**RESULTS**

**Table.2.Molecular properties of molecules used for docking**

| S.NO. | Comp.Name | Mol. Formula | Mol.Wt. (<500) | Clogp (<5) | No.H donors (<5) | No.of H acceptors (<=10) | No.of rotatable bonds (<10) | No.of rings | No.of aromatic rings | Mol. Fractional surface area (<=140 A°) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | BOHYC2A1 | $C_{16}H_{18}N_4O$ | 282 | 3.34 | 0 | 5 | 6 | 3 | 2 | 53.32 |
| 2 | BOHYC2A2 | $C_{17}H_{20}N_4O$ | 296 | 3.87 | 0 | 5 | 7 | 3 | 2 | 53.32 |
| 3 | BOHYC2A3 | $C_{15}H_{21}FN_4O$ | 292 | 2.20 | 0 | 6 | 6 | 3 | 2 | 62.11 |
| 4 | BOHYC2A4 | $C_{15}H_{15}N_5O_3$ | 313 | 3.04 | 0 | 8 | 7 | 3 | 2 | 107.93 |
| 5 | BOHYC3A1 | $C_{17}H_{22}N_4O$ | 298 | 3.53 | 0 | 5 | 7 | 3 | 2 | 53.32 |
| 6 | BOHYC3A2 | $C_{18}H_{24}N_4O$ | 312 | 4.06 | 0 | 5 | 8 | 3 | 2 | 53.32 |
| 7 | BOHYC3A3 | $C_{16}H_{17}FN_4O$ | 300 | 3.44 | 0 | 6 | 7 | 3 | 2 | 62.11 |
| 8 | BOHYC3A4 | $C_{16}H_{17}N_5O_3$ | 327 | 3.39 | 0 | 8 | 7 | 3 | 2 | 107.93 |
| 9 | BOHYC4A1 | $C_{18}H_{22}N_4O$ | 310 | 3.59 | 0 | 5 | 8 | 3 | 2 | 53.32 |
| 10 | BOHYC4A2 | $C_{19}H_{24}N_4O$ | 324 | 4.11 | 0 | 5 | 9 | 3 | 2 | 53.32 |
| 11 | BOHYC4A3 | $C_{17}H_{19}FN_4O$ | 314 | 3.66 | 0 | 6 | 8 | 3 | 2 | 62.11 |
| 12 | BOHYC4A4 | $C_{17}H_{19}N_5O_3$ | 341 | 3.31 | 0 | 8 | 9 | 3 | 2 | 107.93 |
| 13 | MEBOHYC2A1 | $C_{17}H_{20}N_4O$ | 296 | 3.84 | 0 | 5 | 6 | 3 | 2 | 53.32 |
| 14 | MEBOHYC2A2 | $C_{18}H_{22}N_4O$ | 310 | 4.37 | 0 | 5 | 7 | 3 | 2 | 53.32 |
| 15 | MEBOHYC2A3 | $C_{16}H_{17}FN_4O$ | 300 | 3.59 | 0 | 6 | 6 | 3 | 2 | 62.11 |
| 16 | MEBOHYC2A4 | $C_{16}H_{17}N_5O_3$ | 327 | 3.54 | 0 | 8 | 7 | 3 | 2 | 107.93 |
| 17 | MEBOHYC3A1 | $C_{18}H_{24}N_4O$ | 312 | 4.03 | 0 | 5 | 7 | 3 | 2 | 53.32 |
| 18 | MEBOHYC3A2 | $C_{19}H_{26}N_4O$ | 326 | 4.56 | 0 | 5 | 8 | 3 | 2 | 53.32 |
| 19 | MEBOHYC3A3 | $C_{17}H_{19}FN_4O$ | 314 | 3.94 | 0 | 6 | 7 | 3 | 2 | 62.11 |
| 20 | MEBOHYC3A4 | $C_{17}H_{19}N_5O_3$ | 341 | 3.88 | 0 | 8 | 8 | 3 | 2 | 107.93 |
| 21 | MEBOHYC4A1 | $C_{19}H_{24}N_4O$ | 324 | 4.08 | 0 | 5 | 8 | 3 | 2 | 53.32 |
| 22 | MEBOHYC4A2 | $C_{20}H_{26}N_4O$ | 338 | 4.61 | 0 | 5 | 9 | 3 | 2 | 53.32 |
| 23 | MEBOHYC4A3 | $C_{18}H_{21}FN_4O$ | 328 | 3.86 | 0 | 6 | 8 | 3 | 2 | 62.11 |
| 24 | MEBOHYC4A4 | $C_{18}H_{21}N_5O_3$ | 355 | 3.81 | 0 | 8 | 9 | 3 | 2 | 107.93 |
| 25 | NO2BOHYC2A1 | $C_{16}H_{17}N_5O_3$ | 327 | 3.29 | 0 | 8 | 7 | 3 | 2 | 99.14 |
| 26 | NO2BOHYC2A2 | $C_{17}H_{19}N_5O_3$ | 341 | 3.82 | 0 | 8 | 7 | 3 | 2 | 99.14 |
| 27 | NO2BOHYC2A3 | $C_{15}H_{14}FN_5O_3$ | 331 | 3.05 | 0 | 6 | 7 | 3 | 2 | 107.93 |
| 28 | NO2BOHYC2A4 | $C_{15}H_{14}N_6O_5$ | 358 | 2.99 | 0 | 11 | 8 | 3 | 2 | 153.76 |
| 29 | NO2BOHYC3A1 | $C_{17}H_{19}N_5O_3$ | 341 | 3.63 | 0 | 8 | 8 | 3 | 2 | 99.14 |
| 30 | NO2BOHYC3A2 | $C_{18}H_{23}N_5O_3$ | 357 | 4.42 | 0 | 8 | 9 | 3 | 2 | 99.14 |
| 31 | NO2BOHYC3A3 | $C_{16}H_{16}FN_5O_3$ | 345 | 3.39 | 0 | 7 | 8 | 3 | 2 | 107.93 |
| 32 | NO2BOHYC3A4 | $C_{16}H_{16}N_6O_5$ | 372 | 3.34 | 0 | 11 | 9 | 3 | 2 | 153.76 |
| 33 | NO2BOHYC4A1 | $C_{18}H_{21}N_5O_3$ | 355 | 3.54 | 0 | 8 | 9 | 3 | 2 | 99.14 |
| 34 | NO2BOHYC4A2 | $C_{19}H_{23}N_5O_3$ | 369 | 4.07 | 0 | 8 | 10 | 3 | 2 | 99.14 |
| 35 | NO2BOHYC4A3 | $C_{17}H_{18}FN_5O_3$ | 359 | 3.32 | 0 | 7 | 9 | 3 | 2 | 107.93 |
| 36 | NO2BOHYC4A4 | $C_{17}H_{18}N_6O_5$ | 386 | 3.27 | 0 | 11 | 10 | 3 | 2 | 153.76 |

**Inference**: Result output was saved by ADT Tool as PDBQT format and result was analysed in ADT Tool.This file format contains single molecule with multiple conformer which are ranked automatically based on ΔG. 36 molecules were designed and docked out of which NO2BOHYC3A1, NO2BOHYC3A2, MEBOHYC3A1,MEBOH YC3A1 were found to obey the Rule of five and gave the low score.

**Ligand-protein interactions.**

Vina results were analysed in ADT and interaction were also visualized in ADT.

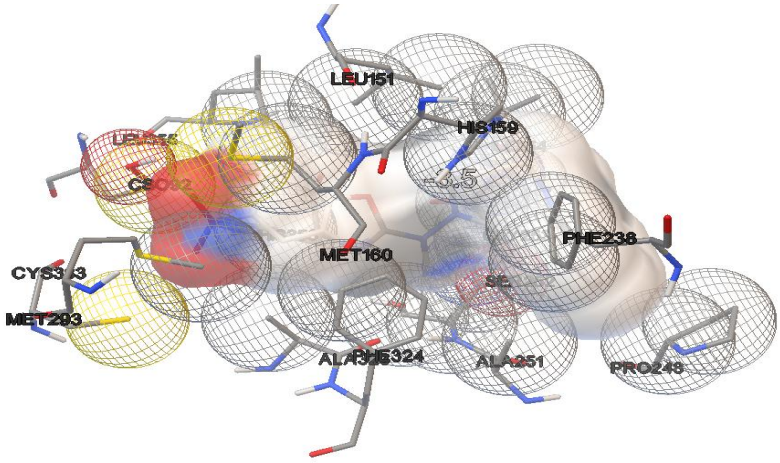


**Fig.3.Interaction of $NO_2$BOHYC3A1with protein (1WL5)**

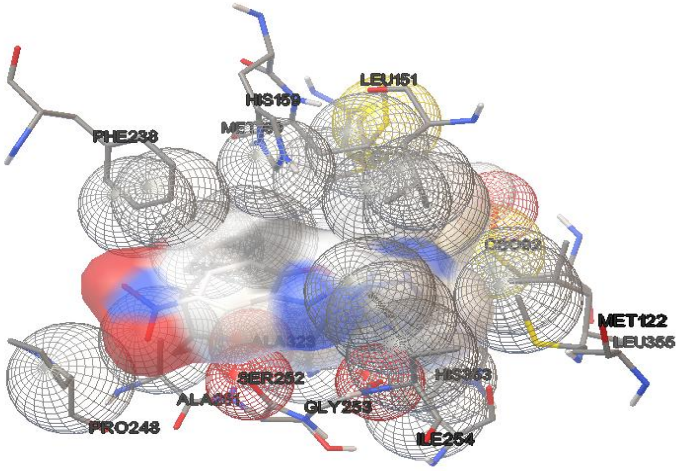


**Fig.4.Interaction of $NO_2$BOHYC3A2 with protein (1WL5)**

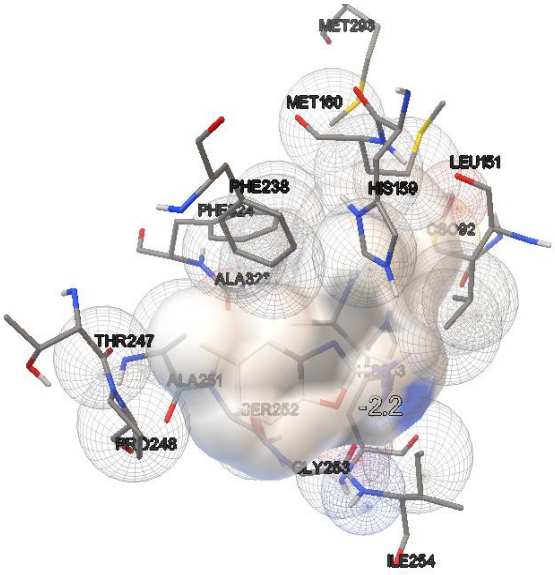


**Fig.5.Interaction of MEBOHYC3A1 with protein (1WL5)**

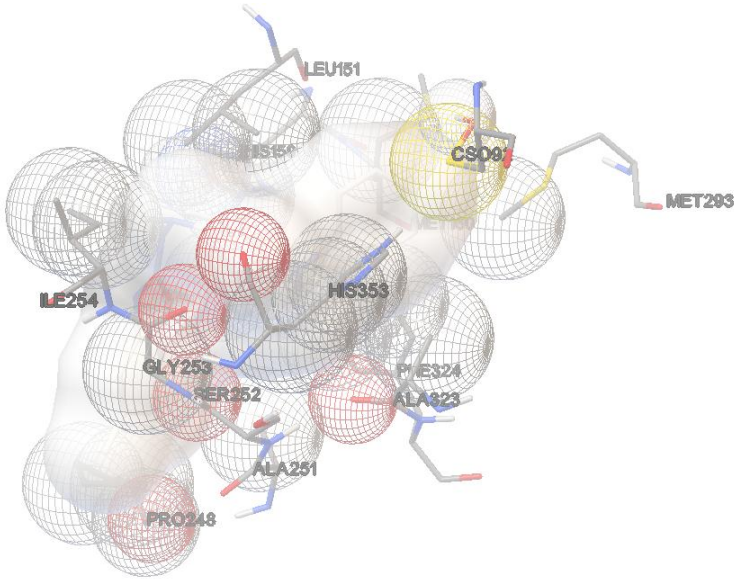


**Fig.6.Interaction of MEBOHYC3A2 with protein (1WL5)**

## CONCLUSION

The major reason for failure of NCEs at latter stages of drug discovery process i.e. drug like pharmacokinetic profile set up, has forced us setting filters like molecular weight, No. of H-bond donors, No. of H-bond acceptors, Polar Surface Area and number of rotatable bonds; so that only drug like NCEs would be generated and resultant NCEs would not have the

pharmacokinetic inadequacies. But the thorough analysis of results of docking studies predicts the safer performance of our designed compounds. The most potent derivatives were subjected to molecular docking studies to get further insights of interactions of NCEs with ACAT. Finally 4 top compounds with good docking score will be subjected to wet lab work viz., synthesis and evaluation using TRITON induced antihyperlipidaemia and ACAT inhibitor assay studies. The results of dry lab work and wet lab work will be analyzed thoroughly to find out correctness of the rational used for the design of NCEs in general and optimization of pharmacophore for inhibition of ACAT.

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