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COMPUTATIONAL INSIGHTS TO THE DRUG DISCOVERY ON MET TYROSINE KINASE INHIBITION IN TUMOR EFFICACY

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ABSTRACT: In this computational study, we employed a multi-faceted approach to investigate potential inhibitors of MET tyrosine kinase for tumor efficacy. Utilizing a combination of structural validation, molecular docking, and structural analysis techniques, we evaluated the binding affinities and structural integrity of candidate compounds against the 6UBW protein structure. Our results revealed promising molecular docking scores foranti-cancer drugs,indicating their potential as ligands with the 6UBW protein. These findings underscore the potential of these compounds in treating aggressive brain cancers, such as glioblastoma multiforme,sarcomas, lymphomas, breast cancer, and leukemia. Through rigorous computational analyses, this study provides valuable insights into the molecular interactions and structural characteristics underlying MET tyrosine kinase inhibition, offering a foundation for further experimental validation and drug development efforts targeting MET-driven malignancies.

KEYWORDS: MET Tyrosine Kinase, Tumor Efficacy, Anti-Cancer Drugs, Computational Drug Discovery, Molecular Docking, Structure Validation, Sequence Alignment

INTRODUCTION

The cells of the human body go through a natural cycle of growth, aging, and death. New cells proliferate andeventually replace the dead ones. However, in some cases, this process goes out of control, and cells start behaving abnormally. These abnormal cells don't die when they should and begin to group, forming a mass called a tumor. Sometimes, these tumors can be cancerous**[1]**.A tumor is an abnormal growth of cancer cells that can occur in any part of the body**[2]**. There are different types of tumors, each with its unique characteristics and treatments. Brain tumors are classified into two categories: primary brain tumors and metastatic brain tumors. Primary brain tumors originate in the brain and tend to stay within the brain. In contrast, metastatic brain tumors begin as cancer in other parts of the body and spread to the brain**[3]**. Brain tumors are classified into four grades based on their behavior under microscopic observation. Grade I tumors, which are benign, closely resemble normal brain cells and grow slowly, representing the earliest stage of tumor development. Grade II tumors, known as low-grade tumors, exhibit slightly abnormal behavior and are malignant. Grade III and Grade IV tumors, termed high-grade tumors, demonstrate significantly different behavior from normal cells, necessitating urgent treatment due to their rapid growth and abnormal characteristics**[4]**. The World Health Organization (WHO) grading system categorizes tumors as low or High-grade based on histopathological features such as cellularity, pleomorphism, mitosis, necrosis,

vascular proliferation, and apoptosis. Low-grade tumors typically have a less aggressive nature and a favorable prognosis, while high-grade tumors are aggressive, grow rapidly, and have a tendency to invade nearby tissues**[5]**.

The Hepatocyte Growth Factor Receptor (HGFR), also known as c-Met Tyrosine Kinase, is a type of receptor tyrosine kinase (RTK) that plays a crucial role in human cancers. It is influenced by its high-affinity native ligand, Hepatocyte Growth Factor (HGF). According to preclinical research, c-Met is amplified, mutated, and over-expressed in a range of human tumor types. As a result, c-Met inhibitors may be useful as targeted cancer therapies to prevent tumor growth and metastasis**[6]**.In mammalian development, cell function, and tissue homeostasis, receptor tyrosine kinases (RTKs) control numerous important processes. These varied processes include, among others, neovascularization, tissue regeneration and repair, organ morphogenesis, and cell growth and survival. Many human cancers have been linked to the development and progression of dysregulation of RTKs through mutation, gene rearrangement, gene amplification, and overexpression of both receptor and ligand**[11]**. The binding of HGF to c-MET induces a conformational change that activates the intracellular protein tyrosine kinase domain through autophosphorylation**[7]**.Although they are expressed in a variety of tissues, HGF and c-MET are typically limited to mesenchymal and epithelial cells. The crucial roles that HGF and c-MET play in normal embryonic development and organogenesis are supported by research on mouse genetics. In contrast, adult HGF and c-MET functions are more limited and primarily related to tissue damage repair and regeneration**[8]**.An important class of biological targets for cancer intervention is represented by oncogenic protein kinases. Among them is the receptor tyrosine kinase (RTK) c-Met, which is dysregulated in many tumor types but exhibits low activity in normal tissues**[9]**. The receptor tyrosine kinase MET is encoded by the MET proto-oncogene, which is situated on chromosome 7q21-31**[10]**.

MATERIALS AND METHODS

The primary source of three-dimensional structural data for proteins, nucleic acids, and complex molecular assemblies is the Protein Data Bank (PDB), accessible through its website (https://www.rcsb.org/). Managed by the Worldwide Protein Data Bank (wwPDB) consortium, the PDB ensures global accessibility to this invaluable dataset, which encompasses information from various experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. This repository is the culmination of collaborative efforts from scientists and biologists worldwide. Utilizing the advanced search and visualization features provided by the Protein Data Bank Japan (PDBj) website, researchers can navigate through individual entries in the PDB with ease. Molecular viewers enable the three-dimensional visualization of PDB structures, facilitating deeper insights into molecular architecture and function. For protein structural validation**[12]**, the ERRAT (or VERIFY3D-ERRAT) application serves as a valuable tool. By assessing non-bonded interactions within protein structures, ERRAT computes an "overall quality factor" or "ERRAT score," providing a statistical measure of structure quality. This tool assists in validating protein structure models generated by various methods, including homology modeling and X-ray crystallography. In the field of bioinformatics, tools and servers, such as ERRAT, play a vital role in validating protein structures, providing researchers with access to a variety of resources for analyzing biological data. These tools form an integral part of the bioinformatics infrastructure, facilitating advancements in molecular biology research. To visualize and analyze molecular structures stored in PDB files, the RasMol software program offers a user-friendly solution**[13]**. Its interactive interface allows users to manipulate molecular models, adjust visual parameters, and explore structural features. RasMol is compatible with the mmCIF format, which makes it more useful for visualizing macromolecular crystallographic data. Moreover, The Molecular Modeling Database (MMDB) is a vast collection of three-dimensional protein structures. It provides valuable insights into the relationship between protein sequence and structure and also aids in making multiple sequence alignments. Updated every week, MMDB contains a comprehensive assortment of molecular complexes obtained from various experimental sources.The National Center for Biotechnology Information (NCBI) is a platform that provides access to genetic and biomedical information, which supports research endeavors in molecular biology. Collaborative efforts with laboratories and databases contribute to the continuous expansion of resources such as the GenBank DNA sequence database. To aid in the interpretation of genetic and protein sequence data, sequence analysis tools like FASTA and BLAST enable rapid homology searches and sequence alignments. BLAST, in particular, offers multiple search programs to cater to diverse research needs, facilitating comparisons between nucleotide and protein sequences. Furthermore, software packages like PyMOL and CB-Dock provide versatile solutions for molecular visualization and docking studies**[14]**. PyMOL isa user-friendly interface and Python-based architecture enhances its accessibility and functionality, making it a popular choice for research tasks in drug discovery and computational biology. CB-Dock, on the other hand, specializes in molecular docking simulations, offering advanced algorithms for predicting ligand-protein interactions and binding affinities.

RESULTS AND DISCUSSION

1. STRUCTURE VALIDATION

The ERRAT structure validation server was employed to assess the quality of the protein sample represented by the PDB entry 6UBW. Upon analysis, an overall quality factor of 96.198 was observed, indicating excellent structural integrity and overall quality of the protein model. This high ERRAT score suggests that the protein sample meets the criteria for robustness and reliability, thereby validating its suitability for further biological research work. In the context of ERRAT analysis, higher values correspond to superior structural quality, affirming the integrity of the protein structure under investigation.

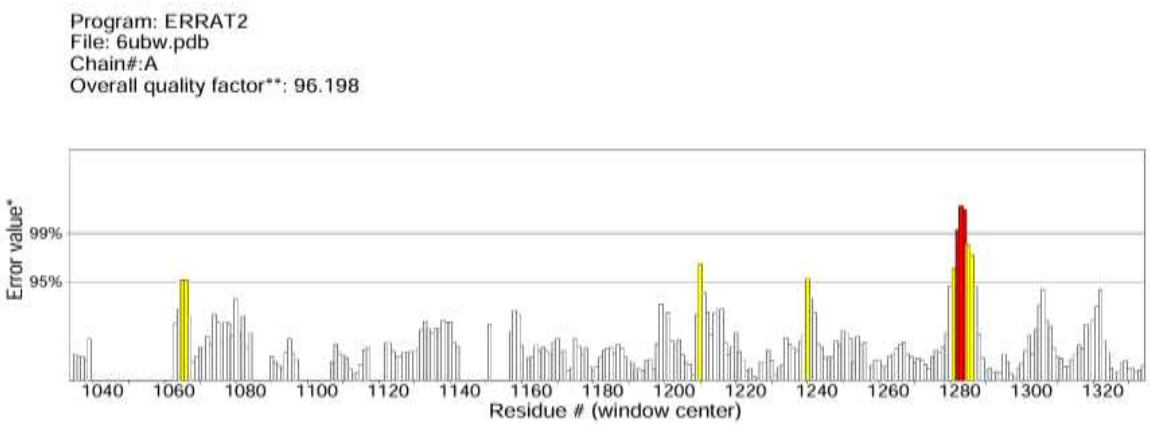


Figure1: ERRAT result of 6UBW

2. RAMACHANDRAN PLOT

The Ramachandran plot analysis for a protein with 296 residues, using the SAVES server, shows that 92.5% of the residues are in the core region, 6.7% in the allowed region, and 0.4% each in the generous and disallowed regions. Three residues were flagged, with five side-chain parameters better than expected. There was one bad contact and bond lengths/angles had minor deviations. All planar groups were within limits, indicating a well-constructed model with a few areas needing further investigation.

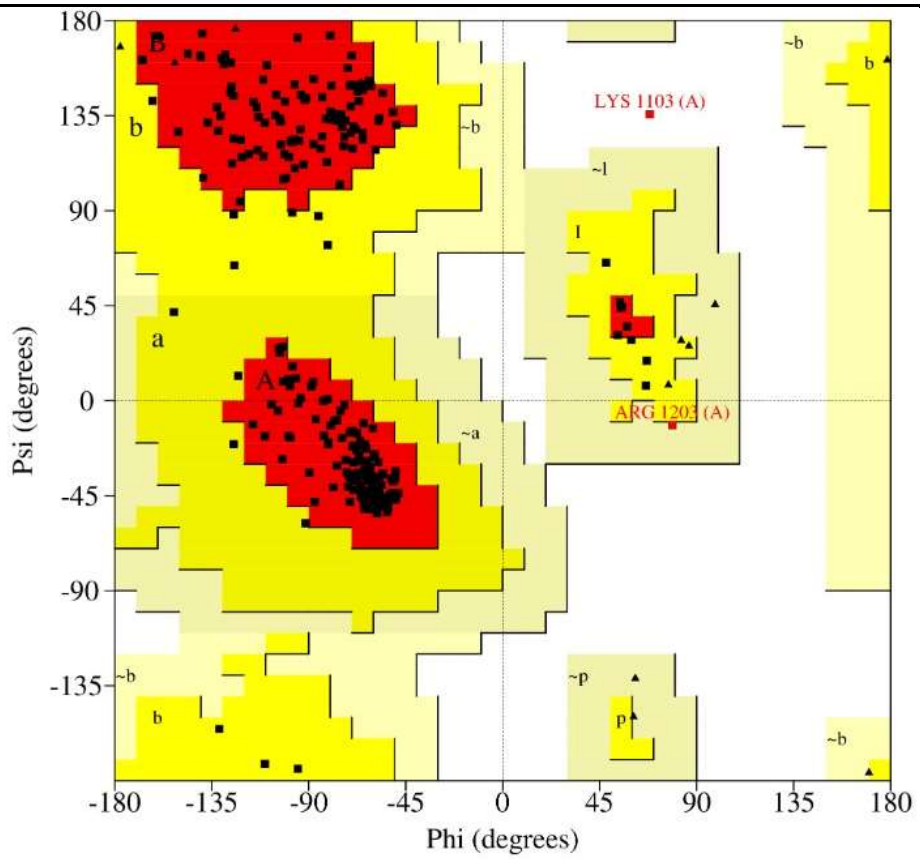


Figure 2: Validation of modeled protein using Ramachandran plot of PROCHECK analysis.

3. Raster molecular (Molecular graphic visualization)

In this investigation, we used a program that can view PDB files and used the four-character PDB ID format, in which the first digit is always a number (6UBW). These IDs are used for organizational purposes and have no intrinsic meaning. We visualized and analyzed the three-dimensional structure of the hepatocyte growth factor receptor (HGFR) using this software. The MET tyrosine kinase protein, commonly referred to as c-MET or simply MET, is produced by the human MET gene.MET is a receptor tyrosine kinase (RTK) that is essential for invasion, migration, growth, and survival, among other cellular processes. When the MET receptor binds to hepatocyte growth factor (HGF), also referred to as scatter factors (SF), it sets off signaling cascades that control important physiological functions in cells.

The 3D structure can be presented to users based on their preferences, with the ability to customize its color for detailed structural analysis. In our study, we assigned red to represent high temperatures and blue for low temperatures. Upon analysis, the predominant blue color in our sample suggests it is characterized by low temperatures.

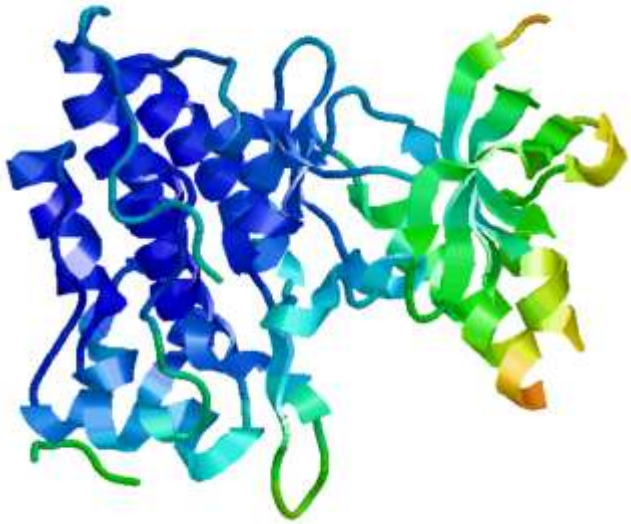


Figure 3: RasMol result observed in temperature form (6UBW)

Using RasMol, we analyzed a protein's structure,with specific amino acids color-coded for clarity. Glutamic acid appeared red, adenine light blue, arginine dark blue, alanine green, proline dark gray, cysteine yellow, leucine olive green, and glycine white [15]. This visualization helped us understand the arrangement of these amino acids in the protein, offering insights into its function and interactions.

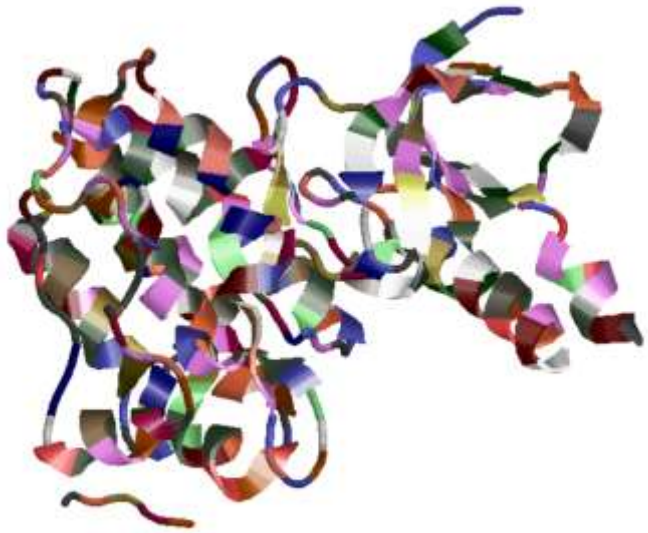


Figure 4: RasMol result observed in shapely form (protein structure)

In this study, RasMol was utilized to analyze the structure of the human MET tyrosine kinase protein. Through a color-coded system, alpha helices were represented in magenta, and beta strands in yellow, aiding in their clear identification. This visualization provided valuable insights into the spatial arrangement of the protein and its potential functional regions.

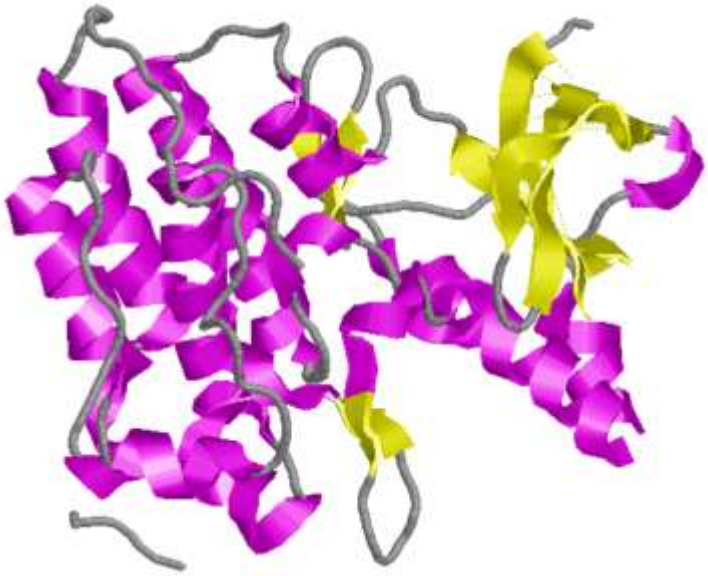


Figure 5:Hydrogen bond on representing (H…192) RasMol command line result

4. Sequence Similarity Search

In our study, we utilized the BLAST software, a powerful sequence similarity search program available in two main forms: Input and Output. The Input form accepts sequences in FASTA format and GenBank format, while the Output form provides results in HTML format, XML format, and plain text format.

In This study, we assigned colors to represent membrane preference: red indicates low membrane preference, while green signifies high membrane preference[19]. This color scheme facilitates the visual analysis of membrane preferences within our experimental data, providing insights into the relative affinity of molecules for membrane interactions.

Figure 6: Comprehensive analysis of Membrane Preference

The Cobalt Hydropathy Scale is a method used to assess the hydrophobicity or hydrophilicityof amino acid residues in a protein sequence. It assigns a numerical value to each amino acidbased on its relativehydrophobic or hydrophilic nature.

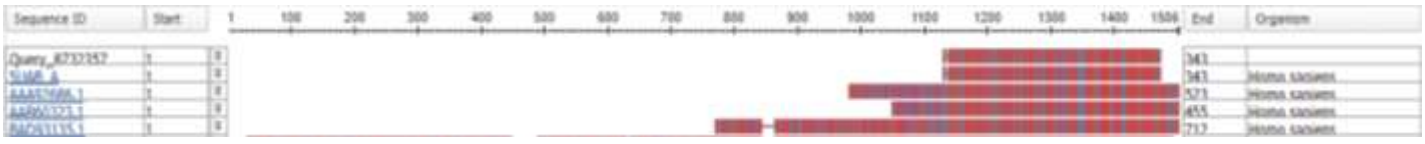


Figure 7: The Cobalt result visualizes the hydropathy scale, with hydrophobic amino acids depicted in red and hydrophilic amino acids depicted in blue.

6.PYMOL

It is a versatile molecular graphics tool utilized for 3D visualization across different platforms. Beyond visualization, it facilitates molecule editing, ray tracing, and more. Notably, Pymoleliminates the need for manual text file editing by seamlessly producing high-quality graphics with Molscript. With its capability to display eight distinct forms, Pymol offers researchers a comprehensive toolkit for exploring and analyzing 3D molecular structures.

In Pymol, a polar contact is depicted as a yellow dash.

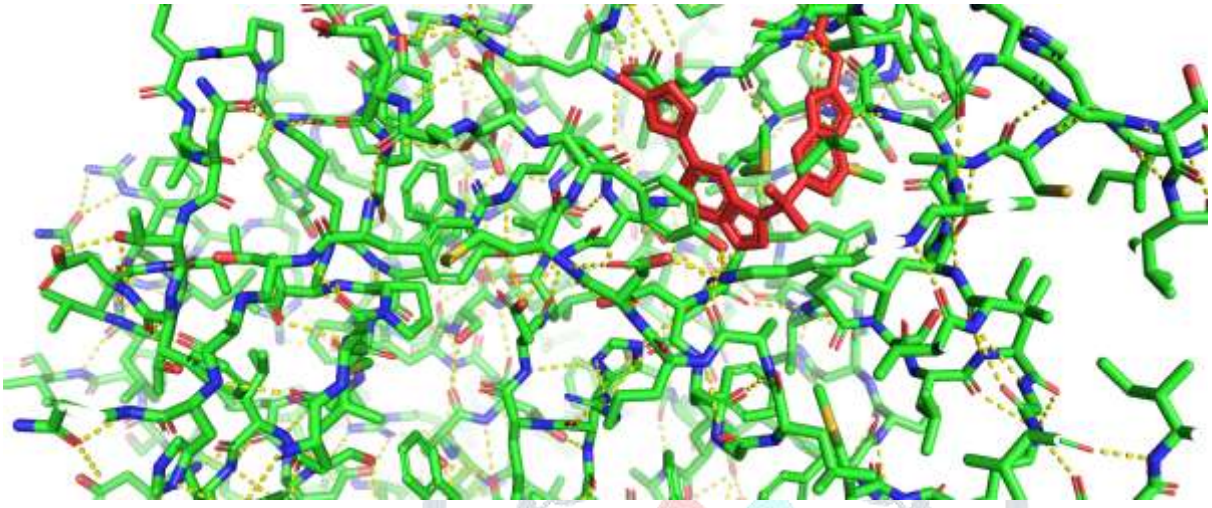


Figure 8: Polar contact analysis showing interaction between potential therapeutic compound and tumor related protein target

In Pymol, active sites of biological samples can be identified and visualized. By employing Pymol tools and features, researchers can pinpoint specific regions within the molecular structure crucial for biological activity. This capability facilitates detailed analysis and interpretation of protein-ligand interactions, ultimately aiding in the discovery of potential drug targets and therapeutic strategies.

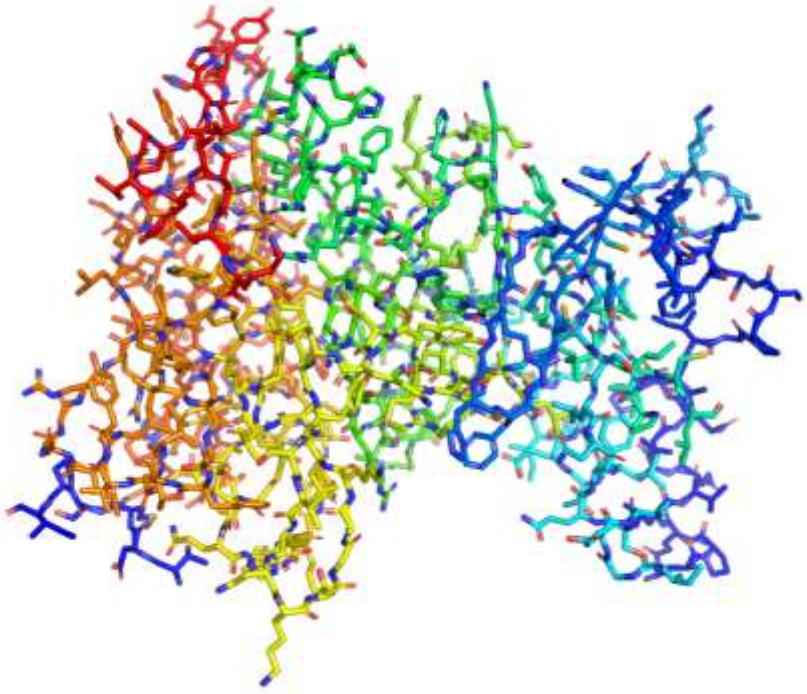


Figure 9: PYMOL active site representation on stick form

In the RMSD analysis, the calculated RMSD value is approximately zero, indicating a value of 0.108Å, which is considered a favorable result. In our analysis, the structure represented by the PDB ID 5UAB is depicted in red, while the tumor efficacy of 6UBW is visualized in yellow. These color representations aid in the clear visualization and interpretation of our findings, facilitating further investigation into the analyzed molecules' structural characteristics and functional properties.

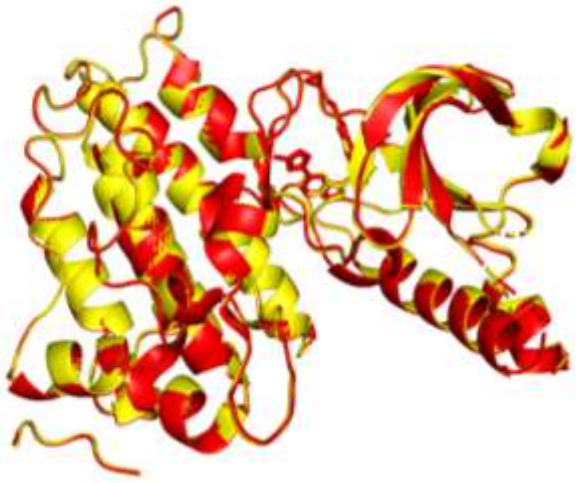
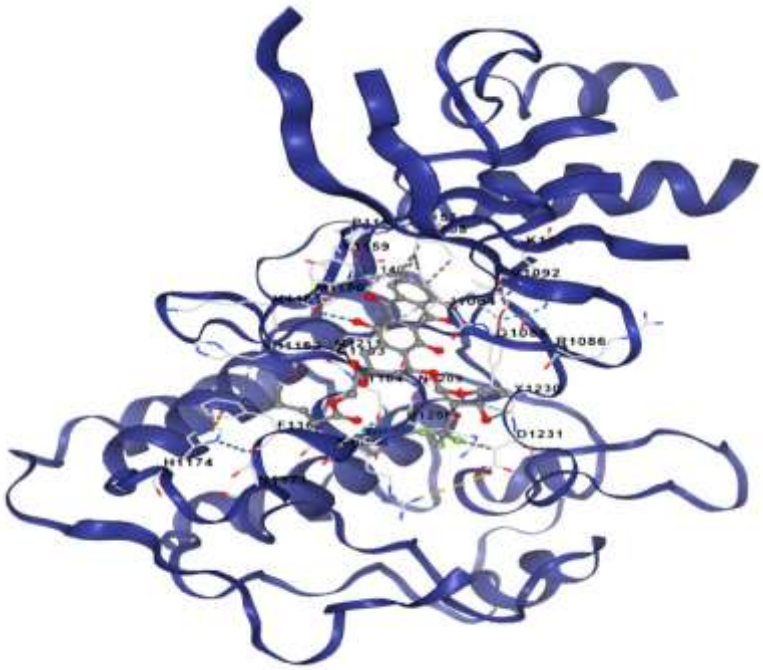


Figure 10: In the RMSD analysis, the RMSD calculation value is nearzero, showing 0.108, a fairly good value. 5UAB is represented by redcolor and 6UBW tumor efficacy shows yellow color.

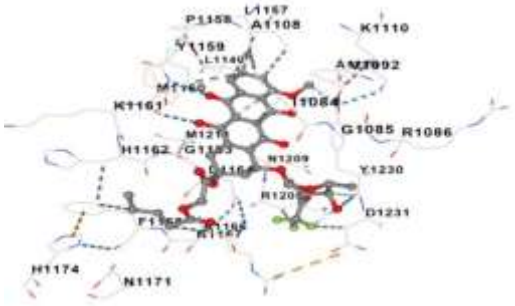
7.MOLECULAR DOCKING

In our study, we used molecular docking, a computer-based method crucial for predicting how drugs interact with proteins. This helps in designing drugs effectively by simulating their interactions with target proteins. We employed CB-DOCK software known for its accuracy in predicting these interactions. This approach aids in discovering potential drugs efficiently, streamlining the drug discovery process for various diseases.

The present study aims to explore the possibility of targeting the brain cancer-associated protein molecule 6UBW with antitumor drugs. One medication that is commonly used to treat bladder cancer, valrubicin, has shown promise in treating glioblastoma multiforme (GBM), a very aggressive type of brain cancer. Although the results of the early trials are encouraging, more study is required to confirm its efficacy against GBM. Another medication under investigation for its ability to treat different tumors, including GBM, is mitoxantrone. These results imply that these medications may be useful in the treatment of brain cancer utilizing adaptable docking mechanisms. Additionally, we are looking at a group of medications known as anthracyclines, which include idarubicin, daunorubicin, doxorubicin, and epirubicin. Currently, anthracyclines are employed in the treatment of various types of cancer, such as sarcomas, lymphomas, breast cancer, and leukemia. They work especially well against acute leukemias, including acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). This emphasizes the possibility of using anthracyclines as a cancer treatment, particularly for leukemia.



(A)

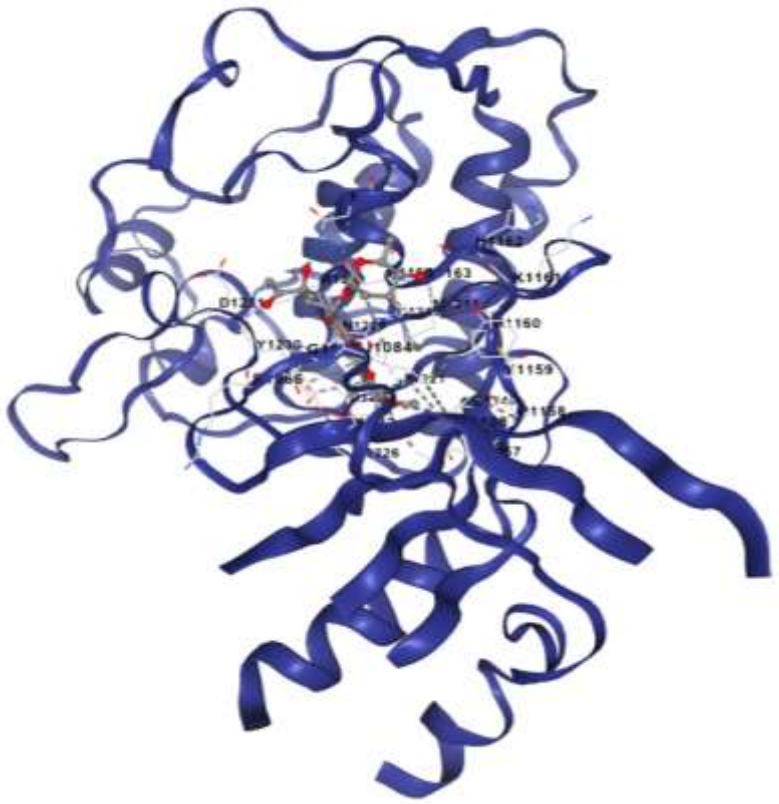


(B)

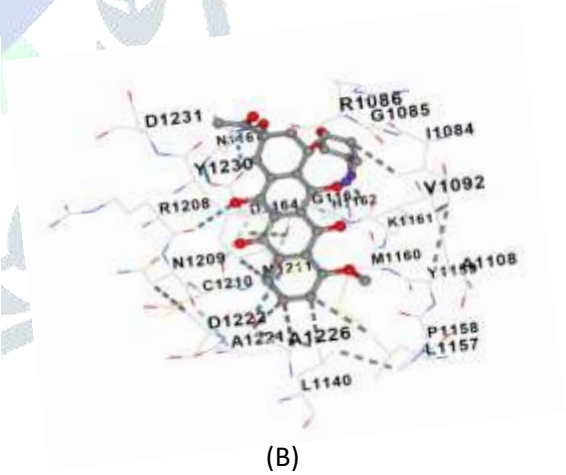
CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
C1	-8.3	929	10, -11, 18	23, 23, 23
C5	-7.5	187	16, 0, 27	23, 23, 23
C2	-6.9	296	24, 1, 1	23, 23, 23
C4	-6.6	223	-4, -8, 16	23, 23, 23
C3	-6.1	266	15, 17, 7	23, 23, 23

(C)

Figure 11 : (A) Molecular Docking of Valrubicin with Protein 6UBW, (B) Contact residues of Valrubicin and (C) Docking Resul



(A)



(B)

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
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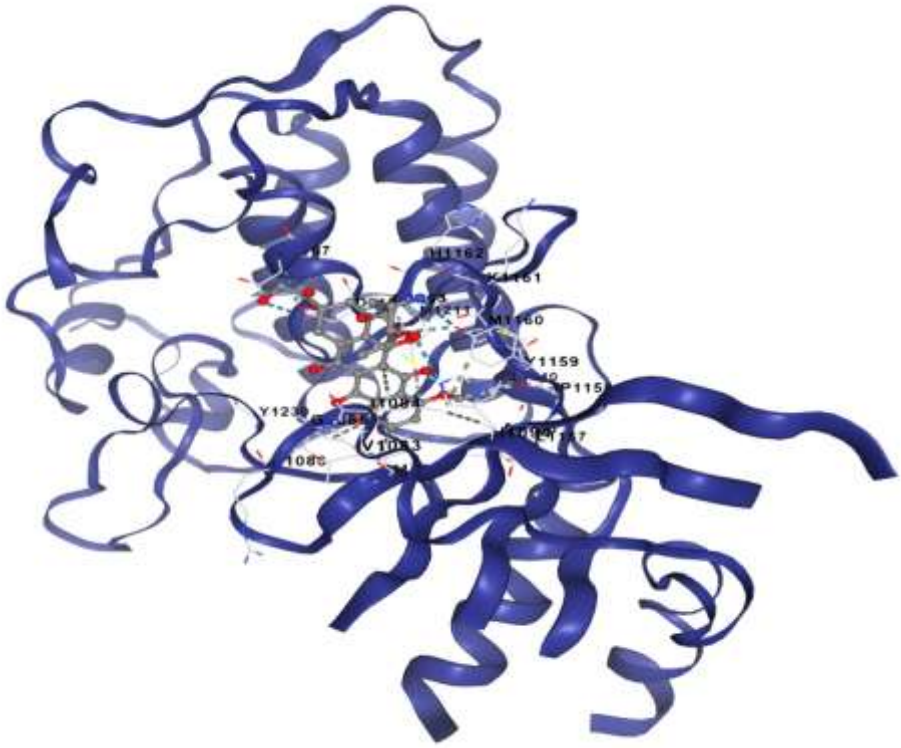
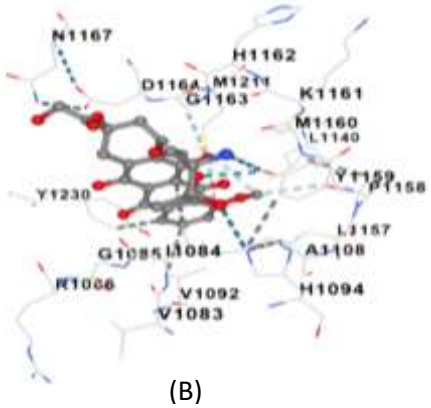
C2	-6.7	296	24, 1, 1	24, 24, 24
C4	-6.5	223	-4, -8, 16	24, 24, 24
C3	-6.2	266	15, 17, 7	24, 24, 24

(C)

Figure 12 : (A) Molecular Docking of Daunorubicin with Protein 6UBW, (B) Contact residues of Daunorubicin, and (C) Docking Results



(A)



(B)

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
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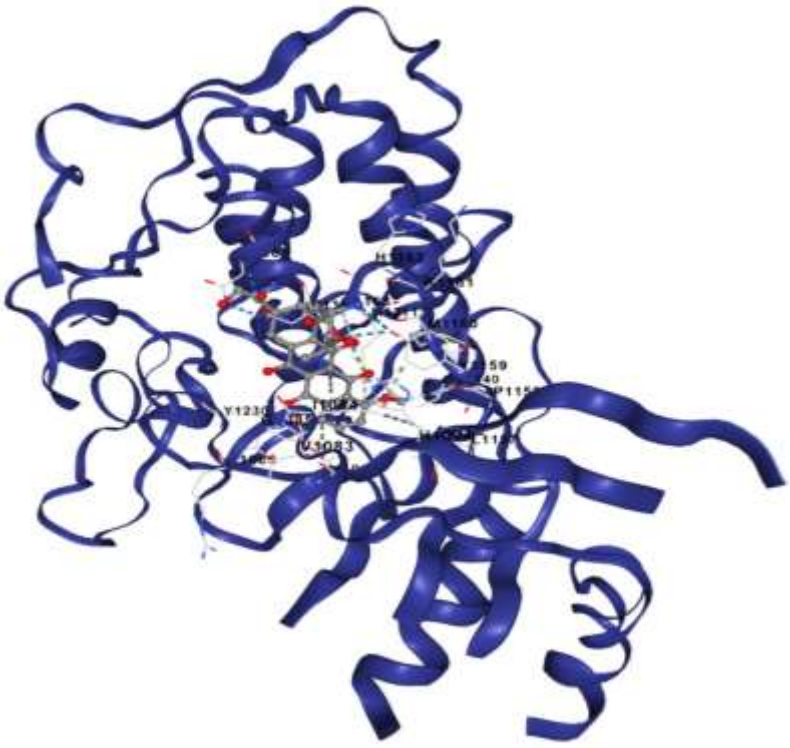
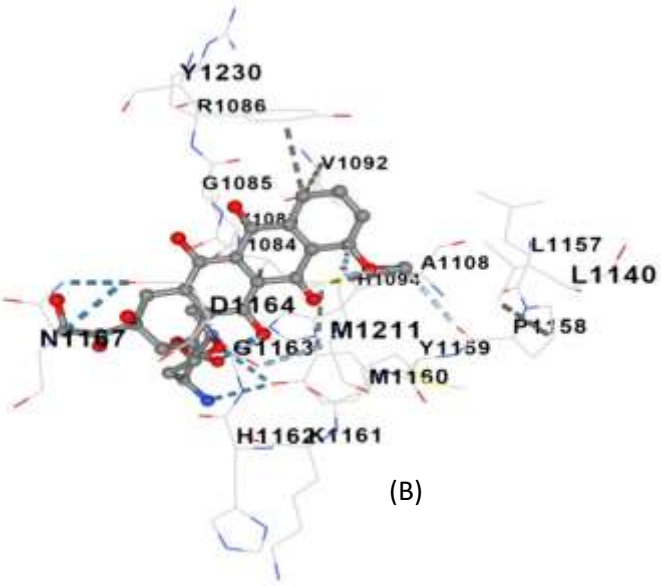
C2	-6.9	296	24, 1, 1	23, 23, 23
C4	-6.6	223	-4, -8, 16	23, 23, 23
C3	-6.1	266	15, 17, 7	23, 23, 23

(C)

Figure 13 : (A) Molecular Docking of Doxorubicin with Protein 6UBW, (B) Contact residues of Doxorubicin, and (C) Docking Result



(A)



(B)

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
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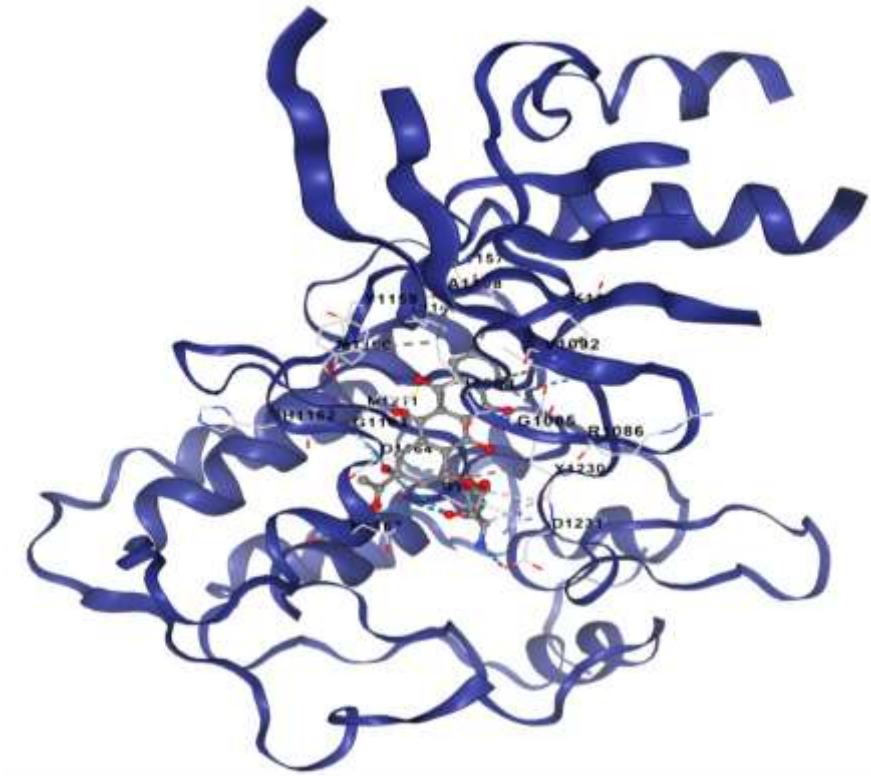
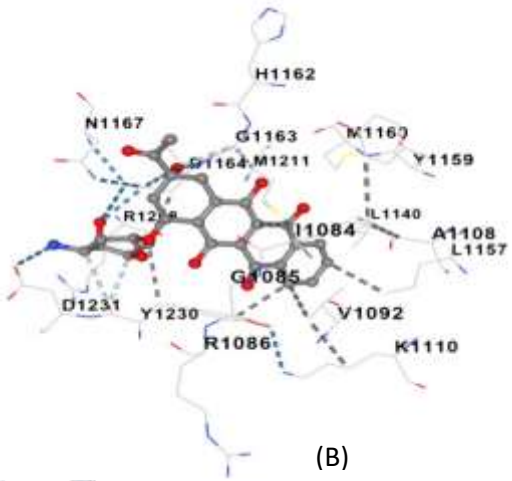
C2	-6.8	296	24, 1, 1	23, 23, 23
C4	-6.7	223	-4, -8, 16	23, 23, 23
C3	-6.3	266	15, 17, 7	23, 23, 23

(C)

Figure 14 : (A) Molecular Docking ofEpirubicinwith Protein 6UBW, (B) Contact residues of Epirubicin, and (C) Docking Results



(A)



(B)

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
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C5	-7.0	187	16, 0, 27	23, 23, 23
C2	-6.8	296	24, 1, 1	23, 23, 23
C4	-6.7	223	-4, -8, 16	23, 23, 23
C3	-6.3	266	15, 17, 7	23, 23, 23

(C)

Figure 15: (A) Molecular Docking of Idarubicin with Protein 6UBW, (B) Contact residues of Idarubicin, and (C) Docking Results

8.INTERPROSCAN

InterProScan is a valuable tool for protein researchers. It combines various methods to analyze protein sequences, identifying key features like functional domains and characteristics. This aids in database matching and enhances understanding of protein functions. For instance, it associates proteins with Gene Ontology (GO) terms, elucidating their cellular roles and aiding in the comprehension of biological processes.

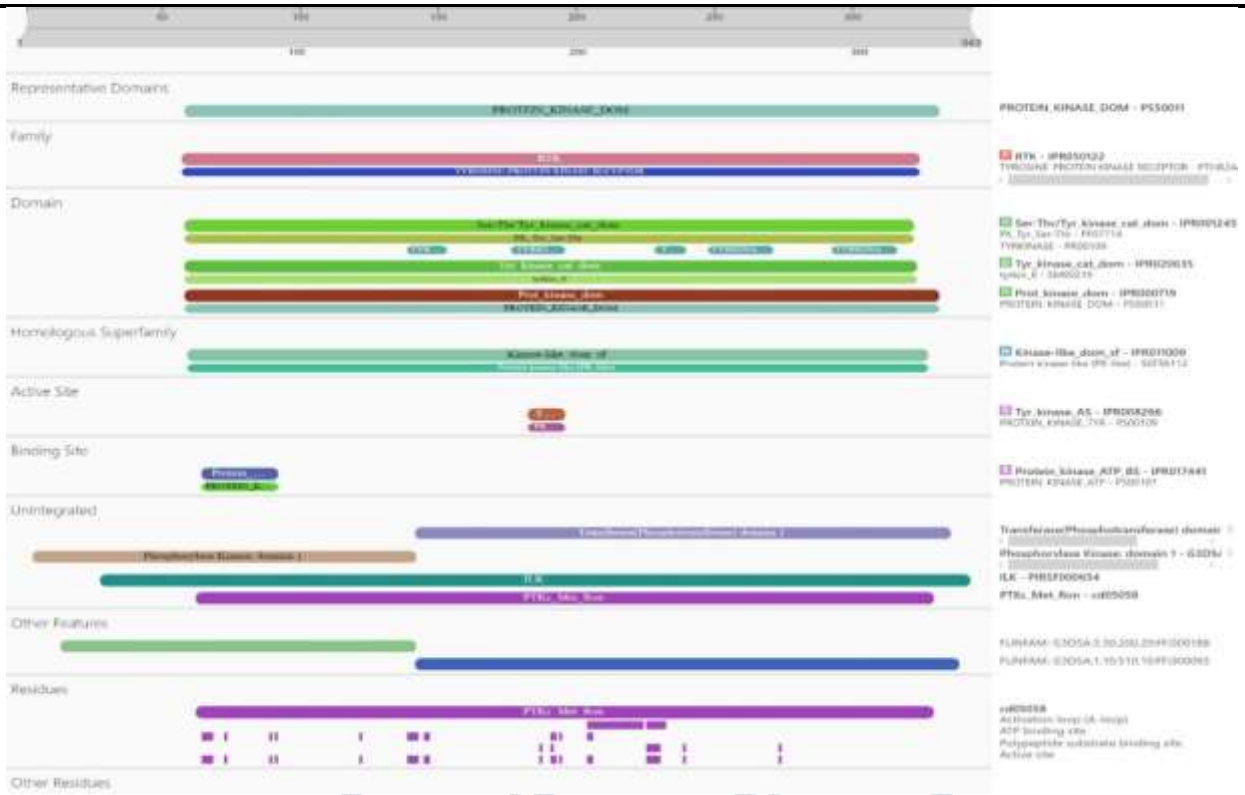

Figure 16: Functional annotation and protein domain identification through protein signature classification and family analysis are possible with Interproscan in biological research(6UBW).

In our study, we use Gene Ontology (GO) to understand protein phosphorylation better. We created a chart showing the connections between different terms related to protein phosphorylation. We're looking at a specific protein from plate number 1. By studying this chart, we can see how different terms are linked, giving us insights into how phosphorylated proteins work and are regulated.

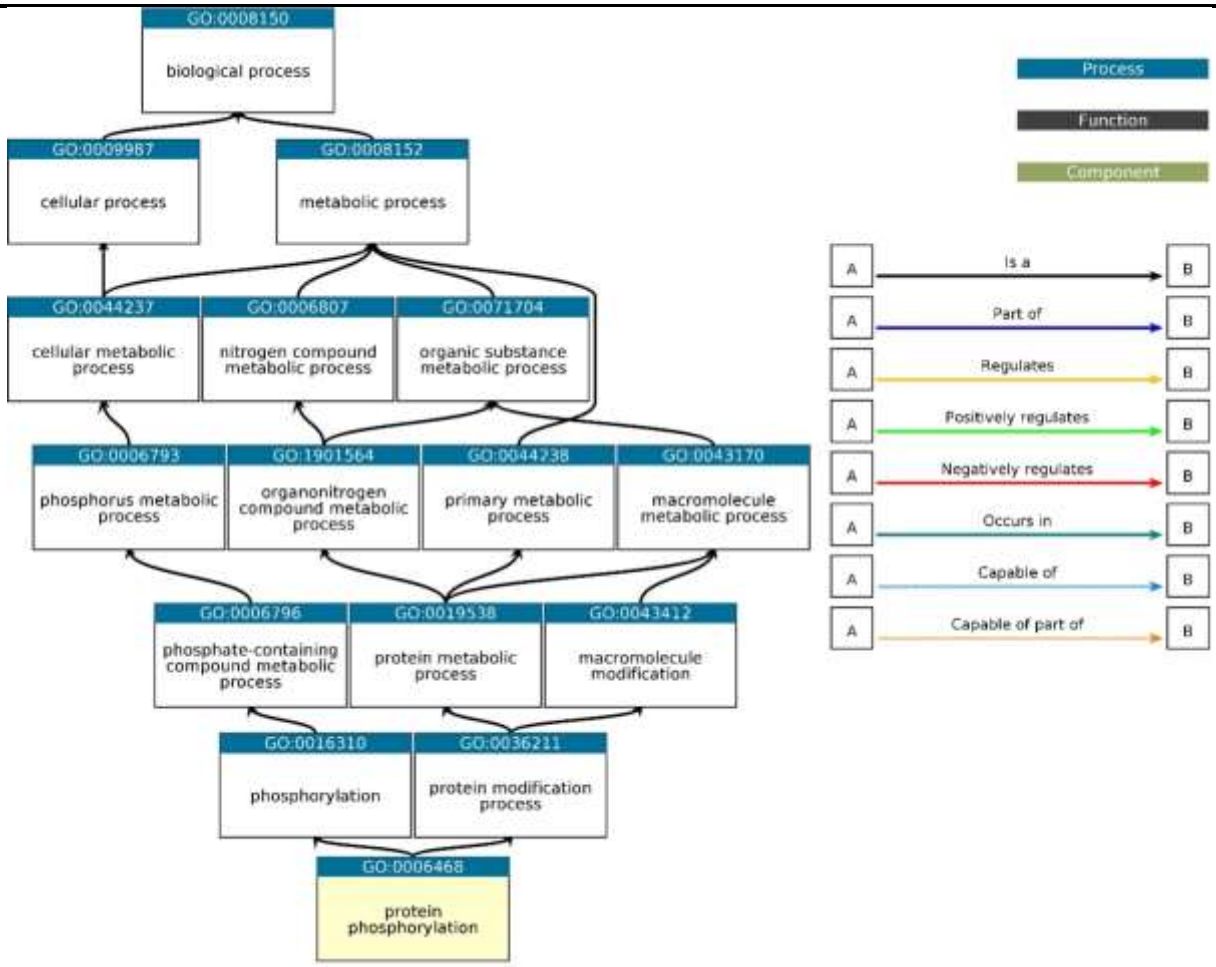

Figure 17: Gene ontology of protein phosphorylation Ancestor chart of protein sample (6UBW)

Conclusion

The investigation into the structural and functional analysis of the MET tyrosine kinase for tumor efficacy to check the binding affinity to the tumor-specific target proteinsshows the strong potential as a therapeutic agent.Tumor efficacy analysis aimed at evaluating the protein domain,active site,binding site,and functional sites associatedwith tumor efficacy has provided valuable interpretation. Experimental validation of polar contact refinement will be essential in translating these computational identificationsinto clinically effective treatment. Active site identification provides molecular targets for potential therapeutic intervention, ERRAT providesa comprehensiveevaluation of the structure quality of tumor-related protein model,highlighting both strengths and areas that need improvement showing experimental validation,leading to the design of inhibitors thatcan advance cancer treatment strategies.

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