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Shape based virtual screening and molecular docking towards designing novel pancreatic lipase inhibitors

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Abstract:
Increase in obesity rates and obesity associated health issues became one of the greatest health concerns in the present world population. With alarming increase in obese percentage there is a need to design new drugs related to the obesity targets. Among the various targets linked to obesity, pancreatic lipase was one of the promising targets for obesity treatment. Using the in silico methods like structure based virtual screening, QikProp, docking studies and binding energy calculations three molecules namely zinc85531017, zinc95919096 and zinc33963788 from the natural database were reported as the potential inhibitors for the pancreatic lipase. Among them zinc95919096 presented all the interactions matching to both standard and crystal ligand and hence it can be further proceeded to drug discovery process.

Background:
Obesity became a serious health concern in the present world population. The latest statistics regarding the obesity by WHO (2014) reported that over 1.9 billion adults, 18 years and above stated as overweight and among them 600 million were obese. Health problems like diabetes, heart-related problems, hypertension, asthma, cancer were associated with the obesity. The rate of population prone to obesity was increasing every day **[1-3]**. Imbalance in the energy metabolism was the major cause of the obesity i.e higher energy consumption with lower expenditure. This leads to the excess deposition of fat in the adipose tissue. Reduction of the dietary fat absorption was one of the approaches aimed in fighting against obesity **[4]**. Pancreatic lipase is one of the key enzyme involved in the triglyceride metabolism. This enzyme was secreted from pancreas, hydrolyses the triglyceride into glycerol and fatty acids **[5]**. Pancreatic lipase in complex with the colipase performs the triglyceride digestion **[6]**. Hydrolysis activity of the enzyme is maintained by the catalytic triad Ser152, Asp176, and His263 amino acids in the active pocket **(Figure 1).** Among the three residues, Ser152 is the important residue for performing lipolysis activity **[7]**. Orlistat; an FDA approved drug as pancreatic lipase inhibitor has shown reducing the fat absorption in humans by reacting with the catalytic serine residue **[8, 9]**.

In the present work, shape based screening of the natural database using the crystalized inhibitor and FDA drug. Screened molecules were further subjected to ADME prediction tool and the passed molecules were progressed to docking studies. Based on the binding scores and interactions the docked complexes were further analyzed and binding free energies were also calculated. The entire schema of the present study was illustrated in the graphical schematic workflow **(Figure 2).**

Methodology:
Database
In the present study ZINC database, a free biomolecules database containing around 1, 69,109 natural compounds was used for virtual screening.

Shape based screening
Shape based screening is one of the standard and significant virtual screening protocol in ligand based computer aided drug design. This was carried out using Phase shape screening application from the Schrodinger suite **[10]**. This technique

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employs the methodology of screening of database based on shape and electrostatic properties of the known molecule to retract similar type of molecules from natural molecule database **[11]**. The screened molecules were predicted to show similar kind of binding modes with active site residues and in turn may produce similar type of activity. The shape based screening requires the known crystalized molecule (generally from PDB) if not the molecule conformers need to be generated and from the generated conformer's, lowest energy exhibiting pose was taken into consideration for the screening process **[12]**. For generating molecule conformers ConfGen module was used **[13]**.

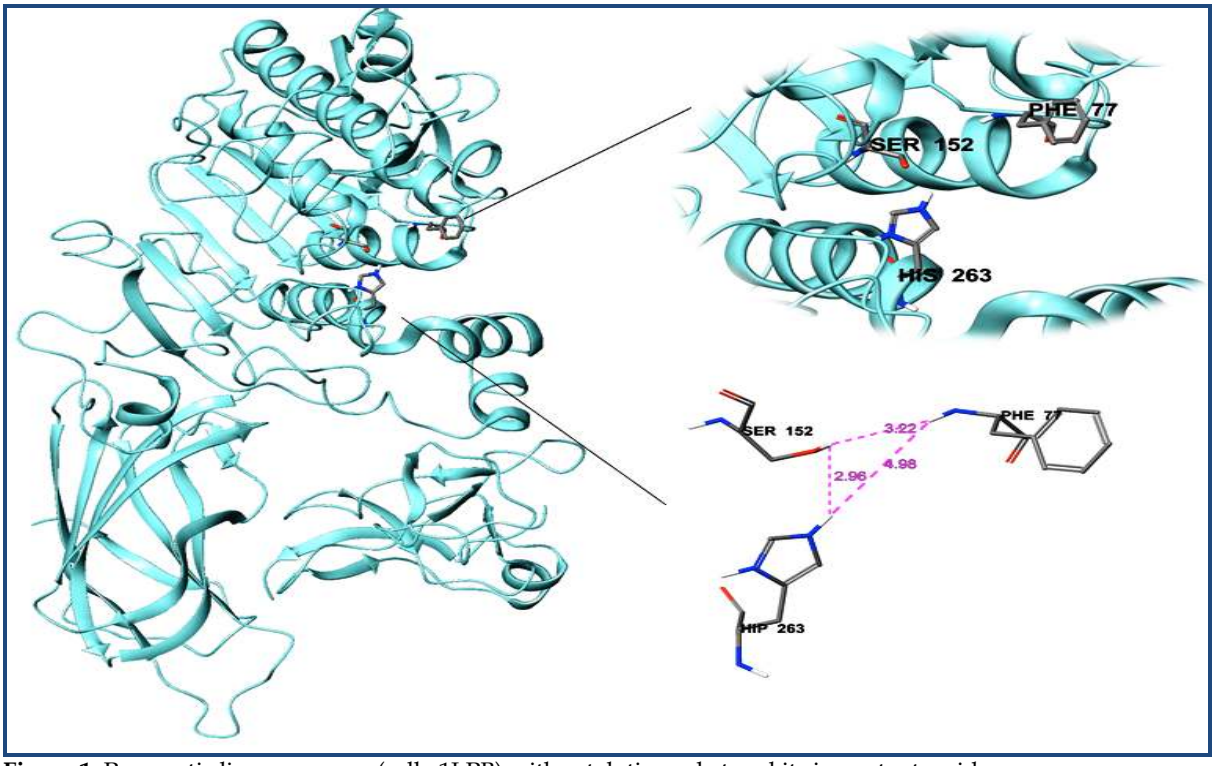

Figure 1: Pancreatic lipase enzyme (pdb: 1LPB) with catalytic pocket and its important residues.

ADME based screening and Ligand preparation

QikProp application was used to calculate ADME properties of the screened molecules **[14]**. This application analyzes the important properties like CNS activity, human oral absorption, octanol/water and water/gas log Ps, log BB, log S,Caco-2, , Lipinski Rule-of-Five, Jorgensen Rule of 3 , etc. referred to as pharmaceutical properties which assist in making the selection of the molecules. CNS activity on a scale of -2 (inactive) to 2 (active) was taken as primary consideration and others like human oral absorption, molecular weight, Donor HB, Accpt HB, QPlog Po/w and Lipinski's rule of 5 as secondary in the present workflow. The retrieved molecules after the ADME based screening were optimized through OPLS 2005 force field **[15]** with LigPrep module.

Protein preparation and receptor grid generation

The 3 dimensional structural coordinates of pancreatic lipase was obtained from protein data bank (pdb id: 1LPB) with a resolution of 3.04 Å. The raw protein was prepared using the protein preparation wizard **[16]** by implying the parameters like assigning bond orders to hydrogen's, zero order bonds were created to metal atoms, capping the termini and desolvation was carried out by deleting the crystallized free water molecules beyond 5Å. Finally the protein hydrogen bonds optimized and minimized using the force field OPLS 2005. With the help of receptor generation programme from the Glide module, the active pocket in the protein was fixed and this was further used for the docking studies.

Glide docking studies

Protein and ligand docking studies were carried out using ligand docking module from the glide application in the Schrodinger suite **[17]**. This module is a grid based method with energetics and gives scores based on the formation of favorable interactions between molecule and protein. The prepared ligands were docked into the grid enclosed active pocket of the protein using the extra precision mode. The protein- ligand complex interactions were calculated based on the quality of geometric contacts and their energy. Ligplot from application menu was adopted to display and study the interactions in the protein-molecule complexes. Ranking was given to the ligands based on their G-scores using the following formulae

G-score = 0.05*vdW + 0.15*Coul + Lipo + Hbond + Metal + Rewards + RotB + Site (1)

vdW was the Van der Waals energy, Coul represents the Coulomb energy, Lipo term explains the Lipophilic, Rewards describes the favorable hydrophobic interactions, Hbond means Hydrogen-bonding term, Metal gives the information about metal-binding RotB tells about penalty for freezing rotatable bonds and Site defines polar interactions in the active site.

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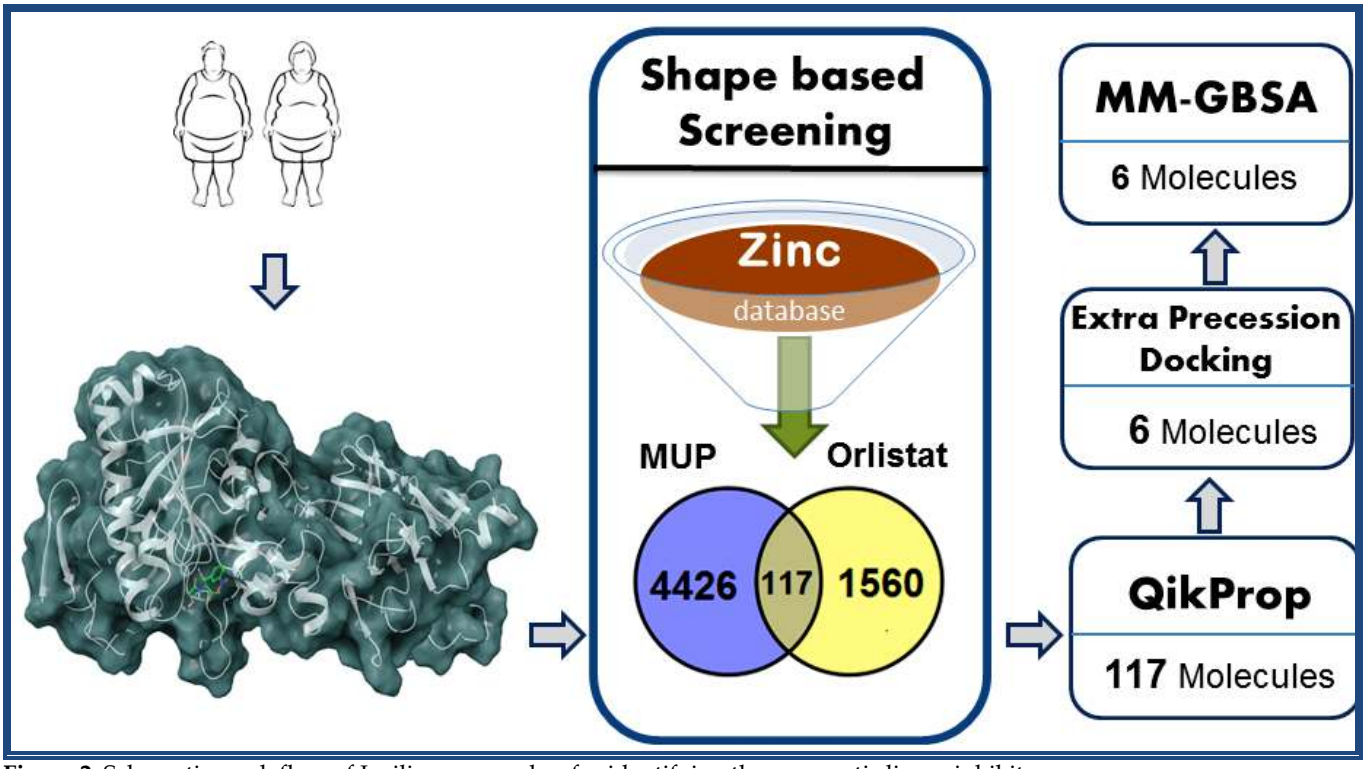

Figure 2: Schematic work flow of Insilico approaches for identifying the pancreatic lipase inhibitors.

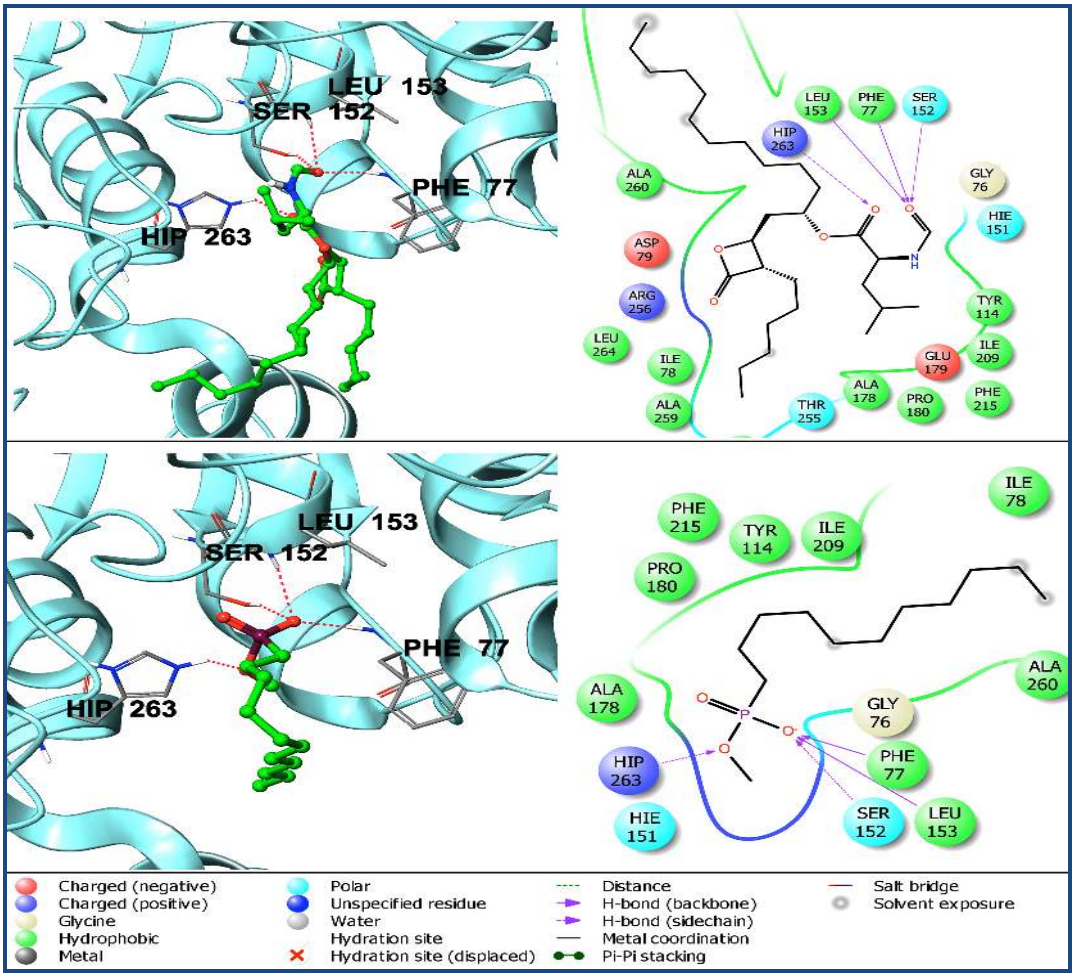

Figure 3: Binding interactions a) Orlistat and b) MUP with in the protein active pocket.

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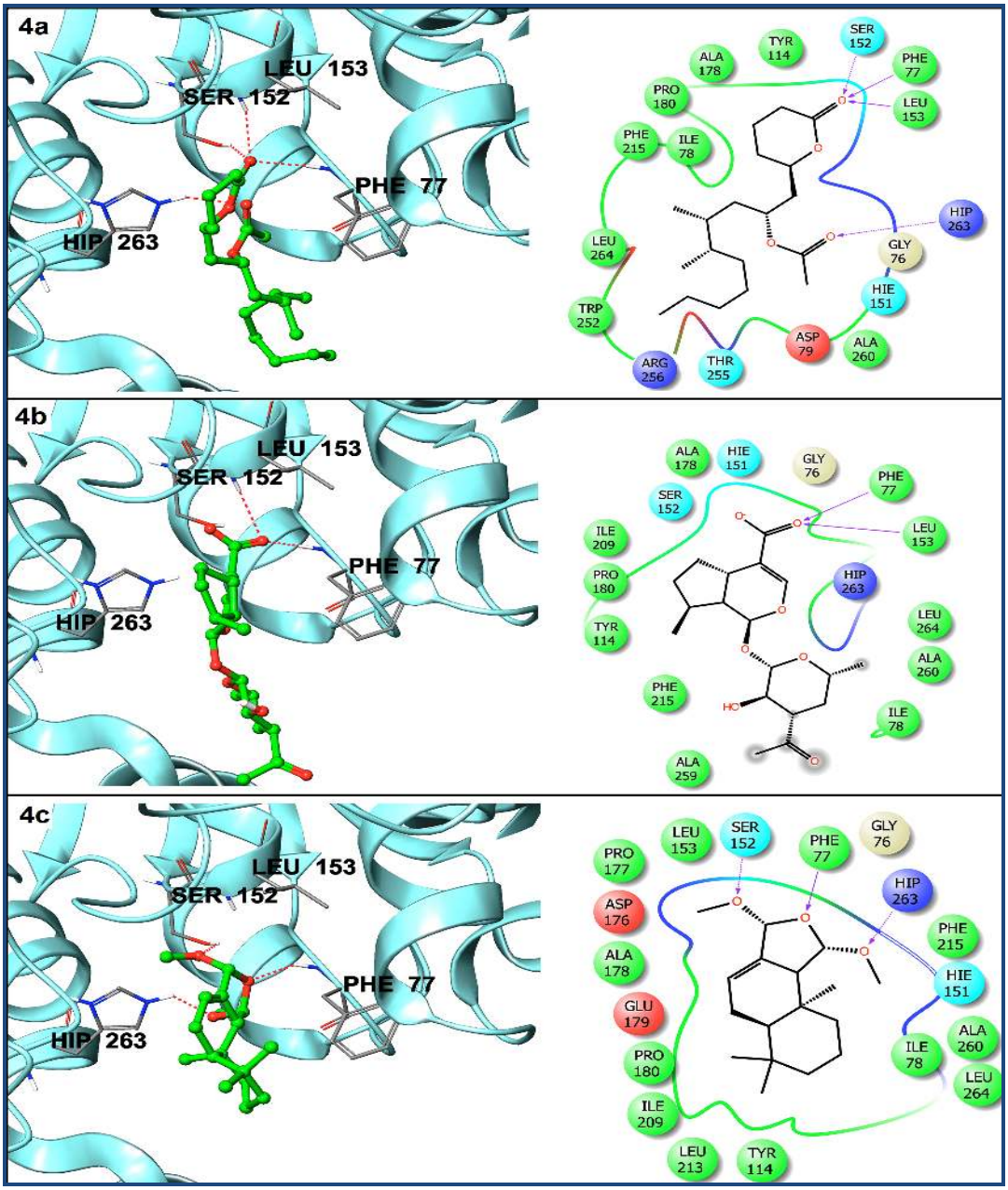

Figure 4: Binding studies of 1LPA with a) zinc85531017, b) zinc 85648552 and c) zinc 27550867

MM-GBSA binding free energy calculations

Prime module in the Schrodinger suite was used to calculate the binding free energies of the complexes. For ΔG binding calculations, output pose viewer files from the docking studies were used. The following equation was used to calculate binding free energies.

$$\Delta G_{binding} = E_{R:L} - (E_R + E_L) + \Delta G_{solv} + \Delta G_{SA} \quad (2)$$

Where $E_{R:L}$ represents the energy of the complex, ER + EL stands sum of energies of the receptor and ligand in the unbound state, ΔG_{solv} is the difference in the solvation energy of the complex and the sum total of solvation energies of unbound receptor and ligand. ΔG_{SA} depicts the difference in surface area energies of the complex and the sum total of surface area energies of unbound receptor and inhibitor.

Results & Discussion:

Virtual screening of the database molecules

The objective of shape-based type virtual screening was to isolate molecules based on the shape and electrostatic properties of a known ligand that binds in specific active site of the protein. Thus the newly screened molecule matching with the same shape and electrostatic properties of the template is expected to bind likewise inside the pocket and perform similar type of activity as well. The methodology evaluates both shape similarities and volume scores. Phase shape based screening was used to screen the hits from the Zinc database using crystal ligand MUP (Methoxyundecyl phosphinic acid) and Orlistat as individual templates to identify the hits with the similar shape. MUP was the crystalized inhibitor chosen from the PDB and the other Orlistat, an FDA approved drug known for the inhibition of pancreatic lipase. As Orlistat was not obtained from the PDB, we have generated conformers using ConfGen.

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Based on the energies, the conformer which was bearing low energy was taken as template for screening. Individually, shape based screening was carried out with the template structures and based on the shape similarity the molecules were ranked. Based on the shape_sim property the results obtained for both templates were tabulated in the descending order. With the cut of range shape_sim ≥ 3.5 the template MUP screened 4426 hits whereas Orlistat screened 1560 hits. Using the online tool Venny, molecules which were commonly reported in the both screening were identified. About 117 hits were found commonly in both the virtual screenings strategies and were further screened based on their ADME parameters.

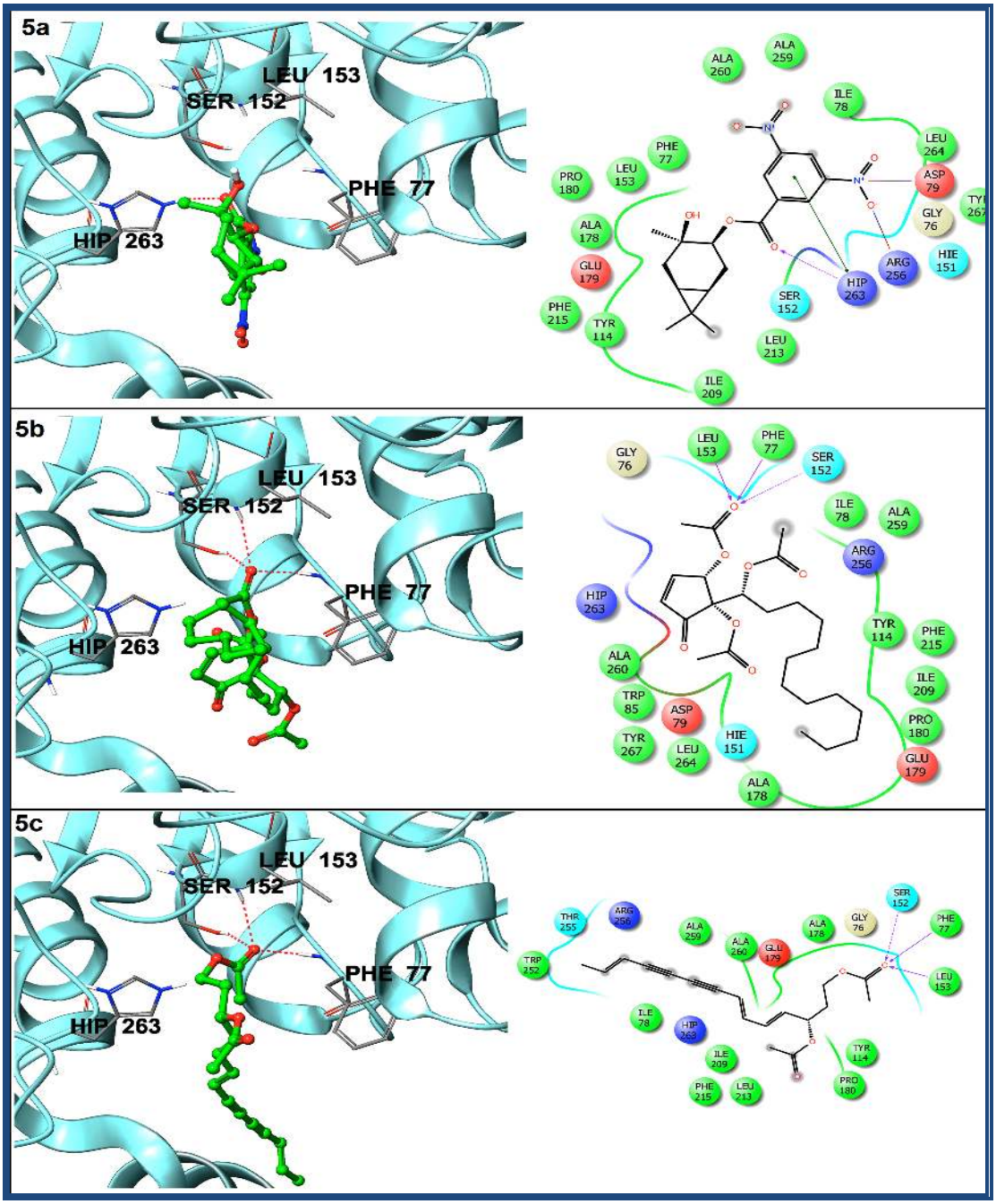

Figure 5: Binding interactions of protein with a) zinc04104767, b) zinc95919096 and c) zinc33963788.

QikProp and ligand preparation

These 117 molecules were further screened using the QikProp module. Among the molecules, the hits which are exhibiting CNS activity as -2 were considered for the further studies. The main interest of our study was to isolate the molecules which are showing activity against lipase and not on the nervous system. Based on this major criterion 9 molecules were screened and were promoted to the preparation. Out of 9, three molecules zinc4104765, zinc85648554 and zinc85648557 were omitted as they are the tautomers of zinc4104767 and zinc85648552. The six finally selected hits along with the ADME properties were tabulated in the **Table 1 (see supplementary material).** All the six hits displayed the properties like molecular weight; percent human oral absorption and Lipinski's rule of five with in the acceptable range. The six selected hits were then geometrically optimized through OPLS

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2005 force field using the LigPrep module of the Schrodinger suite.

Molecular docking studies

Grid based docking study was used to analyze the binding modes of molecules with the amino acids present in the active pocket of the protein. Using the extra precision (XP) mode the hits were docked into the catalytic pocket of the pancreatic lipase. Along with the hits, Orlistat and MUP were also docked for the comparative analysis. To startup the docking studies the grid protocol was initially validated with MUP, a crystalized inhibitor in 1LPB.In the first instance MUP was retracted from the active pocket and then it was redocked into the same pocket. The resultant produced same orientation as it was revealed in the crystalized form and confirming the grid was good enough to continue further. MUP the crystalized inhibitor formed four hydrogen bonds and a salt bridge. The three hydrogen were established between =O of the inhibitor with NH group of Phe77 and Leu153, OH group of Ser152. The NH group of Hip263 formed one hydrogen bond with the oxygen atom of the MUP and also a salt bridge was also observed with another oxygen atom with the same residue. The G-score of this complex was -4.6. 1LPB-Orlistat complex produced three hydrogen bonds by the end of docking with a G-score of -8.5.Two hydrogen bonds were shared between the NH group of Phe77 and OH group of Ser152 with the =O of the Orlistat in the active pocket. The other hydrogen bond was formed between NH group of the Hip263 (Hip: hydrogens on both nitrogens of Histidine) with another =O group of the Orlistat. The interaction profile of both Orlistat and MUP were displayed in the **Figure 3a & 3b**.

Binding affinity in the 1LPB-zinc85531017 complex was maintained with the four hydrogen bonds. Three hydrogen bonds were formed between =O group of the zinc85531017 by NH group of Phe77,OH group of Ser152 and NH group of Leu153.The fourth hydrogen bond was shown between another =O group of the zinc85531017 and NH group of the Hip263.The complex zinc85531017-1LPB maintained a G-score of 9.16 by the end of binding studies. The interaction profile of this complex can be observed in the **Figure 4a**. Zinc85648552-1LPB complex formed two hydrogen bonds in the docking studies with a G-score of 8.6.The two hydrogen bonds were produced via =O of the zinc85648552 molecule with NH group of the Leu153 and Phe77 in the active pocket **(Figure 4b).**

Binding analysis in the 1LPB-zinc27550867 complex formed three hydrogen bonds. The three hydrogen bonds with the three residues Ser152 (OH), Phe77 (NH) and Hip263 (NH) were formed with the three different oxygen atoms of the zinc27550867 and the G-score was -8.0.The interaction profiles of this complex was displayed in **Figure 4c**. Single hydrogen bond interactions was observed in the complexes zinc04104767-1LPB complex between Hip263 (NH) with =O group of the molecule and two salt bridges were observed with the Arg256 and Asp79 residues in the binding pocket with the G-scores of -7.2 **(Figure 5a).** The molecule zinc95919096 and protein 1LPB complex was maintained by the three hydrogen bonds shared with =O group of the molecule with residues Ser152 (OH group), Phe77 (NH group) and Leu153 (NH group) with a G-score of -7.1. The complex 1LPB- zinc33963788 also revealed three hydrogen bonds by the end of docking studies. The three bonds were formed between OH group of the Ser152, NH group of Phe77 and Leu153 with single =O group of the zinc33963788.The G-score of this complex was -6.4.The interaction profiles of the 1LPB-95919096 complex and 1LPB-33963788 were depicted in the **Figure 5b & 5c.**

From the docking results, it was observed that the molecules zinc85531017, zinc95919096, zinc27550867 and zinc33963788 shown hydrogen bond interaction with the important residue Ser152. The two other molecules zinc85648552 and zinc04104767 also showed hydrogen bonds other than Ser152. This residue was the foremost and key residue of the catalytic triad in performing the inhibition activity. This happens by the chemical shift in between the molecule and Ser152 which results in the change of the protein structure and there by inhibition of the lipase enzyme takes place. Based on the G-scores the molecule zinc85531017 showed highest G-scores and it was also greater than the Orlistat and MUP. Comparing the interactions of molecules with the Orlistat and MUP, molecules zinc85531017, zinc33963788 and zinc95919096 displayed interactions similar to MUP and the remaining zinc27550867 interactions were similar to Orlistat. The molecule zinc85531017 G-score was greater than the Orlistat and MUP and the remaining three molecules secured G-score less than the Orlistat but greater than MUP. The six molecules along with MUP and Orlistat in the form of complex individually with 1LPB were further advanced to energy calculations.

The binding free energies were calculated using the Prime MM-GBSA module of the Schrodinger suite. Using the pose viewer file obtained from the docking protocol was used in the calculating the binding free energies of ligand-protein complexes. Through these two studies in correlation it observed that zinc85531017 ranked as highest in the docking studies and the complex stood as third in the energy calculations. Molecule zinc33963788 ranked as least in the docking studies has shown highest binding free energy in complex with 1LPB among the six complexes. Whereas the zinc27550867 molecule disclosed interaction with the important residues but the binding free energies as complex was low when compared to all the complexes as well as with the Orlistat and MUP complexes. Another 1LPB- zinc95919096 complex was the second best in the MM-GBSA calculations. The remaining complexes1LPB-zinc85648552 and zinc04104767 binding free energies were low compared to all complexes except zinc27550867-1LPB complex. The G-scores along with their binding free energies were tabulated in the **Table 2 (see supplementary material).** With these observations the molecules zinc85531017, zinc95919096 and zinc33963788 were proposed as the lead molecules and among these in particular zinc95919096 was suggested more as it was satisfying the molecular interactions displayed by both Orlistat and MUP.

Conclusion:

In this study shape based screening protocol was implied to screen out the similar structures from the natural database based on the shapes of standard drug and crystalized inhibitor. Applications like QikProp, LigPrep, Glide and Prime MM-GBSA were embedded in the work flow and finally three molecules were reported as the potential leads. These three leads produced hydrogen bond interaction with the important residue Ser 152. These hits showed other molecular interactions within the active site of the receptor and were energetically stable. The molecules zinc95919096 was proposed as a good inhibitor for pancreatic lipase based on the interaction profile

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and can be further proceeded to validate them using the Invitro studies.

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Disclosure:
The authors report no conflict of interest regarding this work.

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Supplementary material:

Table 1: Predicted ADME properties of the finally selected six molecules

Molecule	CNS[a]	Molecular Weight [b]	Percent Human Oral Absorption	Donor HB[c]	Accpt HB[d]	QPlog Po/w[e]	Rule Of Five
zinc04104767	-2	364.35	68.90	1	4.7	2.37	0
zinc27550867	-2	280.40	100	0	5.1	1.73	0
zinc33963788	-2	300.35	100	0	4	4.50	0
zinc85531017	-2	326.47	100	0	5	4.20	0
zinc85648552	-2	354.39	68.72	1	9.8	1.59	0
zinc95919096	-2	438.56	100	0	8	4.71	0

a) Predicted central nervous system activity –2 (inactive) to +2 (active); b) Molecular weight between 130.0 and 725.0; c) Number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. 0.0 to 6.0; d) Number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. 2.0 to 20.0; e) Predicted octanol/water partition coefficient scale. –2.0 to 6.5

Table 2: Docking scores and binding free energies of the molecules along with Orlistat

Molecule	G-score	Prime MM-GBSA (Kcal/mol)
zinc85531017	-9.1	-79.52
zinc 85648552	-8.6	-65.35
zinc 27550867	-8.0	-51.39
zinc 04104767	-7.2	-70.35
zinc 95919096	-7.1	-83.41
zinc 33963788	-6.4	-87.36
Orlistat	-6.9	-105.01
MUP	-4.6	-65.62

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