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***Research article***

# COMPUTATIONAL MODELING AND DRUG DESIGNING OF LIPOPROTEIN LIPASE (LPL) INVOLVED IN ISCHEMIC STROKE

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**ABSTRACT:** Homology modeling and flexible docking of Lipoprotein Lipase has been studied in *silico* approach. Blast result was found to have similarity with Lipoprotein Lipase of 83% identity with 1LPA. Active site of LPL protein was identified by CASTP. Large potential drugs were designed for identifying molecules that can likely bind to protein target of interest. The different drug derivatives designed were used for docking with the generated structure, among the 10 derivatives designed, 3rd derivative showed highest docking result. The drug derivatives were docked to the protein by hydrogen bonding interactions and these interactions play an important role in the binding studies. Our investigations may be helpful for further studies.

Key words: Lipoprotein Lipase, Modeling, Drug designing, Docking

## INTRODUCTION

Lipoprotein lipase (LPL) is a member of the lipase gene family, which includes pancreatic lipase, hepatic lipase and endothelial lipase. LPL is water soluble enzyme that hydrolyzes triglycerides in lipoproteins, which are found in chylomicrons and very low-density lipoproteins (VLDL), into two free fatty acids and one monoacylglycerol molecule. It is also plays an important role in promoting the cellular uptake of chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids (Mead et al 2002; Rinninger et al 1998; Ma et al 1994). Lipoprotein lipase which is a hydrolytic enzyme produced by many tissues and is rate-limiting for the removal of lipoprotein triglycerides from the circulation. It also has another important roles in many normal tissues, as well as in certain metabolic diseases, including obesity. More than 40 years ago, Hahn noticed that intravenous heparin totally cleared diet-induced lipemia in dogs. The addition of heparin to plasma in vitro did not reproduce this effect, suggesting that the causative agent was released into plasma by heparin in vivo. This factor, ultimately identified as a triglyceride lipase, was activated in the presence of high-density lipoproteins (HDLs). Lipoprotein lipase (LPL) catalyses hydrolysis of triacylglycerol component of circulating chylomicrons and very low density lipoproteins and provides non-esterified fatty acids and 2-monoacylglycerol for tissue utilization (Mead et al 2002). Due to the large size of LPL substrates, it's physiological site of action is at the luminal surface of blood vessels, to which the enzyme is attached via highly charged heparan sulphate proteoglycans (HSPG) (Braun et al 1992; Enerback et al 1993). In 1973 the role of LPL in the atherosclerotic process was first proposed by Donald Zilversmit (Zilversmit D.B. 1973). LPL-mediated hydrolysis of VLDL leads to the production of LDL, which is the major contributor to the development of atherosclerotic lesions. Frequently, these LDL molecules are oxidized in the initial space by free radicals which, along with other modifications, increase their rate of uptake into macrophages through the scavenger receptors, and thereby promotes further foam cell formation (Ross R, 1999; Glass et al 2001).

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## MATERIALS AND METHODS

### 3D model building

The MODELLER software was used to build the initial model of Lipoprotein Lipase. The first step is searching a number of related sequences to find a related protein as a template by the BLAST program. The high sequence identity between Lipoprotein Lipase (P06858) and the reference protein 1LPA is 83%, which allowed for rather straight forward sequence alignment. In the second step, the backbone coordinates of the residues in Lipoprotein Lipase were generated with the MODELLER software. The structurally conserved regions (SCRs) were determined by multiple sequence alignment, which is based on the Needleman and Wunsch Algorithm, and the coordinates of SCRs in Lipoprotein Lipase were generated by copying from 1LPA template. The structure having the least modeller objective function, obtained from the modeller was improved by molecular dynamics and equilibration methods using NAMD 2.5 software using CHARMM27 force field for lipids and proteins along with the TIP3P model for water. The energy of the structure was minimized with 10,000 steps. A cutoff of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved.

The final structure obtained was checked by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) and environment profile using ERRAT graph (Structure Evaluation server). This model was used for the identification of active site and for docking of the substrate with the protein.

### Binding-site analysis:

The Bindig-site of Lipoprotein Lipase was identified using CASTP server. A new program, CAST, for automatically locating and measuring protein binding pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory.

### Docking of derivatives to Glutamate protein

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the Lipoprotein Lipase. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. During docking, the default algorithm speed was selected and the ligand binding site was defined within a 10 $A^0$ radius. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied.

### Gold Score fitness function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vander Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

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## RESULTS AND DISCUSSION

### Homology modeling of Lipoprotein Lipase

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only the 1LPA which has a high level of sequence identity with the Lipoprotein Lipase domain (83%). Structurally conserved regions (SCRs) for the model and the template were determined by sequence alignment and the SCRs were determined as shown by Fig.1.

```
PROTEIN         -------------------------------------MESKALLVLTLAVWLQSLTASR
TEMP            GIIINLDEGELCLNSAQCKSNCCQHDTILSLLRCALKARENSECSAFTLYGVYYKCPCER
                                                     *..   .:**     . ...*

PROTEIN         GGVAAADQ---------------------------------------------RRDFIDI
TEMP            GLTCEGDKSLVGSITNTNFGICHNVKEVCYERLGCFSDDSPWSGITERPLHILPWSPKDV
                * .. .*:                                              .  *:

PROTEIN         ESKFALRTPEDTAEDTCHLIPGVAESVATCHFNHSSKTFMVIHGWTVTGMYESWVPKLVA
TEMP            NTRFLLYTNEN--PNNFQEVAADSSSISGSNFKTNRKTRFIIHGFIDKGE-ENWLANVCK
                :::* * * *:   :. : :.. :.*:: .:*: . ** ::***:  .*  *.*:.::

PROTEIN         ALYKREPDSNVIVVDWLSRAQEHYPVSAGYTKLVGQDVARFINWMEEEFNYPLDNVHLLG
TEMP            NLFKVES-VNCICVDWKGGSRTGYTQASQNIRIVGAEVAYFVEFLQSAFGYSPSNVHVIG
                 *:* *.  * * *** . ::  *. ::   ::** :** *:::::. *.*. .***::*

PROTEIN         YSLGAHAAGIAGSLTNKKVNRITGLDPAGPNFEYAEAPSRLSPDDADFVDVLHTFTR-GS
TEMP            HSLGAHAAGEAGRRTNGTIGRITGLDPAEPCFQGTPELVRLDPSDAKFVDVIHTDGAPIV
                :******** **  ** .:.******** * *: :    **.*.**.****:**

PROTEIN         PGRSIGIQKPVGHVDIYPNGGTFQPGCNIGEAIRVIAERGLGD-VDQLVKCSHERSIHLF
TEMP            PNLGFGMSQVVGHLDFFPNGGVEMPGCKKNILSQIVDIDGIWEGTRDFAACNHLRSYKYY
                *. .:*:.: ***:*::****.  ***: .   :::   *: : . ::. *.* ** : :

PROTEIN         IDSLLNEENPSKAYRCSSKEAFEKGLCLSCRKNRCNNLGYEINKVRAKR---SSKMYLKT
TEMP            TDSIVNPDG-FAGFPCASYNVFTANKCFPCPSGGCPQMGHYADRYPGKTNDVGQKFYLDT
                 **::* :.   .: *:* :.*  . *:.* .. * ::*:  ::  .*    ..*:**.*

PROTEIN         RSQMPYKVFHYQVKIHFSGTESETHTNQAFEISLYGTVAESENIPFTLPEVSTNKTYSFL
TEMP            GDASNFARWRYKVSVTLSGKKVTGH----ILVSLFGNKGNSKQYEIFKGTLKPDSTHSNE
                 .   :  ::*:*.: :**.:   *    : :**:*. .:*::  :    :..:.*:*

PROTEIN         IYTEVDIGELLMLKLKWKSDSYFSWSDWWSSPGFAIQKIRVKAGETQKKVIFCSREKVSH
TEMP            FDSDVDVGDLQMVKFIWYNNVIN-----PTLPRVGASKIIVET-NVGKQFNFCSPETVR-
                : ::**:*:* *:*: * .:         : * .. .** *:: :. *:. *** *.*

PROTEIN         LQKGKAPAVFVKCHDKSLNKKSG
TEMP            ---EEVLLTLTPC----------
                    :.  .:. *
```

**Fig 1: CLUSTAL 2.0.11 multiple sequence alignment**

In the following study, we have chosen 1LPA as a reference structure for modeling Lipoprotein Lipase domain. Coordinates from the reference protein (1LPA) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeller we will get a 20 PDB out of which we select a least energy.

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The energy unit will be in kilo joule. All side chains of the model protein were set by rotamers. The final stable structure of the Lipoprotein Lipase protein obtained is shown in Figure 2. By the help of SPDBV it is evident that Lipoprotein Lipase domain has 11 helices and 22 sheets.

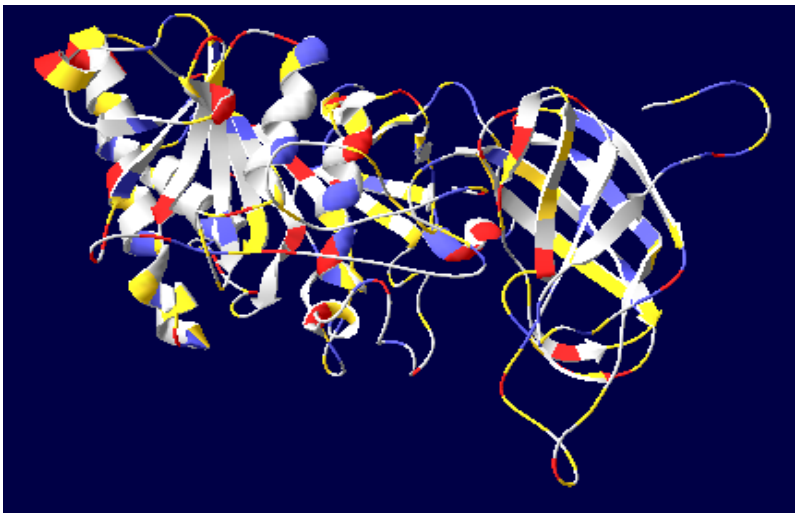

**Fig 2: Three dimensional structure of Lipoprotein Lipase**

The final structure was further checked by verify3D graph and the results have been shown in Figure III: The overall scores indicates acceptable protein environment.

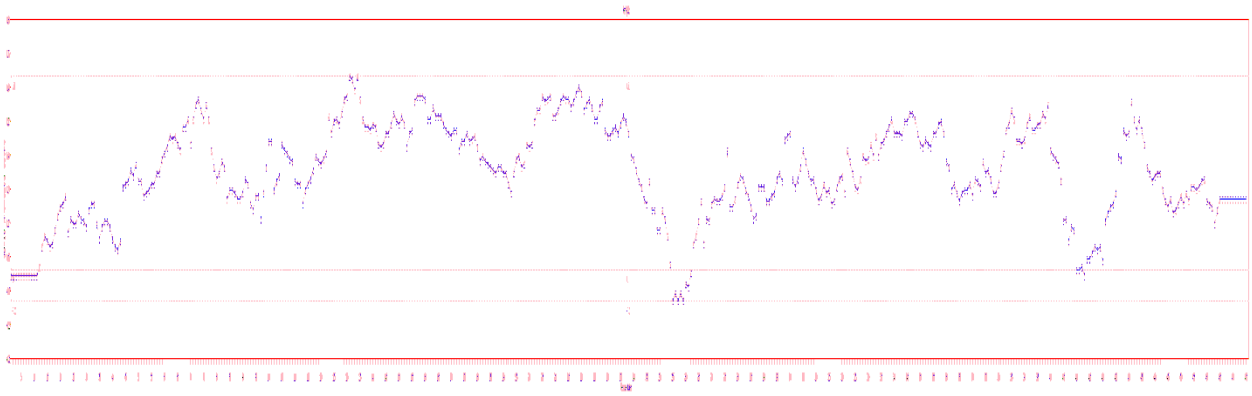

**Fig 3: Verify 3D graph of Lipoprotein Lipase**

## Validation of Lipoprotein Lipase Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program (Figure 4). The distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 1. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of Lipoprotein Lipase was -3.27 and -0.45 Å. Altogether 94.0 % of the residues of Lipoprotein Lipase was in favored and allowed regions. The overall PROCHECK G-factor of Lipoprotein Lipase was – 1.32 and verify3D environment profile was good.

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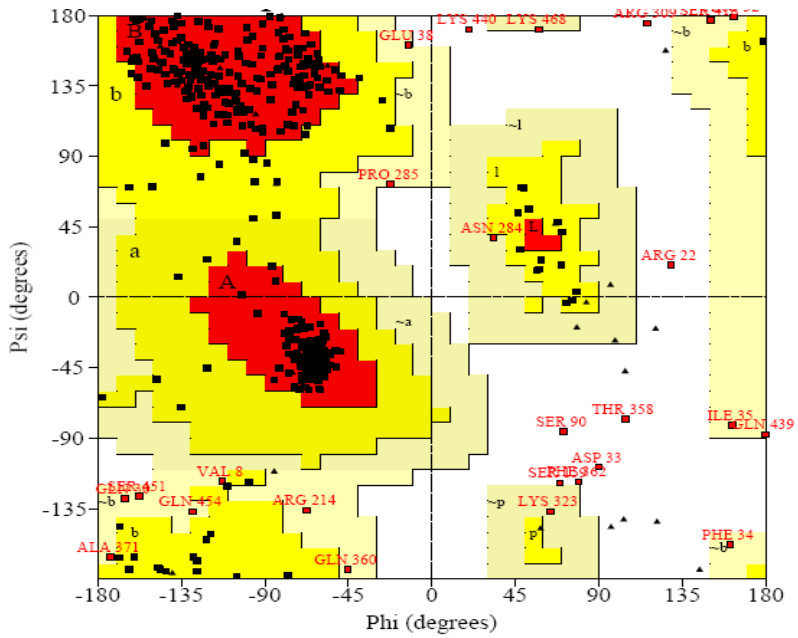


**Fig 4: Ramachandran plot**

## Superimposition of 1LPA with Lipoprotein Lipase domain

The structural superimposition of 1LPA template and Lipoprotein Lipase is shown in Figure 5. The weighted root mean square deviation of trace between the template and final refined models 0.90A$^{o}$. This final refined model was used for the identification of active site and for docking of the substrate with the domain Lipoprotein Lipase.

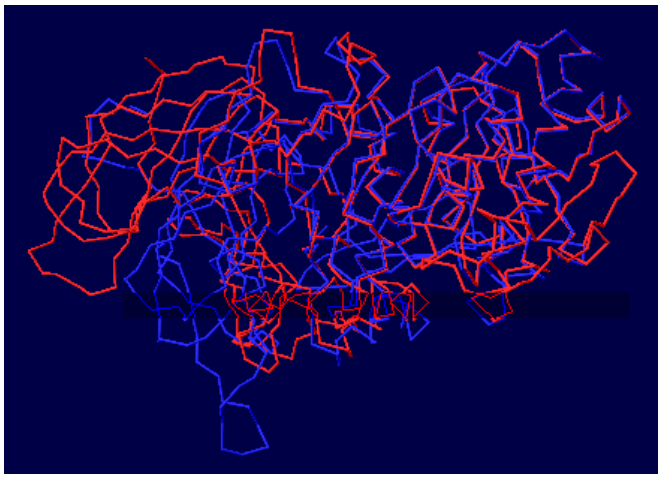

**Fig 5: Superimposition of Lipoprotein Lipase (Blue colour) with Template 1LPA (Red colour)**

## Active site Identification of Lipoprotein Lipase domain

After the final model was built, the possible binding sites of Lipoprotein Lipase was searched based on the structural comparison of template and the model build and also with CASTP server and was shown in Figure 6. Since, Lipoprotein Lipase from Human and the 1LPA are well conserved in both sequence and structure; their biological function should be identical. It was found that secondary structures are highly conserved and the residues, GLY6, ILE8, ILE9, ASN10, GLY14, LEU18, SER20, GLN22, CYS28, THR32, HIS151, PRO180, GLY250, PRO309, VAL321, PHE321.

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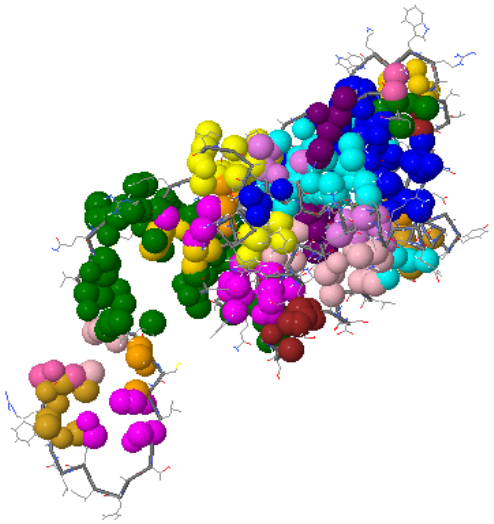
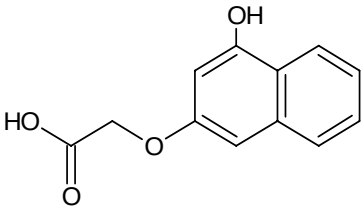


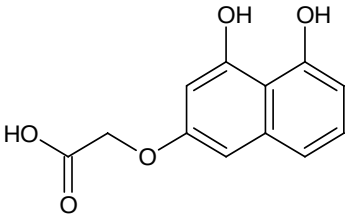
**Fig 6: Active site of protein**

## Docking of inhibitors with the active site of Lipoprotein Lipase

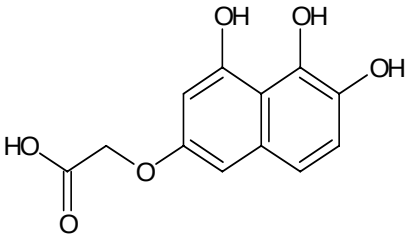
Docking of the inhibitors with Lipoprotein Lipase was performed using GOLD 3.0.1, which is based on genetic algorithimn (Figure 7). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystalized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the modeled protein were added. Docking of best inhibitor with the active site of protein showed the activity of the molecule on protein function (Fig 8).



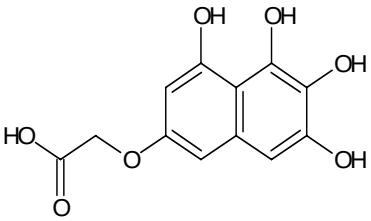

[(4-hydroxy-2-naphthyl)oxy]acetic acid




[(4,5-dihydroxy-2-naphthyl)oxy]acetic acid




[(4,5,6-trihydroxy-2-naphthyl)oxy]acetic acid




[(4,5,6,7-tetrahydroxy-2-naphthyl)oxy]acetic acid

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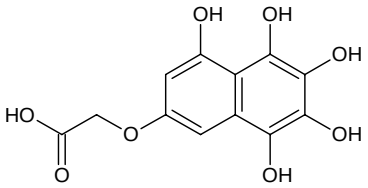
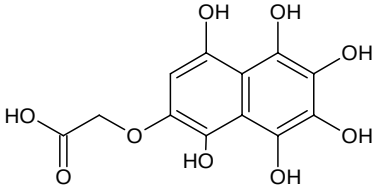
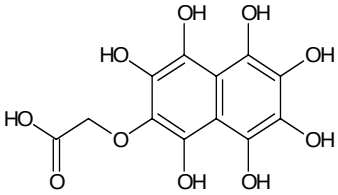


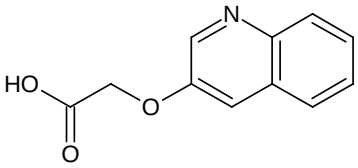

[(4,5,6,7,8-pentahydroxy-2-naphthyl)oxy]acetic acid



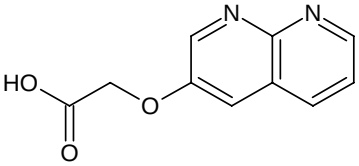

[(1,4,5,6,7,8-hexahydroxy-2-naphthyl)oxy]acetic acid



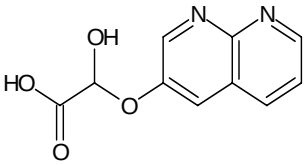

[(1,3,4,5,6,7,8-heptahydroxy-2-naphthyl)oxy]acetic acid




(quinolin-3-yloxy)acetic acid




(1,8-naphthyridin-3-yloxy)acetic acid




hydroxy(1,8-naphthyridin-3-yloxy)acetic acid

**Fig 7: Different compounds were checked for the docking with protein Lipoprotein Lipase.**

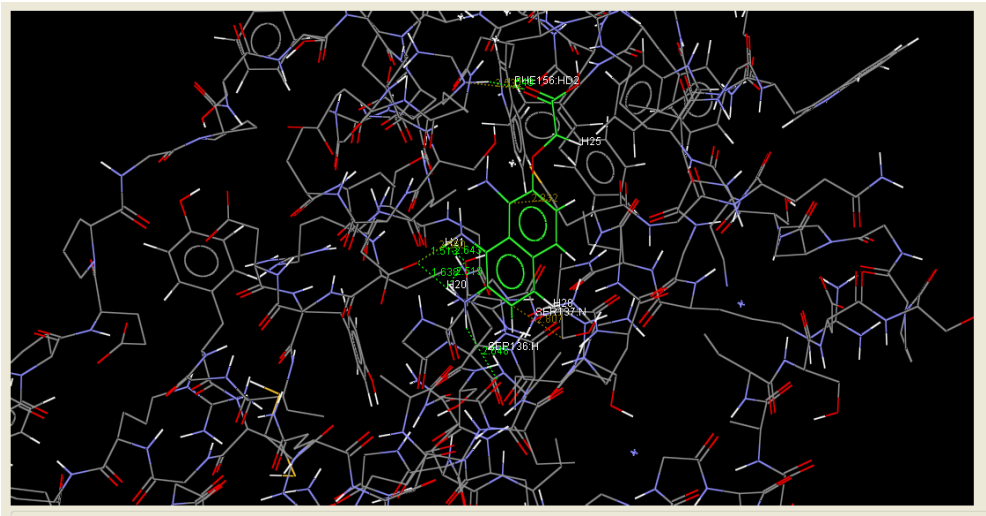



**Fig 8: Docking of best molecule with Lipoprotein Lipase**

## CONCLUSION

In this work, we have amplified and constructed a 3D model of Lipoprotein Lipase domain, from human using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT and PROCHECK program, and the results show that this model is reliable. The stable structure is further used for docking of substrate with the derivatives of target protein inhibitors. Docking results indicate that conserved amino-acid residues in Lipoprotein Lipase main play an important role in maintaining a functional conformation and are directly involved in donor substrate binding.

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The interaction between the domain and the inhibitors proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play important role for the structure and function of biological molecules. In this study it was found that GLY6, ILE8, ILE9, ASN10, GLY14, LEU18, SER20, GLN22, CYS28, THR32, HIS151, PRO180, GLY250, PRO309, VAL321, PHE321 of Lipoprotein Lipase are important for strong hydrogen bonding interaction with the inhibitors. To the best of our knowledge PRO180, GLY250 and PRO309 are conserved in this domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. The 3$^{rd}$ molecule showed best docking results with target protein.

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