

CANONICAL WNT SIGNALING IN **PANCREATIC DUCTAL ADENOMACARCINOMA**

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By

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CERTIFICATE

This is to certify that the project entitled “**Canonical Wnt signaling in Pancreatic Ductal Adenocarcinoma**” submitted by Shashi Kumar of Biotechnology branch, National Institute of Technology, Rourkela, for the degree of bachelor of technology is a record based on the result obtained in the bonafide research work carried out by him under my guidance and supervision.

To the best of my knowledge, the matter embodied in the project has not been submitted to any other university/institute for the award of any degree or diploma.

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ABBREVIATIONS

Wnt	-Wntless
WLS	-Wnt Ligand Secretion
IWP-2	-Inhibitor of Wnt Production-2
Asp	-Aspartic acid
Thr	-Threonine
Phe	-Phenylalanine
Lys	-Lysine
Gln	-Glutamine
His	-Histidine
Arg	-Arginine
Asn	-Asparagine
Ser	-Serine
Leu	-Leucine
Ile	-Isoleucine
Cys	-Cysteine
Pro	-Proline
Met	-Methionine
Val	-Valine
Trp	-Tryptophan
Glu	-Glutamic acid
Tyr	-Tyrosine
Gly	-Glycine
WIF-1	-Wnt inhibitory factor 1
sFRP	-Secreted frizzled-related protein
Dkk	-Dickkopf
SOST	-Sclerostin
KRAS	-Kirsten rat sarcoma viral oncogene homolog
SEER	-Surveillance, Epidemiology, and End Results Program

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Abstract

Cancer is one of the major diseases causing worldwide death of human, related to some different epigenetic dysregulation. Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer due to its rapid clinical progression & resistance to therapies. Recently, PDAC incidences have risen, while 5-year overall survival is about 6%. A primary complicating factor is the remarkable genetic and epigenetic heterogeneity of pancreatic cancer. Vividly, these highly variable alterations also define a core set of twelve signaling pathways along with cellular processes in most of PDAC tumors, including Wnt signaling also. One of the major dysregulation is overexpression of Wnt Proteins. There are 19 set of Wnt proteins found. In this project, novel IWP-2 inhibitor will focus a new light on cancer therapy. The findings indicate that WNT7B can serve as a primary determinant of differential Wnt/b-catenin activation in PDAC. Thereby, disrupting the interaction between Wnt ligands and their receptors may be suitable approach for therapeutic modulation of Wnt/b-catenin signaling in PDAC and other cancer contexts where the Wnt activation is mediated through ligand expression rather than mutations in members of canonical pathway.

CHAPTER 1

INTRODUCTION

Introduction

Cancer is a disease where there is abnormal cell division without control and these cells invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start. For example, cancer that begins in the colon is called colon cancer; cancer that begins in melanocytes of the skin is called melanoma and the cancers that affect pancreatic system are known as Pancreatic Ductal Adenocarcinoma. Cancer is often identified at a later phase. Therefore removing tumor or the organ by surgery is a tedious task. A report from the nation's leading cancer organization, National Cancer Institute shows that rates of death in the United States in 2014. There are 1,665,540 (does not include non-melanoma skin cancers) new cases for cancer with 585,720 deaths.

Pancreatic ductal adenocarcinoma (PDAC) is the most usual type of pancreatic malignancy. PDAC is different from other cancers because of the biological barrier the tumor forms around itself. Pancreatic cancer is known to be the fourth leading cancer killer in the USA. Altogether, just 6% of patients survive 5 years after diagnosis is done. In the year 2012, it has been estimated that there will be 43,920 new diagnoses of pancreatic cancer and 37,390 deaths will attribute due it. Patients whose disease is recognized at an early period have a better scope of survival for long term. Currently there are no early detection tests available for this PDAC. In such cancer, pain occurs in the upper part of abdomen which radiates typically to the back as observed in tail of the pancreas or carcinoma of the body. Also there is poor appetite or nausea along with severe vomiting. Some others symptoms include significant weight loss as well as Diarrohea with loose stools. It generally obstructs the common bile duct as it runs through the pancreas. Also it can cause pale colored stool [3]. Over 30 years, NCI-supported laboratory scientists have been studying a gene known as KRAS (Kirsten rat sarcoma viral oncogene homolog), the genetic driver of pancreatic cancer initiation and propagation. However, presently no therapeutic solutions to KRAS mutations have been developed. Since the death rate are significant, the identification of risk factors & genetic changes, achieving more understanding of the metastatic process, developing better procedures of early detection and treatment offer are the means of better reducing PDAC [1].

KRAS: The KRAS gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the prospective to cause normal cells to convert cancerous. The KRAS gene is in the Ras family of oncogenes, which also comprises two other genes: HRAS and NRAS. The proteins formed from these three genes are GTPases. These proteins play significant roles in cell differentiation, cell division, and the self-destruction of cells (apoptosis). The KRAS gene delivers instructions for making a protein called K-Ras that is involved primarily in regulating cell division. As part of a signaling pathway known as the RAS/MAPK pathway, the protein relays signals from outside the cell to the cell's nucleus. These signals instruct the cell to grow and divide or to mature and take on specialized functions (differentiate). The K-Ras protein is a GTPase, which means it converts a molecule called GTP into another molecule called GDP. The K-Ras protein acts like a switch, and it is turned on and off by the GTP and GDP molecules. To transmit signals, the K-Ras protein must be turned on by attaching (binding) to a molecule of GTP. The K-Ras protein is turned off (inactivated) when it converts the GTP to GDP. When the protein is bound to GDP, it does not relay signals to the cell's nucleus [2].

Objective

1. To study about Wnt Canonical Pathway and overexpression of different Wnt protein in different cancers.
2. To find the inhibitors for the specific target protein i.e. Wnt 7B responsible for Pancreatic Ductal Adenocarcinoma.
3. To carry out molecular docking of inhibitors with Wnt 7B as target protein and assessing binding affinity.
4. To study the interacting residues as well as the interacting bonds between of Wnt7B and the docked inhibitors.
5. To asses toxicity of the small inhibitor molecules.

CHAPTER 2
LITERATURE REVIEW

Wnt Signaling Pathway

The Wnt signaling pathways are assembly of signal transduction paths prepared of proteins that makes the signals flow from outside to inside of a cell over cell surface receptors. Signaling is a way of the system communication among living cells by processing biological information that governs basic cellular activities and co-ordinate cell actions. The signals are transmitted through signaling molecules, and this process ends up with altering of gene transcription in the nucleus resulting in many cellular processes such as differentiation and proliferation. Following three Wnt signaling pathways are considered: a) the Canonical Wnt pathway; b) the Non-canonical planar cell polarity pathway; c) the non-canonical Wnt or Calcium pathway. All 3 Wnt signaling pathways are stimulated because of the binding of a Wnt ligand with a Frizzled family receptor that permits the biological signal to the protein Dsh in the interior of cell [4].

The pathway named Canonical Wnt causes regulation of gene transcript; the non-canonical planar cell polarity pathway controls the cytoskeleton which is accountable for the shaping of the cell, and non-canonical Calcium inside the cell is controlled by Wnt/calcium pathway. Wnt signaling pathways practice either nearby cell-cell communication (paracrine) or through same-cell communication (autocrine). They are greatly evolutionarily conserved, which means they are parallel across many species i.e. from small bugs to humans. Clinical significance of Wnt signaling pathway has been established by changes that lead to a variety of diseases, counting breast and prostate cancer, glioblastoma, type II diabetes and many more [4].

Canonical Wnt Pathway: Generally, vital difference between Canonical Wnt pathway and Non-canonical is that a canonical pathway includes the protein β -catenin whereas a non-canonical pathway works self-sufficiently. A hallmark of Canonical Wnt signaling pathway activation is the enhanced level of cytoplasmic β -catenin protein. Stabilization of cellular β -catenin, leading to elevated protein levels and constitutive gene activation, has been supposed as an important step in many human cancers [4]. Although during the last few years several novel molecular data have contributed to the understanding of the complexity of the Wnt signaling pathway, many of the underlying mechanisms still remain unknown. Both genetic, epigenetic and expression alterations of molecules in the Wnt signaling pathway are characteristic for human solid tumors. Therefore, a future perspective, when it comes to anti-cancer therapeutics, would be to block the β -catenin-Tcf complex and thereby transcription of Wnt target genes[2].

Mechanisms: The Canonical Wnt pathway (or Wnt/ β -catenin pathway) is the Wnt pathway which reasons a gathering of β -catenin within the cytoplasm along with its ultimate translocation into the nucleus acting as a transcriptional co-activator of transcription factors that fit with the T-cell Factor and Lymphoid Enhancing factor family. In case, there is no Wnt signaling, the β -catenin will not collect in the cytoplasm as a destruction complex would generally degrade it. This degradation complex comprises proteins which are: a) Adenomatosis Polyposis Coli (APC), b) Axin, c) protein Phosphatase 2A (PP2A), d) Casein Kinase 1 α and, e) Glycogen Synthase Kinase 3 (GSK3). It cuts down β -catenin by directing it for ubiquitination, which then sends it to the proteasome to get digested [5].

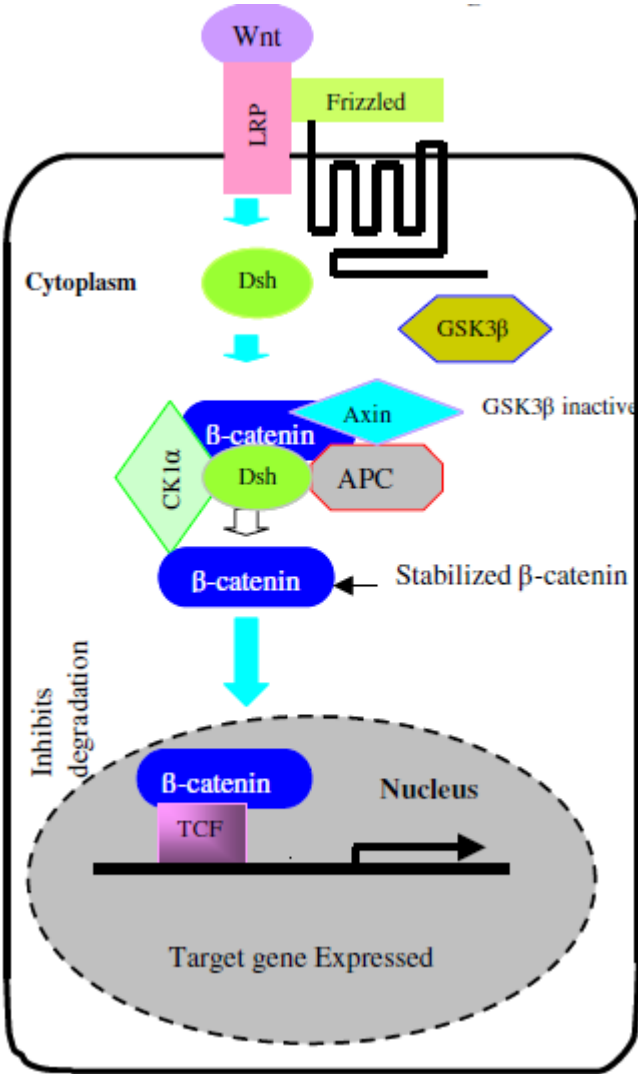


Figure 1. Wnt signaling in cancer [2].

1. In case, the Wnt fixes Fz and LRP-5/6, this leads to the disruption of destruction complex function. It is because of the translocation of both the destruction complex to the plasma membrane caused by Wnt and a negative regulator of Axin.
2. The negative regulator grows regionally in the cytoplasmic tail of LRP- 5/6. Phosphorylation due to other proteins in the destruction complex afterwards combines this tail to the Axin.
3. Axin converts de-phosphorylated, its stability along with levels are diminished.
4. The Dsh turn into activated through phosphorylation and its DIX and PDZ domains prevent the GSK3 activity of the destruction complex.
5. This permits β -catenin to gather and confine to the nucleus and afterwards bring a cellular response through gene transduction beside the TCF/LEF transcription factors.

Therapeutic Approach by targeting Wnt/ β -catenin Signaling Pathway: The main target of all these strategies is to check the β -catenin translocation to nucleus or inhibits its expression or enhances its proteosomal degradation process and thus prevent the expression of Wnt target genes. Some of the approaches includes: i) DKK proteins as anticancer drugs; ii) Regulation at the protein level; iii) Activation of the proteosomal degradation process of β -catenin; iv) Reducing the sign of β -catenin by RNA interference and antisense [6].

Inhibitors of Wnt Signaling:

- i. Wnt inhibitory factor 1 (WIF-1):** It is a extracellular factor which is soluble, binds to Wnt and stops it from interacting with the frizzled receptor.
- ii. Secreted frizzled-related protein (sFRP):** It is a inhibitor which is soluble, binds to Wnt and stops it from associating with the frizzled receptor.
- iii. Dickkopf (Dkk):** Kremen & the lipoprotein receptor-related protein (LRP) co-receptor LRP5/6 binds to it and causes the complex to develop internally. Thus, inactivating the Wnt signaling pathway.
- iv. Sclerostin (SOST):** Wnt signaling is inhibited by it through binding to the lipoprotein receptor-related protein (LRP) co-receptor LRP5/6. Osteoclasts produces it for inhibiting the differentiating and proliferation of osteoblasts.

Wnt signaling pathway generally acts by switching off the GSK-3 β - dependent degradation pathway, thereby making β -catenin to collect in the cytosol and thus to translocate in the nucleus to active transcription of the Wnt target Genes.

Wnt proteins in Various Cancer: The Wnts are secreted glycoproteins & generally comprise a large family of nineteen proteins in human beings thereby hinting to a daunting complexity of signaling regulation, function and biological outputs. Generally, Wnt proteins regulate a dizzying array of cellular processes which include cell fate determination, primary axis formation, motility and organogenesis and in recent researches; this pathway has been implicated in the stem cell renewal. Wnt signaling is the causative factor for a number of pleiotropic human pathologies. Most importantly, these pathologies include cancers of breast, skin and colon, skeleton defects & human birth defect disorders including most common human neural tube closure birth [8].

The Wnt4, Wnt5a and Wnt11 ligands also play a critical role in the early axis formation via the non-canonical, though recently Wnt11 has shown to play a critical role in the early axis formation. The activation of mutation of Canonical Wnt signaling pathway, ultimately lead to the stabilization and accumulation of B-Catenin in the nucleus of a cell. Actually, the mis-regulation of Wnt signal causes many development defects. Some of the Wnt proteins which are responsible for different cancers in human are: a) A proportion of human breast carcinomas express high levels of Wnt2, Wnt-5A, and Wnt-7B; b) Canonical Wnt signaling is only mediated when both the Fz and LRP are complexed with Wnt. Most of Wnt proteins can bind to multiple Fzs and vice versa, suggesting the redundancy in vivo; c) In PDAC cell lines, there is an overexpression of Wnt-7B and it serves as a primary determinant of differential Wnt/B-Catenin activation in the PDAC; d) It has been also found that gene expression profiling correlates an overexpression of Wnt-5A and the concomitant increase in the PKC activity with human melanoma progression [8].

Wnt7B: WNT7B is one of the members of Wnt gene family, which consists of structurally related genes and encoding the secreted signaling proteins. Wnt7B is expressed at high concentrations in the regions of active hyperplasia, metaplasia, and fibrotic change in the idiopathic pulmonary fibrosis patients. Pdgf signaling potentiates the Wnt2 (show WNT2 Antibodies)-Wnt7B signaling for promoting the high levels of Wnt activity in mesenchymal progenitors which is required for proper development of endoderm-derived organs, like the lung. Wnt7B gene is a member of the WNT gene family, consisting of structurally related genes and encoding secreted signaling proteins. Such proteins have therefore been implicated in oncogenesis and in several other developmental processes, which includes regulation of cell fate and patterning, during the process of embryogenesis. Among other members of the human WNT family, this gene product is most likely to WNT7A protein.

IWP-2 (Inhibitor of Wnt Production-2)

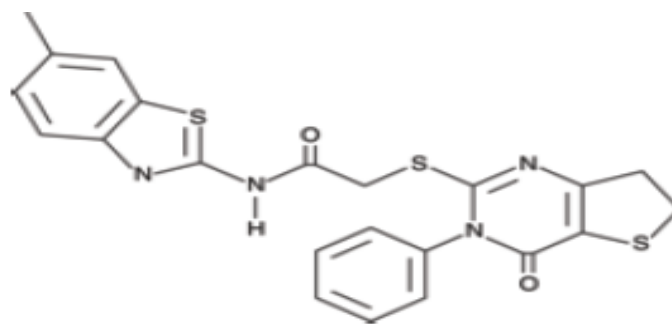


Figure 2. Structure of Inhibitor of Wnt Production-2 (from PubChem).

Wnt signaling proteins are the minor secreted proteins which are dynamic in embryonic development, in tumor genesis and in tissue homeostasis. The Wnt proteins fix to receptors on the cell surface, thus beginning a signaling cascade which clues to β -catenin activation of gene transcription. IWP-2 is one of the inhibitor of Wnt production which impairs Wnt pathway activity in vitro with an IC value of 27 nM. IWP-2 also inactivates the Porcupine, a membrane bound O-acyltransferase which is accountable for palmitoylating Wnt proteins, again which is vital for their signaling ability and the discharge. At 5 μ M, IWP-2 has shown to block Wnt-dependent phosphorylation of frizzled co-receptor and the scaffold protein Dishevelled, prevents the accumulation of β -catenin. This compound has been therefore used to suppress embryonic stem cell self-renewal and thus decrease cancer cell migration, proliferation, & invasion.

WLS (Wntless Wnt Ligand Secretion mediator)

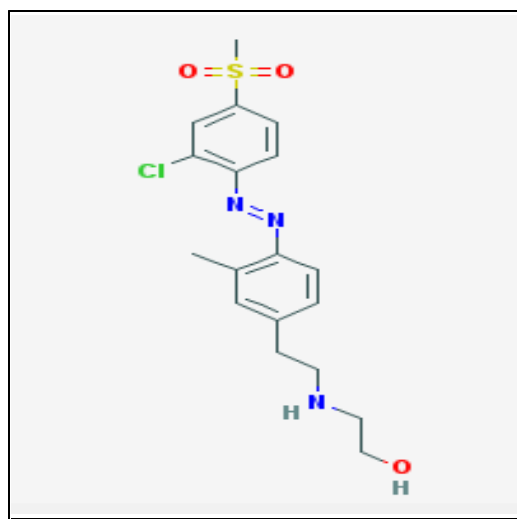


Figure 3. Structure of Wntless Wnt Ligand Secretion mediator molecule (from PubChem).

WLS (Wntless Wnt ligand secretion mediator) is another protein-coding gene. Diseases that are associated with WLS include chronic intestinal vascular insufficiency, and the focal dermal hypoplasia, and among its related different super-pathways are Asparagine N-linked glycosylation and the Signaling through GPCR. GO annotations related to this gene include the Wnt-protein binding & the signal transducer activity.

Canonical Wnt-β-catenin signaling in pancreatic ductal adenocarcinoma.

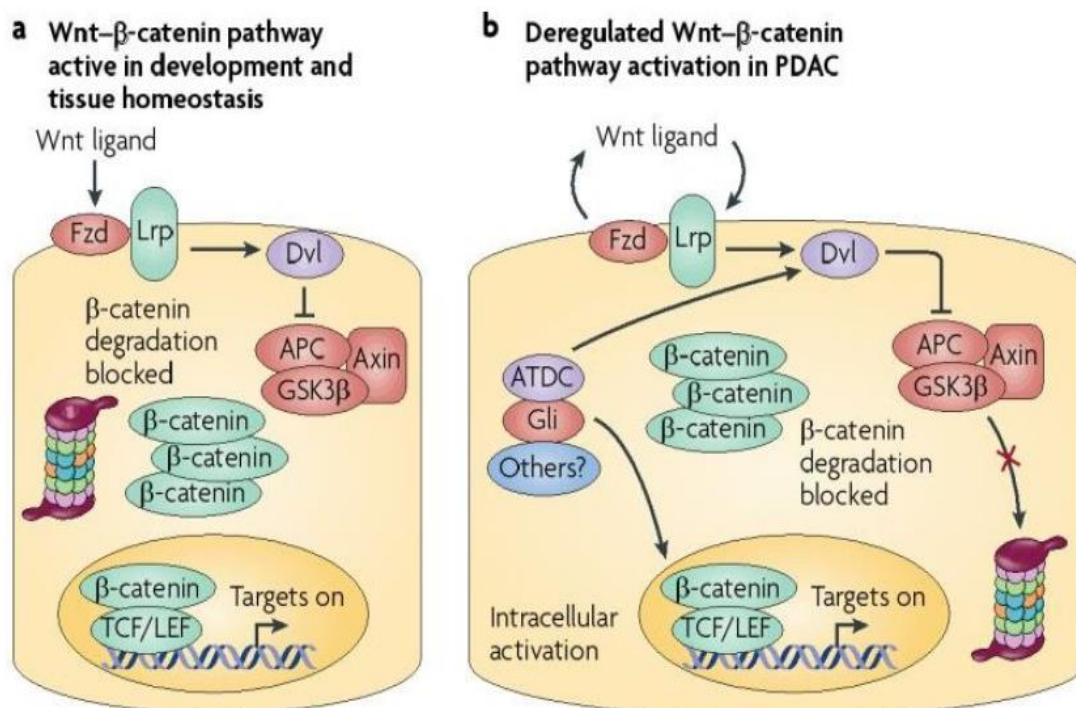


Figure 4. Canonical Wnt-β-catenin signaling in Pancreatic Ductal Adenocarcinoma [8].

In normal development, canonical Wnt-β-catenin signaling rests on secreted ligands (Wnt ligands) that initiate receptors (Frizzled (Fzd)-Lrp complex) that block the proteosomal degradation of β-catenin promoted by the destruction complex (comprising adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β), axin, and other such proteins) through inhibition of Dishevelled (Dvl). β-catenin accumulation is habitually detected in pancreatic ductal adenocarcinoma (PDAC). Accumulated β-catenin can translocate into the nucleus and initiate target genes in performance with TCF/LEF co-factors. Currently, the dominant mechanism of persistent β-catenin accumulation and activity in PDAC is unclear. There is evidence for both autocrine (owing to epithelial-derived Wnt ligands) and cell-autonomous activation (through Gli

signaling and genes such as ataxia telangiectasia group D-associated (ATDC), which activates Dvl). There may also be assistances from the stromal cells and extracellular matrix that may encourage β -catenin accumulation [8,9].

Bioinformatics & Computational Biology

Very easy drug designing has been recently paved in drastic ways by the enhancement of Bioinformatics and Chemo-informatics. The potential to speed up drug discovery processes has been shown by Computational biology & Bioinformatics. This reduces the cost of the processes and thus changes the way of designing of drugs. The drug designing processes involving various methods of identifying novel compounds is facilitated and accelerated by the rational drug design. The docking of the drug molecule / ligand / inhibitor with the target is one such advanced method. The site of action is known as the site where the drug binds. This is ultimately responsible for the pharmaceutical effect in the target.

Two molecules bind to each other in the 3D space by the method of Docking. The free enthalpy of the binding ligand is computed by the use of regression based or knowledge based scoring functions. Several tools, softwares & servers are available for calculations of docking. Docking may be rigid, flexible, & semi-flexible. Different databases are available to store macromolecular 3D structure and ligand structure, extracted from NMR co-ordinates. These are used for simulations and docking. Computational biology or the In Silico approach is day by day developing in more refine manner. This has become a very promising field and with the help of this the time and cost of the biological work related to drug discovery, molecular interaction is reduced.

CHAPTER 3

TOOLS and METHODS

TOOLS AND METHODS

1. Computer Atlas of Surface Topology of Proteins (CASTp): It is a Bioinformatics tool which is an online reserve for identification of some geometric possessions of protein like delineating, locating, and thus computing concave surface regions on 3D structures of proteins attained from the PDB also to study the surface features, functional regions and an active site of proteins. Also, there are measurements of the number of mouth openings (of cavity or pocket), circumference of mouth lips (of void or pocket), area of the openings (of void or pocket), in together Molecular surface (MS) and Solvent Accessible (SA) of each of the pocket.

There can also be request calculation for a certain molecule. Either the results will be exposed on the screen or will be sent by email. The result which is sent by email includes measured parameters for cavities, pockets and mouth openings, along with the listing of wall atoms and the mouth atoms for separate pocket. Additionally, the PyMOL plugin which is downloadable helps in visualizing the pocket of our interest.

2. Phyre and Phyre2 (Protein Homology/AnalogY Recognition Engine): These are web-based services for predicting the structure of protein that are allowed for the non-commercial usage. One of the maximum popular methods for the prediction of protein structure is Phyre cited over more than 1500 times. Other widely used methods like PSI-BLAST cannot generate reliable protein models whereas like other remote homology recognition techniques, it can regularly generate. A user-friendly interface is ensured for the users by the design of Phyre2 in expert in predicting the structure of protein.

3. PubMed: It is one such free search engine, which access primarily the abstracts on life sciences and different biomedical topic the MEDLINE database of references. The the database as part of the Entrez system of information retrieval is maintained by the United States National Library of Medicine (NLM) at the National Institutes of Health. Always free access is provided by this to Medline, NLM's database of citations and abstracts in the fields of medicine, nursing, dentistry, veterinary medicine, health care systems, and preclinical sciences. It links to the full-text articles found in PubMed Central or at publisher web sites, and other related resources. Provides an Advanced search, with Clinical Queries search filters, and Special Queries pages. It links to the related articles and provides discovery tools for other data that may be of interest. It also includes an automatic e-mailing of search updates, the ability for saving records, and filtering

of search results using "My NCBI". It brings a spell checker feature and links to NCBI molecular biology resources. It provides a record for an article prior to being indexed with MeSH® and added to MEDLINE or converted to out-of-scope status.

4. PubChem: It is a database consisting of chemical molecules with their activities against biological assays. National Center for Biotechnology Information (NCBI), which is a component of the National Library of Medicine maintains this system. This is a division of the United States National Institutes of Health (NIH). PubChem is accessible freely by a web user interface. FTP allows to download millions of compound structures and free descriptive datasets. This contains substance descriptions and small molecules with less than about thousand atoms and thousand bonds. More than eighty database vendors add to the growing PubChem database.
Website:- <http://pubchem.ncbi.nlm.nih.gov>.

5. OpenBabel GUI (file format converter): It is a chemical toolbox designed for speaking many languages of chemical data. Basic operation: a) Chemical objects which are presently molecules or reactions are converted from one file format to some another by OpenBabel. An alternative for a command line is this interface having the capabilities which are same. I) The type of the type of the input file is selected from the list which appears dropdown. II) The "." button is clicked and then the file is selected where contents are shown. III) The file and the output format is chosen in a very same manner. The output can be just displayed without being saved by not selecting an output file or by checking "Output below only..". IV) The "Convert" button is clicked. The number of molecules converted is given in the message window which is below the button, and the contents of the output file are shown. If multiple molecules are allowed by the output format then all such molecules in an input file are converted by default.

6. AutoDock: It is a suite of docking tools which are automated. Thereby designed for predicting how small molecules are, like drug candidates or substrates, fix to known 3D structure receptor. **AutoDock Tools** is used for setting up and running AutoDock. It is a docking software which is automated and designed for predicting how molecules of small size, such as drug or substrates candidates generally fix to a known 3D structure receptor.

7. UCSF Chimera: It is one of the greatly extensible programs for visualization in an interactive manner & analyzing structures of molecules and other information that includes density maps, sequence alignments supramolecular assemblies, trajectories, conformational ensembles and

docking results. Animations and images of high quality can be thereby generated. Chimera comprises several tutorials and complete documentation, and thus can be downloaded without any charge for a government, academic, non-profit, or personal use. Resource for Biocomputing, Visualization, and Informatics, developed Chimera and National Institutes of Health (NIGMS P41-GM103311) funded it.

8. LigPlot+ v.1.4.5: It is a computer program which generates the schematic 2-D image of the docked protein–ligand complexes. The 3-D structure of the docked complex is the input in PDB file and the software produces their interacting residues and bonds. In the present study, LigPlot+ was used to identifying interacting residues as well as the interacting bonds between of Wnt7B and the docked inhibitors.

9. The OSIRIS Property Explorer: It helps in drawing of chemical structures & calculation of various drug-relevant properties (solubility, Toxicity Risk Assessment, Molecular Weight, Overall Drug-Score, etc.) when a valid structure is given. The predicted results are thus color coded and valued. Also the high risk properties of any effects which are not desirable like mutagenicity or a poor intestinal absorption are shown in red color. On the other hand, drug-conform behavior is indicated by a green color.

The logP value of a compound, which is the logarithm of its partition coefficient between octanol and water $\log(\text{coctanol}/\text{cwater})$, is a well established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values cause poor absorption or permeation. It has been shown for compounds to have a reasonable probability of being well absorb their logP value must not be greater than 5.0. Optimizing compounds for high activity on a biological target almost often goes along with increased molecular weights. However, compounds with higher weights are less likely to be absorbed and therefore to ever reach the place of action. The drugs should have a molecular weight below 450. In druglikeness property a positive value for the chemicals states that the molecule contains predominantly fragments which are frequently present in commercial drugs. The drug score combines druglikeness, cLogP, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug.

Drug score: It combines druglikeness, cLogP, logS, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug.

Steps For DOCKING using AutoDock Tools

1. First of all the macromolecule 3D structure was downloaded in .pdb format.
2. In case the structure was missing, “Phyre Server” tool was used.
 - The FASTA sequence was pasted in the dialog box.
 - The E-Mail ID was provided.
 - The Validated structure was generated and sent to mail ID.

[Display Settings:](#) FASTA

WNT7B [Homo sapiens]

GenBank: BAB68399.1

[GenPept](#) [Graphics](#)

```
>gi|15721869|dbj|BAB68399.1| WNT7B [Homo sapiens]
MHRNFRKWI FYVFLCFGVLYVKLGALSSVVALGANI ICNKIPGLAPRQRAICQSRPDAI IIVIGEGAQMGI
NECQYQFRFRGRWNC SALGEKTVFGQELRVGSREAAFTYAITAAGVAHAVTAACSQGNLSNCGCDREKQGY
YNQAEGWKGGCSADVRYGIDFSRRFVDARE IKKNARRLMNLHNEAGRKVLDRMQLECKCHGVSGSCT
TKTCWTTLPKFRVGHLLKEKYNAAVQVEVVRASRLRQPTFLRIKQLRSYQKPMETDLVYIEKSPNYCEE
DAATGSVGTQGRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNCCKFWCCFVKCNTCSERVEVFTCK
```



E-mail Address	skumar.gkg@gmail.com
Optional Job description	Wnt7B
Amino Acid Sequence 	MHRNFRKWI FYVFLCFGVLYVKLGALSSVVALGANI ICNKIPGLAPRQRAICQSRPDAI IIVIGEGAQMGINECQYQFRFRGRWNC SALGEKTVFGQELRVGSREAAFTYAITAAGVA HAVTAACSQGNLSNCGCDREKQGYYNQAEGWKGGCSADVRYGIDFSRRFVDARE IKK NARRLMNLHNEAGRKVLDRMQLECKCHGVSGSCTTKTCWTTLPKFRVGHLLKEKY NAAVQVEVVRASRLRQPTFLRIKQLRSYQKPMETDLVYIEKSPNYCEEDAATGSVGTQ GRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNCCKFWCCFVKCNTCSERVEVFTCK
	Or try the sequence finder (NEW!)
Modelling Mode 	Normal <input checked="" type="radio"/> Intensive <input type="radio"/>
	<input type="button" value="Phyre Search"/> <input type="button" value="Reset"/>

Figure 5. Windows for phyre server.

3. The possible Wnt inhibitor molecules were searched using PubChem tools. The ligands' similar structures were then downloaded.
4. The ligands' similar structures were then downloaded in .sdf format.
 - The downloaded .sdf format ligand was then, opened in Open Babel GUI software.
 - Here the .pdf format of the ligands was thus obtained.

STEPS in AutoDock Tools- 1.5.6rc3

1. First the Opening the Wnt macromolecule downloaded.
 - a. File > Read molecule > give .pdb file location.
 - b. Edit > delete water >
 - c. Edit > Add > Hydrogen > Polar Hydrogen
 - d. Edit > Add > Kollman Charges
 - e. Edit > Atoms > Assign AD4 type
 - f. File > save > save as .pdbqt
2. LIGAND
 - a. Ligand > input > open (only .pbf file)
 - b. Ligand > torsion tree > root to be chosen
 - c. Ligand > torsion tree > root to be detected
 - d. Ligand > output > save as .pdbqt
3. GRID
 - a. Grid > Macromolecule > chose (the protein) > select molecule
 - b. Grid > set map type > choose the ligand > select ligand
 - c. Grid > grid box (active site to be inhibited) – select the co-ordinate from .pdf file xyz.
 - d. Centre > on named atom (using CastP)

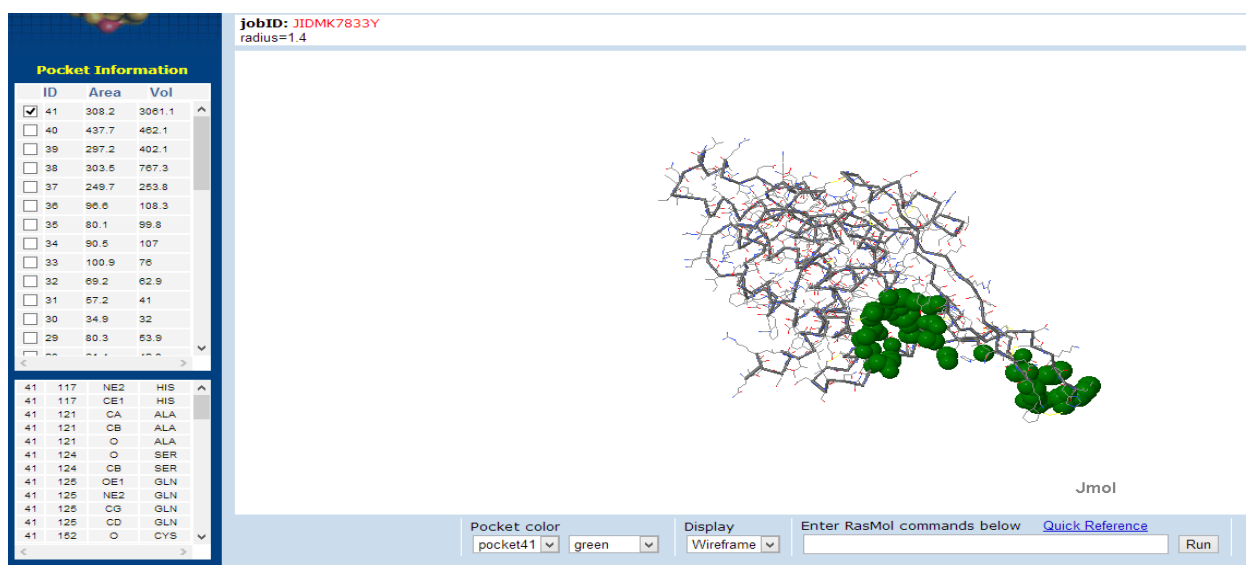


Figure 6. Windows for CastP.

Pocket Information			
ID	Area	Vol	
<input checked="" type="checkbox"/>	41	308.2	3061.1
<input type="checkbox"/>	40	437.7	462.1
<input type="checkbox"/>	39	297.2	402.1
<input type="checkbox"/>	38	303.5	767.3
<input type="checkbox"/>	37	249.7	253.8
<input type="checkbox"/>	36	96.6	108.3
<input type="checkbox"/>	35	80.1	99.8
<input type="checkbox"/>	34	90.5	107
<input type="checkbox"/>	33	100.9	76
<input type="checkbox"/>	32	69.2	62.9
<input type="checkbox"/>	31	57.2	41
<input type="checkbox"/>	30	34.9	32
<input type="checkbox"/>	29	80.3	53.9
<input type="checkbox"/>	28	71.1	10.0

41	117	NE2	HIS
41	117	CE1	HIS
41	121	CA	ALA
41	121	CB	ALA
41	121	O	ALA
41	124	O	SER
41	124	CB	SER
41	126	OE1	GLN
41	126	NE2	GLN
41	126	CG	GLN
41	126	CD	GLN
41	152	O	CYS

Figure 7. Pocket information by CastP.

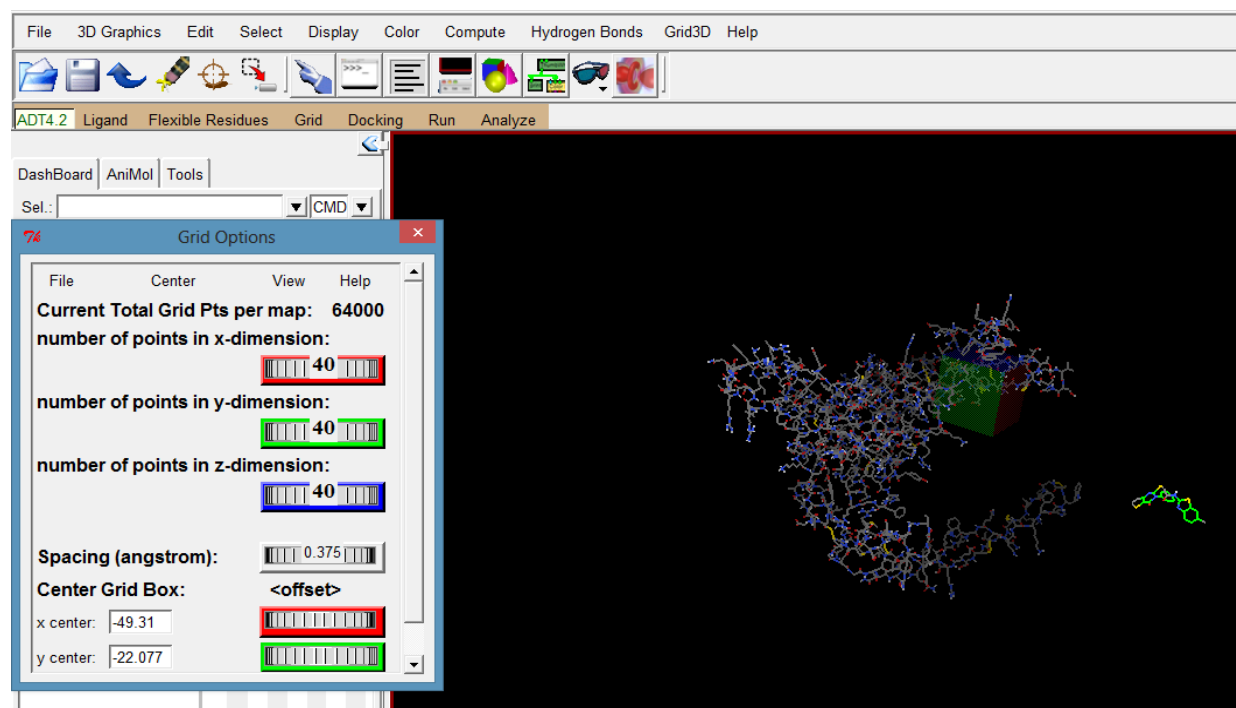


Figure 8. Windows for Docking.

- e. Enter the full name of the atom
- f. File > close saving the current.
- g. Output > save .gpf

4. RUN
 - a. Run > Run Autogrid > select the pathname for the program
 - i. Browse > C:/ > program (86) > The Scripps research Institute > Autogrid4
 - ii. Give the parameter filename by choosing the .gpf file saved
 - iii. Launch
5. DOCKING
 - a. Docking > Macromolecule > Rigid filename to be set > Macromolecule to be selected
 - b. Docking > ligand > choose
 - c. Docking > search parameter > Genetic Algorithm > accept
 - d. Docking > output > Lamarckian GA (4.2) > save as .dpf file (automatically saved)
6. RUN
 - a. Run > Run AutoDock > select the pathname for the program
 - i. Browse > C:/ > program (86) > The Scripps research Institute > Autodock4
 - ii. Give the parameter filename by choosing the .dlg file saved
 - iii. Launch

Here after few minutes, the docking gets completed. Analysis is done using a software tool know as Chimera.

1. Open the .dlg file in wordpad.
2. Now press Ctrl+F to search rmsd.
3. Previous step, leaves to rmsd table which shows binding energy table.
4. The residue number of least binding molecule was pasted in the protein molecule giving rise to the docked molecule.

After this the ligand molecules were tested in Osiris Property Explorer for the Toxicity if any and henceforth using LigPlot+ v.1.4.5 for identifying interacting residues as well as the interacting bonds between Wnt7B and the docked complex.

CHAPTER 4

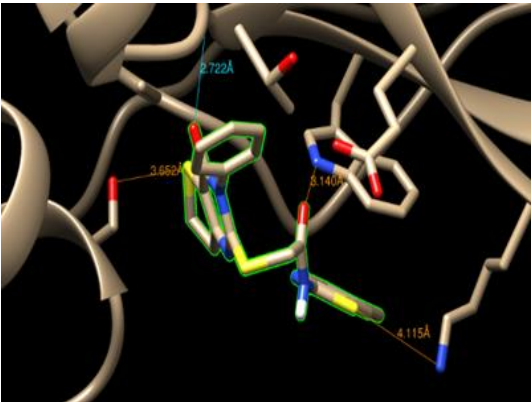
RESULTS AND DISCUSSION

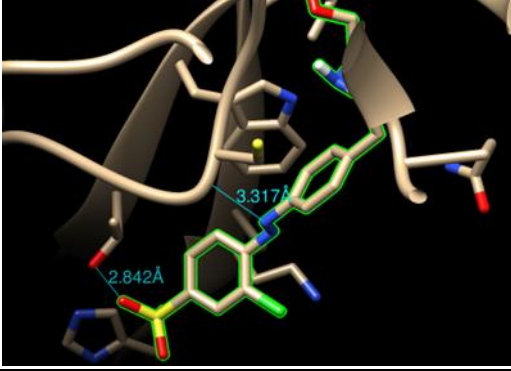
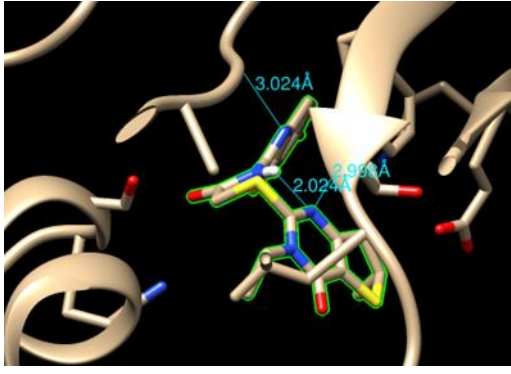
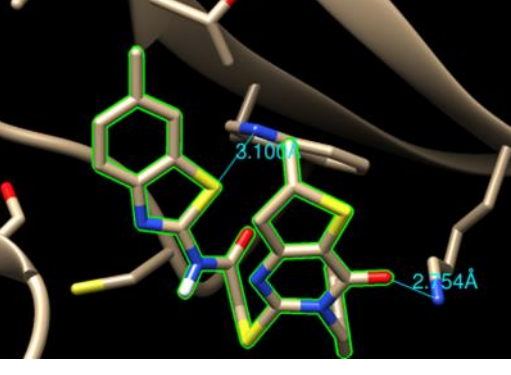
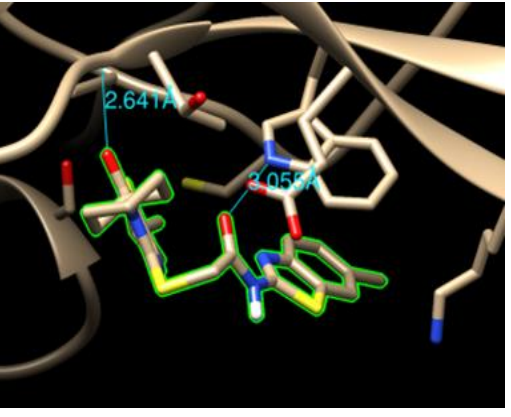
Results for Docking with Ligand IWP-2 and its analogues

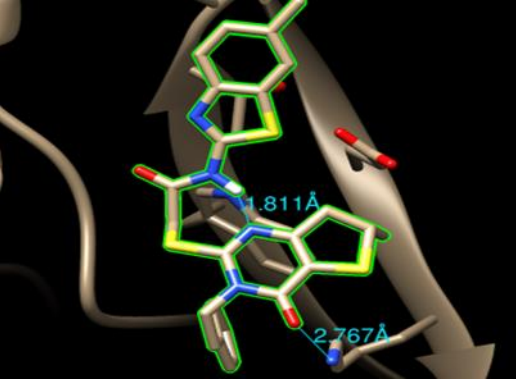
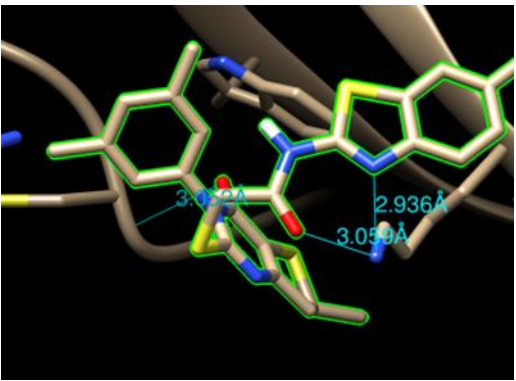
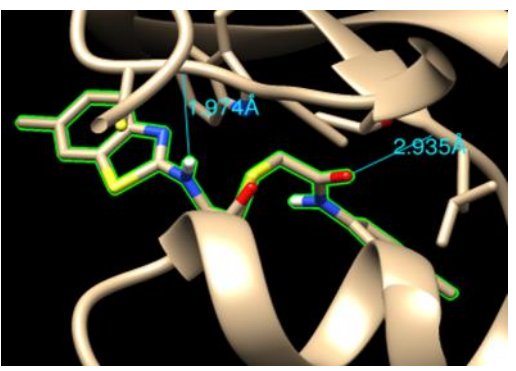
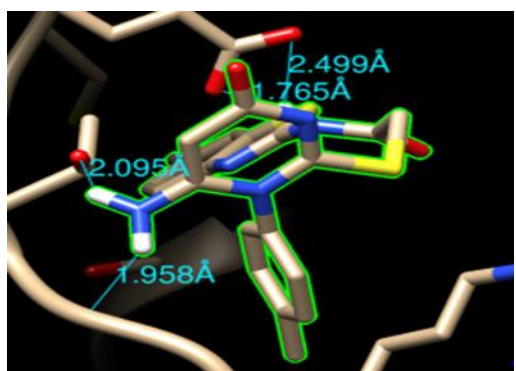
Identifying binding sites of ATP on Wnt7B: The analysis for the identifying binding sites of ATP was performed using various software and online servers. Using CastP server, we obtained the possible ATP binding sites. The results obtained from this server showed that TRP215 was the interacting residue. CastP calculation was done for the validation of results. From CastP results, it was observed that TRP215 was found to be present in the largest binding pocket of Wnt7B. Further, the docking of was performed with possible inhibitors.

Docking of Wnt7B with its existing inhibitors: The docking studies were then carried out using Autodock 4.0 in order to obtain the binding energies upon interaction of the various existing drugs with Wnt7B. The experiment was thus carried out by docking various inhibitors on previously recognized ATP binding residue of i.e. TRP215. The binding energies obtained from the above experiment are tabulated in Table 1. The results clearly showed that 2-[[3-(3,5-dimethylphenyl)-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide demonstrates highest binding energy for all the three sites of ATP interaction. It showed the best binding energy at TRP215 (-7.05 kcal/mol) among all the possible inhibitors under the category of IWP-2.

Table 1. Results for Docking with Ligand IWP-2 and its analogues.

Sl. No.	IUPAC Name of the ligand (IWP-2)	Binding Energy in (kcal/mol)	Docked Complex
1	2-[2-[(6-methyl-1,3-benzothiazol-2-yl)amino]-2-oxoethyl]sulfanyl-N-phenylacetamide	-6.23	

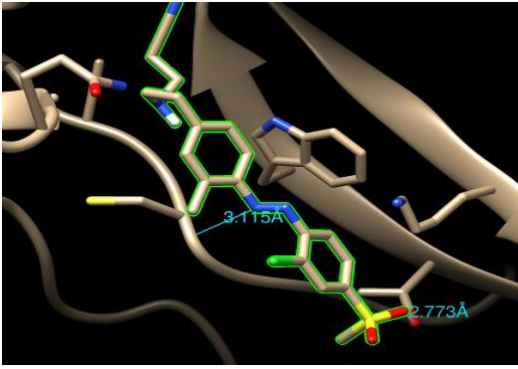
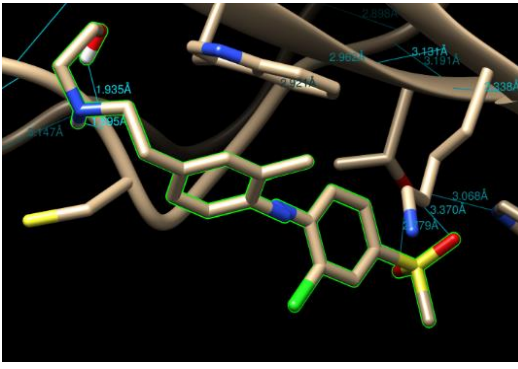
2	2-[(3-cyclohexyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl)sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-5.11	
3	2-[2-[(6-methyl-1,3-benzothiazol-2-yl)amino]-2-oxoethyl]sulfanyl-N-(4-methylphenyl)acetamide	-6.51	
4	2-[6-amino-1-(4-methylphenyl)-4-oxopyrimidin-2-yl]sulfanyl-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-5.82	
5	2-[[3-(3,5-dimethylphenyl)-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-7.05	

6	N-(6-methyl-1,3-benzothiazol-2-yl)-2-[(7-oxo-5-phenyl-1H-[1,2,4]triazolo[4,3-a]pyrimidin-3-yl)sulfanyl]acetamide	-5.84	
7	2-(2-benzylsulfanyl-4-oxo-1H-pyrimidin-6-yl)-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-6.82	
8	2-[[[(6R)-3-ethyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-6.95	
9	N-(1,3-benzothiazol-2-yl)-2-[[[(6S)-3-benzyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]acetamide	-6.29	

Results for Docking with Ligand WLS and its analogues

Similarly, the docking studies were then carried out using Autodock 4.0 in order to obtain the binding energies upon interaction of the WLS inhibitors with Wnt7B. The experiment was thus carried out by docking various inhibitors on previously recognized ATP binding residue of i.e. TRP215. The binding energies obtained from the above experiment are tabulated in Table 2. The results clearly showed that the best binding energy of inhibitor in category of WLS is -5.25 kcal/mol for 2-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]ethanol molecule.

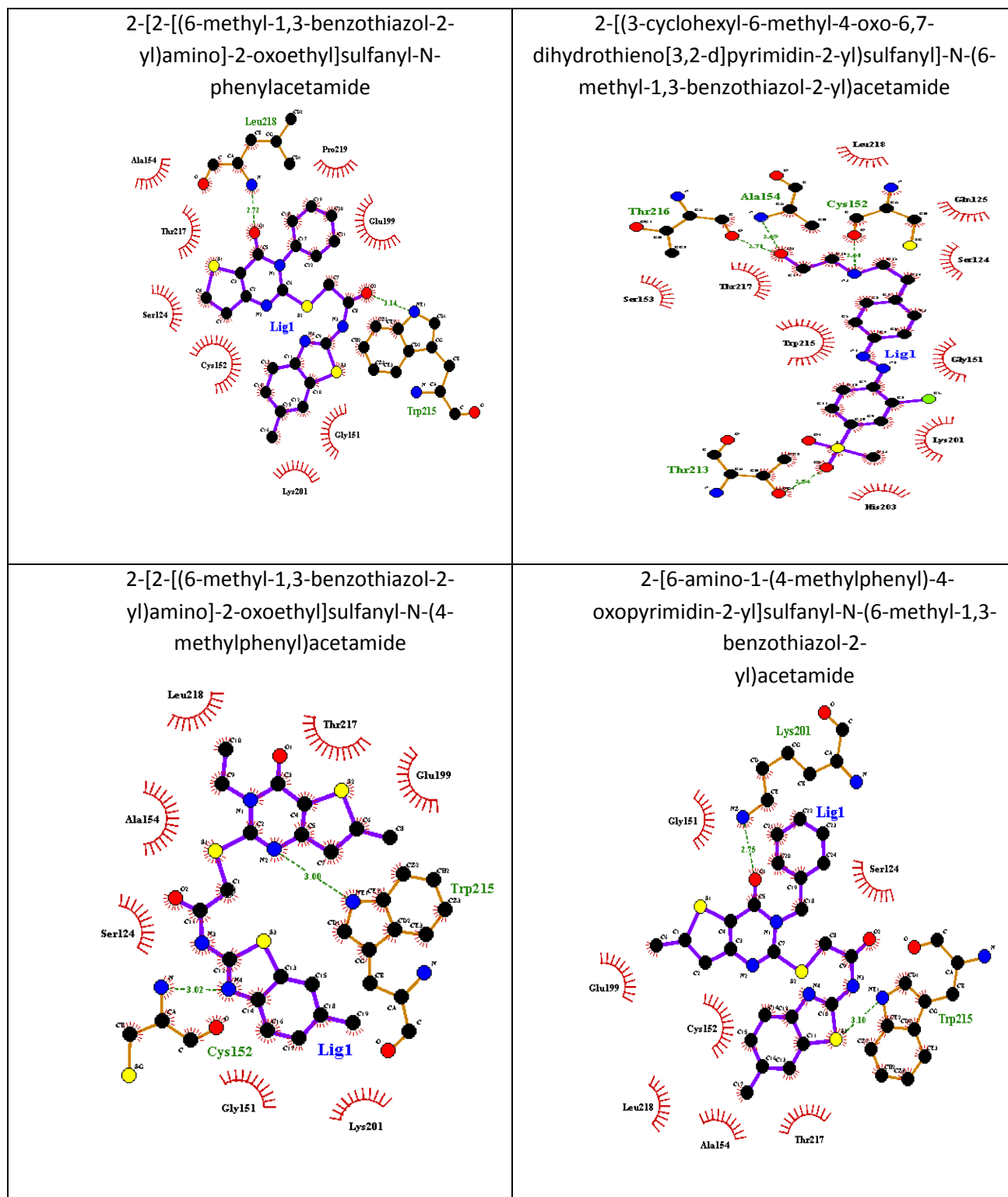
Table 2. Results for Docking with Ligand WLS and its analogues.

Sl. No.	IUPAC Name of the ligand (WLS)	Binding Energy (kcal/mol)	Docked Complex
1	3-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]propanenitrile	-5.19	
2	2-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]ethanol	-5.25	

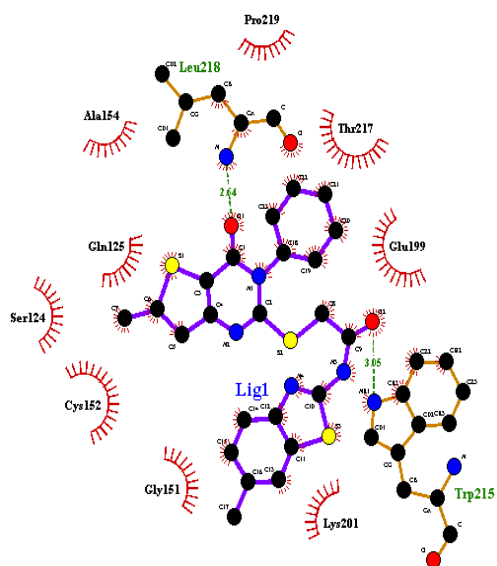
LigPlot Analysis for IWP-2 and its similar structures:

Docked results were analyzed by LigPlot+ v.1.4.5 software. In the LigPlot analysis, generally the residues denoted by red coloured arc show the hydrophobic residues of Wnt7B interacting with the drug molecule. The red, black and blue colour spheres indicate the oxygen, carbon and nitrogen atoms, respectively, of the drug molecule whereas the purple coloured lines represent the bonds present within these drug molecule. The external bonds between the drug and Wnt7B are

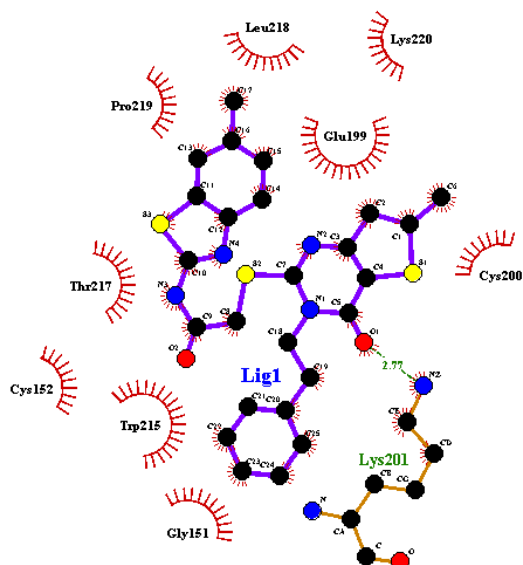
represented by blue colour and the bonds which are coloured green indicate the hydrogen bonds that are formed between the drug and Wnt7B. It was then observed from Ligplot analysis that inhibitors interact with TRP215 which was previously validated by CastP. It further validated the assumption that TRP215 could be the ATP binding residues.



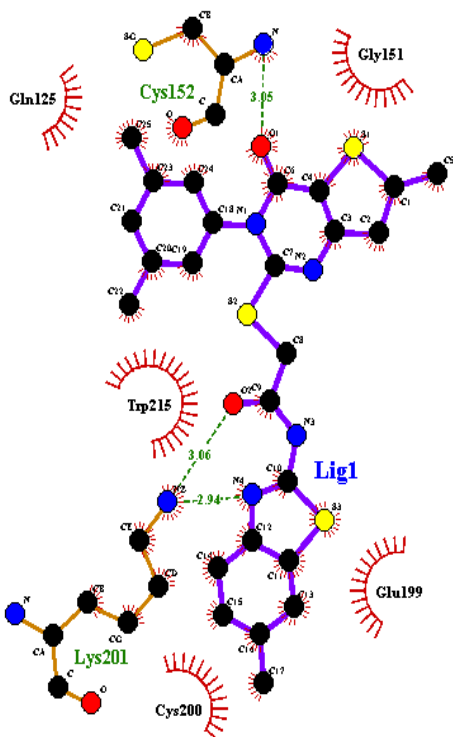
2-[[3-(3,5-dimethylphenyl)-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide



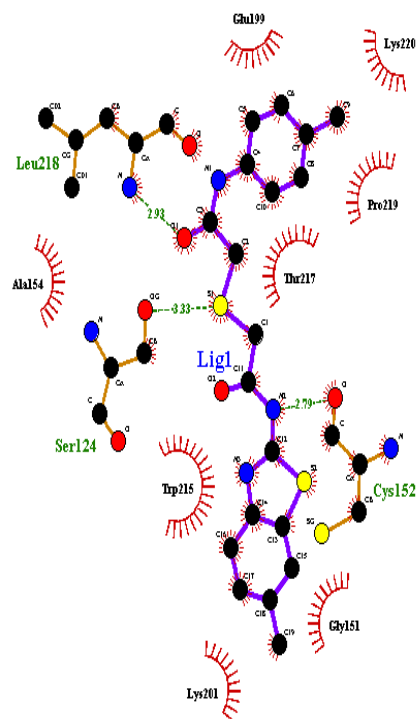
N-(6-methyl-1,3-benzothiazol-2-yl)-2-[(7-oxo-5-phenyl-1H-[1,2,4]triazolo[4,3-a]pyrimidin-3-yl)sulfanyl]acetamide



2-(2-benzylsulfanyl-4-oxo-1H-pyrimidin-6-yl)-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide



2-[[[(6R)-3-ethyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide



N-(1,3-benzothiazol-2-yl)-2-[[[(6S)-3-benzyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl)sulfanyl]acetamide

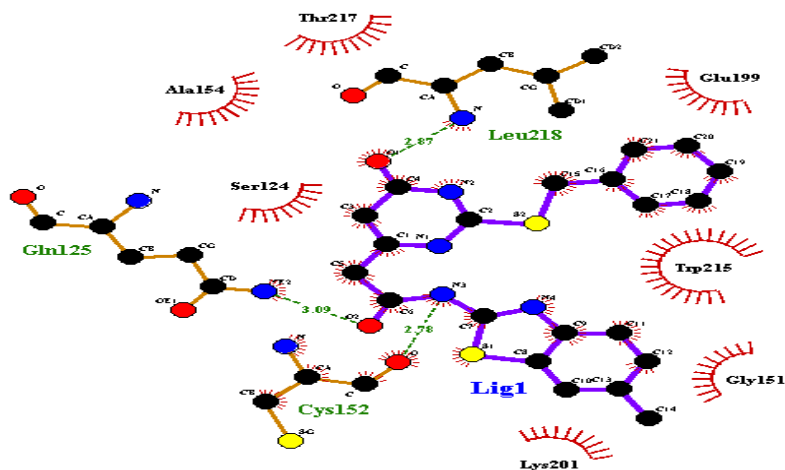
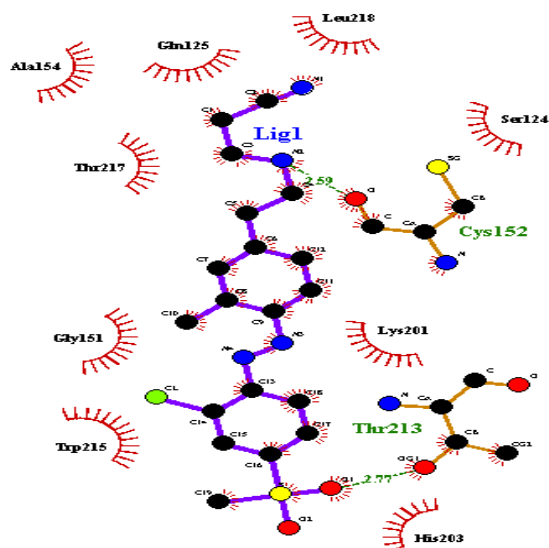


Figure 9. LigPlot Analysis for Inhibitor of Wnt Production-2 and its analogues.

LigPlot Analysis for WLS and its analogues:

3-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]propanenitrile



2-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]ethanol

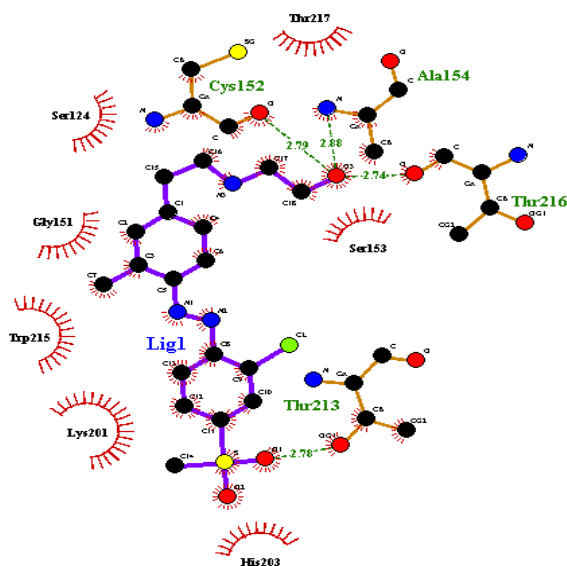


Figure 10. LigPlot Analysis for WLS and its analogues.

Toxicity measure for IWP-2 ligand and its analogues using OSIRIS property calculator:

The OSIRIS Property Explorer was used for measurement of various drug-relevant properties (Toxicity Risk Assessment, Molecular Weight, Overall Drug-Score, etc.) when a valid structure is given. The predicted results are thus color coded and valued. From the property prediction results, it was observed that all the compounds have cLogP values less than 5. The logP value of a compound, which is the logarithm of its partition coefficient between octanol and water $\log(\text{coctanol}/\text{cwater})$, is a well established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values cause poor absorption or permeation. It has been shown for compounds to have a reasonable probability of being well absorbed their logP value must not be greater than 5.0. Optimizing compounds for high activity on a biological target almost often goes along with increased molecular weights.

However, compounds with higher weights are less likely to be absorbed and therefore to ever reach the place of action. So the molecular weight should be around 450g/mol. In druglikeness property a positive value for the chemicals states that the molecule contains predominantly fragments which are frequently present in commercial drugs. All the compounds had a positive value for the drug likeness. The drug score combines druglikeness, cLogP, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug. In case of IWP-2 and its analogues, all the compounds showed green color for all the toxic parameters. Also the high risk properties of any effects which are not desirable like mutagenicity or a poor intestinal absorption are shown in red color. On the other hand, drug-conform behavior is indicated by a green color. All the IWP-2 ligands showed green color for risk to Mutagenicity and Tumorigenicity, which means they exhibit no toxicity. The molecule 2-[[3-(3,5-dimethylphenyl)-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide, was detected to be, not toxic from the Table 3, below.

Table 3. Toxicity measure for IWP-2 ligand and its analogues using ORISIS property calculator.

Sl. No.	IUPAC Name of the ligand (IWP-2)	Binding Energy (kcal/mol)	Drug Likeness Score	Mol. Wt. (g/mol)	cLogP	Toxicity (Mutagenicity)	Toxicity (Tumorigenicity)	Drug Score
1	2-[2-[(6-methyl-1,3-benzothiazol-2-yl)amino]-2-oxoethyl]sulfanyl-N-phenylacetamide	-6.23	0.348	371	3.541	No	No	0.428
2	2-[(3-cyclohexyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl)sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-5.11	0.302	486	4.793	No	No	0.221
3	2-[2-[(6-methyl-1,3-benzothiazol-2-yl)amino]-2-oxoethyl]sulfanyl-N-(4-methylphenyl)acetamide	-6.51	0.436	385	3.885	No	No	0.41
4	2-[6-amino-1-(4-methylphenyl)-4-oxopyrimidin-2-yl]sulfanyl-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-5.82	0.372	437	3.429	No	No	0.286

5	2-[[3-(3,5-dimethylphenyl)-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-7.05	0.309	400	4.748	No	No	0.257
6	N-(6-methyl-1,3-benzothiazol-2-yl)-2-[(7-oxo-5-phenyl-1H-[1,2,4]triazolo[4,3-a]pyrimidin-3-yl)sulfanyl]acetamide	-5.84	0.922	448	3.735	No	No	0.427
7	2-(2-benzylsulfanyl-4-oxo-1H-pyrimidin-6-yl)-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-6.82	0.036	422	3.656	No	No	0.241
8	2-[[[(6R)-3-ethyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide	-6.95	0.995	432	3.644	No	No	0.517
9	N-(1,3-benzothiazol-2-yl)-2-[[[(6S)-3-benzyl-6-methyl-4-oxo-6,7-dihydrothieno[3,2-d]pyrimidin-2-yl]sulfanyl]acetamide	-6.29	0.999	480	4.311	No	No	0.397

Toxicity measure for WLS ligand and its analogues using OSIRIS property

calculator: The WLS ligands showed high risk (red color) to Mutagenicity and Tumorigenicity. They are toxic. So, IWP-2 ligands are less toxic than the WLS ligands on basis of Table 3 and Table 4 (below).

Table 4. Toxicity measure for WLS ligand and its analogues using OSIRIS property calculator Results.

Sl. No.	IUPAC Name for WLS and its Analogues	Binding Energy (kcal/mol)	Score of Drug Likeness	Mol. Wt. (g/mol)	cLogP	Toxicity (Mutagenicity)	Toxicity (Tumorigenicity)	Drug Score
1	3-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]propanenitrile	-5.19	0.007	406	2.922	Yes	Yes	0.067
2	2-[2-[4-[(2-chloro-4-methylsulfonylphenyl)diazenyl]-3-methylphenyl]ethylamino]ethanol	-5.25	0.007	395	3.079	Yes	Yes	0.063

The role of Wnt7B inhibition in the treatment of Pancreatic Ductal Adenoma Carcinoma disease has been evident from various experiments and research carried out till date. Hence, various drugs have been developed which can inhibit Wnt7B by either blocking the ATP binding site, blocking the binding site for client proteins or by obstructing the dimerization site at the protein. The nucleotide binding to Wnt7B alters its conformation and defines the subset of chaperones with which it interacts. In the present study, we have attempted to locate the residues which are involved in the ATP binding site of domain and then blocking this site by various inhibitors and thereby to identify a novel inhibitor.

Hence, through our experiments we attempted to identify the interacting residues of ATP binding site in Wnt7B and a potential inhibitor which can block the identified ATP binding site. The

binding site of the ATP is not known; hence we predicted the possible ATP binding sites present in this domain using various software. CastP server was used which showed TRP215 as the interacting residue between and ATP. CastP calculation was carried out to validate the prediction. It was validated by CastP that TRP215 could be ATP interacting site in as it is present in the largest binding pocket of the structure. This procedure was followed by a docking experiment between with inhibitors. The docked results were analyzed using LigPlot+ v.1.4.5 to identify the interacting residues. It was observed that both ATP and the inhibitor interact with the previously determined ATP binding site of i.e. TRP215. From this result, it was further validated that TRP215 could be the ATP interacting site on domain.

CHAPTER 5

CONCLUSION

CONCLUSION

From above in silico study we can conclude that IWP-2 shows inhibitory activity towards Wnt7B protein involved in overexpression of Pancreatic Ductal Carcinoma (PDAC) as it is interacting with the Tryptophan ion of active site and turn it into inactive form. This experiment is only the in silico approach, which need approval after future experiments (in vivo). We had tested two inhibitor including, IWP-2 and WLS against Wnt7B. If the docking results establish that these bioactive compounds have potent in vivo Wnt inhibition property and will add a new achievement in cancer treatment.

Moreover, toxicity studies to be performed for establishing it as a safe and effective drug. The establishment of ATP binding site in and determining the best inhibitor for Wnt7B could further lead to the effective blockage of Wnt7B which would ultimately causes degrading B-catenin for the treatment in Pancreatic Ductal Adenocarcinoma disease.

REFERENCES

- [1] Luu HH, Zhang R, Haydon RC et al. Wnt/ β -Catenin Signaling Pathway as Novel Cancer Drug Targets. *Current Cancer Drug Targets* 2004; 653–671.
- [2] S. Paul, A. Dey. Wnt signaling and cancer development: therapeutic implication. *Minireview, Neoplasma* 2008; 165-172.
- [3] Wang L, Heidt DG, Lee CJ, Yang H, Logsdon CD, Zhang L et al. Oncogenic function of ATDC in pancreatic cancer through Wnt pathway activation and beta-catenin stabilization. *Cancer Cell* 2009; 207–219.
- [4] Voeller HJ, Truica CI, Gelmann EP. β -catenin mutations in human prostate cancer. *Cancer Res.* 1998; 58.
- [5] Arensman MD et al. Wnt β -Catenin signaling in pancreatic cancer. *Macmillan Publishers Limited* 2013; 1 – 10.
- [6] Polakis P. Wnt signaling and cancer. *Genes Dev.* 2000; 1837–1851.
- [7] Donahue T, Tran L, Hill R, Li Y, Kovochich A, Calvopina J et al. Intergrative survival based molecular profiling of human pancreatic cancer. *Clin Cancer Res* 2012; 1352–1363.
- [8] Vincent A, Omura N, Hong SM, Jaffe A, Eshleman J, Goggins M. Genome-wide analysis of promoter methylation associated with gene expression profile in pancreatic adenocarcinoma. *Clin Cancer Res* 2011; 4341–4354.
- [9] American_Cancer_Society. *Cancer Facts & Figures 2012*. American Cancer Society: New York 2012, 1–66.
- [10] Aram F, Hezel, Alec C. Kimmelman, Ben Z. Stanger, et al. *Genes Dev.* 2006 20: 1218-1249.
- [11] Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; 607–620.

- [12] Hong SM, Park JY, Hruban RH, Goggins M. Molecular signatures of pancreatic cancer. *Arch Pathol Lab Med* 2011; 716–727.
- [13] Maitra A, Hruban RH. Pancreatic cancer. *Annu Rev Pathol* 2008; 157–188.
- [14] American Cancer Society. *Cancer Facts & Figures 2012*. Cancer. American Cancer Society: New York 2012, 1–66.
- [15] Donahue T, Tran L, Hill R, Li Y, Kovochich A, Calvopina J et al. Intergrative survival based molecular profiling of human pancreatic cancer. *Clin Cancer Res* 2012; 1352–1363.
- [16] Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; 1801–1806.
- [17] A.Puratchikody et al. Toxicity Risk Assessment of Some Novel Quinoxaline fused thiazolidinones . *Journal of Pharmacy Research* 2012; 340-342
- [18] Martinico SC, Jezzard S, Sturt NJ et al. Assessment of endostatin gene therapy for familial adenomatous polyposis-related desmoid tumors. *Cancer Res.* 2006; 8233–8240
- [19] Kadoya T, Kishida S, Fukui A et al. Inhibition of wnt signaling pathway by a novel axin-binding protein. *J. Biol. Chem.* 2000; 37030–37037.
- [20] Blanc E, Goldschneider D, Douc-Rasy S et al. Wnt-5a gene expression in malignant human neuroblasts. *Cancer Lett.* 2005; 117–23.