PROTAC-Mediated Proteasomal Degradation of Src Homology 2 Domain–Containing Phosphatase 2 (SHP2) for Solid Tumor Therapy

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Unlike liquid tumors such as leukemia and lymphoma, solid tumors are abnormal masses of tissue that grow in a solid shape. SHP2 protein, encoded by the PTPN11 gene, plays a crucial role in solid tumor development, progression, and metastasis by integrating signals from various membrane receptors and promoting cell survival and reproduction. Proteolysis targeting chimera (PROTACs) are molecules designed to degrade target proteins rather than inhibiting them and can be used in the proteasomal degradation of the SHP2 protein. We hypothesize that PROTAC P1 will bind firmly to the SHP2 and E3 Ligase protein. AlphaFold 3 was used to produce the SHP2 protein structure, while the PROTACs were modeled using YASARA software. Molecular docking simulations using HDOCK were performed to understand the protein-PROTAC interactions. Based on these interactions, chemical mutations were performed using the CReM (Chemically Reasonable Mutations) webserver to generate a PROTAC mutant library. For each PROTAC mutation, PLIP was used to identify hydrophobic interactions and hydrogen bonds, while PRODIGY calculated the binding free energy. The binding energy of the mutant P1 PROTAC was highest (-10.02 kcal/mol) and was selected for further pharmacokinetic and pharmacodynamic analysis. In future studies, we will be performing PROTAC optimization to enhance gastrointestinal absorption and bioavailability. The current research will help in designing novel PROTACs with improved binding affinity and optimized protein ligand interactions for enhanced therapeutic effectiveness against solid tumors.

1. INTRODUCTION

Solid tumors are abnormal masses of tissues that form when cells grow and divide uncontrollably, potentially becoming cancerous (malignant) or remaining non-cancerous (benign). [1] Symptoms are dependent on the location of the solid tumor. However, some common symptoms include fatigue, unexplained weight loss, pain, lumps or swelling, skin changes, and changes in bowel or bladder habits. [2] Solid tumors can develop in various parts of the body, including breast, lung, colon, prostate, brain, pancreas, liver, ovaries, and kidney. [3] Current treatment options for solid tumors include surgery (removal of the tumor), radiation therapy (Uses high-energy rays to kill cancer cells), chemotherapy (drugs that kill fast growing cells), targeted therapy (drugs that target specific mutations), immunotherapy (boosts the immune system to fight cancer), and cellular therapies (regenerative medicine that uses living cells). Side effects for surgery are pain, infection, scarring, and loss of function depending on the organ. Side effects for radiation are fatigue, skin burns, and damage to nearby organs. Side effects for chemotherapy are hair loss, nausea, vomiting, infections, and neuropathy. Side effects for targeted therapy are skin rash, diarrhea, liver issues, and heart damage (sometimes). Side effects for immunotherapy are inflammation of healthy organs (lungs, liver, skin).

SHP2 (Src Homology region 2-containing Protein Tyrosine Phosphatase 2), also known as PTPN11 (Protein Tyrosine Phosphatase, Non-Receptor Type 11). [4] SHP2 can also be involved in drug resistance in cancer, making it a potential target for overcoming resistance to therapies. SHP2 is a non-receptor protein. SHP2 modulates diverse cell signaling events that control metabolism, cell growth, differentiation, cell migration, transcription, and oncogenic transformation. [5] It interacts with diverse molecules in the cell, and regulates key signaling events RAS/ERK, PI3k/AKT, JAK/STAT, and PD-1 pathways downstream of several receptor tyrosine kinases (RTKs) upon stimulation by growth factors and cytokines. [4] SHP2 protein is found inside the cell, primarily in the cytoplasm. SHP2 is a mediator in tumor-related signaling pathways, a suppressor of PD-1, associated with solid tumors.

AlphaFold is a web service that can generate highly accurate biomolecular structure predictions containing proteins, DNA, RNA, ligands, ions, and model chemical modifications for proteins and nucleic acids in one platform. [6] It's powered by the

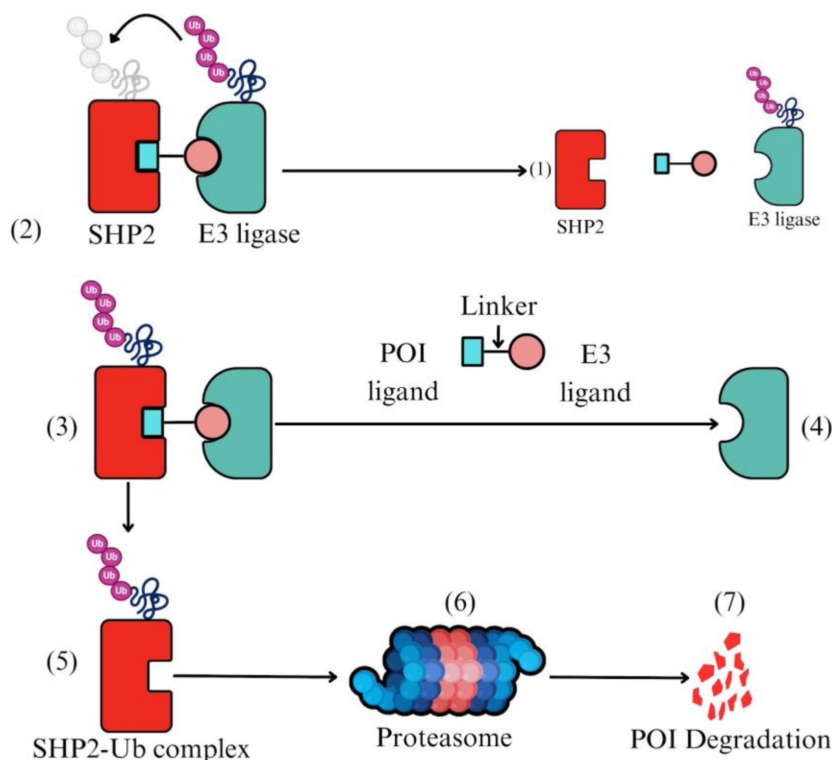

Fig. 1. It shows the mechanism of action of PROTAC step initially in the shp2 and E3 ligase are far. In the presence of PROTAC, PROTAC will help bring SHP2 and E3 ligase close which will result in ubiquitin translocation to the SHP2 protein. Finally, the PROTAC and E3 ligase are released, and the SHP2-UB complex is degraded by Proteasome.

newest AlphaFold 3 model. The initial version was published in 2018. This version demonstrated the potential of deep learning for protein structure prediction, but the code wasn't made broadly available for general use. In 2020, AlphaFold 2 was a breakthrough. AlphaFold 2 achieved unprecedented accuracy in protein structure prediction. While the AlphaFold 2 paper was published in July 2021 alongside open-source software, the initial server access for AlphaFold 3 (later released) drew criticism for its restrictions. Next, in 2021, AlphaFold Protein Structure Database was launched as a collaboration between DeepMind and EMBL-EBI. This database initially provided access to AlphaFold's predicted structures for humans and 20 model organisms. The database has since expanded dramatically. In the same year, AlphaFold-Multimer was launched as an update and expanded AlphaFold's capabilities to predict protein complexes. Finally, in 2024, AlphaFold 3 was co-developed by Google DeepMind and Isomorphic Labs. AlphaFold 3 can predict the structures of complexes involving various biomolecules, including proteins, DNA, RNA, and ligands. Access to AlphaFold 3 was initially restricted through a web server, but DeepMind later made the code and models openly available for non-commercial research. The AlphaFold server was specifically created to provide free access to AlphaFold 3 for non-commercial research.

2. METHOD

Uniprot is a comprehensive and freely accessible resource for protein sequence and functional information. We used this tool to find the bad protein, SHP2, and protein sequence. AlphaFold Server is a free, web-based platform powered by AlphaFold 3 that allows scientists to predict the structures of biomolecular complexes. Furthermore, it can predict the 3D structure of proteins. We used this tool to predict the 3-dimensional structure of the bad protein, SHP2, using the protein sequence from Uniprot. Protter is a web-based tool that allows researchers to visualize protein sequences, their topological features, and experimental proteomic data in an integrated and interactive way. [7] We used this tool to see the location of the protein chain, which is in the cell behind the cell membrane. The Human Protein Atlas is a publicly available database and resource that aims to map the location and abundance of all human proteins in cells, tissue, and organs. [8] We used the subcellular resource of this tool to show the subcellular localization of the bad protein, SHP2, in single cells. Molecular docking simulations are computational techniques used to predict how two molecules interact with each other at the atomic level. [9] Typically, a protein (receptor) and a small molecule or peptide (ligand). The goal for using this tool is to find the best binding orientation and estimate the strength of the interaction. We used this Molecular Docking Simulation to find the best binding orientation between the bad protein (SHP2) and PROTAC; good protein (E3 ligase) and PROTAC; and good protein (E3 ligase) and bad protein (SHP2)

OpenBabel is an open-source chemical informatics tool (it can be used as a software package, command line tool, or through online interfaces/web servers).(O'Boyle et al., 2011) It lets researchers interconvert between many chemical file formats (PDB, MOL2, SMILES, SDF), visualize molecules, and do basic manipulations. We used OpenBabel to convert PROTAC.pdb to SMILES so the molecule could be represented as a string, enabling easier storage, searching, and use in cheminformatics tools. CReM, or Chemically Reasonable Mutations, is a fragment-based, open-

source framework (both a Python module and a web application) for generating chemically valid, and potentially synthetically feasible, structures by modifying input molecules based on a database of interchangeable fragments. [10] The core concept relies on matched molecular pairs with context: fragments that appear in identical local chemical environments are considered interchangeable. We used this tool for the fragment-based context matching to ensure that replacements produce valid molecules, minimizing chemically nonsensical outcomes. A free, online ADMET prediction platform (ADMETlab 2.0) using multi-task graph attention models for accurate, comprehensive chemical profiling. We used this due to the fact it efficiently evaluates multiple molecules' pharmacokinetic and toxicity profiles across a wide range of endpoints all in one place, with no registration required. PLIP stands for Protein–Ligand Interaction Profiler. It's an open-access tool available both as a web service and a command-line/Python package. It automatically identifies and visualizes non-covalent interactions between proteins and ligands. It supports input via PDB IDs or uploaded PDB files. [11] We use PLIP for precise mapping of how your PROTAC molecule engages SHP2, identifying key hydrogen bonds, hydrophobic patches, and other stabilizing interactions which is crucial for understanding binding affinity and specificity. Prodigy (Protein–protein binding energy prediction) is an online bioinformatics tool developed by Utrecht University that estimates the binding affinity between two interacting proteins. We used this tool to estimate how strongly our PROTAC binds to the target protein, helping validate and compare docking results. [12] A more negative binding energy indicates a stronger and more stable interaction.

3. RESULTS

The computational workflow successfully identified and evaluated multiple PROTAC molecules targeting the SHP2 protein for potential solid tumor therapy. Structural prediction, molecular docking, mutation generation, and pharmacokinetic analysis were integrated to identify the most stable and biologically favorable PROTAC candidate. Among all screened molecules, mutant P1 demonstrated the strongest interaction with SHP2 and E3 ligase, suggesting its potential effectiveness in mediating targeted proteasomal degradation of SHP2.

Figure 1 demonstrates the proposed mechanism of PROTAC-mediated degradation of SHP2. Initially, SHP2 and the E3 ligase remain spatially separated inside the cell. Upon introduction of the PROTAC molecule, the bifunctional molecule simultaneously binds SHP2 and E3 ligase, forming a ternary complex. This interaction promotes ubiquitin transfer to SHP2, ultimately leading to proteasomal degradation of the SHP2-ubiquitin complex. The figure highlights the catalytic degradation mechanism of PROTACs rather than simple inhibition of the target protein. The SHP2 protein structure generated using AlphaFold 3 showed a stable folded conformation suitable for molecular docking simulations. Protein localization analysis using Protter and the Human Protein Atlas confirmed that SHP2 is predominantly localized within the intracellular cytoplasmic region, supporting its accessibility to intracellular PROTAC molecules. These findings validate SHP2 as a promising therapeutic target for intracellular degradation strategies in solid tumors. Figure 2 illustrates the intracellular localization of SHP2 protein. The Protter analysis identified the protein topology and intracellular orientation, while Human Protein Atlas data confirmed cytoplasmic localization within the cell. Since PROTAC molecules function intracellularly by recruiting E3 ligases to target proteins, the intracellular localization of SHP2 supports the feasibility of PROTAC-mediated degradation.

Molecular docking simulations revealed successful formation of the SHP2–PROTAC–E3 ligase ternary complex. Docking results demonstrated that the PROTAC molecule could simultaneously interact with both SHP2 and E3 ligase proteins, which is critical for ubiquitination and degradation efficiency. The docking conformations indicated stable spatial orientation and favorable molecular interactions between all three components of the complex. Figure 3 shows the ternary complex consisting of SHP2 (pink), E3 ligase (purple), and the PROTAC molecule (green). The docking simulation generated using HDOCK demonstrated that the PROTAC successfully bridged both proteins, forming a stable interaction interface. The spatial arrangement suggests that the PROTAC linker length and orientation are sufficient to promote ubiquitin transfer from E3 ligase to SHP2.

Docking analysis of the initial PROTAC library demonstrated differences in binding affinity and interaction stability among the designed molecules. The docking simulations identified multiple hydrophobic interactions and hydrogen bonds contributing to stable binding. Among the tested molecules, P1 showed stronger interaction patterns and improved docking orientation relative to the other PROTAC candidates. Figure 4 demonstrates the molecular interaction between the PROTAC and SHP2 protein. HDOCK analysis showed that the PROTAC occupied a stable binding region within the target protein and formed multiple stabilizing interactions. The docking pose suggests favorable ligand accommodation and proper orientation required for efficient ternary complex formation.

Fragment-based chemical mutations generated using the CReM webserver produced several mutant derivatives of the parent PROTAC molecules. Interaction profiling using PLIP identified important hydrogen bonding and hydrophobic interactions that contributed to increased binding stability. PRODIGY binding energy calculations revealed that mutant P1 demonstrated the most favorable binding free energy of approximately -10.02 kcal/mol, indicating the strongest and most stable interaction among all tested compounds. Figure 5 presents the interaction profiling and binding energy analysis of the mutant PROTAC derivatives. PLIP analysis identified key hydrogen bonds and hydrophobic contacts stabilizing the protein-ligand complex. Among all variants, mutant P1 exhibited the highest number of favorable molecular interactions and the lowest binding free energy, suggesting superior binding stability and enhanced degradation potential.

Pharmacokinetic and pharmacodynamic evaluation using ADMET-AI demonstrated that the optimized PROTAC derivatives possessed acceptable drug-like properties. The computational analysis predicted favorable absorption, distribution, metabolism, excretion, and toxicity characteristics for mutant P1. These findings suggest that the optimized PROTAC may possess improved therapeutic potential and could serve as a promising candidate for future experimental validation studies. Figure 6 shows the ADMET properties and derivative analysis of the optimized PROTAC molecules. ADMET-AI predictions indicated that mutant P1 displayed favorable pharmacokinetic properties with acceptable toxicity profiles. The derivative optimization improved several drug-like characteristics, including molecular stability and predicted bioavailability, further supporting its therapeutic potential against solid tumors.

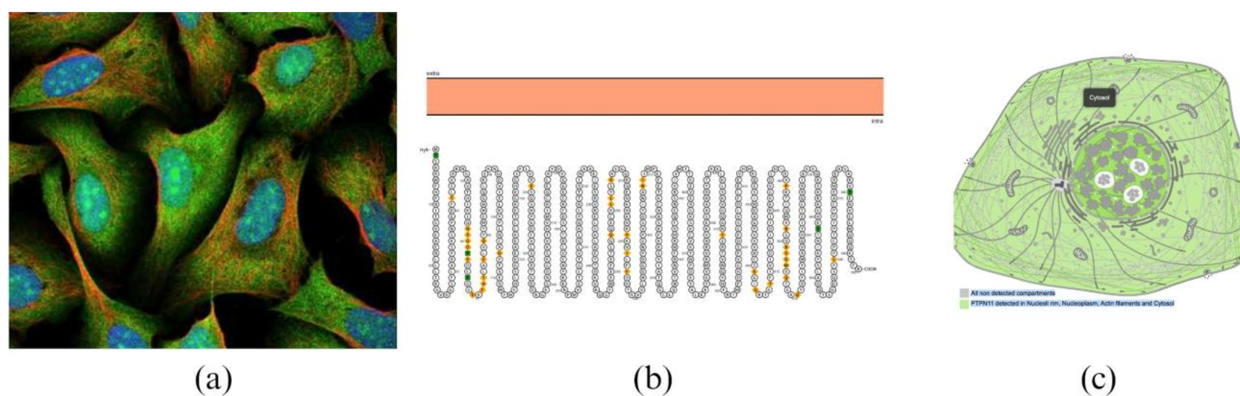

Fig. 2. It shows that the PROTAC is present inside the SHP2 protein which is present in the cell. The figure was made by usingProt-ter and the Human Protein Atlas.

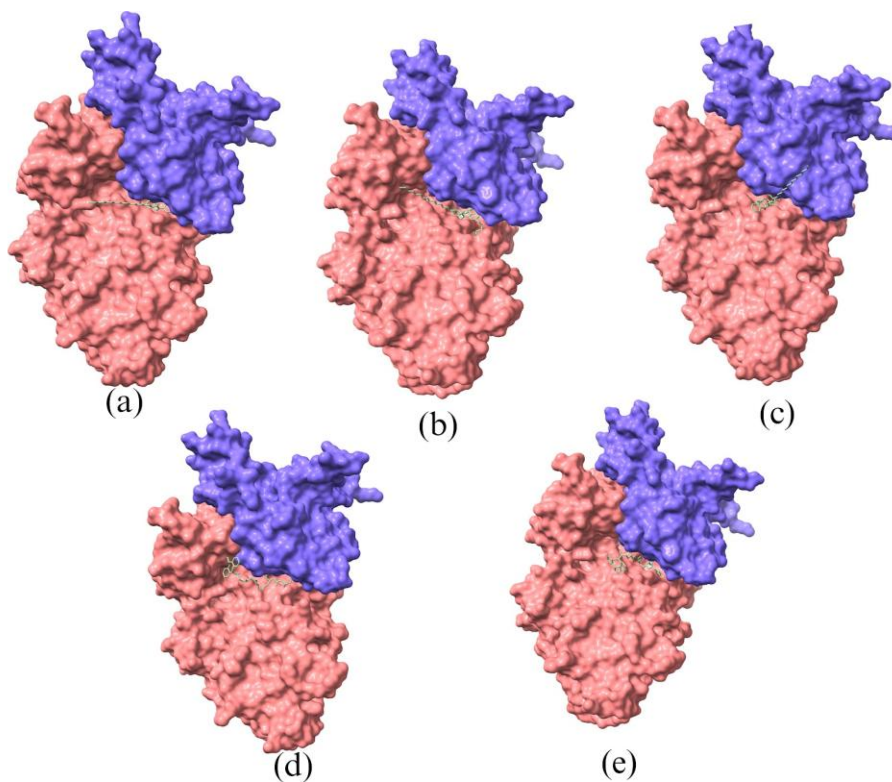

Fig. 3. The figure shows the complex of SHP2 in pink and E3 ligase in purple. The PROTAC is in green color. The figure is obtained by HDOCK and is made by Chimera.

Table 1: The different PROTAC smiles used in this research.

Compound ID	SMILES / Structure
N1	N1(C(=O)CC[C@@H](NCCOCCOCCCCCCCl)O)Cc2c([C@@H]3[C@H](NNN3)c3c1cccc3)cccc2
P1	Cc1cccc(C(=O)Nc2cccc3c2CN(C(=O)CC[C@H](O)NCCOCCOCCCCCCCl)c2ccccc2[C@H]2NNN[C@H]32)c1
P2	CCN1CCN(CCc2ccc3c(c2)N(C(=O)CC[C@H](O)NCCOCCOCCCCCCCl)Cc2ccccc2[C@H]2NNN[C@H]32)CC1
P3	CCOC(=O)c1ccc2c(c1)CN(C(=O)CC[C@H](O)NCCOCCOCCCCCCCl)c1ccccc1[C@H]1NNN[C@H]21
P4	O=C(NC(CCCl)CCCOCCOCCN[C@@H](O)CCC(=O)N1Cc2ccccc2[C@H]2NNN[C@@H]2c2ccccc21)c1ccc(F)cc1
P5	O=C(NC1CCCCC1)NC(CCCCl)CCOCCOCCN[C@@H](O)CCC(=O)N1Cc2ccccc2[C@H]2NNN[C@@H]2c2ccccc21

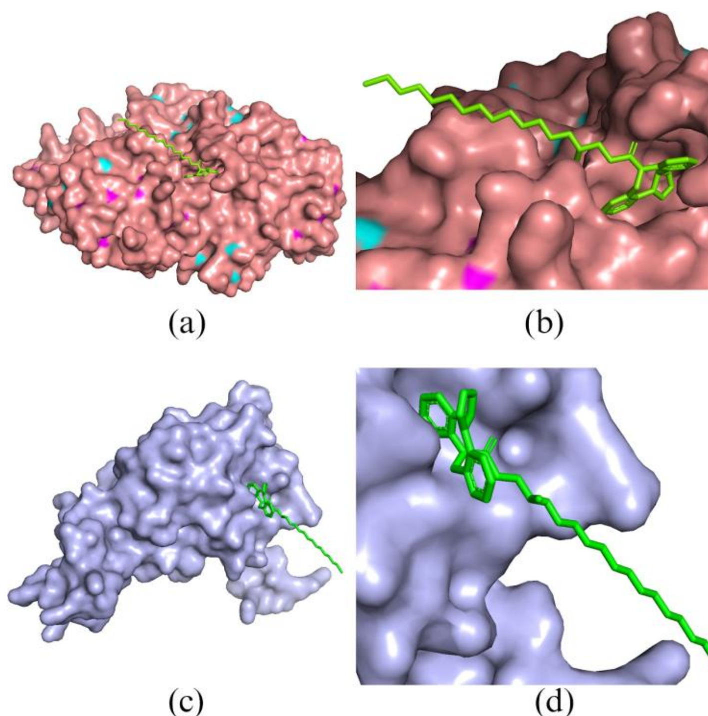

Fig. 4. The figure shows the PROTAC binding to the protein. The figure was made by using

4. DISCUSSION

In recent years, PROTACs have been explored across a wide range of diseases beyond myeloid leukemia, showing great promise in both neurodegenerative and cancer-related conditions. [12] In the realm of brain disorders, several PROTACs have been designed to target pathological proteins involved in diseases like Alzheimer's, Parkinson's, and Huntington's. For example, QC-01-175 and other tau-targeting PROTACs have shown success in degrading toxic tau proteins in Alzheimer's models. Similarly, PROTAC-62 has been developed to eliminate GSK-3β, a kinase linked to memory loss in Alzheimer's. Huntington's disease has seen the development of compounds like PROTAC-63 and its improved versions, which target mutant huntingtin (mHTT). Parkinson's disease has also benefited from the creation of LRRK2-targeting PROTACs, with ARV-102 now entering clinical trials. Additionally, PROTACs have been engineered to degrade alpha-synuclein (implicated in Parkinson's and dementia) and TDP-43, a hallmark of ALS and frontotemporal dementia.

Outside of neurological conditions, PROTACs are being used to modulate immune and inflammatory pathways such as IRAK4, a key player in autoimmune signaling. In cancer, PROTAC technology has advanced significantly. For prostate cancer, AR-targeting drugs like ARV-110, ARV-766, and CC-94676 are in various stages of clinical trials. For ER-positive breast cancer, ARV-471 has shown encouraging results by degrading estrogen receptors. [13] PROTACs have also been developed to target anti-apoptotic proteins like Bcl-XL and Bcl-2, proteins that help cancer cells survive. Compounds like DT-2216 and PZ15227 show strong anti-tumor activity while avoiding toxic side effects like platelet loss. Furthermore, researchers have begun applying PROTACs in infectious disease models. Some experimental PROTACs have shown the ability to degrade viral proteins from hepatitis C and HIV, as well as bacterial and parasitic targets like those involved in tuberculosis and trypanosomiasis, using novel designs like BacPROTACs and TrypPROTACs. Overall, PROTACs are no longer limited to hematological cancers; they are emerging as versatile tools across neurology, oncology, immunology, and infectious disease research. [14]

One major limitation of this study is that it is purely computational, meaning that all the findings are based on simulations

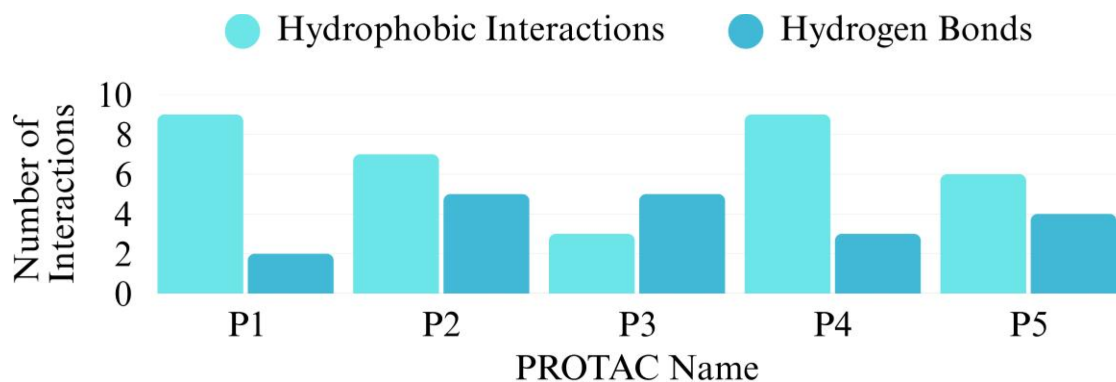

Fig. 5. Comparative analysis of hydrophobic interactions and hydrogen bonds formed between different PROTAC molecules (P1–P5) and the SHP2 protein.

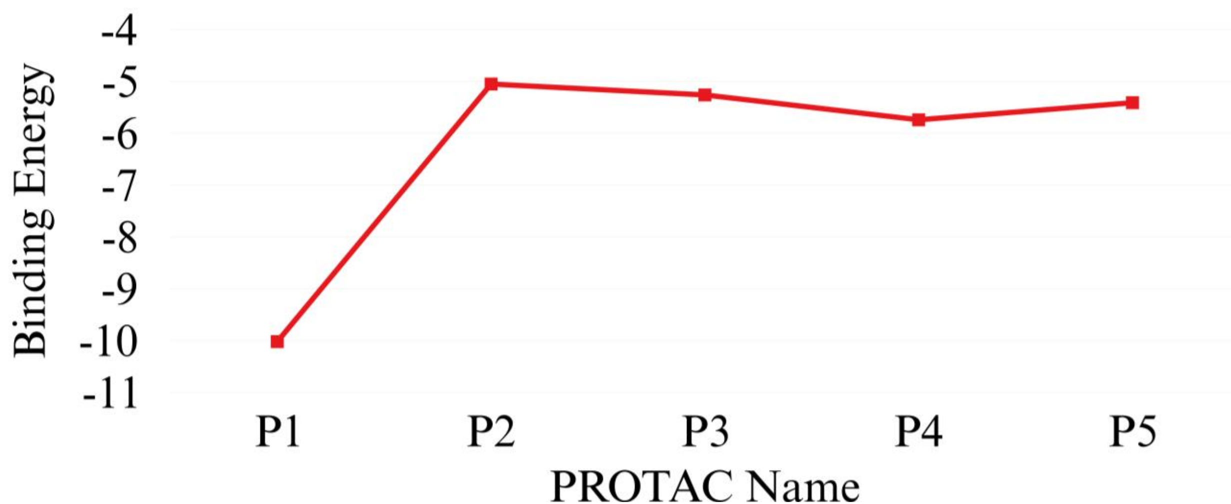

Fig. 6. Binding energy analysis of different PROTAC molecules (P1–P5) interacting with the SHP2 protein. The graph shows that PROTAC P1 exhibited the strongest binding affinity with the lowest binding energy value of approximately -10 kcal/mol, indicating the most stable protein-ligand interaction among all tested PROTAC derivatives.

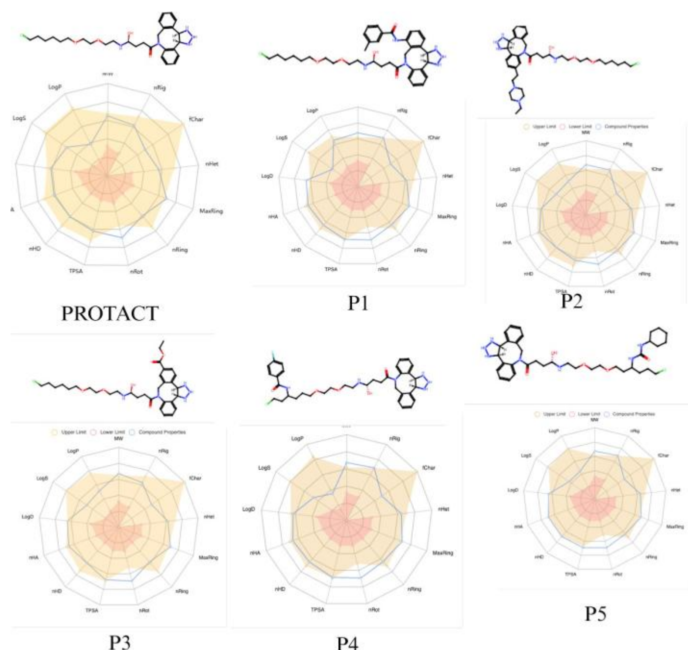

Fig. 7. The figure shows the ADMET property and derivatives of PROTAC. It made by using ADMET-AI webserver.

and modeling rather than laboratory experiments. For instance, using simulations to predict protein-ligand interactions, best binding orientation, or structural confirmations. Even though computational methods like molecular docking provide valuable insights into potential binding between a PROTAC molecule and its target protein, they can't fully account for the complexities of biological systems. For instance, cell permeability, metabolic stability, or actual degradation efficiency in living cells. Therefore, in order to verify their biological significance and therapeutic potential, these predictions need to be carefully understood and experimentally validated using in vitro (cell culture assays) and in vivo (animal models) research.

5. CONCLUSION

This study used computaional tools to design and evaluate PROTACs targeing the SHP2 protein in solid tumors. Through structure prediciton, molecular docking, and interaction analysis, PROTAC P1 emerged as the strongest candidate with the highest binding affinity and favorable and favorable molecualr properties. These results supprt the potential of SHP2-derived PROTACs as a therapeutic stratedy for solid tumors. However, because this work is entirely computational, experiemtnal studies are needed to validate the computational results. The current results provides an important foundation for developing next generation SHP2-targeting PROTACs to imrove solid tumor treatment methods.

REFERENCES

1. D. G. Gilliland, C. T. Jordan, and C. A. Felix, "The Molecular Basis of Leukemia," Hematology pp. 80–97 (2004).
2. L. B. Travis, "Therapy-associated Solid Tumors," Acta Oncologica **41**, 323–333 (2002).
3. D. Luo, K. A. Carter, D. Miranda, and J. F. Lovell, "Chemophototherapy: An Emerging Treatment Option for Solid Tumors," Advanced Science **4**, 1600106–1600106 (2017).
4. S. Yusa and K. S. Campbell, "Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2) can play a direct role in the inhibitory function of killer cell Ig-like receptors in human NK cells," J Immunol **170**, 4539–4547 (2003).
5. M. R. Kandadi, M. S. Stratton, and J. Ren, "The role of Src homology 2 containing protein tyrosine phosphatase 2 in vascular smooth muscle cell migration and proliferation," Acta Pharmacologica Sinica **31**, 1277–1283 (2010).
6. J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, . . Jumper, and J. M, "Accurate structure prediction of biomolecular interactions with AlphaFold 3," Nature **630**, 493–500 (2024).
7. U. Omasits, C. H. Ahrens, S. Müller, and B. Wollscheid, "Protter: interactive protein feature visualization and integration with experimental proteomic data," Bioinformatics **30**, 884–886 (2013).
8. F. Pontén, K. Jirström, and M. Uhlen, "The Human Protein Atlas-a tool for pathology," The Journal of Pathology **216**, 387–393 (2008).
9. Y. Yan, D. Zhang, P. Zhou, B. Li, and S. Y. Huang, "HDOCK: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy," Nucleic Acids Res **45** (2017).
10. P. Polishchuk, "CReM: chemically reasonable mutations framework for structure generation," Journal of Cheminformatics **12**, 28–28 (2020).
11. S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, and M. Schroeder, "PLIP: fully automated protein-ligand interaction profiler," Nucleic acids research **43** (2015).
12. R. Pujari, S. Bhatt, U. Soni, S. Sharma, and S. Patil, "Advantages and Disadvantages of PROTACs," in "PROTAC-Mediated Protein Degradation: A Paradigm Shift in Cancer Therapeutics," M. N. P. Jain, ed. (Springer Nature, 2024), pp. 67–88.
13. H. Xie, J. Liu, D. M. A. Glison, and J. B. Fleming, "The clinical advances of proteolysis targeting chimeras in oncology," Explor Target Antitumor Ther **2**, 511–521 (2021).
14. R. I. Troup, C. Fallan, and M. G. J. Baud, "Current strategies for the

design of PROTAC linkers: a critical review," Explor Target Antitumor
Ther **1**, 273–312 (2020).