

Study of Malignant Melonema Causing Protein “5ixb” Using Multiomics Approach

Devanshi Gupta¹, Dr Uma Kumari²

¹*Trainee at BPRI (Bioinformatics Project and Research Institute)*

²*Bioinformatics Scientist BPRI, Noida, India*

Abstract- It has been scrutinized that skin cancer has become more usual among people all over the world due to sun exposure and an unhealthy diet. According to the reports, every year all over the world more than 14 million people suffer from cancers. Melanoma is the most critical type of skin cancer that occurs in melanocytes. The survival rate of melanoma is 95% in early diagnosis. Malignant melanoma cells can relocate themselves from one position to another position in the human body. That's why malignant melanoma cells are a major threat to humans to develop various types of cancers. In this research paper, we have utilized the multi-omics approach to find the cure for malignant melanoma. Multi-omics is a proposal where the data sets of various omic groups are merge during analysis. The Multi-omics approach includes epigenomics, transcriptomics, proteomics, metabolomics, physiomics, pharmacogenomics, etc. we have performed the structural analysis of MIA protein (5IXB) using pymol and RasMol and identified the ligands in MIA using pymol. After identifying the ligand we have found a potential drug target using molecular docking. To recognize the exact site of binding of the drug ORF analysis is performed and Phylogenetic analysis of the 5IXB is performed using SMARTblast to determine the working pathway of MIA. Structural analysis of the secondary structure of the protein has also been performed using the PDBsum server.

Keywords- Malignant melonema, Sequence analysis, molecular docking, Multiple sequence alignment, homology modeling

INTRODUCTION

It is known that every cell in the body has a certain lifespan. After completing their life-span cells have to

die but due to DNA damage uncontrolled cell growth takes place which causes cancer. The damage in the melanocytes (pigment-producing cells of the human body) leads to melanoma which is detected by MIA. MIA is an extracellular protein with a molecular weight of 11kDa, highly expressed by malignant melanoma cells. After its secretion from melanocytes, it interacts with fibronectin which leads to focal cell detachment from surrounding structures and elevates tumor cell invasion and migration. Thus it works as a biomarker to detect malignant melanoma.[1,2,3,4] In malignant melanoma metastasis is shown at a very early stage and the phenotype of this malignancy is very aggressive. Black people diagnose less than white with melanoma. All over the world, 5% of all new cancer cases are melanoma in men and 4% of all new cancer cases in women. According to some research, the main cause of melanoma is sun exposure which leads to insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, tissue invasion, metastasis, sustained angiogenesis, and self-sufficiency in growth signals. After secreting from melanocytes, MIA binds with extracellular matrix protein like laminin, fibronectin, and tenascin which inhibits the connection of melanocytes with other body parts and elevate the growth of tumor cells. On studying the mutation pathway analysis of MIA (BRAF gene), it is concluded that there is a mutation in p53. It detects damaged DNA and it is involved in 50% of cancers. In DNA damage, p53 responds in two certain ways. Either it will act as a transcription factor to activate the expression of p21, which inhibits CDK/G1 cyclin to halt the cell cycle, then activates DNA repair, or it will trigger apoptosis.[5,6,7,8,9,10]

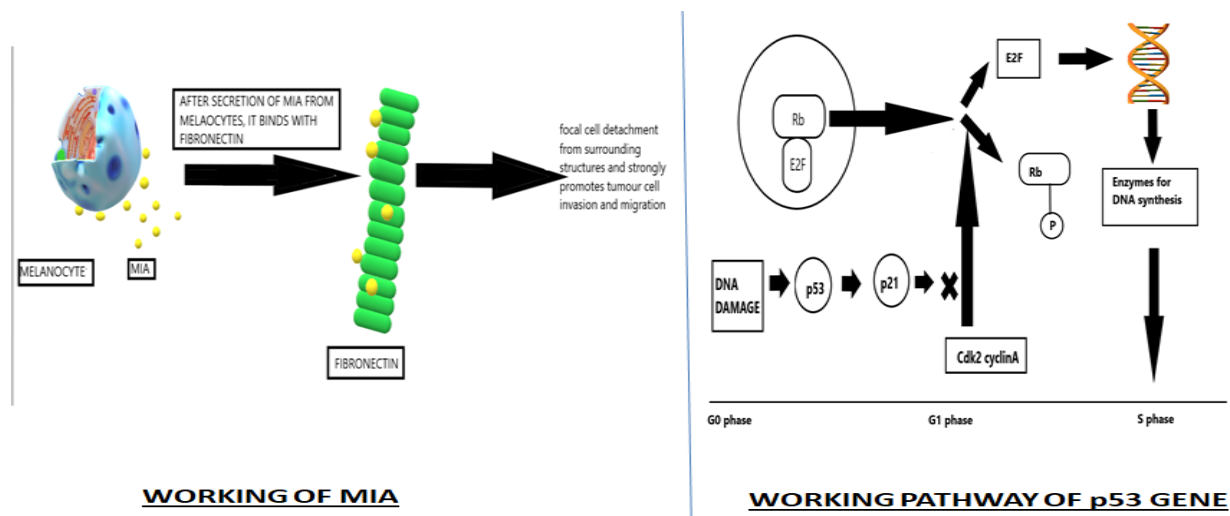


Figure No.-1

To inhibit the activity of MIA we have tried to find a potential drug target using the molecular docking approach and represented a detailed structural analysis of MIA using various bioinformatics tools. Cobimetinib is a commercially acceptable drug for melanoma. First, we have checked its binding affinity towards MIA and represented a comparative study between cobimetinib and phytochemical isobavachin. Phytochemicals are found in plant-originated food which does not contain any nutritive benefit but has health benefits. Examples of phytochemicals are flavonoids, curcumin, phenolic acids, carotenoids,

isothiocyanates, and isoflavones. Among all kinds of phytochemicals, flavonoids are most studied for their anti-cancer activity. cobimetinib is a tyrosine kinase receptor inhibitor. According to some preclinical studies, it is effective to inhibit the growth of carcinoma cells with B-RAF mutation.

We have performed the structural and sequence analysis using multi-omics approach. Multi-omics is a group of different omic groups joined together to obtain a useful result that can be further applied to cure a disease or syndrome.

Some properties of isobavachin and cobimetinib-

Table 1: ligand properties

PROPERTIES	ISOBAVACHIN	COBIMETINIB
MOLECULAR FORMULA	C ₂₀ H ₂₀ O ₄	C ₂₁ H ₂₁ F ₃ IN ₅ O ₂
MOLECULAR WEIGHT	324.4	531.3
HYDROGEN BOND DONOR	2	3
HYDROGEN BOND ACCEPTOR	4	7

MATERIALS AND METHODS

Sequence analysis and phylogenetic analysis of MIA- To analyse the phylogenetic relationship, we have used SMARTBlast and cobalt. Both are online available tools. For sequence analysis we have utilized the tools, Blast and ORF-Finder. Accession no. NM_001202553.2 has been taken to analyse the MIA (melanoma inhibitory activity). We input the accession number in the ORF-Finder and it provides output in the form of graphics. After detecting the ORF Structural Analysis of MIA-

(open reading frame) in the sequence, we draw the cladogram to show the phylogeny for each ORF using SMARTBlast. We have also used cobalt to analyse the phylogenetic relationship for each ORF. In today's era bioinformatics is developing exponentially. Bioinformatics has very powerful tools one of them is Blast. Blast compares a particular nucleotide or protein sequence with the database which results in the form of various similar sequences. BLAST is a heuristic program which means it relies on some shortcuts to perform the search faster.[11,12,16,17]

For structural analysis we have used PyMol and RasMol. Rasmol is a molecular graphics visualization tool which is used to visualize molecular structures like protein, nucleic acid, chemical compounds etc. Using Rasmol, we can visualize our structure in various format like wireframe, spacefill, ball and stick, backbone, ribbon, strands and cartoon. It also works as command-line interface. Pymol is also a visualization tool through which we can visualize the molecular structure. Pymol software is based on the python that is a computer programming language. To analyse the secondary structure of MIA we have utilized PDBsum. It is an online tool on which we input PDB id of MIA, Sixb and it provides us a topology visualization diagram. [13,14,18,19]

Molecular Docking-

We have taken the MIA structure from protein databank and the structure of cobimetinib and isobavachin from the pubchem database. MIA has been downloaded in PDB format and dexamethasone

in SDF format. SDF contains the information about the atoms, bonds, connectivity and coordinates. We have prepared our aptamer and ligand structure using pymol. Autodock is a software program that provides us binding affinity between protein and ligand. [13,14,15,18,19]

RESULT AND DISCUSSION-

1. Sequence similarity and phylogenetic analysis of MIA (5IXB)-

• **BLAST-**

On performing the BLAST we get the conserve domain of MIA. The format for input in BLAST can be FASTA format and GENBANK format. The output can be given in the HTML format, XML format or plain text format. All blast program uses a substitution matrix which is either PAM or BLOSSUM. In our research paper we have used PROTEIN-BLAST that compares a query protein sequence against protein sequence database.[11,12]

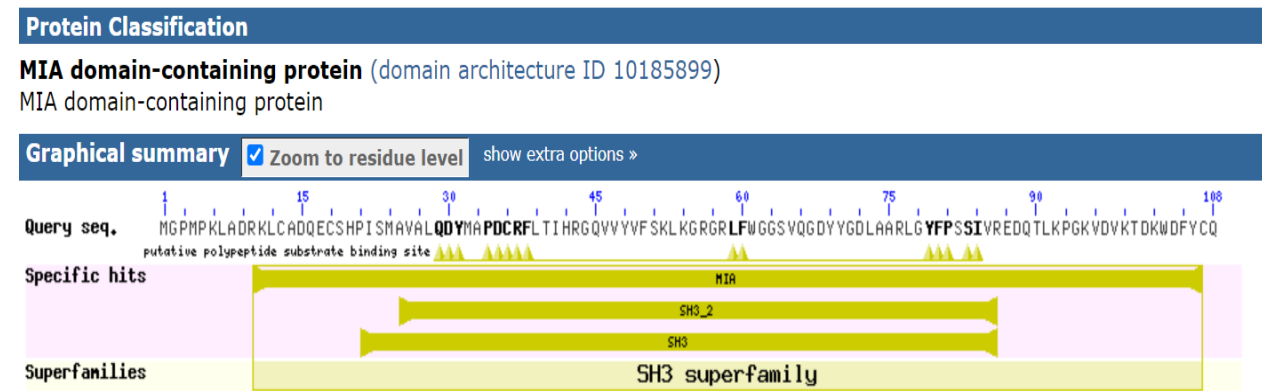


Figure No.-2 BLAST result showing conserve domain

Dark black alphabets show site of mutations in our sequence. These are binding sites for our potential drug target.

Table2: RESULTS OBTAINED FROM Fig. No.- 2

NAME	DESCRIPTION	INTERVAL
MIA	single domain protein that adopts a Src Homology 3 (SH3) domain-like fold; it consist of an additional antiparallel beta sheet and two disulfide bonds compared to classical SH3 domains.	10-107
SH3_2	SH3 (Src homology 3) domains are often indicative of a protein involved in signal transduction related to cytoskeletal organisation. The structure is a partly opened beta barrel.	25-86
SH3	Src homology 3 (SH3) domains bind to target proteins through sequences containing proline and hydrophobic amino acids. Pro-containing polypeptides may bind to SH3 domains in 2 different binding orientations.	21-86

• **ORF-FINDER, SMARTBLAST AND COBALT-**

ORF-FINDER gives the number of open reading frame present in the sequence.It enables the scientists to get their genes of interest and directly show the ORF structure of the query gene which helps in the synthesis of guide RNA

for genome editing .Open reading frames are a stretch of DNA that starts with start codon (ATG) and ends with stop codon (UAA,UAG,UGA).[16,17]



Figure No.-3 ORF-FINDER RESULT

There are 3 ORF found in the sequence.

Table3: Result of ORF-Finder

ORF	START	STOP	LENGTH (Nucleotide Amino acid)
ORF1	433	>522	90 29
ORF2	59	454	396 131
ORF3	198	>1	198 65

SMARTblast shows phylogenetic relationship between different genes. It show how a particular gene evolved from one species to another. It shows ancestral relationship between two organisms.

SMARTblast found no matches for ORF1.

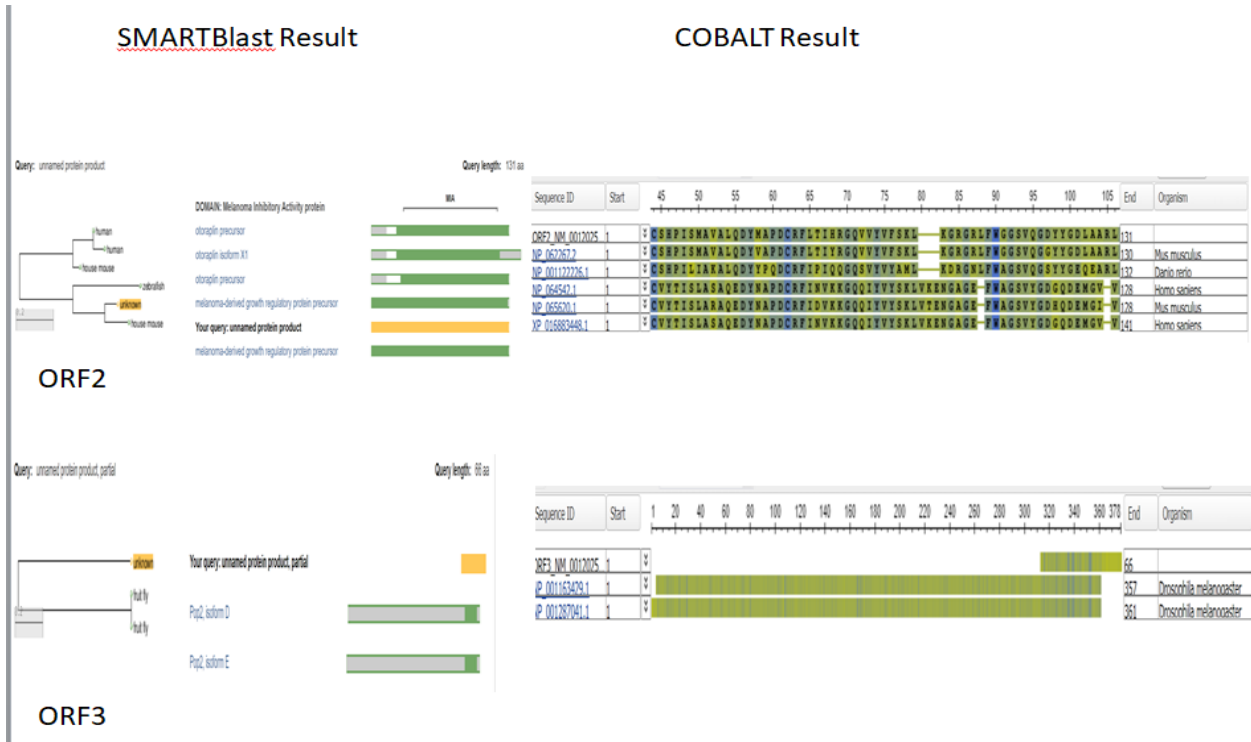


Figure No.-4

DESCRIPTION OF SMART Blast RESULT –

For ORF2-SMARTBlast shows the similarity with the genes of otoraplin precursor of Homo sapiens, house mouse and zebrafish.

For ORF3- SMART Blast shows the similarity with the genes of fruit-fly.

Table4: Description of SMARTBlast

COLOUR	DESCRIPTION
Yellow	Query sequence
Green	Best Matching sequence
White	Deletions in multiple sequence alignment
Gray	Mismatch

DESCRIPTION OF COBALT RESULT-

Cobalt also shows the phylogenetic relationship between two organisms. Green colour shows worst matching while blue colour shows better match.

For ORF2- It shows the similarity with the genes of otoraplin precursor of Homo sapiens and house mouse.

For ORF3- It shows similarity with the gene of fruit-fly (Drosophilamelanogaster).

2. STRUCTURAL ANALYSIS OF MIA-

- RasMol-

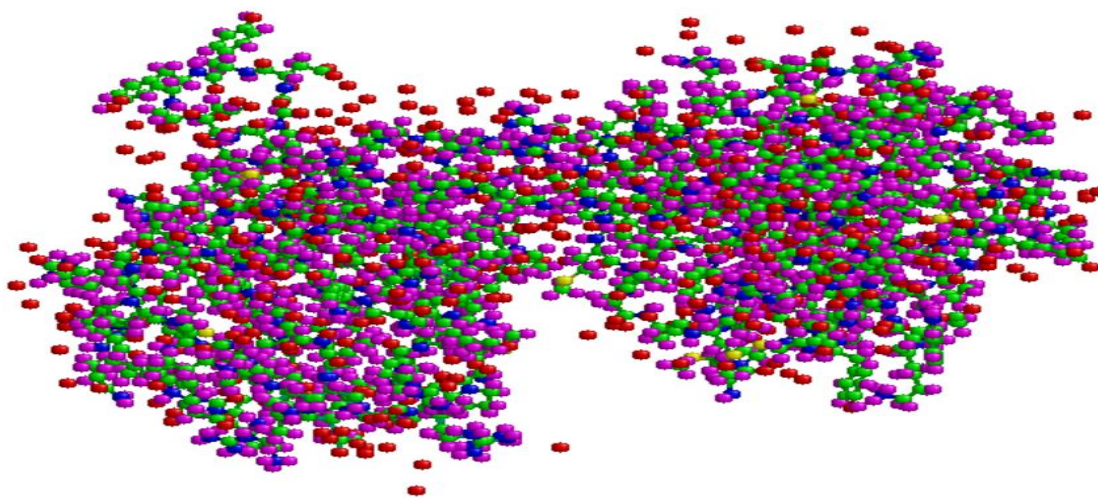


Figure No.-5 - Structure of MIA visualized through RasMol

Table5: Results obtained from RasMol

ATOM NAME	OBSERVED NO. OF ATOMS	ATOM SHOWN BY COLOR
carbon	1144	Green
Oxygen	589	Red
Nitrogen	324	Blue
Hydrogen	1781	Magenta
sulfur	14	Yellow

- PyMol-

PyMol is used to visualize the three- dimensional structure of macromolecules like protein. PyMol is written in python language.

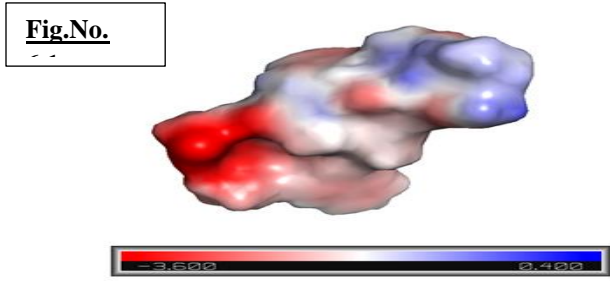


Fig.No.6.1 MIA is visualized through 'electrostatic' scheme in which positively charged region is shown by blue colour, negatively charged region is shown by red colour and neutral region is shown by white.

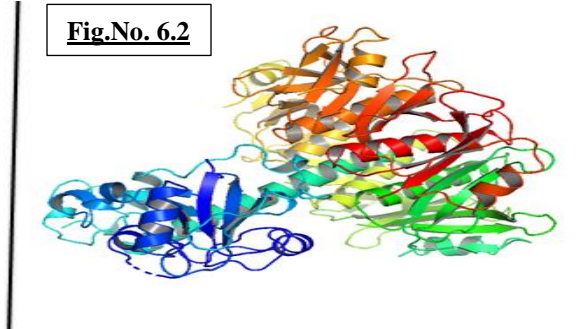
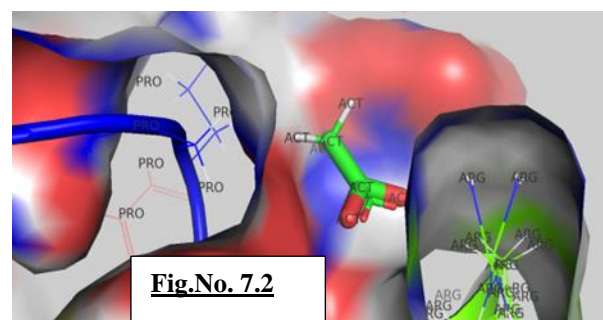
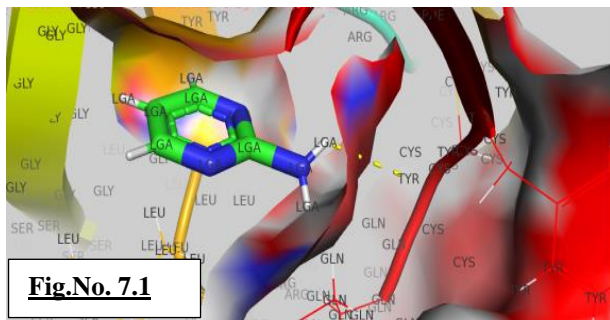


Fig.No. 6.2 MIA is visualized through 'cartoon ribbon' scheme in which high density region is shown by red colour and low density region is shown by blue colour



These images shows the ligand LGA and ACT present on the MIA protein visualize through pymol.

- PDBSum-

It is an online tool through which we can analyse the secondary structure of protein e.g alpha helix, beta-sheets, turns and loops.

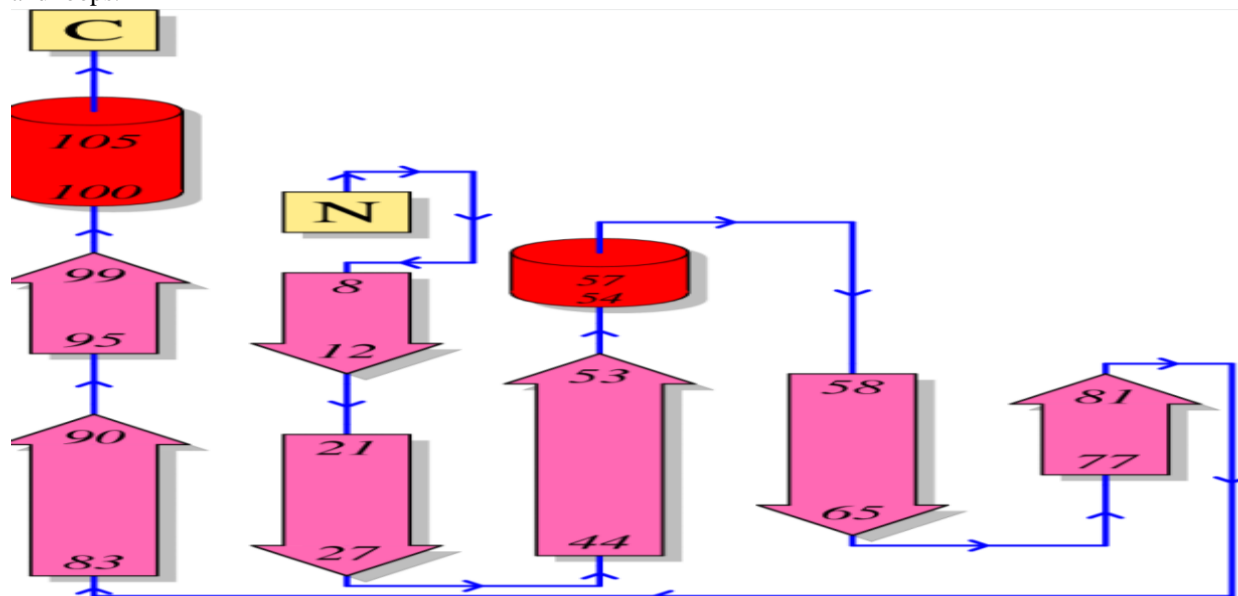


Figure.No.- 8- Topology visualization in PDBSum

The diagram describes that the beta-strands, illustrated by the large arrows, join up, side-by-side, to form the domain's central b-sheet. The diagram represent the relative positions of the alpha-helices, which are represented by the red cylinders. The small arrows shows the directionality of the protein chain, from the N- to the C-terminus. The numbers represent the secondary structural elements correspond to the residue numbering given in the PDB file.

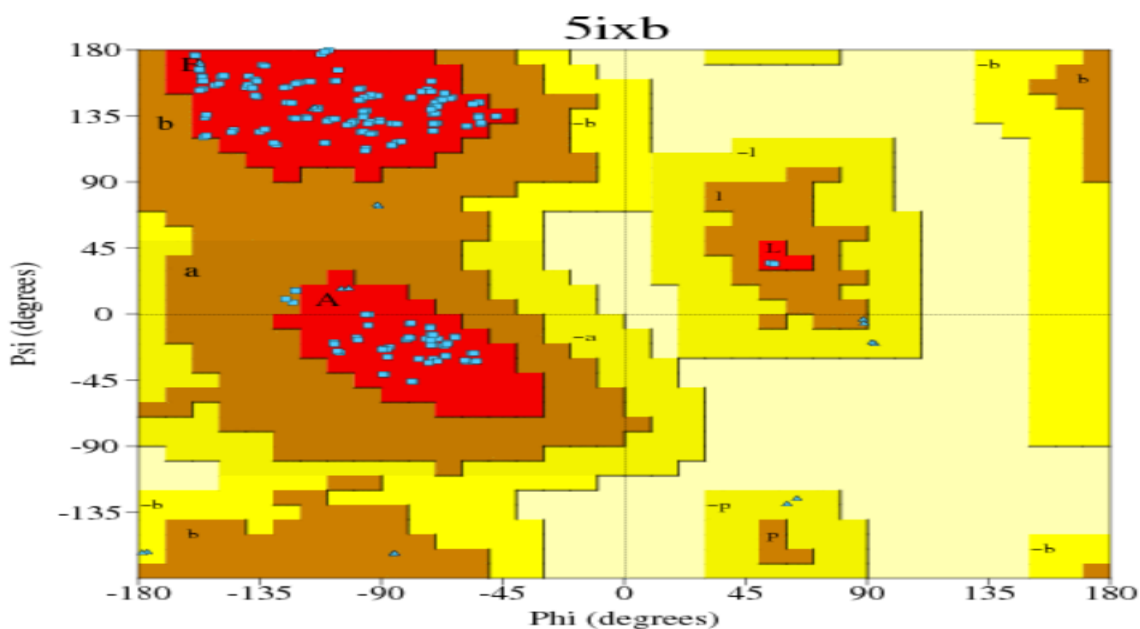


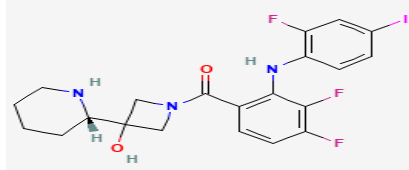
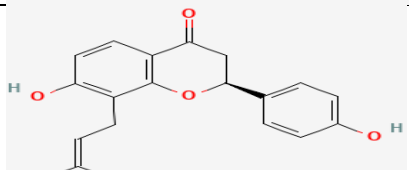
Figure No.- 9 - Ramachandran plot for 5IXB protein

Table6: Results obtained from Ramachandran plot	No. of residues	percentage
Most favoured regions [A,B,L]	174	97.8%
Additional allowed regions [a,b,l,p]	4	2.2%
Generously allowed regions [~a,~b,~l,~p]	0	0.0%
Disallowed regions [XX]	0	0.0%

Ramachandran plots measures the dihedral angle psi and phi for each secondary structure of protein and provides the result which notify that the particular peptide chain will form either beta-sheets or alpha helix.

3. Molecular docking-

Molecular docking shows the binding affinity of protein with a ligand. We have used autodock to check the efficacy of cobimetinib and isobavachin towards MIA to inhibit the activity of MIA. In the process of molecular docking drug is bind to the binding site of the target protein. In MIA, the binding site is situated in the Leucine76, Glycine77 and Tyrosine78.

	Structure	Binding affinity (kcal/mol)	Interacting residues
Cobimetinib		-5.6 kcal/mol	First pocket
Isobavachin		-5.3 kcal/mol	First pocket

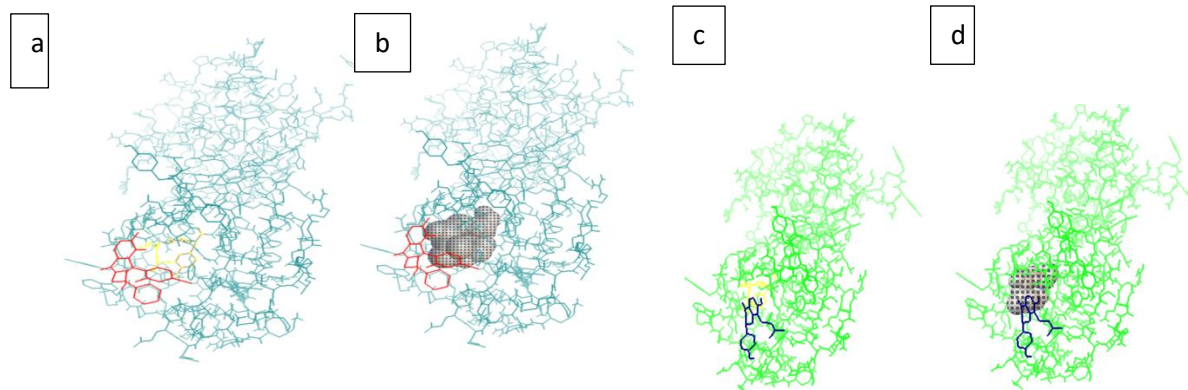


Figure No.- 10

Docking image of Cobimetinib with MIA through autodock. Cobimetinib is shown by red colour and MIA is shown by green colour. Binding pocket is shown by (a) yellow colour and (b) surface structure scheme.

Docked image of isobavachin with MIA through autodock. isobavachin is shown by blue colour and MIA is shown by green colour. Binding pocket is shown by (c) yellow colour and (d) surface structure

CONCLUSION

It is critical to find high affinity drugs that bind to proteins and genes that cause skin cancer. The result of our research has established an alternate paradigm for isobavachin as strong ligands that can bind to 5IXB and might possibly be employed as treatments for skin cancer. Additional analysis can be carried out to confirm their therapeutic potential.

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