

NETWORK PHARMACOLOGY-BASED TARGET DISCOVERY REVEALS MULTITARGET ACTIONS OF OXINDOLE DERIVATIVES IN COLORECTAL CANCER

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ABSTRACT – Objective: Colorectal cancer (CRC) is one of the most common cancers worldwide and a major cause of cancer-related death. This study examined the potential anti-CRC effects of the novel oxindole derivatives GIF-0854-r and GIF-0856-r using network pharmacology-based target identification.

Materials and Methods: The putative molecular targets of GIF-0854-r and GIF-0856-r were identified using SwissTargetPrediction, and CRC-associated genes were collected from GeneCards. Overlapping compound-disease targets were determined using Venn diagrams and subjected to protein-protein interaction analysis using STRING and Cytoscape. The predicted compound targets were analyzed for overlap with CRC genes, revealing 150 gene targets.

Results: The PPI network predicted hub targets including STAT3, CASP3, HIF1 α , GSK3 β , ERBB2, MMP9, PARP1, PRKAC α , PIK3C α , and IGF1R. Pathway enrichment revealed proteoglycans in cancer, pathways in cancer, the HIF1 signaling pathway, apoptosis, responses to oxidative stress, ATP binding, kinase binding, transcription factor binding, and tyrosine kinase activity. The results of disease association revealed predicted hub targets that are linked with colorectal neoplasms and other cancer diseases. Molecular docking showed competent ligand-binding efficiency of GIF-0854-r and GIF-0856-r with the active site of GSK3 β .

Conclusions: The results provide evidence that GIF-0854-r and GIF-0856-r may have multitarget anti-CRC effects by regulating critical oncogenic, apoptotic, hypoxia-, oxidative stress-, and kinase-specific signaling pathways.

KEYWORDS: Network Pharmacology, Drug targets, Colorectal cancer, GSK3 β , CASP3, Functional enrichment.

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent cancer after breast and lung cancers, with an estimated 1.9 million cases in 2020¹. About a quarter of patients are diagnosed at an advanced stage, and about 20% of the remainder develop metastatic disease². Therefore, surgical removal alone is challenging and insufficient to control CRC, resulting in high CRC-related mortality². The current 5-year overall survival rate for localized disease is 91%. For local metastatic disease, the 5-year survival rate is 72%. In patients with metastatic colorectal cancer, the survival rate is 15%, with a median survival of roughly 30 months³. Several biomarkers, including genetic and epigenetic factors, have been identified in CRC⁴. To date, no biomarkers in clinical practice can predict chemotherapy resistance. Clinical practice in CRC relies solely on circulating biomarkers and tumor imaging to track treatment response⁵. This reliance leads to late diagnosis of chemotherapy resistance and poor control⁵. Early-onset CRC in people under 50 is rising worldwide, especially in high-income countries⁶. This surge may be due to Western-style eating, obesity, and physical inactivity. These exposures may modify the genetics, epigenetics, gut microbiota, and host immunity of colorectal epithelial cells.



Colorectal cancer resists chemotherapy through several mechanisms, including increased drug efflux *via* transporter proteins such as ATP-binding cassette (ABC) transporters, genetic and epigenetic changes that alter drug targets or cell death pathways, and the tumor microenvironment⁷. Other mechanisms include activation of DNA damage checkpoints, activation of cancer stem cells, metabolic changes that let tumor cells survive drug pressure, epithelial-mesenchymal transition (EMT), inhibition of antiapoptotic regulatory factor overexpression, and maintenance of reactive oxygen species (ROS) levels^{7,8}.

Standard colorectal cancer therapies vary by stage and tumor type⁹. Surgery to remove the tumor and regional lymph nodes is followed by adjuvant chemotherapy with 5-FU, oxaliplatin, or irinotecan to minimize recurrence. Radiation may be needed for preoperative downstaging or for postoperative local recurrence in rectal tumors. Advanced or metastatic colorectal cancer is treated with systemic chemotherapy and targeted therapies such as bevacizumab and cetuximab, which target specific molecular pathways, or with immunotherapy such as pembrolizumab or nivolumab in microsatellite instability-high tumors⁹.

One of the major obstacles in CRC treatment is the toxicity of current chemotherapeutics. CRC chemotoxicity affects treatment compliance, survival, and patients' quality of life¹⁰. About 45.7% of patients developed moderate to severe toxicity, with gastrointestinal toxicity (22.9%) and neuropathy or neutropenia (17.9%). Malnutrition, poor immunological or hepato-renal function, low gut lactobacillus, age, female sex, aggressive chemotherapy, and low quality of life were risk factors¹⁰. This implies the need to develop more effective and less toxic therapeutic options for the treatment of CRC to minimize side effects and complications and to supplement the above-mentioned limiting therapeutic options.

Recently, GIF-0854-r and GIF-0856-r were identified as oxindole derivatives that suppress ER stress-induced cell death and protein aggregation, including superoxide dismutase (SOD) aggregation¹¹. In a separate study, suppression of MnSOD enhanced the efficacy of CRC treatment in cell lines by inducing apoptosis¹².

Because CRC is multifactorial, network pharmacology aims to systematically unravel the multi-target, multi-pathway therapeutic potential of the oxindole derivatives GIF-0854-r and GIF-0856-r. These compounds reduce amyloidogenic protein aggregation, suppress SOD aggregation, and modulate ER-stress-induced cell death, all of which are linked to CRC progression and therapy resistance. We use an integrated network-based approach to identify the putative molecular targets, signaling pathways, and biological processes influenced by these oxindoles and to map their interactions with CRC-specific networks related to oxidative stress, apoptosis, and DNA damage response.

MATERIALS AND METHODS

Compound Target Identification

Potential molecular targets of the chosen bioactive compounds, GIF-0854-r and GIF-0856-r, were identified using the SwissTargetPrediction database (<https://www.swisstargetprediction.ch/>). Each compound's SMILES structure was uploaded to the platform to predict the most likely human protein targets based on chemical similarity and bioactivity information. The analysis produced a list of predicted protein targets for each compound, which were then combined and normalized to remove overlaps.

Disease Gene Collection

The GeneCards database (<https://www.genecards.org/>) was used to retrieve CRC-associated genes. The query was entered as "Colorectal Cancer" to retrieve all disease-related genes. The gene list was converted to gene symbols to ensure consistency across datasets.

Identification of Overlapping Genes

The predicted compound-associated proteins and CRC-related genes shared targets, identified using the Venny 2.1.0 tool (<https://bioinfogp.cnb.csic.es/tools/venny/>). The two sets were then uploaded to generate a Venn diagram that showed common and unique genes between the two lists. The intersection set represented potential therapeutic targets for the compounds and CRC and served as the basis for network and enrichment analysis.

Protein–Protein Interaction (PPI) Network Construction

The overlapping set of genes was added to the STRING database (<https://string-db.org/>) to build a protein-protein interaction (PPI) network. The organism parameter was set to *Homo sapiens*, and a confidence score threshold of 0.4 was applied. The PPI network file was exported and visualized in Cytoscape (version 3.10). Topological parameters of the network (node degree, clustering coefficient, and network density) were analyzed using the NetworkAnalyzer Cytoscape plugin to assess node connectivity and centrality.

Identification of Hub Genes

CytoHubba, as installed in Cytoscape, was used to identify hub genes. The degree centrality algorithm was used to rank network nodes by their interconnections. Genes with the highest degree values were considered hub targets, important molecular nodes that may contribute to the pathogenesis of CRC. The compound-hub gene interaction network was constructed to illustrate the relationships between GIF-0854-r, GIF-0856-r, and their respective hub proteins.

Functional Enrichment Analysis

The Enrichr platform (<https://maayanlab.cloud/Enrichr/>) was used to enrich functional annotations of the hub genes. Gene Ontology (GO) was used to classify genes into Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) domains. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, built into Enrichr, was used to conduct pathway enrichment. The terms and pathways were then filtered using an adjusted *p*-value threshold of 0.05 or lower to identify highly enriched biological categories. The enrichment results were visualized using cluster maps and bar plots generated by Enrichr.

Disease Association and Perturbation Analysis

The relationship between the hub genes and their respective pathological conditions was assessed using the Enrichr disease-gene association module (DisGeNET-based) to evaluate the relevance of the identified hub genes.

Molecular Docking

Molecular docking was performed using Molegro Virtual Docker (MVD) to investigate binding interactions and predict the affinity of GIF-0854-r and GIF-0856-r for GSK3 β . The three-dimensional crystal structure of GSK3 β complexed with its co-crystallized ligand was obtained from the Protein Data Bank using PDB ID: 8AUZ. The protein structure was prepared in MVD by importing the PDB file, retaining the relevant protein chains and binding-site residues, and removing unnecessary molecules and solvent that could interfere with docking. A template docking approach was used to dock the ligands, and docking was performed using the MolDock scoring function. Ligand efficiency values were recorded to assess predicted binding performance relative to ligand size. The co-crystallized ligand was used as the reference for comparison and was redocked. The docking results were reported as the best-scoring poses for each ligand.

RESULTS

Collection and Integration of Compound and CRC–Associated Targets

Target identification was performed using a multi-database strategy that integrates *in silico* prediction and disease-specific gene mining to identify molecular targets that might be modulated by the active compounds. The SwissTargetPrediction tool was used to identify compound-related targets for both lead ligands, GIF-0854-r and GIF-0856-r. To determine the pathogenic context, CRC-related genes were extracted from the GeneCards database, yielding 20,237 putative CRC-specific targets.

Identification of Common Targets and Protein–Protein Interaction (PPI) Network Analysis

To identify molecular targets common to the compounds and CRC, predicted targets from SwissTargetPrediction were cross-referenced with CRC-related genes from the GeneCards database. The overlap analysis identified 150 common targets (Figure 1).

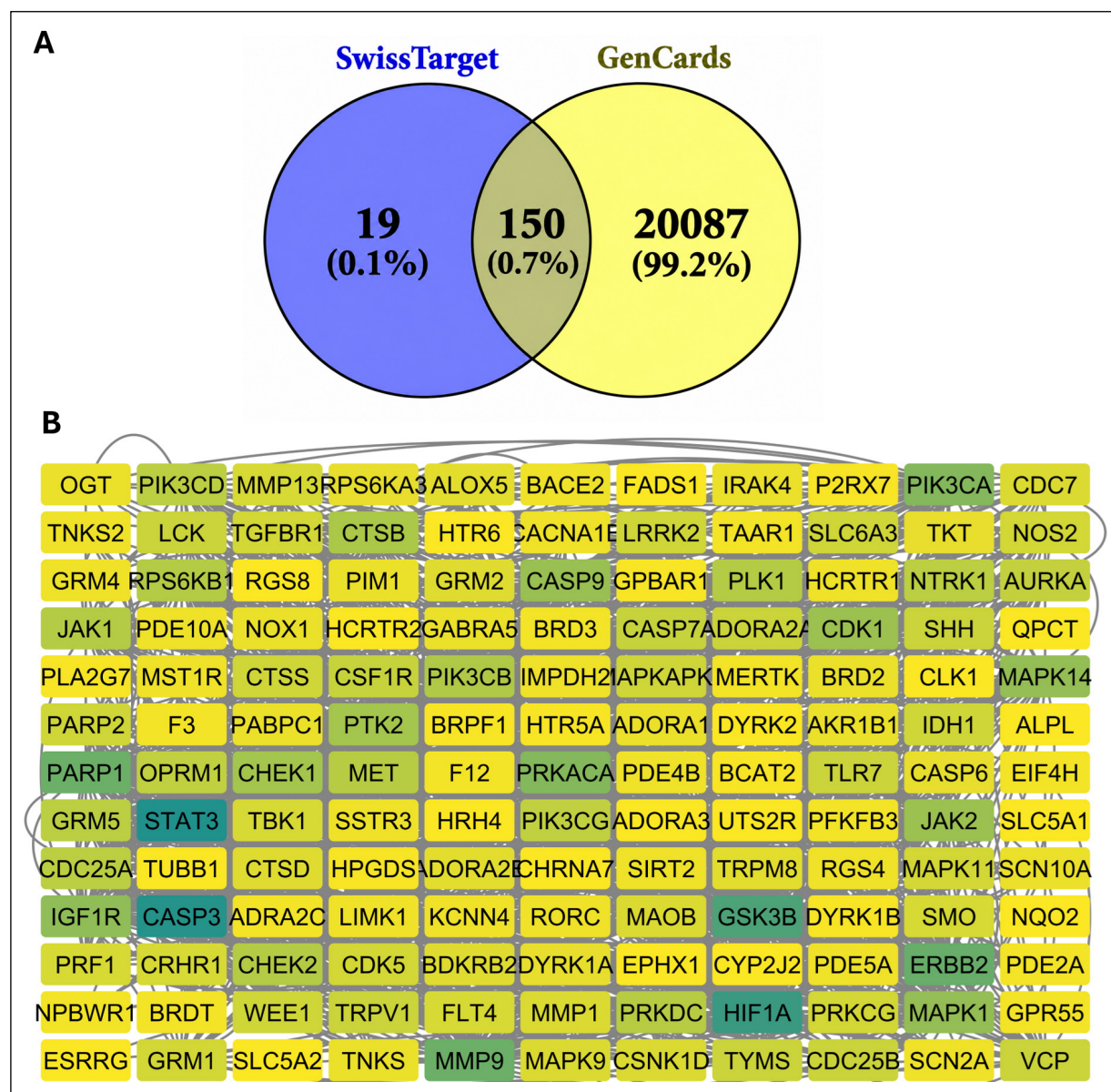


Figure 1. Identification of common targets and construction of the PPI network. **A**, A Venn diagram shows the overlap between predicted compound targets from SwissTargetPrediction and CRC-associated genes from GeneCards. **B**, Cytoscape network visualization highlighting key interacting proteins implicated in CRC pathogenesis.

These overlapping targets were putative therapeutic targets that may mediate the compounds' anti-CRC effects. The overlapping targets were further analyzed using the STRING database, which constructed a PPI network. The resulting network had 150 nodes and 751 edges, with an average node degree of 10 and a local clustering coefficient of 0.519. The anticipated number of edges was 381, yielding a highly significant PPI enrichment p -value of 1.0×10^{-16} . The network showed much higher interconnectivity than expected by chance, indicating strong functional relationships among the identified targets.

Additional topological analysis revealed a few high-degree nodes, or hub genes, that may play a central role in biological processes involved in CRC. The Network Analyzer for the common genes network was also applied, followed by network statistics, as shown in Table 1.

Table 1. The obtained hub targets.

Rank	Name	Degree
1	<i>STAT3</i>	112
2	<i>CASP3</i>	110
3	<i>HIF1α</i>	100
4	<i>GSK3β</i>	90
5	<i>ERBB2</i>	76
6	<i>MMP9</i>	74
7	<i>PARP1</i>	74
8	<i>PRKACα</i>	62
9	<i>PIK3Cα</i>	62
10	<i>IGF1R</i>	56

Identification of Hub Targets and Construction of the Compound–Hub Target Network

To identify significant molecular nodes in the CRC-associated network, we performed a topological analysis in Cytoscape using the CytoHubba plug-in and the degree centrality algorithm. The top ten hub targets identified by the analysis were STAT3 (112), CASP3 (110), HIF1 α (100), GSK3 β (90), ERBB2 (76), MMP9 (74), PARP1 (74), PRKAC α (62), PIK3C α (62), and IGF1R (56) (Table 1 and Figure 2A). Then, the compound-hub target interaction network (Figure 2B) was constructed to visualize the associations between the two active compounds (GIF-0854-r and GIF-0856-r) and their respective predicted hub targets.

The two compounds were identified as potential multitarget binders with putative interactions with a variety of high-degree hubs, including CASP3, STAT3, HIF1 α , GSK3 β , MMP9, PRKAC α , PIK3C α , ERBB2, PARP1, and IGF1R. These multimodal interconnectivities suggest the compounds may have polypharmacological properties and could co-regulate a variety of cancer-associated pathways. Among them, CASP3, STAT3, and GSK3 β appeared to be the most central regulators, suggesting their significance as the main mediators of the compounds' anticancer effects.

Functional Enrichment Analysis of hub Target Genes

To explain the biological significance of the hub genes and CRC, we conducted a comprehensive functional enrichment analysis. We analyzed the results using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and the Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) categories.

The KEGG enrichment results (Table 2) showed that the common hub targets were associated with multiple oncogenic signaling cascades. The most significantly enriched pathway was proteoglycans in cancer, followed by pathways in cancer, prostate cancer, HIF1 signaling pathway, Kaposi sarcoma-associated herpesvirus infection, and Lipid and atherosclerosis. These pathways are mainly implicated in cell proliferation, apoptosis regulation, angiogenesis, and immune signaling, suggesting the broad involvement of the identified targets in CRC pathophysiology.

The GO Biological Process analysis (Table 3) shows that the targets primarily belong to energy metabolism, apoptotic signaling, and oxidative-stress responses. Key enriched processes include regulation of ATP metabolic process, autophagy, response to oxidative stress, and cellular catabolic process, indicating roles in cellular energy homeostasis, mitochondrial function, and adaptive stress response.

GO Cellular Component enrichment (Table 4) showed that most target proteins are in protein kinase complexes and membrane-binding domains, which are required for signal transduction and cell–cell communication.

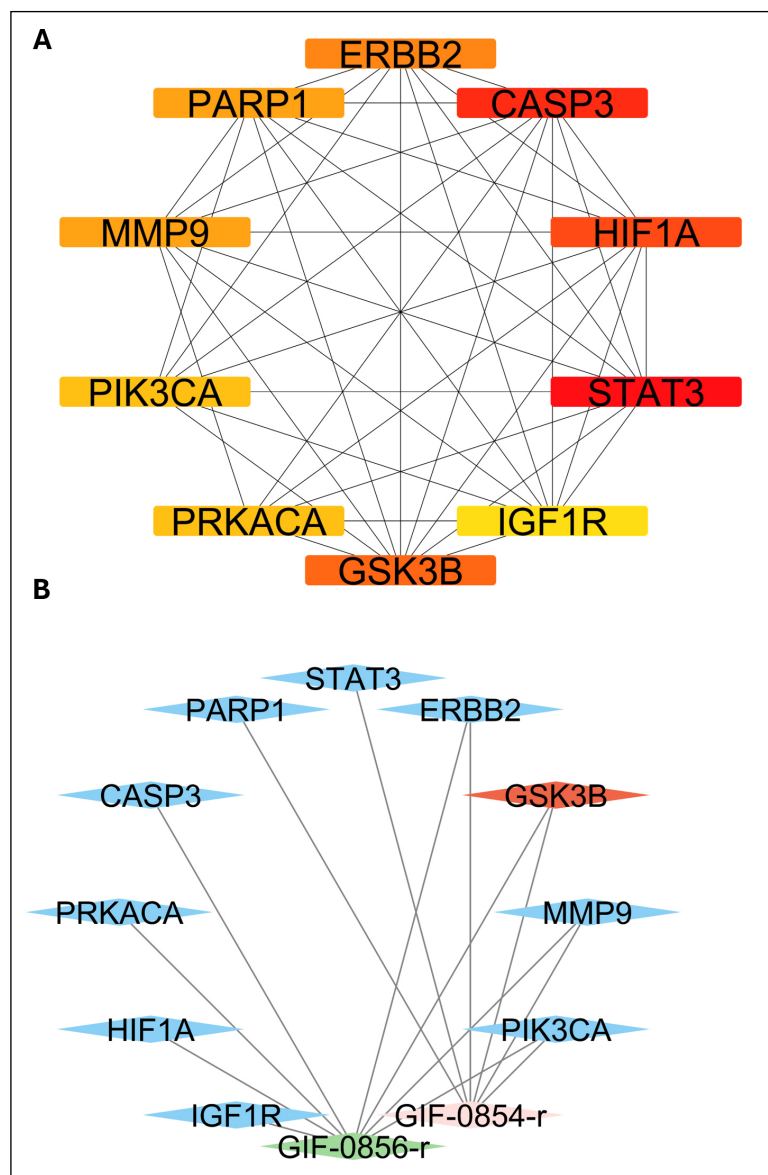


Figure 2. Identification of hub genes and compound–target interaction network. **A**, Top 10 hub genes ranked by degree centrality. **B**, Compound–hub target network showing interactions between GIF-0854-r and GIF-0856-r and CRC targets.

Table 2. The top ten KEGG pathways enriched in the compound–target dataset.

Pathway	Adjusted <i>p</i> -value	<i>p</i> -value	Included hub targets
Proteoglycans in cancer	8.03×10^{-13}	4.69×10^{-15}	PIK3C α , CASP3, STAT3, ERBB2, PRKAC α , HIF1 α , MMP9, IGF1R
Pathways in cancer	5.12×10^{-12}	5.99×10^{-14}	GSK3 β , PIK3C α , CASP3, STAT3, ERBB2, PRKAC α , HIF1 α , MMP9, IGF1R
Prostate cancer	3.41×10^{-8}	5.98×10^{-10}	GSK3 β , PIK3C α , ERBB2, MMP9, IGF1R
HIF1 signaling pathway	4.62×10^{-8}	1.08×10^{-9}	PIK3C α , STAT3, ERBB2, HIF1 α , IGF1R
Kaposi sarcoma–associated herpesvirus infection	6.58×10^{-7}	1.93×10^{-8}	GSK3 β , PIK3C α , CASP3, STAT3, HIF1 α
Lipid and atherosclerosis	9.42×10^{-7}	3.30×10^{-8}	GSK3 β , PIK3C α , CASP3, STAT3, MMP9
Human cytomegalovirus infection	1.01×10^{-6}	4.15×10^{-8}	GSK3 β , PIK3C α , CASP3, STAT3, PRKAC α
MicroRNAs in cancer	4.38×10^{-6}	2.05×10^{-7}	PIK3C α , CASP3, STAT3, ERBB2, MMP9
Growth hormone synthesis and secretion	4.45×10^{-6}	2.43×10^{-7}	GSK3 β , PIK3C α , STAT3, PRKAC α
Thyroid hormone signaling pathway	4.45×10^{-6}	2.60×10^{-7}	GSK3 β , PIK3C α , PRKAC α , HIF1 α

Table 3. The top ten GO Biological Processes associated with CRC targets.

Biological process	Adjusted <i>p</i> -value	<i>p</i> -value	Included hub targets
Regulation of ATP metabolic process	2.33×10^{-5}	6.41×10^{-7}	<i>GSK3B</i> , <i>PIK3Cα</i> , <i>STAT3</i>
Autophagy	6.47×10^{-6}	4.29×10^{-7}	<i>PIK3Cα</i> , <i>PRKACα</i> , <i>HIF1α</i> , <i>IGF1R</i>
Response to oxidative stress	1.04×10^{-4}	8.97×10^{-6}	<i>CASP3</i> , <i>STAT3</i> , <i>HIF1α</i>
Cellular catabolic process	6.69×10^{-4}	3.23×10^{-5}	<i>PRKACα</i> , <i>PARP1</i> , <i>GSK3B</i>
Regulation of apoptotic signaling pathway	9.61×10^{-4}	4.61×10^{-5}	<i>CASP3</i> , <i>PARP1</i> , <i>STAT3</i>
Cellular response to nitrogen compound	1.27×10^{-3}	5.44×10^{-5}	<i>STAT3</i> , <i>HIF1α</i> , <i>MMP9</i>
Cell-surface receptor tyrosine kinase signaling	1.48×10^{-4}	6.19×10^{-6}	<i>ERBB2</i> , <i>PIK3Cα</i> , <i>STAT3</i>
Positive regulation of protein phosphorylation	1.82×10^{-3}	7.13×10^{-5}	<i>GSK3B</i> , <i>PIK3Cα</i> , <i>PRKACα</i>
Mitochondrial energy homeostasis	2.64×10^{-3}	9.22×10^{-5}	<i>PIK3Cα</i> , <i>STAT3</i>
Cellular response to endogenous stimulus	3.01×10^{-3}	1.12×10^{-4}	<i>HIF1α</i> , <i>PARP1</i> , <i>STAT3</i>

Table 4. The top GO Cellular Component significantly associated with the target genes.

Cellular component	Adjusted <i>p</i> -value	<i>p</i> -value	Included hub targets
Phosphatidylinositol 3-kinase complex (Class I)	0.0268	0.00399	<i>PIK3Cα</i>
Protein kinase complex	0.0422	0.0109	<i>IGF1R</i>
Death-inducing signaling complex	0.0268	0.00399	<i>CASP3</i>
Axon	0.0268	0.00497	<i>GSK3B</i> , <i>IGF1R</i>
Intracellular membrane-bounded organelle	0.0313	0.00697	<i>GSK3B</i> , <i>PARP1</i> , <i>CASP3</i> , <i>STAT3</i> , <i>ERBB2</i> , <i>HIF1α</i> , <i>IGF1R</i>
Serine/threonine protein kinase complex	0.0352	0.00752	<i>GSK3B</i> , <i>PRKACα</i>
Plasma membrane-bounded cell projection	0.0403	0.00851	<i>IGF1R</i> , <i>ERBB2</i>
Cell junction	0.0451	0.00947	<i>ERBB2</i> , <i>HIF1α</i>

GO Molecular Function enrichment (Table 5) showed strong enrichment for transcriptional regulation and kinase-binding functions. The first terms in the list, which were RNA polymerase II-specific DNA-binding, transcription factor binding, kinase binding, and protein tyrosine kinase activity, indicated that the hub targets were dual transcription and enzymatic signaling regulators, which were essential to the survival and growth of CRC cells.

Table 5. The top ten GO Molecular Functions associated with CRC targets.

Molecular function	Adjusted <i>p</i> -value	<i>p</i> -value	Included hub targets
RNA polymerase II-specific DNA-binding TF binding	1.96×10^{-4}	3.50×10^{-6}	<i>GSK3B</i> , <i>PARP1</i> , <i>STAT3</i> , <i>HIF1α</i>
DNA-binding transcription factor binding	2.51×10^{-4}	8.96×10^{-6}	<i>GSK3B</i> , <i>PARP1</i> , <i>STAT3</i> , <i>HIF1α</i>
Kinase binding	0.00107	5.74×10^{-5}	<i>GSK3B</i> , <i>PARP1</i> , <i>STAT3</i> , <i>HIF1α</i>
Protein kinase binding	0.00123	8.82×10^{-5}	<i>GSK3B</i> , <i>PARP1</i> , <i>STAT3</i> , <i>HIF1α</i>
Transmembrane receptor protein tyrosine kinase activity	0.00305	2.72×10^{-4}	<i>ERBB2</i> , <i>IGF1R</i>
Protein tyrosine kinase activity	0.0105	0.00113	<i>ERBB2</i> , <i>IGF1R</i>
1-Phosphatidylinositol-4,5-bisphosphate 3-kinase activity	0.0168	0.00250	<i>PIK3Cα</i>
lncRNA binding	0.0168	0.00250	<i>STAT3</i>
Ubiquitin protein ligase binding	0.0247	0.00750	<i>GSK3B</i> , <i>HIF1α</i>
Protein serine kinase activity	0.0249	0.00847	<i>GSK3B</i>

Disease Association Analysis

DisGeNET enrichment analysis of the hub genes revealed a strong disease-association profile, dominated by high-scoring cancer phenotypes (Table 6).

Table 6. The top DisGeNET diseases associated with CRC targets.

Term	Overlap	<i>p</i> -value	Adjusted <i>p</i> -value
Colorectal neoplasms	10/778	7.50E-15	1.43E-11
Chronic lymphocytic leukemia	10/1,120	2.92E-13	2.49E-10
Pancreatic neoplasm	9/665	4.57E-13	2.49E-10
Adenocarcinoma of lung (disorder)	10/1,187	5.23E-13	2.49E-10
Renal cell carcinoma	10/1,348	1.87E-12	6.26E-10
Nasopharyngeal carcinoma	9/782	1.97E-12	6.26E-10
Glioblastoma multiforme	9/854	4.36E-12	1.19E-09
Myeloid leukemia, chronic	9/876	5.48E-12	1.31E-09
Malignant neoplasm of thyroid	8/505	6.73E-12	1.43E-09

The top 10 DisGeNET-enriched disease terms were dominated by malignant and neoplastic conditions, including colorectal neoplasms, chronic lymphocytic leukemia, pancreatic neoplasm, lung adenocarcinoma, and renal cell carcinoma.

Molecular Docking

The molecular docking study showed that the docking poses of the compounds align with the position of the initial co-crystallized ligand (Figure 3). The co-crystallized ligand gave the best MolDock score (-103.723) among all compounds, whereas GIF-0856-r gave a more favorable MolDock score (-95.516) than GIF-0854-r (-90.637) (Table 7). However, GIF-0854-r showed the best ligand efficiency (-4.316), while GIF-0856-r showed (-4.153) and the co-crystallized ligand showed (-4.149). This indicates that GIF-0856-r is closest to the reference ligand, whereas GIF-0854-r shows the best binding efficiency relative to ligand size.

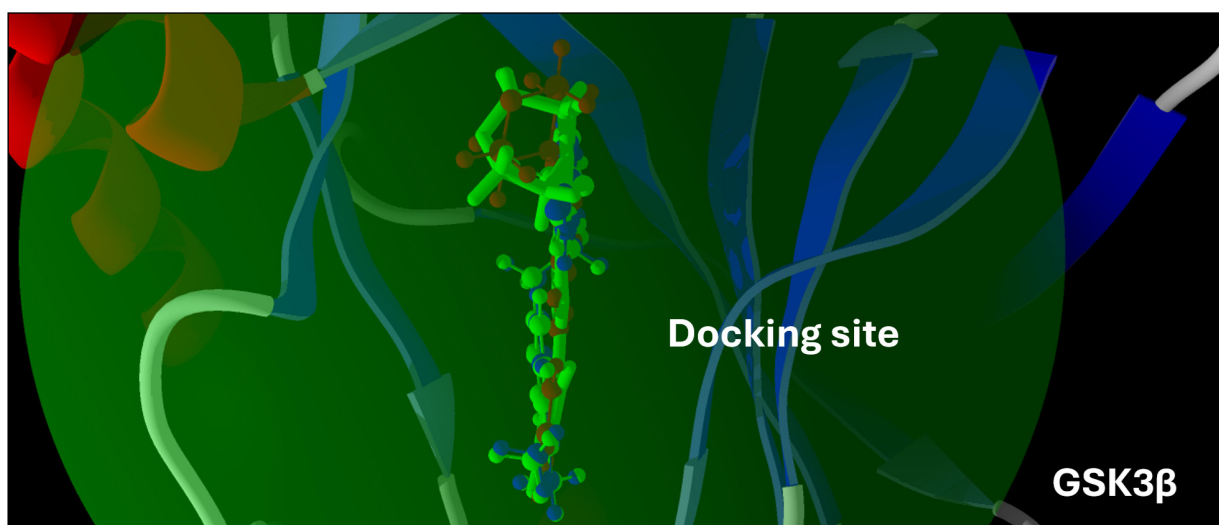


Figure 3. Molecular docking poses of the co-crystallized ligands, GIF-0854-r and GIF-0856-r within the GSK3 β binding site. The three-dimensional ribbon representation illustrates the GSK3 β protein (PDB ID: 8AUZ). The figure highlights the ligands' locations and orientations in the GSK3 β active-site cavity for the co-crystallized ligands (red) GIF-0854-r, (blue), and GIF-0856-r, (green).

Table 7. Molecular docking of the co-crystallized ligand and the two compounds GIF-0854-r and GIF-0856-r using PDB ID 8AUZ of GSK3 β .

Ligand	MolDock Score	Ligand efficiency
Co-crystallized ligand	-103.723	-4.14893
GIF-0854-r	-90.6374	-4.31607
GIF-0856-r	-95.5158	-4.15286

DISCUSSION

A recent study showed that early-onset colorectal cancer incidence rates are increasing, with the rise either exclusive to early-onset disease or faster than the increase in older adults¹³. The complexity of the disease, the fatal outcomes, and the need for effective chemotherapeutics are critical challenges in this field.

In this study, we used a systems pharmacology approach to identify potential CRC mechanisms and the major targets and compounds with therapeutic potential. Our analyses included gene-target networks, biological processes, cellular components, molecular functions, and the identification of overlapping genes between chemical molecules and drug targets. Overall, the findings point to a network-mediated, multi-target mode of action that targets fundamental oncogenic signaling, cellular metabolism, and apoptosis control in CRC. This strategy has been shown to be effective in explaining targets across different diseases and in the discovery of new drugs^{14,15}, including colorectal cancer^{16,17}.

Based on our PPI network constructed from colorectal-cancer-relevant genes and compound targets, we identified a set of hub genes (e.g., *STAT3*, *CASP3*, *HIF1A*, *GSK3B*) that served as central nodes with many neighbors. GSK-3 β activity and levels appear to be perturbed through multiple pathways in CRC tumorigenesis.

STAT3 plays an important role in oncogenesis because it is hyperactive in cancer cells, enhancing cell proliferation and survival¹⁸. In colorectal cancer, the intestinal tumor microenvironment upregulates *STAT3* signaling (primarily through IL-6)¹⁹. Furthermore, activation of *STAT3* is implicated in colorectal cancer stem cell-like properties that support tumor growth and therapy resistance^{20,21}. In colorectal cancer, elevated *STAT3* signaling has been correlated with worse clinical outcomes²². Taken together, these features make *STAT3* and upstream IL-6/JAK signaling appealing candidates for developing targeted therapy for colorectal cancer.

CASP3 is an apoptotic executioner caspase involved in multiple pro- and antitumorigenic processes related to colorectal cancer. *CASP3* activation is widely used as a marker of apoptosis in response to chemotherapy, radiotherapy, and other experimental anticancer treatments in colorectal cancer research²³. However, *CASP3*'s role is not limited to tumor suppression, as evidence indicates tumor progression in cases of *CASP3* activation, suggesting non-apoptotic functions under certain circumstances^{24,25}. *CASP3* is generally considered an apoptotic factor, as its mRNA expression levels were lower in colorectal cancer cells than in normal colorectal cells²⁶. It is thus reasonable to assume that the biological outcome of *CASP3* activation correlates with its potential antitumorigenic role through apoptosis.

GSK3 β has opposing roles, repressing Wnt/beta-catenin signaling on the one hand but maintaining cell survival and proliferation through the NF-kappaB pathway on the other²⁷. Inhibition of GSK3 β activity with chemical inhibitors and RNA interference targeting GSK3 β induced apoptosis and attenuated the proliferation of colorectal cancer cells *in vitro*²⁷. GSK3 β also contributes to the Wnt and Hedgehog (HH) pathways, which regulate cell survival and morphology. These mechanisms are linked to deregulation of GSK3 β in these pathways, which can lead to various cancers in humans²⁸. The role of GSK3 β in neoplastic change and tumorigenesis is disputed, as it has been shown to have both tumor-suppressing and tumor-promoting effects²⁹.

High expression levels of *HIF1A* are a potential predictor of poor prognosis in colorectal cancer. The positive correlation between *HIF1A* levels and prognosis might support the notion that the *HIF1A* signaling pathway promotes more aggressive tumor characteristics, and targeting *HIF1A* might be a viable strategy to treat patients with colorectal adenocarcinoma³⁰. It is suggested that overexpression of *HIF1A* is associated with worse prognosis in patients with colorectal adenocarcinoma, as shown in a study of a large cohort of 731 colorectal cancer patients³¹. Taken together, *HIF1A* can be used as a prognostic predictor for risk stratification and as a therapeutic target for colorectal cancer.

These insights provide a molecular basis for the potential multi-target oxindole hybrids GIF-0854-r and GIF-0856-r as effective anti-CRC agents. We predicted targets for GIF-0854-r and GIF-0856-r, mined a CRC gene signature, constructed a PPI network, identified hub genes, performed enrichment analysis, and suggested synergistic interactions with key regulatory proteins that govern oncogenic and stress-responsive pathways in CRC. Enrichment in signaling pathways associated with cancer, HIF1, apoptosis, oxidative stress, ATP-binding, kinase activity, and transcription factor binding suggests multi-target disruption of several networks critical for CRC survival. This multi-target approach of GIF-0854-r and GIF-0856-r is essential given the complexities of tumor-induced heterogeneity, adaptation to hypoxia, altered apoptosis, and drug resistance to common chemotherapeutic agents, which limit the effectiveness of single-target therapies.

Future work should experimentally validate the predicted compound–target interactions and the biological effects of GIF-0854-r and GIF-0856-r in CRC. To improve the design and future research of oxindole derivatives, further studies could include *in silico* molecular docking and molecular dynamics simulations against the relevant hub targets to determine putative binding interactions and stabilities. Furthermore, *in vitro* and *in vivo* studies should assess selectivity for normal colorectal epithelial cells.

CONCLUSIONS

Based on the integrated network pharmacology approach presented in this study, oxindole derivatives GIF-0854-r and GIF-0856-r are proposed to have multitarget anticancer properties against colorectal cancer. A total of 150 overlapping compound–CRC targets and key hub genes, including *STAT3*, *CASP3*, *HIF1A*, *GSK3B*, *ERBB2*, *MMP9*, *PARP1*, *PRKACA*, *PIK3CA*, and *IGF1R*, indicate that GIF-0854-r and GIF-0856-r could target multiple pathways in CRC, including tumor survival and proliferation, apoptosis, hypoxic adaptation, oxidative stress, kinase activity, and transcription. Disease-gene association and functional enrichment analyses showed that these 150 targets are significantly involved in CRC progression and growth, confirming their pathological role and association with CRC. Taken together, the results provide a computational basis for the multitarget approach of GIF-0854-r and GIF-0856-r in treating CRC; however, further *in silico*, *in vivo*, and *in vitro* validation experiments to assess putative binding interactions, biological activity, selectivity, and therapeutic potential are still warranted.

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The authors declare no conflict of interest.

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Data can be requested from the corresponding author upon reasonable request.

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As this is a computational study, ethics approval and informed consent were not required.

AI DISCLOSURE:

During the preparation of this work, the author(s) used ChatGPT 5.0 and Grammarly to improve readability and language. All data and figures were produced solely by the authors. After using these tools, the authors reviewed and edited the content and take full responsibility for the content of the publication.

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