



RESEARCH

Network-guided identification and multi-targeting of cell cycle regulatory hubs in obesity-linked colorectal cancer

Obezite ilişkili kolorektal kanserde hücre döngüsü düzenleyici merkezlerin ağ rehberliğinde belirlenmesi ve çoklu hedeflenmesi

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Abstract

Purpose: Obesity-associated colorectal cancer (CRC) involves complex molecular networks that limit the effectiveness of single-target therapies. This study aimed to develop an integrative computational pipeline for identifying key regulatory hubs and designing a multi-target-directed ligand (MTDL) capable of simultaneously modulating critical components of the CRC interactome.

Materials and Methods: Differential gene expression analysis between CRC and corresponding healthy tissue-derived samples identified upregulated genes in colon adenocarcinoma. A high-confidence protein-protein interaction network was constructed from these genes to identify central hub proteins using topological analysis. To target these hubs, structure-based de novo ligand design and molecular docking were employed to evaluate binding affinities and multi-target potential. Finally, the candidate MTDL underwent *in silico* pharmacokinetic (ADME) and toxicity profiling, supplemented by a similarity search within natural compound libraries.

Results: A total of 786 upregulated genes were identified, of which 204 formed a dense functional interactome. Three central hubs (CDC6, CCNB1, and CDC25C), all critical to cell cycle regulation, were prioritized as therapeutic targets. The designed MTDL demonstrated favorable binding affinities across all targets, interacting with functionally conserved domains. Furthermore, the ligand exhibited a favorable drug-like profile, characterized by high gastrointestinal absorption and a low toxicity risk. Screening of natural product libraries yielded several

Öz

Amaç: Obezite ile ilişkili kolorektal kanser (CRC), tek hedefli tedavilerin etkinliğini sınırlandıran karmaşık moleküler mekanizmalar içerir. Bu çalışmada, bu mekanizmaların temel düzenleyici merkez proteinlerini belirlemek ve CRC interaktomunun (etkileşim ağı) kritik bileşenlerini eşzamanlı olarak modüle edebilen çok hedefli bir ligand (MTDL) tasarlamak için bütünlük bir hesaplamalı çerçeve geliştirilmesi amaçlanmıştır.

Gereçler ve Yöntem: CRC ve karşılık gelen sağlıklı doku örnekleri arasındaki diferansiyel gen ekspresyon analizi ile kolon adenokarsinomunda ekspresyonu artmış genler belirlenmiştir. Topolojik analiz kullanılarak merkezi (hub) proteinleri saptamak amacıyla, bu genlerden yüksek güvenilirlikli bir protein-protein etkileşim ağı oluşturulmuştur. Bu merkezleri hedeflemek için, yapı tabanlı de novo ligand tasarımı ve moleküler yanıştırma yöntemleri uygulanmıştır. Son olarak, aday MTDL, doğal bileşik kütüphanelerindeki benzerlik taraması ile desteklenen *in silico* farmakokinetik (ADME) ve toksisite profillemesine tabi tutulmuştur.

Bulgular: Toplamda 786 yüksek düzeyde ifade edilen (upregulated) gen belirlenmiş ve bunların 204'ünün yoğun bir fonksiyonel interaktom oluşturduğu görülmüştür. Tamamı hücre döngüsü düzenlemesi için kritik öneme sahip olan üç merkezi hub (CDC6, CCNB1 ve CDC25C), terapötik hedefler olarak önceliklendirilmiştir. Tasarlanan MTDL, fonksiyonel olarak korunmuş alanlarla etkileşime girerek tüm hedeflerde olumlu bağlanma afiniteleri göstermiştir. Dahası, ligand; yüksek gastrointestinal emilim

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analogs with comparable binding profiles, supporting the chemical feasibility of the proposed scaffold.

Conclusion: This study presents a network-guided framework for multi-target drug design in CRC. By shifting from a "one-target, one-drug" model to a systems-level approach, the proposed MTDL and its natural analogs represent promising candidates for experimental validation in the treatment of complex, obesity-associated CRC.

Keywords: Obesity-associated cancer, cell cycle regulation, protein-protein interaction network, multi-target-directed ligand, natural product structural analogs.

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and one of the leading causes of cancer-related mortality, with many patients diagnosed at advanced stages when therapeutic options are limited (WHO: <https://www.who.int/news-room/fact-sheets/detail/colorectal-cancer>; CDC: <https://www.cdc.gov/colorectal-cancer/prevention/index.html>; accessed November 10, 2025). Its incidence continues to rise globally, largely driven by dietary shifts, physical inactivity, and increasing obesity rates. Epidemiological evidence consistently identifies excess body weight as a major modifiable risk factor for CRC. Large meta-analyses demonstrate that obesity - particularly central adiposity - is associated with significantly elevated CRC risk, with clear dose-response relationships between increasing body mass index (BMI) or waist circumference and cancer incidence¹⁻⁵. This association appears stronger in men than in women, potentially reflecting sex-specific fat distribution and the protective effects of estrogens in premenopausal women^{6,7}.

Beyond epidemiological evidence, multiple interrelated biological processes support a causal link between obesity and colorectal carcinogenesis⁸. Metabolic dysregulation represents a central axis: obesity-associated insulin resistance and compensatory hyperinsulinemia chronically activate the insulin/insulin-like growth factor-1 (IGF-1) pathway, promoting proliferation, inhibiting

ve düşük toksisite riski ile karakterize edilen, ilaca benzer uygun bir profile sahip olduğu gözlemlenmiştir. Doğal bileşik kütüphanelerinin taranması, önerilen iskelet yapısının kimyasal uygulanabilirliğini destekleyen ve benzer bağlanma profillerine sahip birkaç analog ortaya çıkarmıştır.

Sonuç: Bu çalışma, obezite ile ilişkili kompleks CRC kanserinde çok hedefli ilaç tasarımı için ağ rehberliğinde (network-guided) bir çerçeve sunmaktadır. "Tek hedef, tek ilaç" modelinden sistem düzeyinde bir yaklaşıma geçişi temsil eden MTDL ve doğal analogları; obezite kaynaklı karmaşık kanserlerin tedavisinde, deneysel doğrulama aşamasına geçilmeye değer, gelecek vadeden aday bileşiklerdir.

Anahtar kelimeler: obezite ilişkili kanser, hücre döngüsü düzenlemesi, protein-protein etkileşim ağı, çok hedefli ligand, doğal bileşik yapısal analogları.

apoptosis, and enhancing survival signaling, which are hallmarks of tumor development^{9,11}.

Adipose tissue also functions as an endocrine and immunological organ. In obesity, hypertrophic adipocytes and infiltrating immune cells secrete elevated levels of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), establishing chronic low-grade systemic inflammation¹². Sustained inflammatory signaling increases reactive oxygen and nitrogen species (ROS/RNS), inducing DNA damage, genomic instability, and oncogenic transformation¹³⁻¹⁵. Concurrently, obesity disrupts adipokine balance¹⁶; leptin levels rise, promoting proliferation, angiogenesis, and anti-apoptotic signaling in CRC^{17,18}, whereas adiponectin - an anti-proliferative and anti-inflammatory hormone - is reduced¹⁹. This imbalance fosters a pro-tumorigenic microenvironment.

Obesity-related alterations in gut biology further contribute to CRC risk^{20,21}. Dysbiosis is characterized by enrichment of pro-inflammatory, potentially oncogenic species, such as *Fusobacterium nucleatum*, which exacerbate mucosal inflammation, impair epithelial barrier integrity, and activate oncogenic pathways²²⁻²⁵. At the same time, beneficial short-chain fatty acid (SCFA)-producing genera, including *Faecalibacterium*, *Roseburia*, and *Eubacterium*, are often reduced. Decreased production of butyrate and other SCFAs compromises epithelial homeostasis and immune regulation, further reinforcing a pro-carcinogenic environment^{26,27}.

Given the rising global burden of obesity and the growing understanding of the molecular networks

linking obesity to CRC, there is a pressing need to identify therapeutic strategies that target their shared pathogenic mechanisms. However, CRC represents a complex, highly interconnected disease system²⁸ where inhibition of a single node often induces the activation of compensatory pathways through feedback loops and signaling crosstalk, thus promoting resistance^{29,30}. In contrast, the simultaneous modulation of multiple critical nodes may more effectively disrupt oncogenic network integrity and yield synergistic outcomes^{31,32}.

Although combination therapy is traditionally employed to achieve multi-target modulation, it is often limited by cumulative toxicity, drug-drug interactions, and increased treatment complexity^{33,34}. An alternative strategy is the rational design of single small molecules capable of interfering with multiple interconnected targets³⁵. Such multi-target-directed ligands (MTDLs) may enhance efficacy while simplifying pharmacokinetic and safety profiles³⁶. While previous studies have independently applied network analysis or molecular docking in colorectal cancer, integrative approaches that simultaneously prioritize disease-associated network hubs and guide multi-target ligand design against those hubs remain limited.

In this context, the present study aims to contribute a comprehensive network pharmacology and computer-aided drug design framework for the identification and prioritization of key proteins within the obesity-associated CRC interactome and the subsequent *de novo* design of multi-target agents directed against them. This study hypothesizes that targeting multiple central nodes within the obesity-CRC molecular network using a single rationally designed ligand will provide a more effective therapeutic strategy than modulation of individual targets alone. Furthermore, we hypothesize that natural product-derived compounds sharing structural similarity with the designed scaffold may possess favorable drug-like characteristics and represent promising lead candidates for future optimization and experimental evaluation.

MATERIALS AND METHODS

All data used in this study were obtained from publicly available databases and resources. As the study relied exclusively on publicly accessible data and did not involve human participants, animal subjects, or identifiable personal information, ethical

approval and informed consent were not required. The research was conducted in accordance with established ethical guidelines and principles of scientific integrity. Appropriate care was taken throughout the study to ensure accurate data handling, proper attribution of sources, and adherence to relevant scientific and ethical standards.

Identification of obesity-related genes

To identify genes associated with obesity, the GeneCards database (<https://www.genecards.org/>; accessed on November 14, 2025)³⁷ was queried using the keyword “obesity”. Genes with a GeneCards Inferred Functionality Score (GIFtS) ≥ 50 were selected for further analysis. The GIFtS score indicates the extent of functional annotation and accumulated knowledge for each gene; thus, this threshold was applied to select genes with substantial biological characterization.

Differential gene expression analysis

Differentially expressed genes (DEGs) between colorectal cancer (CRC) patients and healthy controls were identified using data from The Cancer Genome Atlas (TCGA)³⁸. Since TCGA does not provide a unified CRC dataset, the colon adenocarcinoma (COAD) cohort - the closest available representation - was selected for analysis. Gene expression comparisons between COAD tumor samples (TCGA) and normal colon tissues from both TCGA and the Genotype-Tissue Expression (GTEx) project^{39,40} were performed using GEPIA2 (Gene Expression Profiling Interactive Analysis)⁴¹, an interactive web-based platform integrating TCGA and GTEx RNA-seq data. Differential gene expression analysis was performed using standardized thresholds ($|\log_2FC| \geq 2$; false discovery rate (FDR)-adjusted p-value ≤ 0.01) to ensure reproducibility and comparability across datasets.

In Silico reconstruction of the COAD protein association network and identification of hubs

The upregulated DEGs identified in COAD tumor tissues were used as input for protein-protein interaction (PPI) analysis using STRING database version 12.0⁴². STRING integrates experimentally validated and computationally predicted protein associations derived from curated databases, high-

throughput experiments, and literature mining. To ensure high-confidence interactions and minimize false-positive associations, a stringent confidence score cutoff of 0.85 was applied. Only interactions supported by experimental evidence and curated knowledge evidence were retained. The resulting pairwise PPI data were imported into Cytoscape (v3.10.4)⁴³ for network visualization and topological analysis. Network characteristics, including degree distribution, were examined. Hub proteins were identified using the cytoHubba⁴⁴ plugin, which provides multiple local and global topological ranking algorithms. The top ten hub genes were selected based on the node-degree ranking method. Finally, potential drugs targeting the identified hub proteins were retrieved from DrugBank version 6.0⁴⁵ (<https://go.drugbank.com/>; accessed on December 18, 2025).

Structure-based *de novo* design of an MTDL

The proteins CDC6, CCNB1, and CDC25C were subjected to structure-based *de novo* ligand design and subsequent *in silico* evaluation to identify a potential MTDL.

Preparation of tertiary structures of target proteins

Experimentally resolved three-dimensional (3D) structures of human CCNB1 and CDC6 were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB)⁴⁶; PDB ID: 6GU2 and 8RWV, respectively. Because an experimentally determined structure was unavailable for CDC25C (UniProt ID: P30307), a 3D model was predicted using AlphaFold⁴⁷. Structural quality and completeness were visually inspected prior to downstream analyses.

Multi-target *de novo* drug design

Ligand generation was performed against several structurally distinct targets simultaneously, enabling the exploration of chemical scaffolds capable of accommodating heterogeneous binding environments. *De novo* ligand design was performed using LigBuilder V3⁴⁸, a structure-based platform capable of generating ligands that target multiple proteins or binding sites. Potential binding cavities in CCNB1, CDC6, and CDC25C were identified using the *Cavity* module, which detects druggable pockets based on geometric characteristics, hydrogen-bonding potential, hydrophobicity, and structural

constraints. The entire protein surface was scanned with a probe sphere, and pharmacophore models were constructed from key interaction features. Druggability scores were calculated to prioritize suitable binding sites.

The “*de novo*” design module was applied without a predefined seed. An sp³ carbon atom was randomly positioned within the selected pocket, and molecules were iteratively constructed through fragment-based growth with local energy minimization. Building blocks from the default fragment library were attached using a synchronous growth strategy to generate compounds capable of fitting all three targets.

Ligand optimization was performed using a built-in genetic algorithm involving fragmentation, crossover, and reselection of fitter progeny as new seeds. Iterations continued until convergence and identification of optimal ligand candidates.

The final ligand structure was processed using ChemDraw version 22.2.0 (Revvity Signals Software, Inc.) for chemical nomenclature binding free energies and calculation of basic physicochemical properties.

Molecular docking and binding affinity estimation

Molecular docking analyses were conducted using CB-Dock2⁴⁹, an automated cavity detection-guided blind docking server. This platform identifies potential binding sites, performs docking simulations, and estimates binding affinities expressed as predicted binding free energy values and corresponding pKd scores.

ADME/T prediction

Pharmacokinetic properties and drug-likeness of the designed ligand were evaluated using SwissADME⁵⁰, which predicts key parameters related to absorption, distribution, metabolism, and excretion (ADME), as well as bioavailability and compliance with drug-likeness rules. Toxicological risk assessment was performed using pkCSM⁵¹, which predicts potential toxicity endpoints including hepatotoxicity, cardiotoxicity, mutagenicity, and other safety-related parameters.

Natural compound structural analogs

To support the potential applicability of the novel ligand, we conducted a systematic search for structurally similar analogs derived from natural

products.

Library preparation and filtering

A chemical library containing 695,133 compounds from the COCONUT database (October 2024 release)⁵² was filtered using Lipinski's Rule of Five (RO5) and molecular weight criteria. Compounds were required to have no Lipinski violations (molecular weight ≤ 500 Da, calculated logP ≤ 5 , hydrogen-bond donors ≤ 5 , hydrogen-bond acceptors ≤ 10). Filtering was performed using RDKit version 2024.09.6 (<http://www.rdkit.org/>; accessed on 10 February 2026) with batch processing (10,000 compounds per batch) to manage memory usage.

Query compound

The query molecule ($C_9H_{11}NO_6$, 16 heavy atoms) was loaded from a PDB file, sanitized, and hydrogen atoms were added. Two-dimensional (2D) coordinates were generated for visualization.

Fingerprint-based similarity search

Three fingerprint types were calculated for the query and all filtered library compounds:

- Morgan fingerprints (circular, radius = 2, 2048 bits), which capture functional group connectivity
- RDKit fingerprints (2048 bits), which capture structural features and paths
- MACCS keys (166 bits), which capture predefined structural features

Tanimoto similarity coefficients were calculated between the query and all library compounds using bulk similarity calculations. The top 100 most similar compounds were retained for each fingerprint method.

Molecular docking

From the filtered library, the top 20 compounds identified by each fingerprint method (totaling 60 unique compounds) were selected for docking studies. For each compound, the following preprocessing steps were applied:

- Hydrogen atoms were added
- 3D coordinates were generated (random seed=42)
- MMFF94 force field optimization was applied
- Structures were saved as PDB files

Docking simulations were performed using smina⁵³ (a fork of AutoDock Vina⁵⁴) by focusing on the *de novo*-designed ligand's binding pocket:

- Binding pocket center: x=320.6, y=227.7, z=225.6
- Binding pocket dimensions: $14.0 \times 17.1 \times 16.8$ Å
- Exhaustiveness: default
- Output: 9 binding modes per ligand were generated.

Docking scores were extracted from smina log files using regular-expression (regex) pattern matching. Compounds were categorized relative to the best (lowest) binding affinity (kcal/mol). Subsequent statistical analyses included per-method performance metrics, correlation analysis, and identification of consensus hits.

Visualization of molecular structures

Three-dimensional molecular structures and docking poses were visualized using PyMOL Molecular Graphics System, version 2.5.5 (Schrödinger, LLC). 2D protein-ligand interaction diagrams were generated using LigPlot+ version 2.3.1⁵⁵, enabling detailed analysis of hydrogen-bonds and hydrophobic contacts within each protein-ligand complex.

Identification of amino acid residues mediating multi-target agent-protein interactions

To identify structurally and functionally relevant amino acid residues involved in the interactions between the *de novo*-designed multi-target agent and its target proteins, the protein sequences of CCNB1, CDC6, and CDC25C were analyzed using the NCBI Conserved Domain Database (CDD)⁵⁶. CDD utilizes position-specific scoring matrices to detect conserved domains and annotate residues critical for protein function.

Statistical analysis

Statistical analyses were performed using the algorithms and analytical pipelines implemented in the respective databases and bioinformatics resources, including GEPIA2. The resulting outputs were used for data interpretation and validation throughout the study.

RESULTS

COAD interactome and target selection

A total of 786 genes were found to be significantly upregulated in COAD compared to normal healthy tissue (Table S1). The proteins encoded by these genes may function as key drivers or mediators of COAD progression, rather than acting as mere passive bystanders. Of these, 204 protein-coding genes (PCGs) encode proteins involved in PPIs (Figure 1 and Table S1), forming a large interconnected network, hereafter referred to as the “COAD network,” as well as several smaller subnetworks defined using stringent interaction

criteria. Notably, 70.6% of the nodes within this network are not currently targeted by any marketed or FDA-approved drugs (Figure 1 and Table S2). To identify optimal candidates among the currently undruggable proteins for combinatorial targeting, we concentrated on the most highly connected nodes (hubs), given their central role in maintaining network functionality and structural integrity. Intramodular hubs are characterized by a high number of direct connections within their respective modules, whereas intermodular hubs serve as bridges between distinct modules^{57,58}. Disruption of these critical nodes may therefore perturb COAD network integrity.

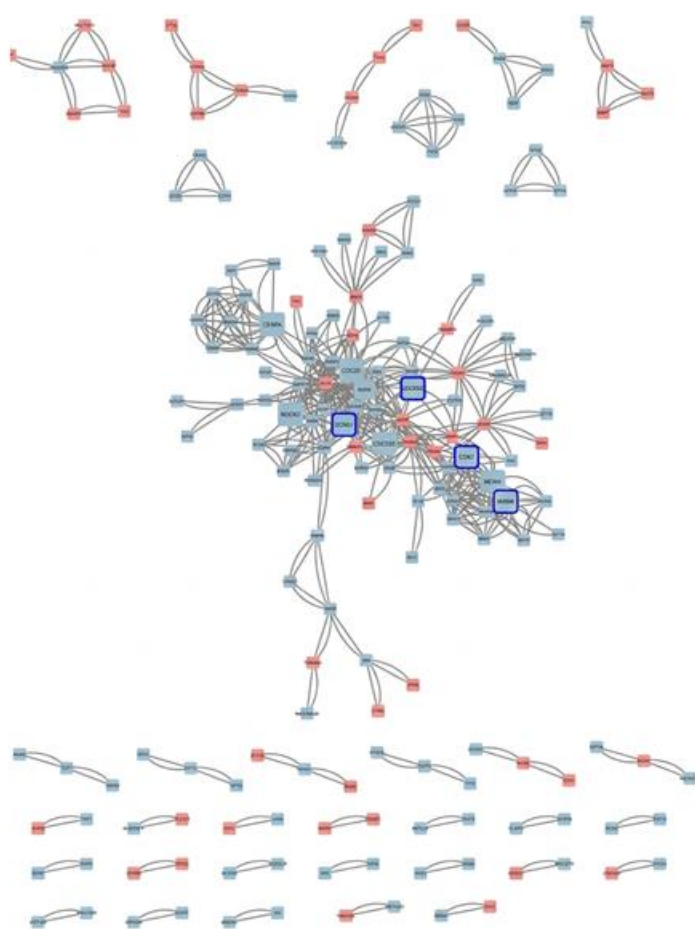


Figure 1. COAD network.

Druggable and undruggable components of the COAD network are depicted in gray and white fill in color, respectively. Nodes represent proteins, while connecting lines (edges) indicate functional associations. The ten selected target proteins are shown as enlarged nodes. The three cell cycle-related proteins prioritized for subsequent de novo multi-target drug design are highlighted with a thicker outline.

To systematically identify the most suitable hubs for *de novo* targeting, the topological characteristics of the entire PPI network were examined. The ten highest-ranking nodes in terms of node degree were: BUB1B, CCNB1, CDC20, CDC25A, CDC25C, CDC6, CENPA, MCM2, MCM4, and NDC80 (Figure 1). Among these, BUB1B, MCM4, CCNB1, CDC6, and CDC25C are also associated with obesity according to GeneCards, based on the predefined selection criteria.

The cell cycle regulatory proteins CCNB1 (Cyclin B1), CDC6 (Cell Division Cycle 6), and CDC25C (M-phase inducer phosphatase 3) were chosen as molecular targets (Figure 1) based on (a) their pivotal roles in cell cycle progression, which is a key process in tumorigenesis^{59,60}, and (b) their central topological positions within the COAD network, acting as both intra- and inter-modular hubs. Simultaneous targeting of these hubs may reduce the likelihood of compensatory network rewiring, a hypothesis that warrants further experimental validation.

Triple-targeting *de novo*-designed agent

We performed multi-target structure-guided *de novo* ligand design to identify a small molecule capable of simultaneously targeting the three cell cycle-related proteins. Although CDC6, CDC25C, and CCNB1 are not considered the classical enzymatic targets with deep catalytic clefts, our structural analysis revealed ligand-accessible cavities suitable for small-molecule binding. Through this approach, the compound (R)-3-hydroxy-2-((3-(hydroxymethyl)-1H-pyrrole-2-carbonyl) oxy) propanoic acid (chemical formula: C₉H₁₁NO₆; molecular weight: 229.06 g/mol) was identified as the top-scoring drug-like candidate for CCNB1, CDC6, and CDC25C (Figure S1).

This *de novo*-designed multi-target agent is hereafter referred to as the “novel ligand.” *In silico* evaluation of pharmacokinetics and drug-likeness indicated the compound had favorable absorption, distribution, metabolism, and excretion (ADME) properties, along with minimal predicted toxicity to vital tissues. According to SwissADME analysis (Figure S2), the compound exhibited high gastrointestinal absorption, no blood–brain barrier (BBB) permeability, no violations of RO5, and a bioavailability score of 0.56. In addition, the synthetic

accessibility score (2.71) suggests that the compound is relatively easy to synthesize.

Toxicity assessment using the pkCSM platform (Figure S2) included evaluation of AMES mutagenicity, maximum tolerated human dose (threshold toxic dose: 0.477 log [mg/kg/day]), hepatotoxicity, and skin sensitization. The novel ligand was predicted to be non-mutagenic, non-hepatotoxic, and non-irritant. Furthermore, toxicity is expected only at substantially elevated doses, indicating a favorable safety profile within the predicted therapeutic range.

To further characterize target engagement, molecular docking simulations were performed to identify the ligand-binding regions and estimate binding affinities. The predicted binding energies (kcal/mol) were -6.8 for CCNB1, -6.6 for CDC6, and -6.3 for CDC25C, suggesting consistent and potentially favorable binding across all three targets and further supporting the multi-target design strategy. The highest-ranked docking poses are shown in Figure 2.

Identification of key protein-ligand interacting residues

Analysis of functional domains using NCBI's CDD revealed that, in CCNB1, all interacting residues are located within the two cyclin box domains, which mediate PPIs essential for cell cycle regulation and transcriptional control⁶¹. In CDC6, the interacting residues are situated in the conserved C-terminal domain, a critical region required for pre-replication complex assembly, origin recognition, and checkpoint regulation⁶².

For CDC25C, key interacting residues (Asp301, Leu302, Lys303, Arg392, Gln408, and Leu409) (Figure 2) are located within the Rhodanese homology domain, which is structurally related to the catalytic core of CDC25 phosphatases and is essential for enzymatic activity⁶³. This domain exhibited a high local confidence score (pLDDT = 92), indicating a highly reliable AlphaFold structural model. Collectively, these interactions within functionally critical domains suggest that the novel ligand may directly interfere with the structural integrity and regulatory functions of these cell cycle proteins.

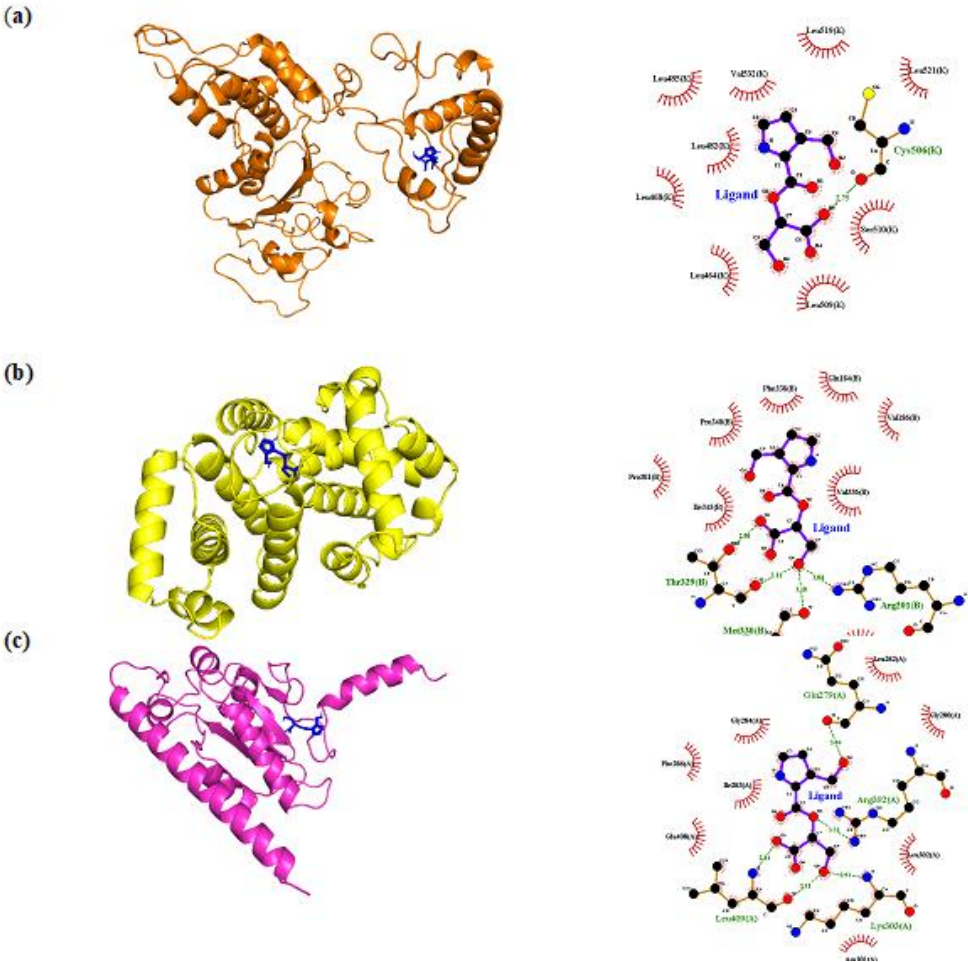


Figure 2. Docking poses of the ligand against CDC6, CCNB1, and CDC25C (a-c).
Left: Cartoon representations of the target proteins with the ligand shown in stick format. Right: 2D schematic views of the corresponding protein-ligand binding interactions. The dotted lines indicate hydrogen-bonds between the ligand and amino acid residues of the target protein. The spoked arcs around residues represent hydrophobic interactions.

Table 1. Top-10 best binding compounds.

Compound (accession identifier)	Method	Binding score (kcal/mol)			Average score
		CDC6	CCNB1	CDC25C	
CNP0002688.1	MACCS	-7.3	-8.9	-7	-7.73
CNP0290056.1	MACCS	-6.9	-8.3	-6.3	-7.17
CNP0258067.2	MACCS	-6.9	-7.6	-6.2	-6.9
CNP0245274.2	MACCS	-7.1	-7.7	-5.9	-6.9
CNP0164427.0	MORGAN	-6.9	-8	-5.8	-6.9
CNP0258067.1	MACCS	-6.8	-7.8	-6	-6.87
CNP0245274.1	MACCS	-6.8	-7.8	-5.9	-6.83
CNP0169230.3	MORGAN	-7.4	-7.6	-5.5	-6.83
CNP0387764.3	MORGAN	-7.4	-7.5	-5.4	-6.77
CNP0230622.2	MACCS	-5.9	-7.5	-5.8	-6.4

CNP: COCONUT Natural Product

Identification of natural structural analogs

A total of 695,133 compounds from the COCONUT database were processed, of which 361,221 compounds (52.0%) satisfied RO5 criteria. Comparative similarity screening revealed no overlap among the three independent top-100 ranked lists (0% overlap) (Table S3), suggesting that the three fingerprinting methods identified substantially different structural neighborhoods. Each fingerprinting approach generated a distinct set of candidate compounds.

Similarity score distributions differed markedly across methods. RDKit and MACCS fingerprints yielded consistently higher similarity scores (0.80-0.83), whereas Morgan fingerprints produced substantially lower values (0.15-0.17).

In total, 60 compounds were selected for docking; 58 were successfully processed, while two failed due to conformer generation errors. The ten structural analogs with the highest predicted binding affinities across all three targets are presented in Table 1, with a median binding score of -6.88 kcal/mol.

DISCUSSION

The global rise in CRC incidence is closely associated with the growing prevalence of obesity, with excess body weight representing a major modifiable risk factor. Conventional therapeutic approaches typically focus on single molecular targets; however, the inherent complexity, redundancy, and adaptive capacity of the CRC interactome²⁸ frequently limit the durability of such interventions. To address this challenge, we implemented an integrated network-based and structure-guided computational strategy to identify and computationally evaluate potential ligands for key nodes at the intersection of obesity and colorectal tumorigenesis. Importantly, the present work does not merely propose a single candidate ligand, but introduces an approach in which disease-specific transcriptomic signals guide network-level target prioritization, which in turn informs structure-based multi-target ligand design.

Transcriptomic analysis revealed 786 significantly upregulated genes in COAD relative to normal colon tissue. Of these, 204 protein-coding genes formed a highly interconnected PPI network. Notably, more than 70% of these nodes are not currently targeted by FDA-approved drugs, highlighting a large therapeutic gap within the CRC interactome.

Topological analysis allowed us to prioritize highly connected hub nodes that play essential roles in maintaining network integrity. Node-degree centrality was selected for hub prioritization since our primary objective was to identify the most interconnected nodes that may function as key regulators within the COAD protein-protein interaction network. Degree centrality is one of the most widely used and intuitive topological metrics for hub identification, as it directly reflects the number of interactions associated with each node. Nodes with high degree values are generally considered biologically important because they can influence multiple molecular pathways simultaneously. Consequently, disrupting such hubs is expected to induce system-wide effects and potentially collapse oncogenic networks^{31,32}.

Notably, several prioritized hubs are implicated in cell cycle regulation and DNA replication and were also associated with obesity-related biological processes, thereby linking metabolic, inflammatory, and microbiome dysregulation to tumor-intrinsic proliferative signaling. The prominence of cell cycle regulators among obesity-associated COAD hubs supports the idea that metabolic and inflammatory inputs reprogram tumor networks toward hyperproliferative states⁶⁴.

Accumulating evidence connects cell cycle regulators to obesity and adipose tissue dysfunction. Beyond their canonical roles in proliferation, cyclin-dependent kinases (CDKs), cyclins, E2F transcription factors, and retinoblastoma (Rb) proteins modulate adipocyte differentiation and adipose biology, with the CDK4-pRb-E2F1 axis driving transcriptional programs that promote adipogenesis⁶⁵. CDK4/6 also regulate adipose metabolism independently of cell division by modulating PPAR γ activity and lipid storage, whereas obesity-associated CDK5 hyperactivation enhances PPAR γ phosphorylation, contributing to both adipogenesis and insulin resistance⁶². Additionally, cell cycle inhibitors such as p21, p27, and p57 play roles in metabolic regulation. For instance, p21 deficiency protects against diet-induced obesity and insulin resistance⁶⁵, while elevated p53 activity in adipose tissue is linked to metabolic dysfunction⁶⁶. Consistently, obese adipose tissue is associated with activation of cell cycle-related gene programs, despite the largely post-mitotic state of mature adipocytes, highlighting non-canonical roles for cell cycle regulators in obesity-associated adipose pathology⁶⁷.

Within this context, CCNB1, CDC6, and CDC25C were selected based on their combined network centrality and biological significance. These proteins orchestrate tightly regulated transitions during cell division: CDC6 is essential for pre-replication complex assembly and proper initiation of DNA synthesis; CCNB1 governs the G2/M transition; and CDC25C activates the CDK1/cyclin B complex through dephosphorylation, thereby enabling mitotic entry^{68,70}. Together, they form a coordinated regulatory axis that ensures faithful DNA replication and mitotic progression, making them attractive targets within obesity-associated CRC networks. Aberrant expression or activity of these proteins is strongly associated with uncontrolled proliferation and tumorigenesis. In particular, CDC6 has been implicated in oncogenic transformation and genomic instability and is considered both a biomarker and functional driver of tumor progression^{71,72}. In CRC, elevated *CDC6* expression correlates with adverse clinicopathological parameters, including metastasis, advanced TNM stage, and reduced overall survival, supporting its diagnostic and prognostic relevance^{73,74}. Moreover, consistent with its pivotal role in G2/M control, CCNB1 is frequently overexpressed in various malignancies, including COAD^{75,76}. Likewise, aberrant CDC25C activity has been linked to enhanced proliferative capacity and aggressive phenotypes in several cancer types⁷⁷.

Although the precise mechanistic connections between these cell cycle regulators and obesity-specific molecular pathways are not fully elucidated, emerging evidence suggests that obesity-associated systemic alterations may influence their expression in tumors. For instance, CDC6 and CDC25C have been found to be differentially expressed in tumors from normal-weight and obese women with invasive breast cancer, indicating that obesity may drive the reprogramming of transcriptional programs, particularly those involved in cell cycle regulation and estrogen-responsive pathways⁷⁸. In adipogenesis models such as 3T3-L1 preadipocytes, transient expression of cell cycle genes including *Cdc25c* occurs during early mitotic clonal expansion prior to terminal differentiation⁷⁹, although this does not imply a direct role in adipogenesis. Cancer genomic analyses further show elevated *CCNB1* expression in tumors from obese compared to normal-weight breast cancer patients, especially in postmenopausal women⁸⁰. Additionally, increased *Ccnb1* expression has been observed in pancreatic β -cells of obese murine models relative to lean controls⁸¹. While these

findings support obesity-related modulation of cell cycle programs, they do not prove a causal role in tumor initiation.

Therapeutically, single-agent inhibition of individual cell cycle components frequently results in compensatory signaling and adaptive resistance²⁹⁻³¹. Given the systems-level complexity of CRC, particularly under obesity-driven metabolic reprogramming^{82,83}, multi-target strategies may offer greater robustness. Obesity-associated tumors may become more dependent on DNA replication and mitotic machinery^{84,85} potentially creating synthetic vulnerabilities within the cell cycle network^{86,87}. Concurrent targeting of CDC6, CCNB1, and CDC25C - and thereby CDK1 activation - could effectively disrupt three core processes of tumor expansion: DNA replication initiation, checkpoint control, and mitotic entry. Compared with combination regimens of separate drugs, a single MTDL may reduce pharmacokinetic incompatibilities, drug-drug interactions, and cumulative toxicity while enabling synchronized target modulation^{35,36}. Accordingly, the MTDL strategy applied herein is intended to explore systems-level modulation of CRC-associated networks rather than isolated linear pathways.

To achieve this, we designed a triple-target agent *ab initio*, with molecular features optimized to fit the structural and physicochemical properties of all three proteins. Analogous to a custom-tailored garment, such compounds may achieve improved binding complementarity and selectivity relative to off-the-shelf alternatives⁸⁸⁻⁹⁰. *In silico* pharmacokinetic profiling predicted favorable ADME properties, drug-like characteristics, and suitability for oral administration, with acceptable preliminary safety parameters and feasible synthetic accessibility, which constitute key factors for translational development. Furthermore, molecular docking analyses demonstrated consistent and favorable binding affinities of the ligand across all three structurally distinct targets. Importantly, the predicted interacting residues were localized within conserved and functionally critical domains, suggesting that the ligand may interfere with key regulatory and enzymatic functions central to tumor cell proliferation.

Although conventional chemotherapy provides measurable clinical benefit, drug resistance and toxicity pose major challenges in CRC management⁹¹. As a result, natural bioactive compounds have gained

attention as complementary or alternative strategies due to their favorable safety profiles and cost-effectiveness⁹¹⁻⁹³. A range of natural products - including bittersweet, curcumin, garlic, ginger, grape, green tea, olives, pomegranate, rosemary, and soybean - have demonstrated anticancer activity against colon cancer⁹⁴⁻⁹⁶. In this context, we explored structural analogs of the *de novo*-designed ligand and identified several natural product-derived candidates with promising predicted interactions. These agents are relatively inexpensive and may offer improved bioavailability, further supporting their relevance as starting leads for future investigation^{97,98}.

However, it is important to explicitly acknowledge that these findings are entirely computational and should therefore be considered hypothesis-generating. Such approaches cannot fully capture the physiological complexity of living biological systems, nor can they definitively assess target engagement, binding kinetics, cellular uptake, biological activity, or safety profiles. Comprehensive *in vitro* and *in vivo* experiments are therefore required to verify the pharmacokinetic stability, off-target selectivity, and therapeutic potential of the designed multi-target agent and its natural analogs. Additionally, mechanistic and translational studies are also warranted to clarify the functional roles of CDC6, CCNB1, and CDC25C in the context of obesity-associated CRC. More broadly, this study highlights the value of integrating disease-context transcriptomics, network topology, and structure-guided ligand generation for the rational development of multi-target therapeutic strategies for complex metabolic malignancies.

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