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In silico molecular docking studies on medicinally active compounds present in *Mollugo cerviana* (L.) Ser

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Abstract
The molecular docking of selective biologically active compounds (ligands) contained in the plant extract of *Mollugo cerviana* with (Protein) Glycogen synthase kinase 3β shortly known as GSK3β a receptor enzyme which is playing an active role in the process of inflammation and fibrosis was carried out and the binding modes, conformations, intermolecular interactions and various docking parameters were evaluated. The ligands preferred under this study are Phytol, Decanoic acid and Farnesol. Auto Dock 4.2 (version 4.2) was used as molecular-docking tool to carry out the docking simulations. The structure of Ligands and protein were retrieved from RCSB PDB (Protein Data Bank) and Pub chem websites. The study revealed the ability of the ligands to bind with the receptor GSK-3β at the binding site generating stable complexes. The Protein–Ligand Interaction studies revealed hydrophobic interactions in all the three complexes. Hydrogen bonding is noticed in Decanoic acid and Farnesol. The negative and low value of free energy of binding indicates a strong favourable binding affinity between the protein and the ligands. The results revealed that Decanoic acid is able to form a stable complex with the receptor enzyme GSK-3β when compared with other two ligands.

Keywords: ligands, protein, glycogen synthase kinase 3β, Auto Dock 4.2, conformations, intermolecular interactions

Introduction
Designing of a medicinal application containing biologically active ingredients isolated from a plant extract, a detailed understanding of the compounds contained in the plant, their ability to bind with the receptors or proteins in the body and their interaction profile are the vital requirements. These interactions between small molecules and proteins may form the basis for rational drug design strategy [1]. *In silico* studies referred to studies performed on computers via computer simulations and they are effective tools in the rational drug designing.
Structure based drug designing (SBDD) employs the use of three dimensional structures to predict the interaction between the ligand (the drug molecule) with particular protein or enzyme. In structure based drug designing the ligand-protein interaction plays a crucial role. Kalyaanamoorthy [2] states that molecular docking, structure-based virtual screening (SBVS) and molecular dynamics (MD) are among the most frequently used structure based drug designing strategies due to their wide range of applications in molecular recognition analysis such as binding energetics, molecular interactions and induced conformational changes. Molecular docking is a key technique in computer assisted drug design. Molecular docking is a bioinformatics modelling technique to study the interaction between two or more molecules to give a stable adduct. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level and which allow us to characterize the behaviour of small molecules in the binding site of target protein and also to elucidate the fundamental biochemical processes [3].
In the present study the molecular docking of three biologically active compounds contained in the plant extract of *Mollugo cerviana* with a receptor enzyme which is playing an active role in the process of inflammation and fibrosis was carried out and the binding modes, conformations, intermolecular interactions and various docking parameters were evaluated.

Materials and methods
Ligands
Among several compounds identified through the GC-MS analysis of *Mollugo cerviana,* following three compounds (ligands) have been chosen for the docking study based on their retention time, activity profile and their ability to bind with the target enzyme. The ligands preferred under this study are Phytol, Decanoic acid and Farnesol. The structure of ligands subjected to docking are shown in Figure 1.

Protein
The protein (receptor) selected and subjected to docking analysis in this study is Glycogen synthase kinase 3β shortly known as GSK3β. GSK-3 exists in two closely related isoforms; GSK-3α (51 kDa) and GSK-3β (47 kDa), both isoforms have nearly identical biochemical functions and substrate affinities but are encoded by separate genes. Geetha Vani Rayasam [4] states that, GSK-3β is a member of the serine/threonine family of protein kinases, known to phosphorylate components of a wide variety in processes such as glycogen synthesis, cell proliferation, embryogenesis, axon growth, and cardiomyocyte hypertrophy. GSK-3 is known to regulate many signalling as well as structural molecules thereby providing multiple

targets for GSK-3 action [5]. A new cellular function regulated by GSK3 was identified and the findings show that GSK3 is a vital factor in the inflammation process [6]. GSK-3β has been implicated in the regulation of a vast array of molecular and cellular functions including cytoskeletal regulation, intracellular vesicular transport, cell cycle progression and apoptosis [7]. According to Doble and Woodgett [8] Glycogen synthase kinase-3 beta (GSK-3β) can able to transfer a phosphate group to either the serine or threonine residues of its substrates. The catalytic activity of GSK-3β is regulated by phosphorylation at two different sites, namely Ser 9 and Tyr 216. Phosphorylation of the Serine 9 site inactivates GSK-3β, whereas phosphorylation at Tyrosine 216 within the activation loop increases its catalytic activity [9]. Glycogen synthase kinase 3 (GSK3) is one of the few master switch kinases that regulate many aspects of cell functions. The studies on cell polarization and migration have shown that GSK3 is essential for proper regulation of these cellular processes [10]. GSK-3β is a negative regulator of glucose homeostasis and is also involved in energy metabolism, inflammation, mitochondrial dysfunction, and apoptotic pathways. GSK-3β phosphorylates MACF1 (Microtubule-Actin Crosslinking Factor 1), inhibiting its binding to microtubules which is critical for its role in bulge stem cell migration and skin wound repair. According to Wu *et al*.,[11] the deletion of the MACF1 gene in skin epidermal cells resulted in a significant delay in wound repair due to impaired focal adhesion dynamics and epidermal migration. Glycogen synthase kinase 3β has been implicated in diverse cellular processes such as cell signalling and survival.

GSK-3β operates as a key protein in the Wnt signalling pathway. Wnts constitute a family of secreted glycoproteins with distinct expression patterns that regulate cell proliferation, migration and specification of cell fate in the embryo and adult organism [12]. Glycogen synthase kinase-3 regulates cell adhesion and migration by regulating the degradation of *β*-catenin in the Wnt pathway [13]. β-catenin-dependent Wnt signalling expression increases shortly after cutaneous wounding, and exogenous rmWnt3a accelerates reepithelialisation, wound matrix maturation and scar formation[14]. GSK-3β is closely associated with the phosphorylation of β-catenin. GSK-3β normally binds with β-catenin and phosphorylates it resulting in its degradation. But when GSK-3β split up from the β-catenin it leads to accumulation of β-catenin in the nucleus and which in turn induces protein synthesis. Precisely GSK-3β activity is inhibited when the Wnt pathway is induced. Inhibition of GSK-3 activates the canonical Wnt pathway in fibroblasts, stimulates the release of collagen from fibroblasts, exacerbates experimental fibrosis and is sufficient to induce fibrosis. GSK-3 is therefore a key regulator of the canonical Wnt signalling in fibroblasts and inhibition of GSK-3 results in fibroblast activation and increased release of collagen [15]. Another reported study by Mohit Kapoor *et al*., [16] recites; GSK-3beta appears to control the progression of wound healing and fibrosis by modulating ET-1 (vasoconstrictive protein endothelin-1) levels. The results suggest that targeting the GSK-3beta pathway or ET-1 may be of benefit in controlling tissue repair and fibrogenic responses *in vivo*, the study further reveals Gsk3b-conditional-Knockout mice (Gsk3b-CKO mice) exhibited accelerated wound closure, increased fibrogenesis, and excessive scarring compared with control mice. In addition, Gsk3b-CKO mice showed elevated collagen production, decreased cell apoptosis, elevated levels of profibrotic α-SMA, and increased myofibroblast formation during wound healing.

Docking Software

Auto Dock 4.2 (version 4.2) was used as molecular-docking tool to carry out the docking simulations with support of MGL tools. The Auto Dock is an automated docking program designed for prediction of the binding among small molecules such as substrates or drug candidates and the receptor with known 3-D structure. MGL tools are a quite large package of different tools and GUIs (Graphical user interface**)** for working with molecular structures, including Auto Dock Tools.

Retrieval of ligand structure

The ligand structures from (RCSB PDB site and Pub chem) were retrieved. The 3 dimensional structure of the ligand (small molecule) was drawn in Chem Draw (software drawing tool for chemical structures) using the Structure Mode. After drawing, the structure was cleaned up in order to standardize the bond lengths and angles. The file format of the ligand was converted to required format before proceed to docking.

Retrieval of protein (target enzyme) structure

The structure of the protein GSK-3-β was retrieved from RCSB PDB (Protein Data Bank) website and converted in to required format. The PDB ID of the protein is 1Q5K. The Protein Data Bank (PDB) is a crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids.

Docking

The prediction of conformation, position and orientation of the ligand molecule within the binding site and assessment of the binding affinity are the steps involved in docking process. In the process of docking first the three dimensional structure of the ligand (the molecule) and the three dimensional structure of the receptor (enzyme) is determined. The ligand is docked in to the binding cavity of the receptor and possible conformations are explored. The most likely binding mode and the intermolecular interactions are examined.

Auto Dock calculations are performed in several steps: 1) preparation of coordinate files using Auto Dock Tools, 2) pre calculation of atomic affinities using Auto Grid, 3) docking of ligands using Auto Dock, and 4) analysis of results using Auto Dock Tools[17]. A brief outline of the methodology carried out in this docking study using Auto Dock 4.2 is as follows:

1. Coordinate files prepared in PDBQT format. This includes a file of coordinates for the receptor and file of coordinates for the ligand.
2. Grid parameter files, grid maps and grid data files were generated. This step is followed by generation of docking parameter file.
3. Based on the grid maps for each atom type in the ligand calculated by Auto Grid, the PDBQT file coordinate for the ligand and the docking parameter file docking programme was run and the Ligands were docked with the protein and the conformations were generated.
4. At the end of a docking simulation, the docking software writes the coordinates for each docked

conformation to the docking log file, along with information on clustering and interaction energies and using Auto Dock tools results were evaluated. Visualization of hydrogen bond formation between protein and ligand were done using protein–ligand interaction profiler (PLIP), a novel web service for fully automated detection and visualization of relevant non-covalent protein–ligand contacts in 3D structures. The result page lists all detected non-covalent interactions (hydrogen bonds, water bridges, salt bridges, halogen bonds, hydrophobic interactions ...etc.) for evaluation.

Results

The best docked conformation of the Phytol (ligand) with GSK 3β the (target enzyme) is the one with lowest binding energy. The cluster analysis chart revealed the binding energy in Kcal/mol against seven conformations. The conformation with lowest binding energy of -1.86 Kcal/mol is the best docked conformation. The energy parameter of the best docked conformation is shown in Table 1. The ligand efficiency is -0.09. (Binding energy of the ligand per atom of a ligand, expressed in kcal per heavy atom). Theintermolecular energy is -6.04 Kcal/mol and the Hydrogen bond desolvation energy is -6.04. The interaction diagram for each binding site generated by (PLIP) the Protein–Ligand Interaction Profiler is shown in Figure 2. The interaction profile of the ligand atom with protein atom is shown in Table 2. The table reveals only hydrophobic interactions.

The best docked conformation of Decanoic acid the (ligand) with GSK 3β the (target enzyme) is the one with lowest binding energy. The cluster analysis chart revealed the binding energy in Kcal/mol against seven conformations. The conformation with lowest binding energy of -4.0 Kcal/mol is the best docked conformation. The energy parameters of the best docked conformation are shown in Table 3. The ligand efficiency is -0.33. (Binding energy of the ligand per atom of a ligand and expressed in kcal per heavy atom). The intermolecular energy is -6.68 Kcal/mol and the Hydrogen bond desolvation energy is -4.69 Kcal/mol. The interaction diagram for each binding site generated by (PLIP) the Protein–Ligand Interaction Profiler is shown in Figure 3. The detailed interaction profile of the ligand atom with protein atom is shown in Table 4. The table shows hydrophobic interactions, hydrogen bonding without side chain and salt bridges between the ligand and protein. A salt bridge is a non-covalent interaction between two ionized sites and it contributes to specificity of interaction of proteins with the surrounding biomolecules.

The best docked conformation of farnesol the (ligand) with GSK 3β the (target enzyme) is the one with lowest binding energy. The cluster analysis chart revealed the binding energy in Kcal/mol against eight conformations. The conformation with lowest binding energy of -1.36 Kcal/mol is the best docked conformation. The energy parameter of the best docked conformation is shown in Table 5. The ligand efficiency is -0.09. (Binding energy of the ligand per atom of a ligand and expressed in kcal per heavy atom). The intermolecular energy is -3.75 Kcal/mol and the Hydrogen bond desolvation energy is -3.69 Kcal/mol. The interaction diagram for each binding site generated by (PLIP) the Protein–Ligand Interaction Profiler is shown in Figure 4. The interaction profile of the ligand atom with protein atoms is shown in Table 6. The table reveals hydrophobic interactions and Hydrogen bonding with side chain between the ligand and protein.

Discussion

The binding interaction between the small molecule (the ligand) and the protein (enzyme) may result in either activation or inhibition of the enzyme. The most common types of interactions are hydrophobic interactions, hydrogen bonding, π-stacking, salt bridges, and amide stacking. The (PLIP) the Protein–Ligand Interaction Profile diagrams generated in this study visualises the interactions in three dimensional views. In the (PLIP) generated diagrams, the protein molecules are shown in blue colour and the ligands are shown in yellow colour. The hydrophobic interactions, hydrogen bonds, salt bridges and other interactions are shown in different colours. The interacting amino acid residues were indicated by their names.

Phytol is an acyclic diterpene alcohol having the molecular formula $C_{20}H_{40}O$ and the IUPAC name is (E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol. Phytol is used as a precursor for the manufacture of synthetic forms of vitamin E and Vitamin K1. The Protein–Ligand Interaction Profiler study in Table 2 revealed two hydrophobic interactions, one between the Alanine residues of the protein with the ligand molecule and another one is between the Leucine residues with the ligand molecule. Visualisation of the interactions is shown in Figure 2. Hydrophobic interactions are the tendency of the hydrocarbons to form intermolecular aggregates in an aqueous medium and analogues intra molecular interactions. These are most common interactions between the ligands and proteins. Hydrophobic interactions are important for folding of proteins and to keep them in stable state. Hydrophobic interactions can increase the binding affinity between target-drug interfaces. It has been reported that the binding affinity and drug efficacy associated with hydrophobic interactions can be optimized by incorporating them at the site of the hydrogen bonding [18].

Decanoic acid (Capric Acid) is a saturated medium-chain fatty acid with a 10-carbon backbone having the molecular formula $C_{10}H_{20}O_2$. Protein–Ligand Interaction Profiler study of the docked complex revealed hydrophobic interactions between the amino acid residues and the ligand molecule in five positions. Table 4 reveals, two hydrophobic interactions each, by lysine and valine with the ligand atoms and one interaction by isoleucine residue with the ligand atom. The Table further reveals the Hydrogen bond between the Arginine residue of the enzyme and the ligand atom. Besides the said interactions the table also reveals salt bridges between the amino acid residues of the protein and the carboxylate group of the ligand, one each by arginine and lysine. Protein–Ligand Interaction Profile diagram for the docked complex visualises the interactions between the ligand and protein. In Figure 3 the hydrophobic interactions between the molecules, the hydrogen bond and the salt bridges were also indicated.

Farnesol is a 15 carbon isoprenoid alcohol having the molecular formula $C_{15}H_{26}O$ and the IUPAC name is (2E, 6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol. The Protein–Ligand Interaction Profiler study of the docked complex revealed hydrophobic interactions between the amino acid residues and the ligand molecule in three pockets at the binding site. Table 6 reveals the hydrophobic interactions between valine, leucine and tyrosine residues with the

ligand atoms. The table also reveals a hydrogen bond between tyrosine residue of the protein and ligand. Figure 4 is the visualisation of Protein–Ligand Interaction for the docked complex.

All the three ligands in this study namely, Phytol, Farnesol and Decanoic acid are able to bind with the receptor GSK-3β at the binding site and generated stable complexes. The docking software based on the search algorithm and cluster analysis and binding energy parameters identified the best docked conformation (pose). The negative values in the binding energy indicate the stability of the docked conformation and the ability of the ligand to bind with the receptor with minimal consumption of energy. More stable is the complex when the binding energy is low. Binding energy is the energy required to separate a docked complex in to two separate parts as protein and ligand. The Protein–Ligand Interaction studies revealed hydrophobic interactions in all the three complexes. Hydrogen bonding is noticed in Decanoic acid and Farnesol. Decanoic acid shows 5 hydrophobic interaction pockets and farnesol shows 3 pockets followed by phytol with 2 hydrophobic interaction pockets. According to Rohan Patil [19] the hydrogen bonding and optimized hydrophobic interactions both stabilize the ligands at the target site and help to alter binding affinity and drug efficacy. According to Meyer *et al*.,[20] hydrogen bonds and salt bridges as contributors to strong physical interactions and are important components in the assessment of binding.

Summary and Conclusion

On the basis of protein ligand interactions, affinity and binding energy values it was evaluated that Decanoic acid can able to form a stable complex upon docking with the receptor enzyme GSK-3β in comparison with other two ligands. The negative and low value of free energy of binding of ligands with the receptor enzyme indicates a strong favourable binding affinity between the protein and the ligands.The ligands were found to be potential inhibitors of GSK-3β. These docking studies are helpful in designing of novel wound healing drug preparations which can act by inhibiting GSK-3β (Glycogen Synthase kinase) activity.

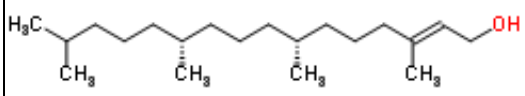
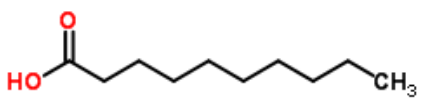
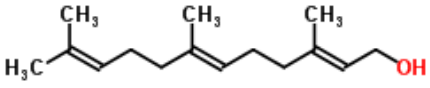
Phytol	
Decanoic acid	
Farnesol	

Fig 1: Structure of Ligands

Table 1: Energy levels of the best docked conformation of Phytol with GSK 3β

Binding energy	-1.86
Ligand efficiency	-0.09
Inhibitory constant	42.96mM
Intermolecular energy	-6.04
Hydrogen bond desolvation energy	-6.04
Electrostatic energy	0
Torsional energy	4.18

Table 2: Interaction profile of the ligand with protein showing Hydrophobic interactions (Phytol with GSK 3β)

Hydrophobic Interactions ••••

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	83A	ALA	3.66	11130	705
2	188A	LEU	3.19	11134	2531

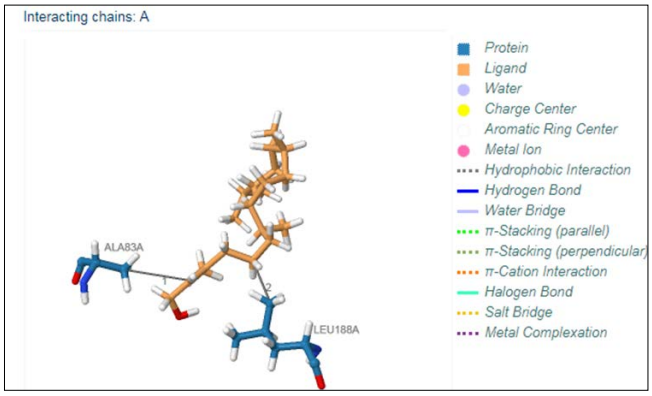
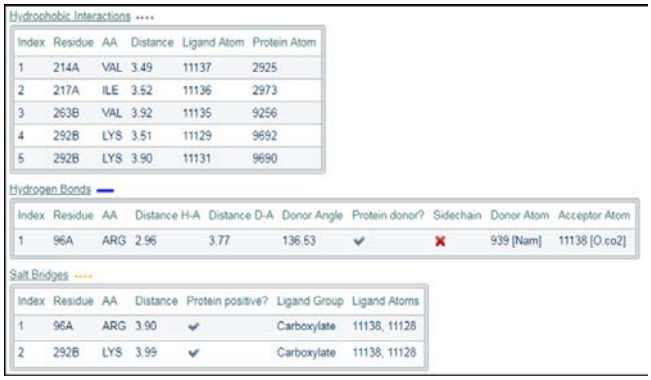


Fig 2: Protein-Ligand Interaction Profile diagrams obtained through PLIP studies (Phytol with GSK 3β)

Table 3: Energy levels of the best docked Conformation of Decanoic acid with GSK 3β

Binding energy	-4.0
Ligand efficiency	-0.33
Inhibitory constant	1.18mM
Intermolecular energy	-6.68
Hydrogen bond desolvation energy	-4.69
Electrostatic energy	-1.99
Torsional energy	2.68

Table 4: Interaction profile of the ligand with protein showing Hydrogen bond, Hydrophobic interactions and Salt bridges (Decanoic acid with GSK 3β)



Hydrophobic Interactions ••••

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	214A	VAL	3.49	11137	2925
2	217A	ILE	3.52	11136	2973
3	263B	VAL	3.92	11135	9256
4	292B	LYS	3.51	11129	9692
5	292B	LYS	3.90	11131	9690

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	96A	ARG	2.96	3.77	136.53	✔	✖	939 [Nam]	11138 [O.co2]

Salt Bridges ••••

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	96A	ARG	3.90	✔	Carboxylate	11138, 11128
2	292B	LYS	3.99	✔	Carboxylate	11138, 11128

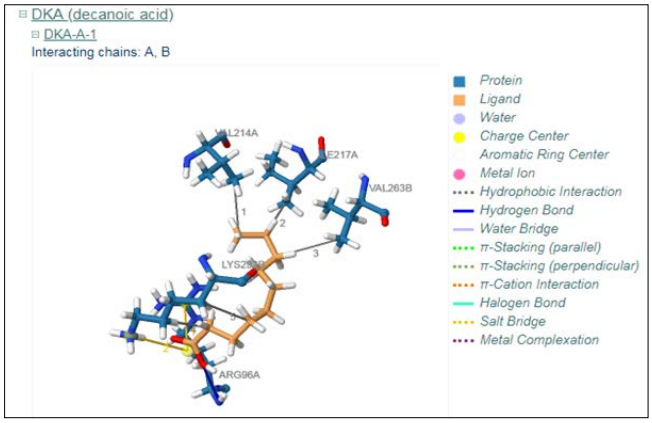


Fig 3: Protein-Ligand Interaction Profile diagrams obtained through PLIP studies

Table 5: Energy levels of the best docked Conformation of Farnesol with GSK 3β

Binding energy	-1.36
Ligand efficiency	-0.09
Inhibitory constant	99.95mM
Intermolecular energy	-3.75
Hydrogen bond desolvation energy	-3.69
Electrostatic energy	-0.06
Torsional energy	2.39

Table 6: Interaction profile of the ligand with protein showing Hydrogen bond and Hydrophobic interactions (Farnesol with GSK 3β)

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	69A	VAL	3.39	11139	495
2	88A	LEU	3.54	11130	794
3	127A	TYR	3.92	11131	1474

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	71A	TYR	2.07	3.01	168.33	✔	✔	532 [O3]	11142 [O3]

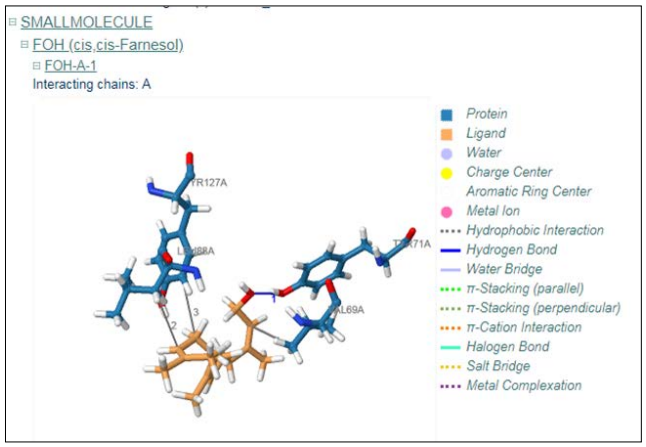


Fig 4: Protein-Ligand Interaction Profile diagrams obtained through PLIP studies

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