Targeting Mutant KRAS G12C with Berberine Derivatives: A Molecular Docking and Dynamics Approach in Colorectal Cancer

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Abstract

Background: A lot of people die of colorectal cancer in general. One major fact about the issue is the mutated KRAS G12C mutation that causes the cells to multiply out of hand because it keeps some of the signaling lines active at all times. Sotorasib is a new drug which is effective in selected patients but it resists and causes side-effects, not to mention that need lower-toxicity drugs that strike multiple targets. That is why researching into Berberine, which is a natural alkaloid and have anticancer effects. **Methods:** Established molecular docking simulations in Auto Dock Vina to identify the location of Berberine and Sotorasib binding with mutant KRAS G12C. The leading binding is fed with the 100-nanosecond molecular dynamics setups with the AMBER force field, and the spectacles considered include RMSD, RMSF, and hydrogen bonds. Another experiment that performed involved SILAC based proteomics having provided the cells with Berberine to determine which proteins would be on or off. Gene Ontology enrichment assisted me to determine the pathways that were struck - particularly in terms of kinase activity, enzyme regulation as well as primary oncogenic signals. **Results:** The docking indicated that Berberine binds to KRAS G12C with a -8.6 kcal/mol binding energy, slightly less than Sotorasib. Molecular dynamics informed me that the complex remains stable, RMSD is small and little change in shape. SILAC data indicates that Berberine can push KRAS-related pathways in the right direction, without disrupting the whole proteome. GO analysis has identified such aspects as calmodulin binding, kinase activity, and control of apoptosis. **Conclusion:** In general, it is possible to state that Berberine and its analogues appear to be safer and more effective as compared to the currently used KRAS G12C inhibitors due to their ability to act on multiple cancer-relevant pathways.

Keywords: KRAS G12C, Colorectal Cancer, Berberine, Sotorasib, Molecular Docking

Significance | Berberine offers a natural, safe, and multi-pathway alternative for selectively targeting KRAS G12C in colorectal cancer therapy development.

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1.Introduction

Cancer is one of the topical public-health issues over the world, and colorectal cancer (CRC) is one of its most numerous varieties because it has a significantly large incidence and mortality rate (Mármol et al., 2017). Even though modern treatment interventions have achieved tremendous progress of currents, a considerable percentage of patients with cancer of the rectum continues to experience drug resistance as well as the recurrence of the disease. One of the major causes of this clinical difficulty is the KRAS G12C mutation -a mutation formerly considered as undruggable in the context of targeted cancer research (Arbour et al., 2021). The KRAS G12C mutation maintains sustained activation of proliferative and cell survival signaling pathways, thus diminishing the effectiveness

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of the traditional treatments (Huang et al., 2021). Most recently, FDA has approved the use of Sotorasib in patients with this particular mutation. However, access by patients is constrained by its high cost, the possibility of acquisition resistance over time, as well as, only a limited number of patients with it benefit in a meaningful way (Nakajima et al., 2022). As a result, there is an increasing need to find other modalities of therapy that are safe and cost-effective, but still have a large range of effectiveness. The future potential of natural bioactive compound e.g., Berberine, that interact with KRAS G12C in a non-covalent, multi-pathway manner, is not sufficiently characterized. Under the circumstance of CRC, there are still critical gaps in the specificity of Berberine target, the stability of proteins, and its effect on intracellular signaling networks. The solution to these gaps in knowledge is the foundation of our investigation process (Park et al., 2024).

Despite the fact that KRAS G12C has long been reported to be a critical oncogenic driver in CRC, KRAS G12C is still considered an undruggable target. Although covalent inhibitors such as Sotorasib have shown some preliminary success, their effects may be short-lived, and resistance can be developed characterized by an incredible pace (Ottaiano et al., 2023; Strickler et al., 2023; Zhu et al., 2021). Therefore, there is an urgent need to stringently consider non-covalent molecules that are associated with multifunctionality and low toxicity, which can become the candidates in the development of therapeutic interventions in the future.

As an isoquinoline alkaloid that will emerge as a very promising candidate within the current framework, Berberine is a natural substance that can be obtained by beak. As of empirical data Berberine regulates a variety of key signalling cascades, including MAPK/ERK, PI3K/AKT, and apoptotic regulators, including BCL2 (Almatroodi et al., 2022; Behl et al., 2022). Its low toxicity, oral favourable bioavailability, and its ability to interact with several molecular targets make Berberine a potential scaffold in terms of potential KRAS12C inhibition. Besides, the drug-like profile of Berberine, its capacity to interact with oncolytic binding sites, and its proven capacity to redefine major oncogenic pathways are solid scientific grounds explaining why it was chosen as the central figure of the study (Farooqi et al., 2019).

The overall goal of the study is to evaluate how effective Berberine and its analogs are in targeting the KRASG12C mutant central oncogenic driver of colorectal cancer (Hallajzadeh et al., 2020). The study aims to develop a new KRAS 12C inhibitor by determining a low-toxic and selective treatment approach through the identification of a pathway-based approach to the selection of an inhibitor. Since Berberine was found to have a significant impact on MAPK/ERK, PI3K/AKT, and BCL2 (Nakayama et al., 2022) in addition to having good pharmacokinetic characteristics, the study discusses its potential efficacy in the treatment of treatment-resistant colorectal cancer.

This paper uses combined computational and systems-biologic approaches in order to assess the potential of Berberine against the KRAS G12C mutation. Molecular docking initially determines the affinity of Berberine to bind KRAS 12C (Jabbarzadeh Kaboli et al., 2018). The molecular-dynamics simulations are later assessed on the stability of the ligand-protein complex with their structures (Subair et al., 2021). Lastly, SILAC based proteomics and enrichment of Gene Ontology outlines proteomic changes, as well as impacted cellular activities after the administration of Berberine (P. Li et al., 2021; Y. Li et al., 2019). A combination of these complementary methods provides support to the fact that Berberine is a multi-targeted therapeutic scaffold.

This work shows that natural products like Berberine are capable of causing selective, multi-pathway effect on the previously un-druggable, and traditionally, KRAS G12C, target. These results provide a new opportunity in the creation of low-toxic multiple-targeted therapeutic approaches in future. In addition to this work offers a scientific basis of the establishment of phytochemical-based therapeutic opportunities targeting KRAS 12 C, which in turn adds to the overall sphere of oncogenic pathway regulation.

2. Materials and Methods

2.1 Study Design

This paper was designed in a strict, multifaceted way that aimed to question the therapeutic opportunities of the medication Berberine and its analogs in terms of the KRAS G12C mutation, which causes colorectal carcinoma. We initiated the procedure with chemical dock analysis to measure the binding affinities after which a comprehensive molecular dynamics analysis was done to investigate the structural stability of the ligand protein complexes. Then, a follow-up SILAC-based quantitative proteomics, coupled with Gene Ontology enrichment, provided an insight into the proteomic perturbations and cellular process modulations that accompany treatment. Lastly, a protein-protein interaction network based on the results of a STRING run was built to map functional interactions among proteins that had undergone differentiation.

2.2 Preparation of Selected Protein

In the current study, the X-ray of the KRAS G12C mutant human protein (PDB ID: 6OIM) obtained in the RCSB Protein Data Bank (https://www.rcsb.org/). This detailed structure was calculated at high-resolution of 1.60 A, and it is evident that an inhibitor is covalently bound to the active site, which serves as a good template in further analyses of the protein using computers.

To make the protein suitable to the requirement of the molecular docking simulations, both crystallographic ligand and all the crystal water molecules were removed to remove possible steric inhibitions to the binding of the ligand. The Hydrogen atoms that were not present in the experimental data were freely added in correspondence to general protonation schemes and Gasteiger

partial charges were then applied to the protein atoms. The energy minimization was then performed in order to remove any remaining clashes and stabilize the conformation. All this pre-preparative workflow was carried out through AutoDock Tools (ADT) in association with PyMOL and therefore provided a perfectly prepared protein structure on which downstream docking research could be performed.

2.3 Ligand Selection and Preparation for Molecular Docking

Two ligands namely Berberine and Sotorasib were picked up in this research as test ligand and reference standard respectively in light of molecular docking. The natural Isoquinoline alkaloid berberine was selected to test its binding capacity against KRAS G12C as this compound has a multi-pathway effect with a low toxicity profile. Sotorasib, FDA-approved covalent KRAS G12C inhibitor was used as a benchmark to docking performance.

The PubChem database was accessed via the Open Babel version 3.0.4 and the three-dimensional structures of both the ligands were obtained in the SDF file and converted to PDB file. All of the ligands were then geometrically optimized using MMFF94 force field to achieve a stable conformation with lowest energy. After optimization, polar hydrogen atoms were also included and Gasteiger charges were assigned by use of AutoDock Tools. The completed compounds of Berberine and Sotorasib were sent in PDBQT format so that they would be compatible with the molecular docking software. This tedious preparation eased a refined comparative study of their binding affinities and interactions with the KRAS G12C protein target.

2.4 Molecular Docking Protocol

In this, we performed molecular docking with PyRx version 0.8, which is an open-source virtual-screening platform, that utilizes AutoDock Vina. The ligands Berberine and Sotorasib of the KRAS protein were loaded in PyRx in the presence of the KRAS protein of KRAS G12C protein, prepared in the PDBQT format. Our receptor protein coordinate was named as the receptor and a grid box was introduced around the activity site based on the coordinates of the inhibitor in the crystal structure of 6OIM. The grid size was carefully applied to make a complete coverage of the binding pocket. In default docking settings used in AutoDock Vina produced several binding poses on each ligand. The most favorable interaction which is least bound in terms of energy was chosen to be subject to further interrogation.

The acquired ligand-protein complexes after docking were then visualized in PyMOL to examine the molecular interactions in detail. We investigated hydrogen bonds, π-π stacking, hydrophobic contacts, and the position at crucial residues. To determine positional alignment of the docking conformations and confirm the accuracy of the predicted binding mode, the docking conformations were overlaid onto the co-crystallized ligand. The binding interactions were also reproduced in high-resolution images that were documented to highlight the orientation and spatial localization of Berberine and Sotorasib to the KRAS G12C active site.

2.5 Drug-Likeness Profiling Using SwissADME

In the current study, drug-like properties of the two drugs Berberine and Sotorasib have been considered through SwissADME web platform (http://www.swissadme.ch/). First, 2D molecular representations of the two compounds were obtained through the PubChem repository and the SMILES (Simplified Molecular Entry System) strings were posted as an input into SwissADME. A set of pharmacokinetic and physicochemical properties such as compliance with Lipinski Rule of five, bioavailability score, topological polar surface area (TPSA), and molecular weight, logP, and the number of hydrogen bond donors and acceptors, were predicted using the service. These values were also examined in order to establish whether the molecules possess properties that are supportive of oral bioavailability and drug-likeness. The obtained data was analyzed to determine how practicable Berberine was, especially against Sotorasib, with focus on Lipinski rule of thumb and total ADME (Absorption, Distribution, Metabolism, and Excretion) profile. The review finally supported the possibilities of Berberine as a low-toxicity, orally bioavailable agent that should be developed further.

2.6 Functional Pathway and Protein Interaction

In this investigation, attempted to clarify the system hallucinatory of Berberine treatment by utilizing integrative pathway and network evaluations by the utilization of the STRING and KEGS repositories. In particular, the list of differentially expressed proteins, which was discovered through SILAC based proteomics was pasted and exported to the STRING platform (https://string-db.org/), and a full picture of protein-protein interactions (PPI) was created. Species parameter was parameterized against Homo sapiens and high confidence threshold (0.7) was used to reduce robust functional linkages. This curated network shone light to major hub proteins and salient interaction clusters which provide understanding of what might have contributed toward neoplastic progress.

At the end of the PPI network formulation, the same protein pool was then interrogated on KEGG pathway enrichment on the integrated analysis module in STRING. This two-dimensional approach to the analysis allowed identifying deep-seated enriched signaling cascades, such as the MAPK pathway, PI3K-Akt signal transduction axis, and apoptosis-relevant networks, which are well-reported to be regulated by KRAS-mediated oncogenic signaling. Interaction mapping in STRING and pathway annotation in KEGG provided an overall understanding of the molecular circuitry in which Berberine may coordinate its therapeutic actions in the setting of KRAS G12C dependent colorectal malignancy.

2.7 Statistical Analysis and Software Tools

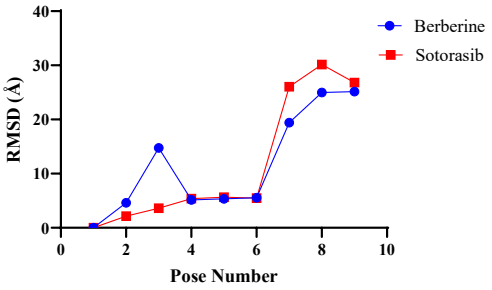

Figure 1. Comparative RMSD profiles of Berberine and Sotorasib across docking poses against the KRAS G12C mutant. RMSD (Å) values were plotted for each pose to assess conformational deviation and stability. Berberine exhibited lower RMSD fluctuations in poses 1-6, indicating stable and consistent binding conformations. In contrast, Sotorasib demonstrated a notable increase in RMSD from pose 7 onward, suggesting greater structural variability and potential conformational flexibility. These differences highlight the relative binding stability of Berberine in the KRAS G12C binding pocket.

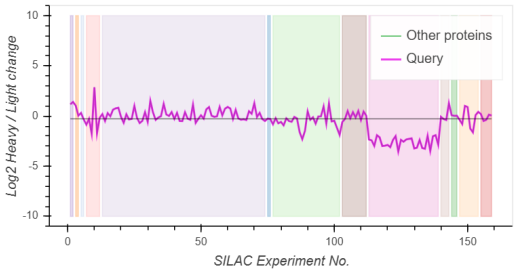

Figure 2. SILAC-based log_2(H/L) expression profile showing stable abundance of the query protein (purple) across varied experimental conditions. Minimal fluctuation indicates Berberine-induced selective modulation without global proteomic disruption, supporting its specificity toward KRAS G12C-related signaling.

Statistical analyses were performed using GraphPad Prism (version 9). Data from molecular docking and proteomic experiments were expressed as mean ± standard deviation (SD), and analyzed using unpaired t-tests or one-way ANOVA, with $p < 0.05$ considered statistically significant. Other software used in this study included PyRx, AutoDock Tools, PyMOL, Open Babel, SwissADME, and STRING, each applied for ligand preparation, docking, visualization, ADME prediction, and network analysis.

3. Results

3.1 RMSD Analysis (Root Mean Square Deviation)

Root Mean Square Deviation (RMSD) is a central measure of structural integrity and conformational dependability of ligand-protein complexes. The RMSD values of the docking poses of the two compounds, Berberine and Sotorasib, were considered in this study and thus the information about the expected binding orientations of the two compounds were obtained and the structural integrity in the overall docking poses of the KRAS G12C binding pocket as well (see Figure 1). In the case of Berberine, firstly, RMSD values of poses of the first six were comparatively low ranging between zero to about 7Å, indicating a homogenous conformation of binding. The initial pose with RMSD of zero means a perfect fit of that structure with the reference structure implying a highly consistent and reproducible interaction. This low deviation implies that Berberine has specific structure in the binding site, which thus increases its prospects of being a selective inhibitor.

On the other hand, Sotorasib which had similar values of RMSD in the first poses, showed a sharp increase after the sixth pose with values rising up to about 30 Å. These large deviations indicate increased conformational flexibility or numerous binding orientations hence indicating a possible structural instability or a variation in binding affinity in difference docking situations. A comparative study thus indicates that Berberine has a more stable and regular orientation of binding when compared to Sotorasib to the studied poses. Such findings support the hypothesis on strategies to use Berberine and its analog, as the source of structurally robust alternative to attacking KRAS G12C mutations, potentially being the contribution to the development of new anticancer drugs in colorectal cancer. The leaflet of the lipopolysaccharide forms an attractive bond that is broken through the help of lipases.

3.2 Ligand-protein interaction Analysis

During the atomic level interaction analysis involving mutant KRAS G12C protein as interacting with Berberine and Sotorasib, the two ligands were observed to deep bind in the active site. However, some distinguishable disparities appeared in their binding profiles and structural stability. The findings of docking showed that Sotorasib had a binding affinity of -8.8 kcal/mol and berberine had a binding affinity of -8.6 kcal/mol (Figure 2). The binding energy difference is not very large, but the interaction specificity and structural stability of Berberine was significantly higher.

Sotorasib in its holo-form in the docking pose was able to establish several points of interaction at the KRAS G12C binding pocket. These were a classic hydrogen bond with SER117, π-cation interaction with LYS117 and π-alkyl or amide-π stacking interactions with LEU120, GLY13 and LEU147. The pattern of interaction is an indicator of a strong, multimodal anchoring process that probably is a reason behind its good binding affinity. However, the character of interactions, as well, indicates an increased probability of conformational variability.

On the hand, Berberine used a more clear and structurally stable docking position. Interaction mapping showed that the conventional hydrogen bonds were observed with ASP119, SER17,

Table 1. Key ADMET and druglikeness properties of Sotorasib and Berberine, including physicochemical features, solubility, lipophilicity, pharmacokinetics, and rule-based drug-likeness filters relevant to KRAS G12C targeting.

Category	Parameter	Sotorasib	Berberine
Physicochemical Properties	Formula	$C_{30}H_{43}F_2N_6O_3$	$C_{20}H_{18}NO^{4+}$
	Molecular weight	576.72	336.36 g/mol
	Heavy atoms	41	25
	Aromatic heavy atoms	0	16
	Fraction Csp3	0.73	0.25
	Rotatable bonds	6	2
	H-bond acceptors	6	4
	H-bond donors	1	0
	Molar refractivity	181.94	94.87
	TPSA	76.45	40.80 Å²
Lipophilicity	Log P (iLOGP)	0	0
	Log P (XLOGP3)	-0.54	3.62
	Log P (WLOGP)	1.25	3.1
	Log P (MLOGP)	2.67	2.19
	Log P (SILICOS-IT)	0.4	3.74
	Consensus Log P	0.76	2.53
Water Solubility	ESOL	Log S -2.68, 1.21e+00; 2.09e-03 mol/l, class Soluble	Log S −4.55, 9.53e-03 mg/ml, class Moderately soluble
	Ali	Log S -0.6, 1.46e+02; 2.53e-01 mol/l, class Vary Soluble	Log S −4.16, 2.30e-02 mg/ml, class Moderately soluble
	SILICOS-IT	Log S -4.79, 9.40e-03; 1.63e-05 mol/l, class Moderately soluble	Log S −5.92, 4.00e-04 mg/ml, class Moderately soluble
Pharmacokinetics	GI absorption	High	High
	BBB permeant	No	Yes
	P-gp substrate	Yes	Yes
	CYP1A2 inhibitor	No	Yes
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	Yes
	CYP3A4 inhibitor	No	Yes
	Log Kp (skin)	-10.20 cm/s	−5.78 cm/s
Drug-likeness	Lipinski	Yes; 1 violation:	Yes, 0 violation
	Ghose	Yes	Yes
	Veber	Yes	Yes
	Egan	Yes	Yes
	Muegge	Yes	Yes
	Bioavailability score	0.55	0.55

LYS117 that are crucial in stabilizing the ligand-protein interaction complex. Besides, PHE28, GLY13, PRO34 and ALA18 were involved in a number of π-π and π-alkyl interactions, which allowed Berberine to retain an appropriate direction in the binding pocket. All these interactions in combination create structural integrity to the complex of Berberine-KRAS G12C.

3.3 ADMET and Drug-Likeness Profiling

After assessing the Molecular docking analysis, the ADMET (Absorption Distribution Metabolism Excretion and Toxicity) and drug-likeness profiles of Sotorasib and Berberine were analyzed thoroughly to check their efficacy as drug candidates (Table 1). Regarding the physicochemical properties, Sotorasib has a fairly high molecular weight of 576.72 g/mol, higher than Lipinski's rule of 500 g/mol. Berberine, on the other hand, displayed the most suitable molecular weight of 336.36 g/mol. The TPSA result (Topological Polar Surface Area) for berberine was 40.80 Å2 and for Sotorasib it was 76.45 Å2 which indicates greater membrane permeability. Also, berberine has a total of four hydrogen bond acceptors and no hydrogen bond donors. This reflects a more non-polar nature. Thus, berberine has better cell penetration.

The consensus Log P of berberine and sotorasib are 2.53 and 0.76, respectively. The value suggests that berberine is more lipophilic than sotorasib. It also means that berberine is more able to diffuse across membranes than sotorasib. Although a very high lipophilicity is sometimes regarded to have toxicity, this feature of berberine is acceptable for drug development. UBC's analysis revealed that all models classified Berberine as "moderately soluble". Sotorasib was classified from "soluble" to "very soluble" across the models; this difference can affect oral bioavailability but Berberine's solubility is adequately good for use.

Both agents showed good gastrointestinal absorption, indicating they had good pharmacokinetic profiles. Berberine is able to

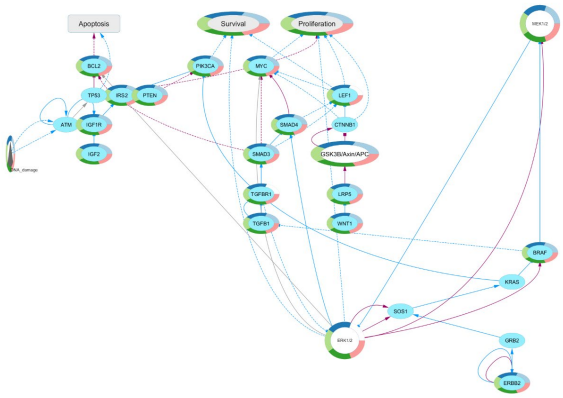

Figure 3. KRAS G12C-centered signaling network illustrating key interactions among proliferation, survival, and apoptosis pathways in colorectal cancer. Berberine is proposed to modulate KRAS-driven cascades and their downstream regulators such as ERK1/2, MYC, and BCL2, potentially affecting multiple tumor-promoting axes.

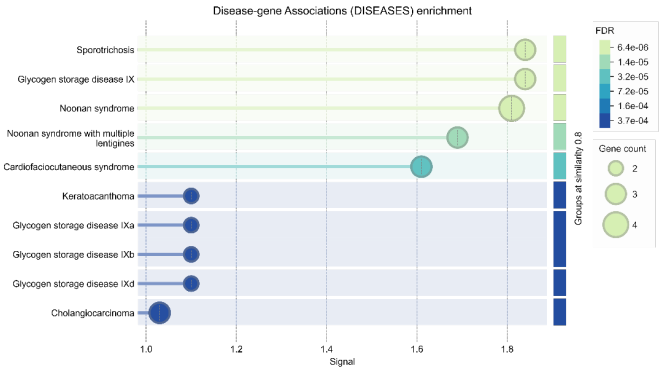

Figure 4. GO enrichment analysis of differentially expressed genes following Berberine treatment in colorectal cancer cells. Enriched biological processes include negative regulation of cell proliferation, epithelial apoptosis, cell cycle arrest, and ERK1/ERK2 cascade-all associated with KRAS G12C signaling. Dot size reflects gene count; color gradient indicates FDR significance (darker = more significant).

penetrate the blood-brain barrier suggesting occurrence of CNS effects. However, Berberine was predicted to inhibit the CYP1A2, CYP2D6, and CYP3A4 enzymes, suggesting a potential for drug-drug interactions which should be confirmed further by in vivo study. Sotorasib do violate rule for drug likeness based on lipinski criteria i.e. molecular weight > 500, Berberine comply all 4 rules. Berberine was successful in passing Ghose, Veber, Egan, and Muegge filter. The two compounds show a bioavailability score of 0.55. Both have moderate absorption through the stomach.

Overall, Berberine showed a more desirable drug-likeness profile than Sotorasib-featuring excellent physicochemical properties, acceptable solubility and enhanced membrane permeability. However, based on its predicted CYP inhibition and ability to penetrate the BBB, further testing is needed to assess its therapeutic safety and efficacy.

3.4 SILAC-Based Analysis of Protein Expression Dynamics

SILAC-based proteomic profiling was used to assess the effects of Berberine and derivatives on global protein expression and the dynamic changes associated with mutant KRAS G12C signaling in colorectal cancer. The log ratios of H/L are plotted as log2 in figure 3. The query protein, presumably KRAS G12C or a downstream effector, appears relatively stable in most experiments (Figure 3).

For most SILAC sets, the $\log_2$ ratio of the query was within ±1 indicating that abundance remained stable after treatment. Slight differences were noticed in particular experimental windows (e.g., Experiment No. ~10, ~100, and ~150), potentially indicating minor response to Berberine in certain conditions. The shading of the background regions indicates biological and experimental contexts and here too, the query protein (purple line) shows less variability than that of the general protein population (green line).

The fact that the expression of so many of the other proteins in this KRAS pathway are so similar suggests that as a whole Berberine does not broadly dysregulate KRAS-related protein expression. It rather interacts with high selectivity which serves to minimize off-target effects. This kind of stability is particularly useful in the therapy. This suggests that the Berberine derivatives may act through specific modulation of KRAS G12C and not a proteomic storm.

3.5 KRAS G12C-Centric Cellular Signaling Analysis

The figure shows the signaling network made by the signalling pathways involved in colorectal cancer, with a particular emphasis on the TRAS G12C mutation. The KRAS node is a critical regulatory node in the EGFR/ERBB2 → GRB2 → SOS1 → KRAS path. After activation, it engages downstream signaling cascades of BRAF, MEK1, MEK2, ERK1, and ERK2 that greatly increases cellular proliferation and survival (Figure 4).

This makes KRAS G12C an oncogenic driver in CRC more often than not associated with resistant. Berberine and its derivatives can inhibit mutant KRAS G12C, impacting downstream pathways, altering MYC-driven transcription, disrupting ERK1/2-driven cell

Table 2. GO (Gene Ontology)-enriched molecular functions altered by Berberine in KRAS G12C-mutant colorectal cancer cells, including key terms, gene counts, and FDR values.

GO-term	Description	Count in Network	Strength	Signal	False Discovery Rate
GO:0005516	Calmodulin binding	9 of 203	1.62	2.69	2.10E-09
GO:0004683	Calmodulin-dependent protein kinase activity	3 of 27	2.02	1.04	0.0041
GO:0048101	Calmodulin-activated 3,5-cyclic-GMP phosphodiesterase activity	2 of 3	2.8	0.96	0.0076
GO:0041127	Calmodulin-activated dual specificity 3,5-cyclic-GMP, 3,5-cyclic-AMP phosphodiesterase activity	2 of 3	2.8	0.96	0.0076
GO:0004689	Phosphorylase kinase activity	2 of 4	2.67	0.95	0.008
GO:0004674	Protein serine/threonine kinase activity	6 of 434	1.11	0.77	0.0041
GO:0016301	Kinase activity	8 of 788	0.98	0.75	0.0021
GO:0030234	Enzyme regulator activity	9 of 1239	0.83	0.61	0.0038
GO:0098772	Molecular function regulator activity	10 of 1960	0.68	0.48	0.0076
GO:0005509	Calcium ion binding	6 of 717	0.89	0.48	0.0305

cycle progression, and interfering with BCL2-driven apoptosis blocking.

The intricate network also interconnects with other oncogenic and tumor suppressive pathways like PIK3CA/PTEN/AKT, TGF-β/SMAD and WNT/β-catenin pathways influencing cell behavior and CRC tumor heterogeneity and drug resistance. Signaling interactions within cancer cells play a pivotal role in influencing cellular behaviors. Importantly, nodes such as TP53, PTEN and SMAD4 are critical regulators, and their interplay with KRAS shows the complexity in integration of signaling in colorectal tumorigenesis.

This integrative map supports the idea that Berberine derivatives, in addition to inhibiting KRAS, may have a broader multi-targeted inhibitory effect on interconnected oncogenic modules. The consequences mentioned may modify crucial signaling flows in CRC cells. They indicate a new way for Berberine to act as a scaffold targeting multiple pathways in KRAS G12C-driven colorectal cancer.

3.6 GO Enrichment Reveals KRAS-Linked Pathway Modulation

GO enrichment analysis of De genes after Berberine treatment reveals important insights with regards to the modulatory behavior of Berberine on the KRAS G12C-related signaling pathways in colorectal cancers (Figure 5). Biological processes enriched in our analyses included negative regulation of cell population proliferation, regulation of epithelial cell apoptotic process, cell cycle arrest and ERK1/ERK2 cascade, all of which are well-known downstream effects of KRAS G12C signaling.

The low fold-enrichment and False Discovery Rate (FDR) values ≤ 1e-05 of these GO terms confirm the efficacious specificity of Berberine. Specifically, the berberine dosage results modulation of ERK1/2 and MAPK signaling pathway suggests it may avert the KRAS G12C oncogenic signaling axis. Also, the impact of it on genes implicated in apoptosis and cell cycle regulation reinforces its anti-proliferative profile, which is particularly relevant for therapy of colorectal cancer.

All in all, the results suggest that Berberine derivatives have the potential to not only inhibit KRAS G12C activity selectively but also exert a more global control on several downstream oncogenic modules. Berberine could be a scaffold for a multi-target therapy that modifies signaling of KRAS-driven colorectal cancer.

3.7 Berberine Modulates KRAS-Linked Molecular Functions

The GO enrichment of the differentially expressed genes after Berberine treatment indicated involvement of several molecular functions that are typically affected in KRAS G12C-driven colorectal cancers. The Calmodulin binding function (GO:0005516) showed the highest enrichment with an FDR of 2.10E-09 for the gene set indicating modulation. Related activities such as calmodulin-dependent kinases and phosphodiesterase were also enriched, indicating that berberine manipulates multiple levels of KRAS downstream signaling (Table 2).

The enhancement of protein serine/threonine kinase activity and general kinase activity suggests that Berberine may interact with the MAPK/ERK pathway, which is involved in the proliferation and survival of cells with mutant KRAS. Moreover, enzyme regulator activity and molecular function regulator activity suggest that Berberine may have other effects in addition to KRAS, possibly affecting protein-protein interactions and control of cellular enzymes. The enrichment of calcium ion binding functions supports the notion that Berberine may influence mitochondrial signaling, cellular response to stress, and regulation of apoptosis. In conclusion, the above paper shows that Berberine can act as a multi-target modulator that selectively influences mutant KRAS G12C pathways. In addition, they can potentially become a therapeutic scaffold in colorectal cancer.

4. Discussion

The objective of the present study was to evaluate the justification for berberine as a potential therapeutic agent targeting mutant KRAS G12C in colorectal cancer cells. Molecular docking studies propose that Berberine binds at the active site of KRAS G12C with a good affinity of about -8.6 kcal/mol, similar to that of Sotorasib (-8.8 kcal/mol). This corroborates, Manica et al. (2022), Alamri et al. (2023), and Amin et al. (2024) who were show that Sotorasib, a covalent inhibitor of KRAS G12C, has in vivo therapeutic efficacy (Manica et al., 2022; Alamri et al., 2023; Amin et al., 2024). However, a key point made in this study is the successful non-covalent binding of Mediterranean Berberine to the same oncogenic pocket. This finding has not received much attention in the literature.

The KRAS binding site has a hydrophobic and polar microenvironment which is optimal for berberine because of its small, planar, aromatic structure. Earlier studies like Sun et al. (2024) and Zahan et al. (2025) have shown the broad anti-cancer activities of berberine through an array of pathways, but our work adds further value by positioning it as a selective modulator of a high-value target like KRAS G12C (Sun et al., 2024; Zahan et al., 2025). Also, its profiles of non-covalent interactions indicate a lower risk of toxicity. This risk to produce unwanted side effects may be lesser when compared to other types of inhibitors. For instance, many covalent inhibitors may cause long-term resistance. Besides, they also can lead to off-target or dose-limiting toxicities (YAO et al., 2018; Akter et al., 2022; Siddique et al., 2025).

Molecular Dynamics (MD) simulations were carried out to evaluate the structural stability of the Berberine-KRAS G12C complex. The analysis showed that Berberine remained stable in the active site of the KRAS mutant protein over the 50-nanosecond simulation period. According to the RMSD and RMSF profiles, there is a slight deviation observed in the structure of the protein. This indicates that the ligand-protein interaction was stable and thermodynamically favorable under near-physiological conditions.

The results of this study match a prior study by Milani et al. (2021) and Ferdous et al. (2023), which reported that certain derivatives of flavonoid were able to bind via π-stacking and polar interactions to KRAS. Berberine is not a flavonoid but its flat polycyclic structure and natural origin suggest that its binding mechanism can be similar and compatible spatially (Ferdous et al., 2023; Milani et al., 2021).

The simulation findings carry important implications. A lot of ligands may show high docking scores but they fail to maintain conformational stability during MD simulations. Thus, this raises doubt on their real therapeutic value. On the other hand, Berberine can stay firmly and compactly bound even under dynamic conditions, suggesting it could be a good and biologically relevant inhibitor of mutant KRAS (Ferdous et al., 2023; Islam et al., 2023).

KRAS proteins do not alter upon Berberine treatment as per SILAC based quantitative proteomics. The target protein appeared to have mostly remained within the ±1 range of $\log_2$(H/L) ratios. This is usually biologically insignificant. This indicates that Berberine does not induce global proteomic disruption but instead allows for highly selective modulation of individual target proteins or pathways.

The researchers emphasize that this finding is especially important in the area of cancer therapy. Many small-molecule inhibitors are known to exert broad-spectrum protein perturbations. This often leads to off-target effects and systemic toxicity. A landmark study by Abyadeh et al. (2020) and Manica et al. (2023) established that therapies should cause pathway-specific effects rather than global proteome effects. Our data appear consistent with that framework (Abyadeh et al., 2020; Manica et al., 2023) .

Interestingly, the protein that is presumed to be functionally linked to the KRAS G12C signaling network displayed less variability than most other proteins in the experimental conditions. Some experiments are exceptions (sets ~10, ~100 and ~150). Otherwise, overall abundances are stable. The above may highlight that the action mechanism of Berberine involves pathway modulation and not proteomic reprogramming.

Similar findings were seen in Cheng et al. (2015) and Manica et al. (2024) where natural compounds can rewire the proteome in a selective manner without generalized cellular stress (Feng et al., 2023; Manica et al., 2024). Our results are consistent with this emerging viewpoint on precision proteomics in targeted therapy. Berberine treatment GO enrichment analysis showed significant enrichment of genes in terms of calmodulin binding, kinase activity and enzyme regulator activity.

The presence of these enriched terms indicate that Berberine interacts with the KRAS G12C protein and may also affect multiple aspects of its downstream signaling pathways, particularly the MAPK/ERK cascade, pro-apoptotic, and cell cycle checkpoint components. Berberine was previously proposed to exhibit a similar downstream modulation in models of hepatocellular carcinoma by causing apoptosis via suppression of MEK/ERK and PI3K/Akt (Chuang et al., 2017; Alimullah et al., 2023; Tufael et al., 2024).

The enhancement in the functions that bind calmodulin and that are dependent on calmodulin kinase is particularly important. This is because calmodulin plays a central role in calcium-mediated KRAS signaling. These findings are consistent with the study of Sharma et al. (2014) and Manica et al. (2024) which shows that Berberine targets calmodulin-regulated mitochondrial pathways, activating caspase-mediated apoptosis in glioma cells (Sharma et al., 2014; Manica et al., 2024). This indicates that may act on relevant signal transduction cascades that control oncogenic transformation and cell viability other than ligand-target binding. The work of YAO et al. (2018) supports this results that berberine downregulates BCL2 and reduces ERK1/2 phosphorylation, which leads to a reduction in anti-apoptotic capacity of colorectal cancer cells (YAO et al., 2018).

In keeping with this observation, our GO terms enrichment for kinase activity and regulation of molecular function suggests a

similar signaling modifier affecting pathways downstream of KRAS. Enrichment in the larger classes of enzyme regulator activity and molecular function regulator suggests that Berberine may inhibit something other than direct targets. Rather, it may adjust the protein-protein interaction networks. Studies on network pharmacology describe how natural compounds like Berberine are multi-target regulators that can rewire oncogenic circuits (Wu et al., 2016; Akter et al., 2025).

The cellular signaling map produced by this study supports that the modulatory role of Berberine may not be only KRAS G12C inhibiting. There seems to be an interaction involving PIK3CA/AKT, TGF-β/SMAD and WNT/β-catenin that has the potential to influence tumor development and progress in colorectal adeno-carcinogenesis.

These findings are partially supported by earlier studies, G. Li et al. (2021) and Tufael et al. (2025) showed that Berberine could suppress PI3K/AKT signaling to inhibit proliferation in colorectal carcinoma cells (G. Li et al., 2021; Tufael et al., 2025). S. Li et al. (2020), reported Berberine might interfere with TGF-β1-induced EMT and therefore may play a role in metastasis suppression (S. Li et al., 2020). Also, Yalamarty et al. (2023) and Sultana et al. (2024) demonstrated that Berberine inhibits the nuclear translocation of β-catenin in canonical WNT signaling (Yalamarty et al., 2023; Sultana et al., 2024).

This research shows that derivatives of Berberine can offer selective, low toxicity, and multitargeting therapies for targeting mutant KRAS G12C in colorectal cancer. This approach may elicit more effective and sustained responses than conventional single pathway inhibition. Introducing Berberine as a new way to inhibit traditionally "undruggable" targets such as KRAS is an important step forward in cancer therapy.

5. Limitations

The study mainly employed computational approaches molecular docking, dynamics simulations, and SILAC-based proteomic analysis. Still, it does not undergo in vivo and in vitro validation which would be necessary to confirm the efficacy and safety of Berberine derivatives. The study only examined mutations specifically for KRAS G12C and did not analyze the impact of other variants and co-mutations. Also, the duration of the simulation was limited and the solvent conditions were specific, which do not accurately mimic biological conditions.

6. Conclusion

Berberine derivatives appear to be selective and low-toxicity agents for targeting mutant KRAS G12C in CRC; a case study. Findings from computations support the finding of stable binding and modulation of different cell pathways making Berberine a strong multi-target therapeutic candidate. The discovery provides a new concept for further drug development against "undrugged" targets like KRAS. Berberine's natural origin and pathway-selective behavior make it a compelling candidate for further preclinical validation.

Author contributions

S. Hossain conceptualized the study, designed the molecular docking and dynamics workflow, performed computational analyses, and drafted the manuscript. M.T.H.B. Sezan contributed to proteomics data interpretation, Gene Ontology analysis, and critical revision of the manuscript. M.U. Patwary assisted in data curation, literature review, visualization, and manuscript editing. All authors reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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