Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/11.2.20250511

Research article
2025 | Volume 11 | Issue 2 | Pages 80-90

ARTICLEINFO

Received
May 11, 2025
Revised
July 13, 2025
Accepted
August 14, 2025

***Corresponding Author**
Jahangir Shafi

E-mail
jahangirshafi8945@gmail.com

Keywords
RASSF4
Cancer
Molecular docking
Drug discovery
Cancer research

How to Cite
Tanveer M, Ullah ZS, Nawaz H, Shafi AJ. Comparative modeling and structure-based identification of drug target sites in RASSF4 using molecular docking approaches. Biomedical Letters 2025; 11(2): 80-90.

SCAN ME

Open Access

Comparative modeling and structure-based identification of drug target sites in RASSF4 using molecular docking approaches

Misha Tanveer[1], Zunaira Saif Ullah[2], Huma Nawaz[3], Muhammad Jahangir Shafi[3,*]

[1]Department of Biotechnology, University of Okara, Okara, Pakistan
[2]Department of Bioinformatics, University of Okara, Okara, Pakistan
[3]Department of Biotechnology, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Abstract

RASSF4, a potential tumor suppressor gene, functions as a KRAS-specific effector protein that may induce apoptosis and cell cycle arrest, playing a significant role in cancer inhibition. In human tumor cells, RASSF4 expression is often suppressed due to promoter methylation. In this study, homology modeling was performed using online servers (I-TASSER, SwissModel, and ModWeb) to generate 3D structures of RASSF4. Models predicted by these web servers were compared with those generated using MODELLER 10.4, and their quality was assessed using standard evaluation tools. The most potent RASSF4 model was selected for molecular docking studies. The structure was visualized and further refined. Binding pockets were identified. Two chemically designed ligands, ANP and GNP, were selected to enhance the activity of the anticancer target protein RASSF4. Molecular docking was conducted to identify potential binding sites. The predicted structure showed high precision, particularly at the active site, indicating that it is suitable for structural and functional analyses. These predicted binding pockets may serve as starting points for further drug discovery in cancer research.

Introduction

Cancer is a genetic disease, caused by abnormal alterations in genes that regulate cell function, particularly growth and replication. It involves uncontrolled cell proliferation with the potential to invade or spread to other body parts [1]. The interaction between different cell types is known as heterotypic signaling [2], and such heterotypic interactions between nascent tumor and normal cells contribute to cancer development. Mutations may lead to uncontrolled tissue growth. In cancer, tumor suppressor genes are often inactivated by mutations, which promote cell growth and inhibit apoptosis [3].

Apoptosis is a distinct form of cell death, playing a crucial role in maintaining tissue homeostasis. It also occurs in specific pathological contexts.

Morphologically, apoptosis is characterized by cell budding and condensation, preserving organelles that are subsequently phagocytosed and digested by surrounding cells [4, 5]. Cancer involves a loss of balance between cell proliferation and cell death [6]. The downregulation of the tumor suppressor genes promotes tumor growth and suppresses apoptosis [7]. Although apoptosis is a key mechanism in cancer treatment, it can also contribute to the complexity of the disease.

Tumors can occur in any part of the body. Some tumor cells manipulate nearby normal cells to continuously release growth-stimulating signals [8]. Instead of eliminating tumor cells, inflammatory cells may further enhance tumor progression [8-10]. Cancer cells also exploit abnormal splicing of mRNA precursors to produce protein isoforms with diverse functions. These isoforms are often tissue-specific and are preferentially regulated in malignant cells, resulting in defects in signaling pathways and cell cycle regulation [11].

The conversion of glucose to lactate generates only two ATP molecules per glucose, whereas oxidative phosphorylation yields 36 ATP molecules. Tumor cells prefer glycolysis, even under oxygen-rich conditions, to rapidly obtain energy and biosynthetic precursors needed for proliferation [11-13]. The Ras Association Domain Family (RASSF) is a group of tumor suppressor proteins that promote apoptosis and regulate the cell cycle to inhibit cancer. The family comprises ten members, RASSF1–RASSF10. Members RASSF1-6 (classical RASSFs) contain C-terminal Ras Association (RA) and SARAH domains, while RASSF7–10 lack SARAH domains and contain N-terminal RA domains [14]. RASSF1–6 possess Ras/Rap association domains that enable binding to GTPases. These are followed by SARAH domains, which mediate interaction with MST1/2 protein kinases. All RASSF proteins function as tumor suppressors and are often epigenetically silenced in cancers. Their proapoptotic and anti-proliferative activities contribute to cancer inhibition [15].

RASSF family proteins are frequently downregulated in various human cancers and exhibit tissue-specific expression, selective cytotoxicity, and differential effects on signaling pathways [16]. RASSF4, a potential tumor suppressor, is also known as AD037. It shares ~60% similarity with RASSF2 and ~25% with RASSF1A [14, 17]. RASSF4 directly binds activated K-Ras in a GTP-dependent manner *via* its effector domain, thus acting as a Ras effector protein.

RASSF4 is frequently downregulated through promoter methylation in tumor cells [17]. Its overexpression induces Ras-dependent apoptosis in 293-T cells and suppresses tumor growth in various cancer cell lines [18]. Decreased RASSF4 mRNA expression has been observed in head and neck squamous cell carcinoma, and promoter hypermethylation was detected in nasopharyngeal carcinoma [19, 20]. In breast and lung tumor cells, RASSF4 overexpression induces apoptosis and inhibits proliferation [20]. Overexpression of RASSF4 in osteosarcoma cells suggests a potential therapeutic role through modulation of signaling pathways [21]. Traditional experimental methods for protein structure determination (e.g., X-ray crystallography, NMR) are time-consuming and costly [22, 23]. Bioinformatics offers efficient alternatives to predict protein 3D structures and identify binding pockets [24-26]. Homology modeling and threading techniques are commonly used for structure prediction when experimental data is unavailable. Since the 3D structure of RASSF4 is not reported in PDB, computational methods were employed to model the structure and identify potential binding sites. Structure prediction is crucial for identifying inhibitor-binding pockets. In the current study, we employed *in silico* methods to identify potential direct activators of RASSF4. These activators may serve as templates for designing direct RASSF4 regulators. This *in silico* analysis provides a

foundation for drug discovery targeting cancer-related pathways involving RASSF4.

Materials and Methods

The amino acid sequence (321 residues) of Ras association domain family member 4 (RASSF4) was retrieved from the UniProt Knowledgebase using the UniProt accession number Q9H2L5, in FASTA format, for 3D structure prediction. The RASSF4 sequence in FASTA format was subjected to protein-protein BLAST against the Protein Data Bank (PDB) to identify suitable templates based on sequence similarity. Two potential templates were retrieved. The template with higher query coverage and identity was selected for structure prediction. Comparative modeling was used to generate the 3D structure of RASSF4. The FASTA sequence was submitted to various structure prediction tools, including SWISS-MODEL[19], MODWEB, MODELLER v10.4 [21], and I-TASSER I-TASSER [27], relies on spatial restraints for structure prediction. Models were evaluated based on the C-score, which indicates the confidence and stability of the predicted structure. The model with the highest C-score was selected.

All predicted models were validated using multiple structure validation tools, including MolProbity was used to assess geometry, ERRAT generated plots indicating model error values, and PROCHECK provided Ramachandran plots, indicating favored, allowed, and disallowed regions. The model with the highest overall validation scores was selected. The selected structure was visualized using UCSF Chimera 1.8, which enabled segmentation and structure standardization [28]. Further refinement of the predicted RASSF4 structure was carried out using WinCoot.

Potential binding pockets of RASSF4 were identified using online tools such as SiteHound and CASTp. Two chemically designed ligands, ANP and GNP, were selected for docking. These compounds are known to enhance the activity of the anticancer target protein RASSF4. Energy minimization was performed prior to docking. Following energy minimization, molecular docking was conducted using PyRx (AutoDock Vina). The selected ligands, ANP and GNP, were docked into the predicted binding sites of RASSF4. High residue-ligand interactions were observed, suggesting enhanced functional potential of RASSF4 in cancer targeting.

Results and Discussion

The present research was based on the *in silico* analyses of RASSF4. RASSF4 has earned considerable appreciation for its involvement in the onset of different human cancers and its probable role as an anti-tumorigenic protein. The 3D structure of RASSF4 has not been predicted yet. This research was intended to predict the 3D structure of RASSF4 and identify novel regulators. In this study, *in silico* techniques (homology modeling, binding site prediction, and docking analyses) were carried out. The 3D structure of RASSF4 was modeled using a crystal structure template. The predicted structure demonstrated a good level of precision, particularly at the active site of the protein. Docking analysis was conducted using AutoDock Vina. A comprehensive docking study of the interactions between RASSF4 and selected ligands (ANP and GNP) highlighted successful interactions with the lowest binding energy, which could potentially contribute to cancer inhibition.

The amino acid sequence of RASSF4 in FASTA format was retrieved from UniProt. BLASTp was used to identify suitable templates against the target sequence, and two potential templates were listed (**Table 1**). 3DDC and 6AMB showed optimal alignment, with the first template having the best overall match. Templates were sorted based on overall quality, query coverage, similarity, and E-value. Low query coverage indicates a match with only a small portion of the target sequence, whereas high query coverage signifies alignment with a larger portion. The 3D structure of RASSF4 was predicted using MODELLER 10.4 and online web servers, including I-TASSER. The predicted models were validated using structural evaluation tools, including ERRAT and RAMPAGE.

The evaluation of the predicted structures was conducted using Ramachandran plots and the overall quality factor. A total of 14 models of RASSF4 were predicted and subsequently analyzed using evaluation tools. The complete analysis results for all 14 models were plotted, and the most reliable model for RASSF4 was identified. The selected high-quality model was then visualized using UCSF Chimera 1.8 (**Fig. 1**).

The Ramachandran plot is a critical analytical plot used to evaluate protein structures by displaying the φ (phi) and ψ (psi) torsion angles of the protein

backbone. Due to the limited conformational space available to protein backbones, they typically adopt specific torsion angles that fall within allowed regions. In contrast, disallowed regions represent conformations that are sterically unfavorable but may occasionally be occupied due to specific molecular interactions. The Ramachandran plots illustrated the distribution of φ and ψ angles for non-glycine and non-proline residues (**Fig. 2**). These plots also featured the overall residue distribution (**Fig. 3**), allowing for the distinction between favorable and unfavorable regions, thereby providing an evaluation of the structural quality of the predicted model [29].

Table 1: Templates for RASSF4 with their score, query coverage, e-value, and identity percentage

Templates	Max score	Total score	Query coverage	E-value	Identity
3DDC	50.1	50.1	26%	7e-07	34.09%
6AMB	34.7	34.7	20%	0.044	35.71%

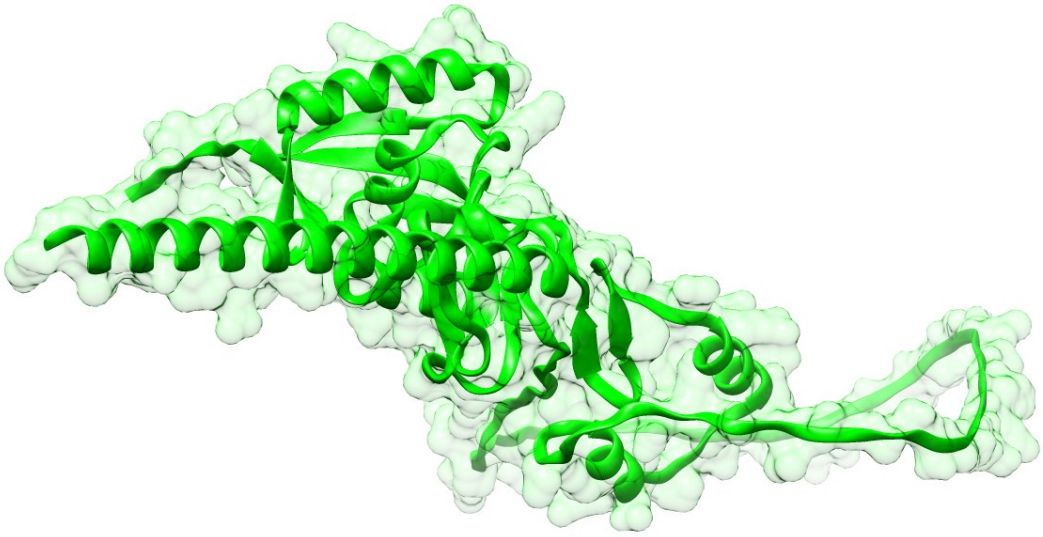

Fig.1: 3D structure of the predicted model

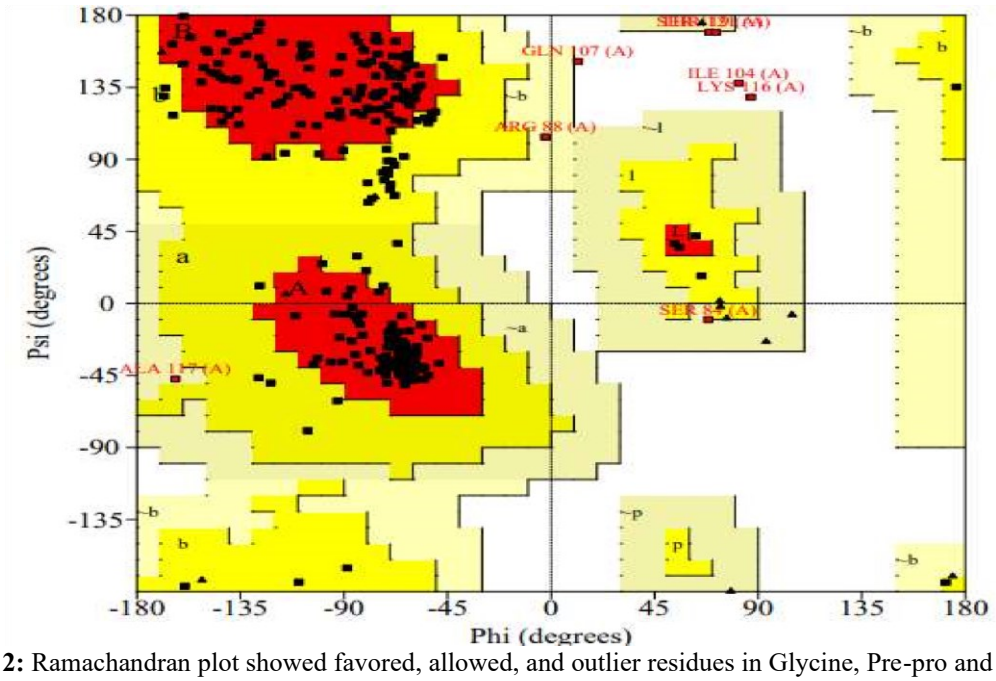

Fig. 2: Ramachandran plot showed favored, allowed, and outlier residues in Glycine, Pre-pro and Proline

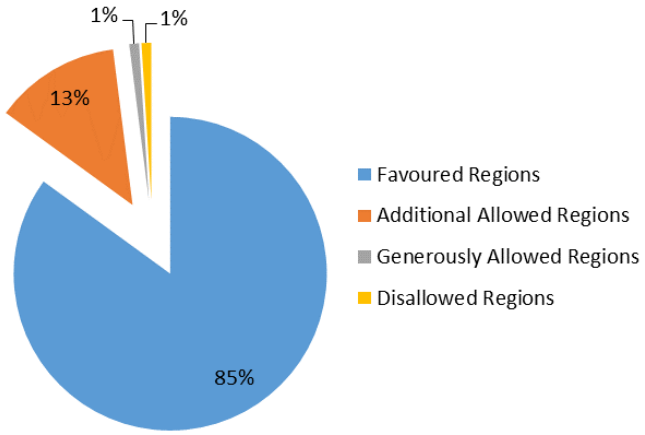

Fig. 3: Graph of Ramachandran plot statistics including favored region and outliers

The predicted structure of RASSF4 was evaluated using the protein structure, which helps researchers identify and correct inaccuracies in modeled protein structures. Overall quality factor of 95.331% (**Fig. 4**), indicating a high level of structural reliability. This analysis primarily focuses on plotting error values derived from non-bonded atom–atom interactions within the structure, which are then compared against a database of reliable, high-resolution protein structures. Poorly defined area was highlighted and regions likely to be rejected at the 95% confidence interval are shown in yellow**,** while those that could be rejected at the 99% confidence level are marked in red. These visual indicators assist in pinpointing structurally uncertain regions. The accuracy of a protein model was evaluated but also illustrates the improvements between the initial and final structures. Therefore, an overall quality factor above 95%, as observed here, is considered to reflect a highly accurate and reliable protein structure.

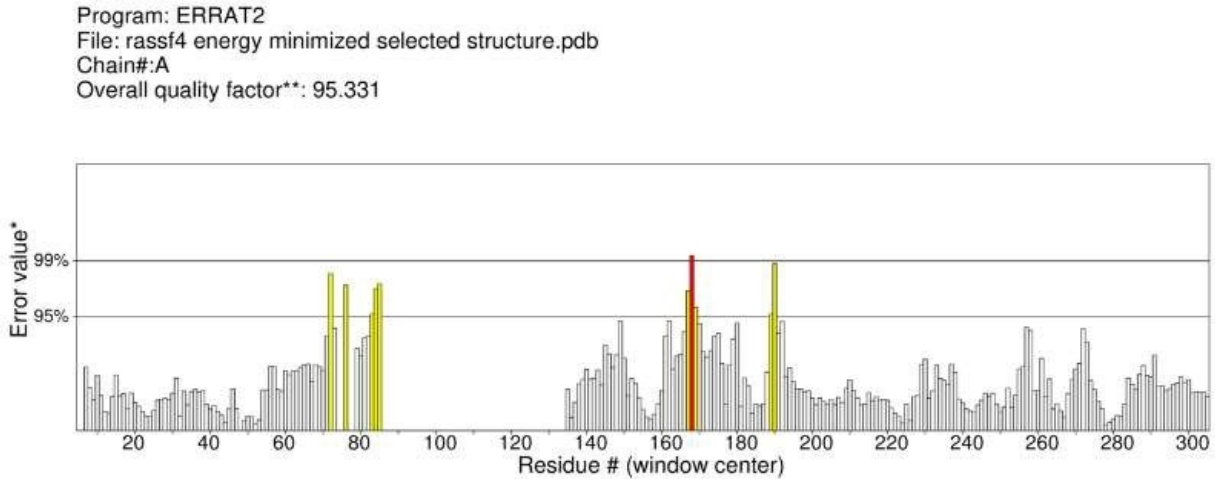

Fig. 4: Overall quality factor of predicted model

Binding pockets are specific regions on the surface of proteins where small molecules, ions, or other proteins can bind. These interactions are essential for numerous biological processes, including enzyme catalysis, signal transduction, and the regulation of molecular activity. Understanding the structure and characteristics of binding pockets is critical for drug development and the design of effective therapeutic agents. In this study, the top ten binding pockets **of** RASSF4 residues were identified (**Table 2**). As the binding pockets of RASSF4 have not been previously reported, *in silico* approaches were employed to predict these binding sites. The energy range of the predicted cavities further illustrates the potential efficiency of these binding pockets. Additionally, mutational analysis revealed that these residues may

serve as potential drug targets in cancer therapy. RASSF4, a potential tumor suppressor, may function as a KRAS effector protein. RASSF4 promotes apoptosis and induces cell cycle arrest. It plays a role in the Hippo signaling pathway, which is crucial for regulating cell proliferation and apoptosis. Dysregulation of this pathway contributes to cancer development. Specifically, the activation of kinases MST1/2 is critical, and their interaction with the SARAH domain through homo- or heterodimerization forms a key activation loop.

ANP and GNP ligands may potentially activate the Hippo signaling pathway, thereby contributing to cancer inhibition. To date, there is no reported evidence in biological databases or literature of direct regulators or activators of RASSF4. In a 2016 study [30] Phosphoaminophosphonic acid adenylate ester (ANP) and Phosphoaminophosphonic acid guanylate ester (GNP) (**Fig. 5**) were used to validate predicted binding pockets of RASSF2. In the present study, ANP and GNP effectively docked with RASSF4, confirming their ability to bind at predicted sites. These compounds are proposed as potential regulators of RASSF4 activity in the Hippo signaling pathway, ultimately contributing to the inhibition of cancer.

Table 2: Binding pockets of RASSF4 with their area and volume

Rank	Area (SA) Å^2	Volume (SA) Å^3	Residues
1	1218.365	3529.732	GLY-62, LEU-63, ARG-64, ARG-65, PRO-66, ARG-68, SER-123, GLU-128, GLU-129, ALA-130, GLU-131, GLU-132, ALA-133, PRO-134, GLN-135, LEU-136, ARG-138, ASP-142, GLN-160, ILE-162, ARG-163, ARG-164, HIS-165, ARG-166, PHE-167, SER-168, GLY-171, HIS-173, TYR-174, THR-178, VAL,180, THR-182, PRO-183, TYR-185, VAL-224, GLY-228, ARG-230, MET-258, GLU-259, LEU-262, GLY-263, VAL-266, ALA-271, ILE-274, LYS,275, PHE-276, GLU-277, MET-278, LEU-281
2	439.268	806.098	MET-71, ASP-73, ASP-74, ARG-75, GLU-76, GLN-77, HIS-79, ALA-143, MET-146, SER-147, ARG-149, ARG-150, LYS-152, ARG-154, HIS-176, LYS-177
3	27.227	578.759	ASN-103, GLN-112, CYS-153, ARG-154, ALA-155, PRO,156
4	110.210	77.767	ILE-13, SER-14, SER-16, LYS-17, ILE-223, HIS,225, GLU,229, THR-231, LYS-232, LEU-233, LYS-234, GLU-237, SER-242, LEU-245, HIS-246, LEU-25, LEU-28, LYS-29, HIS-44
5	5.290	58.488	PRO-99, LYS-152, CYS-153
6	77.802	53.798	GLN-70, MET-71, GLN-72, ASP-74, LYS-140, SER-141, GLU-213
7	-0.329	34.538	SER-120, THR-121, ASP-122, SER, 123, GLN-160
8	63.249	34.169	LEU-63, ARG-65, PRO-66, ILE-67, LEU-136, ASP-214, GLU-218, PHE,219, GLU-259, ASP-261
9	52.530	10.594	SER-8, HIS-10, VAL-11, PRO-12, LYS-21, GLY-49, THR-50, LEU-51, SER-195, THR-196, TYR-238, ILE-241
10	30.979	7.142	THR-99, LEU-200, LEU-203, PRO-216, SER-217, PHE-21, ALA-220, LEU-221, LYS-232, LEU-233, ASP-235

In contrast to RASSF2, RASSF4 does not have clearly defined direct regulators in its signaling pathways. Therefore, in this study, *in silico* methods were employed to identify the binding pockets and potential regulators of RASSF4, building on previous investigations conducted for RASSF5 and RASSF2. The next step involved molecular docking to determine the highest number of interactions between ANP and GNP and the target receptor. Docking experiments were performed utilizing the same

compounds. The resulting complexes were ranked based on their binding affinity, with ANP and GNP exhibiting the lowest binding energies and strongest binding affinities. The 2D structures of the selected compounds were generated and energy-minimized (**Fig. 5**) [31]. Both docking tools revealed interactions occurring within the same binding pockets and involving the same key residues, which were further analyzed.

ANP binds within the SARAH domain of RASSF4, forming a stable complex that inhibits the activity of LATS kinase 1 and LATS kinase 2. This inhibition activates the cell regulatory molecules, which play a role in controlling cell proliferation. ANP contributes to tumor suppression by enhancing the pro-apoptotic function of RASSF4, thereby regulating cellular processes and promoting apoptosis. GNP, a potent stimulator of adenylyl cyclase, influences chromatin accessibility, thereby reducing transcriptional activity and halting the uncontrolled synthesis of proteins. As a result, GNP (GTP) supports tumor suppression by inducing apoptosis and regulating cell proliferation.

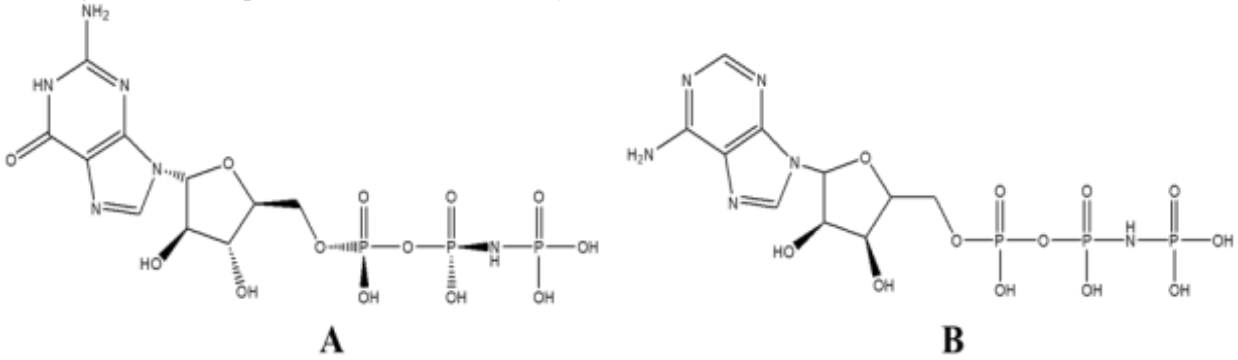

Fig. 5: 2D structures of ligands A] Phosphoaminophosphonic acid adenylate ester (ANP), B] Phosphoaminophosphonic acid guanylate (GNP).

This complex was selected based on its most favorable binding affinity. The subsequent evaluation focused on the number and nature of interactions between the ligands (ANP and GNP) and the target receptor (RASSF4). It was observed that interactions occurring within the same binding pocket and involving the same residues. Notably, ANP and GNP demonstrated the lowest binding energy and highest binding affinity within the top-ranked binding pocket (**Table 3**) of RASSF4.

The ligand–protein interactions of RASSF4–ANP and RASSF4–GNP were analyzed and visualized, revealing that both compounds interacted with the same top-ranked binding pocket. The key interacting residues identified were Arg166, Lys177, His165, Phe167, Phe173, His176, Ala133, Ile162, Arg163, Tyr174, and Asn175 (**Fig. 6**). These identical residues were consistently observed in docking analyses as well, confirming the reliability and reproducibility of the docking results. Furthermore, the residues identified corresponded (**Fig. 7**) to those predicted in the highest-ranked binding domain, thereby validating the docking outcomes through consistent *in silico* evidence.

Table 3: Interacting residues and binding energy of selected ligands against RASSF4

Ligands	Binding energy (kcal/mol)	Residues	Hydrogen Bond
ANP	-5.9	Arg 166, Lys 177, His 165, Phe 167, Phe 173, His 176, Ala 133, Ile 162, Arg 163, Tyr174, Asn175	Tyr174;2.97, Asn175;2.94
GNP	-5.8	Arg 166, Lys 177, His 165, Phe 167, Phe 173, His 176, Ala 133, Ile 162, Arg 163, Tyr174, Asn175	Tyr174;2.97, Asn175;2.98

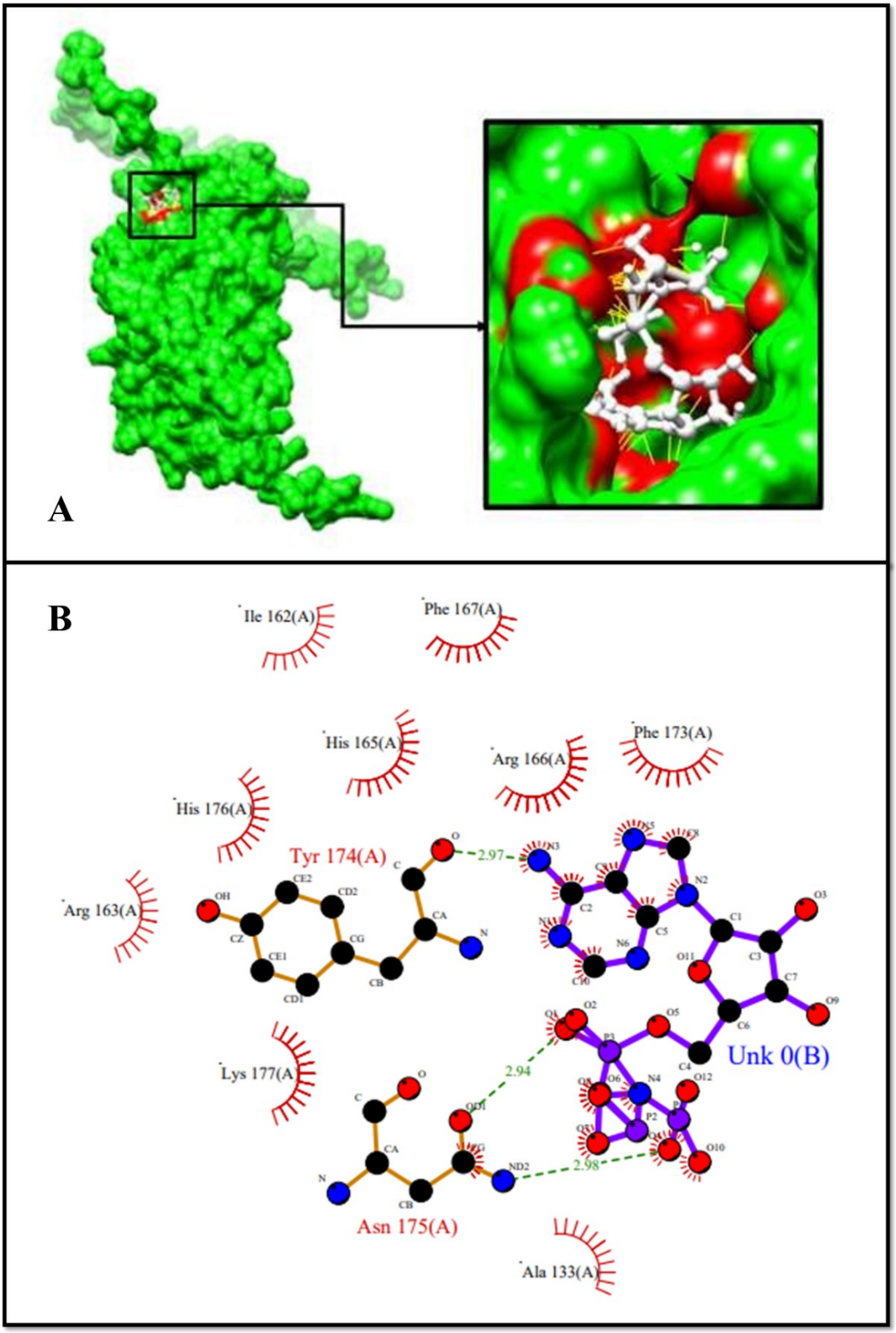

Fig. 6: ANP docking studies. A) Red color highlights the binding domain; B) ANP binding interacting residues.

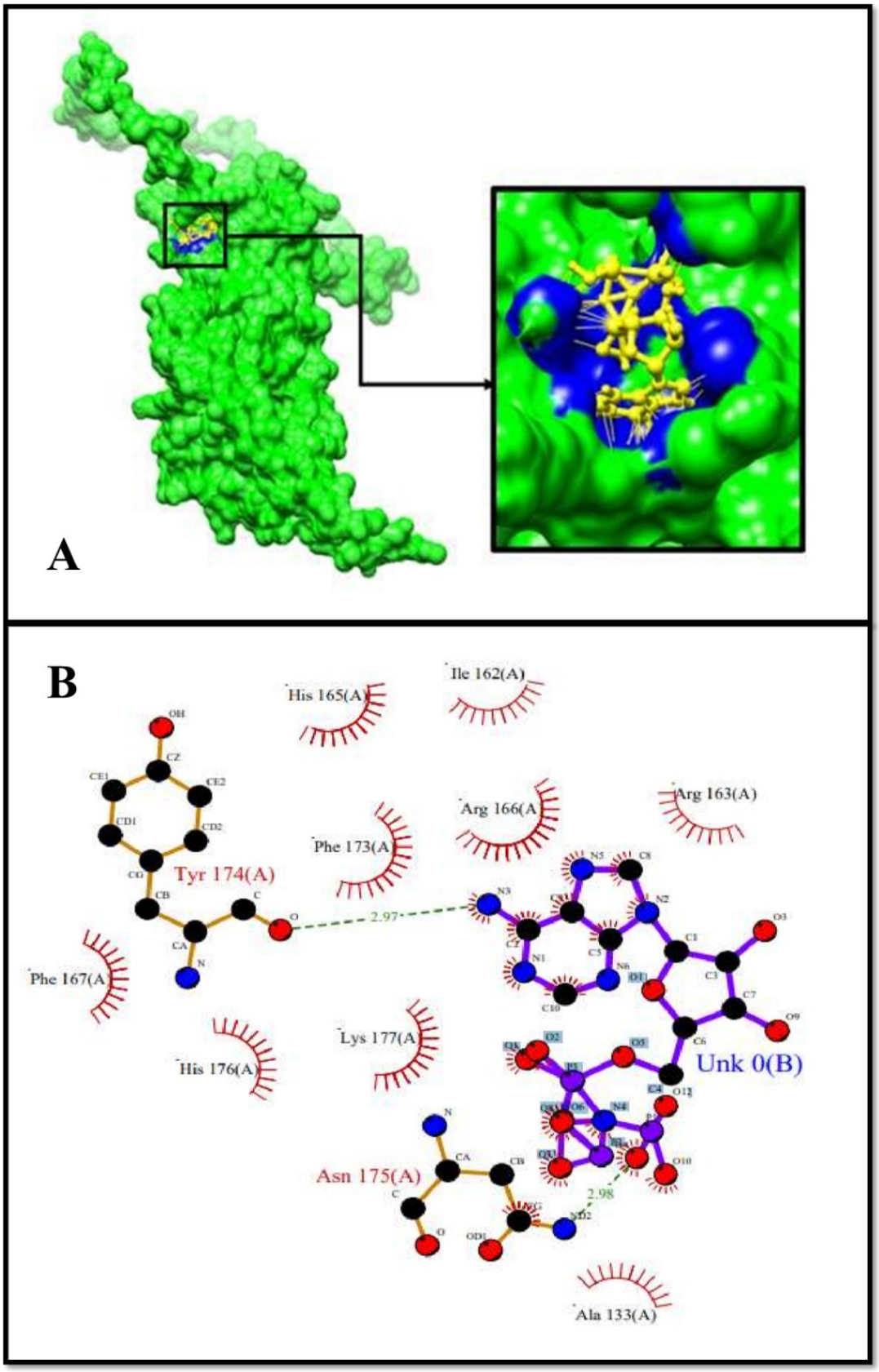

Fig. 7: GNP docking studies. A) Blue color highlights the binding domain; B) GNP binding residues.

The field of bioinformatics holds great promise in oncology [32, 33]. Given the significant attention and resources devoted to cancer research, numerous scientists are actively working to discover effective treatments [24]. Traditional drug design is a costly and time-consuming process [34]. However, bioinformatics methodologies provide powerful alternatives by enabling *in silico* drug discovery approaches [35]. These strategies can significantly reduce development time and facilitate the identification of selective compounds with minimal side effects and superior biological efficacy [36].

Protein function is inherently linked to its structure, and access to the 3D structure of a target protein is crucial for rational drug design [37]. In this study, *in silico* approaches were employed to predict the 3D structure and binding domains of RASSF4. This methodology successfully identified the potential binding pockets and enabled the modeling of the protein's active site.

The compounds ANP and GNP were found to occupy these predicted binding sites, demonstrating favorable interactions with RASSF4. These findings suggest that ANP and GNP could serve as potential regulators or activators of RASSF4, aiding in the development of targeted therapies against cancer.

Conclusion

In this study, the 3D structure of RASSF4 was predicted and potential new inhibitors were identified. The three-dimensional structure of RASSF4 was modeled, and the predicted structure demonstrated a high degree of accuracy, particularly at the protein's active site. A comprehensive docking study of the interactions between RASSF4 and selected ligands (ANP and GNP) revealed strong interactions with the lowest binding energy, suggesting that these ligands may serve as effective regulators capable of inhibiting cancer.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Bu Y, Cao D. The origin of cancer stem cells. Front Biosci (Schol Ed). 2012;4:819-30.

[2] Filipp FV. Spatial cancer systems biology resolves heterotypic interactions and identifies disruption of spatial hierarchy as a pathological driver event. Journal of Investigative Dermatology. 2023;143:1342-7.

[3] Knudson AG. Two genetic hits (more or less) to cancer. Nature Reviews Cancer. 2001;1:157-62.

[4] Häcker G. The morphology of apoptosis. Cell and tissue research. 2000;301:5-17.

[5] Wong RS. Apoptosis in cancer: from pathogenesis to treatment. Journal of experimental & clinical cancer research. 2011;30:87.

[6] Bauer JH, Helfand SL. New tricks of an old molecule: lifespan regulation by p53. Aging cell. 2006;5:437-40.

[7] Cordon-Cardo C, Prives C. At the crossroads of inflammation and tumorigenesis. The Journal of experimental medicine. 1999;190:1367-70.

[8] Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes & development. 1999;13:1382-97.

[9] Hudson JD, Shoaibi MA, Maestro R, Carnero A, Hannon GJ, Beach DH. A proinflammatory cytokine inhibits p53 tumor suppressor activity. The Journal of experimental medicine. 1999;190:1375-82.

[10] David CJ, Manley JL. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. Genes & development. 2010;24:2343-64.

[11] Mazurek S, Boschek CB, Hugo F, Eigenbrodt E. Pyruvate kinase type M2 and its role in tumor growth and spreading. Seminars in cancer biology: Elsevier; 2005. p. 300-8.

[12] Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes & development. 2009;23:537-48.

[13] Avruch J, Xavier R, Bardeesy N, Zhang X-f, Praskova M, Zhou D, et al. Rassf family of tumor suppressor polypeptides. Journal of Biological Chemistry. 2009;284:11001-5.

[14] Allen N, Donninger H, Vos M, Eckfeld K, Hesson L, Gordon L, et al. RASSF6 is a novel member of the RASSF family of tumor suppressors. Oncogene. 2007;26:6203-11.

[15] Eckfeld K, Hesson L, Vos MD, Bieche I, Latif F, Clark GJ. RASSF4/AD037 is a potential ras effector/tumor suppressor of the RASSF family. Cancer research. 2004;64:8688-93.

[16] Tommasi S, Dammann R, Jin S-G, Zhang X-f, Avruch J, Pfeifer GP. RASSF3 and NORE1: identification and cloning of two human homologues of the putative tumor suppressor gene RASSF1. Oncogene. 2002;21:2713-20.

[17] Han Y, Dong Q, Hao J, Fu L, Han X, Zheng X, et al. RASSF4 is downregulated in nonsmall cell lung cancer and inhibits cancer cell proliferation and invasion. Tumor Biology. 2016;37:4865-71.

[18] Zhang M, Wang D, Zhu T, Yin R. RASSF4 overexpression inhibits the proliferation, invasion, EMT, and Wnt signaling pathway in osteosarcoma cells. Oncology research. 2017;25:83.

[19] Federhen S. The NCBI taxonomy database. Nucleic acids research. 2012;40:D136-D43.

[20] Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: an automated protein homology-modeling server. Nucleic acids research. 2003;31:3381-5.

[21] Eswar N, John B, Mirkovic N, Fiser A, Ilyin VA, Pieper U, et al. Tools for comparative protein structure modeling and analysis. Nucleic acids research. 2003;31:3375-80.

[22] Sehgal SA, Mannan S, Ali S. Pharmacoinformatic and molecular docking studies reveal potential novel antidepressants against neurodegenerative disorders by targeting HSPB8. Drug design, development and therapy. 2016:1605-18.

[23] Tahir RA, Bashir A, Yousaf MN, Ahmed A, Dali Y, Khan S, et al. In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1. PloS one. 2020;15:e0228265.

[24] Jin Y, Sehgal SA, Hassan F, Liu G. In Silico Identification of Novel Compounds as Anthelmintics Against Haemonchus contortus Through Inhibiting β-Tubulin Isotype 1 and Glutathione S-Transferase. Animals. 2025;15:1846.

[25] Sehgal SA, Razia IT, Kanwal A, Ahsan M, Tahir RA, Sajid M, et al. Computational Advancement towards the Identification of Natural Inhibitors for Dengue Virus: A Brief Review. Combinatorial Chemistry and High Throughput Screening. 2024;26.

[26] Sehgal SA, Khattak NA, Mir A. Structural, phylogenetic and docking studies of D-amino acid oxidase activator (DAOA), a candidate schizophrenia gene. Theoretical Biology and Medical Modelling. 2013;10:3.

[27] Zhang Y. I-TASSER server for protein 3D structure prediction. BMC bioinformatics. 2008;9:40.

[28] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004;25:1605-12.

[29] Sehgal SA, Tahir RA, Waqas M. Quick Guideline for Computational Drug Design (Revised Edition): Bentham Science Publishers; 2021.

[30] Kanwal S, Jamil F, Ali A, Sehgal SA. Comparative modeling, molecular docking, and revealing of potential binding pockets of RASSF2; a candidate cancer gene. Interdisciplinary Sciences: Computational Life Sciences. 2017;9:214-23.

[31] Mills N. ChemDraw Ultra 10.0 CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www. cambridgesoft. com. Commercial Price: 1910fordownload, 2150 for CD-ROM; Academic Price: 710fordownload, 800 for CD-ROM. ACS Publications; 2006.

[32] Sehgal S, Tahir R, Shafique S, Hassan M, Rashid S. Molecular modeling and docking analysis of CYP1A1 associated with head and neck cancer to explore its binding regions. J Theor Comput Sci. 2014;1:2.

[33] Sehgal SA, Kanwal S, Tahir RA, Khalid Z, Hammad MA. In silico elucidation of potential drug target sites of the Thumb Index Fold Protein, Wnt-8b. Tropical Journal of Pharmaceutical Research. 2018;17:491-7.

[34] Sajid M, Tur Razia I, Kanwal A, Ahsan M, Tahir RA, Khan MS, et al. Computational advancement towards the identification of natural inhibitors for dengue virus: a brief review. Combinatorial Chemistry & High Throughput Screening. 2024;27:2464-84.

[35] Sajid M, Marriam S, Mukhtar H, Sohail S, Sehgal SA. Epitope-based peptide vaccine design and elucidation of novel compounds against 3C like protein of SARS-CoV-2. PloS one. 2022;17:e0264700.

[36] Tahir RA, Wu H, Javed N, Khalique A, Khan SAF, Mir A, et al. Pharmacoinformatics and molecular docking reveal potential drug candidates against Schizophrenia to target TAAR6. Journal of cellular physiology. 2019;234:13263-76.

[37] Sehgal SA. Pharmacoinformatics and molecular docking studies reveal potential novel Proline Dehydrogenase (PRODH) compounds for Schizophrenia inhibition. Medicinal Chemistry Research. 2017;26:314-26.