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PROTEIN-PROTEIN DOCKING ANALYSIS ON WNT3A-FZD4 COMPLEX IN WNT SIGNALING PATHWAY

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ABSTRACT: The binding of WNT protein family to Frizzled transmembrane receptors initiates a signaling cascade that results in transcription of downstream target genes promoting cell proliferation in most cancer types. The present study was initiated in order to analyse the protein-protein interaction between WNT3A and FZD4 via in silico macromolecular docking methods. The three dimensional structure for WNT3A is predicted by Homology modeling from the primary sequence using Swissmodel. The predicted model is validated using ProCheck and Verify3D. The Verify3D score of the model was found to be 104.77 that come within the expected range of a good model. The model was then used as a ligand against the crystal structure of FZ domain of Frizzled (FZD4-CRD) receptor protein Cysteine Rich Domain. The PPI was carried out using ZDock and the top poses from each cluster was ranked with ZRANK. Based on the ZRANK score, the complexes were refined using RDock. The result presented shows the analysis of interface region of the pose with the least E_RDock score and RMSD value as they represent the near native state of the complex.

KEYWORDS: WNT signaling, WNT3A, FZD4, Protein-Protein Docking, Z Dock, Homology Modeling

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1.INTRODUCTION

WNTs (Wingless and Int-1) play a central role in the development of vertebrate and invertebrate, due to their influences on cell proliferation, differentiation, and migration [1]. WNT protein family normally includes cysteine-rich glycoproteins with approximately around 350 amino acid residues. They activate cell surface receptors to initiate at least three different signaling pathways including the canonical β -catenin pathway, and the non-canonical planar cell polarity (PCP) and Ca^{2+} pathways. The seven-pass transmembrane receptor Frizzled (Fz) is crucial for nearly all WNT signaling, and the N-terminal Fz cysteine rich domain (CRD) acts as the WNT binding domain. The WNT/ β -catenin pathway need the Low-density lipoprotein receptor related proteins 5 and 6 (Lrp5/6) co-receptors along with the FZ domain [2-4]. The canonical WNT signaling pathway plays a major role in the hepatic carcinogenesis. Mutation in the Axin1/2, CTNNB1, Adenomatous Polyposis Coli (APC) and glycogen synthase kinase-3 β (GSK-3 β) can activate the canonical WNT signaling. The β -catenin is involved in the various stages of cell development and in maintaining the homeostasis of the cell in adults. Mostly β -catenin is found in the cell membrane bound to E-cadherin in the absence of WNT proteins. The concentration of β -catenin in the cytoplasm is kept low through phosphorylation by kinases glycogen synthase kinase-3 β (GSK-3 β) and casein kinase I (CK1) found in the enzymatic complex that include the translated tumor suppressor genes for adenomatous polyposis coli (APC) and Axins. They facilitate the ubiquitination of the phosphorylated β -catenin resulting in its degradation through proteolysis [5]. The WNT signaling is activated when the secreted growth factors of the WNT family binds to the frizzled receptors at the cell surface which in turn activates the dishevelled protein. This facilitates the dissociation of the cytoplasmic destructive complex and inhibition of GSK-3 β which results in the accumulation and stabilization of cytosolic β -catenin. The β -catenin then enters the nucleus and binds to the TCF/LEF proteins. In the absence of β -catenin, the TCF/LEF proteins are bound to groucho co-repressors along with their cognate DNA recognition elements that ensure the transcriptional silencing of β -catenin target genes that includes Cyclin D1, c-myc and Survivin [5-7]. Activation of WNT/ β -catenin signaling leads to binding of β -catenin to TCF/LEF proteins that result in the subsequent dissociation of groucho co-repressors, and activation of β -catenin target genes including Cyclin D1, c-myc and Survivin, all of which promote cell cycle progression and inhibit apoptosis [7]. Mutation in the β -catenin gene appears to be the most frequent genetic event in many cancers including human HCC and Colorectal cancer [8-11]. WNTs show selective binding to FZDs, and respective WNT-FZD pairs exert functional selectivity in different downstream signaling pathways. WNT-3A belongs to the group of WNTs that normally induce WNT- β -catenin signaling and is a well-known ligand for FZD1–8. WNT3A forms a ternary complex

with overexpressed FZD4 in the presence of endogenously expressed LRP5/6 to bring about WNT- β -catenin signaling and phospho-LRP6 irrespective of the FZD isoform present in the cells [12]. Recently, there is an increasing interest in studying the interface region which consists of grooves in protein complexes that could be a potential active site region for small molecule inhibitors. *Andrographis paniculata* is used in traditional medicine as well as in tribal medicine in India and some other countries for treating various diseases. The plant extracts exhibited anti-typhoid, anti-hepatotoxic, anti-fungal, anti-malarial, anti-biotic, and anti-cancer activities [13,14]. Earlier, three compounds were identified from the methanol leaves extract of *A. paniculata* through GC-MS analysis. The potential of 6-oxa-3-thiooctanoic acid as inhibitor of Thyroid hormone receptor alpha1 was reported [15]. The present study is initiated in order to analyse the protein-protein interaction between the WNT3A and FZD4 protein complexes and to intervene with plant-derived compound in the interface region via in silico methods.

2. MATERIALS AND METHODS

Homology modeling

The three dimensional structure of WNT3A is not found in crystallized form in Protein Data Bank due to its hydrophobic nature. So the structure was predicted based on available template structure using Swiss-Model workspace. Swiss-Model allows the user to predict the unknown structure of a protein based on the non-redundant structural database in automated mode. The sequence of WNT3A is submitted in the workspace of Swiss-Model [16-18]. A pBLAST search was carried out to find a template model. Then a pairwise and multiple sequence alignment of the target and template were carried out automatically. Based on the sequence alignment three models were built and their validity was checked based on the Z-Score and the QMean score. The model with highest QMean is selected for further structure validation using PROCHECK [19] and Verify3D [20]. After validation, the structure was used for further docking analysis.

Macromolecular Docking

In order to study the protein-protein interaction between WNT3A and its receptor protein FZD4 (PDB id:5BPB), a macromolecular docking was carried out using Z-Dock module in Accelrys Discovery Studio v4.1. The Dock Proteins (ZDOCK) protocol provides rigid body docking of two protein structures using the ZDOCK algorithm developed by Chen and Weng 2003, as well as clustering the poses according to the ligand position [21]. The top poses were ranked based on the ZRANK score and then refined by the RDock, a refinement stage algorithm developed by Li *et al.*, 2003 [22,23]. The RMSD of the top poses from each cluster was calculated with XWNT8/FZD as a

reference molecule. Then the pose with the lowest E_RDock score and RMSD which represents the near native pose was chosen for analysing the interface region of the ligand and receptor.

Molecular Docking

With bioactive compounds identified from *Andrographis paniculata* as ligands, a molecular docking was carried out for WNT3A-FZD4 complex in the interface region using CDocker. CDocker is a grid based molecular docking algorithm that employs CHARMM forcefield for carrying out molecular dynamics. Initially random ligand conformations are generated from ligand structure through high temperature molecular dynamics, followed by random rotations. The random conformations are later refined by Grid-based (GRID1) simulated annealing and a final grid-based or full forcefield minimization. The using of force field for the docking in this algorithm enables more reliability of the final docking results. The ligand-receptor docking was carried out with default parameters in CDocker protocol [24].

3. RESULTS AND DISCUSSION

For WNT3A there is no crystallographic data available in the PDB due to hydrophobic nature of the WNTs. The structure was predicted via Template based modeling using SWISS-Model workspace. The sequence of the Human WNT3A was retrieved from UniProtKb with the ID: P56704 (WNT3A_HUMAN) in FASTA format. The sequence was submitted to an interactive modeling workspace in SWISS-Model (available at <http://swissmodel.expasy.org/interactive>). A template search for the target sequence was carried out using BLAST against the SWISS-model template library. Based on the BLAST hits for templates, Protein WNT8 (4F0A_B) with query coverage of 84% and sequence identity of 42.52 was chosen for template based modeling. A pairwise sequence alignment for the target and the template sequence was carried out using Clustal. Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. The global and per-residue model quality has been assessed using the QMEAN scoring function. Three models were built based on the templates and the model with highest QMEAN value was selected. The model validation was carried out using the SAVES online server. The Verify3D measures the compatibility of an amino acid sequence with a 3D protein structure was found to be 104.77 that come within the expected range of a good model. Fig.1 shows the line plot obtained from the Verify 3D score of each amino acid sequence in the modelled structure. The plot shows that 88.81% of the residues had an averaged 3D-1D score ≥ 0.2 (At least 80% of the amino acids have scored ≥ 0.2 in the 3D/1D profile).

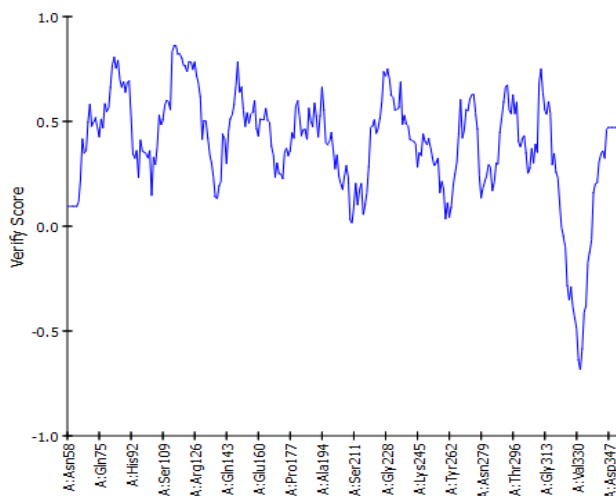


Fig.1 Line plot of Verify3D score of the residues in the predicted model of WNT3A

The stereo chemical properties of the modelled protein was validated using PROCHECK online server that generated a Ramachandran plot for the structure with 88.8% residues in the most favoured region (Fig.2a).

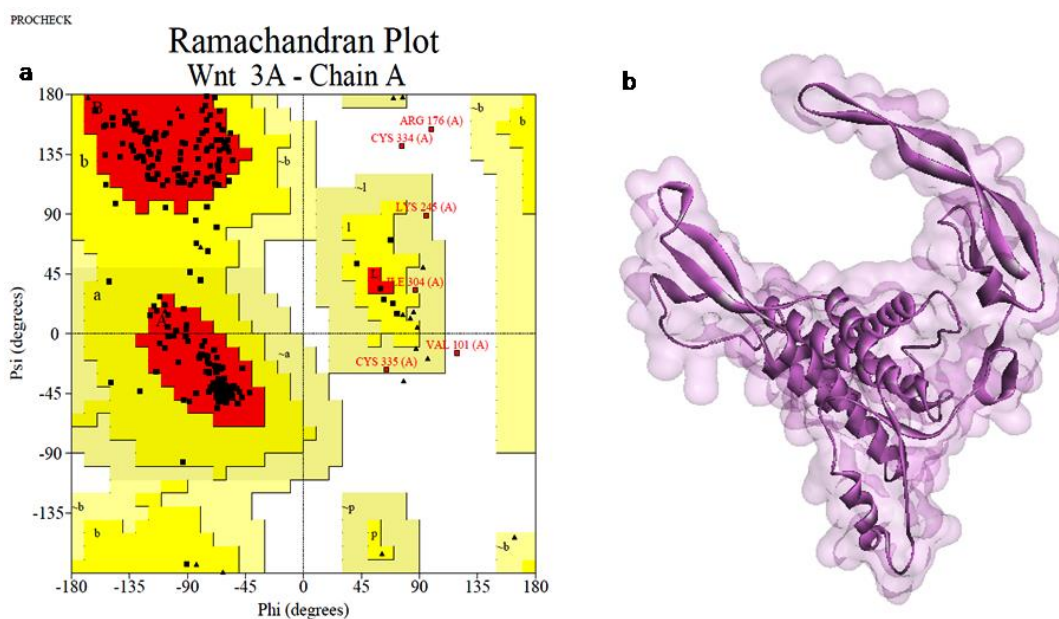


Fig.2 - a- Ramachandran plot for the predicted model using PROCHECK.b –Homology model of WNT3A.

The protein-protein interaction analysis was carried out between WNT3A and FZD4 as the binding of WNT to FZD protein is the crucial event that activates the WNT/ β -catenin signaling pathway. The X-ray crystallographic structure for FZD4 was retrieved from Protein Data Bank with the id: 5BPB and was used as the Receptor. The modelled structure of WNT3A (Fig.2b) was used as ligand in the macromolecular docking analysis using ZDock module in Accelrys Discovery Studio v4.1 with angular step size set to 15 and leaving the rest to default parameters. ZDock performs a full rigid-body search of docking orientations between two proteins based on Fast Fourier Transform correlation technique that is used to explore the rotational and translational space of a protein-protein system and clusters the poses according to the ligand position. Initially 3600 poses were generated and the top 2000 poses were ranked based on the ZDock score. The refinement of the poses was carried out using RDock module. This helps in optimizing and scoring of the docked poses by ZDock algorithm using the CHARMM-based energy minimization. The best E_RDock value of -9.37089Kcal/mol was obtained for the pose 611 from cluster 3 and had the ZDock score of 12.64. The calculated RMSD value for the binding interface region of the poses with XWNT8/FZD as reference protein showed that the pose 611 has the least value of 15.941Å which corresponds with least ZRank value of -25.849. The pose appears to look like the WNT3A grasp the FZD with the extended arms like groove making contact with three sites in FZD4 (Fig.3). The analysis of the interface region in the complex showed the non-bond interactions between the residues of the receptor and the ligand proteins. The Hydrogen bond formed between the FZD4 (A-Chain) and WNT3A (B-Chain) in the interface region consisted of the following residues A:GLN87:HE21 - B:ILE72:O, B:GLN75:HE22 - A:GLN87:O, B:ARG82:HH11 - A:GLY89:O, and B:ARG225:HH21 - A:ILE86:O (Fig.4). The binding interface region can be used as active site for drug intervention while targeting WNT3A-FZD4 with small molecule inhibitors (Table 1).

Table 1- Non-bond interactions obtained in the WNT3a-FZD4 docked complex

Residue name	Distance Å	Category	Type	From Chemistry	To Chemistry
B:LYS71:NZ - A:GLU134:OE2	4.24	Electrostatic	Attractive Charge	Positive	Negative
B:LYS202:NZ - A:GLU76:OE2	4.49	Electrostatic	Attractive Charge	Positive	Negative
B:LYS204:NZ - A:ASP74:OD2	5.55	Electrostatic	Attractive Charge	Positive	Negative
A:GLN87:HE21 - B:ILE72:O	2.84	HBond	Conventional	H-Donor	H-Acceptor
B:GLN75:HE22 - A:GLN87:O	2.99	HBond	Conventional	H-Donor	H-Acceptor
B:ARG82:HH11 - A:GLY89:O	1.99	HBond	Conventional	H-Donor	H-Acceptor
B:ARG225:HH21 - A:ILE86:O	2.17	HBond	Conventional	H-Donor	H-Acceptor

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A:THR83:CB - B:ASP223:OD1	3.48	HBond	Carbon HBond	H-Donor	H-Acceptor
A:PRO150:CD - B:CYS329:O	3.09	HBond	Carbon HBond	H-Donor	H-Acceptor
B:GLN75:OE1 - A:TYR88	2.98	Other	Pi-Lone Pair	Lone Pair	Pi-Orbitals
A:PRO84 - B:ILE72	5.16	Hydrophobic	Alkyl	Alkyl	Alkyl
B:LYS202 - A:LEU77	5.20	Hydrophobic	Alkyl	Alkyl	Alkyl
B:CYS205 - A:LEU71	4.33	Hydrophobic	Alkyl	Alkyl	Alkyl
B:CYS212 - A:LEU71	5.49	Hydrophobic	Alkyl	Alkyl	Alkyl
B:ARG225 - A:MET52	4.73	Hydrophobic	Alkyl	Alkyl	Alkyl
B:ARG225 - A:ILE86	4.78	Hydrophobic	Alkyl	Alkyl	Alkyl
B:LYS326 - A:LEU122	3.98	Hydrophobic	Alkyl	Alkyl	Alkyl
B:CYS329 - A:PRO150	4.77	Hydrophobic	Alkyl	Alkyl	Alkyl
B:TRP218 - A:LEU77	5.11	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl
B:TRP218 - A:LEU77	5.16	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl

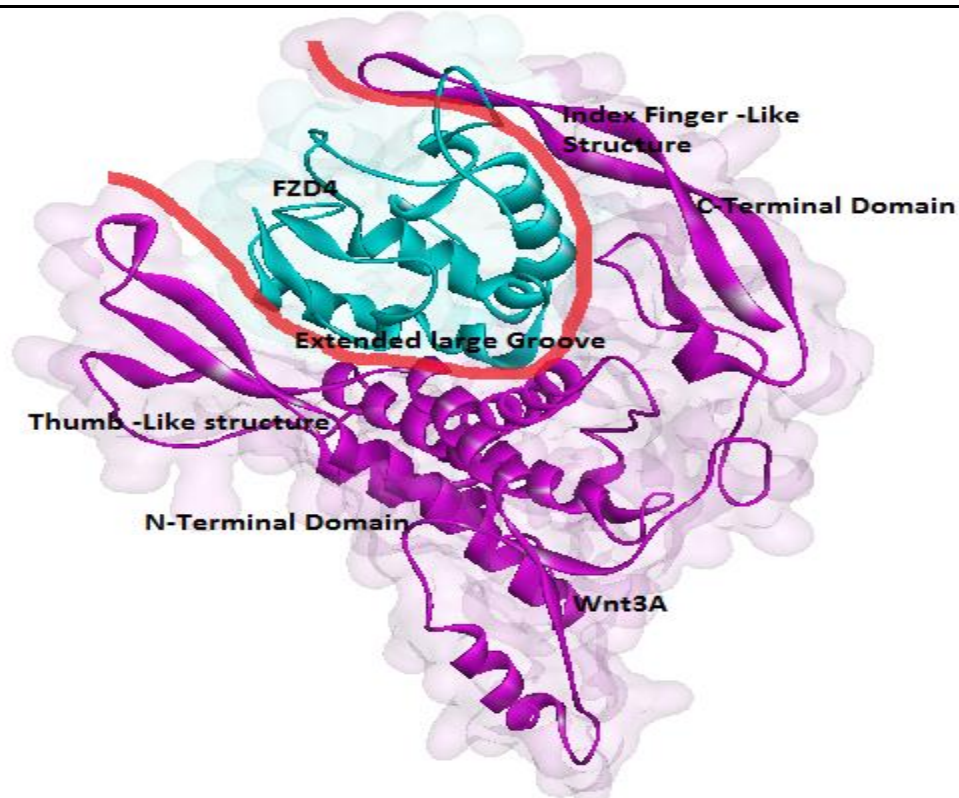


Fig.3 Protein-protein docking of FZD4 (Receptor – Cyan ribbon model) & WNT3A (Ligand – Magenta ribbon model). FZD4 fits into the large groove forming a palm with extended “Thumb” and “index” finger of WNT3A. WNT3A binds to FZD4 by grasping the Cysteine Rich Domain of Frizzled protein.

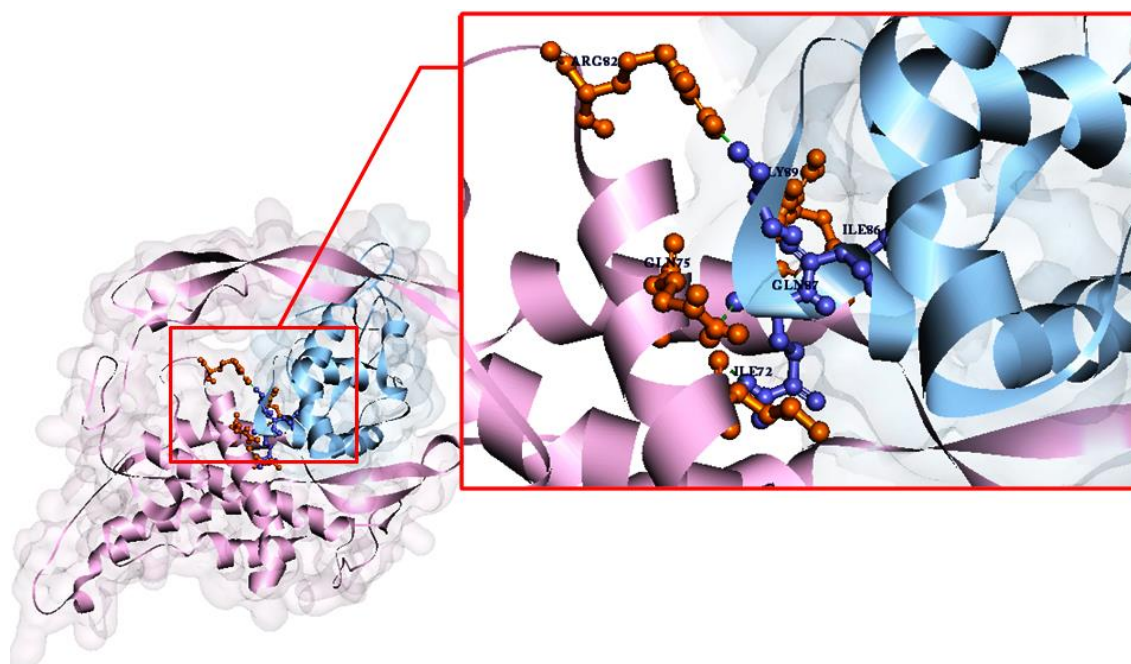


Fig.4- Hydrogen bond interactions observed in the binding interface region of WNT3A and FZD4.

An earlier GC-MS analysis on the methanol leaves extract of *Andrographis paniculata* identified three compounds, of which only two compounds passed the in silico ADMET analysis. The two compounds were used as ligands in a subsequent molecular docking analysis using CDocker against the modelled WNT3A-FZD4 complex. Discovery Studio offers a protocol for predicting the active site based on the cavity on the receptor surface. The active site covering the interface region of the WNT3A-FZD4 complex in the groove with the extended thumb structure on WNT3A was chosen for the docking analysis (Fig.5).

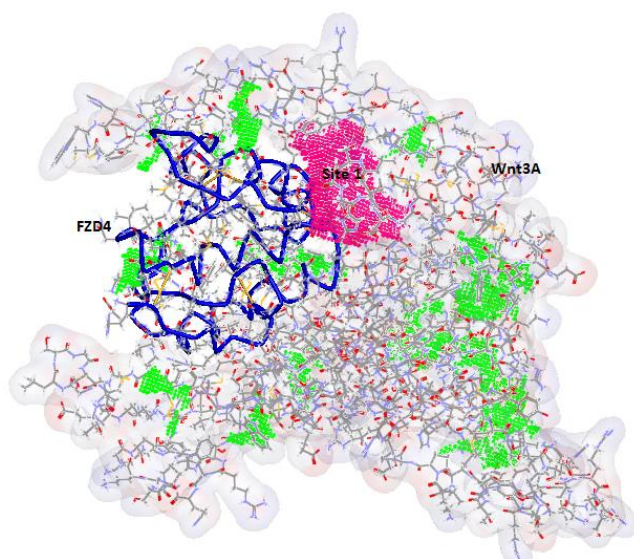


Fig.5 Active site predicted based on the receptor cavity on the surface of the WNT3A-FZD4 complex. Site 1(Magenta) covering the groove where FZD4 (Blue tube) made contact with WNT3A (represented as surface created over atom lines with Carbon atom in grey)

Table 2 shows that compound 1-[3-(Cyclohexylamino)propyl] guanidine had the lowest (best) CDocker Interaction energy of -36.1874Kcal/mol. The binding energy calculated based on the distance dependent dielectrics was found to be -88.7141Kcal/mol. The compound formed interaction with the residues Pro162 and Gly119 of FZD4 and the residue Glu325 of WNT3A (Fig.6a). The second compound 6-oxa-3-thiaoctanoic acid had the lowest CDocker energy value of -26.9737Kcal/mol. It formed 2 conventional hydrogen bonds with the residue Arg82 of WNT3A (Fig.6b). Arg82 formed a hydrogen bond with Gly89 of FZD4 in the interface region (Fig.4). Based on the docking result, it can be concluded that the compound 6-oxa-3-thiaoctanoic acid can be a promising lead in designing a small molecule inhibitor for WNT3A-FZD4 complex.

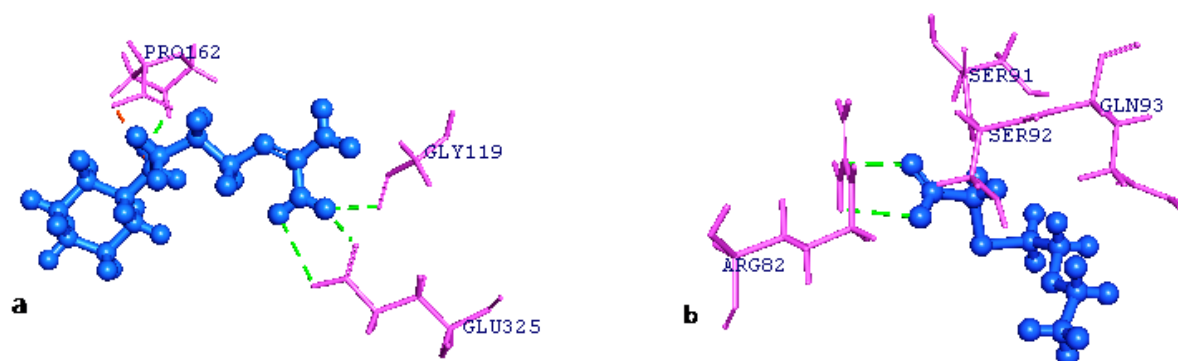


Fig.6 – Receptor-Ligand interaction between Bioactive compounds (Blue ball and stick) from *Andrographis paniculata* and WNT3A-FZD4 complex (interacting residues in Magenta stick). (a) 1-[3-(Cyclohexylamino)propyl] guanidine, (b) 6-oxa-3-thiaoctanoic acid.

Table 2 – Docking results obtained for the bioactive compounds from *A.paniculata* against WNT3a-FZD4 complex

Ligand	CDocker Energy	CDocker Interaction Energy	Favorable	HBond	Binding Energy
1-[3-(Cyclohexylamino)propyl] guanidine	-25.8461	-36.1874	5	4	-88.7141
6-oxa-3-thiaoctanoic acid	-26.9737	-28.3285	8	7	-56.5799

4. CONCLUSION

Binding of WNT to the FZD receptors activates the WNT signaling pathways resulting in the disassociation of the β -catenin destructive complex increasing the cytosolic concentration of β -catenin which then enters the nucleus and activates transcription factors responsible for cell growth and proliferation resulting in tumorigenesis. This made the WNT signaling an attractive target for HCC therapy. The protein-protein interaction analysis on WNT3A-FZD4 provided the molecular insight on the residues in the binding interface region which can be used as active site for designing small molecule inhibitors. The compounds identified from the leaves methanol extract of *Andrographis paniculata* has good binding affinity towards the interface region of the WNT3A-FZD4 complex and can be studied further for their inhibitory activities.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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