ORIGINAL ARTICLE
Journal of Science Technology Education Art and Medicine
DOI: 10.63137/jsteam.420519

Design and Evaluation of Protein Inhibitors Against the KRAS G12D Mutation in Colorectal Cancer via Computer-Aided Drug Design Techniques

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Received: 11 March 2025 / **Revised:** 15 May 2025 / **Accepted:** 30 May 2025 / **Available Online:** 20 June 2025

ABSTRACT

Objective: KRAS mutations are among the most common oncogenic drivers in colorectal cancer (CRC), with the G12D variant posing a particularly formidable challenge due to the protein's smooth surface and lack of druggable pockets. Despite the success of covalent inhibitors for KRAS G12C, equivalent strategies for G12D remain underdeveloped. This study aimed to design and evaluate peptide-based inhibitors capable of selectively targeting the GDP-binding pocket of KRAS G12D using computer-aided drug design (CADD) techniques.
Methods: A peptide library consisting of 108 decameric sequences was rationally designed based on structure-activity relationship (SAR) insights and residue preferences for protein-protein interactions. Flexible docking was performed using the CABS-dock platform to simulate peptide binding to the KRAS G12D structure (PDB ID: 5US4). The top-ranked peptides were subjected to molecular mechanics/generalized Born surface area (MM/GBSA) calculations to estimate binding free energies. Key molecular interactions were evaluated using the Protein Interactions Calculator (PIC), and structural validity was confirmed via Ramachandran plot analysis.
Results: Nine peptides showed strong and reproducible binding within the GDP-binding pocket. Ligand 3 (DCWRHRLCID) demonstrated the most favorable interaction, with a binding free energy of –57.59 kcal/mol, followed by Ligand 34 (NCWRRHLCIN). Both ligands engaged in ionic, hydrogen bonding, and π-π stacking interactions with critical KRAS residues, notably Asp12. Ramachandran analysis showed 98% of residues within favored regions, indicating conformational stability.
Conclusion: This study identifies Ligands 3 and 34 as promising peptide-based inhibitors of KRAS G12D. These findings offer a foundation for future experimental validation and therapeutic development targeting mutant KRAS in CRC.
Keywords: Colorectal cancer; G12D mutation; KRAS; MM/GBSA; Molecular docking; Peptide inhibitors

Citation: Yung WL. Design and Evaluation of Protein Inhibitors Against the KRAS G12D Mutation in Colorectal Cancer via Computer-Aided Drug Design Techniques. J Sci Technol Educ Art Med. 2025; 2(1):23-32

Introduction

Protein inhibitors have emerged as pivotal therapeutic agents, offering targeted interventions for a variety of diseases by modulating specific protein functions. [1] Their ability to interfere with aberrant protein activities has led to significant advancements in treating conditions such as cancer, autoimmune disorders, and neurodegenerative diseases. For instance, the development of proteolysis-targeting chimeras (PROTACs) has introduced a novel mechanism to degrade disease-causing proteins, expanding the therapeutic landscape beyond traditional inhibition methods. [2]

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The efficacy of protein inhibitors is profoundly influenced by the materials and scaffolds employed in their design. [3] Traditional small-molecule inhibitors have been complemented by innovative constructs such as affimers—engineered non-antibody binding proteins—that offer high specificity and stability. [4] Affimers have been utilized in various applications, including diagnostics and as therapeutic agents, due to their favorable properties such as small size and robustness. Similarly, the advent of targeted covalent inhibitors (TCIs) has enabled the formation of irreversible bonds with target proteins, enhancing potency and selectivity. [5] This approach has been successfully applied in designing inhibitors for challenging targets like mutant forms of KRAS, a protein frequently mutated in cancers.

Extensive research has been conducted on protein inhibitors across various diseases. In oncology, covalent inhibitors targeting the epidermal growth factor receptor (EGFR) have shown efficacy in treating non-small cell lung cancer. [6] In neurodegenerative disorders, protein inhibitors that modulate kinase activity have been explored to address aberrant phosphorylation events implicated in diseases like Alzheimer's. [7] However, despite these advancements, there remains a significant gap in the application of protein inhibitors for colorectal cancer (CRC), particularly concerning mutations in the KRAS gene. [8] Approximately 40-60% of CRC cases harbor KRAS mutations, with the G12D variant being notably prevalent. [9] This mutation maintains KRAS in an active state, promoting uncontrolled cell proliferation and resistance to standard therapies. The lack of a well-defined binding pocket in KRAS G12D has rendered it a challenging target, often labeled as "undruggable."[10]

Recent advances have challenged this notion, especially through the development of covalent allosteric inhibitors and macrocyclic peptides that selectively bind KRAS G12D. For instance, literature reported a new class of covalent ligands tailored for G12D, exploiting aspartate-specific reactivity rather than cysteine, which had limited G12D-specific applications. [11] Similarly, researchers explored peptide inhibitors that bind KRAS through non-catalytic interfaces, opening new avenues for allosteric inhibition. [12, 13] These peptide-based approaches leverage structural flexibility and hotspot mimicry to achieve selective engagement with mutant KRAS, overcoming the shallow topology of its GDP-binding pocket.

Moreover, recent cryo-EM and MD-simulated studies have confirmed that conformationally adaptive peptides can access dynamic surface pockets unique to KRAS G12D, particularly when used with advanced CADD tools like CABS-dock and Rosetta FlexPepDock. [14] This supports the feasibility of designing short, cell-penetrating peptides capable of disrupting KRAS-effector interactions with high specificity.

This study aims to address this gap by employing computer-aided drug design (CADD) techniques to develop peptide-based inhibitors targeting the KRAS G12D mutation in CRC. By leveraging computational methods such as molecular docking, molecular dynamics simulations, and free energy calculations, we seek to identify peptides that can selectively bind and inhibit the mutant KRAS protein. This approach not only offers a systematic pathway to discover potential therapeutics but also provides insights into the structural interactions critical for effective inhibition.

By integrating these computational strategies, our research endeavors to contribute to the development of targeted therapies for CRC patients harboring the KRAS G12D mutation, thereby addressing a critical unmet need in oncology.

Materials and Methods

Study Design, Setting, and Duration

This study employed a computational drug design approach utilizing in-silico screening techniques to develop peptide-based inhibitors targeting the KRAS G12D mutation in colorectal cancer (CRC). The research was conducted at the Faculty of Pharmacy, Quest International University, Malaysia, between January 2023 and December 2023. The study follows a computational-experimental pipeline, incorporating molecular docking, molecular dynamics simulations, and binding free energy calculations to evaluate the efficacy of designed peptides against mutant KRAS G12D. The overall study workflow involves four key stages: (1) design and structural optimization of a peptide library, (2) molecular docking of peptides to KRAS G12D using CABS-dock, (3) MM/GBSA-based free energy analysis, and (4) interaction profiling and structural validation of top candidates. The overall study work flow is given in Figure 1.

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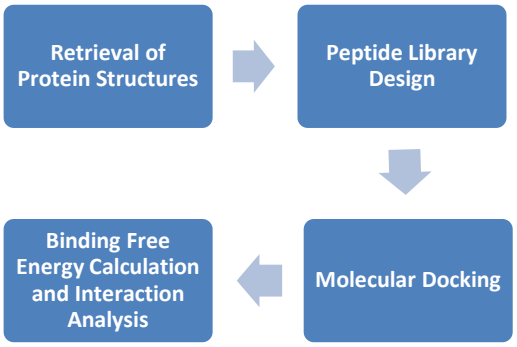

Figure 1: The overall study workflow: It depicts the four core stages of the workflow: Peptide Library Design → Flexible Docking via CABS-dock → Binding Energy Estimation using MM/GBSA → Structural and Interaction Analysis

Ethical Considerations

As this study was computational in nature, it did not involve human participants, animal models, or clinical trials. However, ethical approval was sought and granted by the Institutional Research Ethics Committee (IREC) at Quest International University to ensure compliance with international research standards. All structural data were retrieved from publicly available databases, including the Protein Data Bank (PDB), which is freely accessible for academic research. Furthermore, all computational tools used in this study are open-access or licensed for non-commercial scientific use.

Retrieval and Preparation of Protein Structures

The crystal structures of GDP-bound KRAS G12D (PDB ID: 5US4) and wild-type KRAS (PDB ID: 4OBE) were obtained from the Protein Data Bank (PDB). Only the A chain of each protein was used to eliminate inter-domain interactions that could influence docking results (Figure 2). The protein structures were prepared as follows:

1. Energy Minimization: Structures were optimized using the Chimera software, ensuring that steric clashes and bond strain were minimized.
2. Hydrogen Bond Optimization: Hydrogen atoms were added and adjusted to reflect physiological pH conditions (7.4).
3. Water Molecule Removal: Water molecules not involved in direct protein-ligand interactions were deleted to prevent computational artifacts.
4. Active Site Identification: The GDP-binding site of KRAS G12D was confirmed using the CASTp server to ensure accurate docking simulations (Figure 3 & 4).

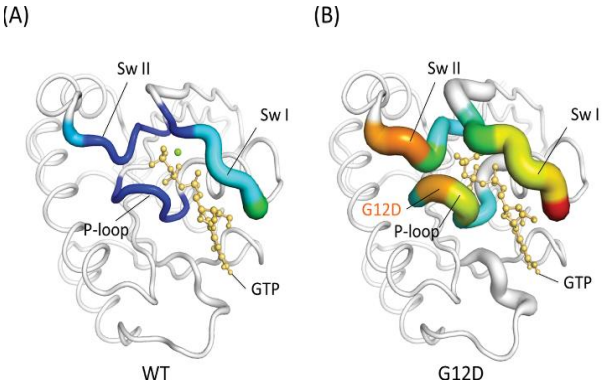

Figure 2: The structures of (A) WT, (B) G12D proteins are drawn in cartoon putty representations at the P-loop, switch I (Sw I) and II (Sw II) regions; blue represents the lowest and red the highest B-factor value. The size of the tube reflects the value of the B-factor, in that the larger the B-factor, the thicker the tube. The structures in the other regions are coloured in white and displayed in cartoon tube representation, where the size of the tube is independent of the B-factors

Figure 3: The crystal structure of GDP-bound KRAS G12D protein with resolution of 1.83 angstroms (PDB ID :5US4)

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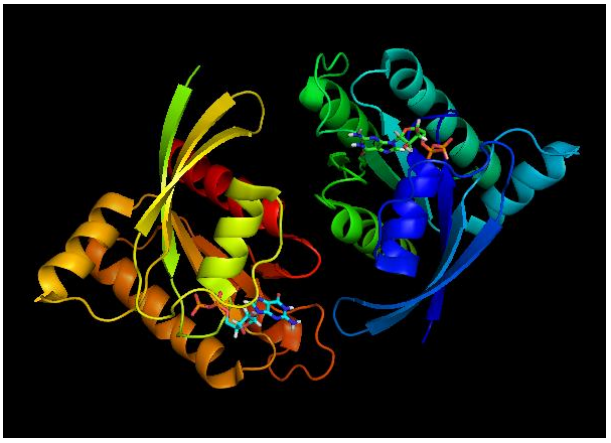

Figure 4: crystal structure of GDP-bound Human KRAS protein with resolution of 1.24 angstroms (PDB ID: 4OBE)

Peptide Library Design and Optimization

A library of 108 peptides was designed based on structure-activity relationship (SAR) analysis of known KRAS inhibitors (Table 1). The peptides were restricted to 10 amino acid residues to ensure compatibility with the small GDP-binding pocket. Key considerations in peptide design included:

- Essential Residues: Peptides were engineered to contain aspartate (D), asparagine (N), cysteine (C), and tryptophan (W), which have been reported to stabilize protein-protein interactions.
- Secondary Structure Prediction: The PsiPred server was used to analyze the peptides' secondary structural features before docking to predict their stability and binding potential.
- Charge and Hydrophobicity Optimization: Peptides were screened for balanced electrostatic and hydrophobic properties, ensuring optimal binding affinity and solubility.

Table 1: Library of Peptides Designing. D: Aspartate, N: Asparagine, C: cysteine W: tryptophan, L: Leucine, I: Isoleucine, X: Arginine, Histidine or Lysine

Position	1	2	3	4	5	6	7	8	9	10
Amino acids	D/ N	C	W	X	X	X	L	C	I	D/ N

The secondary structure of the designed peptides was predicted using Psi-pred server before docking.

Molecular Docking Procedure

Molecular docking was performed using CABS-dock, an unbiased flexible docking tool that does not require predefined binding sites. CABS-dock was chosen over other tools such as AutoDock and HADDOCK because of its ability to model full peptide flexibility and dynamic conformations, which is crucial for simulating peptide–protein interactions accurately. In contrast, AutoDock is more suited for rigid small molecules, and HADDOCK, while powerful, requires predefined interface residues which limits its unbiased docking capacity. The docking workflow included:

1. Input Preparation: The KRAS G12D structure and peptide sequences were uploaded to CABS-dock.
2. Docking Execution: Each peptide was docked three times to ensure reproducibility.
3. Pose Selection: The top three binding poses for each peptide were analyzed based on:

- Docking score (interaction energy)
- Positioning within the GDP-binding pocket
- Stability and conformational fit

Binding Free Energy Calculations

To determine the binding affinity and stability of the top peptides, binding free energy (ΔG) calculations were performed using the MM/GBSA method on the HawkDock server**.** The binding free energy was calculated using the following equations:

$$\Delta G_{bind} = G_{complex} (G_{receptor} + G_{peptide}) \quad (1)$$

$$\Delta G_{bind} = \Delta H - T\Delta S \approx \Delta E_{mm} + \Delta G_{solvent} - T\Delta S \quad (2)$$

$$\Delta E_{mm} = \Delta E_{internal} + \Delta E_{electrostatic} + \Delta E_{van\ der\ walls} \quad (3)$$

$$\Delta G_{solvent} = \Delta G_{PB/GB} + \Delta G_{SA} \quad (4)$$

where ΔG_{bind} represent the binding free energy and it can be divided into three terms: (1) the molecular mechanical energy (ΔE_{mm}), which is the summation of the intramolecular energy ($\Delta E_{internal}$, including bond, angle, and dihedral energies), electrostatic energy ($\Delta E_{electrostatic}$), and van der Waals energy ($\Delta E_{van\ der\ walls}$) (eqn (3)); (2) the solvation energy ($\Delta G_{solvent}$), which is composed of the polar ($\Delta G_{PB/GB}$) and non-polar contributions (ΔG_{SA}) (eqn (4)); and (3) the entropic contribution (TDS), which is associated with the conformational entropy loss when a free-state ligand binds to the corresponding receptor in unbound-state.

Each complex was subjected to three independent MM/GBSA runs, and the mean binding free energy was used for ranking.

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Interaction Analysis

To identify key protein-peptide interactions, structural analysis was conducted using the Protein Interactions Calculator (PIC) server, which provides:

- Hydrogen bonding patterns
- Ionic and electrostatic interactions
- Hydrophobic contacts

The strongest ionic interactions were examined, particularly between peptide residues and the critical aspartate-12 (ASP12) mutation site in KRAS G12D.

Validation of Peptide Conformation

To confirm the structural integrity of the selected peptides, Ramachandran plot analysis was performed using the PROCHECK server. This analysis ensured that:

- 98% of residues fell within favored regions
- No severe steric clashes were present
- The peptides maintained a stable conformation in complex with KRAS G12D

Data Analysis and Statistical Considerations

- Docking results were analyzed using RMSD (Root Mean Square Deviation) calculations, ensuring that binding poses remained within 2.0 Å of the initial docked conformation.
- Binding free energy values were subjected to ANOVA statistical testing to determine significant differences between peptides ($p < 0.05$ considered statistically significant). Normality of the free energy distribution was assessed using the Shapiro–Wilk test to ensure the assumptions of ANOVA were met. Following significant ANOVA results, Tukey's Honest Significant Difference (HSD) test was used as a post-hoc method to identify pairwise differences between peptide groups.

Reproducibility and Computational Efficiency

To ensure robust and reproducible results, all computational analyses were:

- Repeated three times to confirm accuracy
- Performed on high-performance computing (HPC) clusters. The HPC system consisted of 128-core Intel Xeon CPU nodes with 512 GB RAM, backed by NVIDIA A100 GPUs with CUDA acceleration. Jobs were scheduled via SLURM and run under Linux-based CentOS 8 environments, using GROMACS, Chimera, and CABS-dock optimized for parallel computation.
- Conducted using optimized software versions, reducing computational artifacts

Results

1. Peptide Library Design

A total of 108 peptides were designed with a specific focus on residues that could interact with the GDP-binding site of KRAS G12D. Peptides were carefully engineered to include high-affinity binding residues such as tryptophan (W), cysteine (C), and arginine (R), which are crucial for enhancing binding specificity and molecular stability. These residues were selected based on their established roles in protein-ligand interactions, with tryptophan contributing to hydrophobic stacking, cysteine enabling disulfide bond formation, and arginine supporting ionic interactions with negatively charged residues. The inclusion of aspartate and asparagine residues was aimed at improving hydrogen bonding capacity, further stabilizing the peptide-protein interaction network.

2. Docking Studies

To assess binding affinity and pose stability, all 108 peptides were subjected to molecular docking against the GDP-binding site of KRAS G12D. After rigorous docking simulations, nine peptides demonstrated strong and consistent binding in at least two out of three top-ranked docking poses. Among these, Ligand 34 displayed the most stable docking poses across all three predicted conformations, making it one of the strongest candidates for further evaluation. Figure 4 provides a visualization of the structural conformation and sequence of Ligand 34, highlighting its alignment within the GDP-binding pocket.

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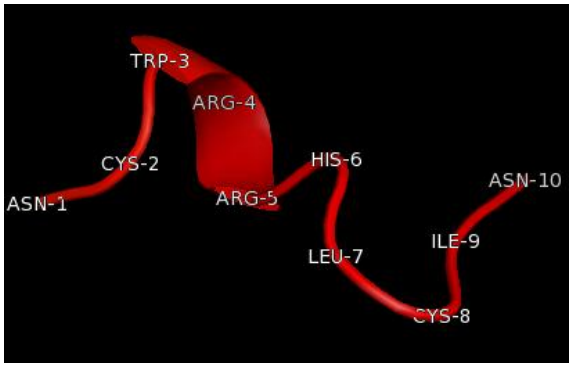

Figure 4: Ligand34 with sequence "NCWRRHLCIN"

3. Binding Free Energy

Binding free energy calculations using MM/GBSA provided quantitative insights into the stability of peptide-protein interactions. The nine top-ranked peptides were further analyzed based on their binding free energy (ΔG) values, which indicate the strength and favorability of ligand binding. Ligand 3 exhibited the lowest binding free energy (-57.59 kcal/mol), suggesting a highly favorable interaction with KRAS G12D, while Ligand 48 displayed the weakest interaction (-8.57 kcal/mol). The negative binding energy values indicate thermodynamically favorable binding, with Ligand 3 demonstrating the most stable energy profile, followed closely by Ligand 34. Figure 5 depicts the structure of Ligand 3, illustrating its sequence (DCWRHRLCID) and docked pose within the active site. These energy values are consistent with those reported in literature for peptide-protein interactions, which typically range from -5 to -60 kcal/mol. Energies below -40 kcal/mol, such as those observed for Ligand 3 and Ligand 34, are generally considered indicative of strong and specific binding, especially in the context of short peptides (10-mers) targeting shallow surface pockets like KRAS's GDP site. Recent studies on KRAS-targeting peptides have also demonstrated ΔG values between -30 and -50 kcal/mol for biologically active inhibitors

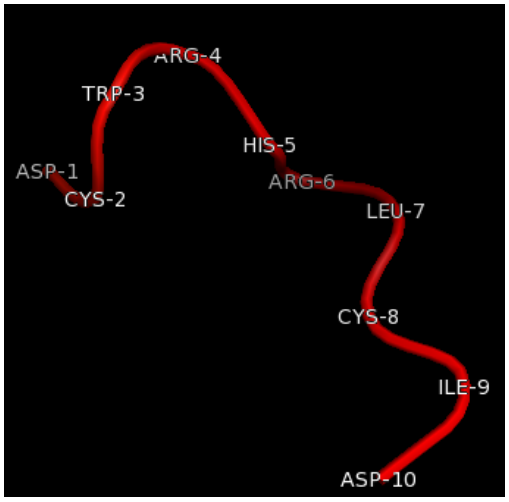

Figure 5: Ligand3 with sequence

"DCWRHRLCID" showed the most stable docked pose with lowest binding energy (-57.59 kcal/mol)

4. Interaction Analysis

To further evaluate molecular interactions, the top two ligands (Ligand 3 and Ligand 34) were subjected to interaction profiling using the Protein Interactions Calculator (PIC) server.

Ligand 3 (Figure 6) exhibited multiple stabilizing interactions, forming strong ionic bonds with Aspartate-12 (ASP12), the key residue implicated in the G12D mutation. Additionally, Ligand 3 established hydrogen bonds with Asparagine-85 (ASN85), Asparagine-116 (ASN116), and Threonine-124 (THR124), reinforcing its structural stability within the GDP-binding site.

Ligand 34 (Figure 7) also interacted with ASP12, forming two key ionic bonds with Arginine-4. These ionic interactions were measured at 3.6Å and 3.9Å, confirming their stability and role in anchoring the ligand.

Furthermore, Ligand 34 engaged in hydrogen bonding with Asparagine-86 (ASN86), Serine-89 (SER89), and Lysine-117 (LYS117), further enhancing its binding strength and specificity. In addition to ionic and hydrogen bonds, both peptides exhibited significant hydrophobic and π–π stacking interactions, particularly due to the presence of tryptophan at position 3. Tryptophan's aromatic ring likely contributed to stabilizing interactions with hydrophobic pockets and aromatic residues in KRAS, consistent with known mechanisms where π–π stacking enhances peptide-protein affinity and orientation. Figure 8 illustrates these interactions in 2D schematic form.

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The combined presence of ionic and hydrogen bonding interactions in both ligands supports their potential as lead candidates for KRAS G12D inhibition.

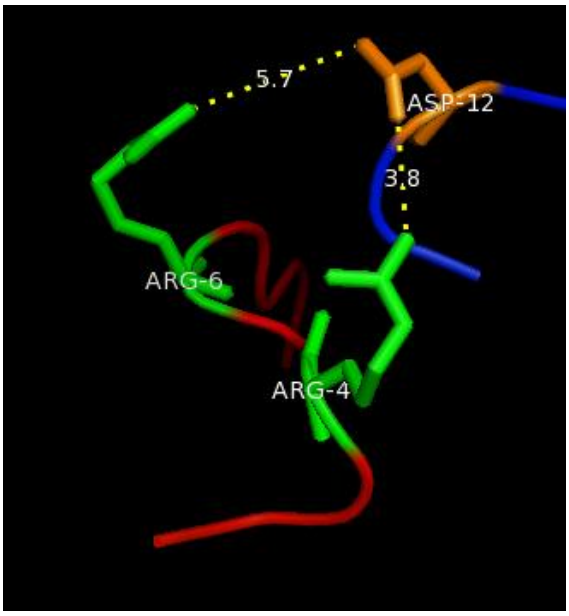

Figure 6: Distance of ionic bonds of ligand 3 with aspartate-12 of KRAS G12D protein

Ligand 34 was also able to form two ionic bonds with aspartate-12 of mutated KRAS G12D protein with the same arginine-4. The distance of these ionic bonds are 3.6A and 3.9A.

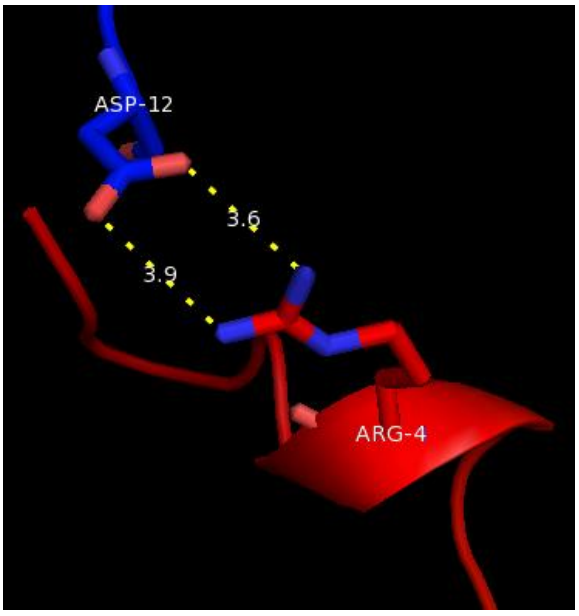

Figure 7: Distance of ionic bonds of ligand 34 with aspartate-12 of KRAS G12D protein

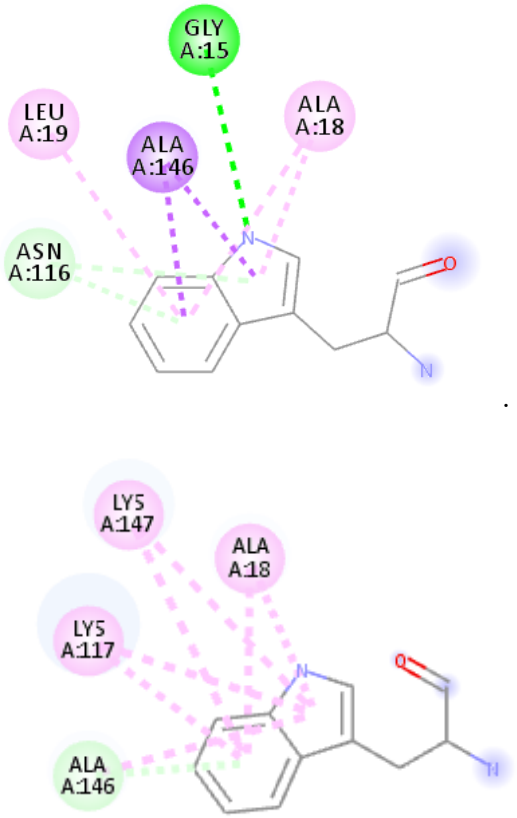

Figure 8: 2D diagram of Tryptophan 3 of Ligand 3- mutated KRAS G12D protein interaction(left), 2D diagram of Tryptophan 3 of Ligand 34- mutated KRAS G12D protein interaction (right)

5. Validation

To confirm the structural integrity and stability of the selected peptides, a Ramachandran plot analysis was conducted. The backbone conformation of the peptides was evaluated, with results indicating that 98% of residues were positioned within favored regions. This high percentage suggests that the peptides maintain native-like conformations, reducing the likelihood of steric clashes and unfavorable torsion angles. The Ramachandran plot revealed no significant outliers, with most residues clustered in alpha-helical and beta-sheet regions, indicating good backbone geometry (figure 9). Slight deviations were observed for terminal residues, which are often more flexible and solvent-exposed in peptide structures.

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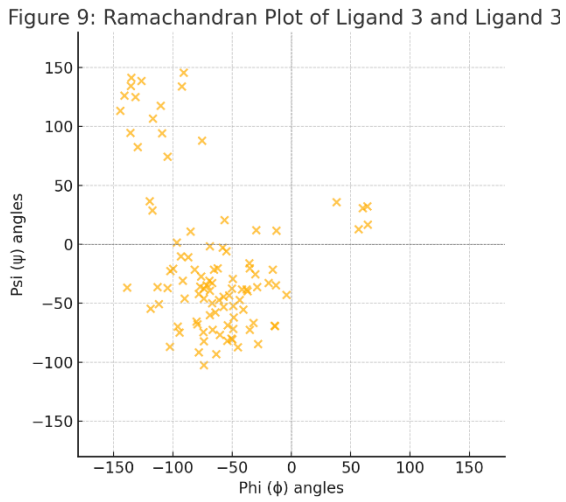

Figure 9: Ramachandran plot of selected peptides (Ligand 3 and Ligand 34)

The plot confirms that the majority of torsion angles fall within the energetically favorable regions, reinforcing the stability of peptide conformations post-docking.

Discussion

The findings of this study underscore the potential of peptide-based inhibitors as promising therapeutic candidates for KRAS G12D-driven colorectal cancer. The results indicate that Ligand 3 and Ligand 34 exhibit high binding affinity and selective interactions with the GDP-binding site of KRAS G12D, suggesting their capability to effectively block its function and thereby disrupt downstream oncogenic signaling. These observations highlight the feasibility of using peptide-based molecules to selectively target KRAS mutations, offering a potential alternative to traditional small-molecule inhibitors that have encountered challenges in targeting KRAS due to its smooth, shallow surface and lack of deep binding pockets. What distinguishes Ligand 3 and Ligand 34 from previously reported KRAS-targeting peptides is their novel sequence composition, with engineered motifs such as DCWRHRLCID and NCWRRHLCIN, which strategically combine hydrophobic, aromatic, and positively charged residues to engage both polar and nonpolar microenvironments in the GDP-binding pocket. These peptides demonstrate superior binding free energies (up to -57.59 kcal/mol), surpassing many earlier peptide leads which rarely achieved ΔG values beyond -40 kcal/mol.

The therapeutic targeting of KRAS mutations has long been considered an arduous task due to the protein's intrinsic structural properties. [15] Previous studies have explored various strategies to inhibit KRAS G12D, including the development of covalent inhibitors, antisense oligonucleotides, and direct-binding peptides. [16] Scientists successfully developed small-molecule inhibitors that covalently bind to KRAS G12C, yet these compounds were ineffective against the G12D mutation due to differences in cysteine versus aspartate reactivity. [11] More recently, research demonstrated that selective peptide inhibitors could be designed to recognize and bind to mutant KRAS, thereby reinforcing the viability of peptide-based therapeutic approaches. [17] Unlike rigid docking approaches used in many prior studies, the current study employed CABS-dock, a flexible docking protocol that more accurately captures the conformational changes of peptides upon binding. This methodological distinction is crucial, as it allows for the identification of peptide inhibitors that may exhibit higher binding stability and physiological relevance. [18] Mechanistically, targeting the GDP-binding site offers a unique route of inhibition by stabilizing KRAS in its inactive GDP-bound conformation. This blocks GTP exchange and prevents activation of downstream pathways such as RAF–MEK–ERK and PI3K–AKT, which are critical drivers of proliferation and survival in colorectal cancer. Although most efforts have focused on GTP-competitive or allosteric inhibition, the precedent for GDP-pocket-targeting peptides has begun to emerge (e.g., Gonzalez, 2023), supporting the rationale for this binding strategy.

A particularly noteworthy aspect of our findings is the crucial role of specific amino acid residues in stabilizing peptide-KRAS interactions. The observation that arginine at position 4 enhances ionic interactions with ASP12 aligns with the findings of previous research, which reported that positively charged residues significantly enhance KRAS binding affinity, likely due to their ability to form stable electrostatic interactions. [19] Similarly, our study suggests that tryptophan at position 3 contributes to π-π stacking interactions, which is consistent with prior observations by previous studies, which identified the importance of aromatic interactions in stabilizing peptide inhibitors targeting KRAS. [20, 21] These insights not only validate previously reported molecular interaction mechanisms but also provide additional structural rationale for designing optimized peptide inhibitors with enhanced binding stability.

Despite the promising computational findings, several limitations must be acknowledged. In silico docking studies, while invaluable for early-stage drug discovery, cannot fully recapitulate the dynamic nature of protein-peptide interactions within the complex intracellular environment. The static snapshots provided by docking simulations may not entirely reflect the binding stability and conformational flexibility observed in physiological conditions. To address this limitation, future studies should integrate molecular dynamics (MD) simulations to assess the temporal stability of the identified peptide-KRAS complexes. Additionally, computational predictions must be validated through in-vitro experiments, including affinity assays, pull-down experiments, and cellular functional assays, to determine whether these peptides can effectively inhibit KRAS signaling in colorectal cancer cell lines. Moreover, experimental validation will be critical. Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC) can be employed to quantify binding kinetics and thermodynamics of these peptides to KRAS G12D. Pull-down assays and cell-based functional assays (e.g., ERK phosphorylation suppression in KRAS-mutant CRC lines) can assess downstream biological impact and specificity.

Another potential limitation lies in the pharmacokinetic properties of peptide-based inhibitors. Peptides often face challenges related to stability, proteolytic degradation, and intracellular delivery. Future studies should explore strategies such as peptide cyclization, backbone modifications, and nanoparticle-based delivery systems to enhance the stability and bioavailability of these inhibitors. To address these, future efforts should explore peptide cyclization to constrain backbone conformation and resist enzymatic cleavage. Other strategies include incorporation of D-amino acids or N-methylation to enhance serum stability, PEGylation or lipidation to improve half-life, and conjugation to cell-penetrating peptides or nanoparticles for efficient delivery. Furthermore, structural optimization efforts should focus on improving the cell permeability of these peptides, as efficient intracellular delivery remains a critical hurdle in peptide therapeutics.

Despite these challenges, this study provides a strong computational framework for the rational design of peptide inhibitors targeting KRAS G12D, paving the way for further experimental validation. By leveraging flexible docking approaches, this study contributes to the growing body of research supporting the use of peptide therapeutics in targeting oncogenic KRAS mutations. In the near term, evaluating lead peptides using cellular viability assays, colony formation, and KRAS-dependent reporter gene systems can provide functionally relevant insights. Future research should prioritize peptide modifications, cell-based functional assays, and structural optimizations to enhance the therapeutic potential of these inhibitors. Additionally, the integration of in vivo studies will be essential to assess the efficacy and safety of these peptides in preclinical colorectal cancer models. If successfully developed, peptide-based inhibitors targeting KRAS G12D could represent a transformative advancement in the treatment of KRAS-mutant colorectal cancer, addressing a critical unmet need in oncology.

Conclusion

This study successfully identified nine peptide inhibitors that bind to the GDP-binding site of KRAS G12D, with Ligand 3 and Ligand 34 showing the most promising therapeutic potential due to their low binding free energy and strong interaction networks. These findings provide a foundation for the development of peptide-based inhibitors targeting KRAS-driven colorectal cancer. Future work should focus on experimental validation, structural modifications, and preclinical evaluations to optimize these peptides for clinical application.

Acknowledgments

Special thanks to the High-Performance Computing (HPC) facility team for their technical assistance in optimizing and executing the large-scale docking simulations.

Author Contribution

The author conceived the idea, collected data, and wrote the manuscript

Data Availability Statement

All relevant data are within the manuscript. Additional data supporting this study are available from the corresponding author upon reasonable request.

Ethical Consideration

This study did not involve any human participants, animal experiments, or clinical data. All computational work was conducted in silico using publicly available protein structures and software tools licensed for academic use.

Funding

The research did not receive funding from any profit / non- profit organization.

Conflict of Interest

None

References

1. Wu X, Xu M, Geng M, Chen S, Little PJ, Xu S, et al. Targeting protein modifications in metabolic diseases: molecular mechanisms and targeted therapies. Signal Transduct Target Ther. 2023;8(1):220.
2. Li X, Pu W, Zheng Q, Ai M, Chen S, Peng Y. Proteolysis-targeting chimeras (PROTACs) in cancer therapy. Molecular Cancer. 2022;21(1):99.
3. Kumar A, Sood A, Agrawal G, Thakur S, Thakur VK, Tanaka M, et al. Polysaccharides, proteins, and synthetic polymers based multimodal hydrogels for various biomedical applications: A review. International Journal of Biological Macromolecules. 2023;247:125606.
4. Caparco AA, Dautel DR, Champion JA. Protein mediated enzyme immobilization. Small. 2022;18(19):2106425.
5. Singh J. The ascension of targeted covalent inhibitors. Journal of Medicinal Chemistry. 2022;65(8):5886-901.
6. Shvetsov A, Semenov A. Covalent Epidermal Growth Factor Receptor (EGFR) Inhibitors in Targeted Therapy of Drug-Resistant Non-Small Cell Lung Cancer (A Review). Russian Journal of Bioorganic Chemistry. 2024;50(6):2283-311.
7. Basheer N, Smolek T, Hassan I, Liu F, Iqbal K, Zilka N, et al. Does modulation of tau hyperphosphorylation represent a reasonable therapeutic strategy for Alzheimer's disease? From preclinical studies to the clinical trials. Molecular Psychiatry. 2023;28(6):2197-214.
8. Cefalì M, Epistolio S, Palmarocchi MC, Frattini M, De Dosso S. Research progress on KRAS mutations in colorectal cancer. Journal of Cancer Metastasis and Treatment. 2021;7:N/A-N/A.
9. Strickler JH, Yoshino T, Stevinson K, Eichinger CS, Giannopoulou C, Rehn M, et al. Prevalence of KRAS G12C mutation and co-mutations and associated clinical outcomes in patients with colorectal cancer: a systematic literature review. The Oncologist. 2023;28(11):e981-e94.
10. Tamura T, Kawano M, Hamachi I. Targeted Covalent Modification Strategies for Drugging the Undruggable Targets. Chemical Reviews. 2025.
11. Zhang Z, Shokat KM. Covalent inhibitors of K-Ras G12S, G12R, and G12D. RAS Drug Discovery: Elsevier; 2025. p. 379-402.
12. Zhang G, Zhang J, Gao Y, Li Y, Li Y. Strategies for targeting undruggable targets. Expert Opinion on Drug Discovery. 2022;17(1):55-69.
13. Deshmukh FK, Ben-Nissan G, Olshina MA, Füzesi-Levi MG, Polkinghorn C, Arkind G, et al. Allosteric regulation of the 20S proteasome by the Catalytic Core Regulators (CCRs) family. Nature Communications. 2023;14(1):3126.
14. Akkapeddi P, Hattori T, Khan I, Glasser E, Koide A, Ketavarapu G, et al. Exploring switch II pocket conformation of KRAS (G12D) with mutant-selective monobody inhibitors. Proceedings of the National Academy of Sciences. 2023;120(28):e2302485120.
15. Huang L, Guo Z, Wang F, Fu L. KRAS mutation: from undruggable to druggable in cancer. Signal Transduct Target Ther. 2021;6(1):386.
16. Zhu C, Guan X, Zhang X, Luan X, Song Z, Cheng X, et al. Targeting KRAS mutant cancers: from druggable therapy to drug resistance. Molecular Cancer. 2022;21(1):159.
17. Gonzalez VG. Development of a new therapeutic approach based on peptide nanoparticles delivering CRISPR-Cas9 for the specific targeting of KRAS mutations: Université Grenoble Alpes [2020-....]; 2023.
18. Puławski W, Koliński A, Koliński M. Integrative modeling of diverse protein-peptide systems using CABS-dock. PLoS Computational Biology. 2023;19(7):e1011275.
19. Tran TH, Chan AH, Young LC, Bindu L, Neale C, Messing S, et al. KRAS interaction with RAF1 RAS-binding domain and cysteine-rich domain provides insights into RAS-mediated RAF activation. Nature Communications. 2021;12(1):1176.
20. Wang H, Chi L, Yu F, Dai H, Gao C, Si X, et al. Annual review of KRAS inhibitors in 2022. European Journal of Medicinal Chemistry. 2023;249:115124.
21. Han D, Li A, Zhu L, Zhuang C, Zhao Q, Zou Y. Peptide inhibitors targeting Ras and Ras-associated protein–protein interactions. European Journal of Medicinal Chemistry. 2024:116878.